

Site: Industriplex
Break: 3.7
Other: 35269

Standard Operating Procedures

Superfund Records Center
SITE: Industrialplex
BREAK: 3.7
OTHER: 35269

C-1

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

FREEZE DRYING

Freeze Drying

1. Method Reference

- 1.1. Methods - Based on procedure developed Lyophilization Services of New England, Inc.

2. Scope and Application

- 2.1. This method is recommended for high moisture content samples from lipids, polymers, proteins, natural resins, cellular components and dispersed high molecular weight compounds.
- 2.2. The intent of the method is to decrease the water content of a sample there by increasing the amount of dry material available for analysis.

3. Summary of Method

- 3.1. Samples collected in appropriate containers. These samples and vegetation may require maceration before the process begins. Samples are place in 8-ounce jars frozen and placed in the freeze-drying unit. The samples remain in the unit under vacuum unit until the desired dryness is achieved.

4. Equipment and Reagents

- 4.1 8 oz. glass-jars with Teflon-lined lids.
- 4.2 Freeze drier model -Hull 8FS12C.

5. Procedure

- 5.1 The 8 oz. jar is filled to no more than half way with the sediment, tissue or vegetation. The lid is placed on the jar loosely to allow for air and water vapor to escape.
- 5.2 Samples are placed onto the pre-cool shelf at -45°C for four hours.
- 5.3 Samples are brought to a temperature of $28 \pm 3^\circ\text{C}$ for a time of 78 hours. The freeze drying apparatus is brought to a pressure of 150 milli-torr for the duration of the process.
- 5.4 Once the samples are at an acceptable dryness, they are removed from the unit and stored at 4°C until sample preparation (extraction or digestion) is performed.
- 5.5 Alternatively, the samples can be frozen at -10°C to preserve the hold time of the samples.

Laboratory Director	<i>Walter J. Phane</i>	Date: 6/7/99
Quality Assurance Manager	<i>John Phane</i>	Date: 6/7/99

Standard Operating Procedures

C-2a

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

Pressurized Fluid Extraction - Method 3545

METHOD 3545

Extraction of Soil, Tissues, Vegetation and Sediments Samples by Pressurized Fluid Extraction

1. Method Reference

- 1.1 Method 3545C and Method 8000B, Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).

2. Scope and Application

- 2.1 This method is applicable for the extraction of soil, sediment, vegetation or tissue samples for the analysis of organic compounds for a variety of analyses.

3. Summary of Method

- 3.1 A measured weight of sample is placed in an extraction vessel. The sample is flushed with hot pressurized solvent. The resulting extract is dried and concentrated to an appropriate extract volume for further cleanup or analysis.

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	Approval Signatures	
Laboratory Director	<i>Peter J. Kane</i>	Date: 5/11/99
Section Supervisor	<i>Nancy a Rose</i>	Date: 5/11/99
Quality Assurance Manager	<i>Eileen Hymus</i>	Date: 5/11/99

4. Sample Handling and Preservation

- 4.1. Samples are collected in pre-cleaned 8-ounce jars with a Teflon lined screw cap. Approximately 15-20g of sample is aliquoted for the extraction. Greater mass can be extracted using multiple cells containing equivalent portions of the sample.

5. Equipment and Reagents

- 5.1 Dionex ASE 200 Accelerated Solvent Extractor
- 5.2 Sample concentrator - apparatus for reducing sample extract volumes (KD Bath, S-Evap, N-Evap or Turbo-Vap Analytical Evaporator).
- 5.3 Diatomaceous earth, pelletized; Ottawa sand cleaned with methylene chloride.
- 5.4 Glassware - glass powder funnels; Kuderna-Danish (KD) evaporator; 10mL concentrator tube; 1mL capacity syringe; Turbo-Vap tubes; Snyder columns and 60mL "VOA-like" collection vials.
- 5.5 Glass wool; boiling chips; dried sodium sulfate; 2-mL capacity glass vials; screw caps and water bath (heated to ~85°C).
- 5.6 Methylene chloride, Acetone and Hexane – Ultra-resi grade. (DI water for rinsing.)
- 5.7 Tissumizer – Ultra-Turrax T-25; stainless steel cups (50mL). Black & Decker model HC3000 chopper/mincer modified with a titanium blade.
- 5.8 Analytical Balance- capable of weighing to 0.01g.

6. Sample Preparation: Soil/Sediment/Tissue Samples/Vegetation (Method 3545)

- 6.1 Tissue samples must be homogenized into a slurry or paste-like consistency before the extraction process can be performed.
- 6.1.1 **Bivalve** samples in most cases are frozen. If required by the project, the lengths of each shell for a sample site may be measured before the sample processing begins.
- 6.1.2 The shells can be shucked from the bivalve best, if it is just partially thawed. Rinse the exterior shell of the bivalve with DI water if there is any extraneous material on it. A stainless steel knife or titanium knife is used to open the bivalve. The entire contents of the bivalve are removed. Multiple specimens from each site are pooled together (unless otherwise specified for the project) into a beaker.
- 6.1.3 If whole **fish** or edible fillets are to be analyzed, care must also be taken to avoid any contact with material that has not been properly cleaned. Whole fish may need to be sectioned into sizes that will fit into the extraction apparatus. After the sections have been reduced to slurry, they are recombined, thoroughly mixed, and the whole fish is now ready for extraction. If fillets are received, they may require sectioning like the whole fish, if they are large. If whole fish are to be filleted, the same care must be taken to avoid any contact with non-cleaned material. The fillet knife, and the surface used for performing the fillet, must be thoroughly cleaned after each fish is processed to avoid cross-contamination between fish samples.

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- 6.1.4 **Blubber** samples must be partially thawed before being cut into sizes that are appropriate for macerating. The same care with regard to contamination in the fish sample must be taken with the blubber samples.
 - 6.1.5 **Target organs** such as the **liver** may require a qualified marine biologist to perform the dissection and identification of the organs of interest. Once removed from the specimen, the organs should be appropriately combined and go through the same maceration process as other materials.
 - 6.1.6 The goal for any tissue is to have 50 to 100 grams of macerated material for the extraction process.
 - 6.1.7 The tissuemizer must be thoroughly rinsed with methylene chloride before use. This will require a stainless steel cup filled half way with methylene chloride to be run on the unit. The mechanical mixing of the solvent will clean the unit. All the spatulas and beakers will also be solvent rinsed.
 - 6.1.8 The actual sample grinding is done with approximately one third of the cup filled with tissue. Run the unit until the tissue is a paste or slurry. Pour the material into a screw-capped jar. Successive sample aliquots may be required to obtain sufficient material.
 - 6.1.9 After the tissue sample homogenizing is complete, the unit must be cleaned again with methylene chloride and inspected for residual tissue or scales clinging to the unit. The unit may require disassembly, rinsing and re-assembly. Then the next sample can be processed.
 - 6.1.10 Alternatively the black & Decker model HC3000 chopper/mincer can be used. This has been modified with titanium blades to minimize the metals contamination for combined samples. The unit is rinsed with detergent and water followed by DI water before use and in between samples. The tissue samples are added to the unit, macerated for one minute or until a homogeneous mix is observed. It can then be transferred to a screw-capped bottle.
 - 6.1.11 Once the tissue is homogenized, it is then processed the by the same protocol as the soil or sediment samples on the ASE extractor.
 - 6.2 Plant materials will have to be reduced in size to fit into the extraction cell. This can be accomplished by cutting the vegetation with a knife on a Teflon board or using solvent cleaned scissors. Enough material must be produced to obtain the project specific reporting limits. Alternatively the plant material may require mechanical maceration using the chopper or the tissuemizer. Examination of the tissue by the project manager in conjunction with the client will determine if this process is required.
 - 6.3 Weigh approximately 20 grams of low moisture sample (soil or vegetation) or 15 grams of high moisture sample (sediment or tissue) into a beaker (10 grams for an Extractable Petroleum Hydrocarbon EPH) and record the actual weight used in the extraction logbook. Remember to decant any water from the top of sediment samples (unless instructed *not* to decant standing water by project specific guidelines) and discard any foreign objects such as rocks and sticks and leaves from all samples prior to determining the percent solids. Add sufficient diatomaceous earth and mix well until dry. Grind if necessary to obtain a free flowing powder. For Method Blanks and Laboratory Control Samples (LCS), aliquot approximately 20g of infusorial/diatomaceous earth and Ottawa sand or equivalent (use a representative sample weight for determining concentrations in blanks).
 - 6.4 Use a prepared stainless steel extraction cell, cleaned by washing with soap and water between each use, rinsing with acetone to remove water, and rinsing with extraction solvent (methylene chloride) immediately prior to use.
 - 6.5 Close the bottom end of the cell, insert the cellulose filter, and use a rod to press the filter into place at the bottom of the cell. Make sure the filter is properly seated.

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- 6.6 Transfer the entire contents of the sample beaker to a 33mL cleaned stainless steel extraction cell fitted with the cellulose filter. Record the sample IDs and the associated cell numbers in the ASE extraction logbook. Do not attach labels to the outside of the cell. Labels may cause misalignment in the system or the label may be damaged since the entire cell is placed in an oven during the extraction.
- 6.7 Insert another cellulose filter using a rod to lightly press it into place on the top of each sample. Spike each cell with 1mL (or the project-specified spiking volume) of the Surrogate mix and any appropriate Matrix/LCS Spike solution.
- 6.8 Close the top of the cell. Caps should be hand-tightened only. Clean the rod between each sample.
- 6.9 Verify that the white O-rings are in place and are in good condition on the ends of the cell.
- 6.10 Place the extraction cells into the autosampler tray on the ASE unit. Record the autosampler position for each sample in the ASE logbook.
- 6.11 Load the collection tray with the appropriate number (up to 24) of 60-mL pre-cleaned, capped "VOA-like" vials with septa, labeled with the corresponding sample IDs. Labels should only be placed from 1 3/8" to 3 1/8" from the top of the closed cap of the vial, to prevent blocking of the sensors on the system.
- 6.12 Select the correct method on the system keypad and press start.
- 6.13 ASE 200 Operating Conditions:
- | | |
|--------------------|--------------------------------|
| System Pressure: | 14 MPa (2000 psi) |
| Oven Temperature: | 100°C |
| Oven Heat-Up Time: | 5 min. |
| Static Time: | 5 min. |
| Solvent: | Methylene chloride |
| Flush Volume: | 60% of extraction cell volume |
| Nitrogen Purge: | 1 MPa (150 psi) for 60 seconds |
| Rinse Cycle | 8 mL |
- 6.14 The sample may now be concentrated and/or solvent exchanged, then brought to the desired final volume for cleanup or analysis. The concentration can be performed using a K-D on the hot-water bath or the Turbo-Vap apparatus.
- 6.15 *K-D Concentration:* Place the KD flask on the KD bath with boiling chips and the Snyder column on top. The bath temperature should be 80-90°C. Macro-concentrate the sample to less than 10mL. This will take approximately 15-20 minutes. As an alternative the S-EVAP may be used to concentrate. Transfer the sample in the 10mL concentrator tube to the N-Evap unit and bring the extract to the final volume required before cleanup (refer to Table 1). *If the sample appears extremely viscous and reduces in volume very slowly then a final volume of 5 or 10mL should be used to salvage any surrogate data. See your supervisor, lab director or project manager.*
- 6.16 If the sample is dark and viscous, an auto-vial can be employed to remove particulate material. This is particularly evident in heavily contaminated petroleum samples. Reduce the sample to <10mL. Remove it from the blow-down apparatus with an appropriate size syringe. Pass it through the auto-vial back into the blow-down apparatus. The extract may concentrate more easily with the particulate matter removed. However, do not force the concentration as this may jeopardize the surrogate and target compound recoveries.

- 6.17 In some instances with heavily contaminated petroleum samples, it is possible to perform an extra step of hexane exchange to remove the asphaltene material that precipitates out in hexane. This decision should be made with the supervisor's approval. It may preserve the integrity of the target analytes and surrogates in the sample. This extract can also be auto-vialed to further remove any unwanted particulate materials.
- 6.18 *Turbo-Vap Concentration:* Place the TurboVap tube in the unit at a pressure of 20 PSI and a temperature of 44°C in the sensor mode of operation and reduce the extract to the final volume required before cleanup (refer to Table 1).
- 6.19 The sample may now undergo any necessary or required cleanups.

7. Percent Moisture Determination

- 7.1 A five to 10 gram aliquot of the sample is loaded into a pre-weighed weighing pan.
- 7.2 The subsample is placed in the oven at 105°C overnight. The dry sample is placed in a dessicator to cool and is re-weighed. The weight due to the pan is subtracted out.
- 7.3 Calculation :

$$\text{Sample percent Moisture (\%)} = ((\text{Wet weight} - \text{Dry weight}) / \text{Wet weight}) \times 100\%$$

8. Percent Lipid Determination

- 8.1 The uncleaned tissue extract is used to measure the lipid content. If the sample contains more than 300 mg of material when calculated using the lipid value, then the extract will require a dilution before proceeding to any chromatographic cleanup process. Thus, lipid content should be measured before sample cleanup.
- 8.2 Measure and record the final volume of the extract before lipid determination.
- 8.3 Using a syringe, remove 50 to 100 uL of the extract and place it in a pre-weighed aluminum pan.
- 8.4 Weigh the extract. Then allow the solvent to evaporate completely. This may take 10 minutes to an hour.
- 8.5 Re-weigh the tin with the dry extract and calculate the lipid content as follows:

Total Extractable Lipid Weight (TELW):

$$\text{TELW} = [\text{Extract volume (uL)} / \text{Aliquot Volume (uL)}] \times \text{Aliquot weight (mg)}$$

Total Extractable Lipid Concentration:

$$\text{Total Lipid Concentration mg/g} = \text{TELW (mg)} / \text{Sample Dry Weight (g)}$$

9. Quality Control

- 9.1 Method Blank - A method blank must be extracted with every batch of samples (extract one for every twenty samples, if a large numbers of samples are to be processed). It consists of Diatomaceous earth and Ottawa sand spiked with 1mL (or the project-specified volume) of the appropriate surrogate solution. This extraction will demonstrate any background contamination

-
- associated with the extraction batch. Method blanks should not contain any analyte at a concentration greater than the reporting limit, or limits defined in the analysis SOP(s). Exceptions may be made for common laboratory contaminants, such as phthalates, however, any contact with plastics should be avoided. Recovery of surrogates must meet the specified limits.
- 9.2 Laboratory Control Samples (LCS) - A Laboratory Control Sample is extracted and analyzed with each batch of samples (extract one for every twenty samples, if a large numbers of samples are to be processed). The LCS is a blank spiked with the LCS spike mix and the surrogate mix for the particular test being evaluated.
- 9.3 Sample Surrogate Recoveries - Recoveries are monitored to evaluate the method performance. If recovery criteria are not met, data should be closely evaluated to determine if laboratory error or matrix effects are the cause. A repeat analysis and/or extraction is recommended to verify recoveries outside of control limits. If it is the judgment of the analyst in consultation with the QA Manager and the project manager, that the recovery is due to matrix interferences, the data may be reported with the surrogate results flagged in the final report.
- 9.4 Matrix Spikes - Extracted and analyzed at the frequency of a pair (Matrix Spike/Matrix Spike Duplicate (MS/MSD) per 20 samples. Recovery and % RPD are calculated and evaluated against established control limits. The Matrix Spike compounds should be representative of the compounds being investigated.
- 9.5 Sample Duplicates - Duplicates analyses can be performed on samples to demonstrate reproducibility. In some instances where a sample is known to contain significant concentrations of target compounds a sample duplicate may be extracted and analyzed in place of a matrix spike duplicate. Evaluate the %RPD of the sample and duplicate (or MS/MSD) against the established control limits.
- 9.6 If recoveries are not within limits, the following action is required: Check to be sure there are no errors in calculations, matrix spike solution and internal standards. Re-calculate the data and/or re-analyze the extract if any of the above checks reveal a problem. If the checks reveal no errors, and recovery of the LCS is within limits for these compound(s), then recovery problems encountered with the spiked sample are judged to be matrix- rather than system- related and the client should be informed of this via narrative in the final report.
- 9.7 Standard reference materials (SRM) are available from NIST to be extracted and analyzed with samples on a project specific basis. These are not used as controls but to evaluate potential matrix effects in associated samples. Advisory criteria: a. $\pm 35\%$ of the accepted value for 80% of the certified results greater than five times the reporting limit. b. Mean %D $\leq 30\%$ across all certified results greater than five times the reporting limit.
- 9.8 Tissue and sediment samples and some other project specific matrices can be preserved as frozen samples. Subsamples can be taken and the sample returned to the freezer for archiving.
- 9.9 Sample extracts in most cases are split before any cleanups are performed. The uncleaned extract is usually in a 4-mL Teflon-capped vial and placed in a freezer.

Table 1

Method	Exchange Solvent for Cleanup	Exchange Solvent for Analysis	Volume before Cleanup (mL)	Volume for Analysis (mL)
8081	DCM	Hexane	10.0	2.0
8082	DCM	Hexane	10.0	2.0
8270	None	None ^a	4.0	1.0
PAH ^b	None	None ^a	4.0	1.0
TPH	None	None ^a	N/A	1.0
EPH	Hexane	None	1.0	1.0

a- Hexane exchange to remove asphaltenes.

b- Including Alkylated PAHs

Note: Final Volumes before cleanup, and for analysis, can change based on project specific specs.

Standard Operating Procedures

C-2b

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

**SULFUR CLEANUP
METHOD 3660B**

Sulfur Cleanup Method 3660B

1. Method Reference

- 1.1. Method 3660B, Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).

2. Method Summary

- 2.1. Sample extracts that are suspected to contain sulfur are treated with elemental copper that has been activated via acid washing. Copper is added to extracts before analysis and turns from a shiny copper color to black in the presence of sulfur. Sequential treatments are required for high levels of sulfur.

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Approval Signatures		
Laboratory Director	<i>Peter J. Kane</i>	Date: 3/2/99
Organic Laboratory Manager	<i>Norman J. Fanning</i>	Date: 3/2/99
Quality Assurance Manager	<i>Eileen Hyman</i>	Date: 3/2/99

3. Equipment and Reagents

- 3.1 Copper – granular (20-30 mesh), Baker analyzed
- 3.2 Hydrochloric Acid – Trace Metal
- 3.3 Methanol – HPLC grade
- 3.4 Deionized water
- 3.5 400 mL beaker
- 3.6 Teflon coated spatula
- 3.7 Methylene Chloride – Ultra Resi grade
- 3.8 Hexane – Ultra Resi Grade
- 3.9 Pasteur Pipettes

4. Copper Preparation

- 4.1 Place approximately ~ 15 - 20 grams of copper powder in the bottom of the 400-mL beaker.
- 4.2 Slowly add ~ 150 mL of 8 molar hydrochloric acid. This acidic solution is prepared by adding 100 mL of concentrated HCL to 50 mL of deionized water. Use a Teflon coated spatula to very carefully mix the copper and the acid, taking care not to splash the acid. In this and all other mixing steps, the mixing should occur for at least 1-2 minutes, then allow the copper to settle to the bottom of the beaker.
- 4.3 Carefully pour off the acid, and replace it with 150 - 200 mL of deionized water. Do not allow the copper to be exposed to the air for any period of time. Neutralize and dispose of the waste acid properly. Allow the water and copper to mix thoroughly (as described above), settle and then pour off water. Repeat the water rinse step one more time.
- 4.4 Pour off the water and add 150 - 200 mL of methanol. Mix, settle, pour off and repeat two more times. Repeat the solvent rinsing step, this time with methylene chloride, two times. If the methylene chloride becomes cloudy, this means there is still water present. Go back to the methanol rinse, and repeat. If the methylene chloride is clear, then continue to rinse with hexane, two times. Upon the addition of the hexane, the copper should look very finely dispersed, with no clumps or lumps. If there is clumping this means that all of the water has not been removed from the copper. Repeat the solvent rinsing starting with methanol. If the copper is not activated (all the water removed) completely it will not react with the sulfur properly. During this entire procedure, expose the copper to the air as little as possible. After activation is complete, always keep the copper under hexane.
- 4.5 The shelf life of the copper may vary. Before usage the copper should be examined for any discoloration. If significant blackening has occurred, discard the copper.

5. Sample Cleanup

- 5.1 The sample extract should be in hexane in a screw top vial. Transfer a small amount of activated copper to the vial (approximately .25 grams), with as little hexane as possible. The copper should turn black upon addition to the sample indicating the formation of copper sulfide. Keep adding until the newly added copper retains its red color indicating that all of the sulfur has been complexed. After treating the extract, transfer to another clean screw top vial using a Pasteur pipette. Be careful not to carry over any copper into the sample.

Standard Operating Procedures

C-2c

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

AMINO-PROPYL CLEANUP OF TISSUES AND SEDIMENTS

Amino-Propyl cleanup of Tissues and Sediments

1. Method Reference

- 1.1 NOAA 1993. Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects, 1984-1992. NOAA Technical Memorandum NOS ORCA 71.

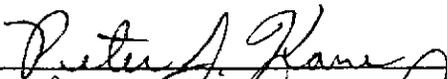
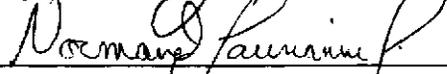
2. Scope and Application

- 2.1. This method is recommended for the cleanup of samples from lipids, polymers, proteins, natural resins, cellular components and dispersed high molecular weight compounds.
- 2.2. The Amino-Propyl interacts with polar material in the extract and allows the target analytes to pass through.

3. Summary of Method

Samples are extracted using the appropriate extraction protocol for the matrix. The sample is concentrated to a final volume of 4 ml if GPC is to be performed. After GPC cleanup the sample is reduced to 1ml final volume before the Amino-Propyl (AP) cleanup. Alternatively the AP can be the primary cleanup tool, particularly for sediment samples.

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	Approval Signatures	
Laboratory Director		Date: 4/14/99
Organic Laboratory Manager		Date: 4/14/99
Quality Assurance Manager		Date: 4/14/99

4. Equipment and Reagents

- 4.1 Varian VAC ELUT SPS-24.
- 4.2 Laboratory Supplies - Syringes, pipettes, autosampler vials, crimper, filter paper, glass filter, glass wool.
- 4.3 Methylene Chloride – Ultra pure
- 4.4 Hexane – Ultra pure
- 4.5 Sep-Pak Vac 6cc (1G), Waters # WAT054605 (Amino-Propyl Cartridge –NH₂)

5. Procedure

- 5.1 Make up a solution of hexane:methylene chloride at 90:10 parts.
 - 5.1.1 Pre-elute (wash) the 1 gram AP cartridge with 20 mls methylene chloride.
 - 5.1.2 Pre-elute (wash) the 1 gram AP cartridge with 20 mls of the 90:10 hexane:methylene chloride mix. Once the solvent has been added to the cartridge and drawn through, the bed of the cartridge must not be exposed to air.
 - 5.1.3 Add 1 ml of the sample extract to the cartridge with the vacuum off. Slowly draw the sample into the cartridge, without exposing the top of the cartridge to air.
 - 5.1.4 Turn the vacuum on and elute 20 mls of the 90:10 solvent mixture to wash the target analytes from the cartridge. The material is collected in the tube inside the VAC –ELUT.
 - 5.1.5 Once the entire 20 mls of solvent has eluted the sample can be concentrated.

6. Method Performance and Interference's

- 6.1 The quality of the reported data is monitored by the Method Blank, Surrogate recoveries, LCS and MS Spike recoveries. Therefore, common sense should always be used in setting up the run batch so that cross-contamination doesn't occur and the characteristics of the entire extraction batch can be evaluated as quickly as possible so that a re-extraction, if needed, can be initiated within holding times
- 6.2 Always run and evaluate the Method Blank from an analytical batch first. If it fails criteria, the entire analytical batch needs to be re-extracted (unless additional sample is not available). *If possible, the Method Blank should be deemed acceptable BEFORE any samples in the batch are analyzed on the instrument. Next check the LCS for acceptability. Pentachlorophenol should pass the criteria for the Semi-volatiles before analysis continues.*
- 6.3 Analyze field samples and their associated QC within the same run sequence at the same dilutions. In other words, if a Sample, MS and MSD were prepared, ensure that they all have the same final dilution prior to analysis and run them in sequence. This will avoid comparability problems in the data that might arise if the Samples and QC are run at different times and at different dilutions.

- 6.4 Method interference can be caused by contaminants in standards, reagents, glassware, solvents, and any other reagents or equipment, which come into contact with samples. All of these materials must be demonstrated to be free of contamination prior to routine use through the analysis of Method Blanks (i.e.. solvent and standard verification must be performed before these reagents can be used for routine sample analysis).
- 6.5 Never use any glassware or other apparatus with the sample if it doesn't appear clean. Ensure that syringes are properly solvent rinsed between uses.
- 6.6 Never use any glassware, or other implements such as syringes, on multiple samples within the batch unless there has been thorough cleaning and decontamination between samples.
- 6.7 Phthalate contamination, from plastics, poses a special problem for analysis since the phthalates can interfere with accurate determination of the target analytes. Never use any plastic material during the handling of the sample extracts.
- 6.8 Any deviations and observations made about the analyses must be documented in the instrument run logbook and or project narrative. If a problem arises during analysis, document the problem and initiate corrective action. If there is a problem with a sample analysis which indicates that re-extraction should be performed, and if there is no additional sample available for re-extraction, the Project Manager needs to be informed immediately so that the client can be involved in the corrective action process. Re-extraction of a sample should be done within holding time whenever possible.

Standard Operating Procedures

C-2d

**WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES
GEL PERMEATION CHROMATOGRAPHY (GPC)**

Gel Permeation Chromatography (GPC)

1. Method Reference

- 1.1. Methods 3640A, and Method 8000B, Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).
- 1.2. NOAA 1993. Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects, 1984-1992. NOAA Technical Memorandum NOS ORCA 71.
- 1.3. "USEPA Contract Laboratory Program Statement of Work for Organics Analysis; Multi-Media, Multi-Concentration" August 1991. Document Number OLM03.1.

2. Scope and Application

- 2.1. This method is recommended for the cleanup of samples from lipids, polymers, proteins, natural resins, cellular components and dispersed high molecular weight compounds.
- 2.2. This method is a size exclusion cleanup using methylene chloride and hydrophobic synthetic gels. In the choice of gels the exclusion range must be larger than the molecular size of the molecules to be separated. A cross-linked divinylbenzene-styrene copolymer is specified for this method.

3. Summary of Method

Samples are extracted using the appropriate extraction protocol for the matrix. The sample is concentrated to a final volume of 4 mL and transferred into a vial. 2mL of the sample are injected into the HPLC systems and 2 mL is maintained for an archive. The sample is carried by a mobile phase of methylene chloride to the calibrated GPC column. A fraction of the sample is collected from the time frame of interest. The solvent before and after the time frame of interest is discarded. The extract is concentrated to the appropriate final volume.

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Approval Signatures		
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Organic Laboratory Manager	<i>Domagala</i>	Date: 3/1/99
Quality Assurance Manager	<i>Edith Hymus</i>	Date: 3/1/99

4. Equipment and Reagents

4.1 Waters 600E Pump System.

4.2 Waters 717plus Autosampler.

4.3 Waters 486 Tunable Absorbance Detector

4.4 Waters Fractionation Collector.

4.5 Spectra-Physics Chrom-Jet Integrator.

4.6 Chromatography Columns

4.6.1 Enviro-Gel 19 x 300 mm GPC Column WAT036554

4.6.2 Enviro-Gel 19 x 150 mm Guard Column WAT036555

4.7 Laboratory Supplies - Syringes, pipettes, autosampler vials, crimper, filter paper, glass filter, glass wool.

4.8 Reagents

4.8.1 Methylene Chloride - HPLC grade or better (Doe & Ingalls).

4.9 Analytical Standards

4.10 GPC Calibration Standard - Prepare a solution of in methylene chloride:

Compound	Concentration (mg/L)
Corn Oil	25,000
bis-(2-Ethylhexyl)phthalate *	1000
Methoxychlor *	200
Perylene *	20
Sulfur	80

* - Optional compound

4.10.1 The Sulfur compound is not very soluble in the methylene chloride. It should be mixed first with warm Corn Oil, then into the overall solution with methylene chloride. The standard should be stored in an amber glass bottle with a Teflon lined screw cap at 4°C. Refrigeration may cause the Corn Oil to precipitate, therefore allow the standard to come to room temperature before use.

5. Procedure

5.1 Calibration procedure - Standard must be in methylene chloride.

5.1.1 Put 4mL of the calibration solution in a sample vial.

5.1.2 The GPC system is setup with a 4 mL/minute flow rate of methylene chloride. When the system is not in use the solvent can be re-cycled into the same container at a lower flow rate to conserve solvent and decrease the stress on the pumping system.

5.1.3 4 mL of the calibration standard is loaded on the autosampler. 2 mL of this standard is injected into the system to act as retention time marker. The order elution of the compounds is: Corn Oil, bis(2-Ethylhexy)phthalate, Methoxychlor, Perylene and Sulfur. The peaks should be resolved to at least 85% and 90% for the Perylene and Sulfur compounds.

5.1.4 The time immediately after the Corn Oil, will be the start collection time for most applications, (Pest/PCBs and Semi-volatile extractables).

5.1.5 The stop time varies. Generally the Pest/PCB stop time is before the Perylene peak. The Semi-volatile extractable stop time is just before the Sulfur peak.

5.1.6 Set the start and stop times in the fractionation collector to match the analytical method being performed.

5.2 Sample Cleanup/Fractionation

5.2.1 Prepare sample extracts for GPC cleanup by concentrating to 4 mL final volume. If the final volume is different, make note of it in the HPLC logbook. Note: extracts must be in methylene chloride. One-half (2 mL) of each extract will be injected onto the GPC column for actual cleanup. The remaining one-half (2mL) split will be labeled, and archived in a sample freezer.

5.2.2 Ensure that the correct start and stop collection times are entered into the fractionation collector for the application being performed.

5.2.3 Run the elution of 100% methylene chloride at 4 ml/min flow rate, collecting sample fractions according to the start and stop times indicated by the calibration standards. Bracket each 10 samples with a GPC standard. If the flow rate is out of this range, corrective action must take place. Changes in pressure, flow rate and room temperature can affect retention times and must be monitored.

5.2.4 Upon completion of the run, evaluate each GPC standard. The retention time of the standards must be within $\pm 5\%$ between calibrations. Only extracts bracketed by acceptable calibration HPLC standards will proceed to analysis. Extracts requiring refractionation will be brought up to 4 mL (a dilution factor of 2, for the archive splits) with 2 mL cleaned.

5.2.5 Cleaned extracts will be concentrated to the intended final volume in preparation for analysis.

5.3 Instrument Setup

5.3.1 Run sequence for GPC analysis:

1. GPC Standard
2. Methylene chloride blank
3. 10 Samples (The method blank should be analyzed before samples, then samples and then spikes).
4. GPC Standard
5. Methylene Chloride Blank
6. 10 samples
7. GPC Standard
8. Methylene Chloride

5.3.2 Autosampler - to run multiple samples press the Auto Key and set to number of desired vials.

5.3.3 Detector:

Turn it on.
Set the wavelength to 254
Allow 15 minutes for the lamp to stabilize
When the system is not in use, shut the lamp off.

5.3.4 Fractionation Collector:

Set the fractionation collection for the GPC with the bottle mode – option #2. See section 5.3.8

5.3.5 Pump Operation -GPC running in Direct Mode (See 600E manual for more information).

The following display will be on the screen for the 600E pump. The bolded parameters must be filled in. Most of the events are controlled through the autosampler, not the pump system. This is why “do not set here” is emphasized.

Autoinjector 0 (Do not set here)	Flow 4ml/min	Current/New
Detector #1 (do not set here)	%A	0
Detector #2 (do not set here)	%B	0
Detector #3 (do not set here)	%C	0
	%D	100

5.3.6 Autoinjector: Up to 48 positions can be loaded

A 2500 ul syringe is used. There must be at least 2.4 mL of sample material in the vial for a 2.0 mL sample to be injected.

On the Stat page, setup for single runs:

Step	From Vial	To Vial	# Injections	Injection Volume	Run Time (min)
1	1	1	1	2000 uL	32

Press start to begin or return to the Main Menu.
Step through each option using the arrow keys.

Autopage: for multiple sample runs – normal operation

Step	From Vial	To Vial	# Injections	Injection Volume	Run Time (min)
1	1	Up to 48	1	2000	32
2	Additional Prog.				

Press start to begin:
When the programming of the samples is complete, the screen will display the # of samples and the end Run Time.

The following screen is displayed:

Status: Auto Run

Vial #	1
Injection	1 of Final Vial # (variable)
Volume (uL)	2000
Runtime (min)	32
Time Remaining (min)	32
Total Run Time (min)	846 (variable)

Auto Page Stat Run Stop run

5.3.7 Detector: Power on and lamp on
The sensitivity of the lamp is set at AUFS – 1.5
The filter of the lamp is set at 0.5

5.3.8 Fractionation collector:

Press C/N – to make any changes or review the current settings.
Select Action Mode #7 (7=Bottle window)
Enter
Select Bottle Arrange #2 (snake mode of collection).
Enter
Set Waiting Time (Bottle Mode)
Wait time 15 min 30 sec (for Alkylated PAH at the time this SOP was written)
Enter
Set window – Window Number 1
Window Starting time – 15 min 31 sec (Alkylated PAH example)
Enter
Set window – Window Number 1
Window Ending time, 27 min 0 sec (Alkylated PAH example)
Enter
Enter
Step to:
Set Fractionation capa (capacity) (bottle mode) request
Time Fractionation 12 Min 30 sec/tube
Enter
Set Conditions #1 (for multi-samples)
Enter
Set Condition (bottle mode)
<peak?> 0=non peak, 1=+ peak Enter 0 for non-peak
Enter
Enter: Hear 2 beeps, Will go back to start

Make sure that the END button is ENTERED. Will ensure the fractionation collector will go to the first bottle.
Collection begins automatically as the autosampler sends a signal to the collector.

Bottle schematic from a top down view: The pattern snakes forth and back.

Back of Fractionation collector as you face it.

Bottle # 1	12	13	24	25	36
2	11	14	23	26	35
3	10	15	22	27	34
4	9	16	21	28	33
5	8	17	20	29	32
6	7	18	19	30	31

Front of Fractionation collector as you face it.

Check to be sure you have the correct vial tray.

6. Method Performance and Interference's

- 6.1 Once weekly, Standards containing the target analytes (Pest/PCB and Semi-volatile) should be run through the GPC system. This is a check for the start and stop times. The recovery for the analytes must be greater than 70%.
- 6.2 The integrity of the chromatography system must be maintained throughout a run sequence. The chromatographer should ensure that all aspects of the system, from injection through data generation are done in a consistent manner across each batch. The GPC standard for the sequence must meet the acceptance criteria of $\pm 5\%$ for retention time shift.
- 6.3 The quality of the reported data is monitored by the Method Blank, Surrogate recoveries, LCS and MS Spike recoveries. Therefore, common sense should always be used in setting up the run batch so that cross-contamination doesn't occur and the characteristics of the entire extraction batch can be evaluated as quickly as possible so that a re-extraction, if needed, can be initiated within holding times. Note that column overload or build up can create active sites that may affect Pentachlorophenol (PCP).
- 6.4 Always run and evaluate the Method Blank first from an analytical batch. If it fails criteria, the entire analytical batch needs to be re-extracted (unless additional sample is available). *If possible, the Method Blank should be deemed acceptable BEFORE any samples in the batch are analyzed on the instrument. Next check the LCS for acceptability. PCP should pass the criteria for the Semi-volatiles before analysis continues.*
- 6.5 Analyze field samples and their associated QC within the same run sequence at the same dilutions. In other words, if a Sample, MS and MSD were prepared, ensure that they all have the same final dilution prior to analysis and run them in sequence. This will avoid comparability problems in the data that might arise if the Samples and QC are run at different times and at different dilutions.
- 6.6 Method interference can be caused by contaminants in standards, reagents, glassware, solvents, and any other reagents or equipment, which come into contact with samples. All of these materials must be demonstrated to be free of contamination prior to routine use through the analysis of Method Blanks (i.e., solvent and standard verification must be performed before these reagents can be used for routine sample analysis).
- 6.7 Never use any glassware or other apparatus with the sample if it doesn't appear clean. Ensure that syringes are properly solvent rinsed between uses.
- 6.8 Never use any glassware, or other implements such as syringes, on multiple samples within the batch unless there has been thorough cleaning and decontamination between samples.

-
- 6.9 Phthalate contamination, from plastics, poses a special problem for analysis since the phthalates can interfere with accurate determination of the analytes of interest. Never use any plastic material during the handling of the sample extracts.
- 6.10 Any deviations and observations made about the analyses must be documented in the instrument run logbook and or project narrative. If a problem arises during analysis, document the problem and initiate corrective action. If there is a problem with a sample analysis which indicates that re-extraction should be performed, and if there is no additional sample available for re-extraction, the Project Manager needs to be informed immediately so that the client can be involved in the corrective action process. Re-extraction of a sample should be done within holding time if at all possible.

Standard Operating Procedures

C-2e

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

METHOD 8082

**POLYCHLORINATED BIPHENYLS (PCBs) AS AROCLORS BY
GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION (GC/ECD)**

METHOD 8082

Polychlorinated Biphenyls (PCBs) as Aroclors By Gas Chromatography/Electron Capture Detection(GC/ECD)

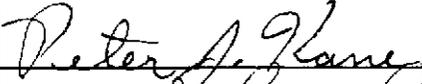
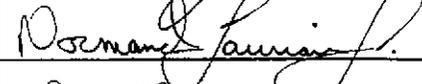
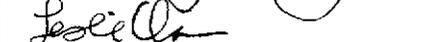
1. Method Reference

- 1.1. Method 8082 and Method 8000B, Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).
- 1.2. "USEPA Contract Laboratory Program Statement of Work for Organics Analysis; Multi-Media, Multi-Concentration" August 1991. Document Number OLM03.1.

2. Scope and Application

- 2.1. This method is applicable to the quantification of PCBs as Aroclors in water, soil, sediment, and sludge samples. The applicable compound list can be found Table 1.
- 2.2. This method is in substantial conformance with the guidelines established in the SW-846 for performing chromatographic analysis as defined in the Method 8000B ("Gas Chromatography"), Method 8082 ("Polychlorinated Biphenyls"), Method 3545 ("Pressurized Fluid Extraction"), Method 3550B ("Sonication Extraction"), Method 3510C ("Separatory Funnel Liquid - Liquid Extraction"), and Method 3520C ("Continuous Liquid - Liquid Extraction"). The data quality objectives of these methods is met or exceeded by this SOP.
- 2.3. The sensitivity of method usually depends on the concentration of interferences rather than on instrumental limitations. If interferences prevent detection of the analytes, Method 8082 may also be performed on samples that have undergone cleanup.
- 2.4. Quantitation of PCBs as Aroclors is appropriate for many regulatory compliance determinations, but is particularly difficult when the Aroclors have been weathered by long exposure in the environment. Therefore, the 8082 method provides procedures for the determination of selected individual PCB congeners and should be used depending on regulatory requirements and project needs. The PCB congener approach potentially affords greater quantitative accuracy when PCBs are known to be present. The congener method is of particular value in determining weathered Aroclors. Caution should be used when using the congener method where regulatory requirements are based on Aroclor concentrations. Please see the 8082 PCB Congeners SOP for laboratory procedures.

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Approval Signatures		
Laboratory Director		Date: 7/15/98
Organic Laboratory Manager		Date: 7/15/98
Quality Assurance Manager		Date: 7/15/98

3. Summary of Method

- 3.1. Aqueous samples are extracted with methylene chloride in a separatory funnel or by continuous liquid/liquid extractors. Soil samples are extracted by sonication in a methylene chloride - acetone mixture or by Dionex Accelerated Solvent Extractor in methylene chloride. Cleanup techniques are applied as necessary. The extract may be treated with Florisil or GPC (for hydrocarbon removal), and/or copper (for sulfur removal), and/or sulfuric acid for the analysis of multicomponent Aroclors. The extract is exchanged into hexane and concentrated to the appropriate volume, usually 10 mL, for analysis. The extract is analyzed on a gas chromatograph which is fitted with two capillary columns of differing polarities each employing separate detectors. The target analytes are resolved on each column and detected using an electron capture detector (ECD). Concentrations are calculated from the ECD response using external standard techniques. Identification of the multiple peak components (Aroclors) are made by comparison to analytical standards.

4.0 Sample Handling and Preservation

- 4.1. Water samples are collected in a pre-cleaned 1-L amber glass bottle with a teflon-lined screw cap. Approximately one liter of sample is required for the analysis. Soil samples are collected in a pre-cleaned 250 mL glass jar with teflon-lined screw cap. Approximately 100 g of soil is required for the analysis although more may be collected to provide a representative sample.
- 4.2. Samples should be preserved by cooling to 4°C. No chemical preservative is required, and would interfere with the analysis.
- 4.3. Water samples should be extracted within seven days of collection. Soil samples should be extracted within 14 days of collection. Sample extracts should be analyzed within 40 days of sample preparation.

5. General Points

- 5.1. Aroclors are multi-component mixtures. When samples contain more than one Aroclor, a higher level of analyst expertise is required to attain acceptable levels of qualitative and quantitative analysis. The same is true of Aroclors that have been subjected to environmental degradation ("weathering") or degradation by treatment technologies. Such weathered multi-component mixtures may have significant differences in peak patterns than those of Aroclor standards. The PCB congener method may be more appropriate for very weathered samples.
- 5.2. The ECD is theoretically a halogen specific detector. However, phthalate esters can be a major source of contamination in that they do respond to the ECD. No forms of plastic or any material containing plasticizers (phthalates) should be used in conjunction with any part of this analysis. These materials can be removed through the use of Method 3665 (sulfuric acid/permanganate cleanup).
- 5.3. Elemental sulfur is readily extracted from soil samples and may cause chromatographic interferences in the determination of PCBs. Sulfur can be removed through the use of Method 3660.
- 5.4. Care must be taken to ensure a complete hexane exchange is performed on the extract, because residual methylene chloride would be an interferent in the analysis.
- 5.5. Oven-drying of glassware used for PCB analysis can increase contamination because PCBs are readily volatilized in the oven and spread to other glassware. Therefore, exercise caution, and do not dry glassware from samples containing high concentrations of PCBs with glassware that may be used for trace analyses, unless glassware has undergone meticulous cleaning.

6. Equipment and Reagents

- 6.1. Glassware - Separatory funnel, 2 L (smaller sizes may sometimes be used) with teflon or glass stopper; Erlenmeyer flask 250 mL; graduated cylinder, 1 L
- 6.2. Liquid Liquid Extractor- 500 mL flat bottom flasks, 1 liter graduated cylinder, heating units.
- 6.3. Sonicator - a Tekmar model TM-600-2 controller equipped with a CV-17 model probe. This unit is capable of a 600 watt output and is operated at a 50% duty cycle.
- 6.4. Dionex ASE 200 Accelerated Solvent Extractor.
- 6.5. Sample Concentrator - Apparatus for reducing prepared sample extract volumes by nitrogen gas (TurboVap 500, Zymark Corporation, Hopkington, MA). Alternate methods for concentration, such as manual introduction of a nitrogen stream or Kuderna-Danish technique, may be utilized in some circumstances
- 6.6. Gas chromatograph - Programmable; heating range from 40°C to 325°C; splitless-type inlet system; electron capture detector (Hewlett-Packard HP 5890 Series II GC or similar).
- 6.7. Chromatography Column - A combination of two of the following.
 - 6.5.1 Restek's proprietary "RTX-CLPesticide 1" Stationary Phase, 30m length x 0.32mm ID, 0.50µm film thickness - Restek Corporation, PN 11139, or equivalent.
 - 6.5.2 Restek's proprietary "RTX-CLPesticide 1" Stationary Phase, 30m length x 0.25mm ID, 0.25µm film thickness - Restek Corporation, PN 11123, or equivalent.
 - 6.5.3 Restek's proprietary "RTX-CLPesticide 2" Stationary Phase, 30m length x 0.32mm ID, 0.25µm film thickness - Restek Corporation, PN 11324, or equivalent.
 - 6.5.4 Restek's proprietary "RTX-CLPesticide 2" Stationary Phase, 30m length x 0.25mm ID, 0.20µm film thickness - Restek Corporation, PN 11323, or equivalent.
 - 6.5.5 Guard column, deactivated fuse silica phenyl-methyl, 5 m x 0.53 mm ID. Restek PN 10045 or equivalent.
 - 6.5.6 The flow from the guard column can be split between the two analytical columns using a press tight Y-splitter . Restek PN 20405 or equivalent.
- 6.6. Data Acquisition System - Computerized system for collecting, storing, and processing detector output (Turbochrom, Perkin-Elmer).
- 6.7. Gases - High purity hydrogen and nitrogen.
- 6.8. Laboratory Supplies - Syringes, pipettes, autosampler vials, crimper, filter paper, glass filter, glass wool
 - 6.8.1 Unilinners for direct injection can be used to decrease the potential for breakdown of compounds in the injector in stead of the standard splitless liners. Uniliner is a product of Restek Corporation, PN 20335.

6.9. Reagents

- 6.9.1 Methylene Chloride - HPLC grade or better. Fisher Scientific is the vendor.
- 6.9.2 Acetone - Optima grade from Fisher Scientific
- 6.9.3 Soil Sample Extraction Solvent - Prepared by mixing acetone and methylene chloride 50:50
- 6.9.4 Sodium sulfate ($\text{Na}_2 \text{SO}_4$) - 10-60 mesh, dried at 400°C for 4 hours. Provided by Fisher scientific at ACS certified purity.
- 6.9.5 Diatomaceous earth - Purified by drying at 400°C for 4 hours, or extraction with methylene chloride to remove interferences.
- 6.9.6 Hexane - Pesticide quality or equivalent, Provided by Fisher Scientific
- 6.9.7 Florisil cartridges provided by J.T. Baker.
- 6.9.8 Copper powder - Copper, fine granular Mallinckrodt 4649 or equivalent.

6.10. Analytical Standards

6.10.1 Surrogate Spiking solution - Two surrogates will be used for this method: 2,4,5,6-tetrachloro-m-xylene(TMx) and decachlorobiphenyl (DCB) (Restek PN 32000-500 at 200 $\mu\text{g}/\text{mL}$ or equivalent. To prepare the spiking solution add one mL of each 200 $\mu\text{g}/\text{mL}$ stock into a 200 mL the flask. Bring to volume to make a 1000 ng/mL solution. 1 mL of this solution is spiked into each sample. The concentration in the final 10 mL extract will be 100 ng/mL .

6.10.2 Matrix spiking solution - is commercially obtained from Restek PN 32035-500 at 2000 $\mu\text{g}/\text{mL}$ and 5000 $\mu\text{g}/\text{mL}$. To prepare the spiking solution add 1 mL of the 2000 $\mu\text{g}/\text{mL}$ solution to a 10 mL volumetric flask and bring it to volume. Perform a 1/1000 dilution on this standard for a 0.2 $\mu\text{g}/\text{mL}$ solution. 1 mL of this solution is spiked into each matrix spike, spike duplicate and blank spike QC sample. The final concentration in a 10 mL extract will be 20 $\mu\text{g}/\text{L}$.

6.10.3 PCB calibration standard solution - commercially available Aroclors are obtained at 100 $\mu\text{g}/\text{mL}$ from Ultra Scientific.

<u>Compound</u>	<u>Part Number</u>
Aroclor 1016	PP-200-1
Aroclor 1260	PP-360-1
Aroclor 1221	PP-290-1
Aroclor 1232	PP-300-1
Aroclor 1242	PP-310-1
Aroclor 1248	PP-340-1
Aroclor 1254	PP-350-1

Aroclors 1016 and 1260 is combined into one standard called AR1660. Individual standards of all other Aroclors are prepared. The following tables describe the initial calibration standard preparation procedure. An intermediate dilution of the surrogate mix is made by diluting 1mL of ampul material with hexane to a 10mL final volume. This yields a concentration of 20 $\mu\text{g}/\text{mL}$. A minimum of 5 levels are analyzed for the curve although more levels may be analyzed.

5-Level Curve Preparation for Aroclors

<u>Calibration Level</u>	<u>Aroclor Conc.</u>	<u>Surrogate Conc.</u>	<u>Volume of Std Added</u>	<u>Volume of Hexane added</u>
Level 1-1*	50	2	0.5mL of Level 3 standard	10 mL
Level 1	100	4	1mL of Level 3 standard	10 mL
Level 2	500	20	1 mL of Level 5 standard	10 mL
Level 3**	1000	40	100 µL of ampul 20 µL of intermed surr mix	10 mL
Level 4	2500	200	250 µL of ampul 50 µL of intermed surr mix	10 mL
Level 4+1*	3500	140	350 µL of ampul 70 µL of intermed surr mix	10 mL
Level 5	5000	200	500 µL of ampul 100 µL of intermed surr mix	10 mL

*optional, additional levels.

** CCV level 3

6.10.4 Florisil Cartridge Check solution - This solution which consists of 2,4,5-trichlorophenol solution which can be obtained from Restek PN 32017-500 at a concentration of 1000 µg/mL. The working solution should be at 0.1 µg/mL or 100 ng/mL in acetone. This can be prepared by adding 10 µL of the stock solution to 100 mL of acetone. (In addition to this solution, the Level 3 calibration standard is also used for Florisil Check.)

7. Procedure

7.1 Water Samples - Separatory Funnel (Method 3510C)

7.1.1 A one liter water sample is measured in a graduated cylinder. This sample is poured into a separatory funnel.

7.1.2 The pH of the sample is determined by dipping a pasture pipette into the separatory funnel and transferring a drop of sample onto a strip of universal pH paper. The pH should be between 5 to 9.

7.1.2.1 If the pH is greater than 9, sulfuric acid solution is added to the sample to reduce the pH to between 5 and 9.

7.1.2.2 If the pH is less than 5, sodium hydroxide solution is added to the sample to increase the pH to between 5 and 9.

7.1.3. Add 50 mL of methylene chloride to the sample.

7.1.4. Add 1 mL of the surrogate spiking solution to the samples, method blanks, matrix spikes, and blank spikes.

7.1.5. Add 1 mL of the matrix spike solution to any matrix spikes or blank spikes that are performed with the analysis.

7.1.6. Seal and shake the separatory funnel vigorously for 1 to 2 minutes with periodic venting of the funnel into a hood. Note: the system should be vented immediately after the initial shaking to avoid pressure build up.

7.1.7. Place funnel in a rack and allow the water and the organic layers to separate for a minimum of 10 minutes after shaking. If an emulsion forms which is greater than one third the size of the organic layer then it must be broken up before continuing. Several procedures can be employed to do this.

7.1.7.1 The emulsion can be broken up using a glass rod in a mixing motion causing the bubbles to break up. The solvent layer is then drained into a flask or TurboVap tube.

7.1.7.2 The organic layer can be drained into a centrifuge cup and spun for several minutes. The non-emulsified organic layer is saved and the emulsion returned to the separatory funnel.

7.1.7.3 The emulsion can be filtered through a funnel with a glass wool plug in it with dried sodium sulfate on that. This is collected into a 250 mL Erlenmeyer flask.

7.1.8. The organic layer in all cases is filtered into a 250 mL Erlenmeyer flask or a TurboVap tube through a glass funnel packed with 20 grams of sodium sulfate in a filter paper cone or on a glass wool plug. The sodium sulfate filtration can be performed for each successive separatory funnel shakes or at the end one time for the entire extract.

7.1.9. Repeat the rinse and shake steps two more times using a fresh portion of 50 mL of methylene chloride each time.

7.1.10. The extract is now ready for concentration.

7.2 Water Samples-Liquid-Liquid Extraction (Method 3520C).

7.2.1. 400 milliliters of methylene chloride is added to the LLE extractor with 500 mL flatbottom flask attached. A water sample is measured in a 1000 mL graduated cylinder, and added to the methylene chloride. Sample volume is recorded in the extraction logbook.

7.2.2. The pH of the sample is determined by dipping a pasture pipette in to the LLE and transferring a drop to pH paper. The pH of the sample (including blanks and QC samples) should now be adjusted to between 5 and 9 by adding sulfuric acid or sodium hydroxide as necessary.

7.2.3. Add one mL of the required surrogate to each sample, blank and QC, and any other required spiking solution.

7.2.4. Turn on all heating units to a setting of four. Turn on cool flow units. Let LLE's extract for 18 to 24 hours.

7.2.5. Turn off heating units, let samples cool. Filter samples through sodium sulfate in a funnel with a glass wool plug. Samples will be filtered into Turbo-Vap tubes, then concentrated as outlined in section 7.5.

7.3. Soil/Sediment Samples - Dionex ASE (Method 3545)

7.3.1. Weigh approximately 20 g of sample into beaker, add sufficient diatomaceous earth until dry, mix well, grind if necessary to obtain a free flowing powder. For method blanks and laboratory control samples (LCS), aliquot approximately 5 g infusorial/diatomaceous earth (use a representative sample weight for determining concentrations in blanks).

7.3.2. Use a prepared cell, cleaned by washing with soap and water between each use, and rinse three times with extraction solvent (methylene chloride) immediately prior to use.

-
- 7.3.3. Close bottom end of cell, insert cellulose filter and use rod to press into place at the bottom of the cell. Make sure filter is properly seated.
- 7.3.4. Transfer entire contents of beaker to a 33mL cleaned stainless steel extraction cell. Record Sample IDs and associated Cell numbers in the ASE logbook. Do not attach labels to the outside of the cell. Labels may cause misalignment in the system or label may be damaged since the entire cell is placed in the oven during extraction.
- 7.3.5. Spike each cell with 1 mL of the surrogate mix and any appropriate matrix/LCS spike.
- 7.3.6. Insert another cellulose filter and use rod to lightly press into place on top of sample. Close the top of the cell. Caps should be handtightened only. Clean rod between each sample.
- 7.3.7. Verify white O-rings are in place and in good condition on the ends of the cell.
- 7.3.8. Place extraction cells into the autosampler tray. Record autosampler position for each sample in the ASE logbook.
- 7.3.9. Load the collection tray with the appropriate number (up to 24) of 60-mL precleaned, capped vials with septa, labeled with corresponding sample IDs. Labels should only be placed from 1 3/8" to 3 1/8" from the top of the closed cap of the vial, to prevent blocking of the sensors on the system.
- 7.3.10. Select correct method on the system keypad and press start.
- 7.3.11. ASE 200 Conditions:
- System Pressure: 14 MPa (2000 psi)
 - Oven Temperature: 100°C
 - Oven heat-up Time: 5 min.
 - Static Time: 5 min.
 - Solvent: Methylene chloride
 - Flush Volume: 60% of extraction cell volume
 - Nitrogen Purge: 1 MPa (150 psi) for 60 seconds
 - Rinse Cycle 8 mL
- 7.3.12. The sample should be transferred to the proper glassware and concentrated as outlined in section 7.5.

7.4. Soil/Sediment Samples - Sonication (Method 3550B)

- 7.4.1. Examine sample and decant any standing water and discard any stick or foreign objects. Weigh a 30 g aliquot of a well mixed sample into a solvent rinsed beaker. A smaller amount may be added if it is known that the sample concentration is high. If the sample contains a water layer on top, decant the water before stirring. Increase sample size if sample is very watery, see Adjusting Sample Size SOP.
- 7.4.2. Add sodium sulfate incrementally to the sample and mix until the sample is "powdery".
- 7.4.3. Add 100 mL of soil sample extraction solvent (acetone and methylene chloride [50:50]) and add one mL of the surrogate spike solution to all samples, blanks, matrix spikes, and matrix spike duplicates.
- 7.4.3.1. One mL of the matrix spiking solution should be added to the matrix spikes and matrix spike duplicates performed with each batch of samples.

7.4.3.2 Place the tip of the sonic disrupter into the sample - 1/2 inch below the solvent layer but above the sediment layer. Note: Rinse the tip with methylene chloride before placing it into the sample to prevent contamination.

7.4.4 Sonicate the sample for 3 minutes at a setting of at least 300 watts on Pulse mode. The analyst should observe very active mixing of the sample and solvent when the ultrasonic pulse is activated.

7.4.5 Transfer the solvent by decanting into a TurboVap tube through a glass funnel packed with glass wool and sodium sulfate for drying the sample.

7.4.6 Repeat the extraction steps twice more with fresh 100 mL portions of the soil extraction solvent.

7.4.7 The extract is now ready for concentration. If particles are present, filter the extract using Whatman No. 41 paper, centrifuge, or autovial prior to concentration.

7.5 Extract Concentration

7.5.1 TurboVap concentration:

- Place the TurboVap tube in the unit at a pressure of 20 PSI and a temperature of 44°C in the sensor mode of operation and reduce the extract to a final volume of 1 mL. (If extract is to be GPC cleaned, concentrate to 10mL, do not hexane exchange, and go to the GPC SOP.)
- Remove the tube from the unit, add 10 to 15 mL of hexane (**the hexane must be squirted into the nipple of the tube to ensure mixing**), and return it to the unit. Evaporate the soil sample extraction solvent to exchange the solvent to hexane and reduce the final volume to 1 mL.
- The extract should be brought up to 10 mL final volume in hexane. The extract may now undergo cleanup if necessary or required. The extract may also be split for separate cleanup procedures if Pesticide analysis is required. ONLY extracts for PCB analysis are to be sulfuric acid cleaned.

7.6 Extract Cleanup - optional

7.6.1 To perform a florisil clean up the lot of florisil cartridges integrity may be verified.

7.6.1.1 Place the 1 gram florisil cartridge into the vacuum manifold and adjust the pressure to 5 to 10 PSI.

7.6.1.2 The cartridge must be conditioned with a hexane/acetone (90:10) solution before use. This is done by passing 5 mL of the solution through the cartridge taking care not to allow the cartridge to go to dryness. Place a 10 mL volumetric flask under the appropriate line to the cartridge.

7.6.1.3 Add 0.5 mL of the 2,4,5-trichlorophenol solution and 0.5 mL of the level 3 calibration standard to 4 mL of hexane. This solution is then reduced to 0.5 mL.

7.6.1.4 The 0.5 mL solution is then placed on top of the cartridge and eluted with the 90:10 hexane/acetone solution. Two additional rinses with one mL of hexane are performed to ensure full removal of the compounds of interest.

7.6.1.5 The solution is reduced to 1.0 mL and analyzed by GC/ECD.

7.6.1.6 The recovery of the pesticides in particular from the pesticide calibration mix should be between 80 to 110 percent. The recovery of the 2,4,5-trichlorophenol should be less than 5%. If these criteria are met the lot of cartridges are deemed acceptable.

7.6.2 Florisil clean up of samples.

7.6.2.1 The set up of the vacuum manifold and conditioning the cartridge is the same as in the cartridge check above, section 7.6.1.1 and 7.6.1.2.

7.6.2.2 One mL of the 10 mL extract is placed at the top of the cartridge. It is eluted with 9 mL of the 90:10 hexane/acetone mixture followed by 2 washes with 1 mL of hexane.

7.6.2.3 This solution is then reduced to 1 mL final volume.

7.6.2.4 If the individual pesticides are to be analyzed then the extract is ready for screening or analysis. If sulfur is suspected the sulfur clean up can be performed.

7.6.3 Sulfur clean up (Method 3660B).

7.6.3.1 Copper must be activated as follows:

- Place approximately 15 - 20 grams of copper powder in the bottom of the 400 mL beaker.
- Slowly add 150 - 200 mL of 8 molar hydrochloric acid. Use a teflon coated spatula to very carefully mix the copper and the acid, taking care not to splash the acid. In this and all other mixing steps, the mixing should occur for at least 1-2 minutes, then allow the copper to settle to the bottom of the beaker.
- Carefully pour off the acid and replace it with 150 - 200 mL of deionized water. Neutralize and dispose of the waste acid properly. Allow the water and copper to mix thoroughly (as described above), settle and then pour off water. Repeat this step several times, it is important that all acid is removed.
- Pour off the water and add 150 - 200 mL of methanol (or acetone). Mix, settle, pour off and repeat. Repeat the solvent rinsing step with methylene chloride, then hexane. Upon the addition of the hexane, the copper should look very finely dispersed, with no clumps or lumps. If there is clumping this means that all of the water has not been removed from the copper. Repeat the solvent rinsing starting with methanol. If the copper is not activated (has all the water removed) completely it will not react with the sulfur properly. During this entire procedure, expose the copper to the air as little as possible. After activation is complete, always keep the copper under hexane. Copper can usually be kept for 24 hours.

7.6.3.2 The sample extract should be in hexane in a screw top vial. Transfer a small amount of activated copper to the vial (approximately .25 grams), with as little hexane as possible. The copper should turn black upon addition to the sample indicating the formation of copper sulfide. Keep adding until the newly added copper retains its red color indicating that all of the sulfur has been complexed. After treating the extract, transfer to another screw top vial using a Pasteur pipette. Be careful not to carry over any copper into the sample.

7.6.4. Sulfuric Acid Cleanup (Method 3665A).

- 7.6.4.5. Carefully add 0.5 mL of concentrated sulfuric acid to 1 mL of the sample extract and shake vigorously. If the sample appears extremely dirty 1 mL of acid may be required. Repeat, if necessary by removing sulfuric acid layer and adding additional acid.
- 7.6.4.6. Allow the extract to settle and remove the top layer (hexane solvent) containing the PCBs from the acid layer and put it into a clean sample vial. The hexane layer should not be colored or have a visible emulsion or cloudiness. It is now ready for analysis.

7.7 Instrument Setup

7.7.1. The instrument used for the analysis is a HP 5890A Series II gas chromatograph or a Perkin-Elmer Autosys. The HP system is equipped with a splitless injector, 7673-type autosampler, and ECD. The instrument is interfaced to the Perkin-Elmer Turbochrom data system for control of the instrument and acquisition of the detector response.

7.7.2. The GC columns used are a combination of two differing phase columns listed in the equipment section. The helium flow or hydrogen is at 10 mL/min.

7.7.3. The basic GC parameters are as follows:

Injector A Temp	220°C
Detector Temp	320°C (PE) or 300°C (HP)
Hold. Time	0.1 minutes
Oven Temp	180°C
Initial Value	180°C
Initial Time	1 minute
Rate	10°/minute to 200°C
Rate	4°/minute to 280°C
Final Value	300°C
Final Time	0 minutes
Purge/Valve A <u>ON</u>	0.6 minutes
Run Time	20.0 minutes

7.7.4. Establish daily retention time windows as the retention time of the component in the opening verification standard \pm .05, .07, or .10 minutes as listed in Table 2. Retention time windows are calculated each time a new GC column is installed. The windows given in Table 2, established by the Contract Laboratory Program based on analysis of real-life environmental samples, are compared with the laboratory calculated windows.

7.8 Initial Calibration

7.8.1 The method of external standard calibration is used. The response of each individual peak and the total response of the PCB in the sample is compared to a calibration curve to determine the analyte concentration in the sample. The calibration curves are generated during initial calibration through the analysis of standards which define the working range of the method.

7.8.2 The following analytical sequence order should be followed for initial calibration of the system. The primer is used at the beginning of the analysis sequence to ensure all sites of activation in the system have been covered or deactivated prior to performing trace level analysis. The sequence below is a general sequence for determination of PCBs as Aroclors and uses a minimum of 5 levels for AR1660 and a single-point initial calibration for the remaining Aroclors near the midpoint of the expected calibration range.

7.8.2.1 For projects where only a few Aroclors are of interest, the analyst may employ a five point initial calibration for each of the Aroclors of interest (i.e. 5 standards for AR1232), and not use the AR1660 mixture or the single point calibration of the other Aroclors.

1. Primer - (20 times more concentrated than Level 3)
2. Hexane
3. AR1660 Level 1
4. AR1660 Level 2
5. AR1660 Level 3
6. AR1660 Level 4
7. AR1660 Level 5
8. AR1221 -1000 ng/mL
9. AR1232 -1000 ng/mL
10. AR1242 -1000 ng/mL
11. AR1248 -1000 ng/mL
12. AR1254 -1000 ng/mL
13. Hexane

7.8.3. Inject 1 μ L of each standard into the GC operated in the splitless mode for trace analyses. Evaluate the standard chromatograms for all compounds contained within the calibration standards.

7.8.4. Using the GC system software (Turbochrom), the analyst must choose 3-5 peaks from the pattern which are characteristic of the Aroclor and are the least subject to degradation and weathering to obtain the response for the component of interest. Choose peaks that are at least 25% of the height of the largest Aroclor peak, the set of 3 to 5 peaks should include at least one peak that is unique to that Aroclor. Use at least 5 peaks for the AR1660, none of which should be found in both. Tabulate the peak area for each analyte against the mass injected of the analyte in the standard analyzed to obtain a calibration curve for each analyte across the working range of the method.

7.8.5. Calibration factors are determined for individual peaks: Choose 3 to 5 peaks in the standard to calculate a calibration factor for that peak, using the total mass of Aroclor in the standard. These calibration factors are then used to calculate the concentration of each corresponding peak in the sample chromatogram and the 3 to 5 resulting concentrations are averaged to provide the final result for the sample.

7.8.6. Once all the components are identified, a linear calibration (average calibration factor) is calculated for the components. The criteria for evaluation are as follows:

7.8.7. For all compounds, %RSD must be less than 20%. If one or more peaks have %RSD greater than 20%, the calibration may still be acceptable if the average %RSD for all analytes in the calibration curve is less than 20%.

*Alternatively, a linear regression model (first order) may be employed, provided that the coefficient of determination (COD or r^2) is ≥ 0.99 . Otherwise, construct a nonlinear calibration of no more than a third order equation. Statistical considerations in developing a non-linear calibration model require more data than the more traditional linear approach. A quadratic (second order) model requires six standards, and a third order polynomial requires seven standards. In setting model parameters, do not force the line through the origin. The COD or r^2 must be ≥ 0.99 . The analyst should select the regression order which introduces the least calibration error into the quantitation.

*See also point 7.10.4.

- 7.8.8. If the curve does not meet criteria, the analyst should check the calculation of the standard preparation that was performed. If the problem appears to be isolated to a single calibration standard, that standard may be reanalyzed and the %RSD may be recalculated. If chromatographic problems are indicated by the standard chromatograms already analyzed, then the injection port should be serviced. This consists of cutting 6 to 12 inches off of the injector end of the column, changing the liner, glass wool and the septa. The detector may be cleaned by raising it to 380°C for the HP GC or 425°C for the Autosys GC for 4 hours or overnight. This may be done with the column removed from the detector and the detector base capped. The make-up gas must be on when the detector is heated. If this does not resolve the problem the column should be replaced and curves re-analyzed.
- 7.8.9. Reference standards (ARI660 mixture) from a separate source or different lot are analyzed after every initial calibration for evaluation against calibration standard solutions. %Difference should not be greater than 25%.

7.9. Continuing Calibration

- 7.9.1. The calibration must be verified with the analysis of the CCV (Level 3 standard) each day at the beginning of the run and each twelve hour shift, or once every 20 samples, whichever is more frequent. However, it is recommended that the CCV is run every ten samples to minimize the number of samples requiring reanalysis. The general daily calibration sequence is as follows:

1. Primer - (20 times more concentrated than Level 3)
2. Hexane
3. ARI660 - CCV
4. 10 samples
5. ARI660 - CCV
6. 10 samples
7. ARI660 - CCV

- 7.9.2. Establish daily retention time windows as the retention time of the component in the opening verification standard \pm window from Table 1.
- 7.9.3. Calculate the %Difference (%Diff) for each analyte in the verification standard relative to the initial calibration curves. If the %Diff $\leq \pm 15\%$, sample analysis may take place following this verification standard. If the %Diff $> \pm 15\%$, the CCV may still be acceptable if ALL components (regardless of whether they have been targeted for a specific project) had an average %D of $\leq \pm 15\%$. In this case CCV data or a detailed narrative must be provided to the client. Otherwise, corrective action must take place. See the calculations section for the %Difference calculation.

7.9.3.1 Corrective Action: If the verification is the beginning standard of the sequence, corrective action may consist of prepping the injection port (replace injection liner, clip column and replace septa) and re-analysis of the standard. If, after re-analyzing the standards, the acceptance criteria are still not met, a new initial calibration must be performed. The system must be in-control with all calibration criteria before sample analysis may proceed.

7.9.3.2 Corrective Action: If the CCV is a closing CCV, the data for the samples run before this CCV may be evaluated for hits. If there are no hits for the compounds that did not meet %D criteria, those samples do not need to be reanalyzed provided that the analyte had increased and would have been detected in the sample if it were present.

7.9.3.3 Corrective Action: The CCV may be reanalyzed once, if %D criteria is still not met, then a new initial calibration must be analyzed following any necessary instrument maintenance. Samples associated with the unacceptable CCV should be reanalyzed.

- 7.9.4. The analyst judgment from continuous daily use of the instrument is important in determining the validity of a continuing calibration. Even if the %Diff criteria are met, the peak shape may have deteriorated to a point where maintenance is required and a new initial calibration is in order. Both columns should meet QC criteria, however, they may be evaluated with the above corrective actions in mind.
- 7.9.5. Confirmation may not be necessary if the composition of the sample matrix is well established by prior analysis, for instance, when a PCB is known to be produced or used in a facility is found in a sample from that facility.

7.10 Sample Analysis

- 7.10.1. Analyze extracts using the same experimental conditions used for the analysis of the calibration standards. Ensure that calibration verification standards are interspersed every 20 samples or every 12 hour period. Area measurements should all be to baseline unless an unresolved complex mixture (UCM) is observed, in which case, the areas measured should skim the top of the UCM.
- 7.10.2. Qualitative identification of multicomponent analytes requires pattern matching between the calibration standards and the response observed in the sample on both columns. Retention time windows should be used as a gauge; however, pattern recognition for the multicomponent analytes is most important.
- 7.10.3. For samples with PCBs positively identified on both columns, compare the responses of the 3 to 5 major peaks in the single point calibration standard for that Aroclor with the peaks observed in the sample extract. The relative peaks and number of peaks in the sample should be similar to that observed in the standard; however, degradation, weathering and interferences may cause the sample pattern to differ from that observed from the standard. The peaks chosen for quantitation must be free from interferences. Calculate the concentration of each corresponding peak in the sample chromatogram and the 3 to 5 resulting concentrations are averaged to provide the final result for the sample.
- 7.10.4. If 5 levels of the AR1660 mixture was used to describe the linear range of the instrument, the single point calibration standard must follow the same calibration model. Also, because one can only perform a linear (point to point) calibration for a single point calibration, then the AR1660 curve must also use a point to point calibration.
- 7.10.5. Calculate the relative percent difference (RPD) between compound concentrations on both columns using the calculation in section 8.7. If the calculated RPD is >40%, check the chromatograms to see if an overlapping peak is causing an erroneously high result. If no overlapping peaks are noted, examine baseline parameters established by the data system (or operator) during peak integration.
- *If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, report the higher result. This approach is conservative relative to protection of the environment. The disparity between the two columns should be noted in the case narrative.
 - *If the high RPD is clearly the result of an interferent on one of the columns, the lower result may be reported.
- 7.10.6. Calculate the recovery for the surrogates TMX and DCB in each field, blank, and quality control sample extract on both columns. Calculate the recovery of the matrix spike and LCS compounds in the appropriate samples on both columns.

7.10.7. Evaluate whether or not a dilution of an extract is needed by ensuring that all target PCBs areas measured fall within the calibration range of the instrument. If the range is exceeded, a dilution analysis is required. Dilute the extract to bring the out-of-range component(s) to within 50-100% of the full calibration range. Also evaluate the sample chromatogram for possible saturation of the detector.

7.10.7.1 **Using micro-syringes to prepare dilutions:** Accuracy and precision are extremely important in extract dilutions; therefore, precision syringes are used. The accuracy range for most syringes is 20-80% of their total marked range (e.g., a 100 μL syringe should only be used for aliquots of 20-80 μL). The size of syringe chosen should ensure that only one volume measurement be made to complete the dilution (e.g., if 750 μL of solvent is needed, a 500 μL syringe should not be used since two volume measurements would have to be done; instead, a 1000 μL syringe should be used).

7.10.7.2 There is a volume associated with every micro-syringe needle, the size of which depends upon the gauge and length of the needle. The error associated with not accounting for the volume of liquid within the needle depends on the size of the syringe being used and whether or not the syringe has been pre-calibrated to account for this volume (i.e., the manufacturer indicates that the volume scale is calibrated to account for the volume contained within the needle). For example, if a 10 μL syringe is used with a needle volume of 2 μL , if the standard is drawn into the syringe up to the 5 μL mark and used, 7 μL of standard are actually delivered in the dilution (40% error in what the dilution should have been and what was prepared). If instead a 1000 μL syringe is used with this same size needle, and 500 μL are drawn up, actually 502 μL (0.4% error) is delivered which will have minimal impact on the accuracy of the dilution.

7.10.7.3 Unless the manufacturer has warranted that the syringe volume has been accounted for in the volume scale on the syringe, always measure the volume required using a meniscus-to-meniscus measurement of the liquid within the syringe (see 7.8.6.4). Determine the needle volume by drawing solvent up to a specific volume on the syringe, then remove the needle from the solvent, and draw the liquid up into the syringe barrel from the needle - measure the new volume from the first meniscus to the second meniscus of the liquid in the syringe. The difference between the first and second volume measurement is the volume from the syringe needle. This test should be repeated several times to ensure that the needle volume is accurately known.

7.10.7.4 Measure all liquids using the meniscus-to-meniscus technique. Prior to inserting the syringe into the extract, draw up a small amount of air (>needle volume) into the syringe. Place the syringe into the extract and draw up the liquid so that the measured volume is the volume required less the volume in the needle (e.g., to measure 10 μL in a 25 μL syringe with a needle volume of 2 μL , 8 μL should be drawn up into the barrel). Remove the syringe from the sample and draw up a small amount of air to force the needle volume into the barrel. Measure the volume from the first meniscus to the second meniscus of the liquid in the syringe. This will give an accurate measurement of the volume of liquid. An added advantage of this technique is that it will ensure that the full volume of liquid is delivered since the air bubble adjacent to the actual syringe plunger, will force all of the liquid out of the needle. Without an air bubble, it's possible that the syringe will deliver all the liquid except the volume contained within the needle.

7.10.7.5 Always add the sample extract to the dilution solvent by injecting the extract directly below the surface of the solvent with a single injection - never draw the solution back up into the syringe to "rinse" the syringe contents into the dilution sample since the syringes are designed to deliver the volume measured without this rinse - if a rinse is done, the dilution will not be accurate.

7.10.8 If a dilution analysis is made, compare this run to the initial analysis to ensure that the chromatography is similar, that compound detection is similar, and that the reported values for detected compounds make sense (i.e., that the final value for any compound detected in both analyses is about the same value). If the dilution run is not comparable to the initial run, corrective action should take place.

7.10.8.1 **Corrective Action:** Re-evaluate both analyses ensuring that integration of all peaks was performed properly and that the correct calibration curves were used for quantitation. Determine whether or not the results are off by a systematic bias which might be indicative of a poor injection or of an inaccurate dilution. If the initial run's injection is determined to have been poor and if this analysis does not need to be reported to the client (see 7.8.7.2), then the results of the dilution analysis only should be reported to the client (unless there are special project requirements to report data at specific reporting limits). If an inaccurate dilution is suspected, a new dilution of the extract should be made and this second dilution analyzed. Ensure that the dilution is done accurately using the techniques described in Section 7.8.6. The results from all three runs should be compared to verify the corrective action and to determine which result should be reported to the client.

7.10.8.2 Reporting of the results for a sample where two different dilution analyses are conducted, depends upon the results of the two runs. If an initial analysis detects a compound that is non-detected in the dilution run, and if the initial result was reported at less than or equal to twice the reporting limit for that compound, only the second analysis should be reported. If however, the initial run has a compound at a concentration greater than twice the reporting limit but it is non-detected in the dilution analysis, the data for both runs should be reported to the client. In this case, a single data sheet should be prepared for reporting the sample with all components reported from the initial run except for the over-range component, which should be reported from the dilution run with a flag indicating that this was the case. The project narrative must accurately explain how the data are reported.

8. Calculations

8.1. Calibration Factor:

$$\text{Calibration Factor (CF)} = \frac{\text{Area of Peak}}{\text{mass injected (ng)}}$$

8.2 % Difference:

$$\% \text{Difference} = (R_{\text{True}} - R_{\text{Predicted}}) / R_{\text{True}} \times 100$$

where: R_{True} = True mass injected of the component within the standard
 $R_{\text{Predicted}}$ = Predicted mass of analyte using initial calibration curve.

8.3. Aqueous Sample Concentration:

$$\text{Concentration of Analyte } (\mu\text{g/L}) = \frac{R_{\text{Predicted}} \times B \times D}{V}$$

8.4. Soil Sample Concentration::

$$\text{Concentration of Analyte } (\mu\text{g/Kg-dry wt.}) = \frac{R_{\text{Predicted}} \times B \times D}{G \times S}$$

where: $R_{\text{Predicted}}$ = μg of Analyte as determined from the calibration curve.

B = final volume (mL) of the extract, usually 10 mL

D = extract dilution factor, if used. If there is no dilution, $D=1$.

V = initial volume of sample (mL) extracted.

G = weight of sample (grams as received) extracted.

S = % solids as a decimal fraction

8.5. Report all results to two (2) significant figures.

8.6. Relative percent difference:

$$\text{RPD} = \frac{|R1 - R2|}{\frac{[R1 + R2]}{2}} \times 100$$

where: R1 = Sample results on column 1, or MS concentration.

R2 = Sample results of column 2., or MSD concentration.

9. Quality Control

9.1 The analytical batch, which was begun in extraction, consists of up to 20 field samples, a method blank, an LCS, a Matrix Spike (MS) and a Duplicate (Dup) or Matrix Spike Duplicate (MSD). All efforts should be made to run the ETR within a single run sequence to save time and effort in project review.

9.2 **Method Blank** - A method blank must be extracted with every batch of samples. The analysis of the method blank extract will demonstrate the background contamination of the apparatus and procedures in the laboratory. The method blank must not contain any analytes of interest at concentrations greater than the reporting limit for the method. *If the method blank is contaminated, as demonstrated by the instrument analysis, the entire analytical batch (all samples and QC) must be re-extracted since there is uncertainty in the results for the batch for the contaminant found.* Re-extraction must be initiated immediately so that the minimum of time is wasted before re-extraction can occur - if at all possible, this re-extraction should take place within holding time. *If it is not possible to re-extract samples due to sample volume problems, the project manager and client need to be contacted immediately to determine if reporting the data as qualified results, with the contaminated method blank, is acceptable.*

9.3 **Surrogate Recoveries** - Surrogate recoveries are monitored to evaluate how well the extraction and analytical system worked for the sample specific matrix. Every sample, blank, and quality control sample (LCS, MS, MSD, Dup) must be spiked with 1.0 mL of the surrogates prior to extraction. The recoveries of the surrogates are determined during instrument analysis and compared to laboratory generated QC limits established through quality control charting. The current QC acceptance limits for the surrogates are given in Table 2. *If the recoveries are not within quality control criteria, the sample must be re-extracted and re-analyzed.* If the second extraction results in the surrogates still being recovered outside of criteria, a matrix effect may be occurring whereby the sample itself is causing interference to recovery of the surrogate(s). If the second extraction results in surrogate recoveries within criteria, then the initial extraction was not in control and the second extraction data should be reported. *All efforts should be made to perform the re-extraction within holding time so that the second analysis is valid and meets all quality control criteria.*

9.4 **Laboratory Control Sample (LCS)** - A Laboratory Control Sample (LCS) is extracted and analyzed with each batch of samples. The laboratory generated acceptance criteria for the spike components is shown in Table 3. If the LCS does not meet quality control criteria for recovery of some of the spike and surrogate compounds, the results for the other samples and quality control samples within the batch must be evaluated to determine if this is an isolated problem for the LCS and whether the data should be reported with the

affected LCS. *If the recovery for all components in the LCS are outside of criteria, the entire analytical batch should be re-extracted and re-analyzed.*

- 9.5 **Matrix spikes** - For every analytical batch, a matrix spike/matrix spike duplicate (MS/MSD) pair should be performed. If specifically requested by the Client, a MS/MSD will be logged in the ETR and must be performed on those samples. If an MS/MSD was not logged in, the extraction analyst should select a sample for spiking. This should be done per 20 samples extracted for this test. The matrix spike is prepared by taking an aliquot of sample and spiking it with 1.0 mL each of the surrogate and matrix spiking solutions. The laboratory generated acceptance criteria for the spike components is shown in Table 3. The recovery of the matrix spikes are evaluated relative to the what was in the unspiked sample to indicate how well the methods worked on extraction of the analytes of interest from the sample matrix. Re-extraction of the sample based on the MS results is rare. If the recovery for the spikes are outside of criteria (Table 3) and if the precision between the MS and MSD is poor, this indicates that there were probably analytical problems during sample extraction; however, this may also indicate a matrix effect. The results for the unspiked and MS/MSD samples need to be compared carefully to determine how well the spiking study worked and if there was any indication of matrix-related problems that might affect the analytical batch.
- 9.6 **Florisil Cartridge Check Sample** - This sample is generated during the Florisil Cleanup if used on the samples and is used to monitor the efficiency of the cleanup technique on the analytes of interest. Table 4 gives the acceptance criteria for this check standard. The florisil check must be prepared and analyzed with each new batch of florisil received by the laboratory to demonstrate that the columns and techniques are appropriate. *Samples should not undergo cleanup unless a valid florisil check has been prepared and analyzed.* If a florisil check is analyzed and the criteria for acceptance is not met, any samples which were processed with, or after, this check standard will need to be re-extracted. Use of the florisil cleanup should only proceed once an acceptable check sample has been obtained.
- 9.7 **Duplicates** - Duplicates are used to evaluate the precision of the method. A laboratory duplicate is two separate aliquots of the same field sample taken through extraction and analysis. Field duplicates are two different samples received by the laboratory; however, these samples should have been collected at the same time using the same techniques. Comparison of the results from a laboratory duplicate analysis, will indicate the precision from extraction through analysis. Evaluation of the field duplicates however, can indicate the precision of the overall process from sampling through analysis and may also give an indication about representativeness of the samples analyzed to the sampling location.
- 10.0 Method Performance and Interferences**
- 10.1. **Method Detection Limit (MDL)** studies must be performed annually or more frequently if significant changes occur to the extraction procedure or instrumentation used for analysis. Method specific MDLs are prepared using the procedure described in 40 CFR Part 136, Appendix B.
- 10.2. The integrity of the chromatography system must be maintained throughout a run sequence. The chromatographer should ensure that all aspects of the system, from injection through data generation is done in a consistent manner across each batch.
- 10.3. The quality of the reported data is monitored by the Method Blank, surrogate recoveries, LCS and MS spike recoveries as described in Section 9. Therefore, common sense should always be used in setting up the run batch so that cross-contamination doesn't occur and the characteristics of the entire extraction batch can be evaluated as quickly as possible so that a re-extraction, if needed, can be done within holding times.
- 10.4. Evaluation of a sample result often requires a knowledge about the results of all of the other samples within an ETR. Therefore, use all of the data, if possible, from an ETR to judge whether or not corrective actions are needed. For example, if a sample is run and the surrogates are low, one might request a re-extraction immediately only to find that a MS/MSD were also done on this sample, with low surrogate and MS

recoveries, confirming a matrix effect. In this case, a re-extraction is not required and the results would be reported with a narration explaining the evidence of a matrix effect on the low surrogate recoveries. The characteristics of the overall ETR may be invaluable in deciding how to report the data to the client.

- 10.5. Always run and evaluate the method blank first from an analytical batch since if it fails criteria, the entire analytical batch needs to be re-extracted (unless additional sample isn't available). *If possible, the Method Blank should be deemed acceptable BEFORE any samples in the batch are analyzed on the instrument.*
- 10.6. Analyze cleanup check standards, if used, immediately following the MB to verify that the column cleanup method was acceptable. If the florisil check fails criteria, many samples may need re-extraction. Once again, the faster a re-extraction is initiated, the better the chance for that re-extraction to meet the sample holding time.
- 10.7. Analyze field samples and their associated QC within the same run sequence at the same dilutions. In other words, if a sample, MS and MSD were prepared, ensure that they all have the same final dilution prior to analysis and run them in sequence. This will avoid comparability problems in the data that might arise if the samples and QC are run at different times and at different dilutions.
- 10.8. Method interferences can be caused by contaminants in standards, reagents, glassware, solvents, and any other reagents or equipment which come into contact with samples. All of these materials must be demonstrated to be free of contamination prior to routine use through the analysis of method blanks (e.g., solvent and standard verification must be performed before these reagents can be used for routine sample analysis).
- 10.9. Never use any glassware or other apparatus with the sample if it doesn't appear clean. Ensure that syringes are properly solvent rinsed between uses.
- 10.10. Ensure that the aldol condensation products (4-methyl-4-hydroxy-2-pentanone and 4-methyl-3-pentene-2-one) which can arise during sonication using methylene chloride:acetone, are not interfering with detection of the analytes of interest.
- 10.11. Never use any glassware, or other implements such as syringes, on multiple samples within the batch unless there has been thorough cleaning and decontamination between samples.
- 10.12. Phthalate contamination, from plastics, poses a special problem for analysis since the phthalates can interfere with accurate determination of the analytes of interest. Never use any plastic material during the handling of the sample extracts.
- 10.13. Any deviations and observations made about the analyses must be documented in the instrument logbook and or project narrative. If a problem arises during analysis, document the problem and initiate corrective action. If there is a problem with a sample analysis which indicates that re-extraction should be performed, and if there is no additional sample available for re-extraction, the Project Manager needs to be informed immediately so that the client can be involved in the corrective action process. Re-extraction of a sample should be done within holding time if at all possible.

Compound	CAS Registry No
Aroclor 1016	12674-11-2
Aroclor 1221	11104-28-2
Aroclor 1232	11141-16-5
Aroclor 1242	53469-21-9
Aroclor 1248	12672-29-6
Aroclor 1254	11097-69-1
Aroclor 1260	11096-82-5

Compound	Retention Time window (minutes)
Aroclor individual peaks	± 0.07
Tetrachloro-m-xylene (TMX)*	± 0.05
Decachlorobiphenyl (DCB)*	± 0.10

* surrogates

*Limits adopted from CLP OLM03.1

Establish daily retention time windows as the retention time of the component in the opening verification standard ± .05, .07, or .10 minutes as listed in Table 2. Retention time windows are calculated each time a new GC column is installed. The windows given in Table 2, established by the Contract Laboratory Program based on analysis of real-life environmental samples, are compared with the laboratory calculated windows (section 7.4.4).

Surrogate	Aqueous (% recovery)	Soil (% recovery)
2,4,5,6-tetrachloro-m-xylene(TMX)	30-150	30-150
Decachlorobiphenyl (DCB)	30-150	30-150

Spiked Component	Aqueous		Soil	
	(% recovery)	RPD	(% recovery)	RPD
AR1016	70-130	50	70-130	50
AR1260	70-130	50	70-130	50

*Limits adopted from CLP OLM03.1

**Advisory limits from Method 8000.

Note: The above limits will be replaced by laboratory limits generated by QC charting, when available.

Standard Operating Procedures

C-2f

WOODS HOLE GROUP ENVIRONMENTAL LABORATORIES

**METHOD 8081A
ORGANOCHLORINE PESTICIDES BY
GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION (GC/ECD)**

METHOD 8081A

Organochlorine Pesticides By Gas Chromatography/Electron Capture Detection(GC/ECD)

1. Method Reference

- 1.1. Method 8081A and Method 8000B, Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).
- 1.2. "USEPA Contract Laboratory Program Statement of Work for Organics Analysis; Multi-Media, Multi-Concentration" August 1991. Document Number OLM03.1.

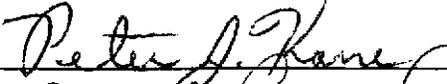
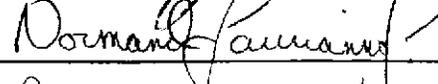
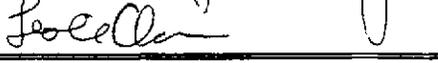
2. Scope and Application

- 2.3. This method is applicable to the quantification of organochlorine pesticides in water, soil, sediment, and sludge samples.
- 2.4. This method is in substantial conformance with the guidelines established in the SW-846 for performing chromatographic analysis as defined in the Method 8000B ("Gas Chromatography"), Method 8081 ("Organochlorine Pesticides"), Method 3545 ("Pressurized Fluid Extraction") Method 3550B ("Sonication Extraction"), Method 3510C ("Separatory Funnel Liquid - Liquid Extraction"), and Method 3520C ("Continuous Liquid - Liquid Extraction"). The data quality objectives of these methods is met or exceeded by this SOP.
- 2.3. The sensitivity of method usually depends on the concentration of interferences rather than on instrumental limitations. If interferences prevent detection of the analytes, Method 8081A may also be performed on samples that have undergone cleanup.

3. Summary of Method

- 3.1. Aqueous samples are extracted with methylene chloride in a separatory funnel or by continuous liquid/liquid extractors. Soil samples are extracted by sonication in a methylene chloride - acetone mixture or by Dionex Accelerated Solvent Extractor. Cleanup techniques are applied as necessary. The extract may be treated with Florisil or GPC (for hydrocarbon removal), and/or copper (for sulfur removal) for the analysis of the individual or multicomponent organochlorine pesticides. The extract is exchanged into hexane and concentrated to the appropriate volume, usually 10 mL, for analysis. The extract is analyzed on a gas chromatograph which is fitted with two capillary columns of differing polarities each employing separate detectors. The target analytes are resolved on each column and detected using an electron capture detector (ECD). Concentrations are calculated from the ECD response using external standard techniques. Identification of the multiple peak components (i.e., toxaphene and chlordane) are made by comparison to analytical standards.

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	Approval Signatures	
Laboratory Director		Date: 7/15/98
Organic Laboratory Manager		Date: 7/15/98
Quality Assurance Manager		Date: 7/14/98

4. Sample Handling and Preservation

- 4.1. Water samples are collected in a pre-cleaned 1-L amber glass bottle with a teflon-lined screw cap. Approximately one liter of sample is required for the analysis. Soil samples are collected in a pre-cleaned 250 mL glass jar with teflon-lined screw cap. Approximately 100 g of soil is required for the analysis although more may be collected to provide a representative sample.
- 4.2. Samples should be preserved by cooling to 4°C. No chemical preservative is required, and would interfere with the analysis.
- 4.3. Water samples should be extracted within seven days of collection. Soil samples should be extracted within 14 days of collection. Sample extracts should be analyzed within 40 days of sample preparation.

5. General Points

- 5.1. The ECD is theoretically a halogen specific detector. However, phthalate esters can be a major source of contamination in that they do respond to the ECD. No forms of plastic or any material containing plasticizers (phthalates) should be used in conjunction with any part of this analysis.
- 5.2. Elemental sulfur is readily extracted from soil samples and may cause chromatographic interferences in the determination of pesticides. Sulfur can be removed through the use of Method 3660.
- 5.3. Care must be taken to ensure a complete hexane exchange is performed on the extract, because residual methylene chloride would be an interferent in the analysis.
- 5.4. The injector system of the gas chromatograph can become contaminated with non-volatile components from prepared samples. This can act to decrease the resolution and responsiveness of the system. The analysis of a DDT/Endrin breakdown standard is the best indicator of the condition of the injector system and the condition of the column. As the percent breakdown increases gradually the system should undergo general maintenance (liner changes, injection port cleaning, etc.) to avoid prolonged down time.

6. Equipment and Reagents

- 6.1. Glassware - Separatory funnel, 2 L (smaller sizes may sometimes be used) with teflon or glass stopper; Erlenmeyer flask 250 mL; graduated cylinder, 1 L
- 6.2. Sonicator - a Tekmar model TM-600-2 controller equipped with a CV-17 model probe. This unit is capable of a 600 watt output and is operated at a 50% duty cycle.
- 6.3. Dionex ASE 200 Accelerated Solvent Extractor.
- 6.4. Sample Concentrator - Apparatus for reducing prepared sample extract volumes by nitrogen gas (TurboVap 500, Zymark Corporation, Hopkington, MA). Alternate methods for concentration, such as manual introduction of a nitrogen stream or Kuderna-Danish technique, may be utilized in some circumstances
- 6.5. Gas chromatograph - Programmable; heating range from 40°C to 325°C; splitless-type inlet system; electron capture detector (Hewlett-Packard HP 5890 Series II GC or similar).
- 6.6. Chromatography Column - A combination of two of the following.
 - 6.6.1 Restek's proprietary "RTX-CLPesticide 1" Stationary Phase, 30m length x 0.32mm ID, 0.50µm film thickness - Restek Corporation, PN 11139, or equivalent.

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- 6.6.2 Restek's proprietary "RTX-CLPesticide 1" Stationary Phase, 30m length x 0.25mm ID, 0.25µm film thickness - Restek Corporation, PN 11123, or equivalent.
 - 6.6.3 Restek's proprietary "RTX-CLPesticide 2" Stationary Phase, 30m length x 0.32mm ID, 0.25µm film thickness - Restek Corporation, PN 11324, or equivalent.
 - 6.6.4 Restek's proprietary "RTX-CLPesticide 2" Stationary Phase, 30m length x 0.25mm ID, 0.20µm film thickness - Restek Corporation, PN 11323, or equivalent.
 - 6.6.5 Guard column, deactivated fused silica phenyl-methyl, 5 m x 0.53 mm ID. Restek PN 10045 or equivalent.
 - 6.6.6 The flow from the guard column can be split between the two analytical columns using a press tight Y-splitter. Restek PN 20405 or equivalent.
 - 6.7. Data Acquisition System - Computerized system for collecting, storing, and processing detector output (Turbochrom, Perkin-Elmer).
 - 6.8. Gases - High purity hydrogen and nitrogen.
 - 6.9. Laboratory Supplies - Syringes, pipettes, autosampler vials, crimper, filter paper, glass filter, glass wool
 - 6.9.1 Unilinners for direct injection can be used to decrease the potential for breakdown of compounds in the injector in stead of the standard splitless liners. Uniliner is a product of Restek Corporation, PN 20335.
 - 6.10. Reagents
 - 6.10.1 Methylene Chloride - HPLC grade or better. Fisher Scientific is the vendor.
 - 6.10.2 Acetone - Optima grade from Fisher Scientific
 - 6.10.3 Soil Sample Extraction Solvent - Prepared by mixing acetone and methylene chloride 50:50
 - 6.10.4 Sodium sulfate ($\text{Na}_2 \text{SO}_4$) - 10-60 mesh, dried at 400°C for 4 hours. Provided by Fisher scientific at ACS certified purity.
 - 6.10.5 Diatomaceous earth - Purified by drying at 400°C for 4 hours, or extraction with methylene chloride to remove interferences.
 - 6.10.6 Hexane - Pesticide quality or equivalent, Provided by Fisher Scientific
 - 6.10.7 Florisil cartridges provided by J.T. Baker.
 - 6.11. Analytical Standards
 - 6.11.1 Surrogate Spiking solution - Two surrogates will be used for this method: 2,4,5,6-tetrachloro-m-xylene(TMx) and decachlorobiphenyl (DCB) (Restek PN 32000-500 at 200 µg/mL or equivalent. To prepare the spiking solution add one mL of each 200 µg/mL stock into a 200 mL the flask. Bring to volume to make a 1000 ng/mL solution. 1 mL of this solution is spiked into each sample. The concentration in the final 10 mL extract will be 100 ng/mL.

6.11.2 Matrix spiking solution - is commercially obtained from Restek PN 32035-500 at 2000 µg/mL and 5000 µg/mL. To prepare the spiking solution add 1 mL of the 2000 µg/mL solution to a 10 mL volumetric flask and bring it to volume. Perform a 1/1000 dilution on this standard for a 0.2 µg/mL solution. 1 mL of this solution is spiked into each matrix spike, spike duplicate and blank spike QC sample. The final concentration in a 10 mL extract will be 20 µg/L.

6.11.3 Standards can be obtained in pre-made commercially available solutions from a variety of vendors. Restek Pest Mix AB #2 PN 32292 (does not contain surrogates) - compounds are at varied concentrations (8 µg/mL, 16 µg/mL and 80 µg/mL). The following tables provide the concentration and volumes added to prepare the calibration standards. The stock standard is prepared by adding 1mL of the Pest AB Mix #2, and 0.08mL of the Surrogate ampul mix, and bringing to a 10mL final volume in hexane.

5-7 Level Curve Preparation for Individual Components

<u>Calibration Level</u>	<u>Volume of Stock Std Added</u>	<u>Volume of Hexane added</u>
Level 1	15 µL	10 mL final volume
Level 2	30 µL	10 mL final volume
Level 3	62.5 µL	10 mL final volume
Level 4	125 µL	10 mL final volume
Level 5*	250 µL	10 mL final volume
Level 6	500 µL	10 mL final volume
Level 7	1000 µL	10 mL final volume

A minimum of 5 levels are required, however up to 7 may be analyzed. Standards chosen should bracket the linear range of the instrument, and establish method quantitation limits (sample reporting limits).

*Level 5 is also the CCV level.

Concentration (µg/L or ppb) of Individual Components in the 5 Levels

<u>Component</u>	<u>Level 1</u>	<u>Level 2</u>	<u>Level 3</u>	<u>Level 4</u>	<u>Level 5</u>	<u>Level 6</u>	<u>Level 7</u>
TMX *	2.4	4.8	10	20	40	80	160
Aldrin	1.2	2.4	5	10	20	40	80
alpha-BHC	1.2	2.4	5	10	20	40	80
gamma-BHC(Lindane)	1.2	2.4	5	10	20	40	80
Heptachlor	1.2	2.4	5	10	20	40	80
beta-BHC	1.2	2.4	5	10	20	40	80
Endosulfan II	2.4	4.8	10	20	40	80	160
delta-BHC	1.2	2.4	5	10	20	40	80
4,4'-DDD	2.4	4.8	10	20	40	80	160
Heptachlor epoxide	1.2	2.4	5	10	20	40	80
Endosulfan I	1.2	2.4	5	10	20	40	80
gamma-Chlordane	1.2	2.4	5	10	20	40	80
alpha-Chlordane	1.2	2.4	5	10	20	40	80
4,4'-DDE	2.4	4.8	10	20	40	80	160
Dieldrin	2.4	4.8	10	20	40	80	160
Endrin	2.4	4.8	10	20	40	80	160
4,4'-DDT	2.4	4.8	10	20	40	80	160
Endrin Aldehyde	2.4	4.8	10	20	40	80	160
Endosulfan Sulfate	2.4	4.8	10	20	40	80	160
Methoxychlor	12	24	50	100	200	400	800
Endrin Ketone	2.4	4.8	10	20	40	80	160
DCB *	2.4	4.8	10	20	40	80	160

* surrogate

6.11.4 Toxaphene Calibration solution - Toxaphene can be obtained from Restek , PN 32005-500, at 1000 µg/mL. A single point calibration is performed at a concentration of 1,000 ng/mL for Toxaphene and 40 ng/mL for the surrogates. This is done by adding 100 µL of the 100 µg/mL stock solution to 10 mL of hexane with 20 µL of the intermediate surrogate standard solution. The intermediate surrogate solution is made by diluting 1mL of the surrogate ampul (200 µg/mL) to 10mL in hexane.

6.11.5 Chlordane Calibration solution - Chlordane can be obtained from Restek ,PN 32021-55, at 1000 µg/mL. A single point calibration is performed at a concentration of 1,000 ng/mL for Toxaphene and 40 ng/mL for the surrogates. This is done by adding 100 µL of the 100 µg/mL stock solution to 10 mL of hexane with 20 µL of the intermediate surrogate standard solution. The intermediate surrogate solution is made by diluting 1mL of the surrogate ampul (200 µg/mL) to 10mL in hexane.

6.11.6 Florisil Cartridge Check solution - This solution which consists of 2,4,5-trichlorophenol solution which can be obtained from Restek PN 32017-500 at a concentration of 1000 µg/mL. The working solution should be at 0.1 µg/mL or 100 ng/mL in acetone. This can be prepared by adding 10 µL of the stock solution to 100 mL of acetone. (In addition to this solution, the Level 5 calibration standard is also used for Florisil Check.)

6.11.7 Breakdown Mix - This solution can be obtained from Restek PN 32032 at 100 µg/mL. It is composed of gamma-BHC, alpha-BHC, 4,4'-DDT, beta-BHC, Endrin, Methoxychlor, TCX, and DCB. A 100 ng/mL solution can be made by adding 100 mL of the stock solution to 100 mL of hexane.

7. Procedure

7.1 Water Samples - Separatory Funnel (Method 3510C)

7.1.1 A one liter water sample is measured in a graduated cylinder. This sample is poured into a separatory funnel.

7.1.2 The pH of the sample is determined by dipping a pasture pipette into the separatory funnel and transferring a drop of sample onto a strip of universal pH paper. The pH should be between 5 to 9.

7.1.2.1 If the pH is greater than 9, sulfuric acid solution is added to the sample to reduce the pH to between 5 and 9.

7.1.2.2 If the pH is less than 5, sodium hydroxide solution is added to the sample to increase the pH to between 5 and 9.

7.1.3. Add 50 mL of methylene chloride to the sample.

7.1.4. Add 1 mL of the surrogate spiking solution to the samples, method blanks, matrix spikes, and blank spikes.

7.1.5. Add 1 mL of the matrix spike solution to any matrix spikes or blank spikes that are performed with the analysis.

7.1.6. Seal and shake the separatory funnel vigorously for 1 to 2 minutes with periodic venting of the funnel into a hood. Note: the system should be vented immediately after the initial shaking to avoid pressure build up.

7.1.7. Place funnel in a rack and allow the water and the organic layers to separate for a minimum of 10 minutes after shaking. If an emulsion forms which is greater than one third the size of the organic layer then it must be broken up before continuing. Several procedures can be employed to do this.

7.1.7.1 The emulsion can be broken up using a glass rod in a mixing motion causing the bubbles to break up. The solvent layer is then drained into a flask or TurboVap tube.

7.1.7.2 The organic layer can be drained into a centrifuge cup and spun for several minutes. The non-emulsified organic layer is saved and the emulsion returned to the separatory funnel.

7.1.7.3 The emulsion can be filtered through a funnel with a glass wool plug in it with dried sodium sulfate on that. This is collected into a 250 mL Erlenmeyer flask.

7.1.8. The organic layer in all cases is filtered into a 250 mL Erlenmeyer flask or a TurboVap tube through a glass funnel packed with 20 grams of sodium sulfate in a filter paper cone or on a glass wool plug. The sodium sulfate filtration can be performed for each successive separatory funnel shakes or at the end one time for the entire extract.

7.1.9. Repeat the rinse and shake steps two more times using a fresh portion of 50 mL of methylene chloride each time.

7.1.10. The extract is now ready for concentration.

7.2 Water Samples-Liquid-Liquid Extraction (Method 3520C).

7.2.1. 400 milliliters of methylene chloride is added to the LLE extractor with 500 mL flatbottom flask attached. A water sample is measured in a 1000 mL graduated cylinder, and added to the methylene chloride. Sample volume is recorded in the extraction logbook.

7.2.2. The pH of the sample is determined by dipping a pasture pipette in to the LLE and transferring a drop to pH paper. The pH of the sample (including blanks and QC samples) should now be adjusted to between 5 and 9 by adding sulfuric acid or sodium hydroxide as necessary.

7.2.3. Add one mL of the required surrogate to each sample, blank and QC, and any other required spiking solution.

7.2.4. Turn on all heating units to a setting of four. Turn on cool flow units. The drip rate of solvent should be 5-10 mL per minute. An experienced analyst should demonstrate this rate to the less experienced. Let LLE's extract for 18 to 24 hours.

7.2.5. Turn off heating units, let samples cool. Filter samples through sodium sulfate in a funnel with a glass wool plug. Samples will be filtered into Turbo-Vap tubes, then concentrated as outlined in section 7.5.

7.3. Soil/Sediment Samples - Dionex ASE (Method 3545)

7.3.1. Weigh approximately 20 g of sample into beaker, add sufficient diatomaceous earth until dry, mix well, grind if necessary to obtain a free flowing powder. For method blanks and laboratory control samples (LCS), aliquot approximately 5 g infusorial/diatomaceous earth (use a representative sample weight for determining concentrations in blanks).

7.3.2. Use a prepared cell, cleaned by washing with soap and water between each use, and rinse three times with extraction solvent (methylene chloride) immediately prior to use.

- 7.3.3. Close bottom end of cell, insert cellulose filter and use rod to press into place at the bottom of the cell. Make sure filter is properly seated.
- 7.3.4. Transfer entire contents of beaker to a 33mL cleaned stainless steel extraction cell. Record Sample IDs and associated Cell numbers in the ASE logbook. Do not attach labels to the outside of the cell. Labels may cause misalignment in the system or label may be damaged since the entire cell is placed in the oven during extraction.
- 7.3.5. Spike each cell with 1 mL of the surrogate mix and any appropriate matrix/LCS spike.
- 7.3.6. Insert another cellulose filter and use rod to lightly press into place on top of sample. Close the top of the cell. Caps should be handtightened only. Clean rod between each sample.
- 7.3.7. Verify white O-rings are in place and in good condition on the ends of the cell.
- 7.3.8. Place extraction cells into the autosampler tray. Record autosampler position for each sample in the ASE logbook.
- 7.3.9. Load the collection tray with the appropriate number (up to 24) of 60-mL precleaned, capped vials with septa, labeled with corresponding sample IDs. Labels should only be placed from 1 3/8" to 3 1/8" from the top of the closed cap of the vial, to prevent blocking of the sensors on the system.
- 7.3.10. Select correct method on the system keypad and press start.
- 7.3.11. ASE 200 Conditions:
- System Pressure: 14 MPa (2000 psi)
 - Oven Temperature: 100°C
 - Oven heat-up Time: 5 min.
 - Static Time: 5 min.
 - Solvent: Methylene chloride
 - Flush Volume: 60% of extraction cell volume
 - Nitrogen Purge: 1 MPa (150 psi) for 60 seconds
 - Rinse Cycle 8 mL
- 7.3.12. The sample should be transferred to the proper glassware and concentrated as outlined in section 7.5.

7.4. Soil/Sediment Samples - Sonication (Method 3550B)

- 7.4.1. Examine sample and decant any standing water and discard any stick or foreign objects. Weigh a 30 g aliquot of a well mixed sample into a solvent rinsed beaker. A smaller amount may be added if it is known that the sample concentration is high. If the sample contains a water layer on top, decant the water before stirring. Increase sample size if sample is very watery, see Adjusting Sample Size SOP.
- 7.4.2. Add sodium sulfate incrementally to the sample and mix until the sample is "powdery".
- 7.4.3. Add 100 mL of soil sample extraction solvent (acetone and methylene chloride [50:50]) and add one mL of the surrogate spike solution to all samples, blanks, matrix spikes, and matrix spike duplicates.
- 7.4.3.1. One mL of the matrix spiking solution should be added to the matrix spikes and matrix spike duplicates performed with each batch of samples.

7.4.3.2 Place the tip of the sonic disrupter into the sample - 1/2 inch below the solvent layer but above the sediment layer. Note: Rinse the tip with methylene chloride before placing it into the sample to prevent contamination.

7.4.4 Sonicate the sample for 3 minutes at a setting of at least 300 watts on Pulse mode. The analyst should observe very active mixing of the sample and solvent when the ultrasonic pulse is activated.

7.4.5 Transfer the solvent by decanting into a TurboVap tube through a glass funnel packed with glass wool and sodium sulfate for drying the sample.

7.4.6 Repeat the extraction steps twice more with fresh 100 mL portions of the soil extraction solvent.

7.4.7 The extract is now ready for concentration. If particles are present, filter the extract using Whatman No. 41 paper, centrifuge, or autovial prior to concentration.

7.5 Extract Concentration

7.5.1 TurboVap concentration:

- Place the TurboVap tube in the unit at a pressure of 20 PSI and a temperature of 44°C in the sensor mode of operation and reduce the extract to a final volume of 1 mL. (If extract is to be GPC cleaned, concentrate to 10mL, do not hexane exchange, and go to the GPC SOP.)
- Remove the tube from the unit, add 10 to 15 mL of hexane (the hexane must be squirted into the nipple of the tube to ensure mixing), and return it to the unit. Evaporate the soil sample extraction solvent to exchange the solvent to hexane and reduce the final volume to 1 mL.
- The extract should be brought up to 10 mL final volume in hexane. The extract may now undergo cleanup if necessary or required. The extract may also be split for separate cleanup procedures if PCB analysis is required. ONLY extracts for PCB analysis are to be sulfuric acid cleaned (Go to 8082 SOP).

7.6 Extract Cleanup - optional

7.6.1 To perform a florisil clean up the lot of florisil cartridges integrity may be verified.

7.6.1.1 Place the 1 gram florisil cartridge into the vacuum manifold and adjust the pressure to 5 to 10 PSI.

7.6.1.2 The cartridge must be conditioned with a hexane/acetone (90:10) solution before use. This is done by passing 5 mL of the solution through the cartridge taking care not to allow the cartridge to go to dryness. Place a 10 mL volumetric flask under the appropriate line to the cartridge.

7.6.1.3 Add 0.5 mL of the 2,4,5-trichlorophenol solution and 0.5 mL of the level 5 calibration standard to 4 mL of hexane. This solution is then reduced to 0.5 mL.

7.6.1.4 The 0.5 mL solution is then placed on top of the cartridge and eluted with the 90:10 hexane/acetone solution. Two additional rinses with one mL of hexane are performed to ensure full removal of the compounds of interest.

7.6.1.5 The solution is reduced to 1.0 mL and analyzed by GC/ECD.

7.6.1.6 The recovery of the pesticides in particular from the pesticide calibration mix should be between 80 to 110 percent. The recovery of the 2,4,5-trichlorophenol should be less than 5%. If these criteria are met the lot of cartridges are deemed acceptable.

7.6.2 Florisil clean up of samples (Method 3620B)

7.6.2.1 The set up of the vacuum manifold and conditioning the cartridge is the same as in the cartridge check above, section 7.6.1.1 and 7.6.1.2.

7.6.2.2 One mL of the 10 mL extract is placed at the top of the cartridge. It is eluted with 9 mL of the 90:10 hexane/acetone mixture followed by 2 washes with 1 mL of hexane.

7.6.2.3 This solution is then reduced to 1 mL final volume.

7.6.2.4 If the individual pesticides are to be analyzed then the extract is ready for screening or analysis. If sulfur is suspected the sulfur clean up can be performed.

7.6.3 Sulfur clean up (Method 3660B).

7.6.3.1 Place approximately 15 - 20 grams of copper powder in the bottom of the 400 mL beaker.

7.6.3.2 Slowly add 150 - 200 mL of 8 molar hydrochloric acid. Use a teflon coated spatula to very carefully mix the copper and the acid, taking care not to splash the acid. In this and all other mixing steps, the mixing should occur for at least 1-2 minutes, then allow the copper to settle to the bottom of the beaker.

7.6.3.3 Carefully pour off the acid and replace it with 150 - 200 mL of deionized water. Neutralize and dispose of the waste acid properly. Allow the water and copper to mix thoroughly (as described above), settle and then pour off water. Repeat these step one more time.

7.6.3.4 Pour off the water and add 150 - 200 mL of methanol. Mix, settle, pour off and repeat. Repeat the solvent rinsing step with methylene chloride, then hexane. Upon the addition of the hexane, the copper should look very finely dispersed, with no clumps or lumps. If there is clumping this means that all of the water has not been removed from the copper. Repeat the solvent rinsing starting with methanol. If the copper is not activated (has all the water removed) completely it will not react with the sulfur properly. During this entire procedure, expose the copper to the air as little as possible. After activation is complete, always keep the copper under hexane. Copper can usually be kept for 24 hours.

7.6.3.5 The sample extract should be in hexane in a screw top vial. Transfer a small amount of activated copper to the vial (approximately .25 grams), with as little hexane as possible. The copper should turn black upon addition to the sample indicating the formation of copper sulfide. Keep adding until the newly added copper retains its red color indicating that all of the sulfur has been complexed. After treating the extract, transfer to another screw top vial using a Pasteur pipette. Be careful not to carry over any copper into the sample.

7.7 Instrument Setup

7.7.1. The instrument used for the analysis is a HP 5890A Series II gas chromatograph or a Perkin-Elmer Autosys. The HP system is equipped with a splitless injector, 7673-type autosampler, and ECD. The instrument is interfaced to the Perkin-Elmer Turbochrom data system for control of the instrument and acquisition of the detector response.

7.7.2. The GC columns used are a combination of two differing phase columns listed in the equipment section. The hydrogen flow is at 10 mL/min.

7.7.3. The basic GC parameters are as follows:

Injector A Temp	220°C
Detector Temp	375°C (PE) or 325°C (HP)
Hold. Time	0.1 minutes
Oven Temp	180°C
Initial Value	180°C
Initial Time	1 minutes
Rate	10°/minute to 200°C
2nd Rate	4°/minute to 280°C
Final Value	300°C
Final Time	0 minutes
Purge/Valve A <u>ON</u>	0.6 minutes
Run Time	20.0 minutes

7.7.4. Establish daily retention time windows as the retention time of the component in the opening verification standard $\pm .05$, $.07$, or $.10$ minutes as listed in Table 1. Retention time windows are calculated each time a new GC column is installed. For multicomponent pesticides, a single peak within the chromatographic pattern should be chosen as the retention time marker. The windows given in Table 1, established by the Contract Laboratory Program based on analysis of real-life environmental samples, are compared with the laboratory calculated windows.

7.8 Initial Calibration

7.8.1 The method of external standard calibration is used. The response of an analyte in the sample is compared to a calibration curve to determine the analyte concentration in the sample. The calibration curves are generated during initial calibration through the analysis of standards which define the working range of the method.

7.8.2 The following analytical sequence order should be followed for initial calibration of the system. The primer is used at the beginning of the analysis sequence to ensure all sites of activation in the system have been covered or deactivated prior to performing trace level analysis. Analysis of two separate mixtures (Individual A and Individual B Mixes) are not required with the GC columns used currently, since all components are resolved on both channels. The sequence below is a general sequence for determination of the analytes by Method 8081A and uses a single-point initial calibration for Toxaphene and Chlordane near the midpoint of the expected calibration range to obtain general retention time and response characteristics (pattern recognition) for the multicomponent analytes. A minimum of 5 levels are analyzed for the single component pesticides.

1. Primer - (20 times more concentrated than Level 5)
2. Hexane
3. Breakdown Mixture
4. Hexane
5. Level 1
6. Level 2
7. Level 3
8. Level 4
9. Level 5
10. Level 6
11. Level 7
12. Chlordane -1000 ng/mL
13. Toxaphene -1000 ng/mL

- 7.8.3. Inject 1 μ L of each standard into the GC operated in the splitless mode for trace analyses. Evaluate the standard chromatograms for all compounds contained within the calibration standards.
- 7.8.4. Using the GC system software (Turbochrom), tabulate the peak area for each analyte against the mass injected of the analyte in the standard analyzed to obtain a calibration curve for each analyte across the working range of the method. For multicomponent pesticides, the analyst must choose 4-6 peaks from the pattern which are characteristic of the component and are the least subject to degradation and weathering to obtain the response for the component of interest.
- 7.8.5. Once all the components are identified, a linear calibration (average calibration factor) is calculated for the components. The criteria for evaluation are as follows:
- 7.8.6. For all compounds, %RSD must be less than 20%. If one or more analytes have %RSD greater than 20%, the calibration may still be acceptable if the average %RSD for all analytes in the calibration curve is less than 20%.
- *Alternatively, a linear regression model (first order) may be employed, provided that the coefficient of determination (COD or r^2) is ≥ 0.99 . Otherwise, construct a nonlinear calibration of no more than a third order equation. Statistical considerations in developing a non-linear calibration model require more data than the more traditional linear approach. A quadratic (second order) model requires six standards, and a third order polynomial requires seven standards. In setting model parameters, do not force the line through the origin. The COD or r^2 must be ≥ 0.99 . The analyst should select the regression order which introduces the least calibration error into the quantitation.
- 7.8.7. If the curve does not meet criteria, the analyst should check the calculation of the standard preparation that was performed. If the problem appears to be isolated to a single calibration standard, that standard may be reanalyzed and the %RSD may be recalculated. If chromatographic problems are indicated by the standard chromatograms already analyzed, then the injection port should be serviced. This consists of cutting 6 to 12 inches off of the injector end of the column, changing the liner, glass wool and the septa. The detector may be cleaned by raising it to 380°C for the HP GC or 425°C for the Autosys GC for 4 hours or overnight. This may be done with the column removed from the detector and the detector base capped. The make-up gas must be on when the detector is heated. If this does not resolve the problem the column should be replaced and curves re-analyzed.
- 7.8.8. Evaluate the percent degradation of 4,4'-DDT (to 4,4'-DDE and 4,4'-DDD) and endrin (to endrin aldehyde and endrin ketone) to monitor the integrity of the injection system (see section 8.3 for calculation). Degradation is not considered to be a problem if the percent degradation of 4,4'-DDT and endrin are less than 15%. If either compound does exceed 15% breakdown, the analysis must be stopped, the injection port serviced and any other maintenance may need to be performed. Subsequently, if breakdown is less than 15% and CCVs meet criteria, and a new initial calibration need not be performed. Otherwise, a new initial calibration may need to be performed.
- 7.8.9. Reference standards from a separate source or different lot are analyzed after every initial calibration for evaluation against calibration standard solutions. %Difference should not be greater than 25%.

7.9. Continuing Calibration

- 7.9.1. The calibration must be verified with the analysis of the CCV (Level 5 standard) each day at the beginning of the run and each twelve hour shift, or once every 20 samples, whichever is more frequent. However, it is recommended that the CCV is run every ten samples to minimize the number of samples requiring reanalysis. In addition, the breakdown mixture must be analyzed daily and every 12 hour

shift to verify that degradation in the system, relative to DDT and Endrin, is not excessive. The general daily calibration sequence is as follows:

1. Primer - (20 times more concentrated than Level 5)
2. Hexane
3. Breakdown Mixture
4. CCV
5. 10 samples
7. CCV
8. 10 samples
9. CCV
10. Breakdown Mixture
11. 10 samples
12. CCV
13. 10 samples
14. CCV
15. Breakdown Mixture
16. 10 samples
17. CCV
18. 10 samples
19. CCV

7.9.2. Evaluate the Breakdown Mixture standard using the same criteria given in section 7.6.7.

7.9.3. Establish daily retention time windows as the retention time of the component in the opening verification standard \pm window from Table 1.

7.9.4. Calculate the %Difference (%Diff) for each analyte in the verification standard relative to the initial calibration curves. If the $\%Diff \leq \pm 15\%$, sample analysis may take place following this verification standard. If the $\%Diff > \pm 15\%$, the CCV may still be acceptable if ALL components (regardless of whether they have been targeted for a specific project) had an average %D of $\leq \pm 15\%$. In this case CCV data or a detailed narrative must be provided to the client. Otherwise, corrective action must take place. See the calculations section for the %Difference calculation.

7.9.4.1 Corrective Action: If the verification is the beginning standard of the sequence, corrective action may consist of prepping the injection port (replace injection liner, clip column and replace septa) and re-analysis of the standard. If, after re-analyzing the standards, the acceptance criteria are still not met, a new initial calibration must be performed. The system must be in-control with all calibration criteria before sample analysis may proceed.

7.9.4.2 Corrective Action: If the CCV is a closing CCV, the data for the samples run before this CCV may be evaluated for hits. If there are no hits for the compounds that did not meet %D criteria, those samples do not need to be reanalyzed provided that the analyte had increased and would have been detected in the sample if it were present.

7.9.4.3 Corrective Action: The CCV may be reanalyzed once, if %D criteria is still not met, then a new initial calibration must be analyzed following any necessary instrument maintenance. Samples associated with the unacceptable CCV should be reanalyzed.

7.9.5. The analyst judgment from continuous daily use of the instrument is important in determining the validity of a continuing calibration. Even if the %Diff criteria are met, the peak shape may have deteriorated to a point where maintenance is required and a new initial calibration is in order. Alternatively, the continuing calibration may indicate that criteria is met for all analytes on one

column but not on the other. In this instance, the column which met criteria may be used for quantitation with the second column used only for qualitative confirmation of the results of the first column. If analysis is done using a single column for quantitation because of a calibration issue on the second column, the narrative to the customer must explain the calibration issues fully.

- 7.9.6. Confirmation may not be necessary if the composition of the sample matrix is well established by prior analysis, for instance, when a pesticide is known to be produced or used in a facility is found in a sample from that facility.

7.10 Sample Analysis

- 7.10.1. Analyze extracts using the same experimental conditions used for the analysis of the calibration standards. Ensure that calibration verification standards are interspersed every 20 samples or every 12 hour period. Area measurements should all be to baseline unless an unresolved complex mixture (UCM) is observed, in which case, the areas measured should skim the top of the UCM.
- 7.10.2. Qualitative identification for pesticides is made when a peak in a sample is observed within the retention time window for a calibrated analyte on both columns. In other words, an analyte is not considered present in a sample unless the analyte is detected on both columns within the retention time windows for that analyte established during calibration.
- 7.10.3. Qualitative identification of multicomponent pesticides requires pattern matching between the calibration standards and the response observed in the sample on both columns. Retention time windows should be used as a gauge; however, pattern recognition for the multicomponent analytes is most important.
- 7.10.4. For samples with toxaphene or chlordane positively identified on both columns, inject an analytical standard at a concentration estimated to be similar to the sample amount, and reinject the sample. Calculate the calibration factors for the standard to quantitate sample concentrations on both columns. Quantitation may be performed using the total area of the pattern, or using 4 to 6 major peaks.

7.10.4.1 In quantitating Toxaphene, the total area should be measured by constructing the baseline between the retention times of the first and last eluting Toxaphene components in the standard. In order to use the total area approach, the pattern in the sample chromatogram must be compared to that of the standard to ensure that all of the major components in the standard are present in the sample. Otherwise, the sample concentration may be significantly underestimated.

7.10.4.2 Toxaphene may also be quantitated on the basis of 4 to 6 major peaks. This approach may avoid difficulties when interferences with Toxaphene peaks are present in the early portion of the chromatogram from compounds such as DDT. The relative peaks and number of peaks in the sample should be similar to that observed in the standard; however, degradation, weathering and interferences may cause the sample pattern to differ from that observed from the standard. The peaks chosen for quantitation must be free from interferences.

The heights or areas of the 4 to 6 peaks should be summed together and used to determine the Toxaphene concentration.

Alternatively, use each peak in the standard to calculate a calibration factor for that peak, using the total mass of Toxaphene in the standard. These calibration factors are then used to calculate the concentration of each corresponding peak in the sample chromatogram and the 4 to 6 resulting concentrations are averaged to provide the final result for the sample.

7.10.5. Calculate the relative percent difference (RPD) between compound concentrations on both columns using the calculation in section 8.7. If the calculated RPD is >40%, check the chromatograms to see if an overlapping peak is causing an erroneously high result. If no overlapping peaks are noted, examine baseline parameters established by the data system (or operator) during peak integration.

- *If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, report the higher result. This approach is conservative relative to protection of the environment. The disparity between the two columns should be noted in the case narrative.
- *If the high RPD is clearly the result of an interferent on one of the columns, the lower result may be reported.

7.10.6. Calculate the recovery for the surrogates TMX and DCB in each field, blank, and quality control sample extract on both columns. Calculate the recovery of the matrix spike and LCS compounds in the appropriate samples on both columns.

7.10.7. Evaluate whether or not a dilution of an extract is needed by ensuring that all target Pesticides areas measured fall within the calibration range of the instrument. If the range is exceeded, a dilution analysis is required. Dilute the extract to bring the out-of-range component(s) to within 50-100% of the full calibration range. For toxaphene or chlordane, evaluate the sample chromatogram for possible saturation of the detector. Historical data has shown toxaphene and chlordane to be linear to 5000ng/mL.

7.10.7.1 **Using micro-syringes to prepare dilutions:** Accuracy and precision are extremely important in extract dilutions; therefore, precision syringes are used. The accuracy range for most syringes is 20-80% of their total marked range (e.g., a 100 μ L syringe should only be used for aliquots of 20-80 μ L). The size of syringe chosen should ensure that only one volume measurement be made to complete the dilution (e.g., if 750 μ L of solvent is needed, a 500 μ L syringe should not be used since two volume measurements would have to be done; instead, a 1000 μ L syringe should be used).

7.10.7.2 There is a volume associated with every micro-syringe needle, the size of which depends upon the gauge and length of the needle. The error associated with not accounting for the volume of liquid within the needle depends on the size of the syringe being used and whether or not the syringe has been pre-calibrated to account for this volume (i.e., the manufacturer indicates that the volume scale is calibrated to account for the volume contained within the needle). For example, if a 10 μ L syringe is used with a needle volume of 2 μ L, if the standard is drawn into the syringe up to the 5 μ L mark and used, 7 μ L of standard are actually delivered in the dilution (40% error in what the dilution should have been and what was prepared). If instead a 1000 μ L syringe is used with this same size needle, and 500 μ L are drawn up, actually 502 μ L (0.4% error) is delivered which will have minimal impact on the accuracy of the dilution.

7.10.7.3 Unless the manufacturer has warranted that the syringe volume has been accounted for in the volume scale on the syringe, always measure the volume required using a meniscus-to-meniscus measurement of the liquid within the syringe (see 7.8.6.4). Determine the needle volume by drawing solvent up to a specific volume on the syringe, then remove the needle from the solvent, and draw the liquid up into the syringe barrel from the needle - measure the new volume from the first meniscus to the second meniscus of the liquid in the syringe. The difference between the first and second volume measurement is the volume from the syringe needle. This test should be repeated several times to ensure that the needle volume is accurately known.

7.10.7.4 Measure all liquids using the meniscus-to-meniscus technique. Prior to inserting the syringe into the extract, draw up a small amount of air (>needle volume) into the syringe. Place the syringe into the extract and draw up the liquid so that the measured volume is the volume required less the volume in the needle (e.g., to measure 10 μL in a 25 μL syringe with a needle volume of 2 μL , 8 μL should be drawn up into the barrel). Remove the syringe from the sample and draw up a small amount of air to force the needle volume into the barrel. Measure the volume from the first meniscus to the second meniscus of the liquid in the syringe. This will give an accurate measurement of the volume of liquid. An added advantage of this technique is that it will ensure that the full volume of liquid is delivered since the air bubble adjacent to the actual syringe plunger, will force all of the liquid out of the needle. Without an air bubble, it's possible that the syringe will deliver all the liquid except the volume contained within the needle.

7.10.7.5 Always add the sample extract to the dilution solvent by injecting the extract directly below the surface of the solvent with a single injection - never draw the solution back up into the syringe to "rinse" the syringe contents into the dilution sample since the syringes are designed to deliver the volume measured without this rinse - if a rinse is done, the dilution will not be accurate.

7.10.8 If a dilution analysis is made, compare this run to the initial analysis to ensure that the chromatography is similar, that compound detection is similar, and that the reported values for detected compounds make sense (i.e., that the final value for any compound detected in both analyses is about the same value). If the dilution run is not comparable to the initial run, corrective action should take place.

7.10.8.1 **Corrective Action:** Re-evaluate both analyses ensuring that integration of all peaks was performed properly and that the correct calibration curves were used for quantitation. Determine whether or not the results are off by a systematic bias which might be indicative of a poor injection or of an inaccurate dilution. If the initial run's injection is determined to have been poor and if this analysis does not need to be reported to the client (see 7.8.7.2), then the results of the dilution analysis only should be reported to the client (unless there are special project requirements to report data at specific reporting limits). If an inaccurate dilution is suspected, a new dilution of the extract should be made and this second dilution analyzed. Ensure that the dilution is done accurately using the techniques described in Section 7.8.6. The results from all three runs should be compared to verify the corrective action and to determine which result should be reported to the client.

7.10.8.2 Reporting of the results for a sample where two different dilution analyses are conducted, depends upon the results of the two runs. If an initial analysis detects a compound that is non-detected in the dilution run, and if the initial result was reported at less than or equal to twice the reporting limit for that compound, only the second analysis should be reported. If however, the initial run has a compound at a concentration greater than twice the reporting limit but it is non-detected in the dilution analysis, the data for both runs should be reported to the client. In this case, a single data sheet should be prepared for reporting the sample with all components reported from the initial run except for the over-range component, which should be reported from the dilution run with a flag indicating that this was the case. The project narrative must accurately explain how the data are reported.

8. Calculations

8.1. Calibration Factor:

$$\text{Calibration Factor (CF)} = \frac{\text{Area of Peak}}{\text{mass injected (ng)}}$$

8.2 % Difference:

$$\% \text{Difference} = (R_{\text{True}} - R_{\text{Predicted}}) / R_{\text{True}} \times 100$$

where: R_{True} = True mass injected of the component within the standard
 $R_{\text{Predicted}}$ = Predicted mass of analyte using initial calibration curve.

8.3. The percentage breakdown for DDT and Endrin are:

$$\% \text{Breakdown DDT} = \frac{(\text{Area DDD} + \text{Area DDE})}{(\text{Area DDD} + \text{Area DDT} + \text{Area DDE})} \times 100$$

$$\% \text{Breakdown Endrin} = \frac{(\text{Area Endrin Ketone} + \text{Area Endrin Aldehyde})}{(\text{Area Endrin} + \text{Area Endrin Ketone} + \text{Area Endrin Aldehyde})} \times 100$$

8.4. Aqueous Sample Concentration:

$$\text{Concentration of Analyte } (\mu\text{g/L}) = \frac{R_{\text{Predicted}} \times B \times D}{V}$$

8.5. Soil Sample Concentration:

$$\text{Concentration of Analyte } (\mu\text{g/Kg-dry wt.}) = \frac{R_{\text{Predicted}} \times B \times D}{G \times S}$$

where: $R_{\text{Predicted}}$ = μg of Analyte as determined from the calibration curve.
B = final volume (mL) of the extract, usually 10 mL
D = extract dilution factor, if used. If there is no dilution, D=1.
V = initial volume of sample (mL) extracted.
G = weight of sample (grams as received) extracted.
S = % solids as a decimal fraction

8.6. Report all results to two (2) significant figures.

8.7. Relative percent difference:

$$\text{RPD} = \frac{|R1 - R2|}{\frac{[R1 + R2]}{2}} \times 100$$

where: R1 = Sample results on column 1, or MS concentration.
R2 = Sample results of column 2., or MSD concentration.

9. Quality Control

9.1 The analytical batch, which was begun in extraction, consists of up to 20 field samples, a method blank, an LCS, a Matrix Spike (MS) and a Duplicate (Dup) or Matrix Spike Duplicate (MSD). All efforts should be made to run the ETR within a single run sequence to save time and effort in project review.

9.2 **Method Blank** - A method blank must be extracted with every batch of samples. The analysis of the method blank extract will demonstrate the background contamination of the apparatus and procedures in the

laboratory. The method blank must not contain any analytes of interest at concentrations greater than the reporting limit for the method. *If the method blank is contaminated, as demonstrated by the instrument analysis, the entire analytical batch (all samples and QC) must be re-extracted since there is uncertainty in the results for the batch for the contaminant found.* Re-extraction must be initiated immediately so that the minimum of time is wasted before re-extraction can occur - if at all possible, this re-extraction should take place within holding time. *If it is not possible to re-extract samples due to sample volume problems, the project manager and client need to be contacted immediately to determine if reporting the data as qualified results, with the contaminated method blank, is acceptable.*

- 9.3 **Surrogate Recoveries** - Surrogate recoveries are monitored to evaluate how well the extraction and analytical system worked for the sample specific matrix. Every sample, blank, and quality control sample (LCS, MS, MSD, Dup) must be spiked with 1.0 mL of the surrogates prior to extraction. The recoveries of the surrogates are determined during instrument analysis and compared to laboratory generated QC limits established through quality control charting. The current QC acceptance limits for the surrogates are given in Table 2. *If the recoveries are not within quality control criteria, the sample must be re-extracted and re-analyzed.* If the second extraction results in the surrogates still being recovered outside of criteria, a matrix effect may be occurring whereby the sample itself is causing interference to recovery of the surrogate(s). If the second extraction results in surrogate recoveries within criteria, then the initial extraction was not in control and the second extraction data should be reported. *All efforts should be made to perform the re-extraction within holding time so that the second analysis is valid and meets all quality control criteria.*
- 9.4 **Laboratory Control Sample (LCS)** - A Laboratory Control Sample (LCS) is extracted and analyzed with each batch of samples. The laboratory generated acceptance criteria for the spike components is shown in Table 3. If the LCS does not meet quality control criteria for recovery of some of the spike and surrogate compounds, the results for the other samples and quality control samples within the batch must be evaluated to determine if this is an isolated problem for the LCS and whether the data should be reported with the affected LCS. *If the recovery for all components in the LCS are outside of criteria, the entire analytical batch should be re-extracted and re-analyzed.*
- 9.5 **Matrix spikes** - For every analytical batch, a matrix spike/matrix spike duplicate (MS/MSD) pair should be performed. If specifically requested by the Client, a MS/MSD will be logged in the ETR and must be performed on those samples. If an MS/MSD was not logged in, the extraction analyst should select a sample for spiking. This should be done per 20 samples extracted for this test. The matrix spike is prepared by taking an aliquot of sample and spiking it with 1.0 mL each of the surrogate and matrix spiking solutions. The laboratory generated acceptance criteria for the spike components is shown in Table 3. The recovery of the matrix spikes are evaluated relative to the what was in the unspiked sample to indicate how well the methods worked on extraction of the analytes of interest from the sample matrix. Re-extraction of the sample based on the MS results is rare. If the recovery for the spikes are outside of criteria (Table 3) and if the precision between the MS and MSD is poor, this indicates that there were probably analytical problems during sample extraction; however, this may also indicate a matrix effect. The results for the unspiked and MS/MSD samples need to be compared carefully to determine how well the spiking study worked and if there was any indication of matrix-related problems that might affect the analytical batch.
- 9.6 **Florisil Cartridge Check Sample** - This sample is generated during the Florisil Cleanup if used on the samples and is used to monitor the efficiency of the cleanup technique on the analytes of interest. Table 4 gives the acceptance criteria for this check standard. The florisil check must be prepared and analyzed with each new batch of florisil received by the laboratory to demonstrate that the columns and techniques are appropriate. *Samples should not undergo cleanup unless a valid florisil check has been prepared and analyzed.* If a florisil check is analyzed and the criteria for acceptance is not met, any samples which were processed with, or after, this check standard will need to be re-extracted. Use of the florisil cleanup should only proceed once an acceptable check sample has been obtained.

9.7 **Duplicates** - Duplicates are used to evaluate the precision of the method. A laboratory duplicate is two separate aliquots of the same field sample taken through extraction and analysis. Field duplicates are two different samples received by the laboratory; however, these samples should have been collected at the same time using the same techniques. Comparison of the results from a laboratory duplicate analysis, will indicate the precision from extraction through analysis. Evaluation of the field duplicates however, can indicate the precision of the overall process from sampling through analysis and may also give an indication about representativeness of the samples analyzed to the sampling location.

10.0 Method Performance and Interferences

10.1. **Method Detection Limit (MDL)** studies must be performed annually or more frequently if significant changes occur to the extraction procedure or instrumentation used for analysis. Method specific MDLs are prepared using the procedure described in 40 CFR Part 136, Appendix B.

10.2. The integrity of the chromatography system must be maintained throughout a run sequence. The chromatographer should ensure that all aspects of the system, from injection through data generation is done in a consistent manner across each batch.

10.3. The quality of the reported data is monitored by the Method Blank, surrogate recoveries, LCS and MS spike recoveries as described in Section 9. Therefore, common sense should always be used in setting up the run batch so that cross-contamination doesn't occur and the characteristics of the entire extraction batch can be evaluated as quickly as possible so that a re-extraction, if needed, can be done within holding times.

10.4. Evaluation of a sample result often requires a knowledge about the results of all of the other samples within an ETR. Therefore, use all of the data, if possible, from an ETR to judge whether or not corrective actions are needed. For example, if a sample is run and the surrogates are low, one might request a re-extraction immediately only to find that a MS/MSD were also done on this sample, with low surrogate and MS recoveries, confirming a matrix effect. In this case, a re-extraction is not required and the results would be reported with a narration explaining the evidence of a matrix effect on the low surrogate recoveries. The characteristics of the overall ETR may be invaluable in deciding how to report the data to the client.

10.5. Always run and evaluate the method blank first from an analytical batch since if it fails criteria, the entire analytical batch needs to be re-extracted (unless additional sample isn't available). *If possible, the Method Blank should be deemed acceptable BEFORE any samples in the batch are analyzed on the instrument.*

10.6. Analyze cleanup check standards, if used, immediately following the MB to verify that the column cleanup method was acceptable. If the florisol check fails criteria, many samples may need re-extraction. Once again, the faster a re-extraction is initiated, the better the chance for that re-extraction to meet the sample holding time.

10.7. Analyze field samples and their associated QC within the same run sequence at the same dilutions. In other words, if a sample, MS and MSD were prepared, ensure that they all have the same final dilution prior to analysis and run them in sequence. This will avoid comparability problems in the data that might arise if the samples and QC are run at different times and at different dilutions.

10.8. Method interferences can be caused by contaminants in standards, reagents, glassware, solvents, and any other reagents or equipment which come into contact with samples. All of these materials must be demonstrated to be free of contamination prior to routine use through the analysis of method blanks (e.g., solvent and standard verification must be performed before these reagents can be used for routine sample analysis).

10.9. Never use any glassware or other apparatus with the sample if it doesn't appear clean. Ensure that syringes are properly solvent rinsed between uses.

- 10.10. Ensure that the aldol condensation products (4-methyl-4-hydroxy-2-pentanone and 4-methyl-3-pentene-2-one) which can arise during sonication using methylene chloride:acetone, are not interfering with detection of the analytes of interest.
- 10.11. Never use any glassware, or other implements such as syringes, on multiple samples within the batch unless there has been thorough cleaning and decontamination between samples.
- 10.12. Phthalate contamination, from plastics, poses a special problem for analysis since the phthalates can interfere with accurate determination of the analytes of interest. Never use any plastic material during the handling of the sample extracts.
- 10.13. Any deviations and observations made about the analyses must be documented in the instrument logbook and or project narrative. If a problem arises during analysis, document the problem and initiate corrective action. If there is a problem with a sample analysis which indicates that re-extraction should be performed, and if there is no additional sample available for re-extraction, the Project Manager needs to be informed immediately so that the client can be involved in the corrective action process. Re-extraction of a sample should be done within holding time if at all possible.

Table 1
Retention Time Windows for Single and Multicomponent Pesticides

Compound	Retention Time window (minutes)
alpha-BHC	± 0.05
beta-BHC	± 0.05
gamma-BHC (Lindane)	± 0.05
delta-BHC	± 0.05
Heptachlor	± 0.05
Aldrin	± 0.05
alpha-Chlordane	± 0.07
gamma-Chlordane	± 0.07
Heptachlor epoxide	± 0.07
Dieldrin	± 0.07
Endrin	± 0.07
Endrin aldehyde	± 0.07
Endrin ketone	± 0.07
4,4'-DDD	± 0.07
4,4'-DDE	± 0.07
4,4'-DDT	± 0.07
Endosulfan I	± 0.07
Endosulfan II	± 0.07
Endosulfan sulfate	± 0.07
Methoxychlor	± 0.07
Toxaphene & Chlordane	± 0.07
Tetrachloro-m-xylene (TMX)*	± 0.05
Decachlorobiphenyl (DCB)*	± 0.10

* surrogates

*Limits adopted from CLP OLM03.1

Establish daily retention time windows as the retention time of the component in the opening verification standard ± .05, .07, or .10 minutes as listed in Table 1. Retention time windows are calculated each time a new GC column is installed. For multicomponent pesticides, a single peak within the chromatographic pattern is chosen as the retention time marker. The windows given in Table 1, established by the Contract Laboratory Program based on analysis of real-life environmental samples, are compared with the laboratory calculated windows (section 7.4.4).

Table 2 Surrogate Recovery Acceptance Criteria*		
Surrogate	Aqueous (% recovery)	Soil (% recovery)
2,4,5,6-tetrachloro- m-xylene(TMx)	30-150	30-150
Decachlorobiphenyl (DCB)	30-150	30-150

Table 3 MS/LCS Recovery Acceptance Criteria*				
Spiked Component	Aqueous		Soil	
	(% recovery)	RPD	(% recovery)	RPD
gamma-BHC	56-123	15	46-127	50
Heptachlor	40-131	20	35-130	31
Aldrin	40-120	22	34-132	43
Dieldrin	52-126	18	31-134	38
Endrin	56-121	21	42-139	45
4,4'-DDT	38-127	27	23-134	50

Table 4
Pesticide Florisil Cartridge Check Criteria*

Spiked Component	Spike Added (ng)	QC Limits (% recovery)
alpha-BHC	20	80-120
gamma-BHC	20	80-120
Heptachlor	20	80-120
Endosulfan I	20	80-120
Dieldrin	40	80-120
Endrin	40	80-120
4,4'-DDD	40	80-120
4,4'-DDT	40	80-120
Methoxychlor	200	80-120
TMX	20	80-120
DBC	40	80-120

*Limits adopted from CLP OLM03.1

Note: The above limits will be replaced by laboratory limits generated by QC charting, when available.

Standard Operating Procedures

C-2g

**TOTAL ORGANIC CARBON IN SOIL/SEDIMENT
MODIFIED FROM EPA METHOD 9060**

Total Organic Carbon in Soil/Sediment Modified from EPA Method 9060

1.0 Method Reference

Modified from USEPA Test Methods for Evaluating Solid Wastes, *Total Organic Carbon Method 9060*, SW846, September 1986 as promulgated in the Final Update, Third Edition, December 1996.

2.0 Scope and Application

- 2.1 This method is applicable to the determination of Total Organic Carbon (TOC) in soils and sediments. Reporting limit is 100 mg/Kg.
- 2.2 This method is most applicable to measurement of TOC at levels above 60 mg/Kg (~ 3x the MDL). If lower levels of TOC need to be measured in a sample, use the Lloyd Kahn TOC method developed for marine sediments.
- 2.3 The organic carbon in the sample consists of a variety of organic compounds in various oxidation states. Some of these compounds may be oxidized by biochemical or chemical processes and can be measured by the BOD₅ or COD tests (see the appropriate SOPs for these methods). The Total Organic Carbon (TOC) is independent of the oxidation state of the organic material in the sample and does not measure other organically bound elements such as nitrogen or hydrogen. To measure the amount of organically bound carbon, the organic carbon molecules are broken down into single carbon units and converted to a form that can be measured quantitatively. The Total Organic Carbon (TOC) analyzer converts the carbonaceous material in a sample to CO₂ by catalytic combustion. The CO₂ formed is then measured by an infrared detector. If the sample is analyzed without pretreatment, the Total Carbon (TC) content is determined. If the sample is treated with phosphoric acid prior to analysis, the Total Organic Carbon (TOC) content is determined. The Inorganic Carbon content can be determined by subtraction of the TOC from the TC values.

3.0 Summary of Method

- 3.1 Organic carbon is measured using combustion analysis on a carbonaceous analyzer. A known weight of dried sample is loaded into a quartz boat and treated with phosphoric acid to remove the inorganic (e.g., carbonate and bicarbonate) carbon (in the form of CO₂) prior to analysis. The sample is then introduced into a furnace and combusted. The carrier gas (O₂) is combined with the carbon content of the sample to form CO₂. The CO₂ is then measured by an infrared detector. The amount of CO₂ derived from a sample is directly proportional to the concentration of organic carbonaceous material in the sample.

Approval Signatures		
Laboratory Director	<i>Peter J. Kane</i>	Date: 7/15/98
Section Manager	<i>Nancy Wade</i>	Date: 7/15/98
Quality Assurance Manager	<i>M. Chesnick</i>	Date: 7/15/98

NEH, Inc. Third-party QAO

4.0 Method Performance

- 4.1 Method performance is determined by successfully meeting the quality control specifications described in section 11 of this SOP. The temperature and flow specifications contained herein have been determined by the laboratory to provide the required quality control (QC) results.
- 4.2 Method detection limits are determined annually by preparation and analysis of seven replicate samples. Samples for MDL analysis are clean sand spiked with 200 mg/Kg TOC. The MDL is calculated using student T statistics as $3.14 \times$ the standard deviation of the mean of the seven replicate results. The MDL should support a laboratory reporting limit of 100 mg/Kg; i.e., be two to five times lower than this reporting limit. As a reference, the current MDL, performed 8/8/97, is 15 mg/Kg.

5.0 Sample Handling and Preservation

- 5.1 The sample should be collected in glass jars. Approximately 50 g solid sample is required. Extra sample volume should be collected for matrix QC (spikes and duplicates).
- 5.2 Samples should be stored in a refrigerator at $4^{\circ} \pm 2^{\circ} \text{C}$ and analyzed within 28 days from collection.

6.0 Interferences

- 6.1 To determine the TOC content, the inorganic fractions must be removed before analysis.
- 6.2 Care must be taken in sample pretreatment to remove the inorganic carbon fraction to minimize the potential of any volatile organic carbon present in the sample.
- 6.3 Large and/or complex organic molecules such as tannins, lignins, or humic acids may be oxidized slowly. If these compounds are suspected to be present it is advisable to check the efficiency of the oxidation procedure with selected representative compounds and adjust the analysis as needed.
- 6.4 Chlorides at a concentration of greater than 0.1% may completely inhibit the oxidation of organic matter.
- 6.5 Oils from your skin can contaminate the analysis. Handle the quartz boats with tweezers.
- 6.6 Sample heterogeneity can be a major source of imprecision in TOC results. Recommend to clients to perform a client-specific duplicate, as requested, to evaluate precision and representativeness of the TOC results to the site.

7.0 Equipment

- 7.1 Instrument: ASTRO Model 2001 Analyzer System 2
- 7.2 Detector: ASTRO Non-Dispersive Infrared (IR) Analyzer
- 7.3 Option for Solids: ASTRO High Temperature Solids Module with quartz sample boats
- 7.4 Instrument Set-points:
- | | |
|----------------|-------------|
| Furnace Temp.: | 550°C |
| Carrier Gas: | Oxygen |
| Flow Rate: | 200 mL/min. |

7.5 Mortar and pestle

7.6 Oven at 103°C

7.7 Desiccator

7.8 Syringes, 25 µL and 50 µL

7.9 Disposable Pipettes and pipettors

7.10 Quartz boats

7.11 Tweezers

7.12 Aluminum weigh dishes

8.0 Reagents

8.1 ASTM Type I Water: Boiled, cooled and capped to eliminate CO₂.

8.2 Phosphoric Acid Solution: Add 1 mL of H₃PO₄ to 19 mL of ASTM Type I water. Mix thoroughly and cool to room temperature before use. Store in a glass bottle and keep at room temperature. This solution is stable for a year.

9.0 Standards

9.1 Potassium Hydrogen Phthalate (KHP), Calibration Solution (10,000 mg/L equivalent to 10,000 mg/Kg carbon): Dissolve 2.128 g of potassium hydrogen phthalate (primary standard grade) in ASTM Type I water and dilute to 100 mL in a volumetric flask. Stocks may be held for up to six months if stored at 4°C. Discard stock solutions if discoloration or any signs of bacterial growth are observed.

9.2 Potassium Hydrogen Phthalate (KHP), Spiking Solution (10,000 mg/L equivalent to 10,000 mg/Kg carbon): Dissolve 2.128 g of potassium hydrogen phthalate (primary standard grade) in ASTM Type I water and dilute to 100 mL in a volumetric flask. Use a different source for the KHP from that used to prepare the calibration standard. Stocks may be held for up to six months if stored at 4°C. Discard stock solutions if discoloration or any signs of bacterial growth are observed.

9.3 Solid Laboratory Control Sample (LCS). Vendor Source Environmental Resource Associates (ERA) or equivalent, catalog # 542.

10.0 Procedure

10.1 Calibration

10.1.1 The balance should be calibrated each day using 1.0 and 100 g weights. Before beginning analysis check the balance with a 100 mg weight and recheck at the end of the analysis. If the balance fails the calibration check, reanalyze the batch.

10.1.2 The ASTRO 2001 with the solids module option is used for this analysis. The flow meter for oxygen should be set at 200 mL/min. Boot up the PC and enter "2001" at the prompt. Select the "manual" mode in the main menu, then select "Solids".

10.1.3 Enter the calibration concentration, (10,000 mg/Kg). Enter the weight to be injected into the boat (40 mg). The required volume of stock solution (40 µL) should be loaded in a syringe and injected onto the quartz boat through the septum.

- 10.1.4 The computer screen will prompt you to slowly push the boat into the furnace. After calibration is completed, pull the boat to the cool zone.
- 10.1.5 Following the daily calibration, load another 40 μL of the CCV stock into the syringe. When prompted, inject this on the boat, enter 40 mg for its weight and press "enter". When prompted, push the boat into the furnace. The instrument will print out the quantified result for the check standard. The recovery of the standard should be within $\pm 20\%$ of its true value (8,000 to 12,000 mg/Kg). If not, examine the instrument to determine the reason for the unstable calibration and re-calibrate. **Do not continue until a stable calibration has been obtained.**
- 10.1.6 A system blank must be performed on one of the boats which will be used during the analysis sequence. Use an empty boat and input a weight of 100 mg for the sample. A value of less than 100 mg/Kg must be obtained prior to using the boat.

If samples are encountered which saturate the detector at any point during the analysis sequence, run system blanks in the affected boat until a value of less than 100 mg/Kg is obtained before continuing with the sequence.

10.2 Sample Pretreatment

This procedure is used to remove the inorganic compounds in the sample prior to the determination of Total Organic Carbon. If Total Carbon is to be determined this procedure is omitted.

- 10.2.1 Weigh approximately 10 g of sample into an aluminum weigh dish.
- 10.2.2 Dry the sample at $103^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for approximately one hour. Cool the sample in a desiccator. When cool, grind with a mortar and pestle to a fine powder. Use this dried sample for all subsequent analyses.
- 10.2.3 Weigh 100 mg of sample into a clean dry quartz boat. If dilutions are necessary, they are prepared by using a smaller weight of sample.
- 10.2.4 Add 2 drops of the phosphoric acid solution to the sample in the boat and check for effervescence. If effervescence is observed, continue adding phosphoric acid drop by drop until the effervescence stops to ensure removal of inorganic carbon compounds.
- 10.2.5 Place the sample boat in the drying oven for approximately 10 minutes.

10.3 Sample Analysis

- 10.3.1 Using the pretreated sample, insert the boat into the sample holder and close the lid.
- 10.3.2 Record and enter the actual sample weight into the computer. When prompted, push the boat into the furnace.
- 10.3.3 If an "IR Overrange" is indicated or if the peak appears not to have been integrated correctly, rerun the sample with an appropriately larger or smaller weight of sample in order to obtain an in-range peak.
- 10.3.4 If an overrange condition is encountered, run "calibration blanks" in the boat until it yields a value of less than 100 mg/Kg before using it again.

12.4 Matrix Duplicates: a matrix duplicate analysis must be performed once per 20 samples, per matrix.

Acceptable RPD of duplicates is $\leq 25\%$. Acceptance criterion is not applicable to sample concentrations less than 5 times the reporting limit.

$$\text{RPD} = \frac{(R1-R2)}{\frac{(R1+R2)}{2}} \times 100$$

Corrective Action: If RPD exceeds acceptance criteria, narrate results to client in final report indicating that a matrix effect has been observed on the precision of the results reported based upon the high relative percent difference of duplicate analyses.

12.5 Matrix Spikes: A matrix spike (MS) must be performed once per 20 samples, per matrix. Acceptable recovery is 75 -125%. Acceptance criterion is not applicable to spike concentrations less than 2 times the sample concentration. Corrective Action: If matrix spike recovery is outside limits examine recovery of the associated LCS. If LCS recovery is acceptable, narrate to client indicating that a matrix effect has been observed on the accuracy of the results reported based upon the poor recovery of the matrix spike. If recovery of LCS also fails to meet acceptance criteria, examine system for cause and repeat preparation and analysis of all associated samples and QC in the batch (including the MS).

12.6 Continuing Calibration Verification (CCV): A CCV must be analyzed every 10 samples to verify the calibration and monitor accuracy during analysis. Acceptable recovery is 80-120%. Corrective Action: If recovery is outside limits examine system for cause. Do not proceed with sample analysis until acceptable recovery is obtained. This may require re-calibration. Re-analyze all samples run since the last acceptable CCV.

12.7 Continuing Calibration Blank (CCB): A CCB (also referred to as a system blank) must be analyzed every 10 samples to monitor drift and contamination during analysis. Acceptable result is $<$ reporting limit. Corrective Action: If CCB result is \geq RL, examine system for cause. Do not proceed with sample analysis until acceptable CCB result is obtained. Re-analyze all samples run since the last acceptable CCB.

12.8 Modifications to SW846 method 9060

- Soil/Sediment samples are analyzed once (not in replicates) and a matrix duplicate is analyzed at a frequency of 5% to evaluate precision.
- An LCS, an independent verification of the calibration, is analyzed daily and a CCV, continuing calibration check, is analyzed every 10 samples to verify accuracy of the calibration; rather than analyzing an independent check standard every 15 samples.
- MS and MD are analyzed at a frequency of 5% , consistent with Chapter One of SW846, rather than a MSD every 10 samples.

Standard Operating Procedures

C-2h

DETERMINATION OF ARSENIC SPECIES IN WATER BY
HYDRIDE GENERATION CRYOGENIC TRAPPING GAS CHROMATOGRAPHY
ATOMIC ABSORPTION SPECTROPHOTOMETRY (FGS-022)

Determination of Arsenic Species in Water by Hydride Generation Cryogenic Trapping Gas Chromatography Atomic Absorption Spectrophotometry (FGS-022)

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August 25, 1997

1.0 SCOPE

1.1 Hydride generation cryogenic trapping gas chromatography atomic absorption spectrophotometry (HG-CT-GC-AAS) is used for sensitive species specific determination of arsenite [As(III)], arsenate [As(V)], monomethyl arsenic [MMAs], and dimethyl arsenic [DMAs]. It can also be used for the determination of total inorganic arsenic [TIAs] at very low concentration levels (below 100 ng) in case the method detection limit of the automated HG-AFS system ("Excalibur") (SOP in preparation) is not sufficiently low. This SOP will also cover sample handling and storage procedures.

2.0 SUMMARY OF METHOD

2.1 In this technique arsenic species are selectively volatilized from solution by controlling the pH of the sample solution and reducing them to their corresponding hydrides with borohydride. The arsines are purged from the solution by a helium gas flow and trapped in a liquid nitrogen cooled 'U' tube packed with 15% OV-3 on Chromasorb WAW-DMCS. Heating the column revolatilizes the arsines and allows for chromatographic separation based on boiling points. Once separated the arsines are carried into a quartz furnace with a hydrogen-air flame where they are atomized and detected via atomic absorption spectrophotometry.

2.2 The typical method detection limit is between 0.003 and 0.020 $\mu\text{g/l}$, depending upon the species sought, for a 15 ml sample.

3.0 INTERFERENCES

3.1 If water is allowed to condense in the trap, multiple and irregular peaks occur in the region where Me_2AsH elutes. This may be overcome by heating the transfer line between the chromatographic column and the furnace. Also, it has been observed that free chlorine (as contained in bad batches of HCl) totally suppresses the signals for all hydrides during cryotrapping. Thus, HCl with obvious green/yellow color must be avoided; if in doubt, it can be tested for free chlorine with iodide solution (formation of red/brown iodine).

3.2 No interferences from environmental matrices (i.e. chlorides, nitrates, organics) have been noted.

4.0 APPARATUS AND MATERIALS

4.1 The apparatus needed for speciation analysis of arsenic is shown in principle in Figure 1. A gas liquid separator (GLS) is inserted between the purging vessel and the GC column to prevent transfer of water droplets and aerosols to the GC column, which causes irreproducible elution of DMAs and gradual decomposition of the stationary phase (packing material of the chromatographic column). However, since Teflon and other plastics seem to absorb small quantities of methylated arsines, the GLS should not be used and all connections should be as short as possible when analyzing for small amounts of MMAs and DMAs (< 100 ng/l). The specific instruments and equipment used in this laboratory is listed below.

4.2 Reaction Vessel made by grafting an 1/4 in outer diameter side-arm inlet onto a 30 ml "Midget Impinger" (Ace Glass #7532-20). The side arm may be fitted with a silicone rubber septa (Ace Glass #9096-31) to allow injection of borohydride into the solution.

4.3 GC Trap and Column is constructed from a 6 mm o.d. borosilicate glass tube about 30 cm long bent into a 'U' shape with appropriate dimensions to fit into a tall wide-mouth Dewar flask. The glass is silanized prior to packing, to minimize active sites, using Sylon-CT (Supelco). The entire column is wrapped in 22 gauge nichrome wire (*ca.* 1.5 turns cm^{-1}) the ends of which are affixed to electrical contacts. The column is packed at the outlet half with 15% OV-3 on 60/80 mesh Chromosorb WAW-DMCS (Supelco). The ends are plugged with silanized glass wool. The entire assembly is then preconditioned by heating to 190°C for 30 min with a helium flow of 150 ml min^{-1} . After this it is silanized by

placing two 100 ml aliquots of Sylon-CT in the inlet end of the column and heating to 190°C after each for 15 min. This conditioning and silanization process may be repeated whenever broadening or a decrease in sensitivity of analyte peaks is observed.

4.4 Atomizer. The furnace/atomizer consists of a quartz tube (9 mm i.d.) with opposing 6 mm o.d. inlets perpendicular to the furnace. A mixture of hydrogen and the carrier gas are admitted via one inlet and air through the opposing inlet. The opposing inlets minimize flame noise and reduce the possibility of extinguishing the flame with surges of hydrogen gas created by the reaction mixture. To light the furnace all gases are turned on and a flame is brought to the furnace ends. After the furnace has warmed up (*ca.* 5 min), a flat metal spatula is placed smoothly, first, over one end of the furnace and, then, the other. This restricts the flame to the center of the tube and reduces flame noise. Check to see that the flame is still burning by placing a mirror near the furnace openings and checking for water condensation. Gas flow rates and pressures are as follows.

<u>Gas</u>	<u>Approximate Flow Rate (ml/min)</u>	<u>flow meter setting</u>	<u>Pressure (psi)</u>
Helium	160	"40"	10
Hydrogen	370	"65"	20
Air	180	"45"	20

4.5 Detector. For this work we use a Perkin Elmer 5000 Atomic Absorption Spectrophotometer fitted with a Perkin-Elmer System II Electroless Discharge Lamp (Arsenic). The 193.7 nm line is used with a 0.7 nm (low) slit width and without background correction.

4.6 Connections. To minimize active sites all connections are either Teflon friction-fit or threaded tubing connectors. All transfer lines are either 1/8 in o.d. Teflon tubing or silanized 6 mm o.d. borosilicate glass. Teflon connections must be kept as short as possible when analyzing for low levels of methylated As species because they seem to be absorbed.

4.7 Recorder. Any multi-range chart recorder or integrator with matching voltage input and variable speeds is acceptable.

5.0 REAGENTS

5.1 Water. 18 M Ω ultra pure deionized water starting from a prepurified (distilled, R.O., etc.) source. To remove any remaining trace metals and organics an activated carbon cartridge is placed between the final ion exchange bed and the 0.2 μ m filter.

5.2 Arsenite (As(III)) Solution. A 1000 ppm stock solution is prepared by dissolving 0.173 g sodium meta-arsenite (>98.7%, Baker Analyzed) in 100 ml 0.1 % ascorbic acid solution. This solution is kept refrigerated in an amber bottle. Working standards (1 ppm, 100 ppb and 1 ppb) are prepared by diluting 100 μ l of the stock solution or respective working standard to 10 ml with water. Millipore[®] water tends to oxidize As(III), so the long term stability of these dilute As(III) solutions is not known and must be tested before use. Stability can be enhanced by preparing the dilutions in 0.1 % ascorbic acid solution also, but in that case, it has to be checked if the addition of ascorbic acid to a sample (e.g. during spiking experiments) reduces any As(V) originally present.

5.3 Arsenate (As(V)) Solution. A 1,000 ppm stock solution is prepared by dissolving 0.416 g sodium arsenate (98.0%) (Baker Analyzed) in 100 ml water. Working standards (1 ppm, 100 ppb and 1 ppb) are prepared by diluting 100 μ l of the stock solution or respective working standard to 10 ml with water.

5.4 Monomethyl arsenic (MMAs) Solution. A 1,000 ppm working standard is prepared by diluting 0.1059 g of a 23.6% solution to 25 ml in water. Working standards (1 ppm, 100 ppb and 1 ppb) are prepared by diluting 100 μ l of the stock solution or respective working standard to 10 ml with water.

5.5 Dimethyl arsenic (DMAs) Solution. A 1,000 ppm stock solution is prepared by dissolving 0.1842 g dimethylarsenic acid (98%, Strem Chemicals, Inc.) in 100 ml water. Working standards (1 ppm, 100 ppb and 1 ppb) are prepared by diluting 100 μ l of the stock solution or respective working standard to 10 ml with water.

5.6 TRIS Buffer. To prepare pH 6.2 solution 39.4 g tris(hydroxymethyl)-aminomethane hydrochloride and 0.25 g reagent grade sodium hydroxide are dissolved in water to make 100 ml of solution.

5.7 Borohydride Solution. A 4% solution is prepared by dissolving 4 g NaBH₄ and 0.1 g NaOH and making up to 100 ml with water. This solution is

stable for up to 3 days when kept covered and stored in the refrigerator overnight. For low level TIAs determinations, KBH_4 (Strem) is preferable over NaBH_4 since it was found to contain lower As blanks, dissolves much faster in water, forms a clear solution and liberates much less hydrogen. It is used in the same concentration and amount as NaBH_4 , but doesn't require the addition of NaOH for stabilization.

5.8 6 M Hydrochloric Acid. Equal volumes of reagent grade HCl and water are mixed to give a 6 M solution. The utilized HCl must be checked before use and should not have any measurable As blank to allow for low MDLs.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Aqueous samples must be collected polyethylene or glass bottles washed in 4 N HCl using accepted clean techniques (nitric acid wash makes the bottles oxidizing to As(III)).

6.2 If only total inorganic As, MMAs, and DMAs concentrations in the $\mu\text{g l}^{-1}$ range are of interest, the suggested storage scheme is to keep samples at 0 to 4°C in polyethylene or glass bottles until analysis. No chemical preservation should be used.

6.3 It is very difficult to preserve the original $\text{As(III)}/\text{As(V)}$ ratio. River water tends to spontaneously reduce As(V) to As(III) and freezing of water tends to induce oxidation of As(III) to As(V) , except in the case of very rapid freezing (in liquid nitrogen).

6.3.1 The best storage scheme is to quick freeze samples to -196°C in liquid nitrogen and store at -80°C (on dry ice) until analysis. Quick freezing is accomplished by filling a 60 ml polyethylene bottle with 55 ml sample, capping tightly (CAUTION: cap should have a 2 mm hole bored in it to avoid explosion when thawing), and dropping the sample into a Dewar flask filled with liquid nitrogen.

6.3.2 An alternate scheme is to refrigerate samples at 4°C in polyethylene or glass bottles and analyze as soon as possible (within 24 h).

7.0 PROCEDURE

7.1 Determination Of Total Inorganic And Methylated Arsenic. Place approximately 15 ml (check for actual volume by weighing and, if necessary, volumetric density determination) of aqueous sample (or sample extract containing between 5 and 25 ng As and dilute to 15 ml with water) and 1 ml 6 M HCl in reaction vessel and replace top. At this time raise the Dewar flask filled with liquid nitrogen so that the trap is immersed. Slowly inject a 2.0 ml aliquot of 4% NaBH₄ through the silicon rubber septum with a disposable 3 ml hypodermic needle. Since all of the substantial As blank comes from the utilized borohydride solution, it is essential that the injected amount is as reproducible as possible if low MDLs are required. Purge the system for 3 min (starting from the beginning of the injection), which is sufficient for quantitative recovery of all species. Longer purging times transfer more water vapor to the GC column, which causes faster column deterioration and has a negative influence on the determination of the methylated arsines, namely lower recoveries and worse reproducibility. As simultaneously as possible, remove the liquid nitrogen from the GC column, turn the chart recorder on, and begin heating the GC column with 36 V (end temperature approximately 150 °C). The volatile arsines elute in order of their boiling points (AsH₃, MeAsH₂, Me₂AsH).

7.2 Arsenite Determination. Arsenite is determined separately from 7.1 by varying the pH so that only As(III) reacts to form AsH₃. The procedure is similar to that in 7.1 except that 1 ml of TRIS buffer is used in place of the 6 M HCl and only 1 ml of NaBH₄ is injected through the septum quickly. Prior to addition of NaBH₄ the pH of the solution is 6.2. At this pH the separation of As(III) from As(V) is quantitative.

7.3 "Total Arsenic" Determination. After suitable oxidative matrix digestion (SOP in preparation), a parameter described as "total arsenic" can be determined as TIAs according to 7.1. While all As species discussed in this SOP will be detected by this procedure, it is known that some of the organic major As species in tissues, e.g. arsenobetaine, do not break down quantitatively under those conditions, thus yielding low results. Interferences on the HG process from oxidizers utilized in the digestion step must be eliminated prior to analysis (if necessary).

8.0 CALIBRATION

8.1 A calibration curve is determined daily from analysis of 4 different amounts (from 0.2 ng to 10 ng for inorganic and 1 to 20 ng for methylated arsenic species). The correlation coefficient for this curve must be greater than 0.995 in order to continue with analysis. Quantitation is based on peak height if a chart recorder is used and peak area if an integrator is used.

8.2 Method detection limits are determined from at least 7 independent runs at one concentration near (between 1 and 5 times the MDL) the method detection limit. They are calculated by multiplying the standard deviation σ_{n-1} of those runs with the corresponding t-factor for n-1 degrees of freedom (3.143 in the case of 7 measurements).

9.0 DATA ANALYSIS

9.1 Concentrations of analytes are determined by comparing peak height to the standard curve and correcting for the volume used.

$$[As] = \frac{S - b}{A \cdot v}$$

where S is the signal height, b is the reagent blank signal height, A is the slope of the regression curve, and v is the volume of sample used.

9.2 The concentration of As(V) is calculated by subtracting the value for As(III) from that of total inorganic As.

10.0 QUALITY CONTROL

10.1 Calibration curve. The calibration curve will be determined daily and again during the day if ongoing calibration standards fall outside of accepted limits (75 - 125% recovery). Minimum acceptable correlation coefficient for the calibration curve is 0.995.

10.2 Duplicates. Maximum acceptable relative deviation is 25%.

10.3 For every 10 samples, 1 duplicate, 1 spike recovery, and 1 blank is run. For every 20 samples a certified reference material (CRM) is run; currently, no CRMs for As species are available, but seawater CRMs (SLEW-2, CASS-3, NASS-4) can be used as CRMs for TIAs in waters. The values for these must be within

specified limits in order to accept the previous batch of data and in order to continue with additional analysis.

10.4 All quality control data will be maintained and available for easy reference and inspection.

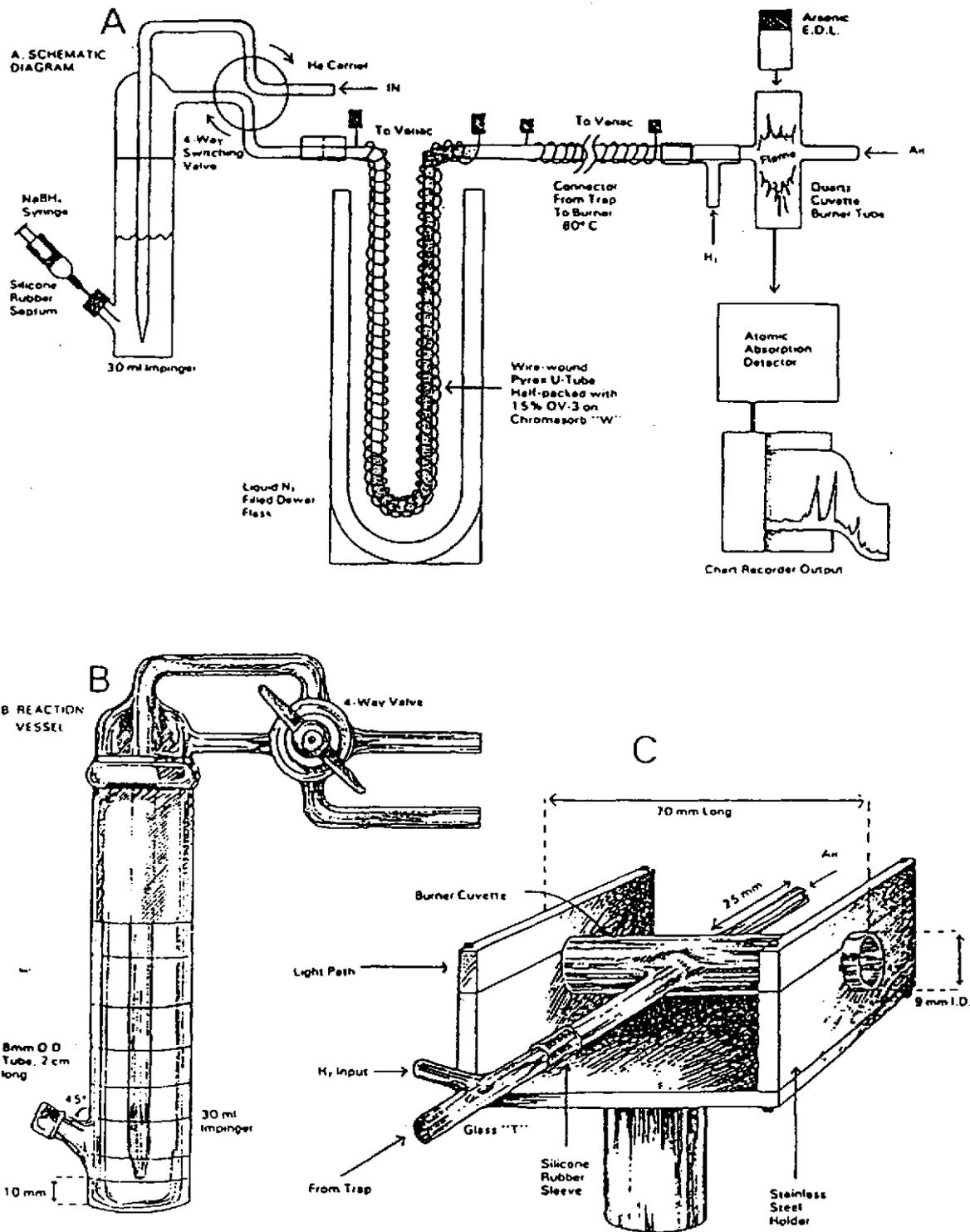


Figure 1. Arsenic Speciation Apparatus: (a) Schematic Diagram, (b) Reaction Vessel, (c) Quartz Cuvette Burner Tube.

Standard Operating Procedures

C-2i

LEACHING OF INORGANIC ARSENIC SPECIES FROM TISSUE SAMPLES

Leaching of Inorganic Arsenic Species from Tissue Samples

July 4, 1999

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1.0 SCOPE

1.1 Arsenic in biogenic samples may be in the form of organic or inorganic species. The organic species are assumed to be far less toxic than the inorganic, so differentiation between the two groups is required for toxicological assessments. This SOP describes the leaching of inorganic arsenic species from tissue samples. We currently have no way of quantifying the relevant organic As species in tissues directly, but in combination with a separate oxidative total tissue digestion (SOP #FGS-058), this procedure permits the calculation of organic arsenic as total arsenic minus total inorganic arsenic.

1.2 This method is designed for arsenic speciation in biogenic tissue (plant and animal) samples. In order to retain speciation, sample preservation is an important facet of this method.

1.3 This SOP is designed to ensure that procedures are followed and the data obtained are verifiable, reproducible, and repeatable.

1.4 The presented method was optimized on a limited number of different types of tissue. Since the leaching behavior of inorganic As species from tissues is potentially extremely matrix-dependent, the analysts performing the sample preparation and analyses are required to provide an evaluation of the method's performance to each project's manager, as well as report any unusual observations. This way, the method will be generally validated for all types of tissues, and minor adjustments will be incorporated, if necessary.

2.0 METHOD SUMMARY

2.1 In this technique, the inorganic species As(III) and As(V), but not organoarsenic compounds, are quantitatively leached from well

homogenized tissue samples with dilute hydrochloric acid. Total inorganic arsenic is determined by HG-CT-GC-AAS, as described in SOP #FGS-022.

2.2 Total arsenic is determined by ICP-MS (SOP #FGS-054) on a separate tissue sample after complete acid digestion (concentrated HNO₃), as described in SOP #FGS-058. The difference between the total arsenic concentration and the inorganic fraction is believed to be organic forms of arsenic.

2.3 The estimated method detection limit is approximately 3 ng/g wet weight of tissue for total inorganic arsenic by HG-CT-GC-AAS.

3.0 INTERFERENCES

3.1 Please refer to the SOP for the analytical methods to be used for specific interferences related to the detection system being used. Typically samples will be analyzed by HG-CT-GC-AAS following SOP #FGS-022 for inorganic arsenic.

4.0 APPARATUS AND MATERIALS.

The apparatus needed for detection of arsenic compounds can be found in SOP #FGS-022. This SOP focuses on the extraction procedures and therefore, includes only the materials needed for that task.

4.1 Borosilicate glass vials (40 mL).

4.2 Equipment for tissue pre-homogenization, such as a cleaned cutting board, knives, razor blades, food processor, etc.

4.3 Centrifuge.

4.4 Cell disruptor, or other equivalent homogenization equipment.

4.5 Calibrated pipettors of various capacities.

4.6 Analytical balance.

5.0 REAGENTS

- 5.1 Water - We use 18 MW ultra pure deionized water starting from a prepurified (distilled, R.O., etc.) source. To remove any remaining trace metals and organics an activated carbon cartridge is placed between the final ion exchange bed and the 0.2 μ M filter.
- 5.2 Arsenate (As(V)) Spiking Solution - Refer to SOP #FGS-022 for instruction for the preparation of an As(V) standard.
- 5.3 Hydrochloric Acid - Reagent grade HCl is used.
- 5.4 Anti-foaming agent ("Antifoam").

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 6.1 Samples must be collected in a way that minimizes contamination. Samples may be placed in plastic zip type bags (double bagged) or cleaned, wide-mouth jars with Teflon® lined lids. Maintain sample at <5°C until it can be frozen (within 24 hours).
- 6.2 Just prior to extraction, samples are thawed and either minced or homogenized in a cleaned food processor. The sample is well mixed to ensure the most representative sample possible.

7.0 PROCEDURE

- 7.1 Approximately 1 g wet tissue, accurately weighed, is placed in a 40-mL borosilicate glass vials with 20 mL of 2 M HCl.
- 7.2 The sample within the vial is emulsified with the cell disruptor until all solids are destroyed. The cell disruptor is only to be used mounted on a stand, and the analyst performing the sample extraction should always wear protective gloves during this step to minimize the risk of injury by breaking the glass vial.
- 7.3 The digestate is diluted up to the 40-mL mark with reagent water.
- 7.4 The vial is centrifuged until the solids are completely settled. The sample is now ready for analysis via HG-CT-GC-AAS (SOP #FGS-022).

7.5 Tissue extracts may foam strongly during the hydride generation step of the analysis. Use Antifoam, as necessary, to suppress the foaming.

8.0 QUALITY CONTROL

8.1 For every batch of 20 samples, one post-extraction spike (analytical spike) and one analytical duplicate are analyzed. The post-extraction spike should be spiked with As(V). Even though no reference material is currently certified for inorganic As, extract one appropriate tissue SRM with every sample batch as a secondary laboratory control sample.

8.2 All other analytical batch QC specified in the determination SOP must also be followed. This includes requirements for the calibration curve, ICV, ICB, CCVs, and CCBs

C-3a

AQUATEC BIOLOGICAL SCIENCES

**AMPHIPOD, *Hyalella azteca*, 10-DAY
SURVIVAL AND GROWTH
TOXICITY TEST FOR SEDIMENTS**

**Standard Operating Procedure
for
Amphipod, *Hyalella azteca*, 10-day Survival and Growth
Toxicity Test for Sediments**

1.0 OBJECTIVE

This SOP describes procedures for performing a ten-day whole sediment survival and growth toxicity test. This test is used to estimate the toxicity of whole sediment samples to the freshwater amphipod, *Hyalella azteca*. When required, toxicity is estimated by statistical comparisons to the control sediment and/or reference sediment. This procedure is based on the guidelines of EPA/600/R-94/024: *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Method 100.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling samples.

2.0 PREPARATION

2.1 Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Ammonia selective electrode and meter
- Mettler M3 Microbalance
- VWR 1320 drying oven

Additional Equipment:

- Test chambers (300-ml beakers, 8 per sample)
- Aeration manifold, tubing, manifold, and pipets
- Automated water-delivery system
- Disposable polyethylene transfer pipets
- Light tables
- Waste collection bucket
- Carolina bowls (assorted sizes)
- Nitex mesh sieves (0.3 mm)

Reagents:

- Reconstituted moderately hardwater (EPA/600/R-94/024)
- Deionized water
- 70 percent Ethanol

Forms and Paperwork:

- Amphipod (*Hyalella azteca*) Water Chemistry Data
- Amphipod (*Hyalella azteca*) Daily Biological Monitoring
- Amphipod (*Hyalella azteca*) 10-Day Survival and Growth Data
- Sediment Characterization Data
- Organism Holding and Acclimation
- Daily Checklist for Automated Delivery System
- Project Documentation Forms

2.2 Test System and Conditions

The test system and environmental conditions for the 10-day survival and growth test are summarized in Figure 1.

2.3 Test Organisms**2.3.1 Procurement and Documentation**

Amphipods are obtained from a commercial supplier or from in-house cultures. If possible, schedule delivery of amphipods at least 48 hours prior to test initiation. They are acclimated to the exposure water used in testing during the the period prior to test initiation. Sources of amphipods include:

Environmental Consulting and Testing: (800) 377-3657

Aquatic BioSystems: (800) 331-6916

Prior to the testing, order sufficient organisms for 10 amphipods per replicate test chamber (80 per test sample) and a surplus for reference toxicant testing. Request that the supplier provide information regarding the age and environmental conditions for the test organisms.

Amphipods are shipped by next-day carrier and delivered to Aquatec Biological Sciences. The amphipods are typically shipped in 500-mL plastic container. Upon receipt, examine the organisms and document their apparent condition, as well as the dissolved oxygen (D.O.), pH, temperature and conductivity of the shipping water. Record the observations on the Organism Data Sheet provided by the supplier. Place a copy of this sheet in the project data package.

2.3.2 Evaluation of Amphipod Condition

If, during examination, it appears that more than 5% of the organisms have died during transport, or if the temperature or other environmental conditions are different from test requirements (e.g., dissolved oxygen <4 mg/L, temperature <15°C), notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document

the reason on the Project Documentation form. Also, it may be necessary to notify the client.

2.3.3 Acclimation and Holding

Transfer the amphipods to a 2-L plastic storage container. Add incremental amounts of laboratory reconstituted water and acclimate to test temperature (23°C). Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, and D.O. during the holding period. Record monitoring data on the Organism Holding and Acclimation form. If more than five percent of the organisms die, contact the Laboratory Manager and arrange for a replacement order.

2.3.4 Food

Feed daily with sufficient *Selenastrum* and YCT to maintain a monolayer of food on the bottom of the container.

2.4 Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-94/024 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is formulated sediment prepared according to the procedure outlined in EPA/600/R-94/024 (Section 7.2.3.2) and consists of 77% fine and medium sand, 17% kaolinite clay, 5% ground peat, and 1% calcium carbonate. The formulated sediment is stored dry and is hydrated by addition of reconstituted moderately hardwater prior to distribution to test chambers.

3.2 Test Sediment Preparation

1. Remove sediment samples from Sample Management refrigerators.
2. Transfer the sample to the ventilation hood in the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic "spaghetti fork-it" spatula or other suitable utensil;
4. Transfer aliquots of the homogenized sediment to a glass tray and examine for indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for amphipods), transfer approximately 100 mL aliquots to each of the replicate test chambers;

6. If indigenous organisms (especially predacious insects or amphipods) are present, remove them with forceps or press sieve sediment through a 0.5 or 1.0 mm Nitex mesh sieve, re-homogenize, then distribute 100-mL aliquots to each of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments. Sieving of sediments should be avoided if possible;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
8. Add overlying water to a final volume of approximately 275 mL;
9. Return the unused sediment sample to Sample Management for storage;
10. Transfer the test chambers to the automated water delivery system and begin the water renewal cycles (noon and midnight). The test replicates remain in the test system overnight without addition of test organisms.

3.2.1 Measuring Initial Overlying Water Chemistry

On the day of test initiation remove an aliquot of overlying water from replicates of each test sample. Measure the following parameters: pH, DO, temperature, and conductivity. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also stored and preserved for Day 0 alkalinity, hardness, and ammonia analyses. The temperature of the exposure water must be within the range of $23 \pm 1^\circ\text{C}$. Dissolved oxygen should be $\geq 40\%$ saturation (3.4 mg/L). Additional water exchanges or aeration may be required if dissolved oxygen levels do not remain above 40% saturation.

3.2.2 Test Initiation: Preparation and Distribution of Test Organisms

1. Place the amphipod holding container over a light table and use a disposable polyethylene transfer pipet to transfer amphipods to 1-oz. (30 mL) disposable cups (Dixie condiment cups) until each cup contains 10 amphipods. Prepare sufficient cups for one per test replicate plus several spares. Sufficient amphipods (60) should be reserved for a standard reference toxicant test and to archive a representative subsample (10-20) of the amphipod test population.
2. Randomly select a cup containing 10 amphipods. Examine them over a light table and replace any apparently unhealthy or injured amphipods.
3. Using a transfer pipet, gently rinse the 10 amphipods into a test replicate with clean exposure water. Check to be sure that all amphipods have been removed from the cup and swim to the sediment in the test replicate. A drop of exposure water can be used to submerge any amphipods that get trapped on the surface.
WARNING: Do not dip condiment cups into the exposure water.
4. Record the date and time of test initiation when amphipods have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
5. After one hour, check all test replicates and replace any amphipods which are floating or are dead.
6. Preserve a representative sample of 10-20 amphipods with 70% ethanol for archiving. After measurement of initial lengths, the amphipods should be stored six months as a reference stock identified by testing group (BTR) and date.

3.3 Daily Monitoring

3.3.1 Environmental Conditions

The environmental conditions monitoring schedule is outlined in Table 1. On Days 0 and 10 preserve a portion of the overlying water sample used for water quality determinations (approximately 100 mL) with 0.3 mL of concentrated H₂SO₄ for ammonia-N analysis. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

3.3.2 Biological Monitoring

Test organism observations are made daily for all test replicates. Position lighting to illuminate the overlying water column and the sediment surface for each replicate. Examine and record observations such as amphipods not buried or dead amphipods (not removed). Replace the test chamber to its assigned position.

3.3.3 Feeding

Provide 1.5 mL of YCT to each test replicate daily.

3.3.4 Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly.

3.4 Termination of the Whole Sediment Toxicity Test

3.4.1 Final Chemistry

Decant an aliquot of exposure water from several test replicates and pool to obtain sufficient water for the Day 10 water chemistry analyses. Measure and record the final chemistry parameters as specified in Figure 1.

3.4.2 Day 10 Survival

1. Transfer a test replicate to a light table equipped with side lighting. Search for amphipods and remove any alive or dead amphipods with a transfer pipet. Decant the overlying water through a 0.3 mm sieve. Rinse the sediment through a 0.3 mm sieve. Pool all amphipods found from a single replicate into a labeled 30-mL disposable cup. Count and record the total number of amphipods surviving on the Survival Data Form. If organisms appear to be dead, examine them under a dissecting microscope. If any movement is detected, the amphipod is considered to be alive.

2. If fewer than 10 amphipods are recovered, transfer all sediment and undieved material back into the test chamber and hold for a possible reexamination. The test material may be preserved with sugar formalin solution and Rose-Bengal Stain for a subsequent re-pick. Stained amphipods found on the repick will be assumed to have been alive when the test was ended if the body tissue is not significantly degraded. The total number surviving will then be corrected.

3.4.2 Day 10 Growth

Growth is based upon the mean dry weight of surviving amphipods, by replicate. Transfer surviving amphipods to pre-weighed weighing boats (data recorded on the Amphipod (*Hyalella azteca*) 10-Day Survival and Growth Data form) and dry overnight in the drying oven at 60°C. Weigh the boats and the dried amphipods to the nearest 0.01 mg. The Mettler M3 microbalance is used for all dry weight determinations.

4.0 QUALITY ASSURANCE

4.1 Blind Sample Analysis

Each sample, including the Control, will be assigned a unique sample number which will be used throughout the test.

4.2 Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-94/024, Table 11.1. Specifically, a test is judged to be acceptable if the average survival of control amphipods is equal to or greater than 80% at the end of the test. The environmental conditions must be within the tolerance limits of *Hyallela azteca*.

4.3 Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

4.4 Reference Toxicant Testing

A water-only 96-hour exposure of amphipods to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of amphipods. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.

Receive verbal and visual instruction.

Demonstrate 90% recovery of 10 amphipods in trial sediments.

Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the amphipod (*Hyalella azteca*) 10-day whole sediment survival toxicity test.

ASSOCIATED PROTOCOLS: EPA 1994. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (EPA/600/R-94/024) Method 100.1.

1. Test type:	Whole-sediment toxicity (static)
2. Temperature:	23 ± 1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Light illuminance:	500 to 1000 lux
5. Photoperiod:	16 hr. light, 8 hr. dark
6. Test chamber size:	300 mL beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	Every 12 hours
10. Age of test organism:	7 - 14 days at the start of the test
11. Number of organisms / test chamber:	10
12. Number of replicate test chambers / treatment:	8
13. Feeding regime:	YCT, 1.5 mL daily per test chamber
14. Aeration:	None, unless D.O. drops below 40% saturation 3.4 mg/L. Additional renewals are preferred to aeration to maintain acceptable D.O. levels
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment	Formulated sediment
17. Test chamber cleaning:	Drainage screens as needed
18. Monitoring:	
Temperature	Daily (overlying water)
Dissolved oxygen	Daily (overlying water)
pH	Daily (overlying water)
Conductivity	Days 0, 5, and 10 (overlying water)
Alkalinity and hardness	Days 0 and 10 (overlying water)
Ammonia	Days 0 and 10 (overlying water)
Organism behavior	Daily
19. Test duration:	10 days
20. End points:	Survival and growth (organism dry weight) by replicate on Day 10
21. Reference toxicant:	Potassium chloride 96-h acute, water only
22. Test acceptability:	Minimum mean control survival of 80%
23. Data analysis:	Hypothesis tests versus the control or the reference site responses

C-3b

AQUATEC BIOLOGICAL SCIENCES

**AMPHIPOD, *Hyalella azteca*, 42-DAY
SURVIVAL, GROWTH AND REPRODUCTION
TOXICITY TEST FOR SEDIMENTS**

**Standard Operating Procedure
for
Amphipod, *Hyalella azteca*, 42-day Survival, Growth and Reproduction
Toxicity Test for Sediments**

1.0 OBJECTIVE

This SOP describes procedures for performing a 42-day whole sediment survival, growth, and reproduction toxicity test. This test is used to estimate the chronic toxicity of whole sediment samples to the freshwater amphipod, *Hyalella azteca*. End points measured include survival (Days 28, 35, and 42); growth (Days 28 and 42), and reproduction (number of neonates produced from Day 28 to 42, assessed on Days 35 and 42). When required, toxicity is estimated by statistical comparisons of survival, growth (dry weight), and reproduction to the organism responses in the control or reference site sediment. This procedure is based on the draft guidelines of EPA/600/R-98/XXX (New number pending): *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* Second Edition, Method 100.4.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

2.0 PREPARATION

2.1 Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Ammonia selective electrode and meter
- Mettler M3 Microbalance
- VWR 1320 drying oven

Additional Equipment:

- Test chambers (300-ml beakers, 8 per sample)
- 0.5 mm Nitex mesh substrate (2 cm x 2 cm) for water-only exposure
- Aeration manifold, tubing, manifold, and pipets
- Automated water-delivery system
- Disposable polyethylene transfer pipets
- Light tables
- Waste collection bucket
- Carolina bowls

Nitex mesh sieves (0.3 mm)

Reagents:

Reconstituted moderately hardwater (EPA/600/R-94/024)
Deionized water
70 percent Ethanol

Forms and Paperwork:

Amphipod (*Hyalella azteca*) Water Chemistry Data
Amphipod (*Hyalella azteca*) Daily Biological Monitoring
Amphipod (*Hyalella azteca*) Day 28 Survival and Growth Data
Amphipod (*Hyalella azteca*) Days 35 Survival and Reproduction Data
Sediment Characterization Data
Organism Holding and Acclimation
Daily Checklist for Automated Delivery System
Project Documentation Forms

2.2 Test System and Conditions

The test system and environmental conditions for the 42-day survival, growth, and reproduction test are summarized in Figure 1.

2.3 Test Organisms

2.3.1 Procurement and Documentation

Amphipods are obtained from a commercial supplier or from in-house cultures. If possible, schedule delivery of amphipods at least 48 hours prior to test initiation. They are acclimated to the exposure water used in testing during the the period prior to test initiation. Sources of amphipods include:

Environmental Consulting and Testing: (800) 377-3657

Aquatic BioSystems: (800) 331-6916

Prior to the testing, order sufficient organisms for 10 amphipods per replicate test chamber (120 per test sample) and a surplus for reference toxicant testing. Request that the supplier provide information regarding the age and environmental conditions for the test organisms.

Amphipods are shipped by next-day carrier and delivered to Aquatec Biological Sciences. The amphipods are typically shipped in 500-mL plastic container. Upon receipt, examine the organisms and document their apparent condition and the dissolved oxygen (D.O.), pH, temperature and conductivity of the shipping water. Record the observations on the Organism Data Sheet provided by the supplier. Place a copy of this sheet in the project data package.

2.3.2 Evaluation of Amphipod Condition

If, during examination, it appears that more than 5% of the organisms have died during transport, or if the temperature or other environmental conditions are different from test requirements (e.g., dissolved oxygen <4 mg/L, temperature <15°C), notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document the reason on the Project Documentation form. Also, it may be necessary to notify the client.

2.3.3 Acclimation and Holding

Transfer the amphipods to a 2-L plastic storage container. Add incremental amounts of laboratory reconstituted water and acclimate to test temperature (23°C). Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, D.O. during the holding period and record the monitoring data on the Organism Holding and Acclimation form. Amphipods should be 7-8 days old when the test is started. If more than five percent of the organisms die during the holding period, contact the Laboratory Manager and arrange for a replacement order.

2.3.4 Food

Feed daily sufficient *Selenastrum* and YCT to maintain a monolayer of food on the bottom of the container.

2.4 Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-94/024 mixed 1:1 with natural river water (Lamoille River, Vermont) is used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is formulated sediment prepared according to the procedure outlined in EPA/600/R-94/024 (Section 7.2.3.2) and consists of 77% fine and medium sand, 17% kaolinite clay, 5% ground peat, and 1% calcium carbonate. The formulated sediment is stored dry and is hydrated by addition of reconstituted moderately hardwater prior to distribution to test chambers.

3.2 Test Sediment Preparation

1. Remove sediment samples from Sample Management refrigerators.
2. Transfer the sample to the ventilation hood in the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic "spaghetti fork-it" spatula;
4. Transfer aliquots of the homogenized sediment to a glass tray and examine for indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for amphipods), transfer approximately 100 mL aliquots to each of the replicate test chambers;
6. If indigenous organisms (especially predacious insects or amphipods) are present, remove them with forceps or press sieve sediment through a 0.5 or 1.0 mm Nitex mesh sieve, re-homogenize, and then distribute 100-mL aliquots to each of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments. Sieving of sediments should be avoided if possible;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
8. Add overlying water to a final volume of approximately 275 mL;
9. Return the unused sediment sample to Sample Management for storage;
10. Transfer the test chambers to the automated water delivery system and begin the water renewal cycles (noon and midnight). The test replicates remain in the test system overnight without addition of test organisms.

3.2.1 Measuring Initial Overlying Water Chemistry

On the day of test initiation, remove an aliquot of overlying water from at least one replicate of each test sample. Measure the following parameters: pH, DO, temperature, and conductivity. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also stored and preserved for Day 0 alkalinity, hardness, and ammonia analyses. The temperature of the exposure water must be within the range of $23 \pm 1^\circ\text{C}$. D. O. should be $\geq 40\%$ saturation (3.4 mg/L). Additional water exchanges or aeration may be required if D.O. levels do not remain above 40% saturation.

3.2.2 Test Initiation: Preparation and Distribution of Test Organisms

1. Place the amphipod holding container over a light table and use a disposable polyethylene transfer pipet to transfer amphipods to 1-oz. (30 mL) disposable cups (Dixie condiment cups) until each cup contains 10 amphipods. Prepare sufficient cups for one per test replicate plus several spares. Sufficient amphipods (60) should be reserved for a standard reference toxicant test and to archive a representative subsample of the amphipod test population (10-20).
2. Randomly select a cup containing 10 amphipods. Examine them over a light table and replace any apparently unhealthy or injured amphipods.

3. Gently rinse the 10 amphipods into a test replicate with clean exposure water using a transfer pipet. Check to be sure that all amphipods have been removed from the cup and swim to the sediment in the test replicate. A drop of exposure water can be used to submerge any amphipods that get trapped on the water surface. **WARNING: Do not dip condiment cups into the exposure water.**
4. Record the date and time of test initiation when amphipods have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
5. After one hour, check all test replicates and replace any amphipods which are floating or are dead.
6. Preserve a representative sample of 10-20 amphipods with 70% ethanol for archiving. After measurement of initial lengths, the amphipods should be stored six months as a reference stock identified by testing group (BTR) and date.

3.3 Daily Monitoring

3.3.1 Environmental Conditions

The environmental conditions monitoring schedule and list of parameters is outlined in Table 1. On Days 0, 28, 35, and 41 preserve a portion of the overlying water sample used for water quality determinations (approximately 100 mL) with 0.3 mL of concentrated H₂SO₄ for ammonia-N analysis and collect subsamples of overlying water for alkalinity and hardness analyses. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

3.3.2 Biological Monitoring

Test organism observations are made daily for all test replicates. Position lighting to illuminate the overlying water column and the sediment surface for each replicate. Examine and record observations such as amphipods not buried or dead (not removed). Replace the test chamber to its assigned position.

3.3.3 Feeding

Provide 1.0 mL of YCT to each test replicate daily. If the D.O. drops below 40% saturation due to the accumulation of uneaten food, feeding may be suspended for 1-2 days. Document these events and increase the water renewal frequency (or aerate), if needed, to maintain acceptable D.O levels.

3.3.4 Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly.

3.4 End-point Determination and Water-only Exposure

3.4.1 Day 28 Survival

1. Transfer each test replicate to a light table equipped with side lighting. Search for amphipods and remove both live or dead amphipods with a transfer pipet. Decant the overlying water through a 0.3 mm sieve. Rinse the sediment through a 0.3 mm sieve. Pool all surviving amphipods from a single replicate into a 30-mL disposable cup. Count and record the total number of surviving amphipods observed on the Survival Data Form. If organisms appear to be dead, examine them under a dissecting microscope. If any movement is detected, the amphipod is considered to be alive.
2. If fewer than 10 amphipods are recovered, transfer all sediment and material that has not passed through the 0.3 mm sieve back into the test chamber and hold the replicates for a possible reexamination. The test material may be preserved with sugar formalin solution and Rose-Bengal Stain for a subsequent re-pick. Stained amphipods found during the repick will be assumed to have been alive on Day 28 if the body tissue is not significantly degraded. The total number surviving will then be corrected.

3.4.2 Day 28 Growth (4 Replicates)

Select four of the 12 replicate cups containing surviving amphipods (e.g., Replicates I, J, K, L) for Day 28 growth analysis. Growth is based upon the mean dry weight of pooled surviving amphipods, for the selected replicates. Transfer surviving amphipods to pre-weighed weighing boats (boat weights recorded on the Amphipod (*Hyalella azteca*) Day 28 Survival and Growth Data form) and dry overnight in the drying oven at 60°C. Weigh the dried amphipods to the nearest 0.01 mg. The Mettler M3 microbalance is used for all dry weight determinations.

3.4.3 Water-only Exposure (8 Replicates)

Decant all sediment and overlying water from the test beakers. Select test replicate beakers A, B, C, D, E, F, G, and H. Rinse them with deionized water, fill with exposure water, and add two squares of Nitex mesh substrate. Transfer the surviving amphipods, replicates A, B, C, D, E, F, G, and H, back into the appropriate replicate test chamber. Daily monitoring activities continue as described (Figure 1).

3.4.4 Day 35 Survival and Reproduction (8 Replicates)

On Day 35 of the test, remove each test replicate to a light table. Count and record the number of surviving adult amphipods, the number of amplexus pairs, and the number of neonates (hatched young). Record data on the Day 35 Survival and Reproduction form. The surviving adult amphipods remain in the test replicate while the neonates are removed. Return the test replicates to the testing system.

3.4.5 Day 42 Survival, Reproduction, and Growth (8 Replicates)

On Day 42 of the test, remove each test replicate to a light table. Count and record the number of surviving adult amphipods, the number of amplexus pairs, number of adult females, and number of adult males, and the number of neonates (hatched young). Record data on the Day 42 Survival, Reproduction, and Growth form.

Growth for Replicates A, B, C, D, E, F, G, and H is based upon the mean dry weight of pooled surviving amphipods, for the selected replicates. Transfer surviving amphipods to pre-weighed weighing boats (boat weights recorded on the Amphipod (*Hyalella azteca*) Day 42 Survival, Reproduction, and Growth Data form) and dry overnight in the drying oven at 60°C. Weigh the dried amphipods to the nearest 0.01 mg using the Mettler M3 microbalance.

4.0 QUALITY ASSURANCE

4.1 Blind Sample Analysis

Each sample, including the Control, will be assigned a unique sample number which will be used throughout the test.

4.2 Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-98/XXX, Table 14.3. Specifically, a test is judged to be acceptable if the average survival of control amphipods is equal to or greater than 80% on Day 28. The environmental conditions must be within the tolerance limits of *Hyalella azteca*.

4.3 Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

4.4 Reference Toxicant Testing

A water-only 96-hour exposure of amphipods to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of amphipods. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.

Receive verbal and visual instruction.

Demonstrate 90% recovery of 10 amphipods in trial sediments.

Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the amphipod (*Hyalella azteca*) 42-day whole sediment chronic toxicity test.

ASSOCIATED PROTOCOLS: EPA 1998. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Ed. (EPA/600/R-94/024) Method 100.4.

1. Test type:	Whole-sediment toxicity (static)
2. Temperature:	23 ± 1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Light illuminance:	500 to 1000 lux
5. Photoperiod:	16 hr. light, 8 hr. dark
6. Test chamber size:	300 mL beaker
7. Sediment volume:	100 mL (Days 0-28). Water-only exposure Days 28-42
8. Overlying water volume:	175 mL (Days 0-28), 275 mL (Days 28-42)
9. Renewal of overlying water:	Every 12 hours
10. Age of test organism:	7 - 8 days
11. Number of organisms / test chamber:	10
12. Number of replicate test chambers / treatment:	12 (4 for 28-day survival and growth, 8 for days 28-42 survival, reproduction, and growth)
13. Feeding regime:	1.0 mL YCT daily per replicate test chamber
14. Aeration:	None, unless D.O. drops below 40% saturation
15. Overlying water:	Reconstituted moderately hard water and natural river water (1:1)
16. Control sediment	Formulated sediment
17. Test chamber cleaning:	Drainage screens daily as needed
18. Monitoring:	
Temperature	Daily, Days 0-42 (overlying water)
Dissolved oxygen	Daily Days 0-28, 3 times weekly Days 29-41 (overlying water)
pH	3 times weekly Days 0-41 (overlying water)
Conductivity	Weekly Days 0-41 (overlying water)
Alkalinity and hardness	Days 0, 28, 25, and 41 (overlying water)
Ammonia	Days 0, 28, 25, and 41 (overlying water)
Organism behavior	Daily
19. Test duration:	42 days
20. End points:	Survival and growth (Day 28); Survival (Day 35), Survival, Reproduction, and Growth (Day 42)
21. Reference toxicant:	Potassium chloride 96-h acute, water only
22. Test acceptability:	Minimum mean control survival of 80% on Day 28
23. Data analysis:	Hypothesis tests versus the control or the reference site responses

Standard Operating Procedures

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AQUATEC BIOLOGICAL SCIENCES

**MIDGE *Chironomus tentans*, 10-DAY
SURVIVAL AND GROWTH
TOXICITY TEST FOR SEDIMENTS**

**Standard Operating Procedure
for
Midge, *Chironomus tentans*, 10-day Survival and Growth
Toxicity Test for Sediments**

1.0 OBJECTIVE

This SOP describes procedures for performing a ten-day whole sediment survival and growth toxicity test. This test is used to estimate the toxicity of whole sediment samples to the freshwater midge, *Chironomus tentans*. When required, toxicity is estimated by statistical comparisons to the control sediment or reference sediment. This procedure is based on the guidelines of EPA/600/R-94/024: *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* Method 100.2.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling samples.

2.0 PREPARATION

2.1 Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Ammonia-selective electrode and meter
- Mettler M3 Microbalance
- VWR 1320 drying oven

Additional Equipment:

- Test chambers (300-ml beakers, 8 per sample)
- Aeration manifold, tubing, manifold, and pipets
- Automated water-delivery system
- Disposable polyethylene transfer pipets
- Light tables
- Waste collection bucket
- Carolina bowls
- Nitex mesh sieves (0.5 mm)

Reagents:

- Reconstituted moderately hardwater (EPA/600/R-94/024)
- Deionized water

70 percent Ethanol

Forms and Paperwork:

Midge (*Chironomus tentans*) Water Chemistry Data
Midge (*Chironomus tentans*) Daily Biological Monitoring
Midge (*Chironomus tentans*) 10-Day Survival and Growth Data
Sediment Characterization Data
Organism Holding and Acclimation
Daily Checklist for Automated Delivery System
Project Documentation Forms

2.2 Test System and Conditions

The test system and environmental conditions for the 10-day survival and growth test are summarized in Figure 1.

2.3 Test Organisms

2.3.1 Procurement and Documentation

Midges are obtained from in-house cultures. Approximately 12 days before testing, adult male and female midges are isolated in mating flasks overnight. The next morning, freshly deposited egg cases are transferred to a petri dish containing culture water. After two days (at 23°C) larvae should begin to hatch from the egg case. Transfer egg cases with hatching larvae to a culture box containing culture water and a monolayer of culture substrate (fine and medium sand). Maintain the culture approximately 8-9 days (post-hatch) until the larvae reach third instar. They are acclimated to the exposure water used in testing during the period prior to test initiation.

Sufficient egg cases should be harvested to obtain 10 midge larvae per replicate test chamber (80 per test sample) and a surplus for reference toxicant testing. Plan on a yield of approximately 200 larvae per egg case. Record culture conditions in the *Chironomus tentans* Culture Log.

2.3.2 Evaluation of Midge Condition

Examine the condition of the organisms to be used in testing, if it appears that more than 5% of the organisms have died or if the temperature or other environmental conditions are different from test requirements (e.g., dissolved oxygen <4 mg/L, temperature <15°C), notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document the reason on the Project Documentation form. Also, it may be necessary to notify the client.

2.3.3 Acclimation and Holding

Midge larvae are held in a 2-L plastic storage container. Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, and dissolved oxygen during the growout/acclimation period. Record monitoring data on the *Chironomus tentans* Culture form.

2.3.4 Food

Feed daily *Selenastrum* for Days 0-1 after larvae begin to hatch. Shift to a 1:1 slurry of Cerophyll and YCT on Day 2 (post-hatch) with increasing amounts (e.g., 1-3 mL) as the larvae grow (evident from increases in the size of the substrate tubes).

2.4 Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-94/024 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is formulated sediment prepared according to the procedure outlined in EPA/600/R-94/024 (Section 7.2.3.2) and consists of 77% fine and medium sand, 17% kaolinite clay, 5% ground peat, and 1% calcium carbonate. The formulated sediment is stored dry and is hydrated by addition of reconstituted moderately hardwater prior to distribution to test chambers.

3.2 Test Sediment Preparation

1. Remove sediment samples from Sample Management refrigerators.
2. Transfer the sample to the ventilation hood in the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic "spaghetti fork-it" spatula or other suitable utensil;
4. Transfer aliquots of the homogenized sediment to a glass tray and examine for indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for midges), transfer approximately 100 mL aliquots to each of the replicate test chambers;
6. If indigenous organisms (especially predacious insects or midges) are present, remove them with forceps or press sieve sediment through a 0.5 or 1.0 mm Nitex mesh sieve, re-homogenize, and then distribute 100-mL aliquots to each

- of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments. Sieving of sediments should be avoided if possible;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
 8. Add overlying water to a final volume of approximately 275 mL;
 9. Return the unused sediment sample to Sample Management for storage;
 10. Transfer the test chambers to the automated water delivery system and begin the water renewal cycles (noon and midnight). The test replicates remain in the test system overnight without addition of test organisms.

3.2.1 Measuring Initial Overlying Water Chemistry

On the day of test initiation, remove an aliquot of overlying water from replicates of each test sample. Measure the following parameters: pH, dissolved oxygen (D.O.), temperature, and conductivity. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also preserved and stored for Day 0 alkalinity, hardness, and ammonia analyses. The temperature of the exposure water must be within the range of $23 \pm 1^\circ\text{C}$. Dissolved oxygen should be $\geq 40\%$ saturation (3.4 mg/L). Additional water exchanges may be required if D.O. levels do not remain above 40% saturation.

3.2.2 Test Initiation: Preparation and Distribution of Test Organisms

1. Place the midge holding container over a light table and use a disposable polyethylene transfer pipet to transfer 10 midge larvae directly to each test replicate. Sufficient midges (60) should be reserved for a standard reference toxicant test and to archive a representative subsample (10-20) of the midge test population.
2. Check to be sure that all midges swim to the sediment in the test replicate. A drop of exposure water can be used to submerge any midges that get trapped on the surface.
3. Record the date and time of test initiation when midges have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
4. After one hour, check all test replicates and replace any midges which are floating or have not burrowed or are dead.
5. Preserve a representative sample of 10-20 midges with 70% ethanol for determination of instar stage by head capsule measurement.

3.3 Daily Monitoring

3.3.1 Environmental Conditions

The environmental conditions monitoring schedule and list of parameters is outlined in Table 1. On Days 0 and 10 preserve a portion of the overlying water sample used for

water quality determinations (approximately 100 mL) with 0.3 mL of concentrated H₂SO₄ for ammonia-N analysis. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

3.3.2 Biological Monitoring

Test organism observations are made daily for all test replicates. Position lighting to illuminate the overlying water column and the sediment surface for each replicate. Examine and record observations such as midges not buried or dead midges (not removed). Replace the test chamber to its assigned position.

3.3.3 Feeding

Provide 1.5 mL of Tetrafin slurry (4.0 mg/mL) to each test replicate daily

3.3.4 Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly.

3.4 Termination of the Whole Sediment Toxicity Test

3.4.1 Final Chemistry

Decant an aliquot of exposure water from several test replicates and pool to obtain sufficient water for the Day 10 water chemistry analyses. Measure and record the final chemistry parameters as specified in Figure 1.

3.4.2 Day 10 Survival

1. Decant the overlying water and sediment into a 0.5 mm sieve. Rinse the sediment through the sieve. Pool all midges from a single replicate into a labeled 30-mL disposable cup. Count and record the total number of midges surviving on the Survival and Growth Data Form. If organisms appear to be immobile and discolored, they are considered to be dead and are not included in the growth analysis. If any movement is detected, the midge is considered to be alive.
2. If fewer than 10 midges are recovered, transfer all sediment and material that has not passed through the 0.5 mm sieve back into the test chamber and hold the replicates for a possible reexamination. The test material may be repicked. If additional surviving midges are found, the total number surviving will then be corrected.

3.4.2 Day 10 Growth

Growth is based upon the mean dry weight of pooled surviving midges, by replicate. Transfer surviving midges to pre-weighed weighing boats (data recorded on the Midge (*Chironomus tentans*) 10-Day Survival and Growth Data form) and dry overnight in the drying oven at 60°C. Weigh the dried midges to the nearest 0.01 mg. The Mettler M3 microbalance is used for all dry weight determinations.

4.0 QUALITY ASSURANCE

4.1 Blind Sample Analysis

Each sample, including the Control, will be assigned a unique sample number which will be used throughout the test.

4.2 Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-94/024, Table 11.1. Specifically, a test is judged to be acceptable if the average survival of control midges is equal to or greater than 70% and the mean weight of the control organisms is >0.6mg/organism at the end of the test. The environmental conditions must be within the tolerance limits of *Chironomus tentans*.

4.3 Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

4.4 Reference Toxicant Testing

A water-only 96-hour exposure of midges to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of midges. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.

Receive verbal and visual instruction.

Demonstrate 90% recovery of 10 midges in trial sediments.

Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the midge (*Chironomus tentans*) 10-day whole sediment toxicity test

ASSOCIATED PROTOCOLS: EPA 1994. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (EPA/600/R-94/024) Method 100.2

1. Test type:	Whole-sediment toxicity (static)
2. Temperature:	23 ± 1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Light illuminance:	500 to 1000 lux
5. Photoperiod:	16 hr. light, 8 hr. dark
6. Test chamber size:	300 mL beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	Every 12 hours
10. Age of test organism:	Third instar or younger (50% or more in third instar)
11. Number of organisms / test chamber:	10
12. Number of replicate test chambers / treatment:	8
13. Feeding regime:	Tetrafin slurry (1 mg/mL), 1.5 mL daily
14. Aeration:	None, unless D.O. drops below 40% saturation (3.4 mg/L). Additional renewals are preferred to aeration to maintain acceptable D.O. levels
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment	Formulated sediment
17. Test chamber cleaning:	Drainage screens daily as needed
18. Monitoring:	
Temperature	Daily (overlying water)
Dissolved oxygen	Daily (overlying water)
pH	Daily (overlying water)
Conductivity	Days 0, 5, and 10 (overlying water)
Alkalinity and hardness	Days 0 and 10 (overlying water)
Ammonia	Days 0 and 10 (overlying water)
Organism behavior	Daily
19. Test duration:	10 days
20. End points:	Survival and growth (organism dry weight) by replicate on Day 10
21. Reference toxicant:	Potassium chloride 96-h acute, water only
22. Test acceptability:	Minimum mean control survival of 70% and mean control weights must be >0.6mg/organisms
23. Data analysis:	Hypothesis tests versus the control or the reference site responses

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AQUATEC BIOLOGICAL SCIENCES

**MIDGE *Chironomus tentans* CHRONIC WHOLE
SEDIMENT TOXICITY TEST**

**Standard Operating Procedure
for
Midge *Chironomus tentans* Chronic Whole Sediment Toxicity Test**

1.0 OBJECTIVE

This SOP describes procedures for performing a chronic whole sediment survival and growth toxicity test. This test is used to estimate the toxicity of whole sediment samples to the freshwater midge, *Chironomus tentans*. Organisms are exposed, for forty or more days, to a whole sediment sample. Endpoint measurements include Day 20 survival and ash-free dry weight, cumulative emergence during the test, adult mortality, and reproduction (which may include number of egg cases deposited, number of eggs per egg case, and number of hatched larvae per egg case). When required, toxicity is estimated by statistical comparisons to the control sediment or reference sediment. This procedure is based on the draft guidelines of EPA/600/R-98/XXX (New number pending): *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* Second Edition, Method 100.5.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling samples.

2.0 PREPARATION

2.1 Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Ammonia-selective electrode and meter

Additional Equipment:

- Test chambers (300-ml beakers, 16 per sample)
- Screened emergence traps
- Aeration manifold, tubing, manifold, and pipettes
- Automated water-delivery system
- Disposable polyethylene transfer pipettes
- Light tables
- Waste collection bucket
- Carolina bowls
- Nitex mesh sieves (0.5 mm)
- Mettler M3 Microbalance
- Drying oven
- Muffle furnace

Compound and dissecting microscopes

Reagents:

Reconstituted moderately hardwater (EPA/600/R-94/024)
Deionized water

Forms and Paperwork:

Midge (*Chironomus tentans*) Water Chemistry Data
Midge (*Chironomus tentans*) Daily Biological Monitoring
Midge (*Chironomus tentans*) 20-Day Survival and Growth Data
Midge (*Chironomus tentans*) Daily Emergence Data
Midge (*Chironomus tentans*) Adult Mortality Data
Midge (*Chironomus tentans*) Egg Case Deposition and Hatching Data
Midge (*Chironomus tentans*) 20-Day Survival and Growth Data
Midge (*Chironomus tentans*) End-of-test Larval Survival Data
Sediment Characterization Data
Chironomus tentans Culture Log
Daily Checklist for Automated Delivery System
Project Documentation Forms

2.2 Test System and Conditions

The test system and environmental conditions for the *Chironomus tentans* chronic toxicity test are summarized in Figure 1.

2.3 Test Organisms

2.3.1 Procurement and Documentation

Midges are obtained from in-house cultures. Approximately 3-4 days before testing, adult male and female midges are isolated in mating flasks overnight. The next morning transfer freshly deposited egg cases to a petri dish containing culture water. After two days (at 23°C) larvae should begin to hatch from the egg cases. Feed each petri dish with approximately 1 ml of a *Selenastrum* food stock. Larvae less than 24-hours old are used for testing. They are acclimated to the exposure water used in testing during the 2 to 3 day period prior to test initiation.

Sufficient egg cases are needed to obtain 12 midge larvae per replicate test chamber (144 per test sample). Additional larvae should be grown out to provide a surplus for reference toxicant testing. Plan on a yield of approximately 200 larvae per egg cases. Record culture conditions in the *Chironomus tentans* Culture Log.

2.3.2 Evaluation of Midge Condition

Examine the egg cases daily prior to testing, to be sure that sufficient larvae are likely to hatch on the day the tests are started. If there is to be a delay in initiating the tests, it may be necessary to notify the client.

2.3.3 Acclimation and Holding

Egg cases are incubated in a Petri dish containing reconstituted moderately hard water during the pre-hatch and pre-test period. The temperature of the culture water should be maintained at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

2.3.4 Food

Provide a monolayer of *Selenastrum* when the larvae begin to hatch and move from the egg case.

2.4 Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-94/024 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is formulated sediment prepared according to the procedure outlined in EPA/600/R-94/024 (Section 7.2.3.2) and consists of 77% fine and medium sand, 17% kaolinite clay, 5% ground peat, and 1% calcium carbonate. The formulated sediment is stored dry and is hydrated by addition of reconstituted moderately hardwater prior to distribution to test chambers.

3.2 Test Sediment Preparation

1. Remove sediment samples from Sample Management refrigerators.
2. Transfer the sample to the ventilation hood in the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic "spaghetti fork-it" spatula;
4. Transfer aliquots of the homogenized sediment to a glass tray and examine for indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for midges), transfer approximately 100 ml aliquots to each of the replicate test chambers;
6. If indigenous organisms (especially predacious insects or midges) are present, remove them with forceps or press sieve sediment through a 0.5 or 1.0 mm Nitex mesh sieve, re-homogenize, and then distribute 100-mL aliquots to each

- of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments. Sieving of sediments should be avoided if possible;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
 8. Add overlying water to a final volume of approximately 275 ml;
 9. Return the unused sediment sample to Sample Management for storage;
 10. Transfer the test chambers to the automated water delivery system and begin the water renewal cycles (noon and midnight). The test replicates remain in the test system overnight without addition of test organisms.

3.2.1 Measuring Initial Overlying Water Chemistry

On the day of test initiation, remove an aliquot of overlying water from replicates of each test sample. Measure the following parameters: pH, dissolved oxygen (D.O.), temperature, and conductivity. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also preserved and stored for Day 0 alkalinity, hardness, and ammonia analyses. The temperature of the exposure water must be within the range of $23 \pm 1^\circ\text{C}$. Dissolved oxygen should be ≥ 2.5 mg/L. Additional water exchanges may be required if D.O. levels do not remain above 2.5 mg/L.

3.2.2 Test Initiation: Preparation and Distribution of Test Organisms

1. Place the Petri dishes holding egg cases with hatching larvae on the stage of a dissecting microscope. Larvae that are actively swimming from the egg case are transferred using a Pasteur pipette directly to a test replicate. Twelve larvae are distributed to each replicate. Sufficient midges should be reserved for a standard reference toxicant test (these will be grown out to third instar) and to archive a representative subsample (10-20) of the midge test population.
2. Check to be sure that all midges swim to the sediment in the test replicate. A drop of exposure water can be used to submerge any midges that get trapped at the water surface.
3. Record the date and time of test initiation when midges have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
4. After one hour, check all test replicates and replace any midges that are floating.
5. Preserve a representative sample of 10-20 midges with 70% ethanol for archival.

3.3 Daily Monitoring

3.3.1 Environmental Conditions

The environmental conditions monitoring schedule and list of parameters are outlined in Table 1. On Days 0, 20, and end of test, preserve a portion of the overlying water sample used for water quality determinations (approximately 100 ml) with 0.3 ml of concentrated H₂SO₄ for ammonia-N analysis. Samples for alkalinity and hardness determinations are collected at the same time intervals. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

3.3.2 Biological Monitoring

Test organism observations are made daily for all test replicates. Position lighting to illuminate the overlying water column and the sediment surface for each replicate. Examine and record observations such as midges not buried or dead midges (not removed) or pupating larvae. Replace the test chamber to its assigned position.

3.3.3 Feeding

Provide 1.0 ml of Tetrafin slurry (4.0 mg/ml) to each test replicate daily. If the D.O. drops below 3.0 mg/L due to accumulation of uneaten food, feeding may be suspended for 1-2 days to stabilize the dissolved oxygen. The frequency of water renewals may also be increased to help maintain acceptable dissolved oxygen concentrations.

3.3.4 Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly.

3.4 Auxiliary Male Production

On Day 9 of the test, an additional four replicates of each test sample are set up. On Day 10 these are inoculated with <24-hour old larvae, 12 per replicate. Egg cases collected from cultures on Days 6 or 7 are used as a source of larvae on Day 10. The auxiliary test beakers will be used as a source of male adults (emerged flies) for continued pairings, by test sample, near the end of the test. Auxiliary males are required because males tend to emerge earlier than females.

3.5 End Point Determinations

3.5.1 Day 20 Survival and Growth

Select Replicates I, J, K, L from each treatment and sieve the sediment to recover the larvae for survival and growth determinations. Record the number of surviving larvae on the Midge (*Chironomus tentans*) 20-Day Survival and Growth Data form. Surviving midges in these replicates will be used to determine ash-free dry weights. Combine the larvae from each replicate on ashed weighing pans and dry the larvae at 60°C for 24 hours. Weigh each replicate weigh pan to 0.01 mg. Ash the replicate pans at 550°C for 2 hours. Re-weigh the ashed larvae. The tissue mass is the difference between the weight of the dried larvae (plus pan) and the weight of the ashed larvae (plus pan).

3.5.2 Emergence

Larvae will begin pupating and emerge as adult flies after Day 20. Install emergence traps on each of the remaining test replicates (Replicates A, B, C, D, E, F, G, and H) on Day 20. Record the number of larvae pupating and the number of males and females emerged each day on the Midge (*Chironomus tentans*) Daily Emergence Data form.

3.5.3 Reproduction and Adult Mortality

Transfer emerged adults daily from individual replicates (of the same sample) to a Reproduction/Oviposit (R/O) chamber using the transfer syringe. Males from a different replicate may be paired with females of replicates where no males have emerged. After Day 33, males collected from the auxiliary male replicates may be needed to create mating pairs. For each emerged female (from any replicate of a sediment sample), at least one male (obtained from the same replicate, or another replicate of the same sample, or from an auxiliary male replicate of the same sample) is transferred to the R/O chamber. Tabulate the number of egg cases deposited daily, and record adult mortality on the Midge (*Chironomus tentans*) Adult Mortality Data form.

3.5.4 Egg Counts and Egg Hatching

Transfer primary egg cases (the first egg case hatched by a female) from the R/O chamber to a petri dish. Estimate the number of eggs per egg case by the "ring method" using a dissecting or compound microscope. Incubate the egg cases from each treatment separately for 6 days. Determine hatching success (proportion hatched) by counting the unhatched eggs and subtracting that value from the original egg count.

3.5.5 Ending the Test

The test is ended after seven consecutive days of no emergence in a given treatment. When no emergence is recorded in a treatment at any time during the test, that treatment can be ended once emergence in the control sediment has stopped (using the 7-day criterion). End the test by sieving to recover surviving larvae or pupae that have not emerged. These data are recorded on the Midge (*Chironomus tentans*) End-of-test Larval Survival Data form.

4.0 QUALITY ASSURANCE

4.1 Blind Sample Analysis

Each sample, including the Control, will be assigned a unique sample number that will be used throughout the test.

4.2 Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-98/XXX, Table 15.3. Specifically, a test is judged to be acceptable if the average survival of control midges (cumulative total of successfully emerged larvae and surviving larvae which do not emerge) is equal to or greater than 70% at the end of the test. The average size of larvae on Day 20 in the Control must be at least 0.6 mg/surviving larva (as dry weight). The environmental conditions must be within the tolerance limits of *Chironomus tentans*.

4.3 Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

4.4 Reference Toxicant Testing

A water-only 96-hour exposure of midges to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of midges. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.

Receive verbal and visual instruction.

Demonstrate 90% recovery of 10 midges in trial sediments.

Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the midge (*Chironomus tentans*) chronic whole sediment survival toxicity test.ASSOCIATED PROTOCOLS: EPA 1998. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (EPA/600/R-94/024) Second Edition, Method 100.5*

1. Test type:	Whole-sediment toxicity (static)
2. Temperature:	23 ± 1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Light illuminance:	500 to 1000 lux
5. Photoperiod:	16 hr. light, 8 hr. dark
6. Test chamber size:	300 ml beaker
7. Sediment volume:	100 ml
8. Overlying water volume:	175 ml
9. Renewal of overlying water:	Every 12 hours
10. Age of test organism:	Larvae, less than 24 hours
11. Number of organisms / test chamber:	12
12. Number of replicate test chambers / treatment:	12
13. Feeding regime:	Tetrafin slurry (1 mg/ml), 1.0 ml daily. Suspended if food accumulates.
14. Aeration:	None, unless D.O. drops below 2.5 mg/L). Additional renewals if needed
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment	Formulated sediment
17. Test chamber cleaning:	Drainage screens daily as needed
18. Monitoring:	
Temperature	Daily (overlying water)
Dissolved oxygen	Daily (overlying water), may be reduced to 3 times weekly after Day 20
pH	3 times weekly (overlying water)
Conductivity	Weekly (overlying water)
Alkalinity and hardness	Days 0, 20 and end of test (overlying water)
Ammonia	Days 0, 20 and end of test (overlying water)
Organism behavior	Daily
19. Test duration:	Until no emergence occurs for 7 days in control or test sediment
20. End points:	Survival and growth (Day 20), and end-of-test emergence, adult mortality, and reproduction
21. Reference toxicant:	Potassium chloride 96-h acute, water only
22. Test acceptability:	Day 20 mean control survival ≥70% and dry weight ≥0.6 mg/larvae
23. Data analysis:	Hypothesis tests versus the control or the reference site responses

C-3e

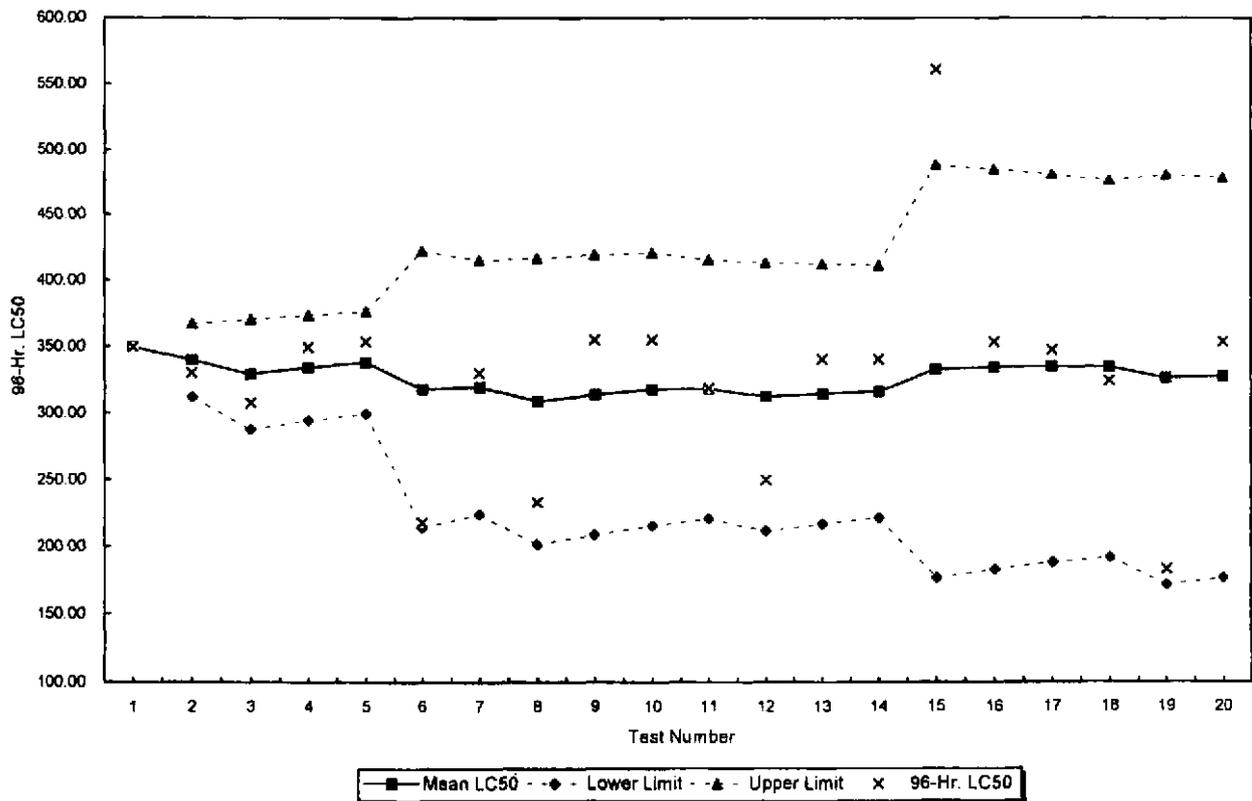
AQUATEC BIOLOGICAL SCIENCES
REFERENCE TOXICANT CONTROL CHART
Hyaella azteca
IN POTASSIUM CHLORIDE (mg/L)

Reference Toxicant Control Chart

Hyalella azteca

in Potassium chloride (mg/L)

Test Number	Test Date	Organism Age (Days)	96-Hr. LC50	Mean LC50	Lower Limit	Upper Limit	Organism Source
1	07/03/96	8	349.35	349.35			Env. Consult & Testing
2	07/12/96	8	329.88	339.62	312.08	367.15	Env. Consult & Testing
3	07/16/96	11	307.79	329.01	287.42	370.59	Env. Consult & Testing
4	07/25/96	8	349.35	334.09	294.51	373.68	Env. Consult & Testing
5	09/06/96	8	353.55	337.98	299.54	376.43	Env. Consult & Testing
6	09/27/96	10	217.64	317.93	213.82	422.03	Env. Consult & Testing
7	10/11/96	12	329.90	319.64	224.17	415.10	Env. Consult & Testing
8	02/14/97	10	233.26	308.84	201.41	416.27	Env. Consult & Testing
9	08/19/97	15	355.00	313.97	208.87	419.07	Env. Consult & Testing
10	08/19/97	15	355.00	318.07	215.64	420.50	Env. Consult & Testing
11	09/26/97	11	318.64	318.12	220.95	415.30	Env. Consult & Testing
12	12/20/97	10	250.00	312.45	211.79	413.10	Env. Consult & Testing
13	04/15/98	8	340.20	314.58	216.99	412.17	Env. Consult & Testing
14	04/17/98	10	340.20	316.41	221.65	411.17	Env. Consult & Testing
15	08/04/98	14	561.23	332.73	176.78	488.68	Env. Consult & Testing
16	08/22/98	10	353.55	334.03	183.01	485.06	Env. Consult & Testing
17	09/13/98	11	347.16	334.81	188.44	481.17	Env. Consult & Testing
18	10/26/98	12	324.21	334.22	192.13	476.30	Env. Consult & Testing
19	11/13/98	10	183.72	326.30	171.91	480.68	Env. Consult & Testing
20	02/19/99	9	353.55	327.66	176.90	478.42	Env. Consult & Testing



C-3f

AQUATEC BIOLOGICAL SCIENCES

REFERENCE TOXICANT CONTROL CHART

Chironomus tentans

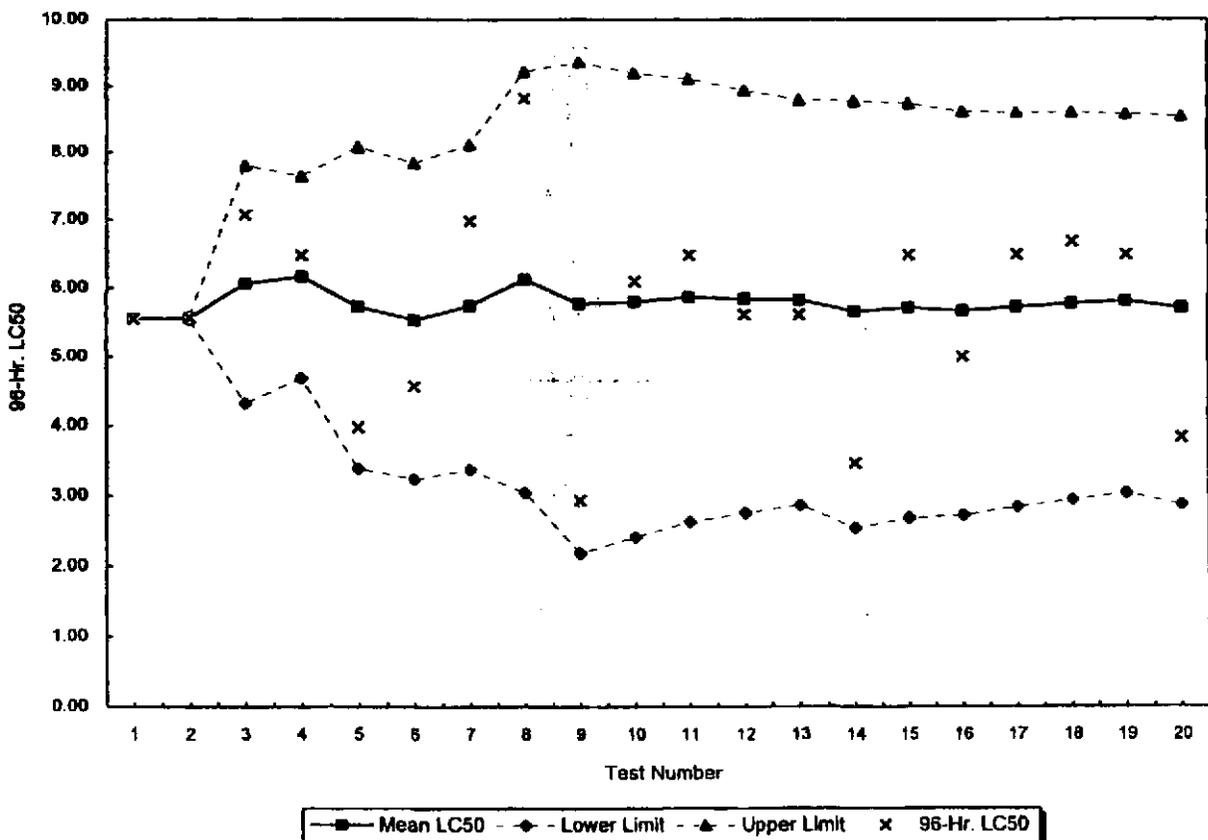
IN POTASSIUM CHLORIDE (g/L)

Reference Toxicant Control Chart

Chironomus tentans

in Potassium chloride (g/L)

Test Number	Test Date	Organism		96-Hr. LC50	Mean LC50	Lower Limit	Upper Limit	Organism Source
		Age (Days)						
1	10/19/96	10		5.55	5.55			Inchcape
2	08/19/97	9		5.57	5.56	5.53	5.59	Env. Consulting & Testing
3	09/17/97	12		7.07	6.06	4.32	7.81	Env. Consulting & Testing
4	09/26/97	10		6.48	6.17	4.68	7.65	Env. Consulting & Testing
5	10/01/97	9		3.98	5.73	3.39	6.07	Aquatec Biological Sciences
6	10/03/97	8		4.56	5.53	3.23	7.84	Aquatec Biological Sciences
7	10/08/97	11		6.98	5.74	3.37	8.11	Aquatec Biological Sciences
8	10/10/97	11		8.82	6.13	3.04	9.21	Aquatec Biological Sciences
9	10/14/97	11		2.93	5.77	2.18	9.36	Aquatec Biological Sciences
10	10/20/97	11		6.10	5.80	2.41	9.19	Aquatec Biological Sciences
11	10/21/97	11		6.48	5.86	2.62	9.11	Aquatec Biological Sciences
12	10/28/97	10		5.61	5.84	2.75	8.94	Aquatec Biological Sciences
13	10/31/97	9		5.61	5.83	2.86	8.79	Aquatec Biological Sciences
14	11/02/97	9		3.47	5.66	2.54	8.77	Aquatec Biological Sciences
15	11/09/97	10		6.48	5.71	2.68	8.75	Aquatec Biological Sciences
16	11/10/97	9		5.00	5.67	2.72	8.62	Aquatec Biological Sciences
17	08/23/98	11		6.48	5.72	2.83	8.60	Aquatec Biological Sciences
18	09/15/98	9		6.67	5.77	2.93	8.60	Aquatec Biological Sciences
19	10/23/98	10		6.48	5.81	3.03	8.58	Aquatec Biological Sciences
20	11/10/98	9		3.83	5.71	2.87	8.55	Aquatec Biological Sciences



C-4

**METHOD FOR LABORATORY SORTING AND IDENTIFICATION OF
MACROINVERTEBRATE SAMPLE**

Method for Laboratory Sorting and Identification of Macroinvertebrate Samples

1.0 OBJECTIVE

This Standard Operating Procedure (SOP) describes the necessary equipment and procedures for macroinvertebrate sample processing and analysis. This SOP is based on the U.S. EPA document "Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish" and "Standard Methods for the Examination of Water and Wastewater 19th Edition" by Andrew D. Eaton, Lenore S. Clesceri, and Arnold E. Greenberg.

2.0 PREPARATION

2.1 Apparatus

Project worksheets
Laboratory Logbook
Benthic Analysis Forms (bench sheets)
Dissecting microscope with reversible black to white stage
2 power magnification hand lens
Compound microscope
Petri dishes
Pyrex pans
Forceps
Probe
2 dram vials with stoppers/caps
Glass slides
Glass cover slips
Slide labels
Slide boxes
Number 30 (600 micron) and Number 35 (500 micron) sieves
Spoon
Counter
Hot plate
Laboratory coat, safety glasses, latex gloves

2.2 Solutions and reagents

70% ethyl alcohol
Deionized water
CMCP-9 Low Viscosity Mountant

2.3 Supporting Methods

- Bell, R.T. 1971. Handbook of the Malacostraca of Vermont and neighboring regions. University of Vermont. Burlington, Vermont.
- Bousfield, E.L. 1967. Freshwater amphipod crustaceans of glaciated North America. Queens Printer and Controller of Stationery. Ottawa, Canada.
- Burks, B.D. 1975. The mayflies, or Ephemeroptera, of Illinois. Illinois Natural History Survey Bulletin 26(1). 216 pp. Reprinted by Entomological Reprint Specialists, Los Angeles, California.
- Elliot, J.M., U.H. Humpesch, T.T. Macan 1988. Larvae of the British Ephemeroptera. Freshwater Biological Association, The Ferry House. Ambleside, Cumbria.
- Klemm, Donald J. 1982. Leeches (Annelida:Hirudinea) of North America. U.S. Environmental Monitoring and Support Lab. Cincinnati, Ohio.
- Mason, William T. Jr. 1973. An introduction to the identification of Chironomid larvae. U.S. Environmental Protection Agency. Cincinnati, Ohio.
- Merritt, R.W., and K.W. Cummins (eds.). 1984. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa. 722 pp.
- Peckarsky, B.L., P. Fraissinet, M. Penton, and D. Conklin, Jr. 1990. Freshwater macroinvertebrates of Northeastern North America. Cornell University Press. Ithaca, New York 14853. 442 pp.
- Pennak, R.W., 1989. Freshwater invertebrates of the United States. Third edition. J. Wiley & Sons, Inc., New York, New York. 628 pp.
- Saether, Ole A. 1970. Nearctic and Palaearctic Chaoborus. Fisheries Research Board of Canada. Ottawa, Canada.
- Savage, A.A. 1989. Adults of the British aquatic Hemiptera Heteroptera. Freshwater Biological Association, The Ferry House. Ambleside, Cumbria.
- Scheffer, Patricia W. and Glenn B. Wiggins 1986. A systematic study of the Nearctic larvae of the Hydropsyche morosa group. Royal Ontario Museum. Toronto, Canada.
- Schuster, Guenter A. and David A. Etnier 1978. A manual for the identification of the larvae of the Caddisfly genera Hydropsyche Pictet and Symphitopsyche Ulmer in

Eastern and Central America. U.S. Environmental Protection Agency. Cincinnati, Ohio.

Simpson, Karl W. 1982. A guide to basic taxonomic literature of the genera of North American Chironomidae (Diptera)-Adults, pupae, and larvae. The University of the State of New York Education Department. Albany, New York.

Simpson, K.W., and R.W. Bode. 1980. Common larvae of Chironomidae (Diptera) from New York State streams and rivers with particular reference to the fauna of artificial substrates. NY Ed. Dept., Bulletin No. 439. New York State Museum, Albany, New York. 105 pp.

Wiggins, G.B. 1978. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto, Canada. 401 pp.

3.0 PROCEDURE

3.1 Sample Custody and Laboratory Log-in

Upon receipt at Aquatec Biological Sciences, samples will be logged into the Laboratory Management System (LMS) and assigned a unique sample identification number. Completed Chain-of-Custody forms will be maintained by the Sample Management Department. Worksheets will be generated for each project which will list all samples to be analyzed, the type of analysis, and client specific information. Project worksheets will be sent along with the samples to the ecological laboratory. A benthic analysis form (bench sheet) is filled out for each sample.

Once in the ecological laboratory, the samples are logged in using a worksheet produced off the LIMS system, to check the compatibility of all information on the project worksheets and the sample labels.

3.2 Laboratory Procedure

3.2.1 Benthic Sample Picking

The field sample is sieved, using a Number 35 (600 micron) sieve. Sieved contents are rinsed with plenty of water to remove as much preservative as possible. The sieved portion is then transferred into a Pyrex dish and preservative is placed back into the field sample jar. Water should be added to cover the sample material so it is prevented from dehydrating.

One spoonful at a time, the sample should be placed in a petri dish and examined under a 2 power magnification hand lens or the lowest power magnification on a dissecting microscope. Macroinvertebrates should be removed with forceps and placed into vials containing 70% ethyl alcohol and separated by order if possible. Chironomid larvae should not only be in a separate vial, but an accurate count should be kept on a counter when they are removed from the sample. This procedure should also be followed with oligochaetes. Be sure while examining the petri dishes contents to switch from black to white backgrounds frequently. Different organisms have a tendency to be more visible on one background as opposed to the other.

After all organisms have been removed from the entirety of the sample and or appropriately subsampled, interior labels should be created for each vial containing the sample identification number; collection station, date, and number; and the project number. All vials for a sample should be secured together with rubber bands and stored on a laboratory shelf until analysis can occur. The caps for the chironomid larvae/oligochaete vials should be labeled with a "C" or and "O" respectively. The benthic debris can be sieved and placed back in the original sample container containing 70% ethyl alcohol. A "P" is placed on the sample jar lid to denote it picked. The biologist should then complete the bench sheet for the sample, recording the number of chironomid larvae/oligochaetes found, their initials, and the date. The project worksheets should also be initialed and dated. After all samples for a project worksheet have been processed, the vials denoted with a "C" or and "O" from the vial groupings on the laboratory shelf should be taken from their groupings and be secured together until slide mounting can occur.

3.2.2 Benthic Sample Subsampling

If rapid bioassessment is the stipulated procedure, the Pyrex pan of sample contents must be distributed evenly in the pan and an equal 16 square grid of the contents must be made. One random square at a time, the contents are placed in a petri dish and macroinvertebrates are removed. If an organism is on the line of two squares, it is considered to be in the square in which its head is in. The same procedures as stated above are followed, except a count is kept of the total organisms picked. Once 100 organisms are procured from the sample, the biologist finishes picking and counting the organisms in the current square of material.

After all organisms have been removed from the entirety of the square(s), interior labels should be created for each vial containing the sample identification number; collection station, date, and number; and the project number. All vials for a sample should be secured together with rubber bands and stored on a laboratory shelf until analysis can occur. Remember, all chironomid larvae/oligochaete vials must be denoted with a "C" or "O" on their caps. The benthic debris should be sieved and placed back in the original sample container containing 70% ethyl alcohol. A "P" is placed on the sample

jar lid to denote it picked. The biologist should then complete the bench sheet for the sample, recording the total number of organisms found, the number of chironomid larvae found, the number of squares picked, their initials, and the date. The project worksheets should also be initialed and dated.

3.2.3 Slide Mounting of Chironomidae Larvae/Oligochaetes

All of the vials containing chironomid larvae/oligochaetes and their associated bench sheets/project worksheets should be taken from the laboratory shelf. Individually, the vials should have all of their contents or 30 chironomids/oligochaetes (whichever is the lesser amount) placed in a petri dish containing 70% ethyl alcohol. Any sample containing more than the 30 specimen subsample, should have the remainder of the organisms kept in the labeled vial and secured with the other macroinvertebrate vials in its collection number. Slide labels should be made containing the sample identification number; collection number, location, and date; project number; and enough space left at the bottom for the taxon to be filled in. The labels should then be placed on empty glass slides.

One at a time, a specimen must be removed from the petri dish with forceps, placed with the head oriented towards the top of the slide and face up on the labeled slide under a dissecting microscope, and covered with enough 70% ethyl alcohol to not desiccate. Two drops of low viscosity mountant should be placed on the specimen and a cover slip placed on top of it. Pressure must be put on the cover glass with a probe (near the head area until all of the teeth are showing and the jaws are open if chironomid larvae). The mountant should be spread out to all corners of the cover slip when finished. If a cover slip breaks while pressure is being exerted, it must be replaced before the mountant dries. No more than two chironomids/oligochaetes should be mounted on one slide (only one chironomid/oligochaete per cover slip).

The mounted specimens should be stored horizontally under ventilation overnight to allow proper drying. Fully dried slides can be placed in a slide box labeled with the contents (chironomids or oligochaetes), box number, project number, and sample identification numbers contained in the box. Slide boxes should be stored on the laboratory shelf until analysis can occur. The staff member should then initial and date the bench sheets, project worksheets, and denote on the bench sheets how many chironomids/oligochaetes were mounted and how many remain for each sample.

3.2.4 Macroinvertebrate Analysis

All vials/slides for a sample number, as well as the bench sheet/project work sheet associated with it, should be obtained from the laboratory shelf. The slides should be examined individually on a compound microscope until the specimen(s) can be identified to the lowest feasible taxonomic level. Oligochaete slides should be placed

on medium heat on a hot plate for 30 seconds to "clear" their inner structures for proper taxon identification. The taxon should be written on the slide label, the slide placed in the original slide box, and the slide box placed back on the laboratory shelf. The total number of each taxon should be recorded on the bench sheet associated with the sample.

The contents of each vial should be analyzed individually placing the contents in a petri dish containing water. Individually, each macroinvertebrate in the petri dish should be examined under a dissecting or compound microscope until the specimen can be identified to the lowest feasible taxonomic level using the appropriate keys (listed above) for the taxonomic group. Identified macroinvertebrates should be placed in vials containing 70% ethyl alcohol with only members of the same taxon in each vial. Vials should be labeled with an internal label containing the vial number; sample identification number; collection date, location, and number; and the project number, as well as an outer label on the cap containing the vial number. Total numbers of all identified taxon and the vial numbers associated with them should be recorded on the bench sheet associated with the sample. All vials for the sample should be secured together with rubber bands and placed back on the laboratory shelf.

The analyst should then initial and date the appropriate bench sheet and project worksheet.

4.0 QUALITY CONTROL

As a quality assurance method, a random five (5) percent of the samples will be re-picked and re-analyzed. Different laboratory personnel will pick/analyze the samples than did so in the previous analysis. New bench sheets will be created and filled out for each QA sample. QA will be the analysis type specified and any organisms found will be specified by total number and taxon on the sheets for each sample undergoing the quality assurance process. Ninety percent or more efficiency is required for the initial sorting of a sample. If the goal is not achieved additional sample(s) will be re-processed until this goal is achieved and/or the QA Officer deems the process acceptable.

5.0 QUALIFICATIONS

To become properly trained in this procedure, the biology staff member must read the entirety of this SOP, as well as work with a trained staff member until both people are confident in the trainee's abilities to perform this procedure adeptly.

6.0 SAFETY

When dealing with samples/preservatives caution must be used to ensure safety of the laboratory personnel. A laboratory coat, latex gloves, and safety glasses are recommended for wear during this procedure. If the staff member is using a microscope, however, the safety glasses become impractical and may be omitted. Review of the MSDS sheets for any solution or reagent used in this procedure should also be conducted prior to use in this procedure.

Standard Operating Procedures

D-1

**DATA USABILITY
STANDARD OPERATING PROCEDURE
NEW ENVIRONMENTAL HORIZONS, INC.**

**DATA USABILITY
STANDARD OPERATING PROCEDURE
New Environmental Horizons, Inc.**

This section describes the QA/QC procedures and the protocols for data usability assessment as it is performed at NEH.

Overview of Data Usability Assessment

At NEH, data usability is performed using an organized approach to reviewing the chemical data as presented in the laboratory's data package as well as the on-site field measurements and quality control. The data assessment evaluates both compliance with specific methods and regulations and technical quality of the data. The goal of the assessment is to provide, to the data users, a complete and understandable report that describes the uncertainties in the results and the effect of these uncertainties on the usability of the data. NEH applies to the data the USEPA Region I standard data validation qualifiers J, U, UJ, and R to help the data user determine, at a glance, the quality and validity of each chemical result. These data validation qualifiers are defined as follows.

- J - The associated numerical value is an estimated quantity due to quality control criteria exceedance(s). The value is usable for project decisions as an estimated result.
- U - The compound was analyzed for, but was not detected. The associated numerical value is the sample-specific reporting limit. The value is usable for project decisions as a nondetect result at the reporting limit.
- UJ - The compound was analyzed for, but was not detected. The associated numerical value is the sample-specific reporting limit and is an estimated quantity. The value is usable for project decisions as a nondetect result at the estimated reporting limit.
- R - Reject data due to severe or cumulative exceedance of quality control criteria. The value is unusable (compound may or may not be present) for project decisions. Re-sampling and reanalysis is recommended for verification.

In addition, for organic data evaluation, the EPA Region I qualifiers of BB (bottle blank), EB (equipment blank), and TB (trip blank) will be added to qualify data if contamination is observed in a blank that is not of the same matrix as the samples they are associated with (e.g., aqueous EB for sediment samples).

NEH Procedure for Data Usability Assessment

Data Assessment in support of Industri-Plex requires the review and evaluation of chemical data based upon EPA Region I guidance for data assessment of inorganic and organic and site-specific requirements as may be defined in the project Quality Assurance Project Plan (QAPP), Section 9.0. The purpose of assessment is to provide information to the data users (e.g., regulators, risk assessors) of the uncertainty and bias in the data for decision making.

A two-stage process for assessment will be performed. The first stage is equivalent to a Region I Tier III validation in scope. The laboratory will submit one full deliverable (Tier III type), including raw data, results, and QC summaries, for each type of analysis they are performing (e.g., Semivolatile Organics Compounds by Method 8270C). This data package will undergo an in-depth evaluation of all of the quality control information provided, as well as a review of the raw data on instrument calibrations, extraction procedures, qualitative and quantitative determinations to ensure that the laboratory is producing data in a manner which is compliant with the methods and with the QAPP. NEH will use a project-specific checklist to conduct the Data Usability Review (included in Attachment A) and a project-specific Data Usability Summary Report (included in Attachment B) to document this Tier III-type data usability review. Data summary spreadsheets, with standard data qualifiers applied to the results, will also be generated, as required by the data users. This process combines the functions of third-party validation with usability assessment for a comprehensive review and evaluation of the data for risk assessment.

Any deficiencies in performance of the work by the laboratory that are uncovered during the Data Usability Review will quickly be brought to the laboratory's attention for corrective action. If these deficiencies prove to be major, the reviewer may request that the laboratory submit another Tier III package of data after all corrective actions have been taken to ensure the integrity of the project.

Once the first stage has been successfully completed, the second stage of the assessment process involves an abbreviated, project-specific Data Usability Checklist Review (an example is provided in Attachment C) which is equivalent in scope to a Region I Tier II validation. The laboratory will provide a Tier II deliverable for assessment, which includes sample results and QC summary data (but no raw data). The checklist will be used to evaluate the key data quality indicators for the samples. The data users will be provided with these Data Usability Checklists and a Data Usability Summary Report (as included in Attachment B). Data summary spreadsheets, with standard data qualifiers applied to the results, will also be generated, as required by the data users.

For the Industri-plex project, approximately 10% of the data will undergo the Region I Tier III-type Data Usability Assessment review and the remainder of the data will undergo the Region I Tier II-type Data Usability Assessment review. 100% of the data generated during this project will be assessed for usability for risk assessment.

The NEH approach to data assessment, either through stage one or stage two, involves the following 12 steps, in the order presented.

1. Receive data package and electronic data deliverables from client or laboratory. Initial and date the front of the data package.
2. Log-in data package using NEH tracking spreadsheet (Excel). This log-in serves to maintain the chain-of-custody. An example NEH tracking spreadsheet is presented in Attachment D.
3. Perform data package Completeness check. (similar to a Region I Tier I validation review). Check that all required reporting forms and associated raw data, if required, are included in the data package. Check that all samples listed on the COC were analyzed by the laboratory. Check that the correct analyses were performed.
4. Issue Resubmittal Requests for any missing or incorrect information. An example resubmittal request form is included in Attachment E.
5. Perform Initial Review of data package report and quality control (QC) forms using appropriate regulatory guidance. The following guidance is used to perform data assessment reviews for Industri-Plex: Region I, EPA-NE Data Validation Functional Guidelines for Evaluating Environmental Analyses; Part II. Volatile/Semivolatile Data Validation Functional Guidelines, 12/96 and Region I Laboratory Data Validation Functional Guidelines for Evaluating Inorganics Analyses, 6/13/88, modified 2/89. For the initial review for each analysis-type, a Region I Tier III-type Data Usability Assessment review report will be executed (Attachments A and B). For all remaining reviews, a Region I Tier II-type Data Usability checklist (Attachment C) will be used in the assessment process. Method- and QAPP-specific requirements will be used to modify the checklists for each type of analyses being performed (e.g., Pesticide and PCB review will follow the Region I guidance, as appropriate, and include any method required criteria as well). Perform data assessments in strict accordance with these checklists. Completed checklists are included in the data assessment report.
6. Perform initial review of data to confirm reported results using the appropriate project-specific Data Usability Review Report formats or checklists (Attachments A, B, C). If any errors are found, contact laboratory immediately and request resubmittal (see Attachment E) and explanation.
7. Apply data qualifiers (J, U, UJ, R, BB, EB, and TB) to data on the data summary excel spreadsheets, as required and provided by Menzie-Cura. Data qualifiers are applied in strict accordance with USEPA Region I SOPs for data validation. In cases where a quality discrepancy is noted that is not covered in the SOPs, professional judgment will be used. This must be detailed in the data assessment narrative report. Note, site-specific requirements (such as detection levels) will over-ride the USEPA Region I SOP criteria.
8. Complete initial review by preparing the Data Usability Summary Report (Attachment B) delineating major and minor quality control exceedances and the affect on the results in the laboratory data package. The results will be assessed based on the following QC parameters and the specific data validation SOPs according to Region I. The data usability narrative will be generated and will include the following items:

Accuracy:

- Holding Times
- Calibration Criteria (tuning, linearity of calibration curves, initial and continuing calibration standards and checks) – for first data package only unless quality issues are uncovered
- Surrogate recoveries
- Matrix Spike/Matrix Spike Duplicate recoveries
- Laboratory Control Sample , Blank Spike Sample, Standard Reference Material recoveries
- Interference Check Sample and serial dilution sample results for metals on ICP and ICP/MS
- Graphite Furnace AA method QC including duplicate burn precision and analytical (post-digestion) spike recoveries

Precision:

- Matrix Spike/Matrix Spike Duplicate relative percent differences (RPDs)
- Field Duplicate RPDs

Representativeness:

- Field Duplicate RPDs
- Dissolved vs. Total metals results for groundwaters
- Evaluation of sampling methods in obtaining samples representative of the site conditions (usually done by field team; choice of sampling method may affect representativeness; but validator may comment if an obvious discrepancy is noted that would affect representativeness of the sample results to the site location).

Sensitivity:

- Review of MDLs compared to laboratory reporting limits
- Low standard evaluation in standard curves (includes evaluation of CRDL standard recoveries for metals) – first data package
- Blank contamination including method blanks, instrument blanks, trip blanks, rinsate blanks

Comparability:

- Review of method compliance; evaluation of method modifications and potential affects on results (define bias, if possible).
- Check sample result calculations from raw data – first data package only unless quality issues are uncovered
- Confirm validity of detection limits for non-detects

Completeness:

- Measure of amount of data planned to be collected compared to amount of valid data obtained for the program. Most programs will require a minimum of 90% completeness. Detail data gaps based on rejected results.

9. Perform senior review of Data Usability Summary Report including narrative, review report or checklist, and data tables with qualifiers. All data usability reports at NEH will have both initial and senior reviewers. Both the initial and senior data assessor's names will appear on the front page of the data usability report with signatures and dates of review. This procedure of a two-level review ensures high quality and accuracy of NEH data assessment reports. Senior review will check narrative report and data summary tables against the data usability checklists to confirm that correct actions were taken for all samples.

10. Submit final report to client with hard-copy. The final report includes the data usability narrative report, the data summary tables with qualifiers applied, as required, and the data assessment checklists and data usability summary reports. Each Sample Delivery Group (SDG) received from the client or laboratory will have a separate data usability report.
11. Return laboratory data packages to client; or, based on client request, hold data packages for specified time period.
12. File/Record Retention: Archive data usability report, data summary tables, all correspondence (including faxes, resubmittals, emails), and NEH tracking COC for project on diskette and store at NEH.

Attachment A
Example – Data Usability Review
Region I Tier III-Type Checklist

Attachment A – Data Usability Review Tier III-Type Checklist

EXAMPLE

Data Usability Review Checklist - Semivolatile Organic Analysis by Modified Method 8270C

Client: Menzi-Cura & Associates, Inc.

Site: Industri-Plex, Woburn, Massachusetts

Laboratory: Woods Hole Group Environmental Laboratory

Case/SDG: _____

of samples/Analyses: _____

Initial Reviewer: Dr. Nancy C. Rothman, New Environmental Horizons, Inc.

Senior Reviewer: Susan D. Chapnick, New Environmental Horizons, Inc.

Date Completed: _____

The Data Usability Review was performed on the data package. The intentions of this review are: 1) to determine if the data were generated and reported in accordance with SW-846 Method 8270C, the Toxicological Surface Water and Sediment Sampling and Fish Sampling Work Plan and Quality Assurance Project Plan for Industri-Plex Site, Woburn, Massachusetts, July 1999, and Region I, EPA-NE Data Validation Functional Guidelines for Evaluating Environmental Analyses; Part II. Volatile/Semivolatile Data Validation Functional Guidelines, 12/96 2) to determine if the data met the program data quality objectives for acceptable accuracy, precision, and sensitivity; 3) to determine and define the technical usability of the data based on the accuracy, precision, and sensitivity QA/QC indicators; and 4) to update the project database with appropriate data quality qualifiers.

The Data Usability Review consists of five main sections. Section I is the Overall Summary of Data Usability Checklist Review including subsections addressing technical usability, accuracy, precision, and sensitivity of the data. Section II is the Data Package Completeness Review. Section III is the Review of the Summary Forms and Additional QA/QC Parameters to determine if the QC requirements met and to determine the affect of exceeded QC requirements on the precision, accuracy, and sensitivity of the data. Section IV is the Review of the Overall Data Package to determine if contractual requirements were met. Section V is Example Sample Calculations to determine if the sample results and reporting limits were correctly calculated and reported by the laboratory.

I. Overall Summary of Data Usability

A. Summary of Technical Usability of Data

B. Summary of Issues Affecting Accuracy

C. Summary of Issues Affecting Precision

D. Summary of Issues Affecting Sensitivity

E. Additional Technical and QA/QC Issues

F. Summary of Documentation and Chain-of-Custody Issues

3. Initial Calibration

The initial calibration data are reviewed to determine if the standards were compliant with the method protocols.

Review the Initial Calibration Data Summary. Check and recalculate the RRFs, \overline{RRF} and %RSD for at least one polynuclear aromatic hydrocarbon (PAH) analyte across the ICAL. Does the RRF and %RSD check back to the raw data? **Yes / No**. Were the RRFs for all analytes in the standard all greater than or equal to 0.05? **Yes / No**

Were at least five concentration levels of each compound analyzed during the initial calibration? **Yes / No** Were all calibration standards analyzed within 12 hours of DFTPP tune? **Yes / No**

Was the lowest initial calibration standard at a concentration equivalent to the sample-specific reporting limit? **Yes / No**

Were retention times for each target analyte stable across the calibration (i.e., minimum drift)? **Yes / No**

Did the initial calibration meet %RSD criteria of $\leq 30\%$ for all analytes (surrogates and targets) across the calibration range? **Yes / No**.

Did the initial calibrations meet %RSD criteria of $\leq 15\%$ for target analytes and surrogates across the calibration range? **Yes / No**. If no, was a calibration curve used for quantitation of results and was the correlation coefficient for the curve ≥ 0.99 ? **Yes / No**. If no, list below all the affected samples.

Action: If the %RSD $>30\%$ and average RRF ≥ 0.05 , qualify positive and non-detected results as estimated (J and UJ). If the %RSD $>30\%$ and average RRF < 0.05 estimate positive results (J) and reject non-detected results (R). If the %RSD $\leq 30\%$ and average RRF < 0.05 estimate positive results (J) and reject non-detected results (R). Sound technical judgment should be used in qualification of the data. The results for each sample associated with ICAL should be evaluated to determine if a result reported would be impacted by the mis-calibration.

Comments:

ICAL Check: Compound Checked _____

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Avg. RRF	%RSD
Concentration								
Response Cpd								
Conc, IS								
Response IS								
RRF								

8270C Data Usability Review

4. Continuing Calibration Check

The continuing calibration data are reviewed to determine if the standards were contractually compliant.

Review the Continuing Calibrations and Summaries. Check and recalculate the RRF and %Difference (%D) for at least one of the PAH in one of the CCALs. Does the RRF and %D check back to the raw data? **Yes / No.** Were the RRFs for all analytes in the standard all ≥ 0.05 ? **Yes / No**

Was a continuing calibration check performed every 12 hours following tuning verification of the instrument? **Yes / No.** If no, list below all the affected samples.

Were the target analytes recovered within the expected retention time window based upon the initial calibration (i.e., drift of instrument was acceptable)? **Yes / No.**

Did the continuing calibrations meet 8270C criteria for verification of $\%D \leq \pm 25\%$? **Yes / No.** If no, list below the outliers and the affected samples.

Action: If the $\%D > \pm 25\%$ and the CCAL RRF ≥ 0.05 , estimate positive and non-detected results (J and UJ) for samples analyzed following this standard for the compound(s) that was outside of calibration. If the RRF < 0.05 , qualify positive results as estimated (J) and reject (R) non-detected results as unusable.

Comments:

CCAL Check: Standard ID _____: Compound Checked _____

Responses	RRF	avg. RRF ICAL	% Difference
Cpd:			
IS:			

8270C Data Usability Review

5. Laboratory and Field Blank Results

Laboratory and field blank results are reviewed to assess the presence of contaminants, which affect the accuracy and sensitivity of the results. See Table 1a. where the Holding Time and Associated QC Table was completed for the samples within this SDG.

Was each sample analysis associated with the appropriate method blank, *i.e.*, correct matrix, correct matrix level, same extraction batch? **Yes / No**. If no, list below affected samples.

Review the reporting forms for each method and field blank. Were any target compounds in the method blanks detected at concentrations above the Reporting Limit (RL)? **Yes / No**. If yes, were these compounds phthalates and were they reported at < 5 times the RL? **Yes / No**

Action: - Blanks should not contain contaminants above the RL except for phthalates that must not be present above 5 times the RL. The Blank Action Level is defined as five times the highest level seen in any of the matrix-matched blanks associated with this SDG, except if phthalates are present, in which case the Blank action is ten times the highest level observed in any matrix-matched blank. The following actions should be taken if conditions warrant:

1. If the blank is not matrix matched, qualify all sample data, for the contaminant associated with this blank, with BB or EB, as appropriate.
2. If the reported result in a sample is below the reporting limit (sample < RL) and if a matrix-matched blank contains a result above the quantitation limit (blank > RL), the result in the sample should be negated (U) and raised to the sample-specific RL for that sample
3. If the sample result is between the reporting limit and the blank Action Level (RL < sample < Action Level), the result for the sample is negated (U) at the level found in the sample. Based on the level of contamination suspected in the sample, the reporting limit may be elevated. Professional judgment will be used in assessing the action needed.
4. If the sample result is greater than the RL and the blank Action Level, no action is taken.

Comments:

Blanks evaluated: _____

Highest Blank: _____

Action taken:

Sample ID	Compound	Reported Result	Result based on Blank Action

IV. Example Sample Calculations

Review of one sample per data package is performed to determine if sample results and quantitation limits were correctly calculated and reported.

Sample ID: _____ was selected for review in this data package.

A. Form 1 Review

1. Were the Form 1s for completed according to the method/QAPP requirements? **Yes / No.** If no, list below the affected fields.
2. Reproduce the reporting limit for SVOC in one of the samples, did the laboratory correctly calculate the quantitation limits? **Yes / No.** If no, list below.

B. Quantitation Review

Reproduce a calculation for one semivolatile analyte in one of the samples that contained a positive result and compare the calculated result to the result reported by the laboratory.

Analyte Checked: _____

Laboratory Result: _____ Calculated Result: _____

Example Calculation:

Data Summary Key for Data Usability Checklist Review

- J - The associated numerical value is an estimated quantity due to quality control criteria exceedance(s). The value is usable for project decisions as an estimated result.
- U - The compound was analyzed for, but was not detected. The associated numerical value is the sample detection/quantitation limit. The value is usable for project decisions as a nondetect result at the reported detection/quantitation limit.
- UJ - The compound was analyzed for, but was not detected. The associated numerical value is the sample detection/quantitation limit and is an estimated quantity. The value is usable for project decisions as a nondetect result at the estimated detection/quantitation limit.
- R - Reject data due to severe or cumulative exceedance of quality control criteria. The value is unusable (compound may or may not be present) for project decisions. Resampling and reanalysis is necessary for verification.
- EB - The compound was detected in an Equipment Blank.
- BB - The compound was detected in a Bottle Blank.
- NA - Not Analyzed

Validation Checklist Review Acronyms

BB	-	Bottle Blank
CCAL	-	Continuing Calibration
CLP	-	Contract Laboratory Program
%D	-	Percent Difference = $(A - B)/A \times 100$
%Drift	-	Percent Drift = Percent Recovery = $((\text{True-Found})/\text{True} \times 100)$
DQO	-	Data Quality Objective
EB	-	Equipment Blank (Rinsate)
EPA	-	Environmental Protection Agency
FB	-	field blank
g	-	gram
GC/MS-		Gas Chromatography/Mass Spectrometry
ICAL	-	Initial Calibration
Kg	-	kilogram
L	-	liter
LCS	-	Laboratory Control Sample
MDL		Method Detection Limit
MS	-	Matrix Spike
MSD	-	Matrix Spike Duplicate
mg	-	milligram
NA	-	not applicable
ND	-	non-detect
QA	-	Quality Assurance
QC	-	Quality Control
RL		Reporting Limit
RPD	-	Relative Percent Difference $((A-B)/\frac{1}{2}(A+B)) \times 100$
%RSD	-	Percent Relative Standard Deviation $(SD/\text{Average Value} \times 100)$
SRM	-	Standard Reference Material
SVOC	-	Semivolatile Organic Compound
TCL	-	Target Compound List
TIC	-	Tentatively Identified Compounds
µg/Kg	-	micrograms per kilogram
µg/L	-	micrograms per liter

Bibliography

Toxicological Surface Water and Sediment Sampling and Fish Sampling Work Plan and Quality Assurance Project Plan for Industri-Plex Site, Woburn, Massachusetts, July 1999.

Region I, EPA-NE Data Validation Functional Guidelines for Evaluating Environmental Analyses; Part II. Volatile/Semivolatile Data Validation Functional Guidelines, 12/96.

Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Updates II and III (USEPA, Office of Solid Waste and Emergency Response, Washington, DC, September 1995 and December 1996).
Methods 8270C.

Attachment B
Example -- Data Usability Summary Report
Region I Tier III and II-Type Narrative Report

Attachment B -- EXAMPLE Data Usability Summary Report
Data Usability Review - Volatile Organic Analysis by Modified
Method 8260A

Client:

Site:

Laboratory:

Case/SDG:

Project Specific Analyte List: VOC Method 8260A (see site QAPP)

of samples/Analyses: 20 low-level and 5 high-level soil samples analyzed on July 7, 1999

Initial Reviewer: Dr. Nancy C. Rothman, New Environmental Horizons, Inc.

Senior Reviewer: Susan D. Chapnick, New Environmental Horizons, Inc.

Date Completed: _____

The Data Usability Review was performed to determine the following: 1) if the data were generated and reported in accordance with the Quality Assurance Project Plan (July 1999) for Volatile Organic compound analysis following EPA Modified Method 8260A (see Bibliography) 2) if the data met the program data quality objectives for acceptable accuracy, precision, and sensitivity; and 3) to define the technical usability of the data based on the accuracy, precision, and sensitivity QA/QC indicators.

The Data Usability Review consists of four main sections. Section I is the Overall Summary of Data Usability Review including subsections addressing technical usability, accuracy, precision, and sensitivity of the data. Section II is the Data Package Completeness Review. Section III is the Review of the Summary Forms and Additional QA/QC Parameters to determine if the QC requirements met and to determine the affect of exceeded QC requirements on the precision, accuracy, and sensitivity of the data. Section IV is Example Sample Calculations to determine if the sample results and quantitation limits were correctly calculated and reported by the laboratory.

Attachment B -- EXAMPLE Data Usability Summary Report

I. Overall Summary of Data Usability

A. Summary of Technical Usability of Data

All soil results for VOCs for samples analyzed on July 7, 1999 are usable for project objectives. The laboratory reported an estimated result for Trichloroethylene in one sample. This result was considered estimated during the usability review. Non-detected results for all of the VOC analytes are considered estimated (J) for four samples due to quality control criteria exceedances based upon this usability review. Data users should note the following uncertainties in the estimated results. The estimated results are usable for project objectives.

B. Summary of Issues Affecting Accuracy

The accuracy for Vinyl Chloride, Carbon Tetrachloride and Trichloroethylene in three low-level samples (X, Y, and Z) was compromised since the samples were analyzed outside of analytical holding time (analyzed beyond 24 hours from sample collection but less than 48 hours). All three samples reported non-detect for the analytes of interest. Based upon this slight holding time exceedance, these non-detected results in these three samples should be considered estimated (UJ) due to a possible low bias in the data.

The accuracy for Vinyl Chloride, Carbon Tetrachloride and Trichloroethylene in one sample (X) was compromised due to poor response of the Internal Standard Fluorobenzene in this sample compared to the Continuing Calibration verification criteria (i.e., the IS response was less than half of the response seen the continuing calibration). This sample reported non-detect for all analytes of interest. Based on the Internal Standard not meeting specification, these non-detected results in this sample should be considered estimated (UJ).

The accuracy for all other samples analyzed met project requirements.

C. Summary of Issues Affecting Precision

The laboratory performed one set of MS/MSD samples for this set of samples: XMS and XMSD. This was a low-level analysis and the precision was acceptable for Vinyl Chloride, Carbon Tetrachloride and Trichloroethylene based upon the matrix spike (MS) and matrix spike duplicate (MSD) results. This is an indication of acceptable precision in the analysis of the low-level samples within this SDG.

Precision of the high-level analysis could not be assessed since the laboratory did not analyze any high-level MS/MSD samples.

The field duplicate samples within this set were X and XFD. Since the results for both samples were non-detect for all analytes of interest, field duplicate precision could not be assessed based on these results.

Attachment B -- EXAMPLE Data Usability Summary Report

D. Summary of Issues Affecting Sensitivity

Five samples were analyzed as high-level samples due to holding time constraints. The laboratory, realizing that they could not analyze the samples within 24 hours, preserved approximately 5g of sample with 5 mL of methanol and then analyzed an aliquot of the methanol in water to obtain results. The reporting limit for these high-level samples, uncorrected for sample solid content of the sample, was 250 ug/kg or 25 times higher than the reporting limits obtained by the low-level method.

E. Additional Technical and QA/QC Issues

Blanks, such as trip blanks, rinsate blanks and field blanks, were not generated in the field for this project. For the samples validated in this set, the lack of field quality control did not affect the results reported since all values for all samples, except for Y, were non-detect for the compounds of interest.

Calibration verification on the instrument, for this set of data, was done once following the tune at the beginning of the analysis sequence. This verification was used for the remaining analyses over the next 24 hours of instrument run time.

The surrogate, 1,2-Dichloroethane, did not meet Initial Calibration criteria (%RSD was 29.9% across the six-levels of standards analyzed as compared to the required %RSD \leq 20%). No action was taken based on this finding other than to note this non-conformance.

The surrogate Dibromofluoromethane, did not meet Continuing Calibration verification criteria in the standard analyzed on the day this set of samples was processed. The %Difference was 20.7% compared to method criteria of %Difference \leq 20%. No action was taken based on this finding other than to note this non-conformance.

One of the Method Blanks (GC file A) analyzed during the sequence did not have acceptable Internal Standard response and did not recover three of the four surrogates. The raw data does not indicate that the laboratory investigated whether the results reported were correct and there is no indication of corrective action as a consequence of this poor blank result. No action was taken based on this finding other than to note this non-conformance.

No high-level Method Blank or LCS was performed for this set of samples. Since the only sample that reported a positive result did so as an estimated value below the sample reporting limit, the lack of this high-level method blank did not impact the results.

Internal Standard variations outside of acceptance criteria were noted for nine samples (i.e., the response for the Internal Standards was below 50% of the continuing calibration Internal Standard's response). For all samples except one, the IS variation did not affect the quantitation of the analytes of interest; therefore, no action for eight samples was taken based on these findings except to note this non-conformance. The Internal Standard variation in one sample may have affected the accuracy for quantitation of the analytes of interest resulting in estimation of the results, as discussed in Section B.

Attachment B -- EXAMPLE Data Usability Summary Report

F. Summary of Documentation and Chain-of-Custody Issues

The original data sent by the laboratory did not properly adjust the reporting limit for samples based on sample preparation and percent solids content of the sample. The laboratory re-issued the data sheets with the low-level samples properly reported; however, a second re-issue of the data was required for the high-level samples since the laboratory did not properly account for sample weight variations between samples in calculating the reporting limits. The revised data sheets for the high-level samples were received on July 1, 1999.

The Chain-of-custody for samples collected on 6/7/99 did not have time of collection for the samples. Since the laboratory analyzed these samples on 6/7/98, this improper chain-of-custody documentation from the field did not impact the results for this sample set. In addition, none of the Chain-of-custody's reviewed noted the Temperature upon receipt of the samples at the on-site trailer.

Sample ID's on five samples were incorrectly reported on the raw data, as verified with the laboratory on 7/5/99. The data sheets, and database, correctly identified these sample ID's. During this assessment, the raw data, including chromatograms and run logs, were changed by the reviewer to reflect the correct ID's.

Attachment C
Example – Data Usability Checklist
Region I Tier II-Type Checklist
(Excel Spreadsheet)

EXAMPLE for Industri-Plex

8270C

Data Usability Review Checklist
(Region I Tier II-type)

Lab Project #: _____

No. Samples _____

Lab: _____

Date Sampled: _____

Method of Analysis: 8270C

Associated Blanks: _____

Matrix: _____

Sediment / Water / Biota

Blank ID	Contaminant / Level	Matrix Related ?	Action Level / Action	Corrected Result

8260C Action Summary:

- T Actions: Waters 7d <HT ≤ 14 d; J det/ J NDs; HT >14 d, J det/R ND
Sediments and Biota 14d <HT ≤ 28 d; J det/ J NDs; HT >28 d, J det/R ND
Analysis 40d < Extract HT ≤ 60d, J det/ J NDs; Extract HT > 60d; J det/ R NDs
- Surrogate Actions: 2 BN or 2 Acids Recovery > Criteria, J det/Accept ND; 10% ≤ 2-All Surrogates < Criteria, J det/J NDs; Recovery < 10%, J det/R NDs. Action taken on Acid and/or BN analytes based on surrogates out.
- Blank Actions: Surrogates outside criteria - Use Judgment if isolated or analysis related
Non-Matrix related Blank contamination, BB or EB contaminant in all samples associated with Blank
Matrix related Blank contamination: Result < RL, U result at RL; RL<Result<Blank Action, U result at level reported
- MS Actions: %Rec<10%, J det/ R NDs; 10% ≤%Rec<Criteria, J det/ J NDs; %Rec >Criteria, J det/Accept NDs for Unspiked Sample only
%Rec<10%, J det/ R NDs; 10% ≤%Rec<Criteria, J det/ J NDs; %Rec >Criteria, J det/Accept NDs for all Batch by Compound
- LCS Actions: Both Conc. ≥ 2xRL, %RPD outside, J det; One result ND, other ≥2 x RL, J det/J NDs; Both Conc. < 2xRL; %RPD out, LCS OK, Accept data
- FD Action: LCS OK, Accept data
- %Solids Action: 10% ≤ % solids ≤ 30%; J det/R ND; %solids < 10% R det/ R NDs

Lab: _____

Date _____

Lab Project #: _____

Data Rev. _____

Attachment D
Example – NEH Tracking Sheet for Data Usability of Laboratory Data Reports

1999 Client: XXX Site: Anywhere, USA Regulatory Protocol: USEPA Region 1

SDG #	Fraction	Number of Samples	Media	Date Rec'd Data Package	Date Rec'd Data Tables	Date Due to Client	Initial Review Completed	Senior Review Completed	Final Sent to Client	Comments
001	TAL metals	18	aqueous	21-Jan	6-Feb	19-Feb	12-Feb	17-Feb	17-Feb	Raw data missing - received 2/5
002	TAL metals	15	soil	21-Jan	21-Jan	9-Feb	27-Jan	31-Jan	2-Feb	Resubmittal NEH #1 Metals
003	TCL organics	20	soil	21-Jan	21-Jan	9-Feb	27-Jan	2-Feb	3-Feb	
004	PAHs	12	soil	21-Jan	21-Jan	9-Feb	27-Jan	2-Feb	3-Feb	
005	PCBs	15	aqueous	23-Jan	26-Jan	9-Feb	27-Jan	31-Jan	2-Feb	Resubmittal NEH #2 PCBs
006	PCBs	18	soil	23-Jan	26-Jan	9-Feb	2-Feb	6-Feb	6-Feb	
007	VOCs	20	aqueous	5-Feb	6-Feb	19-Feb	16-Feb	18-Feb	18-Feb	

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Attachment E
Example -- Resubmittal Request Form

Fax

To: [Insert laboratory contact]	From: Susan D. Chapnick
Fax: [Insert fax #]	Pages: 1
Phone:	Date: February 19, 1998
Re: Resubmittal Request - NEH # 1 [Insert Project Name Here]	CC: [Insert Client Contact]

Urgent **For Review** **Please Comment** **Please Reply** **Please Recycle**

NEH received a single data package (SDG # xxxx) from [laboratory name] on February 19, 1998. This data package contains results and QC summary tables for lead analyses. The package is missing all raw instrument data and raw laboratory preparation logs.

The data validation review of the lead results cannot proceed without the raw data. Please provide this information within 5 business days of this request.

Thank you for your prompt response to this resubmittal.

Please forward your response to:

Susan D. Chapnick
NEH, Inc.
63 College Avenue
Arlington, MA 02174
Tele: (781) 643-4294 ; Fax: same #, call first.