PESTICIDES/PCBS ANALYSIS OF TISSUE SAMPLES BY GC/ECD

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PESTICIDES/PCBS ANALYSIS OF TISSUE SAMPLES BY GC/ECD

1.0 SCOPE AND APPLICATION

This analytical procedure is applicable to the determination of organochlorinated pesticides and polychlorinated biphenyls (PCBs) in tissue matrices, using a gas chromatograph (GC) electron capture detector (ECD) method. The compounds of interest can be found in Appendix A.

2.0 METHOD SUMMARY

Ten-gram aliquots of a tissue homogenate sample are dried with anhydrous sodium sulfate and Soxhlet extracted with methylene chloride solvent. The methylene chloride extract is cleaned-up by Gel Permeation Chromatography (GPC); solvent exchanged to hexane and then concentrated to 1-mL final extract volume. The extracts are analyzed using GC/ECD. A second column is always used for confirmation whether pesticide/PCBs are tentatively identified or not.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

3.1 Sample Storage

Tissue samples must be protected from light and kept frozen in a freezer from the time of receipt, through homogenization until extraction and analysis.

Tissue samples and sample homogenates must be stored in an atmosphere free of all potential contaminants.

Before and after analysis, extracts and unused samples must be protected from light, extracts refrigerated at 4°C (±2°C), unused samples and homogenates frozen at -10°C for the periods specified by the Task Leader and/or Work Assignment Manager.

Samples, sample extracts, and standards must be stored separately.

3.2 Holding Times

Extraction of tissue homogenate samples should be completed within seven (7) days of sampling.

Extracts of tissue homogenate samples must be analyzed within 40 days of sample extraction.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Interferences co-extracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, further cleanup of the sample extract may be necessary.

Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.

Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin, heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse. These items should be hand-rinsed very carefully to avoid this problem.

5.0 EQUIPMENT/APPARATUS

The following equipment/apparatus is required:

- Spatula, stainless steel or Teflon
- Balance capable of weighing 100 g to the nearest 0.01 g
- Vials and caps, 2-mL for GC autosampler
- 500-mL Erlenmeyer Flasks
- Balance - analytical, capable of accurately weighing ± 0.0001 g
- Disposable Pasteur pipettes (1-mL) and Pyrex glass wool prerinsed with hexane
- Test tube rack
- Drying oven
- Dessicator
- Beakers, 250-mL
- 50-mL test tubes
- Filter paper, Whatman No. 541 or equivalent
- Soxhlet Extractor System
- Kuderna-Danish (K-D) apparatus consisting of a 10-mL graduated concentrator tube, 500-mL evaporative flask, and three-ball macro Snyder column.

- Zymark Workstation equipped for Gel Permeation Chromatography (GPC)
- 250 mL Zymark collection/concentration tubes
- Visiprep Solid Phase Extraction Vacuum Manifold (or equivalent) available from Supelco, Inc., Bellefonte, PA (only needed if Envi-Florisil tubes are used for Florisil cleanup).
- Granular silicon carbide boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.
- Water bath - heated, with concentric ring cover, capable of being temperature controlled (±2°C). The bath should be used in a hood.
- Nitrogen evaporation device equipped with a water bath that can be maintained at 35-40°C. The N-Evap by Organomation Associations, Inc., South Berlin, MA (or equivalent) is suitable, as well as the TurboVap II - Concentration Workstation by Zymark Corp., Hopkinton, MA.
- Chromatography column for florisil; 300 mm long and 11 mm ID glass column plugged with a small piece of Pyrex glass wool in the tip. Pyrex glass wool must be prerinsed with methylene chloride to ensure its cleanliness. An alternative is the use of a Florisil Solid Phase Extraction (SPE) tube (Supelclean Envi - Florisil® or equivalent) with an extraction manifold.
- Gas chromatograph - An analytical system complete with gas chromatograph and all required accessories including syringes, analytical columns, gases, an electron capture detector, and strip-chart recorder with recording integrator. A data system is required for measuring peak areas or peak heights and recording retention times. Analytical columns are:

  RTx - 1701 column - 30 m x 0.53 mm ID - 0.5 µm film thickness or equivalent.

  DB-608 column - 30 m x 0.53 mm ID - 0.83 µm film thickness or equivalent.

6.0 REAGENTS

1. Sodium Sulfate - anhydrous granular reagent grade, heated at 400°C for four hours, cooled in a dessicator, and stored in a glass bottle
2. Methylene chloride (pesticide residue analysis grade or equivalent)
3. Hexane (pesticide residue analysis grade or equivalent)
4. Methanol (pesticide residue analysis grade or equivalent)
5. Acetone (pesticide residue analysis grade or equivalent)

6. Ethyl ether (pesticide residue analysis grade or equivalent)

7. 2-Propanol (pesticide residue analysis grade or equivalent)

8. Florisil (pesticide residue grade; 60/100 mesh) or Supelclean Envi-Florisil tubes, which are available from Supelco, Inc., Bellefonte, PA; 6 mL, 1 g size, Cat. No. 5-7053.

9. Tetrabutylammonium (TBA) - sulfite reagent:

   Dissolve 3.39 g tetrabutylammonium hydrogen sulfate in 100 mL reagent water. To remove impurities, extract this solution three times with 20-mL portions of hexane. Discard the hexane extracts, and add 25 g sodium sulfite to the water solution. Store the resulting solution, which is saturated with sodium sulfite, in an amber bottle with a Teflon-lined screw-cap. This solution can be stored at room temperature for at least one month.

10. Stock Standard Solution:

    All compounds of interest must be prepared in acetone and stored in Teflon-sealed containers at 4°C. The solution should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check sample indicates a problem.

11. Pesticide/PCB Surrogate Spiking Solution:

    The compounds specified are decachlorobiphenyl (DCBP) and 2,4,5,6-tetrachloro-meta-xylene (TCMX). Prepare a solution at a concentration of 2 µg/mL in acetone. Store the spiking solutions at 4^0C (±2°C) in Teflon-sealed containers. The solutions should be checked monthly for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicates that the concentration has changed.

12. Pesticide/PCB Matrix Spiking Solution:

    Prepare a spiking solution in acetone that contains the pesticides in the concentrations specified below. For PCB only analysis, prepare Aroclor 1260 (Ar 1260) spike solution in iso-octane at a concentration of 2 µg/mL. Store the spiking solutions at 4°C (±2°C) in Teflon-sealed containers. The solutions should be checked monthly for stability. These solutions must be replaced after six months, or sooner, if comparison with quality control check indicates that the concentration of the standard has changed.

Pesticide	µg/mL
gamma-BHC	2.0
Heptachlor	2.0
Aldrin	2.0
Dieldrin	2.0
Endrin	2.0
4,4'-DDT	2.0

13. Pesticide Calibration Standard Solution:

Prepare pesticide calibration standards containing surrogate compounds at a minimum of five concentration levels: 20 ppb, 50 ppb, 100 ppb, 200 ppb, and 500 ppb, for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. Each standard mixture must contain all compounds listed in Appendix A.

14. Toxaphene and PCB Calibration Standards:

Prepare toxaphene standards at the following concentrations: 100 ppb, 250 ppb, 500 ppb, 1 ppm, and 2 ppm. Prepare PCB calibration standards at a minimum of five concentration levels, 100 ppb, 250 ppb, 500 ppb, 1 ppm, and 2 ppm. The Ar 1221 standards should be at 200 ppb, 500 ppb, 1 ppm, 2 ppm, and 5 ppm. All the toxaphene and PCB standards must also contain surrogate compounds at a concentration of 100 ppb.

15. Resolution Check Mixture

Prepare the mixture of pesticides in hexane or iso-octane at the concentrations listed below. The mixture must be prepared every six months, or sooner if the solution has degraded or concentrated.

- gamma-Chlordane 20 ng/mL
- Endosulfan I 20 ng/mL
- p,p'-DDE 40 ng/mL
- Dieldrin 40 ng/mL
- Endosulfan sulfate 40 ng/mL
- Endrin ketone 40 ng/mL
- Methoxychlor 200 ng/mL
- Tetrachloro-m-xylene 40 ng/mL
- Decachlorobiphenyl 40 ng/mL

16. Performance Evaluation Mixture (PEM)

Prepare the PEM in hexane or iso-octane at the concentration levels listed below. The PEM must be prepared weekly, or more often if the solution has degraded or concentrated.

- gamma-BHC 20 ng/mL
- alpha-BHC 20 ng/mL
- 4,4'-DDT 200 ng/mL
- beta-BHC 20 ng/mL
- Endrin 100 ng/mL
- Methoxychlor 500 ng/mL
- Tetrachloro-m-xylene 40 ng/mL
- Decachlorobiphenyl 40 ng/mL

7.0 PROCEDURE

Tissue samples must be homogenized before pursuing the following steps. See method for tissue sample homogenization (ERT/SERAS SOP #1820, Tissue Homogenization Procedure).

7.1 Sample Preparation and Extraction

1. Open the homogenate sample container in a fume hood. Mix the sample thoroughly.

2. Weigh 10 ± 0.01 g aliquot of homogenized tissue sample into a 250 mL beaker, add up to 120 g anhydrous Na_2SO_4 and mix the tissue sample and Na_2SO_4 thoroughly with a stainless steel (SS) spatula or SS spoon. The sample should have a sandy texture at this point.

3. Determine the total percent solid by following the procedure outlined in Section 7.4.

4. Prepare a method blank by using 120 g Na_2SO_4. A method blank must be prepared every 20 samples.

5. Prepare a matrix spike (MS) and a matrix spike duplicate (MSD) by weighing two additional 10 ± 0.01 g aliquots of homogenized tissue sample that was chosen for that purpose. Add 120 g anhydrous Na_2SO_4 to MS and MSD and mix thoroughly with SS spatula. The MS and MSD should have a sandy texture. Prepare one MS/MSD sample pair for every ten samples.

6. Transfer the blank, MS and MSD, and tissue samples quantitatively to precleaned Soxhlet thimbles for extraction.

7. Place thimbles into soxhlet extractors:

   - Add 0.2 mL of surrogate spike solution to the method blank, the MS and MSD, and all the samples;

   - Add 0.2 mL of pesticide matrix spike solution to each of the MS and MSD samples.

8. Add 250 mL methylene chloride and two boiling chips to each round bottom flask extractors.

9. Connect water cooled condenser and extract for 17 to 24 hours (ca. 60-90 cycles). After the extraction is complete, concentrate the extract using Step 7.2 (TurboVap II) or Step 7.3 (Kuderna-Danish).

7.2 TurboVap II Concentrator Workstation

1. Allow the system to cool and filter the entire sample extract into a 250-mL Zymark concentration tube through a #541 Whatman filter paper with anhydrous Na_2SO_4 packed into a

powder funnel. Rinse the round bottom flask with three 10-mL portions of dichloromethane (DCM). Pour the rinsate through the anhydrous Na_2SO_4 packed funnel and combine the rinsate with the extract.

2. Set the TurboVap II water bath temperature to 45°C. Make sure than the water is at the proper level in the bath. Connect a cylinder of ultra high purity (UHP) nitrogen through a two-stage regulator set at 20 psig.

3. Place the concentration tubes in the workstation openings and begin evaporating with a gentle stream of UHP nitrogen. As the evaporation process progresses, the nitrogen pressure may be increased, however, any splashing of the extract should be avoided. Periodically (2-3 times) during the concentration, rinse down the walls of the tube with DCM.

4. Evaporate the extract to less than 10 mL volume (5-7 mL range). Remove the concentration tube from the TurboVap II.

5. Transfer the extract to a clean (prerinsed with DCM) 12-mL culture tube using a clean disposable pipette. Rinse the concentration tube with two 1-mL portions of DCM and transfer to the culture tube. Keep the total volume of the transferred extract to 10 mL or less.

6. Adjust the final volume in the culture tube to 10 mL with DCM.

7. Take 1 mL for lipid determination as described in Section 7.5.

8. Transfer the remaining 9 mL extract into a 12-mL test tube. The extract is ready for GPC cleanup. If the GPC cleanup is not performed immediately, the extract should be capped, protected from light and refrigerated at 4°C (±2°C). Go to Step 7.6 for GPC cleanup.

7.3 Kuderna-Danish Concentration

1. Allow the system to cool and filter entire sample extract into a 500 mL Erlenmeyer flask through a #541 Whatman filter paper with anhydrous Na_2SO_4 packed into a powder funnel. Rinse the round bottom flask with three 10-mL portions of methylene chloride. Pour the rinsate through anhydrous Na_2SO_4 packed funnel and combine rinsate with sample extracts. The sample extract is ready for concentration.

2. Assemble a Kuderna-Danish (K-D) apparatus by attaching a 10-mL concentrator tube to a 500-mL evaporative flask.

3. Transfer the extract into a K-D concentrator flask; rinse the Erlenmeyer flask with 60 - 100 mL of methylene chloride to complete the quantitative transfer.

4. Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column.

5. Pre-wet the Snyder column by adding 2 - 3 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 - 90^{0}C) 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.

6. Concentrate the extract down to less than 10 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 mL of methylene chloride.

8. Disconnect the concentrator tube and place it on N-Evap with pre-warmed water bath (35^{o}C). Evaporate the extract to final volume of 10 mL with a gentle stream of clean, dry nitrogen.

9. Take 1 mL for lipid determination as described in Section 7.5.

10. Transfer the remaining 9mL extract into a 12-mL test tube. The extract is ready for GPC cleanup. If the GPC cleanup is not performed immediately, the extract should be capped, protected from light and refrigerated at 4^{o}C (± 2^{o}C). Go to Step 7.6 for GPC cleanup.

7.4 Total Solids

Immediately after extracting samples, weigh 3-5 g of the homogenate tissue sample into a tared aluminum dish. Determine the total percent solid by drying in oven placed inside fume hood overnight at 105^{o}C. Before weighing, allow them to cool in a dessicator. Concentrations of individual analytes will be reported relative to the dry weight of the homogenate tissue sample. Calculate the total percent solid using the following equation:

$$\%\,\text{Percent Solids} = \frac{\text{Weight of Dry Sample (g)}}{\text{Weight of Sample Before Drying (g)}} \text{ x } 100$$

7.5 Lipid Determination

1. Transfer 1 mL of the 10-mL extract (Section 7.2, Step 6 or Section 7.3, Step 8) into a preweighed 7.8 g (2 dram) vial and evaporate to dryness overnight.

2. Determine percent lipid gravimetrically. Calculate the percent lipid content using the following equation:

$$\%\ \text{Lipid} = \frac{W_E \times I_E}{V_S \times W_S \times \frac{\%\ \text{solids}}{100}} \times 00$$

where,

W_E = weight of residue after solvent evaporation, in grams
W_S = weight of sample extracted, in grams
V_E = final extract volume, in mL
V_S = volume of extract used to evaporate, in mL

3. Use remaining 9 mL extract for GPC cleanup.

7.6 Gel Permeation Chromatography (GPC) Extract Cleanup

GPC clean-up is required to separate the analytes from biological macromolecules (Lipids).

1. Transfer 5 mL extract onto the GPC column using the Zymark

2. Collect the fraction of extract eluting just after the lipid elution and before the sulfur elution [as determined by injecting a GPC calibration mixture comprised of corn oil, bis-(2-ethylhexyl)phthalate, methoxychlor, perylene, and sulfur] in a 200 mL collecting flask.

3. Transfer the clean extract quantitatively to the TurboVap II system (or a K-D system), solvent exchange with hexane and concentrate to 1 mL final extract volume.

4. If florisil column cleanup or sulfur removal is necessary, proceed to Sections 7.7 or 7.8. Otherwise the extract is ready for GC/ECD analysis (Section 7.9).

7.7 Florisil Cleanup

Follow the instructions in Option A for use of a prepared solid phase extraction tube (Supelclean Envi-Florisil) or Option B for preparation and use of a Florisil-packed chromatography column.

Option A - SPE Tube

1. Use the disposable Supelclean Envi-Florisil SPE tubes in the 6 mL (1 g) size for this cleanup procedure. Inspect each tube before use to be sure that the SPE packing is tightly packed to avoid any channeling.

2. Place SPE tubes in the extraction manifold and condition the tubes with an acetone/hexane (1:9) solvent solution. Rinse with 2 x 6 mL acetone/hexane mixture and discard the rinses. Do not allow the packing to dry out between conditioning and sample addition.

3. Before adding the sample extract to the SPE tube, add a small amount of solvent solution (approximately 1 mm height). Then quantitatively transfer the 1 mL sample extract to the tube using a disposable pipet. Allow the solution to gravity-filter through the Florisil and collect the eluate in a clean 10-mL culture tube. Use 8 mL of acetone/hexane solution for the transfer and

elution through the Florisil tube.

4. Quantitatively transfer the eluate to a concentrator tube and use the TurboVAp II to concentrate the extract to 1 mL final volume as before.

Option B - Florisil Column

1. Bake florisil for three to four hours in an oven at 400°C temperature. Allow it to cool to room temperature in dessicator.

2. Insert glass wool at the tip of chromatography column.

3. Rinse the column with ethyl ether and then with hexane to clean glassware from contamination.

4. Fill 2/3 of the column with baked florisil. Tap the column to settle the florisil. Add sodium sulfate on top of the Florisil until it is 1 cm deep.

5. Rinse the florisil column with ethyl ether followed by hexane. When the last added hexane is just above sodium sulfate, begin to collect eluent into a 50-mL test tube.

6. Add sample extract to the top of sodium sulfate using a disposable pipette. Rinse the extract container with 10 mL of hexane/ethyl ether mixture (1:1) and transfer it into the column. Collect the eluent; add another 40 mL of hexane/ethyl ether mixture to the column and keep collecting the eluent in the 50-mL test tube. Caution: Do not allow the column to go dry during the addition and elution of the sample extract.

7. Place the 50-mL test tube into N-Evap with warm water bath (35°C) and evaporate the solvent to 1 mL using gentle stream of clean, dry nitrogen.

7.8 Tetrabutylammonium (TBA) - Sulfite Cleanup (if needed after GPC & Florisil cleanup)

The solubility of sulfur in various solvents is very similar to the organochlorinated pesticides; therefore, the sulfur interference follows along with the pesticides through the normal extraction techniques. If the gas chromatograph is operated at the normal conditions for pesticide analysis, the sulfur interference can completely mask the region from the solvent peak through aldrin, a target compound. Tetrabutylammonium - sulfite is used to remove the sulfur interference.

1. Transfer the 1 mL extract described in Section 7.6, step 3, or the final extract resulting from the Florisil cleanup (Section 7.7) to a 50-mL clear glass bottle or vial with a Teflon-sealed screw cap.

2. Add 1.0 mL TBA-sulfite reagent and 2 mL 2-propanol; cap the bottle and shake for at least one minute. If the sample is colorless or if the initial color is unchanged, and if clear crystals (precipitated sodium sulfite) are observed, sufficient sodium sulfite is present. If the

precipitated sodium sulfite disappears, add more TBA - sulfite reagent until a solid residue remains after repeated shaking.

3. Add 5 mL distilled water and shake for at least one minute. Allow the sample to stand for 5-10 minutes.

4. For TurboVap II: Transfer the hexane layer (top) to a concentrator tube. Use 1-2 mL additional hexane to complete the transfer and concentrate to 1 mL final volume.

   For N-Evap: Transfer the hexane layer (top) to a 50-mL test tube; add 1-2 mL of hexane and use the N-Evap to concentrate the extract to 1 mL.

5. The 1 mL extract is now ready for GC/ECD analysis (Section 7.9)

7.9 GC/ECD Conditions

Sample analyses are performed concurrently with the use of a Hewlett Packard (HP) 5890A or Hewlett Packard 5890 Series II GC equipped with dual injector, column, and electron capture detector capabilities.

The GC conditions used for the pesticides/PCBs analysis are listed below:

Injector Temperature	250°C
Oven Temperature Program	150°C hold for 1 min 6.5°C/min to 260°C, hold for 22.08 min
Detector Temperature	320°C
Carrier Gas	Helium
Make-up Gas	Argon/Methane
Column Flow Rate	DB-608 3.2 mL/min; Rtx-1701 2.8 mL/min
Head Pressure	DB-608 3.3 psi; Rtx-1701 4 psi 30 second Purge Delay
Data System	HP Chem Station

7.10 Retention Time Window Determination

1. Make three injections of all single component standard mixtures and multi-response products (i.e., PCBs and toxaphene) throughout the course of a 72-hour period. Serial injections over less than a 72-hour period result in retention time windows that are too tight.

2. Calculate the standard deviation of the three absolute retention times for each single component standard. For multi-response products, choose five major peaks from the chromatogram and calculate the standard deviation of the three retention times for each peak. The peaks chosen should be fairly immune to losses due to degradation and weathering in samples.

3. Plus or minus three times the standard deviation of the absolute retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. For multi-response products (i.e., PCBs and toxaphene), the analyst can use the retention time window, but should rely primarily on pattern recognition.

4. In those cases where the standard deviation for a particular standard is zero, the analyst must substitute the standard deviation of a close eluting, similar compound to develop a valid retention time window.

5. The analyst must calculate the standard deviation of the retention times for each standard on each GC column and whenever a new GC column is installed. The data must be retained in the laboratory.

7.11 Standards and Samples Analysis

The analytical sequences listed in Appendix B must be followed.

7.11.1 Pesticide/PCB Analysis

For PCB-only or toxaphene-only, analysis see Section 7.11.3. For toxaphene or PCB quantitation analysis, see Section 7.11.2.

1. Inject 2 µL of the Resolution Check Standard, and calculate the percent resolution between peaks.

$$\%\,\text{Resolution} = \frac{\text{the depth of the valley between the peaks}}{\text{peak height of the smaller peak being resolved}} \times 100$$

The % resolution must be $\geq$ 60% to continue the analysis.

2. Inject 2 µL of the PEM and calculate the percent breakdown (%BD) of 4,4'-DDT and endrin by using the following equations:

$$4,4'\text{-DDT}\,\%\text{BD} = \frac{\text{amount found in ng (DDD + DDE)}}{\text{amount in ng of 4,4'-DDT injected}} \times 100$$

$$\text{Endrin}\,\%\text{BD} = \frac{\text{amount found in ng (endrin aldehyde and endrin ketone)}}{\text{amount in ng of endrin injected}} \times 100$$

$$\text{Combined}\,\%\text{BD} = 4,4'\text{-DDT} + \text{Endrin}\,\%\text{BD}$$

The %BD of 4,4'-DDT must not exceed 20%.
The %BD of endrin must not exceed 20%.
The combined %BD must not exceed 30%.

Should either requirement not be met, corrective actions must be taken before further analysis can be continued.

NOTE: Since the response factors of all analytes are not available at this point, the amount found and the amount injected in the equation can be substituted by peak area or peak height. The percent breakdown must be recalculated by using the above mentioned equation after the average response factors are calculated by the five-point calibration.

3. Inject 2 µL of each of the single component calibration standards (five-point); tabulate peak height or peak area against the standard concentration. Calculate and tabulate the response factor (RF) of each compound at each standard concentration. The average RF and percent relative standard deviation (%RSD) must also be calculated.

$$RF = \frac{\text{Peak Area or Peak Height of the Analyte}}{\text{Mass Injected (ng)}}$$

$$\overline{RF} = \frac{\sum}{n}$$

where,

RF_i = relative response factor for each initial calibration level
n = total number of initial calibration levels

$$\%RSD = \frac{SD}{\overline{RF}} x\,100$$

where,

Std. Dev. = standard deviation of n values of RF (as calculated below)

$$SD = \sqrt{\frac{\sum_{i=}^{n} RF_i - \overline{RF}}{N -}}$$

where,

RRF_i, RF and n are defined as above.

The %RSD of the RF for each analyte must be less than or equal to 20%, except as noted below. The %RSD for the two surrogates must be less than or equal to 30%. Up to two analytes (but not surrogates) per column may exceed the 20% RSD limit, but those analytes must have a %RSD of less than or equal to 30%.

4. Calculate the retention time (RT) windows for each analyte by using the following equation:

RT window = RT from the first calibration standard[(1)] ±3 x SD[(2)]

(1) from step 3 in this section
(2) SD denotes standard deviation; see Section 7.10, step 2.

All compounds (including surrogates) in the standards and in the sample extracts must elute within the specified RT windows.

5. Inject 2 µL of 500 ppb toxaphene standard; choose five dominant peaks to calculate the RT windows.

6. Inject 2 µL each of 500 ppb Ar 1016, Ar 1232, Ar 1248, Ar 1254, and Ar 1260 standards and 1 ppm Ar 1221 standard for the finger prints. Choose five dominant peaks from each Aroclor to calculate the RT windows.

7. Inject 2 µL of PEM; calculate the %BDs for 4,4'-DDT and endrin. Also calculate the combined %BD. The requirements specified in step 2 must be met. In addition, calculate the percent difference (%D) of each compound in the mixture by using the following equation:

$$\%\text{Difference} = \frac{(C_{nom} - C_{calc})}{C_{nom}} \times 100$$

where,

C_{nom} = nominal concentration of each analyte
C_{calc} = calculated concentration from the analysis of the standard

The %D must be less than or equal to 25% for all compounds in the mixture. Tabulate the results.

NOTE: This PEM injection starts the 12-hour clock.

8. Inject 2 µL each of a group of sample extracts. It is a good practice to inject the method blank first to monitor the possible lab contamination. **All sample extracts**

must be injected within 12 hours of the injection of PEM (step 7).

9. At the end of the 12-hour period, inject 2 µL of 100 ppb pesticide standards as another calibration verification (continuing calibration check). Calculate and tabulate the %D for all compounds. All compounds must have a %D less than or equal to 25%.

10. Inject 2 µL each of another group of sample extracts. **They must be injected within 12 hours of the injection of the 100 ppb pesticide standard described in step 9.**

11. Repeat steps 7, 8, 9, and 10 until such a time as any of the PEM or the 100 ppb pesticide standard fails to meet the %BD or the %D requirement. Please note that the PEM is used to check both %BD and %D.

12. End the analytical sequence with a PEM or the alternating standard, a 100 ppb pesticide standard, whichever should be injected next.

7.11.2 Quantitation Analysis of Toxaphene and/or PCBs

Any identified toxaphene and/or Aroclors in the analysis described in Section 7.11.1 must be quantitated by the proper calibration curve (five-point).

1. Inject 2 µL of the Resolution Check Mixture to verify the column performance. See Section 7.11.1, step 1 for QC requirements.

2. Inject 2 µL of PEM to calculate the %BD of 4,4'-DDT and endrin. The combined % BD should also be calculated. See Section 7.11.1, step 2 for QC requirements. Use peak height or peak area to calculate %BD.

3. Inject 2 µL each of the Aroclor standards or toxaphene standard to be quantitated at concentrations specified in Section 6.0, Item 14. Choose a minimum of five dominant peaks to calculate the RFs by using the equation specified in Section 7.11.1, step 3. The average RF and %RSD of each peak can then be calculated. The %RSD of each peak must be less than or equal to 20%. The RF can also be calculated by using the total response of the five peaks. The %RSD must be less than or equal to 20%.

   NOTE: If more than one Aroclor needs to be quantitated, proper calibration (five-point) standards must be injected.

4. Calculate the RT windows of the same dominant peaks as chosen in Section 7.10, step 2. Use the equation specified in Section 7.11.1, step 4.

5. Inject 2 µL of 500 ppb toxaphene standard or 500 ppb specific Aroclor standard (or 1 ppm Ar 1221) to calculate the %D according to the equation specified in Section 7.11.1, step 7. The percent difference must be less than or equal to 25%. This

injection serves as a continuing calibration check and starts the 12-hour clock.

6. Inject 2 µL each of the sample extracts that contain toxaphene and/or specific Aroclor(s). All injections must be made within 12 hours of the continuing calibration check analysis (step 5).

7. Repeat steps 5 and 6 if necessary.

8. End the analytical sequence by injecting 2 µL of the 500 ppb toxaphene standard or 500 ppb specific Aroclor standard (1 ppm Ar 1221).

7.11.3 PCB Only Analysis

1. Follow steps 1 and 2 of Section 7.11.1.

2. Inject 2 µL each of the Aroclor calibration standards at the concentration specified in Section 6.0, step 14. The Aroclor used for matrix spike must be calibrated. Choose five dominant peaks to calculate RFs by following Section 7.11.2, step 3.

3. Inject 2 µL each of the remaining Aroclor standards for fingerprints at the concentration specified in Section 7.11.1, step 6.

4. Calculate the RT windows of each Aroclor injected in steps 2 and 3 by using the equation specified in Section 7.11.1, step 4. The five peaks chosen for calculation must be the same peaks chosen in Section 7.10, step 2.

5. Inject 2 µL of the continuing calibration check standard, which is the mid-point calibration standard in step 2. Calculate and tabulate the %D of each peak. Use the equation specified in Section 7.11.1, step 7. The percent difference must be less than or equal to 25%.

   NOTE: This injection starts the 12-hour period for sample analysis.

6. Inject 2 µL each of a group of sample extracts. It is a good practice to inject a method blank first to monitor any possible lab contamination. All sample extracts must be analyzed within 12 hours of the injection of the continuing calibration standard (step 5).

7. Repeat steps 5 and 6, if necessary, until the %D requirement of the continuing calibration check fails.

8. End the sequence with the continuing calibration check standard. The percent difference requirement is not applied.

7.12 Evaluation of Chromatograms

All standard and sample chromatograms must be evaluated to decide if re-injection and/or dilution is necessary.

7.12.1 Chromatograms of Standards

The following requirements apply to all data presented for single component and multicomponent (toxaphene/PCBs) analytes.

1. The chromatograms that result from the analyses of the standards must display the single component analytes present in each standard at greater than 10% of full scale but less than 100% of full scale.

2. The chromatograms of the standards for the multicomponent analytes must display the peaks chosen for identification of each analyte at greater than 25% and less than 100% of full scale.

3. For any standard containing alpha-BHC, the baseline of the chromatogram must return to below 50% of full scale before the elution time of alpha-BHC, and return to below 25% of full scale after the elution time of alpha-BHC and before the elution time of decachlorobiphenyl.

4. If a chromatogram is replotted electronically to meet requirements, the scaling factor used must be displayed on the chromatograms.

5. If the chromatogram of any standard needs to be replotted electronically to meet these requirements, both the initial chromatogram and the replotted chromatogram must be submitted in the data package.

6. If a chromatogram shows carryover from the previous injection, samples analyzed afterward must be reanalyzed, preferably immediately.

7. The retention time of each single component analyte must fall within the RT windows determined in Section 7.11.1, step 4. If the retention time shifts outside the RT window by more than 0.5 minutes, the analytical sequence (acquisition) must be interrupted for corrective action. After corrective action, the acquisition can only be resumed by an acceptable PEM analysis. A new RT window might need to be defined by injecting a 100 ppb pesticide standard.

7.12.2 Chromatograms of Sample Analyses

The following requirements apply to all data presented for single component and multicomponent analytes.

1. When no analytes are identified in a sample, the chromatograms from the analyses of the sample extract must use the same scaling factor as was used for the low point standard of the initial calibration associated with those analyses.

2. Chromatograms must display single component pesticides detected in the sample at less than full scale.

3. Chromatograms must display the largest peak of any multicomponent analyte detected in the sample at less than full scale.

4. If an extract must be diluted, chromatograms must display single component pesticides between 10% and 100% of full scale.

5. If an extract must be diluted, chromatograms must display the peaks chosen for quantitation of multicomponent analytes between 25% and 100% of full scale.

6. For any samples, the baseline of the chromatogram must return to below 50% of full scale before the elution time of alpha-BHC, and return to below 25% of full scale after the elution time of alpha-BHC and before the elution time of decachlorobiphenyl.

7. If a chromatogram is replotted electronically to meet requirements, the scaling factor used must be displayed on the chromatograms.

8. If the chromatogram of any standard needs to be replotted electronically to meet these requirements, both the initial chromatogram and the replotted chromatogram must be submitted in the data package.

9. If sample chromatograms have interfering peaks, a high baseline, or off-scale peaks, those samples must be reanalyzed following dilution, further cleanup, or reextraction. Samples which cannot be made to meet the given specifications after one reextraction and cleanup must be reported in the case narrative and do not require further analysis. No limit is placed on the number of reextractions of samples that may be required because of contaminated method blanks.

7.12.3 Pesticide/PCB Identification

The identification of single component pesticides by gas chromatographic methods is based primarily on retention time data. The retention time of the apex of the peak can be verified only from an on-scale chromatogram. The identification of multicomponent analytes is based primarily on pattern recognition, which can only be verified from an on-scale chromatogram.

1. Analytes are identified when peaks are observed in the RT windows for the compound on both GC columns. Toxaphene and Aroclors are identified when patterns are

observed on both GC columns.

2. If a peak is just slightly outside any target compound's RT window, examine the retention time of the closest surrogate. Use surrogates to evaluate a possible RT shift. Similar RT shifts of target compounds can be expected in some cases.

3. If a sample contains interfering peaks or a high baseline, a further cleanup might be necessary. Compound identification on this kind of sample can be difficult. Information like surrogate retention time and peak ratio on both GC columns must be evaluated for identification purposes.

7.13 Sample Dilution

No target compound concentrations may exceed the upper limit of the initial calibration range. If analytes are detected at a level greater than the highest calibration standard, samples must be either diluted to a maximum of 1:100,000 or until the analyte response is within the linear range established during calibration. Guidance in performing dilutions and exceptions to this requirement are given below.

1. If the analyst has reason to believe that diluting the final extracts will be necessary, an undiluted run may not be required. However, if no peaks are detected above 25% of full scale, analysis of a 10 times more concentrated sample extract or the undiluted sample extract is required.

2. If the response is still above the highest calibration point after the dilution of 1:100,000, the analyst should contact the group leader immediately for further instruction.

3. The results of the original analysis are to be used to determine the approximate dilution factor required to get the largest analyte peak within the initial calibration range.

4. The dilution factor chosen should keep the response of the largest peak for a target compound in the upper half of the initial calibration range of the instrument.

5. Do not submit data for more than two analyses, i.e., the original sample extract and one dilution, or, if a screening procedure was employed, from the most concentrated dilution analyzed and one further dilution.

6. All chromatograms of dilution analyses must meet the requirements described in Section 7.12.2.

8.0 CALCULATIONS

Quantitation of target compounds and surrogates can be performed on any column that passed all the quality control (QC) criteria specified in this SOP. In order to be quantitated, the detector response (peak area or peak height) of all the analytes must lie within the calibration range.

8.1 Quantitation Limit (QL)

$$QL(\mu/kg) = \frac{C_{std} \times V_t \times DF}{W \times S}$$

where,

C_{std} = Concentration of the lowest standard in the calibration range (µg/mL)
W = Weight of tissue extracted (kg)
DF = Dilution factor
V_t = Volume of the extract (mL)
S = Decimal percent solid

The quantitation limit of each analyte can be found in Appendix A.

8.2 Sample Concentration

$$\text{Concentration, } (\mu/kg) = \frac{A_x \times V_t \times DF}{RF_{Avg} \times W \times V_t \times S}$$

where,

A_x = Peak area or peak height for the compound to be measured
RF_{Avg} = Average response factor
W = Weight of tissue extracted (kg)
V_i = Volume of extract injected (µL)
V_t = Volume of the concentrated extract (mL)
DF = Dilution factor
S = Decimal percent solid

The quantitation of toxaphene or Aroclors must be accomplished by comparing the heights or the areas of each of the five major peaks of the multicomponent analyte in the sample with the average response factor for the same peaks established during the initial calibration sequence. The concentration of multicomponent analytes is calculated by using the above mentioned equation, where A_x is the height or area for each of the major peaks of the multicomponent analyte. The concentration of each peak is determined, and then a mean concentration for five major peaks is calculated.

If more than one multicomponent analyte is observed in a sample, the analyst must choose separate peaks to quantitate the different multicomponent analytes. A peak common to both analytes present in the sample must not be used to quantitate either compound.

NOTE: If any analytes are detected below the quantitation limit, they are to be reported as present below the quantitation limit and flagged as estimated (J).

8.3 Surrogate Spike Recoveries

$$\text{Percent Recovery (\%R)} = \frac{Q_D}{Q_A} \times 100$$

where,

Q_d = Quantity determined by analysis
Q_a = Quantity added to sample

8.4 Matrix Spike Recoveries

The percent recoveries and the relative percent difference (RPD) between the recoveries of each of the six compounds in the matrix spike samples will be calculated and reported by using the following equations:

$$\text{Matrix Spike Recovery (\%R)} = \frac{SSR - SR}{SA} \times 100$$

where,

SSR = Spike sample result
SR = Sample result
SA = Spike added

$$RPD = \frac{|MSR - MSDR|}{(MSR + MSDR)/2} \times 100$$

where,

RPD = Relative percent difference
MSR = Matrix spike recovery
MSDR = Matrix spike duplicate recovery

The vertical bars in the formula above indicate the absolute value of the difference; hence, RPD is always expressed as a positive value.

9.0 QUALITY ASSURANCE/ QUALITY CONTROL

9.1 GC Column Performance

The purpose of this resolution check is to demonstrate that at the time of the initial calibration, the GC column is capable of chromatographically resolving the target compounds. This is accomplished through the analysis of the Resolution Check Mixture, which contains the nine target compounds that are most difficult to resolve.

1. The Resolution Check Mixture must be analyzed at the beginning of every initial calibration

sequence, on each GC column and instrument used for analysis.

2. The percent resolution must be greater than or equal to 60% before standards, samples, or blanks can be analyzed.

9.2 Initial Calibration for Target Compounds and Surrogates

Prior to the analysis of samples and method blank(s), the GC/ECD system must be initially calibrated at a minimum of five concentrations to determine the linearity range of all the target compounds.

1. The concentration of all calibration standards that are specified in Section 6.0 must be used.

2. The standards are to be analyzed according to the procedures given in Section 7.11 using the GC operating conditions in Section 7.9. Appendix B summarizes the specific analysis sequence to be followed.

3. The response factors are determined according to the procedure in Section 7.11.

4. The calibration is evaluated on the basis of the extent of breakdown of endrin and 4,4'-DDT, as described in Section 7.11.1, step 2. The breakdown of each compound must not exceed 20%; the combined breakdown must not exceed 30%.

5. The calibration is also evaluated on the basis of the stability of the response factors of each target compound and surrogate. The %RSD for each target compound must not exceed 20%, except as noted below.

   - The %RSD for the two surrogates must not exceed 30%.

   - Up to two single component analytes (but not surrogates) per column may exceed the 20% RSD limit, but those analytes must have a %RSD less than or equal to 30%.

9.3 Continuing Calibration for Target Compounds and Surrogates

Once the GC/ECD system has been calibrated, the calibration must be verified each 12-hour time period for each GC column and instrument used for analysis. The calibration is verified through the analysis of PEM and the mid point concentrations of pesticide, Aroclor, or toxaphene standards (100 ppb pesticide standard, 500 ppb Aroclor standard, or 500 ppb toxaphene standard).

1. The continuing calibration is evaluated on the basis of the stability of the retention times of the target compounds in the standards. The retention times of all single component analytes and surrogates in the standards must be within the RT windows established in Section 7.11.1, step 4.

2. The continuing calibration is evaluated on the basis of the stability of the instrument response to the target compounds in the PEM and the mid point standard, as judged by the reproducibility of

the determinations of the concentrations of these compounds in the standard, as described in Section 7.11.1, steps 7 and 9.

The %D of all target compounds and surrogates must not exceed 25%.

3. The continuing calibration is evaluated on the basis of the extent of the breakdown of the two target compounds endrin and 4,4'-DDT, in the PEM, as described in Section 7.11.1, step 2.

4. The continuing calibration is evaluated on each GC column and instrument used for analysis.

9.4 Determination of Retention Time Windows

The identification of single component pesticides by gas chromatographic methods is based primarily on retention time data. The identification of multicomponent analytes is based primarily on recognition of patterns of retention times displayed on a chromatogram.
Therefore, the determination of retention time windows is crucial to the provision of valid data for these target compounds.

1. The identification of all target compounds analyzed by this analytical procedure is based on the use of absolute retention time.

2. The retention time window of each target compound peak is determined as described in Section 7.11.1, step 4.

3. The retention time shifts of the surrogates are used to evaluate the stability of the gas chromatographic system during analysis of samples and standards. The retention time of the surrogates must be within the retention time windows determined by Section 7.11.1, step 4.

4. If the confirmation analysis is required for any analytes, retention time windows of those analytes must also be determined and tabulated for the confirmation column by using the procedure described in section 7.11.1, step 4. All the requirements mentioned above must also be met.

9.5 Analytical Sequence

The standards and samples analyzed by this analytical procedure must be analyzed in a sequence described in Section 7.11 (also Appendix B). This sequence includes requirements that apply to the initial and continuing calibrations, as well as to the analysis of samples.

9.6 Method Blank

A method blank is a weight of a clean reference matrix (120g Na_2SO_4) that is carried through the entire analytical procedure. The weight of the reference matrix must be approximately equal to the weight of samples associated with the blank. The purpose of a method blank is to determine the levels of contamination associated with the processing and analysis of samples.

1. A method blank must be prepared each 20 samples and analyzed on each GC/ECD system used to analyze samples.

2. A method blank must not contain any of the compounds listed in Appendix A at greater than the quantitation limit.

3. The surrogate retention times must be within the RT windows calculated from Section 7.11.1, step 4.

4. All samples associated with an unacceptable method blank must be reextracted and then reanalyzed.

9.7 Surrogate Recoveries

The recoveries of the two surrogates are calculated from the analysis of each sample, blank, and MS/MSD. The purpose of the surrogates is to evaluate the preparation and analysis of samples.

1. The surrogates are added to each sample, blank, matrix spike, and matrix spike duplicate prior to extraction at the concentrations described in Sections 6.0 and 7.1

2. The recoveries of the surrogates are calculated according to the procedures in Section 8.3.

3. The quality control limits for surrogate recovery are 60 - 150%. These limits are only advisory, and no further action is required if the limits are exceeded. However, frequent failures to meet the limits for surrogate recovery warrant investigation by the laboratory.

9.8 Matrix Spike and Matrix Spike Duplicate Analysis

The purpose of spiking target compounds into two aliquots of a sample is to evaluate the effects of the sample matrix on the methods used in this analytical procedure.

1. The MS/MSD must be prepared every 10 samples per matrix with each project.

2. The mixture of pesticide standard specified in Section 6.0 must be used to result in the concentration specified in Section 7.1

3. The recoveries of the matrix spike compounds are calculated according to the procedures in Section 8.4. The relative percent difference for each spiked analyte between the results of the matrix spike and the matrix spike duplicate are calculated according to the procedures in Section 8.4.

4. The quality control limits for recovery and relative percent difference are given below. These limits are only advisory at this time, and no further action is required when the limits are

exceeded.

Compound	% Recovery	RPD
gamma-BHC	46 - 127	50
Heptachlor	35 - 130	31
Aldrin	34 - 132	43
Dieldrin	31 - 134	38
Endrin	42 - 139	45
4,4'-DDT	23 - 134	0

9.9 Dilution Analysis

If the concentration of any sample extract exceeds the initial calibration range, that sample extract must be diluted and reanalyzed as described in Section 7.13. If no peaks are detected above 25% of the full scale in the dilution analysis, a more concentrated sample extract must be analyzed.

10.0 DATA VALIDATION

Data validation will be performed by the Analytical Project Control Group, and therefore, it is not applicable to this analytical procedure. However, data is considered satisfactory for submission purposes when ALL the requirements mentioned below are met.

1. All samples must be analyzed as part of a valid analytical sequence, i.e., they must be analyzed under an acceptable peak resolution check, degradation check, initial calibration, and continuing calibration check.

2. Analyte RT windows must be submitted for both analytical columns if confirmation analysis is required.

3. The retention times for both surrogates in every standard and sample must be within the defined RT windows for both columns.

4. All the QC requirements described in Section 9.0 must be met all the time.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, refer to U.S. EPA, OSHA and corporate health and safety practices. More specifically, refer to ERT/SERAS SOP #3013, SERAS Laboratory Safety Program.

12.0 REFERENCES

Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Third Edition, SW-846, September 1986.

U.S. EPA Contract Laboratory Program (CLP), Statement of Work for Organic Analysis, Document Number

OLM01.0 (including revisions through OLM01.8).

U.S. EPA Bioaccumulation Monitoring Guidance: Analytical Methods for U.S. EPA Priority Pollutants and 301(b) Pesticides in Tissues from Estuarine and Marine Organisms (May, 1988). Prepared by Tetra Tech, Inc.

APPENDIX A
Target Compound List and Quantitation Limits
SOP #1822
November, 1994

Target Compound List and Quantitation Limits[1]

COMPOUND	QL[2] (µg/kg)
a-BHC	4
g-BHC	4
b-BHC	4
Heptachlor	4
d-BHC	4
Aldrin	4
Heptachlor epoxide	4
Endosulfan I	4
p,p'-DDE	4
Dieldrin	4
Endrin	4
p,p'-DDD	4
Endosulfan II	4
p,p'-DDT	4
Endrin aldehyde	4
Endosulfan sulfate	4
Methoxychlor	4
Endrin Ketone	4
Toxaphene	20
a-Chlordan	4
g-Chlordan	4
Aroclor 1016	20
Aroclor 1221	40
Aroclor 1232	20
Aroclor 1242	20
Aroclor 1248	20
Aroclor 1254	20
Aroclor 1260	20

[1]On a wet-weight basis
[2]QL denotes Quantitation Limits

APPENDIX B
Analytical Sequences
SOP #1822
November, 1994

Analytical Sequences

Pesticides/PCBs Analytical Sequence (for Section 7.11.1)

Time	Injection #	Material Injected
	1	Resolution Check
	2	Performance Evaluation Mix (PEM)
	3 - 15	Standards; including 5-point std and toxaphene and PCBs; %RSD and RT window will be calculated
0 hr.	16	PEM
	17	1st Sample
	:	
	:	Subsequent Samples
	:	
12 hr.	○	Continuing Calibration Check Std (100 ppb Pesticide Std)
	:	
	:	Samples
	:	
another 12 hr.	○	PEM
	:	
	:	Samples
	:	
another 12 hr.	○	Continuing Calibration Check Std (100 ppb Pesticide Std)
	:	
	:	Samples
	:	
	etc.	
	last	PEM or continuing calibration check std (100 ppb Pesticide Std)

NOTE: All subsequent 12-hour periods are timed from the injection of the PEM or the mid point concentration standard. The analytical sequence must end with a PEM or a continuing calibration check standard.

The toxaphene and PCB standards must be analyzed at concentrations specified in Section 7.11.1, steps 5 and 6.

Analytical Sequence for Toxaphene/PCB Quantitation Analysis (for Section 7.11.2)

Time	Injection #	Material Injected
	1	Resolution Check
	2	Performance Evaluation Mix (PEM) for breakdown check only
	3 - n	5-point of toxaphene Stds or 5-point of Aroclor Stds to be Quantitated
0 hr.	n+1	500 ppb toxaphene Std or 500 ppb Aroclor Std (1 ppm Std for Ar 1221)
	n+2	1st Sample
	:	
	:	Subsequent Samples
	:	
12 hr.	○	500 ppb toxaphene Std or 500 ppb Aroclor Std (1 ppm for Ar 1221)
	:	
	:	Samples
	:	
another 12 hr.	○	500 ppb toxaphene Std or 500 ppb Aroclor Std (1 ppm for Ar 1221)
	:	
	:	Samples
	:	
another 12 hr.	○	500 ppb toxaphene Std or 500 ppb Aroclor Std (1 ppm for Ar 1221)
	:	
	:	Samples
	:	
	etc.	
	last	500 ppb toxaphene Std or 500 ppb Aroclor Std (1 ppm for Ar 1221)

NOTE: All subsequent 12-hour periods are timed from the injection of the mid point concentration standard. The analytical sequence must be ended with a continuing calibration check standard (500 ppb toxaphene Std or 500 ppb Aroclor Std [1 ppm Std for Ar 1221]).

Analytical Sequence for PCB Only Analysis (for Section 7.11.3)

Time	Injection #	Material Injected
	1	Resolution Check
	2	Performance Evaluation Mix (PEM) for breakdown check only
	3 - 13	5-point Stds. of Ar 1260 or spiked Aroclor; other Aroclor at Conc. Specified in 7.11.1, step 6.
0 hr.	14	Continuing calibration check Std (mid point Std) - Ar 1260 or spiked Aroclor
	15	1st Sample
	:	
	:	Subsequent Samples
	:	
12 hr.	○	Continuing calibration check std (mid point Std) - Ar 1260 or spiked Aroclor
	:	
	:	Samples
	:	
another 12 hr.	○	Continuing calibration check std (mid point Std) - Ar 1260 or spiked Aroclor
	:	
	:	Samples
	:	
another 12 hr.	○	Continuing calibration check std (mid point Std) - Ar 1260 or spiked Aroclor
	:	
	:	Samples
	:	
	etc.	
	last	Continuing calibration check std (mid point Std) - Ar 1260 or spiked Aroclor

NOTE: All subsequent 12-hour periods are timed from the injection of the mid point standard. The analytical sequence must be ended with a continuing calibration check standard.