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BATTELLE DUXBURY SOPs

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Battelle Ocean Sciences
Standard Operating Procedures

for

USE OF THE CAHN MODEL 25 AND CAHN MODEL 28 ELECTROBALANCES

Summary of changes in this version: Section 3.0 has been replaced by new text that describes the two current uses of Cahn balances: weighing procedures for neat materials and the gravimetric determination of oil weights.

1.0 APPLICATION

The CAHN 25 and CAHN 28 Electrobalances are very sensitive closed loop electromechanical weight and force measurement instruments that can be used to measure weight changes as small as 100 nanograms (0.0000001g), with a minimum amount of leveling or other special preparations. Operators should note that drafts, direct sunlight and excessive static may adversely affect the sensitivity of the balance. The balances should be used on a solid surface or weighing table, away from drafts and other disturbances.

2.0 CALIBRATION

The CAHN model 25 and CAHN model 28 electrobalances should be calibrated prior to use.

2.1 CAHN 25

1. Turn on the instrument. Allow the balance to warm up for a minimum of 30 minutes. The instrument should normally be left on continuously, however, a 30 minute warm-up is sufficient. The instrument must be recalibrated every time the power is lost, or if the instrument has been shut off.

2. Choose either the small or large weighing pans (the balance must be recalibrated if pans are changed during the weighing procedure). Clean weighing pans with CH_2Cl_2, allow to dry and replace on stirrups. Always use forceps when handling weighing pans, weights, and pans. The stirrups should be lifted from below the yoke for removal.

3. Deactivate the tare mechanism by pressing the tare button twice in rapid succession (the red indicator light above the tare button should go off).

4. Set the instrument to the 200 mg range. Adjust the digital readout to zero using the coarse and fine zero knobs.

5. Reactivate the tare mechanism by pressing the tare button once. The red tare indicator light should go on, wait until the white tare button illuminator light goes off. Now the balance is ready to calibrate. (If the balance does not zero on the first tare, the tare button may be depressed again until the digital readout is zero).

6. Place the 200 mg calibration weight (NBS Class "S") on the left pan (A) and close the weighing chamber door. Adjust the digital readout to 199.99 mg using the calibrate knob. Open the chamber door and carefully remove the calibration weight with forceps. Calibration weights are stored in the NBS supplied container. This helps to keep the weights free from dust.

7. Each time the balance is calibrated, the operator should record the calibration weight (e.g. 199.99 mg), date, and initials on the CAHN Electrobalance Calibration Form (Attachment 1). These records are kept with the balance in a "Balance Operation and Maintenance" book, located with the instrument.

8. Select the desired range and push tare to zero the balance. You are now ready to weigh your sample.

   Note: Procedure to switch ranges: Remove any sample from the pan. Deactivate the tare (two quick pushes of the tare button) and change to the desired range. Push the tare button once. You are now ready to weigh in the new range without recalibration.

9. If you plan to use the CAHN 25 in the "non-tare" mode, by using the zero adjustments or counterbalancing please familiarize yourself with the procedures in the CAHN 25 manual first (located with the balance).

2.2 CAHN 28

1. Turn on the instrument. Allow the balance to warm up for a minimum of 30 min. The instrument should normally be left on continuously; however, a 30-min warm-up is sufficient. The instrument must be recalibrated every time the power is lost, or if the instrument has been shut off.

2. Choose either the small or large weighing pans (the balance must be recalibrated if pans are changed during weighing procedures). Clean the selected weighing pans with methylene chloride, allow to dry, and place on stirrups. Always use forceps when handling weighing pans, weights, and pans. The stirrups should be lifted from below the yoke for removal.

2.2.1 Calibration Of The A Loop

To calibrate the balance on the A loop, it must be in the correct range. The correct range on the A loop is in the 200 mg to 1.0 μg range.

1. Place a weighing pan on the A loop.

2. Place a similar weighing pan on the tare loop.

3. Press the range button until the balance is in the 200 mg or 250 mg range.

4. Zero the balance by pressing the TARE button.

5. Place the 200 mg calibration weight on the A loop pan.

6. Once the digital display has stabilized, push the CAL button once; 200.000 will appear on the meter. Record the calibration weight, date, and initials on the CAHN Electrobalance Calibration Form (Attachment 1).

2.2.2 Calibration Of The B Loop

The B loop is not routinely used at Battelle but does allow for material(s) in a higher weight range to be weighed (1.5 - 3.5 g). The same calibration procedure used for the A loop (Section 2.2.1) should be used when calibrating the B loop. Use a 1000 mg NBS class "S" weight for calibration of the B loop (refer to the balance manual Section 3.3.2, page 7).

3.0 OPERATION

The CAHN model 25 and CAHN model 28 are normally used only in the preparation of analytical standards (from solid neat material) and the gravimetric determination of oil weights.

3.1 PREPARATION OF ANALYTICAL STANDARDS

Clean the weighing pans as described in Section 2.1 (step 2). Push tare button once. Using a micro spatula, weigh a portion of neat material directly onto the left weighing pan (A). Close the weighing chamber door. Allow the balance to equilibrate and read the weight from the digital display. Record the weight on the Solution Preparation Form. Carefully remove the weighing pan and gently rinse the neat material into a volumetric flask, using the appropriate solvent. Use of a small funnel for this transfer step is recommended to minimize material loss during transfer to the volumetric flask.

If the maximum weight limit in a particular weight range is exceeded, the digital display will blink repeatedly. Remove and clean the weighing pan. Change to a higher weight range and weigh a new portion of neat material.

3.2 GRAVIMETRIC DETERMINATION OF OIL WEIGHT

Clean the weighing pans as described in Section 2.1 (step 2). Push tare button once. Remove the weighing pan and place on hotplate. Draw an aliquot (usually 25, 50, or 100 uL) from the sample (total sample volume previously determined by volumetric or syringe measurement) and gently displace it onto the weighing pan. Just before the liquid has completely evaporated, remove the pan and replace it on the stirrups. Close the chamber door and allow the digital readout to equilibrate. Record the weight, remove and clean the weighing pan, tare the balance, and continue.

4.0 MAINTENANCE

The overall calibration and maintenance of the CAHN 25 and CAHN 28 balances is the responsibility of the Organics Sample Preparation Group Supervisor (supervisor) or designee. Daily cleaning and calibration is the responsibility of the operator. All routine and non-routine maintenance should be documented on the CAHN Electrobalance Maintenance Form (Attachment 2), located in the maintenance section of the CAHN Balance Logbook. Routine maintenance generally only includes cleaning the balance weighing chamber. When cleaning the weighing chamber, the operator should remove the weighing pans and stirrups. Using a Kimwipe or a balance cleaning brush, gently remove any dust or spilled sample from the weighing chamber. Replace the stirrups and weighing pans, close the chamber door and calibrate the balance (Section 2). If non-routine maintenance is required then consult the balance manual (CAHN 25: Section 4 Maintenance and Service, CAHN 28: Section 5 Maintenance and Troubleshooting) for assistance. The manual is located in the Organics Laboratory. If inhouse maintenance is not effective then the balance should be professionally serviced.

5.0 TRAINING

The trainee will read this SOP in its entirety. An experienced operator will then demonstrate calibration, operation and routine maintenance procedures to the trainee. Following this demonstration, the experienced operator and trainee will each weigh a series of five NBS Class "S" weights, usually 5, 10, 20, 30, and 50 mg. If the results of the trainee are within $\pm 2\%$ of the readings obtained by the certified instructor, then a Certificate of Training will be issue to the trainee. Percent difference is calculated as:

$$[(W_a - W_b)/W_a] * 100$$

where:

W_a = Weight determined by certified operator
W_b = Weight determined by trainee

The certificate is provided to the Quality Assurance Unit.

6.0 SAFETY

The CAHN 25 and CAHN 28 are very sensitive electrobalances and should be handled carefully if moved. Exercise caution when plugging in the instrument and make sure it is unplugged before removing the cover.

7.0 ATTACHMENTS

Attachment 1. Example of CAHN Electrobalance Calibration Form.

Attachment 2. Example of CAHN Electrobalance Maintenance Form.

Attachment 3. Example of Battelle Ocean Sciences Certificate of Training Form.

APPROVALS

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ATTACHMENT 1

CAHN ELECTROBALANCE CALIBRATION FORM

NBS Class "S" Weight Used	Weight after Calibration	Date and Initials

CAHN ELECTROBALANCE MAINTENANCE FORM

Description	Date and Initials

ATTACHMENT 3

Battelle Ocean Sciences
CERTIFICATE OF TRAINING

SOP No._____

SOP Title_____

Trainee_____

Instructor_____

Date SOP Read_____

Date Training Completed_____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Results

NBS Class "S" Weight	Instructor's Results	Trainee's Results	Percent Difference

Comments

Approved by_____ **Date**_____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedures

for

OPERATION AND MAINTENANCE OF HEWLETT-PACKARD 5970B, 5972A and 5973A GAS CHROMATOGRAPH/MASS SELECTIVE DETECTOR (GC/MS) USING HEWLETT-PACKARD SOFTWARE

Summary of changes in this version: Corrective action documentation for failed sample runs has been addressed. Verification of operation after maintenance has been addressed.

1.0 APPLICATION

This SOP details the daily operation and routine maintenance of the Hewlett-Packard 5970B and 5972A Gas Chromatograph/Mass Selective Detector (GC/MS) which is used for the identification and quantification of volatile and semi-volatile analytes in sample extracts. Additional references to the procedures presented in this SOP can be found in the Hewlett-Packard manuals located in the GC/MS facility.

2.0 MASS SPECTROMETER CALIBRATION

The mass spectrometer must be tuned prior to starting a new sequence to ensure that the electronic mass to charge (m/z) values are precise to within +/- 0.5 atomic mass units (AMU) and that the relative abundances of ions across the mass range 50 - 650 AMU meet accepted criteria. The calibration solution used to tune the mass spectrometer may be perfluorotributylamine (PFTBA) and/or decafluorotriphenylphosphine (DFTPP); both of which are commercially available and accepted as calibration solutions. PFTBA tuning can be performed automatically through autotuning or manually with operator control of tune parameter settings. The instrument is PFTBA tuned prior to DFTPP tuning. All MSDs at Battelle are tuned using PFTBA unless DFTPP is specified in the project work plan.

2.1 PFTBA TUNING

Hewlett-Packard's EnviroQuant software can automatically tune the instrument using PFTBA to achieve certain predefined performance criteria. Use of the autotune program minimizes operator-to-operator variation in tuning procedures and can maximize instrument sensitivity. The autotune program sets target values for six PFTBA ions(m/z 51, 69, 131, 219, 414, 502, and 614) and adjusts the various tuning parameters to achieve the relative values listed in Table 1. These target values are the levels that the masses would have if the instrument were tuned so that a spectrum of DFTPP conformed to EPA criteria.

2.1.1 The AUTOTUNE program is always used as the default tuning method after source cleanings.

2.1.2 AUTOTUNE programs should be run with the "standard values/settings" as a starting point.

2.1.3 The AUTOTUNE program adjusts the voltages of the repeller, ion focus lens, entrance lens, electron multiplier, and x-ray lens. It also adjusts the AMU gain and offset and the mass gain and offset.

TABLE 1. Hewlett-Packard Autotune Settings

m/z	Ion Abundance[a]
51	1 - 6 %
69	100%
131	30 - 50%
219	30 - 60%
414	1 - 2 %
502	1 - 6%
614	detectable

[a]Relative to base peak m/z 69

2.1.4 The relative ion abundances of the **three** masses m/z 69, 219, and 502 from the tune report should be compared to the target abundances listed in Table 1. If the abundances are not within range, tuning should be restarted using standard autotune. If the abundances are still not within range, the instrument can be tuned manually, as described in Section 2.3, below, to achieve the desired ion abundances. In all cases, a hard-copy of the final tune report will be placed in the GC/MS Tune Logbook, as specified in SOP No. 6-011.

2.2 MANUAL TUNING

In all cases, manual tuning is the tune method of choice (except after source cleanings where standard autotuning is used). Manual tuning can be used when PFTBA and/or DFTPP tuning criteria can not be achieved by the procedures in Sections 2.1 and 2.2, above. Manual tuning may be performed as a more time efficient method of generating a tune report. Each of the various instrumental parameters (e.g. repeller, entrance lens) may be varied within the manual tuning procedures. Manual tune parameters are always saved as a file called ATUNE.U on the EnviroQuant system. For more information regarding tuning procedures refer to the section on tuning in the Hewlett-Packard manuals.

2.3 DFTPP TUNING

Certain Projects require tuning of the GC/MS with DFTPP. This tuning procedure is more rigorous than PFTBA tuning, and strict specifications for DFTPP ions and ion abundances (Table 2) must be met prior to the start of a sequence and analysis of samples.

2.3.1 Perform a PFTBA tune as specified in Section 2.1 or 2.2 above.

2.3.2 Set up an acquisition as specified in Section 3.0, below. Inject 2 µL DFTPP (25 µg/mL). Where the acquisition is completed, run EnviroQuant program under Tuner/Evaluate DFTPP. This program will extract the spectra of DFTPP and automatically compare it to the values listed in Table 2.

2.3.3 If all key ions fall within the ranges given in Table 2, the instrument meets DFTPP tuning criteria, and sample analysis and quantitation may proceed. A copy of DFTPP Evaluation Report is kept in the GC/MS Tune Logbook as specified in SOP No. 6-011.

2.3.4 If DFTPP criteria are not met for all key ions, remedial action must be taken. Requantify the DFTPP peak as described in Sections 2.3.2 and 2.3.3, above, taking particular care in the placement of the cursor on the apex of the peak. If key ion abundances are still not met, additional remedial action, adjustment, and re-calibration with PFTBA and DFTPP is necessary before re-analysis.

TABLE 2. DFTPP Tuning Criteria

m/z	Ion Abundance
51	30 - 60%
68	< 2% of m/z 69
69	< 100%
70	< 2% of m/z 69
127	40 - 60%
197	< 1%
198	100%
199	5 - 9%
275	10 - 30%
365	>1%
441	Present < m/z 443
442	> 40%
443	17 - 23% of m/z

[a] Relative to base peak m/z

3.0 OPERATION

3.1 DATA ACQUISITION

Data acquisition software is controlled by the programs METHOD and SEQUENCE. Each different type of analysis performed on the GC/MS requires a specific set of acquisition parameters, including heated zones, and GC temperature, pressure, and autosampler programs. The acquisition parameters are stored in

a unique acquisition parameter file or method file. A hard copy of the acquisition parameter (method) file can be printed on request. The software program under the SEQUENCE menubar allows for creation of the sequence file. The sequence file is a list containing the names of samples to be analyzed, along with their assigned data file names, project numbers, vial positions, and acquisition methods. A hard copy of the sequence list is generated and kept in the GC/MS Logbook as specified in SOP No. 6-011.

If a sample needs to be rerun, (i.e., bad injection, failed calibration, power outage, etc.), this will be documented on the sequence. The analyst's initials and the date should accompany the corrective action taken and the reason a corrective action is needed.

3.2 METHOD - MASS SPECTROMETER CONDITIONS

The GC/MS can acquire data in linear scanning (full-scan) mode where all masses in a given range are scanned equally or in Selected Ion Monitoring (SIM) mode where selected ions are scanned while all other masses are ignored. SIM analysis is used for the detection of trace level analytes in environmental samples. SIM provides greater sensitivity and selectivity than full scan analysis. Full scan analysis is most often used when samples concentrations of target analytes are expected to be high. Full scan analysis is also used to detect, confirm (through library search), and quantify unknown analytes in environmental samples. The mode of acquisition is project-specific and is detailed in the project work plan.

3.2.1 The electron multiplier voltage should be set approximately 100 volts above the value calculated by the TUNE program. A scan-start time/solvent delay will be chosen which allows time for the solvent peak to elute before the first scan occurs. A minimum of three (3) minutes is typically required for a 30 or 60-m GC column. The mass range scanned is 50 - 450 m/z units for full scan analysis, unless stated otherwise in project protocols.

3.2.2 Certain analyses require the increased sensitivity of selected ion monitoring mode (SIM). Operating in SIM, the mass spectrometer monitors a specific m/z value for a predetermined length of time (dwell time), and then switches to another mass, rather than monitoring all the m/z values in a certain mass range.

3.3 GAS CHROMATOGRAPH AND ELECTRONIC PRESSURE CONTROL (EPC) CONDITIONS

The following GC conditions will be used unless stated otherwise in project protocols:

Injection port temp:	300°C
Detector temp:	280°C
Initial oven temp:	40°C
Initial hold time:	1 minute
Ramp rate:	6°C/minute
Final oven temp:	290°C
Final hold time:	20 - 30 minutes or until benzo(g,h,i)perylene elutes
GC Column:	DB5, 60 m, 0.25 id, 0.25 μm film (or equivalent – i.e. 30 m)

Injection port liner:	silanized straight liner (4 mm id) with glass wool
Glass wool position:	~ 1.8 cm from end of liner
Column position:	~ 0.8 cm from end of column nut
Septa:	Merlin microseal (preferable) or 9 mm septa
EPC Initial Pressure:	30 psi (for 60-m column, 15 psi for 30-m column)
EPC Initial hold time:	1.0 minutes
EPC Level 1 rate:	99 psi/min
EPC Final pressure:	psi equivalent to 1 mL/min

3.4 QUANTITATION - DATA PROCESSING

The extent to which acquired data is processed is project-specific, and depends on contract deliverables, analysis type, and acquisition type.

3.4.1 The identification and quantification of analytes in a sample is controlled by the quantitation database file located in the Data Analysis section of the EnviroQuant software. The database file is a three tiered (page) file containing information such as:

- compound names
- expected retention times
- retention time windows
- integration parameters
- quantifiation ions (primary and secondary)
- relative ion ratios
- compound amounts
- compound responses
- internal standard designations
- internal standard amounts
- surrogate standard designations
- surrogate standard amounts
- calculated average response factors (RF)

3.4.2 The retention time window range for all parent compounds in the database file should be ± 0.20 minutes. The retention time window for the alkyl homologues will be set at varying ranges depending on the alkyl homologue pattern. The slope sensitivity in the integration parameter file (rteint.p) is typically set at -0.020.

3.4.3 The first analyte listed in a database file is always an internal standard. Internal standards are designated as compound type "I". All analytes listed after the internal standard will be quantified versus that internal standard. In the case of multiple internal standards, once another analyte is designated as an internal standard, it will be used to quantify all analytes that follow it in the list.

3.4.4 The correct amount of internal standard spiked into the sample is input onto page one of the quantitation database file for the respective internal standard.

3.4.5 If the recovery of the surrogate internal standards are to be calculated, the spike amounts are input onto page two of the respective surrogate compound in the quantitation database file. Designate the surrogate compound as a type "S" on page two of the quantitation database file for the respective surrogate.

3.4.6 The pull down programs *Quant*, and *Calculate and Generate a Report*, allow for sample quantification. The following steps can be performed to quantify a sample or standard:

- Load the sample data file and proper quantitation method into memory by using the *File* pull down menus.
- Confirm that the correct amount of internal standard(s) is entered into the quantitation database file by using the pull down menu *Edit Compounds* located under the *InitCal* menubar.
- Check to see that the proper units are entered into the quantitation database. All data are quantified in total nanogram (ng) unless stated otherwise in project protocols. The units may be changed globally using the pull down menus *InitCal*, *Global Update*, and the option *"Other"*. Type in the runstring -- amount_units$="ng" -- to change the units of all target analytes to ng.
- Save the method by using the *File* pull down menus.
- Quantify the sample by using the *Quant* and *Calculate and Generate a Report* pull down menus. If the file has never been quantified the options *Generate Report* and *QEdit Quant Result* will be grayed out. Select the *summary report* option and select *printer* as the destination. This print out contains the all the analytes (targets + internal standards and surrogates) found in the quantitation database, their respective retention times, area abundances, quantitation ions, and nanogram amounts detected.
- The file naming convention for the quantified file will not change (see SOP 6-011). The only difference will be that there is a quantitation report associated with the file number.

3.4.7 Samples with the same amount of internal and surrogate standards spiked into them can be quantified in a batch mode. The following procedures are followed to quantify a group of similar samples:

- Load one of the sample data files and the proper quantitation method into memory by using the *File* pull down menus.
- Confirm that the correct amount of internal standard(s) is entered into the quantitation database file by using the pull down menu *Edit Compounds* located under the *InitCal* menubar.
- Check to see that the proper units are entered into the quantitation database. All data are quantified in total nanogram (ng) unless stated otherwise in project protocols. Sample sizes, dilutions, and any additional correction factors are applied in spreadsheet. The units may be changed globally using the pull down menus *InitCal* and *Global Update* and selecting *"Other"*. Type in the runstring -- amount_units$="ng" to change the units of all target analytes to ng.
- Save the method by using the *File* pull down menus.
- Using the *Tools* pull down menu, select *DOLIST* followed by *Summary Quant w/Calc* and *OK*. A window appears and allows for files to be selected for quantification. Select the files to be quantified and click *process*.
- The batch of samples will automatically be quantified and spooled to the printer for reporting.

3.4.8 All quantified samples are inspected for proper integration and verification of computer selection by entering the *QEdit Quant Result* program under the *Quant* menu bar. This program allows for reintegration of peaks if necessary and straight baseline integration of the alkyl homologue groups. Analytes that do not meet GC/MS peak criteria (SOP 5-157) may be deleted in this program. QDel's, as they are referred to, are recorded on the hard copy summary report generated above and placed in the Unused Data section of the project data package.

3.4.9 When all the summary reports are reviewed, final reports are generated. At the command line cursor type the runstring -- DOLIST$_{sp}$`QT$_{sp}$0,1,"S"'. Select from the window the files that require final report generation and click *process*. Hard copies of the final reports will be generated to the printer. The reports are spot checked for errors and dated and initialed by the analyst.

3.4.10 The EnviroQuant software allows for the creation of Excel databases to streamline final data table preparation. First a quantified data file is loaded and then a *detail.xls* file is created using the *Quant*, and *Generate Report* pull down menus. Select the *detail, text only* option along with selecting *File*. Type *detail.xls* in the box provided. The database template can then be created from the *Cust Rpt* and *Create/Modify Template/Database* menus. A list of files can be loaded into the database in a batch mode using the *Jobs* and *Queue List of Files* menus. Consult the facility manager for assistance in these procedures.

3.5 DATA STORAGE

Data is stored by the computer on a hard disc with a minimum capacity of 500 megabytes. When the hard disk becomes full, system operation will stop and no files can be acquired. Hard disk space may be made by transferring files from one network disk to another network disc (see SOP 6-011). Data, sequence, method, tune and database files are archived routinely on 60m DDS-1 data cartridges according to procedures outlined in SOP 6-011. Files that have been archived will be purged from the disk drive in order to created more space.

3.6 GC/MS CALIBRATION PROCEDURES

Certain calibration procedures are common to all types of analyses. These procedures include peak identification in standard solutions, generation of a calibration curve, and linearity checks of the initial and continuing calibrations.

3.6.1 Each of the standard solutions that are part of the calibration must be quantified as specified in Section 3.4, above.

3.6.2 Once all of the standards have been quantified correctly, the calibration curve can be generated. The following steps are used to update an existing a calibration curve (consult the facility manager and/or the Hewlett-Packard Software Manuals for assistance in creating a calibration file from scratch):

- Load the proper method and the low standard data file into memory using the *File* pull down menu.
- Input the low standard quantitation results into the quantitation database by using the *InitCal*, followed by *Update Levels* menu bars.

- When the window emerges, select *Recalibrate*, followed by choosing the appropriate standard level from the pull down menubar.
- In the same window select *Replace* for Responses and Retention Times.
- Finally, press *Do Update*.
- Follow the above procedures for each of the remaining calibration levels.
- When all the levels of the curve are loaded into the quantation database save the method using the *File* menubar.
- Generate the response factors to the screen, and then to the printer by using the *InitCal*, *Response Factors to Excel*, and *print* options. Save the method again. This report contains the calculated average response factors that will be used for future standard and sample quantification. The calculated relative standard deviation (RSD) for each of the target analytes in the standard must meet the criteria specified in SOP 5-157 prior to sample quantification.
- If alkyl homologues are to be quantified versus the response factor of the parent compound then the runstring -- $MACRO_{sp}$`BAT_RF,G' -- must be entered at the blinking cursor at the command line.
- Save the method again to ensure the response factors are retained in the quantitation database file. The quantitation database may now be used to quantify samples as specified in Section 3.4 above.

3.6.3 Continuing calibration standards are quantified, reviewed in *Quant*, *QEdit Quant Result* and checked for linearity relative to the initial calibration using the *ConCal*, and *Evaluate Data file as Continuing Cal to Printer* pull down menus. This report lists the percent difference between the average response factor generated in the initial calibration and the response factor determined from the continuing calibration, for each analyte. Results from the continuing calibration should be compared to the calibration criteria specified in SOP 5-157.

4.0 MAINTENANCE

Maintenance will be performed by the facility manager or other trained operators. All routine and non-routine maintenance will be recorded in the GC/MS Maintenance Log as specified in SOP 6-011.

4.1 PUMP MAINTENANCE

The rough pumps, turbomolecular pump (5970B MSD), and diffusion pumps (5972A MSD) should be serviced every six months. The pump maintenance is performed by HP service engineers approximately every six months under the preventative maintenance (PM) service contract. Pump fluid levels may be checked by GC/MS personnel periodically to ensure system performance.

4.2 FORELINE TRAPS (5972A MSD) AND HELIUM GAS TRAPS

The adsorbent pellets (molecular sieve) contained in the foreline traps should be replaced every six to twelve months or as needed. The foreline traps may be inspected during source cleaning events when the mass spectrometer is vented. Gas traps should be inspected routinely for adsorbent discoloration (e.g. black to yellow). The traps should be replaced when this color change is evident.

4.3 GC MAINTENANCE

Several of the GC maintenance procedures require exposing the column, injection port and mass spectrometer to air. To minimize damage of the mass spectrometer and column; the oven, injection port and transfer line should be cooled to room temperature whenever routine maintenance requires opening the system to air. Never leave the heated zones at their setpoints without carrier gas flowing through the column.

4.3.1 The injection port septum is typically a Merlin microseal. The frequency at which the seal is replaced is generally dependent on the number of injections. Replace the Merlin when the EPC no longer maintains pressure. Cool the oven and injection port to ambient temperature. Turn off the EPC. Unscrew the Merlin and remove the microseal with forceps. Using forceps gently insert a new microseal into the top of the port and place the thin Teflon washer into the Merlin nut. Set the EPC pressure to 15 psi. Hand tighten the nut to a position (~ 3 mm) just beyond the point where there is a noticeable pressure change (e.g. <0.2 psi to 15 psi). Tightening the nut too much will cause the seal to leak and the EPC to shut down.

4.3.2 Injection port liners should be changed routinely (every 1 - 3 batches). Cool the injection port. Remove the injection port liner-retaining nut. Remove the injection port liner and silicone o-ring with forceps. Unscrew the column nut from the bottom of the injection port. Unscrew the large bottom injection port nut inside the oven. Remove any debris from the injection port barrel and retaining nut by cleaning them with a cotton swab dipped in solvent. Clean the washer, gold seal, and nut with solvent before reinstalling. Reinstall the bottom injection port nut. Hand tightening the nut and further tighten 1/4 turn with the appropriate wrench. Using forceps, carefully install a clean, silanized liner with a new silicone o-ring into the injection port. Replace the ferrule if it has become disfigured or splintered. Next, clip the column (~ 2 - 8cm) as needed and readjust the column position prior to inserting the column nut into the injection port. Insert the column nut into the bottom injection port and hand tighten followed by tightening 1/4 turn with the appropriate wrench. Reinstall the retaining nut on the top of the port. Hand tighten the nut followed by tightening 1/4 turn with the HP wrench. Do not over tighten this nut as it will cause the top of the glass liner to chip. Wait 5 - 10 minutes before bringing the oven and injection port back up to operating temperature.

4.3.3 Whenever peak shape, resolution or sensitivity warrants, the precolumn (if installed) should be replaced. If this fails to correct the chromatographic problems, 30 - 50 cm of the analytical column should be removed. If column performance is not improved, the analytical column should be replaced. Replacing the column requires the instrument to be vented to the atmosphere, and involves instrument down time. Complete directions for column replacement are found in the Hewlett-Packard manuals.

4.4 MASS SPECTROMETER MAINTENANCE

4.4.1 Approximately every four months, or as needed, inspect and refill the calibration sample vial with PFTBA, in accordance with the instructions in the Hewlett-Packard manuals.

4.4.2 Approximately every 6 months or when needed, vacuum the dust from the grills of the fans on the back of the mass spectrometer.

4.4.3 The ion source should to be cleaned when usage-dependent surface deposits degrade the ion source function below user-determined limits. This will usually be indicated by an increase in the ion focus voltage and a decrease in the repeller voltage necessary to obtain proper abundances of masses m/z 219 and 502 during PFTBA tuning. Detailed, step by step instructions on ion source cleaning are found in the Hewlett-Packard hardware manuals.

4.5 VERIFICATION OF PERFORMANCE

After instrument maintenance has been performed, the analyst must verify that the instrument is back in working order and that any problems requiring maintenance have been corrected. This verification is accomplished by verifying instrument calibration. Either a calibration check standard or a new initial calibration curve that meets the acceptance criteria of this SOP will verify instrument operation.

5.0 TRAINING

1. The trainee must read, understand, and be able to perform all the functions listed in this SOP.

2. An experienced operator must provide a complete demonstration of all aspects of calibration, tuning, acquisition and data processing to the trainee.

3. An experienced operator must supervise and assist the trainee during training and test-sample analysis for each type of analysis to be conducted.

4. A trainee must demonstrate competence by performing independent PFTBA tuning. Furthermore, a previously analyzed sample must be rerun and reprocessed by the trainee. Results should be within +/- 15% of previous values to be acceptable.

5. Upon successful completion of the test sample, the new operator is qualified to operate the GC/MS for that type of sample. A Certificate of Training (Attachment 1) will be issued to the new operator and provided to the QA Office.

6.0 SAFETY

1. Certain areas of the GC and MSD are heated zones. Use caution around these areas and always drop the set temperatures to room temperature before performing hardware maintenance.

2. Proper care should be exercised for the use and disposal of syringes.

3. High voltages exist in several well-marked areas of the GC/MS and data systems and operators should avoid contact with these areas unless the instrument is turned off and power disconnected.

4. Potential health hazards may be associated with analytical standards, solvents, and samples to be analyzed (e.g. PCBs, pesticides, and other carcinogenic compounds). Protective clothing such as safety glasses, lab coats, and gloves may be required. Care should be taken when performing source cleanings as the source parts contain concentrated sample and standard residues. Proper disposal should be followed for used pump oils and used foreline trap pellets. Refer to the project protocol and Material Safety Data Sheets for specific safety guidelines.

5. Proper protocol should be used when handling gas cylinders, high-pressure lines, and gas filters.

7.0 ATTACHMENTS

Attachment 1. Battelle Duxbury Operations Certificate of Training

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APPROVALS

Author:		8.6.99
Laboratory Manager:		8.6.99
Quality Systems Manager:		8-10-99
Environmental Chemistry Section Manager:	AD Uhl for S S Dorell	8-9-99
	Name	**Date**

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

for

SOP No. _____

TITLE: _____

_____

_____

TRAINEE: _____

INSTRUCTOR: _____

DATE OF COMPLETION OF PROFICIENCY TEST: _____

_____

TEST RESULTS

APPROVED BY: _____ **DATE:** _____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedures

for

OPERATION AND MAINTENANCE OF GAS CHROMATOGRAPHS

Summary of Changes in this version: References to the HP LAS Data system have been replaced with the newer Xchrom data system. Internal Standard types have been modified to reflect new analysis methods. Calibration mid check re-occurrence has been changed. References to analysis detectors that are no longer in use have been deleted. This SOP now references the newer 6890 model GC. References to magnetic tape storage have been changed to reflect the data archiving SOP. Corrective Action documentation for failed sample runs has been addressed.

1.0 OBJECTIVE

This Standard Operating Procedure (SOP) covers the daily operation and routine maintenance of the Chemistry Department's non-GC/MS gas chromatographs (GCs). The Department maintains several gas chromatographs, comprised of Hewlett-Packard Model 5890A and 6890 gas chromatographs. These GCs are used for the analysis of a variety of organic compounds including petroleum hydrocarbons, PCBs, pesticides, sterols, and organotins.

This SOP is intended to be used as a guideline for operation of these instruments. For additional information, refer to the specific Instrument Manuals. Additional project-specific information relevant to the operation and maintenance of the GCs may be specified in Quality Assurance Project Plans (QAPPs).

2.0 CALIBRATION

Before the sample is injected into the GC, the detector must be calibrated to determine the response for the analytes of interest. Demonstration of detector response linearity is required before sample analysis can begin. Calibration checks must be analyzed minimally every 10 samples, or 12 hours of run time, whichever is more frequent.

All analytical chromatographic data are quantified by either the internal standard or external standard method. The calibration and quantification method will be by the method of internal standards unless otherwise specified in the QAPP.

The internal standard method of analysis is based on measuring the response of analyte(s) relative to a quantification internal standard (QIS). The QIS(s) is typically a recovery internal standard(s) (RIS) added to the sample after sample processing in the laboratory, but may, if specified in the QAPP, be a surrogate internal standard (SIS) added prior to sample processing is completed and before instrumental analysis. The RIS is used to determine the recovery of the SIS(s), and the thus the efficiency of the sample processing.

In practice, an average response factor, or linear regression calibration equation, is determined using the response of analyte(s) of interest relative to the QIS in a series of calibration standard solutions. Actual samples are fortified with the QIS prior to instrumental analysis. The analysis of the sample is accomplished by measuring the response of the analyte relative to the QIS, and calculating the analyte concentration using the average response factor, or linear regression calibration equation, obtained from the calibration process. The internal standard calibration procedure is discussed in more detail in Section 2.1.

The external standard method of analysis is based on measuring the response of an analyte in a sample, and comparing it to the response of the same compound in the analysis of standard(s) of known concentration(s). In practice, an average response factor, or linear regression calibration equation, is determined for analyte(s) of interest in a series of calibration standard solutions. A sample is prepared to a known volume and analyzed. The concentration of the analyte(s) in the sample is determined by applying the average response factor, or linear regression calibration equation, obtained from the calibration process, to the response of the analyte(s) in the sample analyses. Accurate determination and recording of sample preinjection volumes (PIV) are necessary when using this method. The external standard calibration procedure is discussed in more detail in Section 2.2.

Instrument calibration is the responsibility of the analyst. Calibration records are maintained in project-specific data packages. In the following sections common calibration procedures are described. However, project-specific procedures may be selected and will then be specified in the QAPP.

2.1 INTERNAL STANDARD CALIBRATION

2.1.1 Initial Calibration

Most GC analyses are quantified by the method of internal standards. To use this approach select one or more internal standards that are similar in analytical behavior to the compounds of interest. Further, the measurement of the internal standard should not be affected by method or matrix interferences. Because of these limitations no one internal standard is applicable to all samples. The following listing includes examples of internals standard(s) for the corresponding analyses. However, project-specific internal standards may be selected and will be specified in the QAPP.

Analysis Type	Internal Standard
Petroleum hydrocarbons	*o*-terphenyl, androstane,
PCB's, pesticides	Cl_3(39) 3,4',5 Trichlorobiphenyl
	Cl_3(34) 2',3,5 Trichlorobiphenyl
	Cl_5(103) 2,2',4,5',6 Pentachlorobiphenyl
	Cl_5(112) 2,3,3',5,6 Pentachlorobiphenyl
	Cl_6(166) 2,3,4,4',5,6 Hexachlorobiphenyl
Coprostanol	androstane, androstanol
Organotins	dipropyltin, tripropyltin, dipentyltin, tripentyltin

Calibration standards are prepared at a minimum of one concentration level for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard a known constant amount of the appropriate internal standard(s) is added and the resulting standard diluted

to volume with an appropriate solvent. If only one calibration standard is prepared, it must be at a concentration corresponding to the expected concentration found in real samples.

Typically a five-point calibration is performed, but more or fewer calibration levels may be specified in the QAPP. These standards include the analytes of interest, as well as the appropriate internal standards if an internal standard calibration is to be performed. The concentration of the LOW standard should be approximately 2 to 5 times the detection limit of the instrument. The MID-range calibration standard should be near the expected concentration of the samples. The HIGH concentration standard should be approximately 5 to 10 times the concentration of the LOW standard, or high enough to span the expected concentration range. A wider calibration range may be appropriate for certain applications, and will then be specified in the QAPP. Commonly, calibration standards have the following concentrations (for individual PCB congeners and single peak chlorinated pesticides):

LOW	~ 0.008 ng/µL
MID LOW	~ 0.012 ng/µL
MID	~ 0.048 ng/µL
MID HIGH	~ 0.096 ng/µL
HIGH	~ 0.120 ng/µL

The concentrations of the SIS and RIS should be approximately at the same as the MID-level target analytes, the same in all calibration solutions, and approximately the same concentration as expected in the samples. A multilevel calibration that meets acceptance criteria must be analyzed before sample analysis begins.

Using injections of 1 to 5 µL (1 to 2 µL for most applications), analyze each calibration standard using the same instrumental analysis conditions as will be used for samples. The calibration is established and verified by one of two methods: linear regression or response factor. Instrument calibration is generally accomplished using the linear regression algorithm of the **Xchrom data system**; calibration verification may be performed using the correlation coefficient of the Xchrom data system linear regression calculation or by using response factors (RFs). The following are the applicable equations for the two different methods.

Linear Regression Method

- $Y = mX + b$

  which is equivalent to: $A_A/A_{IS} = [[m \times (C_A/C_{IS})] + b]$

- $C_A = [(A_A/A_{IS}) \times b] \times (C_{IS}/m)$

Response Factor Method

- $RF = (A_A \times C_{IS})/(A_{IS} \times C_A)$

- $C_A = (A_A \times C_{IS})/(A_{IS} \times RF_{AVG})$

where,

C_A = analyte amount
A_A = analyte peak area (or height)
A_{IS} = area (or height) of the QIS
C_{IS} = amount of QIS added to the sample
b = y-intercept of linear regression equation
m = slope of linear regression equation
RF = response factor of the analyte
RF_{AVG} = average response factor of the analyte from the initial multilevel calibration

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked. If the response for a given analyte is acceptably linear over the calibrated range [acceptable correlation coefficient or percent relative standard deviation (%RSD) in RFs], the calibration is considered valid and can be used for sample quantification. Calibration acceptance criteria are discussed in Section 2.5.

The **Xchrom** data system automatically computes and reports the correlation coefficients for the linear regression equations as part of the calibration. However, if the RF calibration check method is used, the variation in the RF for each compound is determined as %RSD using the following equation:

$$\%RSD = \frac{SD_{RF}}{AVG\ RF} \times 100\%$$

%RSD = percent relative standard deviation
SD_{RF} = standard deviation in average RF for the calibration standards
AVG RF = average RF for the calibration standards

2.1.2 Continuing Calibration

The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s). Typically, a MID-level calibration standard is analyzed for every 10 samples analyses. The initial calibration is considered valid if the RF of the calibration check standard (response factor method) is acceptably similar to the average RF determined for the initial calibration. The ongoing acceptance of the calibration can also be verified by quantifying the calibration check standard as a sample and calculating the difference between the measured amount and the known (true) standard amount. Calibration check standard acceptance criteria are discussed in Section 2.5.

In either case, the comparability of the calibration check standard for each analyte versus the initial calibration is computed, and expressed as percent difference (%DIFF), using the following equation:

$$\%DIFF = \frac{(RF_C \times RF_I)}{RF_I} \times 100\%$$

%DIFF = percent difference
RF_I = average RF for the initial calibration standards or true value
RF_C = RF for the calibration check standard or measured value

If a single-point calibration is used, no initial calibration criteria apply but the calibration is continuously verified by determining the %DIFF in the RF or measured value for each analyte in the calibration check

analyses versus the RF or true value from the initial calibration. The RF calculated for a single-point analysis is equivalent to the slope determined in a single-point calibration with the Xchrom datasystem. As with multilevel calibrations, sample analyses should be bracketed by calibrations that are acceptably similar.

2.2 EXTERNAL STANDARD CALIBRATION

Each calibration standard is analyzed using the same instrumental analysis conditions as used for samples and the calibration is established and verified by one of two methods (linear regression or response factor). Instrument calibration is generally accomplished using the linear regression algorithm of the Xchrom data system; calibration verification may be performed using the correlation coefficient of the Xchrom data system linear regression calculation or by using calibration factors (CFs). The following are the applicable equations for the two methods.

Linear Regression Method

- $Y = mX + b$

  which is equivalent to: $A_A = [(m \times C_A) + b]$

- $C_A = (A_A \times b)/(m)$

Response/Calibration Factor Method

- $CF = (A_A)/(C_A)$
- $C_A = (A_A)/(CF_{AVG})$

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked as indicated in Section 2.1. The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s), as described in Section 3.1. Calibration acceptance criteria are discussed in Section 2.5.

2.3 CALIBRATION/QUANTIFICATION OF MULTICOMPONENT COMPOUNDS

Depending upon the QAPP instructions, either a multi- or single-level calibration is analyzed when samples are quantified for multi-component mixtures such as Aroclors, technical chlordane, or toxaphene. The instrument calibration and analysis procedures for multi-level multicomponent standards are discussed in SOP 5-128.

2.4 PREPARATION OF CALIBRATION STANDARDS

The preparation of calibration standards may be performed by properly trained Battelle personnel, in accordance with SOP No. 5-027.

2.5 CALIBRATION ACCEPTANCE CRITERIA

Unless otherwise documented in the QAPP or analysis-specific SOPs, the following acceptance criteria apply.

Initial calibration - Linear Regression Method: correlation coefficient of >0.99 for a 3-point calibration, and >0.995 for a 5-point calibration. Response/Calibration Factor Method: %RSD of the average analyte RF or CF should be ≤25%.

If these criteria are not met corrective action is taken, a new calibration run, and the samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis will be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

Calibration check - Response/Calibration Factor Method: %DIFF between the RF or CF from the calibration check and the average analyte RF or CF of the initial calibration must be ≤25%.

If these criteria are not met, the check standard can be re-analyzed. If the check standard fails to meet the criteria a second time, corrective action will be taken, and the check standard and associated samples re-analyzed. A new initial calibration may need to be run, and affected samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis may be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

3.0 OPERATION

The individual should refer to the H-P 5890/6890 instrument manuals for instruction in the keyboard operation of the appropriate chromatographs and ancillary equipment (including integrators and autosamplers). These manuals have separate sections describing the operation and maintenance of the GCs, including each GC detector, and this source of information should be familiar to the analyst and referred to when needed. It is beyond the scope of this SOP to list step-by-step instructions for the programming, activation, operation, and all possible maintenance of these instruments. Each manual is kept in the instrument room near the appropriate chromatograph.

3.1 EQUIPMENT/SUPPLIES

3.1.1 Instrumentation

The GCs currently used in the GC laboratory are 5890 & 6890 GCs. A separate Instrument Log Book is maintained for each GC. Instrumentation consists of:

> H-P 5890 & 6890 Gas Chromatographs, equipped with combinations of split/splitless and on-column capillary injectors, FIDs, ECDs, flame photometric detector (FPD), and 7673A/6890 series autosamplers. Many of the instruments have dual injection capabilities,

where both injection ports are used simultaneously. The front injection port is connected to the front detector and the back injection port to the back detector.

Xchrom Data System, Digital & Analog data acquisition servers, Dell Power Edge 2100/200 network server, Xchrom chromatography software, disk drives, and printers.

3.1.2 Analytical Columns

A variety of capillary and packed columns may be used in the GC analyses. 30 m X 0.25 or 0.32 mm i.d. (inner diameter) fused silica capillary columns will be used for most applications. DB-1, DB-5, or DB-1701 (J&W Scientific), or equivalent, column stationary phase will generally be used. Other columns (e.g., 0.53 mm i.d. capillary or packed columns) may also be used as specified in QAPPs. Unless otherwise specified in the QAPP or analysis-specific SOPs the following columns will be installed for analysis

FID	30 m DB-5 0.32 mm ID, 0.25 μm film
FPD	30 m DB-5 0.25 mm ID
ECD	Primary column: 60 m DB-5 0.25 mm ID, 0.25 μm film Confirmation column: 60 m DB1701 0.25 mm ID, 0.25 μm film

3.1.3 Compressed Gases

FID, FPD:	Helium, ultra high purity (UHP) grade, or equivalent; Hydrogen, UHP grade, or equivalent; Air, zero grade, or equivalent.
ECD:	Hydrogen or Helium, UHP grade, or equivalent; Argon/Methane (95%/5%), UHP grade, or equivalent.

3.2 STANDARD CONDITIONS

Standard conditions for each type of analysis are specified in the analytical SOPs or in the QAPP. Analysis conditions are recorded on each analysis acquisition schedule and filed in the Instrument Log Book with each analysis sequence, as described in SOP 6-025 and as discussed in Section 3.8.

3.2.1 Injection Mode

All capillary gas chromatographic analyses will be performed in the splitless mode unless otherwise specified in the project protocols.

3.2.2 Flowrates

Optimum GC flowrates will vary with instrument, detector, and the analysis to be performed. Please refer to the applicable instrument manuals and/or analytical SOPs for more information on flowrate and gas pressure settings.

3.2.3 Temperature Programs

Gas chromatograph injector and detector temperature, and oven temperature programs are project/analysis specific. Refer to the appropriate analysis/quantification SOP for specific GC analytical conditions. These conditions may be modified in the QAPP for specific applications.

3.3 INSTRUMENT SETUP AND STARTUP

- Check tank and line pressures. Change compressed gas tanks if any are below 300 psi.
- Make sure all applicable GC equipment are on (including detector flames for FID and FPD) and communicating properly.
- Set GC chromatographic conditions according to applicable analytical SOP or project work plan.
- Place calibration standards and samples in autosampler tray in the order they are to be analyzed.
- Set up the sample data acquisition schedule on the data system for the samples to be analyzed.
- Start the chromatographic analysis by starting the gas chromatograph, autosampler, and data acquisition through the particular controller (e.g., autosampler, integrator, or instrument control) for the GC to be used.

3.4 DATA ACQUISITION

The chromatographic data generated during standard and sample analysis is automatically stored on the Xchrom data system network server hard disk for postrun analysis. The chromatographic data acquired with the Xchrom Data System is automatically dated and time stamped. Hard copies of chromatograms as well as peak area and/or height, and quantification reports may be obtained from the Data System.

Additionally, the GC signal may be plotted in real-time by an integrator connected to the GC or by viewing the acquisition status through the data system software. This will allow for rapid monitoring of the GC analysis, but in most cases will not be used for quantification (unless otherwise specified in QAPP).

The creation of the acquisition schedule is discussed in more detail in SOP 6-025. Information that must be entered include the following:

- Xchrom data system Sequence ID. The Instrument Log Book will be checked to determine which was the last analysis ID used, so that the next ID can be assigned to the first analysis in the sequence.
- Xchrom data system Method ID.
- Laboratory sample ID and project number.

A hard-copy of the data acquisition schedule is printed out, signed by the analyst, and placed in the Instrument Log Book, in accordance with the GC documentation SOP (6-025).

3.5 INSTRUMENTAL ANALYSIS

- Calibrate the GC system prior to analysis (Section 2).
- Immediately after the calibration, begin analyzing samples using the same GC conditions. Ideally, the same sample volume should be introduced into the GC as was used in the analysis of standards. A calibration check standard should be analyzed every 10 samples, as discussed in Section 2, or as specified in the QAPP.
- If a sample needs to be rerun, (i.e., bad injection, failed calibration, power outage, etc.), this will be documented on the sequence. The analyst's initials and the date should accompany the corrective action taken and the reason a corrective action is needed.

3.6 DATA REDUCTION - ANALYTE IDENTIFICATION AND QUANTIFICATION

The chromatographic data is stored on the Xchrom Data System for postrun data reduction. Analyte identification and quantification procedures are described in the applicable analytical SOPs or project plan. The Xchrom-specific procedures for data reduction are discussed in the Xchrom data system manuals and SOP 3-132.

3.7 DATA STORAGE

The chromatographic data generated during standard and sample analysis is initially stored on the Data System hard disk for postrun analysis. The reduced data is later transferred to compact disc for archival by the Data System Manager. For more detailed archival instructions refer to SOP 6-036.

3.8 DOCUMENTATION

Maintaining proper project and instrument specific documentation is critical for GC analysis. The documentation and record keeping procedures associated with GC analysis are described in a separate SOP (6-025), but are summarized below. This section is not intended to replace the documentation and record keeping SOP, and it is essential that the GC analysts are familiar with and adhere to these requirements.

Instrument-Specific Documentation
An Instrument Log Book is maintained for each gas chromatograph. The information in this book will include acquisition schedules of all analyses performed on the instrument, and maintenance logs for the instrument. The instrument-specific documentation is discussed in more detail in the GC documentation SOP (6-025), and the analyst will be familiar with and adhere to these documentation requirements.

The GC conditions are documented on the acquisition schedule. Flow rates are not verified daily, but are recorded as the rate most recently set, preceded by the "~" symbol. The maintenance logs will include

information such as routine maintenance, column changes, service, and major hardware modification/changes.

4.0 MAINTENANCE

All maintenance activities, service, and GC instrument hardware changes/modifications are documented in instrument-specific Log Books located in the GC laboratory. These documentation and record keeping procedures are described in SOP 6-025. All analysts will be trained to performed routine maintenance, and each analyst will perform the maintenance needed to conduct the analysis he/she has been assigned.

Instrument maintenance and troubleshooting is discussed in the instrument manuals under the applicable sections. The manuals also described how to disassemble the various GC components and how to perform maintenance and servicing. Maintenance will be performed to correct a performance problem, but will also be performed routinely to prevent problems. The analysts will be familiar with the routine maintenance procedures described in the following sections.

4.1 SERVICING INJECTION PORT

The injection port of actively used GCs will be serviced routinely, as described below.

- Replace septum weekly after approximately 50 injections, unless a Merlin Microseal is used, or as needed. (The Merlin Microseal can be replaced about every 20,000 injections.)

  Remove top nut on injection port. Septum will either be stuck to this nut or left in injection port. Remove with forceps. Remove any pieces of septa from inside the nut. Install new septum. Replace nut. Tighten to "finger" tight plus ¼ turn.

- Replace injection port liner weekly, after approximately 50 samples, or as needed.

  Loosen the main injection port nut on the top of the injection port until it is free. Place to one side by lifting on attached gas lines. Remove liner with forceps. Remove any small pieces of septa from around liner. Position new o-ring on new liner approximately 1.5 cm from top of clean, silanized liner (o-ring will position itself when injector is reassembled). Insert a small amount of deactivated/silanized glass wool ¼ to ½ the way up from the bottom of the liner, if appropriate for the type of analysis to be performed. Install new liner and o-ring. Reposition nut, screw down until "finger tight" plus ¼ turn.

- Clean rest of injection port monthly, after approximately 100 samples, or as needed.

  With injection liner removed, remove any foreign material from injection port from top to bottom. To clean the lower parts of the injection port the analytical column must be removed from inside the oven, after which the injector nut can be removed. Upon re-installation replace the washer and seal.

.2 COLUMN MAINTENANCE

As a column begins to degrade (i.e., poor peak shape, loss of resolution, or loss of sensitivity) clipping may be needed to remove the compromised front end of the column.

Cool the over to <50°C, then turn off the oven fan. Remove the column and column nut from the injection port from inside the GC oven. To clip a column, place the column (which is already through the column nut and graphite ferrule) on a flat surface and score the column with a diamond tip scribe or similar device (remove ~6 inches of column). Then snap the column to ensure a clean break. Never cut through the column, it will crush it and not leave a perpendicular cut. Inspect the column tip to determine if any burrs or crushing occurred during the cut. If the cut is good, install the column appropriately; if it is not good then repeat the cutting procedure. Check the integrity of the column connections using a leak detector.

4.3 PRECOLUMN INSTALLATION/REPLACEMENT

Capillary analytical columns may be fitted with a 5 m precolumn. The precolumn is a length of deactivated, uncoated fused silica column that is connected to the analytical column by a butt connector. When degradation in chromatography is observed, the precolumn is replaced without any loss in the length or performance of the analytical column.

Cool the oven to <50°C and turn off the oven fan. Remove the column and column nut from the injection port from inside the GC oven. Install a 5 meter length of precolumn. Inspect both ends of the precolumn and the front end of the analytical column to ensure that the ends are trimmed cleanly. Connect one end of the precolumn to the analytical column using a butt connector or instrument specific connector and the other end of the precolumn to the injection port, following the column installation instructions in the appropriate instrument manual. Check the integrity of the column connections prior to use.

4.4 GAS CYLINDER REPLACEMENT

Replace cylinder when tank pressure drops below 300 psi.

4.5 OTHER MAINTENANCE

Other maintenance that will be performed routinely, and/or as needed, include the following:

- Autosampler Rinse Vials - make sure the solvent rinse vials are full prior to starting a new analysis sequence. Replace the solvent weekly and rinse the vials at the same time. These activities do not need to be recorded in the maintenance records.

- Autosampler Syringe - replace and/or align as needed when it does not operate properly (e.g., plunger sticks or aspirates/dispenses air, needle is no longer straight or properly aligned). Consult autosampler manual for more information.

- Ferrule Replacement - several ferrules (mostly graphite ferrules) are installed in the injector, detector, and column interfaces from inside the GC oven. These ferrules ensure a good seal,

- Ferrule Replacement - several ferrules (mostly graphite ferrules) are installed in the injector, detector, and column interfaces from inside the GC oven. These ferrules ensure a good seal, and with time they will need to be replaced. Check for gas leaks on a regular basis, and inspect the ferrules whenever the fitting is disconnected. Replace as needed.

- Gas Drying/Purification Traps - Instrument performance and gas quality will dictate the types of gas purifiers to be used, and when they are to be replaced. The date of installation of the gas purification trap will be recorded on the trap at the time of installation. The following gas purification traps may be appropriate:

  OMI-2 (Supelco), or equivalent - indicating purifier used to remove oxygen, water, and trace contaminants from helium, hydrogen, nitrogen, and argon/methane. This trap is *not* to be used for the air supply. The trap is ideally placed in the gas line prior to the gas distribution manifold. Replace as needed (when indicator turns brown) but at a minimum once a year.

  Hydrocarbon trap - used for air supply. The trap is ideally placed in the gas line prior to the gas distribution manifold. Replace as needed, but at a minimum once a year.

  Moisture trap - used for air supply. The trap is placed in the gas line prior to or after the gas distribution manifold. Replace as needed, but at a minimum once a year.

- Column Bakeout/Cleaning - with time, high molecular weight compounds will deposit onto the column and may create analysis problems. Many of these compounds may be removed by raising the temperature of the oven 10 to 50°C above the highest temperature of the sample analysis temperature used, and allowing the GC to remain at this temperature for 1 h or more. The temperature of the detector should also be raised, ideally to the same or a slightly higher temperature than the oven temperature, to prevent contaminant deposition in the detector. Before raising the temperature consult the manuals to determine column and detector maximum temperatures.

- Column Changing - columns are changed when a different type of column is needed for the analysis to be performed, and when the current column no longer performs adequately. Instructions on column installation, including proper positioning, can be found in the instrument manuals.

- Detector Bakeout and Cleaning - with time compounds will deposit in the detector and may create analysis problems. Some of these compounds may be removed by raising the temperature of the detector to 10 to 50°C above the standard operating temperature, and allowing the GC to remain there for 1 h or more. Significantly longer bakeout periods may sometimes be needed. Before raising the temperature consult the manual to determine detector maximum temperature.

  Should detector "bakeout" not adequately clean the detector it may need to be disassembled and subjected to more rigorous cleaning. Consult the manual for detector cleaning.

For all other maintenance, and for more detail on the above mentioned maintenance, refer to the appropriate instrument manual.

4.6 SERVICE

Battelle has service contracts on all gas chromatographs. If there are operational problems that cannot be resolved by routine maintenance or by consulting the instrument manuals, the analyst will inform the GC Group Leader about the problem. They will jointly review the problem, consider corrective actions, and decide if it is appropriate to call Hewlett-Packard for service. Each instrument problem will be dealt with on a case by case basis, including when to place a service call. All service calls are documented in the instrument-specific Log Books.

4.7 VERIFICATION OF PERFORMANCE

After instrument maintenance has been performed the analyst must verify that the instrument is back in working order and that any problems requiring maintenance have been corrected. This verification is accomplished by verifying instrument calibration. Either a calibration check standard or a new initial calibration curve that meets the acceptance criteria of this SOP will verify instrument operation.

5.0 TRAINING

An analyst performing the procedures described in this document must initially work under the supervision of qualified, trained staff. The analyst must have read and been given proper training and thorough understanding of this SOP, the GC documentation SOP (6-025), and instrument instruction manuals. Appropriate training consists of direct tutorial by the GC Group Leader, or other qualified individual(s) designated by the GC Group Leader. This training will include a demonstration of proficiency in the areas indicated on the Certificate of Training (Attachment 1).

Individuals may work independently once they have satisfactorily completed supervised training. Once training has been completed a Certificate of Training will be completed and provided to the Quality Assurance Unit.

6.0 SAFETY

- Heated zones of the GC are hot and to be avoided.
- Compressed gas cylinders must be secured at all times.
- Specific safety concerns of any particular analysis procedures will be listed in the project work plan or procedure/analysis SOP.

7.0 ATTACHMENTS

Attachment 1. Example of Certificate of Training

APPROVALS

Author:		8-6-99
Laboratory Manager:		8-6-99
Quality Systems Manager:		8-10-99
Environmental Chemistry Section Manager:	for G S Durell	8-9-99
	Name	**Date**

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

FOR

SOP NO. **3-116-04**

TITLE: **OPERATION AND MAINTENANCE OF GAS CHROMATOGRAPHS**

TRAINEE: _____

Task	**Independent Operation Demonstrated**	
Read SOP 3-116	_____	_____
Read SOP 6-025	_____	_____
High Pressure Gas System	_____	_____
Gas Chromatograph Programming, Initialization, and Routine Operation	_____	_____
Routine Maintenance	_____	_____
Data Acquisition	_____	_____
Data Electronic Transfer and Calibration Check	_____	_____
Data Analysis and Quantification	_____	_____
Data Package Compilation	_____	_____
	Date	**Observer/Training Supervisor**

APPROVED: _____ _____
Name **Date**

Battelle Duxbury Operations
Standard Operating Procedure

for

USE OF ELECTRONIC BALANCES

Summary of changes in this version: The use of a computer for direct-capture of data and electronic entry into spreadsheets is added. The acceptance criteria for calibration checks is modified. The identification and labeling of each NIST-traceable weight set is described. Note that this SOP still requires that balances be calibrated using two weights that bracket the expected weight of the samples.

1.0 APPLICATION

Two types of electronic balances are covered by this SOP: top loading and those with a pan in an enclosed chamber. These balances are capable of weighing within a wide range of accuracy (0.01mg to 3000 g), and the selection of the balance to be used for any task should be determined by the precision required. Balances which have a pan in an enclosed chamber, such as the Mettler AC100 and Mettler AT201, are capable of weighing within four or five decimal places (0.0001 to 0.00001 g) in ranges up to 205 g. The top-loading balances, including various models of Ohaus, Mettler, and Sartorius balances, are capable of weighing to one, two, or three decimal places (0.1, 0.01 or 0.001 g) to a maximum weight of 3000 g.

2.0 CALIBRATION

Balances are calibrated annually by a professional balance service using NIST-traceable calibration weights.

Balance calibration must be checked prior to each day's use. Calibration must also be checked if the balance is moved from one location to another. Balances are customarily left plugged in and "on" or in "standby" mode. If the balance has been unplugged or turned "off" then a warm-up period of 30-90 minutes, depending on the balance, is required prior to calibration.

Balance calibration is checked by qualified operators using NIST Class 1 and 2 weights. The weights selected as calibration checks should bracket the anticipated weight of the sample. The results of the calibration checks are recorded on the Balance Calibration form (Attachment 1) located in the Balance Logbook specific to the balance being used. Based on the guidance of ASTM E617-91 most balances at Battelle are used as class 2 or 3 balances. As such, the accuracy of each balance must be verified at the level of accuracy for which measurements will be made. (In other words, to the last readable decimal place).

Example 1:

> The Mettler AC100 is a 5-place balance that reads to 0.00000 g. If weights will be measured at the 5th decimal place then the acceptance criteria for the calibration check is ± 0.00001 g.

Example 2:

The Ohaus PE3600 reads 0.000 g. or 00.00 g. depending on the selected sensitivity setting. The calibration checks for these two settings must be ± 0.001 g or 0.01 g, respectively.

Minor calibration adjustments are possible on some models (see Attachment 2). If the required accuracy level cannot be achieved even after adjustments, contact the Laboratory Manager who will arrange for professional servicing of the balance.

3.0 OPERATION

3.1 Routine Operation

The balance must be located on a stable surface. Most balances have a level indicator; in these cases, adjustments to level the balance are made using adjustable "feet." The balance should also be kept away from drafts, vibrations and direct sunlight.

Zero the balance by pressing "Tare" or "Zero." Place the material/object to be weighed on the pan of the balance. Read the weight and record, if necessary. With many of the electronic balances, it is possible to connect with a computer interface so that weights may be recorded directly into spreadsheets.

To weigh only the contents of a vessel, place the vessel on the balance pan and press "Tare" or "Zero." Add the material to be weighed to the vessel, and record the weight.

Operating options vary from balance to balance, but may include: automatic door operation, vibration compensation, weight units, and computer interface. The manufacturer's instructions, located in the balance logbook for each balance, define the options relative to each instrument. Attachment 2 provides a summary of operations.

3.2 Electronic Data Entry

Balance weights may be sent directly to a spreadsheet for some balances. The program is accessed on Windows-based computers by selecting sequentially:

Start → Programs → WINWEDGE → WINWEDGE → File →Open → Sample Weights or Lipid Weights

then, Activate → Normal Mode → Open spreadsheet for the analysis batch

Once the spreadsheet is open, place the sample on the balance and wait until the balance measurement has stabilized. Place the computer cursor in the correct spreadsheet cell (e.g., Tare Weight) and hit the **ENTER PRINT** bar on the balance. Verify that the weight is entered into the correct cell.

4.0 MAINTENANCE

The balance must be cleaned after each use using a soft brush, a moist, soft cloth, or lint-free tissue. If necessary, remove pan and wash with soapy water. Thoroughly dry the pan before replacing. Other surfaces of the balance should be cleaned if needed as well as the bench area around the balance. Do not use solvents or abrasives on the balance surfaces.

The Lab Manager or designee is responsible for balance maintenance. Each balance is cleaned, inspected, and certified annually by a professional balance service (Bay State Scale and Systems, Inc., Cambridge, MA, (800) 696-8232 or equivalent). A record of this maintenance and calibration is kept in the logbook for each balance. If any other maintenance, such as calibration adjustment (see Attachment 2) is performed, it must be documented on the Balance Maintenance form (Attachment 3). A brief description of the maintenance procedure, date, and initials of the operator should be documented on the form.

Battelle owns several sets of NIST-traceable Class 1 and 2 weights. Each set of weights is uniquely identified with a unique weight set ID (Table 1). One set of weights will be calibrated annually by a professional balance service (Denver Instrument Co., Arvada, CO, (303) 431-7255, or equivalent) and used to verify that all weights in the other sets fall within the acceptable range for their class. A record of this calibration is kept in the Chemistry Section files (Attachment 4). A sticker indicating the calibration date is placed on each set of weights.

Table 1. Weight IDs for the Calibration Weights Sets Used in the Analytical Laboratory

Weight ID	Full Name (manufacturer) [1]	Storage Location
CS#1	Christian Becker set 1	Organic Prep Lab # 1
CS#2	Christian Becker	Organic Prep Lab # 3
P#1	Permas	Oil/weathering
A#1	Ainsworth	Lower Level Chem North

5.0 TRAINING

Trainees must read this SOP. A certified operator will then demonstrate the use of each type of balance: top-loading and enclosed pan. The instructor and trainee will then each weigh a series of five NIST Class 1 or 2 weights on each type of balance and record the results on the Certificate of Training (Attachment 5). If the results of the trainee are within 2% of those of the instructor, a training certificate will be issued. Original copies of each individual's training certificate will be kept in the Quality Assurance Unit training files.

6.0 SAFETY

The use of the balances presents no specific safety hazards. However, care must be exercised when handling hazardous or potentially hazardous materials or samples.

7.0 ATTACHMENTS

Attachment 1. Example of Balance Calibration Form

Attachment 2. Calibration Adjustments for Specific Instruments

Attachment 3. Example of Balance Maintenance Form

Attachment 4. Example for NIST-traceable weight calibration verification form

Attachment 5. Example of Battelle Duxbury Operations Certificate of Training

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APPROVALS

Author	Richard L Motta	11/12/98
Laboratory Manager		11·12·98
QA Coordinator	Rosana Burke	11-12-98
Environmental Chemistry Section Manager	Name	11/19/98 Date

ATTACHMENT 1
Balance Calibration Form

Balance Brand/Model: _____ **Serial Number:** _____

NIST Class 1 or 2 Weight (g) 1	Tared Display (g)	Measured Weight (g)	Recalibration Required (Y/N)2	Results of Recalibration	Date and Initials
Ex: 1 g	0.00000	1.00001	N	NA	

Comments:

1 NIST calibration weight ID _____ assigned to this balance. If another weight set is used this must be documented.

2 Recalibration is required if the measured weight is not ±0.nnn1 g where nnn is equal to the decimal display of the balance.

ATTACHMENT 2

Calibration Adjustments for Specific Instruments

- **Ohaus GT4000, GT4000D:** If after placing a calibrated weight on the pan, the difference between the calibrated weight and the reading is >5%, proceed as follows:

  1. Turn the balance ON and allow it to warm up with no weight on the pan for at least 30 minutes.

  2. Press and hold the ON/rezero switch. Release the switch as soon as CAL is displayed. The display will show "C O", indicating that no weight should be on the platform.

  3. Momentarily push the ON/rezero switch.

  4. The balance will then display a number which corresponds to the value of the calibration weight which should be placed in the center of the pan.

     NOTE: If no calibration weight is available, press the OFF switch to save the existing calibration.

  5. Place the appropriate calibration weight on the center of the pan and momentarily press the ON/rezero switch.

  6. Wait until the balance displays the indicated calibration weight value. This indicates that the balance is now calibrated.

- **Mettler AC100:** Must be calibrated if the balance is moved to a new location. To calibrate, a set of NIST-traceable weights totaling 70 g (50 g + 20 g) is necessary.

  1. Checking the Calibration: The balance must be connected to a power supply for at least 30 minutes and must be leveled.
  - Press control bar (19-Figure 1): 0.0000 g
    (If not exactly zero, press bar again)
  - Place test weights (total of 70 g) on pan. Read display.

  If the Balance indicates 70.0000 g, the calibration is okay; if not, the balance must be recalibrated. Set the weight display using the calibration screw (1-Figure 2) as described in Step 2. After every correction, recheck display. See Step 3.

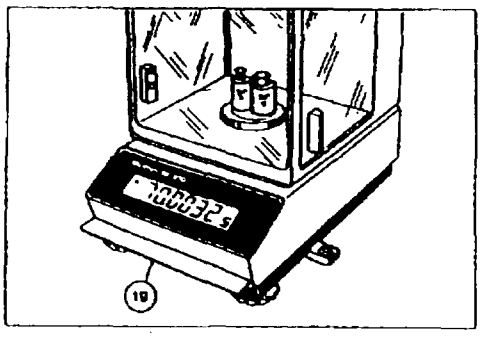


Figure 1

Attachment 2 (continued)

2. Resetting the weight display:
   - Lift off the weights
   - Insert screwdriver into the opening (1-Figure 2) and turn the calibration screw located there: Clockwise if the displayed weight is more than 70.0000 g; Counterclockwise if the displayed weight is less than 70.0000 g.
   One turn of the screw changes the weight display by about 0.006 g.

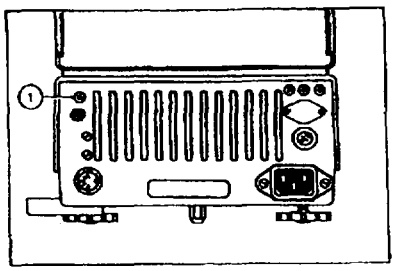


Figure 2

3. Checking the new setting:
   After every correction made using the calibration screw, recheck the result:
   - With test weights removed, press control bar (19-Figure 1): 0.0000 g.
   - Place test weights on pan; read result.
   If the balance now indicates 70.0000 g, it is ready to use-- if not, it must be corrected further by using the calibration screw and checking again until the display is correct.

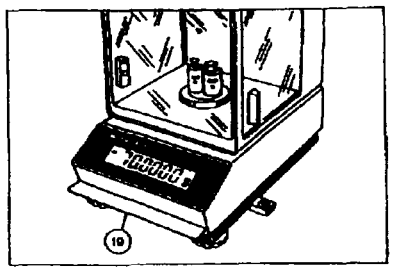


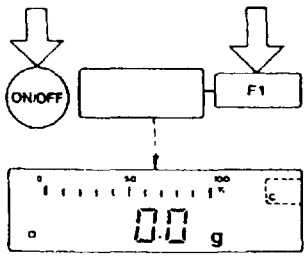
Figure 1

- **Sartorius PT600:** Requires an accurate (certified) calibration weight of 500 g.

1. Remove the protective cap located on the front right of the balance and slide the access switch in the direction of the arrow.

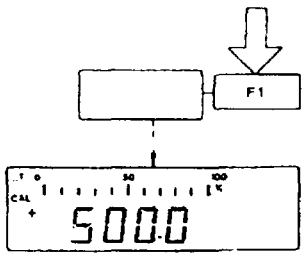
2. With the balance turned off, hold down the F 1 key and briefly press the ON/OFF key.

   Upon completion of the automatic self-test, release the F 1 key as soon as "**C**" is displayed.



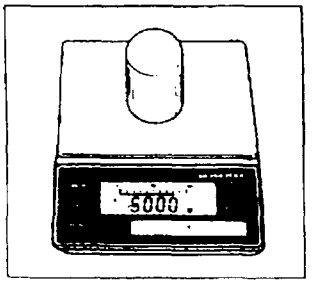
3. Unload the balance and press the tare control to zero the display.

4. When the display shows a zero readout, press the F 1 key. "**CAL**" and the calibration weight readout will now be displayed.



5. Place the calibration weight in the center of the weighing pan. Now the weight unit is displayed which indicates the end of the calibration procedure. Relock the calibration access function by

Attachment 2 (continued)



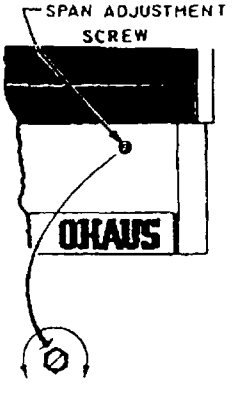
sliding the access switch back to its original setting, and replace the protective cap. After calibration, use the ON/OFF key to turn the balance off then on again so that you will have direct access again to the dedicated application programs preselected by the menu code.

- **Ohaus GT480:** If after placing a calibrated weight on the pan, the difference between the calibrated weight and the reading is >5%, proceed as follows:

  1. Remove all weight from the platform.
  2. Initialize the calibration menu by pressing and holding **ON/TARE** until **CAL** is displayed, then release immediately. **C 0 g** will then be displayed; indicating that no weight should be on the platform.
  3. Momentarily press **ON/TARE.** The display will blank, then show - C - momentarily before showing C followed by the value of the calibration weight to be place on the platform.
  4. Place the required weight on the center of the platform.
  5. Momentarily press the ON/TARE and DO NOT remove the calibration weight until the balance displays a weight reading.
  6. The balance should now be calibrated.

- **Ohaus 3000D (Brainweigh):** Calibration must be checked/adjusted if the balance is moved from one location to another.

  1. Turn the balance **ON** and allow a minimum of twenty minutes for it to thoroughly "warm up".
  2. Zero the balance by pressing the TARE BAR.
  3. Place a known (certified) mass of at least half the capacity of the balance in the center of the platform.
  4. Should the display not agree with the known mass, the **SPAN** must be adjusted. The SPAN **ADJUSTMENT** screw is located above the **TARE BAR** on the right side (when viewed from the front). Using the screwdriver supplied with the balance, slowly rotate the screw until the displayed weight is equal to that of the known mass.



Attachment 2 (continued)

5. Remove the known mass and depress the TARE BAR. Replace the known mass on the platform. If the displayed mass is not equal to that of the known mass, repeat step 4.

- **Mettler PE3600:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to the power source for 30 minutes before calibrating it.

  2. Press the control bar until zero appears in the display.

  3. Place the test weight on the pan and read the display after the ring symbol (stability detector) has been blanked out. If the balance displays the weight of the test weight accurately, the calibration is okay. If not, the calibration must be corrected.

  4. Remove the test weights from the pan. Press the control bar until the display indicates "-----", then release the control bar.

  5. "- C -"appears in the display.

  6. Place test weight (1000 g for the P3600) on the pan. The balance automatically performs its own calibration. After calibration is completed, the display indicates "1000.0 g".

- **Sartorius AC210S:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to a power source for 30 minutes prior to use.

  2. Pressing "T" tares (zeros) the balance.

  3. The weight can be sent to an electronic spreadsheet by pressing ⊙ once the sample weight has stabilized.

- **Ohaus Explorer EOD120:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to a power source for 30 minutes prior to use.

  2. Pressing "O/T" tares (zeros) the balance.
  3. The weight can be sent to an electronic spreadsheet by pressing "Enter" once the sample weight has stabilized.

ATTACHMENT 3
Balance Maintenance Form

Balance Brand/Model:_____ **Serial Number:**_____

Description	Date	Name

ATTACHMENT 4

Verification of NIST-traceable weight ranges

Standard Weights (Nominal weight and acceptable range)	MEASURED WEIGHTS[1]		Acceptable? (Yes/No)
	Standard Weights ID _____	Working Weights ID _____	
CLASS 1			
5 kg ± 12.0 mg			
3 kg ± 7.5 mg			
2 kg ± 5.0 mg			
1 kg ± 2.5 mg			
500 g ± 1.20 mg			
300 g ± 0.75 mg			
200 g ± 0.50 mg			
100 g ± 0.25 mg			
50 g ± 0.120 mg			
30 g ± 0.074 mg			
20 g ± 0.074 mg			
CLASS 2			
10 g ± 0.074 mg			
5 g ± 0.054 mg			
3 g ± 0.054 mg			
2 g ± 0.054 mg			
1 g ± 0.054 mg			
500 mg ± 0.025 mg			
300 mg ± 0.025 mg			
200 mg ± 0.025 mg			
100 mg ± 0.025 mg			
50 mg ± 0.014 mg			
30 mg ± 0.014 mg			
20 mg ± 0.014 mg			
10 mg ± 0.014 mg			
5 mg ± 0.014 mg			
3 mg ± 0.014 mg			
2 mg ± 0.014 mg			
1 mg ± 0.014 mg			

[1] Balance ID used to check weights: _____. Please note that not all weights listed may be applicable.

ATTACHMENT 5

Battelle Duxbury Operations
CERTIFICATE OF TRAINING

SOP No. _____

SOP Title _____

Trainee _____

Instructor _____

Date SOP Read _____

Date Training Completed _____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Results: Enclosed Pan Balance

NIST Class 1 or 2 Weight	Trainee Results	Instructor Results	Difference

Results: Top-Loading Balance

NIST Class 1 or 2 Weight	Trainee Results	Instructor Results	Difference

Approved by_____ **Date**_____

Battelle Duxbury Operations
Standard Operating Procedures

for

QUALITY ASSURANCE FACILITIES INSPECTIONS

Summary of changes in this version: . The reporting procedures (Section 5.0) are identical to those described in SOP 4-015. Specific references to the section number are deleted.

1.0 OBJECTIVE

Facilities inspections are conducted by the Quality Assurance Unit (QAU) to assure management of proper use and maintenance of equipment and facilities as defined by Battelle policies, standard operating procedures (SOP), and government regulations. These facilities may include analytical, database, benthic, histology, and field operations. This SOP describes the policies, procedures, responsibilities, and records associated with the conduct and reporting of facilities inspections.

2.0 PREPARATION

It is the responsibility of the inspector to prepare for each inspection. This preparation should include a thoroughly review of the requirements of this SOP and those of the specific SOPs that are used in the area to be inspected. Compliance with site policies (Section 3.2) should always be assessed.

3.0 PROCEDURES

Facilities inspections are conducted by QAU personnel. During the inspection, selected work areas within the facility are inspected for compliance with the standards discussed below. Areas are chosen by QAU staff based on several criteria, including whether work is being performed in the area and the date of the last inspection of that area. Specific areas may be targeted if a problem is suspected but in general each active facility should be inspected annually.

A facilities audit may include a review of an entire operation or it may focus on one aspect of the facility. In the latter case one or more specific standard operating procedures are used to direct the inspection.

3.1 GENERAL INSPECTION GUIDELINES

Each facility inspection is an evidence-gathering exercise. The job of the inspector is to gather objective evidence that confirms that (1) the facility is in compliance with laboratory policies and procedures, or (2) that it is not. Objective evidence takes the form of exhibits (documentation), direct observation of practice, and interviews with staff. It is the responsibility of inspectors to be objective, to confirm their observations, and to review evidence with the staff to clear up false impressions.

3.2 GENERAL POLICIES

All facilities must comply with the following general site policies.

- Adequate space should be provided for work and storage areas. Laboratory areas should be free from clutter.
- Facilities should be designed and used so that there is a degree of separation that will prevent any function or activity from having an adverse effect on any other function or activity.
- Appropriate personal protective equipment should be worn by personnel.
- Equipment used or involved in data generation activities should be inspected, cleaned, maintained, and calibrated as defined in SOPs or equipment logs. Written records should be kept for all inspection, maintenance, and calibration operations.
- Current, approved SOPs relative to the work being performed should be immediately available to personnel in each work area.
- Personnel should be appropriately qualified or adequately trained to perform their tasks. Written records of qualifications and training should be forwarded to the QAU training files by the appropriate managers.
- Disposal of contaminated or hazardous waste materials should be in accordance with SOPs.
- Raw data entries should be recorded directly, promptly, and legibly in ink. Each entry should be dated on the day of entry and signed or initialed by the person entering the data. Changes to entries should be made so as not to obscure the original entry, should indicate the reason for the change, and should be initialed and dated by the person making the change.

3.3 LABORATORY INSPECTION ELEMENTS

A full laboratory inspection should assess the following elements, in addition to the general requirements described in Section 3.2.

3.3.1 Test Substances, Solutions, Solvents, and Reagents

In analytical areas, any test substances, solutions, and reagents used in a facility are reviewed for conformance to the standards listed below.

- The receipt, handling, preparation, storage, and distribution of test substances, solutions, and reagents should be in accordance with project protocols or QA Project Plans (QAPPs) SOPs, the manufacturer's instructions, and Material Safety Data Sheet (MSDS) information.
- Test substances, reagents, and solutions should be labeled according to SOPs 5-217 and 5-027.

- Deteriorated or outdated materials should not be used and should be discarded on a routine basis in accordance with SOPs 6-010 and 5-114.

- Storage of test substances, reagents, and solutions should be adequate to maintain their purity, strength, and composition.

- The purity of solvents and reagents should be verified according to SOP 5-015.

3.3.2 Sample and Data Control

Sample naming, receipt, storage, and custody records should conform to the requirements of SOPs 6-007 and
6-010 and the following standards (when samples are collected or shipped by other organizations these records may not be available):

- Written records of the history of each sample should exist, including collection, preservation, transfer, storage, processing, analysis, and disposal.

- Written records of sample receipt should be maintained and should include the date and time of receipt, the condition of the samples upon receipt, and the identity of the persons releasing and receiving the samples.

- Each sample should be uniquely identified and should be traceable from collection through reporting.

- Samples should be held according to storage conditions defined in SOPs 6-010, the QAPP, or government regulations. Samples should be discarded according to SOPs 6-010 and 5-114.

3.3.3 Instruments and Equipment

The laboratory contains a wide array of instrument and equipment. Inspection of analytical instruments should be assessed in SOP-specific inspections (Section 3.6). The operation and maintenance of small equipment can be reviewed as part of a routine facilities inspection. This equipment includes, but is not limited to, balances (3-160 and 3-004), refrigerators and freezers (SOP 3-095), thermometers (SOPs 3-018 and 3-151), the deionized water system (SOP 3-084), HPLC (SOP 5-191), and non-data generating equipment (SOP 3-133). The following criteria are reviewed for these types of equipment.

- Standard operating procedures must be prepared and available for all instruments or equipment that generates data.

- Instrument and equipment logs should document calibration, use, and maintenance.

- All staff using the equipment should have documented training in the appropriate SOPs.

3.3.4 *Control Charts*

A review of control charts (SOP 7-028) should be conducted to ensure that they are current, that documentation follows the SOP requirements, that the appropriate quality control samples are being plotted for each analytical batch, and that corrective action is taken and documented when necessary.

3.3.5 Labware Cleaning

The inspector can verify compliance with the labware cleaning SOP (5-216) through observations and discussion with the glassware washer. The inspector should determine that the staff member understands the washing, rinsing, and heat treatments for the various types of labware used in the facility.

3.4 FIELD OPERATIONS INSPECTIONS

Inspections of field operations *in the field* are conducted on a project-specific basis according to SOP 4-027 Section 2.3. An inspector's presence must not jeopardize the field mission or create over-crowding or safety risks. Like laboratory inspections, field inspections focus on general policies (Section 3.2) and SOP requirements.

Shore-based field operations inspections typically review equipment calibration, maintenance and operations inspections. These are based on specific SOPs and are discussed in Section 3.6.

3.5 DATA MANAGEMENT INSPECTIONS

Inspections of data management activities should include a review of the following procedures.

- Receipt records of electronic or hardcopy data submitted for data management should include the date of receipt, login ID, and the submitter.
- Written procedures for data loading, format requirements, and storage should be available.
- Changes to data should be recorded such that the documentation is linked to the project data.

3.6 SOP-SPECIFIC INSPECTIONS

Facilities inspections in the analytical laboratory are often conducted as a series of SOP compliance inspections. For SOP-specific inspections the inspector should verify that

- the procedures described in the SOP are followed exactly as written
- all documentation described in the SOP is maintained as specified
- any quality control requirements specified by the SOP are implemented
- all staff using the SOP have documented training

4.0 SCHEDULE

At least one facilities inspection is conducted quarterly, or as necessary to review facilities operations. The appropriate Resource or Laboratory Manager is notified prior to the inspection to ensure that personnel will be available to answer questions during the inspection and to avoid scheduling conflicts. Unannounced inspections are conducted only if a problem is suspected. Facilities inspections will also be conducted at the request of management (Resource Managers or Laboratory Director).

5.0 REPORTING

Once the inspection is complete, the auditor prepares an inspection report that summarizes the results of the inspection. Any deficiencies are described and corrective actions are recommended. General and project-specific issues are identified as such and discussed separately in the inspection report.

The reporting procedures for project inspections are identical to those described in SOP 4-015. Outstanding issues are resolved through discussions with Lab, Project, or the Resource Manager, as necessary. All original audit reports are maintained in the QAU files.

6.0 TRAINING

An individual who is being trained to perform facilities inspections must first read this SOP. The trainee must then accompany an experienced inspector (instructor) on facilities inspections until it has been established to the instructor's satisfaction that the trainee is able to perform an inspection without supervision. A certificate of training (Attachment 1) is issued upon completion of training and is maintained in the QAU training files.

ATTACHMENTS

1. Example of Certificate of Training

APPROVALS

Author	Donna J Bahl	12-10-99
Quality Systems Manager	Donna J Bahl	12-10-99
Vice President, Battelle Duxbury Operations	Name	12-10-99 Date

Attachment 1

Battelle Duxbury Operations
Certificate of Training

SOP No. 4-009

SOP Title Quality Assurance Facilities Inspections

SOP read and understood _____ _____
Name Date

Description of Training	Date	Instructor

Battelle Duxbury Operations
Standard Operating Procedures

for

QUALITY ASSURANCE AUDITS OF REPORTED DATA

Summary of changes in this version: Define how the year 2000 will be used in assignment audit numbers. Clarify that the results of data audits are characterized as "acceptable," "acceptable once corrections are made," or "rejected" (Section 4). Clarify that data packages are not acceptable until every error is corrected and all documentation is complete.

1.0 INTRODUCTION

Audits of reported data (e.g., tables, figures, spreadsheets, databases) are conducted by the Quality Assurance Unit (QAU) to ensure that the data are accurate, traceable, defensible, complete and generated according to the project plan. This policy applies only to data generated for projects involving environmental measurements (i.e., involving the determination of chemical, physical, or biological factors related to the environment); financial and administrative data are excluded.

2.0 PROCEDURES

2.1 DATA SUBMISSION AND RECEIPT

All data to be audited must be validated (SOP 6-027) before being submitted to the QAU. The data validation process performed by technical personnel may include, but is not limited to, recalculation of reported data, verification of transcriptions, double keypunching, identification and removal of outliers or spurious data points, assessment of quality control (QC) results, performance of electronic QC checks, assessment of compliance with the project work plan, and evaluation of scientific validity.

Once validation is complete, the data are submitted for audit. Any original data and supporting documentation necessary to reconstruct the task or project should be provided. It is the responsibility of the person preparing the spreadsheets, report tables, or database output to ensure that the data are clearly labeled and that supporting data are complete and organized so that the auditor will be able to trace data without assistance. Documentation in the analytical data packages should include explanations of correction factors and all work plan or SOP deviations.

All formulas used in data calculations must be documented in one of the following formats

- full mathematical description in the work plan
- full mathematical description in an SOP and referenced by SOP number in the work plan
- full mathematical description in the data package or referenced by SOP number

Upon receipt in the QAU, the data package is logged in by project and data type and assigned a unique ID number in the format XX-yyyy

where: XX is the year (e.g., 00 for year 2000).
yyyy is a sequential four digit number starting with 0001

An example data tracking form is provided in Attachment 1.

3.0 DATA AUDIT PROCEDURES

Two types of data audits are performed at Battelle. The traditional statistical random audit (Section 3.1) is selected for data sets where the errors are likely to be randomly distributed throughout the data set (key-punched, transcribed, or hand-calculated data). Targeted data audits (Section 3.2) are conducted where the errors are more likely logical or global (unusual sample processing, computer generated or loaded data, data generated in spreadsheets). The project work plan should define the type of audit most appropriate for the data anticipated. If this specification is not made in the work plan then the audit strategy will be determined when the data are submitted to the QA unit for verification. Generally, targeted audits are performed on analytical data and report tables generated at Battelle while statistical audits are performed on subcontractor data, transcribed data, and electronically captured data. Both types of audits involve verifying selected reported values using the original (raw) data, previously audited data, and supporting documentation.

3.1 STATISTICAL RANDOM AUDIT

This procedure, which is based upon the Modified British Standard 6001 described by Hoover and Baldwin (1984)[1], is designed to ensure a 95% chance of detecting whether 1% or more reported values disagree with the original data.

Upon receipt of the data, the auditor photocopies the data to be audited and counts 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. A description of data included in each data set and the number of data points per set is included in the audit report.

Once the data points have been counted and numbered, the auditor determines the number of points to be checked based on the Modified British Standard 6001 (Attachment 2). For example, if the data set contains a total of 303 data points, 50 of those points will be audited. The specific points to be audited are randomly selected using a DOS or Windows 95 random number generator, a calculator, or a random numbers table. These randomly selected points are highlighted on the photocopied data set and attached to it.

Alternative randomizing methods are employed when large data sets of electronic data are transferred from one format to another (e.g., from ASCII files to a client database such as ORACLE). In these cases, the data may be randomized electronically by the analyst prior to submittal. Frequently, these data are randomized by row rather than by individual data point. When an alternative randomizing procedure is appropriate then the randomizing method will be determined by the Project Manager and QA personnel and described in the audit report.

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[1] 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.

3.2 TARGETED AUDIT

This procedure involves the review of specific data based on a knowledge of analytical data manipulation procedures. First, the auditor photocopies the data tables and determines if any data should be verified 100% and where data errors might logically occur (e.g., a sample that was split or re-fractionated after the initial preparation was complete). Minimum verification requirements are

- 100% verification of hand-calculated data, dilution factors, fractions, and all table header information.

- For organic chemistry analyses: at least one target analyte concentration, its relative qualifier, and one surrogate recovery for each sample, being sure to alternate between applicable recovery internal standards, and one complete calculation for each type of QC sample. (For the unusual cases where the target analyte list is less than 10 compounds per sample, either one target compound **or** one surrogate recovery is checked.

- For dissolved inorganic nutrients analyses: one value for each analytical sequence and one complete calculation for each type of QC sample.

- For database audits: verification of loading completeness, accuracy, and logical range checks for each parameter, and (if available) validation of QC checks performed by the analyst.

3.3 AUDITING THE DATA

The data audit is accomplished by checking each selected data point or sample to ensure that it accurately reflects the original data and is traceable throughout its history. For reported values that involve manual, spreadsheet, or instrument calculations where either transcription errors or incorrect manual entries can occur, the auditor repeats the full calculation. Values that result from manipulations involving complex software programs or from high-rate electronic data acquisition systems (e.g., statistical data or BOSS data) are checked vs. results files but are not reconstructed. In these cases, technical validation by someone familiar with the processing procedures is crucial; the type of QA audit performed will be determined jointly by the Project Manager and QA personnel.

The auditor also reviews the Quality Assurance Project Plan (work plan) to ensure that the processing, analytical, and reporting requirements have been met.

3.4 DATA REJECTION

When a data set is unacceptable, the data are returned to the Laboratory or Project Manager who is expected to oversee a review of the entire data set and the necessary corrections. The corrected data are returned to the QAU and the entire data set is re-audited.

3.4.1 Statistical Audits

Errors in reported data are defined as any values that cannot be derived from the original data, including, for example, miscalculations and transcription errors. For random audits (Section 3.1), if the auditor finds the number of errors in the highlighted data points to be greater than the acceptance criteria (as

defined in Attachment 2), the data are judged to be unacceptable ("rejected") and the audit terminated. Errors in data points other than those highlighted are noted by the auditor but are not used in determining the number of errors detected.

3.4.2 Targeted Audits

There are no statistical rejection criteria for target audits, however, a data package is rejected if

- the reported values cannot be traced back to the original data
- if more than 10% of the data are incorrect
- if two or more major error types are noted (e.g., more than one species or compound is missing from the report tables, all reported data are affected by a formula error).

3.5 SCHEDULING

Data audits may be performed at any stage of a project at the discretion of the Project Manager. Project Managers may deliver

- Spreadsheets (e.g., Excel) of all data values. These may be the final deliverable or an intermediate listing in advance of final tables.

- Final tables or appendices that will be inserted directly into report text

- The complete final report including all tables, figures, and text.

Ideally, the audit should be performed after final tables and figures have been prepared but before the accompanying text (if any) has been finalized, so that the text draws from verified data. If intermediate tables are provided for audit then the final product should be submitted to the QAU for verification against the previously audited data.

The Task Leader or Laboratory Manager is responsible for notifying the QAU of data delivery dates and for apprising the QAU 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 the Resource Managers or designees.

4.0 REPORTING AND DOCUMENTATION

4.1 REPORTING

4.1.1 Audit Reports and Audit Files

Upon completion of the audit,

1. The copies of the data tables are marked to indicate the results of the audit as "acceptable," "acceptable once corrections are made," or "rejected." The tables are initialed and dated by the auditor. Any original tables that are "acceptable" are stamped (on the back) to indicate that they have been reviewed and accepted by the QAU.

2. The auditor prepares an audit report for every data audit. The audit report defines the data as acceptable, acceptable once corrections are made, or rejected. The audit report template is illustrated in Attachments 3a and 3b.

- If the data package is considered acceptable once corrections are made then the audit report specifically describes any errors that were found

- If the data were rejected, the audit report will not describe the specific errors identified; it will only describe the type and location of errors (e.g., weight data transcription, percent dominance calculation).

- All audit reports will also identify any documentation issues that must be addressed to ensure the traceability and defensibility of the data.

3. The report and the signature page of the data package are signed and dated by the auditor.

4. Audit reports are returned to the person responsible for generating the data (typically the analyst), who must address each error or documentation issue and document on the audit report the corrective action taken.

5. The audit report with completed responses is then signed and dated by the Project and Resource Managers and returned to the QAU within five working days.

6. Upon receipt of the audit report, the QA auditor verifies any corrections that were necessary and reviews the audit responses to ensure they are complete and appropriate. Final, corrected report tables are stamped on the back to indicate that they are accepted as final by QA.

7. When all audit findings are addressed the auditor signs the QAU Approval line on the data package title page.

8. Unresolved issues are addressed through discussions with the Project or Resource Manager, as necessary. In the Environmental Chemistry Section, the Laboratory Manager is designated to oversee responses to audit reports for the Resource Manager.

9. No audit file is considered "closed" until every error and audit issue has been addressed.

4.1.2 Reports to Management

The audit report constitutes the report to management for each project. Overall quality issues are discussed at quarterly laboratory and field reviews conducted by Battelle's Vice President. In addition, quality issues will be reported directly to the Resource Manager or Vice President whenever project data integrity is threatened.

4.2 DOCUMENTATION

The audited tables and supporting documentation are filed by client, project task, and audit number in the QA Office. The original audit reports are filed by audit number in the QAU audit files. The audit reports are maintained for 10 years unless otherwise specified in a contract.

The Data Package Tracking Log (Attachment 1) provides a record of all data packages received for audit and the audit number. Tracking includes the dates that data are received, relinquished for correction, returned for verification, and accepted (all audit issues addressed). In addition, an Excel spreadsheet of all data packages received for audit is on the Quality System Manager's hard drive (Active\QA\Status Reports). This file is used to track due dates and prompt follow-up. The Project Manager or designee will be contacted if the audit response is not received by the due date. Repeated failure to respond to audit reports is considered a performance issue, prompting the initiation of a formal Request for Corrective Action (SOP 4-035).

A project-specific Audit Log (Attachment 4) is maintained to provide a list of all audits conducted for a project.

5.0 PERSONNEL RESPONSIBILITIES

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

The QA Auditor is responsible for

- conducting a careful, thorough, unbiased audit according to this SOP
- following through on questions to ensure that evidence is identified for each audit comment
- preparing a concise, clearly written audit report that identifies each issue
- completing all QA office documentation described in this SOP

The Project Manager is responsible for

- defining in the project work plan the QAU audit activities, or the alternative validation activities, which will be performed for each project.
- performing a technical review of the data, and verifying that the data meet the requirements of the project.
- reviewing the data package for completeness and acceptable Quality Control results prior to the QA audit.
- reviewing the data package for technical reasonableness prior to submission for audit and for ensuring that documentation, including deviations, is complete.
- ensuring that all issues raised in the audit report are addressed and returning the audit response and corrected data to the QAU within the time specified in the audit report.
- reviewing audit responses and signing the completed audit report.

The Analyst/Task Leader is responsible for

- responding to the audit, addressing issues (after consultation with Project Manager, if necessary)
- ensuring that the data package is complete, validated, paginated,
- ensuring that all SOP or work plan deviations have been documented and approved by the Project Manager

The Laboratory Manager, as the Resource Manager in the Environmental Chemistry Section, is responsible for

- Follow-through on overdue audit reports
- noting general laboratory issues that should be addressed for the facility

The Resource Manager is responsible for reviewing and approving the audit response.

6.0 TRAINING

Personnel who are being trained to perform auditing tasks must first read this SOP. They may then perform specific tasks under the supervision of QAU personnel. Auditing tasks performed by the trainee will be reviewed and co-signed by QAU staff until it has been established that the trainee is able to perform these tasks without supervision. A certificate of training (Attachment 5) will be issued upon completion of training. The original certificate will be maintained in the QAU training files.

ATTACHMENTS

1. Example of Data Package Tracking Form
2. Modified British Standard 6001
3a. Example of Audit Report Cover Sheet
3b. Example of Data Audit Report Form
4. Example of Quality Assurance Audit Log Form
5. Example of Certificate of Training

APPROVALS

Author	Donna Buhl	11-29-99
Quality Systems Manager	Donna Buhl	11-29-99
Vice President Battelle Duxbury Operations	Q Graffeo Name	11-29-99 Date

Attachment 1
Example of Data Package Tracking Form

Initial Audit						Audit Response		
Description	Date in/ Initials	Date out/ Initials	Response Due	Dist'd to	Comments	Date in/ Initials	Date out/ Initials	Comments

Attachment 2

Modified British Standard 6001

Data Points in Data Set	Sample Size to be Inspected	Accepted Number of Errors	Errors for Rejection of Data Set
2-50	100% Inspection	-	-
51-90	12	0	1
91-150	20	0	1
151-280	32	1	2
281-500	50	1	2
501-1200	80	2	3
1201-3200	125	3	4
3201-10,000	200	5	6
10,001-35,000	315	7	8

Attachment 3a
Audit Report Cover Sheet

BATTELLE DUXBURY OPERATIONS
QUALITY ASSURANCE COVER SHEET

PROJECT TITLE:

PROJECT NUMBER:

CLIENT:

PROJECT MANAGER:

STUDY INITIATION DATE:

TYPE OF AUDIT:

PERSONS RESPONSIBLE FOR PHASE BEING AUDITED:

DATE OF AUDIT:

AUDITOR:

NUMBER OF PAGES ATTACHED:

DATE RESPONSE DUE:

DATE RESPONSE RECEIVED:

SUMMARY:

Signature of Auditor: _____

Date: _____

Attachment 3b
Example of Data Audit Report Form

BATTELLE DUXBURY OPERATIONS
AUDIT REPORT

Project Number/Title

Definition of Data Set

Audit Date

Number of Data Points in Data Set: **Number Checked**

Comments	Corrective Action/Date/Initials

Auditor _____ **Date** _____

Laboratory Manager _____ **Date** _____

Project Manager _____ **Date** _____

Attachment 4
Example of Quality Assurance Audit Log Form

BATTELLE DUXBURY OPERATIONS
QUALITY ASSURANCE AUDIT\INSPECTION LOG

Client_____ Project Number_____ Project Manager_____

Project Name_____ Start Date_____ Page ___ of ___

Audit Description	Auditor	Date(s)					Comments
		Audit	Audit Report	Response Expected	Response Received	Report To Mgmnt	

Battelle Duxbury Operations
Standard Operating Procedures

for

NON-CONFORMANCE AND CORRECTIVE ACTION

Summary of Changes in this version: The SOP scope is clarified. A discussion of root cause analysis is added. Responsibilities and authorities are defined. Documentation procedures are updated. The table of non-conformance examples is updated.

1.0 OBJECTIVE

One goal of Battelle's Quality Assurance Program is continuous improvement. The purpose of the corrective action program is to identify practices or activities that are adverse to the generation of quality products and to implement process improvements in response to these issues. This standard operating procedure (SOP) describes general corrective action procedures for all technical sections at Battelle - Field, Laboratory, Information Management, and Ecology. The requirements for corrective action are specific for some activities which are not addressed in this SOP:

- Control charts see SOP 7-028
- Quality control sample non-conformance see SOP 7-029
- SOP deviations see SOP 6-001
- Quality Assurance Project Plan deviations see SOP 6-021

2.0 DEFINITIONS

Observations are defined as incidences that require action or correction but that are not considered ongoing, operational problems.

Findings adverse to quality (findings) are defined as (1) repeated incidences of a deviation, (2) repeated errors due to a flaw in the data generation or validation process, or (3) inspection issues that require a change in laboratory procedures.

Corrective Action is defined as the series of activities undertaken to assess the root cause of a finding and the remedy implemented to minimize re-occurrence. Types of investigation activities, and suggested immediate and long-term corrective action are provided in Attachment 1.

A re-occurring *observation* is considered a *finding*. Normally, if the same error or incorrect procedure is noted during inspections or audits then the issue is considered a *finding* and a Request for Corrective Action (RCA - Attachment 2) is initiated by the QAU. The RCA will cite specific examples of the issue to facilitate investigation and correction.

> Example: If an error is noted in the calculation of a dilution factor, this is an *observation* that requires correction. If dilution factors errors found repeatedly, this is a *finding* that requires *corrective action.* In this example corrective action would include additional training for laboratory staff who must determine dilution factors.

3.0 PROCEDURES

3.1 INITIATION OF REQUESTS FOR CORRECTIVE ACTION

Non-conformance issues are documented by the QAU in audit or inspection findings to Laboratory, Project, and/or Resource Management. In addition, major issues should be discussed in person with management. However, where a pattern of practices or issues adverse to quality continues then a formal Request for Corrective Action (RCA) is initiated.

RCAs are initiated by the Quality Assurance Unit in response to:

- internal or external inspections performed by the QA unit, a client, or a government agency
- the audit of data (note that random errors or unusual occurrences are not considered findings)
- reviews of quality control data by either the QA unit or laboratory personnel
- awareness of an on-going issue

RCAs should be initiated by the Laboratory, Project, or Resource Manages as the result of

- inspections or reviews of laboratory activities
- project reviews by project management
- discussions with laboratory staff
- quality control failures

The person issuing the RCA must provide a complete description of the finding that requires corrective action on the RCA. Each RCA must specify the person responsible for responding and the time period considered adequate to investigate the issue and initiate corrective action. Copies of all RCAs should be forwarded to the QAU where a sequential number is assigned.

3.2 IDENTIFYING THE ROOT CAUSE

The principle of root cause analysis is that every failure has a preventable root cause. Instances of non-conformance may be systematic and indicative of a fundamental problem. The person responsible for addressing the RCA should conduct an informal root cause analysis to determine the reasons that the non-conformance occurred. A root cause analysis consists of isolating the cause and effect relationships for the non-conformance by:

- investigating the specific occurrence
- interviewing staff to determine if the issue is widespread
- defining who, what, when, and where
- repeatedly asking the question "why?"

3.3 RESPONSE TO REQUESTS FOR CORRECTIVE ACTION

Once the root cause of non-conformance is identified then the person responsible for investigating the finding and initiating corrective action must complete all three "response" sections of the RCA. Using the example cited in Section 2:

- The investigation activity performed to determine the cause of the dilution factor errors could be:

recalculated several dilution factors, reviewed spreadsheet templates for possible error.

- The immediate corrective action could be: *discussed dilution factor calculations with analysts, updated spreadsheet formula.*

- The long-term activity implemented to avoid re-occurrence could be: *initiated verification procedure that requires analysts to recalculate one dilution factor per spreadsheet and peer review of all complex dilution factors.*

The RCA is signed by the person responsible for implementing the corrective action and his/her Resource Manager, and returned to the QAU.

If the corrective action involves several staff members (e.g., analysts) or if policy or procedures are changed as a results of corrective action, then these changes must be documented in a memo to the appropriate staff. A copy of the memo is attached to the completed RCA.

3.4 RESPONSIBILITIES AND AUTHORITY FOR CORRECTION OF NON-CONFORMANCE

Management at all levels is responsible for reviewing activities and for identifying areas of repeated non-conformance that require formal RCAs. The Laboratory Manager is responsible for assessing laboratory quality control performance for repeated failures that warrant an RCA. Project Managers are responsible for identifying and investigating repeated failures during the conduct of a study. Resource Managers are ultimately responsible for addressing areas of non-conformance within their sections. They are responsible for

- assigning root cause analysis and RCA responses
- reviewing the results of investigations and the documentation on the RCA
- initiating any section-wide procedural changes required to address the root cause (e.g., including updating SOPs, conducting additional training, reassigning staff, or implementing new policies or procedures)

The Quality Systems Manager is responsible for reviewing audit and inspection reports to identify repeated performance issues and patterns of non-conformance.

The Laboratory, Project, and Resource Managers, and the Quality Systems Manager, are vested with the authority to **stop work** where repeated non-conformance will impact Battelle's ability to meet the Quality Policy defined in the Quality Management Plan.

3.5 DOCUMENTATION

All instances of non-conformance must be documented along with the corrective action implemented and the results of the corrective action. Note that the corrective action is not considered final until the issue is corrected and the affected area has returned to acceptable performance. The respective Laboratory, Resource, and/or Project Management must review this documentation. A copy should be maintained in the project or instrument files, as appropriate.

All formal Request for Corrective Action forms are filed sequentially (i.e., chronologically) in the Corrective Action Log maintained by the QAU. All RCAs that relate to specific projects must be copied to the project manager and included in the project files. Within one week of the receipt of the completed RCA the implementation must be verified by the QAU through a follow-up inspection and the

RCA the implementation must be verified by the QAU through a follow-up inspection and the verification documented on the RCA.

4.0 TRAINING

No specific training is required for this SOP. Project, Laboratory, and Section Managers should understand the principles related to corrective action and continuous improvement. The dated signature on the training form (Attachment 3) documents that this SOP has been read and understood.

ATTACHMENTS

1. Examples of Corrective Action
2. Request for Corrective Action form
3. Training Documentation

APPROVALS

Author		7-31-99
Quality Systems Manager		7-31-99
Vice President, Battelle Duxbury Operations	Name	7-31-99 Date

Attachment 1
Examples of Corrective Action

A. INVESTIGATION

A.1 Quality Control Excursions

1. Review calculations and transcriptions
2. Review original sample preparation and spiking records
3. Verify that instrument is within the calibration criteria
4. Identify any unusual issues related to sample matrix

A.2 Deviations (from SOPs , QAPjPs, etc)

1. Determine whether the deviation is isolated or re-occurring
2. Review the governing document to determine whether a revision should be prepared.
3. Discuss the deviation with lab personnel to determine if additional training is required

B. SHORT-TERM CORRECTIVE ACTION

B.1 Quality Control Excursions

1. Review errors with appropriate personnel; correct calculation or transcription errors
2. Review prep errors/procedures with laboratory personnel; identify practices that could contribute to poor QC results.
3. Perform instrument maintenance, recalibrate, and reanalyze samples
4. Determine with project manager whether reprocessing is required; document QC excursions

B.2 Deviations

1. Document the deviation.
2. Review project requirements and determine the appropriate corrective action (with the client, if appropriate).

C. LONG-TERM CORRECTIVE ACTION

C.1 Quality Control Excursions

1. Conduct additional training
2. Modify and document updated validation procedures
3. Increase frequency of instrument calibration checks
4. Modify clean-up procedures

C.2 Deviations

1. Conduct additional training
2. Update governing document

Attachment 2
Example of Request for Corrective Action Form

Date: Date Response Due: **To:** **From (Signature and Date):**
Description of finding which requires corrective action:
Describe investigation activities performed and the results:
Describe the immediate corrective action to be implemented:
Describe long-term corrective action to be implemented to minimize the re-occurrence of this issue:
Signature and Date: **Approval Signature and Date:** **(Operations Manager)** **Verified by Quality Assurance Office (Signature and Date):**

Attachment 3
Record of Training

for

NON-CONFORMANCE AND CORRECTIVE ACTION

The above mentioned SOP is relevant to your work. Your signature below attests to the fact that you have read this SOP and understand the requirements associated with this procedure.

Name (Printed)	Signature	Date

BATTELLE DUXBURY OPERATIONS
STANDARD OPERATING PROCEDURES

FOR

IDENTIFICATION AND QUANTIFICATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Summary of changes in this version: Justification for manual integration has been added.

1.0 OBJECTIVE

This Standard Operating Procedure describes the identification and quantification of polynuclear aromatic hydrocarbons (PAH) by gas chromatography/mass spectrometry (GC/MS). Please note this method is a modification of EPA methods 8270C and 625 and therefore certain criteria (i.e. initial calibrations and daily verifications) are different than those referenced in EPA methods. Refer to SOPs 5-281 and 5-282 respectively for GC/MS analyses requiring Standard Methods.

1.1 SUMMARY OF METHOD

Polynuclear aromatic hydrocarbons are separated via high resolution capillary gas chromatography, and identified and quantified using electron impact mass spectrometry. A data system interfaced to the GC/MS is used to control acquisition and to store, retrieve, and manipulate mass spectral data. This method provides procedures for the identification and measurement of the selected PAH listed in Table 1 in full-scan mode and selected ion monitoring mode (SIM). Table 1 may be amended to meet project-specific protocols.

2.0 PREPARATION

The GC/MS must be operated and maintained as described in SOP No. 3-092. All documentation in the GC/MS Facility must comply with the procedures described in SOP No. 6-011.

2.1 GAS CHROMATOGRAPH/MASS SPECTROMETER

Prior to the analysis of analytical standards and/or samples the mass spectrometer must be tuned according to the procedures described in SOP No. 3-092. Perfluorotributlyamine (PFTBA) should be used to tune the mass spectrometer, unless otherwise specified in the project protocol.

2.1.1 The gas chromatograph is fitted with a 60-m x 0.25-mm DB-5MS (J & W Scientific, Inc.) or equivalent (0.25-µm film thickness) fused silica capillary column. The GC oven temperature program will be as follows, unless otherwise specified in the project-specific protocol:

Injection Port: 300 °C
Transfer Liner: 280 °C
Initial Temp: 40 °C

Initial Hold:	1 minute
Ramp Rate:	6 °C/min
Final Temp:	290 °C
Final Hold:	20 minutes or until benzo(g,h,i)perylene elutes

2.1.2 All gas chromatographs are fitted with Electronic Pressure Control (EPC). The EPC ramping program will be as follows, unless otherwise specified in the project-specific protocol:

Initial Pressure: 30 psi	Initial Time: 1 min
Level 1 Rate: 99 psi/min	Final Pressure: psi equivalent to flow 1 mL/min
Vacuum Compensation: On	

2.2 PREPARATION FOR FULL-SCAN ANALYSIS

A 1-μL injection volume will be analyzed, unless otherwise specified in the project protocol. The mass spectrometer should be operated in electron impact mode at a manifold pressure of less than 6 x 10^{-5} torr. The mass range scanned will be 50 to 450 atomic mass units (AMU) unless specifically stated otherwise in the project protocols. The quantification and confirmation ions used in the analysis of selected PAH are listed in Table 1.

2.3 PREPARATION FOR SELECTED ION MONITORING (SIM) ANALYSIS

The oven temperatures and electronic pressure programs outlined in sections 2.1.1 and 2.1.2 are used for SIM analysis unless stated otherwise in project protocols. A 2-μL injection volume will be analyzed, unless otherwise specified in the project protocol. The mass spectrometer should be operated in electron impact mode at a manifold pressure of less than 6 x 10^{-5} torr. The electron multiplier voltage should be set a minimum of 100 V above the tune value.

2.3.1 The quantification and confirmation ions used in the analysis of selected PAH are listed in Table 1. If possible ion groups should be selected so that no more than 20 ions are monitored in a single group. It should be noted that as the number of ions scanned per group increases and the individual dwell times decrease, sensitivity will also decrease. Each ion in a group should have identical dwell times to ensure that correct ion ratios are preserved. Total group dwell time should not exceed 400 ms, individual dwell times should be a minimum of 20 ms.

2.3.2 Prior to the first analysis of analytical standards and/or samples in SIM, a 10 ng/μL analytical standard and reference sample (e.g. North Slope Crude Oil) should be analyzed in full-scan mode, under the conditions presented in Section 2.2. The full-scan total ion chromatograms (TIC's) from these analyses should be used to determine the proper group start and stop times in the SIM acquisition method.

TABLE 1. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [% of Base Peak][a]
d_8-naphthalene[b]	136	134 [15]
naphthalene	128	127 [15]
C_1-naphthalenes	142	141 [80]
2-methylnaphthalene	142	141 [80]
1-methylnaphthalene	142	141 [80]
C_2-naphthalenes	156	141
2,6-dimethylnaphthalene	156	141
C_3-naphthalenes	170	155
2,3,5-trimethylnaphthalene	170	155
C_4-naphthalenes	184	169, 141
d_{10}-acenaphthene[b]	164	162 [95]
acenaphthylene	152	153 [15]
acenaphthene	154	153 [98]
biphenyl	154	152 [30]
d_{10}-biphenyl[b]	164	162
d_{10}-fluorene[b]	176	174 [85]
dibenzofuran	168	169 [20]
fluorene	166	165 [95]
C_1-fluorenes	180	165 [100]
C_2-fluorenes	194	179 [25]
C_3-fluorenes	208	193
d_{10}-phenanthrene[b]	188	184
phenanthrene	178	176 [20]
anthracene	178	176 [20]
C_1-phenanthrenes/anthracenes	192	191 [60]
1-methylphenanthrene	192	191 [60]
C_2-phenanthrenes/anthracenes	206	191
C_3-phenanthrenes/anthracenes	220	205
C_4-phenanthrenes/anthracenes	234	219, 191
dibenzothiophene	184	152 [15] 139
C_1-dibenzothiophenes	198	184 [25] 197
C_2-dibenzothiophenes	212	197
C_3-dibenzothiophenes	226	211
fluoranthene	202	101 [15]
pyrene	202	101 [15]
C_1-fluoranthene/pyrenes	216	215 [60]
C_2-fluoranthene/pyrenes	230	215
C_3-fluoranthene/pyrenes	244	229, 215
d_{12}-chrysene[b]	240	236
benz[a]anthracene	228	226 [20]
chrysene	228	226 [30]
C_1-benz[a]anthracenes/chrysenes	242	241
C_2-benz[a]anthracenes/chrysenes	256	241
C_3-benz[a]anthracenes/chrysenes	270	255
C_4-benz[a]anthracenes/chrysenes	284	269, 241
d_{12}-benzo[e]pyrene[b]	264	260 [20]
d_{12}-benzo[a]pyrene[b]	264	260 [20]

TABLE 1 Continued. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [~% of Base Peak][a]
benzo[b]fluoranthene	252	253 [30], 125 [10]
benzo[k]fluoranthene	252	253 [30], 125 [10]
benzo[e]pyrene	252	253 [20]
benzo[a]pyrene	252	253 [30], 125 [10]
perylene	252	253 [20]
indeno[1,2,3-c,d]pyrene	276	277 [25], 138 [30]
dibenz[a,h]anthracene	278	279 [25], 139 [20]
benzo[g,h,i]perylene	276	277 [25], 138 [20]
OTP	230	198
Biomarkers:		
5b-cholane[b]	217	
C30a,b-hopane	191	
C30b,b-hopane	191	
LAB's:		
1-phenylonane[b]	91	92, 105
1-phenyldecane(s)	91	92, 105
1-phenylundecane(s)	91	92, 105
1-phenyldodecane(s)	91	92, 105
1-phenyltridecane(s)	91	92, 105
1-phenyltetradecane(s)	91	92, 105
Phenols:		
d_5-phenol[b]	99	98, 71
phenol	94	66
C_1-phenols	108	107
C_2-phenols	122	107
C_3-phenols	136	107, 77
C_4-phenols	150	135, 107
C_5-phenols	164	149, 121
C_6-phenols	178	163, 135
C_7-phenols	192	177
C_8-phenols	206	107
C_9-phenols	220	107, 135
Phthalates:		
d_4-bis(2-ethylhexyl)phthalate[b]	153	171, 283
bis(2-ethylhexyl)phthalate	149	167, 279
dimethyl phthalate	163	164, 194
diethyl phthalate	149	150, 177
di-n-butyl phthalate	149	150, 104
butyl benzyl phthalate	149	91, 206
di-n-octyl phthalate	149	150, 279

TABLE 1 Continued. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [% of Base Peak][a]
Other:		
decalin	138	
C_1-decalins	152	
C_2-decalins	166	
C_3-decalins	180	
C_4-decalins	194	
benzothiophene	134	
C_1-benzothiophenes	148	
C_2-benzothiophenes	162	
C_3-benzothiophenes	176	
C_4-benzothiophenes	190	
naphthacene	228	226 [30]
chloronaphthalenes	162	127, 126

[a]Relative abundance of ions within any given isomer group will vary considerably, depending on isomer of interest. Relative abundances should be determined from analysis of crude oil solution. [b]Representative spiking compound.

2.3.3 In the event that a section of the analytical column is removed, or the analytical column is replaced, a 10 ng/μL analytical standard and reference sample should be analyzed in SIM mode under the conditions presented in Section 2.3. The extracted ion profiles (EIP's) from these analyses should be used to reassign the proper group start and stop times of the SIM acquisition method.

3.0 PROCEDURES

3.1 CALIBRATION

Demonstration of a linear initial calibration is required prior to the analysis of samples. A continuing calibration is required at the beginning and end of each 12 hour period during which analyses are performed (approximately every eight injections). Sample data may be used if bracketed by a passing continuing calibration. Initial calibrations from previous sequences may be used if the continuing calibrations still show acceptable linearity and no major instrument maintenance has been performed (i.e. source cleaning, column change, etc.).

3.1.1 INITIAL CALIBRATION

Analyze a minimum of five analytical standards that will represent sample concentration range, unless otherwise specified in the project protocol.

3.1.1.1 The concentration of the low standard should be 2 to 3 times the detection limit. The medium concentration standard should be near the concentration range expected in the samples. The high concentration standard should be at least 1 order of magnitude more concentrated than the low concentration standard.

3.1.1.2 For each level of calibration, calculate the response factors for each analyte of interest using the software supplied with the GC/MS data system (see SOP 3-092). For each analyte, calculate the average response factor (RF) and the percent relative standard deviation (RSD). For a valid initial calibration, the percent RSD for each analyte should be less than 25 percent, unless specifically stated otherwise in project protocols (see Section 4.1 for calculation of RF and RSD). The average RSD for all targets should be ≤15%.

3.1.2 CONTINUING CALIBRATION

A continuing calibration will be performed using the following procedure at the beginning and end of each 12 hour period in which samples are analyzed.

3.1.2.1 Analyze a medium concentration analytical standard under the same analytical conditions used in the initial calibration. Calculate response factors for each analyte of interest and compare them to the average response factor calculated in Section 3.1.1.2, above.

3.1.2.2 The percent difference between the average response factor and the continuing calibration response factor is less than 25 percent, the initial calibration is still valid and sample analyses may continue (see Section 4.2 for calculation of percent difference). If the percent difference of a target RF exceeds 25 percent, remedial action should be taken and the medium concentration standard reanalyzed. If the continuing calibration fails again, then sample analyses should be terminated, maintenance performed, and a new initial calibration analyzed, unless specifically stated otherwise in project protocols. In addition the average percent difference of all the targets should be ≤15%. Analysts should be visually monitoring the retention time (RT) deviation (in minutes) and percent area deviation recorded on the continuing calibration reports. Ideally, these values should not exceed 0.5 minutes for RT, and <50% or >200% area deviation.

3.2 SAMPLE ANALYSIS

Samples are analyzed under the same analytical conditions used in the analysis of the analytical standards. A quantification internal standard (QIS, also known as RIS - recovery internal standard) is added to the samples immediately preceding analysis. The type and amount of QIS added is project specific, consult the project protocols.

The criteria presented in Sections 3.2.1 through 3.2.7 must be satisfied to verify identification of an analyte in a sample. Analyte peaks that do not satisfy these criteria in the preliminary quant are deleted from the final quantification reports/files. Original working copies of these reports are maintained with the project files as "QDel Reports" stored in unused data (See SOP No. 3-092).

3.2.1 RELATIVE RETENTION TIME

The sample component relative retention time (RRT) should be within ±0.1 min (6 sec) of the standard component. The standard must have been run within 12 hours of the sample.

3.2.2 SAMPLE SPECTRUM

For each analyte suspected to be present in the sample, the corresponding ions listed in Table 1 must be present and the relative intensities must agree to within ±20 percent of the relative intensities found in spectra of the standard component. This criteria may be modified for trace level analysis and is left to the project manager's/analyst's discretion. Modifications to sample spectrum criteria will be documented. If the intensity of the quantification ion in a sample spectrum is greater than the intensity of the same ion in the high concentration analytical standard the sample should be diluted and reanalyzed.

3.2.3 SIGNAL TO NOISE RATIO

A quantifiable analyte peak *should* (but is not limited to) exhibit a signal:noise ratio of 3:1 or greater unless specified otherwise in the project protocol.

3.2.4 PEAK SYMMETRY

Suspected peaks should be examined for proper shape and symmetry.

3.2.5 PATTERNS

Suspected sample components should display established patterns. See Attachment 1.

3.2.6 MINIMUM AREA

It is recommended that each of the sample components have a minimum area of 1/2 the abundance count of the component in the low standard. Quantitation of a component is not limited to this criteria however. For instance, a component may have an area abundance slightly less than 1/2 the low standard area abundance but exhibit a signal to noise ratio of 5:1. This component would be considered for quantitation.

3.2.7 ALYKL HOMOLOGUES

Certain projects require identification and quantification of the alkyl homologues of selected PAH. This will be detailed in the project-specific protocol. Alkyl homologues are identified by the procedures in Sections 3.2.8. Quantification of alkyl homologues is covered in Section 3.2.10, below (adopting parent response factors is discussed in SOP No. 3-092).

3.2.8 TENTATIVE IDENTIFICATION OF UNKNOWNS AND ALKYL HOMOLOGUES

For components not contained in the analytical standards, a library search may be performed on the sample spectrum for the purpose of tentative identification (Registry of Mass Spectral Data Library). Computer-generated search algorithms should not employ unusual normalization, tilting, or smoothing of the unknown spectrum.

3.2.8.1 Major ions (ions >10 percent of the primary ion) in the reference spectrum should be present in the sample spectrum.

3.2.8.2 Relative intensities of the ions in the sample spectrum should agree with the reference spectrum to within ±20 percent.

3.2.8.3 Quantification of tentatively identified compounds is covered in Section 3.2.11.

3.2.9 QUANTIFICATION OF ANALYTES

Quantification of analytes identified in samples will be performed by the internal standard method, using the average response factor from the initial calibration, unless otherwise specified in the project protocols. See Section 4.3 for additional information regarding calculations used for the determination of target analyte concentrations in samples.

3.2.10 QUANTIFICATION OF ALKYL HOMOLOGUES

Alkyl homologue groups are quantified by the internal standard method. The molecular ion of the alkyl homologue should be extracted, and the areas of the individual isomers summed by a straight line, baseline integration.

3.2.10.1 The response factor used to quantify a specific homologous series (e.g. C_1-naphthalenes) should be the response factor of parent compound (e.g. naphthalene) unless specified otherwise in the project protocols.

3.2.11 QUANTIFICATION OF TENTATIVELY IDENTIFIED COMPOUNDS

Tentatively-identified compounds (see Section 3.2.8) should be quantified by the internal standard method assigning a response factor of 1. The data report should indicate that these compounds are uncorrected for response.

4.0 CALCULATIONS

4.1 INITIAL CALIBRATION

The average response factors from the initial calibration are calculated using the following equation:

$$RF = (A_a/A_b) \times (C_b/C_a)$$

where:

A_a = Area of quantification ion for target analyte in the standard
A_b = Area of quantification ion for internal standard in the standard
C_a = Concentration of target analyte in the standard
C_b = Concentration of internal standard in the standard

The percent relative standard deviation (RSD) is evaluated with following equation:

$$RSD = (SD/\overline{RF}) \times 100$$

where:

SD = standard deviation of the mean RF, calculated as n-1.

See Section 3.1.1.2 for details regarding initial calibration acceptability criteria.

4.2 CONTINUING CALIBRATION

The response factors determined from the continuing calibration are checked against those determined from the initial calibration. The percent difference is calculated using the following

$$\text{Percent Difference} = [(\overline{RF}_i - RF_c)/\overline{RF}_i] \times 100$$

where:

RF_i = Average response factor from initial calibration
RF_c = Response factor from continuing calibration

See Section 3.1.2.2 for continuing calibration acceptability criteria.

4.3 QUANTIFICATION OF SAMPLES

Samples are quantified as detailed in Section 3.2. The concentration of target analytes is determined using the following equation:

$$C_a = [(A_a/A_i) \times (Amt_i/RF_i) \times D]/V_a$$

where:

C_a = Concentration target analyte
A_a = Area quantification ion for target analyte
A_i = Area quantification ion for internal standard
Amt_i = Amount internal standard added to sample
RF_i = Average RF for analyte determined from initial calibration
D = Dilution factor if applicable
V_a = Sample size

Sample size may refer to sample volume or sample dry/wet weight. The project protocol will specify reporting criteria.

5.0 QUALITY CONTROL

The GC/MS Facility is operated and maintained in accordance with SOP No. 3-092 and all GC/MS Facility operations are documented according to SOP No. 6-011. Hardcopies of all initial and continuing calibrations are maintained with the project data. The number of procedural blanks, spiked blanks, matrix spikes, and other types of quality control samples will be specified in individual project protocols. Acceptability criteria and reporting limits will also be outlined in the project-specific protocols. The following quality control procedures have been adapted from relevant procedures in U.S. Environmental Protection Agency (EPA) Method 8270, Gas Chromatography/Mass Spectrometry for Semivolatile Organics: Capillary Column Technique (1986).

5.1 Each day that analysis is performed, the daily calibration standard should be evaluated to determine if the chromatographic system is operating properly.

5.1.1 Peak shape should be evaluated for proper peak shape and symmetry.

5.1.2 The instrumental response should be comparable to previous calibrations.

5.1.3 The system must be recalibrated if the analytical column is replaced.

5.2 There must be an initial calibration and calibration verification of the instrument as specified in Section 3.1.1 and 3.1.2.

6.0 TRAINING

6.1 The operator must be experienced with the operation and maintenance of the GC/MS system, and must have passed the training requirements presented in SOP No. 3-092.

6.2 The trainee must read and fully understand this SOP. The trainee will then be given a demonstration of all aspects of the SOP.

6.3 Individuals may work independently once they have satisfactorily performed the following training test.

6.3.1 The trainee will perform an initial calibration and analyze one sample of known concentration, such as a North Slope Crude check sample.

6.3.2 An experienced operator will then review the same sample results. Successful completion will consist of a valid initial calibration and sample results within 25 percent from historical (certified) values.

6.4 When training is completed, the trainee will be issued a Certificate of Training (Attachment 2). The original completed training certificate and all supporting documentation will be sent to the Quality Assurance Office.

7.0 SAFETY

All GC/MS operators must be experienced with the operation and maintenance of the GC/MS system, and must have passed the training requirements presented in this SOP and SOP No. 3-092 (see additional safety comments in SOP No. 3-092).

7.1 Protective clothing should be worn when appropriate.

7.2 Proper care must be exercised when using syringes.

7.3 Certain areas of the GC/MS system are heated zones and bodily contact with these zones should be avoided.

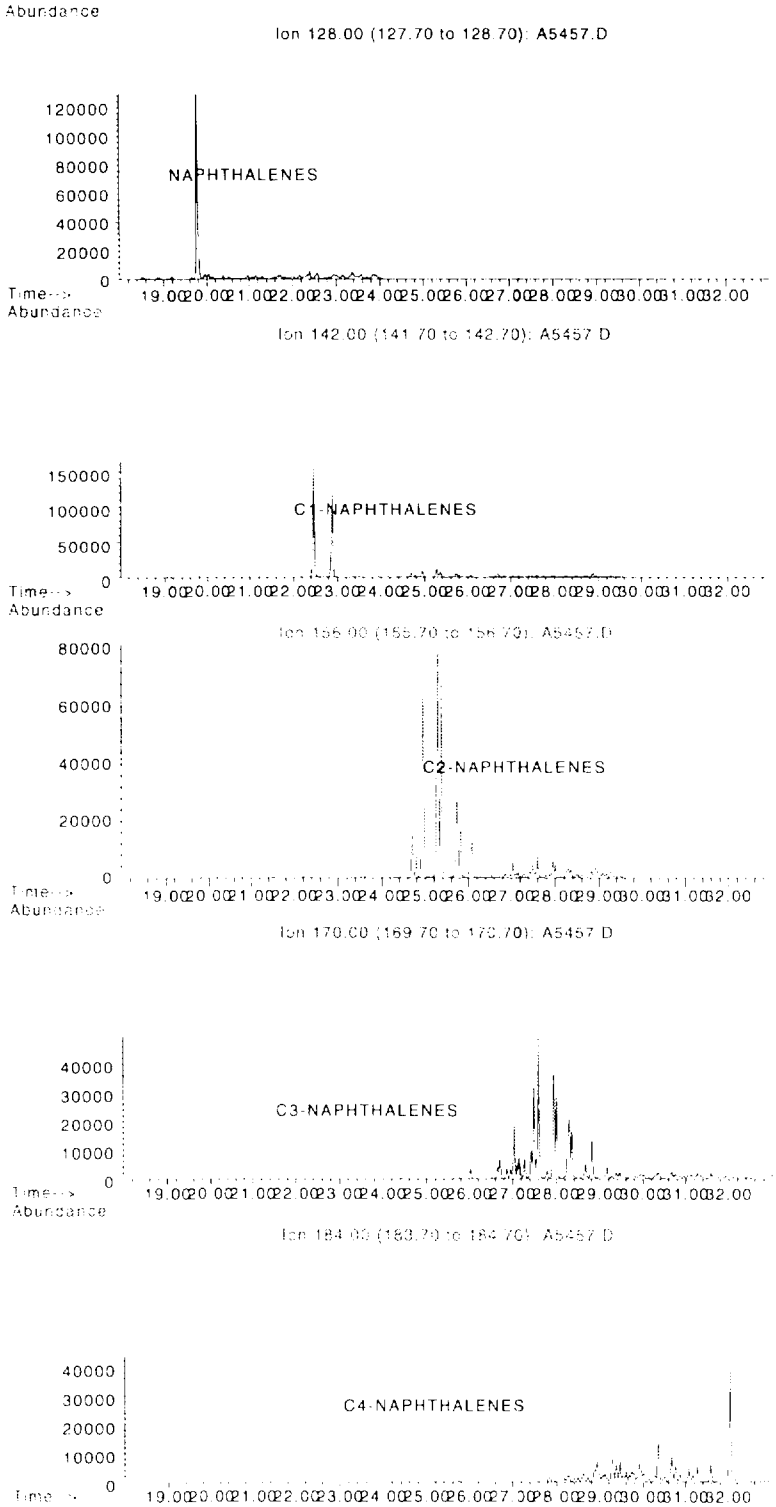
7.4 High voltages exist in certain marked areas of the GC/MS system and bodily contact with these areas is to be avoided.

8.0 ATTACHMENTS

Attachment 1. Alkyl Homologue Patterns
Attachment 2. Certificate of Training

Author	Daniel P. Barker for JAF	8-10-99
Laboratory Manager	Charles S. Fu for RL	8/10/99
QA Coordinator	Karen B. Buhl	8-10-99
Environmental Chemistry Section Manager	ASUH for GSDurell	8-10-99
	Name	**Date**

ATTACHMENT 1



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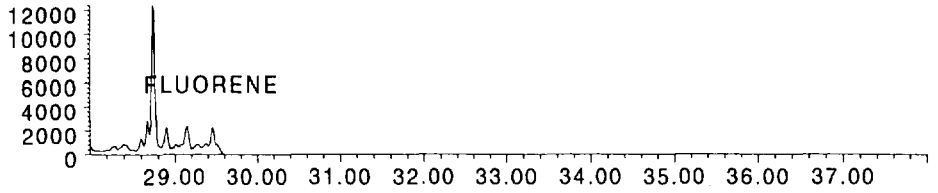

Time-->
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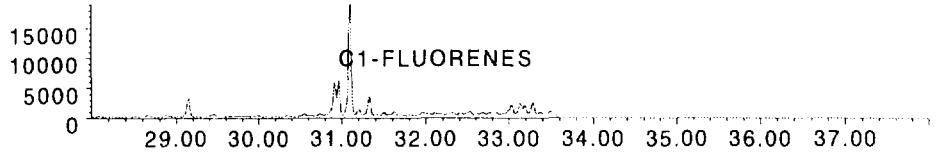

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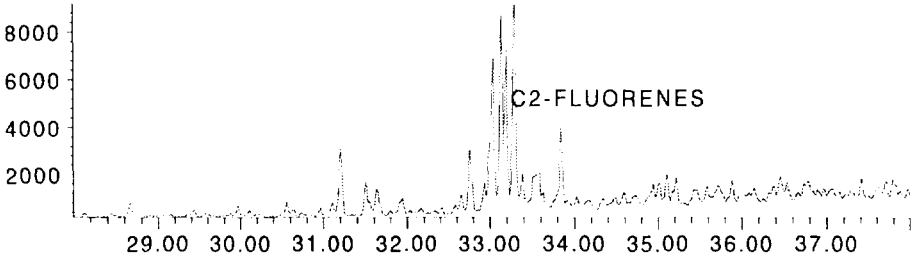

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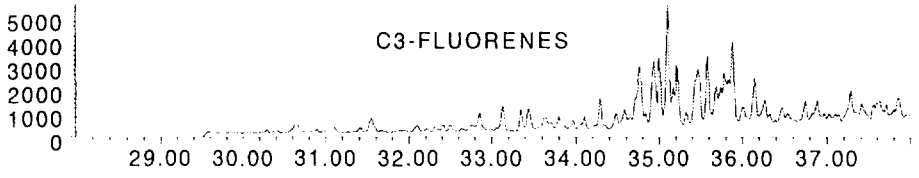

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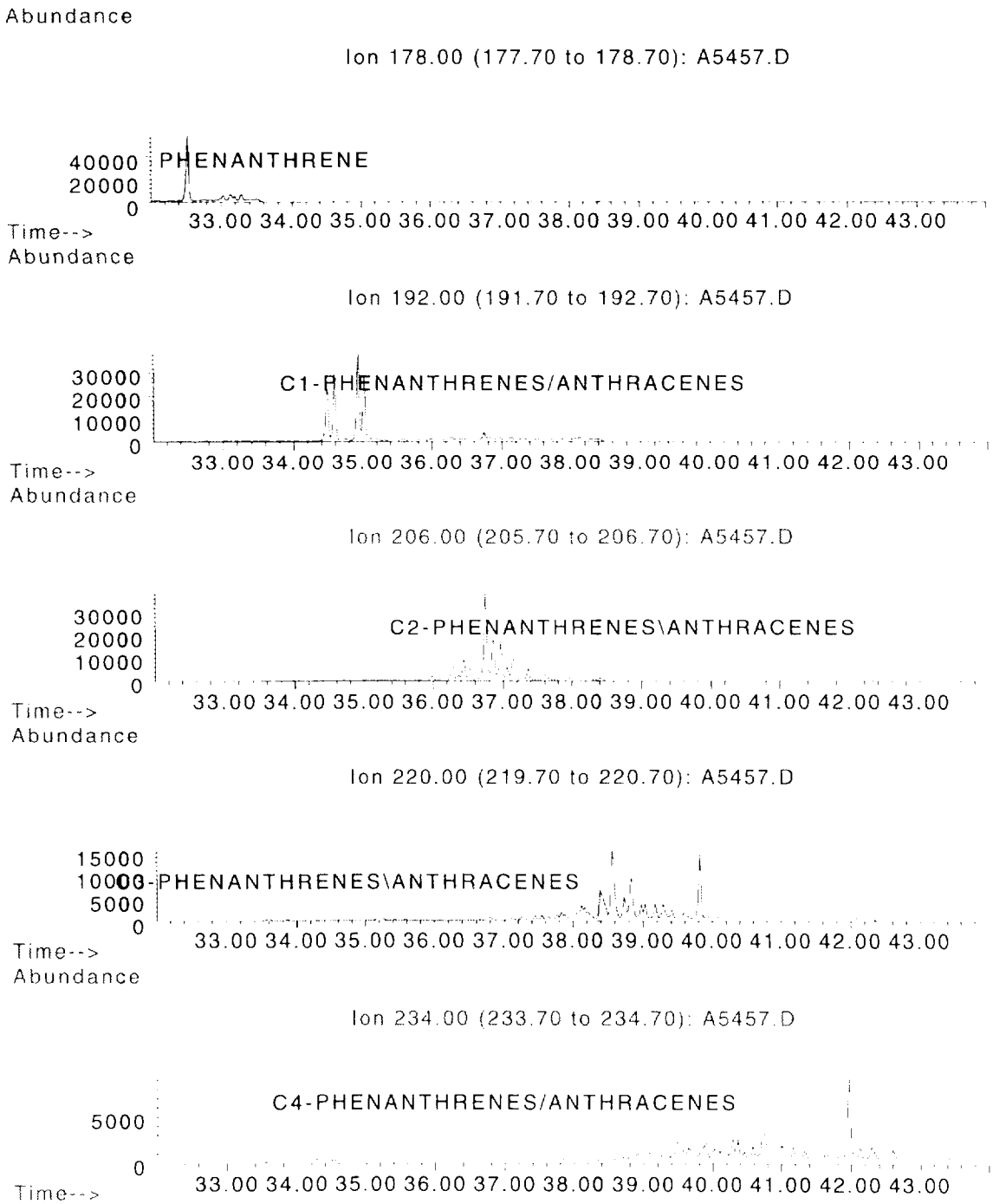

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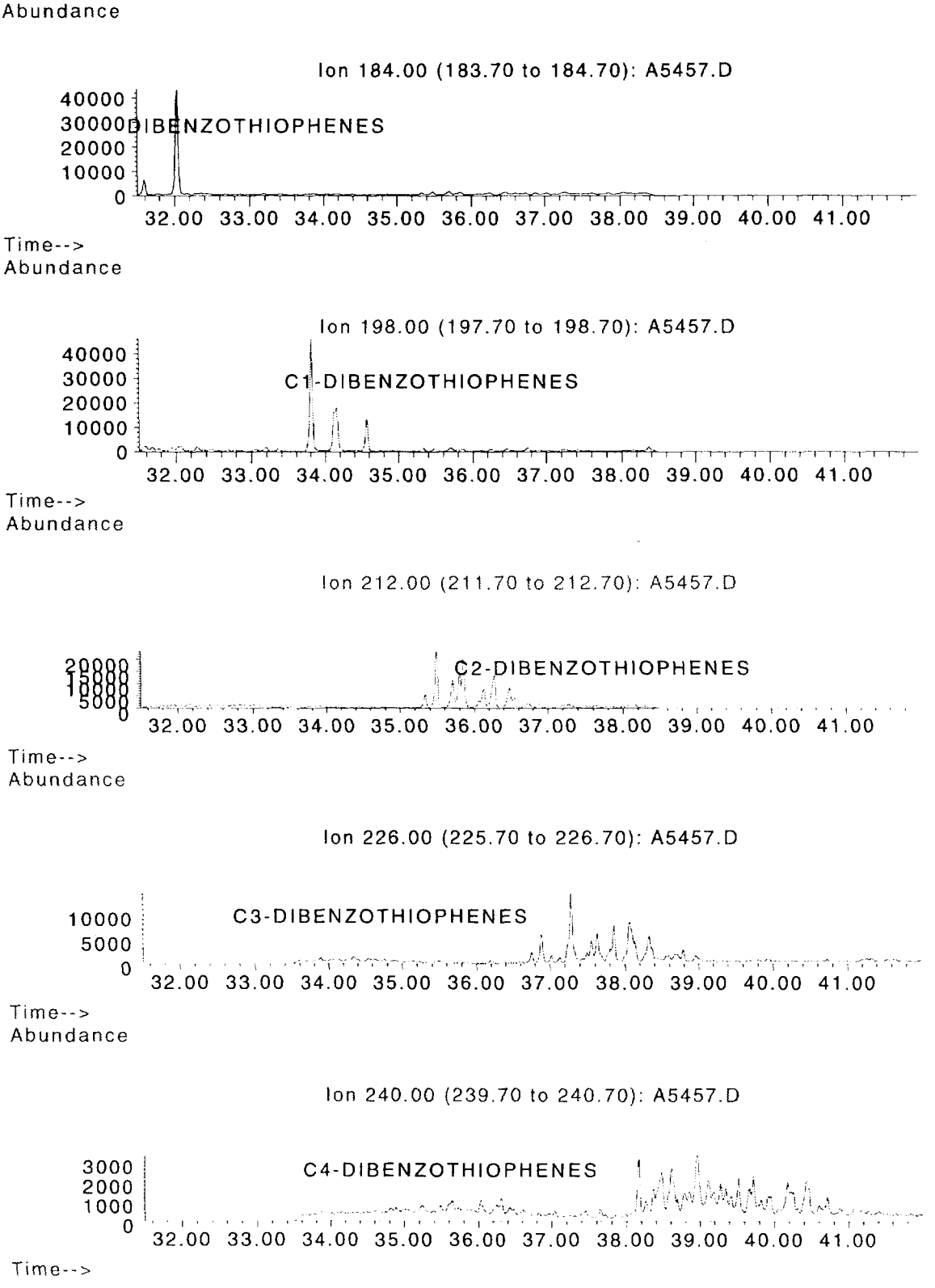

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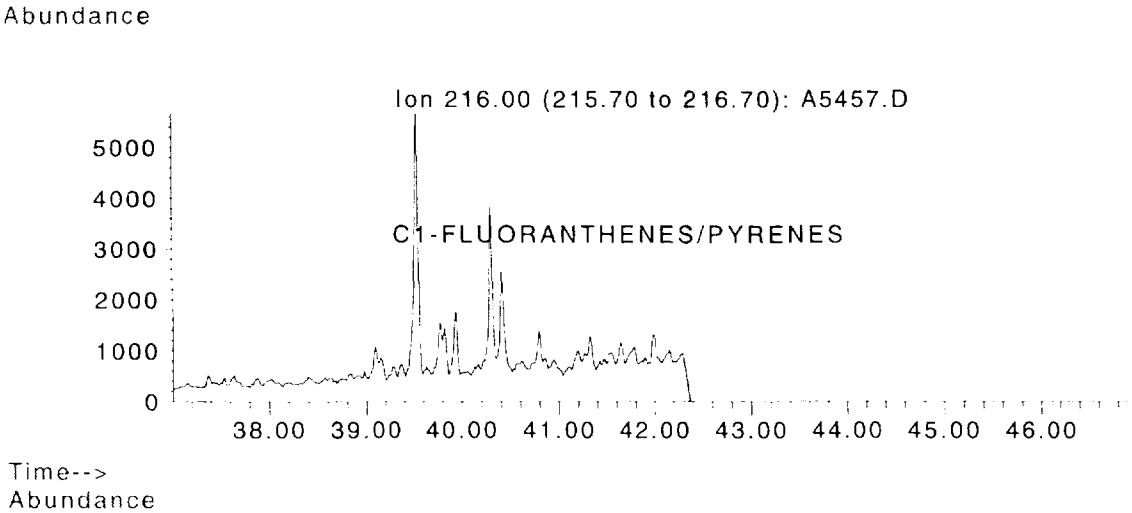

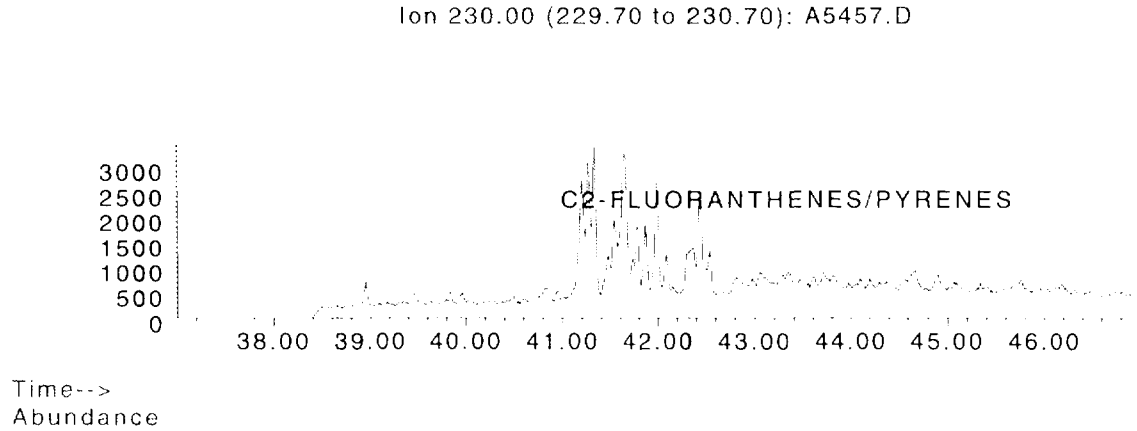

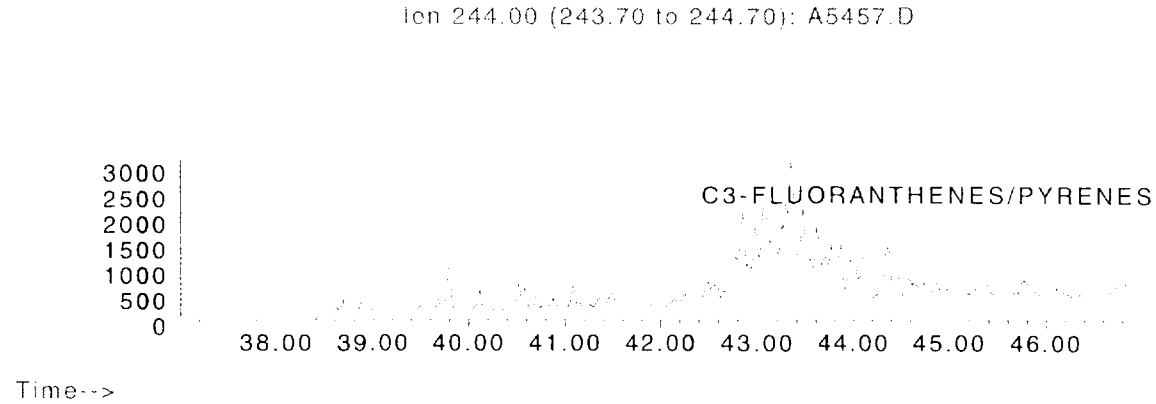

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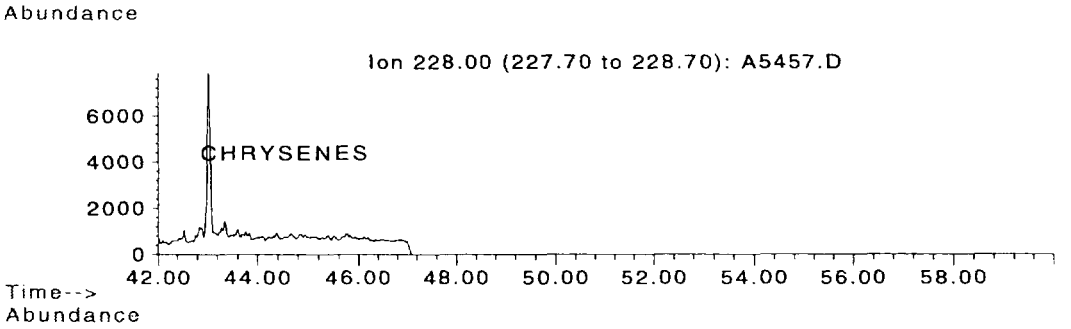

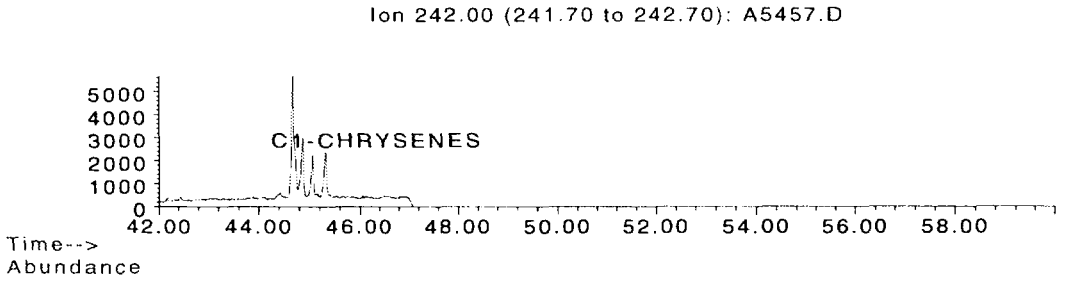

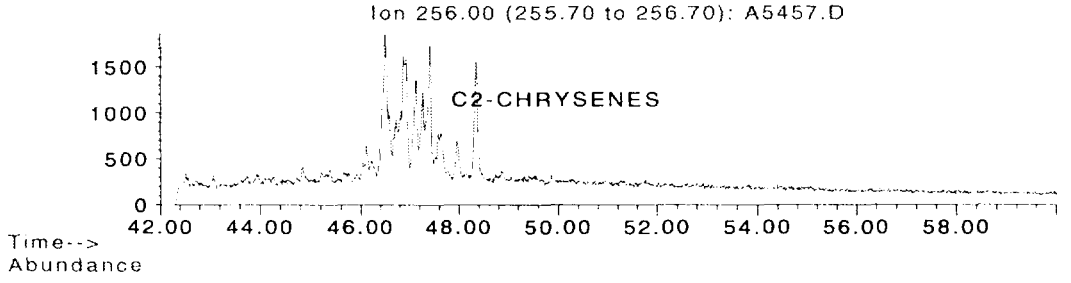

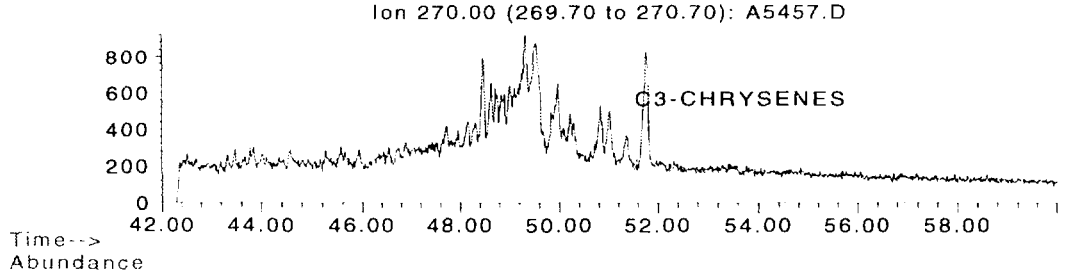

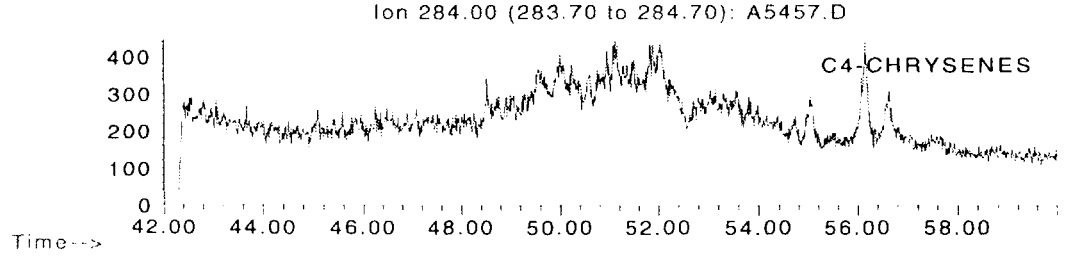

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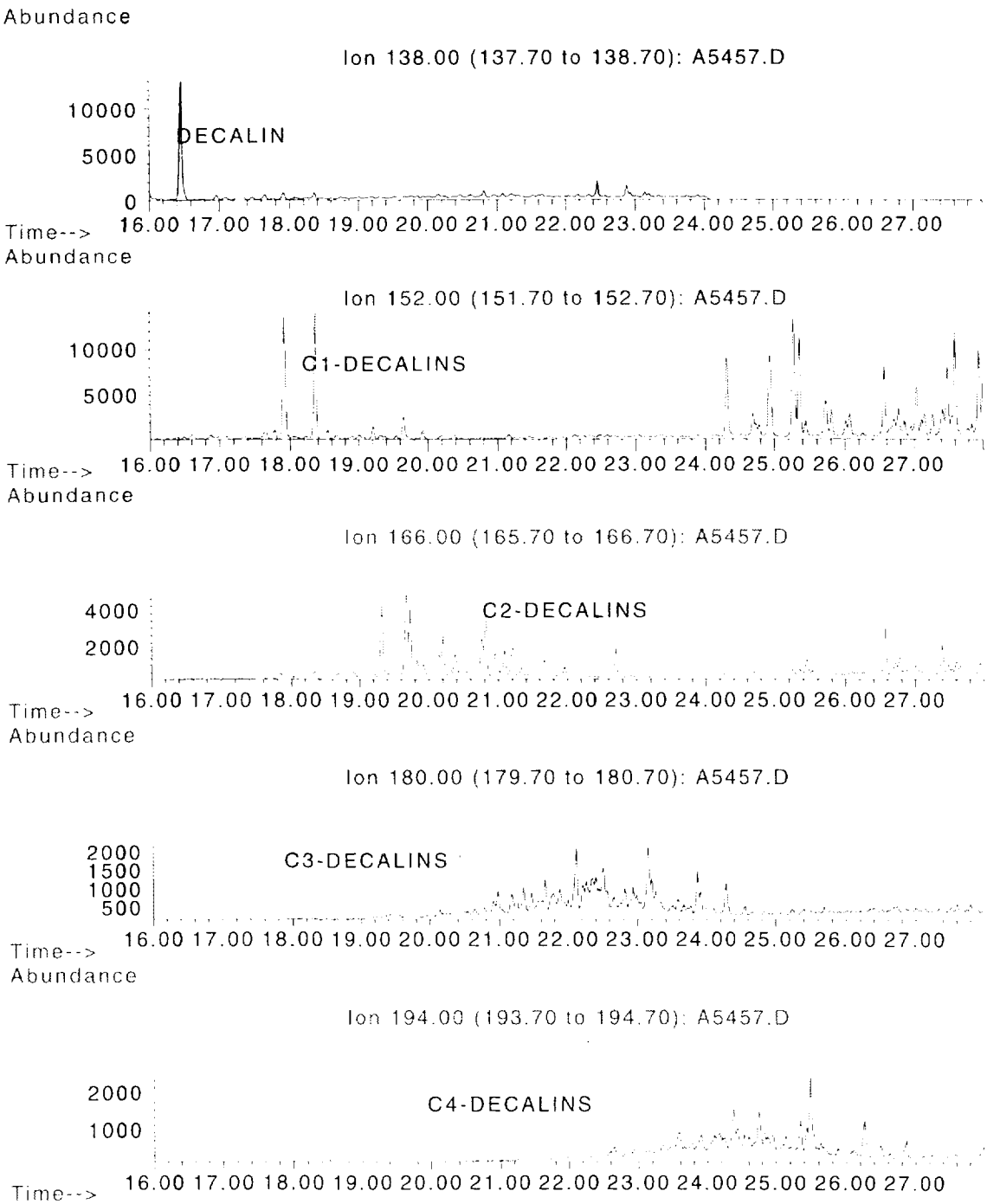


ATTACHMENT 2
BATTELLE DUXBURY OPERATIONS
CERTIFICATE OF TRAINING

FOR

SOP No. _____

Title _____

_____

_____

_____

_____

Trainee: _____

Instructor: _____

Date of Completion
of Proficiency Test _____

_____

TEST RESULTS

APPROVED BY: _____ **DATE:** _____

Battelle Duxbury Operations
Standard Operating Procedures

for

TISSUE EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANT ANALYSIS

Summary of changes in this version: This SOP is the tissue component of what was the combined tissue/sediment extraction SOP. The SOP includes clarifications of the alumina preparation and cleanup steps, and the organization of the SOP has been improved.

1.0 OBJECTIVE

The objective of this document is to define standard procedures for extracting trace levels of semi-volatile organic analytes from tissue matrices for analysis by gas chromatography. The extraction procedures are suitable for analysis of semi-volatile organic pollutants including those listed in EPA Methods 608, 610, 625, 8081, 8082, and 8270. The method may be suitable for other analytes once acceptable extraction efficiency has been demonstrated. Generally, this procedure will be used to prepare extracts that will be further cleaned up by liquid chromatography procedures before analysis.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

Apparatus for homogenizing tissue

- Tekmar Tissuemizer with probes, or equivalent
- Teflon jar or centrifuge bottle, 250-mL capacity
- 500-mL Erlenmeyer flask

Apparatus for determining wet weight and dry weight (~~tissue and sediment~~)

- Top-loading balance capable of weighing to 0.01 g (SOP No. 3-160)
- Aluminum weighing pan, stored in an aluminum foil "package" at 105°C
- Stainless steel spatula
- Drying oven maintained at 105-120 °C, Blue M Model SW-17TA or equivalent

Apparatus for determining lipid weight:

- Class A volumetric pipette or 10 mL syringe
- Aluminum weighing pan

Centrifuge

Balance accurate to 0.01 g

Glass wool heated to 400°C for at least 4 h, then stored in a covered glass container at 105°C, or thoroughly solvent cleaned prior to use if wool is temperature sensitive and becomes brittle at 400°C.

Glass fiber filters heated to 400°C for at least 4 h, then stored in aluminum foil "package" at 105°C or thoroughly solvent cleaned prior to use if filters are temperature sensitive and become brittle at 400°C.

19/21-mm chromatography column with ~200 mL reservoir and Teflon stopcock

Kuderna-Danish (K-D) apparatus (listed below) or Turbovap concentration units and tubes

- Reservoir, 500 mL
- Snyder column, three-ball macro
- Concentrator tube, 10- or 20-mL

Hot water bath capable of reaching 100 °C, located in fume hood.

Boiling chips, solvent rinsed

Nitrogen evaporation apparatus, N-Evap or equivalent, with heated water bath maintained at about 25 °C

Glass funnels

Glass graduated cylinders, 100- and 500-mL

Erlenmeyer flasks, 250- and 500-mL

Microliter syringes

2.2 REAGENTS

Dichloromethane (DCM), pesticide grade or equivalent

Milli-Q water

Sodium sulfate--anhydrous, reagent grade, heated to 400°C for at least 4 h, then cooled and stored in a tightly-sealed glass container at room temperature.

Alumina, F-20 (Aldrich Chemical. CAS #B44-28-1. 80-200 mesh).
Activate the alumina by heating in a shallow dish to 400 °C for at least 4 hr. Allow the alumina to cool in an oven (do not keep in open lab atmosphere for extended periods). Store the alumina in a covered glass container (with an activation date label) at 105 °C, and use within 1 week of activation.

Deactivate the alumina prior to use with 2% water. Prepare the 2% deactivated alumina in batches by adding 5 mL of Milli-Q water (accurately measured with a syringe or volumetric pipette) to 250 g of alumina in a 1,000 mL round bottom glass flask. Seal with a glass or Teflon stopper, shake vigorously by hand for 1 min to begin the process. Vent the flask, put the stopper back on, seal with Teflon tape, and allow to *vigorously* mix on a shaker table for 2 hr (± 15 min). Pack columns within 2 hr of completing the deactivation and use the same day. Unused deactivated alumina should be discarded.

Surrogate Internal Standards (SIS) and Recovery Internal Standards (RIS) are project specific and are defined in the project work plan.

2.3 LABWARE PREPARATION

Unless otherwise specified in the project specific work plan, all glassware contacting samples must be cleaned according to laboratory protocols defined in SOP No. 5-216.

2.4 RECORD KEEPING

Samples will be assigned unique identification numbers and logged in on the Chemistry Laboratory Sample Identification Log according to laboratory protocols (SOP No. 6-007).

Analytical data will be reported on appropriate data forms included in the sample batch books. Information to be recorded includes wet and dry weight information, dates of extraction/processing procedures, initials of laboratory personnel who perform the procedures, types and amount of internal standards added to samples, and, if necessary, comments regarding individual samples.

3.0 PROCEDURES

3.1 GENERAL

Samples should be extracted in batches of 20 or fewer field samples unless otherwise stated in the project specific work plan. Quality control samples accompanying each batch may include a procedural blank, laboratory control sample, matrix spike, reference material and/or field sample duplicate. (See Section 5.0 for more detail).

Each sample, including QC samples, should be spiked with SIS. Generally, the amount of SIS is such that the concentration of SIS compounds in the final extract should be approximately 1 µg/mL for GC/MS analysis, and/or 0.05 µg/mL for GC/ECD analysis.

The tissue samples should be ***thoroughly*** homogenized prior to any aliquotting for chemical or physical characterization, to ensure that a representative sub-sample is taken.

3.2 PERCENT MOISTURE DETERMINATION

Weigh approximately 5 g of well mixed tissue homogenate into a pre-weighed, pre-baked, aluminum weighing pan and record to the nearest 0.01 g. Place the sample in a drying oven and dry overnight at ca. 105 °C. After approximately 24 h, allow the sample to cool at room temperature for at least 30 min. Record dry weight to the nearest 0.01 g in sample batch book. Calculate the percent dry weight and/or percent moisture as in Section 4.0.

3.3 EXTRACTION, CONCENTRATION, AND CLEANUP

Tissue Sample Extraction

- Weigh 20-30 g of well mixed tissue into a 250 mL centrifuge jar (Teflon), weighing the sample to the nearest 0.01 g. Record sample weight.

- Add approximately 50 g sodium sulfate, mix well with spatula. Add more sodium sulfate if lumpy — some sodium sulfate should move freely in the solvent indicating that no more water is available.

- Add appropriate SIS and 75 mL DCM. Macerate/extract the sample with Tissumizer (or equivalent) at high speed for 2 min. Avoid spattering the sample.

- Centrifuge the sample for 5 min. at between 1500 and 2500 rpm. Longer or faster (if below 2000 rpm) centrifugation may be needed for samples containing fine particulates or suspended matter. *Carefully* decant the extract into an Erlenmeyer flask and cap the flask with aluminum foil.

- Repeat the maceration/extraction once more with an additional 75 mL DCM. Centrifuge as above and decant extract into Erlenmeyer, combining the sample extract.

- Add 50 mL DCM to the sample, seal centrifuge bottle and shake on a shaker table for about 30 min. Centrifuge as above, then decant solvent into the Erlenmeyer with the rest of the sample extract.

- Determine the lipid weight (DCM extractable) as follows:

  Gently swirl the Erlenmeyer to mix the extract. Mark the meniscus on the outside of the Erlenmeyer. With a clean 10 mL class "A" volumetric pipette or 10 mL syringe, remove 10 mL of the extract and place in a pre-weighed aluminum weighing pan. Cover weighing pan with foil, and allow extract to air dry overnight or until DCM is no longer visible. After drying, weigh pan and record weight. Record the total extract volume (see under Extract Concentration below) and calculate the lipid content as described in Section 4.

- Add approximately 20-50 g sodium sulfate to the Erlenmeyer and swirl. Add more sodium sulfate if sample is lumpy — some sodium sulfate should move freely in the solvent indicating that no additional water is available. Wait approximately 1 hr.

Note: *The final combined extract should not be stored in the Erlenmeyer flask for more than 1 day. If longer storage is needed before completing the sample preparation, the extract should be concentrated to several milliliters (see below), transferred to a glass vial, securely capped, and stored in darkness in a refrigerator or freezer.*

Extract Concentration — K-D Technique

- Transfer the sample extract to a K-D flask and receiver tube. If there is any evidence of particulates the extract must be filtered, using a glass funnel containing a pre-cleaned glass fiber filter, or pre-cleaned glass wool, topped with sodium sulfate. Rinse the Erlenmeyer twice with about 10 mL of DCM and add to K-D.

- Add 3-5 boiling chips to the K-D receiver and insert a Snyder column. Pre-wet the condenser column with approximately 5-mL DCM. Place the K-D apparatus in a hot water bath maintained at 60-65 ° C (monitored by a mercury thermometer), such that the concentrator tube is partially immersed in hot water and the entire lower rounded surface of the flask is bathed in hot water vapor. At the proper rate of evaporation, the balls of the Snyder column will actively chatter, but will not flood with condensed solvent. Continue concentration until the sample volume is reduced to approximately 10 mL.

- Notify the Lab Manager for instructions on how to proceed if the rate of concentration significantly slows down.

- Remove the K-D apparatus and allow it to drain and cool for at least 10 min. Transfer the concentrator tube to the N-Evap unit and concentrate the sample until the volume is 1-2 mL, maintaining a water bath temperature of approximately 25 °C.

- *Lipid weight extract volume determination*: After extract is poured from the original Erlenmeyer, rinse flask and remove sodium sulfate. Add tap water to the meniscus line drawn prior to removal of aliquot for lipid weight determination, and determine, and record, the original volume by measuring it with a 250 mL graduated cylinder. Calculate the lipid weight according to formulae presented in Section 4.0. Also, determine the lipid weight correction factor so the final data can be corrected for the proportion of extract removed for the lipid determination.

Note 1: The water bath for the K-D is maintained at 60-65 ° C for concentrating DCM, 70-75 ° C for concentrating acetone, and a boiling bath is used for concentrating hexane or toluene.

Note 2: Adjust the flow of nitrogen on the N-Evap to a ***gentle*** *stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.*

Extract Concentration — TurboVap Technique (optional)

If specified in the work plan, TurboVap may be used for concentrating the extracts rather than using K-D.

- Transfer sample extract to a 200 mL TurboVap tube with a 1 mL collection reservoir. TurboVap waterbath temperature should be set at approximately 25 °C; nitrogen pressure should be approximately 4 to 6 psi.

- Follow manufacturers instructions for operation and maintenance. After initial TurboVap concentration the extract should be quantitatively transferred to a 4 mL vial (with DCM rinses), and concentrated until the volume is 1-2 mL.

Extract Cleanup — Alumina

Alumina cleanup is routinely performed on tissue extracts. This may be the only cleanup, but is typically a cleanup step that is performed prior to HPLC/GPC cleanup. Sample extracts must be in DCM when loading onto the column, so solvent exchange may be needed if another extraction solvent is used.

Note: Phenol, and C1-C4 phenols, are retained by the alumina column and this cleanup step cannot be used if these compounds are target analytes.

Column Preparation

- Prepare the 2% deactivated F-20 alumina as described in Section 2.2.

- Pack the tip of the 19/21-mm ("fat") chromatography column with a small piece of glass wool. Add approximately 10 mL DCM and tap glasswool with clean glass rod to remove bubbles. Drain solvent into waste cup.

- Add approximately 25 mL DCM to the column. Weigh 40-g, 2% deactivated alumina into a beaker. Add ~50 mL DCM and swirl to remove bubbles. Slowly pour alumina slurry into the column while rinsing beaker with DCM

- Place approximately 1 g of sodium sulfate on top of the alumina. Tap the column to remove bubbles. Drain column to top of packing and discard solvent. Column is now ready for use; columns must be used the day they are prepared.

Column Elution

- Put a clean glass collection flask under the column (e.g., Erlenmeyer flask, K-D apparatus, or TurboVap tube).

- Load the 1 to 2 mL sample extract (must be in DCM) onto the column.

- Slowly drain and stop at the top of the alumina packing. Rinse the sample vial with about 1 mL DCM and load onto column. Slowly drain and stop at the top of the packing. Repeat vial rinse one time.

- Load with 150 mL of DCM.

Note: 150 mL of DCM will effectively elute our standard analytes (PAH, PCB, chlorinated pesticides, phthalates, and LABs). However, endrin aldehyde ***typically*** *elutes with the rest of the pesticides but sometimes has unpredictable alumina retention and may require 250-275 mL DCM to elute. Additionally, coprostanol and androstanol require 225 mL to elute. The project work plan will specify if more than 150 mL should be used to elute the column.*

- Drain the column slowly (about 2 mL/min), collecting the column eluent containing the target analytes, and stop at the top of the column -- do not drain the column dry into the collection vial.

- Open up and drain the column dry into a solvent waste jar.

Extract Cleanup — GPC

The sample is now ready for further cleanup and processing. Size exclusion HPLC/GPC cleanup (described in SOP No. 5-191) should be performed unless the work plan specifies to omit this step.

- The DCM extract volume should be adjusted to approximately 900 µL and centrifuged, if necessary, to settle out any particulates. The volume is measured with a gas tight syringe (to within 5 µL), the volume recorded, and the sample transferred to a 2-mL GC vial.

- Process the samples as described in SOP No. 5-191.

Final Preparation

If no further processing is required, the samples should be adjusted to the desired final extract volume (specified in work plan) and prepared for instrumental analysis.

- Adjust the final extract volume using N-Evap concentration. Adjust the flow of nitrogen on the N-Evap to a gentle stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.

Note: *Sample extracts at larger volumes (5 mL or more) and in larger vials (e.g., K-D receiver tubes or GPC/HPLC eluent collection tubes) should be concentrated on the N-Evap with the vials in the water bath at a temperature of 20-25°C (room temperature or thermostatically set at 25°C). Once the sample extracts are at lower volumes and in smaller vials (e.g., 4 mL vials), the N-Evap concentration should occur with the vials out of the water bath.*

- Spike the final extract with the appropriate recovery internal standards (RIS), as listed in the work plan, and split the extract as necessary, transferring the samples to GC vials. The GC/ECD split is solvent exchanged with hexane if this analysis is required, and the sample(s) submitted to the instrument analyst(s) for analysis.

4.0 CALCULATIONS

Calculate percent dry weight with the following equation:

$$\%\ dry\ weight = \frac{aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

Calculate percent moisture with the following equation:

$$\%\ moisture = \frac{aliquot\ wet\ wt.^{a} - aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

Calculate sample lipid weight with the following equations:

$$Total\ lipid\ weight\ (mg) = \frac{volume\ of\ sample\ extract\ (mL)}{aliquot\ vol(mL)} * aliquot\ dry\ wt.\ (mg)^{a}$$

[a] corrected for pan weight

Calculate sample dry weight with the following equation:

$$Sample\ dry\ weight\ (g) = \%\ dry\ wt. * sample\ wet\ wt.\ (g)$$

5.0 QUALITY CONTROL

The QC program for each project is defined in the project work plan, which will define the type and amount of internal standards/spiking solutions to be added to the samples, the specific QC samples to be processed, any modifications to the standard QC acceptance criteria, and the corrective action required if QC results do not meet those acceptance criteria.

Quality control methods for this procedure may consist of analysis of procedural blanks (working through procedure without sample; includes extraction solvent, sodium sulfate, copper and SIS), laboratory control samples (like procedural blank but also fortified with target analytes), matrix spikes (tissue fortified with SIS and target analytes; an aliquot of the matrix material will be analyzed separately to determine background levels of contamination), reference materials (SRM, CRM), or field sample duplicates. SOP 7-029 describes the default reporting format and data quality objectives for quality control data.

6.0 TRAINING

Analysts performing the procedure must initially work under the supervision of the Laboratory Manager, task leader, or other designated (trained) individual. Individuals may work independently once they have satisfactorily completed extraction on a set of triplicate laboratory control samples (spiked with a representative suite of analytes), and procedural blank. These samples may be analyzed directly by GC without further processing. Analyses will be considered satisfactory if the recovery of all spiked analytes fall between 70 and 110 percent of the true values and the procedural blank contains less than or equal to

the method detection limit of all analytes of interest, and/or if analytical results demonstrate proficiency as determined and documented by the Lab Manager in the training files.

Upon successful completion of training, a training certificate (Attachment 1) will be issued. The original will be kept on file in the Quality Assurance Office.

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

8.0 ATTACHMENTS

Attachment 1. Example of Battelle Certificate of Training.

9.0 APPROVALS

APPROVALS

Author	Name	Date 12·9·99
Laboratory Manager	Name	Date 12·9·99
QA Coordinator	Name	Date 12-9-99
Environment Chemistry Section Manager	Name	Date 12/9/99

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

Title: **TISSUE EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANTS**

SOP#: **5-190-06**

Trainee: _____

Instructor: _____

Date SOP was read: _____ (*To be completed by trainee*)

Date training was completed: _____ (*To be completed by instructor*)

APPROVED _____, _____
Laboratory Manager Date

Superfund Records Center
SITE:
BREAK:
OTHER:

ORIGINAL

Date Effective: Sept. 17, 1998

Battelle Duxbury Operations
Standard Operating procedure

for

HPLC CLEANUP OF SAMPLE EXTRACTS FOR SEMIVOLATILE ORGANIC POLLUTANTS

Summary of Changes in this Version: Several sections have been updated with clarifications and new information. These include the reference to new equipment and modifications to the fractionation time determinations.

1.0 OBJECTIVE

The objective of this document is to describe the cleanup of sample extracts, and other sample solutions, by High Performance Liquid Chromatography (HPLC) prior to analysis for semi-volatile organic compounds. This document describes the operation of the HPLC as it relates to sample cleanup as well as sample preparation procedures associated with HPLC cleanup.

The HPLC system uses a pump which provides a constant isocratic or gradient flow (depending on the application), a UV detector, an autosampler, a programmable fraction collector, a recorder or integrator for monitoring the UV response, and appropriate HPLC column(s) for the particular cleanup/fractionation to be performed.

This SOP focuses on sample cleanup using gel permeation chromatography (GPC) and silica columns, although other columns and instrumental conditions may be used for particular applications once acceptable performance has been demonstrated.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

1. A liquid chromatography pump capable of providing a constant flow of 5 or 10 mL/min, depending on the application at hand (Thermo-Separations P1000, Spectra-Physics 8800, or equivalent).

2. HPLC columns:
   (1) **GPC**. A 22.5 × 300 mm, 100 Å pore size, Phenogel GPC/size exclusion column, and one 7.8 × 50 mm Phenogel precolumn (Phenomenex, Rancho Palos Verde, CA), or equivalent. Columns are arranged so that the precolumn can be backflushed periodically. Other column dimensions, and columns connected in series, may be used if acceptable performance is demonstrated. The GPC is commonly used for separating PCB, chlorinated pesticide, PAH, and other semivolatile compounds from other tissue and sediment matrix components, thus purifying the extract before instrumental analysis.

(2) **Silica**. A 25 × 100 mm, 10 µm particle size, 125 Å pore size, µPorasil silica column, and a 25 × 10 mm µPorasil precolumn (Waters Chromatography, Milford, MA), or equivalent. Columns are arranged so that they can automatically be backflushed after each sample (e.g. with an electronic Valco valve). Other column dimensions may be used if acceptable performance is demonstrated. The silica column is commonly used for separating/fractionating hydrocarbons [e.g., traditional F_1 (saturated HCs), F_2 (polyaromatic HCs), and F_3 (polar HCs) fractionations], but can also be used for other compound or class specific sample cleanup (e.g., to isolate PCBs from complex matrices).

(3) **Other**. Other columns may be appropriate for other applications. For instance, a cyano column may be suitable to isolate highly polar compounds from other sample components. The suitability of a column and associated chromatographic conditions for the compounds of interest will be verified and documented before being used on actual project samples.

3. Auto sampler capable of injecting 500 to 600 µL of sample with minimum sample loss in transfer lines, loop flushing, or elsewhere (Spectra-Physics AS3000, Gilson 231/401, or equivalent). A 1000 or 2000 µL sample loop is used to assure that none of the 500 to 600 µL sample is flushed to "waste" during sample loading.

4. Programmable fraction collector which is synchronized with the injector (Foxy 200, Gilson 201, or equivalent).

5. Recorder or integrator (H-P 3396, or equivalent) which is synchronized with the injector and records the signal from a UV detector (Spectra-Physics 8450, or equivalent) fixed at a suitable wavelength for the target compounds (typically 254 nm).

6. Microliter syringes

7. Glass vials with Teflon lined caps
   1.8 mL HPLC autosampler vials, with Teflon/Silicone/Teflon septa
   60 mL fraction collector vials/tubes

2.2 REAGENTS

1. Dichloromethane, hexane, and/or methanol, as applicable (standard laboratory grade). These are the three solvents routinely used with the system. The actual solvent delivery programs vary with application, and the most commonly used programs are discussed in Attachment 5. Non-routine applications and solvent programs will be described in the QAPP.

2. HPLC calibration solution compounds. In general, the first and last eluting compounds should be in the calibration solution [e.g., 4,4'-dibromooctafluorobiphenyl (DBOFB) and perylene or benzo(g,h,i)perylene; d_8-naphthalene or naphthalene and perylene or benzo(g,h,i)perylene; Cl_1(1) and Cl_{10}(209)]. Potential interferants/contaminants from which the samples are to be separated and cleaned (e.g., corn oil and sulfur) may also be added.

2.3 LABWARE PREPARATION

Unless otherwise specified in the QAPP, glass materials contacting samples must be washed, baked and solvent rinsed according to SOP 3-019.

2.4 RECORD KEEPING

Samples and standards processed by HPLC cleanup are recorded on HPLC Sample Fractionation Log Forms (Attachment 1) which are kept in a 3-ring binder along with the sample chromatograms. If re-fractionation of samples is necessary, this will be indicated for the affected samples on the original form and a new form will be completed for the samples to be refractionated. Refractionated samples are assigned the suffix "-R" to distinguish them from the original sample. The sample ID recorded must agree with that used in the Sample Preparation Records. The laboratory sample ID (SOP No. 6-007) will be used to track samples through the laboratory, unless otherwise specified in the QAPP.

The HPLC Sample Fractionation Log Form will be completed for each set of fractionations/analyses. Column type, injection volume, flow rate, mobile phase, any automatic valve switching times, and results of the calibration [i.e., retention times of calibration analytes and fractionation window(s)] are also recorded on this form. After completing the HPLC processing the sample IDs will be marked on the UV chromatograms and the chromatograms filed with the Sample Fractionation Form. System maintenance (e.g., column replacement, replacement of tubing, unclogging of system plugging) will be recorded on the appropriate forms in the HPLC System's Log Book.

3.0 PROCEDURE

3.1 GENERAL

Samples are extracted, or otherwise prepared, according to procedures specified in project-specific plans, run through a rapid clean-up, filtering, and/or centrifugation procedure, as needed, concentrated, and submitted for HPLC cleanup. The retention time window is determined and the instrument calibrated using a calibration standard. A portion of the sample is injected on the HPLC, the UV response monitored, and the analyte fraction(s) collected, concentrated and submitted for further instrumental analysis. The QAPP will identify the specific cleanup procedure (e.g., standard GPC column, F_2-hydrocarbons by silica column). Conditions for non-standard HPLC fractionations will be described in the QAPP.

3.2 STANDARDS PREPARATION

Prepare an HPLC fractionation time standard appropriate for the cleanup/fractionation to be performed. The following are examples. HPLC calibration standards are for qualitative determination of retention times, and can therefore be assigned an expiration date of 3 years.

GPC Column – NS&T type PAH, PCB/pesticide analytes. Prepare a standard of approximately 20 µg/mL DBOFB, 20 µg/mL perylene or 10 µg/mL benzo[g,h,i]perylene, 20 mg/mL corn oil, and 40 µg/mL sulfur in dichloromethane. This standard will be used as a retention time (RT) marker

since DBOFB elutes towards the beginning and perylene/benzo[g,h,i]perylene towards the end of the analytes of interest in these analyses, and corn oil and sulfur represent potential interferences. Perylene elutes just before benzo[g,h,i]perylene. Project-specific RT marker compounds can be used, or project-specific fractionation windows determined, and will then be specified in the QAPP.

Silica Column - PAH (F_2) analytes. Prepare a standard of approximately 20 µg/mL d_8-naphthalene or naphthalene and 10 µg/mL perylene or benzo[g,h,i]perylene in approximately the same solvent composition that will be used for the samples. A mixture of approximately 1:1 hexane/dichloromethane is appropriate for most $F_1/F_2/F_3$ hydrocarbon work. The compound d_8-naphthalene elutes just before naphthalene and perylene elutes just before benzo[g,h,i]perylene. These are the first and last eluting PAH of interest in most of our F_2 analyte analyses. F_1 and F_3 target analytes may not have UV detector response (n-alkanes, for instance, do not), but fractionation windows for such target analytes can be established/calibrated relative to the F_2 calibration analytes by collecting multiple fractions of a standard containing all target analytes, along with the HPLC analysis of the F_2 calibration standard, and analyzing the fractions for the target compounds. Should the standard RT marker compounds not be the first and last eluting analytes of interest for a new analysis, the retention time marker analytes will have to be changed accordingly, and will be specified in the QAPP.

Silica or GPC Column - PCB analytes. Prepare a standard of approximately 20 µg/mL each of $Cl_1(1)$ and $Cl_{10}(209)$ — on the silica column the $Cl_{10}(209)$ is the first eluting peak and $Cl_1(1)$ elutes last. Prepare the standard in the solvent that the samples are to be loaded onto the column in (always hexane for the silica column).

3.3 PRE-HPLC SAMPLE PREPARATION

1. Pre-HPLC sample cleanup and/or filtration may or may not be necessary, depending on the lipid content and amount of particulates in the extracts. Specific cleanup/filtration techniques to be used will be determine on a project by project basis by the project team, and documented in the sample preparation records.

   The following is an example of sample cleanup procedure using alumina that may be suitable to remove excess lipid material from some extracts:

   Prepare a preliminary cleanup/filtering column for each sample to be processed by pouring 20 g of 2% deactivated alumina into a 19 mm ID chromatography column with a glass wool plug at the bottom. Alumina and glass wool are cleaned and baked out as for standard column chromatography. Fill column with dichloromethane and drain to the top of the column packing.

   Pour the sample extract through the column and collect the eluent. For pre-concentrated extracts pour an additional 80 mL of dichloromethane through the column and collect with the sample eluent, and for unconcentrated extracts pour 50 mL through and collect with the sample eluent (these elution volumes must be doubled if endrin aldehyde is a target analyte).

   Sample extract centrifugation and filtration may be appropriate to remove particulates, and will be determined on a project by project basis by the Sample Prep Task Leader in consultation with the Project Manager or Laboratory Manager, and will be documented in the sample preparation records.

2. Extracts are concentrated to approximately 900 to 1000 µL using at first a Kuderna-Danish apparatus and then nitrogen evaporation. Transfer the sample to a 1.8 mL HPLC autosampler vial. If particulate matter is present in the sample it may be necessary to filter the sample. Alternatively, the sample may be separated from the particulates by centrifugation and carefully removing the extract with a pipette or syringe, leaving the particulates, and a minimum amount of sample, behind and then dispensing the sample into a 1.8 mL HPLC autosampler vial. It is ***imperative*** that all samples be totally free of particulate matter and suspended solids. The presence of particulates and any project specific sample filtration/separation procedures will be documented in the sample preparation records.

   Measure the sample volume to the nearest 10 µL and record the volume on the HPLC Split Documentation Form (Attachment 2). Place the samples in a refrigerator until shortly before HPLC analysis, but do not store for more than 1 day because evaporation may alter the measured volume.

3.4 HPLC FRACTIONATION TIME DETERMINATION

1. Check the frit and distribution disk on the in-line filter, and replace if needed.

2. Turn on the power for HPLC pump, autosampler, fraction collector, detector, and integrator. Allow at least 15 min for the detector to warm up before use.

3. Rinse out and put fresh solvent in all solvent-holding bottles at least monthly. As needed, de-aerate the mobile phase and autosampler rinse solvent bottle (S-P AS300 only) solvent by bubbling a gentle stream of clean nitrogen or helium through them for at least 1 hour (this can be done while running calibration standards).

4. Purge air out of mobile phase and auto sampler lines by opening the bypass valve and allowing the solvent to be purged into a beaker. If necessary, prime the pump prior to purging.

5. Purge air out of syringe/injector rinse solvent lines and flush several milliliters through the dilutor and sample syringes. *No air bubbles should be visible at top of syringe or elsewhere in sample delivery lines.* Minor bubbles in the solvent deliverey line are generally acceptable.

6. Make sure the waste bottle can hold the volume of mobile phase that will be used.

7. **GPC Column.** Before each day of HPLC analysis with the GPC column, backflush the precolumn with DCM at a flow rate of 2–5 mL/min for 10 to 20 min with the backflushing valve in the RINSE position. Return the valve to the RUN position before analyzing any standards or samples. Alternatively, use an electronic switching valve and program it to backflush the precolumn for 2-4 min at the end of each sample run.

   Note: *Never* backflush the main GPC column. The flow through this column must always be in the forward direction.

   Silica Column. The silica column may need to be backflushed periodically with methanol to remove accumulations of polar compounds, if this is not part of the sample fractionation program. The need, and frequency, of the methanol rinse will depend on the samples analyzed and will be determined by the Project Manager.

8. Program the autosampler (Attachment 3), fraction collector (Attachment 4), HPLC pump (Attachment 5), and integrator for analysis of the HPLC fractionation time standard and the samples to be fractionated. Attachment 5 includes examples of typical solvent delivery conditions for common applications (PAH/PCB/pesticide cleanup by GPC; $F_1/F_2/F_3$ fractionation by silica; PCB cleanup by silica). Attachment 6 summarizes examples of general chromatographic and detector conditions.

   Detailed information on operation and maintenance of the HPLC pump, autosampler, fraction collector, and detector can be found in the respective instrument manuals, which are kept with the instruments.

9. Start the pump. Record the pressure once it has equilibrated. Should the pressure be unacceptably high (over 700 PSI for the GPC column and over 500 for the silica column) it may be necessary to replace the in-line filter, and possibly the precolumn. Other possible causes of elevated pressure are clogged tubing at a fitting (reverse flow through tubing) and accumulations in the detector cell (clean cell with 5-10% nitric acid, as per description in detector manual). Should the pressure be low (under 100 PSI) it may be necessary to replace one of the HPLC pump piston seals.

10. Start the auto sampler program for analysis of HPLC fractionation time standards. Analyze the HPLC fractionation time standard at least twice. If the RT differences between runs is greater than 0.1 min (GPC) to 0.2 min (silica) reanalyze until acceptable RTs are obtained from two consecutive runs. Average the RTs from the reproducible HPLC runs for the retention time marker compounds separately. Pressure readings are recorded on the HPLC Sample Fractionation Log Form at the start of each set of fractionations. Documentation of corrective action and maintenance needed to correct RT drift is kept with the HPLC.

11. Determine the analyte fractionation window, and program the fraction collector accordingly. The programmed fractionation time will be recorded on the HPLC Sample Fractionation Log Form, and the fractionation time programming will be witnessed by another qualified operator, if available. If no-one is available to witness the programming at the beginning of the run then someone should witness what was programmed in at the end of the run before samples are removed from the system.

    The fractionation window depends on the analysis to be performed. The following examples are approximate and actual windows will depend on the column dimensions, analytes, flow rates, solvent composition etc. Other project-specific fractionation times will be documented in the QAPP, or in project records with Project Manager or Laboratory Manager approval.

    GPC. The following table provides general guidelines for establishing a fractionation window for a project. These assume that the commonly used GPC conditions (e.g., single 300 mm long column, pre-column, 5 mL/min flow rate of 100% DCM) are used.

    Attachment 7 lists GPC elution patterns for selected analytes, and this, along with the above table, should be used by Project Managers and other staff to establish project RT windows. If, for instance, the analysis if for PAH only, the fractionation window can be optimized for these compounds.

Example HPLC/GPC Fractionation Windows for Selected Analytes
(Based on Instrument Configuration April, 1998 — applies to both Instruments #1 and #2)

Analytes	Start Time Relative to DBOFB RT	End Time Relative to Perylene RT
PAHs	+0.5 min	+1.5 min
Phenols (C0-C4)	+0.8 min	+0.8 min
PCBs	+0.5 min	+0.5 min
DDTs	–0.5 min	±0 min
LABs	–1.8 min	±0 min
NS&T Pesticides	–0.3 min	+0.5 min
608/8081 Pesticides (without methoxychlor)	–0.8 min	+0.5 min
608/8081 Pesticides (with methoxychlor)	–1.2 min	+0.5 min
NS&T Organic List (PAH/PCB/Pesticides)	–0.5 min	+1.5 min
USACE Organic List (PAH/PCB/Pesticides)[a]	–0.8 min	+1.5 min

[a] Assumes 608/8081 pesticides ***without*** methoxychlor. Would need to start 0.4 min earlier to capture methoxychlor.

The data in the above table and Attachment 7 are for both HPLC System #1 and #2 and are valid on 04/01/98. These values will change with system changes, and should therefore be updated/checked when appropriate, as indicated in Section 5.0.

Silica. Silica-column fractionation windows are highly dependent on HPLC conditions but also on the solvent composition of the sample. Ideally, the standards and the samples will both be of the same solvent composition being delivered by the HPLC pump at the beginning of the run (t_0) — this will give the best chromatography, but is not always practical. Because the silica column solvent delivery program generally begins with 100% hexane, amounts of more polar solvents in the sample (e.g., DCM) can significantly affect the retention times of the analytes. It is therefore best if the RT calibration solutions have the same solvent composition as the samples, but this too is not always practical because the chromatography may suffer and it may be difficult to read the calibration chromatogram. It may therefore be appropriate to run combinations of standards dissolved in hexane and the sample solvent (if different) to obtain an assessment of the column retention.

For most standard $F_1/F_2/F_3$ fractionation work samples and standards will be in a solvent composition of approximately 1:1 hexane:dichloromethane. Standard F_2 fraction collection can be performed by collecting from approximately 0.5 min before the RT of d_8-naphthalene or naphthalene to approximately 1.5 min after the RT of perylene or benzo[g,h,i]perylene, if standards and samples are in 1:1 hexane:dichloromethane. However, if the calibration solution is primarily hexane and the samples are primarily DCM the fractionation window may need to be from 3.0 before to 2.0 min after the RTs of the RT analytes, based on a calibration solution that is in 100% hexane. The Project Manager and/or the Lab Manager should be consulted if the solvent composition of the sample and standard are not the same or where questions regarding RT windows occur.

PCB isolation/fractionation with the silica column will need to be equally carefully calibrated. During standard PCB fractionation (100% hexane mobile phase from the beginning to several minutes past the elution of PCBs) the congeners elute between approximately 5 and 8 minutes, and the collection window should begin approximately 1.5 min before the RT of Cl_{10}(209) and end approximately 2.0 minutes after the RT of Cl_1(1).

Fractionation windows may be modified by the Project Manager depending on analytes, matrix, and other project specific considerations.

3.5 SAMPLE FRACTIONATION/CLEANUP

1. Place a rack with the appropriate number of 50 mL fraction collection tubes on the fraction collector, and place the HPLC samples on the auto sampler. Complete the HPLC Sample Fractionation Log Form. Confirm the order and labeling of the HPLC samples (on the autosampler) and fraction collection tubes (on the fraction collector). Include an HPLC fractionation time standard for analysis approximately every 10–12 samples.

2. Check that the HPLC pump, autosampler, fraction collector, and integrator programs are correct.

3. Start the HPLC sample fractionation/cleanup run by starting the autosampler program. It is important that the HPLC pump runs continuously after the running of the retention time standards used for fractionation window determination, because if the pump is shut off and restarted it may equilibrate at a different pressure and RTs may shift.

3.6 POST HPLC SAMPLE PREPARATION FOR INSTRUMENTAL ANALYSIS

The collected fractions are removed from the fraction collector and concentrated by nitrogen evaporation or TurboVap. Concentrate the samples to the appropriate project-specific final volume, transfer to an autosampler vial, add the internal standard, cap, vortex, and submit for instrumental analysis.

Visually (with a reference vial) check the volumes remaining in the autosampler vials as soon as the analysis sequence is completed. If the volume appears to be significantly different from the expected, measure and record all volumes and adjust the HPLC Injection Volume form, as appropriate. Identify the injection problem and resolve.

3.7 REFRACTIONATION OF SAMPLES

Should the fractionated sample not be usable for final instrumental analysis (e.g., pump shut off in the middle of a run), the unfractionated portion of the original extract can be used for HPLC processing. The unfractionated portion is then brought to a volume of approximately 900 to 1000 µL with the appropriate solvent, the exact volume indicated on an HPLC Split Documentation Form, and the sample analyzed/fractionated as described in previous sections. Unusable sample fractionations will be so indicated on the HPLC Sample Fractionation Form, explained, dated, and initialed. Refractionations will be indicated as such on the new HPLC Sample Fractionation Form that is prepared, and the number of the refractionation will also be documented (e.g., "-R": first refractionation; "-R2": the second time the samples are refractionated).

4.0 CALCULATIONS

Determination of fractionation window is the only calculation required as part of this procedure, and was discussed in Section 3.4 - items #9 and #10. The RT window may be modified by the Project Manager to what is appropriate for the particular project analytes, HPLC conditions, and sample matrices. The portion of the sample processed through the HPLC is calculated and recorded on the HPLC Split Documentation Form using the following equation.

First Fractionation: $[(IV_1)/(EV_1)] \times 100\%$

Re-fractionation: $[1-((IV_1+AV)/EV_1)] \times [(IV_R)/(EV_R)] \times 100\%$

IV_1 = Sample injection volume (µL) in original/first fractionation
EV_1 = Pre-HPLC sample extract volume (µL) in original/first fractionation
AV = Autosampler volume difference between aspiration and dispension (generally 0 µL with the S-P AS3000 and 20 µL with the Gilson 231/401).
IV_R = Sample injection volume (µL) in re-fractionation
EV_R = Pre-HPLC sample extract volume (µL) in re-fractionation

5.0 QUALITY CONTROL

1. Quality control methods for this procedure will consist of establishing the RTs of the fractionation window calibration analytes before each batch of samples are run by multiple injections of an HPLC fractionation time standard until two analyses result in RT differences of less than 0.1 min (0.2 min for silica column). The average RTs from these analyses will be used to program the fraction collector. An HPLC fractionation time check standard will be analyzed every 10–12 samples to check the RTs of the calibration compounds, which should be ±0.5 min of the RTs used to program the fraction collector.

2. After significant HPLC maintenance (e.g., new columns installed or major system"replumbing"), or when performance dictates, the performance will be checked against recently achieve good performance. This will be done qualitatively/semiquantitatively by comparing the chromatography obtained with the calibration standard (e.g., peak shape and absolute and relative retention times of the first and last eluting target analytes are reviewed). From these data it can be determined if the system is performing well (i.e., good peak shape, approximately the same retention times of the peaks, and approximately the same separation between the peaks).

   If the system appears to be performing poorly or differently from before, this should be investigated and resolved before proceeding with sample analysis. If new chromatography/performance appears to be correct, the procedure described under item #3 below should be performed to check/establish retention time windows. If "atypical" system performance is accepted without additional quantitative check (e.g., item #3 below) this should be documented and approved by the Laboratory Manager or Project Manager before proceeding with sample analysis.

3. After major HPLC system changes (e.g., column(s) from different manufacturer, new column dimensions installed, new dimension or length of lines/tubing), or when performance dictates,

calibrations will be done to determine the size of the fractionation window needed to collect >95% of, at a minimum, the first and last eluting target analytes. This calibration will be done by fractionating an injection of the HPLC fractionation time standard and collecting small fractions (e.g., 0.5 min) from several minutes before to several minutes after the HPLC RTs of these compounds (a single larger window can be collected between the two RT marker analytes) and analyzing the fractions by the appropriate instrumental method.

Alternatively, the recoveries of all analytes will be determined by running an analyte standard mixture through the HPLC fractionation procedure and analyzing for the analytes by the applicable instrumental method. This recovery check will always be carried out if the HPLC cleanup procedure is to be used in the analysis of analytes for which the procedure has previously not been used (i.e., first and last eluting compound is not known). Attachment 7 list recent data from such a calibration.

From these data it can be determined how long before to how long after the RTs of the RT marker compounds fraction collection must be performed (see table on page 7; Section 3.4)

The quality control procedure described under item #1 above will be carried out for every batch of samples. The procedure under item #2 when column replacement, or other significant maintenance has been performed. The procedure described under items #3 will be performed when major HPLC system changes have been performed, when performance dictates, when procedures or analytes have changed, and at the discretion of the Laboratory Manager. Additionally, relevant sample and analysis specific information will be recorded on the HPLC Sample Fractionation Log Form. Other project specific quality control programs will be addressed as specified in the QAPP

6.0 MAINTENANCE

This procedural SOP is not intended to include detail on system maintenance. Maintenance considerations and procedures are presented in the various instrument manuals. However, a few specific items that need be considered are listed below. Non-routine maintenance (e.g., pump seal, column or other hardware replacement, or dislodging of a system clog) should be recorded in the HPLC System's Log Book. Routine daily maintenance, such as pre-column backflushing and filter and frit changes, need not be recorded.

Column/Precolumn Cleanup/Conditioning. Before each day of HPLC analysis with the GPC column, backflush the precolumn with DCM at a flow rate of 2–5 mL/min for 10 to 20 min with thebackflushing valve in the RINSE position. Return the valve to the RUN position before analyzing any standards or samples. Alternatively, a 2-4 min automatic backflush may be programmed in to the end of each sample run, if an electronic switching valve is used. In a 30 min GPC run the backflushing would, typically, be from 26 to 29 min, which allows for a 1 min re-equilibration in the normal flow direction.

With time the backpressure due to accumulations in the precolumn (see below) will become unacceptably high, and the precolumn will need to be replaced. It is important to replace it before the main column begins deteriorating. The main column could last several years if the system is maintained well.

The silica column may need to be backflushed periodically with methanol to remove accumulations of polar compounds, if this is not part of the sample fractionation program. The need, and frequency, of

the methanol rinse will depend on the samples analyzed and will be determined by the Project Manager or Laboratory Manager. This is not performed routinely. The use of methanol and other polar mobile phases, as well as samples with much polar material, will shorten the life of the silica column and precolumn. The precolumn should be replaced every 1 to 2 months (~100 samples), depending on the amount of use, types of samples processed, and mobile phases used. The main column should last 6 to 12 months (~500 samples), but this too will depend on use and types of analyses performed. Column deterioration will be evident by shorter retention times, and worsening chromatography. If column performance is suspected of having worsened it should be replaced or checked with target analytes (Section 5), to determine if adequate separation/cleanup is achieved (e.g., adequate separation between F_1 and F_2 analytes).

High/Low Pressure. The system pressure is checked each day before samples are analyzed. Should the pressure be unacceptably high (over 700 PSI for the GPC column and over 400 PSI for the silica column) it may be necessary to clean or replace the in-line filter and/or pre-column. Accumulations in the main column and detector (clean cell with 5-10% nitric acid, as per description in detector manual) may also cause elevated pressure. Other possible causes of elevated pressure are clogged tubing at a fitting (reverse flow through tubing) and accumulations in the detector cell. Should the pressure be low (under 100 PSI) there may be a leak or it may be necessary to replace one of the HPLC pump piston seals.

7.0 TRAINING

An analyst performing the procedures described in this document must initially work under the supervision of Laboratory Manager, task leader, or another individual designated by the Laboratory Manager. Individuals may work independently once they have satisfactorily completed supervised training. Once training has been completed a Certificate of Training (Attachment 8) will be completed and filed in the Quality Assurance Unit (training files).

8.0 SAFETY

As part of the above-mentioned training program, the analyst will be made aware of the safety concerns of this procedure, including:

Use of protective eyeware and clothing

Location and use of laboratory safety devices - eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits.

9.0 ATTACHMENTS

Attachment 1. HPLC Sample Fractionation Log Form

Attachment 2. HPLC Split Documentation Form

Attachment 3. Example Program for the HPLC Auto Sampler

Attachment 4. Example Programs for the Fraction Collector

Attachment 5. Example Programs for the HPLC Pump

Attachment 6. Example HPLC Chromatographic and Detector Conditions

Attachment 7. Example of Approximate GPC Elution Patterns for Selected Analytes

Attachment 8. Certificate of Training

Attachment 9. HPLC Units

APPROVALS

Author: Richard L Motta, 9/11/98

Laboratory Manager: _____, 9-11-98

QA Coordinator: _____, 9-16-98

Environmental Chemistry Section Manager: _____, 9/17/98

Name Date

Battelle Duxbury Operations

HPLC SAMPLE FRACTIONATION LOG FORM

Project Name: _____
Project Number: _____ Page _____ of _____

HPLC System ID: _____ Column Type: _____ Inject Volume (µL): _____
Mobile Phase: _____ Flow (mL/min): _____
First Fractionation (check): _____ Refractionation (and #): _____
Operator Name: _____ Date: _____
Program Witnessed by: _____ Date: _____

Sample ID	Report Number	Time	Pressure (PSI)	Comments/Mobile Phase Program[a]

[a] Record (1) Calibration standard RT marker components, retention times, and fractionation window, (2) pump mobile phase program (if not isocratic), (3) valve switching times (if programmed in), and (4) sample-specific, and otherwise noteworthy, occurrences and observations.

ATTACHMENT 2

BATTELLE - DUXBURY OPERATIONS

HPLC DILUTION DOCUMENTATION

Project Title

Project Number, Batch ID, Matrix

	[illegible]	[illegible]	[illegible]
UN01PB			
UN02BS			
UN03MS			
UN04MSD			
Z0001			
Z0002			
Z0003			
Z0004			
Z0005			
Z0006			
Z0007			
Z0008			
Z0009			
Z0010			
Z0011			
Z0012			
Z0013			
Z0014			
Z0015			
Z0016			
Z0017			
Z0018			
Z0019			
Z0020			
Z0021			
Z0022			
Z0023			
Z0024			
Z0025			
Z0026			
Z0027			
Z0028			
Z0029			
Z0030			
Z0031			

HPLC System ID:_____ Date/Initials:_____

Comments:

EXAMPLE PROGRAM FOR THE HPLC AUTO SAMPLER

Gilson 231/401 Autosampler

LINE #	ENTRY	EXPLANATION
1	RINSE	Moves needle to rinse position
2	DIS 0/5000/9	Rinses needle with 5000 µL solvent
3	TUBE 0/0	Moves needle to injector
4	INJECT 0	Puts injection valve in LOAD position
5	DIS 0/3000/5	Rinses injection port and sample loop with 3000 µL solvent
6	INJECT 1	Puts injection valve into INJECT position
7	RACK CODE 0	Identifies sample rack as type 0
8	FOR B = 1/X	X = the number of rows of sample vials
9	FOR A = 1/Y	Y = the number of sample vials in each row
10	HEIGHT	Lifts needle into air
11	ASPIR 0/50/1	Draws 50 µl of air into sample transfer line
12	WAIT /7/0	Checks to see if pump is running - will not proceed if pump off
13	TUBE A/B	Moves needle to sample vial
14*	ASPIR 0/620/2	Draws 620 µl of sample into sample transfer line *
15	TUBE 0/0	Moves needle to injector
16	INJECT 0	Puts injection valve into LOAD position
17	DIS 0/600/3	Dispenses 600 µl of sample
18	WAIT 5	Waits 0.05 min for pressure to equalize
19	INJECT 1	Injects sample
20	AUXIL 4/1	Sends a start signal to the integrator
21	WAIT 2	Sends a start signal to the integrator
22	AUXIL 4/0	Sends a start signal to the integrator
23	AUXIL 5/1	Sends a start signal to fraction collector
24	WAIT 2	Sends a start signal to fraction collector
25	AUX 5/0	Sends a start signal to fraction collector
26	AUX 6/1	Sends start signal to HPLC pump

27	WAIT 2	Sends start signal to HPLC pump
28	AUX 6/0	Sends start signal to HPLC pump
29	DIS 0/2000/5	Rinses injection port with 2000 µL solvent
30	RINSE	Moves needle to rinse position
31	DIS 0/2000/9	Rinses needle with 2000 µL solvent
32	TUBE 1/5	Moves needle to 1/5 position
33	ASPIR 0/1000/5	Rinses needle with 1000 µL from rinse vial
34	DISP 0/1000/5	Rinses needle with 1000 µL from rinse vial
35**	WAIT 3000	Waits 30 min before continuing **
36	NEXT A	Sends loop back to line #9 of program
37	NEXT B	Sends loop back to line #8 of program
38	TUBE 16/5	Moves needle to row 5 position 16 (position of RT standard)
39	ASPIR 0/520/3	Draws 520 µl of standard into sample transfer line
40	TUBE 0/0	Moves needle to injector
41	INJECT 0	Puts injector in LOAD position
42	DIS 0/500/3	Dispenses 500 µl of standard
43	WAIT 5	Waits 0.05 min for pressure to equalize
44	INJECT 1	Injects sample
45	AUXIL 4/1	Sends start signal to integrator
46	WAIT 2	Sends start signal to integrator
47	AUX 4/0	Sends start signal to integrator
48	AUX 6/1	Sends start signal to HPLC pump
49	WAIT 2	Sends start signal to HPLC pump
50	AUXIL 6/0	Sends start signal to HPLC pump
51	DIS 0/2000/5	Rinses injection port with 2000 µL DCM
52	RINSE	Moves needle to rinse position
53	DIS 0/2000/9	Rinses needle with 2000 µL DCM
54	HOME	Moves needle to "home" position

* Enter ASPIR 0/620/1 for slow aspiration should there be a chance of particulates being present and settling in the sample vial.

** Use approximately 2800-3200 for tissues and approximately 3000-3400 for sediments.

If the electronic backflush valve is used the following lines may be inserted before line 36 of the original program (last page) for backflushing as part of the fractionation program. This would make line 36 in the original program line 40 in a program with the automatic backflushing valve:

LINE #	ENTRY	EXPLANATION
35	WAIT 1300	Enter initial time/duration of flow in forward direction (1300=13 min approximately equal to 14.3 min into the run because of the post-injection valve and syringe rinse routines). This line replaces line 35 in original program
36	AUX 7/1	Sends signal to backflush valve to switch to reverse flow
37	WAIT 1500	Enter time/duration of flow in reverse direction (1500=15 min)
38	AUX 7/0	Sends signal to backflush valve to switch to forward flow
39	WAIT 1100	Enter time/duration of flow in forward direction (1100=11 min) — time remaining of program

EXAMPLE PROGRAM FOR THE HPLC AUTO SAMPLER (continued)

Spectra-Physics AS300 Autosampler

The programming of this autosampler is more straight forward than for the Gilson 231/401 autosampler. The injection conditions are programmed into a method at prompted lines, and the following are the specific considerations that must be addresses and programmed:

- Check and add, as needed, solvent to the solvent rise bottle (same solvent as mobile phase).
- Purge the rinse solvent with helium or nitrogen, and keep a gentle gas steam flowing.
- Flush Prep syringe and Sample syringe thoroughly, in that order. Typically, 5 mL or more is required for each syringe. Make sure there is no air in the syringes or transfer lines — the transfer lines from the rinse bottle are long, so make sure they are completely filled with air bubble-free solvent.
- Check and/or set Injection Volume (typically 600 μL) and Cycle Time (typically 30 min).
- Timed events can be used to automatically backflush the column (typically from 26 min to 29 min in a 30 min GPC run — see Section 3.4).
- Use the "Rainbow" Sample Prep Program (a canned program built into the autosampler) to rinse the syringe between samples and minimize carry-over.
- Use "Viscous" as the sample viscosity and "Normal" as the prep viscosity, and a Flush Volume of 1,000 μL.
- Use the "Pull" injection mode, with an injection range that matches the sample/injection syringe (the syringe on the right when you look at it from the front of the autosampler).
- Use only sample vial crimp caps that have been demonstrated/approved for use with this particular autosampler.

EXAMPLE PROGRAMS FOR THE FRACTION COLLECTOR

Foxy 200 Fraction Collector

PARAMETER SELECT BUTTON	MENU DISPLAYED
Press POWER ON button :	RUN A , EDIT A , LOAD , QUICK REVIEW
Press EDIT A button :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press TIME/DROP/VOLUME :	TIME , DROP , VOLUME , 4 COLUMN
Press TIME button :	Will you use time windows yes or no ?
Press YES button :	Programming for : RACK 1 or RACK 2
Press RACK 1 button :	Tube size 25/28. Tubes/Rack=X (enter # of tubes for X)
Press ENTER :	Peak collection options should be set to Peak = Rack 1 or Rack 1&2 (if more than 36 tubes needed), Nonpeak = Valve
Press ENTER :	Peak fraction size should be set to 20:00 min
Press ENTER :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press PEAK button :	MODE : Time windows only
Press ENTER :	(set time #1 for start of fraction window according to retention time standards) *
Press ENTER :	(set time #2 for end of fraction window according to retention time standards) *
	Add times for subsequent windows if >1 fraction is to be collected
Press EXIT :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press PAGE 2 button :	PAGE 1 , RESTART , INIT. WASTE , PAGE 3
Press RESTART button :	MOMENTARY , LEVEL , TIMED , NONE
Press MOMENTARY :	# of collections before advancing to fresh tubes should be set to 1 (1 is usually blinking)
Press ENTER :	NEXT TUBE , NEXT ROW , SKIP TUBE
Press NEXT TUBE :	PAGE 1 , RESTART , INIT, WASTE , PAGE 3
Press PAGE 3 :	PAGE 2 , OUTPUTS , SERIAL , TECHNICAL
Press TECHNICAL :	EDIT-MENU , DROP-WAIT , PATTERN
Press PATTERN :	STANDARD , ALWAYS LEFT TO RIGHT
Press ALWAYS LEFT TO RIGHT :	EDIT-MENU , DROP-WAIT , PATTERN
Press RUN button :	RUN A , SAVE AS , EDIT A , QUICK REVIEW
Press SAVE AS :	A FULL , B OPEN , C OPEN , D OPEN
Press A FULL button :	READY A , SAVE AS , EDIT A , QUICK REVIEW (PROGRAM IS NOW SAVED)
Press READY A button :	RUN A , EDIT A , LOAD , QUICK REVIEW
Press RUN A or RUN button :	Fraction collector is now waiting for an external start signal

* Times are in minutes and seconds, not in minutes and tenths or hundreds of a minute as with the Gilson autosampler, Gilson fraction collector, and Spectra-Physics pump.

If flashing parameter is desired, ENTER may be pressed as an alternative to the corresponding parameter button.

HITTING STOP TWICE WILL ABORT RUN SHOULD THIS BE NECESSARY!

EXAMPLE PROGRAMS FOR THE HPLC PUMP

The below programs are primarily for the S-P 8800 gradient HPLC pump, but the S-P 1000 can also be used for the isocratic program conditions described.

FRACTIONATION PROGRAM

With the power turned on push the EDIT key for entering your program. Hit ENTER after each entry of a value. The following are example pump programs - other programs may be used, as determined by the Project Manager and specified in the project files:

GPC Column - Isocratic Fractionation/Cleanup

File	1	
MAX PSI	1200	
MIN PSI	100	
TIME 0	%A 100	FLOW 5.0
TIME 30.0*	%A 100	FLOW 5.0

* Slightly longer or shorter run times can be set as needed for any particular sample matrix.

Silica Column - Gradient Fractionation (standard F_1, F_2, F_3 fractionation)

File	4			
MAX PSI	800			
MIN PSI	50			
TIME 0	%A 0*	%B 100*	%C 0*	FLOW 10.0
TIME 1	%A 0.5	%B 99.5	%C 0	FLOW 10.0
TIME 2	%A 2	%B 98	%C 0	FLOW 10.0
TIME 3	%A 4.5	%B 95.5	%C 0	FLOW 10.0
TIME 4	%A 8	%B 92	%C 0	FLOW 10.0
TIME 8	%A 32	%B 68	%C 0	FLOW 10.0
TIME 10	%A 50	%B 50	%C 0	FLOW 10.0
TIME 14	%A 50	%B 50	%C 0	FLOW 10.0
TIME 14.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 29	%A 100	%B 0	%C 0	FLOW 10.0
TIME 29.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 40	%A 0	%B 100	%C 0	FLOW 10.0

* Where A=DCM, B=hexane, C=methanol
Flow direction switched at 14 and 29 minutes, typically.

If a methanol column rinse is needed the following may be an appropriate program modifications from after 14.1 minutes

TIME 20	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20.1	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 35	%A 100	%B 0	%C 0	FLOW 10.0
TIME 35.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 50	%A 0	%B 100	%C 0	FLOW 10.0

Flow direction switched at 14 and 35 minutes, typically.

Silica Column - PCB Isolation/Fractionation

File	4			
MAX PSI	800			
MIN PSI	50			
TIME 0	%A 0*	%B 100*	%C 0*	FLOW 10.0
TIME 15	%A 0	%B 100	%C 0	FLOW 10.0
TIME 15.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20.1	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 30	%A 100	%B 0	%C 0	FLOW 10.0
TIME 30.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 45 (or 50)	%A 0	%B 100	%C 0	FLOW 10.0

* Where A=DCM, B=hexane, C=methanol

Flow direction switched at 15 and 30 minutes, typically.

If the PCBs all elute before 10 minutes all times above can be 5 minutes earlier (i.e., end the 100% hexane time at 10.0 minutes instead of 15.0 minutes), thereby shortening the run by 5 minutes. The flow direction switching times would then have to be adjusted accordingly.

GPC COLUMN MANUAL BACKFLUSH PROGRAM

The GPC precolumn will be backflushed each day before any standards or samples are run on the HPLC. The backflushing is done by switching the Rheodyne valve to the "backflush" position and pumping mobile phase through it at a flow rate of 2 mL/min for 15 to 20 min. HPLC pump file 2 may be used for backflushing. Push the EDIT key for entering your program. Hit ENTER after each entry of a value. The following is an example program for isocratic cleanup runs:

File	2	
MAX PSI	500	
MIN PSI	50	
TIME 0	%A 100	FLOW 2.0

CLEANUP PROGRAM

A CLEANUP file program will always be programmed into the S-P 8800 HPLC pump along with the sample fractionation program. This program is used primarily to shut the pump off at the end of an analysis/fractionation sequence, and to a lesser degree to clean up the column. This program will automatically begin when a specified time has elapsed (see below), after the completion of a run, without receiving a new injection. Use File 9 as the cleanup file. Again, enter EDIT and then the program. An example program is:

File	9	
MAX PSI	500	
MIN PSI	20	
TIME 0	%A 100*	FLOW 2
TIME 10	%A 100	FLOW 2
TIME 10.1	%A 100	FLOW 0
TIME 10.2	%A 100	FLOW 0

* Enter the solvent composition used at the beginning of the run (generally 100% DCM for GPC column and 100% hexane for the silica column).
After 10 min of rinsing the column at 2 mL/min the pump will shut off. It is essential that the last two lines in the cleanup program have a flow of 0 for the pump to stop.

To specify that a cleanup program is in use, and the time the pump will pump for after completing a run before switching to the cleanup file (unless it receives an injection before this time has elapsed), enter the following keys and values:

TEST	
CLEANUP	
ENTER	
TIME 30	FILE 9

STARTING THE PUMP FOR ANALYZING RETENTION TIME STANDARDS AND FRACTIONATION OF SAMPLES

A. Make sure the above programming is completed. Changes in the programs will not be accepted unless the pump is stopped and re-INITIALIZED, and stopping and restarting the pump will usually result in slightly different column pressure and retention times, thus rendering any established fractionation window invalid.

B. Make sure all mobile phase and autosampler/dilutor lines are purged. This may require priming the pump before purging.

C. Press the INITIALIZE key, then the number for the Program File desired, the press ENTER. It is critical to enter the number of the desired file to be used, not simply pressing the enter key even if the correct number shows up in parenthesis (default) on the screen - the pump will not load the new edited file unless the ENTER key is pressed.

Once the ENTER key has been pressed the pump will start at the TIME 0 conditions entered in the specified file.

D. At first the status of the pump will read INIT, but when the pressure has equilibrated the status will change to RDY and the red ready light will be on.

E. Allow the pump to pump for a few minutes before beginning to run the first analyses of the day (generally the HPLC fractionation time standards).

F. Should the pump need to be stopped for any reason simply press the STOP key.

EXAMPLE HPLC CHROMATOGRAPHIC AND DETECTOR CONDITIONS

Columns:	**GPC**	One 7.8 × 50 mm Phenogel 100 Å, precolum. One 22.5 × 300 mm Phenogel 100 Å column (or two 22.5 × 250 mm Phenogel 100 Å columns, in series).
	Silica	One 25 × 10 mm μPorasil silica, precolumn. One 25 × 100 mm μPorasil silica column.
Injection Loop Volume:		1000 or 2000 μL
Injection Volume:		500 to 600 μL
Mobile Phase:		A = 100% dichloromethane B = 100% hexane C = 100% methanol
Flow Rate:		5-10 mL/min, depending on the application (generally 5 mL/min for the GPC column and 10 mL/min for the silica column).
Temperature:		ambient
Column Pressure:		700 PSI maximum for GPC column, but for continuous operation the pressure should ideally be kept below 500 PSI. 600 PSI maximum for silica column, but for continuous operation the pressure should ideally be kept below 400 PSI.
Detector:		254 nm, UV.
Detector Range:		0.10 AUFS
Detector Response Time:		1 sec
Detector Mode:		AU (absorbance units)

The chromatographic conditions may change depending on the specific application. Any changes will be specified by the Project Manager and documented in the Project Files.

GPC SYSTEM CALIBRATION

Approximate % of Compounds Eluting in the Different Fractions During Instrument Calibration
(Calibration on Both Instruments #1 and #2 in March, 1998)

	Elution Fraction #										
	1	2	3	4	5	6	7	8	9	10	11
GC/ECD Analytes											
Dursban/Methoxychlor	0	50	50	0	0	0	0	0	0	0	0
Endosulfan sulfate	0	<5	70	25	0	0	0	0	0	0	0
Endisulfan I/Endrin aldehyde/Endrin ketone	0	0	20	55	25	0	0	0	0	0	0
Heptachlor epoxide	0	0	5	70	20	5	0	0	0	0	0
Oxychlordane	0	0	0	55	40	5	0	0	0	0	0
DDT compounds	0	0	10	40	40	10	0	0	0	0	0
Chlorobenzenes/chloroaliphatics	0	0	0	0	5	70	20	5	0	0	0
Other NS&T/608 pesticides	0	0	0	10	35	35	20	0	0	0	0
PCBs	0	0	0	0	15	80	5	0	0	0	0
GC/MS Analytes											
Di-N-octylphathalate	85	15	0	0	0	0	0	0	0	0	0
Bis-(2-ethylhexyl)phathalate	80	20	<5	0	0	0	0	0	0	0	0
Butylbenzylphthalate	80	20	<5	0	0	0	0	0	0	0	0
Di-N-butylphathalate	75	20	5	0	0	0	0	0	0	0	0
Diethylphathalate	5	85	10	0	0	0	0	0	0	0	0
Dimethylphathalate	0	35	65	0	0	0	0	0	0	0	0
Isophorone	0	10	80	10	0	0	0	0	0	0	0
Phenols (C0-C4)	0	0	0	0	0	60	40	0	0	0	0
Chloronaphthalenes	0	0	0	0	0	75	25	0	0	0	0
"Standard" PAHs (except those listed below)	0	0	0	0	5	80	15	0	0	0	0
Perylene/Benzo(e)pyrene/Benzo(a)pyrene	0	0	0	0	0	30	45	25	0	0	0
Benzo(g,h,i)perylene	0	0	0	0	0	5	40	30	20	0	0
GC/HPLC Analytes											
"Standard" SJRWMD Chlorophenolics	0	0	0	0	10	80	10	0	0	0	0
3,4,6-trichlorocatechol/Tetrachlorocatechol	Do not elute										

Fraction #1 -1.5 min of RT of DBOFB to -1.0 min of RT of DBOFB
Fraction #2 -1.0 min of RT of DBOFB to -0.5 min of RT of DBOFB
Fraction #3 -0.5 min of RT of DBOFB to ±0.0 min of RT of DBOFB
Fraction #4 ±0.0 min of RT of DBOFB to +0.5 min of RT of DBOFB
Fraction #5 +0.5 min of RT of DBOFB to +1.0 min of RT of DBOFB
Fraction #6 +1.0 min of RT of DBOFB to ±0.0 min of RT of Perylene
Fraction #7 ±0.0 min of RT of Perylene to +0.5 min of RT of Perylene
Fraction #8 +0.5 min of RT of Perylene to +1.0 min of RT of Perylene
Fraction #9 +1.0 min of RT of Perylene to +1.5 min of RT of Perylene
Fraction #10 +1.5 min of RT of Perylene to +2.0 min of RT of Perylene
Fraction #11 +2.0 min of RT of Perylene to +2.5 min of RT of Perylene

Work performed on both HPLC Units #1 and #2 with 300×21.2mm main column, 50×7.8mm pre-column, 5 mL/min DCM. Results apply to system configurations on April 1, 1998.

ATTACHMENT 8

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

FOR

SOP NO. 5-191-06

TITLE: HPLC CLEANUP OF SAMPLE EXTRACTS FOR SEMIVOLATILE ORGANIC POLLUTANTS

TRAINEE: _____

INSTRUCTOR: _____

DATE OF COMPLETION OF TRAINING: _____

APPROVED: _____ _____
Name Date

ATTACHMENT 9

Defined Units:

Unit #1: Gilson Model 231 Autosampler (this removes an extra 20 uL during injection)
Thermoseperations P1000 Pump
Spectraphysics SP8450 UV Detector
Foxy 200 fraction Collector
HP integrator

Unit #2: Thermoseperations AS3000 Autosampler
Spectrophysics 8800 Pump
Alltech Model 450 UV detector
Foxy 200 fraction Collector
HP integrator

Battelle Duxbury Operations
Standard Operating Procedure

for

CLEANING OF ORGANIC CHEMISTRY LABWARE

Summary of changes in this version: Minor edits and clarification of the conical cleaning procedures.

1.0 OBJECTIVE

Proper cleaning of glassware and non-glass labware for organic residue analysis is of central importance to the reliability of data for all projects. These procedures must be standardized and documented to assure reproducible results and responsible communications between Battelle and its contractors.

2.0 PREPARATION

2.1 EQUIPMENT

This procedure requires the following equipment:

- Deionized water system,
- Forma Scientific Fury 2000 Washer Model 8825,
- Drying ovens (operating at approximately 150° C),
- Ignition furnaces (operating at approximately 400° C),
- Ultrasonic cleaner bath (200 watt or greater).

2.2 SUPPLIES

This procedure requires the following supplies:

- Micro® Liquid Laboratory Cleaner,
- Forma Scientific Laboratory Cleaning Detergent,
- Acetone, pesticide residue grade,
- Dichloromethane (DCM), pesticide residue grades.

2.3 CLEANING AREAS

The sinks and sink counters are designated for handwashing labware. All other washing occurs in the laboratory glassware preparation room.

Solvent rinsing: All solvent rinsing occurs in exhaust hood areas. No solvent rinsing, solvent transfer of any kind, or storage of solvent bottles of any kind may occur in or near a laboratory sink. All solvent must be evaporated before glassware may enter the washing procedure. Solvent-rinsed glassware should air dry in the exhaust hood until visibly dry.

2.4 GENERAL PREPARATION

Stopcocks and labels are physically removed before placing labware in cleaning area. Stopcock plugs are reassembled to avoid losing pieces. All labware is brought to designated cleaning areas by the technician or analyst. Do not allow dirty labware to remain in work areas.

3.0 PROCEDURES

The cleaning procedure generally consists of washing, rinsing (with water and acetone), drying, and baking (at 400° C). **All glassware should be rinsed with acetone prior to drying in the drying oven.** Open ends of labware are generally covered with aluminum foil during the baking process. The aluminum foil remains on the labware during storage. Labware is generally given a final solvent rinse before use.

Labware may either be washed by hand, or with the automatic washer. Numerous types of labware may not be dried or baked in an oven. Depending on the nature of the labware, a solvent prerinse may also be required.

Separation of labware by project is not required unless specified in the project plan.

Any labware with a visible residue should be either discarded or rinsed thoroughly with solvent by the laboratory technician before it enters the formal cleaning procedure. The Lab Manager (or designee) must be consulted regarding the disposal of any labware. Labware that has not been solvent rinsed should not be accepted by the dishwasher or lab helper for cleaning.

3.1 HANDCLEANING

The following labware should be washed by hand, dried in the drying oven until all traces of solvent or water are removed, and baked in the ignition furnace at ca. 400° C for a minimum of 30 min.

- Beakers of all sizes,
- Snyder columns,
- Glass stoppers.

The following labware should be washed by hand, but not be placed in the drying oven or ignition furnace:

- Volumetric glassware (may be dried but not baked in ignition furnace),
- Homogenizer probes and other homogenizer parts,
- Plastic or Teflon forceps, stoppers, stopcocks, bottles, or caps,
- Metal spatula forceps.

Conical vials are not disposable and as a result they must be scrupulously cleaned prior to reuse. **Avoid cleaning large quantities of conicals together in one beaker. Conicals should be split up into groups of 50 or less for cleaning.** Conical vials are cleaned by first rinsing several times with DCM inside and out. Physically remove any residue that is not removed after the DCM rinses. Next, conicals are sonicated for 15 minutes in acetone and 15 minutes in DCM (2x). Once the solvent sonication steps are

complete the conicals should be dried in the drying oven for approximately 30 minutes and baked at 400° C for a minimum of 30 min..

3.1.1 Handwashing

Handwashing for Snyder columns consists of plain hot tap water rinse (no soap), Deionized water rinse, acetone rinse, and oven drying, in that order.

Handwashing for beakers, graduated cylinders, spatulas, forceps, stoppers, and stopcocks consists of brushing thoroughly with hot, soapy water, rinsing thoroughly with warm tap water until all traces of detergent are removed, and then rinsing with Deionized water. Labware is then moved to the exhaust hood where all inside and outside surfaces are rinsed thoroughly with acetone and air-dried. All homogenizer probes and parts are acetone-rinsed, dried, and stored. They are solvent-rinsed before use. Stopcocks are not placed in the drying oven.

The volumetric flasks and pipettes are rinsed thoroughly with warm tap water and then rinsed thoroughly with Deionized water. The volumetric glassware is then moved to the exhaust hood where all inside and outside surfaces are rinsed thoroughly with acetone and air-dried. Volumetric glassware is sonicated for 15 minutes in acetone, DCM, and hexane. Volumetric glassware is dried in the drying oven, but may not be baked in the ignition furnace.

3.1.2 Drying

When the acetone has evaporated, appropriate labware is placed into the drying oven for at least 30 minutes or until dry.

All labware (with the exceptions mentioned above) must be dried in the drying oven located in the laboratory preparation room and/or labware preparation room. Both ovens have a upper limit button that must be depressed to re-set the heater if the oven temperature has exceeded the upper (pre-set) temperature limit.

3.1.3 Baking

After drying, the glassware may then be prepared for baking in the ignition furnace located in the laboratory preparation room and/or the labware preparation room.

Glass to be baked must be Pyrex or equivalent, and cleaned and dried according to above procedures. All openings must be loosely covered with aluminum foil. Place in furnace leaving spaces between pieces.

Set temperature to 400° C and close door. Refer to the manual (section on operation), located in the labware preparation room. The oven takes approximately 2 h to reach this temperature. Glassware is baked for a minimum of 30 min., unless otherwise specified in the project plan.

3.2 MACHINE WASHING

All other labware (either Teflon or glass) may be washed in the automatic washer located in the labware preparation room. No plastic labware should be washed in this machine because of the possibility of contaminating the washer.

Any visible solid or powdery residue on labware should be removed by brushing in soapy water. Do not place labware with solid or powdery residue into the automatic washer.

Teflon bottles must be washed at a lower temperature than glass; thus the washer must be set at "plastic". Refer to the Forma Scientific Furry 2000 logbook for instructions on the operation and maintenance of the automatic washer.

Machine-washed labware are dried and baked according to procedures presented above.

3.3 FINAL SOLVENT RINSING

After labware has been baked and cooled, most projects require that it be solvent rinsed before use. All surfaces that will be in contact with the sample must be well rinsed with solvent. Using aspirator bottles, rinse inside surfaces with DCM, unless otherwise specified in the project QAPP or SOP. In general, glassware prepared for butyltin analyses will be rinsed with 10% hydrochloric acid, acetone (2x), DCM (2x), and hexane (2x), in that order. The acid rinse procedure is used on all glassware pre-derivitization. Hexane rinses are all that is needed post-derivitization.

This procedure is carried out in exhaust hoods. All rinse solvent is collected and discarded as Liquid Hazardous Waste according to SOP No. 5-114.

4.0 CALCULATIONS

Calculations are not required.

5.0 QUALITY CONTROL

The quality of the cleaning procedures is verified by the analysis of procedural blanks associated with the sample batch. If contamination of samples is apparent and adversely affects the quality of the sample data, then a search for the source commences. If the source is found to be the labware, then all associated labware cleaned following the suspect batch must be recleaned following the procedures outlined in this SOP.

6.0 TRAINING

The Lab Manager (or designee) is responsible for overseeing all glassware cleaning and preparation. The trainee must first read this SOP. The Lab Manager (or designee) then demonstrates proper glassware cleaning procedures, instruct the trainee regarding proper use and handling of solvents, and relay information contained on appropriate Material Safety Data Sheets (MSDS). Once the trainee demonstrates proficiency in the cleaning procedures listed above, a Certificate of Training is issued (Attachment 1). The original of the certificate is sent the Quality Assurance Unit.

7.0 SAFETY

Use of safety glasses and lab coats are required for all procedures.

Use of Nitrile gloves may be necessary. Please consult with the Lab Manager or Safety Officer on the use of gloves.

Instruction in the proper use and disposal of solvents is necessary.

Solvent skin contact. Remove contaminated clothing immediately. Skin should be rinsed immediately with cold water. Pay strict attention in thoroughly rinsing under nails. Eyewashes are available if solvent contacts the eye. Wash eyes immediately with large amounts of water, occasionally lifting upper and lower lids, until no evidence of solvent remains (15 minutes or longer). In case of eye contact, medical attention is warranted.

Notify the Lab Manager and Safety Officer immediately in case of solvent eye contact.

8.0 ATTACHMENTS

1. Example of Battelle Duxbury Operations Certificate of Training.

APPROVALS

Author		8·6·00
Laboratory Manager		8·6·00
Quality Systems Manager		8-7-00
Environmental Chemistry Resource Manager	Name	8/7/2000 Date

CERTIFICATE OF TRAINING
For
SOP No. 5-216-07, Cleaning of Organic Chemistry Labware

The above mentioned SOP is relevant to your work. Your signature below signifies that you have read and understood the requirements associated with this procedure.

Trainee_____

Instructor_____

Date SOP read and understood_____

Comments:

Approval and Date_____

Battelle Duxbury Operations
Standard Operating Procedures

for

CHEMISTRY LABORATORY SAMPLE IDENTIFICATION

Summary of changes in this version: Nomenclature has been updated to reflect current practice.

1.0 OBJECTIVE

Projects performed by the Chemistry Section laboratory at Battelle require a sample tracking system by which uniquely identified samples can be traced from the time of sample collection or receipt to final analysis. This Standard Operating Procedure (SOP) describes the procedure by which the Chemistry Section assigns a unique alphanumeric code (Laboratory Identification Number) to each sample for use within the laboratory. At the discretion of the project manager, field or client identification (ID) numbers can be used in the lab but a Laboratory Identification Number should still be assigned to each sample upon receipt. The use of alternate IDs will be specified in the project work plan.

2.0 PROCEDURE

2.1 FIELD SAMPLES

Samples admitted to the laboratory will be received and logged in by the Laboratory Sample Custodian or the Alternate Laboratory Sample Custodian (SOP 6-010). At the time of receipt, each sample is assigned a Laboratory Identification Number that is indelibly marked on each sample container and cross referenced to the sample's original identification.

- An individual container that is uniquely identified as a specific sample by the client is assigned a separate sample ID number.
- Multiple containers that are uniquely identified as a specific sample by the client and targeted for the same analysis are assigned the same sample ID number.
- Multiple containers that are uniquely identified as a specific sample by the client and targeted for different analyses are assigned unique sample ID numbers.

Laboratory Identification Numbers are assigned sequentially to samples in the following alphanumeric format:

Xnnnn

where: X = a single letter (Z...A)
nnnn = 0001.....9999

2.2 QUALITY CONTROL SAMPLES

Quality control (QC) samples generated within the Chemistry Laboratory are treated identically except that the Laboratory Identification Numbers are assigned by hand by the person who is preparing the sample (Technician, Researcher) and the number sequence can be in the format stated above but is usually in the following format:

XXnn

where: XX = AA,AB,AC,.....ZX,ZY,ZZ
nn = 01,02,03,.....97,98,99

If a sample is split for separate analysis within Battelle (e.g. FID, PAH) both samples will retain the original Laboratory Identification Number. Similarly, if a sample is split for analysis as part of the QC program, the original Laboratory Identification Number will be retained, but will be appended with a suffix to indicate the type of replicate. The following is a list of suffixes:

- 1,-2 etc.: Reextraction number (e.g. first reextraction Z0001-1)
- R: Indicates that sample has been refractionated by HPLC (e.g., Z0001-R, Z0001-R2)
- NF: Indicates that the non-fractionated aliquot from HPLC will be analyzed
- D1 etc.: Indicates that sample has been diluted (e.g., Z0001-D1, Z0001-D2)
- LCS: Laboratory Control Sample (e.g. AB01LCS)
- LCSD: Laboratory Control Sample Duplicate (e.g. AB01LCSD)
- MS: Matrix Spike (e.g. AB01MS or Z0001MS)
- MSD: Matrix Spike Duplicate (e.g. AB01MSD or Z0001MSD)
- PB: Procedural Blank (e.g. AB01PB)
- DUP: Indicates that a second extraction of a sample has been performed (e.g., Z0001DUP)
- TRIP: Indicates that a third extraction of a sample has been performed (e.g., Z0001TRIP)
- REP#: Replicated samples (e.g. Triplicate Analysis: Z0001-Rep1, Z0001-Rep2, Z0001-Rep3)
- F#: Column fractionation (e.g. F1, F2, F3)
- SRM: Standard Reference Material (e.g., AB01SRM)
- GC/MS etc. Indicates that sample has been split for separate analyses (e.g., Z0001-GC/MS. Other analyses may include: GC/ECD, GC/VOA, HPLC)
- -MS-DI: Indicates that a specific split of a sample has been diluted (e.g., Z0001-MS-DI)

3.0 DOCUMENTATION

Every sample received in the Chemistry Section will be logged into the Chemistry Laboratory Sample Receipt Database and assigned a Laboratory ID number (Attachment 1). Information entered at log-in includes collection information, client IDs, matrix, storage location, and sample specific comments.

Records of Laboratory Identification Numbers and their corresponding original field identifications are the responsibility of the Laboratory Sample Custodian. Hard copies of custody forms are stored in a 3-ring binder in the access-controlled custody room. The bound Sample ID Log (QC samples) is kept in the laboratory (Attachment 2).

4.0 TRAINING

All Chemistry Section personnel who log field or quality control samples into the Chemistry Laboratory Sample Receipt Database or Sample Receipt Log are required to read this SOP. Individuals are considered trained once they have read this SOP and signed the associated training certificate (Attachment 3). The original certificate is filed in the Quality Assurance Unit office.

ATTACHMENTS

1. Example of Chemistry Laboratory Sample Receipt Database Laboratory ID Log
2. Example of Sample Identification Log (QC Samples)
3. Example of Training Certificate

APPROVALS

Author		7·10·00
Laboratory Manager		7·10·00
Quality Systems Manager		8-4-00
Environmental Chemistry Section Manager		8/4/2000
	Signature	Date

Attachment 1

Battelle Duxbury Operations

Example of Chemistry Laboratory Sample Receipt Database Laboratory ID Log

BATTELLE Laboratory Sample Login Report

Project # :

Client : EARTH TECH INC.

Receive Date : 08/04/2000 10:30:00 AM

Logged in By : MEARAM

Collection Date	*Login Date*	*Lab ID*	*Client Sample ID*	*#Containers*	*Matrix*	*Preservative*	*Storage Location*	*Login Comments*
08/03/2000 2:15:00 P	08/04/2000 11:20:31	X7222	EA00185HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:00:00 P	08/04/2000 11:20:51	X7223	EA00186HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:20:00 P	08/04/2000 11:21:12	X7224	EA00187HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:25:00 P	08/04/2000 11:21:35	X7225	EA00188HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:30:00 P	08/04/2000 11:21:57	X7226	EA00189HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:35:00 P	08/04/2000 11:22:20	X7227	EA00190HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:45:00 P	08/04/2000 11:22:40	X7228	EA00191HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:55:00 P	08/04/2000 11:22:59	X7229	EA00192HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 4:05:00 P	08/04/2000 11:23:26	X7230	EA00193HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 4:10:00 P	08/04/2000 11:23:54	X7231	EA00194HS	1	SED	NONE	WALK IN FRIDGE	

Attachment 2

Example Sample Identification Log (QC Samples)

Sample ID	Log-in Date/ Initials	Client Name/ Field Sample ID	Sample Type[1]	Comments[2]
OO01				
OO02				
OO03				
OO04				
OO05				
OO06				
OO07				
OO08				
OO09				
OO10				
OO11				
OO12				
OO13				
OO14				
OO15				
OO16				
OO17				
OO18				
OO19				
OO20				
OO21				
OO22				
OO23				
OO24				
OO25				

[1] Sample Type - Matrix, QC Sample Description
[2] Comments - Sample volume (Waters), Number of containers.

Attachment 3

RECORD OF TRAINING
for
SOP No. 6-007, *Chemistry Laboratory Sample Identification*

The above mentioned SOP is relevant to your work. Your signature below signifies that you have read this SOP and understand the requirements associated with this procedure.

Name (printed)	Name (Signature)	Date

Battelle Duxbury Operations
Standard Operating Procedures

for

SAMPLE RECEIPT, CUSTODY, AND HANDLING

Summary of changes in this version: Additions about VOC refrigerator blanks, delivery of samples outside of normal hours, and other minor edits.

1.0 OBJECTIVE

Sample control is a vital aspect of any environmental monitoring program which generates data that may be used for regulatory purposes or as evidence in a court of law. Additionally, the complexity of many environmental sampling programs, which may involve the collection and analysis of samples of various media from different sites to be analyzed for several parameters, makes a sample control system essential. This standard operating procedure (SOP) defines the procedures, organizational responsibilities, and documentation requirements associated with the Laboratory sample control system.

The routine flow of samples through the laboratory is illustrated in Attachment 1. Additional sample control procedures may be required to meet the needs of specific projects. These procedures will be defined in the project plan.

2.0 DEFINITIONS

Custody Records — The administrative records associated with the possession history of each sample from the time of collection, through analysis, to final disposal.

Chain-of-Custody Records — The administrative records associated with the physical possession and/or storage history of each individual sample from the purchase and preparation of each sample container and sampling apparatus to the final analytical result and sample disposal.

Legal or Evidentiary Chain of Custody (COC) – A special type of sample custody which requires that the physical possession, transport and storage of a sample be documented in writing. The records must account for all periods of time from sample container acquisition through sample disposal.

Sample control — The formal system designed to provide sufficient information to reconstruct the history of each sample, including collection, shipment, receipt and distribution within the laboratory, analysis, storage or disposal, and data reporting.

Sample custody — Samples are considered to be in a person's custody if

- The samples are in a person's actual possession
- The samples are in a person's view after being in that person's possession

- The samples were in a person's possession and then were locked or sealed up to prevent tampering
- The samples are in a secure area

3.0 RESPONSIBILITIES

Sample Collector — The person collecting the samples is responsible for

- Collecting and preserving samples in accordance with approved procedures, as specified in the project-specific plan and SOPs
- Adjusting the pH to < 2 if the sample is intended for volatile organics analysis (and also adding sodium thiosulfate if total residual chlorine is present)
- Assigning a number or code at the time of collection that uniquely identifies that sample
- Labeling each sample container with the sample number, project identification, date of collection, collector's initials, and storage requirements (room temperature, frozen, chilled)
- Documenting sample collection and preservation
- Packaging samples for shipment in a manner that minimizes the risk of breaks and leaks and to ensure that the samples are maintained at the appropriate temperature
- Completing and signing the chain-of-custody records accurately and legibly
- Ensuring integrity of the samples by sealing or locking the shipping container(s) and applying custody tape (if required)
- Arranging timely transportation of samples to the laboratory, including identifying on the shipping label the name of the person to whom the samples should be delivered

Laboratory Sample Custodian — The responsibilities of the Laboratory Sample Custodian include:

- Receiving samples (for details see Section 4.1).
- Maintaining records of sample receipt, movement in and out of storage, and release, archival, and disposal
- Distributing completed custody forms according to Section 4.1.5.
- Returning the shipping cooler to the client or shipper.
- Communicating sample custody problems to the project manager and implementing corrective action as directed (Section 4.8).

Alternate Sample Custodian — The Alternate Sample Custodian is responsible for assisting the Laboratory Sample Custodian and for performing the above tasks in the absence of the Laboratory Sample Custodian.

Project Manager — The Project Manager is responsible for communicating

- expected receipt dates and project-specific receipt requirements to the Sample Custodian
- the potential presence of total residual chlorine to the Sample Custodian
- sample custody-related problems to the client
- corrective action to the sample custodian and laboratory manager

Laboratory Manager — The Laboratory Manager is responsible for designating the Laboratory Sample Custodian and the Alternate Sample Custodian and for ensuring that these individuals are trained to perform the tasks specified in this SOP.

4.0 PROCEDURES

4.1 SAMPLE RECEIPT

4.1.1 Hours

Samples that are admitted to the laboratory during normal business hours are either delivered to the front desk or to a designated area in the Chemistry North Building. The Sample Custodian is notified immediately.

It is Battelle policy that samples are not received outside of regular business hours unless the project manager has made specific arrangements with the laboratory manager and the sample custodian in advance. Samples that are delivered outside of regular business hours should be treated equally as samples received during the week. Receipt temperatures should be recorded and the proper storage of the samples should occur. The project manager should coordinate deliveries with the lab manager and the custodian assigned to receive the samples *before such deliveries are scheduled.* The project manager should also make every effort to be available (be able to be reached), in case unexpected problems occur.

- If samples are transported from the field to the laboratory by Battelle sampling personnel, the samples should be placed in a pre-arranged, secure location until they can be formally relinquished to the Laboratory Sample Custodian.

In either case, on the next business day, the Laboratory Sample Custodian logs in the samples. (Note that the receipt form allows for separate entries of receipt and log-in date).

Upon receipt of the samples, the Laboratory Sample Custodian will move the shipping containers to the sample custody room.

4.1.2 Sample Handling

The shipping container should only be opened under the vented hood. The sample custodian must determine whether the sample condition upon receipt is acceptable. That is, that the sample temperatures, pH, and containers, are appropriate for the intended analysis; and that the samples have been received within the required holding times. Attachment 2 defines acceptable sample handling and holding times. If sample containers, preservation, or timely delivery do not meet the criteria in Attachment 2 and section 4.1.3, then the sample custodian must notify the project manager who in turn must notify the client (section 4.8).

The sample custodian must review and document the following for proper receipt of the samples:

1. Method of delivery (*i.e.* commercial carrier, hand delivered) and presence/absence of chains-of-custodies.

2. Inspect the shipping container(s) for the presence/absence and condition of custody seals.

3. Inspect each sample for the presence/absence and condition of samples and custody seals.

4. Inspect each sample for breaks or leaks (see Section 5.0 for safety instructions).

5. Review the accompanying records for completeness and accuracy of sample labels and sample transmittal forms.

6. Measure and record the temperature of each container to document whether or not the samples were maintained at the appropriate temperature (frozen, cool, or room temperature) during shipment. The temperature of a cooler blank (if available), melt water, or the external temperature of the sample containers should be measured and documented. (Thermometers or probes are never inserted into a sample container).

7. Measure and record initial pH of water samples unless otherwise directed by the laboratory manager, project manager, or QAPP (see Section 4.2.1).

8. If the project manager indicates that TRC may be present, measure samples for total residual chlorine (TRC) and treat samples with sodium thiosulfate (Section 4.2.2).

9. Inspect VOC vials for bubbles of sizes greater than 1% of the vial volume. If present, notify the Project Manager immediately.
   *A refrigerator blank must be made for all VOA samples received. This blank should travel with the samples and be included with the sample analysis.

10. Upon completion of the sample inspection, the Laboratory Sample Custodian formally acknowledges receipt of the samples by signing, dating, and noting the current time on the sample transmittal form(s).

11. Log-in and assign unique laboratory identification numbers to each sample (see Section 4.3).

12. Storage of samples in the appropriate storage location until samples are ready to be further processed. VOC samples to be stored in a separate storage location than samples for other organic analyses. This includes releasing samples to the laboratory and to outside contractors.

13. Communicate sample custody problems to the project manager and implement corrective action as directed.

14. Distribute completed custody forms according to Section 4.1.5.

15. Return the shipping coolers to the client or shipper, if necessary.

4.1.3 Sample Acceptance/Rejection Criteria

Under some circumstances Battelle will place itself at risk by accepting samples for analysis because data that are generated from samples that do not meet chain of custody or handling requirements (Section 4.1.1) may be rejected by EPA. Battelle may currently analyze samples for the following regulatory programs:

- Resource Conservation and Recovery Act (RCRA)
- Comprehensive Environmental Response, Compensation and Liability Act (Superfund) (CERCLA)
- Clean Water Act (CWA)

It is the responsibility of the sample custodian to ensure that the following conditions are recorded on the Sample Receipt Form. The Laboratory Sample Custodian will notify the Project Manager and Laboratory Manager in writing (See Section 4.8 and Attachment 3) of sample receipt, condition, and problems (e.g., breakage, leakage, missing samples, excessive temperatures). Upon completion of sample inspection, the Laboratory Sample Custodian formally acknowledges receipt of the samples by signing, dating, and noting the current time on the sample transmittal form(s).

It is the responsibility of the project manager to specify in the QAPP that project samples are being analyzed for compliance monitoring. In these cases samples could be rejected if:

- The integrity of the samples is compromised (leaks, cracks, grossly contaminated container exteriors or shipping cooler interiors, obvious odors, etc.)
- The identity of the container cannot be verified
- The proper preservation of the container cannot be established
- VOC vials contain bubbles of sizes greater than 1% of the vial volume
- Incomplete sample custody forms: the sample collector is not documented or the custody forms are not signed and dated by the person who relinquished the samples
- The sample collector did not relinquish the samples.
- Samples are designated for VOA analysis but no VOA trip blank is provided.

If the sample custodian identifies any of the above conditions the project manager must be notified (Section 4.8).

4.1.4 Documentation

Documentation of sample receipt includes the original sample custody forms (or copies if the originals are returned to the shipper), any additional records of transmittal, the shipper's air bill (if applicable), and the Sample Receipt form. Sample custody records are filed by date in the Custody Logbook which is kept in the access controlled sample custody room. A record of Battelle Laboratory ID numbers, including cross-reference to original field IDs, is entered into the custody database and hard copies are stored in the custody room.

The condition of the samples, integrity of the custody seals, discrepancies between sample labels and transmittal forms, and unusual events or deviations from the project work plan or SOPs are documented in detail on a Sample Receipt Form (Attachment 4). Any problems are also recorded on the original custody forms, if present.

Occasionally, samples are received with only a letter of transmittal or no transmittal forms at all. In these cases the Sample Custodian should complete the sample log-in procedures (Section 4.3) and attach a printout from the Chemistry Laboratory Sample Receipt Database to the Sample Receipt form to provide a record in the Custody Log of the samples received.

4.1.5 Distribution

The sample custodian will provide the Project Manager, the QAU, and Lab Manager with a copy of all documentation that accompanied the samples.

The custodian should make the following distribution of custody forms:

- Copies of the sample receipt and custody forms are provided to the project manager (2 copies), laboratory manager, and the quality assurance officer.
- If the custody forms that are received with the samples are multi-copied then the copies should be distributed as indicated on each copy (e.g., laboratory, customer (client), shipper).
- If the custody forms that are received with the samples are not multi-copied then the custodian should consult the project manager to determine if a copy of the custody records should be returned to the client and/or shipper.

4.2 SAMPLE PRESERVATION ADJUSTMENTS

4.2.1 pH Measurement and Adjustment

If water samples will not be extracted within 72 hours then the pH is typically adjusted according to the preservation requirements in Attachment 2. To measure pH, withdraw a small volume of water (0.1 mL) from the sample container using a baked Pasteur pipette. Place 1 drop on a narrow range pH paper strip and follow the instructions included with the pH paper to read the results. Record the pH on the Sample Receipt form (Attachment 4). If water sample pH is NOT between pH 5 and 9 consult the project and/or laboratory manager and make pH adjustments as directed by these managers. The decision to adjust sample pH is based on the target analyte list and is therefore made on a case-by-case basis.

To adjust the sample pH, sulfuric or hydrochloric acid (1+1) or 10 N NaOH is added until the sample pH is between 5 and 9. (Note that NaOH solutions must be pre-extracted prior to use to prevent sample contamination). The final pH and the volume of solution added in making the pH adjustment is recorded on the Sample Receipt form.

Samples intended for VOA analysis should NOT be opened. pH adjustments must be made in the field.

4.2.2 Total Residual Chlorine Measurement and Treatment.

If the project manager indicates that samples may contain total residual chlorine (TRC) then the sample must be treated with sodium thiosulfate according to Attachment 2 (PCB/Pest, PAH). The sample custodian must work directly with the project manager in these cases. Ideally, the sample is treated for chlorine in the field. However, the sample custodian should verify the absence of TRC using a commercial test kit. If TRC is detected then sodium thiosulfate is added at the ratio of 80 mg/L sample. This treatment is documented on the sample receipt form.

Samples intended for VOA analysis should NOT be opened. Treatment for TRC must be performed in the field.

4.3 SAMPLE LOG-IN

The receipt of all samples received by the Chemistry Laboratory will be recorded in the Chemistry Laboratory Sample Receipt Database (SOP 6-007). Each incoming sample is assigned a unique ID number, which is clearly and indelibly marked on each sample container and the custody form. Samples that contain more than one jar for the same analysis will be labeled with the same Lab ID and the jar number (e.g. 1 of 2 and 2 of 2). Alternatively, a separate ID number can be assigned to each container. The full ID including the jar number will be called out in the preparatory records. Upon completion of log-in procedures, samples are placed in a limited-access area at the appropriate temperature. The storage area is documented on the Sample Receipt form.

4.4 SAMPLE STORAGE

Upon completion of sample log-in procedures, samples are transferred to a secure location for storage. This location may be a room, refrigerator, or freezer, depending on the storage requirements of the samples, but must be an area that can be locked from the outside. The initial storage location is documented on the Sample Receipt form. Only the sample custodian and the facilities manager have keys to these controlled-access areas.

The following storage requirements are applied to samples received at Battelle unless otherwise specified in the QAPP: tissue and sediment samples: ≤20°C; water samples: 4±2°C. Samples collected for compliance monitoring according to EPA regulatory methods are stored according to the conditions specified in Attachment 2 and should be specified in the QAPP.

Samples that are to be analyzed for volatile organic compounds must be stored in a separate storage location from the samples being analyzed for semi-volatile organic compounds.

4.5 SAMPLE TRACKING

Sample custody is transferred from the Sample Custodian to the sample prep Task Leader when sample preparation is initiated. The transfer of custody to laboratory personnel and all sample movement within the laboratory is documented on Daily Sample Tracking forms (Attachment 5) that are maintained with the prep records. Each technician is responsible for the care and appropriate storage of the samples in his/her custody, and for documenting the conditions under which the samples are maintained. Labs/areas which house samples in-progress must be controlled-access (locked) during non-working hours.

4.6 SAMPLE SPLITTING

The aliquotting of samples for multiple analyses is documented on Sample Split and Transfer Logs (Attachment 6). Split samples retain their original Laboratory Sample identification number. Sample Split Logs are maintained with the original sample custody records. Lab Ids are distinguished by the analyses for which the sample was split.

4.7 SHIPMENT OF SAMPLES

Distribution of samples that are aliquotted at Battelle and shipped to external laboratories for analyses is normally documented on the Battelle Sample Split and Transfer Log. If the samples were collected or generated by Battelle, sample custody is tracked on Battelle Custody forms (Attachment 7) that are shipped with the samples. In either case the person who has custody of the samples is responsible for

- packing the samples for shipment such that temperatures requirements are maintained and samples are protected from breaks or leaks
- arranging for transportation.

A *copy* of the transfer or custody form should be retained with the original custody records for tracking purposes. The custodian at the receiving laboratory documents sample receipt and condition on the form and retains the yellow copy. The white (original) and pink copies are returned to Battelle. The original is maintained with the original custody records and the pink copy is sent to the client, if requested. The preparation of field kits, custody of sample containers, and sample packing procedures are defined in SOP 5-210.

4.8 CLIENT NOTIFICATION

The client must be notified immediately if problems are noted during sample receipt and log-in so that corrective action may be initiated. The sample custodian may communicate directly with the client custodian or representative if discrepancies between sample labels and custody forms are noted or if samples are missing. The project manager should communicate other problems (e.g., holding time exceedences, preservation issues, incomplete or improper custody records – see Section 4.1.3). This notification and the clients directions for corrective action is documented on the Corrective Action form (Attachment 3). It must be specifically documented if the client approves analysis of the samples. All corrective action is communicated to the sample custodian or laboratory manager in writing.

Specific samples may include other client notification requirements (e.g., if permit thresh hold limits are exceeded the client must be notified within 24 hours of verified sample data). The project manager should define requirements these in the QAPP.

4.9 SAMPLE ARCHIVAL AND DISPOSAL

Sample extracts and unextracted field samples are returned to the custody of the sample custodian. Once sample analysis is considered final the samples can be archived.

The decision to archive samples should be made by the client and the Project Manager when the project is initiated. Sample disposition and the length of storage should be defined in the project plan. In the absence of other directives, unexpended samples are archived for six months after the delivery of the final data. Unless otherwise specified by the client, the samples will be discarded in the proper waste stream after this period. The project manager will be notified prior to the disposal of samples.

Sample extracts are held for one month after delivery of the final data. Unless otherwise specified by the client the extracts will be discarded in the proper waste stream after this period. The project manager is not notified of extract disposal. It is the responsibility of the project manager to include sample disposition requirements in the QAPP and to communicate them to the sample custodian. The following documentation is required:

1. Samples or extracts for archival are boxed by batch.
2. The project numbers, title/clients, batch numbers, and extract fractions are labeled on the box.
3. A copy of the label is filed in the Sample Archival logbook and the archive location of the box (box number and freezer number) is documented in the logbook.

Sample disposal is documented in the Sample Archival Log Book by documenting "Discarded" with the date and initials of the custodian directly on the label copy. The appropriate handling and disposal procedures for sample and sample extract are discussed in SOP 5-114.

5.0 SAFETY

Sample handling must always assume that samples are potentially "contaminated." Therefore, sample shipping containers are always opened in a vented fume hood, and personnel protective equipment is worn when unpacking samples (safety glasses, lab coat, and gloves).

Occasionally, samples are received broken. Because the potential hazard may be unknown all spills must be treated as if the material is hazardous. Clean-up materials should be maintained in the sample custody room, or easily accessable. These consist of

- absorbent (e.g., speedi-dry)
- dust pan and brush
- glass disposal container
- heavy-duty gloves
- paper towels
- plastic bags
- solid waste stream container

The hazardous waste coordinator should be contacted to determine the proper disposal procedures for spilled sample. In general, water samples are absorbed into chemical absorbent; sediment, soil, or tissues are placed in heavy-duty plastic bags. These are both disposed of in the laboratory's solid waste stream. Broken glass containers are placed in the glass disposal container.

6.0 TRAINING

A person who is being trained as a sample custodian must first read this SOP. The person may then perform specific tasks under the supervision of a qualified instructor (Laboratory Sample Custodian or Alternate). Tasks performed by the trainee are reviewed and co-signed by the Laboratory Sample Custodian or Alternate until it has been established that the trainee is able to perform these tasks without supervision. A certificate of training (Attachment 8) is issued upon completion of training and provided to the Quality Assurance Unit.

ATTACHMENTS

1. Sample Flowchart
2. Sample Handling Requirements
3. Sample Custody Corrective Action Form

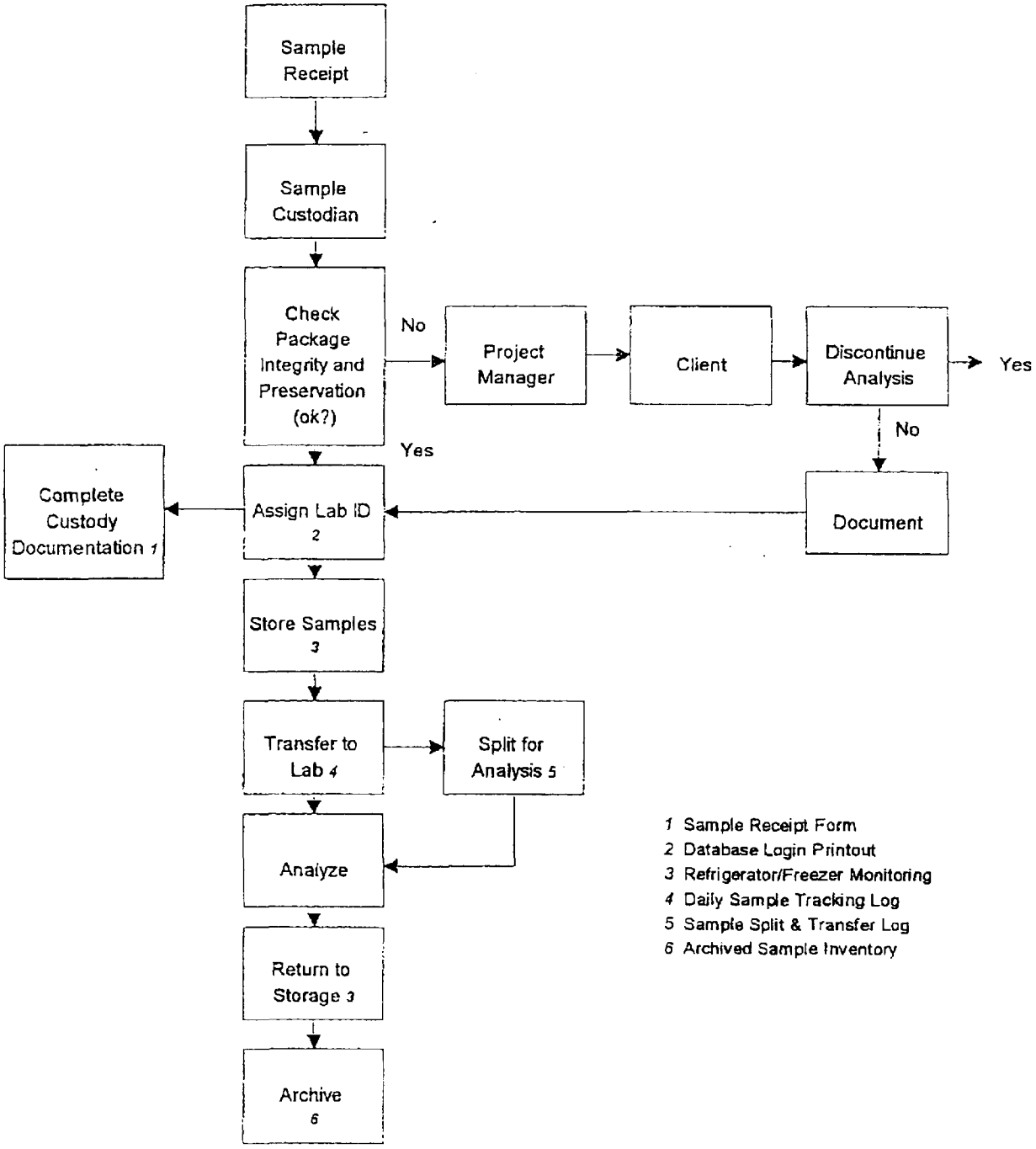
4a. Sample Receipt Form
4b. Sample Receipt Auxiliary Form

5. Daily Sample Tracking Form
6. Sample Split and Transfer Log
7. Battelle Chain-of- Custody Record
8. Certificate of Training

APPROVALS

Author		7·10·00
Laboratory Manager		7·10·00
Quality Systems Manager		8-4-00
Environmental Chemistry Section Manager		8/7/2000
	Name	Date

ATTACHMENT 1
Battelle Duxbury Operations
Sample Flowchart



ATTACHMENT 2
Sample Handling Requirements

WATER

Compound Class	Containers	Preservation		Holding Time
		Temperature	Other	
PESTICIDES[1]	Glass with Teflon lined caps	Cool 4°C ±2°C	pH 5-9 if held longer than 72 hours Store in dark	7 days until extraction, 40 days after extraction
PCBs[1]				
PAH[1]			Store in dark	
Other SVOA • Haloethers • Phthalate Esters • Nitroaromatics • Isophorones • Nitrosamines				
TPH or FINGERPRINT				
VOA[1]			pH<2 Headspace ≤1% of sample	14 days
TBT	Polycarbonate	Freeze ≤20°C		90 days

[1]If Residual Chlorine is present in the sample it must be treated with sodium thiosulfate.

SEDIMENT/SOIL

Compound Class	Containers	Preservation		Holding Time
		Temperature	Other	
PESTICIDES **PCBS**	Glass with Teflon lined caps	Cool 4°C ± 2°C		14 days until extraction, 40 days after extraction
PAH **SVOA**		Freeze ≤20°C		1 Year
VOA		Cool 4°C ± 2°C		14 days
TPH or FINGERPRINT		Cool 4°C ± 2°C		14 days until extraction, 40 days after extraction
TBT		Freeze ≤20°C		1 Year

All tissue samples are stored frozen (≤20°C).

ATTACHMENT 3
Battelle Duxbury Operations
Sample Custody Corrective Action Form

Project Number _____ Client _____

Description of Problem (continue on back, if needed):

The sample custodian must contact the project manager on the day that problems are identified. If the project manager is not in the office the laboratory manager must be notified.

Documentation of project manager notification:

Sample Custodian: _____ _____
Signature Date

Project Manager _____ _____
Signature Date

Documentation of client notification (to be completed by project manager):

On _____ I contacted _____ at _____
Date Name of client contact Name of client organization

Results of communication with client (Describe any corrective action directed by the client):

RETURN THIS ORIGINAL TO THE SAMPLE CUSTODIAN. THE SAMPLE CUSTODIAN WILL PROVIDE COPIES TO THOSE ON THE ORIGINAL SAMPLE CUSTODY DISTRIBUTION LIST.

Date that this form was received by the custodian:_____

ATTACHMENT 4a
Battelle Duxbury Operations
Sample Receipt Form

Project Number _____ Client _____
No. of Shipping Containers _____ Date/Time Received _____

SHIPMENT
Method of Delivery: _____ Commercial Carrier (Air bill No. _____)
_____ Hand Delivered
_____ US Mail (RPS No. _____)
COC Forms: _____ Shipped with samples _____No forms
Cooler(s)\Box(es) were sealed with: _____ Tape _____ Custody Seals_____(Other specify)
Were the seals intact for each shipping container_____Yes_____No
If seals were broken (list impacted samples):

SAMPLES

Sample Labels: _____ Sample labels agree with COC forms _____ Discrepancies (see COC forms)

Container Seals: _____ Tape _____ Custody Seals_____(Other specify)
_____Seals intact for each shipping container
_____Seal broken (list impacted samples):

Condition of Samples:_____ Sample containers intact
_____ Sample containers broken/leaking (see COC forms)

Temperature upon receipt (°C): _____ Temperature blank used _____Yes_____No
(Note: If temperature upon receipt differs from required conditions, list impacted samples):

Initial pH 5 - 9? (Y/N):____ *If no, individual sample adjustments on the Auxiliary Sample Receipt Form.*

Total Residual Chlorine Present?(water) (Y/N): _____
If yes, individual sample adjustments on the Auxiliary Sample Receipt Form.

Head Space <1% in samples for VOC analysis _____Yes_____No
Individual sample deviations listed below.

Sample Containers:
Samples returned in PC-grade sampling jars (Yes/No). _____ Lot No. _____
All but the following samples were returned in Battelle-prepped bottles:

Storage Location: _____ **BDO IDs Assigned**: _____
Additional Comments:

Samples logged in by: _____ Date/Time: _____

ATTACHMENT 4b
Battelle Duxbury Operations
Auxiliary Sample Receipt Form

Sample ID	pH initial	pH Final	Vol. Added Acid/Base	Initial TRC	Final TRC	Vol. Added Sodium Thiosulfate

Work Perform by/Entered by _____ **Date**_____

ATTACHMENT 5
Battelle Duxbury Operations
Example Daily Sample Tracking Form[1]

Samples Relinquished by Custodian :	Date :	Time:
Location from which retrieved :	Date :	Time:
Samples received for sample prep by :	Date :	Time:
Storage until prep initiated :	Date :	Time:
Samples Returned to Custodian :	Date :	Time:
Location Stored :	Date :	Time:

Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
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Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:

Comments:

VALIDATION CHECKLIST

Check sample custudy records to verify client Ids	
Verify all samples in batch are reported and all samples reported are in the prep batch	
Complete surrogate and internal standard spikng forms	
Check sample dilution, grav weights, and correction factors	
Check HPLC tables 100%	
Check dry weight and lipid weight tables	
Ensure sample transfer and documented by proper sign-off	

[1] Separate forms should be initiated for each sample prep batch and for each analysis.
If sample is not consumed, document the storage of the remainder.

ATTACHMENT 6
Battelle Duxbury Operation
Sample Split and Transfer Log

Battelle
. . . Putting Technology To Work

Sample Split and Transfer Log

Project Number _____ Date of Work _____

Project Title _____

Analysis Type(s) _____

Splitting Procedure _____

_____

(include description of amount or weight of split, packaging, storage)

Name _____ Date _____

Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID

Released	Received
Signature/Date:	Signature/Date:
Storage Location/Conditions:	Storage Location/Conditions:
Released	**Received**
Signature/Date:	Signature/Date:
Storage Location/Conditions:	Storage Location/Conditions:

ATTACHMENT 7
Battelle Duxbury Operations
Chain-of-custody Record

Battelle
. . . Putting Technology To Work

Chain of Custody

Proj. No	Proj. Name

SAMPLERS: Signature

ANALYSIS REQUESTED → "NUMBER OF CONTAINERS"

DATE	TIME	BATTELLE ID	CLIENT ID	SAMPLE DESCRIPTION	PEST	PCB	TPH FINGERPRINT	PAH	VOA	TBT	METALS	OTHER	ACIDIFIED	PRESERVED	Total Number of Containers

Relinquished by:	Date/Time	Received by:	Date/Time
Relinquished by:	Date/Time	Received by:	Date/Time

Comments:

ORIGINAL

ATTACHMENT 8
Battelle Duxbury Operations
Certificate of Training

SOP No. 6-010

SOP Title: Chemistry Laboratory Sample Control

Trainee_____

Instructor_____

SOP Read:_____ _____
Signature Date

Date Training Completed:_____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting documentation (if applicable) is attached.

Comments:

Approved By/Date: _____

BATTELLE DUXBURY OPERATIONS
STANDARD OPERATING PROCEDURES

FOR

DOCUMENTATION PROCEDURES IN THE GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS) FACILITY

Summary of changes in this version: Data archival procedures are removed from this SOP. Archival of GC/MS data will be conducted according to SOP 6-036. Reference to a GC/MS tracking logbook is included.

1.0 OBJECTIVE

This SOP is a guide for completing and compiling all the forms and printouts necessary in each logbook. All documentation relating to the operation and maintenance of the GC/MS Facility is compiled and contained in the following logbooks:

1. GC/MS Sample Tracking Logbook
2. GC/MS Logbook
3. GC/MS Tune Logbook

2.0 PROCEDURES

2.1 GC/MS SAMPLE TRACKING LOGBOOK

The GC/MS sample tracking logbook is used to document the type, quantity, and the date on which a batch of samples are received into the GC/MS facility. The logbook documents the date on which GC/MS acquisition of the batch of samples begins and records which instrument is used for GC/MS data acquisition (see Attachment 1).

2.2 GC/MS LOGBOOK

The GC/MS Logbook is divided into five sections:

1. Sample Acquisition Records
2. Maintenance Records
3. Record of Service Calls
4. Service Contracts

2.2.1 All SOPs, which relate to the operation and maintenance of the GC/MS systems, will be kept in separate QA manuals located in designated site areas.

2.2.2 Each Sample Acquisition Record consists of an Acquisition Conditions Form (Attachment 2) and a hardcopy of a sequence file listing (Attachment 3).

2.2.2.1 The Acquisition Conditions Forms are numbered sequentially starting at A001, for GC/MS letter A, B001 for GC/MS letter B, C001 for GC/MS letter C, and V001 for GC/MS/VOC. All pertinent information should be filled out completely and inapplicable information should be noted as NA or dashed (-).

2.2.2.2 Each sample analyzed will be assigned a sequential data file name and the file range will be noted in the comments section of the Acquisition Conditions Form equal to sequence file and data files listed. Naming criteria for data files are as follows:

>xnnnn

where x is the instrument designator "A" for GC/MS letter A, "B" for GC/MS letter B, "C" for GC/MS letter C, "V" for GC/MS/VOC, and nnnn is a sequential number starting at 0001. At the turnover event (ex. >A9999), the numeric portion will be reset to 0001 again with appropriate notation in the comments section.

2.2.2.3 Sequence file listings may contain the following pertinent information: sample name, corresponding data file name, acquisition method used, injection volume, vial location, operator, data path runstrings, and project number/name comments. Sequence files are named as follows:

Sqxnnn

Where "x" denotes GC/MS letter and "nnn" is a sequential number starting at 001. At the turnover event (ex. >SQA999), the numeric portion will be reset to SQx001 again with appropriate notation in the comments section.

2.2.2.4 In the event that a sequence does not run to completion or the data from that sequence will not be used, it will be noted in the comments section of the Acquisition Conditions Form. The data file names allocated to the unused sequence will not be used again and the next sequence will continue sequentially.

2.2.3 Maintenance is performed on the instruments according to SOP 3-092. A record of the routine and non-routine maintenance performed will be kept on a GC/MS Maintenance Log (Attachment 4). The operator must date and initial each form.

2.2.4 Service calls placed for a GC/MS system will be recorded on a Record of Service Calls and Requests Form (Attachment 5).

2.2.4.1 Each form will include the date of the service call, the nature of the problem, and the service call number. The service call number is obtained from Hewlett-Packard support personnel upon placing the call.

2.2.4.2 When the problem with the GC/MS system is resolved, the date and nature of the corrective action taken will be recorded on the Record of Service Calls and Requests Form.

2.2.5 Hardcopies of relevant service contracts are stored in the GC/MS Logbook. Service contracts should be referenced when there are hardware problems. Hewlett-Packard requires model identification and serial numbers upon placing a service call.

2.3 GC/MS TUNE LOGBOOK

The GC/MS Tune Logbook contains two sections for each GC/MS system.

1. PFTBA Tune Hardcopies
2. DFTPP Tune Hardcopies

2.3.1 Prior to the start of a new sequence (or after non-routine maintenance) GC/MS systems are tuned with perfluorotributylamine (PFTBA) according to the procedures in SOP 3-092. Hardcopies of the reports

generated by the tuning programs AUTOTUNE, MANUAL TUNE, or TARGET TUNE will be kept in the GC/MS Tune Logbook.

2.3.2 The GC/MS systems can be tuned with decafluorotriphenylphosphine (DFTPP) according to the procedures in SOP 3-092. Hardcopies of all tune reports will be kept in the GC/MS Tune Logbook.

3.0 RESPONSIBILITY

3.1 Persons operating the Hewlett-Packard GC/MS systems are responsible for following the documentation procedures outlined in this document.

3.2 The GC/MS Logbook will be audited periodically by the system manager and Laboratory Manager to assure that entries are made according to this SOP.

4.0 TRAINING

The trainee must read, understand, and be able to perform all the functions listed in this SOP. Once this has been demonstrated a certificate of training (Attachment 6) will be issued and filed in the Quality Assurance Officer training files.

5.0 ATTACHMENTS

ATTACHMENT 1. GC/MS THROUGHPUT MONITORING SHEET
ATTACHMENT 2. GC/MS DATA ACQUISITION FORM
ATTACHMENT 3. SAMPLE SEQUENCE FILE LISTING
ATTACHMENT 4. GC/MS MAINTENANCE LOG
ATTACHMENT 5. RECORD OF SERVICE CALLS AND REQUESTS
ATTACHMENT 6. CERTIFICATE OF TRAINING

APPROVALS:

Author:	Julie A. Leavey	Dec 11, 1998
Laboratory Manager:	Robert H. J.	12·11·98
QA Coordinator:	Dawn Burke	12-11-98
Environmental Chemistry Section Manager:	Gregory Dull	12/11/98
	Name	**Date**

ATTACHMENT 1

BATTELLE – DUXBURY
GC/MS THROUGHPUT MONITORING SHEET

PROJECT NAME	BATCH ID	MATRIX	No. SAMPLES	DATE RECEIVED	DATE SETUP	INSTR.

ATTACHMENT 2

BATTELLE - DUXBURY Page _____
GC/MS DATA ACQUISITION FORM

Project No:_____ Project Title:_____
Batch ID:_____ Matrix(Circle): Soil H2O Tissue Oil Other
Date: _____ Analyst: _____
Sequence. File: _____ Quantification Method: _____

METHOD FILES: Acq. File _____SIM /Full (Low m/z____ High m/z _____)

InjectionVolume (Circle One) 1 or 2 µL

GC CONDITIONS: Column DB-5 (60m) Carrier Gas: Helium

GC TEMP PRGM: Injector 300 ºC Trans Line 280 ºC Initial Temp 40 ºC

Initial Hold 1.0 min Ramp Rate 1 6 ºC/min Final Temp 290 ºC

Final Time _____min

EPC SETTINGS: Vacuum Compensation ON

Pressure Program (Circle One): Constant or Ramp

Initial Pressure: 30 psi Hold time: 1.0 min

MS ACQ. CONDITIONS: MSD Relative Abundance + ~100 EV above ATUNE.U

TUNING: Tuning Date (s)_____ Circle One: PFTBA DFTPP

MSD Vacuum (Ion Gauge) @ Tune (EPC @ 1 ml/min) _____x 10^{-5} Torr

MSD Vacuum (Tune report) _____ m Torr

Electron Multiplier @ Tune_____volts

Circle one: Autotune Manual Tune saved as: ATUNE.U

Files > _____ through >_____

COMMENTS: _____

TAPE ARCHIVE: CD#: _____

Notes: A hardcopy of the sequence file listing should be inserted in the logbook immediately following this page.

ATTACHMENT 3

SAMPLE SEQUENCE FILE LIST

Sequence Name: C:\HPCHEM\1\SEQUENCE\SQA493.S
Comment: ASE Tests B#98-352/98-352/98-364/98-357
Operator: DPB
Data Path: D:\SQA493\
Pre-Seq Cmd:
Post-Seq Cmd:

Method Sections To Run
(X) Full Method
( ) Reprocessing Only

On A Barcode Mismatch
(X) Inject Anyway
( ) Don't Inject

DPB 12-5-98

Line	Type	Vial	DataFile	Method	Sample Name
1	Sample	1	A5533	MWRATHIA	ET55
2	Sample	2	A5534	MWRATHIA	WA19PB
3	Sample	3	A5535	MWRATHIA	WA20PB
4	Sample	4	A5536	MWRATHIA	WA21PB
5	Sample	5	A5537	MWRATHIA	WA22PB
6	Sample	6	A5538	MWRATHIA	WA23PB
7	Sample	7	A5539	MWRATHIA	WA24PB
8	Sample	8	A5540	MWRATHIA	WA73PB
9	Sample	9	A5541	MWRATHIA	WA74PB
10	Sample	10	A5542	MWRATHIA	WA75PB
11	Sample	11	A5543	MWRATHIA	ET55
12	Sample	12	A5544	MWRATHIA	WA76PB
13	Sample	13	A5545	MWRATHIA	WA77PB
14	Sample	14	A5546	MWRATHIA	WA78PB
15	Sample	15	A5547	MWRATHIA	WA25MDL
16	Sample	16	A5548	MWRATHIA	WA26MDL
17	Sample	17	A5549	MWRATHIA	WA27MDL
18	Sample	18	A5550	MWRATHIA	WA29MDL
19	Sample	19	A5551	MWRATHIA	WA30MDL
20	Sample	20	A5552	MWRATHIA	WA31MDL
21	Sample	21	A5553	MWRATHIA	ET55
22	Sample	22	A5554	MWRATHIA	WA79PB
23	Sample	23	A5555	MWRATHIA	WA80SRM
24	Sample	24	A5556	MWRATHIA	VZ23COMP
25	Sample	25	A5557	MWRATHIA	VZ29COMP
26	Sample	26	A5558	MWRATHIA	VZ31COMP
27	Sample	27	A5559	MWRATHIA	ET55

ATTACHMENT 4

BATTELLE - DUXBURY
GC/MS
MAINTENANCE LOG

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

ATTACHMENT 5

BATTELLE - DUXBURY
GC/MS
RECORD OF SERVICE CALLS
AND REQUESTS

Date of Call: _____ Initials:_____Service Call Number:_____
Description of Problem:_____
_____
_____
_____
Corrective Action Taken or Recommended: _____
_____
_____
_____
Date Corrected/Repaired:_____ Repaired By:_____
Description of How Problem Was Fixed: _____
_____
_____
_____

Date of Call: _____ Initials:_____Service Call Number:_____
Description of Problem:_____
_____
_____
_____
Corrective Action Taken or Recommended: _____
_____
_____
_____
Date Corrected/Repaired:_____ Repaired By:_____
Description of How Problem Was Fixed: _____
_____
_____
_____

ATTACHMENT 6

BATTELLE DUXBURY OPERATIONS
CERTIFICATE OF TRAINING

SOP No. _____

TITLE _____

TRAINEE _____

INSTRUCTOR _____

DATE OF
COMPLETION
OF TRAINING _____

APPROVED _____ _____
Name Date

ORIGINAL

Battelle Ocean Sciences
Standard Operating Procedures

for

DATA RECORDING

Summary of changes in this version: Minor modifications are made throughout the document to reflect current practice: the definition list updated, the use of LRBs de-emphasized, and the requirement for documenting formulas emphasized. Adds one error code. This SOP does not address the specific requirements of EPA Good Automated Laboratory Practices (EPA 2185 8/10/95).

1.0 OBJECTIVE

Data generated during the course of projects must be capable of withstanding challenges to its validity, accuracy, and legibility. Therefore, data must be recorded in standardized formats and in accordance with prescribed procedures. This standard operating procedure (SOP) describes the procedures and personnel responsibilities associated with recording field and laboratory data.

2.0 DEFINITIONS

Raw data are defined as any worksheets, records, memoranda, or notes that result from original observations and activities of a project and that are necessary for the reconstruction and evaluation of the project. Raw data may include, but are not limited to, information recorded in

Field survey logs

Battelle Laboratory Record Books (LRB)

Instrument maintenance logs and calibration records

Sample chain-of-custody forms

Standard or stock solution preparation records

Laboratory data sheets (e.g., sample preparation or spiking sheets, miscellaneous documentation forms)

Project-specific data forms

Taxonomic Species ID forms

Raw data may also include photographs, maps, microfilm or microfiche copies, computer printouts, magnetic media such as dictated observations, recorded data from automated instruments, and correspondence related to planning, conduct, and interpretation of a project.

3.0 PROCEDURES

3.1 GENERAL

Raw data should be recorded on standardized data forms contained in bound notebooks (e.g., three-ring binder), or in Battelle LRBs. Data should be entered directly onto the appropriate form; entries should not be recorded on intermediate materials (e.g., scrap paper) and then transcribed to page. If data must be collected and cannot be entered directly onto the appropriate form the data should be recorded in a coherent and organized manner and attached permanently in the project notebook or LRB.

Procedures for recording data in LRBs are detailed in SOP 6-003. If standard forms are used to record data, the forms must contain enough information to ensure traceability. The minimum information required on each form is the project number, project phase or task (if necessary to distinguish it from other work conducted for that project), descriptive title identifying the type of data to be recorded (e.g., sample weights), date the work was performed, and the name or initials of the person(s) performing the work and recording the data. If the recorded data includes measurements, units must be included. Unused or non-applicable areas of the forms should be deleted or marked "NA".

All entries must be made in ink, preferable black. Felt-tipped pens should be avoided because many of the inks are soluble in water or organic solvents. The use of pencil to record data is not acceptable, except in rare circumstances (e.g., inclement weather conditions in the field). If pencil must be used, the data must be photocopied, stamped or marked as a verified copy, and signed and dated. The photocopies should be maintained with the original data in the project files.

All data must be recorded promptly and legibly. Entries must be signed or initialed by the person performing the work. If another individual recorded the data, that person must also sign or initial. Entries must be dated on the day of entry; times should be recorded for activities for which time intervals are critical.

3.2 DATA CORRECTIONS

Corrections to data should be made by drawing a single line through the original entry and replacing it with the correct value. Original data should not be obliterated or written over. All corrections and changes must be initialed, dated, and justified. Write-overs are considered data changes and must be treated in the same manner as other changes, i.e., the written-over value must be deleted with a single line and replaced with the correct value, and the correction initialed, dated, and justified. Justifications for changes should be clear and concise; vague explanations such as Wrong number" should be avoided. Suggested error codes for the more common changes are provided in Attachment 1. If a code other than the ones listed in Attachment 1 is used, an explanation of the code must be included in the project files.

3.3 DATA TRANSCRIPTIONS

Data that are transcribed from other sources must be traceable to their original source (i.e., either the specific location of the original data must be identified or the data transfer process must be described in an SOP or the project plan). Data entered in spreadsheets are assumed to be transcribed unless clearly marked as direct-entry data. If data are transcribed by hand, vs. instrument transfer, the name of the person transcribing the data and

the date of transcription must be recorded. If the transcription was verified, the name of the verifier and the date of verification should be documented.

3.4 CALCULATIONS

Calculations should be thoroughly documented so that the calculation can be duplicated by a person other than the originator. If the formula applied to data is not documented in an SOP then it must be documented with the data (as miscellaneous documentation, a footnote, etc.). All data sources, methods of calculation, and assumptions should be documented or identified. If data relevant to the calculation have been recorded elsewhere, the location of that data should be specified.

3.5 COMPUTER-DRIVEN DATA COLLECTION AND ANALYSIS

In computer-driven data systems, the individual responsible for entering the data and the date of entry must be identified. All printouts must be initialed and dated by the person responsible. Changes in computer entries must be traceable to the original entry, and the reason for the change, the date of the change, and the name of the individual responsible for the change must be documented.

3.6 EXACT COPIES

For studies conducted under GLPs (EPA FIFRA and TSCA Good Laboratory Practices), exact copies are required for original instrument data recorded on heat-sensitive paper, owing to the tendency of that paper to fade within a relatively short period of time. These photocopies must be stamped or labeled Verified Copy," initialed and dated by the person responsible, and maintained with the original data.

4.0 PERSONNEL RESPONSIBILITIES AND TRAINING

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this SOP are properly trained and that documentation of training exists prior to the performance of those procedures. Individuals whose responsibilities include data recording are responsible for reading and understanding this SOP and for performing the procedures in accordance with the stated requirements.

Individuals are considered trained once they have read this SOP and signed the associated training certificate (Attachment 2). The original certificate is filed in the Quality Assurance Unit office.

ATTACHMENTS

1. Error Code List
2. Example of Record of Training

APPROVALS

Author	Diane F Bahl	5-21-96
QA Coordinator	Diane F Bahl	5-21-96
Vice President, Battelle Ocean Sciences	Andrew L Jeffs	5-22-96
	Name	Date

Attachment 1

ERROR CODE LIST

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

WL Inadvertently recorded in the wrong location (e.g., row, column, page)

CC Changed for greater clarity

WO Write over

SE Spelling error

IR Inadvertently not recorded at the time of initial observation

CE Calculation error

TE Transcription error

RE Rounding 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 page or Misc Doc #)

WP Peak misidentification (gas chromatography only; analyst judged peak to be incorrectly identified by instrument software)

S/B Should be; clarifies correct data

Attachment 2

RECORD OF TRAINING
for
SOP No. 6-017-03, *Data Recording*

The above mentioned SOP is relevant to your work. Your signature below attests to the fact that you have read this SOP and understand the requirements associated with this procedure.

Name (Printed)	Signature	Date

Battelle Duxbury Operations

Standard Operating Procedures

for

LABORATORY VERIFICATION AND VALIDATION OF ANALYTICAL DATA

Summary of changes in this version: All references to "validation" have been changed to "verification" to reflect the currently accepted definitions of these terms. A separate section on laboratory validation is added. Specific verification and validation procedures required for certain Navy programs are addressed.

1.0 INTRODUCTION

Analytical data must be verified by he analyst for accuracy, completeness, compliance with the Quality Assurance Project Plan (QAPP), standard operating procedures (SOPs). Data validation is performed by the project manager by assessing quality control data vs. the project data quality objectives, and by reviewing the data for technical reasonableness prior to submission to the Quality Assurance Office. This SOP describes the procedures and responsibilities for data verification and validation.

2.0 PREPARATION

Prior to data verification and validation, the data package should be completed with the documentation necessary to support the reported analyses. (The contents of a data packages are defined in SOP 6-032). The following items will be required to complete data verification and validation:

Completed data package
QAPP (also called work plan or QA Plan)
Sample prep records
Standard prep forms
Instrument calibration records
Raw data files
Final report tables

3.0 VERIFICATION

Verification is defined as *Confirmation by examination and provision of evidence that specified requirements have been met* (National Environmental Laboratory Accreditation Conference 1999).

3.1 PROCEDURES AND DOCUMENTATION

Data verification proceeds from raw data or the last verified step to the data being reviewed (from the known to the unknown). The Data Verification Checklist (Attachment 1) provides guidance on the types of entries and level of detail to be verified. Manual entries and hand-calculations are 100% verified. At least one spreadsheet calculation per sample is reproduced by hand, verifying the formula components from raw or verified data and then reproducing the reported result. Formulas that are reported with the data are also confirmed in this process. Data for a variety of analytes should be verified during this process, and particular attention should be focused on samples that required individual processing procedures (splits, dilution, additional spiking, or re-extraction).

Data verification is documented on the checklist with a check (✓) and the dated initials of the person who performed the verification. The date of spreadsheet preparation should reflect the most recent version. If a spreadsheet is updated to correct errors then this date should be updated. Validation of the new spreadsheet should consist of verification that the most recent version of the spreadsheet was corrected, that the errors were corrected as anticipated, and that unaffected data are unchanged. Old spreadsheets should either be discarded or filed in the "unused data" section of the data package. In the latter case the spreadsheet should be crossed out with the routine documentation of date, initials, and justification.

4.0 VALIDATION

Validation is defined as *The process of substantiating specified performance criteria* (EPA-QAD). Data validation is performed by the laboratory and project managers unless otherwise specified in the QAPP. This validation consists of a review of analytical quality control results (QC samples and instrument QC) vs. the project data quality objectives and the requirements of the SOPs to determine whether the analytical system was in control for the analyses. If the system is in control then the data meet performance criteria. The signature of the laboratory and project managers on the title page of the data package signify that this validation has been performed.

5.0 RESPONSIBILITIES

In general, verification may be performed by the person who originally performed the work or by another trained analyst. If calculations are unusual or complicated it is advisable to ask for an independent review.

Sample Custodian:

The sample custodian is responsible for comparing

- the sample container IDs vs. the sample custody logs
- the completed Sample Identification Logbook vs the Battelle Duxbury Operations (BDO) and client IDs on the containers

The Sample Receipt Log-in file is verified 100% vs. the chain of custody forms by an independent reviewer. This review is documented directly on the hardcopy Log form.

Sample Prep Task Leader:

The sample prep task leader is responsible for validating

- Project name and project number
- Standard expiration dates
- Sample IDs vs. the custody records
- Assignment of sample suffixes for re-extractions, dilutions, etc.
- All prep summary tables (dry and lipid weights, gravimetric data, etc.)
- Documentation (complete and correct), including deviations from the QAPP and SOPs

Any changes made to the prep records after distribution to the analysts must be reported to the analysts.

Analysis Task Leaders:

The analysis Task Leaders are responsible for verifying the following quantification information:

- Internal standard amounts vs. approved standard prep forms
- Sample multipliers and sample weights
- Project name and number
- The standard amounts of all new calibration standards and methods
- Units
- Documentation (complete and correct)

In addition, the analytical task leader is responsible for report table and spreadsheet verification. The following information should be verified:

- All information in the table headings
- Sample IDs vs. the validated prep records
- All manual entries
- One analyte and one surrogate recovery; at least one value in every column should be checked.
- Qualifiers
- MDLs (including appropriateness - same standards, processing procedures, etc)
- The analyses vs. the QAPP, with all deviations documented.

The task leader documents this verification by signing the title page of the data package.

Laboratory Manager:

The laboratory manager reviews the data package for completeness and reviews the quality control results. This verification is documented by signing the title page of the data package.

Project Manager:

The project manager is responsible for the final review of the data. This review should consist of the following assessment:

- The data are clearly presented, in a manner that facilitates client review. Depending on the purpose of the study, this may include reporting samples from the same station, species, or survey together. The reporting format should be specified in the QAPP.

- The scope of the analyses was completed according to the contract and QAPP

- All documentation (miscellaneous documentation forms and documentation of deviations) in the data package is reviewed and approved.

- The quality control results are acceptable or that corrective action has been performed and documented.

Quality Assurance Auditor:

The QA auditor is responsible for verifying that data have been verified in the laboratory. The independent audit of data may not proceed on unvalidated data without the direct approval of the project

or laboratory manager. Otherwise, if there is no evidence that a package submitted for audit has been validated then the package will be returned to the analytical task leader.

Unless specific exceptions are approved by the Resource Manager, all data packages are audited by the Quality Assurance Unit. These audits are defined in SOP 4-015. This review provides an independent verification of the reported data and the completeness of the data package.

Certain U.S. Navy programs require that defined staff conduct specific review procedures: peer, supervisory, management, and QA. The responsibilities are defined in Attachment 2. The implementation of the 4-tiered review process must be defined in the QAPP.

6.0 TRAINING

All laboratory personnel must understand their responsibilities for data validation and must read this SOP. This is documented on the Documentation of Completion form (Attachment 3) which is signed and dated once this SOP is read. The original is forwarded to the QA Office.

Attachment 1. Validation Checklist
Attachment 2. Verification and Validation Requirements for Certain Navy Programs
Attachment 3. Documentation of Completion Form

APPROVALS

Author	Bonnie Buhl	8-2-99
Laboratory Manager	[illegible]	8·2·99
Quality Systems Manager	Bonnie Buhl	8-3-99
Environmental Chemistry Section Manager	[illegible] Name	8/3/99 Date

Attachment 1
Data Verification Checklist for Data Packages

SAMPLE PREP REVIEW

- Check sample custody records to verify client IDs
- Verify that all samples in batch are reported and that all samples reported are in the prep batch
- Complete surrogate and internal standard spiking forms
- Check sample dilution, gravimetric weights, and correction factors; complete form if more than 1 applies to a sample.
- Check HPLC tables 100%
- Check the dry weight and lipid weight data
- Ensure sample transfers are documented by proper sign-off

CALIBRATIONS

- Spot check the response factors for the initial calibration (1/column and 1 mean) and verify that the standard concentrations are correct and that calibration meets the SOP or work plan criteria.
- Check that mid-check standards bracket all reported samples, and that results meet the SOP or work plan criteria (frequency and acceptance level).
- Verify that the correct method file is used to quantify each sample.
- Check that Q-deletes have been deleted correctly.

DATA TABLES

- Check transcription of weight data onto the report tables
- Check any standard amounts and dilutions entered on the quant reports
- Verify the report table reporting units and header information.
- Check that reported analytes are those called for by the Work Plan.
- Spot-check at least one data point for each sample. Recalculate from scratch using all calibration and prep factors
- Check qualifiers and MDLs vs work plan.
- Spot-check surrogate recoveries, focusing on samples with differing dilutions, etc. and check vs work plan criteria
- In required, verify that reported PCBs have been confirmed.
- Calculate QC data (BS, MS) and check qualifiers vs work plan.

DOCUMENTATION

- Flag questions, documentation ambiguities and errors in the data package
- Review the work plan to ensure that all work plan specifications have been met.

Verification completed by: _____ Date: _____

Attachment 2
Verification and Validation Requirements for Certain Navy Programs[1]

PEER REVIEW
An analyst that is qualified to perform the subject analytical method must perform peer reviews. Peer reviews will include, as appropriate:

- 100% of manual entries and manual calculations;
- spot checks of computer calculations to verify program validity;
- check for compliance with method and project-specific QC requirements;
- check for completeness of raw data or supporting materials;
- confirm spectral assignments and identification of TICs;
- check descriptions of deviations from method or requirements;
- check for appropriate use of significant figures and rounding;
- check reported values for dilutions; and
- evaluate reasonableness of results.

SUPERVISOR REVIEW
Technical reviews by the Project Manager or designated alternate must be performed on 100% of reported data. The supervisory review may be less detailed than the peer review, but must include the following:

- check for compliance with Method and project-specific requirements;
- check the completeness of the reported information;
- check the information for the report narrative, and
- evaluate the reasonableness of results.

MANAGEMENT APPROVAL
The Laboratory Manager must perform a 100% check of all data. All data must receive management approval prior to release. Authority to release data may be delegated to a technical supervisor or other party, if the term of the delegated authority is documented in the QA program file.

QA REVIEW
The QAU audits all reported data prior to submission to the client. Among the review procedures the following will be verified:

- check for compliance with required QC practices;
- check for compliance with approved SOPs; and
- check for compliance with Method and project requirements.

[1] From: Navy Installation Restoration Laboratory Quality Assurance Guide (February 1996).

Attachment 3
Record of laboratory staff who have read SOP 6-027

SOP Title: Laboratory Verification And Validation Of Analytical Data

The above mentioned SOP is relevant to your work. Your signature below attests that you have read this SOP and understand the requirements associated with this procedure, and that a copy of the SOP is available to you at your place of work during performance of this procedure.

Name (printed)	Name (Signature)	Date

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedure

for

PREPARATION OF ANALYTICAL CONTROL CHARTS

Summary of changes in this version: Separation of matrix and method for control charts has been added.

1.0 SCOPE

Control charts are used in the laboratory to monitor and control the accuracy and precision of an analytical method. Control charts provide a graphic representation of on-going laboratory quality through chronological plotting of selected data quality characteristics (e.g., recovery). This standard operating procedure (SOP) defines the laboratory requirements for maintenance of control charts in the organics and metals laboratories. It ensures that the quality of every analytical batch is monitored against statistical levels of confidence. Though requirements for occasional analyses (e.g., DO, chlorophyll) are not defined in this SOP, the same principles apply. EPA-regulated programs require the use of approved analytical methods (e.g., Method 608, 625, and the SW846 series) that describe a rigorous control chart program. There is no attempt to adopt these requirements in this SOP because Battelle does not routinely perform analyses for these programs. Specific project work plans must identify additional requirements for control charts that will be performed in addition to, but not in place of, these requirements.

2.0 PREPARATION

Laboratory performance is monitored though the incorporation of quality control samples into every analytical batch. Quality control (QC) samples are prepared and analyzed according to approved SOPs and are treated identically to authentic field samples. A Laboratory Control Sample (LCS) prepared and analyzed with each batch of samples, will be plotted on control charts:

Separate control charts are maintained by matrix and method type:

PAH	Aqueous
PCBs/pesticides	Sediment/Soil
VOA	Tissue
Alkanes	
Butyltins	

Attachment 1 defines the QC parameter that will be monitored for each analysis. When new or modified procedures are instituted in the laboratory the procedure will incorporate an appropriate quality control sample which will be used to generate baseline data for establishment of control chart statistics.

3.0 PROCEDURES

3.1 Initial Development of Control Charts

Beginning in 1999, all control charts were developed using Control Chart!Pro@Plus. See the software manual for further details about the use of the software. Control charts are prepared to chart performance of routine analytical procedures. The quality control samples defined in Section 2.0 should be analyzed with successive analytical batches according to approved SOPs. Once data are validated, the mean and standard deviation (n-1) of the quality control sample results are determined. Generally, a minimum of 20 data points is needed to establish the control chart limits. However, as few as five values can be used in cases where the analysis is infrequent and less than 20 points have been accumulated over a 6-month period. Additionally, the Laboratory Manager may direct the preparation of interim control charts before 20 data points are generated in order to monitor laboratory performance of a new or infrequent procedure.

A master control chart for each method type (Section 2.0) is prepared at the end of the year using the quality control sample results generated from the last 20 batches of data analyzed during the year. (Or if 20 points are not available, then the values generated during the previous six months will be used). Note that the master charts for 2000 will use available data from 1999. The Laboratory Manager or designee prepares these charts. At a minimum, the QC results [percent recovery] are plotted on the Y-axis and the batch ID is plotted on the X-axis (Attachment 2). Separate charts are prepared for each analyte, matrix, and method combination.

The charts will contain

- A centerline: the calculated QC value average
- Upper and lower warning limits: $\pm 2\sigma$ of the calculated average
- Upper and lower control limits: $\pm 3\sigma$ of the calculated average
  where σ = standard deviation

All standard deviations are calculated for the population (n-1).

The preparation of a typical master control chart is described in the following example.

Example: Twenty blank spike samples (Attachment 1) are analyzed for PAHs in sediments. The summary statistics calculated for the percent recovery of acenaphthylene are

$\bar{x}$ = 107%
SD (σ) = 7.33%
n = 20

Center Line is 107%

Warning Limits (WL) are calculated as the average X2σ

$\bar{x}$ X2(7.33) or 107X14.66, therefore

Upper Warning Limit (UWL) = 107 + 14.66 = 121.66 %
Lower Warning Limit (LWL) = 107 - 14.66 = 92.34 %

Control Limits are calculated as the average 3Xσ

$\bar{X}$ X3(7.33) or 107 ±21.99, therefore

Upper Control Limits (UCL) = 107 + 21.99 = 128.99%
Lower Control Limits (LCL) = 107 - 21.99 = 85.01%

Once established for an analysis, these limits will not be recalculated within a calendar year unless

- A significant drift over time indicates a shift in laboratory or instrument performance. In either case, if corrective action requires changes in procedures or significant instrument maintenance, new control chart values may need to be established.
- It appears that differences exist between instruments or processes which should be segregated and plotted separately.
- The laboratory changes sample processing procedures or new instrument conditions are established for analysis. Separate control charts will not be plotted for each instrument or analyst unless specified by the client.

The control chart limits will be reestablished each year using the last 20 QC values from the previous year.

3.2 Routine Maintenance of Control Charts

Once recovery data are generated and validated, the analyst delivers the electronic data to the Laboratory Manager or designee. The recovery value is formatted to fit the CCPRO+ software. The control chart can then be generated and the data reviewed for trends that could identify an out-of-control process.

3.3 Control Chart Administration and Reporting

The Laboratory Manager or his/her designee reviews control charts weekly to assess data trends. This review is documented by initialing the control chart Logbook. The Logbook is kept with the Laboratory Manager and the electronic control charts are stored on the hard drive of the PC of the generator of the charts. Over 150 control charts are maintained electronically. Control charts will be printed quarterly and as needed for out-of control events.

4.0 QUALITY CONTROL

4.1 Out-of-Control Events

A laboratory procedure is considered out-of-control whenever, at a minimum, if any one of the following conditions is demonstrated by a control chart.

1. any one point is outside of the control limits;
2. any three consecutive points are outside of the warning limits;
3. any eight consecutive points are on the same side of the centerline;
4. any six consecutive points are such that all the points are larger or smaller than their immediate predecessor; and
5. any obvious cyclic pattern is seen in the points.

4.2 Corrective Actions

When any one of the conditions listed in Section 4.1 is identified it is the responsibility of the analyst to notify the Laboratory Manager immediately. Initially, a review of the out-of-control data is performed by the Laboratory Manager and the analyst. They will determine the appropriate corrective action, complete a Corrective Action form (Attachment 3), and notify the QA officer of the out-of-control event by submitting a copy of the corrective action form with a copy of the control chart.

- In some cases corrective action investigations may identify a mathematical error or an isolated incident in the laboratory. If the cause of the out-of-control event is identified (contaminated glassware, degraded standards), then the affected control chart points are circled on the chart with an explanation. These circled points are not included in future Section 4.1 analyses. Calculation errors will be corrected on the chart with appropriate documentation.

- If no apparent cause is found and the out-of-control event(s) appear to be isolated to a specific period, the data generated during that period may be unreliable. In these cases, the quality control data generated with the sample batch should be reviewed.

  (a) If the data for QC samples meet the SOP and work plan criteria then the data are considered acceptable, but corrective action for the analytical system should be initiated and documented in the instrument log.

  (b) If QC data do not meet the SOP or work plan criteria then the reported data associated with the QC check should be flagged as suspect (s). Re-analysis of project data is left to the discretion of the Project Manager. However, the project files must include documentation that the analytical system may have been out of control by documenting the deviation, with justification and approval by the project manager, and a copy of the control chart.

- Generally, data generated in the same batch as a data point which exceeds the Control Limits is considered unreliable. Reanalysis will occur after corrective action has been taken and the procedure is determined to be back in control. If the project manager determines that reanalysis will not occur, the project files must document this deviation, with justification and approval by the project manager, and a copy of the control chart.

- If a trend is observed that appears to be permanent and still within acceptable laboratory operating limits, then it is probably necessary to re-calculate the control limits at this point. The Quality Systems Manager must be consulted prior to this action.

- If routine plotting reveals distinct trends for a particular matrix, instrument, or analyst then these data will be plotted as a separate control chart.

5.0 RESPONSIBILITIES

Each analyst is responsible for

- immediately supplying the results of QC sample analysis to the designated generator of the control charts.

The Laboratory Manager or designate are responsible for

- preparing the master control charts for each analysis type,
- reviewing the control charts weekly,
- reviewing all out-of-control events and determining, with the analyst the appropriate corrective action,
- ensuring that the appropriate corrective action is implemented and documented if warning or control limits are breached, and
- ensuring that any data generated during out-of-control periods is reviewed, the project manager notified, and any affected samples are reanalyzed as directed by the project manager.
- notifying the Quality Systems Manager that an out-of-control event has occurred, and
- collecting, distributing, and filing the control charts.

The Quality Systems Manager is responsible for

- reviewing the results of control charts quarterly,
- working with the Laboratory Manager to determine appropriate corrective action or determining whether new control limits should be established,
- ensuring that out-of-control events and the resulting corrective action are adequately documented, and
- ensuring that the quality control criteria for any reported data generated during out-of-control events are acceptable or flagged appropriately.

6.0 DOCUMENTATION

Current control charts are maintained electronically for immediate update and review. A full set of control charts will be printed on a quarterly basis and kept in the Control Chart Logbook. All historical control charts, with all appropriate documentation of corrective action, will be maintained by the Laboratory Manager or designee in Control Chart Logbook.

7.0 TRAINING

Any individual who will prepare control charts must first read this SOP. Next the analyst must become familiar with Control Chart Pro+. The Laboratory Manager will review data plotting and documentation

procedures with his/her designee about generating control charts. Once these training steps have been completed a certificate of training (Attachment 4) will be completed. The original Certificate of Training will be provided to the QAU.

ATTACHMENTS

Attachment 1. Organics Analyses Control Charts
Attachment 2. Example Control Chart
Attachment 3. Corrective Action Log form
Attachment 4. Certificate of Training

APPROVALS:

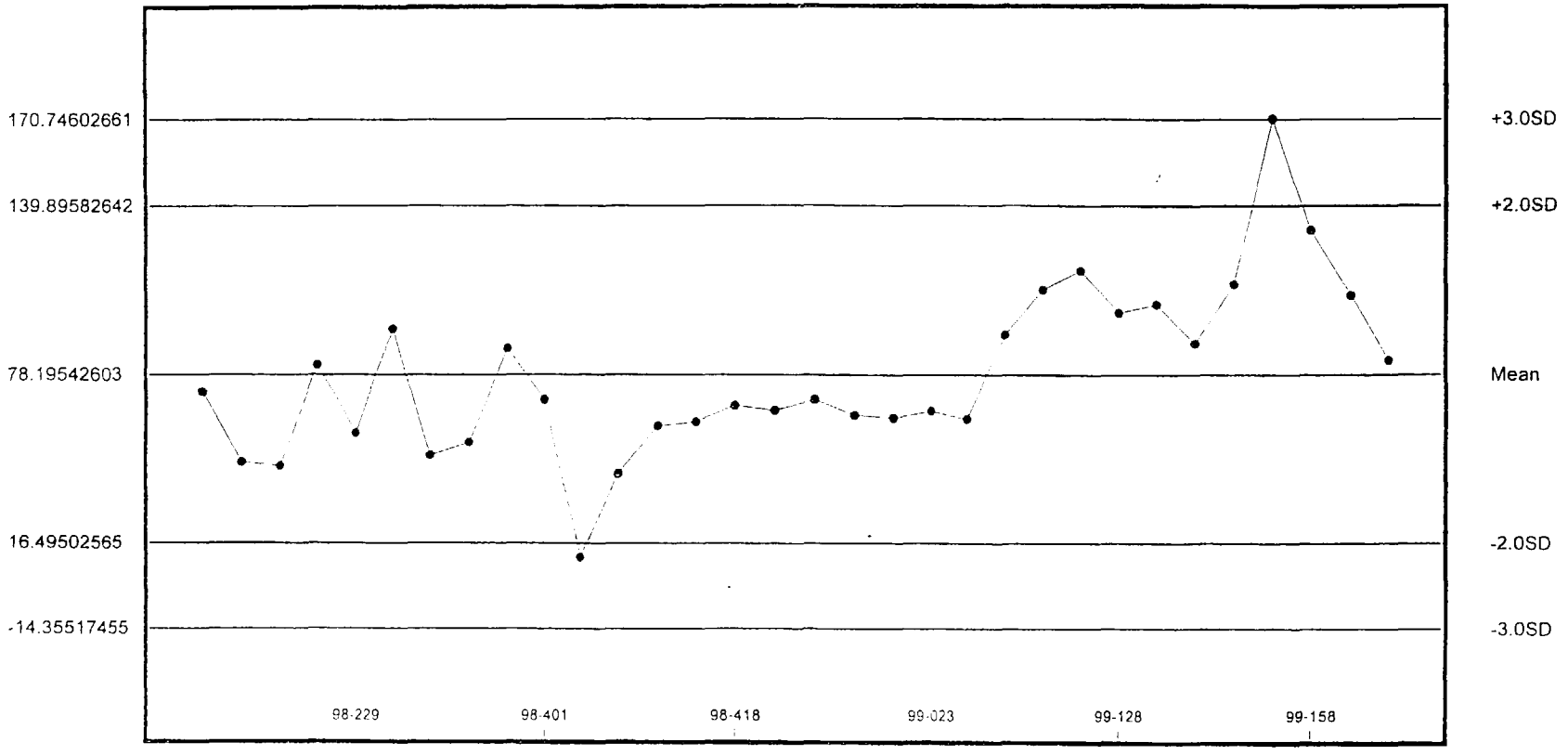
Author		12·10·99
Laboratory Manager		12·10·99
Quality Systems Manager		12-10-99
Environmental Chemistry Section Manager	Signature	12/10/99 Date

Attachment 1. Organics Analyses Control Charts[1]

Analysis Type	QC Sample	QC Statistic
PAH	Laboratory Control Sample	Recovery
Phenols	Laboratory Control Sample	Recovery
Pesticide/PCB	Laboratory Control Sample	Recovery
Alkanes	Laboratory Control Sample	Recovery
Butyltins	Laboratory Control Sample	Recovery
VOAs	Laboratory Control Sample	Recovery

[1]Control charts will track the recovery of all analyzed compounds in LSC samples. All recoveries will be reported vs. the recovery internal standard (RIS). Master Control Chart limits are based on 20 data points, if possible.

Attachment 2
Example Control Chart



n= 32 mean= 78.19542694 SD= 30.85020065 %CV= 39.45% min= 11.16000000 max= 170.94710710

ile: D:\Active\Control Charts\TBT 1999\TBT1999.ccp
Column DBT

JPB 9-20-99

Attachment 3
Corrective Action Log

Battelle Duxbury Operations
Corrective Action Log

Project No. _____

Analyst_____ Analysis Type_____

Date_____

Description of Problem:

Affected Samples:

Corrective Action Plan:

RESOLUTION

Results of corrective action plan:

Was data quality affected?

Was the client contacted?

APPROVAL

Laboratory Manager_____ **Date**_____

Project Manager_____ **Date**_____

QA Officer _____ **Date**_____

Attachment 4

Battelle Duxbury Operations
Certificate of Training

SOP No. 7-028

SOP Title **PREPARATION OF ANALYTICAL CONTROL CHARTS**

SOP read and understood _____ _____
Name Date

Description of Training;

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP.

Approval _____ Date _____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedure

for

PREPARATION, ANALYSIS,AND REPORTING QUALITY CONTROL DATA IN THE CHEMISTRY LABORATORY

Revisions to the SOP: The content is expanded and the title changed to reflect added scope. Sections for sample preparation and analysis are added. Definitions and frequencies of QC samples are provided. Examples of corrective action are added. The discussion of method detection limits and reporting limits was moved to SOP 5-291. Definitions and criteria for several QC samples are added or modified.

1.0 OBJECTIVES

The purpose of this SOP is to describe the preparation, analysis, and standard reporting requirements for quality control (QC) samples. At a minimum, one suite of quality control samples including a procedural blank, a measure of accuracy, and a measure of precision are required with every set of 20 or fewer field samples. Description of common QC samples are described in Attachment 1. The project manager will define the required QC samples in the QAPP.

2.0 PREPARATION

Attachment 1 defines the preparation and frequency of quality control samples that are required routinely. All field and quality control samples are spiked with the same surrogate internal standards prior to processing and with recovery internal standards prior to analysis.

3.0 ANALYSIS

Quality control samples are analyzed using the same calibration, instrument settings, and procedures used for field samples. No extraordinary means may be used in analysis or quantification.

The method blank that was prepared with the processing batch should be analyzed with each analytical sequence (run).

4.0 REPORTING

Project reporting requirements are highly variable. However, the following conventions are applied unless otherwise specified in the QAPP.

4.1 SAMPLE MATRIX

The sample matrix reported with quality control (QC) samples such as the procedural blank (PB) or laboratory control sample (LCS) should be reflective of the associated field samples. For example, the matrix reported for the PB associated with a sediment batch should be "sediment".

4.2 UNITS

Unless otherwise specified in the QAPP, the PB will be reported in the same concentration units as the associated field samples. The LCS sample will be reported in amount units, reflective of the amount of target analyte(s) added to the sample. As a quantitation of analytical accuracy, the percent recovery of each analyte will also be reported.

4.3 SAMPLE SIZE

Unless defined otherwise in the QAPP, the weight/volume of PB samples will be reported as the *approximate* average weight/volume of the field samples in the associated sample batch. This value, an integer, will be determined by the sample preparation Task Leader (or appropriate designee), and documented in the sample preparation records. Sample size will be reported for QC samples as follows:

PB	average field sample weight (described above)
LCS	NA
MS	actual weight

4.4 ANALYTES REPORTED

Only those analytes required by the client, defined in the QAPP, specified in the Method, or otherwise requested and documented by the Project Manager will be reported for QC samples. Any additional analytes quantified or included in the SRM or spike solution will not be reported on the final data tables reported to the client. This applies to PB and LCS samples as well as standard reference materials (SRM) and matrix spike (MS) samples. Exception: In the case of PCBs, if samples are analyzed for Aroclors, and congeners are quantified in the QC samples, the congener data will be reported.

4.5 SRM DATA

The default format for SRM data tables should include the target analytes in concentration units, the certified values or ranges, and the percent difference between the concentration detected and the certified values or range. If the certified value is defined as a range, then the PD based on either the upper or lower concentration limit defined in the Certificate of Analysis, whichever is appropriate. (The appropriate range value is the one closest to the detector value). Values within the range have a PD of 0%.

Unless otherwise requested by the client, only concentrations >10 times MDL will be qualified if they are outside the range stated as the DQO.

4.6 DATA QUALITY CRITERIA AND DATA QUALIFIERS

Default Data Quality Criteria (DQC) for QC samples are presented in Attachment 2. These values/criteria will serve as the DQCs for all projects unless otherwise defined by the client, defined in a method-specific SOP, or unless they do not adequately serve a particular project or matrix. The DQCs are defined in the QAPP. QC sample results that exceed acceptance criteria should be investigated and the appropriate corrective action implemented. Examples of corrective action for quality control samples are provided in Attachment 3.

The use of data qualifiers is often project-specific and defined in the QAPP. In the absence of other specifications the following data qualifiers.

Qualifier	Use
J	Analyte detected below the MDL/RL.
B	Analyte detected at a level greater than 3X MDL/RL in the PB. The qualifier is entered for the PB and affected field samples.
ME	Estimated value; significant Matrix Interference.
U	Analyte not detected. "ND" will be reported in the value column
&	QC value outside the accuracy or precision DQC.

As a default reporting convention, analytes not detected will be reported as "ND" and qualified with a "U." Alternative qualifiers/MDL/RL values will be inserted on a project-specific basis. "NA" is only applicable when a value or descriptor is not available or not applicable (e.g., sampling time is required on report table but is not included, or the Client ID for an internal QC sample).

4.7 SIGNIFICANT FIGURES

Unless specified in the QAPP, sample concentration data are reported to two decimal places. An attempt should be made to limit significant figures to no greater than four. Additional significant figures should be removed after all data manipulations have been completed. All QC data reported as recovery (e.g., surrogate recoveries, analyte recoveries in LSC, MS/MSD samples) should be reported as whole numbers. All QC data reported as a measure of precision (RPD between duplicates; percent difference for SRMs) should be reported to one decimal place.

5.0 CALCULATIONS

5.1 PERCENT RECOVERY

The percent recovery is a measurement of accuracy, where one value is compared with a known/certified value. The formula for calculating this value is:

$$Percent\ \ Recovery = \frac{amount\ \ detected}{amount\ \ expected} x\ 100$$

5.2 PERCENT DIFFERENCE

The percent difference (PD) is a measurement of precision as an indication of how a measured value is difference from a "real" value. It is used when one value is known or certified, and the other is measured. The formula for calculating PD is:

$$Percent\ \ Difference = \frac{X_2 - X_1}{X_1} x\ 100$$

where: X_1 = known value (e.g., SRM certified value)
X_2 = determined value (e.g., SRM concentration determined by analyst)

5.3 RELATIVE PERCENT DIFFERENCE

The relative percent difference (RPD) is a measurement of ***precision***; it is a comparison of two similar samples (matrix spike/matrix spike duplicate pair, field sample duplicates). The formula for calculating RPD is:

$$RPD = \left| \frac{2\ x\ (X_1 - X_2)}{(X_1 + X_2)} \right| x\ 100$$

where: X_1 is concentration or percent recovery in sample 1
X_2 is concentration or percent recovery in sample 2

Note: Report the absolute value of the result -- the RPD is always positive.

5.3.1 Field Sample Duplicates

The RPD between field sample duplicates is a quantitative measure of the variability between duplicates using the native concentrations of target analytes as comparison values. The assumption is that the two samples are exactly alike, and that the concentrations of analytes in the duplicates are expected to be identical.

Step 1: Calculate the concentration of the analytes in the two samples.

Step 2: Substitute the concentrations for X_1 and X_2 in the RPD formula. (An example calculation is presented in Attachment 4).

5.3.2 Matrix Spike/Matrix Spike Duplicate Samples

With MS and MSD duplicate samples there are several variables that must be considered when performing the RPD calculation, including sample size, background concentrations, and amount of

analyte(s) spiked into the samples. To calculate the RPD:

Step 1: Calculate analyte concentration in the MS, MSD, and unspiked background sample (units: weight/weight, e.g. ng/g).

Step 2: Subtract background **concentration** of each analyte detected in the background sample from the MS and MSD. This will, theoretically, leave us with the concentration due to the spike alone. (units: weight/weight, e.g. ng/g).

Step 3: Determine the **amount** of each analyte detected in the MS and MSD samples. To do this, multiply the concentration value of each analyte AFTER background subtraction, by the sample weight. (units: weight, e.g. ng, µg).

Step 4: Calculate percent recoveries of the analytes in the MS/MSD samples:

$$\frac{Amount\ detected\ in\ MS\ (or\ MSD)}{Amount\ expected}\ x\ 100$$

where the amount expected is the amount of the analyte spiked into the sample prior to extraction.

Step 5: Calculate the RPD between the MS and MSD samples. This should be based on the percent recoveries of the analytes detected in each sample. These recoveries will be inserted into the RPD equation as X_1 and X_2. An example calculation is displayed in Attachment 5.

The reason recoveries are calculated based on the **amount** of analyte recovered in the spiked MS/MSD samples rather than the concentration is because the concentrations are not comparable unless the samples are <u>exactly</u> the same size. For example, assume sample A weighs 30 g and sample B weighs 40 g. If they were each spiked with 100 ng acenaphthene prior to extraction, the amount of analyte detected should be the same,

$$Concentration\ in\ A = \frac{100\ \ ng\ acenaphthene}{30\ \ g} = 3.3\ \ ng/g$$

however the concentrations would differ because of the sample size:

$$Concentration\ in\ B = \frac{100\ \ ng\ acenaphthene}{40\ \ g} = 2.5\ \ ng/g$$

5.4 RELATIVE STANDARD DEVIATION

The relative percent difference (RSD) is a measurement of ***precision***; it is a comparison of three or more similar samples (e.g., field sample triplicates, initial calibration, MDLs). The formula for calculating RSD is:

$$\%RSD = \frac{\textit{Standard Deviation of All Samples}}{\textit{Average of All Samples}} \times 100$$

ATTACHMENTS

1. Definitions of Typical Laboratory Quality Control Samples and Frequency
2. Data Quality Criteria
3. Probably Sources and Expected Corrective Actions
4. Field Sample Duplicate Calculations
5. MS/MSD Sample Calculations

APPROVALS:

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Quality Systems Manager	Donna L Buhl	8-8-99
Environmental Chemistry Section Manager	Geoffrey Drell Name	8/6/99 Date

Attachment 1
Definitions of Typical Laboratory Quality Control Samples and Frequency

QC Sample	Definition	Frequency
Method or Procedural Blank (PB)	A combination of solvents, surrogates, and all reagents used during sample processing, processed concurrently with the field samples;. Monitors purity of reagents and laboratory contamination. Matrices: Water (MilliQ); soil or sediment (Ottawa sand or sodium sulfate); tissue (sodium sulfate)	1/sample set [a] For some projects the PB is analyzed with each instrument run.
Trip Blank (TB)	A container of MilliQ water that accompanies sample bottles to the field, during collection, and returned with the field samples. Used to assess contamination that may accumulate during shipment of sample containers and samples. Processed in the lab as a routine field sample.	1/ site location if required by the QAPP
Equipment Blank (EB)	A field sample that consists of MilliQ water that is poured over, around, or into field sampling equipment after routine decontamination procedures to assess the effectiveness of the cleaning and to measure carry-over contamination from the sampling equipment. Processed in the lab as a routine field sample.	Highly variable and specified in the QAPP.
Field Blank (FB)	An empty sample container that is transported to the field and treated as a sampling container but actually left capped throughout the sampling effort. Processed in the lab by adding the appropriate volume of solvent to the container and "extracting" the container. The extracts are added to collection flasks and processed as a routine field sample.	Highly variable and specified in the QAPP.
MDL Verification Spikes	A field sample spiked with the analytes of interest for verification of MDLs and processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate for tissues and soils.	1/sample set if required by the QAPP
Laboratory Control Sample (LCS)	A LCS sample is a matrix-specific sample that is prepared with each processing batch. It is spiked with the analytes of interest and processed identically to the field samples. Matrices are the same as those used for the procedural blank.	1/sample set
Matrix Spike (MS)	A field sample spiked with the analytes of interest at 10 X the MDL, processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate for tissues and soils.	1/sample set

Attachment 1 (continued)
Definitions of Typical Laboratory Quality Control Samples and Frequency

QC Sample/Parameter	Definition	Frequency[a]
Duplicate Sample (DUP) or Matrix Spike Duplicate (MSD)	Second aliquot of a field sample processed and analyzed to monitor precision; each samples set should contain a duplicate. The duplicate may be a second matrix spike sample.	1/batch
Recovery Internal Standards (RIS)	All field and QC samples are spiked with recovery internal standards just prior to analysis; used to quantify surrogates to monitor extraction efficiency on a per sample basis.	Each sample
Surrogate Internal Standards (SIS)	All field and QC samples are spiked with a known amount of surrogates just prior to extraction; recoveries are calculated to quantify extraction efficiency.	Each sample
Standard Reference Material (SRM)	An external reference sample which contain a certified level of target analytes; act as a monitor of accuracy. Extracted and analyzed with samples of a like matrix	1/batch if required by the QAPP
Performance Evaluation (PE) or Performance Test (PT)	Blind sample of unknown composition that is analyzed as a routine sample. The PE is provided by either a government or commercial agency. Results are submitted to the supplier who determines whether the results fall within a statistically acceptable range.	2/year
Independent Instrument Check (IC)	Direct spike of target analytes into solvent where the spike source is independent of that used to prepare the calibration standards to assess instrument performance.	1/analytical run if an independent source is available.
System Performance Check Compounds (SPCC)	System performance check to ensure that minimum RFs are met before calibration curve is used.	1/initial calibration if required by the QAPP
Calibration check compound (CCC)	Check of the integrity of the calibration system.	1/batch if required by the QAPP
Reference Oil	Analysis of an oil standard to assess measurement results against historical data.	1/batch if required by QAPP
Solvent Blank	An injection of straight solvent to assess sample carry-over in GCs (not GC/MSs).	1/every 10 samples
Reagent or Solvent Purity Checks	All reagents are lot-checked prior to use.	Per lot purchase

[a] A batch is defined as 20 field samples processed simultaneously and sharing the same QC samples.

Attachment 2
Data Quality Criteria

QC Sample Type/ QC Parameter	QC Criteria	Comments
PB	<3 x MDL/RL	Or the associated sample analytes are 10 X the PB
LSC	70 to 130% recovery vs. SIS 40 to 120% recovery vs. RIS	
MS/MSD RPD	70 to 130% recovery vs. SIS 40 to 120% recovery vs. RIS <30%	For at least 80% of analytes Spike level must be 5-10 times the background level to be used for MS/MSD assessment.
DUP	<30% RPD [b]	Analyte concentration must be 10 x MDL to be used for DQA
TRIP	<30% RSD [b]	Analyte concentration must be 10 x MDL to be used for DQA
SIS	40 - 120% recovery	
SRM	Within 30% on average and not to exceed 35% for more than 30% of analytes Measured from the upper or lower 95% confidence interval (from certifying agency) as applicable.	Analyte concentration must be 10 x MDL to be used for DQA
PE/PT	NA	Acceptance defined by provider
IC	≤15% PD	
SPCC	Reference SW846 for criteria	
CCC	Reference SW846 for criteria	
Reference Oil	PAH, alkane, THC: PD<25% [b]	
Solvent Blank	No distinguishable signal	
Reagent or solvent purity checks	No distinguishable signal	See SOP 5-015

Attachment 3
Probably Sources and Expected Corrective Actions[1]

1. **BLANKS**
   a. Sources and expected review procedures:
      1. Contaminated reagents – verify reagent sources.
      2. Environmental Contamination (all sample collection, sample and analysis conditions) – review sampling handling protocols.
      3. Improper or incomplete laboratory and/or field decontamination/cleaning procedures – review cleaning protocols.
      4. Contaminated sample containers – verify source and storage conditions.
   b. Expected Corrective Actions:
      1. Review data with respect to reported contamination levels. If sample concentrations are near the reported blanks levels, reprocess (reextract or digest) associated samples or resample. If sample concentrations or the reporting levels are significantly higher than blanks, or contaminants are not detected in the samples, report the sample data and concentrations in blank.
      2. Take measure to eliminate future problems: discard reagents, revise protocols, perform preventative maintenance on system, adjust use of interfering chemicals (solvents, fuels, etc.).

2. **CALIBRATIONS**
   a. Sources and expected review procedures:
      1. Improperly prepared or outdated standards – review preparation logs for calculation/dilution errors and use of expired sources.
      2. Improperly prepared or outdated check standard – verify check standard.
      3. Poor instrument response – determine if preventative maintenance is required.
      4. Incorrect calculations – review and verify all calculations.
      5. Contamination problems (see blanks above).
   b. Expected Corrective Actions:
      1. Recalculate calibration curve.
      2. Prepare fresh standards.
      3. Recalibrate instrument.
      4. Perform preventative maintenance.
      5. Perform mass calibration and retune.
      6. Reanalyze all samples bracketing those from previous ACCEPTABLE QC check through next acceptable QC check.
      7. Take measures to eliminate sources of contamination.

3. **SYSTEM PERFORMANCE CHECKS**
   a. Sources and expected review procedures:
      1. Pesticides:
         Poor column performance – replace/repack column.

[1] From Department of Environmental Protection Standard Operating Procedures for Laboratory Operations and Sample Collection Activities (DEP - QA-001/92 Section 11.0).

Attachment 3 (Continued)
Probably Sources and Expected Corrective Actions

2. Standard Reference Materials and QC Check Samples:
   a. Improper sample preparation or analysis – review all protocols associated with sample preparation and analysis.
   b. Incorrect dilutions or calculations – recheck all calculations.
   c. Contamination (see blanks above).

b. Expected Corrective Actions:
   1. Reanalyze all samples bracketing those from previous ACCEPTABLE QC check through next acceptable QC check.
   2. Reprocess all samples associated with QC check sample or standard reference material (unless the problem is unique to processing of the check sample).
   3. Take measures to eliminate sources of contamination.

4. SPIKES

a. Sources and expected review procedures:
   1. Error in calculation – review/recheck all calculations.
   2. Error in preparing or using spike solutions – review all preparation and/or analytical logs (including sample preparation) for proper dilutions, solvents, buffers, etc.
   3. Outdated standards – review expiration dates and standard preparation logs.
   4. Contamination problems (see blanks above).
   5. Poor instrument response – determine if preventative maintenance is required.

b. Expected Corrective Actions:
   1. Take measures to eliminate contamination problems, reprocess if necessary.
   2. Perform required maintenance and revise pm schedules.
   3. Review preparation, calculation and record keeping to determine if additional training or more stringent protocols are needed.
   4. If the laboratory has no historical data to show that the sample matrix produces consistently unacceptable (out of control) recoveries, and none of the sources discussed above are responsible for the problem, the sample must be reprocessed and reanalyzed. If reanalysis produces the same result, associated samples should be reported with qualified results. If results are different, all associated samples must be reprocessed for analysis.

5. DUPLICATES

a. Sources and expected review procedures:
   1. Nonrepresentative sample – review sample collection and/or sample processing protocols.
   2. Error in calculations – recheck calculations.
   3. Contamination problems (see blanks above).
   4. See matrix spikes above.

b. Expected Corrective Actions:
   1. Report data with qualifiers and explanation.
   2. Revise sample collection/sample processing protocols to assure a representative sample.
   3. Take measures to eliminate contamination problems.
   4. Reprocess and reanalyze sample set (if laboratory generated replicate).

Attachment 3 (Continued)
Probably Sources and Expected Corrective Actions

6. SURROGATE SPIKES

a. Sources and expected review procedures:
   1. See 4.a above.

b. Expected Corrective Actions:
   1. See 4.b above.

7. INTERNAL STANDARDS

a. Sources and expected review procedures:
   1. See 4.a above.

b. Expected Corrective Actions:
   1. See 4.b above.
   2. Reanalyze samples from last acceptable QC check to next acceptable QC check.

8. SPLIT SAMPLES

a. Sources and expected review procedures:
   1. See 5.a above.

b. Expected Corrective Actions:
   1. See 5.b above.

Attachment 4.
Field Sample Duplicate Calculations

Two samples, Tissue A and Tissue B are duplicates of the same field sample. The following table summarizes the characteristics of these samples:

Sample	Dry Weight (g)	Amount of Compound X Detected (ng)
Tissue A	3.6	51.98
Tissue B	3.3	45.01

The concentration of Compound X in Tissue A is 14.44 ng/g
The concentration of Compound X in Tissue B is 13.64 ng/g

Substituting these concentration values into the RPD equation:

$$RPD = \left| \frac{2\,x\,(14.44 - 13.64)}{14.44 + 13.64} \right| x\,100 = 5.70$$

The relative percent difference between the concentrations of Compound X in Tissue A and Tissue B is 5.7%.

Attachment 5
MS/MSD Sample Calculations

MS and MSD samples are spiked with equal amounts of Compound X. The following table presents the information required to calculate percent recoveries, and the RPD between the results:

Sample	Sample Dry Wt. (g)	Amount of Compound X (ng)	Amount of Compound X Spiked (ng)
Background	3.7	62.0	0
MS	3.5	714.0	825
MSD	3.7	667.0	825

Step 1. Calculate concentration

$$Concentration\ in\ background\ sample = \frac{62.0\ ng}{3.7\ g} = 16.76\ ng/g$$

$$Concentration\ in\ MS = \frac{714.0\ ng}{3.5\ g} = 204.0\ ng/g$$

$$Concentration\ in\ MSD = \frac{667\ ng}{3.7\ g} = 180.27\ ng/g$$

Step 2: Subtract background concentration from MS and MSD samples

$$MS\ background\ corrected = 204.0\ ng/g - 16.76\ ng/g = 187.24\ ng/g$$

$$MSD\ background\ corrected = 180.3\ ng/g - 16.76\ ng/g = 163.54\ ng/g$$

Step 3. Re-calculate amount of analyte in MS and MSD based on corrected concentrations

$$Background\ corrected\ amount\ of\ Compound\ X\ in\ MS = 187.24\ ng/g \times 3.5\ g = 655.34\ ng$$

$$Background\ corrected\ amount\ of\ Compound\ X\ in\ MSD = 163.54\ ng/g \times 3.7\ g = 605.10\ ng$$

Step 4. Calculate analyte recovery

$$\%\ Recovery\ of\ Compound\ X\ in\ MS = \frac{655.34\ ng}{825\ ng} \times 100 = 79\%$$

$$\%\ Recovery\ of\ Compound\ X\ in\ MSD = \frac{605.0\ ng}{825\ ng} \times 100 = 73\%$$

Step 5. Calculate RPD

$$RPD = \frac{2 \times (79 - 73)}{79 + 73} \times 100 = 7.9\%$$

BATTELLE MSL SOPs

Battelle
Putting Technology To Work

Marine Sciences Laboratory EFFECTIVE DATE: 4-27-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-002-02

SAMPLE CHAIN-OF-CUSTODY

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	4-26-00
	Signature	Date
TECHNICAL REVIEWER: Carolynn Suslick	Carolynn Suslick	4/27/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4-26-00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	4-27-00
	Signature	Date

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SAMPLE CHAIN-OF-CUSTODY

1.0 SCOPE AND APPLICATION

This procedure defines the methods for establishing the traceability of samples transferred to the Battelle Marine Science Laboratory (MSL) for chemical and/or biological testing. This process ensures the integrity of the samples from the time of collection through sample disposal. The sequential custody of samples will be documented using this procedure. Each custodian of the samples shall comply with the procedures described below.

2.0 DEFINITIONS

- **Custody** - Having control of the sample in one or more of the following manners: 1) physical possession; 2) in person's view after taking possession; 3) secured by a person in a manner that prevents tampering of sample; and/or 4) secured by a person in an area restricted to authorized personnel.
- **Sample Custodian** - The person assigned, at a given field site, laboratory, or testing facility, for having responsibility for custody of the sample.
- **LRB** - Laboratory Record Book
- **CoC** – Chain of Custody

3.0 RESPONSIBLE STAFF

Marine Sciences Laboratory (MSL) Staff as Sample Custodian or as Sample Recipient or as MSL Contact
Project Manager or Task Leader
MSL Manager
MSL Quality Assurance Officer or Representative

4.0 PROCEDURE

4.1 Custody Procedures in the Field or Laboratory

4.1.1 The sample custodian may be a member of the sampling crew or a person that works with those who are collecting the samples. The sample custodian ensures that sample labels are filled out and affixed to the appropriate sample containers before or at the time of sample collection.

Information on the sample labels may include, but not be limited to, a code number identifying the sample, date, time, and location of sample collection, and name of sample collector.

4.1.2 Once the samples are collected, the sample custodian records pertinent sample collection information on required raw data documentation (i.e., sample log, LRB, etc.). Information may include, but not be limited to, a code number identifying the sample, date, time, and location of sample collection, and name of sample collector.

Record in permanent ink all pertinent information about each sample on a Chain-of-Custody Form (Attachment 1 or 2). Press hard when making entries and assure transfer to carbon copies. Multiple samples collected on the same date may be recorded on one Chain-of-Custody Form, provided each sample is identified individually on the form.

Note: The Field Sample Chain of Custody (Attachment 1) is used primarily when transferring samples from the field to the MSL for processing. The Sample Custody Record (Attachment 2) is used when transferring samples from the field or the MSL to another laboratory or testing facility. For the purpose of this SOP, the term "Chain-of-Custody Form" can mean either of the two forms.

4.1.3 If required by a project-specific protocol, the sample custodian attaches custody seals to the samples or to the shipping container (e.g., ice chest) immediately on sample collection. The seal is attached in such a way that the sample cannot be opened without breaking the seal.

4.1.4 If there are special storage requirements (i.e., temperature requirements), the sample custodian ensures that samples are immediately stored using the required method and appropriate containers.

4.1.5 The sample custodian is responsible for the samples during delivery to the MSL, laboratory or testing facility until custody of the samples can be transferred to the sample recipient or until release of the samples during shipment (e.g., if samples have to be shipped via overnight carrier, etc.). If custody of the samples cannot be transferred to the sample recipient or shipped on the same day as sample collection, the samples must be stored in a locked or secured storage area until the transfer can be made.

Note: Chain-of-Custody Forms shall remain with samples during transfer.

4.2 Transferring Custody of Samples to a Laboratory or Testing Facility

4.2.1 Upon arrival at the laboratory or testing facility or just prior to releasing samples for shipment, the sample custodian examines the sample container(s) to ensure that the sample seals are intact and the sample containers have not been damaged.

4.2.2 The sample custodian relinquishes custody by signing, dating, and noting the time in the "Relinquished By" space on the Chain-of-Custody Form. The sample custodian tears off the bottom copy (pink) of the Chain-of-Custody Form and retains it for filing with project files.

4.2.3 The sample recipient takes custody of the samples by signing, dating, and noting the time in the "Received By" space on the Chain-of-Custody Form. The sample recipient now becomes the laboratory sample custodian, completing the transfer of sample custody. The contents of the shipping container must be checked against the information on the chain-of-custody form for anomalies. If any discrepancies are noted, or if laboratory acceptance criteria or project-specific criteria are not met, the laboratory must contact the client's designated point of contact for resolution of the problem. The discrepancy, its resolution, and the identity of the person contacted must be documented in the project file. If any seals have been broken and/or the sample containers are damaged, the sample recipient records the condition of the seals and containers in the remarks section of the Chain-of-Custody Form.

4.2.4 The Chain-of-Custody Forms travel with the samples during the transfer, and are filed in the laboratory or testing facility's project files.

4.3 Internal Chain of Custody

MSL does not routinely invoke a formal internal chain of custody process. Access to the building is limited by requiring all staff members to have electronic access cards to enter the building (refer to MSL-A-011, Marine Sciences Laboratory Access Control.) Visitors are issued daily badges. After hours site access is maintained by a gated fence to the grounds and the presence of a security guard. Non-analytical staff are not encouraged to be in areas when they have no reason to be there.

The laboratories are physically located in close proximity to one another and samples are within the physical control of the analysts during digestion and analysis activities. Samples are received in the shipping and receiving area, and logged in per procedure MSL-A-001, Sample Log-In Procedure, and stored until digestion (if required) and analysis. MSL does not store digestate for re-analysis. Instead, if a sample requires re-analysis it is digested from an archived sample.

Access to sample archive refrigerators and freezers is restricted.

A Log-in Checklist (see Appendix of MSL-A-001) is used to document sample sample receipt activities, verification of field sample preservation, sample filtration and preservation when required, and to document any deviations related to sample receipt and sample log-in.

MSL documentation provides the location of the sample post-receipt on the Sample Log-In Form. Digestion sheets provide a record of sample digestion dates. Analysis times are documented on raw data print outs. Sample disposition is determined by the client and is documented on the Log-In Checklist in the section completed by the Project Manager. Sample disposition processes are documented in MSL-I-026, Use of Laboratory Refrigerators and Freezers. When an internal chain of custody report is desired, it can be generated from data sheets in the sample analysis data file, and verified against the data file documentation by the Project Manager and MSL QA Officer.

4.4 Subdividing Samples

Once at the MSL laboratory or testing facility, if samples have to be subdivided and submitted to a subcontractor laboratory, this information will be noted on the original Chain-of-Custody Form (from sample collection), and a new Chain-of-Custody Form is initiated. With each transaction, the sample custodian relinquishes custody to the sample recipient, who then becomes the next sample custodian. (See Sections 4.2.2 through 4.2.4 above.) The requirements for chain of custody and sample disposition will be noted on the Chain-of-Custody form.

4.5 Disposal of Samples

4.5.1 When samples are disposed of by the subcontractor laboratory:

- If the subcontractor laboratory or testing facility is responsible for disposing of the samples, the subcontractor is asked to notify the MSL Project Manager before final disposition. The MSL Contact will notify the originator that the samples are scheduled to be destroyed, or will define customer requirements for an extended period of storage.

- After destruction of samples, the subcontractor laboratory or testing facility is asked to return a copy of the Chain-of-Custody Form to the MSL Contact for placement in project files. The originator may be forwarded a copy of the final Chain-of Custody documentation if requested.

- The MSL Contact records the date of receipt on the Chain-of-Custody

Form in the "Received by" section of the form space and indicates the samples were destroyed ending the chain of possession.

4.5.2 When samples are disposed of by the Marine Sciences Laboratory (MSL):

- If the laboratory or testing facility is not responsible for disposal of the samples, MSL personnel will obtain custody of the samples from the subcontractor laboratory or testing facility along with the Chain-of-Custody Form.

  For returned samples or samples that have never left MSL custody, the MSL Contact will notify the originator that the samples are scheduled to be destroyed, or will define customer requirements for an extended period of storage.

  If extended storage is not requested, then MSL will dispose of the samples following the guidelines specified in the Pacific Northwest National Laboratory's (PNNL's) Standards-Based Management System (SBMS). This system provides a framework for logging in reagents, chemicals and solutions into the associated Chemical Management System (CMS). This system provides the PNNL Laboratory with the policies and procedures regarding tracking and inventory, storage and disposal of completed samples and analytical wastes, as well as chemical use and disposal. The CMS is used to provide an up-to-date inventory to facilitate emergency response, monitor the location of various classes of materials and identify situations where acceptable limits for the building/facility determined by the assigned chemical hazard group and fire zone might be exceeded before a violation occurs.

- After destruction of samples, MSL personnel responsible for sample destruction returns a copy of the Chain-of-Custody Form to the MSL Contact and the Sample Disposal Log Book entry is updated.

- The MSL Contact records the date of receipt on the Chain-of-Custody Form in the "Received by" space next to the Sample Custodian's signature and indicates the samples were destroyed ending the chain of possession.

4.5.3 When samples are returned to the customer for disposal:

- Samples may be returned to the customer (or the sampling site) by customer request. Samples are shipped to meet Department of Transportation regulations Generally, the samples are shipped in the

same way that they were initially shipped to MSL. Sample disposition should be documented in the central file of each project.

4.5.4 The MSL Contact shall ensure that completed Chain-of-Custody Forms are filed in the appropriate project files. The originator may be forwarded a copy of the final Chain-of Custody documentation if requested.

5.0 DATA ANALYSIS AND CALCULATIONS

There are no calculations applicable to this procedure.

6.0 QUALITY CONTROL

It is the responsibility of each individual taking or relinquishing custody of the samples to ensure that Chain-of-Custody Forms are filled out accurately and completely for each transaction, and that the forms are filed in the appropriate project files.

If the Chain of Custody is broken at any time when the sample is in the control of MSL, this deviation must be documented in the data report narrative.

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

All staff responsible for sample custody (i.e., sample relinquisher or sample recipient) shall first read this procedure and document the training as a completed reading assignment on an Individual Training Assignment Form or a Group Training Documentation Form as described in MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-001	Sample Log-In Procedure
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-011	Marine Sciences Laboratory Access Control
MSL-D-004	Data Reporting, Reduction, Back Up, and Archiving
MSL-I-026	Use of Laboratory Refrigerators and Freezers

Attachment 1

Battelle Marine Sciences Laboratory 1529 W. Sequim Bay Rd. Sequim, WA 98382	**EXAMPLE** **FIELD SAMPLING CHAIN OF CUSTODY**	Page ____ of ____

Shipped To:

Company:
Address:

Method of Shipment:
Shipped From: By:
Telephone:

SPECIAL INSTRUCTIONS:

Container No.: _____
Sampling Location: _____
Samples Collected By: Date: _____
Remarks:

_____
_____

SAMPLE IDENTIFCATION

Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time

ATTACHMENT 2

EXAMPLE
SAMPLE CUSTODY RECORD

Date _____
Page of

Battelle
Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

Project No.:											Laboratory:
Project Name:											Address:
Project Manager:											
Phone:											Attn:
										Number of Containers	
Lab No.	Sample No.	Collection Date	Matrix								Observations, Instructions
											total # of containers

Testing Parameters

Relinquished by: Signature Date Time Printed Name Company	Received by: Signature Date Time Printed Name Company	Shipment Method: **Special Requirements or Conditions:** **Sample Disposition:**
Relinquished by: Signature Date Time Printed Name Company	Received by: Signature Date Time Printed Name Company	Distribution: 1. 2 copies to the Laboratory 2. 1 copy project manager 3. Return completed original to Battelle Marine Sciences Laboratory

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Marine Sciences Laboratory

EFFECTIVE DATE: 4-24-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-C-003-02

PERCENT DRY WEIGHT AND HOMOGENIZING DRY SEDIMENT, SOIL, AND TISSUE

Approvals:		
AUTHOR: Linda Bingler	Linda S. Bingler	April 24, 2000
	Signature	Date
TECHNICAL REVIEWER: Brenda Lasorsa	Brenda Lasorsa	4/24/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4/24/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	4-24-00
	Signature	Date

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PERCENT DRY WEIGHT AND HOMOGENIZING DRY SEDIMENT, SOIL, AND TISSUE

1.0 SCOPE AND APPLICATION

This method is applicable to the determination of percent dry weight and homogenization of dry sediment, soil and tissue samples.

2.0 DEFINITIONS

Desiccator - A sealed environment containing an adsorbing compound (such as silica gel) in which samples are placed while cooling to prevent adsorption of moisture.

3.0 RESPONSIBLE STAFF

- Trained technician-level or higher laboratory personnel.
- QA Officer or Representative- data verification

4.0 PROCEDURE

4.1 Equipment

- Spex jars (125 ml) and Spex balls acid cleaned in accordance with MSL-C-011, Glassware and Equipment Cleaning Procedures.
- Aluminum weigh boats.
- Freeze dryer equipped with a vacuum, capable of maintaining temperatures of –50°C.
- Ultra low freezer, capable of maintaining temperatures near –68°C.
- Analytical balance capable of weighing to 0.001 g.
- Spex Ball Miller/Shaker.
- Oven Maintained at 110°C.

4.2 Percent Dry Weight Determination

Two methods are available to determine percent dry weight.

4.2.1 Freeze-Dried samples

Weigh an appropriately-cleaned and labeled Spex jar and record the tare weight of the jar to the nearest 0.001g on the label under tare. Place 5-20 g of wet soil, sediment, or tissue into the tared jar. Weigh the jar containing the

sample and record the weight on the label as the tare + wet weight. Place the samples in a –68°C freezer for at least 2 hours or until completely frozen. Once frozen, put the samples in the freeze drier with the caps off, but make sure the jars can be associated with their respective caps. Record the project number, matrix, date, time and your initials in the Freezer Logbook (Attachment 1). Allow samples to dry; usually 2-4 days, depending on the sample. Samples are dry when they do not feel cold to the touch. Remove samples, cap, and weigh, recording weight on the label as tare + dry.

NOTE: Make sure the caps and jars are correctly matched to ensure correct weights.

Calculate percent dry weight using a spreadsheet (see Attachment 2 for an example).

4.2.2 Oven-Dried Samples

Weigh empty aluminum boats and record tare weight (see Attachment 2 for an example form). Place 5-20 g of wet soil, sediment, or tissue into the tared boat. Weigh the boat and record as tare + wet. Place samples into a 110°C oven and dry at least 8 hours or overnight. Remove the samples, place in a desiccator, and allow to reach room temperature. Weigh and record weight as tare + dry.

4.2.3 Interferences

Soil, sediment and tissue water content varies from sample to sample. Therefore, the length of time required for complete drying varies from sample to sample. Analysts should take appropriate steps to ensure samples are completely dry before final weight is determined.

4.3 **Homogenizing Dry Samples (Ball Milling)**

4.3.1 If samples are going to be analyzed for metals, after freeze drying, add 2-3 acid-cleaned Spex balls to each sample.

NOTE: Sediments with rocks or gravel need to be ball milled in a Spex ceramic jar with ceramic balls. Place a small amount of sediment in the ceramic jar and add the ceramic ball.

4.3.2 Place the Spex jar or ceramic ball mill on the shaker and shake for 2-15 minutes.

4.3.3 Clean the ceramic Spex mill between each sample by adding approximately 2 grams of clean silica sand, then placing the ball mill on the shaker for 2-5 minutes. Wipe the interior of the mill with a clean Kimwipe to remove any

remaining dust or particles.

4.3.4 Samples ball-milled in Spex jars do not require a cross-contamination blank because each sample is isolated from the other samples during the homogenization process. There is no route for cross-contamination.

4.3.5 Samples ball-milled in the ceramic ball mill must have one cross-contamination sample analyzed per batch (a batch consists of all samples of one matrix type ball-milled for one project). After cleaning the ball mill with silica sand, add approximately 2 grams of standard reference material (SRM) appropriate to the project matrix and ball mill for 2-15 minutes. Place the homogenized SRM into a clean Spex jar and digest and analyze with the samples.

Compare SRM and cross-contamination sample results with the certified mean and range values published for the SRM. Cross contamination in this process is not expected, and the results obtained should verify this assumption. However, cross contamination is indicated when the results vary from each other by greater than 25%. There is no effective corrective action to implement at this point because the samples are consumed by the process. The fact that cross contamination is indicated when the percent difference results are greater than or equal to 25% must be noted and conveyed to the customer in the data report.

5.0 DATA ANALYSIS AND CALCULATIONS

$$\frac{\text{Tare+Dry weight - tare weight}}{\text{Tare+Wet weight - tare weight}} \times 100 = \%\text{Dry Weight}$$

6.0 QUALITY CONTROL

If required by a project, replicate analyses may be performed. The number of replicates per batch or sample set are defined by the customer. Acceptance criteria for duplicate or replicate samples shall be ± 20% relative percent difference (RPD) or relative standard deviation (RSD) respectively, unless otherwise specified by client requirements.

7.0 SAFETY

Laboratory personnel should have access to data on potential risks of working with contaminated sediment and various hazardous substances, and procedures for minimizing accidents with these materials. Appropriate attire, including lab coats, goggles or safety glasses, and protective gloves should be worn when working in the vicinity of samples.

8.0 TRAINING REQUIREMENTS

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

9.0 REFERENCES

MSL-A-006	Marine Sciences Laboratory Training
MSL-C-011	Glassware and Equipment Cleaning Procedures

Attachment 1
EXAMPLE FREEZE DRY LOG

CF No.	Number of Samples/ Description	Matrix	Dryer-1 (left) [check one]	Dryer-2 (right) [check one]	Date IN/ Time IN	Initials	Date OUT/ Time OUT	Initials

Attachment 2
Example Sample Preparation
for Percent Dry Weight Determination Form

Project: _____ Percent Dry Weight

Central File #: _____ Date: _____

Analyst: _____ Matrix: _____

Sample Number	Tare Mass (g)	Tare + Wet Mass (g)	Tare + Dry Mass (g)	Wet Mass (g)	Dry Mass (g)	% Dry Mass	% Wet Mass

Analyst:_____ Peer Reviewer: _____