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Environmental
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Great Lakes
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Lake Michigan Mass Balance Study (LMMB) Methods Compendium

Volume 2: Organic and Mercury Sample Analysis Techniques

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Acknowledgments

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Disclaimer

This document describes sampling and analytical methods used by PIs participating in the LMMB Study. Due to the nature and low concentrations of pollutants monitored in the study, many of the methods used in the LMMB Study represent state-of-the art techniques that will be refined further as new technology is developed and as necessary to resolve matrix interferences. Therefore, the procedures described in this compendium should be considered to accurately reflect procedures in use by the LMMB Study PIs at the time of publication. Users of this document should recognize that these procedures are subject to change.

Users of this document also should recognize that these methods do not constitute "approved EPA methods" for use in compliance monitoring programs. Publication of these methods is intended to assist users of LMMB Study data and to provide a reference tool for researchers interested in building upon LMMB Study findings. Mention of company names, trade names, or commercial products does not constitute endorsement or recommendation for use.

Foreword

The Lake Michigan Mass Budget/Mass Balance (LMMB) Study was initiated in late 1993 as part of the Lakewide Management Plan (LaMP) for Lake Michigan. The Lake Michigan LaMP and the LMMB Study were developed to meet requirements mandated by Section 118 of the Clean Water Act (CWA); Title III, Section 112(m) of the Clean Air Act Amendments; and Annex 2 of the Great Lakes Water Quality Agreement. Organizations participating in the development of these programs included: EPA Region 5, the EPA Great Lakes National Program Office, the National Oceanic and Atmospheric Administration, the U.S. Geological Survey, the U.S. Fish and Wildlife Service, the Michigan Department of Natural Resources, the Wisconsin Department of Natural Resources, the Illinois Department of Natural Resources, and the Indiana Department of Environmental Management. In general, the primary goal of the LaMP and the LMMB Study is to develop a sound, scientific base of information with which to guide future toxic load reduction efforts at the federal, state, and local levels.

This compendium describes the sampling and analytical methods used in the LMMB Study. For ease of use, the compendium is organized into three volumes. Volume 1 describes sampling procedures used in the study; Volumes 2 and 3 describe analytical procedures used by each PI. Because sampling apparatus and techniques are generally geared towards specific matrices, Volume 1 is organized according to sample matrix (e.g., air, water, sediment, tissue, etc). Volumes 2 and 3 are organized by pollutant type (e.g, organics, metals, biologicals) because laboratories and instrumentation are typically set up to address specific pollutants rather than specific matrices.

Each Principal Investigator (PI) was required to follow specific quality control requirements necessary to meet data quality and measurement quality objectives for the LMMB Study. To assist users of this document, Appendix A provides the measurement quality objectives (MQOs) established by each PI for his/her sampling and analysis program.

Finally, EPA has made no attempt to standardize the procedures submitted by PIs for publication in this compendium. Therefore, the methods provided in this document contain varying levels of detail. Appendix B provides names, addresses and phone numbers for each PI and for each EPA Project Officer (PO). Specific questions about the procedures used in the study should be directed to the appropriate PI or PO listed in Appendix B.

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Volume 2

Chapter 1: Organic Analysis

Instrumental Analysis and Quantitation of Polycyclic Aromatic Hydrocarbons and Atrazine:

IADN Project

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July 1996

Version 1.3

Instrumental Analysis and Quantitation of Polycyclic Aromatic Hydrocarbons and Atrazine: IADN Project

1.0 Introduction

This document briefly outlines the instrumental analysis and quantitation of polycyclic aromatic hydrocarbons (PAH) and atrazine collected in air and precipitation samples from three sites on the Great Lakes. This work is conducted at the School of Public and Environmental Affairs, Indiana University-Bloomington as a part of the Integrated Atmospheric Deposition Network (IADN). The following summarizes the gas chromatographic-mass spectrometry (GC-MS) technique used, its pertinent parameters, and analyte quantitation.

PAH analyses are performed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph and a HP 5989 mass spectrometer. Chromatographic resolution is achieved with a 30 m × 250 μm DB-5 capillary column which has a 0.25 μm film thickness (J & W Scientific, Folsom, CA) with helium carrier gas. PAHs are quantified by GC-MS using selected ion monitoring (SIM) and the method of internal standards. The PAHs analyzed in this study are listed in Table 1 along with the primary and secondary ions from their mass spectra.

Table 1. Target compounds and their monitored ions.

Retention Order	Compound	Major Ion	Secondary Ion
1	d10-anthracene*	188	----
2	acenaphthylene	152	151
3	acenaphthene	153	154
4	fluorene	166	165
5	d10-phenanthrene**	188	----
6	phenanthrene	178	176
7	anthracene	178	176
8	fluoranthene	202	101
9	pyrene	202	101
10	retene	219	234
11	d12-benzo (a) anthracene*	240	----
12	benzo (a) anthracene	228	114
13	chrysene	228	114
14	d12-perylene*	264	---
15	benzo (b) fluoranthene	252	126
16	benzo (k) fluoranthene	252	126
17	benzo (e) pyrene	252	126
18	benzo (a) pyrene	252	126
19	indeno (1,2,3,cd) pyrene	276	138
20	dibenzo (a,h) anthracene	278	139

Table 1. Target compounds and their monitored ions.

Retention Order	Compound	Major Ion	Secondary Ion
21	benzo (g,h,i) perylene	276	138
22	coronene	300	150

*internal standard
**surrogate standard

2.0 Performance Evaluation

Prior to analyzing a sample set, the GC-MS system performance and calibration are verified for all analytes. The mass spectrometer is tuned immediately before the running of a sample batch using the system's operating software programs (AUTOTUNE) with perfluorotributylamine (PFTBA) calibration gas. Mass spectrometer parameters are adjusted so that masses 69, 219, and 502 and their respective isotopes meet the target mass-intensity criteria. A sample AUTOTUNE report is included.

Hexane is injected prior to the running of a sample set to insure the system is free from contaminants or interfering peaks. The Relative Percent Difference (RPD) between a calibration standard and a performance standard should be withing 20%.

Sample injections and system maintenance are recorded in the appropriate laboratory logbooks located near the instrument.

3.0 Instrumental Parameters

The GC oven temperature program is given in Table 2. Other significant gas chromatographic parameters are:

Carrier gas:	helium (99.999%; Liquid Carbonic, Chicago)
Injector:	On Column, Constant Flow
Injection volume:	1 μL
Transfer line:	300°C

The mass spectrometer is operated in the electron ionization (EI) mode with ion source and quadrapole temperatures of 250°C and 100°C respectively.

Table 2. GC oven temperature program for PAH analysis

Initial Temperature: 40°C

Initial Time: 3.00 min

Rate (°C/min)	Final Temperature (°C)	Final Hold Time (min)
30.0	240	0.00
50.0	300	11.7

Total Run Time: 22.70 min

4.0 Sample Analysis

The extracted samples, lab blanks and matrix spikes are stored in 4-mL amber vials at -20°C until they are ready for analysis. They contain approximately 1 mL of solvent (hexane) and were previously spiked with 50 µL of the internal standard solution (see Table 3).

Table 3. Internal standard solution.

Compound	Concentration (ng/µL)
d10-anthracene	4.00
d12-benzo(a)anthracene	4.00
d12-perylene	4.00

Standards and samples are brought to room temperature before they are injected. After the hexane blank is injected, a calibration standard (see Table 5) is injected followed by the samples. All injections are performed manually with 1 µL volumes.

The mass spectrometer is turned on after a four minute solvent delay. Data is acquired in selected ion mode. Windows and ion ranges are given in Table 6.

5.0 Data Reduction and Analyte Quantitation

Data is collected and stored within the system's HP Apollo 400 computer. Mass chromatograms are generated, and their peaks are integrated with the accompanying software programs. Chemstation Enviroquant prepares quantitation reports with individual mass chromatograms and spectra for all target analytes.

For each day of samples run, relative response factors (RRFs) for each analyte are determined from the calibration standard's peak areas using Equation 1.

$$RRF_{std} = \left(\frac{mass_a}{area_a}\right)_{std} \div \left(\frac{mass_{istd}}{area_{istd}}\right)_{std}$$

Where $mass_a$ = *the analyte's known mass in the injected amount of calibration standard,*
$area_a$ = *the analyte's peak area,*
$mass_{istd}$ = *the known mass of the appropriate internal standard, and*
$area_{istd}$ = *that internal standard's peak area.*

With reference to Table 1, the response factors for compounds 2-10, 12-13, and 15-22 are calculated relative to the internal standards d10-anthracene, d12-benzo(a)anthracene, and d12-perylene respectively.

An analyte's mass in a sample ($mass_a$) is calculated from the RRF_{std} above and the internal standard response in the sample by the following equation:

$$(mass_a)_{sample} = (area_a)_{sample} \; x \; RRF_{std} \; x \left(\frac{mass_{istd}}{area_{istd}}\right)_{sample}$$

Where $area_a$ = *the analyte's peak area in the sample,*
$mass_{istd}$ = *the mass of internal standard spiked into the sample, and*
$area_{istd}$ = *the internal standard's peak area in the sample.*

The analyte concentrations are tabulated by Enviroquant and transferred to an Excel spreadsheet.

6.0 Quality Assurance

Each daily analytical batch includes at least one calibration standard, one performance standard, one instrument blank, one procedure blank, and one matrix spike. Acceptance criteria are summarized on the attached Table 3-1. Refer to the IADN Quality Assurance Project Plan (QAPjP) for more details.

7.0 Atrazine

Atrazine is analyzed by GC/MS in the selected ion monitoring mode. The GC temperature program is given in Table 4 below. The calibration standard contains both atrazine and the internal standard d10-anthracene at concentrations of 0.4 ng/µL.

The mass spectrometer is turned on after a solvent delay of 8.5 minutes. The ions monitored are m/z 186, m/z 188, m/z 200, and m/z 215. The ions m/z 188 and m/z 200 are the quantitation ions for d10-anthracene and atrazine respectively, and the other two are for confirmation purposes.

Table 4. GC oven temperature program for atrazine analysis.

Initial Temperature: 40°C

Initial Time: 1.00 min

Rate (°C/min)	Final Temperature (°C)	Final Hold Time (min)
25.0	140	0.00
4.0	240	0.00
20.0	290	5.00

Total Run Time: 29.50 min

8.0 Chromatograms

Mass chromatograms from a typical 1 µL injection of calibration standard are given on the following pages in Figures 1a-i. The windows on the left-hand side of each page show the quantitation peak(s) for one or more PAHs, and those on the right give the corresponding confirmation peaks. Similar mass chromatograms (Figure 2) are given for a 1 µL injection of the atrazine calibration standard.

Table 5. Calibration standard.

Retention Order	Compound	Concentration (ng/µL)
1	d10-anthracene*	0.20
2	acenaphthylene	0.20
3	acenaphthene	0.20
4	fluorene	0.20
5	phenanthrene	0.20
6	d10-phenanthrene**	0.20
7	anthracene	0.20
8	fluoranthene	0.20
9	pyrene	0.20
10	retene	0.20
11	d12-benzo (a) anthracene*	0.20
12	benzo (a) anthracene	0.20
13	chrysene	0.20
14	d12-perylene*	0.20
15	benzo (b) fluoranthene	0.20
16	benzo (k) fluoranthene	0.20
17	benzo (e) pyrene	0.19
18	benzo (a) pyrene	0.20
19	indeno (1,2,3,cd) pyrene	0.20
20	dibeno (a,h) anthracene	0.20
21	benzo (g,h,i) perylene	0.20
22	coronene	0.20

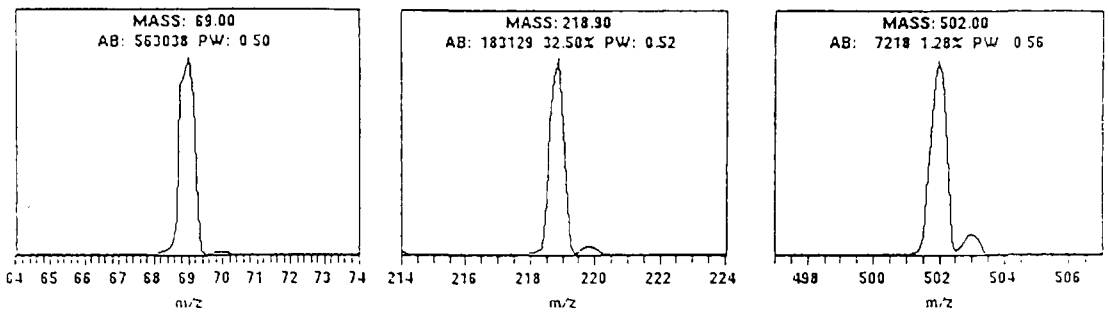
*internal standard
**surrogate standard

Table 6. SIM windows for analyte detection.

SIM Window	Start Time (min)	SIM Mass (m/z)
1	6.50	76, 83, 151, 152, 153, 154, 165, 166
2	9.80	101, 202, 176, 178, 188, 204, 219, 234
3	11.70	114, 126, 152, 228, 240, 264
4	14.80	138, 139, 150, 276, 278, 279, 300

Auto Tune
Tune File: atune.u Tue Apr 11 95 05:08:05 PM
Instrument Name: hp1 Instrument Type: 5989 Ionization Mode: EI

Repeller	7.00	Multiplier	1583	Emission	300	Integration	50
Ion Focus Gain	255	AMU Gain	167	Elec. Energy	70	Samples	16
Ion Focus	34	AMU Offset	106	Polarity	POSITIVE	Averages	1
Entrance Lens	145	Axis gain	63	Source Temp	250	Stepsize	0.12
X Ray	40	Axis Offset	4	Quad. Temp	100		



MASS	Abundance	Rel. Abund	Iso. Mass	Iso. Abund.	Iso. Ratio
69.00	422528	100.00	70.00	4093	0.97
218.90	158464	37.50	219.90	6279	3.96
501.95	6714	1.59	502.95	672	10.01

Scan. 10.00 - 800.00 samples: 16 thresh: 20 Tue Apr 11 95 05:08:16 PM
259 peaks Base. 69.00 Abundance: 422528

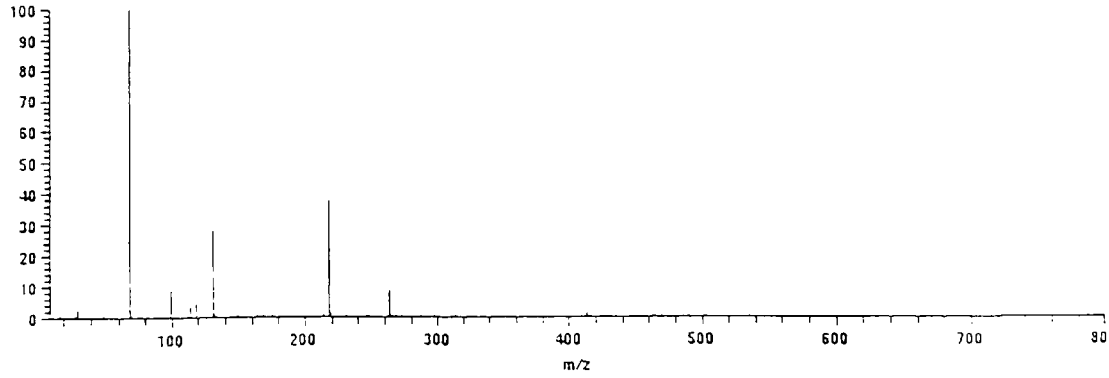
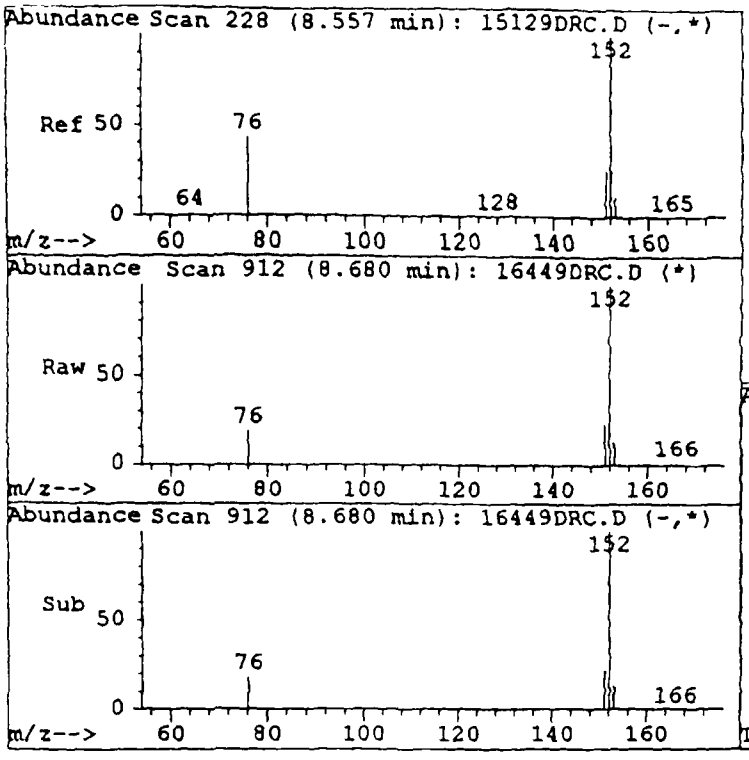


Table 3.1. Data Quality Objectives for Trace Organic Compounds

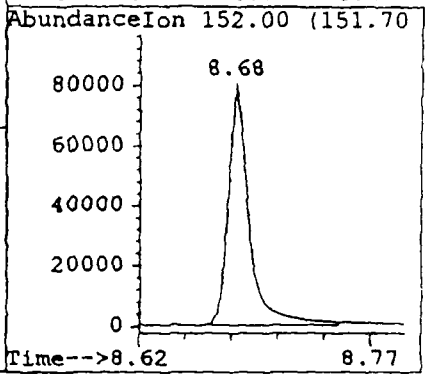
QA Criteria	QC Code	Sample Type	Frequency	Required Objective	Control Action	Units
precision	FD1/ FD2	field; co-located samplers	20%	at <5xMDL, rsd <100% at <5xMDL, rsd <50%	re-analyze if sample available; otherwise flag samples FFD or PTS	%
	LD1/ LD2	laboratory: replicate analyses	10%	at <5xMDL, rsd <50% at <5xMDL, rsd <30%	re-analyze same or alt sample; otherwise flag samples FDL or PTS	%
accuracy	LSS	surrogate spikes	all samples	50% < R <130%	flag PCB congener range FSS; investigate sources of loss	%
	LMS	matrix spikes	1/batch	50% < R < 130% for 70% of individual congeners and for average	flag sample FMS; investigate sources of loss	%
blanks	FRB	field matrix blank	10% (1/month)	<20% of associated sample mass	flag samples FFR; find source of contamination	mass
	LRB	lab matrix blank	10%	<MDL	run 2nd LRB; elminate source of imprecision; flag sample FBS	mass
completeness		field samples		90%	no action; % reported	
calibration	CLM	multiple point calibration; 4 point	annual	r^2 >0.95	reoptimize instrument, repeat calibration	
	CLS	single calibration std	1/10 samples	∑PCBs ± 10% actual mass	reoptimize instrument, repeat calibration	
	LPC	performance std	daily	see Table 3.2	regenerate response factors	
	LCB	lab calibration blank	2/GC run	< MDL	check for contamination; reoptimize instrument	
PCB identification		GCMS confirmation	5%			
detection limits		MDL study	1/project		reported in yearly QA Report	
detectability	RFS	routine field samples	all samples	> MDL	flag BDL	
holding time	RFS	routine field sample	all samples	1 year	flag EHT	

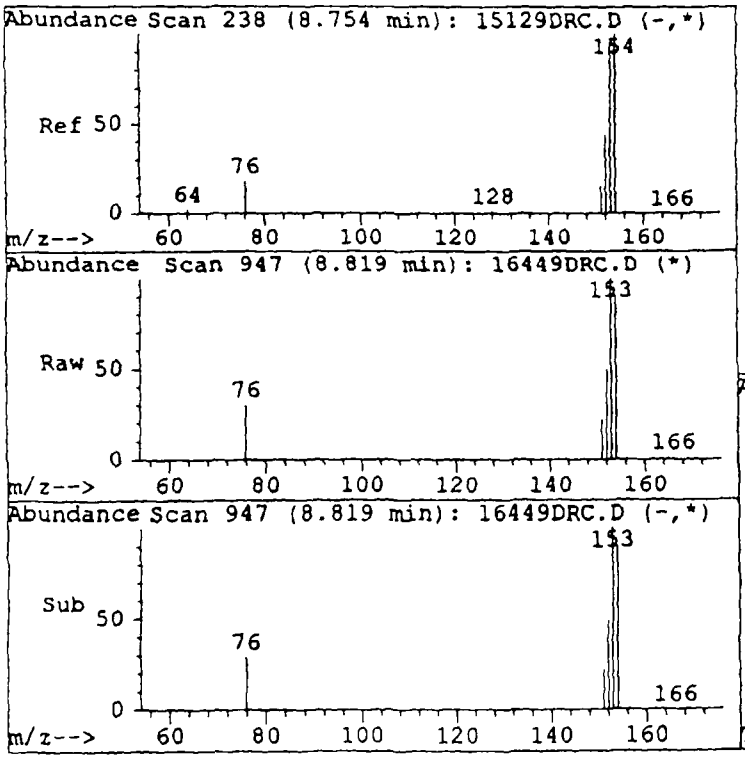


#2
Acenaphthylene
Concen: 374.36 ng
RT: 8.68 min Scan# 912
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:152 Resp: 75932

Ion	Ratio	Lower	Upper
152	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#3
Acenaphthene
Concen: 359.10 ng
RT: 8.82 min Scan# 947
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:153 Resp: 64434

Ion	Ratio	Lower	Upper
153	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

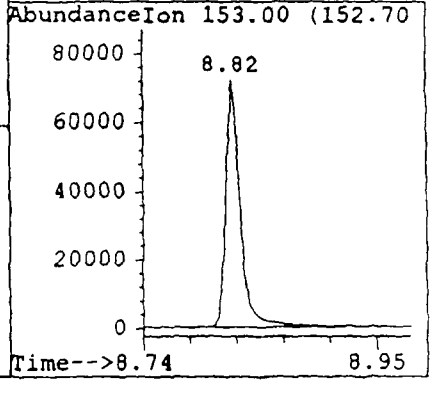
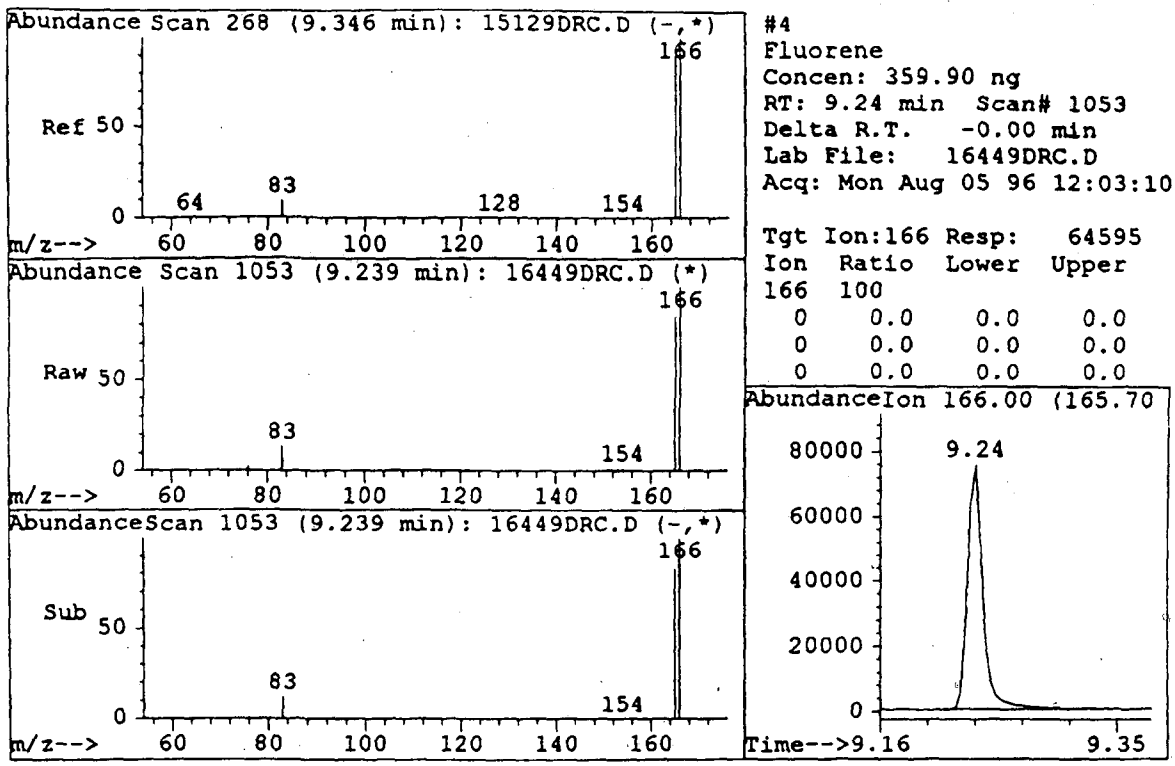


Figure 1a.


Abundance Scan 268 (9.346 min): 15129DRC.D (-,*)
Ref
166
64
83
128
154
m/z-->
Abundance Scan 1053 (9.239 min): 16449DRC.D (*)
Raw
166
83
154
Abundance Scan 1053 (9.239 min): 16449DRC.D (-,*)
Sub
166
83
154
#4
Fluorene
Concen: 359.90 ng
RT: 9.24 min Scan# 1053
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM
Tgt Ion:166 Resp: 64595
Ion Ratio Lower Upper
166 100
0 0.0 0.0 0.0
0 0.0 0.0 0.0
0 0.0 0.0 0.0
Abundance Ion 166.00 (165.70
9.24
Time-->9.16
9.35

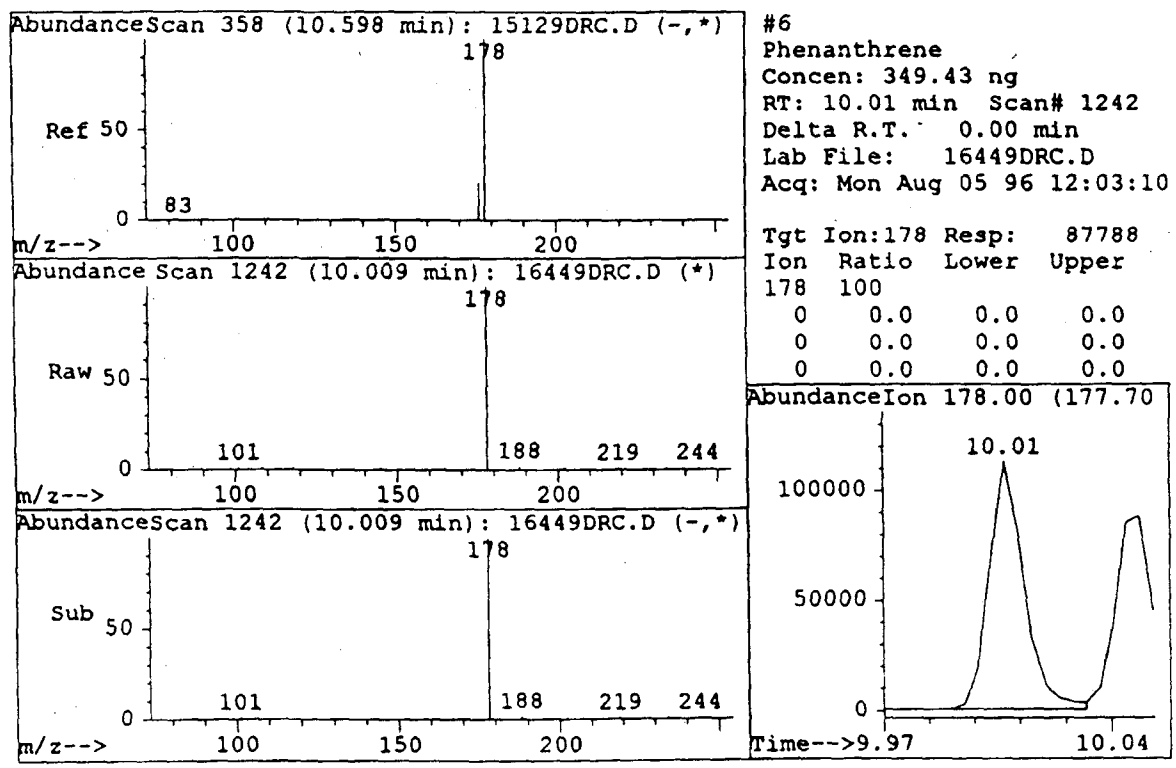
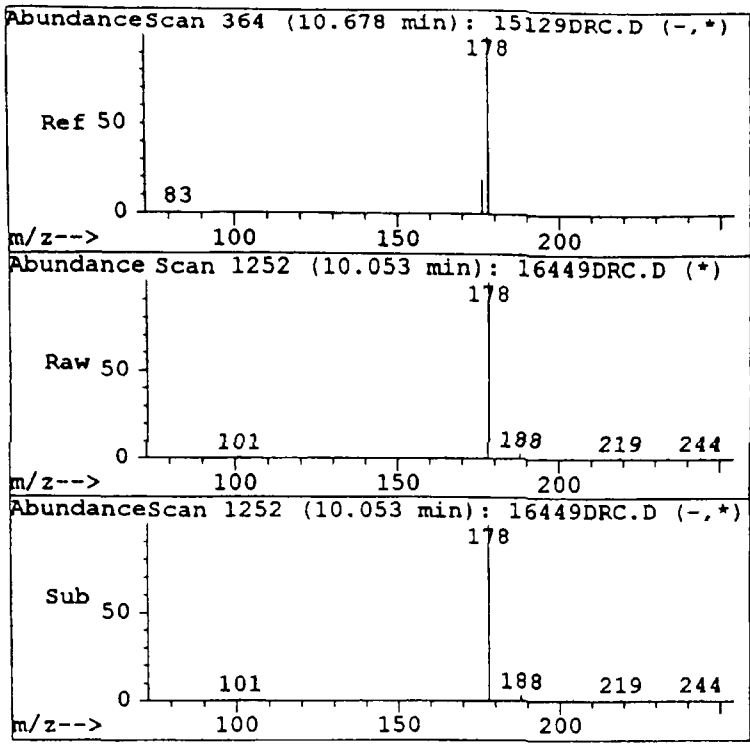

Abundance Scan 358 (10.598 min): 15129DRC.D (-,*)
Ref
178
83
m/z-->
Abundance Scan 1242 (10.009 min): 16449DRC.D (*)
Raw
178
101
188
219
244
Abundance Scan 1242 (10.009 min): 16449DRC.D (-,*)
Sub
178
101
188
219
244
#6
Phenanthrene
Concen: 349.43 ng
RT: 10.01 min Scan# 1242
Delta R.T. 0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM
Tgt Ion:178 Resp: 87788
Ion Ratio Lower Upper
178 100
0 0.0 0.0 0.0
0 0.0 0.0 0.0
0 0.0 0.0 0.0
Abundance Ion 178.00 (177.70
10.01
Time-->9.97
10.04

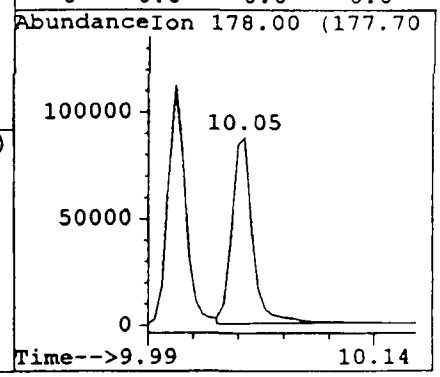
Figure 1b.

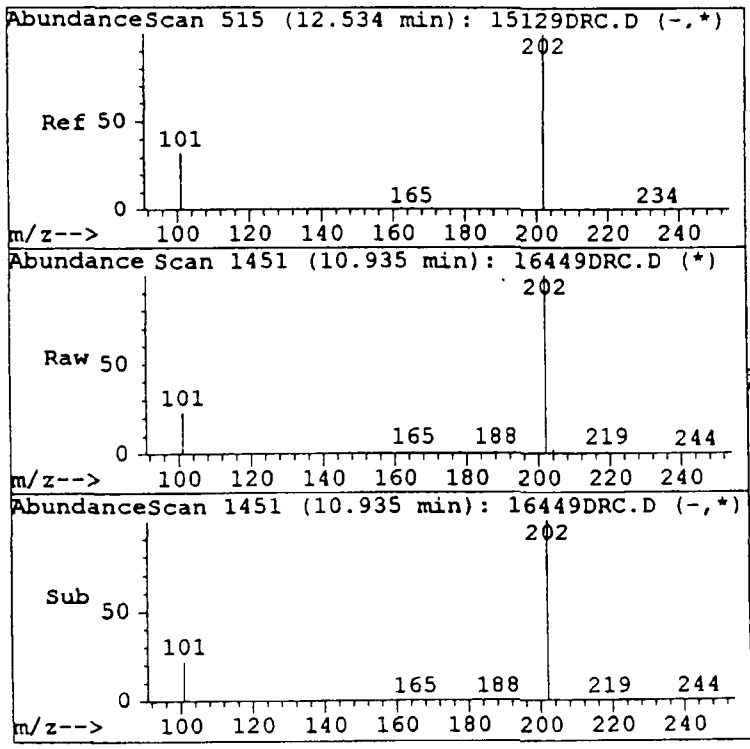


#7
Anthracene
Concen: 370.90 ng
RT: 10.05 min Scan# 1252
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:178 Resp: 80073

Ion	Ratio	Lower	Upper
178	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#9
Fluoranthene
Concen: 351.20 ng
RT: 10.94 min Scan# 1451
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:202 Resp: 88151

Ion	Ratio	Lower	Upper
202	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

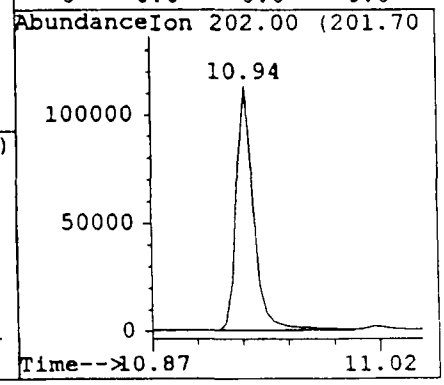
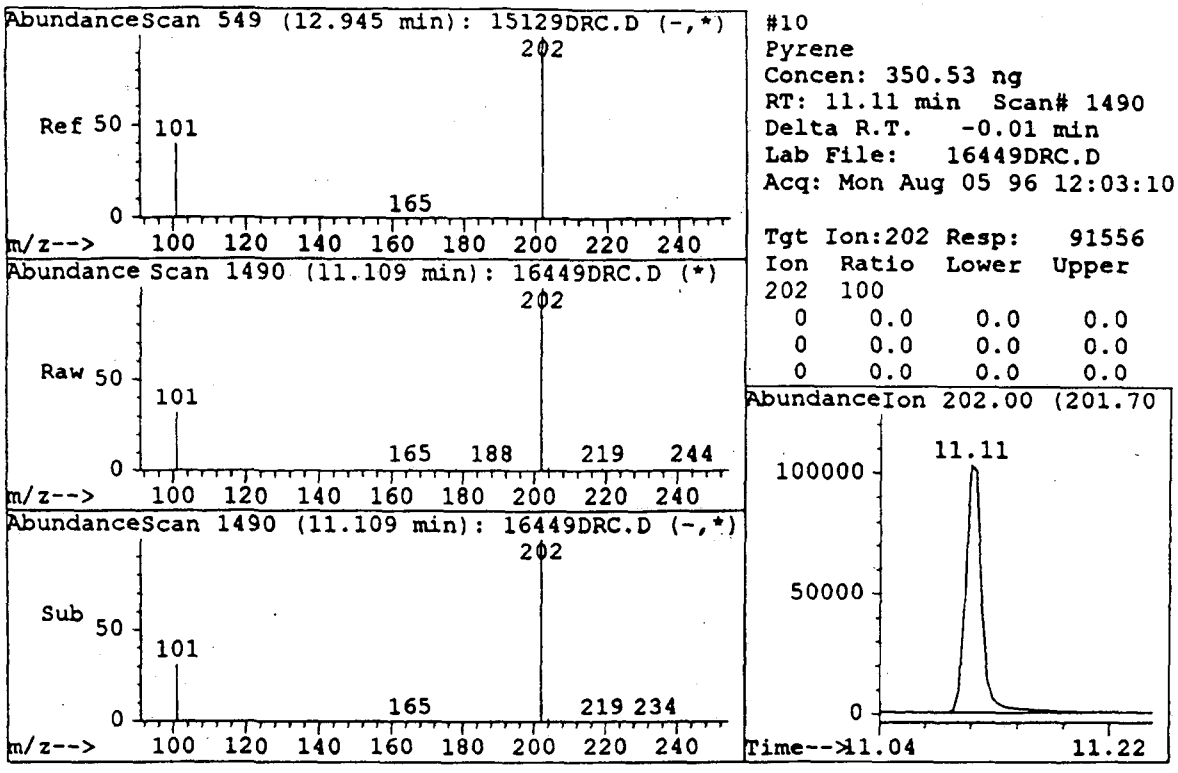


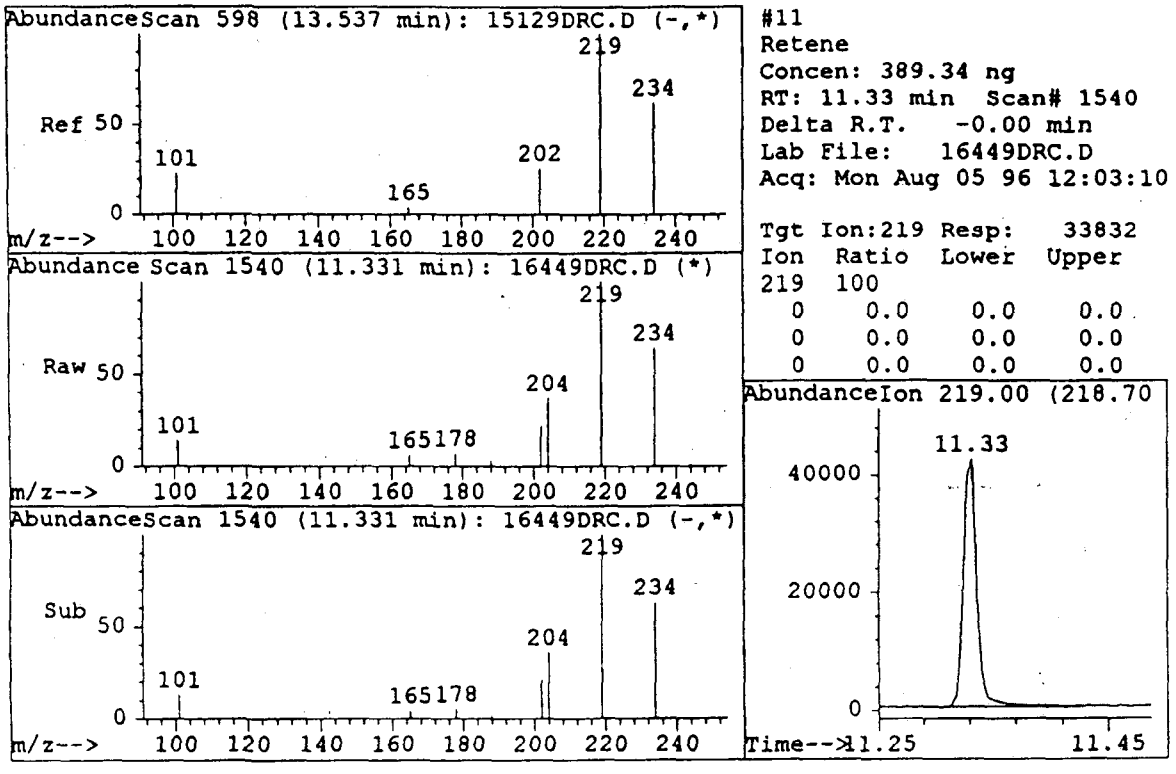
Figure 1c.



#10
Pyrene
Concen: 350.53 ng
RT: 11.11 min Scan# 1490
Delta R.T. -0.01 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:202 Resp: 91556

Ion	Ratio	Lower	Upper
202	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

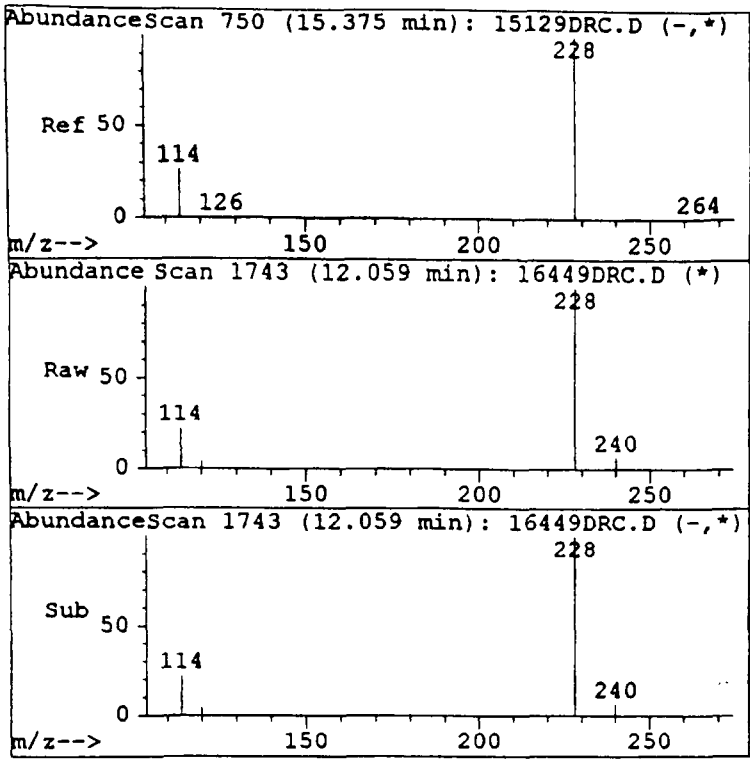


#11
Retene
Concen: 389.34 ng
RT: 11.33 min Scan# 1540
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:219 Resp: 33832

Ion	Ratio	Lower	Upper
219	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

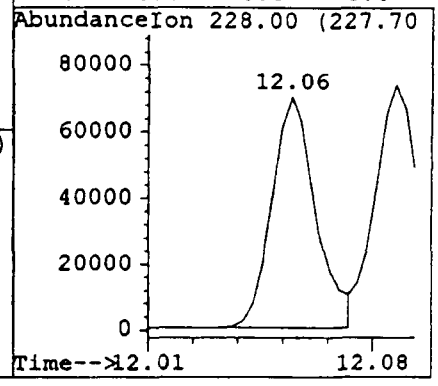
Figure 1d.

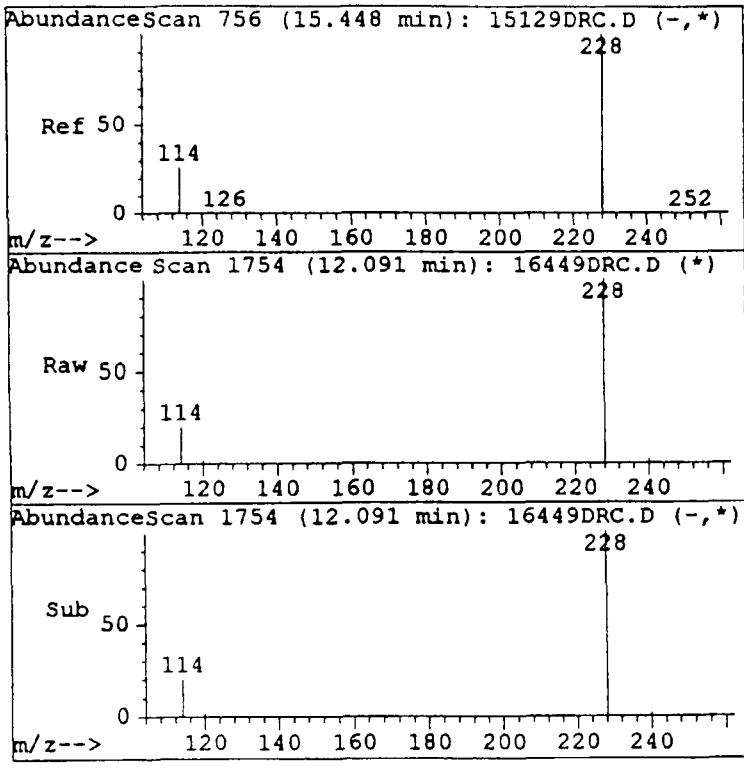


#13
Benzo(a)anthracene
Concen: 351.22 ng
RT: 12.06 min Scan# 1743
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:228 Resp: 66629

Ion	Ratio	Lower	Upper
228	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#14
Chrysene
Concen: 335.06 ng
RT: 12.09 min Scan# 1754
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:228 Resp: 80895

Ion	Ratio	Lower	Upper
228	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

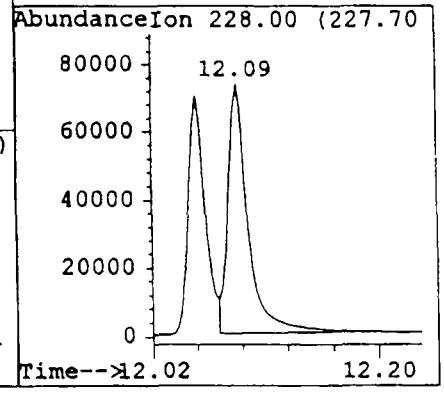
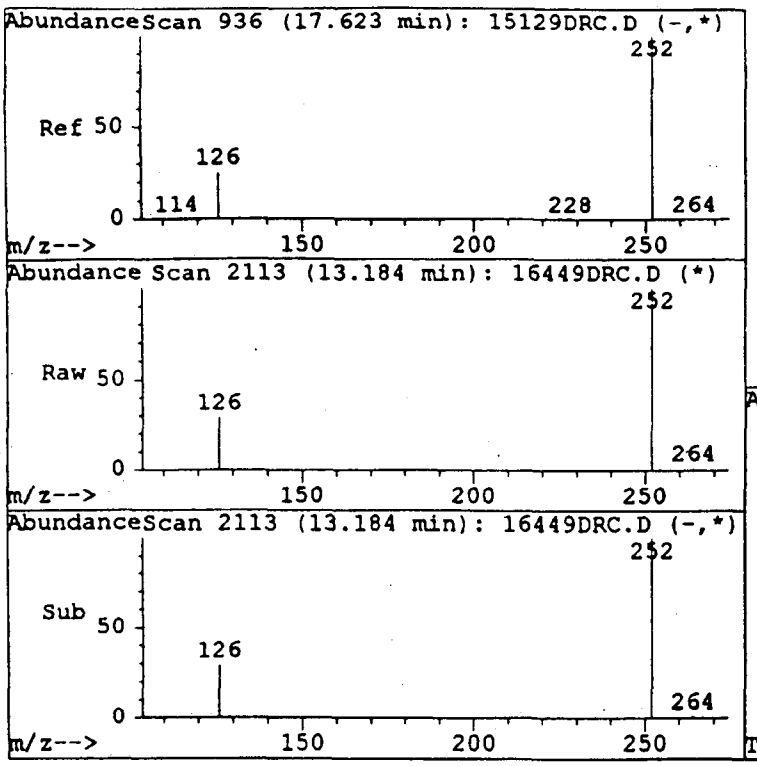


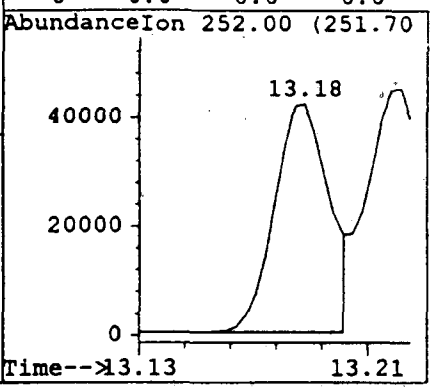
Figure 1e.

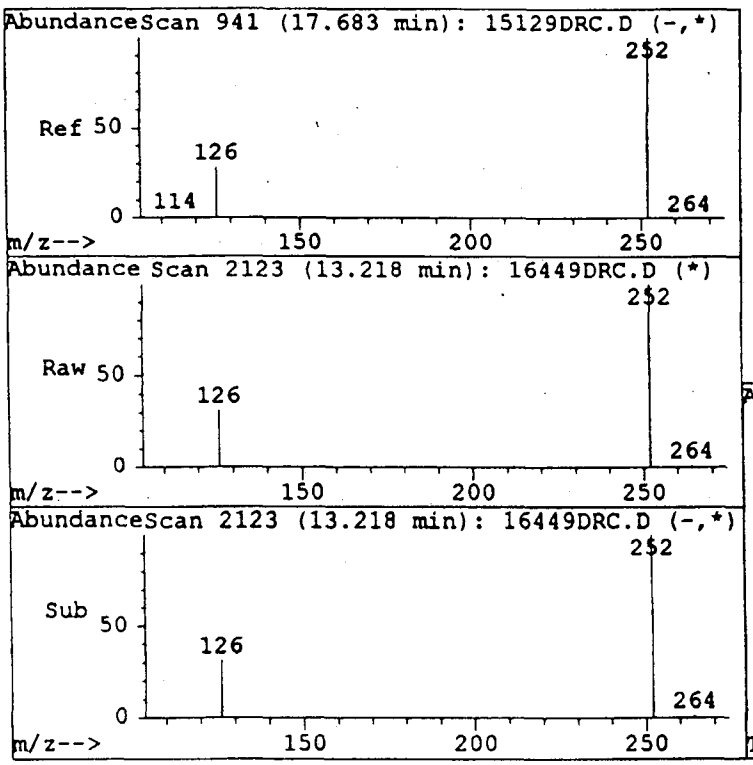


#16
Benzo(b)fluoranthene
Concen: 329.91 ng
RT: 13.18 min Scan# 2113
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:252 Resp: 55725

Ion	Ratio	Lower	Upper
252	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#17
Benzo(k)fluoranthene
Concen: 371.03 ng
RT: 13.22 min Scan# 2123
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:252 Resp: 78719

Ion	Ratio	Lower	Upper
252	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

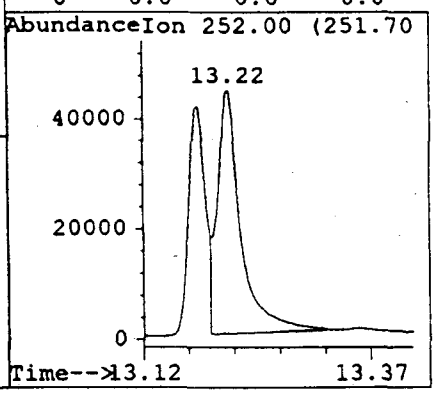
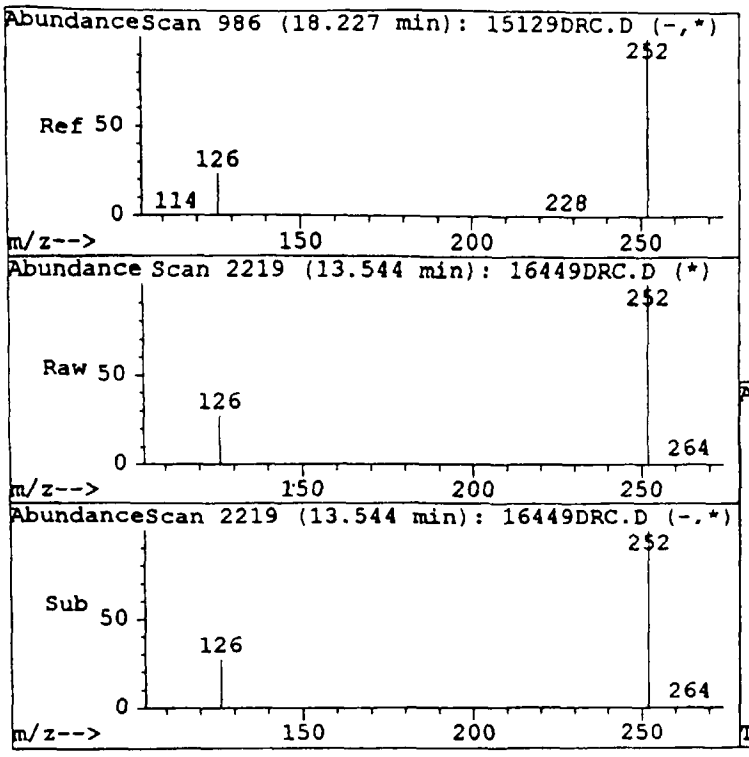


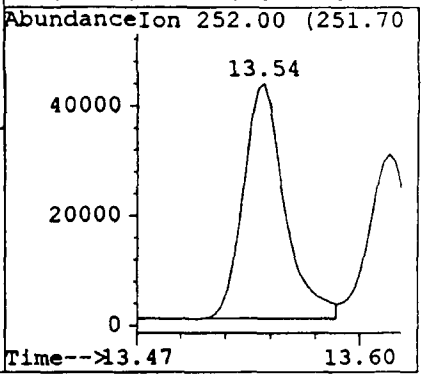
Figure 1f.

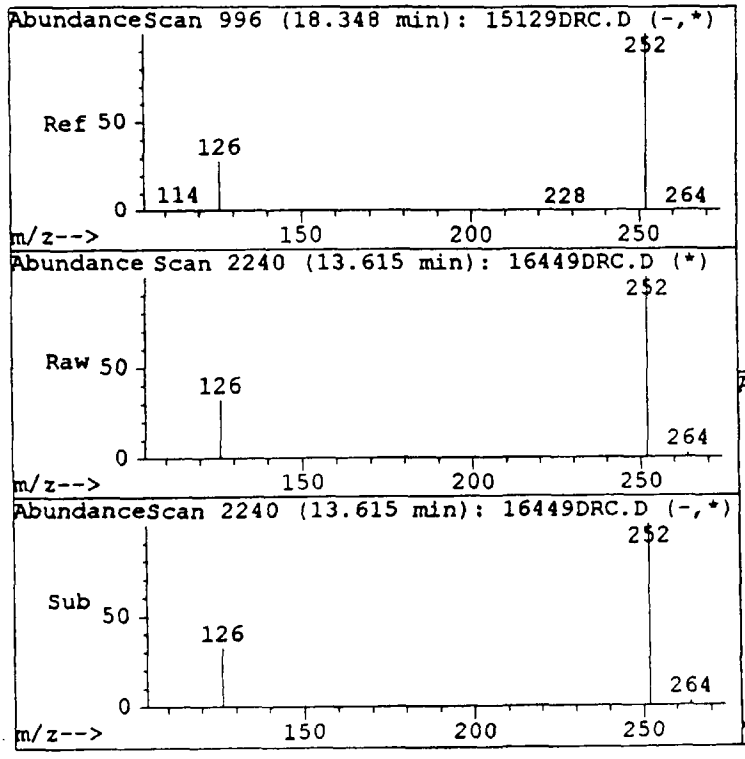


#18
Benzo(e)pyrene
Concen: 337.11 ng
RT: 13.54 min Scan# 2219
Delta R.T. -0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:252 Resp: 69063

Ion	Ratio	Lower	Upper
252	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#19
Benzo(a)pyrene
Concen: 398.13 ng
RT: 13.62 min Scan# 2240
Delta R.T. -0.01 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:252 Resp: 63486

Ion	Ratio	Lower	Upper
252	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

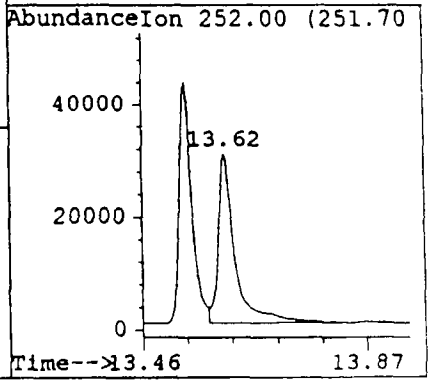
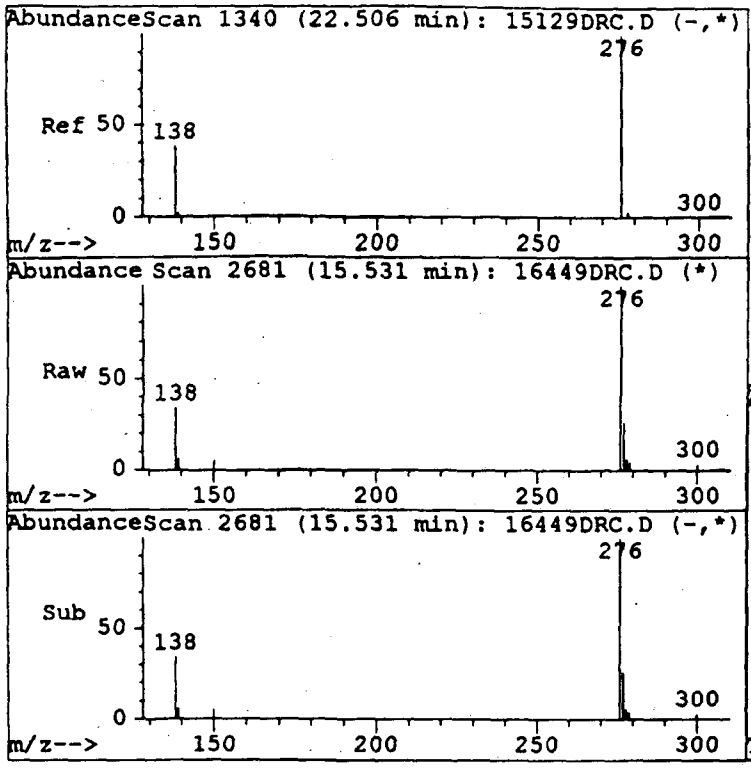


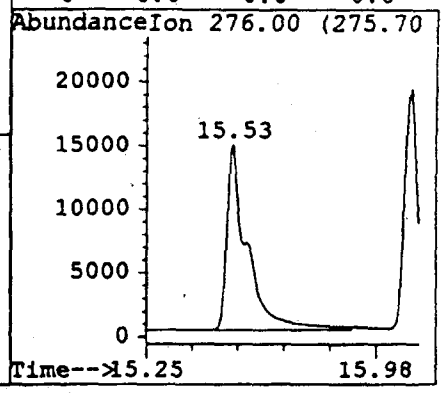
Figure 1g.

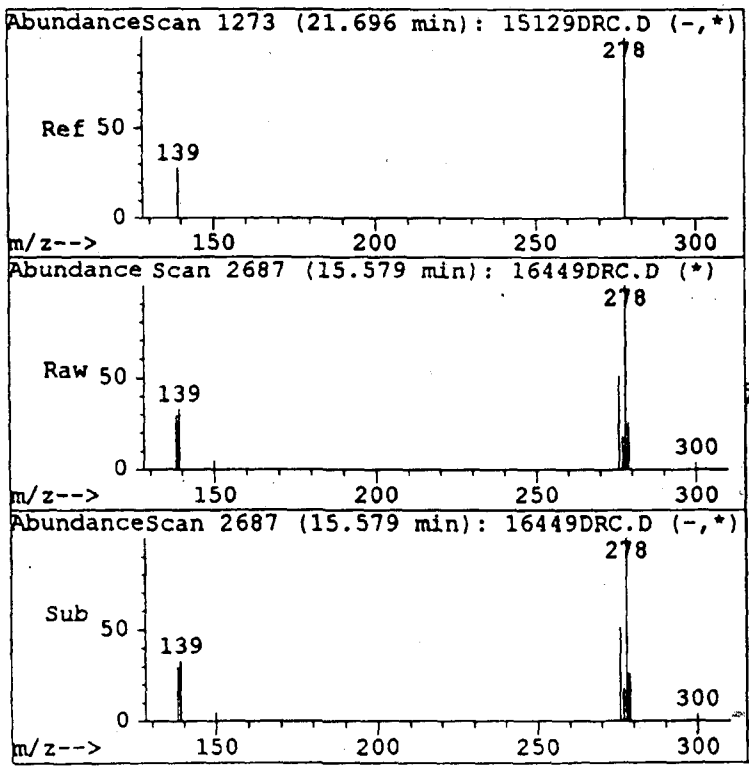


#20
Indeno(1,2,3-cd)pyrene
Concen: 615.39 ng
RT: 15.53 min Scan# 2681
Delta R.T. 0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:276 Resp: 66114

Ion	Ratio	Lower	Upper
276	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#21
Dibenzo(a,h)anthracene
Concen: 464.76 ng
RT: 15.58 min Scan# 2687
Delta R.T. -0.01 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:278 Resp: 47193

Ion	Ratio	Lower	Upper
278	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

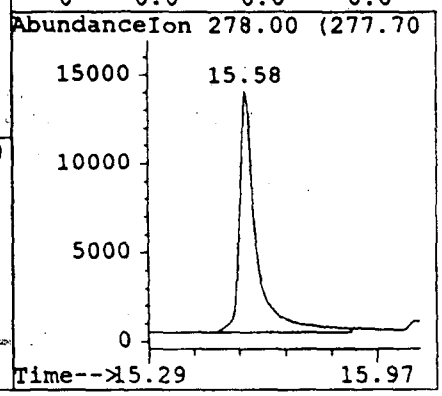
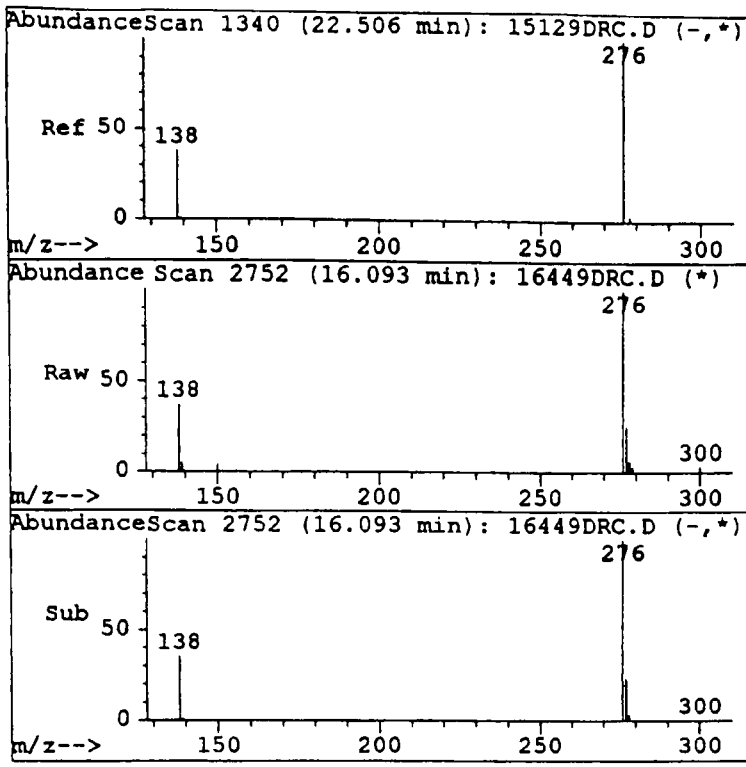


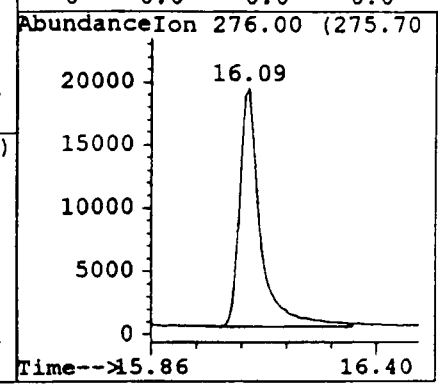
Figure 1h.

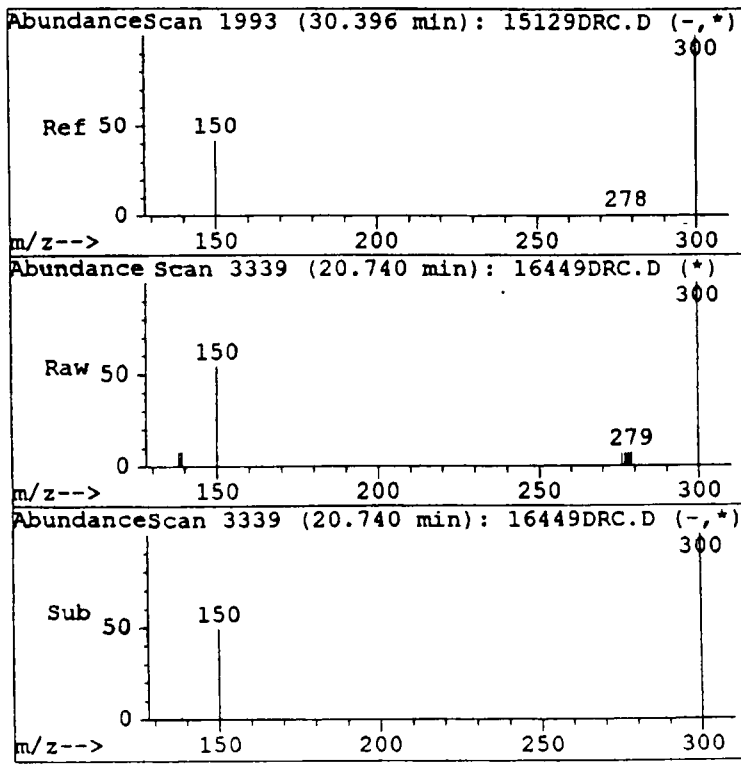


#22
Benzo(g,h,i)perylene
Concen: 363.31 ng
RT: 16.09 min Scan# 2752
Delta R.T. 0.00 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:276 Resp: 64079

Ion	Ratio	Lower	Upper
276	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0





#23
Coronene
Concen: 328.96 ng
RT: 20.74 min Scan# 3339
Delta R.T. -0.01 min
Lab File: 16449DRC.D
Acq: Mon Aug 05 96 12:03:10 PM

Tgt Ion:300 Resp: 44543

Ion	Ratio	Lower	Upper
300	100		
0	0.0	0.0	0.0
0	0.0	0.0	0.0
0	0.0	0.0	0.0

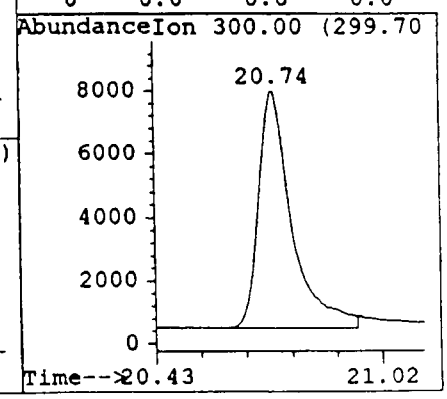


Figure 1i.

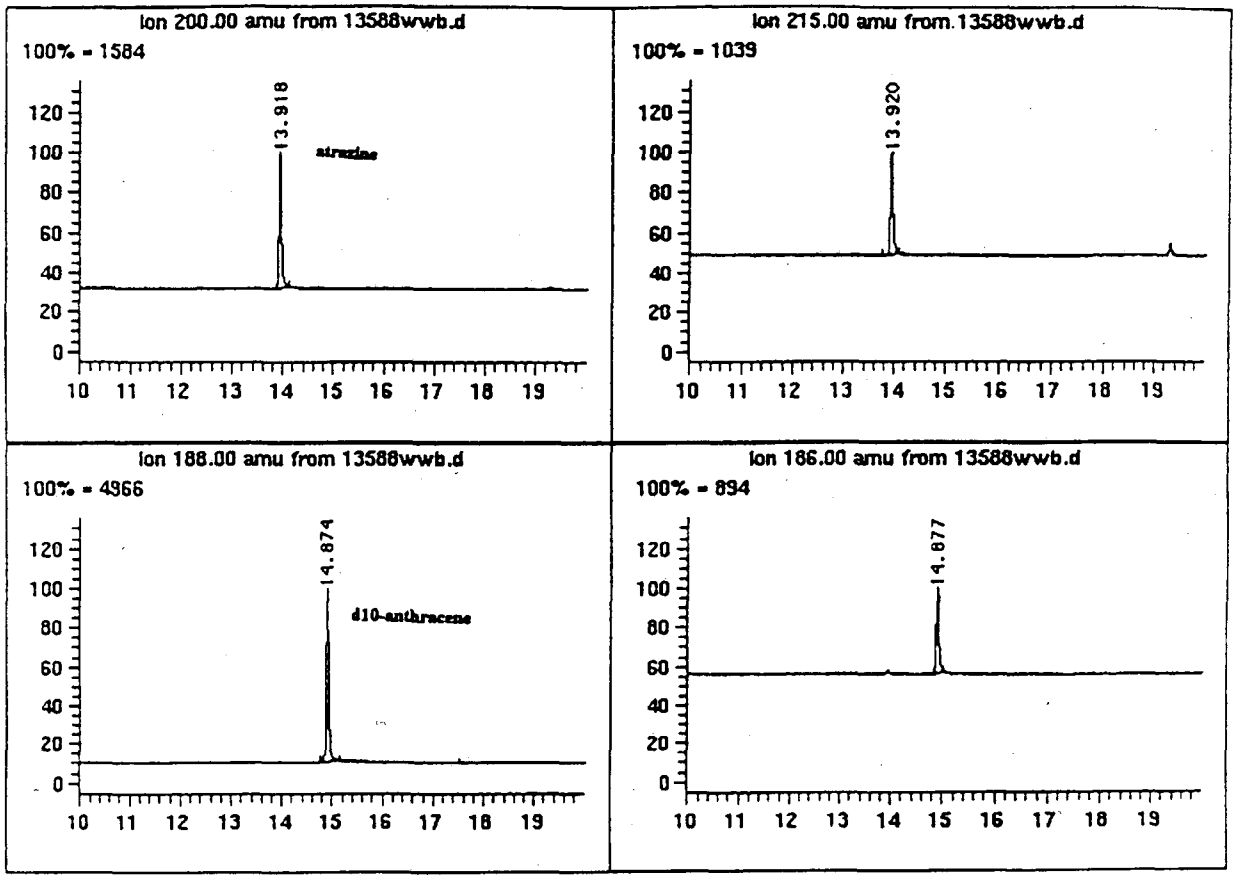


Figure 2. Mass chromatograms for atrazine calibration standard.

Analysis of PCBs and Pesticides in Air and Precipitation Samples:

IADN Project

Gas Chromatography Procedure

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September 1995

Version 1.0

Analysis of PCBs and Pesticides in Air and Precipitation Samples: IADN Project - Gas Chromatography Procedure

1.0 Routine GC Maintenance

1.1 Gas Tanks

1.1.1 Check gas tanks. Tanks should not go dry. While changing the tank, lower the temperature of the oven down to 40°C. Leave it at 40°C for about half an hour after changing the tank to get rid of air or oxygen that was drawn in.

1.1.2 Check column head pressure. It should be at 24 psi. If pressure falls, tighten the septum nut. If the pressure is still low check for leaks and tighten other connections.

1.2 GC Baking

After every GC run bake the oven at 280°C for one hour. After every other run also bake the injector and the detector at 280°C and 380°C.

1.3 Septum

1.3.1 Preparation of Septum

1.3.1.1 Place the septa in a small beaker. Cover with aluminum foil.

1.3.1.2 Place the beaker in GC oven and bake the septa while baking the column at 280°C.

1.3.1.3 Store the clean septa in the beaker keeping the foil lid intact and use as needed. Always use tweezers to get clean septum out.

1.3.2 Changing of Septum

1.3.2.1 After every 60 samples or so change the septum.

1.3.2.2 Cool the oven down to 40°C.

1.3.2.3 Remove autosampler towers.

1.3.2.4 Remove septum nut and take the old septum out. Discard.

1.3.2.5 Using clean Q-tips soaked in hexane, wipe off the septum holder until no more dirt is seen on Q-tips.

1.3.2.6 Put in pre-cleaned septum and replace the nut. Nut should be snug but not too tight. Column head pressure should go up to 24 psi if nut is tight enough. Check the tightness of the nut after injecting the first sample. Make sure that the head pressure is still 24 psi.

1.4 Checking Background

1.4.1 Background signal in GC varies from 28 to 32. Hexane is analyzed at the start of every GC run to monitor the baseline stability. If the signal goes up or hexane run produces noisy chromatogram GC should be cleaned.

1.5 Checking Standard

1.5.1 Mullin 94 standard and Mixed Pesticide Standard should be monitored to check peak detection and peak broadening or tailing. If the peak shapes are not satisfactory, column should be clipped. More than 90 peaks should be detected in PCB standards and cong. 17 and 18 should be separated. If not, install a new column.

1.6 Checking Leaks and Gas Flow

1.6.1 Check leaks once in two weeks with a leak detector. Check around the septum, at the injector end, and at the detector end of the column.

1.6.2 Check the gas flow once in two weeks with a flow meter. Approximate gas flow are as follows :

Split vent	120 mL/min.
Purge vent	2 mL/min.
Total flow through detector	22 mL/min.

2.0 GC Cleaning: Clipping Old Column or Installing a New Column

2.1 Taking Apart

2.1.1 Turn oven, injector and detector off.

2.1.2 Turn hydrogen and nitrogen off. Wait until everything cools down.

2.1.3 Take the autosampler tower off.

2.1.4 Undo the small nut covering the septum and the large nut underneath it to expose the injection liner. Take the liner out.

2.1.5 Open the oven. Take the columns out (by detaching from injector and detector ends).

2.1.6 Unscrew the nuts from both injector and detector ends of columns and plug the column ends with septum. Open end of column should not be exposed to air.

2.1.7 Place the columns on the workbench.

2.1.8 Unscrew the holder nut underneath the injection liner. There is one gold seal and a washer in it. Washer and seal need to be replaced each time it is taken apart. Clean these parts by ultrasonication with Dichloromethane and Hexane.

2.1.9 Put a beaker inside the oven underneath the injection port and pour some hexane through the injection port. Clean the injection port with Q-tips and rinse again with hexane.

2.2 Assembling Injection Port and Liner

2.2.1 After ultrasonication air dry each part. Assemble the holder nut. Place the gold washer first and then the seal. The tapered opening of the seal will face downward. (The tapered end will hold the end of the ferrule from the column.) Screw the nut in before placing the injection liner.

2.2.2 Insert a new liner.

2.2.3 Put a viton O-ring on the liner. Put the big nut on and tighten it. Put a clean septum. Cover the septum with septum nut. Tighten with wrench.

2.3 Clipping Column

2.3.1 Take the nut off the injector end of the column. Carefully scrape out all the ferrules from the column nuts. Clean all different parts with Q-tips soaked in DCM and ultrasonicate these with DCM and Hexane for 10 minutes with each solvent. Onto the column, insert the nut first and then the ferrule with conical end pointing towards the open end of the column.

2.3.2 Clip the column. Make a clean cut with diamond tip score or ceramic square. Examine the hole with magnifying glass. It should be a clean hole without any jagged end. Always clip the column after putting the nut and the ferrule on.

2.3.3 Measure 25mm from the tip of the column. Mark this point with Liquid Paper®.

2.3.4 Carefully insert the column with nut and ferrule through the holder nut and screw it in. As soon as it feels tight, pull the column out gently until the white mark is seen. Hand tighten the screw more and make it tight with wrench ¼ turn after hand tight. Do not overtighten.

2.3.5 Take the nut off the detector end of the column. Put the nut and the ferrule on the column in the same way as in the injector end. Clip the column and check for the nice clean cut. Insert the column all the way up until it does not go any more. Pull down about 1 mm and tighten the screw. Turn hydrogen on and check the flow of gas through the column by inserting the cut end in a beaker of hexane. Turn hydrogen off.

2.4 Checking Leaks and Gas Flow

2.4.1 Turn H_2 and N_2 on. Check leaks with a leak detector. Check around the septum, at the injector and at the detector end of the column. Check that the head pressure is 24 psi.

2.4.2 Check the gas flow with a bubble meter Approximate gas flow are as follows:

Split vent	120 mL/min.
Purge vent	2 mL/min.
Total flow through detector	22 mL/min.

2.5 Assembling

2.5.1 Replace autosampler tower.

2.5.2 Turn heated zones on.

2.5.3 Turn oven on and set the temperature to 40°C for an hour. Change oven temperature to 70°C and leave another hour.

2.5.4 If it is an old column, bake the column, injector and detector for an hour.

Baking temperature :

Oven:	280°C
Injector A:	280°C
Injector B:	280°C
Detector A:	380°C
Detector B:	380°C

2.5.5 If it is a new column, bake injector and detector. Column can be baked by ramping it 1° or 2° per minute to 280°C. Hold there for one hour.

2.5.6 If blank run looks satisfactory, put in a standard and check.

3.0 Routine GC Operation

3.1 GC condition and oven temperature program:

PCBs, Hexachlorobenzene, and DDE are eluted in the hexane fraction, whereas the other chlorinated pesticides are eluted in the 50% dichloromethane in hexane fraction after silica gel column chromatography. The procedure for nitrogen blowdown, spiking with internal standard, and making microvials for the autosampler are described in IADN Project Sample Preparation Procedure, Version 1.0, June 1995.

Gas Chromatograph used for analysis of PCBs and pesticides is Hewlett Packard 5890 with 7673A Autosampler. Operation is controlled by the Integrator Hewlett Packard 3396. Data acquisition is done by Hewlett Packard Peak 96. Finally, data analyses are done in Hewlet Packard 3365 ChemStation.

Carrier gas:	Hydrogen
Make up gas:	Nitrogen
Split vent:	120 mL/min.
Purge vent:	2 mL/min.
Total flow through the detector:	22 mL/min.
Column:	DB-5, 60m, 0.25mm i.d, 0.1μ film thickness

Temperature Program:	Initial temp.100°C
Initial time:	1 min.
Rate:	1°C/min.
Final temp.:	240°C
Rate A:	10°C
Final temp A:	280°C
Final time:	20 min.
Purge on:	3 min.
Purge off:	150 min.
Run time:	165 min.

The GC condition, column type, and the oven temperature program was specified by Mike Mullin. The method name is Mullin.met.

3.2 GC Pre-run

3.2.1 Check to see if there is sufficient H_2 for operation. If not, change tanks.

3.2.2 If necessary, change septum.

3.2.3 Bake oven at 280°C for one hour. Bake injector and detector at 280°C and 380°C respectively about every other time the oven is baked.

3.2.4 Cool oven to 100°C, injector to 250°C, and detector to 350°C.

3.2.5 Make the samples ready in microvials and load the autosampler tray.

3.3 Preparing Sequence in ChemStation

3.3.1 Editing Sequence

3.3.1.1 Sequence Parameters

Sequence	Edit Sequence Parameters

Type in operator's name and subdirectory name. Type in information about calibration standard and spikes in comment.

3.3.1.2 Sequence Table

Sequence	Edit Sequence Table

Enter "From Vial" and "To Vial" number.

3.3.1.3 Sample Table

Sequence	Edit Sample Table

Type in sample ID's. First vial should be hexane. Second vial should be calibration standard. Third vial should be a Performance standard. Other vials are actual samples. At the end of each sample ID indicate whether the sample is a hexane fraction or 50% fraction (H,F1). Repeat hexane blank and a fresh standard after every set of samples.

3.3.1.4 Print Sequence

Sequence	Print

x Sequence Parameters
x Sample Table

3.4 Starting a GC run

3.4.1 Programming integrator

Set runnum 1
Set Date mo/day/year
Shift edit seq

The integrator will ask questions about method to load and autosampler sequence. Enter all information.

3.4.2 Programming peak 96

Integrator 2

Follow the keys:

Data Acquisition	Set up integrator	Select old method
Mullin.met	Download method	Mullin.met

3.4.3 Set up PC

Follow the keys:

Generate new

Data prefix J3095 (June 30, 95. It will pick up the vial number from the integrator)

Check export path/peak/export 2

Data to ChemStation Y

3.4.4 Transfer information from integrator to PC

Follow the keys:

Utilities	Transfer	Integrator to PC	IBCOL.SEQ

3.4.5 Start a GC run

Follow the keys:

Data Acquisition	Start Seq

3.4.6 Post GC run

After the GC run is over copy the .d files on floppy disks.

Sequence

27 Apr 95 05:05 PM
age 1
Sequence: C:\HPCHEM\1\SEQUENCE\DEFAULT.SEQ

Operator: Ilora Basu

Sequence preparation date: 15 Jun 92 09:14 AM

Data File Subdirectory: ON94CH

Part of methods to run: full method

On a barcode mismatch: inject anyway

Comment:
was spiked with 8 ng of 30 and 6 ng of 204

Sequence Table

Inj.	Seq. Line	Cal. Line	Method Name	From Vial	To Vial	Inj/ Vial
FRONT						
	1			1	16	1
REAR						
	1					

Sample Table

Vial Num.	Sample Name	Sample Amount	Multiplier	ISTD Amount
1	hexane blank			
2	pcbcalst950427			
3	pcbperfst950427			
4	LBC 950113,H			
5	MSC 950112,H			
6	EH02C1 941024,H			
7	EH02C2 941024,H			
8	EH01C 941105,H			
9	SH01C 941024,H			
10	SH02C 941025B,H			
11	SH01C 941105,H			
12	TH01C 941024,H			
13	TH02C 941024,H			
14	TH01C 941105,H			
15	PCBCALST950427			
16	Hexane blank			

Chart 1

4.0 HP 3365 Chemstation Initialization and Baseline Correction

4.1 Copy the .d files from Peak 96 on to the floppy disks.
Copy the .d files to HP 3365 ChemStation.

4.2 Load IBPCBN.MTH or IBPEST2.MTH

4.3 Loading Chromatogram

Files → Load Data File

Sub directory → Sample Vial Number

Chromatogram will appear on screen.

4.4 Baseline Correction

4.4.1 By Integration Event

Go to "command line" and type in Clrevents.
Correct starting parameters and then make baseline corrections by using Baseline Now, and Area Sum.

Starting Parameters

Integration → Integration Events

Initial Area Reject	10	Initial
Initial Peak Width	0.04	Initial
Shoulder Detection	OFF	Initial
Initial Threshold	-4	Initial
Integrator On	0.0	
Negative peak on	0.0	

4.4.2 Integrate the chromatogram

Change the scale axis for close view of each peak.

4.4.3 Baseline Now:
This command is used to get a straight base line

Integration → Possible Events → Baseline Now

Click cursor where you want the baseline.

Integration | Integrate

If do not like changes go to and delete. Integration Events

4.4.4 Area Sum and Negative Peak:

This command is used to split a peak.

Integration | Possible Events | Neg. Peak

Turn negetive peak on and off in the area to split the peak

Integration | Possible Events | Area Sum

Move the cursor and click on the area to split.

Notice: Area Sum On\Off does not work with Neg. Peak On.

5.0 Pesticide Data Reduction in 50% Fraction

5.1 Standard

5.1.1 Integration and Peak Identification

Call standard chromatogram and correct baseline and integrate

Compounds	GC Retention time min.(approx.)	concentration ng/mL
α-HCH	35	20
γ-HCH	40	20
Cong 65(ISTD)	59	20

Compounds	GC Retention time min.(approx.)	concentration ng/mL
γ-Chlordane	70	20
cong 155(ISTD)	72	20
α-Chlordane	73	20
t-Nonachlor	74	20
Dieldrin	77	20
DDD	86	20
DDT	93	20
Dibutylchlorendate	110	10

5.1.2 Printing Report and Saving Text File

[Graphics] [Annotate]

Type in the sample name and add it on the chromatogram.

Check Calibration Settings under Reports.

Mixed Pest.	Standard.
Ref. Window	1
Nonref. Window	1
Units of Amount	ng
Sample ISTD	20
Fit: Linear	Origin: Force

Check report on screen before printing

[Reports] [Specify Report]

X File (X Auto)	X Area
X Screen	X ISTD (if calib. is replaced).
X Report	X Percent (if new calib is made).

If the report looks alright, print the report.

[Reports] [Print Report]

To print report on paper go back to Specify Report and add Printer and Chromatogram to the options selected.

Print integration events and calibration settings

File | Return to Top

Method | File | Integration Events

5.1.3 Preparing New Calibration

Set up initial calibration table by identifying the standard peaks in the area percent report.

Reports | Prp Calib/Recalib. | New Table

Manually type in the amount and name of each analyte with the retention times from the area percent printout.

Highlight "yes" in Reference ISTD peak boxes for Congener 65

OK

Do you want to delete all the peaks with the amount of zero? Yes

Save to method? Yes

You will get a printout for calibration table.

5.1.4 Replacing Previous Calibration

For subsequent standard runs with analyte peaks all correctly identified in ISTD report the calibration table is replaced.

Reports | Prep Calib/Recalib.

Recalibrate | Replace | OK

Save to method? Yes

Will get a printout of calibration table.

If the GC column has been clipped or running conditions have been changed the analyte peaks shift so much that they are not found in the internal standard report and then a new calibration table will have to be created.

5.1.5 Saving Calibration on Data File

File	Command Line

Type in: SAVETBL C:\HPCHEM\1\DATA\SUBDIR.\0**R0101.CAL

5.1.6. Saving Event on Data File

File	Command Line

Type in: SAVE C:\HPCHEM\1\DATA\SUBDIR.\0**R0101.EVT

5.2 Samples, 50% Fraction

5.2.1 Integration - See Section 4.4.

5.2.2 Printing Report and Saving Text File.
See previous Section 5.1.2 with the following exceptions:

Note: Sometimes it is necessary to increase the window more than 0.25% to find internal standard. If it goes more than 0.5%, rerun the sample in GC.

5.2.3 Saving Events - See Section 5.1.6

5.3 Copying .Txt Files

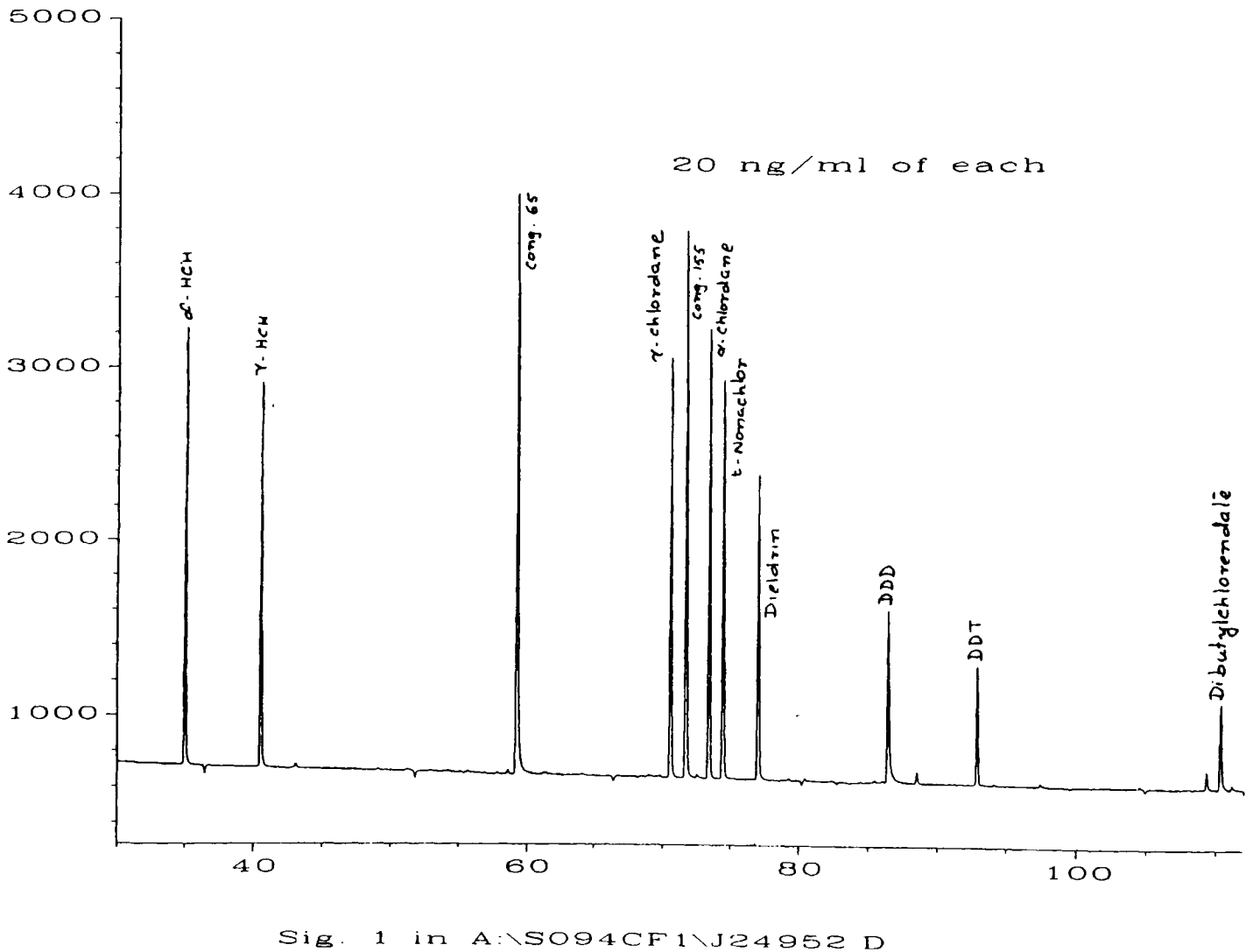
After one set of data is reduced write comments in the text files.

Program manager	Accessories	Note pad

Click on C, HPCHEM 1, data, subdirectory and .txt files.

Call .txt file one by one and write down comments about spike, dilution, reinjection etc.

Save .txt files after writing comments.



Chromatogram 1. Pesticide Calibration Standard

09 Aug 95 08:33 AM
Method: C:\HPCHEM\1\METHODS\IBPEST2.MTH

Calibration Table

Pk#	RT	Lvl	ng	Amt/Area	Ref Istd	I#	Name
1	34.931	1	20.0	1.2744e-003		1	A-HCH
2	40.469	1	20.0	1.3924e-003		1	G-HCH
3	59.180	1	20.0	7.8613e-004	Ref ISTD	1	CONG 65
4	70.521	1	20.0	1.0422e-003		1	G-CHLORDANE
5	71.658	1	20.0	8.3836e-004		1	CONG 155
6	73.377	1	20.0	9.9121e-004		1	A-CHLORDANE
7	74.405	1	20.0	1.0712e-003		1	T-NONA
8	76.972	1	20.0	1.4431e-003		1	DIELDRIN
9	86.359	1	20.0	2.4822e-003		1	DDD
10	92.832	1	20.0	3.8777e-003		1	DDT
11	110.300	1	10.0	2.2105e-003		1	DBC

Calibration Settings

Title:

Reference window:	0.500 %
Non-reference window:	0.500 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

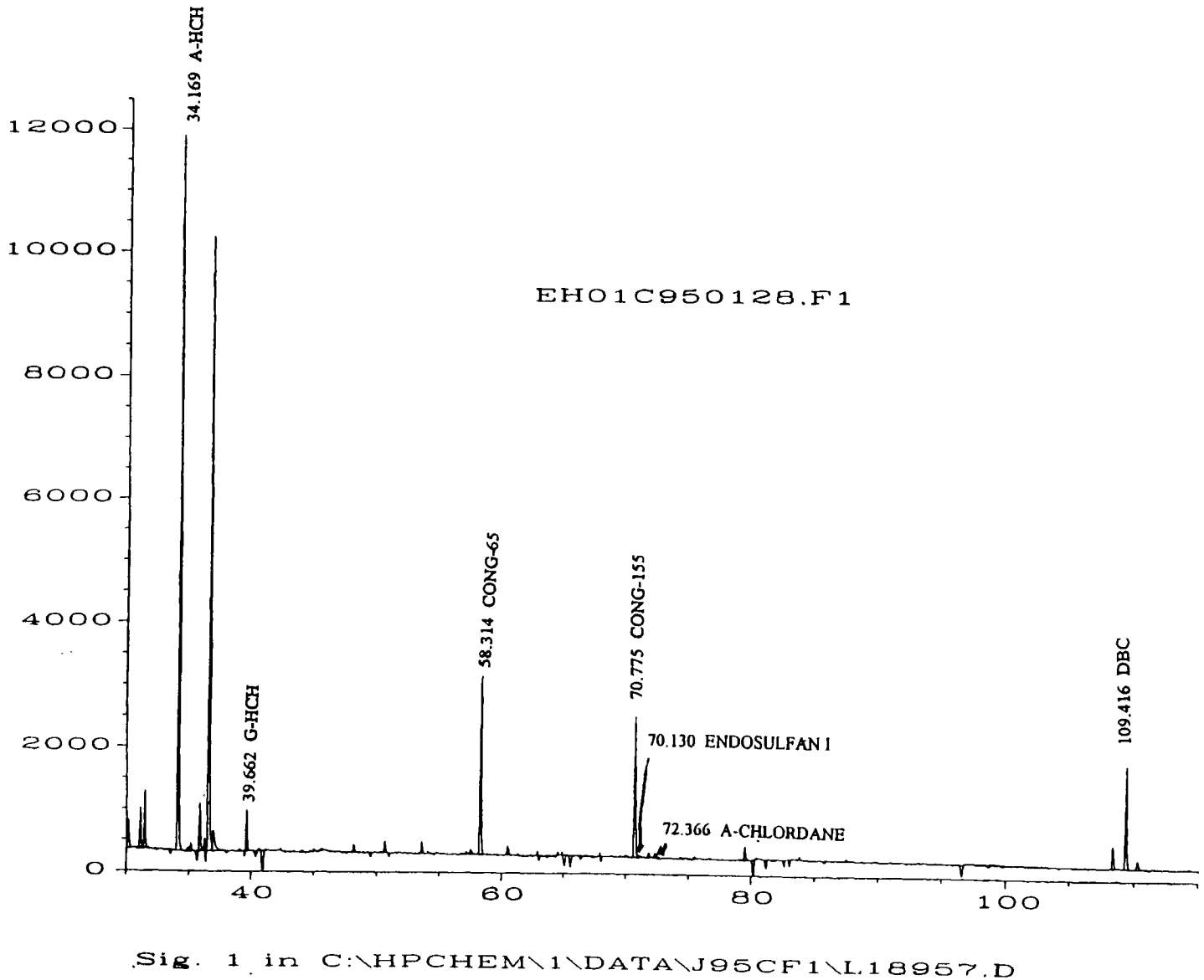
Sample ISTD Information

I#	Amount
1	20.0

Multilevel Information

Fit: Linear
Origin: Force

Chart 2



Chromatogram 2. Pesticides in Vapor Sample

Internal Standard Report

Data File Name	: C:\HPCHEM\1\DATA\J95CF1\L18957.D		
Operator	:	Page Number	: 1
Instrument	: HP5890A	Vial Number	: 0
Sample Name	:	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: JUL 19, 1995 02:43:09	Instrument Method	:
Report Created on	: 09 Aug 95 12:33 PM	Analysis Method	: IBPEST2.MTH
Last Recalib on	: 09 AUG 95 10:20 AM	Sample Amount	: 0
Multiplier	: 1	ISTD Amount	: 20

Sig. 1 in C:\HPCHEM\1\DATA\J95CF1\L18957.D

Ret Time	Area	Type	Width	Ref#	ng	Name
34.169	70370	PV	0.093	1	71.797	A-HCH
39.662	3866	PV	0.096	1	4.038	G-HCH
58.314	22586	VV	0.123	1	20.759	CONG 65
69.780	142	VV -	0.000	1	0.142	G-CHLORDANE
70.775	19005	BV	0.132	1-IR	20.000	CONG 155
72.366	545	BVA	0.109	1	0.549	A-CHLORDANE
73.506	* not found *			1		T-NONACHLOR
76.043	* not found *			1		DIELDRIN
85.473	* not found *			1		DDD
91.939	* not found *			1		DDT
109.416	13402	PV	0.128	1	25.822	DBC

Time Reference Peak	Expected RT	Actual RT	Difference
5	70.785	70.775	-0.0%

Not all calibrated peaks were found

Chart 3

09 Aug 95 12:33 PM
Method: C:\HPCHEM\1\METHODS\IBPEST2.MTH

Integration Events

Events:	Value:	Time:
Initial Area Reject	50	INITIAL
Initial Peak Width	0.040	INITIAL
Shoulder Detection	OFF	INITIAL
Initial Threshold	-4	INITIAL
Baseline Now		0.000
Negative Peak ON		0.000
Baseline Now		33.943
Baseline Now		62.680
Negative Peak OFF		72.300
Area Sum ON		72.400
Area Sum OFF		72.722
Negative Peak ON		72.730
Baseline Now		81.033
Baseline Now		82.107
Baseline Now		87.837
Baseline Now		88.403
Baseline Now		89.180

Calibration Settings

Title:

Reference window:	0.500 %
Non-reference window:	0.500 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

Sample ISTD Information

I#	Amount
1	20.0

Multilevel Information

Fit: Linear
Origin: Force

Chart 4

6.0 PCB and Pesticide Data Reduction in Hexane Fraction

6.1 Mullin 94 Standard

6.1.1 Integration and Peak Identification

Load standard chromatogram and correct baseline according to Section 4.2 and 4.3. Integrate and divide the chromatogram in five to six sections. Identify PCBs from Mullin's 94 chromatogram and pesticides (HCB and DDE) from Mixed Pesticide Standard.

6.1.2 Printing Report and Saving Text Files

Graphics | Annotate

Click in text box and type in sample name. Click on | Add |, and then on chromatogram where you want sample name to be placed.

Check Calibration Settings under Reports.

Ref. Window	1.0
Nonref. Window	1.0
Units of Amount	ng
Sample ISTD #1	8
Sample ISTD #2	6

Fit: Linear Origin: Force

Check report on screen before printing

Reports | Specify Reports

X File (X Auto)	X Area
X Screen	X ISTD (if calib. is replaced).
X Report	X Percent (if new calib is made).

Report | Print Reports

To print report on paper go back to Specify Report and add Printer and Chromatogram to the options selected.

Print integration events and calibration settings:

File | Return to Top

Method | Print | Integration Events

Calibration Setting

6.1.3 Preparing New Calibration

Set up initial calibration table by identifying congener peaks from Mullin's chromatogram in the area percent report.

Reports | Prep Calib/Recalib

New Table | OK

Manually type in and enter the amount and name of each analyte with the retention times from the area percent printout.

Highlight "yes" in Reference ISTD peak boxes for Congener 30 and 204.

Ref.ISTD#1 will be used up to cong.110.
Ref.ISTD#2 will be used from cong.82.

| OK |

Do you want to delete all the peaks with the amount of zero?

| Yes |

Save to method?

| Yes |

6.1.4 Replacing Previous Calibration

For subsequent standard runs with analyte peaks all correctly identified in ISTD report the calibration table is replaced.

Reports | Prep Calib/Recalib

Recalibration | Replace | OK

Save to method?

| Yes |

Will get a printout of calibration table.

If the GC column has been clipped or running conditions have been changed, the retention times of analyte peaks shift and they are not identified in the ISTD report. A new calibration table will have to be created. This can be done either by following Section 6.1.3 or by inserting the new retention time in old calibration table and creating a temporary calibration table. Get ISTD report of the new standard with temporary calibration table and replace the calibration according to Section 6.1.4.

6.1.5 Saving Calibration on Data File

File	Command Line

Type in SAVETBL C:\HPCHEM\1\DATA\SUBDIRECTORY\0**R0101.CAL

6.1.6 Saving Event

File	Command Line

Type in: SAVE C:\HPCHEM\1\DATA\SUBDIR.\0**R0101.EVT Enter with keyboard.

6.2 Samples, Hexane Fraction

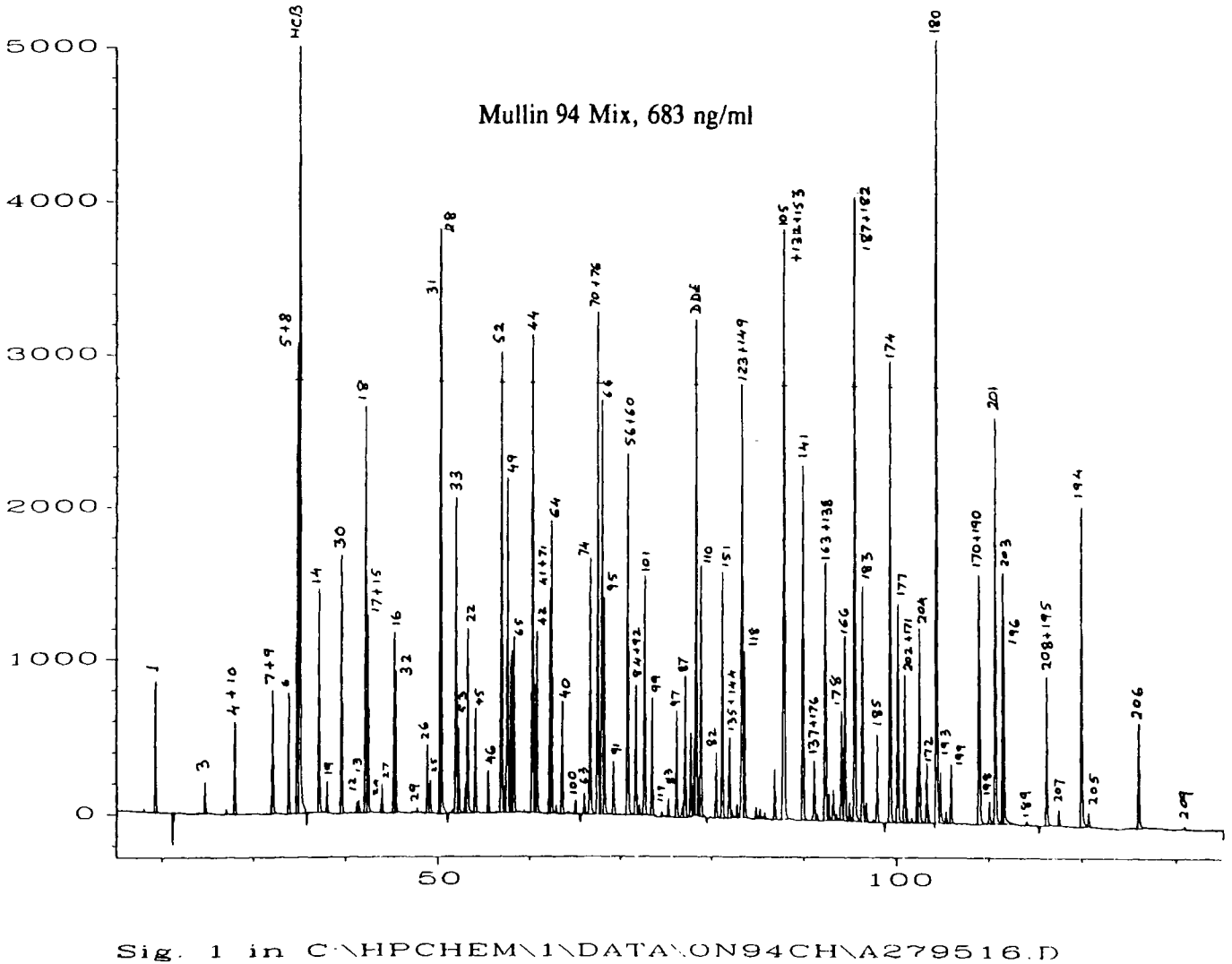
6.2.1 Integration - See previous Section 4.4

6.2.2 Printing Report and Saving Text File

See previous Section 5.1.2 with the following exceptions:
Calibration Settings Ref. and Nonref. Windows should be changed to 0.25%.

Note: Sometimes it is necessary to increase the window more than 0.25% to find internal standard. If it goes more than 0.5% rerun the sample in GC.

6.2.3 Saving Events - See previous Section 5.1.6



Chromatogram 3. PCB Calibration Standard 950427

11 Jul 95 02:52 PM
Method: C:\HPCHEM\1\METHODS\IBPCBN.MTH

Calibration Table

Pk#	RT	Lvl	ng	Amt/Area	Ref Istd	I#	Name
1	19.187	1	48.0	1.0132e-002		1	1
2	24.672	1	28.0	2.2807e-002		1	3
3	27.943	1	13.6	3.6808e-003		1	4+10
4	32.039	1	4.8	9.3258e-004		1	7+9
5	33.775	1	7.6	1.6816e-003		1	6
6	34.701	1	56.0	3.0596e-003		1	5+8
7	34.958	1	20.0	3.6399e-004		1	HCB
8	37.077	1	20.0	2.2436e-003		1	14
9	37.944	1	1.12	9.3145e-004		1	19
10	39.526	1	8.0	8.4154e-004	Ref ISTD	1	30
11	41.222	1	0.68	1.734e-003		1	12
12	41.420	1	0.39	8.1106e-004		1	13
13	42.141	1	14.8	8.6819e-004		1	18
14	42.378	1	14.8	1.7505e-003		1	17+15
15	43.782	1	0.52	2.2385e-003		1	24
16	43.928	1	0.52	4.5844e-004		1	27
17	45.198	1	8.0	1.2027e-003		1	16
18	45.372	1	7.6	1.3979e-003		1	32
19	47.724	1	0.21	1.0343e-003		1	29
20	48.843	1	2.8	1.1252e-003		1	26
21	49.151	1	1.3	1.0363e-003		1	25
22	50.195	1	18.8	1.0569e-003		1	31
23	50.297	1	18.8	1.107e-003		1	28
24	51.959	1	13.2	1.0978e-003		1	33
25	52.245	1	2.6	7.7852e-004		1	53
26	53.008	1	0.72	7.3206e-004		1	51
27	53.246	1	11.6	1.682e-003		1	22
28	54.093	1	3.56	9.1343e-004		1	45
29	55.492	1	1.6	1.0223e-003		1	46
30	56.914	1	18.0	9.9944e-004		1	52
31	57.246	1	1.1	1.0759e-003		1	43
32	57.567	1	9.2	7.1717e-004		1	49
33	57.945	1	4.0	8.0619e-004		1	47
34	58.045	1	4.0	6.8621e-004		1	48
35	58.295	1	5.0	7.5344e-004		1	65
36	60.304	1	17.2	9.4466e-004		1	44
37	60.650	1	4.8	1.6582e-003		1	37
38	60.774	1	5.6	7.105e-004		1	42
39	62.234	1	9.2	1.1125e-003		1	41+71
40	62.395	1	7.2	6.3997e-004		1	64
41	63.646	1	3.76	9.4652e-004		1	40
42	65.123	1	0.44	8.0299e-004		1	100
43	66.067	1	0.84	1.127e-003		1	63
44	66.707	1	7.6	7.905e-004		1	74
45	67.504	1	13.6	7.1319e-004		1	70+76
46	67.988	1	20.8	1.3476e-003		1	66
47	68.205	1	8.0	9.4067e-004		1	95
48	69.243	1	2.04	9.5141e-004		1	91
49	70.795	1	14.0	9.7113e-004		1	56+60
50	71.665	1	7.2	1.4746e-003		1	84+92
51	72.076	1	0.4	8.7453e-004		1	89
52	72.627	1	7.2	7.9384e-004		1	101
53	73.429	1	2.96	6.6508e-004		1	99
54	74.518	1	0.112	2.6071e-004		1	119
55	75.234	1	0.6	6.7352e-004		1	83
56	76.129	1	2.24	5.4267e-004		1	97

Chart 5

11 Jul 95 02:52 PM
Method: C:\HPCHEM\1\METHODS\IBPCBN.MTH

57	76.768	1	0.64	1.1439e-003		1	81
58	77.055	1	4.0	7.372e-004		1	87
59	77.665	1	2.8	9.0102e-004		1	85
60	78.061	1	3.0	1.1743e-003		1	136
61	78.260	1	20.0	1.1061e-003		1	DDE
62	78.596	1	0.92	0.00125		1	77
63	78.788	1	7.6	7.6996e-004		1	110
64	80.467	1	1.8	7.0807e-004		2	82
65	81.135	1	6.8	7.4529e-004		2	151
66	81.903	1	3.56	1.0635e-003		2	135+144
67	82.187	1	0.22	5.1262e-004		2	124+147
68	82.730	1	0.52	7.6193e-004		2	107
69	83.249	1	11.2	6.8246e-004		2	123+149
70	83.524	1	4.8	6.9706e-004		2	118
71	84.783	1	0.29	3.3027e-004		2	134
72	85.727	1	0.56	1.5268e-003		2	114+131
73	86.796	1	1.56	9.0246e-004		2	146
74	87.813	1	17.2	6.8456e-004		2	105+132+153
75	89.856	1	6.8	5.0933e-004		2	141
76	91.096	1	1.04	4.7746e-004		2	137+176
77	91.398	1	0.3	1.3091e-003		2	130
78	92.301	1	10.8	9.6416e-004		2	163+138
79	92.663	1	1.0	9.9319e-004		2	158
80	93.503	1	0.052	2.8924e-004		2	129
81	94.033	1	4.4	1.163e-003		2	178
82	94.411	1	5.0	7.4955e-004		2	166
83	94.927	1	0.8	1.0108e-003		2	175
84	95.471	1	14.4	6.2431e-004		2	187+182
85	96.296	1	6.8	8.1456e-004		2	183
86	96.711	1	0.4	4.7325e-004		2	128
87	97.464	1	0.196	1.9914e-003		2	167
88	97.877	1	1.9	6.255e-004		2	185
89	99.284	1	12.8	7.7724e-004		2	174
90	100.118	1	6.8	8.8769e-004		2	177
91	100.850	1	3.16	6.067e-004		2	202+171
92	101.080	1	0.26	5.3533e-004		2	156
93	101.616	1	0.152	1.4242e-003		2	173
94	102.194	1	1.56	8.4592e-004		2	157+200
95	102.445	1	6.0	8.7429e-004	Ref ISTD	2	204
96	103.240	1	2.24	1.1557e-003		2	172
97	103.432	1	0.44	1.2268e-003		2	197
98	104.309	1	24.4	7.6752e-004		2	180
99	104.737	1	1.68	8.6603e-004		2	193
100	105.361	1	0.48	1.4826e-003		2	191
101	105.905	1	1.72	8.9833e-004		2	199
102	108.970	1	6.8	6.393e-004		2	170+190
103	110.083	1	0.48	5.7375e-004		2	198
104	110.714	1	16.8	1.1407e-003		2	201
105	111.510	1	8.6	9.3343e-004		2	203
106	111.732	1	8.6	1.5565e-003		2	196
107	114.055	1	0.16	1.5706e-003		2	189
108	116.223	1	3.2	6.1544e-004		2	208+195
109	117.547	1	0.37	6.9444e-004		2	207
110	120.001	1	7.2	6.306e-004		2	194
111	120.770	1	0.44	7.3461e-004		2	205
112	126.056	1	2.72	7.3361e-004		2	206
113	130.915	1	0.048	4.7233e-004		2	209

Chart 5 (Cont'd)

11 Jul 95 02:52 PM
Method: C:\HPCHEM\1\METHODS\IBPCBN.MTH

Calibration Settings

Title:

Reference window:	0.500 %
Non-reference window:	0.500 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

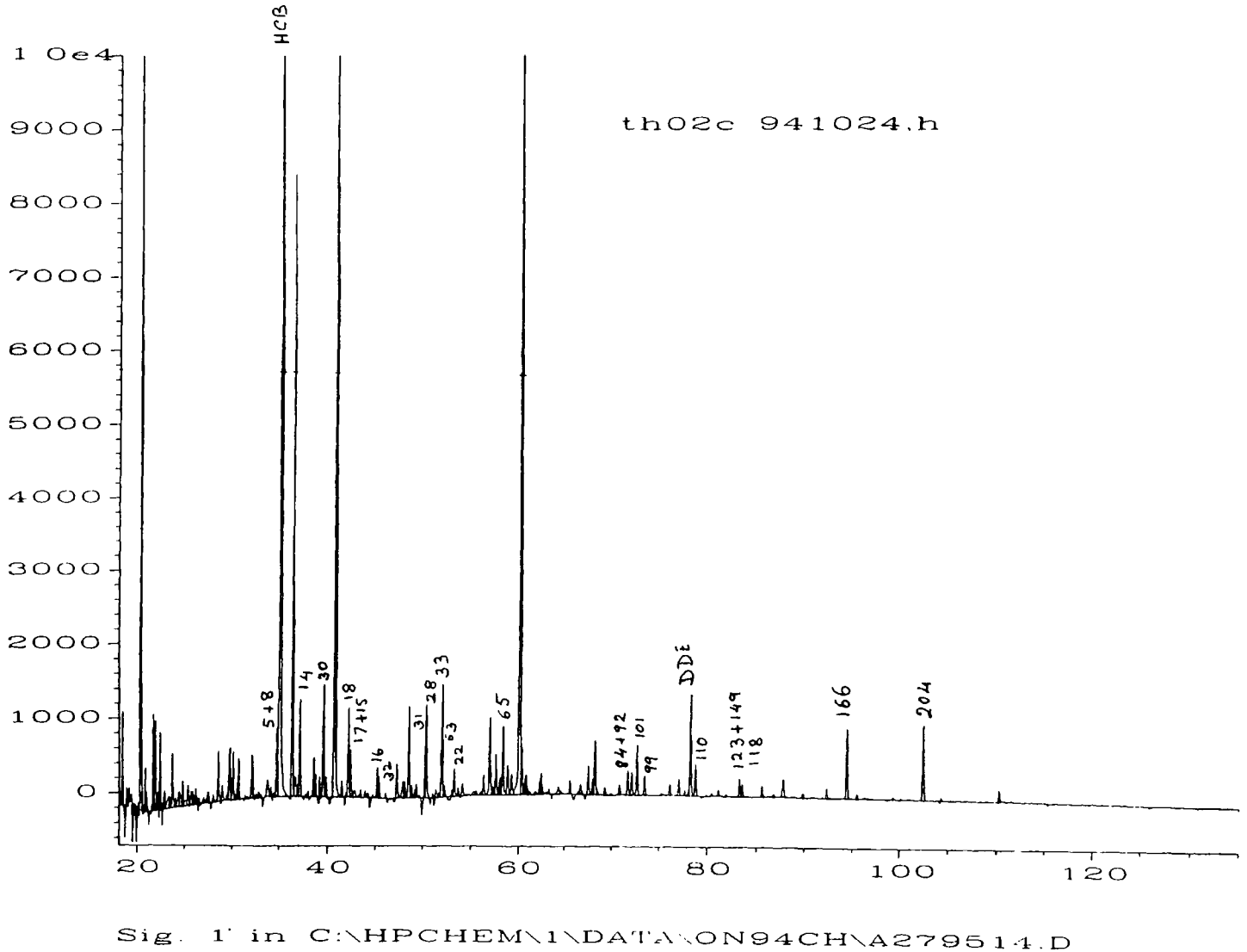
$ample ISTD Information

I#	Amount
1	8.0
2	6.0

Multilevel Information

Fit: Linear
Origin: Force

Chart 5 (Cont'd)



Chromatogram 4. PCBs in Vapor Sample

```
==============================================================================
                          Internal Standard Report
==============================================================================

Data File Name    : C:\HPCHEM\1\DATA\ON94CH\A279514.D
Operator          :                                  Page Number       : 1
Instrument        : HP5890A                          Vial Number       : 0
Sample Name       :                                  Injection Number  :
Run Time Bar Code:                                   Sequence Line     :
Acquired on       : APR 28, 1995  17:32:09           Instrument Method:
Report Created on: 22 Jun 95  01:39 PM               Analysis Method   : IBPCBN.MTH
Last Recalib on   : 21 JUN 95 11:36 AM               Sample Amount     : 0
Multiplier        : 1                                ISTD Amount       : 8

Sig. 1 in C:\HPCHEM\1\DATA\ON94CH\A279514.D
Ret Time     Area      Type Width Ref#  ng                    Name
|-------|------------|----|-----|-----|--------|----------------------------|
  19.225          50 VBA  0.039  1        0.496 1
  24.692 * not found *           1              3
  27.971         868 PV   0.110  1        3.027 4+10
  32.111        4297 PVA  0.110  1        3.839 7+9
  33.752        2840 PP   0.168  1        4.459 6
  34.697        6752 PV   0.113  1       19.228 5+8
  34.958      235283 VV   0.116  1       80.586 HCB
  37.071        9754 VP   0.116  1       20.720 14
  37.950         398 PP   0.095  1        0.327 19
  39.522       10418 PP   0.109  1-IR     8.000 30
  41.195         250 VP   0.110  1        0.393 12
  41.464        1313 PP   0.095  1        0.835 13
  42.141        9515 VV   0.123  1        7.438 18
  42.378        5080 VV   0.122  1        8.112 17+15
  43.807 * not found *           1              24
  43.922         662 PV   0.104  1        0.272 27
  45.191        3020 PV   0.115  1        3.319 16
  45.367        2080 VV   0.108  1        2.622 32
  47.763 * not found *           1              29
  48.833        1350 VV   0.113  1        1.390 26
  49.142         890 PV   0.119  1        0.804 25
  50.160        3441 PV + 0.000  1        3.219 31
  50.304       11483 VP   0.137  1       11.717 28
  51.964       12077 VV   0.125  1       11.951 33
  52.238        1138 VP   0.113  1        0.779 53
  53.020         710 PV   0.108  1        0.446 51
  53.236        3123 VV   0.131  1        4.763 22
  54.087        1466 PV   0.142  1        1.178 45
  55.485         625 VV   0.138  1        0.555 46
  56.910        9430 VV   0.137  1        8.396 52
  57.204         908 VV   0.110  1        0.866 43
  57.566        4527 VV   0.122  1        2.889 49
  57.941        1458 VV   0.110  1        1.044 47
  58.058        1937 VV   0.115  1        1.181 48
  58.291        7669 VV   0.125  1        5.113 65
  60.300        4996 VV   0.118  1        4.236 44
  60.588        1785 VV + 0.000  1        2.658 37
  60.756        2317 VVA  0.133  1        1.532 42
  62.234        1399 PV   0.116  1        1.401 41+71
  62.393        2446 VVA  0.128  1        1.405 64
  63.560         306 PP   0.084  1        0.252 40
  65.155 * not found *           1              100
  65.997        1094 PP   0.178  1        1.692 63
  66.702        1344 VV   0.135  1        0.977 74
  67.507        3229 PVA  0.130  1        2.106 70+76
```

Chart 6

67.986	1823	VV	0.127	1	2.236	66
68.207	6262	VV	0.132	1	5.290	95
69.243	1042	PV	0.137	1	0.924	91
70.796	1228	PV	0.136	1	1.131	56+60
71.659	2814	VV	0.134	1	3.904	84+92
72.058	2434	VVA	0.122	1	2.361	89
72.621	5809	PV	0.028	1	4.210	101
73.438	2358	BV	0.138	1	1.450	99
74.559	74	BV	0.087	1	0.0427	119
75.229	205	PV	0.088	1	0.205	83
76.122	1239	PV	0.126	1	0.708	97
76.759	111	BV	0.097	1	0.191	81
77.050	1895	PV	0.129	1	1.371	87
77.661	730	BV	0.140	1	0.658	85
78.056	507	VV	0.095	1	0.572	136
78.257	10906	VV	0.125	1	11.329	DDE
78.605	* not found *			1		77
78.786	3549	VV	0.131	1	2.766	110
80.467	300	PB	0.116	2	0.224	82
81.131	679	PV	0.107	2	0.420	151
81.906	252	PV	0.080	2	0.219	135+144
82.216	* not found *			2		124+147
82.713	94	PV	0.088	2	0.0876	107
83.246	2093	PV	0.128	2	1.156	123+149
83.522	1475	VV	0.133	2	0.924	118
84.792	152	PV	0.111	2	0.0981	134
85.586	1290	BV	0.125	2	2.220	114+131
86.783	480	VV	0.150	2	0.333	146
87.809	2728	BB	0.163	2	1.495	105+132+153
89.850	723	BV	0.127	2	0.303	141
91.130	* not found *			2		137+176
91.387	109	BB	0.112	2	0.117	130
92.311	1195	BV	0.131	2	0.944	163+138
92.655	157	PB	0.103	2	0.117	158
93.488	72	PV	0.116	2	0.0122	129
94.054	111	BV	0.097	2	0.108	178
94.404	7584	PV	0.127	2	4.854	166
94.960	* not found *			2		175
95.468	562	BV	0.125	2	0.294	187+182
96.299	202	PV	0.128	2	0.141	183
96.698	170	PV	0.115	2	0.0841	128
97.488	* not found *			2		167
97.911	* not found *			2		185
99.281	305	PB	0.138	2	0.202	174
100.114	123	BV	0.094	2	0.0918	177
100.853	112	PV	0.104	2	0.0569	202+171
101.108	* not found *			2		156
101.644	* not found *			2		173
102.226	* not found *			2		157+200
102.442	8529	BV	0.133	2-IR	6.000	204
103.270	* not found *			2		172
103.458	* not found *			2		197
104.311	361	PB	0.132	2	0.242	180
104.764	* not found *			2		193
105.299	106	PB	0.112	2	0.0882	191
105.932	* not found *			2		199
108.986	225	PV	0.167	2	0.139	170+190
110.270	1318	PBA	0.132	2	0.924	198
110.709	105	PB	0.100	2	0.107	201
111.539	* not found *			2		203
111.712	* not found *			2		196
114.085	* not found *			2		189

Chart 6 (Cont'd)

116.256	* not found *		2		208+195
117.576	* not found *		2		207
120.031	* not found *		2		194
120.791	* not found *		2		205
125.982	306	BV	2	0.215	206
130.854	92	BV	2	0.0140	209

Time Reference Peak	Expected RT	Actual RT	Difference
10	39.556	39.522	-0.1%
95	102.477	102.442	-0.0%

Not all calibrated peaks were found

==========

Chart 6 (Cont'd)

22 Jun 95 01:41 PM
Method: C:\HPCHEM\1\METHODS\IBPCBN.MTH

Integration Events

Events:	Value:	Time:
Initial Area Reject	50	INITIAL
Initial Peak Width	0.040	INITIAL
Shoulder Detection	OFF	INITIAL
Initial Threshold	-4	INITIAL
Negative Peak ON		0.000
Baseline Now		18.230
Baseline Now		18.483
Baseline Now		19.250
Baseline Now		20.610
Baseline Now		31.027
Negative Peak OFF		31.886
Area Sum ON		32.158
Area Sum OFF		32.279
Negative Peak ON		32.302
Baseline Now		33.447
Baseline Now		43.747
Baseline Now		45.733
Negative Peak OFF		50.081
Area Sum ON		50.092
Area Sum OFF		50.224
Negative Peak ON		50.558
Negative Peak OFF		60.026
Area Sum ON		60.479
Area Sum OFF		60.687
Area Sum ON		60.885
Area Sum OFF		61.037
Negative Peak ON		61.386
Negative Peak OFF		62.010
Area Sum ON		62.492
Area Sum OFF		62.643
Negative Peak ON		62.803
Negative Peak OFF		67.328
Area Sum ON		67.663
Area Sum OFF		67.850
Negative Peak ON		68.652
Negative Peak OFF		71.281
Area Sum ON		71.318
Area Sum OFF		71.523
Area Sum ON		72.138
Area Sum OFF		72.325
Negative Peak ON		73.705
Baseline Now		92.057
Baseline Now		92.080
Baseline Now		110.463
Baseline Now		111.157

Calibration Settings

Title:

Chart 7

```
22 Jun 95  01:41 PM
Method: C:\HPCHEM\1\METHODS\IBPCBN.MTH


Reference window:                    0.500 %
Non-reference window:                0.500 %
Units of amount:                        ng
Multiplier:                            1.0
RF uncal peaks:                        0.0
Sample Amount:                         0.0

                          Sample ISTD Information

I#        Amount
 1            8.0
 2            6.0

                           Multilevel Information

Fit:     Linear
Origin:  Force
```

Chart 7 (Cont'd)

7.0 Creating Excel File for PCB Analysis

7.1 Call *.txt files

7.2 Select "fixed width" and click on "next"

7.3 Click on line and "finish"

7.4 Delete a1..a5

7.5 Insert six rows

7.6 Delete Column C

7.7 Change column width b to 10

7.8 Edit and replace *not found* to 0

7.9 Copy 0's to C

7.10 Type at A1

Subdirectory
Sample name
Vial id
Date

7.11 Delete rows A124 to I131

7.12 Delete negative peaks amount

7.13 At 124 type "total pcbs"

7.14 At C124 put formula =((sum(c9..c121))-(c15+c16+c43+c69+c90)

7.15 At A126 type % recovery of 14 = c16/20*100

7.16 At A127 type % recovery of 65 = c43/5*100

7.17 At 128 type % recovery of 166 = c90/5*100

7.18 At 130 type ratio of 30:204 = B18/B103

7.19 At A132 type HCB = c15

7.20 At A133 type DDE = c69

Write comments

Save as .xls files

subdirectory		on94ch			
sample name		th02c941024,h			
vial id		14			
date		22-Jun-95			
ret time	Area	f# ng	Name		
19.225	50	0.496	1		
24.692	0	0	3		
27.971	868	3.027	4+10		
32.111	4297	3.839	7+9		
33.752	2840	4.459	6		
34.697	6752	19.228	5+8		
34.958	235283	80.586	HCB		
37.071	9754	20.72	14		
37.95	398	0.327	19		
39.522	10418	-IR 8.000	30		
41.195	250	0.393	12		
41.464	1313	0.835	13		
42.141	9515	7.438	18		
42.378	5080	8.112	17+15		
43.807	0	0	24		
43.922	662	0.272	27		
45.191	3020	3.319	16		
45.367	2080	2.622	32		
47.763	0	0	29		
48.833	1350	1.39	26		
49.142	890	0.804	25		
50.16	3441	3.219	31		
50.304	11483	11.717	28		
51.964	12077	11.951	33		
52.238	1138	0.779	53		
53.02	710	0.446	51		
53.236	3123	4.763	22		
54.087	1466	1.178	45		
55.485	625	0.555	46		
56.91	9430	8.396	52		
57.204	908	0.866	43		
57.566	4527	2.889	49		
57.941	1458	1.044	47		
58.058	1937	1.181	48		
58.291	7669	5.113	65		
60.3	4996	4.236	44		
60.588	1785	2.658	37		
60.756	2317	1.532	42		
62.234	1399	1.401	41+71		
62.393	2446	1.405	64		
63.56	0	0	40		
65.155	0	0	100		
65.997	0	0	63		

Chart 8

66.702	1344	0.977	74		
67.507	3229	2.106	70+76		
67.986	1823	2.236	66		
68.207	6262	5.29	95		
69.243	1042	0.924	91		
70.796	1228	1.131	56+60		
71.659	2814	3.904	84+92		
72.058	2434	2.361	89		
72.621	5809	4.21	101		
73.438	2358	1.45	99		
74.559	74	0.0427	119		
75.229	205	0.205	83		
76.122	1239	0.708	97		
76.759	111	0.191	81		
77.05	1895	1.371	87		
77.661	730	0.658	85		
78.056	507	0.572	136		
78.257	10906	11.329	DDE		
78.605	0	0	77		
78.786	3549	2.766	110		
80.467	300	0.224	82		
81.131	679	0.42	151		
81.906	252	0.219	135+144		
82.216	0	0	124+147		
82.713	94	0.0876	107		
83.246	2093	1.156	123+149		
83.522	1475	0.924	118		
84.792	152	0.0981	134		
85.586	1290	2.22	114+131		
86.783	480	0.333	146		
87.809	2728	1.495	105+132+153		
89.85	723	0.303	141		
91.13	0	0	137+176		
91.387	109	0.117	130		
92.311	1195	0.944	163+138		
92.655	157	0.117	158		
93.488	72	0.0122	129		
94.054	111	0.108	178		
94.404	7584	4.854	166		
94.96	0	0	175		
95.468	562	0.294	187+182		
96.299	202	0.141	183		
96.698	170	0.0841	128		
97.488	0	0	167		
97.911	0	0	185		
99.281	305	0.202	174		
100.114	123	0.0918	177		
100.853	112	0.0569	202+171		
101.108	0	0	156		
101.644	0	0	173		
102.226	0	0	157+200		

Chart 8 (Cont'd)

102.442	8529	IR 6	204		
103.27	0	0	172		
103.458	0	0	197		
. 104.311	361	0.242	180		
104.784	0	0	193		
105.299	106	0.0882	191		
105.932	0	0	199		
106.986	225	0.139	170+190		
110.27	1318	0.924	198		
110.709	105	0.107	201		
111.539	0	0	203		
111.712	0	0	196		
114.085	0	0	189		
116.256	0	0	208+195		
117.576	0	0	207		
120.031	0	0	194		
120.791	0	0	205		
125.982	0	0	206		
130.854	0	0	209		
total PCBs		159.0276			
%rec 14		103.6			
%rec 65		102.26			
%rec 166		97.08			
30:204		1.22148			
hcb		80.586			
dde		11.329			
comments		sample saver worked properly			

Chart 8 (Cont'd)

8.0 Data Storage and Data Retrieval

8.1 Copy the complete files (*.d, *.txt, *.evt,*.cal, and *.xls) on to the floppy disks. Leave one copy on hard disks too.

8.2 Loading Old Chromatogram

Same as Loading chromatogram in Section 4.3

8.3 Loading Old Calibration

Program Manager	File Manager

Click on c:\hpchem\1\subdirectory\0**R0101.cal

File | Copy

To C:\hpchem\1\methods\appropriate method.cal

Replace Calib | Yes

Close File manager and Return to TOP
Click on Method and Load proper method.
Make sure that you got correct calibration table by clicking on

Report | Edit Calibration Table

8.4 Loading Old Integration Event

Program Manager | File Manager

Click on c:\hpchem\1\data\subdirectory\0**R0101.evt

File | Copy

8.5 Data Storage and Data Retrieval

To C:\hpchem\1\methods\appropriate method.evt

Replace Event | Yes

Close File manager and Return to TOP
Click on Method and Load proper method.
Make sure that you got correct integration event by clicking on

Integration | Event

Close File manager and Return to TOP
Click on Method and Load proper method.
Make sure that you got correct calibration table by clicking on

Report	Edit Calibration Table

Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples:

IADN Project

Sample Preparation Procedure

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June 1995

Version 1.0

Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples: IADN Project - Sample Preparation Procedure

1.0 Summary

This guide is written for chemists and technical assistants so that everybody follows the same details in preparation of IADN samples. We process Air (both vapor and particle phase) and Precipitation samples for analysis of PCBs, pesticides and PAHs. A brief description of the 12 sections covered in this volume of SOP follows:

1.1 Section I: Cleaning

This section describes the procedures of soap and water cleaning, muffling, and ultrasonic cleaning of glassware and other tools.

1.2 Section II: Precleaning

This section covers the long procedure of precleaning XAD_2 with different types of solvents. The procedure was originally standardized by Steve Eisenreich and later on it was modified in our laboratory. Besides XAD_2, we have also described the precleaning procedure of glass wool, silica gel, boiling chips, and glass fibre filters.

1.3 Sections III and IV: Extraction

Soxhlet extraction of air vapor, air particle and precipitation samples from XAD_2 cartridges and GFF are described in these two sections. Detailed procedures for concentration by rotary evaporation, solvent exchange and back extraction are documented. We also mention QC samples and spiking samples with recovery standard etc.

1.4 Section V: Silica Column Chromatography

After extraction, the extracts are cleaned up from interfering compounds and fractionated into three different fractions through silica gel deactivated to 4%. First fraction is collected with Hexane which contains all PCBs and pesticides like HCB and DDE. The second fraction which is collected with 50% CH_2Cl_2 in Hexane contains all PAHs and pesticides like α and γ HCHs, Dieldrin, DDD, DDT, γ Chlordane, α Chlordane, and T-Nonachlor. The third fraction is collected in Methanol and contains atrazine.

1.5 Sections VI and VII: Transfer and Nitrogen Blow Down

These two sections describe the procedure for final transfer of prepared samples from flasks to 4 mL amber vials. The samples are then concentrated to desired volume by a slow stream of ultra-pure nitrogen. Final volumes are adjusted depending on types of samples and time of collection to ensure GC chromatograms are not off scale.

1.6 Sections VIII and IX: Spiking and Making Microvials

After proper concentration by Nitrogen blow down, each sample is spiked with known amount of internal standard or quantitation standard. Subsamples are then transferred to autosampler microvials for GC analysis.

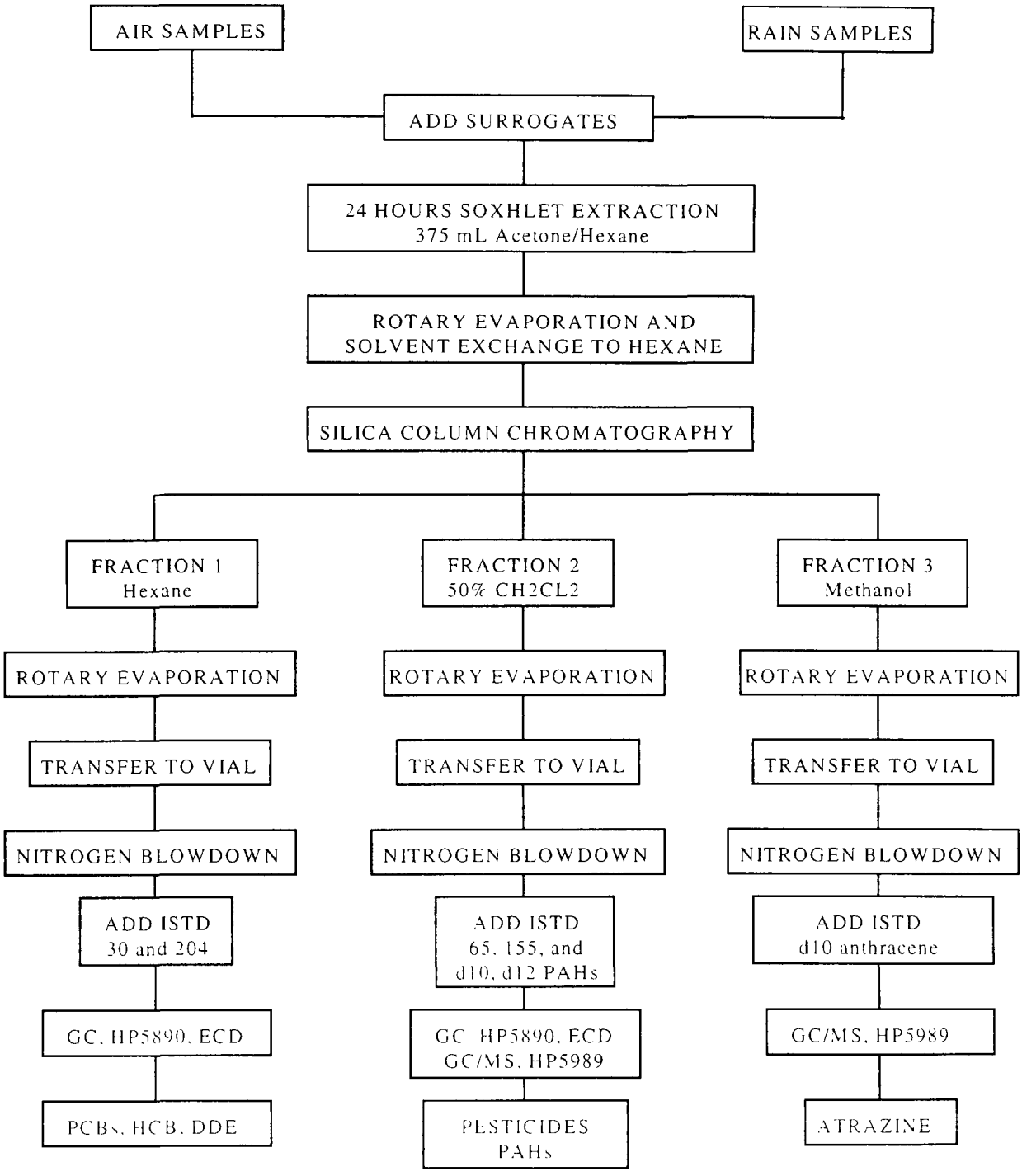
1.7 Section X: Standards

Procedures for preparation of all stock standards, working standards, calibration standards, recovery standards, and spiking internal standards are compiled in this section.

1.8 Section XI: Safety

Some of the safety rules that we follow for day to day laboratory work are mentioned here. Procedure for waste disposal is also included in this section.

2.0 The Flow Chart of Sample Preparation



3.0 Cleaning

3.1 Glassware

3.1.1 Supplies

3.1.1.1 *Glassware:*

Assemble what is to be cleaned

3.1.1.2 *Non-glassware:*

Micro cleaning solution
DI water
Dish washing brush

3.1.1.3 *Equipment:*

Drying oven
Muffle furnace
Acid bath: 50/50 H_2SO_4 and HNO_3

3.1.2 Procedures

1) Wash/Dry
Wash glassware thoroughly with soap and water. Use brush if necessary.
Glassware with bad stains should be rinsed with MeOH or CH_2Cl_2 before using the soap and water procedure. If still not clean, soak in acid bath overnight, then wash thoroughly with soap and water.
Volumetric pipettes used for standards **must** soak in acid bath overnight.
Rinse glassware thoroughly with tap water.
Rinse glassware thoroughly with DI water.
Dry glassware in air.
Cover all open ends with foil.
2) Muffle glassware at 450°C for four hours. If glassware is not clean after muffling at 450°C for four hours, muffle at 500°C for four hours.
3) Allow glassware to cool to 100°C before removing from furnace.
4) Store.

3.1.3 Comment

Always use dull side of foil towards glassware.

3.2 Stainless Steel Tools

3.2.1 Supplies

3.2.1.1 *Glassware:*
-none-

3.2.1.2 *Non-glassware:*

Items to be cleaned: forceps, spatula, scissors, etc.
CH_2Cl_2 in teflon bottle
Cl^- waste bottle

3.2.1.3 *Equipment:*

drying oven

3.2.2 Procedures

1) Wash with soap and water.
2) Rinse well with tap water.
3) Rinse thoroughly with DI water.
4) Dry at room temperature overnight.
5) Wrap each tool separately in foil.
6) Store.

3.2.3 Comment

ALWAYS rinse with CH_2Cl_2 before use.

3.3 Amber Glass Vials and Pasteur Pipettes

3.3.1 Supplies

3.3.1.1 *Glassware:*

400 mL beaker

3.3.1.2 *Non-glassware:*

Foil

3.3.1.3 *Equipment:*

Muffle furnace

3.3.2 Procedure

1) Wrap glass in foil or place in beaker and cover beaker with foil
2) Muffle at 450°C for four hours.
3) Cool to 100°C; remove from oven.
4) Insert teflon liner into vial cap and cap the vial as soon as the vial comes out of the oven.
5) Store in a beaker covered with foil.

3.4 Teflon liners

3.4.1. Supplies

3.4.1.1 *Glassware:*

400 mL beaker

3.4.1.2 *Non-glassware:*

Foil
CH_2Cl_2
Cl^- waste bottles

3.4.1.3 *Equipment:*

Ultrasonicator

3.4.2 Procedures

1) Place teflon liners in glass beaker; cover with CH_2Cl_2.
2) Ultra-sonicate for 15 minutes. Drain CH_2Cl_2.
3) Repeat.
4) Place in 70°C drying oven for two hours.
5) Store in sealed jar.

3.5 Micropipette Tubes, GC Microvials, and Stainless N_2 Blowdown Needles

3.5.1 Supplies

3.5.1.1 *Glassware:*

400 mL or larger beaker

3.5.1.2 *Non-glassware:*

CH_2Cl_2
Cl waste bottle

3.5.1.3 *Equipment:*

Muffle furnace

3.5.2 Procedures

1) Micropipette tubes
Before using, rinse with CH_2Cl_2 and air dry.
2) GC microvials
Place microvials, open end up, in a clean beaker. Cover vials with CH_2Cl_2,

making sure **NO** air bubbles remain in the microvials. Cover loosely with foil.
Sonicate microvials for 10 minutes.
Drain solvent, and repeat twice more. (The microvials should be sonicated a total of three times.)
Drain all solvent and transfer microvials to clean beaker; cover with foil. Muffle at 450°C for four hours. After furnace returns to 100°C (or the next morning) remove vials from furnace.
Store in sealed container.

3) Stainless N_2 blowdown needles
Place needles in a clean beaker and cover with CH_2Cl_2. Cover loosely with foil.
Sonicate needles for 10 minutes.
Drain solvent, and repeat twice more. (The needles should be sonicated a total of three times.)
Drain all solvent and transfer needles to clean beaker. Cover beaker with foil.
Label beaker "CLEAN"; store near the N_2 blowdown unit.

3.6 Teflon Stopcocks and Lids for Sample Jars

3.6.1 Supplies

3.6.1.1 *Glassware:*

-none-

3.6.1.2 *Non-glassware:*

Alconox
DI water
kimwipes

3.6.1.3 *Equipment*

-none-

3.6.2 Procedure

1) Wash stopcocks and lids with Alconox and tap water.
2) Rinse stopcocks and lids with DI water.
3) Air dry on kimwipes.
4) Storage
Store the stopcocks in muffled jar or beaker covered with foil.
Place lids on muffled sample jars or wrap them in foil.

4.0 Precleaning

4.1 Glass Wool

4.1.1 Supplies

4.1.1.1 *Glassware:*

Sample jar and lid
Glasswool

4.1.1.2 *Non-glassware:*

Foil

4.1.1.3 *Equipment:*

Scissors
Muffle furnace

4.1.2 Procedures

1) Cut glass wool into 2" pieces.
2) Put into muffled glass sample jar; cover jar with foil.
3) Muffle at 450°C for four hours.
4) Screw lid on jar (do not remove foil).
5) Store.

4.2 Teflon Boiling Chips

4.2.1 Supplies

4.2.1.1 *Glassware:*

Soxhlet extractor: extra large (71/60 and 29/42 joints)
large (55/50 and 24/40 joints)
Condenser: 71/60 joint for extra large soxhlet
55/50 joint for large soxhlet
Round bottom flask: 1 liter for extra large soxhlet
500 ml for large soxhlet
Adaptor (for extra large soxhlet, converts 29/42 joint to 24/40 joint)
Sample jar and lid
1 L beaker

4.2.1.2 *Non-glassware:*

Boiling chips
CH_2Cl_2

CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste container
Non-Cl^- solvent waste container
Cellulose thimbles: 60 x 180 mm for extra large soxhlet
43 x 123 mm for large soxhlet
Foil
Cork ring for round bottom flask

4.2.1.3 *Equipment:*

Variable autotransformer (aka variac)
Heating mantle for either 1 L or 500 mL round bottom flask
Drying oven

4.2.2. Procedures

Day 1

1) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
2) Add five or six boiling chips to flask. Add appropriate amount of CH_2Cl_2 to flask.
3) Place new teflon boiling chips in appropriate cellulose thimble. Place thimble in soxhlet extractor.

	Thimble Size	Flask Size (mL)	CH_2Cl_2 (mL)
large soxhlet	43 x 123	500	300
extra large soxhlet	60 x 180	1000	600

4) Assemble flask/soxhlet/condenser/adaptor (if necessary) apparatus.
5) Turn on heater to give proper boiling (set variac to 40-45).
6) Turn on chilled water for condenser.
7) Extract for 18 to 24 hours.

Day 2

1) Turn heat off; let cool 15 to 30 minutes.
2) Turn off condenser water.
3) Drain as much solvent from soxhlet as possible.
4) Remove thimble from soxhlet, place upside down in a 1 L beaker, cover loosely with foil.

5) Place boiling chips in a 70°C oven: Every 10 to 15 minutes, check boiling chips, shaking beaker to determine if all solvent has evaporated. Let boiling chips remain in oven two to four hours, until dry.
*****WARNING: BEWARE OF SOLVENT FUMES.*****
6) Wrap thimble in foil and store for future use.
7) Place in boiling chips in clean sample jar; cover with foil and lid.
8) Store on shelf.

Note: Boiling chips can be directly placed in soxhlet plugged with glasswool instead of using cellulose thimble.

4.3 Sodium Sulfate (Na_2SO_4)

4.3.1 Supplies

4.3.1.1 *Glassware:*

500 mL beaker
Sample jar and lid

4.3.1.2 *Non-glassware:*

Sodium sulfate (Na_2SO_4)
Foil

4.3.1.3 *Equipment:*

Muffle oven
Drying oven
Desiccator

4.3.2 Procedures

1) New Na_2SO_4
Put Na_2SO_4 in a clean muffled beaker and bake at 450°C for four hours or overnight.
Cool to 100°C in oven. Remove.
Place in clean sample jar; cover with foil and lid.
Store in desiccator.
2) Reconditioning Na_2SO_4 (to be done every two weeks):
Place Na_2SO_4 in 100°C drying oven overnight.
Remove from oven; cover with foil and lid.
Store in desiccator.

4.4 XAD_2

4.4.1 Supplies

4.4.1.1 *Glassware:*

Soxhlet extractor and condenser 71/60 and 29/42 joints
Six 1 L round bottom flasks with 24/40 joint
Six glass stoppers (24/40 joint)
One 1 L beaker
Two 400 mL beakers (one need not be clean)
Adaptor to convert 29/42 to 24/40

4.4.1.2 *Non-glassware:*

Boiling chips
CH_2Cl_2
Hexane
Methanol
Acetone
HPLC grade water: EM Science
CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste container
Non-Cl^- solvent waste container
Foil
Glass wool
Six cork rings

4.4.1.3 *Equipment:*

Heating mantle for 1 L flask
Variable autotransformer (aka variac)
Refrigerator or freezer

4.4.2 Procedures for dry XAD_2 for air sample cartridges

Day 1

1) Place XAD_2 in extractor plugged with glass wool.
2) Rinse XAD_2 with tap water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with small amount of methanol three times to remove water.
4) Add 500 mL of methanol to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac to 60-65 for methanol)
8) Turn on chilled water for condenser.
9) Cover soxhlet and flask with foil.
10) Extract with methanol for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL hexane to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL CH_2Cl_2 to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-50 for CH_2Cl_2).
6) Cover soxhlet and flask with foil.
7) Extract with Ch_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible.
3) Add 100 mL hexane to the soxhlet. Wait 15 minutes, then hand flush. Repeat at least three times, until the level of the solvent in the siphon tube is the same as in the soxhlet.
4) Add 500 mL hexane to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Turn on heater (set variac to 40-45 for hexane).
7) Cover soxhlet and flask with foil.
8) Extract with hexane for 24 hours. Flushing may need to be induced twice before it flushes on its own.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL 50% acetone/50% hexane to 3 L flask.
4) Add boiling chips to flask.
5) Turn on heater (set variac to 40-45 for acetone/hexane).
6) Cover soxhlet and flask with foil.
7) Extract with acetone/hexane for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone/hexane from soxhlet as possible.
3) Pour XAD_2 in a beaker and dry overnight in 65°C oven.
4) Store in amber bottle in freezer at -20°C for up to three months.
5) Keep subsample in separate jar for checking lab blank and matrix spike.

4.4.3 Procedures for wet XAD_2 for precipitation sample cartridges

Day 1

1) Place XAD_2 in soxhlet plugged with glass wool.
2) Rinse XAD_2 with water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with small amount of methanol three times to remove water.
4) Add 500 mL methanol to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac at 60-65 for methanol).
8) Turn on chilled water for condenser.
9) Cover soxhlet and flask with foil.
10) Extract for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL hexane to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL CH_2Cl_2 to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40 for CH_2Cl_2).
6) Cover soxhlet and flask with foil.
7) Extract with CH_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible.
3) Add 100 mL hexane mixture to the soxhlet. Wait 15 minutes, then drain solvent. Repeat at least three more times, until level of solvent in the siphon tube is the same as in the soxhlet.
4) Add 500 mL hexane to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Turn on heater (set variac at 40-45 for hexane).
7) Cover soxhlet and flask with foil.
8) Extract with hexane for 24 hours.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac at 40-45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL methanol to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 60-65 for methanol).
6) Cover soxhlet and flask with foil.
7) Extract with methanol for 24 hours.

Day 8 or so

1) Turn off heater; cool 15 to 30 minutes.
2) Turn off condenser water.
3) Flush as much methanol from soxhlet as possible.
4) Rinse XAD_2 at least three times with EM Science HPLC grade water (until XAD_2 does not smell of methanol).
5) Store the clean XAD_2 in DI water in amber bottle in the refrigerator at 4°C. (The resin may be stored in this manner for up to three months.)

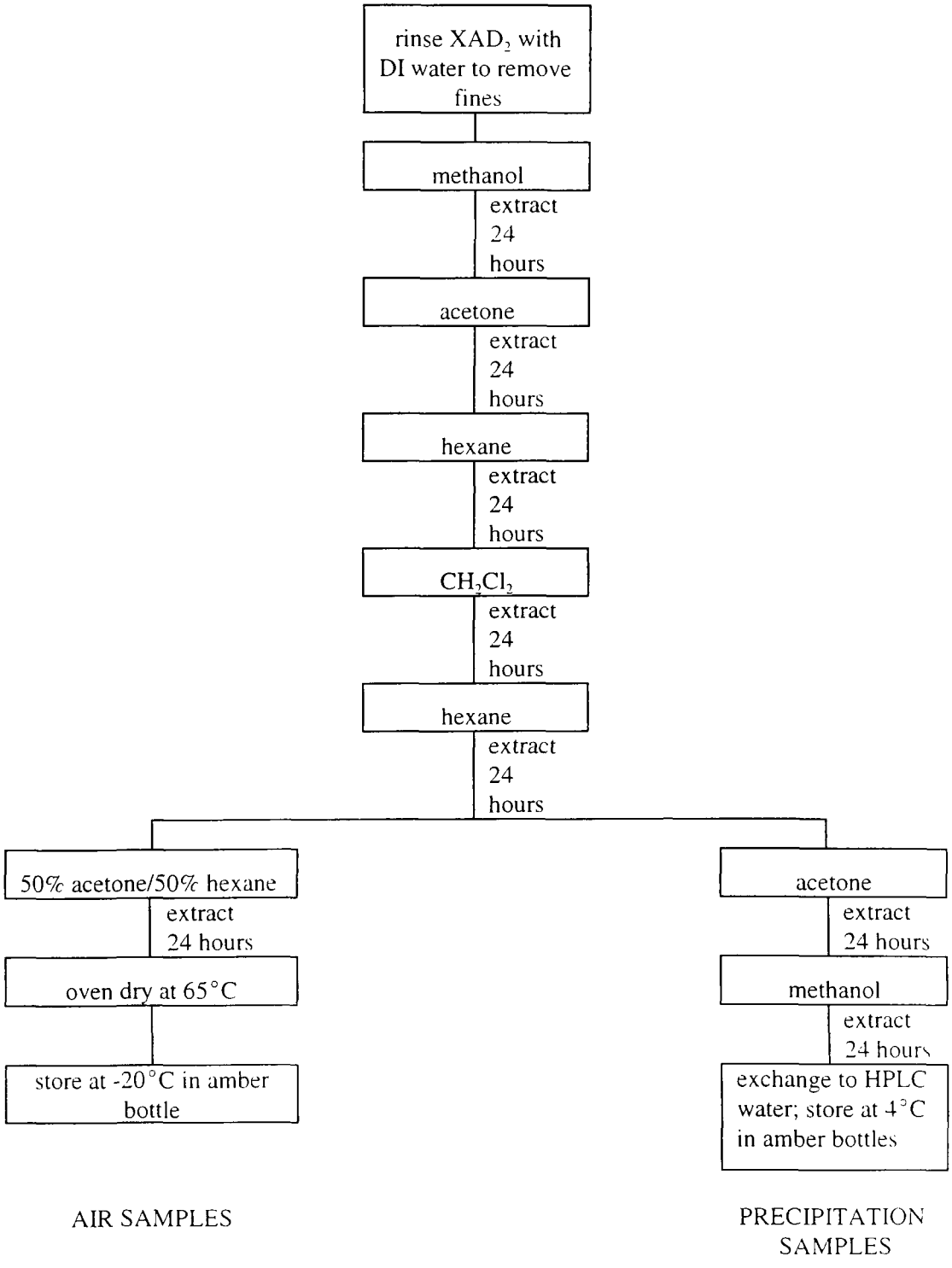
4.4.4 Comments

4.4.4.1 Variac settings may vary from autotransformer to autotransformer. Check that the solvent is boiling properly (nice rolling boil).

4.4.4.2 If XAD_2 is re-used after sample extraction, it is not necessary to rinse with DI water before extracting. The cleaning process can begin by extracting with methanol.

4.4.4.3 Sometime solvent does not siphon very well. Induce siphoning by hand as many times as possible. Allow extra time in case of improper flushing.

4.4.5 Flowchart of XAD_2 precleaning procedure



4.5 Silica and Quartz Fiber Filters (QF)

4.5.1 Silica

It has been determined silica is adequately cleaned during the activation process therefore no additional processing is necessary.

4.5.2 Quartz fiber filters (QF)

Each QF is wrapped up by aluminum foil separately and then muffled to 450°C for four hours. After it reaches ambient temperature, about 25 are wrapped again in aluminum foil and stored in freezer in a sealed plastic bag.

5.0 Air Samples, Particle and Vapor Phase: QF and XAD_2 Cartridges

5.1 Extraction

5.1.1 Supplies

5.1.1.1 *Glassware:*

Large soxhlet extractor (55/50 and 24/40 joints)
Condenser (55/50 joint)
500 mL round bottom flask (24/40 joint)
Glass stopper (24/40 joint)
400 mL beaker
Micro-dispenser (50 or 100 µL) and 1 mL pipette

5.1.1.2 *Non-glassware:*

Boiling chips
Acetone
Hexane
Spiking standards:

Standard	Concentration
PCB surrogate standard	Congener 14: 200 ng/mL
	Congener 65: 50 ng/mL
	Congener 166: 50 ng/mL
pesticide recovery standard	100 ng of each pesticide/mL
PAH recovery standard	2 µg of each PAH/mL
Dibutylchlorendate	500 ng/mL
Terbutylazine	5600 ng/mL
Atrazine	2000 ng/mL
d_{10} Phenanthrene	2 µg/mL
PCB recovery standard	683 ng of PCBs/mL

CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste bottle
Non-Cl^- solvent waste bottle
Cork ring (size #2)
Glasswool
12" rod (glass or metal)
Large tweezers
Small tweezers
Foil
Scissors

5.1.1.3 *Equipment:*

Heating mantle and variable autotransformer or multi-unit extraction heater

5.1.2 Procedures

One set of sample is extracted in two days. The set includes 10-12 samples (including one duplicate), one field blank, one lab blank, and one combination matrix spike. In combination matrix spike, the matrix is spiked with known amount of PCBs, Pesticides,PAHs, and atrazine to calculate recovery of each compound. A name will be assigned to each set on the day of extraction (month, year and type of sample), such as S94C, in which:

S = Month of sample collection, such as September
94 = year of sample collection
C = Type of sample, such as cartridge

Day 1

1) Remove spiking standards from freezer. Standards **must** be at ambient temperature before using. (Ambient temperature is achieved in about two hours.)
   surrogate PCB standard- PCB #14, 65, 166
   surrogate pesticide standard- dibutylchlorendate
   surrogate atrazine standard- trbutylazine
   surrogate PAH standard- d_{10} phenenthrene
   pesticide recovery standard
   PAH recovery standard
   PCB recovery standard
   Atrazine recovery standard
2) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
3) Assemble supplies and samples under hood and/or utility cart. Label flasks.
4) Add five to six clean teflon chips into 500 mL round bottom flask.
5) Pour solvent into round bottom flask: 175 mL of acetone and 175 mL of hexane.
6) Transfer sample to soxhlet extractor:
   Vapor sample - XAD_2
   Place glass wool plug at the siphon tube opening of the soxhlet extractor using glass or metal rod.

Carefully pour XAD_2 in soxhlet extractor. Rinse container with solvent (50% acetone/50% hexane) to remove all XAD_2; pour solvent rinse into soxhlet.

Assemble flask/soxhlet/condenser. Place on heating mantle.

Particle sample-Composite QF

Unwrap one QF at a time.

Trim off the number at the corner with clean scissors.

Use two pairs of blunt tweezers to fold one QF; place in soxhlet.

Rinse tweezers and scissors with CH_2Cl_2.

Repeat procedure for all QFs in composite sample.

Assemble flask/soxhlet/condenser. Place on heating mantle.

7) Spike extraction:

XAD_2 and QF Samples

Using a micropipette dispenser, spike each sample with 100 µL of the PCB surrogate standard. (One standard solution contains all three congeners.) 50 µL of dibutylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenenthrene

Lab Blank

Using a micropipette dispenser, spike the extraction medium with 100µL of the PCB surrogate standard, 50 µL of dibutylchlorendate, 100 µL of terbuylazine, and 200 µL of d_{10} phenenthrene.

Combination matrix spike:

Spike sample medium with 1 mL of PCB recovery standard (683 ng of PCBs), 200 µL of Mixed Pesticide Recovery standard (20 ng of each), 200 µL of mixed PAH congeners (400 ng of each), 500 µL of atrazine (1000ng), 100 µL of PCB surrogate standard (14=20 ng, 65=5 ng, 166=5 ng), 50 µL of dibutyl chlorendate (25 ng), 100 µL of terbutylazine (560 ng), and 200 µL of d_{10} phenanthrene (400 ng). PCB recovery standard contains 683 ng of PCB in 1 mL (diluted from Michael D. Mullin 94 mix). These data are used for the recovery of individual PCB congeners, individual pesticides and PAHs.

8) Assemble flask/soxhlet/condenser unit. Place on heating mantle.

9) Turn on heating mantles: set Staco heating mantles to 45 or the multi-unit extraction heater to 5.

10) Turn on condenser water.

11) Cover soxhlet and flask with foil.

12) Extract for 18 to 24 hours.

Day 2

1) Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from soxhlet extractor into flask as possible.

2) Detach the flask and insert stopper.

3) Turn off condenser water.

4) Store in cool dark place.

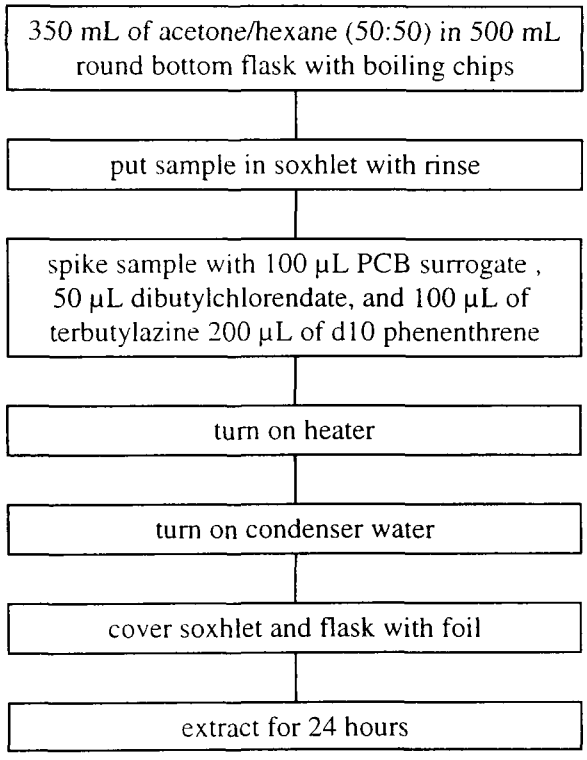
5.1.3 Comments

5.1.3.1 If XAD_2 gets into the flask, see Section 5.2.2. Removing XAD_2 from flask.

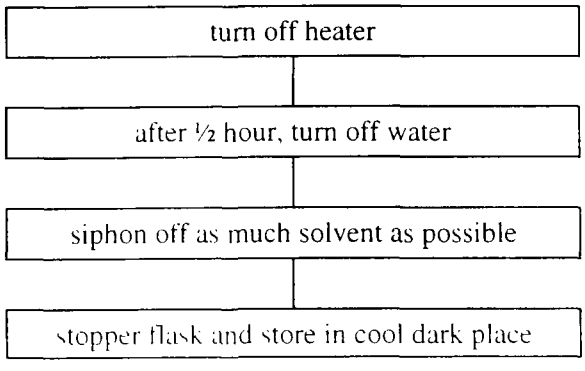
5.1.3.2 If condensation is a problem, wrap condensers with foil wrapped insulation or with kimwipes.

5.1.4 Flow charts for air sample extraction

5.1.4.1 Setting-up extraction



5.1.4.2 Taking down extraction



5.2 Rotary Evaporation

5.2.1 Supplies

5.2.1.1 After extraction/before column clean-up

5.2.1.1.1 *Glassware:*

Splash guard with 24/40 joint
100 or 200 mL beaker
Waste container for used boiling chips

5.2.1.1.2 *Non-glassware:*

Hexane
Clean large forceps
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

5.2.1.1.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

5.2.1.2 After column clean-up

5.2.1.2.1 *Glassware:*

Splash guards: one with 24/40 joint and one with 14/20 joint
25 mL beaker

5.2.1.2.2 *Non-glassware:*

Hexane
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

5.2.1.2.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

5.2.1.3 Removing XAD_2 from Flask

5.2.1.3.1 *Glassware:*

500 mL round bottom flask

5.2.1.3.2 *Non-glassware:*

Cork ring
Hexane

5.2.1.3.3 *Equipment:*

-none-

5.2.2 Procedures

5.2.2.1 Set-up

1) Fill chamber with DI water.
2) Turn on the chiller circulator.
3) Set bath temperature:

Solvent	Temperature (°C)
hexane	30-32
acetone	30-32
acetone/hexane	30-32
methanol	40
CH_2Cl_2	30

4) Rinse joint of steam duct with CH_2Cl_2.
5) Attach appropriate splash guard(s) to steam duct. Clamp each joint.
6) Turn on vacuum. Check vacuum of system.

5.2.2.2 Evaporation

1) Remove boiling chips with large forceps. If XAD_2 is in flask, see Section 5.2.2.4. Removing XAD_2 From Flask.
2) Attach flask to splash guard. Clamp joint.
3) Turn on motor of rotator to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
4) Evaporate sample down to approximately 2 mL (in a 500 mL round bottom flask, area of liquid should be about the size of a quarter).
5) Open stopcock of rotary evaporator to release vacuum.
6) Detach the flask:

   If exchanges are necessary, add specified amount of hexane from Section 5.2.2.3. Solvent Exchanges, then return flask to splash guard and clamp.

   If additional exchanges are not necessary, stopper flask. Store flask under cabinet.

7) Empty receiving flask into proper waste bottle as needed.
8) Rinse splash guard with CH_2Cl_2 before using with a different sample. Muffle splash guard at the end of a set of samples. Splash guards should be washed and muffled after every three or four sets of samples.

5.2.2.3 Solvent exchanges

	Fraction	Amount of Hexane to Add	No. of Exchanges	Total # of Rotary Evaporations	Final volume
after extraction	----	75 mL	2	3	2-5 mL
after column clean-up	hexane	----	0	1	1 mL
	50%	25 mL	1	2	1 mL

5.2.2.4 Removing XAD_2 from flask
1) Label another 500 mL flask with sample ID.
2) Decant sample from original flask into clean flask.
3) Rotary evaporate new flask using above procedures.
4) Add hexane for the exchanges to original flask with XAD_2; swirl hexane in flask to remove any remaining items of interest.
5) Decant hexane wash from original flask into new flask as needed to complete exchanges.

5.2.2.5 Clean-up
1) Turn off heater on rotary evaporator.
2) Turn off motor on rotary evaporator.
3) Turn off chiller.
4) Empty receiving flask into proper waste solvent bottle.
5) Cover steam duct with foil.

6.0 Rain Samples

6.1 Extraction

6.1.1 Supplies

6.1.1.1 *Glassware:*

Large soxhlet extractor (55/50 and 24/40 joints)
Condenser (55/50 joint)
500 mL round bottom flask
Glass stopper (24/40 joint)
Micro-dispenser (50 or 100 µL) and 1 mL pipette
200 mL (or larger) beaker

6.1.1.2 *Non-glassware:*

Boiling chips
Acetone
Hexane
CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste bottle
Non-Cl^-solvent waste bottle
Cork ring for 500 mL flask
Glasswool
12" rod (glass or metal)
Large tweezers
Small tweezers
Foil
Spiking standards:

Standard	Concentration
PCB surrogate standard	Congener 14: 200 ng/ml
	Congener 65: 50 ng/mL
	Congener 166: 50 ng/mL
pesticide recovery standard	100 ng of each pesticide/mL
dibutylchlorendate	500 ng/mL
terbutylazine	5600 ng/mL
atrazine	2000 ng/mL
PAH recovery standard	2 µg of each PAH/mL
d_{10} phenanthrene	2 µg/mL
PCB recovery standard	683 ng of PCBs/mL

6.1.1.3 *Equipment:*

Heating mantle for 500 mL round bottom flask
Variable autotransformer or multi-unit extraction heater

6.1.2 Procedures

6.1.2.1 Extraction of Rain Samples from XAD_2 cartridges

One set of samples (usually one month's sample from all different site) is extracted on Day 1. A name is assigned to that set. An example of set name is Au94P- month of collection, year, and type of sample. In this case, P stands for precipitation sample. One set will include approximately six to eight samples, at least one duplicate sample, one field blank, one lab blank, and one combination matrix spike.

Day 1

1) Remove spiking standards from freezer. Standards **must** be at ambient temperature before using. (Ambient temperature is achieved in about two hours.)

   surrogate PCB standard
   surrogate pesticide standard - dibutylchlorendate
   surrogate atrazine standard - terbutylazine
   surrogate PAH standarad - d_{10} phenanthrene
   pesticide recovery standard
   PAH recovery standard
   PCB recovery standard
   Atrazine recovery standard

2) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
3) Assemble supplies and samples under hood and/or utility cart. Label flasks.
4) Add five to six clean teflon chips into 500 mL round bottom flask.
5) Measure 175 mL acetone in a beaker.
6) Place glass wool plug at the siphon tube opening of the soxhlet extractor using glass or metal rod. Assemble soxhlet extractor and flask.
7) Put XAD_2 sample in soxhlet extractor. Rinse container with acetone from beaker; add this and remaining acetone from beaker to soxhlet.
8) Add 175 mL hexane to top of soxhlet.
9) Spike extraction:

   Samples

   Using micropipette dispenser, spike each sample with 100 µL of the PCB surrogate (One standard solution contains all three congeners), 50 µL of dibuylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenanthrene.

   Lab Blank

   Using micropipette dispenser, spike approximately 8 g of clean XAD_2 with 100 µL of the PCB surrogate standard, 50 µL of dibuylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenanthrene.

   Combination Matrix Spike or MS

   Spike sample medium with 1 ml of PCB recovery standard (683 ng of PCBs), 200 µL of Mixed Pesticide Recovery standard (20 ng of each), 200 µL of mixed PAH standard (400 ng of each), 500 µL of atrazine(1000 ng), 100 µL of PCB surrogate standard (14=20 ng, 65=5 ng, 166=5 ng), 50 µL of dibutyl chlorendate (25 ng), 100 µL of terbutylazine (560 ng), and 200 µL of d_{10} phenanthrene (400 ng). PCB recovery standard contains 683 ng of PCB in 1 mL (diluted from Michael D. Mullin 94 mix). These data are used for the recovery of individual PCB congeners, individual pesticides, each PAHs and atrazine.

10) Assemble flask/soxhlet/condenser apparatus. Place on heating mantle.
11) Turn on heating mantles: set Staco heating mantle to 45 or the multi-unit extraction heater to 5.

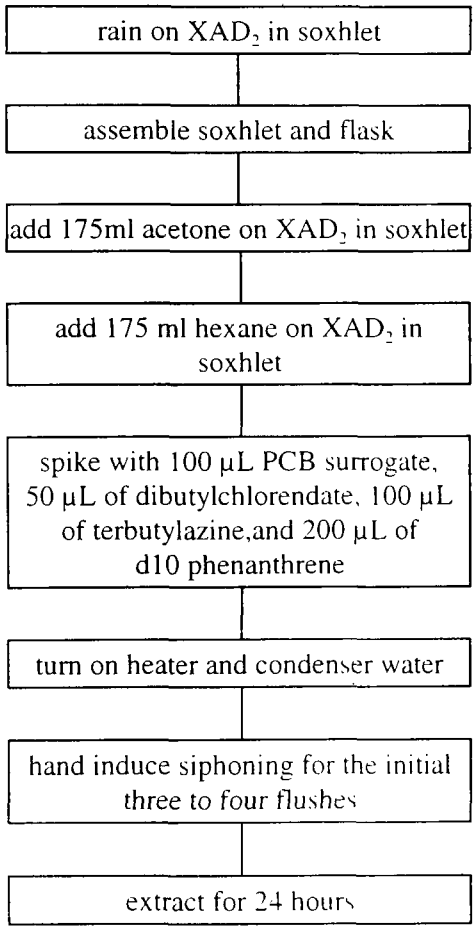
12) Turn on condenser water.
13) Cover soxhlet and flask with foil.
14) Extract for 24 hours.

Note: The sample has water in it, thus it may not siphon on its own the first two or three times depending on the amount of water present. Induce siphoning until the level of solvent in the soxhlet and in the syphon tube are the same.

Day 2

1) Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from soxhlet extractor into flask as possible.
2) Detach the flask and insert stopper.
3) Turn off condenser water.
4) Store in cool dark place.

6.1.3 Flow chart for the extraction of rain samples



6.2 Rotary Evaporation

6.2.1 Supplies

6.2.1.1 After extraction/before column clean-up

6.2.1.1.1 *Glassware:*

Splash guard with 24/40 joint
100 mL or 200 mL beaker
Waste container for used boiling chips

6.2.1.1.2 *Non-glassware:*

Hexane
Clean large forceps
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

6.2.1.1.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

6.2.1.2 After column clean-up

6.2.1.2.1 *Glassware:*

Splash guards: one with 24/40 joint and one with 14/20 joint.
25 mL beaker

6.2.1.2.2 *Non-glassware:*

Hexane
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

6.2.1.2.3 *Equipment:*

Rotary evaporator
Chiller circulator

6.2.1.3 Back Extraction (in addition to the items listed in Section 6.2.1, Supplies. After extraction/before column clean-up)

6.2.1.3.1 *Glassware:*

125 mL separatory funnel
Pasteur pipettes
10 mL graduated pipette

6.2.1.3.2 *Non-glassware:*

Rubber pipette bulb

6.2.1.3.3 *Equipment:*

Three-prong clamp with support

6.2.2 Procedures

6.2.2.1 XAD_2 Cartridges

6.2.2.1.1 Set-up

1) Fill chamber with DI water.
2) Turn on the chiller circulator.
3) Set bath temperature:

Solvent	Temperature (°C)
hexane	30-32
methanol	40
acetone/hexane	30-32
CH_2Cl_2	30

4) Rinse joint of steam duct with CH_2Cl_2.
5) Attach appropriate splash guard(s) to steam duct. Clamp each joint.
6) Turn on vacuum. Check vacuum of system.

6.2.2.1.2 Evaporation

1) Remove boiling chips with large forceps. If XAD_2 is in flask, see Section 6.2.2.1.3. Removing XAD_2 from flask.
2) Attach flask to splash guard. Clamp joint.
3) Turn on motor of rotator to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
4) Evaporate sample until the evaporation slows down. **Note:** If rate of evaporation slows down, **DO NOT** continue. There is water in the sample.

6.2.2.1.3 Removing XAD_2 from the flask

1) Label another 500 mL flask with the samples ID
2) Decant sample from original flask into the clean flask; wash with 10 mL hexane twice.
3) Rotary evaporate the new flask until evaporation begins to slow down.

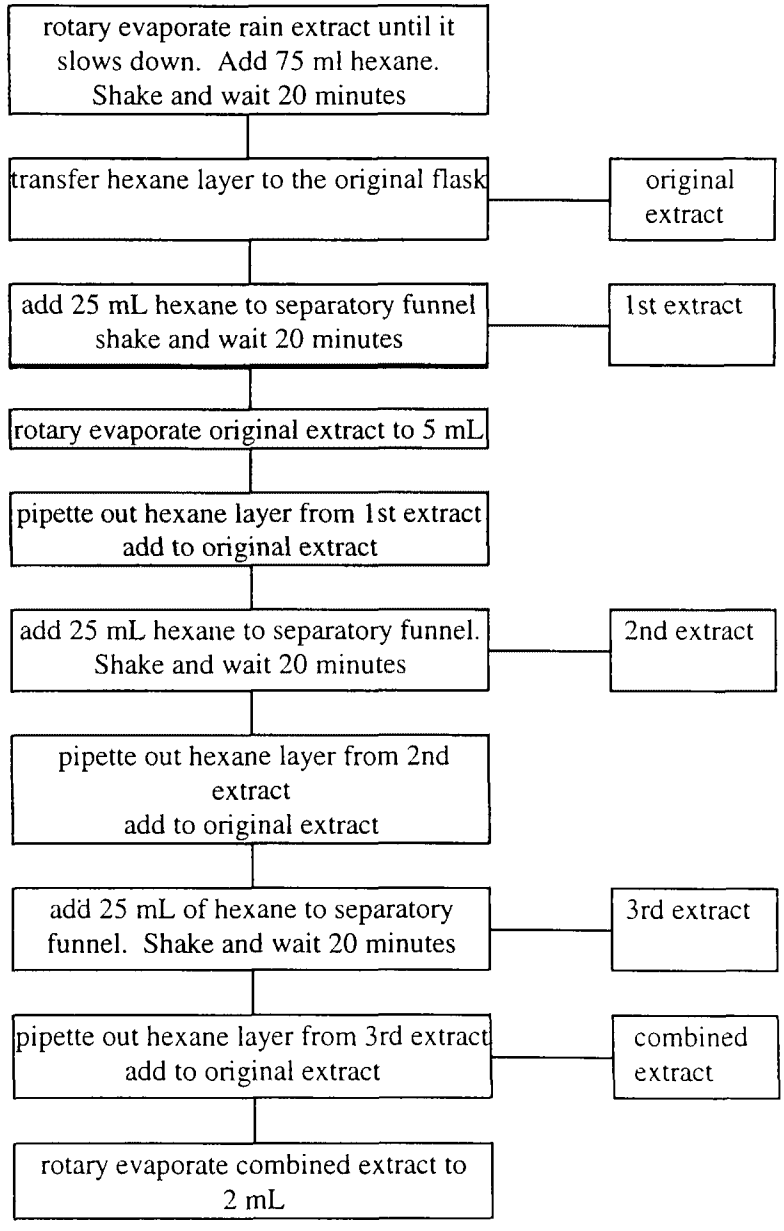
6.2.2.1.4 Back extraction and solvent exchanges

1) Add 75 mL hexane to sample flask. Rotavap again to 100 mL. Transfer the content to separatory funnel. Add 1 gm of sodium sulfate. Shake vigorously. Wait for 20 minutes.
2) First Extract:
   Transfer the original hexane layer to the flask. Add 25 mL hexane to the water in separatory funnel. Then add approximately 1 g of Na_2SO_4 Shake vigorously; let stand at least 20 minutes. While waiting for first extract to separate, rotary evaporate the original sample to approximately 5 mL.
   After 20 minutes or so, pipette the hexane out and add it to the original sample flask.
3) Second Extract:
   Add 25 mL hexane to the water layer in the separatory funnel. Shake vigorously; let stand at least 20 minutes.
   Pipette out the hexane layer from the separatory funnel; add it to the original flask.
4) Third Extract:
   Add 25 mL hexane to the water layer in the separatory funnel. Shake vigorously; let stand at least 20 minutes.
   Pipette out the hexane layer from the separatory funnel; add it to the original flask.
5) Rotary evaporate the combined extract to 2 mL.
   Note: *More water may separate out after the addition of the first and second extract. Pipette the water out and add it to the separatory funnel.
   *It is possible trace amounts of water may be in the final sample - ignore it! The $NaSO_4$ on the top of the silica column will take care of it.
   *If an emultion forms in the separatory funnel, add extra Na_2SO_4 to the funnel. This will facilitate the separation of the water.

6.2.2.1.5 Clean-up

1) Turn off heater on rotary evaporator.
2) Turn off motor on rotary evaporator.
3) Turn off chiller.
4) Empty receiving flask into proper waste solvent bottle.
5) Cover steam duct with foil.

6.2.2.1.6 Flow Chart of Rotary Evaporation and Back Extraction



6.2.2.2 Rotary evaporation after column chromatography

1) Attach flask to splash guard. Clamp joint.
2) Turn on motor of rotator to pre-determined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
3) Evaporate sample down to approximately 2 mL.
4) Open stopcock of rotary evaporator to release vacuum.
5) Detach the flask:
   If exchanges are necessary, add specified amount of hexane as listed in Section 5.2.2.3, Solvent Exchanges, then return flask to splash guard and clamp.
   If additional exchanges are not necessary, stopper flask and store it under the cabinet.
6) Empty receiving flask into proper waste bottle as needed.
7) Rinse splash guard with CH_2Cl_2 before using with a different sample. Muffle splashguard a the end of a set of samples. Splash guards should be washed and muffled after every three or four sets of samples.

6.2.2.3 Solvent Exchanges

	Fraction	Amount of Hexane to Add	No. of Exchanges	Total # of Rotary Evaporations	Final Volume
after column chromatography	hexane	----	0	1	1 mL
	50%	25 mL	1	2	1 mL
	meth.	-----	0	0	1 mL

7.0 Silica Column Chromatography

7.1 Supplies

7.1.1 Activation/Deactivation

7.1.1.1 *Glassware:*

100 mL or 250 mL beaker
Powder funnel
250 mL or 500 mL round bottom flask
Stopper to fit round bottom flask
1 mL graduated pipette
25 mL beaker

7.1.1.2 *Non-glassware:*

*Silica
Pipette bulb
Cork ring to fit round bottom flask

7.1.1.3 *Equipment:*

Muffle furnace
Desiccator
Calculator
Balance
Particle mask

7.1.2 Column clean-up (for a three-fraction column clean-up of one sample)

7.1.2.1 *Glassware:*

Column
Three 100 mL pear shaped flasks with 14/20 joints
Three glass stoppers with 14/20 joints
Pasteur pipettes (9½ inch and/or 5¼ inch): minimum of one for each sample and six additional pipettes for each set of samples fractionated
Graduated cylinders: 50 mL and 10 mL
Funnel
100 mL beaker
250 mL beaker OR waste jar (need not be clean)
Three 250 mL beakers

7.1.2.2 *Non-glassware:*

Rubber pipette bulbs
Hexane
50% hexane/50% CH_2Cl_2
CH_2Cl_2
Methanol
Two cork rings for 100 mL pear shaped flasks (size #1)
Rubber hammer
Stainless steel spatula
20" rod
Teflon stopcock
Glass wool
4% deactivated silica
$NaSO_4$

7.1.2.3 *Equipment*

Ultrasonicator

7.1.3 Supply chart for each sample

Item	Air Particle (QF)	Air Vapor (XAD_2)	Rain (XAD_2)
amount of silica to activate/deactivate	4-6 g	4-6 g	4-6 g
column size	3.5"	3.5"	3.5"
amount of $NaSO_4$	0.5	0.5"	1.5"
elution volume (1st and 2nd fraction)	25 mL	25 mL	30 mL
switching volume	4 mL	4 mL	5 mL
elution volume (3 rd fraction)	30 mL	30 mL	35 mL

7.2 Procedures

7.2.1 General procedures

7.2.1.1 Activation/Deactivation of silica

Day 1

1) Place approximate amount of silica needed in a beaker. Cover beaker with foil.
2) Place beaker in 100°C oven, turn thermostat to 300°C; keep in oven overnight.
DO NOT PUT SILICA INTO 300°C OVEN!

Day 2

1) Turn oven temperature down to 100°C;
DO NOT REMOVE SILICA FROM OVEN.
2) When oven has cooled to 100°C, remove beaker from oven; let cool on counter top until warm (approximately 5 to 10 minutes); place in desiccator.
3) When silica has reached ambient temperature (approximately two hours), deactivate it:

Working quickly, weigh out desired amount of silica in the round bottom flask. Stopper flask **immediately** after pouring silica.Add 4% 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 desiccator overnight for equilibration.
Use deactivated silica in desiccator within three days. Any unused silica may be reused after re-activating and re-deactivating).

7.2.1.2 Preparation and packing of column(s)

1) Assemble stopcock(s) on column(s).
2) Stuff glass wool plug (approximately 1 cm) into lower end of the each column with 20" rod.
3) Measure and mark appropriate distance from top of glass wool plug.
4) Clamp column(s) securely onto frame in ventilation hood. Place empty glass container under each column (100 mL minimum size; it need not be clean).
5) Close stopcock(s); fill column(s) half full with hexane.
6) Make a slurry of hexane and deactivated silica. Pour slurry into each column. **DO NOT ALLOW SILICA TO DRY OUT**: rinse column and beaker with hexane via Pasteur pipette. (Use of a funnel may facilitate process.) Open stopcock(s). Tap column(s) with rubber hammer to pack silica. Add silica/hexane as needed until desired length is loaded.
7) Cap column(s) with ½" Na_2SO_4 for XAD_2 and QF samples, 0.5" Na_2SO_4 for rain samples.
8) Wash column(s) with 25 mL hexane for conditioning.
9) When hexane level reaches 1 cm above top of Na_2SO_4, close stopcock(s) to prevent further dripping. **NEVER LET COLUMN RUN DRY.**
10) If column(s) is/are not going to be used immediately, stopper column(s) and cover tip(s) of column(s) with foil.

7.2.1.3 Set-up

1) Label one 100 mL pear-shaped flask for each fraction which is to be collected.
2) On a cart, assemble pear shaped flasks and remaining supplies listed in Section 7.1, Supplies.
3) Place sample flask in front of column.
4) Place a 50 mL or 100 mL beaker in front of sample flask.
5) Add hexane to 50 mL or 100 mL beaker; cover with foil. (For volume of hexane, see chart in Section 7.1.3, Supply Chart.)

7.2.1.4 Column chromatography

7.2.1.4.1 First fraction

1) Ultrasonicate sample flask before loading the sample onto the column to detach the particles which are sticking to the walls of the flask.

2) Remove stopper from sample flask. Assemble pipette and rubber bulb; place pipette in sample flask.
3) Place Fraction #1 (hexane) pear shaped flask under the column.
4) Open stopcock and let column drip until hexane level is at the top of the Na_2SO_4.
5) Load sample into column with Pasteur pipette.
6) Set drip rate to approximately one drip per second. Add approximately 5 mL hexane to sample flask from the beaker. Swirl solvent in flask..
7) When sample has drained down to the top of the Na_2SO_4, add the hexane from the sample flask to the column. Add an additional 5 mL hexane to the sample flask from the beaker. Swirl solvent in flask.
8) When solvent has drained down to the top of the Na_2SO_4, add the second 5 mL hexane to the column. Add the remaining hexane from the beaker to the sample flask. Swirl solvent in sample flask.
9) When solvent has drained down to the top of the Na_2SO_4, add the remaining hexane from the sample flask. (If reservoir on top of the column cannot hold entire amount, add as much as possible, then refill as space becomes available.)
Note: Stagger the timing of the column loadings such that the changing of the flasks are not concurrent.

7.2.1.4.2 Second fraction

1) While the hexane is dripping (from the first fraction), measure the hexane/CH_2Cl_2 and put it into the appropriate containers.
2) When the hexane drips down to the top of the Na_2SO_4, add the switching volume hexane/CH_2Cl_2 from the sample flask to the column.
3) Transfer the hexane/CH_2Cl_2 from the beaker to the sample flask. Swirl the solvent in the flask.
4) Place the appropriate pear shaped flask (labeled '50%' fraction) next to the flask under the column.
5) When the hexane/CH_2Cl_2 level in the column is to the top of the $NaSO_4$, quickly switch flasks and pour as much of the remaining hexane/CH_2Cl_2 into the column as possible. Add hexane/CH_2Cl_2 to the column as space permits.
6) Continue to monitor the rate of drip (approximately one drip per minute).
7) Place the pear shaped flask from the first fraction on the supply cart. Stopper the flask.
8) Once the column has stopped dripping, remove flask from second fraction, stopper it, and put it on the supply cart.

9) collect another fraction with 30 ml of methanol in case of air samples and 35 mL of methanol in case of rain samples.

7.2.1.4.3 Clean-up

1) Remove stopcock from column.
2) Turn column upside down and secure it with clamps. Place container under column to catch $NaSO_4$ and silica.
3) After column has dried out, use vacuum (air or water) to remove glass wool plug.
4) Pour silica and $NaSO_4$ into used glove or foil before discarding into trash can.

7.2.2 Specific procedures by sample type

7.2.2.1 XAD_2 (vapor) and GFF

Follow General Procedures.

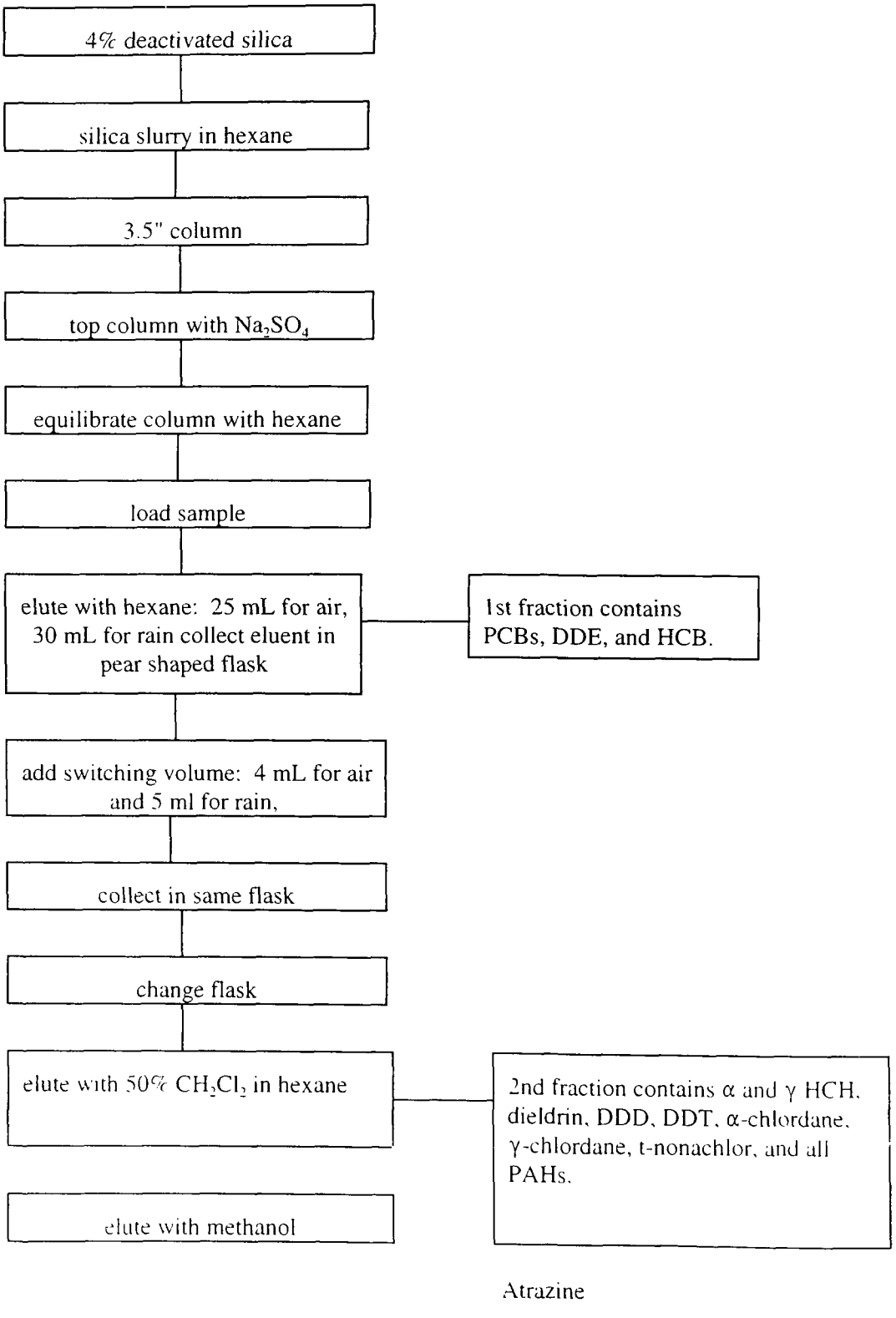
7.2.2.2 Rain samples

Procedure is essentially the same as XAD_2 (vapor and particles) and QF with the following exceptions:

1) $NaSO_4$ should be activated no more than 2 to 3 days before using; cap should be 1.5"
2) Elution volumes:

Solvent	Solvent needed (mL) for rain samples
hexane	30
50% CH_2Cl_2 in hexane	30
switching volume	5
methanol	35

7.2.3 Summary flow-chart



8.0 Transfer of Solution

8.1 Supplies for Each Sample

8.1.1 *Glassware:*

Pasteur pipettes (9½ inch and/or 5¼ inch): minimum of one for each fraction and one additional pipette for hexane
4 mL amber glass vial for each fraction
50 mL beaker

8.1.2 *Non-glassware:*

Vial file for 4 mL vials
Rubber pipette bulbs
Hexane (minimum 2 mL for each fraction)

8.1.3 *Equipment:*

-none-

8.2 Procedures

8.2.1 Preparation
1) Label each amber vial with sample ID and fraction ID.
2) Put hexane in 50 mL beaker (approximately 2 mL per sample).
3) Concentrate all fractions by rotary evaporation to 1 mL. 50% CH_2CL_2 fraction needs to be solvent exchanged to hexane (see Section 5.2.3).

8.2.2 Transferring sample
1) Using a Pasteur pipette, transfer entire sample from flask to amber vial.
2) Add approximately 1 mL hexane to flask and swish solvent around to clean out flask; transfer to amber vial.
3) Repeat.
Note: Do not add so much solvent as to fill the vial. If it is too full, there is a chance of splashing at the time of N_2 blowdown.
4) Close amber vial tightly, place in vial file, and store in freezer. Label the vial file with sample set name, type of samples, and site of collection.

9.0 N_2 Blowdown

9.1 Supplies

9.1.1 *Glassware:*

-none-

9.1.2 *Non-glassware:*

CH_2Cl_2
Sample in amber vial

9.1.3 *Equipment:*

N_2 blowdown unit

9.2 Procedures

9.2.1 Set-up

1) Remove all nozzle plugs from unit.
2) Turn on N_2 at tank and trap (do NOT touch primary or secondary controls on regulator). Let N_2 flush out for approximately five minutes.
3) Turn heater on LOW.
4) Attach clean needle to each nozzle to be used.

9.2.2 Blowdown

1) Place amber vials in slot; adjust N_2 flow such that there are gentle (barely detectable) ripples in the vials.
2) Evaporate down to the approximate predetermined volume. (See following chart.)

Type of sample		Approximate volume after N_2 blowdown (mL)		
		hexane fraction	50% fraction	methanol fraction
rain	winter	0.4	0.4	0.4
	summer	0.4	0.4	0.4
QF	winter	0.4	0.4	0.4
	summer	0.4	0.8	0.4
XAD_2	winter	0.4-0.8	1.0-2.0	0.4
	summer	0.8-1.0	1.5-2.0	0.4

9.2.3 Closing-up unit

1) Turn off N_2 at trap and near the regulator.
2) Replace the nozzle caps.
3) Rinse nozzle extension tubes with CH_2Cl_2. After three to four uses or after a highly contaminated batch of samples, ultrasonicate nozzle extension tubes.

10.0 Spiking Samples with ISTD

10.1 Supplies

10.1.1 *Glassware:*

-none-

10.1.2 *Non-glassware:*

Samples in 4 mL amber glass vials
Internal standards (see next page)
Hexane
CH_2Cl_2
Cl^- and non-Cl^- waste containers

10.1.3 *Equipment:*

25, 50, and 100 µL microdispensers

10.2 Procedures

1) Remove ISTDs from freezer; equilibrate to ambient temperature (approximately two hours).

Fraction	Compound	Type of sample	Internal Standard	Spike Volume (µL)	Final Mass in Sample (ng)	Color of Dot on Label
hexane	PCBs and pesticides	vapor, particle, and rain	PCB 30	100	8	red
			PCB 204		6	
50%	PAHs	vapor, particle, and rain	D_{10} anthracene,	50	200	black
			D_{12} benzo(a)anthracene		200	
			triphenylmethane		134.6	
			d_{12} perylene		180	
50%	Pesticide	vapor particle rain	PCB 65	100	20	blue
			PCB 155		20	
methanol	atrazine	vapor particle rain	d_{10} anthracene	50	200	green

2) Clean micropipette
Remove glass tube used to cover plunger.
Rinse plunger with CH_2Cl_2. Wave pipette to evaporate solvent.

Without touching glass tubes, insert plunger into new glass capillary; tighten tube in place.
Rinse the capillary with dichloromethane twice and air dry. Draw spiking standard. Make sure that there is no air bubble.

3) Spike sample vial (see above chart for surrogate and amount).
4) Mark each amber vial label with a appropriate color of dot (use a water-proof marker).
5) Replace glass tube used to cover plunger of micropipette. Store micropipette.

11.0 Making Microvials for GC Analysis

11.1 Supplies

11.1.1 *Glassware:*

Conical microvials
Pasteur pipettes

11.1.2 *Non-glassware:*

Vial racks
Septa (vial caps)

11.1.3 *Equipment:*

Crimper

11.2 Procedures

1) Label conical microvials with sample Ids. In addition, label extra microvial for hexane and the appropriate calibration standard for every set of samples.
2) Using a Pasteur pipette, remove approximately 200 µL of each sample and put in labeled conical microvial. (The level of liquid will be at the shoulder of the microvial.) Also place 200 µL of hexane and 200 µL of the appropriate standard into the labeled microvials. (See following chart for the appropriate standard.)

Fraction	Target Compounds	Calibration Standard
hexane	PCBs	Mullin 94: 683 ng/mL
50%	pesticides	mixed pesticide standard: 20 ng/mL ea
50%	PAH	mixed PAH standard: 200 ng/mL ea (approx)
methanol	atrazine	1000 ng/mL

Note: Make one vial with performance standard for each set of analyte.

3) Crimp septa onto microvial.
4) Load microvials into autosampler or store in freezer.

12.0 Standards

12.1 PCB Standards

12.1.1 Mullin's 94 mix: 170.8 µg/mL: Mixture of 1232, 1248, and 1262 in 25:18:18.

12.1.2. Surrogate standards

12.1.2.1 Congener 14: Primary stock: 100 µg/mL in isooctane: Accustandard.

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.2 Congener 65: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.3 Congener 166: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.4 PCB mix surrogate recovery standard: To be used for spiking each sample.

Congener	Stock Concentration	Mix	Final Concentration
14	1000 ng/mL	10 mL	200 ng/mL
65	1000 ng/mL	2.5 mL	50 ng/mL
166	1000 ng/mL	2.5 mL	50 ng/mL

Volume was made up to 50 mL with hexane.

12.1.3 Internal Standards (ISTD)

12.1.3.1 Congener 30: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	0.5 mL (50 µg)	50 mL	1000 ng/mL

12.1.3.2 Congener 204: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	0.5 mL (50 µg)	50 mL	1000 ng/mL

12.1.3.3 PCB spiking standard:

Congener	Stock Concentration	Mix	Final Concentration
30	1000 ng/mL	8 mL	80 ng/mL
204	1000 ng/mL	6 mL	60 ng/mL
Volume was made up to 100 mL with hexane.			

12.1.4 PCB calibration standard for PCBs

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	400 µL	683.2 ng/mL
14	1000 ng/mL	2 mL	20 ng/mL
65	1000 ng/mL	0.5	5 ng/mL
166	1000 ng/mL	0.5	5 ng/mL
30	1000 ng/mL	0.8	8 ng/mL
204	1000 ng/mL	0.6	6 ng/mL
DDE	2000 ng/mL	1 mL	20 ng/mL
HCB	2000ng/mL	1 mL	20 ng/mL
Volume made up to 100 mL with hexane.			

12.1.5 PCB recovery standard for PCBs: used for matrix spike.

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	400 µL	683.2 ng/mL

12.1.6 PCB performance standard: used for instrument calibration check.

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	300 µL	512.4 ng/mL
14	1000 ng/mL	1 mL	10 ng/mL
65	1000 ng/mL	1 mL	10 ng/mL
166	1000 ng/mL	1 mL	10 ng/mL
30	1000 ng/mL	0.8	8 ng/mL
204	1000 ng/mL	0.6	6 ng/mL
DDE	2000 ng/mL	0.5 mL	10 ng/mL
HCB	2000ng/mL	0.5 mL	10 ng/mL
Volume made up to 100 mL with hexane			

12.2 Pesticide Standards

12.2.1 Stock solutions

12.2.1.1 Primary stock

12.2.1.2 Stock

Pesticide	Ultra Sc. ampule concentration	Dilution	Stock Concentration
dieldrin	100 µg/mL in MeOH	1 mL - 50 mL hexane	2 µg/mL
α-HCH	100 µg/mL in MeOH	1 mL - 50 mL hexane	2 µg/mL
γ-HCH	100 µg/mL in MeOH	1 mL - 50 mL hexane	2 µg/mL
HCB	100 µg/mL in methylene chloride	1 mL - 50 mL hexane	2 µg/mL

Pesticide	Ultra Sc. ampule concentration	Dilution	Stock Concentration
4-4'DDT	100 µg/mL inMeOH	1 mL → 50 mL hexane	2 µg/mL
4-4'DDD	100 µg/mL in MeOH	1 mL → 50 mL hexane	2 µg/mL
4-4'DDE	100µg/mL in MeOH	1 mL → 50 mL hexane	2 µg/mL
α-chlordane	100 µg/mL in MeOH	1 mL → 100 mL hexane	1 µg/mL
γ-chlordane	100 µg/mL in MeOH	1 mL --- 100 mL in hexane	1 µg/mL
t-nonachlor	100 µg/mL in MeOH	1 mL ---- 100 mL in hexane	1 µg/mL
atrazine	100 µg/mL MeOH	1 mL → 50 mL hexane	2 µg/mL

12.2.2 Pesticide spiking standard: Cong 65, 155

Compound	Stock Concentration	Mix	Final Concentration
Congener 65	1000 ng/ mL	10 mL	200 ng/mL
Congener 155	1000 ng/ mL	10 mL	200 ng/mL
Volume made up to 50 mL with hexane			

12.2.3 Pesticide surrogate standard

Dibutylchlorendate: 100 µg/ mL in methanol
Stock: 1 mL of above diluted to 100 mL in hexane = 1000 ng/ mL
Spiking standard: 25 mL of stock solution diluted to 50 mL with hexane = 500 ng/ mL

Terbutylazine: 2.8 mg was weighed and diluted to 100 mL with MeOH
Stock: 28000 ng/ mL
Spiking standard: 10 mL of stock was diluted to 50 mL with CH_2CL_2 = 5600 ng/mL

12.2.4 Mixed pesticide calibration standard: MPS 65, 155

This is used for analysis of pesticides in 50% CH_2Cl_2 fraction.

Compound	Stock Concentration	Mix	Final Concentration
α-HCH	2000 ng/mL	1 mL	20 ng/mL
γ-HCH	2000 ng/mL	1 mL	20 ng/mL
dieldrin	2000 ng/mL	1 mL	20 ng/mL
DDT	2000 ng/mL	1 mL	20 ng/mL
DDD	2000 ng/mL	1 mL	20 ng/mL
α-chlordane	1000 ng/mL	2 mL	20 ng/mL
γ-chlordane	1000 ng/mL	2 mL	20 ng/mL
t-nonachlor	1000 ng/mL	1 mL	20 ng/mL
Cong. 155	1000 ng/mL	2 mL	20 ng/mL
Cong. 65	1000 ng/mL	2 mL	20 ng/mL
Volume made up to 100 mL with hexane			

12.2.5 Mixed pesticide performance standard

Compound	Stock Concentration	Mix	Final Concentration
Pest Recovery standard B5 from ISWS	100 ng of each / mL	5 mL	10 ng of each/ mL
Dibutyl chlorendate	1000 ng/ mL	0.5 mL	10 ng/ mL
cong 65	1000 ng/ mL	0.5 mL	10 ng/ mL
cong 155	1000 ng/ mL	0.5 mL	10 ng/ mL

Volume was made up to 50 mL with hexane.

12.2.6 Pesticide recovery standard

Compound	Stock Concentration	Mix	Final Concentration
HCB	2000 ng/mL	2.5 mL	100 ng/mL
α-HCH	2000 ng/mL	2.5 mL	100 ng/mL
γ-HCH	2000 ng/mL	2.5 mL	100 ng/mL
dieldrin	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDE	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDD	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDT	2000 ng/mL	2.5 mL	100 ng/mL
α-chlordane	1000 ng/mL	5 mL	100 ng/mL
γ-chlordane	1000 ng/mL	5 mL	100 ng/mL
t-nonachlor	1000 ng/mL	25 mL	100 ng/mL
Volume made up to 50 mL with hexane			

12.3 PAH Standard

12.3.1 PAH mixed GC/MS calibration standard
solvent = hexane

PAH	Stock Conc. (µg/mL)	mL stock	Final Volume (mL)	Final Conc. (µg/mL)
acenapthene	1.97	10	100	0.20
acenapthylene	1.97	10	100	0.20
anthracene	1.97	10	100	0.20
benzo(a)anthracene	1.97	10	100	0.20
benzo(a)pyrene	1.97	10	100	0.20
benzo(b)fluoranthene	1.97	10	100	0.20
benzo(e)pyrene	1.91	10	100	0.19
benzo(g,h,i)perylene	1.97	10	100	0.20
benzo(k)fluoranthrene	1.97	10	100	0.20
chrysene	1.97	10	100	0.20
coronene	1.93	10	100	0.19
d_{10}anthracene-ISTD	4.00	4.2	100	0.17

PAH	Stock Conc. (µg/mL)	mL stock	Final Volume (mL)	Final Conc. (µg/mL)
d_{10} perylene	3.6	4.2	100	0.15
d_{12}benzo(a)anthracene-ISTD	4.00	4.2	100	0.17
dibenzo(a,h)anthracene	1.97	10	100	0.20
fluoranthene	1.97	10	100	0.20
fluorene	1.97	10	100	0.20
indeno(1,2,3,cd)pyrene	1.97	10	100	0.20
napthalene	1.97	10	100	0.20
phenanthrene	1.97	10	100	0.20
pyrene	1.97	10	100	0.20
retene	1.98	10	100	0.20
triphenylmethane-ISTD	2.69	4.2	100	0.11

12.3.2 PAH matrix spike recovery standard, Batch 2A

Analyte	Stock Conc. (µg/mL)	Stock Amt. (mL)	Final Conc. (µg/mL)
Acenapthene	100	1.97	1.97
Acenapthylene	100	1.97	1.97
Anthracene	100	1.97	1.97
Benzo(a)anthracene	100	1.97	1.97
Benzo(b)fluoranthene	100	1.97	1.97
Benzo(k)fluroanthene	100	1.97	1.97
Benzo(a)pyrene	100	1.97	1.97
Benzo(e)pyrene	96.5	1.98	1.91
Benzo(g,h,i)perylene	100	1.97	1.97
Chrysene	100	1.97	1.97
Coronene	98.2	1.97	1.93
Dibenz(a,h)anthracene	100	1.97	1.97
Fluoranthene	100	1.97	1.97
Fluorene	100	1.97	1.97

Analyte	Stock Conc. (µg/mL)	Stock Amt. (mL)	Final Conc. (µg/mL)
Indeno(1,2,3,cd)pyrene	50	1.97	1.97
Naphthalene	95.24	1.97	1.97
Phenanthrene	100	1.97	1.97
Pyrene	100	1.97	2.0
Retene	158.95	1.98	1.98

Volume was made up to 100 mL with hexane.

12.3.3 PAH internal standard

Compound	Stock (µg/mL)	Mix (mL)	Final Concentration (µg/mL)
d_{10}anthracene	1000	0.2	4
d_{12}benzo(a)anthracene	1000	0.2	4
d_{12} perylene	2000	0.09	3.60
triphenylmethane	136	0.99	2.69
Volume was made up to 50 mL with hexane			

12.3.4 PAH surrogate standard

d10 Phenanthrene: 2.13 ug/ mL of hexane

13.0 Safety

13.1 Emergency Numbers

Name	*Telephone numbers*
IU Fire Department	911
Ronald A. Hites	812-855-0193 (O) 812-334-1323 (H)
Jeffery White	812-855-1466 (O) 812-336-1462 (H)

13.2 Chemists Numbers

Name	*Telephone Numbers*
Ilora Basu	812-855-5040 (O) 812-855-2926 (O) 812-334-2184 (H)
Barbara Hillery	812-855-1005 (O) 812-334-4151 (H)
James M. O'Dell	812-855-5040 (O) 812-824-7962 (H)
Tom Stanko	812-855-2926 (O) 812-336-8546 (H)
Mary Tankard	812-855-5035 (O) 812-824-1863 (H)
Mike Wassouf	812-855-2926 (O) 812-330-1517 (H)
Charles Alan Long	812-855-2926 (O) 812-333-9535 (H)

13.3 Working in the Laboratory

Chemists working in the laboratory should follow certain safety rules :

1) Individual is required to wear a lab coat whenever working in the lab.
2) Eye protection with splash resistant safety glasses or safety goggles is required. Contact lens is forbidden.
3) Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.
4) All solvent work should be done inside fume hood.
5) Open shoes are not allowed in the laboratory.
6) Particle mask is required when using dry silica.
7) Generally nobody should work alone in the laboratory. If work must be performed after hours or in the weekend inform supervisor or other lab mates so that your presence is known and will be accounted for in case of an emergency.
8) Chemicals and solvents are stored in separate storage area. One week's supply is kept in the lab. Solvents are stored in special solvent cabinet. Acids must be separated from bases. A rubber bucket needs to be used to carry any chemical.
9) Gas cylinders should be well secured at all times. Flammable gases are stored in separate cage.
10) Wash your hands well after work. Protective hand cream "Soft guard" is supplied.
11) No food or drink is allowed in the laboratory.

12) 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 evacuated.
13) MSDS are filed in a three ring binder.
14) All chemicals and standard should be labeled properly with scientific name, date, and initials of person to contact.
15) Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

13.4 Safety Equipment

13.4.1 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.

13.4.2 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.

13.4.3 Eye Wash

Emergency eye wash is located in the laboratory. It is operated by pushing the lever backward.

13.5 Waste Disposal

13.5.1 Solvents

1) Label 2 containers, '**CHLORINATED WASTE**' and '**NON-CHLORINATED WASTE**'.
2). Containers may be empty glass bottles from solvents or poly jericans (10 L or less).
3) When in use they are to be placed inside a fume hood with the sash pulled down.
4) University Health and Safety Department will pick up the waste solvent on Friday. Label the bottle properly and sign it.

13.5.2 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.

13.5.3 Teflon boiling chips

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

13.5.4 Glass

Place in 'Broken Glass Disposal Containers'. When containers are full, close according to directions on box; leave for janitors to pick-up or take out to the trash dumpster.

13.5.5 Foil

Place in trash can.

13.5.6 Fiberglass

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

13.5.7. XAD_2 and QF

Leave in soxhlet under hood until solvent has evaporated. Pour XAD_2 into container labeled '**USED XAD_2**'. Discard QF into trash can.

14.0 References

Following publications were consulted for the development of methods of PCB, pesticides and PAHs analysis in Air and Precipitation samples. Experimental procedure was modified according to our need.

Baker, J.E.; Eisenreich, S.J. PCBs and PAHs as Tracers of Particulate Dynamics in Large Lakes. J. Great Lakes Res., 1989, 15(1),84-103.

Bidleman, T.F.; Mathews, J.R.; Olney, C.E.; and Rice, C.P Separation of Polychlorinated Biphenyl, Chlordane and p-p DDT from Toxaphene by silicic acid column chromatography. J. Ass. off. analyt. chem., 1978, 61, 820-828.

Hermanson, M.H. and Hites, R.A. Long-Term Measurement of Atmospheric Polychlorinated Biphenyls in the Vicinity of Superfund dumps. Environ. Sci. Technol., 1989, 23. No. 10, 1253-1258.

Marti, E.A. Armstrong D.E. Polychlorinated Biphenyls In Lake Michigan Tributaries. J. Great Lakes Res., 1990, 16(3): 396-405

Mc Veety, B.D. and Hites, R.A. Atmospheric Deposition of Polycyclic Aromatic Hydrocarbons to Water Surfaces: A Mass Balance Approach. Atmos. Environ., 1988, 22, 511-536.

Mullin, M.D. PCB Workshop, U.S. EPA Large Lakes Research Station, Grosse Ile, MI, June 1985.

Murphy, T. J. and Rzeszutko, C.P. Precipitation inputs of PCBs to Lake Michigan. J. Great Lakes Res., December 1977. Internat. Assoc. Great Lakes Res., 3(3-4): 305-312.

Swackhamer, D.L.; Mc Veety, B.D.; and Hites R.A. Deposition and Evaporation of Polychlorinated Biphenyl congeners to and from Siskiwit Lake, Isle Royale, Lake Superior. Environ. Sci. Technol., 1988, 22, 664-672.

Sweet, C.W.; Vermette, S.J.; and Gatz, D.F. Atmospheric Deposition of Toxic Materials: A Compound of the Green Bay Mass Balance Study. 1992, Contract Report 530, Illinois State Water Survey, Champaign, IL 61820.

Personal communication with:
Hites, R.A. and his group from Indiana University, 1990-1993
Eisenreich S.J. and his group, 1990-1993
Swackhamer, D.L., 1990-1993

Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples: Sample Preparation Procedures

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Champaign, IL 61820

SOP #CH-PR-001.3

March 1995

Revision 3.0

Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples: Sample Preparation Procedures

SOP #CH-PR-001.3, Revision 3.0

1.0 Scope and Applications

1.1 This procedure details the sample preparation methods utilized at the ISWS, Office of Atmospheric Chemistry, Trace Organic Toxicants Lab as applied to the Lake Michigan Mass Balance (LMMB) and Lake Michigan Loading Study (LMLS) projects. The procedures apply to XAD-2 cartridge, filter, and XAD-2 precipitation samples. The following analytes are measured by this SOP:

Polychlorinated Biphenyls (PCBs) - Total and 105 congener peaks

congener (BZ)	CAS #
1	2051-60-7
3	2051-62-9
4+10	13029-08-8, 33146-45-1
6	25569-80-6
7+9	33284-50-3, 34883-39-1
8+5	34883-43-7, 16605-91-7
12	2974-92-7
13	2974-90-5
15+17	2050-68-2, 37680-66-3
16	28444-78-9
18	37680-65-2
19	39444-73-4
21	55702-46-0
22	38444-85-8
27	38444-76-7
25	55712-37-3
24	55702-45-9

Polychlorinated Biphenyls (PCBs) Continued

congener (BZ)	CAS #
26	38444-81-4
29	15862-07-4
31+28	16606-02-3, 7012-37-5
32	38444-77-8
33	38444-86-9
37	38444-90-5
40	8444-93-8
41+71	52663-59-9, 41464-46-4
42	36559-22-5
43	70362-46-8
44	41464-39-5
45	70362-45-7
46	41464-47-5
47	2437-79-8
48	70362-47-9
49	41464-40-8
51	68194-04-7
52	35693-99-3
53	41464-41-9
56+60	41464-43-1, 33025-41-1
63	74472-34-7
64	52663-58-8
66	32598-10-0
70+76	32598-11-1, 70362-48-0
74	32690-93-0
77	32598-13-3
81	70362-50-4
82	52663-62-4

Polychlorinated Biphenyls (PCBs) Continued

congener (BZ)	CAS #
83	60145-20-2
87	38380-02-8
89	73575-57-2
91	68194-05-8
92+84	52663-61-3, 52663-60-2
95	38379-99-6
97	41464-51-1
99	38380-01-7
100	39485-83-1
101	37680-73-2
107	70424-68-9
110	38380-03-9
114+131	74472-37-0, 61798-70-7
118	31508-00-6
119	56558-17-9
123+149	65510-44-3, 38380-04-0
128	38380-07-3
129	55215-18-4
130	52663-66-8
132+153+105	38380-05-1, 35065-27-1, 32598-14-4
134	52704-70-8
135+144	52744-13-5, 68194-14-9
136	38411-22-2
137+176	35694-06-5, 52663-65-7
141	52712-04-6
146	51908-16-8
151	52663-63-5
156	38380-08-4
157+200	69782-90-7, 52663-73-7
158	74472-42-7
163+138	74472-44-9, 35065-28-2

Polychlorinated Biphenyls (PCBs) Continued

congener (BZ)	CAS #
167	52663-72-6
170+190	35065-30-6, 41411-64-7
174	38411-25-5
175	40186-70-7
177	52663-70-4
178	52663-67-9
180	35065-29-3
183	52663-69-1
185	52712-05-7
201	40186-71-8
202+171	2136-99-4, 52663-71-5
196	42740-50-1
203	52663-76-0
205	4472-53-0
206	40186-72-9
207	52663-79-3
208+195	52663-77-1, 52663-78-2
209	2051-24-3

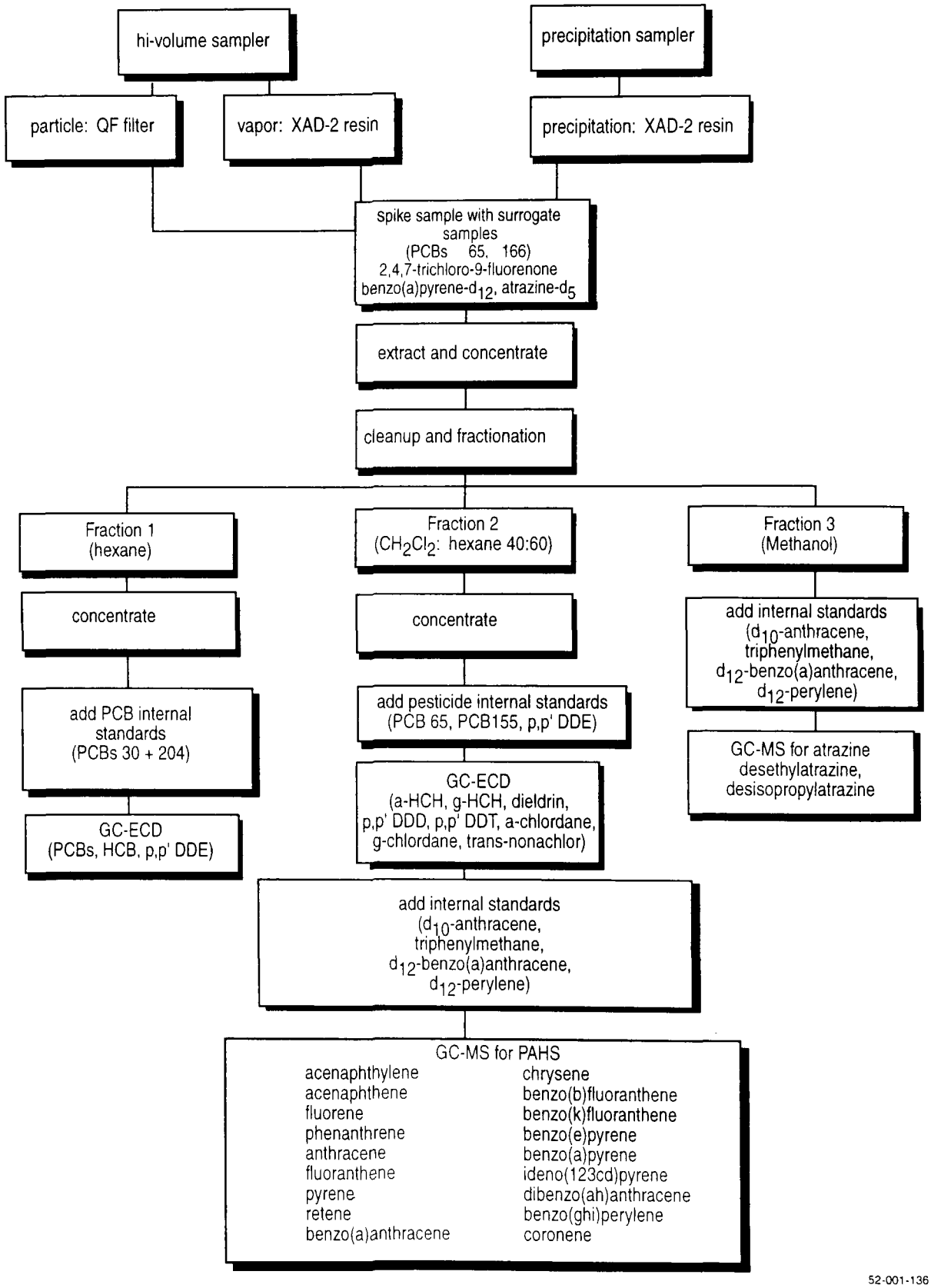
Pesticide	CAS #
atrazine	1912-24-9
desethylatrazine (DEA)	6190-65-4
desisopropylatrazine (DIA)	1007-28-9
dieldrin	60-57-1
a-chlordane	5103-71-9
g-chlordane	5103-74-2
t-nonachlor	39765-80-5
a-hexachlorocyclohexane (a-HCH)	319-84-6
g-hexachlorocyclohexane (g-HCH)	58-89-9
hexachlorobenzene (HCB)	118-74-1
p'p'-DDD	72-54-8
p,p'-DDE	72-55-9
p,p'-DDT	50-29-3

Polycyclic Aromatic Hydrocarbons (PAHs)	CAS #
acenaphthene	83-32-9
acenaphthylene	208-96-8
anthracene	120-12-7
benzo(a)anthracene	56-55-3
benzo(a)pyrene	50-32-8
benzo(b)fluoranthene	205-99-2
benzo(e)pyrene	192-97-2
benzo(ghi)perylene	191-24-2
benzo(k)fluoranthene	207-08-9
chrysene	218-01-9
coronene	191-07-1
dibenzo(a,h)anthracene	53-70-3
fluoranthene	206-44-0
fluorene	86-73-7
indeno(123cd)pyrene	193-39-5
phenanthrene	85-01-8
pyrene	129-00-0
retene	483-65-8

1.2 Method detection limits (MDL) are defined in CFR, Vol 49, No. 209, October 26, 1984, Appendix B to Part 136. Matrix specific MDLs are determined by spiking 7-10 clean matrix samples with the analytes of interest and processing them through the entire extraction, cleanup, and analysis procedure.

1.3 The instrument detection limit (IDL) refers to the smallest signal above background noise that an instrument can reliably detect. The IDL is determined from a data set comprised of three separate chromatographic runs of a low level calibration standard; each run contains 7-10 analyses of the standard. The IDL equals the Student's t value (n-1) multiplied by the standard deviation of this data set.

1.4 Method Flow Diagram



2.0 Summary of Method

The analytes are extracted from XAD-2 resin or filter samples by Soxhlet extraction with hexane: acetone (50:50) followed by concentration by rotary evaporation. After extraction, interfering compounds are removed and analytes separated into different fractions with silica gel (3% deactivated). The first fraction (hexane) contains all PCBs and the pesticides HCB and DDE. The second fraction (40% DCM, 60% hexane) contains all PAHs and pesticides α and γ HCHs, dieldrin, DDD, DDT, γ chlordane, α chlordane, and t-nonachlor. Fraction three (methanol) contains atrazine and two metabolites (DEA, DIA). The samples are then concentrated to the desired volume with a slow stream of ultra-pure nitrogen. Final volumes depend on sample matrix, site, and date. Each sample is spiked with a known amount of internal standard. Subsamples are then transferred to autosampler microvials for capillary GC-ECD or GC-Ion Trap MS analysis.

3.0 Definitions

3.1 *Internal Standard (IS)* -- A pure analyte(s) added to a sample extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution.

3.2 *Surrogate Analyte (SA)* -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 All other terms are defined in the QAPjP, Revision 5, July 1995.

4.0 Interferences

Method interferences may be caused by contaminants in solvents, the sampling matrix, reagents, glassware, and other sample processing apparatus that lead to anomalous peaks or elevated baselines in gas chromatograms. Laboratory equipment and reagents will be monitored by the inclusion of quality control samples with each batch of samples prepared. Individual samples may contain interferences which will require additional sample preparations. All sample preparation details will be documented.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined. However, each one must be treated as a potential health hazard, and exposure to these chemicals should be minimized. Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.

5.2 Chemists working in the laboratory should follow ISWS safety rules :

5.2.1 A lab coat is required when working in the lab.

5.2.2 Eye protection with splash resistant safety glasses or safety goggles are required.

5.2.3 Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.

5.2.4 All solvent work should be done in fume hoods.

5.2.5 Open shoes are not allowed in the laboratory.

5.2.6 Particle mask is required when using dry silica.

5.2.7 Avoid working alone in the laboratory. If work must be performed after hours or in the weekend inform the supervisor or other staff so that your presence is known and will be accounted for in case of an emergency.

5.2.8 Chemicals and solvents are stored under the hoods. Acids must be separated from bases. A rubber bucket is required to transport any chemical.

5.2.9 Gas cylinders should be well secured at all times. Flammable gases are stored in separate storage areas.

5.2.10 Wash hands well after work.

5.2.11 No food or drink is allowed in the laboratory.

5.2.12 In case of minor spillage, get spillage kit to clean the area. A major spill requires the University of Illinois Fire Department to be contacted and the working area evacuated.

5.2.13 MSDS sheets are stored in the laboratory and a copy placed on file with the office administrator.

5.2.14 All chemicals and standards must be labeled with chemical name, date, and initials of person to contact.

5.2.15 Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

5.3 Waste disposal

5.3.1 Solvents

Label waste containers, *Chlorinated Waste* and *Non-Chlorinated Waste*. Glass bottles used for waste are placed under hoods for convenience. When full, transfer waste to 10 L carboy containers in solvent cabinet. Contact the ISWS Waste Coordinator for removal.

5.3.2 Silica

After solvent has evaporated, pour silica into a disposable glove and discard.

5.3.3 Teflon Boiling Chips

Allow solvent to evaporate then discard.

5.3.4 Glass

Place in `Broken Glass Disposal Containers'. When containers are full, close according to directions on box and discard per university instructions.

5.3.5 Aluminum Foil

Recycle

5.3.6 Glass Wool

Allow solvent to evaporate then discard.

5.3.7 XAD-2 and Filters

Leave XAD from air sample in Soxhlet under hood until solvent has evaporated then pour into container labeled *Used XAD-2*. Allow filter to dry in hood before discarding. XAD-2 (precip) is discarded.

6.0 Equipment and Supplies

6.1 Glassware -- General requirements

All glassware must be meticulously cleaned. Large glassware is thoroughly washed with laboratory detergent and hot water. Glassware with bad stains should be rinsed with MeOH or CH_2Cl_2 before using the soap and water procedure. If still not clean, soak in H_2SO_4:HNO_3 (50:50) acid bath overnight, then wash thoroughly with soap and water. Volumetric pipettes used for standards *must* soak in acid bath overnight. Glassware is thoroughly rinsed with tap water, then with DI water and allowed to air dry. The glassware is foil wrapped and heated 450°C for four hours. If glassware is not clean after muffling at 450°C for four hours, muffle at 500°C for four hours. The glassware is cooled to ambient temperature and stored in a clean location.

Small glassware such as stoppers, vials, and disposables are wrapped in foil or placed into a beaker and covered with foil and heated to 450°C for four hours, cooled to ambient temperature, and stored in a clean location. Vials are capped as soon as they are removed from the oven. Note: Always use dull side of foil towards glassware. Set initial temperature of furnace to 200°C if possible.

6.2 Sample vials and bottles -- amber glass vials with Teflon-lined screw caps. Amber colored glass is used for extract and standard storage since some of the method analytes are sensitive to light.

6.3 Volumetric flasks -- Various sizes.

6.4 Volumetric and graduated pipets -- Various sizes.

6.5 Forceps, spatulas, scissors and other stainless steel laboratory supplies. Metal supplies are washed with soap and water, rinsed with tap water, then rinsed with DI water and allowed to air dry. They are then rinsed with CH_2Cl_2, wrapped in foil, and stored in a clean location.

Note: Always rinse with CH_2Cl_2 immediately before use.

6.6 Micropipettor (Drummond or equiv.), glass capillaries -- Various sizes. Preclean capillaries with CH_2Cl_2 prior to use.

6.7 Teflon stopcocks and Teflon lined caps. Stopcocks and caps are washed with laboratory detergent and hot water, rinsed with tap then DI water, and air dried on kimwipes. The stopcocks are stored in a clean jar or beaker and covered with foil. Caps are rinsed with hexane, air dried, then placed onto their associated bottle or vial.

6.8 Cork Rings

6.9 Muffle Oven

6.10 Drying Oven

6.11 Soxhlet extraction apparatus, glassware and heating assembly.

6.12 Desiccator

6.13 Analytical and top loading balances with check weights.

6.14 Rotary evaporator with aspirator pump and chiller circulator

6.15 Separatory funnel, various sizes

6.16 Round bottom flask and stoppers, various sizes

6.17 Chromatography column for silica cleanup - large columns for cartridge samples are 11 x 300 mm (Kimax # 1780011300) with removable PTFE stopcocks, replaceable large bore glass tips, and 100 mL reservoirs. Small columns for rain and filters samples are 11 x 300 mm (Kimax # 4205300214) with size 2 PTFE stopcock plug. A 22 x 155 mm joint (# 668500-1922) is added to the top of the column.

6.18 Misc. lab supplies including: Pasteur pipets, beakers, funnels, pipet bulbs, glass rods, rubber hammer

6.19 Ultrasonic bath

6.20 Pierce Reactitherm, Model 18800, with stainless steel needles

6.21 Autosampler vials, caps and inserts

7.0 Reagents and Standards

7.1 Solvents - Pesticide quality or equivalent

Methylene Chloride (CH_2Cl_2, DCM)
Methanol (MeOH)
Acetone
Hexane

7.2 Reagents - Residue grade or equivalent

7.2.1 Organic free DI water

7.2.2 Glass Wool -- Cut glass wool into 2" pieces, place into a muffled beaker and cover with foil. Muffle at 450°C for four hours. Store in a clean location.

7.2.3 Teflon Boiling Chips -- Boiling chip cleanup requires a Soxhlet extraction step as follows:

Thoroughly rinse inside of condenser and outside of joint with methanol then CH_2Cl_2 from wash bottles. Cover joint and exhaust tube with foil.
Add five or six boiling chips to flask. Add appropriate amount of CH_2Cl_2 to flask.
Place glasswool plug at the siphon tube opening of the Soxhlet extractor using large tweezers.
Place new teflon boiling chips in Soxhlet extractor.
Assemble flask/Soxhlet apparatus.
Turn on heater to give proper boiling (set variac to 40-45).
Turn on chilled water for condenser.
Extract for 18 to 24 hours.
Turn heat off; let cool 15 to 30 minutes.
Turn off condenser water.
Drain as much solvent from Soxhlet as possible.
Remove boiling chips from Soxhlet and place in a 1 L beaker, cover loosely with foil.
Place boiling chips in a 70°C oven:
Every 10 to 15 minutes, shake beaker to accelerate solvent evaporation.
Let boiling chips remain in oven two to four hours, until dry.
****Warning: Beware of Solvent Fumes.****
Place boiling chips in clean sample jar; cover with foil and lid.
Store on shelf.

7.2.4 Sodium Sulfate (Na_2SO_4) -- Place Na_2SO_4 into a clean muffled beaker and heat to 450°C for four hours or overnight, cool to 100°C in the oven. Place into a clean sample jar, cover with foil and cap. Store in a desiccator. If not used within two weeks, recondition in a 100°C drying oven overnight. Remove from oven; cover with foil and cap then store in desiccator.

7.2.5 Silica Gel (Davisil, grade 634, 100-200 mesh or equivalent)

7.3 Standards

7.3.1 PCB Stock Standards

7.3.1.1 LMMB PCB Intermediate PCB Stock Standard (provided by Mullin June 1994).

Stock solution provided in sealed ampules with a total congener concentration of 183 µg/mL. (Aroclor 1232 = 75 µg/mL, Aroclor 1248 = 54 µg/mL, Aroclor 1262 = 54 µg/mL). Per M. Mullin the PCB concentration of the intermediate PCB Stock Std is 170.8 µg/mL due to the presence of biphenyl and other components in the mix.

7.3.1.2 Custom PCB Standard (Ultra Scientific #CUS-937) for Method Detection Limit studies (provided by US EPA July 1994). Stock solution provided in sealed ampules with a certificate of analysis as follows: Lot # 0858

PCB 1 = 12.00 µg/mL
PCB 6 = 14.20 µg/mL
PCB 29 = 6.30 µg/mL
PCB 49 = 5.86 µg/mL
PCB 101 = 4.93 µg/mL
PCB 141 = 2.19 µg/mL
PCB 180 = 2.21 µg/mL
PCB 194 = 1.69 µg/mL
PCB 206 = 2.05 µg/mL
PCB 209 = 1.36 µg/mL

7.3.1.3 2-chlorobiphenyl (PCB 1), Ultra Scientific (RPC-0069) Stock Standard for enhancement of PCB 1 in the Ultra CUS-937 mix described above. Stock Solution provided by EPA in a sealed ampule with a concentration for PCB 1 = 100 µg/mL (Lot No. H 0039)

7.3.2 Pesticide and PAH Stock Standards

Stock standard solutions are purchased from commercial sources (Ultra Scientific, Accustandard, Chem Service, Cresent Chemical) or are obtained from the USEPA repository. When stock solutions are not available, pesticides are purchased as the neat material and gravimetrically prepared in house.

7.3.3 Surrogate Standard Solutions

7.3.3.1 Surrogate standards are purchased from commercial sources (Aldrich Chemical, Cambridge Isotope) or are obtained from the USEPA repository. The following surrogate standards are utilized.

PCB 65 (PCB, HCB, DDE surrogate)
PCB 166 (PCB surrogate)
2,4,7-trichloro-9-fluorenone (pesticide surrogate)
atrazine-d5 (atrazine surrogate)
benzo-(a)pyrene-d12 (PAH surrogate)

7.3.3.2 If stock solutions are not commercially available, they are gravimetrically prepared from the neat material. Individual stock solutions are serially diluted in volumetric flasks to obtain the surrogate spike standard/s. A combined surrogate spiking standard may be prepared to save sample preparation time during the extraction procedure.

7.3.3.3 All samples are spiked with surrogate standards prior to extraction using volumetric pipets or a Drummond pipet and the spike volumes recorded on the sample preparation log.

7.3.4 Internal Standard Solutions (ISTDs)

7.3.4.1 Internal Standards are purchased commercially (Ultra Scientific) as a stock standard or as the neat material. The following ISTDs are utilized.

PCB 30 (PCB ISTD)
PCB 204 (PCB ISTD)
PCB 65 (Pesticide ISTD)
PCB 155 (Pesticide ISTD)
DDE (Pesticide ISTD)
anthracene-d10 (PAH and atrazine ISTD)
benzo(a)anthracene-d12 (PAH ISTD)
perylene-d12 (PAH ISTD)
triphenylmethane (PAH ISTD)

7.3.4.2 If stock solutions are not commercially available, they are gravimetrically prepared from the neat material. Individual stock solutions are serially diluted in volumeteric flasks to obtain the ISTD spiking standard/s.

7.3.4.3 ISTDs are added to the appropriate sample fraction (PCBs in hexane, pesticides and PAHs in 40% DCM, and atrazine in MeOH) prior to GC-ECD or GC-MS analysis. A Drummond micropipet is used for ISTD addition.

7.3.5 Chromatographic Calibration Standards

Combined instrument calibration standards are prepared from the individual stock standards by volumetric dilution to obtain five concentration levels. The calibration standard concentrations bracket the expected analyte amounts in samples assayed and are within the working linear range of the detectors. Calibration mixes are prepared specifically for the appropriate instrument and fraction analyzed. The following calibration mixes are prepared.

PCBs, DDE, HCB in the hexane fraction, with surrogate and ISTDs
chlorinated pesticides in the 40% DCM fraction, with surrogate and ISTDs
PAHs in the 40% DCM fraction, with surrogate and ISTDs
atrazine, DEA, and DIA in the MeOH fraction, with surrogate and ISTDs.

7.3.6 Matrix Spiking Solutions

7.3.6.1 Combined matrix spiking solutions are prepared from the individual stock standards by volumetric dilution. Combined matrix spike solutions are prepared for each analyte group. The following matrix spike mixes are prepared.

PCBs
chlorinated pesticides
PAHs
atrazine, DEA, DIA

7.3.6.2 The matrix spike solutions will be added to clean sample matrix material prior to extraction to calculate the recovery of individual analytes. One matrix spike will be extracted with each batch of samples. The matrix spike will be added to the sample using a Drummond micropipet or a volumetric pipet and the spiking amounts reported in the sample preparation log.

7.3.7 Standard Evaluation

New working standards will be assayed prior to use by comparison with existing standards. Standards must agree within 10% prior to use.

8.0 Sample Collection, Preservation and Storage

8.1 Sample collection, storage, and storage limits are defined in the QAPjP, Revision 5.0, July 1995.

8.2 Extracts are stored in amber vials at -10 to -20 °C before and after GC analyses.

9.0 Quality Control/Quality Assurance (QC/QA)

9.1 QA/QC requirements are described in the QAPjP, Revision 5.0, July 1995.

9.2 Quality control samples include: field and laboratory blanks, laboratory matrix spikes, laboratory surrogate spikes, and field and laboratory duplicate samples. The laboratory maintains all sample preparation and data records to document the quality of the data generated.

10.0 Calibration

All instrument calibration and analysis are detailed in the following ISWS SOPs:

Standard Operating Procedure for the Analysis of PAHs and Atrazine by GC/Ion Trap MS, July 1995.

Standard Operating Procedure for the Analysis of PCBs and Organochlorine Pesticides by GC-ECD, Revision 3.0, November 1995.

11.0 Filter and XAD-2 Resin Precleaning Procedure

11.1 XAD-2 Resin

11.1.1 The following supplies are required:

Soxhlet extractor and condenser custom made by Crown Glass Company
six 3 L round bottom flasks with 29/42 joint
six glass stoppers (29/42 joint)
one 1L beaker
two 400 mL beakers
heating mantle for 3 L flask
variable autotransformer

11.1.2 Procedure for vapor sample cartridges

Day 1

1) Place approximately 1 kg XAD-2 in extractor plugged with glass wool.
2) Rinse XAD-2 with tap water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with a small amount of methanol three times to remove water.
4) Add 2000 mL of methanol to 3 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/Soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac to 60-65 for methanol).
8) Turn on chilled water for condenser.
9) Cover Soxhlet and flask with foil.
10) Extract with methanol for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from Soxhlet as possible.
3) Add 2000 mL acetone to 3 L flask.
4) Add about 20 boiling chips to flask.

5) Turn on heater (set variac to 45 for acetone).
6) Cover Soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from Soxhlet as possible.
3) Add 2000 mL hexane to 3 L flask.
4) Add five or six boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover Soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from Soxhlet as possible.
3) Add 2000 mL CH_2Cl_2 to 3 L flask.
4) Add five or six boiling chips to flask.
5) Turn on heater (set variac to 40-50 for CH_2Cl_2).
6) Cover Soxhlet and flask with foil.
7) Extract with CH_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from Soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible through stopcock (remove stopcock if necessary).
3) Add 300 mL hexane to the Soxhlet. Wait 15 minutes, then drain through stopcock. Repeat at least three times, until the level of the solvent in the siphon tube is the same as in the Soxhlet.
4) Add 2000 mL hexane to 3 L flask.
5) Add five or six boiling chips to flask.
6) Turn on heater (set variac to 40-45 for hexane).
7) Cover Soxhlet and flask with foil.
8) Extract with hexane for 24 hours. Flushing may need to be induced twice before it flushes on its own.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from Soxhlet as possible.
3) Add 2000 mL 50% acetone/50% hexane to 3 L flask.
4) Add five or six boiling chips to flask.
5) Turn on heater (set variac to 40-45 for acetone/hexane).
6) Cover Soxhlet and flask with foil.
7) Extract with acetone/hexane for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone/hexane from Soxhlet as possible.
3) Pour XAD-2 in a beaker and dry overnight in 65°C oven.
4) Store in amber bottle in freezer at -20°C for up to three months.
5) Keep subsample in separate jar for use in preparation of lab blank and matrix spike.

11.1.3 Procedure for precipitation sample cartridges

Day 1

1) Place XAD-2 in Soxhlet plugged with glass wool.
2) Rinse XAD-2 with water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with small amount of methanol 3 times to remove water.
4) Add 2000 mL methanol to 3 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/Soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac at 60-65 for methanol).
8) Turn on chilled water for condenser.
9) Cover Soxhlet and flask with foil.
10) Extract for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from Soxhlet as possible.
3) Add 2000 mL acetone to 3 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for acetone).
6) Cover Soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from Soxhlet as possible.
3) Add 2000 mL hexane to 3 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover Soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from Soxhlet as possible.
3) Add 2000 mL CH_2Cl_2 to 3 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40 for CH_2Cl_2).
6) Cover Soxhlet and flask with foil.
7) Extract with CH_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from Soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible through stopcock (remove stopcock if necessary).
3) Add 300 mL hexane mixture to the Soxhlet. Wait 15 minutes, then drain solvent through stopcock. Repeat at least three more times, until level of solvent in the siphon tube is the same as in the Soxhlet.
4) Add 2000 mL hexane to 3 L flask.
5) Add about 20 boiling chips to flask.
6) Turn on heater (set variac at 40-45 for hexane).
7) Cover Soxhlet and flask with foil.
8) Extract with hexane for 24 hours.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from Soxhlet as possible.
3) Add 2000 mL acetone to 3 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac at 40-45 for acetone).
6) Cover Soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from Soxhlet as possible.
3) Add 2000 mL methanol to 3 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 60-65 for methanol).
6) Cover Soxhlet and flask with foil.
7) Extract with methanol for 24 hours.

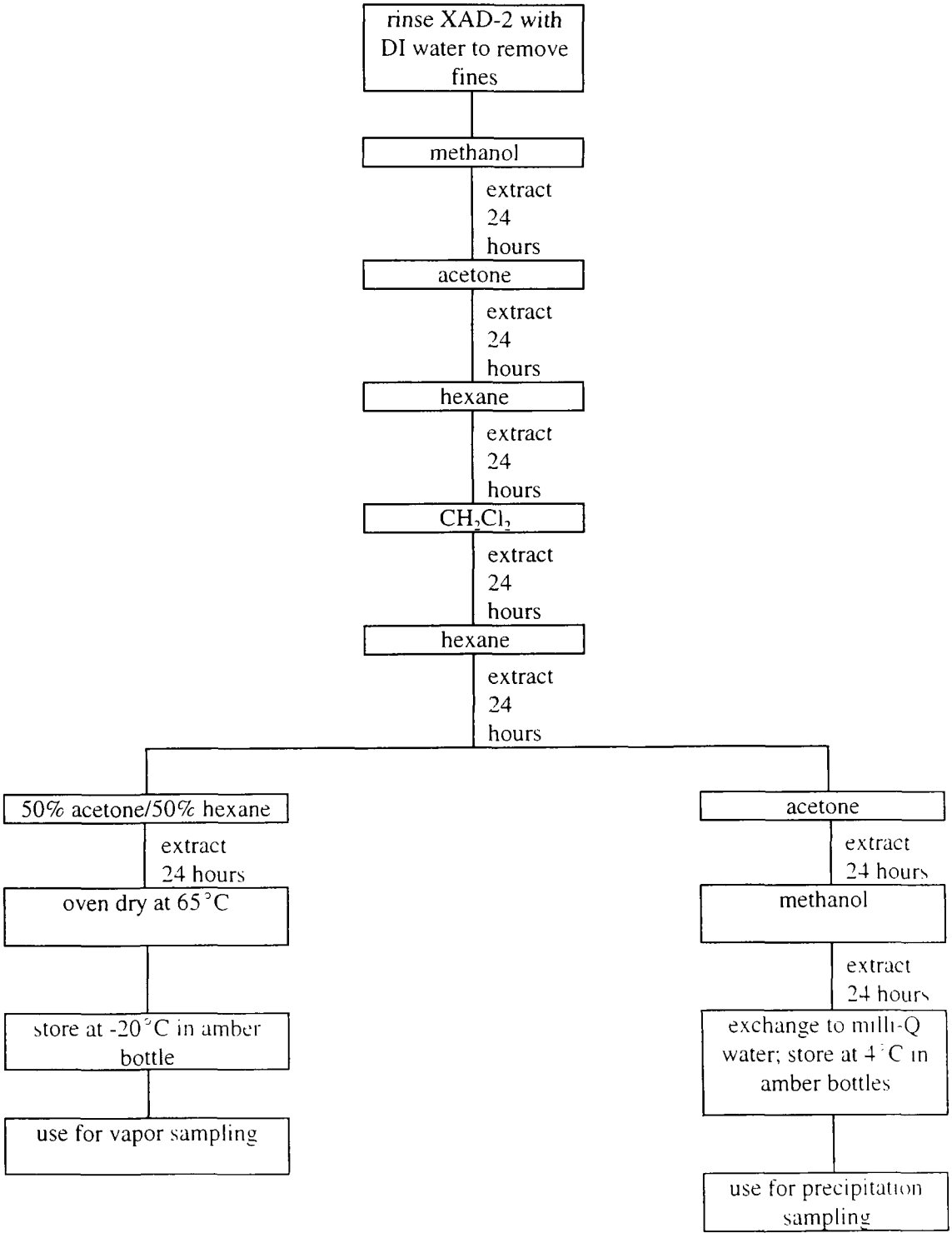
Day 8

1) Turn off heater; cool 15 to 30 minutes.
2) Turn off condenser water.
3) Flush as much methanol from Soxhlet as possible.
4) Rinse XAD-2 at least three times with organic-free DI water.
5) Store the clean XAD-2 in DI water in amber bottle in the refrigerator at 4°C. (The resin may be stored in this manner for up to three months.)
6) Vacuum filter about 50 gm of XAD-2 for use in the preparation of lab blank and matrix spike. Store it in a separate jar at 4°C.

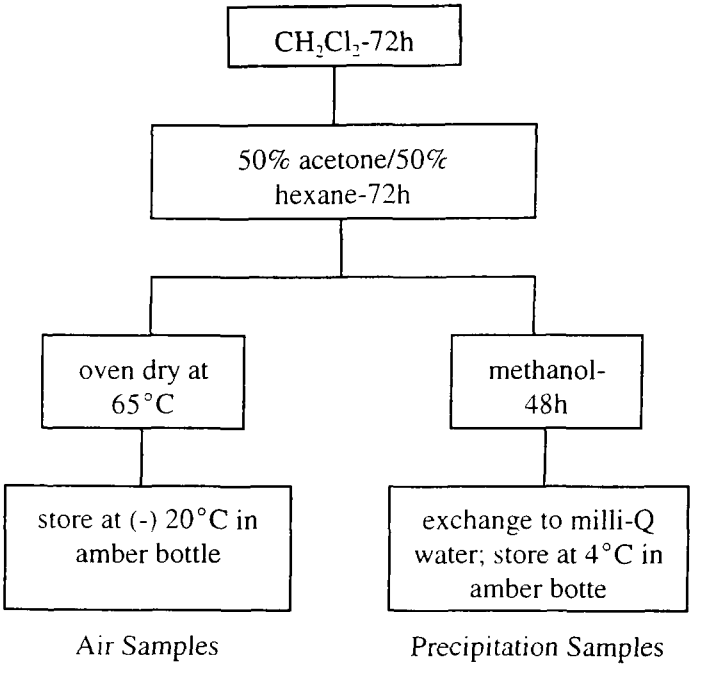
11.1.4 **Notes:** Settings may vary from autotransformer to autotransformer.
Check that the solvent is boiling properly (nice rolling boil).
Solvent may not siphon well. Induce siphoning as many times as possible. Allow extra extraction time when improper syphoning occurs.
If XAD-2 is re-used after sample extraction, it is not necessary to rinse with DI water before extracting.

Extract used XAD with CH_2Cl_2 and acetone:hexane (1:1) for three days each. XAD for precipitation collection requires an additional extraction in methanol for two days.

11.1.5 Flowchart of XAD-2 Precleaning Procedure



11.1.6 Flow Chart of Used XAD_2 Precleaning Procedure



11.2 Glass fiber (GFF) or Quartz fiber (QFF) Filters

Each filter is wrapped with aluminum foil separately, and muffled to 450°C for four hours. After returning to ambient temperature, about 25 are wrapped in aluminum foil and stored.

12.0 Sample Preparation - Extraction

12.1 Air Samples, Particulate and Vapor Phase (Filter and XAD-2 Cartridges)

12.1.1 Samples are extracted as a set. A sample set includes up to 12 samples (including one duplicate, one field blank, one lab blank, and one matrix spike). The matrix spike is spiked with known amount of PCBs, Pesticides, PAHs, and atrazine and is used to calculate the recovery of each analyte for that set. A batch code is assigned to each sample set based on the date of extraction (YY, MM, DD) and sample matrix, such as 951107C (where C = cartridge). All information about the sample set will be recorded on sample preparation log sheets (see Appendix A & B).

12.1.2 Sample extraction requires the following:

large Soxhlet extractor (55/50 and 24/40 joints)
condenser (55/50 joint)
500 mL round bottom flask (24/40 joint)
glass stopper (24/40 joint)
400 mL beaker

Remove spiking standards from freezer. *Standards must be at ambient temperature before using.* (Ambient temperature is achieved in about two hours.)
Thoroughly rinse inside of condenser and outside of joint with methanol then CH_2Cl_2 from wash bottles. Cover joint and exhaust tube with foil.
Assemble supplies and samples under hood and/or utility cart. Label flasks.
Add five to six clean teflon chips into 500 mL round bottom flask.
Pour solvent into round bottom flask: 175 mL of acetone and 175 mL of hexane.

12.1.3 Procedure for compositing XAD-2 samples.

Composite information is included on the Sample Log worksheet. Group the samples to be composited for each site on the lab cart.
Label a 400mL beaker with the same site, year and month as the individual samples with "00" in place of the day. eg. VH01C950300.
Check and record the balance calibration using a 50g and 10g weight. If off by greater than 0.1 g, recalibrate.
Tare the labelled beaker.
Weigh out approximately the same amount of each individual sample so the composite final weight is close to 40g.
Record the initial and final weights of the individual samples as well as the total weight of the composite sample on the sample prep. sheet.
Immediately cover beaker with aluminum foil.

XAD-2 Procedure:

Place glass wool plug at the siphon tube opening of the Soxhlet extractor using glass or metal rod.
Carefully pour XAD-2 into Soxhlet extractor. Rinse container with solvent (acetone/hexane 1:1) to remove all XAD-2; pour solvent rinse into Soxhlet.
Assemble flask/Soxhlet/condenser apparatus. Place on heating mantle.

12.1.4 Procedure for Compositing Filters

Unwrap one filter at a time.
Trim off the black number at the corner with clean scissors.
Use two pairs of blunt tweezers to fold one filter: place in Soxhlet.
Repeat procedure for all filters in composite sample.
Assemble flask/Soxhlet/condenser apparatus. Place on heating mantle.
Rinse tweezers and scissors with CH_2Cl_2.

12.1.5 Surrogate Standard Addition

Using a micropipette dispenser, spike each sample with the PCB, Pesticide, PAH, and atrazine surrogate standards. Record the amount spiked.

12.1.6 Matrix Spike (CMS)

Spike sample medium with the PCB, PAH, Pesticide and atrazine matrix standards. Add surrogate standards used in 12.1.5. Record the amounts added.

12.1.7 Lab Blank (LB)

Spike sample medium with surrogate standards (12.1.5). Record amount added.

12.1.8 Assemble flask/Soxhlet/condenser unit. Place on heating mantle.

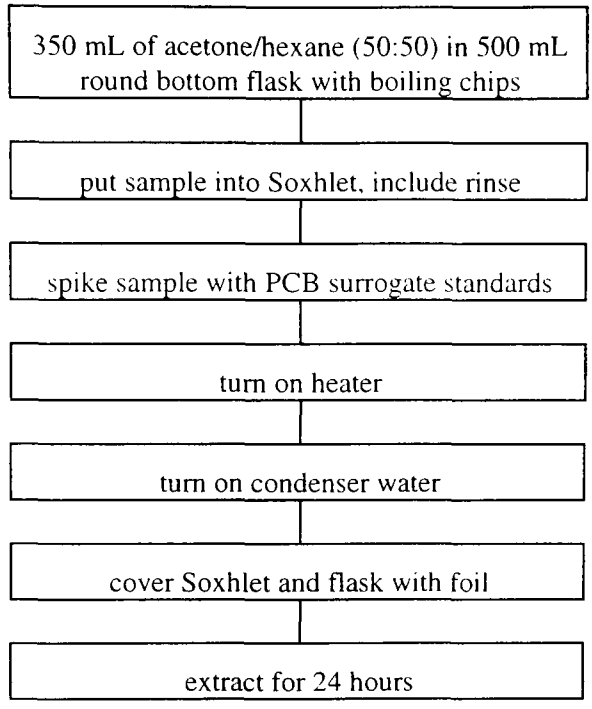
Turn on heating mantles: set Staco heating mantles to 45 or the multi-unit extraction heater to 5. Turn on condenser water. Cover Soxhlet and flask with foil. Extract for 24 hours.

12.1.9 Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from Soxhlet extractor into flask as possible. Detach the flask and insert stopper. Turn off condenser water. Store in cool dark place.

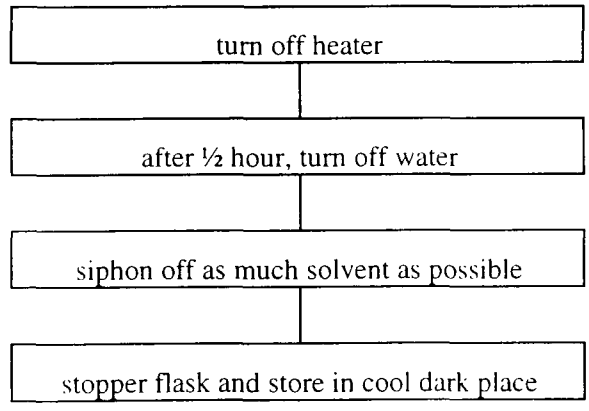
Notes: If XAD-2 gets into the flask, see Removing XAD-2 from flask (12.1.10.3). If condensation is a problem, wrap condensers with foil wrapped insulation or with kimwipes.

12.1.9.1 Flow Charts for Air Sample Extraction

Setting-up extraction:



Taking down extraction:



12.1.10 Rotary Evaporation

12.1.10.1 Fill chamber with DI water. Turn on the chiller circulator.

Set bath temperature:

SOLVENT	TEMPERATURE (°C)
hexane	30-32
acetone	30-32
acetone/hexane	30-32
CH_2Cl_2	30
Methanol	40

Rinse joint of steam duct with CH_2Cl_2. Attach appropriate splash guard(s) to steam duct. Clamp each joint. Turn on and check vacuum system.

12.1.10.2 Evaporation

Remove boiling chips with large forceps. If XAD-2 is in flask, remove it as described in 12.1.10.3.

Attach flask to splash guard. Clamp joint.

Turn on motor to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Evaporation should begin in approximately one minute; solvent should *not* boil.

Evaporate sample down to approximately 2 mL (in a 500 mL round bottom flask, area of liquid should be about the size of a quarter).

Open stopcock of rotary evaporator to release vacuum.

Detach the flask
If exchanges are necessary, add specified amount of hexane from table below, then return flask to splash guard and clamp.

If additional exchanges are not necessary, stopper flask. Store flask under cabinet.

If vacuum unit get hot, turn on cold tap water and allow it to cycle through the bath.

Empty receiving flask into proper waste bottle as needed.

Rinse splash guard with CH_2Cl_2 before using with a different sample. Splash guards should be washed and muffled after every set of samples.

	Fraction	Amount of hexane to add	# of exchanges	Total # of rotary evaporations	Final volume
After extraction		75 mL	2	3	2-5 mL
After column cleanup	hexane	----------	0	1	1 mL
	40%	25 mL	1	2	1 mL
	methanol	25 mL	2	3	1 mL

12.1.10.3 Removing XAD-2 From Flask

Label another 500 mL flask with sample ID. Decant sample from original flask into clean flask. Hexane rinse the XAD-2 remaining in flask and add rinse to new flask. Rotary evaporate new flask using above procedures.

12.1.10.4 Clean-up

Turn off heater and motor on rotary evaporator. Turn off chiller. Empty receiving flask into proper waste solvent bottle. Cover steam duct with foil. Turn off water supply to the vacuum unit, if used.

12.2 Precipitation Samples

12.2.1 Sample extraction requires the following:

large Soxhlet extractor (55/50 and 24/40 joints)
condenser (55/50 joint)
500 mL round bottom flask
glass stopper (24/40 joint)
200 mL (or larger) beaker

12.2.2 Samples are extracted as a set. A sample set will include approximately 12 samples (including at least one duplicate sample, one field blank, one lab blank, and one matrix spike). All information about the sample set will be recorded on the sample preparation log sheets (see Appendix A & B). An example of a set name is 941107P (year, month, day of sample extraction; P = precipitation sample).

12.2.3 Remove spiking standards from freezer. *Standards must be at ambient temperature before using.* (Ambient temperature is achieved in about two hours.)

Thoroughly rinse inside of condenser and outside of joint with methanol then CH_2Cl_2 from wash bottles. Cover joint and exhaust tube with foil.
Assemble supplies and samples under hood and/or utility cart. Label flasks.
Add five to six clean teflon chips into 500 mL round bottom flask.
Measure 175 mL acetone in a beaker.
Place glass wool plug at the siphon tube opening of the Soxhlet extractor using glass or metal rod. Assemble Soxhlet extractor and flask.
Put XAD-2 sample into Soxhlet extractor. Rinse container with acetone from beaker; add this and remaining acetone from beaker to Soxhlet.
Add 175 mL hexane to top of Soxhlet.

12.2.4 Surrogate Standard Addition

Using micropipette dispenser, spike each sample with the PCB, Pesticide, PAH and Atrazine surrogate standards.

12.2.5 Matrix Spike (CMS)

Spike clean XAD-2 with the PCB, Pesticide, PAH, and Atrazine matrix standards. Add surrogate standards as in 12.2.4 above. Record the amount added.

12.2.6 Lab Blank (LB)

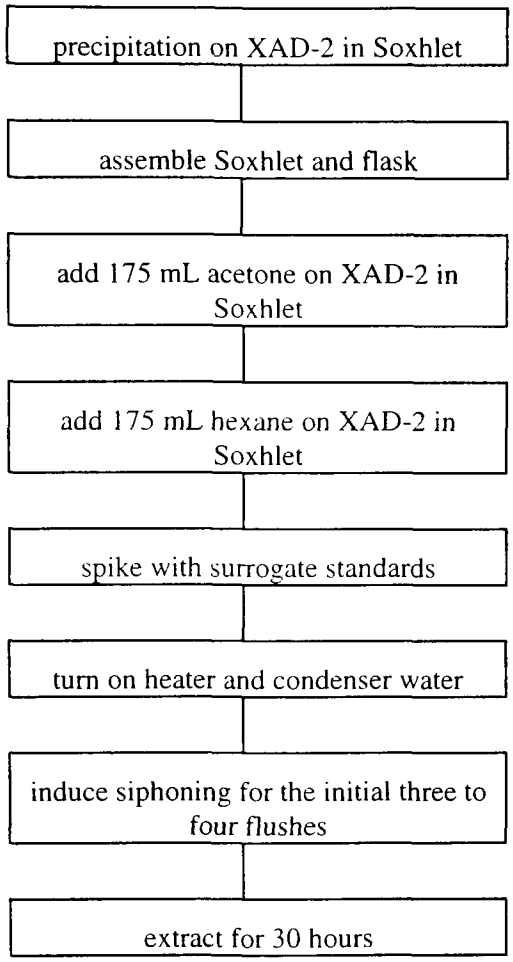
Spike clean matrix with the surrogate standards (12.2.4). Record the amount added.

12.2.7 Assemble flask/Soxhlet/condenser apparatus. Place on heating mantle. Turn on heating mantles: set Staco heating mantle to 45 or the multi-unit extraction heater to 5. Turn on condenser water. Cover Soxhlet and flask with foil. Extract for 30 hours.

Note: The sample has water in it, thus it may not siphon on its own the first two or three times depending on the amount of water present. Induce siphoning until the level of solvent in the Soxhlet and in the syphon tube are the same.

12.2.8 Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from Soxhlet extractor into flask as possible. Detach the flask and insert stopper. Turn off condenser water. Store in cool dark place.

12.2.9 Flow Chart for the Extraction of Precipitation Samples



12.2.10 Rotary Evaporation

12.2.10.1 Fill chamber with DI water.
Turn on the chiller circulator.
Set bath temperature:

SOLVENT	TEMPERATURE (°C)
hexane	30-32
acetone	30-32
acetone/hexane	30-32
CH_2Cl_2	30
methanol	40

Rinse joint of steam duct with CH_2Cl_2. Attach appropriate splash guard(s) to steam duct. Clamp each joint. Turn on and check vacuum system.

12.2.10.2 Evaporation

Remove boiling chips with large forceps. If XAD-2 is in flask, remove it as described in 12.2.10.3. Attach flask to splash guard. Clamp joint. Turn on motor of rotator to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should *not* boil. Evaporate sample until sample is at half the original volume. If vacuum unit gets hot, turn on cold tap water and allow it to cycle through the bath.

Note: If rate of evaporation slows down, *do not* continue. There is water in the sample.

12.2.10.3 Removing XAD-2 from the Flask.

Label another 500 mL flask with the sample ID. Decant sample from original flask into the clean flask; wash with 10 mL hexane twice. Rotary evaporate the new flask with rinses until evaporation begins to slow down.

12.2.10.4 Back Extraction and Solvent Exchanges

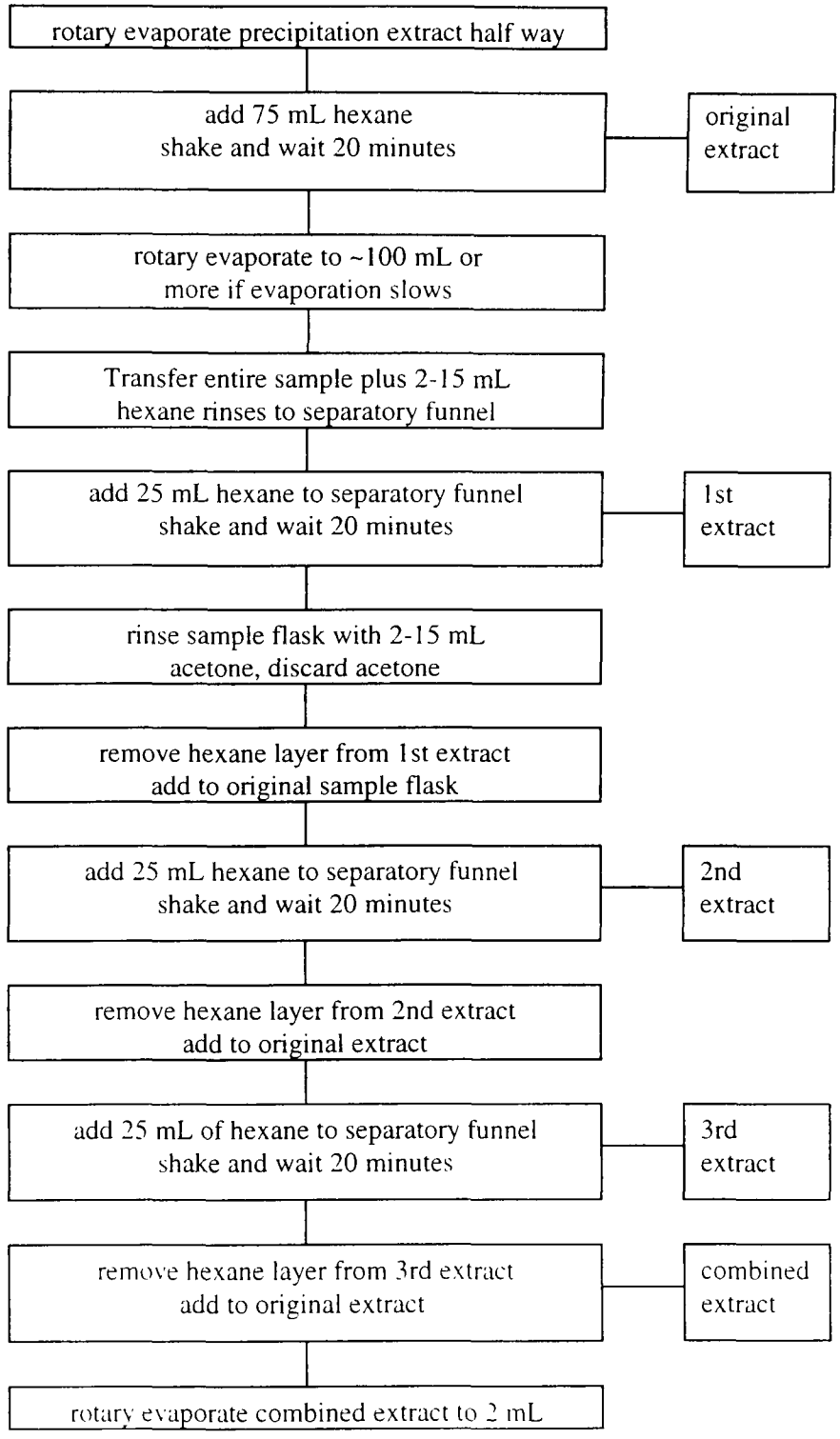
Add 75 mL hexane to sample flask. Shake vigorously; let stand *at least* 20 minutes. Rotary evaporate to a volume of approximately 100 mL or more if evaporation slows. Transfer entire sample plus 2-15 mL hexane rinses to a separatory funnel using a pipet. Rinse sample flask with 2-15 mL acetone and discard. Allow flask to dry. Add 25 mL hexane to separatory funnel. Add approximately 1 gm Na_2SO_4. Shake vigorously. Let stand *at least* 20 minutes then transfer the upper hexane layer back to original flask. Back extract the water layer twice more with hexane. Combine all hexane extracts.

12.2.10.5 Rotary evaporate the combined extract to 2 mL.

12.2.10.6 Clean-up

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

12.2.10.7 Flow Chart of Rotary Evaporation and Back Extraction



13.0 Sample Preparation - Silica Cleanup

13.1 Glassware required:

columns
three 100 mL pear shaped flasks with 14/20 joints
three glass stoppers with 14/20 joints
Pasteur pipettes (9½ inch)
graduated cylinders: 50, 25, and 10 mL
funnel
100 mL beaker
250 mL beaker OR sample jar (need not be clean)
three 250 mL beakers
50 mL beakers

13.2 Column packing and elution amounts

Item	Air Particulate (filter)	Air Vapor (XAD-2)	Precipitation (XAD-2)
amount of silica to activate/deactivate	4-6 gms	8-10 gms	4-6 gms
column size	3½"	7"	3½"
amount of Na_2SO_4	½"	½"	1½"
elution volume (hexane)	23 mL	50 mL	23 mL
switching volume	4 mL	8 mL	4 mL
elution volume (40% DCM)	23 mL	50 mL	23 mL
DCM	15	30	15
switching volume	4	8	4
elution volume (methanol)	23	50	23

13.3 Silica gel activation

Place approximate amount of silica needed in a beaker. Cover beaker with foil. Place beaker in 100°C oven, turn thermostat to 300°C; keep in oven overnight.
Do Not Put Silica Into 300°C Oven!
Turn oven temperature down to 100°C;

Do Not Remove Silica From Oven.
When oven has cooled to 100°C, remove beaker from oven; let cool on counter top until warm (approximately five to 10 minutes); place in desiccator.
When silica has reached ambient temperature (approximately two hours), deactivate it:

13.4 Silica gel deactivation

Working quickly, weigh out desired amount of silica into a round bottom flask. Stopper flask *immediately* after pouring silica.

Add 3% weight/volume of DI water to silica, using the following equation:

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

Shake Well. Shake flask until all clumps are broken-up.
Store in desiccator overnight for equilibration.
Use deactivated silica in desiccator within three days. Any unused silica may be reused after re-activating and re-deactivating.

13.5 Preparation and packing of column(s)

Assemble stopcock(s) on column(s).
Stuff glass wool plug (approximately 1 cm) into lower end of the each column with 20" rod.
Measure and mark appropriate distance from top of glass wool plug for silica and Na_2SO_4 with information from 13.2.
Clamp column(s) securely onto frame in ventilation hood. Place empty glass container under each column (100 mL minimum size; it need not be clean).
Close stopcock(s); fill column(s) half full with hexane.
Make a slurry of hexane and deactivated silica. Pour slurry into each *column. Do Not Allow Silica to Dry Out*: rinse column and beaker with hexane via Pasteur pipette. (Use of a funnel may facilitate process.) Open stopcock(s). Tap column(s) with rubber hammer to pack silica. Add silica/hexane as needed until desired length is loaded.
Cap column(s) with ½" Na_2SO_4 for XAD-2 and filter samples, 1½" Na_2SO_4 for precipitation samples.
Wash column(s) with 25 or 50 mL hexane for equilibrium.
When hexane level reaches top of Na_2SO_4, close stopcock(s) to prevent further dripping. *Never Let Column Run Dry.*
If column(s) is/are not going to be used *immediately*, stopper column(s) and cover tip(s) of column(s) with foil.

13.6 Cleanup procedure

13.6.1 Label one 100 mL pear-shaped flask for each fraction which is to be collected.
On a cart, assemble pear shaped flasks and remaining supplies.
Place sample flask in front of column.

Place a 50 or 100 mL beaker in front of sample flask.
Add hexane to 50 or 100 mL beaker; cover with foil (see 13.2 for hexane volume required).

13.6.2 Column chromatography

13.6.2.1 First Fraction (hexane)

Sonicate sample flask before loading the sample onto the column to detach the particles adhering to the walls of the flask.
Remove stopper from sample flask. Assemble pipette and rubber bulb; place pipette in sample flask.
Place fraction #1 (hexane) pear shaped flask under the column.
Load sample into column with Pasteur pipette.
Set drip rate to approximately one drip per second. Add approximately 5 mL hexane to sample flask from the beaker. Swirl solvent in flask.
When sample has drained down to the top of the Na_2SO_4 add the hexane from the sample flask to the column. Add an additional 5 mL hexane to the sample flask from the beaker. Swirl solvent in flask.
When solvent has drained down to the top of the Na_2SO_4 add the second 5 mL hexane to the column. Add the remaining hexane from the beaker to the sample flask. Swirl solvent in sample flask.
When solvent has drained down to the top of the Na_2SO_4 add the remaining hexane from the sample flask (If reservoir on top of the column cannot hold entire amount, add as much as possible, then refill as space becomes available).

Note: Stagger the timing of the column loadings such that the changing of the flasks are not concurrent.

13.6.2.2 Second Fraction (40% CH_2Cl_2/60% hexane)

While the hexane is eluting (from the first fraction), measure the hexane/CH_2Cl_2 and put it into the appropriate containers. (See the following table.) Swirl the hexane/CH_2Cl_2 in the sample flask.

column size (in)	hexane/CH_2Cl_2 in beaker (mL)	switching volume in flask (mL)
3½	23	4
7	50	8

When the hexane level reaches the top of the Na_2SO_4 add the switching volume hexane/CH_2Cl_2 from the sample flask to the column.
Transfer the hexane/CH_2Cl_2 from the beaker to the sample flask. Swirl the solvent in the flask.

Place the appropriate pear shaped flask (labeled '40%' fraction) next to the flask under the column.
When the hexane/CH_2Cl_2 level in the column is to the top of the Na_2SO_4 quickly switch flasks and pour as much of the remaining hexane/CH_2Cl_2 into the column as possible. Add hexane/CH_2Cl_2 to the column as space permits.
Continue to monitor the rate of drip (approximately one drip per minute).
Place the pear shaped flask from the first fraction on the supply cart.
Stopper the flask.
DCM column conditioning: While the hexane/CH_2Cl_2 is dripping, measure out 15 mL DCM for 3½" columns or 30 mL for 7" columns and put into beaker.
Place a waste jar next to the flask under the column.
When the hexane/CH_2Cl_3 reaches the top of the Na_2 SO_4, add the DCM to the column and quickly switch the flask and waste jar.
Stopper the second fraction flask and place on the supply cart.

13.6.2.3 Third Fraction (MeOH)

While the DCM is eluting, measure the methanol and put into the appropriate containers (See the following table).

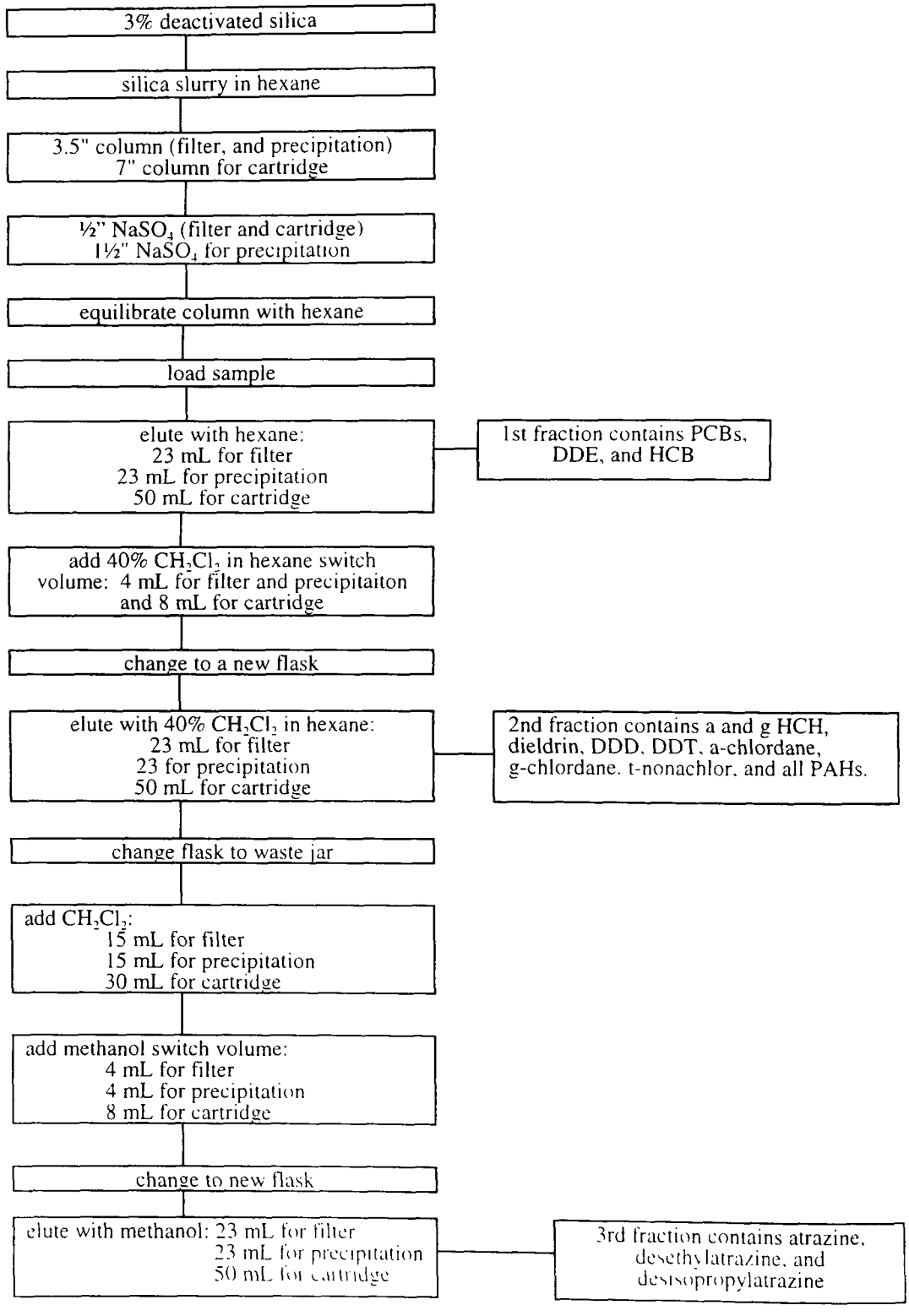
column size (in)	methanol in large beaker	switching volume in small beaker
3½	23	4
7	50	8

When DCM reaches the top of Na_2SO_4, add the switching volume to the column.
Place the appropriate flask (labeled "MeOH fraction") next to the waste jar under the column.
When the methanol level in the column is to the top of the Na_2SO_4, quickly switch the flask and waste jar and pour the remaining methanol onto column.
Open the stopcocks fully so the methanol elutes as fast as possible.
Discard DCM from the waste jars.
Once the column has stopped dripping, remove flask and stopper it.

13.7 Clean-Up

Remove stopcock from column.
Turn column upside down and secure it with clamps. Place container under column to catch Na_2SO_4 and silica.
After column has dried out, use vacuum (air or water) to remove glass wool plug.
Pour silica and Na_2SO_4 into used glove or foil before discarding into trash can.

13.8 Flow-Chart



14.0 Rotary Evaporation after Column Chromatography

14.1 Attach flask to splash guard. Clamp joint.
Turn on motor of rotator to pre-determined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Evaporation should begin in approximately one minute; solvent should *not* boil.
Evaporate sample down to approximately 2 mL.
Open stopcock of rotary evaporator to release vacuum.
Detach the flask:
If vacuum unit gets hot, turn on cold tap water and allow it to cycle through the bath.
If exchanges are necessary, add specified amount of hexane as listed in 14.2, then return flask to splash guard and clamp.
If additional exchanges are not necessary, stopper flask and store it under the cabinet.
Empty receiving flask into proper waste bottle as needed.
Rinse splash guard with CH_2Cl_2 before using with a different sample. Splash guards should be washed and muffled after every set of samples.

14.2 Solvent Exchange

	fraction	amount of hexane to add	# of exchanges	total # of rotary evaporations	final volume
after column chromatography	hexane	----	0	1	1 mL
	40%	25 mL	1	2	1 mL
	methanol	25 mL	2	3	1 mL

15.0 Extract Transfer

15.1 Label each 4 mL amber vial with sample ID and fraction ID.

15.2 Using a Pasteur pipette, quantitatively transfer entire sample from flask to amber vial rinsing with hexane.
Note: Do not add so much solvent as to fill the vial. If it is too full, there is a chance of splashing at the time of N_2 blowdown.

Close amber vial tightly, place in vial file, and store in freezer. Label the vial file with sample set name, type of samples, and site of collection.

16.0 N_2 Blow Down

16.1 Set-up

Let N_2 flush the system for approximately five minutes. Ensure the N_2 cylinder pressure is >500 psi.
Turn heater on *Low*, 5.5 setting
Attach clean needle to each nozzle to be used.

16.2 Blow down

Place amber vials in heater block; adjust N_2 flow such that there are *gentle* (barely detectable) ripples in the vials.
Evaporate samples down to a final volume of 0.5 to 4.0mL according to sample site, matrix, and season. Document the actual amount on the sample preparation log sheet.
Note: Volumes may be changed as analyte amounts and/or interferences fluctuate.

16.3 Cleanup

Turn off N_2.
Replace the nozzle caps.
Soak used needles in MeOH. Sonicate needles with CH_2Cl_2 three times prior to reuse.
Store in a foil covered beaker.

17.0 Spiking Samples with ISTD

17.1 Remove ISTDs from freezer; equilibrate to ambient temperature (approximately two hours).

17.2 Clean micropipette

Remove glass tube used to cover plunger.
Rinse plunger with CH_2Cl_2. Allow to dry
Without touching glass tubes, insert plunger into new glass tube;
Rinse pipette tube:

1) Draw-up CH_2Cl_2 into pipette and discard in solvent waste container.
2) Draw-up hexane into pipette and discard in solvent waste container. Repeat five times.
3) Vortex standard bottle to mix solution.
4) Draw standard into pipette and discard in solvent waste container. Repeat two times.

17.3 Spike sample vial (as described).

fraction	analyte group	ISTDs	color code
hexane	PCBs	PCB 30 PCB 204	red
40%	OC pesticides	DDE PCB 65 PCB 155	blue
40%	PAHs	anthracene-d10 benzo(a)anthracene-d12 perylene-d12 triphenylmethane	black
MeOH	atrazine	anthracene-d10	black

17.4 Mark each amber vial label with a appropriate color of dot (use a water-proof marker).

17.5 Rinse tip of micropipet with DCM and hexane, replace glass tube used to cover plunger. Store micropipette.

17.6 Label vial file with the following information (use same color of pen as dots on microvials): date microvials spiked fraction spiked initials of chemist spiking

17.7 Mix samples vigorously after spiking.

18.0 Preparing Samples for GC Analysis

18.1 Glassware required
disposable GC vials
200 µL disposable inserts

18.2 Label GC vials with sample IDs. In addition, label an extra GC vial for hexane and the appropriate calibration and performance standards for every set of samples.
Place inserts into the GC vials.
Using a Pasteur pipette, remove approximately 200 µL of each sample and put into the appropriate vials. (The level of liquid should be at least half the volume of the insert.) Also place 200 µL of hexane and 200 µL of the appropriate standards into individual vials (See following chart for the appropriate standard).

fraction	target compounds	calibration standard
hexane	PCBs	PCB calibration standard with DDE and HCB
40%	PAHs	PAH standards
MeOH	atrazine	atrazine standards
40%	OC pesticides	mixed pesticide standard

Cap microvial.
Load microvials into autosampler.

19.0 References

19.1 *Baker, J.E., Eisenreich, S.J.* PCBs and PAHs as Tracers of Particulate Dynamics in Large Lakes. *J. Great Lakes Res.*, 1989, 15(1), #84-103.

19.2 *Bidleman, T.F.; Mathews, J.R.; Olney, C.E.; Rice, C.P* Separation of Polychlorinated Biphenyl, Chlordane and p-p DDT from Toxaphene by silicic acid column chromatography. *JAOAC*, 1978, 61, 820-828.

19.3 *Harlin, K.S.; Surratt, K; and Peters, C.* Standard Operating Procedure for the Analysis of PCBs and Organochlorine Pesticides by GC-ECD. Illinois State Water Survey, Champaign, IL 61820, November 1995.

19.4 *Hermanson, M.H.; Hites, R.A.* Long-Term Measurement of Atmospheric Polychlorinated Biphenyls in the Vicinity of Superfund dumps. *Environ. Sci. Technol.*, 1989, 23. No. 10, 1253-1258.

19.5 Lake Michigan/Superior Load Monitoring Quality Assurance Program Plan (QAPP), Feb. 1993.

19.6 *Marti, E.A.; Armstrong D.E.* Polychlorinated Biphenyls In Lake Michigan Tributaries. *J. Great Lakes Res.*, 1990, 16(3): 396-405

19.7 *McVeety, B.D., Hites, R.A.* Atmospheric Deposition of Polycyclic Aromatic Hydrocarbons to Water Surfaces: A Mass Balance Approach. *Atmos. Environ.*, 1988, 22, 511-536.

19.8 *Mullin, M.D.* Personal Communication, June 1995

19.9 *Mullin, M.D.* PCB Workshop, U.S. EPA Large Lakes Research Station, Grosse Ile, MI, June 1985.

19.10 *Murphy, T.J.; Rzeszutko, C.P* Precipitation inputs of PCBs to Lake Michigan. *J. Great Lakes Res.*, December 1977. *Internat. Assoc. Great Lakes Res.*, 3(3-4): 305-312.

19.11 *Peters, C. and Harlin, K.S.* Standard Operating Procedure for the Analysis of PAHs and Atrazine by GC/Ion Trap MS. Illinois State Water Survey, Champaign, IL 61820, July 1995.

19.12 Quality Assurance Project Plan (QAPjP) Atmospheric Monitoring for the Lake Michigan Mass Balance and the Lakes Michigan and Superior Loading Studies, July 1995, Revision 5.

19.13 *Swackhamer, D.L.; Mc Veety, B.D.; Hites R.A.* Deposition and Evaporation of Polychlorinated Biphenyl congeners to and from Siskiwit Lake, Isle Royale, Lake Superior. *Environ, Sci. Technol.*, 1988, 22, 664-672.

19.14 *Sweet, C.W., Vermette, S.J.; Gatz, D.F* Atmospheric Deposition of Toxic Materials: A Compound of the Green Bay Mass Balance Study. 1992, Contract Report 530, Illinois State Water Survey, Champaign, IL 61820.

Appendix A.

Sample Preparation Log Form
Filter and Precipitation Samples

LAKE MICHIGAN LOADING STUDY (LMLS)
SAMPLE PREPARATION LOG

SAMPLE SET NAME: _____

FIELD ID	LAB ID	COMMENTS

NOTES:
SAMPLE SET NAME: _____

LAB PROCEDURES	DATE PERFORMED	BY (INITIALS)	COMMENTS
Extraction			
Extract Concentration (hexane/acetone)			
Back Extraction (if Appl.)			
Silica Column Cleanup			
Concentration of Fraction	_____	_____	
hexane	_____	_____	
40%	_____	_____	
MeOH	_____	_____	
Nitrogen Blowdown	_____	_____	
hexane	_____	_____	
40%	_____	_____	
MeOH	_____	_____	

ADDITIONAL COMMENTS:

_____

_____

_____

Appendix B.

Preparation Log Form
Composited Cartridge Samples

LAKE MICHIGAN LOADING STUDY (LMLS)
SAMPLE PREPARATION LOG

SAMPLE SET NAME: _____

FIELD ID/WT (g)	SUB SAMPLE WT (g)	LAB ID/TOTAL WT EXTRACTED (g)	COMMENTS

NOTES:

SAMPLE SET NAME: _____

LAB PROCEDURES	DATE PERFORMED	BY (INITIALS)	COMMENTS
Extraction			
Extract Concentration (hexane/acetone)			
Back Extraction (if Appl.)			
Silica Column Cleanup			
Concentration of Fraction	_____	_____	
hexane	_____	_____	
40%	_____	_____	
MeOH	_____	_____	
Nitrogen Blowdown	_____	_____	
hexane	_____	_____	
40%	_____	_____	
MeOH	_____	_____	

ADDITIONAL COMMENTS:

_____

_____

_____

Standard Operating Procedure for the Analysis of PAHs and Atrazine by GC/Ion Trap MS

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SOP # CH-IN-003.1

July 1995

Standard Operating Procedure for the Analysis of PAHs and Atrazine by GC/Ion Trap MS SOP # CH-IN-003.1

1.0 Scope And Application

This method is used to determine the concentrations of polycyclic aromatic hydrocarbons (PAHs), atrazine, and degradation products in extracts from vapor, particulate, and precipitation samples. The method is specific for the IADN and LMMB/LMLS projects. The following analytes are measured by this Standard Operation Procedure (SOP).

Analyte	*CAS#*
atrazine	1912-24-9
desethylatrazine (DEA)	6190-65-4
desisopropylatrazine (DIA)	1007-28-9
acenaphthene	83-32-9
acenaphthylene	208-96-8
anthracene	120-12-7
benzo(a)anthracene	56-55-3
benzo(a)pyrene	50-32-8
benzo(b)fluoranthene	205-99-2
benzo(e)pyrene	192-97-2
benzo(ghi)perylene	191-24-2
benzo(k)fluoranthene	207-08-9
chrysene	218-01-9
coronene	191-07-1
dibenzo(a,h)anthracene	52-70-3
fluoranthene	206-44-0
fluorene	86-73-7
indeno(123cd)pyrene	193-39-5
phenanthrene	85-01-8
pyrene	129-00-0
retene	483-65-8

2.0 Summary of Method

This method describes equipment and procedures for operating the GC-Ion trap MS, and instrument optimization specific for PAHs, atrazine and atrazine metabolites. The method is specific for the IADN and LMLS/LMMB atmospheric deposition research projects.

2.1 Personnel Restrictions

This method is restricted to use by or under the supervision of an analyst trained and experienced in the operation of gas chromatographs, mass spectrometers, mass spectral and capillary chromatogram interpretation, and data reduction. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.2 Working Linear Range

A multipoint calibration curve will be constructed for each analyte to document the working linear range.

2.3 Limit of Detection

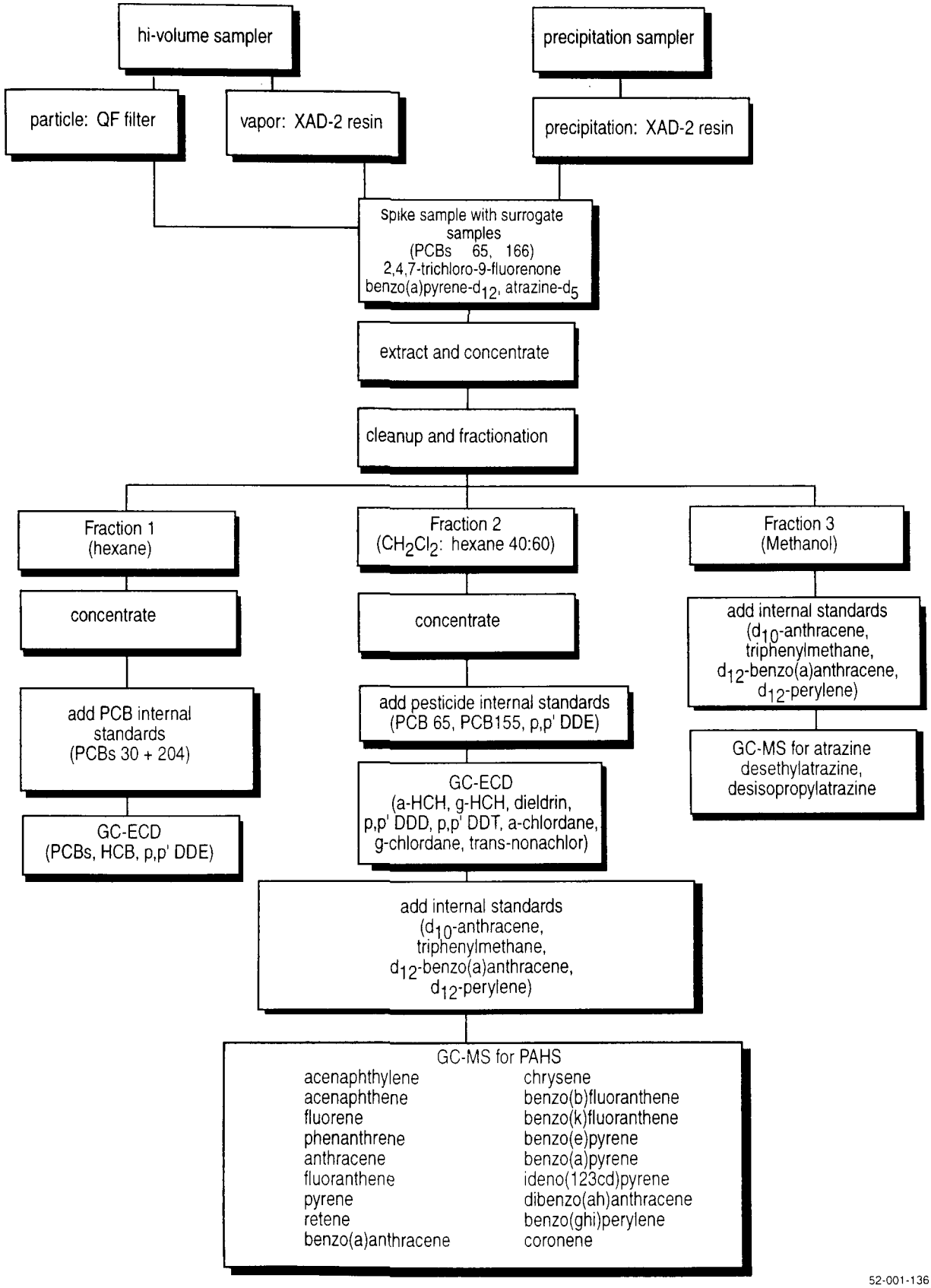
2.3.1 IDL

The instrument detection limit (IDL) refers to the smallest signal above background noise that an instrument can reliably detect. The IDL is determined from a data set comprised of three separate chromatographic runs of a low level calibration standard; each run contains 7-10 analyses of the standard. The IDL equals the Student's t value (n-1) multiplied by the standard deviation of this data set.

2.3.2 MDL

Method detection limits (MDL) are defined in CFR, Vol 49, No. 209, October 26, 1984, Appendix B to Part 136. Matrix specific MDLs are determined by spiking 7-10 clean matrix samples with the analytes of interest and processing them through the entire extraction, cleanup, and analysis procedure.

2.4 Flow Diagram



3.0 Safety Precautions

3.1 The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined. However, each one must be treated as a potential health hazard, and exposure to these chemicals should be minimized. Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.

3.2 Chemists working in the laboratory should follow ISWS safety rules :

3.2.1 A lab coat is required when working in the lab.

3.2.2 Eye protection with splash resistant safety glasses or safety goggles are required.

3.2.3 Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.

3.2.4 All solvent work should be done in fume hoods.

3.2.5 Open shoes are not allowed in the laboratory.

3.2.6 Particle mask is required when using dry silica.

3.2.7 Avoid working alone in the laboratory. If work must be performed after hours or in the weekend inform the supervisor or other staff so that your presence is known and will be accounted for in case of an emergency.

3.2.8 Chemicals and solvents are stored under the hoods. Acids must be separated from bases. A rubber bucket is required to transport any chemical.

3.2.9 Gas cylinders should be well secured at all times. Flammable gases are stored in separate storage areas.

3.2.10 Wash hands well after work.

3.2.11 No food or drink is allowed in the laboratory.

3.2.12 In case of minor spillage, get spillage kit to clean the area. A major spill requires the University of Illinois Fire Department to be contacted and the working area evacuated. MSDS sheets are stored in the laboratory and a copy placed on file with the office administrator.

3.2.13 All chemicals and standards must be labeled with chemical name, date, and initials of person to contact.

3.2.14 Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

3.3 Waste Disposal

Solvents: Label waste containers, *Chlorinated Waste* and *Non-Chlorinated Waste*. Glass bottles used for waste are placed under hoods for convenience. When full, transfer waste to 10 L carboy containers in solvent cabinet. Contact the ISWS Waste Coordinator for removal.

4.0 Apparatus and Materials

4.1 Glassware: General Requirements

All glassware, must be meticulously cleaned. Large glassware is thoroughly washed with laboratory detergent and hot water. Glassware with bad stains should be rinsed with MeOH or CH_2Cl_2 before using the soap and water procedure. If still not clean, soak in H_2SO_4:HNO_3 (50:50) acid bath overnight, then wash thoroughly with soap and water. Volumetric pipettes used for standards *must* soak in acid bath overnight. Glassware is thoroughly rinsed with tap water, then with DI water and allowed to air dry. The glassware is foil wrapped and heated 450°C for four hours. If glassware is not clean after muffling at 450°C for 4 hours, muffle at 500°C for four hours. The glassware is cooled to ambient temperature and stored in a clean location. Small glassware such as stoppers, vials, and disposables are wrapped in foil or placed into a beaker and covered with foil and heated to 450°C for four hours, cooled to ambient temperature, and stored in a clean location. Vials are capped as soon as they are removed from the oven. Note: Always use dull side of foil towards glassware. Set initial temperature of furnace to 200°C if possible.

4.2 Vortex Mixer

4.3 Volumetric flasks and pipets - Class A, various sizes

4.4 Auto sample vials, amber, 2 mL with 200 µL inserts, caps and teflon-lined septa

4.5 Positive displacement micro pipet, glass capillaries (Drummond or equivalent)

4.6 GC-Ion Trap MS (see Section 9.0 for detailed information)

4.6.1 Model: Varian Saturn 3, capillary, GC/Ion trap-MS system

4.6.2 Injector: SPI, 8200 autosampler, LB2 Thermogreen septa (Supelco)

4.6.3 Column: 30 m x 0.25 µm x 0.25 mm, DB-5 MS (J & W Scientific)

4.7 Chemicals/Standards
Pesticide residue quality or equivalent. All reagents are evaluated for interferences using laboratory blanks.

4.7.1 Solvents, EM Omnisolve or equivalent

4.7.1.1 Hexane

4.7.1.2 Methanol

4.7.1.3 Acetone

4.7.2 Standards

4.7.2.1 Atrazine and PAH Stock Standards

Stock standard solutions are purchased from commercial sources (Ultra Scientific, Accustandard, Chem Service, Cresent Chemical) or are obtained from the USEPA repository. When stock solutions are not available, pesticides are purchased as the neat material and gravimetrically prepared in house.

4.7.2.2 Surrogate Standard Solutions

4.7.2.2.1 Surrogate standards are purchased from commercial sources (Aldrich Chemical, Cambridge Isotope) or are obtained from the USEPA repository. The following surrogate standards are utilized:

atrazine-d5 (atrazine surrogate)
benzo-(a)pyrene-d12 (PAH surrogate)

4.7.2.2.2 If stock solutions are not commercially available, they are gravimetrically prepared from the neat material. Individual stock solutions are serially diluted in volumetric flasks to obtain the surrogate spike standard/s. A combined surrogate spiking standard may be prepared to save sample preparation time during the extraction procedure.

4.7.2.2.3 All samples are spiked with surrogate standards prior to extraction using volumetric pipets or a Drummond pipet and the spike volumes recorded on the sample preparation log.

4.7.2.3 Internal Standard Solutions (ISTDs)

4.7.2.3.1 Internal Standards are purchased commercially (Ultra Scientific) as a stock standard or as the neat material. The following ISTDs are utilized:

anthracene-d10 (PAH and atrazine ISTD)
benzo(a)anthracene-d12 (PAH ISTD
perylene-d12 (PAH ISTD)
triphenylmethane (PAH ISTD)

4.7.2.3.2 If stock solutions are not commercially, available they are gravimetrically prepared from the neat material. Individual stock solutions are serially diluted in volumetric flasks to obtain the ISTD spiking standard/s.

4.7.2.3.3 ISTDs are added to the appropriate sample fraction (PAHs in 40% DCM, and atrazine in MeOH) prior to GC-MS analysis. A Drummond micropipet is used for ISTD addition.

4.7.2.4 Chromatographic Calibration Standards

Combined instrument calibration standards are prepared from the individual stock standards by volumetric dilution to obtain five concentration levels. The calibration standard concentrations bracket the expected analyte amounts in samples assayed and are within the working linear range of the detectors. Calibration mixes are prepared specifically for the appropriate instrument and fraction analyzed. The following calibration mixes are prepared.

PAHs with surrogate and ISTDs
atrazine, DEA, and DIA with surrogate and STDs.

4.7.2.5 Matrix Spiking Solutions

4.7.2.5.1 Combined matrix spiking solutions are prepared from the individual stock standards by volumetric dilution. Combined matrix spike solutions are prepared for each analyte group. The following matrix spike mixes are prepared:

PAHs
atrazine, DEA, DIA.

4.7.2.5.2 The matrix spike solutions will be added to clean sample matrix material prior to extraction to calculate the recovery of individual analytes. One matrix spike will be extracted with each batch of samples. The matrix spike will be added to the sample using a Drummond micropipet or a volumetric pipet and the spiking amounts reported in the sample preparation log. Detailed sample preparation procedures can be found in SOP #CH-PR-001.3, March 1995.

4.7.2.6 Standard Evaluation

New working standards will be assayed prior to use by comparison with existing standards. Standards must agree within 10% prior to use.

4.7.3 Gases

4.7.3.1 Helium, carrier gas, 99.9999% chromatographic grade

4.7.3.2 Carbon Dioxide, SPI coolant, general grade

4.7.3.3 Air, autosampler pressurizing gas, general grade

5.0 GC/MS System Evaluation

Prior to each run the GC/IT-MS system performance and calibration are verified for all analytes. A mass spectrometer tune is performed prior to each run using system software with perfluorotributylamine (PFTBA) calibration gas. Adjustments are made or maintenance is performed such that selected calibration masses and their respective isotopes meet the target mass-intensity criteria.

Hexane is injected immediately prior to each run to ensure the system is free of contaminants or interfering peaks.

Records of daily system performance and maintenance are maintained.

Note: *The system is evaluated and tuned with the column and injector set at the highest temperature attained during a normal acquisition (i.e., column at 300 °C and injector at 289 °C). This is to assure optimal conditions for the high-boiling compounds (such as coronene).*

5.1 Daily Evaluation

5.1.1 Check air/water for leaks.
Set mass range to 10-45 m/z; insure AGC is off; set ion time to 300μsec; set filament emission current to 10 µA; turn on the filament, multiplier, and RF; normalize the peaks. The following conditions indicate there is no evidence of a significant air leak or water background present:

a) There are discrete peaks at 18, 28, and 32.
b) The peak at 28 is higher than that at 18 and the 28:32 ratio is about 4:1.
c) The ratio of peak 18 to peak 19 is 10:1 or greater.
d) The 100% counts value is less than 500.
e) The TIC value is less than 2000.

5.1.2 Check PFTBA calibration gas level.
The ionization time should read between 500-1000 µsec.

5.1.3 Check the valley to isotope % of ions 131 and 132.
The value should be around 25%. This means the 132 isotope peak is four times the height of the valley between ions 131 and 132. Adjust tune parameters, if necessary, to obtain the proper valley/isotope %:

a) Begin by setting the mass range to 127-137 m/z. Turn on the multiplier, filament, RF, calibration gas, and AGC. Set the AGC target value to 20000.
b) Adjust the A/M (axial modulation) voltage until the valley/isotope % appears to be close to 25%. The axial modulation value is typically between 2.5 and 5 volts.
c) Run the *Set AGC Target* program. The AGC target should be optimally set to 20000 - 25000. If not, the A/M voltage and/or the electron multiplier voltage may need to be (re)adjusted.

5.1.4 Run a mass calibration with PFTBA calibration gas. Verify valid calibration prior to proceeding.

5.2 Weekly Evaluation

5.2.1 Check integrator zero.
When both the RF and the multiplier are on, the measured signal should be between 0.2 and 0.8 ADC counts. If the integrator zero is not within these limits, it must be adjusted.

5.2.2 Set AGC target.
The *Set AGC Target* program should be run to verify the valley/isotope % is actually 25%. This program is also a check on the electron multiplier value. The optimal target value is 20000.

5.3 Monthly Evaluation

5.3.1 Check multiplier voltage.

5.3.2 Check filament emission current.
The filament emission current is set to 10 μA. The *Set Filament Emission Current* program should be ran to verify instrument performance. If the program wants to set the filament emission current higher, this is usually indicative of a high level of background.

5.3.3 Check RF voltage ramp
The RF voltage ramp should rise gradually in a generally straight line from low mass to high mass throughout the entire mass range, without any sudden rises in the ramp. The *average* response value should be 300 - 500 ADC counts and the *highest* response value should be 500-900 ADC counts.

5.3.4 Check carrier gas flow rate.
The optimum carrier gas volumetric flow rate into the ion trap is 1 mL/min. at the maximum temperature reached during an analysis. This is determined by measuring the time required for an unretained compound, such as air, to elute from the column, and then calculating the volumetric flow rate using the following formula:

$$\textit{Volumetric flow rate } (mL/\text{min}) \quad \frac{\pi r^2 l}{t}$$

Where:

π is 3.14
r is the radius of the column (cm)
l is the length of the column (cm)
t is the retention time of the unretained compound (min)

6.0 Periodic GC/MS Maintenance

6.1 Change injector septa after approximately 100 injections.

6.2 Vent rough pump a minimum of two times per month.

6.3 Change hexane in injector rinse reservoir every month.

6.4 Change waste arm septa every month.

6.5 Change rough pump oil every three to six months.

7.0 Preparation of Autosampler Vials for GC/MS Analysis

7.1 Remove standards and samples from freezer; equilibrate to ambient temperature (approximately two hours).

7.2 Initiate sample log sheet for GC/MS run. Each analytical run consists of a hexane blank, standards to establish a calibration curve, a performance check standard if available, samples, a calibration check standard every five to seven samples, and a final calibration check and hexane blank.

7.3 Label autosampler vials for samples, standards, and hexane blanks.

7.4 Insert 200 µL glass insert into each vial (except in cases of sample dilutions).

7.5 Spike samples with ISTD if necessary. (See Section 8.0 for spiking procedure.)

7.6 Before transferring samples and standards to the corresponding labeled autosampler vials, mix contents of each vial and bottle well by holding on a vortex mixer for 5-10 seconds. Use muffled Pasteur pipettes for transfer. The glass insert should be filled approximately half full.

7.7 Place cap onto autosampler vial after it is filled.

7.8 Load autosampler vials onto autosampler.

8.0 Spiking Samples with ISTD

8.1 Remove ISTDs from freezer; equilibrate to ambient temperature (approximately two hours). Vortex ISTD solution to mix.

8.2 Clean micropipette:

Remove glass tube used to cover plunger.Rinse plunger with CH_2Cl_2. Allow to dry. Without manually touching glass tubes, insert plunger into a new glass tube. Tighten tube in position.

Rinse pipette tube:

1) Draw up CH_2Cl_2 into pipette and discard into solvent waste container.
2) Draw up hexane into pipette and discard into solvent waste container. Repeat five times. Allow to dry.
3) Draw up internal standard into pipette and discard into solvent waste container. Repeat two times.
4) Fill micropipette.

8.3 Spike sample vial. See chart below for internal standard and amount.

Fraction	Compound	Type of Sample	Internal Standard	Spike Volume (µL)	Final Mass in Sample (ng)*	Color of Dot on Label
Hexane	PCBs and Pesticides	vapor, particle, and rain	PCB 30	100	8	Red
			PCB 204		6	
40%	Pesticides	vapor, particle, and rain	PCB 65	100	23.7	Blue
			PCB 155		17.5	
			DDE		20	
40%	PAHs	vapor, particle, and rain	d10-Anthracene	100**	200	Black
			Triphenylmethane		100	
			d12-Benzo(a)anthracene		200	
			d12-Perylene		200	
MeOH	Atrazine	vapor, particle, and rain	d10-Anthracene	100	200	Black

*Values in this column are approximate and may change slightly depending on the exact concentrations of the compounds in the stock solutions.
**If the sample is expected to be high in concentration of PAHs or if the final sample volume is greater than 2 mL, more ISTD solution may be added in increments of 100µL.

8.4 Mark each sample vial label with the appropriate color of dot to verify sample has been spiked. (Use water-proof marker.)

8.5 Rinse with solvent and replace glass tube used to cover plunger of micropipette. Store micropipette.

8.6 Mark sample box with the following information using the same color of pens as dots on vial labels:

Date sample vials spiked.
Fraction spiked.
Initials of chemist spiking.

9.0 Normal Operating Parameters for Saturn 3 GC/MS

9.1 Operating software: Version 5.0 or later

9.2 Operating mode: EI (electron impact)

9.3 AGC (automatic gain control): ON

9.4 AGC prescan factors:

Background mass:	98
Data steps:	50
Weight factor:	1

9.5 Scan rate variables:

Scan time (msec):	1000
Micro-scans:	15

9.6 EI/AGC parameters:

EI background mass (m/z):	98
EI maximum ionization time (µsec):	25000
AGC prescan ionization time (µsec):	100
AGC prescan storage level (m/z):	20.0
Data steps in AGC prescan:	50
RF dump value (m/z):	650.0
AGC weight factor:	1

9.7 Scan segment breaks:

Seg masses	Seg RF	Seg time
Segment 1: 10-98	10	100
Segment 2: 99-310	10	100
Segment 3: 311-399	10	100
Segment 4: 400-650	10	100

9.8 PAH Operating Conditions

9.8.1 *SPI Injector*

Injection volume:	1 µL
Hot needle time:	0.03 minutes
Injection time:	0.01 minutes
Injection rate:	0.5 µL/second
Lower air gap	yes
Upper air gap	yes
Needle depth	90%
Initial temperature:	50°C, hold 0.10 minutes
Ramp 1:	200°C/min. to 290°C, hold 48.70 minutes

9.8.2 The GC temperature program is optimized to achieve >50% resolution for all analyte peaks and any known interferant. Typical conditions are as follows:

Oven Program:	50 minutes
Initial temperature:	75°C, hold 2.0 minutes
Ramp 1:	25°C/min. to 150°C
Ramp 2:	4°C/min. to 235°C
Ramp 3:	3°C/min. to 265°C
Ramp 4:	50°C/min. to 300°C, hold 11.94 minutes

9.8.3 Miscellaneous Parameters

Column head pressure:	15 psi
Transfer line:	300°C
Column linear velocity:	1.0 mL/min. at 300°C
Septum purge flow:	4.25 mL/min.
Manifold set temperature:	240°C
Mass range:	98-310 m/z
Mass defect:	50 mu/100μ

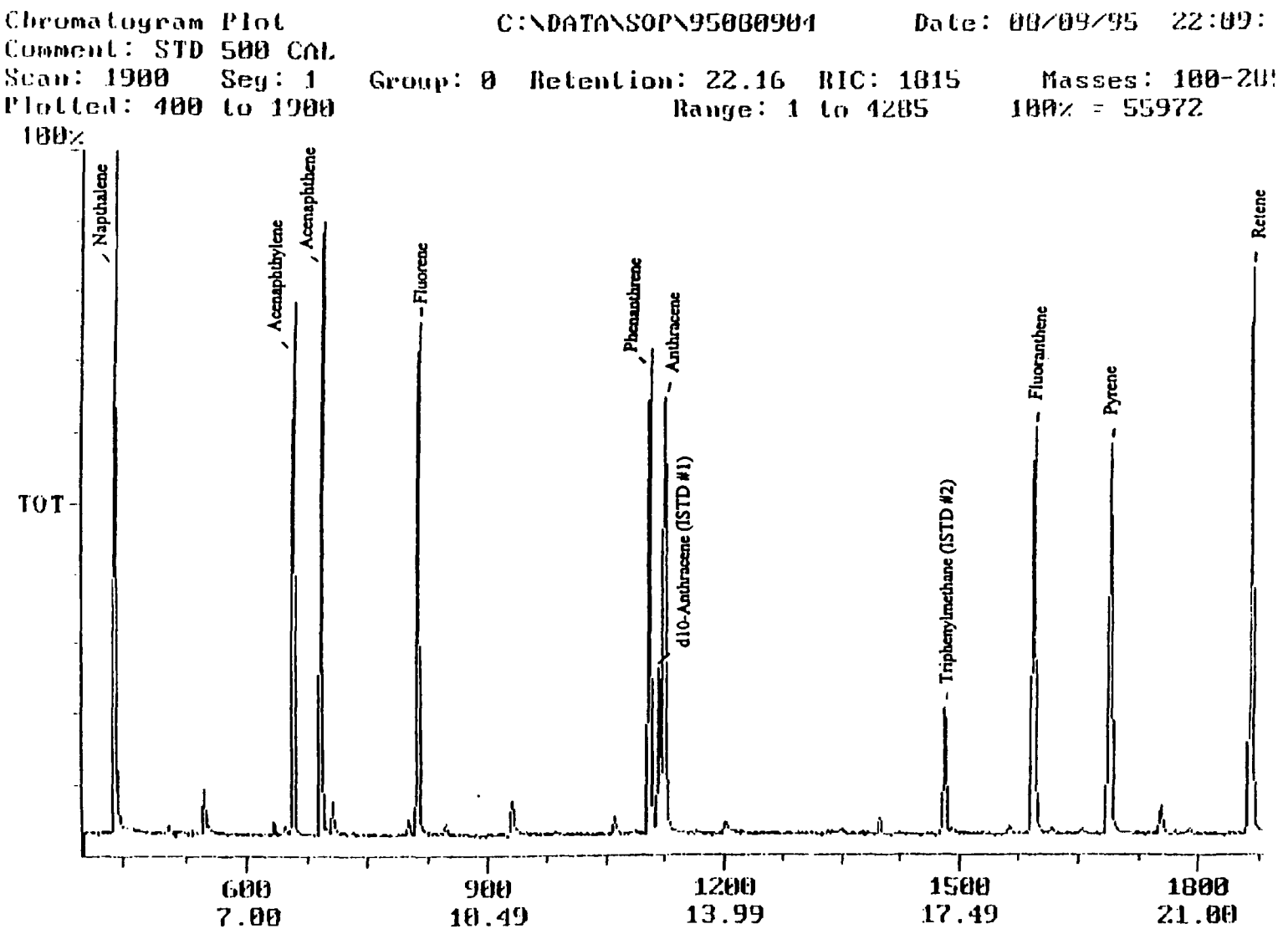
9.8.4 Calibration Information

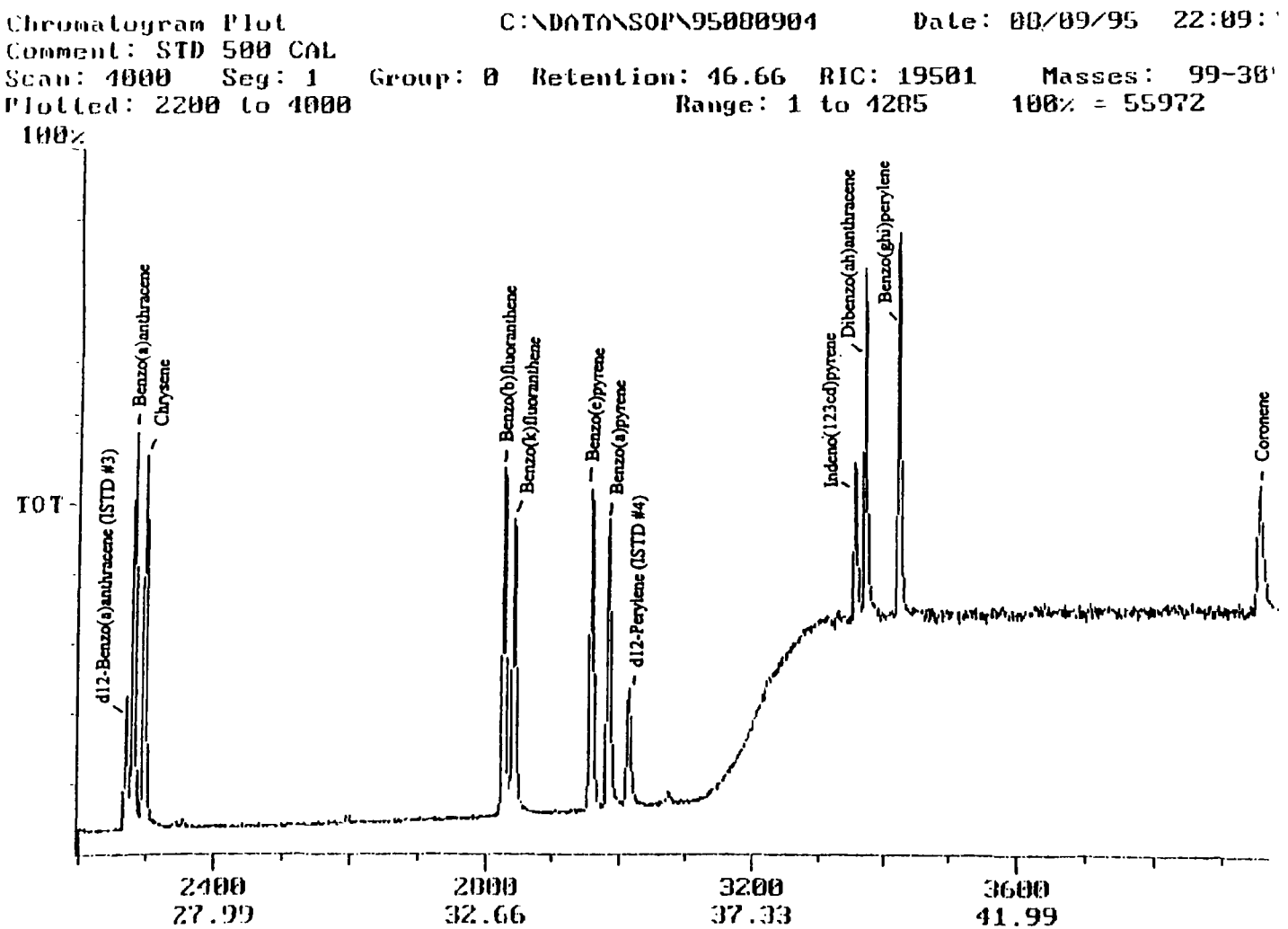
A multipoint calibration curve is prepared for each analyte prior to each run. A calibration check is performed every 5-10 samples and at the end of each run. Recalibration is required if a shift of >20% is observed. The method of internal standard calibration is utilized with four internal standards for PAHs and one for atrazine. The chromatogram is divided into four time segments with each segment calibrated relative to one internal standard response for the PAH runs.

Data is collected in the full-scan mode, however, selected ions are used for quantitation.

Retention Time	ISTD #	Name of Compound	Quantitating Masses
8.03	1	Acenaphthylene	152
8.46	1	Acenaphthene	153
9.95	1	Fluorene	165+166
13.54	1	Phenanthrene	178
13.68	1	d10-Anthracene (ISTD #1)	188
13.76	1	Anthracene	178
18.02	2	Triphenylmethane (ISTD #2)	165+244
19.36	2	Fluoranthene	202
20.5	2	Pyrene	202
22.58	3	Retene	219+234
27.38	3	d12-Benzo(a)anthracene (ISTD #3)	240
27.5	3	Benzo(a)anthracene	228
27.7	3	Chrysene	228
34.25	4	Benzo(b)fluoranthene	252
34.44	4	Benzo(k)fluoranthene	252
35.89	4	Benzo(e)pyrene	252
36.09	4	d12-Benzo(a)pyrene (surrogate)	264
36.21	4	Benzo(a)pyrene	252
36.59	4	d12-Perylene (ISTD #4)	130+132+264
40.68	4	Indeno(123cd)pyrene	138+276+277
40.87	4	Dibenzo(ah)anthracene	139+278+279
41.56	4	Benzo(ghi)perylene	138+276+277
48.51	4	Coronene	150+300+301

9.8.5 PAH chromatogram- full scan mode




Chromatogram Plot
C:\DATA\SOP\95080904
Date: 08/09/95 22:09:
Comment: STD 500 CAL
Scan: 4000 Seg: 1 Group: 0 Retention: 46.66 RIC: 19501 Masses: 99-30
Plotted: 2200 to 4000
Range: 1 to 4285
100% = 55972
100%
TOT
d12-Benzo(a)anthracene (ISTD #3)
Benzo(a)anthracene
Chrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(e)pyrene
Benzo(a)pyrene
d12-Perylene (ISTD #4)
Indeno(123cd)pyrene
Dibenzo(ah)anthracene
Benzo(ghi)perylene
Coronene
2400
27.99
2800
32.66
3200
37.33
3600
41.99

9.9 Atrazine Operating Conditions

9.9.1 SPI Injector

Injection volume:	2 µL
Hot needle time:	0.03 minutes
Injection time:	0.10 minutes
Injection rate:	0.2 µL/second
Lower air gap	yes
Upper air gap	yes
Needle depth	90%
Initial temperature:	50°C, hold 0.85 minutes
Ramp 1:	200°C/min. to 290°C, hold 29.95 minutes

9.9.2 *Oven Program:* 32 minutes

Initial temperature:	75°C, hold 2.0 minutes
Ramp 1:	25°C/min. to 150°C
Ramp 2:	4°C/min. to 190°C
Ramp 3:	50°C/min. to 300°C, hold 14.80 minutes

9.9.3 Miscellaneous Parameters

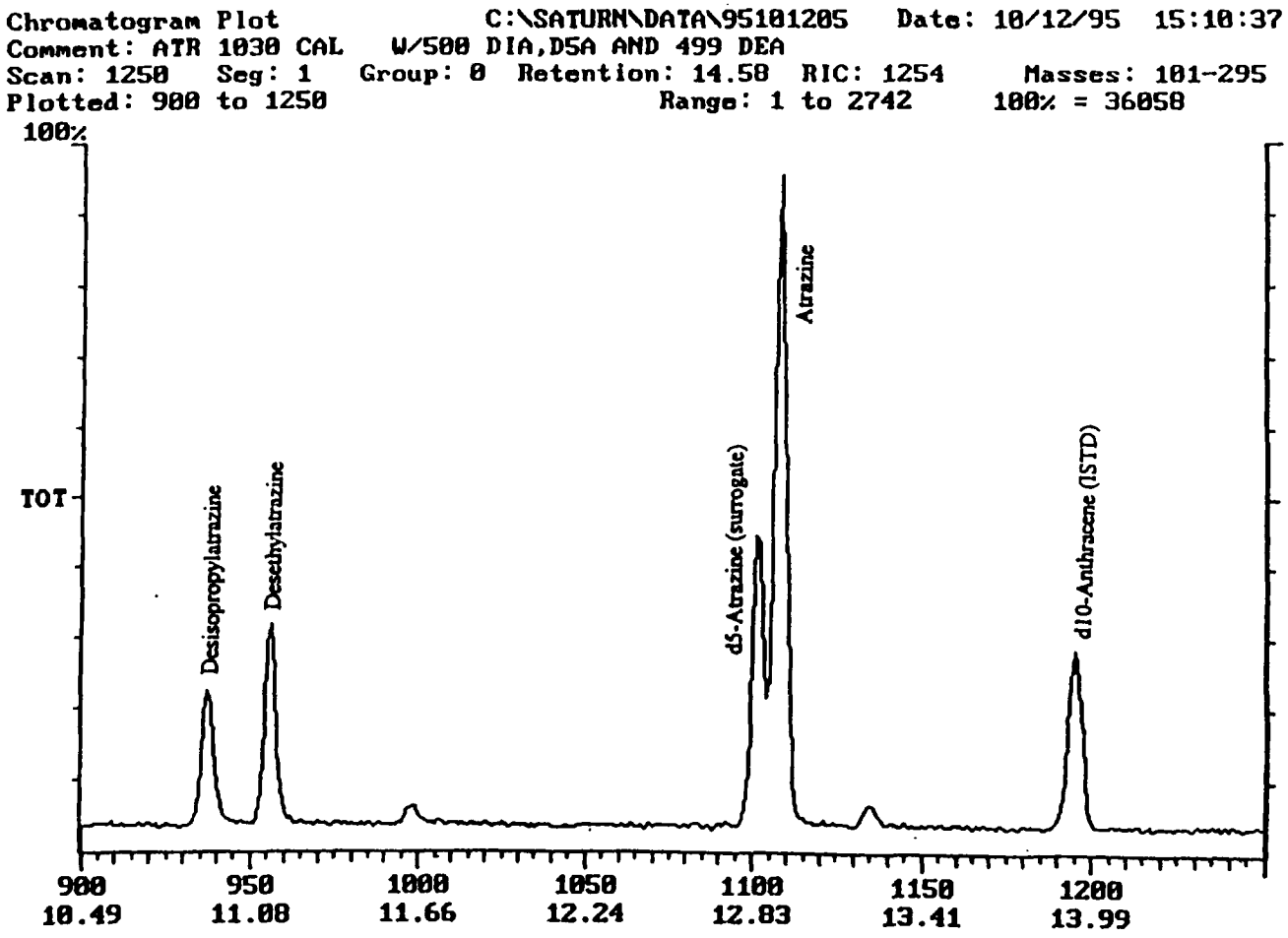
Column head pressure:	15 psi
Transfer line:	300°C
Column linear velocity:	1.0 mL/min. at 300°C
Septum purge flow:	4.25 mL/min.
Manifold set temperature:	240°C
Mass range:	98-310 m/z
Mass defect:	50 mu/100µ

9.9.4 Calibration

See Section 9.8.4 for a general discussion of the calibration procedure.

Retention Time	Name of Compound	Quantitating Masses
10.73	Desisopropylatrazine	158+173
10.93	Desethylatrazine	172
12.61	d5-Atrazine (surrogate)	205+220
12.69	Atrazine	200+215
13.64	d10-Anthracene (ISTD)	188

9.9.5 Atrazine chromatogram - full scan mode



10.0 Data Evaluation

10.1 View the hexane blank total ion chromatogram to determine if the system is clean.

10.2 Integrate each standard using the previous calibration curve. Print a hard copy of each standard data report.

10.3 Build a new calibration curve daily or for each sample set using a minimum of three standards.

10.4 Evaluate the performance check standard (US 106) for PAHs. Print a hard copy of the data report.

10.5 Integrate each sample, peak observing each individual compound. Print a hard copy of compounds that are manually integrated and a hard copy of the final sample report.

10.6 Continue evaluating each sample, each calibration check (every five to seven samples), and the final hexane blank chromatogram.

10.7 Print a hard copy of the tune conditions for the run.

10.8 After the entire run has been integrated and evaluated, run the macro *Print.prc* (see Appendix A) to convert Saturn data files to a format for import into Quattro® Pro.

10.9 Import the Saturn data to a Quattro® Pro worksheet.

10.10 Check the data in the worksheet against the Saturn hard copies. Correct the worksheet and add extraction batch codes and any necessary comments.

10.11 Print out a hard copy of the Quattro® Pro worksheet and compare the data again to the Saturn hard copies. Initialize the folder containing the Saturn hard copies as being checked.

10.12 Copy the worksheet data into the appropriate spreadsheet.

10.13 Send the Saturn hard copies and a disk copy of the updated Quattro® Pro spreadsheet to the lab supervisor for final review.

10.14 Saturn data files must be backed up before they are deleted from the Saturn system hard drive. Make two backups using optical disks or magnetic tapes.

10.15 The data will be considered valid if the calibration check standards and the performance check standard are within 20%.

10.16 Formula for manual-calculations of GC/MS Data

Some over-range peaks may require manual calculations to determine the analyte concentration

$$Mass_{samp} \quad Area_{samp} \; x \left\{\frac{Mass}{Area}\right\}_{cal} \; x \left\{\frac{Area}{Mass}\right\}_{\substack{cal \\ ISTD}} \; x \left\{\frac{Mass}{Area}\right\}_{\substack{samp \\ ISTD}}$$

Appendix A. Macros

PRINT.PRC MACRO

```
screen 1:
\ PRINT
# $pathname = "D:\DATA\"
# $areafile = "B:\AR"
# $amntfile = "B:\AM"

screen 2:
\ PRINT
CLS
ROW 10
COLUMN 10
PRINT "Enter subdirectory: "
INPUT-STRING
CHOP-TRAILING-BLANKS-FROM $STRING
JOIN-STRINGS $pathname $STRING
JOIN-STRINGS $pathname "\"
JOIN-STRINGS $areafile $STRING
JOIN-STRINGS $areafile ".PRN"
JOIN-STRINGS $amntfile $STRING
JOIN-STRINGS $amntfile ".PRN"

screen 3:
\ PRINT
CLS
ROW-COL 3 15
FILE-LIST-OF "*.QD"
$FILE-LIST-PATH = $pathname
FILE-LIST-TITLE "Select Quant. files"
FILE-LIST-SIZE = 10
SHOW-FILE-LIST

screen 4:
\ PRINT
CREATE-FILE $areafile
WRITE-TO-FILE
FOR J = 1 TO #FILES-IN-LIST
USE-DATA-FILE $FILE-LIST-NAME# J
PRINT 1 "
PRINT $DATA-SAMPLE-ID
PRINT 1 "
PRINT 1 ,
PRINT 1 '
PRINT $DATA-FILE-NAME
```

Appendix A. Macros (Cont'd)

```
PRINT 1 "
PRINT 1 ,
PRINT 1 "
PRINT-DATE(1)-OF DATA-FILE-DATE
PRINT 1"
PRINT ", ,"
USE-QUAN-FILE $FILE-LIST-NAME# J

screen 5:
\ PRINT
FOR I = 1 TO #QUAN-COMPOUNDS
READ-QUAN-COMPOUND# I
FIELD L7.0 PRINT QUAN-PEAK-AREA
PRINT 1 ,
NEXT
CR
NEXT
CLOSE-FILE
```

Standard Operating Procedure for the Analysis of PCBs and Organochlorine Pesticides by GC-ECD

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Standard Operating Procedure CH-IN-002.3

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Standard Operating Procedure for the Analysis of PCBs and Organochlorine Pesticides by GC-ECD

1.0 Scope and Application

This procedure details the analysis and data reduction methods utilized to determine polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in vapor, particulate, and precipitation samples. The method is specific to the Lake Michigan Mass Balance (LMMB) and Lake Michigan Loading Study (LMLS) projects. The following analytes are measured by this SOP using gas chromatograph-electron capture detection methods:

Polychlorinated Biphenyls (PCBs)-Total and 105 congener peaks

congener (BZ)	CAS #
1	2051-60-7
3	2051-62-9
4+10	13029-08-8, 33146-45-1
6	25569-80-6
7+9	33284-50-3, 34883-39-1
8+5	34883-43-7, 16605-91-7
12	2974-92-7
13	2974-90-5
15+17	2050-68-2, 37680-66-3
16	38444-78-9
18	37680-65-2
19	38444-73-4
21	55702-46-0
22	38444-85-8
24	55702-45-9
27	38444-76-7
25	55712-37-3
26	38444-81-4
29	15862-07-4
31+28	16606-02-3, 7012-37-5
32	38444-77-8
33	38444-86-9
37	38444-90-5

congener (BZ)	CAS #
40	8444-93-8
41+71	52663-59-9, 41464-46-4
42	36559-22-5
43	70362-46-8
44	41464-39-5
45	70362-45-7
46	41464-47-5
47	2437-79-8
48	70362-47-9
49	41464-40-8
51	68194-04-7
52	35693-99-3
53	41464-41-9
56+60	41464-43-1, 33025-41-1
63	74472-34-7
64	52663-58-8
66	32598-10-0
70+76	32598-11-1, 70362-48-0
74	32690-93-0
77	32598-13-3
81	70362-50-4
82	52663-62-4
83	60145-20-2
85	65510-45-4
87	38380-02-8
89	73575-57-2
91	68194-05-8
92+84	52663-61-3, 52663-60-2
95	38379-99-6
97	41464-51-1
99	38380-01-7
100	39485-83-1
101	37680-73-2
107	70424-68-9
110	38380-03-9
114+131	74472-37-0, 61798-70-7

congener (BZ)	CAS #
118	31508-00-6
119	56558-17-9
123+149	65510-44-3, 38380-04-0
128	38380-07-3
129	55215-18-4
130	52663-66-8
132+153+105	38380-05-1, 35065-27-1, 32598-14-4
134	52704-70-8
135+144	52744-13-5, 68194-14-9
136	38411-22-2
137+176	35694-06-5, 52663-65-7
141	52712-04-6
146	51908-16-8
151	52663-63-5
156	38380-08-4
157+200	69782-90-7, 52663-73-7
158	74472-42-7
163+138	74472-44-9, 35065-28-2
167	52663-72-6
170+190	35065-30-6, 41411-64-7
172	52663-74-8
173	68194-16-1
174	38411-25-5
175	40186-70-7
177	52663-70-4
178	52663-67-9
180	35065-29-3
183	52663-69-1
185	52712-05-7
187+182	52668-68-0, 60145-23-5
189	39635-31-9
191	74472-50-7
193	69782-91-8
194	35694-08-7
197	33091-17-7

congener (BZ)	CAS #
198	68194-17-2
199	52663-75-9
201	40186-71-8
202+171	2136-99-4, 52663-71-5
196	42740-50-1
203	52663-76-0
205	4472-53-0
206	40186-72-9
207	52663-79-3
208+195	52663-77-1, 52663-78-2
209	2051-24-3

Pesticides	CAS #
dieldrin	60-57-1
a-chlordane	5103-71-9
g-chlordane	5103-74-2
t-nonachlor	39765-80-5
a-hexachlorocyclohexane (a-HCH)	319-84-6
g-hexachlorocyclohexane (g-HCH)	58-89-9
hexachlorobenzene (HCB)	118-74-1
p'p'-DDD	72-54-8
p,p'-DDE	72-55-9
p,p'-DDT	50-29-3

2.0 Summary of Method

This method describes equipment and procedures for performing gas chromatographic analysis with an electron capture detector (GC-ECD). It includes instrument optimization specific for PCBs and OCs and data reduction.

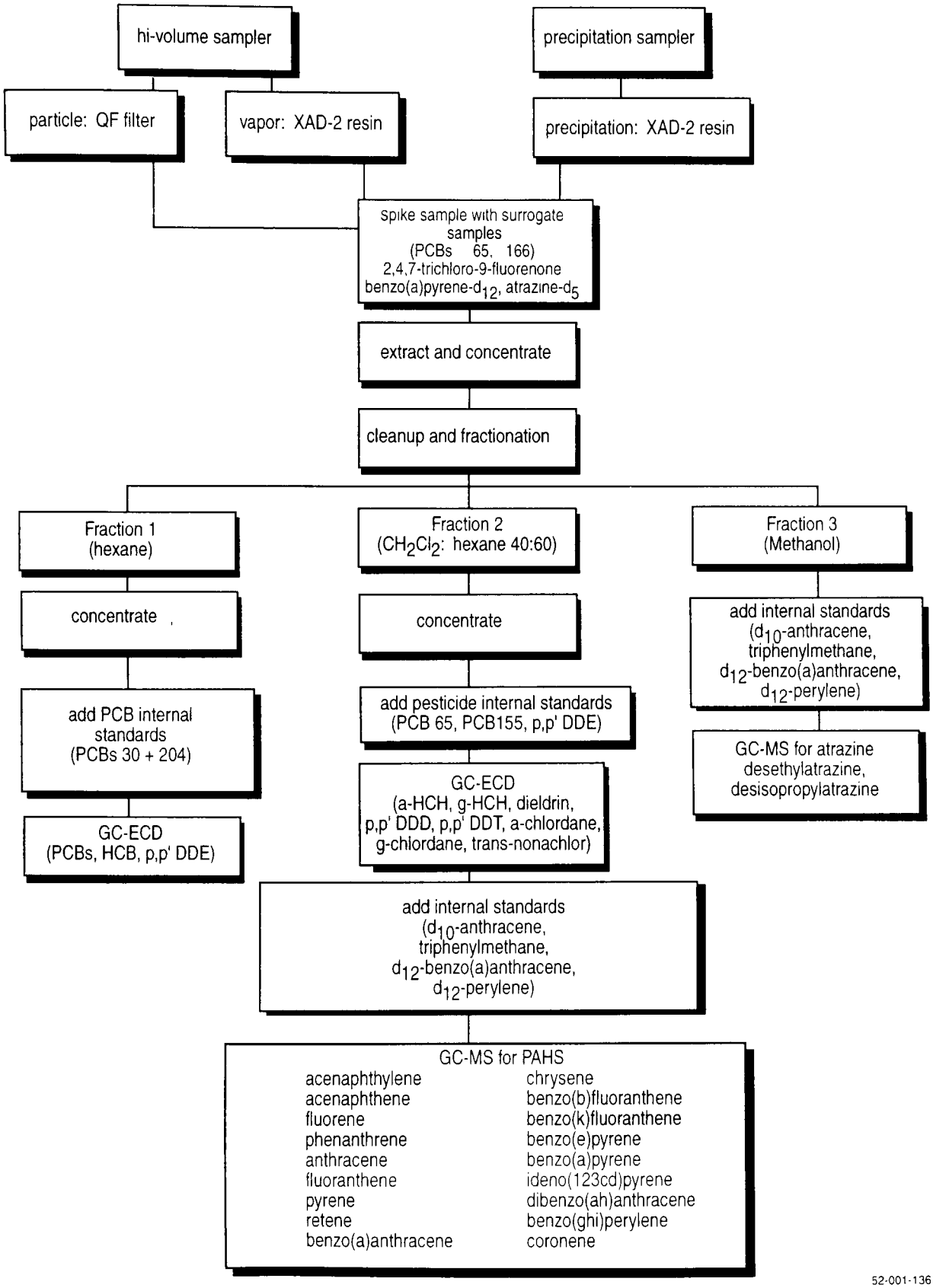
2.1 Personnel Restrictions

This method is restricted to use by or under the supervision of an analyst trained and experienced in the operation of gas chromatographs, electron capture detectors, and capillary chromatogram interpretation, and data reduction. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.2 Working Linear Range

A multi point calibration curve will be constructed for each analyte to document the working linear range.

2.3 Method Flow Diagram



3.0 Definitions

3.1 Limits of Detection

3.1.1 Instrument detection limit

The instrument detection limit (IDL) refers to the smallest signal above background noise that an instrument can reliably detect. The IDL is determined from a data set comprised of three separate chromatographic runs of a low level calibration standard; each run contains 7-10 analyses of the standard. The IDL equals the Student's t value (n-1) multiplied by the standard deviation of this data set.

3.1.2 Method detection limit

Method detection limits (MDL) are defined in CFR, Vol 49, No. 209, October 26, 1984, Appendix B to Part 136. Matrix specific MDLs are determined by spiking 7-10 clean matrix samples with the analytes of interest and processing them through the entire extraction, cleanup, and analysis procedure.

3.2 Internal standard (ISTD)

A pure analyte(s) added to a sample extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution.

3.3 Laboratory surrogate spike (LSS)

A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the LSS is to monitor method performance with each sample.

3.4 All other terms are defined in the QAPjP, Revision 5, July 1995.

4.0 Interferences

Method interferences may be caused by contaminants in solvents, the sampling matrix, reagents, glassware, and other sample processing apparatus that lead to anomalous peaks or elevated baselines in gas chromatograms. Laboratory equipment and reagents will be monitored by the inclusion of quality control samples with each batch of samples prepared. Individual samples may contain interferences which will require additional sample preparations. All sample preparation details will be documented.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined. However, each one must be treated as a potential health hazard, and exposure to these chemicals should be minimized. Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.

5.2 Chemists working in the laboratory should follow ISWS safety rules :

5.2.1 A lab coat is required when working in the lab.

5.2.2 Eye protection with splash resistant safety glasses or safety goggles are required.

5.2.3 Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.

5.2.4 All solvent work should be done in fume hoods.

5.2.5 Open shoes are not allowed in the laboratory.

5.2.6 Particle mask is required when using dry silica.

5.2.7 Avoid working alone in the laboratory. If work must be performed after hours or in the weekend inform the supervisor or other staff so that your presence is known and will be accounted for in case of an emergency.

5.2.8 Chemicals and solvents are stored under the hoods. Acids must be separated from bases. A rubber bucket is required to transport any chemical.

5.2.9 Gas cylinders should be well secured at all times. Flammable gases are stored in separate storage areas.

5.2.10 Wash hands well after work.

5.2.11 No food or drink is allowed in the laboratory.

5.2.12 In case of minor spillage, get spillage kit to clean the area. A major spill requires the University of Illinois Fire Department to be contacted and the working area evacuated.

5.2.13 MSDS sheets are stored in the laboratory and a copy placed on file with the office administrator.

5.2.14 All chemicals and standards must be labeled with chemical name, date, and initials of person to contact.

5.2.15 Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

5.3 Waste Disposal

Solvents: Label waste containers, *'chlorinated waste'* and *'non-chlorinated waste'*. Glass bottles used for waste are placed under hoods for convenience. When full, transfer waste to 10 L carboy containers in solvent cabinet. Contact the ISWS Waste Coordinator for removal.

6.0 Equipment and Supplies

6.1 Glassware, General Requirements

All glassware, must be meticulously cleaned. Large glassware is thoroughly washed with laboratory detergent and hot water. Glassware with bad stains should be rinsed with MeOH or CH_2Cl_2 before using the soap and water procedure. If still not clean, soak in H_2SO_4:HNO_3 (50:50) acid bath overnight, then wash thoroughly with soap and water. Volumetric pipettes used for standards *must* soak in acid bath overnight. Glassware is thoroughly rinsed with tap water, then with DI water and allowed to air dry. The glassware is foil wrapped and heated 450°C for 4 hours. If glassware is not clean after muffling at 450°C for 4 hours, muffle at 500°C for 4 hours. The glassware is cooled to ambient temperature and stored in a clean location. Small glassware such as stoppers, vials, and disposables are wrapped in foil or placed into a beaker and covered with foil and heated to 450°C for 4 hours, cooled to ambient temperature, and stored in a clean location. Vials are capped as soon as they are removed from the oven. Note: Always use dull side of foil towards glassware. Set initial temperature of furnace to 200°C if possible.

6.2 Vortex Mixer

6.3 Volumetric flasks and pipets - Class A, various sizes

6.4 Autosampler vials, 2 mL, caps, teflon-lined septa, and 200 μL glass inserts.

6.5 Positive displacement micro pipet, glass capillaries (Drummond or equivalent)

6.6 GC-ECD (see section 11.0 for detailed information)

6.6.1 Hewlett-Packard 5890A capillary gas chromatograph with split/splitless capillary inlet, and electron capture detector (15 m Curie ^{63}Ni source)

6.6.2 Hewlett-Packard 3365 ChemStation, Version B.01.02

6.6.3 Autosampler: Hewlett-Packard 7673 or 7673A autosampler

6.6.4 Column:

For OC pesticides--30 m x 0.25 μm x 0.25 mm, DB-5 (J & W Scientific)

For PCBs--60 m x 0.1 μm x 0.25 mm, DB-5 (J & W Scientific)

6.7 J & W AccuRATE™ 1000 flowmeter (PN2201170, Folson, CA)

6.8 GL Sciences Inc.,LD223 flowmeter (Tokyo, Japan)

7.0 Reagents and Standards

7.1 All reagents will be pesticide residue quality or equivalent. All reagents are evaluated for interferences using laboratory blanks.

7.2 Solvents, EM Omnisolve or equivalent

7.2.1 Hexane

7.2.2 Methylene chloride

7.3 Standards

7.3.1 PCB and pesticide stock standards

Stock standard solutions are purchased from commercial sources (Ultra Scientific, Accustandard, Chem Service, Cresent Chemical), are obtained from the USEPA repository, or from the USEPA (PCB Mullin stock standard, 1994). When stock solutions are not available, analytes are purchased as the neat material and gravimetrically prepared in house. Stock solutions and mixed working standards are prepared volumetrically by serial dilution in volumetric flasks. A standards preparations are detailed in laboratory log books.

7.3.2 Chromatographic Calibration Standards

7.3.2.1 Mixed instrument calibration standards are prepared from the individual stock standards by volumetric dilution to obtain five concentration levels. The calibration standard concentrations bracket the expected analyte amounts in samples assayed and are within the working linear range of the detectors. Calibration mixes are prepared specifically for the appropriate instrument and fraction analyzed. Calibration mixes are described in "Standard Operating Procedure for the Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples," SOP #CH-PR-001.3, Revision 3.0, March 1995, Harlin and Surratt, ISWS, Champaign, IL 61820).

7.3.2.2 Standard Evaluation : New working standards will be assayed prior to use by comparison with existing standards. Standards must agree within 10% prior to use.

7.4 Gases

7.4.1 Hydrogen, carrier gas, 99.9995% chromatographic grade

7.4.2 Make-up gas, argon/methane (P5)

8.0 GC-ECD System Evaluation and Maintenance

8.1 System Evaluation

Before each run GC-ECD system performance and calibration are verified for all analytes. Hexane is injected at the beginning of each run to ensure the system is free from contaminants or interfering peaks. Records of daily system performance and maintenance are maintained.

8.1.1 Conditioning

A conditioning program is run prior to every analytical GC run with a hexane injection. The GC oven is set to 280°C, the injector at 280°C, and the ECD at 380°C for about half an hour (See Conditioning method, Appendix A; Method 1).

8.1.2 Injector

8.1.2.1 Septum: Prior to every analytical GC run the septum is changed. Prior to removing the septum the heated zones are cooled and the column oven cooled to 40°C.

Note: Many septa are rated to last for about 100 injections, however many are soft and small fragments frequently break off and enter the injection port or the split/splitless weldment. More frequent changes, such as before each run, help minimize this problem.

8.1.3 ECD Baseline Signal

The ECD baseline signal is usually below 30. After conditioning the system, hexane is analyzed at the start of every GC run to monitor the baseline stability. If the baseline signal is elevated or the hexane run produces a noisy chromatogram, the system should be evaluated for leaks or contamination.

8.1.4 Calibration standard performance

Inject a calibration standard to evaluate peak resolution, peak shape, and identification using the reference chromatograms in this SOP for evaluation. If the peak shapes are not satisfactory inspect the system for proper flow rates and leaks. If these are acceptable, remove 0.5-1 meter from the injector end of the column or install a new column.

8.1.4.1 PCBs: Over 90 peaks should resolved with congeners 77 and 110 separated from adjacent peaks. Monitor the ratio of the areas of the 30/204 ISTD peaks. This ratio will increase as the sensitivity for peaks in the later portion of the chromatogram are reduced (observe the peak area of congener 209). When this ratio exceeds 1.2 (a value of about 1 or less is desirable), the injection insert should be changed. In a run with a new injection insert, the peak area of 209 in a 595 ng/mL standard is easily seen. With repeated injections this peak area will decrease and can be used in addition to the 30/204 area ratio for determining the need for an injection liner change.

8.1.5 Leak checking and gas flows (See Appendix B for detailed instructions)
When the chromatography performance deteriorates check for gas leaks. Check around the septum, the injector connection, and at the detector connection of the column. Use of a leak detection fluid is discouraged because of the possibility of drawing the fluid into the system. A pure solvent such as isopropyl alcohol may be used as a leak detection fluid, however, an electronic leak detector is recommended such as the J & W AccuRATE™, 1000 flowmeter (PN2201170, Folson, CA)

Check the gas flow after any routine maintenance with a flowmeter. An electronic flowmeter is recommended such as the GL Sciences Inc., LD223 flowmeter (Tokyo, Japan).

8.2 Gases

Replace all tanks at 500 psig. Prior to changing a gas tank, lower GC oven temperature to 40° C for approximately 30 min. Maintain a 40°C oven temperature for approximately 30 min. after changing a tank to eliminate air from the system prior to heating the column and detector.

8.2.1 Carrier gas (hydrogen) flow rate.

The optimum carrier gas volumetric flow rate is specified by the column manufacturer. This is determined by measuring the time required for an unretained compound to elute from the column (such as methylene chloride vapor for an ECD), and then calculating the volumetric flow rate using the following formula:

$$\textit{Volumetric flow rate (mL/min)} = \frac{\pi r^2 l}{t}$$

where:
π is 3.14
r is the radius of the column (cm)
l is the length of the column (cm)
t is the retention time of the unretained compound (min)

8.2.2 Column head pressure should be at the preset value, usually 20/25 psi for 30/60 m columns. If the pressure is low, tighten the septum nut. If the pressure is still low check for leaks and tighten other connections.

9.0 Preparation of Autosampler Vials for GC-ECD Analysis

9.1 Initiate sample sequence table for the GC run. Each analytical run consists of a hexane blank, standard/s to establish a calibration table, a performance check standard (LPC), samples, a calibration check standard every 7-10 samples (if necessary), and a final calibration check (CLC).

9.2 Label autosampler vials for samples, standards, and hexane blanks; add inserts to vials.

9.3 Spike samples with ISTD if necessary (See Section 10.0 for spiking procedure).

9.4 Before transferring samples and standards to the corresponding labeled autosampler vials, mix contents of each vial and bottle well by holding on a vortex mixer for 5-10 seconds. Use muffled Pasteur pipettes for transfer.

10.0 ISTD Addition

10.1 Remove ISTDs from freezer; equilibrate to ambient temperature (approximately two hours). Vortex ISTD solution to mix.

10.2 Clean micropipette as follows: Remove glass capillary used to cover plunger. Rinse plunger with CH_2Cl_2 and allow to dry. Without manually touching glass capillaries, insert plunger into a new capillary, position and tighten. Rinse capillary in the following order: CH_2Cl_2, hexane (five times), air dry, ISTD solution (two times). Fill micropipette with the ISTD solution and dispense into sample vial.

10.3 Internal standard amount:

Fraction	Compound	ISTD	Spike Volume (μL)	Amount in Sample (ng)*	Color code
Hexane	PCBs and Pesticides	PCB 30	100	8	Red
		PCB 204		6	
40%	Pesticides	PCB 65	100	23.7	Blue
		PCB 155		17.5	
		DDE		20	
40%	PAHs	d10-Anthracene	100**	200	Black
		Triphenylmethane		100	
		d12-Benzo(a)anthracene		200	
		d12-Perylene		200	
MeOH	Atrazine	d10-Anthracene	100	200	Black

*Values in this column are approximate and may change slightly depending on the exact concentrations of the compounds in the stock solutions.
**If the sample is expected to be high in concentration of PAHs or if the final sample volume is greater than 2 mL, more ISTD solution may be added in increments of 100μL.

10.4 Mark each sample vial label with the appropriate color code verify ISTD addition with a water-proof marker.

10.5 Rinse the micropipette with solvent and replace glass capillary used to cover plunger.

10.6 Label the sample set with the following information (using the same color code as dots on vial labels):
Date sample vials spiked.
Fraction spiked.
Initials of chemist spiking.

11.0 GC-ECD Analytical Run Conditions

11.1 Initial Check

Ensure sufficient carrier (hydrogen) and make-up gas (argon/methane, P5) to complete the run. Change the septum as described in 8.0. Change the solvent in the autosampler wash vials.

11.2 ChemStation Control

The ChemStation software controls the GC. Analyzer 1 controls GC1 and Analyzer 2 controls GC2. Load the analysis method. "MULLIN.MTH" for PCBs and "ISWS'PES.MTH" for OC pesticides. Verify method parameters are correct. Edit the method if changes are necessary and resave the method. Copies of these methods are included in Appendix A- Method 2 or Method 3.

11.3 Load the method sequence. "ISWS'PCB.SEQ" for PCBs and "ISWS'PEST.SEQ" for OC pesticides. Edit the sequence *parameters* to include:

- operator's name
- subdirectory name
- calibration standard information
- spike target levels
- final sample volume
- sample batch code
- comments

In the *sequence table* ensure the correct injector is selected (rear vs front) and enter the "from vial" and "to vial" numbers for selected methods. The first hexane injection will utilize the "BAKE.MTH" method to condition the system.

Edit the sequence *sample table* to enter sample ID numbers (the ID#=sample name with a ", hex" or ", 40" suffix to indicate the hexane or 40%DCM fraction respectively). Hexane is always the first vial followed by two vials of the calibration standard, a calibration performance check (LPC). Samples usually begin with the 5th vial. Each sample batch will include a hexane blank, a calibration standard, a LPC, and an end of run calibration check.

Verify the correct method name is entered in the *sample log table* and the multiplier is entered for each sample (usually 1). Verify the start and end vial numbers. Save the method and sequence on disk using the path for the directory created for this sample batch. Print copies of the sequence for the sequence log and the raw data file for the sample batch. An example of a PCB sequence is shown in Appendix C.

11.4 GC Conditions, typical run conditions are listed below.

PCBs:

Oven Program: 158 minutes

Initial temperature: 100°C

Ramp 1: 1°C/min. to 235°C

Ramp 2: 15°C/min. to 280°C, hold 20 min.

Injector temp. 250°C

ECD: 350°C

OC pesticides:

Oven Program: 147 minutes

Initial temperature: 70°C, hold 1 min.

Ramp 1: 5°C/min. to 140°C

Ramp 2: 0.2°C/min. to 160°C

Ramp 3: 10°C/min. to 280°C, hold 20 min.

Injector temperature 250°C

ECD: 350°C

11.5 Calibration

A multipoint calibration curve is prepared yearly for each analyte to document the linear range. A single point calibration standard is injected in duplicate immediately prior to a sample run. Compare the duplicate injections of the calibration standard to ensure the system has stabilized and is reproducible. Inject a calibration performance standard (LPC) immediately after the calibration standard. The acceptable ranges for the LPC are defined in the QAPjP for the project. Inject a calibration standard every 5-10 samples (for multi-batch runs) and at the end of each run. Recalibration is required if a shift of >20% is observed for OC pesticides and >25% for congeners 1, 6, 29, 49, 101, 141, 180, 194, 206, and 209 for PCBs. The methods of internal standard calibration is utilized. The chromatogram is divided into two time segments with each segment calibrated relative to one internal standard response for the PCB runs.

12.0 Data Reduction

12.1 Data Files

The electronic data are converted to compacted (zipped) format for storage and transfer to other computers for data reduction.

12.2 Chromatogram and ISTD report

The data files are expanded (unzipped) and the appropriate method (*.MTH) and data files loaded to view the chromatogram, generate calibration tables, and ISTD reports. Each analyte peak is evaluated for proper peak integration, baseline correction, and peak identification. The final integrated data and all associated files are saved on removable disks. The chromatograms and ISTD reports are also printed as hard copy reports and compiled as a sample batch data file (filed by the GC sequence name). The hard copy files are initialed by the data analyst. PCB and OC pesticide macro programs convert the ChemStation data into a spreadsheet format for further data storage and retrieval. All data are reviewed by the laboratory supervisor prior to release.

A properly integrated and identified chromatograms for a 595 ng/mL PCB standard and a 20 ng/mL OC pesticide standard are presented in Appendix C. Calibration tables and sample data are included.

12.3 Data Storage

All chromatography data are archived including: ChemStation raw data files, processed data files, and associated calibration and integration files along with associated spreadsheet files. These files are stored on 5.25" and 3.5" data disks and are filed by the GC sequence name. Duplicate copies of the raw data disks are stored in two separate buildings.

Appendix A. GC Methods

METHOD 1- System Conditioning

Injector Information

Injection Source:	Auto
Injection Location:	Dual

Front:

Sample Washes:	3
Sample Pumps:	5
Sample Volume:	1 stop
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No

Rear:

Sample Washes:	3
Sample Pumps:	5
Sample Volume:	1 stop
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No

Purge A/B:

	Init Value	On Time (Min.)	Off Time (Min.)
A (Valve 3)	On	0.00	40.00
A (Valve 4)	On	0.00	40.00

Temperature Information

Zone Temperatures:

	Set Point
Inl. A	280 C.
Inl. B	280 C.
Det. A	380 C.
Det. B	380 C.

Oven Parameters:

Oven Equib. Time:	1.00 Min.
Oven Max:	300 C.
Oven	On
Cryo	Off

Appendix A. GC Methods (Cont'd)

METHOD 1 (Cont'd)

Oven Program:

	Set Point
Initial Temp:	280 C.
Initial Time:	20.00 Min.

Level	Rate (C./Min.)	Final Temp. (C.)	Final Time. (Min.)
1	0.00		
2 (A)			
3 (B)			

Next Run Time: 20.00 Min.

Signal Information

Save Data: Both

Signal 1

Source:	Det. A
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz.
Data Storage:	All

Signal 2

Source:	Det. B
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz
Data Storage:	All

Detector Information

Detector	Type	State
A	ECD	On
B	ECD	On

Signal Plot Information

Signal	Attn. (2^)	Offset (%)	Time (Min.)
1	2	20	20
2	2	20	20

Appendix A. GC Methods (Cont'd)

METHOD 2- PCBs

Method Information

This method is for IL H2O Survey.

Run Time Checklist

Pre-Run Program:	None
Name:	
Parameter:	
Data Acquistion:	On
Data Analysis:	On
Sig. 2 Mth:	None
Post-Run Program:	None
Name:	
Parameter:	

Injector Information

Injection Source:	Auto
Injection Location:	Dual
Front:	
Sample Washes:	3
Sample Pumps:	5
Sample Volume:	2 stops
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No
Rear:	
Sample Washes:	3
Sample Pumps:	5
Sample Volume:	2 stops
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No

Appendix A. GC Methods (Cont'd)

METHOD 2 (Cont'd)

Purge A/B:

	Init Value	On Time (Min.)	Off Time (Min.)
A (Valve 3)	On	0.00	40.00
A (Valve 4)	On	0.00	40.00

Temperature Information

Zone Temperatures:

	Set Point
Inl. A	250 C.
Inl. B	250 C.
Det. A	350 C.
Det. B	350 C.

Oven Parameters:

Oven Equib. Time:	3.00 Min.
Oven Max:	280 C.
Oven	On
Cryo	Off

Oven Program:

	Set Point
Initial Temp:	100 C.
Initial Time:	0.00 Min.

Level	Rate (C./Min.)	Final Temp. (C.)	Final Time. (Min.)
1	1.00	235	0.00
2 (A)	15.0	280	20.0
3 (B)	0.00		

Next Run Time: 158.00 Min.

Signal Information

Save Data: Both

Signal 1

Source:	Det. A
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz.
Data Storage:	All

Appendix A. GC Methods (Cont'd)

METHOD 2 (Cont'd)

Signal 2

Source:	Det. B
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz
Data Storage:	All

Detector Information

Detector	Type	State
A	ECD	On
B	ECD	On

Sequence Recalibration Table

Cal. Line	Cal. Level	Update Response Factor	Update Retention Times	Recalib Interval

Signal Plot Information

Signal	Attn. (2^)	Offset (%)	Time (Min.)
1	2	20	20
2	2	20	20

Appendix A. GC Methods (Cont'd)

METHOD 3- OC Pesticides

Method Information

This method is for IL H_2O Survey.

Run Time Checklist

Pre-Run Program:	None
Name:	
Parameter:	
Data Acquistion:	On
Data Analysis:	On
Sig. 2 Mth:	None
Post-Run Program:	None
Name:	
Parameter:	

Injector Information

Injection Source:	Auto
Injection Location:	Dual
Front:	
Sample Washes:	3
Sample Pumps:	5
Sample Volume:	1 stop
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No
Rear:	
Sample Washes:	3
Sample Pumps:	5
Sample Volume:	1 stop
Viscosity Delay:	0 sec.
Solvent A Washes:	6
Solvent B Washes:	6
On-Column:	No

Appendix A. GC Methods (Cont'd)

METHOD 3 (Cont'd)

Purge A/B:

	Init Value	On Time (Min.)	Off Time (Min.)
A (Valve 3)	Off	0.50	140.00
A (Valve 4)	Off	0.50	140.00

Temperature Information

Zone Temperatures:

	Set Point
Inl. A	250 C.
Inl. B	250 C.
Det. A	350 C.
Det. B	350 C.

Oven Parameters:

Oven Equib. Time:	1.00 Min.
Oven Max:	300 C.
Oven	On
Cryo	Off

Oven Program:

	Set Point
Initial Temp:	70 C.
Initial Time:	1.00 Min

Level	Rate (C./Min.)	Final Temp. (C.)	Final Time. (Min.)
1	5.00	140	0.00
2 (A)	0.20	160	0.00
3 (B)	10.0	280	20.0

Next Run Time: 147.00 Min.

Signal Information

Save Data: Both

Signal 1

Source:	Det. A
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz.
Data Storage:	All

Appendix A. GC Methods (Cont'd)

METHOD 3 (Cont'd)

Signal 2

Source:	Det. B
Peak Width:	0.053 Min.
Data Rate:	5.000 Hz
Data Storage:	All

Detector Information

Detector	Type	State
A	ECD	On
B	ECD	On

Sequence Recalibration Table

Cal. Line	Cal. Level	Update Response Factor	Update Retention Times	Recalib Interval

Signal Plot Information

Signal	Attn. (2^)	Offset (%)	Time (Min.)
1	2	20	20
2	2	20	20

Appendix B. GC-ECD Maintenance

B.1 Injection Port Cleaning and Liner Installation

B.1.1 Turn oven, injector and detector off

B.1.2 After everything cools, turn hydrogen off

B.1.3 Remove autosampler towers

B.1.4 Remove the septum retainer nut and the split/splitless weldment (the large nut underneath the septum) to expose the injection liner. Remove the liner

B.1.5 Open the oven and disconnect the column from injector end of the GC. The open end of the column should not be exposed to air so plug the open end with a septum

B.1.6 Unscrew the reducing nut (inside the oven, below the injection liner). There is one gold seal and a washer in it. Washer and seal need to be replaced each time it is taken apart

B.1.7 Put a beaker inside the oven underneath the injection port and rinse the injector with hexane. Clean the injection port with Q-tips and rinse again with hexane

B.1.8 Check inside the split/splitless weldment, below where the septum sits. Some septa particles may be inside. Complete removal may be impossible, however some particles may be removed using a small tool similar to a dental tool. Also washing hexane through the opening with a Pasteur pipet helps, being careful not to break pipet tips off inside this cavity

B.1.9 Place a new washer followed by a new gold seal in the reducing nut. The tapered opening of the seal will face downward (the tapered end will be fitted to the ferrule from the column). Tighten this reducing nut before placing the injection liner in the injection port

B.1.10 Insert a new liner. Some liners are not symmetrical but require a specific orientation. Take care to insert properly

B.1.11 Place a viton O- ring (rated for the injector temperature range) on the liner. Put the split splitless weldment (big nut) on and tighten it. Install in a new septum. Replace and tighten the septum nut

B.2 Column Installation

B.2.1 Remove the column from the injector end. Remove the ferrule and all particles from the column nut. Before removing a section of the column, insert a clean column nut onto the column, followed by a new ferrule, with conical end pointing towards the open end of the column (small pieces of ferrule material can get inside the column, therefore, a new section of column must be exposed after the ferrule is on).

Appendix B. GC-ECD Maintenance (Cont'd)

B.2.2 Cut off the injector end of the column. Remove 0.5-1 meter from an existing column. For a new column, remove a few inches. Make a clean cut with diamond tip score or ceramic wafer. Examine the cut under a magnifier to ensure it is square

B.2.3 Measure 23 mm from the tip of the column. Mark this point with Liquid Paper® Before marking, the ferrule should be between the end of the column and the 23 mm point

B.2.4 Carefully insert the column, fitted with nut and ferrule, through the reducing nut into the injection port. Insert column far enough that the white mark is at least even with the end of the column nut, or a little further in so that white mark is up inside column nut, and tighten slightly. As soon as the ferrule starts gripping the column, pull the column out gently just until the white mark is seen. Hand tighten column nut then turn at least ¼ additional turn

B.2.5 Detector end of the column (if needed): Remove the column from the detector end. If a portion of the ferrule is stuck inside the makeup gas adaptor remove by turning a threaded tool (such as a small file) into the ferrule and pulling it out. The makeup gas adaptor may need to be removed to remove the ferrule. When replacing the makeup gas adaptor, a 1/4 inch vespel ferrule is used. Place a column nut and ferrule on the column in the same way as described for the injector end. Remove a portion of the column and check for cut as described above. Measure 70 mm from the end of the column and put a white mark on the column, place the ferrule between the end of the column and the 70 mm point before marking. Turn hydrogen on and check the flow of gas through the column by inserting the cut end in a beaker of hexane. Turn hydrogen off

B.2.6 Carefully fit the column into the detector, slightly tighten the column nut and pull the column out until you see the white mark. Tighten with wrench ¼ turn after hand tight.

B.3 Leak Checking and Gas Flow Measurement

B.3.1 Turn hydrogen and argon/methane on. Check leaks with a leak detector An electronic leak detector is recommended such as the J & W AccuRATE™, 1000 flowmeter (PN2201170, Folson, CA). Check around the septum, and at the injector and at the detector end of the column. Check the column head pressure for the appropriate reading

B.3.2 Check the gas flow with a flowmeter. An electronic flowmeter is recommended such as the GL Sciences Inc.,LD223 flowmeter (Tokyo, Japan).Approximate gas flow in both GCs are as follows :

Split vent	130 mL/min
Purge vent	2 mL/min.
Total flow through detector	22 mL/min

Appendix B. GC-ECD Maintenance (Cont'd)

B.4 Reassembly

B.4.1 Replace autosampler towers

B.4.2 Turn heated zones on

B.4.3 If injector was allowed to cool, retighten the septum retainer nut to avoid a leak at that point

B.4.4 Turn oven on and set to 40°C for about an hour; then increase oven temperature to 70°C for an hour.

B.4.5 If a used column, bake the column, injector and detector until baseline stabilizes. If the baseline has not stabilized within an hour, other problems probably require identification and correction.

B.5 System conditioning

B.5.1 Set the temperature setting to the following (see Appendix A for GC method):

Oven: 280°C
Injector A: 280°C
Injector B: 280°C
Detector A: 380°C
Detector B: 380°C

B.5.2 Run approx. 6 hexane blanks by the instrumental method being evaluated

B.5.3 If a new column, bake injector and detector by ramping oven temp 1-2°/min. to 280°C and hold there for 1 hour. After conditioning, run 6 hexane blanks by the method being evaluated

B.5.4 If the hexane blank runs look satisfactory, evaluate a calibration standard before resuming analysis of samples.

Appendix C-1. PCB Sequence

Sequence: C:\HPCHEM\1\SEQUENCE\ISWS'PCB.SEQ

Operator: MONTE

Sequence preparation date: 03 Mar 95 03:56 PM

Data File Subdirectory: 950303H

Part of methods to run: full method

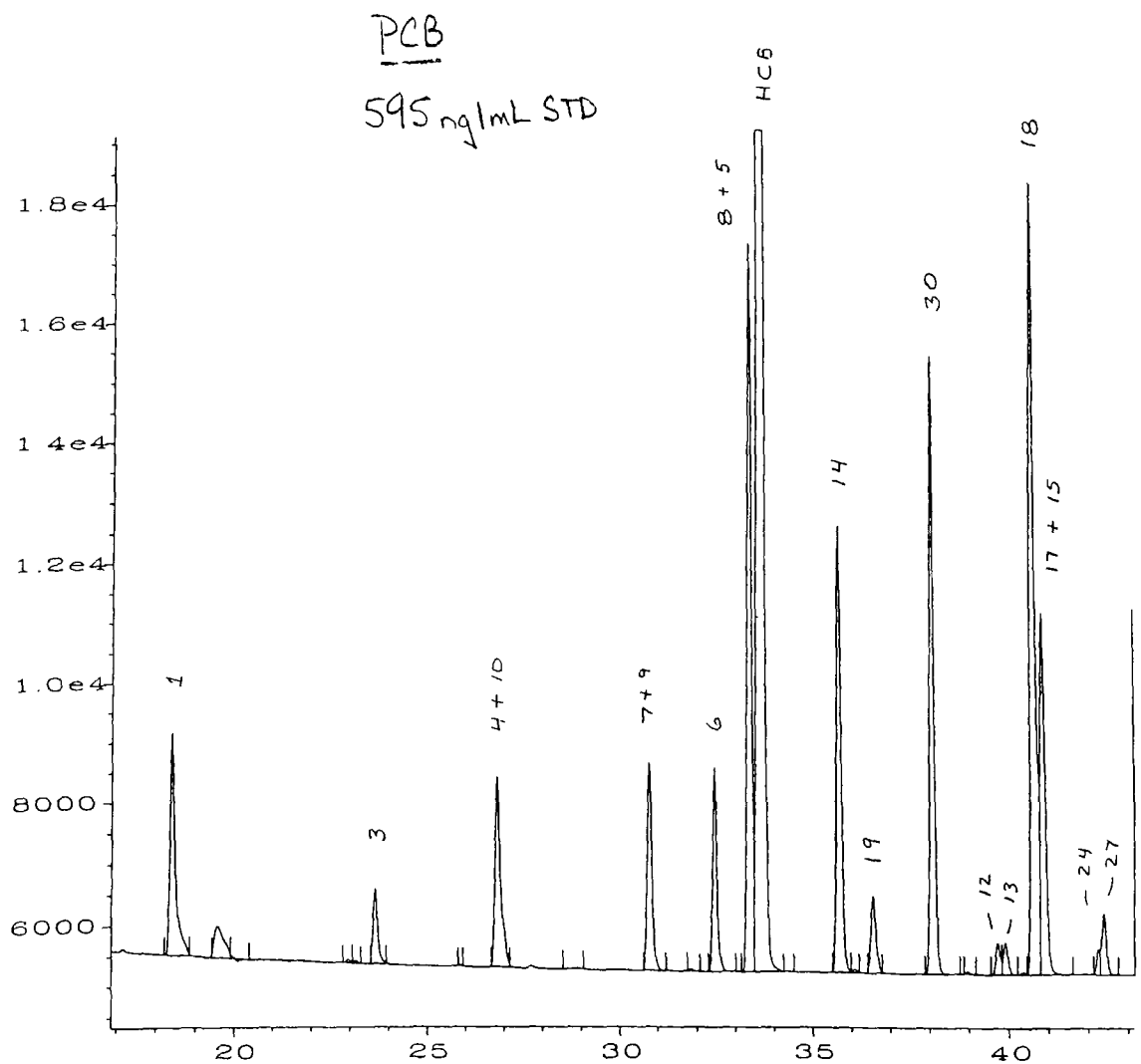
On a barcode mismatch: inject anyway

Comment:
Preparation dates: 595 + PES STD = 05 AUG 94
30,204 I.S. = 21 NOV 94
342 QC CHK = 05 AUG 94
Blowdown volumne: 1 ml
Injection: 2 ul, column: DB5, 60 m.
Sample set: 941222F

Sample Log Table

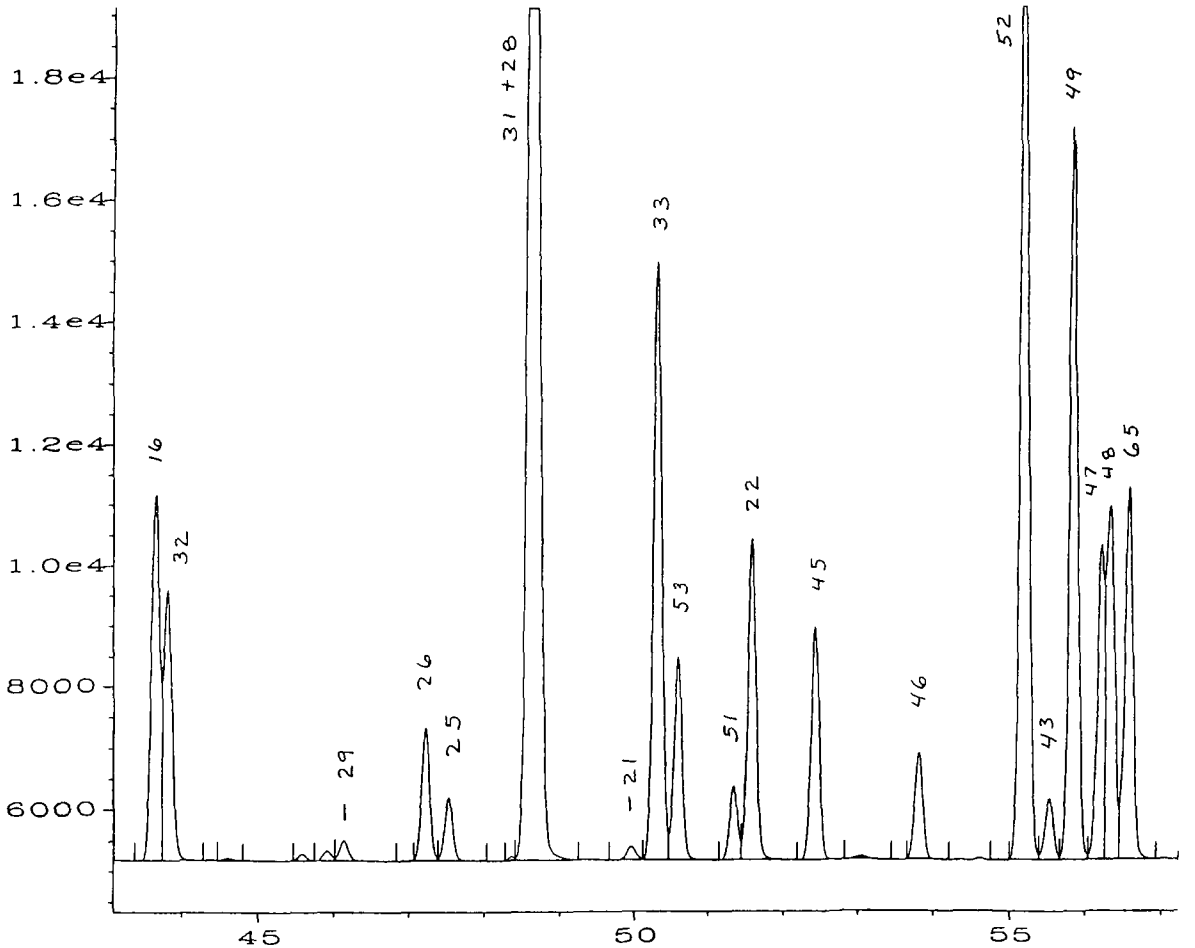
Seq. line	Vial Num.	Sample Name	Sample Multiplier Amount	ISTD Amount	Cal. Line	Method Name	Inj/ Vial
FRONT							
1	21	HEXANE	1			BAKE	1
2	21	HEXANE	1			MULLIN	1
2	22	595 STD 950303	1			MULLIN	1
2	23	595 STD 950303	1			MULLIN	1
2	24	342QCCHK 950303	1			MULLIN	1
2	25	XPFB 950227 HX	1			MULLIN	1
2	26	XP01 950227 HX	1			MULLIN	1
2	27	LB 950206C HX	1			MULLIN	1
2	28	LBXT4 950206C HX	1			MULLIN	1
2	29	LBX14 950206C HX	1			MULLIN	1
2	30	HEXANE	1			MULLIN	1
2	31	595 STD 950303	1			MULLIN	1
2	32	CMS 941222F HX	1			MULLIN	1
2	33	LB 941222F HX	1			MULLIN	1
2	34	BH01F 941100 HX	1			MULLIN	1
2	35	CH01F 941100 HX	1			MULLIN	1
2	36	IH01F 941100 HX	1			MULLIN	1
2	37	IH01F 941117 HX	1			MULLIN	1
2	38	IH02F 941117 HX	1			MULLIN	1
2	39	JH01F 941100 HX	1			MULLIN	1
2	40	MH01F 941100 HX	1			MULLIN	1
2	41	UH01F 941100 HX	1			MULLIN	1
2	42	VH01F 941100 HX	1			MULLIN	1
2	43	WH01F 941100 HX	1			MULLIN	1
2	44	HEXANE	1			MULLIN	1
2	45	595 STD 950303	1			MULLIN	1

Appendix C-2. PCB 595 ng/mL Standard Chromatogram



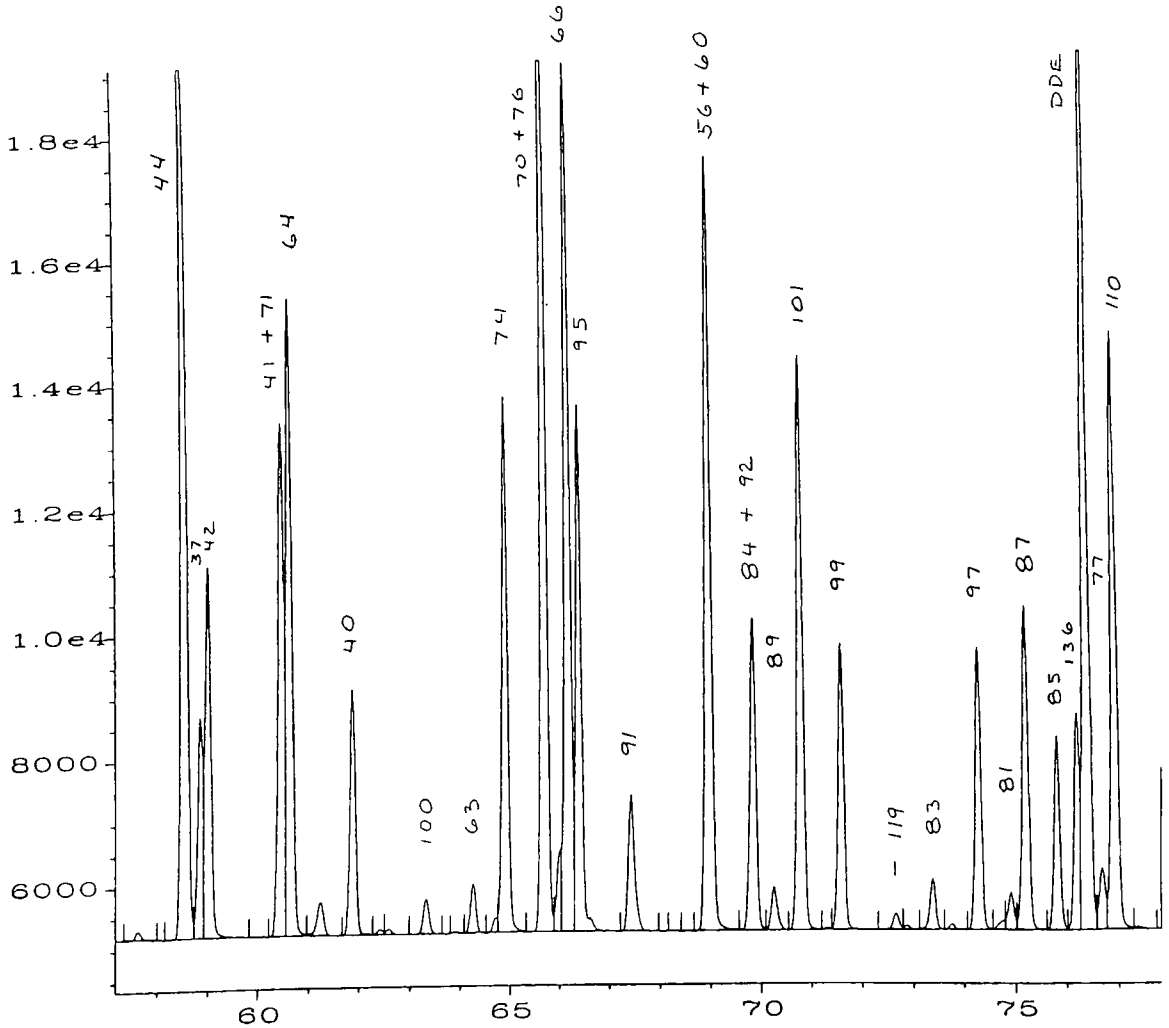
Sig. 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-2. (Cont'd)



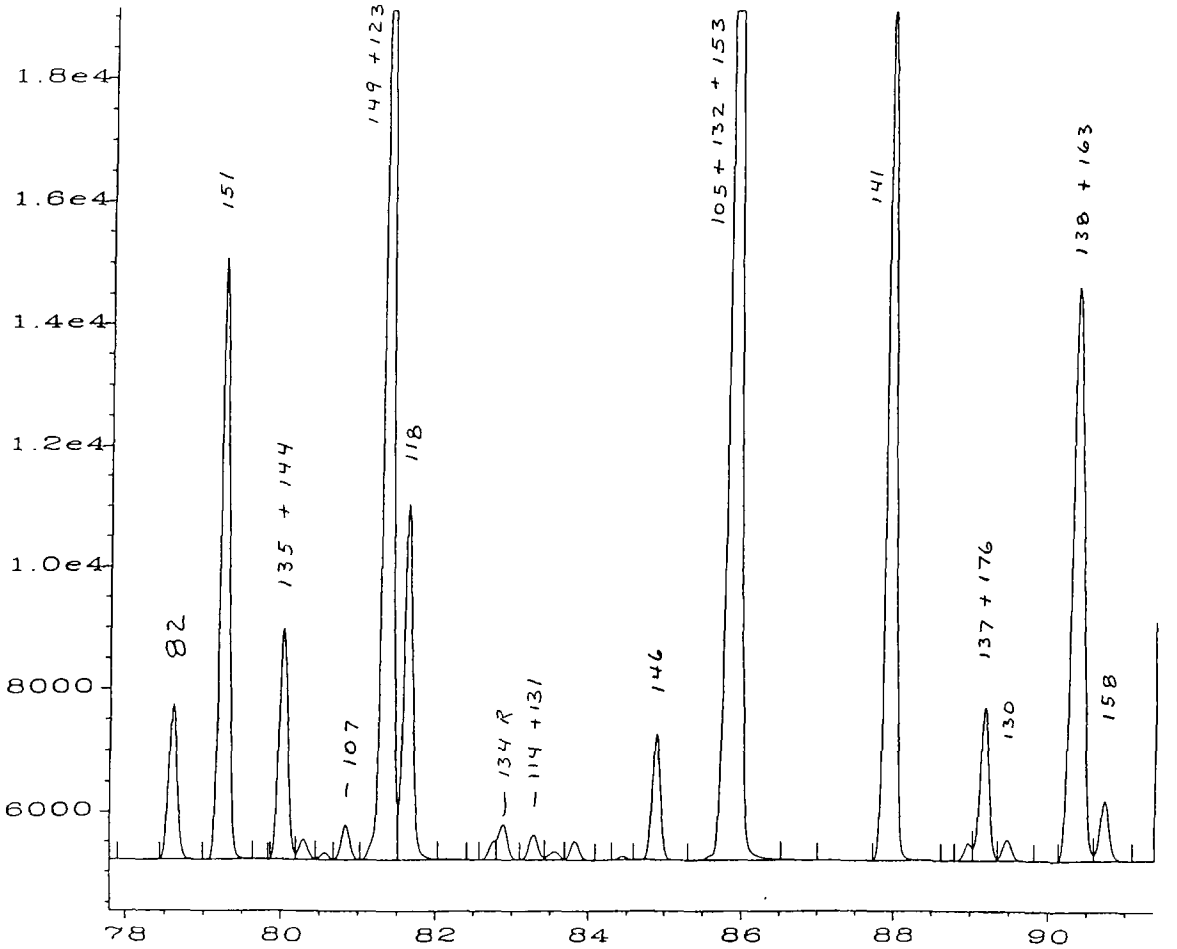
Sig. 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-2. (Cont'd)



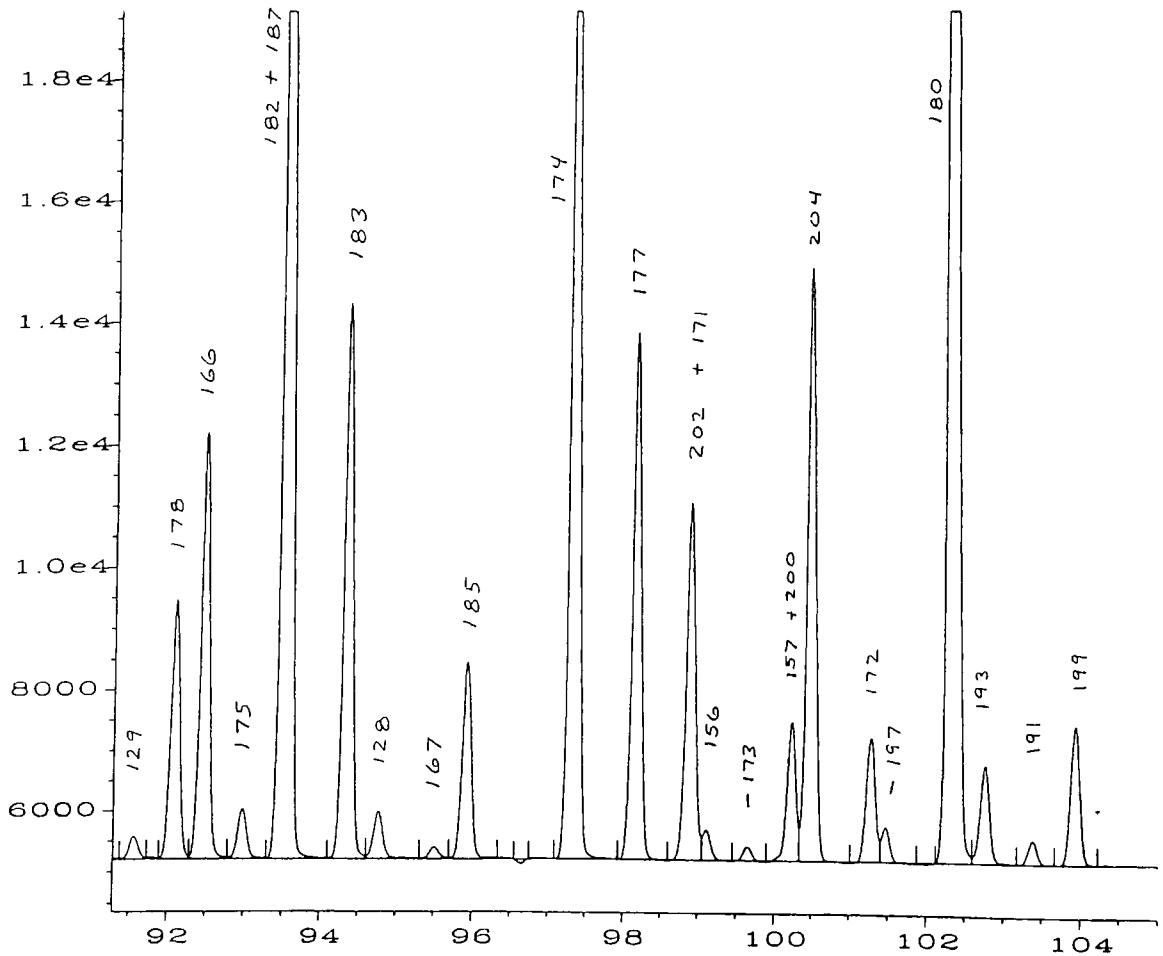
Sig. 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-2. (Cont'd)



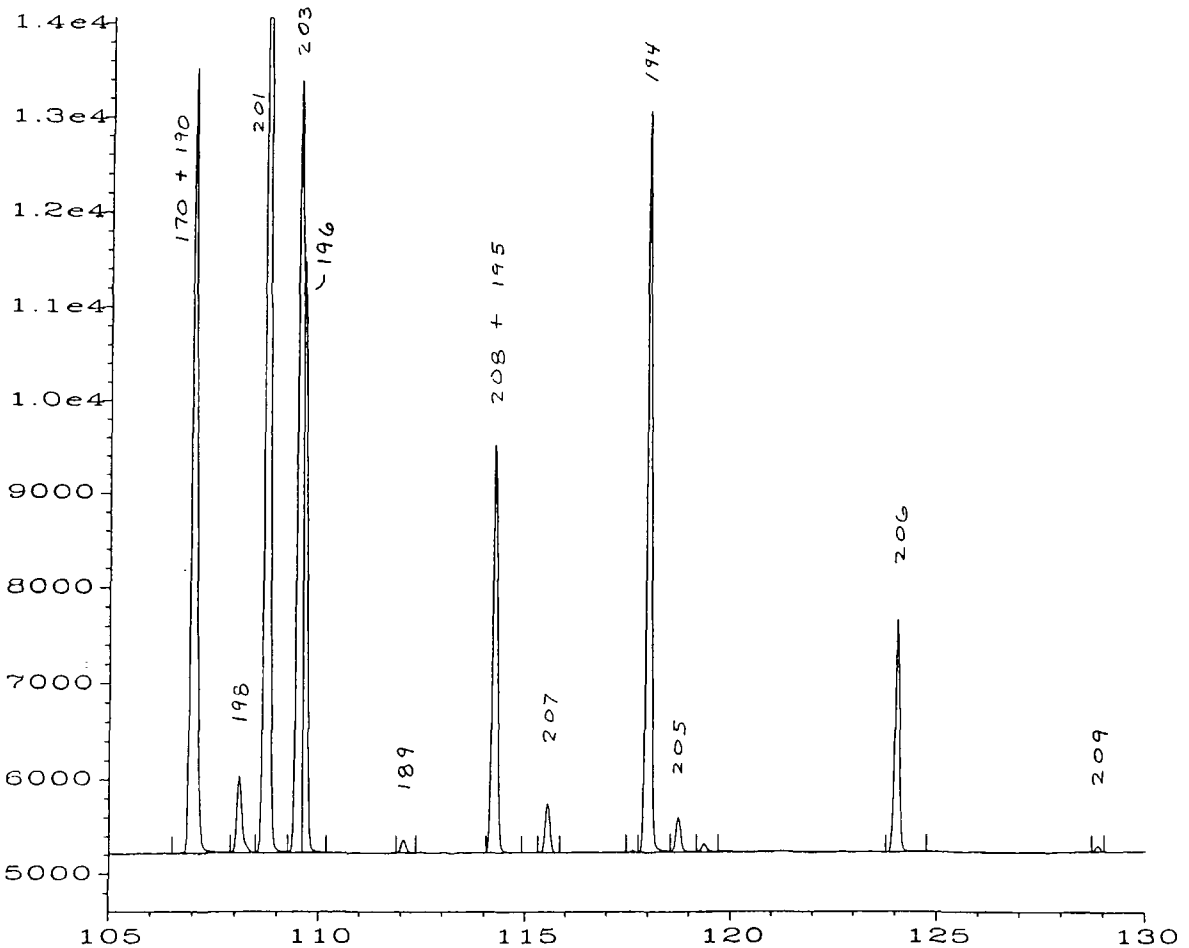
Sig. 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-2. (Cont'd)



Sig 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-2. (Cont'd)



Sig. 1 in C:\HPCHEM\1\DATA\941219H\023F0201.D

Appendix C-3. PCB Calibration Table

Method: C:\HPCHEM\1\METHODS\PCB1.MTH

Calibration Table

#	RT	Lvl	ng	Amt/Area	Ref Istd	I#	Name
1	18.270	1	42.0	1.3056e-003		1	1 (14)
2	23.510	1	24.5	3.0529e-003		1	3
3	26.667	1	11.9	3.9365e-004		1	4 + 10
4	30.633	1	4.2	1.6441e-004		1	7 + 9
5	32.320	1	6.65	2.8359e-004		1	6
6	33.226	1	49.0	6.1031e-004		1	8 + 5
7	33.481	1	40.0	7.0083e-005		1	HCB
8	35.533	1	22.19	4.4475e-004		1	14 surrogate
9	36.402	1	0.98	8.7173e-005		1	19
10	37.938	1	9.12	1.3793e-004	Ref ISTD	1	30 internal std
11	39.578	1	0.595	1.8906e-004		1	12
12	39.772	1	0.3395	1.0793e-004		1	13
13	40.501	1	12.95	1.3403e-004		1	18
14	40.738	1	12.95	2.9468e-004		1	15 + 17
15	42.112	1	0.175	9.177e-005		1	24
16	42.259	1	0.735	1.0768e-004		1	27
17	43.516	1	7.0	1.6519e-004		1	16
18	43.678	1	6.65	2.2082e-004		1	32
19	45.988	1	0.1855	1.1757e-004		1	29
20	47.085	1	2.52	1.8308e-004		1	26
21	47.393	1	1.12	1.7135e-004		1	25
22	48.504	1	32.9	1.8577e-004		1	31 + 28
23	49.827	1	0.112	8.6874e-005		1	21
24	50.173	1	11.55	1.7372e-004		1	33
25	50.457	1	2.24	9.398e-005		1	53
26	51.210	1	0.63	7.6311e-005		1	51
27	51.444	1	10.15	2.8382e-004		1	22 (65)
28	52.288	1	3.115	1.1769e-004		1	45
29	53.676	1	1.4	1.1351e-004		1	46
30	55.064	1	15.75	1.4022e-004		1	52
31	55.403	1	0.945	1.3413e-004		1	43
32	55.714	1	8.05	1.0043e-004		1	49
33	56.089	1	3.5	1.1243e-004		1	47
34	56.199	1	3.5	8.9794e-005		1	48
35	56.441	1	4.74	1.1166e-004		1	65 surrogate
36	58.432	1	15.05	1.3076e-004		1	44
37	58.756	1	4.2	1.967e-004		1	37
38	58.905	1	4.9	1.1316e-004		1	42
39	60.351	1	8.05	1.4252e-004		1	41 + 71
40	60.510	1	6.3	9.1908e-005		1	64
41	61.758	1	3.29	1.204e-004		1	40
42	63.198	1	0.385	1.0325e-004		1	100
43	64.142	1	0.735	1.4191e-004		1	63
44	64.781	1	6.65	1.1965e-004		1	74
45	65.576	1	11.9	1.0377e-004		1	70 + 76
46	66.055	1	18.2	2.0515e-004		1	66
47	66.278	1	7.0	1.1703e-004		1	95
48	67.309	1	1.785	1.0315e-004		1	91
49	68.855	1	12.25	1.4375e-004		1	56 + 60
50	69.720	1	6.3	1.713e-004		1	92 + 84
51	70.133	1	0.35	6.4548e-005		1	89
52	70.667	1	6.3	9.7475e-005		1	101
53	71.463	1	2.59	8.1995e-005		1	99

Appendix C-3. (Cont'd)

Method: C:\HPCHEM\1\METHODS\PCB1.MTH

54	72.547	1	0.098	5.2775e-005		1	119	
55	73.267	1	0.525	8.7674e-005		1	83	
56	74.158	1	1.96	6.4062e-005		1	97	
57	74.786	1	0.56	1.424e-004		1	81	
58	75.081	1	3.5	9.1511e-005		1	87	
59	75.690	1	2.45	1.1276e-004		1	85	
60	76.087	1	2.625	1.1117e-004		1	136	
61	76.281	1	20.0	2.0671e-004		1	DDE	
62	76.557	1	0.805	1.189e-004		1	77	
63	76.807	1	6.65	1.0175e-004		1	110	
64	78.489	1	1.54	8.6984e-005		2	82 (166)	
65	79.137	1	5.95	8.3983e-005		2	151	
66	79.903	1	3.115	1.094e-004		2	135 + 144	
67	80.720	1	0.455	1.1219e-004		2	107	
68	81.247	1	9.8	7.5132e-005		2	123 + 149	
69	81.512	1	4.2	1.0255e-004		2	118	
70	82.777	1	0.252	6.6548e-005		2	134R	
71	83.171	1	0.49	1.6698e-004		2	114 + 131	
72	84.778	1	1.365	9.2233e-005		2	146	
73	85.791	1	15.05	7.9865e-005		2	132 + 153 + 105	
74	87.838	1	5.95	5.3325e-005		2	141	
75	89.077	1	0.91	4.633e-005		2	137 + 176	
76	89.377	1	0.2625	9.995e-005		2	130	
77	90.273	1	9.45	1.0607e-004		2	163 + 138	
78	90.627	1	0.875	1.0594e-004		2	158	
79	91.476	1	0.0455	1.6136e-005		2	129	
80	91.996	1	3.85	1.1609e-004		2	178	
81	92.377	1	4.76	9.2456e-005		2	166	surrogate
82	92.886	1	0.7	1.0278e-004		2	175	
83	93.430	1	12.6	6.7425e-005		2	187 + 182	
84	94.251	1	5.95	8.6864e-005		2	183	
85	94.673	1	0.35	5.5002e-005		2	128	
86	95.409	1	0.1715	1.1549e-004		2	167	
87	95.834	1	1.645	6.5655e-005		2	185	
88	97.241	1	11.2	7.9148e-005		2	174	
89	98.073	1	5.95	8.9665e-005		2	177	
90	98.802	1	2.765	5.7744e-005		2	202 + 171	
91	99.011	1	0.231	6.1043e-005		2	156	
92	99.560	1	0.133	6.771e-005		2	173	
93	100.141	1	1.365	7.299e-005		2	157 + 200	
94	100.394	1	6.03	7.8638e-005	Ref ISTD	2	204	internal std
95	101.182	1	1.96	1.1516e-004		2	172	
96	101.376	1	0.385	8.7529e-005		2	197	
97	102.248	1	21.35	8.7835e-005		2	180	
98	102.667	1	1.47	9.8563e-005		2	193	
99	103.291	1	0.42	1.1757e-004		2	191	
100	103.846	1	1.505	7.8345e-005		2	199	
101	106.907	1	5.95	7.8308e-005		2	170 + 190	
102	108.001	1	0.42	5.5328e-005		2	198	
103	108.642	1	14.7	1.2464e-004		2	201	
104	109.427	1	7.35	1.0391e-004		2	203	
105	109.532	1	7.0	1.6017e-004		2	196	
106	111.955	1	0.14	9.8705e-005		2	189	
107	114.145	1	2.8	7.1185e-005		2	208 + 195	
108	115.463	1	0.3255	6.4941e-005		2	207	
109	117 906	1	6.3	8.7518e-005		2	194	

Appendix C-3. (Cont'd)

Method: C:\HPCHEM\1\METHODS\PCB1.MTH

110	118.652	1	0.385	9.8299e-005	2	205
111	123.947	1	2.38	9.5285e-005	2	206
12	128.795	1	0.042	5.172e-005	2	209

Calibration Settings

Title:

Reference window:	0.250 %
Non-reference window:	0.250 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

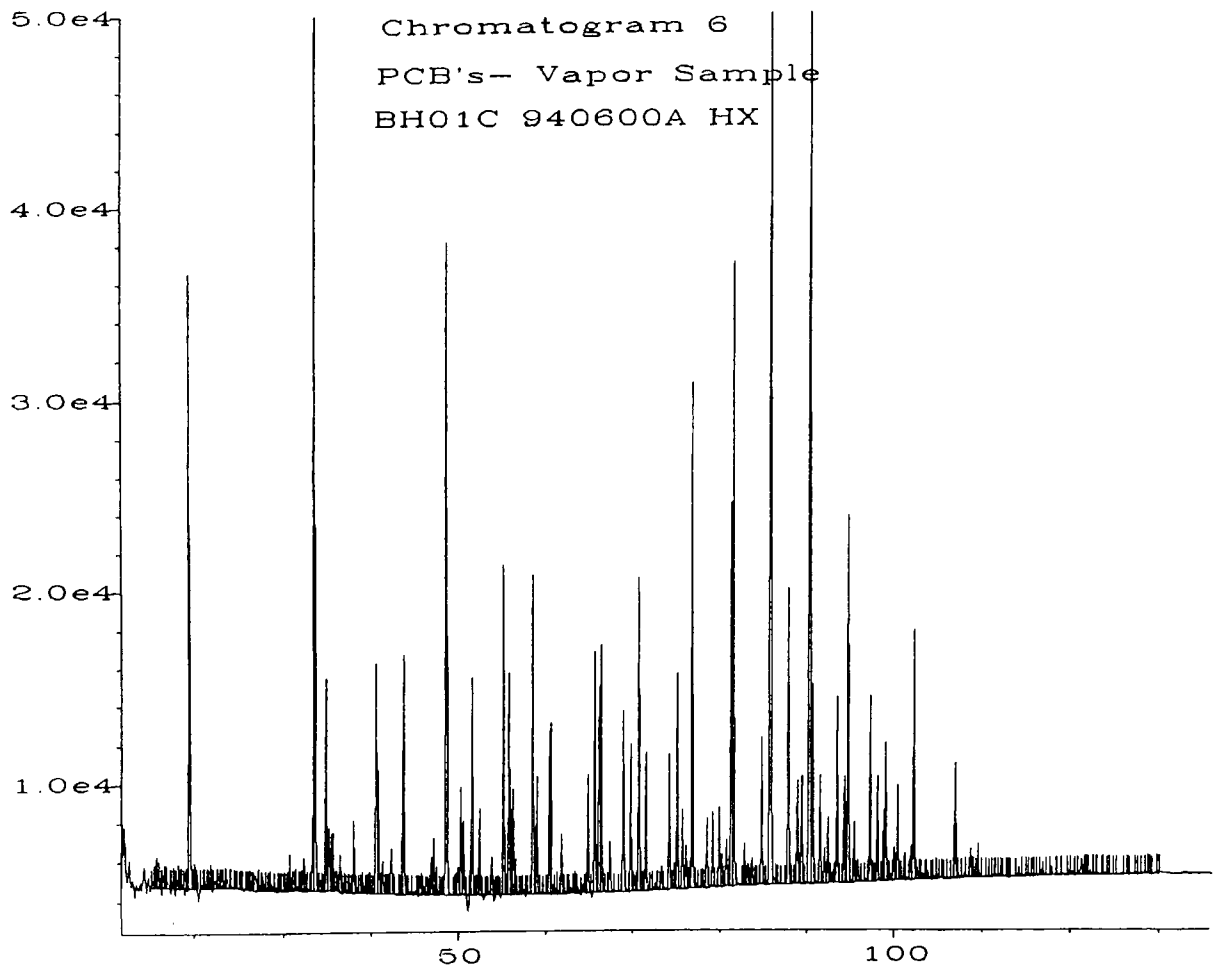
Sample ISTD Information

I#	Amount
1	9.12
2	6.03

Multilevel Information

Fit: Linear
Origin: Force

Appendix C-4. PCB Sample Chromatogram



Sig. 1 in C:\HPCHEM\CHECKING\JN94CH\027F0201.D

Appendix C-5. PCB Sample Report

Internal Standard Report

Data File Name	C:\HPCHEM\1\DATA\JN94CH\027F0201.D		
Operator	MONTE	Page Number	1
Instrument	ANALYZER1	Vial Number	27
Sample Name	BH01C 940600A HX	Injection Number	1
Run Time Bar Code:		Sequence Line	2
Acquired on	12 Nov 94 09:52 AM	Instrument Method:	MULLIN.MTH
Report Created on:	26 Jan 95 11:00 AM	Analysis Method	ISWS'PCB.MTH
Last Recalib on	18 JAN 95 02:25 PM	Sample Amount	0
Multiplier	1	ISTD Amount	8.09

Sig. 1 in C:\HPCHEM\1\DATA\JN94CH\027F0201.D

Ret Time	Area	Type	Width	Ref#	ng	Name
18.270	* not found *			1		1 (14)
23.510	* not found *			1		3
26.667	* not found *			1		4 + 10
30.633	* not found *			1		7 + 9
32.345	12677	VP	0.142	1	8.013	6
33.256	31808	VV	0.126	1	43.271	8 + 5
33.516	412806	VP	0.114	1	64.487	HCB
35.564	22467	VV	0.114	1	22.272	14 surrogate
36.429	16004	PP	0.125	1	3.110	19
37.976	26312	PP	0.110	1-IR	8.090	30 internal std
39.651	1799	PVA	0.123	1	0.758	12
39.812	1324	VV +	0.000	1	0.318	13
40.533	96535	VV	0.122	1	28.841	18
40.775	52131	VV	0.124	1	34.242	15 + 17
42.146	3918	PV	0.089	1	0.801	24
42.291	17953	VP	0.116	1	4.309	27
43.558	33925	PV	0.105	1	12.492	16
43.718	96117	VV	0.117	1	47.309	32
46.040	3301	VP	0.131	1	0.865	29
47.130	21909	VV	0.121	1	8.940	26
47.436	10143	VP	0.116	1	3.874	25
48.597	328313	VVA	0.141	1	135.949	31 + 28
49.908	12809	VV	0.137	1	2.480	21
50.234	46002	VV	0.128	1	17.813	33
50.509	30698	VBA	0.126	1	6.431	53
51.268	10702	PV	0.109	1	1.820	51
51.499	77745	VP	0.107	1	49.184	22 (65)
52.337	33277	VP	0.117	1	8.730	45
53.723	15803	VP	0.126	1	3.998	46
55.119	129655	PV	0.118	1	40.523	52
55.454	7688	VV	0.118	1	2.299	43
55.770	84873	VP	0.116	1	18.999	49
56.143	32633	PV	0.100	1	8.178	47
56.256	41157	VV	0.114	1	8.238	48
56.499	12988	VP	0.113	1	3.233	65 surrogate
58.485	120051	VV	0.112	1	34.990	44
58.807	26628	VV	0.111	1	11.675	37
58.958	47016	VV	0.116	1	11.859	42
60.398	64729	PV	0.116	1	20.564	41 + 71
60.559	66442	VV	0.113	1	13.612	64
61.805	22329	BV	0.114	1	5.993	40

Appendix C-5. (Cont'd)

63.240	4783	VVA	0.116	1	1.101	100
64.196	2246	PV	0.092	1	0.711	63
64.831	47206	VP	0.122	1	12.590	74
65.628	93861	PV	0.118	1	21.712	70 + 76
66.107	80996	VV	0.118	1	37.039	66
66.322	100355	VV	0.121	1	26.180	95
67.353	23132	VBA	0.131	1	5.319	91
68.895	75078	VV	0.127	1	24.056	56 + 60
69.759	60811	VV	0.124	1	23.220	92 + 84
70.168	6948	VV	0.133	1	1.00	89
70.710	126376	VV	0.122	1	27.458	101
71.508	56295	VV	0.121	1	10.289	99
72.586	3491	PV	0.146	1	0.411	119
73.304	9134	PV	0.118	1	1.785	83
74.194	53186	VV	0.118	1	7.595	97
74.825	4815	VV	0.111	1	1.528	81
75.119	85185	VV	0.115	1	17.376	87
75.725	32140	VV	0.123	1	8.078	85
76.121	17911	VV	0.127	1	4.438	136
76.315	5312	VV	0.117	1	2.448	DDE
76.585	10803	VV	0.123	1	2.863	77
76.846	205206	VV	0.122	1	46.543	110
78.515	27812	VV	0.121	2	4.722	82 (166)
79.173	30603	VV	0.124	2	5.016	151
79.935	34593	PV	0.135	2	7.386	135 + 144
80.750	19518	VVA	0.127	2	4.274	107
81.280	158360	VV	0.125	2	23.222	123 + 149
81.545	263380	VVA	0.128	2	52.714	118
82.806	16415	VV	0.121	2	2.132	134R
83.195	8442	VV	0.123	2	2.751	114 + 131
84.812	59396	PP	0.122	2	10.692	146
85.828	640338	PVA	0.156	2	99.814	132 + 153 + 105
87.851	122682	PV	0.126	2	12.768	141
89.103	8579	VV	0.112	2	0.776	137 + 176
89.401	43527	VV	0.125	2	8.491	130
90.310	643704	PV	0.148	2	133.262	163 + 138
90.659	82819	VVA	0.123	2	17.124	158
91.500	43235	VVA	0.121	2	1.362	129
92.025	14459	VV	0.126	2	3.276	178
92.404	27226	VV	0.126	2	4.913	166 surrogate
92.911	4843	VV	0.130	2	0.972	175
93.462	74608	VV	0.123	2	9.818	187 + 182
94.280	43770	BVA	0.126	2	7.421	183
94.696	150638	VVA	0.124	2	16.171	128
95.429	25738	VV	0.128	2	5.802	167
95.852	9196	VV	0.127	2	1.178	185
97.268	76068	VV	0.123	2	11.751	174
98.100	42381	PV	0.124	2	7.417	177
98.817	29417	PV	0.127	2	3.315	202 + 171
99.033	57397	VVA	0.125	2	6.838	156
99.584	3408	VV	0.139	2	0.450	173
100.160	3235	VV	0.104	2	0.461	157 + 200
100.420	39158	VV	0.123	2-IR	6.010	204 internal std
101.204	10858	VV	0.126	2	2.440	172
101.391	1154	VV	0.141	2	0.197	197
102.272	100809	VV	0.120	2	17.282	180
102.694	5435	VV	0.138	2	1.045	193
103.310	3085	BV	0.149	2	0.708	191

Appendix C-5. (Cont'd)

103.866	2834	VV	0.118	2	0.433	199	
106.925	51283	VV	0.135	2	7.838	170	+ 190
107.987	2283	PV	0.141	2	0.247	198	
108.661	12476	VV	0.130	2	3.035	201	
109.446	7106	BV	0.118	2	1.441	203	
109.558	6104	VV	0.112	2	1.908	196	
111.969	1011	VV	0.130	2	0.195	189	
114.161	4010	PV	0.134	2	0.557	208	+ 195
115.458	561	VV	0.141	2	0.0710	207	
117.920	4071	PV	0.135	2	0.695	194	
118.720	392	PV	0.167	2	0.0752	205	
123.962	760	PV	0.117	2	0.141	206	
128.801	153	VV	0.100	2	0.0155	209	

Time Reference Peak	Expected RT	Actual RT	Difference
10	37.938	37.976	0.1%
94	100.394	100.420	0.0%

Not all calibrated peaks were found

Appendix C-6. PCB Integrator Event Report

Method: C:\HPCHEM\1\METHODS\PCB1.MTH

Integration Events

Events:	Value:	Time:
Initial Area Reject	200	INITIAL
Initial Peak Width	0.040	INITIAL
Shoulder Detection	OFF	INITIAL
Initial Threshold	-6	INITIAL
Integrator OFF		0.000
Area Reject	100	1.150
Integrator ON		15.107
Negative Peak ON		15.107
Baseline Now		19.190
Baseline Now		24.907
Baseline Now		25.960
Baseline Hold ON		25.960
Baseline Hold OFF		26.549
Baseline Now		26.550
Baseline Now		28.887
Baseline Now		30.360
Negative Peak OFF		30.422
Baseline Now		30.513
Area Sum ON		30.517
Area Sum OFF		30.693
Negative Peak ON		30.956
Baseline Now		31.797
seline Now		32.950
Negative Peak OFF		32.967
Area Sum ON		32.968
Area Sum OFF		33.129
Negative Peak ON		33.855
Baseline Now		36.273
Baseline Now		37.730
Negative Peak OFF		39.559
Area Sum ON		39.726
Area Sum OFF		39.894
Negative Peak ON		40.085
Baseline Now		43.413
Baseline Now		48.157
Negative Peak OFF		48.160
Area Sum ON		48.837
Area Sum OFF		49.329
Negative Peak ON		49.432
Baseline Now		50.727
Baseline Now		61.550
Negative Peak OFF		63.083
Area Sum ON		63.329
Area Sum OFF		63.554
Negative Peak ON		63.862
seline Now		67.777
Negative Peak OFF		74.509
Area Sum ON		74.550
Area Sum OFF		74.756
Negative Peak ON		77.176
Negative Peak OFF		79.801
Area Sum ON		80.950

Appendix C-6. (Cont'd)

Method: C:\HPCHEM\1\METHODS\PCB1.MTH

Area Sum ON	81.893
Area Sum OFF	82.499
seline Now	82.500
Area Sum ON	82.522
Area Sum OFF	82.706
Negative Peak ON	83.975
Negative Peak OFF	85.388
Area Sum ON	86.060
Area Sum OFF	86.408
Negative Peak ON	88.425
Negative Peak OFF	90.025
Area Sum ON	90.860
Area Sum OFF	91.300
Area Sum ON	91.602
Area Sum OFF	91.857
Negative Peak ON	92.204
Baseline Now	93.977
Negative Peak OFF	93.981
Area Sum ON	94.384
Area Sum OFF	94.496
Area Sum ON	94.876
Area Sum OFF	95.234
Negative Peak ON	96.107
Negative Peak OFF	98.456
Area Sum ON	99.149
ea Sum OFF	99.440
Area Sum ON	100.828
Area Sum OFF	101.074
Negative Peak ON	101.700
Baseline Now	103.053
Baseline Now	109.260
Baseline Now	120.780
Integrator OFF	130.000

Calibration Settings

Title:

Reference window:	0.250 %
Non-reference window:	0.250 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

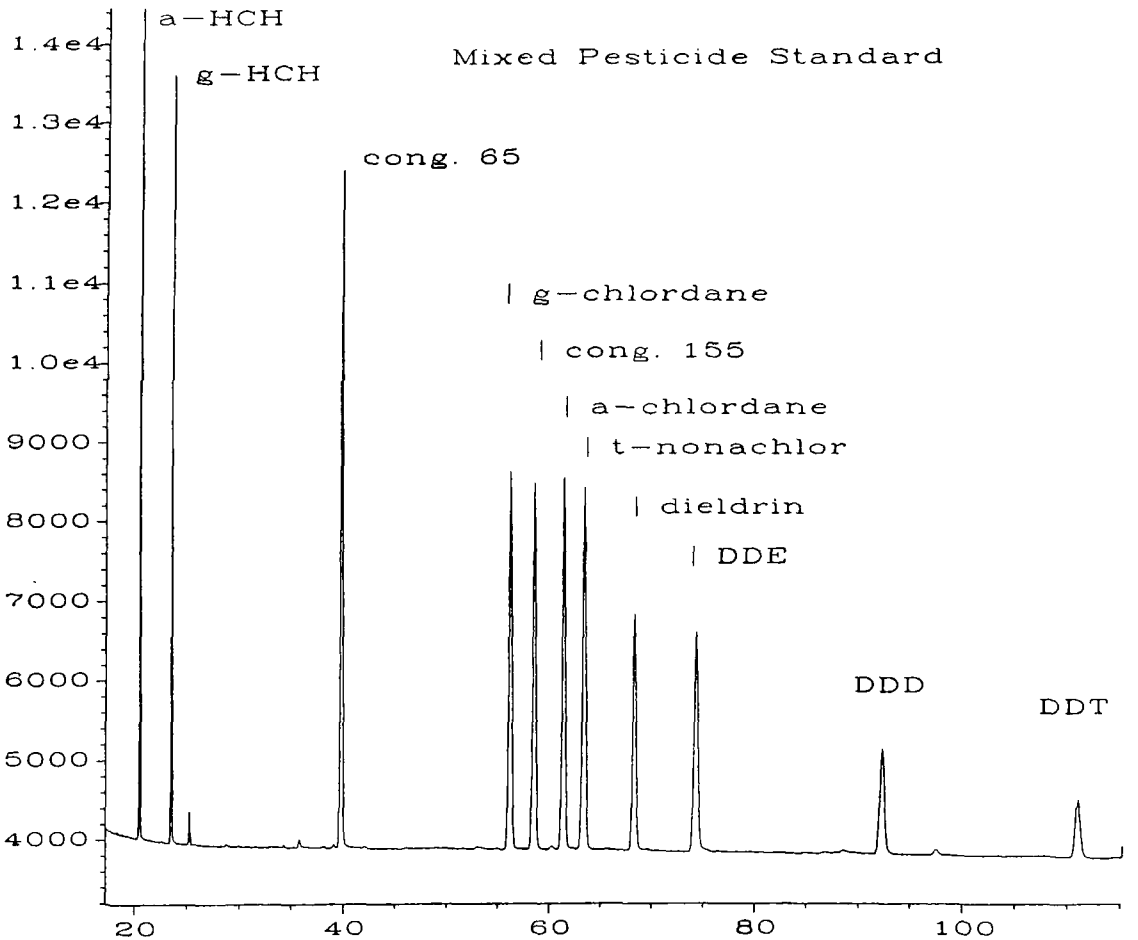
Sample ISTD Information

I#	Amount
1	9.12
2	6.03

Multilevel Information

Fit: Linear

Appendix C-7. Pesticide 20 ng/mL Standard Chromatogram



Sig. 1 in C:\HPCHEM\CHECKING\9503034F\043F0201.D

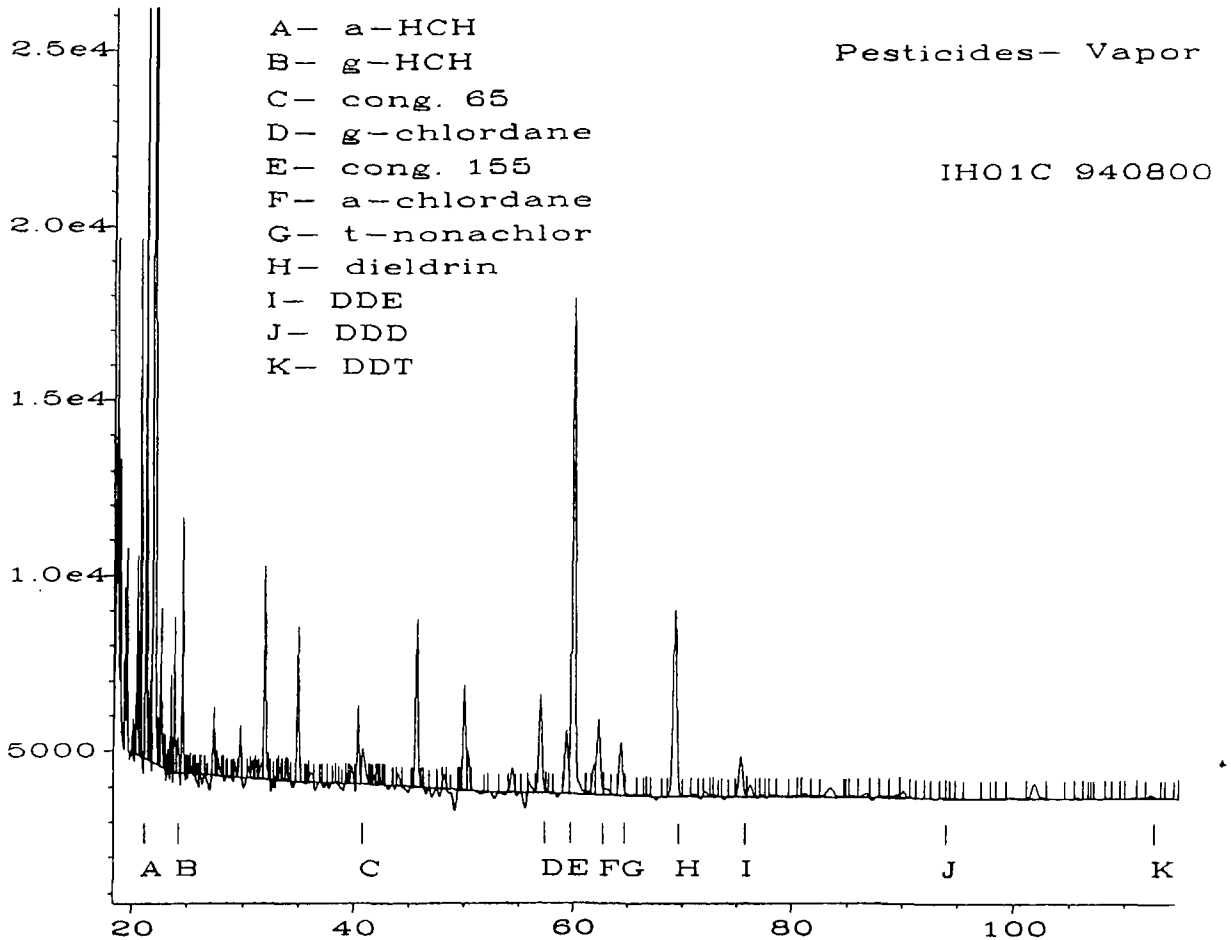
Appendix C-8. Pesticide Calibration Table

Method: C:\HPCHEM\1\METHODS\PEST1.MTH

Calibration Table

Pk#	RT	Lvl	ng	Amt/Area	Ref Istd	I#	Name
1	20.477	1	20.0	3.4963e-004		1	A-HCH
2	23.517	1	20.0	3.9724e-004		1	G-HCH
3	39.765	1	4.74	4.2976e-005		1	CONG 65(I.S)
4	56.179	1	20.0	2.2182e-004		1	G-CHLORDANE
5	58.529	1	14.99	1.5123e-004		1	CONG 155(I.S)
6	61.385	1	20.0	2.0852e-004		1	A-CHLORDANE
7	63.406	1	20.0	2.0738e-004		1	T-NONACHLOR
8	68.317	1	20.0	2.816e-004		1	DIELDRIN
9	74.299	1	20.0	2.6304e-004	Ref ISTD	1	DDE(I.S)
10	92.310	1	20.0	4.7227e-004		1	DDD
11	111.059	1	20.0	8.9441e-004		1	DDT

Appendix C-9. Pesticide Sample Chromatogram



Sig. 1 in C:\HPCHEM\CHECKING\941025C4\049F0201.D

Appendix C-10. Pesticide Sample Report

Internal Standard Report

```
Data File Name    : D:\HPCHEM\2\DATA\941025C4\049F0201.D
Operator          : MONTE                          Page Number       : 1
Instrument        : ANALYZER2                      Vial Number       : 49
Sample Name       : IH01C 940800 40                Injection Number  : 1
Run Time Bar Code:                                 Sequence Line     : 2
Acquired on       : 06 Nov 94  02:21 PM            Instrument Method: ISWS'PES.MTH
Report Created on: 14 Feb 95  03:44 PM             Analysis Method   : PEST2.MTH
Last Recalib on   : 14 FEB 95 03:19 PM             Sample Amount     : 0
Multiplier        : 1                              ISTD Amount       : 20

Sig. 1 in D:\HPCHEM\2\DATA\941025C4\049F0201.D
Ret Time     Area      Type Width Ref#  ng                    Name
|-------|-----------|----|-----|-----|--------|-----------------------------|
  20.676       77562 VP   0.088  1      51.647 A-HCH
  23.773       27066 PV   0.094  1      19.116 G-HCH
  40.294       25633 PV   0.181  1      13.009 CONG 65
  56.960       57957 VV   0.328  1      28.780 G-CHLORDANE
  59.336       35618 PV   0.311  1      15.320 CONG 155
  62.249       50086 VV   0.347  1      23.779 A-CHLORDANE
  64.293       32679 PP   0.340  1      15.738 T-NONACHLOR
  69.273      127351 PV   0.371  1      92.475 DIELDRIN
  75.279       29322 PV   0.373  1-IR   20.000 DDE
  93.453         936 PP   0.278  1       1.133 DDD
 112.349        2646 PP   0.374  1       5.127 DDT

       Time Reference Peak      Expected RT    Actual RT     Difference
                  9                 75.220        75.279          0.1%
```

Appendix C-11. Pesticide Integrator Event Report

Method: C:\HPCHEM\1\METHODS\PEST2.MTH

Integration Events

Events:	Value:	Time:
Initial Area Reject	100	INITIAL
Initial Peak Width	0.040	INITIAL
Shoulder Detection	OFF	INITIAL
Initial Threshold	-6	INITIAL
Integrator OFF		0.000
Integrator ON		19.845
Negative Peak ON		20.000
Baseline Now		23.647
Baseline Now		25.503
Baseline Now		28.757
Baseline Now		35.383
Baseline Now		43.350
Baseline Now		52.060
Baseline Now		68.057
Integrator OFF		115.000

Calibration Settings

Title:

Reference window:	0.250 %
Non-reference window:	0.250 %
Units of amount:	ng
Multiplier:	1.0
RF uncal peaks:	0.0
Sample Amount:	0.0

Sample ISTD Information

I#	Amount
1	4.74

Multilevel Information

Fit: Linear
Origin: Force

Standard Operating Procedure for Isolation, Extraction and Analysis of Atrazine, DEA and DIA

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1994

Standard Operating Procedure for Isolation, Extraction and Analysis of Atrazine, DEA and DIA

1.0 General Principal

Atrazine, DEA and DIA will be isolated from Lake Michigan water samples using Carbopak SPE cartridges. Analytes will be extracted from SPE cartridges with 7 mL of 85% dichloromethane (DCM), 15% methanol (MeOH) solution (vol:vol), followed by 2 mL of MeOH. Concentrated extracts will be spiked with internal standard and analyzed by gas chromatography-mass spectrometry.

2.0 Isolation

Samples will be collected on board the EPA research vessel Lake Guardian according to the Standard Operating Procedure for sample collection. Samples will be transported to the University of Minnesota and stored at 4°C until processing. Samples will be stored for less than 30 days until extraction.

Carbopak (Supelco) solid phase extraction cartridges (SPE) will be used for herbicide isolation. SPE cartridges (250 mg) will be cleaned prior to use with 3 mL of 85% DCM, 15% MeOH solution (vol:vol). Just prior to isolation cartridges will be conditioned ~ 2 mL of MeOH, followed by ~2 mL of Milli Q water. Conditioning the cartridge is critical to achieving complete herbicide isolation, and facilitating the passing of sample water. Conditioning of SPE cartridges should be done less than 15 minutes before passing sample water through the cartridge.

Solvents and water are pulled through the SPE cartridge under vacuum. Samples are interfaced to the SPE cartridge using Teflon tubing and a rubber stopper. One end of the Teflon tubing is inserted through the stopper about one inch. The stopper and protruding tubing are then pressed into the reservoir end of the SPE cartridge. The other end of the Teflon tubing is placed in the sample bottle. The leur end of the extraction cartridge is connected to vacuum, and sample water is pulled through the cartridge at ~ 20 mL/min. Flow rate is controlled by adjusting the vacuum.

Teflon tubing is cleaned before each extraction by placing one end of the tubing into a reservoir of MeOH, and inserting the stopper end into a "dummy" extraction cartridge as though it were a sample. MeOH is then pulled through the tubing under the vacuum. This procedure is repeated with Milli-Q water as a rinse.

Prior to isolation the sample will be spiked with 50 µL of a surrogate solution containing terbutylazine. The sample should be spiked on the same day it is to be passed through the SPE cartridge. A 50 µL micro-pipetter fitted with a glass capillary tube is used for surrogate spiking. The same micro-pipetter should be used for spiking all samples.

Sample volumes are determined using an E2000 Mettler top loading balance. The full sample bottle is placed on the balance and the balance is tared to zero. After the sample has been passed through the SPE cartridge the empty sample bottle is re-weighed and the sample volume calculated by difference.

Once the sample has been passed through the SPE cartridge, the cartridge is labeled, disconnected from the vacuum line, wrapped in aluminum foil, and stored at 4°C. Cartridges are labeled with all information on the sample bottle including: Lake, station, date, depth and code number. The volume of the sample is also put on the label. All extraction information is also entered into the lab log book. The lab log book should contain all information transferred from the field sampling log book, along with the data of sample isolation procedure, volume of sample, and amount of surrogate added.

3.0 Extraction

SPE cartridges are removed from cold storage, the aluminum foil removed, and allowed to reach room temperature.

Herbicides are extracted from the SPE cartridge by passing 7 mL of 85% DCM, 15% MeOH (vol:vol) solution, followed by 2 mL of MeOH through the cartridge. Extraction solvent pass through the cartridge by gravity. The small amount of solvent remaining in the cartridge after extraction is forced through using a syringe fitted with a rubber stopper. The bored out stopper is inserted into the reservoir end of the cartridge and remaining solvent is forced through using the syringe.

As the extraction solvent moves through the SPE cartridge, a small spatula full of anhydrous sodium (0.75 g) sulfate is added to the extractant in the centrifuge tube. The sodium sulfate removes any water remaining from sample isolation. The solvent is then collected by the centrifuge tube. Care must be taken to insure that the solvent flows evenly through each step of the extraction, and does not build up and overflow the pipette. Care must also be taken to insure that the leur end of the cartridge is centered in the pipette, if it is not solvent may drip along the outsides of the pipette.

Once all the extraction solvent has been collected in the centrifuge tube, the tube is labeled with all information on the SPE cartridge, sealed with aluminum foil, lined, capped, and stored at 4°C. If any solvent is lost, or spilled during extraction this should be noted on the label, and in the lab log book.

Extracted samples should be stored no longer than two weeks in centrifuge tubes. In less than two weeks the extracted sample should be solvent reduced and concentrated. Sample volume is reduced by placing the centrifuge tube in a Supelco Visidry Evaporation Manifold, attached to zero graded nitrogen gas source. Solvent is evaporated under a slow steady stream of nitrogen. About 25 pounds of pressure is desirable, but all samples should be visually inspected to insure that solvent is not mixing violently or splashing.

Samples should be blown down to 0.3 mL in the centrifuge tube. When the sample has been reduced to 0.3 mL it should be removed from the evaporation manifold, capped and placed in a test tube rack. The sample should then be transferred immediately to a 2 mL amber vial. The sample should not be stored in the reduced volume condition as it may evaporated to dryness. The sample should be transferred to a 2 mL vial using a 9 inch disposable pipette and pipette bulb. The centrifuge tube should be rinsed with 85% DCM: 15% MeOH solution three times, and the rinse also transferred to the amber vial

The amber vial should be labeled with all information on the centrifuge tube, and stored at 4°C. If any solvent is spilled or lost during transfer this should be noted on the label and in the lab log book.

4.0 Analysis

Approximately 15 samples at a time should be prepared for analysis. Amber vials should be removed from cold storage and allowed to reach room temperature before uncapping. It is important that samples be allowed to reach room temperature before uncapping, otherwise condensation forming on the vials may enter the sample, and contaminated the sample with water.

Once the samples have reached room temperature they can be uncapped and placed in the Supelco Visidry Evaporation Manifold. The 2 mL vials should be concentrated to ~150 µL under a slow steady stream of nitrogen.

After the samples have been concentrated they should be spiked with 2 µL of the internal standard solution containing deuterated, $d_5$ethyl atrazine and 4,4'-dibromobiphenyl. Samples should be spiked using a 2 µL Hamilton syringe fitted with a cheny syringe guide. The cheny syringe guide assures that every injection spike is identical. The cheny adapter has a stopper fitted on the guide so that each injection is automatically set to deliver 2 µL. The stopper guide is calibrated at the beginning of the project to properly set the syringe for exactly a 2 µL injection. This is accomplished by making repeated injections of 20°C water onto an analytical balance, and adjusting the guide stop until the syringe consistently deliver 2 µL. It is very important the internal standard solution spikes are accurate and consistent since quantitation is based on these injection volumes (or mass per injection volume). Once a sample has been spiked with internal standard, the date and amount should be recorded in the lab log book.

Samples that have been spiked with internal standard should be run within one week after spiking. As long as samples are stored at 4°C, and in amber vials the internal standards are stable, but prompt analysis of the samples is recommended to lessen the chance of errors, sample evaporation, breakage, loss, and to enhance analytical consistency.

When a set of samples has been spiked and is ready for analysis the following steps should be followed:

1. Remove spiked samples and calibration standards from cold storage, and allow to reach room temperature.

2. Initiate AutoTune calibration function of GC-ms *chem station*.

3. Transfer ~ 50 µL of each sample and each calibration standard to a 2 mL auto sampler vial with 250 µL micro vial insert.

4. Four calibration standards are run daily. Calibration standards are preparedfrom a stock solution, and are the same calibration standards used by Schottler and Eisenreich.

5. Load samples and calibration standards into auto sampler tray, and log samples into GC-MS *chem station* sequence function.

6. Enter sample order, sample identification, and Adaption values in GC-MS sample run log book.

7. Fill auto sampler solvent wash vials with DCM and MeOH.

8. Run calibration standard (1) twice, and inspect to make sure all peaks are present and peak shape is good.

9. Initiate auto sampler and run *chem station* sequence function.

10. Acquire data and use internal standard method to quantitate, see Section 10.0 of QAPjP.

5.0 Blanks

5.1 Procedural Blanks

One procedural blank should be run with every 20-25 samples.

A procedural blank is a SPE cartridge that is processed identical to a sample with the exception of water being passed through the cartridge. A procedural blank is cleaned, eluted, concentrated and analyzed identical to a sample.

5.2 Solvent Blanks

Once every six months, or any time a procedural or field blank shows contamination a solvent blank should be run.

A solvent blank is simply the analytical reagents analyzed by GC-MSD for possible contaminants.

If a solvent blank shows contamination the auto sampler injection syringe should be changed and second solvent blank run. Blank contamination often results from dirty syringe needles, and should be tested as described to eliminate or confirm solvent as contaminated.

6.0 Sample Locations

Remember:

Rinse three times
Fill two bottles per one sample
Label bottle and cap

6.1 Open Water Stations:

6.1.1 If Stratified

*Mid Epi
*Mid Hypo (If possible, sample hypo at depth that corresponds to mean particle mass as measured by transmissometery)

6.1.2 If Not Stratified

*2 ft. below surface
*Mid-water column

Collect Duplicates of two open water stations. One station in Northern LM and one in Southern LM. Put "DUP" on label.

6.2 Master Stations:

6.2.1 If Stratified

Stations 18 and 41
*2 ft below surface plus duplicate
*5 ft below surface
*Mid Epi
*Thermo
*Mid Hypo
*5 ft off bottom plus duplicate
*plus duplicates of all depths at St. 23 and put "BE DUP" on label

6.2.2 If Not Stratified

All Master Stations: (18,23,27,41,47)
*2 ft below surface
*Mid water column
*5 ft off bottom
*Duplicates of all depths at Stations 18,23,41
(Put BE on St. 23 label)

Mark St. 18 and St. 41 Duplicates with "DUP," St. 23 with "BE DUP" in addition to regular sample label.

Standard Operating Procedures for Semivolatile Organic Compounds in Dry Deposition Samples

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1994

Standard Operating Procedures for Semivolatile Organic Compounds in Dry Deposition Samples

1.0 Overview

1.1 Tare weights and final weights of dry deposition plates are determined at the Illinois Institute of Technology (IIT).

1.2 Immediately after the final weight is determined at IIT, two to four deposition plates from each site during each sampling period will be placed into precleaned 120 mL wide-mouth amber bottles, capped, labeled and stored frozen in the dark until shipped overnight unfrozen to Rutgers University/University of Minnesota (RU/UMN) via Federal Express.

1.3 Upon receipt at RU/UMN, samples are again stored frozen in the dark until analyzed.

1.4 Storage of samples will be limited to about one to two years prior to extraction.

2.0 Sample Extraction at RU/UMN

2.1 General Notes

All laboratory glassware and utensils are washed with Alconox and rinsed with tap water, Milli-Q® water (Millipore) and acetone and baked overnight at 450°C. All equipment is wrapped in aluminum foil during storage in the laboratory. Only pesticide grade solvents (Baxter) and reagent grade solids are employed as required during analytical procedures. Whenever clean glassware is used, the aluminum foil is first removed and the glassware rinsed with dichloromethane (DCM) prior to use. If at all possible, glassware openings are re-covered with aluminum foil during usage to minimize exposure to laboratory air. At all transfer steps, sample codes and descriptions are similarly labeled on the next vial containing that sample. Thus, the same sample code is passed from the original sample bottle to the final vial containing the final extract for instrument injection and final archiving. All procedures and sample codes are similarly recorded in laboratory notebooks and instrument logbooks. Detailed records of daily laboratory progress with all pertinent observations must be noted in the laboratory notebook by the individual performing the analytical tasks.

2.2 Specific Procedure

2.2.1 Using forceps, place all deposition strips from an individual amber bottle, representing one site exposed for one sample cycle (≈ one month) or two to three month composites, into a clean 35 mL screw-top amber glass vial.

2.2.2 Rinse the original sample bottle with two 1 mL aliquots of DCM and add to the extraction vial.

2.2.3 With syringe(s), add an appropriate amount of analytical surrogate standards (3,5-dichlorobiphenyl [IUPAC #14], 2,3,5,6-tetrachlorobiphenyl [IUPAC #65], and 2,3,4,4',5,6-hexachlorobiphenyl [IUPAC #166] for PCBs and t-nonachlor; d_8-naphthalene, d_{10}-fluorene, d_{10}-fluoranthene and d_{12}-perylene for PAHs) to the extraction vial. (The subset of samples for atrazine analysis will be additionally spiked with the surrogate, terbutylazine).

2.2.4 Add 30 mL of DCM to the extraction vial to cover the greased deposition strips.

2.2.5 Place the extraction vials in an ultrasonic bath, sonicate for 30 min and store vials overnight in a freezer.

2.2.6 Transfer the DCM to another clean amber 35 mL vial with two 1 mL hexane (HEX) rinses of the extraction vial.

2.2.7 Concentrate the DCM extract to ≈ 1 mL under a gentle N_2 gas stream.

2.2.8 Meanwhile, add approximately 30 mL of HEX to the original extraction vial with the dry deposition strips, sonicate for 15 min., and store vials overnight in a freezer.

2.2.9 After the DCM fraction is blown down to ≈ 1 mL, add the HEX fraction to the DCM vial with two 1 mL HEX rinses of the original extraction vial.

2.2.10 Discard the original extraction vial or reclean.

2.2.11 Concentrate the HEX to ≈ 1 mL under a gentle N_2 gas stream.

2.2.12 The HEX sample is now ready for cleanup (unless selected for atrazine analysis).

2.3 If samples are selected for *atrazine analysis:*

2.3.1 General Notes: Since atrazine is *not* expected to be observed in most dry deposition samples, only a select set of samples will be analyzed for atrazine. These samples will primarily include the spring samples (April, May, June) when atrazine is most likely to be detected on atmospheric particles. Because atrazine may not be recovered from silica gel/alumina column during sample cleanup for PCBs and PAHs, samples selected for atrazine analysis will be analyzed prior to clean-up. For this subset of samples, the following steps apply immediately following Step 12 above (otherwise skip to Sample Cleanup below).

2.3.2 Specific Procedure

2.3.2.1 Using a syringe, add an appropriate amount of d_5-atrazine to the uncleaned sample (≈ 1 mL HEX in 35 mL amber vial) as internal quantification standard LIS).

2.3.2.2 Concentrate the HEX to 200-500 µL under gentle N_2 gas stream.

2.3.2.3 Transfer the extract to an autosampler vial with a 250 µL glass insert and cap.

2.3.2.4 Place the autosampler vials within an autosampler tray and analyze on an HP-5890 GC with an HP-5971A MSD using a 30 m DB-5, 0.25 mm i.d., 25 μm film thick glass capillary column (J&W Scientific). The MSD is operated in selective ion monitoring mode with at least one confirmation ion.

2.3.2.5 Record sample codes in the instrument logbook in the order in which they are injected.

2.3.2.6 After samples are injected, transfer the samples from the autosampler vial back to the original HEX vial with four to five complete HEX rinses (≈ 1-1.5 mL total) of the glass insert within the autosampler vial.

3.0 Sample Cleanup

3.1 General Notes

Concentrated samples will be cleaned up to remove the grease from the dry deposition plates and other potential chemical interferences prior to instrumental analysis, using a 0.5 cm i.d. x 20 cm glass column with a 50 mL glass reservoir on top. Usually, enough columns are assembled to clean an entire sample set or at least half of a sample set in the course of one day.

3.2 Specific Procedure

3.2.1 Secure glass column to ring stand using clamps, being careful not to break column.

3.2.2 Install teflon stop-cocks.

3.2.3 Place a small clean glasswool plug into bottom of column.

3.2.4 Add pinch (≈ 0.5 cm) of clean anhydrous Na_2SO_4 to column.

3.2.5 With stopcock open, rinse column with ≈5-10 mL of DCM, letting the column drain completely.

3.2.6 Rinse column with ≈5 mL HEX and let drain to top of Na_2SO_4.

3.2.7 Close stopcock and add ~ 5 mL HEX.

3.2.8 Add 5 cm 3% deactivated silica gel as slurry in HEX while slowly letting HEX drain from column and while tapping the column with rubber hose to help silica gel settle evenly within column. Do NOT allow HEX to drain below level of silica gel. (Silica gel: reagent grade, 60-200 mesh, Baker, activated overnight at 200°C, deactivated with MilliQ water by weight and stored overnight prior to use within dessicator.)

3.2.9 Add another pinch of clean anhydrous Na_2SO_4 to top of silica gel in column.

3.2.10 Add 5 mL HEX to column.

3.2.11 Add ≈ 5 cm 5% deactivated alumina (as dry solid) while slowly letting HEX drain from column and while tapping the column with rubber hose to help alumina settle evenly within column. Do not allow HEX to drain below level of alumina. (Alumina: neutral Brockman activity 1, 60-325 mesh, Fisher Scientific, activated overnight at 450°C, deactivated with MilliQ water by weight and stored overnight prior to use within dessicator).

3.2.12 Add ≈0.5 cm clean anhydrous Na_2SO_4 to top of column.

3.2.13 Add ~ 10 mL HEX to clean and condition column and let drain to top of Na_2SO_4.

3.2.14 Place 25 mL amber vial beneath column. This vial must be labeled with same sample code as on sample vial to be cleaned.

3.2.15 Add sample extract to top of column and drain solvent to top of column while collecting eluant in vial. At no time allow solvent to drain beyond level of Na_2SO_4 at top of column.

3.2.16 When HEX reaches top of column, add two 1 mL HEX rinses of sample vial.

3.2.17 Again, when HEX reaches top of column, add 5 mL HEX to top of column and let drain to top of column.

3.2.18 When HEX reaches top of column, add 9 mL 1:4 DCM:HEX (v/v) and collect eluant in the same 25 mL amber vial as above.

3.2.19 When DCM:HEX drains completely from column, cleanup is complete.

3.2.20 Concentrate eluant to ≈ 1 mL under gentle N_2 gas stream.

3.2.21 Transfer sample to a 4 mL amber vial with ≈ 2-1 mL HEX rinses of 25 mL vial.

3.2.22 With syringe(s), add appropriate amounts of internal quantification standards (2,4,6-trichlorobiphenyl [IUPAC #30] and 2,2',3,4,4',5,6,6'-octachlorobiphenyl [IUPAC #204] for PCBs and t-nonachlor; and d_{10}-acenaphthalene, d_{10}-phenanthrene, d_{10}-pyrene, d_{12}-benzo(e)pyrene and d_{12}-benzo(g,h,i)perylene) for PAHs.

3.2.23 Concentrate the final extract ≈25-100 μL under gentle N_2 gas stream prior to injection on GC-ECD (PCBs and t-nonachlor) or GC-MSD (PAHs).

Extraction and Cleanup of XAD-2 Resin Cartridges for Polychlorinated Biphenyls and Trans-Nonachlor

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Standard Operating Procedure MSL-M-091-00

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Extraction and Cleanup of XAD-2 Resin Cartridges for Polychlorinated Biphenyls and Trans-Nonachlor

1.0 Scope and Application

This SOP is applicable to the extraction and analysis of polychlorinated biphenyl compounds extracted from fresh water using XAD-2 polymeric resin cartridges. The target compounds which can be determined by this method are, generally speaking, the polychlorinated biphenyls (PCBs) found in Aroclors 1232, 1248, and 1262 and trans-Nonachlor. It may be applied to other compounds once acceptable method recovery has been demonstrated.

Up to 200 liters of filtered freshwater is passed through a glass column containing approximately 400 grams of pre-cleaned XAD-2 resin at a flow rate of approximately 1 liter per minute. This resin has been shown to efficiently extract non-polar organic compounds, including PCBs, from freshwater. The columns are sealed, labeled, and stored under refrigeration until analysis.

At analysis the resin is transferred from the column to an extraction apparatus and rinsed with acetone to remove external water. The surrogate compounds are then added and the resin is then extracted with acetone to remove organics and interstitial water. Next the resin is extracted with a mixed solvent of 50% hexane 50% acetone to remove the remaining organic compounds. The acetone rinse and acetone extract are combined in a separatory funnel, 300 mL of reagent water is added, and the mixture is then extracted once with 200 mL of hexane then twice with 100 mL of hexane to remove PCBs into the hexane extracts. These hexane extracts are then combined with the hexane-acetone extracts, reduced in volume and exchanged into hexane to a final volume of approximately 1 mL. The extract is then applied to a cleanup column which is eluted with 60 mL of hexane. The hexane is reduced in volume to 0.9 mL at which point 0.1 mL of internal standard is added to complete the sample preparation.

Extracts are then subjected to gas chromatographic analysis with electron capture detection (see SOP MSL-093-00). The chromatographic separation is performed using a 60 meter capillary column. Identification and quantitation of the PCB compounds is accomplished by comparison to calibration standards containing a large number of PCB congeners in known concentration.

2.0 Definitions

The following terms and acronyms are associated with this procedure:

DCM	Dichloromethane
GC-ECD	Gas chromatography with electron capture detection
K-D	Kuderna-Danish
NA_2SO_4	Sodium Sulfate
rpm	Revolutions per minute
SRM	Standard reference material

3.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory, 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.
Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper handing of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) reporting results to the Project Manager; and 5) participating in QA audits.

Quality Assurance Representative: A qualified staff member assigned to the Quality Assurance Unit. Responsible for monitoring the project activities and conducting Quality Assurance Audits to ensure that 1) analysts have conducted the analysis according to the SOP and that deviations from the SOP have been noted in project files; 2) instrument use and maintenance records are kept correctly; and 3) data have been reported and presented accurately.

4.0 Procedure

4.1 Apparatus and Reagents

4.1.1 Chromatography column 50 mm x 300 mm (Ace Glass Co. #5820-50 or equivalent)

4.1.2 Nylon column end caps with FETFE O-Ring (Ace Glass Co. #5845-50 or equivalent)

4.1.3 Teflon column end cap with 1/4" NPT threaded hole (Ace Glass Co. #5844-78)

4.1.4 Roller apparatus capable of rolling 250 mL Qorpak jars

4.1.5 Separatory funnels (sized to fit sample)

4.1.6 Erlenmeyer flasks, various sizes

4.1.7 Chromatography column, 15 x 250 mm with 250 mL reservoir and No. 2 Teflon stopcock (Kontes #42080-0222)

4.1.8 Kuderna-Danish (K-D) evaporator apparatus: 250 mL and/or 500 mL reservoir; 3-ball macro Snyder column; 2 or 3 ball micro Snyder column; 10 mL or 25 mL concentrator tube

4.1.9 Hot water bath capable of reaching 100°C, located in a fume hood

4.1.10 Water aspirator vacuum source (Bucchi #B169 or equivalent)

4.1.11 1 Liter Soxhlet extraction apparatus complete with flask and condenser (Ace #6810-10 modified to a 1 L size or equivalent)

4.1.12 1 Liter round-bottom boiling flasks (Ace #6887-53 or equivalent)

4.1.13 Reducing Glass Joint Bushings 34/45 to 24/40 (Ace #5023-21 or equivalent)

4.1.14 Pre-Cleaned XAD-2 Resin, 20-60 mesh, 330 m^2 surface area, 90Å pore diameter

4.1.15 Boiling chips, carborundum, soxhlet extracted or baked at >400° C

4.1.16 Glass wool, soxhlet extracted ≥4 hrs in 50:50 hexane-acetone

4.1.17 Nitrogen evaporation apparatus, N-Evap or equivalent, heated with a water bath maintained at 25-35°C

4.1.18 RapidVap Evaporation System (Labconco)

4.1.19 Glass graduated cylinders

4.1.20 Stainless steel and teflon forceps

4.1.21 Steel rod, 3 mm x 50 cm

4.1.22 Microliter syringes or micropipets

4.1.23 Concentrated sulfuric acid

4.1.24 Solvents – pesticide grade or equivalent

Dichloromethane (DCM)
Hexane
Acetone
Reagent Water (Barnstead Organic-Free or equivalent)

4.1.25 Sodium sulfate – anhydrous, reagent grade, heated to 400°C for ≥4 hr, then cooled to room temperature and stored in a desiccator

4.1.26 Alumina, Sigma F-20 or equivalent, 80-200 mesh

4.1.27 Silica, Amicon Matrix Silica pore diameter 60 Å, particle size 105 µm

4.1.28 Internal standard solution – a hexane solution containing 100 ng/mL of PCB 30, PCB 204, and PCB 103

4.1.29 Surrogate solution – a hexane solution containing 200 ng/mL PCB 14, 50 ng/mL PCB 65, 50 ng/mL PCB 166, and 100 ng/mL dibutyl chlorendate (DBC). PCB numbers refer to their IUPAC designations.

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

4.1.31 Acid silica gel – 30% (w/w) sulfuric acid

4.2 Sample Handling

Samples shall be kept cold (2-6°C) until analysis. Samples shall be extracted within 12 months of receipt at the lab unless specified otherwise by the Project Manager or project-specific plans. Refer to the project-specific sampling plan for sample collection, preservation, and handling methods.

4.3 Labware Preparation

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

4.4 XAD-2 Resin Preparation

Prior to use, the XAD-2 resin must be solvent extracted sequentially with a number of solvents to remove manufacturing impurities. This process is fully described in Battelle SOP MSL-M-090-00 but is also briefly described here. The resin should be placed in a large soxhlet apparatus (1 liter pot size or bigger) and sequentially extracted for 24 hours with each solvent, first with methanol, then with acetone, hexane, and finally with dichloromethane. Then the resin is sequentially extracted for four hours with each solvent, first with hexane, acetone, and finally with methanol. The methanol is then displaced from the resin by numerous rinses with reagent water and then stored under reagent water in the dark at cool temperatures. This procedure must be followed closely with no shortcuts. At this point the resin is ready to be packed into columns.

4.5 XAD-2 Sampling Column Preparation

A detailed description of how the resin-containing columns used for field sampling are prepared is contained in Battelle SOP MSL-M-090, but a condensed description is also given here. Briefly, the column is fitted with a teflon end cap adapter which has a 1/4" FPT threaded hole through it, to which PVC vacuum tubing is connected. The tubing is then connected to a water aspirator vacuum source. First, several inches of pre-cleaned glass wool is added and then the column is filled with reagent water. The resin is then added to the column with additional water rinses, and the vacuum source is activated. The resin is not allowed to go dry and more resin is added with water washing until approximately 400 cc of resin has been added to the column. Additional pre-cleaned glass wool is added to the top of the resin, forcing out the water, so as to avoid the

formation of air pockets. More water is added if needed and a nylon end plug is screwed onto the column which displaces water. This is done to avoid creating any air pockets. The column is then inverted and the opposite end is capped in a similar fashion. The columns are then appropriately labeled and stored horizontally in a cool dark place.

4.6 Resin Sample Extraction

4.6.1 A small wad of pre-cleaned glass wool is first added to the soxhlet extractor body to prevent any resin from escaping up the siphon tube exit. Next, a column is opened and the glass wool removed and placed into a soxhlet extractor body. The resin is then transferred, with acetone rinsing, into the same soxhlet body. The last plug of glass wool is then added to the soxhlet and the soxhlet is drained of its contents into a 2 liter separatory funnel. This acetone is saved and put aside until later. The total volume of acetone used for this process is 400 to 800 mL. The smallest volume that effectively transfers the resin is the volume that should be used. The soxhlet body containing the resin is then connected to a 1 liter boiling flask containing several boiling stones, 800 mL of acetone is slowly poured into the soxhlet body onto the resin bed and allowed to siphon into the boiling flask. A 100 μL aliquot of surrogate standard (see Sec. 4.1) is then added to the resin in the soxhlet body and the soxhlet condenser is attached. If the sample is to be spiked, 100 μL of matrix spiking solution is added at this time. Coolant water and the heating mantle are activated and the resin is extracted for approximately 4 hours, the heat is then shut off and the system is allowed to cool to room temperature. During extraction the soxhlet should cycle 4 times per hour. The acetone extraction step is important in removing water from the resin, which is critical in making the resin hydrophobic extractable by the non-polar extraction solvent used in the next step.

4.6.2 The soxhlet apparatus is removed from the condenser and tilted to force the acetone extract to siphon into the 1 liter boiling flask. This extract is then combined with the rinse acetone in the 2 liter separatory funnel. *Note: To avoid contamination of the sample from material on the exterior of the neck, before transferring extracts from containers with ground glass joints always rinse the exterior of the necks with the same solvent as that being transferred.* At this point fresh boiling stones and 800-850 mL of 50:50 hexane-acetone are added to the boiling flask, the flask is connected to the soxhlet body, the condenser is connected and the cooling water and heating mantle is activated. The resin is extracted with this solvent system for ≥16 hrs after which the heat is then shut off and the system is allowed to cool to room temperature. During the extraction the soxhlet should cycle 4 times per hour. After extraction the soxhlet body is tilted to cause the solvent to be siphoned over into the boiling flask, where it will be evaporated.

4.7 Acetone Extract Back Extraction

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

4.8 Extract Evaporation

Several fresh boiling stones are added to the hexane-acetone extract contained in the boiling flask, a 24/40 3-ball macro Snyder column is connected to the flask by means of a reducing bushing, the Snyder column is wetted with hexane, and the extract is evaporated to approximately 300 mL. This extract is then quantitatively transferred with several hexane rinses (see note in Sec. 4.6.2) to the boiling flask containing the hexane back-extract of the acetone rinse-extract. Several fresh boiling stones are added, a 3-ball macro Snyder column is attached and wetted, and the solvent is evaporated to a volume of approximately 200 mL. The contents are then transferred to a 250 mL K-D apparatus, fresh boiling stones are added, and the extract is reduced to an apparent volume of 1-5 mL. The K-D is allowed to cool and the 250 mL flask and macro Snyder column are removed from the concentrator tube. Fresh boiling stones are added, a micro Snyder column is attached and wetted with hexane, and the volume is reduced to approximately 1 mL. To effect a complete exchange into hexane fresh boiling stones and 10 mL of hexane are added and the volume is again reduced to 1 mL. This final step is then repeated once more and the volume brought to approximately 1 mL. Any evaporation device (such as the Labconco Rapidvap) that can be demonstrated to yield acceptable spike recoveries, acceptable precision, and acceptable levels of contamination can be used in place of the K-D apparatus.

4.9 Column Chromatography Clean-up of Extract

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

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

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

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

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

4.9.3 Prepare a column by placing a small portion of glass wool at the bottom of a chromatography column. Pour 70 mL hexane into the column filling it to a level that is approximately 1/3 the volume of the reservoir. Place a powder funnel in the column and pour 10 g of the 10% deactivated alumina into a column, swirling the hexane and the alumina allowing the alumina time to completely settle. Add 3 g of 6% deactivated silica

to the column in the same manner. Before adding the sodium sulfate open the stopcock and allow the hexane to slowly drain, then add enough sodium sulfate to result in a plug approximately 1 cm high. The acid silica gel must be slurry packed in order to prevent trapping bubbles in the column. Into a small beaker containing 8 g of acid silica gel, pour a sufficient amount of hexane to immerse the adsorbent. Swirl the beaker to release most of the bubbles, then with the aid of a squeeze bottle of hexane, pour the slurry into the column, swirling it to aid settling. Again using the same technique as described above, add enough sodium sulfate to result in a plug approximately 1 cm high. Drain the solvent level down to just above the top of the sodium sulfate. Place a 250 mL K-D apparatus under the column to collect the eluent.

4.9.4 The 1 mL sample extract from 4.8.1 is carefully transferred to the column with several small hexane rinses. A small portion of hexane should be used to rinse down the sides of the column. Allow each rinse to drain to just above the sodium sulfate layer. Carefully add a *total* of 60 mL of hexane to the column (including rinses) and allow the column to drain completely, collecting all the eluent.

4.9.5 Add boiling stones to the K-D containing the eluent and reduce in volume to 0.9 mL (as measured in the 10 mL concentrator tube) using macro and micro Snyder columns followed by nitrogen evaporation using a stream of ultra high purity nitrogen. At this point add 100 μL of internal standard solution to bring the volume to 1.0 mL and transfer to an autosampler vial for analysis.

4.10 Quality Control Sample Frequency

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

4.10.1 Lab Procedural Blank – prepare one per batch. Prepared by working through the sample preparation procedure using only solvents and reagents.

4.10.2 Lab Matrix Blank – prepare one per batch. This is a non-field exposed resin sample prepared in a manner identical to that used for field samples.

4.10.3 Spiked Matrix Blank – prepare one per batch. This is a lab matrix blank fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.4 Spiked Procedural Blank – prepare one per batch. This is a lab procedural blank which is fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.5 Sample Replicates – analyze one sample per batch in duplicate.

4.11 Data Recording and Storage

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

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

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

5.0 Quality Control

Results of quality control samples (e.g., blanks, spikes, intercomparison samples, and replicate samples) prepared using this procedure will meet the criteria given in the project specific QAPjP. Recovery of the surrogates will be used to monitor for extraction efficiency, unusual matrix effects, or sample processing errors. Surrogate recovery criteria will be given in the project specific QAPjP.

Solvents, reagents, glassware, and other sample processing hardware may cause artifacts or interferences to sample analysis. The analyst must demonstrate that these materials are free from interferences under the conditions of the procedure by analyzing method blanks.

6.0 Safety

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

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

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

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

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

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

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

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

7.0 Training Requirements

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

8.0 References

J. I. Gomez-Bellinchon, Grimalt, J. O., and Albaiges, J., "Intercomparison Study of Liquid-Liquid Extraction and Adsorption on Polyurethane and Amberlite XAD-2 for the Analysis of Hydrocarbons, Polychlorobiphenyls, and Fatty Acides Dissolved in Seawater," Environ. Sci. Technol. 1988, 22, 677-685.

Quality Assurance Plan Green Bay Mass Balance Study "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

Analytical Quality Assurance Project Plan (QAPjP) for the EPA Lake Michigan PCB Mass Balance Study, DRAFT, dated October 25, 1994.

ASTM Method D4059-91, "Standard Test Method for Analysis of Polychlorinated Biphenyls in Insulating Liquids by Gas Chromatography."

EPA 660/4-81-045, "The determination of Polychlorinated Biphenyls in TQuality Assurance Plan Green Bay Mass Balance Study" "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

"Analytical Quality Assurance Plan for the Lake Michigan PCB Mass Balance Study."

Attachment 1.

XAD-2 Resin and Filter Extraction Data Sheet, EPA PCB Mass Balance Study

DATE _____ ANALYST _____ SOP# _____

ALUMINA LOT # _____
SILICA LOT# _____
Na2SO4 LOT# _____
SURROGATE STD, no/vol _____
INTERNAL STD, no/vol _____
SPIKE SOLN A, no/vol _____
SPIKE SOLN B, no/vol _____

PROJECT/CF# _____
CRIUSE _____
BATCH# _____
RESIN/FILTER _____

GLASS WOOL LOT# and MFG _____
HEXANE LOT# and MFG _____
ACETONE LOT# and MFG _____

SAMPLE EXTRACTION					COMMENTS
SAMPLE ID	EXTRACTION DATE	COLUMN BATCH ID	NO OF FILTERS	WATER VOLUME (L)	

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

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Standard Operating Procedure MSL-M-092-00

June 1994

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

1.0 Scope and Application

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

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

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

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

2.0 Definitions

The following terms and acronyms are associated with this procedure:

DCM	Dichloromethane
GC-ECD	Gas chromatography with electron capture detection
K-D	Kuderna-Danish
NA_2SO_4	Sodium Sulfate
rpm	Revolutions per minute
SRM	Standard reference material
GFF	Glass fiber filter (see description below)

3.0 Responsible Staff

3.1 *Project Manager.* A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory, 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.

3.2 *Laboratory Supervisor.* A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

3.3 *Analyst.* A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper handing of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) reporting results to the Project Manager; and 5) participating in QA audits.

3.4 *Quality Assurance Representative.* A qualified staff member assigned to the Quality Assurance Unit. Responsible for monitoring the project activities and conducting Quality Assurance Audits to ensure that 1) analysts have conducted the analysis according to the SOP and that deviations from the SOP have been noted in project files; 2) instrument use and maintenance records are kept correctly; and 3) data have been reported and presented accurately.

4.0 Procedure

4.1 Apparatus and Reagents

4.1.1 Separatory funnels (sized to fit sample)

4.1.2 Erlenmeyer flasks, various sizes

4.1.3 Chromatography column, 15 x 250 mm with 250 mL reservoir and No. 2 Teflon stopcock (Kontes #42080-0222)

4.1.4 Roller apparatus capable of rolling 250 mL Qorpak jars

4.1.5 Kuderna-Danish (K-D) evaporator apparatus: 250 mL and/or 500 mL reservoir; 3 ball macro Snyder column; 2 or 3 ball micro Snyder column; 10 mL or 25 mL concentrator tube

4.1.6 Hot water bath capable of reaching 100°C, located in a fume hood

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

4.1.8 0.5 Liter Soxhlet extraction apparatus complete with flask and condenser (Ace # 6810-10 or equivalent)

4.1.9 1 Liter round-bottom boiling flasks (Ace #6887-53 or equivalent)

4.1.10 Reducing Glass Joint Bushings 34/45 to 24/40 (Ace #5023-21 or equivalent)

4.1.11 Pre-Cleaned 293 mm Whatman GFF, 0.7μm pore size, Whatman # 1825-293 or equivalent

4.1.12 Boiling chips, carborundum, soxhlet extracted or baked at >400° C

4.1.13 Glass wool, soxhlet extracted ≥4 hrs in 50:50 hexane-acetone

4.1.14 Nitrogen evaporation apparatus, N-Evap or equivalent, heated with a water bath maintained at 25-35°C

4.1.15 RapidVap Evaporation System (Labconco)

4.1.16 Glass graduated cylinders

4.1.17 Stainless steel and teflon forceps

4.1.18 Steel rod, 3 mm x 50 cm

4.1.19 Microliter syringes or micropipets

4.1.20 Concentrated sulfuric acid

4.1.21 Solvents–pesticide grade or equivalent
- Dichloromethane (DCM)
- Hexane
- Acetone
- Reagent Water (Barnstead Organic-Free or equivalent)

4.1.22 Sodium sulfate–anhydrous, reagent grade, heated to 400°C for ≥4 hr, then cooled to room temperature and stored in a desiccator

4.1.23 Alumina, Sigma F-20 or equivalent, 80-200 mesh

4.1.24 Silica, Amicon Matrix Silica pore diameter 60Å , particle size 105 μm

4.1.25 Surrogate solution- a hexane solution containing 200 ng/mL PCB 14, 50 ng/mL PCB 65, 50 ng/mL PCB 166, and 100 ng/mL dibutyl chlorendate (DBC). PCB numbers refer to their IUPAC designations

4.1.26 Internal standard solution- a hexane solution containing 100 ng/mL of PCB 30, PCB 204, and PCB 103

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

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

4.2 Sample Handling

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

4.3 Labware Preparation

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

4.4 Filter Preparation

Prior to use, the filters must be muffled at 450 ± 20°C for 4 hrs to remove manufacturing impurities. This process is fully described in Battelle SOP MSL-M-090-00.

4.5 Pentaplate Filtration Apparatus Preparation

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

4.6 Filter Sample Extraction

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

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

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

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

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

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

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

4.7 Acetone Extract Back Extraction

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

4.8 Extract Evaporation

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

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

4.9 Column Chromatography Clean-up of Extract

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

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

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

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

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

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

4.9.4 The 1 mL sample extract from 4.8.1 is carefully transferred to the column with several small hexane rinses. A small portion of hexane should be used to rinse down the sides of the column. Allow each rinse to drain to just above the sodium sulfate layer. Carefully add a total of 60 mL of hexane to the column (including rinses) and allow the column to drain completely, collecting all the eluent.

4.9.5 Add boiling stones to the K-D containing the eluent and reduce in volume to 0.9 mL (as measured in the 10 mL concentrator tube) using macro and micro Snyder columns followed by nitrogen evaporation using a stream of ultra high purity nitrogen. At this point add 100 μL of internal standard solution to bring the volume to 1.0 mL and transfer to an autosampler vial for analysis.

4.10 Quality Control Sample Frequency

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

4.10.1 Lab Procedural Blank – prepare one per batch. Prepared by working through the sample preparation procedure using only solvents and reagents.

4.10.2 Lab Matrix Blank – prepare one per batch. This is a non-field exposed filter sample prepared in a manner identical to that used for field samples.

4.10.3 Spiked Matrix Blank – prepare one per batch. This is a lab matrix blank fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.4 Spiked Procedural Blank – prepare one per batch. This is a lab procedural blank which is fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.5 Sample Replicates – analyze one sample per batch in duplicate.

4.11 Data Recording and Storage

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

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

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

5.0 Quality Control

5.1 Results of quality control samples (e.g.,blanks, spikes, intercomparison samples, and replicate samples) prepared using this procedure will meet the criteria given in the project specific QAPjP. Recovery of the surrogates will be used to monitor for extraction efficiency, unusual matrix effects, or sample processing errors. Surrogate recovery criteria will be given in the project specific QAPjP

5.2 Solvents, reagents, glassware, and other sample processing hardware may cause artifacts or interferences to sample analysis. The analyst must demonstrate that these materials are free from interferences under the conditions of the procedure by analyzing method blanks.

6.0 Safety

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

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

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

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

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

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

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

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

7.0 Training Requirements

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

8.0 References

8.1 J. I. Gomez-Bellinchon, Grimalt, J. O., and Albaiges, J., "Intercomparison Study of Liquid-Liquid Extraction and Adsorption on Polyurethane and Amberlite XAD-2 for the Analysis of Hydrocarbons, Polychlorobiphenyls, and Fatty Acids Dissolved in Seawater," Environ. Sci. Technol. 1988, 22, 677-685.

8.2 Quality Assurance Plan Green Bay Mass Balance Study "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

8.3 Analytical Quality Assurance Project Plan (QAPjP) for the EPA Lake Michigan PCB Mass Balance Study, DRAFT, dated October 25, 1994.

8.4 MSL-D-001. Recording Data on Data Sheets and Laboratory Notebooks.

8.5 MSL-A-006. MSL Training.

8.6 MSL-M-056. Stock and Standard Solution Preparation.

8.7 MSL-M-093 PCB Congener Analysis of XAD Resins and GFIF Filters Using GC/ECD.

8.8 ASTM Method D4059-91, "Standard Test Method for Analysis of Polychlorinated Biphenyls in Insulating Liquids by Gas Chromatography."

8.9 EPA 660/4-81-045, "The determination of Polychlorinated Biphenyls in TQuality Assurance Plan Green Bay Mass Balance Study" "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

8.10 "Analytical Quality Assurance Plan for the Lake Michigan PCB Mass Balance Study."

Attachment 1.
XAD-2 Resin and Filter Extraction Data Sheet, EPA PCB Mass Balance Study

DATE _____ ANALYST _____ SOP# _____

ALUMINA LOT # _____
SILICA LOT# _____
Na2SO4 LOT# _____

PROJECT/CF# _____
CRIUSE _____
BATCH# _____
RESIN/FILTER _____

GLASS WOOL LOT# and MFG _____
HEXANE LOT# and MFG _____
ACETONE LOT# and MFG _____

SURROGATE STD, no/vol _____
INTERNAL STD, no/vol _____
SPIKE SOLN A, no/vol _____
SPIKE SOLN B, no/vol _____

SAMPLE EXTRACTION					COMMENTS
SAMPLE ID	EXTRACTION DATE	COLUMN BATCH ID	NO OF FILTERS	WATER VOLUME (L)	

PCB Congener Analysis of XAD-2 Resins and GFF Filters Using GC/ECD

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Standard Operating Procedure MSL-M-093-00

June 1994

PCB Congener Analysis of XAD-2 Resins and GFF Filters Using GC/ECD

1.0 Application and Scope

1.1 This SOP is applicable to the analysis of GFF filter and XAD-2 resin extracts prepared according to SOPs MSL-M-091 and MSL-M-092 for polychlorinated biphenyls (PCB) and trans-nonachlor by capillary gas chromatography with ^{63}Ni electron-capture detection.

1.2 This procedure provides typical gas chromatography (GC) conditions for the detection of trace levels of PCBs and trans-nonachlor, methods for identifying the analytes, and methods for analyte quantitation using the internal standard method. Attachment 1 lists the most frequently analyzed compounds. However, this list may be amended to meet requirements of specific projects.

2.0 Definitions

The following terms and acronyms may be associated with this procedure:

ECD	Electron capture detector or detection
GC	Gas chromatography
PCB	Polychlorinated biphenyl
RF	Response factor
RRF	Relative response factor; response factor of analyte normalized to the response factor of the internal standard.
RSD	Relative standard deviation (%)
RT	Retention time
IS	Internal standard - compound(s) added just prior to analysis on instrument.
Surrogate	Compound(s) added prior to extraction to assess efficiency of method.

In addition, it should be noted that the numbering scheme used for PCB congeners, e.g. PCB 3, is that used by Ballschmiter and Zell.

3.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory; 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.

Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of the GC. Responsible for 1) ensuring that analysts are trained in operation of the GC; 2) appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper use and maintenance of the GC; 2) recording information regarding instrument use and maintenance in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) tabulating all sample and QC data and reviewing the quality of the data based on QC guidelines presented in this SOP and any other project-specific QC guidelines; 5) reporting results to the Project Manager; and 6) defending the data during an audit.

Quality Assurance Representative: A qualified staff member assigned to the Quality Assurance Unit. Responsible for monitoring the project activities and conducting Quality Assurance Audits to ensure that 1) analysts have conducted the analysis according to the SOP and that deviations from the SOP have been noted in the appropriate log book or in the project files; 2) instrument use and maintenance records are kept correctly; and 3) data have been reported and presented accurately.

4.0 Procedures

The GC must be maintained and operated as described in Battelle SOP No. MSL-M-075.

4.1 GC Preparation

The primary quantitation GC column is a 60 meter x 0.25 mm (i.d.) fused silica capillary column coated with a 5% phenyl-, 95% methyl-polysiloxane film of 0.25 µm thickness. (J&W Scientific, Inc., 60 meter DB-5 or equivalent). The confirmation column is a 60 m x 0.25 mm (i.d.) fused silica capillary column coated with a 14% cyanopropylphenyl-86% methyl polysiloxane film of 0.25 m thickness (J&W Scientific, Inc. 60 meter DB-1701 or equivalent). Both columns are installed in a single splitless injection port using a 2-hole ferrule.

4.2 Sample Collection, Preservation, and Handling

To conduct this analysis, the analyst should receive the samples as solvent extracts reduced to an appropriate volume (as specified in SOP MSL-M-091 or MSL-M-092). Holding times to be followed are those specified in the project specific QAPjP; normally the holding time for extracts is 40 days from date of extraction. If holding times have been exceeded, the Project Manager should be notified immediately. Refer to project-specific plans or protocols for sample collection, preservation, and handling methods. .

4.3 Sample Specifications

Sample preparation methods may vary depending on the sample matrix and project needs; refer to project-specific protocols. Normally, this method is used to analyze extracts prepared according to Battelle SOPs MSL-M-091 and MSL-M-092. Samples and standards for analysis using this SOP should be prepared in hexane.

Table 1: Suggested Instrument Conditions for PCB and Chlorinated Pesticide Analysis

Primary Column (DB-5) Conditions:

Injection port type	Splitless
Injection port temperature	250°C
Detector temperature	300°C
Initial temperature	50°C
Initial hold	1.5 min
Ramp 1 rate	10°C/min
Final temp 1	105°C
Final time 1	0.0 min
Ramp 2 rate	1°C/min
Final temp 2	225°C
Ramp 3 rate	10°C/min
Final temp 3	280°C
Final time 3	20 min
Carrier gas	Hydrogen
Carrier gas velocity	60 cm/sec
ECD make-up gas	Nitrogen
Make-up gas flow	30 cc/min
Split vent flow	215 cc/min
Split vent on-time	1.5 min
Injection volume	5 µL
Injection speed	10 µL/sec
Hot needle time	0.07 min
Needle residence time	0.2 min

4.4 Analyte Identification

Prior to sample analysis, the elution order of the analytes of interest must be determined by analyzing the analytes individually or in combination with other analytes having known or predetermined retention times. The retention times of the analytes have all been verified on both the quantitation (DB-5) and confirmation columns (DB-1701) specified above under the GC conditions listed in Table 1, and are listed in Attachments 1 and 2. The elution order of the congeners was determined from data provided by another laboratory using similar GC conditions (Mullins et al. 1985, 1994 personal communications). Additional information on analyte identification is discussed in Section 4.6.1.

4.5 Instrument Calibration

Before the sample is injected into the GC, the detectors must be calibrated to determine the response of the detector to the analytes of interest. Demonstration of linearity of detector response is required before sample analysis. Calibration checks must be analyzed at a minimum frequency of once every 10 samples during sample analysis.

4.5.1 *Initial Calibration:* Prior to initiating any sample analyses initial calibration is performed by analyzing calibration standards at a total of seven levels spanning the concentration range of 9.1 ng to 1830 ng as "Total PCBs" Not all the congeners respond or are clearly resolved from other congeners at all these levels. On the DB-5 quantitation column, eleven congeners (BZ #s 12, 13, 100, 147 & 124, 134, 130, 129, 167, 173, and 189) are only determinable at three of these concentration levels. The calibration curves for these consist of the three concentrations plus zero. All other congeners are calibrated using a minimum of four concentrations. Calibration standards include the appropriate surrogates (Surr.) and internal standards (IS) at concentrations identical to their concentrations in the samples (see MSL-M-091 and MSL-M-092). An initial calibration must also be run if any GC conditions have changed significantly or if continuing calibration acceptance criteria (CCAC) are not met. Initial calibration acceptance criteria are a correlation coefficient (r^2) of 0.95 for a minimum 4 point curve using a quadratic or linear fit.

4.5.2 *Continuing Calibration (CCAC):* A mid-level calibration solution is analyzed as a calibration check minimally every 10 samples while samples are being analyzed. All sample analyses must be bracketed by two calibration check standards that meet calibration criteria. Continuing calibration acceptance criteria for the primary quantitation column are a 75-125% recovery relative to the total PCB value expected.

4.5.2.1 *Performance Criteria*: Additional performance criteria that will be evaluated within each CCAC include: a recovery of 50-150% for PCBs 6 & 205 (which represent small peaks), and a recovery of 75-125% for PCBs 101, 185, 44, & 180 (which represent average and large peaks). To ensure proper identification, the retention time of the internal standard reference peaks, PCB 30 and PCB 204, cannot shift by more than ±0.4 min (see Section 4.6.1).

4.5.2.2 *Internal Standard Criteria:* To ensure that the internal standards are not interfered with the area or height ratios between the internal standards PCB 30 and PCB 204 in the samples are monitored. If the area or height ratios observed in the samples differ from those observed during initial calibration by more than ±15% relative percent difference (RPD), all congeners must be quantitated relative to the uncontaminated internal standard and the data flagged accordingly.

4.6 Sample Analysis Procedure

Samples are analyzed under the same analytical conditions as the calibration standards. Samples must be bracketed by acceptable calibrations (see acceptance criteria in Section 4.5.2). Criteria for accepting peaks as analytes of interest are explained in Sections 4.6.1 through 4.6.4.

4.6.1 *Retention Time Windows:* Analytes are identified by the data system by setting allowable time windows for reference peak and analyte peak retention times. Initial retention times are set during the initial calibration of the instrument. From that point forward, the system utilizes retention time windows in which to "look" for peaks of interest and reference peaks. The reference peak windows are set at ±0.4 min. and the analyte peak windows are set at ±0.10 min. Since the internal standard reference peaks are large and clearly resolved using a larger window for recognizing them is justified. The data system then uses the

actual retention time of the internal standard reference peaks to adjust the position of the analyte peak windows, which are much narrower, to compensate for any minor chromatographic drift. One internal standard reference peak shall also be designated an RRT reference peak to further assist in proper peak identification through the use of relative retention times.

4.6.2 *Second Column Confirmation.* Since each injection is split between the quantitation column (DB-5) and confirmation column (DB-1701) all sample results will be confirmed by whether or not a peak is observed at the appropriate retention time on both columns. The retention time criteria described in Section 4.6.1 apply to the confirmation column also. Trans-nonachlor can only be quantitated on the DB-1701 since it is interfered with by PCB 99 on the DB-5. As a result of this, second column confirmation of trans-nonachlor is not possible except as PCB 99 and trans-nonachlor on the DB-5. When trans-nonachlor is confirmed in this manner the PCB 99 value must be flagged accordingly.

4.6.3 *Resolution.* Resolution on the primary (DB-5) column should be sufficient to separate congeners #17 and #18 into two peaks with a valley less than half the height of PCB #17. If this cannot be accomplished a new column must be installed or the instrument conditions optimized further.

4.6.4 *Minimum Area or Height*: Peaks with a signal-to-noise ratio of three or less should be regarded as not detected unless otherwise noted in a specific project plan and/or documented by project management.

5.0 Data Analysis and Reporting

5.1 Data Recording

Data quantitation and calculations will be performed on personal computers using commercial software such as Varian Star Chromatography Software (Version 4.0 or higher), Microsoft Excel (Version 4.0 or higher) or database software (Access Version 2.0). All transfers of data to forms and data reductions (e.g., concentration calculations, means, standard deviations) will be checked by the analyst and approved by the Laboratory Supervisor. Hard copies of GC printouts of calibrations and sample data and spreadsheet reports will be kept in the GC/ECD files. A copy of the summary sheets and extraction logs will be placed in the appropriate project file in the Chemistry Group Central Files. Hard copies of chromatograms from each sample and all calibrations will be kept in the GC/ECD files unless otherwise noted in a specific project plan. All GC data files will be archived on magnetic tape.

5.2 Sample Quantitation

The internal standard method is used for quantitation. PCB 30 is used to quantify all PCB congeners with retention times up to and including PCB 110, all other congeners are quantitated vs. PCB 204. PCB 30 is used to quantitate the surrogates PCB 14 and PCB 65 and PCB 204 is used to quantitate the surrogate PCB 166. In addition, the results reported are corrected for the recovery of the surrogates PCB 65 and PCB 166; the surrogate PCB 14 is not used because it is oftentimes interfered with. The recovery of PCB 65 is used to correct the congeners quantitated vs. PCB 30 and the recovery of PCB 166 is used to correct the congeners quantitated vs. PCB 204.

If the ratio of internal standard areas exceeds the criteria set in Section 4.5.2.2, all congeners must be quantitated relative to the uncontaminated internal standard and the data must be flagged accordingly.

The concentration of a specific analyte in a sample is calculated by the Varian Star chromatography workstation. The system uses the concentration amounts for the individual analytes entered into the peak table and the results from the calibration standards to generate the coefficients of a polynomial curve fit that is, in turn, used to calculate the analytical concentrations. This result is then adjusted for internal standard and surrogate recoveries in an Excel spreadsheet:

$Star\ result = Amt_{(star)}$

Where:

$Amt_{(star)}$ *is obtained by solving:*

$Area(or\ height)_{(unk)} = A * (Amt_{(star)}^2 + B * Amt_{(star)} + C)$

Where:

A, B & C = coefficients of the polynomial equation
$Amt_{(star)}$ *= amount of compound present in extract unadjusted for internal or surrogate stds*
$Area(or\ height)_{(unk)}$ *= area or height of the peak for the selected compound found in the analysis run.*

At this point the result is not corrected by any internal standards. The results are then imported to an Excel spreadsheet using various star macros and adjusted for internal and surrogate standard recoveries.

$Result = Amt_{(ca)} / (Sample\ Volume)$

Where:

$Rec_{(istd)} = Amt_{(istd)} / Amt\ expected_{(istd)}$
$Rec_{(surr)} = Amt_{(surr)} / Amt\ expected_{(surr)} / Rec_{(istd)}$
$Amt_{(adj)} = Amt_{(star)} / Rec_{(istd)}$
$Amt_{(ca)} = Amt_{(adj)} / Rec_{(surr)}$

Where:

$Amt_{(ca)}$ *= Amount of compound present in analysis run*
$Amt_{(istd)}$ *= Amount of internal standard present in extract, calculated by Varian Star*
$Amt\ expected_{(istd)}$ *= Amount of internal standard added to extract*
$Amt_{(surr)}$ *= Amount of surrogate standard present in extract, calculated by Varian Star*
$Amt\ expected_{(surr)}$ *= Amount of surrogate standard added to extract*

5.3 Confirmation Data

The identification of particular PCB congeners is confirmed in all the samples by analysis on a column of dissimilar polarity (DB-1701). Confirmation is strictly by retention time only. The same criteria for retention time windows as described for the quantitation column (Section 4.6.1) are applied to the confirmation column.

5.4 Surrogate and Spike Recovery Calculation

Surrogate and Spike recoveries are calculated from the quantitation column. Calculations are as follows:

$$\text{Surrogate \% Recovery} = \frac{Q_d}{Q_a} \times 100$$

Q_d = *Quantity determined by analysis*
Q_a = *Quantity added*

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

SSR = Spike sample result
SR = Sample result
ESR = Expected sample result = Q_d/Divisor, where Divisor = amount sample analyzed
The relative percent difference (RPD) between replicates is calculated as follows:

$$RPD = \frac{|SR - SDR|}{1/2\,(SR + SDR)} \times 100$$

SDR = Sample Duplicate Result

6.0 Quality Control

Some quality control considerations associated with this SOP are described in the individual sections to which they apply. The following additional quality control criteria apply unless otherwise specified in a project specific QAPjP:

Field and Method Blanks	≤10 ng Total PCB
Surrogate Recoveries	40-140% (excluding PCB 14)
Matrix Spike Recoveries	50 to 150% Total PCB & 70% of analytes within 50-150% range
Blank Spike Recoveries	70-130% Total PCB, & 70% of analytes within 60-140% range
Sample Duplicate RPD	≤50% Total PCB and ≤100% for analytes ≥5X MDL

7.0 Safety

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

1. Protective clothing and eyeglasses should be worn when appropriate
2. Proper care must be exercised when using syringes
3. Certain areas of the GC system are heated. Avoid bodily contact with these areas and use care in handling flammable solvents in and around the GC system.

8.0 Training

All analysts following this procedure will be directly supervised by the Principal Investigator, qualified analyst, or laboratory supervisor until they have demonstrated to the satisfaction of the supervisor that they are capable of operating the GC independently. At a minimum, the analyst trainee should be competent in operation and maintenance of the GC (SOP No. MSL-M-075). The analyst trainee should also be able to analyze and quantify a multi-point calibration and quantitate a sample of known concentration (e.g., a reference material or matrix spike) within established control limits. Documentation of training will be recorded on training assignment and on-the-job training forms from SOP MSL-A-006. Records of this training will be kept by the laboratory Quality Assurance Representative.

9.0 References

9.1 MSL-A-006 Marine Sciences Laboratory Training.

9.2 MSL-M-075 Routine GC Maintenance.

9.3 MSL-M-091 Extraction and Cleanup of Resin Cartridges for Polychlorinated Biphenyls and trans Nonachalor.

9.4 MSL-M-092 Extraction and Cleanup of Glass Fiber Filters for Polychlorinated Biphenyls and Nonachlor.

9.5 Quality Assurance Plan Green Bay Mass Balance Study "Cleaning Methods for XAD-2 Resin and Filters," U.S. Environmental Protection Agency (EPA). 1986.

9.6 Analytical Quality Assurance Project Plan (QAPjP) for the EPA Lake Michigan PCB Mass Balance Study, DRAFT, dated October 25, 1994.

9.7 K Ballschmiter and Zell, "Analysis of Polychlorinated Biphenyls by Capillary Gas chromatography," Fresenius Z. Analytical Chemistry, #302 pp. 20-31 (1980)

Attachment 1: Mixed Congener Standard on DB-5

(Page 1 of 5)

```
Title          7 pt pp-376 GLNPO june using height for cal
Run File       C:\STAR\MODULE16\728JN4\7284013.RUN
Method File :  C:\STAR\MODULE16\728JN4\7284014.MTH
Sample ID    . PP-376C 183

Injection Date: 26-MAY-95 4:51 PM    Calculation Date: 22-JUN-95 2:56 PM

Operator      TJF                    Detector Type: ADCB (10 Volts)
Workstation:                         Bus Address    16
Instrument : Varian Star #1          Sample Rate    5.00 Hz
Channel    : A - 5                   Run Time       152.503 min

********** Star Chromatography Software ********** Version 4.01 **************

Chart Speed =    0.39 cm/min   Attenuation = 10          Zero Offset = 35%
Start Time  =   10.000  min     End Time    = 128.000 min  Min / Tick  = 5.00
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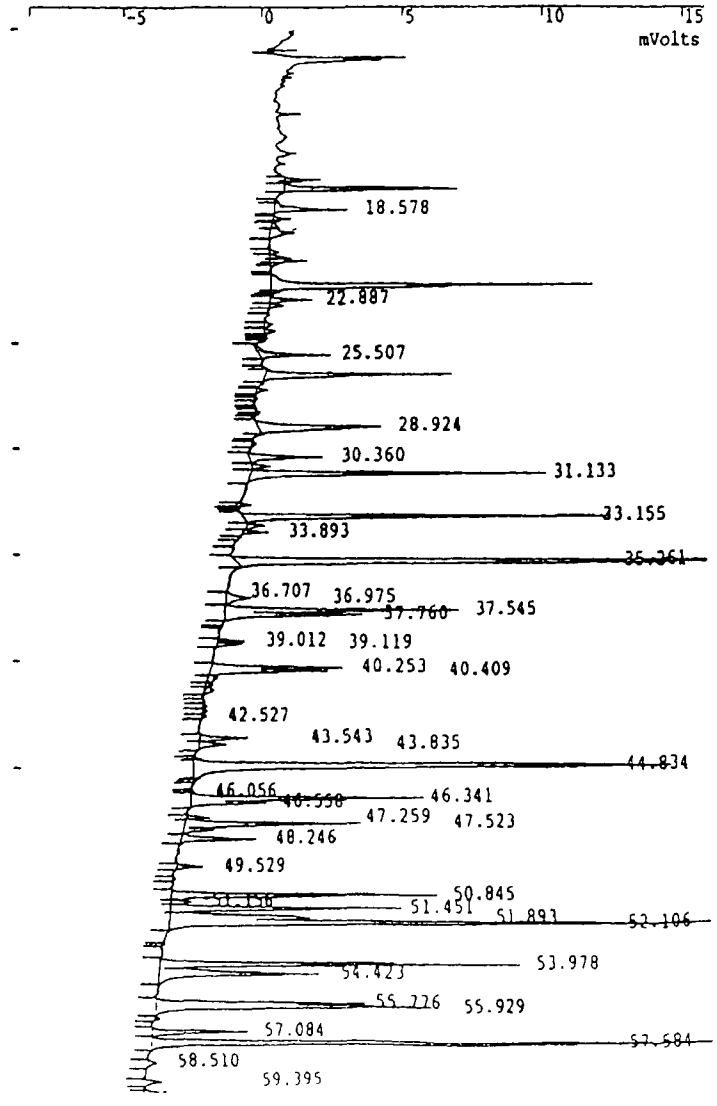
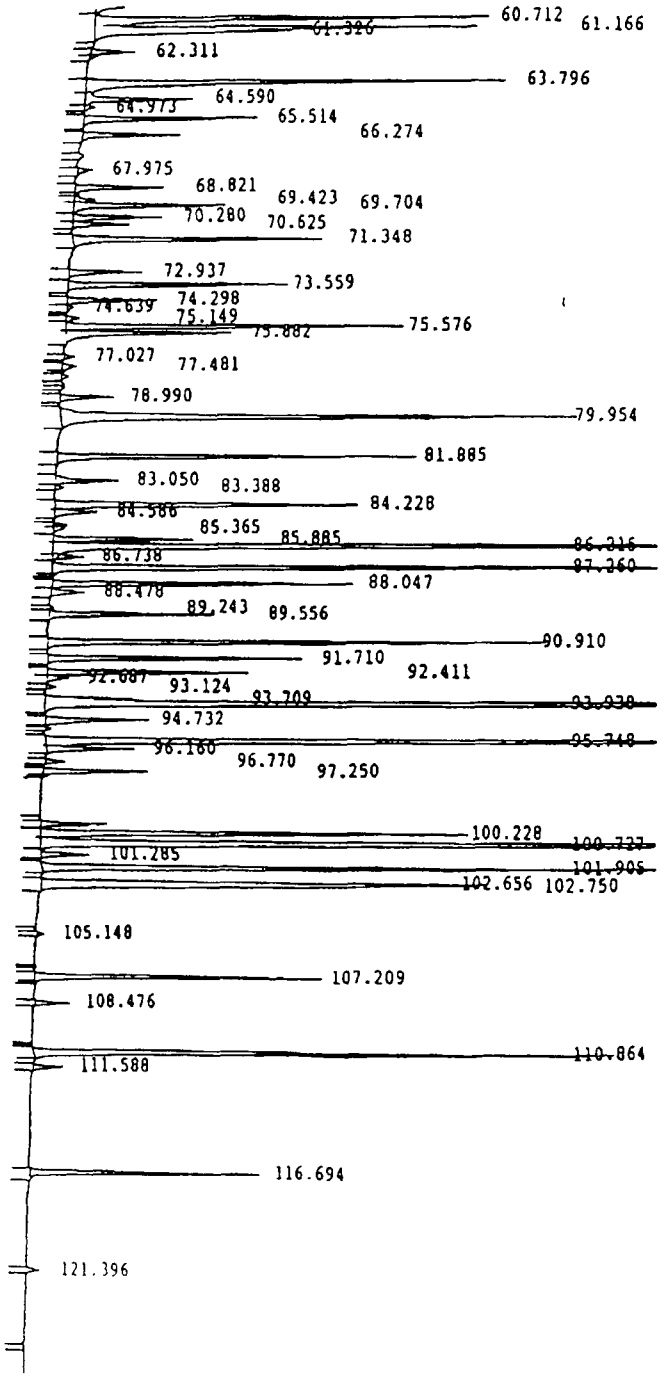


Attachment 1: Mixed Congener Standard on DB-5

(Page 2 of 5)



Attachment 1: Mixed Congener Standard on DB-5

(Page 3 of 5)

Title : 7 pt pp-376 GLNPO june using height for cal
Run File : C:\STAR\MODULE16\728JN4\7284013.RUN
Method File : C:\STAR\MODULE16\728JN4\7284014.MTH
Sample ID : PP-376C 183

Injection Date: 26-MAY-95 4:51 PM Calculation Date: 22-JUN-95 2:56 PM

Operator : TJF Detector Type: ADCB (10 Volts)
Workstation: Bus Address : 16
Instrument : Varian Star #1 Sample Rate : 5.00 Hz
Channel : A = 5 Run Time : 152.503 min

********** Star Chromatography Software ********** Version 4.01 **********

Run Mode : Analysis
Peak Measurement: Peak Height
Calculation Type: External Standard

Peak No.	Peak Name	Result (NG/L)	Ret. Time (min)	Time Offset (min)	Height (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1	1	12.452705	18.578	0.006	264	VB	6.2	
2	3	7.748489	22.887	0.005	154	BV	6.1	
3	4+10	3.673396	25.507	0.009	257	BB	6.6	
4	7+9	1.111898	28.924	0.030	437	BB	11.9	
5	6	1.810442	30.360	0.010	269	BB	7.5	
6	8+5	14.648691	31.133	0.006	1047	BB	7.3	
7	14(SURR)	19.450569	33.155	0.001	1303	BB	7.4	
8	19	0.278382	33.893	0.003	92	BB	6.3	
9	30(ISTD)	10.014711	35.261	0.041	3154	BB	7.3	R
10	12	0.075297	36.707	0.033	12	BV	1.8	
11	13	0.178523	36.975	-0.013	92	VB	10.1	
12	18	3.918240	37.545	0.007	831	BV	7.0	
13	15+17	3.817946	37.760	0.008	501	VB	7.8	
14	24	0.052955	39.012	0.005	100	BV	7.1	
15	27	0.200533	39.119	-0.011	94	VB	8.5	
16	16	2.078020	40.253	0.005	478	BV	7.3	
17	32	2.000064	40.409	0.008	430	VB	8.7	
18	29	0.051388	42.527	0.000	16	VB	5.6	
19	26	0.683344	43.543	0.019	174	BV	7.4	
20	25	0.299107	43.835	0.014	99	VB	8.1	
21	31+28	10.189798	44.834	0.014	1700	VB	12.0	
22	21	0.031160	46.056	0.023	10	BV	9.7	
23	33	3.577584	46.341	0.021	829	VV	7.8	
24	53	0.672404	46.558	0.002	272	VB	9.7	
25	51	0.179196	47.259	-0.000	82	BV	6.9	
26	22	2.948125	47.523	0.020	637	VV	7.8	
27	45	0.937357	48.246	0.009	272	VB	6.8	
28	46	0.377848	49.529	0.004	95	BB	6.5	
29	52	4.857748	50.845	0.008	941	BV	7.5	
30	43	0.266150	51.156	0.001	73	VV	8.0	
31	49	2.580539	51.451	0.012	828	VV	7.4	
32	48+47	1.112766	51.893	0.006	505	VV	0.0	
33	65(SURR)	4.909873	52.106	0.003	2146	VB	7.5	
34	44	4.793020	53.978	0.013	1277	BV	7.7	
35	42+37	1.452151	54.423	0.009	580	VB	9.1	
36	41+71	2.459767	55.776	0.009	751	BV	8.2	
37	64	1.899905	55.929	0.012	974	VB	10.2	
38	40	0.956243	57.084	0.004	345	BV	7.5	

Attachment 1: Mixed Congener Standard on DB-5

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39	103(ISTD)	9.980792	57.584	0.039	2275	VB	7.6	R
40	100	0.102683	58.510	0.003	33	BB	9.2	
41	63	0.205672	59.395	0.015	68	BV	8.2	
42	74	2.026874	59.972	0.021	767	VV	7.7	
43	70+76	3.643651	60.712	0.018	1401	VV	8.0	
44	66	5.537148	61.166	0.017	1361	VV	9.2	
45	95	2.298095	61.326	-0.000	719	VB	11.5	
46	91	0.537131	62.311	0.005	164	BV	7.2	
47	56+60	3.821698	63.796	0.023	1497	BV	8.2	
48	92+84	1.896827	64.590	0.004	391	VV	7.7	
49	89	0.109377	64.973	0.012	43	VB	6.5	
50	101	1.932718	65.514	0.007	625	BB	8.0	
51	99	0.758792	66.274	0.008	358	BB	7.6	
52	83	0.143149	67.975	0.004	57	BB	7.6	
53	97	0.568289	68.821	0.006	327	BV	7.9	
54	81	0.184326	69.423	0.010	81	VV	7.9	
55	87	1.073545	69.704	0.013	551	VV	8.3	
56	85	0.709927	70.280	0.005	328	VV	8.0	
57	136	0.778711	70.625	0.005	210	VB	7.4	
58	110+77	2.019769	71.348	0.013	911	BB	8.4	
59	82	0.469813	72.937	0.006	273	BB	7.5	
60	151	1.803968	73.559	0.008	801	BB	8.0	
61	135+144	0.948432	74.298	-0.000	333	BV	8.1	
62	147+124	0.395914	74.639	0.028	23	VB	7.6	
63	107	0.124099	75.149	0.013	51	BV	7.6	
64	123+149	3.043263	75.576	0.008	1224	VV	8.1	
65	118	1.255572	75.882	0.020	597	VB	8.3	
66	134	0.068224	77.027	0.006	40	BB	6.4	
67	114+131	0.141561	77.481	0.020	55	BV	8.3	
68	146	0.400207	78.990	0.016	204	BB	7.8	
69	132+153+105	4.593065	79.954	0.013	1858	BB	9.5	
70	141	1.841368	81.885	0.009	1285	BB	8.1	
71	137+176	0.271340	83.050	0.005	236	BV	7.9	
72	130	0.071601	83.388	0.015	33	VB	7.7	
73	163+138	2.926401	84.228	0.009	1103	BV	10.6	
74	158	0.252452	84.586	0.010	160	VB	8.8	
75	129	0.015677	85.365	-0.003	43	VP	17.4	
76	178	1.193907	85.885	0.006	518	PV	8.1	
77	166(SURR)	4.808515	86.215	0.000	3252	VV	8.4	
78	175	0.194360	86.738	0.002	100	TS	0.0	
79	187+182	4.078599	87.260	0.001	2366	VB	8.4	
80	183	1.905587	88.047	0.002	1101	BV	8.1	
81	128	0.094070	88.478	0.023	126	VB	9.5	
82	167	0.041623	89.243	0.014	15	BV	6.6	
83	185	0.494288	89.556	0.007	605	VB	8.0	
84	174	3.529921	90.910	-0.001	1802	BB	8.6	
85	177	1.909970	91.710	0.005	934	BB	8.0	
86	202+171	0.871405	92.411	0.005	731	BV	8.0	
87	156	0.065165	92.687	0.011	82	VB	8.2	
88	173	0.034467	93.124	-0.000	35	BB	7.0	
89	157+200	0.439246	93.709	-0.002	326	BV	8.4	
90	204(ISTD)	9.931970	93.938	0.050	5983	VB	8.3	R
91	172+197	0.595701	94.732	0.004	385	BB	8.2	
92	180	6.918325	95.748	0.012	4264	BV	8.5	
93	193	0.444705	96.160	0.003	323	VB	8.4	
94	191	0.110430	96.770	0.002	70	BB	6.9	
95	199	0.430443	97.250	-0.007	388	BB	8.1	
96	170+190	1.942022	100.228	0.006	1556	BV	9.1	
97	DBC(SURR)	10.149161	100.727	-0.013	5274	VB	8.4	
98	198	0.125405	101.285	-0.002	179	TS	0.0	

Attachment 1: Mixed Congener Standard on DB-5

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99	201	4.806856	101.905	-0.002	2272	BB	8.3	
100	203	2.407035	102.656	-0.012	1611	BV	8.3	
101	196	1.977705	102.750	-0.013	1323	VB	10.8	
102	189	0.039184	105.148	-0.020	26	BB	8.1	
103	208+195	0.896954	107.209	0.005	1064	BB	7.9	
104	207	0.093248	108.476	-0.015	133	BB	7.4	
105	194	2.084478	110.864	-0.002	2088	PB	8.2	
106	205	0.112808	111.588	-0.003	112	BB	7.7	
107	206	0.777460	116.694	-0.007	849	BB	7.9	
108	209	0.013712	121.396	0.000	48	BB	7.2	
	Totals:	249.217190		0.826	82548			

Status Codes:
R - Reference peak

Total Unidentified Counts . 5777 counts

Detected Peaks: 196 Rejected Peaks: 0 Identified Peaks: 108

Amount Standard: N/A Multiplier: 1.000000 Divisor: 1.000000

Baseline Offset: -11 microVolts

Noise (used): 140 microVolts monitored before this run

Rack: 1 Vial: 12 Injection Number: 1 Injection Volume: 5.0 ul

Original Notes:

MULLIN CAL STD W SINGLE LEVEL SURROGATES FOR SURROGATE
INTERNAL STD CALIBRATION

Appended Notes:

Attachment 2. Mixed Congener Standard on DB-1701

(Page 1 of 5)

```
Title       : june cal DB-1701 5 ul inj using height for cal
Run File    : C:\STAR\MODULE16\728JN4\7284013.RUN
Method File : C:\STAR\MODULE16\728JN4\7284014.MTH
Sample ID   : PP-376C 183

Injection Date: 26-MAY-95 4:51 PM    Calculation Date: 22-JUN-95 3:05 PM

Operator    : TJF                    Detector Type: ADCB (10 Volts)
Workstation:                         Bus Address  : 16
Instrument : Varian Star #1          Sample Rate  : 5.00 Hz
Channel     : B = 01                 Run Time     : 152.503 min

*********** Star Chromatography Software ********** Version 4.01 *************

Chart Speed =   0.42 cm/min   Attenuation = 12          Zero Offset = 25%
Start Time  =  30.000  min     End Time    = 140.000 min  Min / Tick  = 5.00
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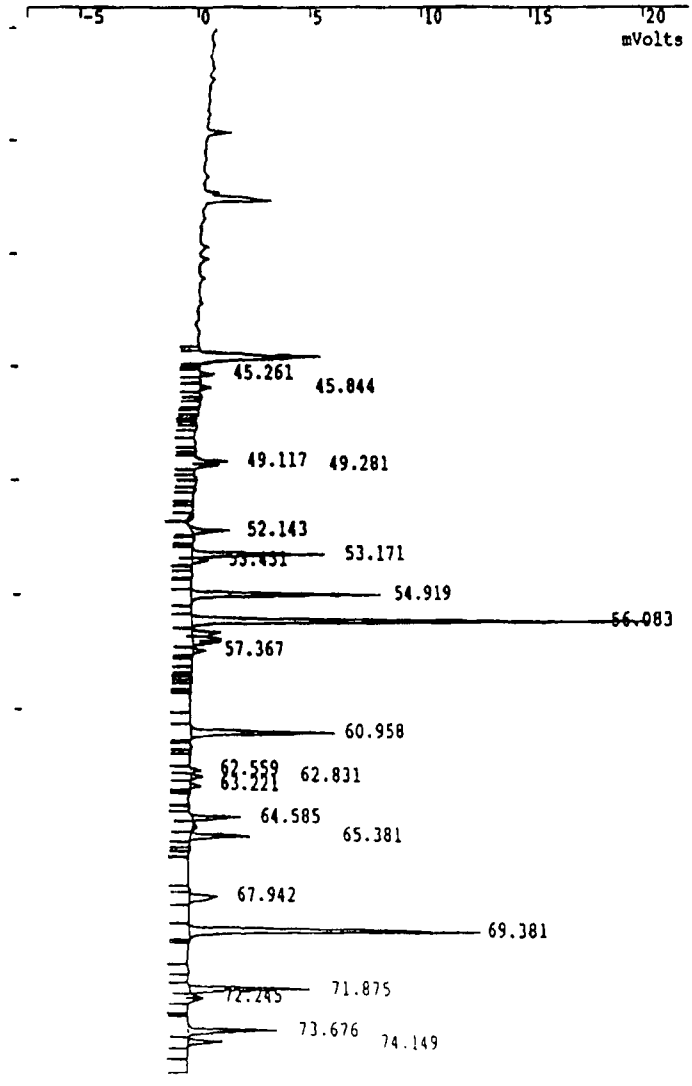
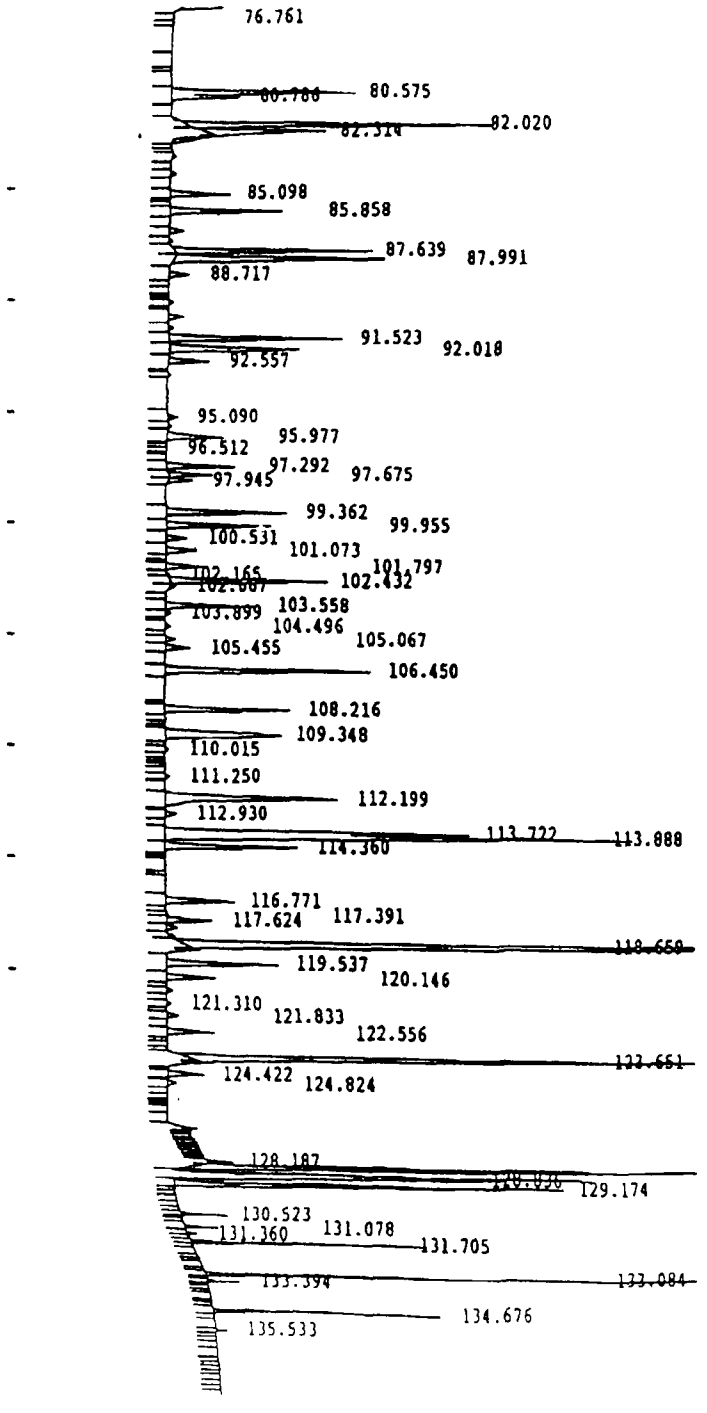


Attachment 2: Mixed Congener Standard on DB-1701

(Page 2 of 5)



Attachment 2: Mixed Congener Standard on DB-1701

(Page 3 of 5)

Title : june cal DB-1701 5 ul inj using height for cal
Run File : C:\STAR\MODULE16\728JN4\7284013.RUN
Method File : C:\STAR\MODULE16\728JN4\7284014.MTH
Sample ID : PP-376C 183

Injection Date: 26-MAY-95 4:51 PM Calculation Date: 22-JUN-95 3:05 PM

Operator : TJF Detector Type: ADCB (10 Volts)
Workstation: Bus Address : 16
Instrument : Varian Star #1 Sample Rate : 5.00 Hz
Channel : B = 01 Run Time : 152.503 min

********** Star Chromatography Software ********** Version 4.01 **********

Run Mode : Analysis
Peak Measurement: Peak Height
Calculation Type: External Standard

Peak No.	Peak Name	Result (NG/L)	Ret. Time (min)	Time Offset (min)	Height (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1	10	0.176037	45.261	0.000	65	BB	6.2	
2	4	3.018902	45.844	0.014	50	BB	6.1	
3	7	0.265841	49.117	0.018	95	BB	7.8	
4	9	0.335552	49.281	0.010	57	BB	9.7	
5	6	1.816117	52.143	0.011	174	BB	6.8	
6	8	14.159795	53.171	0.005	593	BV	7.3	
7	5	0.278926	53.431	-0.002	77	VB	7.2	
8	14SURR	5.036743	54.919	0.005	841	BB	7.5	
9	30ISTD	10.156128	56.083	0.039	2043	BP	7.2	R
10	19	0.281359	57.367	0.004	59	BB	7.2	
11	12+13+17+18	6.391715	60.958	0.010	643	BB	8.7	
12	24	0.052619	62.559	0.017	49	BB	7.0	
13	15	1.490831	62.831	0.014	54	BB	8.2	
14	27	0.203341	63.221	0.006	51	BB	7.3	
15	32	1.854917	64.585	0.004	223	BB	7.5	
16	16+29	2.099022	65.381	0.011	273	BB	7.6	
17	26+25	1.021916	67.942	0.008	128	BB	13.1	
18	28+31	10.125882	69.381	0.022	1314	BB	9.0	
19	21+33+53	4.102441	71.875	0.018	542	BB	8.1	
20	51	0.023595	72.245	-0.019	10	BB	11.4	
21	22	2.925104	73.676	0.015	402	BB	7.8	
22	45	0.928908	74.149	0.016	156	BB	7.6	
23	43+46+52	4.576473	76.102	0.011	476	BB	8.1	
24	65+47+49	3.705014	76.477	0.004	1378	BB	9.5	
25	48	0.847172	76.761	0.003	172	BB	8.0	
26	44	4.734031	80.575	0.016	803	BV	8.2	
27	42	1.447829	80.786	0.009	296	VB	9.7	
28	103ISTD	9.717182	82.020	-0.002	1354	BB	7.7	
29	41+71+100+64	3.625286	82.314	0.016	534	BB	11.7	
30	40+63	1.256547	85.098	0.004	260	BB	7.5	
31	74	2.065444	85.858	0.014	494	BB	7.9	
32	70	3.283874	87.639	0.017	864	BB	8.0	
33	66+95	5.817988	87.991	0.017	911	BB	9.9	
34	91	0.533801	88.717	0.009	90	BB	7.7	
35	60+101	5.634208	91.523	0.005	760	BB	8.2	
36	56+99	3.559681	92.018	0.014	572	BB	9.8	
37	89+84	1.446028	92.557	0.001	174	BB	7.9	
38	83	0.158861	95.090	0.004	46	BB	8.1	

Attachment 2: Mixed Congener Standard on DB-1701

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39	92+97	1.070954	95.977	-0.002	245	BB	8.7	
40	trans nonach	0.006340	96.512	-0.013	2	BB	2.6	
41	87	1.052906	97.292	0.005	296	BB	7.8	
42	85	0.695014	97.675	0.004	187	BB	7.8	
43	136	0.726305	97.945	0.001	104	BB	7.9	
44	110	2.009048	99.362	0.009	530	BB	8.0	
45	151	1.796553	99.955	0.003	458	BB	7.6	
46	144	0.630569	100.531	0.002	92	BB	7.2	
47	77	0.228873	101.073	-0.003	133	BB	8.4	
48	82	0.464388	101.797	0.015	161	BB	7.6	
49	135	0.156599	102.165	0.014	10	BB	8.0	
50	149	3.115006	102.432	0.011	686	BB	8.1	
51	107+123	0.071140	102.667	0.011	22	BB	11.3	
52	118	1.281861	103.558	0.019	385	BB	7.8	
53	114	0.356901	103.899	-0.008	24	BB	8.1	
54	134	0.076659	104.496	0.006	28	BB	8.3	
55	131+137	0.035547	105.067	0.012	45	BB	7.7	
56	146	0.403681	105.455	0.019	106	BB	7.9	
57	153	2.840417	106.450	0.015	895	BB	8.3	
58	132	0.684852	108.216	0.016	555	BB	8.3	
59	141+176+105	2.702293	109.348	-0.009	515	BB	14.4	
60	129	0.014328	110.015	0.013	16	BB	7.1	
61	130	0.081114	111.250	0.007	20	BB	6.9	
62	163+138+158+	4.417425	112.199	0.010	757	BB	10.6	
63	182	0.697081	112.930	0.018	48	BB	8.5	
64	187	3.900236	113.722	0.023	1328	BV	9.6	
65	166SIS	4.921273	113.888	-0.006	2041	VV	8.8	
66	183	1.816817	114.360	0.019	569	VB	8.3	
67	185	0.465152	116.771	0.018	302	BB	8.2	
68	167	0.037056	117.391	-0.003	9	BB	6.7	
69	202+167+128	0.311932	117.624	-0.006	191	BB	8.0	
70	174+200+204I	11.663553	118.659	0.045	3932	BB	9.5	R
71	177	1.840270	119.537	0.014	492	BB	8.3	
72	171+197	0.363120	120.146	0.008	214	BB	8.2	
73	173	0.037543	121.310	0.023	19	BB	8.2	
74	156	0.070713	121.833	0.019	54	BB	7.5	
75	172	0.576366	122.556	0.028	204	BB	7.6	
76	157+180+199	7.206979	123.651	0.021	2487	BB	8.1	
77	193	0.435179	124.422	0.021	159	BB	8.2	
78	191	0.124112	124.824	0.021	41	BB	7.6	
79	198	0.068043	128.187	0.003	109	BB	0.0	
80	170+190+201	0.939771	128.836	0.007	209	BB	6.4	
81	196+203	4.480081	129.174	-0.008	1660	BB	6.6	
82	208	0.101339	130.523	-0.013	206	BB	4.3	
83	207	0.095886	131.078	-0.015	148	BB	4.7	
84	189	0.036086	131.360	-0.016	47	BB	0.0	
85	195	0.703326	131.705	-0.016	1035	BB	3.5	
86	194	1.901737	133.084	-0.020	2564	BB	3.2	
87	205	0.093067	133.394	-0.020	146	BB	0.0	
88	206	0.724105	134.676	-0.020	995	BB	3.6	
89	209	0.010731	135.533	-0.019	55	BB	3.3	
	Totals:	187.695437		0.651	42619			

Status Codes:
R - Reference peak

Total Unidentified Counts : 6147 counts

Detected Peaks: 293 Rejected Peaks: 6 Identified Peaks: 89

Attachment 2: Mixed Congener Standrd on DB-1701

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```
Amount Standard: N/A        Multiplier: 1.000000    Divisor: 1.000000
Baseline Offset: -14 microVolts
Noise (used): 50 microVolts - monitored before this run
Rack: 1       Vial: 12       Injection Number: 1     Injection Volume: 5.0 ul
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PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction

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Revised January 1, 1996

PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction

1.0 Application

1.1 This method is used to determine congener specific PCB and pesticide concentrations at trace levels in surface water. A multi-plate filtration system for collection of particulates, and an XAD-2 resin column for collection of dissolved PCBs is used. Water volumes of 80 L to 160 L can be analyzed by this method.

1.2 The following limits of detection (method detection limits) were determined for the resin ("dissolved") PCBs and the filters ("particulate") PCBs for sample sizes of 80 L and 160 L:

BZ # (1)	80 L		160 L	
	LOD (MDL) ng/L	LOQ ng/L	LOD (MDL) ng/L	LOQ ng/L
#3	0.43	1.4	0.22	0.72
#4/10	0.050	0.17	0.025	0.083
#7/9	0.011	0.037	0.0055	0.018
#6	0.022	0.073	0.011	0.037
#8/5	0.049	0.16	0.024	0.080
#19	0.0070	0.023	0.0035	0.012
#18	0.014	0.047	0.0070	0.023
#15/17	0.030	0.10	0.015	0.050
#24/27	0.0070	0.023	0.0035	0.012
#16/32	0.022	0.073	0.011	0.037
#26	0.014	0.047	0.0070	0.023
#25	0.012	0.040	0.0060	0.020
#28/31	0.040	0.13	0.020	0.070
#33	0.015	0.050	0.0075	0.025
#53	0.0080	0.027	0.0040	0.013
#51	0.0070	0.023	0.0035	0.012
#22	0.022	0.073	0.011	0.037
#45	0.0090	0.030	0.0045	0.015
#46	0.0090	0.030	0.0045	0.015
#52	0.015	0.050	0.0075	0.025
#49	0.010	0.033	0.0050	0.017
#47/48	0.018	0.060	0.0090	0.030
#44	0.013	0.043	0.0065	0.022
#37/42	0.020	0.067	0.010	0.033
#41/71/64	0.020	0.067	0.010	0.033
#40	0.010	0.033	0.0050	0.017
#63	0.025	0.083	0.012	0.040
#74	0.013	0.043	0.0065	0.022
#70/76	0.025	0.083	0.012	0.040

BZ # (1)	80 L		160 L	
	LOD (MDL) ng/L	LOQ ng/L	LOD (MDL) ng/L	LOQ ng/L
#66	0.023	0.076	0.012	0.040
#95	0.012	0.040	0.0060	0.020
#91	0.011	0.037	0.0055	0.018
#56/60	0.016	0.053	0.0080	0.027
#92/84	0.024	0.080	0.012	0.040
#89	0.0060	0.020	0.0030	0.010
#101	0.011	0.037	0.0055	0.018
#99	0.0080	0.027	0.0040	0.013
#83	0.0090	0.030	0.0045	0.015
#97	0.0060	0.020	0.0030	0.010
#87	0.010	0.033	0.0050	0.017
#85	0.011	0.037	0.0055	0.018
#136	0.030	0.10	0.015	0.050
#77/110	0.022	0.073	0.011	0.037
#82	0.0070	0.023	0.0035	0.012
#151	0.010	0.033	0.0050	0.017
#135/144	0.013	0.043	0.0065	0.022
#123/149	0.010	0.033	0.0050	0.017
#118	0.016	0.053	0.0080	0.027
#146	0.011	0.037	0.0055	0.018
#132/153/105	0.020	0.067	0.010	0.033
#141	0.0080	0.027	0.0040	0.013
#137/176	0.013	0.043	0.0065	0.022
#163/138	0.022	0.073	0.011	0.037
#158	0.015	0.050	0.0075	0.025
#178	0.014	0.047	0.0070	0.023
#187/182	0.010	0.033	0.0050	0.017
#183	0.011	0.037	0.0055	0.018
#128	0.0090	0.030	0.0045	0.015
#167	0.012	0.040	0.0060	0.020
#185	0.0070	0.023	0.0035	0.012
#174	0.011	0.037	0.0055	0.018
#177	0.012	0.040	0.0060	0.020
#202/171	0.0080	0.027	0.0040	0.013
#172	0.015	0.050	0.0075	0.025
#180	0.013	0.043	0.0065	0.022
#193	0.015	0.050	0.0075	0.025
#199	0.0090	0.030	0.0045	0.015
#170/190	0.011	0.037	0.0055	0.018
#198	0.015	0.050	0.0075	0.025
#201	0.018	0.060	0.0090	0.030
#203/196	0.028	0.093	0.014	0.047

BZ # (1)	80 L		160 L	
	LOD (MDL) ng/L	LOQ ng/L	LOD (MDL) ng/L	LOQ ng/L
#208/195	0.0080	0.027	0.0040	0.013
#207	0.0070	0.023	0.0035	0.012
#194	0.011	0.037	0.0055	0.018
#206	0.0070	0.023	0.0035	0.012

1.3 Pesticide LODs and LOQs: 80 L and 160 L Water

Compound	80 L		160 L	
	LOD (MDL) ng/L	LOQ ng/L	LOD (MDL) ng/L	LOQ ng/L
alpha-BHC	0.050	0.16	0.025	0.082
gamma-BHC (Lindane)	0.050	0.16	0.025	0.082
oxychlordane	0.019	0.063	0.010	0.033
gamma-chlordane	0.022	0.073	0.011	0.037
alpha-chlordane	0.021	0.070	0.010	0.033
trans-nonachlor	0.017	0.057	0.0085	0.028
p,p'DDD	0.050	0.16	0.025	0.082
cis-nonachlor	0.021	0.070	0.010	0.033
p,p'DDT	0.050	0.16	0.025	0.082
toxaphene	10.0	33.0	5.0	16.0
hexachlorobenzene (HCB)	0.0060	0.020	0.0030	0.010
p,p'DDE	0.030	0.10	0.015	0.050

2.0 Sampling

2.1 Water samples are filtered and pumped through XAD-2 resin columns in the field. (See the field standard operating plan.)

2.2 Foil-wrapped filters and resin columns received in the lab from the field are refrigerated at about 4°C until time for laboratory extraction.

3.0 Reagents

3.1 Hexane, acetone, ethyl ether, methylene chloride, methanol Pesticide Grade

3.2 Sodium sulfate ACS granular; stored at 130°C.

3.3 Silica Gel Davison Grade 923, 100-200 mesh activated at 130°C, deactivated with 3.5% water for an hour prior to use.

3.4 Florisil PR Grade 60-100 mesh. Dried at 130°C, stored in air tight container at room temperature.

3.5 Glass wool Soxhlet extracted in acetone/hexane 50:50 for eight hours.

3.6 Glass fiber filters: 293 mm diameter, 0.7 micron mesh from Microfiltration system, Dublin, CA; wrapped in aluminum foil and heated for four hours at 450°C, stored and sent to the field in the foil packets.

3.7 Resin columns: 5.0 cm X 30.0 cm glass chromatographic columns, with threaded ends, 50 mm thread size; heated for four hours at 450°C.

3.8 Nylon plugs, two per column, 50 mm thread size, and nylon adaptor plugs with swagelock fittings; soaked overnight in a 50/50 mixture of acetone/hexane prior to use. O-rings are soaked in hexane overnight.

3.9 Amberlite XAD-2, 20-60 mesh, Sigma Chemical Company, cleaned as described below.

3.10 HCl-Reagent grade, diluted to 50% and extracted with hexane three times.

4.0 XAD-2 Resin Column Preparation

4.1 The XAD-2 resin is cleaned in the lab by a series of solvent extractions in a large Soxhlet apparatus. Approximately 2.5 kg of resin is extracted sequentially for 24 hrs each in methanol, acetone, hexane, and methylene chloride. This is followed by sequential six hour extractions in acetone, hexane, and acetone. This sequence cycles the resin back to a water-miscible solvent, which is displaced from the resin by rinsing with several volumes of organic free water. Cleaned resin is stored under organic free water in amber bottles for one to three months, until column preparation. The hexane from the 6 hour extraction is used as a resin quality control blank. The final six hour acetone extract can be used as the first acetone on the next batch of resin.

4.2 XAD-2 resin columns are prepared by first attaching one nylon adaptor with a Swagelock fitting and a 3" length of latex tubing to one end of the glass column, and pushing a large plug of cleaned glass wool into the bottom. The column is filled about ½ full with organic free water and clean resin is poured into the column in a slurry to a final packed length of 19.5 cm (400 mL). The resin is packed by pumping excess water out from the bottom using a water aspirator or peristaltic pump but maintaining enough water in the column to cover the resin. The column should not contain air bubbles or channels. The top of the column is plugged with wet glass wool and a nylon plug. The nylon adaptor at the bottom is replaced with a nylon plug. Columns are wrapped for shipping and stored in the lab until picked up by United States Geological Survey sampling crews.

4.3 A log is kept of resin batches as they are being cleaned, and of columns as they are prepared and sent to the field so that traceability of samples to individual columns and to batches of cleaned resin is maintained.

5.0 Surrogate and Matrix Spikes

5.1 Surrogate standards are added to each sample and blank prior to extraction to monitor analytical recoveries of PCB congeners. The surrogates are PCB congeners #14, #65, and #166 at nominal concentrations of 20, 5, and 5 ng/mL respectively. They are added to the Soxhlet extractor of every sample and blank at the beginning of the analytical procedure.

5.2 The matrix spike solution consists of the following Aroclor mixture: Aroclors 1232, 1248, and 1262 at 0.25, 0.18, and 0.18 mg/L, respectively, in acetone. It does not contain internal standards. With each batch of samples analyzed, an appropriate amount of this spike solution is added by Class A volumetric pipet to a Soxhlet containing clean resin and to a Soxhlet containing a clean filter. Surrogates are also added. These spikes are extracted and analyzed along with the samples.

5.3 With each batch of samples, a separate spike of chlorinated pesticides is added to a Soxhlet containing clean resin and to a Soxhlet containing a clean filter.

6.0 Sample Extraction - Resin and Filters

6.1 The resin, representing the dissolved portion of a surface water sample, is analyzed by extracting the resin and glass wool plugs in two Soxhlets, each with a 500 mL mixture of 50% acetone/50% hexane, for 16 hours. Excess water at the top of the resin column is first poured off into an Erlenmeyer flask. Resin is transferred to the Soxhlets in an acetone slurry, and rinsed at least twice with acetone to remove as much water as possible. This acetone-water "rinsate" (400 to 450 mL) is added to the water in the Erlenmeyer and set aside. Surrogate spike solution is added to each resin Soxhlet and to the rinsate.

6.2 The Soxhlet extract will still contain some water which will result in a two-layer acetone-and-water/hexane system. After extraction is complete, the extract is reduced in volume on a rotary evaporator to approximately 300 mL and transferred to a 500 mL separatory funnel. The water layer is drawn off and combined with the rinsate from that sample in a 1 L or 2 L separatory funnel. The hexane layer is saved. Then 300 mL of organic-free water is added to the rinsate. Five (5) mL of 50% HCl is added to minimize emulsion. The rinsate is then extracted three times with 100 mL, 75 mL and 75 mL of hexane. The hexane extracts are combined with the hexane layer from the Soxhlet extract and concentrated to approximately 5 mL using 15 mL of iso-octane as a keeper. Sodium sulfate (approximately 10g) is added to absorb residual water.

6.3 Filters from each sample are combined and extracted in a Soxhlet (separately from the resin) with a 600 mL mixture of 50% acetone/50% hexane for 16 hours. Surrogate spike is added to the Soxhlet at the beginning of the extraction.

6.4 The filter extract also contains some water which will form a separate layer. The filter extract is concentrated to approximately 300 mL on a rotary evaporator, and transferred to a 500 mL separatory funnel. The water layer is drawn off, transferred to a second separatory funnel, and 100 mL of organic-free water is added. Five (5) mL of 50% HCl is added to minimize emulsion. The rinsate is then extracted three times with hexane (75 mL, 50 mL, 50 mL). The hexane extracts are combined with the filter Soxhlet hexane layer and concentrated on a rotary evaporator to approximately 5 mL for clean-up, using 15 mL of iso-octane as a keeper. Sodium sulfate (approximately 10g) is added to absorb residual water.

7.0 Sample Clean-up and Fractionation

7.1 Florisil and silica gel column chromatography are employed as clean-up techniques prior to GC-EC analysis. The fractionations are required to separate PCBs from as many other parameters as possible. This facilitates identification and analysis by GC-EC. The florisil procedure is performed first, followed by silica gel.

7.2 Florisil columns are prepared by placing 1 cm of anhydrous sodium sulfate in a 1 cm i.d. X 30 cm chromatography column fitted with a 75 mL reservoir. The column should be previously filled to slightly above the reservoir base with hexane. Eight grams of 60/100 mesh Florisil (Floridin Company), activated at 130°C for 16 hours, is then added and topped with another 1 cm layer of sodium sulfate. Avoid entrapping air bubbles when pouring the column. Adjust the hexane level to within a few mm of the top layer of sulfate and discard the excess solvent (this also serves as a column wash).

7.3 When hexane reaches the top of the upper sodium sulfate layer, the sample extract is quantitatively transferred to the column and allowed to drain onto the bed of Florisil. The sample container is washed with 5 mL of hexane and added to the column as the original extract has just reached the top layer of sulfate. This also serves to wash down the walls of the column. When the hexane rinse reaches the top of the Florisil, the elution solvent is added, and the eluate is collected for further separation. The volume and makeup of the elution solvent is determined from the Florisil elution check. Currently 50 mL of 94/6 hexane/ethyl-ether is used. This eluate is concentrated under a gentle stream of air to about 5 mL and cleaned up on silica gel. See Section 7.4

7.4 The eluate from the Florisil column must be further fractionated through silica gel to separate PCBs and chlorinated pesticides. Prepare the silica gel by heating at 130°C overnight and deactivating before use by equilibrating one hour with 3.5% distilled water. (The percentage of deactivation may change with different lots of silica gel.) Prepare silica gel columns (1cm i.d. x 30 cm) by first filling with hexane. Add 1 cm of anhydrous sodium sulfate, 5 gm of deactivated silica gel and another 1 cm of sodium sulfate layer and quantitatively add the first florisil fraction. Start collecting the eluate and elute the PCBs, HCB, and p,p'DDE with 50 mL of hexane. Two to 3 mL of iso-octane is added to this SG1 fraction, and it is concentrated down under a gentle stream of air to approximately 5 mL, then transferred to a centrifuge tube and further concentrated to 1.0 mL for GC analysis. A final clean-up is done by adding 1.0 mL concentrated sulfuric acid to the SG1 extract.

7.5 A second fraction is eluted from the silica gel column using 60 mL of 25% ethyl ether in hexane. This will contain alpha-BHC, lindane, the chlordanes, nonachlors, p,p'DDD, p,p'DDT and toxaphene. Two to 3 mL of iso-octane is added to this SG2 fraction and it is concentrated as in Section 7.4 above, transferred to a centrifuge tube and further concentrated to 1.0 mL. A final clean-up of this fraction is done by adding 1 mL of concentrated sulfuric acid to the extract, mixing thoroughly, and allowing to sit for up to 24 hours.

8.0 Gas Chromatography for PCB Congeners, HCB, and p,p'DDE

8.1 GC Conditions

HP 5890-II Gas Chromatograph
60M DB5 column, 0.2 mm ID, 0.1 um film
Hydrogen carrier gas
Electron Capture Detector; 300°C
Pressure Programmable Injector; 265°C
Initial Pressure 40 psi, 1.0 min hold
Programmed from 40 psi to 20 psi at 20 psi/min, then go to constant flow mode for remainder of run
Splitless injection; purge on at 0.70 min
Injector volume 1 µL

Oven Temperature Profile:
Initial Temp 100°C, hold for 1.0 min
100°C to 150°C at 3°C/min
150°C to 220°C at 1°C/min
220 C to 280 C at 5°C/min, hold for three min

8.2 Standards

The single point calibration standard consists of a dilution of a stock solution of Aroclors 1232, 1248,and 1262 at 183 μg/mL which was supplied by M. Mullein in June 1994. See Table 1293.8b1 for congener composition of the stock solution. The diluted standard contains Aroclors 1232, 1248, and 1262 at .225, .162, and .162 ng/L for a total of .549 μg/mL PCB. Quantitation of congeners #128 and #167 requires the addition of individual standards of these congeners to the calibration mix, at nominal concentrations of 4 ng/mL and 2 ng/mL, respectively. The total concentration of these congeners in the calibration mix must also include the contribution from the Aroclors. This contribution is 0.30 ng/mL of #128 and 0.15 ng/mL of #167. This standard also contains PCB congener #30 at a nominal concentration of 0.012 mg/L (12. ng/mL), and PCB congener #204 at 0.013 mg/l (13. ng/mL) which are used as retention time reference peaks and as internal standards for quantitation. Congeners eluting prior to and including #77/110 use congener #30 as internal standard, those eluting after #77/110 use congener #204 as internal standard. The calibration table contains the concentration in ng/mL of each congener in the mix, including internal standards, as well as surrogates #14, #65, and #166 at nominal concentrations of 32, 7, and 8 ng/mL. See Table 1293.8b2.

8.3 A three-level calibration is performed yearly to verify detector response linearity, using the single-point standard and standards at 0.5x and at 2x the single point standard. The RSD of the three response factors for each congener shall be less than 25%. Alternatively, linearity may be demonstrated by a correlation coefficient of at least 0.95.

8.4 Pesticide standards containing HCB, trans-nonachlor, and p,p'-DDE at concentrations of 4-8 ng/mL and containing internal standards #30 and #204 are also run with each batch of PCB extracts.

8.5 Instrument Performance

Congener response factors are generated daily from a run of the single point calibration standard. This standard will also be run every 12 hours as a performance standard and evaluated for resolution, reproducibility, and sensitivity. In addition, a PCB performance standard at either the .5x or 2x concentration will be run at a nominal frequency of every other sample batch. The calculated concentrations of congeners #44, #101, #185 and #180 shall not differ from their known concentrations by more than 25%. The calculated concentrations of congeners #6 and #198 shall not differ from their known concentrations by more than 50%. If these limits are exceeded, response factors will be regenerated, or the necessary instrument maintenance will be performed.

8.6 Samples

Some samples may need to be screened by packed column GC-EC. This is to insure that there has been adequate clean-up, and that samples are diluted or concentrated to an appropriate volume for injection onto the capillary column. Exactly known amounts of internal standards are added to the cleaned-up sample extract just prior to capillary column gas chromatography. An appropriate amount (usually 25 µL) of a standard containing congeners #30 and #204 is added to the sample extract to bring their concentrations in the extract to approximately the same as they are in the calibration standard.

8.7 Calculations

Calculations for PCB Congeners are done by the HP3396 Integrator using the formula for internal standard quantitation:

$$Conc. = \frac{Height\ (y)}{Height(IS)} \times \frac{RF\ (y)}{RF(IS)} \times Amount\ (IS) \times Mult.$$

Where: *y = analyte*
IS = internal standard
RF = response factor = mass/peak ht.
Amount(IS) = mass of internal standard added to the sample
Mult. = multiplier = 1/sample volume

Response factors are generated from a daily run of the calibration standard. Calculations for HCB and p,p'-DDE are done manually using the same internal standard formula, and using congener #30 as internal standard.

8.8 Confirmation

Confirmation of correct PCB and pesticide identification is done on 5% of the SG1 extracts by retention time agreement on a 60M DB-1column, using the same GC conditions and standards as given in Sections 8.1 and 8.2. Table 1293.8h1 contains the concentration in ng/mL of each congener in the mix.

9.0 Gas Chromatography for Pesticides in the Second Silica Gel Fraction

9.1 GC Conditions

HP 5890 Gas Chromatograph or equivalent
60M DB1 column, 0.2 mm i.d., 0.1 um film
Hydrogen carrier gas
Electron Capture Detector
Oven Temperature Profile:
- 90 °C Initial temperature
- 90 °C to 120 °C at 10 °C/min
- 120 °C to 245 °C at 4 °C/min
- 245 °C to 280 °C at 15 °C/min, hold for 6 min

Injector temperature 265°C
Detector temperature 320°C
Pressure Programmable Injector
Initial Pressure 25 psi, 1 min hold
Programmed from 25 psi to 16.5 psi at
10 psi/min, hold at 16.5 psi 42 min
Splitless injection, 1 µL; purge on at 0.80 min

Retention times are given in the following table:

Compound	RT, min.	RRT vs #204
alpha-BHC	15.90	.459
gamma-BHC (lindane)	17.36	.502
#30 (Int. Std)	17.80	.514
oxychlordane	25.08	.725
gamma-chlordane	25.94	.749
alpha-chlordane	26.76	.773
trans-nonachlor	27.20	.786
p,p'DDD	29.57	.854
cis-nonachlor	29.87	.863
p,p'-DDT	31.49	.910
#204 (RT Reference)	34.61	1.000
toxaphene compounds	27 to 36 min.	---

9.2 Standards

A mixed standard containing the chlorinated pesticides at 4 to 8 ng/mL and also containing congeners #30 and #204 as internal standards and retention time reference peaks is run with each set of samples. Standards at 2x or 3x those concentrations will be run if high concentrations are expected in samples. A standard containing toxaphene at 0.80 mg/L is also run with each batch of samples.

9.3 Instrument performance

Response factors for pesticides are generated daily from a run of the 4 to 8 ng/mL standard. This standard, or the 2x or 3x standard, will be run daily as a performance standard.
Calculated concentrations of pesticides in the performance standard should not differ from the known concentration by more than 20%.

9.4 Samples

Internal standards PCB #30 and #204 are added to the second silica gel fraction, which has been cleaned up with sulfuric acid (See Section 7.5), just prior to capillary column gas chromatography.

9.5 Calculations for pesticides

Calculations for pesticides are done using peak heights, and the same internal standard formula given in Section 8.7. PCB congener #30 is used as the internal standard for all pesticides; there is often an interference in this fraction which co-elutes with congener #204.
Note: Trans-nonachlor may split on the silica gel and be found in both fractions. If this occurs it is quantitated in both fractions and the sum is reported.

9.6 Calculations for toxaphene
Toxaphene is a multi-component mixture of chlorinated camphenes. Identification of toxaphene in a sample requires a minimum of five sample peaks matching the retention times of standard peaks. Quantitation is done by summing peak heights of five or more matching peaks in the sample and in the standard, and using the internal standard formula:

$$Conc = \frac{\text{Sum of peak heights (sample)}}{\text{Ht IS (sample)}} \times \frac{RF(y)}{RF(IS)} \times \frac{\text{Amount IS}}{\text{sample vol.}} \times \frac{1}{L}$$

Where y = toxaphene

$$RF(y) = \frac{\text{mass of standard}}{\text{Sum of peak heights (std)}}$$

9.7 Confirmation

Confirmation of SG2 pesticides is done on 5% of the samples by retention time agreement on a second column (e.g. 60M DB-5), or by GC/MS.

10.0 References

10.1 Ballschmiter, K. and Zell, M., 1980. Fresenius Z. Anal. Chem., 302, 20-31.

10.2 M. Mullein, File=C:\QPR04\QC\LMMBPCB1.WQ1 21-June 1994 (Table 1293.8b1).

Table 1293.8b1 PCB Stock Solution Concentrations 183 µg/mL

FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd		FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd	
Peak Name	**Congener Conc'ns µg/mL**	**Peak Name**	**Congener Conc'ns µg/mL**
PCB-000	4.1	PCB-097	0.56
PCB-001	12	PCB-099	0.74
PCB-003	7.0	PCB-100	0.11
PCB-004+010 (SUM)	3.4	PCB-101	1.8
PCB-006	1.9	PCB-107	0.13

Table 1293.8b1 PCB Stock Solution Concentrations 183 µg/mL

FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd		FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd	
Peak Name	**Congener Conc'ns µg/mL**	**Peak Name**	**Congener Conc'ns µg/mL**
PCB-007+009 (SUM)	1.2	PCB-110	1.9
PCB-008+005 (SUM)	14	PCB-114+131 (SUM)	0.14
PCB-012	0.17	PCB-118	1.2
PCB-013	0.097	PCB-119	0.028
PCB-015+017 (SUM)	3.7	PCB-123+149 (SUM)	2.8
PCB-016	2.0	PCB-128	0.10
PCB-018	3.7	PCB-129	0.013
PCB-019	0.28	PCB-130	0.075
PCB-021	0.032	PCB-132+153+105 (SUM)	4.3
PCB-022	2.9	PCB-134R	0.072
PCB-024+027 (SUM)	0.26	PCB-135+144 (SUM)	0.89
PCB-025	0.32	PCB-136	0.75
PCB-026	0.72	PCB-137+176 (AVE)	0.26
PCB-029	0.053	PCB-141	1.7
PCB-031+028 (SUM)	9.4	PCB-146	0.39
PCB-032	1.9	PCB-151	1.7
PCB-033	3.3	PCB-156	0.066
PCB-037	1.2	PCB-157+200 (AVE)	0.39
PCB-040	0.94	PCB-158	0.25
PCB-041+071 (AVE)	2.3	PCB-163+138 (SUM)	2.7
PCB-042	1.4	PCB-167	0.049
PCB-043	0.27	PCB-170+190 (SUM)	1.7
PCB-044	4.3	PCB-172	0.56
PCB-045	0.89	PCB-173	0.038
PCB-046	0.40	PCB-174	3.2
PCB-047	1.0	PCB-175	0.20
PCB-048	1.0	PCB-177	1.7
PCB-049	2.3	PCB-178	1.1
PCB-051	0.18	PCB-180	6.1
PCB-052	4.5	PCB-183	1.7
PCB-053	0.64	PCB-185	0.47
PCB-056+060 (AVE)	3.5	PCB-187+182 (AVE)	3.6
PCB-063	0.21	PCB-189	0.040

Table 1293.8b1 PCB Stock Solution Concentrations 183 µg/mL

FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd		FILE=C:\QPRO4\QC\LMMBPCB1.WQ1 21-Jun-94 2 Sig.Fig. 15:01 LMMB CALCULATED AVERAGE Calc'd	
Peak Name	**Congener Conc'ns µg/mL**	**Peak Name**	**Congener Conc'ns µg/mL**
PCB-064	1.8	PCB-191	0.12
PCB-066	5.2	PCB-193	0.42
PCB-070+076 (SUM)	3.4	PCB-194	1.8
PCB-074	1.9	PCB-197	0.11
PCB-077	0.23	PCB-198	0.12
PCB-081	0.16	PCB-199	0.43
PCB-082	0.44	PCB-201	4.2
PCB-083	0.15	PCB-202+171 (AVE)	0.79
PCB-085	0.70	PCB-203+196 (SUM)	4.3
PCB-087	1.0	PCB-205	0.11
PCB-089	0.10	PCB-206	0.68
PCB-091	0.51	PCB-207	0.093
PCB-092+084 (SUM)	1.8	PCB-208+195 (SUM)	0.80
PCB-095	2.0	PCB-209	0.012

Table 1293.8b2 PCB Congener Calibration Concentrations - DB-5 Column

CAL #	Name	Amount ng/mL	CAL #	Name	Amount ng/mL
1	#1	3.6000E+01	42	#83	4.5000E-01
2	#3	2.1000E+01	43	#97	1.6800E+00
3	#4/10	1.0200E+01	44	#87	3.0000E+00
4	#7/9	3.6000E+00	45	#85	2.1000E+00
5	#6	5.7000E+00	46	#136	2.2500E+00
6	#8/5	4.2000E+01	47	#77/110	6.4000E+00
7	#14	3.1600E+01	48	#82	1.3200E+00
8	#19	8.4000E-01	49	#151	5.1000E+00
9	ISTD1#30	1.4200E+01	50	#135/144	2.6700E+00
10	#18	1.1100E+01	51	#123/149	8.4000E+00
11	#15/17	1.1100E+01	52	#118	3.6000E+00
12	#24/27	7.8000E-01	53	#146	1.1700E+00
13	#16/32	1.1700E+01	54	#132/153/105	1.2900E+01
14	#26	2.1600E+00	55	#141	5.1000E+00
15	#25	9.6000E-01	56	#137/176	7.8000E-01

Table 1293.8b2 PCB Congener Calibration Concentrations - DB-5 Column

CAL #	Name	Amount ng/mL	CAL #	Name	Amount ng/mL
16	#28/31	2.8200E+01	57	#163/138	8.1000E+00
17	#33	9.9000E+00	58	#158	7.5000E-01
18	#53	1.9200E+00	59	#178	3.3000E+00
19	#51	5.4000E-01	60	#166	8.4000E+00
20	#22	8.7000E+00	61	#187/182	1.0800E+01
21	#45	2.6700E+00	62	#183	5.100E+00
22	#46	1.2000E+00	63	#128	4.3000E+00
23	#52	1.3500E+01	64	#167	1.9000E+00
24	#49	6.9000E+00	65	#185	1.4000E+00
25	#47/48	6.0000E+00	66	#174	9.6000E+00
26	#65	7.8000E+00	67	#177	5.1000E+00
27	#44	1.2900E+01	68	#202/171	2.3700E+00
28	#37/42	7.800E+00	69	#157/200	1.1700E+00
29	#41/71/64	1.2300E+01	70	#ISTD 2 #204	1.5600E+01
30	#40	2.8000E+00	71	#172	1.6800E+00
31	#63	6.3000E-01	72	#180	1.8300E+01
32	#74	5.7000E+00	73	#193	1.2600E+00
33	#70/76	1.0200E+01	74	#199	1.3000E+00
34	#66	1.5600E+01	75	#170/190	5.1000E+00
35	#95	6.0000E+00	76	#198	3.6000E-01
36	#91	1.5300E+00	77	#201	1.3000E+01
37	#56/60	1.0500E+01	78	#203/196	1.3000E+01
38	#92/84	5.4000E+00	79	#208/195	2.4000E+00
39	#89	3.0000E-01	80	#207	2.8000E-01
40	#101	5.4000E+00	81	#194	5.4000E+00
41	#99	2.2200E+00	82	#206	2.0000E+00

Table 1293.8h1 PCB Congener Calibration Concentrations - DB-1 Column

CAL #	Name	Amount ng/mL	CAL #	Name	Amount ng/mL
1	#1	3.6000E+01	44	#97	1.7000E+00
2	#3	2.1000E+01	45	#87	3.0000E+00
3	#4/10	1.0000E+01	46	#85	2.1000E+00
4	#7/9	3.6000E+00	47	#136	2.3000E+00
5	#6	5.7000E+00	48	#110	5.7000E+00
6	#8/5	4.2000E+01	49	#82	1.3000E+00
7	#14	3.1600E+01	50	#151	5.1000E+00

Table 1293.8h1 PCB Congener Calibration Concentrations - DB-1 Column

CAL #	Name	Amount ng/mL	CAL #	Name	Amount ng/mL
8	ISTD1 #30	1.4200E+01	51	#135	8.4000E-01
9	#18	1.1000E+01	52	#144	1.8000E+00
10	#15/17	1.1000E+01	53	#123/149/118	1.2000E+01
11	#24/27	7.8000E-01	54	#105/146/132	5.3000E+00
12	#16	6.0000E+00	56	#153	8.1000E+00
13	#32	5.7000E+00	57	#141	5.1000E+00
14	#26	2.2000E+00	58	#137/130	1.0000E+00
15	#25	9.6000E-01	59	#138/168	8.1000E+00
16	#31	1.4000E+01	60	#158	7.5000E-01
17	#28	1.3000E+01	61	#166	8.4000E+00
18	#33/53	1.1800E+00	62	#178	3.3000E+00
20	#22	8.7000E+00	64	#182/187/128	1.5000E+01
21	#45	2.7000E+00	65	#183	5.1000E+00
22	#46	1.2000E+00	66	#167	1.9000E+00
23	#52	1.3500E+01	67	#185	1.4000E+00
24	#49	6.9000E+00	68	#174	9.6000E+00
25	#48	3.0000E+00	69	#177	5.1000E+00
26	#47	3.0000E+00	70	#171/156	1.5000E+00
27	#65	7.8000E+00	71	#173	1.1000E-01
28	#44	1.3000E+01	72	#200	1.5000E+00
29	#42	4.2000E+00	73	#ISTD 2 #204	1.5600E+01
30	#41/71/64	1.2300E+01	74	#172	1.7000E+00
31	#40	2.8000E+00	76	#180	1.8000E+01
32	#63	6.3000E-01	77	#193	1.3000E+00
33	#74	5.7000E+00	78	#199	1.3000E+00
34	#70/76	1.0000E+01	79	#170	3.6000E+00
35	#66	1.6000E+01	80	#190	1.4000E+00
36	#95	6.0000E+00	81	#198	3.6000E-01
37	#91	1.5000E+00	82	#201	1.3000E+01
38	#56/60	1.0000E+01	83	#203/196	1.3000E+01
39	#84	3.9000E+00	84	#195	2.0000E+00
40	#89	3.0000E-01	85	#207	2.8000E-01
41	#101	5.4000E+00	86	#194	5.4000E+00
42	#99	2.2000E+00	88	#206	2.0000E+00
43	#83	4.5000E-01			

Lake Michigan Tributary Study Measurement Quality Objectives

QC Description	PCBs QC Objective	Trans-Nonachlor QC Objective
Holding Time & Storage To extraction After Extraction Storage Conditions	 NA NA 4° C.	 NA NA 4° C.
Sample Set	≤8	≤8
Reporting Units	ng/L Dissolved and Particulate	ng/L Dissolved and Particulate
XAD Cleanliness Check Frequency Criteria	 1 per resin batch <MDL	 1 per resin batch <MDL
Method Detection Limit (MDL) Frequency Criteria Source	 1/year +3sd, n=7 Ultra 10 mix	 1/year +3sd, n=7
System Detection Limit Frequency Criteria	 EPA to calculate	 EPA to calculate
Initial Calibration Levels Frequency Criteria Source	3 point 1/year RF RSD<25% Mullin (1994)	3 point 1/year RF RSD<25%
Continuing Calibration Frequency Criteria Source	1 point daily #101,185,44,180<25% #6,198<50% Mullin (1994)	1 point daily ±20%

Lake Michigan Tributary Study Measurement Quality Objectives

QC Description	PCBs QC Objective	Trans-Nonachlor QC Objective
Blanks		
Field Blanks (FRB)		
Frequency	1:20	1:20
Criteria	<MDL x 3.3	<MDL x 3.3
Lab Reagent Blank (LRB)		
Frequency	1 per set	1 per set
Criteria	<MDL	<MDL
Trip Blank (FTB)		
Frequency	1:20	1:20
Criteria	<MDL	<MDL
Lab Dry/Procedural Blank (LDB)		
Frequency	1:20	1:20
Criteria	<MDL	<MDL
Performance Standard (LPC)	Mullin (1994)	
Frequency	every 12 hours	daily
Criteria	#101,185,44,180<25% #6,198<50%	±20%
Surrogate Standards (LSS)	#14,65,166	None to date
Frequency	all samples	
Criteria	50-130%	
Matrix Spike Standards (LMS)		
Frequency	1/set	1/set
Criteria	mean of all congeners: 50-125%	50-120%
Source	Mullin (1994)	
Internal Standards	#30, 204	#30
Frequency	every sample	every sample
Criteria	none	none
Duplicates Field (FDI)		
Frequency	1:20	1:20
Criteria	RPD<100% for conc <5xMDL RPD<50% for conc>5xMDL	RPD<100% for conc<5x MDL RPD<50% for conc>5x MDL
Confirmation (CON)	5% by DB-1 GC/EC	5% by DB-5 GC/EC

Extraction and Cleanup of Sediments for Semivolatile Organics Following the Internal Standard Method

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Standard Operating Procedure GLERL - M - 401 - 01

May 10, 1996

Version 1.0

Extraction and Cleanup of Sediments for Semivolatile Organics Following the Internal Standard Method

1.0 Scope and Application

This SOP is applicable to the extraction of semivolatile organic compounds from sediment matrices for analysis by gas chromatography (GC). Samples processed using this extraction method can be analyzed for semivolatile organic compounds listed in EPA Methods 608, 610, or 625, 8080, 8270, 8310 (EPA 1984, 1986). This method may be applied to other analytes once acceptable extraction efficiency has been demonstrated.

In this procedure, approximately 15-30 g wet weight sediment is extracted with dichloromethane (DCM) in a 30°C sonication bath. The extracts are dried over sodium sulfate and passed through a cleanup column. Column chromatography fractionation allows for separation of pesticides (PESs) from the majority of polychlorinated biphenyls (PCBs). Polyaromatic hydrocarbons (PAHs) elute along with the PESs. After a concentration step, internal standards are added and the sample is ready for analysis.

2.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory; 3) defending the data in a Quality Assurance Audit; and 4) reporting results to the client.

Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of target compound concentrations; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using the procedure. Responsible for 1) understanding the proper handling of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality control samples for each batch of samples analyzed; 4) reporting results to the Project Manager; 5) participating in QA Audits.

3.0 Procedure

3.1 Apparatus and Reagents

3.1.1 Branson model 8210 ultrasonic cleaner with variable temperature water bath

3.1.2 Nitrogen evaporator, N-Evap or equivalent, heated with a water bath maintained at 35°±1°C

3.1.3 Rotary evaporator heated with a water bath maintained at 30°±1°C

3.1.4 Glassware

3.1.4.1 250 mL Erlenmeyer flasks

3.1.4.2 100 mL graduated cylinders

3.1.4.3 1000 mL separatory funnels

3.1.4.4 50 mL, 150 mL, and 250 mL beakers

3.1.4.5 Glass rod, 20" long

3.1.4.6 500 mL round bottom flasks

3.1.5 Apparatus for determining sample wet and dry weight

3.1.5.1 Top-loading balance, accurate to 0.001 grams

3.1.5.2 Drying oven maintained at 90°C

3.1.5.3 Aluminum weighing pans

3.1.5.4 Stainless steel spatulas

3.1.6 Long-tipped Pasteur pipettes (9.5" long)

3.1.7 Wide-bore Pasteur pipettes

3.1.8 Chromatography columns, 310 x 11 mm, with Teflon stopcocks

3.1.9 Drying oven maintained at 130°C

3.1.10 Desiccator

3.1.11 Muffle furnace

3.1.12 Sample vials, 2 mL capacity

3.1.13 Purified water (Millipore filtration system)

3.1.14 Saturated sodium chloride solution

3.1.15 Microliter syringes with replaceable needles

3.1.16 Glass wool, cleaned by soxhlet extraction using DCM

3.1.17 Sodium sulfate, anhydrous (Mallinkrodt-AR), heated to 450°C for at least 10 hrs, stored in 130°C oven

3.1.18 Silica gel (Aldrich grade 634, 100 - 200 mesh, 60 Å, 99+%), stored in a 130°C oven

3.1.19 Aluminum foil

3.1.20 Solvents: pesticide grade or equivalent

3.1.20.1 Acetone (DMK)

3.1.20.2 Methylene chloride (DCM)

3.1.20.3 Hexane

3.1.20.4 Diethyl Ether

3.1.21 Copper, activated by treating with concentrated HCl and washing with purified water twice, methanol twice, methylene chloride twice, and hexane twice, then storing in hexane.

3.1.22 Ring weights for securing flasks in water bath

3.2 Standards

3.2.1 PAH surrogate standard - containing 2-4 mg/mL each of naphthalene-d_8, anthracene-d_{10}, benz(a)anthracene-d_{12}, benzo(e)pyrene-d_{12}, and benzo(ghi)perylene-d_{12} in DMK.

3.2.2 PAH internal standard - containing 20 mg/mL each of acenapthylene-d_{10}, fluorene-d_{10}, chrysene-d_{12}, and perylene-d_{12} in hexane.

3.2.3 PCB surrogate standard - containing 400 ng/mL of PCB 14, 100 ng/mL PCB 65, and 80 ng/mL of PCB 166 in DMK.

3.2.4 PCB internal standard - containing 170 ng/mL of PCB 30, and 130 ng/mL of PCB 204 in hexane.

3.2.5 Matrix spiking standard - containing 2 mg/mL NIST SRM 1491 PAH standard mixture, 730 ng/mL total PCBs (Mullin's 1994 Aroclor mix), 660 ng/mL trans nonachlor, and 800 ng/mL of other individual pesticides in DMK.

3.3 Sample Handling

Samples shall be kept frozen at approximately -15°C until extraction, unless specified differently on a project-specific basis. All extracts held overnight shall be covered and refrigerated at 4°C. Extracts shall be saved and stored at 4°C in case re-analysis is required.

3.4 Labware Preparation

Prior to use, all glassware, Teflon, and other labware should be washed with hot, soapy water and rinsed with tap water, followed by purified water. Additionally, all non-volumetric glassware should be combusted in a muffle furnace @ 450°C for at least 16 hours. All volumetric glassware (i.e., graduated cylinders) should be solvent rinsed twice prior to use.

3.5 Dry Weight Determination

Dry-to-wet weight ratios of sediment samples should be analyzed in triplicate at the time of sediment sample extractions. Aluminum weighing pans are weighed, a sample of wet sediment is added, and the pans are weighed again. All samples are dried in the 90°C oven overnight or until a constant weight is obtained (no change in weight for a period of 3 minutes). Dry-to-wet weight ratios are determined as follows:

$$\frac{\text{Dry Wt}}{\text{Wet Wt}} = \frac{(\text{g dry sample} + \text{pan}) - (\text{g pan})}{(\text{g wet sample} + \text{pan}) - (\text{g pan})}$$

The dry weight of the sample to be extracted is determined as follows:

Sample Dry Wt = Sample Wet Wt x Mean Dry-to-Wet Wt Ratio

Information for dry weight determinations shall be recorded in the Sediment Extraction Data Sheet (Attachment 1) for the individual samples. The following criteria for the coefficient of variation for four measurements were used: if dry/wet ratio ≤ 20%, then c.v. < 25%
if dry/wet ratio > 20%, then c.v. < 15%.

3.6 Sample Extraction

Weigh the appropriate amount of well-mixed wet sediment onto a piece of tared alumina foil to the nearest 0.01 g. Fifteen to 30 g wet weight sediment shall be weighed out when trace (nanogram per gram dry weight) quantities of contaminants are expected. Mix sediment with appropriate amount of sodium sulfate and then scoop mixture into a 250 mL Erlenmeyer flask. One hundred microliters of the PAH surrogate standard and 62 microliters of the PCB surrogate standard are pipetted directly onto the sediment sample. Sample weights and standard volumes shall be recorded in the Sediment Extraction Data Sheet (Attachment 1).

Add 150 mL DCM to the flask, mixing thoroughly. Place ring weight around flask, and cover opening with foil. Place all batch samples in 30°C ultrasonic bath and sonicate for 60 minutes. After sonication, let stand in 30°C bath overnight (24 hours). The next day, sonicate samples again for 60 minutes at 30°C. Remove flasks from bath.

Set up filtration column as follows: plug the inside of a wide-bore Pasteur pipette with a piece of clean glass wool (3 cm in length). Clamp pipette filter above a 500 mL round bottom flask. Filter solvent phase from the Erlenmeyer flask through the glass wool via transfer Pasteur pipette.

3.7 Extract Evaporation

Reduce solvent in the flask to 15 mL using the rotary evaporator apparatus. Transfer the extract to a conical centrifuge tube and further reduce solvent to approximately 1 mL using the nitrogen evaporator apparatus. Exchange solvent by adding 5 mL hexane, then evaporate to approximately 1 mL and repeat this step twice more. Transfer extract from the centrifuge tube to a pre-combusted 2 mL sample vial, rinse with 1 mL hexane, add to vial, and cap securely.

3.8 Pre-Column Preparation and Extract Clean-up

3.8.1 Preparation of 3% deactivated silica gel and 10% deactivated alumina

Day 1
Place silica in a 130°C oven overnight (at least 18 hrs). Place alumina in a shallow ceramic dish and activate it in a muffle furnace @ 450°C for at least 16 hours.

Day 2
Remove silica and alumina from oven and let cool on counter top until room temperature is reached (approximately 5 - 10 minutes). When silica and alumina have reached ambient temperature, deactivate it as follows:

--Working quickly, weigh out desired amount of silica and alumina in separate round-bottom flasks. Stopper immediately.

--Add 3% and 10% weight/volume of deionized water to silica and alumina respectively, using the following equation:

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

--Shake for 10 minutes. Store in desiccator overnight for equilibration. Use deactivated silica and alumina within three days. Any unused silica and alumina may be reused after re-activating and re-deactivating.

3.8.2 Preparation of Sodium Sulfate

Pour aliquot of sodium sulfate into a shallow ceramic dish. Heat at 450°C for 16 hrs. Cool, then store in 130°C drying oven until ready for use.

3.8.3 Column Preparation

Assemble stopcock on column. Stuff glass wool plug (approximately 1 cm) into lower end of the column with a glass rod. Clamp column securely onto stand. Place empty 150 mL beaker under column. Close stopcock; fill column half full with hexane. Make a slurry of hexane and 3-grams deactivated silica gel. Open stopcock to partially drain excess hexane dislodging any trapped bubbles in glass wool plug.

Pour slurry into column tapping column gently with a glass rod or spatula. Rinse column and beaker with hexane via Pasteur pipette. Make a slurry of hexane and 10 grams of

deactivated alumina. Pour slurry into column. Cap column with 2 inches sodium sulfate. Wash column with 25 mL hexane for equilibration. When hexane level reaches 1 cm above top of sodium sulfate, close stopcock to prevent further dripping. Never let column run dry. If column is not going to be used immediately, cover column and tip with foil.

3.8.4 Extract Clean-Up

First fraction: PCBs, HCB, 4,4'-DDE, aldrin, and heptachlor

Sonicate sample for 10 seconds before loading onto column. Open stopcock and let drip until hexane level is at the top of the sodium sulfate. Place a conical centrifuge tube under the column to catch eluate of first fraction. Measure 35 mL of hexane into a graduated cylinder. Load sample onto column via Pasteur pipette. Open stopcock and let sample flow to just the top of the sodium sulfate. Rinse sample vial with approximately 5-mL hexane in the cylinder and load onto column. Set drip rate to approximately 2 drops per second. When rinse reaches the top of the sodium sulfate, add the rest of hexane to the column.

Second fraction: Alpha-BHC, gamma-BHC, heptachlor epoxide, alpha-chlordane, gamma-chlordane, trans-nonachlor, dieldrin, cis-nonachlor, 4,4'-DDT, 4,4'-DDD and all PAHs.

While the hexane is dripping from the first fraction, measure 50 mL of 10% diethyl ether in hexane into the graduated cylinder. When the hexane level from the first fraction reaches the top of the sodium sulfate layer, add the 10% solution to the column. Replace conical centrifuge tube with another for collection of the second fraction. Once the column has stopped dripping, remove the tube and evaporate the solvent.

3.8.5 Evaporation of First and Second Solvent Fractions and Sample Preparation

Place the centrifuge tubes from the first and second fractions in the nitrogen evaporator water bath. Reduce solvent in the flasks to 1 mL using the nitrogen evaporator apparatus. The second fraction requires solvent exchange into hexane by adding 5 mL hexane. Rinse sample tubes with a small amount of hexane and transfer to sample vials, keeping volume of solvent less than 1 mL. Add the appropriate amount of internal standards. Record the amount and ID of the IS in the Sediment and Tissue Extraction Data Sheet. The first fraction requires sulfur cleanup by adding activated copper beads. Add more if all the copper beads in sample vial turn black. Cap sample vials, mix, and store at -15°C (first fraction samples) or at 4°C (second fraction samples) until gas chromatography analysis

4.0 Quality Control Sample Frequency

Samples prepared using this procedure should be processed in batches of approximately 10 samples plus quality control samples.

Laboratory Reagent Blank (LRB) - analyze one per batch Prepare by working through the sample procedure without a sample matrix, but including Na_2SO_4, extraction solvents, and appropriate surrogates and internal standards.

Laboratory Matrix Spike (LMS) - analyze one per batch. Prepare by fortifying a matrix known to be below detection limits for an analyte(s) (e.g. pre-industrial sediment) with known amount of surrogates, target analyte(s), and internal standards.

Laboratory Environmental Matrix Blank (MSB) - analyze less frequently, once or twice per field trip. Prepare by working a matrix known to be below detection limits for an analyte(s) through the sample procedure including surrogate and internal standard additions.

Laboratory Duplicate (LD1) - analyze one per batch. Prepare by splitting a sample and treating identically throughout the analytical procedure.

Laboratory Performance Check (LPC) - run one per batch. A calibration solution used to verify whether the initial calibration data are currently valid.

Field Duplicate (FD1) - analyze one per batch if possible, otherwise as often as logistics allow.

Field Reagent Blank (FRB) - collect one per field trip. Collect sediment of pre-industrial age for subcores from several sites (~ 7). This "clean" older sediment will then be run through the entire collection and analytical systems just as routine field samples.

Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection

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Standard Operating Procedure GLERL - M - 501 - 02

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Version 2.0

Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection

1.0 Scope and Application

This SOP is applicable to the analysis of environmental sample extracts for polychlorinated biphenyls (PCBs) as Aroclors and individual congeners and chlorinated pesticides by capillary gas chromatography with ^{63}Ni electron-capture detection.

This procedure provides typical gas chromatography (GC) conditions for the detection of trace levels of PCBs and pesticides, methods for identifying the analytes, and methods for analyte quantification using the internal standard method. Tables 1 and 2 list the most frequently analyzed compounds and formulations. However, this list may be amended to meet requirements of specific projects.

2.0 Definitions

The following terms and acronyms may be associated with this procedure:

ECD	Electron capture detector or detection
GC	Gas chromatography
PCB	Polychlorinated biphenyl
RF	Response factor
RRF	Relative response factor; response factor of analyte normalized to the response factor of the internal standard.
RSD	Relative standard deviation (%)
RT	Retention time
IS	Internal standard - compound(s) added just prior to analysis on instrument.
SS	Surrogate standard - Compound(s) added prior to extraction to assess efficiency of method.

3.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory; 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.

Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of the GC. Responsible for 1) ensuring that analysts are trained in operation of the GC; 2) appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit, and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper use and maintenance of the GC; 2) recording information regarding instrument use and maintenance in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) tabulating all sample and QC data and reviewing the quality of the data based on QC guidelines presented in this SOP and any other project-specific QC guidelines; 5) reporting results to the Project Manager; and 6) defending the data during an audit.

Table 1. PCB and Chlorinated Pesticide Analyte List

PCBs (Aroclors)	**Suggested Internal Standards**
Aroclor 1232	PCB-030
Aroclor 1248	PCB-204
Aroclor 1262	
Aldrin	**Suggested Surrogate Standards**
alpha-BHC	PCB-014
beta-BHC	PCB-065
gamma-BHC (Lindane)	PCB-166
delta-BHC	PBB-153
4,4'-DDE	
4,4'-DDD	
4,4'-DDT	
(cis)alpha-Chlordane	
(trans)gamma-Chlordane	
Tech. Chlordane	
Dieldrin	
Endosufan I	
Endosufan II	
Endrin	
Endrin Aldehyde	
Endrin ketone	
Heptachlor	
Heptachlor Epoxide	
Endosulfan Sulfate	
Hexachlorobenzene	
Mirex	
Trans-Nonachlor	
Cis-nonachlor	

Table 2. PCB Congener List

CB Number[a]	CAS Nomenclature[b]	CAS Registry Number[b]
8	2,4'-dichlorobiphenyl	34883-43-7
18	2,2',5-trichlorobiphenyl	37680-65-2
28	2,4,4'-trichlorobiphenyl	7012-37-5
29	2,4,5-trichlorobiphenyl	15862-07-4
44	2,3',3,5'-tetrachlorobiphenyl	41464-29-5
49	2,2',4,5'-tetrachlorobiphenyl	41464-40-8
50	2,2',4,6-tetrachlorobiphenyl	62796-65-8
52	2,2',5,5'-tetrachlorobiphenyl	35693-99-3
66	2,3',4,4'-tetrachlorobiphenyl	32598-10-0
77	3,3',4,4'-tetrachlorobiphenyl	32598-13-3
87	2,2',3,4,5'-pentachlorobiphenyl	38380-02-8
101	2,2',4,5,5'-pentachlorobiphenyl	37680-73-2
104	2,2',4,6,6'-pentachlorobiphenyl	56558-16-8
105	2,3,3',4,4'-pentachlorobiphenyl	32598-14-4
118	2,3',4,4',5-pentachlorobiphenyl	31508-00-6
126	3,3',4,4',5-pentachlorobiphenyl	57465-28-8
128	2,2',3,3',4,4'-hexachlorobiphenyl	38380-07-3
138	2,2',3',4,4',5-hexachlorobiphenyl	35065-28-2
153	2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1
154	2,2',4,4',5,6'-hexachlorobiphenyl	60145-55-4
170	2,2',3,3',4,4',5-heptachlorobiphenyl	35065-30-6
180	2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-3
183	2,2',3,4,4',5',6-heptachlorobiphenyl	52663-69-1
184	2,2',3,4,4',6,6'-heptachlorobiphenyl	74472-48-3
187	2,2',3,4',5,5',6-heptachlorobiphenyl	52663-68-0
188	2,2',3,4',5,6,6'-heptachlorobiphenyl	74487-85-7
195	2,2',3,3',4,4',5,6-octachlorobiphenyl	52663-78-2
200[c]	2,2',3,3',4,5',6,6'-octachlorobiphenyl	40186-71-8
206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	40186-72-9
209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	2051-24-3

[a] Ballschmiter and Zell numbering scheme.

[b] Chemical Abstracts, Tenth Collective Inex, Index Guide, American Chemical Society, Columbus, Ohio, 1982.

[c] CB 200 in the Ballschmiter and Zell numbering scheme.

Table 3. Suggested Instrument Conditions for PCB and Chlorinated Pesticide Analysis

Injection port temperature	250°C
Detector temperature	325°C
Initital temperature	100°C
Initial hold	0 min
Ramp 1 rate	1°C/min to 265°C
Ramp 2 rate	20°C/min to 300°C
Final hold	0 min
Carrier gas flow (linear velocity)	45 cm/sec
Makeup gas flow	40 mL/min
Purge Vent	3 mL/min
Split vent	60 mL/min
Purge on after 1 min	

4.0 Procedures

4.1 GC Preparation

The GC is typically fitted with one column; a DB-5 60-m x 0.25 mm (i.d.) fused silica capillary column with a 0.1-μm film thickness (J&W Scientific, Inc.). Suggested instrumental conditions are listed in Table 3. Other columns or GC conditions may be specified in individual project plans.

4.2 Sample Collection, Preservation, and Handling

To conduct this analysis, the analyst should receive the samples as solvent extracts reduced to an appropriate volume. All organic extracts are normally analyzed within 40 days from extraction. Refer to project-specific plans or protocols for sample collection, preservation, and handling methods. If holding times have been exceeded, the Project manager should be notified immediately.

4.3 Sample Specifications

Sample preparation methods may vary depending on the sample matrix and project needs; refer to project-specific protocols. Samples and standards for analysis using this SOP should be prepared in hexane unless otherwise specified. Methylene chloride injected into the GC/ECD system should be limited, as it may damage the detector. Unless otherwise specified, standard and sample aliquots of 2-μL volumes will be injected.

4.4 Analyte Identification

Prior to sample analysis, the elution order of the analytes of interest must be determined by analyzing the analytes individually or in combination with other analytes having known or predetermined retention times.

4.5 Instrument Calibration

Before the sample is injected into the GC, the detector must be calibrated to determine the response of the detector to the analytes of interest. Demonstration of linearity of detector response is required before sample analysis. Calibration checks must be analyzed at a minimum frequency of once every 10 samples during sample analysis.

4.5.1 Initial Calibration

The initial calibration consists of the analysis of a minimum of five calibration solutions, each at different concentrations that span the expected concentration range of the samples. These standards include the analytes of interest as well as the appropriate surrogates (SS) and internal standards (IS). The concentration of the LOW standard should be approximately 2-5 times the detection limit of the instrument. The MID-range calibration standard should be near the expected concentration of the samples. The HIGH concentration standard should be approximately 4-10 times the concentration of the MID standard, or high enough to span the expected concentration range. The range of concentrations of these standards are as follows:

Pesticides	PCB Congener Mixture
1.0 - 100 ng/mL	1.0 -100 ng/mL

The concentrations of IS and SS should be the same in all calibration solutions and in the same concentration range as they are spiked in the samples, typically at a concentration 2 to 5 times below the highest calibration standard. Initial calibration standards must be analyzed prior to initiating sample analyses. An initial calibration should also be run if any GC conditions have changed. If GC conditions have not changed since the previous initial calibration, a continuing calibration standard may be analyzed and if it falls within acceptable criteria, the previous initial calibration may be used.

4.5.2 Continuing Calibration

The upper mid-level calibration solution is analyzed as a calibration check minimally every 10 samples while samples are being analyzed. All sample analyses must be bracketed by two calibration check standards that meet calibration criteria (see acceptance criteria in section 4.5.4.3, Relative Response Factors).

4.5.3 Calibration for Analysis of Aroclors and Multi-Peak Pesticides

A multilevel calibration is analyzed when samples are to be quantified for multicomponent mixtures such as Aroclors, or toxaphene. Calibration solutions are analyzed minimally at the beginning of each analysis run or sample batch unless otherwise noted in project protocols.

4.5.4 Relative Response Factors

The relative response factor (RRF) of each analyte is calculated as follows:

$$RRF_A = (H_A) \times (C_{IS})/(H_{IS}) \times (C_A)$$

where: H_A = *Analyte Peak Height*
H_{IS} = *Internal Standard Peak Height*
C_{IS} = *Concentration of Internal Standard*
C_A = *Concentration of Analyte*

4.5.4.1 Initial Calibration Response Factors

Individual Relative Response factors are generated for each analyte at each calibration level. A weighted average RRF (X_{RRF}) and correlation coefficient are calculated from the linear regression of the ratio of responses (H_A/H_{IS}) versus the ratio of amounts (C_A/C_{IS}) for a multipoint calibration. The correlation coefficient must be >0.95 for each individual analyte unless otherwise specified in project plans. If any correlation coefficient does not meet the acceptable criteria, the initial calibration must be repeated and all samples associated with that calibration re-run (unless otherwise specified in a specific project plan and/or documented by the Project Manager).

4.5.4.2 Response Factors for Six Mixture Components

For multi-peak analytes such as polychlorinated biphenyls, up to 110 significant peaks or specific predetermined components are chosen. An RRF is calculated for each peak as described above. A similar method is used for quantifying the technical mixture of chlordane. The acceptance criteria for the multicomponent mixture RRF linear regression is the same as stated for single peak components (r^2 >0.95).

4.5.4.3 Continuing Calibration RFs

Continuing calibration checks are considered acceptable if the % difference between the concentration of the analyte and the known calibration concentration is less than 25% for four selected medium to large peaks and less than 50% for two selected small peaks. If the newly generated concentrations are acceptable, the initial calibration is still valid and sample analysis may continue. If the percent difference exceeds the acceptable criteria, remedial action should be taken and the continuing calibration check solution should be reanalyzed. If the calibration fails again, the analyses should be terminated, remedial action taken, a new initial calibration should be performed, and the affected samples reanalyzed. The percent difference is calculated as follows:

$$\%\ difference = (C_{vi} - C_s)/C_{vi} \times 100\%$$

where: C_{vi} = *Concentration of the analyte from initial calibration*
C_s = *Concentration of the analyte from continuing calibration check*

4.6 Evaluation of DDT and Endrin Degradation

DDT and endrin are easily degraded in the injection port, if the injection port or front of the column is contaminated with buildup of high boiling residue from sample injection. Check for degradation problems by injecting a mid-concentration standard containing only 4,4'-DDT and endrin prior to sample analyses. Look for the degradation products of 4,4'-DDT (4,4'-DDE and 4,4'-DDD) and endrin (endrin ketone and endrin aldehyde).

If degradation of either DDT or endrin exceeds 20% or the combined breakdown of DDT and endrin is greater than 20%, then take corrective action before proceeding with calibration. Corrective action includes cleaning and deactivating the injection port, breaking off at least 0.5 m of column and remounting it. Lowering the injection port temperature may also be an option. This should be determined by the laboratory Supervisor. Calculate percent breakdown as follows:

$$\text{\% breakdown for 4,4'-DDT} = \frac{\text{Response (peak height) (DDE + DDD) x 100}}{\text{Response (peak height) DDT injected}}$$

$$\text{\% breakdown for endrin} = \frac{\text{Response (peak height) endrin aldehyde + endrin ketone x 100}}{\text{Response (peak height) endrin}}$$

Combined % breakdown = % breakdown DDT + % breakdown endrin

4.7 Sample Analysis Procedure

Samples are analyzed under the same analytical conditions as the calibration standards. Samples must be bracketed by acceptable calibrations. Criteria for accepting peaks as analytes of interest are explained in Sections 4.7.1 through 4.7.2.

4.7.1 Relative Retention Time

Retention time (RT) windows for each analyte may be determined daily or by batch of samples. Relative retention time (RRT) for each analyte shall be determined from the ratio of the RT of the analyte and the RT of a time reference compound, usually the internal standard for a designated time interval. RRT for a particular analyte shall be within 1% of RRT determined during initial calibration for a peak to be identified.

4.7.2 Minimum Height

Peaks with a signal-to-noise ratio of three or less should be regarded as not detected unless otherwise noted in a specific project plan and/or documented by project management.

5.0 Data Analysis and Reporting

5.1 Data Recording

Data quantification and calculations will be performed on personal computers using commercial spreadsheet software such as *HP CHEM* version A03.01 and *Microsoft Excel*. All transfers of data to forms and data reductions (e.g., concentration calculations, means, standard deviations) will be checked by the analyst and approved by the Laboratory Supervisor. Hard copies of GC printouts of calibrations and sample data and spreadsheet reports will be kept in the GC/ECD files. A copy of the summary sheets and extraction logs will be placed in the appropriate project file in the Laboratory Supervisor's Central Files. Hard copies of chromatograms from each sample and all calibrations will be kept in the GC/ECD files unless otherwise noted in a specific project plan

5.2 Sample Quantification

The internal standard method is used to quantify PCBs and chlorinated pesticides in environmental samples. The internal standards added to the samples prior to GC analysis are the basis for sample quantification.

5.2.1 Single-Peak Analytes

The concentration of a specific analyte in a sample is calculated as follows:

$$\text{Concentration (ng/sample amt)} = [H_A \times Amt_{IS} / [H_{IS} \times X_{RRF}]]/\text{Sample Amt}$$

where: H_A = *Peak Height of analyte in sample*
H_{IS} = *Peak Height of IS*
X_{RRF} = *Relative response factor of the analyte based on the linear regression of the initial calibration*
Amt_{IS} = *Amount of the IS added (ng)*
Sample Amt = g (sediment) or L (water)

5.2.2 Multicomponent Analytes

The same calculation as above is used for quantifying multi-peak analytes.

Multicomponent analyses are performed as follows:

Quantification

1. Analyze samples as described previously.
2. Using RRF identify the peaks of interest in the samples.
3. Calculate concentration for each identified peak in the mixture as described above (Section 5.2.1).

5.3 Dual Column Confirmation Data

Data from the confirmation column is treated exactly the same as that obtained from the primary column as described above. QA criteria outlined in Section 4.0 also applies. Quantitative comparison of the values obtained from both columns should be performed for the single peak chlorinated pesticides. In the absence of interferences, values obtained from each column should be within approximately a factor of two of each other to be considered acceptable. If the criteria are not met, the value from the primary column is reported with a "G" flag. This criteria is only a guideline and should not be applied as an absolute rule, especially as concentration values approach detection limits. The value reported should be that obtained from the primary column unless chromatographic interferences would indicate that a more accurate value has been obtained from the confirmation column.

In addition, a number of compounds may co-elute when multi-component mixtures are present in the samples. The quantitative confirmation/comparison guidelines cannot be strictly adhered to in these cases. Likewise, if chromatographic interferences (i.e., poor peak shape, baseline drift etc.) are present on one of the columns this quantitative comparison is not required. These decisions are based in a large part on the judgement of the analyst and any decisions made in regards to this should be noted on the analytical reports and on copies of the chromatograms.

5.4 Surrogate and Matrix Spike Recovery Calculations

Calculation of Surrogate recovery is as follows:

$$\% \text{ Surrogate Recovery} = Q_d / Q_a \times 100$$

Q_d = *Quantity determined by analysis*
Q_a = *Quantity added*

The matrix spike recovery is determined as follows:

$$\text{Matrix Spike Recovery} = (SSR/SR) \times 100/SA$$

SSR = Spike sample result
SR = Fraction surrogate recovery
SA = Spike added

Note that the matrix spike recovery is a surrogate corrected calculation.

The Relative Percent Difference (RPD) between spike and spike duplicates is calculated as follows:

$$RPD = \frac{|MSR - MSDR| \times 100}{\frac{1}{2}(MSR + MSDR)}$$

MSR = Matrix Spike % Recovery
MSDR = Matrix Spike Duplicate % Recovery

The following recovery criteria apply:

Surrogate Recoveries -	50 to 130%
Spike Recoveries -	50 to 130%
MS/MSD RPD -	≤30%

These values cover the recoveries suggested by the EPA CLP These recovery limits are only guidelines. Data exceeding these values should be flagged as described below, however, no corrective action is advised. Frequent failure to meet the limits warrant investigation by the laboratory. Specific actions or limits may also be imposed by QAPPs associated with individual projects.

6.0 Quality Control

Most quality control consideration associated with this SOP are described in the individual sections to which they apply:

Instrument calibration	Section 4.5.1 - 4.5.3
Acceptance criteria for instrument calibration	Section 4.5.4
Acceptance criteria for sample peak recognition	Section 4.7.1 - 4.7.2
Data recording	Section 5.0
Surrogate and Spike Recovery Limits	Section 5.4

Minimum requirements for quality control samples, such as method blanks, matrix spikes, and standard reference materials (SRM), intended to monitor precision and accuracy of the analytical method, are specified in the extraction SOPs. In addition, project specific guidelines may be specified which differ from those outlined in the SOPs.

7.0 Safety

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

1. Protective clothing and eyeglasses should be worn when appropriate
2. Proper care must be exercised when using syringes
3. Certain areas of the GC system are heated. Avoid bodily contact with these areas and use care in handling flammable solvents in and around the GC system.

8.0 Training

All analysts following this procedure will be directly supervised by the Principal investigator, qualified analyst, or laboratory supervisor until they have demonstrated to the satisfaction of the supervisor that they are capable of operating the GC independently. At a minimum, the analyst trainee should be competent in operation and maintenance of the GC. The analyst trainee should also be able to analyze and quantify a multi-point calibration and quantitate a sample of known concentration (e.g., a reference material or matrix spike) within established control limits

9.0 References

EPA Method 8000. U.S. Environmental Protection Agency (EPA). 1988. Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods. EPA-600-4-79-020. 3rd Edition. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Standard Operating Procedure for the Analysis of PCB Congeners by GC/ECD and Trans-Nonachlor by GC/MS/ECNI

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Revision 3

Standard Operating Procedure for the Analysis of PCB Congeners by GC/ECD and Trans-Nonachlor by GC/MS/ECNI

1.0 Scope and Application

1.1 Scope

This method is used to determine the concentrations of PCB congeners and trans-nonachlor in extracts from phytoplankton, zooplankton, *Mysis*, *Diporeia*, detritus and dissolved phase of lake water samples. As the detritus and dissolved phase parameters are not funded by this project, procedures relating to these parameters are provided as information only. The following analytes are measured by this Standard Operating Procedure (SOP):

Analyte	CAS #
trans-nonachlor	39765-80-5
PCB Congener #	**CAS #**
1	2051-60-7
3	2051-62-9
4	13029-08-8
5	16605-91-7
6	25569-80-6
7	33284-50-3
8	34883-43-7
9	34883-39-1
10	33146-45-1
12	2974-92-7
13	2974-90-5
14	34883-41-5
15	2050-68-2
16	38444-78-9
17	37680-66-3
18	37680-65-2
19	38444-73-4
21	55702-46-0
22	38444-85-8
24	55702-45-9
25	55712-37-3

PCB Congener #	CAS #
26	38444-81-4
27	38444-76-7
28	7012-37-5
29	15862-07-4
30	35693-92-6
31	16606-02-3
32	38444-77-8
33	38444-86-9
37	38444-90-5
40	8444-93-8
41	52663-59-9
42	36559-22-5
43	70362-46-8
44	41464-39-5
45	70362-45-7
46	41464-47-5
47	2437-79-8
48	70362-47-9
49	41464-40-8
51	68194-04-7
52	35693-99-3
53	41464-41-9
56	41464-43-1
60	33025-41-1
63	74472-34-7
64	52663-58-8
65	33284-54-7
66	32598-10-0
70	32598-11-1
71	41464-46-4
74	32690-93-0
76	70362-48-0
77	32598-13-3
81	70362-50-4
82	52663-62-4
83	60145-20-2

PCB Congener #	CAS #
84	52663-60-2
85	65510-45-4
87	38380-02-8
89	73575-57-2
91	68194-05-8
92	52663-61-3
95	38379-99-6
97	41464-51-1
99	38380-01-7
100	39485-83-1
101	37680-73-2
105	32598-14-4
107	70424-68-9
110	38380-03-9
114	74472-37-0
118	31508-00-6
119	56558-17-9
123	65510-44-3
124	70424-70-3
128	38380-07-3
129	55215-18-4
130	52663-66-8
131	61798-70-7
132	38380-05-1
134	52704-70-8
135	52744-13-5
136	38411-22-2
137	35694-06-5
138	35065-28-2
141	52712-04-6
144	68194-14-9
146	51908-16-8
147	68194-13-8
149	38380-04-0
151	52663-63-5
153	35065-27-1

PCB Congener #	CAS #
156	38380-08-4
157	69782-90-7
158	74472-42-7
163	74472-44-9
166	41411-63-6
167	52663-72-6
170	35065-30-6
171	52663-71-5
172	52663-74-8
173	68194-16-1
174	38411-25-5
175	40186-70-7
176	52663-65-7
177	52663-70-4
178	52663-67-9
180	35065-29-3
182	60145-23-5
183	52663-69-1
185	52712-05-7
187	52663-68-0
189	39635-61-9
190	41411-64-7
191	74472-50-7
193	69782-91-8
194	35694-08-7
195	52663-78-2
196	42740-50-1
197	33091-17-7
198	68194-17-2
199	52663-75-9
200	52663-73-7
201	40186-71-8
202	2136-99-4
203	52663-76-0
204	74472-52-9
205	4472-53-0

PCB Congener #	CAS #
206	40186-72-9
207	52663-79-3
208	52663-77-1
209	2051-24-3

1.2 Method Optimization

The analyst selects columns and calibration procedures most appropriate for the specific analytes of interest in a study. Matrix-specific performance data are established and the stability of the analytical system and instrument calibration are established for each new matrix.

1.3 Resolution

The analytes listed in Section 1.1 are detected in a clean matrix. Some analytes co-elute with other analytes so that one peak is identified as more than one PCB congener. Analytes that can be detected and quantified in individual samples may differ. This is due to the possible chemical and chromatographic behavior of many of these analytes in real environmental matrices. Cleanup/fractionation schemes are provided in this method.

1.4 Compound Identification

Identification based on single column GC analysis should be supported by one other qualitative technique. Qualitative support for PCB identification will be provided by GC/MS/ECNI. Confirmation of selected PCB congeners with more than four chlorines will be conducted on 5% of the samples. The GC/MS/ECNI method for the analysis of trans-nonachlor directly confirms the identity of the compound, so external confirmation is not needed.

1.5 Method Usage

This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph (GC), mass spectrometer (MS) and in the interpretation of gas chromatograms and mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.6 Working Linear Range

It is important to ensure linearity between a range of PCB concentrations. To demonstrate linearity, five calibration standard solutions ranging from approximately 300 ng/mL to 3000 ng/mL will be run on the GC and compared on a congener basis.

1.7 Limit of Detection Terminology

This study will incorporate three terms describing detection limits - Instrument Detection Limit (IDL), Method Detection Limit (MDL) and System Detection Limit (SDL) - see Section 13.2 for specific calculations. Definitions for each term follows:

IDL = y-intercept of initial calibration curve

MDL = three standard deviations of seven injections of low level homolog spikes, adjusted for congener specific relative response factors (RRF)

SDL = three standard deviations of seven injections of field matrix blanks

2.0 Summary of Method

All samples will undergo methanol rinse, four hour Soxhlet extraction with methanol and 16 to 24 hour Soxhlet extraction with dichloromethane (DCM). Surrogate compounds (congeners 14, 65, 166 for PCBs and ^{13}C-chlordane or ^{37}Cl-nonachlor for trans-nonachlor) are added at the beginning of the extraction to monitor the efficiency of the extraction process. The methanol fraction is batch extracted with hexane. Water/methanol phase is discarded. The hexane fraction containing PCBs is saved, combined with the DCM fraction, solvent exchanged to hexane and volume reduced to approximately 15 mL.

Extracts have lipids removed by passing them over a column containing:

1g Na_2SO_4
13g 6% deactivated Al
1g Na_2SO_4
Washed with 2 x 60 mL hexane

The columns are eluted with 150 mL hexane, and the extracts are volume reduced to approximately 10 mL.
The extracts undergo column chromatography to separate PCBs from nonachlor and toxaphene and to aid in further cleanup. The extracts are loaded onto the following column:

3g Na_2SO_4
4.5g 0% deactivated Si
1g Na_2SO_4
6g 1% deactivated Al
1g Na_2SO_4
Washed with 2 x 50 mL 2% DCM/hexane, 2 x 50 mL 40% DCM/hexane, 2 x 60 mL 100% hexane.

The columns are then eluted with 95 mL 100% hexane followed by 105 mL 40% DCM/hexane.

Both fractions are solvent exchanged and volume reduced to approximately 1 mL. The final extracts are stored in amber vials in the freezer until analysis. Just prior to instrumental analysis, extracts are reduced to approximately 200-300 µL by a gentle stream of nitrogen and internal standards are added. Internal standards used are PCB congeners 30 and 204 for PCBs and 204 for trans-nonachlor.

PCBs will be quantified by individual congener using the method of Mullin (1985). The 1994 congener standard supplied by Mullin will be used as the quantitation, performance, and spiking standard. Analysis will be accomplished using a Hewlett Packard GC equipped with an ^{63}Ni electron capture detector (ECD) and a 60 m high resolution capillary column (DB-5) with H_2 carrier gas. Individual congener data are processed by Millennium Chromatographic Management System (Waters Corporation). Concentrations will be reported by individual congener, homologe distributions and total PCBs.

Trans-nonachlor will be analyzed by electron capture negative ionization (ECNI) GC/MS with selected ion monitoring (SIM). The GC utilizes a 60m DB-5 MS column with He carrier gas. Methane is the reagent gas. Further instrument specifics can be located in Section 5.19. Mass spectra are acquired and processed by Hewlett Packard (HP) RTE-A and Aquarius software.

3.0 Interferences and Corrective Action

3.1 Sources of Interference

Sources in this method can be grouped into three broad categories: contaminated solvents, reagents, XAD resin, or sample processing hardware; contaminated GC carrier gas, parts, column surfaces or detector surfaces; and the presence of coeluting compounds in the sample matrix to which the ECD will respond. Interferences coextracted from the samples will vary considerably from sample to sample. While general cleanup techniques are provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation.

3.2 Interferences by Phthalate Esters

These interferences which are introduced during sample preparation can pose a major problem in PCB determinations. These materials may be removed prior to analysis using the silica gel/alumina cleanup. Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from materials during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials. Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

3.3 Glassware

Glassware must be scrupulously cleaned. Used glassware is cleaned with Alconox detergent in hot water, rinsed with tap water followed by deionized water. The glassware is allowed to dry, is foil wrapped, and ashed for a minimum of four hours at 450°C. It is then stored in a clean environment.

3.4 Toxaphene

The presence of toxaphene will result in a series of peaks that interfere with the detection of PCBs. Toxaphene and PCBs will be separated by column chromatography using a silica gel/alumina column. PCBs will be recovered in the first elution fraction while toxaphene will elute in the second fraction.

4.0 Safety Precautions

4.1 Safety Attire

Latex disposable gloves and lab coats are worn when making up stock solutions of standards. Lab coats are preferred but optional during other activities. Gloves will not be worn during other steps in the method due to the possibility of sample contamination resulting from materials in the gloves.

4.2 Equipment Testing

Testing of the ^{63}Ni ECD is performed bi-annually by the University of Minnesota Radiation Protection Division. Testing of the hoods is performed by the University of Minnesota Environmental Health and Safety Division on a bi-annual basis.

5.0 Apparatus and Materials

5.1 Soxhlet Extractor

Soxhlet extractors used are 50 mm ID with 500 mL round bottom flask. This will be used with all matrices except for XAD which will use 68 mm ID with 1000 mL round bottom flasks. A plug of ashed glass wool is placed in the bottom of the Soxhlet before the sample is introduced.

5.2 Kuderna-Danish (KD) Concentrator

The KD apparatus consists of three parts as described below.

5.2.1 Receiver

Either 10 mL or 15 mL. Ground glass stoppers of the appropriate size are used to prevent evaporation of extracts after volume reduction.

5.2.2 KD Body

Either 500 mL or 250 mL. The evaporation flask is attached to the receiver with delrin clamps.

5.2.3 Snyder column

Three ball macro. Ground glass stoppers of the appropriate size are used to prevent contamination if volume reduction must be interrupted before completion.

5.3 Lipid Removal

An initial cleanup column (1 x 50 cm) is used to remove lipids comprised of 6% deactivated alumina and anhydrous sodium sulfate.

5.3.1 Sodium sulfate

Anhydrous granular, Mallinckrodt, pre-ashed at 450°C for four hours.

5.3.2 Alumina

60-325 mesh, Fisher Scientific, pre-ashed at 450°C for four hours.

5.4 Cleanup Columns

Cleanup columns 1 cm x 50 cm. Columns are comprised of a small plug of ashed glass wool, ashed anhydrous sodium sulfate, 1% deactivated alumina and 0% deactivated silica gel.

5.4.1 Sodium sulfate

Anhydrous granular, Mallinckrodt, pre-ashed at 450°C for four hours.

5.4.2 Alumina

60-325 mesh, Fisher Scientific, pre-ashed at 450°C for four hours.

5.4.3 Silica gel

60-200 mesh, Baker Analyzed, pre-ashed at 300°C for four hours.

5.5 Nitrogen Evaporation Apparatus

Pierce Reacti Therm Model 18780 Evaporating Unit. Ultra-pure Carrier grade nitrogen is used at a flow setting of 7 psi.

5.6 Boiling Chips

Eight to 12 mesh, Cargille Laboratory, ashed at 450°C for a minimum of four hours.

5.7 Heating Mantle

Electrothermal Soxhlet Apparatus heater. Individual solid-state controllers control the heat for each of six individual heating bays.

5.8 Steam Bath

Heated, concentric ring cover. Steam bath is used in the hood.

5.9 Extract Vials

Extracts are stored in 4 mL amber vials which have been wrapped in aluminum foil and ashed at 450°C for a minimum of four hours.

5.10 Drummond Pipets

Drummond pipettes are used to deliver surrogates and internal standards in amounts of 10 µL, 25 µL, 50 µL, and 100 µL.

5.11 Vortex Mixer

Deluxe Mixer by Scientific Products

5.12 Apparatus for Determining Percent Dry Weight:

5.12.1 Sartorius MC1 Balance

Self calibrating.

5.12.2 Drying oven

Precision Scientific, drying is performed at 60°C.

5.13 Apparatus for Determining Percent Lipid:

5.13.1 Sartorius MC1 Balance

Self calibrating.

5.13.2 Drying oven

Precision Scientific, drying is performed at 60°C.

5.13.3 Aluminum weighing tins

Fisherbrand.

5.14 SPM Determination:

5.14.1 0.4 µm Nuclepore filters, 47 mm diameter

5.14.2 Sartorius MC1 Balance

5.15 POC Apparatus

Leko Total Carbon Analyzer

5.16 DOC Analyzer

Ionics Model 555, Thermal Combustion, Total Carbon Analyzer.

5.17 Gas Chromatograph

Following are the gas chromatograph parameters.

Model:	HP 5890A
Injector:	Splitless
Injector Temp.:	225°C
Detector Temp.:	325°C

Temperature Program:	100°C hold for 10 minutes 100°C-130°C at 10°/min. 130°C-255°C at 1°/min. 255°C-285°C at 10°/min. 160 minutes for a run
Injection Volume:	1 µL
Carrier Gas:	H_2
Processing System:	Millennium 2010 Chromatography Manager

5.18 Narrow-bore columns:

5.18.1 GC Column 1

60 m x 0.25 mm internal diameter (ID) fused silica capillary column DB-5 (J & W Scientific) chemically bonded with 5%-Phenyl Methylpolysiloxane, 0.25 µm film thickness.

5.18.2 GC/MS/ECNI Column

60m x 0.25 mm ID fused silica capillary column DB-5 MS (J & W Scientific), 0.25 µm film thickness.

5.19 GC/MS/ECNI

Following are the parameters for the MS system.

Model:	Hewlett Packard 5988A
Autosampler:	HP 7637
Mode:	Single Ion Monitoring (SIM), Negative Ion Mode
Injector:	Splitless
Injector Temp.:	270°C
Temperature Program:	80°C hold for one minute 80°C-210°C at 10°/min. 210°C-250°C at 0.8°/min. 250°C-290°C at 10°/min.
Injection Volume:	1 µL
GC Carrier Gas:	He
NI Reagent Gas:	Methane
Transfer Line Temp:	290°C
Source Temp:	100°C
Source Pressure:	1.2 Torr
Electron Energy:	240 EV
Emission Current:	300 A
Mass Range:	1-800 m/z
Mass Accuracy:	0.3 ± dalton
Scan Start Time:	24 minutes

6.0 Reagents

6.1 Chemicals

Reagent or pesticide grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

Note: Standard solutions (stock, calibration, internal, and surrogate) are stored at -16 ±4°C in amber glass containers. When stock standard solutions are prepared, it is recommended that aliquots of that stock solution be stored in individual small vials for use as working solutions. Standard solution must be replaced if routine QC indicates a problem.

6.2 Solvents

All solvents should be pesticide quality or equivalent. Solvents must be exchanged to hexane prior to analysis.

6.2.1 Hexane

Fisher Optima.

6.2.2 DCM

Fisher Optima.

6.2.3 Methanol

Fisher Optima.

6.2.4 Acetone

Fisher Optima.

6.3 Stock Standard Solutions

6.3.1 Mullins mix: 183 µg/mL

Aroclors 1232 = 75 µg/mL
1248 = 54 µg/mL
1262 = 54 µg/mL

6.3.2 Ultra mix:

Congeners 001 = 12 µg/mL
006 = 14.2 µg/mL
029 = 6.3 µg/mL
049 = 5.86 µg/mL
101 = 4.93 µg/mL

141 = 2.19 µg/mL
180 = 2.21 µg/mL
194 = 1.69 µg/mL
206 = 2.05 µg/mL
209 = 1.36 µg/mL

Additional Congener 001 will be added to the ultra mix due to it's low ECD response. Congener 001 was supplied by Ultra Scientific.

6.3.3 Trans-nonachlor

Accustandard solid.

6.3.4 Stable isotopes

Liquid, as is from Cambridge Isotopes.

6.3.5 Initial calibration standards

Dilutions of the Mullins mix listed above. Concentrations are listed under Section 6.8.

6.4 Sodium Sulfate

Anhydrous, granular - Mallinckrodt. Ash at 450°C for a minimum of four hours.

6.5 Alumina

60-325 mesh - Fisher Scientific. Ash at 450°C for a minimum of four hours. Store in oven at 105°C. When ready to use cool to room temperature. Weigh out needed mass into a round bottom flask. Add water to provide the necessary deactivation on a mass to mass basis. Stopper the flask with the appropriate sized ground glass stopper. Shake for two minutes. Wrap stopper and top of flask with parafilm and set in desiccator for 24 hours.

6.6 Silica Gel

60-200 mesh - Baker Analyzed. Ash at 300°C for a minimum of four hours. Store in oven at 105°C. When ready to use cool to room temperature. No deactivation is required for the procedure used in this method.

6.7 Calibration Standards

Standards are prepared at five concentrations by dilution of the stock standard with hexane. Concentrations correspond to the expected range of concentrations found in real samples and are in the linear range of the detector. Concentrations for the initial calibration standards are: 366 ng/mL, 586 ng/mL, 732 ng/mL, 1464 ng/mL, and 2928 ng/mL. The continuing calibration standard is 732 ng/mL.

6.8 Internal Standards

6.8.1 Source

Solids purchased from Ultra Scientific.

6.8.2 Concentration

#030 = 82.73 ng/mL
#204 = 59.16 ng/mL

6.8.3 Preparation

Individual stock solution of Congeners 30 and 204 were prepared in concentrations of 16,545 ng/mL and 11,831 ng/mL respectively in hexane. To a 100 mL volumetric flask 0.5 mL of each solution was dispensed via a 0.5 mL volumetric pipet. Hexane was added to the flask to bring to volume.

6.8.4 Procedure for addition to extracts

The 4 mL vial of internal standard solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of internal standard to extracts. All extracts will be vortexed to ensure proper mixing.

6.8.5 Storage

Internal standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

6.9 Surrogate Standards

6.9.1 Source

PCB congener solids purchased from Ultra Scientific; stable isotope solutions purchased from Cambridge Isotopes.

6.9.2 Concentration

#014 = 439.2 ng/mL
#065 = 106.4 ng/mL
#166 = 125.04 ng/mL
^{13}C chlordane = 1317 ng/mL
^{37}Cl nonachlor = 1300 ng/mL

6.9.3 Preparation

Individual stock solution of Congeners 14, 65, and 166 were prepared in concentrations of 5.490 µg/mL, 5.320 µg/mL and 5.210 µg/mL respectively in hexane. To a 50 mL volumetric flask 4.0 mL of #14, 1.0 mL of #65 and 1.2 mL of #166 was dispensed via volumetric pipets. Hexane was added to the flask to bring to volume. The stable isotope solutions were diluted in hexane to appropriate concentrations in hexane.

6.9.4 Procedure for addition to samples

The 4 mL vial of surrogate solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of surrogate solution to samples prior to extraction.

6.9.5 Storage

Surrogate standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

6.10 Matrix Spike Standard

6.10.1 Source

Mullins Mix.

6.10.2 Concentration

2928 ng/mL stock solution.

6.10.3 Preparation

The 2928 ng/mL Stock Spiking Solution will be diluted with hexane in the preparation of matrix spikes with concentrations that are within a factor of five of the media of interest. An appropriate amount of nonachlor will be added during this dilution step to be within a factor of five of the media of interest.

6.10.4 Procedure for addition to samples

The 4 mL vial of matrix spike solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of matrix spike solution to the matrix prior to extraction.

6.10.5 Storage

Matrix spike standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

7.0 Sample Collection, Preservation and Handling

7.1 Collection

Details of sample collection are found in the SOP "Sampling Procedures for the Lake Michigan Lower Pelagic Foodchain for PCBs, Nonachlor and Mercury", Revision 1, 31 August, 1994.

7.2 Zooplankton

7.2.1 Definition

The zooplankton fraction is operationally defined as particulate matter greater than 100 µm (excluding fish). Approximately 10 g (wet weight) is needed for the analyses.

7.2.2 Collection

Wet zooplankton is removed from the net container and transferred to a clean glass bottle. The slurry in the glass bottle is poured through a piece of 100 µm Nitex netting supported by either a funnel or seive. The material remaining on the netting is removed by a spatula to an ashed glass jar.

7.2.3 Storage

Jars containing zooplankton are labeled and frozen until extraction.

7.3 Phytoplankton

7.3.1 Definition

The phytoplankton fraction is operationally defined as particulate matter between 10 and 100 µm. Approximately 10 g (wet weight) is needed for the analyses.

7.3.2 Collection

Wet phytoplankton will be quantitatively transferred from the net container to a clean glass graduated bottle. After a small subsample is provided for Hg analysis the remaining slurry is taken to the next nearest hundred mL with filtered lake water (i.e. slurry volume reads between 600 mL and 700 mL the volume is increased to 700 mL.).

7.3.3 Subsamples

The suspension is homogenized and subsampled for mass, taxonomic identification, organic carbon and stable isotopes. The remainder of the suspension is quantitatively transferred using filtered lake water and filtered through an ashed 125 mm GF/F glass fiber filter in a Buchner funnel by a gentle vacuum.

7.3.4 Storage

The glass fiber filter containing the phytoplankton will be folded in quarters, wrapped in foil, sealed in a freezer bag, labeled and frozen until extraction.

7.4 Detrital Fraction

7.4.1 Definition

The detrital fraction is operationally defined as the material collected between 0.7 and 10 µm, as isolated by a 293 mm GF/F glass fiber filter after passing through a piece of 10 µm Nitex netting. Approximately 1000 L of water must be processed for this size fraction.

7.4.2 Storage

The GF/F filters containing the detrital sample will be individually stored. Each filter will be folded in quarters, wrapped in aluminum foil, sealed in a freezer bag, and labeled and frozen until extraction.

7.5 Mysis Relicta

7.5.1 Samples of *Mysis relicta* are hand picked to ensure clean collections. Approximately 10 g (wet weight) is needed for the analyses.

7.5.2 Collection

Mysis are collected by vertical tows with 500 µm nets or by benthic sled tows. Material from these methods of collection are transferred to a pan for hand-picking of organisms. Organisms are placed in ashed glass jars.

7.5.3 Storage

Jars containing *Mysis* are labeled and frozen until extraction.

7.6 Diporeia sp.

7.6.1 Samples of *Diporeia sp.* are hand picked to ensure clean collections. Approximately 10 g (wet weight) is needed for the analyses.

7.6.2 Collection

Diporeia sp. are collected by benthic sled tows. Material from this method of collection are transferred to a pan for hand-picking of organisms. Organisms are placed in ashed glass jars.

7.6.3 Storage

Jars containing *Diporeia sp.* are labeled and frozen until extraction.

7.7 Holding Times

Samples are to be extracted within one year of collection, beginning after approval of the QAPjP. Extracts are stored in the freezer at -16 ±4°C in the dark and analyzed within three years after extraction.

8.0 Sample Preparation Procedure

8.1 Sample Selection

An appropriate sample is removed from the freezer and allowed to thaw. For biota samples aliquots are taken for weight wet and dry weight determinations.

8.2 Wet Weight Determination

An ashed glass beaker is placed on the balance and tared. An appropriate amount of sample is weighed into the beaker (between 5 and 10 g). The wet weight of the sample is recorded in the Lake Michigan Pelagic Foodchain notebook.

8.3 Dry Weight Determination

8.3.1 A small aliquot is taken from the remaining sample and placed in a tared aluminum foil cup. The wet weight is recorded. These aliquots are placed in a drying oven until they reach a consistent weight which is recorded in the notebook.

8.3.2 Phytoplankton dry weights are determined from SPM subsamples collected in the field. Nuclepore filters (0.4 µm) are allowed to air dry and their final consistent weight is recorded in the SPM field notebooks.

8.4 Methanol Wash

8.4.1 Set up a 1000 mL separatory funnel with a conical funnel on top. The conical funnel will hold a plug of ashed glass wool.

8.4.2 Transfer the whole wet sample from the beaker onto the glass wool with methanol (MeOH). Wash the sample with approximately 5-20 mL MeOH.

8.5 Methanol Extraction

8.5.1 Transfer both the sample and glass wool from the conical funnel to a Soxhlet extractor apparatus containing a plug of ashed glass wool. Sample is transferred with methanol

8.5.2 In the case of filters, the filters will be carefully cut into pieces and the pieces will be added to the Soxhlet extractor. Cutting will be done over the aluminum foil the filters were wrapped in and the aluminum foil will be rinsed with methanol with the methanol rinse added to the Soxhlet.

8.5.3 Add approximately 1 tablespoon ashed boiling stones to a 500 mL round bottom flask.

8.5.4 Connect the round bottom to the Soxhlet extractor.

8.5.5 Add approximately 300 mL MeOH through the Soxhlet so it completely covers the sample and drains to the round bottom flask.

8.5.6 Add the appropriate amount of surrogate standard solution. This is dependent on the matrix being extracted - it will vary from 50-200 L. The lab procedural blank will be spiked with an analogous amount of surrogate.

8.5.7 Connect the Soxhlet extractor to the condenser.

8.5.8 Turn on the heating mantel. Let cycle for four hours.

8.5.9 After four hours turn the heating mantel off and let sample cool until round bottom flasks are warm.

8.5.10 Quantitatively transfer the MeOH extract from the round bottom flask to the separatory funnel set up in Section 8.4.

8.6 Dichloromethane Extraction (DCM)

8.6.1 Recharge the round bottom flask with approximately 300 mL DCM.

8.6.2 Reattach the round bottom to the Soxhlet extractor.

8.6.3 Cycle for 16-24 hours.

8.6.4. Allow the extract to cool to room temperature after the extraction is complete.

8.7 Batch Extraction of Methanol Fraction

8.7.1 To the separatory funnel containing the MeOH fractions add the following:

100 mL Barnstead Nanopure water
50 mL saturated NaCl solution
50 mL hexane

8.7.2 Shake separatory funnel for three minutes. Vent funnel often.

8.7.3 Drain lower water layer to a large bottle.

8.7.4 Drain hexane layer into a KD body connected to a receiver holding a funnel containing approximately 150 g sodium sulfate (Na_2SO_4) on top of a plug of ashed glass wool. Wash the Na_2SO_4 with approximately 2 x 15 mL hexane.

8.7.5 Pour the water from Section 8.7.3 back into its corresponding separatory funnel

8.7.6 Rinse the bottle with 50 mL hexane and add to separatory funnel.

8.7.7 Shake separatory funnel for three minutes, and drain lower layer as above. Add the hexane layer to the KD from Section 8.7.4. Repeat one more time starting at Section 8.7.5. Wash separatory funnel with 3 x 15 mL hexane on the last extraction.

8.8 Volume Reduction and Solvent Exchange

8.8.1 Attach a three-ball Snyder column to the KD assembly. Place on steam bath and adjust the vertical position of the assembly so that the appropriate flux is accomplished At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

8.8.2 Reduce the MeOH fraction to approximately 10-15 mL.

8.8.3 Quantitatively transfer the DCM extract obtained from Section 8.6 to the KD apparatus.

8.8.4 Volume reduce to approximately 15 mL.

8.8.5 Solvent exchange the extract to hexane by adding 30 mL hexane, reducing volume to 15 mL, adding another 30 mL hexane, reducing volume to 15 mL and adding a final 15 mL hexane.

8.8.6 Reduce the volume to 10-15 mL. Turn off the steam tables, cool the KD assembly to room temperature and stopper with a ground glass stopper of appropriate size.

8.8.7 Remove the KD body from the receiver - rinse the ground glass joint with hexane into the receiver.

8.8.8 Volume reduce the extract to less than 10 mL using a Nitrogen evaporation apparatus. Needles are sonicated in hexane prior to use.

8.8.9 Transfer, with hexane, the extract to a 25 mL graduated cylinder.

8.9 Lipid Content Determination

8.9.1 Add hexane to the graduated cylinder to bring extract to 10 mL. Using a 1 mL volumetric pipet, transfer 1 mL of the extract to a pre-weighed aluminum tin.

8.9.2 Evaporate solvent from the aluminum tin by air exposure.

8.9.3 After solvent has evaporated, weigh the aluminum tin to constant weight using the Sartorius MC1 balance. Residue remaining in the tin is the lipid.

8.10 Lipid Removal

8.10.1 Column is assembled in the following fashion from top to bottom.

2 g ashed sodium sulfate
13 g 6% deactivated alumina
1 g ashed sodium sulfate

Plug of ashed glass wool
Wash columns with 2 x 60 mL hexane

8.10.2 Deactivation

See Section 6.6 for alumina deactivation procedure.

8.10.3 Columns

1 x 50 cm columns.

8.10.4 Quantitatively load the extract to the top of the column using hexane.

8.10.5 Elute with 3 x 50 mL hexane and collect in a 250 mL KD body attached to a receiver containing two to three ashed boiling chips.

8.10.6 Attach three ball Snyder columns to the KD body and place the assembly on the steam table. Adjust vertical height for appropriate flux.

8.10.7 Reduce the extracts to approximately 5-10 mL using the same procedure described in Sections 8.8.4 through 8.8.7.

8.11 Column Cleanup/Fractionation

8.11.1 Cleanup/fractionation column is assembled in the following fashion from top to bottom.

3 g ashed sodium sulfate
4.5 g 0% deactivated silica
1 g ashed sodium sulfate
6 g 1% deactivated alumina
1 g ashed sodium sulfate
Plug of ashed glass wool
Wash columns with 2 x 50 mL 2% DCM/hexane, 2 x 50 mL 40% DCM/Hexane, 2 x 60 mL 100% hexane

The silica is made into a slurry using hexane before it is poured into the column. Pouring silica in this fashion results in columns that pack better and have fewer air channels

8.11.2 Deactivation

See Section 6.6 for alumina deactivation procedure.

8.11.3 Columns

1 x 50 cm columns.

8.11.4 Quantitatively load the extract to the top of the column using hexane.

8.11.5 Elute with 95 mL 100% hexane and collect in a 250 mL KD body attached to a receiver containing two to three ashed boiling chips. This is labeled the F1 fraction containing PCBs.

8.11.6 Elute again with 105 mL 40% DCM in hexane and collect in a different 250 mL KD body attached to a receiver containing two to three ashed boiling chips. This is labeled the F2 fraction containing trans-nonachlor and toxaphene.

8.12 Final Solvent Exchange

8.12.1 Attach three ball Snyder columns to the KD body and place the assembly on the steam table. Adjust vertical height for appropriate flux.

8.12.2 Reduce the extracts to approximately 5-10 mL using the same procedure described in Sections 8.8.4 through 8.8.7.

8.12.3 Reduce to a final volume of 2-3 mL using the procedure described in Section 8.8.8.

8.12.4 Quantitatively transfer the extract using hexane from the receiver to ashed 4 mL amber vials.

8.13 Storage

Store extract in the freezer at -16 ±4°C until internal standards addition and analysis.

8.14 Internal Standards Addition

8.14.1 Remove extracts from the freezer.

8.14.2 Remove internal standards solution from freezer and allow to come to room temperature.

8.14.3 Reduce extract to approximately 300 µL using nitrogen evaporation apparatus.

8.14.4 Add 50-200 µL internal standard solution (#30, #204) depending on the matrix to the PCB extract fraction using a Drummond pipet and 50-200 µL of chlordane (matrix dependent) to the trans-nonachlor extract fraction via Drummond pipet.

8.14.5 Vortex the extracts. The extract are now ready for instrumental analysis.

9.0 Instrument Calibration and Quantitation

9.1 GC/ECD Analysis for PCB Congeners

9.1.1 Annual Initial Calibration

The GC/ECD will be calibrated at the beginning of the project (or after any major instrument repairs such as replacements of column, detector, or injector assembly) by using calibration standards prepared at five different concentrations as listed in Section 6.8. The concentrations correspond to the expected range of concentrations in the

samples or define the working range of the detector. Each calibration standard is injected using the technique that will be used for the injection of environmental extracts. Peak areas are tabulated against the mass injected for each congener and associated internal standard area and mass.

Note: Because of the sensitivity of the electron capture detector, the injection port and column should always be cleaned prior to performing the initial calibration.

9.1.1.1 Relative Response Factor (RRF)

Millennium Chromatographic Management System automatically calculates the RRF each time it calibrates a calibration standard. The calculation used for this is as follows:

$$RRF = (C_s/A_s) \ / \ (C_{is}/A_{is})$$

Where A_s = *area of the congener*
A_{is} = *area of the internal standard*
C_{is} = *mass (ng) of the internal standard*
C_s = *mass (ng) of the congener*

9.1.1.2 Relative Standard Deviation (RSD)

The math for this calculation across all five RRFs is as follows:

$$RSD = sd \ / \ X_{RRF} \ x \ 100$$

Where sd = *standard deviation of the five RRF measurements*
X_{RRF} = *mean RRF across all five RRFs.*

9.1.1.3 If RSD value for more than 5% of the congeners exceeds 25%, the GC system will be reoptimized and calibration will be performed under the new conditions.

9.1.2 For calibration verification a midrange concentration standard containing all congeners, surrogates and internal standards will be injected as a Continuing Calibration Standard with each set of samples run on the GC.

9.1.2.1 Each congener RRF calculated by Millennium is compared to the mean RRF from the initial calibration. A relative percent difference (RPD) of the continuing calibration RRF from the initial calibration mean RRF for each congener is determined as follows:

$$RPD = (RRF_{cc} - X_{RRF\ ic} \ / \ X_{RRF\ ic}) \cdot 100$$

Where RRF_{cc} = *RRF for continuing calibration standard*
$X_{RRF\ ic}$ = *mean RRF from the initial calibration*

9.1.2.2 If the % difference exceeds 100% for more than 30% of the congeners, the GC system will be inspected to determine the cause. Any required maintenance will be performed and calibration will be reverified. If routine maintenance does not correct the GC performance based on the annual initial calibration, a new initial calibration will be performed.

9.2 Retention Time Windows

9.2.1 Retention time (RT) windows can be established only when the GC is operating under optimum conditions. Any drift in retention times result in meaningless RT windows. The width of RT windows is established on a run by run basis. Each calibration standard run at the beginning of a sample set is used to establish RT windows. These RT windows are confirmed on the performance standard run at the end of the sample set.

9.2.2 If the RT windows determined from the calibration standard fail to identify peaks correctly in the performance standard, it will be necessary to perform maintenance steps.

9.2.3 Check for leaks throughout the GC system. This problem can cause large and continuing shifts of all peak retention times. Check for a column blockage leading to skewed and/or deformed peaks.

9.2.4 Once maintenance has been performed the sample set will be rerun. If the problem is still not corrected it may be necessary to change the column.

9.2.5 Retention time windows are recalculated when columns are clipped or new columns are installed.

9.3 GC Analysis of Samples

9.3.1 Samples are analyzed in a set referred to as a sample set. The sequence will always begin with a hexane injection followed by a continuing calibration check, sample extracts, field blanks, procedural blank and a performance standard. A typical run sequence would look like this:

Vial	Description
1	Hexane
2	Standard
3	Sample
4	Sample
5	Sample
6	Sample
7	Sample
8	Proced. Blank
9	Performance Standard

9.3.2 Continuation of sample injection may continue for as long as the daily continuing calibration standard and Mullin mix standard interspersed with the samples meet QC requirements. It is recommended that standards be analyzed at least after every 20 environmental samples and at the end of a set.

9.3.3 Each sample set will be bracketed with an acceptable continuing calibration standard and a performance standard.

9.3.4 Baselines for the continuing calibration standard are carefully drawn, peaks are identified and daily absolute RT windows for each congener are established. Congener identification occurs when a peak from a sample extract falls within the daily retention time window.

9.3.5 Millennium automatically performs calculation of peak mass using the internal standard calibration procedure as follows:

$$PCB_{mass} = Area_{PCB} \; x \; RRF \; x \; [(mass_{istd})/(area_{istd})]_{sample}$$

Where PCB_{mass} = *pg PCB congener detected in sample*
$Area_{PCB}$ = *area of PCB congener detected in sample*
RRF = RRF of PCB congener from continuing calibration std
$mass_{istd}$ = *pg applicable internal standard added to sample*
$area_{istd}$ = *area of internal standard response in sample*

9.3.6 If the peak response is less than the SDL the validity of the quantitative result may be questionable. The sample will be analyzed to determine if further concentration is warranted.

9.4 GC/MS/ECNI Analysis of Trans-nonachlor

9.4.1 Tuning

The GC-MS is tuned approximately every two to three weeks. The decision to re-tune the instrument is based on evaluating a daily injection of the performance standard octafluoronaphthalene (OFN). Peak area, shape, and electron multiplier setting (sensitivity) are evaluated by a trained operator. If re-tuning is necessary the instrument is re-tuned in negative chemical ionization mode.

9.4.2 Initial calibration

Consists of three solutions of perfluorotributylamine (PFTBA) at concentrations ranging from 10 ng/mL to 75 ng/mL. Criteria include m/z 633 = 50,000 and m/z 452 should equal 3-15% of m/z 633. The calibration is done at the beginning of the project, and anytime after source vacuum has been lost and re-gained due to cleaning or repairs.

9.4.3 Continuing Calibration

A trans-nonachlor standard will be run with each sample set.

9.4.4 RT Window and SIM criteria - retention time window is set based on the trans-nonachlor standard run as the continuing calibration standard. The standard is run under full scan and the retention time for trans-nonachlor is determined. Within this retention time the program monitors for ions corresponding to the mass of trans-nonachlor - 444 m/z and 442 m/z. The m/z 444 is the quantitation ion, m/z 442 is the confirmation ion. Samples will be checked within the retention time window for the appropriate ions.

9.4.5 Analysis sequence

Samples are analyzed in sample sets. A typical run sequence appears as follows:

Vial	Description
1	Hexane
2	Standard
3-8	Samples
9	Standard
10-15	Samples
16	Standard

9.4.6 Baselines

Baselines are manually set using the HP Aquarius software program.

9.4.7 Quantitation

Areas are determined by Aquarius, and the mass of trans-nonachlor is calculated using the internal standard method as described in Section 9.3.5.

10.0 Preventative Maintenance

10.1 GC

10.1.1 Columns

When installing a column, it is very important to scrutinize the cut very carefully as jagged or angled cuts can result in poor chromatography or gas leaks.

10.1.2 Gases

Gases go through a series of filters before reaching the GC including; moisture trap, hydrocarbon trap, oxygen trap, indicating oxygen trap and chemical filter. Only Ultra-Pure Carrier grade hydrogen and helium with purity of 99.999% should be used.

10.1.3 Septa

Septa should be changed with each run. Only teflon septa should be used with the GC/ECD.

10.1.4 Injector

If chromatography indicates an injection side problem it will be necessary to clean the injector port and replace the injector liner. At this time it is also a good idea to clip the column and reinstall.

10.1.5 ECD

Since the ECD contains a radioactive compound (^{63}Ni) it is regulated by the Nuclear Regulatory Commission. All maintenance relating to the ECD is handled by the University of Minnesota Radiation Protection Division.

10.2 MS

10.2.1 Mechanical pump oil is changed bi-annually.

10.2.2 Refrigerant coolant level is checked once every three months.

10.2.3 The source is cleaned as needed determined by chromatography quality.

10.2.4 Poles and ceramics are cleaned as needed.

10.2.5 Fan screens are cleaned and the area surrounding the instrument is vacuumed on a monthly basis.

10.2.6 Cleaning contacts on the component boards and vacuuming component boards is covered though an HP service contract for the instrument.

10.3 Balances

10.3.1 Sartorius MC1 balance has internal calibration ability. Before weighing, an internal calibration is performed. Weights ranging from 2 mg - 1 g are used to double check the calibration.

10.3.2 Balances are checked before weighing for cleanliness. Solids wedged under the weighing pan can result in unstable readings.

11.0 Quality Control Requirements

11.1 Surrogate Recovery

11.1.1 Surrogate standard recovery in all samples, blanks, and spikes will be calculated. Samples with average surrogate spike recoveries <50% or >125% will be re-analyzed or flagged. The following steps will also be investigated.

11.1.2 Confirm that there are no errors in amounts of surrogate solutions or internal standards added to the solution.

11.1.3 Examine chromatograms for baseline determination and interfering peaks.

11.1.4 Recalculate the data if any of the above checks reveal a problem.

11.1.5 Reextract and reanalyze the sample if none of the above are a problem. If an individual surrogate standard exceeds the stated limits, the surrogate will be flagged as FSS. If two of three surrogates exceed the QA limits, all data for that sample will be flagged FSS.

11.2 Calibration QC

11.2.1 Initial Calibration

An initial calibration will be performed annually. Five concentrations of all congeners will be used and the RRF RSD across all five concentrations will be <25% for 95% of the congeners in order for the calibration to be considered valid. If this criteria is not met, recalibration must occur until it is. If problems persist, the stability of the standards used and the dilution techniques should be checked.

11.2.2 Continuing Calibration

A continuing calibration standard will be analyzed and compared against the initial calibration with each run. The RRF RSD of each congener from the initial calibration RRF must be <100% in order for sample analyses to occur. If this criteria is not met, action must be taken to achieve an acceptable calibration prior to resuming sample analysis.

11.3 Internal Standards Performance

Internal standard RF and 204/30 ratio in each sample, blank and standard will be evaluated for acceptance. The change in each internal standard's RF in each sample compared to the continuing calibration RF must be <50%. The change in ratio of 204/30 area response for each sample compared to the continuing calibration ratio must be < 50%. If the ratio fails this criteria, then the absolute RF of each internal standard is examined to determine of one or both internals standards are compromised (change in RF >50%). If one internal standard in the PCB analysis is compromised, the other internal standard should be used for quantitation. If both fail the sample will be rerun or all sample data will be flagged FIS. If #204 for GC/MS/ECNI analysis of trans-nonachlor fails the criteria of the change in RF compared to the continuing calibration (RF <50%) the sample will be rerun or all data will be flagged FIS.

11.4 Blanks

11.4.1 Lab Procedure Blank

A lab procedure blank will be prepared with every six samples. This blank consists of all reagents, surrogates and internal standards used in the extraction of environmental samples at the volumes used in these extractions. The procedure blank is carried through the entire analytical procedure in the same manner as a sample. Any detected congeners at a concentration <SDL will be flagged FBK. If the blank exceeds this criteria for more than 30% of the congeners in the samples, the samples may be flagged following evaluation of

the data by the analyst. Also, all reagents will be checked before proceeding with additional analyses and the associated sample sets will be checked against previous ones for self consistency. If the sample data or reagent purity are questionable, samples will be re-extracted or flagged FKB if no further sample is available. If sample data are consistent with previous data and reagent blanks are acceptable then the data will be accepted without flagging.

11.4.2 Field Blanks

Collection of field blanks will occur at a frequency of two blanks per 11 phytoplankton/detrital/XAD fraction samples collected. Any detected congeners at a concentration <SDL will be flagged FBK. If the blank exceeds this criteria for more than 30% of the congeners in the samples, the samples may be flagged following evaluation of the data by the analyst, as above.

11.5 Duplicates

11.5.1 Lab Duplicates

Zooplankton samples will have a lab duplicate prepared at a frequency of one per 11 samples/cruise. One zooplankton sample from each cruise will be split in the lab and analyzed as a duplicate. The Relative Percent Difference (RPD) between the sample and its lab duplicate must be <30% for 70% of all congeners. If this RPD is exceeded, associated sample data will be flagged as FDL.

11.5.2 Field Duplicates

Collection of field duplicates will occur at a frequency of two per 11 samples/cruise except for *Diporeia* and *Mysis* which have a frequency of one per 11 samples/cruise. The RPD between the sample and its field duplicate must be <30% for 70% of all congeners. If this RPD is exceeded, associated sample data will be flagged as FFD.

11.6 Matrix Spikes

Mullins Mix solution will be added to result in a concentration within a factor of five of the media of interest to one sample out of every 24 extracted and analyzed. The percent recovery of 70% of all congeners will be between 50-125%. If recovery is outside of these limits, the associated sample data will be flagged as FMS.

11.7 Performance Standard

Mullins mix (1994) at a concentration comparable to the quantitation standard will be analyzed with every sample set. The recoveries of the congeners must be between 90-110% for 70% of all congeners for the associated sample data to be considered valid. If recovery is outside of these limits, associated sample data for that congener should be flagged as FPC.

11.8 Retention Time Window

Analytes reported as detected must be within the retention time window as described in Section 9.2. If the analyst believes that a retention time window shift has occurred and the analytes are present outside the statistically established window, maintenance on the instrument will be performed and the sample set rerun.

12.0 Data Reporting

12.1 Units

All PCB congeners and trans-nonachlor will be reported as pg/g dry weight calculated as:

$$pg/g\ PCB\ or\ trans\text{-}nonachlor = [A_s \times RRF \times M_{is}] / [A_{is} \times W_s]$$

Where A_s = *area counts of the analyte in the sample*
RRF = *relative response factor of the analyte based on the continuing calibration standard*
M_{is} = *pg of internal standard added to the sample*
A_{is} = *area counts of internal standard in the sample*
W_s = *weight of sample extracted, in g dry weight*

12.2 PCBs

12.2.1 Total PCBs as a sum of all congeners, the sum of each homologe series and each congener concentration will be reported.

12.2.2 A surrogate correction value will be reported in the appropriate field of the "Laboratory Reporting Standard" Raw data will be divided by this value to provide the reported result value.

Surrogate Recovery Correction: Each congener concentration will be corrected to the recovery of the surrogate that best represents that congener. This is determined by running duplicate procedural spikes, and correcting the recoveries to each of the three surrogate standards. The region of the chromatogram that is corrected closest to 100% by a given surrogate would be the most appropriate surrogate for those congeners. In past projects, the first third of the chromatogram was best corrected to Congener 14, the middle third to Congener 65, and the final third to Congener 166 (see Table 6, QAPjP). This study will be conducted specifically for this project.

12.3 Data

Data from Millennium and GC/MS/ECNI data system Aquarius will be electronically transferred to spreadsheets in Excel for QA review and data reduction/calculation.

12.4 Associated QC

Results of lab/field blanks, lab/field duplicates, matrix spike recoveries, surrogate spike recoveries, and performance standard recoveries will be reported with each sample batch.

13.0 Method Validation Procedures

13.1 Analyst Proficiency

Analysts associated with this project have reviewed the Sampling SOP, the Sample and Analysis Quality Assurance Project Plan and this Analysis SOP Analysts working with either the GC or the GC/MS/ENCI have extensive experience in operating, maintenance, and repair of the instrument. Additionally, analysts have a strong working knowledge of the software used in analyzing the data associated with each instrument.

13.2 Detection Limit Determinations

13.2.1 IDL

The annual initial five-point calibration curve will be constructed and extrapolated to determine the y axis intercept. This intercept will be considered the Instrument Detection Limit (IDL).

13.2.2 MDL

The Method Detection Limit (MDL) for each homologue series will be determined once during the project using the Ultra standard mix at a low level concentration in a procedural spike. A mean value and standard deviation for each congener in the Ultra mix will be established across seven spikes. The Ultra MDLS are equal to three times the standard deviation. The Ultra MDL for a given homolog will be proportioned against each congener's RRF within a homolog to establish a congener specific MDL. The congener specific RRF will be determined from PCB calibration standards run on the GC the same day as the MDL spiked samples. These MDLs will be used in data reporting.

13.2.3 SDL

The System Detection Limit (SDL) will be calculated annually. This will occur once seven field blanks have been analyzed. The SDL will be set as +3sd of the mean field blank concentration.

14.0 References

14.1 Mullin, M. 1985. PCB Congener Workshop, Large Lakes Research Station, U.S. EPA, Grosse Ile, Michigan.

14.2 Pearson, R.F., K.C. Hornbuckle, K.A. Golden, S.J. Eisenreich, and D.L. Swackhamer. 1996. PCBs in Lake Michigan Water: Comparison to 1980 and mass budget for 1991. *Environ. Sci. Technol.* 30:1429-1436.

Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination

Standard Operating Procedure SOP No. HC521A

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Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination

1.0 Scope and Application

This method covers the extraction of fish samples for organic analysis by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). For analysis of PCBs and the chlorinated pesticides currently reported by this laboratory (as of the above date), a 90/10 mixture by volume of petroleum ether/ethyl acetate is used for the extraction. If the samples are to be analyzed for general contaminants a 80/20 mixture of the same solvents is used. If more polar pesticides (such as atrazine) are analyzed by this laboratory in the future, a more polar extracting solvent mixture will probably be needed.

2.0 Summary of Method

This method covers only the extraction and cleanup portions of the testing procedure and is applicable to fish only. Analytical procedures for PCBs and pesticides in fish are already in place and are covered by the appropriate NBS/GLSC methods.

3.0 Interferences

3.1 Interferences from sample preparation glassware and reagents are routinely monitored by running method blanks. The method blank is run through the entire extraction process along with the samples, except that it consists only of sodium sulfate, the compound that is mixed with fish tissue before extraction.

3.2 All glassware is cleaned as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing is followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware is then drained dry and heated in a muffle furnace at 400°C for two hours. The glassware is solvent rinsed with acetone, hexane, and the solvent or solvent mixture used for a given operation immediately prior to glassware use. Volumetric ware is not heated in a muffle furnace. After cooling and drying, glassware is sealed and stored in a clean environment to prevent accumulation of dust and other contaminants. Glassware is stored inverted or capped with aluminum foil.

4.0 Safety

PCBs and pesticides have been tentatively classified as known or suspected, human or mammalian carcinogens. The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined, although each chemical compound should be treated as a potential health hazard. The NBS Great Lakes Science Center maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets is also available to all personnel involved in chemical analysis.

5.0 Apparatus

5.1 Glass chromatography columns -- 330 mm x 23 mm I.D., each fitted with a removable teflon stopcock. Kontes K-420540-0234 or equivalent.

5.2 Beakers -- 150 mL Pyrex Berzelius beakers.

5.3 Glass wool -- Corning Pyrex brand #3950 or equivalent. Solvents -- Petroleum ether, ethyl acetate, and iso-octane -- Pesticide quality or equivalent.

5.4 Zymark Turbovap II Concentrator.

5.5 Zymark Turbovap Concentration Tubes, 200 mL with 1 mL endpoint.

5.6 Zymark Benchmate Workstation robot equipped with Rheodyne 700 L switch valve with a 2 mL loop.

5.7 HPLC solvent pump, Waters, or equivalent.

5.8 ISCO Foxy 200 fraction collector.

5.9 Glass GPC column (51 x 1.5 cm i.d) packed with 200-400 mesh SX-3 biobeads.

5.10 Jones disposable 0.5 g micro silica gel columns.

5.11 Concentration Tubes, Kadurna-Danish - 10 mL. graduated. Kontes k-570050-1025 or equivalent.

5.12 Organomation N-Evap Concentrator Model III.

5.13 Argon gas, purified grade.

5.14 Vials, 15 mL glass, with Teflon-lined screw cap.

5.15 Sodium sulfate -- (ACS) Granular, anhydrous. Purified by heating at 400°C. for 4 h in a shallow tray. Supelco 2-0296 or equivalent.

6.0 Extraction Procedure

6.1 Sample Preparation

Samples are processed in sets of about 5-14 samples including all the necessary QA/QC samples specified in the QAPjP for this work. Fish tissue, previously homogenized according to the fish processing method HC500A.SOP, is thawed and thoroughly mixed. Ten g of tissue is weighed into a contaminant-free beaker and mixed with 40.0 g of Na_2SO_4 which has been previously dried by heating to 140 C overnight. The mixture is stirred frequently until it is dry and free flowing, containing no large lumps. The beaker is then labelled with the appropriate sample number and weight. Necessary surrogates specified in the QAPjP Plan for this study.

6.2 Extraction

6.2.1 The extraction columns are prepared by inserting a small glass wool plug into the bottom of each chromatography column, and then the column is rinsed twice with 15 mL of petroleum ether. Air is removed from the glass wool by lightly tapping it with a clean glass stirring rod. A Zymark concentration tube is placed under each column and the appropriate labels are transferred to these tubes. The sample mixture is then poured into the column, after which 50 mL of the appropriate solvent is added to the sample beaker, stirred, and transferred to the column. The solvent is allowed to pass through the column, but as it begins to elute into the concentration tube, the stopcock is closed. At this point the column is lightly stirred with a glass rod to remove trapped air. Elution is then continued at the rate of 1-2 mL/min. until the solvent level reaches the beginning of the sample mixture.

6.2.2 Another 50 mL of the appropriate solvent mixture is added and elution is continued at the same rate. The columns are allowed to drain completely after the second 50 mL of solvent is added. The stopcock tips are rinsed with ethyl acetate to wash any residual lipid and analytes into the concentration tube.

6.3 Extract Concentration

The eluant is concentrated by placing the Zymark concentrator tubes in the Turbovap concentrator. The concentrator's water bath is kept at 40°C, and the argon sweep gas pressure is set to 10-12 psi with the Turbovap's control knob. Samples are concentrated to 1 mL or the minimum amount allowed by a sample's lipid content, whichever volume is greater. The residual solvent from this process should be mostly ethyl acetate. Each extract is then transferred to a graduated (or marked) culture tube and diluted to volume (10 or 20 mL depending on lipid and contaminant concentration) with ethyl acetate. The tubes are sealed with a teflon-lined screw cap and the labels are transferred to the appropriate tubes.

6.4 Lipid Determination

Prior to cleanup by gel permeation chromatography, a volume of sample extract equivalent to 1 g of tissue is pipetted into a preweighed (after acetone rinsing and drying) aluminum drying pan. Preweighing is to the nearest 0.1 milligram. The extract is allowed to evaporate under static conditions in a fume hood for 2 hours. The pan is again weighed to the nearest 0.1 milligram and the percent extractable lipid is computed as 100 x (1-weight of residual lipid).

7.0 Lipid Separation/Removal

7.1 The GPC column is initially calibrated by taking 5 mL fractions as the elution proceeds through the dump cycle, during which the lipids are routed to a waste bottle, and then through the collect cycle, during which the compounds of interest are routed to the appropriate collecting station. The endpoint of lipid removal is monitored by the gravimetric procedure detailed in Section 6.4. The starting point of compound collection is monitored by GC/GCMS analysis of the 5 mL fractions. The normal dump fraction is about 43 mL, followed by a collect fraction of 45 mL.

7.2 The status of the GPC column is monitored by either a pre- or post-run procedure for each batch extraction. While a batch is proceeding, or processing put on hold until the spike sample (run first in a batch) is brought from 45 to 50 mL and 2 μL of this are injected into a GC/EC to estimate recovery before advancing to the next sample. The peak height ratios of transnonachlor (elutes at beginning of collect cycle) and p,p'-DDE (elutes toward the end of collect cycle) should closely match that in the spike standard. The GPC can be prevented from advancing to the next sample or stopped at any point in the sample set. But once a sample is loaded on the column that sample will need to be rerun in case of a problem. If the original calibration conditions are changed after the spike sample any samples in the batch processed by GPC before correcting the problem must be rerun. Usually the GPC monitoring will not indicate a problem so frequently the spike will be checked during or after the entire batch has been processed or when recoveries are determined from previous sample sets to be low.

7.3 For PCB and/or pesticides analysis, a 2 mL portion of sample extract is loaded into the calibrated loop of the gel permeation system (attachment to the Benchmate Robot) and lipid/contaminants are separated with ethyl acetate through 200-400 mesh SX-3 Biobeads. The first fraction contains the lipids is discarded and the second fraction containing the contaminants is collected in a clean, appropriately labelled Zymark concentration tube. This fraction will be greater than 93% lipid-free. Consecutive loops of each extract can be processed when greater amounts of contaminants are needed (i.e. GC/MS). Refer to GPC technical manual for complete details of GPC setup and operation. The extracts are concentrated with the Zymark Turbovap set to the same conditions as specified in Section 6.3 above. The extracts are brought to 1 mL with the Zymark concentrator and then brought up to final volume with iso-octane.

7.4 Each sample is passed through a 0.5 g acidified micro-column of silica gel to remove the last 7% of the lipid. The silica gel column is designed to work with the Zymark Benchmate Workstation Robot. Fifty μL of 1:1 concentrated sulfuric and water is added manually to a silica gel column for each sample in the set being processed. Thereafter, the Benchmate will automatically rinse, load the sample (extract from 1 g of fish), weight amount loaded on the column, and elute the desired analytes from the column. Samples are then transferred to a graduated culture tube and evaporated using the Organomation N-Evap Concentrator to about 0.5 mL. The sample is then diluted to volume (usually 1-5 mL) with iso-octane and then transferred to a GC or GC/MS autosampler vial and stored until ready for analysis.

7.5 This is a manual method for removal of remaining lipids after GPC and was used for most of the mass balance fish samples beginning because with the automated method lipid was causing problems quantitating several PCB congeners. One mL of iso-octane is added to the GPC extract (equivalent to g fish tissue) and samples are concentrated to a final volume of 1 mL with the Zymark TurboVap set to conditions as specified in Section 6.3. Each sample is passed through a borosilicate glass column, 30 cm X 1.5 cm i.d., assembled as follows from bottom to top:

a. Pyrex wool plug
b. 1.0 g dried sodium sulfate
c. 7.0 g of a 10% (w/w) mixture sodium bicarbonate and sodium sulfate
d. 3.6 g 2% deactivated silica gel
e. 0.2-0.3 mL concentrated sulfuric acid

Each column is conditioned with 5 mL of the elution solvent (5:95 ethyl acetate:hexane) before loading sample onto the column. Zymark tubes are rinsed with 5 mL of elution solvent which is passed through the eluant from the column is collected in 12 mL calibrated centrifuge tube. Three additional 2 mL aliquots are passed through the column and collected in the same receiving tube. Each sample is then concentrated to the final volume necessary for analysis using a stream of argon gas, approx. 10 psi, and a water bath at 38°C.

Analysis of Total PCBs and PCB Congeners and Trans-nonachlor in Fish by Gas Chromatography/ Negative Chemical Ionization Single Ion Mass Spectrometry

Standard Operating Procedure SOP No. HC 519.D
(Replaces: No. HC 519C)

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Version 2.0

Analysis of Total PCBs and PCB Congeners and Trans-nonachlor in Fish by Gas Chromatography/Negative Chemical Ionization Single Ion Mass Spectrometry

1.0 Scope and Application

1.1 This method covers the determination of total PCBs, PCB congeners and Trans-nonachlor developed at the National Biological Service/Great Lakes Science Center (NBS/GLSC) by gas chromatography using negative chemical ionization single ion monitoring mass spectrometry (GC/NCI/SIM) for the U.S. EPA Mass Balance Study. The parameters presently reported by this method are given in Table 1. The parameters listed are qualitatively and quantitatively determined as target compounds by this method. The NCI reagent gas used is methane.

1.2 The method detection limits (MDL) for selected congeners are also listed in Table 1. These were determined according to EPA rule Appendix B of 40 CFR Part 136 on method blanks spiked with one congener at each chlorine level (one through ten) at very low levels (3-10X signal to noise ratio) on the GC/MS just before extraction. The value for the other congeners at a given level of chlorination were calculated using the ratio of the normal response factor for the congener used in the MDL study for that level of chlorination to the normal response factor of the congener in question, multiplied by the detection limit of that congener representing the chlorine level.

2.0 Summary of Method

This method covers only the analytical portion of the testing procedure applicable to fish. Sampling and sample preparation procedures for PCBs and trans-nonachlor in these matrices are already in place and are covered by the appropriate NBS/GLSC methods. Qualitative identification the parameters in the resulting extracts is performed using the retention time and the relative abundance of two characteristic masses (m/z). Quantitative analysis is performed using an internal standard technique with a single characteristic m/z.

3.0 Interferences

3.1 Interferences from sample preparation glassware and reagents are routinely monitored by running method blanks. The method blank is run through the entire extraction process along with the samples, except that it consists only of sodium sulfate, the compound that is mixed with fish tissue before extraction.

3.2 Matrix interferences may be caused by compounds that are co-extracted from the sample, and may vary considerably from source to source. The level of interference using GC/NCI/SIM is far less, however, than when using standard positive electron-impact (EI) mass spectrometry.

3.3 Two congeners of interest, numbers 77 and 126, are not separated completely from interfering PCBs. For the Mass Balance Study, they will be quantitated by subtracting out the typical contribution of the interfering PCB and will thus be quantitated somewhat less accurately than the other target congeners. Neither compound contributes significantly to total PCBs in biota.

4.0 Safety

4.1 PCBs have been tentatively classified as known or suspected, human or mammalian carcinogens. Primary standards of these toxic compounds must be handled in a manner to avoid direct contact.

4.2 The toxicity or carcinogenicity of each chemical and reagent used in this method has not been precisely defined, although each chemical compound should be treated as a potential health hazard. The NBS/GLSC maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets is also available to all personnel involved in chemical analysis.

5.0 Apparatus

5.1 Gas Chromatograph The NBS/GLSC uses an HP5890 gas chromatograph (GC) equipped with an HP7673A robotic autosampler. All data are acquired using computer controlled batching of sample, standard, and quality control runs. This approach is critical to obtaining the retention time reproducibility needed for doing PCB congener work, even though relative retention times are used. Members of a given congener elute closely enough together that tight control of chromatographic conditions is necessary to avoid misidentification, as the ion ratios for a given congener series are very similar. The HP gas chromatograph is capable of multi-stage temperature programming (ramping) and is equipped for splitless/split capillary injection.

5.2 Column - A 30 meter DB-5 fused silica capillary column is used, with an I.D. of .25 mm and a coating thickness of .25 micron.

5.3 Mass Spectrometer - The NBS/GLSC uses an HP5988A research-grade low resolution mass spectrometer (MS) equipped with positive and negative chemical ionization capability. The instrument can perform single ion monitoring (SIM), analyzing up to 999 ion groups of 20 ions per group during each run. The GC capillary column is interfaced directly into the MS source with no splitting of carrier gas.

5.4 Data System The HP5988A mass spectrometer is equipped with an RTE-A data system, capable of doing automatic identification and quantification of target compounds using a reverse search for identification and an internal standard method for quantitation. This system also controls data acquisition, including automatic operation of the GC, MS, and autosampler, and any other required manipulation of the raw data or processed files. The HP Enviroquant software is used for data reduction, which will necessitate transfer of raw data files from RTE to PC/DOS by RS-232 using a procedure file.

6.0 Analytical Procedure

6.1 Tuning and Calibration--The source is operated in the CI mode using methane reagent gas. When the source pressure has stabilized at a value known to produce satisfactory results (.5 to .8 Torr), the instrument is manually tuned and calibrated using an NCI customized tuning file. Pressure stabilization usually takes about 45 minutes. Tuning is checked daily by using at least two ions which span the approximate range of interest for PCBs/trans-nonachlor. Using FC-43, the ions currently selected are 235 and 452. A third ion, 633, is always present in the display, but is not used for tuning. The parameter ramp program is run for purposes of maximizing NCI response, with the HP lenses known as repeller, drawout, ion focus, and entrance lens being adjusted so that their settings are near the displayed curve maxima for the two ions. Since the lens settings are interactive, the lens sequence should be rechecked until no changes to the settings are needed. The manufacturer's manual contains detailed instructions on manual tuning in NCI. Once tuning is accomplished, a half-page profile scan is printed out. The source pressure and any unusual conditions is noted on this display, and the exact masses of ion 452 is checked to assure that it has not changed more than 0.15 amu from 452.0. Any centroid change greater than this may require alteration of the exact masses as given in the ion groupings (presented in Table 3) of this method. It is much simpler to adjust the centroid in the tuning file to within 0.15 of 452.0 with the Mass Offset (B/b) control in the manual tuning procedure. Once proper tuning and calibration have been obtained, the manual tune is saved before exiting the manual tuning program. Note that tuning is most needed the first few weeks after the source has been cleaned. The system becomes very stable thereafter, and usually no tuning operations are necessary other than printing out and archiving a copy of the tuning display.

6.2 Calibration and Linear Range

6.2.1 GC and MS conditions are set by the analytical method. These conditions are given in Table 2 for the GC parameters and in Table 3 for the MS parameters. It is important to note that the method file controls if there is a disparity between it and the tuning file with respect to source temperature, multiplier voltage, and/or emission current. The column head pressure is manually controlled using the GC oven pressure controller and the oven may be baked by setting the oven temperature from the keyboard if no method is running. Source pressure (reagent gas flow) is controlled manually using the CI reactant gas flow controller. All other parameters are established through the terminal and keyboard.

6.2.2 Standards of a 25:18:18 ratio of Aroclor 1232, 1248, and 1262 are run at concentrations of 500, 2500, and 5000 ng/g to demonstrate linear response over this range. Using these concentrations, we obtain three concentrations for all congeners which do not saturate at the highest concentration and do not go below the detection limit for the least concentrated congener. A linear three-point curve is established when the standard deviation of the relative response ratios at the three concentrations is less than 25%.

6.2.3 The 2500 PPB concentration is designated as the calibration standard. The concentrations in the ID file are based on this standard. It is run at the beginning of each batch run. The performance standard is be run immediately following the calibration standard. Congener concentrations in the performance standard are calculated from the calibration standard response factors. A subset of six congeners are used to evaluate the current response factors. These include #44, #207 (small peaks), #101, #185 (average peaks, and #151, #180 (large peaks). Calculated concentrations of these congeners are compared to their known concentrations. Deviations from actual concentrations of greater than 50% for the small peaks and greater than 10% for the average and large peaks result in flagging of all samples in the data set for the failed congener.

6.2.4 Because a single calibration standard is being used to generate RRFs, the calibration standard concentration should be within a factor of five of the concentrations of PCBs in the sample extracts. Sample extracts that fall outside this range are either diluted or concentrated to bring them to within this range.

6.3 Sample Run

Once the source pressure has stabilized at the desired value and a manual tune has been performed (or checked), a batch data acquisition (sample run) can be initiated. A batch data acquisition is begun by accessing the batch sequence menu. Every batch includes a method blank, standards, a spike standard, a surrogate standard, a check sample, a background fish extract, then actual samples and spikes. The latter will vary with the number of samples extracted at one time. Information on each sample is entered sequentially into the menus as they come up until the batch edit. The proper tuning file, method file, allowable space per run, and total cartridge space (minimum 600 tracks for a normal batch) for the batch are also inputted to the batch file. When finished, the batch sequence listed and checked for correctness, the batch is started. Before the start command is given, a final check is made as to condition of column, solvent wash vials, septum, injection port liner, and leak-free status of the injection area. Internal standards should be spiked into the samples and spikes just before the beginning of the batch data acquisition run.

6.4 Data Reduction and Reporting

6.4.1 Quantification of PCBs is congener specific and done by the internal standard method. The internal standards that will be used are congeners #136 and #204. Congeners eluting prior to and including #110 are quantitated relative to internal standard #136, and those after #110 are quantitated relative to #204.

6.4.2 Data analysis begins with the execution of AUTSFX (Single ion monitoring background subtraction program) for the entire batch and then BATCH3 using ID file MBSUR for the entire batch. This will provide assurance that the batch completed without gross problems such as syringe lugging or failure of GC temperature controlled zones. The CENTROIDS procedure file is the first injection (DOSE) to verify calibration of mass axis. A procedure file is then run on the standard PCB solution calibration standard to check source condition and GC resolution. The appropriate data are recorded in a log book. The batch program is then executed to produce the QT output file needed to perform a calibration check. If many compounds are absent, it usually means the internal standard retention times must be adjusted in the ID file. Ion ratios almost never have to be adjusted. Even if all compounds are present after adjusting the ID file, the retention times should be closely checked to assure that they are no more than 0.2 minute from expected. Once the QT output file is acceptable for all standards runs, the calibration is checked.

6.4.3 At this time, spike and surrogate recoveries are calculated and examined for acceptability insofar as method performance is concerned. Surrogates (PCBs #65 and #166) are spiked into every sample and blank. The matrix spike is spiked into a hatchery fish at 30 times background PCB levels. These spikes are made directly into the fish/sodium sulfate mixture immediately before solvent extraction. For a given sample set, acceptance values for spike recoveries are specified in Table 7.1 of the Mass Balance QAPjP If matrix spike recoveries do not meet these standards, then data from that sample set are flagged. If surrogate spike recoveries do not meet these standards, then that sample must be evaluated according to the QAPjP If the flags are determined to be serious samples must be re-extracted and analyzed. The spike ID file is MBSPK. The surrogate ID file is MBSUR.

6.4.4 Once checking of standards runs is satisfactorily completed, the ID file is updated for a final time using the response factors from the calibration standard by executing the QCAL command file. The final QT reports are then produced. The value for the internal standards in the ID file may have to be adjusted to reflect differing final volumes or initial weights.

6.4.5 At this point a comparison of duplicates is made, and results for the check fish run during the batch are compared to values representing an average of six or more check fish ran previously. The acceptance values for duplicates and for the check fish are specified in Table 7.1 of the NBS/CLSO QAPjP.

7.0 Method Performance

As part of NBS-GLSC internal ongoing quality control and performance monitoring, check fish samples are run as deemed to be necessary (usually one per sample batch). These are run as ordinary samples and the results are tracked for consistency over time. Baseline data for check fish samples are generated by a one time extraction of six replicate lab reference fish samples. The replicates are processed through sample preparation and analyzed in the normal manner. The concentration and standard deviations (N=6) are calculated for the parameters routinely analyzed. Variation in the check fish samples over the course of many extraction batches can be expected to be no better, at best, than this baseline data.

Table 1. PCB Congeners/trans-nonachlor to be Determined by GC/NCI/SIM.

	Compound	Instrument Detection Limit using 1 g sample (ng/g)
1.	PCB Congener #31+#28	9
2.	PCB Congener #33	4
3.	PCB Congener #22	4
4.	PCB Congener #52	12
5.	PCB Congener #49	18
6.	PCB Congener #47+#48	6
7.	PCB Congener #44	25
8.	PCB Congener #42	4
9.	PCB Congener #41+#71	18
10.	PCB Congener #64	4
11.	PCB Congener #40	7
12.	PCB Congener #63	0.4
13.	PCB Congener #74	2
14	PCB Congener#70 + #76	1
15.	PCB Congener #66	2
16.	PCB Congener #95	6
17.	PCB Congener #91	7
18.	PCB Congener #56+#60	1
19.	PCB Congener #84+#92+#89	1
20.	PCB Congener #101	0.2
21.	PCB Congener #99	0.4
22.	Trans-nonachlor	0.08
23.	PCB Congener #119	0.1
24.	PCB Congener #83	0.6
25.	PCB Congener #97	0.9
26.	PCB Congener #81 + #87	0.6
27.	PCB Congener #85	0.3
28.	PCB Congener #77	0.2
29.	PCB Congener #110	0.5
30.	PCB Congener #82	1
31.	PCB Congener #151	0.02
32.	PCB Congener #144 + #135	0.03
33.	PCB Congener #107	0.3
34.	PCB Congener #123	0.1
35.	PCB Congener #149	0.04
36.	PCB Congener #118	0.3
37.	PCB Congener #134	0.02
38.	PCB Congener #114	0.4
39.	PCB Congener #131	0.01
40.	PCB Congener #146	0.01
41.	PCB Congener #132 + #153	0.02
42	PCB Congener #105	0.02
43	PCB Congener #141	0.1

Table 1. PCB Congeners/trans-nonachlor to be Determined by GC/NCI/SIM. (Cont'd)

	Compound	Instrument Detection Limit using 1 g sample (ng/g)
44.	PCB Congener #137 + #176	0.08
45.	PCB Congener #138 + #163	0.04
46.	PCB Congener #158	0.03
47.	PCB Congener #129	0.01
48.	PCB Congener #126	0.03
49.	PCB Congener #178	0.1
50.	PCB Congener #175	0.1
51.	PCB Congener #187 + #182	0.08
52.	PCB Congener #183	0.06
53.	PCB Congener #128	0.02
54.	PCB Congener #167	0.03
55.	PCB Congener #185	0.04
56.	PCB Congener #174	0.09
57.	PCB Congener #177	0.1
58.	PCB Congener #202	0.2
59.	PCB Congener #171	0.1
60.	PCB Congener #156	0.04
61.	PCB Congener #173	0.06
62.	PCB Congener #157	0.03
63.	PCB Congener #200	0.2
64.	PCB Congener #172	0.04
65.	PCB Congener #197	0.04
66.	PCB Congener #180	0.07
67.	PCB Congener #193	0.08
68.	PCB Congener #191	0.1
69.	PCB Congener #199	0.2
70.	PCB Congener #170 + #190	0.09
71.	PCB Congener #198	0.1
72.	PCB Congener #201	0.3
73.	PCB Congener #203 + #196	0.4
74.	PCB Congener #189	0.1
75.	PCB Congener #195	0.1
76.	PCB Congener #208	0.07
77.	PCB Congener #207	0.1
78.	PCB Congener #194	0.1
79.	PCB Congener #205	0.2
80.	PCB Congener #206	0.2
81.	PCB Congener #209	0.07

Table 2. GC and Autosampler Operating Parameters

1. Column - 30 meter DB-5 (J&W Scientific), .25 mm I.D., .25 micron film thickness.

2. GC Temperature Program Initial temperature 80°C, hold for one minute, then program to 150° at 20°/minute, then program to 250° at 2°/minute, hold five minutes. Post-run bakeout is 300° for six minutes.

3. Oven Equilibration Time - three minutes
   Total Run Time - 59 minutes
   Scanning Start Time - four minutes
   Splitless Operation Time - two minutes

4. Injection Port Temperature - 280°C
   GC/MS Interface Temperature - 280°C

5. Sample Injection Volume 2 microliters. Data Cartridge Space - Minimum 600 tracks available space for a 24-run batch. Minimum Blocks Reserved for CR Check 3000.

6. Carrier Gas - Helium at 10-15 psi column head pressure. This can vary, depending mostly on column age.

Table 3. Mass Spectrometer Operating Parameters

1. Source Temperature - 110°C

2. Multiplier Voltage - 1400-2800V (depending on stage of multiplier life)

3. Emission Current - 300 µA

4. Electron Energy - 200 eV

5. Reagent Gas Methane

6. Source Pressure - (0.5 to 0.8 Torr).

7. SIM Groupings - Group 1, run from 4.0 to 16.3 minutes
   Group 2, run from 16.3 to 21.5 minutes
   Group 3, run from 21.5 to 26.0 minutes
   Group 4, run from 26.0 to 30.0 minutes
   Group 5, run from 30.0 to 35.5 minutes
   Group 6, run from 35.5 to 39.85 minutes
   Group 7, run from 39.85 to 45.6 minutes
   Group 8, run from 45.6 to 46.3 minutes
   Group 9, run from 46.3 to 50.0 minutes
   Group 10, run from 50.0 to 59.0 minutes

Table 3. Mass Spectrometer Operating Parameters (Cont'd)

Group 1	Exact masses 254.8, 254.9, 255, 256.8, 256.9, 257 (Lindane) each at dwell times of 150 milliseconds.
Group 2	Exact masses 255.86, 255.87, 255.88, 255.89, 256.9 256.91, 256.92, 256.93, 256.94, 256.95, 257.86, 257.87, 257.88, 257.89, 257.9, 257.91, 257.92, 257.93, 257.94, 257.95 (Trichlorobiphenyls) each at dwell times of 45 milliseconds.
Group 3	Exact masses 289.86, 289.87, 289.88, 289.89, 289.9 289.91, 289.92, 289.93, 289.94, 289.95, 291.86, 291.87, 291.88, 291.89, 291.9, 291.91, 291.92, 291.93, 291.94, 291.95 (Tetrachlorobiphenyls) each at dwell times of 45 milliseconds.
Group 4	Exact masses 289.79, 289.8, 289.9, 291.79, 291.8, 291.9 (Tetrachlorobiphenyls), Exact masses 325.79, 325.8, 325.9, 327.79, 327.8, 327.9 (Pentachlorobiphenyls), Exact masses 441.6, 441.7, 443.6, 443.7 (Trans-nonachlor) each at dwell times of 55 milliseconds.
Group 5	Exact masses 289.79, 289.8, 291.79, 291.8 (Tetrachlorobiphenyls), Exact masses 315.8, 315.9, 317.8, 317.9(4,4'-DDE), Exact masses 325.8, 325.9, 327.8, 327.9 (Pentachlorobiphenyls), Exact masses 359.7, 359.8, 358.9, 361.8 (Hexachlorobiphenyls), Exact masses 379.7, 379.8, 381.7, 381.8 (Dieldrin) each at dwell times of 45 milliseconds.
Group 6	Exact masses 325.77, 325.78, 325.79, 325.8, 325.81, 325.82, 327.77, 327.78, 327.79, 327.8, 327.81, 327.82, (Pentachlorobiphenyls), Exact masses 359.7, 359.8, 361.7, 361.8 (Hexachlorobiphenyls), Exact masses 393.6, 393.7, 395.6, 395.7 (Heptachlorobiphenyls) each at dwell times of 45 milliseconds.
Group 7	Exact masses 359.69, 359.7, 359.8, 361.69, 361.7, 361.8, (Hexachlorobiphenyls), Exact masses 393.59, 393.6, 393.7, 395.59, 395.6, 395.7 (Heptachlorobiphenyls), Exact masses 427.59, 427.6, 427.7, 429.59, 429.6, 429.7 (Octachlorobiphenyls) each at dwell times of 50 milliseconds.
Group 8	Exact masses 359.66-359.74 (10 ions) and exact masses 359.76-359.84 (10 ions), each at dwell times of 45 milliseconds (PCB #169).
Group 9	Same exact masses and dwell times as Group 7, except add exact masses 463.6, 465.6 (Nonachlorobiphenyls) and reduce each dwell time to 45 milliseconds.
Group 10	Exact masses 427.68, 427.69, 427.7, 427.71, 427.72, 429.68, 429.69, 429.7, 429.71, 429.72 (Octachlorobiphenyls), Exact masses 463.59, 463.6, 463.61, 465.59, 465.6, 465.61 (Nonachlorobiphenyls), Exact masses 497.49, 497.50, 499.49, 499.50 (Decachlorobiphenyl), each at dwell times of 55 milliseconds.

Volume 2

Chapter 2: Mercury Analysis

Standard Operating Procedure for Analysis of Vapor Phase Mercury

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Version 2.0

Standard Operating Procedure for Analysis of Vapor Phase Mercury

1.0 Introduction to Principals of Vapor Phase Mercury Sampling and Analysis

Mercury in the atmosphere exists predominantly in the gas phase in the form of elemental mercury (Schroeder, 1982). Other species of mercury found in the gas phase include methyl and dimethylmercury, and mercuric chloride, mercuric hydroxide and free divalent mercury. Vapor phase mercury is quantitatively removed from an air stream by amalgamation onto gold. While the amalgamation process is believed to remove most vapor phase mercury species with >99% efficiency, the analytical procedure employed determines whether or not 'total mercury' or predominantly elemental mercury is quantified. At the University of Michigan Air Quality Laboratory (UMAQL) vapor phase mercury is collected onto gold-coated borosilicate glass bead traps by drawing air at a low flow rate through a baked glass fiber pre-filter followed by the gold-coated borosilicate glass bead trap. The air is prefiltered to eliminate particles from the gas phase collection traps. After sampling, vapor phase mercury is quantified by cold vapor atomic florescence spectrometry (CVAFS).

In the past, methods for collection of vapor phase mercury have dictated long sampling duration, often from 24 hours up to a week. The collection method employed for the Lake Michigan Loading Study and described in this protocol uses gold-coated borosilicate glass bead traps, which UMAQL has determined to be >99% efficient at collection of vapor phase mercury (at a flow rate <1 lpm). Dual-amalgamation and subsequent analysis by cold-vapor atomic florescence, allows detection of mercury at picogram levels. After thermal desorption, gold-coated bead traps are re-used since they do not exhibit memory effects. Due to the collection efficiency of gold-coated beads and the ability to detect picogram amounts of mercury, sampling strategies using gold-coated bead traps can employ much shorter duration samples than have previously been possible. Short sampling duration provides the resolution necessary to use receptor models in determining sources and source contributions of measured vapor phase mercury.

Preparation and collection of accurate and reliable data on mercury concentrations in environmental samples requires that ultra clean procedures are used. All sampling supplies with which a sample will come into contact must be acid cleaned in a Class 100 Clean Room. At the sampling site, precautions taken to avoid contamination of the sample include storing samples at an outdoor staging area and special operator handling. These and other techniques employed to minimize contamination of the samples are described in detail in this protocol.

2.0 Sample Preparation

2.1 Acid Cleaning Procedure

All Teflon filter packs, Teflon jars, Teflon tubing, gold trap fittings and end plugs (referred to below as 'supplies') are cleaned using an 11 day procedure described by Rossmann and Barres (1991).

Supplies to be acid cleaned are first rinsed in reagent grade acetone under a fume hood, then washed in hot tap water and diluted Alconox. Supplies are rinsed five times in cold tap water then rinsed three times in DI water. The supplies are then heated in 3M hydrochloric acid (EM Science Tracepur HCl in Milli-Q water (18.2 MΩ/cm)) for six hours at 80° C. One liter of 3M HCl is prepared by adding 750 mL of Milli-Q water to 250 mL of concentrated EM Science Tracepur HCl. The 3M HCl can be used several times and is stored for reuse in a polyethylene carboy dedicated for this purpose. The supplies are placed into clean polyethylene tubs which are then filled with the 3M HCl, making sure that all of the surfaces are submersed in the HCl. The tubs are covered and placed in a water bath which is heated to 80°C in a fume hood. The water in the bath is maintained at the level of the acid inside the tubs. After the water in the bath reaches 80°C, the supplies in the tubs are allowed to soak for 6 hours.

After the 6 hour, 80°C soak, the tubs are removed from the water bath and allowed to cool in the fume hood. When cool, the 3 M HCl is poured back into its polyethylene carboy. The supplies are rinsed in the tubs three times with Milli-Q water. The supplies are then soaked in a 0.56M nitric acid solution (Baker Instra-Analyzed HNO_3 in Milli-Q water) for 72 hours at room temperature in the same polyethylene tubs in which they were heated with HCl. The nitric acid solution is made by adding 35 mL Baker Instra-Analyzed HNO_3 to 965 mL of Milli-Q water. Nitric acid is reused for up to 6 months and is stored in a carboy dedicated for HNO_3. At the end of the 3 day soak, the supplies being cleaned are rinsed three times with Milli-Q water and transferred into a Class 100 Clean Room.

Inside the clean room, the supplies are again rinsed three times with Milli-Q water. The tubs containing the supplies are filled with 0.56 M Baker Instra-Analyzed HNO_3 that is kept in the clean room and is dedicated for this final step only. The supplies are then allowed to soak in this acid for 7 days. This acid is prepared by adding 35 mL of the Instra-Analyzed HNO_3 to 965 mL of Milli-Q water. At the end of the seven day acid soak inside the clean room, the supplies are rinsed five times with Milli-Q water and allowed to air dry on a clean surface. When the supplies are dry, they are triple bagged in new polyethylene bags and removed from the clean room, ready for use in sampling.

2.2 Preparation of Gold-Coated Bead Traps and Pre-Filters

Gold-coated borosilicate glass bead traps are constructed at The University of Michigan Air Quality Laboratory and tested prior to use in the field. The gold-coated beads used in the traps are made by generating a gold plasma under vacuum conditions that uniformly deposits onto the surface of the beads. The thickness of the coating generated using this process is about 300 Å. The gold-coated beads are contained in a quartz tube which is 10 cm long with an inner diameter of 5 mm and an outer diameter of 7 mm. Teflon heat-shrink tubing is attached to both ends of the tube into which Teflon endplugs are placed when the trap is in storage or connectors when the trap is being used to collect a sample. Each trap contains approximately 0.7 g of gold-coated borosilicate glass beads which are held in place using quartz wool and two sets of three radial indentations in the quartz tube. The gold-coated beads, quartz tubes and quartz wool are baked at 600°C for one hour prior to making the trap. In addition, Teflon endplugs and heat shrink tubing are acid cleaned as previously described. After each trap is made, it is given a unique number identifier in order to chart the history and performance of the trap. New traps are first conditioned by drawing approximately 0.4 m³ of air through the trap then heating the trap to 500°C for 5 minutes. Inert gas is purged through the traps at 300 cc/min during heating procedure to remove moisture and other volatile constituents. The conditioning procedure is performed twice prior to

testing the trap. The trap is then tested by injecting a known amount of elemental mercury vapor and comparing the result to an analytical standard (standard generation is described on page 9). The trap must exhibit duplicate measurements that are within 5% of the standard and the replicate measurements must also be within 5% of each other. Following this test, the trap is then blanked (described below) and stored for 7 days. After 7 days, the trap is analyzed for a storage blank (sample analysis is described in Section 3.0). The storage blank must be less than 15 pg for the trap to be accepted for use in field sampling. Gold traps are stored with endplugs in place, triple bagged in polyethylene before and after sampling.

Just before going into the field to collect vapor phase mercury samples, gold-coated bead traps are blanked again. Blanking a trap removes all mercury from the gold-coated bead surface and will also remove water vapor and other unwanted constituents. Traps are blanked by placing them in the analytical train and heating them to 500°C for 2 minutes, identical to a normal sample analysis.

Vapor phase mercury samples collected onto gold-coated borosilicate glass bead traps must be prefiltered to exclude particles. Glass fiber filters (Gelman Sciences) are pre-treated to remove all mercury prior to use in sampling. Glass fiber filters, 47 mm in diameter, are placed in a clean crucible with a lid. The crucible is placed in a muffle furnace which is heated to 500°C and the filters are allowed to bake at this temperature for one hour. While hot, filters are removed from the crucible with acid-cleaned Teflon-coated forceps and placed in an acid-cleaned Teflon jar which is closed and sealed with Teflon tape. The Teflon jar is triple bagged and stored at -40°C until use. Filters are stored no more than 3 months prior to use and frequent blanks are taken to ensure the filters remain clean.

3.0 Sample Analysis

3.1 Summary

Vapor phase mercury collected onto a gold-coated bead trap is thermally desorbed and carried by a mercury-free helium stream into a cold-vapor atomic fluorescence (CVAFS) analyzer where the mercury atoms are excited by light of wavelength 253.7 nm. These excited atoms fluoresce in the same wavelength which is detected by a photomultiplier tube and the signal is converted to voltage which is sent to an integrator.

Gold-coated bead traps are >99% efficient at collecting mercury in its various forms in the atmosphere. In sampling locations away from local sources the predominant form of mercury in the atmosphere is elemental mercury, however, in source regions, there may be an important fraction of other mercury species present. The temperature used to desorb mercury from the traps determines whether or not a mercury compound (other than elemental mercury) released from the gold is in elemental form or a molecular form. Typically, when gold-coated bead traps are employed, a desorption temperature of 500° C or less is used since the melting point of borosilicate glass is typically about 650° C (rendering the trap unusable). At 500° C mercury compounds will not quantitatively be converted into elemental mercury. Published values for temperatures required to break methyl mercury bonds approach 1000° C. Therefore, in environments in which there may be mercury species other than elemental mercury, in the atmosphere, it is incorrect to term this CVAFS procedure an analysis of "total gaseous phase mercury". At UMAQL a desorption temperature of 500° C is employed and results of this analysis are referred to simply as "vapor phase mercury".

3.2 Sample Analysis and Data Acquisition

The CVAFS analyzer used for vapor phase mercury analysis is operated in a normal laboratory setting (outside of the clean room) because the risk of contamination of the samples is minimal. The instrument is keep on at all times, since this has been shown to stabilize the UV lamp and maintain consistency from one day to the next. The power supplied to the CVAFS analyzer is modulated by a line tamer (Shape Magnetronics) to prevent power fluctuations. It is imperative that the mercury lamp not experience wide temperature fluctuations or power surges since both of these drastically affect the sensitivity of the instrument. During operation of the instrument the helium carrier gas flow rate is regulated upstream of the analyzer using a mass flow controller (Tylan) which is set to maintain a 35 cc/min flow rate. This flow rate has been determined by UMAQL to yield the optimal peak characteristics for mercury standards. The regulator on the helium cylinder is set at 50 Kilopascals. The helium stream is prefiltered using a gold-coated trap before entering the analytical train in order to remove any mercury. In the analytical train, mercury is thermally desorbed from the sample trap, and amalgamated onto the analytical trap which is subsequently thermally desorbed into the CVAFS analyzer where the mercury atoms are detected. Traps are desorbed by heating a nichrome coil which is wrapped around the trap covering the gold-coated beads. Application of 12 volts of current to the coil is sufficient to achieve a temperature of 500° C inside the gold bead trap (voltage may vary due to variations in length and thickness of nichrome wire). Two fans supply cool air to the sample and analytical trap separately in order to speed analysis time.

For comparability between laboratories UMAQL sets the gain on the CVAFS analyzer to yield an approximate 1000 mV net response for a 1 ng mercury standard. The background on the CVAFS analyzer is set at 5.0 and maintained in that position in order to track the drift in the baseline of the analyzer. A Hewlett Packard Integrator is connected to the analyzer to convert output signal into an integrated area of the detected response. Area units are used for all sample calculations since area is much more reliable than peak height.

Particle-free clean room gloves are worn during all procedures. To analyze a sample trap, the trap is placed snugly into the analytical train using friction fit Teflon connectors and Teflon sleeves. The nichrome coil used specifically for the sample trap is slid over the trap and moved to completely cover the quartz wool plugs and the gold-coated beads contained between the plugs. Helium is allowed to flow through the sample trap for 2 minutes before analysis begins in order to purge air and water vapor from the analytical train. A circuit controller (ChonTrol) is employed which is programmed to turn on the variable transformers and fans in a precise and reproducible manner. First, the sample coil is heated for 2 minutes, then it is cooled while the analytical trap is heated for 2 minutes. The analytical trap is then cooled for 2.5 minutes and the fan to the sample trap is turned off. While the analytical trap is cooling, a new sample trap is installed in the analytical train and helium is passed through this trap until the analytical trap is cool and ready for another sample. When the analytical trap begins heating, the integrator is turned on and the ambient temperature, time and base mV are recorded in a log book and the LCD display on the analyzer is set to record the peak mV (by depressing the Peak button on the face of the analyzer). After the sample is analyzed and the peak height and area reported by the CVAFS and integrator respectively, these values are recorded in the log book.

A standard curve is analyzed at the beginning of each day of analysis and a control standard which yields a response in the range of the samples being analyzed is run every six samples. Criteria for the standard curves and control standards are described below in Section 3.3. All sample analysis is recorded in a log book specific to the analyzer with which samples are being quantified and also in a lab notebook specific to the study for which the samples were collected. At the end of the day of analysis all results from the log sheet are entered into an Excel spreadsheet for subsequent checking and processing by a statistical software program, SAS (Cary, NC).

3.3 Standard Curve and Control Standards

A standard curve, generated by injecting different volumes of mercury saturated air, is analyzed before each day of analysis. The amount of mercury per volume of mercury saturated air depends on the temperature of the mercury saturated air. This relationship is described by the Ideal Gas Law. The amount of mercury injected for the calibration curve is tailored to the expected value of the samples to be analyzed. At UMAQL, a typical calibration curve consists of five injection volumes: 0 mL, 20 mL, 40 mL, 80 mL and 160 mL of mercury saturated air, which represent mercury masses shown in Table 1.

Standards for the calibration curve are generated by withdrawing known volumes of gaseous elemental mercury from the headspace of a closed 150 mL flask containing about 2-3 mL of metallic mercury. The flask is maintained below ambient temperature by being immersed in a constant temperature (+\- 0.1° C) circulating water bath. The temperature of the circulating water bath and thus the flask of mercury saturated air is usually maintained at a temperature of 16.6° C while ambient temperature is about 22-24° C. The temperature of the flask must be maintained below ambient temperature otherwise, the mercury injected will condense on the walls of the injection apparatus. Also, the flask is purged periodically with N_2 in order to displace oxygen which may oxidize the surface of the mercury. The mercury is withdrawn from the flask using a gas tight syringe (Hamilton) and is injected onto and quantitatively captured by a gold coated bead trap. The trap is then thermally desorbed and analyzed using the CVAFS analyzer.

At the start of each day of analysis, the syringe is "conditioned" for 15 minutes. The syringe is conditioned by flushing it three times with mercury saturated air, then the maximum amount of mercury saturated air is pulled into the syringe and the needle is left inside the flask for 15 minutes. While the syringe is conditioning, the injection port is inserted into the analytical train and is connected to a gold coated bead test trap using friction fit Teflon connectors. The injection port is fitted with a Minnert valve and Teflon coated silicon septum. After 15 minutes, the syringe is flushed with mercury saturated air three times and 80 mL is withdrawn and injected onto the test trap to "condition" the injection port. The injection port is conditioned for a total of three times before the syringe and injection port are ready to be used for a calibration curve. This conditioning process is necessary before each day of analysis to ensure precise and reproducible results.

The standard curve is generated starting with the zero point (0 mL) and continued in ascending order to the highest volume, usually 160 mL. First, a blanked gold-coated bead trap is attached to the downstream end of the injection port. Prior to withdrawing the necessary volume of mercury saturated air for each injection, the temperature of the flask is recorded and the syringe is flushed three times with mercury saturated air. After flushing the syringe, the required volume of mercury saturated air is withdrawn. The syringe is then removed from the flask and the mercury saturated air is injected onto the gold trap via the injection port as quickly as possible to avoid diffusion of

mercury from the needle tip. After the injection is complete, the syringe is returned to the flask and the maximum amount of mercury saturated air is pulled into the syringe and allowed to sit until the next injection. After the trap is loaded with a standard amount of mercury, the injection port is removed from the analytical train and the trap is analyzed as described above. The injection port is kept sealed in a polyethylene bag between injections.

The zero point of the calibration curve (0 mL) is generated in the same manner described above except that no mercury saturated air is withdrawn into the syringe. Instead, the syringe is flushed three times and fully depressed prior to removing the syringe from the flask and the needle is promptly inserted and removed from the injection port. The zero point indicates the amount of mercury that comes from the needle tip and the injection apparatus and is usually between 1-6 pg The 0 mL injection is also a good indication of when the septum in the injection port needs to be changed. If the 0 mL injection is higher than 10 pg then the septum is changed. Otherwise, the septum is changed after loading 30 traps for standards or controls.

At any given temperature the vapor density of mercury can be calculated using the Ideal Gas Law and the saturation vapor pressure of mercury. A table of associated vapor densities versus temperature has been compiled and is used to determine the amount of mercury injected for each standard. Table 1 lists the amount of mercury delivered for the 5 injection volumes used to generate the standard curve at a temperature of 16.6° C.

Table 1. Amount of Mercury Injected for a Typical Calibration Curve

Volume of Mercury Saturated Air Injected (mL)	Amount of Mercury Injected (ng)
0	0
20	0.198
40	0.396
80	0.793
120	1.190

Flask temperature = 16.6 °C
Vapor density = 9.912 ng/cm^3
1 cm^3 = 1000 mL

After the standards for the calibration curve have been analyzed, a linear regression is calculated to establish the coefficient of determination (r^2), the slope of the line and how well the slope of the curve predicts each of the points in the calibration curve. The slope of the line is forced through

zero and the 0 mL injection area is subtracted from each of the points on the curve. The r^2 must be >0.999 and each of the points on the curve must be predicted by the slope within 5% of their true value (Table 2). If these criteria are not met, specific points which are errant are repeated and the linear regression recalculated.

Table 2. Example Calibration Curve and Calculation of Slope:

Standard (ng Hg)	Response Area Units	Response - Zero Point	Predicted Value (ng Hg)
0	71,219	0	0
0.198	2,286,400	2,215,181	0.197
0.396	4,495,200	4,423,981	0.393
0.793	9,089,300	8,946,862	0.794
1.190	13,597,000	13,383,343	1.190

Slope = 8.8886E-8 ng/AU
$Slope^{-1}$ = 11,250,357 AU/ng
r^2 = 0.9999

This curve is accepted and sample analysis commences.

Control standards are analyzed every sixth sample. The control standards are generated in the same manner as described above and are chosen to be representative of the samples being analyzed, usually an 80 mL injection. The integrated area from each of the control standards must be within 5% of the slope of the calibration curve in order to continue analyzing. If this is not the case, a second control is analyzed immediately. If the second control indicates that analyzer sensitivity has changed a second calibration curve is generated and sample analysis is continued.

The mercury flask is checked weekly to determine if the surface of the mercury is oxidized. If there is visible discoloration on the surface of the mercury then the flask is purged for 15 minutes with N_2 to reduce the oxidized layer. Also, to determine if water has entered the flask the mercury is gently swirled around the bottom of the flask. If the mercury moves around the flask without adhesion to the glass then there is no water in the flask.

3.4 Calculation of Mercury Concentration in Sample

The vapor phase mercury concentration from a set of gold-coated bead traps is calculated in ng/m^3 First, the amount of mercury detected on each of the sample traps (A & B) is calculated by multiplying the integrator response from the individual trap by the slope of the calibration curve which is in ng/Area Unit (AU). The product is ng of mercury (Table 3). Each trap is field blank corrected and the results are summed to yield the total mercury mass collected. The calculated value, in nanograms of mercury is converted to ng Hg/m^3 by calculating the total volume of air drawn through the sample and dividing the ng of mercury by the cubic meters of air sampled.

Table 3. Calculation of ng Hg/m^3 in a Vapor Phase Sample.

1. Calculation of ng of mercury recovered from a set of sample traps

ng Hg on the Sample Trap (A) = Response (AU) x Slope of the calibration curve (ng/AU)
7,135,900 x 8.8886E -8 = 0.634 ng Hg

ng Hg on the Sample Trap (B) = Response (AU) x Slope of the calibration curve (ng/AU)
281,560 x 8.8886E -8 = 0.025 ng Hg

2. Field blank correction and summation of traps
Corrected ng Hg on Sample Traps = (ng on trap A + ng on trap B) - 2(mean ng of field blanks)
(0.634 + 0.025) - 2(0.016) = 0.627

3. Calculation of m^3 sampled at a flow rate of 300 cc/min and a sample duration of 24 hours:

Flow Rate x Duration of Sample = Volume of Air Sampled

$$\frac{300ml}{\text{min}} \; x \; 24\ hr \; x \; \frac{1{,}440\ \text{min}}{24\ hr} \; x \; \frac{m^3}{1{,}000{,}000\ ml} = 0.432\ m^3$$

4. Concentration of Vapor Phase Mercury in Sample = ng Hg/m^3

$$\frac{0.627\ \text{ng Hg}}{0.432\ m^3} = 1.451\ \text{ng Hg} / m^3$$

3.5 Trouble-Shooting

A source of irreproducible results may be due to faulty gold-coated bead traps. These traps are numbered with discrete identifiers. All samples must be identified with a particular trap number and trap performance must be tracked. Contact with halogen fumes, organic fumes or overheating of the trap during analysis can damage the trap, rendering it unusable. If performance of a gold trap is suspect, at least two consecutive standards are analyzed from this trap to determine its ability to amalgamate and release mercury.

Another source of irreproducible results could be from water or water vapor seeping into the flask containing mercury. If this is the case, the mercury in the flask is properly discarded and the flask is rinsed with 0.56M HNO_3 and allowed to thoroughly dry. After drying, the flask is replenished with about 2-3 mL of triple distilled metallic mercury and purged with clean N_2 for 5 minutes. The flask is then be returned to the constant temperature water bath and allowed to thermally equilibrate and to reach saturation for at least 3 hours.

If a low response is observed, the trap loading apparatus is checked for leaks, especially the plunger tip in the syringe or the where the needle contacts the plunger tip. If the plunger tip is worn, the syringe will no longer be gas tight and needs to be carefully replaced without damaging the plunger tip. If there is any material (Teflon shavings, etc.) in the base of the needle where it contacts the plunger tip, the material is wiped away with a particle-free clean wipe. Also, the side port hole in the needle tip is checked to make sure that it is not clogged with any foreign material (Teflon from the septum, etc.).

If peak-broadening is observed or no peak is detected in a sample, the analytical train is checked for leaks. Peak broadening is often the result of low gas flow, water vapor on the gold-coated bead trap, inadequate heating of analytical trap or an analytical trap damaged by exposure to halogen fumes or overheating. Analytical traps are replaced only when a potential problem is suspected with the trap.

If a broad peak is observed directly after the sharp mercury peak then the problem is most likely a thermally or chemically damaged trap and the line from the analytical trap to the detector is replaced as gold atoms may have been liberated from the trap and migrated down stream.

If the baseline drifts more than 10% the UV lamp is replaced. After replacement, the analyzer is allowed to equilibrate for 24 hours. If the problem persists, sources of power fluctuation, drafts or air currents that may be changing the temperature of the UV lamp are investigated.

Room temperature in which the CVAFS is located is maintained between 20-22 C, however, if the temperature exceeds 26° C analysis is stopped, since instrumental noise increases significantly.

4.0 Performance Criteria, Quality Assurance and Quality Control

4.1 Field operators are carefully instructed in the techniques of contaminant-free vapor phase mercury collection. All of the operators are currently operating sampling equipment for either the National Dry Deposition Network, the National Atmospheric Deposition Program, the Integrated Atmospheric Deposition Network or the Great Lakes Acid Deposition Network.

4.2 Every 6 months UMAQL personnel will inspect the sampling sites to audit the sampling equipment and make all necessary repairs or adjustments.

4.3 Co-located samples are collected from one sampling site during the study to quantify method precision. Reported concentrations are based on the mean of the two co-located samples.

4.4 Precision and accuracy levels will be set and maintained for each type of analysis. A relative precision for total mercury of less than 10% is maintained for samples with values at least 3 standard deviations greater than the detection limit. Analysis of standards and controls is within 5% of the stated value.

4.5 A minimum of 25% of all samples analyzed are field blanks or analytical blanks to ensure that samples are collected in a contaminant-free manner.

4.6 Every 3 months maintenance on the CVAFS analyzer is conducted, including replacement of the UV lamp, the Teflon tubing, and the detection cell.

4.7 Gold traps are checked prior to every sample with 0.8 ng of mercury in order to ensure that their use during the previous sample collection has not diminished trap performance.

5.0 References

5.1 Bloom, N.S. and Fitzgerald, W.F (1988) Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection. *Anal. Chem. Acta.* 208, 151.

5.2 Dumarey, R., Temmerman, E., Dams, R. and Hoste, J. (1985) The Accuracy of the Vapour-Injection Calibration of Mercury by Amalgamation/Cold-Vapour Atomic Absorption Spectrometry. *Anal. Chem.. Acta.* 170, 337-340.

5.3 Dumarey, R., Dams, R., and Hoste, J. (1985) Comparison of the collection and desorption efficiency of activated charcoal, silver, and gold for the determination of vapor-phase atmospheric mercury. *Anal. Chem.* 57, 2638-2643.

5.4 Fitzgerald, W.F., and Gill, G.A. (1979) Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis. *Anal. Chem.* 15, 1714.

5.5 Lindberg, S.E. (1981) Author's Reply 'Mercury partitioning in a power plant plume and its influence on atmospheric removal mechanisms.' Atmos. Environ. 15, 631-635.

5.6 Rossmann, R. and Barres, J. (1991) Trace element concentrations in near-surface waters of the Great Lakes and methods of collection, storage, and analysis *J. Great Lakes Res.* 14,:188.

5.7 Schroeder, W.H. (1982) Sampling and analysis of mercury and its compounds in the atmosphere. *Environ. Sci.. Technol.* 16, 394-400.

APPENDIX A: FACILITIES, EQUIPMENT AND REAGENTS

Following is a list of the required facilities, equipment, supplies and reagents for sample preparation, sample collection and sample analysis that are outlined in this document. The make and model of the following items are those used at The University of Michigan Air Quality Laboratory. Many of these items are available from a variety of sources.

1. Preparation of Field Supplies

   Class 100 Clean Room, Work Stations
   Clean Room Gloves
   Particle-free Wipes
   Clean Room Cap, Gown and Boots
   Milli-Q Water (18.2MΩ/cm)
   Exhaust Hood
   Acetone
   Alconox
   Polyethylene Tubs
   EM Science Tracepur and Suprapur Hydrochloric Acid
   Polytherm Water Bath (Science/Electronics)
   Baker Instra-Analyzed or EM Science Suprapur Nitric Acid
   New Polyethylene Bags
   20 Liter Polyethylene Carboys

2. Sample Collection

   Mass Flow Controlled Vacuum Pump (URG, Model 3000-02M)
   Calibrated 300 cc/min Rotameter (Matheson)
   HDPE Tubing with quick connects
   Black Latex Tubing
   Mercury Sampling Box (UMAQL, See Appendix B)
   Acid-Cleaned 47 mm Teflon Filter Holders (Savillex, PFA Labware)
   47 mm Preheated Glass Fiber Filters (Gelman Sciences A/E)
   Acid-Cleaned Teflon Jars (Savillex, PFA Labware)
   Teflon-Coated Forceps
   'Blanked' Gold-Bead Traps (UMAQL)
   Teflon Endplugs
   Trap Heater & Variable Transformer
   Acid-Cleaned Teflon Tubing
   Particle-Free Gloves
   Teflon Tape
   Sample Labels
   Field Operator Log Book
   Shipping Boxes

3. Sample Analysis

Cold Vapor Atomic Florescence Detector (Brooks Rand, LTD.)
Line Tamer/Conditioner (Shape Magnetronics Model PCLT 150)
Integrator (Hewlett-Packard Model 3390A)
Helium, Ultra High Purity Grade (99.999%)
Mass Flow Controller (Tylan)
Nichrome Coils (UMAQL)
Electric Leads
Variable Transformers (Staco Energy Products Co. Type 3PN1010)
Cooling Fans
Gold-Coated Glass Bead Traps (UMAQL)
Gas Tight Syringe (Hamilton series 1800)
Injection Port (UMAQL)
Constant Temperature Circulating Water Bath (Fisher Model 901)
Instrument Grade Metallic Mercury (Triple Distilled)
Mercury Flask (UMAQL)
Certified Immersion Thermometer (Kessler Instruments, Inc. 15041A)

Standard Operating Procedure for Analysis of Mercury in Precipitation

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Version 2.0

Standard Operating Procedure for Analysis of Mercury in Precipitation

1.0 Introduction/Overview

The objective of the Lake Michigan Loading Study is to assess the contribution of atmospheric deposition to the concentration of mercury and other toxic trace species found in Lake Michigan The atmosphere has been implicated as one of the dominant sources of mercury and trace elements to bodies of water and it is clear from investigations in remote regions of the globe that long range transport of mercury and other toxics from source regions is occurring. By quantifying the wet deposition and ambient concentrations of mercury it will be possible to determine the relative importance of precipitation and dry deposition in accounting for the atmospheric loading of mercury to Lake Michigan. In addition, investigating other ambient trace species will aid in the identification of significant mercury sources.

2.0 Sample Handling and Processing

2.1 Sample Handling

Precipitation samples contain exceptionally low levels of trace metals and therefore these samples are processed with careful attention to avoid contamination. Blanks are routinely analyzed to ensure extremely low analyte levels and operating conditions.

When samples are received at UMAQL they are logged in, the tracking form sent with the sample is catalogued and the sample is taken to a staging area to be moved into the clean room. At the staging area outside the clean room, the outer polyethylene bag is removed from the sample. The double-bagged sample is placed in the outer room of the clean room, and the analyst suits up to enter the clean room according to laboratory protocol. As the sample is brought into the clean room the second outer bag is removed and left in the outer room of the clean room. The inner bag is removed inside the clean room and discarded.

2.2 Sample Processing

The precipitation volume of each sample is determined gravimetrically. Mercury samples are oxidized to 1% bromine monochloride volume/volume and are stored in a dark cold room overnight before being analyzed according to the following procedure.

3.0 Sample Analysis and Data Acquisition

3.1 Summary

Mercury is purged from solution in a mercury-free nitrogen stream after appropriate sample pretreatment and reduction. Mercury liberated from solution is concentrated on a gold-coated bead trap. A mercury-free pretreated soda lime trap is utilized in the purge system to capture acid gases

that may damage the gold-coated bead trap. The gold-coated bead trap is subsequently thermally desorbed and carried by a mercury-free helium stream into a cold-vapor atomic fluorescence (CVAFS) analyzer where the mercury atoms are excited by light of wavelength 253.7 nm. These excited atoms fluoresce in the same wavelength which is detected by a photomultiplier tube and the signal is converted to voltage which is sent to an integrator.

All analytical procedures for determination of mercury in precipitation are carried inside a Class 100 Clean Room. Volatilization/Recapture (Section 3.3.1) is carried out in a class 100 laminar flow exhaust hood inside the clean room. Nitrogen utilized for purging is 99.998% pure and is stripped of any mercury using a gold coated trap before use in the purge system. Clean room gloves are worn at all times and all labware with which the samples and reagents comes into contact is cleaned weekly using the acid cleaning procedure described in *Standard Operating Procedure for Sampling of Mercury in Precipitation*, Section 2.1.

3.2 Reagents and Materials

All reagent lot numbers, preparation dates and procedures are recorded for each new batch of reagent used. A reagent blank is obtained after each new batch of reagent has been prepared. Bromine monochloride (BrCl), stannous chloride ($SnCl_2$) and hydroxylamine hydrochloride ($NH_2OH{\bullet}HCl$) are prepared fresh monthly.

Solid reagents (potassium bromide, potassium bromate, hydroxylamine hydrochloride and stannous chloride) are stored in the clean room in a desiccator containing silica gel and an open bed of activated charcoal. The caps of all reagent bottles are Teflon taped to reduce entry of vapor phase compounds. Even with these precautions, reagents will nevertheless absorb mercury over time and must be replaced. All reagents are made in the clean room, except the working standard solution.

3.2.1 Hydrochloric Acid

EM Science Suprapur hydrochloric acid is used to prepare BrCl and $SnCl_2$. This acid characteristically has a very low blank value (20 pg/mL).

3.2.2 Bromine Monochloride

Bromine monochloride is prepared in a class 100 laminar flow exhaust hood by adding 11.0 mg KBr per mL of HCl while the solution is stirred using an acid-cleaned Teflon-coated magnestir. When the KBr is dissolved, 15.0 mg $KBrO_3$ per mL of HCl is added slowly and the solution is allowed to continue stirring. This process produces chlorine and bromine gas and must be performed slowly in a functioning fume hood. After addition of the salts the solution is a deep yellow color. If there is no color (or very faint) then the BrCl has been substantially reduced and will not have enough oxidizing power for use. In this case, the solution is remade. Bromine monochloride is stored at room temperature in the clean room. Fresh bromine monochloride is be prepared monthly or as needed.

3.2.3 Hydroxylamine Hydrochloride

30 grams of $NH_2OH.HCl$ is dissolved in MQ-water to make 100 mL in an acid-cleaned 100 mL volumetric flask. This solution is purified by adding 0.5 mL of $SnCl_2$ and purging overnight with Hg-free N_2. The solution is stored in an acid-cleaned, dark Teflon bottle in the refrigerator. Fresh hydroxylamine solution is prepared every month or as needed.

3.2.4 Stannous Chloride

20.0 grams of $SnCl_2.H_2O$ is placed into an acid-cleaned 100 mL volumetric flask. Working in a fume hood, 10 mL of concentrated HCl is added and the solution is then brought to 100 mL with Milli-Q water. The solution is stored in an acid-cleaned, dark Teflon bottle in the refrigerator. Fresh stannous chloride is prepared every month or as needed.

3.2.5 Milli-Q Water

Deionized water, with a resistivity of 18.2 MΩ/cm, is prepared using a Milli-Q system from a pre-purified (reverse osmosis) water source. Milli-Q water is used for reagent preparation.

3.2.6 Soda Lime Traps

High purity grade soda lime (EM Science) is utilized in an acid-cleaned glass tube with glass wool endplugs and Teflon connectors. After packing, this trap is conditioned by purging a 0.5 M HCl solution through the trap for 30 minutes. The soda lime trap is changed after analysis of 30 samples.

3.2.7 Preparation of Working Standard Solution

100 µL of the stock Hg solution (1 mg/mL in nitric acid) is pipetted into a 1 L volumetric flask. 5 mL of concentrated BrCl is added and the flask is brought up to volume with MQ-water and thoroughly mixed. This is the Secondary Standard solution (100 ng Hg/mL). Replace this solution as needed (it is stable for at least one year).

The Working Standard (2 ng Hg/mL) is prepared from the Secondary Standard solution by placing 2 mL of Secondary Standard into a 100 mL volumetric flask, adding 1 mL of BrCl and bringing the solution to volume with MQ. The Working Standard is replaced monthly.

3.3 Sample Analysis and Data Acquisition

3.3.1 Volatilization/Recapture

Volatilization of mercury from solution is accomplished using a glass impinger assembly manufactured at the University of Michigan. A 100 mL graduated bubbler attaches to an impinger via a ground glass fitting. N_2 flow is regulated using a Teflon stopcock. A soda lime trap is incorporated into the system to prevent damage of the gold-coated bead traps by capturing acid gases liberated during the purging procedure.

Total mercury is quantified by oxidizing all mercury forms using bromine monochloride. Bromine monochloride is a strong oxidizing agent, capable of breaking organic bonds with mercury, thus liberating the divalent form of mercury. Approximately 10 mL of the oxidized precipitation sample to be analyzed is poured into an acid-cleaned or previously purged glass bubbler. The bubbler is rinsed by gently swirling the solution. After rinsing, the solution is discarded. 100 mL of the oxidized precipitation sample is carefully poured into the graduated glass bubbler and 250 µL of hydroxylamine hydrochloride is added. A stopper is then inserted into the bubbler, its swirled briefly and allowed to react for 5 minutes to reduce the excess bromine monochloride from solution. Bromine monochloride is reduced from solution since halogens liberated from solution will quickly damage the gold-coated bead traps onto which the purged elemental mercury is amalgamated.

A blanked gold-coated bead sample trap is affixed to the end of the soda lime trap. The bubbler is opened, 500 µL of stannous chloride is added, and the bubbler is quickly attached to the impinger. The N_2 flow is adjusted to 450 cc/min using a calibrated rotameter and the solution is purged for 7 minutes. The stannous chloride reduces the divalent mercury to Hg^0 which is quantitatively captured on the goad-coated bead trap.

3.3.2 Analysis of Total Mercury

The CVAFS analyzer used for mercury in precipitation determinations is kept on at all times, since this has been shown to stabilize the UV lamp and maintain consistency from one day to the next. The power supplied to the CVAFS analyzer is modulated by a line tamer (Shape Magnetronics) to prevent power fluctuations. It is imperative that the mercury lamp not experience wide temperature fluctuations or power surges since both of these drastically affect the sensitivity of the instrument. During operation of the instrument the helium carrier gas flow rate is regulated upstream of the analyzer using a mass flow controller (Tylan) which is set to maintain a 35 cc/min flow rate. This flow rate has been determined by UMAQL to yield the optimal peak characteristics for mercury standards. The regulator on the helium cylinder is set at 50 Kilopascals. The helium stream is prefiltered using a gold-coated trap before entering the analytical train in order to remove any mercury. In the analytical train, mercury is thermally desorbed from the sample trap, and amalgamated onto the analytical trap which is subsequently thermally desorbed into the CVAFS analyzer where the mercury atoms are detected. Traps are desorbed by heating a nichrome coil which is wrapped around the trap covering the gold-coated beads. Application of 12 volts of current to the coil is sufficient to achieve a temperature of 500 °C inside the gold bead trap (voltage may vary due to variations in length and thickness of nichrome wire). Two fans supply cool air to the sample and analytical trap separately in order to speed analysis time.

For comparability between laboratories UMAQL sets the gain on the CVAFS analyzer to yield an approximate 1000 mV net response for a 1 ng mercury standard. The background on the CVAFS analyzer is set at 5.0 and maintained in that position in order to track the drift in the baseline of the analyzer. A Hewlett Packard Integrator is connected to the analyzer to convert output signal into an integrated area of the detected response. Area units (AU) are used for all sample calculations since area is much more reliable than peak height.

To analyze a sample trap, the trap is placed snugly into the analytical train using friction fit Teflon connectors and Teflon sleeves. The nichrome coil used specifically for the sample trap is slid over the trap and moved to completely cover the quartz wool plugs and the gold-coated beads contained between the plugs. Helium is allowed to flow through the sample trap for 2 minutes before analysis begins in order to purge air and water vapor from the analytical train. A circuit controller (ChonTrol) is employed which is programmed to turn on the variable transformers and fans in a precise and reproducible manner. First, the sample coil is heated for 2 minutes, then it is cooled while the analytical trap is heated for 2 minutes. The analytical trap is then cooled for 2.5 minutes and the fan to the sample trap is turned off. While the analytical trap is cooling, a new sample trap is installed in the analytical train and helium is passed through this trap until the analytical trap is cool and ready for another sample. When the analytical trap begins heating, the integrator is turned on and the ambient temperature, time and base mV are recorded in a log book and the LCD display on the analyzer is set to record the peak mV (by depressing the Peak button on the face of the analyzer). After the sample is analyzed and the peak height and area reported by the CVAFS and integrator respectively, these values are recorded in the log book.

A standard curve is analyzed at the beginning of each day of analysis and a control standard which yields a response in the range of the samples being analyzed is run every six samples. Criteria for the standard curves and control standards are described below in Section 3.3.4. All sample analysis is recorded in a log book specific to the analyzer with which samples are being quantified and also in a lab notebook specific to the study for which the samples were collected. At the end of the day of analysis all results from the log sheet are entered into a computer spreadsheet file for subsequent checking and processing by a statistical software program, SAS (Cary, NC).

3.3.3 System Purge and Blanks

At the start of each day of analysis, each impinger system is purged after the soda lime trap is conditioned. First 25 mL of Milli-Q water is added to an acid cleaned bubbler, then 1.0 mL of $SnCl_2$ is added and the solution is purged at 450 cc/min for 15 minutes. After each system is purged a System Blank is generated to ensure the impinger assembly is free of contamination.

System Blank (Bubbler Blank): This blank is generated by adding 1.0 mL of $SnCl_2$ to the system purge solution and purging the solution onto a blanked gold-coated bead trap at 450 cc/min for 5 minutes. After the System Blanks have been completed, one of the purged bubblers is dedicated for generation of standards.

A Total Reagent Blank is generated on each day of analysis for each analytical aliquot volume. The Total Reagent Blank is used to calculate the method detection limit (presently 0.1 ng/L for a 100 mL aliquot) and to calculate sample concentration (Section 3.3.5).

Total Reagent Blank: A Total Reagent Blank is generated in sample solution that has been purged of mercury. Bromine monochloride is pipetted into the solution, allowing reduction of halogens by hydroxylamine for 5 minutes then mercury is purged from the solution after adding $SnCl_2$. Purged mercury is captured on a gold-coated trap and analyzed as described above in Section 3.3.2. For a 100 mL aliquot the following reagent volumes are used: 1.0 mL of BrCl, 0.25 mL $NH_2OH{\cdot}HCl$, and 0.5 mL of $SnCl_2$.

3.3.4 Standard Curve and Control Standards

A standard curve, generated by bubbling different volumes of mercury working standard solution, is analyzed before each day of analysis. The amount of mercury bubbled for the calibration curve is tailored to the expected value of the samples to be analyzed. At UMAQL, a typical calibration curve consists of five standards: 0 ng, 0.1 ng, 0.2 ng, 0.5 ng and 1.0 ng. The volumes of working standard solution required to achieve the five standard concentrations are shown in Table 1.

Table 1. Volumes of Working Standard Required to Generate Aqueous Standards

Hg Delivered to Trap	Volume of Hg Standard
0 ng	0 mL
0.1 ng	0.05 mL
0.2 ng	0.10 mL
0.5 ng	0.25 mL
1.0 ng	0.50 mL

Standards for the calibration curve are generated starting with the zero point (0 mL of working standard solution) and continued in ascending order to the highest volume, usually 0.50 mL of working standard solution. First, a blanked gold-coated bead trap is attached to the end of the soda lime trap. Then 1.0 mL of $SnCl_2$ is added to the standard bubbler followed by the appropriate volume of working standard solution. The standard bubbler is quickly attached to the impinger and the solution is purged at 450 cc/min for 5 minutes. The gold-coated bead trap is analyzed immediately after purging.

After each of the standards for the calibration curve has been analyzed, a linear regression is calculated to establish the coefficient of determination (r^2), the slope of the line and how well the slope of the curve predicts each of the points in the calibration curve. The 0 mL standard area is subtracted from each of the other points which are then regressed against the expected values using no intercept (line is forced through zero). The r^2 must be >0.995 and each of the points on the curve must be predicted by the slope within 10% of their true value (Table 2). If these criteria are not met, specific points which are errant are repeated and the linear regression recalculated.

Table 2. Example Calibration Curve and Calculation of Slope

Standard (ng Hg)	Response (AU)	Response (AU)- Zero Point (AU)	Predicted Value (ng Hg)
0	56,714	0	0
0.100	1,364,600	1,307,886	0.103
0.200	2,571,000	2,514,286	0.199
0.500	6,357,800	6,301,086	0.498
1.000	12,709,000	12,652,286	1.001

Slope = 7.909E-8 ng/AU
$Slope^{-1}$ = 12,643,823 AU/ng
$r^2 = 0.9999$

This curve is accepted and sample analysis commences.

Control standards are analyzed every sixth sample. The control standards are generated in the same manner as described above and are chosen to be representative of the samples being analyzed. The integrated area from each of the control standards must be within 10% of the slope of the calibration curve in order to continue analyzing. If this is not the case, a second control is analyzed immediately. If the second control indicates that analyzer sensitivity has changed a second calibration curve is generated and sample analysis is continued.

3.3.5 Calculation of Mercury Concentration

Mercury concentration in precipitation is calculated in ng/L. The total reagent blank response is subtracted from the analytical aliquot response and the difference is multiplied by the slope of the calibration curve which is in ng/AU. The mass of Hg for the entire sample is then calculated by dividing the analytical aliquot Hg mass by the analytical aliquot volume and multiplying the result by the total volume of sample. Once the ng of mercury in the entire sample is determined, the concentration is then adjusted for the (i) Teflon sample bottle blank, (ii) HCl preservative blank, (iii) HCl preservative dilution factor and finally converted to ng/L for reporting purposes (Table 3). The Hg concentration is calculated using Eqn. (1).

(1) $$Hgpcp = \{(CAU*Slope*TV/AV) \quad (AB+BB)\}*(1000/PV)$$

where:
Hgpcp = Hg concentration in precipitation (ng/L)
CAU = Total reagent blank corrected sample area units
Slope = Slope of the five point calibration curve (forced through zero)
TV = Total volume of sample (precipitation volume + HCl volume)
AV = Volume of analytical aliquot
AB = HCl acid preservative blank
BB = Mean Teflon sample bottle check blank
PV = Volume of precipitation collected

Table 3. Calculation of ng Hg/L in a Precipitation Sample.

1. Calculation of ng of mercury recovered from the analytical aliquot

*ng Hg = (sample response (AU)-total reagent blank (AU)) * slope of calibration curve (ng/AU)*
*(8,657,600 - 74,543) * 7.909E-8 = 0.679 ng Hg*

2. Calculation of ng of mercury for total sample volume

*ng Hg = (ng Hg from analytical aliquot * total volume of sample)/ volume of analytical aliquot*
*(0.679 ng Hg * 439 mL) / 100 mL = 2.980 ng Hg*

3. HCl acid preservative blank and Bottle blank correction

ng Hg = ng Hg for total sample volume - (HCl acid blank + Bottle blank)
2.980 - (0.004 + 0.020) = 2.956 ng Hg

4. Conversion to ng Hg/L

*ng Hg/L = ng Hg for total sample volume * (1000 / precipitation volume collected)*
*2.956 * (1000 / 419) = 7.1 ng Hg/L*

3.3.6 Trouble-Shooting

- A source of irreproducible results may be due to faulty gold-coated bead traps. These traps are numbered with discrete identifiers. Contact with halogen fumes, organic fumes or overheating of the trap during analysis can damage the trap, rendering it unusable. If performance of a gold trap is suspect, at least two consecutive standards are analyzed from this trap to determine its ability to amalgamate and release mercury.

- If a low response is observed, the impinger assembly is checked for leaks. Teflon compression fittings on the soda lime trap and the Teflon nut on the Teflon stopcock are the most common location of leaks.

- If peak-broadening is observed or no peak is detected in a sample, the analytical train is checked for leaks. Peak broadening is often the result of low gas flow, water vapor on the gold-coated bead trap, inadequate heating of analytical trap or an analytical trap damaged by exposure to halogen fumes or overheating. Analytical traps are replaced only when a potential problem is suspected with the trap.

- If the baseline drifts more than 10% the UV lamp is replaced. After replacement, the analyzer is allowed to equilibrate for 24 hours. If the problem persists, sources of power fluctuation sources, drafts or air currents that may be changing the temperature of the UV lamp are investigated.

- Room temperature in which the CVAFS is located is maintained between 20-22°C, however, if the temperature exceeds 26°C analysis is stopped, since instrumental noise increases significantly.

4.0 Performance Criteria, Quality Assurance and Quality Control

4.1 Field operators are carefully instructed in the techniques of contaminant-free precipitation sample collection for mercury determination. All of the operators are currently operating sampling equipment for either the National Dry Deposition Network, the National Atmospheric Deposition Program, the Integrated Atmospheric Deposition Network or the Great Lakes Acid Deposition Network and are experienced with other precipitation collectors.

4.2 Every 6 months UMAQL personnel inspect each of the sampling sites to audit the performance of the equipment and to make all necessary repairs or adjustments.

4.3 Co-located samples are collected from three sampling sites during the study to quantify method precision. Reported concentrations for co-located samples are based on the mean of the two samples.

4.4 In order to confirm that the collection funnel assemblies are free of mercury, funnel blank samples are collected throughout the study.

4.5 Precision and accuracy levels will be set and maintained for each type of analysis. A relative precision for total mercury of less than 15% is maintained for samples with values at least 3 standard deviations greater than the detection limit. Analysis of standards and controls is within 10% of the stated value.

4.6 A minimum of 25% of all samples are analyzed in duplicate. Reported concentrations will be based on the mean of the replicates. Analytical precision averages better than 7%.

4.8 Every 3 months maintenance on the CVAFS analyzer is conducted, including replacement of the UV lamp, the Teflon tubing, and the detection cell.

4.9 The analytical trap is not changed unless it begins to demonstrate poor recovery or release of amalgamated mercury.

4.10 Teflon sleeves heat-sealed onto the gold trap are replaced frequently to maintain a gas tight seal.

5.0 Clean Room Procedures

5.1 Entering the Clean Room

Shoes are taken off outside the clean room by all UMAQL personnel. Personnel then enter the outer vestibule (changing room). Once inside, the hood is put on followed by the clean room suit and clean room boots. The boots are snapped to the suit at the back of the leg (to hold up the boots) and are buckled in the front. Personnel then step over a dividing bench where they put on clean room gloves and snap the clean room suit at the wrist. Now fully clothed they enter the clean room making sure to securely close the door behind.

5.2 Taking Supplies into the Clean Room

All supplies to be taken into the clean room are double bagged in polyethylene. The supplies to be taken into the clean room are placed in the outer dressing room. Upon entering the clean room, the outer bag is removed and left in the entry room. All supplies that enter the clean room that have not been bagged are rinsed with MQ-water and wiped off with particle-fee wipes.

Appendix A: Facilities, Equipment and Reagents

Following is a list of the required facilities, equipment, supplies and reagents for sample preparation, sample collection and sample analysis that are outlined in this document. The make and model of the following items are those used at The University of Michigan Air Quality Laboratory. Many of these items are available from a variety of sources.

1. Preparation of Field Supplies

Class 100 Clean Room, Work Stations
Clean Room Gloves
Particle-free Wipes
Clean Room Cap, Gown and Boots
Milli-Q Water (18.2MΩ/cm)
Exhaust Hood
Acetone
Alconox
Polyethylene Tubs
EM Science Tracepur and Suprapur Hydrochloric Acid
Polytherm Water Bath (Science/Electronics)
Baker Instra-Analyzed or EM Science Suprapur Nitric Acid
New Polyethylene Bags
20 Liter Polyethylene Carboys

2. Sample Collection

MIC-B Wet-Only Precipitation Collector (MIC)
UMAQL Modified Acrylic Insert
Digital Indoor/Outdoor Recording Thermometer
BSG Collection Funnels
Polypropylene Collection Funnels
Teflon & Polypropylene Precipitation Adapters
Glass P-trap Vapor Lock
1 Liter Teflon & Polypropylene Sample Bottles
Funnel Heat Tape Nests & Variable Transformer
Ceramic Space Heater
2 Gallon HDPE Bucket
Particle-Free Gloves
Teflon Tape
Sample Labels
Permanent Label Markers
Field Operator Log Book
Shipping Crates

3. Sample Analysis

Cold Vapor Atomic Florescence Detector (Brooks Rand, LTD.)
Line Tamer/Conditioner (Shape Magnetronics Model PCLT 150)
Integrator (Hewlett-Packard Model 3390A)
Helium, Ultra High Purity Grade (99.999%)
Mass Flow Controller (Tylan)
Nichrome Coils (UMAQL)
Electric Leads
Variable Transformers (Staco Energy Products Co. Type 3PN1010)
Cooling Fans
Gold-Coated Glass Bead Traps (UMAQL)
Pre-purified (99.998%), Analyzed Nitrogen
Glass Impingers
Resin-coated Ring Stand
100 mL Graduated Glass Bubblers
12.7 cm lengths of 1.27 cm OD Glass Tubing (Soda Lime Trap)
Automatic Pipettes
Teflon Reagent Bottles (Clear and Opaque)
Teflon Reagent Vials
Resin-coated Wire Rack (Support Bubblers)
Refrigerator

Standard Operating Procedure for Analysis of Particulate Phase Mercury

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Version 2.0

Standard Operating Procedure for Analysis of Particulate Phase Mercury

1.0 Introduction/Overview

The objective of the Lake Michigan Loading Study is to assess the contribution of atmospheric deposition to the concentration of mercury and other toxic trace species found in Lake Michigan. The atmosphere has been implicated as one of the dominant sources of mercury and trace elements to bodies of water and it is clear from investigations in remote regions of the globe that long range transport of mercury and other toxics from source regions is occurring. By quantifying the wet deposition and ambient concentrations of mercury it will be possible to determine the relative importance of precipitation and dry deposition in accounting for the atmospheric loading of mercury to Lake Michigan. In addition, investigating other ambient trace species will aid in the identification of significant mercury sources.

Particle-phase mercury, Hg(p), generally represents a small but significant fraction of total atmospheric mercury. Recent advances in analytical chemistry have made quantification of the extremely low levels of Hg(p) possible, however, tremendous care must be exercised in all phases of sample handling and analysis. This protocol describes analysis of 'acid-extractable' total mercury from atmospheric particulate samples.

2.0 Sample Analysis

2.1 Summary

The technique described by this protocol is designed for use with glass-fiber or quartz fiber filter media. When used in conjunction with an open-faced filter pack, these media demonstrate a low pressure drop and have a very low background level of Hg with proper pretreatment. Sample filters are stored at -40°C before analysis to prevent volatilization of the collected Hg(p). Particulate mercury is extracted into a 1.6 M nitric acid solution utilizing a microwave digestion technique. The mercury forms are then oxidized with bromine monochloride, to Hg^{2+} Oxidized mercury forms are subsequently reduced to Hg0 with stannous chloride ($SnCl_2$). In this volatile form, the metal is purged from solution using an Hg-free nitrogen stream and collected on a gold-coated bead trap. A mercury-free pretreated soda lime trap is utilized in the purge system to capture acid gases that may damage the gold-coated bead trap. Quantification is accomplished using a dual amalgamation technique followed by cold vapor atomic fluorescence spectroscopy (CVAFS).

All analytical procedures for determination of particulate phase mercury are carried out in a class 100 laminar flow exhaust hood inside a Class 100 Clean Room. Nitrogen utilized for purging is 99.998% pure and is stripped of any mercury using a gold coated trap before use in the purge system. Clean room gloves are worn at all times and all labware with which the samples and reagents comes into contact is cleaned weekly using the acid cleaning procedure described in *Standard Operating Procedure for Sampling of Particulate Phase Mercury*, Section 2.1.

2.2 Reagents and Materials

All reagent lot numbers, preparation dates and procedures are recorded for each new batch of reagent used. A reagent blank is obtained after each new batch of reagent has been prepared. Bromine monochloride (BrCl), stannous chloride ($SnCl_2$) and hydroxylamine hydrochloride ($NH_2OH{\bullet}HCl$) are prepared fresh monthly.

Solid reagents (potassium bromide, potassium bromate, hydroxylamine hydrochloride and stannous chloride) are stored in the clean room in a desiccator containing silica gel and an open bed of activated charcoal. The caps of all reagent bottles are Teflon taped to reduce entry of vapor phase compounds. Even with these precautions, reagents will nevertheless absorb mercury over time and must be replaced. All reagents are made in the clean room, except the working standard solution.

2.2.1 Hydrochloric Acid

EM Science Suprapur hydrochloric acid is used to prepare BrCl and $SnCl_2$. This acid characteristically has a very low blank value (20 pg/mL).

2.2.2 Bromine Monochloride

Bromine monochloride is prepared in a class 100 laminar flow exhaust hood by adding 11.0 mg KBr per mL of HCl while the solution is stirred using an acid-cleaned Teflon-coated magnestir. When the KBr is dissolved, 15.0 mg $KBrO_3$ per mL of HCl is added slowly and the solution is allowed to continue stirring. This process produces chlorine and bromine gas and must be performed slowly in a functioning exhaust hood. After addition of the salts the solution is a deep yellow color. If there is no color (or very faint) then the BrCl has been substantially reduced and will not have enough oxidizing power for use. In this case, the solution is remade. Bromine monochloride is stored at room temperature in the clean room. Fresh bromine monochloride is be prepared monthly or as needed.

2.2.3 Hydroxylamine Hydrochloride

30 grams of $NH_2OH{\bullet}HCl$ is dissolved in MQ-water to make 100 mL in an acid-cleaned 100 mL volumetric flask. This solution is purified by adding 0.5 mL of $SnCl_2$ and purging overnight with Hg-free N_2. The solution is stored in an acid-cleaned, dark Teflon bottle in the refrigerator. Fresh hydroxylamine solution is prepared every month or as needed.

2.2.4 Stannous Chloride

20.0 gm of $SnCl_2{\bullet}H_2O$ is placed into an acid-cleaned 100 mL volumetric flask. Working in a fume hood, 10 mL of concentrated HCl is added and the solution is then brought to 100 mL with Milli-Q water. The solution is stored in an acid-cleaned, dark Teflon bottle in the refrigerator. Fresh stannous chloride is prepared every month or as needed.

2.2.5 Milli-Q Water

Deionized water, with a resistivity of 18.2 MΩ/cm, is prepared using a Milli-Q system from a pre-purified (reverse osmosis) water source. Milli-Q water is used for reagent preparation.

2.2.6 Soda Lime Traps

High purity grade soda lime (EM Science) is utilized in an acid-cleaned glass tube with glass wool endplugs and Teflon connectors. After packing, this trap is conditioned by purging a 0.5 M HCl solution through the trap for 30 minutes. The soda lime trap is changed after analysis of 30 samples.

2.2.7 Preparation of Working Standard Solution

100 µL of the stock Hg solution (1 mg/mL in nitric acid) is pipetted into a 1 L volumetric flask. 5 mL of concentrated BrCl is added and the flask is brought up to volume with MQ-water and thoroughly mixed. This is the Secondary Standard solution (100 ng Hg/mL). Replace this solution as needed (it is stable for at least one year).

The Working Standard (2 ng Hg/mL) is prepared from the Secondary Standard solution by placing 2 mL of Secondary Standard into a 100 mL volumetric flask, adding 1 mL of BrCl and bringing the solution to volume with MQ. The Working Standard is replaced monthly.

2.2.8 Nitric Acid Extraction Solution.

The extraction solution is a 10% dilution of concentrated nitric acid (1.6M). A 1000 mL volumetric flask is filled with about 800 mL of Milli-Q water. *In a hood*, 100 mL of suprapur HNO_3 (EM Science) is measured using a graduated cylinder and poured into the flask. The solution is mixed completely and allowed to cool in the hood with a glass stopper closing the top. When cool, the flask is brought up to volume with Milli-Q water. After the extraction solution is thoroughly mixed it is poured into an acid-cleaned re-pipetting dispenser.

2.3 Sample Handling and Preparation

2.3.1 Sample Handling

If samples are not analyzed immediately, they are stored triple bagged in a dark freezer (-40°C) to avoid exposure to laboratory air. Particle free gloves are worn whenever vials, filters or dishes are handled or transferred.

2.3.2 Sample Preparation

Eighteen samples, five standards and one vessel check are prepared for each day of particulate mercury analysis. Eighteen acid-cleaned Teflon vessel liners are inserted into outer vessel bodies and are placed in a laminar flow work station. The vessel bodies are then labeled with complete sample identifications. The petri dishes containing the

samples to be analyzed are taken out of their polyethylene bags and the Teflon sealing tape is removed. Each sample filter is carefully folded into quarters and placed in its corresponding vessel using Teflon-coated forceps. The forceps are rinsed in a beaker of HNO_3 extraction solution followed by a beaker of Milli-Q water and are dried using a particle-free wipe before handling the next filter. After each filter has been placed in a vessel it is capped and is moved into a laminar flow exhaust hood.

2.4 Sample Filter Extraction

The HNO_3 extraction solution is made on the same day that it is used and is dispensed using a calibrated Repipet II dispenser. 20 mL of extraction solution is dispensed into each of the Teflon vessels containing the sample filters. The Teflon vessel liners are then weighted and inserted back into their digestion vessel body and tightly capped. An acid-cleaned rupture disc membrane is placed into an acid-cleaned vent stem which is attached to the top of each microwave vessel cap. Each capped vessel is swirled lightly.

Note: Vigorous shaking of the vessel will cause the filter to disperse in solution which will make it very difficult to pipette solution for analysis.

Only twelve samples and/or standards can be microwave digested at a time. One vessel from each of the two digestion runs is outfitted with a Teflon thermowell cap into which a Teflon-coated Pyrex thermowell is inserted. Eleven regular vessels and one thermocouple vessel are loaded in the carousel tray. The carousel tray is then removed from the clean room and is placed in the microwave digester. The fiber optic temperature probe is carefully inserted into the vessel with the thermowell. A pressure monitoring and control probe is also attached to the same vessel. The digestion program for the particulate mercury filters is then initiated. The program heats the samples to 160°C (approximately 70 psi) for 20 minutes.

After the samples are heated, the microwave digester fan will remain on to help cool the Teflon vessels. The vessels are allowed to cool until the pressure inside the control vessel is 1-2 psi (approximately 60 minutes). The fiber optic probe and pressure probe are carefully removed and the carousel is transferred back into the clean room. The vessels are then reweighed to confirm no loss during digestion. 0.5 mL of BrCl is added to each vessel. The vessels are then gently swirled to collect liquid droplets from the side of the liner. The vessels are allowed to react for one hour prior to analysis.

2.5 Sample Analysis and Data Acquisition

2.5.1 Volatilization/Recapture

Volatilization of mercury from solution is accomplished using a glass impinger assembly manufactured at the University of Michigan. A 25 mL graduated bubbler attaches to an impinger via a ground glass fitting. N_2 flow is regulated using a Teflon stopcock. A soda lime trap is incorporated into the system to prevent damage of the gold-coated bead traps by capturing acid gases liberated during the purging procedure.

Total mercury is quantified by oxidizing all mercury forms using bromine monochloride. Bromine monochloride is a strong oxidizing agent, capable of breaking organic bonds with mercury, thus liberating the divalent form of mercury (Hg^{2+}). A 5 mL aliquot of the

oxidized filter sample solution is carefully pipetted into a graduated glass bubbler containing 20 mL of a previously purged sample and 100 µL of hydroxylamine hydrochloride is added. A stopper is then inserted into the bubbler, its swirled briefly and allowed to react for 5 minutes to reduce the excess bromine monochloride from solution. Bromine monochloride is reduced from solution since halogens liberated from solution will quickly damage the gold-coated bead traps onto which the purged elemental mercury is amalgamated.

A blanked gold-coated bead sample trap is affixed to the end of the soda lime trap. The bubbler is opened, 500 µL of stannous chloride is added, and the bubbler is quickly attached to the impinger. The N_2 flow is adjusted to 450 cc/min using a calibrated rotameter and the solution is purged for 7 minutes. The stannous chloride reduces the divalent mercury to Hg^0 which is quantitatively captured on the gold-coated bead trap.

2.5.2 Analysis of Total Mercury

The CVAFS analyzer used for particulate mercury analysis is kept on at all times, since this has been shown to stabilize the UV lamp and maintain consistency from one day to the next. The power supplied to the CVAFS analyzer is modulated by a line tamer (Shape Magnetronics) to prevent power fluctuations. It is imperative that the mercury lamp not experience wide temperature fluctuations or power surges since both of these drastically affect the sensitivity of the instrument. During operation of the instrument the helium carrier gas flow rate is regulated upstream of the analyzer using a mass flow controller (Tylan) which is set to maintain a 35 cc/min flow rate. This flow rate has been determined by UMAQL to yield the optimal peak characteristics for mercury standards. The regulator on the helium cylinder is set at 50 Kilopascals. The helium stream is prefiltered using a gold-coated trap before entering the analytical train in order to remove any mercury. In the analytical train, mercury is thermally desorbed from the sample trap, and amalgamated onto the analytical trap which is subsequently thermally desorbed into the CVAFS analyzer where the mercury atoms are detected. Traps are desorbed by heating a nichrome coil which is wrapped around the trap covering the gold-coated beads. Application of 12 volts of current to the coil is sufficient to achieve a temperature of 500°C inside the gold bead trap (voltage may vary due to variations in length and thickness of nichrome wire). Two fans supply cool air to the sample and analytical traps separately in order to speed analysis time.

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To analyze a sample trap, the trap is placed snugly into the analytical train using friction fit Teflon connectors and Teflon sleeves. The nichrome coil used specifically for the sample trap is slid over the trap and moved to completely cover the quartz wool plugs and the gold-coated beads contained between the plugs. Helium is allowed to flow through the sample trap for 2 minutes before analysis begins in order to purge air and water vapor from the analytical train. A circuit controller (ChonTrol) is employed which is

programmed to turn on the variable transformers and fans in a precise and reproducible manner. First, the sample coil is heated for 2 minutes, then it is cooled while the analytical trap is heated for 2 minutes. The analytical trap is then cooled for 2.5 minutes and the fan to the sample trap is turned off. While the analytical trap is cooling, a new sample trap is installed in the analytical train and helium is passed through this trap until the analytical trap is cool and ready for another sample. When the analytical trap begins heating, the integrator is turned on and the ambient temperature, time and base mV are recorded in a log book and the LCD display on the analyzer is set to record the peak mV (by depressing the Peak button on the face of the analyzer). After the sample is analyzed and the peak height and area reported by the CVAFS and integrator respectively, these values are recorded in the log book.

A standard curve is analyzed at the beginning of each day of analysis and a control standard which yields a response in the range of the samples being analyzed is run every six samples. Criteria for the standard curves and control standards are described below in Section 2.5.4. All sample analysis is recorded in a log book specific to the analyzer with which samples are being quantified and also in a lab notebook specific to the study for which the samples were collected. At the end of the day of analysis all results from the log sheet are entered into a computer spreadsheet file for subsequent checking and processing by a statistical software program, SAS (Cary, NC).

2.5.3 System Purge and Blanks

At the start of each day of analysis, each impinger system is purged after the soda lime trap is conditioned. First 20 mL of Milli-Q water is added to an acid-cleaned bubbler, then 1.0 mL of $SnCl_2$ is added and the solution is purged at 450 cc/min for 15 minutes. After each system is purged a System Blank is generated to ensure the impinger assembly is free of contamination.

System Blank (Bubbler Blank): This blank is generated by adding 500 µL of $SnCl_2$ to the system purge solution and purging the solution onto a blanked gold-coated bead trap at 450 cc/min for 5 minutes. After the System Blanks have been completed, one of the purged bubblers is dedicated for generation of standards.

Total Reagent Blank: This blank is synonymous with the 0 pg filter standard (Section 2.5.4), which is generated on each day of analysis. The Total Reagent Blank is used to calculate the method detection limit (presently 1.0 pg/m^3) and to calculate sample concentration (Section 2.5.5).

2.5.4 Standard Curve and Control Standards

A standard curve, generated by bubbling five different filter standard solutions, is analyzed before each day of analysis. The concentration of the 5 mL filter standard solution aliquots for the calibration curve are tailored to the expected value of the samples to be analyzed. At UMAQL, a typical calibration curve consists of five filter standards: 0 pg, 100 pg, 200 pg, 500 pg and 1000 pg. Because of the BrCl dilution factor imparted onto the filter standards, the nominal pg of mercury delivered by each 5 mL aliquot is slightly less than standard name indicates. The volumes of standard working solution added to each filter standard vessel to achieve the five standard concentrations in each 5 mL aliquot are shown in Table 1.

Table 1. Calibration Curve for Bubbled Hg Standards

Hg in 5 mL of Filter Standard Solution	Volume of Standard Working Solution
0 pg	0 µL
96.6 pg	200 µL
191.4 pg	400 µL
465.1 pg	1000 µL
888.9 pg	2000 µL

The filter standard solutions are prepared at the same time that the field samples are extracted. Baked glass fiber filters are folded and placed into acid-cleaned Teflon vessel liners and the appropriate volume of standard working solution is pipetted directly onto the filter. The filters are then extracted in the same manner as the sample filters (described in Section 2.4)

Standards for the calibration curve are generated starting with the zero point and continued in ascending order to the highest, usually 1000 pg. First, a blanked gold-coated bead trap is attached to the end of the soda lime trap. Then 5.0 mL of filter standard solution is pipetted into the standard bubbler followed by 100 µL of $NH_2OH{\bullet}HCl$. After the solution has reacted for 5 minutes 500 µL of $SnCl_2$ is added. The standard bubbler is quickly attached to the impinger and the solution is purged at 450 cc/min for 5 minutes. The gold-coated bead trap is analyzed immediately after purging.

After each of the standards for the calibration curve has been analyzed, a linear regression is calculated to establish the coefficient of determination (r^2), the slope of the line and how well the slope of the curve predicts each of the points in the calibration curve. The 0 pg standard area is subtracted from each of the other points which are then regressed against the expected values using no intercept (line is forced through zero). The r^2 must be >0.995 and each of the points on the curve must be predicted by the slope within 10% of their true value (Table 2). If these criteria are not met, specific points which are errant are repeated and the linear regression recalculated.

Table 2. Example Calibration Curve and Calculation of Slope

Standard (pg Hg)	Response (AU)	Response (AU)-Zero Point (AU)	Predicted Value (pg Hg)
0	141,510	0	0
96.6	2,156,000	2,010,490	92.4
191.4	4,126,400	3,984,890	183.2
465.1	10,214,000	10,072,490	463.0
888.9	19,548,000	19,406,490	892.1

Slope = 4.5969E-5 ng/AU
$Slope^{-1}$ = 21,754 AU/ng
$r^2 = 0.9998$

This curve is accepted and sample analysis commences.

Control standards are analyzed every sixth sample. The control standards are generated in the same manner as described above and are chosen to be representative of the samples being analyzed. The integrated area from each of the control standards must be within 10% of the slope of the calibration curve in order to continue analyzing. If this is not the case, a second control is analyzed immediately. If the second control indicates that analyzer sensitivity has changed a second calibration curve is generated and sample analysis is continued.

2.5.5 Calculation of Particulate Phase Mercury Concentration

The particulate phase mercury concentration from a glass fiber or quartz fiber filter is calculated in pg/m^3 The total reagent blank response is subtracted from the analytical aliquot response and the difference is multiplied by the slope of the calibration curve which is in pg/AU. The mass of Hg for the entire sample is then calculated by dividing the analytical aliquot Hg mass by the analytical aliquot volume and multiplying the result by the total volume of sample. The calculated value, in picograms of mercury is converted to pg Hg/m^3 by calculating the total volume of air drawn through the filter and dividing the pg of mercury by the cubic meters of air sampled (Table 3).

Table 3. Calculation of pg Hg/m^3 in a Particle Phase Sample.

1. Calculation of pg of mercury recovered from the analytical aliquot

*pg Hg = (sample response (AU)-total reagent blank (AU)) * slope of calibration curve (pg/AU)*
*(5,944,100 - 141,510) * 4.5969E-5 = 266.7 pg Hg*

2. Calculation of ng of mercury in entire sample volume

*pg Hg = (pg Hg from analytical aliquot * total volume of sample)/ volume of analytical aliquot*
*(266.7 pg Hg * 20.5 mL) / 5.0 mL = 1093.4 pg Hg*

3. Calculation of m^3 sampled at a flow rate of 30 lpm and a sample duration of 24 hours:

*Volume of Air Sampled = (DTM Reading Off - DTM Reading On) * DTM Calibration Curve*
*(1982.597 1937.864) * 0.97886 -0.00024 = 43.787 m^3*

4. Calculation of Particle Phase Mercury Concentration in Sample = pg Hg/m^3

1093.4 pg Hg / 43.787 m^3 = 25 pg Hg/m^3

2.5.6 Trouble-Shooting

A source of irreproducible results may be due to faulty gold-coated bead traps. These traps are numbered with discrete identifiers. Contact with halogen fumes, organic fumes or overheating of the trap during analysis can damage the trap, rendering it unusable. If performance of a gold trap is suspect, at least two consecutive standards are analyzed from this trap to determine its ability to amalgamate and release mercury.

If a low response is observed, the impinger assembly is checked for leaks. Teflon compression fittings on the soda lime trap and the Teflon nut on the Teflon stopcock are the most common location of leaks.

If peak-broadening is observed or no peak is detected in a sample, the analytical train is checked for leaks. Peak broadening is often the result of low gas flow, water vapor on the gold-coated bead trap, inadequate heating of analytical trap or an analytical trap damaged by exposure to halogen fumes or overheating. The analytical trap is not replaced unless it begins to demonstrate poor recovery or release of amalgamated mercury.

If the baseline drifts more than 10% the UV lamp is replaced. After replacement, the analyzer is allowed to equilibrate for 24 hours. If the problem persists, sources of power fluctuation, drafts or air currents that may be changing the temperature of the UV lamp are investigated.

Room temperature in which the CVAFS is located is maintained between 20-22 C, however, if the temperature exceeds 26 °C analysis is stopped, since instrumental noise increases significantly.

3.0 Performance Criteria, Quality Assurance and Quality Control

3.1 Field operators are carefully instructed in the techniques of contaminant-free particulate phase mercury sample collection. All of the operators are currently operating sampling equipment for either the National Dry Deposition Network, the National Atmospheric Deposition Program, the Integrated Atmospheric Deposition Network or the Great Lakes Acid Deposition Network.

3.2 Every 6 months UMAQL personnel inspect each of the sampling sites to audit the performance of the equipment and to make all necessary repairs or adjustments.

3.3 Co-located samples are collected from one sampling site during the study to quantify method precision. Reported concentrations for co-located samples are based on the mean of the two samples.

3.4 Precision and accuracy levels will be set and maintained for each type of analysis. A relative precision for total mercury of less than 15% is maintained for samples with values at least 3 standard deviations greater than the detection limit. Analysis of standards and controls is within 10% of the stated value.

A minimum of 25% of all samples are analyzed in duplicate. Reported concentrations are based on the mean of the replicates. Analytical precision averages better than 6%.

3.5 Every 3 months maintenance on the CVAFS analyzer is conducted, including replacement of the UV lamp, the Teflon tubing, and the detection cell.

4.0 Clean Room Procedures

4.1 Entering the Clean Room

Shoes are taken off outside the clean room by all UMAQL personnel. Personnel then enter the outer vestibule (changing room). Once inside, the hood is put on followed by the clean room suit and clean room boots. The boots are snapped to the suit at the back of the leg (to hold up the boots) and are buckled in the front. Personnel then step over a dividing bench where they put on clean room gloves and snap the clean room suit at the wrist. Now fully clothed they enter the clean room making sure to securely close the door behind.

4.2 Taking Supplies into the Clean Room

All supplies to be taken into the clean room are double bagged in polyethylene. The supplies to be taken into the clean room are placed in the outer dressing room. Upon entering the clean room, the outer bag is removed and left in the entry room. All supplies that enter the clean room that have not been bagged are rinsed with MQ-water and wiped off with particle-free wipes.

Appendix A: Facilities, Equipment and Reagents

Following is a list of the required facilities, equipment, supplies and reagents for sample preparation, sample collection and sample analysis that are outlined in this document. The make and model of the following items are those used at The University of Michigan Air Quality Laboratory. Many of these items are available from a variety of sources.

1. Preparation of Field Supplies

Class 100 Clean Room, Work Stations
Clean Room Gloves
Particle-free Wipes
Clean Room Cap, Gown and Boots
Milli-Q Water (18.2 MΩ/cm)
Exhaust Hood
Acetone
Alconox
Polyethylene Tubs
EM Science Tracepur and Suprapur Hydrochloric Acid
Polytherm Water Bath (Science/Electronics)
Baker Instra-Analyzed or EM Science Suprapur Nitric Acid
New Polyethylene Bags
20 Liter Polyethylene Carboys

2. Sample Collection

Vacuum Pump (URG, Model 3000-02M)
Calibrated Dry Test Meter (DTM)
Calibrated 30 lpm Rotameter (Matheson)
HDPE Tubing with quick connects
Black Latex Tubing
Mercury Sampling Box (UMAQL, See Appendix B)
Acid-Cleaned 47 mm Teflon Filter Holders (Savillex, PFA Labware)
47 mm Preheated Glass Fiber Filters (Gelman Sciences A/E)
Acid-Cleaned Teflon Jars (Savillex, PFA Labware)
Teflon-Coated Forceps
Particle-Free Gloves
Teflon Tape
Sample Labels
Field Operator Log Book
Sample Tracking Forms
Shipping Boxes

3. Sample Analysis

Cold Vapor Atomic Florescence Detector (Brooks Rand, LTD.)
Line Tamer/Conditioner (Shape Magnetronics Model PCLT 150)
Integrator (Hewlett-Packard Model 3390A)
Helium, Ultra High Purity Grade (99.999%)

Mass Flow Controller (Tylan)
Nichrome Coils (UMAQL)
Electric Leads
Variable Transformers (Staco Energy Products Co. Type 3PN1010)
Cooling Fans
Gold-Coated Glass Bead Traps (UMAQL)
Pre-purified (99.998%), Analyzed Nitrogen
Glass Impingers
Resin-coated Ring Stand
25 mL Graduated Glass Bubblers
12.7 cm lengths of 1.27 cm OD Glass Tubing (Soda Lime Trap)
Automatic Pipettes
Repipet II Dispenser (Labindustries)
Reagents (Section 4.2)
Magnetic Stir Plate
Class A Volumetric Flasks
Teflon Reagent Bottles (Clear and Opaque)
Teflon Reagent Vials
Resin-coated Wire Rack (Support Bubblers)
Refrigerator
Freezer (-40°C)
Microwave Digester (CEM)
Microwave accessories:
24 Teflon Lined Vessels
Rupture Disks
Thermowell
Fiber Optic Temperature Probe
Pressure Tubing
Carousel

Standard Operating Procedure for Mercury Analysis

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June 26, 1996

University of Maryland Standard Operating Procedures for the Lake Michigan Mass Balance Project

Calibration Frequency And Procedures (Taken from QAPjP)

The detection limits of validated EPA methods for mercury quantification are orders of magnitude too high for Lake Michigan waters. For this reason, a peer reviewed method will be employed.

University of Maryland lab personnel will employ a Brooks-Rand Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS) Model-2 (Brooks-Rand, Ltd, Seattle, WA) to measure mercury. Commercially available, traceable $HgNO_3$ standard (from Fisher Scientific or comparable supplier), diluted using Class A volumetric equipment, will be used to calibrate the CVAFS. Working standards will be replaced at least monthly. An intercalibration with outside laboratories will be performed yearly to check our analytical procedure.

During each analysis session, the CVAFS will be initially calibrated with a four point curve spanning the anticipated range of sample signals. This calibration is achieved using a vapor mercury standard. As mercury is a liquid at room temperature it has a well-defined vapor pressure, which is temperature dependent. A temperature-controlled sealed container containing liquid mercury will therefore have a defined air concentration of mercury. This mercury-saturated air can be subsampled via a septum port using a gas tight chromatography syringe. Known aliquots of the air are then injected into the gas stream of the dual gold trap-CVAFS system, and trapped on the gold column just prior to the analyzer. Heating the column releases the Hg into the analyzer allowing quantification of the injected concentration. This method allows calibration of the instrument independent of the wet chemical manipulation-sparging techniques associated with mercury determination in water samples and allows a calibration curve to be established based on instrument sensitivity and linearity. Changes in CVAFS sensitivity will be monitored with single point standard injections after every five sample analyses. If CVAFS sensitivity drifts more than 10% during an analysis session - this would be atypical of the instrument - these single point standards will be used to recalibrate the analyzer.

Due to infrequent rapid temperature changes in the laboratory, obtaining a standard curve using the temperature dependent vapor mercury standard may be difficult. The technician under these circumstances is advised to proceed with the calibration verification (liquid standard) and sample analysis and wait until later in the day when the temperature has stabilized to run a vapor mercury standard curve. The calibration verification standard results are then checked using this curve. If specifications are not met, samples analyzed prior to the curve will be reanalyzed.

If the calibration QC requirement is not met, corrective measures will be taken. These include checking the integrity of the septum injection port and replacing if necessary; changing the injection needle to insure that this is not a source of error; check the stability of the gas flow within the analyzer and check and replace any tubing that might have become contaminated and might be leaching mercury into the system. The lamp and photomultiplier do deteriorate over the long-term (years) and this could be another source of variability. If none of these procedures results in a sufficient improvement in the calibration, the instrument will be returned to the manufacturer for checking and recalibration. All maintenance, calibration and repair will be logged in an equipment notebook. As the analyzer is kept on constantly, no specific recording of usage is maintained. Field equipment will be checked before and at the end of each cruise. The pump will be calibrated for flowrate at the beginning of the study. As filtration volume will be determined volumetrically, small changes in the pump flowrate are not critical. The pump head and

peristaltic tubing will be checked weekly during the cruises, and additional supplies will be on board. For the Go-Flo bottles, O-rings and the rubber tubing and the associated closing mechanisms are checked on a deployment basis. Spares are kept on board as maintenance can be performed, in the majority of cases, on board. In addition, there will be at least one additional Go-Flo, besides those being used, as a spare.

Analytical Procedures (Taken from QAPjP)

See Appendix 1 of our QAPjP for more detailed laboratory protocol for total mercury analyses. The protocol as written by Steve Claas for the University of Wisconsin-Madison Water Chemistry Program is essentially the same as that to be used at the University of Maryland. As this is the most recent detailed method, this should be considered the reference method. The CVAFS techniques have been developed based on techniques first initiated through the University of Connecticut (Bloom and Fitzgerald, 1988) and are generally used throughout the mercury research community for the analysis of mercury in environmental samples. The PI has been using similar techniques for the analysis of open ocean seawater, estuarine and freshwater samples for the last six years. The methods used are based on sample digestion methods described in Bloom and Crecelius (1983) and the CVAFS total mercury method of Bloom and Fitzgerald (1988). Deviations from these methods are described below.

1) The analyzer is kept on usually, so no warmup time is required.

2) A Hewlett-Packard integrator will be used instead of a strip chart recorder.

3) The calibration procedure is different from that used by the University of Wisconsin. See details above for the calibration procedure.

4) Analysis of water samples: Subsamples to be analyzed will be decanted from the 2 L Teflon bottle into a smaller, preweighed Teflon bottle for analysis. After tarring the sample, it will be wet oxidized with 100 µL of BrCl solution for every 100 mL of sample. Samples will be sealed in double polyethylene bags and heated for at least an hour, and preferably overnight, at 70°C before prereduction and analysis. Aliquots of 125-500 mL, depending on expected mercury concentration, will be purged and trapped. Argon will be used as the carrier gas and the purge time will be such that 20 volumes of argon are purged through each sample volume.

5) Filters containing particulate will be unfrozen. Two mL of MilliQ water and 2 mL of BrCl reagent will be added and the bottle sealed. From this point the samples are treated in a similar fashion to the water sample, except 2 mL of hydroxylamine HCl is used to produce samples. The detection limit for both methods are similar, as the analytical techniques are comparable and the BrCl reagent is the primary source of the blank. For similar amounts of added BrCl, the blank should be comparable. The method has a mean detection limit of approximately 0.1 ng/L. Detection limits depend to a large extent on volume of sample analyzed and can be lowered by increasing the volume of the purged aliquot.

Standard Operating Procedure for Total Hg (Mason Lab Detailed Procedure) Analytical System

1.0 At start of day, turn on argon tanks for bubblers and analyzer. Pressure into analyzer should be b/w 50-60 units.

2.0 A column should be connected in line with crimped side facing integrator (First on, First off concept). Make sure that coils are covering gold and quartz wool or improper heating will result.

3.0 Run a standard curve by spiking you sample column with 20, 30, and 50 cc of mercury. Turn the integrator on by pressing <start> before each spike.

4.0 Turn on heating program by pressing <Program>, <1>, <On> on the Control Programmer. It will run through the heating program for 8 min.

5.0 Spikes of 20 to 50 cc should be run for most low level detection. For each spike, you must record the temperature and convert temperature to vapor pressure with the conversion chart on wall. A standard curve including 0 as a point should yield a correlation of 0.999 or better.

6.0 When done analyzing a sample, integrator is shut off by pressing <stop>.

7.0 Hg Collection System (Bubbler System)

7.1 While standard curve is being run, the bubbler blanks can be run with the second set of columns.

7.2 Rinse bubblers two to three times with Q-water, then fill to mark. Rinse spargers with Q-water, then replace bubblers.

7.3 Stannous Chloride: The $SnCl_2$ pipette is labeled and should only be used for this reagent. Rinse the tip three to four times in 6N HCl then rinse one time with $SnCl_2$. All waste should be placed in labeled waste container. Spike 0.5 mL into each bubbler, then rinse tip again three to four times with HCl. Rinsing keeps the pipette tip clean (we do not change this tip) and it keeps the $SnCl_2$ from precipitating (it dissolves under acid conditions).

7.4 Place lids firmly on bubblers so that no leaking occurs. Place clean gold traps onto bubblers, next to soda lime columns. (Columns should have crimped end facing bubbler-First on, First off). Turn the gas on to 60 units for larger bubblers and ~ 20 for small bubblers. Samples should bubble for about 20 min.

7.5 After running Q-water blanks, a standard Hg spike should be run using the now blanked water. Standard Hg (~1ng/mL) is kept in the dark in the refrigerator. Only remove for use. Use 1 mL pipette labeled Hg Std only and take a pipette tip from the acid bath. Rinse with Q-water, then rinse once with standard before dispensing into bubblers. Hg waste and tip should go in flask labeled Hg waste. Ultimately, any Hg waste should be placed in labeled acid bottle at back of hood.

7.6 After spiking with standard, spike bubblers with $SnCl_2$.

7.7 After standard is run, a blank should be run using the same blanked water. Only $SnCl_2$ should be added.

7.8 If standard and blank values are acceptable, then you are ready to run samples.

8.0 Water samples

8.1 For Reactive Hg, just add a known volume to each bubbler and spike with $SnCl_2$.

8.2 For Total Hg, rinse out acid cleaned 500 mL bottles with Q-water. (I usually rinse out about six and re-use them over again). This is done if your sample is in a 2 L bottle, as you only need 500 mL for analysis. If you are using smaller bottles this step is unnecessary. When running a duplicate, you will need two bottles per sample.

8.3 Rinse bottles once with sample, then fill with sample. Spike with BrCl. (Use pipette labeled BrCl and keep same tip; rinse with Q-water several times then one time with reagent prior to use). The volume of BrCl used will depend on how much mercury is in the sample (ie. rain samples usually get 1 mL BrCl). Tighten lids on samples, shake, then let sit for at least ½ hour.

8.4 After time has expired, add the same volume of Hydroxylamine HCl as BrCl to your samples, rinsing the tip in the same manner as your BrCl addition. Shake well; samples are ready for analysis.

8.5 To analyze samples, empty bubblers of old water. All waste from bubblers must be placed in container labeled $SnCl_2$. Rinse bubbler with sample. Record sample volume using balance. Again, samples need to be treated with $SnCl_2$ prior to analysis.

9.0 Filter samples

9.1 Filters should be placed into acid cleaned vials prior to analysis.

9.2 To each vial add 2 mL Q-water and 2 mL BrCl. Use Teflon coated forceps to push any unexposed filters into reagent and tighten caps. Shake gently and let stand for ½ hour.

9.3 Add 2 mL of Hydroxylamine HCl and shake well to neutralize.

9.4 To blanked bubblers, add 2 mL of sample (try to avoid getting filter paper pieces in pipette tip). Spike with $SnCl_2$.

10.0 End of the day

10.1 When finished running samples, empty bubblers into $SnCl_2$ waste container and rinse several times with Q-water. Fill again with Q-water and replace lids. If you are not going to use bubblers for an extended time period, top off with concentrated acid to clean.

10.2 All $SnCl_2$ waste should be placed in labeled flask in hood. When flask is full add some dissolved NaOH pellets to precipitate the $SnCl_2$. Let stand overnight. Before next use, decant clear liquid into sink being careful not to let any precipitate escape. When a substantial amount of precipitate has accumulated, empty flask into $Hg/SnCl_2$ bottle for proper waste disposal.

10.3 Shut off gas cylinders.

Total Mercury Analysis in Aqueous Samples

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April 1994

Revision 2

Total Mercury Analysis in Aqueous Samples

1.0 Introduction

This manual contains instructions for measuring total mercury in aqueous samples. Both experienced chemists and neophyte technicians will find these protocols understandable and useful. Besides dictating each step in the total mercury analysis, this manual provides explanations, suggestions, precautions, definitions, tips and helpful background information. Although the stepwise protocols are complete, this manual cannot replace a knowledgeable, experienced, and friendly human instructor.

Environmental Mercury Pollution

After new sampling and analytical techniques were developed and applied during the mid 1980s, researchers discovered environmental mercury concentrations were up to 1000 times lower than previously reported. For many years, scientists had unknowingly contaminated their samples during collection and analysis. Scientists now believe the concentration of mercury in our atmosphere, oceans, and lakes is often quite low. In some cases, concentrations are almost too low to measure.

Because mercury is found in paints, batteries, fluorescent lights, electric switches, and many other human inventions, concentrations of the element tend to be quite high inside and near buildings. Unfortunately, the situation is often worse in laboratories which frequently house mercurial reagents and mercury equipped instruments. Mercury's ubiquity, along with some of its chemical properties, make contaminating environmental samples virtually inevitable. For this reason, special techniques must be implemented when analyzing total mercury samples.

2.0 Clean Techniques

The following guidelines were developed to minimize the probability of sample contamination. These Clean techniques should be followed when analyzing mercury samples.

2.1 Never open mercury sample bottles indoors unless working in a trace-metal clean lab.

2.2 Keep sample bottles sealed in plastic bags except when adding to or taking from the bottles.

2.3 Minimize sample contact time with atmosphere. Avoid breathing into samples.

2.4 Wear a lint free suit.

2.5 Wear plastic gloves when handling mercury samples and analytical equipment such as gold traps and bubblers.

2.6 Do not place potentially contaminated objects such as pipettors, balances, and lab benches into contact with mercury sample bottles. Cover equipment and benches with plastic bags, gloves or particle free towels if necessary.

2.7 Work in a laminar flow or HEPA-filtered hood whenever possible.

2.8 Keep lab work areas uncluttered.

2.9 Clean the trace-metal clean lab frequently.

3.0 Definitions

3.1 *Analytical Trap*: This is the gold trap that captures the mercury from a sample trap and subsequently releases that mercury into the analyzer. It is the trap farthest down-stream and is not routinely removed from the analytical system.

3.2 *Analyzer:* The mercury analyzer is a cold vapor atomic fluorescence spectrophotometer (CVAFS). In the CVAFS, light from a small mercury vapor lamp is shined through a quartz flow cell that contains mercury in a stream of argon carrier gas. This light excites the mercury atoms which subsequently emit more light. The amount of light emitted by the mercury is proportional to the amount of mercury passing through the cell. The light emitted by the mercury atoms passes through a filter (254nm) and into a photomultiplier tube (PMT) which converts the light into an electrical signal. This signal is received and plotted by a stripchart recorder or integrator.

3.3 *Blank* (noun): The mercury that is associated with equipment and reagents must be measured; this quantity of mercury must subsequently be subtracted from the mercury measured in samples. This measured quantity of mercury from background levels is called a blank. Sometimes blanks are associated with specific objects, such as a bubbler blank or a BrCl blank. Sometimes blanks are associated with specific processes, such a traveling blank or a digestion blank. The term blank can also be used in a more generic sense to refer to the sum of background contamination from all potential sources.

3.4 *Blank* (verb):

1) The process of measuring the quantity of mercury that is associated with equipment and reagents is called blanking. For example, during an analysis session, the analyst must blank the bubblers.

2) The process of thermally desorbing mercury from a gold trap is called blanking. This is sometimes also referred to as analyzing a trap.

3.5 *Bubbler:* A flask and stopper system used to purge aqueous samples. Typically, 250 mL, flat bottomed, spherical, 24/40 jointed, boiling flasks are used for total mercury analyses.

3.6 *Bubbler Stopper:* This is a modified ground glass joint which fits into the bubbler flask. The stoppers have a glass tube which extends from a vertical gas inlet on the top of the stopper and terminates in a frit near the bottom of the bubbler flask. An outlet extends horizontally near the top of the stopper.

3.7 *Bubbling Matrix*: An aqueous mixture which may contain any combination of deionized water, HCl, $SnCl_2$, BrCl, $NH_2OH{\cdot}HCl$, total mercury standard, and/or water sample. This is the solution out of which elemental mercury is purged.

3.8 *Coil:* Coils are made from nichrome wire. These coils are used to desorb mercury from gold traps. Variable autotransformers are used to apply a potential of approximately 10 VAC to the coils; this heats the gold traps desorbing elemental mercury.

3.9 *FEP:* A type of Teflon (Fluorinated Ethylene-Propylene).

3.10 *Glass Wool:* Fibrous strands of silanized glass. Plugs of glass wool are used to hold soda-lime chips in soda-lime traps.

3.11 *Gold Trap:* Gold traps are made from a 9 cm length of 4 mm I.D. quartz tube. The tubes contain approximately 1 g of gold coated beads held in place with silanized glass wool plugs. A constriction in the quartz tube holds all the packing materials in place. Because elemental mercury forms an amalgam with gold, these gold coated bead traps are used to preconcentrate mercury purged from aqueous samples.

3.12 *Prereduce:* The addition of $NH_2OH{\bullet}HCl$ to a brominated sample. Chemically reduces excess BrCl which could damage the gold traps.

3.13 *PTFE:* A type of Teflon (Poly Tetra Fluoro Ethylene).

3.14 *Purge:* To pass N_2 bubbles through an aqueous matrix to remove and trap the elemental mercury contained within that matrix.

3.15 *Regulator Units:* These consist of the single or two stage regulators on the N_2 and Ar cylinders and any needle valves and other brass or Teflon fittings connected to these regulators.

3.16 *Sample Traps:* These are the gold traps that are first attached to the purging apparatus to capture elemental mercury and then connected to the analytical system for mercury measurement. These traps are connected to the Tenax TA® pretraps or soda-lime traps during bubbling and to the argon line and analytical trap during blanking.

3.17 *Scrubbing Traps:*These are the gold traps attached to the purging and analytical systems that are intended to remove contaminant mercury from the N_2 and Ar gases.

3.18 *Soda-lime Trap:* Soda-lime traps are made from 10 cm lengths of 0.9 cm I.D. Teflon tubing. These traps are packed with non-indicating, 8-14 mesh, reagent grade soda-lime; the ends are plugged with glass wool. These traps neutralize acid fumes and trap water vapor during the purging process.

3.19 *Tenax TA®:* A porous polymer based on 2,6-diphenyl-p-phylene oxide used to trap matrix-interferents associated with some tributary samples. Placed between the gold trap and soda lime trap.

3.20 *Timer Controller:* A device used to switch coils and fans on and off at appropriate times during gold trap blanking.

3.21 *Total Mercury:* The sum of all the different chemical forms of mercury (includes inorganic and organic forms).

3.22 *Working Standard:* A solution with a known concentration of Hg^{2+} This solution, mixed form more concentrated primary and secondary standards, is used to calibrate the analyzer. It typically has a concentration of 10 ng/mL.

4.0 Cautions

The following list of warnings and prescriptions is incomplete. Consult the material safety data sheets that are shipped with all hazardous chemicals.

4.1 *BrCl:* Extremely corrosive. Contact with any body part will cause severe injury. The BrCl solution releases toxic and extremely caustic Cl_2, Br, and BrCl fumes which will cause severe damage to the respiratory system if inhaled. Flush affected areas with water and mild soap. Always use BrCl under a well operating fume hood.

4.2 *Glass Wool:* Harmful if inhaled. May irritate skin. Handle with gloves.

4.3 *HCl:* Can cause severe burns. Fumes can cause severe damage to respiratory system. Flush affected areas with large amounts of water. Always work with concentrated HCl under a fume hood.

4.4 *$NH_2OH \cdot HCl$*: Harmful if inhaled or swallowed. Avoid contact with eyes and skin. Flush affected areas with water and mild soap. Has caused mutagenic effects in laboratory animals.

4.5 *Nichrome Coils:* Coils heat up to 450-500°C during a blanking cycle. Under normal room light, hot coils look no different than cold coils. Always approach coils tentatively.

4.6 *$SnCl_2$*: Can cause eye and skin irritation. Rinse affected areas with large amounts of water and mild soap. Persons with a history of skin disease may be at an increased risk from exposure.

4.7 *Soda-Lime:* Can cause burns. Avoid contact with skin and eyes. Rinse affected areas with large amounts of water.

4.8 *Total Mercury Standard*: Mercury in the standard can damage the nervous system. Avoid contact with skin. The working standard contains 1 to 5% BrCl.

5.0 Total Mercury Analysis Sessions

A typical total mercury analysis session often lasts from 8 to 12 hours. Depending on the number of standards, blanks, and replicates that are analyzed, 9 to 18 samples can be analyzed during one session.

Although instructions in this manual are written as linear, stepwise protocols, procedures must often be performed concurrently. Below is a suggested sequence of events for one analysis session.

5.1 Begin each analysis session by removing any mercury that may have accumulated on the six sample traps since the last analysis session. Follow the trap blanking procedure in Section 8.0. While the traps are being blanked, prepare the bubblers for blanking and analysis as prescribed in Section 8.3.

5.2 After the bubblers have been filled with an initial matrix and have started bubbling, install the soda-lime traps (Section 9.3.3) and Tenax TA® pretraps (Section 8.3.5). Note that no sample traps are attached at this time; this initial 20 minute purging serves to preclean the matrix, soda-lime and Tenax TA® pretraps. Continue the initial sample trap blanking procedure while bubbling.

5.3 After the bubblers have purged for at least 20 minutes, blank the bubblers (Section 8.4). Continue the initial sample trap blanking procedure if not already completed.

5.4 After the 20 minute purging period, analyze the gold traps from the bubbler blanks as prescribed in Section 10.0. By now the last of the sample traps should have undergone an initial blanking. Purge and trap standards to calibrate the analyzer (Section 8.4) while analyzing the bubbler blank traps. You should also prereduce the first set of three samples. (Prereduce fewer if you want to replicate analyses, of course.)

5.5 After the bubbler blank traps have been analyzed and the standards have finished purging, analyze one or two gold traps with standards. Assess the analytical and purging systems. Are the bubbler blanks low? Do the standards indicate sufficient analyzer sensitivity? If all is well, run another set of standards so trap efficiency for all traps can be assessed and also run a set of second set of bubbler blanks. If traps are behaving similarly and bubbler blanks are low and seem stable, purge and trap the prereduced samples (Section 9.2).

5.6 By this time you should have established a cycle in which one set of samples can be purged and trapped while another set is being analyzed. Continue this cycle for two to four more rounds. Don't forget to prereduce samples a few minutes before pouring them into the bubblers.

5.7 Continue to purge and analyze samples. At minimum, a set of standards and bubbler blanks should be run midsession and at the end of an analysis session.

6.0 Protocol Organization

The stepwise protocols are organized as follows.

6.1 Step 1 These sections describe major tasks

Caution: These sections list dangerous equipment and reagents

6.2 These sections provide detailed prescriptions on equipment use and sample manipulation.

These sections expand on the instructions given above and include precautions, reminders, and helpful tips. Background information and explanations are sometimes also included in this section

7.0 How to Prepare the Analytical System

7.1 Step 1 Analyzer is normally left on at all times.

7.1.1 The analyzer should be switched on at least 24 hours before beginning analyses. For a Brooks Rand Model 2 analyzer, turn on by the red rocker switch on the front panel.

Analyzer sensitivity is correlated to operating temperature. Turning the analyzer on in advance allows electronic components to warm and stabilize. If the analyzer is not switched on in advance, be certain to calibrate frequently between analyses.

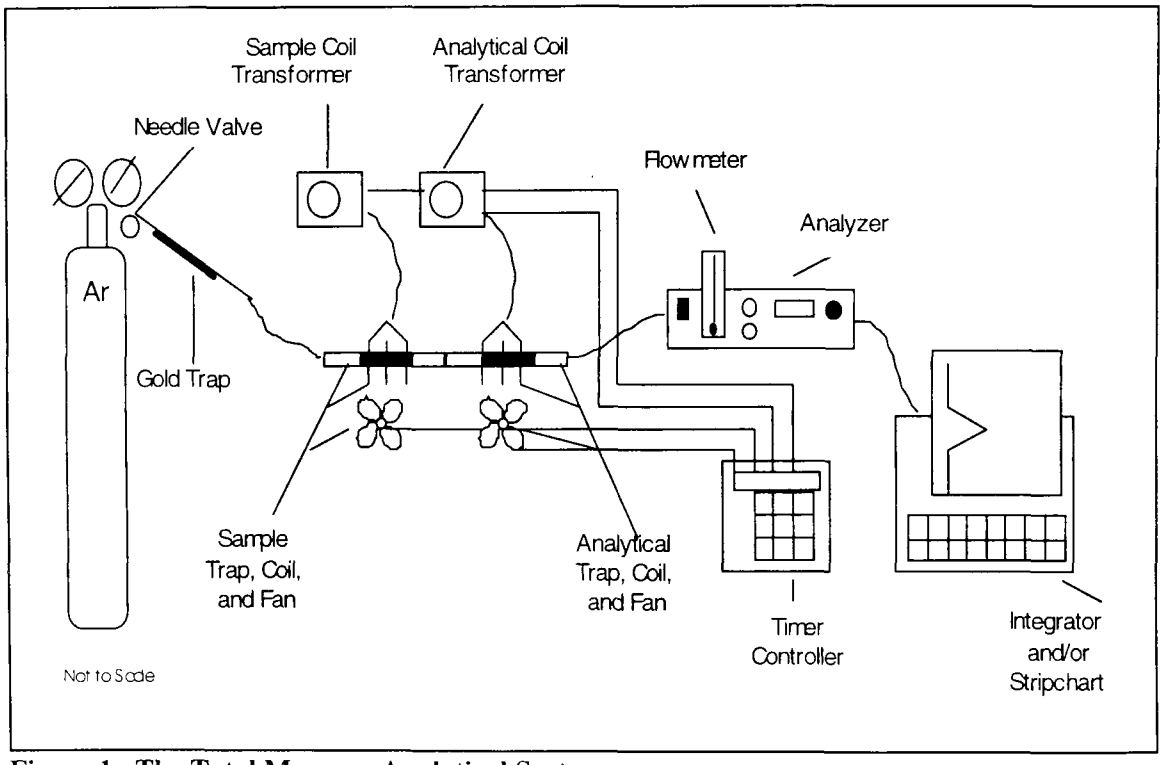

Figure 1. The Total Mercury Analytical System

7.2 Step 2 Adjust the systems settings

7.2.1 Switch the variable transformers that power the sample and analytical nichrome coils to the 140 V position. The transformer dials should be set at approximately 10%.

These settings control the temperature ramp used to thermally desorb mercury from the gold traps. Low coil temperatures allow mercury to remain on the trap. High temperatures can compromise trapping efficiency and may vaporize the gold; which then plates out further downstream destroying tubing, fittings and the analyzer's quartz cell. If a coil emits a slight reddish glow in a dimly lighted room but does not glow under normal room light, the coil temperature is likely within the acceptable range. When in doubt, test the traps with Hg injections to determine efficiency and reburn the traps to assess carryover.

7.2.2 Turn on the Ar gas flow with the small needle valve feeding into the gold trap on the regulator unit. If necessary, adjust the Ar flow to about 60 units using the needle valve on the analyzer flow-meter.

The rate of Ar flow controls peak shape which should be symmetrical. If a suitable Ar flow cannot be established using the flow-meter, carefully adjust the Ar regulator valve. Adjust carefully since high pressures can cause friction connections to leak or burst.

7.2.3 Make necessary adjustments to the system settings. Consult the Appendix for appropriate total mercury analysis settings.

Some settings, such as analyzer gain, rarely need adjustment. Others, such as stripchart settings, change for various mercury species and average sample concentrations. Always check every setting before beginning analyses. Figure 1 illustrates the critical components of the analytical system.

8.0 How to Blank Traps & Bubblers and Calibrate the Analyzer

8.1 Step 1 Blank the gold traps

8.2 *Caution*: Nichrome coils

8.2.1 Remove the end-plugs from a gold trap and orient the trap so the constriction is on the downstream or right side of the gold beads. Slip the sample nichrome coil around the trap's quartz barrel. See Figure 2.

It is important to properly orient the trap before inserting it into the coil. Twisting the trap after it is nested in the coil can break the coil or connections.

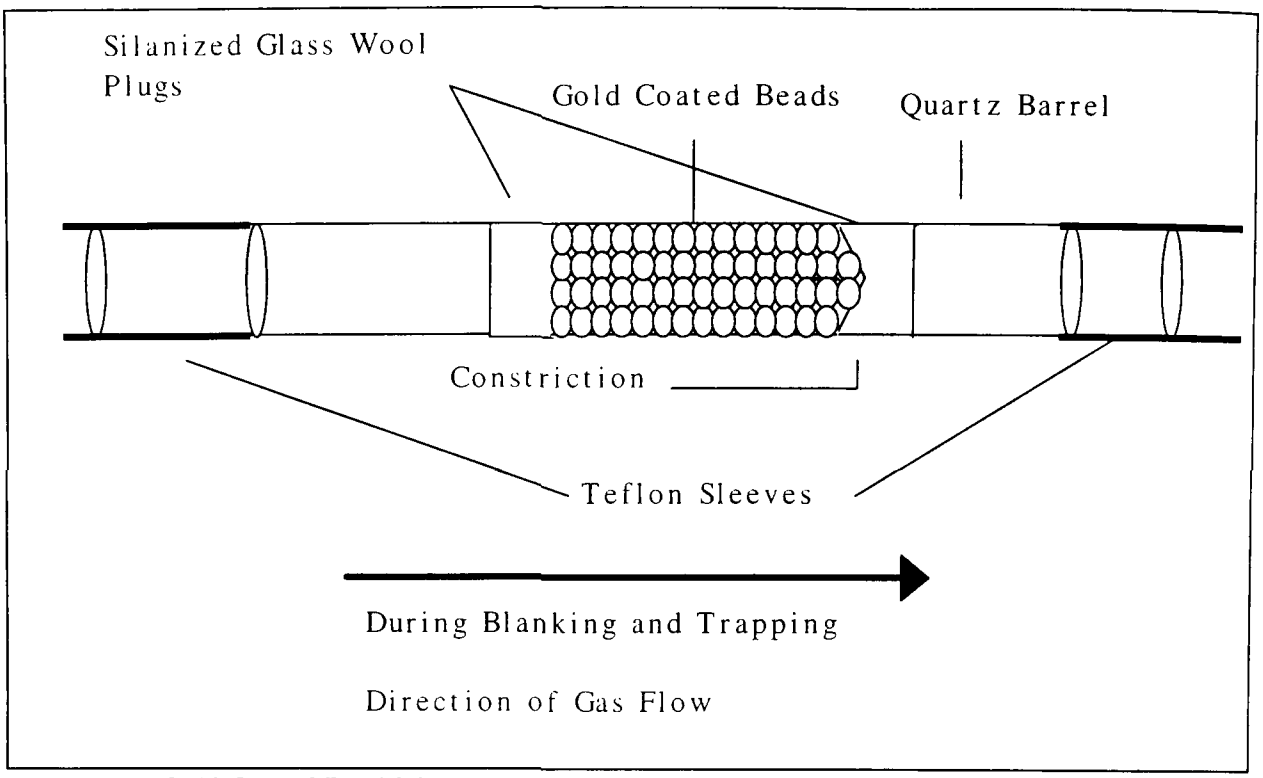

Figure 2. Gold Coated Bead Mercury Amalgamation Trap.

8.2.2 First connect the trap to the analytical (right) side of the system, then plug the Ar line into the left side of the trap.

Improperly connecting a trap to the Ar line can cause the packing material to shift or be blown out of the quartz tube.

8.2.3 Carefully adjust the position of the nichrome coils so they are centered over the gold bead region of the traps.

The coils should be placed in precisely the same position every time a trap is heated. Mercury can plate out on the inside of the traps barrel during normal use. This plated mercury is subsequently desorbed into the sample stream if the coil extends over a section of the trap that is not normally heated.

8.2.4 Press the sequence <PROGRAM> <1> <ON> on the timer controller.

This program switches the sample coil on for 4 minutes (Figure 3). This blanking procedure is intended only to clean residual mercury from the traps which may have accumulated since last use. Because this is not a quantitative step, the analytical trap may be purged just twice while blanking the last two traps in the set of sample traps. This will be explained in G.

8.2.5 After the 4-minute sample trap heating cycling is complete, press the sequence <CIRCUIT> <3> <ON> on the timer controller.

This switches on the sample trap cooling fan. Allow the coil and trap to cool for about 2 minutes before proceeding.

8.2.6 When the sample nichrome coil and trap are cool, press the sequence <CIRCUIT> <3> <OFF> on the timer controller. *Remove the sample gold trap; disconnect the analytical (right) side first.*

This switches off the sample trap cooling fan. Improperly disconnecting a trap from the Ar line can cause the packing material to shift or be blown out of the quartz tube.

8.2.7 Repeat Step 1 for four traps. For the last two traps, follow the above blanking procedures through C, then press the sequence <PROGRAM> <8> <ON> on the timer controller.

Program 8 appropriately controls coils and fans over an 8 minute period; both the sample and analytical traps are blanked. See Figure 3.

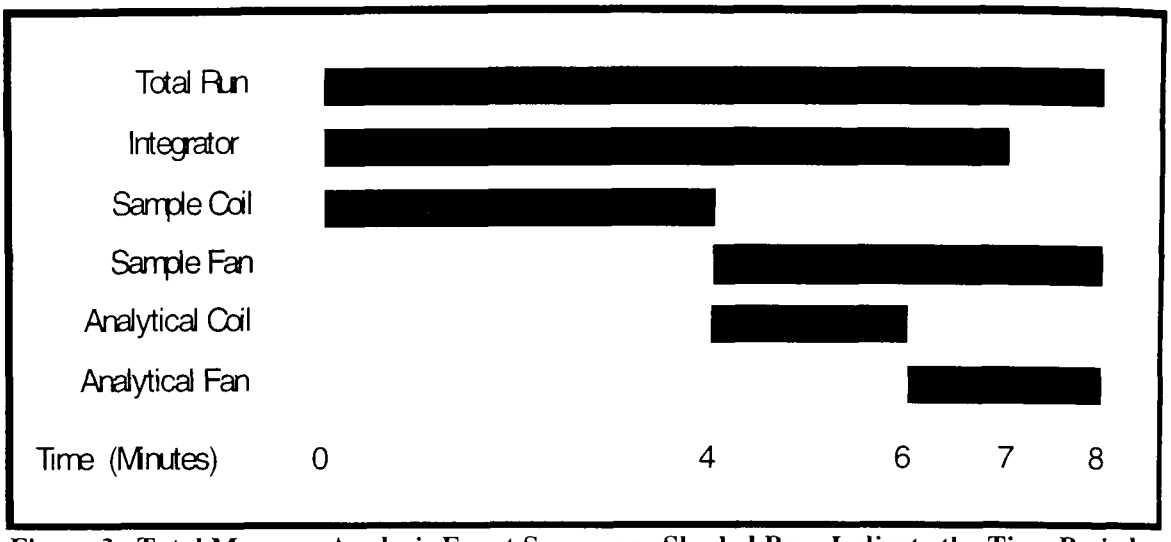

Figure 3. Total Mercury Analysis Event Sequence. Shaded Bars Indicate the Time Periods When Each Device Is Switched On.

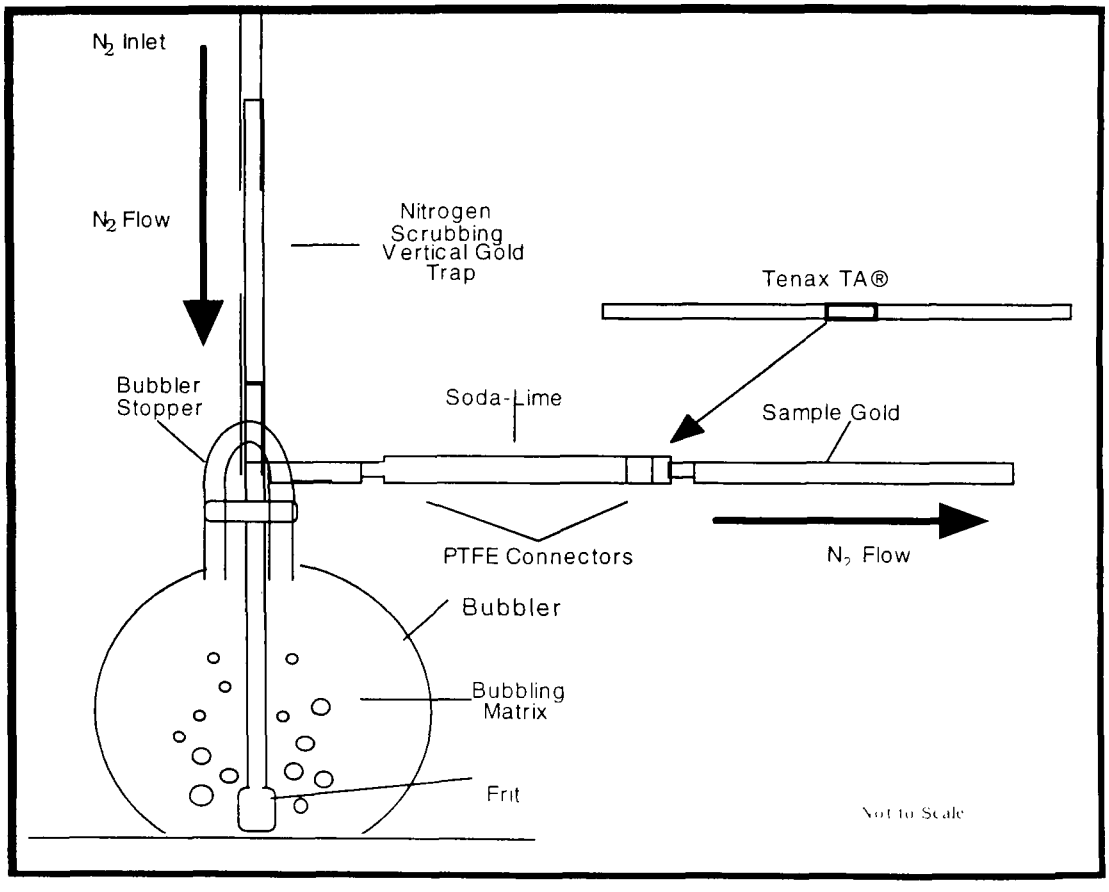

Figure 4. Total Mercury Purging and Trapping System.

8.3 Step 2 - Prepare bubblers for blanking and analyses

Caution: HCl, $SnCl_2$

8.3.1 Using clean deionized water, thoroughly rinse and partially fill each bubbler (about half full, or 125 mL).

Deionized water from a Milli-Q system is quite clean (0.1-0.3 ng/L) if the plumbing has been completely flushed.

8.3.2 Clear the HCl repipetor by pumping a few strokes into a small beaker and disposing this acid. Dispense 5 mL of clean acid into each bubbler.

Avoid producing acid fumes by filling the beaker halfway with water before pumping the repipetor.

8.3.3 Dispense 0.5 mL of $SnCl_2$ into each bubbler.

Remember to rinse the pipette tip.

8.3.4 After removing the end-plugs, attach the gold traps to the vertical inlets on the bubbler stoppers. Plug the N_2 lines into the gold traps. See Figure 4.

These gold traps scrub residual elemental mercury from the N_2. Remove the Teflon sleeve and plug from the end of the trap nearest the constriction; carefully insert the trap into the sleeve on the bubbler top. Remove just the plug from the other end of the trap and connect the N_2 line.

8.3.5 Attach soda lime traps - see Section 9.3.3 for description of soda lime traps.

8.3.6 Attach Tenax TA® pretraps (if used) downstream of the soda lime traps.
Some tributary samples, particularly early spring samples, exhibited a pronounced matrix effect which is eliminated by using these pretraps. It is recommended that the pretraps be used for all tributary samples.

8.3.7 Turn on the N_2 with the small needle valve feeding into the gold trap attached to the regulator unit. Adjust the N_2 flow into the bubblers if necessary. All flow rates should be about 50 units. See Figure 5.

The exact flow rate is unimportant. Make certain that all bubblers have approximately the same flow rate. If an appropriate N_2 flow cannot be established with the flow-meters, carefully adjust the N_2 regulator valve. Monitor the back pressure. Excessive pressure will cause the bubbler stoppers to pop off.

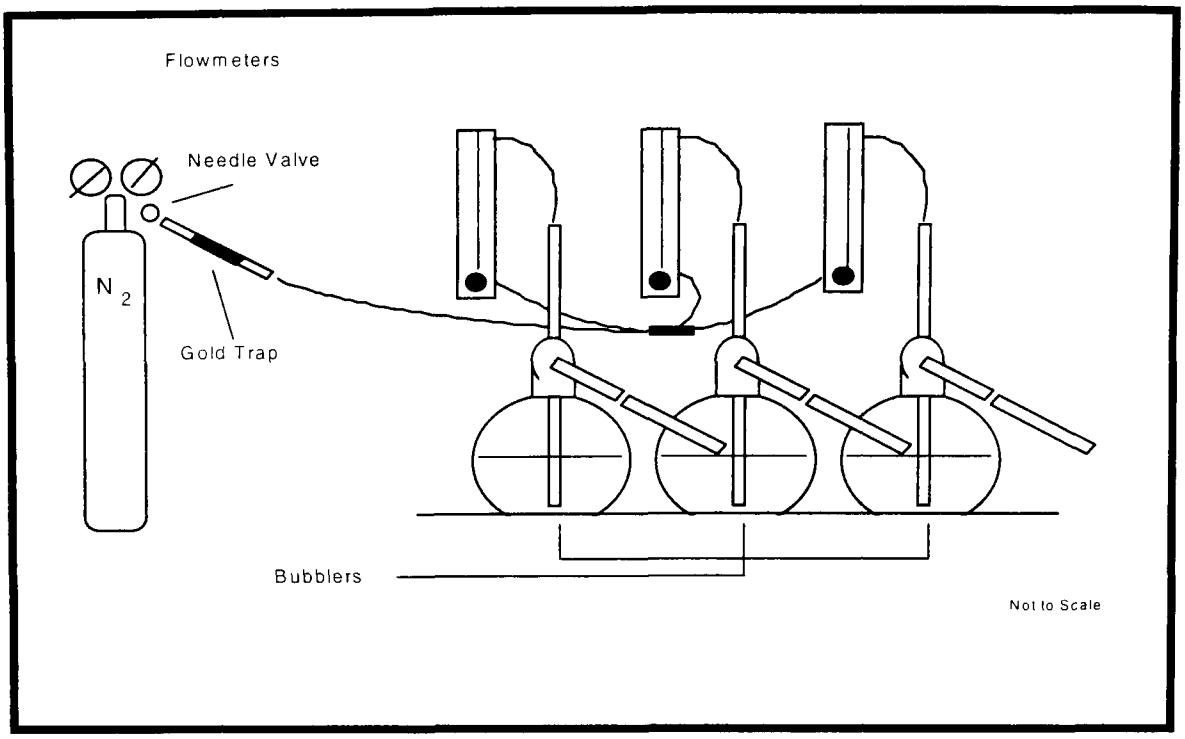

Figure 5. Total Mercury Purging and Trapping System

8.4 Step 3 Blank the bubblers

Caution: $SnCl_2$

8.4.1 Dispense 0.5 mL of $SnCl_2$ into each bubbler containing the pre-bubbled solution from Steps 2 and 3. After removing the end-plugs, attach a gold sample trap to each Tenax TA® pretrap (if used) or directly to each soda-lime trap.

Because 0.5 mL of $SnCl_2$ is added to every sample independent of sample volume, the $SnCl_2$ is considered part of the bubbler blank. Attach the gold trap so the constriction is farthest from the Tenax TA® pretrap or soda lime trap *i.e.*, on the down-stream end.

8.4.2 Bubble for 20 minutes.

This process purges and traps the ubiquitous residual mercury from the glassware and bubbling matrix. Assume this amount of mercury is released every time a sample is purged. Because it is an artifact, this quantity of mercury must be subtracted from the standard and samples.

8.4.3 Remove and plug the sample traps; analyze each gold trap.

Turn to How to Analyze Gold Traps on Section 10.0 to continue

8.5 Step 4 - Calibrate the Analyzer

Caution: $SnCl_2$ total mercury standard

8.5.1 Add 0.5 mL $SnCl_2$ and a series of volumes (usually 25, 50 and 100 μL) of total mercury working standard to the pre-bubbled matrix from Steps 2, 3 and 4. Attach a gold sample trap to each of the soda-lime traps connected to the bubblers.

The concentration of the total mercury working standard is 10 mg/mL; a 100 µL aliquot has 1 mg of mercury in it. If you suspect that bubbled samples will have significantly more or less mercury choose a volumes of standard that will more closely match the sample concentrations.

8.5.2 Bubble for 20 minutes.

This process purges and traps a known mass of mercury.

8.5.3 Turn off N_2; twist bubbler stopper to release seal; remove and plug the gold traps. Analyze each gold trap.

To prevent water from being sucked into the traps that scrub mercury from the N_2, always turn off the N_2 before removing the sample traps. Turn to How to Analyze Gold Traps on Section 10.0 to continue.

9.0 How to Prepare Water Samples for Analysis

9.1 Step 1 Digest the water samples

Caution: BrCl

9.1.1 Add excess BrCl to every water sample. This will be indicated by a persistent yellow color. For tributary samples generally 5 to 8 mL per 500 mL bottle are required. Record the amount of BrCl added to the sample.

Mercury in any of its chemical forms is oxidized to Hg^{2+} by BrCl. Remember to record the amount of BrCl in each sample so the appropriate reagent blank can be subtracted.

9.1.2 Repackage sample bottles in two plastic bags. Place in a 70 °C oven overnight. If the yellow color disappears, continue to add BrCl until the yellow color remains. Always wrench tighten bottles after they have been removed from the oven and cooled.

Some samples contain compounds that compete with mercury for the BrCl. These compounds effectively neutralize all the BrCl before the mercury is oxidized. For this reason, BrCl should be added in excess, indicated by a persistent yellow color. Wrench tightening is necessary, since the bottle caps become loose during the heating and cooling process.

9.2 Step 2 Prereduce samples

Caution: $NH_2OH{\cdot}HCl$

9.2.1 To each digested sample, add 30 μL of $NH_2OH{\cdot}HCl$ solution for every 1 mL of BrCl added in Step 1. Swirl the sample.

The $NH_2OH{\cdot}HCl$ reduces (*i.e.* neutralizes) the excess BrCl in the sample.

9.2.2 Allow the sample to react for 5 minutes.

The yellow color from the excess BrCl should disappear.

9.3 Step 3 - Purge samples and capture mercury on gold traps

Caution: $SnCl_2$

9.3.1 Place a bubbler on a pan balance and tare. The bubbler may be empty or contain a purged solution from bubbler blanks, standards or a previous sample. Dispense about 100 to 125 mL of sample into the bubbler. Try to keep the same overall volume in the bubbler during the day. Record the exact volume dispensed.

Because the matrix from the blanks, standards and bubbled samples has been purged of mercury, sometimes it is more efficient to simply add another sample without emptying the bubbler. Make sure, however, that the new sample will fit into the bubbler before pouring. Of course the volume of sample dispensed into the bubbler can be more or less than prescribed here, depending on the suspected mercury concentration. By keeping the bubbled volume within a 1 mL range for an entire analysis session, you can facilitate easy concentration calculations. Also, by keeping the same overall liquid level in the bubbler constant you minimize the possibility of cleaning bubbler surfaces not cleaned initially at the beginning of the day.

9.3.2 Add 0.5 mL of $SnCl_2$ to the sample and cap.

The $SnCl_2$ reduces the Hg^{2+} in the sample to Hg^0. Hg^0 is volatile and can be purged from the water sample onto gold traps.

9.3.3 Attach a gold sample trap to each soda-lime trap or Tenax TA ® pretrap. Reconnect the N_2 lines.

Attach the gold trap so the constriction is farthest from the soda lime or Tenax TA® pretrap, *i.e.*, on the down-stream end.

9.3.4 Bubble for 20 minutes.

This process purges mercury from the sample water; the mercury is trapped on the gold coated beads.

9.3.5 Turn off N_2, twist bubbler stoppers to release seal; remove and plug the sample traps. Analyze each gold trap.

To prevent water from being sucked into the vertical gold traps that scrub mercury from the N_2, always disconnect the N_2 lines before removing the sample traps. Turn to How to Analyze Gold Traps on Section 10.0 to continue

10.0 How to Analyze Gold Traps

10.1 *Caution*: Nichrome coils

10.1.1 Remove the end-plugs from a gold trap and orient the trap so the constriction is on the downstream or right side of the gold beads. Slip the nichrome coil around the trap's quartz barrel.

It is important to properly orient the trap before inserting it into the coil. Twisting the trap after it is nested in the coil can break the coil or connections.

10.1.2 First connect the trap to the analytical (right) side of the system, then plug the Ar line into the left end of the trap

Improperly connecting a trap to the Ar line can cause the packing material to shift or be blown out of the quartz tube.

10.1.3 Carefully adjust the position of the nichrome coils so they are centered over the gold bead region of the traps.

The coils should be placed in precisely the same position every time a trap is heated. Mercury can plate out on the inside of the traps barrel during normal use. This plated mercury is subsequently desorbed into the sample stream if the coil extends over a section of the trap that is not normally heated.

10.1.4 Press the sequence <Program> <8> <ON> on the timer controller. If you are using an integrator.

Program 8 appropriately controls coils and fans over an 8 minute period; both the sample and analytical traps are heated. See Figure 3.

10.1.5 When the cooling fans turn off (after about 8 minutes), write the sample identification next to the peak on the chart or integrator paper. Remove the sample gold trap; disconnect the analytical (right) side first.

Improperly disconnecting a trap from the Ar line can cause the packing material to shift or be blown out of the quartz tube.

10.1.6 Repeat Step 1 for the other two traps.

With a system of three bubblers, sample bubbling time is approximately equal to the time needed to analyze three traps. Establish a cycle where one set of three traps is being analyzed while the other set is capturing purged mercury.

11.0 How to Prepare Soda Lime Traps

11.1 *Caution*: Glass wool, soda-lime

11.1.1 Assemble materials on the lab bench before beginning. You'll need FEP tubes, PTFE machined connectors (10 mM to 5 mM), glass wool, teflon tape and soda-lime.

The FEP tubes and PTFE connectors can be used more than once between cleanings. Repack the traps with fresh soda-lime at least every other day of analysis.

11.1.2 Create six small balls of glass wool to plug the ends of the traps.

The balls of glass wool should be about 0.5 cm in diameter. They need not be very dense.

11.1.3 Place one of the glass wool balls into an FEP tube. Carefully wrap a piece of teflon tape around a machined connector. Make sure the tape is free of wrinkles, as this can be a conduit for leaks, and is not covering the bore hole for gas flow. Insert this machined connector carefully so the teflon tape is not bunched or wrinkled.

The connector should fit tightly in the tube. Take care not to pinch an excess of glass wool fibers between the outside of the connector and the inside of the tube. This can create also leaky connections.

11.1.4 Holding the connector, scoop soda-lime into the tube. Fill the tube to 0.25 cm of the unplugged end.

To avoid spilling soda-lime around the Clean Lab, stand over a trash can while filling the traps.

11.1.5 Place another glass wool plug into the tube and insert a connector wrapped with teflon tape. Wipe any soda-lime dust off the outside of the trap with a towel.

Both the soda-lime and the glass wool will compress to allow insertion of the connector.

11.1.6 After building 2 more traps, place one trap on the horizontal outlet of each bubbler.

The soda-lime traps are symmetrical so orientation is not important.

11.1.7 After the soda-lime traps have been installed, bubble overnight in a MilliQ matrix with mL HCl and a few mL $SnCl_2$ at a flow rate of 5 to 10 units.

This process cleans mercury from the soda-lime traps and the matrix in the bubblers.

Note: Soda lime may be reused once. After the first use, dry the traps by attaching directly to N_2 pretraps overnight applying a N_2 flow rate of about 5 units

12.0 How to Prepare Reagents

12.1 Stannous Chloride

To prepare the stannous chloride solution, 200 g of $SnCl_2$ is measured using a large weighing boat and Teflon spatula. The crystals are poured into a clean 1 L Teflon bottle and then 100 mL of concentrated Tracepure HCl is added to the 1 L bottle with the $SnCl_2$ crystals. This can be done either using the HCl repipetor or a type of clean volumetric glassware. Milli-Q water is then added to bring the solution to 1 L. The solution is purged with N_2 overnight and then labeled $SnCl_2$ and the date the solution was purged. The $SnCl_2$ solution is stored doubled-bagged in the refrigerator.

12.2 Hydroxylamine Hydrochloride

To prepare $NH_2OH{\cdot}HCl$ solution, 300 g of $NH_2OH{\cdot}HCl$ crystals are measured using a large weighing boat and Teflon spatula. The crystals are poured into a clean Teflon 1 L bottle and then filled to 1L with Milli-Q water. (To make 125 mL of solution use 37.5 g of $NH_2OH{\cdot}HCl$ crystals and 125 mL bottle.) Cap the bottle and shake until all crystals are dissolved. For each 1 L of solution, add 1 mL of $SnCl_2$ and purge the solution overnight with N_2.

12.3 Bromine Monochloride

Caution: To prepare BrCl solution, all work must be done in the fume hood and wearing safety goggles.

First 8.6 g of KBr is measured in the hood using a weighing boat and Teflon spatula. The KBr is poured in the 1L Teflon bottle (BrCl stock solution bottle) and the bottle is then filled with 800 mL of concentrated Tracepure HCl and a clean magnetic stir bar added. For approximately an hour the solution is stirred in the fume hood with a stir plate. After the hour is up, *very slowly* add the $KBrO_4$ crystals while stirring slowly. Add small amounts of crystals and only add more when fizzing has stopped. Then allow the solution to stir with the cap on loosely for another hour. Smaller amounts of this stock solution (about 50 mL) are purified in a teflon, sub-boiling distillation apparatus (Savillex®).

12.4 Mercury Standards

To prepare the mercury secondary standard use a stock mercury standard of 1000 mg/L. In a clean 100.0 mL class A volumetric flask, pipette 100.0 μL of the stock solution and 5 mL of BrCl solution into the volumetric flask and dilute to 100.0 mL with Milli-Q water. This pipetting must be done extremely accurately and should be redone if pipette error or contamination occurs. After mixing, the solution is poured into a clean 125 mL Teflon bottle and labeled with Hg 2, ID of stock solution, the date, and your initials. This can be stored in a refrigerator for up to one year. To prepare the working standard, dispense 1.00 mL of the mercury secondary standard and 1 to 5 mL of BrCl solution into another clean 100 mL class A volumetric flask and bring to volume with MilliQ water. This pipetting must also be done with great accuracy. After mixing the solution, pour it into a clean 125 mL Teflon bottle and label with Hg Working Standard, the date, and your initials. This solution should be replaced monthly.

Standard Operating Procedure for Analysis of Sediment for Total Mercury Using the Cold Vapor Technique with the Leeman Labs, Inc. Automated Mercury System

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October 9, 1996

Revision 1

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[1] Current affiliation is SoBran, Inc./Pathology Associates International.

Standard Operating Procedure for Analysis of Sediment for Total Mercury Using the Cold Vapor Technique with the Leeman Labs, Inc. Automated Mercury System

1.0 Introduction

Elemental concentrations of mercury in sediment and water are determined by the PS200 system, and its operation is based upon cold vapor AAAS. The prepared sample enters the system in the divalent phase, and is mixed with stannous chloride to form elemental mercury vapor. This mixture moves to the liquid gas separator, and argon carries the mercury vapor through a drying tube for vapor removal. The vapor enters one path of the cell optimized. The mercury lamp emits light at 254 nm, and absorbance is measured by the detector.

2.0 Materials Required

2.1 Chemicals

Reagents needed include the following ultra pure grade chemicals:

2.1.1 Leeman Labs 100ppm Mercury Standard

2.1.2 Leeman Labs Hydrochloric, Nitric Acids

2.1.3 Leeman Labs Ultra Pure Water

2.1.4 Liquinox

2.1.5 J.T. Baker Brand Hydrochloric, Nitric Acids

2.1.6 J.T. Baker Brand Stannous Chloride or Leeman Labs Stannous Chloride

2.1.7 Hydroxylamine Hydrochloride

2.1.8 Magnesium Perchlorate 10-20 size mesh

2.1.9 Potassium Permanganate

2.2 Equipment and Supplies

2.2.1 Supplies Needed for analyses include:

2.2.1.1 PS200 Automated Mercury Analyzer with autosampler, with associated data acquisition system

2.2.1.2 AP200 Automated Preparation System with associated data acquisition system

2.2.1.3 Analytical Balance in Biology Lab, Mettler 2100T

2.2.1.4 EDP pipettors and associated disposable tips

2.2.1.5 Associated pump tubing for sample drainage, tin chloride

2.2.1.6 Polyethylene tubes 12 mL capacity, and caps

2.2.1.7 Polyethylene tubes 45mL capacity

2.2.1.8 Teflon or polyethylene beakers

2.2.1.9 Teflon wash bottles

2.2.1.10 Teflon bottles (60 mL)

2.2.1.11 Electronic balance

2.2.1.12 PVC gloves

2.2.1.13 Paper towels, clean wipes

2.2.1.14 Lubricating oil for autosampler

2.2.1.15 Quartz wool and quartz glass drying tubes

2.2.1.16 Teflon spatula

2.2.2 Supplies needed before sample analyses if not using automated preparation system:

2.2.2.1 CEM microwave digester with associated Teflon PFA vessels

2.2.2.2 Low density 30-mL polyethylene bottles for sample storage

2.3 Reference Documents

The user of this method must be familiar with the following established standard operation procedures:

LLRS-MET-SOP-001 Standard Operating Procedures for the Preparation of Materials used for Ultra-low Trace Element Analyses

LLRS-MET-SOP-003	Standard Operating Procedures for the Maintenance of the LLRS Trace Metal Laboratories
LLRS-MET-SOP-010	Standard Operating Procedures for Analysis of Total Mercury in Tissue and Sediment using the Cold Vapor Technique with the Perkin-Elmer Model MHS-20 Gold Amalgam System
LLRS-QA-001	Minimum Analytical Quality Assurance Objectives for U. S. EPA Large Lakes Research Station
LLRS-QA-SOP-001	Standard Operating Procedures for the Release of Data
LLRS-QA-SOP-002	Standard Operating Procedures for the Routine Review of Data Quality and Quantity

3.0 Reagent Preparation

3.1 5% Nitric Acid Solution

This acid solution is used for rinsing materials used for cleaning materials used in the analysis of samples.

3.4.1 Rinse a pre-cleaned graduated cylinder (100 mL) with three rinses of MSQ.

3.4.2 To the graduated cylinder, add 95 mL of MSQ.

3.4.3 Carefully add 5 mL of concentrated reagent grade nitric acid.

3.4.4 Transfer the solution to a squirt bottle.

3.4.5 Repeat steps 3.4.1 through 3.4.3 but add five milliliters of reagent grade acid. (For preparation of 5% nitric acid rinses).

3.4.6 Repeat step 3.4.4 but add to separate.precleaned teflon bottle.

3.2 10% Hydrochloric Acid Rinse Solution

Use reagent grade J.T. Baker or Leeman Labs hydrochloric acid. The amount needed for a one day run is 300 mL. Approximately 2 L will be needed to be prepared weekly.

WARNING

Hydrochloric acid is highly corrosive and incompatible with metals, hydroxides, amines, and alkalis. Handle it while wearing personal protection gear. A full face shield is recommended. Always use the concentrated acid under a fume hood. Store it in an appropriate place. Store concentrated acid in a corrosives cabinet.

3.2.1 Use a precleaned 1000 mL polyethylene graduated cylinder. Carefully rinse the inside of the cylinder with 5% reagent grade nitric acid using a Teflon (precleaned) squirt bottle. Follow this rinse with three rinses of Millipore Super-Q water (MSQ).

3.2.2 Add 900 mL of MSQ to the graduated cylinder.

3.2.3 Carefully add 100 mL of reagent grade hydrochloric acid to the cylinder.

3.2.4 Transfer the mixture to a high density polyethylene bottle from Leeman Labs.

3.3 1:1 Nitric Acid Solution

As recommended by the manufacturer, polyethylene autosampler cups must soak in 1:1 nitric acid before running to ensure acceptable results. Soak the cups for at least two hours. Use J.T. Baker trace metal grade nitric acid for preparation. This solution can be recycled, provided the autosampler cups are adequately rinsed with MSQ a minimum of ten times before and after use. Dispose of 1:1 nitric after one month to eliminate the possibility of any residual contamination.

WARNING

Nitric acid is corrosive and incompatible with combustible materials, metallic powders, hydrogen sulfide, carbides, and alcohols. Handle it with personal protection. A full face shield is recommended for the concentrated acid. Always use the acid under a fume hood. Store the concentrated acid in a corrosives cabinet.

3.3.1 Use an appropriate pre-cleaned container. An empty Suprapure hydrochloric acid bottle has been used. Rinse the container at least three times with MSQ before addition of nitric acid.

3.3.2 Using the graduations on the glass bottle, add 400 mL MSQ.

3.3.3 Carefully add 400mL of J.T. Baker nitric acid to the glass bottle.

3.3.4 Swirl the contents of the bottle and , if necessary, label.

3.4 10%(w/v) Stannous Chloride Solution

The volume of tin chloride solution required to run is dependent upon the daily run time. To run for six hours approximately 250 mL are required.

WARNING

Stannous chloride should be handled with care. Avoid contact with eyes, skin, and clothing. Avoid breathing its dust. Handle solid chemical under a fume hood. Handle while wearing personal protection gear for eyes and skin.

3.4.1 Rinse a precleaned wide mouth teflon bottle three times with quartz distilled water.

3.4.2 Tare the bottle and add 25 g of tin chloride to the bottle.

3.4.3 Rinse a precleaned graduated cylinder with 5% reagent grade nitric acid solution followed by three rinses of MSQ.

3.4.4 Carefully add 25 mL of reagent grade hydrochloric acid to the graduated cylinder. Carefully pour the cylinder's contents into the bottle containing the stannous chloride.

3.4.5 Swirl the contents of the bottle vigorously to dissolve the stannous chloride.

3.4.6 After the stannous chloride has been dissolved, add 200 mL of MSQ using the same graduated cylinder.

3.4.7 Replace the cover and vigorously shake the bottle to ensure all of the tin chloride is dissolved.

3.5 10% Nitric Acid Solution

This acid solution is used for preparation of standards and for dilution of samples. Historically, this solution is used for samples that have undergone microwave digestion.

3.5.1. Rinse a pre-cleaned graduated cylinder (100 mL) with 5% reagent grade nitric acid solution followed by three rinses of MSQ.

3.5.2 To the graduated cylinder, add 90 mL of MSQ.

3.5.3 Carefully add 10 mL of concentrated Seastar or reagent grade nitric acid (whichever matches the matrix of samples).

3.5.4 Transfer the solution to a teflon bottle.

3.6 Working Mercury Standard Solution

Commercial 100 ppm Leeman Labs mercury standard is used to prepare the working standard. The working standard is prepared at a concentration of 100 ppb or 0.1 µg Hg/mL and is made fresh weekly.

WARNING

Mercury is a poison. Handle it, its compounds, and its solutions with personal protection. Mercury can form a vapor and be inhaled. It also is absorbed through the skin. Use this material in a fume hood. Always wear personal protection gear.

3.6.1 Rinse a pre-cleaned teflon bottle (LLRS-MET-SOP-001) three times with MSQ and air dry.

3.6.2 Using a precleaned graduated cylinder, rinse three times with 5% nitric and three times with MSQ.

3.6.3 Using two EDP separate automatic pipettors, rinse 2-1000 mL tips three times with a 5% reagent grade nitric solution followed by three rinses with MSQ.

3.6.4 Tare a dry bottle on the electronic scale.

3.6.5 Using an additional EDP pipettor, rinse a 100 µL tip in the same manner as 3.6.2

3.6.6 Using the cleaned 100 µL tip, carefully add 50 µL of the commercial 100 ppm Leeman Labs mercury standard solution to the bottle.

3.6.7 Transfer 45mLs of the 10% nitric acid solution using the cleaned graduated cylinder.

3.6.8 Using an EDP pipettor, with a 1000 µL precleaned tip, add 10% nitric acid solution to the bottle until the weight is 50 g.

3.7 Preparation of Recommended Range of Calibration Standards

The calibration standards are prepared fresh semiweekly.

3.7.1 Microwave Digestion Standard Range

The following range of standards has been used for analyses of Green Bay Sediment samples and bracket the samples well. 0.250 ppb, 0.500 ppb, 1.00 ppb 2.00 ppb, 5.00 ppb are used (0.00025 µg/mL, 0.00050 µg/mL, 0.001 µg/mL, 0.002 µg/mL, 0.005 µg/mL). It may be possible to go below 0.250 ppb depending upon instrument performance. The lowest concentration above background noise

is approximately 0.087 ppb (0.000087 µg/ mL). 60 mL of each calibration standard will last for two daily runs. Prepare double of the desired standard that will be run as a check standard. The autosampler cups must be filled to at least 40 milliliters, (60 mL total capacity).

3.7.1.1 Rinse six pre-cleaned teflon bottles three times with MSQ and allow to air dry.

3.7.1.2 Prepare two separate EDP pipettors each with a precleaned 1000 µL tip. (Rinse each pipette tip three times with 5% nitric acid followed by three rinses with MSQ) .

3.7.1.3 Rinse a precleaned graduated cylinder with 5% nitric acid followed by three rinses of MSQ.

3.7.1.4 For each standard, tare each bottle individually.

3.7.1.5 For the 0.250 ppb standard, pipette 150 µL of the 100 ppb working standard to the bottle.

3.7.1.6 Using the graduated cylinder, bring the total volume up to 55 mL by the careful addition of prepared Seastar 10% nitric acid. The weight of the bottles contents should now be roughly 55 g.

3.7.1.7 Add prepared Seastar 10% nitric acid with the other EDP pipettor and precleaned tip until the total weight is 60 g.

3.7.1.8 Repeat steps 3.7.1.6 3.7.1.9 for the remaining standards by the addition of 300 µL of the 100 ppb standard for a 0.500 ppb calibration standard, 600 µL of the 100 ppb standard for the 1.00 ppb calibration standard, 1200 µL of the 100 ppb standard for the 2.00 ppb calibration standard, and 3000 µL of the 100 ppb standard for the 5.00 ppb calibration standard.

3.7.1.9 Do not recycle the standards remaining in the autosampler cups at the end of the day. Properly dispose of these daily. Attempts to recycle the standards from autosampler cups have given diminished intensities.

3.7.2 Automated Digester Standard Range

Use the following range of standards: 0.000 ppb, 0.125 ppb, 0.250 ppb, 0.500 ppb, 1.00 ppb, 2.00 ppb. Prepare in a 2% hydrochloric acid matrix in precleaned Teflon bottles. Use Leeman Labs or J.T. Baker hydrochloric acid. Prepare 50mL of each standard weekly or as needed.

3.7.2.1 0 ppb Standard

3.7.2.1.1 Rinse a precleaned graduated cylinder with 5% nitric acid followed by three rinses of MSQ. Dispose of waste in an appropriate container.

3.7.2.1.2 Rinse a precleaned 500mL teflon bottle three times with MSQ.

3.7.2.1.3 Add 245 mL MSQ to the bottle.

3.7.2.1.4 Carefully add 5 mL of concentrated hydrochloric acid to the bottle.

3.7.2.1.5 Transfer the solution to a teflon bottle.

3.7.2.2 0.125 ppb Standard

3.7.2.2.1 Prepare one EDP pipettor with a precleaned 100 µL tip.

3.7.2.2.2 Prepare two separate EDP pipettors each with a precleaned 1000 µL tip.

3.7.2.2.3 Rinse a teflon bottle three times with MSQ that will be used for each standard.

3.7.2.2.4 Tare the bottle on the balance.

3.7.2.2.5 Pipette 62.5 µL of the 100 ppb working standard into the bottle

3.7.2.2.6 Use the rinsed graduated cylinder to transfer 45 mL of the 0 ppb standard to the bottle.

3.7.2.2.7 Slowly pipette 5 mL of 0 ppb standard to the bottle until the bottle contents weight 50 g.

3.7.2.3 0.250 ppb Standard

For the 0.250 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 125 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.4 0.500 ppb Standard

For the 0.500 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 250 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.5 1.00 ppb Standard

For the 1.00 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 500 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.6 2.00 ppb Standard

For the 2.00 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 1000 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.8 Reagents Needed for Preparation of Sediment Samples using Protocol PRP7471

3.8.1 50% Aqua Regia (3:1 Hydrochloric : Nitric)

3.8.1.1 Use the glass container that previously held the Suprapure hydrochloric acid, this is supplied with graduations. Rinse three times with MSQ to eliminate any residual contamination.

3.8.1.2 To this glass container add 400 mL of MSQ water.

3.8.1.3 To the glass container add 300 mL of Leeman Labs hydrochloric acid to the bottle.

3.8.1.4 Finally add to the bottle 100 mL of Leeman Labs nitric acid.

3.8.1.5 Carefully transfer solution to instrument bottle.

3.8.2 Potassium Permanganate Solution

3.8.2.1 Rinse a 100 mL precleaned finely graduated cylinder three times with 5% reagent grade nitric acid followed by three rinses of MSQ.

3.8.2.2 Dispose of the instrument bottle's contents if applicable and rinse three times with MSQ.

3.8.2.3 Add 40mLs of the Leeman Labs potassium permanganate solution to the rinsed 100 mL graduated cylinder.

WARNING

Potassium permanganate is a strong oxidizer. Keep it from contact with clothing or combustible materials. Avoid contact with eyes or skin. Avoid breathing the dust. Handle solid chemical under a fume hood. Handle it while wearing personal protection gear.

3.8.2.4 Transfer to the instrument bottle.

3.8.2.5 Add 760 mL of MSQ to the bottle.

3.8.2.6 Swirl the contents of the bottle to ensure a mixed solution.

3.8.2.7 Attach to the instrument.

3.8.3 Hydroxylamine Sulfate Solution

3.8.3.1 Rinse a 100 mL precleaned finely graduated cylinder three times with 5% reagent grade nitric acid followed by three rinses of MSQ.

3.8.3.2 Dispose of the instrument bottle's contents if applicable and rinse three times with MSQ.

WARNING

Hydroxylamine sulfate is an eye, skin, inhalation, and ingestion hazard. It will cause skin irritation and may be absorbed through the skin. Always wear eye and skin protection.

3.8.3.3 Add 96mL of the Leeman Labs Hydroxylamine Sulfate solution to the 100mL graduated cylinder.

3.8.3.4 Transfer the Hydroxylamine Sulfate to the instrument bottle and continue to add 704 mL of MSQ to the instrument bottle.

3.8.3.5 Swirl bottle to ensure a mixed solution.

3.8.3.6 Attach bottle to instrument.

NOTE

This is a modification of the protocol that calls for full strength concentrations of Potassium Permanganate and Hydroxylamine Sulfate. Full strength concentrations of these chemicals were attempted and the fumes generated were very strong. There was no difference in instrument performance in using some higher concentrations of reagents in the protocol. Both gave acceptable recoveries on the SRM material and similar instrumental intensities.

4.0 Microwave Sample Preparation

4.1 Preparation of Teflon Digester Vessels

Teflon digester vessels are cleaned following the procedure in section 4 of LLRS-MET-SOP-010. An alternate method proposed by the manufacturer to increase the life of the digestion vessels is under consideration. It may not be used until it is verified that blanks are equally low for the two methods. Upon verification, the following alternate steps may be substituted for steps 4.4 through 4.7 in LLRS-MET-SOP-010. Steps are taken directly from CEM manual with slight variations to fit constraints of laboratories (Gilman 1988).

4.1.1 Add 20 mL of concentrated nitric acid to the digestion flask. Place the safety disk on the vessel and tighten finger tight only. Place the vessel in the turntable, and attach a venting tube.

4.1.2 Repeat step until the turntable contains 12 vessels.

4.1.3 Turn the MDS-81D exhaust onto the maximum fan speed. Ensure the turntable is rotating.

4.1.4 Program the instrument for five minutes and 100% power. Depress the start key and allow the acid to heat.

4.1.5 Allow the acid to cool to room temperature and manually vent each vessel. Open vessels and pour the acid into an appropriate waste container.

4.1.6 Rinse the vessels three times with MSQ water and allow them to dry in a clean area.

4.2 Extraction of Samples

The maximum weight of samples extracted in CEM vessels without venting and still obtaining acceptable recoveries for all metals has been two grams. For mercury, an average weight of 0.300g has been used. The total volume is 25mL in 10% Seastar nitric acid. Follow steps 5.1.1 through 5.1.11 in LLRS-MET-SOP-010 for preparation of sediments

5.0 AUTOMATED DIGESTER (AP200) SAMPLE PREPARATION

5.1 Daily Instrument Setup

5.1.1 Clean autosampler rails with isopropyl alcohol.

5.1.2 Lubricate rails with oil daily

NOTE

Only if reagents are low or instrument was in shutdown will following steps need to be followed (5.1.3 through 5.)

5.1.3 Open cover and carefully disconnect each bottle separately from the interior of the instrument.

5.1.4 Rinse each bottle three times with MSQ

5.1.5 Fill bottles numbered 1, 5, 6 with MSQ up to the 800ml mark.

5.1.6 Prepare and fill bottle #3 with the Potassium Permanganate Solution.

5.1.7 Prepare and fill bottle #4 with Hydroxylamine Sulfate Solution

5.1.8 Carefully prepare and fill bottle #2 with the 3:1 Aquaregia solution

5.1.9 Check conditions of all fittings, caps and bottles

5.1.10 Close cover and pressurize system (turn gas on).

5.1.11 Set gas pressure = 20 psi

CAUTION

Do not exceed 25 psi

5.1.12 Turn instrument on

5.1.13 Turn computer and monitor on.

5.1.14 Turn printer on and press the online button.

5.1.15 After the computer has booted up, at the C: prompt, type apps.

5.1.16 Follow instructions on p4-4 of manual revision c.

5.2 Software and Instrument Setup

Follow the instruction in system startup section 4-3, What follows is a summary. The user should review the following sections prior to analyses.

References to AP200 Manual

Section 3- System Testing
Section 4- System Operation
Section 5- Routine Maintenance
Section 6- Troubleshooting

5.2.1 Select Protocal and Get PRP7471, defines method, (sec 4-4 of manual)

5.2.2 Check reagent pressure of system, ensure it is > then 5.5psi and within 6.5. (section 2-10 of manual)

5.2.3 Go to menu, F1, select Utility, select Diagnostics

5.2.4 Select Reagent Pressure

5.2.5 Run the change rinse solution macro, @CHRINS

5.2.6 Rinse the autosampler rinse tray 3-4 times with MSQ

5.2.7 Replace the rinse tray

5.2.8 Run the wake up macro @WAKEUP (section 4-3 of manual)

5.2.9 Check centering of autosampler tip over cups, adjust if necessary (section 3-1)

5.2.10 Check precision of dispenser 1X/week (section 3-3), or if there is a pressure change of .2 psi in system at step 14.3.3

5.2.11 Set up autosampler sequence, and start finish sequence (section 4.6)

5.3 Preparation for Sample Digestion

5.3.1 Soak the 45mL standard cups and sample cups in 1:1 HNO_3 for at least 2.0 hours.

5.3.2 Recycle the acid from the cups back into the Suprapure Acid bottle.

5.3.3 Rinse each cup three to six times with MSQ and allow to air dry.

5.4 Preparation of Standards for Automated Digestion

5.4.1 Prepare an EDP pipettor with a precleaned 1000ul tip.

5.4.2 Using the electronic scale, tare the beaker, and then the standard cup within the beaker.

5.4.3 Record the weight of the empty cup on the extraction sheet.

5.4.4 Under the laboratory hood, pipette 5mls of the 0 ppb std to the cup.

5.4.6 Weigh the cup to confirm volume delivered is 5mls, and adjust accordingly with the EDP pipettor.

5.4.7 Repeat steps 16.2 16.5 to extract multiple 0 standards.

5.4.8 Repeat steps 16.1 to 16.5 for the 0.125ppb std, 0.250ppb std, 0.500ppbstd. 1.00ppb std, and 2.00ppb std. Each time use a new precleaned 1000ul tip.

5.4.9 Rinse polyethylene vapor covers 3x with MSQ and place over sample cups.

5.4.10 Snap in place aluminum guard over polyethylene vapor barrier.

5.5 Preparation of Sediment Samples for Automated Digestion

Note

Please read the instruction manual for the Mettler Analytical Balance before proceeding.

5.5.1 Rinse a precleaned teflon spatula with 5% nitric acid.

5.5.2 Rinse the spatula three times with MSQ.

5.5.3 Use the analytical balance in the Biology Lab, Mettler 2100T

5.5.4 Tare the 100mL polyethylene beaker provided.

5.5.5 Add the 45mL sample cup to the beaker and tare the scale again.

5.5.6 Record the weight of the sample cup on the digestion sheet.

5.5.7 Carefully open the Whirlpak bag of sediment.

5.5.8 Open the Whirlpak bag partially, so only a small circular opening exists (about the size of a nickel). Caution: if the bag is open all the way, dust from the bag tends to migrate upwards.

5.5.9 Using the precleaned teflon spatula, carefully scoop an aliquot of sample = 0.100g.

5.5.10 Transfer this aliquot to the sample cup. Note: sand like samples will require a very small aliquot, and silty samples will require a larger aliquot.

5.5.11 Record the weight of the sample in the most significant digits available on the extraction log sheet.

5.5.12 Place the cup in its designated slot in a sample rack.

5.5.13 Zero the scale with the polyethylene beaker on it.

5.5.14 Rinse the teflon spatula with MSQ water three times.

5.5.15 Wipe teflon spatula dry with a fresh clean wipe square between samples.

5.5.16 Repeat steps 16.6.5 till 16.6.15 for the desired amount of samples, usually fourteen.

5.5.17 Load dummy cups into any space not occupied by actual sample.

5.5.18 Rinse polyethylene vapor covers 3x with MSQ and place over sample cups.

5.5.19 Snap in place aluminum guard over polyethylene vapor barrier.

5.6 Initiation of the Digestion Procedure

5.6.1 Load autosampler racks into PS200

5.6.2 Confirm autosampler start to finish sequence is correct.

5.6.3 Go to Main Menu, F1, press MACRO key, and type in @ PRP7471

5.6.4 Method will begin to run at this point.

5.6.5 User will be prompted the following: Wait before reducing with KMNO4. answer Y

CAUTION

User will be prompted near end of 5 hr procedure: Add Hydroxylamine Sulfate, answer Y. Method will not proceed without this input.

5.7 Shutdown of AP200

5.7.1 After protocol is completed, wipe down bath to remove any remaining water.

5.7.2 Run an @APNAP two or three times to clean the dispenser with water.

5.7.3 Remove chemicals and replace reagents with MSQ if it will not be run for an extended period, and run an @CLEAN twice. This cleans not only the dispenser, but all reagent lines.

6.0 Automated Analysis of Digested Extracts

6.1 Preparation of the Instrument

6.1.1 Preparation of Drying Tube

6.1.1.1 Rinse a quartz drying tube three times with MSQ, followed by a dilute rinse of Liquinox, if it previously contained perchlorate.

6.1.1.2 Rinse several times with MSQ to eliminate Liquinox residuals.

6.1.1.3 Allow tube to air dry.

6.1.1.4 Rinse the teflon spatula three times with MSQ and dry with a fresh clean wipe.

6.1.1.5 Gently place a small plug of quartz wool into one end of drying tube.

6.1.1.6 Carefully pour the Leeman Labs Magnesium Perchlorate into the plugged drying tube. Try to fill with coarse grained perchlorate. Do not overfill the drying tube. Overfilling will cause the drying tube to become blocked more easily once it becomes moistened by the gas stream. When filled, the perchlorate should be able to move within the tube when gently moving the drying tube from side to side.

WARNING

Magnesium perchlorate is moderately toxic and a strong oxidizing material. It is a dangerous fire and explosion risk in contact with organic materials. It is an inhalation hazard, and contact with skin or eyes can cause irritation. Work with it in a fume hood while wearing skin and eye protection.

6.1.2 Autosampler Tray Rinse with 10% Hydrochloric Acid

6.1.2.1 Rinse the autosampler rinse tray three times with MSQ.

6.1.2.2 Fill the tray with the 10% hydrochloric rinse prepared in step 3.1.

6.1.3 Tin Chloride Rinse

6.1.3.1 Rinse the tin chloride bottle out three times with MSQ.

6.1.3.2 Fill it with the 10% tin chloride prepared in step 3.3.

6.1.4 Check Tubing Condition and Adjust to Appropriate Tension

6.1.4.1 Check condition of tubing for flattening, abrasion or other signs of wear. If flattened, replace it.

6.1.4.2 Adjust tension on clamps to a halfway point. The sample line should be halfway minus one notch.

CAUTION

Do not over tighten clamps. Too much tension will cause tube flattening and a decrease in overall sensitivity.

6.1.5 Clean and Oil Autosampler Rails

6.1.5.1 Using a clean wipe or clean a paper towel, wipe the autosampler rails with isopropyl alcohol.

6.1.5.2 Place a small amount of oil on bottom of each rail.

6.1.5.3 Complete a high stress maintenance cleaning monthly.

6.1.6 Warmup Period

6.1.6.1 Allow the instrument to warmup for at least one hour time before analyses. If the instrument was in SHUTDOWN mode for an extended period allow it to warm up for several hours.

6.1.6.2 Software commands for warmstrt or coldstrt are found on page 16 of the manual.

6.1.6.3 Perform a COLDSTRT or WARMSTRT

6.1.7 Optimize Optics with an Aperture Test

6.1.7.1 Go to Main Menu, F1

6.1.7.2 Select Diagnostics, Select Aperture Test

6.1.7.3 Unscrew screw which is furthest out until the minimum absorbance is obtained. An acceptable value is 0-100.

6.1.7.4 Select Test Optics from Diagnostics menu, and confirm gain, or intensities are in the range of 500000-1100000 (= to voltage on lamp).

6.1.7.5 Difference between the two beams must be less than 100,000.

6.2 Software Setup for Routine Analysis

Consult Leeman Labs Automated Mercury Analyzer Manual pp 17-29 for guidance on the software setup. What follows are the software and instrumental parameters used to date. Complete the following steps before analyses.

6.2.1 Establishing a Protocol = Method file that Contains All Instrumental Parameters

6.2.1.1 From the Main Menu, select Protocol and then select **G**et.

6.2.1.2 To create a new Protocol, enter its name.

6.2.1.3 Suggested protocol naming is as follows: YYMMDD (Year, Year, Month, Month, Day, Day. Example 960916F = September 16, 1996.

Note
Limitation is 8 Characters

6.2.1.4 Computer will guide you through prompts (PS200 Manual pp. 18-19).

6.2.2 Creating a Folder=Data File

6.2.2.1 Press F1, to be at the Main Menu.

6.2.2.2 Select Data output and select Open Folder.

6.2.2.3 Type in a folder name, suggested name = same name as protocol to avoid confusion.

6.2.2.4 Follow instructions on page 19 of the manual.

6.2.3 Entering Instrumental Parameters

6.2.3.1 Follow instructions in PS200 instrument manual on pp 20-21.

6.2.3.2 The following are parameters used to date:

Integrations:	1
Uptake Time:	10
Weight:	Y
Dilution:	Y
Percent Recovery:	N
On/Off:	10 (higher integration time generates a nonlinear curve)
Flow Rate:	0.30 L/min

6.2.4 Standard Concentration Calculations

For those standards that are prepared with the automated digestion system, the appropriate concentration to be used for keying in standard concentration data must be calculated. Do not do this for microwave digested samples.

6.2.4.1 Tare a 100ml polyethylene beaker on the electronic scale.

6.2.4.2 Weigh a sample cup.

6.2.4.3 Record the weight on the extraction log, and zero the scale.

6.2.4.4 Repeat 6.1.1 through 6.1.3 for all sample cups.

6.2.4.5 Apply the following formula to calculate the final concentration of the extracted standards:

$$C_2 = C_1 * V_1/V_2 ;$$

where,

C_1 is the prepared standard concentration,
C_2 is the final concentration in the standard,
V_1 is the volume of prepared standard used,
and V_2 is the total volume of the extract

For example if 5 mL of 0.500 ppb standard was added to the digestion tube and the final volume of the extract is 43 mL, the resulting standard concentration is as follows:

$$C_2 = 0.500 \text{ ppb} * 5 \text{ mL}/43 \text{ mL} = 0.058 \text{ ppb}.$$

6.2.4.6 Record this concentration on the extraction log.

6.2.5 Entering Standard Concentrations

6.2.5.1 Follow instructions on p. 21 of the manual. Enter units in ppb, not ppm (i.e. 0.500 not 0.00050).

6.2.5.2 Calculations are only carried out to three decimal points. 0.00050 will be truncated to 0.000.

6.2.5.3 Do not enter terms of units, i.e. ppb, ppm. The final calculation will be in ug/g.

6.2.5.4 The following are ranges of standards used for analysis. These ranges have been successful in bracketing low level samples.

Microwave Digestion Standards (ppb)	Automated Prepared Standards (ppb) (Dependent upon total volume of extract. See section 6.2.4)
0.000	0.0000
0.250	~0.0140
0.500	~0.0280
1.000	~0.0570
2.000	~0.1140
4.000	~0.2280

6.2.6 Reset Calibration Intensity Data

Follow instructions on page 22 of manual.

6.2.7 Autosampler Rinse Time

6.2.7.1 Follow instructions on page 23 of manual.

6.2.7.2 Use a rinse time of 60, not 50 seconds. This is the rinse time between samples in the analyses mode.

6.2.8 Autosampler Rack Entry

6.2.8.1 For basic entry information, refer to page 25 of the manual. For extended information on macros and advanced command see reference section A-B-7.

6.2.8.2 What follows is an example of an autosampler rack file. It was used for analyses of Green Bay sediment. Prepare the file before analyses.

NOTE

Actual sample weight must be multiplied by 1000 to obtain results in ug/g.
Total volume = extraction volume * dilution factor.

1 0PPB	1.000		
2 GII25F102SQ*	323.00	250.00	SRM diluted 10x extracted in 25mls
3 GB88-71	280.00	125.00	Sample diluted 5x extracted in 25mls
4 GB89-73	241.00	125.00	
5 GB89-74	5.700	125.00	

A microwave digested SRM will need to be diluted 10 to 20 times depending upon the weight of the sample to be within range of standards. Certified value for SRM2704 = 1.47 ug/g. For a sample that has a extraction weight of 0.250g in 25mL, (1.47*.250)/25=0.018375 ppm=18.37 ppb. This is diluted 20X = .918 ppb

6.3 Analysis of Extracts

6.3.1 Filling Autosampler Cups

6.3.1.1 Recycle the 50% acid rinse used in the autosampler cups. Place it in the glass SUPRAPURE acid bottle.

6.3.1.2 Rinse each cup 5-10 times with MSQ.

6.3.1.3 Allow cups to air dry

6.3.1.4 Fill each standard cup with its designated standard.

6.3.1.5 Using two separate EDP pipettors, prepare a clean 10mL tip and 1000uL tip. Rinse each tip three times with 5% nitric acid followed by three rinses of MSQ.

6.3.1.6 For samples and SRMS requiring dilution, dilute to at least half their capacity of the autosampler tips (6mL). First add the required volume of diluent with the 10mL tip and then the required amount of the sample. An SRM will need to be diluted 10 to 20 times depending on weight of sample. For dilutions of auto-digested samples use 0 ppb standard that has undergone digestion. For microwave digested samples use 10% Seastar nitric acid.

6.3.1.7 Mix the sample 3-5 times with the 1000uL tip.

6.3.1.8 Use a new precleaned 1000uL tip for each sample.

6.3.2 Calibrate the Instrument

Calibrate the instrument using the Macro CAL245 (p. 26 in PS200 instrument manual). Use a 5-point calibration curve that includes a zero standard. If an acceptable correlation coefficient is obtained (0.995) and a standard's intensity is within a the range expected, continue with SRM analyses. See Appendix A for historic performance of the instrument

6.3.3 Analyze the SRM

Analyze the SRM. Refer to page 25 in manual for autosampler start to finish sequence and reference section A-B 7.

6.3.4 Check Standards

6.3.4.1 Run check standards every 10 samples to ensure the instrument has not drifted from its calibration range.

6.3.4.2 Acceptable Check Standard Ranges are:

Microwave Digestion	Automated Digestion
0.250ppb = 15%	~0.014ppb = 15-20%
0.500ppb=10%	~0.028 ppb = 15%
1.00ppb=10%	~0.058ppb = 10%
2.00ppb=10%	~0.115 ppb = 10%

6.3.4.3 Refer to reference section c-11 for more information.

CAUTION

If check standards fail, recalibrate the instrument. Do not use update slope or intercept.

6.4 Data File Preparation

Refer to the reference sections D-1, E-1-5, R-3, and R-5 for preparation of post-run data and computer files in the AP200 Manual. These data references apply to digested and samples prepared on the Automated Digester.

6.5 Instrument Shutdown

6.5.1 Dispose of 10% Hydrochloric acid rinse in an appropriate container.

6.5.2 Rinse the tray out three times with MSQ.

6.5.3 Transfer remaining tin chloride to teflon bottle in which it was earlier prepared.

6.5.4 Rinse out tin chloride bottle three times with MSQ.

6.5.5 Fill autosampler tray and tin chloride bottle with MSQ, flush for ten minutes.

6.5.6 Use OVERNITE, or SHUTDOWN modes to shutdown the instrument.

6.5.7 If using OVERNITE MODE, check condition of drying tube, to ensure it is not saturated with moisture.

6.5.8 Repack a new drying tube if necessary.

7.0 Suggestions for Successful Analyses

7.1 Allow the autosampler cups and standard cups to rest in 50% nitric acid for at least two hours before analyses.

7.2 For best results prepare tin chloride and standards as described.

7.3 Prepare a loosely packed drying tube daily. After use, dispose of perchlorate in an appropriate container. Rinse a drying tube with MSQ and flush lightly with Liquinox. Rinse several times to eliminate any residual Liquinox.

7.4 Periodically check tin chloride line and liquid gas separator for any blockage.

7.5 Check at seals of teflon tubing of drying tube connection for gas leaks.

7.6 Change tin chloride line and sample line weekly or after four days of continuous use.

7.7 Change drain tubing every two weeks as needed.

7.8 Clean autosampler rails with isopropyl alcohol weekly and oil rails daily. If not sufficiently lubricated, the autosampler arm will encounter snags or stops.

7.9 Biweekly calibrate the autosampler tip to ensure it is picking up more than three milliliters.

8.0 Literature Cited

Gilman, L. B., 1988. *General Guidelines for Microwave Sample Preparation.* Revision of July 1988. CEM Corporation, Matthews, NC.

Leeman Labs, Inc., 1991. *PS200 Automated Mercury Analyzer Set-up and Operating Manual.* Version of November 1991.

Leeman Labs, Inc., 1993. *AP200 Automated Mercury Preparation System Manual.* Revision C (11/20/93).

Rossmann, R. And K. A. Rygwelski, 1996. *Standard Operating Procedures for the Release of Data.* Revision 1 (1/18/96) LLRS-QA-SOP-001.

Rossmann. R., 1992. *Standard Operating Procedures for the Routine Review of Data Quality and Quantity.* Revision 0 (12/2/92) LLRS-QA-SOP-002.

Rossmann, R., 1992. *Minimum Analytical Quality Assurance Objectives for U. S. EPA Large Lakes Research Station.* Revision 1 (7/28/92) LLRS-QA-001.

Rossmann, R., 1993. *Standard Operating Procedures for the Preparation of Materials used for Ultra-low Trace Element Analyses.* Revision 1 (8/9/93) LLRS-MET-SOP-001

Rossmann, R., 1993. *Standard Operating Procedures for the Preparation of Materials used for Ultra-low Trace Element Analyses.* Revision 1 (8/9/93) LLRS-MET-SOP-001

Rossmann, R., 1994. *Standard Operating Procedures for the Maintenance of the LLRS Trace Metal Laboratories.* Revision 1 (5/4/94) LLRS-MET-SOP-003.

Rossmann, R. and T. Uscinowicz, 1994. *Standard Operating Procedures for Analysis of Total Mercury in Tissue and Sediment using the Cold Vapor Technique with the Perkin-Elmer Model MHS-20 Gold Amalgam System.* Revision 1 (5/6/94) LLRS-MET-SOP-010.

APPENDIX: HISTORIC PERFORMANCE FOR STANDARDS ON PS200.

Daily Standard Intensities and Percent Drift for Standards. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) 0.250	0.500	1.000	2.000	5.000
01/17/96		30807	62450	128462	319499
01/18/96	11567	24577	52846	112763	289297
01/24/96	15405	27507	62220	127897	309450
01/25/96	14521	25177	55494	117531	279308
01/29/96	12899	30207	54795	108733	283705
01/31/96	12153	25133	47097	110427	280385
02/01/96	13360	26287	52552	113649	286800
02/05/96		28271	55116	107236	262538
02/05/96		28294	57208	116260	287149
02/06/96		21563	43415	95356	258884
2/6/96		25966	47569	103997	263870
2/6/96		24807	49621	102217	259967
2/6/96		23963	48835	99624	251241
02/08/96	15429	31433	62122	124122	312746
2/8/96		32989	62558	121403	316603
2/8/96		31222	60391	120489	312282
02/13/9	13614	27169	59876	121738	299635
2/13/96		30254	63307	125174	304689
2/13/96b	13675	26879	58308	121548	297642
2/13/96b		28977	60081	122166	278297
02/15/96	13628	24557	57516	118139	286801
2/15/96			54759	111736	268327
2/15/96b	12642	21928	53348	112076	270167
Begin New Lamp					
03/04/96	32237	44465	127713	233412	620712
03/04/96	31507	46352	130143	226759	626601
03/04/96	29918	41993	119879	217858	598765
03/05/96	22484	65754	124625	264984	628674
03/05/96		64815	114069	259107	609113
03/06/96	22151	45823	90596	180965	422997

APPENDIX: Continued.

Daily Standard Intensities and Percent Drift for Standards. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) 0.250	0.500	1.000	2.000	5.000
03/07/96	21132	44232	95743	194749	478754
03/07/96	19282	39037	79923	202894	
03/12/96		37940	79106	166230	411516
03/14/96	23058	46864	96048	192849	457716
03/14/96	24152	47468	95645	188087	469761
03/14/96	24206	47189	96993	188049	454648
03/14/96	24008	47491	94850	187538	453447
03/15/96	25333	48936	98592	195526	485709
03/15/96	23274	48272	98146	197376	473520
03/19/96		35727	81247	178190	469406
03/22/96	23430	43189	90404	194519	493374
03/26/96b	22930	45156	84083	188764	465947
03/28/96[1]	23663	44270	79330	184093	457756
03/28/96[2]	27619	53606	109573	212636	523307
03/29/96	22912	47100	96970	194573	483750
03/29/96				191256	474460
03/29/96	21975	44362	90593	196257	
03/29/96		44504			
04/03/96	20843	42173	85748	167910	429912
04/09/96	20494	41875	83552	169354	419310
04/09/96	42012			167949	
04/09/96	40964		81646	160894	401227
04/11/96	21043	42323	89779	178299	444557

[1] Old Tubing
[2] New Tubing

APPENDIX: Continued.

Daily Standard Intensities and Percent Drift for Standards Prepared with Automated Digestion System. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) ~0.014	~0.028	~0.056	~0.114	~0.228
06/11/96			4278	8331	
06/13/96			4275	8689	17993
06/18/96			4138	8979	17995
07/03/96			5043	8057	15743
07/09/96				8369	18117
07/12/96a		1937	4098	8088	17318
07/12/96b		2162		8118	17009
07/16/96		1867	4308	8488	17285
07/17/96	927	1973	4457	8454	16962
07/24/96	1175	2124	4146	8043	16190
07/25/96	980	1923	4492	7546	
08/01/96	600	1728	3989	7908	17072
08/15/96	895	1678	3910	7977	15964
08/16/96	1069		4189	8468	
09/18/96	816	1819	360	7382	15077

Mercury in Plankton

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Mercury in Plankton

1.0 Subsample Collection

Samples were collected from the sampler cup at the base of the plankton net or the phytovibe nets by backwashing with lake water. The portion of the plankton suspension to be analyzed for mercury was then transferred to a 500 mL PFA Teflon jar and covered with its screw-on lid. This sample was carried into the clean room where a 20-30 mL subsample of the plankton suspension was collected using cleanroom techniques. A 10 mL automatic pipetter was used to transfer the sample to a cleaned, tared 30 mL PFA Teflon sample vial, which was sealed in a pre-marked polyethylene zipper bag. The bagged sample was then placed in a second polyethylene zipper bag which had been marked with the sample identifier. Once the log book had been filled out with sample number, location, type, date, initials, and observations, the sample was frozen.

2.0 Sample Containers

Samples were collected and stored in PFA Teflon vials and jars. These containers produce negligible Hg contamination of samples and can withstand extremely rigorous cleaning methods. New containers were purchased for this study.

3.0 Container Labeling

PFA Teflon is difficult to mark: laboratory ink markers do not produce permanent markings and adhesive labels do not stick well to PFA Teflon. Consequently, sample bottles were marked by engraving a unique sample number on both the bottle and the cap. These unique identifying numbers consisted of a letter followed by a two digit number (e.g., A07). The unique identifying number was used to track samples through all phases of sample collection, storage, processing, and analysis, and was cross-referenced to all sample attributes.

Polypropylene containers were used for storage of some reagents and solutions, and polyethylene bags were used for sample bottle storage; all were marked with permanent laboratory markers.

4.0 Cleaning of Sample Containers

Sample containers and other plasticware that may come in contact with samples were cleaned by one of the following two methods, depending on their composition.

4.1 Cleaning Procedure A (PFA Teflon and Other Fluoropolymers)

Items (sample bottles, stirring rods, large sample containers) were:

- Washed in hot tap water with a laboratory detergent (Alconox or similar product) in combination with a laboratory surfactant (Vers-A-Kleen or similar product).
- Rinsed repeatedly in hot tap water.
- Rinsed thoroughly in doubly-deionized water

- Acid-washed in 50% HCl maintained at 70°C overnight.
- Rinsed thoroughly in doubly-deionized water.
- Soaked for four hours (minimum) in doubly-deionized water.
- Either:
  - dried in an inverted position on clean polystyrene racks in a dust-free environment; or
  - filled with 1% (v/v) HCl and sealed until use.
- Sealed with its own cap.
- Sealed in a polyethylene zipper bag subjected to cleaning Procedure B below. This bag was marked with the unique sample bottle identifier.
- Once sealed in the "inner" bag, the sealed sample bottle plus inner bag was sealed in a second, "outer", zipper bag subjected to cleaning Procedure B. This bag was also marked with the unique sample bottle number.
- Sample bottles sealed in inner and outer bags were stored in large polyethylene bags in cabinets in the laboratory. They were stored in clean coolers (ice chests) or PVC dry bags (the kind kayakers use) for transfer to, and during use on, the EPA lakes sampling vessel.

4.2 Cleaning Procedure B (Polypropylene, Polyethylene)

Items (pipette tips, polyethylene zipper bags) were:

- Washed in hot tap water with a laboratory detergent (Alconox or similar product) in combination with a laboratory surfactant (Vers-A-Kleen or similar product).
- Rinsed repeatedly in hot tap water.
- Rinsed thoroughly in doubly-deionized water.
- Acid-washed overnight in room temperature 30% HCl.
- Rinsed thoroughly in doubly-deionized water.
- Soaked four hours minimum in reagent-grade water.
- Dried in an inverted position on clean polystyrene racks in a dust-free environment.
- Sealed in polyethylene bags that have been similarly cleaned.

5.0 Example Sample Label

Samples were uniquely coded at the time of collection including the unique sample bottle number, date, location, phase collected, sample volume, and whether they are duplicates or field blanks. An example of a sample label would be the following:

A07	LM	5	6-16-94	Z	30 mL	a
identifier	lake	station	date	phase	volume	replicate

The above example indicates a sample placed in sample bottle A07, from lake Michigan, Station 5, taken on June 16, 1994, zooplankton, a 30 mL subsample, and is replicate "a". All pertinent station information were cross-referenced and recorded in the field notebook. Identification of preservation methods on the label were not necessary as all samples were preserved in the same manner.

6.0 Calibration Procedures and Frequency

All field equipment, with the exception of the plankton nets, was maintained and cleaned by U of MN personnel. Field notebooks noted problems with field equipment. The cold vapor atomic fluorescence spectrometer (CVAFS) used for Hg analysis in this study was subjected to checks for resolution, sensitivity, and reproducibility of response factors (mV/mass Hg) before and during analysis of each batch of samples. Instrument calibration curves were produced before and during analysis of each batch of samples. All standards were obtained from commercial sources. Logbooks recorded dates of calibration, names and concentrations of standards used, result of calibration, and any corrective action needed and taken.

7.0 Sample Preparation

Prior to analysis, samples were prepared by lyophilization (freeze-drying). The operational status of the freeze-dryer was checked before sample preparation began. Double-bagged sample bottles were removed from the freezer and carried into the cleanroom. Label information was recorded for each sample placed in the freeze-dryer. Using clean-hands/dirty-hands techniques, the samples were removed from their outer bags and the sample and its inner bag were placed in a sample tray. In the cleanroom the inner bags were opened and the lids of the samples were loosened but remained on top of the samples. The status of the dryer and the samples was checked at least once during the first half hour of the process and then daily thereafter.

Upon removal from the freeze-dryer (usually five days) the sample bottles were tightly sealed and the inner bags closed. The samples were prepared for digestion back in the cleanroom.

8.0 Sample Digestion

A portion of the lyophilized sample (20 ± 10 mg) was transferred to a clean, pre-weighed 5 mL conical-bottom PFA Teflon digestion vessel and the weight recorded to ±0.1 mg. For zooplankton samples, 4 mL of a 1:1 concentrated sulfuric (H_2SO_4) and nitric (HNO_3) acid mixture was added to the digestion vessel which was tightly sealed. For phytoplankton samples and zooplankton samples weighing less than 10 mg, 2 mL of a 1:1 acid mixture was added. The digestion vessels were triple-bagged, and then placed in a hot (70°C) water bath overnight.

9.0 Sample Analysis

Samples were analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) using the double amalgamation technique of Bloom and Crecelius (1983) as described in Claas (1996). The oxidizing acids, H_2SO_4 and HNO_3, were pre-reduced by reaction with hydroxylamine hydrochloride, which was added to the bubbler flasks prior to the introduction of the sample.

One mL of the digestate was added to the bubbler and allowed to react with the hydroxylamine hydrochloride for five minutes prior to the addition of 0.5 mL of the stannous chloride ($SnCl_2$) reducing agent. A sample gold trap was placed on the bubbler behind a soda-lime trap and 0.5 mL of stannous chloride to the sample. The bubbler was sealed and the N_2 gas was turned on at 350 mL min^{-1} The sample was then allowed to bubble for 20 minutes.

The sample gold trap was then removed from the bubbler and placed in the analytical system upstream of the analytical gold trap. The argon (Ar) flow was turned on and calibrated to 50 mL min^{-1} The automatic sequence controller was then turned on, which heats the sample trap to thermally release its Hg onto the analytical trap, then heated in turn to release the Hg from the analytical trap into the CVAFS analyzer. The peak height and area were recorded for each sample.

10.0 QA

Standards, blanks, and bubbler blanks were run at the beginning and during each set of samples. Knowns (NIST standard reference materials, generally citrus leaves or apple leaves) were run along with the standards. Blanks, duplicates, and spiked samples were also interspersed with the samples such that the total number of QA samples constituted 25% of all samples run.

11.0 Data Reduction

Bubbler blank peak areas were subtracted from the peak areas of the standards and a response factor (ng peak $area^{-1}$) was calculated for each of the standards (0.2, 0.4, and 0.8 ng Hg). The mean response factor was then used to convert peak areas for the samples into quantities of Hg analyzed. Values were then converted to concentrations of Hg per dry weight of plankton (ng g^{-1}) by using the dilution factors and sample weights.

Versatile Combustion-Amalgamation Technique for the Photometric Determination of Mercury in Fish and Environmental Samples

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Permission has been granted by AOAC International to reprint this method as a part of the Lake Michigan Mass Balance Methods Compendium.

Versatile Combustion-Amalgamation Technique for the Photometric Determination of Mercury in Fish and Environmental Samples

1.0 Overview

Total mercury in a variety of substances is determined rapidly and precisely by direct sample combustion, collection of released mercury by amalgamation, and photometric measurement of mercury volatilized from the heated amalgam. Up to 0.2 g fish tissue is heated in a stream of O_2 (1.2 L/min) for 3.5 min in one tube of a two-tube induction furnace. The released mercury vapor and combustion products are carried by the stream of O_2 through a series of traps (6% NaOH scrubber, water condenser, and $Mg(ClO_4)_2$ drying tube) and the mercury is collected in a 10 mm diameter column of 24-gauge gold wire (8 g) cut into 3 mm lengths. The resulting amalgam is heated in the second tube of the induction furnace and the volatilized mercury is measured with a mercury vapor meter equipped with a recorder integrator. Total analysis time is approximately 8 min./sample. The detection limit is less than 0.002 µg and the system is easily converted for use with other biological materials, water, and sediments.

Concern over mercury contamination in the environment has resulted in a rapid proliferation of methods in which the principle of "flameless atomic absorption" is used for the determination of mercury (1-4). Common to all of the flameless methods is the production of an elemental mercury vapor which can be measured photometrically. Methods generally differ only in the means by which the mercury is released from the sample and in the steps taken to remove interferences. Mercury is commonly released by acid digestion, followed by reduction and aeration (5, 6) or amalgamation and heating (7, 8); direct combustion (9, 10); or various combinations of these and other less common techniques (11-15). Our experience has shown that present flameless methods tend to be deficient in one or more of the following areas: sensitivity, accuracy and precision, effort or time required for analysis, ease of adaptability to various sample matrices, freedom from error due to sample or reagent contamination, and safety of operation.

This paper describes a method in routine use at the Great Lakes Fishery Laboratory for the determination of total mercury in fish and other environmental samples. The method combines several proven techniques into a unit that is simple in design and operation and adequately meets the criteria defined above. Mercury is volatilized from the sample in a stream of oxygen by means of combustion in a high frequency induction furnace. The mercury vapor is carried along with combustion products and other volatilized materials from the sample by a gas stream through a series of traps (sodium hydroxide, water condenser, and magnesium perchlorate) to reduce interferences. Final separation of mercury from possible interferences is accomplished by amalgamation on gold. The amalgam is then heated in the induction furnace and the released mercury is measured in a mercury vapor meter. Total analysis time is about 8 min./sample and a single analyst can make up to 40 determinations in eight hours.

2.0 Method

2.1 Apparatus and Reagents

2.1.1 High frequency induction furnace.--Laboratory Equipment Corp. Model 523000 (Leco) two-tube induction furnace equipped with Type "L" conversion for combustion sulfur analyses of hydrocarbons and Type "C" conversion for gasometric or gravimetric carbon analyses of iron and steel (Type "C" combustion tube removed). See Fig. 1.

2.1.2 *Induction furnace accessories.*--Leco No. 84 variable transformer for control of power input to induction furnace. Ceramic crucibles (Leco No. 528035) and Vycor insert crucibles (Leco No. 550183) with silicon carbide crucible covers (Leco No. 763212).

2.1.3 *Spectrophotometer.*--Laboratory Data Control Model 1235 mercury monitor, with Beckman Model 1005, 10 mv recorder equipped with Disc integrator.

2.1.4 Sodium *hydroxide gas wash* trap.--125 mL gas washing bottle (Corning No. 31770) containing ca 35 mL 6 % (w/v) NaOH in distilled water. Renew daily. Solution should be prepared before use (1 L) and aerated to remove traces of mercury found in reagent NaOH.

2.1.5 *Water condenser.*--28 X 200 mm od separable vacuum trap (Corning No. 7729) with tube portion immersed in ice bath. Empty as needed to prevent bubbling.

2.1.6 *Drying tube.*--18 X 150 mm od drying tube filled with anhydrous magnesium perchlorate ($Mg(ClO_4)_2$. Replace daily.

2.1.7 Amalgamator.--Quartz tube (Fig. 2) containing 8 g 24-gauge gold wire cut into short lengths (ca 3 mm) supported by a very coarse glass disk over a layer of silicon carbide chips (used to preheat gas stream).

2.1.8 Flow meters.--Two gas controller-flow meters with 0-5 L/min. capacity (Matheson Model 62OBBU).

2.1.9 Heating tape.--2.5 X 610 cm "Briskeat" heating tape (96 watts) connected to STACO, Inc., Type 500B (120 v) variable transformer.

2.1.10 Mercury standard solution.--(1) Stock solution.--1000 µg/mL. Dissolve 0.1358 g $HgCl_2$ in 100.0 mL water or use commercially available standard solution. *(2) Working solution.*--As required to give desired µg in 0.05-0.2 mL water for particular range of sensitivity.

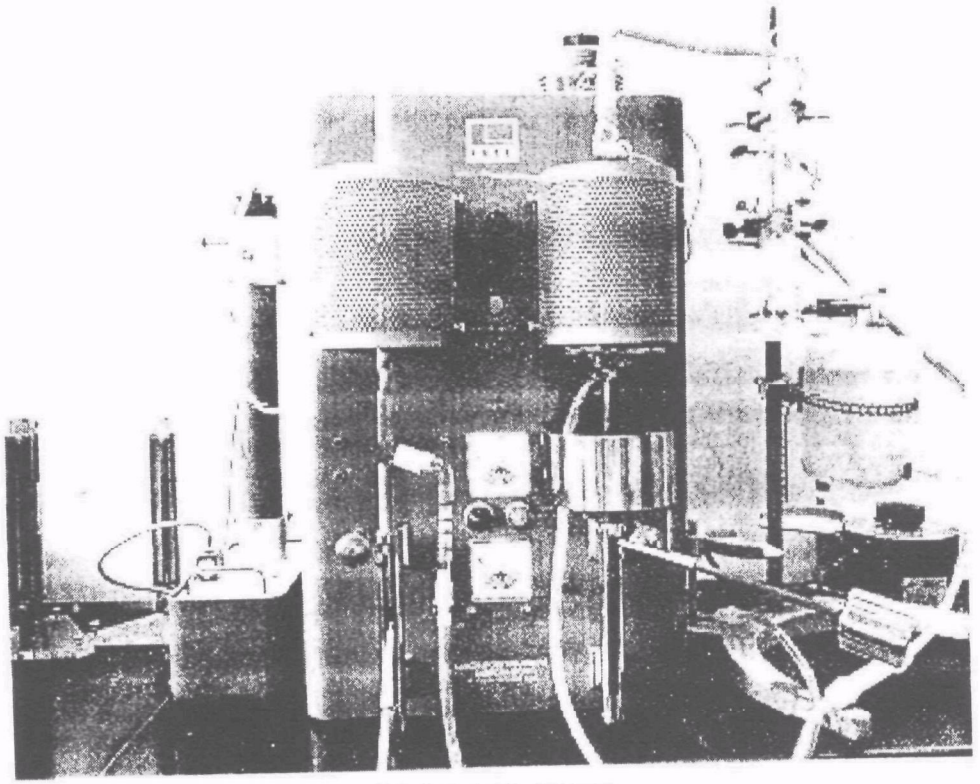

FIG. 1—Induction furnace.

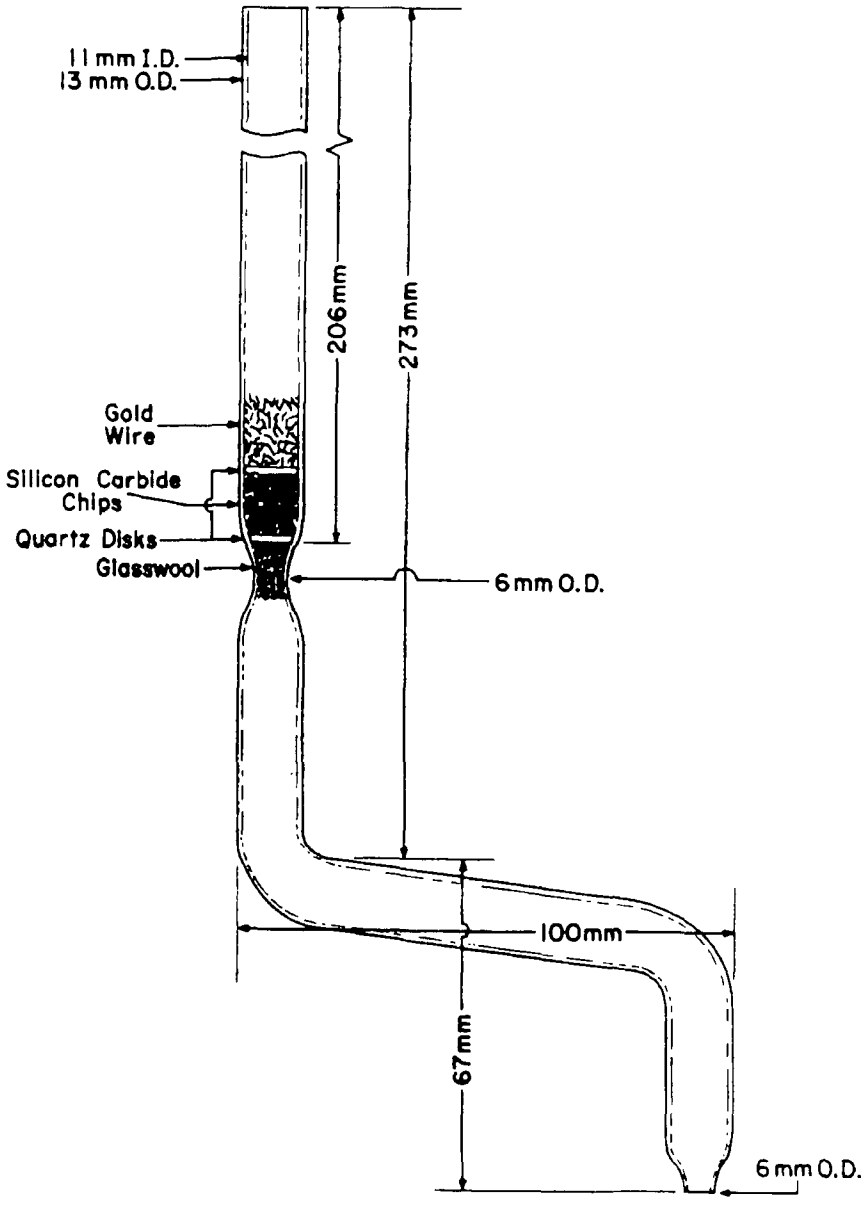

Fig. 2—Detailed drawing of gold amalgamator.

3.0 Assembly of Apparatus

Connect a regulated cylinder of compressed oxygen, using flexible tubing so that the gas stream passes through controller-flow meter, the reference cell of the mercury vapor meter, and the gas inlet post of the tray assembly on the Type "L" arm of the induction furnace (Fig. 3). Connect the Type "L" tube, with ignitor installed, to the NaOH gas wash bottle with Teflon or glass tubing. Wrap exposed portion of combustion tube and connecting tube to gas wash with heat tape controlled by a 110v variable transformer. Maintain internal temperature of connecting tube in excess of 100°C to prevent condensation in line leading to the NaOH gas wash. Connect NaOH gas wash in series to the water condenser, drying tube, and bottom of the amalgamator, using Tygon tubing.

Remove lower guard, tray assembly, and combustion tube from Type "C" arm (carbon analysis) of induction furnace. Install amalgamator in modified arm by attaching it to center post of raising-and-locking assembly for that arm. Curved metal spatula ("Scoopula") bent at 90° and inserted into center post serves as adequate bed for attachment of amalgamator. Adjust height of amalgamator on center post so that entire column of gold and silicon carbide is completely within induction coil when locked in raised position. Connect top of amalgamator to absorption cell of mercury analyzer with flexible tubing and run exhaust tube from outlet of absorption cell to fume hood; install second flow meter in this exhaust tube to detect possible leaks in system.

Before using system, ensure that all connecting lines and glassware are free of mercury contamination or residues of acid. Flush sample combustion tube and connecting line to NaOH gas wash bottle with 6% (w/v) NaOH daily. This precaution is required to prevent accumulation of acids in line which may trap mercury and reduce recoveries.

4.0 Determination

Establish 1.2 L/min flow of oxygen in system, using flow meter on gas inlet line. Check for gas leaks by comparing readings with exhaust flow meter. Weigh 0.05-0.1 g sample into silica crucible and place silicon carbide cover over crucible. Place covered crucible on ceramic pedestal of Type "L" arm of induction furnace. Adjust setting on variable transformer that controls power to induction furnace to read 80. Lift raising mechanism on Type "L" arm, locking sample into position in induction coil, and heat for 3.5 min. Second arm of furnace (amalgamator) must be in lowered position with gold out of induction coil during this period. After combustion of the sample, lower crucible from coil and place crucible and cover in safe place to cool.

Re-establish gas flow in system by locking raising mechanism of Type "L" arm into raised position without crucible. Adjust variable transformer to setting of 60, raise and lock gold amalgamator into position, and heat until recorder peak returns to baseline (1-2). Lower gold amalgamator and allow to cool (ca 1 min). Count integrator sweeps under peak and compare with standard curve prepared by heating standard solutions, using same operating procedures as above. Because of porosity of silica crucibles, use of Vycor liner is required for standard solutions and other liquid samples. It is recommended that volume of standards used in system not exceed 0.2 mL.

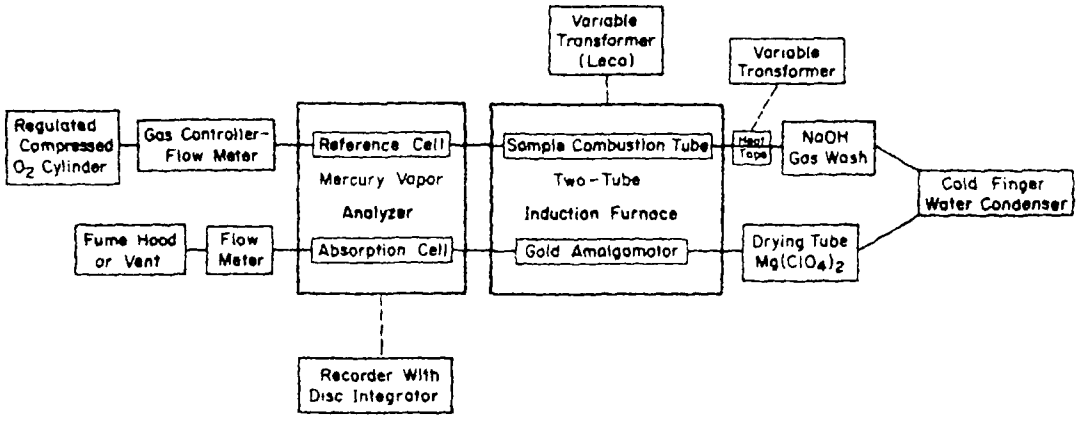

Fig. 3—Combustion amalgamation system for the photometric determination of mercury.

Silicon carbide covers can be used for an indefinite number of samples. For rapid analysis of several samples, two covers are alternated to allow adequate time for cooling between samples. Silica crucibles are also reusable; however, it is recommended they be fired at 800°C for two hours in a muffle furnace between uses, to prevent accumulation of organic residue.

5.0 Results and Discussion

Operating Procedures

The described rate of oxygen flow and settings used on the variable transformer that controls the rate of heating in the induction furnace give a good balance between sensitivity, precision, and time of analysis for samples of fish containing 0.02-5.0 ppm mercury. Samples in this range are analyzed by attenuating the mercury analyzer and adjusting the sample size. Typical analytical ranges used are 0.002-0.01, 0.01-0.05, and 0.05-0.25 µg. Fish samples larger than 0.2 g can overload the system and greatly reduce accuracy of the results.

For analysis of samples outside the range of 0.02-5.0 ppm a change of operating procedure is required. Sensitivity can be increased by reducing the rate of oxygen flow or reduced by increasing the rate. This technique, within limits, effectively increases or decreases the concentration arid the retention time of the mercury vapor in the absorption cell of the mercury analyzer, resulting in the altered sensitivity. Installation of a stream splitter in the gas line between the sample combustion tube and sodium hydroxide gas wash also effectively reduces sensitivity. Precision may be reduced and time required for analysis increased, however, at altered flow rates.

The heating rate and temperature of the gold amalgamator can be varied by adjusting the variable transformer attached to the induction furnace; this results in a large alteration of observed peak height on the recorder for a given amount of mercury atid oxygen flow rate. The integrator counts vary only slightly, however, for settings on the transformer between the values of 50 and 100% It is this characteristic which makes it mandatory to use area measurement (integrator counts) in place of peak height to ensure reproducible results. The heating rate and temperature obtained in the induction field at a given power setting are functions of geometry, mass, density, and purity of the amalgamator. After repeated use, the heating characteristics of the amalgamator slowly

change, as evidenced by increased peak width and lowered height. This change is probably due to a gradual accumulation of oxides and organics on the amalgamator (8, 13). Periodic removal of the amalgamator (semi-monthly) and firing of the gold in a muffle furnace at 800°C for two hours generally restores the amalgamator to its original characteristics. The use of an integrator compensates for the changing characteristics of the amalgamator while it is in use.

6.0 Precision and Accuracy

The average recoveries of mercury ($HgCl_2$) from fortified samples of fish muscle, lake sediment, aquatic vegetation, and bituminous coal were 94-105% for fish muscle and 94-112% for all materials tested (Table 1). The average recovery for all materials and levels tested was 102.7% (coefficient of variation, 5.5%). Our inability to obtain truly homogenous sediments of constant mercury background undoubtedly contributed to the wider variation in recoveries from sediment. Limited testing of fish samples fortified with methyl mercuric chloride gave recoveries within the range obtained with mercuric chloride. The accuracy of the method was determined on samples of fish flesh and sediments analyzed concurrently by several laboratories (Table 2). Values obtained by the described method (Method CA) agree well with average values obtained by other laboratories using acid-digestion, flameless atomic absorption, and neutron activation techniques.

To determine the reproducibility of the method over a period of time, we collected, composited, and homogenized large amounts of fish muscle containing different levels of mercury contamination to form a source of uniform reference material for analysis. Samples of this material were analyzed daily along with unknown samples. Table 3 presents a statistical treatment of data accumulated from these repeated analyses. The values given should include nearly every source of routine error that can be expected when this method is used, since they represent over 100 days of testing. Sample replication on any given day generally gave a coefficient of variation less than 10% and routinely 5% or less, as indicated in Table 1. Failure of standard solutions or check samples to give at least ±10% agreement indicates that the analytical system should be dismantled and cleaned before routine analyses are performed.

Table 1. Recoveries of mercury ($HgCl_2$) added to samples of fish muscle, lake sediment, vegetation, and coal

Sample and amount of mercury added, μg	No. of replicates	Av. amount recovered,[a] %	Coeff. of var., %
Fish muscle (coho salmon)			
0.01	6	94.4	5.8
0.03	6	104.9	3.7
0.06	6	98.5	3.1
0.20	6	104.0	4.7
Sediment (Lake Michigan)			
0.03	3	112.2	2.1
0.06	3	96.2	11.5
Vegetation (chara)			
0.03	3	102.2	5.8
0.06	3	106.8	0.6
Coal (bituminous)			
0.03	3	109.8	2.4
0.06	3	99.3	3.1

[a] Values corrected for following background (ppm) in samples: fish muscle, 0.093; sediment, 0.032; vegetation, 0.016; and coal, 0.081.

Table 2. Average mercury content (ppm) in fish flesh and sediments as determined by the described method and other methods of analysis

Sample[a]	Method[b] CA	FAAS	NAA
Fish flesh			
1	0.22 (1)	0.17 (12)	---
2	0.83 (1)	0.76 (12)	0.92 (2)
3	2.1 (1)	2.4 (12)	2.5 (2)
Sediment			
4	0.18 (1)	0.17 (9)	---
5	107 (1)	110 (20)	120 (1)
6	45 (1)	45 (20)	44 (1)

[a] Samples 1-4: Mercury in Fish and Sediment Round-Robin-1971, Ontario Water Resources Commission, Division of Laboratories. Samples 5 and 6: Mercury in Sediment Round-Robin-1972, Environmental Protection Agency, Region IV, Surveillance and Analysis Division.
[b] Methods used: CA, combustion-amalgamation; *FAAS*, flameless atomic absorption (variety of digestion mixtures); *NAA*, neutron activation analysis. Numbers in parentheses show the number of laboratories participating.

Table 3. Accumulated data on the precision of replicate analyses of fish flesh during several weeks of testing

Sample	No. of Analyses	No. of Days	Av. Concn, PPM	Std dev.	Range	Coeff. var %
A	258	58	0.0883	0.0123	0.0563 - 0.141	13.9
B	60	16	0.399	0.0485	0.310 0.569	12.2
C	65	16	0.774	0.0837	0.596 1.09	10.8
D	186	49	2.82	0.214	2.24 3.20	7.6

7.0 Sample Adaptation

The data given in this paper were derived primarily from "clean" samples of fish muscle, vegetation, lake sediments, and coal. We found, however, that some samples of individual tissues, as well as of whole fish, caused an acidification in the lines, resulting in low recoveries of mercury. The problem was eliminated by air-drying the samples overnight at room temperature to remove most of the water. We have adopted this practice for all samples of whole fish. Care must be taken, however, to ensure that no mercury contaminated air or dust reaches the samples during this drying period.

Since the heat supplied by the silicon carbide cover used during sample combustion may not be adequate for samples other than those tested (e.g., geological materials or metals), we also tested a quartz-enclosed silicon carbide crucible (Leco No. 550182) in place of the silica crucible. This crucible was satisfactory for samples of low organic content but not for biological materials because combustion was apparently too rapid and caused erratic results. The addition of "activators" of metal (copper and iron) to the sample gave the same results; they, too, were unsatisfactory for biological materials and, in addition, had high and variable background levels of mercury.

Water can be analyzed directly in the system, with a sensitivity as low as about 10 P.B., by using the same analytical procedure used for standard solutions. However, a superior and much more sensitive method is a modification of the procedure described by Kala (13). In this modification, a 100 mL water sample is heated with sulfuric acid, nitric acid, potassium permanganate, and potassium persulfate; decolorized with hydroxylamine hydrochloride; reduced with stannous chloride; and aerated in a gas wash bottle (16). The released mercury vapor is passed through magnesium perchlorate and amalgamated on gold as described earlier in this paper. A sensitivity of less than 0.02 P.B. can be obtained with this technique.

Of the various materials we have tried in the system, only those which are volatile or explosive in nature could not be analyzed. Samples of volatile solvents have been analyzed after evaporation to dryness, but this technique is subject to loss of mercury from the sample. Extraction and concentration of the mercury into an aqueous system which can be analyzed similarly to standard solutions is a useful technique.

8.0 Conclusions

The described method has been found to be a reliable and rapid technique for the precise determination of total mercury in a variety of samples. Materials that do not lend themselves to direct analysis by the method as described can be accommodated by minor changes in equipment or sample preparation. Routine use of the method at the Great Lakes Fishery Laboratory has shown it to be superior to previously tested methods for use with fish tissue, and preliminary work strongly suggests that it is superior for numerous other types of samples as well.

Major advantages of the method over the normal acid-digestion, flameless atomic absorption techniques include: simplicity of operation; speed of complete analysis; high sensitivity, precision, and accuracy; small sample size required; freedom from rigorous and sometimes hazardous acid digestion procedures; freedom from reagent and glassware contamination; and comparatively low cost of equipment.

Disadvantages of the method as described include: somewhat limited usable range of sensitivity (0.02-5.0 ppm); a general inability to analyze highly contaminated samples (>5.0 ppm) without the use of a gas stream splatter or an extremely small sample; necessity for frequent changes in attenuation of mercury vapor meter, unless previous knowledge permits grouping of samples within ranges having less than a five-fold difference, in concentration; and increased emphasis on the need for well homogenized representative samples because of the small sample size used. It is expected that minor changes in the system or substitution of a less sensitive mercury vapor meter would overcome many of these disadvantages when they are restrictive for a particular use.

9.0 Acknowledgments

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10.0 References

10.1 Manning, D.C. (1970) *At. Absorption Newsletter* 9, 97-99

10.2 Slavin, S. (1971) *At. Absorption Newsletter* 10, 17-39

10.3 Slavin, S. (1972) *At. Absorption Newsletter* 11, 7-32

10.4 Slavin, S. (1972) *At. Absorption Newsletter* 11, 74-88

10.5 Hatch, W.R., and Ott, W.L. (1968) *Anal. Chem.* 40, 2085-2087

10.6 Munns, R.K., and Holland, D.C. (1971) *JAOAC* 54@ 202-205

10.7 Fishman, M.J. (1970) *Anal. Chem.* 42, 14621463

10.8 Okuno, I., Wilson, R.A., and White, R,. E. (1972) *JAOAC* 55, 96-100

10.9 Herrmann, W.J., Jr., Butler, J.W., and Smith, R.G. (1970) in *Laboratory Diagnosis of Diseases Caused by Toxic Agents*, F. W Sunderman and F W. Sunderman, Jr. (eds.), Warren H. Green, Inc., St. Louis, Mo., pp. 379-386

10.10 Thomas, R.J., Hagstrom, R.A., and Kuchar, E. (1972) J. *Anal. Chem.* 44, 512-515

10.11 Thilliez, G., (1968) *Chim. Anal.* 50, 226-232

10.12 Lidums, V., and Ulfvarson, U. (1968) *Acta Chem. Scand.* 22, 2150-2156

10.13 Kala, G.W (1970) *At. Absorption Newsletter* 9, 84-87

10.14 Ukita, T., Osawa, T., Imura, N., Tonomura, M., Sayato, Y., Nakamura, K., Kanno, S., Fukui, S., Kaneko, M., Ishikura, S., Yonaha, M., and Nakamura, T (1970) *J. Hyg. Chem.* 16, 258-266

10.15 Joensuu, O.I. (1971) *Appl. Spectry.* 25, 526-528

10.16 *Methods for Chemical Analysis of Water* and *Waste*, (1971) Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio, pp. 121-130

Analysis of Fish for Total Mercury

Standard Operating Procedure SOP No. HC520B.SOP

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Version 1.0

Analysis of Fish for Total Mercury
SOP # HC520B.SOP

1.0 Scope and Application

This method is only for total mercury measurement after dissolving the fish tissue in concentrated nitric acid under high pressure and temperature using microwave digestion system. The mercury in solution will be analyzed using a nondispersive atomic fluorescence spectrometer. Digestion procedure for samples is described by Feng et al. (1994) and the procedure for instrumental measurement of mercury is given by Bloom and Fitzgerald. Procedures for collection, homogenization, and data reporting are covered by other appropriate NBS/GLSC SOPs.

Note: This method replaces HC520A.SOP.

2.0 Summary of Method

This method is only for total mercury measurement after dissolving the fish tissue in concentrated nitric acid under high pressure and temperature using a microwave digestion system. The mercury in solution will be analyzed using a nondispersive atomic fluorescence spectrometer. Digestion procedure for samples is described by Feng et al. (1994) and the procedure for instrumental measurement of mercury is given by Bloom and Fitzgerald. Procedures for collection, homogenization, and data reporting are covered by other appropriate NBS/GLSC methods

3.0 Interferences

3.1 Contamination in the laboratory will be minimized by processing the samples in an epoxy-coated plastic chamber equipped with HEPA filers to achieve Class 100 laminar flow conditions. Milli-Q water and quartz-redistilled acids will be used for sample digestion.

3.2 All the labware (glass or teflon will be decontaminated using the 9-step procedure described by Nriagu et al. (1993). Some of the key steps include degreasing with soap, sequential washing with acetone, concentrated nitric and hydrochloric acid, soaking in warm (40-50°C) 2 M nitric acid for 3 days, and rinsing thoroughly with Milli-Q water. After use, the labware is soaked in 6 M hydrochloric acid for three days, followed by warm nitric acid for three days and then rinsed with Milli-Q water. The final rinse is done in the HEPA-equipped chamber, and all the containers are stored in acid-washed, triple plastic bags. Cleaned volumetric flasks will be filled with 1.0 M nitric acid and triple bagged for storage.

4.0 Safety

Mercury in the pure form is toxic. Both nitric and sulfuric acid in the concentrated form will cause severe chemical burn on tissue and require use of safety glasses when handling, even at dilutions containing 20% of the concentrated form. The hazards of each chemical and reagent used in this method have been generally defined, but each chemical compound used should be treated as a potential health hazard. A reference file of material safety data sheets is available at the U of M Lab and in NBS to all personnel involved in chemical analysis.

5.0 Apparatus and Chemicals

5.1 Teflon-lined pressure bombs, 90 mL

5.2 Sphex CDS 7000 Microwave digestion unit

5.3 Cold vapor atomic fluorescence spectrometer, Rand Corp.,Seattle, WA.

5.4 Freeze dryer, model 25 SRC, Virtis, Gardiner, NY

5.5 Balance, Top loading, Sartorius, 1204 (0.01 g) or better

5.6 Ultra pure acids (nitric and hydrochloric)

5.7 Milli-Q water

5.8 Stannous chloride, ACS grade, Fisher Scientific

5.9 Mercury standard stock solution, Perkin Elmer

5.10 Aluminum weighing pan (6 X 2 cm)

5.11 Whatman quartz 47 mm filter membrane, acid washed, #1851-047

6.0 Digestion Procedure

6.1. Homogenized study fish samples are stored frozen in glass screw cap containers until analyzed. Fish samples are thawed just prior to being weighed for digestion. For each sample from the study, check, duplicate, or spiked samples about one gram of homogenized tissue is weighed into a pre-weighed digestion tube. Record both empty weighing pan and weighing pan plus wet tissue weights which will be used to determine dry weight.

6.2 Digestion

6.2.1 Add 10 mL of concentrated nitric acid to the sample in the digestion vessel and leave the mixture at room temperature for 30 minutes. Then place the teflon vessel containing the sample into the double outer liner of the digestion bomb, cap with a sensor head and pressure rupture disc. Place the sealed vessel in the microwave carousel. Prepare the remaining samples of the set in the same manner The set must also contain a blank, spike, duplicate, and reference samples.

6.2.2 After connecting the sensor cables to a port in the oven cavity, the power level and time for each digestion stage are programmed into the computer controller. To minimize violent reactions the oven temperature is slowly ramped to the set temperature over 20-30 minutes. When the temperature reaches 190°C and pressure 180 psi, heat the sample for an additional 15 minutes. After the digestion step is completed digestion bombs are cooled to room temperature.

6.2.3 Each cooled digestion bomb is opened and the clear liquid (fish dissolved in nitric acid) is diluted to 25 mL using mercury free Milli-Q water. Residues in sample vessel after digestion is often indicative of incomplete or improper digestion and the sample batch are re-digested. Sample filtration is not required in completely digested fish tissues.

7.0 Analysis

7.1 An aliquot (0.5 to 1.0 mL) of the digested sample is added to 10 mL of Milli-Q water in a glass reduction chamber. The mixture is purged with ultra-pure argon for 10 minutes. One mL of stannous chloride is then added to the mixture to reduce the mercury, and the elemental mercury formed is stripped (with argon) and collected on gold-coated quartz grains. The trapped mercury is subsequently desorbed thermally and measured on a Tekran CVAFS Mercury Analyzer Model 2500. The output, as peak height or peak area, is recorded by means of an HP 3396A Integrator.

7.2 Calibration

7.2.1 The calibration curve is prepared by reducing standard solutions containing 1, 2, 4 and 6 ng of mercury. This is done by using 0.5, 1.0, 2.0 and 3.0 mL of standard solution containing 2.0 μg/L (or 2.0 ng/mL) Hg. Each aliquot of the standard solution is added to 10 mL of Milli-Q water in a glass reduction chamber. The mixture is purged with ultra-pure argon for 10 minutes. One mL of stannous chloride is then added to the mixture to reduce the mercury, and the elemental mercury formed is stripped (with argon) and collected on gold-coated quartz grains. The trapped mercury is subsequently desorbed thermally and measured on a Tekran CVAFS Mercury Analyzer Model 2500. The outputs for the four standard samples, recorded by an HP 3396A Integrator, are used to derive the average slope for the standard curve in terms of atomic fluorescence units per 1.0 ng of Hg.

7.2.2 Calibration of the spectrometer is with a minimum of four serial diluted standards of mercury covering the range expected in the samples that have been taken through the digestion and reduction procedure. The calibration range for most samples is expected to be from about 0.1 3 ng of Hg. If concentrations in the fish are lower the calibration range will be moved downward since the detection limit of the instrument is 0.01 ng/L of dissolved Hg.

7.3 Determining the method detection limit

7.3.1 The method detection limit will be determine using the USEPA method of seven (40 CFR) using spiked fish tissue or other acceptable matrix containing less than 1 ng of mercury.

7.3.2 When a peak is visible below the established method detection limit a concentration will be reported but result will be flagged using established EPA codes. When no peak is detected for mercury the results will be reported as zero.

7.4 Result

7.4.1 Results will be reported in ng/g (wet weight) or as stated in the data standards being established by USEPA. Mercury data, including results from calibration, check, duplicates, spikes, and reference samples will be submitted to NBS-GLSC where they will be checked by Jim Hickey for consistency with EPA standards and completeness. Acceptance criteria is shown in Tables 7.1 and 7.2 of the NBS Analytical Quality Assurance Project Plan for IAG DW14947692-02-0. NBS will submit mercury results to USEPA using data standards that are currently being finalized.

8.0 References

8.1 Bloom, N.S. and W.F. Fitzgerald. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatograph with cold-vapor atomic fluorescence detection. Anal. Chim. Acta 208: 151-161

8.2 Feng, Y. and R.S. Barratt. 1994. Digestion of dust samples in a microwave oven. Sci. total Environ. 143: 157-161

8.2 Nriagu, J.O., Lawson, G., Wong, H.K.T. and J.M. Azcue. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters. J. Great Lakes Res. 19: 175-182