70004

Phase 2A
Sampling and Analysis Plan/Quality Assurance Project Plan
Hudson River PCB Reassessment
RI/FS

EPA Work Assignment No. 013-2N84
Revision 1 March 10, 1992

Appendices

Prepared for
EPA Region II
Alternative Remedial Contracting Strategy (ARCS)
for
Hazardous Waste Remedial Services

EPA Contract No. 68-S9-2001

Prepared by
TAMS Consultants, Inc.
and
Gradient Corporation

Appendix A

Appendix A-1

EXTRACTION AND CLEAN-UP OF WATER SAMPLES FOR PCB CONGENER ANALYSIS

1.0 Scope and Application

1.1 This is a procedure for the extraction and clean-up of polychlorinated biphenyls (PCBs) from aqueous samples. The procedure which is a modified version of NYSDEC's Analytical Service Protocols for PCB Congeners, is based on EPA Methods 3510A (separatory funnel liquid-liquid extraction), 3630 (silica gel cleanup), and 3620 (Florisil column cleanup) (Ref. 9.1) with some modifications.

1.2 This extraction and clean-up procedure is meant to be followed by congener specific PCB analysis by GC-ECD.

2.0 Summary of Method

2.1 A measured volume of sample, approximately one-liter, is solvent extracted with hexane using a separatory funnel or a continuous liquid-liquid extractor. The hexane extract is concentrated in a Kuderna-Danish (K-D) Evaporator, and adjusted to a final volume of 1 to 2 mL.

2.2 The concentrated extract from the hexane extraction is applied to an activated Florisil column and eluted with 6% ether/hexane (v/v). The eluted fraction is concentrated with a K-D apparatus, added to a silica column and eluted with hexane to separate PCBs from chlorinated pesticides. The eluate is concentrated once again using a K-D apparatus.

2.3 The concentrated eluate from the silica column clean-up may be further purified with sulfuric acid or alcoholic potassium hydroxide to remove matrix interferences.

2.4 The final extract is suitable for gas chromatographic analysis.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, and glassware that lead to false positive peaks and/or elevated baselines in gas chromatograms. All of the reagents and glassware used must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks. Interferences by phthalate esters can pose a major problem in PCB congener analysis when using the electron capture detector. These compounds generally appear in the chromatogram as broadly eluting peaks. Common, flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled. Interferences from phthalates can best be minimized by avoiding the use of all plastics, except Teflon, in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination once it becomes a problem.

3.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry, dry in a 100°C oven or, if necessary, heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

4.0 Apparatus and Materials

4.1 2-L separatory funnel
1-L graduated cylinder
100-ml graduated cylinder
250-ml Erlenmeyer flask (or equivalent)

4.2 Kuderna-Danish (K-D) apparatus:

4.2.1 Concentrator tube - 10 ml, graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.2.2 Evaporation Flask - 500 ml (Kontes K-5700-1-500 or equivalent). Attach to concentrator tube with springs.

4.2.3 Snyder column - Three ball macro (Kontes K-503000-121 or equivalent).

4.2.4 Snyder column - Two ball micro (Kontes K-569001-219 or equivalent).

4.3 Drying Column - 20 mm ID Pyrex chromatographic column with coarse frit. A small pad of disposable, hexane washed Pyrex glass wool is substituted for the frit to help prevent cross contamination of sample extracts).

4.4 Boiling Chips - Silicon carbide or equivalent, approximately 10/40 mesh, solvent extracted with hexane for approximately one hour and heated to 400 °C for 30 minutes.

4.5 Water or steam bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.6 Vials - Amber glass 10 to 15 ml capacity, with Teflon lined screw caps.

4.7 Continuous Liquid-Liquid Extractors - Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication. (Hershberg-Wolf Extractor - Ace Glass Company, Vineland, NJ P/N 6841-10 or equivalent).

4.8 Bottle or test tube. 50 ml with Teflon lined screw cap for sulfur removal.

4.9 Balance - Analytical capable of accurately weighing ± 0.01 mg.

4.10 Apparatus for Silica Column clean-up procedure:

4.10.1 Glass chromatographic column, 10 mm ID with Teflon stopcock and reservoir.

4.10.2 Erlenmeyer flask (250 ml).

4.10.3 Glass stoppers.

4.10.4 Long glass rod.

4.10.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.11 Apparatus for Florisil clean-up procedure:

4.11.1 Glass chromatographic column, 20 mm ID with Teflon stopcock and reservoir.

4.11.2 Erlenmeyer flask (250 ml).

4.11.3 Glass stoppers.

4.11.4 Long glass rod.

4.11.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.12 Nitrogen source with regulator and low pressure manifold.

5.0 Reagents

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

5.2 ASTM Type II Water (ASTM D-1193-77) or equivalent. All references to reagent water in this method refer to ASTM Type II unless otherwise specified.

5.3 Sodium sulfate (granular, anhydrous) purification is by washing with acetone/hexane followed by heating at 400 °C for four hours in a shallow tray.

5.4 Organic solvents for extraction and clean-up:

5.4.1 Acetone - Pesticide quality or equivalent.

5.4.2 Hexane - Pesticide quality or equivalent.

5.4.3 Methylene Chloride - Pesticide quality or equivalent.

5.4.4 Diethyl Ether - Pesticide quality or equivalent. Must be free of peroxides as indicated by test strips.

5.5 Silicic acid (Mallinckrodt A.R. 100 mesh or equivalent) Before use, activate each batch at least 16 hours at 135°C in a shallow glass tray, loosely covered with aluminum foil.

5.6 Florisil - Pesticide residue (PR) grade (60/100 mesh). Purchase activated at 1250 °F (677 °C), stored in glass containers with ground glass stoppers or foil-lined screw caps.

5.6.1 Deactivation of Florisil - Just before use, weigh out 1000 g of Florisil into a glass container and heat at 130°C for 16 hours. After heating, cool in a desiccator. Pour into a 4-L solvent bottle, close with a Teflon lined cap and place on a tumbler or roller. Dropwise add 40 ml of water while shaking to deactivate. Tumble for at least 4 hours. There should be no lumps present. Store in a tightly closed amber bottle. Let stand overnight before using.

5.6.2 Florisil activity check - Before each batch of Florisil is first used and once a week thereafter test the Florisil by adding 1 ml of GC/EC CAL STD-3 and following 7.13. All resolvable PCB congeners should be present in the extract after column elution. Some batches of Florisil may require 4% deactivation to allow all PCB congeners to be eluted by 50 ml of hexane.

5.7 Copper powder - activate copper powder by treating with diluted nitric acid, rinse with free reagent water to remove all traces of acid, rinse with acetone and dry under a stream of nitrogen.

5.8 Sodium hydroxide solution (10N) - Dissolve 40 g NaOH (ACS reagent grade) in reagent water and dilute to 100 ml.

5.9 Alkali Solution - Prepare fresh daily as needed. Dissolve eight pellets of potassium hydroxide into 6 ml of ethanol in a glass stoppered test tube.

5.10 Sulfuric acid solution - Slowly add, with rapid stirring, 50 ml concentrated sulfuric acid (sp. gr. 1.84) to 50 ml of reagent water.

5.11 PCB Surrogate Standard Spiking Solution

5.11.1 The surrogate standards are added to all samples, blanks, matrix spikes, and calibration standards before extraction. The surrogate compounds are tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB). Other surrogates may prove suitable, but it is the responsibility of the laboratory to demonstrate their acceptability as additional surrogates, not substitutes.

5.11.2 Prepare the surrogate standard spiking solution at a concentration of 0.2 μg/1.00 ml of each of the two compounds in acetone. Store the spiking solutions at 4°C (±2°C) in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicates a problem. **CAUTION**: Analysts must allow all spiking solutions to equilibrate to room temperature before use.

5.12 PCB Congener Matrix Standard Spiking Solution

5.12.1 Prepare a matrix spike standard solution that contains each of the congeners listed in Table 1 in acetone. Place each solution in a 10 - 15 ml clean glass vial with a Teflon-lined screw cap and store at 4°C (±2°C) and protect from the light. Stock solutions must be replaced after twelve months, or sooner if comparison with check standards indicates a problem. **Caution**: Each time a vial containing small volumes of solutions is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible headspace, and opening vials should be minimized.

5.12.2 Matrix spikes are also to serve as duplicates by spiking two equal portions from the one sample chosen for spiking.

6.0 Sample Collection, Preservation, and Handling

6.1 Sample collection is according to the study protocol and relevant standard operating procedures.

6.2 Preservation - In the field and during transport, all samples collected must be kept cool on wet ice (approximately 4 °C or under). In the laboratory, all samples must be kept in the refrigerated storage room at a temperature ranging from 2 ° - 5 °C before extraction.

6.3 Samples must be extracted within 5 days of verified time of sample receipt (VTSR) and extracts analyzed within 40 days following VTSR.

7.0 Procedure

7.1 Samples may be extracted using separatory funnel techniques. If emulsions prevent acceptable solvent recovery with separatory funnel extractions, continuous liquid-liquid extraction (paragraph 7.) may be used. The separatory funnel extraction scheme described below assumes a sample volume of one liter.

7.2 Pour the entire sample into a 2-liter separatory funnel. (If a sample larger than 1 liter is extracted, the funnel size and solvent volume for samples and blanks must be adjusted also.) Check the pH of the sample with wide range pH paper and adjust to between 5 and 9 pH with 10N sodium hydroxide or 1:1 sulfuric acid solution. Pipet 1.0 mL of PCB surrogate standard spiking solution into the separatory funnel and mix well. Add 1.0 mL of PCB congener matrix spiking solution to each of two 1-liter portions from the sample selected for spiking.

7.3 Add 60 mL of hexane to the sample bottle and shake for two minutes, with periodic venting to release excess pressure. Transfer to the separatory funnel. Shake the separatory funnel for two minutes, with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, and may include: stirring, filtration of the emulsion through glass wool, centrifugation, or other physical means. Return the

water layer to the sample bottle and collect the hexane layer in a 250 mL Erlenmeyer flask containing enough granular anhydrous sodium sulfate to allow for free flowing crystals. Swirl. When drying is complete, indicated by the clarity of the extract, decant the hexane phase into a 500 mL Kuderna-Danish evaporator with a 1 mL collection tube and three-ball Snyder column.

7.4 The aqueous layer is returned to the separatory funnel and the sample bottle and sample are extracted again as in 7.3, the organic layer being collected in the Erlenmeyer flask, dried, and combined in the evaporator. Perform a third extraction in the same manner. Run the aqueous layer into a graduated cylinder to determine the sample volume.

7.5 Assemble a K-D concentrator by attaching a 10 ml concentrator tube to a 500 ml evaporation flask.

7.6 Add one or two clean boiling chips to the flask and attach a three ball Snyder column. Prewet the Snyder column by adding 1.0 ml of hexane to the top of the column. Place the K-D apparatus on a hot water bath (15 ° - 20 °C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the hot water temperature, as required, to complete the concentration in 10 - 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid has reached 1 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. DO NOT ALLOW THE K-D TUBE TO GO DRY.

7.7 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.8 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners present in the calibration solutions. Nitrogen blow-down is not recommended, since its employment may result in intermittent loss of the more volatile PCB congeners.

7.9 Sample Extraction - Continuous Extractor

7.9.1 When experience with a sample from a given source indicates that a serious emulsion problem will result, or if an emulsion is encountered in paragraph 6.3

using a separatory funnel, a continuous extractor may result in a successful extraction.

7.9.2 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the continuous extractor. Rinse the sample container with hexane and add rinsate to the continuous extractor. (If a sample larger than 1 liter is extracted, the size of the extractor and solvent volume for samples and blanks must be adjusted also. Pipet 1.0 mL of PCB Surrogate Standard Spiking-Solution into the continuous extractor and mix well. Check the pH of the sample with wide range pH paper and adjust to between 5 and 9 pH with 10N sodium hydroxide and/or 1:1 sulfuric acid solution.

7.9.3 Add 500 mL of hexane to the distilling flask. Add sufficient reagent water to ensure proper operation and extract for 18 hours. Allow to cool, then detach the boiling flask and dry. Concentrate the extract as in paragraphs 7.5 through 7.7.

7.10 If crystals of sulfur are evident or sulfur is expected to be present, an optional sulfur clean-up step may be performed.

7.11 Sulfur Cleanup

7.11.1 Transfer the extract from paragraph 7.7 to a 15 mL clear glass bottle or vial with a Teflon-lined screw cap. Rinse the concentrator tube with 1.0 mL of hexane, adding the rinsings to the 15 mL bottle. If only a partial set of samples requires sulfur cleanup, set up a new reagent blank with 1.0 mL of hexane and take it through the sulfur cleanup. Include the surrogate standards.

7.11.2 Add activated copper powder to the sample and seal. Agitate vial in a vortex mixer before quantitative transfer to the Florisil column.

7.12 The extract obtained must now be cleaned up on a Florisil column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.13 Florisil column clean-up. Fill the chromatography column to about two-thirds full with hexane. Add a piece of glass wool to the bottom of the column with the help of a long glass rod. Remove any air bubbles which become trapped in the glass wool. Add 20 g of the deactivated Florisil to a 20 mm ID chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 ml of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

7.14 Transfer the sample extract from the K-D concentrator tube or vial to the Florisil column. Rinse the tube twice with 1 - 2 ml hexane, adding each rinse to the column.

7.15 Place a 250 - 500 ml erlenmeyer or round bottom flask under the chromatographic column. Drain the column into the flask until the sodium sulfate is nearly exposed. Elute the column with 200 ml of 6% ethyl ether in hexane (v/v). Elute at a 5 ml/min rate.

7.16 The eluate is then concentrated with the K-D apparatus to an apparent volume of 1 ml. DO NOT ALLOW THE SAMPLE TO GO TO DRYNESS.

7.17 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.18 The concentrated eluate may now be further cleaned up on a Silica column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.20 Silica column clean-up. After drying the silicic acid overnight at 135°C, weigh 3.0 g portions into vials and redry for 1 hour. When ready to prepare column, remove vial of silicic acid from the oven and immediately seal with a Teflon-lined cap. When the vial has cooled to room temperature, add 0.10 ml of water and then reseal. Mix the contents thoroughly and let the adsorbent equilibrate for approximately one hour with occasional mixing.

NOTE: The silica column chromatography is included in the procedure to remove pesticides from the analysis. The optimum percent water on the silica used in the column

chromatography must be determined by running standards. The percent water added to the activated silica should be adjusted to obtain optimum separation between PCBs and pesticides. For calibration purposes, a mixture of the PCB calibration standard with chlordane, op'-DDT, pp'-DDT, pp'-DDE, and pp'-DDD may be used. The pesticide pp'-DDE will not be separated from the PCB fraction. Under optimum conditions, not more than 20-60% op'-DDT will elute with the PCB fraction.

7.21 Empty the vial of equilibrated silicic acid into a chromatographic column and tap until the adsorbent is well settled. Top column with 1-2 cm sodium sulfate (Na_2SO_4).

7.22 Wash the column with 20 ml of methylene chloride, forcing solvent through the column at 2-3 ml per minute with approximately 6 psi nitrogen pressure. Stop the flow when the level of the solvent reaches the sodium sulfate layer. At this point, the silicic acid portion should have a uniform translucent appearance, without air pockets or channels.

7.23 Wash the methylene chloride from the column with 20 ml of hexane, again stopping the flow when the solvent level reaches the sodium sulfate layer. The column should have reverted back to its original opaque appearance.

7.24 Add the concentrated eluate from the Florisil column and rinse onto the column with hexane.

7.25 Elute column at 2-3 ml per minute with hexane, collecting the first 50 ml of eluate. Concentrate this fraction on the K-D apparatus to a suitable volume for gas chromatographic analysis.

7.26 Optional Sulfuric Acid Cleanup. Using a syringe or a volumetric pipette, transfer the hexane extract solution to a 10 ml vial and carefully add 5 ml of concentrated sulfuric acid. This procedure must always be done in a fume hood. CAUTION: Make sure that there is neither an exothermic reaction nor evolution of gas prior to proceeding.

7.27 Cap the vial tightly and agitate using a vortex mixer for one minute. A vortex must be visible in the vial. CAUTION: Stop agitating immediately if the vial leaks. **AVOID CONTACTING THE SOLUTION WITH BARE SKIN. SULFURIC ACID WILL BURN.**

7.28 Allow the phases to separate for at least one minute. Examine the top (hexane) layer. It should not be highly colored nor should it have a visible emulsion or cloudiness. If a clean phase separation is achieved, proceed to paragraph 7.29. If the hexane layer is colored or the emulsion persists for several minutes, remove the sulfuric acid layer from the vial via a glass pipette and dispose of it properly. Add another 5 ml of clean sulfuric acid. NOTE: Do not remove any hexane at this stage of the procedure. Agitate the sample using a vortex mixer and allow the phases to separate as described previously.

7.29 Transfer the hexane layer to a clean 10 ml vial.

7.30 Add an additional 1 Ml of hexane to the sulfuric acid layer, cap the vial securely and shake. This second extraction is done to ensure quantitative transfer of all analytes. Remove the second hexane layer and combine with the hexane from paragraph 7.29.

7.31 Final Extract Concentration

7.32 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 ml hexane to the top. Place the K-D apparatus on a hot water or steam bath so that the concentrator tube is partially immersed and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 to 15 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 ml, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.33 Remove the Snyder column, rinse the flask and its lower joint into the concentrator tube with 1 to 2 ml of hexane. Reconnect the concentrator tube and concentrate the extract to a final volume of 1.0 ml. Transfer the 1.0 ml to a GC vial and label as PCB fraction. The extract is ready for GC/ECD analysis. Store the extracts at 4°C ($\pm$2°C) in the dark until analyses are performed.

7.34 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners present in Calibration Standard #3 (see Appendix A-4). Air or nitrogen blow-down is not recommended since its employment results in intermittent loss of the more volatile PCB congeners.

7.35 Optional Alkali Solution Clean-up. If the gas chromatography reveals that additional sample clean-up is required, this optional alkali solution clean-up step may be performed on a portion of the stored extract (7.33).

7.36 Assemble a K-D concentration apparatus by attaching a 10 ml concentrator tube to a 500 ml evaporation flask. Transfer approximately one half the volume (0.5 ml) of the stored extract (7.33) to the K-D apparatus. Then add one or two clean boiling chips and 2 ml of freshly prepared alkali solution. Attach a three ball snyder column to the apparatus. Pre-wet the snyder column by adding 1 ml of hexane to the top of the column. Immerse the apparatus in a hot water bath and reflux the solution for approximately 30 minutes taking care not to boil the contents to dryness.

7.37 Remove the K-D apparatus from the water bath, remove the condenser and allow the solution to cool. Add 4 ml of reagent water and 2 ml of hexane to the K-D apparatus. Carefully remove the 10 ml concentrator tube from the apparatus and place a glass stopper on the tube. Shake the tube for approximately one minute. Remove the stopper occasionally during this time to vent the contents of the tube.

7.38 Replace the stopper and allow the phases to separate for approximately 10 minutes. Attach a second 10 ml graduated concentrator tube to the K-D flask and transfer the hexane to the apparatus. Repeat steps 7.32 and 7.33.

8.0 Quality Control

8.1 All reagents should be checked prior to use to verify that interferences do not exist. New solvents and other reagents should be run in a method blank prior to use on actual samples. These method blanks should be included in the GC run just prior to their intended use.

8.2 Surrogate standards must be added to all samples, blanks, and standards.

8.3 To evaluate the performance of the analytical method, the method blank, duplicate sample and matrix spike sample should be extracted and cleaned-up according to the procedures as described. The QC check samples must be performed at a frequency of 5% of samples with each sample delivery group (a sample delivery group consists of a maximum of 20 samples collected over no more than a 7 day period).

8.4 The spike standard should contain the most representative compounds at a concentration appropriate to the anticipated sample concentrations.

9.0 References

9.1 USEPA "Test Methods for Evaluating Solid Waste" - Third Edition (SW-846) Revision 1, 1990. Methods 3500A, 3510A, 3600A. 3620A, and 3630A.

Table 1

PCB Congener Matrix Spiking Solution

Congener	Concentration (μg/mL)
2,4'-Dichlorobiphenyl	0.2
2,2',5-Trichlorobipheny	0.2
2,4,4'-Trichlorobiphenyl	0.2
2,2',3,5'-Tetrachlorobiphenyl	0.2
2,2',5,5'-Tetrachlorobiphenyl	0.2
2,3',4,4'-Tetrachlorobiphenyl	0.2
3,3',4,4'-Tetrachlorobiphenyl	0.2
2,2',4,5,5'-Pentachlorobiphenyl	0.2
2,3,3',4,4'-Pentachlorobiphenyl	0.2
2,3',4,4',5-Pentachlorobiphenyl	0.2
3,3',4,4',5-Pentachlorobiphenyl	0.2
2,2',3,3',4,4'-Hexachlorobiphenyl	0.2
2,2',3,4,4',5'-Hexachlorobiphenyl	0.2
2,2',4,4',5,5'-Hexachlorobiphenyl	0.2
2,2',3,3',4,4',5-Heptachlorobiphenyl	0.2
2,2',3,4,4',5,5'-Heptachlorobiphenyl	0.2
2,2',3,4',5,5',6-Heptachlorobiphenyl	0.2
2,2',3,3',4,4',5,6-Octachlorobiphenyl	0.2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	0.2

Appendix A-2

EXTRACTION AND CLEAN-UP OF LARGE VOLUME WATER SAMPLES FOR PCB CONGENER ANALYSIS

1.0 Scope and Application

1.1 This is a procedure for the extraction and clean-up of polychlorinated biphenyls (PCBs) from large volume (20 liter) aqueous samples. The procedure which is a modified version of NYSDEC's Analytical Service Protocols for PCB Congeners, is based on EPA Methods 3510A (separatory funnel liquid-liquid extraction), 3630 (silica gel cleanup), and 3620 (Florisil column cleanup) (Ref. 9.1) with some modifications.

1.2 This extraction and clean-up procedure is meant to be followed by congener specific PCB analysis by GC-ECD.

2.0 Summary of Method

2.1 A measured volume of sample, approximately twenty liters collected in 6 four liter amber glass bottles, is solvent extracted with 85:15 (v/v) hexane/methylene chloride solvent mixture using a separatory funnel. Each bottle is extracted individually. Roughly 400 ml of the solvent mixture are required per sample. The hexane/methylene chloride extract is concentrated in a Kuderna-Danish (K-D) Evaporator, and adjusted to a final volume of 1 to 2 mL.

2.2 The concentrated extract from the hexane/methylene chloride extraction is applied to an activated Florisil column and eluted with 6% ether/hexane (v/v). The eluted fraction is concentrated with a K-D apparatus, added to a silica column and eluted with hexane to separate PCBs from chlorinated pesticides. The eluate is concentrated once again using a K-D apparatus.

2.3 The concentrated eluate from the silica column clean-up may be further purified with sulfuric acid or alcoholic potassium hydroxide to remove matrix interferences.

2.4 The final extract is suitable for gas chromatographic analysis.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, and glassware that lead to false positive peaks and/or elevated baselines in gas chromatograms. All of the reagents and glassware used must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks. Interferences by phthalate esters can pose a major problem in PCB congener analysis when using the electron capture detector. These compounds generally appear in the chromatogram as broadly eluting peaks. Common, flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled. Interferences from phthalates can best be minimized by avoiding the use of all plastics, except Teflon, in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination once it becomes a problem.

3.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry, dry in a 100°C oven or, if necessary, heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

4.0 Apparatus and Materials

4.1 5-L separatory funnel
1-L graduated cylinder
100-ml graduated cylinder
500-ml Erlenmeyer flask (or equivalent)

4.2 Kuderna-Danish (K-D) apparatus:

4.2.1 Concentrator tube - 10 ml, graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.2.2 Evaporation Flask - 500 ml (Kontes K-5700-1-500 or equivalent). Attach to concentrator tube with springs.

4.2.3 Snyder column - Three ball macro (Kontes K-503000-121 or equivalent).

4.2.4 Snyder column - Two ball micro (Kontes K-569001-219 or equivalent).

4.3 Drying Column - 100 mm x 20 mm ID Pyrex chromatographic column with coarse frit and reservoir (Supelco drying column 6-478M or equivalent). A small pad of disposable, hexane washed Pyrex glass wool is substituted for the frit to help prevent cross contamination of sample extracts.

4.4 Boiling Chips - Silicon carbide or equivalent, approximately 10/40 mesh, solvent extracted with hexane for approximately one hour and heated to 400 °C for 30 minutes.

4.5 Water or steam bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.6 Vials - Amber glass 10 to 15 ml capacity, with Teflon lined screw caps.

4.8 Bottle or test tube. 50 ml with Teflon lined screw cap for sulfur removal.

4.9 Balance - Analytical capable of accurately weighing ± 0.01 mg.

4.10 Apparatus for Silica Column clean-up procedure:

4.10.1 Glass chromatographic column, 10 mm ID with Teflon stopcock and reservoir.

4.10.2 Erlenmeyer flask (250 ml).

4.10.3 Glass stoppers.

4.10.4 Long glass rod.

4.10.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.11 Apparatus for Florisil clean-up procedure:

4.11.1 Glass chromatographic column, 20 mm ID with Teflon stopcock and reservoir.

4.11.2 Erlenmeyer flask (250 ml).

4.11.3 Glass stoppers.

4.11.4 Long glass rod.

4.11.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.12 Nitrogen source with regulator and low pressure manifold.

5.0 Reagents

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

5.2 ASTM Type II Water (ASTM D-1193-77). All references to reagent water in this method refer to ASTM Type II unless otherwise specified.

5.3 Sodium sulfate (granular, anhydrous) purification is by washing with acetone/hexane followed by heating at 400 °C for four hours in a shallow tray.

5.4 Organic solvents for extraction and clean-up:

5.4.1 Acetone - Pesticide quality or equivalent.

5.4.2 Hexane - Pesticide quality or equivalent.

5.4.3 Methylene Chloride - Pesticide quality or equivalent.

5.4.4 Dicthyl Ether - Pesticide quality or equivalent. Must be free of peroxide as indicated by test strips.

5.5 Silicic acid (Mallinckroct A.R. 100 mesh or equivalent) Before use, activate each batch at least 16 hours at 135°C in a shallow glass tray, loosely covered with aluminum foil.

5.6 Florisil - Pesticide residue (PR) grade (60/100 mesh). Purchase activated at 1250 °F (677 °C), stored in glass containers with ground glass stoppers or foil-lined screw caps.

5.6.1 Deactivation of Florisil - Just before use, weigh out 1000 g of Florisil into a glass container and heat at 130°C for 16 hours. After heating, cool in a desiccator. Pour into a 4-L solvent bottle, close with a Teflon lined cap and place on a tumbler or roller. Dropwise add 40 ml of water while shaking to deactivate. Tumble for at least 4 hours. There should be no lumps present. Store in a tightly closed amber bottle. Let stand overnight before using.

5.6.2 Florisil activity check - Before each batch of Florisil is first used and once a week thereafter test the Florisil by adding 1 ml of GC/EC CAL STD-3 and following Part C, paragraph 1.2. All resolvable PCB congeners should be present in the extract after column elution. Some batches of Florisil may require 4% deactivation to allow all PCB congeners to be eluted by 50 ml of hexane.

5.7 Copper powder - activate copper powder by treating with diluted nitric acid, rinse with free reagent water to remove all traces of acid, rinse with acetone and dry under a stream of nitrogen.

5.8 Sodium hydroxide solution (10N) - Dissolve 40 g NaOH (ACS reagent grade) in reagent water and dilute to 100 ml.

5.9 Alkali Solution - Prepare fresh daily as needed. Dissolve eight potassium hydroxide pellets into 6 ml of ethanol in a glass stoppered test tube.

5.10 Sulfuric acid solution - Slowly add, with rapid stirring, 50 ml concentrated sulfuric acid (sp. gr. 1.84) to 50 ml of reagent water.

5.11 PCB Surrogate Standard Spiking Solution

5.11.1 The surrogate standards are added to all samples, blanks, matrix spikes, and calibration standards before extraction. The surrogate compounds are tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB). Other surrogates may prove suitable, but it is the responsibility of the laboratory to demonstrate their acceptability as additional surrogates, not substitutes.

5.11.2 Prepare the surrogate standard spiking solution at a concentration of 0.2 μg/1.00 ml of each of the two compounds in acetone. Store the spiking solutions at 4°C (±2°C) in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicates a problem. **CAUTION:** Analysts must allow all spiking solutions to equilibrate to room temperature before use.

5.12 PCB Congener Matrix Standard Spiking Solution

5.12.1 Prepare a matrix spike standard solution that contains each of the congeners listed in Table 1 in acetone. Place each solution in a 10 - 15 ml clean glass vial with a Teflon-lined screw cap and store at 4°C (±2°C) and protect from the light. Stock solutions must be replaced after twelve months, or sooner if comparison with check standards indicates a problem. **Caution:** Each time a vial containing small volumes of solutions is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible headspace, and opening vials should be minimized.

5.12.2 Matrix spikes are also to serve as duplicates by spiking two equal portions from the one sample chosen for spiking.

6.0 Sample Collection, Preservation, and Handling

6.1 Samples will be collected in six 4 liter amber glass bottles. Five bottles will be filled to about 3.8 liters, not 4, so as to allow room for the hexane additions to be mixed in the collection bottle itself. The sixth bottle will contain about 1.0 to 1.25 liters. The set of six 4 liter bottles constitutes one 20 liter sample.

6.2 Preservation - In the field and during transport, all samples collected must be kept cool on wet ice (approximately 4 °C or under). In the laboratory, all samples must be kept in the refrigerated storage room at a temperature ranging from 2 ° - 5 °C before extraction.

6.3 Samples must be extracted within 5 days of verified time of sample receipt (VTSR) and extracts analyzed within 40 days following VTSR.

7.0 Procedure

7.1 Add 20 ml of 85:15 (v/v) hexane-methylene chloride mixture to the first bottle. Swirl the mixture thoroughly, developing a vortex which extends at least half way down the bottle. Allow the mixture to settle one minute and pour the hexane/methylene chloride mixture off the top into a 5-liter separatory flask along with a few hundred ml of water. Leave the bulk of the water in the sample bottle. Return most of the water from the separatory flask to the sample bottle leaving about 0.1 to 1.0 ml of water in the flask. In this first step of the extraction for each 4 liter sample bottle, some of the hexane-methylene chloride mixture is not recovered due to dissolution in the sample.

7.2 Add a second 20 ml aliquot of the hexane-methylene chloride mixture to the 4 liter sample bottle and repeat step 7.1.

7.3 Add a third 20 ml aliquot of the hexane-methylene chloride mixture to the 4 liter sample bottle and swirl as described in step 7.1.

7.4 Pour the entire contents of the 4 liter sample bottle into the 5 liter separatory flask and whirl thoroughly. Wait five minutes for the phases to separate and draw off water from the bottom, leaving behind enough water (about 0.1 ml) to fill the stopcock volume. Measure the volume of water removed to the nearest ml. Do not do a complete

separation. At this point the separatory flask will contain the hexane-methylene chloride mixture, any emulsion, and a very small amount of water.

7.5 Rinse and swirl the 4 liter sample bottle with 5 ml of the hexane-methylene chloride mixture and add to separatory flask. Repeat with additional 5 ml and add to separatory flask.

7.6 Collect the contents of the separatory flask in a glass-stoppered 500 ml erlenmeyer flask. Cap erlenmeyer.

7.7 Repeat steps 7.1 to 7.6 with each of the remaining four 4 liter sample bottles containing 2.8 liter each, being sure to measure the volume of water drawn off each time with a graduated cylinder. The sixth bottle is extracted with 10 ml solvent aliquots instead of 20 ml since it contains only 1 liter of sample. Steps 7.1 to 7.6 are otherwise the same. Be sure to measure the volume of water in the sixth bottle as well. Use the same separatory flask for all six sample bottles (constituting one sample) without cleaning it in between. Collect all hexane-methylene chloride extracts into the same 500 ml erlenmeyer flask.

7.8 Upon completion of the sixth 4 liter sample bottle extraction, rinse the separatory flask with 5 ml of the hexane-methylene chloride mixture and add the rinsate to the erlenmeyer flask. Repeat this rinse with a second 5 ml aliquot of the hexane-methylene chloride mixture and add to the erlenmeyer.

7.9 Slowly add granular sodium sulfate to the extract in the erlenmeyer flask and swirl. This will serve to remove all water in the extract. The initial granules will become crusted. Continue adding crystals until the granules flow freely in the flask without crusting or sticking together. This step should remove nearly all of the emulsion.

7.10 Store the mixture overnight in a freezer. This will serve to break up any additional emulsions. In the morning check for free flow of sodium sulfate. Also check for the absence of a second phase. If a second phase is present or if all sodium sulfate is crusted, additional sodium sulfate should be added until it flows freely and the extract should be placed in the freezer for an additional four hours.

7.11 Assemble a K-D concentrator by attaching a 10 ml concentrator tube to the K-D evaporation flask.

7.12 After breaking up any crusted sodium sulfate with a scoupula, pour the extract through a packed column of purified, granular sodium sulfate. Collect the extract in a K-D evaporation flask to concentrate the sample. Rinse the erlenmeyer flask with 5 ml of hexane/methylene chloride mixture and collect the rinsate in the K-D flask. Repeat this step two more times. Then rinse the column with 15 ml of hexane/methylene chloride mixture and collect the rinsate in the K-D flask.

7.13 Add one or two clean boiling chips to the flask and attach a three ball Snyder column. Prewet the Snyder column by adding 1.0 ml of hexane to the top of the column. Place the K-D apparatus on a hot water bath (15 ° - 20 °C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the hot water temperature, as required, to complete the concentration in 10 - 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid has reached 1 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. DO NOT ALLOW THE K-D TUBE TO GO DRY.

7.14 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.15 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners present in the calibration solutions. Nitrogen blow-down is not recommended, since its employment may result in intermittent loss of the more volatile PCB congeners.

7.16 Sulfur Cleanup

7.16.1 Transfer the extract from paragraph 7.14 to a 15 ml clear glass bottle or vial with a Teflon-lined screw cap. Rinse the concentrator tube with 1.0 ml of hexane, adding the rinsings to the 15 ml bottle. If only a partial set of samples requires sulfur cleanup, set up a new reagent blank with 1.0 ml of hexane and take it through the sulfur cleanup. Include the surrogate standards.

7.16.2 Add activated copper powder to the sample and seal. Agitate vial in a vortex mixer before quantitative transfer to the Florisil column.

7.17 The extract obtained may now be cleaned up on a Florisil column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.18 Florisil column clean-up. Fill the chromatography column to about two-thirds full with hexane. Add a piece of glass wool to the bottom of the column with the help of a long glass rod. Remove any air bubbles which become trapped in the glass wool. Add 20 g of the deactivated Florisil to a 20 mm ID chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 ml of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

7.19 Transfer the sample extract from the K-D concentrator tube or vial to the Florisil column. Rinse the tube twice with 1 - 2 ml hexane, adding each rinse to the column.

7.20 Place a 250 - 500 ml erlenmeyer or round bottom flask under the chromatographic column. Drain the column into the flask until the sodium sulfate is nearly exposed. Elute the column with 200 ml of 6% ethyl ether in hexane (v/v). Elute at a 5 ml/min rate.

7.21 The eluate is then concentrated with the K-D apparatus to an apparent volume of 1 ml. DO NOT ALLOW THE SAMPLE TO GO TO DRYNESS.

7.22 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.23 The concentrated eluate may now be further cleaned up on a Silica column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.24 Silica column clean-up. After drying the silicic acid overnight at 135°C, weigh 3.0 g portions into vials and redry for 1 hour. When ready to prepare column, remove vial of silicic acid from the oven and immediately seal with a Teflon-lined cap. When the vial has cooled to room temperature, add 0.10 ml of water and then reseal. Mix the

contents thoroughly and let the adsorbent equilibrate for approximately one hour with occasional mixing.

NOTE: The silica column chromatography is included in the procedure to remove pesticides from the analysis. The optimum percent water on the silica used in the column chromatography must be determined by running standards. The percent water added to the activated silica should be adjusted to obtain optimum separation between PCBs and pesticides. For calibration purposes, a mixture of the PCB calibration standard with chlordane, op'-DDT, pp'-DDT, pp'-DDE, and pp'-DDD may be used. The pesticide pp'-DDE will not be separated from the PCB fraction. Under optimum conditions, not more than 20-60% of the op'-DDT will elute with the PCB fraction.

7.25 Empty the vial of equilibrated silicic acid into a chromatographic column and tap until the adsorbent is well settled. Top column with 1-2 cm prepared sodium sulfate.

7.26 Wash the column with 20 ml of methylene chloride, forcing solvent through the column at 2-3 ml per minute with approximately 6 psi nitrogen pressure. Stop the flow when the level of the solvent reaches the sodium sulfate layer. At this point, the silicic acid portion should have a uniform translucent appearance, without air pockets or channels.

7.27 Wash the methylene chloride from the column with 20 ml of hexane, again stopping the flow when the solvent level reaches the sodium sulfate layer. The column should have reverted back to its original opaque appearance.

7.28 Add the concentrated eluate from the Florisil column and rinse onto the column with hexane.

7.29 Elute column at 2-3 ml per minute with hexane, collecting the first 50 ml of eluate. Concentrate this fraction on the K-D apparatus to a suitable volume for gas chromatographic analysis.

7.30 Optional Sulfuric Acid Cleanup. Using a syringe or a volumetric pipette, transfer the hexane extract solution to a 10 ml vial and carefully add 5 ml of concentrated sulfuric acid. This procedure must always be done in a fume hood. CAUTION: Make sure that there is neither an exothermic reaction nor evolution of gas prior to proceeding.

7.31 Cap the vial tightly and agitate using a vortex mixer for one minute. A vortex must be visible in the vial. CAUTION: Stop agitating immediately if the vial leaks. **AVOID**

CONTACTING THE SOLUTION WITH BARE SKIN. SULFURIC ACID WILL BURN.

7.32 Allow the phases to separate for at least one minute. Examine the top (hexane) layer. It should not be highly colored nor should it have a visible emulsion or cloudiness. If a clean phase separation is achieved, proceed to paragraph 7.33. If the hexane layer is colored or the emulsion persists for several minutes, remove the sulfuric acid layer from the vial via a glass pipette and dispose of it properly. Add another 5 ml of clean sulfuric acid. NOTE: Do not remove any hexane at this stage of the procedure. Agitate the sample using a vortex mixer and allow the phases to separate as described previously.

7.33 Transfer the hexane layer to a clean 10 ml vial.

7.34 Add an additional 1 ml of hexane to the sulfuric acid layer, cap the vial securely and shake. This second extraction is done to ensure quantitative transfer of all analytes. Remove the second hexane layer and combine with the hexane from paragraph 7.29.

7.35 Final Extract Concentration

7.36 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 ml hexane to the top. Place the K-D apparatus on a hot water or steam bath so that the concentrator tube is partially immersed and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 to 15 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 ml, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.37 Remove the Snyder column, rinse the flask and its lower joint into the concentrator tube with 1 to 2 ml of hexane. Reconnect the concentrator tube and concentrate the extract to a final volume of 1.0 ml. Transfer the 1.0 ml to a GC vial and label as PCB fraction. The extract is ready for GC/EC analysis. Store the extracts at 4°C (±2°C) in the dark until analyses are performed.

7.38 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners present in Calibration Standard #3. Air or

nitrogen blow-down is not recommended since its employment may result in intermittent loss of the more volatile PCB congeners.

7.39 Optional Alkali Solution Clean-up. If the gas chromatography reveals that additional sample clean-up is required, this optional alkali solution clean-up step may be performed on a portion of the stored extract (7.37).

7.40 Assemble a K-D concentration apparatus by attaching a 10 ml concentrator tube to a 500 ml evaporation flask. Transfer approximately one half the volume (0.5 ml) of the stored extract (7.37) to the K-D apparatus. Then add one or two clean boiling chips and 2 ml of freshly prepared alkali solution. Attach a three ball snyder column to the apparatus. Pre wet the snyder column by adding 1 ml of hexane to the top of the column. Immerse the apparatus in a hot water bath and reflux the solution for approximately 30 minutes taking care not to boil the contents to dryness.

7.41 Remove the K-D apparatus from the water bath, remove the condenser and allow the solution to cool. Add 4 ml of reagent water and 2 ml of hexane to the K-D apparatus. Carefully remove the 10 ml concentrator tube from the apparatus and place a glass stopper on the tube. Shake the tube for approximately one minute. Remove the stopper occasionally during this time to vent the contents of the tube.

7.42 Replace the stopper and allow the phases to separate for approximately 10 minutes. Attach a second 10 ml graduated concentrator tube to the K-D flask and transfer the hexane to the apparatus. Repeat steps 7.32 and 7.33.

8.0 Quality Control

8.1 All reagents should be checked prior to use to verify that interferences do not exist. New solvents and other reagents should be run in a method blank prior to use on actual samples. These method blanks should be included in the GC run just prior to their intended use.

8.2 Surrogate standards should be added to all samples.

8.3 To evaluate the performance of the analytical method, the method blank, duplicate sample and matrix spike sample should be extracted and cleaned-up according to the procedures as described. The QC check samples must be performed at a frequency of 5% of

samples with each sample delivery group (a sample delivery group consists of a maximum of 20 samples collected over no more than a 14 day period).

8.4 The spike standard should contain the most representative compounds at a concentration appropriate to the anticipated sample concentrations.

9.0 References

9.1 USEPA Test Methods for Evaluating Solid Waste (SW-846) Revision 1, 1990. Methods 3500A, 3510A, 3600A. 3620A, and 3630A.

9.2 Bopp, R.F. The geochemistry of polychlorinated biphenyls in the Hudson River. Ph.D. Dissertation, Columbia University, 1979.

Table 1
PCB Congener Matrix Spiking Solution

Congener	Concentration (µg/mL)
2,4'-Dichlorobiphenyl	0.2
2,2',5-Trichlorobipheny	0.2
2,4,4'-Trichlorobiphenyl	0.2
2,2',3,5'-Tetrachlorobiphenyl	0.2
2,2',5,5'-Tetrachlorobiphenyl	0.2
2,3',4,4'-Tetrachlorobiphenyl	0.2
3,3',4,4'-Tetrachlorobiphenyl	0.2
2,2',4,5,5'-Pentachlorobiphenyl	0.2
2,3,3',4,4'-Pentachlorobiphenyl	0.2
2,3',4,4',5-Pentachlorobiphenyl	0.2
3,3',4,4',5-Pentachlorobiphenyl	0.2
2,2',3,3',4,4'-Hexachlorobiphenyl	0.2
2,2',3,4,4',5'-Hexachlorobiphenyl	0.2
2,2',4,4',5,5'-Hexachlorobiphenyl	0.2
2,2',3,3',4,4',5-Heptachlorobiphenyl	0.2
2,2',3,4,4',5,5'-Heptachlorobiphenyl	0.2
2,2',3,4',5,5',6-Heptachlorobiphenyl	0.2
2,2',3,3',4,4',5,6-Octachlorobiphenyl	0.2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	0.2

Appendix A-3

EXTRACTION AND CLEAN-UP OF SEDIMENTS AND PARTICULATES FOR PCB CONGENER ANALYSIS

1.0 Scope and Application

1.1 This is a procedure for the extraction and clean-up of polychlorinated biphenyls (PCBs) from river sediments. The procedure is based on EPA Methods 3540A (soxhlet extraction), 3630 (silica gel cleanup), and 3620 (Florisil column cleanup) (Ref. 9.1) with some modifications.

1.2 This extraction and clean-up procedure is meant to be followed by congener specific PCB analysis by GC-ECD.

2.0 Summary of Method

2.1 A portion of the sediment sample is mixed with anhydrous sodium sulfate to dryness, placed in an extraction thimble or between two plugs of glass wool, and extracted with a 50:50 (v/v) acetone/hexane solvent mixture with a soxhlet extractor. The extract is then concentrated. If necessary, sulfur can be removed from the extract using a copper clean-up procedure.

2.2 The concentrated acetone/hexane extract is applied to an activated Florisil column and eluted with 6% ether/hexane (v/v). The eluted fraction is concentrated with a Kuderna Danish (K-D) apparatus and added to a silica column and eluted with hexane to separate PCBs from chlorinated pesticides. The eluate is then concentrated using a K-D apparatus.

2.3 The concentrated eluate from the silica column clean-up may be further purified with sulfuric acid to remove matrix interferences.

2.4 The final extract is suitable for gas chromatographic analysis.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, and glassware that lead to false positive peaks and/or elevated baselines in gas chromatograms. All of the reagents and glassware used must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks. Interferences by phthalate esters can pose a major problem in PCB congener analysis when using the electron capture detector. These compounds generally appear in the chromatogram as broadly eluting peaks. Common, flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled. Interferences from phthalates can best be minimized by avoiding the use of all plastics, except Teflon, in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination once it becomes a problem.

3.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry, dry in a 100°C oven or, if necessary, heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

4.0 Apparatus and Materials

4.1 Soxhlet Extractor - 40 mm ID, with 500 ml round bottom flask.

4.2 Kuderna-Danish (K-D) apparatus:

4.2.1 Concentrator tube - 10 ml, graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.2.2 Evaporation Flask - 500 ml (Kontes K-5700-1-500 or equivalent). Attach to concentrator tube with springs.

4.2.3 Snyder column - Three ball macro (Kontes K-503000-121 or equivalent).

4.2.4 Snyder column - Two ball micro (Kontes K-569001-219 or equivalent).

4.3 Boiling Chips - Silicon carbide or equivalent, approximately 10/40 mesh, solvent extracted with hexane for approximately one hour and heated to 400 °C for 30 minutes.

4.4 Water Bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.5 Vials - Glass, 12 and 2 ml capacity, with Teflon lined screw or crimp top.

4.6 Glass thimble or glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.7 Heating mantle - Rheostat controlled

4.8 Disposable glass pasteur pipet and bulb.

4.9 Apparatus for determining percent dry weight.

4.9.1 Oven - Drying.

4.9.2 Desiccator.

4.9.3 Crucibles - Porcelain or disposable aluminum.

4.10 Analytical balance - 0.0001 g.

4.11 Apparatus for Silica Column clean-up procedure:

4.11.1 Glass chromatographic column, 10 mm ID with Teflon stopcock and reservoir.

4.11.2 Erlenmeyer flask (250 ml).

4.11.3 Glass stoppers.

4.11.4 Long glass rod.

4.11.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.12 Apparatus for Florisil clean-up procedure:

4.12.1 Glass chromatographic column, 20 mm ID with Teflon stopcock and reservoir.

4.12.2 Erlenmeyer flask (250 ml).

4.12.3 Glass stoppers.

4.12.4 Long glass rod.

4.12.5 Glass wool - Soxhlet extracted for at least two hours with 50:50 (v/v) acetone/hexane solvent mixture.

4.13 Nitrogen source with regulator and low pressure manifold.

5.0 Reagents

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

5.2 ASTM Type II Water (ASTM D-1193-77) or equivalent. All references to reagent water in this method refer to ASTM Type II unless otherwise specified.

5.3 Sodium sulfate (granular, anhydrous) purification is by washing with acetone/hexane followed by heating at 400 °C for four hours in a shallow tray.

5.4 Organic solvents for extraction and clean-up:

5.4.1 Acetone - Pesticide quality or equivalent.

5.4.2 Hexane - Pesticide quality or equivalent.

5.4.3 Diethyl ether - Pesticide quality or equivalent. Must be free of peroxide as indicated by test strips.

5.4.4 Methylene Chloride - Pesticide quality or equivalent.

5.5 Silicic acid (Mallinckroct A.R. 100 mesh or equivalent) Before use, activate each batch at least 16 hours at 135°C in a shallow glass tray, loosely covered with aluminum foil.

5.6 Florisil - Pesticide residue (PR) grade (60/100 mesh). Purchase activated at 1250 °F (677 °C), stored in glass containers with ground glass stoppers or foil-lined screw caps.

5.6.1 Deactivation of Florisil - Just before use, weigh out 1000 g of Florisil into a glass container and heat at 130°C for 16 hours. After heating, cool in a desiccator. Pour into a 4-L solvent bottle, close with a Teflon lined cap and place on a tumbler or roller. Dropwise add 40 ml of water while shaking to deactivate. Tumble for at least 4 hours. There should be no lumps present. Store in a tightly closed amber bottle. Let stand overnight before using.

5.6.2 Florisil activity check - Before each batch of Florisil is first used and once a week thereafter test the Florisil by adding 1 ml of GC/ECD CAL STD-3 (see Appendix A-4) and following 7.13. All resolvable PCB congeners should be present in the extract after column elution. Some batches of Florisil may require 4% deactivation to allow all PCB congeners to be eluted by 50 ml of hexane.

5.7 Copper powder - activate copper powder by treating with diluted nitric acid, rinse with free reagent water to remove all traces of acid, rinse with acetone and dry under a stream of nitrogen.

5.8 PCB Surrogate Standard Spiking Solution

5.8.1 The surrogate standards are added to all samples, blanks, matrix spikes, and calibration standards before extraction. The surrogate compounds are

tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB). Other surrogates may prove suitable, but it is the responsibility of the laboratory to demonstrate their acceptability as additional surrogates, not substitutions.

5.8.2 Prepare the surrogate standard spiking solution at a concentration of 0.2 μg/1.00 ml of each of the two compounds in acetone. Store the spiking solutions at 4°C (±2°C) in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicates a problem. **CAUTION**: Analysts must allow all spiking solutions to equilibrate to room temperature before use.

5.9 PCB Congener Matrix Standard Spiking Solution

5.9.1 Prepare a matrix spike standard solution that contains each of the congeners listed in Table 1 in acetone. Place each solution in a 10 - 15 ml clean glass vial with a Teflon-lined screw cap and store at 4°C (±2°C) and protect from the light. Stock solutions must be replaced after twelve months, or sooner if comparison with check standards indicates a problem. **Caution**: Each time a vial containing small volumes of solutions is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible headspace, and opening vials should be minimized.

5.9.2 Matrix spikes are also to serve as duplicates by spiking two equal portions from the one sample chosen for spiking.

5.10 Sulfuric Acid Solution - Slowly add, with rapid stirring, 50 ml concentrated sulfuric acid (sp. gr. 1-84) to 50 ml of reagent water.

5.11 Alkali Solution - Prepare fresh daily as needed. Dissolve eight pellets of potassium hydroxide into 6 ml of ethanol in a glass stoppered test tube.

6.0 Sample Collection, Preservation and Handling

6.1 Sample collection is according to the study protocol and relevant standard operating procedures.

6.2 Preservation - In the field and during transport, all samples collected must be kept cool on wet ice (approximately 4 °C or under). In the laboratory, all samples must be kept in the refrigerated storage room at a temperature ranging from 2 ° - 5 °C before extraction.

6.3 Samples must be extracted within 5 days of verified time of sample receipt (VTSR) and extracts analyzed within 40 days following VTSR.

7.0 Procedure

7.1 Sediment samples. Mix sample thoroughly, especially samples which have been field composited. Discard foreign objects such as sticks, leaves, and rocks.

7.2 Determination of sample % dry weight - In all cases sample results must be reported on a dry weight basis. When such data are desired, a portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination (see 7.3).

7.3 Weigh out a 2.0 g sediment sample for extraction into a tared 250 ml beaker. Immediately after weighing the sample for extraction, weigh 1 - 2 g, weight to nearest 0.001 g accuracy of the sample into a tared crucible or aluminum weighing dish. Determine the % dry weight of the sample by drying overnight at 105 °C. Allow to cool in a desiccator before weighing.

% dry weight = g dry sample/g sample x 100

7.4 Blend 2 g of sediment with 2 g anhydrous sodium sulfate (the sodium sulfate should be added in small aliquots). For filtered particulate samples, the filter with particulates is placed in the extractor. A small amount of water, equal to the weight of the filter, must be added to filtered particulate samples prior to extraction. Place in a pre-cleaned extraction thimble. The thimble and glass wool should be soxhlet extracted for approximately six cycles prior to using. The extraction thimble must drain freely for the duration of the extraction period. A glass wool plug above and below the sample in the Soxhlet extractor is an acceptable alternative for the thimble. Add surrogate standard spiking solution onto the sample. For the sample in each analytical batch selected for spiking, add 1.0 ml (50 μg/l) of the matrix spiking standard.

7.5 Place 300 ml 50:50 (v/v) of acetone/hexane solvent mixture into a 500 ml round bottom flask containing one or two clean boiling chips. Attach the flask to the extractor and extract the sample for approximately 16 hours at 4 - 6 cycles/hr.

7.6 Allow the extract to cool after the extraction is complete.

7.7 Assemble a K-D concentrator by attaching a 10 ml concentrator tube to a 500 ml evaporation flask.

7.8 Add one or two clean boiling chips to the flask and attach a three ball Snyder column. Prewet the Snyder column by adding 1.0 ml of hexane to the top of the column. Place the K-D apparatus on a hot water bath (15 ° - 20 °C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the hot water temperature, as required, to complete the concentration in 10 - 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid has reached 1 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. DO NOT ALLOW THE K-D TUBE TO GO DRY.

7.9 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.10 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners present in the calibration solutions. Nitrogen blow-down is not recommended, since its employment may result in intermittent loss of the more volatile PCB congeners.

7.11 Add a sufficient quantity of copper shavings to scavenge the sulfur. It is anticipated that all sediment extracts will require sulfur removal.

7.12 The extracts obtained may now be cleaned up on a Florisil column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.13 Florisil column clean-up. Fill the chromatography column to about two-thirds full with hexane. Add a piece of glass wool to the bottom of the column with the help of a long glass rod. Remove any air bubbles which become trapped in the glass wool. Add 20 g of the deactivated Florisil to a 20 mm ID chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 ml of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

7.14 Transfer the sample extract from the K-D concentrator tube or vial to the Florisil column. Rinse the tube twice with 1 - 2 ml hexane, adding each rinse to the column.

7.15 Place a 250 - 500 ml erlenmeyer or round bottom flask under the chromatographic column. Drain the column into the flask until the sodium sulfate is nearly exposed. Elute the column with 200 ml of 6% ethyl ether in hexane (v/v). Elute at a 5 ml/min rate.

7.16 The eluate is then concentrated with the K-D apparatus to an apparent volume of 1 ml. DO NOT ALLOW THE SAMPLE TO GO TO DRYNESS.

7.17 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of hexane.

7.18 The concentrated eluate must now be further cleaned up on a Silica column. If the clean-up of the extract will not be performed immediately, transfer the concentrate to a 10 ml Teflon-lined screw-cap vial with a pasteur pipet (rinsing concentrator tube with several aliquots of hexane into the vial) and store refrigerated.

7.20 Silica column clean-up. After drying the silicic acid overnight at 135°C, weigh 3.0 g portions into vials and redry for 1 hour. When ready to prepare column, remove vial of silicic acid from the oven and immediately seal with a Teflon-lined cap. When the vial has cooled to room temperature, add 0.10 ml of water and then reseal. Mix the contents thoroughly and let the adsorbent equilibrate for approximately one hour with occasional mixing.

NOTE: The silica column chromatography is included in the procedure to remove pesticides from the analysis. The optimum percent water on the silica used in the column

chromatography must be determined by running standards. The percent water added to the activated silica should be adjusted to obtain optimum separation between PCBs and pesticides. For calibration purposes, a mixture of the PCB calibration standard with chlordane, op'-DDT, pp'-DDT, pp'-DDE, and pp'-DDD may be used. The pesticide pp'-DDE will not be separated from the PCB fraction. Under optimum conditions, not more than 20-60% of the op'-DDT will elute with the PCB fraction.

7.21 Empty the vial of equilibrated silicic acid into a chromatographic column and tap until the adsorbent is well settled. Top column with 1-2 cm sodium sulfate (Na_2SO_4).

7.22 Wash the column with 20 ml of methylene chloride, forcing solvent through the column at 2-3 ml per minute with approximately 6 psi nitrogen pressure. Stop the flow when the level of the solvent reaches the sodium sulfate layer. At this point, the silicic acid portion should have a uniform translucent appearance, without air pockets or channels.

7.23 Wash the methylene chloride from the column with 20 ml of hexane, again stopping the flow when the solvent level reaches the sodium sulfate layer. The column should have reverted back to its original opaque appearance.

7.24 Add the concentrated eluate from the Florisil column and rinse onto the column with hexane.

7.25 Elute column at 2-3 ml per minute with hexane, collecting the first 50 ml of eluate. Concentrate this fraction on the K-D apparatus to a suitable volume for gas chromatographic analysis.

7.26 Optional Sulfuric Acid Cleanup. Using a syringe or a volumetric pipette, transfer the hexane extract solution to a 10 ml vial and carefully add 5 ml of concentrated sulfuric acid. This procedure must always be done in a fume hood. CAUTION: Make sure that there is neither an exothermic reaction nor evolution of gas prior to proceeding.

7.27 Cap the vial tightly and agitate using a vortex mixer for one minute. A vortex must be visible in the vial. CAUTION: Stop agitating immediately if the vial leaks. **AVOID CONTACTING THE SOLUTION WITH BARE SKIN. SULFURIC ACID WILL BURN.**

7.28 Allow the phases to separate for at least one minute. Examine the top (hexane) layer. It should not be highly colored nor should it have a visible emulsion or cloudiness. If a clean phase separation is achieved, proceed to paragraph 7.29. If the hexane layer is colored or the emulsion persists for several minutes, remove the sulfuric acid layer from the vial via a glass pipette and dispose of it properly. Add another 5 ml of clean sulfuric acid. NOTE: Do not remove any hexane at this stage of the procedure. Agitate the sample using a vortex mixer and allow the phases to separate as described previously.

7.29 Transfer the hexane layer to a clean 10 ml vial.

7.30 Add an additional 1 ml of hexane to the sulfuric acid layer, cap the vial securely and shake. This second extraction is done to ensure quantitative transfer of all analytes. Remove the second hexane layer and combine with the hexane from paragraph 7.29.

7.31 Final Extract Concentration

7.32 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 ml hexane to the top. Place the K-D apparatus on a hot water or steam bath so that the concentrator tube is partially immersed and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 to 15 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 ml, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.33 Remove the Snyder column, rinse the flask and its lower joint into the concentrator tube with 1 to 2 ml of hexane. Reconnect the concentrator tube and concentrate the extract to a final volume of 1.0 ml. Transfer the 1.0 ml to a GC vial and label as PCB fraction. The extract is ready for GC/ECD analysis. Store the extracts at 4°C ($\pm$2°C) in the dark until analyses are performed.

7.34 Other concentration devices or techniques may be used in place of the K-D if equivalency is demonstrated for all the PCB congeners evaluated. Air or nitrogen blow-down is not recommended since its employment may result in intermittent loss of the more volatile PCB congeners.

7.35 Optional Alkali Solution Clean-up. If the gas chromatography reveals that additional sample clean-up is required, this optional alkali solution clean-up step may be performed on a portion of the stored extract (7.33).

7.36 Assemble a K-D concentration apparatus by attaching a 10 ml concentrator tube to a 500 ml evaporation flask. Transfer approximately one half the volume (0.5 ml) of the stored extract (7.33) to the K-D apparatus. Then add one or two clean boiling chips and 2 ml of freshly prepared alkali solution. Attach a three ball snyder column to the apparatus. Pre wet the snyder column by adding 1 ml of hexane to the top of the column. Immerse the apparatus in a hot water bath and reflux the solution for approximately 30 minutes taking care not to boil the contents to dryness.

7.37 Remove the K-D apparatus from the water bath, remove the condenser and allow the solution to cool. Add 4 ml of reagent water and 2 ml of hexane to the K-D apparatus. Carefully remove the 10 ml concentrator tube from the apparatus and place a glass stopper on the tube. Shake the tube for approximately one minute. Remove the stopper occasionally during this time to vent the contents of the tube.

7.38 Replace the stopper and allow the phases to separate for approximately 10 minutes. Attache a second 10 ml graduated concentrator tube to the K-D flask and transfer the hexane to the apparatus. Repeat steps 7.32 and 7.33.

8.0 Quality Control

8.1 All reagents should be checked prior to use to verify that interferences do not exist. New solvents and other reagents should be run in a method blank prior to use on actual samples. These method blanks should be included in the GC run just prior to their intended use.

8.2 Surrogate standards should be added to all samples, standards, and blanks.

8.3 To evaluate the performance of the analytical method, the method blank, duplicate sample and matrix spike sample should be extracted and cleaned-up according to the procedures as described. The QC check samples must be performed at a frequency of 5% of samples with each sample delivery group (a sample delivery group consists of a maximum of 20 samples collected over no more than a 7 day period).

8.4 The spike standard should contain the most representative compounds at a concentration appropriate to the anticipated sample concentrations.

9.0 References

9.1 USEPA "Test Methods for Evaluating Solid Waste" - Third Edition (SW-846) Revision 1, 1990. Methods 3500A, 3540A, 3600A. 3620A, and 3630A.

9.2 Bopp, R.F. The geochemistry of polychlorinated biphenyls in the Hudson River. Ph.D. Dissertation, Columbia University, 1979.

Table 1

PCB Congener Matrix Spiking Solution

Congener	Concentration (µg/mL)
2,4'-Dichlorobiphenyl	0.2
2,2',5-Trichlorobipheny	0.2
2,4,4'-Trichlorobiphenyl	0.2
2,2',3,5'-Tetrachlorobiphenyl	0.2
2,2',5,5'-Tetrachlorobiphenyl	0.2
2,3',4,4'-Tetrachlorobiphenyl	0.2
3,3',4,4'-Tetrachlorobiphenyl	0.2
2,2',4,5,5'-Pentachlorobiphenyl	0.2
2,3,3',4,4'-Pentachlorobiphenyl	0.2
2,3',4,4',5-Pentachlorobiphenyl	0.2
3,3',4,4',5-Pentachlorobiphenyl	0.2
2,2',3,3',4,4'-Hexachlorobiphenyl	0.2
2,2',3,4,4',5'-Hexachlorobiphenyl	0.2
2,2',4,4',5,5'-Hexachlorobiphenyl	0.2
2,2',3,3',4,4',5-Heptachlorobiphenyl	0.2
2,2',3,4,4',5,5'-Heptachlorobiphenyl	0.2
2,2',3,4',5,5',6-Heptachlorobiphenyl	0.2
2,2',3,3',4,4',5,6-Octachlorobiphenyl	0.2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	0.2

Appendix A-4

CONGENER SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCB's) IN HEXANE EXTRACTS BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

1.0 Scope and Application

1.1 This method, which is a modified version of NYSDEC's Analytical Service Protocols for PCB Congeners, describes procedures for the determination of polychlorinated biphenyl (PCB) congeners in hexane extracts by fused silica capillary column gas chromatograph with electron capture detector (FSCC/GC/ECD). The method is applicable to samples containing PCBs as single congeners or as complex mixtures, such as commercial Aroclors. PCBs are identified and quantitated by congener. A concentration is determined for each PCB congener.

2.0 Summary of Method

2.1 Hexane extracts are analyzed for PCB congeners on a fused silica capillary column gas chromatograph with electron capture detector (FSCC/GC/ECD). If PCB congeners are tentatively identified, a second GC/ECD analysis is recommended using an alternate column. Prior to sample analysis the method requires the laboratory to use individual congener standards to identify the retention time order of the resolvable congener peaks for each column that is used in the FSCC/GC/ECD analysis. The laboratory must also determine the method detection limit using FSCC/GC/ECD for each of the resolvable PCB congener peaks.

2.2 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.

3.0 Apparatus and Materials

3.1 Gas chromatograph - An analytical system complete with gas chromatograph and all required accessories including syringes, analytical columns, gases, electron capture detector, and strip-chart recorder with recording integrator.

3.1.1 Quantitation and/or Confirmation Columns

- Capillary Column 1 - 40 m x 0.18 mm ID, 0.4 micron film thickness, fused silica capillary column (J&W Scientific DB-5 or equivalent).

- Capillary Column 2 - 60 m x 0.25 mm ID, 0.25 micron film thickness, fused silica SP-2100 (or equivalent), splitless mode

3.2 A data system capable of handling information on the congener peaks is required for measuring peak areas or peak heights, recording retention times, and calculating data.

3.3 Balance - analytical capable of accurately weighing ± 0.01 mg.

4.0 Reagents

4.1 Solvents

4.1.1 Hexane - Pesticide quality or equivalent.

4.1.2 Acetone - Pesticide quality or equivalent.

4.1.3 Toluene - Pesticide quality or equivalent.

4.2 Stock Standard Solution. Prepare a stock standard solution of each of the PCB congeners listed in Table 1 and any additional individual PCB congeners that the laboratory has identified to be resolvable and commercially available. These should be at a concentration 10,000 times more concentrated than Calibration Standard #1 (4.4.1). Place the solution in a clean glass vial with a Teflon-lined screw cap and store at 4°C (± 2°C) and protect from the light. The stock solution must be replaced after twelve months, or sooner if comparison with check standards indicates a problem.

4.3 Primary Dilution Standard Solution. Accurately measure 100 µL aliquots of the PCB congener stock solution (4.2) and dilute to 10 mL in a volumetric flask with hexane.

4.4 Calibration Standard Solutions. Prepare calibration standards, diluted from the Primary Dilution Standard Solution (4.3) with hexane, at a minimum of 5 concentrations levels, such that Calibration Standard #1 contains each of the appropriate PCB congeners at a concentration 5 times the MDL for that congener (assume absolute detection limits of 5 to 10 nanograms per congener). Include the tetrachloro-m-xylene and decachlorobiphenyl surrogates in each standard (see accompanying SOPs for extraction of PCBs from sediment and water). Table 1 is a suggested list of PCB congeners to be used that are currently available. Place each solution in a clean glass vial with a Teflon-lined screw cap and store at 4°C (±2°C) and protect from the light.

4.4.1 Calibration Standard #1 (CAL STD-1). Dilute 100 µL of the Primary Dilution Standard Solution to 10 mL using hexane.

4.4.2 Calibration Standard #2 (CAL STD-2). Dilute 200 µL of the Primary Dilution Standard Solution to 10 mL using hexane.

4.4.3 Calibration Standard #3 (CAL STD-3). Dilute 400 µL of the Primary Dilution Standard Solution to 10 mL using hexane.

4.4.4 Calibration Standard #4 (CAL STD-4). Dilute 600 µL of the Primary Dilution Standard Solution to 10 mL using hexane.

4.4.5 Calibration Standard #5 (CAL STD-5). Dilute 800 µL of the Primary Dilution Standard Solution to 10 mL using hexane.

5.0 Initial Calibration

5.1 The gas chromatographic system must initially be calibrated using the external standard technique for all columns used for quantitation. Table 2 provides operating conditions for the gas chromatograph. Tables 3 and 4 list the identification and the typical relative retention times for the resolvable peaks using these columns. These are provided for information only. The Laboratory must determine the relative retention times for these peaks for the specific analytical system used.

5.1.1 Prepare calibration standards as described in 4.4 above.

5.1.2 Inject 1 to 2 μL of the standard extract using the solvent-flush technique or auto samplers. Smaller volumes can be injected only if automatic devices are employed. Record the volume injected to the nearest 0.05 μL and the total extract volume.

5.1.3 Inject each of the five Calibration Standards and a hexane blank. To establish the RT window for the PCB congener peaks, use the mean of the absolute RT from the above chromatograms as the mid-point, and ± 3 times the standard deviation as calculated below for each congener. CAL STD-3 is analyzed intermittently throughout the analysis. Any PCB congener outside of its established retention time window requires immediate investigation and correction before continuing the analysis. The laboratory must reanalyze all affected samples.

SD = *Standard Deviation of the retention time for each congener*

Where:

$$SD = \sqrt{\frac{\sum_{i-1}^{N} (x_i - \bar{x}_i)^2}{N - 1}}$$

$\bar{x}_i$ = mean retention time for congener(i)

5.1.4 Tabulate peak height or area responses against the mass injected for each Calibration Standard. The results can be used to prepare a calibration curve for each PCB congener peak. A first or second order regression equation may be used to fit to the data. No higher order of regression is allowed. The correlation coefficient must be >0.990. A plot of the calibration curve and standards must be supplied with the sample results.

NOTE: Use of a computerized statistical data package is highly recommended, with the data being electronically transmitted to the computer from the GC.

5.1.5 This should be done on each quantitation column and each instrument before any samples are analyzed by this procedure, each time a new column is installed, or whenever quality control samples indicate a calibration problem.

5.1.6 This full range calibration must be done whenever significant adjustments to the GC system are made (e.g., column changes, cleaning or changing the detector).

6.0 Method Detection Limit (MDL) and Extraction Efficiency Determinations

Prior to receiving environmental samples, the laboratory must determine method detection limits for the suite of PCB congener matrix spiking compounds identified in the accompanying SOPs for the extraction and clean-up of sediment and water samples. The laboratory must also demonstrate that acceptable PCB congener extraction efficiencies can be obtained using the extraction and analysis procedures defined in this and the accompanying SOPs.

6.1 MDL Determination for 1 Liter Water Samples

6.1.1 Prepare seven low level PCB congener MDL standards by spiking a seven liter volume of reagent water with 700 μL of the PCB congener matrix spiking solution (see accompanying SOP "Extraction and Clean-Up of Water Samples for PCB Congener Analyses").

6.1.2 Extract these low level PCB MDL standards following the procedures outlined in the accompanying SOP "Extraction and Clean-Up of Water Samples for PCB Congener Analyses."

6.1.3 Analyze the low level PCB MDL standards as described in section 7 of this SOP.

6.1.4 Calculate the standard deviation of the results for each congener for the seven analyses as follows:

SD = *Standard Deviation of the results for each congener*

Where:

$$SD = \sqrt{\frac{\sum_{i-1}^{N} (x_i - \bar{x}_i)^2}{N - 1}}$$

x_i = mean result time for congener(i)

N = 7

6.1.5 Calculate the MDL for each congener as follows:

MDL = 3.14 x SD

6.2 MDL Determination for 20 Liter Water Samples

6.2.1 Prepare seven low level PCB congener MDL standards by spiking 100 µl of the PCB congener matrix spiking solution into each of seven 20 liter volumes of reagent water. Divide each 20 liter volume of spiked solution equally between six 4 liter amber glass containers.

6.2.2 Extract these low level PCB MDL standards following the procedures outlined in the accompanying SOP "Extraction and Clean-Up of Large Volume Water Samples for PCB Congener Analyses."

6.2.3 Repeat steps 6.1.3 to 6.1.5.

6.3 MDL Determination for Sediment Samples

6.3.1 Prepare seven low level PCB congener MDL standards by spiking 150 µl of the PCB congener matrix spiking solution into each of seven 2 gram samples EPA certified clean sand.

6.3.2 Extract these low level PCB MDL standards following the procedures outlined in the accompanying SOP "Extraction and Clean-Up of Sediment Samples for PCB Congener Analyses."

6.2.3 Repeat steps 6.1.3 to 6.1.5.

6.4 Extraction Efficiency Determinations

6.4.1 Extraction efficiency determinations will be evaluated on environmental samples supplied to the laboratory prior to initiation of the project. Four sediment, four large volume filtered water, and four 1 liter whole water samples will be supplied to the laboratory.

6.4.2 The laboratory must extract and analyze each sample according to this and accompanying SOPs.

6.4.3 The laboratory must save the extracted samples and then re-extract those samples using the same procedures as specified in 6.4.2.

6.4.4 Extraction efficiencies will be evaluated as the mass of congener removed in the first extraction divided by the sum of the mass of congener removed in the first and second extractions.

$$EE = \frac{M_f}{M_f + M_s} \times 100$$

Where:

EE = extraction efficiency

M_f = mass of congener removed in first extraction

M_s = mass of congener removed in second extraction

6.4.5 Extraction efficiencies must be calculated for each congener and for each sample analyzed. Extraction efficiencies must be at least 95%. If any extraction efficiency for any congener is below 95%, the laboratory must contact the Quality Assurance Officer for the project and review the analyses.

7.0 FSCC/GC/ECD Primary Analysis

(Quantitation may be performed on primary or confirmation analyses).

7.1 Sample analysis of extracts can begin when linearity QA/QC requirements specified in 10.4.3.4 have been met. **NOTE:** The 10.0% RSD linearity criterion is only required on the column(s) being used for PCB quantitation.

7.2 Analyze samples in groups of no more than 5 samples. After the analysis of each group of up to 5 samples, analyze CAL STD-3. The PCB analytical sequence must end with CAL STD-3 regardless of the number of samples analyzed (see 7.8.1).

7.3 If the samples are split between 2 or more instruments, the complete set of standards must be analyzed on each instrument with the same calibration requirement. All standards must be analyzed prior to the samples to avoid the effects of poor chromatography caused by the unsuspected injection of a highly concentrated sample.

7.4 Paragraphs 7.6 and 7.7 contains GC performance criteria. If it is determined during the course of the analytical sequence that one or more of the criteria have been violated, stop the run and take corrective action. After corrective action has been taken, the analytical sequence may be restarted as follows.

7.4.1 If a standard violated the criterion, restart the sequence with the previous standard, determine that the criteria have been met and continue with sample analyses, according to 7.8.1.

7.4.2 If a sample violated the criterion, restart the sequence with the standard that preceded that group of samples (thereby preserving the sequence of standards in 7.8.1), determine that the criteria have been met and continue with sample analysis, according to 7.8.1.

7.5 If it is determined after completion of a analytical run that one or more criteria have been violated, proceed as follows.

7.5.1 If a standard violated the criterion, all samples analyzed after the previous good standard must be re-analyzed as part of a new analytical sequence.

7.5.2 If a subsequent standard in the original sequence met all the criteria, then only those samples analyzed between the standard previous to the standard that did not meet the criterion and the standard that did meet the criterion must be re-analyzed as part of a new analytical sequence.

7.5.3 If only samples violated the criteria, then those samples must be re-analyzed as part of a new analytical sequence.

7.6 Differences in the Calibration Factors for each CAL STD-3 must not exceed 15.0% during the Primary Analysis. Calculate % difference using the initial CAL STD-3 versus all subsequent CAL STD-3 analyzed during the analytical sequence. **NOTE:** To determine that no PCB congeners are present at or above the practical quantitation limit is a form of quantitation.

7.7 The retention time shift of the tetrachloro-m-xylene and decachlorobiphenyl surrogates in any standard or sample must be less than 0.3% difference.

7.8 Inject 1 to 2 μL of the sample or standard extract using the solvent-flush technique or auto samplers. Smaller volumes can be injected only if automatic devices are employed. Record the volume injected to the nearest 0.05 μL and the total extract volume.

7.8.1 The analytical sequence must be as follows:

Analytical Sequence for PCB Analysis:

1. CAL STD-1
2. CAL STD-2
3. CAL STD-3
4. CAL STD-4
5. CAL STD-5
6. Instrument Blank
7. 5 samples
8. CAL STD-3
9. Instrument Blank
10. 5 samples
11. Repeat the above sequence starting with CAL STD-3 (step 8 above).

12. PCB congener analysis sequence must end with the analyses of CAL STD-3

7.8.2 Inject the method blank (extracted with each set of samples) on every GC and GC column on which samples are analyzed.

7.9 Evaluate chromatograms according to the following guidance.

7.9.1 Consider the sample negative when all PCB congener peaks, depending on the congener's response factor, results in concentration less than the method detection limit. The sample is complete at this point. Confirmation is not required.

7.9.2 Tentative identification is when the unknown's retention time matches the retention time of a corresponding standard congener peak from the previous CAL STD-3.

7.9.3 Determine if any PCB congeners are present.

7.9.3.1 If the response for all of these compounds is less than or equal to the response of CAL STD-5, the extract is ready for confirmation and quantitation.

7.9.3.2 If the response for any compound is greater than the response for CAL STD-5, dilute the extract so that the peak will be approximately midway in the calibration range and reanalyze. Use this dilution also for confirmation and quantitation.

7.9.4 Quantitation may be performed on the primary analysis. See Section IV for special QC requirements for quantitation.

7.9.5 If identification of compounds of interest are prevented by the presence of interferences, further cleanup is required.

7.9.6 When selecting a GC column for confirmation and/or quantitation, be sure as few as possible of the PCB congeners to be confirmed/quantitated overlap. When samples are very complex, it may be necessary to use more than two columns to achieve adequate separation (>75% resolution) for as many as possible of the

PCB congeners being quantitated. Two recommended capillary columns are listed in Table 2. The analyst should be aware that coeluting PCB congeners on one column may coelute with different PCB congeners on a different column.

8.0 GC/ECD Confirmation Analysis

8.1 Confirmation Analysis is performed to confirm the identification and quantification of all PCB congeners tentatively identified in the Primary Analysis.

8.2 Table 2 provides examples of operating conditions for the gas chromatograph. All QC specified in Section 10 must be adhered to, i.e., the specified criteria for degradation, linearity, calibration factor for standards, and retention time shift for tetrachloro-m-xylene.

8.3 Inject 1-2 μL of the sample or reagent blank extract and standards using the solvent-flush technique or auto samplers. A volume of 1 μL can be injected only if automatic devices are employed. Record the volume injected to the nearest 0.05 μL and the total extract volume. The detector attenuation must provide peak response equivalent to the Primary Analysis response for each compound to be confirmed.

8.3.1 Begin the Confirmation Analysis GC sequence with the five concentration levels of the CAL STDs.

8.3.2 After injection of the calibration standards, begin injection of samples. Analyze groups of 5 samples. Analyze CAL STD-3 and a hexane Instrument Blank after the first group of 5 samples followed by the analysis of another CAL STD-3 and Instrument Blank. Continue analyzing groups of 5 samples, alternately analyzing CAL STD-3 and an Instrument Blank between groups of 5 samples. The alternating CAL STD-3 calibration factors must be within 15.0% of the initial CAL STD-3 if quantitation is performed. Deviations larger than 15.0% require the laboratory to repeat the analyses of samples which were analyzed after the standard that exceeded the criterion. The 15.0% criterion only pertains to compounds being quantitated.

If the samples are split between 2 or more instruments, all required calibration standards and the method blanks pertaining to those samples must be analyzed on each instrument.

8.3.3 Inject the method blank (extracted with each set of samples) on every GC and GC column on which the associated samples are analyzed.

8.4 Evaluate chromatograms according to the following guidance.

8.4.1 A compound tentatively identified in the primary analysis is confirmed if the retention time from the confirmation analysis falls within the retention time window of a corresponding standard that was chromatographed on the same instrument within an analytical sequence.

8.4.2 Quantitation must be performed and reported on all columns used for PCB congener identification. **NOTE:** To determine that no PCB congeners are present at or above the method detection limit is a form of quantitation.

8.4.3 If identification of compounds of interest are prevented by the presence of interferences, further cleanup is required. If sulfur is evident refer to accompanying SOPs for sulfur clean-up.

If unknown interferences or poor chromatography are noted only in the sample chromatogram, extract cleanup procedures should be applied.

8.4.4 Calculate surrogate standard recoveries on all samples, blanks, and spikes unless the surrogate was diluted out. See formula for calculation in 9.4.

8.4.5 If PCB congeners were identified in the unspiked sample from which the matrix spike and matrix spike duplicate were prepared, confirmation analysis is required for the matrix spike and matrix spike duplicate. If PCB congeners were not identified in the unspiked sample, confirmation of the matrix spike and matrix spike duplicate is not required. Calculate matrix spike duplicate recoveries.

9.0 GC/MS Confirmation Analysis

9.1 A certain percentage of samples which have PCB congeners detected by dual column GC/ECD, shall be qualitatively and quantitatively confirmed by GC/MS. The analytical approach shall entail modifying EPA Method 680. GC/MS analysis will be performed on an aliquot of the original extract. The extract may require further concentration (possibly by a factor of 10) to facilitate detection of the analytes by GC/MS. The GC

operational and QC criteria shall mimic the requirements for GC/ECD analysis (Section 7) and quality control (Section 11). Operational and QC criteria for the MS shall adhere to Method 680 requirements. GC retention times and MS characteristic ions will be determined using the appropriate congener standard mixes used for the GC/ECD analysis. Quantitative deviations in the results of the two methods should be less than 75%.

10.0 Calculations

10.1 Calculate the concentration of congeners in water samples using the following equation for external standards. Response can be measured by the manual peak height technique or by automated peak height or peak area measurements from an integrator.

$$\textit{Concentration of each congener } C_x \text{ } (\mu g/L) = \frac{(A_x)(I_s)(V_t)}{(A_s)(V_i)(V_s)}$$

Where:

A_x = Response for the parameter to be measured.

A_s = Response for the external standard.

V_t = Volume of total extract (μL) (take into account any dilutions).

I_s = Amount of standard injected in nanograms (μg).

V_i = Volume of extract injected (μL).

V_s = Volume of water extracted (L).

10.2 Calculate the concentration of congeners in the sediment/particulate samples using the following equation for external standards. Response can be measured by the manual peak height technique or by automated peak height or peak area measurements from an integrator.

$$\textit{Concentration of each congener } C_x \text{ } (\mu g/g) = \frac{(A_x)(I_s)(V_t)}{(A_s)(V_i)(W_s)}$$

Where:

A_x = Response for the parameter to be measured.

A_s = Response for the external standard.

V_t = Volume of total extract (μL) (take into account any dilutions).

I_s = Amount of standard injected in nanograms (μg).

V_i = Volume of extract injected (μL).

W_s = Weight (dry) of sample extracted (g).

10.3 Match retention times of peaks in the standards with peaks in the sample. Calculate the concentration of every identifiable congener peak unless interference with individual peaks persist after cleanup.

10.4 Calculation for Surrogate and Matrix Spike Recoveries

$$\textit{Percent Recovery} = \frac{Qd}{Qa} \times 100\%$$

Where:

Q_d = quantity determined by analysis

Qa = quantity added to sample.

Be sure all dilutions are taken into account.

10.5 Report results in micrograms per liter for water samples and micrograms per gram for sediment/particulate samples without correction for recovery data. If the concentration for any resolvable PCB congener peak, as calculated using the secondary column analysis, differs by greater than ±25% from the value calculated using the primary column, report the primary column result, qualified with a "P" flag.

11.0 Quality Control

11.1 Method Blanks

11.1.1 A method blank is a volume of deionized, distilled laboratory water , carried through the entire analytical scheme (extraction, concentration, and analysis). The method blank volume must be approximately equal to the sample volumes being processed.

11.1.2 Method blank analysis must be performed at the following frequency:

- each 20 samples in a Sample Delivery Group that are of similar matrix, OR

- whenever 20 or fewer samples from the same Sample Delivery Group are extracted by the same procedure, whichever is more frequent, on each GC system used to analyze samples.

11.1.3 It is the Laboratory's responsibility to ensure that method interferences caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms be minimized.

11.1.4 For the purposes of this protocol, an acceptable laboratory method blank must contain no PCB congener at or above 5 times the MDL determined by the laboratory.

11.1.5 If a laboratory method blank exceeds these criteria, the Laboratory must consider the analytical system to be out of control. The source of the contamination must be investigated and appropriate corrective measures MUST be taken and documented before further sample analysis proceeds. All samples processed with

a method blank that is out of control (i.e., contaminated) MUST be reextracted and reanalyzed. The reextraction should be performed within the 5 days of sample collection, whenever possible. The Laboratory Manager, or his designee, must address problems and solutions in a Case Narrative.

11.1.6 The Laboratory must report results of method blank analysis.

11.1.7 The Laboratory must report ALL sample concentration data as UNCORRECTED for blanks.

11.2 Surrogate Spike (SS) Analysis

11.2.1 Surrogate standard determinations are performed on all samples and blanks. All samples and blanks are fortified with surrogate spiking compounds before purging or extraction in order to monitor preparation and analysis of samples.

11.2.2 Each sample, matrix spike, matrix spike duplicate, and blank are spiked with a surrogate compound prior to extraction. The surrogate spiking compounds, tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB), are used to fortify each sample, matrix spike, matrix spike duplicate, and blank with the proper concentration. Performance based criteria are generated from laboratory results. Therefore, deviations from the spiking protocols will not be permitted.

11.2.3 Surrogate spike recovery must be evaluated by determining whether the concentration (measured as percent recovery) falls inside the advisory recovery limits of 60 to 150 percent. If the laboratory uses different surrogate compounds, recovery should be evaluated against statistical limits developed from the laboratory's historical data.

11.2.4 The Laboratory shall report surrogate recovery data for the following:

- Method Blank Analysis
- Sample Analysis
- Matrix Spike/Matrix Spike Duplicate Analyses

11.3 Matrix Spike/Matrix Spike Duplicate Analysis (MS/MSD)

11.3.1 In order to evaluate the matrix effect of the sample upon the analytical methodology, the PCB congener matrix spiking solution (see the accompanying SOPs for extraction of PCBs from sediment and water), is to be used for matrix spike and matrix spike duplicate analyses. These compounds are subject to change depending upon availability and suitability for use as matrix spikes.

11.3.2 A matrix spike and matrix spike duplicate must be performed on a sample once:

- each 20 samples in a Sample Delivery Group of a similar matrix, OR
- each 7 calendar day period during which samples in a Sample Delivery Group were received (said period beginning with the receipt of the first sample in that Sample Delivery Group), whichever is most frequent.

11.3.3 The analytical protocols in the accompanying SOPs for the extraction and clean-up of PCBs from sediments and water stipulate the amount of matrix spiking solution to be added to the sample aliquots prior to extraction. Each method allows for optional dilution steps which must be accounted for when calculating percent recovery of the matrix spike and matrix spike duplicate samples. Samples requiring optional dilutions and chosen as the matrix spike/matrix spike duplicate samples, must be analyzed at the same dilution as the original unspiked sample.

11.3.4 Individual component recoveries of the matrix spike are calculated using the equation below. Advisory recovery limits for all PCB matrix spiking compounds are 60 to 150 percent.

$$\textit{Matrix Spike Percent Recovery} = \frac{SSR - SR}{SA} \times 100$$

Where:

SSR = Spike Sample Results

SR = Sample Result

SA = Spiked Added from spiking mix

11.3.5 The Laboratory is required to calculate the relative percent difference between the matrix spike and matrix spike duplicate. The relative percent differences (RPD) for each component are calculated using the following equation. The advisory limit for MS/MSD relative percent difference is 40 percent.

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where:

RPD = Relative Percent Difference

D_1 = First Sample Value

D_2 = Second Sample Value (duplicate)

11.3.6 The matrix spike (MS) results (concentrations) for nonspiked PCB congeners shall be reported along with the matrix spike percent recoveries. This will assist data users in assessing analytical precision of non-spiked congeners.

11.4 Matrix Spike Blank (MSB)

11.4.1 A Matrix Spike Blank (MSB) is a volume of reagent water or EPA certified clean sand that has been spiked with the PCB congener matrix spiking solution (see the accompanying SOPs for extraction of PCBs from sediment and water for composition of PCB congener matrix spiking solution) and subject to the entire extraction and analysis procedure. The MSB is used to assess the performance of the method.

11.4.2 A Matrix Spike Blank must be extracted and analyzed once:

- each 20 samples in Sample Delivery Group of a similar matrix, OR

- each 7 calendar day period during which samples in a Sample Delivery Group were received (said period beginning with the receipt of the first sample in that Sample Delivery Group) whichever is most frequent.

11.4.3 The PCB congener matrix spiking solution should be added to the blank so that the resultant concentrations of PCB congeners in the Matrix Spike Blank are approximately 10 to 15 times the MDL determined for each congener.

11.4.4 The Matrix Spike Blank must be evaluated by determining whether the concentration (measured as percent recovery) falls inside the advisory recovery limits of 60 to 150 percent.

11.4.5 The Laboratory shall report Matrix Spike Blank recovery data for all matrix spiking compounds.

11.5 PCB Calibration, Quantitation, and Confirmation QA/QC Requirements

Section 11.5 summarizes ongoing QC activities involved with PCB congener analysis that were detailed in Parts 11.1, 11.2, 11.3, and 11.4, and describes the additional QA/QC procedures required during the analysis of PCB congeners that are not covered in Parts 11.1, 11.2, 11.3, and 11.4.

11.5.1 The Laboratory must perform the following:

- Method Blank analysis as per Part 11.1.
- Spike all standards, samples, blanks, matrix spike and matrix spike duplicate samples with the surrogate spike compounds (TCMX and DCB) as per Part 11.2.
- Matrix Spike/Matrix Spike duplicate analysis as per Part 11.3.
- Matrix Spike Blank as per Part 11.4.

11.5.2 The external standard quantitation method must be used to quantitate all parameters. Before performing any sample analysis, the laboratory is required to determine the retention time window for each PCB congener to be determined

and the surrogate spike compounds. These retention time windows are used to make tentative identification of the PCBs during sample analysis.

11.5.2.1 Establish retention time windows as follows:

11.5.2.2 At the beginning of the project and each time a new GC column is installed, make seven consecutive injections of PCB CAL STD-3.

11.5.2.3 Verify the retention time shifts for the TCMX and DCB in each analytical standard. The retention time shifts between the initial and subsequent standards must be less than 0.3 percent. If this criterion is not met, continue injecting replicate standards to meet criteria.

11.5.2.4 Calculate the standard deviation of the three absolute retention times for each PCB congener in each CAL STD.

11.5.2.5 The standard deviations determined in 10.5.2.4 shall be used to determine the retention time windows for a particular analytical sequence. Apply plus or minus three times the standard deviations in 11.5.2.4 to the retention time of each PCB congener determined for the first analysis of the PCB standard in a given analytical sequence. This range of retention times defines the retention time window for the compound of interest for that analytical sequence. **Note:** By definition, the retention time of a PCB congener from the first analysis of that compound in the analytical sequence (11.5.4.1) is the center of the retention time window. Do not use the retention time measured in 11.5.2.2 as the center of the retention time window. The experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.5.2.6 In those cases where the retention time window for a particular PCB congener is less than ± 0.01 minutes, the laboratory may substitute of the following formula. For narrow bore capillary columns (ID less than 0.32 mm), the retention time window of the particular PCB congener shall be calculated as $\pm 0.15\%$ of the initial retention time of the compound in the analytical sequence.

11.5.2.7 Regardless of how the retention time windows are calculated, the retention time windows must be reported as a range of values, not as, for example, 1.51 minutes ±1%.

11.5.2.8 The Laboratory must calculate retention time windows for each PCB congener on each GC column used at the beginning of the program and whenever a new GC column is installed. The data must be retained by the Laboratory and made available during an on-site laboratory evaluation.

11.5.3 Primary GC Column Analysis

11.5.3.1 Primary Analysis establishes whether or not PCB congeners are present in the sample, and establishes a tentative identification of each PCB congener. Quantitation may be performed on the primary analysis if the analysis meets all of the QC criteria specified for quantitation. **NOTE:** To determine that no PCB congeners are present at or above the method detection limit is a form of quantitation.

11.5.3.2 Prepare the Calibration Standards at the 5 concentration levels described in 4.4. Analyze the five Calibration Standards sequentially at the beginning of each analytical sequence (see 11.5.4.1).

11.5.3.3 Calculate the Calibration Factor (ratio of the total area to the mass injected) for each PCB congener in each CAL STD using the following equation:

$$\textit{Calibration Factor} = \frac{\textit{Total Area of Peak}}{\textit{Mass Injected (in nanograms)}}$$

11.5.3.4 Using the Calibration Factors from 11.5.3.3 above, calculate the percent relative standard deviation (%RSD) for each compound at the five concentration levels using the following equation. The relative standard deviation for TCMX and DCB must be less than 10.0 percent.

Note: The 10.0% RSD linearity criteria pertains only to columns being used for PCB quantitation. If a column is used only for surrogate quantitation, the 10.0% RSD is only required for TCMX and DCB.

$$\%RSD = \frac{SD}{\bar{x}} \times 100$$

Where:

RSD = Relative Standard Deviation

SD = Standard Deviation of initial relative response factors (per compound)

Where:

$$SD = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \bar{x}_i)^2}{N - 1}}$$

$\bar{x}_i$ = mean of initial relative response factors (per compound)

11.5.4 Sample Analysis (Primary GC Column)

11.5.4.1 Samples are analyzed per the sequence described below.

Analytical Sequence for PCB Analysis

1. CAL STD-1
2. CAL STD-2
3. CAL STD-3
4. CAL STD-4
5. CAL STD-5
6. Instrument Blank

7. 5 samples
8. CAL STD-3
9. Instrument Blank
10. 5 samples
11. Repeat the above sequence starting with CAL STD-3 (step 8 above). Continue as long as quality control requirements are met and no significant adjustments are made to the analytical system.
12. PCB analysis sequence must end with the analyses of CAL STD-3

11.5.4.2 The retention time shift for the TCMX and DCB surrogate standards must be evaluated after the analysis of each sample. The retention time shift may not exceed a 0.3% difference for capillary GC columns between the initial standard analysis and any sample analyzed during the 72 hour period.

Calculate the percent difference in the retention time for each of the surrogate standards using the following equation:

$$\textit{Percent Difference (\%D)} = \frac{RT_1 - RT_s}{RT_1} \times 100$$

Where:

RT_I = absolute retention time of the surrogate standard in the initial standard (CAL STD-1).

RT_S = absolute retention time of the surrogate standard in the sample.

11.5.4.3 If one or more compounds have a response greater than full scale, the extract requires dilution according to the specifications in Section IV. If the dilution of the extract causes any compounds tentatively identified in the first analysis to be undetectable in the second analysis, then the results of both analyses shall be reported.

11.5.5 Confirmation Analysis (GC/ECD)

11.5.5.1 Confirmation Analysis is to confirm the identification and quantitation of all PCB congeners tentatively identified in the Primary Analysis.

11.5.5.2 Separation should be >75 percent resolution between peaks for BZ#28 and BZ#31. This criteria must be considered when determining whether to quantitate on the Primary Analysis or the Confirmation Analysis. When this criteria cannot be met, quantitation is adversely affected because of the difficulty in determining where to establish the baseline.

Calculate the percent resolution as follows:

$$\textit{Percent Resolution} = \frac{D_v}{H_p} \times 100$$

Where:

D_v = the minimum depth of the valley between the peaks

H_p = The maximum height of the peaks

11.5.5.3 All QC specified previously must be adhered to, i.e., linearity, calibration factor for standards, and retention time shifts for the TCMX and DCB surrogate standards.

11.5.5.4 Begin the Confirmation Analysis GC sequence with the five concentration levels of CAL STDs.

11.5.5.5 After the linearity standards required in 11.5.5.4 are injected, continue the confirmation analysis injection sequence with all compounds tentatively identified during primary analysis to establish the daily retention time windows during primary analysis. Analyze all confirmation standards for a case at the beginning, at intervals specified in 11.5.5.6 and at the end. Any PCB congener outside of its established retention time window requires immediate investigation and correction

before continuing the analysis. The laboratory must reanalyze all samples between the standard that exceeds the criterion and a subsequent standard that meets the criterion. This reanalysis must be performed within the holding times specified in Appendix A-1, A-2, and A-3.

11.5.5.6 Begin injection of samples at this point of the Confirmation Analysis sequence. Analyze groups of 5 samples with a CAL STD-3 after each group. The alternating CAL STD-3's calibration factors must be within 15.0 percent of each other if quantitation is performed. Deviations larger than 15.0 percent require the laboratory to repeat the samples analyzed following the standard that exceeds the criteria. The reanalysis must be performed within 40 days from extraction. The 15.0 percent criteria only pertains to compounds being quantitated.

If the samples are split between 2 or more instruments, all appropriate standards and method blanks pertaining to those samples must be analyzed on each instrument.

11.5.5.7 Inject the method blank (extracted with each set of samples) on every GC and GC column on which the samples are analyzed.

12.0 References

12.1 Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, "Trace Analyses for Wastewaters", *Environmental Science & Technology*, Vol 15, pg. 1426, 1981.

12.2 Ballschmiter, K. and M. Zell, *Fresenius Z. Anal. Chem.*, 302, 20, 1980.

12.3 "Carcinogens – Working with Carcinogens", Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

12.4 "OSHA Safety and Health Standards, General Industry", 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

12.5 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

12.6 Mullin, M.D., C. Pochini, S. McCrindle, M. Romkes, S.H. Safe and L.M. Safe, "High Resolution PCB Analysis: Synthesis and Chromatographic Properties of All 209 PCB Congeners", *Environmental Science & Technology*, Vol 18, pg. 466, 1984.

12.7 Gehart, J.E., Hayes, T.L., Alford-Stevens, A.L., and W.L. Budde, "Mass Spectrometric Determination of Polychlorinated Biphenyls as Isomer Groups", *Analytical Chemistry*, Vol 57, pg. 2458, 1985.

12.8 Slivon, L.E., J.E. Gebhart, T.L. Hayes, A.L. Alford-Stevens, and W.L. Budde, "Automated Procedures for Mass Spectrometric Determination of Polychlorinated Biphenyls as Isomer Groups", *Analytical Chemistry*, Vol. 57, pg. 2464, 1985.

12.9 Rote, J.W. and W.J. Morris, "Use of Isotopic Abundance Ratios in Identification of Polychlorinated Biphenyls by Mass Spectrometry", Journal of the Association of Official Analytical Chemists, Vol 56(1), pg 188, 1973.

12.10 Schulz, D.E., Petrick, G., and Duinker, J.C., "Complete Characterization of Polychlorinated Biphenyl Congeners in Common Aroclor and Clophen Mixtures by Multidimensional Gas Chromatography-Electron Capture Detection", *Environmental Science & Technology*, Vol. 23, No. 7, pg. 852, 1989.

12.11 "Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11", Federal Register, Vol. 49, No. 209, pg. 43430.

12.12 Alford-Stevens, A., Bellar, T.A., Eichelberger, J.W., and Budde, W.L., "Method 680 - Determination of Pesticides and PCBs in Water and Soil/Sediment by Gas Chromatograph/Mass Spectrometry", November 1985.

Table 1

PCB Congeners for Calibration Standards

BZ#	Structure
1	2-Chlorobiphenyl
3	4-Chlorobiphenyl
4	2,2'-Dichlorobiphenyl
5	2,3-Dichlorobiphenyl
6	2,3'-Dichlorobiphenyl
7	2,4'-Dichlorobiphenyl
9	2,5-Dichlorobiphenyl
12	3,4-Dichlorobiphenyl
15	4,4'-Dichlorobiphenyl
16	2,2',3-Trichlorobiphenyl
18	2,2',5-Trichlorobiphenyl
19	2,2',6-Trichlorobiphenyl
22	2,3,4'-Trichlorobiphenyl
25	2,3',4-Trichlorobiphenyl
26	2,3',5-Trichlorobiphenyl
27	2,3',6-Trichlorobiphenyl
28	2,4,4'-Trichlorobiphenyl
29	2,4,5-Trichlorobiphenyl
31	2,4',5-Trichlorobiphenyl
37	3,4,4'-Trichlorobiphenyl
40	2,2',3,3'-Tetrachlorobiphenyl
41	2,2',3,4-Tetrachlorobiphenyl
44	2,2',3,5'-Tetrachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl
53	2,2',5,6'-Tetrachlorobiphenyl
56	2,3,3',4'-Tetrachlorobiphenyl
66	2,3',4,4'-Tetrachlorobiphenyl
70	2,3',4',5-Tetrachlorobiphenyl
75	2,4,4',6-Tetrachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl

Table 1 - cont'd

BZ#	Structure
82	2,2',3,3',4-Pentachlorobiphenyl
83	2,2',3,3',5-Pentachlorobiphenyl
84	2,2',3,3',6-Pentachlorobiphenyl
85	2,2',3,4,4'-Pentachlorobiphenyl
87	2,2',3,4,5'-Pentachlorobiphenyl
91	2,2',3,4',6-Pentachlorobiphenyl
92	2,2',3,5,5'-Pentachlorobiphenyl
95	2,2',3,5',6-Pentachlorobiphenyl
97	2,2',3',4,5-Pentachlorobiphenyl
99	2,2',4,4',5-Pentachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
107	2,3,3',4',5-Pentachlorobiphenyl
115	2,3,4,4',6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
119	2,3',4,4',6-Pentachlorobiphenyl
122	2',3,3',4,5-Pentachlorobiphenyl
123	2',3,4,4',5-Pentachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
128	2,2',3,3',4,4'-Hexachlorobiphenyl
129	2,2',3,3',4,5-Hexachlorobiphenyl
136	2,2',3,3',6,6'-Hexachlorobiphenyl
137	2,2',3,4,4',5-Hexachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
141	2,2',3,4,5,5'-Hexachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
170	2,2',3,3',4,4',5-Heptachlorobiphenyl
171	2,2',3,3',4,4',6-Heptachlorobiphenyl

Table 1 - cont'd

BZ#	Structure
177	2,2',3,3',4',5,6-Heptachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
183	2,2',3,4,4',5',6-Heptachlorobiphenyl
185	2,2',3,4,5,5',6-Heptachlorobiphenyl
187	2,2',3,4',5,5',6-Heptachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
190	2,3,3',4,4',5,6-Heptachlorobiphenyl
191	2,3,3',4,4',5',6-Heptachlorobiphenyl
193	2,3,3',4',5,5',6-Heptachlorobiphenyl
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
196	2,2',3,3',4,4',5',6-Octachlorobiphenyl
198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
199	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
200	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
201	2,2',3,3',4',5,5',6-Octachlorobiphenyl
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl
208	2,2',3,3',4,,5,5',6,6'-Nonachlorobiphenyl
209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl

Note: BZ# = Ballschmiter and Zell System

Table 2

Capillary column 1 conditions:	40 m x 0.18 mm ID, 0.4 micron film thickness, fused silica DB-5 (or equivalent) splitless mode
Carrier gas:	Helium
Makeup gas:	95% Argon/5% Methane
Septum purge:	15 mL/minute
Split vent:	None
Initial temperature:	90°C, initial hold - 2 minutes
Temperature program 1:	10°C/minute for 6 minutes
Temperature program 2:	3°C/minute to 290°C.
Final temperature:	290°C, final hold - 4 minutes
Injection port temperature:	210°C
Detector temperature:	300°C

Capillary column 2 conditions:	60 m x 0.25 mm ID, 0.25 micron film thickness, fused silica SP-2100, splitless mode
Carrier gas:	Helium
Makeup gas:	95% Argon/5% Methane
Septum purge:	15 mL/minute
Split vent:	None
Initial temperature:	150°C, initial hold - 5 minutes
Temperature program 1:	15°C/minute to 180°C - hold for 1 minute
Temperature program 2:	2°C/minute to 190°C
Final temperature:	190°C
Injection port temperature:	200°C
Detector temperature:	300°C

Table 3

Relative Retention Times for Column 1

BZ #	RRT*	BZ #	RRT*	BZ #	RRT*
1	0.7968	63	1.8021	138	2.4866
3	0.9096	74	1.8214	158	2.5043
10,4	0.9700	70	1.8337	126,129	2.5241
Surrogate	1.0000	66	1.8487	187	2.5850
7,9	1.0545	95	1.8540	128	2.6037
6	1.0850	91	1.8818	183	2.6096
8,5	1.1048	56,60	1.9187	167	2.6316
Hexachlorobenzene	1.1230	92,84	1.9439	185	2.6610
19	1.1717	90,101	1.9658	174	2.6877
12	1.2465	99	1.9888	177	2.7091
18	1.2551	119	2.0209	156,171	2.7310
15,17	1.2652	83	2.0332	202	2.7465
24,27	1.2952	97	2.0572	201	2.7829
16,32	1.3241	87	2.0770	157.172.197	2.7952
29	1.3882	115 + DDE	2.0882	180	2.8262
26	1.3979	85	2.0957	193	2.8417
25	1.4086	136	2.1107	191	2.8620
31	1.4278	77,110	2.1225	200	2.8882
28	1.4348	82	2.1684	170	2.9524
20,33,53	1.4733	151	2.1957	190	2.9690
22	1.4984	135	2.2112	Mirex	2.9797
45	1.5235	107	2.2257	198	3.0080
51	1.5529	123	2.2374	199	3.0182
52	1.5765	118,149	2.2492	196,203	3.0460
49	1.5963	146	2.2957	189	3.1021
47	1.6096	122	2.3096	195	3.1754
48,75	1.6171	105	2.3615	208	3.1914
44	1.6594	132,153	2.3684	207	3.2289
37,42,59	1.6743	141,179	2.4235	194	3.2786
41,64	1.7123	130	2.4342	205	3.3128
40	1.7433	137	2.4524	206	3.4770
67	1.7840				

* Relative Retention Times to surrogate compound tetrachloro-m-xylene

Table 4

Relative Retention Times for Column 2

BZ #	RRT*	BZ #	RRT*	BZ #	RRT*
1	0.8018	63	1.7934	138	2.4723
3	0.9141	74	1.8134	158	2.4876
10,4	0.9736	70	1.8266	126,129	2.5076
Surrogate	1.0000	66	1.8419	187	2.5688
7,9	1.0543	95	1.8466	128	2.5867
6	1.0854	91	1.8730	183	2.5925
8,5	1.1060	56,60	1.9093	167	2.6136
Hexachlorobenzene	1.1244	92,84	1.9346	185	2.6431
19	1.1713	90,101	1.9568	174	2.6705
12	1.2441	99	1.9794	177	2.6911
18	1.2599	119	2.0095	156,171	2.7127
15,17	1.2625	83	2.0221	202	2.7285
24,27	1.2926	97	2.0469	201	2.7644
16,32	1.3221	87	2.0659	157.172.197	2.7760
29	1.3843	115 + DDE	2.0770	180	2.8076
26	1.3943	85	2.0843	193	2.8213
25	1.4038	136	2.1002	191	2.8413
31	1.4249	77,110	2.1118	200	2.8682
28	1.4312	82	2.1566	170	2.9320
20,33,53	1.4697	151	2.1834	190	2.9478
22	1.4939	135	2.1993	Mirex	2.9573
45	1.5187	107	2.2124	198	2.9868
51	1.5477	123	2.2246	199	2.9974
52	1.5720	118,149	2.2372	196,203	3.0242
49	1.5909	146	2.2826	189	3.0796
47	1.6036	122	2.2941	195	3.1523
48,75	1.6115	105	2.3463	208	3.1687
44	1.6537	132,153	2.3548	207	3.2056
37,42,59	1.6679	141,179	2.4085	194	3.2551
41,64	1.7059	130	2.4196	205	3.2889
40	1.7359	137	2.4365	206	3.4528
67	1.7765				

* Relative Retention Times to surrogate compound tetrachloro-m-xylene

Table 5
Numbering System For PCB Congeners

BZ#	Structure
-	Biphenyl
1	2-Chlorobiphenyl
2	3-Chlorobiphenyl
3	4-Chlorobiphenyl
4	2,2'-Dichlorobiphenyl
5	2,3-Dichlorobiphenyl
6	2,3'-Dichlorobiphenyl
7	2,4-Dichlorobiphenyl
8	2,4'-Dichlorobiphenyl
9	2,5-Dichlorobiphenyl
10	2,6-Dichlorobiphenyl
11	3,3'-Dichlorobiphenyl
12	3,4-Dichlorobiphenyl
13	3,4'-Dichlorobiphenyl
14	3,5-Dichlorobiphenyl
15	4,4'-Dichlorobiphenyl
16	2,2',3-Trichlorobiphenyl
17	2,2',4-Trichlorobiphenyl
18	2,2',5-Trichlorobiphenyl
19	2,2',6-Trichlorobiphenyl
20	2,3,3'-Trichlorobiphenyl
21	2,3,4-Trichlorobiphenyl
22	2,3,4'-Trichlorobiphenyl
23	2,3,5-Trichlorobiphenyl
24	2,3,6-Trichlorobiphenyl
25	2,3',4-Trichlorobiphenyl
26	2,3',5-Trichlorbiphenyl
27	2,3',6-Trichlorobiphenyl
28	2,4,4'-Trichlorobiphenyl
29	2,4,5-Trichlorobiphenyl
30	2,4,6-Trichlorobiphenyl
31	2,4',5-Trichlorobiphenyl

Table 5 - cont'd

BZ#	Structure
32	2,4',6-Trichlorobiphenyl
33	2',3,4-Trichlorobiphenyl
34	2',3,5-Trichlorobiphenyl
35	3,3',4-Trichlorobiphenyl
36	3,3',5-Trichlorobiphenyl
37	3,4,4'-Trichlorobiphenyl
38	3,4,5-Trichlorobiphenyl
39	3,4',5-Trichlorobiphenyl
40	2,2',3,3'-Tetrachlorobiphenyl
41	2,2',3,4-Tetrachlorobiphenyl
42	2,2',3,4'-Tetrachlorobiphenyl
43	2,2',3,5-Tetrachlorobiphenyl
44	2,2',3,5'-Tetrachlorobiphenyl
45	2,2',3,6-Tetrachlorobiphenyl
46	2,2',3,6'-Tetrachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl
48	2,2',4,5-Tetrachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl
50	2,2',4,6-Tetrachlorobiphenyl
51	2,2',4,6'-Tetrachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl
53	2,2',5,6'-Tetrachlorobiphenyl
54	2,2',6,6'-Tetrachlorobiphenyl
55	2,3,3',4-Tetrachlorobiphenyl
56	2,3,3',4'-Tetrachlorobiphenyl
57	2,3,3',5-Tetrachlorobiphenyl
58	2,3,3',5'-Tetrachlorobiphenyl
59	2,3,3',6-Tetrachlorobiphenyl
60	2,3,4,4'-Tetrachlorobiphenyl
61	2,3,4,5-Tetrachlorobiphenyl
62	2,3,4,6-Tetrachlorobiphenyl
63	2,3,4',5-Tetrachlorobiphenyl
64	2,3,4',6-Tetrachlorobiphenyl

Table 5 - cont'd

BZ#	Structure
65	2,3,5,6-Tetrachlorobiphenyl
66	2,3',4,4'-Tetrachlorobiphenyl
67	2,3',4,5-Tetrachlorobiphenyl
68	2,3',4,5'-Tetrachlorobiphenyl
69	2,3',4,6-Tetrachlorobiphenyl
70	2,3',4',5-Tetrachlorobiphenyl
71	2,3',4',6-Tetrachlorobiphenyl
72	2,3',5,5'-Tetrachlorobiphenyl
73	2,3',5',6-Tetrachlorobiphenyl
74	2,4,4',5-Tetrachlorobiphenyl
75	2,4,4',6-Tetrachlorobiphenyl
76	2',3,4,5-Tetrachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl
78	3,3',4,5-Tetrachlorobiphenyl
79	3,3',4,5-Tetrachlorobiphenyl
80	3,3',5,5'-Tetrachlorobiphenyl
81	3,4,4',5-Tetrachlorobiphenyl
82	2,2',3,3',4-Pentachlorobiphenyl
83	2,2',3,3',5-Pentachlorobiphenyl
84	2,2',3,3',6-Pentachlorobiphenyl
85	2,2',3,4,4'-Pentachlorobiphenyl
86	2,2',3,4,5-Pentachlorobiphenyl
87	2,2',3,4,5'-Pentachlorobiphenyl
88	2,2',3,4,6-Pentachlorobiphenyl
89	2,2',3,4,6-Pentachlorobiphenyl
90	2,2',3,4',5-Pentachlorobiphenyl
91	2,2',3,4',6-Pentachlorobiphenyl
92	2,2',3,5,5'-Pentachlorobiphenyl
93	2,2',3,5,6-Pentachlorobiphenyl
94	2,2',3,5,6'-Pentachlorobiphenyl
95	2,2',3,5',6-Pentachlorobiphenyl
96	2,2',3,6,6'-Pentachlorobiphenyl
97	2,2',3',4,5-Pentachlorobiphenyl

Table 5 - cont'd

BZ#	Structure
98	2,2',3',4,6-Pentachlorobiphenyl
99	2,2',4,4',5-Pentachlorobiphenyl
100	2,2',4,4',6-Pentachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
102	2,2',4,5,6'-Pentachlorobiphenyl
103	2,2',4,5',6-Pentachlorobiphenyl
104	2,2',4,6,6'-Pentachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
106	2,3,3',4,5-Pentachlorobiphenyl
107	2,3,3',4',5-Pentachlorobiphenyl
108	2,3,3',4,5'-Pentachlorobiphenyl
109	2,3,3',4,6-Pentachlorobiphenyl
110	2,3,3',4',6-Pentachlorobiphenyl
111	2,3,3',5,5'-Pentachlorobiphenyl
112	2,3,3',5,6-Pentachlorobiphenyl
113	2,3,3',5',6-Pentachlorobiphenyl
114	2,3,4,4',5-Pentachlorobiphenyl
115	2,3,4,4',6-Pentachlorobiphenyl
116	2,3,4,5,6-Pentachlorobiphenyl
117	2,3,4',5,6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
119	2,3',4,4',6-Pentachlorobiphenyl
120	2,3',4,5,5'-Pentachlorobiphenyl
121	2,3',4,5',6-Pentachlorobiphenyl
122	2',3,3',4,5-Pentachlorobiphenyl
123	2',3,4,4',5-Pentachlorobiphenyl
124	2',3,4,5,5'-Pentachlorobiphenyl
125	2',3,4,5,6'-Pentachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
127	3,3',4,5,5'-Pentachlorobiphenyl
128	2,2',3,3',4,4'-Hexachlorobiphenyl
129	2,2',3,3',4,5-Hexachlorobiphenyl
130	2,2',3,3',4,5'-Hexachlorobiphenyl

Table 5 - cont'd

BZ#	Structure
131	2,2',3,3',4,6-Hexachlorobiphenyl
132	2,2',3,3',4,6'-Hexachlorobiphenyl
133	2,2',3,3',5,5'-Hexachlorobiphenyl
134	2,2',3,3',5,6-Hexachlorobiphenyl
135	2,2',3,3',5,6'-Hexachlorobiphenyl
136	2,2',3,3',6,6'-Hexachlorobiphenyl
137	2,2',3,4,4',5-Hexachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
139	2,2',3,4,4',6-Hexachlorobiphenyl
140	2,2',3,4,4',6'-Hexachlorobiphenyl
141	2,2',3,4,5,5'-Hexachlorobiphenyl
142	2,2',3,4,5,6-Hexachlorobiphenyl
143	2,2',3,4,5,6'-Hexachlorobiphenyl
144	2,2',3,4,5',6-Hexachlorobiphenyl
145	2,2',3,4,6,6'-Hexachlorobiphenyl
146	2,2',3,4',5,5'-Hexachlorobiphenyl
147	2,2',3,4',5,6-Hexachlorobiphenyl
148	2,2',3,4',5,6'-Hexachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl
150	2,2',3,4',6,6'-Hexachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl
152	2,2',3,5,6,6'-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
154	2,2',4,4',5,6'-Hexachlorobiphenyl
155	2,2',4,4',6,6'-Hexachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl
159	2,3,3',4,5,5'-Hexachlorobiphenyl
160	2,3,3',4,5,6-Hexachlorobiphenyl
161	2,3,3',4,5',6-Hexachlorobiphenyl
162	2,3,3',4',5,5'-Hexachlorobiphenyl
163	2,3,3',4',5,6-Hexachlorobiphenyl

Table 5 - cont'd

BZ#	Structure
164	2,3,3',4',5',6-Hexachlorobiphenyl
165	2,3,3',5,5',6-Hexachlorobiphenyl
166	2,3,4,4',5,6-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
168	2,3',4,4',5',6-Hexachlorobiphenyl
169	3,3',4,4',5,5'-Hexachlorobiphenyl
170	2,2',3,3',4,4',5-Heptachlorobiphenyl
171	2,2',3,3',4,4',6-Heptachlorobiphenyl
172	2,2',3,3',4,5,5'-Heptachlorobiphenyl
173	2,2',3,3',4,5,6-Heptachlorobiphenyl
174	2,2',3,3',4,5,6'-Heptachlorobiphenyl
175	2,2',3,3',4,5',6-Heptachlorobiphenyl
176	2,2',3,3',4,6,6'-Heptachlorobiphenyl
177	2,2',3,3',4',5,6-Heptachlorobiphenyl
178	2,2',3,3',5,5',6-Heptachlorobiphenyl
179	2,2',3,3',5,6,6'-Heptachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
181	2,2',3,4,4',5,6-Heptachlorobiphenyl
182	2,2',3,4,4',5,6'-Heptachlorobiphenyl
183	2,2',3,4,4',5',6-Heptachlorobiphenyl
184	2,2',3,4,4',6,6'-Heptachlorobiphenyl
185	2,2',3,4,5,5',6-Heptachlorobiphenyl
186	2,2',3,4,5,6,6'-Heptachlorobiphenyl
187	2,2',3,4',5,5',6-Heptachlorobiphenyl
188	2,2',3,4',5,6,6'-Heptachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
190	2,3,3',4,4',5,6-Heptachlorobiphenyl
191	2,3,3',4,4',5',6-Heptachlorobiphenyl
192	2,3,3',4,5,5',6-Heptachlorobiphenyl
193	2,3,3',4',5,5',6-Heptachlorobiphenyl
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
196	2,2',3,3',4,4',5',6-Octachlorobiphenyl

Table 5 - cont'd

BZ#	Structure
197	2,2',3,3',4,4',6,6'-Octachlorobiphenyl
198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
199	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
200	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
201	2,2',3,3',4',5,5',6-Octachlorobiphenyl
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl
203	2,2',3,4,4',5,5',6-Octachlorobiphenyl
204	2,2',3,4,4',5,6,6'-Octachlorobiphenyl
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl
208	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl
209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl

Appendix A-5

CONGENER SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCB's) IN HEXANE EXTRACTS BY CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROSCOPY - CONFIRMATION ANALYSES

February 26, 1992

1.0 Scope and Application

1.1 This method, which is a modified version of EPA Method 680, describes procedures for confirmation of polychlorinated biphenyls (PCBs) in hexane extracts by fused silica capillary column gas chromatography with selected-ion-monitoring (SIM) mass spectrometric detection. This method is applicable to samples containing PCBs as single congeners or as complex mixtures, such as commercial Aroclors. The preparation procedures for all the extracts are described in Appendices A-1 through A-3. The extracts should be analyzed according to the procedures in Appendix A-4 prior to G C/MS analysis to determine the appropriate concentration or dilution.

1.2 Detection limits vary among method analytes and with sample matrix, sample preparation, procedures, condition of the GC/MS system, type of data acquisition, and individual samples. Detection limits for individual PCB congeners increase with increasing number of chlorine atoms, with the detection limit for decachlorobiphenyl being about 5-10 times higher than that of a monochlorobiphenyl. A monochlorobiphenyl can be identified and accurately measured when the injected extract aliquot contains 1 ng and full-range data are acquired. The detection limit for total PCBs will depend on the number of individual PCB congeners present. SIM data acquisition procedures reduce the detection limit for PCBs by at least a factor of three.

2.0 Summary of Method

2.1 Sample extract components are separated with fused silica capillary column gas chromatography (GC) and identified and measured with low resolution, electron ionization, selected-ion-monitoring mass spectrometry (MS). An interfaced data system (DS) to control data acquisition and to store, retrieve, and manipulate mass spectral data is essential.

2.2 Nine selected PCB congeners are used as calibration standards, and two internal standards, chrysene-d_{12} and phenanthrene-d^{10}, are used to calibrate MS response to PCBs.

3.0 Interferences

3.1 Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing equipment. All of these materials must be demonstrated to be free of interferences under the analytical conditions by routine analysis of laboratory method blanks.

3.2 For PCBs, interference can be caused by the presence of much greater quantities of other sample components that overload the capillary column; additional sample extract preparation procedures must then be used to eliminate interferences. Capillary column GC retention times and the compound-specific characteristics of mass spectra eliminate many interferences that formerly were of concern with PCB determinations with electron capture detection. The approach and identification criteria used in this method for PCBs eliminate interference by most chlorinated compounds other than other PCBs.

4.0 Apparatus and Materials

4.1 Computerized GC/MS System

4.1.1 The GC must be capable of temperature programming and be equipped with all required accessories, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns.

4.1.2 SIM mass spectral data are obtained with electron ionization at a nominal electron energy of 70 eV. To ensure sufficient precision of mass spectral data, the required MS scan rate must allow acquisition of at least or five data points for each monitored ion while a sample component elutes from the GC. The MS must produce a mass spectrum meeting all criteria for $\leq$20 ng of decafluorotriphenylphosphine (DFTPP) introduced through the GC inlet.

4.1.3 An interfaced data system (DS) is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of searching a data file for specific ions and plotting ion abundances versus time or spectrum number to produce selected ion current profiles (SICPs). Also required is the capability to obtain chromatographic peak areas between specific times or spectrum numbers in SICPs. Total data acquisition time per cycle should be $\geq$0.5 s and must not exceed 1.5 s.

4.1.4 For SIM data acquisition, the DS must be equipped with software capable for acquiring data for multiple groups of ions, and the DS must allow automated and rapid changes for the set of ions being monitored. To acquire all PCB data needed for implementation of two currently-available automated interpretation procedures, the SIM program must be capable of acquiring data for four groups (or mass ranges) each consisting of $\leq$35 ions or for five groups of $\leq$20 ions each. The times spent monitoring ions during sample analyses must be the same as the times used when calibration solutions were analyzed.

4.2 GC COLUMN -- A 40 m x 0.18 mm ID fused silica capillary column coated with a 0.4 um or thicker film crosslinked phenyl methyl silicone (such as Durabond-5 (DB-5), J and W Scientific, Rancho Cordova, CA) or polydiphenyl vinyl dimethyl siloxane (such as SE-54, Alltech Associates, Deerfield, IL) is required. Operating conditions known to produce acceptable results with these columns are shown in Table 1. Retention times have been reported (6) for all 209 PCB congeners with an SE-54 column, which provides the same retention order for PCBs and essentially the same separation capabilities as a DB-5 column.

5.0 Reagents

5.1 Solvents

5.1.1 High purity, distilled-in-glass hexane. For precise injections with splitless injectors and capillary columns, all samples and standards should be contained in the same solvent. Effects of minor variations in solvent composition (i.e., small percentage of another solvent remaining in hexane extracts) are minimized with the use of internal standards. (External standard calibration is not acceptable.)

5.1.2 Nanograde ethyl ether shown to be free of peroxides.

5.2 MS Performance Check Solution -- Prepare a 10 ng/ul solution of decafluorotriphenylphosphine (DFTPP) in an appropriate solution.

5.3 Internal Standards -- Chrysene-d_{12} and phenanthrene-d_{10} are used as internal standards. They are added to each sample extract just before analysis and are contained in all concentration calibration and performance check solutions.

5.4 PCB Concentration Calibration Compounds - The nine individual PCB congeners listed in Table 2 are used as concentration calibration compounds for PCB determinations. One isomer at each level of chlorination is used as the concentration calibration standard for all other isomers at that level of chlorination, except that decachlorobiphenyl (Cl_{10}) is used for both Cl_9 and Cl_{10} isomer groups.

5.5 PCB Retention Time Congeners for SIM Data Acquisition -- Knowledge of the retention times of certain congeners is necessary to determine when to acquire data with each ion set. Two concentration calibration congeners also serve as retention time congeners; the first eluting Cl_1-PCB indicates the times when data acquisition must have been initiated for Ion Set #1, and the Cl_{10}-PCB indicates when all PCBs have eluted. Two or three additional PCB congeners are used to establish times to initiate data acquisition with other Ion Sets (Sect. 6.1.3).

5.6 PCB Solutions

5.6.1 Stock Solutions of PCB Calibration Congeners - Prepare a stock solution of each of the nine PCB concentration calibration congeners and each of the three retention time congeners at a concentration of 1 ug/ul in hexane. Place each solution in a clean glass vial with a Teflon-lined screw cap and store at 4°C if solutions are not to be used right away. Solutions are stable indefinitely is solvent evaporation is prevented.

CAUTION: Each time a vial containing small volumes of solutions is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible volume of headspace, and opening vials should be minimized.

5.6.2 PCB Primary Dilution Standard - Take aliquots of the stock solutions of the nine PCB concentration calibration congeners and mix together in the proportions of one part of each solution of the Cl_1 (#1), Cl_2 (#5), and Cl_3 (#29) congeners, two parts of each solution of the Cl_4 (#50), Cl_5 (#87), and Cl_6 (#154) congeners, three parts of each solution of the Cl_7 (#188) and Cl_8 (#200) congeners, and five parts of the Cl_{10} (#209) congener solution. (Note: The retention time congeners described in Sect. 5.6 are <u>not</u> included in the PCB primary dilution standard because they are not needed for full-range data acquisition.) This will provide a primary dilution standard solution of the composition shown in Table 3. Calculate the concentration in ug/ul; use three significant figures. Place each solution in a clean glass vial with a Teflon-lined screw cap and store at 4°C. Mark the meniscus on the vial wall to monitor solution volume during storage; solutions are stable indefinitely if solvent evaporation is prevented.

5.7 Internal Standard (IS) Solution

5.7.1 Weigh 0.75 mg $\pm$ 0.001 mg each of phenanthrene-d_{10} and chrysene-d_{12}; dissolve in hexane and dilute to 10 ml in a volumetric flask. (Concentration of each IS = 75 ng/ul.)

5.8 CALS for SIM Data Acquisition – One set of five solutions for determination of PCB congeners is needed. Appropriate concentrations of SIM CALs are given in Table 4. The solutions are prepared by diluting appropriate primary dilution standards and adding appropriate volume of IS solution.

CAUTION: The PCB SIM CALs must include the three PCB retention time congeners that are used to establish conditions for SIM data acquisition.

5.9 Calculate the concentration (two significant figures) of each component in each solution. Note: Concentrations presented in tables are only approximate.

5.10 Laboratory Performance Check Solution – The Medium CAL is used as the laboratory performance check solution (LPC) to verify response factors and to demonstrate GC resolution and MS performance.

6.0 Calibration

Initial calibration is required before any samples are analyzed and is required intermittently throughout sample analyses as dictated by results of continuing calibration checks. No samples are to be analyzed until acceptable initial calibration is demonstrated and documented. After initial calibration is successfully performed, a continuing calibration check is required at the beginning and end of each 12-h period during which analyses are performed.

6.1 Initial Calibration

6.1.1 Calibrate and tune the MS with standards and procedures prescribed by the manufacturer with any necessary modifications to meet USEPA requirements.

6.1.2 Inject a 1- ul or 2-ul aliquot of the 10 ng/ul DFTPP solution and acquire a mass spectrum that includes data for m/z 45-450. If the spectrum does not meet all criteria (Table 5), the MS must be hardware tuned to meet all criteria before proceeding with calibration.

6.1.3 SIM Calibration - Acquire at least five data points for each ion during elution of each GC peak. Total cycle time should be $\geq$0.5 s and $\leq$1.5 s.

CAUTION: When acquiring SIM data, GC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times; if not, ions will not be monitored at the appropriate times.

6.1.3.1 SIM calibration for PCB determinations

6.1.3.1.1 Two options for SIM data acquisition are provided. Data can be acquired for four sets of $\leq$6 mass range ($\leq$35 ions each as shown in Table 6a) or with the five ion sets ($\leq$20 ions each) shown in Tables 6b and 6c.

6.1.3.1.2 The time (scan number) for initiation of data acquisition with each ion set must be carefully determined from the retention times (scan numbers) of the retention time congeners. Approximate relative retention times of calibration congeners and approximate relative retention time windows for PCB isomer groups are shown in Table 7. The GC conditions are different than those recommended herein, and should be used as guidelines only.

6.1.3.1.3 SIM data acquisition with four ion sets. Begin data acquisition with Ion Set #1 before elution of PCB congener #1, the first eluting Cl_1-PCB. Stop acquisition with Ion Set #1 and begin acquisition with Ion Set #2 just (approximately 10 s) before elution of PCB congener #104, the first eluting Cl_5-PCB. Stop acquisition with Ion Set #2 and begin acquisition with Ion Set #3 just (approximately 10 s) after elution of PCB congener #77, the last eluting Cl_4-PCB. Stop acquisition with Ion Set #3 and begin

acquisition with Ion Set #4 just (approximately 10 s) after elution of $^{13}C_{12}$-4,4'-DDT.

6.1.3.1.4 SIM data acquisition with five ion sets. Acquire data with the four Ion Sets described in Sect. 6.1.3.1.3 and add a fifth Ion Set beginning data acquisition with that set just (approximately 10 s) before elution of PCB congener #208, the first eluting Cl_9-PCB.

6.1.4 Performance Criteria

6.1.4.1 SIM PCB Data

6.1.4.1.1 GC separation -- Baseline separation of PCB congener #87 from congeners #154 and #77, which may coelute.

6.1.4.1.2 MS sensitivity -- Signal/noise ratio of ≥ 5 for m/z 499 of PCB congener #209, Cl_{10}-PCB, and for m/z 241 of chrysene-d_{12}.

6.1.4.1.3 MS calibration -- Abundance of $\geq 70\%$ and $\leq 95\%$ of m/z 500 relative to m/z 498 for congener #209, Cl_{10}-PCB.

6.1.5 Replicate Analyses of CALS -- If all performance criteria are met, analyze each of the other four concentration calibration solutions.

6.1.6 Response Factor Calculation

6.1.6.1 Calculate response factors (RFs) for each PCB calibration congener and surrogate compound relative to both ISs, phenanthrene-d_{10} and chrysene-d_{12}:

$$RF = A_x Q_{is} / A_{is} Q_x$$

where A_x = integrated ion abundance of quantitation ion for a PCB calibration congener or a surrogate compound,

A_{is} = integrated ion abundance of m/z 240, the quantitation ion when chrysene-d_{12} is used as the internal standard or m/z 188, the quantitation ion when phenanthrene-d_{10} is used as the internal standard,

Q_{is} = injected quantity of chrysene-d_{12} or phenanthrene-d_{10},

Q_x = injected quantity of PCB calibration congener or surrogate compound. RF is a unitless number, units used to express quantities must be equivalent.

6.1.7 Response Factor Reproducibility -- For each PCB calibration congener and surrogate compound, calculate the mean RF from analyses of each of the five CALs. When the RSD exceeds 20%, analyze additional aliquots of appropriate CALs to obtain an acceptable RSD of RFs over the entire concentration range, or take action to improve GC/MS performance.

6.1.8 SIM Analyte Retention Time Reproducibility

6.1.8.1 PCB determination -- Absolute retention times of PCB congeners #77 and #104 should not vary by more than ± 10 s from one analysis to the

next. (Retention time reproducibility is not as critical for congeners #1 and #209 as for #77 and #104, which are used to determine when ion sets are changed.)

6.1.9 Record a spectrum of each CAL component.

6.2 Continuing Calibration Check

6.2.1 With the following procedures, verify initial calibration at the beginning and end of each 12-h period during which analyses are to be performed.

6.2.2 Calibrate and tune the MS with standards and procedures prescribed by the manufacturer.

6.2.3 Analyze a 1-ul or 2-ul aliquot of the DFTPP solution and ensure acceptable MS calibration and performance (Table 5).

6.2.4 Inject a 1-ul or 2-ul aliquot of the Medium CAL and analyze with the same conditions used during Initial Calibration.

6.2.5 Demonstrate acceptable performance for criteria described in Sect. 6.1.4.

6.2.6 Determine that neither the area measured for m/z 240 for chrysene-d_{12} nor that for m/z 188 for phenanthrene-d_{10} has decreased by more than 30% from the area measured in the most recent previous analysis of a calibration solution or by more than 50% from the mean area measured during initial calibration.

6.2.7 Response Factor Reproducibility – For an acceptable Continuing Calibration Check, the measured RF for each analyte/surrogate compound must be within $\pm$20% of the mean value calculated (Sect. 6.1.6) during Initial Calibration. If not, remedial action must be taken; recalibration may be necessary.

6.2.8 SIM Analyte Retention Time Reproducibility -- Demonstrate and document acceptable (Sect. 6.1.8) reproducibility and absolute retention times of appropriate PCB congeners.

6.2.9 Remedial actions must be taken if criteria are not met; possible remedies are:

6.2.9.1 Check and adjust GC and/or MS operating conditions.

6.2.9.2 Clean or replace injector liner.

6.2.9.3 Flush column with solvent according to manufacturers instructions.

6.2.9.4 Break off a short portion (approximately 0.33 m) of the column; check column performance by analysis of performance check solution.

6.2.9.5 Replace GC column; performance of all initial calibration procedures then required.

6.2.9.6 Adjust MS for greater or lesser resolution.

6.2.9.7 Calibrate MS mass scale.

6.2.9.8 Prepare and analyze new concentration calibration/performance check solution.

6.2.9.9 Prepare new concentration calibration curve(s).

7.0 Quality Control

7.1 Laboratory Method - The method blanks associated with the samples must be analyzed by this method.

7.2 Calibration -- Included among initial and containing calibration procedures are numerous quality control checks to ensure that valid data are acquired (See Sect. 6). Continuing calibration checks are accomplished with results from analysis, the medium level calibration solution.

7.2.1 If some criteria are not met for a Continuing Calibration Check after a 12-h period during which samples were analyzed, those samples must be reanalyzed. Those criteria are: GC performance (Sect. 6.1.4), MS calibration as indicated by DFTPP spectrum, and MS sensitivity as indicated by area of internal standards.

7.2.2 When other criteria in Sect. 6.2 are not met, results for affected analytes must be labeled as suspect to alert the data user of the observed problem. Included among those criteria are: response factor check for each analyte or PCB calibration congener and retention time reproducibility for SIM data acquisition.

7.3 Laboratory Surrogate Spike

7.3.1 Measure the concentration of the decachlorobiphenyl (DCB) surrogate compound in every sample and blank if there is sufficient sensitivity. The tetrachloro-m-xylene (TCMX) surrogate will not be monitored as there is insufficient information on mass spectral characteristics at this time.

7.3.2 Until performance based acceptance limits have been established for surrogate compounds, the advisory limits of 60-150% will be used.

8.0 Procedures

8.1 GC/MS Analysis

8.1.1 Remove the sample extract or blank from storage and allow it to warm to ambient laboratory temperature if necessary. With a stream of dry, filtered nitrogen, reduce the extract/blank volume to the appropriate volume, depending on anticipated analyte

concentrations. Add an appropriate volume of the internal standard stock solution. Internal standard concentration for SIM data acquisition = 0.75 ng/ul of extract.

8.1.2 Inject a 1-ul or 2-ul aliquot of the extract into the GC operated under conditions used to produce acceptable results during calibration.

8.1.3 Acquire mass spectral data with SIM conditions. Use the same data acquisition time and MS operating conditions previously used to determine response factors.

8.1.4 Examine data for saturated ions in mass spectra of target compounds, if saturation occurred, dilute and reanalyze the extract after the quantity of the internal standards is adjusted appropriately.

8.1.5 For each internal standard, determine that the area measured in the sample extract has not decreased by >30% from the area measured during the most recent previous analysis of a calibration solution or by >50% from the mean area measured during initial calibration. If either criterion is not met, remedial action must be taken to improve sensitivity, and the sample extract must be reanalyzed.

8.2 Identification Procedures

8.2.1 Using the ions shown in Table 6 for PCBs examine selected ion current profiles (SICPs) to locate internal standards and PCBs for each isomer group. Use the RRT window data in Table 7 as guidelines for location of PCB isomers. (A reverse search software routine can be used to locate compounds of concern.)

8.2.2 SIM Data -- Obtain appropriate SICPs for IS quantitation and confirmation ions and for the quantitation and confirmation ions for each PCB isomer group.

8.2.3 PCB Analytes

8.2.3.1 For all PCB candidates, confirm the presence of an $(M-70)^+$ ion cluster by examining SICPs or spectra for at least one of the most intense ions in the appropriate ion cluster.

8.2.3.2 For Cl_3-CL_7 isomer groups, examine SICPs or spectra for intense $(M+70)^+$ ions that would indicate a coeluting PCB containing two additional chlorines. (GC retention time data shown that this is not a potential problem for other PCB isomer groups.) If this interference occurs, a correction can be made. Obtain and record the area of the appropriate ion (Table 8) for the candidate PCB isomer group. Use the information in Table 9 to correct the measured abundance of M^+. For example, if a Cl_7-PCB and a Cl_5-PCB candidate coelute, the Cl_7-PCB will contribute to the ion measured for m/z 326 and m/z 324, the quantitation and confirmation ions, respectively, for a Cl_5-PCB. Obtain and record the area for m/z 322 (the lowest mass ion in the $M+-70)^+$ ion cluster of a Cl_5-PCB fragment produced by a Cl_7-PCB). To determine the m/z 326 and m/z 324 areas produced by the Cl_5 PCB, calculate the Cl_7-PCB contribution to each and subtract it from the measured area. In this example, 164% of the area measured for m/z 322 should be subtracted from the area measured for m/z 324, and 108% of the m/z 322 area should be subtracted from the area measured for m/z 326 (Table 9).

8.2.3.3 For Cl_2-Cl_8-PCB candidates, examine SICPs or spectra for intense $(M+35)^+$ ions that would indicate a coeluting PCB containing one additional chlorine. This coelution causes interferences because or the natural abundance of ^{13}C. (This interference will be small and can be neglected except when measuring the area of a small amount of PCB coeluting with a large amount of another PCB containing one more chlorine.) To correct for this interference, obtain and record the area for

the appropriate ion (Table 10) from the $(M-1)^+$ ion cluster, and subtract 13.5% of the area measured for the $(M-1)^+$ ion from the measured area of the quantitation ion. For example, for Cl_5-PCB candidates, obtain and record the area of m/z 325; subtract 13.5% of that area from the measured area of m/z 326.

8.2.4 All Analytes -- Use SICP data to calculate the ratio of the measured peak areas of the quantitation ion and confirmation ion(s), and compare to the acceptable ratio (Table 8). If acceptable ratios are not obtained, a coeluting or partially coeluting compound may be interfering. Examination of data from several scans may provide information that will allow application of additional background corrections to improve the ion ratio.

8.3 Identification Criteria

8.3.1 Internal Standards

8.3.1.1 Chrysene -d_{12} -- the abundance of m/z 241 relative to m/z 240 must be $\geq$15% and $\leq$25%, and these ions must maximize simultaneously. The area measured for m/z 240 must be within 30% of the area measured during the most recent calibration.

8.3.1.2 Phenanthrene-d_{10} -- the abundance of m/z 189 relative to m/z 188 must be $\geq$10% and $\leq$22%, and these ions must maximize simultaneously. The area measured for m/z 188 must be within 30% of the area measured during the most recent acceptable calibration.

8.3.1.3 Retention time must be within $\pm$10 s of that observed during the most recent acceptable calibration.

8.3.2 SIM Data for PCBs

8.3.2.1 Absolute retention times of surrogate compounds must be within ± 10 s of that measured during the last previous continuing calibration check.

8.3.2.2 Quantitation and confirmation ions for each PCB isomer group must maximize within ± 1 scan of each other.

8.3.2.3 The integrated ion current for each quantitation and confirmation ion must be at least three times background noise and must not have saturated the detector.

8.3.2.4 For each PCB isomer group candidate, the ratio of the quantitation ion area to the confirmation area must be within limits shown in Table 8; at least one ion in the $(M-70)^+$ ion cluster must be present.

9.0 Calculations

9.1 From appropriate SICPs of quantitation ions, obtain and record the spectrum number of the chromatographic peak apex and the area of the entire chromatographic peak.

9.2 For PCBs, sum the areas for all isomers identified at each level of chlorination (e.g., sum all quantitation ions areas for Cl_4-PCBs).

9.3 Calculate the concentration of each surrogate compound and PCB isomer group using the formula:

$$C_x = (A_x \cdot Q_{is}) / (A_{is} \cdot RF \cdot W)$$

where C_x = concentration (micrograms per kilogram or micrograms per liter) of surrogate compound or a PCB isomer group,

A_x = the sum of quantitation ion areas for all PCB isomers at a particular level of chlorination,

A_{is} = the area of the internal standard quantitation ion, m/z 240 for chrysene-d_{12} or m/z 188 for phenanthrene-d_{10},

Q_{is} = quantity (micrograms) of internal standard added to the extract before GC/MS analysis,

RF = calculated response factor for the surrogate compound or the PCB calibration compound for the isomer group (level of chlorination), and

W = weight (kilograms) of sample extracted. If a liquid sample was extracted, W becomes V, the volume (liters) of water extracted, and concentration units become micrograms per liter.

9.3.1 Use the grand mean RF calculated during Initial Calibration. CAUTION: For PCB analyses with automated data interpretation a linear fit algorithm will produce erroneous concentration data.

9.3.2 For PCBs, use the RF relative to chrysene-d_{12} unless an interference makes the use of the RF relative to phenanthrene-d_{10} necessary.

9.4 Report calculated values to two significant figures.

9.5 When standards of known composition are analyzed, calculate the percent method bias using the equation:

$$B = 100 (C_s - C_t) / C_t$$

where C_s = measured concentration (in micrograms per kilogram or micrograms per liter),

C_t = theoretical concentration (i.e., the quantity added to the sample aliquot/weight or volume of sample aliquot).

Note: The bias value retains a positive or negative sign.

10.0 Automated Identification and Measurement

10.1 Special software can be used for automated identification and measurement of PCBs (7) and pesticides. Unprocessed GC/MS data are handled without human interaction with the software operating on the dedicated computer.

11.0 References

11.1. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde, "Trace Analyses for Wastewaters," Environ. Sci. Technol. 15, 1426, 1981.

11.2. Ballschmiter, K. and M. Zell, Fresenius Z. Anal. Chem., 302, 20, 1980.

11.3. "Carcinogens -- Working with Carcinogens," Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

11.4. "OSHA Safety and Health Standards, General Industry," 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

11.5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

11.6. Mullin, M.D., C. Pochini, S. McCrindle, M. Romkes, S.H. Safe, and L.M. Safe, "High Resolution PCB Analysis: Synthesis and Chromatographic Properties of All 209 PCB Congeners," Environ. Sci. Technol. 18, 466, 1984.

11.7. Slivon, L.E.; Gebhart, J.E.; Hayes, T.L.; Alford-Stevens, A.L.; Budde, W.L. "Automated Procedures for Mass Spectrometric Determination of Polychlorinated Biphenyls as Isomer Groups," Anal. Chem. 1985, 57, accepted for publication.

11.8. Rote, J.W. and W.J. Morris, "Use of Isotopic Abundance Ratios in Identification of Polychlorinated Biphenyls by Mass Spectrometry," J. Assoc. Offic. Anal. Chem. 56(1), 188, 1973.

Table 1
Recommended GC Operating Conditions

Capillary column:	40 m x 0.18 mm ID, 0.4 micron film thickness, fused silica DB-5 (or equivalent) splitless mode
Initial temperature:	90°C, initial hold - 2 minutes
Temperature program 1:	10°C/minute for 6 minutes
Temperature program 2:	3°C/minute to 290 C
Final temperature:	290°C, final hold - 4 minutes
Injection port temperature:	210°C
Detector temperature:	300°C

Table 2
PCB Congeners Used as Calibration Standards

PCB Isomer Group	Congener Number[a]	Chlorine Substitution
Concentration Calibration Standard		
Monochlorobiphenyl	1	2
Dichlorobiphenyl	5	2,3
Trichlorobiphenyl	29	2,4,5
Tetrachlorobiphenyl	50	2,2', 4,6
Pentachlorobiphenyl	87	2,2', 3,4,5'
Hexachlorobiphenyl	154	2,2', 4,4', 5,6'
Heptachlorobiphenyl	188	2,2', 3,4', 5,6,6'
Octachlorobiphenyl	200	2,2', 3,3', 4,5', 6,6'
Nonachlorobiphenyl[b]	--	--
Decachlorobiphenyl	209	2,2', 3,3', 4,4', 5,5', 6,6'
Retention Time Calibration Standards		
Tetrachlorobiphenyl	77	3,3', 4,4'
Pentachlorobiphenyl	104	2,2', 4,6,6'
Nonachlorobiphenyl	208	2,2', 3,3', 4,5,5', 6,6'

[a] Numbered according to the system of Ballschmiter and Zell (2).
[b] Decachlorobiphenyl is used as the calibration congener for both nona- and decachlorobiphenyl isomer groups.

Table 3
PCB Primary Dilution Solution

PCB Cong.	Isomer Group	Stock Sol. Conc. mg/ml	Proportion for Primary Dil. Sol.	Primary Dil. Std. Conc. ng/ul
#1	Cl_1	1.0	1 part	50
#5	Cl_2	1.0	1 part	50
#29	Cl^3	1.0	1 part	50
#50	Cl_4	1.0	2 parts	100
#87	Cl_5	1.0	2 parts	100
#154	Cl_6	1.0	2 parts	100
#188	Cl_7	1.0	3 parts	150
#200	Cl_8	1.0	3 parts	150
#209	Cl_{10}	1.0	5 parts	250
			Total 20 parts	

Table 4
Composition and Approximate Concentrations of
Calibration Solutions for SIM Data Acquisition for PCB Determinations

	Concentration (ng/ul)				
Compound	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5
Cal. Congeners					
Cl_1 (#1)	0.1	0.5	1	2	5
Cl_2 (#5)	0.1	0.5	1	2	5
Cl_3 (#29)	0.1	0.5	1	2	5
Cl_4 (#50)	0.2	1	2	4	10
Cl_5 (#87)	0.2	1	2	4	10
Cl_6 (#154)	0.2	1	2	4	10
Cl_7 (#188)	0.3	1.5	3	6	15
Cl_8 (#200)	0.3	1.5	3	6	15
Cl_{10} (#209)	0.5	2.5	5	10	25
RT Congeners					
Cl_4 (#77)	0.2	1	2	4	10
Cl_5 (#104)	0.2	1	2	4	10
Cl_9 (#208)	0.4	2	4	8	20
Internal Standards					
Chrysene-d_{12}	0.75	0.75	0.75	0.75	0.75
Phenanthrene-d_{10}	0.75	0.75	0.75	0.75	0.75

Table 5
Criteria for DFTPP Spectrum

m/z	Relative Abundance
127	40-60%
197	< 1%
198	100% (Base Peak)
199	5-9%
275	10-30%
365	> 1%
441	Present and < m/z 443
442	> 40%
443	17-23% of m/z 442

Table 6a
Ions for Selected Ion Monitoring to Determine PCBs by Acquiring Data for Four Sets of ≤35 Ions Each

PCB Isomer Group/ Int. Std.	Nominal Mol. Wt.	Mass or Range to be Monitored	No. of Ions	Ion Sets #1	#2	#3	#4
Monochlorobiphenyls	188	152; 186-190	6	6			
Dichlorobiphenyls	222	220-224	5	5			
Trichlorobiphenyls	256	254-260	7	7	7	1[a]	
Tetrachlorobiphenyls	290	288-294	7	7	7	1[b]	
Pentachlorobiphenyls	324	322-328	7		7	7	
Hexachlorobiphenyls	358	356-362	7		6[c]	7	7
Heptachlorobiphenyls	392	390-396	7			6[d]	7
Octachlorobiphenyls	426	424-430	7				7
Nonachlorobiphenyls	460	460-466	7				7
Decachlorobiphenyl	494	496-500	5				5
Chrysene-d_{12}	240	240-241	2				2
Phenanthrene-d_{10}	188	188-189	2	2[e]			
			Total # ions	23	27	22	35

[a] Monitor m/z 254 to confirm presence of $(M-70)^+$ for Cl_5-PCBs.
[b] Monitor m/z 288 to confirm presence of $(M-70)^+$ for Cl_6-PCBs.
[c] Begin range at m/z 357 in Ion Set #2.
[d] Begin range at m/z 391 in Ion Set #3.
[e] M/z 188 and 189 included among ions used to detect and measure monochlorobiphenyls.

Table 6b
Ions for Selected Ion Monitoring to Determine PCBs by Acquiring Data for Five Sets of ≤20 Ions Each

Ion Set	Isomer Group/ IS/Surrogate	Quant. Ion	Confirm. Ions	M-70 Ions	M+70 Ions	M+35 Ions	Ion Measured[a] for Correction	
1	Cl_1	188	190	152, 153[b]	256, 258	222, 224	–	–
	Cl_2	222	224	152, 153, 186, 188[c]	290, 292, 294	256, 258	–	221
	Cl_3	256	258	186, 188	–	290, 292, 294	–	255
	Cl_4	292	290, 294	220, 222	–	–	–	–
	Phenanthrene-d_{10}	188	189	–	–	–	–	–
2	Cl_3	256	258	186, 188	324, 326, 328	290, 292, 294	254	255
	Cl_4	292	290, 294	220, 222	360, 362	324, 326, 328	288	289
	Cl_5	326	324, 328	254, 256, 258	–	360, 362	–	323
	Cl_6	360	358, 362	288, 290, 292	–	–	–	–
3	Cl_5	326	324, 328	254, 256	392, 394, 396, 398	360, 362	322	323
	Cl_6	360	358, 362	288, 290	–	392, 394, 396, 398	–	357
	Cl_7	394	392, 396	322, 324, 326	–	–	–	–
4	Cl_6	360	358, 362	288, 290	426, 428, 430, 432	392, 394, 396	356	357
	Cl_7	394	392, 396, 398	322, 324	–	428, 430, 432	–	391
	Cl_8	430	428, 432	356, 358, 360	–	–	–	–
	Chrysene-d_{12}	240	241	–	–	–	–	–
5	Cl_8	430	426, 428, 432	356, 358, 360	494, 496, 498, 500	462, 464, 466	–	425
	Cl_9	464	460, 462, 466	390, 392, 394	–	496, 498, 500	–	–
	Cl_{10}	498	494, 496, 500	424, 426, 428, 430	–	–	–	–

[a] See Tables 8-9.
[b] Cl_1-PCBs lose HCl.
[c] Some Cl_2-PCBs lose Cl_2 and some lose HCl.

Table 6c
Ions for Selected Ion Monitoring to Determine PCBs by Acquiring Data for Five Ion Sets of ≤20 Ions

Ion Set No. 1[a]	Ion Set No. 2[b]	Ion Set No. 3[c]	Ion Set No. 4[d]	Ion Set No. 5[e]
152	186		240	356
153	188		241	358
186	220	254	288	360
187	222	256	290	390
188	254	288	322	392
189	255	290	324	394
190	256	322	326	424
220	258	323	356	425
221	288	324	357	426
222	289	326	358	428
224	290	328	360	430
255	292	357	362	432
256	294	358	391	462
258	323	360	392	464
290	324	362	394	466
292	326	392	396	496
294	328	294	398	498
	358	396	428	499
	360	398	430	500
	362		432	502
17 ions	20 ions	17 ions	20 ions	20 ions

[a] Ions to identify and measure Cl_1-Cl_4-PCBs, and phenanthrene-d_{10}.
[b] Ions to identify and measure Cl_3-Cl_6-PCBs.
[c] Ions to identify and measure Cl_5-Cl_7-PCBs.
[d] Ions to identify and measure Cl_6-Cl_8-PCBs and chrysene-d_{12}.
[e] Ions to identify and measure Cl_8-Cl_{10}-PCBs.

Table 7
Retention Time Data for PCB Isomer Groups and Calibration Congeners

Isomer Group	RRT Window[a]	Cal. Cong. Number	Cal. Cong. RRT[a]
Monochlorobiphenyls	0.30-0.35	1	0.30
Dichlorobiphenyls	0.38-0.50	5	0.43
Trichlorobiphenyls	0.46-0.64	29	0.54
Tetrachlorobiphenyls	0.55-0.82	50	0.56
Pentachlorobiphenyls	0.64-0.92	87	0.80
Hexachlorobiphenyls	0.75-1.1	154	0.82
Heptachlorobiphenyls	0.88-1.2	188	0.88
Octachlorobiphenyls	0.99-1.21	200	1.03
Nonachlorobiphenyls	1.16-1.28	–	–
Decachlorobiphenyl	1.3	209	1.3

[a] Retention time relative to chrysene-d_{12} with a 30 m X 0.31 mm ID SE-54 fused silica capillary column and the following GC conditions: splitless injection at 80°C; hold for 1 min; heat rapidly to 160°C and hold 1 min; increase at 3°C/min to 310°C.

Table 8
Quantitation, Confirmation, and Interference Check Ions for PCBs and Internal Standards

Analyte/ Internal Std.	Nom. MW	Quant. Ion	Confirm. Ion	Expected Ratio[a]	Accept. Ratio[a]	M-70 Confirm. Ion	Interference Check Ions M+70	Interference Check Ions M+35
PCB Isomer Group								
Cl_1	188	188	190	3.0	2.5-3.5	152[b]	256	222
Cl_2	222	222	224	1.5	1.3-1.7	152	292	256
Cl_3	256	256	258	1.0	0.8-1.2	186	326	290
Cl_4	290	292	290	1.3	1.1-1.5	220	360	326
Cl_5	324	326	324	1.6	1.4-1.8	254	394	360
Cl_6	358	360	362	1.2	1.0-1.4	288	430	394
Cl_7	392	394	396	1.0	0.8-1.2	322	464	430
Cl_8	426	430	428	1.1	0.9-1.3	356	498	464
Cl_9	460	464	466	1.3	1.1-1.5	390	--	498
Cl_{10}	494	498	500	1.1	0.9-1.3	424	--	--
Internal Standards								
Chrysene-d_{12}	240	240	241	5.1	4.3-5.9	--	--	--
Phenanthrene-d_{10}	188	188	189	6.6	6.0-7.2	--	--	--

[a] Ratio of quantitation ion to confirmation ion.
[b] Monodichlorobiphenyls lose HCl to produce anion at m/z 152.

Table 9
Correction for Interference of PCB Containing Two Additional Chlorines

Candidate Isomer Group	Quant. Ion	Confirm. Ion	Ion Measured to Determine Interference	% of Meas. Ion Area to be Subtracted from Quant. Ion Area	% of Meas. Ion Area to be Subtracted from Confirm. Ion Area
Trichlorobiphenyls	256	258	254	99%	33%
Tetrachlorobiphenyls	292	290	288	65%	131%
Pentachlorobiphenyls	326	324	322	108%	164%
Hexachlorobiphenyls	360	362	356	161%	71%
Heptachlorobiphenyls	394	396	390	225%	123%

Table 10
Correction for Interference of PCB Containing One Additional Chlorine

Candidate Isomer Group	Quant. Ion	Ion Measured to Determine Interference	% of Meas. Ion Area to be Subtracted from Quant. Ion Area
Dichlorobiphenyls	222	221	13.5%
Trichlorobiphenyls	256	255	13.5%
Tetrachlorobiphenyls	292	289	17.4%
Pentachlorobiphenyls	326	323	22.0%
Hexachlorobiphenyls	360	357	26.5%
Heptachlorobiphenyls	394	391	30.9%
Octachlorobiphenyls	430	425	40.0%

Appendix A-6

DATA VALIDATION GUIDELINES FOR CONGENER SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBs)

I. METHOD SUMMARY

The analytical methods described in Appendices A-1 through A-4 of the Quality Assurance Project Plan (QAPP) for the Hudson River PCB Reassessment RI/FS are modifications of the NYSDEC's Analytical Services Protocols for PCB Congeners. The polychlorinated biphenyl (PCB) congeners are extracted from water and sediment samples, and the resulting hexane extracts are analyzed by fused silica capillary column gas chromatography with electron capture detector (FSCC/GC/ECD). The PCBs are identified and quantitated by congener. A subset of the sample extracts (approximately 10% of the sediments and 5% of the water column samples) will be analyzed by a modified EPA Method 680 as described in Appendix A-5 of the QAPP to confirm the congener identification by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode. The GC/MS analyses will also provide quantitative confirmation of the congeners.

The data deliverables for these methods will include, at a minimum:

- Case narrative
- Chain-of-Custody records
- Sample and method blank results summaries
- Surrogate recovery summaries for each matrix
- Matrix spike/matrix spike duplicate recovery summaries for each matrix
- Retention time window summaries
- Initial and continuing calibration summaries
- Raw data for each sample, blank, and QC sample, including chromatograms, quantitation reports, and sample preparation logbook summaries
- Mass spectrometer tuning (DFTPP) summaries and raw data, including mass list and mass spectra (for GC/MS confirmations only)
- GC/MS calibration summaries, including raw data (selected ion current profiles, SICPs)
- GC/MS data for each confirmed sample, including SICPs and quantitation reports.

The specific sample weight, final volume, and clean-up procedures used for each sample should be clearly identified in the data.

These guidelines are subject to revision, based on the actual methodology used and the reporting formats utilized by the laboratory.

II. HOLDING TIMES

A. Objective

The objective is to ascertain the validity of results based on the holding time of the sample from time of collection to time of sample preparation or analysis, as appropriate.

B. Criteria

Technical holding times have been established for water matrices only. The water holding time requirements will be used to evaluate soil/sediments results until actual holding times are established. On October 26, 1984 in Volume 49, Number 209 of the Federal Register, page 43260, the holding time requirements were established under 40 CFR 136 (Clean Water Act). Both samples and extracts must preserved at 4° C. Samples must be extracted within 7 days of sample collection and the extract must be analyzed within 40 days.

C. Evaluation Procedure

Technical holding times are established by comparing the sampling date on the EPA Sample Traffic Report and/or Chain-of-Custody records with dates of extraction and analysis listed on Form I.

D. Action

If the 40 CFR 136 holding times are exceeded, flag all positive results as estimated (J) and sample quantitation limits as estimated (UJ) and document in the validation report that holding times were exceeded. If the holding times are grossly exceeded, either upon initial analysis or upon re-analysis, the reviewer must use professional judgment to determine the reliability of the data. The reviewer may determine that the nondetect data are unusable.

III. INSTRUMENT PERFORMANCE

A. Objective

These criteria are established to ensure that adequate chromatographic resolution is achieved, which is crucial for qualitative identification of the congeners. These are method criteria and therefore all criteria should be met in all circumstances.

B. Criteria

1. Resolution Check

   Separation should be greater than 75 percent resolution (% RES) between peaks BZ#28 and BZ#31. This criterion must be met on the quantitation column. When this criteria

cannot be met, quantitation may be adversely affected because of the difficulty in establishing a baseline.

$$\% \text{ RES} = \frac{D_v}{H_p} \times 100$$

where,

D_v = the depth of the valley between the peaks

H_p = the maximum height of the smaller peak

2. Retention Time Windows

The laboratory must report retention time window data for each congener for each GC column used to analyze the sample.

3. Surrogate Retention Time Check

The retention time of the surrogates tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB) in each analysis must be compared to the retention time of TCMX in CAL STD-1. The Percent Difference (%D) must not exceed 0.3% for narrow-bore capillary columns.

$$\%D = \frac{RT_I - RT_S}{RT_I} \times 100$$

where,

RT_I = Absolute retention time of the surrogate in the initial standard CAL STD-1

RT_S = Absolute retention time of the surrogate in subsequent analyses.

C. **Evaluation Procedure**

1. Check the raw data to verify that the resolution of BZ#28 and BZ#31 is greater than 75%. Note any other congener pairs that have resolutions less than 75%.

2. Check the raw data to verify that the retention time windows are correctly calculated and reported, and that all PCB standards are within the established retention time window.

3. Check the raw data to verify that the %D in retention time is less than 0.3% in all standards and samples.

D. **Action**

1. If the % RES between BZ#28 and BZ#31 is less than 75%, a close examination of the chromatography is necessary to ensure adequate separation of the congeners. If adequate separation (>75%) of any congener pair is not achieved, flag all affected congener data as unusable (R). The reviewer may use professional judgment, based on the chromatography review, to estimate (J) the positive congeners results for a poorly resolved pair if there is sufficient evidence that the congeners are present in the sample.

2. Retention time windows are used in qualitative identification. If the standards do not fall within the retention time windows, the associated sample results should be carefully evaluated. All samples after the last in-control standard are potentially affected.

   a. For the affected samples, check to see if chromatograms contain any peaks within an expanded window surrounding the expected retention time window of the congener(s) of concern. If no peaks are present either within or close to the retention time window of the deviant congener, there is no effect on the data. Nondetect results can be considered valid.

   b. If the affected sample chromatograms contain peaks which may be of concern (close or within the retention time window), the reviewer may use the following information to confirm or refute the presence of the deviant congeners:

      - pattern recognition, based on other samples in the SDG;
      - the congener retention times of other standards to create "revised" windows, based on possible retention time shifts; and
      - matrix spike results which confirm the magnitude of the retention time shift.

      If the results (positive or nondetect) cannot be confirmed, the data should be flagged as unusable (R).

   c. The data validation narrative should identify any additional efforts taken by the reviewer and the resultant impact on data usability. In addition, the supporting documentation in the data validation report should contain all calculations and comparisons generated by the reviewer.

3. If the retention time shift for TCMX or DCB is greater than 0.3%, the analysis may be flagged as unusable (R) for that sample, but qualification of the data is left up to the professional judgment of the reviewer. The chromatograms from both columns should be carefully reviewed to determine if any co-eluting interferences are present.

IV. CALIBRATION

A. **Objective**

Compliance requirements for satisfactory instrument calibration are established to ensure that the instrument is capable of producing acceptable quantitative data. Initial calibration demonstrates

that the instrument is capable of acceptable performance prior to the analysis of samples, and the continuing calibration checks document satisfactory maintenance and adjustment of the instrument over a specific time period.

B. **Criteria**

1. Initial Calibration

The Percent Relative Standard Deviation (%RSD) of the congener calibration factors must not exceed 10%.

$$\text{Calibration Factor} = \frac{\text{Total Area of Peaks}}{\text{Mass Injected (ng)}}$$

$$\%\text{RSD} = \frac{(\text{sigma})}{\text{CF}} \times 100$$

where,

(sigma) = Standard Deviation of the calibration factors

CF = Mean calibration Factor

The 10% RSD linearity check is required only for the column which is used for quantitation. Quantitation of the surrogate requires the use of a column shown to meet the 10% linearity criterion. Columns used for qualitative confirmation are not required to meet this criterion.

2. Analytical Sequence

At the beginning of each sequence a five concentration level calibration must be performed. The CAL STD-3, the mid-level calibration standard, must be analyzed after every five sample analyses as a continuing calibration check. A hexane instrument blank must be analyzed after each standard, immediately prior to the analysis of the next five samples.

3. Continuing Calibration

The calibration factor for each congener must be within 15% of the mean calibration factor from the five-level calibration at the beginning of the sequence.

C. **Evaluation Procedure**

1. Inspect the initial calibration summary forms and verify agreement with the raw GC data. Recalculate 10% of the data to verify the reported %RSDs. If errors are detected, a more comprehensive recalculation must be performed.

2. Verify that the appropriate analytical sequence was followed.

3. Check the raw data to verify the %D, using the following formula, for approximately 10% of the reported values by recalculation.

$$\%D = \frac{CF_I - CF_S}{CF_I} \times 100$$

where,

CF_I = Mean calibration factor from initial calibration

CF_S = Calibration factor from continuing standard.

D. **Action**

1. If the criteria for linearity are not met, flag all associated quantitated results as estimated (J).

2. If the standards or instrument blanks have not been analyzed at the required frequency, the reviewer must use professional judgment to determine the severity of the effect and qualify the data accordingly.

3. If the %D between the continuing calibration factor and the mean calibration factor of a congener is greater than 15%, flag associated positive quantitative results as estimated (J) for that congener. If the %D grossly exceeds 15%, the reviewer should use professional judgment to determine the extent and direction of the bias, and estimate the nondetect results if deemed necessary. If, in the opinion of the reviewer, the variability in instrument response is so great that all positive and nondetect results are suspect, the data may be unusable (R).

   The reviewer must carefully document the qualification of data, with detailed descriptions of potential biases.

V. BLANKS

A. **Objective**

The assessment of blank analysis results is to determine the existence and magnitude of contamination problems. The criteria for evaluation of blanks apply to any blank associated with the samples. If problems with any blank exist, all associated data must be carefully evaluated to determine whether or not there is an inherent variability in the data, or if the problem is an isolated occurrence not affecting other data.

B. **Criteria**

No congeners should be present in the blanks.

C. **Evaluation Procedure**

Review the results of all associated blanks (method, field, and instrument blanks), including all raw data. Verify that the blanks contain less than reporting quantitation limits for all congeners as determined in the Method Detection Limit Study. The laboratory may not report a blank contaminant that does not meet the requirements for congener confirmation. However, the presence of unknown peaks in a blank within the retention time windows of congeners may result in false positives or results which may be biased high.

Verify that a method blank was extracted for each matrix for each SDG. Verify that instrument blanks have been analyzed after each continuing calibration standard.

D. **Action**

Action in the case of unsuitable blank results depends on the circumstances and the origin of the blank. No positive results should be accepted unqualified unless the concentration of the compound in the sample exceeds 5 times the amount in the blank. In instances where more than 1 blank is associated with a given sample, qualification should be based on comparison with the associated blank having the concentration of the contaminant. The results must not be corrected by subtracting the blank value. Specific actions are as follows:

1. If a congener is found in the blank but not found in the sample(s), no action is taken.

2. Any congener detected in a sample and also detected in any associated blank, must be qualified when the sample concentration is less than 5 times the blank concentration. The positive results qualified as nondetect (followed by U).

   The reviewer should note that the blank analyses may not involve the same weights, volumes, or dilution factors as the associated samples. These factors must be accounted for when applying the 5x criteria, such that a comparison of the total amount of contamination is made.

   In addition, there may be instances where little or no contamination was present in the associated blanks, but qualification of the sample was deemed necessary. Contamination introduced through dilution hexane is one example. Such occurrences can be determined when contaminants are not present in the initial analyses but are present in dilutions. If the reviewer determined that the source of the contamination is not from the sample itself, then the data should be qualified. In these cases, the 5x rule does not apply and the sample value should be reported as a nondetect (value followed by U).

3. Non-congener contamination can also occur, such as phthalate contamination introduced by pierced vial septa between initial and re-analyses, or other contaminants that are not removed during the sample clean-up procedures. The contamination may result in false positives or higher calculated concentrations on one column due to co-elution of the contaminant with a native congener. False negatives are also possible, as a contaminant may obscure the detection of a congener within the retention time windows.

   The reviewer should carefully document all actions taken due to contamination.

VI. SURROGATE RECOVERY

A. Objective

Laboratory performance on individual samples is established by spiking surrogate compounds in all samples prior to sample preparation. The evaluation of surrogate spikes is not necessarily straightforward. The sample itself may produce effects due to such factors as interferences and high concentrations of analytes. Since the effects of the sample matrix are frequently outside the control of the laboratory and may present unique problems, the review and validation of data based on specific sample results is frequently subjective and demands analytical experience and professional judgment. Accordingly, this section consists primarily of guidelines.

B. Criteria

Sample and blank recoveries of TCMX and DCB must be within 60 - 150%.

C. Evaluation Procedure

Check the raw data to verify the reported recoveries on the surrogate recovery form. If recoveries are not within limits, check the raw data for possible interferences which may have affected surrogate recoveries.

D. Action

If the surrogate recoveries are outside of the control limits, the associated sample results and quantitation limits may be estimated (J and UJ, respectively). However, professional judgment may be used to apply the qualifiers. Low recovery of TCMX but not DCB may indicate a problem with the concentration procedures, and therefore the possibility of a negative bias is more likely for the more volatile congeners, such as mono- and di-chlorobiphenyl. The DCB is less volatile than the other PCB congeners, and therefore is more likely to be unaffected by reported extraction concentration procedures. Low recoveries of both TCMX and DCB may indicate matrix effects if the blanks are unaffected, or a general method bias within the laboratory if all samples and blanks are affected.

If high recoveries are obtained, professional judgment should be used to determine appropriate action. A high bias may be caused by co-eluting interferences or by a laboratory method bias.

If zero recoveries are reported, the reviewer should examine the sample chromatogram to determine if the surrogates may be present, but slightly outside of the retention time windows. If this is the case, in addition to assessing surrogate recovery for quantitative bias, the reviewer should investigate the qualitative validity of the analysis due to possible retention time shifts.

If the surrogate is not present, flag all negative results as unusable (R).

VII. MATRIX SPIKE/MATRIX SPIKE DUPLICATE

A. Objective

These data are generated to determine long-term precision and accuracy of the analytical method on various matrices. These data alone cannot be used to evaluate the precision and accuracy of individual samples.

B. Criteria

The advisory limits for congener recovery is 60 - 150%. The advisory limit for relative percent difference (RPD) is 40%.

C. Evaluation Procedure

Verify the reported recoveries by reviewing the raw data and verify the calculations.

D. Action

No action is taken on the matrix spike/matrix spike duplicate (MS/MSD) data alone to qualify an entire SDG. However, using informed professional judgment, the reviewer may use the MS/MSD information in conjunction with other QC criteria to determine the need for some qualification of the data.

In those instances where it can be determined that the results of the MS/MSD affect only the sample spiked, then qualification should be limited to this sample alone. However, it may be determined through the MS/MSD results that a lab is having a systematic problem in the analysis of one or more congeners, which affects all associated samples.

VIII. MATRIX SPIKE BLANK

A. Objective

This QC check sample is equivalent to a laboratory control sample (LCS) and matrix effects are assumed to minimal. These data are generated to determine long-term precision and accuracy of the analytical method. These data alone cannot be used to evaluate the precision and accuracy of individual samples.

B. Criteria

The advisory limits for congener recovery is 60 - 150%.

C. Evaluation Procedure

Verify the reported recoveries by reviewing the raw data and verify the calculations.

D. **Action**

No action is taken on the matrix spike blank data alone to qualify an entire SDG. However, using informed professional judgment, the reviewer may use the information in conjunction with other QC criteria to determine the need for some qualification of the data. All matrix spike blank samples should be evaluated for the project to determine if there is any systematic bias in the laboratory procedures.

IX. FIELD DUPLICATES

A. **Objective**

Field duplicate samples will be taken and analyzed as an indication of overall precision. These analyses measure both field and laboratory precision; therefore, the results may have more variability than laboratory duplicates (or MS/MSD) which only measure laboratory performance. It may also be expected that soil and sediment duplicates will have a greater variance than water matrices due to the difficulties associated with collecting representative field samples.

B. **Criteria**

The Quality Assurance Project Plan requires that field duplicate results have less than 40% RPD.

C. **Evaluation Procedure**

Samples which are field duplicates should be identified using EPA Sample Traffic Reports and/or Chain-of-Custody records. The reviewer should compare the results reported for each sample and calculate the RPD.

D. **Action**

Any congeners that have a RPD > 40% should be estimated (J). If a congener is reported in one field sample but is a nondetect in the duplicate, estimate the positive result (J) in the original sample and the nondetect result (UJ) in the field duplicate.

Significant differences in the concentrations or type of congeners reported in a field duplicate pair may indicate poor sample handling, possible mislabelling of sample containers, and/or high variability in the sample matrix.

X. COMPOUND IDENTIFICATION

A. **Objective**

Qualitative criteria for compound identification have been established to minimize the number of erroneous identifications of congeners. An erroneous identification can either be a false positive (reporting a compound present when it is not) or a false negative (not reporting a compound that is present).

B. **Criteria**

Retention times of reported congeners must fall within the calculated retention time windows for the two chromatographic columns.

Selected samples will be analyzed by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring (SIM) mode by EPA Method 680 (modified).

C. **Evaluation Procedure**

Review the reported results and the associated raw data (chromatograms and data system printouts). Confirm the reported positive detects, using appropriate retention times and retention time windows, and verify that the congeners listed as "not detected" are correct.

Verify that positive identifications were confirmed on dissimilar columns. Verify that GC/MS confirmation was performed on the specified samples.

Verify that the congeners identified by GC/ECD were confirmed by GC/MS/SIM. The retention time for each m/z of a congener must maximize within 1 scan of each other, and the relative ratio of the m/z must meet the criteria listed in Appendix A-5.

D. **Action**

If the qualitative criteria for two column confirmation are not met, all reported positive detects should be considered nondetects. The reviewer should use professional judgment to assign an appropriate quantitation limit. If the misidentified peak poses an interference with potential detection of a target congener, then the reported value should be considered and flagged as an estimated quantitation limit (UJ).

As PCBs are multi-component mixtures, the reviewer can use professional judgment and knowledge of Aroclor congener composition to aid in congener identification and to qualify data based on the results of the GC/MS analysis.

XI. COMPOUND QUANTITATION AND REPORTED DETECTION LIMITS

A. **Objective**

The objective is to ensure that the reported quantitation results and reporting limits are accurate.

B. **Criteria**

Compound quantitation, as well as the adjustment of the reporting limits, must be calculated according to the appropriate SOP.

The results of the GC/MS confirmation analyses should be within 75% RPD for each congener.

C. **Evaluation Procedure**

1. Raw data should be examined to verify the correct calculation of all sample results reported by the laboratory. Quantitation reports, chromatograms, and sample preparation log sheets should be compared to the reported positive results and quantitation limits.

2. Verify that the reporting limits have been adjusted to reflect all sample dilutions, concentrations, splits, clean-up activities, and dry weight factors that are not accounted for in the method. Verify that the reporting limits are consistent with the MDL studies performed by the laboratory.

3. Verify that the GC/MS congener results are within 75% RPD of those from the GC/ECD analyses.

D. **Action**

The reviewer should use professional judgment to decide whether a significantly (>25% RPD) larger concentration on one column versus the other indicates the presence of an interfering compound. If an interfering compound is indicated, the lower of the two values should be reported. However, if the laboratory reported the higher value which was on the quantitation column, the reviewer should determine if there are any potential positive or negative biases for the analyses on the confirmation column before using either value. Likewise, the data validation narrative should indicate the presence of interferences that obscured the attempt at a second column confirmation.

Quantitation limits affected by large peaks should be flagged as unusable (R). The reviewer may also elect to provide an estimated detection limit for the effected congeners.

If the RPD between the GC/ECD and GC/MS analyses exceed 75% for a given congener, the positive result should be estimated (J). If the congener was detected in the sample extract by GC/ECD at sufficient concentration to be analyzed by GC/MS, but is not found during the GC/MS analysis, the positive results should be estimated (J). However, the reviewer may use professional judgment to determine that the result is a false positive and therefore the result may be changed to a nondetect with an elevated detection limit.

Appendix B

ORGANIC CARBON, TOTAL

Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680
Dissolved 00681

1. Scope and Application
   1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.
   1.2 The method is most applicable to measurement of organic carbon above 1 mg/1.
2. Summary of Method
   2.1 Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion or wet chemical oxidation. The CO_2 formed can be measured directly by an infrared detector or converted to methane (CH_4) and measured by a flame ionization detector. The amount of CO_2 or CH_4 is directly proportional to the concentration of carbonaceous material in the sample.
3. Definitions
   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:
      A) soluble, nonvolatile organic carbon; for instance, natural sugars.
      B) soluble, volatile organic carbon; for instance, mercaptans.
      C) insoluble, partially volatile carbon; for instance, oils.
      D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.
   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

Approved for NPDES
Issued 1971
Editorial revision 1974

4. Sample Handling and Preservation

4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples.
NOTE 1: A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.

4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified ($pH \leq 2$) with HCl or H_2SO_4.

5. Interferences

5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.

5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.

6. Apparatus

6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.

6.2 Apparatus for total and dissolved organic carbon:

6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.

6.2.2 No specific analyzer is recommended as superior.

7. Reagents

7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.

7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.
NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.

7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.

7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as Bias, %	Accuracy as Bias, mg/liter
4.9	3.93	+15.27	+0.75
107	8.32	+ 1.01	+1.08

(FWPCA Method Study 3, Demand Analyses)

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 2574–79, p 469 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 532, Method 505, (1975).

Appendix C

Appendix C

MEASUREMENT OF DISSOLVED ORGANIC CARBON IN RIVER WATER SAMPLES VIA PERSULFATE DIGESTION

1.0 Scope and Application

1.1 This method describes a procedure for the determination of dissolved organic carbon (DOC) in field-filtered water samples. The procedure is designed to measure natural water levels of dissolved organic carbon on a per unit volume basis. Method will be performed by Lamont Doherty Geological Observatory.

2.0 Summary of Method

2.1 Samples are field-filtered and acidified with sulfuric acid. Then, 0.1 g potassium persulfate is added to a sample aliquot and purged of all CO_2 with a stream of CO_2-free helium. The ampoule is sealed heated to 90°C for 4 hours. A portion of the sample is then withdrawn, mixed with an equal volume of helium, and shaken. The helium, now containing sample CO_2, is injected into a gas chromatograph and the CO_2 level is measured by a thermal conductivity detector.

2.2 Method interferences may result from the loss of volatile organic compounds during the initial stripping of CO_2 from the sample. Additional interferences may occur from contaminated laboratory glassware if care is not taken with low-level samples.

3.0 Apparatus and Materials

3.1 Field-filtering Apparatus

The apparatus for field-filtering consists of a stainless steel filter holder (47 mm diameter) using a pre-combusted 0.7 um Whatman GFF glass fiber filter or a 0.45 um Gelman membrane filter. This filter holder is coupled to a positive pressure system used to push the water through the filter.

3.2 Gas Chromatograph

An analytical system complete with gas chromatograph and all required accessories including syringes, analytical columns, thermal conductivity detector and a strip-chart and/or an electronic recording device. The chromatograph must have a loop injection system to ensure a reproducible injection volume.

3.3 Hot Water Bath

Bath is needed to maintain samples at 90°C. Temperature control must be accurate to ± 5°C.

4.0 Reagents

4.1 Sample Preservation

4.1.1 2N H_2SO_4 to be added to filtered field samples or preservation at pH ≤2.

4.1.2 Wide range pH paper - to test samples in field and ensure proper preservation.

4.2 Helium - CO_2-free for stripping samples of CO_2 before beginning digestion.

4.3 Potassium persulfate - Reagent grade

4.4 Calibration standards

Glucose solution in five different concentrations: 100, 200, 500, 1000, and 2000 umol/l (1.2, 2.4, 6.0,12, and 24 mg/l) made with high purity distilled deionized water.

4.5 Distilled, deionized water, 18 Mohm purity, minimum dissolved organic carbon content.

5.0 Initial Calibration

5.1 The gas chromatographic system must be initially calibrated using the five standard solutions described in Section 4. All standard solutions are prepared for analysis on the gas chromatograph following the procedure described in Section 6 with the exclusion of the filtration step. Prior to the analysis of these standards, the laboratory must determine the retention time of CO_2 on the instrument.

5.2 Tabulate peak height or area responses against concentration for each standard corrected for the distilled deionized water blank. This correction is necessary since the distilled deionized water is used to make up the standards. (It must be demonstrated by the laboratory that the distilled deionized water blank is not a result of the analysis method prior to beginning sample analysis (see Section 9.1).

5.3 Fit a first or second order regression equation to the calibration data. No higher order of regression is permitted. The correlation coefficient must be ≥ 0.995. A plot of the calibration curve and standards must be supplied with the sample results.

5.4 The full range calibration must be done for every group of 20 samples or less.

6.0 Procedure

6.1 Field Procedure

About 80 to 100 ml of river water is filtered through a glass fiber or membrane filter and collected in two 40 ml glass vials. The vials will be prepared with 0.5 ml of 2N H_2SO_4 prior to adding sample. A small air bubble is left in the vial to allow for expansion and mixing. After the sample has been placed in the vial, the vial is sealed with a teflon coated cap liner and cap and shaken. To ensure that the sample is sufficiently acidified, one vial is reopened and a small drop of solution is tested with wide-range pH paper. Solution must be at $\leq$ pH 2. If not, additional sulfuric acid solution is added to achieve the proper pH. Keep sample refrigerated at $\leq 4°C$.

6.2 Laboratory Procedure

6.2.1 Measure out 24 ml of sample from one 40 ml VOA vial, add 0.1 gm of potassium persulfate and place in a 25 ml ampule.

6.2.2 Strip CO_2 from sample for 3 minutes using a thin diameter tube and CO_2-free helium. The helium flow should be about 0.5 cc/minute.

6.2.3 Remove tube and immediately flame-seal the ampule.

6.2.4 Place sample in hot water bath at 90°C for at least four hours.

6.2.5 Break ampule and remove about 18 cc of sample using a 30 cc plastic syringe fitted with a syringe valve. Expel all air bubbles and some water until 15 cc of sample are left in syringe.

6.2.6 Fill syringe to 30 cc with CO_2-free helium, yielding a syringe half filled with water and half with helium. Close syringe valve and shake vigorously for two minutes. Place syringe in shaker bath at 25°C for at least five minutes.

6.2.7 Inject gas into gas chromatograph. Inject about 8 cc or sufficient gas to purge injection loop.

6.2.8 Inject gas filled loop into gas chromatograph and record response.

7.0 Calculations

7.1 Calculate the mass of dissolved carbon in each of the samples using the response curve generated in Section 5.

7.2 Calculate the relative percent difference (RPD) for duplicate pairs:

$$RPD = \frac{Sample\ Level - Sample\ Duplicate\ Level}{\left(\frac{-Sample\ Level + Sample\ Duplicate\ Level}{2}\right)} \times 100\%$$

Compare % recovery of ICV and CCV to "true" value. % recovery criteria is 90-110%.

8.0 Precision and Accuracy

8.1 The precision of the method is expected to be ≤10% (RPD) on duplicates.

8.2 The minimum detection limit is anticipated to be 25 ug/l.

8.3 The anticipated accuracy of the measurement is 90-110% or better.

9.0 Quality Control

9.1 Method Blanks

Since most distilled deionized water contains some dissolved organic carbon, a reliable method blank can only be generated using a previously digested distilled, deionized water blank.

9.1.1 To prepare a method blanks, two previously analyzed distilled deionized water blanks are required. Approximately 12 ml of solution are taken from each water blank and combined in a clean, unused ample for a total of 24 ml.

9.1.2 0.1 gm of potassium persulfate are added.

9.1.3 The solution in bubbled with CO_2-free helium for at least 3 minutes at 0.5 cc/sec using a thin tube.

9.1.4 The tube is removed and the ample quickly sealed with a flame.

9.1.5 The remainder of the preparation and analysis follows steps 6.2.4 to 6.2.8.

9.1.6 One method blank will be run for every twenty samples or for every sample delivery group, whichever is more frequent.

9.2 Calibration

A five point calibration will be performed for every twenty samples or for every sample delivery group, whichever is more frequent.

9.3 Laboratory Calibration Verification

In addition to the five point calibration to be run with every sample delivery group, an independent laboratory calibration standard (ICV) will be run. This standard will be derived from a separate source or vendor than that for the five calibration standards. The measured value of the standard must recover within 90-110% calculated as:

$$\%\ recovery = \frac{measured\ value\ of\ ICV}{true\ value\ of\ ICV\ standard} \times 100\%$$

9.4 Continuing Calibration Verification

The mid-range standard (CCV) will be repeated during the analytical process after every 10 samples and at the end of the day's operation to check instrument drift. This standard must agree to within 10% of the true value. If this is not achieved, all samples run since the last time the containing calibration check was in control or since the last five point calibration must be rerun. The laboratory must first re-establish control by recalibrating the instrument and rerunning the ICV prior to continuing sample analyses.

Appendix D

RESIDUE, NON-FILTERABLE

Method 160.2 (Gravimetric, Dried at 103–105°C)

STORET NO. 00530

1. Scope and Application
   - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   - 1.2 The practical range of the determination is 4 mg/1 to 20,000 mg/1.
2. Summary of Method
   - 2.1 A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103–105°C.
   - 2.2 The filtrate from this method may be used for Residue, Filterable.
3. Definitions
   - 3.1 Residue, non-filterable, is defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103–105°C.
4. Sample Handling and Preservation
   - 4.1 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
   - 4.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
   - 5.1 Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.
   - 5.2 Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.
6. Apparatus
   - 6.1 Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.
     NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.
   - 6.2 Filter support: filtering apparatus with reservoir and a coarse (40–60 microns) fritted disc as a filter support.

Approved for NPDES
Issued 1971

NOTE: Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

6.3 Suction flask.

6.4 Drying oven, 103–105°C.

6.5 Desiccator.

6.6 Analytical balance, capable of weighing to 0.1 mg.

7. Procedure

7.1 Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103–105°C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.

7.2 Selection of Sample Volume

For a 4.7 cm diameter filter, filter 100 ml of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm^2 of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five ml increments for timing are suggested. Continue to record the time and volume increments until fitration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

7.3 Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.

7.4 Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected in 7.2 to the filter using a graduated cylinder. Remove all traces of water by continuing to apply vacuum after sample has passed through.

7.5 With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of wash water used should equal approximately 2 ml per cm. For a 4.7 cm filter the total volume is 30 ml.

7.6 Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103–105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

8. Calculations

8.1 Calculate non-filterable residue as follows:

$$\text{Non-filterable residue, mg/l} = \frac{(A - B) \times 1{,}000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg
B = weight of filter (or filter and crucible) in mg
C = ml of sample filtered

9. Precision and Accuracy

9.1 Precision data are not available at this time.

9.2 Accuracy data on actual samples cannot be obtained.

Bibliography

1 NCASI Technical Bulletin No. 291, March 1977. National Council of the Paper Industry for Air and Stream Improvement, Inc., 260 Madison Ave., NY.

Appendix E

3. References

1. LONGHURST, A.R. & D.L.R. SEIBERT. 1967. Skill in the use of Folsom's plankton sample splitter. *Limnol. Oceanogr.* 12:334.
2. MCEWEN, G.F., M.W. JOHNSON & T.R. FOLSOM. 1954. A statistical analysis of the Folsom sample splitter based upon test observations. *Arch. Meteorol. Geophys. Bioklimatol.*, Ser. A. 6:502.
3. ALDEN, R.W., III, R.C. DAHIYA & R.J. YOUNG, JR. 1982. A method for the enumeration of zooplankton samples. *J. Exp. Mar. Biol. Ecol.* 59:185.
4. GANNON, J.E. 1971. Two counting cells for the enumeration of zooplankton micro-crustacea. *Trans. Amer. Microsc. Soc.* 90:486.
5. DODSON, A.N. & W.H. THOMAS. 1964. Concentrating plankton in gentle fashion. *Limnol. Oceanogr.* 9:455.

10200 H. Chlorophyll

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass.[1,2] All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of planktonic algae. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins, and carotenes. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides, and pheophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups.

The three methods for determining chlorophyll *a* in phytoplankton are the spectrophotometric,[3–5] the fluorometric,[6–8] and the high-performance liquid chromatographic (HPLC) techniques.[9] Fluorometry is more sensitive than spectrophotometry, requires less sample, and can be used for in-vivo measurements.[10] These optical methods can significantly under- or overestimate chlorophyll *a* concentrations,[11–18] in part because of the overlap of the absorption and fluorescence bands of co-occurring accessory pigments and chlorophyll degradation products.

Pheophorbide *a* and pheophytin *a*, two common degradation products of chlorophyll *a*, can interfere with the determination of chlorophyll *a* because they absorb light and fluoresce in the same region of the spectrum as does chlorophyll *a*. If these pheopigments are present, significant errors in chlorophyll *a* values will result. Pheopigments can be measured either by spectrophotometry or fluorometry, but in marine and freshwater environments the fluorometric method is unreliable when chlorophyll *b* co-occurs. Upon acidification of chlorophyll *b*, the resulting fluorescence emission of pheophytin *b* is coincident with that of pheophytin *a*, thus producing underestimation and overestimation of chlorophyll *a* and pheopigments, respectively.

HPLC is a useful method for quantifying photosynthetic pigments[9,13,15,16,19–21] including chlorophyll *a*, accessory pigments (e.g., chlorophylls *b* and *c*), and chlorophyll degradation products (chlorophyllides, pheophorbides, and pheophytins). Pigment distribution is useful for quantitative assessment of phytoplankton community composition and zooplankton grazing activity.[22]

1. Pigment Extraction

Conduct work with chlorophyll extracts in subdued light to avoid degradation. Use opaque containers or wrap with aluminum foil. The pigments are extracted from the plankton concentrate with aqueous acetone and the optical density (absorbance) of the

extract is determined with a spectrophotometer. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve consistently the complete extraction of the pigments, disrupt the cells mechanically with a tissue grinder.

Glass fiber filters are preferred for removing algae from water. The glass fibers assist in breaking the cells during grinding, larger volumes of water can be filtered, and no precipitate forms after acidification. Inert membrane filters such as polyester filters may be used where these factors are irrelevant.

a. Equipment and reagents:

1) *Tissue grinder:** Successfully macerating glass fiber filters in tissue grinders with grinding tube and pestle of conical design may be difficult. Preferably use round-bottom grinding tubes with a matching pestle having grooves in the TFE tip.

2) *Clinical centrifuge.*

3) *Centrifuge tubes,* 15-mL graduated, screw-cap.

4) *Filtration equipment,* filters, glass fiber† or membrane (0.45-μm porosity, 47-mm diam); vacuum pump; solvent-resistant disposable filter assembly, 1.0-μm pore size;‡ 10-mL solvent-resistant syringe.

5) *Saturated magnesium carbonate solution:* Add 1.0 g finely powdered $MgCO_3$ to 100 mL distilled water.

6) *Aqueous acetone solution:* Mix 90 parts acetone (reagent grade BP 56°C) with 10 parts saturated magnesium carbonate solution.

b. Extraction procedure:

1) Concentrate sample by centrifuging or filtering as soon as possible after collection. If processing must be delayed, hold samples on ice or at 4°C and protect from exposure to light. Use opaque bottles because even brief exposure to light during storage will alter chlorophyll values. Samples on filters taken from water having pH 7 or higher may be placed in airtight plastic bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation. Use glassware and cuvettes that are clean and acid-free.

2) Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, and macerate at 500 rpm for 1 min. Use TFE/glass grinder for a glass-fiber filter and glass/glass grinder for a membrane filter.

3) Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to a constant level, 5 to 10 mL, with 90% aqueous acetone. Use solvent sparingly and avoid excessive dilution of pigments. Steep samples at least 2 h at 4°C in the dark.

4) Clarify by filtering through a solvent-resistant disposable filter (to minimize retention of extract in filter and filter holder, force 1 to 2 mL air through the filter after the extract), or by centrifuging in closed tubes for 20 min at 500 *g*. Decant clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure total volume. Proceed as in 2, 3, or 4 below.

2. Spectrophotometric Determination of Chlorophyll

a. Equipment and reagents:

1) *Spectrophotometer,* with a narrow band (pass) width (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow. At a spectral band width of 20 nm the chlorophyll *a* concentration may be underestimated by as much as 40%.

2) *Cuvettes,* with 1-, 4-, and 10-cm path lengths.

3) *Pipets,* 0.1- and 5.0-mL.

*Kontes Glass Co., Vineland, N.J. 08360: Glass/glass grinder, Model No. 8855: Glass/TFE grinder, Model 886000; or equivalent.

†Whatman GF/F (0.7 μm), GFB (1.0 μm), Gelman AE (1 μm),[23] or equivalent.

‡Gelman Acrodisc or equivalent.

4) *Hydrochloric acid.* HCl, 0.1*N*.

b. Determination of chlorophyll a *in the presence of pheophytin* a: Chlorophyll *a* may be overestimated by including pheopigments that absorb near the same wavelength as chlorophyll *a*. Addition of acid to chlorophyll *a* results in loss of the magnesium atom, converting it to pheophytin *a*. Acidify carefully to a final molarity of not more than $3 \times 10^{-3} M$ to prevent certain accessory pigments from changing to absorb at the same wavelength as pheophytin *a*.[13] When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption-peak-ratio (OD664/OD665) of 1.70 is used in correcting the apparent chlorophyll *a* concentration for pheophytin *a*.

Samples with an OD664 before/OD665 after acidification ratio ($664_b/665_a$) of 1.70 are considered to contain no pheophytin *a* and to be in excellent physiological condition. Solutions of pure pheophytin show no reduction in OD665 upon acidification and have a $664_b/665_a$ ratio of 1.0. Thus, mixtures of chlorophyll *a* and pheophytin *a* have absorption peak ratios ranging between 1.0 and 1.7. These ratios are based on the use of 90% acetone as solvent. Using 100% acetone as solvent results in a chlorophyll *a* before-to-after acidification ratio of about 2.0.[3]

Spectrophotometric procedure—Transfer 3 mL clarified extract to a 1-cm cuvette and read optical density (OD) at 750 and 664 nm. Acidify extract in the cuvette with 0.1 mL 0.1*N* HCl. Gently agitate the acidified extract and read OD at 750 and at 665 nm, 90 s after acidification. The volumes of extract and acid and the time after acidification are critical for accurate, consistent results.

The OD664 before acidification should be between 0.1 and 1.0. For very dilute extracts use cuvettes having a longer path length. If a larger cell is used, add a proportionately larger volume of acid. Correct OD obtained with larger cuvettes to 1 cm before making calculations.

Subtract the 750-nm OD value from the readings before (OD 664 nm) and after acidification (OD 665 nm).

Using the corrected values calculate chlorophyll *a* and pheophytin *a* per cubic meter as follows:

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = \frac{26.7\,(664_b - 665_a) \times V_1}{V_2 \times L}$$

$$\text{Pheophytin } a,\ \text{mg/m}^3 = \frac{26.7\,[1.7\,(665_a) - 664_b] \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, L,
V_2 = volume of sample, m^3,
L = light path length or width of cuvette, cm, and
664_b, 665_a = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.7 is the absorbance correction and equals $A \times K$

where:

A = absorbance coefficient for chlorophyll *a* at 664 nm = 11.0, and
K = ratio expressing correction for acidification,

$$= \frac{\left(\frac{664_b}{665_a}\right)_{\text{pure chlorophyll a}}}{\left(\frac{664_b}{665_a}\right)_{\text{pure chlorophyll a}} - \left(\frac{664_b}{665_a}\right)_{\text{pure pheophytin a}}}$$

$$= \frac{1.7}{1.7 - 1.0} = 2.43$$

c. Determination of chlorophyll a, b, *and* c *(trichromatic method):*

Spectrophotometric procedure—Transfer extract to a 1-cm cuvette and measure optical density (OD) at 750, 664, 647, and 630 nm. Choose a cell path length or di-

lution to give an OD664 between 0.1 and 1.0.

Use the optical density readings at 664, 647, and 630 nm to determine chlorophyll *a*, *b*, and *c*, respectively. The OD reading at 750 nm is a correction for turbidity. Subtract this reading from each of the pigment OD values of the other wavelengths before using them in the equations below. Because the OD of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, adhere closely to the 90 parts acetone:10 parts water (v/v) formula for pigment extraction. Turbidity can be removed easily by filtration through a disposable, solvent-resistant filter attached to a syringe or by centrifuging for 20 min at 500 *g*.

Calculate the concentrations of chlorophyll *a*, *b*, and *c* in the extract by inserting the corrected optical densities in following equations:[5]

a) $C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$

b) $C_b = 21.03(OD647) - 5.43(OD664) - 2.66(OD630)$

c) $C_c = 24.52(OD630) - 7.60(OD647) - 1.67(OD664)$

where:

C_a, C_b, and C_c = concentrations of chlorophyll *a*, *b*, and *c*, respectively, mg/L, and

OD664, OD647, and OD630 = corrected optical densities (with a 1-cm light path) at the respective wavelengths.

After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = \frac{C_a \times \text{extract volume, L}}{\text{Volume of sample, m}^3}$$

3. Fluorometric Determination of Chlorophyll *a*

The fluorometric method for chlorophyll *a* is more sensitive than the spectrophotometric method and thus smaller samples can be used. Calibrate the fluorometer spectrophotometrically with a sample from the same source to achieve acceptable results. Optimum sensitivity for chlorophyll *a* extract measurements is obtained at an excitation wavelength of 430 nm and an emission wavelength of 663 nm. A method for continuous measurement of chlorophyll *a* in vivo is available, but is reported to be less efficient than the in-vitro method given here, yielding about one-tenth as much fluorescence per unit weight as the same amount in solution. Pheophytin *a* also can be determined fluorometrically.[24]

a. Equipment and reagents: In addition to those listed under 1*a* and 2*a* above:

Fluorometer,§ equipped with a high-intensity F4T.5 blue lamp, photomultiplier tube R-446 (red-sensitive), sliding window orifices 1×, 3×, 10×, and 30×, and filters for light emission (CS-2-64) and excitation (CS-5-60). A high-sensitivity door is preferable.

b. Extraction procedure: Prepare sample as directed in 1*b* above.

1) Calibrate fluorometer with a chlorophyll solution of known concentration as follows: Prepare chlorophyll extract and analyze spectrophotometrically. Prepare serial dilutions of the extract to provide concentrations of approximately 2, 6, 20, and 60 µg chlorophyll *a*/L. Make fluorometric readings for each solution at each sensitivity setting (sliding window orifice): 1×, 3×, 10×, and 30×. Using the values obtained, derive calibration factors to convert fluorometric readings in each sensitivity level to concentrations of chlorophyll *a*, as follows:

§Model 111, Sequoia-Turner Corp., 755 Ravendale Dr., Mountain View, Calif. or equivalent.

$$F_s = \frac{C_a'}{R_s}$$

where:

F_s = calibration factor for sensitivity setting S,
R_s = fluorometer reading for sensitivity setting S, and,
C_a' = concentration of chlorophyll a determined spectrophotometrically, μg/L.

2) Measure sample fluorescence at sensitivity settings that will provide a midscale reading. (Avoid using the 1× window because of quenching effects.) Convert fluorescence readings to concentrations of chlorophyll *a* by multiplying the readings by the appropriate calibration factor.

c. Determination of chlorophyll a *in the presence of pheophytin* a: This method normally is not applicable to freshwater samples. See discussion under 10200G and 1*c* above.

1) Equipment and reagents—In addition to those listed under 1*a* and 2*a* above, pure chlorophyll *a*‖ (or a plankton chlorophyll extract with a spectrophotometric before-and-after acidification ratio of 1.70 containing no chlorophyll *b*).

2) Fluorometric procedure—Calibrate fluorometer as directed in ¶ 3*b*1). Determine extract fluorescence at each sensitivity setting before and after acidification. Calculate calibration factors (F_s) and before-and-after acidification fluorescence ratio by dividing fluorescence reading obtained before acidification by the reading obtained after acidification. Avoid readings on the 1× scale and those outside the range of 20 to 80 fluorometric units.

3) Calculations—Determine the "corrected" chlorophyll *a* and pheophytin *a* in sample extracts with the following equations:[8,24]

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = F_s \frac{r}{r-1}(R_b - R_a)$$

$$\text{Pheophytin } a,\ \text{mg/m}^3 = F_s \frac{r}{r-1}(rR_a - R_b)$$

where:

F_s = conversion factor for sensitivity setting S (see ¶ 2*b*. above),
R_b = fluorescence of extract before acidification,
R_a = fluorescence of extract after acidification, and
r = R_b/R_a, as determined with pure chlorophyll *a* for the instrument. Redetermine r if filters or light source are changed.

d. Extraction of whole water, nonfiltered samples: Alternatively, to prevent cell lysis during filtration, extract whole water sample.

1) Equipment and reagents—Fluorometer equipped with a high-sensitivity R928 phototube⁼ with output impedance of 36 ma/W at 675 nm and a high-sensitivity door. Place neutral density filter (40-60N) in the rear light path**, selected to permit reagent blanking on the highest sensitivity scale.

2) Extraction procedure—Decant 1.5 mL sample into screw-cap test tube and add 8.5 mL 100% acetone. Mix with vortex mixer and hold in the dark for 6 h at room temperature. Filter through glass fiber filter†† or centrifuge. Measure fluorescence as described in Section 10200H.3 and estimate concentrations as in ¶ 3*c*. Because humic substances interfere, if they are present filter a sample portion (see 10200H. 1*b*) and process filtrate with sample. Subtract filtrate (blank) fluorescence from that of sample.

‖ Purified chlorophyll *a*, Sigma Chemical Company, St. Louis, Mo., or equivalent.

⁼ Hammamatsu Corp., Middlesex, N.J., or equivalent.
** If using Model 10-005, Sequoia-Turner Corp., or equivalent.
†† Whatman GF/F or equivalent.

Appendix F

Appendix F

WEIGHT LOSS-ON-IGNITION ANALYSIS

1.0 Scope and Application

1.1 This method describes the procedure for the determination of weight loss via combustion of sediment or of non-filterable suspended solids retained by a glass fiber filter. This procedure will be performed by Lamont Doherty Geological Observatory.

2.0 Method Summary

2.1 A small (0.2 to 0.5 gm) amount of sediment or a glass fiber filter containing suspended solids weighed after drying is heated to 375° C in air for 14 to 16 hours to remove all organic material and reweighed.

2.2 Method interferences may be caused by loss of water by dehydroxylation of clays in the sediments if the heating temperature is brought to 500° C.

3.0 Apparatus and Materials

3.1 Precision Balance - The balance must be capable of weighing to 0.1 mg for samples weighing several grams.

3.2 Autoclave or Muffle Furnace - This unit must be capable of controlling the combustion temperature to $\pm$ 10° C at 375° C.

3.3 For suspended solids, the filter must consist of glass fiber. Membrane filters are not acceptable since they will combust.

4.0 Reagents

4.1 Internal laboratory standard (LCS) of Hudson River sediment, containing about 5% organic materials (5% loss on ignition).

5.0 Initial Calibration

5.1 Balances must be calibrated daily using "S" class weights. The balance calibration is checked using the internal standard at least once per day. The balance is allowed to return to zero between each sample weighing.

5.2 The internal laboratory standard (LCS) is run once for each group of 20 samples or sample delivery group, whichever is more frequent.

6.0 Methodology

6.1 Sediments

6.1.1 A sediment sample of about 0.2 to 0.5 gm is dried to a constant weight in an incubator oven at 110° C for approximately 2 hours. Historical data show that 2 hr is sufficient to provide a constant weight (i.e. successive weighings meet a relative percent different (RPD) of <0.5%).

6.1.2 Sample is placed in a muffle furnace or autoclave at 375°C for 14 to 16 hours.

6.1.3 Sample is allowed to cool in a drying cabinet and then reweighed.

6.2 Filter Sample

6.2.1 The mass of suspended matter on the filter must be previously determined before determining weight loss-on-ignition. This is accomplished by using a pre-fired (500° C) pre-weighed glass fiber filter, filtering the water sample, and drying to a constant weight (following step 6.1.1 above). The mass of suspended matter is the difference between the preweighed filter weight and the dried filter weight.

6.2.2 Follow steps 6.1.2 and 6.1.3 as defined for sediments.

7.0 Calculations

7.1 Sediments

7.1.1 Calculate the relative percent difference (RPD) for the dried sample as:

$$RPD = \frac{Wt\ 1 - Wt\ 2}{\left(\frac{Wt\ 1 + Wt\ 2}{2}\right)} \times 100\%$$

where: Wt 1 and Wt 2 are the sample weights at two successive weighings separated by at least 4 hours. These weighings are taken prior to baking at 375° C.

7.1.2 Calculate the weight loss on ignition (LOI) as follows:

$$LOI = \frac{Wt\ 2 - Wt\ 3}{Wt\ 2} \times 100\%$$

where: Wt 3 is the weight of the sample after heating for 14 to 16 hours at 375°. Wt 2 is the last weight of the sample taken prior to heating at 375° C.

7.1.3 Calculate the RPD for sample duplicates and laboratory standards as follows:

$$RPD = \frac{LOI\ 1 - LOI\ 2}{\left(\frac{LOI\ 1 + LOI\ 2}{2}\right)} \times 100\%$$

where: LOI 1 = the loss on ignition calculated for the first sample analysis or, in the case of the laboratory standard, the established loss on ignition value.

LOI 2 = the loss on ignition calculated for the duplicate sample or standard analysis.

8.0 Precision and Accuracy

Precision criteria for a sediment sample is ≤1 mg/sample or ≤1% on a 1 gm sample containing ≥10% organic material. Accuracy criteria of the recovery of the internal laboratory standard (LCS) is 90-110% recovery.

9.0 Quality Control

9.1 Method Blank - A method blank is analyzed with each group of 20 samples or sample delivery group (SDG), whichever is more frequent. The method blank consists of a previously fired sand or sediment sample. The % LOI must be ≤1%.

9.2 The laboratory control standard (LCS) is equivalent to an initial calibration check (ICV) for this analysis, and is run with each sample batch must be compared to a previously established weight loss on ignition value for the standard. The criteria for the % recovery is 90-110%.

9.3 If either of these criteria are exceeded the entire sample batch must be reanalyzed from new sample material. For filter samples, reanalysis cannot be performed as the entire sample is used in the preparation. Problems should be reported in the data package narrative and to the Project Quality Assurance Officer.

9.4 Sample Duplicates - One laboratory duplicate analysis will be run for at least every 20 samples or SDG, whichever is more frequent. Duplicates must meet an RPD of 20%. If this criteria is not met a third sample portion should be determined, where possible. All three values must be reported.

Appendix G

Appendix G

TOTAL CARBON AND TOTAL NITROGEN ANALYSIS OF RIVER SEDIMENT

1.0 Scope and Application

1.1 This procedure determines the total carbon content and total nitrogen content of a small (<0.5 g) sediment sample. The procedure is based on Verardo, et. al., 1990. The procedure measure the combined masses of the inorganic and organic forms of carbon and nitrogen.

2.0 Summary of Method

2.1 A measured mass of dried sediment, approximately 5 - 10 mg, is combusted at 1050° C. The resulting gases pass over a solid catalyst (chromium trioxide (Cr_2O_3) on a silvered cobalt oxide base (Co_3O_4+Ag)). The resulting gases pass over a second catalyst (metallic copper) to convert nitrogen oxides to N_2. The exhaust stream then passes through a column containing magnesium perchlorate to remove water vapor. The cleaned gas stream is then analyzed via gas chromatography for CO_2 and N_2 using a thermal conductivity detector.

2.2 The instrument used is a modified Carlo Erba NA-1500 Analyzer.

2.3 No method interferences are known.

3.0 Apparatus and Materials

3.1 Carlo Erba NA-1500 Analyzer - modified for this method.

3.2 50-unit sample delivery carousel

3.3 Catalytic column containing a porous layer of chromium trioxide (Cr_2O_3) overlaying silvered cobalt oxide.

3.4 Catalytic column containing metallic copper.

3.5 Absorption column containing magnesium perchlorate.

3.6 Aluminum sample cups.

4.0 Reagents

4.1 Calibration Standard: $CH_3CON\ (CH_3)C_6H_4OH$, a derivative of Acetanilide, an NBS standard (no 141C) with a C/N weight ratio of 7.71 and a C/N molar ratio of 9. This standard is weighed out into 3 or 4 standard weight aliquots to completely encompass the anticipated range of sample C and N levels.

5.0 Initial Calibration

5.1 The measurement system is calibrated once per 50-sample carousel run. Each calibration consists of four measured amounts of the NBS standard containing approximately 35, 100, 150 and 200 ug of carbon and a standard blank (empty sample cup). Since the instrument measures total carbon and total nitrogen mass generated, the standardization consists of measurements of known amounts of carbon and nitrogen.

5.2 The initial calibration is followed by a method blank (MB), consisting of an empty sample cup.

5.3 The method blank is followed by analysis of a primary laboratory standard (acetanilide) at a total carbon level comparable to that anticipated for the samples. This is the laboratory control standard (LCS) or the initial calibration sample (ICV). A second laboratory standard is also analyzed, consisting of a recent Hudson River sediment.

5.4 The remaining carousel slots are filled with about 37 samples including two duplicates, two additional blanks and 2 additional primary/secondary laboratory standard pairs.

The trio of primary laboratory standard, secondary laboratory standard and method blank analyses will be done after every 18 to 19 sample analyses.

6.0 Sample Preparation

6.1 All sediment samples will be dried in an incubator under a controlled atmosphere. ("Controlled atmosphere" is defined in Appendix L - Radionuclides.) After drying, the sediments are ground with a mortar and pestle to remove all lumps and minimize sample heterogeneity. The anticipated sample size is 5 to 10 mg, although this size may be adjusted as necessary to meet detection limit and precision goals.

7.0 Calculations

7.1 The results of the initial calibration, including the 4 standards and blank, are used to prepare a calibration curve. A first or second order regression equation may be used to fit the data. No higher order of regression is allowed. The correlation coefficient for the fit must be ≥ 0.995. A plot of the calibration curve must be supplied with the sample results.

7.2 Repeat procedure 7.1 for the nitrogen results.

7.3 Calculate the mass of carbon and the mass of nitrogen for each of the samples, internal laboratory control checks and blanks using the established initial calibration curve for that run.

7.4 Calculate the concentration of carbon in a sample as follows:

$$\textit{Carbon Concentration (\%)} = \frac{\textit{mass of carbon generated (ug)}}{\textit{mass of sample (mg)}} \times \frac{1\ mg}{1000\ ug} \times 100\%$$

7.5 Similarly calculate the concentration of nitrogen in a sample:

$$\textit{Nitrogen Concentration (\%)} = \frac{\textit{mass of nitrogen generated (ug)}}{\textit{mass of sample (mg)}} \times \frac{1\ mg}{1000\ ug} \times 100\%$$

7.6 Calculate carbon to nitrogen weight ratio for each sample, internal standard and blank as follows:

$$C/N\ (wt) = \frac{Carbon\ Concentration\ (\%)}{Nitrogen\ Concentration\ (\%)}$$

7.7 Calculate the carbon to nitrogen moles ratio for each sample, internal standard and blank as follows:

$$C/N\ (Mole) = \frac{\left(\frac{Carbon\ Concentration\ (\%)}{12}\right)}{\left(\frac{Nitrogen\ Concentration\ (\%)}{14}\right)}$$

7.8 Calculate the relative percent difference (RPD) for all duplicate pairs for the nitrogen concentration, carbon concentration, and the C/N ratio as follows:

$$RPD = \frac{Sample\ Level - Sample\ Duplicate\ Level}{\left(\frac{Sample\ Level + Sample\ Duplicate\ Level}{2}\right)} \times 100\%$$

8.0 Precision and Accuracy

8.1. The precision of the carbon and nitrogen determination is largely dependent on the precision of the sample weighing. The criteria for precision is $\leq 10\%$ on laboratory duplicate pairs.

8.2 The minimum detection limits for carbon and nitrogen are 0.01% and 0.001% by weight respectively.

8.3 The criteria for accuracy of the LCS measurement is 90-100% recovery for the NBS standard.

9.0 Quality Control

9.1 Method Blank

9.1.1 A method blank, consisting of an empty sample cup, will be analyzed at the frequency of one for every 20 samples or sample delivery group (SDG), whichever is more frequent. Three blanks are included for each 50 slot carousel run.

9.1.3 Method blanks must not exceed the minimum detection limits for carbon and nitrogen and will be reported in the final data report.

9.1.4 If a laboratory method blank exceeds these criteria, the laboratory must consider the analytical system to be out of control. The source of the contamination must be investigated and appropriate corrective measures must be taken and documented before further sample analysis proceeds. All samples processed with a method blank that is out of control (i.e., contaminated) must be reanalyzed. The Laboratory Manager, or his designee, must address problems and solutions in the Case Narrative, which will accompany the data report.

9.1.5 The laboratory must report all sample concentration data as uncorrected for blanks contamination.

9.2 Laboratory Standards

9.2.1 All internal laboratory standards (LCS) must recover within 90-110% for carbon concentration, nitrogen concentration or the C/N ratios. If any laboratory standard exceeds criteria, all samples analyzed since the last time the criteria was met must be reanalyzed.

10.0 References

10.1 Verardo, D.J., P.N. Froelich, and A. McIntyre. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer, *Deep Sea Research* 37, 157-165, (Instruments and Methods Section), 1990.

Appendix H

Appendix H

TOTAL INORGANIC CARBON ANALYSIS IN HUDSON RIVER SEDIMENTS

1.0 Scope and Application

This procedure determines the total inorganic carbon content of a small (<100 mg) sediment sample. The procedure is applicable to sediments containing 0.01 to 10% carbon in an inorganic form. This procedure will be performed by Lamont Doherty Geological Observatory.

2.0 Summary of Method

An aliquot of dried sediment of about 50 mg is placed in a reaction vessel. The sample is then treated with an excess of concentrated acid and the resulting CO_2 is swept from the reaction chamber to a coulometer which determines the moles of CO_2 generated. From these results the amount of inorganic carbon in total μg can be calculated.

3.0 Apparatus and Materials

3.1 CO_2 - coulometer made by Coulometrics, Inc. with associated reaction chamber and electrolytic cell.

3.2 Electrolyte solution from Coulometrics, Inc. containing monorethanolamine.

3.3 Precision balance - with an accuracy of ±0.05 mg.

3.4 Aluminum reaction vessels.

4.0 Reagents

4.1 Concentrated hydrochloric (HCL) or sulfuric acid (H_2SO^4).

4.2 Carrier Gas - Nitrogen or air, CO_2-free.

4.3 Calibration Standard - Pure $CaCO_3$ as calcite weighed out in three increments designed to bracket the anticipated sample range.

5.0 Initial Calibration

5.1 The initial calibration will consist of a one-point calibration points derived from a standard weight of calcite plus a standard blank (empty reaction vessel).

5.2 After standardizing the instrument, analyze an initial calibration verification (ICV) standard. This ICV must be a separate source form the calibration standard and must recover within 90-110% of the true value.

6.0 Procedure

6.1 Place the weighed aliquot of sediment sample ($\leq$ 50 mg) in reaction vessel.

6.2 Purge reaction vessel with CO_2 - free carrier gas for approximately 2 minutes.

6.3 Add sufficient concentrated acid to ensure complete conversation of inorganic carbon to CO_2.

6.4 Record total µg of carbon as measured by the instrument.

7.0 Calculations

7.1 Calculate the sample value for total inorganic carbon using the initial calibration curve generated with the samples.

7.2 Calculate the relative percent difference (RPD) for duplicate pairs as follows:

$$RPD = \frac{Sample\ Level - Sample\ Duplicate\ Level}{\left(\frac{Sample\ Level + Sample\ Duplicate\ Level}{2}\right)} \times 100\%$$

7.3 Calculate % recovery of the initial calibration verification (ICV) and the continuing calibration verification (CCV) as follows:

$$\%\ Recovery = \frac{Observed\ Value}{True\ Standard\ Value} \times 100\%$$

8.0 Precision and Accuracy

8.1 The criteria for duplicate precision of the method is ≤10% RPD.

8.2 The minimum detection limit is anticipated to be 0.02% total inorganic carbon (200 mg/kg) on a 50 mg sample.

8.3 The criteria for accuracy of the ICV and CCVs are 90 to 110% recoveries.

9.0 Quality Control

9.1 Method Blank

9.1.1 Method blanks are generated using an empty reaction vessel. The method blank is then analyzed via the procedure given in Section 6.

9.1.2 The method blank should not exceed the detection limit given in Section 8. If this occurs, all samples analyzed since the last acceptable blank level was obtained must be reanalyzed.

9.1.3 Method blanks are analyzed for every 20 samples or each sample delivery group which ever is more frequent.

9.2 Initial Calibration

The calibration must be performed daily. If the ICV does not meet criteria, the instrument must be recalibrated and the ICV reanalyzed until criteria is met.

9.3 Continuing Calibration Verification (CCV)

A mid-range standard, CCV, will be analyzed after every 10 samples and at the end of the day's operation to check instrument drift. This standard must agree to within 10% of the true value. If this is not achieved, all samples analyzed since the last time the CCV was in control must be rerun. The laboratory must first re-establish control by recalibrating the instrument and rerunning the ICV as an initial check prior to continuing sample analyses.

Appendix I

NITROGEN, KJELDAHL, TOTAL

Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

STORET NO. 00625

1. Scope and Application
   1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogeneous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/1 TKN. The range may be extended with sample dilution.
2. Summary of Method
   2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.
3. Definitions
   3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.
   3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
   4.1 Samples may be preserved by addition of 2 ml of conc H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.
5. Apparatus
   5.1 Block Digestor–40
   5.2 Technicon Manifold for Ammonia (Figure 1)
   5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)
6. Reagents
   6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10 ml conc H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.
   6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled water and 200 ml of conc H_2SO_4. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Pending approval for NPDES
Issued 1978

6.3 Sulfuric Acid Solution (4%): Add 40 ml of conc. sulfuric acid to 800 ml of ammonia free distilled water, cool and dilute to 1 liter.

6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 ml of ammonia-free distilled water and dilute to 1 liter.

6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.

6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) in about 800 ml of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.

6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (6.5) to 200 ml of stock buffer solution (6.6) and mix. Add xx ml sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.

6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.

6.9 Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled water.

6.10 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml = 1.0 mg NH_3-N.

7. Procedure

Digestion

7.1 To 20 or 25 ml of sample, add 5 ml of digestion solution (6.2) and mix (use a vortex mixer).

7.2 Add (4–8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.

7.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

7.4 Cool sample and dilute to 25 ml with ammonia free water.

Colorimetric Analysis

7.5 Check the level of all reagent containers to ensure an adequate supply.

7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

7.7 Flush the Sampler IV wash receptacle with about 25 ml of 4.0% sulfuric acid (6.3).

7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

TABLE 1

CONCENTRATION RANGES
(NITROGEN)

No.	Dilution loops: Initial sample — Sample line	Dilution loops: Initial sample — Diluent line	Dilution loops: Resample — Resample line	Dilution loops: Resample — Diluent line	Approx. std. cal. setting	Range PPM N (±10%)	ml stock NaOH per liter working buffer solution
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0–2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0–10	80

7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

7.10 After a stable baseline has been obtained start the Sampler.

8. Calculations

8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/l, the precision was ±0.07, ±0.03 and ±0.15, respectively.

9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/l, the recoveries were 99 and 99%, respectively.

Bibliography

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of Total Kjeldahl Nitrogen in Estuarine Water", Technicon Symposia, pp. 362–367, Vol. 1, 1967.
2. Gales, M.E., and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.
3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May, 1974.
4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August, 1974.
5. Gales, M.E., and Booth, R.L., "Evaluation of the Block Digestion System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600/4–78–015, Environmental Monitoring and Support Laboratory, Cinncinnati, Ohio.

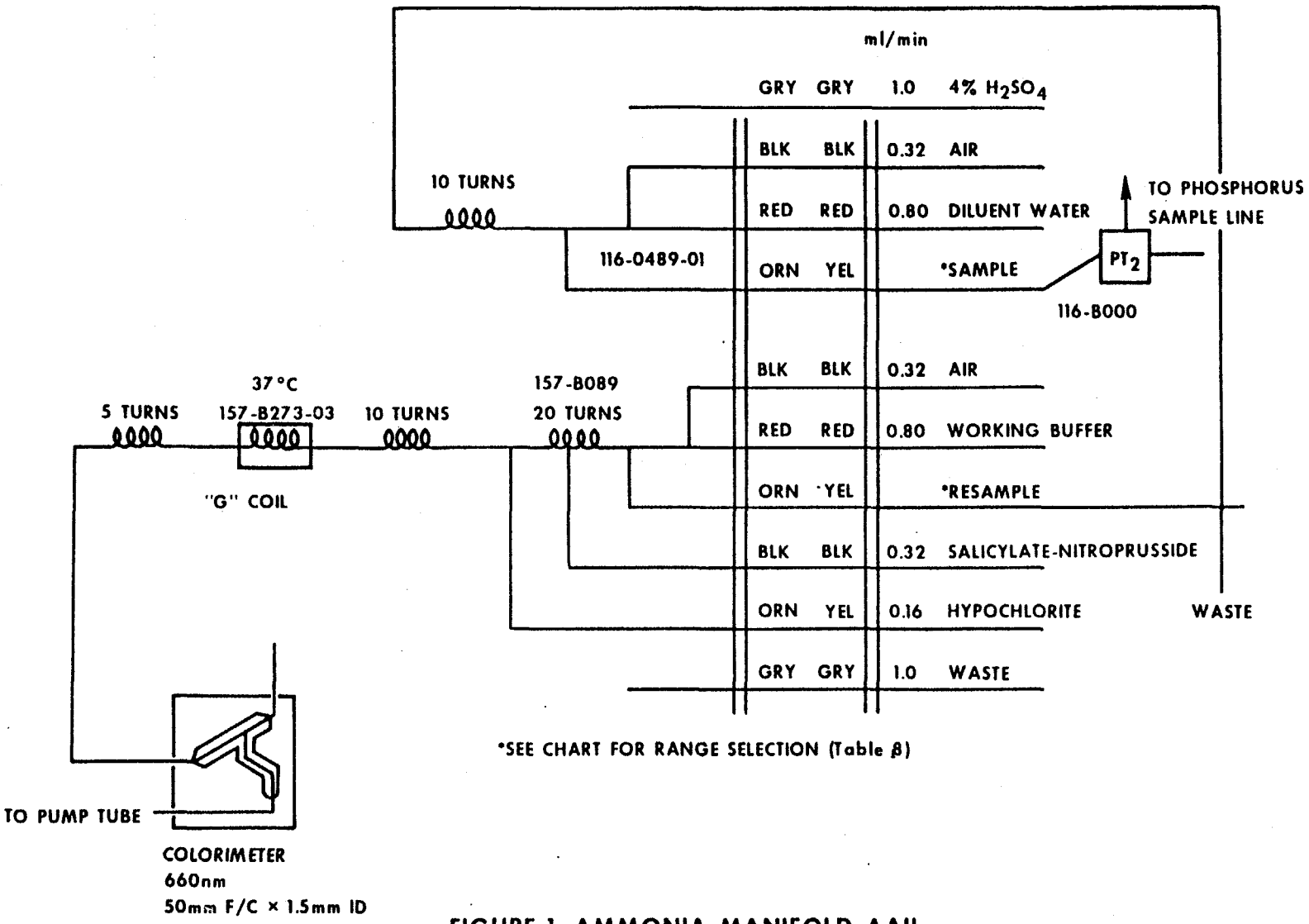


FIGURE 1. AMMONIA MANIFOLD AAII

NITROGEN, KJELDAHL, TOTAL

Method 351.3 (Colorimetric; Titrimetric; Potentiometric)

STORET NO. 00625

1. Scope and Application
   1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, surface and saline waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.
   1.2 Three alternatives are listed for the determination of ammonia after distillation: the titrimetric method which is applicable to concentrations above 1 mg N/liter; the Nesslerization method which is applicable to concentrations below 1 mg N/liter; and the potentiometric method applicable to the range 0.05 to 1400 mg/1.
   1.3 This method is described for macro and micro glassware systems.
2. Definitions
   2.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.
   2.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.
3. Summary of Method
   3.1 The sample is heated in the presence of conc. sulfuric acid, K_2SO_4 and $HgSO_4$ and evaporated until SO_3 fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by Nesslerization, titration or potentiometry.
4. Sample Handling and Preservation
   4.1 Samples may be preserved by addition of 2 ml of conc. H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Preserved samples should be analyzed as soon as possible.
5. Interference
   5.1 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. The reaction between nitrate and ammonia can be prevented by the use of an anion exchange resin (chloride form) to remove the nitrate prior to the TKN analysis.

Approved for NPDES
Issued 1971
Editorial revision 1974 and 1978

6. Apparatus

6.1 Digestion apparatus: A Kjeldahl digestion apparatus with 800 or 100 ml flasks and suction takeoff to remove SO_3 fumes and water.

6.2 Distillation apparatus: The macro Kjeldahl flask is connected to a condenser and an adaptor so that the distillate can be collected. Micro Kjeldahl steam distillation apparatus is commercially available.

6.3 Spectrophotometer for use at 400 to 425 nm with a light path of 1 cm or longer.

7. Reagents

7.1 Distilled water should be free of ammonia. Such water is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

NOTE 1: All solutions must be made with ammonia-free water.

7.2 Mercuric sulfate solution: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10.0 ml conc. H_2SO_4 : 40 ml distilled water) and dilute to 100 ml with distilled water.

7.3 Sulfuric acid-mercuric sulfate-potassium sulfate solution: Dissolve 267 g K_2SO_4 in 1300 ml distilled water and 400 ml conc. H_2SO_4. Add 50 ml mercuric sulfate solution (7.2) and dilute to 2 liters with distilled water.

7.4 Sodium hydroxide-sodium thiosulfate solution: Dissolve 500 g NaOH and 25 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 liter.

7.5 Mixed indicator: Mix 2 volumes of 0.2% methyl red in 95% ethanol with 1 volume of 0.2% methylene blue in ethanol. Prepare fresh every 30 days.

7.6 Boric acid solution: Dissolve 20 g boric acid, H_3BO_3, in water and dilute to 1 liter with distilled water.

7.7 Sulfuric acid, standard solution: (0.02 N) 1 ml = 0.28 mg NH_3–N. Prepare a stock solution of approximately 0.1 N acid by diluting 3 ml of conc. H_2SO_4 (sp. gr. 1.84) to 1 liter with CO_2-free distilled water. Dilute 200 ml of this solution to 1 liter with CO_2-free distilled water. Standardize the approximately 0.02 N acid so prepared against 0.0200 N Na_2CO_3 solution. This last solution is prepared by dissolving 1.060 g anhydrous Na_2CO_3, oven-dried at 140°C, and diluting to 1 liter with CO_2-free distilled water.

NOTE 2: An alternate and perhaps preferable method is to standardize the approximately 0.1 N H_2SO_4 solution against a 0.100 N Na_2CO_3 solution. By proper dilution the 0.02 N acid can the be prepared.

7.8 Ammonium chloride, stock solution: 1.0 ml = 1.0 mg NH_3–N. Dissolve 3.819 g NH_4Cl in water and make up to 1 liter in a volumetric flask with distilled water.

7.9 Ammonium chloride, standard solution: 1.0 ml = 0.01 mg NH_3–N. Dilute 10.0 ml of the stock solution (7.8) with distilled water to 1 liter in a volumetric flask.

7.10 Nessler reagent: Dissolve 100 g of mercuric iodide and 70 g potassium iodide in a small volume of distilled water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 ml of distilled water. Dilute the mixture to 1 liter. The solution is stable for at least one year if stored in a pyrex bottle out of direct sunlight.

NOTE 3: Reagents 7.7, 7.8, 7.9, and 7.10 are identical to reagents 6.8, 6.2, 6.3, and 6.6 described under Nitrogen, Ammonia (Colorimetric; Titrimetric; Potentiometric-Distillation Procedure, Method 350.2).

8. Procedure

8.1 The distillation apparatus should be pre-steamed before use by distilling a 1:1 mixture of distilled water and sodium hydroxide-sodium thiosulfate solution (7.4) until the distillate is ammonia-free. This operation should be repeated each time the apparatus is out of service long enough to accumulate ammonia (usually 4 hours or more).

8.2 Macro Kjeldahl system

8.2.1 Place a measured sample or the residue from the distillation in the ammonia determination (for Organic Kjeldahl only) into an 800 ml Kjeldahl flask. The sample size can be determined from the following table:

Kjeldahl Nitrogen in Sample, mg/l	Sample Size ml
0–5	500
5–10	250
10–20	100
20–50	50.0
50–500	25.0

Dilute the sample, if required, to 500 ml with distilled water, and add 100 ml sulfuric acid-mercuric sulfate-potassium sulfate solution (7.3). Evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Continue heating for 30 additional minutes. Cool the residue and add 300 ml distilled water.

8.2.2 Make the digestate alkaline by careful addition of 100 ml of sodium hydroxide – thiosulfate solution (7.4) without mixing.

NOTE 5: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause heavier solution to underlay the aqueous sulfuric acid solution without loss of free-ammonia. Do not mix until the digestion flask has been connected to the distillation apparatus.

8.2.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (7.6) in the receiving flask.

8.2.4 Distill 300 ml at the rate of 6–10 ml/min., into 50 ml of 2% boric acid (7.6) contained in a 500 ml Erlenmeyer flask.

8.2.5 Dilute the distillate to 500 ml in the flask. These flasks should be marked at the 350 and the 500 ml volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks. For concentrations above 1 mg/l, the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/l.

8.3 Micro Kjeldahl system

8.3.1 Place 50.0 ml of sample or an aliquot diluted to 50 ml in a 100 ml Kjeldahl flask and add 10 ml sulfuric acid-mercuric sulfate-potassium sulfate solution (7.3). Evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Then digest for an additional 30 minutes. Cool the residue and add 30 ml distilled water.

8.3.2 Make the digestate alkaline by careful addition of 10 ml of sodium hydroxide-thiosulfate solution (7.4) without mixing. Do not mix until the digestion flask has been connected to the distillation apparatus.

8.3.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (7.6) in the receiving flask or 50 ml short-form Nessler tube.

8.3.4 Steam distill 30 ml at the rate of 6–10 ml/min., into 5 ml of 2% boric acid (7.6).

8.3.5 Dilute the distillate to 50 ml. For concentrations above 1 mg/l the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/l.

8.4 Determination of ammonia in distillate: Determine the ammonia content of the distillate titrimetrically, colorimetrically, or potentiometrically, as described below.

8.4.1 Titrimetric determination: Add 3 drops of the mixed indicator (7.5) to the distillate and titrate the ammonia with the 0.02 N H_2SO_4 (7.7), matching the endpoint against a blank containing the same volume of distilled water and H_3BO_3 (7.6) solution.

8.4.2 Colorimetric determination: Prepare a series of Nessler tube standards as follows:

ml of Standard 1.0 ml = 0.01 mg NH_3–N	mg NH_3–N/50.0 ml
0.0	0.0
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
5.0	0.050
8.0	0.080
10.0	0.10

Dilute each tube to 50 ml with ammonia free water, add 1 ml of Nessler Reagent (7.10) and mix. After 20 minutes read the absorbance at 425 nm against the blank. From the values obtained for the standards plot absorbance vs. mg NH_3-N for the standard curve. Develop color in the 50 ml diluted distillate in exactly the same manner and read mg NH_3-N from the standard curve.

8.4.3 Potentiometric determination: Consult the method entitled Nitrogen, Ammonia: Potentiometric, Ion Selective Electrode Method, (Method 350.3) in this manual.

8.4.4 It is not imperative that all standards be treated in the same manner as the samples. It is recommended that at least 2 standards (a high and low) be digested, distilled,

and compared to similar values on the curve to insure that the digestion-distillation technique is reliable. If treated standards do not agree with untreated standards the operator should find the cause of the apparent error before proceeding.

9. Calculation

9.1 If the titrimetric procedure is used, calculate Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{TKN, mg/l} = \frac{(A - B)N \times F \times 1{,}000}{S}$$

where:
A = milliliters of standard 0.020 N H_2SO_4 solution used in titrating sample.
B = milliliters of standard 0.020 N H_2SO_4 solution used in titrating blank.
N = normality of sulfuric acid solution.
F = milliequivalent weight of nitrogen (14 mg).
S = milliliters of sample digested.

If the sulfuric acid is exactly 0.02 N the formula is shortened to:

$$\text{TKN, mg/l} = \frac{(A - B) \times 280}{S}$$

9.2 If the Nessler procedure is used, calculate the Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{TKN, mg/l} = \frac{A \times 1{,}000}{D} \times \frac{B}{C}$$

where:
A = mg NH_3–N read from curve.
B = ml total distillate collected including the H_3BO_3.
C = ml distillate taken for Nesslerization.
D = ml of original sample taken.

9.3 Calculate Organic Kjeldahl Nitrogen in mg/l, as follows:
Organic Kjeldahl Nitrogen = TKN –(NH_3–N.)

9.4 Potentiometric determination: Calculate Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{TKN, mg/l} = \frac{B}{D} \times A$$

where:
A = mg NH_3–N/l from electrode method standard curve.
B = volume of diluted distillate in ml.
D = ml of original sample taken.

10. Precision

10.1 Thirty-one analysts in twenty laboratories analyzed natural water samples containing exact increments of organic nitrogen, with the following results:

Increment as Nitrogen, Kjeldahl mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Accuracy as Bias, mg N/liter
0.20	0.197	+15.54	+0.03
0.31	0.247	+ 5.45	+0.02
4.10	1.056	+ 1.03	+0.04
4.61	1.191	- 1.67	-0.08

(FWPCA Method Study 2, Nutrient Analyses)

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 437, Method 421 (1975).
2. Schlueter, Albert, "Nitrate Interference In Total Kjeldahl Nitrogen Determinations and Its Removal by Anion Exchange Resins", EPA Report 600/7–77–017.

Appendix I

TABLE 4500-N_{org}:I. PRECISION AND BIAS DATA FOR ORGANIC NITROGEN, MACRO-KJELDAHL PROCEDURE

			Relative Standard Deviation			Relative Error		
Sample	No. of Laboratories	Organic Nitrogen Concentration $\mu g/L$	Nessler Finish %	Titrimetric Finish %	Calculation of Total Kjeldahl N Minus NH_3-N %	Nessler Finish %	Titrimetric Finish %	Calculation of Total Kjeldahl N Minus NH_3-N %
1	26	200	94.8	—	—	55.0	—	—
	29	200	—	104.4	—	—	70.0	—
	15	200	—	—	68.8	—	—	70.0
2	26	800	52.1	—	—	12.5	—	—
	31	800	—	44.8	—	—	3.7	—
	16	800	—	—	52.6	—	—	8.7
3	26	1500	43.1	—	—	9.3	—	—
	30	1500	—	54.7	—	—	22.6	—
	16	1500	—	—	45.9	—	—	4.0

Kjeldahl determination of nitrogen: A critical study of digestion conditions. *Aust. J. Chem.* 7:55.

MORGAN, G.B., J.B. LACKEY & F.W. GILCREAS. 1957. Quantitative determination of organic nitrogen in water, sewage, and industrial wastes. *Anal. Chem.* 29:833.

BOLTZ, D.F., ed. 1978. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.

4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N_{org}.B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus* equipped with heating elements to accomodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 365 to 380°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater† (Figure 4500-N_{org}:1).

c. pH meter.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH_3.B.3) and Nitrogen (Organic) macro-kjeldahl (Section 4500-N_{org}.B.3) are required. Prepare all reagents and dilutions with ammonia-free water.

*Rotary kjeldahl digestion unit, Kontes, Model K551100, or equivalent.

†ASTM E-147 or equivalent.

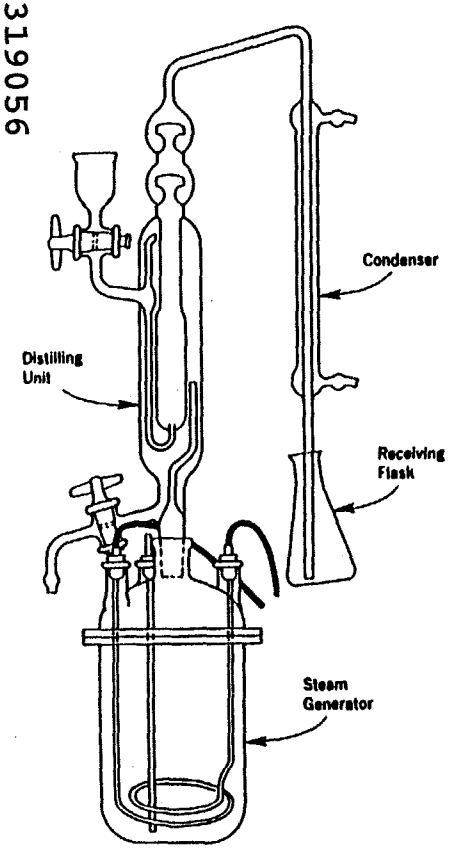


Figure 4500-N_{org}:1. Micro-kjeldahl distillation apparatus.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample *mg/L*	Sample Size *mL*
4–40	50
8–80	25
20–200	10
40–400	5

[r] For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add 3 mL borate buffer and adjust to pH 9.5 with 6*N* NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl flask and boil off 30 mL.

Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ *c* below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add 5 or 6 glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO_3. Continue to boil briskly until solution clears (becomes colorless or a pale straw color) and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL boric acid solution contained in a 125-mL erlenmeyer flask. Use plain boric acid solution when ammonia is to be determined by nesslerization and use indicating boric acid for a titrimetric finish. Use 10 mL 0.04*N* H_2SO_4 solution for collecting distillate for the phenate, nessler, or electrode methods. Extend tip of condenser well below level of boric acid solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with delivery tube and continue distillation during last 1 or 2 min to cleanse condenser.

e. Blank: Carry a reagent blank through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Determine ammonia by nesslerization, manual phenate, titration, or ammonia-selective electrode method.

5. Calculation

See Section 4500-NH_3.C.5, D.5, E.5, or F.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N_{org}.B.7.

Appendix J

Designation: D 421 – 85

Standard Practice for Dry Preparation of Soil Samples for Particle-Size Analysis and Determination of Soil Constants[1]

This standard is issued under the fixed designation D 421; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the dry preparation of soil samples as received from the field for particle-size analysis and the determination of the soil constants.

1.2 *This standard may involve hazardous materials, operations, and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of whoever uses this standard to consult and establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 2217 Practice for Wet Preparation of Soil Samples for Particle-Size Analysis and Determination of Soil Constants[2]

E 11 Specification for Wire-Cloth Sieves for Testing Purposes[3]

3. Significance and Use

3.1 This practice can be used to prepare samples for particle-size and plasticity tests where it is desired to determine test values on air-dried samples, or where it is known that air drying does not have an effect on test results relative to samples prepared in accordance with Practice D 2217.

4. Apparatus

4.1 *Balance*, sensitive to 0.1 g.

4.2 *Mortar and Rubber-Covered Pestle*, suitable for breaking up the aggregations of soil particles.

4.3 *Sieves*—A series of sieves, of square mesh woven wire cloth, conforming to Specification E 11. The sieves required are as follows:

No. 4 (4.75-mm)
No. 10 (2.00-mm)
No. 40 (425-μm)

4.4 *Sampler*—A riffle sampler or sample splitter, for quartering the samples.

5. Sampling

5.1 Expose the soil sample as received from the field to the air at room temperature until dried thoroughly. Break up the aggregations thoroughly in the mortar with a rubber-covered pestle. Select a representative sample of the amount required to perform the desired tests by the method of quartering or by the use of a sampler. The amounts of material required to perform the individual tests are as follows:

5.1.1 *Particle-Size Analysis*—For the particle-size analysis, material passing a No. 10 (2.00-mm) sieve is required in amounts equal to 115 g of sandy soils and 65 g of either silt or clay soils.

5.1.2 *Tests for Soil Constants*—For the tests for soil constants, material passing the No. 40 (425-μm) sieve is required in total amount of 220 g, allocated as follows:

Test	Grams
Liquid limit	100
Plastic limit	15
Centrifuge moisture equivalent	10
Volumetric shrinkage	30
Check tests	65

6. Preparation of Test Sample

6.1 Select that portion of the air-dried sample selected for purpose of tests and record the mass as the mass of the total test sample uncorrected for hygroscopic moisture. Separate the test sample by sieving with a No. 10 (2.00-mm) sieve. Grind that fraction retained on the No. 10 sieve in a mortar with a rubber-covered pestle until the aggregations of soil particles are broken up into the separate grains. Then separate the ground soil into two fractions by sieving with a No. 10 sieve.

6.2 Wash that fraction retained after the second sieving free of all fine material, dry, and weigh. Record this mass as the mass of coarse material. Sieve the coarse material, after being washed and dried, on the No. 4 (4.75-mm) sieve and record the mass retained on the No. 4 sieve.

7. Test Sample for Particle-Size Analysis

7.1 Thoroughly mix together the fractions passing the No. 10 (2.00-mm) sieve in both sieving operations, and by the method of quartering or the use of a sampler, select a portion weighing approximately 115 g for sandy soils and approximately 65 g for silt and clay soil for particle-size analysis.

8. Test Sample for Soil Constants

8.1 Separate the remaining portion of the material passing the No. 10 (2.00-mm) sieve into two parts by means of a No. 40 (425-μm) sieve. Discard the fraction retained on the No. 40 sieve. Use the fraction passing the No. 40 sieve for the determination of the soil constants.

---

[1] This practice is under the jurisdiction of ASTM Committee D-18 on Soil and Rock and is the direct responsibility of Subcommittee D18.03 on Texture, Plasticity, and Density Characteristics of Soils.

Current edition approved July 26, 1985. Published September 1985. Originally published as D 421 – 35 T. Last previous edition D 421 – 58 (1978)[ε1].

[2] *Annual Book of ASTM Standards*, Vol 04.08.

[3] *Annual Book of ASTM Standards*, Vol 14.02.

Designation: D 422 – 63 (Reapproved 1990)ε1

Standard Test Method for Particle-Size Analysis of Soils[1]

This standard is issued under the fixed designation D 422; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε1 NOTE—Section 19 was added editorially in September 1990.

1. Scope

1.1 This test method covers the quantitative determination of the distribution of particle sizes in soils. The distribution of particle sizes larger than 75 μm (retained on the No. 200 sieve) is determined by sieving, while the distribution of particle sizes smaller than 75 μm is determined by a sedimentation process, using a hydrometer to secure the necessary data (Notes 1 and 2).

NOTE 1—Separation may be made on the No. 4 (4.75-mm), No. 40 (425-μm), or No. 200 (75-μm) sieve instead of the No. 10. For whatever sieve used, the size shall be indicated in the report.

NOTE 2—Two types of dispersion devices are provided: (*1*) a high-speed mechanical stirrer, and (*2*) air dispersion. Extensive investigations indicate that air-dispersion devices produce a more positive dispersion of plastic soils below the 20-μm size and appreciably less degradation on all sizes when used with sandy soils. Because of the definite advantages favoring air dispersion, its use is recommended. The results from the two types of devices differ in magnitude, depending upon soil type, leading to marked differences in particle size distribution, especially for sizes finer than 20 μm.

2. Referenced Documents

2.1 *ASTM Standards:*

D 421 Practice for Dry Preparation of Soil Samples for Particle-Size Analysis and Determination of Soil Constants[2]

E 11 Specification for Wire-Cloth Sieves for Testing Purposes[3]

E 100 Specification for ASTM Hydrometers[4]

3. Apparatus

3.1 *Balances*—A balance sensitive to 0.01 g for weighing the material passing a No. 10 (2.00-mm) sieve, and a balance sensitive to 0.1 % of the mass of the sample to be weighed for weighing the material retained on a No. 10 sieve.

3.2 *Stirring Apparatus*—Either apparatus A or B may be used.

3.2.1 Apparatus A shall consist of a mechanically operated stirring device in which a suitably mounted electric motor turns a vertical shaft at a speed of not less than 10 000 rpm without load. The shaft shall be equipped with a replaceable stirring paddle made of metal, plastic, or hard rubber, as shown in Fig. 1. The shaft shall be of such length that the stirring paddle will operate not less than ¾ in. (19.0 mm) nor more than 1½ in. (38.1 mm) above the bottom of the dispersion cup. A special dispersion cup conforming to either of the designs shown in Fig. 2 shall be provided to hold the sample while it is being dispersed.

3.2.2 Apparatus B shall consist of an air-jet dispersion cup[5] (Note 3) conforming to the general details shown in Fig. 3 (Notes 4 and 5).

NOTE 3—The amount of air required by an air-jet dispersion cup is of the order of 2 ft^3/min; some small air compressors are not capable of supplying sufficient air to operate a cup.

NOTE 4—Another air-type dispersion device, known as a dispersion tube, developed by Chu and Davidson at Iowa State College, has been shown to give results equivalent to those secured by the air-jet dispersion cups. When it is used, soaking of the sample can be done in the sedimentation cylinder, thus eliminating the need for transferring the slurry. When the air-dispersion tube is used, it shall be so indicated in the report.

NOTE 5—Water may condense in air lines when not in use. This water must be removed, either by using a water trap on the air line, or by blowing the water out of the line before using any of the air for dispersion purposes.

3.3 *Hydrometer*—An ASTM hydrometer, graduated to read in either specific gravity of the suspension or grams per litre of suspension, and conforming to the requirements for hydrometers 151H or 152H in Specifications E 100. Dimensions of both hydrometers are the same, the scale being the only item of difference.

3.4 *Sedimentation Cylinder*—A glass cylinder essentially 18 in. (457 mm) in height and 2½ in. (63.5 mm) in diameter, and marked for a volume of 1000 mL. The inside diameter shall be such that the 1000-mL mark is 36 ± 2 cm from the bottom on the inside.

3.5 *Thermometer*—A thermometer accurate to 1°F (0.5°C).

3.6 *Sieves*—A series of sieves, of square-mesh woven-wire cloth, conforming to the requirements of Specification E 11. A full set of sieves includes the following (Note 6):

[1] This test method is under the jurisdiction of ASTM Committee D-18 on Soil and Rock and is the direct responsibility of Subcommittee D18.03 on Texture, Plasticity, and Density Characteristics of Soils.

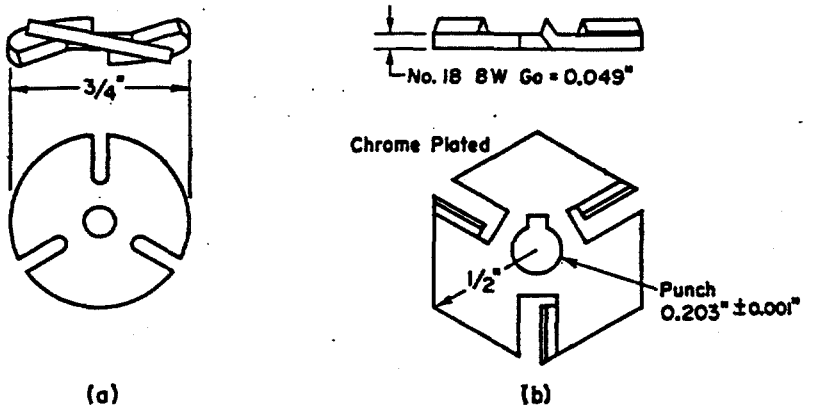
Current edition approved Nov. 21, 1963. Originally published 1935. Replaces D 422 – 62.

[2] *Annual Book of ASTM Standards*, Vol 04.08.

[3] *Annual Book of ASTM Standards*, Vol 14.02.

[4] *Annual Book of ASTM Standards*, Vol 14.03.

[5] Detailed working drawings for this cup are available at a nominal cost from the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103. Order Adjunct No. 12-404220-00.



Metric Equivalents					
in.	0.001	0.049	0.203	½	¾
mm	0.03	1.24	5.16	12.7	19.0

FIG. 1 Detail of Stirring Paddles

3-in. (75-mm)	No. 10 (2.00-mm)
2-in. (50-mm)	No. 20 (850-μm)
1½-in. (37.5-mm)	No. 40 (425-μm)
1-in. (25.0-mm)	No. 60 (250-μm)
¾-in. (19.0-mm)	No. 140 (106-μm)
⅜-in. (9.5-mm)	No. 200 (75-μm)
No. 4 (4.75-mm)	

NOTE 6—A set of sieves giving uniform spacing of points for the raph, as required in Section 17, may be used if desired. This set consists the following sieves:

3-in. (75-mm)	No. 16 (1.18-mm)
1½-in. (37.5-mm)	No. 30 (600-μm)
¾-in. (19.0-mm)	No. 50 (300-μm)
⅜-in. (9.5-mm)	No. 100 (150-μm)
No. 4 (4.75-mm)	No. 200 (75-μm)
No. 8 (2.36-mm)	

3.7 *Water Bath or Constant-Temperature Room*—A ater bath or constant-temperature room for maintaining e soil suspension at a constant temperature during the ydrometer analysis. A satisfactory water tank is an insulated nk that maintains the temperature of the suspension at a nvenient constant temperature at or near 68°F (20°C). ch a device is illustrated in Fig. 4. In cases where the work performed in a room at an automatically controlled nstant temperature, the water bath is not necessary.

3.8 *Beaker*—A beaker of 250-mL capacity.

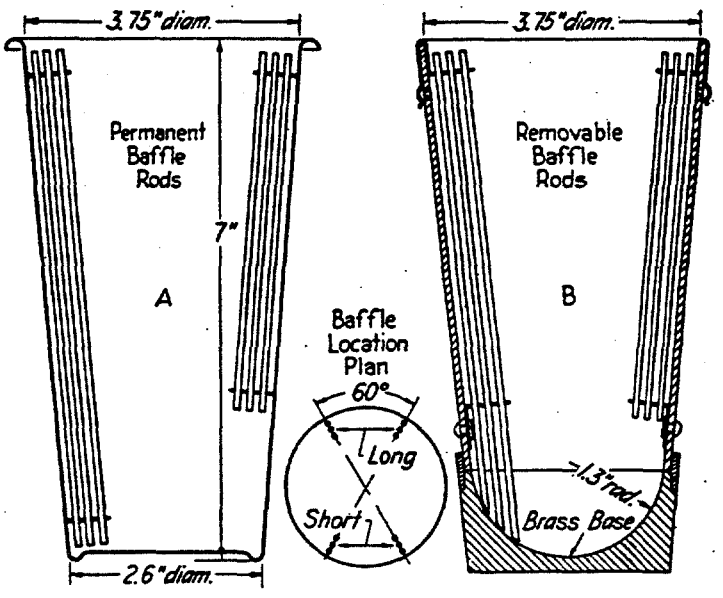
3.9 *Timing Device*—A watch or clock with a second and.

Dispersing Agent

4.1 A solution of sodium hexametaphosphate (sometimes lled sodium metaphosphate) shall be used in distilled or mineralized water, at the rate of 40 g of sodium xametaphosphate/litre of solution (Note 7).

NOTE 7—Solutions of this salt, if acidic, slowly revert or hydrolyze ck to the orthophosphate form with a resultant decrease in dispersive tion. Solutions should be prepared frequently (at least once a month) adjusted to pH of 8 or 9 by means of sodium carbonate. Bottles ining solutions should have the date of preparation marked on

4.2 All water used shall be either distilled or mineralized water. The water for a hydrometer test shall be brought to the temperature that is expected to prevail during the hydrometer test. For example, if the sedimentation cylinder is to be placed in the water bath, the distilled or demineralized water to be used shall be brought to the temperature of the controlled water bath; or, if the sedimentation cylinder is used in a room with controlled temperature, the water for the test shall be at the temperature of the room. The basic temperature for the hydrometer test is 68°F (20°C). Small variations of temperature do not introduce differences that are of practical significance and do not prevent the use of corrections derived as prescribed.



Metric Equivalents			
in.	1.3	2.6	3.75
mm	33	66	95.2

FIG. 2 Dispersion Cups of Apparatus

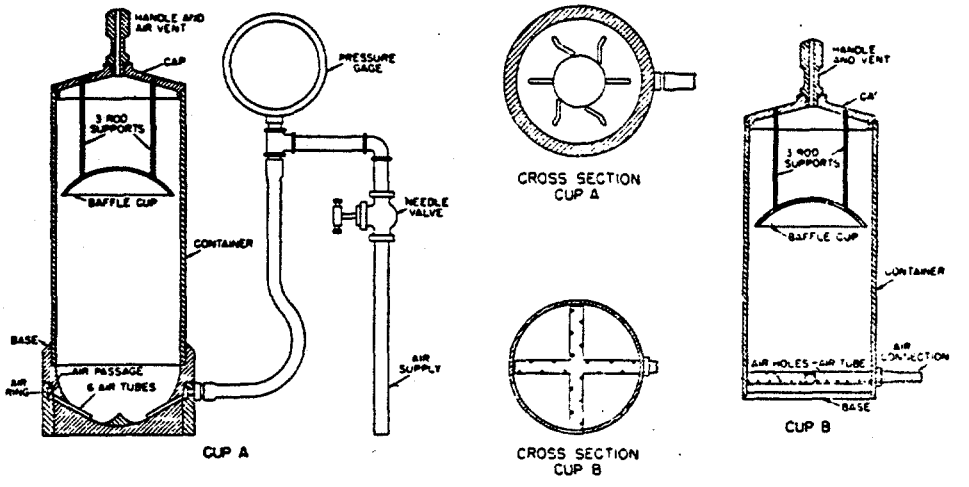


FIG. 3 Air-Jet Dispersion Cups of Apparatus B

5. Test Sample

5.1 Prepare the test sample for mechanical analysis as outlined in Practice D 421. During the preparation procedure the sample is divided into two portions. One portion contains only particles retained on the No. 10 (2.00-mm) sieve while the other portion contains only particles passing the No. 10 sieve. The mass of air-dried soil selected for purpose of tests, as prescribed in Practice D 421, shall be sufficient to yield quantities for mechanical analysis as follows:

5.1.1 The size of the portion retained on the No. 10 sieve shall depend on the maximum size of particle, according to the following schedule:

Nominal Diameter of Largest Particles, in. (mm)	Approximate Minimum Mass of Portion, g
⅜ (9.5)	500
¾ (19.0)	1000
1 (25.4)	2000
1½ (38.1)	3000
2 (50.8)	4000
3 (76.2)	5000

5.1.2 The size of the portion passing the No. 10 sieve shall be approximately 115 g for sandy soils and approximately 65 g for silt and clay soils.

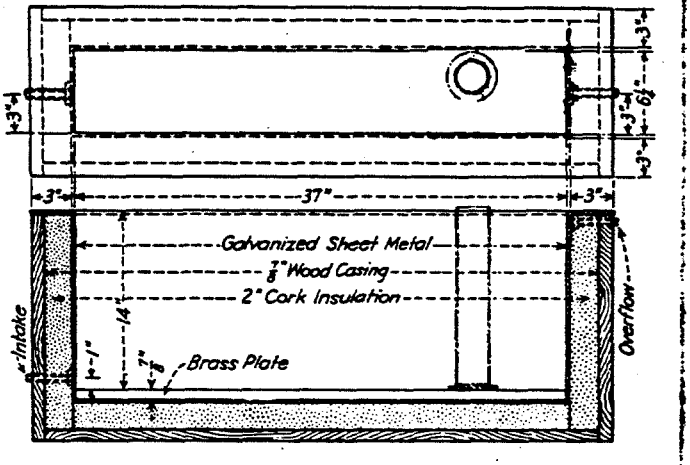
5.2 Provision is made in Section 5 of Practice D 421 for weighing of the air-dry soil selected for purpose of tests, the separation of the soil on the No. 10 sieve by dry-sieving and washing, and the weighing of the washed and dried fraction retained on the No. 10 sieve. From these two masses the percentages retained and passing the No. 10 sieve can be calculated in accordance with 12.1.

NOTE 8—A check on the mass values and the thoroughness of pulverization of the clods may be secured by weighing the portion passing the No. 10 sieve and adding this value to the mass of the washed and oven-dried portion retained on the No. 10 sieve.

SIEVE ANALYSIS OF PORTION RETAINED ON NO. 10 (2.00-mm) SIEVE

6. Procedure

6.1 Separate the portion retained on the No. 10 (2.00-mm) sieve into a series of fractions using the 3-in. (75-mm), 2-in. (50-mm), 1½-in. (37.5-mm), 1-in. (25.0-mm), ¾-in. (19.0-mm), ⅜-in. (9.5-mm), No. 4 (4.75-mm), and No. 10 sieves, or as many as may be needed depending on the sample, or upon the specifications for the material under test.



Metric Equivalents

in.	⅞	1	3	6¼	14	37
mm	22.2	25.4	76.2	158.2	356	940

FIG. 4 Insulated Water Bath

6.2 Conduct the sieving operation by means of a lateral and vertical motion of the sieve, accompanied by a jarring action in order to keep the sample moving continuously over the surface of the sieve. In no case turn or manipulate fragments in the sample through the sieve by hand. Continue sieving until not more than 1 mass % of the residue on a sieve passes that sieve during 1 min of sieving. When mechanical sieving is used, test the thoroughness of sieving by using the hand method of sieving as described above.

6.3 Determine the mass of each fraction on a balance conforming to the requirements of 3.1. At the end of weighing, the sum of the masses retained on all the sieves used should equal closely the original mass of the quantity sieved.

HYDROMETER AND SIEVE ANALYSIS OF PORTION PASSING THE NO. 10 (2.00-mm) SIEVE

. Determination of Composite Correction for Hydrometer Reading

7.1 Equations for percentages of soil remaining in suspen-on, as given in 14.3, are based on the use of distilled or :mineralized water. A dispersing agent is used in the water, however, and the specific gravity of the resulting liquid is appreciably greater than that of distilled or demineralized ater.

7.1.1 Both soil hydrometers are calibrated at 68°F (20°C), and variations in temperature from this standard tempera-ure produce inaccuracies in the actual hydrometer readings. he amount of the inaccuracy increases as the variation om the standard temperature increases.

7.1.2 Hydrometers are graduated by the manufacturer to : read at the bottom of the meniscus formed by the liquid n the stem. Since it is not possible to secure readings of soil suspensions at the bottom of the meniscus, readings must be taken at the top and a correction applied.

7.1.3 The net amount of the corrections for the three :ms enumerated is designated as the composite correction, and may be determined experimentally.

7.2 For convenience, a graph or table of composite rrections for a series of 1° temperature differences for the nge of expected test temperatures may be prepared and used as needed. Measurement of the composite corrections ay be made at two temperatures spanning the range of cted test temperatures, and corrections for the interme-: temperatures calculated assuming a straight-line rela-tionship between the two observed values.

7.3 Prepare 1000 mL of liquid composed of distilled or mineralized water and dispersing agent in the same proportion as will prevail in the sedimentation (hydrometer) test. Place the liquid in a sedimentation cyclinder and the linder in the constant-temperature water bath, set for one the two temperatures to be used. When the temperature of the liquid becomes constant, insert the hydrometer, and, er a short interval to permit the hydrometer to come to the nperature of the liquid, read the hydrometer at the top of e meniscus formed on the stem. For hydrometer 151H the composite correction is the difference between this reading d one; for hydrometer 152H it is the difference between : reading and zero. Bring the liquid and the hydrometer to me other temperature to be used, and secure the composite correction as before.

-. Hygroscopic Moisture

8.1 When the sample is weighed for the hydrometer test, igh out an auxiliary portion of from 10 to 15 g in a small :tal or glass container, dry the sample to a constant mass in an oven at 230 ± 9°F (110 ± 5°C), and weigh again. Record the masses.

-. Dispersion of Soil Sample

When the soil is mostly of the clay and silt sizes, weigh a sample of air-dry soil of approximately 50 g. When the l is mostly sand the sample should be approximately 100

9.2 Place the sample in the 250-mL beaker and cover with 125 mL of sodium hexametaphosphate solution (40 g/L). Stir until the soil is thoroughly wetted. Allow to soak for at least 16 h.

9.3 At the end of the soaking period, disperse the sample further, using either stirring apparatus A or B. If stirring apparatus A is used, transfer the soil - water slurry from the beaker into the special dispersion cup shown in Fig. 2, washing any residue from the beaker into the cup with distilled or demineralized water (Note 9). Add distilled or demineralized water, if necessary, so that the cup is more than half full. Stir for a period of 1 min.

NOTE 9—A large size syringe is a convenient device for handling the water in the washing operation. Other devices include the wash-water bottle and a hose with nozzle connected to a pressurized distilled water tank.

9.4 If stirring apparatus B (Fig. 3) is used, remove the cover cap and connect the cup to a compressed air supply by means of a rubber hose. A air gage must be on the line between the cup and the control valve. Open the control valve so that the gage indicates 1 psi (7 kPa) pressure (Note 10). Transfer the soil - water slurry from the beaker to the air-jet dispersion cup by washing with distilled or demineralized water. Add distilled or demineralized water, if necessary, so that the total volume in the cup is 250 mL, but no more.

NOTE 10—The initial air pressure of 1 psi is required to prevent the soil - water mixture from entering the air-jet chamber when the mixture is transferred to the dispersion cup.

9.5 Place the cover cap on the cup and open the air control valve until the gage pressure is 20 psi (140 kPa). Disperse the soil according to the following schedule:

Plasticity Index	Dispersion Period, min
Under 5	5
6 to 20	10
Over 20	15

Soils containing large percentages of mica need be dispersed for only 1 min. After the dispersion period, reduce the gage pressure to 1 psi preparatory to transfer of soil - water slurry to the sedimentation cylinder.

10. Hydrometer Test

10.1 Immediately after dispersion, transfer the soil - water slurry to the glass sedimentation cylinder, and add distilled or demineralized water until the total volume is 1000 mL.

10.2 Using the palm of the hand over the open end of the cylinder (or a rubber stopper in the open end), turn the cylinder upside down and back for a period of 1 min to complete the agitation of the slurry (Note 11). At the end of 1 min set the cylinder in a convenient location and take hydrometer readings at the following intervals of time (measured from the beginning of sedimentation), or as many as may be needed, depending on the sample or the specification for the material under test: 2, 5, 15, 30, 60, 250, and 1440 min. If the controlled water bath is used, the sedimentation cylinder should be placed in the bath between the 2- and 5-min readings.

NOTE 11—The number of turns during this minute should be approximately 60, counting the turn upside down and back as two turns.

Any soil remaining in the bottom of the cylinder during the first few turns should be loosened by vigorous shaking of the cylinder while it is in the inverted position.

10.3 When it is desired to take a hydrometer reading, carefully insert the hydrometer about 20 to 25 s before the reading is due to approximately the depth it will have when the reading is taken. As soon as the reading is taken, carefully remove the hydrometer and place it with a spinning motion in a graduate of clean distilled or demineralized water.

NOTE 12—It is important to remove the hydrometer immediately after each reading. Readings shall be taken at the top of the meniscus formed by the suspension around the stem, since it is not possible to secure readings at the bottom of the meniscus.

10.4 After each reading, take the temperature of the suspension by inserting the thermometer into the suspension.

11. Sieve Analysis

11.1 After taking the final hydrometer reading, transfer the suspension to a No. 200 (75-μm) sieve and wash with tap water until the wash water is clear. Transfer the material on the No. 200 sieve to a suitable container, dry in an oven at 230 ± 9°F (110 ± 5°C) and make a sieve analysis of the portion retained, using as many sieves as desired, or required for the material, or upon the specification of the material under test.

CALCULATIONS AND REPORT

12. Sieve Analysis Values for the Portion Coarser than the No. 10 (2.00-mm) Sieve

12.1 Calculate the percentage passing the No. 10 sieve by dividing the mass passing the No. 10 sieve by the mass of soil originally split on the No. 10 sieve, and multiplying the result by 100. To obtain the mass passing the No. 10 sieve, subtract the mass retained on the No. 10 sieve from the original mass.

12.2 To secure the total mass of soil passing the No. 4 (4.75-mm) sieve, add to the mass of the material passing the No. 10 sieve the mass of the fraction passing the No. 4 sieve and retained on the No. 10 sieve. To secure the total mass of soil passing the ⅜-in. (9.5-mm) sieve, add to the total mass of soil passing the No. 4 sieve, the mass of the fraction passing the ⅜-in. sieve and retained on the No. 4 sieve. For the remaining sieves, continue the calculations in the same manner.

12.3 To determine the total percentage passing for each sieve, divide the total mass passing (see 12.2) by the total mass of sample and multiply the result by 100.

13. Hygroscopic Moisture Correction Factor

13.1 The hydroscopic moisture correction factor is the ratio between the mass of the oven-dried sample and the air-dry mass before drying. It is a number less than one, except when there is no hygroscopic moisture.

14. Percentages of Soil in Suspension

14.1 Calculate the oven-dry mass of soil used in the hydrometer analysis by multiplying the air-dry mass by the hygroscopic moisture correction factor.

14.2 Calculate the mass of a total sample represented by the mass of soil used in the hydrometer test, by dividing the oven-dry mass used by the percentage passing the No. 10 (2.00-mm) sieve, and multiplying the result by 100. This value is the weight W in the equation for percentage remaining in suspension.

TABLE 1 Values of Correction Factor, a, for Different Specific Gravities of Soil Particles[A]

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

[A] For use in equation for percentage of soil remaining in suspension when using Hydrometer 152H.

14.3 The percentage of soil remaining in suspension at the level at which the hydrometer is measuring the density of the suspension may be calculated as follows (Note 13): For hydrometer 151H:

$$P = [(100\,000/W) \times G/(G - G_1)](R - G_1)$$

NOTE 13—The bracketed portion of the equation for hydrometer 151H is constant for a series of readings and may be calculated first and then multiplied by the portion in the parentheses.

For hydrometer 152H:

$$P = (Ra/W) \times 100$$

where:

a = correction faction to be applied to the reading of hydrometer 152H. (Values shown on the scale are computed using a specific gravity of 2.65. Correction factors are given in Table 1),

P = percentage of soil remaining in suspension at the level at which the hydrometer measures the density of the suspension,

R = hydrometer reading with composite correction applied (Section 7),

W = oven-dry mass of soil in a total test sample represented by mass of soil dispersed (see 14.2), g,

G = specific gravity of the soil particles, and

G_1 = specific gravity of the liquid in which soil particles are suspended. Use numerical value of one in both instances in the equation. In the first instance any possible variation produces no significant effect, and in the second instance, the composite correction for R is based on a value of one for G_1.

15. Diameter of Soil Particles

15.1 The diameter of a particle corresponding to the percentage indicated by a given hydrometer reading shall be calculated according to Stokes' law (Note 14), on the basis that a particle of this diameter was at the surface of the suspension at the beginning of sedimentation and had settled to the level at which the hydrometer is measuring the density of the suspension. According to Stokes' law:

$$D = \sqrt{[30n/980(G - G_1)] \times L/T}$$

where:

D = diameter of particle, mm,

n = coefficient of viscosity of the suspending medium (in this case water) in poises (varies with changes in temperature of the suspending medium),
= distance from the surface of the suspension to the level at which the density of the suspension is being measured, cm. (For a given hydrometer and sedimentation cylinder, values vary according to the hydrometer readings. This distance is known as effective depth (Table 2)),
T = interval of time from beginning of sedimentation to the taking of the reading, min,
G = specific gravity of soil particles, and
G_1 = specific gravity (relative density) of suspending medium (value may be used as 1.000 for all practical purposes).

NOTE 14—Since Stokes' law considers the terminal velocity of a single sphere falling in an infinity of liquid, the sizes calculated represent the diameter of spheres that would fall at the same rate as the soil particles.

15.2 For convenience in calculations the above equation may be written as follows:

$$D = K\sqrt{L/T}$$

where:

K = constant depending on the temperature of the suspension and the specific gravity of the soil particles. Values of K for a range of temperatures and specific gravities are given in Table 3. The value of K does not change for a series of readings constituting a test, while values of L and T do vary.

15.3 Values of D may be computed with sufficient accuracy, using an ordinary 10-in. slide rule.

NOTE 15—The value of L is divided by T using the A- and B-scales, the square root being indicated on the D-scale. Without ascertaining the value of the square root it may be multiplied by K, using either the C- or CI-scale.

16. Sieve Analysis Values for Portion Finer than No. 10 (2.00-mm) Sieve

16.1 Calculation of percentages passing the various sieves used in sieving the portion of the sample from the hydrometer test involves several steps. The first step is to calculate the mass of the fraction that would have been retained on the No. 10 sieve had it not been removed. This mass is equal to the total percentage retained on the No. 10 sieve (100 minus total percentage passing) times the mass of the total sample represented by the mass of soil used (as calculated in 14.2), and the result divided by 100.

16.2 Calculate next the total mass passing the No. 200 sieve. Add together the fractional masses retained on all the sieves, including the No. 10 sieve, and subtract this sum from the mass of the total sample (as calculated in 14.2).

16.3 Calculate next the total masses passing each of the other sieves, in a manner similar to that given in 12.2.

16.4 Calculate last the total percentages passing by dividing the total mass passing (as calculated in 16.3) by the total mass of sample (as calculated in 14.2), and multiply the result by 100.

17. Graph

17.1 When the hydrometer analysis is performed, a graph of the test results shall be made, plotting the diameters of the particles on a logarithmic scale as the abscissa and the percentages smaller than the corresponding diameters to an

TABLE 2 Values of Effective Depth Based on Hydrometer and Sedimentation Cylinder of Specified Sizes[A]

Hydrometer 151H		Hydrometer 152H			
Actual Hydrometer Reading	Effective Depth, L, cm	Actual Hydrometer Reading	Effective Depth, L, cm	Actual Hydrometer Reading	Effective Depth, L, cm
1.000	16.3	0	16.3	31	11.2
1.001	16.0	1	16.1	32	11.1
1.002	15.8	2	16.0	33	10.9
1.003	15.5	3	15.8	34	10.7
1.004	15.2	4	15.6	35	10.6
1.005	15.0	5	15.5		
1.006	14.7	6	15.3	36	10.4
1.007	14.4	7	15.2	37	10.2
1.008	14.2	8	15.0	38	10.1
1.009	13.9	9	14.8	39	9.9
1.010	13.7	10	14.7	40	9.7
1.011	13.4	11	14.5	41	9.6
1.012	13.1	12	14.3	42	9.4
1.013	12.9	13	14.2	43	9.2
1.014	12.6	14	14.0	44	9.1
1.015	12.3	15	13.8	45	8.9
1.016	12.1	16	13.7	46	8.8
1.017	11.8	17	13.5	47	8.6
1.018	11.5	18	13.3	48	8.4
1.019	11.3	19	13.2	49	8.3
1.020	11.0	20	13.0	50	8.1
1.021	10.7	21	12.9	51	7.9
1.022	10.5	22	12.7	52	7.8
1.023	10.2	23	12.5	53	7.6
1.024	10.0	24	12.4	54	7.4
1.025	9.7	25	12.2	55	7.3
1.026	9.4	26	12.0	56	7.1
1.027	9.2	27	11.9	57	7.0
1.028	8.9	28	11.7	58	6.8
1.029	8.6	29	11.5	59	6.6
1.030	8.4	30	11.4	60	6.5
1.031	8.1				
1.032	7.8				
1.033	7.6				
1.034	7.3				
1.035	7.0				
1.036	6.8				
1.037	6.5				
1.038	6.2				

[A] Values of effective depth are calculated from the equation:

$$L = L_1 + \tfrac{1}{2}\,[L_2 - (V_B/A)]$$

where:
L = effective depth, cm,
L_1 = distance along the stem of the hydrometer from the top of the bulb to the mark for a hydrometer reading, cm,
L_2 = overall length of the hydrometer bulb, cm,
V_B = volume of hydrometer bulb, cm^3, and
A = cross-sectional area of sedimentation cylinder, cm^2
Values used in calculating the values in Table 2 are as follows:
For both hydrometers, 151H and 152H:
L_2 = 14.0 cm
V_B = 67.0 cm^3
A = 27.8 cm^2
For hydrometer 151H:
L_1 = 10.5 cm for a reading of 1.000
= 2.3 cm for a reading of 1.031
For hydrometer 152H:
L_1 = 10.5 cm for a reading of 0 g/litre
= 2.3 cm for a reading of 50 g/litre

TABLE 3 Values of K for Use in Equation for Computing Diameter of Particle in Hydrometer Analysis

Temperature, °C	Specific Gravity of Soil Particles								
	2.45	2.50	2.55	2.60	2.65	2.70	2.75	2.80	2.85
16	0.01510	0.01505	0.01481	0.01457	0.01435	0.01414	0.01394	0.01374	0.01356
17	0.01511	0.01486	0.01462	0.01439	0.01417	0.01396	0.01376	0.01356	0.01338
18	0.01492	0.01467	0.01443	0.01421	0.01399	0.01378	0.01359	0.01339	0.01321
19	0.01474	0.01449	0.01425	0.01403	0.01382	0.01361	0.01342	0.1323	0.01305
20	0.01456	0.01431	0.01408	0.01386	0.01365	0.01344	0.01325	0.01307	0.01289
21	0.01438	0.01414	0.01391	0.01369	0.01348	0.01328	0.01309	0.01291	0.01273
22	0.01421	0.01397	0.01374	0.01353	0.01332	0.01312	0.01294	0.01276	0.01258
23	0.01404	0.01381	0.01358	0.01337	0.01317	0.01297	0.01279	0.01261	0.01243
24	0.01388	0.01365	0.01342	0.01321	0.01301	0.01282	0.01264	0.01246	0.01229
25	0.01372	0.01349	0.01327	0.01306	0.01286	0.01267	0.01249	0.01232	0.01215
26	0.01357	0.01334	0.01312	0.01291	0.01272	0.01253	0.01235	0.01218	0.01201
27	0.01342	0.01319	0.01297	0.01277	0.01258	0.01239	0.01221	0.01204	0.01188
28	0.01327	0.01304	0.01283	0.01264	0.01244	0.01255	0.01208	0.01191	0.01175
29	0.01312	0.01290	0.01269	0.01249	0.01230	0.01212	0.01195	0.01178	0.01162
30	0.01298	0.01276	0.01256	0.01236	0.01217	0.01199	0.01182	0.01165	0.01149

arithmetic scale as the ordinate. When the hydrometer analysis is not made on a portion of the soil, the preparation of the graph is optional, since values may be secured directly from tabulated data.

18. Report

18.1 The report shall include the following:

18.1.1 Maximum size of particles,

18.1.2 Percentage passing (or retained on) each sieve, which may be tabulated or presented by plotting on a graph (Note 16),

18.1.3 Description of sand and gravel particles:

18.1.3.1 Shape—rounded or angular,

18.1.3.2 Hardness—hard and durable, soft, or weathered and friable,

18.1.4 Specific gravity, if unusually high or low,

18.1.5 Any difficulty in dispersing the fraction passing the No. 10 (2.00-mm) sieve, indicating any change in type and amount of dispersing agent, and

18.1.6 The dispersion device used and the length of the dispersion period.

NOTE 16—This tabulation of graph represents the gradation of the sample tested. If particles larger than those contained in the sample were removed before testing, the report shall so state giving the amount and maximum size.

18.2 For materials tested for compliance with definite specifications, the fractions called for in such specifications shall be reported. The fractions smaller than the No. 10 sieve shall be read from the graph.

18.3 For materials for which compliance with definite specifications is not indicated and when the soil is composed almost entirely of particles passing the No. 4 (4.75-mm) sieve, the results read from the graph may be reported as follows:

(1) Gravel, passing 3-in. and retained on No. 4 sieve ..... %
(2) Sand, passing No. 4 sieve and retained on No. 200 sieve ..... %
  (a) Coarse sand, passing No. 4 sieve and retained on No. 10 sieve ..... %
  (b) Medium sand, passing No. 10 sieve and retained on No. 40 sieve ..... %
  (c) Fine sand, passing No. 40 sieve and retained on No. 200 sieve ..... %
(3) Silt size, 0.074 to 0.005 mm ..... %
(4) Clay size, smaller than 0.005 mm ..... %
  Colloids, smaller than 0.001 mm ..... %

18.4 For materials for which compliance with definite specifications is not indicated and when the soil contains material retained on the No. 4 sieve sufficient to require a sieve analysis on that portion, the results may be reported as follows (Note 17):

SIEVE ANALYSIS

Sieve Size	Percentage Passing
3-in.	.....
2-in.	.....
1½-in.	.....
1-in.	.....
¾-in.	.....
⅜-in.	.....
No. 4 (4.75-mm)	.....
No. 10 (2.00-mm)	.....
No. 40 (425-μm)	.....
No. 200 (75-μm)	.....

HYDROMETER ANALYSIS

0.074 mm	.....
0.005 mm	.....
0.001 mm	.....

NOTE 17—No. 8 (2.36-mm) and No. 50 (300-μm) sieves may be substituted for No. 10 and No. 40 sieves.

19. Keywords

19.1 grain-size; hydrometer analysis; hygroscopic moisture; particle-size; sieve analysis

Appendix K

Method Summary:

Samples are well-mixed prior to analysis in order to obtain a representative sub-sample. The methodology will combine measurements made by standard sieving techniques (as referenced above) for the gravel fraction where necessary and the measurements obtained on a Malvern 2600L laser particle sizer (see manufacturer's instructions for QC and operation).

The Malvern instrument is calibrated at the factory. It employs lenses of different focal lengths to measure the concentration of particles within a maximum size range of 1800 um to 1 um. Sub-samples are introduced into the Malvern by first disaggregating in a waterbath by mechanical stirring and ultrasonic dispersion. It may be required with certain samples to perform two laser measurements: one to cover the sand fraction and the other for the silts. In these cases, the separate distributions and the sieve data are "merged" together using an algorithm to reproportion the weight per cents into a single, complete distribution.

The merging algorithm must be mathematically correct; there must be appropriate overlap in the tails of the different distributions in order for the merging to be consistent. This approach, or an equivalent, should be employed to give the required complete distribution from gravel (4mm) to clay (0.001 mm) particles. For the smaller size particles, 0.120 mm to 0.001 mm, 16 sizes fractions must be defined.

Analysis: Particle size distribution for 500 g and 5 g sample sizes must encompass range of 4 mm down to 0.001 mm (1 um).

Method	**QC Requirements**
ASTM D421-85 or equivalent for sieve analysis of larger particles (4mm down to approx. 1 mm)	Duplicate performed at 5% frequency Duplicate RPD criteria: < 20%
merged with:	Field Duplicates as delivered (estimate 5% frequency)
Laser Sizer Methodology (recommend Malvern 2600L) or equivalent for particle sizes down to 0.001 mm (1 um) and small sample aliquots.	

Notes:

RPD = Relative Percent Difference between a sample and sample duplicate. Calculated as:

$$\frac{\text{(Sample value(s) - Sample Duplicate value(s))}}{\frac{\text{(Sample value(s) + Sample Dup. value(s))}}{2}} \times 100\%$$

Note: RPD and duplicates refer to a single size fraction as a measure of the precision of the data for that size fraction.

For each size fraction analyzed, the duplicate frequency and RPD criteria must be met.

Appendix L

Appendix L

RADIONUCLIDES: ACTINIUM-228, BERYLLIUM-7 BISMUTH-214, CESIUM-134, CESIUM-137, COBALT-60 POTASSIUM-40: LDGO METHOD

1.0 Scope and Application

1.1 The method describes the procedure to determine the activities of several radionuclides in Hudson River sediments. These radionuclides are derived from natural and anthropogenic sources. The technique is non-destructive. This procedure will be performed at the Lamont-Doherty Geological Observatory.

2.0 Method Summary

2.1 A sample of river sediment, 40 to 120 g, is dried in a controlled atmosphere and then homogenized using a mortar and pestle. The weight of the sample is determined and recorded. The homogenized sample is then placed in a small can (100 cc volume), and the can is placed inside a gamma spectrometer. The number of gamma decays are recorded by the spectrometer. The presence of a given radionuclide is determined from its gamma decay energy signature. The level of activity for a given radionuclide is proportional to the number of decays detected per unit time.

2.2 The principal radionuclides to be measured are beryllium - 7 and cesium - 137. Data on other radionuclides including actinium - 228, bismuth - 214, cesium - 134, cobalt - 60 and potassium - 40 will also be obtained.

2.3 Method interferences may be caused by cosmic radiation if sufficient shielding of the sample and counter is not provided.

3.0 Apparatus and Materials

3.1 Drying Chamber - The drying chamber consists of an incubator maintained at 35°C or less. The "PCB-free" atmosphere in the incubator is maintained by using a purging system which consists of a teflon piston pump and a flourosil column for removing PCBs from air. The use of this drying system ensures that samples are suitable for gamma spectrometry as well as for long term storage for potential PCB analysis at some time in the future.

3.2 Precision Balance. The balance must be capable of weighing a 100.0 $\pm$ 0.1g.

3.3 Gamma spectrometer. The spectrometer consists of a germanium - drifted - lithium detector [Ge(Li)] capable of measuring gamma decay radiation energy at high resolution. The detector is connected to a computer-based data logger for final data output and calculations.

4.0 Reagents

4.1 Primary Standard - National Bureau of Standards #4350 river sediment containing certified activities of Ac-228, K-40, Cs-137, Co-60, Mn-54, Zn-65, Sr-90, En-152, Eu-154 and Pu-239, 240. The standard also contains Ra-226, Tl-208 and Bi-214 at uncertified activity levels.

4.2 Internal Laboratory Standard (CCV) - Containing measured levels of Cs-137, Cs-134 and Co-60. The activities of this standard as measured by several laboratories is shown in Table 1 of this appendix.

5.0 Initial Calibration

Each of the gamma spectrometers to be used will be calibrated using the NBS standard and various amounts of the internal standard to establish a calibration curve for each of the radionuclides of concern. This complete calibration will be done once for the entire analysis program since this procedure involves about one and one half weeks of counting time per instrument per calibration. The initial calibration will include analysis of background by counting empty sample containers. This calibration is used to determine the instrument efficiency over a range of activities, decay energies and sample sizes. No calibration standard exists for beryllium-

7. The levels will be estimated based on a historic calibration of the instrument and the decay energy vs detection efficiency curve generated by the calibration.

6.0 Procedure

6.1 Dry the sectioned sediment core samples at low temperature in the drying chamber. Dry samples are necessary for the gamma counting method because all of the detector calibrations are performed with dry materials. This also permits the highest and most uniform counting efficiencies to be achieved by eliminating absorption of gamma rays by variable quantities of water in the samples.

6.2 Grind the dry sediments to a fine powder with a large mortar and pestle. This step is essential to achieving a well-mixed uniform sample texture for gamma counting and for representative subsampling for other analyses.

6.3 Remove the material necessary for other analyses. Determine the remaining sample weight and place in an aluminum can. Seal the dry sediment powder in an air-tight aluminum can to obtain a reproducible geometry for gamma spectrometry and to permit the daughter products of Rn-222 (half-life = 3.8 days) and Rn-220 (half-life = 54 seconds), both of which are gases, to achieve secular equilibrium with the critical gamma-emitting daughter products in the uranium and thorium decay series. Aluminum cans of 100 cc volume will be used for the gamma spectrometry measurements.

6.4 Set the sealed aluminum cans aside for a minimum of approximately 5 half-lives of Rn-222 (20 days) to permit a achievement of secular equilibrium of U-238 decay series nuclides.

6.5 Place a sealed equilibrated aluminum can on the top surface of a Ge(Li) detector, after enclosing the can in a plastic bag to prevent introduction of dust into the shielded counting space around the detector.

6.6 Start accumulation of the spectrum of gamma emissions as a function of energy of each gamma ray detected.

6.7 Accumulation of the gamma spectrum should be terminated after enough counts have been recorded for the critical nuclides, Cs-137 and Be-7, to yield a statistical counting error of $\leq 10\%$, (1σ) or until 8 hrs of counting time have elapsed. Technical judgment

may be used in the final determination of counting endpoints, especially for samples with extremely low activity levels.

6.8 Record the counts in each photo peak of interest from the gamma spectrum on a data sheet, along with other relevant data necessary for calculation of the radionuclide activities per unit weight of sample: sample weight (dry), sample collection date, sample counting date, proportion of can filled by the sample powder, total counting time, code information for sample identification (e.g. core mile point, depth interval, laboratory control number).

6.9 Enter the data discussed above in the calculation program to obtain concentrations for each radionuclide of interest, expressed in terms of activity units, picoCuries (pCi), per kilogram of dry weight of sediment. One pCi is equivalent to 2.22 disintegrations per minute (dpm), or 0.037 Becquerels (1 Becquerels = 1 disintegration per second, dps). One Curie equals the number of decays per gram of pure radium-226, and one pCi equals 10^{12} Ci.

6.10 Store sealed aluminum cans containing dry sediment powder after completion of gamma counting. In this mode the samples can be saved indefinitely without risk of contamination or need to retain at low temperature to prevent microbial alteration of any important labile constituents. The container can be opened at a later time for further sampling and sealed in the same can.

7.0 Calculations

7.1 Counting Statistics - The rough counting statistics error is calculated as given below.

$$\% \ error = \frac{\sqrt{Total\ Sample\ Counts}}{Total\ Sample\ Counts} \times 100\%$$

$$= \frac{1}{\sqrt{Total\ Sample\ Counts}} \times 100\%$$

7.2 The complete calculation of the sample activity for an individual radionuclide is given by the following example for Cs-137 in a laboratory standard. Note that the branching ratio is obtained form the scientific literature and is the ratio of the proportion of decays represented by the measured gamma radiation to all decays undergone by the

radionuclide. For the example, 0.851 or 85.1% of all Cs-137 atoms decay via the gamma decay mode at about 650 KeV.

Sample SLOSH III 1000 I-2 (LDGO internal standard):

Counts in peak	=	7773 (13 channels, ch.)
Counts in background	=	946 (20 channels)
Count time	=	1387 minutes
Sediment weight	=	92 grams
Detector efficiency	=	0.012372576
Branching ratio	=	0.851
cpm	=	counts per minute
dpm	=	decays per minute

$$(1)\ cpm\ in\ peak = \frac{7773 \pm \sqrt{7773}}{1387} = 5.60 \pm 0.06$$

(2) *Bkg./20 ch.* = 946; *bkg./13 ch.* = 615

$$cpm\ in\ bkg. = \frac{615 \pm \sqrt{615}}{1387} = 0.44 \pm 0.018$$

$$(3)\ Net\ cpm\ in\ peak = (5.6 - 0.44) \pm \sqrt{(0.06)^2 + (0.018)^2} = 5.16 \pm 0.066$$

$$(4)\ Net\ cpm/g = \frac{5.16 \pm 0.066}{92} = 0.056 \pm 0.0007\ (error\ 1.3\%)$$

$$(5)\ Net\ dpm/g = \frac{cpm/g}{Eff} \times \frac{1}{B.R.} = \frac{0.056}{0.012372576} \times \frac{1}{0.851} = 5.33$$

(6) *Error calculation for dpm/g must include:*

sample counting error (1.3%)
std. counting error (1.4%)
statistical error in std. (0.2%) given with std.

$$\%\ error = \sqrt{(1.3\%)^2 + (1.4\%)^2 + (0.2\%)^2} = \sim 2\%$$

(7) 5.33 dpm/g $\pm$ (5.33 x 2%) = 5.33 $\pm$ 0.107 dpm/g

(8) *Conversation to pCi/kg*: $\frac{(1000g/kg)}{(222\ dpm/pCi)}$ (5.33±0.107 *dpm/g*) = 2400±48 *pCi/kg*

7.3 For radionuclides exhibiting two or more modes of gamma decay, the activity will be reported as the mean of each individual mode of decay calculated in pCi/kg.

7.4 All short lived isotopes (half life less than 50 years) must be corrected to the date of sample collection using the following formula:

$$A_o = \frac{A}{e^{-\lambda(t-t_o)}}$$

where:
- A_o = activity of the radionuclide at time of collection (t_o).
- A = measured activity of the radionuclide.
- t_o = time of collection.
- t = time of analysis.
- λ = decay constant for the radionuclide in units consistent with t and t_o.

8.0 Precision and Accuracy

8.1 Because of the low level of activity for the radionuclide of interest and the small sample size, precision and accuracy will be constrained by time limitations.

8.2 All sediment samples should be counted for at least 8 hours each or until the statistical counting error is ≤10% for both Be-7 and Cs-137. No other radionuclides will be used to determine the sample counting end point; however, technical judgment may be used, especially incases where samples have extremely low activity levels. For samples where the ≤10% threshold is reached, anticipated overall precision and accuracy is 20%. However, no sample radionuclide results will be rejected based upon counting a statistics alone. The final decision as to data usability will be depend upon the interpretation of the radionuclide chronologies for an entire core.

8.3 The minimum detection limits for Cs-137 and Be-7 are 60 pCi/kg and 600 pCi/kg, respectively although these limits are also subject to the 18 hour counting time limits.

9.0 Quality Control

9.1 Calibration - The calibration discussed in Section 5 of this appendix will be performed once for each counter at the beginning of the analytical program. This calibration will not be repeated unless the program lasts more than 6 months. The calibration will determine the instrument detection efficiency for each radionuclide of concern and the effect of variable sample size on detection efficiency. Background measurements will be made during this calibration as well.

9.2 Background Measurement - Background measurements will be made using empty cans on a regular basis, roughly once for every twenty samples run on an individual instrument. Due to the large number of samples and the long analytical period, background levels are expected to vary over time due to the seasonal dependence of the incidence of cosmic radiation at the earth's surface but not by more than 50%. Greater variation than this will result in a reevaluation of instrument performance and shielding and if necessary a recalibration. The Quality Assurance Officer must be notified if discrepancies in the background measurements require corrective action.

9.3 Continuing Calibration Verification

An internal laboratory standard (CCV) will be run once for every twenty samples on an individual instrument. The CCV recovery criteria is 80-120% after correcting for background based on the following formula:

$$\% \ Recovery = \frac{Observed \ Standard \ Value}{Expected \ Standard \ Value} \times 100\%$$

If the CCV does not meet criteria, the Quality Assurance Officer must be contacted. A recalibration of the individual instrument may be necessary.

9.4 Duplicate Analysis - Duplicate analyses will be performed for one in every twenty samples. The relative percent difference (RPD) as given below must not exceed 20% for either Cs-137 or Be-7. If the RPD threshold is exceeded, three samples from the group of twenty must be recounted and compared to the original value. All duplicate analyses must be separated from the original analyses by at least one week. If the duplicate analyses still do not agree, contact the Quality Assurance Officer.

$$RPD = \frac{Sample\ Activity - Sample\ Duplicate\ Activity}{\left(\frac{Sample\ Activity + Sample\ Duplicate\ Activity}{2}\right)} \times 100\%$$

where activity is in pCi/kg.

10.0 References

10.1 Olsen, C.R., Radionuclides, Sedimentation and the Accumulation of Pollutants in the Hudson Estuary, Ph.D. dissertation, Columbia University, 343 pp., 1979.

10.2 Olsen, C.R., H.J. Simpson, T.-H. Peng, R.F. Bopp and R.M. Trier, Sedimentation mixing and accumulation rate effects on radionuclide depth profiles in Hudson estuary sediments, J. Geophys. Res. 86, 11020-11028, 1981.

Table 1

Laboratory Intercomparison Data for SLOSH III
Lamont-Doherty Geological Observatory Internal Standard

Sample	$^{239,240}Pu$ (pCi/kg)	^{238}Pu (pCi/kg)	^{137}Cs (pCi/kg)	^{134}Cs (pCi/kg)	^{60}Co (pCi/kg)
LDGO					
1000 Q-1	–	–	2630±54	340±40	320±16
-2	26.0±2.0	1.5±0.2	2920±65	500±54	385±29
-3	26.3±2.3	0.9±0.2	2780±76	430±52	505±43
-4	25.0±1.6	1.0±0.1	2700±72	345±42	400±37
-6	–	–	2610±50	410±50	300±20
WHOI					
1000 Q-1	25.3±1.4	1.08±0.23	2720±30	483±38	340±20
"	24.5±1.0	0.79±0.14	2720±30	445±62	–
HASL					
1000 Q-6	–	–	2680±30	440±50	320±20

LDGO - Lamont-Doherty Geological Observatory
WHOI - Woods Hole Oceanographic Institution
HASL - Health and Safety Laboratory of ERDA

Appendix M

1. OBJECTIVE

This guideline details the steps required to measure the pH of an aqueous sample while in the field using either a pH meter or pH paper. It is important to obtain a pH measurement soon after taking a sample and thus avoid sample changes such as precipitation, temperature fluctuation, or oxidation, which can affect the pH of the sample.

2. LIMITATIONS

This guideline is applicable to aqueous samples such as potable well water, monitoring well water, surface water, leachate, and drummed wastewater.

3. DEFINITIONS

"pH" -- The negative logarithm (base 10) of the hydrogen ion activity. The hydrogen ion activity is related to the hydrogen ion concentration, and, in relatively weak solution, the two are nearly equal. Thus, for all practical purposes, pH is a measure of the hydrogen ion concentration.

"pH Paper" -- Paper that turns different colors depending on the pH of the solution to which it is exposed. Comparison with color standards supplied by the manufacturer will then give an indication of the solution pH.

4. GUIDELINES

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment such as acid-base neutralization, water softening, and corrosion control is pH dependent.

Two methods are given for pH measurement: the pH meter, and pH indicator paper. The indicator paper is used when only a rough estimate of the pH is required. For this program pH paper will be used to verify sample preservation when pH <2 must be maintained. The pH meter is used when a more accurate measurement is needed, usually within 0.01 to 0.1 pH units. All pH water analyses will employ the pH meter method. To use the pH meter, the meter with electrode are standardized using pH 7, pH 4, and pH 10 buffers. The probe is then immersed in the unknown sample aliquot to obtain a pH reading. No standardization is required when using pH paper. A small aliquot of acidified sample will be used to moisten the pH paper for verification of acidification.

4.1 RESPONSIBILITIES

The project team leader is responsible for deciding when a pH measurement should be taken.

The field samplers are responsible for measuring the pH and for recording and reporting the results.

4.2 EQUIPMENT

The following equipment is needed for taking field pH measurements:

4.2.1 pH Measurements by paper

pH indicator paper, such as Hydrion or Alkacid, to cover the pH range 1 through 12. pH paper is available in a variety of ranges, depending on the accuracy required. pH paper is typically used when checking the proper preservation of aqueous samples.

4.2.2 pH Measurements by Instruments:

1. Portable pH meter or equivalent; model #HI 9025, Hanna; Model #103, Corning.

2. Combination electrode with polymer body to fit the meter.

3. pH buffer (standard) solutions (recommended standards are 4.0, 7.0, and 10.0)

4. Clean glass or plastic containers (e.g., disposable 50 ml beakers)

4.3 FIELD pH MEASUREMENT

4.3.1 pH Meter

The following procedure is used for measuring pH with a pH meter:

1. Immerse the tip of the electrode in pH 7 buffer vernight. If this is not possible due to field conditions, immerse the electrode tip in pH 7 buffer for at least an hour before use. (Probes should be stored with the plastic liquid cup attached so as to minimize the stabilization period required and to prevent the wick from drying out.)

2. Rinse the electrode with demineralized water.

3. Immerse the electrode in pH 7 buffer solution.

4. Adjust the temperature compensator to the sample temperature.

5. Follow directions for 2 point calibration using pH 4 and pH 7 buffer. Record the calibration on the logsheet for the pH meter.

6. Remove the electrode from the buffer and rinse thoroughly with demineralized (deionized) water.

7. Pour a small volume (25 ml- 50 ml) of sample into a beaker and do not immerse the electrode in any sample portion which is being sent to a laboratory for chemical analysis. Immerse the electrode in the unknown solution.

8. Read and record the pH of the solution, after adjusting the temperature compensator to the sample temperature.

9. Rinse the electrodes with demineralized water.

10. Keep the electrode immersed in water, or covered with protective cap filled with pH 7 buffer, at all times when not in use.

11. Prior to each pH measurements, check calibration with pH buffer. Criteria for acceptance = I0.1 pH units. Also see SOP for Corning Model 103 pH meter.

4.3.2 Indicator Paper

The following procedure is used for measuring pH with pH indicator paper:

1. Immerse a one-inch strip of indicator paper into 25 ml to 50 ml of the unknown solution, immediately withdraw it, or pour a portion of the sample over a small piece of pH paper. Do not allow the indicator paper to contact any sample being sent to a laboratory for chemical analysis.

2. Compare the color of the wet pH paper with the indicator colors given on the pH paper container.

3. Record the pH value which most closely resembles the colors shown. (Note: If the indicator paper is suspected of being old or deteriorated, immerse it in an appropriate buffer and check the color that develops against the standards given. Discard all rolls of deteriorated paper.)

4.4 RECORDS

All results are to be recorded in the field logbook.

Appendix N

1. OBJECTIVE

This guideline summarizes the steps necessary to use the Corning Model 103 pH meter.

2. LIMITATIONS

This guideline is applicable to all aqueous samples such as potable well water, monitoring well water (groundwater), surface water, wastewater, leachate, and other water samples. Measurements should be taken as soon as possible after sample collection to avoid sample changes which can affect the apparent pH reading.

3. DEFINITIONS

"pH" — The negative log (base 10) of the hydrogen ion concentration [H+] in a sample.

4. GUIDELINES

4.1 GENERAL

The 103 pH meter is a precision instrument compatible with pH electrodes and is designed for use as a hand-held instrument. The 103 can be used with a 9V battery for portable applications. The digital display provides a direct readout in pH units or absolute millivolts.

4.2 RESPONSIBILITIES

The project team leader or site manager is responsible for determining when conductivity/temperature measurements should be taken in accordance with the site-specific work plans. Generally, field measurements of temperature and conductivity are made whenever an aqueous sample is taken. The field samplers are responsible for taking the measurements and recording and reporting the results.

4.3 OPERATING INSTRUCTIONS

4.3.1 Initial Use

4.3.1.1 Unpacking

Unpack the instrument and check that all items and accessories are present and undamaged. (This should be checked prior to use in the field.) The following items should be present:

— Model 103 pH meter

— Combination electrode

— Slope adjustment tool (approximately 3 inch long black plastic rod with a phillips head screwdriver on one end; the other end is used for slope adjustment)

— Instruction manual

4.3.1.2 Battery Installation/Change

To install the battery, unclip the battery compartment cover (refer to Figure 42-1) and fit the battery connector onto the battery. Place the battery inside the compartment and replace the battery compartment cover.

4.3.1.3 Electrode Installation

Disconnect the shorting plug from the pH socket (see Figure 42-2), and connect the combination electrode.

4.3.2 Instrument Description

4.3.2.1 Display and Controls (refer to Figure 42-3)

Readout: The liquid crystal display will read from 0.00 to 14.00 in pH mode and from -1999 to 1999 in mV mode. A low battery indicator is also integral to the display and will be sent as LO BAT.

O pH mV: Power and mode switch.

CALIBRATE: This control sets the LCD readout to the value of the first calibration buffer, and operates in pH mode only.

cal 2 Δ %: This control compensates for deviations from the theoretical value of the Nernst slope of the electrode. Used to set the second calibration point when carrying out a 2-point calibration. This control operates in pH mode only. The cal 2 Δ % control is accessible via a hole in the connector panel and is adjusted using the slope adjustment tool supplied.

TEMPERATURE: This control compensates for the slope versus temperature characteristics of electrodes and operates in pH mode only.

4.3.2.2 Input/Output Connectors (refer to Figures 42-1 and 42-2)

pH: Standard BNC socket that will accept a wide range of electrodes. A positive millivolt input at this socket results in a positive mV reading on the display. Adjust TEMPERATURE control to the temperature of the unknown solution.

ref (blue): Standard ref. jack socket that will accept a wide range of reference electrodes.

a.c./d.c. Adapter Socket: Socket for the 9V d.c. power supply provided by the a.c./d.c. adapter. (Note: TAMS does not have this accessory.)

Battery Compartment: Rear panel compartment for 9V battery (NE1604 or equal).

4.3.3 Operating Instructions

NOTE: Refer to Section 4.3.1.2 for information on connecting electrodes and to Section 4.3.3.3 for information on recommended operating procedures.

4.3.3.1 pH Measurements

Calibration:

1. Select pH mode.
2. Rinse electrode with pH 7.00 buffer or deionized water.
3. Immerse the electrode in pH 7.00 buffer and adjust TEMPERATURE control to the temperature of the buffer.
4. When the reading stabilizes, adjust the CALIBRATE control to set the display to the value of the buffer.

   NOTE variations in pH values for changes in buffer temperature are shown on Corning buffer solution bottles.
5. For a 2-point calibration, continue with Step 6; ~~if a 1-point calibration is being performed, go to Step 9.~~

2-Point Calibration

6. Rinse electrode with the second buffer or deionized water.
7. Immerse electrode in the second buffer and adjust TEMPERATURE control to the temperature of the buffer.
8. When the reading stabilizes, adjust the cal 2 Δ % control, with the slope adjustment tool, to set the display to the value of the second buffer.

Measuring Samples

9. Rinse electrode with unknown solution or deionized water.
10. Immerse electrode in the unknown solution and adjust TEMPERATURE control to the temperature of the unknown solution.
11. Allow time for the display to stabilize, then note the reading.
12. Repeat Steps 9 to 11 for further samples.
13. Recalibrate periodically. The frequency will depend on the degree of accuracy required and the condition of the electrode.

4.3.3.2 Absolute mV Measurement

1. Select mV mode.

2. Rinse the electrode with the unknown solution or deionized water.

3. Immerse the electrode in the unknown solution.

4. Allow time for the display to stabilize, then note the reading.

5. Repeat Steps 2 to 4 for further samples.

4.3.3.3 Operating Hints

To make accurate measurements, it is essential that care is exercised when using electrodes.

The following points will help to obtain the optimum performance of the Corning Model 103 pH meter 103 and the electrode system:

1. Conditioning requires that glass pH and combination electrodes are presoaked for at least eight hours in pH 7 buffer solution. Distilled or deionized water is not recommended. As a general rule, the electrode should be stored in a buffered medium around pH 7 and should not be allowed to dry out. A pH electrode with a slope value of less than 50mV per decade should not be used. If the slope value fails rapidly, the pH bulb should be cleaned. Refer to the electrode manufacturer's instructions.

2. Make sure that reference and combination electrodes are filled with the recommended fill solution and that when in use the fill hole cap is removed from the fill hole. Failure to remove it will result in drifting readings because the flow of the fill solution will be impaired.

3. Avoid handling the electrode membrane. Any damage to its surface caused by abrasion will lead to inaccuracy and slow response.

4. Retain the manufacturer's instructions supplied with electrodes, and refer to these for operating, conditioning, storage and maintenance information.

5. Do not use buffer solutions after the expiration date shown on the label and do not pour solutions back into the bottle.

6. When transferring electrodes from one solution to another, the electrode should be rinsed with the solution that is to be measured or deionized water. Then blot the electrode with tissue paper. Do not wipe the electrodes with tissue paper as it may result in slight polarization and consequent sluggishness of response.

7. Response time (i.e., the time required for the electrode to reach equilibrium when transferred from one solution to another) is a function of both the electrode and the solutions. With some solutions, very rapid

equilibration times are possible, while other solutions, particularly those of low ionic strength, may exhibit response times of several minutes.

8. When measuring low ionic strength solutions, either of high or low pH, some drift may be observed. Equilibration times are normally longer in such solutions and greater care should be exercised when taking measurements. To decrease the response time of measurements of distilled or deionized water, one drop of saturated KCl may be added to 50 ml of water sample.

4.4 MAINTENANCE AND PROBLEM SOLVING

4.4.1 Routine Maintenance

The 103 has been designed for a long, troublefree life without the need for regular maintenance. For optimum performance, carry out the electrode maintenance as detailed in the manufacturer's instructions (supplied with the electrode). If required, clean the external casework with a slightly damp cloth — do not use solvents to clean the 103. For information on changing the battery, refer to Section 4.3.1.2.

4.4.2 Instrument Check Procedure

1. Select mV mode.
2. Disconnect the electrode and connect the shorting plug to the pH socket.
3. Check that the display reads between -002 and 002.
4. Select pH mode.
5. Set TEMPERATURE control to 25°C.
6. Check that the display can be set to 7.00 using the CALIBRATE control.
7. Check that the display can be set to 8.00 by clockwise rotation of the CALIBRATE control.
8. Check that the display can be set to 6.00 by counterclockwise rotation of the CALIBRATE control.

4.4.3 Problem Solving

If a fault occurs, it must be isolated to the electrode or the instrument.

For the following faults, carry out the action indicated:

Fault	Action
1. Slow Response Unstable response	Refer to Section 4.3.3., paragraph 7 Condition/substitute electrode. (Refer to electrode manufacturer's instructions.)
2. LO BAT indicated	Replace battery (Refer to Section 4.3.1.2.)
3. Displays blank	Check that pH or mV mode is selected. Check that the battery is fitted correctly or that the a.c./d.c. adapter is connected to the a.c. supply.
4. CALIBRATE control inoperative cal 2 Δ % control inoperative TEMPERATURE control inoperative	Check that pH mode is selected.
5. Display shows: Negative reading or 1 in pH mode, other digits blank -1 or 1 in mV mode, other digits blank	Overrange indicated; check that electrode is connected and immersed in solution. Check that wetting cap has been removed. Substitute connection lead, if used.

4.5 SPECIFICATIONS

Display

Liquid crystal display (LCD), 12.7 mm high.

Operating Ranges

pH
0 to 14.00 pH

Absolute mV
0 to ± 1999 mV

Resolution

0.01 pH : 1 mV

Relative Accuracy

±0.01 pH : ±1 mV, plus one least significant digit

Precision

±1 least significant digit

Input Conditions

Impedance, 10^{12} ohms
Current, typically 1 pA

Drift

±0.01 pH : ±1 mV, plus one least significant digit. Measured over a 24 hour period. Assumes a maximum ambient temperature change of ±4°C and is specified for the instrument only.

Isopotential Point

Set at pH 7.00

Temperature Control Range

0 to 100°C

Power Requirements

Battery, 9 volt (NE 1604) or optional a.c./d.c. adapter (voltage range 90 to 127V a.c.). (TAMS does not have the adapter.)

FIGURE 42-1

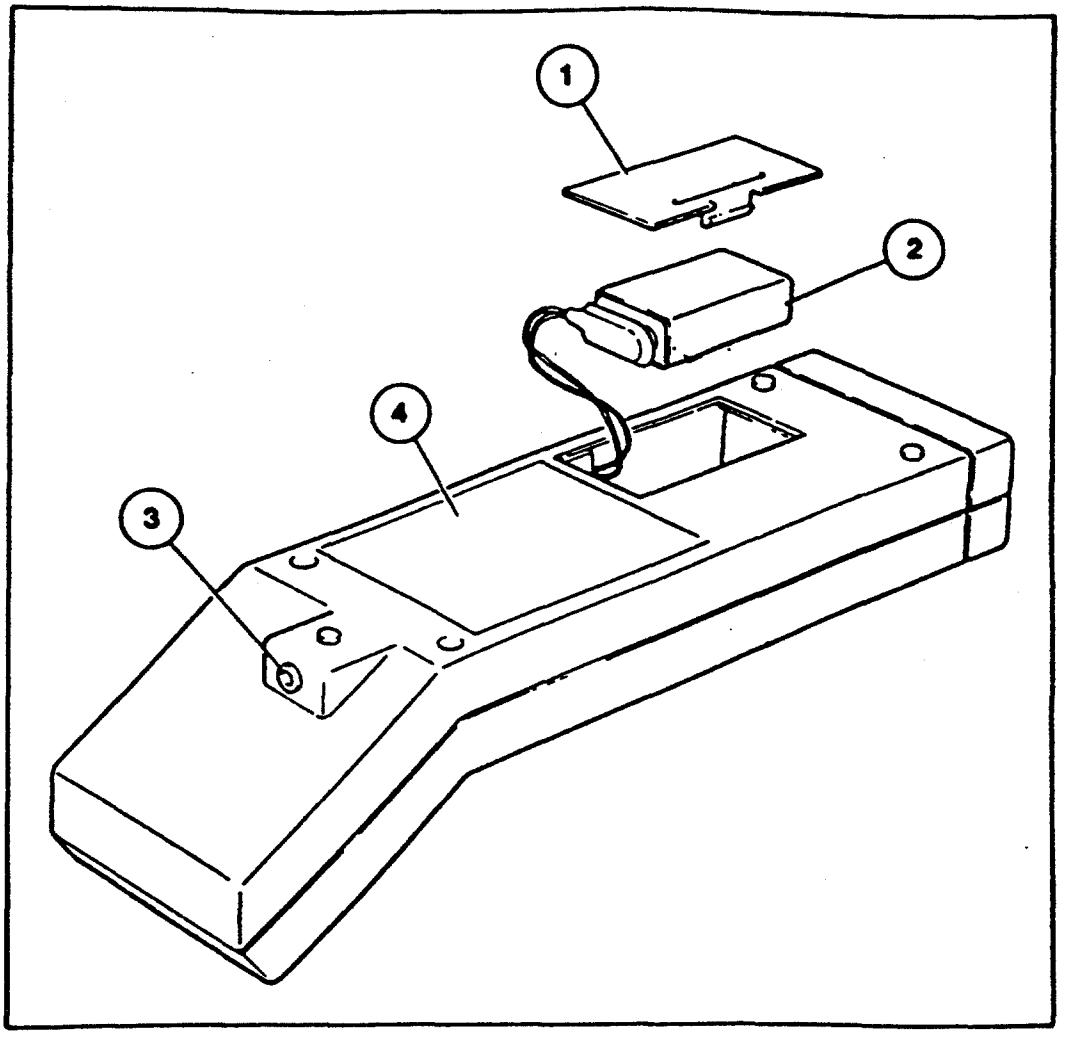


FIGURE 42-1 REAR PANEL

1 - Battery compartment cover
2 - 9V Battery
3 - Socket for a.c./d.c. adapter
4 - Serial number plate

FIGURE 42-2

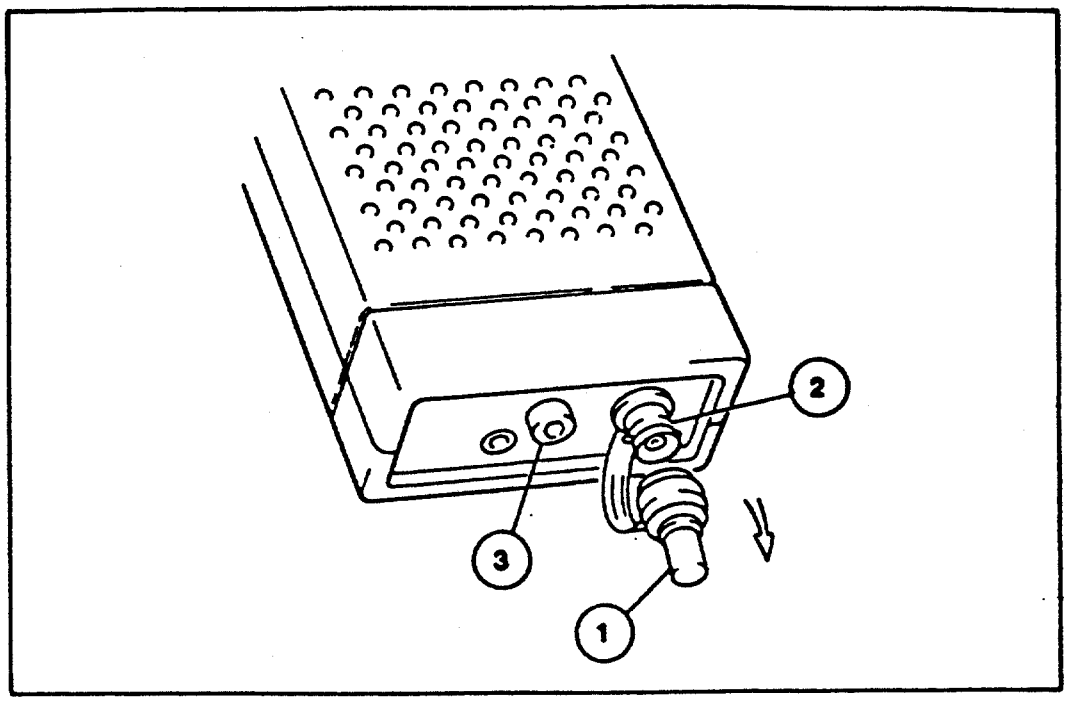


FIGURE 42-2 CONNECTOR PANEL

1 - Shorting plug
2 - pH socket
3 - ref socket

FIGURE 42-3

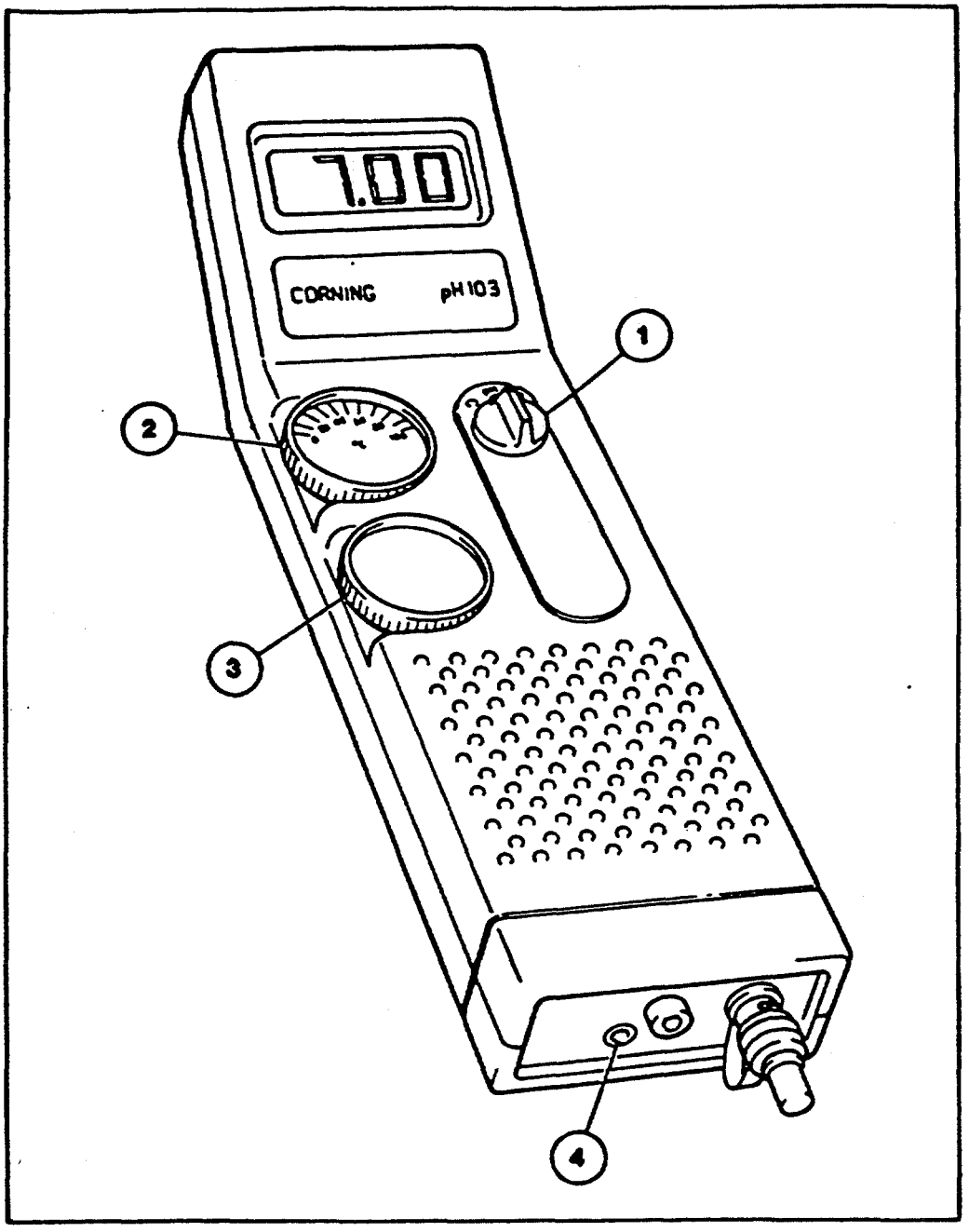


FIGURE 42-3 DISPLAYS AND CONTROLS

1 - Power and mode swithch
2 - TEMPERATURE control
3 - CALIBRATE control
4 - cal 2Δ% control

1. OBJECTIVE

This guideline details the steps required to measure the oxidation-reduction potential (ORP) of an aqueous sample in the field. ORP is an important measure of the ratio of the activities of the oxidized to the reduced species in the sample. In this program, the Eh potential will be taken for pore waters of river sediment cores by placing probe in the core.

2. APPLICABILITY

This guideline is applicable to nearly all aqueous solutions and in general is not subject to solution interference from color, turbidity, colloidal matter, or suspended matter.

3. DEFINITIONS

"Oxidation-Reduction Potential" -- The electromotive force (emf) developed by a noble metal electrode immersed in water, referred to the standard hydrogen electrode. This measurement is also referred to as Eh.

4. GUIDELINES

When an inert metal electrode, such as platinum, is immersed in a solution, a certain potential is developed at the electrode, depending on the ions present in the solution. If a reference electrode is placed in the same solution, the potential difference between the two electrodes can be measured and will be dependent on the concentration of the ions in the solution.

The ORP measurement establishes the ratio of oxidants and reductants prevailing within a solution of water or wastewater. The measurement is nonspecific in contrast, for example, to the pH measurement. The ORP electrode pair senses the prevailing net potential of a solution. By this measurement, the ability to oxidize or reduce species in solution may be determined. Supplemental measurements such as dissolved oxygen may be correlated with ORP to provide a knowledge of the quality of the solution, water, or wastewater.

4.1 RESPONSIBILITIES

The Project Team Leader is responsible for deciding when the oxidation-reduction potential of a sample should be measured. Sampling Plans will provide the details.

The field samplers are responsible for performance of the procedure, recording, and reporting of the results.

4.2 OXIDATION-REDUCTION MEASUREMENT

The following procedure is used for measuring oxidation reduction:

1. Condition and maintain ORP electrodes as recommended by the manufacturer. Keep the ends of the electrodes in water between measurements.

2. Clean the electrode using aqua regia, nitric acid, or chromic acid, according to the instructions given by the manufacturer.

3. Thoroughly rinse the electrode with deionized water.

4. Verify the sensitivity of the electrodes by noting the change in millivolt reading when the pH of the test solution is altered. The ORP will increase when the pH of the test solution decreases and the ORP will decrease if the test solution pH is increased. Place the sample in a clean glass beaker and agitate the sample. Insert the electrodes and note the ORP or millivolt reading. Add a small amount of a dilute NaOH solution and note the value of the ORP. If the ORP drops sharply when the caustic is added, the electrodes are sensitive and operating properly. If the ORP increases sharply when the caustic is added, the polarity is reversed and must be corrected in accordance with the manufacturer's instructions. If the ORP does not respond as above when the caustic is added, the electrodes should be cleaned and the above procedure repeated.

5. After the assembly has been checked for sensitivity, wash the electrodes with three changes of water or by means of a flowing stream from a wash bottle. Place electrode directly into core sediment. Read the milllivolt potential allowing sufficient time for the system to stabilize. Measure successive portions of the sample until readings on two successive portions differ by no more than 10 mV. A system that is very slow to stabilize probably will not yield a meaningful ORP.

4.4 MAINTENANCE AND TROUBLESHOOTING

The instrument used to measure Eh is the Corning Model 103 pH/mv meter, equipped with a specific electrode.

4.3 CALIBRATION

The instrument measures directly in millivolts and is not subject to calibration.

4.5 RECORDS

All results are to be recorded in the field logbook.

A field notebook should be kept by each individual sampler. Report the oxidation-reduction potential to the nearest 10 mV (interpolating the meter scale as required), the temperature at which the measurement was made, and the pH at the time of measurement.

Appendix O

1. OBJECTIVE

This guidelines summarizes the steps necessary to use the YSI Model 57 Dissolved Oxygen Meter.

2. LIMITATIONS

This guideline is applicable to all aqueous samples. Measurements should be taken as soon as possible after sample collection to avoid sample changes which can affect the apparent DO reading.

3. DEFINITIONS

"Dissolved Oxygen (DO)"--The amount of oxygen dissolved in water.

4. GUIDELINES

4.1 GENERAL

The YSI Model 57 oxygen meter is a precision instrument for measuring dissolved oxygen and temperature in water. The sensing element is a Clark-type, membrane-covered polarographic probe. It is battery-operated and completely portable.

4.2 RESPONSIBILITIES

The project team leader or site manager is responsible for determining when the DO should be taken, in accordance with the site-specific work plans. Generally, field measurement of DO and temperature are made whenever an aqueous sample is taken. The field samplers are responsible for taking the measurements and recording and reporting the results.

4.3 OPERATING INSTRUCTIONS

4.3.1 Initial Use - Preparing the Oxygen Probe

All YSI 5700 series oxygen probes (see specifications) have similar sensors and should be cared for int he same manner. They are precision devices and require good treatment if high accuracy measurements are to be obtained.

The procedure for preparing the probe is an follows (see Figure 38-2):

1. Add distilled water to the KCl crystals and dissolved completely. (Tap water introduces harmful contaminants to the probe.)

2. Transfer a part of the KCl solution to the eyedropper bottle.

3. Remove sensor guard from the probe (YSI 5739 probe).

4. Remove the protective membrane and "O" ring and thoroughly rinse the sensor with KCl solution.

5. Fill the Probe with Electrolyte as follows:

   Grasp the probe in your left hand. When preparing the YSI 5739 probe the pressure compensating vent should be to the right. Successively fill the sensor body with electrolyte while pumping the diaphragm with the eraser end of a pencil or similar soft, blunt tool. Continue filling and pumping until no more air bubbles appear. (With practice you can hold the probe and pump with one hand while filling with the other.) When preparing the YSI 5720A and 5750 probes, simply fill the sensor body until no more air bubbles appear.

   Secure a membrane under your left thumb. Add more electrolyte to the probe until a large meniscus completely covers the gold cathode. NOTE: Handle membrane material with care, keeping it clean and dust free, touching it only at the ends.

   With the thumb and forefinger of your other hand, grasp the free end of the membrane.

   Using a continuous motion stretch the membrane UP, OVER, and DOWN the other side of the sensor. Stretching forms the membrane to the contour of the probe.

   Secure the end of the membrane under the forefinger of the hand holding the probe.

   Roll the "O" ring over the end of the probe. There should be no wrinkles in the membrane or trapped air bubbles. Some wrinkles may be removed by lightly tugging on the edges of the membrane beyond the "O" ring.

Trim off excess membrane with scissors or sharp knife. Check that the stainless steel temperature sensor is not covered by excess membrane.

6. Shake off excess KCl and reinstall the sensor guard.

7. A bottomless plastic bottle is provided with the YSI 5739 probe for convenient storage. Place a small piece of moist towel or sponge in the bottle and insert the probe into the open end. This keeps the electrolyte from drying out. The YSI 5720A and 5750 probes can be stored in B.O.D. bottle containing about 1" water.

8. Membranes will last indefinitely, depending on usage. Average replacement is 2-4 weeks. However, should the electrolyte be allowed to evaporate and an excessive amount of bubbles form under the membrane, or the membrane become damaged, thoroughly flush the reservoir with KCl and install a new membrane.

9. Also replace the membrane if erratic readings are observed or calibration is not stable.

10. "Home brew" electrolyte can be prepared by making a saturated solution of reagent grade KCl and distilled water, and then diluting the solution to half strength with distilled water. Adding two drops of Kodak Photo Flo per 100 ml of solution assures good wetting of the sensor, but is not absolutely essential.

11. The gold cathode should always be bright and untarnished. If it is tarnished (which can result from contact with certain gases) or plated with silver (which can result from extended use with a loose or wrinkled membrane), return it to the factory for service or else clean it with the YSI 5680 Probe Reconditioning Kit. Never use chemicals or any abrasive other than that supplied with this kit.

12. It is also possible that the silver anode may become contaminated, which will prevent successful calibration. Try soaking the probe overnight in a 3% ammonia solution; rinse with deionized water, recharge with electrolyte, and install a new membrane. If still unable to calibrate, return the probe for service.

13. H_2S, SO_2, Halogens, Neon, Nitrous Oxide and CO are interfering gases. If you suspect erroneous readings, it may be necessary to determine if these are the cause. These gases have been tested for response.

100% Carbon Monoxide-Less than 1%	100% Helium-none
100% Carbon Dioxide-Around 1%	100% Nitrous Oxide-1/3 O_2 response
100% Hydrogen-Less than 1%	100% Ethylene-none
100% Chlorine-2/3 O_2 response	100% Nitric Oxide-1/3 O_2 response

4.3.2 Preparing the Instrument

It is important that the instrument be placed in the intended operating position (vertical, titled, or on its back) before it is prepared for use and calibrated. Readjustment may be necessary when the instrument operating position is changed. After preparing the probe proceed as follows:

1. With switch in the OFF position, adjust the meter pointer to Zero with the screw in the center of the meter panel. Readjustment may be necessary if the instrument position is changed.

2. Switch to RED LINE and adjust the RED LINE knob until the meter needle aligns with the red mark at the 31°C position.

3. Switch to ZERO and adjust to zero with zero control knob.

4. Attach the prepared probe to the PROBE connector of the instrument and adjust the retaining ring finger tight.

5. Before calibrating allow 15 minutes for optimum probe stabilization. Repolarize whenever the instrument has been OFF or the probe has been disconnected.

4.3.3 Instrument Description

4.3.3.1 Specifications

1. Instrument

   Ranges: 0-5, 0-10, and 0-20 mg/l (0-2.5, 0-5 and 0-10 mg/l with YSI 5776 High Sensitivity Membrane)

   Accuracy: ±1% of full scale at calibration temperature (±0.1 mg/l on 0-10 scale), or 0.1 mg/l (whichever is larger).

Temperature Measurement

Range: -5° to +45°C
Accuracy: ±0.5°C plus probe which is ±0.1°C
Readability: 0.25°C

Temperature Compensation: ±1% of D.O. reading for measurement made within ±5°C of calibration temperature; ±3% of D.O. reading over entire range of -5 to +45°C probe temperature.

System Response Time:

Typical response for temperature and D.O. readings is 90% in 10 seconds at a constant temperature of 30°C with YSI 5775 Membranes. D.O. response at low temperature and low D.O. is typically 90% in 30 seconds. YSI 5776 High Sensitivity Membranes can be used to improve response at low temperature and low D.O. concentrations. If response time under any operating conditions exceeds two minutes, probe service is indicated.

Operating Temperature Range:

Instrument and probe operating range is -5° to +45°C. Large ambient temperature changes will result in 2% loss of accuracy unless Red Line and Zero are reset.

Recorder Output:

0 to 114 to 136 mV. Recorder should have 50,000 ohms minimum input impedance.

Power Supply:

The YSI Model 57 is powered by two disposable "C" size carbon zinc batteries (Eveready 935C or equal) providing approximately 1000 hour operation.

2. Probe

Cathode: Gold
Anode: Silver
Membrane: 001" FEP Teflon
Electrolyte: Half saturated KCl

Temperature Compensation (See SPECIFICATIONS, 1. Instrument)

Pressure Compensation Effective 1/2% of reading with pressures to 100 psi (230 ft. sea water)
Polarizing Voltage: 0.8 volts normal
Probe Current: Air at 30°C = 19 microamps nominal
Nitrogen at 30°C = .15 microamps or less

4.3.4 Measurement Procedure

With the instrument prepared for use and, the probe calibrated, place the probe in the sample to be measured and provided stirring.

1. Stirring for the 5739 Probe can best be accomplished with a YSI submersible stirrer. Turn the STIRRER knob ON. If the submersible stirrer is not used, provide manual stirring by raising and lowering the probe about 1 ft. per second. If the 5075A Calibration Chamber is used, the entire chamber may be moved up and down in the water at about 1 ft. per second.

2. Adjust the SALINITY knob to the salinity of the sample.

3. Allow sufficient time for probe to stabilize to sample temperature and dissolved oxygen. Read dissolved oxygen.

4.3.5 Calibration

The operator has a choice of three calibration methods: Winkler Titration; Saturated Water; and Air. Experience has shown that air calibration is quite reliable, yet far simpler than the other two methods. Only the air method is presented here.

Air Calibration

1. Place the probe in moist air. BOD probes can be placed in partially filled (50 mL) BOD bottles. Other probes can be placed in the YSI 5075A Calibration Chamber (refer to the following section describing calibration chamber) or the small storage bottle (the one with the hole in the bottom) along with a few drops of water. The probe can also be wrapped loosely in a damp cloth taking care the cloth does not touch the membrane. Wait approximately 10 minutes for temperature stabilization.

2. Switch to TEMPERATURE and read Refer to Table 38.1 - Solubility of Oxygen in Fresh Water, and determine calibration value.

3. Determine altitude or atmospheric correction factor from Table 38.2.

4. Multiply the calibration value from Table 38.1 by the correction factor from Table 38.2.

   EXAMPLE: Assume temperature = 21°C and altitude = 1000 feet. From Table 38-1, the calibration value for 21°C is 8.9 mg/l. From Table 38-2 the correction factor for 1000 feet is about 0.96. Therefore, the correct calibration value is 8.9 mg/l X 0.96 = 8.54 mg/l.

5. Switch to the appropriate mg/l range, set the SALINITY knob to zero and adjust the CALIBRATE knob until the meter reads the correct calibration value from Step 4. Wait two minutes to verify calibration stability. Readjust if necessary.

   The probe is now calibrated and should hold this calibration value for many measurements. Calibration can be disturbed by physical shock, touching the membrane, or drying out of the electrolyte. Check calibration after each series of measurements and in time you will develop a realistic schedule for recalibration. For best results when not in use, follow the storage procedures recommended for the probes described under probe Maintenance (Section 4.4.1). This will reduce drying out and the need to change membranes.

4.4 MAINTENANCE AND TROUBLESHOOTING

4.4.1 Probe Maintenance

Keep the probe clean and avoid letting the KCl dry in the probe cavity. When changing the membrane, flush out the probe cavity with KCl solution several times. Do not use abrasives to polish the surface of the gold and plastic. Wipe gently with a soft, lint-free cloth (if required).

It should be noted that some other gases can be reduced at the cathode at the polarizing voltage for oxygen. Included are SO_2 and Halogens. H_2S reacts with the metals and poisons the cell. This poisoning can usually be overcome by periodic wiping of the gold surface with a clean, lint-free, coarse cloth or a hard paper. Do

not use any form of abrasive. All poisoning shows as tarnish on the gold and polishing should continue until the gold is shiny.

4.4.2 Instrument Maintenance

Model 57 contains two separate circuits: (1) A temperature bridge circuit; and (2) an amplifier for oxygen measurement. The amplifier is a six resistor balanced-feedback amplifier featuring good temperature stability, low voltage power requirements, and long battery life. Current from the oxygen probe develops a voltage across a resistor network which includes a thermistor (kept at O_2 probe temperature). This voltage is applied to the input of the circuit. A portion of the amplifier output is applied to the amplifier input in a standard negative feedback configuration.

4.4.3 Instrument Batteries

The instrument batteries are two "C" size carbon-zinc cells located inside the instrument on the meter end. These should be replaced when the RED LINE know is at its extreme adjustment or at least annually. The amount of remaining adjustment is an indication of the battery condition. the batteries are replaced by removing the screws on the rear cover of the instrument and removing the two batteries at the end of the instrument near the meter. When installing the new batteries the plus (+) end fits into the red washer on the battery holding.

5. SUMMARY OF OPERATING INSTRUCTIONS

5.1 Calibration

A. Switch instrument to OFF and adjust meter mechanical zero.
B. Switch to RED LINE and adjust.

C. Prepare probe for operation, plug into instrument, wait up to 15 minutes for probe to stabilize. Probe can be located in calibration chamber (see instruction manual) or ambient air.
D. Switch to ZERO and adjust.
E. Adjust SALINITY know to FRESH.
F. Switch to TEMP and road.
G. Use probe temperature and true local atmospheric pressure (or feet above sea level) to determine correct calibration values from Table 38.1 and 38.2.

EXAMPLE: Probe temperature = 21°C; Altitude = 1000 feet. From Table 38-1 the calibration value for 21°C is 8.9 mg/l. From Table 38-2 the altitude factor for 1000 feet is approximately .96. The correct calibration value is:

8.9 mg/l X. 96 factor = 8.54 mg/l

H. Switch to desired dissolved oxygen range 0-5, 0-10, or 0-20 and with calibrate control adjust meter to correct calibration value determined in Step G.

NOTE: It is desirable to calibrate probe in a high humidity environment. See instruction manual for more detail on calibration and other instrument and a probe characteristics.

5.2 MEASUREMENT

A. Adjust the SALINITY know to the salinity of the sample.
B. Place the probe and stirrer in the sample and switch and STIRRER control to ON.
C. When the meter has stabilized switch to the appropriate range and read D.O.
D. It is recommended that the instrument be left on between measurements to avoid necessity for repolarizing the probe.

5.3 GENERAL CARE

A. Replace the instrument batteries when unable to adjust to red line. Use two, Eveready No. 935 "C" size or equivalent.
B. In the BATT CHECK position the voltage of the stirrer batteries is displayed on the red 0-10 scale. Do not discharge below 6.0 volts. Recharge for 14-16 hours with YSI No. 5728 charger.
C. Membrane will last indefinitely, depending on usage. Average replacement is 2-4 weeks. Probe should be stored in humid environmental to prevent drying out.
D. Calibrate daily.

TABLE 38-1 - Solubility of Oxygen in Fresh Water

Table I shows the amount of oxygen in mg/l that is dissolved in air saturated fresh water at sea level (760 mmHg atmospheric pressure) as temperature varies from 0° to 45°C.

Table I — Solubility of Oxygen in Fresh Water

Temperature °C	mg/l Dissolved Oxygen	Temperature °C	mg/l Dissolved Oxygen
0	14.60	23	8.56
1	14.19	24	8.40
2	13.81	25	8.24
3	13.44	26	8.09
4	13.09	27	7.95
5	12.75	28	7.81
6	12.43	29	7.67
7	12.12	30	7.54
8	11.83	31	7.41
9	11.55	32	7.28
10	11.27	33	7.16
11	11.01	34	7.05
12	10.76	35	6.93
13	10.52	36	6.82
14	10.29	37	6.71
15	10.07	38	6.61
16	9.85	39	6.51
17	9.65	40	6.41
18	9.45	41	6.31
19	9.26	42	6.22
20	9.07	43	6.13
21	8.90	44	6.04
22	8.72	45	5.95

Source: Derived from 15th Edition "Standard Methods for the Examination of Water and Wastewater."

TABLE 38-2 - Altitude Correction Factor

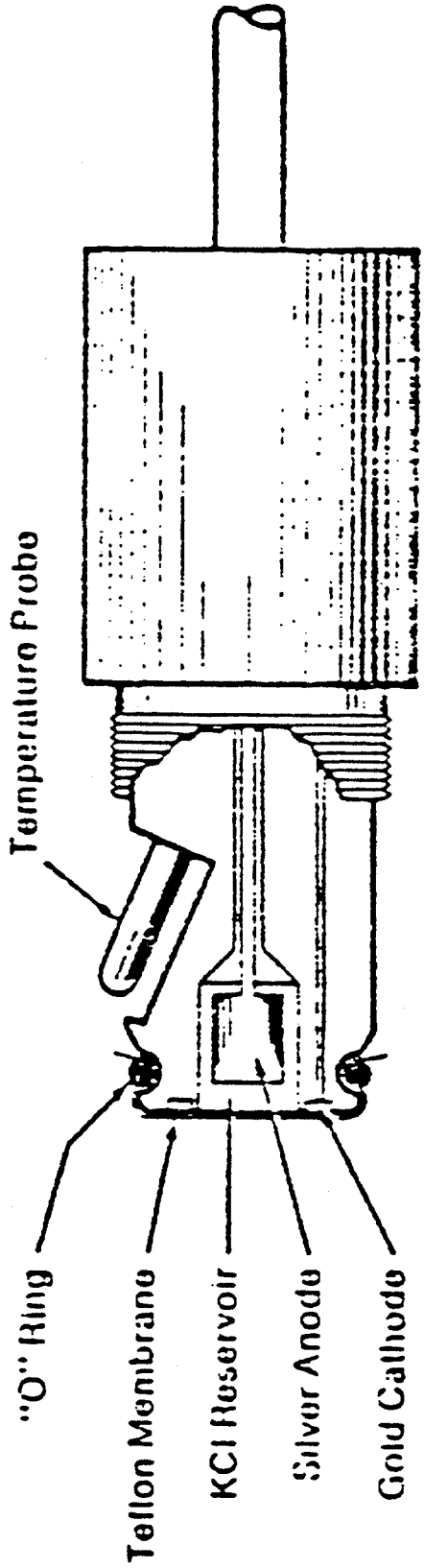
Table II shows the correction factor that should be used to correct the calibration value for the effects of atmospheric pressure or altitude. Find true atmospheric pressure in the left hand column and read across to the right hand column to determine the correction factor. (Note that "true" atmospheric pressure is as read on a barometer. Weather Bureau reporting of atmospheric pressure is corrected to seal level.) If atmospheric pressure is unknown, the local altitude may be substituted. Select the altitude in the center column and read across to the right hand column for the correction factor.

Table II — Altitude Correction Factor

Atmospheric Pressure mmHg	or	Equivalent Altitude Ft.	=	Correction Factor
775		540		1.02
760		0		1.00
745		542		.98
730		1094		.96
714		1688		.94
699		2274		.92
684		2864		.90
669		3466		.88
654		4082		.86
638		4756		.84
623		5403		.82
608		6065		.80
593		6744		.78
578		7440		.76
562		8204		.74
547		8939		.72
532		9694		.70
517		10472		.68
502		11273		.66

Source: Derived from 15th Edition "Standard Materials for the Examination of Water and Wastewater."

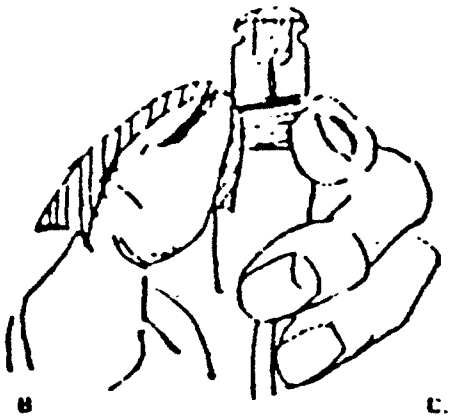
FIGURE 38-1



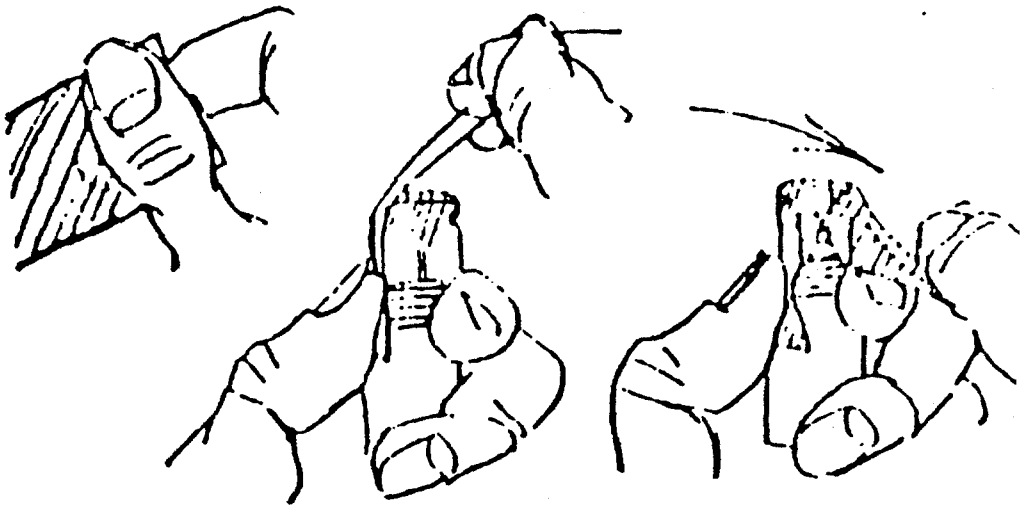
TYPICAL YSI 5400 SERIES O_2/TEMPERATURE ELECTRODE

FIGURE 38-2

A
Grasp threaded section of probe between left thumb and fore finger.

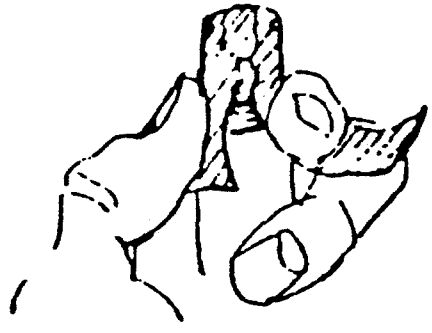


B
Secure one end of membrane under thumb. Use eyedropper to fill probe and wet "O" ring groove with KCl solution.



C.
With right thumb and forefinger grasp free end of membrane.

D.
Using a continuous motion stretch the membrane up over down the ot side. Stretching forms the membrane down the sides of the probe.

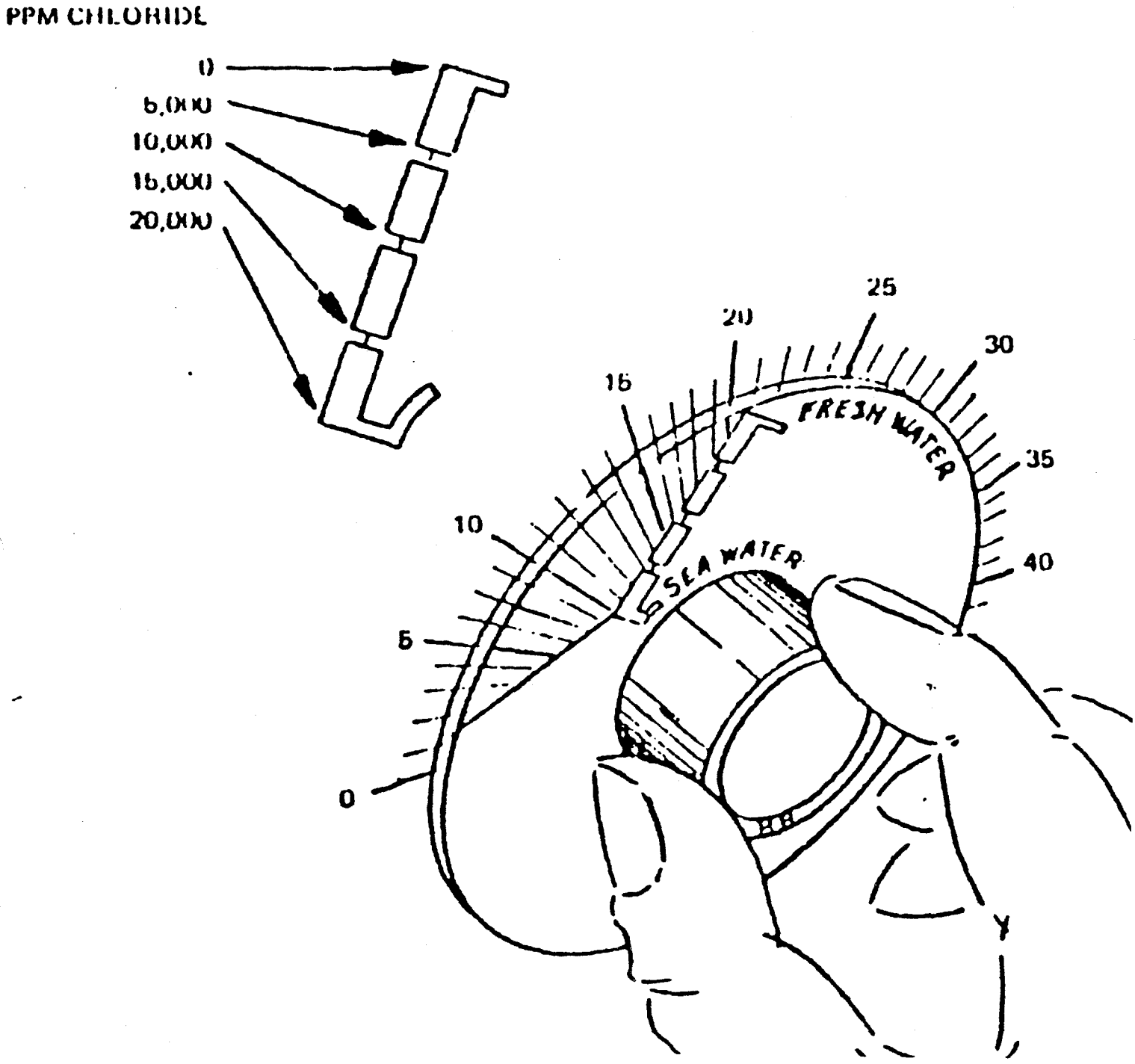


E
Secure the membrane under the left forefinger

F.
Roll on the "O" ring. Inspect to see there are no trapped air bubbles or wrinkles in the membrane.

G
Trim off excess membrane near the "O" ring. Temperature sensor must be exposed for rapid response.

FIGURE 38-3


PPM CHLORIDE
0
5,000
10,000
15,000
20,000
0
5
10
15
20
25
30
35
40
FRESH WATER
SEA WATER

Appendix P

1. OBJECTIVE

This guideline summarizes the steps necessary to use the YSI Model 33 S-C-T (Salinity, Conductivity, and Temperature) Meter. For this program, conductivity and temperature will be recorded using this instrumentation.

2. LIMITATIONS

This guideline is applicable to all aqueous samples. Measurements should be taken in-situ or as soon as possible after sample collection to avoid sample changes which can affect the readings.

4. GUIDELINES

4.1 GENERAL

The YSI Model 33 S-C-T meter is a portable, battery powered, transistorized instrument designed to accurately measure salinity, conductivity, and temperature. It uses a probe consisting of a plastic conductivity cell and a thermistor temperature sensor combined in a single unit.

4.2 RESPONSIBILITIES

The project team leader or site manager is responsible for determining when the readings should be taken, in accordance with the site-specific work plans. Generally, field measurements of salinity/conductivity and temperature are made whenever an aqueous sample is taken. The field samplers are responsible for taking the measurements and recording and reporting the results.

4.3 OPERATING INSTRUCTIONS

4.3.1 INITIAL USE

4.3.1.1 UNPACKING

Unpack the instrument and check that all items and accessories are present and undamaged. (This should be checked prior to use in the field.) The following items should be present:

-- YSI MODEL 33 S-C-T METER

-- Combination conductivity/temperature probe

-- Instruction manual

4.3.1.2 BATTERY INSTALLATION/CHANGE

To install the battery, unscrew the rear cover and fit the two "D" batteries (alkaline) into the battery holder. Replace the rear cover.

4.3.1.3 ELECTRODE INSTALLATION

Connect the probe to the side of the unit.

4.3.2 INSTRUMENT DESCRIPTION

4.3.1.2 DESCRIPTION

Conductivity

-- Ranges: 0 to 500 (x 1), 0 to 5,000 (x 10), and 0 to 50,000 micromhos/cm (x 100). The "micromho" designations on the meter are a shorthand form for "micromhos/cm".

-- Accuracy: See Error Section in Manual.

±2.5% maximum error at 500, 5,000 and 50,000 plus probe.
±3.0% maximum error at 250, 2,500 and 25,000 plus probe.

-- Readability: 2.5 micromhos/cm on 500 micromho/cm range.
25 micromhos/cm on 5,000 micromho/cm range.
250 micromhos/cm on 50,000 micromho/cm range.

-- Temperature Compensation: None.

Temperature Range -2° to +50°C

-- Accuracy: ±0.1°C at -2°C, ±0.6°C at 45°C, plus probe error. See Error Section in Manual.

-- Readability: 0.15°C at -2° to 3°C at 45°C.

-- Power: Supplied by two D-size alkaline batteries. Eveready E95 or equivalent, provide approximately 200 hours of operation.

-- Instrument Ambient Range: -5° to +45°C. A maximum error of ±0.1% of the reading per °C change in instrument temperature can occur. This error is negligible if the instrument is readjusted to redline for each reading.

YSI 3000 SERIES CONDUCTIVITY/TEMPERATURE PROBE

-- Nominal Probe Constant: K = 5/cm (K = 500/m).

-- Accuracy: ±2% of reading for conductivity and salinity. Error of ±0.1°C at 0°C and ±0.3°C at 40°C.

4.3.3 OPERATION

Setup

1. Adjust meter zero (if necessary) by turning the bakelite screw on the meter face so that the meter needle coincides with the zero on the conductivity scale.

2. Calibrate the meter by turning the MODE control to REDLINE and adjusting the REDLINE control so the meter needle lines up with the redline on the meter face. If this cannot be accomplished, replace the batteries.

3. Plug the probe into the probe jack on the side of the instrument.

4. Put the probe in the solution to be measured. (See PROBE Use.)

Temperature

Set the MODE control to TEMPERATURE. Allow time for the probe temperature to come to equilibrium with that of the water before reading. Read the temperature on the bottom scale of the meter in degrees Celsius.

Conductivity

1. Switch to x 100. If the reading is below 50 on the 0-500 range (5.0 on the 0-50 mS/m range), switch to x 10. If the reading is still below 50 (5.0 mS/m), switch to the x 1 scale. Read the meter scale and multiply the reading appropriately. The answer is expressed in micromhos/cm (mS/m). Measurements are not temperature compensated.

Example: Meter Reading	:	247 (24.7 mS/m)
Scale	:	x 10
Answer	:	2470 micromhos/cm (247.0 mS/m)

2. When measuring on the x 100 and x 10 scales, depress the CELL TEST button. The meter reading should fall less than 2%; if greater, the probe is fouled and the measurement is in error. Clean the probe and re-measure.

   Note: The CELL TEST does not function on the x 1 scale.

Conductivity Error

Figure 48-2 shows the worst-case conductivity error as a function of the conductivity reading for the probe and instrument combined.

Example:	Meter Reading	:	360 micromhos/cm (36 mS/m)
	Scale	:	x 10
	% Reading Error	:	±4.5%
	Accuracy	:	3600 ±162 micromhos/cm (360 ±16.2 mS/m) for probe and instrument

Instrument Maintenance

The only maintenance required is battery replacement. Two "D" size alkaline batteries will provide 200 hours of operation. Accuracy will not be maintained if zinc-carbon "D" cells are used. Battery replacement is indicated when the redline adjustment cannot be accomplished.

Replace batteries every six months to reduce the danger of corrosion due to leaky batteries. To replace batteries, remove the screws from the rear cover. The battery holders are color coded. The positive end must go on red.

Recalibration

Recalibration should be done at the factory.

YSI 3300 SERIES CONDUCTIVITY/TEMPERATURE PROBES

Description

These probes are designed and constructed for rugged, accurate service in field use. The conductivity cell constant is 5.0/cm (500.0m) ±2%. Each probe contains a precision YSI thermistor temperature sensor of +0.1°C accuracy at 0°C and ±0.3°C at 40°C. The low capacity cable assembly terminates in a three terminal 0.25 inch diameter phone plug.

Cleaning and Storage

When the cell test indicates low readings, the probable cause is dirty electrodes. Hard water deposits, oils and organic matter are the most likely contaminants.

For convenient normal cleaning, soak the electrodes for 5 minutes with a locally available bathroom tile cleaning preparation such as Lysol Brand "Basin, Tub, Tile Cleaner."

For stronger cleaning, a 5 minute soak in a solution made of 10 parts distilled water, 10 parts isopropyl alcohol, and 1 part HCl can be used.

Always rinse the probe thoroughly in tap water, then in distilled or deionized water after cleaning and before storage.

Caution: Do not touch the electrodes inside the probe. Platinum black is soft and can be scraped off.

If cleaning does not restore the probe performance, replatinizing is required.

Storage

It is best to store conductivity cells in deionized water. Cells stored in water require less frequent platinization. Any cell that has been stored dry should be soaked in deionized water for 24 hours before use.

Replatinization

Return to factory for replatinization.

Probe Use and Precautions

1. Obstructions near the probe can disturb readings. At least two inches of clearance must be allowed from non-metallic underwater objects. Metallic objects such as piers or weights should be kept at least six inches from the probe.

2. Weights are attached to the cable of the YSI 3310 and 3311 probes. The YSI 3327 weights are supplied in pairs with a total weight of 4 ounces per pair. Should it become necessary to add more weight to overcome water currents, limit the total weight to two pounds (8 pairs). For weights in excess of two pounds, use an independent suspension cable. In either case, weights must be kept at least six inches away from the probe.

3. Gentle agitation by raising and lowering the probe several times during a measurement improves flow of specimen solution through the probe and improves the time response of the temperature sensor.

Conductivity and Salinity Corrections for Long Cables

The additional length of wire in long cables adds capacitance and resistance which will affect readings. The recommended way to correct for this influence is by the use of YSI Conductivity Calibrator Solutions which will permit an estimate of correction factors.

Cell Calibration and Standard Solutions

The cell constant of a conductivity cell may vary slightly with the conductivity of the solution being measured. Cell calibration may also be affected by electrode fouling, replatinization, or by mechanical shock. A cell and meter can be calibrated together, as a system with conductivity calibrator solutions.

YSI conductivity calibrator solutions are supplied with a technical discussion and instructions for use. Directions for calibration at temperatures other than 25° are included with the conductivity calibration solutions. In calculating the cell constant in absolute terms, the uncertainty of the meter calibration must be added to the tolerance of the conductivity calibrator solution.

FIGURES 48-1

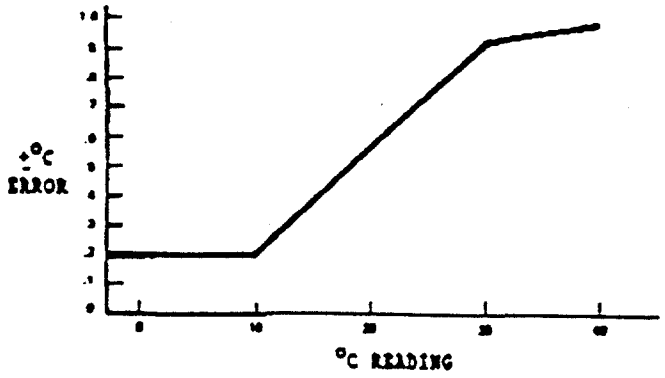


FIGURE 48-2

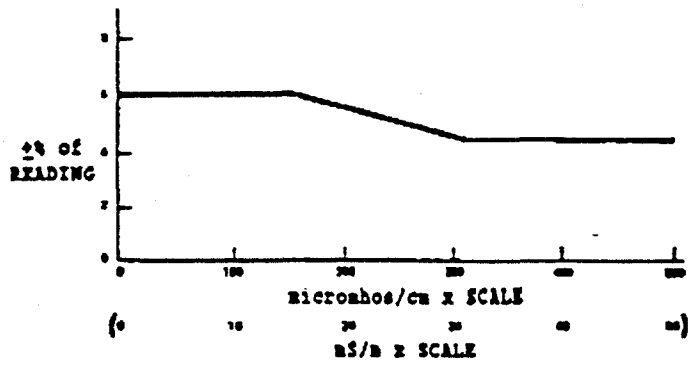
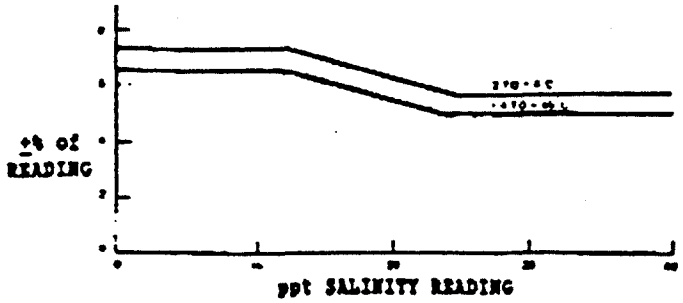


FIGURE 48-3



Appendix Q

Appendix Q

SURVEYING OF SHORELINE CONTROL POINTS AND RIVER SAMPLING POINTS

1.0 Scope and Application

In order to ascertain the location of sediment sampling points and geophysical measurements in the river, a surveyor will be required to establish shoreline control points to which riverine measurement points can be referenced. Shoreline control points will be established along the Hudson in all areas of study. These points will be used for sediment sampling and geophysical work only. The column station locations coincide with established bridge and dam locations along the Hudson River.

2.0 Shoreline Control Points

The shoreline control points will be referenced to the New York State Plane Coordinate System, North American Datum, (NAD 1927) since most of the historic work in the Hudson was referenced to this coordinate system. The vertical datum will be based upon water level reported from the nearest downstream lock for areas of the Upper Hudson. All vertical datum will be referenced to the National Geodetic Vertical Datum of 1929 (NGVD 1929). To the extent possible, the shoreline control points as established by General Electric in 1991 (G.E., 1991) will be used. Five to ten percent of these points will be reconfirmed. Horizontal control for existing and new shoreline control points will be established to a minimum of Third Order, Class II (FGCC, 1984) under the supervision of certified inshore hydrographers (certification by the American Congress on Surveying and Mapping).

3.0 River Sampling Point Surveying

Sampling locations established for sediment collection and geophysical data collection will be surveyed from the shoreline control points described in Section 2 of this appendix. The surveying will be performed under the supervision of a certified inshore hydrographer. The method employed will use a laser based range/angle system accurate to $\pm$ 1m and capable of

a rapid update rate (0.7 sec). The geophysical investigation will require the rapid rate of update since the data is collected continuously while the geophysical survey boat is underway.

4.0 References

4.1 General Electric (GE), 1991. Draft Horizontal Control-Hudson River Project. Corporate Environmental Programs.

4.2 Federal Geodetic Control Committee (FGCC), 1984. Standards and Specifications for Geodetic Control Networks, National Oceanic and Atmospheric Administration Dept. of Commerce, Rockville, MD, PB85-166478.

Appendix R

1. OBJECTIVE

This guideline provides instructions for sample packaging and shipping of Contract Lab Program (CLP) samples in accordance with USEPA guidelines and U.S. Department of Transportation (USDOT) regulations.

2. APPLICABILITY

The guideline is applicable to shipment of all samples taken from controlled or uncontrolled hazardous substance sites for analysis at laboratories away from the site.

3. LIMITATIONS

These guidelines are to be used for low and medium concentration samples collected from hazardous substance sites. High concentration hazardous substance samples and radiation samples are not covered by this SOP.

4. DEFINITIONS

"Carrier" -- A person or firm engaged in the transportation of passengers or property.

"N.O.S." -- Not otherwise specified.

"N.O.I." -- Not otherwise indicated.

"ORM" -- Other regulated material.

"Environmental Samples" -- Samples with medium or low contaminant concentrations such as ambient air, streams, groundwater, leachates, ditches, soil, and sediments collected at a distance from direct sources of contaminants.

"USDOT Classifications for Hazardous Materials" -- Classifications used to classify materials for shipment are set forth by the USDOT in the Code of Federal Regulations (49 CFR 173.2):

5. GUIDELINES

Samples collected at controlled or uncontrolled hazardous substance sites usually are transported elsewhere for analysis. Samples shall be transported so as to protect their integrity, as well as to protect against any detrimental effects from

leakage or breakage. Regulations for packaging, marking, labeling, and shipping hazardous materials and wastes are promulgated by the U.S. Department of Transportation and described in the Code of Federal Regulations (49 CFR 171 through 177, in particular 172.402h, Packages Containing Samples collected at controlled or uncontrolled hazardous waste sites or samples collected during emergency responses). However, the USEPA has agreed through a memorandum of agreement to package, mark, label, and ship samples observing USDOT procedures.

5.1 RESPONSIBILITIES

The field SMO and ultimately the team leader is responsible for determining that the hazardous substance site samples are properly packaged and shipped. Sampling personnel are responsible for implementing the packaging and shipping requirements. The Chain-of-Custody procedures and requirements are described in TAMS' Standard Operating Procedure No. 60.

5.2 EQUIPMENT

The following equipment is used in packaging and shipping low concentration samples:

1. Sample bottles (provided by TAMS).

2. Polyethylene bags (2 mil or thicker).

3. Packing materials such as vermiculite, or "slick wick".

4. Picnic coolers or ice chests (preferably constructed of metal) capable of withstanding impact caused by a 4-foot drop.

The following additional equipment is used in packaging and shipping medium concentration samples:

1. Metal paint cans and lids (1 gallon or other sizes as appropriate).

5.3 ENVIRONMENTAL SAMPLES

5.3.1 Low Concentration Environmental Samples

5.3.1.1 Packing

Each environmental sample is packaged in a separate sealable polyethylene bag (both VOA vials may be put in one bag) and packed in metal (picnic cooler-type) containers. Sufficient noncombustible, absorbent cushioning material such as vermiculite will be used to minimize the possibility of sample container breakage. Ice is added to the cooler when sample preservation is required.

Ice cubes are put in sealable polyethylene bags and placed around samples before the packing material covers the bottles.

Organic and inorganic fractions are sent to separate laboratories. These fractions may be further split by matrix such that organic water samples may go to one lab and organic soil samples to another. SAS samples will also be shipped to a separate laboratory. If only soil samples are collected, field blanks and trip blanks will be sent, with the soil samples, to their respective organic and inorganic labs.

5.3.1.2 Marking and Labeling

A complete sample identification tag (as explained in SOP No. 60) shall be affixed to sample containers.

An address label shall be affixed to the shipping container, along with proper labels (e.g., "This End Up").

No USDOT marking or labeling is required.

5.3.1.3 Shipping Papers

No USDOT shipping papers are required for environmental samples. However, the appropriate Chain-of-Custody forms shall be included with the shipment.

5.3.1.4 Transportation

There are no USDOT restrictions on the mode of transportation for low concentration environmental samples.

5.3.2 Medium Concentration Environmental Samples

The procedures to be used to pack, label, mark, and ship hazardous waste samples are presented below.

5.3.2.1 Packaging

Packaging procedures are as follows:

a. After collection of sample in a properly labeled bottle (see TAMS SOP No. 60), seal sample bottle with a custody seal and place in 2 mil thick (or thicker) sealable polyethylene bag (one sample bottle per bag except VOA vials). Tags shall be positioned to enable visibility through the bag.

b. Place sealed bag inside a metal can with incombustible, absorbent cushioning (e.g., vermiculite) to deter breakage (one bag per can). Pressure-close the can and use clips, tape or other means to secure the lid tightly and effectively. Two VOA vials may be placed in one metal can.

c. Mark and label this container with CLP sample number and date.

d. Place one or more metal cans, surrounded by incombustible packaging material for stability during transport, into a metal picnic cooler. Place the ice (in sealed polyethylene bags) adjacent to the metal cans.

e. Mark and label the shipping container and complete shipping documents as described below.

5.3.2.2 Marking and Labeling

Use abbreviations only where specified. Place the following information (either hand-printed or on preprinted labels) on the cooler: laboratory name and address and "Flammable Liquid, N.O.S." (if not liquid, write "Flammable Solid, N.O.S."). Place the following labels on the outside of the cooler: "Cargo Aircraft Only" and "Flammable Liquid" (if not liquid, "Flammable Solid"). ("Dangerous When Wet" label should be used if the solid has not been exposed to wet environment.) Using "Flammable" does not convey the certain knowledge that a sample is in fact flammable, or how flammable, but is intended to prescribe the class of packaging in order to comply with DOT regulations.

The cooler shall also have "Laboratory Samples" and "THIS SIDE UP" (or "THIS END UP") marked on the top of the shipping cooler, and upward-pointing arrows should be placed on all four sides of the shipping cooler.

5.3.2.3 Shipping Papers

Complete the shipper's certification section of the airbill (Fig. 61.1) in the following manner:

1. Check "49 CFR"
2. Fill in number of coolers to be shipped
3. Fill in proper shipping category
   -- "Flammable Solids N.O.S." or "Flammable liquids N.O.S."
   -- Limited quantity; cargo aircraft only

   "Limited quantity" indicates:

Solids: the fraction of solids inside the glass container shall not exceed one pound net weight and the net quantity of solids in each cooler shall not exceed 25 lbs.

Liquids: liquids in volume of 32-oz or less are placed in metal cans and then packed in a durable outside (exterior) container. These are shipped cargo aircraft only, as the total volume in the cooler exceeds the one quart limit set for passenger aircraft (49 CFR173.118). Coolers shall not exceed 10 gallons flammable liquid N.O.S. when shipped by cargo aircraft.

4. Class or Division
   Fill in "Flammable Liquid or Flammable Solid" N.O.S.

5. UN or ID Number
   Flammable Liquid UN 1993 or Flammable Solid UN 1325

6. Subsidiary Risk
   (Leave Blank)

7. Total Net Quantity

   For Solids: State the number of coolers and the net quantity of flammable solid N.O.S. contained in each cooler (e.g., 2 @ 5 lbs.).

   For Liquids: State the number of coolers and then state the net quantity of flammable liquids in each cooler (e.g., 1@ 2 gallons).

   The net volume for each cooler cannot exceed 10 gallons of flammable liquid N.O.S.

8. Packing Instructions
(Leave Blank)

9. Authorization
(Leave Blank)

10. Additional Description Requirements for Radioactive Materials
(Leave Blank)

11. Shipping Limitations
Circle: "Cargo Aircraft Only"

12. Airport Departure/Destination
(Leave Blank: Federal Express agent will fill in

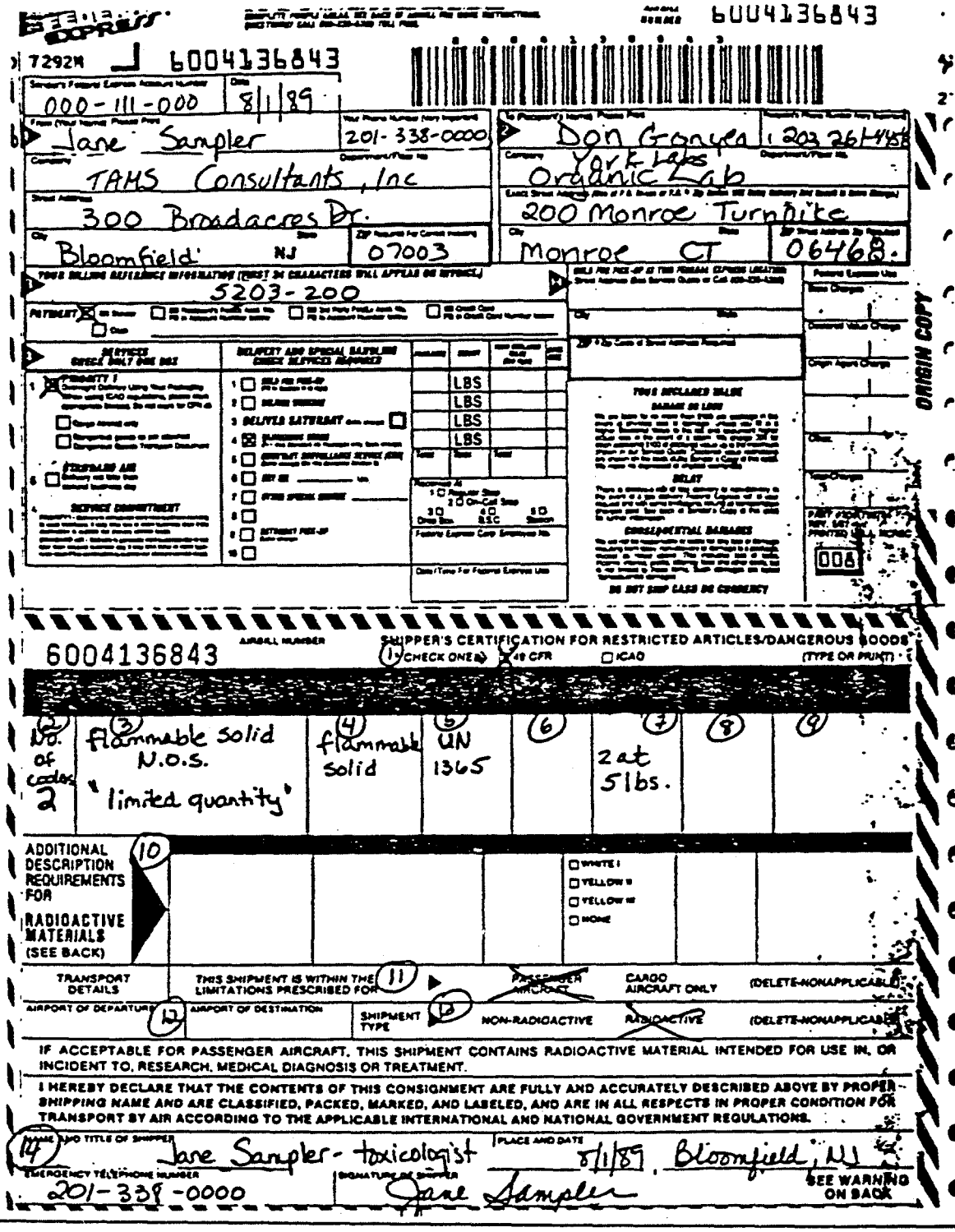
13. Shipment Type
Circle: "Non-radioactive"

14. Print Name & Title
TAMS Telephone Number
Signature of Shipper

Refer to Figure 61-1 for completed airbill/shipper's certification form. A Chain-of-Custody form (see Standard Operating Procedure No. 60) shall be executed and placed in the exterior container.

FIGURE 61-1 - AIRBILL/SHIPPER'S CERTIFICATION FORM

FIGURE 61-1 - AIRBILL/SHIPPER'S CERTIFICATION FORM



6004136843

7292M 6004136843

000-111-000 | 8/1/89

Jane Sampler | 201-338-0000

TAMS Consultants, Inc

300 Broadacres Dr.

Bloomfield NJ 07003

Don Graves | 1 203 261 4567

York Lakes Organic Lab

200 Monroe Turnpike

Monroe CT 06468

5203-200

ORIGIN COPY

6004136843

SHIPPER'S CERTIFICATION FOR RESTRICTED ARTICLES/DANGEROUS GOODS

(1) CHECK ONE ☒ 49 CFR ☐ ICAO (TYPE OR PRINT)

(2) No. of codes	(3)	(4)	(5)	(6)	(7)	(8)	(9)
2	flammable solid N.O.S. "limited quantity"	flammable solid	UN 1365		2 at 5 lbs.		

(10) ADDITIONAL DESCRIPTION REQUIREMENTS FOR RADIOACTIVE MATERIALS (SEE BACK)

☐ WHITE I ☐ YELLOW II ☐ YELLOW III ☐ NONE

TRANSPORT DETAILS: (11) THIS SHIPMENT IS WITHIN THE LIMITATIONS PRESCRIBED FOR ~~PASSENGER AIRCRAFT~~ CARGO AIRCRAFT ONLY (DELETE-NONAPPLICABLE)

(12) AIRPORT OF DEPARTURE | AIRPORT OF DESTINATION | (13) SHIPMENT TYPE: NON-RADIOACTIVE ~~RADIOACTIVE~~ (DELETE-NONAPPLICABLE)

IF ACCEPTABLE FOR PASSENGER AIRCRAFT, THIS SHIPMENT CONTAINS RADIOACTIVE MATERIAL INTENDED FOR USE IN, OR INCIDENT TO, RESEARCH, MEDICAL DIAGNOSIS OR TREATMENT.

I HEREBY DECLARE THAT THE CONTENTS OF THIS CONSIGNMENT ARE FULLY AND ACCURATELY DESCRIBED ABOVE BY PROPER SHIPPING NAME AND ARE CLASSIFIED, PACKED, MARKED, AND LABELED, AND ARE IN ALL RESPECTS IN PROPER CONDITION FOR TRANSPORT BY AIR ACCORDING TO THE APPLICABLE INTERNATIONAL AND NATIONAL GOVERNMENT REGULATIONS.

(14) NAME AND TITLE OF SHIPPER: Jane Sampler - toxicologist | PLACE AND DATE: 8/1/89, Bloomfield, NJ

EMERGENCY TELEPHONE NUMBER: 201-338-0000 | SIGNATURE OF SHIPPER: Jane Sampler

SEE WARNING ON BACK

Appendix S

Appendix S

Gradient Corporation

Field Quality Assurance Audit

Program: _____

Date of Audit: _____

Audit Conducted By: _____

On-Site Sampling Personnel: _____

_____

Audit Conducted on the Following:

_____ Sediment Sampling (Grab and Cove)

_____ River Water Sampling

_____ Decontamination Procedures

_____ Sample Handling, Labeling, Aliquoting Procedures

Y = Yes N = No N/A = Not Applicable N/D = Not Determined

I. Overall Sample Collection

		Yes	No
1.	Do sampling locations agree with those specified in the Work Plan/Sampling Plan?		
2.	Is the sampling location either documented sufficiently or marked to allow it to be found/sampled again in the future?		
3.	Are sampling times, Traffic Report Numbers and sample description noted in the Field Note Book (FNB)?		

	Yes	No
4. Have all field measurements been properly taken as per Sampling Plan? • pH • conductivity/temperature • Redox potential • dissolved oxygen		
5. Have sample bottles been labeled properly?		
6. Have proper containers and preservatives been used?		
7. Are proper sample volumes procured?		
8. Have sampling times been recorded in order to complete documentation on holding times per sample per parameter?		
9. Have weather conditions been recorded?		
10. Have MS and MSD(s) and MD(s) been collected as per QAPP frequencies?		
11. Have field blanks been collected as per QAPP frequencies?		
12. Have field duplicates been collected as per QAPP frequencies?		
13. Are samples being refrigerated/iced immediately after collection?		
14. Has condition of sample been recorded in the FNB and in the traffic report?		
15. Does the potential for sample cross-contamination exist based on procedures observed?		
16. Have samples been properly packaged and labeled for shipment to appropriate laboratory for analysis?		

	Yes	No
17. Have legal seal(s) been properly filed out and attached to the shipping containers?		
18. Has the Chain-of-Custody Form been properly filled out and a copy included with sample shipments?		

II. Sediment Sampling

1. Type: core _____ If core, state type: gravity core _____
hand core _____
vibracore _____

grab _____

	Yes	No
2. Is a description of sediment cores being logged?		
3. Have sediments been homogenized where applicable prior to aliquoting (specified by the Sampling Plan)?		
4. Are proper slicing/aliquoting procedures being followed as per QAPP?		
5. Has Redox been measured as per QAPP?		

Comments:

III. River Water Sampling

	Yes	No
1. Are PCB samples collected directly into precleaned bottles?		
2. Are samples for dissolved or particulate analyses filtered within 4 hours of collection?		
3. Are filtering procedures being followed as per QAPP?		
4. Are dissolved organic carbon samples being filtered and preserved?		
5. Are chlorophyll a samples being filtered and the filter properly placed in container for analysis?		
6. Are field pH, conductivity, and temperature and dissolved oxygen being measured and documented? Is there documentation of calibrating the instruments?		

Comments:

IV. Decontamination Procedures

		Yes	No
1.	Have all sampling materials (spatulas, spoons, etc.) and equipment (e.g. filter apparatus) been decontaminated properly for the given analytes as per QAPP?		
2.	Have the proper decontamination solutions been used?		
3.	Has decontamination water/solution been collected for proper disposal?		
4.	Has disposable equipment that is contaminated been properly deconned and disposed of?		
5.	Have decon samples been taken from the sampling equipment as per Sampling Plan?		

Comments:

V. General

	Yes	No
1. Has all appropriate information been recorded in the FNB?		
2. Are employees conducting the investigation in a professional manner?		
3. Are the objectives of the sampling activities understood by the field personnel?		
4. Are weather conditions affecting sample quality?		

Comments:

Audit Summary and Comments:

Signed by:

Sampler: _____ Print Name: _____

Auditor: _____ Date: _____

Appendix T

Appendix T

Gradient Corporation

Laboratory Being Audited: _____

Date of Audit: _____

Audit Conducted By: _____

Laboratory Evaluation Checklist

I. Orientation Meeting

A. Organization and Personnel

1. Laboratory or Project Manager (individual responsible for overall technical effort): _____

2. Inorganic Laboratory Supervisor (BS or BA in science and 3 years related experience, including 1 year as a supervisor): _____

3. Organic Laboratory Supervisor (BS or BA in science and 3 years experience including 1 year as a supervisor): _____

4. Analyst(s) for Inorganic analyses (BS or BA in science and 1 year direct experience for each technique or instrumental analysis): _____
_____

5. Analyst(s) for Organic Analyses (BS or BA in science and 1 year direct experience for each technique or instrumental analysis): _____
_____

6. Inorganic Sample Preparation Specialist(s) (High School Diploma and college level course in chemistry and 6 months related experience): _____
_____

7. Organic Sample Preparation Specialist(s) (High School Diploma and college level course in chemistry and 6 months related experience): _____
_____

8. Back-up Technical Personnel (BS or BA in science and 1 year experience in each technique or instrumental analysis including: GC, classical wet chemistry techniques, biological properties, radiochemistry, geophysical properties as applicable to the program): _____
_____
_____

9. Quality Assurance Director: _____

10. Glassware Preparation Technician: _____

11. Sample Custodian: _____

12. Is the organization adequately staffed to meet project commitments in a timely manner? _____

13. Are resumes for all listed personnel available? Do the resumes demonstrate that the personnel meet education and experience requirements? _____

_____

14. Were all key personnel available during the on-site visit? _____

15. To whom does the QA Officer report? Senior Management?

_____

B. Documentation

1. Is the written QA Manual available and satisfactory for the following:

Personnel _____
Facilities and equipment _____
Operation of instruments _____
Documentation of procedures _____
Preventive maintenance _____
Reliability of data _____
Feedback and corrective action _____
Archival of outdated SOPs _____

Comments:

2. Are written SOPs available and satisfactory for the following?

Receipt and storage	_____	Data package preparation	_____
Security	_____	Standards preparation	_____
Glassware cleaning	_____	Technical review of data	_____
Sample preparation	_____	Instrument maintenance	_____
Analytical methods	_____	QA/QC self inspection	_____

Comments:

II. Laboratory Tour - When touring facilities, give special attention to: (a) the overall appearance of organization and neatness, (b) the proper maintenance of facilities and instrumentation, (c) the general adequacy of the facilities to accomplish the required work.

A. Sample Receipt and Storage Area

	Yes	No
1. Is the sample receiving area secure?		
2. Are coolers opened in a contamination free area (preferably in a functional hood)?		

		Yes	No
3.	Does the observed sample log-in procedure agree with the written SOP?		
4.	Is the appropriate portion of the SOP available to the Sample Custodian? Is the temperature of the cold storage recorded daily?		
5.	Are adequate facilities provided for storage of samples, including cold storage?		
6.	Are the sample receipt/storage and temperature records maintained in a manner consistent with good laboratory practice (GLP)?		
7.	Is there a periodic document review by the supervisor?		

Comments:

B. Preparation Area

1. General Operation	Yes	No
a. Does the laboratory appear to have adequate workspace (120 sq. feet, 6 linear feet of unencumbered bench space per analyst)?		
b. Is the laboratory maintained in a clear and organized manner that is appropriate for trace level analysis?		
c. Are the hoods functional and periodically checked?		
d. Does the laboratory have source of distilled/ demineralized water and is the water quality routinely checked and documented?		
e. Is the analytical balance located away from drafts and areas subject to rapid temperature changes?		
f. Has the balance been calibrated within 1 year by a certified technician?		
g. Is the balance routinely checked with the appropriate range of class S weights before use and are the results recorded in a logbook?		
h. Is an adequate drying oven available with a temperature measurement device? Is the temperature being monitored?		

Comments:

2. Glassware Washing — Yes — No

   a. Is the SOP for glassware washing posted and is it followed by the technician(s)?

   b. Does the SOP prescribe an adequate amount of acid or solvent treatment of the glassware and DI water rinses?

Comments:

3. Standards Preparation — Yes — No

   a. Is the appropriate SOP available in the area for preparation and traceability?

   b. Are unexpired standards used to prepare instrument calibration standards? Are standards dated upon receipt?

   c. Are fresh analytical standards prepared at a frequency consistent with good QA?

		Yes	No
d.	Are standards properly labeled with concentrations, date of preparation, date of expiration, and the identity of the person preparing the standard?		
e.	Is the reference/spiking/ calibration standards preparation and tracking logbook(s) maintained?		
f.	Does the laboratory use automatic pipets for preparing their standards? If yes, are these pipet calibrated on a routine basis?		
g.	Are reagent grade or higher purity chemicals used to prepare standards?		
h.	Are the primary standards traceable to EPA reference standards?		

Comments:

4. Sample Preparation Area	Yes	No
a. Is the Sample Preparation SOP available in the area? Do the procedures followed by the analyst agree with those documented in the SOP?		
b. Are the digestion logbooks/bench sheets maintained in a manner consistent with the QAPP or contractual requirements?		
c. Is the pH of the samples recorded and available for data review?		
d. Are standards stored separately from the digestates or extracts?		
e. Do the digested/extracted sample batches examined contain method blanks, LCSs, duplicates, and matrix spikes where applicable for inorganic parameters? Contain LCS, MS, MSD, method blanks for organic parameters? List SDG# examined in Audit: _____		
f. Are the samples measured and transferred to the beakers or extraction vessels as per method SOP (by volume, weight, etc.)?		
g. Is a consistent procedure used for transferring the sample numbers from the sample bottles to the extraction/digestion beakers or vials?		
h. Is the QC digested/extracted with the samples?		

		Yes	No
i.	Can the laboratory supervisor document that organic-free water or inorganic analyte free water is available for preparation of standards and blanks? (Method blank data must be available for confirmation.)		
j.	Are solvent storage cabinets vented or located in such a way as to prevent possible laboratory contamination? (Confirm by method blank data.)		

Comments:

		Yes	No
k.	Are analytical reagents dated upon receipt?		
l.	Are solvents and acids and other reagents used in preparations checked routinely per lot number received by vendor prior to routine use in the lab?		
m.	Are the temperatures of the refrigerators/freezers recorded daily?		

		Yes	No
n.	Are temperature excursions noted and appropriate actions taken when required?		

Comments:

C.	Sample Analysis Instrumentation	Yes	No
1.	Are manufacturer's operating manuals readily available to the operators?		
2.	Are extensive in-house replacement parts available? Does the laboratory maintain a service contract?		
3.	Is a permanent service maintenance record maintained in a logbook? For routine, and non-routine maintenance?		
4.	Is the instrument properly vented or are appropriate traps in place?		
5.	Is raw data being archived properly (i.e. magnetic tape storage)?		
6.	Is a log of the contents of the raw data magnetic tapes available?		

		Yes	No
7.	Can the instrument operator demonstrate, using the instrument run log, that corrective actions have been taken when required (e.g., reruns)?		
8.	Are reruns performed when internal standard areas are out?		
9.	Are analytical blanks run when the previous sample showed saturation?		
10.	Has two column verification of PCB congeners been performed?		

Comments:

_____

_____

_____

_____

_____

_____

_____

_____

III. Data Handling and Review		Yes	No
1.	Does the laboratory analyst perform a primary review of the data as stated in the program QAPP?		
2.	Are data calculations spot-checked by a secondary reviewer?		
3.	Do records indicate that appropriate corrective action has been taken when analytical results fail to meet QC criteria?		
4.	Are computer programs validated before use?		

	Yes	No
5. Do supervisory personnel review the data and QC results?		
6. Does the laboratory QA Director check 5% of all projects generated?		

Comments:

IV. Quality Control/Quality Assurance - General

	Yes	No
1. Can the Quality Assurance Officer document the analysis of blind laboratory QA or PE samples?		
2. Does the laboratory maintain a project specific Quality Control Manual?		
3. Are outdated portions of the QC Manual properly archived?		
4. Does the manual address the important elements of a QC program, including the following?		
a. Personnel?		
b. Facilities and equipment?		
c. Operation of Instruments?		
d. Documentation of procedures?		
e. Preventive Maintenance?		
f. Reliability of Data?		

		Yes	No
g.	Data Validation?		
h.	Feedback and corrective action?		
i.	QA/QC self-inspection?		

Comments:

_____
_____
_____
_____
_____
_____
_____
_____

V.	Summary Checklist	Yes	No
1.	Do responses to the evaluator indicate that project and supervisory personnel are aware of QA/QC and its applications to the project?		
2.	Do project and supervisory personnel place positive emphasis on QA/QC?		
3.	Have responses with respect to QA/QC aspects of the project been open and direct?		
4.	Has a cooperative attitude been displayed by all project and supervisory personnel?		
5.	Have any QA/QC deficiencies been discussed before leaving?		
6.	Have corrective actions recommended during previous evaluations been implemented? If not, provide details below.		

Comments:

Table 9-3

PCB Congeners - Required Detection Limits

Matrix	Homolog	Detection Limit
Particulates	Monochlorobiphenyl	2 µg/filter
	Dichlorobiphenyl through Hexachlorobiphenyl	1 µg/filter
	Heptachlorobiphenyl through Decachlorobiphenyl	1-2 µg/filter
Sediment (approx. 2 grams)	Monochlorobiphenyl	1 µg/kg
	Dichlorobiphenyl through Hexachlorobiphenyl	0.5 µg/kg
	Heptachlorobiphenyl through Decachlorobiphenyl	0.5-1 µg/kg
Water (20 liters)	Monochlorobiphenyl	0.1 ng/l
	Dichlorobiphenyl through Hexachlorobiphenyl	0.05 ng/l
	Heptachlorobiphenyl through Decachlorobiphenyl	0.05-0.1 ng/l
Water (1 liter)	Monochlorobiphenyl	1.0 ng/l
	Dichlorobiphenyl through Hexachlorobiphenyl	0.5 ng/l
	Heptachlorobiphenyl through Decachlorobiphenyl	0.5-1 ng/l

Gradient Corporation

March 20, 1992

Mr. Douglas Tomchuk
USEPA Region II
26 Federal Plaza
Room 747
New York, NY 10278

Subject: Replacement page for Hudson River, Phase 2A SAP/QAPP, Revision 1

Dear Mr. Tomchuk:

Please find enclosed a replacement Table 9 for the Hudson River RI/FS, Phase 2A SAP/QAPP, Revision 1, previously submitted to EPA on 3/18/92. We discovered a typographical error in the units listed for the water PCB congener detection limits. Please discard Table 9 in the original document and insert this corrected version. We regret any inconvenience this may cause you.

If there are any questions about the content of this SAP/QAPP revision, please do not hesitate to contact me or Dr. A. Dallas Wait at 617-576-1555.

Sincerely,

GRADIENT CORPORATION

Susan D. Chapnick

Susan D. Chapnick
Senior Associate, Analytical Chemistry

Enclosure

cc: Ms. Laura Scalise, USEPA Region II, Edision, NJ
Dr. A. Dallas Wait, Gradient Corp.
Dr. Edward Garvey, TAMS, Inc.

CC: D.Merrill, Gradient
E.Garvey, TAMS

44 Brattle Street, Cambridge, MA 02138 • (617)576-1555 • Telefax (617)864-8469
Environmental Consultants with Offices in Cambridge, MA and Boulder, CO