

Quality Assurance Document

Standard Operating Procedure  
Analytical Method

TITLE: Analysis of Polychlorinated Biphenyls (PCBs) in Soil and Sediment Samples by Immunoassay

DEPARTMENT: Immunoassay

APPLICATION: This method is used to determine the total concentration of PCBs in soil or sediment samples. The following PCB Aroclors contribute to the total PCB value reported.

Aroclor 1016      Aroclor 1221      Aroclor 1232      Aroclor 1242  
Aroclor 1248      Aroclor 1254      Aroclor 1260      Aroclor 1262

REFERENCES: Hybrizyme DELFIA PCB Test Kit - Instructions and User Guide  
Test Methods for Evaluating Solid Waste, 3rd. Ed.  
SW846 Method 4000, December 1996  
SW846 Method 4020, December 1996

PROCEDURE SUMMARY:

The soil or sediment sample is dried by adding sodium sulfate and the PCBs are extracted by shaking the sample with methanol. An aliquot of the sample extract is incubated with PCB antibody. Any PCB present is bound to the PCB antibody. A second antibody which is attached to the microtiter plate wells bonds with and traps the antibody-PCB complex. The microtiter plate wells are washed to remove matrix interferences that may be present in the sample extract. A Europium-labeled PCB compound (PCB Tracer) is added and allowed to bind to any PCB antibody sites that are empty. A second wash step removes any unbound PCB tracer. Enhancement solution is added and forms highly fluorescent chelates with the europium ions. The amount of fluorescence produced is inversely proportional to the concentration of PCB in the sample.

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Annual Review

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## SAFETY PRECAUTIONS:

The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure be as low as reasonably achievable. Laboratory staff should observe all safety procedures as outlined in the Laboratory Health and Safety Manual. Staff should consult Materials Safety Data Sheets (MSDS) for information on specific chemicals.

PCBs have been tentatively classified as known or suspected human or mammalian carcinogens. Primary standards of these toxic compounds should be prepared in a fume hood.

## APPARATUS AND MATERIALS:

**Note: Equivalent apparatus and materials to those listed may be used.**

Multilabel Counter:	Wallac (Hybrizyme) 1420 Victor 2
Data System:	Minimum Requirements of system include: Windows 95, Intel Pentium Processor, 16 MB RAM, 30 MB free hard disk space, CD-ROM Drive, Super VGA Drive, Wallac Instrument Interface Board and printer
Microtiter Strip Washer:	Bio-Tek Model ELx50 Automated Strip Washer or equivalent
Multi-channel Pipet:	Wheaton Calibra, 20-200 $\mu$ L, 12 Channel or equivalent and multichannel pipet basins
Micropipettes:	Gilson P200 Pipetman, P1000 Pipetman and M10 Microman or equivalent and tips
Balance:	Ohaus Model CT200-S, 200g x 0.01g or equivalent
Bottle-Top Dispenser:	Brinkmann Dispensette, 5-25 mL or equivalent
Sample Containers:	Wide-mouth 2 oz. glass jars with teflon-lined lids
Misc. Items	Spatulas, timer and waste containers

## REAGENTS:

Solvents:	Methanol, ACS Reagent grade or better
Desiccant:	Sodium sulfate, ACS Reagent grade, preheated at 400 C for 4 Hrs
PCB Test Kit:	Hybrizyme DELFIA PCB Test Kit containing: Wash Concentrate, Assay Buffer, PCB Antibody, PCB Tracer, Enhancement Solution and Anti-Mouse IgG Microtitration Strips (1 plate 8 x 12 wells). <b>Store the test kit between 2 and 8 C.</b>
Aroclor Stock Solutions:	Stock solutions of Aroclors in methanol. Various Aroclor stock solutions (i.e. Aroclor 1248) can be used to prepare standards or be used for spiking samples to determine percent recoveries.

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**SAMPLE EXTRACTION:**

- 1.) A Laboratory Control Blank (LCB) and Laboratory Control Spike (LCS) are prepared and carried through the entire process with each set of samples. Label a 2 oz. glass jar for each. The LCS is prepared by weighing 5.0 g of clean sand into a 2 oz. jar and spiking with an appropriate amount of PCB spike to yield a concentration near the ED50 value of the calibration curve. The LCB and LCS receive all of the same reagents as the samples and are carried through all of the extraction and analysis steps.
- 2.) Weigh 5.0 g of each soil or sediment sample into a 2 oz. glass jar with a teflon-lined lid. Record weight of samples to 0.01 g. If a sample is to be spiked with Aroclors, the spike is added at this time. The spiking solution is pipetted directly onto the sample.
- 3.) A percent solids determination needs to be done on each sample if the PCB value is to be reported on a dry weight basis. Follow the En Chem Percent Solids SOP for this analysis.
- 4.) Add 10 to 20 g of sodium sulfate to each jar containing a sample, the LCB and LCS. Stir until sample is dry and appears homogeneous. More sodium sulfate is added if the sample is not dry and granular.
- 5.) Add 25.0 mL of methanol to each jar. Seal the jars by screwing the lid on tightly.
- 6.) Shake each jar vigorously for 3 minutes to extract the PCBs from the sample.
- 7.) Sample jars are to sit for a minimum of 10 minutes to allow the methanol layer to clear. The sample can be analyzed with some particulates in the methanol extract.

**IMMUNOASSAY PROCEDURE:**

- 1.) All immunoassay reagents are to be allowed to warm to room temperature before using.
- 2.) Prepare the PCB Antibody solution in the ratio of 50 uL of the Antibody to 1.5 mL of the Assay Buffer. This prepares enough Antibody for one strip (12 wells) of the microtiter plate. The solution should be prepared in a disposable multichannel pipette basin. Agitate basin to ensure that reagents are thoroughly mixed. Use the PCB Antibody solution within one hour of preparation.
- 3.) Place the number of required microtitration strips in a strip frame. Wash the strips using the "PREWASH" program (see Strip Washer SOP).
- 4.) After the strips have been carried through the "PREWASH" procedure, tap the strip frame on a paper towel to remove any liquid that remains from the prewash procedure. Add 100 uL of the PCB Antibody solution (prepared in Step #2) to each well by means of the multi-channel pipet. Draw the solution up into the pipet tips and then eject the solution back into the tray by pressing the push-button to the first stop. Without releasing the button, draw the solution back up into the tips of the multi-channel pipet. Carefully transfer the Antibody solution into the wells of the strip(s) while holding the tips of the pipet against the walls of the microtitration strip wells. Discard the pipet tips.
- 5.) Add 4 uL (8 uL for Aroclor 1242 analysis) of methanol to wells 1 and 2 of each strip. Add 4 uL (8 uL for Aroclor 1242 analysis) of each sample to two consecutive wells (i.e. A3 & A4). Filling order for the wells is "well #1" through "well #12" of each strip required for the analysis. Draw the sample up into the pipet tip and eject the first volume back into the sample container. Dispense the following aliquots of sample into the appropriate wells of the microtitration strips. Use a new pipet tip for each sample.

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- 6.) After all the standards, QC solutions and samples have been added to the wells, place the strip frame into the multilabel counter. Select the 15 minute shake option and start the shake program (see Multilabel Counter SOP).
- 7.) While the samples are incubating, prepare the PCB Tracer Solution. Mix the reagents in the ratio 50 uL of PCB Tracer with 1.5 mL of Assay Buffer. This is enough Tracer for one strip of wells (12 wells). Prepare enough Tracer for the number of strips being analyzed. The solution should be prepared in a disposable multichannel pipette basin. Agitate basin to ensure that reagents are thoroughly mixed.
- 8.) When the shake program has finished, remove the strip frame from the multilabel counter and place it into the strip washer. Wash the wells using the "3 Washes" procedure (see Strip Washer SOP).
- 9.) Remove the strip frame from the strip washer and tap the strip frame on a paper towel to remove any liquid that remains from the wash procedure. Add 100 uL of the PCB Tracer Solution to each well in the strip frame using the multi-channel pipet. Follow the procedure described in Step #4 for the pipetting.
- 10.) Place the strip frame back into the multilabel counter. Select the 5 minute shake option and start the shake program (see Multilabel Counter SOP).
- 11.) When the shake program has finished, remove the strip frame from the multilabel counter and place it into the strip washer. Wash the wells using the "3 Washes" procedure (see Strip Washer SOP).
- 12.) Remove the strip frame from the strip washer and tap the strip frame on a paper towel to remove any liquid that remains from the wash procedure. Add 150 uL of the Enhancement Solution to each well using the multi-channel pipet. Follow the procedure described in Step #4 for the pipetting. It is very important not to form bubbles on the surface of the solutions in the wells in doing this pipetting. Bubbles will cause problems when making the fluorescence readings.
- 13.) Place the strip frame into the multilabel counter. Select the PCB QUANT protocol to determine the PCB concentrations of the samples (see Multilabel Counter SOP-Reading The Plate). This protocol will shake the samples for two minutes, determine the fluorescence readings of each of the samples and provide an Excel spreadsheet with the "raw" results of the analysis.

CALCULATIONS:

- 1.) Fluorescence readings from the daily calibration standards are entered into the "4 or 5 Point Standard Curve Modified" program depending upon the number of standards used. The program calculates the slope and ED50 values for the calibration curve and plots the "S" shaped curve.
- 2.) The slope and five times the ED50 (corrects for dilution made during sample extraction) determined in number 1 above are entered into the "raw" data spreadsheet. The program automatically updates the concentrations of PCBs in the samples. The concentration reported on the "final" Excel spreadsheet is the total PCB concentration in the original soil/sediment sample. This is based on a 5.0 g sample extracted with 25 mL of methanol. If any dilutions were made to the sample extract before carrying it through the immunoassay procedure, a correction needs to be made to obtain the concentration in the original sample. To make this correction, multiply the value from the "final" Excel spreadsheet times the dilution factor that was used.

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- 3.) To correct the PCB concentration to a dry weight basis, the value obtained in the previous step needs to be divided by the percent solids value for the sample and then multiplied by 100.
- 4.) Sensitivity of the various Aroclors in this immunoassay varies depending on the particular Aroclor. The varying sensitivities provided by Hybrizyme are found in the following table of cross reactivities:

<u>Aroclor</u>	<u>% Reactivity</u>
1262	110
1260	130
1254	160
1248	100
1242	40
1016	25
1232	20
1221	10

Aroclor 1248 is used as the base in making the comparisons of the sensitivities. If the % reactivity for an Aroclor is greater than 100%, that Aroclor is more sensitive in the immunoassay than Aroclor 1248 and those Aroclors with % reactivities less than 100% are less sensitive than Aroclor 1248. The % reactivities can be used to correct for differences in sensitivities of the Aroclors present in a sample. For example, to make a concentration correction for a sample containing Aroclor 1242 when using Aroclor 1248 for the calibration curve, the value obtained from the Excel spreadsheet would have to be divided by 0.40. This would correct the concentration to the value that would have been obtained if the calibration curve had been prepared using Aroclor 1242 standards. It is helpful to know what Aroclors are present in a sample so that corrections for cross reactivities can be made to provide the most accurate values for the PCB concentration of a sample.

## QUALITY CONTROL

## Initial Multipoint Calibration (IMC)

The IMC shall be run daily before the analysis of samples. The slope and ED50 values must be 70-130% of average values of the previous 5 daily calibration curves. If IMC fails, correct problem and repeat initial calibration.

## Calibration Check (CC)

One CC standard (200 ug/L) shall be analyzed per analytical batch of 20 samples or less. The analyzed value must be within 80-120% of the actual value. If CC fails, correct problem. Repeat initial calibration verification and reanalyze all samples since last successful calibration verification.

## Method Blank (MB)

An Ottawa sand MB shall be run with each analytical batch of 20 samples or less. The result for the MB must be <RL (0.50 mg/kg). If the MB fails, correct the problem and repeat prep and analysis of MB and all samples analyzed since last successful MB.

## Lab Control Spike (LCS)

An Ottawa sand LCS shall be run with each analytical batch of 20 samples or less. The LCS will be spiked at 1.0 mg/kg Aroclor 1242. Percent recovery for the LCS must be within 70-130% of the spike concentration. If the LCS fails, correct the problem and repeat prep and analysis of LCS and all samples analyzed since last successful LCS.

## Lab Duplicate (LD)

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One sample from each analytical batch of 20 samples or less shall be analyzed in duplicate. The RPD for the LD must be <30%. If the LD fails, qualify the data and note in case narrative.

### Fox Control Sediment (FCS)

A selected Fox sediment sample, the FCS, will be analyzed daily. The analytical result will be compared to acceptance limits set based on the 95% and 99% confidence limits determined by regression analysis between SW846 8082 and Hybrizyme PCB immunoassay results. If the FCS falls outside the 99% limit, all associated samples will be reanalyzed.

### Matrix Spike/Matrix Spike Duplicate (MS/MSD)

One MS/MSD pair will be analyzed with each analytical batch of 20 samples or less. The sediment used for the MS/MSD will be selected so that the spiked sample concentrations will fall in the mid-range of the calibration curve. The MS/MSD spike will be 1.0 mg/kg Aroclor 1242. The percent recoveries of the MS and MSD must be within 60-120%. If both the MS and MSD recoveries are out of the specified limits, qualify the data and note in the case narrative a suspected matrix problem.

## POLLUTION PREVENTION and WASTE MANAGEMENT:

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Laboratory staff should order and prepare only those quantities of reagents that will be used prior to the expiration date. Other appropriate measures to minimize waste generation should be brought to the attention of laboratory management. All laboratory waste shall be handled as directed by the Laboratory Waste Management Plan and Hazardous Waste Contingency Plan.