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Marine Sciences Laboratory

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-Q-003-01

QUALITY ASSURANCE AUDITS OF REPORTS

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	11-24-99
	Signature	Date
TECHNICAL REVIEWER: Richard M. Ecker	RM Ecker	11/24/99
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	11-24-99
	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	RM Ecker	11/24/99
	Signature	Date

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SDMS DocID 25649

QUALITY ASSURANCE AUDITS OF REPORTS

1.0 SCOPE AND APPLICATION

This procedure describes the methods and guidelines for performing quality assurance (QA) audits of Good Laboratory Practice (GLP) reports, and of general MSL reports. Reports must be complete and traceable to the raw data generated.

GLP regulations specify that the QA Unit (QAU) shall "Review the final study report to assure that such report accurately described the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the nonclinical laboratory". So, for GLP projects, all reports shall be reviewed by the QAU.

For gernal MSL reports, the same review policy applies. For non-GLP projects, the QAU will review a report when required by the client, project manager or by project planning documents.

2.0 DEFINITIONS

None.

3.0 RESPONSIBLE STAFF

Study Director
Project Manager
Quality Assurance Unit, or MSL QA Officer(or Represenative) for non-GLP studies

4.0 PROCEDURE

Report Audits are concerned with two primary GLP issues. First, the fulfillment of all requirements as stated in protocols, QA Plans, procedures, test and reporting guidelines, or any pertinent regulation, and secondly, reconciliation of the report to the raw data records for accuracy, traceability and completeness.

4.1 Submission of the Report to QAU

4.1.1 Previous internal iterations and peer reviews must be completed prior to submission of the report to QAU so that the audit is conducted on the actual deliverable report.

4.1.2 Final report submissions should incorporate all previously audited reports and data packages, all un-audited study activity records and the Final or Draft Final Report (see section 4.4.3) as it is to be sent to the sponsor.

4.2 QAU Audit Process for Reports

4.2.1 *For GLP projects* - The QAU shall audit all Final Report packages for the following:

1) 100 percent of the Protocol or regulatory requirements to be addressed in reports will be audited.

2) Attachment 1 will be used as the checklist to verify that all GLP requirements are included in the Final Report. Omission of any of the parameters listed in Attachment 1 may cause rejection of the report package by the QAU.

3) 100 percent of the report text will be checked with data presented in the form of tables or summary statements.

4) 100 percent of the documentation required for Amendments and deviations will be checked.

5) Raw data records submitted with report packages will be audited at the frequency described in SOP MSL-Q-005.

6) **For an amended final report:** The final report must be reissued with

   a) title page referencing the amended report

   b) a page in the front of the report entitled "Amendment(s) to Final Report" that describes the specific part(s) of the report being amended <u>and</u> the reasons for the amendment. This page must be signed and dated by the Study Director, and indicated in the report table of contents.

   c) insertion of the corrected pages or additions in the proper order and correctly numbered.

7) **For terminated or aborted studies:** A final report is required according to GLP regulations for studies that are terminated or aborted. These reports should include name and address of the testing facility, initiation and termination dates of the study, a summarization of data collected to date, the reasons for termination or aborting of the study, compliance, archival and QA statements and be signed and dated by the Study Director.

4.2.2 *For non-GLP projects* - When it is required by the client, project manager of project planning documents, the QAU shall audit the Final Report for the following:

1) The report test will be checked with data presented in the form of tables or summary statements.

2) Raw data records submitted with report packages will be audited at the frequency described in SOP MSL-Q-005.

4.2.3 If, after the audit is initiated, it is the judgement of the QAU auditor that an adequate QC review has not been performed on the submitted report package, the audit will be terminated. The entire package will be returned to the Study Director/Project Manager and management with a written summary describing the reasons for its rejection by the QAU.

4.3 Correction Process for Reports

4.3.1 For GLP projects, the correction process for report packages involves submitting those reports with any discrepancies noted in a QAU Report Review Checklist (Attachment 1) along with the corrected pages. The entire report should then be assembled for a final check by the QAU for completeness and page numbering. Once the correction process is complete, the QA Statement is signed and the draft or final report is ready for release as described in section 4.4.

4.3.2 For non-GLP projects, the correction process for reports involves submitting the reports with any discrepancies and/or comments noted either in a memroandum reconciling the review comments or as an attachment to the report.

4.4 Release of GLP Reports by the QAU

4.4.1 No study report will be released by the QAU while there are still outstanding audit comment sheets from QAU Inspection Reports. All issues needing a corrective action or response must have been resolved prior to QAU signature approval.

4.4.2 All Final Reports shall include a QA Statement that is signed by the QAU. This statement (see Attachment 2 for an example) will be drafted by the QAU based upon the QA inspections performed for that particular study. This statement will include a complete list of laboratory, field and data audits by brief description, when the phase was inspected and when the inspection reports were reported to the Study Director and to Management.

4.4.3 All Final Reports shall also include a Good Laboratory Practices Compliance Statement. (see Attachments 3 and 4 for examples) signed by the Study Director.

4.4.4 Draft Final Reports may be sent to the client for review and comments before the actual final report is issued by the Study Director. This is in response to the regulation specifying that any corrections or additions to a final report shall be made in the form of an amendment by the Study Director. A report amendment must clearly identify that part of the final report that is being added to or corrected and the reasons for the correction or addition, and shall be signed and dated by the person responsible.

Draft Final Reports must have the same elements as the official final report document, including a signed Quality Assurance Statement. However, because the signature of the Study Director on the Final Report indicated the completion date for that study, the Draft Final is sent to the sponsor without that signature in place on the title page or on the GLP Compliance Statement.

4.5 Initiation of the Archive Process for GLP Projects

4.5.1 According to regulations, all raw data, documentation, protocols, specimens and final reports are transferred to the archives prior to the signing of the GLP Compliance Statement by the Study DIrector. While materials may be submitted to the QAU Archives during the conduct of a study, the completion date should indicate that the archive process for a given study has been completed.

4.5.2 When the Final Report is signed by the Study DIrector, a copy of the signature page should be sent to the QAU. This will serve to place the completion date for the study on the Master Schedule and also alert the Archivist that study records, data, Protocol, Amendments and all Records to be maintained should already have been submitted to the archives and that the only item outstanding is a copy of the final report.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Not applicable.

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

Quality Assurance Unit personnel who will be performing QA audits of reports and all Study Directors must first read this SOP prior to performing the audits and issuing the final report. Documentation of training will be recorded on a reading assignment form from procedure MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

None.

ATTACHMENT 1
EXAMPLE REPORT REVIEW CHECKLIST

TEST ARTICLE _____ REPORT TYPE _____ _____

STUDY TITLE _____

SPONSOR _____ STUDY NO. _____

QA AUDITOR _____ DATE _____

_____ Name and address of the facility performing the study and the dates on which the study was initiated and completed.

_____ Objectives and procedures stated in the approved protocol, including any changes to the original protocol.

____ Statistical methods employed for analyzing the data.

____ The test and control articles identified by name, chemical abstract number or code number, strength, purity, and composition or other appropriate characteristics.

____ Stability of the test and control articles under the conditions of administration. EPA reports must include solubility of the test and control article under the conditions of administration when this information is relevant to the conduct of the study.

____ A description of the methods used.

____ A description of the test system used. Where applicable, the final report shall include the number of animals used, sex, body weight range, source of supply, species, strain and substrain, age, and procedure used for identification.

____ A description of the dosage, dosage regimen, route of administration, and duration.

____ A description of all circumstances that may have affected the quality or integrity of the data.

____ The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.

____ A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

____ The signed and dated reports of each of the individual scientists or other professionals involved in the study.

____ The location where all specimens, raw data, and the final report are to be stored.

____ The statement prepared and signed by the QAU.

____ Signature and date of the study director.

____ Corrections or additions to a final report shall be in the form of an amendment.

ATTACHMENT 2

EXAMPLE QUALITY ASSURANCE STATEMENT

STUDY NUMBER: _____

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management

_____

Quality Assurance Unit Date

ATTACHMENT 3
EXAMPLE GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted according to the principles of Good Laboratory Practices and is in compliance with **(reference GLP regulation)**.

_____

(Name) Date
Study Director

SPONSOR CERTIFICATION OF GOOD LABORATORY PRACTICE*

To the best of my knowledge, the Good Laboratory Practice Compliance Statement signed by the study director is truthful and accurate.

_____

Sponsor Date

_____

Submitter Date

* The sponsor certification is a requirement of EPA GLP Standards only, as found in 40 CFR Parts 160.12 and 792.12.

ATTACHMENT 4
EXAMPLE GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The following two examples apply only to EPA GLP studies to which full GLP compliance cannot be assigned. Both examples are referenced in 40 CFR parts 160.12 and 792.12. Headers and signatures would be identical to those indicated in Attachment 3.

Example 1.

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted according to the principles (or requirements) of 40 CFR Part 160 with the following exceptions:

1. _____

2. _____

3. or whatever number of exceptions are required to be listed.

Example 2.

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The submitter of this study was neither the sponsor of this study, nor conducted the study, and does not know whether the study has been conducted in accordance with 40 CFR Part 160.

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EFFECTIVE DATE: 5-15-00

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-Q-004-02

QUALITY OF TESTING WATER AND FEED

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	5-11-00
	Signature	Date
TECHNICAL REVIEWER: Jeff A. Ward		05-15-00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	5-11-00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff A. Ward		05-15-00
	Signature	Date

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QUALITY OF TESTING WATER AND FEED

1.0 SCOPE AND APPLICATION

This procedure describes the minimum requirements and criteria for ascertaining that the natural seawater and/or freshwater used in toxicological investigations is of an acceptable quality to assure that healthy test animals will survive in it for the duration of acclimation, holding, and testing, without showing signs of stress, such as discoloration or unusual behavior. When applicable, this procedure also applies to the analysis of feed used during Good Laboratory Practices (GLP) regulated bioassay and toxicological tests.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

Study Director
Quality Assurance Unit or MSL QA Officer

4.0 PROCEDURE

4.1 Water Analysis

4.1.1 Sequim Bay seawater is used in bioassay and toxicity tests as a natural dilution water. Seawater is considered to be of constant quality when the weekly range of salinity is less than 6 $^{o}/_{oo}$, and when the monthly range of pH measuremnts is less than 0.8 pH units (ASTM 1980). At the Battelle Marine Sciences Laboratory (MSL), natural seawaterhas beem monitored over a long period and has been shown to consistently be well within these ranges. Therefore, the Sequim Bay seawater can be considered to be of constant demonstable quality.

4.1.2 Both fresh and seawater used in bioassay and toxicity tests should be acceptable to test organisms to ensure that 1) test organisms are not stressed during holding acclimation and during the test period, and 2) that test results are not affected by water quality. These criteria are satisfied if it can be demonstrated that test organisms exposed to the water will survive in the control containers at acceptable mortality levels during toxicity tests. At the MSL, this criterion has been met consistently, as acceptable test organism survival in control water has been observed since 1980.

4.1.3 The following parameters are monitored routinely during specific bioassays: pH, salinity, dissolved oxygen, and temperature. The following procedures provide the necessary steps for instrument calibration and use:

- MSL-W-001 Calibration and Use of pH Meters
- MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
- MSL-W-003 Calibration and Use of Thermometers, and
- MSL-W-004 Calibration and Use of Refractometers
- MSL-W-007 Routine Water Quality Measures for Toxicity Tests

Specific tests may require additional analyses such as ammonia which is conducted using the MSL procedure,

- MSL-W-008 Determination of Ammonia

To verify further that contaminants are not present in fresh and seawater at levels which may be harmful to sensitive marine organisms, the MSL Project Manager and QA Officer (QA Unit for GLP studies) shall ensure that representative fresh and seawater samples be collected every 12 months in a manner described below:

1. Containers are cleaned and preserative pre-added according to the analyte being measured (refer to Table 1.)

2. Water samples are taken from a glass aquarium (cleaned according to SOP MSL-C-011, Glassware and Equipment Cleaning Procedures) in which water flows continuously for at least 24 hours prior to sampling. This water should be passed through the same sand filter, boiler, pipes, etc. as the water used during the toxicity tests to ensure that it is representative of the water used in static or flow-through bioassays.

3. As a minimum, fresh and seawater samples collected will be analyzed for the constituents listed in Table 1. The maximum allowable concentrations listed in Table 2. for metals and ammonia are based on EPA's Water Quality Criteria Summary table (Marine & Fresh Chronic Criteria).
   The constituents were chosen because they have the highest potential of all constituents of being in the water due to the unlikely chance of an oil spill or degradation of pipes/equipment in the sea and freshwater systems. Other constituents can be analyzed at the discretion of the MSL Project Manager.

4. A Chain of Custody form shall accompany all samples that are submitted for chemical analysis. Refer to the form and the requirements for its use in procedure, MSL-A-002, Sample Chain of Custody.

5. If the sampling of fresh and seawater is applicable to a GLP study, then the sample container preparation procedure, sample collection, and sample ID are documented on a sample analysis form (Attachment 1) which is kept in the QAU files along with the analytical results. In addition, the GLP Study number and the fact that the samples are GLP-study related should appear on the Chain of Custody form.

Table 1. Sample Preservation Requirements

Analysis	Container	Preservation
Metals	Fluoropolymer (FEP; Teflon™), conventional or linear polyethylene (PTFE; Teflon™), polycarbonate or polypropylene 500 or 1,000 ml bottles with lids	10% Ultrapure Nitric Acid (HNO_3)
TOC, Ammonia	Fluoropolymer (FEP; Teflon™), conventional or linear polyethylene (PTFE; Teflon™), polycarbonate or polypropylene 500 or 1,000 ml bottles with lids	Sulfuric Acid (H_2SO_4)
PAHs	Amber Glass with Teflon™, Lid	Hydrochloric Acid (HCl)

Table 2. Minimum Fresh and Seawater Sample Analysis Parameters

Chemical Parameter	Maximum Allowable Concentration
SEAWATER	
PAHs[1]	50 µg/L
Ammonia	2 mg/L
Total Organic Carbon (TOC	5 mg/L
Metals	
Cd	9.3 µg/L
Cr	50 µg/L
Cu	2.9 µg/L
Fe	--
Hg	0.025 µg/L
Ni	8.3 µg/L
Pb	5.6 µg/L
Zn	86 µg/L
FRESHWATER	
PAHs[1]	50 µg/L
Ammonia	2 mg/L
TOC	5 mg/L
Metals	
Cd	1.1 µg/L
Cr	11 µg/L
Cu	12 µg/L
Fe	1000 µg/L
Hg	0.012 µg/L
Ni	160 µg/L
Pb	3.2 µg/L
Zn	110 µg/L

[1] PAH Polycyclic Aromatic Hydrocarbon

4.2 Feed Analysis

4.2.1 When required by the sponsor or GLP regulations the Study Director shall have the feed used to feed organisms during acclimation and testing analyzed to help ascertain that contaminants are not present.

4.2.2 As a minimum, the feed shall be analyzed for the following constituents: PCBs, Pesticides, PAHs, Cd, Cr, Cu, Fe, Hg, Ni, Pb, and Zn. Other constituents can be analyzed at the discretion of the Study Director or as otherwise specified by the sponsor. Contact the selected analytical laboratory for recommendations regarding sample collection, containers, preservation, and shipping requirements.

4.2.3 All feed samples shall be accompanied to the analytical laboratory with a Chain-of-Custody Form (as per MSL-A-002).

4.2.4 All records pertaining to the feed analysis shall be kept with the study files and shall be traceable to feed manufacturer and feed lot number, if applicable.

5.0 DATA ANALYSIS AND CALCULATIONS

None

6.0 QUALITY CONTROL

If samples are analyzed by a laboratory other than Battelle MSL, the contract laboratory must be able, upon request, to provide evidence of being capable of performing high-quality chemical analyses. The laboratory must be able to provide records of methods used, calibration curves, method and instrument detection limits, and data sheets. The final data report received from the outside analytical laboratory should contain a reference of the methods used for chemical analyses, quality control data, and the results of the analyses. All documentation obtained will be kept in the QAU files. Analytical services are to be obtained following the requirments specified in MSL-A-012, Procurement.

7.0 SAFETY

None

8.0 TRAINING REQUIREMENTS

All Study Directors, Quality Assurance Unit personnel and anyone who will be responsible for obtaining or submitting water or feed to analytical labs for analysis shall first read this

procedure and document the training as a reading assignment on an Individual Training Assignment Form or a Group Training Documentation Form as described in MSL-A-006.

9.0 REFERENCES

MSL-A-002 Sample Chain-of-Custody
MSL-A-006 Marine Sciences Laboratory Training
MSL-A-012 Procurement
MSL-C-011 Glassware and Equipment Cleaning Procedures
MSL-W-001 Calibration and Use of pH Meters
MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
MSL-W-003 Calibration and Use of Thermometers
MSL-W-004 Calibration and Use of Refractometers
MSL-W-007 Routine Water Quality Measures for Toxicity Tests
MSL-W-008 Determination of Ammonia

ASTM 1980Committee E-35. Standard Practices for Conducting Static Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. ASTM Method E-729.

ATTACHMENT 1

BATTELLE MARINE SCIENCES LABORATORY
WATER OR FEED ANALYSIS FORM

Analysis of: Seawater Freshwater Feed

Sample Number:

Each sample collection period is identified with a unique code number **as follows:**

1) MSL
2) Sea Seawater
   F - Freshwater
   Feed- Feed
3) Month Number (1-12)
4) Year

Example: A seawater sample collected in February, 2001 would be **identified as either MSL-S-02-2001** or MSL-S-02-00.

Purpose of Sample:

Collection Method:

Sample Collected By: Date:

Method of Delivery: Date of Pick-up:

Container Preparation:

Results Attached:

Date Received: Number of Pages:

EFFECTIVE DATE: 10-13-99

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Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-Q-005-01

QUALITY ASSURANCE DATA AUDITS

Author:	Deborah Coffey Printed Name	Deborah Coffey *Signature*	10-1-99 Date
Technical Reviewer:	Rob Cuello Printed Name	*Signature*	10/4/99 Date
QA Officer:	Deborah Coffey Printed Name	Deborah Coffey *Signature*	10-13-99 Date
Technical Group Manager:	Dick Ecker Printed Name	RM Ecker *Signature*	10/13/99 Date

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QUALITY ASSURANCE DATA AUDITS

1.0 SCOPE AND APPLICATION

Audits of reported data (e.g., tables, figures, spreadsheets, databases) are conducted by the Marine Science Laboratory (MSL) Quality Assurance (QA) staff to ensure that the data are accurate, traceable, defensible, and complete, as compared to the planning documents and/or project requirements. This procedure describes the methods and guidelines that are to be used when conducting final QA data audits. QA data audits are required for all Good Laboratory Practice (GLP) regulated studies and whenever requested by MSL management and/or the sponsor.

2.0 DEFINITIONS

None.

3.0 RESPONSIBLE STAFF

- MSL Quality Assurance Staff
- Project Manager/Study Director

4.0 PROCEDURE

4.1 Data Receipt and Log In

4.1.1 All data to be audited must be validated and reviewed by the technical staff before being submitted to the QA staff. The data validation process performed by technical personnel may include, but is not limited to, verification of transcription, double keyboarding, identification and removal of outliers or spurious data points with a reason for removal, assessment of quality control (QC) results, and evaluation of scientific validity.

4.1.2 The data package should contain all of the original data necessary for the reconstruction of the task or project. Documentation of dates of sample receipt and analysis are key for determining holding time compliance. Formulas for all calculations must be documented in standard operating procedures (SOPs), the planning documents, or in the data package. It is the responsibility of the Project Manager/Study Director to ensure that the data are complete and organized in such a manner that the auditor will be able to trace data without assistance. Upon receipt, the data package is logged in by description (e.g., project, task, type of data) and date of receipt.

4.2 Selection of Data Points to be Audited

The audit procedure is a randomized check that involves comparing selected reported values to the original data. This check can either be performed randomly (4.2.1) or on a statistical basis (4.2.2).

4.2.1 Random Check of Data

4.2.1.1 For routine data audits that don't have specific customer- designated data validation requirements, the auditor randomly chooses the number of data points to be audited.

4.2.1.2 Upon receipt of the data, the auditor determines the total number of data points to be audited based on the type of data set. Factors that may influence this determination include, but are not limited to, type of analyses, electronic transfer of data throughout the analytical and data management process, and level of preliminary data validation by technical staff.

4.2.2 Statistical Check of Data

4.2.2.1 For GLP projects or when a statistically-valid check of data is requested by the Project Manager/Study Director or client, the sampling process will be based on Modified British Standard 6001 as described by Hoover and Baldwin (1984). This approach is designed to ensure a 95 percent chance of detecting whether one percent or more reported values disagree with the original data.

4.2.2.2 Upon receipt of the data, the auditor determines the total number of data points in the data set. A data set may be defined as all values reported for a project or task, or any subset of those values, at the discretion of the auditor or on request of the Project Manager/Study Director. A description of data included in each data set and number of data points per set is noted.

4.2.2.3 Once the total number of data points have been defined, the auditor determines the number of points to be checked (see Attachment 1). For example, if the data set contains a total of 300 data points, 50 of those points will be audited. The specific points to be audited may be randomly selected or selected using a random number table or a random number generator. These randomly selected points are highlighted on the documentation.

4.3 Auditing the Data

4.3.1 Each highlighted reported value is checked to ensure that it accurately reflects the original data and is traceable throughout its history. For reported values that involve calculations, the auditor repeats the calculation. Values that result from manipulations involving complex software, or that are derived from high-rate electronic data acquisition system are not covered by this SOP.

4.3.2 Errors in reported data are defined as any values that cannot be derived from the original data, including, for example, miscalculations and transcription errors. The reviewer may request clarification from staff during the review to resolve questions. If the auditor finds the number of errors in the highlighted data points to be greater than the acceptance criteria (as defined in Attachment 1), the data are judged to be unacceptable and the audit terminated. The audit may also be terminated if the reported values cannot be traced back to the original data. Errors in data points other than those highlighted are noted by the auditor, but are not used as the basis for rejecting data.

4.3.3 If a data set is determined to be unacceptable, the data are returned to the Project Manager/Study Director who is then expected to review, or have someone else review, the entire data set and make the necessary corrections. The corrected data are next returned to the auditor, who must complete the audit process again.

4.4 Scheduling

4.4.1 Reported data audits may be performed at any stage of a project. Ideally, the audit should be performed after final tables and figures have been prepared, but before the accompanying text (if any) has been finalized. However, audits may also be performed on interim reported values (e.g., spreadsheets, hand-written tables). If this occurs, the final product must be submitted to the MSL QA staff for verification against the previously audited interim data.

4.4.2 The Project Manager/Study Director is responsible for notifying the MSL QA staff of data delivery dates and for informing all affected persons of any major changes in the schedule. Data are usually audited on a first-come, first-serve basis; however, project deadlines and staff availability are also considered when scheduling audits. Any conflicts are resolved through discussions with Line Management.

4.5 Documentation and Reporting

4.5.1 Upon completion of the audit, the data tables are marked to indicate the results of the audit (acceptable, acceptable with corrections, or rejected) and are

initialed and dated by the auditor. The auditor may then prepare an audit report (Attachment 2) in the case of GLP studies, or a Data Package Checklist (Attachment 3) for routine sample analyses. Both documents will specify whether the data were accepted or rejected and, when accepted, will describe any errors that were found. If the data were rejected, the audit report does not identify specific errors; however, the type of error (e.g., transcription, calculation) may be identified. The report also describes any documentation issues that must be addressed to ensure the traceability and defensibility of the data. If a report is prepared. the report is signed and dated by the auditor and sent, with the data package, to the Project Manager/Study Director. The Project Manager/Study Director is responsible for assigning project personnel to respond in writing to audit findings. The completed audit report, including responses, is then signed and dated by the Project Manager/Study Director and must be returned to the MSL QA staff within a predetermined time period (approximately 10 days). Upon receipt of the audit report, the MSL QA staff will verify any corrections that were necessary and review the audit responses to ensure they are complete and appropriate. Outstanding issues are resolved through discussions with the Project Manager/Study Director or appropriate Line Manager, if necessary. When a checklist is used and there are no unresolved issues, the checklist is filed by the QA staff. Issues that can not be resolved will be documented as deviations as per MSL-A-005, Deviations from Stated Requirements.

4.5.2 The original audit reports are maintained by the MSL QA staff. Project staff maintain files of the audited data package. Copies of the audit reports may be distributed to the Laboratory Manager, Project Manager/Study Director and appropriate Line Managers.

4.6 **Personnel and Responsibilities**

Audits are performed by the MSL QA staff. Technical personnel may perform specific audit procedures (e.g., selecting and highlighting random numbers, checking calculations) under the direction of the MSL QA staff. Technical personnel who have been involved in the generation of a specific data set may not perform audits on that data.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Not applicable

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

Personnel who are being trained to perform auditing tasks must first read this SOP. They may then perform specific tasks under the supervision of the MSL QA staff. Auditing tasks performed by the trainee will be reviewed and co-signed by the MSL QA staff until it has been established that the trainee is able to perform these tasks without supervision.

9.0 REFERENCES

Hoover, B.K. and J.K. Baldwin. 1984. *Meeting the Quality Assurance Challenges of the 1980s: Team Auditing by Toxicologists and QA Professionals*. J. Am. Coll. of Tox. 3:129-139.

Attachment 1

Modified British Standard 6001

Total Data Points in Data Set	Sample Size to be Inspected	Acceptable Number Number of Errors	Errors for Rejection of Data Set
2-50	100% Inspection	-	-
51-90	13	0	1
91-150	20	0	1
151-280	32	1	2
281-500	50	1	2
501-1,200	80	2	3
1,201-3,200	125	3	4
3,201-10,000	200	5	6
10,001-35,000	315	7	8

Note: This sampling approach assures that the data set is acceptable 95% of the time if the data set is 1% defective (or less).

Attachment 2

DATA AUDIT No. 9X-XXX **Page 1 of 2**

BATTELLE MARINE SCIENCES LABORATORY

EXAMPLE QUALITY ASSURANCE DATA AUDIT COVER SHEET

PROJECT TITLE:

PROJECT NUMBER:

CLIENT:

PROJECT MANAGER:

PERSON(S) RESPONSIBLE FOR PHASE BEING AUDITED:

DATE OF AUDIT:

AUDITOR:

DATE RESPONSE DUE:

DATE RESPONSE RECEIVED:

COMMENTS:

_____ _____

Signature of Auditor Date

Corrective Action Approval

Auditor: _____ Date _____

Project Manager _____ Date _____

Attachment 2

DATA AUDIT No. 9X-XXX **Page 2 of 2**

BATTELLE MARINE SCIENCES LABORATORY

EXAMPLE DATA AUDIT REPORT

Project:

Definition of Data Set:

Audit Date: **Audit Procedure:** MSL-Q-005-01

No. of Points in Data Set: **No. Checked:**

Comments	Response/Initials/Date

Attachment 3

BATTELLE MARINE SCIENCES LABORATORY

EXAMPLE DATA PACKAGE CHECKLIST

Sampling Event: _____ Central File #: _____

Data Type: _____ WP #: _____

Auditor: _____ Date(s): _____

Notes: Time to Complete: _____

SAMPLE RECEIPT AND PREPARATION

[__] Review Chain of Custody forms for completeness and to identify the date of sample receipt.
[__] Verify that all samples were logged in and the all samples are reported on the sample preparation sheets.
[__] Review sample preparation records; check all pages for completeness.
[__] Review any miscellaneous documents included.
[__] Check for surrogate and internal standard spiking information.
[__] Check for sample dilution and/or correction factor information.
[__] Verify that samples were digested and/or analyzed within established or required holding times.

CALIBRATIONS

[__] Compare sequence and data tables to verify that correct standards were used for calibration.
[__] Check initial calibration and verify that the $r^2 > 0{,}995$.
[__] Check that continuing calibration standards are analyzed at the specified frequency and within the required criteria.

DATA TABLES

[__] Determine if blank corrected data are reported.
[__] Check for dilutions and correct dilution factors.
[__] Verify the report table reporting units and header information.
[__] Verify that data are reported to the correct number of significant figures (usually 3).
[__] Calculate and verify sample data (highlighted values). Check data qualifiers.
[__] Verify reported method detection limits (MDLs).
[__] Calculate quality control (QC) data:
_____blanks _____matrix spikes [MSs]
_____duplicates _____standard reference materials (SRMs)
_____other
and check qualifiers against stated criteria.

OTHER

[__] Review QA/QC summary

COMMENTS

[__]

Battelle
Putting Technology To Work

Marine Sciences Laboratory

EFFECTIVE DATE: 5-11-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-Q-009-00

METHOD DEVELOPMENT, VALIDATION, AND IMPLEMENTATION

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	5/11/00
	Signature	Date
TECHNICAL REVIEWER: Brenda Lasorsa	B. Lasorsa	5/11/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	5/11/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	5-11-00
	Signature	Date

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METHOD DEVELOPMENT, VALIDATION, AND IMPLEMENTATION

1.0 SCOPE AND APPLICATION

This procedure describes the standard procedure for method development, validation, and implementation and it includes determining initial method detection limits (MDLs), instrument detection limits (IDLs) and quantitation limits (QLs) as described in the Federal Register (40 CFR Part 136, Appendix B). This procedure is used for developing and testing new procedures for routine analytical data collection and reporting by the Battelle Marine Sciences Laboratory (MSL) and for using new instruments using existing methods.

2.0 DEFINITIONS

IDL	Instrument Detection Limit – The IDL is the minimum concentration of an analyte that a particular instrument can measure and report with 99% confidence that the analyte concentration is greater than zero.
MDL	Method Detection Limit - The MDL is defined as the minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. A matrix-specific MDL is experimentally determined through analysis of replicate samples containing the target analyte.
QL	Quantitation Limit – The QL is the maximum or minimum levels, concentrations, or quantities of a target analyte that can be quantified with the confidence required by the data user.
99% CL	99% Confidence Limit – The 99% CL represents an acceptance criteria for determining whether or not an analytical system is in control.

3.0 RESPONSIBLE STAFF

Analyst
MSL Data Coordinator
MSL QA Officer or Representative
MSL Managers

4.0 PROCEDURE

The following steps apply to using a new instrument to collect data using an existing standard method or to developing a new method with new or existing equipment.

Methods development may be based on use of an existing published method or may be the basis for defining a new method as a product of the work activity.

4.1 Instrument Operations

Generally when new instruments are delivered the manufacturer provides initial set up and testing of the instrument, initial training and often an intensive training course. Intensive training usually covers analytical theory and instrument capabilities, and use of the equipment software. Initial manufacturer set-up testing might involve running a set of test standards or standard reference materials (SRMs).

Staff are encouraged to take advantage of all training opportunities (manufacturer-provided, in-house) to understand instrument operation. Training documentation (e.g., certificate of completion) should be submitted to the MSL training file maintained by the MSL QA Officer.

4.2 Training

Once training courses are completed, hands-on training is essential and is generally obtained as on-the-job training while optimizing and testing instrument sensitivity, and running calibration standards, samples of known concentration and test samples. On-the-job training shall be documented and submitted to the MSL training file maintained by the MSL QA Officer. The Individual Training Assignment form from MSL-A-006, Marine Sciences Laboratory Training should be used for this purpose and a summary of training activities performed and the number of hours required should be noted.

4.3 Optimization and Sensitivity Testing

The manufacturer can provide suggestions for approaches to optimize and test instrument sensitivity and performance. Analyzing blanks, serial dilution samples, and test samples may be part of this step. It is important to keep good records of this process for the documentation file.

At this point in the process, if this has not been done already, a preventive maintenance logbook should be initiated to document instrument set up, testing, optimization, testing, and any corrective actions required to optimize the test system.

4.4 Calibration

A series of calibration standards shall be analyzed to help set the range for the standard set of calibration standards to be used in routine analyses. The standard range will be determined from suggestions from the manufacturer, the instrument (IDL) and method

detection limits (MDL), and required quantitation limits (QL). A determination of linear range should be part of this step.

4.5 Verification

The precision and accuracy of the instrument or method shall be determined based on the replicate analysis of known concentration samples. Precision and accuracy capabilities in many matrices are required for marketing of the capability of the instrument and/or analysis method. Verification of linear range can also be made using known concentration samples.

4.5.1 SRM Analysis

SRMs for all matrices to be tested (sediment or soil, water, tissues) should be obtained so that a wide range of sample types of known concentration can be tested. Once the capabilities of the instrument have been investigated and are known, appropriate unknown samples can be purchased. The results from this exercise can be used to internally validate the instrument operation or method. Acceptance criteria should be the published method acceptance criteria, or in the case of new method development should be set between 10 and 20% and may be set based on performance evaluation (PE) sample analysis acceptance ranges. Poor results can be used to trouble shoot the instrument or the method and may require interaction with the manufacturer to ensure that the instrument is performing at full capability. . Initial SRM sample results need to become part of the file for instrument or method validation.

4.5.2 PE Sample Analysis

PE samples are required to be analyzed in as many matrices as possible to provide an external, unbiased assessment of instrument performance and accuracy. Initial PE sample results need to become part of the file for instrument or method validation. Routine PE sample analyses should be conducted as per the requirements specified in MSL-A-013, Laboratory Accreditation and PE Sample Analysis.

4.6 Limit Determinations

The IDL, MDL, and QL of the instrument or method in all matrices of interest must initially be determined prior to actual sample analysis to demonstrate the capability of the instrument and/or analysis method. . Initial IDL, MDL, and QL (if different) results need to become part of the file for instrument or method validation. In addition, these results must be forwarded to the Data Coordinator for inclusion in the MDL notebook used to prepare client reports. MSL-Q-007, Procedure for Determining Method Detection Limits must be followed in order to derive these values.

4.6.1 Method Detection Limits

The MDL study itself should ideally be performed on each matrix of interest for each analyte to be quantified. This is sometimes not practical, especially when a wide range of different matrices are to be analyzed. At a minimum, a representative water, sediment or soil, and tissue sample should be chosen to use as the matrix of interest for MDL studies. MDL studies for the determination of metals in sediment need not be performed; an appropriate sediment MDL may be computed from the experimentally determined MDL for metals in aqueous solution.

In general, the MDL study should be conducted as follows:

- Prepare or obtain the matrix of interest that is as free of the analyte as possible.
- If necessary, the analyte(s) of interest is then spiked into the matrix at a level between 1 and 5 times the estimated MDL.
- A minimum of 7 aliquots of the spiked or unspiked sample is analyzed by processing each aliquot through the entire analytical method, including sample digestion.

Gas chromatography MDL studies require the determination of MDLs for the primary columns. MDLs on confirmation columns must be equal to or less than the MDL for the corresponding primary column.

The laboratory must perform method detection limit studies at least annually on a method, instrument, matrix, and analyte-specific basis.

4.6.2 Instrument Detection Limits

IDL is determined from an analysis of at least seven replicates of an undigested deionized water sample and is calculated from the standard deviation of the mean x the appropriate Students' t value.

IDL studies must be performed at least annually for each analyte for methods using for example for inorganics, ICP-MS, ICP-AES, , CVAF, GFAA, FIAS (and other instruments when appropriate).

4.6.3 Quantitation Limits

The lower end of the QL is defined as the minimum level, concentration, or quantity of a target analyte that can be quantified with the confidence required by the data user. In practice, the QL is often equivalent to the method limit and the project quantitation limit, both terms that are sometimes used to describe QL. QL is mathematically-based on the calculation 3.18 x MDL. The 3.18 value is

based on the Students' T value for 7-10 replicates, the number generally analyzed in this type of study. The upper end of the QL must be determined experimentally and is related to the upper linear range.

4.6 Sample Analysis

Initial sample analysis will ideally include a comparison of the instrument or method results with sample results derived from another instrument or method. There are no criteria for this type of comparison. The comparison is necessary to 1) bridge from an old instrument to a new instrument, in order to demonstrate comparability between the instruments, or 2) to demonstrate comparability between an existing accepted method and a new method, or between SRM results and the new method. This comparison is qualitative in nature, especially for multi-element analyses. Any documentation related to comparison of sample results should be included in the method validation file.

4.7 Required Data Documentation

As stated throughout this document, there are documents that should be included in the method validation file. The required documentation is summarized below: Training documentation is not included.

- Procedure – either an existing procedure or the procedure developed from the activities associated with implementing this procedure. Refer to MSL-A-003, Guidelines for SOP Format and Control.
- Optimization and sensitivity testing records
- Calibration records
- Method or instrument verification records
- Limit determination results - IDL, MDL, QL

The method validation file should be surrendered to the MSL Data Coordinator for archiving.

5.0 DATA ANALYSIS AND CALCULATIONS

The calculations associated with determining IDL and MDL are found in MSL-Q-007.

6.0 QUALITY CONTROL

The goal of this procedure is to provide data to determine method and/or instrument precision and accuracy expressed quantitatively and to define initial IDL, MDL and QL for each sample matrix of interest.

7.0 SAFETY

There are no safety requirements associated with implementing this procedure.

8.0 TRAINING REQUIREMENTS

The Analyst, the Data Coordinator, the Technical Group Manager, and the MSL QA Officer or Representative shall be assigned this procedure for reading in accordance with MSL-006, Marine Sciences Laboratory Training. Documentation of training will be recorded on a training assignment form from procedure MSL-A-006.

9.0 REFERENCES

MSL-A-003	Guidelines for SOP Format and Control
MSL-A-005	Deviations from Established Requirements
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-013	Laboratory Accreditation and PE Sample Analysis.
MSL-Q-007	Procedure for Determining Method Detection Limits

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 2/4/92

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-~~M-054-00~~ D. Coffey
T-001-00 2/16/00

WATER AND TISSUE SAMPLE COLLECTION

Approvals:

Lisa M Karle	1-17-92
AUTHOR	DATE
Michael E. Barrows	1-17-92
TECHNICAL REVIEWER	DATE
R Ellis	1/16/92
QUALITY ASSURANCE	DATE
RM Ecker	2/4/92
MARINE SCIENCE LABORATORY MANAGER	DATE

WATER AND TISSUE SAMPLE COLLECTION

1.0 APPLICATION AND SCOPE

This procedure details the methods for collecting water and tissue samples from toxicity tests and establishing the traceability of samples that will be transferred to laboratories for chemical and/or biological testing.

2.0 DEFINITIONS

- **Custody** - custody of a sample requires assuming control of the sample in one or more of the following manners: 1) physical possession; 2) in his/her view after taking possession; 3) secured by him/her in a manner that prevents tampering of sample; and/or 4) secured by him/her in an area restricted to authorized personnel.
- **Sample Custodian** - responsible person in custody of sample.

3.0 RESPONSIBLE STAFF

- Technical Staff Collecting Samples
- Sample Custodian
- Sample Recipient
- Reviewer (Project Manager or designee)

4.0 PROCEDURE

During the process of toxicity testing, water and tissue samples are often required in order to assess the progress and validity of the study. Check with the project specific protocol or work plan to obtain the number and frequency of samples required, and the location in the test container from which to obtain the sample.

4.1 Water Sampling

Protocol/Work Plans will give specifics as to the quantity of water to be sampled and how often samples need to be removed from test containers.

4.1.1 Label all sample jars prior to collecting the samples. Labels should include the following information:

- Test compound name and concentration
- Any preservative, solvent, or chemical included with the sample (i.e. 10% ethanol in seawater)
- Date and initials
- Battelle name and sample number
- Study number
- Sampling interval and time or sampling time

4.1.2 Use the appropriate pipette for the accuracy of the sample quantity required (see SOP MSL-M-050 for pipette calibration) and remove the water sample from the center (midpoint of the width, length and depth) of the test container.

4.1.3 Transfer the water from the pipette to the prelabeled sample jar. Complete all label information, if necessary.

4.1.4 After all the samples have been taken, a sample chain of custody sheet (see SOP MSL-A-002) must be filled out prior to any sample transfer.

4.1.5 Upon completion of the sample chain of custody sheets, The chemistry department representative (Project Manager or Study Director will supply this information) can be contacted and a time needs to be arranged for the relocation of the samples to the chemistry department.

4.1.6 If the samples are not to be transferred to the chemistry department immediately, the water samples must be placed in a labeled cooler, with a chain of custody seal closing the cooler cover. Place the cooler in the wet lab cold room (or other freezer storage) until they are to be transferred for analysis.

4.1.7 At the time of sample transmittal, the Staff Member acting as custodian (either the sample collector or group representative) must sign, date, and record the time of transfer of the samples to the appropriate testing facility. This information is entered in the section marked **RELINQUISHED BY**, while the Staff Member accepting the samples fills out the Sample Chain of Custody section **RECEIVED BY**.

4.1.8 The relinquishing sample custodian keeps one copy of the Sample Chain of Custody and is to place this copy in the project files or designated data accumulation notebook.

4.2 Tissue Sampling

The most common tissue used for bioaccumulation studies is that of bivalves (clams, mussels, and oysters) and marine worms. The number of tissues required and the frequency of these samples is information obtained from each particular study's protocol. The following is a general procedure for collecting tissues; specifics must be supplied by the Protocol/Work Plan.

4.2.1 All sample jars should be labeled prior to the collection of tissues. Labels, at a minimum, should include the following information:

- Test compound name and required analysis
- Any preservative, solvent, or chemical included with the sample (i.e. 10% ethanol in seawater)
- Date and initials
- Battelle name and sample number
- Study number
- Sampling interval and time

4.2.2 Carefully identify the **POSITION** number of the test aquarium, and choose the prelabeled sample jar which corresponds to the test position number.

4.2.3 Sample jars for some analyses are preweighed, and it is very important that these jars are matched with the same jar lid with which they were weighed.

4.2.4 When several sample jars require the allocation of tissues from a test container, each specific jar should have a brief explanation of the quantity of tissue needed for each particular jar/analysis. If this information is not available, the Project Manager should be consulted for details.

4.2.5 For marine worm samples, assess the worms as being alive or dead, remove the dead animals, and place the remaining test worms in the appropriate sample jar(s). If mortality is greater than 25% for a particular aquarium, the worm tissue may not be abundant enough for adequate analysis. At this circumstance, consult the Project Manager.

4.2.6 For the bivalve samples, pry apart the two shell halves using a titanium scalpel. Scrape the soft tissue away from the shell, making sure to include the abductor muscle fastened to the mantle.

4.2.7 Allocate the proper amounts of tissue to the various sample jars for each test container's position number. Double check that all the jars for each test container were filled with the necessary quantity.

4.2.8 After all the samples have been taken, a sample chain of custody sheet (see SOP MSL-A-002) must be filled out prior to any sample transfer.

4.2.9 Upon completion of the sample chain of custody sheets, the chemistry department representative (Project Manager will supply this information) can be contacted and a time needs to be arranged for the relocation of the samples to the chemistry department.

4.2.10 If the samples are not to be transferred to the chemistry department immediately, the tissue samples must be placed in a labeled cooler, with a chain of custody seal closing the cooler cover. Place the cooler in the wet lab cold room (or other freezer storage) until they are to be relocated to the appropriate testing facility.

4.2.11 At the time of sample transmittal, the Staff Member acting as custodian (either the sample collector or group representative) must sign, date, and record the time of transfer of the samples to chemistry. This information is entered in the section marked **RELINQUISHED BY**, while the Staff Member accepting the samples fills out the Sample Chain of Custody section **RECEIVED BY**.

4.2.12 The relinquishing sample custodian keeps one copy of the Sample Chain of Custody and is to place this copy in the project files or designated data accumulation notebook.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Each project's QA Plan or Protocol/Work Plan should be consulted for the individual chemical analytical requirements of a project. Usually samples are broken down into various batches of a specific size, and each batch is analyzed separately along with an analytical blank, sample duplicate or triplicate, matrix spike/matrix spike duplicate, and standard reference material for the overall measurement of equipment and procedure accuracy and precision. The QA Plan or Protocol/Work Plan for each project will list these various specifications along with the acceptable limits for these determined values.

7.0 SAFETY

For each compound to be tested in the laboratory, it is required by law that all individuals have information concerning possible hazards of a given material made accessible to them. A Material Safety Data Sheet (MSDS) detailing all pertinent information about specific chemicals or solvents utilized in the laboratory may be found in a binder in the lowlands wet lab facility (room 202).

8.0 TRAINING REQUIREMENTS

To initiate training, all persons must read this SOP and demonstrate proficiency in these methods, prior to application in a project. A staff member unfamiliar with these procedures will receive a verbal and visual demonstration by a qualified employee. Following this, the staff member will be tested for proficiency using non-test organisms and/or water. Once proficiency has been verified by a certified co-worker, documentation of training will be recorded on a training assignment from SOP MSL-A-006.

9.0 REFERENCES

- MSL-M-050 Calibration and Use of Pipettes
- MSL-A-002 Sample Chain of Custody
- MSL-A-006 Marine Sciences Laboratory Training

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Marine Sciences Laboratory

EFFECTIVE DATE: 04-26-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-T-002-01

ANIMAL RECEIPT, ACCLIMATION, AND HOLDING

Approvals:		
AUTHOR: Jeff Ward		04.25.00
	Signature	Date
TECHNICAL REVIEWER: Meg Pinza	Meg Pinza	04.26.00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	04-024-00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		04.25.00
	Signature	Date

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ANIMAL RECEIPT, ACCLIMATION, AND HOLDING

1.0 APPLICATION AND SCOPE

This procedure provides guidelines for receiving, acclimating and holding aquatic animal populations prior to testing in the Battelle Marine Sciences Laboratory (MSL) wet laboratory facility located in Sequim, Washington. This procedure also describes guidelines for water quality testing and provides information for determining acceptable conditions; discusses organism acclimation to the holding facilities; and defines guidelines for animal feed procurement, receipt, storage, and use. This procedure replaces MSL-M-055-00.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

Marine Sciences Laboratory (MSL) Manager
Project Manager and Task Leader
Staff Members
Quality Assurance Officer or Representative

4.0 PROCEDURE

All information concerning the introduction of potential test organisms to MSL must be fully documented. This includes:

- ensuring that quality-affecting requirements are specified when organisms are ordered from the source,
- recording the date of receipt,
- completing any Chain of Custody documentation,
- noting the apparent physical condition of organisms upon arrival, and the details of the transfer (e.g., condition of animals, cooler temperatures), and
- daily and weekly documentation collected during holding of aquatic organisms at MSL prior to testing.

Test conditions are specified in specific MSL procedures for testing and include:

- acceptable sources of seawater, and/or freshwater
- guidelines for test animals and test preparation,
- acceptable water quality ranges prior to test initiation, and
- procedures for test initiation, monitoring, and termination

4.1 General Facilities Requirements

The immediate environment into which organisms are received and held must be clean, and well maintained. Care is required to ensure that no contaminated

waters or sediments come in contact with the animals, the containers in which they are held, or the water or substrates in the containers. Ensure that the work rooms are neat, with no trash on floors, shelves or overflowing waste baskets. The work rooms should be maintained to be orderly and professional in appearance. It is important to maintain an environment that provides assurance that organisms and being cared for in a healthy environment.

4.2 Animal Receipt

Suppliers of aquatic organisms to MSL should be requested to send along the following information with the shipment of animals:

- an invoice or shipping label describing the item and quantity (including number of containers shipped),
- Chain of Custody documentation,
- if applicable, information about the environmental conditions under which the animals were cultivated prior to shipment, and
- any special acclimation procedures required for the organism.

Although it may be difficult to ensure that suppliers comply with MSL requests, providing an MSL Chain of Custody form (refer to MSL-A-002, Chain of Custody) for use during shipment may facilitate compliance.

4.2.1 Upon receipt of each shipment of animals, note the appearance of the container; look for possible shipping damage, liquid leaks, or holes in the outside of the shipping container. Locate the supplier's invoice or shipping label on the container (this information may be inside the shipping container), record your initials and the date, and place in the project files or designated notebook.

4.2.2 Upon opening the shipped container, note the medium in which the organisms arrived (i.e. animals may be shipped "dry" with wet towels or ice packs in the container, or in water) and record this information on a Test Organism Receipt and Acclimation Record (Attachment 1).

4.2.3 Observe the animals in the container. The animals must be alive and appear undamaged by shipping. If the animals are in water, perform water quality measurements such as salinity, pH, temperature, and dissolved oxygen (DO) content, and record these on the Test Organism Receipt and Acclimation Record. In addition, note and record the following:

- Presence and number of dead or moribund animals
- Observation of abnormal behavior
- Discrepancies from ordered quality, quantity or age/weight specifications

Refer to the following procedures for instrument calibration and use instructions for water quality measures:

- MSL-W-001 Calibration and Use of pH Meters

- MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
- MSL-W-003 Calibration and Use of Thermometers, and
- MSL-W-004 Calibration and Use of Refractometers
- MSL-W-007 Routine Water Quality Measures for Toxicity Tests
- MSL-W-008 Determination of Ammonia

4.3 Animal Acclimation and Holding

If visual inspection of the animals is satisfactory, the shipment will be accepted. The animals will be transported to a holding area where they will be maintained in separate holding tanks. If the shipment is unacceptable, the Project Manager and/or Task Leader will be contacted immediately for further instructions. The supplier will also be informed.

4.3.1 Carefully transfer the animals to the prepared holding area. The shipment of organisms may be divided into separate holding groups according to the number of animals and the amount of space they each require.

4.3.2 The transfer of each group of animals to the holding tank should be performed as gently as possible to avoid any undue stress and to maintain a constant wet environment.

4.3.3 Perform water quality measurements on the MSL holding water (refer to Section 4.2.3), and record this information on the Test Organism Receipt and Acclimation Record (Attachment 1). Compare these values to those obtained for the shipping water. Marked differences between shipping and acclimation conditions guide the acclimation step discussed in Section 4.3.4.

4.3.4 Acclimation from the shipping water to the holding conditions at MSL should be performed without any abrupt changes in temperature (not to exceed 2°C/24 hr) or salinity (not to exceed 5 ppt/24 hr). Gentle aeration of the shipping water will drive off any excess dissolved oxygen (super-saturation). The pH should be adjusted to normal conditions. See the MSL testing procedure or plan and/or consult with the Project Manager and/or Task Leader for the appropriate water quality levels that need to be obtained for each particular project.

4.3.5 During the acclimation and holding period, acclimatipn to temperature should be obtained by raising or lowering the temperature of the water in which the animals are maintained until the desired temperature has been obtained. Rate of temperature increase or decrease will depend on the particular species. Refer to the MSL testing procedure or plan and/or consult with the Project Manager and/or Task Leader for the appropriate rates.

4.3.6 Acclimation to salinity should be gradually obtained by raising or lowering

the salinity of the water in which the animals are maintained until the desired salinity has been obtained. Salinity may be adjusted downward using untreated well water. Salinity may be adjusted upward using a slow seawater drip (the usual case; when $\leq$ 32 ppt is required)or using concentrated brine (an exceptional case; when > 32 ppt is required). Refer to MSL-T-007, Suspended Particulate Phase Preparation, for more detail regarding salinity adjustments. Record all salinity adjustment calculations on the Test Organism Receipt and Acclimation Record. Rate of salinity increase or decrease will depend on the particular species. Refer to the MSL testing procedure or plan and/or consult with the Project Manager and/or Task Leader for the appropriate rates.

4.3.7 During the holding period, water quality measurements will be made daily for all holding containers. These data will be recorded on a Test Organism Care and Maintenance Form (Attachment 2). Holding tanks will be free of animal waste buildup and tank surfaces will be clean.

4.3.8 The time, quantity, and type of food given to animals in holding will also be recorded on the Test Organism Care and Maintenance Form. Feed procurement, quality, and expiration are discussed below in Section 4.3. Subject to research protocol requirements, fish will be fed from 0.5 to 3% of body weight daily.

4.3.9 Holding tanks will be observed daily for organism health and mortality and dead animals will be removed daily. Additional required information included on the Test Organism Care and Maintenance Form is the observation and removal of any dead animals as well as any unusual observations or occurrences. The Comments section of the Test Organism Care and Maintenance Form (which is actually the Daily Log form) may be used for this purpose.

4.4 Animal Feed Procurement, Receipt, Use and Testing

The procurement of animals and feed for bioassays should include requirements for chain of custody of animals during shipping and documentation of any available feed analyses, feed storage recommendations, and expiration dates so that feed quality can be monitored. Animal shippers should be requested to document conditions of animals and environmental parameters (temperature) at the time of shipping for comparison with conditions encountered at the time of receipt. In some cases, it might be important to include QA requirements for a minimum/maximum thermometer or temperature strip in the cooler at the time of shipping. Feed shippers shall be requested to ship feed under specified conditions such as at recommended storage temperatures. Requirements regarding common carriers, Saturday delivery acceptability and locations, and other details might also be specified in QA requirements documents. Procurement for quality-affecting work is discussed in MSL-A-012, Procurement. Monitoring of feed

quality and testing water is described in more detail in MSL-Q-004, Quality of Testing Water and Feed. Some studies will have requirements for the analysis of feed. It is useful to attempt to provide any baseline information or analyses from the supplier.

When feed is received, shipping conditions must be assessed before the shipment is accepted. Shipping conditions must match any pre-specified requirements. Feed receipt conditions can be documented using an MSL Chain of Custody form (refer to MSL-A-002, Chain of Custody) or on the shipping invoice. This documentation shall become part of the project record. When feed has an expiration date, this date will be transferred to all containers holding the feed. Any lot numbers appearing on the feed shall also be tracked and transferred to all containers holding the feed.

4.5 Feed Storage

Feed shall be stored in such a way as to eliminate or minimize any possibility of contamination. Sources of contamination that should be considered are insects, rodents, chemicals, molds, and bacteria. Feed shall be stored in closed, air-tight containers, utilizing a container-within-a container approach whenever possible. Feed that is required to be frozen or stored under refrigerated conditions must be stored in the appropriate refrigerator or freezer and the temperature monitored periodically and recorded. When feed has an expiration date, this date will be transferred to all containers holding the feed. The feed will be discarded when the expiration date is reached. However, a feed sample for archiving may be retained after the expiration date, if freeze-dried or frozen. Feeding requirements during toxicity tests are specified in individual testing procedures or plans.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable

6.0 QUALITY CONTROL

Test organisms being received at MSL must meet certain criteria in order for the shipment to be accepted. The animals must be alive and appear undamaged by shipping, any shipping water the organisms may have arrived in must be evaluated for temperature, pH, salinity, and dissolved oxygen values. All information regarding the shipment and the organisms' appearance must be recorded.

During the test organism holding period, all water quality measurements must be performed and recorded daily, along with feeding information. Salinity and temperature acclimation will be accomplished at a rate required for the particular species in order to avoid undue stress on test animals.

7.0 SAFETY

No specific safety requirements result from the implementation of this procedure.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this procedure are properly *trained prior to beginning work*, and that documentation of the training exists prior to the performance of those procedures.

Persons participating in the receipt, handling and acclimation of test organisms for toxicological testing must first read this procedure. Documentation of training shall be recorded on a training assignment form provided in procedure MSL-A-006, Marine Sciences Laboratory Training. Project specific modifications to this procedure will be documented in the project work/QA plan, which shall be made available to all laboratory staff prior to beginning a test.

9.0 REFERENCES

MSI-A-002	Sample Chain of Custody
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-012	Procurement
MSL-Q-004	Quality of Testing Water and Feed
MSL-T-007	Suspended Particulate Phase Preparation
MSL-W-001	Calibration and Use of pH Meters
MSL-W-002	Calibration and Use of Dissolved Oxygen Meters
MSL-W-003	Calibration and Use of Thermometers, and
MSL-W-004	Calibration and Use of Refractometers
MSL-W-007	Routine Water Quality Measures for Toxicity Tests
MSL-W-008	Determination of Ammonia

ATTACHMENT 1

Battelle Marine Sciences Laboratory

Example Test Organism Receipt and Acclimation Record

Project Name: _____ **Project No.**_____

TEST SPECIES: _____

Received From: _____
Address: _____
_____
_____
_____

Received By: _____
Date Received: _____
Time Received: _____
Estimated Quantity: _____
Shipping Containers: _____

INITIAL CONDITIONS:

INSTRUMENTATION: _____ _____ _____ _____

Container #	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

Acclimate to: Test Conditions	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

Acclimation Record: Record Procedures; include date, time and initials

INSTRUMENTATION: _____ _____ _____ _____

DO (mg/L) pH Salinity (%) Temp (°C)

Container #	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

SEE TEST ORGANISM CARE AND MAINTENANCE FORM FOR FURTHER ACCLIMATION

REVIEWED BY: _____ DATE: _____

ATTACHMENT 2

Battelle Marine Sciences Laboratory

Example Test Organism Care and Maintenance Form

Project Name: _____ **Project No.**_____

TEST SPECIES: _____ **Page** _____ **of** _____

Date	Tank Desig.	DO mg/L	M#	pH	M#	Sal%	M#	Temp °C	M#	Time	INIT

record comments on reverse side

YSI Meter# _____
or
DO Meter# _____ DO 1_____ 2_____ 3_____
pH Meter# _____ pH 1_____ 2_____ 3_____
Refract. Meter# _____ S% 1_____ 2_____ 3_____
T Meter# _____ T 1_____ 2_____ 3_____

REVIEWED BY:_____ DATE: _____

ATTACHMENT 2 (continued)
Battelle Marine Sciences Laboratory
Daily Log/Observations

Date/Time	Initial All Entries

REVIEWED BY:____________________ DATE: ___________

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Marine Sciences Laboratory

EFFECTIVE DATE: 12-1-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-T-003-00

TEST ORGANISM OBSERVATIONS

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	12/1/00
	Signature	Date
TECHNICAL REVIEWER: Nancy Kohn	Nancy P Kohn	12/1/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	12/1/00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		12.01.00
	Signature	Date

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TEST ORGANISM OBSERVATIONS

1.0 SCOPE AND APPLICATION

This procedure describes the method used for conducting daily observations of various organisms in toxicity tests. All information must be completely recorded on data sheets. For any event or observation which the recorder notes as unusual or important should be transmitted to the laboratory lead or project manager and documented on the daily log sheet in the project notebook.

2.0 ABBREVIATIONS AND DEFINITIONS

Attachment 1 provides a list of the common abbreviations used to record observations for various species.

3.0 RESPONSIBLE STAFF

MSL Staff
Project Manager, Laboratory Leader
MSL Manager
Quality Assurance Officer or Representative

4.0 PROCEDURE

Observation methods are different for several aquatic animals. The following sections describe the behavior and distinct observations noted for the most common aquatic organisms used in MSL toxicity tests.

4.1 Bivalves

Bivalves include adult mussels (*Mytilus*), oysters (*Crassostrea*), and clams (*Macoma*). All three of these animals tend to remain on the sediment surface, buried, or partially buried in sediment.

4.1.1 Fill out a Daily Observation Form (See Attachment 2 for an example) specific for the organism requiring daily observation monitoring. At a minimum, forms for *Macoma* observations will generally have categories for recording the number of animals on the surface, the number of siphons exposed, and the number of animals removed. Forms for *Mytilus* and *Crassostrea* will show fields for recording information regarding the number of animals alive and the number of animals feeding.

4.1.2 Fill in the space on the Daily Observation Form titled **POSITION** with the position number of the test chamber being observed.

4.1.3 Observe the animals in the chamber. Fill in all the requested information on the Daily Observation Form, crossing out any errors with a single line, dating, initialing, and giving an error reason (refer to Attachment 3 for suggested error codes.)

4.1.4 When performing daily observations on *Macoma* (clams) in toxicity tests, the number of siphons exposed helps to give an indication of how many animals are feeding. Count the number of individual siphons that can be seen on the surface of the sediment and fill in the category of **# Siphons Exposed** on the Daily Observation Form.

4.1.5 Note if any animals have a shell gaping open. Gently touch the soft tissue of the bivalve with a disposable pipette or glass rod and record whether or not the gaping animal responds to the touch. Remarks describing the actions of any animal seemingly exhibiting the effects of toxicity may be noted in the **COMMENTS** section of the Daily Observation Form (i.e. slow to respond, unresponsive, unusual color or change in color, etc.).

4.1.6 If a gaping bivalve responds slowly to touch, note this fact and leave the animal in the test aquarium. Any gaping animal which fails to respond at all to being touched may be removed from the test chamber and disposed of in the trash or frozen for later incineration, unless otherwise specified in the project toxicity testing plan. The removal of any dead organisms must be noted on the Daily Observation Form. If mortality is uncertain, leave the gaping animal in the test chamber until the next day's observations.

4.1.7 The **COMMENTS** section can be made easier with abbreviations of words frequently used. Attachment 1 lists abbreviations for common or routine observations. For any new or test-specific abbreviations, write a key at the bottom of each Daily Observation Form indicating the word or phrase replaced by the abbreviation.

Example: Toxicity tests with sediments may have days when observations are difficult due to turbidity in the water. A code such as STW indicating that the water was slightly turbid, MTW for moderately turbid, or the abbreviation VTW to indicate that the water was very turbid and observations were difficult or unable to be performed.

4.1.8 Toxicity tests without sediments should have different observation forms in which the **# on Surface** grouping is eliminated. Most observations for bivalves in non-sediment tests include the number of live or dead animals, and the number of animals feeding.

4.2 Marine Worms

The most common marine worms used in toxicity tests are *Nephtys* and *Neanthes* species. Both of these organisms require fairly careful handling; manipulation should be avoided if possible during daily observations. If manipulation is necessary to determine a response or mortality, it should be done very gently and carefully. Again, some toxicity tests are conducted with sediments and others are not, so different observation forms exist for the various test media.

4.2.1 Fill out a Daily Observation Form (See Attachment 2 for an example) adequate for the organism you will be observing. Toxicity tests using sediments will have categories to include the number of animals on the surface, and the number dead removed. Tests without sediments would only require the number of dead/removed worms to be reported daily.

4.2.2 Marine worms normally remained buried in sediment. When a worm is noted on the surface of the sediment, record this information under **# on Surface** on the Daily

Observation Form, and gently touch the animal with a disposable pipette or glass rod. Note in the **COMMENTS** section whether the animal is responsive and reburies or not. Occasionally, a worm may be seen partially sticking out of the sediment. When this is noticed, record if the portion of the worm visible is the head or tail section of the animal (identification of these animals is available as on-the-job training).

4.2.3 Marine worms are only removed from test aquaria when they are known to be dead. The most reliable indication of mortality is a completely colorless (white) worm body on the sediment surface. At this point, the animal may be removed with forceps from the test container and disposed of as required (usually incineration).

4.2.4 The **COMMENTS** section can be abbreviated by using one or two letters as representatives of frequently used comments (see Attachment 1). After prodding a worm on the sediment, abbreviations such as DNR may indicate that the animal did not rebury or RB for reburied. If symbols other than those listed in Attachment 1 are to be utilized, it is good practice to provide a key at the bottom of each Daily Observation Form explaining what each abbreviation means.

4.3 Marine Fish

Some of the marine fish commonly used in toxicity testing are sanddabs (*Citharichthys*), sheepshead minnows (*Cyprinodon*), silversides (*Menidia*), and salmon (*Oncorhynchus*). Observations on marine fish should include information describing any unusual behavior (e.g., unusual feeding, swimming), and any complications such as hemorrhaging or discoloration, with mortality being the primary indicator.

4.3.1 Fill out a Daily Observation Form appropriate for the species you will be observing. The most important observation to be made when looking at toxicity in fish is the number of mortalities. The **COMMENTS** section of a Daily Observation Form can be used for any other observations such as how many fish might be at the bottom of the test vessel and/or the number of animals at the surface.

4.3.2 Notes on the loss of equilibrium or erratic and jerky movements are important and can also be recorded in the **COMMENTS** section.

4.3.3 Mortality in marine fish is defined by the lack of movement and unresponsiveness of the animal to touching the caudal peduncle. If no opercular (gill cover) movement is noted, and the fish does not respond to being touched with a disposable pipette or glass rod, it may be removed and properly discarded.

4.4 Marine Amphipods

Amphipods are small crustaceans which usually burrow or build tubes near the sediment surface. . Some species may emerge and spend some time in the water column, but typical behavior will be close association with the sediment. While most amphipods are associated with sediment, some tests are performed with other media (e.g., water only, artificial substrate). Some frequently used amphipod species include *Rhepoxynius*, *Corophium*, *Ampelisca*, *Grandidierella*, and *Eohaustorius*.

4.4.1 Fill out a Daily Observation Form (See Attachment 4 for an example) specific for the organism you will be observing. Write in the position number of the test vessel to be

observed under **POSITION** on an appropriate (sediment test or not) Daily Observation Form.

4.4.2 Lift the cover on the amphipod test vessel and look down into the container (usually a mason jar). Record the number of animals on the sediment surface (if applicable) and record this information under the category titled **NUMBER ON SEDIMENT**. Count the number of amphipods floating on the water surface and place this data in the column marked **NUMBER FLOATING**.

4.4.3 When amphipods are observed floating on the water surface, they should be gently poked with a disposable pipette until they are pushed slightly under the surface of the water. Note in the **COMMENTS** section how many of the floating animals swam down to the test container bottom and reburied in the sediment and how many did not rebury.

4.4.4 For toxicity tests that don't utilize sediments, the Daily Observation Form categories would require the observations of mortality and the number of animals floating on the surface. Animals on the surface in these tests should also be gently pushed down and their reactions recorded.

4.4.5 Mortality in amphipods is best identified by a colorless, unresponsive animal lying on the surface of the sediment or at the bottom of the container. When mortality is certain, the dead animal may be removed with a disposable pipette and released in the wastewater treatment drain of the wet lab.

4.5 Marine Shrimp

Toxicity tests involving marine shrimp rarely include sediments, so the most important daily observation required for these animals is the number of mortalities. The most commonly used marine shrimp in toxicity testing are mysid species.

4.5.1 Fill out a Daily Observation Form specific for the organism you will be observing. Record the test container number to be observed in the **POSITION** section of the Daily Observation Form.

4.5.2 Observe the animals in the test vessel and note the number of shrimp alive and the number dead in the appropriate columns.

4.5.3 The **COMMENTS** section of the Daily Observation Form can be used to record any behavior observed by the test animals. Erratic swimming behavior, lethargy, or any other descriptions of test animal conduct should be noted. Cannibalism can be common in these animals, and if this phenomenon is observed, it should be noted in the **COMMENTS** section.

4.5.4 Death is determined by the lack of response to prodding and a colorless body, usually curled up on the bottom of the test container. When mortality is certain, the dead shrimp should be noted on the Daily Observation Form, and disposed of down the wastewater treatment drain in the wet lab.

4.6 Test Animal Replacement

If mortality is observed within the first few hours of test initiation, some forms of toxicological testing require the replacement of dead animals with live ones. This replacement is due to the fact that some organisms are more sensitive to handling and transfer and mortality or damage may result from test initiation rather than from toxic effects of the test compound. However, this replacement is very specific to certain organisms and only in a few projects. Consult the test-specific procedure and the Project Manager prior to any test organism's study initiation.

5.0 DATA ANALYSIS AND CALCULATIONS

There are no applicable data analyses or calculations related to the implementation of this procedure.

6.0 QUALITY CONTROL

It is important to note any unusual events in test organism response and the test system. All out of ordinary events and observations need to be documented so that any seemingly unexplainable results may be accounted for in the study, and the possibility of false responses due to any particular test compound are eliminated or avoided. In addition, all errors need to be documented for the same reason. A good rule of thumb to follow is to document everything; when in doubt, write it down in the daily log..

7.0 SAFETY

There are no applicable safety concerns related to the implementation of this procedure.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the project managers to ensure that all staff performing the activities described in this procedure are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all persons must read this procedure and demonstrate proficiency in the above described methods. A staff member unfamiliar with the procedures of test organism observations will receive a verbal and visual demonstration by a certified co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this SOP are followed. Upon successful completion of training, documentation of training will be recorded on a training assignment form obtained from SOP MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

ATTACHMENT 1
Example MSL Observation Codes

General Observations Used in Most Tests

AB	All Buried
BH	Burrow Holes (note presence or absence)
MT	Mucous Trails Present
TP	Tubes Present (write qualifier if needed, i.e. "no TP")
PDF	Pushed Down Floating Organisms
RB	(number that) Reburied
DNR	(number that) Did Not Rebury
WC	(number) in Water Column
A	Active
L	Lethargic
AD	Appears to be Dead
AM	Appears to be Molt
FR	Food Remaining (could be brine shrimp, Tetramarin, algae)
STW	Slightly Turbid Water
MTW	Moderately Turbid Water
VTW	Very Turbid Water
SD	Sediment Discoloration (due to fungus/bacteria/etc., note color/size)

ATTACHMENT 2

Battelle Marine Sciences Laboratory
Example Daily Observation Form for Marine Bivalves

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Project Name: _____ Test Day: _____
Project Number: _____ Date: _____
Test Type: _____ Page __ of __
Species: _____

Position	# on Surface	# Siphons	# Removed	Position	# on Surface	# Siphons	# Removed	Comments

Comments:

Recorder: _____ Reviewed By: _____ Date: _____

ATTACHMENT 3

SUGGESTED ERROR CODES

Error codes should be written near the line-out correction and must be accompanied by the initials of the person making the change and the date of the change.

Symbol	Explanation of Correction
WL	Inadvertently recorded in the wrong location
CC	Changed for greater clarity
O	Write over
SE	Spelling error
IR	Inadvertently not recorded at time of initial observation
CE	Calculation error
TE	Transcription error
RE	Recording error
EI	Entry not initialed, dated, and/or justified at the time of entry
TI	Incorrect time
DA	Incorrect date
UN	Incorrect units
ID	Incorrect sample ID
MD	See miscellaneous documentation form (refer to date of the form)

ATTACHMENT 4

Battelle Marine Sciences Laboratory
Example Daily Observation Form for Amphipods

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Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, WA 98382
(360) 683-4151

Observations

Project Title: _____ Observer: _____
Project No. _____ Date: _____
Duration: _____ Test day: _____
Test Type: _____ Page: _____ of _____
Species: _____

AB = All Burried
AD = Appears to be Dead
AM = Appears to be Molt
STW = Slightly Turbid Water
MTW = Moderately Turbid Water
VTW = Very Turbid Water
PDF = Pushed Down Floaters
RB = (number) Reburied
BH = Burrow Holes present

Position	Number Floating	Number on Sediment	Comments

Reviewed By: _____ Date: _____

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Marine Sciences Laboratory

EFFECTIVE DATE: 05.09.00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-T-032-00

RECEIPT, HOLDING AND TESTING OF FISH

Approvals:		
AUTHOR: Ann Skillman		5-8-00
	Signature	Date
TECHNICAL REVIEWER: Rhonda Karls	Rhonda Karls	5.9.00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4-28-00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		05.09.00
	Signature	Date

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RECEIPT, HOLDING AND TESTING OF FISH

1.0 APPLICATION AND SCOPE

This procedure provides guidelines for the receipt, holding and testing of fish at the Battelle Marine Sciences Laboratory (MSL) wet laboratory facility located in Sequim, Washington. This procedure describes guidelines for water quality measurement; defines guidelines for animal feed procurement, receipt, storage, and use; and discusses daily observations and test termination.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

Project Manager and Task Leader
Staff Members
Quality Assurance Officer or Representative
Marine Sciences Laboratory (MSL) Manager

4.0 PROCEDURE

4.1 General Facilities Requirements

The immediate environment in which the fish are held and/or tested must be clean, and well maintained. Care is required to ensure that no contamination comes in contact with the fish or tanks in which they are held. The laboratory space must be neat with no trash on floors, cluttered shelves or overflowing waste baskets. The area should be maintained in an orderly and professional manner. It is important to provide an environment that assures good health and well-being for the organisms being cared for.

4.2 Animal Receipt

Suppliers of aquatic organisms to MSL are requested to send along the following information with the shipment of animals:

- an invoice or shipping label describing the item and quantity (including number of containers shipped),
- Chain of Custody documentation,
- when applicable, information about the environmental conditions under which the animals were cultivated prior to shipment, and
- any special acclimation procedures required for the organism.

Fish will be obtained from licensed aquaculture facilities or from state hatchery systems.

Upon receipt of the shipment of fish, observe the animals in the transport container(s). The animals must be alive and appear undamaged by transport. Perform water quality measurements such as salinity, pH, temperature, and dissolved oxygen (DO) content, and record these on the Test Organism Receipt and Acclimation Record. In addition, note and record the following:

- Presence and number of dead or moribund animals
- Observation of abnormal behavior
- Discrepancies from ordered quality, quantity or age/weight specifications

Refer to the following procedures for instrument calibration and use instructions for water quality measures:

- MSL-W-001 Calibration and Use of pH Meters
- MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
- MSL-W-003 Calibration and Use of Thermometers
- MSL-W-004 Calibration and Use of Refractometers
- MSL-W-007 Routine Water Quality Measures for Toxicity Tests
- MSL-W-008 Determination of Ammonia

4.3 Animal Acclimation and Holding

If visual inspection of the fish is satisfactory, the shipment will be accepted. The animals will be transported to a holding area where they will be maintained in separate holding tanks. If the shipment is unacceptable, the Project Manager and/or Task Leader will be contacted immediately for further instructions.

4.3.1 Perform water quality measurements on the MSL holding water (refer to Section 4.2), and record this information on the Test Organism Receipt and Acclimation Record (Attachment 1). Compare these values to those obtained for the shipping water. Transfer from the transport tanks to the holding tanks at MSL should be performed without any abrupt changes in temperature. If necessary, add water from the MSL holding tanks to the transport tanks to bring the temperature to within 2° C of the temperature in the MSL holding tanks. Rate of temperature increase or decrease will depend on the particular species. Refer to the MSL testing procedure or plan and/or consult with the Project Manager and/or Task Leader for the appropriate rates.

4.3.2 Carefully transfer the fish to the prepared holding tanks. The shipment of fish may be divided into separate holding groups according to the number of fish and the amount of space they each require. The transfer of each group of fish to the holding tank should be performed as gently as possible

to avoid any undue stress and to maintain a constant wet environment.

4.3.3 During the holding and testing period, daily water quality measurements will be made for all holding containers. These data will be recorded on a Test Organism Care and Maintenance Form (Attachment 2). Holding tanks will be free of animal waste buildup and tank surfaces will be clean.

4.3.4 The time, quantity, and type of food given to the fish will be recorded on the Test Organism Care and Maintenance Form. Feed procurement, quality, and expiration are discussed below in Section 4.4. Subject to research protocol requirements, fish will be fed from 0.5 to 3% of body weight daily.

4.3.5 Holding and test tanks will be observed daily for fish health and mortality and dead animals will be removed daily. Additional required information included on the Test Organism Care and Maintenance Form is the observation and removal of any dead animals as well as any unusual observations or occurrences. The Comments section of the Test Organism Care and Maintenance Form (which is actually the Daily Log form) may be used for this purpose.

4.3.6 If an off-normal event should occur during the holding or testing of the fish, immediately contact the Project Manager and/or Task Leader for guidance.

4.4 Animal Feed Procurement, Receipt, Use and Testing

The procurement of fish and feed for bioassays should include requirements for chain of custody of animals during transfer and documentation of any available feed analyses, feed storage recommendations, and expiration dates so that feed quality can be monitored. Feed shippers shall be requested to ship feed under specified conditions such as at recommended storage temperatures. Procurement for quality-affecting work is discussed in MSL-A-012, Procurement. Monitoring of feed quality and testing water is described in more detail in MSL-Q-004, Quality of Testing Water and Feed. Some studies will have requirements for the analysis of feed. It is useful and cost-effective to obtain any available baseline information or feed analysis data from the supplier.

When feed is received, shipping conditions must be assessed before the shipment is accepted. Shipping conditions must match any pre-specified requirements. Feed receipt conditions can be documented using an MSL Chain of Custody form (refer to MSL-A-002, Chain of Custody) or on the shipping invoice. This documentation shall become part of the project record. When feed has an expiration date, this date will be transferred to all containers holding the feed. Any lot numbers appearing on the feed shall also be tracked and transferred to all containers holding the feed.

4.5 Sources of Feed Contamination

Minimize any possibility of feed contamination. Sources of contamination that should be considered are insects, rodents, chemicals, molds, and bacteria. Feed shall be stored in closed, air-tight containers, utilizing a container-within-a container approach whenever possible. Feed that is required to be frozen or stored under refrigerated conditions must be stored in the appropriate refrigerator or freezer and the temperature monitored periodically and recorded. For Good Laboratory Practice (GLP) studies, refrigerator or freezer temperatures must be monitored and documented daily. When feed has an expiration date, this date will be transferred to all containers holding the feed. The feed will be discarded when the expiration date is reached. However, a feed sample for archiving may be retained after the expiration date, if freeze-dried or frozen. Feeding requirements during tests are specified in individual testing procedures or plans.

4.6 Water Quality Measures

During the holding and testing period, water quality measurements will be made daily for all holding and test tanks. These data will be recorded on a Test Organism Care and Maintenance Form (Attachment 2). Holding tanks will be free of animal waste buildup and tank surfaces will be clean.

4.7 Daily Observations

Holding and test tanks will be observed daily for fish health and mortality and dead animals will be removed daily. Additional required information included on the Test Organism Care and Maintenance Form is the observation and removal of any dead animals as well as any unusual observations or occurrences. The Comments section of the Test Organism Care and Maintenance Form (which is actually the Daily Log form) may be used for this purpose.

4.8 Equipment Cleaning

Equipment used for fish studies including buckets, brushes, nets, gloves, and tanks should be cleaned in a 1:2000 bleach to water solution and then well rinsed before use or re-use.

4.9 Test Termination

Upon termination of the study, the fish will be euthanised with an overdose of anesthesia. The fish carcasses from testing will be kept frozen until incineration.

5.0 DATA ANALYSIS AND CALCULATIONS

There are no calculations applicable to the implementation of this procedure.

6.0 QUALITY CONTROL

Fish received at MSL must meet certain criteria in order for the shipment to be accepted. The animals must be alive and appear undamaged by transport and temperature, pH, salinity, and dissolved oxygen values in the transport tanks must be recorded.

Temperature acclimation will be accomplished at a rate required for the particular species in order to avoid undue stress on test animals. During the holding and test period, all water quality measurements must be performed and recorded daily, along with feeding information.

7.0 SAFETY

No specific safety requirements result from the implementation of this procedure.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this procedure are properly *trained prior to beginning work*, and that documentation of the training exists prior to the performance of those procedures.

Persons participating in the receipt, holding and testing of fish must first read this procedure. Documentation of training shall be recorded on a training assignment form provided in procedure MSL-A-006, Marine Sciences Laboratory Training. Project specific modifications to this procedure will be documented in the project work/QA plan, which shall be made available to all laboratory staff.

9.0 REFERENCES

MSI-A-002	Sample Chain of Custody
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-012	Procurement
MSL-Q-004	Quality of Testing Water and Feed
MSL-W-001	Calibration and Use of pH Meters
MSL-W-002	Calibration and Use of Dissolved Oxygen Meters
MSL-W-003	Calibration and Use of Thermometers, and
MSL-W-004	Calibration and Use of Refractometers
MSL-W-007	Routine Water Quality Measures for Toxicity Tests
MSL-W-008	Determination of Ammonia

ATTACHMENT 1

Battelle Marine Sciences Laboratory

Example Test Organism Receipt and Acclimation Record

Project Name: _____ **Project No.**_____

TEST SPECIES: _____

Received From: _____
Address: _____
_____
_____
_____

Received By: _____
Date Received: _____
Time Received: _____
Estimated Quantity: _____
Shipping Containers: _____

INITIAL CONDITIONS:

INSTRUMENTATION: _____ _____ _____ _____

Container #	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

Acclimate to: Test Conditions	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

Acclimation Record: Record Procedures; include date, time and initials

INSTRUMENTATION: _____ _____ _____ _____

DO (mg/L) pH Salinity (%) Temp (°C)

Container #	DO (mg/L)	pH	Salinity (%)	Temp (°C)	Time	Initials

SEE TEST ORGANISM CARE AND MAINTENANCE FORM FOR FURTHER ACCLIMATION

REVIEWED BY: _____ DATE: _____

ATTACHMENT 2

Battelle Marine Sciences Laboratory

Example Test Organism Care and Maintenance Form

Project Name: _____ **Project No.**_____

TEST SPECIES: _____ **Page** _____ **of** _____

Date	Tank Desig.	DO mg/L	M#	pH	M#	Sal%	M#	Temp °C	M#	Time	INIT

record comments on reverse side

YSI Meter# _____
or
DO Meter# _____ DO 1_____ 2_____ 3_____
pH Meter# _____ pH 1_____ 2_____ 3_____
Refract. Meter# _____ S% 1_____ 2_____ 3_____
T Meter# _____ T 1_____ 2_____ 3_____

REVIEWED BY:_____ DATE: _____

ATTACHMENT 2 (continued)
Battelle Marine Sciences Laboratory
Daily Log/Observations

Date/Time	Initial All Entries

REVIEWED BY:____________________ DATE: __________

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Marine Sciences Laboratory

EFFECTIVE DATE: 7/14/00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-T-034-00

REFERENCE TOXICANT STOCK SOLUTION PREPARATION

Approvals:		
AUTHOR: Trudy Davis	Trudy Davis	7-14-00
	Signature	Date
TECHNICAL REVIEWER: Jeff A. Ward		7.14.00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	7.14-00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		07.14.00
	Signature	Date

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REFERENCE TOXICANT STOCK SOLUTION PREPARATION

1.0 SCOPE AND APPLICATION

This procedure describes the steps for preparing cadmium (Cd), copper (Cu), and ammonia (NH_3) stock solutions to be used in reference toxicity assays. It includes the calculations needed to prepare the proper concentration. Accuracy in preparation of these stock solutions is required because the results of the reference toxicity assays are used to support project testing results.

2.0 DEFINITIONS

Stock solution: A prepared solution of a chemical of a particular concentration. The stock solution is used to spike reference toxicity test containers to the required chemical concentration.

3.0 RESPONSIBLE STAFF

Project Manager and Task Leader
Staff Members
Quality Assurance Officer or Representative
Marine Sciences Laboratory (MSL) Manager

A Science and Engineering Associate (SEA) or Scientist in the Marine Science Laboratory with expertise in laboratory operations is responsible for the preparation of stock solutions, recording of information in stock solution preparation, and defending the data in a quality assurance (QA) review. The SEA or Scientist may train technicians in the preparation of stock solutions including the proper handling of toxic chemicals and the associated hazards.

A Technician properly trained by an SEA or Scientist may be assigned to prepare stock solutions using this procedure. The Technician is responsible for understanding the proper handling of chemicals and recording the information on stock solution preparation in the appropriate logbooks.

4.0 PROCEDURE FOR PREPARATION OF STOCK SOLUTIONS

4.1 Cd Stock Solution using Cadmium Chloride ($CdCl_2$) Reagent

4.1.1 Determine amount of chemical reagent needed to make the appropriate concentration stock solution as described in Section 5.1. $CdCl_2$ reagent is located in MSL 1, Room 121. Label a clean 100 mL volumetric flask with chemical name, date prepared, concentration, storage requirements, reagent batch or lot number, reagent expiration date, and initials. Refer to MSL-A-008-01, Control of Standards, Reagents, Solutions,

Test/Control Articles and Specimens, for guidance on assignment of expiration dates.

4.1.2 Check the calibration logbook to ensure that the balance to be used is within the calibration interval.

4.1.3 Transfer weighing pan to balance and tare with balance doors closed. Carefully add crystals from chemical container to the weighing pan using the designated metal scoop. Accuracy within ± 0.01 g is desired.

4.1.4 Insert a small glass funnel into the labeled flask (flask should sit inside a tri-pour beaker to minimize spill potential). Pour content of weighing pan into funnel.

4.1.5 If crystals are trapped in funnel, slowly add deionized (DI) water to dissolve crystals in funnel. Any crystals that are not dissolved can be probed with the metal scoop.

4.1.6 Finish filling flask to 100 mL line. Insert top on flask and mix thoroughly by inverting the flask.

4.1.7 Store labeled flask and chemical container in designated cabinet in MSL 1, Room 121.

4.2 Cu Stock Solution Preparation from $CuSO_4$ Reagent

4.2.1 Determine amount of $CuSO_4$ reagent needed to make the appropriate concentration stock solution as described in Section 5.2. Reagent is located in chemical cabinet in MSL 1, Room 121. Label a clean 100 mL volumetric flask with chemical name, date prepared, concentration, storage requirements, reagent batch or lot number, reagent expiration date, and initials. Refer to MSL-A-008-01, Control of Standards, Reagents, Solutions, Test/Control Articles and Specimens, for guidance on assignment of expiration dates.

4.2.2 Follow same steps as outlined in Section 4.1.

4.3 Cu Stock Solution Preparation from Cu Nitrate [$Cu(NO_3)_2$] Standard Solution

4.3.1 Determine amount of $Cu(NO_3)_2$ standard needed to make the appropriate concentration stock solution as described in Section 5.3. Standard is located in chemical cabinet in MSL 1, Room 121. Label a clean 100 mL volumetric flask with chemical name, date prepared, concentration, storage requirements, standard batch or lot number, standard expiration date, and initials. Refer to MSL-A-008-01, Control of Standards, Reagents, Solutions, Test/Control Articles and Specimens, for guidance on assignment of expiration dates.

4.3.2 Use a calibrated pipettor to measure $Cu(NO_3)_2$ needed for stock solution.

4.3.3 Add to labeled flask and fill with DI water to 100 mL mark. Mix thoroughly by inverting flask.

4.3.4 Store labeled flask and chemical container in designated cabinet in MSL 1, Room 121.

4.3 Ammonia Stock Solution Preparation from Ammonium Chloride (NH_4Cl) Reagent

4.3.1 Determine amount of NH_4Cl reagent needed to make the appropriate concentration stock solution as described in Section 5.4. Reagent is located in chemical cabinet in MSL 1, Room 121. Label a clean 100 mL volumetric flask with chemical name, date prepared, concentration, storage requirements, reagent batch or lot number, reagent expiration date, and initials. Refer to MSL-A-008-01, Control of Standards, Reagents, Solutions, Test/Control Articles and Specimens, for guidance on assignment of expiration dates.

4.3.2 Follow same procedures as outlined in Section 4.1.

5.0 DATA ANALYSIS AND CALCULATIONS

Concentrations of stock solutions will vary depending on the chemical and its use. Stock solutions must be prepared in cleaned and labeled 100 mL volumetric flasks.

5.1 Calculations for Cd stock solutions prepared from $CdCl_2$ reagent

5.1.1 Using the molecular weight (MW) of Cd, determine the number of moles of Cd needed for the stock solution concentration desired.

5.1.2 Multiply the number of moles of Cd by the formula weight (FW) of the $CdCl_2$ reagent to obtain the weight of $CdCl_2$ reagent to add to the desired volume. (FW is used because reagents may be hydrated.)

5.1.3 A more direct calculation would be to use the ratio of the FW of the $CdCl_2$ reagent to the MW of Cd to obtain the weight of $CdCl_2$ reagent to use.

5.1.4 Sample calculations for preparation of 100 mL of 10 mg/mL Cd stock solution

10 mg Cd/mL = 1 g Cd needed for 100 mL of stock solution

FW of $CdCl_2 \cdot 2.5\ H_2O$ = 228.35
MW of Cd = 112.4

Step 1:

$$\frac{1\text{ g Cd}}{\text{MW of Cd}} = \text{\# moles needed}$$

$$\frac{1\ g\ Cd}{112.4\ g} = 8.89 \times 10^{-3} \text{ moles Cd needed}$$

Step 2:

moles Cd x FW $CdCl_2$ = g $CdCl_2$ reagent

8.89×10^{-3} X 228.35g = g $CdCl_2$

Alternative Method:

Use the ratio of the FW and MW to determine amount of $CdCl_2$ needed when 1 gram of Cd is need for stock solution.

$$\frac{FW\ CdCl_2}{MW\ Cd}$$

$$\frac{228.35\ g}{112.4\ g} = \frac{2.032\ g\ of\ CdCl_2\ reagent}{1\ g\ Cd}$$

5.2 Calculations for Cu stock solutions prepared from $CuSO_4$ reagent

Follow the same procedures as outlined in Section 5.1.

5.3 Calculations for copper stock solutions prepared from $Cu(NO_3)_2$ standard solution

The $Cu(NO_3)_2$ standard contains 1 mg Cu/mL. To prepare 100 mL of 10 ug Cu/mL stock solution:

10 ug Cu/mL x 100 mL = 1 mg Cu = 1 mL of $Cu(NO_3)_2$ standard

100 mL Cu stock solution – 1 mL $Cu(NO_3)_2$ standard = 99 mL of DI water

5.4 Calculations for preparation of ammonia stock solution using ammonium chloride (NH_4Cl)

Follow the same procedures as outlined in Section 5.1.

6.0 QUALITY CONTROL

6.1 Expiration dates of stock solutions

Expiration dates of stock solutions are generally the same as the chemical standard or reagent used. Solutions cannot be used past the expiration date unless steps outlined in MSL-A-008-01, Control of Standards, Reagents, Solutions, Test/Control Articles and Specimens, Sections 4.7.2 and 4.7.3 are followed.

6.2 Review of calculations

All calculations and dilutions will be reviewed and approved by the laboratory supervisor.

6.3 QA review

Routine inspection will be conducted by MSL QA officer or representative to ensure that stock solution preparation is performed in accordance with this procedure.

7.0 SAFETY

Laboratory personnel are required to have access to information on potential risks of working with laboratory chemicals and various hazardous substances, and be trained in procedures for minimizing accidents with these materials. Appropriate attire, including laboratory coats, goggles or safety glasses, and protective gloves, should be worn when working in the vicinity of chemicals and chemical solutions. It is important to use a fume hood when working with volatile or toxic chemicals. Package, store, and dispose of chemical solutions following appropriate local, state, and federal regulations.

Before handling any potentially toxic chemical compounds, the individual preparing the solutions must be aware of any hazards and employ the appropriate safety precautions. Material Safety Data Sheets (MSDSs) are available from the MSL ES&H Representative and are electronically available on the internal PNL web at

http://w3.pnl.gov/safety/msdata.htm

and for recently procured chemicals, hard copies are in the MSDS Notebook located in the entry suite near room 320, MSL 5 (Uplands Facility).

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the work described in this procedure are properly trained *prior to beginning work*, and that documentation of the training exists prior to the performance of those procedures.

The individual preparing the solutions must read this procedure. Individuals must show proficiency for preparing stock solutions. This may be demonstrated by comparison of results for the new stock solution to the results from the old stock solutions. Documentation of training shall be recorded on a training assignment form provided in procedure MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training
MSL-A-008 Control of Standards, Reagents, Solutions, Test/Control Articles and Specimens
MSL-C-009 Use and Performance Checks of Balances

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Marine Sciences Laboratory

EFFECTIVE DATE: 11-20-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-T-036-00

DRY WEIGHT MEASUREMENTS

Approvals:		
AUTHOR: Brian Gruendell	Brian Gruendell	11-20-00
	Signature	Date
TECHNICAL REVIEWER: Roy Kropp	Roy K. Kropp	11-20-00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	11-17-00
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		11.17.00
	Signature	Date

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DRY WEIGHT MEASUREMENTS

1.0 SCOPE AND APPLICATION

This procedure describes the dry weight and ash-free dry weight measurements that are required for various invertebrate tests at the Marine Sciences Laboratory (MSL). These measurements are made by using a drying oven, a muffle furnace, and an analytical balance with an accuracy to five significant figures. This procedure describes the method to perform the listed measurements.

2.0 DEFINITIONS

Dry Weight – The weight of a biomass sample, which does not include the weight of moisture in tissues. Moisture content is a variable component of any biomass sample and therefore can bias mass measurements unless it is accounted for as in the measurement of dry weight.

Ash-Free Dry Weight – The weight of the organic contents of a biomass sample (actually measurement of dry weight minus ash weight). Inorganic content of a biomass sample is a component that can interfere with the relationship between dry weight and organic content of a biomass sample. Ash-free dry weight is considered a more accurate measurement of the actual amount of organic matter in a biomass sample.

3.0 RESPONSIBLE STAFF

MSL Staff
Project Manager, Task Leader
MSL Manager
Quality Assurance Officer or Representative

4.0 PROCEDURES

4.1 Dry Weight

Dry weights are commonly used in measuring growth of amphipods (e.g. *Leptocheirus plumulosus*) and polycheates (e.g. *Neathes arenaceodentata*). The general procedure for performing dry weight measures is to first measure the tare weight of the sample container, heat the sample to remove moisture, measure the dried sample, and then subtract the container tare weight.

4.1.1 Prepare Weighing Pans

a) Either obtain commercial aluminum weighing pans or make weighing pans out of small pieces of aluminum foil formed into cup shapes.
b) Label aluminum weighing pans and place them in drying oven (~60-70 °C) overnight.

c) Remove weighing pans from the drying oven and allow them to cool to room temperature in a desiccator (about 1 hour).

4.1.2 Record Tare Weight of Weighing Pans

a) Measure tare weight to five significant figures using an analytical balance (to nearest 0.01 mg) and record on data sheet.
b) If weighing pans are not to be used immediately, store them in a desiccator or drying oven.

Note: Record the unique identifier for the balance used (serial number or MSL ID number) and ensure that the balance is within its calibration interval before use, record the interval or interval expiration date on the data sheet, and then measure a calibration verification check weight (from a calibrated, certified weight set) within the same range as the measures being made. Record this on the data sheet. The check weight should agree within 2% with the expected weight value.

4.1.3 Add Animals to Weighing Pans

a) Select animals to be weighed and remove any debris adhering to animal; place specified number of animals in each weighing pan.
b) If animals are in seawater, rinse with freshwater prior to placing animals into weighing pan to avoid the weight of salts in seawater, complicating the measurement.
c) Excess water in the weighing pan may be removed by using a Kim-Wipe™ as a wick, being careful to avoid small animals adhering to the Kim-Wipe™. Alternatively, a fine glass pipet may be used to draw up excess water.

4.1.4 Oven Dry Filled Weighing Pans

a) Set drying oven temperature between 50°C and 110°C depending on project requirements (usual temperature setting is ~60°C to ~70°C). Place filled weighing pans in drying oven for 24 hr (a longer initial drying time may be necessary for larger invertebrates).
b) Remove samples from drying oven and allow them to cool to room temperature in a desiccator (~1 hr).

4.1.5 Measure Dry Weight of Filled Pans

a) Obtain the first dry weight estimate by weighing samples on a five-place analytical balance to the nearest 0.01 mg; record weight on data sheet.
b) Return the samples to drying oven for 24 hr for further drying.
c) Continue the drying and weighing process (steps 4.1.4 and 4.1.5) until weights are "constant" (i.e. mass of samples varies by 0.1 mg or less from the previous weighing.)

4.1.6 Calculate Dry Weight of Samples

a) On data record sheet, subtract the tare weight from the final dry weight, and record result.
b) The resulting number is the "dry weight" of the animal tissues placed in the weighing pan.

4.2 Ash-Free Dry Weight

Ash-free dry weights are commonly used in measuring growth of insect larvae (e.g. *Chironomus tetans*). The general procedure for performing ash-free dry weight measures is to first measure the tare weight of the container, measure the dry weight of the sample, ash the sample, measure the ash weight, and then subtract the ash weight from the tared dry weight.

4.2.1 Prepare Crucibles or Weighing Pans

a) Label pre-cleaned crucibles or commercial aluminum weighing pans and place them in a pre-heated muffle furnace (usually ~500 °C depending on project protocols) for 2 hr. The commercial aluminum weighing pans are preferred to aluminum foil due to the heavier gauge aluminum, which withstands the high temperature of the muffle furnace.
b) Remove containers from the muffle furnace and allow them to cool to room temperature in a desiccator.
c) **Caution: The muffle furnace operates at very high temperatures; always wear heat resistant gloves, safety glasses, and use tongs when placing crucibles or weight pans in the furnace or when removing them. Do not set the crucible or weight pans on flammable materials.**

4.2.2 Record Tare Weight of Crucibles or Weighing Pans

a) Measure the tare weight of crucibles or aluminum weighing pans to five significant figures using an analytical balance (to nearest 0.01 mg) and record on data sheet.
b) Crucibles must be returned to muffle furnace for 1 hr (at ~500 °C), removed to cool in a desiccator and re-weighed. Record the weight. Repeat this step until the crucible weight is "constant" (i.e. mass of crucible varies by 0.1 mg or less from the previous weighing.)
c) For aluminum weighing pans, do not perform step b as "constant" weight will be achieved with one heating in the muffle furnace.
d) If crucibles or weighing pans are not to be used immediately, store them in a desiccator.

4.2.3 Add Animals to Crucibles or Weighing Pans

a) Select animals to be weighed and remove any debris adhering to animal; place specified number of animals in each crucible or weighing pan.

b) If animals are in seawater, rinse with freshwater prior to placing animals into weighing pan to avoid the weight of salts in seawater complicating the measurement.
c) Excess water in the crucible or weighing pan may be removed by using a Kim-Wipe™ as a wick, being careful to avoid small animals adhering to the Kim-Wipe™. Alternatively, a fine glass pipet may be used to draw up excess water.

4.2.4 Oven Dry Filled Crucibles or Weighing Pans

a) Set drying oven temperature between 50°C and 110°C depending on project requirements (usual temperature setting is ~60°C to ~70°C). Place filled crucibles or weighing pans in drying oven for 24 hr (a longer initial drying time may be necessary for larger invertebrates).
b) Remove samples from drying oven and allow them to cool to room temperature in a desiccator (~1 hr).

4.2.5 Measure Dry Weight of Filled Crucibles or Pans

a) Obtain the first dry weight estimate by weighing samples on a five-place analytical balance to the nearest 0.01 mg; record weight on data sheet.
b) Return filled crucibles or weighing pans to drying oven for 24 hr for further drying.
c) Continue drying and weighing process (steps 4.2.4 and 4.2.5) until weights are "constant" (i.e. mass of samples varies by less than 0.1 mg from the previous weighing.)

4.2.6 Ignite Filled Crucibles or Weighing Pans

a) Place filled crucibles or aluminum weighing pans in a pre-heated muffle furnace (usually ~500 °C depending on project protocols) for 2 hr.
b) Remove samples from muffle furnace and allow them to cool to room temperature in a desiccator.
c) **<u>Caution</u>: The muffle furnace operates at very high temperatures; always wear heat-resistant gloves, safety glasses, and use tongs when placing crucibles in the furnace or when removing them. Do not set the crucible on flammable materials.**

4.2.7 Re-Wet Ash in Crucibles or Weighing Pans

a) Re-wet ash with a couple of drops of deionized water.
b) Only use enough deionized water to re-wet sample, keep amount of water used to a minimum.
c) This step reintroduces water of hydration for minerals, which is not driven off in the drying oven but is lost in the muffle furnace. If not corrected for, this water loss will be recorded as volatile organic matter.

4.2.8 Oven Dry Ash in Crucibles or Weighing Pans

a) Return re-wetted, filled crucibles or weighing pans to drying oven for 24 hr (usual temperature setting is ~60°C to ~70°C, but can be between 50°C and 110°C depending on project requirements).
b) Remove samples from drying oven and allow them to cool to room temperature in a desiccator (~1 hr).

4.2.9 Measure Dry Weight of Ash in Crucibles or Pans

a) Obtain the first ash dry weight estimate by weighing samples on a five- place analytical balance to the nearest 0.01 mg; record weight on data sheet.
b) Return the samples to the drying oven for 24 hr for further drying.
c) Continue the drying and weighing process (steps 4.2.8 and 4.2.9) until weights are "constant" (i.e. mass of samples varies by less than 0.1 mg from the previous weighing.)

4.2.10 Calculate Ash-Free Dry Weight of Samples

a) On data record sheet, subtract the tare weight from the final dry weight, and record result.
b) The resulting number is the "dry weight" of the animal tissues placed in the weighing pan.
c) Then subtract the ash dry weight (as obtained in step 4.2.9) from the "dry weight" of the animal tissues to obtain the "ash-free dry weight" and record result on data record sheet.

5.0 DATA ANALYSIS AND CALCULATIONS

5.1 Documentation

Document each value obtained for the container of interest. Report the "constant" weight of the measurements. Keep all laboratory records, test results, measurements, and other supporting documentation for each toxicity test in a project file dedicated to that purpose.

In some cases, the weight measurement may be expressed as weight per the number of individuals used in the determination. In this case, the weight should be divided by the total number of animals used. In other cases, the weight is based on a standard number of animals used, and division of the weight obtained by the number of animals is not necessary. Contact the laboratory lead or project manager to determine which value should be reported.

Two example data sheets are provided Attachments 1 and 2. Documentation must include the "constant" weight measurements as mentioned above, as well as the serial number of the balance used, balance calibration expiration date, and balance calibration verification checks using a certified weight within the range of the measures.

5.2 Calculations

Dry weight (mg) and ash-free dry weight (mg) are calculated using the following formulas:

$$DW = W_s - W_t$$

dry weight = (sample with tare weight) minus (tare weight)

$$DWA = W_a - W_t$$

dry weight of sample ash = (ash with tare weight) minus (tare weight)

$$AFDW = DW - DWA$$

ash-free dry weight = (dry weight) minus (dry weight of sample ash)

where:

W_t = weight of weighing pan, or crucible, after heating in drying oven (tare weight)

W_s = weight of sample and weighing pan, or crucible, after drying oven (sample with tare weight)

DW = dry weight of sample

W_a = weight of weighing pan, or crucible, after ignition in muffle furnace, re-wetting, and re-heating in drying oven (ash with tare weight)

DWA = dry weight of sample ash

AFDW = ash-free dry weight

Dry weight (mg) <u>per</u> <u>individual</u> and ash-free dry weight (mg) <u>per</u> <u>individual</u> may be calculated, if required, using the following formulas:

$$DW(perIndividual) = DW \div N$$

dry weight per individual animal = (dry weight) divided by (number of animals)

$$AFDW(perIndividual) = AFDW \div N$$

ash-free dry weight per individual animal = (ash-free dry weight) divided by (number of animals)

where:

N = number of animals (individual samples) that were placed in that weigh pan or crucible.

6.0 QUALITY CONTROL

6.1 Whenever using MSL balances, record the unique identifier for the balance used (serial number or MSL ID number) and ensure that the balance is within its calibration interval before use, record the interval or interval expiration date on the data sheet, and then measure a calibration verification check weight in the same range as the measures being made. Record this on the data sheet. The check weight should agree within 2% with the expected weight value. Verification checks should be made at the beginning and end of each weighing session.

6.2 Periodic surveillances should be conducted by the MSL's Quality Assurance Officer or Representative to ensure that all aspects of the testing accurately reflect the work that is planned and in-process, and that all necessary information, as defined by regulations, procedures and program-specific requirements, is included. Results of periodic surveillances may become a part of the project files.

7.0 SAFETY

The major safety concern associated with the implementation of this procedure is during the operation and use of the muffle furnace. For this procedure the furnace operates at or near 500°C (932°F), which can cause severe burns if proper safety measures, care, and caution are not implemented. Always wear safety glasses and proper heat-resistant gloves when using the muffle furnace. In addition, tongs must be used to handle the crucibles or weighing pans. When items are removed from the muffle furnace they must be placed on an appropriate heat-resistant surface and not on any flammable surface.

8.0 TRAINING REQUIREMENTS

All staff members who will be performing toxicity test dry weight measurements shall first read this procedure prior to performing the measurements. Documentation of training shall be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

US EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA 600/R-99/064, U. S. Environmental Protection Agency, Office of Research and Development, Duluth, Minnesota.

American Public Health Association, American Water Works Association, Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Paine, R.T. 1971. The Measurement and Application of the Calorie to Ecological Problems. Annual Review of Ecology and Systematics. 2:145-164.

Battelle
Putting Technology to Work
Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, WA 98382
(360) 683-4151

Dry Weight Form

Project: _____ Page: _____ of _____

Project No. _____ Balance Serial No. _____

Sample: _____ Balance Calibration Expiration Date: _____

Oven Temp. _____ Oven Serial No. _____

Note: Prior to using the balance each day, a calibration verification check using a certified weight should be measured. Record this verification check on a separate line below.

(continue until variation < 0.0001g)

Weigh Pan Number	Number of Animals	Tare Weight (grams)	Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)
	Date:	Date:					
	Initials:	Initials:					

ATTACHMENT 1
Example Dry Weight Record From

Battelle
Putting Technology to Work
Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, WA 98382
(360)683-4151

Ash-Free Dry Weight Form

Project: _____ Page: _____ of _____

Project No. _____ Balance Serial No. _____

Sample: _____ Balance Calibration Expiration Date: _____

Oven Temp. _____ Oven Serial No. _____

Furnace Temp. _____ Muffle Furnace Serial No. _____

Note: Prior to using the balance each day, a calibration verification check using a certified weight should be measured. Record this verification check on a separate line below.

Weigh Pan Number	Number of Animals	Tare Weight (grams)	*(continue until variation < 0.0001g)* Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)	Dry Weight of Pan with Animals (grams)	*(continue until variation < 0.0001g)* Dry Weight of Pan with Ash (grams)	Dry Weight of Pan with Ash (grams)	Dry Weight of Pan with Ash (grams)
	Date:	_____	Date: _____	_____	_____	Date: _____	_____	_____
	Initials:	_____	Initials: _____	_____	_____	Initials: _____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____

ATTACHMENT 2
Example Ash-Free Dry Weight Form

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Marine Sciences Laboratory

EFFECTIVE DATE: 2-22-01

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-W-003-00

CALIBRATION AND USE OF THERMOMETERS

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	2-19-01
	Signature	Date
TECHNICAL REVIEWER: Gary Dennis	Gary Dennis	2-19-01
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	2-19-01
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	2-22-01
	Signature	Date

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CALIBRATION AND USE OF THERMOMETERS

1.0 SCOPE AND APPLICATION

This procedure describes the methods needed to calibrate and use temperature measurement equipment including glass thermometers, digital thermometers, thermocouples, thermistors, and the devices used to read and record temperature such as chart recorders and data loggers. Most of the devices are used for the routine measurement and monitoring of water, solution, or air temperature during laboratory testing and to monitor temperature of cold storage facilities. There are also other approaches for temperature measurement (labels, color-change crayons, temperature cones that melt a certain temperatures, and Galileo thermometers, that are not routinely used or covered by this procedure. This procedure does not address the calibration and use of temperature controllers. MSL-I-026, Use of Laboratory Refrigerators and Freezers should be consulted regarding the routine monitoring of these items. This procedure replaces MSL-M-047.

> **Note: Mercury (Hg) –filled thermometers are no longer acceptable for test measurements at Battelle facilities. Because MSL analyzes Hg in samples at ultra-low levels, Hg thermometers at MSL can be a source of container or sample contamination and should not be used.**

2.0 DEFINITIONS

Enclosed Thermometer – At MSL, enclosed thermometers (generally spirit-filled) are used to monitor temperatures in cold room, refrigerators, freezers and incubators. The devices are compared with a National Institute of Standards and Technology (NIST)-calibrated standard when fabricated. A substitute for spirit-filled thermometers is a water-filled container, which is used for devices above the freeze point.

Metrology –Metrology is the science that deals with measurement.

NIST – The National Institute of Standards and Technology, formerly known, as the National Bureau of Standards (NBS) is an agency of the U.S. Department of Commerce's Technology Administration. Established in 1901, NIST strengthens the U.S. economy and improves the quality of life by working with industry to develop and apply technology, measurements, and standards. It carries out this mission through a portfolio of four major programs: Measurements and Standards Laboratories, Advanced Technology Program, Manufacturing Extension Partnership, and the Baldrige National Quality Award.

NIST Traceability - NIST traceability for temperature measuring equipment is a measure of *system* accuracy and provides the capability to compensate or calibrate the instrument to increase accuracy.

Thermistor - A resistor made of semiconductors having resistance that varies rapidly and predictably with temperature.

Thermocouple - A thermoelectric device used to measure temperatures accurately, especially one consisting of two dissimilar metals joined so that a potential difference

generated between the points of contact is a measure of the temperature difference between the points.

3.0 RESPONSIBLE STAFF

Staff Members
Project Manager or designee
MSL QA Officer or Representative

4.0 PROCEDURE

4.1 Necessary Equipment

- Laboratory thermometer, thermocouple, or thermistor
- NIST-certified thermometer (with correction factor documentation). Be sure that the type of thermotemter is known. MSL has an immersion-type and a regular-type thermometer available. NIST-certified thermometers have an assigned calibration interval. The interval is provided when these thermometers are initially purchased and is usually good for 1-2 years. After this time calibration traceability needs to be certified by a metrology laboratory.
- Ice water bath
- Room temperature water bath

- **Note: When using the immersion NIST-traceable thermometer, the thermometer is immersed to the depth specified by the manufacturer.**

4.2 Calibration

Thermometers used for the routine measurement and monitoring of water or air temperature during laboratory testing or chemical tests shall be calibrated at least semi-annually. The client or testing program may specify a greater calibration frequency.

Thermometers used to monitor the temperature of cold storage facilities shall be calibrated annually. When performing calibration of thermometers, staff members shall comply with the following.

Digital thermometers with detachable probes must be calibrated as one unit. The probe needs to be labeled along with the digital read out unit with appropriate markings.

4.2.1 Place the certified thermometer in an ice water bath.

4.2.2 When the temperature has stabilized, record the temperature (with any appropriate correction factor as required) on the Calibration Record as the Certified Reading (see Attachment 1 for an example).

Example: If the thermometer reads 24 °C and the correction factor at that temperature is -0.2 °C, then the temperature to record is 23.8 °C.

4.2.3 Place the Laboratory thermometer, (glass thermometers, digital thermometers, thermocouples, thermistors) next to the certified thermometer and read the temperature of the Laboratory thermometer after a stable reading is observed and record on the Calibration Record as the Lab Reading.

4.2.4 If the two thermometers do not read within 0.2 °C for thermometers that are used for the routine measurement and monitoring of water or air temperature or within 1.0 C for thermometers used to monitor the temperature of cold storage facilities, do either of the following:

1) If possible, adjust the Laboratory thermometer to match the crtified thermometer, or

2) Attach a label to the Laboratory thermometer informing the operator of the two following status items:

a) The correction factor to be applied (e.g. add 0.5 °C to the reading for true temperature)

b) The calibration expiration date/initial

c) If the two thermometers read within the established criteria, attach a label to the laboratory thermometer informing the operator of the two following status items:

(1) No correction factor is required, and

(2) the calibration expiration date/initial

4.2.5 Repeat steps 4.2.2 through 4.2.4 at ambient temperature (e.g., water bath, room air).

4.2.6 Errors shall be crossed out with a single line, initialed, dated and the reason for the error shall be noted (error codes are listed in procedure MSL-D-001).

4.2.7 Upon completion of the Calibration Record, staff members shall transfer them to a labeled three-ring binder or place them in the project files.

4.3 Verifying Temperature Recording Devices

The temperature of devices used to read and record temperature such as chart recorders and data loggers can be verified using the NIST-traceable thermometer. Place the thermometer in the water, refrigerator, freezer, etc. (ensuring that the range of the NIST-traceable thermometer is compatible with the range of the temperature-recording device) and compare the reading of the NIST-traceable thermometer against the temperature-recording device. Use a data sheet similar to that provided as

Attachment 1. Refer to manufacturer's specifications for precision and accuracy. The rule-of-thumb agreement when no manufacturer's recommendations are provided should be within 1% or 0.2 °C, and if not, either adjust (when possible) or post a correction factor for the measuring device following Step 4.2.4 above.

4.4 Use of Thermometers

When using thermometers, staff members shall make sure thermometer is calibrated and then shall comply with the following:

4.3.1 Once the temperature has stabilized, read the temperature to the nearest 0.1 °C when measuring water and air temperatures for laboratory testing and to the nearest 1.0 °C when measuring cold storage facility temperatures.

4.3.2 Once the temperature has been accurately measured, apply the correction factor, if any, noted on the thermometer. Record the temperature, with corrections applied, on the appropriate data sheet or logbook.

5.0 DATA ANALYSIS AND CALCULATIONS

$$\frac{\text{Observed T}}{\text{Expected T}} * 100 = \%\ \text{difference}$$

6.0 QUALITY CONTROL

Thermometers shall be calibrated using a certified thermometer and the documentation available in the laboratory and a copy provided to the MSL QA Officer. Copies may be required to also be placed in the project files. The calibration information such as date, initials of staff member conducting the calibration and any necessary correction factor should be written on a label and be easily visible on the instrument.

7.0 SAFETY

Identify and replace Hg thermometers when ever possible. If a Hg thermometer is broken, use a Hg spill kit to clean it up following the kit instructions.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this procedure are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all staff must read this procedure and demonstrate proficiency in the use of this instrument prior to application in project use. A staff member unfamiliar with the use of thermometers will receive a verbal and visual demonstration by a certified staff

member. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this procedure are followed. Once proficiency has been verified, documentation of training will be recorded on a training assignment form obtained from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training
MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks
MSL-I-026 Use of Laboratory Refrigerators and Freezers

ATTACHMENT 1
Example Thermometer Calibration Form

Date: _____

Technician: _____

Notes:

Temperature Measuring Device ID – unique number or serial number plus Manufacturer/Model	Ice bath	Ice bath	Comparison within 0.2°C or 1% for temperature recording devices[1]? Yes or no?	Correction factor	Room T bath or ambient air T (circle one)	Room T bath or ambient air T (circle one)	Comparison within 0.2°C or 1% for temperature recording devices[1]? Yes or no?	Correction factor
NIST-traceable Thermometer		----- ----- --				----------		
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			
	------ ------				---------- -			

1 Or other project specific criterion – list the different criteria limit on the data sheet

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Effective Date: 10-29-99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-W-007-01
DETERMINATION OF AMMONIA

Approvals:		
AUTHOR: Laurie Niewolny	Laurie Niewolny	10.29.99
	Signature	Date
TECHNICAL REVIEWER: Rhonda Karls	Rhonda Karls	10.25.99
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	10-29-99
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		10.29.99
	Signature	Date

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Determination of Ammonia

1.0 SCOPE AND APPLICATION

This procedure describes the methods needed to measure ammonia in overlying water and/or interstitial water used in toxicity tests. When required, measurement of ammonia is necessary during the conduct of various types of toxicity tests.

2.0 DEFINITIONS

D.I. water Deionized water

3.0 RESPONSIBLE STAFF

Laboratory Staff Members
Project Manager, Task Leader
MSL Manager
Quality Assurance Officer or Representative

4.0 PROCEDURE

Ammonia can be determined either by using the ion-selective electrode methods (4.1, 4.2) or the salicylate-hypochlorite method (4.2).

4.1 High-Range Ammonia Calibration and Operation Using Ammonia Ion-Selective Electrode and Orion 900A Meter

4.1.1 Meter Calibration

4.1.1.1 Set-up

a) The ammonia meter (Orion 900A) and ion-selective electrode set-up does not generally require a "warm up" period; the meter is always left on and the electrode is at room temperature. If it is off, a 30-minute warm up period is necessary before use.

b) The ammonia standards and all samples should be at the same temperature, which is 20-30°C, before calibration or any ammonia measurements are attempted. This temperature range requirement is to ensure that the maximum efficiency and accuracy of the electrode are attained. The required temperature range can be reached by placing the glass beakers containing the ammonia standards and samples in a heated water bath.

c) There are two ammonia standard concentrations used for a two-point calibration: However, the calibration range must be selected before beginning, and is based on the expected concentration of ammonia in the

samples. Refer to the expected ranges listed in Table 1 below. There are four possible standard concentrations 500, 100, 50.0, and 10.0 mg/L (or ppm) as NH_3. The preparation of each standard is as follows:

- **500.0 mg/L** = 29.412 ml of 1700 ppm (0.1 M) stock solution + 70.588 mL seawater
- **100 mg/L** = 5.88 mL of 1700 ppm (0.1 M) stock solution + 94.12 mL seawater
- **50 mg/L** = 10 mL of 500 ppm stock solution + 90 mL seawater (serial dilution)
- **10.0 mg/L** = 10.0 ml of 10 ppm stock solution + 90 mL seawater (serial dilution)

Note: Fresh seawater is required for standard preparation. The target temperature for the seawater is 20°C. The source of fresh seawater is the filtered seawater hose supply or filtered seawater in one of the various headtanks in the Wet Lab.

Table 1. Estimating Ammonia Concentration Ranges

Type of Test	Ammonia Concentration Range
Larval Mysids	1-10 mg/L (ppm) calibration range
Overlying water	test dependent
Porewater	10-100 mg/L (ppm) calibration range
Reference toxicant test	50-500 mg/L (ppm) and/or 10-100 mg/L (ppm) calibration range

4.1.1.2 Calibration

See Attachment 1 for an example of the form that is to be used to record ammonia electrode calibration information.

a) First, warm-up the water bath to 20-30°C.

b) Fill the electrode with ammonia- electrode filling solution and rinse the outer surface of the electrode thoroughly with D.I. water.

c) Shake down the electrode (like a clinical thermometer) to remove internal bubbles.

d) Place 30.0 ml of the lowest standard in a clean glass beaker, and start the magnetic stir bar.

- Ensure that the other standard and any samples to be measured are near 20°C. A 1°C difference between the standards and the samples will result in about 2% measurement error.

- Place 30 mL of the next highest standard in the chosen range in another beaker.
- Place any ammonia water samples into 50 mL plastic (S-P) beakers (the minimum volume for a sample is 20.0 mL).

e) Place the electrode in the beaker and add 1 mL (measured with a repipettor) of the pH/ISA solution (Ionic Strength Adjuster: 5M NaOH/ 0.5 M Disodium EDTA/10% methanol with color indicator).

NOTE: If the magnetic stirrer and stir bar do not fully mix the sample when the pH/ISA solution is added, swirl the solution to ensure adequate mixing.

f) Press "calibrate" button, and enter 2 at the "number of standards" prompt.

g) Allow to sit until a stable reading is obtained and the meter says "enter value". At this point enter the calibration standard nominal value and press "Yes".

h) Remove the electrode from the solution, rinse the electrode with D.I. water, and repeat steps e-g above using the next highest standard. Enter the calibration information on the calibration data sheet (refer to Attachment 1 for an example).

i) The meter automatically prints out the two-point calibration data. The slope must be between –54 to –60 MeV/decade when the solution is between 20-25 °C. The slope is provided as part of the data print-out. The slope is defined as the change in millivolts observed with every tenfold change in concentration. The slope is the best means for checking electrode calibration and should be monitored.

4.1.2 Taking Sample Measurements

4.1.2.1 Once the calibration is complete, water samples can now be measured. The procedure is similar to taking standard readings. See Attachment 1 for an example of a form used to record calibration and Attachment 2 for an example form for recording ammonia sample measurements.

4.1.2.2 Rinse the electrode with D.I. water and place in the sample container and stir until the meter reading is stable. The meter reading says "Meas" while it is measuring and then "RDY" [ready] when it is done (stable). Add the necessary amount of pH/ISA solution to the sample; 1 mL per 30 mL of sample), then swirl to mix. At this point press the "print" button to record the final reading in mg/L (see Attachment 1.) The strips from the instrument are fixed onto the data sheet (refer to Attachment 2) and become part of the data record.

4.1.2.3 The ammonia meter should be re-calibrated after two hours of use to limit any possibility of drift. Re-calibration is done by following the procedure explained in Section 4.1.1.2.

4.1.2.4 Do not allow the electrode to dry out. Store it in 10 ppm standard when taking short breaks in the measurement process.

4.1.3 Long-Term Electrode Storage

Store the electrode in 100 ppm standard solution.

4.2 Low-Range Ammonia Calibration and Operation Using Ammonia Ion-Selective Electrode and Orion 900A Meter

4.2.1 Meter Calibration

4.2.1.1 Set-up

a) The ammonia meter (Orion 900A) and ion-selective electrode set-up do not generally require a "warm up" period; the meter is always left on and the electrode is at room temperature. If it is off, a 30-minute warm up period is necessary before use.

b) The ammonia standards and all samples should be at the same temperature, which is 20-30°C, before calibration or any ammonia measurements are attempted. This temperature range requirement is to ensure that the maximum efficiency and accuracy of the electrode are attained. The required temperature range can be reached by placing the glass beakers containing the ammonia standards and samples in a heated water bath.

c) There are two ammonia standard concentrations used for a two-point calibration: 10.0, and 1.00 mg/L. The preparation of each is done as follows:

- **100 mg/L** = 5.88 ml of 1700 ppm (0.1 M) stock solution + 94.12 mL seawater.

  NOTE: this standard is used to make serial dilutions for the two standards and is therefore made in a larger volume.

- **10.0 mg/L** = 10.0 ml of the 100 mg/L ammonia standard brought to a final volume of 100.0 ml with D.I. water to make 100.0 ml of 10 mg/L ammonia standard.

- **1.00 mg/L** = 10.0 ml of the 10 mg/L ammonia standard brought to a final volume of 100.0 ml with D.I. water to make 100.0 ml of 1 mg/L ammonia standard.

  NOTE: These standard concentrations are used assuming that the approximate ammonia range in the samples is between 0 mg/L and 10 mg/L. If the expected ammonia range is higher than this, then the procedures described in Section 4.1 should be used.

4.2.1.2 Calibration

See Attachment 1 for an example of the form that is to be used to record ammonia electrode calibration information.

a) First, warm-up the water bath to 20-30°C.

b) Fill the electrode with ammonia- electrode filling solution and rinse the outer surface of the electrode thoroughly with D.I. water.

c) Shake down the electrode (like a clinical thermometer) to remove internal bubbles.

d) Place 30.0 ml of 1.00 mg/L ammonia standard in a clean beaker.

- It is a good idea to place the two standards and any samples to be measured into the water bath at this time to allow for temperature equilibrium. Place 30.0 ml of the 10.0 mg/L ammonia standard in another beaker.

- Place any ammonia water samples into glass beakers or any other small plastic containers (the minimum volume for a sample is 20.0 mL), and then into the water bath as well.

e) Place the electrode in the beaker and add 1 mL (measured with a repipettor) of the pH/ISA solution (Ionic Strength Adjuster: 5M NaOH/ 0.5 M Disodium EDTA/10% methanol with color indicator).

NOTE: If the magnetic stirrer and stir bar do not fully mix the sample when the pH/ISA solution is added, swirl the solution to ensure adequate mixing.

f) Press "calibrate" button, and enter 2 at the "number of standards" prompt.

g) Allow to sit until a stable reading is obtained and the meter says "enter value". At this point enter the calibration standard nominal value and press "Yes".

h) Remove the electrode from the solution, rinse the electrode with ph 4.0 buffer, and repeat steps e-g above using the next highest standard. Enter the calibration information on the calibration data sheet (refer to Attachment 1 for an example).

i) The meter automatically prints out the two-point calibration data. The slope must be between –54 to –60 MeV/decade when the solution is between 20-25°C. The slope is provided as part of the data print-out. The slope is defined as the change in millivolts observed with every tenfold change in concentration. The slope is the best means for checking electrode calibration and should be monitored.

4.2.2 Taking Sample Measurements

4.2.2.1 Once the calibration is complete, water samples can now be measured. The procedure is similar to taking standard readings. See Attachment 1 for an example of a form used to record calibration and Attachment 2 for an example form for recording ammonia sample measurements.

4.2.2.2 Rinse the electrode with pH 4.0 buffer and place in the sample container and stir until the meter reading is stable. The meter reading says "Meas" while it is measuring and then "RDY" [ready] when it is done (stable). Add the necessary amount of pH/ISA solution to the sample; 1 mL per 30 mL of sample), then swirl to mix. At this point press the "print" button to record the final reading in mg/L (see Attachment 1.) The strips from the instrument are fixed onto the data sheet (refer to Attachment 2) and become part of the data record.

4.2.2.3 The ammonia meter should be re-calibrated after two hours of use to limit any possibility of drift. Re-calibration is done by following the procedure explained in Section 4.1.1.2.

4.2.2.4 Do not allow the electrode to dry out. Store it in pH 4.0 buffer when taking short breaks in the measurement process.

4.2.3 Long-Term Electrode Storage

Store the electrode in 100 ppm standard solution.

4.3 Salicylate-Hypochlorite Method for Ammonia Determination in Seawater

REFERENCE: *Bower, C. E., and T. Holm-Hansen. 1980. Can. J. Fish. Aquat. Sci. 37: 794-798*

The operating range of this method is 0.01 to 1.00 mg/L NH_4-H (total ammonia).

4.3.1 Procedure

Attachments 3 and 4 are examples of forms that are to be used to record ammonia preparation documentation and ammonia as part of water quality measurements.

4.3.1.1 Turn-on the spectrophotometer for at least 15 minutes prior to use. (See manufacturer procedures for operation of the Bausch & Lomb 710 spectrophotometer.)

4.3.1.2 Take out the Salicylate-Catalyst solution and let it warm to room temperature IN THE HOOD. (**DANGER**: This catalyst contains cyanide - use only in a hood. It is also light sensitive and must be stored IN THE DARK)

4.3.1.3 Dilution series of the samples to be measured are done according to the following criteria. The diluted samples should then be placed in a disposable container (i.e. plastic centrifuge tubes).

a) If the concentration of the sample is known to be >100 mg/L ammonia, then a 1:1000 dilution is needed - this is 0.1 ml or 100 uL of sample in a 100.0 ml volumetric flask. Next, a 10.0 ml subsample is taken and placed in a centrifuge tube. As a substitute, 10 uL in 10.0 ml of D.I. water can be used.

b) If the concentration of the sample is known to be ≤ 100 mg/L ammonia, then a 1:100 dilution is needed. This dilution is 0.1 ml or 100 uL of sample in a 10.0 ml volumetric flask. Next, a 10.0 ml subsample is taken and placed in a centrifuge tube. As a substitute, 100 uL in 10.0 ml of D.I. water can be used.

c) For a sample with an expected zero concentration of ammonia, a 1:10 dilution is needed. A 1:10 dilution is 1.0 ml of sample in a 10.0 ml volumetric flask. The entire 10.0 ml sample is placed in a centrifuge tube or other disposable container.

NOTE: Any other dilution scheme that results in the same dilutions as mentioned above can be used if at least 10.0 ml of sample is available for analysis.

4.3.1.4 The reagent blank used is 10.0 ml of DI. water.

4.3.1.5 The other reagents used in this procedure are:

a) a 0.1 mg/L ammonia standard solution,
b) an Alkaline-Citrate solution,
c) a Salicylate-Catalyst solution (mentioned above), and
d) a Sodium-Hypochlorite solution or bleach (Clorox)

The information on how each of these reagents are prepared can be found in section 3.0 as well as in the reference publication cited (Bower and Holm-Hansen, 1980).

4.3.1.6 A 1:10 dilution of the Alkaline-Citrate solution to Bleach is made and placed in a beaker (10 parts of Alkaline-Citrate solution and 1 part of Bleach, both added and mixed in a volumetric flask). This is the Alkaline-Hypochlorite solution and it must be used within one hour of preparation.

4.3.1.7 Add **1.2 ml** of the Salicylate-catalyst and **2.0 ml** of the Alkaline-hypochlorite solution (IN THIS ORDER!) to each sample, to each tube containing the 0.1 mg/L ammonia standard (3 tubes for three different measurements), and to the D.I. water reagent blank IN THE HOOD!

4.3.1.8 A time window of at least 1 hour and a maximum of 3 hours is needed for the reaction to take place. The samples should be allowed to react IN THE DARK.

4.3.2 Measuring Samples

4.3.2.1 A sample of the reagent blank (D.I. water) is placed in a calibration cuvet (glass) and then the cuvet is placed in the center rack and read (the reading should be zero; if not, adjust the instrument until it reads zero) each time before a sample or a calibration standard is analyzed. A sample from each 0.1 mg/L ammonia standard tube prepared (3) is read and a mean absorbance value is taken. The formula,

$$F = 100/ (E_{cs} - E_b)$$

where E_{cs} is the mean absorbance of the calibration standard and E_b is the mean blank absorbance,is used to obtain the conversion factor (F). F is used in the conversion formula for converting absorbance to ammonia concentration. It represents the quotient of the concentration of ammonia in the calibration standards (100 ug/L) and the corrected absorbance of the calibration standards – refer to Bower and Holm-Hansen (1980) for more detail.

NOTE: Because the blank absorbance is zero (DI water), the formula is reduced to:

$$F = 100/ E_{cs}$$

4.3.2.2 Once the conversion factor (F) is calculated, each sample can now be measured and the ammonia concentration determined. Before each sample is measured, the reagent blank is checked to ensure that a zero reading is still obtained. The blank should be changed (fresh DI water) approximately every seven to ten samples, or when it is found that the measurement is drifting significantly away from zero when checked.

Fill a disposable cuvet to the fill line with an aliquot of sample and place in the spectrophotometer. Check the zero and then read and record the absorbance of the sample. This absorbance value can then be used to convert to ammonia concentration in the sample. It is recommended that replicates of each sample be measured and a mean absorbance be used. A different cuvet should be used for every three measurements made.

4.3.2.3 The conversion formula for converting absorbance to ammonia concentration is the following:

$$\text{ug NH4-N/L} = (E_s - E_b) * F$$

where E_s is the mean sample absorbance and E_b is the mean blank absorbance (which should be zero). Therefore, the formula reduces to:

$$\text{ug NH4-N/L} = E_b * F.$$

Because the formula calculates concentration in micro-grams, a conversion to mg/L is then needed, if this is the required report unit.

4.3.3 List of Reagent Preparations

These weights and volumes correspond to the glassware and volumes used in the laboratory; the volumes used in the original referenced procedure can be found in Bower and Holm-Hansen (1980), pp. 795.

4.3.3.1 **Salicylate-Catalyst solution:**

- 220.25 grams of Sodium Salicylate, and
- 0.14 grams Sodium Nitroprusside,

- brought to a final volume of 500 ml with D.I. water

Store this solution in a brown glass bottle (or cover volumetric flask with dark paper) and at 5.0 degrees Celsius. This solution is stable for three months.

4.3.3.2 Alkaline-Citrate solution:

- 18.5 grams Sodium Hydroxide, and
- 100.0 grams Sodium Citrate
- brought to a final volume of 1.0 L with D.I. water

This solution is stable indefinitely

4.3.3.3 Sodium-Hypochlorite solution:

- Any stable commercial bleach (Clorox)

4.3.3.4 Alkaline-Hypochlorite solution:

- A 1:10 dilution of the Alkaline-Citrate solution and Sodium-hypochlorite solution - store at 5.0 °C for up to one hour only.

4.3.3.5 Ammonia Standard:

- Preferably made by using ammonium sulfate; can be made using an ammonium chloride solution
- 4.72 grams of ammonium chloride in 1.0 Liter of D.I. (volumetric)
- This stock solution is then diluted 1:10 with D.I. water and stored in a brown glass bottle and at 5.0 degrees Celsius

4.3.4 Qualitative Observations

As mentioned in Bower and Holm-Hansen (1980), there are qualitative observations that can be made for the samples. After the reagents have been added and allowed to react for sufficient time, different colors will be present in each sample tube, corresponding to the amount of ammonia in each sample. A dark blue will indicate more ammonia present than in a tube with a light green color for example. As stated in the paper, these color changes can be used for quick checks on the relative or qualitative (high, medium, low) concentrations of ammonia in each sample, when a number is not necessary to quantify the difference.

5.0 DATA ANALYSIS AND CALCULATIONS

The required calculations have been described in previous sections of this procedure.

6.0 QUALITY CONTROL

6.1 High-Range Ion-Selective Electrode and Meter Method

The ammonia meter is re-calibrated every two hours to limit any possibility of drift, however, no drift has been observed using this meter.

Re-calibrate and note the re-calibration on the data form.

6.2 Low-Range Ion-Selective Electrode and Meter Method

The ammonia meter is checked for excessive drift after the analysis of every ten samples. This is done by analyzing a 10.0 mg/L continuing calibration standard check sample. If the result is within the range of 10.0 ± 0.5 , then no re-calibration is needed. If the result exceeds this range, then re-calibration is necessary. Discuss the need to re-analyze samples with the Project Manager when drift is observed.

It is also recommended that the ammonia meter be re-calibrated after every hour of use in addition to when drift is observed in the measurement of the continuing calibration standard (10.0 mg/L) after every 10 samples analyzed

Another option, provided that the meter/probe are functioning properly within the hour of use, is to do a "one-point" calibration. The one-point calibration is done by re-calibrating with only the 1.00 mg/L standard. A one-point calibration is generally adequate unless the operator suspects the meter/probe has drifted excessively.

Re-calibrate and note the re-calibration on the data form. Any needed re-measurement of samples will be at the discretion of the Project Manager.

6.3 Salicylate-Hypochlorite Method

Before each sample is measured, the regent blank should be checked to see if it still registers as zero. The blank should be changed (fresh D.I. water) approximately every seven to ten samples or when it is found that the measurement is drifting significantly away from zero when checked.

A sample from each 0.1 mg/L ammonia standard (three total) is prepared, measured, and a mean value is taken. This mean value is used to obtain the conversion factor.

A duplicate sample is measured once every 10 samples.

7.0 SAFETY

Care must be taken in the use of the Salicylate-Catalyst solution: This catalyst contains cyanide - use only in a hood and wearing appropriate personal protection (gloves, goggles).

8.0 TRAINING REQUIREMENTS

It is the responsibility of the Technical Group Manager to ensure that all staff performing the procedures described in this procedure are properly trained and that documentation of the training exists prior to the performance of this procedure.

To initiate training, all staff must first read this procedure and demonstrate proficiency in measuring ammonia prior to application for project use. A staff member unfamiliar with the measurement of ammonia will receive a verbal and visual demonstration by an experienced co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this procedure are followed. Documentation of training shall be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

Bower, C. E., and T. Holm-Hansen. 1980. Can. J. Fish. Aquat. Sci. 37: 794-798.

MSL-A-006 Marine Sciences Laboratory Training.

ATTACHMENT 1

Battelle Marine Sciences Laboratory

Example High or Low-Range Ammonia Meter Calibration Record

Battelle Marine Sciences Laboratory | **Ammonia Measurements**

Project No.: _____ Date: _____

Project Name: _____ Analyst: _____

Test Species: _____ Time of Sample Collection: _____

Duration of Test: _____ Day of Test: _____

Ammonia Meter Serial Number: _____ Ammonia Probe Number: _____

Ammonia Stock Solution Lot Number: _____ Ammonia Stock Solution Expiration Date: _____

Initial calibration: Standards: _____ Slope: _____

_____ Acceptable? Y N Recorded in Project Notebook: _____

Sample Type*: Seawater Freshwater Overlying Water Porewater Other: _____

*** Use separate pages for different sample types**

Strips from Instrument Attached:

NOTE: The use of this specific form is not required, however, as a minimum, the information shown must appear on any form used as an alternative.

ATTACHMENT 2

Battelle Marine Sciences Laboratory

Example Form

DATA COLLECTION FORM: Ammonia Measurements with Associated Water Quality Parameters

Project Name: _____ Data Entered By: _____

Project Number: _____ Reviewed By/Date: _____

Sample Type: _____ Day: _____

Associated Water Quality Measurements

					Meter #	Meter #	Meter #	Meter #	Meter #	
Date	Sample ID	Vol Sed/ Spin Time	(Spin) Temp °C	Sample Vol (mL)	Ammonia Conc. (mg/L)	Sample Temp °C	pH	D.O. (mg/l)	Salinity %	Initials

Comments: _____

NOTE: The use of this specific form is not required, however, as a minimum, the information shown must appear on any form used as an alternative.

ATTACHMENT 3

Battelle Marine Sciences Laboratory
Example Ammonia Meter Calibration Record

Date	Time	S/N	Probe ID	Cal. 1 STM/mV/ACT	Cal. 2 STM/mV/ACT	Cal. 3 STM/mV/ACT	Comments	Initials

NOTE: The use of this specific form is not required, however, as a minimum, the information shown must appear on any form used as an alternative.

ATTACHMENT 4
Battelle Marine Sciences Laboratory
Example Form
Ammonia via the Spectrophotometer – Bower and Holm-Hansen Method

Project Name:_____ Test Day: _____
Project Number: _____ Date: _____
Test Type: _____ Page __ of __
Species: _____

Sample #	Sample ID	Absorbance	Factor	Dilution	Concentration (mg/L)

0.10 mg/L NH_4-N Standard Absorbance	1 _____ 2 _____ 3 _____
	average _____

Factor Calculations:

Comments: _____

Recorder: **Reviewed By:** **Date:**

NOTE: The use of this specific form is not required, however, as a minimum, the information shown must appear on any form used as an alternative.

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EFFECTIVE DATE: 10-29-99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-W-008-00

ROUTINE WATER QUALITY MEASURES FOR TOXICITY TESTS

Approvals:		
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QA OFFICER: Deborah Coffey	Deborah Coffey	10-29-99
	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward	[illegible]	10.26.99
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ROUTINE WATER QUALITY MEASURES FOR TOXICITY TESTS

1.0 SCOPE AND APPLICATION

This procedure describes the routine water quality measurements [dissolved oxygen (DO), pH, conductivity/salinity, and temperature (T)] that are required for Marine Sciences Laboratory (MSL) toxicity testing. These measurements are made using a sonde combination sensor/probe system (600 XL) and a YSI Model 610-DM data logger. This procedure describes the calibration and use of the instrumentation to make the listed measures.

2.0 DEFINITIONS

Salinity – Salinity is defined as the weight of inorganic salts in one Kg of seawater. Salinity is determined by an indirect method involving a measurement of the physical property of conductivity. From an empirical relationship of salinity and conductivity measured for a standard solution, it is possible to calculate salinity. The precision of the method is the precision of the conductivity method.

3.0 RESPONSIBLE STAFF

MSL Staff
Project Manager, Task Leader
MSL Manager
Quality Assurance Representative

4.0 PROCEDURES

4.1 Calibration

Calibration for temperature is done on a monthly basis following procedure MSL W-003, Calibration and Use of Thermometers. Ensure that the probe is within calibration by noting when the calibration period expires on the daily calibration form (Attachment 1). Calibrations for DO, salinity, and pH are to be performed each day before water quality measurements are made. See Attachment 1 for an example of the form that is to be used to record YSI instrument calibration information. It is recommended that calibration be completed for all parameters, in the order described below.

4.1.1 Dissolved Oxygen Saturated Air Calibration

a) Remove calibration cup. Put enough deionized (DI) water in cup to just cover the bottom of the cup, (< 1cm), and replace calibration cup loosely.
b) Check to ensure that the sensors do not make contact with the water, and allow to sit for 10 minutes. Contact between the sensor and the water will result in a false reading.
c) After 10 minutes, turn on meter, press "Esc" to get to main menu, scroll to "Calibration mode", press "Enter".
d) Scroll to "Dissolved Oxy". Press "Enter".
e) Scroll to "DO %". Press "Enter".

f) Input pressure as 760 mg Hg[1]. Press "Enter".
g) Wait for a stable DO reading and press "Enter".

4.1.2 Conductivity/Salinity Calibration

a) Press "Esc" to return to CALIBRATE menu. Scroll to "Conductivity" and press "Enter".
b) Remove the calibration cup, dry thoroughly with Kim-Wipes™. Fill to the 50 mL mark with 50 mS/cm conductivity standard solution and replace conductivity cup snugly. Ensure that the standard solution is not expired and record its lot number and expiration date.
c) Scroll to "SpCond". Press "Enter".
d) Input the calibration standard value. Input an SpCond of 50mS/cm. Press "Enter".
e) Wait for a steady salinity reading. Press "Enter".

4.13 Two-Point pH Calibration

a) Press "Esc" repeatedly to return to CALIBRATE menu. Scroll to "ISE1 pH" and press "Enter".
b) Scroll to "2 Point" and press "Enter".
c) Verify that the calibration standards to be used are not expired and record their lot numbers and expiration dates. Mark the buffers to be used on the data sheet. Calibration buffers are chosen to bracket the expected range of the measurements to be made.
d) Remove the calibration cup. Rinse thoroughly with DI water, fill to the 25 mL mark with the lower pH standard (usually 7.00) and replace cup snugly.
e) Input first pH standard as it is labeled (usually 7.00). Press "Enter".
f) Wait for a stable pH reading. Press "Enter".
g) Remove the calibration cup and rinse thoroughly with DI water. Fill to the 25 mL mark with the second (usually 10.01) pH standard.
h) Press "Enter", (not "Esc"), until prompted to enter 2nd pH. Input second pH standard as it is labeled (usually 10.01). Press "Enter".
i) Wait for a stable pH reading. Press "Enter".

4.2 Operation

There are two distinct methods of entering data. Data may be added to more permanent designated files such as *Leptocheirus* culture files, or it may be placed in the more generic "marked" file by test position number. This second method is used for ongoing tests, and requires that the data be downloaded and filed between uses, as the data logger is only capable of storing data from 145 samples. Because the operator has to manually select file numbers for each sample, the operator can keep track of when the 145 sample limit is reached.

---

[1] Ideally, a weather station barometric pressure should be obtained. Smith Island Weather Station readings are appropriate and can be obtained via the web. However, when readings are not available, the default of 760 mg Hg will be used.

4.2.1 Operation for bioassay test samples ("marked" file).

a) After daily calibration is completed, remove calibration cup and screw sonde guard to sonde.
b) Turn power on. Data logger should be in "Run" mode, but if not, escape to main menu and scroll to "Run Mode". Press "Enter".
c) Submerge sonde in sample to black line and wait for a steady reading. Press "M" (for mark).
d) Scroll to number corresponding to sample position number. Press "Enter".
e) Press "A" (for add).
f) If you are prompted "New File yn?", press "Y". This and the following will occur only on the first sample.
g) If prompted "Enter Weather[2] yn?", press "N".
h) Rinse sonde and proceed to next sample.
i) Repeat steps c) through e) for each sample.

4.2.2 Operation for *Leptocheirus* culture and culture renewal water

a) Remove calibration cup and screw sonde guard to sonde.
b) Turn power on. Data logger should be in "Run" mode, but if not, escape to main menu and scroll to "Run Mode". Press "Enter".
c) Submerge sonde in sample to black line and wait for a steady reading. Press "A" (for Add).
d) Scroll to "Lepto cult" or "Lepto watr" as appropriate. Press "Enter".
e) If prompted "New File yn?" press "Y".
f) If prompted "Enter Weather[2] yn?" press "N". This and the above prompt will appear only on the first measurement.
g) Place sonde in the next culture tray to the black line. When readings have steadied, press "A".
h) At prompt, press "Enter".

4.2.3 Instrument Storage/Charging

a) When not in use, the sonde should be stored vertically with approximately 1 cm DI water in the calibration cup, which should be screwed on snugly.
b) The instrument should be plugged into its charger once a week overnight, but never for more than 48 hours.

4.3 **Download Data**

4.3.1 Disconnect the data logger from the sonde and connect to the PC in the wet laboratory office using the 610-DM download cable attached to the PC.
4.3.2 Turn data logger power on and escape to main menu.

---

2 The system can be used both in the laboratory and in the field. The MSL system is generally used in the laboratory and weather entries are not necessary. If the instrument is to be used in the field, consult the operation manual for instructions for entering weather data *prior to use*.

4.3.3 On PC, open **Eco Watch** program.
4.3.4 Click on the "Sonde" icon on the tool bar and select "Com 1". Click "OK".
4.3.5 On data logger, scroll to "Communication". Press "Enter".
4.3.6 Scroll to "Kermit 610→PC". Press "Enter".
4.3.7 Scroll to the file you are downloading. Press "Enter". When the computer indicates that the download is complete, turn data logger power off to conserve batteries.
4.3.8 Open the file you have downloaded and print data and data summary. Check to make sure the sample numbers and the total number of observations are appropriate for the test that was monitored.
4.3.9 Label these 8 ½" x 11" printouts with the project name, test species and type of test, (e.g. Ampelisca 10-d Sediment), test day and replicate # as appropriate, and place in the project data notebook.
4.3.10 Export file from **EcoWatch** to **Excel** and save data to the appropriate **Excel** workbook for the test being measured.

5.0 DATA ANALYSIS AND CALCULATIONS

5.1 Documentation

Document calibration data prior to making routine measures.

Document each value obtained for the container of interest. Report the range for the measurements of water-quality parameters (DO, pH, temperature, and salinity). When a value is outside the specified range, mark this value with an asterisk (*) and note if any corrective action was required and if so, what was done to address the problem.

Keep all laboratory records, test results, measurements, and other supporting documentation for each toxicity test in a project file dedicated to that purpose.

5.2 Reporting

A summary project report should be prepared and may include, but is not limited to, the following water quality information:

--Summary of water quality conditions for each treatment
--Summary of any deviations from the project test plan
--Copies of raw data, observations, or data forms generated during the test

6.0 QUALITY CONTROL

6.1 Quality Assurance Verification Activities

Periodic surveillance should be conducted by the MSL's Quality Assurance Representative to ensure that all aspects of the testing accurately reflect the work that was planned and completed, and that all necessary information, as defined by regulations, procedures and program-specific plans, is included. Results of periodic surveillances may become a part of the project files.

6.2 Performance Evaluation Samples

Some projects or state accreditation programs may require the analysis of blind performance evaluation samples. Analyze these samples in exactly the same way that calibration standards or routine samples are analyzed, and report the results to the MSL QA Representative. The data obtained will be submitted to the supplier providing the statistical analysis of the results. The results will be transmitted to the managers, analysts and other interested staff when received. Analysis data are statistically evaluated against the data provided by other participating laboratories and ranked, as well as compared to the group mean.

7.0 SAFETY

There are no safety concerns associated with the implementation of this procedure.

8.0 TRAINING REQUIREMENTS

All staff members who will be performing toxicity tests shall first read this procedure prior to working on the test. Documentation of training shall be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006	Marine Sciences Laboratory Training
MSL-W-003	Calibration and Use of Thermometers

ATTACHMENT 1

Battlle Marine Sciences Laboratory

YSI Multiparameter Probe Daily Calibration Record

Model No: _____ Serial No: _____

D.O. Membrane Change Date:		
4 pH Buffer Lot# and Expiration Date:		
7 pH Buffer Lot# and Expiration Date:		
10 pH Buffer Lot# and Expiration Date:		

Conductivity Standard:	mS/cm	mS/cm	mS/cm
Lot# and Expiration Date:			

Date	Time	pH Calibration	Conductivity Calibration	D.O. Calibration

Comments: _____

Reviewed by: _____ Date: _____

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Marine Sciences Laboratory

Effective Date: 3-06-01

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-W-009-00
DETERMINATION OF SOLUBLE SULFIDE IN AQUEOUS SAMPLES

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	Signature	Date
TECHNICAL GROUP MANAGER: Jeff Ward		03.05.01
	Signature	Date

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Determination of Soluble Sulfide in Aqueous Samples

1.0 SCOPE AND APPLICATION

This procedure describes the measurement of sulfide in overlying water and/or interstitial water. When required, measurement of sulfide is necessary during the conduct of various types of toxicity tests usually as a screening test for sulfide as a confounding factor. This procedure is for the use of an Orion combination silver/sulfide electrode used in conjunction with an Orion Model 290A meter. Sulfide concentrations between 0.032 and 100 mg/L can be measured by this method. If a lower detection limit is needed, then MSL-C-001, Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals (SEM) in Sediments and Total Dissolved Sulfides in Aqueous Samples by a Colorimetric Method, should be used. For higher concentrations, sample dilution is required. With careful technique, titration of sulfide is accurate to ± 0.1% of the total sulfide ion concentration of the sample.

Humic substances may interfere with the measurements. For highly colored samples (high concentration of humic substances), the method of standard additions may be used to check results. Temperature changes may affect the results. Therefore, **be sure standards and samples are at the same temperature (ambient room temperature) before making measurements.**

Sulfide samples must be buffered to pH above 12 with SAOB II so that HS^- and H_2S are converted to S^{-2}. **(NOTE: It is critical to measure pH of sample before adding buffer, so that [H_2S] can be calculated from the total).**

For high ionic strength samples, (e.g. seawater) prepare standards with composition similar to that of the standard.

2.0 DEFINITIONS

Deaerated deionized water and deaerated filtered seawater	Deaerated water is generated by purging it with argon (Ar) or helium (He) gas.
CCV	Continuing calibration verification sample
ICV	Initial calibration verification sample
Titration endpoint	The titration endpoint (also called the inflection point) for the determination of the sulfide concentration in the stock solution is defined as the point of greatest slope.

3.0 RESPONSIBLE STAFF

Laboratory Staff Members
Project Manager, Task Leader
MSL Manager
Quality Assurance Officer or Representative

4.0 PROCEDURE

4.1 Sample Collection, Preservation, and Storage

4.1.1 Samples should be collected with minimum aeration. Sulfide may be volatilized by aeration and inadvertently converted to an immeasurable form. Samples should be analyzed immediately.

4.1.2 SAOB II Solution Sample Preservation -When samples are not to be immediately analyzed, they should be preserved with SAOB II solution. This is the preferred MSL method. Sample preservation is done by combining equal amounts of sample and SAOB II solution (1:1 ratio). The SAOB II solution results in a sample pH of greater than 12 pH units. Check pH with pH paper.

4.1.3 Zinc Acetate Sample Preservation – Another alternative for sample preservation is to use zinc acetate. Sample preservation is done either by adding 4 drops of 2N zinc acetate solution per 100 mL of sample. The final pH of the sample must be at least 9 pH units. Use 6N NaOH solution for pH adjustment when zinc acetate is used. If the sample concentration is expected to be greater than 64 mg/L , add more zinc acetate solution. The recommended holding time limit for preserved samples is 7 days.

4.2 Apparatus/Equipment and Reagents

4.2.1 Apparatus/Equipment

- Orion combination silver/sulfide electrode
- Orion Model 290A meter (pH, ISET/Temperature/mV)
- Magnetic stirrer and stir bar(s); insulate from beaker using a piece of Styrofoam or cardboard
- Beakers or sample cups
- Electrode Filling Solution (Optimum Results B)
- Pipette or burette for titrations
- 1 mL syringe with a fine needle tip
- Graph paper or data sheet (see Attachments 2 and 3)
- Polishing strips (from Orion)

4.2.2 Reagents

- 2 N Zinc acetate [$Zn(C_2H_3O_2)_2$] solution: Dissolve 220 g $Zn(C_2H_3O_2)_2 \cdot H_2O$ in 870 mL DI water; this makes a 1 liter solution.

- Sulfide anti-oxidant buffer (SAOB II): **This is a caustic solution**. Measure 80 g sodium hydroxide (NaOH) and place in a 1L volumetric flask. Add 74.4 g EDTA (use the di-sodium salt). Fill the flask to the 1 L volume mark with DI water **or deaerated filtered seawater if the samples to be tested are of seawater salinity.** Measure 475 mL of the solution into a bottle and add 18 g ascorbic acid. SAOB II solution can also be purchased.

- Lead perchlorate [Pb $(ClO_4)_2$] Solution (0.1 M): Dissolve 4.60 g Pb $(ClO_4)_2 \cdot 3\ H_20$ in 100 mL reagent water or use commercially available solution.

- Stock Sulfide standard (100 mg/L (ppm) as S^{2-}: Dissolve 12.5 g reagent grade Na_2S in 25 mL deaerated, deionzed water. Shake the solution and let stand capped overnight in a fume hood. This solution is good for up to one week, or less.

- Deionized (DI) deaerated water or deaerated filtered seawater – Generate by purging water with an inert gas such as Ar or He. The deaerated water should be good for up to seven days.

4.3 Step-by-Step Procedure

4.3.1 Sample Preparation

4.3.1.1 All samples and standards should be at the same temperature (preferably room temperature).

4.3.1.2 It is critical to measure the pH of the samples before adding buffer, so that [H_2S] can be calculated from the total.

4.3.2 Instrument Start Up

4.3.2.1 Refer to Attachment 1 for a checklist to assist with electrode and meter use. The electrode should be filled with Optimum Results B filling solution and checked for salt deposits and rinsed with DI before use. Soak the electrode for up to one hour in DI before use. Rinse the electrode between samples with DI. Never rub or wipe the end of the electrode; this causes electrode polarization. The electrode can be blotted after rinsing by touching the tip lightly to a KimWipe. Stir all samples during measurement. The magnetic stirrer generates heat that may affect the determination of the sulfide concentration. Insulate the sample from the stirrer by use of a piece of Styrofoam or cardboard between them.

4.3.2.2 The model 290A meter and electrode are shown in Figure 1.

4.3.3 Calibration

4.3.3.1 The weekly working sulfide standard is prepared fresh weekly or when measurements are to be made (whichever is applicable). Pipette 1 mL of the stock solution (see Section 4.2.2) into a 100 mL volumetric flask. Add 50 mL of the SAOB II solution and bring up to volume with deaerated seawater.

4.3.3.2 Thirty (30) ml of the weekly working standard is titrated with a 0.1 M lead perchlorate solution using the electrode mV reading as the endpoint indicator to determine ***the exact standard concentration***. The end point is the point of greatest slope, which is determined to be the point at which the mV reading drops the most with the smallest addition of lead perchlorate (refer to Attachment 2). Add lead perchlorate using a 1 ml syringe with a fine needle tip to allow addition of small amounts of volume. The weekly standard concentration before dilution is determined by:

$$C = 3206\ (V_t/V_{sample})$$

C = concentration as mg/L (ppm) sulfide
V_t = $Pb(ClO_4)_2$ volume added to reach endpoint
V_{sample} = sample volume (10 mL)

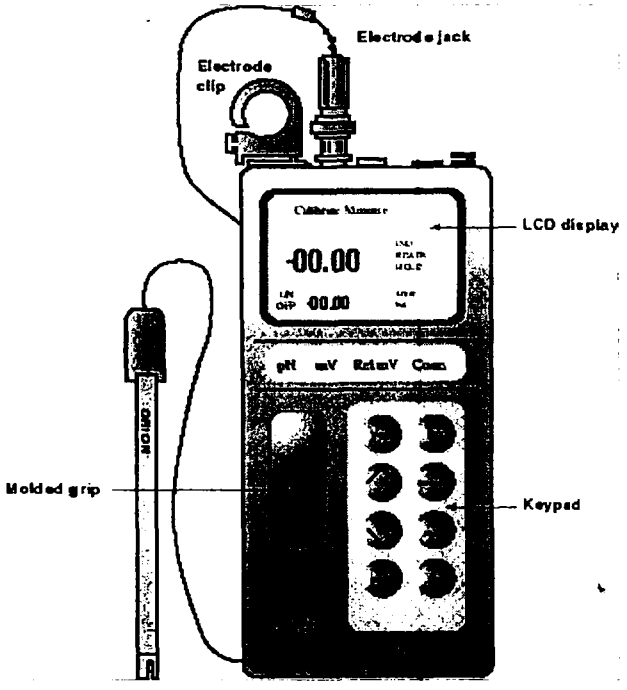

Figure 1. Orion® Model 290A portable pH/ISE/Temperature/mV meter with Model 9107 Electrode

4.3.3.3 From the weekly working sulfide standard which was titrated, prepare the daily calibration standard series by using aliquots of the weekly standard and diluting it to volume (100 mL). First use the formula at the bottom of Attachment 2 to obtain the concentration of the weekly working sulfide standard. From this concentration, the amount of standard to be used to make a 10 mg/L or 100 mg/L working standard can be calculated. Use the relationship $C_1/V_1 = C_2/V_2$. An example calculation is shown at the bottom of Attachment 2.

4.3.3.4 The routine MSL daily calibration standards used for past work are 0.1, 1.0, and 10.0 mg/L. However, alternatives can be selected based on the expected range of sample concentrations. One alternative is to use a 4-point curve of 0.1, 1.0, 10.0 and 100.0 mg/L concentrations.

4.3.3.5 Use the chart below as a guide to prepare the calibration standard series.

Want	Use	Dilute to
10.0 mg/L	This working sulfide standard is made using the concentration determined in the titration step. Make a 10 mg/L (or 100 mg/L) solution using the relationship $C_1/V_1 = C_2/V_2$ illustrated in the example at the bottom of Attachment 2.	No dilution is needed
0.1 mg/L	Use 1 mL of the 10 mg/L working sulfide standard	100 mL
1.0 mg/L	Use 10 mL of the 10 mg/L working sulfide standard	100 mL
ICV/CCV 0.5 mg/L	Use 5 mL of the 10 mg/L working sulfide standard	100 mL

4.3.3.6 A known standard will be measured after calibration (ICV – initial calibration verification) and every 10 samples (CCV-continuing calibration verification). The acceptance criterion for both ICV and CCV samples is $\pm$ 20%. If the criterion is not met, the instrument must be recalibrated before continuing and samples between the unacceptable CCV and the prior CCV must be re-measured. Re-calibrate every 2 hours

4.3.4 <u>Setting up the Calibration Curve</u>

4.3.4.1 Measure 10 mL of the most dilute standard into a small beaker. Add 10 mL of SAOB II solution and stir. Rinse the electrode, and place in the beaker. Wait for a stable reading then calibrate the meter to display the value of the standard using the "Conc" mode of the meter. Always start with the most dilute standard.

4.3.4.2 Press "2nd cal." "Calibrate" and the time and date of the last calibration will be displayed. After a few seconds, "P1" will be displayed indicating that the meter is ready for the first standard.

4.3.4.3 When the reading is stable, use the scroll keys to enter the value of the standard starting with the first digit. After this is done, press "Yes". Continue for each digit. The display will freeze for 3 seconds, then "P2" will be displayed in the lower left field.

4.3.4.4 Rinse the electrode and place into the second standard. Adjust the value.

4.3.4.5 Repeat this for all standards in progressively higher concentrations.

4.3.4.6 At the end of this process, press "Measure". The slope of the standard curve will be displayed and the measurement of the last standard. The slope of the curve must be between –30 to –35, in discussions with Orion –25 to-30 is permissible. If the slope is out of range, perform the slope electrode check described in the electrode manual.

4.3.5 Sample Measurement

4.3.3.1 Place 5 mL of the sample into a small beaker.

4.3.3.2 Add 5 mL of SAOB II into the sample beaker* and stir thoroughly.
* unless it has already added as a preservative

4.3.3.3 Place the electrode in the sample and stir.

4.3.3.4 Press the "Measure" button on the meter and when the meter stabilizes, record the reading (given in mg/L) on the data sheet (Attachment 3).

4.3.3.5 Remove the probe, rinse and move on to the next sample.

4.3.3.6 As stated above, check the CCV every 10 samples to assess instrument accuracy and performance. If the 20% accuracy criterion is not met, re-calibrate.

$$100 - \frac{\text{Observed reading}}{\text{Expected reading}} \times 100 = \text{percent difference}$$

4.3.6 Preventive Maintenance

4.3.6.1 The electrode must be stored in DI water. If the electrode is to be stored longer than a week before the next use, it should be drained, flushed internally with DI water and stored dry with the cap on.

4.3.6.2 Before use inspect the electrode for scratches, cracks, salt crystal build up or membrane/junction deposits. The membrane normally allows some of the fill solution to leak out. So a slight deposit is not reason to change the membrane.

4.3.6.3 To clean the electrode, place a drop of liquid dish detergent on a moist cloth or tissue and gently rub over the sensing element. Rinse well with DI.

4.3.6.4 The electrode requires "polishing" when its response becomes sluggish or drifty and the cleaning procedure does not improve electrode response.

4.3.6.5 Cut off one inch of polishing strip. Hold the electrode with the sensing element facing upwards and place a few drops of DI on the surface.

4.3.6.6 Place the polishing strip on the sensing element (with the frosted side down) using a light finger pressure.

4.3.6.7 Rotate the electrode for 30 seconds.

4.3.6.8 Rinse and soak the electrode in a 1 mg/L (ppm) standard solution for about 2 minutes and check the electrode response.

4.3.6.9 If this procedure does not improve electrode response, the electrode likely needs to be replaced.

5.0 DATA ANALYSIS AND CALCULATIONS

The MSL meter used for this analysis provides the sample concentration in mg/L when calibrated as per instructions in Section 4.3.2. There are no additional calculations other than those already provided in the procedure.

The critical calculation for determining the concentration of the working standard as mg/L sulfide (ppm) from the titration with lead perchlorate and the associated calculation for preparing a 10 mg/L stock solution may be found at the bottom of Attachment 2.

6.0 QUALITY CONTROL

6.1 General Requirements

6.1.1 The sulfide meter is re-calibrated every two hours to limit any possibility of drift. Re-calibrate and note the re-calibration data on the data form (attachment 3) under the "Comments" section.

6.1.2 ICV and CCV results must agree within ± 20% of the known concentration. If this is not attained, the corrective action is to re-calibrate and re-check the ICV.

6.1.3 If the ICV is not acceptable after re-calibration, electrode performance should be evaluated by performing the specified steps in Section 4.3.4.

7.0 SAFETY

7.1 Care must be taken in the use of the SAOB II solution: This solution is caustic – prepare and use wearing appropriate personal protection (gloves, goggles), and dispose of in a properly labeled container

7.2 Sulfide stock and weekly working standard should be made and stored in a fume hood because of their sulfur smell. Be sure that containers are appropriately labeled.

7.3 Wear gloves when working with lead perchlorate solution.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the Technical Group Manager to ensure that all staff performing the activities described in this procedure are properly trained and that documentation of the training exists prior to the performance of this procedure. To initiate training, all staff must first read this procedure and demonstrate proficiency in measuring ammonia prior to application for project use. A staff member unfamiliar with the measurement of sulfide will receive a verbal and visual demonstration by an experienced co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this procedure are followed. Documentation of training shall be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

Orion manufacturer's instruction manual. 1996. Model 9416 Silver/Sulfide Half-cell, Model 9616 Sure-Flow™ Combination Silver/Sulfide Electrodes

Orion manufacturer's instruction manual. 1999. Benchtop pH/ISE Meters Models 210A, 230A, 250A, 290A.

Standard Methods for the Examination of Water and Wastewater. 20th Ed. 1998. American Public Health Association. Pp. 4-168-169. Method 4500-S^{2-} G. Ion-Selective Electrode Method.

MSL-A-006 Marine Sciences Laboratory Training.

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ATTACHMENT 1
Battelle Marine Sciences Laboratory
Electrode and Meter Checklist

Electrode Preparation

1. Remove the protective shipping cap from the sensing element and save for storage.

2. Clean any salt deposits from the exterior by rinsing with DI.

3. Fill the electrode body with Optimum Results B filling solution.

   a. Insert the spout of the filling solution bottle into the fill hole in the outer sleeve and add a small amount of filling solution to the chamber.

   b. Tip the electrode to moisten the O-ring at the top and return the electrode to a vertical position.

   c. Holding the electrode by the barrel and one hand, use the thumb to push down on the electrode cap, allowing a few drops of filling solution to drain and wet the inner core.

4. Soak the electrode in DI water for up to one hour.

2.2.2 Meter and Electrode Setup

1. The meter operates on either one 9V alkaline battery, one 9V lithium battery, or an AC line adapter (not provided).

2. Attach the electrode to the top of the meter.

2.3 Instrument Startup

1. Turn on the meter by pressing the "power" button.

2. Select the mV mode (press the "mode" button and it will cycle through four mode choices) or ensure that the mV mode is selected.

ATTACHMENT 2

Battelle Marine Sciences Laboratory

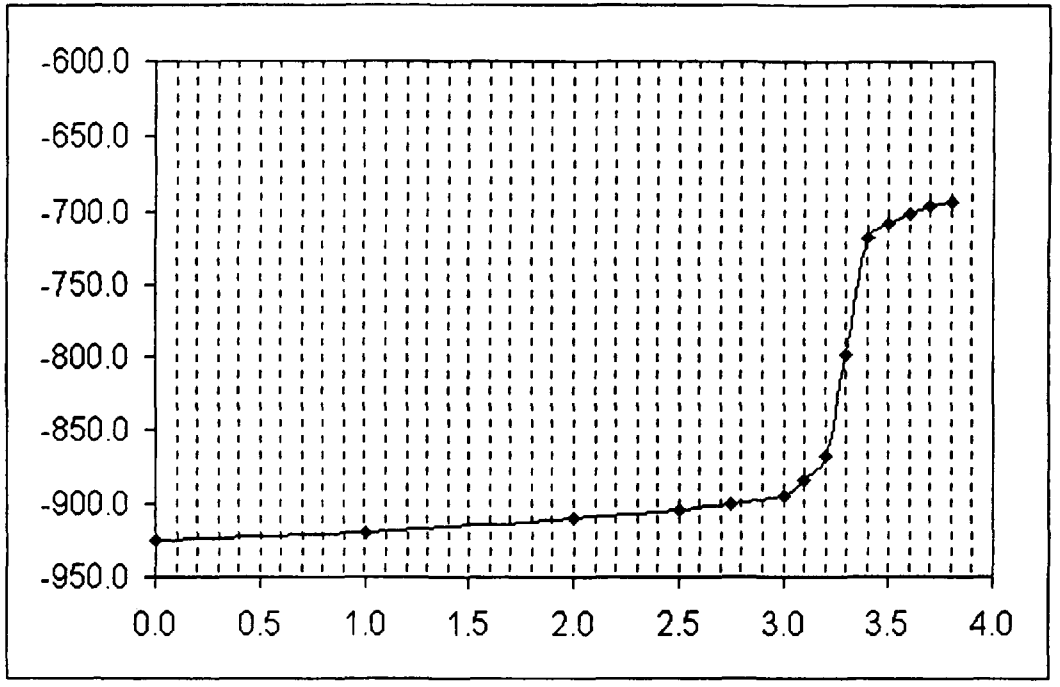
Example Working Standard Concentration Calculation Worksheet

Working Standard Concentration Calculation Worksheet

WS made from Stock Solution Dated 9/3/97

DATE: 26-Sep-97

mL of Lead Perchlorate	mV
0.00	-925.1
1.00	-919.7
2.00	-910.1
2.50	-905.1
2.75	-900.0
3.00	-894.4
3.10	-884.5
3.20	-867.5
3.30	-798.8
3.40	-719.1
3.50	-709.3
3.60	-702.2
3.70	-697.0
3.80	-693.9



Calculation of Working (Weekly) Standard Concentration: $C=3206(V_t/V_{sample})$

3.2 = V_t = lead perchlorate volume added to reach endpoint (mL)

20 = V_{sample} = sample volume (mL)

512.96 = C = concentration of working standard as mg/L sulfide (ppm)

10mg/L S^{2-} Std Calculation	1.949mL of Working Standard in 100mL volumetric add equal volumes (~48 mL ea) SAOB and deaerated seawater	That is 1000/512.96 = 1.949

ATTACHMENT 3

Battelle Marine Sciences Laboratory

Example Sample Measurement Data Sheet

BATTELLE MARINE SCIENCES LABORATORY

Total Sulfide Measurements

PROJECT NAME: _____ ANALYST: _____

PROJECT NUMBER: _____ REVIEWED BY/ DATE: _____

SAMPLE TYPE/DESCRIPTION: _____

SAMPLE ID	TEST DAY and DATE SAMPLE TAKEN	DATE MEASURED	Vol Sed/ Spin time	SAMPLE VOLUME (total mL with SAOB)	Meter # TOTAL SULFIDES Conc (mg/L)	Meter # TEMP °C	INITIALS

COMMENTS: _____

APPLIED MARINE SCIENCES SOPs

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 1301**
Written By: Mike A. Seymour

GUIDELINES FOR SAMPLE RECEIPT, LOGIN, AND STORAGE

Prepared By: _____ **Date:** 3/27/01

Approved By: _____ **Date:** 3/27/01
Laboratory Manager

_____ **Date:** 4/12/01
QA Officer

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 1301**
Written By: Mike A. Seymour

GUIDELINES FOR SAMPLE RECEIPT, LOGIN, AND STORAGE

1.0 INTRODUCTION

This procedure is designed to act as a guide in ensuring that all chain of custody requirements are met in receiving, handling, and storing samples.

2.0 APPLICATION

While there are differences in the chain of custody (hereafter COC) for each client, the general requirements are the same. In all cases, AMS has in-house procedures which follow each sample from the time of receipt, through the analytical procedure, and until it leaves AMS to be reported as analyzed data. This is a written document trail, which verifies the handling and analysis of each sample, and ensures that AMS meets or exceeds all applicable Federal, State, and Local regulations and requirements for that sample.

3.0 REFERENCES

Applied Marine Sciences, Inc. 1997. Quality Assurance Plan. AMS, League City, TX.

Applied Marine Sciences, Inc. 2001. Quality Management Plan (QMP). AMS, League City, TX.

4.0 PROCEDURE

4.1 Receiving the Sample: Shipping Documentation

Virtually all samples that arrive at AMS are shipped via Federal Express, Airborne, or UPS. In all cases, count the number of sample shipping containers and verify with the delivery person. Sign for the samples, and move them into the laboratory check-in area.

4.2 Opening the Cooler, Verifying the Contents

The technician should initially open the cooler or shipping package and measure the temperature of the cooler and samples. Record the temperature on the Sample Receipt Checklist (Appendix B). All shipments should have paper documentation called a Chain of Custody (COC) listing each sample I.D. number, collection date, project number/name, and procedure(s) requested. Remove the samples, and verify that each sample to be tested matches the client's COC document. Note any discrepancies on the client's COC form, and notify the Laboratory Manager so that they may contact the client. After sample verification, sign the client COC, with the date and time. This officially releases the samples into AMS custody.

4.3 Assigning a Project Number

Each project that comes to AMS is assigned a unique Project Number. This system assigns a number that identifies the year, client, and sequential project amount for that year. For example:

2001-00-01 AMS Blank Client Code

The first series of numbers designates the year
The second series designates the client code
The third series designates the sequential project number for that client

The technician simply needs to "build" a project number by assigning the year, client, and project number. Take a manila file folder, and write this number across the file marker at the top. This will hold all of the documentation for the duration of the project. Place the signed COC and the shipping document in the project folder. Your Laboratory Manager can assist you with any questions you may have.

4.4 Assigning an AMS Sample I.D. Number

After assigning the project number, assign AMS numbers to each sample. AMS uses a spreadsheet created in Microsoft Excel 2000 to catalog sample I.D. numbers. Each sample that arrives at AMS is given a blind, sequential, and unique number that isolates the sample from its client number to ensure scientific integrity. To assign numbers, the technician should go to the main laboratory computer, and open the Excel 2000 program.

The project sample I.D. files are located in:

> C:\mydocuments\sample_identification_log_200x file, where x is the current year.

Open the first available file with unassigned numbers and enter the date received, the client code, client project title, AMS Project Number (from 4.3), the client sample I.D., the analysis requested, and your initials. Appendix A contains a hypothetical AMS sample log for the project 2001-00-01, which should act as a guide. Print two copies of the updated file and place one copy in the project folder. Sign the document with your initials next to your typed initials. Place the second copy in the Master Sample Check-In Log, so that the entire file may be viewed. Ask the Laboratory Manager for assistance if you have any questions concerning the data entries or with operating Microsoft Excel.

4.5 Completing the AMS Sample Receipt Checklist

The next AMS document to complete is the Sample Receipt Checklist (See Appendix B). This is the document of record for the receipt of the samples at AMS. Fill it out completely and accurately, using Appendix B as a guide. Be sure to record the shipper information, and any discrepancies from the client COC. Be sure to bring these to the attention of the Laboratory Manager, as he/she will need to contact the client immediately.

4.6 Completing the AMS Project Work Order Sheet

AMS uses a work order system to accurately track all procedures requested by a client for a project. This Work Order Sheet will remain in the project file to act as the timeline for sample analysis. Check the box for the procedure(s) requested by the client. Fill out all known information, using Appendix C as a guide. If any information is unknown, leave it blank and notify your Laboratory Manager.

4.7 Sample Storage

Once samples have been logged in and all COC requirements are satisfied, the samples should be stored at 4°C until analysis.

5.0 QUALITY CONTROL

The entire Chain of Custody must be maintained for each sample from the field to the client, from the client to AMS, and during the entire analysis process while at AMS. The COC is an assurance that the highest ethical and analytical standards were met. Each and every part of the COC must withstand both legal and scientific scrutiny. To this end, AMS has a proactive system that:

1. Provides individual and blind sample identification. Data tampering is discouraged because no obvious sample identity exists that could jeopardize the results.

2. Provides a multi-level documentation trail. Each project has supporting documents that track the sample from beginning to end with instructional cover sheets, detailed Laboratory Bench Sheets, and structured Standard Operating Procedures for each and every procedure authorized by the client.

3. Provides dual oversight of projects by a trained laboratory technician and their Laboratory Manager, with most projects receiving multi-level oversight and review before the results are reported.

6.0 APPENDICES

6.1 Appendix A: AMS Sample I.D. Log (Document 1301:01)

6.2 Appendix B: AMS Sample Receipt Checklist (Document 1301:02)

6.3 Appendix C: AMS Project Work Order Sheet (Document 1301:03)

Appendix A: Applied Marine Sciences, Inc.
Sample I.D. Log

Date Received	Client Code	Client Project Title	Client Project #	AMS Project #	Client Sample I.D.	AMS Sample I.D.	Analysis Requested	Initials
1/1/01	AMS	Hypothetical	H123456	2001-00-01	abc1	0001	SOP 2101	MAS
"	"	"	"	"	abc2	0002	"	"
"	"	"	"	"	abc3	0003	"	"
"	"	"	"	"	abd1	0004	"	"
"	"	"	"	"	abd2	0005	"	"
"	"	"	"		abd3	0006	"	"
"	"	"	"	"	abe1	0007	"	"
"	"	"	"	"	abe2	0008	"	"
"	"	"	"	"	abe3	0009	"	"
						0010		
						0011		
						0012		
						0013		
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						0027		
						0028		
						0029		
						0030		

MAS

AMS 1301:01

Laboratory Technician: _____. **Laboratory Manager:** _____

Appendix B: Applied Marine Sciences, Inc.

Sample Receipt Checklist

Date: *1/1/01* Client No. *00*

Time: *09:00 A.M.* Contract No. *2001-00-01*

Client Sample Nos. *abc-1, -2, -3; abd-1, -2, -3; abe-1, -2, -3*

AMS Sample Nos. *0001, 0002, 0003, 0004, 0005, 0006, 0007, 0008, 0009*

		YES	NO
1.	Is a Chain-of-Custody form present?	X	
2.	Is the COC properly completed?	X	
	If no, please provide details:		
3.	Is airbill/packing list/bill of laden present?	X	
	If yes, ID#: *1234-5678-9012 Federal Express*		
4.	Are custody seals present on package?	X	
	If yes, were they intact upon receipt?	X	
5.	Are all samples tagged or labeled?	X	
6.	Do the sample tags/labels match the COC?	X	
	If no, has the client been contacted?		
	(Attach subsequent documentation from client regarding this matter.)		
7.	Do all shipping documents agree?	X	
	If no, describe the nonconformity:		
8.	Condition/temperature of shipping container:	*Good, Ambient*	
9.	Condition/temperature of sample containers:	*Good, < 4° C (On Ice)*	
10.	Sample Disposal?: AMS X	Return to client	

Notes:

Attest: Date: 1/1/01

Corrective Action: Date:

Resolved: Date:

AMS 1301:02: Revised 1/01

Appendix C: Applied Marine Sciences, Inc.
Project Work Order Sheet

AMS Project No.: *2001-00-01* **Date Received:** *1/1/01*

Turn Around Time: *14 days* **Report Deadline:** *1/15/01*

AMS Sample ID Numbers: *0001, 0002, 0003, 0004, 0005, 0006, 0007, 0008, 0009*

Testing and Procedure Requested:

X SOP 2101: Grain Size, Full-Phi Pipette Method (Plumb, 1981)

____ SOP 2102: Grain Size, Abbreviated Pipette Method (Plumb, 1981)

____ SOP 2103: Grain Size, Sieve and Hydrometer Method (ASTM D422/D2216)

____ SOP 2104: Grain Size, Sieve Only Method (ASTM D422/D2216)

____ SOP 2105: Grain Size, Wet Sieve (4,10,40,200) (ASTM D422/D1140/D2216)

____ SOP 2201: TOC, Sediments by Coulometer (EPA Method 9060/Kahn 1986)

____ SOP 2202: TOC, Water by Coulometer (EPA Method 415.1/ASTM D4129)

____ SOP 2301: Moisture Content, ASTM D2216

____ SOP 2302: Specific Gravity, ASTM D854

____ SOP 2303: Total Solids, Plumb, 1981

____ SOP 2304: Bulk Density, Plumb, 1981

____ SOP 2401: Flex Wall Permeability, ASTM D5084

____ SOP 2402: Rigid Wall Permeability, ASTM D2434

____ SOP 2501: Swell, ASTM D4546

____ SOP 2502: Consolidation, ASTM D2435

____ SOP 2601: Atterberg Limits: Liquid, Plastic Limits, Plasticity, ASTM D4318

____ Other - _____

____ Other - _____

Project Manager: Allison D.

AMS 1301:03: Revised 2/01

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2103**
Written By: Mike A. Seymour

WET PREPARATION OF SOIL SAMPLES FOR THE QUANTITATIVE DETERMINATION OF PARTLE SIZE ANALYSIS (SIEVE AND HYDROMETER METHOD)

Prepared By: _____ **Date:** 3/29/01

Approved By: _____ **Date:** 3/29/01
Laboratory Manager

_____ **Date:** _____
QA Officer

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2103**
Written By: Mike A. Seymour

WET PREPARATION OF SOIL SAMPLES FOR THE QUANTITATIVE DETERMINATION OF PARTICLE SIZE ANALYSIS (SIEVE AND HYDROMETER METHOD)

1.0 INTRODUCTION

This document describes a standard grain size procedure to determine the particle size distribution in marine and terrestrial sediments. This method, taken from ASTM D-422, uses physical measurements of the coarse fraction of the soil (larger than 75μm) by using sieves, and a gravimetric method of determining the fine fraction of the soil (smaller than 75μm).

2.0 APPLICATION

This procedure applies to all field and laboratory-based activities involving the analysis of grain size. It addresses theory, chain of custody, and laboratory-specific operations.

3.0 REFERENCES

ASTM D-422, 1963. Particle Size Analysis of Soils. American Society for Testing and Materials.

ASTM D-2216, 1980. Laboratory Determination of Water (Moisture) Content of Soil, Rock, and Soil-Aggregate Mixtures. American Society for Testing and Materials.

4.0 SAMPLE COLLECTION, STORAGE, AND HOLDING TIMES

4.1 Sample Collection

Sediment grain size samples should be collected according to project-specific sampling plans and quality-assurance guidelines. See SOP 1301 for more information.

4.2 Sample Storage

Sediment grain size samples can be stored in plastic or glass containers. For grain size, storage in a refrigerator or cooler at 4oC until analysis can be performed is adequate.

4.3 Sample Holding Time

Samples stored at 4°C have holding times of six (6) months. Holding times longer than six (6) months should be avoided, due to the possibility of altered water content values or chemical degradation of the samples due to metals or other contaminants held within the soil.

4.4 Sample Batching

Samples to be analyzed by hydrometer may be arranged in batches of no more than fourteen (14) samples. As with pipette sampling, it is suggested that batches contain no more than eleven (11) samples.

5.0 APPARATUS AND MATERIALS

Due to the wide range of particle sizes that occur within samples, analysis of sediments often requires utilization of two different methods: sieving of the fraction larger than 75μm, and hydrometer analysis of the fraction smaller than 75μm. Sections 5.1 and 5.2 list the equipment and materials necessary to perform this analysis.

5.1 Labware and Apparatus

1. 1-Liter Mason Jars, Lids, and Retaining Rings
2. Analytical Balance (0.0001g Sensitivity)
3. 150 ml Beakers
4. U.S. Standard Sieve #200 (0.075 mm) for Wet Sieving
5. Stainless Steel Mixing Bowl
6. 1000 ml Graduated Cylinders
7. Plastic Funnel, 8" or larger
8. Hydrometers, Gilson Co. Model SA-1, SA-2
9. Nylon Brushes (Coarse and Fine)

10. Stirring Plunger
11. Precision Timer
12. Drying Oven (Maintain 105°C, +/- 0.5°C)
13. Sieve Shaking Apparatus
14. 500 ml Wash Bottles
15. Aluminum Weighing Boats (Reynolds Baking Tins #00290)
16. U.S. Standard Sieves, Numbered as Below:

3 Inch	75 mm
2 Inch	50 mm
1 1/2 Inch	37.5 mm
1.0 Inch	25.4 mm
3/4 Inch	19 mm
3/8 Inch	9.51 mm
No. 4	4.75 mm
No. 10	2.0 mm
No. 40	0.425 mm
No. 200	0.075 mm

5.2 Reagents

1. Dispersant Solution: 4.0 g/L $(NaPO_3)_6$: Sodium Hexametaphosphate
2. 30% H_2O_2: Hydrogen Peroxide
3. Reverse Osmosis/Deionized (RO/DI) Water

6.0 PROCEDURE

6.1 Sample Preparation

6.1.1 Labware and Bench Preparation

Create Laboratory Bench Sheets for each sample. An example of this may be found in Appendix A. For each sample, label a 1000 ml graduated cylinder, 1000 ml Mason jar, 150 ml beaker, and an aluminum boat.

6.1.2 Sample Preparation

Remove the sample from the refrigerator and allow it to equilibrate to ambient temperature. Using a scoopula or spoon, stir the sample until it is homogeneous. Two sub-samples will be removed at the same time, one for moisture content and one for grain size analysis as noted below in 6.2 and 6.3.

6.2 Moisture Content Analysis

Sieve/hydrometer analysis requires that a moisture content analysis be performed on all samples. This will determine the percentage of moisture contained within the material that is actually used in the grain size analysis.

6.2.1 Sampling and Procedure for Moisture Content

Weigh the aluminum boat and record the value on the Laboratory Bench Sheet. Use a scoopula to sub-sample approximately 10 g of material into the boat. As soon as practical, record the weight of the moist sample and boat. This should be representative of the actual moisture present within the sample. Repeat (along with grain size aliquot) for the remainder of the samples. Dry the moisture content samples at 105°C for 24 hours. After drying, weigh and record the dry sample weights on the Laboratory Bench Sheets. A computerized spreadsheet will be used to calculate the water content of the sample at the time of reporting. Further information may be found in SOP 2301.

6.3 Grain Size Analysis: Wet Sieve

6.3.1 Sampling for Grain Size

Tare or zero a 150 ml Teflon cup on the precision balance. At the same time as moisture content sampling, scoop 50 g to 100 g of the sediment sample into the tared cup. Weigh the sample on the balance, and record the weight on the Laboratory Bench Sheet. Use a 500 ml wash bottle filled with dispersant to rinse the contents of the cup into a Mason jar. Add approximately 5 ml of concentrated hydrogen peroxide to be Mason jar to oxidize the organic material in the sample. Place a lid and retaining ring onto the jar, and shake to disaggregate the sample. Unscrew the retaining ring to vent the lid and allow the sample to oxidize for 1 hour. Repeat for the remaining samples.

6.3.2 Wet Sieving

Place the No. 200 sieve in the stainless mixing bowl. Pour the sample from the Mason jar onto the sieve, and thoroughly rinse all material from the jar with dispersant. Using minimal volume, wash all fine material through the sieve with dispersant. Tilt the sieve at a 45° angle and concentrate the coarse material against the lower rim of the sieve. Rinse this coarse material into the labeled 150 ml beaker using RO/DI water. The fine-grained material and dispersant contained within the mixing bowl should then be poured into the labeled 1000 ml graduated cylinder using a funnel. Rinse all sediment and residue from the bowl and funnel using dispersant. Bring the cylinder to within ~25 ml of the being full using dispersant. Use a stirring plunger to thoroughly mix and homogenize the fine material within the cylinder. Cover the cylinder with the Mason jar lid pending hydrometer analysis. Place the coarse fraction 150 ml beakers in the oven and dry at 105°C for 24 hours.

6.4 Dry Sieve Analysis

6.4.1 Loading and Sieving the Sample

Arrange the sieves in descending order (i.e. 3/4", 3/8", No. 4, No. 10, from largest to smallest in size) onto the bottom pan. Pour the dried sample from the 150 ml beaker onto the top sieve in the stack. Use a brush to clean the beaker of all remaining sediment. Cover the stack with a sieve cover, and place the stack in the shaker cabinet. Secure the stack with the retaining clamp, and close the outer door. Turn on the shaker by rotating the timer dial to 15 minutes. After completion, move the stack to the analysis bench.

6.4.2 Weighing the Sample Retained on the Sieves

Invert the top sieve onto a clean sheet of heavy cotton paper. Use a back-and-forth pattern to brush all sediment and material from the screen. Turn over the sieve and brush any fine sediment from the sides of the sieve onto the paper. Pour or brush the material into a clean and tared 250 ml Teflon beaker or cup. Weigh the material and record the value into the corresponding data block on the Laboratory Bench Sheet. Repeat the process for each sieve in the stack. After the sediment from each sieve has been weighed and recorded onto the Laboratory Bench Sheet, brush the fine sediment from the bottom pan into the 1000 ml graduated cylinder for the sample. Discard the sample from the weighing cup and proceed with all samples until finished with the batch or project.

6.5 Hydrometer Analysis

6.5.1 Preparation

Prepare for hydrometer analysis by assembling the Laboratory Bench Sheets in sequential order on the laboratory counter. Place each cylinder on top of its worksheet. Other supplies necessary will be a precision timer, stirring plunger, rinse bucket, 500 ml wash bottles filled with dispersant, hydrometers, and drying towels. Assign the hydrometer to be used for each sample, and record its identification number on the Bench Sheet. Note and record the ambient temperature in the laboratory on each sample's Bench Sheet. The first hydrometer analysis will be performed at 2, 5, 15, 30, 60, 240, and 1440 minutes. The next sample will be done at 3, 6, 16, 31, 61, 241, and 1441 minutes. The next will be at 4, 7, 17, 32, etc. Write the hydrometer analysis times on each Bench Sheet, increasing each sample time by one (1) minute in sequence.

6.5.2 Calculation of Hydrometer Correction Factor

Both hydrometer types used (Density, 152H; Specific Gravity, 151H) are designed to generate accurate readings at exactly 20°C from the bottom of the meniscus line. Due to the high amount of suspended solids encountered in sediment analysis, an accurate determination of the location of the bottom of the meniscus may not be available. A correction factor is determined experimentally to allow readings to be taken from the top of the solution/water meniscus. For hydrometer 151H, this correction value is the difference between the reading and one. For hydrometer 152H this correction value is the difference between the reading and zero. Actual determination of the correction factor is detailed in SOP 1104 and SOP 1302.

6.5.3 Hydrometer Analysis

This procedure requires that two people be present at the beginning of the hydrometer run through the fourteen-minute analysis (14:00).

6.5.3.1 First technician: sample stirring

Pre-stir all samples, and bring each cylinder up to full volume using dispersant. Begin by stirring the first sample for one (1) minute; begin the timer at the exact moment the plunger is withdrawn. Carefully rinse the plunger in a wash bucket with dispersant. The second sample in sequence should be stirred until the timer reads 1:00 minutes, the third until 2:00, in that order through the end of the batch.

6.5.3.2 Second technician: hydrometer analysis

At about 1:30, the second technician should carefully lower the hydrometer into the cylinder, making sure that it is centered and not touching the sides. Do not drop the hydrometer too far into the sample, as droplets of dispersant on the stem can add to the weight of the hydrometer and give an inaccurate reading. At exactly 2:00, read the hydrometer by locating the highest meniscus of fluid on the stem. Record the value onto the Laboratory Bench Sheet. Withdraw the hydrometer, being careful not to disturb the sample. Wash the hydrometer with dispersant, dry it with a clean paper towel, and lower into the next sample for the 3:00 reading. Continue in sequence, taking readings exactly 60 seconds apart. At the 5:00 time, an overlap reading will need to be taken on the first and fourth samples in sequence. This requires two hydrometers; they must be rinsed, dried, floated, and read within 60 seconds of time. Overlap readings will continue on the second and fifth samples, third and sixth, through the end of the batch, until the first technician is finished with stirring and can assist with hydrometer readings.

6.5.3.3 Hydrometer readings after 15:00

At the 15:00 point, the second technician is no longer necessary. One person may take all further readings. Continue in sequence until the 24-hour reading.

6.5.4 Measuring Dispersant Concentration

Completely fill a 1000 ml graduated cylinder with dispersant. Use a hydrometer as described above to determine the g/L concentration of sodium hexametaphosphate in solution. This should be recorded on each Laboratory Bench Sheet in the appropriate box.

7.0 STANDARDIZATION AND CALCULATIONS

7.1 Sieve Analysis Values for the Portion Coarser than the No. 200 (0.074) Sieve

7.1.1 Calculations for Percentage Passing and Mass Retained on No. 200 Sieve

Calculate the percentage of material passing the No. 200 sieve by dividing the mass passing by the mass of soil originally split on the No. 200 sieve, and multiplying the result by 100. To obtain the mass passing the No. 200 sieve, subtract the mass retained sieve from the original mass.

7.1.2 Calculations for Percentage Passing, Mass Retained on Additional Sieves

To obtain the total mass of soil passing the No. 140 sieve, add to the mass of the material passing the No. 200 sieve the mass of the fraction passing the No. 140 sieve and retained on the No. 200 sieve. To obtain the total mass of soil passing the 60 sieve, add to the total mass of soil passing the No. 60 sieve, the mass of the fraction passing the 140 sieve and retained on the No. 200 sieve. For the remaining sieves, continue the calculations in the same manner. To determine the total percentage passing for each sieve, divide the total mass passing (as calculated in Section 7.1.1) by the total mass of sample and multiply the result by 100.

7.2 Percentages of Soil in Suspension

7.2.1 Oven Dry Mass of Soil Used in Analysis

Calculate the oven-dry mass of soil used in the analysis by dividing the aliquot wet weight by the water content, as demonstrated in Sections 6.2.1 and 6.3.1.

7.2.2 Calculating Percentage of Sample Remaining in Suspension by Using Actual Sample Weights.

To calculate the percentage of the sample remaining in suspension, divide the oven-dry mass of the total sample used (Section 7.2.1) by the percentage passing the No. 200 sieve (Section 7.1.1), and multiply the result by 100.

7.2.3 Calculating Percentage of Sample Remaining in Suspension by Using Hydrometer 151H (Density (g/L))

An equation for determining the percentage of soil remaining in suspension at the level that the 151H hydrometer is measuring is shown in Equation 1.

Equation 1: Percentage of Soil In Suspension For Hydrometer 151H

$$P = \left(\frac{100{,}000}{W} \times \frac{G}{G - G_l} \right) (R - G_l)$$

Where:

P	=	Percentage of soil remaining in suspension at the level of the hydrometer reading
R	=	Hydrometer reading with composite correction applied
W	=	Dried mass of soil in a total test sample, as mass of soil dispersed
G	=	Specific gravity of soil particles
G_l	=	Specific gravity of the liquid in which the particles are suspended. Use numerical value of one (1) on both instances in the equation. In the first bracketed use of G_l, any possible variation produces no significant effect, and in the second instance the composite correction for R is based on a value of one for G_l.

Note: The bracketed portion of Equation 1 for hydrometer 151H is constant for a series of readings and may be calculated first and then multiplied by the portion in the parenthesis.

7.2.4 Calculating Percentage of Sample Remaining in Suspension by Using Hydrometer 152H (Specific Gravity)

An equation for determining the percentage of soil remaining in suspension at the level that the 152H hydrometer is measuring is shown in Equation 2.

Equation 2: Percentage of Soil in Suspension for Hydrometer 152H

$$P = \frac{Ra}{W} \times 100$$

Where:

a	=	Correction factor to be applied to the reading of hydrometer 152H. Values on the hydrometer scale are calculated using a specific gravity of 2.65. Correction factors are listed in Table 1).

All other variables are consistent with Equation 1 (Section 7.2.3).

Table 1

Values of Correction Factor, a, for Different Specific Gravities of Soil Particles*

Specific Gravity	Correction Factor*
2.95	0.94
2.90	0.95
2.85	0.96
2.80	0.97
2.75	0.98
2.70	0.99
2.65	1.00
2.60	1.01
2.55	1.02
2.50	1.03
2.45	1.05

* For use in equation for percentage of soil remaining in suspension when using hydrometer 152H.

7.3 Diameter of Soil Particles

7.3.1 Stokes' Law

The diameter of a particle corresponding to the percentage indicated by a given hydrometer reading shall be calculated according to Stokes' Law, on the basis that a particle of this diameter is at the surface of the suspension at the beginning of sedimentation and had settled to the level at which the hydrometer measures the density of the solution. Stokes' Law is represented mathematically below:

Equation 3: Stokes' Law

$$D = \sqrt{\left[\frac{30n}{980}(G - G_1)\right]} \times \frac{L}{T}$$

Where:

D	=	Diameter of particles, (mm)
n	=	Coefficient of viscosity of the suspending medium (water) in poises (variable depending on the temperature of the suspending medium)
L	=	Distance form the surface of the suspension to the level at which the density of the suspension is being measured, (cm). For a given hydrometer and sedimentation cylinder, values vary according to the hydrometer readings. This distance is known as effective depth, and is listed in Table 2.
T	=	Interval of time from the beginning of sedimentation to the reading time, (min)
G	=	Specific gravity of soil particles, and
G_1	=	Specific gravity (relative density) of suspending medium (value may be used as 1.00 for all practical purposes).

7.3.1.1 Refinement of Stokes' Law

Since Stokes' Law considers the terminal velocity of a single sphere falling in an infinity of liquid, the sizes calculated represent the diameter of spheres that would fall at the same rate of soil particles. For convenience in calculations the above equation may be written as follows:

Equation 4: Stokes' Law With Effective Depth Constant

$$D = K\sqrt{\frac{L}{T}}$$

Where:

K	=	Constant depending on the temperature of the suspension and the specific gravity of the soil particles. Values of K for a range of temperatures and specific gravities are given in Table 3. The value of K does not change for a series of readings constituting a test, while values of L and T do vary.

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2103**
Written By: Mike A. Seymour

Table 2

Values of Effective Depth Based on Hydrometer and Sedimentation Cylinder of Specified Sizes**

Hydrometer 151H		Hydrometer 152H			
Actual Hydrometer Reading	Effective Depth L, cm	Actual Hydrometer Reading	Effective Depth L, cm	Actual Hydrometer Reading	Effective Depth L, cm
1.000	16.3	0	16.3	31	11.2
1.001	16.0	1	16.1	32	11.1
1.002	15.8	2	16.0	33	10.9
1.003	15.5	3	15.8	34	10.7
1.004	15.2	4	15.6	35	10.6
1.005	15.0	5	15.5	36	10.4
1.006	14.7	6	15.3	37	10.2
1.007	14.4	7	15.2	38	10.1
1.008	14.2	8	15.0	39	9.9
1.009	13.9	9	14.8	40	9.7
1.010	13.7	10	14.7	41	9.6
1.011	13.4	11	14.5	42	9.4
1.012	13.1	12	14.3	43	9.2
1.013	12.9	13	14.2	44	9.1
1.014	12.6	14	14.0	45	8.9
1.015	12.3	15	13.8	46	8.8
1.016	12.1	16	13.7	47	8.6
1.017	11.8	17	13.5	48	8.4
1.018	11.5	18	13.3	49	8.3
1.019	11.3	19	13.2	50	8.1
1.020	11.0	20	13.0	51	7.9
1.021	10.7	21	12.9	52	7.8
1.022	10.5	22	12.7	53	7.6
1.023	10.2	23	12.5	54	7.4
1.024	10.0	24	12.4	55	7.3
1.025	9.7	25	12.2	56	7.1
1.026	9.4	26	12.0	57	7.0
1.027	9.2	27	11.9	58	6.8
1.028	8.9	28	11.7	59	6.6
1.029	8.6	29	11.5	60	6.5
1.030	8.4	30	11.4		
1.031	8.1				
1.032	7.8				
1.033	7.6				
1.034	7.3				
1.035	7.0				
1.036	6.8				
1.037	6.5				
1.038	6.2				

** Values of effective depth are calculated from Equation 5:

7.3.1.2 Calculating Effective Depth Constant, K

The equation for calculating the effective depth constant for both hydrometer types 151H and 152H is shown below as Equation 5.

Equation 5: Calculation of Effective Depth Constant, K

$$L = L_1 + \left(\frac{L_2 - \frac{V_B}{A}}{2} \right)$$

Where:

L	=	Effective depth, cm
L_1	=	Distance along the stem of the hydrometer from the top of the bulb to the mark for a hydrometer reading, cm
L_2	=	Overall length of the hydrometer bulb, cm,
V_B	=	Volume of hydrometer bulb, cm^3, and
A	=	Cross-sectional area of sedimentation cylinder, cm^2

Values used in calculating the values in Table 2 are as follows:

For both hydrometers, 151H and 152H:

L_2	=	14.0 cm
V_B	=	67.0 cm^3
A	=	27.8 cm^2

For hydrometer 151H

L_1	=	10.5 cm for a reading of 1.000
		2.3 cm for a reading of 1.031

For hydrometer 152H

L_1	=	10.5 cm for a reading of 0 g/L
		2.3 cm for a reading of 50 g/L

Table 3

Values of K for Use in Equation for Computing
Diameter of Particle in Hydrometer Analysis

	Temperature			Specific Gravity of Soil Particles					
°C	2.45	2.50	2.55	2.60	2.65	2.70	2.75	2.80	2.85
16	0.01510	0.01505	0.01481	0.01457	0.01435	0.01414	0.01394	0.01374	0.01356
17	0.01511	0.01486	0.01462	0.01439	0.01417	0.01396	0.01376	0.01356	0.01338
18	0.01492	0.01467	0.01443	0.01421	0.01399	0.01378	0.01359	0.01339	0.01321
19	0.01474	0.01449	0.01425	0.01403	0.01382	0.01361	0.01342	0.01323	0.01305
20	0.01456	0.01431	0.01408	0.01386	0.01365	0.01344	0.01325	0.01307	0.01289
21	0.01438	0.01414	0.01391	0.01369	0.01348	0.01328	0.01309	0.01291	0.01273
22	0.01421	0.01397	0.01374	0.01353	0.01332	0.01312	0.01294	0.01276	0.01258
23	0.01404	0.01381	0.01358	0.01337	0.01317	0.01297	0.01279	0.01261	0.01243
24	0.01388	0.01365	0.01342	0.01321	0.01301	0.01282	0.01264	0.01246	0.01229
25	0.01372	0.01349	0.01327	0.01306	0.01286	0.01267	0.01249	0.01232	0.01215
26	0.01357	0.01334	0.01312	0.01291	0.01272	0.01253	0.01235	0.01218	0.01201
27	0.01342	0.01319	0.01297	0.01277	0.01258	0.01239	0.01221	0.01204	0.01188
28	0.01327	0.01304	0.01283	0.01264	0.01244	0.01255	0.01208	0.01191	0.01175
29	0.01312	0.01290	0.01269	0.01249	0.01230	0.01212	0.01195	0.01178	0.01162
30	0.01298	0.01276	0.01256	0.01236	0.01217	0.01199	0.01182	0.01165	0.01149

7.4 Graphing of Test Results

A graph of the test results shall be made that includes both the sieve and hydrometer results. For the sieve results, plot the diameter of the particles (the sieve opening in mm) on the logarithmic scale as the abscissa and the percentages passing corresponding to that diameter on the arithmetic scale as the ordinate. For the hydrometer results, plot each reading taken during the hydrometer test. Plot the diameter of the particles on a logarithmic scale as the abscissa and the percentage of soil remaining in suspension, corresponding to that diameter, on the arithmetic scale as the ordinate. Draw a line between plotted points to show the natural progression between the diameter of soil particles and the percentages passing.

7.5 Calculation of Relative Percent Difference

In addition to the analyses in the above sections, all duplicate samples will be analyzed using relative percent difference (RPD) as shown below:

$$\frac{|DuplicateA - DuplicateB|}{\left(\frac{DuplicateA + DuplicateB}{2}\right)}$$

8.0 QUALITY CONTROL

Changes in the distribution of the grain sizes of sediments could be a signal of short and/or long-term influence on the sedimentation process. Because any effects of these processes could be subtle with long-range implications, it is important to maintain a procedure that has the highest precision and accuracy and minimizes the effects of multiple analysts.

8.1 Standard Reference Material (SRM)

At the time of revision of this SOP, there is no Standard Reference Material available. Strict attention to QA/QC standards and the guidelines provided by in-house operating procedures insures the highest standards of accuracy and precision.

8.2 Duplicate Analyses

One duplicate sample will be analyzed with every batch of 10 samples, or with every sample set, whichever is more frequent. Duplicates will be below 25% RPD (See Section 7.5).

8.3 Equipment

Constant attention to details is necessary to assure that short-term shifts in the grain size distribution are not confused with faulty or flawed procedures. To minimize error, strict adherence is made to standard laboratory practices. All equipment is checked to assure quality performance, such as daily temperature measurements (ambient and oven), and daily calibration of the balance using NIST-traceable weights.

8.4 Method Blank

The usage of a method blank is not applicable to grain size analysis.

9.0 REPORTING AND PERFORMANCE CRITERIA

9.1 Reporting Units

Reporting units are in percent gravel, sand, silt, and clay submitted in columns within a computerized spreadsheet.

9.2 Minimum Method Performance Criteria

The minimum method performance standard for the determination of grain size is 0.0001 g per total weight of sample, expressed as a percentage of the total weight.

9.3 Significant Figures

All results are reported to three significant figures

9.4 Duplicate Analysis

All duplicates are reported. A duplicate is created for every 10 samples, or sample batch, whichever is more frequent.

10.0 SAFETY

The technician will be instructed in and made aware of the safety considerations in using this method including the following:

* Protective clothing and eyewear is to be worn while in the laboratory.
* The location and use of eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits will be given.
* The proper handling and disposal of samples and reagents necessary to the procedure will be followed.

This is in addition to standard laboratory practices, including but not limited to, the guidelines specified in the AMS Laboratory Safety Guide and SOP 1304.

11.0 APPENDICES

11.1 Appendix A: Laboratory Data Sheet

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2201**
Written By: Kenneth S. Davis, Revision By: Mike A. Seymour

TOTAL ORGANIC CARBON CONTENT OF SEDIMENTS BY COULOMETRIC DETECTION

Prepared By: _____ **Date:** 3/23/01

Approved By: _____ **Date:** 3/23/01
Laboratory Manager

_____ **Date:** _____
QA Officer

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2201**
Written By: Kenneth S. Davis, Revision By: Mike A. Seymour

TOTAL ORGANIC CARBON CONTENT OF SEDIMENTS BY COULOMETRIC DETECTION

1.0 INTRODUCTION

This Standard Operating Procedure (SOP) describes a method to determine the total organic carbon content (TOC) of sediments. Total organic carbon concentrations are useful parameters when interpreting trace organic data.

TOC measurements are determined on dry samples using a high-temperature tube furnace to combust the material to carbon dioxide (CO_2) in a pure oxygen atmosphere. The combustion product gas flows through a Balston filter to remove water vapor. The gas is then flowed through two reaction chambers: the first containing magnesium perchlorate [$Mg(ClO_4)_2$] to remove water vapor, the second containing both acid dichromate on Silocel [as $K_2Cr_2O_7$ and H_2SO_4] and manganese dioxide [as MnO_2] to adsorb SO_x and NO_x products. The CO_2 gas stream then passes to a UIC 5012 Carbon Dioxide Coulometer that determines the CO_2 within the gas stream by coulometric titration via a photoelectric detection cell. Data output is sent to a computer that calculates the TOC present in the sample and prints the results.

2.0 REFERENCES

EPA, 1995. SW-846 Method 9060. Total Organic Carbon

EPA Region II, 1986. Determination of Total Organic Carbon in Sediment (Kahn Method).

3.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

3.1 Sample Collection

Sediment samples should be collected in pre-cleaned glass jars with Teflon-lined lids, aluminum foil, or core liners. Cool samples to 4°C for shipment to laboratory.

3.2 Sample Preservation and Storage

Samples should be shipped to the laboratory and stored at 4°C until analysis. After subsampling, excess material is archived at 4°C in darkness. The samples should be analyzed within 28 days of the date of collection. See SOP 1301 for more information.

4.0 SUPPLIES AND EQUIPMENT

4.1 Laboratory Equipment and Apparatus

The following labware and equipment is needed to perform the TOC analysis:

* UIC Model 5012 Carbon Dioxide Coulometer
* Bottled Pure Oxygen, Laboratory Grade
* Platinum Electrode (UIC No. CM101-135)
* Silver Electrode (UIC No. CM101-033)
* Combustion Tube (UIC No. CM211-005)
* Analytical Balance, (0.0001 g sensitivity)
* Drying Oven (Maintain 70°C, +/- 0.5°C)
* Mortar and Pestle
* Glazed Porcelain Boats (Coors 60028)

4.2 Reagents

The following reagents are required to perform the total organic carbon analysis:

* Distilled Water
* Carbon anode solution (UIC No. CM300-002).
* Carbon cathode solution (UIC No. CM300-001).
* Potassium Iodide (CAS No. 7681-11-0, Certified ACS Grade)
* Magnesium Perchlorate (CAS No. 10034-81-8, Reagent Grade)
* Potassium Hydroxide Solution (CAS No. 1310-58-3, Certified ACS Grade)
* Acid Dichromate on Silocel (UIC No. CM300-008)
* Hydrochloric Acid (CAS No. 7647-01-0, NF/FCC Grade)
* Barium Chromate (UIC No. CM211-011)
* Barium Chromate with Reduced Silver (UIC CM211-002)
* 4.8% Carbon Standard (NIST SRM 1941a)

* 12.0% Carbon Standard (LECO No. 501-034)
* 42.1% Carbon Standard (LECO No. 501-046)

5.0 PROCEDURE

5.1 Sample Preparation

Samples with no material larger than 2.0 mm can be aliquoted into a clean 20 ml scintillation vial. Materials larger than 2.0 mm should be removed by sieving through a #10 (2.00 mm) sieve. If samples are highly oiled and/or clumped, remove large particles by hand with clean tweezers. After removing any material larger than the #10 sieve, aliquot a small portion (~10 g) into the 20 ml scintillation vial, and dry the samples at 70°C for 12 hours. After the samples are dry, grind the sample using a clean mortar and pestle. Pour the ground sample back into its scintillation vial and acidify by adding 2M HCl until no reaction is observed. Allow the sample to equilibrate for one hour, and then wash using deionized water until the sample is neutral in pH (usually 7-8 times). Dry the sample in the oven at 70°C for 12 hours. If the sample is to be removed from the oven, store it in a desiccator to prevent absorption of atmospheric moisture.

5.2 Coulometer System Preparation

5.2.1 Preparing the Coulometer Furnace

Change the solution in the oxygen gas pre-scrubber, using fresh potassium hydroxide solution. Fill the scrubber apparatus to approximately 40% full. Reinstall the scrubber, and attach the oxygen lines. Turn the master control valve on the oxygen tank counter clockwise to allow gas flow to the coulometer. Using the left-side flow meter on the coulometer, turn the gas control valve counter clockwise until approximately 125 ml/min is flowing to the combustion tube. Using the control pad in the center of the furnace module, press the up arrow to turn on the furnace module and adjust the temperature to 900°C. Retrieve the post-combustion scrubber tubes from the desiccator, and change the magnesium perchlorate (white and granular in color). Check that the barium chromate in the second post-combustion tube is brownish-gold and not "green" in color. This indicates exhaustion of the material and the contents should be changed before samples are run. Be sure the magnesium perchlorate is placed before the barium chromate when finishing the assembly.

5.2.2 Preparing the Coulometer Module

The coulometer module consists of two parts; a larger section called the body and the smaller section called the side arm. Begin with the preparation of the detection cell. Place a small stir bar in the larger cell body. Fill the larger cell with 100 to 125 ml of the carbon cathode solution. Pour potassium iodide into the side cell until it fills to a depth of 0.25". Then pour carbon anode solution into the cell until it is 1/8" below the level of the cathode level. Place the silver and platinum electrodes and caps onto the detection cell. Place the cell inside the coulometer body, and rotate cell in the body until the light path is not blocked by the electrodes. Plug in the electrode leads, and screw the CO_2 gas feed lines onto their fittings. Check that the cell current switch is in the neutral position, and turn the main power to "on". Adjust the %T knob clockwise to its stopping point. Readjust the oxygen flow on the furnace module to 125 ml/min. Rotate the detection cell in the main body so that the highest %T reading is obtained. Slowly adjust the %T knob until the digital display reads "100". Turn the cell current switch to the "on" position and allow the coulometer to equilibrate. After equilibration, the display should read 29 to 30 and the cell current should read zero.

5.2.3 Computer Start Up and Printer Set

The computer should be turned on and program selected to receive and analyze data. Please see Appendix A for a detailed instruction sheet for the computer and program currently in use at Applied Marine Sciences Inc.

5.3 Total Organic Carbon Determination

5.3.1 Initial Blank Analysis

An initial blank calibration will be run before analyzing carbon standards. The initial blank is determined by combusting a series of empty boats until the final three (3) results are within 10% RPD.

5.3.2 Initial Three-Level Calibration

A calibration analysis will be performed before any samples are analyzed. A 4.8%, 12%, and 42.1% carbon standard will be analyzed to determine the calibration of the coulometer. The calibration check must fall within 5.0% RPD of the known carbon content of the standard for all three values before sample analyses will be accepted.

In addition, a continuing calibration check will be performed with each group of 10 field samples using the 4.8% NIST standard. All values must be within 5.0% RPD. See SOP 1103 for more information.

5.3.3 Sample Analysis

Tare the combustion crucible to a zero value on the balance. Using a clean scoopula, place approximately 0.0200 g of sample into the crucible. Record the weight value in the laboratory notebook. Insert the sample I.D. number in the computer, and input the weight. Form feed the printer to a clean sheet of paper. Place the crucible into the combustion tube. Close the combustion tube, and insert the crucible ladle into the furnace using the magnetic slide. Hit the return key on the computer to start sample analysis. The coulometer analysis is complete when two consecutive readings fall within a preset difference. After the sample analysis is complete, record the %C, μg CO_2, and blank values into the laboratory notebook. Repeat this process for all samples.

5.3.4 Continuing Blank and Continuing Calibration Standard

A new blank will be analyzed with every ten (10) samples as noted in 5.3.1. In addition, a 4.8% carbon standard should be analyzed with every ten (10) samples.

6.0 STANDARDIZATION AND CALCULATIONS

6.1 Standardization

The accuracy of the coulometer is verified by performing an initial three-level calibration with 4.8%, 12%, and 42.1% carbon standards. The standard analysis is the same as for sample analysis. The results of standard analysis are compared to the known values, and RPD is calculated. No sample analysis is to be done unless the RPD values for all three standards is within 5% of the known value. The calibration is to be done on a weekly basis.

6.2 Calculations

6.2.1 Calculation of Total Organic Carbon (TOC)

$$TOC = \left(\left[\frac{\text{Result} - \text{Blank}}{W} \right] \times 0.27 \right) \times 100$$

Where:

Result	=	μg CO_2
Blank	=	μg CO_2
W	=	Weight of Sample (μg)

6.2.2 Calculation of Relative Percent Difference (RPD)

$$\frac{|DuplicateA - DuplicateB|}{\left(\frac{DuplicateA + DuplicateB}{2} \right)}$$

7.0 QUALITY CONTROL

Quality control samples are processed in the same manner as samples. See SOP 1103 and the AMS Quality Manual for more information.

7.1 Standard Reference Material (SRM)

To document accuracy, one SRM (NIST 1941a) is analyzed with each batch of 10 field samples. The result must be within 5% RPD of the average value (4.8%C +/- 1.2%) for acceptable accuracy.

7.2 Method Blank

One method blank will be processed with each batch of 10 samples, or with every sample set, whichever is more frequent. A method blank consists of following all preparation and analytical steps conducted with a sample except that the sample (i.e. sediment) is not added to the combustion crucible. An acceptable procedural blank must be less than one-tenth of the lowest sample signal (S:N=10.1) for the batch of samples.

7.3 Duplicate

One duplicate sample will be run with each batch of 10 samples, or with every sample set, whichever is more frequent. Duplicates should be within 5% RPD. Duplicates may be less precise for inhomogeneous samples (i.e. peat, and samples containing twigs, grasses, algae, etc.).

8.0 REPORTING AND PERFORMANCE CRITERIA

8.1 Reporting Units

Reporting units are in percent total organic carbon on a dry weight basis. All sample weights, instrument readings, analysis date, and calculated percent TOC are recorded in the project notebook.

8.2 Minimum Method Performance Criteria

The minimum method performance standard for the method is detection of 0.01% carbon in a sample.

8.3 Significant Figures

All results are reported to three significant figures.

8.4 Duplicate Analysis

All duplicates are reported. Duplicates are analyzed with every 10 field samples or one per batch, whichever is more frequent.

8.5 Standard Reference Materials

Standard reference materials include the NIST 1941a (4.8% C), and the LECO 12% and 42.1% C samples. Initial and continuing calibrations will be reported.

8.6 Method Blank

Method blanks will be reported. Blank runs are analyzed with every 10 field samples or one per batch, whichever is more frequent.

9.0 SAFETY

The technician will be instructed in and made aware of the safety considerations in using this method including the following:

* Protective clothing and eyewear is to be worn while in the laboratory, especially due to the use of caustic and acidic chemicals.

* The location and use of eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits will be given.

* The proper handling and disposal of samples and reagents necessary to the procedure will be followed.

* The proper handling of gas tanks.

This is in addition to standard laboratory safety practices, including but not limited to, the guidelines specified in the AMS Laboratory Safety Guide and SOP 1304.

10.0 APPENDICES

10.1 Appendix A: Use of the Computer Worksheet

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2301**
Written By: Kenneth S. Davis, Revision By: Mike A. Seymour

LABORATORY DETERMINATION OF MOISTURE CONTENT OF SOILS

Prepared By: _____ **Date:** 4/19/01

Approved By: _____ **Date:** 4/19/01
Laboratory Manager

KSD for CSD **Date:** 4/19/01
QA Officer

APPLIED MARINE SCIENCES, INC.
Standard Operating Procedure **SOP 2301**
Written By: Kenneth S. Davis, Revision By: Mike A. Seymour

LABORATORY DETERMINATION OF MOISTURE CONTENT OF SOILS

1.0 INTRODUCTION

This procedure describes the laboratory determination of the moisture content of soils. The moisture content is defined as the ratio of the mass of water to the mass of the solid particles within a defined amount of material.

2.0 REFERENCE

ASTM, 1996. D2216: Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil, Rock, and Soil-Aggregate Mixtures. Annual Book of ASTM Standards, Volume 4.08 Soil and Rock; Building Stone.

3.0 APPARATUS AND MATERIALS

* Laboratory Drying Oven, Maintain 105°C +/- 0.5°C
* Laboratory Balance, Precision of 0.0001g for samples less than 200g mass
* Laboratory Balance, Precision of 0.1 g for samples between 200 and 1000 g mass
* Laboratory Balance, Precision of 1.0 g for samples greater than 1000 g mass.
* Aluminum Weighing Boats, Reynolds #00290
* Spatula or Other Laboratory Apparatus for Aliquot of Sample
* Laboratory Desiccator

4.0 SAMPLE COLLECTION, STORAGE, AND HOLDING TIMES

4.1 Sample Collection and Storage

Samples should be collected and stored in airtight plastic or glass containers at a temperature of 4°C. To prevent loss of moisture from the sample, laboratory analysis should be done as soon as practicable after sampling.

4.2 Sample Holding Time

Samples have a holding time of no more than six (6) months. Holding times longer than this should be avoided, due to the possibility of altered water content values, compaction due to long-term storage, or chemical degradation of the samples due to metals or other contaminants held within the soil.

5.0 PROCEDURE

5.1 Preparation

Print a Laboratory Bench Sheet for each batch of samples to be analyzed. Label and tare an aluminum boat for each sample, and record its mass on the data sheet. Remove the samples from refrigeration and allow them to equilibrate to ambient temperature.

5.2 Analysis

Using the spatula, stir the sample until it is homogeneous. Scoop a portion of the sample into the tared aluminum boat. Place the boat with wet sample on the balance, and record the mass on the Laboratory Bench Sheet. Dry the sample in the laboratory oven at 105°C until a constant mass is observed. Remove the container from the oven and store in desiccator. Allow the material and container to cool to ambient temperature. Weigh the dried sample and boat on the laboratory balance, and record the value on the Bench Sheet.

5.3 Duplicate Analysis

One duplicate sample will be analyzed with every batch of 10 samples, or with every sample set, whichever is more frequent. Duplicates will be below 25% RPD (See 6.2).

6.0 CALCULATIONS

6.1 Water Content

Calculate the water content of the material as follows:

$$w = \left(\frac{(W_1 - W_2)}{W_2 - W_C} \right) \times 100$$

where:

w	=	water content, %
W_1	=	mass of container and moist specimen, g
W_2	=	mass of container and oven-dried specimen, g
W_C	=	mass of container, g

6.2 Relative Percent Difference

Calculate Relative Percent Difference as shown below:

$$\frac{|DuplicateA - DuplicateB|}{\left(\frac{DuplicateA + DuplicateB}{2} \right)}$$

7.0 QUALITY CONTROL

7.1 Duplicate Analysis

The duplicate analysis will have an RPD value not to exceed 25%.

7.2 Precision and Accuracy

There is no standard for precision and accuracy with this method. Unless a standard is specified in the QAPP for the samples, an arbitrary precision of 25% RPD will be followed and reported.

7.3 Equipment

Laboratory balances are calibrated daily using NIST traceable weights. This ensures that the results generated will be scientifically valid and accurate.

8.0 REPORTING CRITERIA

8.1 Report Data

The report shall include the following: sample identification, moisture content to the nearest 0.1%, and indication of any material excluded from test specimen.

8.2 Significant Figures

All results are reported to three significant figures where applicable.

8.3 Duplicate Analysis

All duplicates are reported.

9.0 SAFETY

The technician will be instructed in and made aware of the safety considerations using this method including the following:

* Protective clothing and eyewear is to be worn while in the laboratory.
* The location and use of eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits will be given.
* The proper handling and disposal of samples and reagents necessary for the procedure will be followed.

This is in addition to standard laboratory practices, including but not limited to, the guidelines specified in the AMS Laboratory Safety Guide and SOP 1304.

SEVERN TRENT LABORATORY (STL) SOPs

SEVERN TRENT LABORATORIES BALTIMORE

STANDARD OPERATING PROCEDURE

Number: STL-SOP-035 **Rev. No.:** 6

Sample Log-in Procedures

Approved By: S.A. Corum, Sample Management Officer — Date: 6/9/99

Approved By: M. M. Uhlfelder, Quality Services Manager — Date: 6/9/99

Approved By: A. R. Karimi, General Manager — Date: 6/9/99

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SEVERN TRENT LABORATORIES BALTIMORE

STANDARD OPERATING PROCEDURE

Number: STL-SOP-035 **Rev. No.:** 6

Sample Log-in Procedures

Revisions

Rev. No.	Date	Description
0 - 3	May 8, 1989	Initial distribution, historical information on file
4	January 31, 1996	Revised Section 3.2 to include internal COC reference, Section 3.7 for the Naval Quality Assurance sample container identification requirement.
5	August 15, 1996	Revised Section 3.7 to describe methods of numbering multiple bottles of the same sample.
6	June 1, 1999	Edited to reflect new laboratory ownership, incorporated example Cooler Receipt Checklist.

1 PURPOSE

This SOP describes the procedures for sample log-in is to assign unique accession numbers to each sample received by the laboratory, and to initiate the Internal Custody procedures and documentation (see STL-SOP-039).

2 SCOPE

All samples received by STL Baltimore.

3 PROCEDURE

3.1 Shipment Inspection

3.1.1 Samples are either delivered by a commercial delivery service or are hand carried to the laboratory. Hand-carried deliveries consist of samples from local clients. The Sample Custodian signs and dates the chain-of-custody form accepting custody of the samples.

3.1.2 Samples received from a commercial courier are accepted by signing the bill-of-lading sheet. This sheet serves as an intermediate form of custody and must stay attached to all chain-of-custody documents. The Sample Custodian or designee signs the chain-of-custody (COC) form (See EAL-SOP-051) in the space labeled "Received by Laboratory".

3.1.3 Hand-carried samples will have only the COC to provide documentation of delivery. The deliverer will relinquish samples to the Sample Custodian or designee by signing and dating the form in the space labeled "Relinquished by". The Sample Custodian or designee will then sign for receipt of samples for the laboratory as described in 3.2 above.

3.1.4 The individual receiving the samples must check to determine whether or not the "tamper evident" seal, if present, on the sample shipping container is unbroken. The state of this seal must be noted on the COC, and if damaged, broken, or disturbed, the Laboratory Project Manager (LPM) is notified immediately and a Non-conformance Record (STL-SOP-072) is generated. Verbal authorization must be received from the client to proceed with sample log-in in spite of the seal tampering.

3.1.5 The sample shipping container is then opened in a hooded environment. A calibrated mercury or digital thermometer is placed into the sample shipping container near the samples until the temperature stabilizes and temperature readout is obtained. The temperature is recorded on the chain-of-custody (COC) and the Cooler Receipt Form (See Figure 1).

3.1.5.1 If the temperature is 2-6C, the temperature is noted on the COC and sample log-in may proceed.

3.1.5.2 If the temperature is <2 or > 6C, or if the samples exhibit any signs of icing, the client must be contacted for approval to proceed despite the temperature non-conformance. A NCR is initiated and signed by the Sample Management supervisor. It is then given to the appropriate LPM for documentation of the communication with the client about the cooler temperature, and given to the Quality Services Manager for approval.

3.1.5.3 If samples are not in coolers with ice when received, no shipping temperature is recorded. The situation is noted on the COC and sample log-in proceeds. A NCR is completed, signed by the Sample Management supervisor, given to LPM for documentation of any client communication, and then given to the Quality Services Manager for approval.

3.1.6 The samples are removed from the coolers and compared with the accompanying chain-of-custody form to ensure that the shipment is complete. The samples are lined up in the same order as that on the chain-of-custody form. Each sample is checked as well as each bottle type.

3.1.7 If any samples or bottle types are missing or the information on the chain-of-custody form does not agree with that on the bottle labels, a Nonconformance Record is initiated and the Laboratory Project Manager is notified.

4 Sample Log-in

4.1 The Laboratory Information Management System (LIMS) is used to generate labels with report number, sample accession numbers, client/site information, and project number.

4.2 Labels are printed and affixed to the Internal Custody Transfer Log (STL-SOP-039), the Report Review Logsheet (STL-SOP-187), and the Cooler Receipt Checklist

4.3 Sample Accession Numbers: A unique STL Baltimore accession number is assigned to each sample. The accession number is a seven-digit number that begins with **00001 (** indicates the year) at the start of each calendar year and follows sequentially until the end of each year. The accession numbers are recorded on the chain-of-custody form to provide a cross-reference between the accession number and the client sample designation. The beginning and ending numbers in the sample batch identification are recorded in the SMO logbook.

4.4 Container Labels: The field samples may include various sample containers with different types of preservations for the requested analysis. In order to account for each client sample container, a unique sample number will be assigned and displayed on each container. The sample container number will begin with the STL Baltimore assigned accession number **00001 and a sequential hyphenated number (-1, -2, -3...etc.) for each container submitted. The laboratory would be responsible for listing the individual container that was used to perform the analysis, which is documented on the internal COC. Please refer to SOP-STL-039, Internal Custody Transfer of Samples.

4.5 A locator code is assigned (see STL-SOP-038, Assigning Sample Storage Locations) to the sample set if any of the sample aliquots are to be stored in the walk-in refrigerators. The aliquots received in the set are checked off by bottle type in the logbook.

4.6 The individual STL sample numbers and locator codes are written on labels that are put on each of the bottles.

4.7 The Sample Custodian initials the logbook to complete the manual log-in procedure.

4.8 The designated Laboratory Project Manager is then notified to report to the Sample Management Office to review the client samples and related documentation before samples are released to the laboratory for analysis.

4.9 The samples are then put into storage. Internal custody transfers are performed according to the procedure STL-SOP-039.

4.10 The samples are then logged into the laboratory computer system (see STL-SOP-036, LIMS sample log-in).

Committed to Your Success

SEVERN TRENT LABORATORIES COOLER RECEIPT FORM
Discrepancy Facsimile and Chains-of-Custody

PLEASE DELIVER TO:
Company/Department:
Fax Number:
SENT BY:
Number of Pages (including cover page): 1 of

A. **COOLER INSPECTION:**
Date cooler was opened

by(print) (sign)

1	Did cooler come with a shipping slip, airbill, etc?	YES	NO	N/A	NCR# _____
	If Yes circle courier Federal Express UPS Handcarried Other:				
	Enter Airbill Number on Chain-of-Custody				
2	Were custody seals present on outside of cooler?	YES	NO	N/A	NCR# _____
	Seal Date Seal Name				
3	Were custody seals unbroken and intact at the time of arrival?	YES	NO	N/A	NCR# _____
4	Were the Chain-of-Custody records sealed in a plastic bag and taped inside of the lid?	YES	NO	N/A	NCR# _____
5	Were the Chain-of-Custody records filled out properly(ink, signed, etc.)?	YES	NO	N/A	NCR# _____
6	Are all signatures dates, and times present to establish the chain-of-custody?	YES	NO	N/A	NCR# _____
7	Was ice used? Type of ice Wet Blue Dry	YES	NO	N/A	NCR# _____
8	Was cooler temperature < 6C? Enter Cooler Temperature:	YES	NO	N/A	NCR# _____

B. **SAMPLE INSPECTION**

9	Did all bottles arrive unbroken and were labels in good condition?	YES	NO	N/A	NCR# _____
10	Were all bottles labels complete(I.D., date, time, signature, preservative, etc.)?	YES	NO	N/A	NCR# _____
11	Were correct containers used for the analysis indicated on the Chains-of-Custody?	YES	NO	N/A	NCR# _____
12	Were preservatives checked (except VOA vials)?	YES	NO	N/A	NCR# _____
13	Was a sufficient amount of sample sent for the analyses indicated on the Chain-of-Custody?	YES	NO	N/A	NCR# _____
14	Were bubbles absent in Volatiles samples?	YES	NO	N/A	NCR# _____

C. **SUBCONTRACT INFORMATION**

15	Were any samples subcontracted to an outside laboratory?	YES	NO	N/A	NCR# _____
	If YES enter LSOA#				
	Subcontracting Laboratory Name				
	STL Accession Numbers of subcontracted samples				

Special Notes

Please respond within 24 hours of receipt of this facsimile if any changes are required to the Chain-of-Custody. Failure to notify STL Baltimore within 24 hours of receipt of this facsimile of any required changes could result in additional premiums and may not guarantee samples analyzed within holding time if changes to the analysis are required.

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Figure 1 Cooler Receipt Checklist

Severn Trent Laboratories Baltimore

STANDARD OPERATING PROCEDURE

Number: STL-SOP-062 **Rev. No.: 4**

Procedures for Glassware Cleaning

Approved By: P.A. Conley — 3-23-2000
P.A. Conley, Operations Manager — Date

Approved By: M.M. Uhlfelder — 3.22.00
M.M. Uhlfelder, Quality Services Manager — Date

Approved By: A.R. Karimi — 3/23/00
A.R. Karimi, Ph.D., General Manager — Date

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Severn Trent Laboratories Baltimore

STANDARD OPERATING PROCEDURE

Number: STL-SOP-062 **Rev. No.: 4**

Procedures for Glassware Cleaning

Revisions		
Rev. No.	*Date*	*Description*
0-1	Not available	Initial distribution
2	September 16, 1998	Revised to reflect current practice and per USACE audit.
3	March 3, 1999	Revised to reflect current practices per D. Hartman.
4	March 22, 2000	1. Revised to reflect change in ownership and update per annual review. 2. Added operation procedures for annealing oven. 3. Removed Section 5.6 (SW846 General Guidance).

1.0 PURPOSE

This describes the procedures to be used for cleaning laboratory glassware.

2.0 SCOPE

It applies to all glassware used in the analytical process for all analytical protocols performed by STL Laboratories. These procedures are followed by all STL Laboratories staff assigned to clean glassware. An annealing oven, capable of maintaining a temperature of 400 C, is in an area adjacent to the glassware cleaning room.

3.0 BACKGROUND

In the analysis of samples containing components in the parts per billion range, the preparation of scrupulously clean glassware is necessary. Failure to do so can lead to a myriad of problems in the interpretation of the data due to the presence contamination. Particular care must be taken with any glassware coming in contact with an extract or digestate that will be evaporated to a smaller volume. The process of concentrating the compounds of interest in this operation may similarly concentrate the contamination substance(s), which may seriously distort the results.

4.0 EQUIPMENT AND SUPPLIES

4.1 Lancer dishwasher (Model 1400)
4.2 Lancer Dryer (Model 1400)
4.3 Labline Instruments Annealing Oven (Model 3844A)
4.4 Drying oven, 105°C ± 2°C
4.5 Lancer Detergent - KOH (25%)
4.6 Lancer Acid - Acetic Acid
4.7 Scrubbing brushes
4.8 Liqui-Nox detergent (for hand washing)
4.9 Tap water
4.10 DI water
4.11 Acetone, reagent grade (for organic glassware rinse)
4.12 1:1 Nitric Acid, ultra pure
4.13 Pipet washer
4.14 Alconox pipet detergent tablets (for pipet washer)
4.15 Gloves, nitrile and cut resistant
4.16 Oven mitts

5.0 SAFETY AND CHEMICAL HYGIENE

5.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

5.2 The laboratory maintains a reference file of Material Safety Data Sheets for the chemicals specified in this method. Additional information on general laboratory safety is available in the laboratory Safety and Health Plan or from the Laboratory Safety Officer.

5.3 Laboratory safety glasses, gloves, and lab coats are required personal protective equipment for all laboratory staff working in the area. Safety glasses are required for all visiting personnel, including non-operational laboratory staff, auditors, and touring visitors.

6.0 *PROCEDURE*

6.1 Metals Glassware

6.1.1 Fill sink with hot soapy water, and then with proper brush, scrub each piece of glassware both inside and out. After glassware is washed, begin to rinse. Rinse three times with tap water (water should be lukewarm), and then three times with DI water. If glassware was very dirty, then double rinse. Metals beakers used for digestion, delicate glassware, or glassware with small openings must be hand washed and rinsed, not put in the dishwasher. BOD bottles used for mercury analysis may be washed in the dishwasher in place of hand washing and rinsing. Refer to section 6.5 for dishwasher instructions.

Note: Exceptionally dirty glassware, including beakers, may be placed in the dishwasher first, then hand washed and rinsed after the dishwasher.

6.1.2 All metals glassware, whether washed and rinsed by hand or by the dishwasher, must be soaked in 1:1 nitric acid for a minimum of 1 hour.

6.1.3 Return acid to carboy for reuse.

6.1.4 Rinse 1:1 nitric acid bath glassware 3 times with tap water and then 3 times with DI water. (Treat wastewater according to STL-SOP-086, Sink Neutralization Stations, prior to dumping down the drain.)

6.1.5 Beakers and watch glasses are returned to the lab wet. All other metals glassware is dried in the Lancer dryer, being careful not to mix metals glassware with general glassware. General oven drying is not necessary as long as items are fairly well air dried.

6.1.6 Do not let clean items remain in glassware room overnight.

6.1.7 Cover openings of flasks with paraffin and return all metal items to the metals lab.

6.2 General Chemistry

6.2.1 Fill sink with hot soapy water, and then with proper brush, scrub each piece of glassware both inside and out. After glassware is washed, begin to rinse. Rinse three times with tap water (water should be lukewarm), and then three times with DI water. If glassware was very dirty, then double rinse. With the exception of delicate glassware, or glassware with small openings, the dishwasher may be used in place of hand washing and rinsing. Exceptionally dirty glassware may be placed in the dishwasher first, then hand washed and rinsed after the dishwasher.

6.2.2 Dry non-volumetric glassware in annealing oven. Volumetric glassware should be dried in Lancer dryer

6.2.3 Do not let clean items remain in glassware room overnight.

6.3 General Use Pipets

6.3.1 Pipets should be put in the pipet basket, tip up, in a soaking container filled with 10% Nitric Acid.

6.3.2 When basket is full and pipets have soaked for at least ½ hour, remove basket slowly from container.

6.3.3 Put the basket with the pipets in the pipet washer, and add one detergent tablet.

6.3.4 Connect the pipet washer to the hot water spigot on the sink, making sure the discharge hose is directed into the sink.

6.3.5 Turn on hot water at a moderate rate. The pipet washer should fill and empty by itself. Make sure water is operating properly before leaving unattended.

6.3.6 Let washer run about 1 hour to thoroughly rinse pipets.

6.3.7 Turn off hot water and allow pipets to drain.

6.3.8 Attach pipet washer to DI water faucet for final rinse of about 15 minutes.

6.3.9 Remove pipets from basket, oven dry in rack, and then put away.

6.4 Organic Glassware

6.4.1 Fill sink with hot soapy water, and then with proper brush, scrub each piece of glassware both inside and out. After glassware is washed, begin to rinse. Rinse three times with tap water (water should be lukewarm), and then three times with DI water. If glassware was very dirty, then double rinse. With the exception of delicate glassware, or glassware with small openings, the dishwasher may be used in place of hand washing and rinsing. Exceptionally dirty glassware may be placed in the dishwasher first, then hand washed and rinsed after the dishwasher.

Note: Large "tumbler" jars used to prepare TCLP or other extracts for metals analysis must be washed in the dishwasher to provide an acid rinse during washing.

6.4.2 Using clean acetone, rinse one more time.
6.4.3 Place glassware in annealing oven at 735°F (400°C) for 1 hour.
6.4.4 Follow section 6.5 below for oven procedures.
6.4.5 Allow glassware to cool.
6.4.6 After glassware is touchable, store glassware on proper shelf or in drawers.

6.5 Dishwasher Procedure

6.5.1 The Lancer dishwasher programs consist of the programmable steps listed in Table 1. Each of the individual cycles uses 18 liters of water (tap or deionized, at user-specified temperatures). For example, a wash or pre-wash cycle consists of 18 L of water in the machine, with a specified amount of "detergent" (Lancer 25% KOH), circulating over the glassware for the specified amount of time. The washer then empties and fills with 18 L of the next specified water type and any additives such as detergent or acid. Rinses also use 18 L.
6.5.2 Table 1 presents programs 1 and 2. Program 2 has longer wash and rinse times, and stronger detergent and acid doses than program 1. Program 2 is used for glassware requiring extra cleaning.
6.5.3 To access the programs for review or changes: Power on machine, press "00" for program number, press START, screen will say "VALIDATION", enter "308", then enter desired program # to review or change. Use +, -, number keys to change programmed values. Press START to accept each change or step to the next item in program. Note the detergent and acid are dispensed by a time setting. The detergent and acid pumps dispense at 5 mL/second. Thus, a setting of 10 seconds equals 50 mL of acid or detergent being dispensed into the machine.

Table 1: Lancer Dishwasher Programs

FUNCTION	SELECTION	PROGRAM #1	PROGRAM #2
PREWASH 1 TIME	0 to 30 min	2 min	2 min
FILLING	hot/cold	HOT	HOT
PREWASH 1 TEMP	0 to 95 c	55 c	55 c
PREWASH 1 DETERGENT	0 to 360 sec (pump time at 5 mL/sec)	0 sec	0 sec
OPTION: PREWASH ADDITIVE	0 to 6 min	0	0
PREWASH 2 TIME	0 to 30 min	0	2 min

Table 1: Lancer Dishwasher Programs

FUNCTION	SELECTION	PROGRAM #1	PROGRAM #2
FILLING	hot/cold	0	HOT
PREWASH 2 TEMP	0 to 95 c	0	55 c
PREWASH 2 DETERGENT	0 to 360 sec (pump time at 5 mL/sec)	0	0 sec
OPTION: PREWASH 2 ADDITIVE	0 to 6 min	0	0
PREWASH 3 TIME	0 to 30 min	0	0
FILLING	hot/cold	0	0
PREWASH 3 TEMP	0 to 95 c	0	0
PREWASH 3 DETERGENT	0 to 360 sec (pump time at 5 mL/sec)	0	0
OPTION: PREWASH 3 ADDITIVE	0 to 6 min	0	0
WASH TIME	0 to 30 min	4 min	5 min
FILLING	hot/cold	Hot	Hot
WASH TEMP	0 to 95 c	55 c	55 c
WASH DETERGENT	0 to 360 sec (pump time at 5 mL/sec)	12 sec	18 sec
OPTIONAL: WASH ADDITIVE	0 to 6 min	0	0
RUNNING WATER RINSE 1	0 to 9 time (30 seconds each)	1	1
ACID RINSE TIME	0 to 30 min	2 min	4 min
NEUTRALIZING ACID	0 to 360 sec (pump time at 5 mL/sec)	10 sec	18 sec
RUNNING WATER RINSE 2	0 to 9 time (30 seconds each)	1	1
COLD DI TIME 1	0 to 30 min	2	2
COLD DI TIME 2	0 to 30 min	2	2
COLD DI TIME 3	0 to 30 min	0	0
COLD DI TIME 4	0 to 30 min	0	0
HOT DI TIME	0 to 30 min	0	0

Table 1: Lancer Dishwasher Programs			
FUNCTION	SELECTION	PROGRAM #1	PROGRAM #2
HOT DI TEMP	0 to 95 c	0	0
HOT DI ADDITIVE	0 to 360 sec (pump time at 5 mL/sec)	0	0
DRYING	0 to 90 min	0	0

6.6 Annealing Oven Operation

6.6.1 Load non-volumetric organic glassware into the oven. Close door.
6.6.2 Turn oven on by pressing large red button on control panel.
6.6.3 Press start. Oven will begin heating to 735°F (400°C).
6.6.4 When oven has been at this temperature for 1 hour, turn red know to off.
6.6.5 When oven cools to 300-350°F, open oven door to expedite cooling time.
6.6.6 When oven cools to approximately 150°F, glassware may be removed.

7.0 REFERENCES

7.1 United States Environmental Protection Agency. June 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition. including UPDATE III. U.S. EPA, Washington, D.C.

Severn Trent Laboratories Baltimore

Standard Operating Procedure

Number: STL-SOP- 180 **Rev. No.:** 4

Technical Training Requirements

Approved By: P.A. Conley, Operations Manager — Date: 3-23-2000

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 3.22.00

Approved By: A.R. Karimi, Ph.D., General Manager — Date: 3/22/00

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Severn Trent Laboratories Baltimore

Standard Operating Procedure

Number: STL-SOP-180 **Rev. No.:** 4

Technical Training Requirements

Revisions		
Rev. No.	*Date*	*Description*
0	December 19, 1995	Initial distribution.
1	July 25, 1997	Added procedures for employees with prior experience, further described acceptable documentation to demonstrate proficiency.
2	March 16, 1999	1. Added requirement for annual on-going proficiency demonstration (Section 4) 2. Revised analytical training records requirements. (Section 3.2)
3	July 23, 1999	Revised to reflect change in laboratory ownership, and revise documentation requirements to be consistent with STL QMP and with NELAC.
4	March 13, 2000	Revised IDC requirements to be consistent with STL QMP, Revision 2, Section 5.1.2 (September 1999)

1 *PURPOSE*

This procedure provides training requirements for all analysts to demonstrate competency in performing a particular analytical method.

2 *SCOPE*

The procedure applies to the technical training of all new and current analysts assigned new analytical methods. This procedure also applies to any employee performing a method that he/she has not performed in the last twelve months.

3 *PROCEDURE*

3.1 Trainers

Section Supervisors shall assign trained personnel to conduct training. Trainer personnel are defined as staff members who meet any of the following:

3.1.1 Previously certified and qualifications are on file.
3.1.2 BS/BA in relevant field
3.1.3 Prior experience with procedure at Severn Trent Laboratories Baltimore or previous employer.

3.2 Chemist

Persons in each senior technical position shall have a bachelor's degree in a relevant scientific field or equivalent experience. Successful training in specific methods used in the laboratory shall be verified and documented as Initial Demonstration of Capability (Section 3.4).

3.3 Technician

3.3.1 Junior staff (non-degreed personnel) must work under the direct supervision of the Section Supervisor or a Chemist who has performed successfully in the relevant analyses using the same technologies being applied for the analysis of environmental samples. The laboratory personnel must comply with the following minimum levels of experience required for independent operation:

3.3.1.1 Sample Preparation: 1 month per method used.
3.3.1.2 Routine Sample Analysis: 1 month per method used.

3.3.2 Technicians-in-training may perform work on samples submitted for environmental analysis as long as the following conditions are met:

3.3.2.1 They have demonstrated the ability to produce reliable results through performance of a successful Initial Demonstration of Capability.
3.3.2.2 Their immediate supervisor or instructor is readily available in their work area when they are preparing and/or analyzing the samples (A2LA, 1996).

3.3.3 Technicians may not perform any preparation or analyses of client samples independently until they have met the minimum experience level. Technicians-in-training will be allowed to work on client samples during this period as long as they follow the Method SOP and have performed the Initial Demonstration of Capability - just like the Chemist - to demonstrate their ability to produce acceptable data. The additional requirement for Technicians is that the Section Supervisor or a designated Chemist must be available when working on client samples. In the event that a

Technician in training is performing analyses on an evening or weekend shift, the Section Supervisor or a designated Chemist should be available by phone.

3.4 Initial Demonstration of Capability (IDC)

3.4.1 For all technical training, the requirement for performing analyses can be satisfied by an Initial Demonstration of Capability (IDC). Each analyst shall complete an IDC prior to independent analysis of any client samples.

3.4.2 The IDC shall consist of four LCS prepared and analyzed according to the laboratory Method SOP which meet the acceptance criteria for precision and accuracy in the reference test method. Where no reference method criteria are available, the results shall be within the laboratory-generated acceptance criteria.

3.4.3 The required documentation shall consist of the following:

3.4.3.1 A copy of the preparation and/or analysis log with identification of all contributing analysts.

3.4.3.2 A copy of the summary of the LCS recovery showing the acceptance criteria for the specified method. If the four LCSs are analyzed in different sequences, the required data from each sequence must be included, as applicable.

3.4.3.3 A copy of the Technical Data Review Checklist showing that all required review has been completed, and that the LCS recoveries were obtained during a compliant analysis.

3.4.4 Where method performance is the result of a group of analysts that together constitute the method analysis, the group of analysts, as a unit, must meet the requirements for the IDC.

3.4.5 All IDC documentation must be included with the form provided for certification of demonstration of capability. The documentation is reviewed and approved by the Section Supervisor and Quality Services Manager.

3.5 Re-training

Re-training for a procedure is required for any of the following reasons:

3.5.1 The procedure is not conducted within a twelve month period.

3.5.2 A failing score is achieved on any Performance Evaluation Sample.

For re-training, all the requirements for initial training must be met.

During the training or re-training period, if the Trainee performs analyses on client samples, both the Trainee and Section Supervisor must sign the Technical Data Review Sheet .

3.6 **On-going Annual Demonstration of Proficiency**

All trained Chemists and Technicians are required, on a minimum annual basis, to demonstrate their proficiency through successful analysis of a single blind PT sample which can be prepared by the Section Supervisor or Chemist as an unknown sample prepared from the QC stock, or purchased from an outside vendor. Successful results from external PT programs may also be used to satisfy this requirement.

3.7 **Documentation of Proficiency**

On satisfactory completion of training or re-training (Section 3), the Initial Demonstration of Capability Certification Statement (Figure 1) is completed and signed by the Section Supervisor and the Quality Services Manager, and included in the employee's Training Record.

3.7.1 **Employee Training Records** are maintained by the Quality Services staff. Copies of the training certificate showing successful mastery of each task are included. Each record is reviewed and updated during Internal Audits (STL-SOP-296).

3.7.2 **External Performance Test (PT) Samples** The quality Services Manager updates the employee Training Records with Performance Testing sample results from formal programs (e.g., WS/WP, NYDOH). When PT results are submitted to Quality Services, the analyst shall be identified for each analyte. When Quality Services receives the scores from the PT providers, the scores will be placed into the employee's training record within thirty days of receipt.

4 *REFERENCES*

4.1 American Association for Laboratory Accreditation. April 1996. *General Requirements for Accreditation of Laboratories.* A2LA, Fredrick, MD.

4.2 National Bureau of Standards/ February 1985. *Principles of Quality Assurance of Chemical Measurement.* NBSIR 85-3105. NBS, Gaithersburg MD 20899.

4.3 United Stated Environmental Protection Agency. July 1999. *National Environmental Laboratory Accreditation Conference. Constitution, Bylaws, and Standards.* EPA600/R-99/151. USEPA, Office of Research and development, Washington, D.C. 20460.

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STL Baltimore

19 Loveton Circle
Sparks, Maryland 21152
(410) 771-4920

Initial Demonstration of Capability
Certification Statement

Date: . . . . . ***Matrix:*** . . . . . .
Analyst Name: . ***Method:*** . . . .

We the undersigned CERTIFY that:

1. The analyst identified above, using the cited test method, which is in use at this facility for the analysis of samples under the National Environmental Laboratory Accreditation Program, have met the Initial Demonstration of Capability.

2. The test method was performed by the analyst identified on this certification.
3. A copy of the test method and laboratory Method SOP are available for all personnel on site.

4. The data associated with the IDC are true, complete and representative.

5. All raw data (including a copy of this certification form) necessary to reconstruct and validate these analyses have been retained at the facility, and that the associated information is available for review by authorized inspectors.

Section Supervisor Date

Quality Services Manager Date

Figure 1 Initial Demonstration of Capability Certification Statement

Severn Trent Laboratories Baltimore

STANDARD OPERATING PROCEDURE

Number: STL-SOP- 293 **Rev. No.:** 3

Employee Orientation and Training Program

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 7/26/99

Approved By: A.R. Karimi, General Manager — Date: 7/26/99

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Revisions		
Rev. No.	Date	Description
0	May 10, 1995	Initial distribution
1	May 22, 1995	Revised to correct errors
2	June 30, 1995	Revised to reflect actual practice
3	July 26, 1999	Revised to reflect actual practice and change in laboratory ownership

1 PURPOSE

This describes the procedures for STL Baltimore' Employee Orientation and Training Programs

2 SCOPE

This applies to all STL Baltimore employees. Temporary employees, hired though an employment agency, have a one week evaluation period to determine their capabilities.

STL Baltimore has implemented a formal training program which consists of orientation, technical training, and other job-related training activities.

3 ORIENTATION

3.1 Before Arrival

For all new hires, permanent and temporary, the Office Administrator provides following information to QS at least two days prior to the start date:

- Employee Name
- Date of Hire
- Position
- Section
- Shift (Times for Evening Shift)
- Status (Part Time, Full Time, Agency Temp)

3.2 Reception

All new employees report to the Reception Desk upon arrival. The receptionist will notify the Section Chief and the Quality Services Manager. General orientation is the responsibility of the Quality Services Manager who conducts the initial session with the new employee. In the event that the Quality Services Manager is unavailable, the Section Chief is responsible for the initial session.

3.2 Introduction for Temporary Employees

The initial session consists of the basic information required to work in a laboratory environment:

- View the laboratory video on chemical safety
- Review the laboratory dress code, and any Personal Protective Equipment requirements
- Discuss Material Safety Data Sheets
- Review evacuation procedures

The employee is then turned over to the Section Chief. After a one week evaluation period, the Section Chief contacts the Quality Services Manager to schedule orientation.

3.3 New Employee Orientation

3.3.1 A Training Record, prepared by the Quality Services Manager, contains the following orientation documentation:

3.3.1.1 Personnel Information

- Resume, if available

- Education/Experience Summary
- Laboratory Position Description
- Orientation and Training Form (Figure 1)

3.3.1.2 Quality Assurance

- STL Ethics Agreement (Figure 2)
- STL Confidentiality Agreement (Figure 3)
- QAMP - uncontrolled copy
- QA Acknowledgment Record (Figure 4)
- List of applicable SOPs
- List of applicable Method SOPs

3.3.1.3 Safety and Health

- SHP - Uncontrolled copy
- Safety and Health Acknowledgment Record (Figure 5)

3.3.2 Quality Services Manager's Responsibilities

During the initial orientation session, the employee works with the Quality Services manager to complete all header information on the forms included in the Training Record. The Quality Services Manager will provide an overview of the Quality Assurance and Safety and Health programs and instruct the employee to read the manuals and to sign the acknowledgment forms.

In addition, the Quality Services Manager will discuss the Ethics Agreement and the Client Confidentiality Agreement to ensure that the employee understands the requirements and the rationale behind them.

3.3.3 Section Chief's Responsibilities

The Section Chief ensures that

- All information on the Orientation and General Training Record is completed
- The Ethics Agreement and the Client Confidentiality Agreement are discussed
- Required SOPs and Method SOPs are identified
- Training checklists are identified and discussed

4 TECHNICAL TRAINING

Technical training related to job function is addressed in EAL-SOP-180.

5 OTHER TRAINING

Additional training is available at the discretion of the Section Chief with the approval of the General Manager, and may include any of the following:

- In-house training to introduce new and revised procedures, equipment, software or manuals.
- STL Sponsored Training Programs.
- Outside seminars and courses.

6 TRAINING RECORDS

All training and proficiency demonstrations are documented in the employee's Training Record. These

records are to be updated in a timely manner to accurately reflect each employee's capabilities.

6.1 Training Records consist of the following information:

Training Record Sections	Contents
Employee Information	• Resume • Education/Experience Summary • Position Description
Quality Assurance	• Orientation and General Training Record • STL Ethics Agreement • Confidentiality Agreement • Quality Assurance Acknowledgment Record
Health and Safety	• Safety and Health Acknowledgment Record • Documentation of all Safety training.
Technical Training	For operations sections: • Initial Demonstration of Capability Record (EAL-SOP-180) • On-going proficiency documentation For support sections: • Training checklists

6.2 Training Records are maintained by the Quality Services Manager
6.3 Training Records are reviewed during internal audits by the Quality Services staff to ensure that documentation is accurate, complete and current.
6.4 In the case of resignation or termination, the Training Record is archived for a period of five years.

SEVERN TRENT LABORATORIES BALTIMORE

ORIENTATION AND GENERAL TRAINING RECORD

Employee _____ Job Title _____

Date of Hire _____ Section _____

Status _____ Agency (if applicable) _____

1. Reception

_____ Contact Section Chief/supervisor, Quality Services Manager
_____ Give employee Orientation and Training Packet

The trainer checks each item as it is covered with the employee. After each session, the trainer signs and dates the form.

2. Section Chief - *Trainer* _____ *Date* _____

_____ Safety glasses required (direct employee to Laboratory Safety Officer)
_____ Laboratory coat required (as needed).
_____ Location of Material Safety Data Sheets (MSDS) discussed.
_____ Explained how to complete the timesheet.
_____ Explained how work assignments will be given.
_____ Discussed reporting relationship, how feedback occurs, formal and informal.
_____ Identified tools and resources available to the employee.
_____ Explained need for confidentiality and sense of professionalism.
_____ Explained STL Ethics Agreement, and obtained employee's signature
_____ Explained that STL operates under formal QA program with Standard Operating Procedures.
_____ Discussed location and contents of STL Baltimore's Methods SOP Manual (STL-005), and identified required Method SOPs. (Copy of list attached).

2. Information Systems -*Trainer* _____ *Date* _____

_____ Passcard issued.
_____ Sonitrol identification issued.
_____ LAN, LIMS and e-mail accounts opened (as necessary).

3. Health and Safety - *Trainer* _____ *Date* _____

_____ Overview of Safety Program provided, issued copy of Safety and Health Plan (STL-004).
_____ Training needs assessed.
_____ Respirator needs assessed.
_____ Medical monitoring needs assessed.
_____ Viewed Safety video

4. Quality Assurance - *Trainer* _____ *Date* _____

_____ Overview of STL Baltimore Quality Assurance Program provided.
_____ Location and contents of STL Baltimore SOP Manual (STL-002) discussed.
_____ Issued copy of STL Baltimore Quality Assurance Manual (STL-001).
_____ Employee signed Signature Logbook.
_____ Discussed location and contents of STL Baltimore SOP Manual (STL-002), and identified required SOPs.

Figure 1 Orientation and General Training Record

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Severn Trent Laboratories
STL Baltimore

19 Loveton Circle
Sparks, Maryland 21152
(410) 771-4920

STL ETHICS AGREEMENT

I, _____(print name) understand that high standards of integrity are required of me with regard to the duties I perform and the data I report in connection with my employment at the Company. I agree that in the performance of my duties at the Company:

- ***I will not intentionally report data values that are not the actual values obtained;***
- ***I will not intentionally report the dates, times, sample or QC identifications, or method citations of data analyses that are not the actual dates, times, sample or QC identifications, or method citations;***
- ***I will not intentionally misrepresent another individual's work; and***
- ***If a supervisor or a member of STL management requests me to engage in or perform an activity that I feel is compromising data validity or quality, I will not comply with the request and report this action immediately to a member of the upper management, up to and including the President of Severn Trent Laboratories, Inc.***
- ***I will not intentionally report data values that do not meet established quality control criteria as set forth in the Method and/or Standard Operation Procedures, or as defined by Company Policy.***
- ***I agree to inform my Supervisor of any accidental reporting of non-authentic data by me in a timely manner.***
- ***I agree to inform my Supervisor of any accidental or intentional reporting of non-authentic data by other employees.***

I have read this Ethics Agreement and understand that failure to comply with the conditions stated above will result in disciplinary action, up to and including termination from the Company.

Compliance with this policy of business ethics and conduct is the responsibility of every STL employee. Disregard or failing to comply with this standard of business ethics and conduct could lead to disciplinary action, up to and including possible termination of employment.

_____

Employee Signature Date

Figure 2 Ethics Agreement

Committed to *Your* Success

Severn Trent Laboratories
STL Baltimore

19 Loveton Circle
Sparks, Maryland 21152
(410) 771-4920

CLIENT CONFIDENTIALITY AND PROPRIETARY RIGHTS AGREEMENT

Data and sample materials provided by the client or at the client's request, and the results obtained by STL, shall be held in confidence (unless such information is generally available to the public or is in the public domain or client has failed to pay STL for all services rendered or is otherwise in breach of the terms and conditions set forth in the STL and client contract) subject to any disclosure required by law or legal process. STL's reports, and the data and information provided therein, are for the exclusive use and benefit of client, and are not released to a third party without written consent from the client.

I, _____ (print name), understand that all work we perform is confidential. This includes both sample results and whether or not we are performing work for a particular client. *We must have the client's permission before we can disclose any information to a third party* validator, regulatory agency, or any outside party.

Compliance with this policy of business ethics and conduct is the responsibility of every STL employee. Disregard or failing to comply with this standard of business ethics and conduct could lead to disciplinary action, up to and including possible termination of employment.

_____

Employee Signature Date

Figure 3 Client Confidentiality and Proprietary Rights Agreement

SEVERN TRENT LABORATORIES BALTIMORE

QUALITY ASSURANCE ACKNOWLEDGMENT RECORD

Employee _____ Job Title _____

Date of Hire _____ Section _____

Status _____ Agency (if applicable) _____

I HAVE READ AND UNDERSTAND THE QA PROGRAM REQUIREMENTS STATED IN STL BALTIMORE QUALITY ASSURANCE MANUAL (EAL-001).

Employee _____ Date _____

I HAVE READ AND UNDERSTAND THE RECORD KEEPING REQUIREMENTS IN STL BALTIMORE QUALITY ASSURANCE MANUAL (EAL-001, Section 7.4.1): "All data entries are made in indelible, water-resistant ink. The date of the entry and the observer is clear on each entry. The observer uses his/her full name or initials. An initial and signature log is maintained so that the recorder of every entry can be identified.

All information is recorded in a notebook or on other records at the time the observations are made. Recording information on loose pieces of paper is not allowed.

When a mistake is made, the wrong entry is crossed out with a single line, initialed and dated by the person making the entry, and the correct information recorded. Obliteration of an incorrect entry or writing over is not allowed; neither is the use of correction tape or fluid on any laboratory records."

Employee _____ Date _____

I HAVE RECEIVED A LIST OF THE STANDARD OPERATING PROCEDURES (STL-002) AND METHOD SOPs (STL-005) ASSOCIATED WITH MY JOB FUNCTION. I UNDERSTAND THAT I AM RESPONSIBLE FOR FOLLOWING THESE PROCEDURES.

Employee _____ Date _____

Quality Services Manager _____ Date _____

Figure 4 Quality Assurance Acknowledgment Form

SEVERN TRENT LABORATORIES BALTIMORE

SAFETY AND HEALTH ACKNOWLEDGMENT RECORD

Employee _____ Job Title _____

Date of Hire _____ Section _____

Status _____ Agency (if applicable) _____

I HAVE READ AND UNDERSTAND THE PROGRAM REQUIREMENTS STATED IN EA LABORATORIES LABORATORY SAFETY PLAN (EAL-003)

Employee _____ *Date* _____

Laboratory Safety Officer _____ *Date* _____

Figure 5 Chemical Hygiene Plan Acknowledgment Form

Severn Trent Laboratories Baltimore

STANDARD OPERATING PROCEDURE

Number: STL-SOP-296 **Rev. No.:** 3

Internal Audit Program

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 2/18/00

Approved By: A.R. Karimi, General Manager — Date: 2/22/00

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Revisions		
Rev. No.	*Date*	*Description*
0	March 29, 1996	Initial release
1	May 20, 1997	Revised to comply with A2LA On-site Audit, Item 4 of the *Assessor Deficiency Report*", submitted 04 February 1997.
2	30 August, 1998	Revised to reflect current practice.
3	February 18, 2000	Revised to reflect change in laboratory ownership and for compliance with NELAC standards (July 1999)

1 *PURPOSE*

To describe STL Baltimore policy concerning laboratory audits.

2 *SCOPE*

This applies to all audits conducted by STL Baltimore personnel including performance, systems, and data audits.

3 *RESPONSIBILITIES*

3.1 The Quality Services Manager is responsible for development and implementation of STL Baltimore's internal audit program.

3.2 The Laboratory Director and Section Supervisors are responsible for timely implementation of corrective actions.

4 INTRODUCTION

Audits are systematic checks to determine the quality of operation of some activity or function. . Audits are of four types: performance, system, focused, and report audit. :Audits of any type may be conducted at any time at the discretion of the QSM and the Laboratory Director

4.1 ***Performance Tests (PTs)*** are independent checks made by the Quality Services Manager (QSM) to arrive at a quantitative measure of the quality of the data produced by the measurement process. PT samples are purchased from an accredited PT Provider[1], and are introduced into the laboratory as single or double "blinds."

4.1.1 Single "blinds" are defined when the laboratory knows that the samples are for audit purposes but does not know the analytes and/or the concentrations present.

4.1.2 Double "blind" samples are defined when the laboratory does not know the samples are audit samples. These may be introduced as a field sample by clients or the STL Corporate QA Manager.

4.2 ***System Audits*** are on-site qualitative inspections and reviews of the quality assurance system used by some part of or the entire measurement system. The audits are performed against a set of requirements, which may be a QA project or program plan, a standard method, or a project statement of work.

Typical systems audits include, but are not limited to, the following:

- Assessment of degree of compliance with a Quality Assurance Program including the laboratory QA program, federal and state certification programs, client programs.
- Compliance with SOPs, QAPPs.
- Assessment of documentation and record keeping procedures.
- Evaluation of personnel training programs and documentation.
- Detailed performance audits of selected analytical programs.
- Calibration procedures and documentation.
- Chain-of-custody procedures.

---

[1]Under NELAC PT samples are to be analyzed and reported for all analytes/methods/matrices included in the laboratory scope of accreditation. PT providers for traditional Water Supply and Water Pollution samples are accredited by the National Institute for Standards and Technology (NIST). PT providers for other analytes should be obtained from ANSI/RAB accredited providers.

4.3 *Focused Audits* are evaluations of an individual aspect of the quality system within a single area of service in the laboratory. These audits are not preceded by formal pre-audit meetings, but are conducted to evaluate a specific process *in-situ* to determine conformance. STL Baltimore can perform focused audits as a result of nonconformances (STL-SOP-072), client complaints, or at the directions of the General Manager or Laboratory Director.

4.4 *Report Audits* are performed on 10% of the final analytical reports prepared by the laboratory. Each report is checked for format and content (Appendix A). This percentage may increase at the discretion of the QSM if more than 80% of the reports audited have one or more findings.

4.4.1 Reports forwards all Laboratory Data Report ending in "0" to QS for audit.

4.4.2 QS must initiate the audit within 24 hours of receipt of the report.

4.4.3 If the audit findings affect on-time delivery (OTD), QS will notify the LPM and General Manager, and work with them to decide how to resolve any issues.

5 *PROCEDURE*

5.1 The Quality Services Manager (QSM) has responsibility for all audits conducted by STL Baltimore. Each audit is assigned a unique number in the format:

XXX-YY-ZZZ

where

XXX denotes the audit type LAB for system or focused audits, REP for report audits.
YY denotes the last two digits of the calendar year in which the audit occurs.
ZZZ is a three digit sequential number beginning with 001 for the first audit in the specified calendar year.

All audits are tracked using an Audit Logbook (Figure 1).

5.1.1 ***Systems Audits***: A systems audit of each operational and support area is conducted by the QS staff annually. Prior to a systems audit, the designated auditor performs the following activities:

5.1.2 *Prepare an Audit Plan* (Figure 2) against the program or project requirements which becomes the basis for the audit. Schedule the audit with the Section Supervisor. In some cases, the audit may be conducted over a period of several days or weeks depending upon the auditor's schedule. The auditor should discuss timing with the Section Supervisor.

5.1.3 *Audit Checklist*: An Audit Checklist (Appendix B) is prepared and a copy given to the Section Supervisor with the Audit Plan.

5.1.4 *Review of Documentation*: The auditor reviews all applicable documentation during the audit. This includes, bu this not limited to: SOPs, training records, MDLs, Initial Demonstrations of Capability (IDCs) for each analyst, external audit reports, internal audit reports, etc.

5.1.5 *Laboratory Inspection*: The auditor will go through the laboratory following the sample flow, i.e. receipt, log-in, standard and sample preparation, analysis, data reduction, reporting. One or Method SOPs are reviewed with a responsible analyst to verify compliance with the SOPs. Standards prep logs, balance logs and instrument logs are checked.

5.1.6 *Audit Finding Report*: Any deficiencies noted during the audit are summarized in an Audit Finding Report (Appendix C) within 10 working days and distributed to the appropriate Section Supervisor, and Laboratory Director.

5.1.7 *Response to Findings:* The Section Supervisor is responsible for submitting a corrective action plan to the auditor within five working days of receipt by completing the Corrective Action Section of the Audit Finding Report. All corrective actions must address the finding and have a completion/implementation date.

5.1.8 *Verification of Corrective Actions:* It is the responsibility of the auditor to verify implementation and effectiveness of the corrective action, and to document it in the Audit Finding Report and to submit it to the QSM for acknowledgment.

5.1.9 Completed Audit Finding Reports are kept on file in the QS office. Audits are closed upon completion of all corrective actions.

5.2 ***Reports to Management***:

5.2.1 The QSM summarizes audit activities in a monthly report to the General Manager, Laboratory Director and all Section Supervisors. This includes audits performed and findings as well as status of corrective actions for open audits.

5.2.2 The QSM summarizes internal, external, and report audit activities in a monthly report to the STL Corporate QA Manager.

STL Baltimore
INTERNAL AUDIT LOG

Audit Number	Group	Section Supervisor	Auditor	Audit Date	Audit Type	Finding Number	Obs Number	CAP Due	CAP Rec'd	Response Accepted	CAP Deadline	CA Verified	Audit Closed

Figure 1. Example Audit Logbook

STL Baltimore QUALITY ASSURANCE AUDIT PLAN

AUDIT NUMBER:	AUDIT REPORT NUMBER:
AUDIT DATE(S):	LEAD AUDITOR:
CHECKLISTS TO BE USED: GENERIC . . . . . . AUDIT SPECIFIC	
AUDIT TEAM: _____ _____	LABORATORY/AREA TO BE AUDITED: _____
AUDIT TYPE: PREQUALIFICATION . . . . . . LABORATORY . . . . . . IN-PROCESS PROJECT . . . . . . PROJECT DATA REPORT . . . . . . OTHER	APPLICABLE DOCUMENTS: QA MANUALS . . . . . . PROJECT QA PLAN . . . . . . SPECIFIC/GENERIC PROCEDURES . .
ACTIVITIES AND MATERIALS TO BE AUDITED: SUBCONTRACTOR CAPABILITIES . . . . . . QA/QC PROGRAMS . . . . . . ANALYTICAL REPORTS . . . . . . LABORATORY ACTIVITIES & IN-PROCESS RECORDS . . . . . . EQUIPMENT CALIBRATION & RECORDS . . . . . . PRODUCTION ACTIVITIES & IN-PROCESS RECORDS . . . . . . COMPUTER PROGRAM VERIFICATION & RECORDS . . . . . . COMPLETED RECORDS & CHECKLISTS: SUBCONTRACTOR . . . . . . LABORATORY . . . . . . TRANSMITTAL OF INFORMATION TO EA: QA MANUAL . . . . . . SOP MANUAL . . . . . . PE SAMPLE RESULTS . . . . . . TRANSMITTAL OF INFORMATION FROM EA: CHECKLIST . . . . . . REPORT . . . . . . OTHER	

Figure 2. Example Audit Plan

APPENDIX A

Report Audit Checklist

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SEVERN TRENT LABORATORIES BALTIMORE
Laboratory Data Reports
Audit Finding Report

Audit Number: REP-00-000

Report Number:	LPM:	Date Rec'd by QS:	Audit Date:	Auditor:
Date Report Due to Client:			Client:	Date Report Released by QS:

FINDINGS AND CORRECTIVE ACTIONS

Section	*Finding*	*Required Corrective Action (CA)*	*Date CA Completed*

COMPLETED BY AUDITOR

Date Corrective Actions Verified	Auditor Signature:

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SEVERN T NT LABORATORIES BALTIMORE
Laboratory Data Reports
Audit Finding Report

Audit Number: REP-00-000

	CRITERIA	YES	NO	COMMENTS
	ANALYTICAL DATA			
A1a	Chain-of-custody (COC) is complete with no break in custody documentation.			
A1b	Cooler Receipt checklist completed			
A2	Analyses performed consistent with COC for method, analyte list, and matrix.			
A3	QC analyses performed on designated samples, or as specified by QAPP or method			
A4	Analyses and QC consistent with Project Summary.			
A5	Interrelated analyses are consistent and logical (Section 10.4.3 in EAL-001).			
A6	Manual integrations initialed/dated/coded on quant report/chromatogram or on Review Checklist.			
A7	Laboratory Logs included, complete, and accurate for all Level 4 reports.			
A8	Review Checklists completed and signed by Analyst, Peer Reviewer, and Section Chief.			
A9	EDD file included with hardcopy results, or noted indicating shipment under separate cover			
A10	Sample receipt nonconformances addressed in report narrative			
	REPORT			
R1	Client's full name, title and address in cover letter, client's full name in narrative header			
R2	Report contains all required signatures			
R3	Report dates consistent in cover letter and narrative header			
R4	Methods table is included and consistent with COC (Required for reports with 3 or more methods).			
R5	Data Qualifiers and Manual Integration Codes are included in narrative section of the report, if applicable.			
R6	Client Report Level consistent with COC			
R7	Narrative format compliant with EAL-SOP-305			
R8	Spelling, spacing, or grammar are accurate in both narrative and cover letter.			
R9	Table of contents is consistent with pagination			
R10	Backup data is included, if applicable			
R11	Invoice included and consistent with COC.			

Additional Comments:

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3520C

Rev. No.: 0

Continuous Liquid-Liquid Extraction

Approved By: G.M. Gregory, Semivolatiles Section Chief — Date: 1/24/00

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 1/26/00

Approved By: P.A. Conley, Operations Manager — Date: 1/24/2000

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Controlled Distribution

Name	Manual No.
Walter Miller	2
Mimi Uhlfelder	4
Glen Gregory	6
Glen Gregory	7
Natasha Sullivan	8
Phyllis Conley	9

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3520C Rev. No.: 0

Continuous Liquid-Liquid Extraction

Revisions		
Rev. No.	Date	Description
0	January 20, 2000	Initial Distribution

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating organic compounds from aqueous samples. The method also describes concentration techniques suitable for preparing the extract for the appropriate determinative methods.

1.2 This method is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures.

1.3 This method is restricted to use by or under supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample, usually 1 liter, is placed into a continuous liquid-liquid extractor, adjusted, if necessary, to a specific pH (see Table 2), and extracted with organic solvent for 18-24 hours.

2.2 The extract is dried, concentrated, and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method to be used (see Table 2 for appropriate exchange solvents).

2.3 Samples of interest are organized into analytical batches by the Section Chief and Group Leader based on specific project summaries. At a minimum, each batch will contain the required quality control samples consisting of one method blank, one LCS, and one MS/MSD for every 20 samples.

2.4 Sample amounts and spiking amounts are prepared by the Section Chief and Group Leader based on specific project summaries.

3.0 DEFINITIONS

3.1 **Organic-free reagent water** refers to water in which no target analyte is observed at the Reporting Limit of the compounds of interest. Severn Trent Laboratories-Baltimore uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water.

3.2 **Method Blank** is a reagent water which is taken through the entire analytical procedure.

3.3 **Laboratory Control Sample (LCS)** is an aliquot of reagent water spiked with the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes and compared to laboratory or project specified control limits for precision and accuracy.

3.4 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample aliquots spiked with the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix.

3.5 **Surrogate** is a non-target compound spiked into all samples and QC samples and taken through the entire analytical procedure to determine matrix bias and overall system performance.

4.0 SAFETY AND CHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.3 All wastes must be disposed of following the procedures outlined in STL-SOP-018.

5.0 *SAMPLE HANDLING AND PRESERVATION*

5.1 Sample container, preservation and holding time requirements are given in Table 1.

5.2 While sample extracts are in the custody of the laboratory, they are stored in the semivolatiles laboratory at 4°C ± 2°C prior to and during analysis.

5.3 After extraction is completed, samples are stored by the Sample Management Office in laboratory walk-ins until disposal.

5.4 Custody control is maintained throughout the process according to the requirements in STL-SOP-035.

Table 1. Recommended Samples Containers, Preservation Techniques, and Holding Times

Parameter	Container	Preservative	Holding Times
No Residual Chlorine Present	Amber glass, Teflon liner	Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.
Residual Chlorine Present	Amber glass, Teflon liner	Add 3 mL of 10% $Na_2S_2O_3$/gal. Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.

6.0 *INTERFERENCES*

6.1 Solvents, reagent, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to the specific determinative method for specific guidance on quality control procedures.

6.2 Interferences coextracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, further cleanup of the sample extract may be necessary. Refer to determinative method for guidance on cleanup procedures.

6.3 Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.

6.4 Glassware contamination resulting in analyte degradation: Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin, heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse (e.g., 500-mL K-D flask). These items should be hand-rinsed very carefully to avoid this problem.

6.5 The decomposition of some analytes has been demonstrated under basic extraction conditions required to separate analytes. Organochlorine pesticides may dechlorinate, phthalate esters may exchange, and phenols may react to form tannates. These reactions increase with increasing pH.

7.0 *APPARATUS*

7.1 Continuous liquid-liquid extractor - Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication.

7.2 Condenser, Friedrich.

7.3 Drying column - 20 mm ID Pyrex® column containing sodium sulfate with Pyrex® glass wool at bottom.

7.4 Kuderna-Danish (K-D) apparatus:

7.4.1 Concentrator Tube - 10-mL, graduated (Kontes K-570050-1025 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

7.4.2 Evaporation flask - 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with clamps or equivalent.

7.4.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent)

7.5 Boiling chips - Solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent).

7.6 Water bath - Heated, with concentric ring cover, capable of temperature control (±5°C). The bath should be used in a hood.

7.7 Hotplates - variable temperature controls.

7.8 Vials - Glass, 2-mL capacity with PTFE-lined screw-caps or crimp tops.

7.9 pH indicator paper - pH range including the desired extraction pH.

7.10 Erlenmeyer flask - 500-mL.

7.11 Disposable volumetric pipettes, 1.0-mL and 2.0-mL.

7.12 Graduated cylinder - 1 liter.

8.0 *STANDARDS AND REAGENTS*

8.1 **Reagents:** Reagent grade chemicals shall be used in all tests. Other grades may be used, provided its first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.1.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water defined as water in which no target analyte is observed at the Reporting Limit of the compounds of interest. STL-Baltimore uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water which meets or exceeds the specifications of ASTM Type II water.

8.1.2 Sodium hydroxide solution (10N), NaOH. Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL.

8.1.3 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours in a shallow tray.

8.1.4 Sulfuric acid solution (1:1 v/v), H_2SO_4. Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of organic-free reagent water.

8.1.5 Extraction/exchange solvents - All solvents must be pesticide quality or equivalent.

8.1.5.1 Methylene chloride, CH_2Cl_2

8.1.5.2 Hexane, C_6H_{14}

8.1.5.3 Acetonitrile, CH_3CN

8.2 Standards

8.2.1 Stock Solutions: Refer to the appropriate determinative Method SOP

9.0 *PROCEDURES*

9.1 Retrieve samples according to STL-SOP-039; *Internal Custody Transfer of Samples*.

9.2 Prepare the continuous liquid-liquid extractor

9.2.1 Add 300 mL of methylene chloride and several boiling chips to the distilling flask.

9.2.2 Add 200 mL of methylene chloride to the extraction vessel. Place labels on continuous liquid-liquid extractor with sample identifications.

9.3 Using a 1-liter graduated cylinder, measure 1 liter (nominal) of sample. If high analyte concentrations are anticipated, a smaller volume may be taken and diluted to 1-L with organic free-reagent water.

9.4 Quantitatively transfer the sample from the graduated cylinder to the liquid-liquid extractor.

9.5 Check the pH of the sample with wide-range pH paper and adjust the pH, if necessary, to the pH indicated in Table 2, using 1:1 (v/v) sulfuric acid or 10N sodium hydroxide.

9.6 Pipet the surrogate standard spiking solution into each sample in the liquid-liquid extractors below the surface of the sample and mix well.

9.7 Add the matrix spiking standard to the sample(s) selected for use as the matrix spike/matrix spike duplicate sample or the LCS. The spike must be added below the surface of the sample and must be mixed well.

Note: The amount of surrogate spiking solution and matrix spiking solution to be added are determined by the Section Chief and/or the Group Leader and will be indicated on the extraction sheet.

9.8 Attach the distillation flask, with boiling chips and solvent, to the continuous liquid-liquid extractor. Securely hang the apparatus in the hood so that the bottom of the distillation flask is in contact with the top surface of a hotplate.

9.9 Add reagent water, if necessary, to samples to ensure proper operation. Attach condensers to the top of the liquid-liquid extractors.

9.10 Once all samples have been connected to the condensers, check that hoses connected to condensers are not kinked. Check that water is running. Turn on hot plates and extract samples for 18-24 hours. Verify the extraction rate once started.

9.11 Turn off hotplates and allow extracts to cool. Check the sample pH to ensure that it has not changed since starting the extraction. If the pH has changed, adjust the pH using NaOH or H2SO4 (as appropriate), and extract the sample for six additional hours.

9.12 If a secondary pH adjustment and extraction is required, adjust the pH of the aqueous phase, while stirring the sample, to the desired pH indicated in Table 2. Next, extract the sample for another 18-24 hours as outlined in Sections 9.9 - 9.11.

9.13 When performing GC/MS semivolatiles analysis (STL-M-8270), the acid and base/neutral extractions are performed using the same distillation flask of solvent.

9.14 Transfer the label from the continuous liquid-liquid extractors to the distillation flasks. Remove the distillation flasks. It may be necessary to gently tap the connection points with a small wooden hammer if any of the glass joints have adhered to one another

9.15 Perform concentration using the Kuderna-Danish (K-D) technique outlined below.

9.16 K-D technique

9.16.1 Assemble a Kuderna-Danish (K-D) apparatus by attaching a 10-mL concentrator tube to a 500-mL evaporation flask. Rinse with CH_2Cl_2 and add boiling chips.

9.16.2 Using large tweezers, insert a piece of pre-baked glass wool into the drying column. Scoop

anhydrous sodium sulfate with a beaker and pour into the column. Fill the column approximately 2/3 full. Rinse with $MeCl_2$.

9.16.3 Inspect the extracted sample for water before pouring into the K-D apparatus. If water is present, add anhydrous sodium sulfate directly into the Erlenmeyer flask and swirl. Pass the extract through the drying column into the K-D apparatus. Rinse the flask twice with CH_2Cl_2 using the appropriate extraction solvent and pass each rinse through the column. Rinse the column to complete the quantitative transfer.

Note: Excessive water in extract must be pipetted out before passing through the drying column.

9.17 Transfer the label from the distillation flask to the K-D apparatus.

9.18 Concentrating the Sample

9.18.1 Pre-wet a Snyder column by rinsing about 5 mL of methylene chloride through the bottom of the column and into a waste solvent container. Insert the Snyder column into the K-D apparatus. Place on a hot water bath (15 to 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 10 -15 mL, remove the K-D apparatus from the water bath and allow it to drain and cool.

Note: Before placing apparatus on hot water bath, check temperature of water bath and ensure that boiling chips have been added to the K-D apparatus.

9.18.2 If a solvent exchange is required, remove the K-D apparatus from the water bath, pour approximately 50 mL of the exchange solvent into the top the Snyder column, and return K-D apparatus to the water bath. Concentrate the extract as described above (Section 9.18.1), raising the temperature of the water bath, if necessary, to maintain proper distillation. When the apparent volume again reaches 10-15 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for several minutes. If GPC cleanup is required, the extract must remain in $MeCl_2$. Solvent exchange will occur after this cleanup.

Note: It is important that the concentrator tubes do not become dry as this may severely affect recoveries.

9.18.3 Remove the Snyder column, and separate the K-D flask from the concentrator tube. Transfer the label from the K-D flask to the concentrator tube.

9.19 Nitrogen blowdown technique

9.19.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent to the required level using a gentle stream of clean, dry nitrogen. (Proper flow should just ripple the surface of the extract.)

9.19.2 Rinse the internal wall of the tube several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into sample (i.e., the solvent level should be below the level of the water bath). The extract should **NOT** be allowed to become dry.

9.19.3 Transfer the extract from the concentrator tube to an appropriate volumetric flask once it has reached slightly below final volume. Rinse the concentrator tube with appropriate exchange solvent. Add the rinse to the volumetric flask and bring to final volume (or other appropriate volume).

9.20 See the appropriate Method SOPs for the clean-up methods that are required.

9.21 Transfer the extract from the volumetric flask to a labeled PTFE-lined screw or crimp top vial. Amber-colored vials should be used to store extracts for PAH or BNA analysis.

9.22 When finished, complete the extraction sheet and have it reviewed and signed by the Group Leader or Section Chief before transferring samples and internal chain of custody to the instrumentation laboratory.

9.23 Relinquish custody of the extracted samples to the instrumentation laboratory for analysis by signing and dating the extraction sheet after the instrumentation analyst has inspected the sample extracts. The receiving instrumentation analyst will then sign and date the extraction sheet. Make a copy of the sheet to accompany the sample extracts in the instrumentation laboratory. Keep the original extraction sheet on file in the Instrumentation Laboratory.

9.24 Table 2 below lists the appropriate volumes and solvents for the determinative methods.

Table 2. Determinative Method Volumes and Solvents

Method STL-M-	Initial Sample Volume	Final Extract Volume	Extraction pH		Initial Extraction Solvent	Solvent Exchange	Final Extract Solvent
			Initial	Secondary			
8015B	1000 mL	5 mL	as received	none	$MeCl_2$	N	$MeCl_2$
8081A	1000 mL	10 mL	5-9	none	$MeCl_2$	Y	Hexane
8082	1000 mL	10 mL	5-9	none	$MeCl_2$	Y	Hexane
8100	1000 mL	1 mL	as received	none	$MeCl_2$	N	$MeCl_2$
8141A	1000 mL	1 mL	as received	none	$MeCl_2$	N	$MeCl_2$
8270C	1000 mL	1 mL	≥11	< 2	$MeCl_2$	N	$MeCl_2$
8310	1000 mL	1 mL	as received	none	$MeCl_2$	Y	Acetonitrile

10.0 QUALITY CONTROL

10.1 Method blanks, LCS, and MS/MSD samples are be subjected to exactly the same procedures as those used upon actual samples.

10.2 Method Blank: A method blank is extracted once per analytical batch of 20 or fewer samples to determine whether or not the analysis has introduced any contamination to the samples.

10.3 Laboratory Control Sample: Extracted once per analytical batch of 20 or fewer samples. A LCS Duplicate is required if insufficient sample is available to perform a MS/MSD with the analytical batch.

10.4 Matrix Spike/Matrix Spike Duplicate: Extracted with each analytical batch of 20 or fewer samples.

10.5 Surrogate: Added to every sample, LCS, MS, and MSD.

11.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

11.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

11.3 Refer to STL-SOP-018 for waste management procedures.

12.0 REFERENCES

12.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition, including Update III. U.S. EPA, Washington, D.C.

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3540C **Rev. No.:** 2

Soxhlet Extraction

Approved By: G.M. Gregory, Semivolatiles Section Chief — Date: 1/24/00

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 1/24/00

Approved By: P.A. Conley, Operations Manager — Date: 1-21-2000

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Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3540C Rev. No.: 2

Soxhlet Extraction

Revisions		
Rev. No.	Date	Description
0	September 5, 1997	Initial Distribution
1	March 25, 1998	Revised to document current laboratory practices. Update to 3540C
2	January 20, 2000	Revised to reflect change in ownership and document current laboratory practices.

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for extracting nonvolatile and semivolatile organic compounds from solids such as soils, sludges, tissues, and wastes. The Soxhlet extraction process ensures intimate contact of the sample matrix with the extraction solvent.

1.2 This method is restricted to use by or under supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 The solid sample is mixed with anhydrous sodium sulfate and extracted using an appropriate solvent in a Soxhlet extractor for 16-24 hours.

2.2 The extract is dried, concentrated, and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method to be used (see Table 2 for appropriate exchange solvents).

2.3 Samples of interest are organized into analytical batches by the Section Chief and Group Leader based on specific project summaries. At a minimum, each batch will contain the required quality control samples consisting of one method blank, one LCS, and one MS/MSD for every 20 samples.

2.4 Sample amounts and spiking amounts are prepared by the Section Chief and Group Leader based on specific project summaries.

3.0 DEFINITIONS

3.1 **Method Blank** is a standard solid matrix which is taken through the entire analytical procedure.

3.2 **Laboratory Control Sample (LCS)** is an aliquot of a standard solid matrix spiked with the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes and compared to laboratory or project specified control limits for precision and accuracy.

3.3 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample aliquots spiked with the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix.

3.4 **Surrogate** is a non-target compound spiked into all samples and QC samples and taken through the entire analytical procedure to determine matrix bias and overall system performance.

4.0 SAFETY AND CHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.3 All wastes must be disposed of following the procedures outlined in STL-SOP-018.

5.0 SAMPLE HANDLING AND PRESERVATION

5.1 Sample container, preservation and holding time requirements are given in Table 1.

5.2 While sample extracts are in the custody of the laboratory, they are stored in the semivolatiles laboratory at 4°C ± 2°C prior to and during analysis.

5.3 After extraction is completed, samples are stored by the Sample Management Office in laboratory walk-ins until disposal.

5.4 Custody control is maintained throughout the process according to the requirements in STL-SOP-035.

Table 1. Recommended Samples Containers, Preservation Techniques, and Holding Times

Parameter	Container	Preservative	Holding Times
Soil, Sediments and Sludges	8 oz widemouth glass, Teflon liner	Cool to 4°C	Extract samples within 14 days; analyze extracts within 40 days of extraction.
Tissue	8 oz widemouth glass, Teflon liner or wrapped in solvent rinsed aluminum foil	Frozen	Extract samples within 1 year (within 14 days of thawing); analyze extracts within 40 days of extraction.

6.0 INTERFERENCES

6.1 Solvents, reagent, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to the specific determinative method for specific guidance on quality control procedures.

6.2 Interferences coextracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, further cleanup of the sample extract may be necessary. Refer to determinative method for guidance on cleanup procedures. GPC cleanup (STL-M-3640) should be performed on all tissue extracts to remove lipid interferences.

6.3 Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.

6.4 Glassware contamination resulting in analyte degradation: Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin, heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse (e.g., 500-mL K-D flask). These items should be hand-rinsed very carefully to avoid this problem.

7.0 APPARATUS

7.1 Soxhlet extractor - 40 mm ID, with 500-mL Erlenmyer flask.

7.2 Condenser, Friedrich.

7.3 Drying column - 20 mm ID Pyrex® column containing sodium sulfate with Pyrex® glass wool at bottom.

7.4 Kuderna-Danish (K-D) apparatus:

7.4.1 Concentrator Tube - 10-mL, graduated (Kontes K-570050-1025 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

7.4.2 Evaporation flask - 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with clamps or equivalent

7.4.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent).

7.5 Boiling chips - Solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent)
7.6 Water bath - Heated, with concentric ring cover, capable of temperature control (±5°C). The bath should be used in a hood.
7.7 Hotplates - variable temperature controls
7.8 Vials - Glass, 2-mL capacity with PTFE-lined screw-caps or crimp tops.
7.9 Analytical balance - capable of weighing to 0.01 g.
7.10 Disposable volumetric pipettes, 1.0-mL and 2.0-mL.

8.0 STANDARDS AND REAGENTS

8.1 **Reagents:** Reagent grade chemicals shall be used in all tests. Other grades may be used, provided its first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.
8.1.1 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours in a shallow tray.
8.1.2 Extraction/exchange solvents - All solvents must be pesticide quality or equivalent.
8.1.2.1 Methylene chloride, CH_2Cl_2
8.1.2.2 Hexane, C_6H_{14}
8.1.2.3 Acetonitrile, CH_3CN

8.2 **Standards**

8.2.1 Stock Solutions: Refer to the appropriate determinative Method SOP.

9.0 PROCEDURES

9.1 Retrieve samples according to STL-SOP-039; *Internal Custody Transfer of Samples*
9.2 Prepare the soxhlet extractor:

9.2.1 Rinse beakers, flasks and Soxhlet extractors with CH_2Cl_2. Check glassware for cracks.
9.2.2 Pour approximately 300 mL of extraction solvent into the 500-mL Erlenmeyer flasks and add several boiling chips.
9.2.3 Prepare Soxhlet extractors by plugging the bottom of the Soxhlet with glass wool. The glass wool should cover the arm of the extractor to prevent sample particles from clogging the arm and decreasing efficiency of the extraction.
9.2.4 Place labels on soxhlet extractors with sample identifications.
9.2.5 Calibrate the scale(s) which will be used to weigh the sample and sodium sulfate (STL-SOP-015). Use weights that approximate the amount of sample and sodium sulfate that will be added. Place labels on continuous liquid-liquid extractor with sample identifications.

9.3 Sediment/soil samples - Decant and discard any water layer on sediment/soil samples. Mix sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks.
9.4 Waste samples - Samples consisting of multiphases must be prepared by phase separation. This extraction procedure is for solid matrices only.
9.5 Tissue samples are homogenized prior to extraction. See STL-SOP-289.
9.6 Using a top loader balance or equivalent, weigh the appropriate amount of sample (see Table 2) into a beaker and add sufficient amount of anhydrous sodium sulfate to dry sample. The method blank and LCS consist of an appropriate aliquot of sodium sulfate (equal to the weight of the associated samples).
9.7 Mix sample and sodium sulfate with a spatula until a loose pouring consistency is achieved. Pour into the Soxhlet extractor, and rinse beaker into extractor. Rinse the spatula with CH_2Cl_2 after

each sample to prevent potential contamination.

9.8 Pipet the surrogate standard spiking solution into each sample in the soxhlet extractors (Refer to STL-SOP-331).

9.9 Add the matrix spiking standard to the sample(s) selected for use as the matrix spike/matrix spike duplicate sample and the LCS.

Note: The amount of surrogate spiking solution and matrix spiking solution to be added are determined by the Section Chief and/or the Group Leader and will be indicated on the extraction sheet.

9.10 Attach the Erlenmyer flask, with boiling chips and solvent, to the bottom of the soxhlet extractor. Securely hang the apparatus in the hood so that the bottom of the Erylenmyer flask is in contact with the top surface of a hotplate.

9.11 Attach condensers to the top of the soxhlet extractors.

9.12 Once all samples have been connected to the condensers, check that hoses connected to condensers are not kinked. Check that water is running. Turn on hot plates and extract samples for 16-24 hours. Verify the extraction rate once started.

9.13 Turn off hotplates and allow extracts to cool.

9.14 Transfer the label from the soxhlet extractors to the Erlenmyer flasks. Remove the distillation flasks. It may be necessary to gently tap the connection points with a small wooden hammer if any of the glass joints have adhered to one another.

9.15 Perform concentration using the Kuderna-Danish (K-D) technique outlined below.

9.16 K-D technique:

9.16.1 Assemble a Kuderna-Danish (K-D) apparatus by attaching a 10-mL concentrator tube to a 500-mL evaporation flask. Rinse with CH_2Cl_2 and add boiling chips.

9.16.2 Using large tweezers, insert a piece of pre-baked glass wool into a drying column. Scoop anhydrous sodium sulfate with a beaker and pour into the column. Fill the column approximately 2/3 full. Rinse with $MeCl_2$.

9.16.3 Inspect the extracted sample for water before pouring into the K-D apparatus. If water is present, add anhydrous sodium sulfate directly into the Erlenmeyer flask and swirl. Pass the extract through the drying column into the K-D apparatus. Rinse the flask twice with CH_2Cl_2 using the appropriate extraction solvent and pass each rinse through the column. Rinse the column to complete the quantitative transfer.

Note: Excessive water in extract must be pipetted out before passing through the drying column.

9.17 Transfer the label from the distillation flask to the K-D apparatus.

9.18 Concentrating the Sample:

9.18.1 Pre-wet a Snyder column by rinsing about 5 mL of methylene chloride through the bottom of the column and into a waste solvent container. Insert the Snyder column into the K-D apparatus. Place on a hot water bath (15 to 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 10 -15 mL, remove the K-D apparatus from the water bath and allow it to drain and cool.

Note: Before placing apparatus on hot water bath, check temperature of water bath and ensure that boiling chips have been added to the K-D apparatus.

9.18.2 If a solvent exchange is required, remove the K-D apparatus from the water bath, pour approximately 50 mL of the exchange solvent into the top the Snyder column, and return K-D apparatus to the water bath. Concentrate the extract as described above (Section 9.16.1), raising the temperature of the water bath, if necessary, to maintain proper distillation. When the apparent volume again reaches 10-15 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for several minutes. If GPC cleanup is required, the extract must remain in $MeCl_2$. Solvent exchange will occur after this cleanup.

Note: It is important that the concentrator tubes do not become dry as this may severely affect recoveries.

9.18.3 Remove the Snyder column, and separate the K-D flask from the concentrator tube. Transfer the label from the K-D flask to the concentrator tube.

9.19 Nitrogen blowdown technique:

9.19.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent to the required level using a gentle stream of clean, dry nitrogen. (Proper flow should just ripple the surface of the extract.)

9.19.2 Rinse the internal wall of the tube several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into sample (i.e., the solvent level should be below the level of the water bath). The extract should **NOT** be allowed to become dry.

9.19.3 Transfer the extract from the concentrator tube to an appropriate volumetric flask once it has reached slightly below final volume. Rinse the concentrator tube with appropriate exchange solvent. Add the rinse to the volumetric flask and bring to final volume (or other appropriate volume).

9.20 See the appropriate Method SOPs for the clean-up methods that are required.

9.21 Transfer the extract from the volumetric flask to a labeled PTFE-lined screw or crimp top vial. Amber-colored vials should be used to store extracts for PAH or BNA analysis.

9.22 When finished, complete the extraction sheet and have it reviewed and signed by the Group Leader or Section Chief before transferring samples and internal chain of custody to the instrumentation laboratory.

9.23 Relinquish custody of the extracted samples to the instrumentation laboratory for analysis by signing and dating the extraction sheet after the instrumentation analyst has inspected the sample extracts. The receiving instrumentation analyst will then sign and date the extraction sheet. Make a copy of the sheet to accompany the sample extracts in the instrumentation laboratory. Keep the original extraction sheet on file in the Instrumentation Laboratory.

9.24 Table 2 lists the appropriate weights, volumes, and solvents for the determinative methods.

Table 2. Determinative Method Weights, Volumes and Solvents					
Method SOP (STL-M-)	Inital Sample Weight	Fianl Extract Volume	Initial Extraction Solvent	Solvent Exchange	Final Extract Solvent
8015B	20 grams	5 mL	$MeCl_2$	N	$MeCl_2$
8081A **	30 grams	10 mL	$MeCl_2$	Y	Hexane
8082 **	30 grams	10 mL	$MeCl_2$	Y	Hexane
8100	30 grams	1 mL	$MeCl_2$	N	$MeCl_2$
8141A	30 grams	1 mL	$MeCl_2$	N	$MeCl_2$
8270C	30 grams	1 mL	$MeCl_2$	N	$MeCl_2$
8310	30 grams	1 mL	$MeCl_2$	Y	Acetonitrile

** To reduce lipid interferences, the initial weight for tissue samples will be 6 grams and the final extract volume will be 2 mL for these methods.

10.0 QUALITY CONTROL

10.1 Method blanks, LCS, and MS/MSD samples are be subjected to exactly the same procedures as those used upon actual samples.

10.2 Method Blank: A method blank is extracted once per analytical batch of 20 or fewer samples to determine whether or not the analysis has introduced any contamination to the samples.

10.3 Laboratory Control Sample: Extracted once per analytical batch of 20 or fewer samples. A LCS Duplicate is required if insufficient sample is available to perform a MS/MSD with the analytical batch.

10.4 Matrix Spike/Matrix Spike Duplicate: Extracted with each analytical batch of 20 or fewer samples.

10.5 Surrogate: Added to every sample, LCS, MS, and MSD.

11.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

11.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

11.3 Refer to STL-SOP-018 for waste management procedures.

12.0 REFERENCES

12.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition, including Update III. U.S. EPA, Washington, D.C.

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3640A Rev. No.: 1

Gel-Permeation Cleanup

Approved By: G.M. Gregory, Semivolatiles Section Chief Date

Approved By: M.M. Uhlfelder, Quality Services Manager 2/2/2000 Date

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Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-3640A Rev. No.: 1

Gel-Permeation Cleanup

Revisions		
Rev. No.	*Date*	*Description*
0	September 10, 1996	Initial Distribution
1	January 21, 2000	1. Edited to update methods references and to reflect actual practice. 2. Edited to change laboratory name and formatting

1.0 SCOPE AND APPLICATION

1.1 Gel-permeation chromatography (GPC) is a size exclusion cleanup procedure using organic solvents and hydrophobic gels.

1.2 GPC is recommended for the elimination of lipids, polymers, copolymers, and other high molecular weight compounds from the sample extracts. GPC can be effectively used to cleanup extracts containing a broad range of analytes.

1.3 GPC will not remove interferences that are of similar molecular size as the target analytes. Other cleanup procedures may be performed to remove those interferences.

1.4 This method is restricted to use by or under supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 The GPC column is calibrated to determine the collection range for the analytes of concern.

2.2 Extracts to be GPC cleaned must be in methylene chloride.

2.3 The extracts are brought to 4.0 mL (or multiple of 4.0 mL) and are loaded onto the GPC column. The column is eluted with methylene chloride and the appropriate fraction is collected.

2.4 Since only one half of the extract is loaded onto the GPC column, the final extract volume after GPC cleanup needs to be adjusted to one half of the target final volume to correct for the effective GPC dilution factor of 2 (i.e., if the target final extract volume is 10.0 mL and GPC cleanup is performed on the extracts, the final extract volume after GPC should be 5.0 mL).

3.0 DEFINITIONS

3.1 **Method Blank** is a reagent water or standard solid matrix which is taken through the entire analytical procedure.

3.2 **Laboratory Control Sample (LCS)** is an aliquot of reagent water or standard solid matrix spiked with the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes and compared to laboratory or project specified control limits for precision and accuracy.

3.3 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample aliquots spiked with the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix.

4.0 SAFETY AND CHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.3 All wastes must be disposed of following the procedures outlined in STL-SOP-018.

5.0 SAMPLE HANDLING

5.1 Extracts should have met the method holding time requirements prior to this cleanup procedure.

5.2 While sample extracts are in the custody of the laboratory, they are stored in the semivolatiles laboratory at 4°C ± 2°C prior to and during analysis.

5.3 After extraction is completed, samples are stored by the Sample Management Office in laboratory

walk-ins until disposal.

5.4 Custody control is maintained throughout the process according to the requirements in STL-SOP-035.

6.0 INTERFERENCES

6.1 Solvents, reagent, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks.

6.2 Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.

6.3 Glassware contamination resulting in analyte degradation: Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin and heptachlor will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse.

7.0 APPARATUS

7.1 Collection tubes - 18x150 mm tube

7.2 Vials - 4 mL autosampler vials with Teflon wafer and open center cap.

7.3 Waters Gel Permeation Chromatography System which includes a Waters 510 pump, 717 autosampler, column, UV detector (254 nm wavelength), a fraction collector, and data acquisition system (Hewlett-Packard (HP)). GPC Columns are Waters Envirogel 19x150mm and 19x300mm in series. The columns are packed with 15 µm (100 Å pore size) particles of styrene divinylbenzene.

8.0 STANDARDS AND REAGENTS

8.1 Reagents

8.1.1 Solvents - all solvents must be pesticide quality or equivalent.

8.1.1.1 Methylene Chloride, CH_2Cl_2

8.2 Standards

8.2.1 A GPC check solution containing the following analytes at the following concentrations: (in methylene chloride)

Corn Oil	25.0 mg/mL
Bis(2-ethylhexyl)phthalate	0.5 mg/mL
Methoxychlor	0.1 mg/mL
Perylene	0.02 mg/mL
Sulfur	0.08 mg/mL

8.2.2 Pesticide check solution - Prepare a solution containing the following analytes in methylene chloride:

Aldrin	0.10 ug/mL	Dieldrin	0.20 ug/mL
Heptachlor	0.10 ug/mL	Endrin	0.20 ug/mL
gamma-BHC	0.10 ug/mL	4,4'-DDT	0.20 ug/mL
Tetrachloro-m-xylene	0.10 ug/mL	Decachlorobiphenyl	0.10 ug/mL

8.2.3 PCB check solution - Prepare a solution containing Aroclors 1016 and 1260 at 0.25 ug/mL and the surrogate analytes tetrachloro-m-xylene and decachlorobiphenyl at 0.10 ug/mL in methylene chloride

9.0 CALIBRATIONS

9.1 The GPC cleanup of all sample extracts must be performed within seven calender days of an acceptable GPC calibration check.

9.1.1 Refer to Section 10.1 for GPC set-up and equilibration. Inject the GPC check solution (see Section 8.2.1) onto the GPC column and obtain a UV chromatogram showing a discrete peak for each component.

9.1.2 The check solution chromatogram must meet the following requirements:

9.1.2.1 Peaks must be observed and should be symmetrical for all compounds in the calibration solution

9.1.2.2 Corn oil and phthalate peaks must exhibit >85.0 percent resolution.

9.1.2.3 Phthalate and methoxychlor peaks must exhibit >85.0 percent resolution.

9.1.2.4 Methoxychlor and perylene peaks must exhibit >85.0 percent resolution.

9.1.2.5 Perylene and sulfur peaks must exhibit >90.0 percent resolution.

9.1.3 The retention times for each of the components in the GPC Check solution should be evaluated against the previous UV trace. The retention times should not differ by more than ± 5% If the retention times differ by > 5%, an instrument problem may be indicated, and corrective action may be required.

9.1.4 The collection range for semivolatiles (includes GC/MS Semivolatiles and PAHs) begins after the elution of corn oil and ends at the elution of sulfur (approximately 12 to 23 minutes). Note: it has been observed that on high resolution columns such as the Waters Envirogel small phenol molecules are prone to adsorption and elute very close to sulfur. It is recommended that for GC/MS Semivolatiles analysis, 4-nitrophenol (the last eluting target analyte) be substituted for sulfur in the check solution and collection times adjusted to include the resulting peak.

9.1.5 The collection range for chlorinated pesticides and PCBs (aroclors and congeners) begins after the elution of phthalate and ends prior to the elution of sulfur (approximately 14 to 22 minutes). This collection range must be verified as follows:

9.1.5.1 A GPC Blank (methylene chloride), the Pesticide Check solution (Section 8.2.2) and the PCB Check solution (Section 8.2.3) are injected onto the GPC column. An appropriate fraction (based on the GPC Check solution calibration) is collected for each of these QC samples.

9.1.5.2 The collected fractions are concentrated and solvent exchanged to hexane, concentrated to a final volume of 5 mL, vialed in a 2 mL autosampler vial with crimp-cap, and analyzed by GC/ECD.

9.1.5.3 The collection range is verified when 1) no target analytes are detected at or above the laboratory reporting limit in the GPC Blank, 2) the recoveries for the six pesticide analytes in the Pesticide Check solution are from 80 to 120%, and 3) the aroclor patterns in the PCB Check solution are consistent with the instrument calibration standards.

NOTE: The acceptance limits for the Pesticide Check solution by the USEPA Contract Laboratory Program (CLP) methodologies is 80-110%.

10.0 PROCEDURES

10.1 GPC set-up and equilibration

10.1.1 Initialization

10.1.1.1 Turn on power to the pump, autosampler, detector, and fraction collector.

10.1.1.2 Depending on the time elapsed since last use, the column may be connected to autosampler and detector lines or it may be stored with end plugs in place.

10.1.2 Pump Priming and Autosampler Purge

10.1.2.1 Place inlet line with frit into methylene chloride reservoir and tap frit against glass to remove trapped air.

10.1.2.2 If the column is capped, pump priming should take place before the column is connected in order to avoid pumping air into the column.

10.1.2.3 Refer to operator's manual for correct pump priming procedure and prime pump.

10.1.2.4 Configure system with column in line and slowly raise flow rate to 5.0 mL/min.

10.1.2.5 Set autosampler purge time to five minutes, include compression check, and start purge. High compression values indicate the need for an additional purge. Acceptable values range about 0-20 µL. Multiple purges may be required if the system has been idle for an extended time.

10.1.2.6 Allow the system to equilibrate until a stable baseline is obtained.

10.2 Handling sample extracts

10.2.1 The sample extracts to be GPC cleaned **must** be in methylene chloride.

10.2.2 The extracts are brought to a volume of 4.0 mL prior to GPC cleanup. If the extract cannot be concentrated to 4.0 mL or is viscous at 4.0 mL, the extract should be taken to a volume that is a multiple of 4.0 mL (i.e., 8.0 mL, 12.0 mL, 16.0 mL, etc.). Extracts taken to higher volumes are separated into 4.0 mL aliquots, and each aliquot is injected onto the GPC column separately. The collected fractions for each of the aliquots will be combined after GPC cleanup.

10.2.3 Filter the extracts (or aliquots of extracts) for all samples, including QC, through a 0.45 µm Teflon filter into a 4 mL autosampler vial and cap with a Teflon wafer and open center cap.

10.2.4 Establish a sequence on the HP data system to acquire UV data.

10.2.5 Program the autosampler to inject 2000 uL with a run time of 30 minutes using the Auto function. Load vials into autosampler tray and arrange sample fraction collection test tubes into fraction collector corresponding to the HP sequence.

10.2.6 Verify fraction collector parameters by accessing the C/N function.

10.2.7 Use Window Mode 3, non-peak, and multi modes.

10.2.8 Collection time per tube may be set up to 5 min 24 sec for an 18x150 mm tube at 5.0 mL/min.

10.2.9 For proper sequencing, it is essential that the total tube collection time be LESS than (NOT equal to) the collection window time. For example, a command to collect three tubes for one minute each over a window of three minutes will not sequence correctly (the collector will complete collection on the third tube and position to the next tube before ending the window, thereby skipping a tube between samples).

10.2.10 Begin the sequence by pressing "Start Auto".

10.2.11 The collected fractions are to be concentrated and solvent exchanged (if applicable) following the procedures in the appropriate extraction SOP. For samples that were separated into multiple aliquots prior to GPC, the collected fractions are to be combined and concentrated as one extract.

NOTE: Since only one half of the extract is loaded onto the GPC column, the final extract volume after GPC cleanup needs to be adjusted to one half of the target final volume to correct for the GPC dilution factor of 2 (i.e., if the target final extract volume is 10.0 mL and GPC cleanup is performed on the extracts, the final extract volume after GPC should be 5.0 mL).

10.2.12 The date of the GPC cleanup and the instrument ID should be recorded on the extraction sheet.

10.2.13 The remaining 2 mL pre-GPC extracts should be saved and stored in the Extraction Lab refrigerator.

10.2.14 If a problem should occur with the original GPC extract, the extract is to be reinjected onto the GPC column a second time as follows:

10.2.14.1 Dilute the remaining 2 mL pre-GPC extract up to 4.0 mL (or multiple of 4.0 mL) with methylene chloride.

10.2.14.2 GPC clean this extract as indicated above.

NOTE: Since only one fourth of the original extract is loaded onto the GPC column for the second cleanup, the final extract volume after GPC cleanup needs to be adjusted to one fourth of the target final volume to correct for the GPC dilution factor of 4 (i.e., if the target final extract volume is 10.0 mL and GPC cleanup is performed a second time on the extracts, the final extract volume after GPC should be 2.5 mL).

11.0 QUALITY CONTROL

11.1 Method blanks, LCSs, and MS/MSD samples are be subjected to exactly the same procedures as those used upon actual samples.

11.2 The GPC cleanup of all sample extracts must be performed within seven calender days of an acceptable GPC calibration check.

11.3 The GPC check solution chromatogram must meet the acceptance criteria specified in Sections 9.1.2 and 9.1.3.

11.4 The chlorinated pesticide and PCB collection range is verified when all of the acceptance criteria specified in Section 9.1.5.3 are met.

12.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

12.3 Refer to STL-SOP-018 for waste management procedures.

13.0 REFERENCES

13.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition, including Update III. U.S. EPA, Washington, D.C.

13.2 United States Environmental Protection Agency. March, 1996. USEPA Contract Laboratory Program Statement of Work for Organics Analysis. Multi-Media, Multi-Concentration, OLM03.2.

Sample Preparation for the Analysis of Volatile Organics by a Purge-and-Trap Procedure in Water Samples

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1.0 SCOPE AND APPLICATION

1.1 This method describes sample preparation for the analysis of volatile organics in water and high level concentrations of organics in soil by a purge-and-trap procedure. The gas chromatographic determinative steps are found in STL-M-8015M-GRO and STL-M-8021B. This purge-and-trap procedure is already incorporated into Method 8260B.
1.2 Method STL-M-5030B can be used for most volatile organic compounds that have boiling points below 200°C and are insoluble or slightly soluble in water. Volatile water-soluble compounds can be included in this analytical technique; however, quantitation limits (by GC or GC/MS) may be higher due to poor purging efficiency.
1.3 Water samples can be analyzed directly for volatile organic compounds by purge-and-trap extraction and gas chromatography. High concentrations of these analytes in soil can be determined by extracting the soil with methanol and adding a portion of that extract to DI water and purging the sample.
1.4 This method also describes the preparation of water-miscible liquids for analysis by the purge-and-trap procedure.
1.5 Any changes in the analytical procedures must be approved by the Section Chief, Operations Manager and the Quality Services Manager before samples can be analyzed.

2.0 SUMMARY OF METHOD

The purge-and-trap process: An inert gas is bubbled through the solution and the volatile components are efficiently transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent column where the volatile components are adsorbed. After purging is completed, the sorbent column is heated and back flushed with an inert gas to desorb the components onto a gas chromatographic column.

3.0 DEFINITIONS

3.1 An **Analytical Batch**, either a sample preparation batch or an analytical sequence, is defined as a group of samples, including required quality control samples, that is processed as a unit through an entire preparation and/or determinative procedure. A preparation batch includes no more than 20 field samples.

3.2 **Analyte-free reagent water** refers to water in which no target analyte is observed at the Reporting Limit of the compounds of interest. EA Laboratories uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water.

3.6 A **Method Blank** is reagent water or standard soil which is processed through the entire analytical procedure.

3.7 A **Laboratory Control Sample (LCS)** is reagent water or standard soil spiked with a known concentration of the analyte(s) of interest and processed through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes are compared to laboratory or project specified control limits for precision and accuracy.

3.10 **Surrogate** is a non-target compound spiked into all samples and QC samples and taken through the entire analytical procedure to determine purging efficiency, and any possible matrix bias.

3.11 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample duplicates spiked with a representative subset of the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix. Recoveries of target analytes are compared to LCS control limits.

4.0 INTERFERENCES

4.1 Impurities in the purge gas, and from organic compounds out-gassing from the plumbing ahead of

the trap, account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks. The use of non-TFE plastic coating, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

4.2 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal of the sample vial during shipment and storage. A trip blank prepared from organic-free reagent water and carried through sampling and handling protocols serves as a check on such contamination.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed sequentially. Whenever an unusually concentrated sample is analyzed, it should be followed by an analysis of organic-free reagent water (instrument blank) to check for cross-contamination. The trap and other parts of the system are subject to contamination. Therefore, frequent bake-out and purging of the entire system may be required.

5.0 APPARATUS AND MATERIALS

5.1 Syringes - 5.0 mL and 10.0 mL, gas-tight.

5.2 Purge-and-trap device: The purge-and-trap device consists of three separate pieces of equipment: the autosampler and the concentrator. The purging device may either be a Tekmar ALS2016 or a Varian Archon Autosampler. The concentrators are Tekmar LSC2000s.

5.2.1 The recommended purging chamber is designed to accept 5 mL samples with a water column at least 3 cm deep. The purge gas must pass through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin. The purge gas must be introduced no more than 5 mm from the base of the water column.

5.2.2 The trap must be at least 25 cm long and have an inside diameter of at least 0.105 in. STL Baltimore currently uses a Vocarb 3000 (K trap). The trap should be conditioned prior to use by baking it at 270°C for at least one hour while backflushing with an inert gas at a flow rate of at least 20 mL/min.

5.2.3 The desorber should be capable of rapidly heating the trap to 250°C for desorption. The polymer section of the trap should not be heated higher than 250°C, and the remaining sections should not exceed 260°C during bake-out mode.

6.0 REAGENTS

6.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water.

6.2 Methanol, CH_30H - Purge and Trap grade or equivalent. Store away from other solvents.

7.0 SAMPLE PRESERVATION, AND HANDLING

Samples should be stored in capped bottles, with minimum headspace, at 4°C or less.

8.0 PROCEDURE

8.1 All standards, blanks and samples must be prepared in the same manner. (See Table 1 for Purge and Trap Operating Parameters.)

NOTE: Screening of samples prior to analysis using a headspace analyzer will provide guidance on whether sample dilutions are necessary and may prevent contamination of the purge-and-trap system.

8.2 [redacted]

8.3.1 [redacted]
8.3.2 [redacted]
8.3.3 Remove the plunger from a 5 mL or 10 mL syringe.

8.3.3.1 (For standards purged using a TEKMAR ALS2016): Fill the 5.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 5.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 5.0 mL syringe. Slowly add the standard/water solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

8.3.3.2 (For standards purged using a VARIAN ARCHON): Fill the 10.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 10.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 10.0 mL syringe. Slowly add the standard/water solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

8.3.4 (For samples purged using a TEKMAR ALS2016): Open the sample vial, which has been allowed to come to ambient temperature, and carefully pour the sample into the 5.0 mL syringe barrel to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 5.0 mL. Pull the plunger back to allow volume for the addition of the sample extract and standards. Standard additions using a micro syringe should be added directly into the tip of the 5.0 mL syringe. Slowly add the sample solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

8.3.5 (For samples purged using a VARIAN ARCHON): Open the sample vial, which has been allowed to come to ambient temperature, and carefully pour the sample into the 10.0 mL syringe barrel to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 10.0 mL. Pull the plunger back to allow volume for the addition of the sample extract and standards. Standard additions using a micro syringe should be added directly into the tip of the 10.0 mL syringe. Slowly add the sample solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

NOTE: ***The process of taking an aliquot destroys the validity of the liquid sample for future analysis. Therefore, if there is only one VOA vial, the analyst must fill a second sample bottle at this time to protect against possible loss of sample integrity. This second sample is maintained only until such time when the analyst has determined that the first sample has been analyzed properly.***

8.3.6 The following procedure is appropriate for diluting purgeable samples. All steps must be performed without delay until the diluted sample is in a gas-tight syringe.

8.3.6.1 Dilutions may be made in volumetric flasks (10 mL to 100 mL). Select the volumetric flask that will allow for the necessary dilution. Intermediate dilutions may be necessary for extremely large dilutions.

8.3.6.2 Calculate the approximate volume of organic-free reagent water to be added to the volumetric flask selected and add slightly less than this quantity of organic-free reagent water to the flask.

8.3.6.3 Inject the proper aliquot of sample from the syringe prepared in Sections 8.3.4/8.3.5 into the flask. Aliquots of less than 1 mL are not recommended. Dilute the sample to the mark with organic-free reagent water. Cap the flask, invert, and shake three times. Repeat the above procedure for additional dilutions.

8.3.6.4 Fill a syringe with the diluted sample and continue as in Sections 8.3.4/8.3.5.

8.3.7 At the conclusion of the purge time, switch the concentrator device to the desorb mode, and begin the gas chromatographic temperature program and GC data acquisition. This will introduce the trapped materials onto the gas chromatographic column by rapidly heating the trap to 260°C while backflushing the trap with an inert gas at 40 mL/min.

8.3.8 At the conclusion of the desorb time, switch the concentrator device to the bake mode. The trap temperature should be maintained at 260°C for 7 minutes. Turn off the trap heater and allow trap to cool. When cool, the trap is ready for the next sample.

8.4 High-concentration method: Designed for sample concentrations which exceed 2000 ug/Kg for PID analysis and 10000 ug/Kg for FID in soil. This method is based on extracting the sediment/soil with methanol and adding a portion of that methanol extract to DI water for purging.

8.4.1 The sample consists of the entire contents of the sample container. Do not discard any supernatant liquids. Mix the contents of the sample container with a narrow metal spatula. Weigh 4 g (wet weight) of sample into a tared 20 mL vial using a top-loading balance. Note and record the actual weight to 0.1 gram. Quickly add 10.0 mL of methanol. Cap and shake for 2 min.

NOTE: Section 8.4.1 must be performed rapidly and without interruption to avoid loss of volatile organics. These steps must be performed in a laboratory free from solvent fumes.

8.4.2 Transfer approximately 1 mL of the extract into a GC vial for storage. The remainder may be discarded. The extract should be stored at 4°C in the dark, prior to analysis.

8.4.3 The GC system should be set up according to the specific determinative method. This should be done prior to the addition of the methanol extract to organic-free reagent water.

8.4.4 Estimate the concentration range of the sample from either the low-concentration analysis or the screening analysis to determine the appropriate volume of methanol extract to use. If the sample was submitted as a high-concentration sample, start with 100 μL. All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the calibration range of the curve.

8.4.5 (For samples purged using a TEKMAR ALS2016): Fill the 5.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the appropriate volume. Pull the plunger back to allow volume for the addition of the sample extract and standards. Sample and standard additions using a micro syringe should be added directly into the tip of the 5.0 mL syringe. Slowly add the standard/water solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

8.4.6 (For samples purged using a VARIAN ARCHON): Fill the 10.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the appropriate volume. Pull the plunger back to allow volume for the addition of the sample extract and standards. Sample and standard additions using a micro syringe should be added directly into the tip of the 10.0 mL syringe. Slowly add the standard/water solution to the 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

8.5.7 Proceed with the sample analysis as described in the specific determinative method. The daily method blank and LCS should also contain 100 μL of methanol to simulate the sample conditions.

9.0 QUALITY CONTROL

9.1 Method blanks, LCSs, and MS/MSD samples are to be subjected to exactly the same procedures as those used upon actual samples.

9.2 Initial Demonstration of Precision and Accuracy: Prior to analysis of samples, each analyst is required to demonstrate the ability to generate data of acceptable bias and precision by meeting the acceptance criteria specified in the determinative method by analyzing four LCSs. The documentation of this demonstration is included in the analyst's training records.

10.0 REFERENCES

10.1 U.S. EPA 40 CFR Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule, " October 26, 1984.

10.2 U.S. EPA, "Test Methods for Evaluating Solid Waste," SW-846, 1995, third edition, Office of Solid Waste and Emergency Response, Washington, D.C.

TABLE 1. PURGE-AND-TRAP OPERATING PARAMETERS

	ANALYSIS METHOD		
	8015M using a TEKMAR 2016	*8015M using a VARIAN ARCHON*	*8021B using a VARIAN ARCHON*
Purge gas	Helium	Helium	Helium
Purge gas flow rate (mL/min)	40	40	40
Purge time (min)	11	11	11
Purge temperature (°C)	30	30	30
Desorb temperature (°C)	260	260	260
Backflush inert gas flow (mL/min)	20-60	20-60	20-60
Desorb time (min)	4	4	4

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-8151A Rev. No.: 1

Determination of Chlorinated Herbicides in Water and Solid Samples by Gas Chromatography using an Electron Capture Detector

Approved By: G.M. Gregory, Semivolatiles Section Chief — Date: 1/26/2000

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 1/26/2000

Approved By: P.A. Conley, Operations Manager — Date: 1/26/2000

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Revisions		
Rev. No.	*Date*	*Description*
0	July 1, 1999	Initial Distribution
1	January 24, 2000	1. Edited to change laboratory name and reformat 2. Edited to reflect actual practice and to add section on pollution prevention.

1.0 SCOPE AND APPLICATION

1.1 This method is a capillary gas chromatographic (GC) method for determining certain chlorinated acid herbicides and related compounds in aqueous and solid matrices. Table 1 lists the compounds that are routinely determined by this method, and the laboratory Reporting Limit for each analyte by matrix. These are provided for guidance and may not always be achievable. Sample reporting limits are highly matrix-dependent.

1.2 Because these compounds are produced and used in various forms (i.e., acid, salt, ester, etc.), this method describes a hydrolysis step that must be used to convert herbicide esters into the acid form prior to analysis. Herbicide esters generally have a half-life of less than one week in soil.

1.3 Modifications to the analyte list or procedural changes to reach lower Reporting Limits are allowed if required by client, project or program. Any changes in the analytical procedures must be approved by the Section Chief and the Quality Services Manager before samples can be analyzed.

Table 1. Method Analyte List

Analyte	CAS Number	Aqueous Reporting Limit (ug/L)	Solid Reporting Limit (ug/kg)
2,4 -D	94-75-7	5.0	100
2,4 -DB	94-82-6	10	200
2,4,5 -TP (Silvex)	93-72-1	1.0	20
2,4,5 - T	93-76-5	1.0	20
Dalapon	75-99-0	10	200
Dicamba	1918-00-9	1.0	20
Dichloroprop	120-36-5	10	200
Dinoseb	88-85-7	5.0	100
MCPA	94-74-6	750	15000
MCPP	93-65-2	750	15000
Pentachlorophenol	87-86-5	1.0	20
Picloram	1918-02-1	10	200

2.0 SUMMARY OF METHOD

2.1 This method provides extraction, esterification, and gas chromatographic conditions for the analysis of chlorinated acid herbicides in water, soil, and waste samples. An option for the hydrolysis of esters is also described.

2.2 Soil and water samples are extracted and esterified with diazomethane. The derivatives are determined by gas chromatography with an electron capture detector (GC/ECD). The results are reported as the acid equivalents.

3.0 DEFINITIONS

3.1 **Organic-free reagent water** refers to water in which no target analyte is observed at the Reporting Limit of the compounds of interest. Severn Trent Laboratories-Baltimore uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water.

3.2 **Initial Calibration Verification (ICV)** is a second source calibration standard used to verify the initial calibration and evaluate method performance. It contains all the analytes listed in Table 1. The stock used to prepare the ICV must be from a source that is different from the stocks used to prepare the initial calibration standards.

3.3 **Continuing Calibration Verification(CCV)** is a mid-level calibration standard, which may be prepared from the same source as the initial calibration standards, used to verify the initial calibration throughout the analytical sequence.

3.4 **Method Blank** is a reagent water or standard solid matrix which is taken through the entire analytical procedure.

3.5 **Laboratory Control Sample (LCS)** is an aliquot of reagent water or standard solid matrix spiked with the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes and compared to laboratory or project specified control limits for precision and accuracy.

3.6 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample aliquots spiked with the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix.

3.7 **Reference** the terminology used to identify the native sample used for matrix spiking purposes.

4.0 SAFETY AND CHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Only experienced analysts should be allowed to work with diazomethane due to the potential hazards associated with its use (the compound is explosive and carcinogenic).

4.2.1 **CAUTION: Diazomethane, used in the esterification step of this procedure, is a known carcinogen and is explosive under certain conditions. The analyst shall exercise the additional cautions listed when handling and disposing of this reagent:**

4.2.1.1 Use a safety screen.
4.2.1.2 Use mechanical pipetting aides.
4.2.1.3 Do not heat above 90-C -- EXPLOSION may result.
4.2.1.4 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers -- EXPLOSION may result.
4.2.1.5 Store away from alkali metals -- EXPLOSION may result.
4.2.1.6 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

4.2.2 **CAUTION: Diethyl ether can be extremely explosive if peroxides are allowed to form during**

storage. Test strips (EM Quant, or equivalent) shall be used to determine the absence of peroxides. Procedures for removal of peroxides are provided with the test strips.

4.3 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.4 All wastes must be disposed of following the procedures outlined in STL-SOP-018.

5.0 SAMPLE HANDLING AND PRESERVATION

5.1 Sample container, preservation and holding time requirements are given in Table 2.

5.2 While sample extracts are in the custody of the laboratory, they are stored in the semivolatiles laboratory at 4°C ± 2°C prior to and during analysis.

5.3 After analysis is completed, samples are stored by the Sample Management Office in laboratory walk-ins until disposal.

Table 2. Sample Containers, Preservation Techniques, and Holding Times

Matrix	Container	Preservative	Holding Time
Concentrated Waste Samples:	8 oz wide mouth glass with Teflon liner	None	Extract samples within 14 days; analyze extracts within 40 days of extraction.
Liquid Samples: No Residual Chlorine Present	Amber glass, Teflon liner	Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.
Liquid Samples: Residual Chlorine Present	Amber glass, Teflon liner	Add 3 mL of 10% $Na_2S_2O_3$/gal. Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.
Soil/Sediments and Sludges:	8 oz wide mouth glass with Teflon liner	Cool to 4°C	Extract samples within 14 days; analyze extracts within 40 days of extraction.

6.0 INTERFERENCES

6.1 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, the injection syringe must be rinsed between samples with solvent. Whenever an unusually concentrated sample is encountered, the following sample(s) may need to be reanalyzed to determine if carryover contamination had occurred.

6.2 Analytical interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. All of these materials must be routinely demonstrated to be free of interferences, under the conditions of the analysis, by running laboratory reagent blanks.

6.3 Interferences coextracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, the Section Chief and Laboratory Project Manager should be notified before any further actions are taken.

6.4 Organic acids, especially chlorinated acids, cause the most direct interference with the determination by methylation. Phenols, including chlorophenols, may also interfere with this procedure.

6.5 Alkaline hydrolysis and subsequent extraction of the basic solution removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture

analysis. However, hydrolysis may result in the loss of Dinoseb and the formation of aldol condensation products if any residual acetone remains from the extraction of solids

6.6 The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Therefore, glassware and glass wool must be acid rinsed and then rinsed to constant pH with organic -free reagent water. Sodium sulfate must be acidified.

6.7 Glassware contamination

6.7.1 Clean all glassware as soon as possible after use by detergent washing with hot water, and rinses with tap water and organic-free reagent water. The glassware is rinsed with acetone, drained and air-dried for several hours.

6.7.2 Glassware contamination resulting in analyte degradation may include soap residue on glassware.

7.0 *APPARATUS/INSTRUMENTATION*

7.1 Apparatus

7.1.1 Kuderna-Danish (K-D) apparatus.

7.1.1.1 Concentrator tube - 10-mL graduated (Kontes K-570050-1025 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

7.1.1.2 Evaporation flask - 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with clamps or equivalent.

7.1.1.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent)

7.1.1.4 Springs - ½ inch or equivalent (for K-D apparatus)

7.1.2 Diazomethane generator

7.1.2.1 Assemble from two 20 x 150 mm test tubes, two Neoprene rubber stoppers, and a source of nitrogen. Use Neoprene rubber stoppers with holes drilled in them to accommodate glass delivery tubes. The exit tube must be drawn to a point to bubble diazomethane through the sample extract.

7.1.2.2 The generator assembly is shown in Figure 1.

7.1.3 Erlenmeyer flasks - 250-mL and 500-mL, with a ground-glass joint at the neck.

7.1.4 Beaker - 400-mL, thick-walled.

7.1.5 Vials - 10-mL, glass with PTFE-lined screw caps.

7.1.6 Separatory funnel - 2000 mL, 500-mL with polytetrafluoroethylene (PTFE) stopcock.

7.1.7 Funnel - 75 mm diameter

7.1.8 Centrifuge bottle - 500-mL, Pyrex® 1260 or equivalent.

7.1.9 Disposable Pasteur pipets (140 mm x 5 mm ID).

7.1.10 Volumetric flasks, Class A - 10-mL to 1000-mL.

7.1.11 Glass wool - acid washed

7.1.12 Boiling chips - Solvent extracted with methylene chloride, approximately 10/40 mesh silicon carbide or equivalent).

7.1.13 Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

7.1.14 Balance - Analytical, capable of accurately weighing to 0.0001 g.

7.1.15 Centrifuge

7.1.16 pH paper - wide range.

7.1.17 Microsyringe - 10 µL
7.1.18 Wrist-shaker - Burrell Model 75 or equivalent.
7.1.19 Drying column

7.2 Instrumentation

7.2.1 Gas chromatograph

7.2.1.1 Analytical system complete with gas chromatograph suitable for on-column injections and all required accessories, including detectors, column supplies, data system, gases, and syringes.
7.2.1.2 Hewlett Packard HP5890 dual injector/dual column/dual detector system equipped with Hewlett Packard HP7673 auto injector system.

7.2.2 Columns: Wide bore capillary column (30 m x 0.53 mm ID)

7.2.2.1 Column 1 - RTX-5, 30 m x 0.53 mm ID x 0.5 micron film thickness.
7.2.2.2 Column 2 - RTX-35, 30 m x 0.53 mm ID x 0.5 micron film thickness.

7.2.3 Electron capture detector (ECD).
7.2.4 Data Collection System: Enviroquant with Enviroforms.

8.0 STANDARDS AND REAGENTS

8.1 Reagents

8.1.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water as defined in section 3.1.
8.1.2 Sulfuric Acid (1:1): Dilute 500 mL of concentrated sulfuric acid to 1000 mL of organic-free reagent water.
8.1.3 Sodium hydroxide (10N): Dissolve 400 g NaOH in organic-free reagent water and dilute to 1000 mL.
8.1.4 Potassium hydroxide solution (KOH) - 37% aqueous solution (w/v). Dissolve 37 g potassium hydroxide pellets in organic-free reagent water, and dilute to 100 mL.
8.1.5 Phosphate buffer (0.1 M), pH = 2.5

8.1.5.1 Dissolve 12 g sodium phosphate (NaH_2PO_4) in organic-free reagent water and dilute to 1.0 L.
8.1.5.2 Add phosphoric acid to adjust the pH to 2.5.

8.1.6 N-Methyl-N-nitroso-p-toluenesulfonamide (Diazald) - High purity (Aldrich Chemical Co., or equivalent).
8.1.7 Silicic acid, H_2SiO_5 - 100-mesh powder, store at 130°C
8.1.8 Potassium carbonate, K_2CO_3.
8.1.9 Sodium sulfate (granular, acidified, anhydrous), Na_2SO_4.

8.1.9.1 Purify by heating at 400°C for 4 hours in a shallow tray.
8.1.9.2 Acidify by slurrying 100 g sodium sulfate with enough diethyl ether to just cover the solid; then add 0.1 mL of concentrated sulfuric acid and mix thoroughly. Remove the ether under a vacuum. Mix 1 g of the resulting solid with 5 mL of organic-free reagent water and measure the pH of the mixture. It must be below a pH of 4. Store the remaining solid at 130°C.

8.1.10 Sodium chloride, NaCl, - Purify by heating at 400°C for 30 minutes in a shallow tray
8.1.11 Methylene chloride, CH_2Cl_2, pesticide grade or equivalent.
8.1.12 Acetone, CH_3COCH_3, pesticide grade or equivalent.
8.1.13 Methanol, CH_3OH, pesticide grade or equivalent.
8.1.14 Diethyl Ether, $C_2H_5OC_2H_5$ Must be free of peroxides as indicated by test strips (EM Quant. or equivalent). Procedures for removal of peroxides are provided with the test strips.

Note: Diethyl ether used for this procedure should be stabilized with BHT, not with ethanol, as when ethanol-stabilized ether is used, the methylation reaction may not proceed efficiently, leading to low recoveries of target analytes.

8.1.15 Isooctane, $(CH_3)_3CH_2CH(CH_3)$, pesticide grade or equivalent.
8.1.16 Hexane, C_6H_{14}, pesticide grade or equivalent.
8.1.17 Carbitol (diethylene glycol monoethyl ether), $C_2H_5OCH_2CH_2OCH_2CH_2O$ - optional, for producing alcohol-free diazomethane.

8.2 Standards

8.2.1 The Standards Log Book must be filled out completely (see STL-SOP-299).
8.2.2 Stock standard solutions are prepared from neat materials or are purchased as certified solutions. These stock materials/solutions must be replaced after the manufactures expiration date or sooner if checks indicate a problem.
8.2.3 All intermediate or working standards must be replaced after 6 months, or sooner if comparison with check standards indicates a problem or the parent standard expiration date is sooner.
8.2.4 Certificates of analysis for all purchased standards will be kept on file.

9.0 CALIBRATIONS

9.1 Calibration Standards: Calibration standards are prepared at a minimum of five concentration levels. One of the concentration levels should be at or below the laboratory Reporting Limit. The remaining concentration levels should correspond to the expected range of concentrations found in samples or should define the working range of the GC.

9.1.1 Stock Standards of the methyl esters of each analyte are purchased as commercially prepared solutions and as the pure product as follows:

9.1.1.1 2,4-Dichlorophenylacetic acid (DCAA) is purchased as a 100 μg/mL solution in methyl-tert-butyl ether.
9.1.1.2 MCPA and MCPP are purchased as certified solids. 0.02g are weighed into a 10 mL volumetric flask and diluted to volume with toluene to provide a 2000 ug/L stock.
9.1.1.3 2,4,5-T and 2,4,5-TP are purchase as stock solutions at 1000 ug/L concentration. 200 uL are diluted to 2 mL to provide an intermediate stock solution of 100 ug/L concentration.
9.1.1.4 The balance of standards are purchased as stock concentrations as indicated in Table 3.

9.1.2 Instrument calibration standards are prepared by serial dilutions of the stock standard as outlined in Table 3 and 4.

9.1.2.1 Preparation of Initial Calibration Standard Concentration 6 is outlined in Table 3. Add the listed volume of each stock to a small amount of hexane in a 10 mL volumetric flask. After all stocks are added, bring to volume with hexane.

Table 3. Preparation of Initial Calibration Standard -- CONCENTRATION 6				
Compound	Stock Conc. (µg/mL)	Volume (µL)	Final Vol. (mL)	Final Conc. (µg/mL)
2,4-D	1000	50	10	5.0
2,4-DB	200	250	10	5.0
2,4,5-TP (Silvex)	100	50	10	0.50
2,4,5-T	100	50	10	0.50
Dalapon	1000	120	10	12.5
Dicamba	200	25	10	0.50
Dichloroprop	200	250	10	5.0
Dinoseb	200	125	10	2.5
MCPA	2000	1000	10	500
MCPP	2000	1000	10	500
Pentachlorophenol	1000	5	10	0.5
Picloram	1000	50	10	5.0
DCAA (surrogate)	100	500	10	5.0

9.1.2.2 The other initial calibration standards are prepared by serial dilutions as identified in Table 4.

Table 4. Preparation of Other Initial Calibration Standards					
Initial Cal. Std.	Conc. 5	Conc. 4	Conc. 3	Conc. 2	Conc. 1
Stock Used	Conc. 6	Conc. 5	Conc. 4	Conc. 3	Conc. 2
µL stock	750	750	750	750	750
µL hexane	750	750	750	750	750

9.1.2.3 The concentrations of all 6 initial calibration standards are outlined in Table 5.

Table 5. Initial Calibration Standard Concentrations-- (ug/mL)						
Compound	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6
2,4-D	0.15625	0.3125	0.625	1.25	2.5	5.0
2,4-DB	0.15625	0.3125	0.625	1.25	2.5	5.0
2,4,5-TP (Silvex)	0.015625	0.03125	0.0625	0.125	0.25	0.50
2,4,5-T	0.015625	0.03125	0.0625	0.125	0.25	0.50
Dalapon	0.390625	0.78125	1.5625	3.125	6.25	12.5

Table 5. Initial Calibration Standard Concentrations-- (ug/mL)

Compound	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6
Dicamba	0.015625	0.03125	0.0625	0.125	0.25	0.50
Dichloroprop	0.15625	0.3125	0.625	1.25	2.5	5.0
Dinoseb	0.078125	0.15625	0.3125	0.625	1.25	2.5
MCPA	15.625	31.25	62.5	125	250	500
MCPP	15.625	31.25	62.5	125	250	500
Pentachlorophenol	0.015625	0.03125	0.0625	0.125	0.25	0.50
Picloram	0.15625	0.3125	0.625	1.25	2.5	5.0
DCAA (surrogate)	0.15625	0.3125	0.625	1.25	2.5	5.0

9.1.3 Initial Calibration Verification (ICV) Standard: Prepared the same as CONC 4 of the initial calibration standards using a different stock standard source.

9.1.4 Continuing Calibration Verification (CCV) Standard: Prepared the same as CONC 4 of the initial calibration standards.

9.1.5 LCS/Matrix Spike Preparation

9.1.5.1 Stock standards of the acid form of each analyte are purchased as commercially-prepared solutions at concentrations between 10-10,000 µg/mL.

9.1.5.2 The matrix spiking solution is prepared from purchased solution "Ultra-Herb" which contains all of the spiking compounds in appropriate concentrations. 4.0 mL of Ultra-Herb are diluted to 25 mL in a volumetric flask with acetone. Final concentrations of herbicides are 1.6 µg/L for Dicamba, 2,4,5-T and 2,4,5-TP; 8 µg/mL for Dinoseb; 16 µg/mL for 2,4-D, 2,4-DB, and Dichloroprop; 40 µg/mL for Dalapon; and 1600 µg/mL for MCPA and MCPP. For PCP, stock concentration is 100 µg/mL, 400 µL is added to the 25 mL volumetric flask for a final concentration of 1.6 µg/mL. For picloram, stock concentration is 1000 µg/mL, 400 µL is added to the 25 mL volumetric flask for a final concentration of 16 µg/mL.

9.1.5.3 1.0 mL of matrix spike stock working solution is added to the LCS and specified MS/MSD sample, resulting in concentrations ranging 0.16 -160 µg/mL in the final extract.

9.1.5.4 Unless specified by project or program, the method control analytes are 2,4-D and 2,4,5-TP.

9.1.6 Surrogate Additions

9.1.6.1 Stock standards of the acid form of the surrogate 2,4-Dichlorophenylacetic acid (DCAA) are purchased as commercially-prepared solutions at 2000 µg/mL.

9.1.6.2 The working surrogate standard is prepared from the stock standard at 20 µg/mL by measuring 2 mL of the stock standard into a 200 mL volumetric flask containing a small amount of acetone. The solution is brought to volume with acetone.

9.1.6.3 1.0 mL of surrogate working standard are added to every sample, resulting in 2.0 ug/mL in the final extract.

9.1.6.4 The surrogate working standard for high concentration waste samples will be prepared at a concentration of 200 µg/mL (ex., 1 mL of stock to 10 mL of acetone). 1 mL of this solution is added to each sample.

9.2 **Initial Calibration**

9.2.1 The laboratory may use average calibration factors or a linear regression analysis to establish the curve and use for quantitation.

9.2.1.1 **Calibration Factors:** Using peak responses and mass injected, the software calculates a calibration factor (CF) for each compound for each concentration in the standard curve, and calculates the percent relative standard deviation (%RSD)of the responses for each compound.

$$CF = \frac{A_x}{C_x}$$

where:

CF	=	Calibration factor
A_x	=	Area of the compound being measured
C_x	=	Concentration of the compound being measured (ng on column)

9.2.2 **Relative Standard Deviation (RSD)** Calculation:

$$\%RSD = \frac{SD}{CF_A} \times 100$$

where:

%RSD	=	Percent Relative Standard Deviation
SD	=	Standard Deviation (n-1)
CF_A	=	Average Calibration Factor

9.2.3 If the percent relative standard deviation (%RSD) of the response factors is ≤ 20% over the working range, linearity through the origin can be assumed, and the average response factor can be used in place of a calibration curve.

9.2.4 If the %RSD is >20%, the following may apply:

9.2.4.1 Review the results (area counts, response factors) for those analytes which failed the %RSD acceptance criteria to determine if the problem is with just one of the standards. Should this be the case, the analyst has the option to reanalyze and replace the standard in question.

9.2.4.2 The analyst also has the option of narrowing the calibration range by replacing one or more of the standards with standards of different concentrations. Since this option may change the quantitation limit, the Section Chief and Project Manager must approve this option before proceeding.

9.2.4.3 In instances where the %RSD for one or more analytes exceed 20%, the initial calibration may still be acceptable if the following conditions are met:

9.2.4.3.1 The mean of the %RSD values for <u>all</u> analytes in the calibration is ≤20%.

9.2.4.3.2 The mean %RSD is calculated by summing %RSD values for each analyte and dividing by the total number of analytes.

9.2.4.3.3 The mean %RSD criteria applies to **all analytes in the standards**, regardless of whether or not they are of interest for a specific project.

9.2.4.3.4 If an individual %RSD or the mean %RSD is not less than or equal to 20%, the linear regression calibration option may be performed.

9.2.4.4 **Linear Regression:** a regression analysis to establish the calibration curve is performed. Make certain that the instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). The regression will produce the slope and intercept terms for a linear equation in the form:

$$y = mx + b$$

where:
y = Instrument response
m = Slope of the line (also called the coefficient of x)
x = Concentration of the calibration standard (ng)
b = The y intercept

9.2.4.4.1 The line must not be forced through the origin. Do not include the origin (0,0) as a calibration point.

9.2.4.4.2 The regression calculation will generate a correlation coefficient (r) that is a measure of the "goodness of fit" of the regression line to the data. A value of 1.00 indicates a perfect fit. The correlation coefficient (r) must be $r \geq 0.990$ ($r^2 \geq 0.980$).

9.2.4.4.3 The linear regression plot needs to be evaluated to ensure that the y-intercept does not exceed the method reporting limit. If this situation should occur, a new initial calibration may be required.

9.2.4.4.4 Another approach is to raise the reporting limit to above the y-intercept (or at least meet the y-intercept). ***Note: The Section Chief and Project Manager must approve this option before proceeding.***

9.2.4.4.5 If the r^2 value does not meet the acceptance criteria, and using average %RSD criteria is not an option, the initial calibration is not acceptable for that analyte and must be repeated.

9.2.4.4.6 If the y-intercept is higher than the method reporting limit, and raising the reporting limit or using average %RSD criteria is not an option, the initial calibration is not acceptable for that analyte and must be repeated.

9.3 Calibration Verification

9.3.1 The calibration is verified immediately following the initial calibration by injecting the ICV. The calibration is also verified at the beginning of every analytical sequence (when no initial calibration starts the sequence), every 12 hours, or 20 samples [every 10 samples may cut down on repeat analysis], and at the end of the analytical sequence by injecting the CCV standard.

9.3.2 The average %D (absolute values) calculated from the initial calibration curve must be ±15% of the true value.

$$\%Difference = \frac{EXP - FND}{EXP} \times 100$$

where:
EXP = Expected or True concentration of the CCV standard
FND = Found or Detected Concentration of CCV standard

9.3.3 If the ICV or CCV fails to meet acceptance criteria, the standard should be reanalyzed

immediately.

9.3.4 Should the standard fail a second time, the analysis is stopped and a new initial curve is analyzed.

9.3.5 All samples since the previous acceptable CCV standard may need to be reanalyzed.

9.3.6 If the %Ds for the analyte(s) in the CCV are >15% and a positive bias is indicated (increased responses), and those analytes are not detected in the associated samples, the data can be reported with discussion in the report narrative.

10.0 PROCEDURE

10.1 Sample Extraction

10.1.1 Extraction of high concentration waste samples

10.1.1.1 Follow the Waste Dilution Method (STL-M-3580A), with the following exceptions:

10.1.1.1.1 Use diethyl ether as the dilution solvent.

10.1.1.1.2 Use acidified anhydrous sodium sulfate and acidified glass wool

10.1.1.1.3 Spike the sample with surrogate according to Section 9.1.6.4.

10.1.1.1.4 Transfer 1.0 mL (a smaller volume or a dilution may be required if herbicide concentrations are high) to a 250-mL Erlenmeyer flask with a ground-glass joint at the neck.

10.1.1.1.5 Proceed to Section 10.1.3 for hydrolysis and continue with method.

10.1.1.2 If the analysis is for acid herbicides only, proceed to section 10.1.6 for derivitization by diazomethane.

10.1.2 Shaker extraction of soil, sediment, and other solid samples.

10.1.2.1 Add 50 g of the well mixed, moist solid sample to a 500-mL wide-mouth Erlenmeyer flask. Record the weight to the nearest 0.1 grams. Adjust the pH to 2 with 1:1 sulfuric and monitor the pH for 15 minutes with occasional stirring. If necessary, add additional 1:1 sulfuric acid until the pH remains at 2. Spike the samples with 1.0 mL of the surrogate solution.

10.1.2.1.1 Prepare matrix spike and matrix spike duplicate QC samples by measuring two additional 50 g aliquots of the selected sample into two additional 500 mL Erlenmeyer flasks, adding 1.0 mL of the surrogate spiking solution and 1.0 mL of matrix spiking solution to both the MS and MSD using a 1 mL glass disposable pipette.

10.1.2.1.2 Prepare an LCS QC sample in the same manner as the matrix spike sample, adding 1.0 mL of the surrogate and matrix spiking solutions directly to 50 grams sodium sulfate already in an Erlenmeyer flask.

10.1.2.1.3 Prepare a method blank by adding 1.0 mL of the surrogate spiking solution to 50 grams anhydrous sodium sulfate in an Erlenmeyer flask.

10.1.2.2 Add 20 mL of acetone and 80 mL diethyl ether to each flask and shake for 20 minutes. Decant solvent into a 250 mL Erlenmeyer flask.

10.1.2.3 Extract the samples two more times and combined in the 250 mL flask..

10.1.2.4 After the third extraction, the volume of extract recovered should be at least 75% of the volume of added solvent. If this is not the case, additional extractions may be necessary. Combine the extracts in a 2-L separatory funnel containing 800 mL of reagent water. If an emulsion forms, slowly add 5 g increments of acidified sodium sulfate up to the weight of the sample.

10.1.2.5 Add 2 mL of 1:1 sulfuric acid and check the pH of the water. If it is not at or below pH 2, add more 1:1 sulfuric acid until stabilized at the desired pH. Gently mix the contents of the separatory funnel for 1 minute and allow the layers to separate. Collect the aqueous phase in a clean beaker and the extract phase (top layer) in a 500-mL ground glass-stoppered Erlenmeyer flask. Place the aqueous phase back into the separatory funnel and re-extract using 25 mL of diethyl ether. Allow the layers to separate and discard the aqueous layer. Combine the ether extracts in a 500-mL Erlenmeyer flask.

10.1.3 Hydrolysis of soil, sediment, or other solid sample extracts

10.1.3.1 Use this step only if herbicide esters in addition to herbicide acids are to be determined.

10.1.3.2 Add 5 mL of 10N sodium hydroxide to 1 liter of reagent water. Add 30 mL of this basic water to each of the extracts. Add additional boiling chips to the Erlenmeyer flask. Attach a Snyder column to the Erlenmeyer flask and reflux the mixture on a water bath at 60 - 65°C until the hydrolysis step is completed (usually 1 - 2 hours). Remove the flasks from the water bath and cool to room temperature.

CAUTION: The presence of residual acetone will result in the formation of aldol condensation products which will cause GC interference.

10.1.3.3 Transfer the hydrolyzed aqueous solution to a 500-mL separatory funnel and extract the solution two times with 100 mL portions of methylene chloride. Discard the methylene chloride phase. At this point the basic (aqueous) solution contains the herbicide salts.

10.1.3.4 Adjust the pH of the solution to <2 with cold (4°C) sulfuric acid (1:1) and extract once with 40 mL of diethyl ether and twice with 20 mL portions of ether. Combine the extracts in a 500-mL Erlenmeyer flask (with a 24/40 joint) containing 20 g of acidified anhydrous sodium sulfate. Periodically, vigorously shake the extract and drying agent and allow the drying agent to remain in contact with the extract for a minimum of 2 hours. See NOTE in Section 10.1.4.9 that emphasizes the need for a dry extract prior to esterification. Quantitatively transfer the contents of the flask to a 500-mL Kuderna-Danish flask with a 10-mL concentrator tube attached when the extract is known to be dry (see 10.1.4.10).

10.1.3.5 Proceed to Section 10.1.5 for extract concentration.

10.1.4 Preparation of Aqueous Samples

10.1.4.1 Using a graduated cylinder, measure a 1-L sample and transfer it into a 2-L Separatory funnel. Spike the samples with 1.0 mL of the surrogate spiking solution below the surface of the sample.

10.1.4.1.1 Prepare matrix spike and matrix spike duplicate QC samples by measuring two additional 1-L aliquots of the selected sample into two additional 2-L separatory funnels, add 1.0 mL of the surrogate spiking solution and 1.0 mL of the matrix spiking solution to both the MS and MSD, below the surface of the samples, using a 1 mL glass disposable pipette.

10.1.4.1.2 Prepare an LCS QC sample in the same manner as the matrix spike sample, adding 1.0 mL of the surrogate and matrix spiking solutions, below the surface of the sample, to a 1-L aliquot of reagent water already in a 2-L separatory funnel

10.1.4.1.3 Prepare a method blank by adding 1.0 mL of the surrogate spiking solution, below the surface of the sample, to a 1-L aliquot of reagent water already in a 2-L separatory funnel.

10.1.4.2 Add 250 g of NaCl to the sample, seal, and shake to dissolve the salt.

10.1.4.3 Use this step only if herbicide esters, in addition to herbicide acids, are to be determined:

10.1.4.4 Add enough of 10N NaOH to the sample to adjust the pH to ≥12, seal, and shake. Check the pH of the sample with pH paper. If sample does not have a pH greater than or equal to 12, adjust the pH by adding more 10N NaOH. Let the sample sit at room temperature until the hydrolysis step is completed (usually 1 to 2 hours), shaking the Separatory funnel and contents periodically.

10.1.4.5 Add 60 mL of methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 minutes, with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between the layers is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Discard the methylene chloride phase.

10.1.4.6 Add a second 60 mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, discarding the methylene chloride layer.

10.1.4.7 Add enough cold (4°C) 1:1 sulfuric acid to the sample (or hydrolyzed sample) to adjust the pH to ≤2, seal, and shake to mix. Check the pH of the sample with pH paper: if the sample does not have a pH less than or equal to 2, adjust the pH by adding more acid.

10.1.4.8 Add 120 mL diethyl ether to the sample, seal, and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, employ mechanical techniques to complete the phase separation. The optimum techniques to complete the phase separation depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Remove the aqueous phase (bottom layer) to a 2-L Erlenmeyer flask and collect the ether phase in a 500-mL Erlenmeyer flask containing approximately 20 g of acidified anhydrous sodium sulfate. Periodically, vigorously shake the extract and drying agent.

10.1.4.9 Return the aqueous phase to the Separatory funnel, add 60 mL of diethyl ether to the sample, and repeat the extraction procedure a second time, combining the extracts in the 500-mL Erlenmeyer flask. Perform a third extraction with 60 mL diethyl ether in the same manner. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hours.

NOTE: The drying step is very critical to ensuring complete esterification. Any moisture remaining in the ether will result in low herbicide recoveries. The amount of sodium sulfate is adequate if some free flowing crystals are visible when swirling the flask. If all of the sodium sulfate solidifies in a cake, add a few additional grams of acidified sodium sulfate and again test by swirling. The 2 hour drying time is a minimum; however, the extracts may be held in contact with the sodium sulfate overnight.

10.1.4.10 Pour the dried extract into a K-D flask with concentrator tube. Use a glass rod to crush any caked sodium sulfate during the transfer. Rinse the Erlenmeyer flask and funnel with 20 to 30 mL of diethyl ether to complete the quantitative transfer. Proceed to Section 10.1.5 for extract concentration.

10.1.5 Extract Concentration

10.1.5.1 Add several clean boiling chips to the K-D flask and attach a three-ball Snyder column. Pre-wet the Snyder column by rinsing about 5 mL of diethyl ether through the bottom of the column and into a waste solvent container. Place the K-D apparatus on a hot water bath (15 to 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration

in 10-20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 5-10 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.1.5.2 Remove the Snyder column and separate the K-D flask from the concentrator tube. The extract may be further concentrated by using the nitrogen blowdown technique.

10.1.5.3 Nitrogen Blowdown Technique

10.1.5.3.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to approximately 0.5 mL using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

10.1.5.3.2 Rinse the internal wall of the tube several times with diethyl ether during the operation. During the evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

10.1.5.4 Dilute the extract with 1 mL of isooctane and 0.5 mL of methanol. The extract volume should be approximately 2 mL. The sample is now ready for methylation with diazomethane.

10.1.6 Esterification

10.1.6.1 Diazomethane Derivatization

10.1.6.1.1 The diazomethane derivatization procedures will react efficiently with all of the chlorinated herbicides described in this method and should be used only by experienced analysts, due to the potential hazards associated with its use.

10.1.6.1.2 Review the safety precautions outlined in Section 4, Safety and Chemical Hygiene.

10.1.6.2 Bubbler method

10.1.6.2.1 Assemble the diazomethane bubbler as indicated in Figure 1.

10.1.6.2.2 Add 5 mL of diethyl ether to the first test tube.

10.1.6.2.3 Add 1 mL of diethyl ether, 1 mL of Carbitol, 1.5 mL of 37% KOH, and 0.1-0.2 g of Diazald to the second test tube.

10.1.6.2.4 Immediately place the exit tube into the concentrator tube containing the sample extract.

10.1.6.2.5 Apply nitrogen flow (10 mL/min) to bubble diazomethane through the extract for 10 minutes or until the yellow color of diazomethane persists.

10.1.6.2.6 The amount of Diazald used is sufficient for esterification of approximately three sample extracts. An additional 0.1-0.2 g of Diazald may be added (after the initial Diazald is consumed) to extend the generation of the diazomethane. There is sufficient KOH present in the original solution to perform a maximum of approximately 20 minutes of total esterification.

10.1.6.2.7 Remove the concentrator tube and seal it with a Neoprene or Teflon stopper. Store at room temperature in a hood for 20 minutes.

10.1.6.2.8 Destroy any unreacted diazomethane by adding 0.1-0.2 g of silicic acid to the concentrator tube.

10.1.6.2.9 Allow to stand until the evolution of nitrogen gas has stopped. Adjust the sample volume to 10.0 mL with hexane. Transfer approximately 1 mL of each extract to a GC vial, and cap. Extracts should be stored at 4°C away from light.

10.1.6.2.10 When finished, complete the extraction sheet and have it reviewed and signed by the Group

Leader or Section Chief before transferring samples and internal chain of custody to the instrumentation laboratory.

10.1.6.2.11 Relinquish custody of the extracted samples to the instrumentation laboratory for analysis by signing and dating the extraction sheet after the instrumentation analyst has inspected the sample extracts. The receiving instrumentation analyst will then sign and date the extraction sheet. Make a copy of the sheet to accompany the sample extracts in the instrumentation laboratory. Keep the original extraction sheet on file in the Instrumentation Laboratory

10.2 Instrumental Analysis

10.2.1 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection. Hewlett Packard 5890.

10.2.2 Gas chromatography conditions: The recommended chromatographic operating conditions for the instrument are:

10.2.2.1 Temperature program:

Helium column flow	5.0 mL/min
Nitrogen make up flow	40-50 mL/min
Injection Temp	240 °C
Detector Temp	325 °C
Initial Temp	100 °C
Initial Time	0.5 min
Level 1	20 °C/min to 220 °C and hold for 1 min
Level 2	20 °C/min to 300 °C and hold for 8 min

10.2.3 Automated Data Acquisition System: Enviroquant with Enviroforms. The system allows for the continuous acquisition and storage of data obtained throughout the duration of the chromatographic program.

10.3 Retention Time Windows : A default value of ±0.1 minutes from the average retention time of the initial calibration is used when analyzed after an initial calibration. The retention time window can be updated to ±0.1 minutes from the retention time of the daily CCV standard when appropriate.

10.4 Analytical Sequence

10.4.1 Column Conditioning

10.4.2 Because of the low concentration of herbicide standards injected on a GC/ECD, column adsorption may be a problem when the GC has not been used for a day.

10.4.3 Therefore, the GC column(s) must be primed or deactivated by injecting a chlorinated herbicide standard mixture approximately 20 times more concentrated than the mid-level standard. Inject this prior to beginning initial or daily calibration.

10.4.4 Introduce 1 µL of each calibration standard, sample, LCS, MS, and MSD into the gas chromatograph.

10.4.5 The analytical sequence must start with an initial calibration or a daily CCV standard.

10.4.6 The initial calibration must be verified every 12 hours or 20 samples (whichever is more frequent) and at the end of the analytical sequence.

11.0 QUALITY CONTROL

11.1 Quality control frequency, criteria and corrective actions for this method are summarized in Table 6.

11.2 **Reporting Conventions**

11.2.1 Unless specified by client or project, quantitated results below the laboratory reporting limit are not reported as detected results but are reported as less than the reporting limit (RL U).

11.2.2 When both column analyses are compliant and the percent difference (%D) between the two column results for an analyte is greater than 40%, the analyst should closely review the results for obvious causes for the high %D (i.e., coeluting interferences on one column, improper integration on one or both columns, negative baseline deflection on one column, etc.). It is laboratory policy that unless there is an obvious cause to reject the lower value, the lower value is reported. This policy assumes that if the analysis is bracketed by compliant calibration standards (indicating acceptable instrument performance), and there are no obvious instrumental anomalies associated with the sample analysis in question, then the lower detected value is the more accurate result. If there is reason to reject the lower detected value, then the higher value will be reported.

11.2.3 If one column analysis is compliant and the other is not, the compliant column analysis will be designated the primary analysis (values from this column will be reported), while the noncompliant column analysis will be designated the confirmation analysis (if needed).

11.3 Identification/Quantitation:

11.3.1 An analyte is considered present when there is a peak response within the defined retention time window on two dissimilar GC columns.

11.3.2 Analytes are quantitated by comparing the measured response in the sample against the initial standard curve.

11.3.3 If the extract concentration for any sample exceeds the initial calibration range, the extract concentration must be diluted to within calibration range and reanalyzed.

11.4 Method Blank: A method blank is analyzed once per analytical batch of 20 or fewer samples to determine whether or not the analysis has introduced any contamination to the samples.

11.5 Laboratory Control Sample: Analyzed once per analytical batch of 20 or fewer samples. Calculate the recovery (%R) using the following equation:

$$\%\ LCS\ Recovery\ (\%R) = \frac{Found}{True} \times 100$$

where:
Found = Calculated concentration of the analyte
True = Concentration of the analyte added

11.6 Matrix Spike (Matrix Spike Duplicate)

11.6.1 With each analytical batch of 20 or fewer client samples an MS/MSD pair must be analyzed.

11.6.2 Spike each aliquot and analyze in the same manner as the reference sample

11.6.3 Report the percent recovery (%R) in the MS and MSD samples and the relative percent difference (RPD) between the MS and MSD results. Note QC outliers in the report narrative.

11.6.4 Spike Recovery Calculation

$$\%\ Spike\ Recovery\ (\%R) = \frac{SSR - SR}{SA} \times 100$$

where:
SSR = Spiked Sample Result
SR = Sample Result
SA = Spike Added

11.6.5 Relative Percent Difference Calculation

$$\%\ RPD = \frac{MSR - MSRD}{(MSR + MSRD)/2} \times 100$$

where:
MSR = Matrix spike recovery
MSRD = Matrix spike duplicate recovery

(The vertical bars in the formula above indicate the absolute value of the difference. therefore, RSD is always expressed as a positive value.)

11.7 **Surrogate**: Added to every sample, LCS, MS, and MSD. Calculate the recovery (%R) using the following equation:

$$\%\ Surrogate\ Recovery\ (\%R) = \frac{Found}{True} \times 100$$

where:
Found = Calculated concentration of the surrogate
True = Concentration of the surrogate added

11.8 **Confirmation** is performed using two dissimilar GC columns.

12.0 CALCULATIONS

12.1 Water (Calibration Factor option)

$$Conc_{sample} = \frac{A_x \times V_t \times DF}{\overline{CF} \times V_i \times V_s}$$

where:

$Conc_{sample}$	=	Sample concentration (ug/L)
A_x	=	Area of the peak for the compound to be measured.
CF	=	Calibration factor from the initial calibration (area per ng)
V_t	=	Volume of the concentrated extract in milliliters (mL)
V_i	=	Volume of extract injected in microliter (uL).
V_s	=	Volume of sample extracted in milliliters (mL)
DF	=	Dilution Factor. If no dilution is performed, DF = 1.0.

The dilution factor is defined as follows:

$$DF = \frac{V_e + V_s}{V_e}$$

where:

V_e	=	Volume of extract used to make dilution (uL)
V_s	=	Volume of clean solvent (uL)

12.2 Water (Linear Regression option)-The extract concentration is determined using the linear equation in section 9.2.4.4 as follows:

X=(y-b)/m

where:

y	=	Instrument response
m	=	Slope of the line (also called the coefficient of x)
x	=	Concentration of the extract (ng)
b	=	The y intercept

then,

$$Conc_{sample} = \frac{X \times V_t \times DF}{V_i \times V_s}$$

where:

$Conc_{sample}$	=	Sample concentration (ug/L)
X	=	Concentration of the extract (ng)
V_t	=	Volume of the concentrated extract in milliliters (mL)
V_i	=	Volume of extract injected in microliter (uL).
V_s	=	Volume of sample extracted in milliliters (mL)
DF	=	Dilution Factor. If no dilution is performed, DF = 1.0.

12.3 Solid (Calibration Factor option)

$$Conc_{sample} = \frac{A_x \times V_t \times DF}{\overline{CF} \times V_i \times W_s}$$

where:

$Conc_{sample}$	=	Sample concentration on wet weight basis (ug/kg)
A_x	=	Area of the peak for the compound to be measured.
CF	=	Calibration factor from the initial calibration (area per ng)
V_t	=	Volume of the concentrated extract in milliliters (mL)
V_i	=	Volume of extract injected in microliter (uL). If a single injection is made onto two columns, use one half the volume in the syringe as the volume injected onto each column.
W_s	=	Weight of sample extracted in grams (g)
DF	=	Dilution Factor. If no dilution is performed, DF = 1.0.

12.4 Solid (Linear Regression option)-The extract concentration is determined using the same equation as in section 12.2.

then,

$$Conc_{sample} = \frac{X \times V_t \times DF}{V_i \times W_s}$$

where:

$Conc_{sample}$	=	Sample concentration on wet weight basis (ug/kg)
X	=	Concentration of the extract (ng).
V_t	=	Volume of the concentrated extract in milliliters (mL)
V_i	=	Volume of extract injected in microliter (uL). If a single injection is made onto two columns, use one half the volume in the syringe as the volume injected onto each column.
W_s	=	Weight of sample extracted in grams (g)
DF	=	Dilution Factor. If no dilution is performed, DF = 1.0.

12.5 If solid sample concentrations are to be reported on a dry weight basis, the following equation is used:

$$Conc_{dry} = \frac{Conc_{sample}}{\%Solid} \times 100$$

where:

$Conc_{dry}$	=	Concentration of the sample on a dry weight basis (ug/kg)
$Conc_{sample}$	=	Concentration of the sample on a wet weight, or "as received" basis (ug/kg)
%Solid	=	Percent solids determined gravimetrically.

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

13.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

13.3 Refer to STL-SOP-018 for waste management procedures.

14.0 REFERENCES

14.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition, including Update III. U.S. EPA, Washington, D.C.

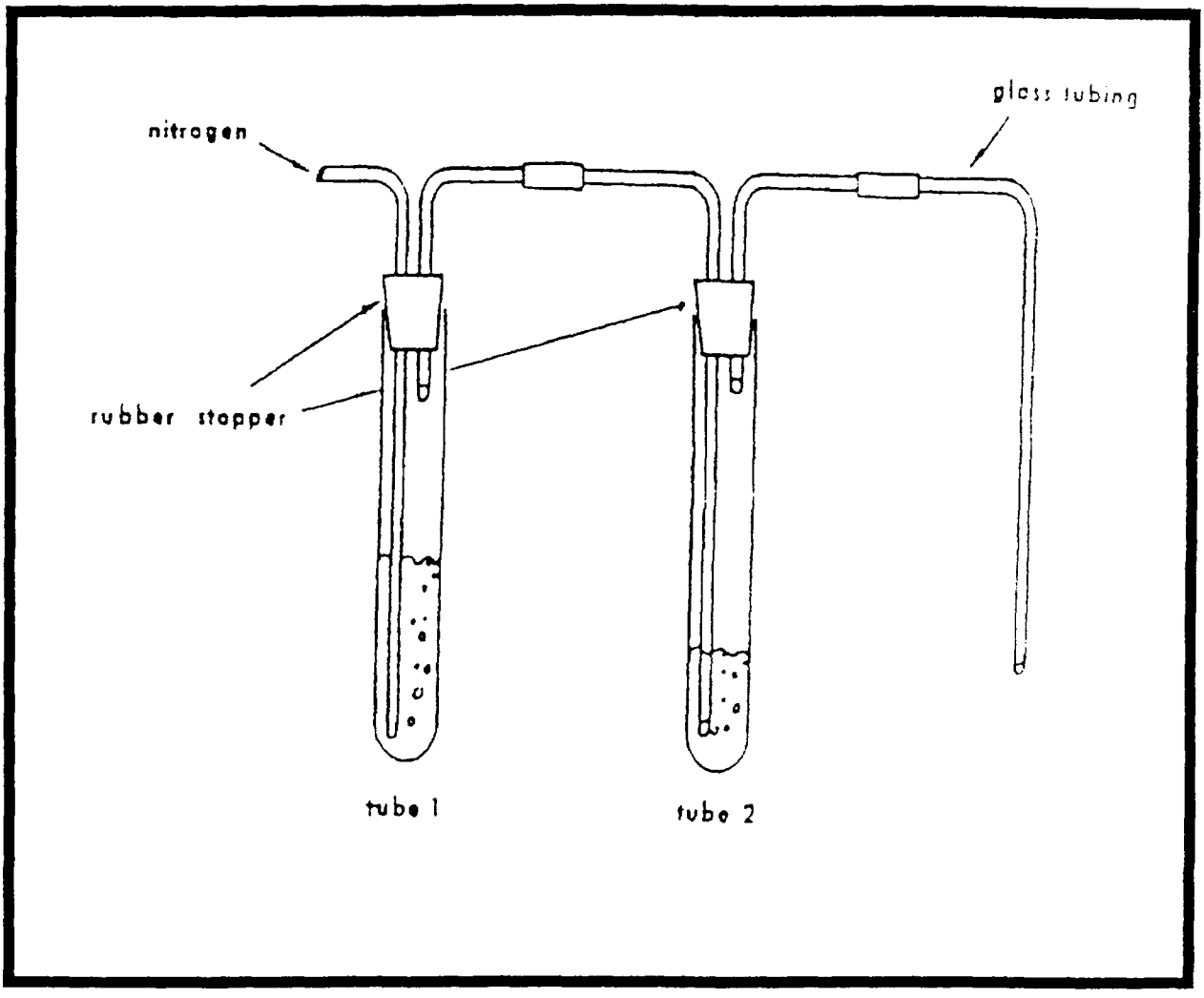

Figure 1 - Diazomethane Bubbler Set Up

Table 6. Quality Control Criteria and Corrective Actions

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Holding time	Aqueous samples extracted within 7 days; Solid samples extracted within 14 days; Analyze all extracts within 40 days of extraction.	Extraction and analysis must be completed within holding time.	Notify client to determine if laboratory is to proceed with analysis or if client will resample
Initial Calibration (ICAL)	A minimum of 5 concentration levels Low standard < RL.	Initial calibration for all target analytes ≤20 %RSD; or mean %RSD for all analytes in the standard < 20%; or use linear regression (r ≥ 0.990).	1. Check standard preparation and instrument operations, correct problem. 2. Recalibrate instrument. 3. Document actions taken.
Initial Calibration Verification (ICV)	Immediately following each ICAL.	Average %D from initial calibration no greater than ±15%.	1. Check standard preparation and instrument operations, correct problem. 2. Reanalyze standard. If same results, repeat initial calibration.
Continuing Calibration Verification (CCV)	At the beginning of each analytical sequence (if no ICAL), after every 20 samples (10 recommended) and at end of analytical sequence.	Average %D from initial calibration no greater than ±15%.	1. If %D>15 with a positive bias indicated, and no target analytes are found, no further action is required. 2. Check standard preparation and instrument operations, correct problem. 3. Repeat initial calibration if problem cannot be identified. 4. Document actions taken.
Method Blank	1 per analytical batch of 20 or less samples. Must be analyzed on each instrument used to analyze samples.	All target compound concentrations must be <RL.	1. Determine source of contamination, i.e. instrument, reagent water, reagents. 2. Take appropriate corrective action and document. If preparation in error reanalyze or prepare analytical batch. 3. If samples cannot be reanalyzed or reprepared, qualify data 4. Document actions taken.

Table 6. Quality Control Criteria and Corrective Actions

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Laboratory Control Sample (LCS)	1 per analytical batch of 20 or less samples.	Values are within project specified control limits (or lab determined) for precision and accuracy.	1. Reanalyze the LCS. 2. Check instrument parameters, sensitivity and linearity. Correct any problems. 3. Validate LCS preparation. If error is found, reextract and reanalyze the method blank, LCS and all field samples in the batch. 4. If LCS is valid, evaluate against project specific DQOs and report data if there is no impact on data usability. 5. If data is not usable, reextract and reanalyze the method blank, LCS and all field samples in the batch. 6. If repreparation of samples is not possible, qualify data, and note in the report narrative. 7. Document all actions taken in a NCR and in the report narrative.
Surrogate spike	All field and QC samples	Values are within project specified control limits (or lab determined) for recoveries.	1. Examine all QC (including but not limited to LCS, MB). 2. If surrogate in LCS and/or MB is out-of-control, check quantitation. If quantitation is correct reprepare and reanalyze the batch. 3. If samples cannot be reprepared, qualify data. 4. If surrogate spike in LCS and MB are acceptable but out-of-control for samples, review preparation of samples. If no errors or problems are discovered for sample preparation, qualify data. 5. If errors are discovered in preparation of samples, reprepare QC samples and all affected samples. 6. Document actions taken in a Nonconformance Record and in the report narrative.
MS/MSD	1 set per analytical batch of 20 or less samples.	Values are within project specified control limits (or lab determined) for precision and accuracy	1. If analyte recovery is outside control limits in LCS and data is judged unusable, reextract and reanalyze the analytical batch. 2. If LCS is acceptable but recovery is outside control limits in MS/MSD, validate preparation of samples. If no errors or problems are discovered for sample preparation, a matrix effect is assumed and the data is reported with discussion in report narrative.

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-8260B **Rev. No.:** 4

Determination of Volatile Organic Compounds in Water, Waste, Soil and Sediment Samples by Gas Chromatography/Mass Spectrometry (GC/MS)

Approved By: Craig S. Schenning — 1/27/2000
C.S. Schenning, Volatiles Section Supervisor — Date

Approved By: M.M. Uhlfelder — 1/27/2000
M.M. Uhlfelder, Quality Services Manager — Date

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P.A. Conley, Operations Manager — Date

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Severn Trent Laboratories Baltimore

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M- 8260B Rev. No.: 4

Determination of Volatile Organic Compounds in Water, Waste, Soil and Sediment Samples by Gas Chromatography/Mass Spectrometry (GC/MS)

Controlled Distribution

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Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M- 8260B **Rev. No.: 4**

Determination of Volatile Organic Compounds in Water, Waste, Soil and Sediment Samples by Gas Chromatography/Mass Spectrometry (GC/MS)

Revisions		
Rev. No.	**Date**	**Description**
0	August 14, 1996	Initial distribution.
1	December 27, 1997	Revised per Naval Auditor response CTO#004.
2	October 7, 1998	Revised per annual review
3	August 2, 1999	Revised to reflect change in ownership and current procedures
4	January 27, 2000	Revised Reporting Limits and Corrected typos. Added Freezing Preservation option for Solid Matrices. Clarified ICV/CCV acceptance criteria. Added Pollution Prevention and Waste Management section.

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine the concentration of volatile organic compounds in a variety of matrices. This method is applicable to nearly all types of samples, regardless of water content, including ground water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments.

1.2 Table 1 lists the compounds and reporting limits which can be routinely determined by this method. Modifications to the analyte list or procedural changes to reach lower reporting limits are allowed if required by client, project or program. Any changes in the analytical procedures must be approved by the Section Chief and the Quality Services Manager before samples can be analyzed.

1.3 This method is based upon a purge-and-trap, gas chromatographic/mass spectrometric (GC/MS) procedure. It is restricted to use by, or under the supervision of, analysts experienced in the use of purge-and-trap systems and gas chromatograph/mass spectrometers, and skilled in the interpretation of mass spectra and their use as a quantitative tool.

1.4 Reporting Limits are the lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The Reporting Limit is derived from the MDL and the sensitivity of the analytical technique. Sample reporting limits are highly matrix-dependent and may not always be achievable. The reporting limits for solid matrices are "wet weight" limits. Normally data are reported on a dry weight basis; therefore, RL will be higher, based on the percent dry weight in each sample.

Table 1. Method Analytes with Characteristic Masses (M/Z) and Reporting Limits

Analyte	CAS#	Primary/ Secondary Characteristic Ions for the HP5970 MSD analyses	Primary/ Secondary Characteristic Ions for the HP5973 MSD analyses	Aqueous Reporting Limits (ug/L)		Solid Reporting Limits (ug/Kg)
				5 mL purge	25 mL purge	
Acetone	67-64-1	43/58	43/58	10	5	10
Acetonitrile	75-05-8	41/40,39	41/40,39	100	30	100
Acrolein	107-02-8	56/55	56/55	50	20	50
Acrylonitrile	107-13-1	53/52	53/52	50	20	50
Allyl chloride	107-05-1	41/39,76	41/39,76	5	1	5
Benzene	71-43-2	78/50	77/50	5	1	5
Bromobenzene	108-86-1	156/158,77	156/158,77	5	1	5
Bromochloromethane	74-97-5	128/49,130	128/49,130	5	1	5
Bromodichloromethane	75-27-4	83/85,127	83/85,127	5	1	5
Bromoform	75-25-2	173/175,254	173/175,254	5	1	5
Bromomethane	74-83-9	94/96	94/96	5	1	5
2-Butanone (MEK)	78-36-3	43/72	72/43	10	5	10
n-Butylbenzene	104-51-8	91/92,134	134/92	5	1	5
sec-Butylbenzene	135-98-8	105/134	134/91	5	1	5
tert-Butylbenzene	98-06-6	119/41	134/41	5	1	5
Carbon disulfide	75-15-0	76/78	76/78	5	1	5
Carbon tetrachloride	56-23-5	117/119	117/119	5	1	5
Chlorobenzene	108-90-7	112/114,77	114/112,77	5	1	5
Chloroethane	124-48-1	64/66	64/66	5	1	5
2-Chloroethyl vinyl ether	110-75-8	63/65	63/65	10	5	10

Table 1. Method Analytes with Characteristic Masses (M/Z) and Reporting Limits

Analyte	CAS#	Primary/ Secondary Characteristic Ions for the HP5970 MSD analyses	Primary/ Secondary Characteristic Ions for the HP5973 MSD analyses	Aqueous Reporting Limits (ug/L)		Solid Reporting Limits (ug/Kg)
				5 mL purge	25 mL purge	
Chloroform	75-00-3	83/85	83/85	5	1	5
1-Chlorohexane	544-10-5	91/55,41	91/55,41	5	1	5
Chloromethane	67-66-3	50/52	50/52	5	1	5
Chloroprene	126-99-8	53/88	53/88	10	2	5
2-Chlorotoluene	74-87-3	91/126	126/91	5	1	5
4-Chlorotoluene	95-49-8	91/126	126/91	5	1	5
1,2-Dibromo-3-chloropropane	106-43-4	75/157,155	75/157,155	5	1	5
Dibromochloromethane	96-12-8	129/127	129/127	5	1	5
1,2-Dibromoethane	106-93-4	107/109	107/109	5	1	5
Dibromomethane	74-95-3	93/95,174	93/95,174	5	1	5
1,2-Dichlorobenzene	95-50-1	146/111,148	111/146,148	5	1	5
1,3-Dichlorobenzene	541-73-1	146/111,148	111/146,148	5	1	5
1,4-Dichlorobenzene	106-46-7	146/111,148	111/146,148	5	1	5
trans-1,4-Dichloro-2-butene	110-57-6	53/75,89	53/75,89	20	5	20
Dichlorofluoromethane	75-43-4	67/69	67/69	5	1	5
Dichlorodifluoromethane	75-71-8	85/87	85/87	5	1	5
1,1-Dichloroethane	75-34-3	63/65,83	63/65,83	5	1	5
1,2-Dichloroethane	107-06-2	62/98	62/98	5	1	5
1,1-Dichloroethene	75-35-4	96/61,63	96/61,63	5	1	5
cis-1,2-Dichloroethene	156-59-2	96/61,98	96/61,98	5	1	5
trans-1,2-Dichloroethene	156-60-5	96/61,98	96/61,98	5	1	5
1,2-Dichloropropane	78-87-5	63/112	63/112	5	1	5
1,3-Dichloropropane	142-28-9	76/78	76/78	5	1	5
2,2-Dichloropropane	594-20-7	77/97	77/97	5	1	5
1,1-Dichloropropene	563-58-6	75/39	75/39	5	1	5
cis-1,3-Dichloropropene	10061-01-5	75/77	75/77	5	1	5
trans-1,3-Dichloropropene	10061-02-6	75/77	75/77	5	1	5
Diisopropyl ether	67-63-0	45/43,87	45/43,87	5	1	5
Ethyl acetate	141-78-6	43/73	43/73	10	2	5
Ethyl ether	60-29-7	59/45,74	59/45,74	5	1	5
Ethyl methacrylate	97-63-2	69/41	69/41	5	1	5
Ethylbenzene	100-41-4	106/91	106/91	5	1	5
Hexachlorobutadiene	87-68-3	225/227,223	225/227,223	5	1	5
2-Hexanone	591-78-6	43/100	43/100	10	5	10
Iodomethane	74-88-4	142/127	142/127	5	1	5
Isobutyl alcohol	78-83-1	43/42	43/42	200	50	100
Isopropylbenzene	98-82-8	105/120	120/105	5	1	5
p-Isopropyltoluene	99-87-6	119/134,91	91/134	5	1	5
Methacrylonitrile	126-98-7	67/41	67,41	100	20	100
4-Methyl-2-pentanone (MIBK)	108-10-1	43/100	43/100	10	5	10
Methylene chloride	75-09-2	84/49,86	84/49,86	5	1	5
Methyl methacrylate	80-62-6	69/41,39	69/41,39	5	1	5
Methyl tertiary butyl ether	1634-04-4	73/57	73/57	5	1	5
Naphthalene	91-20-3	128/102	128/102	5	1	5
2-Nitropropane	79-46-9	43/42	41/42	10	2	20
Pentachloroethane	76-01-7	167/130,165	167/130,165	10	1	5

Table 1. Method Analytes with Characteristic Masses (M/Z) and Reporting Limits

Analyte	CAS#	Primary/ Secondary Characteristic Ions for the HP5970 MSD analyses	Primary/ Secondary Characteristic Ions for the HP5973 MSD analyses	Aqueous Reporting Limits (ug/L)		Solid Reporting Limits (ug/Kg)
				5 mL purge	25 mL purge	
Propionitrile	107-12-0	54/55	54/55	100	20	100
n-Propylbenzene	103-65-1	91/120	120/91	5	1	5
Styrene	100-42-5	104/78	103/78	5	1	5
1,1,1,2-Tetrachloroethane	630-20-6	131/133,119	131/133,119	5	1	5
1,1,2,2-Tetrachloroethane	79-34-5	83/85	83/85	5	1	5
Tetrachloroethene	127-18-4	166/168,129	166/168,129	5	1	5
Tetrahydrofuran	109-99-9	42/41,72	42/41,72	30	10	20
Toluene	108-88-3	92/91	65/91	5	1	5
1,2,3-Trichlorobenzene	87-61-6	180/182,145	180/182,145	5	1	5
1,2,4-Trichlorobenzene	120-82-1	180/182,145	180/182,145	5	1	5
1,1,1-Trichloroethane	71-55-6	97/99,61	97/99,61	5	1	5
1,1,2-Trichloroethane	79-00-5	97/99	83/99	5	1	5
Trichloroethene	79-01-6	130/132,95	130/132,95	5	1	5
Trichlorofluoromethane	75-69-4	101/103	101/103	5	1	5
1,1,2-Trichloro-1,2,2-trifluoroethane	76-13-1	101/151,85	101/151,85	5	1	5
1,2,3-Trichloropropane	96-18-4	110/75	110/75	5	1	5
1,2,3-Trimethylbenzene	526-73-8	105/120	120/105	5	1	5
1,2,4-Trimethylbenzene	95-63-6	105/120	120/105	5	1	5
1,3,5-Trimethylbenzene	108-67-8	105/120	119/120	5	1	5
Vinyl acetate	108-05-4	43/86	86/43	10	5	10
Vinyl chloride	75-01-4	62/64	62/64	5	1	5
o-Xylene	95-47-6	106/91	92/91	5	1	5
m&p-Xylenes	108-38-3 /106-42-3	106/91	92/91	5	1	5
INTERNAL STANDARDS/SURROGATES:						
Bromofluorobenzene	460-00-4	174/95	174/95	NA	NA	NA
Dibromofluoromethane	1868-53-7	113/111	113/111	NA	NA	NA
Toluene-d8	2037-26-5	98/100	99/98	NA	NA	NA
Pentafluorobenzene	363-72-4	168/99	99/168	NA	NA	NA
1,4-Difluorobenzene	540-36-3	114/63	88/114	NA	NA	NA
Chlorobenzene-d5	3114-55-4	54/117	54/117	NA	NA	NA
1,4-Dichlorobenzene-d4	2199-69-1	152/150	152/150	NA	NA	NA
1,2-Dichloroethane-d4	17060-07-0	65/102	67/102	NA	NA	NA

2.0 *SUMMARY OF METHOD*

2.1 Volatile compounds are introduced into the gas chromatograph by the purge-and-trap method. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and back flushed with helium to desorb trapped sample components. The analytes are desorbed directly to a large bore capillary for analysis. The column is temperature programmed to separate the analytes which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph.

2.2 Qualitative identifications are confirmed by analyzing standards under the same conditions used for samples and comparing resultant mass spectra and GC retention times. Each identified component is quantitated by relating the MS response for an appropriate selected ion produced

by that compound to the MS response for another ion produced by an internal standard.

3.0 DEFINITIONS

3.1 **Organic-free reagent water** refers to water in which no target analyte is observed at the reporting limit of the compounds of interest. STL Baltimore uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water.

3.2 **Initial Calibration Verification (ICV)** is a second source calibration standard used to verify the initial calibration and evaluate method performance. It should contain all the analytes of interest. The stock used to prepare the ICV must be from a source that is different from the stocks used to prepare the calibration standards.

3.3 **Continuing Calibration Verification (CCV)** is a mid-level calibration standard used to verify the initial calibration throughout the analytical sequence.

3.4 **Method Blank** is a reagent water or standard solid matrix spiked with all surrogates of interest and taken through the entire analytical procedure.

3.5 **Surrogate** is a non-target compound spiked into all samples and QC samples and taken through the entire analytical procedure to determine purging efficiency, and any possible matrix bias.

3.6 **Laboratory Control Sample (LCS)** is an aliquot of reagent water or a standard solid matrix, e.g. Na_2SO_4 or sand, spiked with a representative subset of the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes are compared to laboratory or project specified control limits for precision and accuracy.

3.7 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample duplicates spiked with a representative subset of the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix. Recoveries of target analytes are compared to LCS control limits.

3.8 **Reference** the terminology used to identify the native sample used for matrix spiking purposes.

4.0 SAFETY AND ALCHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.3 All wastes must be disposed of following the procedure outlined in STL-SOP-018.

5.0 SAMPLE HANDLING AND PRESERVATION

5.1 Samples are stored in the laboratory at 4°C ± 2°C .

5.2 Prior to analysis the analyst should check all water samples for air bubbles. If air bubbles are present, the analyst should notify the laboratory Project Manager (LPM) immediately. The sample analysis may proceed if holding times will be affected.

5.3 Three vials are sent to the laboratory for 5 mL purge analysis; four vials are needed for 25 mL purge. The pH is determined for all samples received at the time of analysis. Notify the LPM if the pH > 2. Three Encore samplers should be sent for each sample where appropriate.

Table 2. Recommended Sample Containers, Preservation Techniques, and Holding Times			
Parameter	**Container**	**Preservative**	**Holding Time**
Concentrated Waste Samples	8-oz. wide mouth glass with Teflon liner	None	14 days
Liquid Samples: No Residual Chlorine Present	2 40-mL vials with Teflon lined septum caps	4 drops conc. HCl, Cool to 4°± 2°C	14 days
Liquid Samples: Residual Chlorine Present	2 40-mL vials with Teflon lined septum caps	Collect sample in a 4 oz. soil VOA container which has been pre-preserved with 4 drops of 10% sodium thiosulfate. Gently mix sample and transfer to a 40-mL VOA vial that has been pre-preserved with 4 drops conc. HCl, Cool to 4°C ± 2°C	14 days
Soil/Sediments and Sludges (STL-M-5030A)	4 oz (120-mL) wide mouth glass with Teflon liner	Cool to 4°± 2°C	14 days
Soil/Sediment and Sludges (STL-M-5035)	3-Encore Samplers	0.2 grams of sodium bisulfate for each 1.0 gram of sample, Cool to 4°C or freeze samples. *	48 hrs from time of collection to preservation, then analyze within 14 days

* Recent studies have shown that acetone may be produced through biological degradation in the presence of the sodium bisulfate preservative. These same studies indicate that freezing the samples is comparable to acidification as an appropriate preservation technique. The freezing preservation option is recommended when acetone is an analyte of concern.

6.0 *INTERFERENCES*

6.1 Major contaminant sources are volatile materials in the laboratory and impurities in the inert purging gas and in the sorbent trap. The use of non-polytetrafluoroethylene (PTFE) thread sealants, plastic tubing, or flow controllers with rubber components should be avoided since such materials out-gas organic compounds which will be concentrated in the trap during the purge operation. Analyses of calibration and reagent blanks provide information about the presence of contaminants. When potential interfering peaks are noted in blanks, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter. Subtracting blank values from sample results is not permitted. If reporting values not corrected for blanks result in what the laboratory feels to be a false positive for that sample, an detailed explanation with accompany the uncorrected data.

6.2 Interfering contamination may occur when a sample containing low concentrations of volatile organic compounds is analyzed immediately after a sample containing high concentrations of volatile organic compounds. After each sample batch is complete, the auto-sampler is purged to remove residual water. To assure that any contamination is eliminated the auto-sampler position that contained samples that may have been saturated is purged with 1:1 methanol/water and the instrument is programed to bake for 9 minutes and 30 seconds. Prior to and after analyses all glassware is rinsed and baked in an oven at 250°C.

6.3 Special precautions must be taken to analyze for methylene chloride. The analytical and sample storage area should be isolated from all atmospheric sources of methylene chloride. Otherwise

random background levels will result. Since methylene chloride will permeate through PTFE tubing, all gas chromatography carrier gas lines and purge gas plumbing should be constructed from stainless steel or copper tubing. Laboratory clothing worn by the analyst should be clean since clothing previously exposed to methylene chloride fumes during liquid/liquid extraction procedures can contribute to sample contamination.

6.4 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal into the sample during shipment and storage. A trip blank prepared from organic-free reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

7.0 *APPARATUS/INSTRUMENTATION*

7.1 Microsyringes - 5, 10, 25, 100, 250, 500, and 1,000 uL.

7.2 Syringes - 5, 10, 25, and 50 mL gas-tight.

7.3 Balance - Analytical , 0.0001 g, and top-loading, 0.01 g.

7.4 Glass scintillation vials, 1 and 2-mL, with Teflon lined screw-caps

7.5 Disposable Pasteur pipets.

7.6 Volumetric flasks, Class A - 10 mL, 40 mL, 100 mL, 200 mL, 250 mL, and 1000 mL with ground-glass stoppers. .

7.7 Spatula - Stainless steel.

7.8 Purge-and-Trap device - The purge-and-trap device consists of two separate pieces of equipment: the sample purger, and the concentrator. Both pieces are commercially available in automated units. Either a Varian Archon Autosampler or a Tekmar ALS2016 autosampler in conjunction with a Tekmar LSC2000 concentrator are used at STL Baltimore.

7.8.1 Sample Purger: The purging chamber is designed to accept both 5 mL and 25 mL samples.

7.8.2 The traps are Supelco K (VOCARB 3000) with Carbopack B/Carboxen 1000 & 1001.

7.8.3 The desorber should be capable of rapidly heating the trap to 250°C for desorption. The trap bake-out temperature should not exceed 260°C

7.9 Heater - The heater used is capable of maintaining the purging chamber to within 1°C over the temperature range of ambient to 100°C.

7.10 Gas chromatograph

7.10.1 The Hewlett Packard Gas Chromatographs are temperature programmable and suitable for split/splitless injections. They are equipped with all required accessories, including syringes, analytical columns, and gases.

7.10.2 Gas chromatographic columns: Rtx 502.2, 105 m x 0.53 mm ID, 3.0 µm film thickness (or equivalent) or DB-624, 75 m x 0.53 mm ID, 3.0 µm film thickness (or equivalent).

7.11 Mass spectrometer - Capable of scanning from 35 to 300 amu every 2 seconds or less, using 70 volts (nominal) electron energy in the electron impact ionization mode.

7.12 GC/MS interface - The GC is interfaced to the MS with an all glass enrichment device and an all glass transfer line that gives acceptable calibration points at 50 ng or less per injection for each of the analytes and achieves all acceptable performance criteria (see Table 7) .

7.13 Data system - HP Enviroquant data system allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that allows searching any GC/MS data file for ions of a specified mass and plotting

such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library is used.

8.0 *STANDARDS AND REAGENTS*

8.1 Reagents

8.1.1 The Reagent log book will be filled out completely following STL-SOP-299.

8.1.2 Organic Free Water, laboratory reagent water purged with helium.

8.1.3 Methanol, CH_3OH - Purge and Trap Grade or equivalent, demonstrated to be free of analytes. Store apart from other solvents.

8.2 Standards

8.2.1 Stock Calibration Standards

8.2.1.1 Stock standard solutions for all method analytes are purchased from either Supelco or Accustandard as certified solution kits at a concentration of 2000 ppm.

8.2.1.2 Gas standards should be replaced after 1 week.

8.2.1.3 Other standards should be replaced after 6 months.

8.2.1.4 All standards should be stored at -10°C to -20°C in amber bottles with Teflon lined-screw caps.

8.2.2 Intermediate Calibration Standards

8.2.2.1 Intermediate calibration standards can be prepared by diluting the stock standard as appropriate (See Table 3).

8.2.2.2 Intermediate standards are stored with no headspace at -10°C to -20°C, and should be replaced after 1 week.

Table 3. Intermediate Standard Preparation

	Stock Conc.	Initial Wt./Vol.	Final Volume	Final Conc.	Solvent
8260#1	2000ppm	250 uL	10 mL	50 ppm	Methanol
8260#2	2000ppm	250 uL	10 mL	50 ppm	Methanol
8260#3	2000ppm	250 uL	10 mL	50 ppm	Methanol
8260#4	10000ppm	500 uL	10 mL	500 ppm	Methanol
ADD	2000ppm	250 uL	10 mL	50 ppm	Methanol
GASES	2000ppm	250 uL	10 mL	50 ppm	Methanol
8260IS	200ppm	1 mL	4 mL	50 ppm	Methanol
8260SS	200ppm	1 mL	4 mL	50 ppm	Methanol
KETONES	2000ppm	250 uL	10 mL	50 ppm	Methanol
8260MS	200ppm	1 mL	4 mL	50 ppm	Methanol

8.2.3 Working Calibration Standards

8.2.3.1 Calibration standards are prepared at a minimum of five concentrations (preferably six concentrations) as directed in Table 4.

8.2.3.2 To prepare a calibration standard, add an appropriate volume of a secondary standard solution to an aliquot of DI water in gas tight syringe. Use a microsyringe and rapidly inject the standard into the tip of the syringe.

8.2.3.3 Mix by inverting the syringe three times.

Table 4. Working Calibration Standard Preparation

50 ppm Stock Standard (uL)	50 ppm Surrogate Standard (uL)	50 ppm Internal Standard (uL)	Final Volume (mL)	Standard Concentration (ng/mL)
For 5 mL Purge Volumes				
0.4	0.4	5	5	4
1	1	5	5	10
2	2	5	5	20
5	5	5	5	50
10	10	5	5	100
20	20	5	5	200
For 25 mL Purge Volumes				
0.4	0.4	5	25	.8
1	1	5	25	2
2	2	5	25	4
5	5	5	25	10
10	10	5	25	20
20	20	5	25	40

8.2.4 Surrogate standards

8.2.4.1 The recommended surrogates are toluene-d8, bromofluorobenzene, dibromofluoromethane, and 1,2-dichloroethane-d4.

8.2.4.2 A stock surrogate solution in methanol is purchased as a vendor certified solution at a concentration of 200 ppm.

8.2.4.3 An intermediate surrogate standard spiking solution is prepared from the stock at a concentration of 50 ppm in methanol.

8.2.4.4 Each calibration standard, blank, LCS and sample undergoing GC/MS analysis should be spiked with 50 ppb of the surrogate spiking solution prior to analysis.

8.2.5 Internal standards

8.2.5.1 The recommended internal standards are chlorobenzene-d5, 1,4-difluorobenzene, 1,4-dichlorobenzene-d4, and pentafluorobenzene.

8.2.5.2 A stock surrogate solution in methanol is purchased as a vendor certified solution at a concentration of 200 ppm.

8.2.5.3 An intermediate internal standard spiking solution is prepared from the stock at a concentration of 50 ppm in methanol.

8.2.5.4 Each calibration standard, blank, LCS and sample undergoing GC/MS analysis should be spiked with 50 ppb of the internal standard spiking solution prior to analysis.

8.2.6 Initial Calibration Verification (ICV)

A second source calibration standard is purchased as a vendor certified solution and is analyzed after each new initial calibration and prior to sample analysis. This is typically a 50 ppb standard made from a standard other the 8260MIX.

8.2.7 Tuning Standard

8.2.7.1 Bromofluorobenzene (BFB) neat solution is purchased from the vendor.

8.2.7.2 BFB Intermediate Solution: Mix 0.1 g neat BFB to 10 mL methanol.

8.2.7.3 A working standard solution containing 50 ng/uL of BFB in methanol is prepared by diluting 125 μL of the intermediate solution into 25 mL of methanol.

8.2.8 LCS and Matrix Spiking Standards

8.2.8.1 Prepared from separate solutions as the calibration standards.

8.2.8.2 Matrix spiking standards should be prepared from VOC mixes which are representative of the compounds being investigated. At a minimum, the matrix spike should include:

1,1-dichloroethene
trichloroethene
chlorobenzene
toluene
benzene

The analyst should review the Project Summary for information on spiking compounds. It is desirable to perform a matrix spike using compounds found in samples. Some projects may require spiking specific compounds of interest, especially if they are polar and would not be represented by the above listed compounds. The standard should be prepared in methanol, with each compound present at a concentration of 50 ppb.

8.2.9 Great care must be taken to maintain the integrity of all standard solutions. It is recommended all standards in methanol be stored at -10°C to -20°C in amber bottles with Teflon lined screw-caps.

9.0 PROCEDURE

9.1 Instrument Set Up

9.1.1 Instrument Tuning

9.1.1.1 For each 12 hour shift and prior to any analysis a GC/MS tuning standard must be analyzed.

9.1.1.2 Each GC/MS system must be hardware-tuned to meet the criteria in Table 5 for a 50 ng injection of bromofluorobenzene (1 uL injection of the BFB standard). Analyses cannot begin until these criteria are met.

Table 5. BFB Mass - Intensity Specifications (Bromofluorobenzene) (a)

Mass	Intensity Required (relative abundance)
50	8 to 40% of mass 95
75	30 to 66% of mass 95
95	base peak, 100% relative abundance
96	5 to 9% of mass 95
173	less than 2% of mass 174
174	50 to 120% of mass 95
175	4 to 9% of mass 174
176	93 to 101% of mass 174
177	5 to 9% of mass 176

(a) USEPA CLP Statement of Work, OLMO3.2

9.1.1.3 The BFB should be evaluated using three scans, the peak apex scan and the scans immediately preceding and following the apex. These scans are acquired and averaged. A scan no more than 20 scans prior to the elution of BFB should be chosen for background subtraction. Background subtraction is required.

9.1.1.4 All subsequent standard, sample, MS/MSDs and blanks associated with a BFB analysis must use the identical mass spectrometer instrument conditions.

9.1.2 Purge and Trap

9.1.2.1 Assemble a purge-and-trap device according to manufacturers instructions. New traps should be conditioned at 260°C in the purge mode with an inert gas flow of at least 20 mL/min for 30 minutes.

9.1.2.2 Sample desorption - After the purge, adjust the purge and trap system to the desorb mode and initiate the temperature program sequence of the gas chromatograph and start data acquisition. Introduce the trapped materials onto the GC column by rapidly heating the trap and back flushing with an inert gas. If the non-cryogenic cooling technique is followed, the trap must be preheated just prior to desorption.

9.1.2.3 Hold the column temperature at 10°C for 5 minutes, then program at 6°C/min to 160°C and hold until all analytes elute. After desorbing the sample for 4 minutes, bake trap for 9.5 minutes at 260°C using "bake gas by-pass" mode for 120 seconds.

Table 6. Purge and Trap Operating Parameters

Instrument	ALS2016/LSC2000/5970B	Archon/LSC2000/5970B	Archon/LSC2000/5973
Purge gas	Helium	Helium	Helium
Purge gas flow rate (mL/min)	40	40	40

Table 6. Purge and Trap Operating Parameters

Instrument	ALS2016/LSC2000/5970B	Archon/LSC2000/5970B	Archon/LSC2000/5973
Purge time (min)	11	11	7
Purge temperature (°C) for waters	Ambient	Ambient	Ambient
Purge temperature (°C) for soils	40	40	40
Desorb preheat (°C)	245	245	245
Desorb temperature (°C)	250	250	250
Backflush inert gas flow (mL/min)	20 -60	20 - 60	20 - 60
Desorb time (min)	4	4	2
Bake time (min)	9	6	3
Bake temperature (°C)	260	260	260

9.2 Initial Calibration

9.2.1 Prepare each calibration standard (See Table 4) in a gas tight syringe.

9.2.2 (For 5.0 mL standards purged using a TEKMAR ALS2016): Fill the 5.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 5.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 5.0 mL syringe. Slowly add the standard/water solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

9.2.3 (For 25.0 mL standards purged using a TEKMAR ALS2016): Fill the 25.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 25.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 25.0 mL syringe. Slowly add the standard/water solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

9.2.4 (For 5.0 mL standards purged using a VARIAN ARCHON): Fill the 10.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 10.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 10.0 mL syringe. Slowly add the standard/water solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

9.2.5 (For 25.0 mL standards purged using a VARIAN ARCHON): Fill the 50.0 mL syringe barrel with purged DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 50.0 mL. Standard additions using a micro syringe should be added directly into the tip of the 50.0 mL syringe. Slowly add the standard/water solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

9.2.6 Carry out the purge-and-trap analysis procedure for each standard.

9.2.6.1 The purge-and-trap of aqueous samples is performed at ambient temperature, while purging of soil/solid samples is performed at 40°C, to improve purging efficiency.

9.2.6.2 Aqueous and soil/solid samples may also be purged at temperatures above those being recommended as long as all calibration standards, samples, and QC samples are purges at the same temperature, appropriate trapping material is used to handle the excess water, and the laboratory demonstrates acceptable method performance for the project. Purging of aqueous samples at elevated temperatures (e.g., 40°C) may improve the purging performance of many of the water soluble compounds which have poor purging efficiencies at ambient temperatures.

9.2.7 Tabulate the area response of the characteristic ions (see Table 1) against concentration for each compound and each internal standard. Calculate relative response factors (RRFs) for each compound relative to one of the internal standards. The internal standard selected for the calculation of the RRF for a compound should be the internal standard that has a retention time closest to the compound being measured. Calculate relative response factors (RRFs) for each compound as follows:

$$RRF = \frac{A_x C_{IS}}{A_{IS} C_x}$$

where:

RRF = Relative response factor
A_x = Area of the characteristic ion for the compound being measured
A_{IS} = Area of the characteristic ion for the specific internal standard
C_x = Concentration of the compound being measured (ng on column)
C_{IS} = Concentration of the specific internal standard (ng on column)

9.2.8 The percent relative standard deviation (%RSD) is calculated for each compound:

$$\%RSD = \frac{SD}{RRF_A} \times 100$$

where:

%RSD = Percent Relative Standard Deviation
SD = Standard Deviation
RRF_A = Average Relative Response Factor

9.2.8.1 If the percent relative standard deviation (%RSD) of the response factor for any target analyte is ≤ 15% over the working range, linearity through the origin can be assumed, and the average response factor can be used in place of a calibration curve.

9.2.8.2 If the average %RSD of all spiked analytes in the initial calibration is >15%, the laboratory has the following options:

9.2.8.2.1 Review the results (area counts, response factors) for those analytes which failed the %RSD acceptance criteria to determine if the problem is with just one of the standards. Should this be the case, the analyst has the option to reanalyze and replace the standard in question.

9.2.8.2.2 The calibration range may be narrowed by replacing one or more of the standards with standards

of different concentrations.

9.2.8.2.3 If the high standard is dropped, more dilutions may be required.

9.2.8.2.4 If the low standard is dropped, the analyst must verify that changing the low standard concentration will not effect any client DQO's (or that the new quantitation level is at least as low as any required regulatory limits or action levels).

9.2.8.3 For those analytes with RSD > 15%, the laboratory may use a linear regression analysis to establish the curve and use for quantitation. Make certain that the instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). The regression will produce the slope and intercept terms for a linear equation in the form:

$$y = mx + b$$

where:

y	=	Instrument response (ratio of standard response to IS response) [Ax/Ais]
m	=	Slope of the line (also called the coefficient of x)(ratio of standard concentration to IS concentration) [Cis/Cx]
x	=	Concentration of the calibration standard
b	=	intercept

9.2.8.3.1 <u>The line must not be forced through the origin</u>. (Do not include the origin [0,0] as the sixth point!) The regression calculation will generate a correlation coefficient (r) that is a measure of the "goodness of fit" of the regression line to the data. A value of 1.00 indicates a perfect fit. The correlation coefficient must be $r \geq 0.990$ ($r^2 \geq 0.980$).

9.2.8.3.2 The calculated intercept value must also be evaluated before reporting sample results. A positive value for the intercept indicates that there is some threshold instrument response which is the limiting factor in establishing linearity. A negative intercept value can be transformed into an x-intercept value that represents a threshold concentration which is the limitation. If the intercept is positive, then, as a general rule, results where the instrument response is less than three times (3x) the intercept value may be unreliable. This will afford some protection against false positive results. If the intercept is negative, results below the concentration of the lowest concentration calibration standard may be unreliable. These adjustments to the quantitation limits will apply to all samples analyzed using the regression line. In calculating sample concentrations, the regression equation is rearranged to solve for the concentration (x), as shown below.

$$x = \frac{y-b}{m}$$

9.2.8.3.3 An acceptable approach is to raise the reporting limit to above the y-intercept (or at least meet the y-intercept) if it would not change required client reporting limits. ***The analyst must first check the Project Summary to determine if raising the RL exceeds action levels. This must be approved by the Section Chief, Quality Service Manager, and the Laboratory Project Manager prior to reporting of the data package.***

9.3 **Relative Retention Time** The relative retention times (RRT) of each compound in each

calibration run should agree to ±0.06 relative retention time units.

9.3.1 Target Analytes - The relative retention times of the target analyte in each calibration standard should agree within ±0.06 relative retention time units.

9.3.2 Internal standard - The retention times of the internal standards in the calibration verification standard must be evaluated immediately after or during data acquisition. If the retention time for any internal standard changes by more than 30 seconds from the mid-level standard of the most recent initial calibration, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. After corrections are made, reanalysis of the affected samples is required.

9.4 System Performance Check Compounds (SPCC)

9.4.1 A system performance check must be made before the calibration curve is used. Five system performance check compounds (SPCCs) are evaluated for a minimum average response factor.

Compound	Minimum RRF
Chloromethane	0.10
1,1,-Dichloroethane	0.10
Bromoform	0.10
Chlorobenzene	0.30
1,1,2,2,-Tetrachloroethane	0.30 (0.1 for 25 mL purge)

9.4.2 These compounds are used to check compound instability and to check for degradation caused by contaminated lines or active sites in the system.

9.4.2.1 Chloromethane is the most likely compound to be lost if the purge flow is too fast.

9.4.2.2 Bromoform is one of the compounds most likely to be purged very poorly if the purge flow is too slow. Cold spots and/or active sites in the transfer lines may adversely affect response. Response of the quantitation ion (m/z 173) is directly affected by the tuning of BFB at ions m/z 174/176. Increasing the m/z 174/176 ration relative to m/z 95 may improve bromoform response.

9.4.2.3 Tetrachloroethane and 1,1-Dichloroethane are degraded by contaminated transfer lines in purge-and-trap systems and/or active sites.

9.4.3 If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the from end of the analytical column, and active sites in the column or chromatographic system.

9.5 Calibration Check Compounds (CCC)

9.5.1 The purpose of the CCCs are to evaluate the calibration from the standpoint of the integrity of the system. High variability for these compounds may be indicative of system leaks or reactive sites on the column. ***Meeting the CCC criteria is not a substitute for successful calibration of the target analytes.***

9.5.2 The RSD for each individual Calibration Check Compound (CCC) should be ≤15%. The CCCs are:

1,1-Dichloroethene	Toluene
Chloroform	Ethylbenzene
1,2-Dichloropropane	Vinyl chloride

9.5.3 If a % RSD of greater than 30% is measured for any CCC, then corrective action to eliminate a system leak and/or column reactive sites is necessary before reattempting calibration. The relative retention times of each compound in each calibration standard should agree within 0.06 relative retention time units.

9.6 Initial Calibration Verification

9.6.1 The initial calibration curve must be verified after the calibration is performed with a mid-level Initial Calibration Verification (ICV) standard.

9.6.2 The percent difference from the initial calibration must be within ± 20% for CCC compounds. If the ICV standard fails the acceptance criteria the standard should be reanalyzed immediately. If the standard fails again, the analysis is stopped and a new initial calibration is performed.

$$\%\ Difference = \frac{\overline{RRF_A} - RF}{\overline{RRF}} \times 100$$

where:

RF = Calibration factor from the analysis of the verification standard

RRF_A = Mean calibration factor from the initial calibration

Note: If linear regression is used as the quantitation technique, then concentrations are used in place of calibration factors.

9.6.3 System Performance Check Compounds (SPCCs) - Each ICV must be evaluated to ensure that the SPCC criteria are met (see Section 9.4.1) If the minimum relative response factors are not met, then the system must be evaluated, and corrective action must be taken before sample analysis can begin.

9.6.4 The internal standard responses and retention times in the ICV must be evaluated immediately after or during data acquisition.

9.6.4.1 If the retention time for any internal standard changes by more than 30 seconds from the mid-level standard of the initial calibration, the chromatographic system must be inspected for malfunctions and corrections must be made, as required.

9.6.4.2 If the EICP area for any of the internal standards changes by a factor of two (-50% to +100%) from the mid-level standard of the initial calibration, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. After corrections are made, reanalysis of the affected samples is required.

9.7 Continuing Calibration Verification (CCV)

9.7.1 The calibration curve for each compound of interest must be verified daily or once every 12 hours of analysis time. This is accomplished by analyzing a mid-level calibration standard and evaluating it against the following SPCC and CCC criteria.

9.7.2 System Performance Check Compounds (SPCCs) - Each CCV must be evaluated to ensure that the SPCC criteria are met (see Section 9.4.1) If the minimum relative response factors are not met, then the system must be evaluated, and corrective action must be taken before sample analysis can begin.

9.7.3 Calibration Check Compounds (CCCs) - Each CCV must be evaluated to ensure that the CCC criteria are met. If the percent deviation for each CCC is ≤ 20%, the initial calibration is assumed to be valid. If the criterion is not met (>20%D), for any one CCC, remake and reanalyze the CCV. If the CCV still fails to meet acceptance criteria, the instrument must be recalibrated prior to sample analysis.

9.7.4 The internal standard responses and retention times in the CCV must be evaluated immediately after or during data acquisition.

9.7.4.1 If the retention time for any internal standard changes by more than 30 seconds from the last calibration check (12 hours), the chromatographic system must be inspected for malfunctions and corrections must be made, as required.

9.7.4.2 If the EICP area for any of the internal standards changes by a factor of two (-50% to +100%) from the mid-level standard of the initial calibration, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. After corrections are made, reanalysis of the affected samples is required.

9.8 Sample Analysis

9.8.1 Screening of the sample, using a headspace analysis by gas chromatography with a FID, prior to purge-and-trap analysis will provide guidance on whether sample dilutions are necessary and will prevent contamination of the purge-and-trap system.

9.8.2 All samples and standard solutions must be allowed to warm to ambient temperature before analysis.

9.8.3 Analysis of Water Samples

9.8.3.1 (For 5.0 mL samples purged using a TEKMAR ALS2016): Fill the 5.0 mL syringe barrel with the sample to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the volume to 5.0 mL. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 5.0 mL syringe. Slowly add the standard/sample solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

9.8.3.2 (For 25.0 mL samples purged using a TEKMAR ALS2016): Fill the 25.0 mL syringe barrel with the sample to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 25.0 mL. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 25.0 mL syringe. Slowly add the standard/sample solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

9.8.3.3 (For 5.0 mL samples purged using a VARIAN ARCHON): Fill the 10.0 mL syringe barrel with the sample to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 10.0 mL. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and

matrix spikes, should be added directly into the tip of the 10.0 mL syringe. Slowly add the standard/sample solution to the 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

9.8.3.4 (For 25.0 mL samples purged using a VARIAN ARCHON): Fill the 50.0 mL syringe barrel with sample to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting the volume to 50.0 mL. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 50.0 mL syringe. Slowly add the standard/sample solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

9.8.4 Analysis of Sediment/Soil and Waste Samples

9.8.4.1 Use the screening data to determine whether to use the low-concentration method (0.005-1 mg/Kg) or the high-concentration method (> 1 mg/Kg).

9.8.4.2 Low-concentration method - This is designed for samples containing individual purgeable compounds of <1 mg/Kg. The low-concentration method is based on purging a heated sediment/soil sample mixed with DI water containing the surrogate and internal standards. Analyze all blanks and standards under the same conditions as the samples. Use a 5 g sample if the expected concentration is < 0.1 mg/Kg or a 1 g sample for expected concentrations between 0.1 and 1 mg/Kg. A heated purge calibration curve must be prepared and used for the quantitation of all samples analyzed with the low-concentration method. Follow the initial and daily calibration instructions, except for the addition of a 40°C purge temperature.

9.8.4.3 (For soils using a TEKMAR 2016) - Fill the 5.0 mL syringe barrel with the sample to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the volume to 5.0 mL. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 5.0 mL syringe. Mix the contents of the sample container with a narrow metal spatula. Weigh the soil sample into a tared purge device. Slowly add the standard/sample solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube. Heat and purge the sample.

9.8.4.4 (For 5030 soils using a VARIAN ARCHON) - Remove the plunger from a 5.0 mL syringe and fill until overflowing with purged DI water. Replace the plunger and compress the water to vent trapped air. Pull the plunger back to allow volume for the addition of the standard solutions. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 5.0 mL syringe. Mix the contents of the sample container with a narrow metal spatula. Weigh the soil sample into a 40 mL VOA vial. Slowly add the standard/sample solution to the VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage. Heat, stir and purge the sample.

9.8.4.5 (For 5035 soils using a VARIAN ARCHON) - Place the 40 ml VOA vial, which contains the preserved sample, in the appropriate slot on the autosampler. The autosampler should be programmed to add 10.0 ml of DI water as well as 50 ug/Kg of surrogate and internal standard solutions. Heat, stir and purge the sample.

9.8.5 High-concentration method for sediment/soil - This is designed for samples containing individual purgeable compounds of > 1 mg/Kg. The high concentration method is based on extracting the soil with methanol and a portion of the extract being added to DI water containing the surrogates and internal standards. The sample is then purged at ambient temperature, just like a water

sample.

9.8.5.1 Mix the contents of the sample container with a narrow metal spatula. For sediment/soil and solid wastes that are insoluble in methanol weigh 4 g (wet weight) of sample into a tared 20 mL vial. Using a top-loading balance note and record the actual weight to 0.1 g. Quickly add 10.0 mL of methanol to the vial. Cap and shake for 2 minutes.

9.8.5.2 Pipet approximately 1 mL of the extract to a 2-mL screw cap vial for storage, using a disposable pipet. The remainder may be disposed. Transfer approximately 1 mL of appropriate solvent to a separate GC vial for use as the method blank for each set of samples. These extracts may be stored at 4° ± 2°C in the dark, prior to analysis.

9.8.5.3 (For samples purged using a TEKMAR ALS2016): Fill the 5.0 mL syringe barrel with DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the volume to 4.9 mL. Pull the plunger back to the 5.0 mL mark to allow volume for the addition of the standard solutions and sample. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 5.0 mL syringe. Add 100 uL of the sample extract into the tip of the 5.0 mL syringe. Slowly add the standard/sample solution to a purging tube and assemble on the autosampler. Ensure that the purging tube is tight so as to avoid leakage. However, do not over tighten as this may crack the glass purging tube.

9.8.5.4 (For samples purged using a VARIAN ARCHON): Fill the 5.0 mL syringe barrel with DI water to just short of overflowing. Replace the syringe plunger and compress. Vent any residual air while adjusting to the volume to 4.9 mL. Pull the plunger back to the 5.0 mL mark to allow volume for the addition of the standard solutions and sample. Standard solutions, which include surrogates, internal standards and matrix spikes, should be added directly into the tip of the 5.0 mL syringe. Add 100 uL of the sample extract into the tip of the 5.0 mL syringe. Slowly add the standard/sample solution to a 40 mL VOA vial and place in the appropriate slot on the autosampler. Ensure that the vial cap is tight so as to avoid leakage.

NOTE: The associated method blank and LCS should also contain 100 uL of methanol in order to better mimic the analysis of the samples.

9.8.5.5 If a screening procedure was followed, use the estimated concentration to determine the appropriate volume of solvent extract. Otherwise, estimate the concentration range of the sample from the low concentration analysis to determine the appropriate volume of the methanol extract to be added to the DI water. If the sample was submitted as a high-concentration sample, start with 100 uL. Note: if less than 100 uL of the methanol extract is added to the DI water, then an additional amount of methanol must be added so that the total volume of methanol (sample plus additional methanol) added is equal to 100 uL. All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the calibration curve.

10.0 ANALYTE IDENTIFICATION AND QUANTITATIVE CALCULATIONS

10.1 Identification

10.1.1 The qualitative identification of compounds determined by this method is based on retention time, and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest relative intensity, or any ions over 30% relative intensity if less than three such ions occur in the reference spectrum. Compounds are

identified as present when the following criteria are met.

10.1.2 The intensities of the characteristic ions of a compound maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

10.1.3 The relative retention time (RRT) of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

10.1.4 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum.

EXAMPLE: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%

10.1.5 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

10.1.6 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

10.1.7 Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

10.1.8 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The analyst must review the Project Summary or contact the LPM if there are any questions. Data system library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. Use the following guidelines for making tentative identifications:

10.1.8.1 Relative intensities of major ions in the reference spectrum (ions greater than 10% of the most abundant ion) should be present in the sample spectrum.

10.1.8.2 The relative intensities of the major ions should agree within +/- 20%. For example, an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%.

10.1.8.3 Molecular ions present in the reference spectrum should be present in the sample spectrum.

10.1.8.4 Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

10.1.8.5 Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

10.2 Quantitative Determination

10.2.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. Quantitation will take place using the internal standard technique. The internal standard used shall be the one nearest the retention time of the given analyte.

10.2.2 Calculate the concentration of each identified analyte in the sample as follows:

10.2.2.1 Water and Water-Miscible Wastes:

$$Conc_{sample} = \frac{A_{sample} \times C_{is}}{A_{is} \times \overline{RRF}} \times DF$$

where

$Conc_{sample}$	=	Sample concentration in ug/L
A_{sample}	=	Area of characteristic ion for compound being measured in the sample.
C_{is}	=	Concentration of internal standard injected (ng/uL).
A_{is}	=	Area of characteristic ion for the internal standard.
RRF	=	Average relative response factor of compound being measured from initial calibration.
DF	=	Dilution factor

10.2.2.2 Sediment/Soil, Sludge, and Waste

$$Conc_{sample} = \frac{A_{sample} \times C_{is}}{A_{is} \times \overline{RRF}} \times DWF \times DF$$

where:

$Conc_{sample}$	=	Sample concentration in ug/kg
A_{sample}	=	Area of characteristic ion for compound being measured.
C_{is}	=	Concentration of internal standard injected (ug/kg).
A_{is}	=	Area of characteristic ion for the internal standard.
RRF	=	Average relative response factor of compound being measured from initial calibration.
DWF	=	Dry Weight Factor
DF	=	Dilution factor

10.2.2.2.1 Sediment/soil samples are reported on a dry weight basis, while sludges and wastes may be reported on a wet weight basis.

10.2.2.2.2 The dry weight factor (DWF) of the sample is calculated using the following equation.

$$DWF = \frac{100}{100 - \%Moisture}$$

10.2.3 Where applicable, an estimate of concentration for noncalibrated components in the sample should be made. The formulae given above should be used with the following modifications: The areas A_{sample} and A_{is} should be from the total ion chromatograms, and the RRF for the compound should be assumed to be 1. The concentration obtained should be reported indicating (1) that the value is an estimate and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

11.0 QUALITY CONTROL

11.1 Quality Control Acceptance Criteria for this method, including the frequency and corrective actions are shown in Table 7.

11.2 In order to produce acceptable sample results, the response of the analyte must be within the working range established by the initial calibration.

11.3 The extrapolation of the calibration to concentrations above or below those of the actual calibration standards is not permitted.

11.4 The initial calibration is verified using a second source standard (ICV).

11.5 The initial calibration is verified once for every 12 hour analytical sequence using a continuing calibration verification standard (CCV).

11.6 A method blank is analyzed once for every 12 hour analytical sequence to determine whether or not the analysis has introduced any contamination to the samples.

11.7 Results from the method blank ***are not*** subtracted from the samples.

11.8 When method or solvent blanks are not used after the analysis of high level samples, the results for at least two of the following samples are carefully reviewed to determine if there was any contamination. If the analytes are not present in the samples following the highly concentrated sample, that data is usable. Otherwise, the samples are reanalyzed.

11.9 A Laboratory Control Sample is analyzed once for every 12 hour analytical sequence.

11.10 Spiked analyte recoveries are calculated to determine whether the operating method is in compliance.

$$\%\ LCS\ Recovery\ (\%R) = \frac{Found}{True} \times 100$$

11.11 Matrix Spike/Matrix Spike Duplicates are analyzed for every 12 hour analytical sequence.

11.12 Report the results of the both the %R in the MS and MSD samples and the %RPD between the MS and MSD.

11.12.1 Spike Recovery

$$\%\ Spike\ Recovery\ (\%R) = \frac{SSR - SR}{SA} \times 100$$

where:

SSR = Spiked Sample Result
SR = Sample Result
SA = Spike Added

11.12.2 %RPD

$$\% \ RPD = \frac{|MSR - MSRD|}{(MSR + MSRD)/2} \times 100$$

where:
MSR = Matrix spike recovery
MSRD = Matrix spike duplicate recovery

(The vertical bars in the formula above indicate the absolute value of the difference, therefore, RPD is always expressed as a positive value.)

12.0 *POLLUTION PREVENTION AND WASTE MANAGEMENT*

12.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

12.3 Refer to STL-SOP-018 for waste management procedures.

13.0 *REFERENCES*

13.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd Edition, Method 8260B. U.S. EPA, Washington, D.C.

13.2 American Public Health Association (APHA), American Water Works Association, and Water Environmental Federation. 1992. Standard Methods For The Examination of Water and Wastewater (18th edition). APHA, Washington, D.C.

13.3 United States Environmental Protection Agency. 1995. Contract Lab Program , Statement of Work OLM03.2. U.S. EPA, Washington, D.C.

TABLE 7. SUMMARY OF LABORATORY QUALITY CONTROL REQUIREMENTS AND CORRECTIVE ACTION PROCEDURES FOR SW-846 METHOD 8260

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Holding time	See Table 2	Analysis is completed within holding time.	Notify LPM, determine if laboratory is to proceed or if client will resample.
Tuning	Every 12 hours	Within limits of method	Adjust instrument parameters.
Calibration curve	Established initially at a minimum of 5 concentration levels	1. %RSD for all CCCs must be ≤ 30%. 2. Average %RSD for all analytes ≤ 15%. 3. RRF for SPCCs must meet criteria in section 9.4.1. If average %RSD >15%, there are several options: a. Reanalyze any outlying standards, b. Linear regress analytes with %RSD over 15%, however, must meet r≥0.990	1. Recalibrate instrument. 2. Reanalyze samples since last criteria met. 3. Document actions taken.
Initial Calibration Verification	After each initial calibration using second source standard	1. %D for each CCC must be ≤ 20% 2. RRF for SPCCs must meet criteria in section 9.4.1. 3. Must meet retention time and internal standard area criteria in section 9.6.4.	1. Verify ICV preparation and reanalyze the ICV standard once to verify. 2. If still fails, recalibrate the instrument.
Continuing Calibration Verification	Verified each 12 hour analytical shift at mid-level	1. %D for each CCC must be ≤ 20% 2. RRF for SPCCs must meet criteria in section 9.4.1. 3. Must meet retention time and internal standard area criteria in section 9.7.4.	1. If acceptance criteria is not met, reanalyze the CCV.. 2. If still fails, recalibrate the instrument.
Method Blank	1 per analytical batch	1. Analyte concentrations must be less than the reporting limit, except that the common laboratory contaminates methylene chloride and acetone must be less than five times the reporting limit	1. Determine source of contamination, i.e. instrument, blank water, reagents. 2. Take appropriate corrective action and document. 3. Reanalyze or prepare analytical batch. 4. If samples cannot be reanalyzed or reprepared, qualify data. 5. Document actions taken.

TABLE 7. SUMMARY OF LABORATORY QUALITY CONTROL REQUIREMENTS AND CORRECTIVE ACTION PROCEDURES FOR SW-846 METHOD 8260

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
LCS	1 per analytical batch	All recoveries must be within established laboratory control limits.	1. Examine instrument parameters, sensitivity and linearity. Correct problems and document. 2. Review standard and LCS preparation. Correct any problems and document. 3. Evaluate against project specific DQOs and report data if there is not impact on data usability. 4. If data is not usable, reprepare/reanalyze the method blank, LCS and all field samples the batch. 5. If repreparation of samples is not possible, qualify data. 6. Document all actions taken in a Nonconformance Record and in the report narrative.
Internal Standard Responses and Retention Times	Internal standards are added to all calibration standards, LCS, samples and blanks	1. Retention times for any internal standard in the CCV must be within 30 seconds of the mid-level standard of the most recent initial calibration. Retention time for any internal standard in a sample must be within 30 seconds of the continuing calibration verification standard. 2. The area counts for all internal standards in the CCV must be within a factor of two (-50% to +100%) of the initial calibration. The area counts for all internal standards in the sample must greater than -25% but less than +100% of the initial calibration.	1. Inspect the mass spectrometric system for malfunction and correct. 2. Reanalyze affected samples. If the areas meet criteria, report data from the compliant analysis. 3. If reanalysis of the sample does not solve the problem, submit data from both runs, an document all inspection and corrective actions taken in the analytical narrative.
Surrogate spike	All field and QC samples	Surrogate recoveries must be within established laboratory limits.	1. Examine all QC, including calibration and quantitation. 2. If surrogates in LCS and/or MB are out-of-control reprepare/reanalyze the entire batch. 3. If samples cannot be reprepared, qualify data. 5. If surrogate spike in LCS and MB are acceptable but out-of-control for samples, exami preparation of samples. If no errors or problems are discovered for samples preparatio a matrix effect is assumed. 6. If errors are discovered in preparation of samples, reprepare/reanalyze the entire batch 7. Document actions taken in a Nonconformance Record, and in the analytical report.

TABLE 7. SUMMARY OF LABORATORY QUALITY CONTROL REQUIREMENTS AND CORRECTIVE ACTION PROCEDURES FOR SW-846 METHOD 8260

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
MS/MSD	1 set per 20 samples	Recoveries and RPDs are evaluated against laboratory LCS limits for precision and accuracy.	1. Examine all QC, including calibration and quantitation. 2. If spike recoveries in LCS are out-of-control reprepare/reanalyze the entire batch. 3. If samples cannot be reprepared, qualify data. 5. If spike recoveries in LCS are acceptable but out-of-control for samples, examine preparation of samples. If no errors or problems are discovered for samples preparatic a matrix effect is assumed and data is reported. 6. If errors are discovered in preparation of samples, reprepare/reanalyze the *entire* batc 7. Document actions taken in a Nonconformance Record, and in the analytical report.

Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-8270C **Rev. No.:** 2

Semivolatile Organic Compounds By Gas Chromatography/ Mass Spectrometry (GC/MS): Capillary Column Technique

Approved By: G.M. Gregory, Semivolatiles Section Chief — Date: 1/24/00

Approved By: M.M. Uhlfelder, Quality Services Manager — Date: 1/24/00

Approved By: P.A. Conley, Operations Manager — Date: 1-21-2000

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Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-8270C **Rev. No.: 2**

Semivolatile Organic Compounds By Gas Chromatography/ Mass Spectrometry (GC/MS): Capillary Column Technique

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Severn Trent Laboratories Baltimore

Analytical Method

Number: STL-M-8270C **Rev. No.:** 2

Semivolatile Organic Compounds By Gas Chromatography/ Mass Spectrometry (GC/MS): Capillary Column Technique

Revisions		
Rev. No.	**Date**	**Description**
0	September 25, 1998	Initial Distribution
1	January 21, 2000	Edited Table 6 to change Retention Time criteria to agree with SOP text and reference method.

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine the concentration of semivolatile organic compounds in extracts prepared from all types of aqueous and solid matrices.

1.2 This method can be used to quantify most neutral, acidic, and basic organic compounds that are soluble in methylene chloride and capable of being eluted without derivatization as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds, and phenols, including nitrophenols.

1.3 The Reporting Limits (RLs) and characteristic ions for the method analytes are listed in Table 1. Since sample RLs are highly matrix-dependent, the RLs listed herein are provided for guidance and may not always be achievable. RLs will be proportionately higher for sample extracts that require dilution to avoid saturation of the detector. Modifications to the analyte list or procedural changes to reach lower Reporting Limits are allowed if required by client, project or program. Any changes in the analytical procedures must be approved by the Section Chief and the Quality Services Manager before samples can be analyzed.

1.4 The following compounds may require special treatment when being determined by this method:

1.4.1 Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition.

1.4.2 N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine.

1.4.3 Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, benzoic acid, 2-nitroaniline, 3-nitroaniline, 4-chloroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

TABLE 1. REPORTING LIMITS AND CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

Compound	CAS # (a)	Aqueous (ug/L)	Solid (ug/Kg)	Primary Ion	Secondary Ion (s)
Phenol	108-95-2	10	330	94	65,66
Bis(2-chloroethyl)ether	111-44-4	10	330	93	63,95
2-Chlorophenol	95-57-8	10	330	128	64,130
1,3-Dichlorobenzene	541-73-1	10	330	146	148,111
1,4-Dichlorobenzene	106-46-7	10	330	146	148,111
Benzyl alcohol	100-51-6	10	330	108	79,77
1,2-Dichlorobenzene	95-50-1	10	330	146	148,111
2-Methylphenol (o-cresol)	95-48-7	10	330	108	107,79
bis(2-Chloroisopropyl)ether	39638-32-9	10	330	45	77,121
4-Methylphenol (p-cresol)	106-44-5	10	330	108	107,79
N-Nitrous-Di-n-propyl amine	621-64-7	10	330	70	42,101,130
Hexachloroethane	67-72-1	10	330	117	201,199
Nitrobenzene	98-95-3	10	330	77	123,65
Isophorone	78-59-1	10	330	82	95,138
2-Nitrophenol	88-75-5	10	330	139	109,65
2,4-Dimethylphenol	105-67-9	10	330	122	107,121
Benzoic acid	65-85-0	100	3300	122	105,77
Bis(2-chloroethoxy)methane	111-91-1	10	330	93	95,123
2,4-Dichlorophenol	120-83-2	10	330	162	164,98
1,2,4-Trichlorobenzene	120-82-1	10	330	180	182,145

TABLE 1. REPORTING LIMITS AND CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

Compound	CAS # (a)	Aqueous (ug/L)	Solid (ug/Kg)	Primary Ion	Secondary Ion (s)
Naphthalene	91-20-3	10	330	128	129,127
4-Chloroaniline	106-47-8	20	330	127	129
Hexachlorobutadiene	87-68-3	10	330	225	223,227
4-Chloro-3-methylphenol	59-50-7	10	330	107	144,142
2-Methylnaphthalene	91-57-6	10	330	142	141
Hexachlorocyclopentadiene	77-47-4	10	330	237	235,272
2,4,6-Trichlorophenol	88-06-2	10	330	196	198,200
2,4,5-Trichlorophenol	95-95-4	50	1700	196	198,200
2-Chloronaphthalene	91-58-7	10	330	162	127,164
2-Nitroaniline	88-74-4	50	1700	65	92,138
Dimethylphthalate	131-11-3	10	330	163	194,164
Acenaphthylene	208-96-8	10	330	152	151,153
3-Nitroaniline	99-09-2	50	1700	138	108,92
Acenaphthene	83-32-9	10	330	154	153,152
2,4-Dinitrophenol	51-28-5	50	1700	184	63,154
4-Nitrophenol	100-02-7	50	1700	139	109,65
Dibenzofuran	132-64-9	10	330	168	139
2,4-Dinitrotoluene	121-14-2	10	330	165	63,89
2,6-Dinitrotoluene	606-20-2	10	330	165	63,89
Diethylphthalate	84-66-2	10	330	149	177,150
4-Chlorophenyl phenyl ether	7005-72-3	10	330	204	206,141
Fluorene	86-73-7	10	330	166	165,167
4-Nitroaniline	100-01-6	50	1700	138	108,92
4,6-Dinitro-2-methylphenol	534-52-1	50	1700	198	51,105
N-Nitrosodiphenylamine	86-30-6	10	330	169	168,167
Hexachlorobenzene	101-55-3	10	330	284	142,249
Pentachlorophenol	118-74-1	50	1700	266	264,268
Phenanthrene	87-86-5	10	330	178	179,176
Anthracene	85-01-8	10	330	178	176,179
Di-n-butylphthalate	120-12-7	10	330	149	150,104
Fluoranthene	84-74-2	10	330	202	101,203
Pyrene	206-44-0	10	330	202	200,203
Butyl benzyl phthalate	129-00-0	10	330	149	91,206
3,3'-Dichlorobenzidine	85-68-7	20	330	252	254,126
Benzo(a)anthracene	91-94-1	10	330	228	229,226
Bis(2-ethylhexyl)phthalate	53-55-3	10	330	149	167,279
Chrysene	117-81-7	10	330	228	226,229
Di-n-octylphthalate	218-01-9	10	330	149	167,43
Benzo(b)fluoranthene	117-84-0	10	330	252	253,125
Benzo(k)fluoranthene	205-99-2	10	330	252	253,125
Benzo(a)pyrene	207-08-9	10	330	252	253,125
Indeno(1,2,3-cd)pyrene	50-32-8	10	330	276	138,227
Dibenz(a,h)anthracene	193-39-5	10	330	278	139,279
Benzo(g,h,i)perylene	53-70-3	10	330	276	138,277

(a) = Chemical Abstract Services Registry Number

2.0 *SUMMARY OF METHOD*

2.1 Prior to using this method, the samples should be prepared for chromatography using appropriate sample preparation and cleanup methods.

2.2 The semivolatile compounds are introduced into the GC/MS by injecting the sample extract into a gas chromatograph (GC) with a narrow-bore fused silica capillary column. The GC column is

temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph.

2.3 Analytes eluted from the capillary column are introduced into the mass spectrometer by direct connection. Identification of target analytes is accomplished by comparing their mass spectra with the electron impact spectra of authentic standards. Quantification is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard with a five-point calibration curve.

3.0 DEFINITIONS

3.1 **Organic-free reagent water** refers to water in which no target analyte is observed at the Reporting Limit of the compounds of interest. Severn Trent Laboratories-Baltimore uses a Culligan reverse osmosis (R/O) water purification system to generate organic-free deionized water.

3.2 **Initial Calibration Verification (ICV)** is a second source calibration standard used to verify the initial calibration and evaluate method performance. It contains all the analytes listed in Table 1. The stock used to prepare the ICV must be from a source that is different from the stocks used to prepare the initial calibration standards.

3.3 **Continuing Calibration Verification(CCV)** is a mid-level calibration standard, which may be prepared from the same source as the initial calibration standards, used to verify the initial calibration throughout the analytical sequence.

3.4 **Method Blank** is a reagent water or standard solid matrix which is taken through the entire analytical procedure.

3.5 **Laboratory Control Sample (LCS)** is an aliquot of reagent water or standard solid matrix spiked with the analytes of interest and taken through the entire analytical procedure. It is used to monitor the analytical process and recoveries of target analytes and compared to laboratory or project specified control limits for precision and accuracy.

3.6 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)** are two sample aliquots spiked with the analytes of interest and taken through the entire analytical procedure. Results are used to evaluate measurement bias due to the sample matrix.

3.7 **Reference** the terminology used to identify the native sample used for matrix spiking purposes.

4.0 SAFETY AND CHEMICAL HYGIENE

4.1 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, and exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a reference file of material safety data sheets for the chemicals specified in this method. Additional information on general laboratory safety is available from the Laboratory Safety Officer.

4.2 Good laboratory technique dictates the use of appropriate dermal protection. A laboratory coat, eye protection, and gloves are the minimum requirements.

4.3 All wastes must be disposed of following the procedures outlined in STL-SOP-018.

5.0 SAMPLE HANDLING AND PRESERVATION

5.1 Sample container, preservation and holding time requirements are given in Table 2.

5.2 While sample extracts are in the custody of the laboratory, they are stored in the semivolatiles laboratory at 4°C ± 2°C prior to and during analysis.

5.3 After analysis is completed, samples are stored by the Sample Management Office in laboratory

walk-ins until disposal.

Table 2. Recommended Sample Containers, Preservation Techniques, and Holding Times			
Matrix	**Container**	**Preservative**	**Holding Time**
Concentrated Waste Samples	8-oz. wide mouth glass with Teflon liner	None	Extract samples within 14 days; analyze extracts within 40 days of extraction.
Liquid Samples (No Residual Chlorine Present)	Amber glass , Teflon liner	Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.
Liquid Samples (Residual Chlorine Present)	Amber glass , Teflon liner	Add 3 mL of 10% $Na_2S_2O_3$/gal. Cool to 4°C	Extract samples within 7 days; analyze extracts within 40 days of extraction.
Soil/Sediments and Sludges	8 oz wide mouth glass with Teflon liner	Cool to 4°C	Extract samples within 14 days; analyze extracts within 40 days of extraction.

6.0 *INTERFERENCES*

6.1 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, the injection syringe must be rinsed between samples with solvent. Whenever an unusually concentrated sample is encountered, the following sample(s) may need to be reanalyzed to determine if carryover contamination had occurred.

6.2 Analytical interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. All of these materials must be routinely demonstrated to be free of interferences, under the conditions of the analysis, by running laboratory reagent blanks.

6.3 Interferences coextracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, the Section Chief and Laboratory Project Manager should be notified before any further actions are taken.

6.4 Samples containing lipids/fats/greases (*i.e.*, tissue samples), large molecular weight interferences (*i.e.*, cellulose in plant tissues or heavy petroleum distillates), or have a history of poor analytical performance (*i.e.*, low surrogate recoveries or low internal standard responses) should be GPC "cleaned" following STL-M-3640 prior to analysis of those samples.

6.5 Glassware contamination:

6.5.1 Clean all glassware as soon as possible after use by detergent washing with hot water, and rinses with tap water and organic-free reagent water. The glassware is rinsed with acetone, drained and air-dried for several hours.

6.5.2 Glassware contamination may include soap residue on glassware.

7.0 APPARATUS/INSTRUMENTATION

7.1 Gas chromatograph/mass spectrometer system:

7.1.1 Gas chromatograph consisting of a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases. Equipped with Hewlett Packard HP7673 auto injector system.

7.1.1.1 The capillary column should be directly coupled to the source.

7.1.1.2 Mass spectrometer: Capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts

(nominal) electron energy in the electron impact ionization mode.

7.1.1.3 Data system: HPEnviroquant with Enviroforms. The system allows for the continuous acquisition and storage of all mass spectra obtained throughout the duration of the chromatographic program. The data system has the capability to search any GC/MS data file for ions of a specific mass and plot such ion abundances versus time or scan number (Extracted Ion Current Profile (EICP)). The most recent version of the EPA/NISTMass Spectral Library is used.

7.2 Column: 30-m x 0.25-mm I.D. (or 0.32-mm I.D.) 1-um film thickness silicon-coated fused-silica capillary column (J&W Scientific DB-5 or equivalent).

7.3 Volumetric flasks: Class A, various sizes, ground-glass stopper.

7.4 Microsyringe: various sizes

8.0 STANDARDS AND REAGENTS

8.1 Reagents

8.1.1 Methylene chloride, CH_2Cl_2, pesticide grade or equivalent.

8.1.2 Methanol CH_3OH, pesticide grade or equivalent

8.2 Standards

8.2.1 The Standards Log Book must be filled out completely (see STL-SOP-299).

8.2.2 Stock standard solutions are prepared from neat materials or are purchased as certified solutions. These stock materials/solutions must be replaced after the manufactures expiration date or sooner if checks indicate a problem.

8.2.3 All intermediate or working standards must be replaced after 6 months, or sooner if comparison with check standards indicates a problem or the parent standard expiration date is sooner.

8.2.4 Certificates of analysis for all purchased standards will be kept on file.

9.0 CALIBRATIONS

9.1 Calibration Standards: Calibration standards are prepared at a minimum of five concentration levels. One of the concentration levels must be at or below the laboratory Reporting Limit. The remaining concentration levels should correspond to the expected range of concentrations found in samples or should define the working range of the GC.

9.1.1 **Stock Standards:**

9.1.1.1 Stock Calibration Standards are prepared from vendor certified standard solutions sold as multicomponent standard "kits" (Supelco, 2000 ug/mL for each mix except for a 10000 ug/mL acid surrogate mix and a 5000 ug/mL base/neutral surrogate mix).

9.1.1.2 Stock standards are stored at <10°C when not in use.

9.1.2 **Intermediate Standards:** Prepare a 200 ug/mL intermediate standard solution by accurately transferring 1000 uL of each of the 2000 ug/mL multicomponent mixes, 200 uL of the 10000 ug/mL acid surrogate mix, and 400 uL of the 5000 ug/mL base/neutral surrogate mix to a 10 mL volumetric flask. Bring up to volume with methylene chloride.

9.1.3 **Working Standards:** Preparation of the Initial Calibration Standards from the 200 ug/mL Intermediate standard is outlined in Table 3.

TABLE 3. INITIAL CALIBRATION STANDARD PREPARATION

Calibration Standard	200 ppm Secondary Standard (uL)	Methylene Chloride (uL)	Final volume (mL)	Concentrations (ug/mL)
1	25	975	1	5
2	50	450	0.5	20
3	125	375	0.5	50
4	200	300	0.5	80
5	250	250	0.5	100
6	300	200	0.5	120

9.1.4 Initial Calibration Verification (ICV) Standard: Prepared the same as CONC. 3 (50 ug/mL) of the initial calibration standards using a different stock standard source. The ICV is prepared weekly and stored at 4°C.

9.1.5 Continuing Calibration Verification (CCV) Standard: Prepared the same as CONC. 3 (50 ug/mL) of the initial calibration standards. The CCV is prepared weekly and stored at 4°C.

9.1.6 Internal standard solution: The internal standards used for this method are 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (purchased from vendor at 2000 µg/mL). 20 uL of this solution is added to 1 mL of the standard or sample extract (i.e., 20 uL to a 1 mL extract, 10 uL to a 0.5 mL extract). This results in a final concentration of 40 ug/mL for each of the internal standards.

9.1.7 GC/MS Tuning Standard:

9.1.7.1 The stock standard is a purchased solution containing 2000 ug/mL each of decafluorotriphenylphosphine (DFTPP), pentachlorophenol, benzidine and 4,4"-DDT in methylene chloride(Supelco).

9.1.7.2 A 50 ug/mL working standard is prepared from the 2000 ug/mL stock in methylene chloride.

9.1.8 LCS/Matrix Spike Preparation:

9.1.8.1 The LCS/Matrix Spike Stock standards are from a different source than those used for calibration.

9.1.8.2 The LCS/Matrix spike solution is prepared from purchased solutions at a concentration of 100 ug/mL for each method analyte in methanol.

9.1.8.3 1.0 mL of LCS/Matrix spike solution is added to the LCS and specified MS/MSD samples, resulting in concentrations for each method analyte at 100ug/mL in the final extract.

9.1.8.4 Unless specified by project, the control analytes for this method are:

Acid Extractables	Base/Neutral Extractables
Phenol	1,4-Dichlorobenzene
2-Chlorophenol	N-Nitroso-di-n-propylamine
4-Chloro-3-methylphenol	1,2,4-Trichlorobenzene
4-Nitrophenol	Acenaphthene
Pentachlorophenol	2,4-Dinitrophenol
	Pyrene

9.1.9 Surrogate Spike Preparation:

9.1.9.1 Stock surrogate standards are prepared from vendor certified standard solutions (Restek, 7500

µg/mL for the acid surrogates mix and 5000 µg/mL for the base/neutral surrogates mix).

Acid Surrogates	Base/Neutral Surrogates
Phenol-d6	Nitrobenzene-d5
2-Fluorophenol	2-Fluorobiphenyl
2,4,6-Tribromophenol.	Terphenyl-d14.

9.1.9.2 The working Surrogate Spike standard is prepared by adding 2.67 mL of the 7500 ug/mL acid surrogate mix and 4.0 mL of the 5000 ug/mL base/neutral surrogate mix to a 200 mL volumetric flask and brought up to volume with methanol. The final concentration is 100 ug/mL for each surrogate analyte.

9.1.9.3 1.0 mL of working Surrogate Spike standard is added to every sample, resulting in 100 ug/mL of each surrogate in the final extract.

9.2 Initial Calibration

9.2.1 Analyze 1 uL of each calibration standard (containing Internal standards at 40 ng/µL) and tabulate the area of the primary characteristic ion for each analyte and internal standard (Table 1 and Table 6) (Table 5 identifies the correct internal standard to use for each target analyte.)

9.2.2 Calculate relative response factors (RRFs) for each compound as follows:

$$RRF = \frac{A_x C_{is}}{A_{is} C_x}$$

where:

RRF	=	Relative response factor
A_x	=	Area of the characteristic ion for the compound being measured.
A_{is}	=	Area of the characteristic ion for the specific internal standard.
C_x	=	Concentration of the compound being measured (ng on column)
C_{is}	=	Concentration of the specific internal standard (ng on column).

9.2.3 The average RRF is calculated for each compound, and the percent relative standard deviation (%RSD) is calculated for each compound:

$$\%RSD = \frac{SD}{RRF_A} \times 100$$

where:

%RSD	=	Percent Relative Standard Deviation
SD	=	Standard Deviation (n-1)
RRF_A	=	Average Relative response factor

9.2.4 System performance check compounds (SPCC)

9.2.4.1 The following method SPCC compounds must meet a minimum RRF of 0.05 or corrective action must be taken prior to sample analysis: (n-Nitrosi-di-n-propylamine, Hexachlorocyclopentadiene; 2,4-Dinitrophenol; 4-Nitrophenol).

9.2.4.2 These SPCC typically have a very low RRFs (0.1 - 0.2) and tend to decrease in response as the chromatographic system begins to deteriorate or the standard material begins to deteriorate. They are usually the first to show poor performance. They must meet the minimum requirement when the system is calibrated.

9.2.4.3 If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the front end of the column, and active sites in the column or chromatographic system.

9.2.5 Calibration Check Compounds (CCC)

9.2.5.1 The purpose of the CCCs are to evaluate the calibration from the standpoint of the integrity of the system. High variability for these compounds may be indicative of system leaks or reactive sites on the column. Meeting the CCC criteria is not a substitute for successful calibration of the target analytes.

9.2.5.2 The %RSD for each individual Calibration Check Compound (CCC) must be ≤ 30%. The CCCs are:

Base Neutral Fraction	Acid Fraction
Acenaphthene	4-Chloro-3-methylphenol
1,4-Dichlorobenzene	2,4-Dichlorophenol
Hexachlorobutadiene	2-Nitrophenol
n-Nitrosodiphenylamine	Phenol
Di-n-octylphthalate	Pentachlorophenol
Fluoranthene	2,4,6-Trichlorophenol
Benzo(a)pyrene	

9.2.6 Calibration Evaluation

9.2.6.1 The relative retention times of each compound in each calibration standard should agree within 0.06 relative retention time units.

9.2.6.2 If the average percent relative standard deviation (%RSD) of the response factors of all analytes in the standard (target and non-target analytes) is ≤ 15% over the working range, linearity through the origin can be assumed, and the average response factor can be used in place of a calibration curve.

9.2.6.3 If the %RSD criteria for initial calibration is >15%, the laboratory has the following options:

9.2.6.4 Review the results (area counts, response factors) for those analytes which failed the %RSD acceptance criteria to determine if the problem is with just one of the standards. Should this be the case, the analyst has the option to reanalyze and replace the standard in question.

9.2.6.5 The calibration range may be narrowed by replacing one or more of the standards with standards of different concentrations. **(This option can only be applied with the prior approval from the Section Chief and Laboratory Project Manager.)**

9.2.6.5.1 If the high standard is dropped, more dilutions may be required.

9.2.6.5.2 If the low standards are dropped, the analyst must verify that changing the low standard concentration would not effect any client DQOs (that the new quantitation level is at least as low as any required regulatory limits or action levels).

9.2.6.6 The laboratory may use a linear regression analysis to establish the curve and use for

quantitation.

9.2.6.6.1 Make certain that the instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). The regression will produce the slope and intercept terms for a linear equation in the form:

$$y = mx + b$$

where:

y	=	Instrument response (ratio of standard response to IS response) [Ax/Ais]
m	=	Slope of the line (also called the coefficient of x)
x	=	Concentration ratio of the calibration standard concentration to the IS concentration [C_X/C_{IS}]
b	=	intercept

9.2.6.6.2 **<u>The line must not be forced through the origin</u>**. (Do not include the origin [0,0] as a calibration point!).

9.2.6.6.3 The regression calculation will generate a correlation coefficient (r) that is a measure of the "goodness of fit" of the regression line to the data. A value of 1.00 indicates a perfect fit. The correlation coefficient must be $r \geq 0.990$ ($r^2 \geq 0.980$).

9.2.6.6.4 The linear regression plot needs to be evaluated to ensure that the y-intercept does not exceed the method reporting limit. If this situation should occur, a new initial calibration may be required.

9.2.6.6.5 Another approach is to raise the reporting limit to above the y-intercept (or at least meet the y-intercept) if it would not change required client reporting limits. **The analyst must first check the project summary to determine if raising the RL exceeds action levels. This must be approved by the QSM and the LPM prior to reporting of the data package.**

9.3 Calibration Verification

9.3.1 The calibration is verified immediately following the initial calibration by injecting the ICV. The calibration is also verified at the beginning of every 12 hour analytical sequence (when no initial calibration starts the sequence).

9.3.2 The %D for all CCCs must be ≤20% and the RRF for all the SPCCs must be ≥0.05.

$$\%\ Difference = \frac{RRF_A - RF}{RRF_A} \times 100$$

where:

RF	=	Calibration factor from the analysis of the verification standard
RRF_A	=	Mean relative response factor from the initial calibration

Note: concentrations are used when linear regression is the quantitation technique

9.3.3 If the ICV or CCV fails to meet acceptance criteria, the standard should be reanalyzed immediately.

9.3.4 Should the standard fail a second time, the analysis is stopped and a new initial curve is analyzed.

9.3.5 Internal Standards

9.3.5.1 IS Retention Time - The retention times of the internal standards in the calibration verification standard are evaluated immediately after or during data acquisition. If the RT for any internal standard changes by more than 30 seconds from that in the mid-point standard level of the most recent initial calibration sequence, then the chromatographic system is inspected for malfunctions and corrections must are made. Any sample that was analyzed when the instrument was not in compliance are rerun.

9.3.5.2 IS Response - If the EICP area for any of the internal standards in the calibration verification standard changes by a factor of two (-50% to +100%) from that in the mid-point standard level of the most recent initial calibration sequence, the mass spectrometer are inspected for malfunctions and corrections are made. Any sample that was analyzed when the instrument was not in compliance are rerun.

9.3.5.2.1 If the IS response for any sample changes by more than a factor of two (-50% to 100%) from the response of the preceding CCV (or from the mid-point standard if analyzed after an initial calibration), a bias may be indicated for analytes quantitated using that IS. This will be discussed in the report narrative. If the response is <25% of the referenced standard, the extract will be reanalyzed to determine if a matrix effect is indicated. If the reanalysis confirms a matrix effect, both analyses will be reported and the results will be discussed in the report narrative. Reanalysis for low IS response (<25%) may not be performed for complex matrices (i.e., tissue samples) and where the potential for equipment damage may occur due to very high concentrations. Note: All IS responses in Method Blanks and Laboratory Control Samples (LCS) must be within a factor of two from the referenced calibration standard.

10.0 PROCEDURES

10.1 Sample Extraction

10.1.1 Samples must be extracted prior to instrumental analysis. Refer to the appropriate STL Extraction Method SOP as indicated below:

Matrix	Extraction Procedure	STL SOP ID
aqueous	Separatory Funnel	STL-M-3510C
aqueous	Continuous Liquid-Liquid	STL-M-3520C
solid	Soxhlet	STL-M-3540C
solid	Ultrasonic	STL-M-3550B
waste	Waste Dilution	STL-M-3580A

10.2 Instrumental Analysis

10.2.1 Gas chromatograph/mass spectrometer system recommended operating conditions:

Mass range:	35-500 amu
Scan time:	1 sec/scan
Initial column temperature and hold time:	40°C for 4 min
Column temperature program:	40-310°C at 10°C/min
Final column temperature hold:	270°C (until benzo[g,h,i]perylene has eluted)
Injector temperature:	250-300°C
Transfer line temperature:	250-300°C

Source temperature:	According to manufacturer's specifications
Injector:	Grob-type, splitless
Sample volume:	1 uL
Carrier gas:	Hydrogen at 50 cm/sec or helium at 30 cm/sec.

10.2.2 Automated Data Acquisition System: Enviroquant with Enviroforms. The system allows for the continuous acquisition and storage of data obtained throughout the duration of the chromatographic program.

10.3 **Retention Time Windows**: The retention time for each analyte must be within ±30 seconds of the mid-point standard (50 ng/μL) of the initial calibration.

10.4 **Analytical Sequence:**

10.4.1 For each 12 hour shift, prior to initial calibration of the instrument, daily calibration of the instrument, and analysis of samples, a GC/MS tuning standard must be analyzed.

10.4.2 A 50-ng injection of DFTPP must result in a mass spectrum for DFTPP which meets the criteria given in Table 4. This standard must also include Benzidine, Pentachlorophenol, and DDT.

10.4.3 Three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP.

NOTE: All subsequent standard, sample, MS/MSDs and blanks associated with a DFTPP analysis must use the identical mass spectrometer instrument conditions.

TABLE 4. DFTPP KEY IONS AND ION ABUNDANCE CRITERIA(a)

Mass	Ion Abundance Criteria
51	30-80% of mass 198
68	<2% of mass 69
70	<2% of mass 69
127	25 - 75% of mass 198
197	<1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	>0.75% of mass 198
441	Present but less than mass 443
442	40 - 110% of mass 198
443	15 - 24% of mass 442

(a) USEPA CLP Statement of Work. OLMO3.2

10.4.4 The tuning standard is also used to assess GC column performance and injection port inertness.

10.4.4.1 Degradation of DDT to DDE and DDD must not exceed 20%.

$$\%Breakdown\ of\ DDT = \frac{total\ of\ degradtion\ peak\ area\ (DDD + DDE)}{total\ of\ all\ peak\ areas\ DDT + DDE + DDD)} \times 100$$

10.4.4.2 Benzidine and Pentachlorophenol should be present at their normal response, and no peak tailing should be visible. (Refer to Figure 1 for Calculation of Peak Tailing.)

10.4.4.2.1 Benzidine - tailing factor must be <3.

10.4.4.2.2 Pentachlorophenol - tailing factor must be <5.

10.4.4.2.3 Results of evaluation are indicated on the daily run logs.

10.4.4.2.4 If degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6-12 inches of the capillary column. The use of a guard column between the injection port and the analytical column may help prolong analytical column performance.

10.4.5 Sample extracts are warmed to room temperature prior to analysis.

10.4.5.1 Just prior to analysis, add 20 µL of the internal standard solution per 1 mL of the sample extract.

10.4.5.2 After the initial calibration or verification of the initial calibration, a 1 uL aliquot of the sample extracts is injected into the GC/MS system using the exact operating conditions as for the calibration standards.

10.4.5.3 All sample extracts must be injected within 12 hours of the injection time of the preceding GC/MS tune standard.

11.0 QUALITY CONTROL

11.1 **Quality Control Acceptance Criteria** for this method, including the frequency and corrective actions are shown in Table 7.

11.2 Identification/Quantitation:

11.2.1 Qualitative Identification:

11.2.1.1 Qualitative identifications of compounds by this method are based on retention time and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference spectrum are generated by the laboratory using the conditions specified in this SOP. The characteristic ions from the reference mass spectrum are defined as the three ions of greatest relative intensity, or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. Compounds are considered identified when the following criteria are met:

11.2.1.1.1 The intensities of the characteristic ions of a compound must maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

11.2.1.1.2 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the referenced calibration standard.

11.2.1.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Using as an example: For an ions with an abundance of

50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%).

11.2.1.1.4 Structural isomers that produce very similar mass spectra are only identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between the two isomers peaks is less than 25% of the sum of the two peaks heights. Otherwise, structural isomers are identified as isomeric pairs. Diastereomeric pairs that may be separable by the GC should be identified, quantitated and reported as the sum of both compounds by the GC.

11.2.1.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The analyst should review the Project Summary or contract the LPM if there are any questions. Guidelines for tentative identifications are as follows:

11.2.1.2.1 Relative intensities of major ions in the reference spectrum (ions >10% of the most abundant ion) should be present in the sample spectrum.

11.2.1.2.2 The relative intensities of the major ions should agree within ±20%. (For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%).

11.2.1.2.3 Molecular ions present in the reference spectrum should be present in the sample spectrum.

11.2.1.2.4 Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

11.2.1.2.5 Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction form the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

11.2.2 Once a compound has been identified, the quantitation of that compound is based on the integrated abundance of the primary characteristic ion form the EICP.

11.2.3 Where applicable, the concentration of any non-target analytes identified in the sample are estimated. The same formulae are used with the following modifications: the areas Ax and As are from the total ion chromatograms (the total area of the internal standard closes to the non-target analyte is used for As), and the RF for the compound should be assumed to be 1.

11.2.4 If the extract concentration for any sample exceeds the initial calibration range, the extract concentration must be diluted to within calibration range and reanalyzed.

11.2.4.1 Additional internal standard must be added to the diluted extracteto maintain the same concentration as the calibration standards (40 ng/µL).

11.2.4.2 The extrapolation of the calibration to concentrations above or below those of the actual calibration standards must be qualified as estimated values (*i.e.*, "E" for results above calibration range and "J" for results below the laboratory reporting limit).

11.3 **Method Blank:** A method blank is analyzed once per analytical batch of 20 or fewer samples to determine whether or not the analysis has introduced any contamination to the samples.

11.3.1 When samples that are extracted together are analyzed on separate instruments, the method blank associated with those samples must be analyzed at least once on each of those instruments.

11.3.2 When method or solvent blanks are not used after the analysis of high level samples, the results for at least two of the following samples are carefully reviewed to determine if there was any contamination. If the analytes are not present in the samples following the highly concentrated

sample, that data is usable. Otherwise, the samples are reanalyzed.

11.4 **Laboratory Control Sample**: Analyzed once per analytical batch of 20 or fewer samples. Calculate the recovery (%R) using the following equation:

$$\%\ LCS\ Recovery\ (\%R) = \frac{Found}{True} \times 100$$

where:
Found = Calculated concentration of the analyte
True = Concentration of the analyte added

11.5 **Matrix Spike (Matrix Spike Duplicate)**

11.5.1 With each analytical batch of 20 or fewer client samples an MS/MSD pair must be analyzed.
11.5.2 Spike each aliquot and analyze in the same manner as the reference sample.
11.5.3 Report the percent recovery (%R) in the MS and MSD samples and the relative percent difference (RPD) between the MS and MSD results. Note QC outliers in the report narrative.
11.5.4 Spike Recovery Calculation

$$\%\ Spike\ Recovery\ (\%R) = \frac{SSR - SR}{SA} \times 100$$

where:
SSR = Spiked Sample Result
SR = Sample Result
SA = Spike Added

11.5.5 Relative Percent Difference Calculation

$$\%\ RPD = \frac{|MSR - MSRD|}{(MSR + MSRD)/2} \times 100$$

where:
MSR = Matrix spike recovery
MSRD = Matrix spike duplicate recovery

(The vertical bars in the formula above indicate the absolute value of the difference, therefore, RSD is always expressed as a positive value.)

11.6 **Surrogates**: Added to every sample, LCS, MS, and MSD. Calculate the recovery (%R) using the following equation:

$$\%\ Surrogate\ Recovery\ (\%R) = \frac{Found}{True} \times 100$$

where:
Found = Calculated concentration of the surrogate
True = Concentration of the surrogate added

12.0 CALCULATIONS

12.1 Water (Relative Response Factor option)

$$Conc_{sample} = \frac{A_x \times I_s \times V_t}{A_{is} \times RRF_A \times V_o \times V_i}$$

where:
$Conc_{sample}$ = Sample concentration in ug/L
A_x = Area of characteristic ion for compound being measured.
I_s = Amount of internal standard injected (ng).
V_t = Volume of total extract, taking into account dilutions (i.e., a 1-to-10 dilution of a 1-mL extract will mean V(t) = 10,000 uL.
A_{is} = Area of characteristic ion for the internal standard.
RRF_A = Average relative response factor for compound being measured (from ICAL)
V_o = Volume of water extracted (mL).
V_i = Volume of extract injected (uL).

12.2 Water (Linear Regression option)-The extract concentration is determined using the linear equation in section 9.2.6.6.1 as follows:

$$X=(y-b)/m$$

where:
y = Instrument response (ratio of sample response to IS response) [Ax/Ais]
m = Slope of the line (also called the coefficient of x)
X = Concentration ratio of the calibration standard concentration to the IS concentration [C_X/C_{IS}]
b = The y intercept

then,

Cx = X x Cis

where:
Cx = Concentration of the extract (ng)
X = Concentration ratio of the calibration standard concentration to the IS concentration [C_X/C_{IS}]

Cis = Concentration of internal standard injected (ng)

then,

$$Conc_{sample} = \frac{Cx \times V_t \times DF}{V_i \times V_s}$$

where:

$Conc_{sample}$	=	Sample concentration (ug/L)
Cx	=	Concentration of the extract (ng)
V_t	=	Volume of the concentrated extract in milliliters (uL)
V_i	=	Volume of extract injected in microliter (uL).
V_s	=	Volume of sample extracted in milliliters (mL)
DF	=	Dilution Factor. If no dilution is performed, DF = 1.0.

12.3 Solid (Relative Response Factor option)

$$Conc_{sample} = \frac{A_x \times I_s \times V_t}{A_{is} \times RRF_A \times V_i \times W_s \times D}$$

where:

$Conc_{sample}$	=	Sample concentration in ug/kg
A_x	=	Area of characteristic ion for compound being measured.
I_s	=	Amount of internal standard injected (ng).
V_t	=	Volume of total extract, taking into account dilutions (i.e., a 1-to-10 dilution of a 1-mL extract will mean V(t) = 10,000 uL.
A_{is}	=	Area of characteristic ion for the internal standard.
RRF_A	=	Average relative response factor for compound being measured (from ICAL)
V_i	=	Volume of extract injected (uL).
W(s)	=	weight of sample extracted or diluted in grams.
D	=	Percent dry weight (100 - % moisture in sample)/100, or 1 for a wet-weight basis.

12.4 Solid (Linear Regression option)-The extract concentration is determined using the same equation as in section 12.2.

then,

$$Conc_{sample} = \frac{Cx \times V_t \times DF}{V_i \times W_s}$$

where:

$Conc_{sample}$	=	Sample concentration on wet weight basis (ug/kg)
Cx	=	Concentration of the extract (ng).

V_t = Volume of the concentrated extract in milliliters (uL)
V_i = Volume of extract injected in microliter (uL).
W_s = Weight of sample extracted in grams (g)
DF = Dilution Factor. If no dilution is performed, DF = 1.0.

12.5 If solid sample concentrations are to be reported on a dry weight basis, the following equation is used:

$$Conc_{dry} = \frac{Conc_{sample}}{\%Solid} \times 100$$

where:
$Conc_{dry}$ = Concentration of the sample on a dry weight basis (ug/kg)
$Conc_{sample}$ = Concentration of the sample on a wet weight, or "as received" basis (ug/kg)
%Solid = Percent solids determined gravimetrically.

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

13.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever, feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

13.3 Refer to STL-SOP-018 for waste management procedures.

14.0 REFERENCES

14.1 United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods. EPA SW-846, 3rd edition, including Update III. U.S. EPA, Washington, D.C.

TABLE 5. SEMIVOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES ASSIGNED FOR QUANTITATION

1,4-Dichlorobenzene-d(4)

Benzyl alcohol
Bis(2-chloroethyl)ether
Bis(2-chloroisopropyl)ether
2-Chlorophenol
1,3-Dichlorobenzene
1,4-Dichlorobenzene
1,2-Dichlorobenzene
2-Fluorophenol (surr.)
Hexachloroethane
2-Methylphenol
4-Methylphenol
N-Nitroso-Di-n-propyl amine
Phenol-d(6)(surr.)

Naphthalene-d(8)

Benzoic acid
Bis(2-chloroethoxy)methane
4-Chloroaniline
4-Chloro-3-methylphenol
2,4-Dichlorophenol
2,4-Dimethylphenol
Hexachlorobutadiene
Isophorone
2-Methylnaphthalene
Naphthalene
Nitrobenzene
Nitrobenzene-d(5) (surr.)
2-Nitrophenol
1,2,4-Trichlorobenzene

Acenaphthene-d(10)

Acenaphthene
Acenaphthylene
2-Chloronaphthalene
4-Chlorophenyl phenyl ether
Dibenzofuran
Diethyl phthalate
Dimethyl phthalate
2,4-Dinitrophenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Fluorene
2-Fluorobiphenyl (surr.)
Hexachlorocyclopentadiene
2-Nitroaniline
3-Nitroaniline
4-Nitroaniline
4-Nitrophenol
2,4,6-Tribromophenol (surr.)
2,4,6-Trichlorophenol
2,4,5-Trichlorophenol

Phenanthrene-d(10)

Anthracene
4-Bromophenyl phenyl ether
Di-n-butyl phthalate
4,6-Dinitro-2-methylphenol
Fluoranthene
Hexachlorobenzene
N-Nitrosodiphenylamine
Pentachlorophenol
Phenanthrene

Chrysene-d(12)

Benzo(a)anthracene
Bis(2-ethylhexyl)phthalate
Butylbenzylphthalate
Chrysene
3,3'-Dichlorobenzidine
Pyrene
Terphenyl-d(14)(surr.)

Perylene-d(12)

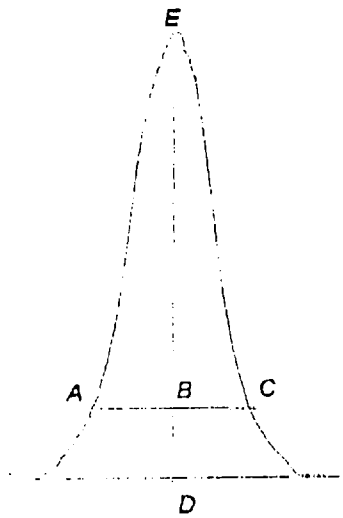
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(g,h,i)perylene
Benzo(a)pyrene
Dibenz(a,h)anthracene
Di-n-octylphthalate
Indeno(1,2,3-cd)pyrene

(surr.) = surrogate

TABLE 6. CHARACTERISTIC IONS FOR THE INTERNAL STANDARDS

Internal Standard	Primary Ion	Secondary Ion(s)
1,4-Dichlorobenzene-d4	152	150, 115
Naphthalene-d8	136	68
Acenaphthene-d10	164	162, 160
Phenanthrene-d10	188	94, 80
Chrysene-d12	240	120,236
Perylene-d12	264	260, 265

FIGURE 1. TAILING FACTOR CALCULATION



$$TAILING\ FACTOR = \frac{BC}{AB}$$

where AC = peak width at 10% of peak height

Table 7. Summary of Laboratory Quality Control Requirements and Corrective Action Procedures for Semivolatiles by GC/MS

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Holding time	See Table 2	Extraction and analysis are completed within holding time.	Notify LPM, determine if laboratory is to proceed or if client will resample.
Tuning	Every 12 hours	Within limits of method - See Table 4 for DFTPP Criteria DDT breakdown <20% Tailing Factors: Benzidine <3 Pentachlorophenol <5	Adjust instrument parameters and reanalyze tuning standard.
Calibration	Established initially at a minimum of 5 concentration levels	1. Initial calibration %RSD must be ≤15% on average for all compounds; however, %RSD for each individual CCCs must be ≤30%. 2. If %RSD fails several options: a. Reanalyze any outlier standards b. Average of all analytes ≤15%, ICAL valid c. Linear regression with r≥0.990 and y-intercept is ≤ the reporting limits, ICAL valid 3. The relative retention times (RRT) of each compound in each calibration run should agree within 0.06 relative retention time units.; 4. RF for SPCC is ≥0.05.	If acceptance criteria are not met, correct the problem and recalibrate.
Initial Calibration Verification	After each initial calibration using second source standard	%Ds for CCCs must be ≤ 20% RRF for SPCC must be ≥0.05	1. Verify ICV preparation and reanalyze the ICV standard once to verify. 2. If still out, recalibrate the instrument.
Continuing Calibration Verification	Verified every 12 hour analytical shift at mid level	1. %D for each CCC must ≤ 20%, then the initial calibration is assumed to be valid if the criterion is not met (> 20% difference) for any one CCC, then corrective action must be taken. 2. RF for SPCC is >0.05.	1. If acceptance criteria not met, reanalyze CCV. 2. If still out, recalibrate instrument.

Table 7. Summary of Laboratory Quality Control Requirements and Corrective Action Procedures for Semivolatiles by GC/MS

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Internal Standard Responses and Retention Times	Internal standards are added to all calibration standards, LCS, samples and blanks	• The retention times of the internal standards in the calibration verification standard must be within 30 seconds from that in the mid-point standard level of the initial calibration. • The EICP area for any of the internal standards in the calibration verification standard must be within a factor of two (-50% to +100%) from that in the mid-point standard level of the initial calibration.	1. Inspect the mass spectrometric system for malfunction and correct. 2. Reanalyze affected samples. If the areas meet criteria, report data from the compliant analysis. 3. If reanalysis of the sample does not solve the problem, submit data from both runs, and document all inspection and corrective actions taken in the analytical narrative.
Method Blank	1 per analytical batch	Analyte concentration is ≤ RL, except that the common laboratory contaminate bis-2-ethylhexylphthalate is ≤ 5X RL.	1. Determine source of contamination, i.e. instrument, blank water, reagents. 2. Take appropriate corrective action and document. 3. Reanalyze or prepare analytical batch. 4. If samples cannot be reanalyzed or reprepared, qualify data. 5. Document actions taken.
LCS	1 per analytical batch	All recoveries must be within laboratory control limits.	1. Examine instrument parameters, sensitivity and linearity. Correct problems and document. 2. Review standard and LCS preparation. Correct any problems and document. 3. Evaluate against project specific DQOs and report data if there is no impact on data usability. 4. If data is not usable, reprepare/reanalyze the method blank, LCS and all field samples in the batch. 5. If repreparation of samples is not possible, qualify data,. 6. Document all actions taken in a Nonconformance Record and in the report narrative.

Table 7. Summary of Laboratory Quality Control Requirements and Corrective Action Procedures for Semivolatiles by GC/MS

QC Check	Frequency	Acceptance Criteria	Laboratory Corrective Action
Surrogate spike	All field and QC samples	Surrogate recoveries must be within laboratory limits	1. Examine all QC, including calibration and quantitation. 2. If surrogates in LCS and/or MB are out-of-control reprepare/reanalyze the entire batch. 3. If samples cannot be reprepared, qualify data. 4. If surrogate spike in LCS and MB are acceptable but: (a) any 2 base/neutral or any 2 acid surrogates are out-of-control, or (b) any one surrogate recovery is less than 10% in the samples, reprepare and reanalyze the samples. If similar results are obtained, verifies matrix effects. [6] 5. If errors are discovered in preparation of samples, reprepare/reanalyze the entire batch. 6. Document actions taken in a Nonconformance Record, and in the analytical report
MS/MSD	1 set per 20 samples	Recoveries and RPD's are evaluated against laboratory LCS limits for precision and accuracy.	1. Examine all QC, including calibration and quantitation. 2. If spike recoveries in LCS are out-of-control reprepare/reanalyze the entire batch. 3. If samples cannot be reprepared, qualify data. 4. If spike recoveries in LCS are acceptable but out-of-control for samples, examine preparation of samples. If no errors or problems are discovered for samples preparation, a matrix effect is assumed. 5. If errors are discovered in preparation of samples, reprepare/reanalyze the entire batch.. 6. Document actions taken in a Nonconformance Record, and in the analytical report

(A) Abbreviations: **CCC**, calibration check compound; **CCV**, continuing calibration verification standard; **LCS**, laboratory control sample; **MDL**, method detection limit; **MS/MSD**, matrix spike/matrix spike duplicate; **NCR**, Nonconformance Record; **IS**, internal standard; **QC**, quality control; **RL**, reporting limit; **RPD**, relative percent difference; **SD**, standard deviation; **SPCC**, system performance check compound.

(B) Reextractions for surrogate recovery outliers may not be performed for samples with complex matrices (*i.e.*, tissues) and samples with obvious matrix interferences (*i.e.*, required higher than standard final volumes, dilutions, high concentration of target or non-target analytes).