4.4 ORGANICS

4.4.1 SCOPE

The organic pollutants studied at EML are combustion related. Of particular interest are pollutants which are toxic, resistant to natural degradation, and that accumulate in the environment. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) fit into this category and have been analyzed at EML in sediment samples (Tan et al., 1993).

The anthropogenic PAHs in the environment are formed primarily by pyrolysis of carbonaceous materials and the major source is the combustion of fossil fuels. Various combustion processes, especially those involving chlorinated aromatic compounds, generate PCDD/Fs. These compounds are of particular interest to DOE because, besides being combustion originated, they are quite persistent chemicals and can spread throughout the environment. Some PAH and PCDD/F isomers are highly carcinogenic. The study of these compounds in sediments is important as they can reveal the magnitude of contamination, as well as their origins and historical inputs.

OR-01

PAH AND PCDD/Fs IN SEDIMENT - GC/MS

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APPLICATION

This procedure can be applied to the analysis of sediment for three-ring to six-ring polycyclic aromatic hydrocarbons (PAHs) and tetra- to octa- polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs).

Many organic compounds, including PAHs and PCDD/Fs, that exist in complex compositions in sediments can be extracted into organic solvents such as methylene chloride. From the extract, the PAHs can be isolated from other organics by silica gel adsorption column chromatography and Sephadex LH-20 gel permeation column chromatography, and the PCDD/Fs can be isolated by silica gel adsorption column chromatography, acid/base wash, and alumina adsorption column chromatography. Individual PAHs and PCDD/Fs in these isolated fractions can be further separated and identified with a gas chromatograph/mass spectrometer (GC/MS) by GC retention times and mass spectra. Internal standards of labeled stable isotopes serve the purpose of quantification and quality control.

SPECIAL APPARATUS

1. Hewlett-Packard (3000-T Hanover St., Palo Alto, CA 94304) 5988 GC/MS with on-column injector.

2. HP 5890 GC/Kratos CONCEPT 1S (Barton Dock Road, Unmston, Manchester, M31 2LD UK) high resolution MS with on-column injector.

3. Labconco (8111-T Prospect, Kansas City, MO 64132) chemical carcinogen glove box.

4. Cahn Instruments, Inc. (16207-T S. Carmenita Rd., Cerritos, CA 90701), automatic electrobalance.

5. Whatman Soxhlet extraction thimbles, 33 x 80 mm, cleaned by Soxhlet extraction with CH_2Cl_2.

6. Buchi rotary evaporator (Brinkmann, Westbury, NY 11590).

7. Fused silica capillary columns, bonded with DB-5, 30 m x 0.25 mm i.d., 0.25 µm film (J & W Scientific Inc., Folsom, CA 95630).

8. On-column syringes.

9. Amberized microflex tubes, 5 mL and 1 mL.

10. Milli-Q reagent grade water system (Millipore Corp., 80-T Ashby Rd., Bedford, MA 01730).

11. Volumetric flasks, 25 mL, l50 mL, 300 mL.

12. Narrow mouth amber glass bottles, 60 mL, with Teflon-lined screw caps.

13. Glass columns (40 cm x 19 mm i.d.), for gel permeation chromatography, with Teflon stopcocks and 250-mL reservoirs having 24/40 female joints for 24/40 stoppers.

14. Filter paper, Whatman No. 41, 2.5 cm, cleaned by Soxhlet extraction with CH_2Cl_2.

15. Soxhlet extractors, medium size.

16. Heavy-duty explosion proof centrifuge, Model EXD (Forma Scientific, Inc., Box 649, Millcreek Rd., Marietta, OH 45750).

17. Baker Bond SPE glass Teflon columns and adapters (J. T. Baker, 222-T Red School Lane, Phillipsburg, NJ 08865).

18. 60-mL separatory funnels with Teflon stopcocks and stoppers.

19. Separatory funnel shaker.

20. Glass columns (14 cm x 5.5 mm i.d.), for alumina column chromatography, with Teflon stopcocks and 50-mL reservoirs.

SPECIAL REAGENTS

1. Distilled in glass - methylene chloride, hexane, and toluene (Burdick and Jackson, Muskegon, MI 49442).

2. PAH solutions - in the Labconco glove box, put about 100 µg each of the following standard PAHs (from various suppliers: Aldrich, P.O. Box 335, Milwaukee, WI 53201; Analabs, North Haven, CT 06473; Ultra Scientific, North Kingston, RI 02852; and Community Bureau of Reference (BCR), Brussels, Belgium) into separate 5-mL amberized microflex tubes:

   phenanthrene, anthracene, 1-methylphenanthrene, fluoranthene, pyrene, 1-methylpyrene, benzo(ghi)fluoranthene, benz(a)anthracene, chrysene, triphenylene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenz(a,c)-anthracene, dibenz(a,h)anthracene, benzo(ghi)perylene.

   Dissolve each of the PAHs into 5 mL of CH_2Cl_2.

3. SRM-1647 (priority pollutant PAHs, NIST).

4. PAH standard solution - using the Cahn 25 automatic electrobalance in the Labconco glove box, quantitatively weigh an amount of about 5 mg each of the following PAHs into a 50-mL volumetric flask:

   phenanthrene, anthracene, 1-methylphenanthrene, fluoranthene, pyrene, 1-methylpyrene, benzo(ghi)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(e)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(ghi)perylene.

Dissolve the weighed PAHs with 50 mL of toluene so that the concentration of this primary standard solution contains about 100 $\mu g\ mL^{-1}$ for each PAH. Transfer the solution to a 60-mL narrow mouth amber glass bottle with a Teflon-lined screw cap and store in a refrigerator. Mix quantitative amounts of the primary standard and SRM-1647. The mixture is analyzed as PAH calibration solutions by measuring the molecular ions of the PAHs with GC/MS according to Steps 1-8 of **Determination**, Section A. Calibrate the concentrations of each PAH in the standard solution based on the PAH concentrations in SRM-1647. Use the method of standard additions for PAHs that are present in both solutions. For PAHs present only in the standard solution, response factors of neighboring PAHs in the gas chromatogram are used.

5. Labeled PAH solution - Using the Cahn 25 automatic electrobalance in the Laconco glove box, quantitatively weigh an amount about 2 mg each of the following deuterated PAHs into a 50-mL volumetric flask:

   anthracene, fluoranthene, pyrene, chrysene, benzo(e)pyrene, perylene, benzo(ghi)perylene

   Dissolve the weighed deuterated PAHs in 50 mL of toluene.

6. PAH calibration solutions - Mix and dilute the labeled PAH solution and the PAH standard solution with toluene to make five calibration solutions. All five calibration solutions contain 5 $\mu g\ mL^{-1}$ of each deuterated PAHs with various concentrations of native PAHs at 1 $\mu g\ mL^{-1}$, 2 $\mu g\ mL^{-1}$, 5 $\mu g\ mL^{-1}$, 10 $\mu g\ mL^{-1}$, and 25 $\mu g\ mL^{-1}$.

7. Native PCDD/F stock solutions containing: 2378-TCDF, 12378-PnCDF, 123478-HxCDF, 1234678-HpCDF, OCDF, 2378-TCDD, 12378-PnCDD, 123678-HxCDD, 1234678-HpCDD, and OCDD, 1 $mg\ mL^{-1}$ each in toulene.

8. PCDD/F spiking solution - mix and dilute labeled PCDD/F solutions:

   $^{13}C_6$ 2378-TCDF, $^{13}C_6$ 23478-PnCDF, $^{13}C_6$ 123478-HxCDF, $^{13}C_{12}$ 1234678-HpCDF, $^{13}C_{12}$ 2378-TCDD, $^{13}C_{12}$ 12378-PnCDD, $^{13}C_{12}$ 123678-HxCDD, $^{13}C_{12}$ 1234678-HpCDD, and $^{13}C_{12}$ OCDD from Cambridge Isotope Laboratory (Woburn, MA 01810) with toluene so that the spiking solution contains 100 $ng\ mL^{-1}$ each of the labeled PCDD/Fs.

9. PCDD/F calibration solutions - mix and dilute the PCDD/F spiking solution and the native PCDD/F stock solutions with toluene to make six calibration solutions. All six calibration solutions contain 10 ng mL^{-1} of each labeled PCDD/Fs in the PCDD/F spiking solution with various concentrations of each of the native PCDD/Fs listed in Step 7 at 1 ng mL^{-1}, 2 ng mL^{-1}, 5 ng mL^{-1}, 10 ng mL^{-1}, 25 ng mL^{-1}, and 50 ng mL^{-1}.

10. Sulfuric acid, ACS reagent (Fisher Scientific, 52 Faden Rd., Springfield, NJ 07081).

11. KOH solution, 20% by weight in Milli-Q water.

12. Copper powder - electrolytic purified (Fisher Scientific, 52 Faden Rd., Springfield, NJ 07081), freshly activate before use by soaking in 6N HCl for 5 min then rinsing thoroughly with Milli-Q water, followed by methanol.

13. Sephadex LH-20, Sigma Chemicals Co. (P.O. Box 14508, St. Louis, MO 63178) - swell overnight in 1:1 methanol-methylene chloride before column packing.

14. Silica gel, 100-200 mesh, EM Science (480-T Democrat Rd., Gibbstown, NJ 08027), Grade 923, cleaned by Soxhlet extraction with methylene chloride, activated at 120°C overnight, then stored in a desiccator.

15. ICN alumina B, activity 1 (ICN Pharmaceuticals, Inc., 3300-T Hyland Ave., Costa Mesa, CA 92626) cleaned by Soxhlet extraction with methylene chloride, activated at 150°C overnight, then stored in a desiccator.

16. Glass beads, 0.5 mm, cleaned by Soxhlet extraction with CH_2Cl_2.

17. Zero gases, He, H_2, air, and N_2 (Matheson Gas Products, 30-T Seaview Dr., P.O. Box 1587, Secaucus, NJ 07096).

18. Perfluorotributylamine, Hewlett-Packard calibration compound.

SAMPLE PREPARATION

A. Sediment handling.

1. Centrifuge the watery sediment and decant off the excess water.

2. Dry the sediment in a freeze dryer.

3. Pulverize the dried sediment in a mortar and pestle and then sieve with a No. 40 (0.425 mm) sieve to remove stones and extraneous material. The fines are collected for analyses.

B. Extraction.

1. Place the fine sediment in a cleaned Whatman extraction thimble, 33 x 80 mm.

2. Spike appropriate amounts of labeled PAH and labeled PCDD/F solutions close to those of the estimated analytes.

3. Soxhlet extract the sample with CH_2Cl_2 for 30 h.

4. Concentrate the extract to dryness with a rotary evaporator at room temperature.

C. Silica gel adsorption chromatography and sulfur removal.

1. Pack 250 mg of clean activated silica gel in an empty SPE glass Teflon column between two Teflon fritted disks. Tap gently for a tight packing and make sure the top of the column is evenly packed and closely held by the Teflon-fritted disk.

2. Pack 1000 mg of cleaned and activated silica gel in another empty SPE column as above.

3. Reactivate the two columns overnight at 120°C.

4. Prior to using, allow the columns to cool in a desiccator.

5. Place a piece of Whatman No. 41 filter paper, ca. 1 cm I.D., onto the top of the Teflon disk of the 250 mg silica gel column, add about 0.5 g of activated copper powder, place a second piece of filter paper on top of the powder.

6. Transfer the dried residue (Step B4 of **Sample Preparation**) onto the copper powder topped 250 mg silica gel column with 2 x 0.5 mL of CH_2Cl_2. Dry the wetted column thoroughly with dry N_2 after each transfer.

7. Wet the 1000 mg column with hexane.

8. Attach sampled 250 mg column to the top of the 1000 mg column with a Teflon column adapter.

9. Wash the two-column assembly with 3 mL of hexane, and discard the wash.

10. Elute the columns with 10 mL of hexane followed by 7 mL of 60% CH_2Cl_2 in hexane into two separate 25-mL round bottom flasks for PCDD/F and PAH fractions, respectively.

11. Evaporate the PAH fraction to dryness at 35°C under reduced pressure with a rotary evaporator.

D. Acid/base wash.

1. Transfer the PCDD/F fraction in 10 mL of hexane from Step 10 of Section C, into a 60-mL separatory funnel, add 10 mL of concentrated H_2SO_4.

2. Shake the funnel and contents with a separatory funnel shaker for 2 min.

3. Centrifuge the funnel for 30 s.

4. Drain off the lower aqueous layer.

5. Add second 10 mL of H_2SO_4 into the funnel and repeat Steps 2-4.

6. Add 10 mL of Milli-Q water, shake briefly by hand, and repeat Steps 3-4.

7. Add 10 mL of 20% KOH solution into the funnel, and repeat Steps 2-4.

8. Add second 10 mL of 20% KOH solution and repeat Steps 2-4 once and Step 6 twice.

9. Transfer the hexane layer into a 25-mL round bottom flask and concentrate to dryness with a rotary evaporator at 35°C.

E. Alumina column chromatography.

1. Place a small amount of glass wool at the bottom of the glass column for alumina column chromatography.

2. Pack 2.5 mL of activated alumina into the column and reactivate overnight at 150°C.

3. Prior to use, cool the column in a desiccator. Once cool, add a 3-mm thick layer of dry Na_2SO_4.

4. Transfer the residue from Step 9 of Section D with 2 x 0.5 mL of hexane onto the top of the Na_2SO_4 layer. Drain the column until the meniscus reaches the sodium sulfate layer after each transfer.

5. Wash the column with 45 mL of hexane and discard the wash.

6. Elute the column with 4 mL of 60% CH_2Cl_2 in hexane.

7. Collect the eluent and concentrate to dryness on a rotary evaporator at room temperature.

F. Gel permeation chromatography.

1. Place a piece of Whatman No. 41 filter paper, 17 mm in diameter, on the fritted glass disc of the gel permeation column. Wet the filter paper with a few drops of methanol so that the paper adheres to the fritted glass disc.

2. Pour in a layer of about 1 mL of glass beads to hold the filter paper onto the fritted glass disc.

3. Fill half the column with the eluting solvent, 1:1 methanol-methylene chloride.

4. Pour in the swelled Sephadex LH-20 to form a 37-cm tall column. After the solvent is drained to the top of the gel, place a piece of Whatman No. 41 filter paper, 17 mm in diameter, on top of the column and add a 1-cm thick layer of glass beads.

5. Transfer, dropwise, the PAH fraction (Step 11 of Section C) with three to five portions of 0.5 mL each of the eluting solvent onto the column. Spread the drops evenly over the top of the column and drain off each transferring solution before adding the succeeding portions.

6. Elute with 1:1 methanol-methylene chloride and discard the first 75 mL of the eluent. Collect the following 30 mL of eluent and dry it with a rotary evaporator at room temperature.

7. Regenerate the column by washing with an additional 50 mL of eluting solvent. Keep the column in the solvent and seal the reservoir with a 24/40 stopper.

DETERMINATION

A. PAHs.

1. Set the HP5988 GC/MS parameters as follows:

Parameters	Description
GC Column	J & W Scientific, Inc., fused silica capillary column (see **Special Apparatus**)
Oven temperature program	from initial 60° to 150°C, at 32°C min^{-1}, then from 150° to 280°C at 4°C min^{-1}, and hold at 280°C for 30 min
Carrier gas	He, head pressure 20 psi.
Injection mode	On-column
Ionization mode	Electron impact
Emission	300 µA
Electron energy	70 eV
Ion source temperature	150°C

2. Tune the MS with a calibration compound, perfluorotributylamine.

3. Set the MS at scan mode and mass scan range at 50-500.

4. Inject 0.5 µL of each PAH solutions (Step 2 of Special Reagents).

5. Immediately start the GC oven temperature program, and acquire the data during the GC run.

6. Determine the GC retention times of each PAH from the total ion chromatograms.

7. Switch the MS to the SIM mode, set the masses of the molecular ions of labeled and native PAHs in calibration solutions (Step 6 of Special Reagents) to be acquired in their retention windows.

8. Inject 1 µL of each PAH calibration solution and acquire the ion abundances of the selected ions at the SIM mode. Repeat five times.

9. Establish the calibration curve for each native PAH by plotting the molecular ion abundance ratios of native to labeled PAHs vs. their concentration ratios in the calibration solutions. For native PAHs without labeled counterparts, data from neighboring labeled PAHs in the total ion chromatograms are used. The slopes of these curves are the response factors, F.

10. Repeat Step 3.

11. Redissolve the residue of the PAH fraction (Step 6 of Section F) into a small amount of methylene chloride and inject 1 µL of the solution into the GC/MS.

12. Repeat Step 5.

13. Identify the PAHs in the PAH fraction by their retention times and mass spectra, considering there are superimposed spectra of spiked labeled PAHs on some of the native PAHs.

14. Repeat Step 7.

15. Inject 1 µL solution of PAH fraction and repeat Step 5.

16. Calculate the molecular ion abundance ratios of native to labeled PAHs, r.

17. Determine the concentrations of native PAHs, C, from the weight of the sample, W, amount of spiked labeled PAHs, w, the ion abundance ratios and response factors, F, as:

$$C = \frac{w \cdot r \cdot F}{W}$$

B. PCDD/Fs.

1. Set the Kratos 1S CONCEPT parameters as follows:

Parameters	Description
GC column	J & W Scientific, Inc.; fused silica capillary column (see Special Apparatus)
Oven temperature program	Hold at initial 80°C for 1 min, program from 80-200°C at 30°C min^{-1}, then go to 280°C at 5°C min^{-1} and to 300°C at 15°C min^{-1}
Carrier gas	He, head pressure 20 psi
Injection mode	On column
Ionization mode	Electron impact
Electron energy	30-40 eV
Source temperature	250°C
Trap current	500 µA
Resolution	10,000 (10% valley definition)

2. Tune the MS with a calibration compound, perfluorotributylamine.

3. Under the GC oven temperature program, acquire the mass spectra of the native PCDD/Fs in stock solution (Step 7 of **Special Reagents**) at scan mode, mass range 50-500. Establish the retention windows of PCDD/Fs with the acquired spectra and published results (Ryan et al., 1991).

4. Set the MS at the SIM mode and acquiring ions with masses M (molecular mass with all ^{35}Cl) and M+2 (molecular mass with one ^{37}Cl and the rest ^{35}Cl) for both native and

labeled tetra- to penta- PCDD/Fs isomers and M+2 and M+4 (molecular mass with two ^{37}Cl and the rest ^{35}Cl) for both native and labeled hexa- to octa- PCDD/Fs isomers in their respective retention windows.

5. Inject 0.5 µL of each PCDD/F calibration solutions (Step 9 of **Special Reagents**), start the GC oven temperature program, and acquire the ion abundances of the selected ions at the SIM mode.

6. Establish the calibration curves for each native PCDD/F by plotting the ion abundance ratios of native to labeled PCDD/F vs. their concentration ratios in the calibration solutions. The slopes of these curves are response factors, F_1.

7. Redissolve the residue of PCDD/F fraction (Step 7 of Alumina Column Chromatography) into a small amount of toluene and inject 0.5 µL of the solution into the GC/MS.

8. Repeat Step 4.

9. Inject 0.5 µL of solution made in Step 7 and start GC/MS run as Step 5.

10. Identify the PCDD/Fs in the PCDD/F fraction by their retention times (Ryan et al., 1991) and ion abundances.

11. Calculate the ion abundance ratios of native to labeled PCDD/Fs, r_1.

12. Determine the concentrations of native PCDD/Fs, C_1, from the weight of the sample, W, amount of spiked labeled PCDD/Fs, w_1, the ion abundance ratios of native to labeled PCDD/Fs, r_1, and F_1, as:

$$C_1 = \frac{w_1 \cdot r_1 \cdot F_1}{W}$$

LOWER LIMIT OF DETECTION

The recovery of PAHs and PCDD/Fs from the whole sample preparation is ~ 50%. The detection limit varies from 1 to 3 pg for three-ring to six-ring PAHs on the HP5988 GC/MS, and 0.2 to 0.5 pg for tetra- to octa-PCDD/Fs on the HP5890 GC/Kratos CONCEPT 1S MS.

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