

**Extraction and Cleanup of  
Glass Fiber Filters for Polychlorinated  
Biphenyls and Trans-Nonachlor**

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**Standard Operating Procedure MSL-M-092-00**

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# Extraction and Cleanup of Glass Fiber Filters for Polychlorinated Biphenyls and Trans-Nonachlor

## 1.0 Scope and Application

This SOP is applicable to the extraction and analysis of polychlorinated biphenyl compounds from glass fiber filters used to collect particulates from fresh water. The target compounds which can be determined by this method are, generally speaking, the polychlorinated biphenyls (PCBs) found in Aroclors 1232, 1248, and 1262 and trans-Nonachlor. It may be applied to other compounds once acceptable method recovery has been demonstrated.

Up to 400 liters of freshwater is passed through a series of pre-cleaned 293 mm glass fiber filters with 0.7 $\mu$ m pore size that are contained in a "Pentaplate" filtration apparatus. The flow rate used is such that the back pressure on the filters will not exceed 5 psi. The filtrate thus collected is operationally defined as the suspended particulate phase. The filters are removed from the Pentaplate device, replaced in their original pre-cleaned foil pouches, which are sealed, labeled, and stored frozen until extraction.

At analysis, up to five filters pertaining to a single sampling site are allowed to thaw, removed from their pouches, placed in an extraction apparatus. Surrogate compounds are then added and the filters are extracted with acetone to remove organics and interstitial water. The filters are then extracted with a mixed solvent of 50% hexane, 50% acetone to remove the remaining organic compounds from the filter. The acetone extract is combined in a separatory funnel with 300 mL of reagent water and the mixture is then extracted once with 200 mL of hexane, then twice with 100 mL of hexane, to remove PCBs into the hexane extracts. These hexane extracts are then combined with the hexane-acetone extracts, reduced in volume, and exchanged into hexane to a final volume of approximately 1 mL. The extract is then applied to a cleanup column which is eluted with 60 mL hexane. The hexane is reduced in volume to 0.9 mL, at which point 0.1 mL of internal standard is added to complete the sample preparation.

Extracts are then subjected to gas chromatographic analysis with electron capture detection (see SOP MSL-M-093). The chromatographic separation is performed using a 60 meter capillary column. Identification and quantitation of the PCB compounds is accomplished by comparison to calibration standards containing a large number of PCB congeners in known concentration.

## 2.0 Definitions

The following terms and acronyms are associated with this procedure:

DCM	Dichloromethane
GC-ECD	Gas chromatography with electron capture detection
K-D	Kuderna-Danish
NA <sub>2</sub> SO <sub>4</sub>	Sodium Sulfate
rpm	Revolutions per minute
SRM	Standard reference material

GFF Glass fiber filter (see description below)

### **3.0 Responsible Staff**

- 3.1 *Project Manager.* A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory, 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.
- 3.2 *Laboratory Supervisor.* A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.
- 3.3 *Analyst.* A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper handling of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) reporting results to the Project Manager; and 5) participating in QA audits.
- 3.4 *Quality Assurance Representative.* A qualified staff member assigned to the Quality Assurance Unit. Responsible for monitoring the project activities and conducting Quality Assurance Audits to ensure that 1) analysts have conducted the analysis according to the SOP and that deviations from the SOP have been noted in project files; 2) instrument use and maintenance records are kept correctly; and 3) data have been reported and presented accurately.

### **4.0 Procedure**

- 4.1 Apparatus and Reagents
- 4.1.1 Separatory funnels (sized to fit sample)
- 4.1.2 Erlenmeyer flasks, various sizes
- 4.1.3 Chromatography column, 15 x 250 mm with 250 mL reservoir and No. 2 Teflon stopcock (Kontes #42080-0222)
- 4.1.4 Roller apparatus capable of rolling 250 mL Qorpak jars
- 4.1.5 Kuderna-Danish (K-D) evaporator apparatus: 250 mL and/or 500 mL reservoir; 3 ball macro Snyder column; 2 or 3 ball micro Snyder column; 10 mL or 25 mL concentrator tube
- 4.1.6 Hot water bath capable of reaching 100°C, located in a fume hood

4.1.7 Water aspirator vacuum source (Buchi #B169 or equivalent)

- 4.1.8 0.5 Liter Soxhlet extraction apparatus complete with flask and condenser (Ace # 6810-10 or equivalent)
- 4.1.9 1 Liter round-bottom boiling flasks (Ace #6887-53 or equivalent)
- 4.1.10 Reducing Glass Joint Bushings 34/45 to 24/40 (Ace #5023-21 or equivalent)
- 4.1.11 Pre-Cleaned 293 mm Whatman GFF, 0.7 $\mu$ m pore size, Whatman # 1825-293 or equivalent
- 4.1.12 Boiling chips, carborundum, soxhlet extracted or baked at >400° C
- 4.1.13 Glass wool, soxhlet extracted  $\geq$ 4 hrs in 50:50 hexane-acetone
- 4.1.14 Nitrogen evaporation apparatus, N-Evap or equivalent, heated with a water bath maintained at 25-35° C
- 4.1.15 RapidVap Evaporation System (Labconco)
- 4.1.16 Glass graduated cylinders
- 4.1.17 Stainless steel and teflon forceps
- 4.1.18 Steel rod, 3 mm x 50 cm
- 4.1.19 Microliter syringes or micropipets
- 4.1.20 Concentrated sulfuric acid
- 4.1.21 Solvents—pesticide grade or equivalent
  - Dichloromethane (DCM)
  - Hexane
  - Acetone
  - Reagent Water (Barnstead Organic-Free or equivalent)
- 4.1.22 Sodium sulfate—anhydrous, reagent grade, heated to 400° C for  $\geq$ 4 hr, then cooled to room temperature and stored in a desiccator
- 4.1.23 Alumina, Sigma F-20 or equivalent, 80-200 mesh
- 4.1.24 Silica, Amicon Matrix Silica pore diameter 60Å , particle size 105  $\mu$ m
- 4.1.25 Surrogate solution- a hexane solution containing 200 ng/mL PCB 14, 50 ng/mL PCB 65, 50 ng/mL PCB 166, and 100 ng/mL dibutyl chlorendate (DBC). PCB numbers refer to their IUPAC designations
- 4.1.26 Internal standard solution- a hexane solution containing 100 ng/mL of PCB 30, PCB 204, and PCB 103

4.1.27 Matrix spiking solution- a hexane solution having a nominal concentration of 1830 ng/mL as total PCBs (prepared from the 1994 Aroclor mixture provided by M. Mullins) and 100 ng/mL trans Nonachlor

4.1.28 Acid silica gel - 30% w/w sulfuric acid

## 4.2 Sample Handling

Samples shall be kept frozen (-10 to -30°C) until analysis. Samples shall be extracted within 9 months of receipt at the lab unless specified otherwise by the Project Manager or project-specific plans. Refer to the project-specific sampling plan for sample collection, preservation, and handling methods.

## 4.3 Labware Preparation

Prior to use, all glassware, Teflon, and other labware should be washed with hot, soapy water and rinsed with tap water, followed by deionized distilled water. Teflon should be solvent rinsed, teflon stopcocks are sonicated for 30 minutes in dichloromethane. Additionally, glassware must be baked at 450°C for at least 4 hours.

## 4.4 Filter Preparation

Prior to use, the filters must be muffled at  $450 \pm 20^\circ\text{C}$  for 4 hrs to remove manufacturing impurities. This process is fully described in Battelle SOP MSL-M-090-00.

## 4.5 Pentaplate Filtration Apparatus Preparation

A detailed description of how the "Pentaplate" apparatus is loaded with filters and used to collect samples is contained in the project specific sampling QAPjP.

## 4.6 Filter Sample Extraction

4.6.1 A small wad of pre-cleaned glass wool is first placed over the siphon tube entrance port in the soxhlet extractor body to prevent any particulate filter material from escaping.

**Note:** Soxhlet body used for the resin extract, SOP# MSL-M-091-00 is a modified soxhlet with a capacity greater than 500 mL, using this soxhlet may cause "bumping" or other problems with the filters.

The filters pertaining to a single sampling site are allowed to thaw, removed from their foil pouches, folded, and placed in the soxhlet extractor body using clean teflon or stainless steel forceps. Any water associated with the thawed samples is also added to soxhlet. The extractor body containing the filters is then connected to a 1 liter boiling flask containing several boiling stones, and 800-850 mL of acetone is added and allowed to siphon over into the boiling flask below. Next, 100  $\mu\text{L}$  of surrogate solution is added to the filters in the extractor body and, if the sample is to be spiked, 100  $\mu\text{L}$  of matrix spiking solution is

also added. The condenser is then connected, the cooling water and heating mantle are activated, and the filters are extracted for 4 hours. During extraction the soxhlet should cycle 4 times per hour. The acetone extraction step is important in removing water from the filters as it is critical to ensuring efficient extraction during the next extraction step.

- 4.6.2 The soxhlet apparatus is removed from the condenser and tilted to force the acetone extract to siphon into the 1 liter boiling flask. The extract is then transferred from the boiling flask into a 2 liter separatory funnel.

**Note:** To avoid contamination of the sample from material on the exterior of the neck, before transferring extracts from containers with ground glass joints always rinse the exterior of the necks with the same solvent as that being transferred.

At this point fresh boiling stones and 800-850 mL of 50:50 hexane-acetone are added to the boiling flask, the flask is connected to the soxhlet body, the condenser is connected and the cooling water and heating mantle are activated. The filters are extracted with this solvent system for  $\geq 16$  hrs, after which the mantle is shut off and the system is allowed to cool to room temperature. During extraction the soxhlet should cycle at least 4 times per hour. After extraction the soxhlet body is siphoned over to remove the extraction solvent into the boiling flask where it will be evaporated.

#### 4.7 Acetone Extract Back Extraction

To accomplish this step 300 mL of reagent water is added to the acetone extract contained in the separatory funnel and the mixture is then extracted once with 200 mL hexane and twice more with 100 mL hexane. This step is performed in order to reduce the amount of polar or water soluble interferences being carried through the procedure and to remove water from the acetone prior to evaporation. The hexane extracts are collected in a 1 liter boiling flask for evaporation. If emulsions are encountered or the acetone:water and hexane phases do not cleanly separate, additional reagent water and/or a small amount of baked sodium chloride may be added to facilitate separation of the phases.

#### 4.8 Extract Evaporation

- 4.8.1 Several fresh boiling stones are added to the hexane-acetone extract contained in the boiling flask, a 24/40 3-ball macro Snyder column is connected to the flask by means of a reducing bushing, the Snyder column is wetted with hexane, and the extract is evaporated to approximately 300 mL. This extract is then quantitatively transferred with several hexane rinses (see note in Sec. 4.6.2) to the boiling flask containing the hexane back-extract of the acetone rinse/extract. Several fresh boiling stones are added, a 3-ball macro Snyder column is attached and wetted, and the solvent is evaporated to a volume of approximately 200 mL. The contents is then transferred to a 250 mL K-D apparatus, fresh boiling stones are added, and the extract is reduced to an apparent volume of 1-5 mL. The K-D is allowed to cool and the 250 mL flask and macro Snyder column are removed from the concentrator tube. Fresh boiling stones are added, a micro Snyder column is attached and wetted with hexane, and the volume is reduced to approximately 1 mL. To effect a complete exchange into hexane fresh boiling stones and 10 mL of hexane is added

and the volume is again reduced to one mL. This final step is then repeated once more and the

volume brought to approximately 1 mL. Any evaporation device (such as the Labconco Rapidvap) that can be demonstrated to yield acceptable spike recoveries, acceptable precision, and acceptable levels of contamination can be used in place of the K-D apparatus.

#### 4.9 Column Chromatography Clean-up of Extract

A silica/alumina cleanup column is used to remove polar interfering compounds remaining in the extract prior to GC analysis.

4.9.1 Prepare 10% deactivated Alumina and 6% deactivated silica by activating a portion of each by heating to 400°C for at least 4 hours and allowing to cool to room temperature in a desiccator. Weigh a portion of the alumina or the silica into a glass jar with TFE-lined lid. Add a weight of water equal to the percent deactivation desired (either 10% or 6%) based upon the weight of the portion used. Place jar on roller for 30 minutes. Store in a sealed glass container. The deactivated material must be used within 24 hours of preparation or this procedure must be repeated. After initial heating to 400°C both the alumina and silica gel must be stored either at approximately 130°C or in a desiccator prior to deactivation.

4.9.2 Prepare acid silica gel (40% w/w) by thoroughly mixing the appropriate portions of concentrated sulfuric acid and activated silica gel together in a clean container. The amount of concentrated sulfuric acid to be used for any weight of activated silica gel can be calculated by using the following equation:

$$(0.36) X (\text{gms Silica}) = \text{mLs conc. } H_2SO_4$$

Break up aggregates with a stirring rod or place the container on a roller table until a uniform mixture is obtained. Store in a glass jar with the TFE-lined lid.

4.9.3 Prepare a column by placing a small portion of glass wool at the bottom of a chromatography column. Pour 70 mL hexane into the column filling it to a level that is approximately 1/3 the volume of the reservoir. Place a powder funnel in the column and pour 10 g of the 10% deactivated alumina into a column, swirling the hexane and the alumina allowing the alumina time to completely settle. Add 3 g of 6% deactivated silica to the column in the same manner. Before adding the sodium sulfate open the stopcock and allow the hexane to slowly drain, then add enough sodium sulfate to result in a plug approximately 1 cm high. The acid silica gel must be slurry packed in order to prevent trapping bubbles in the column. Into a small beaker containing 8 g of acid silica gel, pour a sufficient amount of hexane to immerse the adsorbent. Swirl the beaker to release most of the bubbles, then with the aid of a squeeze bottle of hexane, pour the slurry into the column, swirling it to aid settling. Again using the same technique as described above, add enough sodium sulfate to result in a plug approximately 1 cm high. Drain the solvent level down to just above the top of the sodium sulfate. Place a 250 mL K-D apparatus under the column to collect the eluent.

- 4.9.4 The 1 mL sample extract from 4.8.1 is carefully transferred to the column with several small hexane rinses. A small portion of hexane should be used to rinse down the sides of the column. Allow each rinse to drain to just above the sodium sulfate layer. Carefully add a total of 60 mL of hexane to the column (including rinses) and allow the column to drain completely, collecting all the eluent.
- 4.9.5 Add boiling stones to the K-D containing the eluent and reduce in volume to 0.9 mL (as measured in the 10 mL concentrator tube) using macro and micro Snyder columns followed by nitrogen evaporation using a stream of ultra high purity nitrogen. At this point add 100  $\mu$ L of internal standard solution to bring the volume to 1.0 mL and transfer to an autosampler vial for analysis.
- 4.10 Quality Control Sample Frequency

Samples prepared using this procedure should be processed in batches sized in accordance with the project analytical QAPjP. The QA/QC samples described below and their frequency are guidelines; the project specific QAPjP should be consulted prior to beginning any analysis of sample preparation. Additional QA/QC samples may be required as specified in the project specific QAPjP.

- 4.10.1 Lab Procedural Blank – prepare one per batch. Prepared by working through the sample preparation procedure using only solvents and reagents.
- 4.10.2 Lab Matrix Blank – prepare one per batch. This is a non-field exposed filter sample prepared in a manner identical to that used for field samples.
- 4.10.3 Spiked Matrix Blank – prepare one per batch. This is a lab matrix blank fortified with target analytes and prepared in a manner identical to that used for field samples.
- 4.10.4 Spiked Procedural Blank – prepare one per batch. This is a lab procedural blank which is fortified with target analytes and prepared in a manner identical to that used for field samples.
- 4.10.5 Sample Replicates – analyze one sample per batch in duplicate.
- 4.11 Data Recording and Storage

All standard preparation data will be recorded in accordance with SOP MSL-M-056.

All extraction data and sample extraction information will be recorded on the XAD-2 Resin and Filter Extraction Data Sheet (Attachment 1).

All transfers of data to forms and data reductions (e.g., concentration calculations, means, standard deviations) will be checked by the analyst and approved by the project manager. Hard copies of GC printouts of calibrations and sample data and spreadsheet reports will be kept in the Chemistry Group Central Files. Analytical electronic data will be archived on magnetic tape.

## **5.0 Quality Control**

- 5.1 Results of quality control samples (e.g., blanks, spikes, intercomparison samples, and replicate samples) prepared using this procedure will meet the criteria given in the project specific QAPjP. Recovery of the surrogates will be used to monitor for extraction efficiency, unusual matrix effects, or sample processing errors. Surrogate recovery criteria will be given in the project specific QAPjP.
- 5.2 Solvents, reagents, glassware, and other sample processing hardware may cause artifacts or interferences to sample analysis. The analyst must demonstrate that these materials are free from interferences under the conditions of the procedure by analyzing method blanks.

## **6.0 Safety**

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

Protective clothing and eyeglasses must be worn at all times when handling samples and chemicals.

Proper care must be exercised when handling solvents and acids, and when using syringes.

Extractions of filter samples are only to be performed in the walk-in fume hood located in Room MSL 114 or in the fume hood in Room MSL 231. Both of these hoods are equipped with heating mantles that are equipped with safety earthed ground screens and with recirculating water chillers that have been equipped with flow sensing devices.

The purpose of the safety earthed ground screens is to shut off power to the heating mantle unit should the screen (which is located just above the heating element) become electrically connected to the mantle housing, as in the case of a boiling vessel rupturing.

The flow sensing device on the recirculating chiller will shut off the chiller and the heating mantle unit should coolant water flow be interrupted, as in the case of a hose breaking. This will prevent the boiling flask from boiling dry.

In addition to these features each hood is equipped with a liquid sensor, which if it detects liquid present in the hood or in the containment trays (used in the walk-in fume hood), will shut down power to both the chiller and the heating mantles.

If overnight unattended extractions are to be performed the project manager must make arrangements with the Building Director to ensure that at least once during the off-shifts a security guard checks for any problems in the extraction areas.

## **7.0 Training Requirements**

All staff performing extractions of GFF filter samples for analysis of PCBs and trans-Nonachlor must first read this SOP and then demonstrate proficiency in the process prior to performing the work.; Proficiency will include demonstrating that 1) a blank having an acceptable low level of contaminants can be produced and 2) that blank spike recoveries are within acceptable recovery range. Documentation of training will be recorded on training assignment and on-the-job training forms from SOP MSL-A-006. Records of this training will be kept by the laboratory Quality Assurance Representative.

## **8.0 References**

- 8.1 J. I. Gomez-Bellinchon, Grimalt, J. O., and Albaiges, J., "Intercomparison Study of Liquid-Liquid Extraction and Adsorption on Polyurethane and Amberlite XAD-2 for the Analysis of Hydrocarbons, Polychlorobiphenyls, and Fatty Acids Dissolved in Seawater," *Environ. Sci. Technol.* 1988, 22, 677-685.
- 8.2 Quality Assurance Plan Green Bay Mass Balance Study "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.
- 8.3 Analytical Quality Assurance Project Plan (QAPjP) for the EPA Lake Michigan PCB Mass Balance Study, DRAFT, dated October 25, 1994.
- 8.4 MSL-D-001. Recording Data on Data Sheets and Laboratory Notebooks.
- 8.5 MSL-A-006. MSL Training.
- 8.6 MSL-M-056. Stock and Standard Solution Preparation.
- 8.7 MSL-M-093 PCB Congener Analysis of XAD Resins and GFIF Filters Using GC/ECD.
- 8.8 ASTM Method D4059-91, "Standard Test Method for Analysis of Polychlorinated Biphenyls in Insulating Liquids by Gas Chromatography."
- 8.9 EPA 660/4-81-045, "The determination of Polychlorinated Biphenyls in TQuality Assurance Plan Green Bay Mass Balance Study" "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.
- 8.10 "Analytical Quality Assurance Plan for the Lake Michigan PCB Mass Balance Study."



