MEASURING AND MODELLING THE DYNAMIC RESPONSE
OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO
ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PROTOCOLS FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN

SEDIMENTS
WET-ONLY DEPOSITION
DRY DEPOSITION
BULK DEPOSITION
SNOW
WATER
AIR

Joan O. Grimalt
Pilar Fernandez
Rosa Vilanova
Lourdes Berdie
Department of Environmental Chemistry (CID-CSIC). Barcelona. Catalonia.

1. SEDIMENT CORES

1.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH: Parent compounds: fluorene, phenanthrene, anthracene, fluoranthene, acephenanthrylene, pyrene, benzo[*a*]fluorene, benzo[*ghi*]fluoranthene, cyclopenta[*cd*]pyrene, benz[*a*]anthracene, chrysene+triphenyene, benzo[*b+j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[7,1,2,3-*cdef*]chrysene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene, dibenz[*aj*]anthracene, dibenz[*ah*]anthracene, dibenz[*ac*]anthracene, benzo[*b*]chrysene, coronene.

  Methyl derivatives: 3-methylphenanthrene, 2-methylphenanthrene, 4H-cyclopenta-[*def*]phenanthrene, 4-methylphenanthrene, 1-methylphenanthrene, dimehtyl-phenanthrenes (10 individual isomers), retene, methylfluoranthenes/pyrenes (7 individual isomers).

  Sulphur derivatives: naphto[1,2-*b*]thiophene, dibenzothiophene, naphto[2,1-*b*]-thiophene, 4-methyldibenzothiophene, 3+2-methyldibenzothiophene, 1-methyldibenzothiophene, benzo(*b*)naphto[1,2-*d*]thiophene, benzo(*b*)naphto-[2,1-*d*]thiophene, benzo(*b*)naphto[2,3-*d*]thiophene.

1.2 Other measurements.

* Total organic carbon

1.3 Materials and reagents.

-Cutting tools
-Tweezers
-Aluminum foil
-Sulphuric acid for analysis grade
-Distilled water
-Milli-Q water
-Potassium hydroxide (for analysis)
-*n*-Hexane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-*Iso*-octane (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-alumina (70-230 mesh, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

1.4 Cleaning.

1. The material to be used for core sampling and sub-sectioning must be:
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10
minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40ºC for drying.

2. Prior to sampling they have to be rinsed with Milli Q water and acetone.

1.5 Sampling and sub-sectioning.

1. After sampling the core must be kept upright to avoid any disturbance of the sediment profile.

2. The sediment sections (0.5-1 cm) for trace organic analyses should be obtained from the central core area to avoid contamination from the walls and cross-contamination from other core sections.

3. The collected sediment in each section should be wrapped in aluminum foil. Two aluminum sheets must be used: one in direct contact with the sediment (previously rinsed), another to wrap the first. This last one should be labelled. A paper label than a water-resistant mark is preferred. Sediment storage in glass jars is not recommended because of breaking during frozening and de-frozening and transport to the laboratory.

1.6 Storage.

Sample sediments should be stored at -20ºC after sub-sectioning. These frozening conditions should also be kept during transport.

1.7 Sample amount.

0.1-1 g. of sediment are needed.

1.8 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

1.9 Analyses (extraction and fractionation).

About 0.1-1 g of wet sediment are extracted by sonication with methanol (1 x 20 ml; 20 min) to separate the interstitial water. Subsequent extractions are performed with (2:1, v/v) dichloromethane-methanol (3 x 20 ml; 20 min). The extracts are combined and spiked with d_{10}-pyrene, d_{12}-benzo[*ghi*]perylene and PCB 30). The combined extracts are vacuum evaporated to 10 ml and hydrolyzed overnight with 20 ml of (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

1.10 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310ºC at 4ºC/min, held for 10 min. Injection and transfer line temperatures are 280 and 300ºC, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

1.11 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310ºC, respectively. Oven temperature is programmed from 80 to 150ºC at 10ºC/min and to 300ºC at 4ºC/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

1.12 Quantitation.

Authentic standards of hexachlorobenzene pp'DDE and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

1.13 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120ºC, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

2. WET, DRY, BULK AND SNOW DEPOSITION

2.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

2.2 Other measurements.

* Weight of total particles
* Total organic particulate carbon (when sufficient material will be collected)

2.3 Materials and reagents.

- Dry and wet deposition sampler.
- Millipore 47 mm filtration apparatus.
- 0.45 mm (GF/B) glass microfibre filters.
- Glass bottles.
- Octadecylsilane membrane extraction disks (47 mm diameter, 0,5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
- Measuring cylinders.
- Pasteur pipettes.
- Tweezers.
- Aluminium foil.
- Milli-Q water.
- Distilled water.
- Cyclohexane (for trace organic analysis, Merck).
- Dichloromethane (for trace organic analysis, Merck).
- Methanol (for trace organic analysis, Merck).
- Acetone (for trace organic analysis, Merck).
- Soap (Extran-AP 13, alkaline, Merck).

2.4 Cleaning.

- PLASTIC SHOULD BE AVOIDED IN ANY INSTANCE.

- THE CLEANING OF THE SAMPLING MATERIAL IS THE MOST IMPORTANT STEP OF THE SAMPLING WORK.

2.4.1. Wet-dry deposition reservoirs.

- THE CLEANING OF THE WET-DRY DEPOSITION RESERVOIRS MUST BE DONE BETWEEN EVERY SAMPLE.

- The bulk and wet-dry deposition reservoirs should be cleaned with 5% inorganic alkaline (e.g.EXTRAN AP 13, Merck) in distilled water (stirring 10 minutes).

- Rinse with distilled water, milli-Q water and acetone.

2.4.2. Cleaning with ultrasonic bath.

- TWEEZERS AND EXTRACTION SYSTEM MUST BE CLEANED BETWEEN EVERY SAMPLE.

- Add 5% inorganic alkaline (e.g.EXTRAN AP 13, Merck) in distilled water into the ultrasounds reservoir.

- Put all the material inside the ultrasonic bath trying not to have bubbles of air into the material.

- Connect the ultrasonic for 15 minutes.

- Empty and rinse the material with abundant tap water.

- Rinse with distilled water, milli-Q water and finally with acetone.

- Wrap the cleaned material with aluminium foil and let it in the oven at 60 ºC for drying.

2.4.3. Cleaning without ultrasonic bath.

- Add 5% inorganic alkaline (e.g. EXTRAN MA 01, Merck) in distilled water and fill all the material that it has to be cleaned with.

- Let it stand for 24 hours.

- Rinse and dry the material as the last step.

2.4.4. Pasteur pipettes.

- Wrap the Pasteur pipettes with aluminium foil and let them remain into a oven at 400 ºC for 12 hours.

- Once it has been finished remain them always wrapped with aluminium foil.

2.5 Collection of the sample.

- THE GLASS BOTTLE COLLECTOR OF THE SAMPLE MUST BE CLEANED BETWEEN EVERY SAMPLE.

2.5.1. Wet deposition.

- Take a glass bottle and clean as the rest of the material.

- Put the wet deposition inside and record the volume collected of the sample.

- Rinse and stir adequately with some milli-Q water three times in order to make sure that all the suspended particles and compounds in the wet reservoir are transferred to the glass bottle.

2.5.2 Dry deposition.

- Take a glass bottle and clean as the rest of the material.

- Rinse the dry reservoir collector 400 ml of milli-Q water and collect it inside the glass bottle. Repeat this step three times.

2.6 Filtration on glass microfibre filters.

- It is very important to **FILTRATE THE SAMPLE THE SAME DAY THAT YOU COLLECT IT**, 20% of the compounds analysed can be lost because of the adsortion in the bottle.

- Record the water volume collected.

- Rinse the filtration apparatus with Milli-Q water.

- Record the filter label (glass microfibre filters are pre-weighted in the Department of Enviromental Chemistry).

- Take a glass microfibre filter with cleaned tweezers and place it in the filtration apparatus.

- Check whether the upper and lower parts of the apparatus are well assembled.

- Connect the apparatus to the vacuum.

- Pour the water sample in the upper part and connect the vacuum.

- Make sure that all the suspended particles in the glass bottle are transferred to the filtration apparatus. Rinse and stir adequately with some milli-Q water three times in order to make sure that all the suspended particles and compounds in the wet reservoir are transferred to the glass

bottle.

- Take the glass microfibre filter with cleaned tweezers and store it as is indicated in the section number 6.

2.7 Adsorption on membrane extraction disks.

- Transfer the filtered sample from the filtration apparatus into a cleaned glass bottle, in order to filter it through a membrane extraction disk (C_{18} empore disk).

- Take a membrane extraction disk with cleaned tweezers and place it in the filtration apparatus.

- Check whether the upper and lower parts of the apparatus are well assembled.

- Connect the apparatus to the vacuum.

- With the vacuum off, wash the membrane extraction disk with 10 ml of dichloromethane:cyclohexane (1:1) and allow to stand for 3 minutes.

- With the vacuum on, draw the remaining solvent through the disk. Let the vacuum for 1 minute until the disk become dry.

- With the vacuum off, add 10 ml of methanol to the membrane extraction disk and let stand for 3 minutes. **DO NOT ALLOW DISK TO RUN DRY.** If the disk remains dry in this step, replace the disk for a new one and start the process from the beginning.

- Pour the sample to the methanol that remains on the disk.

- Start the extraction. Water extraction should be slow. Adjust the vacuum trying to have a flow of 2 litres/hour.

- **NEVER ALLOW THE MEMBRANE EXTRACTION DISK GOES TO DRYNESS DURING EXTRACTION**. If it happens by accident, take out the membrane extraction disk and store it as it is indicated below. The rest of the water, must be filtered with a new membrane extraction disk, which should be cleaned as it has been previously described.

- When the extraction is finished, let the vacuum on for five minutes.

- Store the membrane extraction disk wrapping it in aluminium foil as it is indicated in the section number 6. **DO NOT BEND IT**.

2.8 Storage.

2.8.1. Glass microfibre filter.

- **BEND** the glass microfibre filter with the tweezers. The two parts containing the particulate material should face each other.

- Wrap it in aluminium foil.

2.8.2. Membrane extraction disk.

- **DO NOT BEND** the membrane extraction disk, just wrap it in aluminium foil.

- Indicate which is the upper part of the disk where the sample went through.

- Record the filter label and give a sample identification to the filter and membrane. This information must be included together with the period of collection, date, site, temperature range during the collection and precipitation, collected volume, in a sample protocol form.

- Store the wrapped and labelled filters and membrane extraction disks at -20🗁C.

2.9 Blank of the wet-dry and bulk reservoirs.

- **THE BLANK OF THE WET-DRY AND BULK RESERVOIRS MUST TO BE DONE MONTHLY.**

- The wet-dry and bulk reservoirs must be cleaned with 5% inorganic alkaline (e.g.EXTRAN AP 13, Merck) in abundant distilled water (stirring 10 minutes). Rinse with distilled water and milli-Q water.

- Add 1L of milli-Q water into the wet-dry and bulk reservoirs. Cover it with aluminium foil. Let it stand inside the station (not outside) as much as possible (at least one day).

- Filter the blank of the reservoirs as a sample.

- Wrap, store and record the filter and membrane extraction disks as a sample.

- Clean the wet-dry and bulk reservoirs and put them in the sampling place.

2.10 Transport.

Samples should be sent within boxes containing dry ice to the address and contact people indicated below. Samples should be sent monthly.

Contact people: Dr. Joan Grimalt.
Guillem Carrera.

Address: Department of Environmental Chemistry.
C.I.D.-C.S.I.C.
Jordi Girona 18-26
08034 Barcelona

Phone: 34-3-4006100
Fax: 34-3-2045905
E-mail: jgoqam@cid.csic.es
gcrqam@cid.csic.es

3. WATER (DISSOLVED + COLLOID AND PARTICULATE MATTER)

3.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

3.2 Other measurements.

* Weight of total particles

3.3 Materials and reagents.

-Tweezers
-Glass bottles
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Infiltrex 2 Pump
-1 mm GF/B glass fiber filters (14.2 cm diameter)
-XAD-2 resin columns
-Sodium sulphate (analysis grade)
-Milli-Q water
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

3.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40ºC for drying.

2. Glass bottles should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters and the Pasteur pipettes are kiln-fired at 400ºC.
4. Prior to sampling the XAD-2 columns are cleaned with 200 ml of methanol and 200 ml of dichloromethane. Then, further 200 ml of dichloromethane are collected to determine blank levels. If blanks are acceptable the columns are left moist with methanol not being allowed to dry out to prevent cracking of the extraction material. Columns should be stored in the refrigerator. Cleaning must be continued until low blank levels are achieved.

3.5 Sampling

The pump is introduced in the water column. The water is impelled through the filter and XAD2 column. Flow rate should be between 300-400 ml/min and the volume sampled about 100 liters.

3.6 Storage

The filters should be wrapped in aluminum foil and frozen (-20ºC) until analysis. The XAD-2 columns should be stored in the refrigerator but never freezed. These columns should be extracted within one week after sampling.

3.7 Analysis of particulate organic matter

The filter is freeze dried in an oil-free freeze-drier and weighed. Then, it is cut in small pieces and spiked with PCB 30, octachloronaphtalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The mixture is extracted by sonication with (2:1) dichloromethane:methanol (3 x 20 ml, 20 ml). The extract is vacuum evaporated to 2 ml and hydrolyzed overnight with 20 ml of (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

3.8 Analysis of dissolved+colloidal organic matter

The columns are eluted in the inverted direction to sampling with 200 ml of methanol and 200 ml of dichloromethane. The methanol fraction is extracted with 3 x 30 ml of *n*-hexane. This *n*-hexane extract is combined with the dichloromethane eluate and spiked with PCB 30, octachloronaphthalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The combined extracts are

hydrolyzed with 20 ml (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

3.9 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310ºC at 4ºC/min, held for 10 min. Injection and transfer line temperatures are 280 and 300ºC, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

3.10 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310ºC, respectively. Oven temperature is programmed from 80 to 150ºC at 10ºC/min and to 300ºC at 4ºC/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

3.11 Quantitation.

Authentic standards of hexachlorobenzene, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

3.12 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used.

Transfer line and ion source temperatures are 300 and 120ºC, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass

units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

4. AIR

4.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

4.2 Other measurements.

* Weight of total particles

4.3 Materials and reagents.

-Glass microfiber (GF/A) 1.6 mm (20.3 x 25.4 cm)
-Polyurethane foam density 0.022 g/cm^3
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-*n*-Hexane (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Hydrochloric acid (25% w/w; for analysis; Merck)
-neutral silica gel (Kieselgel 40, 70-230 mesh, Merck)
-alumina (aluminum oxide 90 active, 70-230 mesh, Merck)

4.4 Cleaning.

1. The filters are kiln-fired at 400ºC for 12 h and then wrapped into solvent rinsed aluminum foil until use.

2. The individual polyurethane plugs (height 7.5 cm, diameter 5 cm) are compressed/decompressed in water and acetone (10 times). Then, they are Soxhlet extracted with acetone (24 h), dried in a vacuum dessicator containing phosphorous pentoxide. Each plug is wrapped with aluminum foil and stored in a clean Teflon sealed glass jar.

3. Silica gel and alumina are extracted with (2:1, v/v) dichloromethane:methanol in a Soxhlet apparatus for 24 h. After Soxhlet evaporation they are respectively heated at 120ºC and 350ºC for 12 h. A total of 3% (w/w) of Milli-Q grade water is then added to the chromatographic absorbents for deactivation.

4.5 Sampling

The sampling system is composed by a high-vol MCV pump Mod. CAV-P, a filtering system equipped with Wathman GF/A filters and a Teflon column filled with 2 foam plugs. Air volumes of 40 m^3 are collected at a flow rate of 20 m^3/h. After air sampling the polyurethane plugs are removed from the Teflon columns, wrapped in aluminum foil, and stored in Teflon sealed glass jars at -20ºC.

4.6 Analyses (extraction and fractionation)

An internal standard containing perdeuterated naphthalene and anthracene are added to the polyurethane plugs before Soxhlet extraction with 700 ml of *n*-hexane for 24 h. The extract is vacuum evaporated to 0.3 ml and then fractionated by column chromatography. A column containing 2 g of alumina is used. The aliphatic and aromatic hydrocarbons are respectively eluted with 3 ml of *n*-hexane and 6 ml of (4:1, v/v) *n*-hexane:dichloromethane. These fractions are concentrated under a current of nitrogen to a small volume (0.1 ml) and reconstituted to 2 ml with *n*-hexane.

Filters are stored at -20ºC until analysis. Before extraction the above described internal standard mixture is added and then the filters are cut into *ca.* 1 cm^2 pieces. These pieces are Soxhlet extracted with 375 ml of (2:1, v/v) dichloromethane:methanol for 24 h. The extract is vacuum evaporated to 0.3 ml and fractionated by column chromatography following the same procedure described above.

4.7 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310ºC at 4ºC/min, held for 10 min. Injection and transfer line temperatures are 280 and 300ºC, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

4.8 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310ºC, respectively. Oven temperature is programmed from 80 to 150ºC at 10ºC/min and to 300ºC at 4ºC/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

4.9 Quantitation.

Authentic standards of hexachlorobenzene, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

4.10 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120ºC, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.