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Analytical Protocol for Hazardous Organic Chemicals in Environmental Samples

Division of Chemistry and Toxicology, Virginia Institute of Marine Science

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Virginia Institute of Marine Science
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College of William and Mary
Gloucester Point, Virginia 23062

2nd Edition: 1991

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Acknowledgements

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The revisions for this edition were prepared by J. Greaves, C. L. Smith and R. C. Hale.

Abbreviations

ARI	aromatic retention index (for PAH and substituded PAH)
BHC	hexachlorocyclohexane
DCM	dichloromethane (methylene chloride)
DDD	dichloro-diphenyl-dichloroethane
DDE	dichloro-diphenyl-dichloroethylene
DDT	dichloro-diphenyl-trichloroethane
ECD	electron capture detector
ELCD	electrolytic conductivity detector
FID	flame ionization detector
FPD	flame photometric detector
GC	gas chromatography (gas liquid chromatography)
GC-MS	gas chromatography - mass spectrometry
GPC	gel permeation chromatography
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HRI	halogen retention index (for organohalogenated compounds)
LC	liquid chromatography
MS	mass spectrometry
MW	molecular weight
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate (anhydrous)
NPD	nitrogen/phosphorus detector
PAH	polynuclear aromatic hydrocarbon(s)
PCB	polychlorinated biphenyl(s)
PCT	polychlorinated terphenyl(s)
PRI	polar retention index (for polar aromatic compounds, e.g. carbazole derivatives)

QUSO®	synthetic amorphous precipitated silica (silicon dioxide) (Degussa Corp., Telerboro, NJ)
RRI	relative retention index (for specific RRI the first letter is changed to designate the fraction analyzed, e.g. ARI, HRI)
S8	standard containing specific PAH (see glossary)
UV	ultra-violet

Introduction

The Virginia Institute of Marine Science has been involved in the analysis of environmental samples for hazardous organic chemicals for the past twenty years. During this time a wide variety of compounds have been assayed. The research has involved both the analysis of individual compounds and survey type analyses for large classes of compounds. Examples of the former group are the insecticide Kepone, and the antifouling agent tributyltin. Included in the second group are complex classes of compounds such as PAH and organochlorines. In the course of developing methodologies for these classes of compounds, a variety of techniques have been utilized. This manual is intended to provide the analyst with information pertaining to these assay procedures. These methodologies for analysis of semi-volatile organic contaminants should be viewed as a guide. The manual can be followed verbatim and will produce satisfactory results in most instances. However, relatively minor tailoring to a particular application may provide much improved results.

A glossary has been attached (p. 40) which describes various terms (e.g., internal standard) and methodologies (e.g., sample transfer) which are referred to frequently in the text. The reader is advised to consult this section prior to using the methodologies described.

The single instrumental technique that has produced the most information on organic contaminants is capillary GC in combination with an array of detectors. GC is a technique that separates the components of a mixture on the basis of their molecular weight, vapor pressure and polarity. Environmental sample extracts are always complex mixtures, and so it is important to separate them into their component parts to ensure accurate identification and quantification. The most sophisticated of the detectors is MS which allows identification of the greatest number of compounds in a single analysis. The sophistication of MS can also reduce its usefulness because of the need for skilled operators and the cost of the instruments both in terms of purchase and operating expenses. There are many other types of detectors ranging from the almost universal FID to those with increased levels of specificity, e.g. ECD for halogens, ELCD for halogens or nitrogen or sulfur, NPD for nitrogen and phosphorus, FPD for sulfur, tin and other elements which emit light of specific wave lengths when excited in a flame. The combination of the above with computer storage of data provide highly sophisticated means of obtaining and handling very large amounts of data on environmental contaminants.

The combination of mass spectra and GC retention data, i.e. the time that it takes for a compound to elute from the GC expressed either on an absolute or relative basis, provides a highly reliable method of identifying compounds. The inclusion of retention data is necessary because MS does not usually distinguish isomeric structures. GC retention times may vary due to a variety of factors including column length, temperature program, and differences in operator technique. These variations can, however, be compensated for because they affect the retention of a compound with respect to an arbitrarily defined time, i.e. the time a sample is injected. The behavior of the analytes is, however, normally consistent with respect to each other. If, therefore, a method is

devised that relates analytes one to another rather than to time of injection, variations in results are reduced. The method used is to correlate of the retention times of the analytes with the retention times of either known components of a mixture or co-injected standards. This gives a system of relative retention times. Injection of known compounds and mass spectrometry are used to generate a RRI naming table. This can then be used, in combination with the relative retention times of components of an unknown mixture, to provide tentative identification for the compounds analyzed.

The Kovats index (Kovats, 1958) was a RRI designed to correct for differing operating conditions and liquid phases when aliphatic fractions were being analyzed. It was, however, noticed that when aromatic compounds were analyzed the Kovats index did not remain accurate (Bieri *et al.*, 1978; Lee *et al.*, 1979). This led to the introduction of unsubstituted PAH as retention standards (Bieri *et al.*, 1978; Lee *et al.*, 1979). This solved the problems associated with the application of the Kovats index to aromatic compounds and indicated the importance of using retention standards that are similar in structure, and therefore have similar physical properties, to the compounds being analyzed. It is often possible to choose retention standards that are substantial peaks in gas chromatograms obtained from extracts of environmental samples. These standards can often be identified by their elution pattern, thus eliminating the need to inject a separate set of standards.

RRI act as an independent parameter that can be used to: 1) tentatively identify a compound, 2) perform a reverse search of MS data, 3) retrieve information from a data bank or 4) correlate data from separate gas chromatographic injections. For example, suppose a gas chromatographic peak in an aromatic fraction with an ARI of 4804 must be characterized. If the sample has been analyzed by GC only, the ARI naming table (Appendix C) would narrow down the identity of the compound to 2 likely choices: benzo(ghi)fluoranthene and benzo(c)phenanthrene. Analysis of the same aromatic fraction by GC-MS should show a mass spectrum corresponding to one, or a combination, of these compounds, plus any other compound with an ARI at or very near 4804 that may be present but not listed in the ARI naming table. Appropriate computer software would allow the direct access to the MS data by requesting spectra in the vicinity of ARI 4804. In a reverse search, a precise ARI would allow a drastic reduction of the time required to search for the presence or absence of a specific compound in GC-MS files. For isomers which frequently have indistinguishable mass spectra, the ARI quickly allows the identification of the specific isomer if the ARI of that isomer has been determined by a standard. If GC is used to determine concentration, while GC-MS is used to derive qualitative identification information, the ARI is invaluable in correlating the output from the two instruments. Finally, there is the case of the unidentifiable mass spectrum and hence unidentifiable compound. This would result in a data file listing a concentration and an ARI, but not a compound name. Although the identity of the mass spectrum remains unknown, it still can be loaded with its ARI into a library of results as compound X having ARI Y and mass spectrum Z. This compound X can be tracked using these criteria until such time as it can be formally identified and the database updated.

Some of the principle benefits of this analytical method that emphasizes the combination of GC-MS and GC-FID (or other detectors) with RRI and a computerized data storage and retrieval system are given below. Some of these benefits are of immediate practical use; others (f, g, h) are more speculative future benefits.

a. The RRI are easily computed by simple software once the peaks of the retention standards have been identified in the chromatogram. If these standard compounds are not present in a sample, their positions are determined by co-injection with an appropriate reference standard. The positions may also be estimated from a previously (same day) analyzed set of standards.

b. Once the primary data have been converted to the RRI scale, the effects on retention times of small changes in either the gas chromatographic conditions or the properties of the gas chromatographic column are minimized.

c. For GC-MS systems, the combination of mass spectral and RRI data provides an increase in confidence of the identification of a compound over the separate use of RRI and MS.

d. The relative insensitivity of RRI to changes in chromatographic conditions may, in some instances, allow the analyst to save time, effort and expense by utilizing GC data instead of GC-MS data. Alternatively, it may reduce the number of compounds that require identification by MS. This applies especially to areas with a nearly homogenous sample character, where it may be sufficient to analyze only every 5th or 10th sample by GC- MS.

e. The RRI can serve as a preliminary or temporary surrogate name for a compound in cases where the identity cannot be derived from the mass spectrum. Although the identity remains unknown, the combination of the ARI with the mass spectrum is still very specific. Such data can be reprocessed whenever new standard spectra and RRI become available.

f. A RRI/MS data bank could be queried not only by the researcher performing the analysis, but by any agency or research institution possessing the code to the bank. The RRI, along with a code describing the sample and the desired file, could allow one to directly gain access to information on the existence and abundance of a compound in a sample, or geographical sampling area, of interest.

g. The automatic transfer of raw GC, GC-MS or any other data to a computerized data bank could guarantee that no data are lost. Such data should, however, be protected from outside access until the originator of the data has checked them.

h. If organized properly, such a system on a statewide, regional, or national basis would save significant time and effort, prevent duplication and foster the dissemination of information about the occurrence of organic compounds in the environment.

Extraction Methodologies

The essentials of the procedures for the extraction of samples and fractionation of extracts are given in Figures 1 and 2. Details of the methodologies are given below. The glossary (p. 40) should be consulted in conjunction with these methodologies.

Effluent Water

Samples may or may not be filtered prior to extraction depending upon whether differentiation between the soluble and particulate-bound fractions of the compounds of interest is required. Many organic compounds are preferentially associated with the suspended solids present in a sample. This methodology separates the compounds into two categories: the Base/Neutral fraction which contains neutral compounds, such as PAH, and basic compounds, such as the carbazoles. More acidic components, such as phenolic compounds, are extracted as the Acid fraction after the Base/Neutral compounds have been recovered.

1. Samples should be stored in clean solvent rinsed glassware. (See glossary for cleaning, p. 41, and solvent rinsing, p. 43, procedures.) Empty, dry, organic reagent solvent bottles are ideal. Storage should be between 1°C and 5°C and analysis should be performed as soon as possible (EPA-625 requires analysis within 72 hrs.).

2. Transfer 1 liter of the effluent into a 2 liter separatory funnel.

3. Add 6M NaOH until the pH ≥ 12.

4. Add 1 ml solution of internal standard of choice in acetone and shake to mix. (See glossary for internal standard composition and discussion of the use of an external standard.)

5. Add 100 ml DCM.

6. Shake the funnel vigorously for 3 min, venting as required to prevent pressure build up.

7. Let the mixture settle in order to separate layers.

8. Drain the DCM layer (lower layer) into a 500 ml round-bottom flask leaving any emulsion in the funnel.

9. If a severe emulsion occurs, freeze/thaw the sample to break the emulsion and then drain the DCM into the 500 ml flask. (Centrifugation and/or addition of pre-extracted sodium chloride may also be used to break emulsions.)

10. Add 50 ml DCM to the separatory funnel and repeat procedures 6 through 8.

Figure 1. Flow diagram of essentials of sample extraction procedure.

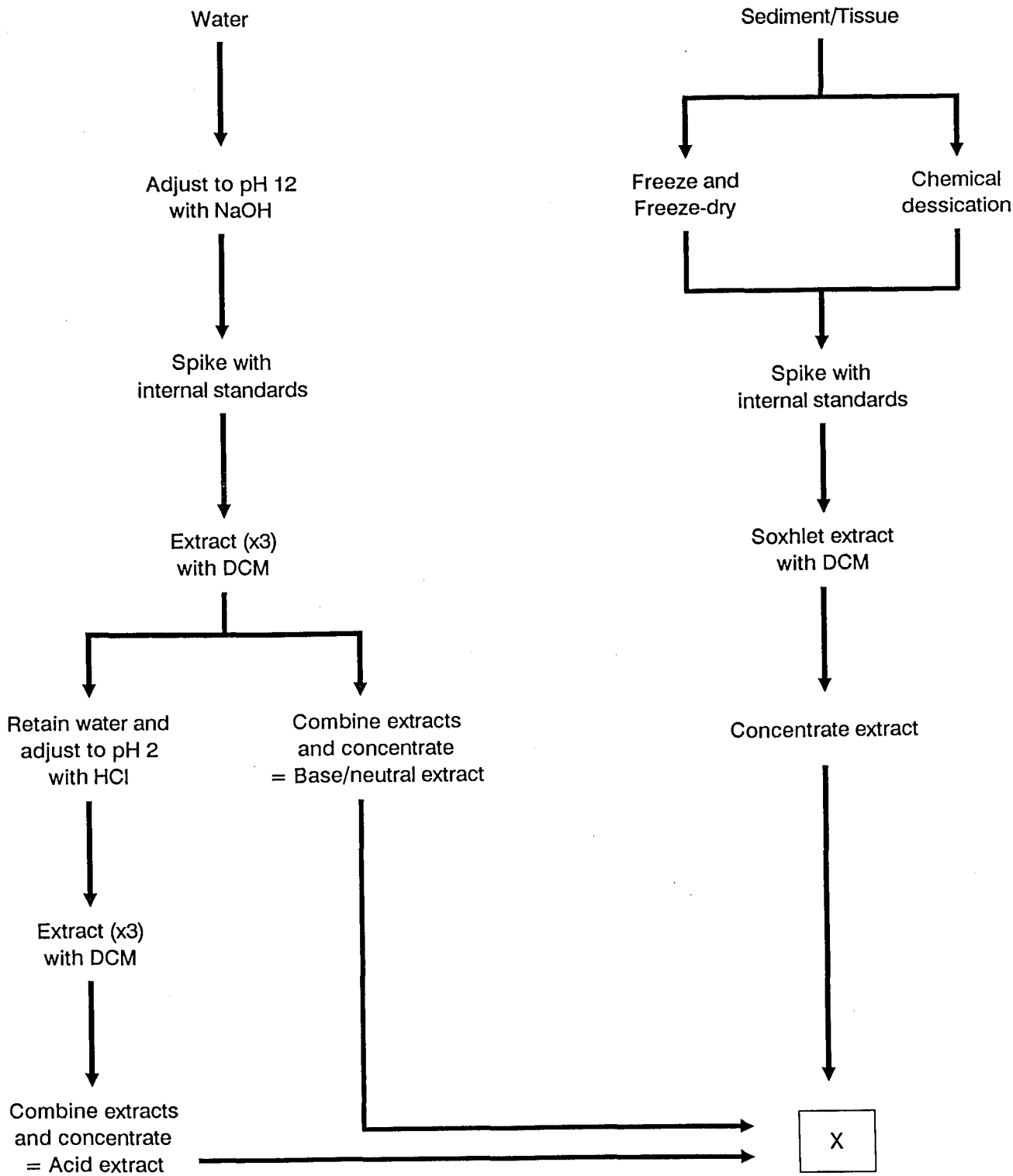
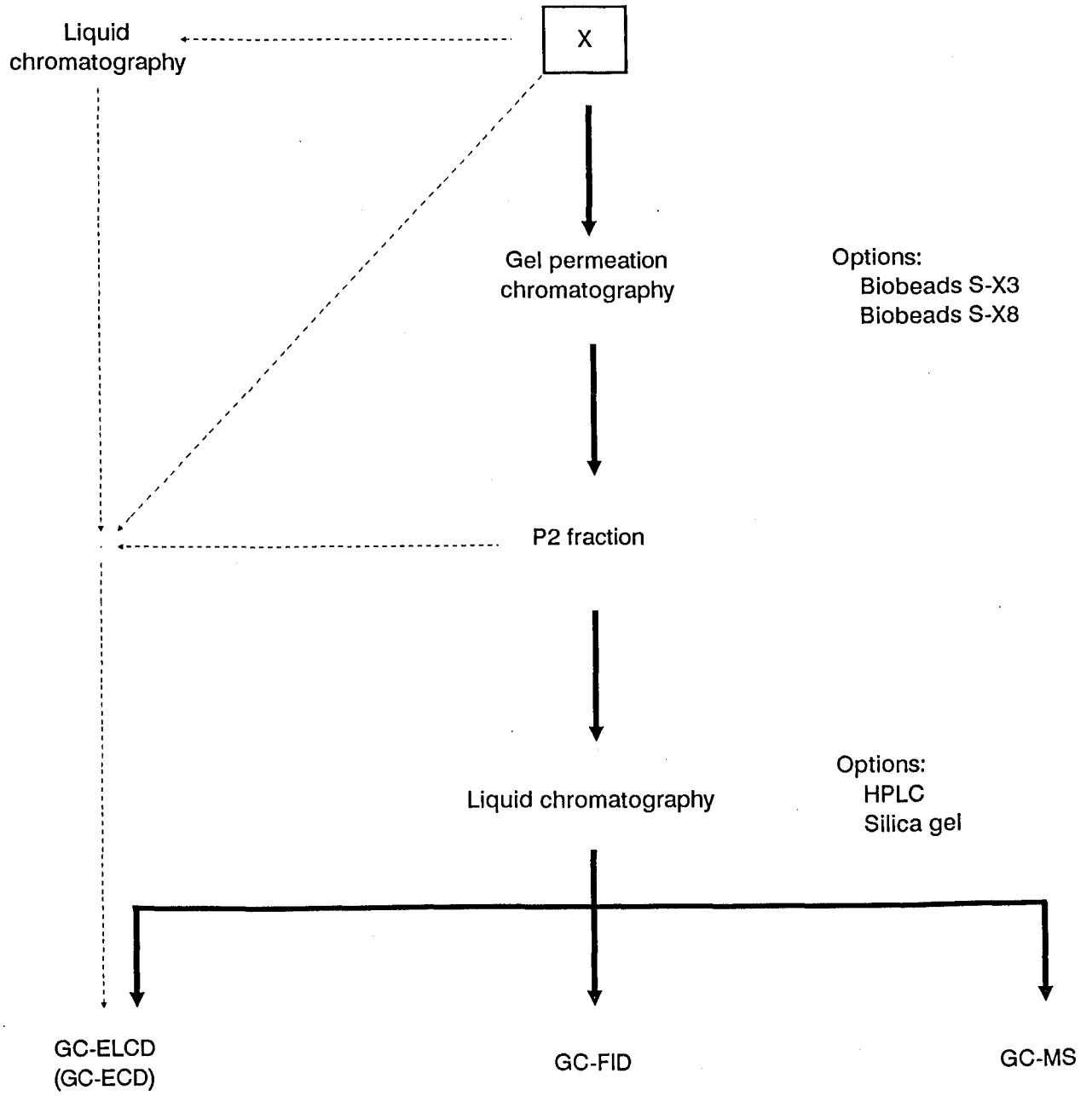


Figure 2. Flow diagram of essentials of extract fractionation procedure.



11. If the emulsion persists, repeat step 9.

12. Add 50 ml DCM to the separatory funnel and repeat procedures 6 through 8.

13. Retain the aqueous layer for further extraction (step 18).

14. Combine the DCM extracts and reduce the volume to ~2 ml by roto-evaporation at 35°C.

15. Transfer (see glossary for description of transfer methodology) the extract to a graduated sample tube.

NOTE: This will increase the volume of the extract.

16. Reduce the volume of the extract to 6 ml under a gentle stream of nitrogen in a water bath at 35°C.

17. This is the Base/Neutral fraction. It is ready for GPC separation (p. 20).

18. Add 6N HCl to the aqueous layer (from step 13) until pH $\leq$ 2.

19. Repeat steps 5 to 16, discarding the aqueous layer afterward.

20. This is the Acid fraction. It is ready for GPC separation (see p. 20).

Sediment

1. Sediment samples should be stored in glass jars (subjected to cleaning as described in glossary). Ordinary Mason jars may be used. Tops should be lined with teflon. Storage should be in a freezer at < -10°C until processing.

2. If wet weight concentrations are required, then the water content of a sample should be determined, at this stage, by drying a sub-sample to constant weight.

3. Samples should be dehydrated either by freeze drying (lyophilization) (steps 4-7) or chemical dessication (steps 8-10).

4. Stored samples are thawed, any water is decanted, and sediments transferred to appropriate containers, refrozen and freeze dried in a suitable freeze drier according to manufacturer's instructions. The freeze drier should be equipped with a continuous nitrogen bleed to avoid back- streaming of the pump oil. Typical operating pressure of the chamber = 400 microns.

5. Break up the dried sediment cake with either a spatula or a mortar and pestle.

6. Transfer the entire sample to a clean jar and shake to homogenize it. At this stage the sample may be stored for an extended period in a freezer.

7. Add 20-60 g of dried sediment to a glass soxhlet thimble equipped with a coarse porosity frit. Place the thimble in a soxhlet apparatus with tongs or large forceps. If desired, a layer of solvent-rinsed glass wool can be placed in the thimble before the sample. This reduces the potential for clogging of the glass frit. (Go to step 11.)

8. For chemical dessication add a 9:1 mixture of Na_2SO_4:QUSO to wet sediment in a 2:1 ratio and mix thoroughly. Usually a maximum of 30g wet sediment is accommodated by this method.

9. Freeze the mixture for 1 hr to assist the dessication process. Afterwards, if necessary, break up the mixture with a spatula or mortar and pestle.

10. Transfer the mixture to a glass soxhlet thimble with a coarse porosity frit. Place the thimble in a soxhlet apparatus with tongs or large forceps. If desired, a layer of solvent-rinsed glass wool can be placed in the thimble before the sample. This will sometimes help to reduce clogging of the glass frit. (Go to step 11).

11. Add the internal standard solution using a volumetric pipet.

12. Wet the sediment with a few ml of DCM to prevent spattering of the sediment by drops of the condensed extraction solvent. Alternatively, place a layer of solvent-rinsed glass wool on top of the sample to reduce spattering of the sediment.

13. Soxhlet extract the sediment for 48 h with 300 ml DCM in a 500 ml round bottom flask at a cycling rate of 4-5 cycles per hour.

14. Remove the flask with the extract, being careful to collect any solvent remaining in the Soxhlet extractor, and roto-evaporate at 35°C to ~2 ml.

15. Transfer (see glossary for transfer methodology) the extract to a 10-15 ml graduated glass stoppered tube with a Pasteur pipet.

NOTE: This will increase the volume of the extract.

16. Reduce the volume to 6 ml under a gentle stream of nitrogen at 35°C.

NOTE: If difficulty is encountered in reducing the volume to 6 ml, or if the sample is extremely viscous, dilute it to a larger volume (e.g., 10 ml) and work with an accurate aliquot in the GPC stage.

17. The sample is ready for GPC separation (p. 20).

Biota

1. Homogenization of samples depends on a number of factors including type of organism, size of sample, number of individuals to be pooled and organs to be analyzed. If sufficient sample size is available, the sample should be thoroughly homogenized to a uniform consistency with a tissue homogenizer. Small samples may be simply cut into pieces.

2. Biota should be either freeze-dried or chemically dessicated as described in steps 3-9 for sediment samples. The dessicant:sample ratio may need to be increased to 3:1 for biota with high water content, e.g. oysters.

3. Place ~5 g of the lyophylized tissue or ~40 g of the dessicant:tissue mixture in the soxhlet thimble. (These quantities are somewhat arbitrarily determined and can be varied as required.) If desired, layers of solvent rinsed glass wool can be placed below and above the sample as noted for sediments (p. 16, steps 6-9 and 11).

4. Place the thimble in the soxhlet with tongs or large forceps.

5. Add an internal standard solution with a volumetric pipet.

6. Soxhlet extract with 300 ml DCM in a 500 ml boiling flask for 48 h at 4- 5 cycles/h.

7. Remove the extraction flask, being careful to collect any solvent remaining in the soxhlet extractor, and roto-evaporate the extract to ~2 ml at 35°C.

8. Transfer the concentrated extract to a 10-15 ml graduated sample tube with a Pasteur pipet.

9. Reduce the sample volume to 6 ml under a gentle stream of nitrogen with the tube in a 35°C water bath. For organisms with a high lipid content, a 6 ml volume may not be reached. If this is the case, record the final volume.

10. The sample is now ready for GPC separation (p. 20).

Sample Fractionation and Cleanup

Fractionation and cleanup steps are important because they isolate the compounds within an extract into categories appropriate for analysis in conjunction with RRI naming tables. This reduces the potential for false identifications. In addition, the removal of high molecular weight and highly polar biogenic compounds extends the life of GC columns. It is, however, not always essential that every step be carried out provided that the analyst is aware of the resulting limitations. For instance, because of the selectivity of a GC detector, it may be possible to go directly from extracting a water sample to analysis on a GC-ELCD if only organochlorines are targeted. The naming convention for the sample fractions is given in Table 1. Individuals familiar with the naming convention used in the first edition of this document will notice that the naming has been streamlined. For comparison purposes, the old naming system has been included.

Table 1

Sample extract fraction naming convention

New Names	Old Names	Examples of compounds present
After Gel Permeation chromatography		
P1	G1/G2	High molecular weight biogenics
P2	G3	Xenobiotics
After HPLC of P2		
P2H1	G3.1	Aliphatics
P2H2	G3.2	Aromatics
P2H3	G3.3/3.4	Polar aromatics
P2H4	G3.5/3.6	Fatty acids other polar biogenics
After Silica gel chromatography of P2		
P2S1	—	Aliphatics
P2S2	—	Aromatics

Note that the new naming system is sequential according to the sequence of sample fractionation. The assigned fraction name can be extended if further fractionation is conducted, e.g. if a Florisil column is used to purify a P2H2 sample, then the fractions from the Florisil chromatography would be P2H2F1, P2H2F2, etc.

GPC

1. More detailed information, beyond that given below, concerning items such as GPC equipment used, column packing and column calibration methodology is given in Appendix A (p. 48).

2. The GPC separation of the samples is accomplished on columns containing either of two GPC column materials, Biobeads S-X8 or Biobeads S-X3. Both materials consist of a styrene-divinylbenzene copolymer. The solvent systems are DCM for Biobeads SX-8 and DCM or a 1:1 mixture of DCM and cyclohexane for Biobeads S-X3. In our laboratory Biobeads S-X3 is now the resin of choice because of improved resolution of biogenic compounds, particularly lipids, from the xenobiotics of interest.

3. Adjust the sample volume to exactly 6 ml (for 5 ml loop), draw the sample into a syringe and inject the sample into the loop.

> NOTE: Due to the dead volume of the sampling valves and the injector, all of the sample does not enter the loop. Hence, a volume larger than the loop volume must be injected to ensure that the loop is filled. This injection volume must be determined for the particular injection system used, and will be the final volume in the sample tube. The GPC correction factor (f_{gpc}) is the loop volume divided by the total volume of the extract and is the actual fraction of the sample introduced to the column. The use of internal standards accounts for this loss when concentrations are calculated. The f_{gpc} is required for calculation of internal standard recoveries.

4. For lipid rich tissues no more than 1 gm of lipoidal material should be placed on the column. Record the exact volume placed on the column. The use of internal standards will account for the changes in volume placed on the column. The fraction of the sample injected into the GPC is necessary for calculation of the recovery of the internal standard.

5. Fractions should be collected according to individual column calibration.

> NOTE: For an S-X8, the first fraction collected is P1 and contains some compounds which may be of interest (see Appendix B, p. 51). At the present time the distribution of compounds in Appendix B on an S-X3 column is being evaluated.

6. After the end of elution of the P2 fraction, inject a full sample loop of a wash solution of 20% methanol in DCM and rinse the column with a volume of DCM equal to the whole amount used for P1 and P2 fractions. Rinses such as this may not be applicable with new instrumentation such as automated GPC.

> NOTE 1: Most elemental sulfur extracted from sediments elutes 10 ml after the S8 is entirely eluted; therefore, no specific sulfur cleanup is required. Sulfur is

eluted in the wash stage. For methodology on removal of sulfur using activated copper see "sulfur" in the glossary (p. 45).

NOTE 2: The methanol wash is designed to remove adsorbed material not eluted by DCM alone.

CAUTION: More than 20% methanol in the wash solution may change the column performance.

Liquid Chromatographic Cleanup and Fractionation

Two options are available for the liquid chromatographic fractionation of the P2 fraction.

Option 1: HPLC fractionation of P2 fraction.

The HPLC system must be capable of 3 solvent delivery. Figure 3 shows the basic layout of the HPLC.

Column = semipreparative cyano-amino bonded to 10u silica normal phase column (e.g. 250 mm long x 9.4 mm i.d. Whatman: Magnum 9).

Guard Column = 60 mm long x 2 mm i.d. packed with cyano-amino phase bonded to 30-38u glass beads.

Detector = UV absorbance (λ = 254 nm).

Solvent program = see Figure 4.

1. Add 1 ul of toluene to the sample and inject onto the system. If a Rheodyne type injector is being used, the injection procedure should follow the methodology recommended by the manufacturer, i.e., injection of < 0.5x of the loop volume or injection of > 2x loop volume. The fraction of sample injected onto the HPLC (f_{hplc}) is required for calculation of internal standard recovery. If all the sample reaches the column, then f_{hplc} = 1.

2. Solvent A = hexane, Solvent B = DCM, Solvent C = methanol.

3. Program the HPLC according to Figure 4, collecting the fractions as indicated.

> NOTE: The distribution of compounds into the fractions being collected should be checked with standards.

4. The P2H1 fraction contains mostly aliphatic compounds. These compounds are generally not significant toxicologically. The P2H2 fraction contains slightly more polar compounds including PAH, polycyclic hetero (sulfur) aromatics, PCB, pesticides, etc. The P2H3 fraction contains moderately polar compounds such as carbazoles. The P2H4 fraction contains fatty acids and other polar biogenic compounds (see Table 2).

5. Fractions P2H1 and P2H4 are generally not analyzed. They can be reduced in volume and archived or discarded. Fractions P2H2 and P2H3 are reduced in volume, by roto-evaporation at 35°C, to ~2 ml and transferred to 10-15 ml calibrated glass tubes.

6. Volumes of P2H2 and P2H3 are reduced under a gentle stream of nitrogen at 35°C. Final volume is usually 0.2 ml.

7. Samples are ready for GC and/or GC-MS analysis.

Figure 3. Basic layout of a HPLC system.

* sample injection port.

___ valve position for sample loading

- - - valve position for sample injection (rotate counter-clockwise)

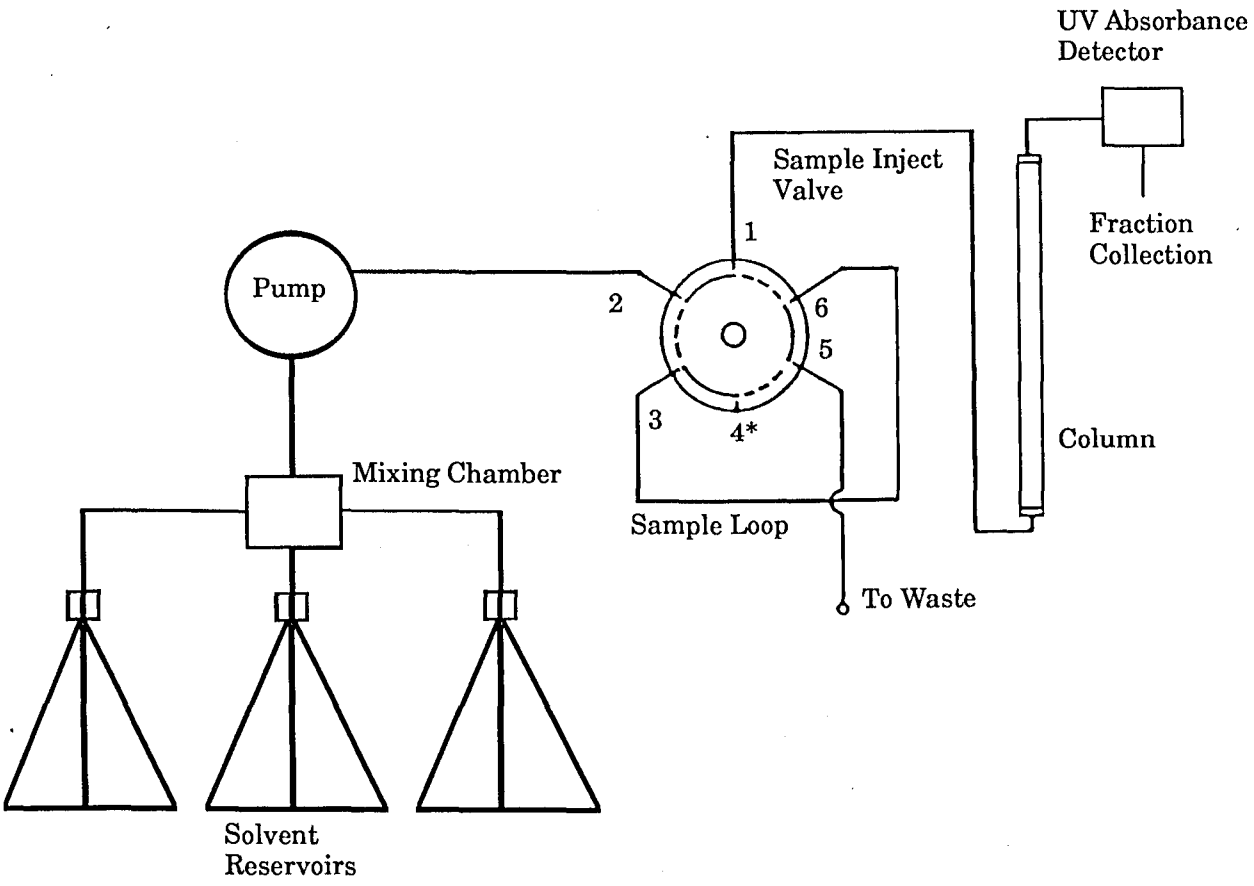


Figure 4. HPLC solvent program for fractionation of GPC P2 fraction.

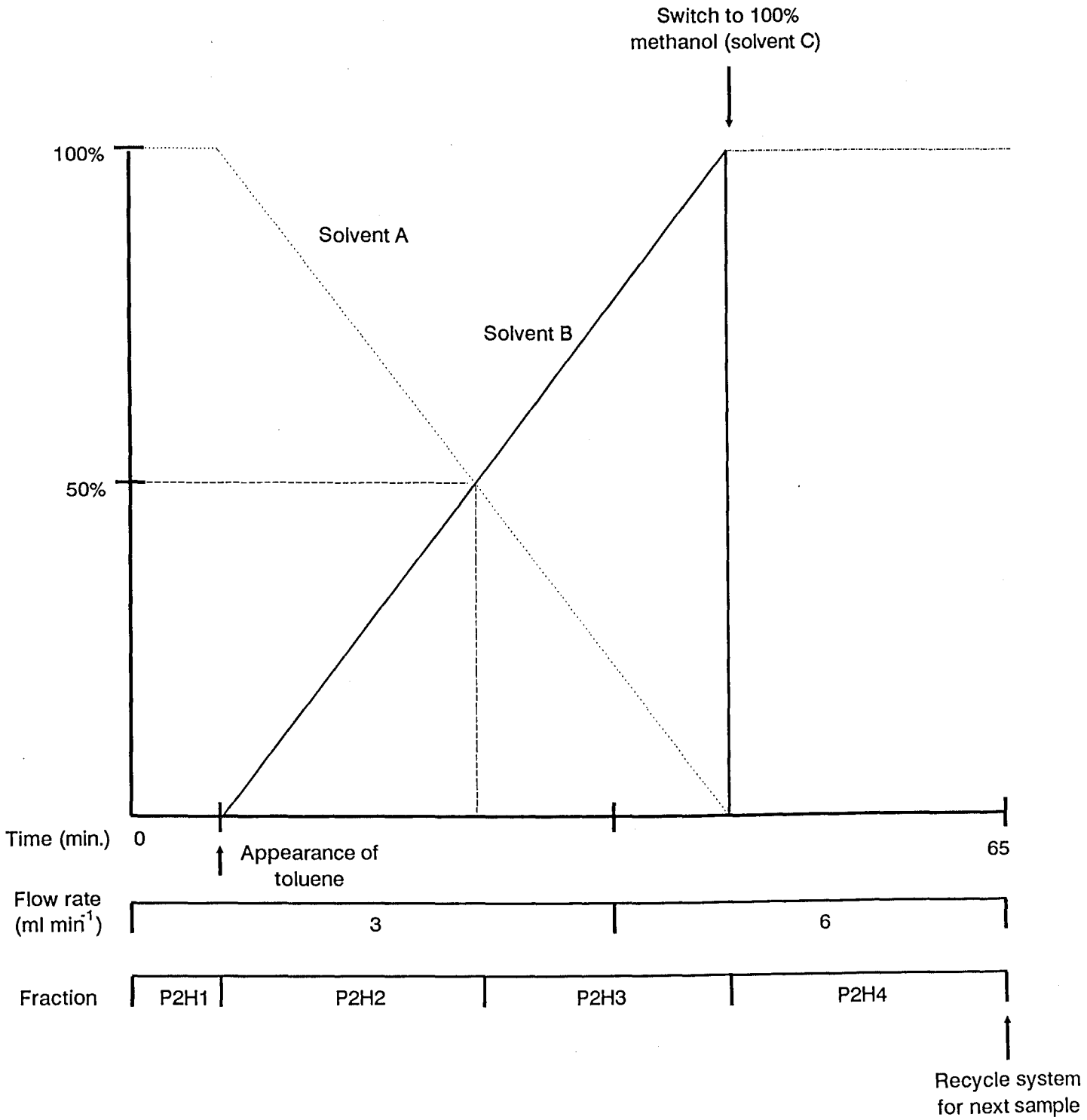


Table 2

Compound distribution resulting from HPLC fractionation

Fraction	Compound Class	Representative Compound Groups
P2H1	Aliphatic	Parafins
P2H2	Aromatic	PAH PCB DDT, DDE and DDD Mononitro-PAH
P2H3	Polar	Cyano PAH Ketones Amines Carbazoles Aldehydes Hydroxy PAH Azaarenes Phenols
P2H4	Highly Polar	Fatty acids (and other biogenic compounds)

Option 2: Silica gel fractionation of P2 fraction.

The silica gel should be pre-extracted before use.

> NOTE: Prior to use of this method, sample must be solvent exchanged into hexane (see glossary for method).

1. Slurry pack a column with 10g silica gel using hexane as the mobile phase. Do not allow the column to dry out.

2. Pass 10 ml hexane through column to settle column bed.

3. Place sample (1 ml volume) on column.

4. Rinse sample tube with 0.5 ml hexane and add to column.

5. Elute column with 25 ml hexane (P2S1 fraction).

6. Elute column with 40 ml of 80:20 (v/v) hexane:DCM (P2S2 fraction). This fraction contains the aromatic compounds and organohalogens such as the PCB.

7. The P2S1 and P2S2 fractions are reduced in volume to ~2 ml at 35°C.

8. The P2S1 and P2S2 fractions are transferred to glass stoppered graduated tubes and reduced in volume to 0.2 ml, while standing in a water bath at 35°C under a gentle flow of nitrogen.

9. The P2S2 fraction is analyzed for the aromatic compounds of interest by GC-FID and the organohalogens by GC-ELCD.

NOTE 1: The behavior of polar compounds, e.g. carbazoles, on the column has not been verified. They do not elute with 20% DCM in hexane. Further studies are required concerning the fractionation of the polar compounds on silica gel.

NOTE 2: There may be a need to remove inorganic sulfur, particularly if sediments are purified by this method. See glossary for technique (p. 45).

Gas Chromatography

GC is used for the final separation in this analytical protocol. Figure 5 shows the basic layout and necessary gas supplies for a GC system. Various detectors may be used with the GC. FID is used because of its response to carbon and thus widespread applicability. MS is used because of its ability to provide structural information. ELCD is our detector of choice for organochlorines when maximum sensitivity is not required. The sensitivity of the ELCD is not as high as for the ECD. However, the selectivity is much improved. If ECD is used, additional cleanup steps may be required, beyond those given, to achieve a sample extract that can be effectively analyzed. One step may be the use of Florisil columns (see glossary, p. 40).

There are a number of other GC detectors that can be used depending on the type of sample to be analyzed. Such applications, combined with GC-MS data, can result in the creation of other RRI naming tables. For instance, complex mixtures of nitrogen containing compounds might be analyzed on a nitrogen-phosphorus detector, while sulfur containing compounds may be analyzed with a flame photometric detector or a reconfigured ELCD.

Typical GC Configuration

Column: 30 m long x 0.32 mm i.d. fused silica capillary 0.25µm liquid phase film thickness.

Liquid phase: 95% methyl-, 5% phenyl-silicone, crosslinked (e.g. DB-5).

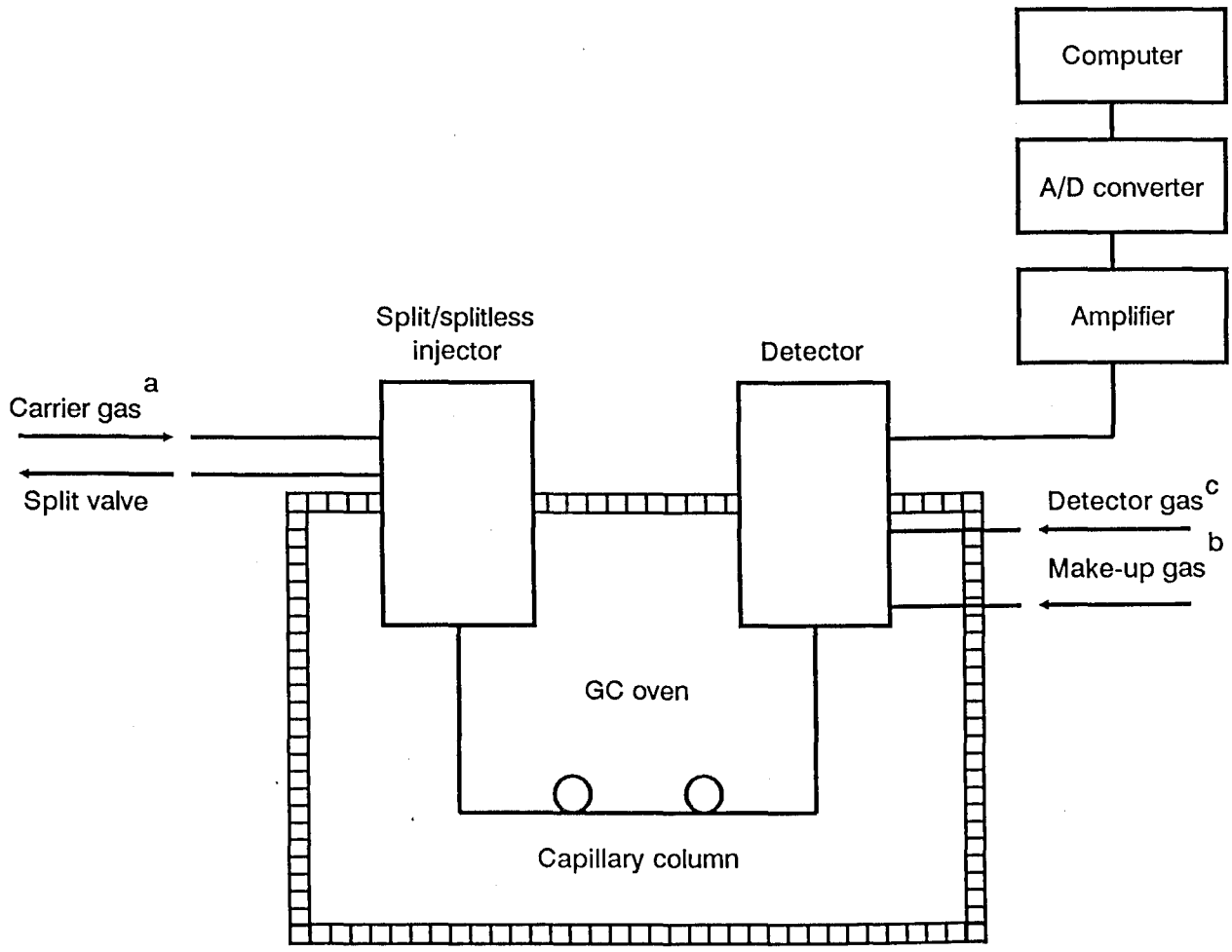
Carrier gas: helium

Injector: split/splitless

NOTE 1: The length, i.d. and liquid phase film thickness can all be varied as necessary to achieve the required separation. The RRI system will accommodate these changes. However, the liquid phase composition must not be altered as this may affect the relative retention times of individual compounds.

NOTE 2: The use of hydrogen as the carrier gas may cause changes in relative retention times of certain compounds, particularly the hexachlorocyclopentadiene based insecticides.

Figure 5. Basic layout and gas supplies for a GC system.



a. Carrier gas.	Helium.	(Hydrogen may be used but may require changes in the RRI naming table to avoid inaccuracies in identifications due to changes in RRI caused by the different properties of this carrier gas.)
b. Make-up gas.	FID.	Helium.
	ELCD.	Helium.
	ECD.	Nitrogen or Argon/Methane (5-10%).
c. Detector gases.	FID.	Hydrogen and air.
	ELCD.	Hydrogen.

GC-FID Operating Conditions

Injector temperature: 300°C - 310°C

Detector temperature: 300°C - 310°C

Initial column oven temperature: 75°C

Final column oven temperature: 310°C

Temperature program rate: 6°C min^{-1}

Final column oven temperature hold time: 10 min.

Injection technique: solvent effect (cold trapping)

GC-ELCD Operating Conditions

Injector temperature: 300°C

Detector temperature: 300°C

Detector reaction temperature: 950°C

Initial column oven temperature: 90°C

Final column oven temperature: 310°C

Temperature program rate: 4°C/6°C min^{-1}

Final column oven temperature hold time: 10 min.

Injection technique: solute focussing

NOTE: The initial temperature of the GC oven is determined by the nature of the solvent plug and the injection technique. Thus for a solvent effect (cold trapping) injection, the temperature should be at least 10°C below the boiling point of the solvent plug. For a solute focussing injection, the initial temperature of the GC oven should be 10°C above the boiling point of the solvent plug.

Sample Introduction (GC)

1. Splitless injection should use the hot needle technique and be at a moderate, steady plunger speed.

* For GC-FID, follow steps 2-4.

2. Draw 1 ul of toluene into the syringe next to the plunger, then leave a small air gap (0.2-0.4 ul) and draw an accurately measured aliquot (1-3 ul) of sample.

3. Inject the sample.

4. When the solvent elutes, open the split valve, begin the temperature program and start the data collection.

* For GC-ELCD, use steps 5-8.

5. It is essential that there be no DCM present in a sample prior to injection onto the GC-ELCD or other halogen selective detectors. If DCM is present, it should be removed by solvent exchange (see glossary, p. 44).

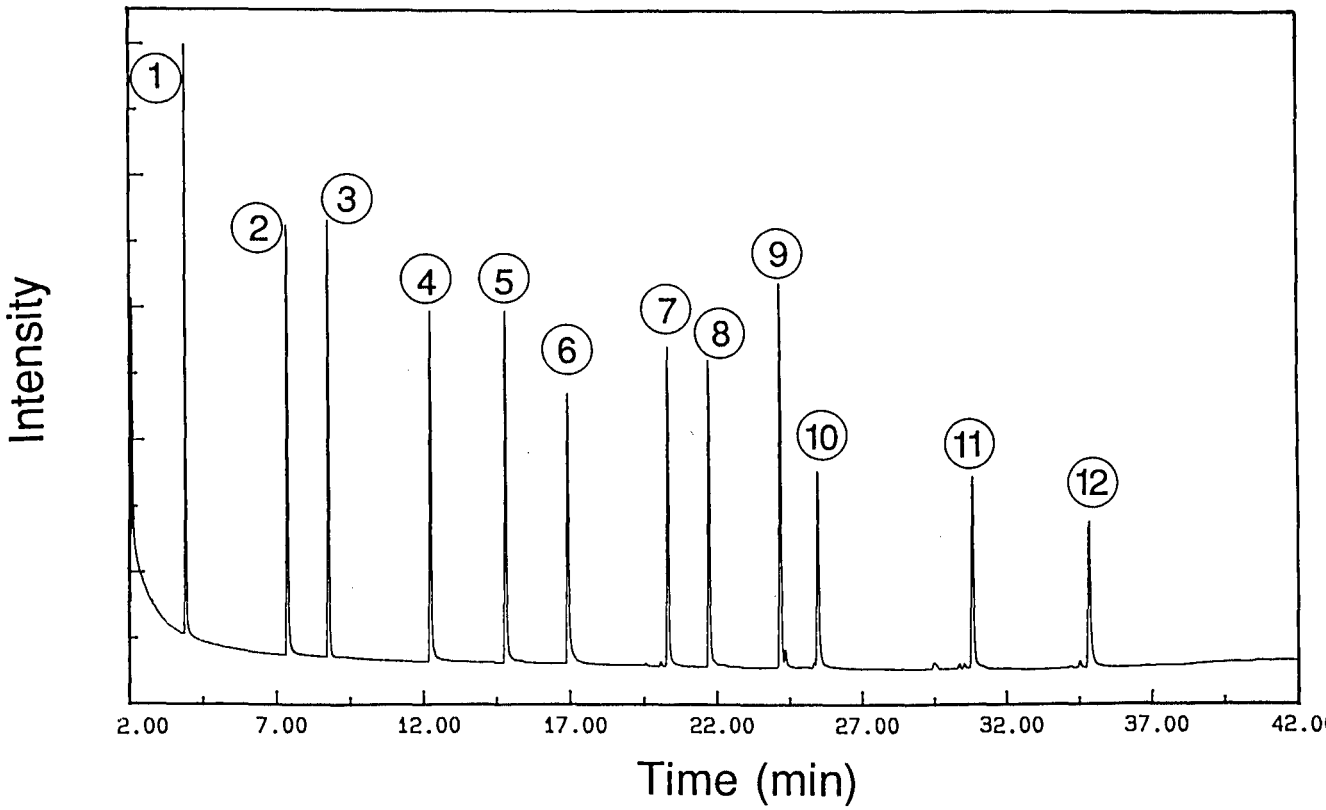
6. Draw 1 ul of hexane (not toluene) into the syringe next to the plunger, followed by a small air gap (0.2-0.4 ul) and a measured aliquot (1-3 ul) of sample.

7. Inject the sample and open the detector vent valve. This valve prevents excessive solvent from passing through the detector and reduces the potential for carbon build-up.

8. When the solvent elutes, open the split valve, close the vent valve and start the temperature program and the data collection. The split valve can be closed again 3-4 minutes into the temperature program in order to conserve helium.

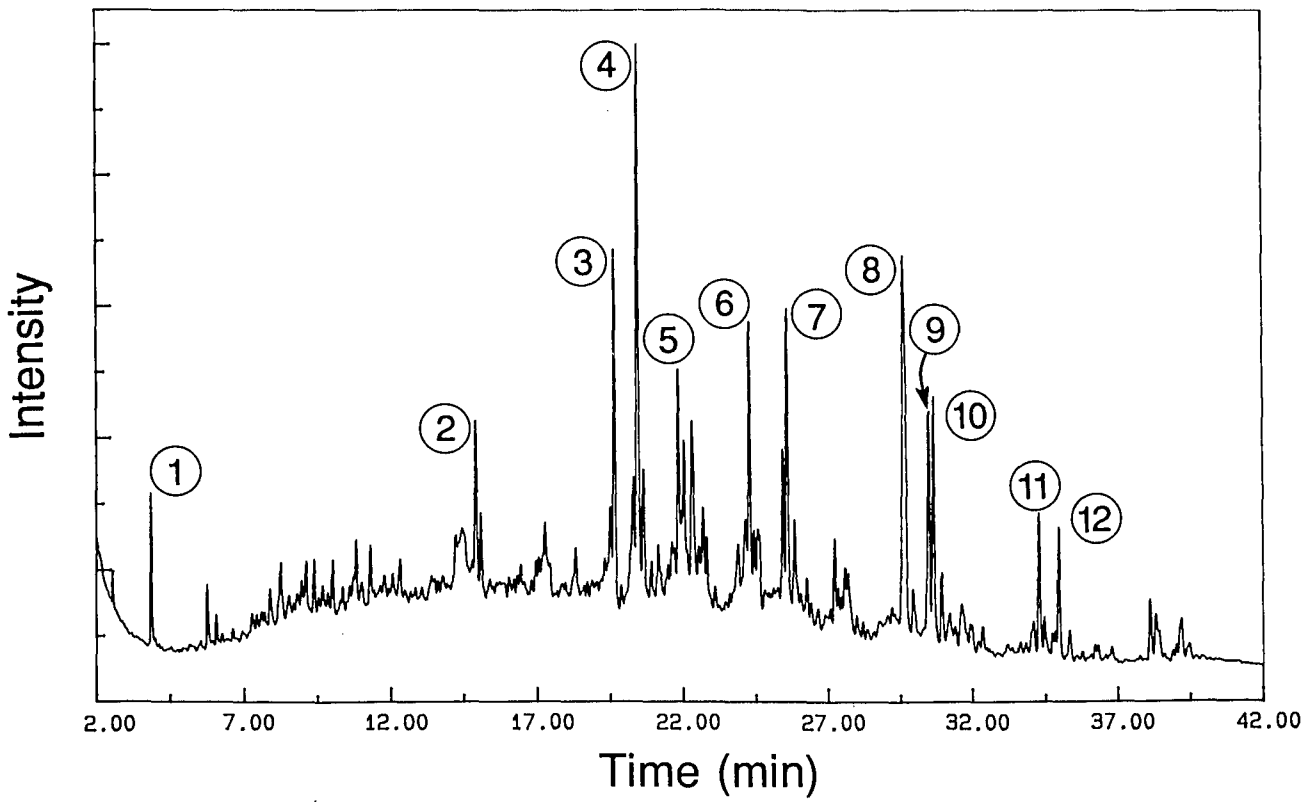
Typical chromatograms obtained in these types of analyses are shown in the next three figures. Figure 6 is a GC-FID chromatogram of standards. It includes S8, 1,1′-binaphthyl, perinaphthenone, tribromophenol and terphenyl. Figure 7 is a GC-FID chromatogram of the aromatic fraction (P2H2) of an extract of sediment contaminated with creosote. The use of the GC-ELCD is shown in Figure 8 which is a chromatogram of an extract of an effluent from an industrial wastewater treatment plant that contained polychlorinated biphenyls (Aroclors 1254 and 1260).

Figure 6. GC-FID chromatogram of standards (S8, 1,1′-binaphthyl, perinaphthenone, tribromophenol and terphenyl).



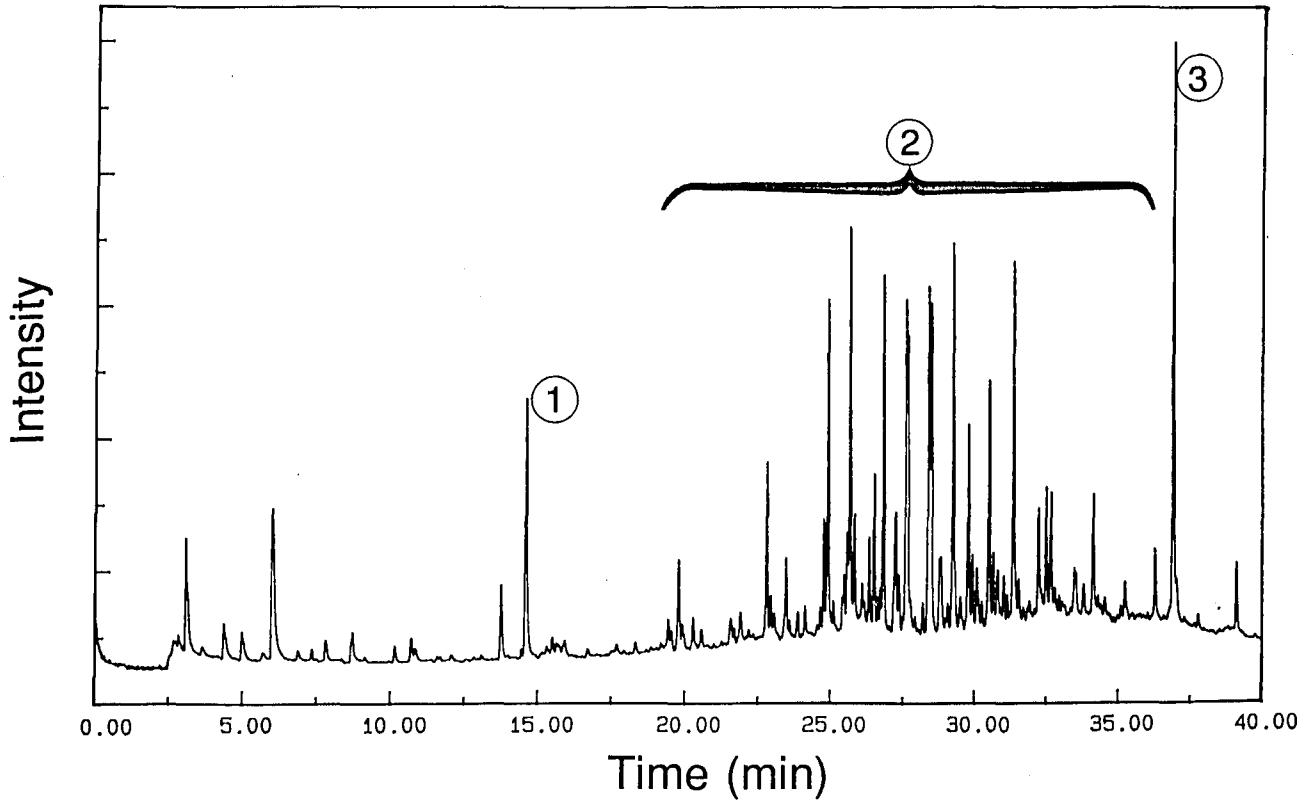
1. Napathalene
2. Biphenyl
3. Hexamethylbenzene
4. Tribromophenol
5. Phenanthrene
6. Perinaphthenone
7. Pyrene
8. Terphenyl
9. 1,1′-Binaphthyl
10. Chrysene
11. Perylene
12. Benzo(ghi)perylene

Figure 7. GC-FID chromatogram of the aromatic fraction (P2H2) of an extract of sediment contaminated with creosote.



1. Naphthalene
2. Phenanthrene
3. Fluoranthene
4. Pyrene
5. Terphenyl (std.)
6. 1'1'-Binaphthyl (std)
7. Chrysene
8. Benzo(b/j/k)fluoranthene
9. Benzo(e)pyrene
10. Benzo(a)pyrene
11. Indeno(1,2,3-cd)pyrene
12. Benzo(ghi)perylene

Figure 8. GC-ELCD of polychlorinated biphenyls extracted from the effluent of an industrial wastewater treatment plant (contains primarily Aroclors 1254 and 1260).



1. Pentachlorobenzene (std.)
2. PCB
3. Decachlorobiphenyl (std.)

Mass Spectrometry

Ionization:

Electron ionization is suitable for most compounds except PCB and other highly halogenated compounds. Usually these compounds are present at concentrations which are lower than can be detected by this technique.

Negative chemical ionization is suitable (electron capture mode, methane = moderator gas) for PCB and other highly halogenated compounds.

Positive chemical ionization may be useful if the electron ionization spectra do not produce an obvious molecular ion. Regent gases commonly used are methane, isobutane and ammonia.

For electron ionization the MS source is operated at ~250°C. For chemical ionization (positive or negative) a typical source temperature is 100°C.

GC configuration:

As for GC-FID operation.

GC-MS interface:

An open-split interface is the interface of choice because it allows the GC column to elute at atmospheric pressure, thus reducing any effects of the interface on the elution times of the analytes. Other means of interfacing, such as running the GC column directly up to the source of the MS, can be used without difficulty provided the analyst is aware that minor changes in retention times can occur. An open-split interface is generally located inside the GC oven and operates at the temperature of the oven. The transfer line between the GC and MS is maintained at ~250°C.

NOTE: Temperatures of the ion source in the electron ionization mode and of the GC-MS interface can be varied depending on instrument limitations and the compounds being analyzed. They may need to be raised for higher molecular weight and less volatile compounds, but care should be taken to avoid sample degradation.

Quantification

In our laboratory it is our normal practice to quantify compounds with respect to internal standards. External standards are discussed in the glossary (p. 44). The internal standards are:

1,1-binaphthyl - for the aromatic fraction including the base/neutral aromatic fraction for water extracts.

Tribromophenol - for the acid fraction of water extracts.

Perinaphthenone - for the polar aromatic fraction.

Decachlorobiphenyl - for halogenated compounds.

An assumption is made for the GC-FID analyses that the response factors for all compounds are the same as that of the internal standard. This is not ideal, however, for many of the compounds encountered, e.g. PAH, it is a good approximation. For a round-robin comparison of analysis of hydrocarbons in marine sediments see MacLeod *et al.* (1982). This laboratory was "Laboratory D" in the PAH analysis. For compounds where a high degree of accuracy is required, extraction and purification methodologies specifically targeted at that compound are normally developed.

For the GC-ELCD analyses the response of the detector is directly proportional to the number of halogen atoms present on a molecule. These responses are also dependent upon which halogen is present. For GC-ECD, detector response is dependent on both the halogen content and the stereochemistry of the molecule. As such, it is necessary to calculate a response factor for each compound analyzed if quantification of individual compounds is to be achieved.

The equation for the concentration of compound X is

$$C_x = \left(\frac{M_{is}}{A_{is}}\right) * A_x * \frac{1}{S} * \frac{1}{rf}$$

where

C_x = concentration of compound X (in ng/g)

M_{is} = mass of internal standard added at the beginning of the extraction process (in ng)

A_{is} = peak area of internal standard

A_x = peak area of compound X

S = quantity of starting material (in g)

rf = response factor (1 on FID, variable on ELCD and other detectors).

The response factor (rf) is determined by injections of a mixture of the target compound and the internal standard. These injections are of pure compounds. They have not been subject to the extraction and purification processes.

$$rf = \frac{\frac{A_x}{M_x}}{\frac{A_{is}}{M_{is}}}$$

where

A = peak area

M = mass of compound injected

x = target compound

is = internal standard

NOTE: In all the above calculations it may be possible to substitute peak height for peak area. The use of height is usually less accurate because it does not accurately reflect peak degradation.

Detection Limits

In general, for compounds quantified by GC-FID, the methodologies described have detection limits of 1 ug/l in water and 5-10 ug/kg for sediment and biota. For the organohalides using GC-ELCD detection limits are improved by an order of 10^3.

Recovery of an Internal Standard

The recovery of an internal standard is considered to be equal to the recovery of the target compounds from the matrix being extracted. This is not an ideal system because it does not consider differences in binding between the internal standard, which is spiked into the matrix immediately prior to extraction, and the compounds of interest which have had longer contact with the matrix and may have become more highly bound to the medium. It should be noted that there is no ideal way of determining the absolute recoveries of these compounds and, therefore, concentrations based on the recovery of the internal standard must suffice.

To determine the recovery of an internal standard, a recovery standard (see glossary, p. 45) must be either co-injected into the GC or spiked into the sample immediately prior to injection into the GC. The following equations are used to determine the percent recovery of the internal standard.

1. Calculation of amount of internal standard injected on column assuming a theoretical recovery of 100%.

$$M_{is\text{-}initial} * f_{gpc} * f_{hplc} * f_{inj} = M_{is\text{-}calc}$$

where:

$M_{is\text{-}initial}$ = mass of internal standard added to sample.

f_{gpc} = fraction of sample extract injected into the GPC. (This will be 1 if all sample is injected.)

f_{hplc} = fraction of GPC subfraction injected into the HPLC. (This will be 1 if all sample is injected.)

f_{inj} = fraction of final extract injected into GC, e.g. 0.01 if 1 ul is injected out of a final sample volume of 100 ul.

$M_{is\text{-}calc}$ = mass of internal standard injected on column assuming 100% recovery.

2. Percentage recovery of internal standard.

$$\% \text{ recovery} = \frac{A_{is}}{M_{is\text{-}calc}} * \frac{M_{rs}}{A_{rs}} * rf_{is} * 100$$

where:

A_{is} = peak area of internal standard.

$M_{is\text{-}calc}$ = ng of internal standard injected on column assuming a theoretical 100% recovery. (From equation 1 above.)

M_{rs} = ng of recovery standard injected on column.

A_{rs} = peak area of recovery standard.

rf_{is} = response factor for internal standard with respect to recovery standard.

The response factor for the internal standard (rf_{is}) is determined by injections of a mixture of the internal standard and the recovery standard. These injections are of pure compounds. They have not been subject to the extraction and purification process.

$$rf_{is} = \frac{\frac{A_{rs}}{M_{rs}}}{\frac{A_{is}}{M_{is}}}$$

where:

A = peak area

M = mass of compound injected

rs = recovery standard

is = internal standard

NOTE: Peak height may be used instead of area in certain circumstances, though this may prove to be less accurate as changes in peak shape are not effectively monitored when peak height is used.

Calculation of Relative Retention Indices

1. The RRI of a compound is defined in relation to the adjacent marker compounds.

2. The marker peaks may or may not be found in the sample. If they are not present in the sample, then they should be coinjected.

3. The RRI of a peak X can be calculated with the formula:

$$RRI_X = \left[\frac{t_X - t_{MP}}{t_{MF} - t_{MP}}\right] \cdot 1000 + RRI_{MP}$$

where:

t_X = retention time of peak X

t_{MP} = retention time of last marker compound preceeding X

t_{MF} = retention time of next marker compound following X

RRI_{MP} = RRI defined for last marker preceeding X

4. Tentative peak identification can be made at this point by comparing the calculated RRI with established RRI data.

5. Examples of RRI naming tables are provided in Appendices C-H. It must be emphasized that these are examples and should be used in conjunction with mass spectrometric data. The tables can then be modified or new ones created to suit a particular environment or set of samples.

Glossary

Blank - Procedural (extraction/reagent)

The procedural blank is a blank extracted at the same time as the samples of interest as a check on contamination during sample preparation. A procedural blank should be extracted with each set of samples and accompany that set of samples through all the analytical stages. For water samples use "Milli-Q" water. For solid samples use pre-extracted sand.

Blank - Trip

The trip blank is a blank that is taken to the site from which samples of interest are obtained. The blank is then manipulated in the same way as are the samples, returned to the laboratory and extracted in parallel with the samples. For water samples use "Milli-Q" water. For solid samples use pre-extracted sand.

Control

A control is a sample that is collected in parallel with the samples of interest. This sample should be obtained from a site that does not include or has low levels of the contaminants being examined. The physical composition of the control should match that of the samples as closely as possible.

Florisil Column Purification

Methodology should be tailored to the compounds of interest, but a good starting point is the EPA methodology for organochlorine analysis. The reader should consult EPA publications for details of the method given here in brief. Florisil, which may require pre-extraction, should be activated at 130°C. A column should be dry-packed with 20g and then flushed with 60 ml hexane at a flow rate of ~5 ml min^{-1}. The column must not dry out. The sample should be placed on the column with appropriate rinsing of the sample container. Compounds are eluted first with 200 ml of 6% diethylether in hexane followed by 200 ml of 15% diethylether in hexane. PCB and many pesticides elute in the first fraction while some more polar pesticides such as endosulfan and endrin elute in the second fraction.

Gases

All gases used should be of high purity. Considerable time, money and effort can be lost if less than the best quality gases are used. This is particularly the case for GC where impure gases can lead to improper instrument performance and excessive down time. From our experience, gases that are not of sufficiently high quality can lead to irreversibly contaminated gas lines and GC components. Recommended purities of gases are provided in Table 3.

Table 3

Recommended purities of gases used in analytical procedures

Gas	Nitrogen Pre-Purified	Nitrogen Ultra High Purity	Hydrogen Pre-Purified	Hydrogen Ultra High Purity	Air (dry)	Helium High Purity	Helium Extra High Purity
Usage	Solvent Evaporation	ECD	FID FPD NPD	ELCD	FID FPD NPD	FID ECD FPD NPD	ELCD GC-MS
Purity (%)	99.998	99.999 (Excluding Ar)	99.99	99.999	Dry** Breathing Quality	99.995	
Maximum Impurities ppm							
H_2O	< 3	< 3	< 5	< 3	< 3	< 5	*
O_2	< 5	< 1	< 10	< 2			*
N_2				< 6		< 5	*
THC		< 1		< 1			*

*Less than 5 ppm total impurities

**Dew point = -92°F

No gas should contribute organic residues to samples or instrument systems. The grade names given in the table are as per Union Carbide. Grade names are not universal. The grading of the extra high purity helium is that of the U.S. Department of the Interior, Bureau of Mines (Amarillo, TX).

Glassware - Cleaning

All glassware, and other items coming in contact with the samples or their extracts, such as stainless steel trays and spatulas, must be rigorously cleaned prior to use. Cleaning consists of:

a) rinsing with the last solvent that the item contained;

b) washing in hot water with laboratory detergent (preferably non-phosphate);

c) soaking overnight in 5% solution of Contrad-70 (we have found this to be a very effective cleaning product. It can be re-used a number of times.);

d) rinse thoroughly in running tap water;

e) rinse with 3N HCl;

f) rinse thoroughly with deionized water (minimum of 3 rinses);

g) rinse with acetone;

h) allow to air dry;

i) bake at 100°C;

j) bake non-volumetric items at 400°C;

k) cap all items with solvent rinsed (see p. 43) aluminum foil prior to storage;

l) immediately prior to use all items should be solvent rinsed (p. 43).

HCl

Formulate a 6M (6N) solution of reagent grade HCl with "Milli-Q" or equivalent water. This can be accomplished by making a 2:1 (50%) dilution of the concentrated acid. Safety note: Remember to add the acid to water and not the reverse. If "Milli-Q" or equivalent water is not available, the water should be pre-extracted prior to use.

NaOH

Formulate a 6M (6N) solution of reagent grade NaOH with "Milli-Q" or equivalent water ($240gL^{-1}$ = 6M). If "Milli-Q" water is not available, the water should be pre-extracted with DCM prior to use. Purities of solid NaOH do vary, so this should be checked by extraction and analysis of a solution during method development.

Na_2SO_4

Use reagent grade anhydrous Na_2SO_4. This must be pre-extracted by soxhlet extraction with DCM prior to use. This step is essential to avoid contamination of samples. As an alternative to pre-extraction, it may be possible to achieve the necessary cleanup of the Na_2SO_4 by pre-igniting it at 550°C for 1 h.

Plastic

Except for particular applications where their suitability has been assessed, plastics should not be used at any stage of sample preparation because of the compounds, e.g. phthalates, that may be leached out of them. Teflon is the only medium, apart from glass and stainless steel, which should be allowed contact with the sample, although this should also be kept to a minimum.

QUSO

Synthetic amorphous precipitated silica (silicon dioxide), should be purified by being pre-extracted or pre-ignited at 550°C for 1 h prior to use.

S8

A standard containing hexamethylbenzene and the following PAH: naphthalene, biphenyl, phenanthrene, pyrene, chrysene, perylene and benzo(ghi)perylene. When PAH are being analyzed it is used extensively, as a GC retention standard, and as GPC and silica gel column calibration marker, etc. The concentrations of the components are 20 ng/ul (20 µg/ml).

Sample Evaporation

Samples should not be allowed to go to dryness at any stage of sample preparation because this may result in analyte loss. Samples are evaporated by purging with nitrogen while standing in a water-bath. The temperature of the water-bath should be set as low as practicality allows, e.g. 35°C for DCM and 40°C for hexane.

Sand

Sand is used as a matrix for procedural blanks when sediments and biota are extracted. The sand (e.g., sea sand from any major laboratory supply house) must be pre-extracted with DCM prior to use.

Solvent Rinsing

Immediately prior to use, all glassware, spatulas, aluminum foil, etc. which may come in contact with the sample, should be rinsed with solvents. The recommended sequence of solvents is:

toluene

methanol

acetone

dichloromethane

and/or hexane (not necessary unless hexane is to be used as the final sample solvent)

Solvent

It is essential that all solvents used be of high quality. HPLC grade Burdick and Jackson solvents are employed in our laboratory.

Solvent Exchange

A solvent can be exchanged for another by the following method, provided the second solvent has a higher boiling point than the first. For example, when replacing DCM with hexane:

1) reduce sample volume to 0.2 ml under a gentle stream of nitrogen;

2) add 2 ml of hexane;

3) reduce sample volume to 0.2 ml under a gentle stream of nitrogen;

4) repeat steps 2 and 3 twice more;

5) adjust final volume of sample as necessary.

Standard - External

A quantification standard that is injected into the GC independently of the sample containing the compounds to be quantified. It should be the same compound as is being quantified. A series of different concentrations of the external standard are injected and a calibration curve obtained. Samples can then be quantified against this regression line.

This methodology has the advantage that the same compound as is being analyzed provides the calibration and there is therefore no need for response factors to be determined or for consideration of whether the chromatographic behavior of the internal standard and the analyte will remain consistent. The disadvantage of the method is that no account is made for losses during sample preparation. This means that accurate volumes must be recorded at all stages of the extraction process including the final injection volume. In our laboratory, external standardization is avoided whenever possible.

If external standardization is used, then the steps in the methodologies concerned with internal standards should be omitted.

Standard - Internal

A standard added at the beginning of the extraction process against which the compounds of interest are quantified. The current internal standards used in our laboratory are:

1,1'-binaphthyl, 25 µg/ml, used for aromatic fraction of all extracts including for base/neutral extracts of water;

Perinaphthenone, 25 µg/ml, used for polar fraction of all extracts;

Decachlorobiphenyl, 1 µg/ml, used for organohalides of all extracts;

Tribromophenol, 100 µg/ml, used for acid fraction for aqueous samples.

Standards are made up in acetone when used for water samples so that they are miscible with the medium, or in DCM for sediment and biota samples. Typically 1 ml of each is used (0.5 ml for water), but this should be adjusted to suit the expected concentrations of the analytes. Ideally, the internal standard should approximate the area/height of the largest peaks in the sample.

Standard - Recovery

A standard co-injected or spiked into the purified sample extract immediately prior to GC analysis to determine recovery of the internal standard. The ones currently being used in our laboratory are:

Terphenyl - for recovery of 1,1'-binaphthyl, perinaphthenone, tribromophenol;

Pentachlorobenzene, or hexabromobiphenyl - for recovery of decachlorobiphenyl.

Standard - Retention

A standard used to standardize GC retention data for use in RRI systems. These can be run independently or co-injected with samples. They can also be used as the first sample of the day as an indicator of instrument performance. The first set are used for all RRI except the HRI for which the second set are used.

Marker	Molecular Weight	RRI
		0000
1,3,5-trimethylbenzene	120	1000
naphthalene	128	2000
biphenyl	154	3000
phenanthrene	178	4000
pyrene	202	5000
chrysene	228	6000
perylene	252	7000
benzo(ghi)perylene	276	

Markers for HRI

Marker	Molecular Weight	Formula Weight	RRI
		162.5	1000
2-chloronaphthalene	162	291	2000
alpha-BHC	288	320	3000
2,4'-DDD	318	499	4000
decachlorobiphenyl	494	444	5000
2,2",5,5"-tetrachloroquaterphenyl	442		

Sulfur

The GPC procedure should remove elemental sulfur from the sample, but there are occasions when sulfur may have to be removed from a sample prior to analysis (e.g., if GPC is not used in the sample cleanup). The sulfur can be removed with activated cop-

per using a small column filled with copper wool or capping another column, e.g. the silica gel cleanup column, with powdered copper. To activate copper it should be treated with 3M HCl and then thoroughly rinsed with water, methanol, acetone, DCM, and hexane. This activation should be undertaken immediately prior to use and once activated the copper should not be exposed to the atmosphere.

Transferring

In order to minimize sample loss, any transfer of sample from one vessel to another should include triplicate rinsing of the first vessel into the second. The solvent used should be that in which the sample is dissolved.

For example, to transfer a ~2 ml sample in DCM from 500 ml round-bottom flask to a graduated glass tube:

1) Using pasteur pipette, use the sample to rinse the inside walls of the flask, and then transfer to a graduated glass tube.

2) Add 1-2 ml DCM to the flask running the solvent down the walls of the flask.

3) Using the same pasteur pipette, rinse the inside walls of the flask and then transfer the DCM to the graduated glass tube containing the sample.

4) Repeat steps 2) and 3) twice.

5) Transfer is now complete.

This procedure should be used on all occasions when a sample is being transferred between vessels.

Water

A high quality supply of water is required. If available, "Milli-Q" or equivalent water gives good results. If less pure water is used, then it must either be pre-extracted prior to use as a procedural blank or evaluated for the presence of other contaminants.

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Appendix A

Gel Permeation Chromatography

The basic set-up of a GPC system is shown in Figure 9.

Column Preparation

A. Choice of resin and associated solvent. Examples:

1. 100g Biobeads S-X3 elution solvent = 1:1 (v/v) cyclohexane:DCM

2. 70g Biobeads S-X3 elution solvent = DCM

3. 60g Biobeads S-X8 elution solvent = DCM

(Biobeads are available from Bio-Rad Laboratories, Richmond, CA.) They consist of a styrene-divinylbenzene copolymer with controlled pore dimensions for GPC separation of lipophilic compounds. Molecular weight range: S-X3 = up to 2,000 daltons; S-X8 = up to 1,000 daltons.

B. Pump

The pump should be capable of delivering solvent at 5 ml/min. at a pressure of ~5-10 psi (35-70 kPa).

C. Packing a column

1. The column is a standard 75 cm x 2.5 cm low pressure glass column.

2. Gently sieve the resin on a 250 mesh (63 μm) sieve to remove fines (i.e. retain the material that remains in the sieve).

3. Slurry the resin of choice with 500 ml of solvent required (see A above).

4. Let the resin swell overnight.

5. Degas the slurry in an ultrasonic bath for 30 min.

6. Pack the column with mild vacuum on the outlet to obtain a uniform bed. Do not allow the column to go dry at the top.

7. Pump solvent from bottom to top, taking care not to let air get into the system; the pump pressure should be between 5 and 10 psi (35-70 kPa).

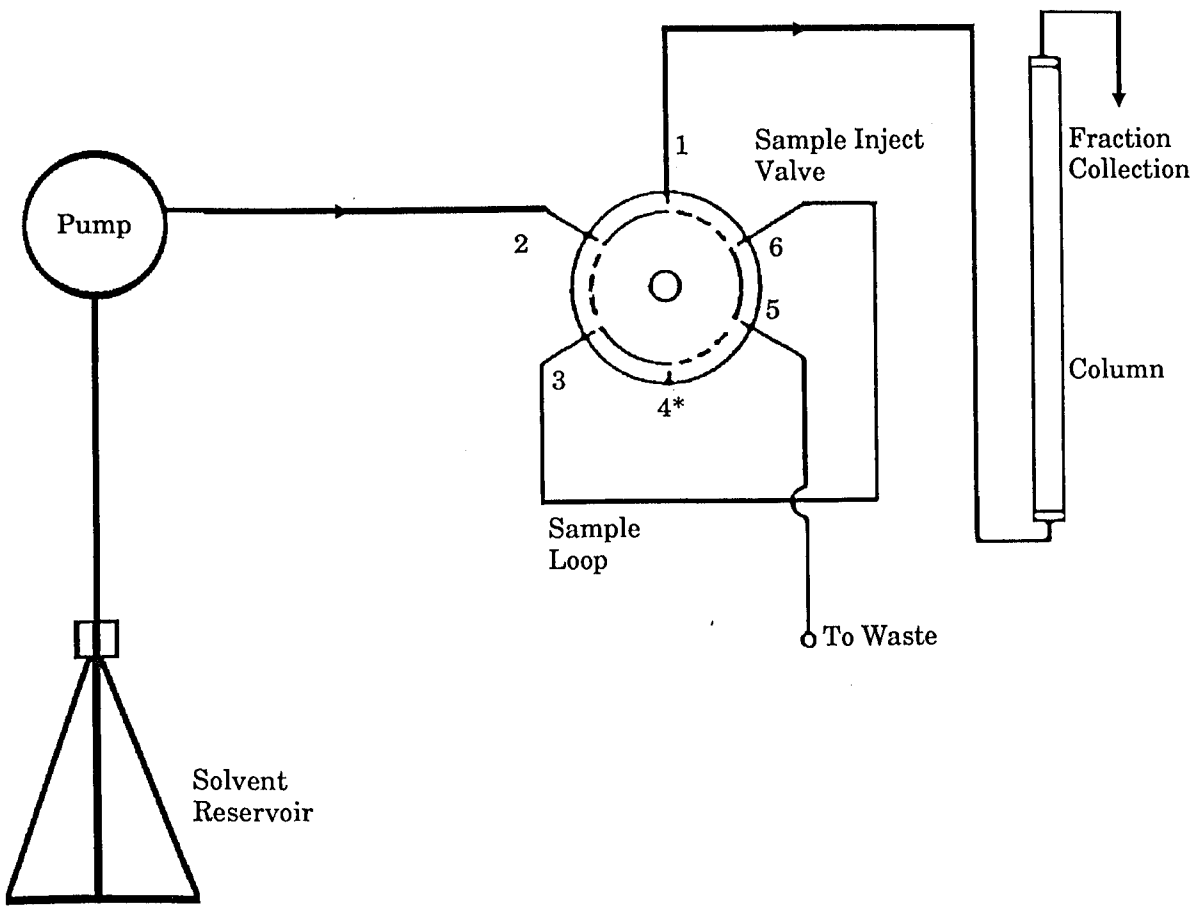
8. The flow rate should be approximately 5 ml/min.

Figure 9. Basic layout of a GPC system.

* sample injection port.

___ valve position for sample loading

- - - valve position for sample injection (rotate counter-clockwise)



D. Column calibration

1. Injection is via a sample loop in a six way valve.

2. The column is calibrated with standards that encompass the molecular weight range of the compounds of interest. Typically, the calibration is with S8 (aromatic standard), but other compounds can be used such as PCB and PCT depending on the interests and needs of the analyst.

3. The following is an example based on use of S8 to calibrate a S-X3 column (70g, 100% DCM eluent) for aromatic compounds:

a. Inject 1 ml of S8 onto the GPC. Collect 5 ml fractions corresponding to elution volumes 170-175 ml, 175-180 ml, 180-185 ml, 185-190 ml, 190-195 ml, 195-200 ml and 270-275 ml, 275-280 ml, 280-285 ml, 285-290 ml, 290-295 ml, 295-300 ml.

b. These fractions are reduced in volume to 0.2 ml each and analyzed by GC for the presence of S8 components.

4. The first GPC fraction, designated P1, is from injection to the point where S8 compounds are first detected, (e.g., if a compound in S8 is detected in the 180-185 ml fraction, P1 will be from 0-180 ml). The P2 fraction will be from the end of P1 until the end of the last 5 ml fraction containing S8 components (e.g., if there are components in 275-280 ml but not 280-285 ml, P2 ends at 280 ml).

NOTE: Elution characteristics vary with the individual column, injection system, resin (including batch to batch variation) and exact packing density. Each column must be calibrated prior to use. A column that is well prepared and maintained may last for hundreds of samples.

Appendix B

Gel Permeation Chromatography of Standard Compounds on a Biobeads S-X8 Column.

Compound Type	Standard	Percentage Found in P1	Percentage Found in P2
Alkanes	n-C16	67	33
	n-C15	0	100
	n-Decyl cyclohexane	2	98
Aromatics	Acenaphthene	0	100
	Acenaphthylene	0	100
	Anthracene	0	100
	Benzo(a)anthracene	0	100
	Benzo(b)fluoranthene	0	100
	Benzo(k)fluoranthene	0	100
	Benzo(e)pyrene	0	100
	Benzo(a)pyrene	0	100
	Benzo(ghi)perylene	0	100
	Biphenyl	15	85
	Chrysene	0	100
	Dibenzo(a,h)anthracene	0	100
	Dibenzothiophene	0	100
	2,6-Dinitrotoluene	0	100
	2,4-Dinitrotoluene	0	100
	Fluoranthene	0	100
	Fluorene	0	100
	Hexamethylbenzene	0	100
	Indeno(1,2,3-cd)pyrene	0	100
	1-Methylphenanthrene	0	100
	Naphthalene	0	100
	Phenanthrene	0	100
	Pyrene	0	100
	m-Quaterphenyl	30	70
	p-Quaterphenyl	30-50	50-70
	1,3,5-Tri-isopropylbenzene	30	70
Phenols	p-Cloro-m-cresol	0	100
	2,4-Dichlorophenol	0	100
	2,4-Dimethylphenol	0	100
	2,6-Dimethylphenol	0	100
	4,6-Dinitro-o-cresol	0	100
	2,4-Dinitrophenol	0	100
	2-Nitrophenol	0	100
	Pentachlorophenol	0	100
	2,4,6-Trichlorophenol	0	100
	2,4,5-Trichlorophenol	0	100

Phthalates	Bis(2-ethylhexyl)Phthalate	100	0
	Butylbenzyl phthalate	100	0
	Dibutyl phthalate	100	0
	Dimethyl phthalate	100	0
	Dioctyl phthalate	100	0
Ethers	4-Bromophenyl ether	0	100
	4-Chlorophenyl ether	0	100
	Phenyl ether	10	90
Hydrazines	1,2-Diphenylhydrazine	0	100
Chlorinated hydrocarbons	Aldrin	0	100
	BHC	0	100
	Captofol	65	35
	Chlordane	0	100
	2-Chloronaphthalene	0	100
	2,4'-DDD	0	100
	4,4'-DDD	0	100
	2,4'-DDE	0	100
	4,4'-DDE	0	100
	2,4'-DDT	0	100
	4,4'-DDT	0	100
	Dibenzo-p-dioxin	0	100
	3,3'-Dichlorobenzidine	0	100
	Dieldrin	0	100
	Endosulfan	0	100
	Endrin	0	100
	Heptachlor	0	100
	Heptachlor epoxide	0	100
	Hexachlorobenzene	0	100
	Kepone	0	100
	Trichlorodibenzo-p-dioxin	0	100
PCB	Decachlorobiphenyl	0	100
	Aroclor 1242	0	100
Carbamates	Carbaryl	0	100
	Chlorpropham	0	100
	Aldicarb	100	0
	Butylate	50	50
	CDEC (sufallate)	0	100
Phosphate Esters	Temephos	100	0
	Malathion	100	0
	Dichlofenthion	80	20
	Trichlorfon	0	100
Triazines	Atrazine	50	50
	Ametryn	0	100

RRI Naming Tables

Selected RRI naming tables are given in Appendices C-H. These are intended to be examples of RRI tables and should not be used without verification of applicability to a particular set of samples. For Appendices C, D, G and H, which are based on the use of GC-FID (with MS verification), a response factor of 1 is used and the window of acceptance is the delta value (±) for variation in peak position that is acceptable in the tentative identification of a compound. For Appendices E and F, which are based on the use of GC-ELCD (with MS verification) for the detection of organohalides, the response factors, which are directly proportional to the number of halogens present in a compound, are given in the table (NOTE: these do not apply to GC-ECD). The window of acceptance for tentative identification of a compound is ±5 in Appendices E and F. Windows of acceptance are based on experience gained through multiple analyses. On occasion they are deliberately set wide to encompass isomers with the same generic identification.

Appendix C

ARI - aromatic retention index. For P2H2 fraction. Suitable for water/sediment samples contaminated with PAH. Response factor = 1. Generic naming of substituent groups is used when the isomeric structure of a compound is unknown. *Compound used as retention marker or standard.

ARI Value	Window of Acceptance ±	Compound Name
0000	05	*1,3,5-trimethylbenzene
1000	05	*Naphthalene
1035	15	Benzothiophene
1510	35	Naphthalene,2-methyl-
1605	35	Naphthalene,1-methyl-
2000	05	*Biphenyl
2030	10	Naphthalene,ethyl-
2060	10	Naphthalene,C2H5-
2100	15	Naphthalene,C2H5-
2150	10	Naphthalene,C2H5-
2165	10	Acenaphthylene
2195	10	Benzene,hexamethyl-
2263	10	Acenaphthene
2280	10	Biphenyl,4-methyl-
2300	10	Biphenyl,3-methyl-
2325	10	Naphthalene,C3H7-
2345	10	Dibenzofuran
2360	10	Naphthalene,C3H7-
2380	10	Bibenzyl
2395	10	Naphthalene,C3H7-

2455	25	Naphthalene,C3H7-
2518	15	Fluorene
2595	10	Acenaphthene,methyl-
2620	10	Dibenzofuran,methyl-
2810	10	Fluorene,methyl-
2935	10	Dibenzothiophene
3000	05	*Phenanthrene
3030	10	Anthracene
3235	10	Dibenzothiophene,methyl-
3310	20	Dibenzothiophene,methyl-
3360	10	Phenanthrene,3-methyl-
3375	10	Phenanthrene,2-methyl-
3425	10	Cyclopenta(def)phenanthrene,4H-
3440	20	Phenanthrene,methyl-
3605	15	Anthraquinone
3610	10	Naphthalene,2-phenyl-
3740	10	Phenanthrene,C2
3790	15	C2-178
3860	15	Fluoranthene
4000	05	*Pyrene
4035	20	Naphthalene,phenyl-,methyl-
4130	10	Naphthalene,phenyl-,methyl-
4235	10	*Terphenyl
4300	19	Benzo(a)fluorene
4335	15	Retene
4366	16	Benzo(b)fluorene
4440	20	Methyl-202
4735	20	*Binaphthyl,1,1'-
4777	12	Benzo(b)naphtho(2,1-d)thiophene
4800	10	Benzo(ghi)fluoranthene
4811	10	Benzo(c)phenanthrene
4910	10	Benzonaphthothiophene
4971	10	Benz(a)anthracene
5000	05	*Chrysene
5060	55	Chrysene,tetramethyloctahydro-
5130	10	Methyl-228
5310	20	Methyl-228
5390	10	Phenanthrene,1-phenyl-
5430	50	Chrysene,trimethyltetrahydro-
5745	25	Benzofluoranthene
5805	15	Benzo(a)fluoranthene
5909	20	Benzo(e)pyrene
5946	15	Benzo(a)pyrene
6000	15	*Perylene
6060	20	Methyl-252
6820	20	Indeno(1,2,3-cd)pyrene
6880	20	Dibenz(a,h)anthracene
7000	05	*Benzo(ghi)perylene

Appendix D

PRI - Polar retention index. RRI naming table for P2H3 fraction compounds. Suitable for water/sediments contaminated with more polar aromatic compounds, particularly those containing heteroatoms. Response factor = 1. In this table the positional isomers structures are currently unassigned. Samples should be co-injected with S8.

PRI Value	Windows of Acceptance ±	Compound Name
3185	14	Benzoquinoline
3210	20	Carbazole,9H-
3460	10	Carbazole,9H-,methyl-
3495	10	Carbazole,9H-,methyl-
3900	20	Carbazole,9H-,C2H5-
3920	20	MW203 (nitrogen heterocyclic)
3980	20	MW203 (nitrogen heterocyclic)
5040	20	Benzocarbazole
5110	20	Benzanthrone/Benzofluorenone
5175	20	Benzocarbazole
5215	20	Benzocarbazole
5680	20	MW253 (nitrogen heterocyclic
5755	20	MW253 (nitrogen heterocyclic
5920	20	Benzacridine,C2H5-
5955	20	Cyclopenta(def)chrysen-4-one
6100	90	Benzacridine,C2H5-
6100	90	Benzacridine,C2H5-
6635	20	Dibenzocarbazole
6750	20	Dibenzocarbazole
6980	20	Dibenzocarbazole
7030	20	Indenylanthracenone
7090	20	Dibenzocarbazole
7145	20	Dibenzocarbazole
7175	20	Dibenzocarbazole
7245	20	Dibenzocarbazole,methyl-
7310	20	Dibenzocarbazole,methyl-
7350	20	Dibenzocarbazole,methyl-
7370	20	Dibenzocarbazole,methyl-

Appendix E

HRI.1 - Halogen retention index 1. RRI table for PCB and a few assorted pesticides (based on Edstrom, 1989). Suitable for water/sediment/biota samples. Windows of acceptance = ±5. PCB are numbered according to IUPAC convention. Numbering on pesticides, e.g. Chlordane I-VIII, is for components in technical grade preparations. *Compound used as retention markers.

HRI Value	Response Factor (on ELCD)	Compound Name
1000	.220	*2-Chloronaphthalene
1405	.270	PCB-1
1447	1.000	Pentachlorobenzene
1620	.270	PCB-2
1643	.270	PCB-3
1770	.450	PCB-4,10
1913	.450	PCB-7,9
1973	.450	PCB-6
2000	1.030	*alpha-BHC
2009	.450	PCB-8,5
2018	1.060	Hexachlorobenzene
2086	.580	PCB-19
2123	1.030	beta-BHC
2136	1.030	gamma-BHC
2181	.580	PCB-15
2189	.580	PCB-17,18
2227	.580	PCB-24,27
2247	1.030	delta-BHC
2261	.580	PCB-16,32
2300	.580	PCB-34
2315	.580	PCB-29
2338	.580	PCB-26
2345	.580	PCB-25
2347	.883	Chlordane (C)
2380	.580	PCB-28,31
2414	.580	PCB-33,20
2417	.680	PCB-53
2424	.935	Heptachlor
2424	.883	alpha-Chlordene
2444	.580	PCB-22,51
2464	.680	PCB-45
2495	.680	PCB-46
2499	.680	PCB-69
2519	.680	PCB-52
2534	.680	PCB-49
2548	.680	PCB-47,75,48
2552	.829	Aldrin
2570	.883	gamma-Chlordene
2578	.580	PCB-35

2580	.883	beta-Chlordene
2598	.680	PCB-44
2608	.580	PCB-37,42,59
2644	.680	PCB-41,64
2674	.680	PCB-40
2688	.935	Chlordane (1)
2699	.680	PCB-67,100
2712	.900	Heptachlor epoxide
2717	.940	Oxychlordane
2721	.680	PCB-63
2736	.680	PCB-74
2758	.680	PCB-70
2762	.680	PCB-66
2768	.760	PCB-95
2771	.760	PCB-88
2777	.760	PCB-121
2791	.760	PCB-91
2792	.935	Chlordane (3)
2809	.974	trans-Chlordane
2827	.680	PCB-60,56
2836	.760	PCB-92
2842	.974	Chlordane (5)
2846	.760	PCB-84
2857	.760	PCB-90,101
2869	.974	cis-Chlordane
2876	.760	PCB-99
2890	.990	trans-Nonachlor
2899	.760	PCB-119,150
2909	.740	Endosulfan I
2918	.760	PCB-83
2824	.974	Chlordane (7)
2936	.760	PCB-97
2945	.790	Dieldrin
2956	.760	PCB-87,115
2971	.760	PCB-85
2972	.630	4,4'-DDE
2979	.830	PCB-136
2989	.680	PCB-77
2993	.760	PCB-110
3000	.630	*2,4'-DDD
3021	.790	Endrin
3034	.830	PCB-82,151
3052	.830	PCB-135
3060	.740	Endosulfan II
3067	.830	PCB-107
3077	.760	PCB-149
3091	.760	PCB-118
3111	.830	PCB-134
3115	.883	Chlordane (K)
3119	.712	4,4'-DDD
3123	.760	PCB-122,131
3135	1.000	Nonachlor
3143	.830	PCB-146
3162	.830	PCB-153,132
3177	.760	PCB-105

3207	.890	PCB-179,141
3229	.830	PCB-130
3233	.890	PCB-176,137
3245	.700	4,4′-DDT
3255	.830	PCB-138,158
3283	.890	PCB-178,129
3302	.890	PCB-175
3312	.890	PCB-187
3328	.890	PCB-183
3346	.830	PCB-128
3356	.830	PCB-167
3362	.890	PCB-185
3389	.890	PCB-174
3407	.890	PCB-177
3422	.890	PCB-171,156
3444	.930	PCB-157,201,176
3458	.430	Methoxychlor
3463	.890	PCB-172
3483	.890	PCB-180
3519	.930	PCB-193
3533	1.100	Mirex
3580	.930	PCB-170,190
3607	.930	PCB-199
3625	.930	PCB-196,203
3676	.890	PCB-189
3721	.960	PCB-208,195
3742	.960	PCB-207
3771	.260	cis-Permethrin
3788	.930	PCB-194
3797	.260	trans-Permethrin
3841	.930	PCB-205
3906	.960	PCB-206
4000	1.000	*PCB-209 (Decachlorobiphenyl)
4275	.650	Octachlorodibenzo-p-dioxin
5000	.451	*2,2″,5,5″-Tetrachloro-quaterphenyl

Appendix F

HRI.2 - Halogen retention index 2. RRI table for PCB, PCT, and a few assorted pesticides. Suitable for water/sediment/biota samples where PCT eluting prior to DCB have been identified. Window of acceptance = ±5. PCB are numbered according to IUPAC convention. Numbering on pesticides, e.g. Chlordane I-VIII, is for components in technical grade preparations. PCT structures cannot currently be assigned. *Compound used as retention marker.

HRI Value	Response Factor (on ELCD)	Compound Name
1000	.220	*2-Chloronaphthalene
1405	.270	PCB-1
1447	1.000	Pentachlorobenzene
1620	.270	PCB-2
1643	.270	PCB-3
1770	.450	PCB-4,10
1913	.450	PCB-7,9
1973	.450	PCB-6
2000	1.030	*alpha-BHC
2009	.450	PCB-8,5
2018	1.060	Hexachlorobenzene
2086	.580	PCB-19
2123	1.030	beta-BHC
2136	1.030	gamma-BHC
2181	.580	PCB-15
2189	.580	PCB-17,18
2227	.580	PCB-27,24
2247	1.030	delta-BHC
2261	.580	PCB-16,32
2300	.580	PCB-34
2315	.580	PCB-29
2338	.580	PCB-26
2345	.580	PCB-25
2347	.883	Chlordane (C)
2380	.580	PCB-28,31
2414	.580	PCB-33,20
2417	.680	PCB-53
2424	.935	Heptachlor
2424	.883	alpha-Chlordene
2444	.580	PCB-22,51
2464	.680	PCB-45
2495	.680	PCB-46
2499	.680	PCB-69
2519	.680	PCB-52
2534	.680	PCB-49
2548	.680	PCB-47,75,48
2552	.829	Aldrin
2570	.883	gamma-Chlordene
2578	.580	PCB-35

2580	.883	beta-Chlordene
2598	.680	PCB-44
2608	.580	PCB-37,42,59
2644	.680	PCB-41,64
2674	.680	PCB-40
2688	.935	Chlordane (1)
2699	.680	PCB-67,100
2712	.900	Heptachlor epoxide
2717	.940	Oxychlordane
2719	.680	PCB-63
2736	.680	PCB-74
2752	.680	PCB-70,76
2758	.680	PCB-70
2762	.680	PCB-66
2768	.760	PCB-95
2771	.760	PCB-88
2777	.760	PCB-121
2790	.760	PCB-91
2792	.935	Chlordane (3)
2809	.974	trans-Chlordane
2827	.680	PCB-60,56
2836	.760	PCB-92
2842	.974	Chlordane (5)
2846	.760	PCB-84
2857	.760	PCB-90,101
2869	.974	cis-Chlordane
2876	.760	PCB-99
2890	.990	trans-Nonachlor
2899	.760	PCB-119,150
2909	.740	Endosulfan I
2918	.760	PCB-83
2824	.974	Chlordane (7)
2936	.760	PCB-97
2945	.790	Dieldrin
2956	.760	PCB-87,115
2971	.760	PCB-85
2972	.630	4,4'-DDE
2979	.830	PCB-136
2989	.680	PCB-77
2993	.760	PCB-110
3000	.630	*2,4'-DDD
3021	.790	Endrin
3033	.400	PCT
3034	.830	PCB-82,151
3047	.400	PCT
3052	.830	PCB-135
3060	.740	Endosulfan II
3067	.830	PCB-107
3077	.830	PCB-149
3091	.760	PCB-118
3111	.830	PCB-134,143
3116	.400	PCT
3119	.712	4,4'-DDD
3123	.760	PCB-122,131
3135	1.000	Nonachlor

3143	.890	PCB-146
3150	.400	PCT
3162	.830	PCB-153,132
3163	.400	PCT
3171	.400	PCT
3177	.760	PCB-105
3195	.400	PCT
3207	.890	PCB-179,141
3217	.400	PCT
3222	.400	PCT
3229	.830	PCB-130
3230	.400	PCT
3233	.890	PCB-176,137
3245	.700	4,4'-DDT
3255	.830	PCB-138,158
3259	.400	PCT
3273	.400	PCT
3283	.890	PCB-129,178
3292	.400	PCT
3302	.890	PCB-175
3308	.400	PCT
3312	.890	PCB-187
3319	.400	PCT
3328	.890	PCB-183
3332	.400	PCT
3337	.400	PCT
3346	.830	PCB-128
3356	.830	PCB-167
3352	.400	PCT
3362	.890	PCB-185
3387	.500	PCT
3389	.890	PCB-174
3393	.500	PCT
3407	.890	PCB-177
3418	.500	PCT
3422	.890	PCB-171,156
3444	.930	PCB-157,201,176
3450	.500	PCT
3458	.430	Methoxychlor
3462	.500	PCT
3463	.890	PCB-172
3480	.500	PCT
3483	.890	PCB-180
3489	.500	PCT
3496	.500	PCT
3511	.500	PCT
3519	.930	PCB-193
3520	.500	PCT
3533	1.100	Mirex
3537	.500	PCT
3548	.500	PCT
3562	.500	PCT
3568	.500	PCT
3580	.930	PCB-170,190
3584	.500	PCT

3599	.500	PCT
3607	.930	PCB-199
3616	.500	PCT
3625	.930	PCB-196,203
3628	.500	PCT
3655	.500	PCT
3667	.600	PCT
3672	.600	PCT
3676	.890	PCB-189
3699	.600	PCT
3711	.600	PCT
3719	.600	PCT
3721	.960	PCB-208,195
3733	.600	PCT
3741	.600	PCT
3742	.960	PCB-207
3757	.600	PCT
3764	.600	PCT
3771	.260	cis-Permethrin
3781	.600	PCT
3788	.930	PCB-194
3789	.600	PCT
3797	.260	trans-Permethrin
3799	.600	PCT
3821	.600	PCT
3830	.600	PCT
3841	.930	PCB-205
3851	.600	PCT
3866	.600	PCT
3886	.600	PCT
3894	.600	PCT
3904	.600	PCT
3906	.960	PCB-206
3919	.600	PCT
3934	.600	PCT
3942	.600	PCT
3961	.600	PCT
3970	.600	PCT
3988	.600	PCT
4000	1.000	*PCB-209 (Decachlorobiphenyl)
4275	.650	Octachlorodibenzo-p-dioxin
5000	.451	*2,2″,5,5″-Tetrachloro-quaterphenyl

Appendix G

CSRI - *Callinectes sapidus* retention index. RRI naming table for biota samples. Suitable where biota (e.g., *C. sapidus*) have been exposed to PAH and dissolved fuel oil components. Response factor = 1. PCB are identified only by level of chlorination. *Compound used as retention marker or standard.

CSRI Value	Window of Acceptance	Compound Name
0000	05	*1,3,5-trimethylbenzene
1000	05	*Naphthalene
1035	15	Benzothiophene
1510	35	Naphthalene,2-methyl-
1605	35	Naphthalene,1-methyl-
2000	05	*Biphenyl
2030	10	Naphthalene,ethyl-
2060	10	Naphthalene,C2H5-
2100	15	Naphthalene,C2H5-
2150	10	Naphthalene,C2H5-
2165	10	Acenaphthylene
2195	10	Benzene,hexamethyl-
2263	10	Acenaphthene
2280	10	Biphenyl,4-methyl-
2300	10	Biphenyl,3-methyl-
2325	10	Naphthalene,C3H7-
2345	10	Dibenzofuran
2360	10	Naphthalene,C3H7-
2380	10	Bibenzyl
2395	10	Naphthalene,C3H7-
2455	25	Naphthalene,C3H7-
2485	05	Naphthalene,C3H7-
2518	15	Fluorene
2545	10	Naphthalene,C3H7-
2570	15	Naphthalene,C3H7-
2595	10	Acenaphthene,methyl-
2620	10	Dibenzofuran,methyl-
2640	05	Naphthalene,C4H9-/ Biphenyl,C2H5-
2715	40	Naphthalene,C4H9-
2770	10	Naphthalene,C5H11-
2796	05	Naphthalene,C4H9-
2810	10	Fluorene,methyl-
2830	05	Fluorene,methyl-
2860	05	Fluorene,methyl-/ Naphthalene,C5H11-
2870	10	Naphthalene,C4H9-/ Biphenyl,C2H5-
2900	05	Naphthalene,C4H9-
2935	10	Dibenzothiophene
3000	05	*Phenanthrene
3030	10	Anthracene/ Naphthalene,C5H11-
3095	10	Naphthalene,C7H15-

3125	10	Acenaphthene,C3H7-/ Fluorene,C2H5-
3150	05	Acenaphthene,C4H9-
3165	05	Acenaphthene,C3H7-/ Fluorene,C2H5-
3200	10	Acenaphthene,C3H7-
3240	10	Acenaphthene,C4H9-
3265	10	Acenaphthene,C4H9-/ Biphenyl,C4H9-
3330	10	Naphthalene,C5H11-
3360	10	Phenanthrene,3-methyl-
3375	15	Phenanthrene,2-methyl-
3425	10	Cyclopenta(def)phenanthrene,4H-
3440	20	Phenanthrene,methyl-
3605	15	Anthraquinone
3610	10	Naphthalene,2-phenyl-
3740	10	Phenanthrene,C2H5-
3790	15	MW 178,C2H5-
3830	10	Cyclopentaphenanthrene,methyl-
3860	15	Fluoranthene
4000	05	*Pyrene
4035	20	Naphthalene,phenyl-,methyl-
4065	10	Phenanthrene,C3H7-/Cl5- PCB
4130	10	Naphthalene,phenyl-,methyl-
		/Phenanthrene,C3H7-
4180	10	Phenanthrene,C3H7-
4220	10	DDE,4,4'-
4235	10	*Terphenyl
4290	10	Methyl-202
4300	19	Benzo(a)fluorene
4335	15	Retene
4366	16	Benzo(b)fluorene
4400	10	Cyclopentaphenanthrene,C4H9-
4420	10	Cl5-PCB
4440	10	Methyl-202
4495	10	Cyclopentaphenanthrene,C4H9-
4420	10	Cl5-PCB
4530	10	Cl5-PCB/Phenanthrene,C4H9-
4565	10	Cl6-PCB
4655	10	MW 202,C2H5-
4735	20	*Binaphthyl,1,1'/Cl6- PCB
4777	12	Benzo(b)naphtho(2,1-d)thiophene
4800	10	Benzo(ghi)fluoranthene
4811	10	Benzo(c)phenanthrene
4835	10	Cl7-PCB
4865	10	Cl7-PCB/MW 202,
		C2H5-
4895	10	C17-PCB
4910	10	Methyl-228
4971	10	Benz(a)anthracene
5000	05	*Chrysene
5055	10	Benzanthracene/MW 202,C4H9-
5095	10	Benzanthracene/MW 202,C4H9-
5130	10	C17-PCB
5265	10	Methyl-228
5290	10	Methyl-228/Cl7- PCB
5310	20	Methyl-228
5370	20	Methyl-226

5390	10	Phenanthrene,1-phenyl-
5440	30	Binaphthyl,2,2'- /Dihydrobenzofluoranthene
5630	10	MW 228,C2H5-
5745	25	Benzofluoranthene
5805	16	Benzo(a)fluoranthene
5909	20	Benzo(e)pyrene
5946	15	Benzo(a)pyrene
6000	15	*Perylene
6060	10	Methyl-252
6140	10	Methyl-252
6230	10	Methyl-252
6650	10	Dibenzanthracene
6820	20	Indeno(1,2,3-cd)pyrene
6880	20	Dibenz(a,h)anthracene
7000	05	*Benzo(ghi)perylene

Appendix H

BTRI - Biogenic terpenoid retention index. RRI naming table for samples abundant in biogenic terpenoid hydrocarbons. Response factor = 1. *Compound used as retention marker or standard.

BTRI Value	Window of Acceptance	Compound Name
0780	10	Terpene alcohol,MW 154
0805	10	Terpene hydrocarbon,MW 134
0985	10	Terpene alcohol,MW 150
1000	05	*Naphthalene
1030	05	Styrene deriv.
1045	10	Terpene alcohol,MW 150
1245	05	Anisole,C4H9-
1515	10	Isoborneol
1540	15	Naphthalene,2-methyl-
1605	15	Naphthalene,1-methyl-
1745	15	Sesquiterpene hydrocarbon,C15H24
1845	15	Sesquiterpene hydrocarbon,C15H24
1950	10	Sesquiterpene hydrocarbon,C15H24
1980	05	Sesquiterpene hydrocarbon,C15H24
2000	05	*Biphenyl
2030	05	Naphthalene,ethyl-
2045	10	Sesquiterpene hydrocarbon,C15H24
2060	10	Sesquiterpene hydrocarbon,C15H24
2060	10	Naphthalene,C2H5-
2080	05	Sesquiterpene hydrocarbon,C15H24
2090	15	Naphthalene,C2H5-
2110	10	Sesquiterpene hydrocarbon,C15H24
2150	10	Naphthalene,C2H5-
2165	05	Acenaphthylene
2175	10	Sesquiterpene hydrocarbon,C15H24
2185	10	Sesquiterpene hydrocarbon,C15H24
2195	10	Benzene,hexamethyl-
2235	05	Sesquiterpene hydrocarbon,C15H22
2240	05	Tetralin,methyl-
2263	10	Acenaphthene
2280	05	Biphenyl,4-methyl-
2285	10	Sesquiterpene hydrocarbon,C15H22
2300	10	Sesquiterpene hydrocarbon,C15H24
2330	10	Sesquiterpene hydrocarbon,C15H22
2350	10	Sesquiterpene hydrocarbon,C15H22
2345	10	Dibenzofuran
2360	10	Naphthalene,C3H7-
2380	10	Bibenzyl
2395	10	Tetralin,alkyl-
2410	10	Tetralin,alkyl-
2425	10	Tetralin,alkyl-
2455	25	Naphthalene,C3H7-

2490	05	Naphthalene,C3H7-
2520	15	Fluorene
2550	05	Naphthalene,C3H7- + Tetralin,alkyl-
2565	10	Biphenyl,methyl-
2620	10	Tetralin,alkyl-
2640	10	Tetralin,alkyl-
2690	10	MW 204
2785	05	Naphthalene,C5H11-
2820	20	Fluorene,methyl-
2910	10	Fluorenone
2935	10	Dibenzothiophene
3000	05	*Phenanthrene
3030	10	Anthracene
3090	10	MW 182
3235	10	Dibenzothiophene,methyl-
3310	20	Phenanthrene,methyl-
3360	10	Phenanthrene,3-methyl-
3375	10	Phenanthrene,2-methyl-
3390	05	MW 258
3425	10	Cyclopenta(def)phenanthrene,4H-
3440	20	Phenanthrene,methyl-
3550	10	Diterpene hydrocarbon,MW 272
3610	10	Diterpene hydrocarbon,MW 272
3670	10	Diterpene hydrocarbon,MW 256
3700	10	Diterpene hydrocarbon,MW 272
3730	05	Phenanthrene,C2H5-
3760	10	Diterpene hydrocarbon,MW 256
3775	10	Diterpene hydrocarbon,MW 256
3790	10	Diterpene hydrocarbon,MW 272
3820	05	Phenanthrene,C2H5-
3860	15	Fluoranthene
3900	10	Dehydroabietane
4000	05	*Pyrene
4035	20	Naphthalene,phenyl-,methyl-
4130	10	Naphthalene,phenyl-,methyl-
4170	10	Naphthalene,phenyl-,methyl-
4275	15	*Terphenyl
4300	19	Benzo(a)fluorene
4335	15	Retene
4366	16	Benzo(b)fluorene
4440	20	Methyl-202
4735	15	*Binaphthyl,1,1'-
4777	12	Benzo(b)naphtho(2,1-d)thiophene
4800	10	Benzo(ghi)fluoranthene
4811	10	Benzo(c)phenanthrene
4910	10	Benzonaphthothiophene
4971	10	Benz(a)anthracene
5000	05	*Chrysene
5060	55	Chrysene,tetramethyloctahydro-
5130	10	Methyl-228
5310	20	Methyl-228
5390	10	Phenanthrene,1-phenyl-
5430	50	Chrysene,trimethyltetrahydro-
5740	25	Benzofluoranthene
5805	15	Benzo(a)fluoranthene

5909	20	Benzo(e)pyrene
5946	15	Benzo(a)pyrene
6000	15	*Perylene
6050	20	Methyl-252
6820	20	Indeno(1,2,3-cd)pyrene
6880	20	Dibenz(a,h)anthracene
7000	05	*Benzo(ghi)perylene