Standard Operation Procedure (SOP) for the Extraction and Clean-up of PCBs and OH-PCBs in Sediment

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1. Scope and Application

This method covers the extraction and cleanup of OH-PCBs in sediment. Samples are prepared for instrumental analysis using gas chromatography (GC) with tandem mass spectrometry (MS/MS). Approximately 4 - 6 g of sediment is extracted using pressurized solvent extraction with a mixture of hexane and acetone. OH-PCBs are separated from PCBs by liquid/liquid partitioning using basic and then acidic solutions. The samples are cleaned up by mixing with sulfuric acid and then passing through sulfuric acid-impregnated silica gel columns.

2. Quality Assurance and Control (QA/QC)

2.2 Method blanks

Interferences from glassware, solvent and chemicals are monitored by running one method blank with every batch of samples. The method blank is spiked with surrogate standards and run through the entire extraction process and clean up steps in parallel with the samples. The method blank is prepared by crushing 30 - 50 g DE with mortar and pestle and adding it to an ASE extraction cell.

2.1 Cleaning glassware, ASE supplies, and plastic caps

Everything is washed as soon as possible after use. All glassware and ASE cells are heated in a furnace at 450 °C overnight. After cooling, the glassware and ASE cells are capped with aluminum foil and stored in a clean environment to be protected from dust and other contaminants. ASE collection bottles, extraction cell caps, and plastic caps are washed in the same method as the glassware and ASE cells followed by a triple-rinsing of methanol, hexane, and acetone instead of furnace combustion. These supplies are also stored in a clean environment protected from dust and other contaminants.

2.3 Reference

Before starting the extraction procedure all samples are spiked with surrogate standards (SS). At the same time, one empty test tube is also spiked with the same surrogate standard to serve as the reference. The reference represents 100% of what has been added to the samples and will be used for the calculation of the recovery of the SS in the samples. The reference contains OH-PCBs and must be derivatized with the samples but otherwise does not go through any of the other extraction and cleanup steps. After transfer to GC vials, the samples AND the reference are spiked with the internal standard.

2.4 Recovery

Before starting the extraction procedure all samples are spiked with both PCB and OH-PCB surrogate standards (SS). At the same time, a GC vial is spiked with the same PCB SS to serve as the PCB reference and one empty test tube is also spiked with the same OH-PCB SS to serve as the OH-PCB reference. The reference represents 100% of what has been added to the samples and will be used for the calculation of the recovery of the SS in the samples. The OH-PCB reference must be derivatized with the samples but otherwise does not go through any of the other extraction and cleanup steps. After transfer to GC vials, the samples and both references are spiked with internal standards.

2.4 Standards

2.4.1 Surrogate Standards (SS)

OH-PCBs — Wellington Laboratories, Guelph, ON, Canada

3',4'-dichloro-4-[13C]biphenylol	13C 4'-OH-PCB 12
2,4,5-trichloro-4'-methoxy[13C]biphenylol	13C 4'-OH-PCB 29
2,3,4,5-tetrachloro-4'-methoxy[13C]biphenylol,	13C 4'-OH-PCB 61
2',3,4',5,5'-pentachloro-4-[13C]biphenylol	13C 4'-OH-PCB 120
2,3,3',4,5,5'-hexachloro-4'-methoxy[13C]biphenylol	13C 4'-OH-PCB 159
2,2',3,3',4,5,5'-heptachloro-4'-methoxy[13C]biphenylol	13C 4'-OH-PCB 172
2,2',3,4',5,5',6-heptachloro-4-[13C]biphenylol	13C 4-OH-PCB 187

PCBs

4-monochlorobiphenyl (13C)	13C PCB 3
4,4'-dichlorobiphenyl (13C)	13C PCB 15
2,4,4'-trichlorobiphenyl (13C)	13C PCB 28
2,2',4,4'-tetrachlorobiphenyl (13C)	13C PCB 52
2,3',4,4',5-pentachlorobiphenyl (13C)	13C PCB 118
2,2',4,4',5,5'-hexachlorobiphenyl (13C)	13C PCB 153
2,2',3,4,4',5,5'-heptachlorobiphenyl (13C)	13C PCB 180
2,2',3,3',4,4',5,5'-octachlorobiphenyl (13C)	13C PCB 194
2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl (13C)	13C PCB 208
2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl (13C)	13C PCB 209

2.4.2 Internal Standards (IS)

Source: Cambridge Isotope Laboratories, Inc, Andover, MA, USA

2,4,6-trichlorobiphenyl-2',3',4',5',6'-d5 (C/D/N Isotopes, Pointe-Claire, QC, Canada) d-PCB 30
2,2',3,4,4',5,6,6'-octachlorobiphenyl (Cambridge Isotope Labs, Andover, MA, USA) PCB 204

2.4.3 Calibration Standards

OH-PCBs

Wellington Laboratories, Guelph, ON, Canada

2,4,5-trichloro-4'-methoxy[13C]biphenyl	13C 4'-MeO-PCB 29
2,3,4,5-tetrachloro-4'-methoxy[13C]biphenyl,	13C 4'-MeO-PCB 61
2',3,4',5,5'-pentachloro-4-[13C]biphenyl	13C 4'-MeO-PCB 120
2,3,3',4,5,5'-hexachloro-4'-methoxy[13C]biphenyl	13C 4'-MeO-PCB 159
2,2',3,3',4,5,5'-heptachloro-4'-methoxy[13C]biphenyl	13C 4'-MeO-PCB 172
2,2',3,4',5,5',6-heptachloro-4-[13C]biphenyl	13C 4-MeO-PCB 187

2,4,6-trichlorobiphenyl-2',3',4',5',6'-d5 (C/D/N Isotopes, Pointe-Claire, QC, Canada) d-PCB 30
2,2',3,4,4',5,6,6'-octachlorobiphenyl (Cambridge Isotope Labs, Andover, MA, USA) PCB 204

4-methoxy-2-chlorobiphenyl	AccuStd, New Haven, CT, USA	4MeO PCB1
4-methoxy-3-chlorobiphenyl	AccuStd	4MeO PCB2
2-methoxy-5-chlorobiphenyl	AccuStd	6MeO PCB2
4-methoxy-4'-chlorobiphenyl	AccuStd	4'MeO PCB3
2-methoxy-2',3'-dichlorobiphenyl	AccuStd	2'MeO PCB5
3-methoxy-2',5'-dichlorobiphenyl	AccuStd	3'MeO PCB9
4-methoxy-2',5'-dichlorobiphenyl	AccuStd	4'MeO PCB9
2-methoxy-3',4'-dichlorobiphenyl	AccuStd	2'MeO PCB12
4-methoxy-3,5-dichlorobiphenyl	AccuStd	4MeO PCB14
4-methoxy-2,2',5'-trichlorobiphenyl	AccuStd	4'MeO PCB18
4-methoxy-2',3,5'-trichlorobiphenyl	AccuStd	4'MeO PCB26
2-methoxy-2',5,5'-trichlorobiphenyl	AccuStd	6'MeO PCB26
2-methoxy-2',4',6'-trichlorobiphenyl	AccuStd	2'MeO PCB30
3-methoxy-2',4',6'-trichlorobiphenyl	AccuStd	3'MeO PCB30
4-methoxy-2',4',6'-trichlorobiphenyl	AccuStd	4'MeO PCB30
3-methoxy-2,2',6,6'-tetrachlorobiphenyl	AccuStd	3MeO PCB54
2-methoxy-2',3',4',5'-tetrachlorobiphenyl	AccuStd	2'MeO PCB61
3-methoxy-2',3',4',5'-tetrachlorobiphenyl	AccuStd	3'MeO PCB61
2-methoxy-2',3',5',6'-tetrachlorobiphenyl	AccuStd	2'MeO PCB65
3-methoxy-2',3',5',6'-tetrachlorobiphenyl	AccuStd	3'MeO PCB65
4-methoxy-2',3',4',6'-tetrachlorobiphenyl	AccuStd	4'MeO PCB69
2-methoxy-2',4',5,6'-tetrachlorobiphenyl	AccuStd	6'MeO PCB69
4-methoxy-2',3,5,5'-tetrachlorobiphenyl	AccuStd	4'MeO PCB72
2-methoxy-2',3',5,5',6-pentachlorobiphenyl	AccuStd	6'MeO PCB83
4-methoxy-2,2',3',4',5'-pentachlorobiphenyl	AccuStd	4'MeO PCB86
4-methoxy-2,2',3',5',6'-pentachlorobiphenyl	AccuStd	4'MeO PCB93
2-methoxy-2',3,4',5',6-pentachlorobiphenyl	AccuStd	6'MeO PCB101
2-methoxy-2',3',4',5,5'-pentachlorobiphenyl	AccuStd	2'MeO PCB106
5-methoxy-2,2',3,4,4',5'-hexachlorobiphenyl	AccuStd	5MeO PCB138
5-methoxy-2,2',3,4,4',5'6'-heptachlorobiphenyl	AccuStd	5MeO PCB183

2-methoxy-3-chlorobiphenyl	AccuStdCustom	2-MeO PCB2
3-methoxy-5-chlorobiphenyl	AccuStdCustom	5-MeO PCB2
2-methoxy-3'-chlorobiphenyl	AccuStdCustom	2'-MeO PCB2
3-methoxy-3'-chlorobiphenyl	AccuStdCustom	3'-MeO PCB2
4-methoxy-3'-chlorobiphenyl	AccuStdCustom	4'-MeO PCB2
4-methoxy-2,3,5,6-tetrachlorobiphenyl	AccuStdCustom	4-MeO PCB 65
4-methoxy-2',3',5',6'-tetrachlorobiphenyl	AccuStdnew	4'-MeO PCB 65
2,3,4,5-tetrachloro-4'-methoxybiphenyl	WellMixA	4'MeO PCB 61
2,3',4,5,5'-pentachloro-4'-methoxybiphenyl	WellMixA	4'MeO PCB120
2,2',4,4',6,6'-hexachloro-3,3'-dimethoxybiphenyl	WellMixA	3,3'diMeO PCB155
2,2',3,4,4',6,6'-heptachloro-3'-methoxybiphenyl	WellMixA	3'MeO PCB184
2,2',3,3',5,5',6,6'-octachloro-4-methoxybiphenyl	WellMixA	4MeOPCB202
3,3',4',5-tetrachloro-4-methoxybiphenyl	WellMiXB	4'MeO PCB79
2,2',4,5,5'-pentachloro-4'-methoxybiphenyl	WellMiXB	4'MeO PCB101
2,2',3,3',5,6-hexachloro-4-methoxybiphenyl	WellMiXB	4MeO PCB134
2,2',3,3',5,5',6-heptachloro-4-methoxybiphenyl	WellMiXB	4MeO PCB178
2,2',3,3',4',5,6,6'-octachloro-4-methoxybiphenyl	WellMiXB	4'MeO PCB201
2,3,4,4',5-pentachloro-2'methoxybiphenyl	WellMixC	2'MeO PCB114
2,2',3,4',5,5'-hexachloro-4-methoxybiphenyl	WellMixC	4MeO PCB146
2,2',3,4,4',5,6'-heptachloro-3'-methoxybiphenyl	WellMixC	3'MeO PCB182
2,2',3,4,4',5,5',6-octachloro-3'-methoxybiphenyl	WellMixC	3'MeO PCB203
2,2',3,3',4,5,5',6,6'-nonachloro-4'-methoxybiphenyl	WellMixC	4'MeO PCB208
2,3',4,4',5-pentachloro-3-methoxybiphenyl	WellMixD	3MeOPCB118
2,2',3',4,4',5-hexachloro-3-methoxybiphenyl	WellMixD	3'MeO PCB138
2,2',3',4,4',5,6'-heptachloro-3-methoxybiphenyl	WellMixD	3'MeO PCB183
2,2',3,3',4,5,5',6-octachloro-4'-methoxybiphenyl	WellMixD	4'MeO PCB198
2,3,3',4,5'-pentachloro-4'-methoxybiphenyl	WellMixE	4'MeO PCB108
2,2',3,3',4',5-hexachloro-4-methoxybiphenyl	WellMixE	4'MeO PCB130
2,2',3,4',5,5',6-heptachloro-4-methoxybiphenyl	WellMixE	4MeO PCB187
2,2',3,3',4',5,5',6-octachloro-4-methoxybiphenyl	WellMixE	4'MeO PCB199
2,3,3',4',5-pentachloro-4-methoxybiphenyl	WellMixF	4MeO PCB107
2,3,3',4',5,6-hexachloro-4-methoxybiphenyl	WellMixF	4MeO PCB163
2,2',3,3',4',5,6-heptachloro-4-methoxybiphenyl	WellMixF	4MeO PCB177
2,2',3,3',4,5,6,6'-octachloro-4'-methoxybiphenyl	WellMixF	4'MeO PCB200
2,2',3,4',5'-pentachloro-4-methoxybiphenyl	WellMiXG	4'MeO PCB97
2,3,3',4,5,5'-hexachloro-4'-methoxybiphenyl	WellMiXG	4'MeO PCB159
2,2',3,4,4',5,5'-heptachloro-3'-methoxybiphenyl	WellMixG	3'MeO PCB180
2,2',3,3',5,5',6,6'-octachloro-4,4'-dimethoxybiphenyl	WellMiXG	4,4'diMeO PCB202
3,3',4,5,5'-pentachloro-4'-methoxybiphenyl	WellMixH	4'MeO PCB127
2,2',3,3',4,5,5'-heptachloro-4'-methoxybiphenyl	WellMixH	4'MeO PCB172
2,3,3',4',5,5'-hexachloro-4-methoxibiphenyl	WellMixI	4MeOPCB162
2,3,3',4',5,5',6-heptachloro-4-methoxybiphenyl	WellMixI	4MeOPCB193

PCBs

All 209 PCBs as 5 mixes from AccuStandard, New Haven, CT, USA
The 10 13C PCB SS
IS d-PCB 30 and PCB 204

3. Safety

All laboratory personnel are expected to pass required safety courses as directed by the EES Lab Manager. Safety precautions for use of diazomethane are detailed here.

3.1 Diazomethane

Uses, Safety Hazards, and Exposure Symptoms. Diazomethane (CH_2N_2) occurs as a very toxic, explosive yellow gas and it is prepared as a solution in diethylether. It is used for converting the OH-PCBs into their methyl esters. Diazomethane is toxic by inhalation or by contact with the skin or eyes. Exposure symptoms include chest discomfort, headache, weakness, and in severe cases, collapse. CH_2N_2 may explode when in contact with ground-glass joints or when heated to about 100.0 °C. Consequently specialized, scratch-free and rounded-edge glassware and a blast shield should be employed for its use. Glass pipettes, not plastic pipettes, will be used to aliquot the diazomethane to samples. Transport of diazomethane (or other forms of shock) should be minimized. Diazomethane vials with crystals present should NOT be used and should be reported to the Synthesis Core Director immediately.
Usage Overview. The diazomethane is prepared in the laboratory of IREH by the Synthesis Core. When diazomethane is needed the samples will be transported to the Oakdale campus and the derivitization procedure will take place there. The evaporation of excess diazomethane from samples will also be performed at Oakdale before the samples can be transported back to the Environmental Engineering lab.
Protective Equipment. All diazomethane usage will take place in a well-ventilated fume hood with the sash in the down, protective position. Protective clothing such as long pants and closed-toed, impervious shoes need to be worn. Doubled Nitrile gloves, protective glasses, face shield, and a lab coat need to be worn at all times when handling the diazomethane.
Used equipment like gloves and pipettes will sit in the hood to allow the diazomethane to completely evaporate before being discarded.
Additional Information. Additional information on diazomethane safety can be found in the Standard Operating Procedure for Diazomethane Generation available through the Synthesis Core. **All personnel handling diazomethane must be certified by the Synthesis Core Director.**

4. Apparatus, Glassware, Supplies, Chemicals, and Reagents

4.1 Apparatus

Barnstead|Thermolyne Rotisserie (custom)
Fisher Scientific Mini-Vortexer
The Meyer N-Evap Analytical Evaporator equipped with Popper non-sterile pipetting needles with blunt end and standard hub, ref 7936,18x4"
Mettler Toledo AG245 analytical balance
Thermolyne 30400 Furnace
Beckman Coulter Model J2-21M Induction Drive Centrifuge equipped with a FiberLite F14-6x250 rotor and Sorvall adapters, cat. No. 00456
Silica column holder (custom)
ASE 200 Accelerated solvent extractor (Thermo scientific/Dionex)
TurboVap II, 50 and 200 mL size (Biotage/Dionex)

4.2 Glassware and supplies

Pasteur pipettes, borosilicate & non-sterile, 9" Fisher Cat. No. 13-678-20D
Pasteur pipettes, borosilicate & non-sterile, 5 3/4" Fisher Cat. No. 13-678-20B
16x125 mm Pyrex test tubes w/ Teflon-lined screw caps
Small glass funnel (custom to fit into the top of the Pasteur pipettes)
GC sample vials (2mL capacity)
GC vial inserts, Wheaton Cat. No. 225350-631, 0.35 mL glass flat bottom limited volume insert
Teflon aluminum crimp caps with Teflon septa, SUN-Sri 200 100 TFE/RUB 11MM seal
Glass wool, Pyrex borosilicate, Fisher Cat. No. 11-388 from Corning, Inc. X3950
Solvent reservoirs (custom)
2 small tweezers (for stuffing glass wool into Pasteur pipettes)

4.3 Chemicals

Diatomaceous earth ASE® Prep DE	Thermo Scientific/Dionex
Silica gel 70-230 Mesh, S826-1	Fisher Scientific
Potassium Chloride EP/BP/USP/FCC, P333-500	Fisher Scientific
Potassium Hydroxide NF/FCC Pellets, P251-3	Fisher Scientific
Acetone, pesticide	
Hexanes, pesticide, H300-4	Fisher Scientific
2-Propanol, histological, A426P-4	Fisher Scientific
Methyl-tert Butyl Ether, HPLC, E127-4	Fisher Scientific
Methylene Chloride, pesticide, D142-4	Fisher Scientific
Methanol, optima, A454-4	Fisher Scientific
Ethanol, absolute, 200 proof , 99.5% A.C.S reagent	Sigma-Aldrich
Water optima, W7-4	Fisher Scientific
Sulfuric Acid certified ACS plus, A300-212	Fisher Scientific
Hydrochloric Acid 6N, SA56-500	Fisher Scientific

4.4 Reagents

1:1 Hx:MTBE (v/v)
1% KCl (w/w): 5 g KCl, 495 g reag. H2O
KOH: 100 mL reagent water, 5.622 g KOH, 100 mL EtOH
9:1 Hx:MTBE (v/v): 50 mL MTBE, 450 mL Hx
2 M HCl: 1 part 6 N HCl to 2 parts reagent water (50 mL acid + 100 mL H_2O)

5. Preparation

5.1 Diatomaceous earth (DE) activation

1. Heat approximately 400 g of DE at 450 °C for at least 12 h. Cover it with aluminum foil.
2. Before using it, allow to cool.
3. Keep it in a beaker with cap (no more than 20 days).

5.2 Sediment preparation

1. Weight 4 – 6 g of sediment sample, using a small aluminum weighing dish, and record the weight.
2. Using the mortar and pestle, place the sample and start adding activated DE, until a free-flowing powder is obtained. The quantity added will depend on the water content of the sample, but it will be around 30 - 50 g.
3. Place the dry sample in an amber jar and weigh it, and record final weight of sample with DE.
4. After each preparation, the mortar and pestle have to be cleaned. Clean them with water and then apply triple rinse using methanol, hexane and finally, acetone. Let it dry.

5.3 Silica gel

1. Heat 600 g of silica gel at 450 °C for at least 12 h. Do not cover the beaker with aluminum foil.
2. Allow to cool
3. Transfer to a container with a screw-top cap

5.3.1 Silica gel for ASE cells

1. Weigh a portion of the cooled silica gel into a beaker with cap
2. Add 3% (w/w) water to the cooled silica gel
3. Mix together
4. Keep it in a beaker with cap (no more than 20 days)

5.5 Extraction cell preparation

Prior to extraction, prepare a 100-mL cell as follows:

1. Remove top of the ASE 300 cell (bottom is designed as the side of the cell that has the cell number) and insert one filter paper onto the bottom of the cell (outlet end) using the rubber tube provided.
2. Add a layer of silica gel, around 10 g.
3. Add the dehydrated sediment from the amber jar into the cell, 25 - 35 g. Leave a space of around 1 cm in the top.
4. Rinse syringe 3x with DCM followed by 3x with hexane.
5. Add PCB surrogate standard, rinse the syringe 3x with DCM followed by 3x with hexane, then add OH-PCB surrogate standard.
6. Repeat step 5.5.4 to rinse the syringe.
7. Tap filter on top of the cell with rubber tube.
8. Place the cell top back on and secure tightly.
9. Add the same amount of surrogate standard to an empty clean test tube (reference). Then, add 20 drops of methanol.

6. Extraction

6.1 ASE extraction method

1. Press tray button on ASE 300 to release tray lock (light should appear on right side of the button when unlocked).
2. Place cell onto top rack of ASE.
3. Place corresponding glass vials that have been combusted and labeled with cell ID number into bottom tray.
4. Place glass vial onto rinse (R1) spot onto bottom tray.
5. Press tray button to lock tray.
6. Fill solvent jar in ASE 300 with 1:1 acetone: hexane mix until full.
7. Press rinse button and wait until rinse is finished.
8. Press 1 on menu screen to load method.
9. Load Method 1 and press Enter – DO NOT load a sequence – only the method should be loaded.
10. Press start button (light should be on the right side of button when method is running).

6.2 ASE extraction conditions

1. Extraction solvent: Acetone/n-hexane (1:1, v/v), CH_3COCH_3/CH_2Cl_2
2. Pressure: 1,500 psi
3. Temperature: 100 °C
4. Static time: 5 min
5. Static cycles: 1
6. Flush: 60%
7. Purge: 90 s
8. Cell size: 100 mL

6.3 Concentration

1. Transfer the sample extract from the ASE bottle to a large TurboVap tube. For each sample rinse the ASE bottle with 2 mL of hexane and transfer the rinse to the TurboVap tube.
2. Concentrate in the TurboVap tube until ~0.5 mL.
3. Transfer each sample to a test tube, rinsing with ~3.5 mL hexane for a total volume of ~4.0 mL.
4. Vortex

CAN STOP HERE OVERNIGHT

CHECK WITH RACHEL OR ANDRES BEFORE PROCEEDING TO ENSURE DIAZOMETHANE AVAILABILITY FOR DERIVATIZATION

7. PCB/OH-PCB Separation

7.1 PCB extraction

1. Add 2 mL KOH solution (0.5 M in 50% EtOH).
2. Mix by inversion for 3 min.
3. Centrifuge 3 min. (rotor: 10.0, speed: 3000, temp: 20)
4. Transfer the organic solvent phase (top) to a new test tube (this is the PCB fraction).
5. Re-extract any remaining PCBs from the alkaline solution:
    a. Add 3 mL hexane.
    b. Mix by inversion for 3 min.
    c. Centrifuge 3 min. using above settings.
    d. Transfer top (solvent) layer containing PCBs.
6. Repeat re-extract step for a total of 3 top layer transfers to the test tube.
7. PCB fraction can be stored overnight in the freezer.

7.2 OH-PCB extraction

1. Acidify the alkaline solution with 1.0 mL HCl (2 M).
2. Check pH of a random sample (not blank) by vortexing, dipping pipette into sample, and streaking a pH strip. It should be acidic, pH 1.
3. Extract the OH-PCBs from the acidic solution:
    a. Add 4 mL hexane/MTBE (9:1).

CAN STOP FOR A BREAK BUT NOT OVERNIGHT

    b. Mix by inversion for 3 min.
    c. Centrifuge 3 min. using above settings.
    d. Transfer the organic (top) phase to a small TurboVap tube. This is the OH-PCB fraction.

4. Re-extract any remaining OH-PCBs from the acidic solution:
   a. Add 3 mL hexane/MTBE (9:1).
   b. Mix by inversion for 3 min.
   c. Centrifuge 3 min. using above settings.
   d. Transfer the organic phase to the TurboVap tube.
   e. Repeat steps a-d for a total of 3 top layer transfers to the test tube.
5. The bottom (aqueous) layer containing the HCl and KOH in ethanol should be dumped into the waste jug in the flammables cabinet.
6. Concentrate the phenolic fraction to ~0.5 mL, rinsing TurboVap tube once with ~0.5 mL hexane. Check for water in the bottom of the test tube!
7. Add 5 drops of MeOH (MeOH increases the efficiency of the derivitization process).
8. Vortex.
9. Mark sample level on test tube with a Sharpie.
10. Also mark the 4 mL level using the test tube guide taped to the fume hood.

8. OH-PCB Derivatization to MeO-PCB Using Diazomethane

THIS STEP IS DONE IN SYNTHESIS CORE LAB BY APPROVED PERSONNEL ONLY!

8.1 Supplies

1. samples and reference test tubes
2. evaporation needles
3. Erlenmeyer flask with 4mL hexane per test tube
4. 2 combusted Pasteur pipettes
5. 2 dropping bulbs

8.2 Diazomethane Addition

1. Add 0.5 mL of diazomethane to samples and reference.
2. Keep in fridge at 4-8 °C for at least 3 hours or preferably overnight.

8.3 Diazomethane Evaporation

1. Evaporate the excess diazomethane under a gentle flow of N_2 and concentrate to 1 drop. DO NOT LET SAMPLE GO DRY.
2. Add 1 mL hexane to the reference and 4 mL hexane to the other test tubes and mix by inversion by hand.

CAN STOP HERE OVERNIGHT

9. OH-PCB Fraction: Removing Interferences

DO NOT TAKE THE REFERENCE THROUGH THE INTERFERANCE REMOVAL STEPS

9.1 Mixing with Sulfuric Acid

1. Add 2 mL concentrated H_2SO_4.
2. Invert 2 min and centrifuge 5 min.
3. Transfer the organic solvent (top) phase containing MeO-PCBs to a small TurboVap tube.
4. Re-extract any remaining MeO-PCBs:
   a. Add 3 mL hexane

CAN STOP FOR A BREAK AT THIS POINT IF NEEDED, BUT NOT OVERNIGHT

   b. Invert 2 min.
   c. Centrifuge 5 min.
   d. Transfer the top (solvent) layer containing the MeO-PCBs
5. Test tubes containing the concentrated H_2SO_4 should stand in the hood overnight to evaporate the residual hexane layer on top, then dump the concentrated H_2SO_4 into the waste hug in the corrosive cabinet. Rinse the test tubes 3 times into the waste jug using the water squeeze bottle.

CAN STOP HERE OVERNIGHT

9.2 Preparing Silica Gel Columns

MAKE THE COLUMNS IMMEDIATELY PRIOR TO USE WITH SAMPLES

1. Use combusted tweezers to stuff a small amount of combusted glass wool into the bottom of a 9" Pasteur pipette
2. Use a small glass funnel to add 0.1 g of combusted, pure activated silica gel.
3. Gently flick the pipette over a black surface to make sure the glass wool is tight enough to hold the silica gel without leaking out of the pipette into the sample.
4. Add 1 g H_2SO_4-acidified silica gel (2 silica gel:1 acid, w:w) on the top.

9.3 Using Silica Gel Columns

1. Concentrate the organic solvent to 1 mL.
2. Transfer to an acidified silica gel column, rinsing TurboVap tube with 2x10 drops of hexane.
3. Elute with 10 mL DCM into a new small TurboVap tube.

CAN STOP FOR A BREAK AT THIS POINT IF NEEDED, BUT NOT OVERNIGHT

4. Change sample and reference solvent to Hx before GC analysis

a. evaporate to ~0.5 mL DCM
b. add 2 mL hexane
c. evaporate to ~0.5 mL
d. add 2 mL hexane
e. evaporate to ~0.5 mL
f. Add 2 mL hexane
g. evaporate to ~0.5 mL
h. Concentrate reference test tube to ~0.5 mL
i. DO NOT LET SAMPLES GO DRY

CAN STOP HERE OVERNIGHT

10. PCB Fraction: Removing Interferences

Repeat all of Step 9 except:

1. Step 9.3.3 elute column with hexane instead of DCM
2. Do not perform solvent exchange in step 9.3.4. Do concentrate to ~0.5 mL hexane.

11. Internal Standard Spiking

11.1 OH-PCB Fraction

1. Evaporate the hexane from each sample, blank, and reference in the TurboVap until ~0.1 mL. DO NOT LET SAMPLES GO DRY
2. Transfer the samples, blank, and reference each to a GC vial with insert, rinsing TurboVap tube twice with 10 drops hexane each time. Final volume in the insert has to be less than 1 mL mark. GC vials can stand open in fume hood to reach this level.
3. Rinse syringe 3x with DCM followed by 3x with hexane.
4. Spike each sample, blank, and reference with 50 µL of internal standard.
5. Repeat step 11.1.3 to rinse the syringe.

11.2 PCB Fraction

1. Evaporate the hexane from each samples in the TurboVap until ~0.5 mL. DO NOT LET SAMPLES GO DRY.
2. Transfer the samples to GC vials, rinsing TurboVap tube twice with 10 drops hexane each time. Final volume ~1 mL.
3. Rinse syringe 3x with DCM followed by 3x with hexane.
4. Spike each sample, blank, and reference with d-PCB30 and PCB 204 IS, 100 uL of ~100 ng/mL = ~10 ng per congener.

5. Repeat step 11.2.3 to rinse the syringe.