Analysis of PCBs, Pesticides, PAHs, and Flame Retardants in Air and Precipitation Samples

IADN Project

Sample Preparation Procedure

Prepared by
Team IADN

School of Public and Environmental Affairs
Indiana University
Bloomington, Indiana 47405

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INTRODUCTION

This document describes the detailed laboratory procedure for extraction and chromatographic cleanup of air and precipitation samples collected for the Integrated Atmospheric Deposition Network (IADN) from six sampling stations near the Great Lakes. It includes routine operation for cleaning glassware and pre-cleaning sampling media such as XAD-2, quartz fiber filter (QFF), and laboratory chemicals. The procedure requires meticulous attention and extreme care at each step to avoid interference caused by contaminants in the solvents, sampling matrix, and reagents. These methods are strictly followed in the Environmental Chemistry Laboratory, School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana. Any deviation from the procedure is documented in the laboratory notebooks.

Laboratory personnel are often required to handle chemicals and standards, which may be toxic and carcinogenic. Proper safety protection should be taken to handle these chemicals. Indiana University offers a training program for laboratory safety rules and personal protection. All laboratory employees are required to take this training.

The target compounds in this project are 84 polychlorinated biphenyl (PCB) congeners, 22 organochlorine pesticides (OCs), 16 polycyclic aromatic hydrocarbons (PAHs), and 47 flame retardants (FRs) including 36 polybrominated diphenyl ether (PBDE) congeners, and 11 non-PBDEs. Most recently a pilot project on analyzing 12 organophosphate flame retardants (OPEs) was started.

A complete list of all compounds is given in Table 1.

TABLE 1. LIST OF ANALYTES

PCB	
4+10	101
7+9	99
6	119
8+5	83
19	97
12	81
13	87
18	85
15+17	77
16	110
32	135+144
26	123
31	149
28	118
33	114
53	131
22	132+153+105
45	163+138
52	126
49	128
47	167
48	174
44	202+171
37	156
42	172
41+71	180
64	199
100	169
74	170+190
70+76	201
66	207
95	194
91	205
56+60	206
92+84	PCB-11
89	**Total**

Pesticides
HCB
alpha-HCH
beta-HCH
gamma-HCH
heptachloroepoxide
alpha-chlordane
gamma-chlordane
oxychlordane
trans-nonachlor
methoxychlor
endosulfan I
endosulfan II
endosulfan sulfate
p,p'-DDT
p,p'-DDE
p,p'-DDD
o,p'-DDT
o,p'-DDD
Aldrin
Endrin
dieldrin
Octachlorostyrene

PAHs:
Fluorine
phenanthrene
anthracene
fluoranthene
Pyrene
Retene
benz[*a*]anthracene
chrysene+
benzo[*b*]fluoranthene
benzo[*k*]fluoranthene
benzo[*e*]pyrene
benzo[*a*]pyrene
indeno[1,2,3-
dibenz[*a,h*]anthracene
benzo[*ghi*]perylene
coronene

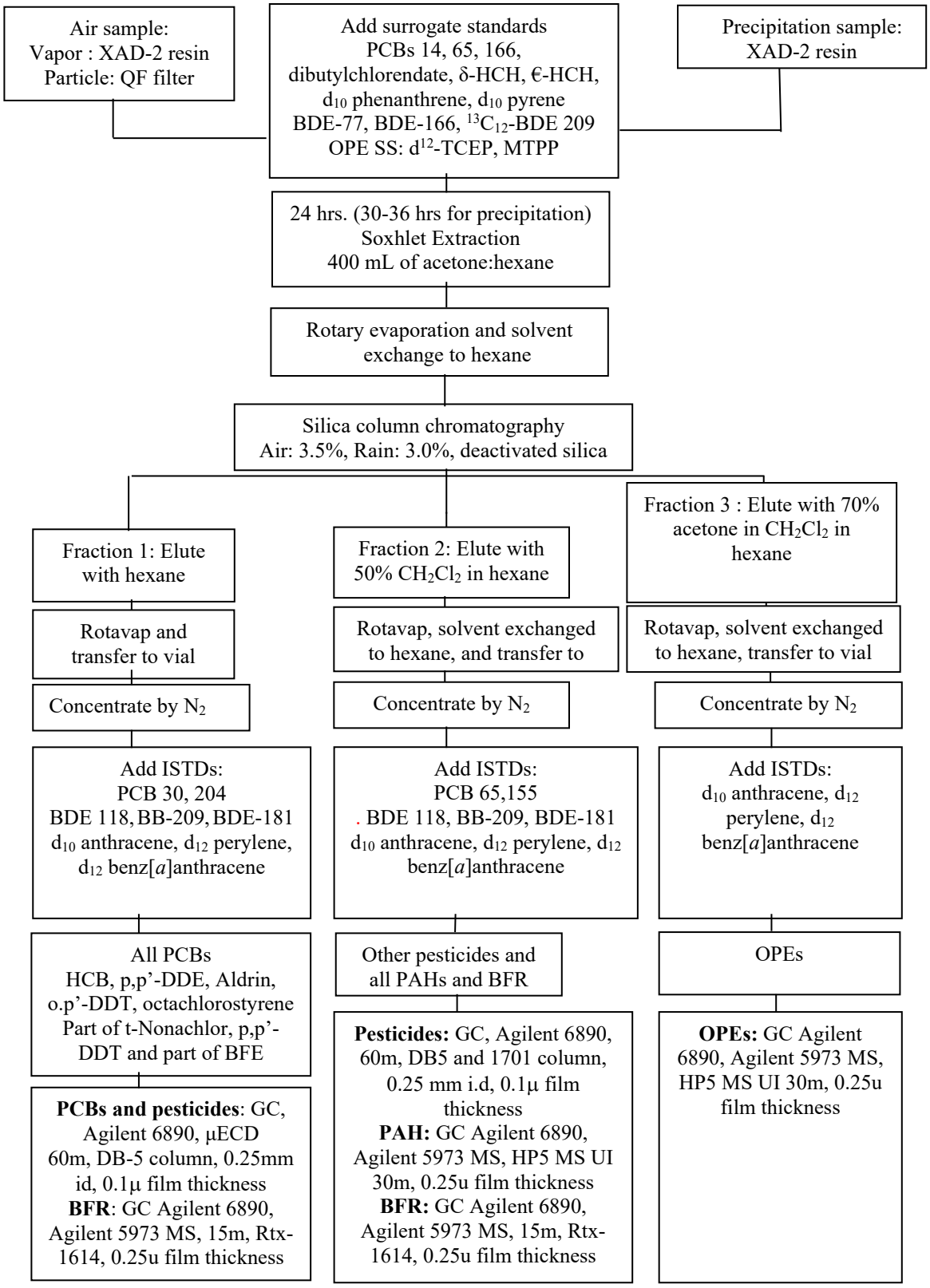
Brominated FRs (BFRs)	
BDE-7	BDE-184
BDE-10	BDE-191
BDE-15	BDE-196
BDE-17	BDE-197
BDE-28	BDE-201
BDE-30	BDE-203
BDE-47	BDE-204
BDE-49	BDE-205
BDE-66	BDE-206
BDE-71	BDE-207
BDE-85	BDE-208
BDE-99	BDE-209
BDE-100	DBDPE
BDE-119	HBCD
BDE-126	TBE
BDE-138	Syn-DP
BDE-139	Anti-DP
BDE-140	PBBZ
BDE-153	pTBX
BDE-154 + BB-153	EHTBB
BDE-156+169	BEHTBP
BDE-180	PBEB
BDE-183	HBB

Meteorological
Temperature
Wind speed speed
Wind direction
Solar radiation
Relative humidity
Barometric pressure

Organophosphate esters (OPE)
TIPRP
TPRP
TNBP
TCEP
TCIPP
TPEP
TDCIPP
TPHP
TBOEP
EHDP
TEHP
TOTP
TMTP
TPTP
TIPPP
TDMPP
TDBPP
TBPP

Others
Total suspended

FLOW CHART 1. SUMMARY OF SAMPLE PREPARATION



I. CLEANING: GENERAL LABWARE

General Cleaning Supplies

Micro cleaning solution (Micro-90, International Products Corporation)
Glassware washing brushes
Deionized (DI) water, Barnstead, E-Pure series 1090, 4 module water system
Muffle furnaces: Thermolyne 30400
Ultra sonicator
Aluminum foil
Solvents: dichloromethane, hexane (Omnisolv, EMD)
Teflon squirt bottle with solvents
Beakers
Kimwipes

Procedure

1. Glassware

Wash general glassware like soxhlet extractors, round bottom flasks, beakers, pear shaped flasks, centrifuge tubes, separatory funnels, etc. thoroughly with micro-90 soap and water using brushes.
Rinse glassware with tap water and with organic free DI water from E-Pure system. DI water system should be turned on and 2 liters drawn off before use. Check resistivity. Change cartridges when resistivity gets to 2.
Dry the glassware at room temperature.
Cover all open ends with foil. Always use dull side of the foil towards glassware.
Muffle glassware in furnace at 500^0C for 8 hours.
Allow glassware in furnace to cool to 100^0C (usually it takes 10-12 hrs) before removing from furnace.
Store them in cabinets.

The volumetric flasks and the pipettes are not muffled. Volumetric flasks are cleaned with soap and water then ultrasonicated with dichloromethane 3 times, 15 minutes each time. Volumetric pipettes are initially solvent rinsed and then ultrasonicated with dichloromethane 3 times, 15 minutes each time.

2. Stainless Steel Tools

Wash forceps, spatulas, stainless steel air cartridges, and aluminum cartridge rings with micro-90 soap and water using brushes.
Rinse well first with tap water and then with DI water from the E-Pure system. DI water system should be turned on and 2 liters drawn off before use.
Dry at room temperature for minimum of 2 hours.
Put in a drying oven overnight at 100^0 C.
Rinse with dichloromethane.
Wrap each tool separately in multiple foils, shiny side towards the outside.
Store them in drawers.
Rinse with dichloromethane before use.

Air sampling cartridges and screen meshes are wrapped in aluminum foil (shiny side out) and muffled in the furnace at 500^0C for 8 hours before storing.
***Aluminum rings cannot be muffled and must be solvent rinsed with dichloromethane, wrapped in foil (shiny side out), and stored in drawers.**

3. Amber glass vials and Pasteur pipettes

Put the pipette or the vials in beakers and cover beakers with foil. Always use dull side of the foil towards glassware.
Muffle beakers containing vials or pipettes in furnace at 500^0C for 8 hours.
Cool glassware in furnace to 100^0C (usually next morning); remove from oven. Insert clean Teflon liners (see below) into vial caps.
Cap the vials within 24 hours and store in a beaker covered with foil.

4. Teflon liners

Place Teflon liners in glass beaker; cover with dichloromethane.
Ultra-sonicate for 15 minutes. Drain dichloromethane.
Repeat 2 more times.
Put the beaker in a drying oven for 2 hours at 70^0C.
Store in sealed jar (covered with foil, lid screwed on).

5. Microdispenser capillaries, GC vials, and stainless N_2 blow down needles

a) Microdispenser capillaries

Before using rinse with dichloromethane and air dry.

b) GC autosampler vials

Place the vials in beakers. Cover with Al foil. Always use dull side of the foil towards glassware.
Muffle in furnace at 500^0C for 8 hours.
Dispose of them after use.

c) Stainless Steel N_2 blow down needles

Place needles in a clean beaker and cover with dichloromethane. Cover loosely with foil. Always use dull side of the foil towards glassware.
Sonicate needles for 10 minutes.
Drain solvent and repeat twice more.
Drain all solvent and transfer needles to clean beaker. Cover beaker with foil and store them for future use.
Just before use, squirt dichloromethane solvent through these needles.
Pass nitrogen through them for 10 minutes before use.

6. Teflon Stopcocks and Lids for Sample Jars

Wash stopcocks with micro-90 soap and water. Rinse with DI water from E-Pure system. Make sure the stopcock adjuster on the side is turned to the open position (vertical).
Lids are wiped with a damp Kimwipe soaked in tap water, and then are wiped with a damp Kimwipe soaked in DI from E-Pure system.
Air dry on Kimwipes.
Rinse the Teflon stopcocks (without washers) with dichloromethane.
Store the stopcocks in muffled jars.
Place the clean lids on muffled sample jars or wrap them in foil, shiny side out.

II. PRECLEANING: SAMPLING MEDIA AND CHEMICALS

1. Glass Wool

Supplies

Beaker (1 Liter)
Glass wool
Scissors
Muffle furnace

Procedure

Cut glass wool into 2" pieces.
Put them in muffled beaker.
Cover with foil. Always use dull side of the foil towards glassware.
Muffle in furnace at 500^0C for 8 hours. Cool furnace down to 100^0C.
Store on a shelf in a beaker covered with foil.

2. Teflon Boiling Chips

Supplies

Soxhlet extractor
Condenser
Sample jar and lid
500 mL round bottom flask
Boiling chips
Dichloromethane
Dichloromethane in squirt bottle
Methanol in squirt bottle
Cork ring for round bottom flask
Variable autotransformer
Heating mantle for either 1 liter or 500 mL round bottom flask
Drying oven

Procedure

Day 1

Thoroughly rinse inside of the condenser and outside joint with solvent from squirt bottles: first with methanol, then with dichloromethane.
Put 10 to 12 boiling chips in round bottom flask. Add 350 mL of dichloromethane to flask.
Place Teflon boiling chips to be cleaned in soxhlet extractor with glass wool plug at the bottom.
Assemble flask, soxhlet, and condenser.
Turn on heater to give proper boiling (set variac to 40-45 (48 for DCM) and heating mantle to dial 3).
Turn on cold water for condenser.
Extract for 18 to 24 hours.

Day 2

Turn heat off and cool it down for 15 to 30 minutes.

Turn off condenser water.
Drain as much solvent from soxhlet as possible.
Place boiling chips in muffled sample jar; cover loosely with foil. Always use dull side of the foil towards glassware.
Put sample jar in drying oven at 70^0C with Al foil cover cracked open.

Label sample jar with date cleaned and store the jar on shelf (covered with foil, lid screwed on).

3. SODIUM SULFATE

Supplies

Sodium sulfate (anhydrous, granular, mesh 12-60, Fisher Scientific)
Muffled sample jar, muffled beaker
Aluminum foil
Muffle furnace
Desiccator

Procedure

Do this each time silica gel is muffled for column work.
Put some sodium sulfate in a muffle beaker.
Cover with Aluminum foil with the shiny side out.
Crack open the foil before placing in muffle furnace for approximately 24 hours at 300^0C.
Reduce the temperature to 100^0C after the sodium sulfate has been muffled for approximately 18 hours.
Once the temperature reaches 250^0C crack open muffle furnace, then when the temperature reaches 150^0C take out the sodium sulfate and cover jar completely with the Aluminum foil.
Allow to sit on bench to cool until warm.
Once cool, transfer sodium sulfate to a jar, cover it with a lid, label with date cleaned and put it in a desiccator for storage.

4. XAD-2: (Supelpak -2SV, catalog #13682-U, 20-60 mesh size, pore diameter 90Å, Fisher Scientific)

Supplies

Soxhlet extractor and condenser 71/60 and 29/42 joints
One liter round bottom flasks with 24/40 joint
Glass stoppers (24/40 joint)
1 or 2 liter beakers
Adapter to convert 29/42 to 24/40
Boiling chips
Dichloromethane
Hexane
Methanol
Acetone
HPLC grade water: EMD, Omni Solv
Squirt bottle
Methanol in squirt bottle
Foil
Glass wool
Cork rings
Heating mantle for 1 liter flask

Variable autotransformer
XAD-2

Procedure

i) Dry XAD-2 for air sample cartridges:

Day 1

Rinse XAD-2 with tap water several times, stirring to remove the foam and the small particles.
Sometimes it is necessary to add about 100 mL of methanol.
Use Kimwipes to remove the foam.
Place XAD-2 in soxhlet extractor plugged with glass wool.
Rinse with small amount of methanol 3 times to remove water.
Add 800 mL of **methanol** to 1-liter flask.
Add about 20 boiling chips to flask.
Assemble flask/soxhlet/condenser.
Turn on heater to give proper boiling (set variac to 70 for methanol).
Turn on cold water for the condensers.
Cover soxhlet and flask with foil.
Extract with methanol for 24 hours.

Day 2

Turn heater off. Cool down for 15 to 30 minutes.
Flush as much methanol from soxhlet as possible.
Add 800 mL **acetone** to 1-liter flask.
Add about 20 boiling chips to flask.
Turn on heater (set variac to 55 for acetone).
Cover soxhlet and flask with foil.
Extract with acetone for 24 hours.

Day 3

Follow the procedure of Day 2 but use **hexane** as solvent. Use a new flask. Set variac at 50.
Extract for 24 hours.

Day 4

Follow the procedure of Day 2 but use **acetone:dichloromethane (70:30 by volume).** Set variac at 55.
Extract for 24 hours.

Day 5

Follow the procedure of Day 2 but use **dichloromethane** as solvent. Use a new flask. Set variac at 48.
Extract for 24 hours.

Day 6

Turn off heater; cool 15 to 30 minutes.
Flush as much dichloromethane as possible from soxhlet as possible.
Pour XAD-2 in a large funnel plugged with muffled glass wool and partially cover with Al-foil. Allow remaining Dichloromethane to filter into a muffled 1 L beaker until funnel stops dripping. Get rid of dichloromethane and transfer XAD-2 to the beaker.
Dry XAD-2 in a drying oven at 75^0C for 8 hrs.
Store in amber bottle in freezer at -20^0C for up to three months.

Keep subsample in separate jar for checking lab blank and matrix spike.

Note: Recycled XAD-2 is already free from foam and fine particles. To pre-clean this, omit the water rinsing and the methanol extraction steps. Start extraction with acetone and then follow the whole procedure. For recycled XAD-2, extraction period for each solvent can be reduced to 24 hours.

ii) Wet XAD-2 for precipitation sample cartridges:

Day 1

Rinse XAD-2 with tap water many times, stirring to remove foam and small particles.
Place XAD-2 in extractor plugged with glass wool.
Rinse with small amount of methanol 3 times to remove water.
Add 800 mL of **methanol** to 1-liter flask.
Add about 20 boiling chips to flask.
Assemble flask/soxhlet/condenser.
Turn on heater to give proper boiling (set variac to 70 for methanol).
Turn on cold water for condenser.
Cover soxhlet and flask with foil.
Extract with methanol for 24 hours.

Day 2

Turn heater off. Cool them down for 15 to 30 minutes.
Flush as much methanol from soxhlet as possible.
Add 800 mL **acetone** to 1-liter flask.
Add about 20 boiling chips to flask.
Turn on heater (set variac to 55 for acetone).
Cover soxhlet and flask with foil.
Extract with acetone for 24 hours.

Day 3

Follow the procedure of Day 2 but use **hexane** as solvent. Use a new flask.
Set variac at 50.
Extract with hexane for 24 hours.

Day 4

Follow the procedure of Day 2 but use **acetone:dichloromethane (70:30 by volume)** as solvent.
Set variac at 55.
Extract for 24 hours.
Day 5

Follow the procedure of Day 2 but use **dichloromethane** as solvent. Use a new flask.
Set variac at 48.
Extract for 24 hours.

Day 6

Follow the procedure of Day 2 but use **acetone** as solvent.
Set variac at 55.
Extract for 24 hours.

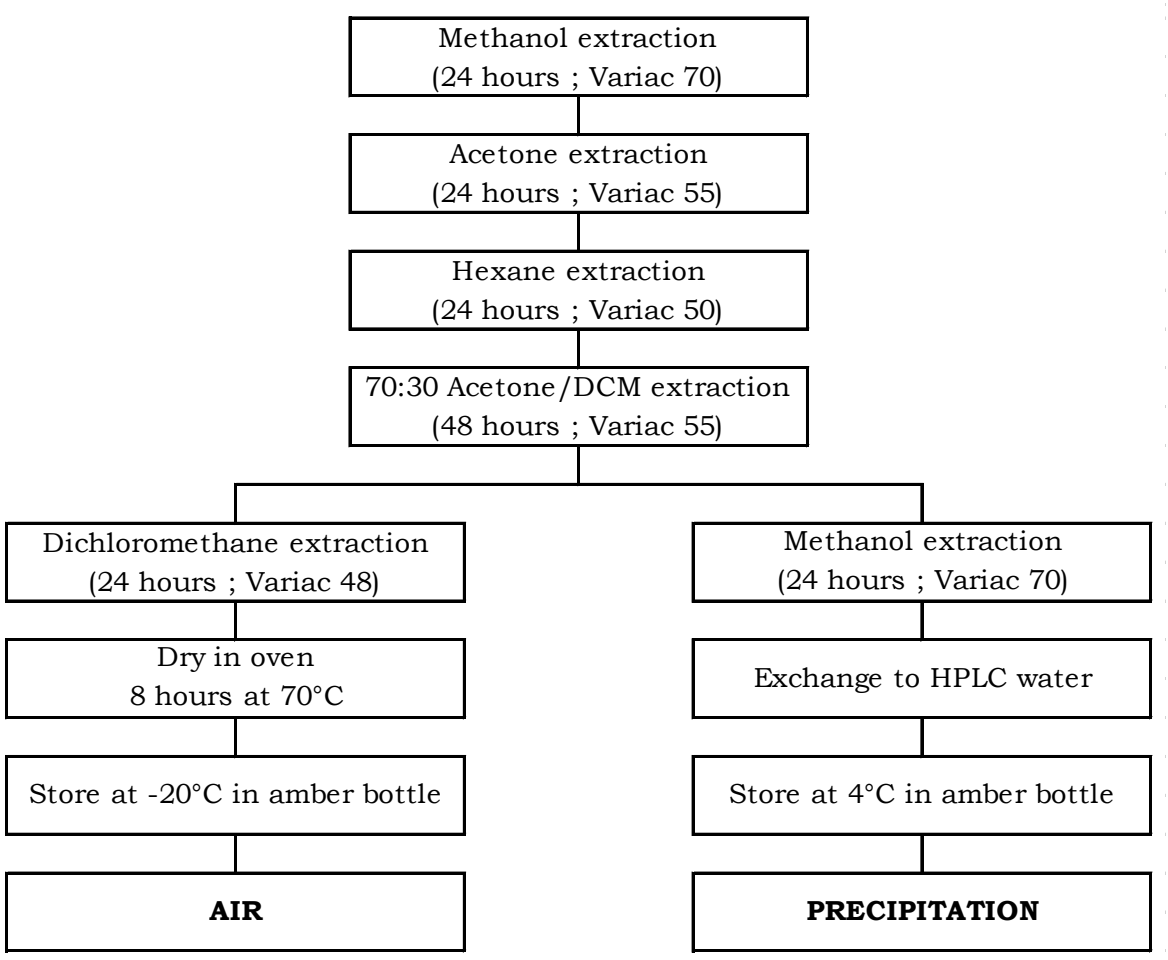
Day 7

Follow the procedure of Day 2 but use **methanol** as solvent. Use a new flask.
Set variac to 70.
Extract for 24 hours.

Day 8

Turn off heater; cool 15 to 30 minutes.
Flush as much methanol from soxhlet as possible.
Rinse XAD-2 with EMD HPLC grade water until XAD-2 does not smell of any solvent.
Store clean XAD-2 in an amber bottle at 4^0C. It can be used up to three months.
Keep subsample in separate jar for checking lab blank and matrix spike after making a column and drying it for extraction use.

FLOW CHART 2. SUMMARY OF XAD-2 PRECLEANING



5. Silica and quartz fiber filters (QFF)

i) Silica: Silica gel sorbent 100-200 mesh, Fisher Scientific. It has been determined that the silica is adequately cleaned during the activation process therefore no additional processing is necessary.

ii) Quartz fiber filters (QFF): 0.8 micron, VWR

Wrap up each QFF by aluminum foil separately, shiny side out.
Muffle at 450^0C for 6 hours.
After cooling put them individually in plastic bag and store them in freezer at -20^0C.

iii) Glass Fiber Filters (GFF): 1.0 micron, VWR
Wrap up each GFF by aluminum foil separately shiny side out.
Muffle at 450^0C for 6 hours.
After cooling put them in plastic bag and store them in freezer at -20^0C.

III. PREPARATION OF SAMPLING CARTRIDGES

1. Precipitation Columns for MIC sampler

Supplies:

Glass rain columns: Chromatographic columns (Ace Glass Inc. 5820-16)
15 mm threaded Teflon plugs
"O" rings for the Teflon plugs
Teflon adapter with valves
Muffled glass wool
Water: EMDSolv grade
Beakers
Tweezers
Aluminum foil
Stand and clamp
Pre-cleaned wet XAD-2: Supelpak -2SV, 20-60 mesh size, pore diameter 90Å

Procedure:

Attach the column to a clamp stand so that the red arrow points up.
Attach the Teflon valve at the bottom end to control the flow.
Pack glass wool to about 1/4".
Pour water in to check, and adjust the flow.
Fill the column with wet XAD-2 (11-14 cm. in length) and let it settle. Tap the column gently to get better packing. Never let the XAD-2 get dry.
Put another plug of glass wool on the top.
Put water on the top of the column and screw in the Teflon plug with "O" ring.
Turn it upside down, take the adapter valve off and put another Teflon plug in place of the valve.
Make sure that the o-ring on the Teflon plug makes a good seal.
Cover it first with Aluminum foil and then with bubble wrap.
Store them at 4^0 C until shipping.
Make one extra column for laboratory blank and matrix spike.

2. Quartz Fiber Filter for High-vol Air Samplers

Supplies

Quartz fiber filter: Whatman 8x10 inch, QM-A
Humidity chamber: Lab Line Descicab No 1477 with saturated solution of Lithium.
Nitrate to maintain 50% relative humidity.
Balance: Mettler AE50 with a filter chamber and a hanger underneath.
Muffle furnace
Gallon size plastic Ziploc bags
Aluminum foil
Tweezers

Procedure

Wrap quartz fiber filters with aluminum foil and make sure that the sides are not damaged.
Heat the wrapped filters at 450^0 C for 6 hours in the muffle furnace.
Store them at -20^0 C.
Take them out of the freezer 48 hours before shipping and put them in the designated humidity chamber for 24 hours, with the aluminum foil slightly opened.
After it has been equilibrated with 50% humidity for 24 hours, put a filter ID on the upper right hand corner of the filter with a pencil. Put it into the filter chamber of the balance, using tweezers, and take the weight. Take three weights to get a good average.
Record the filter ID and the initial weight in the filter book.
Wrap the filter again in the same foil. Write the filter ID on the aluminum foil with a marker.
Put the filter in Aluminum foil in a Ziploc plastic bag and store it at -20^0 C until shipping.
Place the filter in a book mailer for mailing to the site.

Calibrate the balance with a set of external weights ranging from 2mg to 200mg once a month. Check the internal calibration once every two weeks. Company calibration is done annually.

Avoid touching the filter. Always use tweezers.

3. XAD-2 Cartridges for Hi-vol Air samplers

Supplies

Pre-cleaned dry XAD-2
Stainless steel cartridges, wrapped in aluminum foil and muffled
Screens, wrapped in foil and muffled
Aluminum rings for the cartridges solvent cleaned and wrapped in foil (Do not muffle the aluminum rings)
Tweezers
Tin cans
Teflon tape
Black electric tapes

Procedure

Take a muffled stainless steel cartridge and carefully un-wrap and remove the foil.
Put a screen and retainer ring at one end. Pour 40-42g of pre-cleaned XAD-2. Put another screen and retainer ring on the other end. Check to make sure no XAD-2 is leaking. Always handle the screens with tweezers to avoid contamination.
Wrap the XAD-2 cartridge in the same foil it was muffled in. If necessary, use some extra foil. Place the whole cartridge in a tin ointment can rinsed with solvent. Seal the cover first with Teflon tape and then with black electrical tape.
Store them at -20^o C until shipping.
Record the batch number of the XAD-2 used for making the cartridges in the sampling protocol book.

Use new pre-cleaned XAD-2 for summer months' cartridges (April through October) and recycled XAD-2 for the winter months' cartridges (November through March).

Chicago and Cleveland recycled XAD-2 should be used only for Chicago and Cleveland.

IV. SAMPLE HANDLING AND STORAGE

1. Air vapor samples or XAD-2 cartridges

Check the sample packaging and the integrity of the samples very carefully. If it is not done properly write them down in Field Data Sheet and in sample log file.
Unwrap the aluminum foil carefully.
Unscrew the retainer nut and remove the screen with clean and solvent rinsed tweezers.
Transfer all XAD-2 into a previously cleaned and muffled glass jar.
Put the cap tightly after covering the jar with aluminum foil.
Label the jar with sample ID (Site_Sampler#_Sample type_Date): eg. SH 01C 031230.
Store in the freezer at -20^0C until analysis.
Sign and date the field data sheet and write comments, if any. File the field data sheets in folders.
Enter the field data in Laboratory log file.
File site visit data sheet on weekly basis.

If the samples cannot be transferred immediately they can be stored in refrigerator (4^0C) temporarily.

2. Air particle sample or quartz fiber filter

Check the sample packaging, sample integrity, and write comments in Filed Data Sheet and in the sample log file.
Unwrap the filters slightly and place in the humidity chamber (Boekel Scientific) for approximately 24 hours.
Sign, date, and file the field data sheet with comments.
File site visit sheet.
Enter field data into sample log file.
Next day take the final weight along with the sample ID code and record alongside the corresponding filter ID numbers in filter folder.
Rewrap the filters in the foil, put in a plastic bag and store at -20^0 C in a freezer until extraction.
Subtract the initial weight from the final weight and record the total suspended particle (TSP) of the filter in micrograms per meter^3. Enter this information in sample log file.

If the samples cannot be put in the humidity chamber immediately, they can be stored in cold room (10^0C) temporarily.

3. Precipitation Column

Check the sample packaging and the integrity of the samples very carefully. If it is not done properly write them down in the Field Data Sheet and sample log file.
Unscrew the bottom Teflon cap and put the Teflon valve on the bottom side.
Clamp the column securely.
Put a drain jar underneath the column.
Drain extra water from the column.
Aspirate all water out of the column (15 minutes per sample).
By gentle tapping transfer all XAD-2 in a clean pre muffled jar.
With the help of a Pasteur pipette rinse the inside of column with acetone and collect it in the same jar.
Label the jar with sample ID. Site_sample type_ Sampler# _Collection date. Example SP 01 030508.
Store the sample in freezer at -20^0C until analysis.
Sign, date, and file the field data sheet.
Enter the data into sample log file.

If the samples cannot be transferred immediately, they can be stored in refrigerator (4^0C) temporarily.

V. EXTRACTION

1. Air samples (vapor phase and particle phase) and precipitation samples

XAD-2 cartridges, Quartz fiber filter (QFF), and XAD-2 rain columns

Supplies

Large soxhlet extractor (55/50 and 24/40 joints)
Condenser (55/50 joint)
Round bottom flask (24/40 joint) 500 mL
Glass stopper (24/40 joint)
Beakers
Micro-dispenser (50 or 100 µl) and 1 mL pipette
Boiling chips
Acetone
Hexane
Surrogate Recovery standards: Table 2
One matrix spike vial (MS vial) with recovery standards: PCB (683.2 ng), pesticides (20 ng each), PAHs (400 ng ea), BFR (PBDE) Recovery Standard (40-200 ng/ml), and OPEs.
Waste solvent bottle
Cork rings (one per each 500ml round bottom flask)
Glass wool
12" rod (glass or metal)
Large tweezers
Small tweezers
Al foil
Scissors
Heating mantle and variable autotransformer or multi-unit extraction heat
Clean XAD-2 or QFF for blank

TABLE 2. SURROGATE RECOVERY STANDARDS

PCBs	Congener 14: 200 ng/mL
	Congener 65: 50 ng/mL
	Congener 166: 50 ng/mL
Pesticides	Dibutylchlorendate: 200 ng/mL
	δ-HCH: 200 ng/mL
	€-HCH 200ng/ml
PAHs	d_{10} phenanthrene: 4 µg/mL
	d_{10} pyrene: 4 µg/mL
PBDE	BDE-77: 60 ng/mL
	BDE-166: 100 ng/mL
	$^{13}C_{12}$-BDE-209: 80 ng/mL
OPEs	d12-TCEP: 5 µg/mL
	MTPP: 5 µg/mL

Procedure

i) Setting up

One batch of samples generally include:

Regular samples: 8-15 (This usually includes field blanks and / or field duplicates)
Laboratory duplicate (usually once a month): One air vapor sample split into two equal parts in laboratory **(No laboratory duplicate for filter and precipitation samples).**
Laboratory blank (alternate batches): Sampling media spiked with **surrogate standards**
Matrix spike (alternate batches): Sampling media spiked with **recovery standards**

On the day of extraction a unique **Batch ID** is assigned to a batch of sample with month, year, and sample type. Example: Batch IDs of the cartridge, filter, and precipitation samples from September 2010 will be S10C, S10F, and S10P, respectively.

Day 1

Remove standards from freezer. Standards **must** be at ambient temperature before using. (Ambient temperature is achieved in about 2 hours).
Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with dichloromethane.
Label flasks with sample IDs.
Add 10-12 clean Teflon chips into 500 mL round bottom flask.
Pour solvent into round bottom flask: 200 mL of acetone and 200 mL of hexane (for vapor and particle only).

Vapor sample: XAD-2
Place glass wool plug at the bottom of the soxhlet extractor using large tweezers, glass or metal rod.

Carefully pour XAD-2 in soxhlet extractor. Rinse the container twice with solvent (50% acetone/50% hexane) to remove all XAD-2; pour solvent rinses into soxhlet.

Particle sample: QFF
Unwrap one QFF at a time.
Trim off the number at the corner with clean scissors rinsed with dichloromethane.
Use 2 pairs of blunt tweezers to fold one QFF; place it all the way down in soxhlet so that the top part of the QFF remains below the top level of the small siphon tube.
Label the soxhlet with the sample ID
Rinse tweezers and scissors with dichloromethane before starting the next sample.

Precipitation sample: XAD-2 column
Place glass wool plug at the bottom of the soxhlet extractor using large tweezers, glass or metal rod.
Keep a beaker with 200 mL of acetone in front of soxhlet extractor.
Carefully transfer XAD-2, and glass wool plug in the soxhlet extractor. Rinse the container twice with acetone to remove all XAD-2; Pour about 150 mL of acetone into soxhlet and let the solvent stand there for 15 min.
Hand flush the solvent. Add rest of acetone from beaker to soxhlet and flush again.
Add 200 mL of hexane to soxhlet and siphon.

Note: The precipitation samples have water in them and may not siphon on its own. Induce siphoning first 2-3 times by hand until the level of solvent in the soxhlet and in the siphon tube are the same.

Matrix spike:
Take about 20-30g of dry XAD-2 (vapor set), or muffled QFF (filter set), or 8g of wet XAD-2 (precipitation set) in a soxhlet extractor plugged with glass wool.
Add: A vial containing all recovery standards.

PCB recovery standard: complete suite of PCB congeners (683.2 ng, from Michael D. Mullin 94 mix)
Pesticide recovery standard: Calibration Reference Standard (CRS), S-8206A fortified with 3 other pesticides**,** all pesticides 20 ng each.
PAH recovery standard: all PAHs 400 ng each (Laboratory mix)
PBDE recovery standard: Mixture of 25 selected PBDE listed in Table 27 (40-200 ng/mL)
OPE recovery standard (used only with filter sets): all OPFRs 500 ng each (Laboratory mix)

Make sure the matrix spike vial used is recorded in the sample prep book.
The recoveries of each compound will show the extraction efficiency of that batch.

Laboratory blank
Take about 20-30g of dry XAD-2 (vapor set), or muffled QFF (filter set), or 8g of wet XAD-2 (precipitation set) in a soxhlet extractor plugged with glass wool.

Laboratory duplicate: for Chicago air vapor only
Shake the sample from Chicago in the jar to mix it thoroughly.
Weigh out the whole XAD-2.
Weigh out approximately 10-12g of it and carefully transfer it in a Soxhlet extractor plugged with glass wool. Weigh out another 10-12g of the same sample and transfer it in a second Soxhlet extractor. Label the 1st one as CH-02C1-yy-mm-dd and the 2nd one as CH-02C2-yy-mm-dd. Record mass in sample prep book

ii) Spiking with Surrogate Standards

Using a 100 µL micro dispenser, spike each sample with mix surrogate standard that contain:

PCB 14:	200 ng/mL
PCB 65:	50 ng/mL
PCB 166:	50 ng/mL
Dibutylchlorendate:	200 ng/mL
δ-HCH:	200 ng/mL
ε-HCH	200 ng/mL
d_{10} phenanthrene:	4 µg/mL
d_{10} pyrene:	4 µg/mL
d_{12}-TCEP	5 µg/mL
MTPP	5 µg/ml

Using a 50 µL micro dispenser, spike each sample with:

BDE-166	100 ng/mL
BDE 77	60 ng/ml
$^{13}C_{12}$-BDE 209	80 ng/mL

Make sure to rinse dispenser with DCM and change tip between each standard. Recovery of each surrogate standard will show the extraction efficiency of individual sample.

iii) Extraction

Day 1

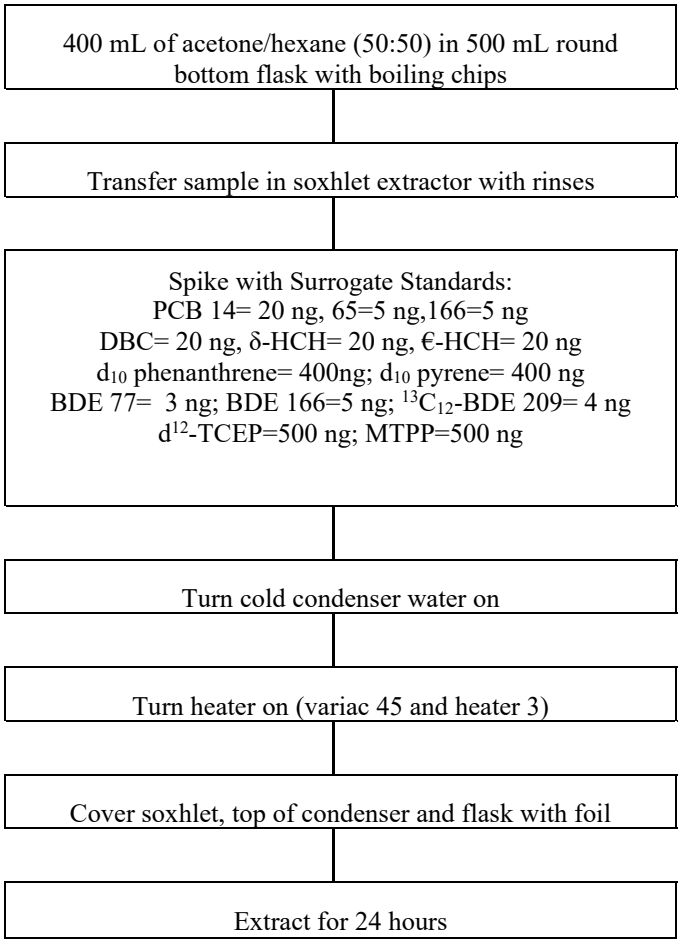
Assemble flasks, soxhlets, and condensers. Place on heating mantles.
Turn on heating mantles. Set the heater at 3 and or the variac at 45.
Turn on condenser cold water on.
Cover soxhlet, top of the condenser and flask with foil.
Extract for 18 to 24 hours (30 hours for precipitation).

Day 2

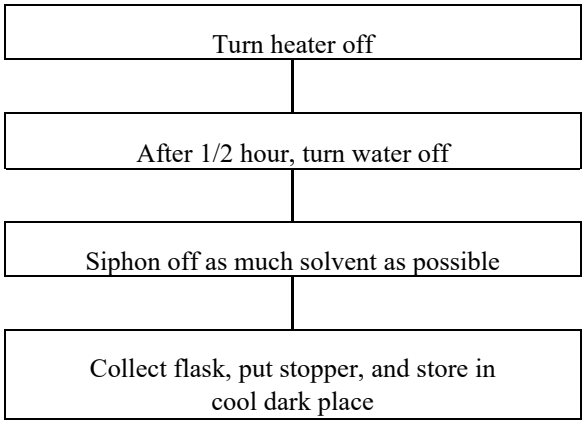
Turn heating mantle off. Let them cool down for 30 minutes. Turn off condenser water.
Siphon off as much solvent from soxhlet extractor into flask as possible.
Detach the flask and insert stopper. Store the extracts in cool dark place.

FLOW CHART 3. SUMMARY OF EXTRACTION OF AIR SAMPLES

Setting up extraction: Day 1

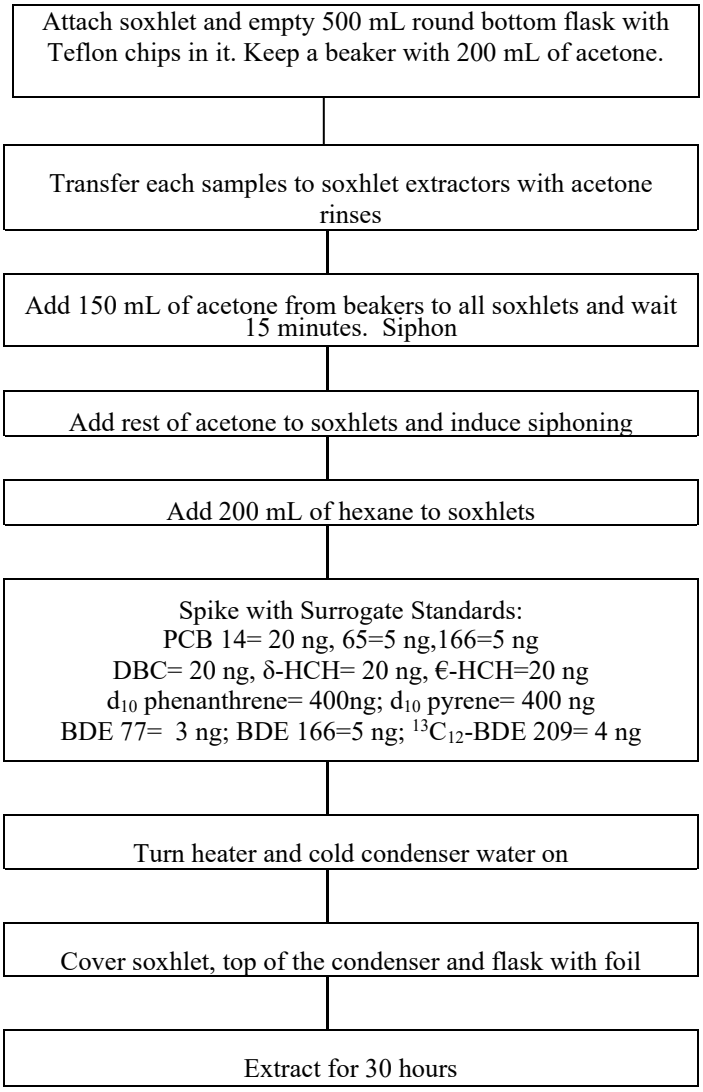


Taking extraction down: Day 2

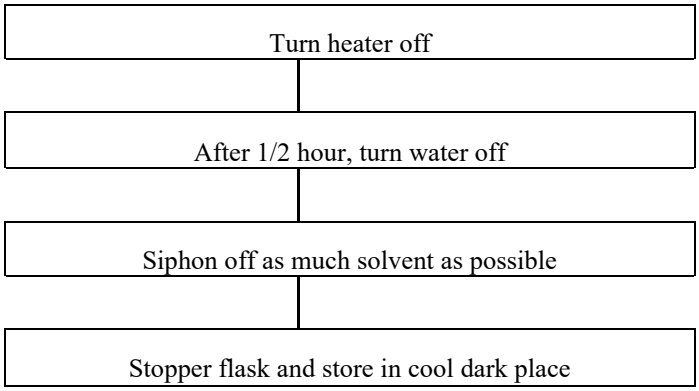


FLOW CHART 4. SUMMARY OF EXTRACTION OF PRECIPITATION SAMPLES

Setting up extraction: Day 1



Taking extraction down: Day 2



VI. ROTARY EVAPORATION

1. Air (XAD-2 and QFF) extract

After extraction, the extracts need to be concentrated and solvent exchanged to hexane before silica gel chromatography.

Supplies

Splashguard with 24/40 joint or 20/14 joint
Beaker 100 or 200 mL
Waste container for used boiling chips
Hexane
Clean large forceps
Squirt bottle
Rotary Evaporator (Buchi Rotavapor, R-110)
Faucet aspirator
Chiller circulator (Neslab, CoolFlow, CFT-25)
Kimwipes

Procedure

i) Setting up

Fill chamber with DI water.
Turn the chiller circulator on.
Set bath temperature 30^0C -35^0C.
Rinse joint of steam duct with dichloromethane or hexane. Wipe it off with Kimwipe.
Attach appropriate splashguard to steam duct. Rinse the joint of splashguard with dichloromethane.
Clamp each joint.
Turn vacuum on with the faucet aspirator.

ii) Evaporation

Remove boiling chips from the extract with large clean forceps.
Attach flask to splashguard. Clamp joint.
Turn motor on to rotate the flask. The sample should **not** boil.
Evaporate the extract down to approximately 2 mL.
Open stopcock of the rotary evaporator to release vacuum.
Detach the flask.

iii) Solvent exchange

Add 75 mL of hexane and rotavap down to 2 mL again.
Repeat the process once more.
Rinse the splashguard with dichloromethane or hexane before next sample.

iv) Completion

Empty the receiving flask into proper waste bottle.
Turn the heater, motor, chiller, and the aspirator off.
Cover steam duct and stopcock with foil.

2. Rotary Evaporation and Back Extraction of Precipitation Extracts

Supplies

Splashguard with 24/40 joint
Waste container for used boiling chips
Hexane
Clean large forceps
Waste bottles
Dichloromethane in Teflon bottle
Separatory funnel with stopcock
50mL Centrifuge tubes with stoppers
Pasteur pipettes
Rotary evaporator (Buchi Rotavapor, R-110)
Chiller circulator (Neslab, Cool Flow, CFT-25)
Kimwipes

Procedure

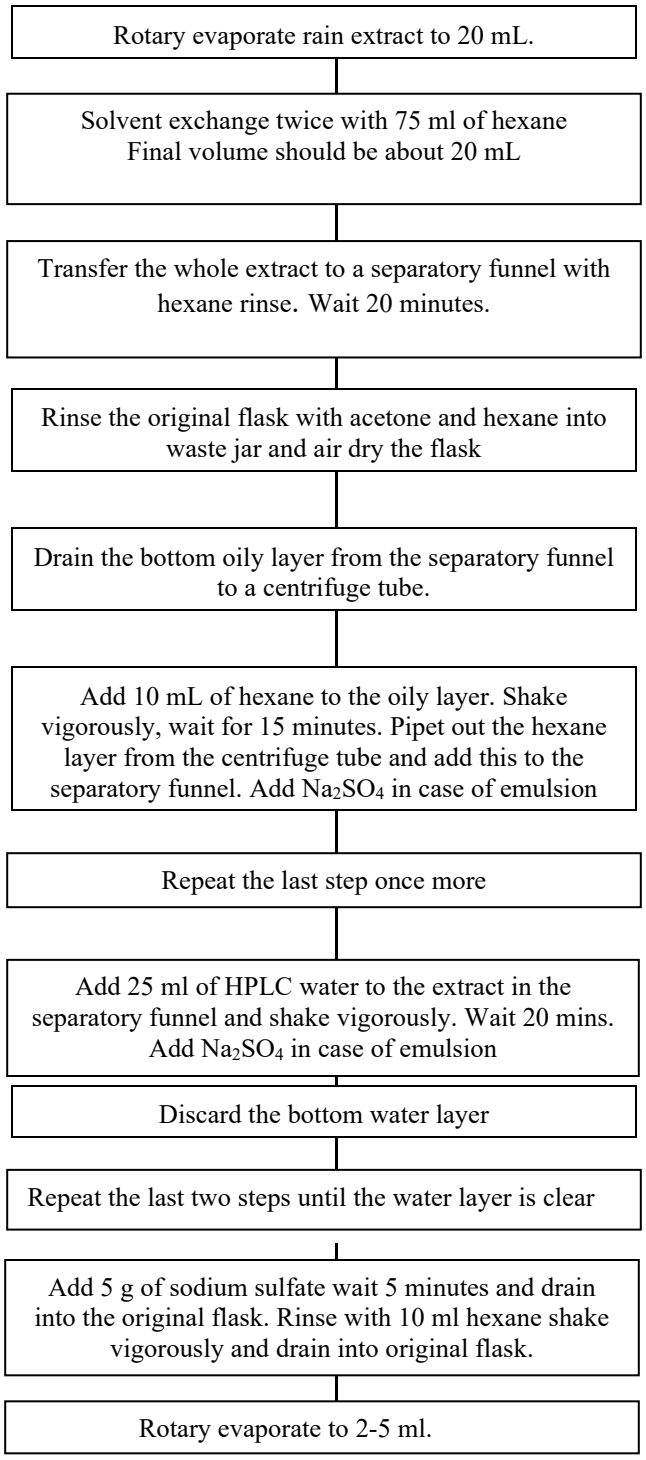
i) Evaporation

Rinse the joint of the steam duct with dichloromethane or hexane.
Attach splashguard to steam duct. Clamp joint.
Turn vacuum on with faucet aspirator.
Remove boiling chips from the extract with large clean forceps.
Attach flask to splashguard after rinsing the joint with dichloromethane.
Clamp joint.and turn motor on. Turn water bath on. The temperature should be 30-35^{0}C.
Turn chiller on.
The solvent should start evaporating within 2 minutes. The sample **should not boil**.
Concentrate the samples to about **20 mL** until the two layers are separated.
Add 75 mL of hexane and rotavap down to about 20 mL. The separation mark will be clearer.
Add 75 mL of hexane again and go down to about 20 mL. The two phases should be clearly visible.

ii) Back Extraction

Transfer the whole extract to a 125 mL separatory funnel.
Rinse the original flask with 10 mL of hexane and add this to the separatory funnel. Wait 20 minutes.
Rinse the original flask with acetone and hexane into waste jar and air-dry the flask.
Drain the bottom oily layer from the separatory funnel to a 50 mL centrifuge tube.
Add 10 mL of hexane to the centrifuge tube and shake vigorously. Wait for 15 minutes.
If it forms an emulsion, add Na_2SO_4 and shake.
Pipet out the hexane layer from the centrifuge tube and add this to the separatory funnel.
Repeat last three steps once more.
Add 25 mL of HPLC water to the extract in the separatory funnel, shake vigorously, and let it stand for 20 minutes.
Drain the bottom water layer.
Repeat last two steps until the water layer is clear.
Vigorously shake the separatory funnel again and settle.
Drain off any amount of water that forms at the bottom.
Measure out 5 grams of sodium sulfate and place in separatory funnel.
Shake vigorously and set for 5 minutes. Drain the sample into original flask.
Add 10 mls of hexane to separatory funnel and shake vigorously. Drain into original flask.
Rotary evaporate to 2-5 mL.

FLOW CHART 5. ROTARY EVAPORATION AND BACK EXTRACTION OF PRECIPITATION EXTRACTS



VII. SILICA COLUMN CHROMATOGRAPHY

1. Activation and Deactivation of silica

Supplies

Beakers
Powder funnel
Round bottom flask 250 mL with stopper and cork ring
Pipet and pipet filler
Silica gel, Davisil, Grade 634, 100-200 mesh, 60Å
Muffle furnace
Desiccator
Calculator
Balance
Particle mask

Procedure

i). Activation

Day 1
Place approximate amount of silica needed in a beaker. Cover the beaker with foil loosely.
Place beaker in 100^0C oven, turn thermostat to 300^0C; keep in oven overnight.

Day 2
Turn the oven temperature down to 100^0C.
Crack the door of the oven open when the oven has cooled down to 250^0C.
When the oven temperature is 150^0C remove silica from the oven and make the Al foil tightly closed.
Let it cool on the counter top until warm.
Store in a desiccator for 2 hours to allow silica to reach ambient temperature.

ii). Deactivation

After the silica has cooled in the desiccator for 2 hours, deactivate it:
Working quickly, weigh out desired amount of silica in the round bottom flask. Stopper the flask **immediately** after pouring silica.
Add 3.5% weight/volume of DI water to silica, using the following equation:

$$\frac{\%\ deactivation}{100 - \%\ deactivation} = \frac{ml\ DI\ water}{weight\ of\ silica\ (gm)}$$

For precipitation samples use 3% deactivation.

SHAKE WELL. Shake flask until all clumps are broken-up.
Store in a desiccator overnight for equilibration.
Use deactivated silica in desiccator within 3 days.

2. Column chromatography

Supplies:
(for a 2 fraction column clean-up of one sample)

For each sample:
Column - 1
Pear shaped flasks100 mL with 14/20 joints - 3
Glass stoppers 14/20 - 3
Pasteur pipettes (92 inch and 53 inch):
Graduated cylinders: 50 mL and 10 mL
Beaker, 50 mL - 1
Waste jar - 1
Beakers, 400 mL - 3
Rubber pipette bulbs
Hexane
50% hexane:50% dichloromethane
70% acetone:30% dichloromethane
Cork rings for each 100 mL pear shaped flasks - 1
Rubber hammer - 1
Stainless steel spatula - 1
20" rod - 1
Teflon stopcock
Glass wool
3.5% or 3% deactivated silica
Sodium sulfate
Ultrasonicator

TABLE 3. COLUMN SIZE AND AMOUNT OF SILICA

Item	Air Particle (QFF)	Air Vapor (XAD-2)	Rain (XAD-2)
Amount of silica to activate/deactivate	4-6 gm	4-6 gm	4-6 gm
Column size	3.5"	3.5"	3.5"
Na_2SO_4	0.5"	0.5"	1.5"
Elution volume	25 mL	25 mL	30 mL
Switching volume	4 mL	4 mL	5 mL

Procedure

i) Packing Columns

Put stopcocks on columns.
Stuff glass wool (approximately 1 cm) into lower end of the each column with 20" rod.
Measure and mark 3.5" from glass wool plug for silica packing and 0.5" for sodium sulfate cap. For rain sample the sodium sulfate cap should be 1.5".
Clamp columns securely onto frame in ventilation hood. Place empty glass container under each column.
Close stopcocks; fill columns half full with hexane. Tap columns to get out air bubbles before packing columns.
Make slurry of hexane and deactivated silica. Pour slurry into each column. **DO NOT ALLOW SILICA TO DRY OUT.** Open stopcocks.
Tap columns with rubber hammer to pack silica to desired length.
Cap columns with 0.5" Na_2SO_4 for XAD-2 and QFF samples, 1.5" Na_2SO_4 for precipitation samples.
Wash columns with 25 mL hexane for conditioning.
Close stopcocks to prevent further dripping when hexane level reaches 1 cm above the top of Na_2SO_4. **NEVER LET THE COLUMN RUN DRY.**

Set up

Label 100 mL pear-shaped flask for each sample for hexane, 50% dichloromethane in hexane, 70% acetone in dichloromethane fraction. Place the flasks for the hexane fraction underneath the columns.
Place sample flasks in front of columns.
Place a 50 mL beaker in front of sample flask for elution solvents either hexane, 50% dichloromethane in hexane and 70% acetone in dichloromethane. For 1st fraction add 25 mL of hexane in beaker for air samples and 30 mL of hexane for precipitation samples. Measurement of the elution and switching solvents can be done ahead of time.

Loading samples and collection of Fraction 1

Ultrasonicate each filter and vapor sample in the flask and load the sample on column with Pasteur pipet. DO NOT ultrasonicate the precipitation samples. Once precipitation samples are loaded on the column, place the flask in the wash bin as no rinses are added to the precipitation flasks.
Open stopcock and let the column drip at a rate of 1 drop per second in the pear-shaped flask.
When the sample touches the top of the Na_2SO_4, add a small portion of hexane from the beaker to the sample flask and rinse the sample flask. Add the rinse to the column.
When the rinse touches the top of the Na_2SO_4 add rest of the solvent to the column after rinsing the flask. Collect the 1st fraction.

Fraction 2

After the first fraction is completely collected, add 50% dichloromethane in hexane (4mL in case of air sample and 5 mL in case of precipitation samples) in sample flask and then on the top of the column. This is the switch volume.
After the switch volume is collected in the same flask containing hexane fraction, get the pear-shaped flask with elute and stopper it.
Put a new pear shape flask for collection of 2nd fraction.
Add 25 mL (for air samples) or 30 mL (for precipitation samples) of 50% dichloromethane in hexane on the column after rinsing the flask and elute.
When the rinse touches the top of the Na_2SO_4 add rest of the solvent to the column after rinsing the flask. Collect the 2nd fraction.

Fraction 3

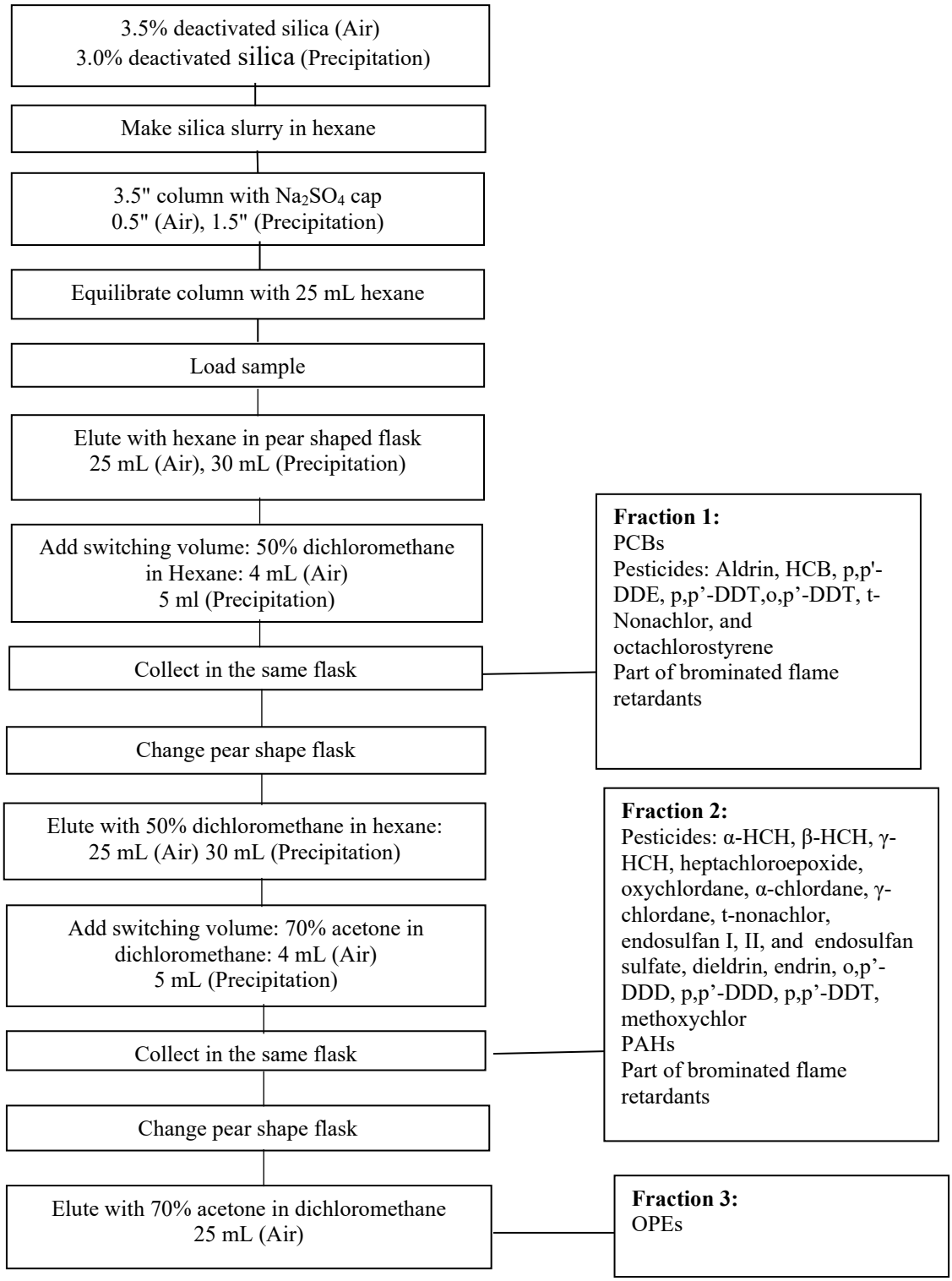
After the second fraction is completely collected, add 70% acetone in dichloromethane (4mL in case of air sample and 5 mL in case of precipitation samples) in sample flask and then on the top of the column. This is the switch volume.

After the switch volume is collected in the same flask containing 50% fraction, get the pear-shaped flask with elute and stopper it.
Put a new pear shape flask for collection of 3rd fraction.
Add 25 mL (for air samples) or 30 mL (for precipitation samples) of 70% acetone in dichloromethane on the column after rinsing the flask and elute.
Once the elute reaches the top of sodium sulfate, collect `15 more drops and remove flask with 3rd fraction, stopper it, and store in dark place.

iii) Clean-Up

With a jet of air get the dry silica out of the column. The silica should be treated as solid waste.

FLOW CHART 6. SUMMARY FOR SILICA COLUMN CHROMATOGRAPHY



iv). Re-cleaning of Fraction 1 (hexane fraction) and Fraction 2 (50% fraction)

Sometimes the chromatograms are not clean enough for correct analysis. This may be due to overloading of the silica column by the concentrated extracts. In these cases, the fractions need to be recleaned through silica column for the 2nd time. Take a subsample of the sample before recleaning. This will need to be saved for PBDE analysis. Put the subsample in a box labeled for PBDEs in the freezer.

Procedure for Fraction 1 (hexane fraction) recleaning:

Activate and deactivate the silica in the usual way.
Pack up the slurry in the same way topping it with sodium sulfate.
Directly load the Fraction 1 from the vial. Rinse the vial with 1 ml of hexane twice and load the rinsing on the column.
Let the column drip at the usual rate. Elute and collect in a pear shape flask.
Elute with 30 ml of hexane and collect.

It is not necessary to collect the Fraction 2.

Procedure for Fraction 2 (50% fraction) re-cleaning:

Transfer the Fraction 2 from the vial to a pear shape flask. Exchange the fractions to hexane by rotavapping and solvent exchanging with 25 mL of hexane once.
Activate and deactivate the silica in the usual way.
Pack up the slurry in the same way topping it and cap with sodium sulfate.
Load the exchanged extract from the pear shape flask on to the silica column.
Wash the flask twice with 1 mL 50% dichloromethane in hexane.
Elute and collect in another pear shape flask.
Elute with additional 25 mL of 50% dichloromethane in hexane and collect in the same flask.

VIII. ROTARY EVAPORATION OF FRACTION 1, FRACTION 2, AND FRACTION 3

Supplies

Splashguard with 14/20 joint
Beaker 50 mL and 100 mL
Hexane
Squirt bottle
Rotary Evaporator
Faucet aspirator
Chiller circulator
Kimwipes

Procedure

i) Setup

Fill chamber with DI water.
Turn on the chiller circulator.
Set bath temperature 30^0C -35^0C.
Rinse joint of steam duct with dichloromethane.
Attach appropriate splashguard to steam duct after rinsing them with dichloromethane. Clamp each joint.
Turn vacuum on with the faucet aspirator.

ii) Evaporation

Attach flask to splashguard. Clamp joint.
Turn motor on to rotate the flask. The sample should **not** boil.
Evaporate sample down to approximately 1 mL.
Open stopcock of rotary evaporator to release vacuum.
Detach the flask.
Hexane fraction is ready to be transferred.

iii) Solvent exchange

For Fraction 2, solvent exchange once with 25 mL of hexane.
Rinse splashguard with dichloromethane before using with a different sample.

For Fraction 3, solvent exchange twice with 25 mL of hexane.
Rinse splashguard with dichloromethane before using with a different sample.

iv) Completion

Empty receiving flask into proper waste bottle as needed.
Turn off heater on rotary evaporator.
Turn motor off on rotary evaporator.
Turn chiller off.
Cover steam duct and stopcock with foil.

IX. TRANSFER OF SAMPLES

Supplies (each sample)

Pasteur pipettes (9.2 inch and/or 5.3 inch):
Amber glass vial (4 mL) for each fraction
Beaker
Vial file for 4 mL vials
Rubber pipette bulbs
Hexane

Procedure

Label each amber vial with sample ID and fraction ID.
Transfer entire sample volumetrically from flask to amber vial with 2 hexane rinses using a pasteur pipette.
Close amber vial tightly, place in vial file, and store in freezer at -20^0C. Label the vial file with Batch ID.

X. NITROGEN BLOW DOWN

Supplies

Samples in amber vials
Nitrogen blow down unit
Dichloromethane

General procedure

Remove all nozzle plugs from unit.
Turn on N_2 at tank and let N_2 flush out for approximately 5 minutes.
Turn heater on LOW.

Squirt DCM solvent in each needle.
Attach clean needle to each nozzle to be used.

Pass nitrogen through them for 10 minutes.
Place amber vials in slot; adjust N_2 flow such that there are gentle ripples in the vials.
Evaporate down all samples and all fractions to approximately 1mL. For summer samples it may be changed to 1.5 to 2 mL especially for 50% fraction.

If the chromatograms look dirty in GC run, dilute the extracts and analyze again.

Completion

Turn off N_2 at trap.
Replace the nozzle caps.
Place needles in a clean beaker and cover with dichloromethane.
Cover loosely with foil with dull side down.
Sonicate needles for 15 minutes.
Drain solvent, and repeat twice more.
Drain all solvent and transfer needles to clean beaker.
Cover beaker with foil and store them for future use.

XI. SPIKING SAMPLES WITH INTERNAL STANDARDS (ISTD)

Supplies

Samples in 4 mL amber glass vials
Internal standards (ISTD)
Hexane
Dichloromethane
Waste containers
Microdispensers: 50 and 100 µl

Procedure

Remove internal standards from freezer; equilibrate to ambient temperature (approximately two hours).
Clean microdispenser by rinsing with dichloromethane.
Insert a new glass capillary.
Rinse the capillary with hexane twice and air dry. Draw spiking standard. Make sure that there are no air bubbles in the capillary.
Spike the sample.
Mark each amber vial label with an appropriate color of dot to denote that they have been spiked:
red for PCBs
blue for pesticides
black for PAHs
purple for PBDEs
Rinse the dispenser with solvent
Replace glass tube used to cover plunger of microdispenser before storing.

TABLE 4. INTERNAL STANDARDS AND MASS PER FRACTION

Fraction	Compounds	Internal Standard	Concentration	Spike	Vial	Box
Fraction 1	PCB	Cong, 30, 204	30=80 ng/ml, 204=60 ng/ml	100 µl	●	✓
	PAH	d_{10}anthracene, d_{12}benz[a]anthracene, d_{12}perlyene	4 µg/ml each	50 µl	●	✓
	PBDE	BDE-118, BDE-181, BB-209	BDE-118= 0.1 µg/ml, BDE-181= 0.2 µg/ml, BB-209= 0.2 µg/ml	50 µl for air 100 µl for precip	●	✓
Fraction 2	Pesticide	cong. 65,155	65=20 ng/ml, 155=20 ng/ml	100 µl	●	✓ (DB-5) ✗ (1701)
	PAH	d_{10}anthracene, d_{12}benz[a]anthracene, d_{12}perlyene	4 µg/ml each	50 µl	●	✓
	PBDE	BDE-118, BDE-181, BB-209	BDE-118= 0.1 µg/ml, BDE-181= 0.2 µg/ml, BB-209= 0.2 µg/ml	50 µl for air 100 µl for precip	●	✓
Fraction 3	PAH	d_{10}anthracene, d_{12}benz[a]anthracene, d_{12}perlyene	4 µg/ml each	100 µl	●	✓ (OPE)

XII. MAKING MICROVIALS FOR GC ANALYSIS

Supplies

Disposable microvials with inserts
Pasteur pipettes
Vial racks
Septa (vial caps)
Crimper

Procedure for PCBs, pesticides, and PAHs

Label microvials with sample IDs and fractions. Arrange for 2 hexane blanks, 2 Calibration Standards and 1 reference standard.
Put the insert in the vial.
Using a pasteur pipette, put approximately 200 µL of each sample, hexane, appropriate Calibration Standards, and Reference Standard in the inserts.
Use different pasteur pipette for different sample and standard.
Crimp septa onto the microvials.
Load the microvials into GC or GC/MS autosampler.

Procedure for brominated flame retardants:

Brominated flame retardants analysis is usually done after PCB, pesticides, and PAH analyses.
Label microvials with sample IDs and fractions.
Put the insert in the vial.
N_2 blow down the amber vials to approximately 200 µL (make sure that the sample has been analyzed for PCBs, pesticides, and PAHs).
Using a Pasteur pipet, transfer all the liquid from amber vials to the inserts.
Crimp septa onto the microvials.
Load the microvials into GC/MS autosampler.

TABLE 5. CALIBRATION STANDARDS

Fraction	Target compounds	Calibration standards
Fraction 1	PCBs	S-8074A-R1, S-8074B-R1 S-8074C-R1 (0.5 to 1 ug/mL- supplied by EPA and Env. Canada) fortified with 5 pesticides
Fraction 2	Pesticides	Mixed pesticide standard: 20 ng/mL each
Fraction 2	PAH	Mixed PAH standard 200 ng/mL each (approximately)
Fractions 1 and 2	Brominated flame retardants	Mixed flame retardants standard: 20-400 ng/mL
Fraction 3	OPEs	Mixed OPE standard: 2 µg/mL