

**Analysis of PCBs, Pesticides, PAHs, and PBDE
in
Air and Precipitation Samples**

IADN Project

Sample Preparation Procedure

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TABLE OF CONTENTS

Introduction.....	1
Chapters	Page
I. Cleaning: General Labware.....	4
II. Precleaning: Sampling media and chemicals.....	6
III. Preparation of Sampling Cartridges.....	12
IV. Sample Handling and Storage.....	14
V. Extraction.....	15
VI. Rotary Evaporation.....	20
VII. Silica Column Chromatography.....	23
VIII. Rotary Evaporation of Fraction 1 and Fraction 2.....	28
IX. Transfer of Samples.....	29
X. N ₂ Blow Down.....	30
XI. Spiking Samples with Internal Standards.....	31
XII. Making Micro vials.....	32
XIII. Standards.....	33
XIV. Safety.....	49

Flow-Charts	Page
1. Summary of Sample Preparation.....	3
2. Summary of XAD-2 Precleaning.....	10
3. Summary of Extraction of Air Samples.....	18
4. Summary of Extraction of Precipitation Samples.....	19
5. Rotary Evaporation and Back Extraction of Precipitation.....	22
6. Summary of Silica Column Chromatography.....	26

Tables

1. List of Analytes.....	2
2. Surrogate Standards.....	15
3. Column Size and Amount of Silica.....	24
4. Internal Standards and Mass per Fraction.....	31
5. Calibration standards.....	32
6. PCB Calibration Standard (CCS): stock.....	33

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 precleaning 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 notebook.

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.

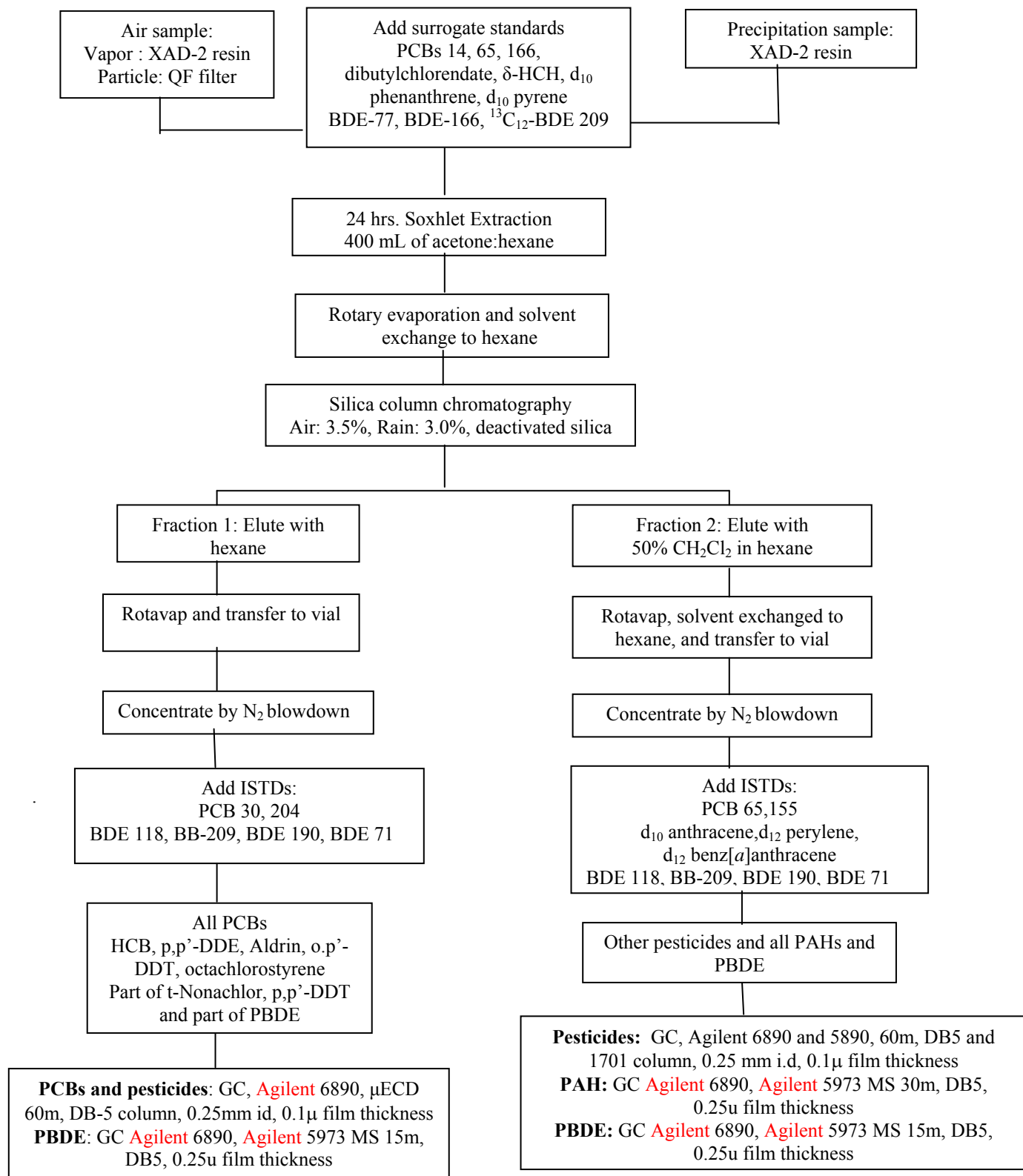
The compounds that are analyzed in this project are total and 84 polychlorinated biphenyl (PCB) congeners, 22 organochlorine pesticides (OCs), 17 polycyclic aromatic hydrocarbons (PAHs), and 43 polybrominated diphenyl ether (PBDE).

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

TABLE 1. LIST OF ANALYTES

PCB from 2005		Pesticides from 1999	PBDE from 2005
4+10	101	HCB	7
7+9	99	alpha-HCH	10
6	119	beta-HCH	15
8+5	83	gamma-HCH	17
19	97	heptachloroepoxide	28+30
12	81	alpha-chlordane	PBEB
13	87	gamma-chlordane	HBB
18	85	oxychlordane	47
15+17	77	<i>trans</i> -nonachlor	49
16	110	methoxychlor	66
32	135+144	endosulfan I	85
26	123	endosulfan II	99
31	149	endosulfan sulfate	100
28	118	p,p'-DDT	119
33	114	p,p'-DDE	126
53	131	p,p'-DDD	138
22	132+153+105	o,p'-DDT	139
45	163+138	o,p'-DDD	140
52	126	aldrin	153
49	128	endrin	154 + BB-153
47	167	dieldrin	156+169
48	174		BTBPE
44	202+171	PAHs:	180
37	156	fluorene	183
42	172	phenanthrene	184
41+71	180	anthracene	191
64	199	fluoranthene	196
100	169	pyrene	197
74	170+190	retene	201
70+76	201	benz[a]anthracene	203
66	207	chrysene+ triphenylene	204
95	194	benzo[b]fluoranthene	205
91	205	benzo[k]fluoranthene	206
56+60	206	benzo[e]pyrene	207
92+84	Total	benzo[a]pyrene	208
89		indeno[1,2,3- <i>cd</i>]pyrene	209
		dibenz[a,h]anthracene	DBDPE
		benzo[ghi]perylene	HBCD
		coronene	TBE
		Others	
		Total suspended particles	
		Meteorological	
		Temperature	
		Wind speed	
		Wind direction	
		Solar radiation	
		Relative humidity	
		Barometric pressure	

FLOW CHART 1. SUMMARY OF SAMPLE PREPARATION



I. CLEANING: GENERAL LABWARE

General Cleaning Supplies

Micro cleaning solution (Micro-90, International Products Corporation)
Dish washing brushes
Deionized (DI) water, Millipore, Milli-Q water system
Muffle furnaces: Thermolyne 30400
Ultra sonicator
Aluminum foil
Solvents: dichloromethane, hexane
Teflon squirt bottle with solvents
Beakers

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 hot tap water and with organic free DI water from Milli-Q system. DI water system should be turned on 10 minutes before use.

Dry glassware in air overnight.

Cover all open ends with foil. Always use dull side of the foil towards glassware.

Muffle glassware in furnace at 450⁰C for 6 hours.

Allow glassware in furnace to cool to 100⁰C (usually it takes 10-12 hrs) before removing from furnace.

Store 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. 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 nuts with micro-90 soap and water using brushes.

Rinse well first with hot tap water and then with DI water from the Milli-Q system. DI water system should be turned on 10 minutes before use.

Dry at room temperature in air overnight.

Rinse with dichloromethane.

Wrap each tool separately in foil, shiny side towards the outside.

Store them in drawers.

Air sampling cartridges and screen meshes are wrapped in aluminum foil (shiny side out) and muffled in the furnace at 450⁰C for 6 hrs 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 450°C for 6 hours.

Cool glassware in furnace to 100°C (usually next morning); remove from oven. Insert clean Teflon liners (see below) into vial caps.

Cap the vial 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.

Place in 70°C drying oven for 2 hours.

Store in sealed jar (covered with foil, lid screwed on).

5. Microdispenser capillaries, GC vials, and stainless N₂ blowdown needles

a) Microdispenser capillaries

Before using rinse with dichloromethane and air dry.

b) GC autosampler vials and inserts (disposable)

Place the vials and the inserts in beakers. Cover with Al-foil. Always use dull side of the foil towards glassware.

Muffle in furnace at 450°C for 6 hours.

Dispose off after use.

c) Stainless N₂ blowdown 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 some solvent through these needles and dry them in drying oven at 70°C for 15 minutes.

6. Teflon Stopcocks and Lids for Sample Jars

Wash stopcocks with micro-90 soap and water. Rinse with DI water from Milli-Q system. Make sure the stopcock adjuster on the side is turned to the open position (vertical).

Lids are wiped with a damp Kim wipe soaked in tap water, and then are wiped with a damp Kim wipe soaked in DI from Milli-Q 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 450°C for 6 hours. Cool furnace down to 100°C
Store

2. Teflon Boiling Chips and Sodium Sulfate

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 or sodium sulfate 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 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 or sodium sulfate in muffled sample jar; cover loosely with foil. Always use dull side of the foil towards glassware.
Place jar containing boiling chips in a drying oven at 70°C for approximately 2 hours.
Bake sodium sulfate at 100°C overnight.
Cover the jars with lids.
Store the boiling chips on shelf.
Store sodium sulfate in a desiccator.

3. XAD-2

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: EM Science 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.
Sometime 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 500 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 500 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 dichloromethane.
Set variac at 48.
Extract for 24 hours.

Day 5

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

Day 6

Follow the procedure of Day 2 but use acetone:hexane 50:50 (vol:vol) as solvent.
Set the variac at 45
Extract for 24 hours.

Day 7

Turn off heater; cool 15 to 30 minutes.
Flush as much acetone/hexane from soxhlet as possible.
Pour XAD-2 in a beaker loosely covered with foil and dry in an oven at 75°C for 8 hours.
Store in amber bottle in freezer at -20°C 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 preclean this, omit the water rinsing and the methanol extraction steps. Start extraction with acetone and then follow the whole procedure. For new XAD-2, extraction period for each solvent can be extended to 48 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 500 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 500 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 dichloromethane as solvent.

Set variac at 48.

Extract for 24 hours.

Day 5

Follow the procedure of Day 2 but use hexane as solvent . Use a new flask.

Set variac at 50

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.

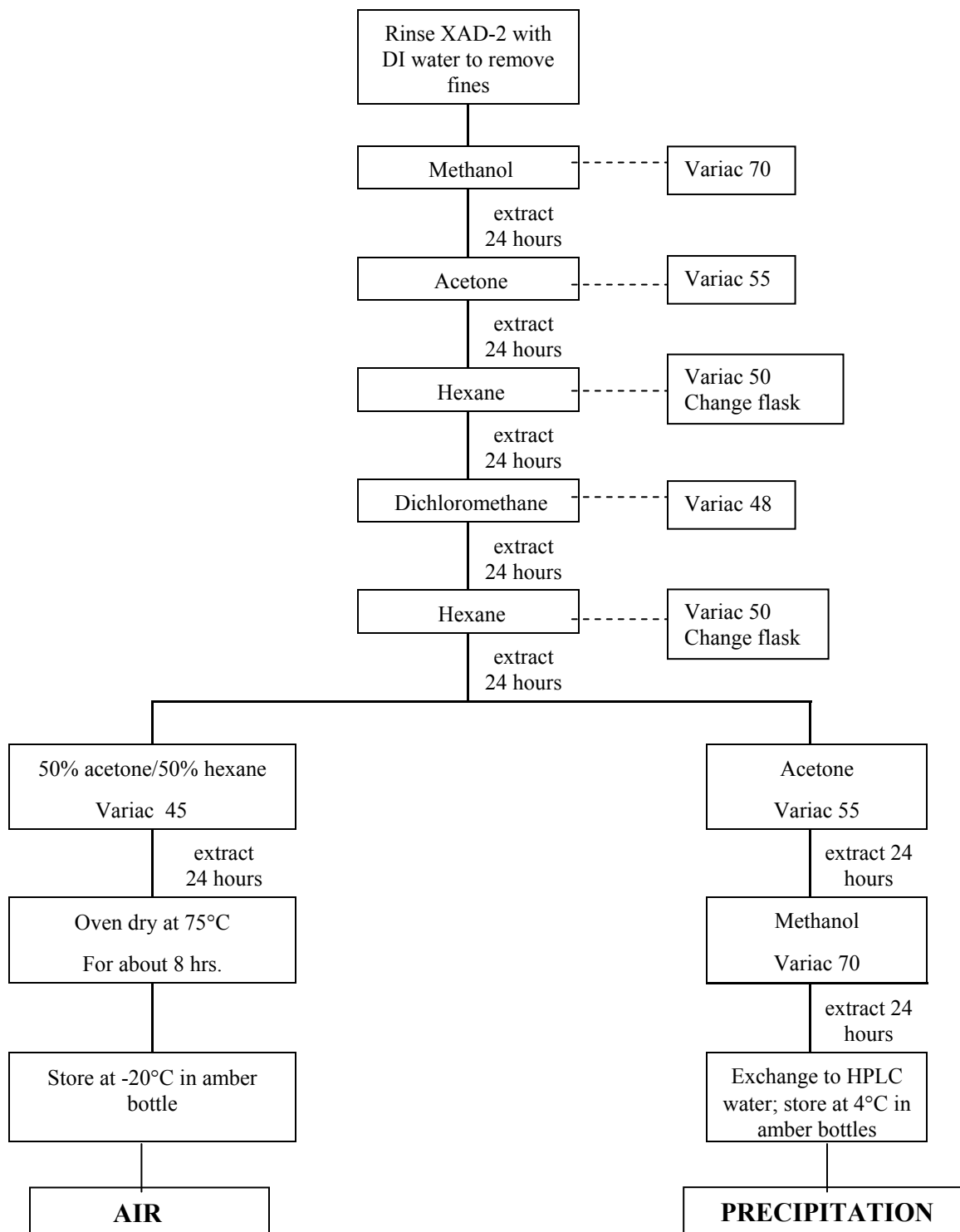
Flush as much methanol from soxhlet as possible.

Rinse XAD-2 with EM Science HPLC grade water until XAD-2 does not smell of any solvent.

Store clean XAD-2 in an amber bottle at 4⁰C. It can be used up to three months.

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

FLOW CHART 2. SUMMARY OF XAD-2 PRECLEANING



4. Silica and quartz fiber filters (QFF)

i) Silica

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)

Wrap up each QFF by aluminum foil separately, shiny side out

Muffle at 450°C for 6 hours.

After cooling put them individually in plastic bag and store them in freezer at -20°C.

iii) Glass Fiber Filters (GFF)

Wrap up each GFF by aluminum foil separately shiny side out

Muffle at 450°C for 6 hours.

After cooling put them in plastic bag and store them in freezer at -20°C.

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	EM Science Omni Solv grade
Beakers	
Tweezers	
Aluminum foil	
Stand and clamp	
Pre-cleaned wet XAD-2	Amberlite XAD-2 resin, 20-60 mesh size, pore diameter 90A ⁰

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° 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:	Thermolyne 30400
Gallon size plastic ziplock 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° C for 6 hours in the muffle furnace.
Store them at -20° C.

Take them out of the freezer 48 hours before shipping and put them in the 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 Al-foil in a ziplock plastic bag and store it at -20° 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.

Carefully unwrap the foil.

Put a screen and retainer ring at one end. Pour 40-42g of precleaned 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° C until shipping.

Record the batch number of the XAD-2 used for making the cartridges in the sampling protocol book.

Use new precleaned 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.

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°C 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 site visit data sheet on weekly basis.

If the samples cannot be transferred immediately they can be stored in cold room (10°C) temporarily.

2. Air Particle sample or Quartz Fiber Filter

Check the sample packaging, sample integrity and write comments

Unwrap the filters slightly and place in the humidity chamber for approximately 24 hours.

Sign, date, and file the field data sheet with comments

File site visit sheet

Enter field data into Log book

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°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 meter³.

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

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°C until analysis.

Sign, date, and file the field data sheet

Enter into log book

If the samples cannot be transferred immediately they can be stored in cold room (10°C) 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 (490 ng), pesticides (20 ng each, b-HCH 30 ng), PAHs (400 ng ea), and PBDE Recovery Standard (60-200 ng/ml).
 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
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

Procedure

i) Setting up

One batch of samples generally include:

Regular field samples: 10-12. It usually includes field blank (5% of all samples) and field duplicate (5% of all

samples)

Laboratory duplicate (5% of samples, 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 (5%, alternate batch): Sampling media spiked with Surrogate Standards or a

Matrix spike (5%, alternate batch): Sampling media spiked with Recovery Standards with known amount of all compounds of interest.

On the day of extraction a unique Batch ID is assigned to a batch of sample with month, year, and sample type. Thus the Batch IDs of the cartridge and filter samples from September 03 will be S03C and S03F. The Batch ID for precipitation samples from 03 August will be AU03P.

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.

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. The complete list of compounds are:

PCB recovery standard: complete suit of PCB (490 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, beta-HCH 30 ng

PAH Recovery Standard: all PAHs 400 ng each (Laboratory mix)

PBDE Recovery Standard: Mixture of 14 selected PBDE listed in Table 27(60-200 ng/mL)

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 20g of it and carefully transfer it in a soxhlet extractor plugged with glass wool. Weigh out another 20g 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 which contain:

PCB 14:	200 ng/mL
PCB 65:	50 ng/mL
PCB 166:	50 ng/mL
Dibutylchloroendate:	200 ng/mL
δ-HCH:	200 ng/mL
d ₁₀ phenanthrene:	4 µg/mL
d ₁₀ pyrene:	4 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.

Day 2

Turn heating mantle off. Let them cool down for 30 minutes. Turn off condenser water.

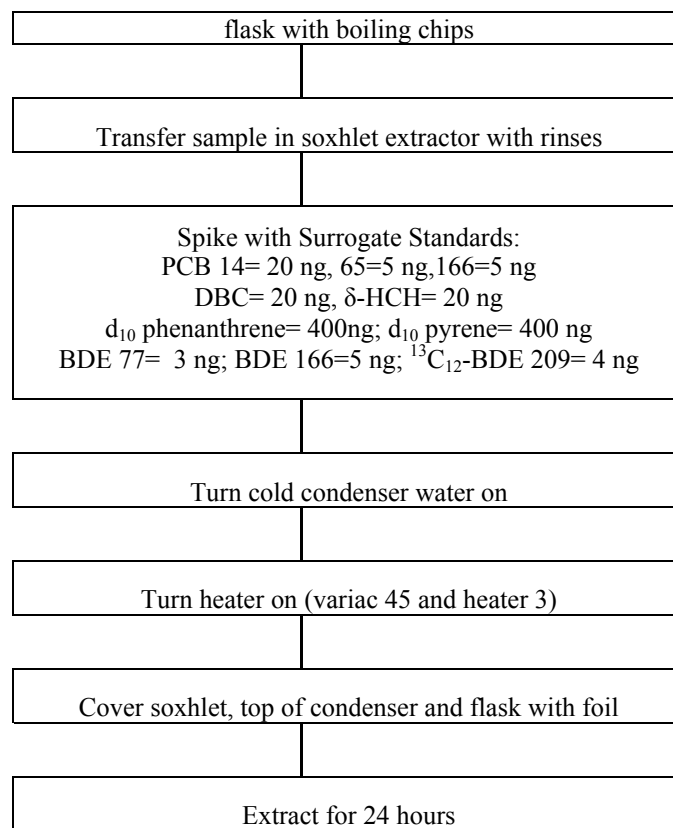
Siphon off as much solvent from soxhlet extractor into flask as possible.

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

400 mL of acetone/hexane (50:50) in 500 mL round bottom

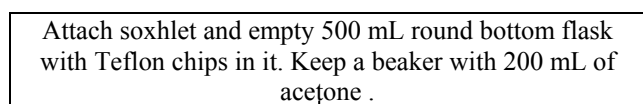


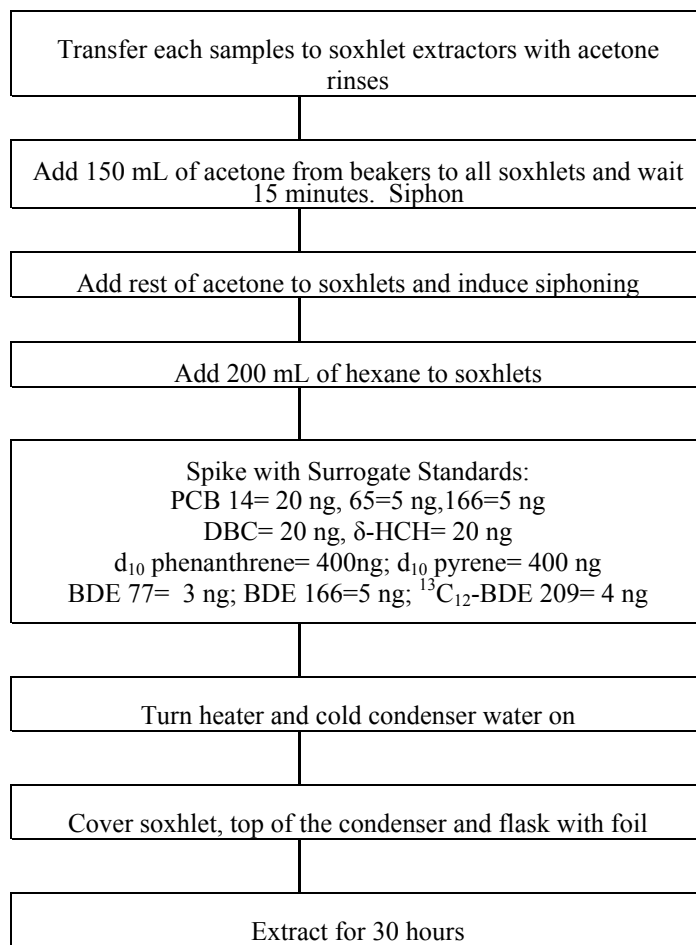
Taking extraction down: Day 2



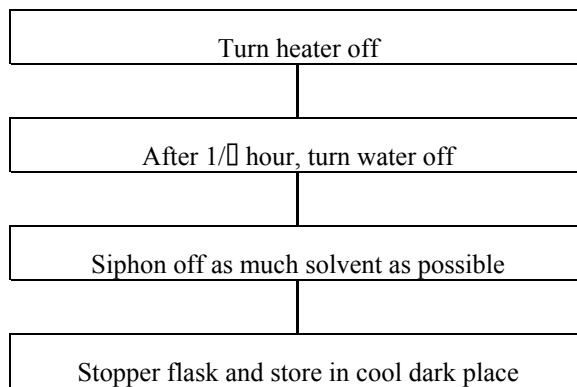
FLOW CHART 4. SUMMARY OF EXTRACTION OF PRECIPITATION SAMPLES

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

Splash guard 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-114)
Faucet aspirator
Chiller circulator (Neslab, CoolFlow, CFT-25)

Procedure

i) Setting up

Fill chamber with DI water.
Turn the chiller circulator on.
Set bath temperature 30^oC -35^oC.
Rinse joint of steam duct with dichloromethane or hexane.
Attach appropriate splash guard to steam duct. 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 with foil

2. Rotary Evaporation and Back Extraction of Precipitation Extracts

Supplies

Splash guard 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-114)
Chiller circulator (Neslab, Cool Flow, CFT-25)

Procedure

i) Evaporation

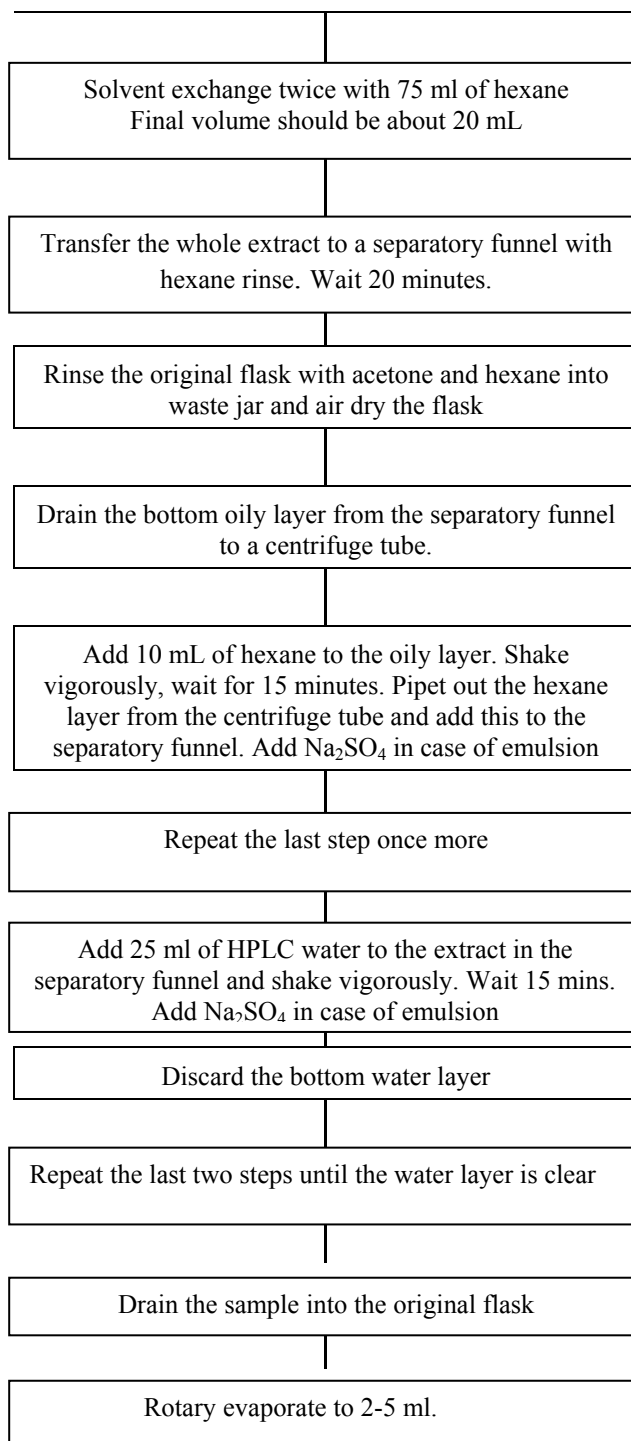
Rinse the joint of the steam duct with dichloromethane or hexane.
Attach splash guard to steam duct. Clamp each joint.
Turn vacuum on with faucet aspirator.
Remove boiling chips from the extract with large clean forceps.
Attach flask to splashguard. Clamp joint.
Turn motor on.
Turn water bath on. The temperature should be 30-35°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 Na₂SO₄ 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 15 minutes
Drain the bottom water layer
Repeat last two steps until the water layer is clear.
Drain the sample into the original flask
Rotary evaporate to 2-5 mL.

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

Rotary evaporate rain extract to 20 mL.



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°C oven, turn thermostat to 300°C; keep in oven overnight.

Day 2

Turn the oven temperature down to 100°C.
Crack the door of the oven open when the oven has cooled down to 250°C.
When the oven temperature is 150°C 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:

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

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 flasks 100 mL with 14/20 joints- 2

Glass stoppers 14/20- 2
 Pasteur pipettes (9 inch and 5 inch):
 Graduated cylinders: 50 mL and 10 mL
 Beaker, 50 mL -1
 Waste jar -1
 Beakers, 250 mL-3
 Rubber pipette bulbs
 Hexane
 50% hexane:50% dichloromethane
 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 ₂ SO 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 1/4" Na₂SO₄ for XAD-2 and QFF samples, 1.5" Na₂SO₄ for precipitation samples.

Add glass tips to columns and check for leaks, use teflon tape if needed

Wash columns with 25 mL hexane for conditioning.

Close stopcocks to prevent further dripping when hexane level reaches 1 cm above the top of Na₂SO₄.

NEVER LET THE COLUMN RUN DRY.

ii) Fractionation

Set up

Label 100 mL pear-shaped flask for each sample for hexane and 50% dichloromethane in hexane 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 or 50% dichloromethane in hexane. For 1st fraction add 25 mL of hexane in beaker for air samples and 30 mL of hexane for precipitation samples.

Loading samples and collection of first fraction

Ultrasonicate each sample in the flask and load the sample on column with Pasteur pipet.

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 Na₂SO₄, 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 Na₂SO₄ add rest of the solvent to the column after rinsing the flask. Collect the 1st fraction.

Second Fraction

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.

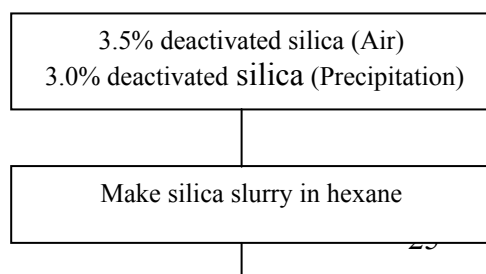
Once the elute reaches the top of sodium sulfate, collect 15 more drops and remove flask with second fraction, stopper it, and store in dark place.

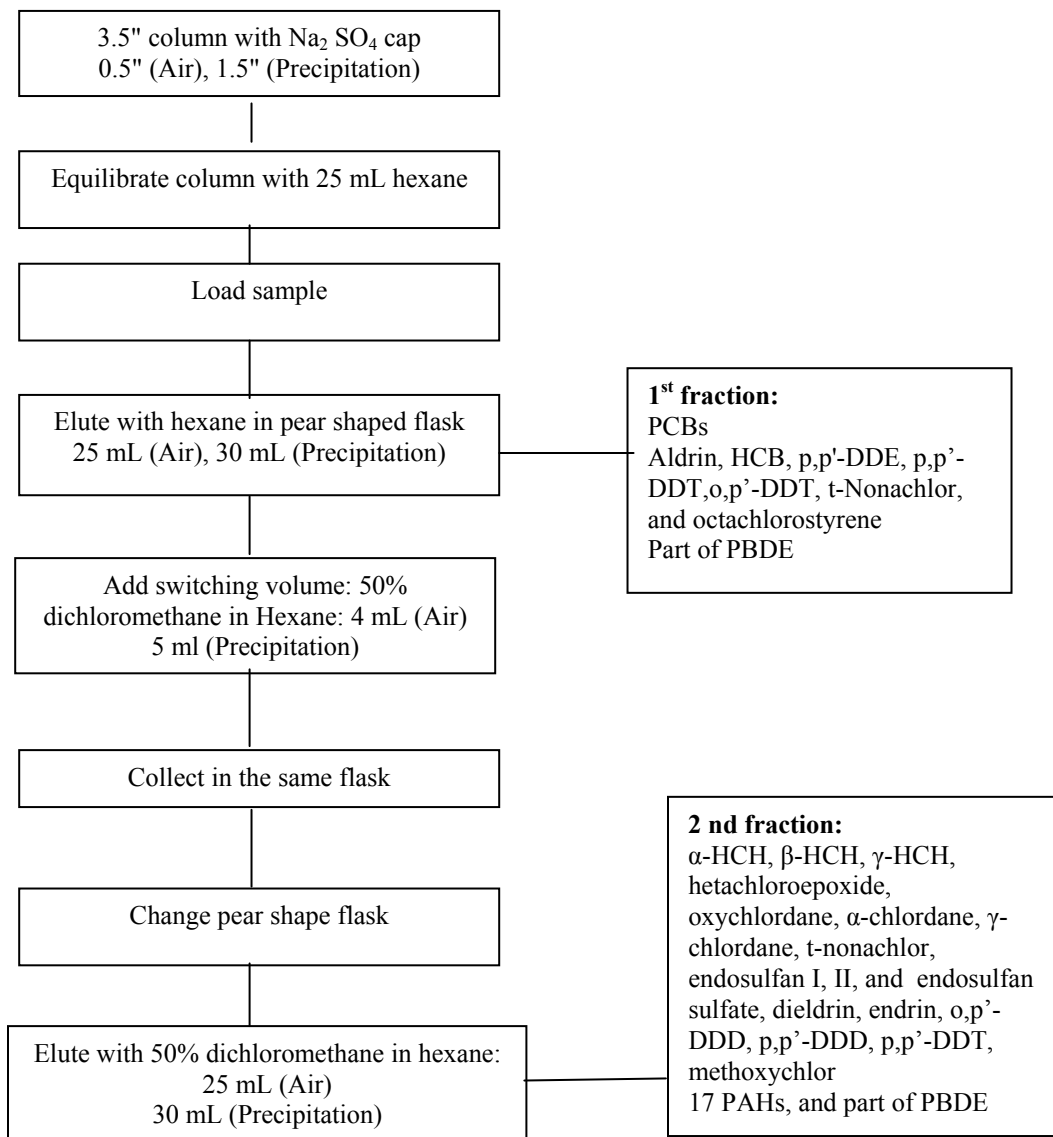
iii) Clean-Up

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

Measurement of the elution and switching solvents can be done ahead of time.

FLOW CHART 6. SUMMARY FOR SILICA COLUMN CHROMATOGRAPHY





iv). Recleaning of hexane fraction and 50% fraction of samples

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.

Procedure for 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 hexane fraction 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 2nd fraction.

Procedure for 50% fraction recleaning:

Transfer the 50% fraction 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 AND FRACTION 2

Supplies

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

Procedure

i) Setup

Fill chamber with DI water.
Turn on the chiller circulator.
Set bath temperature 30⁰C -35⁰C.
Rinse joint of steam duct with dichloromethane.
Attach appropriate splashguard to steam duct. 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 50% dichloromethane fraction, solvent exchange once 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 with foil.

IX. TRANSFER OF SAMPLES

Supplies (each sample)

Pasteur pipettes (9.0 inch and/or 5.0 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°C. Label the vial file with Batch ID

X. N₂ BLOW DOWN

Supplies

Samples in amber vials
N₂ blow down unit
Dichloromethane

Procedure

Remove all nozzle plugs from unit.
Turn on N₂ at tank and let N₂ flush out for approximately 5 minutes.
Turn heater on LOW.
Squirt some solvent through these needles and dry them in drying oven at 70⁰C for 15 minutes.
Attach clean needle to each nozzle to be used.
Place amber vials in slot; adjust N₂ 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 N₂ 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 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.

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).

TABLE 4. INTERNAL STANDARDS AND MASS PER FRACTION

Fraction	Compound	Internal Standards	Spiking Volume	Mass ISTD in ng
Hexane	PCBs	PCB cong. 30 and 204	100 ul	8 and 6 ng
Hexane	PBDE	BDE 118, BB-209, BDE 190, BDE 71	50 ul	5, 10, 5, and 6 ng
50% fraction	Pesticides	PCB cong 65, 155	100 ul	20 and 20 ng
50% fraction	PAH	d ₁₀ anthracene	50 ul	400 ng ea
		d ₁₂ benz[a]anthracene		
		d ₁₂ perylene		
50% fraction	PBDE	BDE 118, BB-209, BDE 190, BDE 71	50 ul	5, 10, 5, and 6 ng

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 is no air bubble.

Spike sample.

Mark each amber vial label with an appropriate color of dot: red for PCBs, blue for pesticides, black for PAHs, and purple for PBDEs to denote that they have been spiked.

Rinse the dispenser with solvent

Replace glass tube used to cover plunger of microdispenser before storing.

XII. MAKING MICROVIALS FOR GC ANALYSIS

Supplies

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

Procedure

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.

TABLE 5. CALIBRATION STANDARDS

Fraction	Target compounds	Calibration standards
Hexane	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
50%	pesticides	Mixed pesticide standard: 20 ng/mL each
50%	PAH	Mixed PAH standard 200 ng/mL each (approximately).
Hexane and 50%	PBDE	Mixed PBDE standard: 20-400 ng/mL

Crimp septa onto the microvials.

Load the microvials into GC or GC/MS autosampler.

XIII. STANDARDS

The standards are procured from Ultra Science, Inc. or AccuStandards, Inc. They are further diluted in the laboratory with hexane to make stock and working standards. There are mainly 5 different type of working standards for each group of compounds:

1. Calibration standard (CCS): used for instrument calibration

2. Calibration Reference standard (CRS): used for checking the instrument calibration
3. Recovery standard: used for matrix spiking and checking overall recovery
4. Surrogate standards: used for checking recovery of each sample
5. Internal standard: used for quantitation of compounds

A. PCBs:

1. Common Calibration Standard (CCS)

3 ampoules of PCBs, custom made by Accu Standard were distributed by Peter Fowlie on 3/7/05. Each ampoule has 28 congeners from **suitPCB**. The congeners and the original concentrations are tabulated below. All IADN participating laboratories are using this standard.

TABLE 6: PCB COMMON CALIBRATION STANDARD (CCS): STOCK

Ampoule #1 S-8074A-R1 Lot# B5020104		Ampoule #2 S-8074B-R1 Lot # B5020105		Ampoule #3 S-8074C-R1 Lot # B5020115	
PCB Congener	certified ug/mL	PCB Congener	certified ug/mL	PCB Congener	certified ug/mL
4	1.012	5	1.006	6	1.003
7	1.009	8	1.005	9	0.999
10	1.011	12	1.01	13	0.999
15	1.01	16	0.502	17	0.5
18	0.501	19	0.503	22	0.499
28	0.505	26	0.499	31	0.504
32	0.5	33	0.499	37	0.503
41	0.5	42	0.504	44	0.499
45	0.503	47	0.506	48	0.504
52	0.499	49	0.5	53	0.5
56	0.499	60	0.499	64	0.499
66	0.505	70	0.5	71	0.505
74	0.504	76	0.499	77	0.499
81	0.505	83	0.507	84	0.504
85	0.499	87	0.505	89	0.499
91	0.502	92	0.502	95	0.506
97	0.499	99	0.502	100	0.5
101	0.502	105	0.499	110	0.502
114	0.505	118	0.504	119	0.504
123	0.503	126	0.506	128	0.502
131	0.504	132	0.503	135	0.498
138	0.503	144	0.508	149	0.5
153	0.502	156	0.5	163	0.499
167	0.5	169	0.504	170	0.499
171	0.502	172	0.505	174	0.504
180	0.503	190	0.5	194	0.505
200	0.505	199	0.5	202	0.495
205	0.502	206	0.506	207	0.504

**TABLE 7. PCB COMMON CALIBRATION STANDARD
WORKING SOLUTION**

Compounds	Concentration
-----------	---------------

S-8074A-R1	5-10 ng/mL
S-8074B-R1	5-10 ng/mL
S-8074C-R1	5-10 ng/mL
14 (Surrogate)	10 ng/mL
65 (Surrogate)	5 ng/mL
166 (Surrogate)	5 ng/mL
30 (ISTD)	8 ng/mL
204 (ISTD)	6 ng/mL
Congener 11	10 ng/mL
Congener 126	5 ng/mL
Congener 169	5 ng/mL
p,p'-DDE	10 ng/mL
HCB	20 ng/mL
<i>trans</i> -nonachlor	5 ng/mL
p,p'-DDT	5 ng/mL
o,p'-DDT	5 ng/mL
octachlorostyrene	5 ng/mL
aldrin	5 ng/mL

**TABLE 8: PCB COMMON CALIBRATION STANDARD (CCS)
PCB CONGENERS AND PESTICIDES: WORKING STANDARD
WHOLE LIST**

PCB Congener	ng/mL	PCB Congener	ng/mL	PCB Congener	ng/mL
-------------------------	--------------	-------------------------	--------------	-------------------------	--------------

4+10	20	44	5	135+144	10
7+9	20	37	5	123+149	10
6	10	42	5	118	5
8+5	20	41+71	10	114	5
HCB	20	64	5	131	5
14	10	100	5	o,p'-DDT	5
19	5	Octachlorostyrene	5	132+153+105	15
30	8	74	5	p,p'-DDT	5
12	10	70+76	10	163+138	10
13	10	66	5	126	5
18	5	95	5	166	5
15+17	15	91	5	128	5
16	5	56+60	10	167	5
32	5	92+84	10	174	5
26	5	89	5	202+171	10
31	5	101	5	156	5
28	5	99	5	204	6
33	5	t-Nona	5	172	5
53	5	119	5	180	5
22	5	83	5	199	5
45	5	97	5	169	5
52	5	81	5	170+190	10
49	5	87	5	201	5
Aldrin	5	85	5	207	5
47	5	p,p'-DDE	10	194	5
48	5	77	5	205	5
65	5	110	5	206	5

This standard is used for sample analysis everyday

2. Common Reference Standard (CRS)

Three standards in glass ampoules are distributed by Peter Fowlie, s-8074a, s-8074b, s-8074c. Eighty four PCB congeners (suitPCBs), abundant in air samples are present in these 3 standards. Each original ampoule contains 30 µg/mL of each PCB congener. Each was diluted to 300 ng/mL in hexane. Aliquots of each standard are mixed together to get a final concentrations of each 7.5 ng/mL and spiked with cong 30 and 204.

TABLE 9. PCB COMMON REFERENCE STANDARD (CRS)

Compounds	Final concentrations
Standard S-8074A	7.5 ng /mL
Standard S-8074B	7.5 ng/mL
Standard S-8074C	7.5 ng/mL
Congener 30	8 ng/mL
Congener 204	6 ng/mL

TABLE 10. LIST FOR SUIT PCBS

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

3. Recovery Standard

TABLE 11. PCB RECOVERY STANDARD: USED FOR MATRIX SPIKE

Compounds	Concentrations (in Hexane)
Mullin 94	490 ng/mL
Cong. 11	20 ng/mL
Cong. 169	20 ng/mL

Cong. 126	20 ng/mL
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4. Surrogate Standard

TABLE 12. PCB SURROGATE STANDARDS

Standards	AccuStandard Ampule	Stock concentrations (in hexane)
PCB Congener 14	100 µg/mL in Isooctane	1 µg/mL or 2 µg/mL
PCB Congener 65	100 µg/mL in isooctane	1 µg/mL or 2 µg/mL
PCB Congener 166	100 µg/mL in isooctane	1 µg/mL or 2 µg/mL

5. Internal Standard

TABLE 13. PCB INTERNAL STANDARDS (ISTD)

Standards	AccuStandard Ampule	Stock concentrations (in hexane)	Working concentrations (in hexane)
Congener 30	100 µg/mL in Isooctane	1 µg /mL	80 ng/mL
Congener 204	100 µg /mL in isooctane	1 µg /mL	60 ng/mL

B. Pesticide Standards

1. Calibration Standard

Stock Standards

A composite of 17 pesticides, US112B (2000 µg/mL), was bought from Ultra Scientific, Inc. and used as stock pesticide standard.

Additional individual pesticide standards were also purchased from Ultra Science, Inc. or AccuStandard Inc. and diluted in the laboratory with hexane to desired concentrations. US 112B is fortified with these additional standards. Calibration standard was prepared from these stock standards.

US 112B ampoule =2000 µg /mL

100 μ L is diluted to 100 mL of hexane. Final concentration is 2000 ng/mL

TABLE 14. PESTICIDE STANDARDS: US 112B STOCK

Pesticides	Final Concentration ng/mL
Aldrin	2000 ng/mL
α -HCH	2000 ng/mL
β -HCH	2000 ng/mL
γ -HCH	2000 ng/mL
δ -HCH	2000 ng/mL
p,p'-DDD	2000 ng/mL
p,p'-DDE	2000 ng/mL
p,p'-DDT	2000 ng/mL
dieldrin	2000 ng/mL
endosulfan I	2000 ng/mL
endosulfan II	2000 ng/mL
endosulfan Sulfate	2000 ng/mL
endrin	2000 ng/mL
endrin aldehyde	2000 ng/mL
heptachlor	2000 ng/mL
heptachlorepoxyde	2000 ng/mL
methoxychlor	2000 ng/mL

TABLE 15. PESTICIDE CALIBRATION STANDARD WORKING STANDARD

Compounds	Concentrations (in Hexane)
α -HCH	20 ng/mL
β -HCH	20 ng/mL
γ -HCH	20 ng/mL
δ -HCH (surrogate)	20 mg/mL
heptachloroepoxide	20 ng/mL
dieldrin	20 ng/mL
p,p'-DDT	20 ng/mL
p,p'-DDD	20 ng/mL
o,p'-DDD	20 ng/mL
P,p'-DDT	20 ng/mL
α -chlordane	20 ng/mL
γ -chlordane	20 ng/mL
t-nonachlor	20 ng/mL
endosulfan I	20 ng/mL
endosulfan II	20 ng/mL
endosulfan sulfate	20 ng/mL
endrin	20 ng/mL
heptachloroepoxide	20 ng/mL
oxychlordane	20 ng/mL
dibutylchlorodate(Surrogate)	20 ng/mL
cong. 155 (ISTD)	20 ng/mL
cong. 65 (ISTD)	20 ng/mL
Methoxychlor	20 ng/mL

2. Reference

**TABLE 16.
COMMON
STANDARD**

Standard

**PESTICIDE
REFERENCE
(CRS, S-8206A)**

Compound	Concentrations (Hexane)
alpha-HCH	25 ng/mL
HCB	25 ng/mL
beta-HCH	10 ng/mL
gamma-HCH	25 ng/mL
aldrin	25 ng/mL

PCB 65 (IS)	20 ng/mL
heptachloroepoxide	25 ng/mL
gamma-chlordane	25 ng/mL
PCB 155 (IS)	20 ng/mL
endosulfan I	25 ng/mL
alpha-chlordane	25 ng/mL
trans-nonachlor	25 ng/mL
dieldrin	25 ng/mL
p,p'-DDE	25 ng/mL
endrin	25 ng/mL
endosulfan II	25 ng/mL
p,p'-DDD	25 ng/mL
o,p'-DDT	25 ng/mL
endosulfan sulfate	25 ng/mL
p,p'-DDT	25 ng/mL
methoxychlor	25 ng/mL

3. Recovery Standard

TABLE 17. PESTICIDE RECOVERY STANDARD: STOCK

Compounds	Concentrations ng/mL of Hexane
Pesticide common reference standard S-8206A	100 ng/mL of each Pesticide (b-HCH 50 ng/mL)
octachlorostyrene	100.5 ng/mL
oxychlordane	100 ng/mL
o,p'-DDD	100 ng/mL

4. Surrogate Standard

TABLE 18. PESTICIDE SURROGATE STANDARD

Surrogate Standards	Ultra Sc. ampule	Stock Concentrations (µg/mL of Hexane)
Dibutylchloroendate	2000 µg/mL Methanol	1 µg/mL
δ-HCH	100 µg/mL Hexane	2 µg/mL

5. Internal Standard

TABLE 19. PESTICIDE INTERNAL STANDARDS

compound	Ultra/AccuStandard Ampoule Concentration	stock concentration	Working concentration (ng/mL of Hexane)
Congener 65	100 ug/mL	1 µg/ mL	200 ng/mL
Congener 155	35 µg/ mL	1 µg/ mL	200 ng/mL

C. PAH Standard

1. Calibration Standard

TABLE 20. PAH CALIBRATION STANDARD

PAH	Concentrations (ng/mL of Hexane)
acenaphthene	200
acenaphthylene	200
anthracene	200

benz[<i>a</i>]anthracene	200
benzo[<i>a</i>]pyrene	200
benzo[<i>b</i>]fluoranthene	200
benzo[<i>e</i>]pyrene	200
benzo[<i>ghi</i>]perylene	200
benzo[<i>k</i>]fluoranthrene	200
triphenylene+chrysene	400
coronene	200
d ₁₀ anthracene-istd	200
d ₁₂ perylene-istd	200
d ₁₂ benz[<i>a,h</i>]anthracene-istd	200
dibenz[<i>a,h</i>]anthracene	200
fluoranthene	200
fluorene	200
indeno[1,2,3- <i>cd</i>]pyrene	200
phenanthrene	200
pyrene	200
retene	200
d ₁₀ pyrene (surrogate)	200
d ₁₀ phenanthrene (surrogate)	200

2. Reference Standard:

2 standards were distributed by the IADN QC officer Peter Fowlie in 2001. The 1st one has 15 PAHs, S-8206B and the 2nd one has only retene, H-250S. These standards were purchased from Accustandard Inc. Original stock was diluted 500X.

Original stock

S-8206B ampoule = 100ug/ml

H-250S ampoule = 50ug/ml

TABLE 21. PAH COMMON REFERENCE STANDARD A

Compound	Concentrations in ng/mL Hexane
fluorene	200
phenanthrene	200

anthracene	200
fluoranthene	200
pyrene	200
d10-pyrene (Surr)	200
benz[<i>a</i>]anthracene	200
triphenylene+chrysene	400
benzo[<i>b</i>]fluoranthene	200
benzo[<i>k</i>]fluoranthene	200
benzo[<i>e</i>]pyrene	200
benzo[<i>a</i>]pyrene	200
indeno[1,2,3- <i>cd</i>]pyrene	200
dibenz[<i>a,h</i>]anthracene	200
benzo[<i>g,h,l</i>]perylene	200
coronene	200
d10-Phenanthrene (Surr)	200

TABLE 22. PAH COMMON REFERENCE STANDARD B

Compound	Concentrations in ng/mL Hexane
retene	200

3. **Recovery Standard:**

TABLE 23. PAH RECOVERY STANDARD

Compounds	Concentrations ($\mu\text{g/mL}$ of Hexane)
acenaphthene	2
acenaphthylene	2
anthracene	2
benz[<i>a</i>]anthracene	2
benzo[<i>b</i>]fluoranthene	2
benzo[<i>k</i>]fluroanthene	2
benzo[<i>a</i>]pyrene	2
benzo[<i>e</i>]pyrene	2
benzo[<i>ghi</i>]perylene	2
tryphenylene+chrysene	2
coronene	2
dibenz[<i>a,h</i>]anthracene	2
fluoranthene	2
fluorene	2
indeno[1,2,3- <i>cd</i>]pyrene	2
naphthalene	2
phenanthrene	2
pyrene	2
retene	2

4. **Surrogate Standard:**

TABLE 24. PAH SURROGATE STANDARD

Compound	Final Concentration ($\mu\text{g}/\text{mL}$ of Hexane)
d ₁₀ phenanthrene	4
d ₁₀ pyrene	4

5. **Internal Standard Standard:**

TABLE 25. PAH INTERNAL STANDARD

Compound	Concentration $\mu\text{g}/\text{mL}$ hexane
d ₁₀ anthracene	4
d ₁₂ benz[a]anthracene	4
d ₁₂ perylene	4

D. PBDE standards

1. Calibration Standard

TABLE 26. PBDE CALIBRATION STANDARD

Component	Concentration (ug/ml in hexane)	Component	Concentration (ug/ml in hexane)
7	0.02	153	0.04
10	0.02	154	0.04
15	0.02	156	0.04
17	0.02	169	0.04
25	0.02	BB-153	0.04
28	0.02	BTBPE	0.04
30	0.02	171	0.08
HBB	0.02	180	0.08
PBEB	0.02	183	0.08
47	0.04	184	0.08
49	0.04	191	0.08
66	0.04	196	0.08
71	0.04	197	0.08
77	0.04	201	0.08
85	0.04	203	0.08
99	0.04	204	0.08
100	0.04	205	0.08
119	0.04	206	0.2
126	0.04	207	0.2
138	0.04	208	0.2
139	0.04	209	0.2
140	0.04	DBDPE	0.4

2. Recovery Standard

TABLE 27. PBDE RECOVERY STANDARD

Compounds	Stock (µg/mL)
BDE-47	0.06 µg/mL
BDE-99	0.06 µg/mL
BDE-100	0.06 µg/mL
BDE-153	0.06 µg/mL
BDE-154	0.06 µg/mL
BDE-181	0.1 µg/mL
BDE-183	0.08 µg/mL
BDE-196	0.08 µg/mL
BDE-197	0.08 µg/mL
TBE	0.08 µg/mL
BDE-206	0.1 µg/mL
BDE-207	0.1 µg/mL
BDE-209	0.2 µg/mL
HBCD	0.096 µg/mL

3. Surrogate Standard

TABLE 28. PBDE SURROGATE STANDARD

Compounds	Stock (µg/mL)
BDE-77	60 ng/mL
BDE-166	100 ng/mL
¹³ C ₁₂ -BDE-209	80 ng/mL

4. Internal Standard

TABLE 29. PBDE INTERNAL STANDARD

Compounds	Stock (µg/mL)
BDE-118	0.1 µg/mL
BB-209	0.2 µg/mL
BDE-190	0.1 µg/mL
BDE-71	0.12 µg/mL

Matrix Spike Vial (MS Vial) :

One MS vial is prepared from PCB Recovery standard, Pesticide Recovery Standard, PAH Recovery Standard, PBDE Recovery Standard and β-HCH. This is used for spiking the matrix in the soxhlet for matrix spike recovery experiment.

TABLE 30. MATRIX SPIKE VIAL (MS VIAL)

Standards	Stock	MS-vial
PCB Recovery Standard	490 ng/mL	490 ng
Pesticide Recovery Standards	100 ng/mL	20 ng for each pesticide beta-HCH=10 ng
Octachlorostyrene	100.5 ng/mL	20.1 ng/mL
β-HCH	200 ng/mL	20 ng/mL
PAH Recovery Standards	2 µg/mL	400 ng of each PAH
PBDE Recovery Standards	0.05-0.2 µg/mL	5-20 ng/mL

XIV. SAFETY

1. Emergency Numbers

Name	Telephone numbers
IU Fire Department	911
Environmental Health and Safety	812-855-3234
Ronald A. Hites	812-855-0193 (O) 812-334-1323 (H)
Control Center and Physical Plant	812-855-9514

2. Chemists' Telephone Numbers

Name	Telephone Numbers
Ilori Basu	812-855-2926 (O) 812-334-2184 (H) 812-322-1099 ©
Karen Arnold	812-856-5485 (O) 812-275-8273 (H)
James C. Bays	812-855-4364 (O) 812-339-3660 (H)
Jennifer Kelley	812-855-4364 (O) 812-339-5413 (H)

3. Working in the Laboratory

Chemists working in the laboratory should follow certain safety rules:

- Individual is required to wear a lab coat whenever working in the lab.
- Eye protection with splash resistant safety glasses or safety goggles is required. **Contact lenses are forbidden.**
- Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.
- All solvent work should be done inside fume hood.
- Open shoes are not allowed in the laboratory.
- Particle mask is required when using dry silica.
- Generally, nobody should work alone in the laboratory. If work must be performed after hours or on the weekends inform supervisor or other laboratory personnel so that your presence is known.
- Chemicals and solvents are stored in separate storage areas. One week's supply is kept in the laboratory. Solvents are stored in special solvent cabinet. Acids must be separated from bases. A rubber bucket is used to carry any chemicals.
- Gas cylinders should be well secured at all times. Flammable gases are stored in separate cage.
- Hands should be washed thoroughly after work. Protective hand cream "Soft guard" is supplied.
- No food or drink is allowed in the laboratory.

- l) In case of minor spillage get spillage kit to clean the area. A major spill requires the University Health and Safety Division to be contacted and the working area needs to be evacuated.
- m) MSDS and safety manuals are filed in a three ring binders and kept in book case near the laboratory main entrance
- n) All chemicals and standard should be labeled properly with scientific name, date, and initials of person to contact.
- o) Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.
- p) All employees should take the Safety training offered by Indiana University

4. Safety Equipment

a) Fume Hood

IADN sample preparation requires frequent use of solvent. Therefore, all extraction, column chromatography, standard preparation, sample transfer, nitrogen blow down and preparation of microvials should be done in the hood. It is real important to check hood from time to time to ensure that it is working properly. A flow of 80-120 linear feet per second must cross the hood.

b) Safety Showers

Emergency showers are located in strategic areas of the laboratory to provide to provide immediate emergency protection against fire or chemical injury. It is operated by pulling the hanging ring down. It delivers 30 gallons of water per minute. It is checked periodically by authorized personnel

c) Eye Wash

Emergency eyewash is located in the laboratory. It is operated by pushing the lever backward. It is checked every month to ensure that is functioning properly

5. Waste disposal

a) Solvents

Label 2 containers, 'CHLORINATED WASTE' and 'NON-CHLORINATED WASTE'.

Containers may be empty glass bottles from solvents or 5 gallon metal cans

When in use they are to be placed inside a fume hood with the sash pulled down.

University Health and Safety Department will pick up the waste solvent.

Label the container properly and sign it. The request for waste pick up should be done on line.

b) Silica

After solvent has evaporated, pour silica into a separate bottle. When the bottle is full label it. University Health and Safety will pick it up together with the waste solvent.

c) Teflon Boiling Chips

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trash can.

d) Glass

Place in 'Broken Glass Disposal Containers'. Close the container according to directions when the containers are full. Leave for the custodial services to pick-up or take out to the trash dumpster.

e) Foil

Place in trash can.

f) Fiberglass wool

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trash can.

g) Sharp objects

Sharp objects like needle and syringes should be disposed off in special container designed for sharp objects

h) XAD-2 and QFF

Leave in soxhlet under hood until solvent has evaporated. Pour XAD-2 into container labeled '**USED XAD-2**'. Discard QF into trash can.