REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME DECEMBER 1996

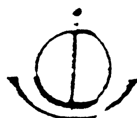
Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment

DRAFT-NOT TO BE CITED
PROJET-NE PAS DIVULGUER

Reference Methods For Marine Pollution Studies 71

Prepared in co-operation with

IAEA



IOC

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then, the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it (1), (2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment, its resources and the sources and trends of the pollution and its impact on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The Methods recommended for adoption by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialised bodies of the United Nations system as well as other organisations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines, the style used by the International Organisation for Standardisation (ISO) has been followed as closely as possible.

The methods and guidelines published in UNEP's series of Reference Methods for Marine Pollution Studies are not considered as definitive. They are planned to be periodically revised taking into account the new developments in analytical instrumentation, our understanding of the problems and the actual need of the users. In order to facilitate these revisions, the users are invited to convey their comments and suggestions to :

Marine Environmental Studies Laboratory
IAEA Marine Environment Laboratory
19, avenue des Castellans
MC 98000 MONACO

which is responsible for the technical co-ordination of the development, testing and inter-calibration of Reference Methods.

References:

(1) UNEP : Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No 1, UNEP 1982.

(2) P. HULM : A strategy for the Seas. The Regional Seas Programme Past and Future, UNEP, 1983.

(3) UNEP/IAEA/IOC : Reference Methods and Materials : A Programme of comprehensive support for regional and global marine pollution assessment. UNEP, 1990.

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1. SCOPE AND FIELD OF APPLICATION

This reference method is intended for use in monitoring programmes and pilot research studies. The document describes procedures for the isolation of purified fractions amenable for the determination of DDTs and PCBs in marine sediments and marine organisms by capillary GC/ECD. It is assumed that most of the participants in the UNEP Regional Seas Programmes are equipped with advanced high resolution capillary gas chromatographs and will be able to implement most, if not all, of the procedures described in Reference Method No 40, "Determination of DDTs and PCBs by capillary gas chromatography and electron capture detection" (UNEP 1988). Assuming consistent results are routinely being obtained with these methods by the analytical laboratory, the determination of specific compounds (as opposed to generic mixture of PCBs) opens up the possibility not only of identifying environmental "hot spots", but also for characterising sources, elucidating transport pathways and developing data of greater toxicological relevance. The organisation and content of this document, however, deserves further comment. Under the sections devoted to SEDIMENTS and ORGANISMS, subsections are provided relating to procedures for : 1) Sampling, 2) Extraction and 3) Clean-up and fractionation. In each subsection, several alternative procedures are described. These various procedures have been previously tested and are provided to accommodate the range of capabilities in participating laboratories. For example, laboratories which have access to an HPLC may consider the benefits of using HPLC fractionation procedures in lieu of more conventional low pressure column chromatographic method. Participants are generally encouraged to implement the most effective procedures within the constraints of their individual laboratories.

Several other halogenated pesticides and other electron capturing organic compounds may be present in environmental samples and many of these compounds could also be isolated by the methods described here. However, not all residues will be stable to the clean-up procedures applied for the determination of PCBs and DDTs. Consequently, every analyst must test for analyte recovery and analytical reproducibility prior to applying these methods for other analytes on a routine basis. Primary emphasis should be placed on obtaining the cleanest possible purified fraction for capillary GC/ECD analysis so that interferences and misidentification are minimised, if not eliminated.

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3. PRINCIPLES

Following collection of sediment or biota samples using appropriate techniques, samples are stored in trace organic free vessels at -20 °C until analysis. For analysis, the samples are defrosted and prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons,

lipids), extracts are fractionated using column chromatography. Detailed protocols for absorption chromatographic fractionation are described for both low and high pressure systems, using Florisil and silica gel respectively. (Additional information concerning alternative techniques including gel permeation chromatography is provided).

4. REAGENTS AND MATERIALS

4.1. Reagents

4.1.1. List of reagents

- Demineralized distilled water produced by distillation over potassium permanganate (0.1 g/l $KMnO_4$) or equivalent quality, demonstrated to be free from interfering substances.
- Detergent.
- Potassium dichromate.
- Concentrated HCl. (d 20°C: 1.19 g/ml).
- Concentrated H_2SO_4 (d 20°C: 1.84 g/ml).
- Sulfochromic cleaning solution made from concentrated sulphuric acid and potassium dichromate.
- KOH.
- Hexane, Dichloromethane, Methanol, Pentane, Cyclohexane, Toluene and Ethyl Acetate, all "distilled in glass" quality.
- Tetrahydrofuran (THF).
- Dimethylformamide (DMF).
- Hexamethyldisilazane (HMDS).
- Anhydrous sodium sulphate.
- $CuSO_4.5H_2O$.
- Carborundum.
- Hg.
- Finely divided Zinc.
- Glass wool
- Alumina (200-240).
- Silica gel (60-100).
- Florisil PR (60-100).
- Bio-Beads SX-3 (200-400).
- Sephadex LX-20.
- PCB congeners: 29, 30, 121, 198.
- ε HCH.
- n-$C_{32}H_{66}$.
- Octadeca-1-ene ($C_{18}H_{36}$).
- 9,10-dihydroanthracene.
- Deuterated $C_{24}H_{50}$.
- DDT reference solutions - Prepare a stock solution of the DDT series (pp' DDT, op DDT, pp' DDD, op DDD, pp' DDE, op DDE) by dissolving 50 mg of each compound in 100 ml of hexane. Store stock solution in sealed glass ampoules.
- Other reference solutions - should be prepared if other residues are to be quantified in these procedures.

NOTES :

Working solutions obtained from the stock reference solutions should be prepared on a regular basis depending on their use and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that the concentrations of the standards have not altered due to solvent evaporation.

In order to achieve acceptable accuracy for the standard solutions, at least 50 mg of pure individual compound should be weighed and dissolved into 100 ml of hexane. This will give stock solutions of 500 ng/µl.

Example of preparation of stock solutions:

Preparation of a stock solution of pp' DDE at approximately 500 ng/µl:

The pp' DDE stock solution is prepared by dissolving approximately (but weighed accurately) 50 mg of pp' DDE in hexane in a 100 ml volumetric flask and bringing the volume to exactly 100 ml with hexane. If the actual weight of pp' DDE is 52 mg, then

$$\frac{52\text{ mg DDE}}{100\text{ ml solvent}} \times \frac{1000\ \mu g}{mg} \times \frac{ml}{1000\ \mu l} = \frac{52\text{ mg DDE}}{100\text{ ml of solution}}$$

52 mg/100 ml => 0.52 mg/ml => 520 µg/ml => 520 ng/µl

The concentration of the stock solution will be: 520 ng/µl

Preparation of an intermediate solution:

Use the stock solution to prepare the intermediate solution. The concentration of pp' DDE intermediate solution should be approximately 5 ng/µl. To prepare the 5 ng/µl intermediate solution, transfer 1 ml of the pp' DDE stock solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1\text{ ml DDE Stock soln}}{100\text{ ml final volume}} \times \frac{520\text{ ng DDE}}{\mu l} = \frac{5.2\text{ ng}}{\mu l\text{ interm. soln}}$$

The concentration of the intermediate solution will be: 5.2 ng/µl

Preparation of the working solution :

Use the intermediate solution to prepare the working solution. The concentration of pp' DDE in the working solution could be approximately 50 pg/µl.

To prepare the 50 pg/µl working solution, transfer 1 ml of the pp' DDE intermediate solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1\text{ ml DDE interm. soln}}{100\text{ ml final volume}} \times \frac{5.2\text{ ng}}{\mu l} \times \frac{1000\text{ pg}}{ng} = \frac{52\text{ pg}}{\mu l\text{ working soln}}$$

The concentration of the working solution will be: 52 pg/µl

4.1.2. Cleaning of solvents

All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances, their concentrations vary from batch to batch and with supplier. Reagent quality should be checked by injection of 2 µl of a 100 ml batch of solvent, after concentration to 50 µl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 - 250 °C) should be larger than that for 1 pg of lindane. Otherwise, the solvent must be distilled. The following procedure has been found to be both efficient and cost effective, as it allows the use of technical grade solvents as the basic material (reducing the cost by one order of magnitude). 130 - 150 cm height

columns are required, the packing material must be glass (to allow subsequent cleaning with an oxidising acid). The entire equipment is cleaned prior to use by 2 consecutive distillation procedures with 500 ml water in each case. It is essential that a current of nitrogen gas (15 ml/min) flows from the distillation flask during distillation of the organic solvents: the condenser serves as exhaust. Ambient air is not in contact with the solvent in this way. Problems are associated with other methods of excluding room air (e.g., active carbon or molecular sieves), the most important one being discontinuity. The condensate is distilled into a 1 litre flask at a 1:20 ratio. This large volume allows for direct transfer into the appropriate solvent containers which should be made of glass and of a sufficient size to provide solvent for not more than 6 analysis. A bottle with sufficient solvent for 10 - 15 analysis has to be opened and closed many times and even when kept closed, when not in use, contamination from the surrounding atmosphere takes place. For more detailed information, consult the Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low-level contaminant monitoring.

4.1.3. Cleaning of reagents and adsorbents

4.1.3.1. Cleaning of reagents

Powdered or crystalline reagents, such as anhydrous sodium sulfate (Na_2SO_4)*, copper sulfate ($CuSO_4.5H_2O$), potassium hydroxide (KOH), finely divided zinc (Zn), glass wool * and carbon or carborundum boiling chips *, must be thoroughly cleaned before use. They should be extracted with hexane in a Soxhlet apparatus for 8 hours and then with methanol or dichloromethane for another 8 hours. For those items indicated by an * , this will require pre-combustion in a muffle furnace at approximately 400 °C.

4.1.3.2. Cleaning of adsorbents

Silica gel, alumina and Florisil have to be solvent extracted. Each reagent is first refluxed with methanol or dichloromethane in a Soxhlet apparatus for 8 hours, then with n-hexane for the same period. The solvent is removed by a rotary evaporator operating at low speed, until the sorbent starts falling down as fine particles. Reagents are dried in a drying oven at 0.01 mbar. If this is not available, they are dried in a normal oven at 120 °C for 4 hours. This serves to activate silica and alumina. Florisil has to be activated at 130 °C for 12 hours. The sorbent is allowed to cool in the oven (if possible under vacuum to avoid uptake of contaminants from the atmosphere) or alternatively, in a dessicator. As active sorbents attract water and contaminants from the atmosphere, controlled deactivation should be carried out by adding water to the fully active sorbent (5 % by weight to silica, 2 % by weight to alumina, 0.5 % by weight to Florisil). The deactivation procedure should be carried out by adding the water to the sorbent and mixing by gentle shaking for a few minutes. The equilibration takes one day. The activity can be maintained for longer periods of time by sealing the required amount of sorbent in glass ampoules. Otherwise, the activation/deactivation has to be done the day before use.

4.2. List of apparatus

The laboratory used for organic trace analysis must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc. The emergency cut-off switch should be accessible from both inside and

outside the laboratory. Fire fighting equipment should be mounted in obvious places and lab personnel trained in their use.

4.2.1. List of materials

- A coring device with liners and plunger or a grab sampler. (thoroughly cleane detergents and solvents before use).
- Glass jars and aluminium foil, stainless steel knives, scoops , forceps, labels, markin log book.
- Insulated plastic boxes for transporting samples. Ice or dry ice.
- Deep freezer (-18 to -20 °C) for sample preservation (frost free type freezers heat to zero during frost removal cycles and they cannot be used for long term storage).
- Rotary evaporator.
- Kuderna-Danish (or similar) concentrator and heater.
- Soxhlet extraction apparatus and heaters.
- Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer separatory funnels, centrifuge tubes, weighing bottles, pipettes, tissue grinders.
- Drying oven (temperature range up to at least 300 °C) for determining sample dry we baking of contaminant residues from glassware and reagents.

Note : A muffle furnace is better for baking materials at greater than 300 required.

- Centrifuge and tubes.
- Freeze-dryer and porcelain pestle and mortar.
- Analytical balance with an accuracy of 0.1 mg and an electro-balance with an accur at least 1 µg.
- Stainless steel tweezers and spatulas.
- Dessicator - completely free of organic contamination and with no grease applied to se edges.
- Supply of clean, dry nitrogen.
- Columns for silica gel, alumina and Florisil chromatography.
- Mechanical blender (food mixer).
- Vacuum pump (water-jet air pump).

4.2.2. Cleaning of glassware

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse five times tap water and twice with distilled water. Rinse with acetone or methanol followed by hexa petroleum ether. Bake overnight in an oven at 300 °C. All glassware should be stored in dus cabinets and tightly sealed with pre-cleaned aluminium foil when not in use. Ideally glassware sl be cleaned just before use.

For more detailed information, consult Reference Method No 65: UNEP/IOC/IAEA: Re and laboratory ware clean-up procedures for low level contaminant monitoring.

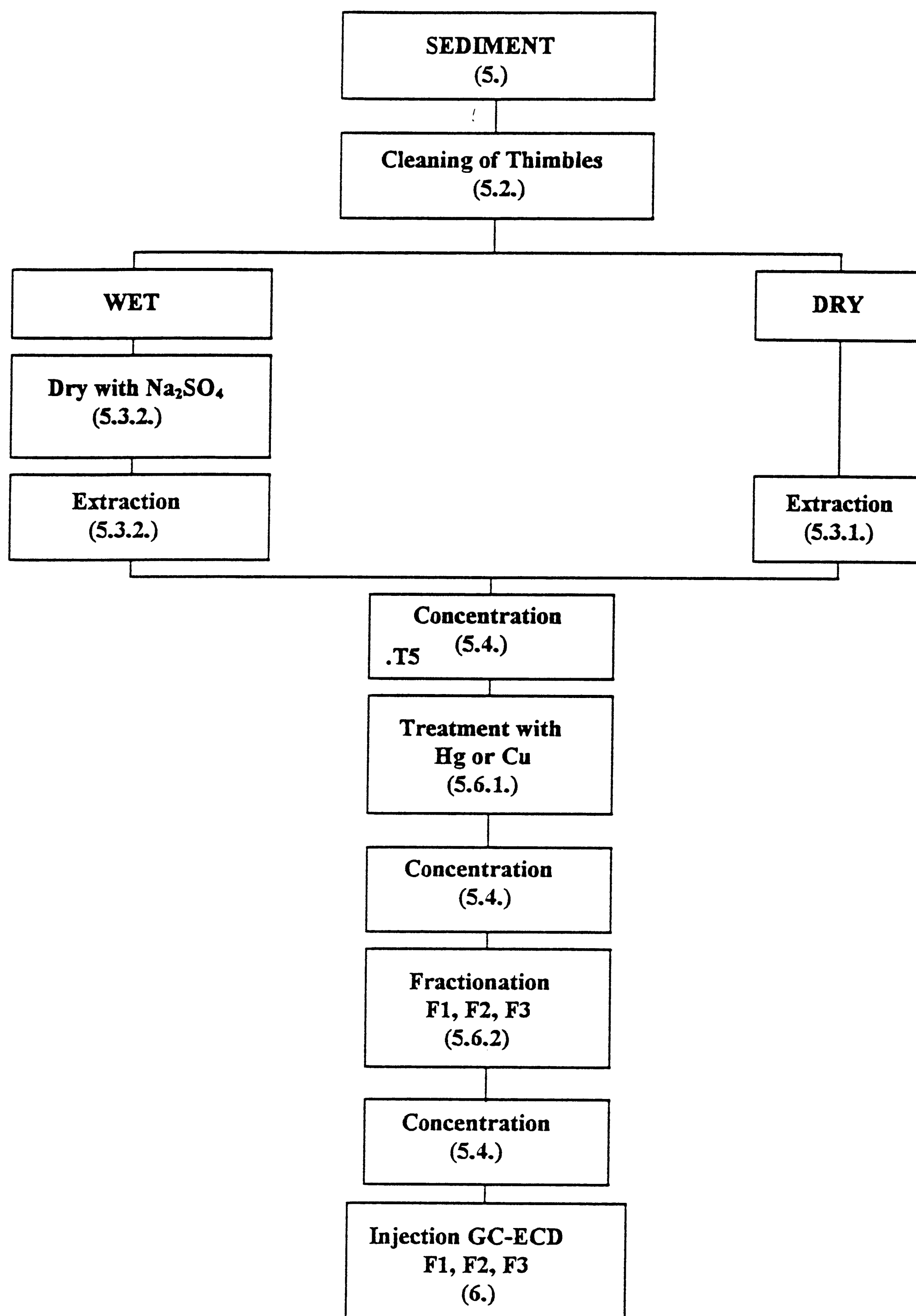


Diagram of the extraction procedure for sediment samples.

5. SEDIMENTS

5.1. Sampling

Many contaminants have a high affinity for particles, therefore, a large proportion of contaminants end up in the marine sediments through particle transport. Studies of the distribution of concentrations of contaminants in sediments have been shown to reflect inputs of contaminants and therefore represent an important element in marine monitoring programs. Organic contaminants generally accumulate in fine grained, organic rich sediments which are characteristic of depositional environments. For this reason, it is often useful to express analytical results in tandem with sedimentology data. The scheme is based on a factor-of-two scale. The categories for wet sieved sediments are summarised below:

Sedimentology classes

	Class	Size	Suggested sieves(μm)
		2000	1000
sand	very coarse	1000	
	coarse	500	
	medium	250	
	fine	125	125
	very fine	62	63
silt	very coarse	31	
	coarse	16	
	medium	8	
	fine	4	
	very fine	2	
clay		< 2	

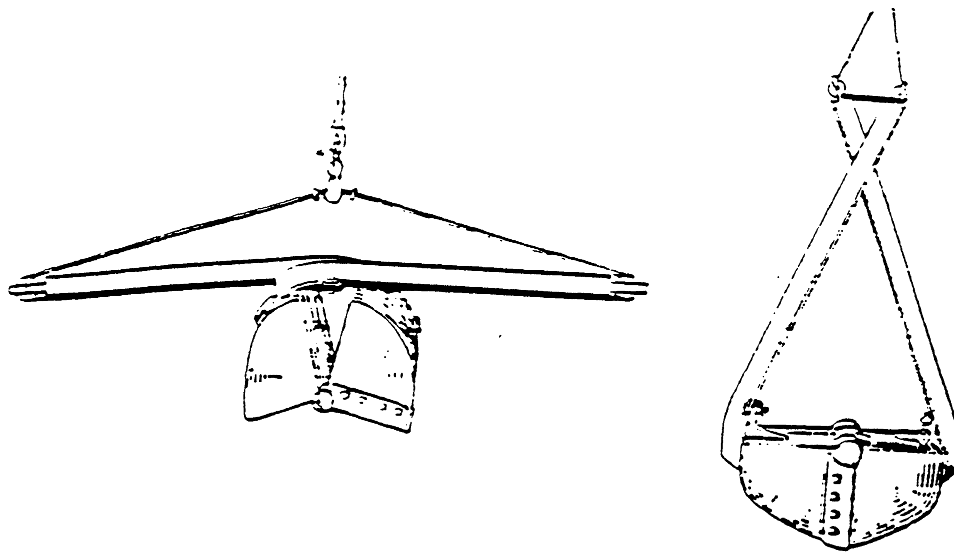
The homogenised sediment used for dry weight determination should be sieved to estimate size composition. This information together with data on the total extractable organic matter, will be useful for the interpretation of relative pollution loads in sediments. If the instrumentation is available, further information may be gained by analysis of total organic carbon. This is normally done on the < 63 μm sediment fraction.

Consideration of the goals of the monitoring program is essential in determining the sampling strategy. Aspects that are involved are : choice of area and choice of sampling equipment.

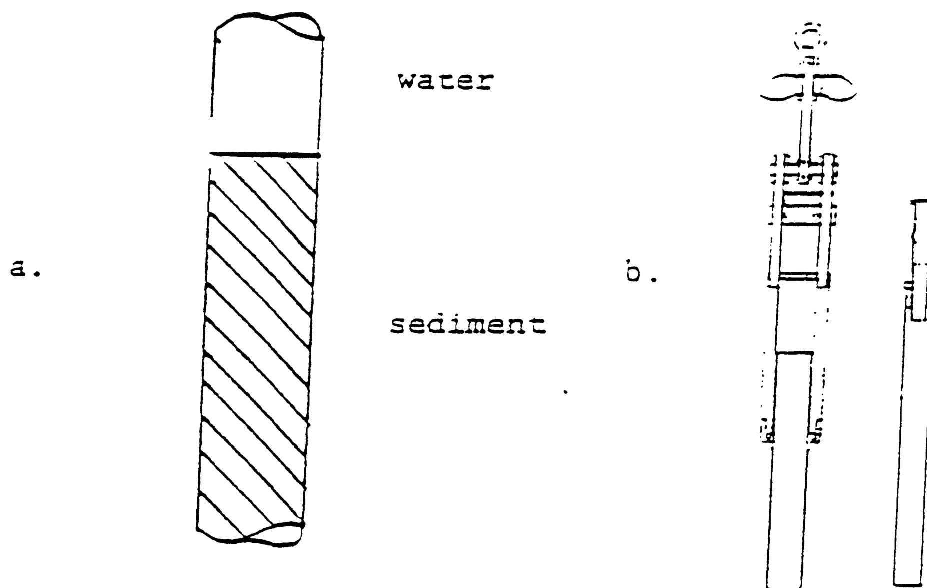
Two approaches to sampling may be considered : first to provide information on spatial distribution and temporal trends in surface sediment; second to provide information on the sedimentary record of contamination and environmental fluxes of contaminants.

In spatial surveys, samples should be distributed throughout the area of interest, with consideration of likely point sources of contaminants including areas of sediment accumulation and dispersive areas. Sampling sites are normally chosen on a broad grid network which covers the whole geographical area. Care should be taken in selection of both the numbers of samples collected and the number of sampling sites. The rationale to this may be a statistical evaluation of the sampling scheme in order to achieve goals at optimum labour costs.

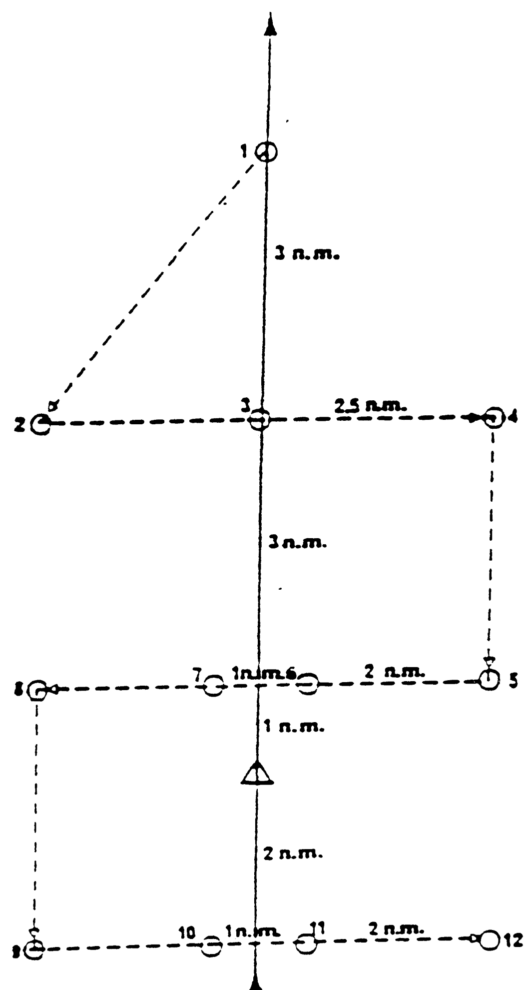
In order to assess the variability of contaminant concentrations, replicate samples and analysis should be carried out whenever possible. Analysis of replicates will allow the variance in the measured parameters to be determined. The number of replicates calculated for statistical purposes assumes that the values are normally distributed. Careful and precise positioning of each sampling site should be advised in order to minimise large variance of results introduced by a patchy distribution of sedimentary contaminants. Monitoring for assessment of temporal trends may be achieved through repeated sampling of the site of interest over the long-term.



Van Veen grab for sediment sampling (after H. Friedrich).



a. Glass liner from a gravity corer.
The sediment from the upper part may be used for trend-analyses.
b. Schematic of bottom corer.



Grid system for sediment sampling. O- sampling stations, Δ-drilling position and the arrow is pointing in the direction of the residual current. (distances in nautical miles).

Retrospective assessment of temporal changes in the accumulation of refractory contaminants in one place may be realised by the examination of contaminant concentrations in the surface and subsurface sediment cores. Samples should be taken using sampling devices which cause the least disturbance to the sediment e.g., a box corer or large diameter gravity corer (one should bear in mind that grab samplers disturb the surface of the sediment). Measurements of sediment accumulation rates by radiochemical (^{210}Pb or ^{214}Th) or other techniques are required. Sampling should be done in flat bottom basins, normally at the deepest part of the area (see also Reference Method No 20: UNEP/IOC/IAEA: Monitoring of petroleum hydrocarbons in sediments).

5.2. Cleaning of extraction thimbles

Paper extraction thimbles should be cleaned prior to sample extraction. For use in the extraction of sediment samples, the extraction can be performed in two different ways : for chlorinated pesticides and PCBs, without toxaphene, one extraction with hexane is enough, so, the extraction thimbles should be extracted with hexane only. In this case, extract the thimbles in the Soxhlet apparatus with 250 ml of hexane for 8 hours. If toxaphene is of interest, extraction thimbles will be extracted first with 250 ml of dichloromethane for 8 hours in the Soxhlet apparatus, then the dichloromethane is removed and replaced by 250 ml of hexane and extracted again for 8 hours. Doing this will avoid the mixture of the remaining of dichloromethane used for cleaning with the hexane used for the extraction which could cause some problems in the Soxhlet for the cycling of the solvent.

The use of disposable paper thimbles for the extraction procedure rather than re-usable glass fibre thimbles is recommended due to the difficulties encountered in cleaning the latter.

5.3. Extraction of sediments

5.3.1. Extraction of freeze-dried samples

Select a 50-100 g sub-sample of the sediment, weigh this sub-sample and freeze-dry it. When dried, re-weigh it and calculate the dry to wet ratio. Then pulverise the sample using a pestle and mortar and sieve it using a 125 μm stainless steel sieve. Accurately weigh about 20 g of ground sample and place it in the pre-cleaned extraction thimble. Add 1 ml of a solution of 25 pg/μl of 2,4,5 trichlorobiphenyl (PCB No 29) and 20 pg/μl of ε HCH as internal standards and extract for 8 hours in a Soxhlet apparatus with hexane (250 ml). Alternatively (or in addition), PCB congeners No 30, 121, 198 or octachloronaphthalene and PCB congeners can be used as internal standards. If toxaphene is of interest, after the extraction with hexane, exchange the flask for one containing 250 ml of dichloromethane and extract for another 8 hours. Evaporate the dichloromethane to a few ml with a rotary evaporator and add a small volume of hexane (in order to keep the compounds of interest) to the flask. Transfer the contents of the flask to the first flask rinsing again with hexane. Prepare a procedural blank by extracting an empty thimble using the same procedure as for the samples.

5.3.2. Extraction of wet samples

The sediment is thawed, sieved at 125 μm and homogenised manually with a stainless steel spatula or clean glass rod. A sub-sample of 1-2 g is weighed into a flask and placed in a drying oven at 105 °C for 24 hours, then allowed to cool to room temperature and re-weighed. Calculate the dry to wet ratio and discard the dry sediment (unless it is being used for other analysis e.g. TOC, total organic carbon).

Place a 30-40 g sub-sample of thawed, homogenised sediment into a blender. Slowly, add 100 g of anhydrous sodium sulphate (desiccant) and blend the mixture at high speed for 10 minutes. Transfer the dried sample quantitatively to the pre-cleaned extraction thimble in the Soxhlet apparatus, add the internal standard solution (see above) and apply the same extraction procedure as above. Extract the same amount of sodium sulphate as a procedural blank, making sure to add an appropriate amount of internal standard solution.

5.3.3. Example of determination of percent moisture

Many environmental measurements require the results to be reported on a dry weight basis. The percent moisture or water content in the sample is determined by weighing an aliquot, not used for analysis, of the sample before and after drying. The drying can be done by heating a few grammes (1-2 g) of the sample in an oven to constant weight.

Weigh an empty glass beaker that will be used to hold the sample while it is dried.

Empty beaker weight = 10.4417 g

Add the wet sample to the beaker and reweigh. Calculate the wet weight of the sample.

Empty beaker weight + wet sample = 12.2972 g
Wet sample weight = 12.2972 g - 10.4417 g = 1.8555 g

Dry the sample to constant weight: dry the sample for 24 hours, weigh it, dry again for 12 hours, re-weigh it, when the difference in weight is less than 5 %, it means that the sample is dried.

Empty beaker weight + dry sample weight = 10.9396 g
Dry sample weight = 10.9396 g - Empty beaker weight
Dry sample weight = 10.9396 g - 10.4417 g = 0.4979 g

Calculate the percent dry sample weight.

$$\% \text{ sample weight} = \frac{\text{Sample dry weight}}{\text{Sample wet weight}} \times 100$$

$$= \frac{0.4979}{1.8555} \times 100 = 26.8 \%$$

Calculate the percent moisture.

Water content = wet weight - dry weight
= 1.855 g - 0.4979 g = 1.3576 g

$$\% \text{ moisture} = \frac{\text{Sample water weight}}{\text{Sample wet weight}} \times 100$$

$$\% \text{ moisture} = \frac{1.3576}{1.8555} \times 100 = 73.2 \%$$

5.4. Concentration of the extract

For both extraction procedures, the extracts are concentrated in a rotary evaporator to about 15 ml. The temperature of the water bath must not exceed 30 °C. Dry the extract with anhydrous sodium sulphate (when the sodium sulphate moves freely in the flask it means that the extract is dried). Collect the dried extract in the graduated tube of a Kuderna-Danish concentrator. Concentrate the extract to approximately 5 ml with the Kuderna-Danish concentrator and adjust the volume to exactly 1 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen. The sample extract will be analysed gravimetrically for extractable organic matter (EOM) content at the 1 ml volume as a starting point. If measurements of the EOM are outside the calibration range of the

balance, the total volume of the extract is adjusted accordingly using either dilution with h evaporating under a stream of nitrogen gas.

5.5. Extractable organic matter

Before carrying out the clean-up procedure, it is advisable to determine the ex organic matter.

The EOM is determined in the following manner. On the weighing pan of an electro- evaporate a known volume of the sediment or biota extract (up to 100 µl) and weigh the residu precision of about ± 1 µg. If the residue is less than 2 µg, pre-concentration of the original e required. The quantity of EOM is:

$$\text{EOM } (\mu g/g) = \frac{\text{Weight of residue } (\mu g) \times \text{volume of the extract (ml)} \times 1000}{\text{volume evaporated } (\mu l) \times \text{quantity of sample extracted (g)}}$$

Note that extreme care must be taken to ensure balance and pans are clean, dry and obtain accurate readings at the ± 1 µg level. A small hot plate is used to warm pans and for thus keep these instruments dry after solvent cleaning. If no electro-balance is available, a volume of the extract can be transferred into a clean pre-weighed beaker. The solvent is ev with dry and clean nitrogen until a constant weight of about 1 mg is reached. Calculate the a "lipids" in the sample taking into account the volume of the lipid extract which was dried.

Example of calculation of E.O.M.

The extractable organic matter content of a sample is operationally defined as the v material extracted with the solvent employed (H.E.O.M. in case hexane is used as solvent). A of the sample extract is taken (few µl), the solvent is evaporated and the residue is we determine the quantity of lipids extracted in the aliquot and from it to the total sample. The re normally reported in mg lipids per gramme dry weight extracted.

A 1 µl aliquot is removed from a 2.5 ml sample extract for determination of E.O. 1 µl aliquot is evaporated on the pan of an electro-balance and the residue is weighe determinations are made and the average taken.

Measurements :

Sample dry weight extracted: 4.443 g
Total volume of the extract: 2.5 ml
Sample aliquot removed: 1 µl
(1) Weight of a 1 µl aliquot after solvent evaporation: 32.2 µg
(2) Weight of a 1 µl aliquot after solvent evaporation: 32.1 µg
(3) Weight of a 1 µl aliquot after solvent evaporation: 32.3 µg
Average weight of a 1 µl aliquot : 32.2 µg

Total volume of the extract: 2.5 ml

Total quantity of lipids in the sample:

$$32.2\ \mu g/\mu l \times 2.5\ \text{ml} \times \frac{1000\ \mu l}{\text{ml}} = 80500\ \mu g \text{ or: } 80.5\ \text{mg}$$

With 4.443 g of sample extracted:

80.5 mg/ 4.443 g = 18.1 mg lipids/g

5.6. Clean-up procedure and fractionation

Purposes of the clean-up: removal of lipids, whenever present at a significant amount; removal of elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation.

5.6.1. Sulphur and sulphur compounds removal

Elementary sulphur and sulphur compounds such as mercaptans should be removed from the extract. This could be done by using either mercury or activated copper.

a) Mercury method.

Add one drop (a few ml) of mercury to the sediment extract and shake vigorously for one minute. Centrifuge and carefully recover and transfer the extract in another tube with a Pasteur pipette. If the mercury is still tarnished, repeat the treatment with another drop of mercury, shake, transfer the hexane into another tube. Repeat this treatment until the mercury stays brilliant in the extract. Rinse the mercury with 5 ml of hexane and combine the extracts. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of nitrogen.

Cleaning of mercury:

Caution: When removing mercury from the sample, always use a plastic tray to keep the glassware in and work under a fume hood.

Fit a folded filter paper in a 10 cm diameter conical glass funnel and fix the funnel over a 250 ml glass beaker. Using a needle, make a small hole in the bottom of the filter paper. Carefully put the mercury onto the funnel. The mercury flows through the small hole in the filter paper leaving the solid impurities on its surface. The mercury collected is washed three times by shaking it carefully with dichloromethane and by removing dichloromethane layer with the help of a clean glass syringe. Allow the rest of dichloromethane evaporate and store the clean mercury in a thick walled glass bottle with a ground glass stopper. In order to avoid escape of mercury vapour, store the mercury under methanol.

Another way of cleaning the mercury involves sucking the dirty mercury through a capillary tube, such as a Pasteur pipette, connected to a guard-flask and then to a vacuum pump. The mercury will pass through the Pasteur pipette and will be collected and cleaned in the guard-flask. Then it should be transferred into a thick wall glass bottle with a ground glass stopper. The mercury is covered with a layer of methanol to protect it from oxidation.

b) Activated copper method.

Procedure for the preparation of activated copper: Activated copper can be prepared as follows : In a 250 ml beaker dissolve 10 g $CuSO_4.5H_2O$ in 100 ml distilled water and 4 ml 2 N HCl and keep the solution cold for 20 minutes (an ice bath over a magnetic stirrer is useful). Make a slurry of 3 g powered Zinc and 5 ml distilled water. Slowly add the slurry to the $CuSO_4$ mixture while it is being stirred over the ice. Remove the beaker and let it stand on the bench for 1 hour at room temperature. Then stir gently until all the blue of the $CuSO_4$ has disappeared from the solution. The Cu forms a red flocculent precipitate. Treat it gently to avoid breaking the particles. Decant most of the liquid allowing the very fine Cu particles to escape. Wash the larger particles several times with distilled water. Never allow it to be exposed to air as it will oxidise the surface and prevent it from reacting with sulphur. The activated Cu is now ready for use or can be stored in the freezer until needed.

Add 1 g of activated copper to the sediment extract and shake for 1 minute. Centrifuge and carefully recover the extract. If the copper turns black, repeat the procedure. Rinse the copper with 5 ml of hexane, centrifuge and combine the extracts. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of pure nitrogen.

5.6.2. Fractionation

An adsorption chromatography step is used to remove interfering lipids and to fractionate the extract into classes of compounds. Many variations of adsorption chromatography clean-up procedures have been published to date. Four procedures are reported here in order of increasing complexity.

Preparation of the columns: Glass burettes (1 cm diameter) with Teflon stopcocks make convenient adsorption columns. If not equipped with sintered glass disks, the column can be plugged with pre-cleaned cotton or glass wool. Prepare separate columns for each sample and blank determination. The column is partially filled with hexane. The appropriate amount of sorbent is mixed with hexane in a small beaker to form a slurry. A glass funnel and a glass rod are used to pour the adsorbent into the column. Several rinses with hexane are necessary to fill the column to the desired height. Tap with a pencil or a hard silicone tube against the column in order to settle the adsorbent into an even bed. Flush the material adhering to the wall of the column down to the bed with solvent. Prepare each column freshly immediately before use. Never let the column get dry.

5.6.2.1. Florisil

A Florisil column is used for this fractionation which is prepared in the following way. The Florisil should be pre-extracted in the Soxhlet apparatus to remove any contaminants, using methanol or dichloromethane for 8 hours, followed by hexane for another 8 hours. It is then dried in an oven. Activation is achieved by heating the dried Florisil at 130 °C for 12 hours. It is then partially deactivated with 0.5 % water by weight and stored in a tightly sealed glass jar with ground glass stopper. The water should be well mixed into the Florisil and the mixture should be allowed to equilibrate for one day before use. The activation/deactivation procedure should be carried out one day before use. A 1 cm burette with Teflon stopcock is plugged with pre-cleaned glass wool. A column with a sintered glass disk could also be used. 17 grammes of Florisil are weighed out in a beaker and covered with hexane. A slurry is made by agitation and poured into the glass column. The Florisil is allowed to settle into an even bed and any Florisil adhering to the column is rinsed down with hexane. The solvent is drained to just above the Florisil bed. It should be rinsed with a further 5 ml of hexane, one gramme of anhydrous sodium sulphate is added to the top of the column in order to protect the surface of the Florisil from any disturbance. The column should never run dry. Individual columns should be prepared immediately before use and a new column of Florisil used for each sample.

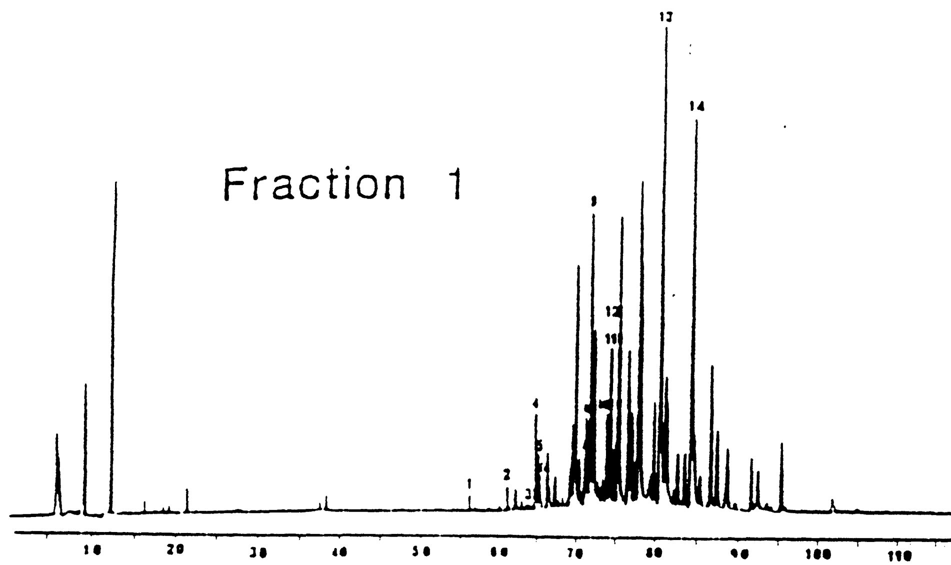
The extract, reduced to 1 ml, is put onto the Florisil column. It is carefully eluted with 70 ml of hexane and the first fraction collected. Then the column is eluted with 50 ml of a mixture containing 70 % of hexane and 30 % of dichloromethane and the second fraction collected. The third fraction will be eluted with 40 ml of pure dichloromethane.

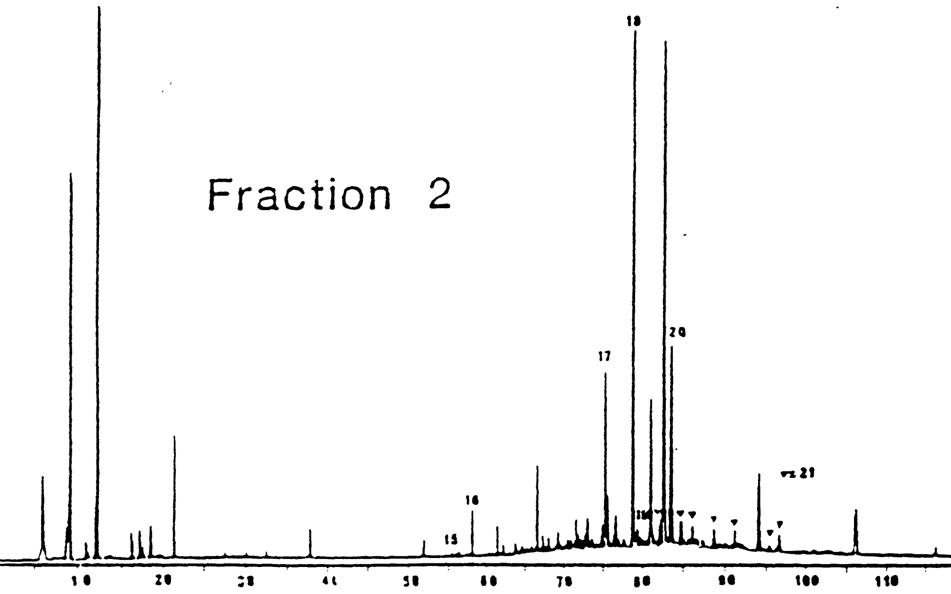
Fraction one will contain the PCBs, pp' and op DDE and some other pesticides such as HCB, aldrin, heptachlor, DDMU.

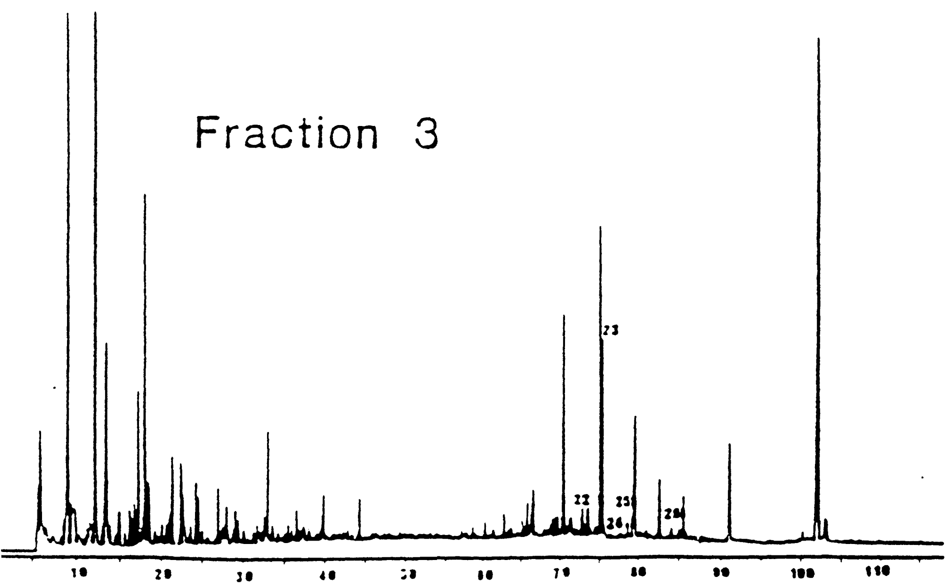
Fraction two will contain the DDTs, DDDs, most of the toxaphene, and some pesticides such as the HCH isomers and chlordane components.

Fraction three will contain mainly dieldrin, endrin and endosulfan components. Typical chromatograms obtained are shown below.

cGC-ECD organohalogen analyses







Retention time (min.)

Peak Number	Compounds
1	H.C.B
2	Internal Standard 2, 4, 5 trichlorobiphenyl (IUPAC No : 29)
3	Heptachlor
4	2,2',5,5' tetrachlorobiphenyl (IUPAC No : 52)
5	2,2',4,5' tetrachlorobiphenyl (IUPAC No : 49)
6	Aldrin
7	DDMU
8	op DDE
9	2,2',4,5,5'pentachlorobiphenyl (IUPAC No : 101)
10	2,2',3',4,5 pentachlorobiphenyl (IUPAC No : 97)
11	pp' DDE
12	2,2',3,3',6,6' hexachlorobiphenyl (IUPAC No : 136)
13	2,2',4,4',5,5' hexachlorobiphenyl (IUPAC No : 153)
14	2,2',3,4,4',5' hexachlorobiphenyl (IUPAC No : 138)
15	Alpha-HCH
16	Lindane
17	op DDD
18	pp' DDD
19	op DDT
20	pp' DDT
21	Toxaphene
22	Alpha-endosulfan
23	Dieldrin
24	Endrin
25	Beta-endosulfan
26	Endosulfan sulfate

5.6.2.2. Gel permeation chromatography

Low pressure GPC can be used as an alternative clean-up technique to remove high molecular weight co-extractable lipidic material from polycyclic aromatic compounds and halogenated aromatics. Concurrently, elemental sulphur could be also removed from the whole organic extract.

The main feature of the semi-preparative-GPC as a clean-up technique relies on the compatibility of this analytical procedure with labile components of the extract (i.e. DDTs, chlorinated cyclohexadiene derivatives) which are not stable in other types of extract clean-up procedures. Further, GPC as a clean-up technique has already been automated, enabling a high sample throughput, taking into account the short analysis time involved.

The GPC retention mechanism may involve adsorption, partition and size exclusion mechanisms. The predominance of one mechanism over the others is largely determined by the choice of the mobile phase and the pore size of the packing. In the case of GPC packings with large pore size (1000-2000 daltons) size exclusion and adsorption mechanisms prevail (Bio-Beads SX-3 using cyclohexane, dichloromethane-hexane, dichloromethane-cyclohexane, toluene-ethylacetate and ethylacetate-cyclohexane) (Ericksson *et al.*, 1986). On the other hand, when smaller pore sizes (400 daltons) are used in combination with highly polar solvents, (THF, DMF) size exclusion predominates (Lee *et al.*, 1981). While using the first approach, a chemical class fractionation could be obtained, however, if smaller pore sizes are used it should be combined with another fractionation technique (i.e. adsorption chromatography) to achieve this selectivity. It has yet to be demonstrated that using GPC as a single clean-up step produces a completely clean extract for GC-ECD determination. Nevertheless, taking into account the increasing availability of high-resolution low molecular weight exclusion packings, they could definitively integrate fractionation and clean-up in a single step.

Low resolution packings (Sephadex LH and Bio-Beads SX, 200-400 mesh size) are the most widely used because they are inexpensive and afford relatively high sample loading (500 mg in 10 mm i.d. columns). The implementation of low resolution GPC requires a solvent delivery system and a UV detector and may be useful. For method development, it is advisable to inject a broad range of standard compounds covering the whole range of molecular weights of the analytes to be determined in order to determine the cut-off points to fractionate real samples. Reported recoveries of PCBs and PAHs range from 60 to 80 % for the concentration level (ng) injected. (Fernandez and Bayona, 1992).

5.6.2.3. Alumina and HPLC (silica column)

The first step in this clean-up procedure is an adsorption step using an alumina column to remove most of the lipid material. Prepare an alumina column (4 x 0.5 cm i.d., made from a Pasteur pipette). Apply the concentrated extract to the top of the column and elute with 10 ml hexane. Concentrate the eluate to about 200 µl. It is followed by a second step to more completely remove interfering compounds and at the same time to separate the compounds of interest into different fractions, containing aliphatics, PCBs, PAHs, pesticides and toxaphene. Between 20 and 200 µl of the extract (after alumina clean-up) are eluted on a stainless steel column (200 x 4 mm i.d.), packed with Nucleosil 100-5 with n-pentane, 20 % dichloromethane in n-pentane and finally dichloromethane. The eluate is collected in fractions containing 1) n-hydrocarbons, 2) PCBs, 3) PAHs and toxaphene, 4) pesticides and toxaphene and 5) acids, etc. (polar compounds). The size of the fractions has to be determined with standard solutions containing the compounds of interest, collecting the eluate in 0.5 ml fractions. Each fraction is then analysed by GC-ECD. Full details have been given in the literature (Petrick *et al.*, 1988 and IOC, 1993).

5.6.2.4. High pressure chromatography

High pressure liquid chromatography (HPLC) columns packed with microparticles are available and have the advantages of high reproducibility, low consumption of solvents, high efficiency and high sample loading capacity.

This method can be used to separate fractions containing aliphatic hydrocarbons, PCBs a
aromatic hydrocarbons from interfering compounds. These fractions can then be analysed separat
for their constituents by GC-FID and/or GC-ECD.

HPLC methods have been developed using synthetic solutions of n-alkanes, PAHs, pesticid
PCBs and toxaphene and have been applied to samples in which interfering substances were prese
in such high concentrations as to render the analysis of HC and PCBs extremely difficult without t
clean-up procedure (e.g. sediments and biological tissues with OCs in the ng/g range). The samp
are eluted with n-hexane, subjected to clean-up over alumina, concentrated down to 20-200 µl a
treated by HPLC. With the use of n-hexane, n-pentane and 10 %, 20 % and 50 % dichloromethane
n-hexane, respectively, the following five fractions are obtained : 1) n-hydrocarbons and alkenes,
PCBs and alkylbenzenes; 3) PAHs and toxaphene, 4) pesticides, 5) acids, etc.(polar compound
(Petrick *et al.* 1988).

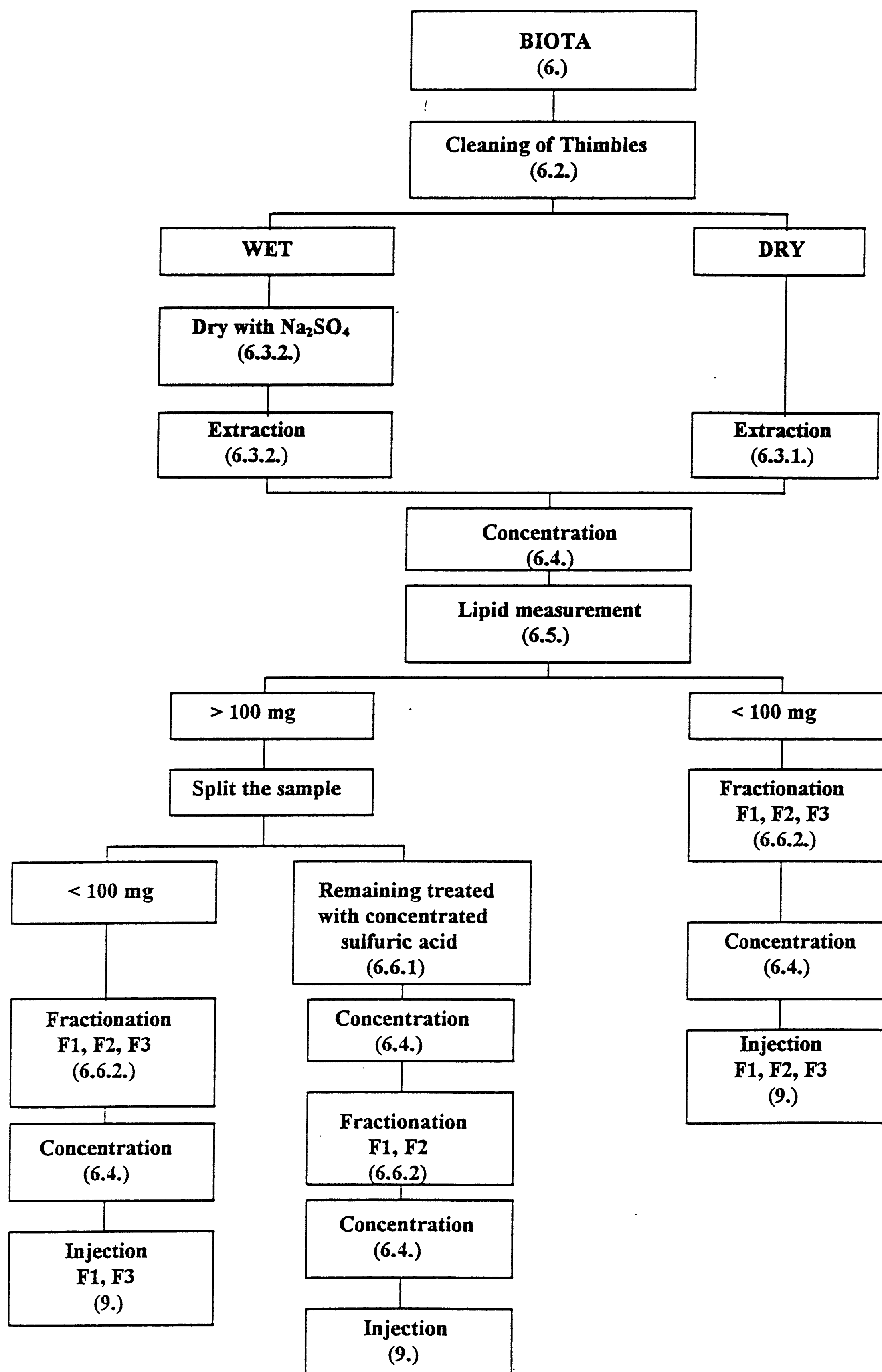


Diagram of the extraction procedure for biota samples.

6. BIOTA

6.1. Sampling

Organisms accumulate many contaminants from their environment (i.e., from sea water, suspended particulate matter, sediment and food). Field and laboratory studies have shown that contaminant concentrations in some marine plants and animals reflect concentrations in their environment. Scientists use this process (termed bio-accumulation) to assess marine contamination resulting from human activity (e.g., pipeline discharges, dumping from ships).

There are problems with using biota as bio-accumulators (bio-indicators). For example, tissues from individuals of a species exposed to the same contaminant concentration may contain different levels of contamination after the same exposure time. These deviations reflect individual differences in factors such as age, sex, size, and physiological and nutritional states. Also, various species show different contaminant concentrations following identical exposure; differences in elimination rates may partially account for this. These factors must be considered when planning a monitoring programme in order to control their effects on the precision of the analysis (by reducing the variances). Variance reduction is necessary in order to detect smaller differences in mean contaminant concentrations observed in monitoring programmes.

For proper sampling and sample preparation, refer to Reference Method No 6 "Guidelines for monitoring chemical contaminants in the sea using marine organisms" and Reference Method No 12 Rev.2 " Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons".

6.2. Cleaning of extraction thimbles

As for extraction of sediment samples, thimbles should be extracted first with the same solvent used for the extraction of the sample. As the extraction of biota sample is achieved with hexane, a pre-extraction of these thimbles is made with 250 ml of hexane for 8 hours in the Soxhlet apparatus.

6.3. Extraction of tissues

6.3.1. Extraction procedure for freeze-dried samples.

Take a 50 to 100 g fresh weight sub-sample from the sample. Weigh this sub-sample and freeze-dry it. When the sub-sample appears to be dry, re-weigh it and freeze-dry it for a further 24 hours and then re-weigh it. If the difference between the two dry weights is greater than 5 %, continue the freeze-drying process. Special care must be taken to ensure that the freeze-drier is clean and does not contaminate the samples. The freeze drying procedure should be tested by drying 100 g Na_2SO_4 as a blank and extracting this as a sample. Pulverise the freeze-dried sub-sample carefully using a cleaned pestle and mortar. Accurately weigh about 5 to 10 g of this pulverised material, note the exact weight to be extracted, and place it into a pre-cleaned extraction thimble in a Soxhlet apparatus. The size of the sub-sample should be adjusted so that about 100 mg of extractable organic matter ("lipid") will be obtained. Smaller sub-samples should be used if residue concentrations are expected to be high. Add a known amount of internal standard to the sub-sample in the thimble before Soxhlet extraction. It is important to spike the sample at levels that are near to that of the analyte concentrations in the samples. If, in the end, the analyte and the internal standard concentrations do not fall within the established calibration range of the GC-ECD, the analysis must be repeated. Consequently, it may be advisable to perform range-finding analysis for samples of unknown character beforehand. Candidate internal standards are the same as for sediment samples (see 5.3.). Add about 200 ml of hexane or petroleum ether to the extraction flask with a few carborundum boiling chips, and extract the sample for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Extract an empty thimble as a procedural blank, making sure to spike it with internal standards in the same fashion as the sample. If unacceptable procedural blanks are found, the source

of contamination must be identified and eliminated rather than subtracting high blank values from the analytical results.

6.3.2. Extraction procedure without freeze-drying

Select a 25 to 100 g fresh weight sub-sample and place in a blender. Add anhydrous sodium sulphate to the sample, manually homogenise and determine whether the sample is adequately dried. If not, more sodium sulphate should be added until a dry mixture is obtained. Normally, 3 times by the sample weight used should be enough. Once this has been achieved, blend the mixture at high speed for 1 or 2 minutes until the mixture is well homogenised and the sample appears to be dry. Transfer the mixture to a pre-cleaned extraction thimble, add internal standards as described above and extract the dehydrated tissue with about 200 ml hexane or petroleum ether for 8 hours in a Soxhlet apparatus, cycling 4 to 5 times per hour. Extract the same amount of sodium sulphate as the procedural blank, making sure to add internal standards in the same fashion as the sample.

6.4. Concentration of the extract

Refer to section (5.4.)

6.5. Extractable Organic Matter (EOM)

Refer to section (5.5.)

6.6. Clean-up procedure and fractionation

6.6.1. Removal of lipids by concentrated sulphuric acid

If the lipid content of the extracts is higher than 100-150 mg, a preliminary step for the removal of the lipids is necessary before further sample purification. This can be carried out by using concentrated sulphuric acid. Treatment with sulphuric acid is used when chlorinated hydrocarbons are to be determined. However, sulphuric acid will destroy dieldrin and endrin so that an aliquot of the untreated extract must be set aside for the determination of these compounds.

CAUTION : During all this procedure it is very important to wear safety glasses.

Take an aliquot of the concentrated extract, containing about 200 mg of "lipids", transfer into a separatory funnel and add to this extract enough hexane in order to dilute the sample (40 to 50 ml should be enough), this will allow recovery of the hexane after acid treatment, because if the sample is too concentrated, the destroyed "lipids" will become almost solid and it will be difficult then to recover the hexane from this solid mass. Add 5 ml concentrated sulphuric acid to the extract and tightly fit the glass stopper and shake vigorously. Invert the funnel and carefully vent the vapours out through the stopcock. Repeat this procedure for several minutes. Place the separatory funnel in a rack and allow the phases to separate. Four or five samples and a spiked blank are convenient to process at one time. The extract should be colourless. Recover the hexane phase into a glass beaker. Dry with sodium sulphate and transfer the hexane into a Kuderna-Danish concentrator. Reduce the volume of the extract by evaporating the solvent with a gentle stream of pure nitrogen to about 1 ml.

6.6.2. Fractionation

Refer to section (5.6.2.)

7. GAS CHROMATOGRAPHIC DETERMINATIONS

7.1. Packed column GC

7.1.1. Gas chromatographic conditions

The samples are analysed on a gas chromatograph equipped with an electron capture detector. The glass column (1.8 m x 0.4 cm i.d.) should be filled with acid-washed and dried Chromosorb W (HP) 80-100 mesh, coated with 5 % OV-101 or with mixture consisting of 1.5 % OV-17 + 1.95 % OV-210. It is easy to prepare a phase in the laboratory by dissolution of the liquid phase in the appropriate solvent and transferring this into a flask containing the solid support, then using a rotary evaporator the solvent is eliminated slowly. In order to obtain a good phase homogeneity it is recommended to use 10 % of the liquid phase on the support (10 % OV-101 on Chromosorb W (HP) 80-100).

The column is held at 200 °C to give pp' DDT a retention time relative to Aldrin of 3.03. The injector is held at 200 °C and the detector at 300 °C (for ^{63}Ni only). The carrier gas flow is set to 30 ml per minute.

7.1.2. Column preparation

The following procedure is recommended for the preparation of columns for the gas chromatograph.

- Fill the column with concentrated HCl and leave it for one hour.

- Wash with distilled water, then with acetone and finally with toluene.

- Fill the column with a toluene solution of Hexamethyldisilazane (HMDS, 10 %).

WARNING: use fume hood and do not touch the HMDS reagent.

- Wash the column with toluene, then with methanol and finally with acetone.

- Dry the column either with compressed air, or in an oven.

- Put about 10 mm of glass wool into the bottom end of the column and weigh the column.

- Attach a funnel to the inlet of the column and fill at least half a coil with the packing material before connecting the other end to a vacuum pump.

- Fill the column by gentle tapping. If the packing material gets stuck before the column is filled, disconnect the vacuum pump and put the column in an oven at 100 °C for a few minutes and then continue the process with the warm column.

- When the column is full, put about 10 mm glass wool in the inlet end.

- Weigh the packed column and label it. Keep records of the weight of packing material and the column performance.

The column has to be conditioned before it is connected to the detector. Connect the inlet to the injector in a gas chromatograph (but do not connect the detector end) and set a carrier gas flow of about 60 ml per minute. Heat the column to the allowed maximum temperature for the stationary phase. This temperature should be 50 °C above the normal working temperature of the column. The column should be conditioned overnight.

7.1.3. Column test

When the column has been connected to the detector, the carrier gas flow is set to 30 ml per minute for a column with 4 mm internal diameter. The column performance is then measured according to the criteria of the "number of theoretical plates" for a specific compound and can be achieved according to the following procedure.

- Set injector and detector temperatures at 200 and 300 °C respectively and the column oven temperature at 180 °C.

- Inject pp' DDT standard and measure the retention time (Tr). Adjust the column temperature to get a pp' DDT retention time relative to Aldrin of 3.03.

- Measure the width of the pp' DDT peak at its half height ($b_{1/2}$), in minutes and the retention time (Tr) also in minutes.

- Calculate the number of theoretical plates using the formula:

$$N = 5.54 \left(\frac{Tr}{b_{1/2}}\right)^2$$

- A parameter which is independent of the column length is the height equivalent to a theoretical plate (HETP):

$$HETP = \frac{L}{N}$$

Where L is the column length. Adjust the flow rate of the carrier gas to obtain optimum performance. The HETP should be as low as possible (i.e. the number of theoretical plates should be as great as possible).

7.1.4. Electron capture detector

High-energy electrons, emitted by a radioactive source within the detector (e.g. a ^{63}Ni foil), are subject to repeated collisions with carrier gas molecules, producing secondary electrons. These electrons, upon returning to their normal state, can be captured by sample molecules, eluting from a GC column. The resulting reduction in cell current is the operating principle of an electron capture detector. The detector current produced is actually a non-linear function of the concentration of electron-capturing material. However, the useful linear range of an ECD may be greatly improved if the instrument is operated at a constant current, but in a pulsed mode, i.e. with short voltage pulses being applied to the cell electrodes. The current in the cell is kept constant by varying the frequency of the pulses.

The optimum flow for an ECD is about 30 ml per minute. Contamination of the detector (and thus lower sensitivity) may result from high-boiling organic compounds eluting from the column. Periodic heating to 350 °C may overcome this problem. The ^{63}Ni ECD can be used at 320 °C under normal operational conditions, in order to limit such contamination.

7.1.5. Quantification

PCBs represent a complex mixture of compounds that cannot all be resolved on a packed column. Also there is no simple standard available for their quantification. Each peak in a sample chromatogram might correspond to a mixture of more than one individual compound. These difficulties have led to the recommendation of various quantification procedures. The usual method to

quantify PCBs is to compare packed-column chromatograms of commercially available industrial formulations (Aroclors, Clophens, Phenoclors) with the sample chromatogram. Most commonly, it is possible to match one single formulation, such as Aroclor 1254 or Aroclor 1260 with the sample chromatogram. An industrial formulation (or mixture of formulations) should be chosen to be as close a match as possible and in the case of sample extracts from sediment or organisms, Aroclor 1254 and Aroclor 1260 are most frequently chosen.

For the second fraction obtained on Florisil separation, it is possible to quantify DDTs after comparison with the retention times of peaks in the sample chromatogram to those in the corresponding standard, the peak heights (or peak areas) are measured and related to the peak height (or peak area) in the standard according to the formula:

$$[\text{Concentration}] = \frac{h \times C \times V \times 1000}{h' \times V(inj) \times M \times R} \text{ ng/g (or pg/g)}$$

Where:

V= total extract volume (ml)
M= weight of sample extracted (g)
h= peak height of the compound in the sample
h'= peak height of the compound in the standard
C= quantity of standard injected (ng or pg)
V(inj)= volume of sample injected (µl)
R= Recovery of the sample

7.2. Capillary column GC

7.2.1. Gas chromatographic conditions

- Gas chromatograph with a split/splitless injection system, separate regulation system for inlet and column pressures and temperatures; multi-ramp temperature programming facilities (preferably microprocessor controlled), electron capture detector interfaced with the column with electronic control unit and pulsed mode facilities. An integrator with a short response time (0.25 s) is essential.

- Narrow-bore (0.22 mm internal diameter), 25 m long, fused silica open tubular column, coated with SE-54 (0.17 µm film thickness, preferably chemically bonded) with sufficient resolution to separate the relevant peaks in the standards provided for PCB analysis.

- Carrier gas should be high purity H_2. If this is not available or if the GC is not equipped with a special security system for hydrogen leak, He may be used. Gas purification traps should be used with molecular sieves to remove oxygen, moisture and other interfering substances.

- High purity nitrogen gas (99.995 %) as ECD make-up gas can be used (Argon/methane high purity gas is another option).

Conditions :

- H_2 or He carrier gas at inlet pressure of 0.5 to 1 Kg/cm^2 to achieve a flow rate of 1 to 2 ml/min.

- Make-up gas N_2 or Ar/CH_4 at the flow rate recommended by the manufacturer (between 30 and 60 ml/min.).

- ECD temperature: 300 °C

- Injector temperature: 250 °C

- Septum purge: 1 ml/min.

- Injector purge 20 ml/min. (valve to be opened 50 s after injection)

- Temperature programme: 70 °C for 2 min., then increase at a rate of 3 °C/min. up to 260 °C, then maintain at isothermal conditions until the end of the analysis (15 to 20 min.)

7.2.2. Column preparation

Fused silica columns are the columns of choice for their inertness and durability (they are extremely flexible). They are made of material that is stable up to 360 °C. The 5 % phenyl methyl silicone gum (SE-54) liquid phase, is present as a thin, (0.17 µm), uniform film which can tolerate temperatures up to 300 °C. SE-54 is relatively resistant to the detrimental effects of solvents, oxygen and water, at least at low temperatures. These columns are even more resistant and durable if the liquid phase is chemically bonded to the support by the manufacturer.

For GC/MS work, it is advised to restrict the film thickness to 0.17 µm because with thicker films some of the phase could be released, resulting in an increase of the noise signal in the GC/MS.

The flexible fused silica columns can be conveniently connected directly to the inlet and outlet systems without the transfer lines used in conventional glass capillary chromatography which often lead to increased dead volume. Low bleed graphite or vespel ferrules provide a good seal.

The presence of extraneous peaks and elevated baseline drift will result in poor detector performance. This can be caused by components which elute from the column, such as residual solvents and low molecular weight liquid phase fractions on new columns and build-up of later eluting compounds on old columns. Conditioning is a necessary step to remove these contaminants. New columns are connected to the inlet (while left unconnected to the detector). Columns are flushed with carrier gas at low temperature for 15 min. to remove the oxygen, then heated at 70-100 °C for 30 min. and finally at 170 °C overnight. The column can be then connected to the detector. Old columns can be heated directly to elevated temperatures overnight. The final temperature is selected as a compromise between time required to develop a stable baseline and expected column life. Thus, it may be necessary for older columns to be heated to the maximum temperature of the liquid phase resulting in shorter column life. The temperature of the ECD, when connected to the column, should always be at least 50 °C higher than the column, in order to avoid condensation of the material onto the detector foil. It is essential that carrier gas flows through the column at all times when at elevated temperatures. Even short exposure of the column to higher temperature without sufficient flow will ruin the column.

CAUTION : if H_2 is used as a carrier gas, position the column end outside of the oven to avoid explosion risk.

7.2.3. Column test

A column performance test should be carried out at regular intervals according to the procedure described in 7.1.3.

The column remains in optimum condition as long as the liquid phase exists as a thin, uniform film. The quality of the film at the inlet side may be degraded as a result of repeated splitless injections. Decreased column quality may be remedied by the removal of the end of the column (10 to 20 cm) at the inlet side. Chemically bonded liquid phases require less maintenance.

7.2.4. Electron capture detector

Refer to section 7.1.4.

The optimum flow for an ECD (30 to 60 ml/min.) is much higher than carrier gas flow through the column of one or two ml/min. Thus an additional detector purge flow is necessary (N_2 or Ar/CH_4). Once leaving the outlet of the column, the compounds have to be taken up into an increased gas flow in order to avoid extra-volume band broadening within the detector. Thus, the detector purge flow also serves as the sweep gas.

7.2.5. Quantification

The most widely used information for identification of a peak is its retention time, or its relative retention time (i.e., the adjusted retention time relative to that of a selected reference compound). Retention behaviour is temperature dependent and comparison of retention times obtained at two or more temperatures may aid in determining a peak's identity. However, retention times are not specific and despite the high resolution offered by capillary columns, two compounds of interest in the same sample may have identical retention times.

One way of using retention indexes could be to inject di-n-alkyl-phthalates such as a mixture containing di-n-methyl-phthalate, di-n-ethyl-phthalate, di-n-propyl-phthalate, di-n-butyl-phthalate, di-n-hexyl-phthalate and di-n-heptyl-phthalate, which will cover the elution range from 70 °C to 260 °C. An arbitrary index of 100 is given to the di-n-methyl phthalate, 200 to the di-n-ethyl phthalate, and so on up to 700 to the di-n-heptyl phthalate, it is possible to identify all chlorinated pesticides by a proper retention index. This will be used also for unknown compounds which can be found easily on the GC/MS using the same index and so, identified. (Villeneuve J.P. 1986).

For quantification refer to section 7.1.5.

8. COMPUTERIZED GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

8.1. Operating conditions

The chemical ionisation source of a mass spectrometer can be used to produce negative ions by electron capture reactions (CI-NI-MS) using a non-reactive enhancement gas such as methane or argon. CI-NI has the advantage of being highly selective, permitting the detection of specific compounds in complex matrices. Under CI-NI conditions, methane (99.99 %) is used as the reagent gas. Samples are introduced through a SE-54, 30 m x 0.25 mm i.d., fused silica column. The film thickness used is 0.17 µm in order to minimise the bleeding of the phase into the system. Helium is used as carrier gas with an inlet pressure of 13 psi, which gives a carrier flow of 1.5 ml/min. or a gas velocity of 44 cm/sec.

The temperature of the injection port is held at 250 °C.

The temperature of the source is set at 240 °C, the quadrupole at 100 °C and the interface at 285 °C.

Injections of 1-3 µl are made in the splitless mode.

The temperature programme of the oven starts at 70 °C, for 2 minutes, then it is increased at 3 °C/min. to 260 °C and kept under isothermal conditions for 40 minutes.

File : C:\HPCHEM\1\DATA\AR1254.D
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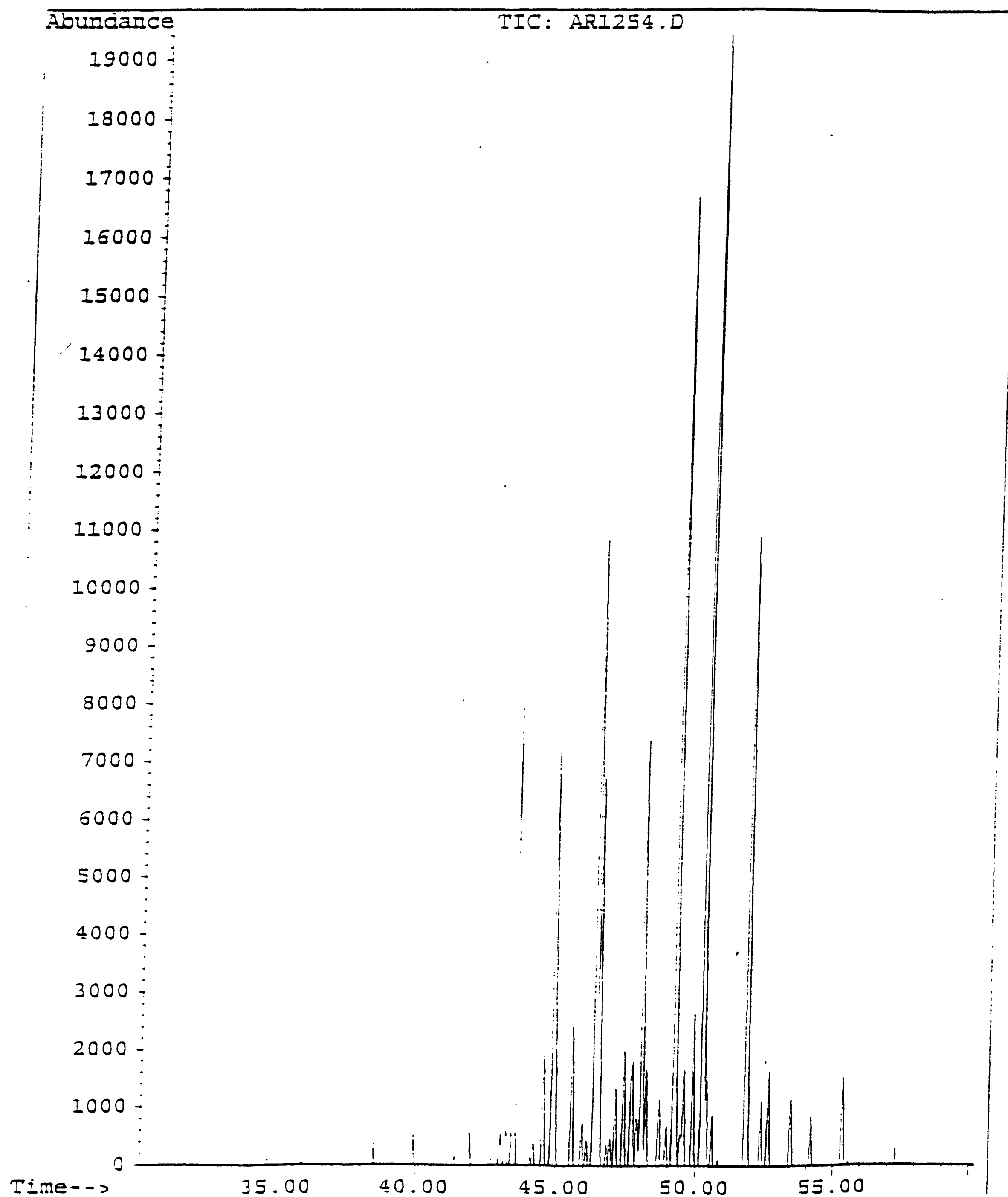
Acquired : 12 Jul 95 8:02 am using AcqMethod OC
Instrument : 5989B
Sample Name: standard ar1254
Misc Info :
Vial Number: 1



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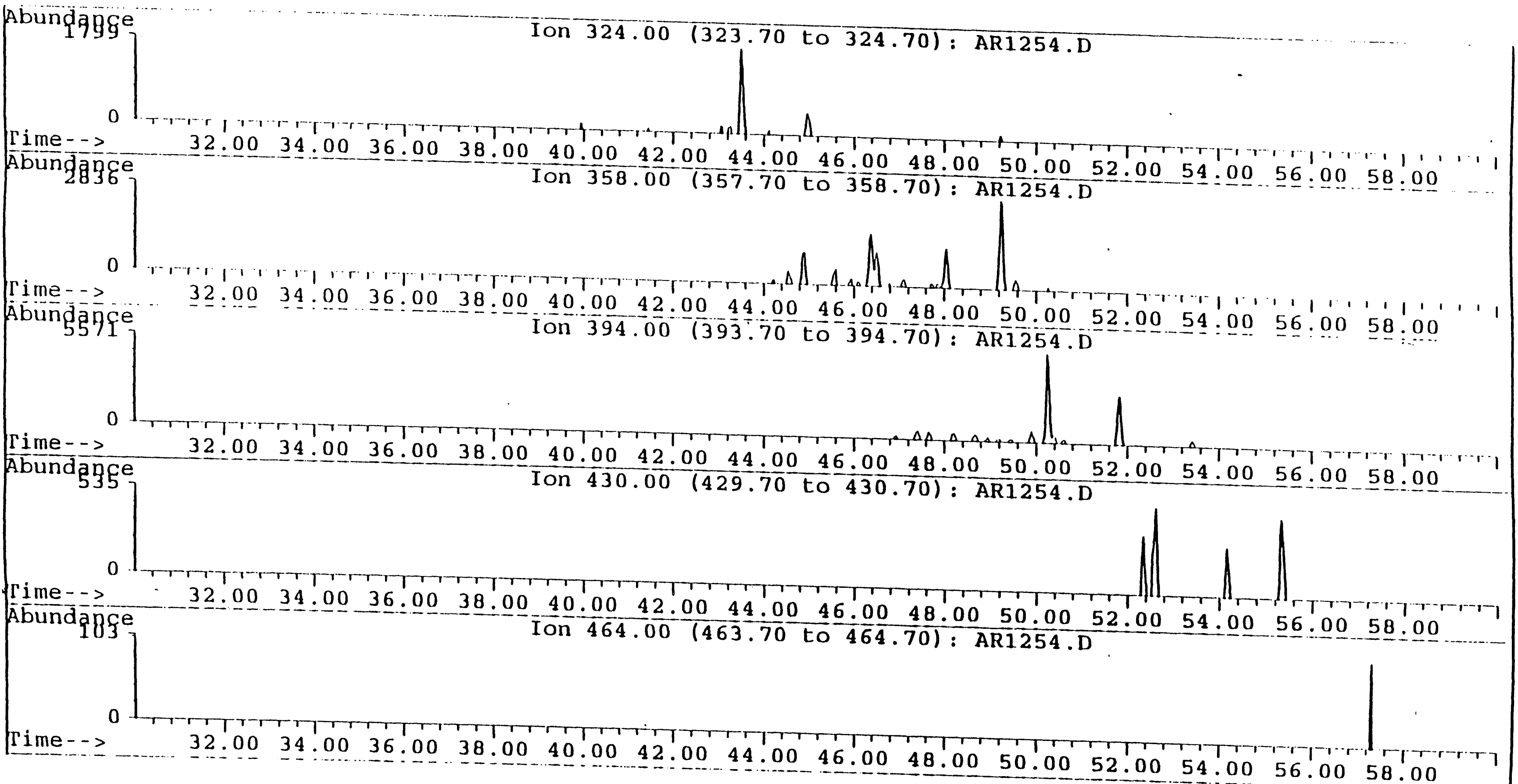

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Instrument : 5989B
Sample Name: standard ar1260
Misc Info :
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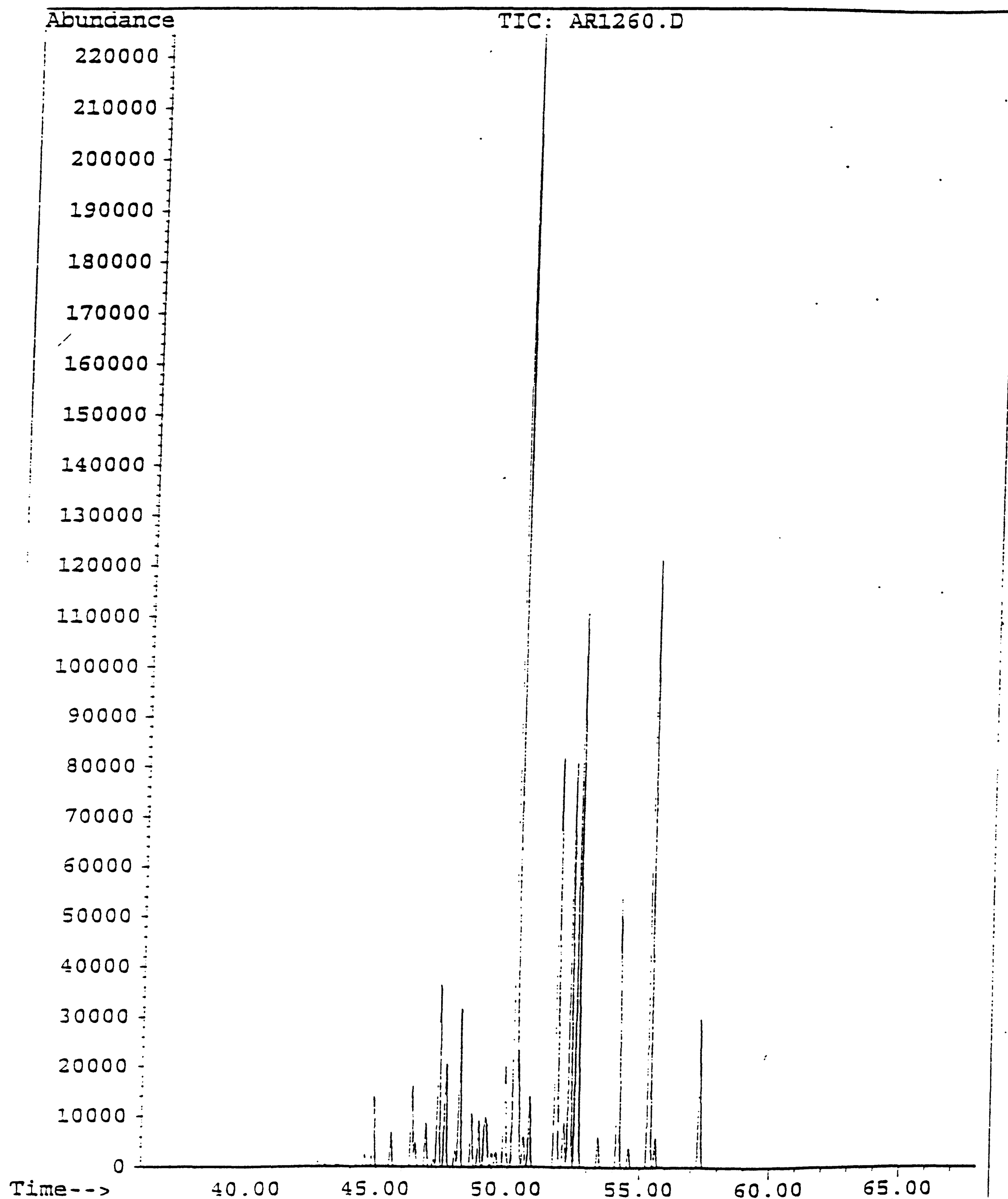

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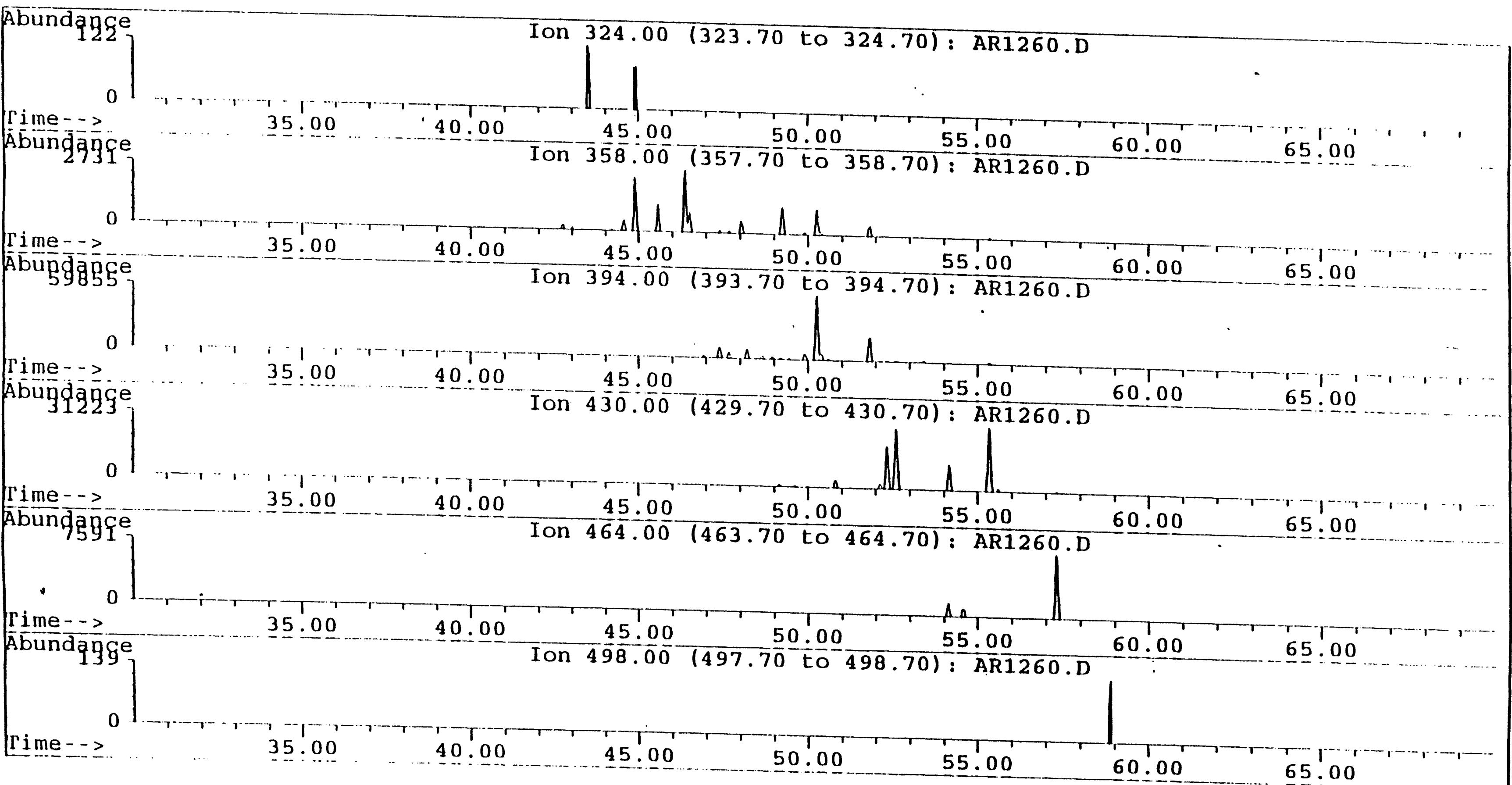
Instrument : 5989B
Sample Name: standard ar1260
Misc Info :
Vial Number: 1



8.2. **Example of a selected ion monitoring programme useful for quantitative analysis of chlorinated compounds.**

Compounds	Fraction No on Florisil	Retention Time (min.)	Target Ion (daltons)
HCB	1	37-38	284
Heptachlor	1	44-45	266
Aldrin	1	46-48	237
op DDE	1	51-53	246
Transnonachlor	1	52-54	444
pp' DDE	1	53-55	281
PCBs			
3 Cl	1		258
4 Cl	1		292
5 Cl	1	40-55	324
6 Cl	1	40-55	358
7 Cl	1	45-55	394
8 Cl	1	45-60	430
9 Cl	1	50-60	464
10 Cl	1	58-60	498
α HCH	2	37-39	255
β HCH	2	39-41	255
γ HCH (Lindane)	2	39-41	255
δ HCH	2	41-43	255
γ Chlordane	2	51-53	410
α Chlordane	2	52-54	266
op DDD	2	54-56	248
pp' DDD	2	56-58	248
op DDT	2	56-58	246
pp' DDT	2	58-60	283
Heptachlor epox.	3	49-51	318
α Endosulfan	3	52-54	406
Dieldrin	3	53-55	346
Endrin	3	55-57	346
β Endosulfan	3	55-57	406
Endosulfan sulfate	3	58-60	386

9. NOTES ON WATER ANALYSIS

The levels of lipophilic compounds in tissues of aquatic organisms and organic fractions of sediments are determined to a large extent by the levels of these compounds in the surrounding water (marine mammals are an obvious exception). Data for CBs and hydrocarbons in sea water is therefore extremely useful for an understanding of the levels in organisms. However, the levels in sea water are extremely low and consequently, their determination needs considerable experience. Large volumes of water are required and extreme care has to be taken in order to avoid contamination during sampling, extraction and clean-up of the samples. Details are described in Manual and guide No 27 of IOC, 1993 and Villeneuve J.P. (1986).

10. ALTERNATIVE PROCEDURES

10.1. Combining sample preparation and extraction for chlorinated and petroleum hydrocarbons

In the event that analysis for petroleum hydrocarbons and chlorinated compounds (and/or sterols) are of interest, the following extraction procedure can be used. To the freeze-dried sample

introduce internal standards for each compound class. The following are suggested: 1) aliphatic hydrocarbons: n-C_{32}, $C_{18:1}$ (octadec-1-ene), 2) polycyclic aromatic hydrocarbons: 9,10 dihydroanthracene (deuterated PAHs are also useful), 3) organochlorine compounds: PCB congeners 29, 30, 121 or 198 and ε HCH, 4) sterols: 5 α (H)-androstan-3β-ol. These standards are used for quantifying the recovery of the total procedure. Samples are Soxhlet extracted for 8 hours using 250 ml hexane. After cooling to room temperature, the flask containing hexane is removed, and the sample is re-extracted with 250 ml of dichloromethane for 8 hours. The dichloromethane is concentrated down to approximately 1 ml using a rotary evaporator. The flask is then rinsed with hexane and transferred to the flask containing the hexane extract. The combined extract is concentrated by rotary evaporation down to 15 ml and transferred to a Kuderna-Danish tube. It is then further concentrated down to 5-6 ml under nitrogen gas. Following removal of sulphur and water, the extract is separated into aliquots : 1/3 for petroleum hydrocarbons and sterols and 2/3 for chlorinated hydrocarbons.

10.2. Supercritical fluid extraction (SFE) of marine samples

Sample preparation is probably the most time-consuming and labor-intensive analytical task performed in a laboratory. Studies shows that 60 % of the overall sample analysis time is spent in sample preparation which is the main source of error and of contamination. In addition, the amount of hazardous chemicals used for sample preparation is a continuous source of concern. Due to safe handling and disposal requirements, the reduction of their use is a priority for laboratories world-wide.

Supercritical fluids are gases (i.e. N_2O and CO_2) at room temperature and pressures above the critical point. The SFE technique allows an efficient extraction of a variety of contaminants with considerable reduction in the analysis cost, sample amount and allows the extraction of the thermal sensitive substances, reducing the amount of environmentally hazardous solvents.

A small change in the pressure of a supercritical fluid results in a big change in its density and the solvent strength of the fluid changes with changing density. As a result, one supercritical fluid easily performs the work of many solvents. If this is not enough, it is possible to add a modifier, such as methanol (a few per cent) to increase the solvating range of the fluid. Therefore, SFE should speed up the sample preparation process, minimising the wastes associated with the analysis.

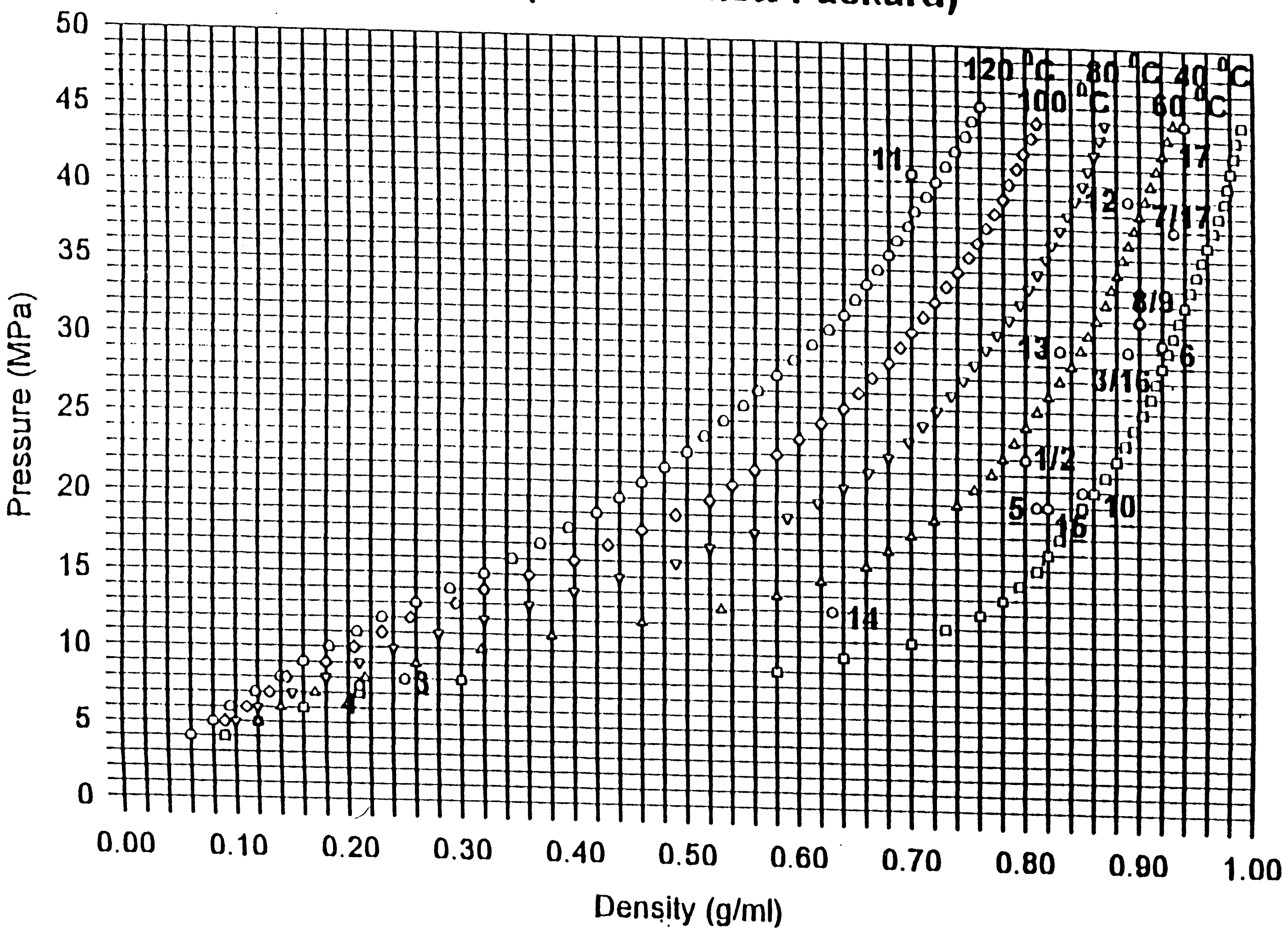
Until now, the main fields of analytical applications of SFE are related to environmental studies and to the food-processing industry (Hawthorne, 1990, Bayona, 1993). A method using carbon dioxide (80 °C-340 atm) for the extraction of total petroleum hydrocarbons has been approved as an EPA standard method. The extraction efficiency of modified CO_2 for the recovery of 41 organochlorine and 47 organophosphorus pesticides spiked on sand at different pressures and temperatures were higher than 80 %. Furthermore, by increasing the extraction temperature up to 200 °C, PCBs and PAHs can be extracted from naturally occurring samples with neat CO_2. Nam *et al.* (1991), have developed a method for rapid determination of polychlorinated organics in complex matrices. The method is based on direct coupling of supercritical fluid extraction with tandem supercritical fluid chromatography and gas chromatography. The on-line system permits simultaneous extraction and analysis with high reproducibility and accuracy.

11. DATA INTERPRETATION

11.1. DDT

The residence time of total DDT in the environment is relatively short (t1/2 = 3-5 years), so, at least 75-80 % of the current total DDT should be in the form of DDE or DDD if it was introduced into the environment before the 1975 ban. Values of Henry's law constant indicate that these compounds can reach the troposphere as vapour. These vapours are little adsorbed by airborne particulate matter and represent the major component in atmospheric chlorinated hydrocarbon levels. Vapour movements of these pollutants suggest that restrictions and regulations operating in the more technically advanced countries could only be partially effective on a world wide basis.

Selection guide for CO2 extraction of common pollutants
(from Hewlett-Packard)



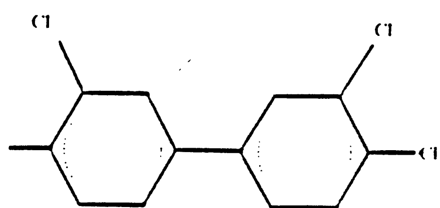
	Compounds	Matrix	Modifier
1	Triazines	Sediment	MeOH
2	Chlorophen.	Sediment	Water
3	PAH	Diesel ex.	-
4	Hydrocarb.	Diesel ex.	-
5	PAH	Tenax	-
6	TCDD	Sediment	MeOH
7	PAH	PUF	-
8	2,4,5 T Herb.	Soil	-
9	Alachlor	Soil	-
10	PCB	Sediment	MeOH
11	Diuron/Lin.	Soil	MeOH
12	PCDD/PCDF	Fly ash	Benzene
13	Organochlor. Pesticides	Soil	-
14	PAH	Wood tar	-
15	PAH	Coal tar	-
16	Phenol	Wood smoke	-
17	PCB	PUF	-

The presence of the op DDT together with anomalous pp' DDT values in environmental samples indicates a recent treatment with this insecticide.

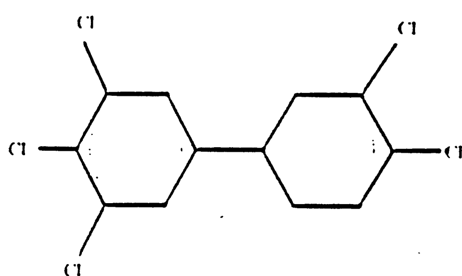
11.2. PCBs congeners

Among the 209 possible PCB congeners, seven of them: 28, 52, 101, 118, 138, 153 and 180, were selected as the most relevant because of their distribution in the chromatogram and in the chlorination range.

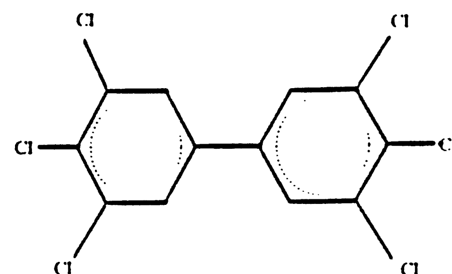
Recently, attention has been paid to congeners having 2 para-chlorines and at least 1 meta-chlorine. These congeners are called "coplanar" PCBs. Among the 209 congeners, 20 members attain coplanarity due to non-ortho chlorine substitution in the biphenyl ring. Three of these show the same range of toxicity as the 2,3,7,8 tetrachlorodibenzo-p-dioxin and the 2,3,7,8 tetrachlorodibenzofuran, these are the IUPAC No: 77, 126 and 169. These compounds should be identified and quantified in the environmental samples with high priority. They can be separated using fractionation with carbon chromatography (Tanabe *et al.*, 1986).



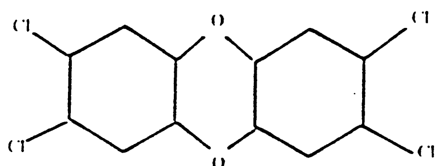
3,3',4,4' tetrachlorobiphenyl
IUPAC No : 77



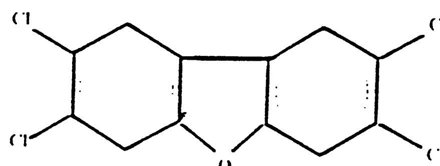
3,3',4,4',5 pentachlorobiphenyl
IUPAC No : 126



3,3',4,4',5,5' hexachlorobiphenyl
IUPAC No : 169



2,3,7,8 tetrachlorodibenzo-p-dioxin



2,3,7,8 tetrachlorodibenzofuran

11.3. Typical profiles of commercial mixtures

Formulations available in different countries are slightly different in their composition (Aroclor in USA, Kanechlor in Japan, Clophen in Germany, Phenoclor in France, Fenclor in Italy or Sovol in Russia). For the same global composition, such as Aroclor 1254, KC-500 or Phenoclor DP-5, the composition of individual congeners differs by 5-10 %. If a sample is collected on the French coast (therefore, contaminated with DP-5), and is quantified with DP-5 and Aroclor 1254, the difference observed in concentration could be in the order of 5-10 %. This shows the importance of choosing one common standard for the quantification of global industrial formulations or the importance of quantifying with individual congeners.

12. QUALITY ASSURANCE / QUALITY CONTROL

Guidelines on the QA/QC requirements for analysis of sediments and marine organisms are detailed in Reference Method No 57, "Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice". Brief descriptions of issues that must be addressed in the course of understanding the procedures described here are given below.

Percent contribution of individual chlorobiphenyls to Clophen A 50 and Aroclor 1254.

PCB No	Clophen A50	Aroclor 1254	PCB No	Clophen A50	Aroclor 1254
17	0	0.19	115	0.28	0.3
18	0	0.41	118	10.9	6.39
28	0.05	0.25	119	0.19	0.14
31	0.05	0.22	122	0.19	0.5
33	0.11	0.14	123	0.85	0.81
40	0.28	0.2	126	0.08	0
41	0.83	0.64	128	3.04	2.07
42	0.13	0.23	129	0.83	0.23
44	2.46	2.03	130	0.83	0.63
47	0.18	0.11	131	0.06	0.16
48	0.17	0.14	132	2.57	1.98
49	1.96	1.64	134	0.52	0.49
52	5.53	5.18	135	1.61	1.62
53	0.06	0.09	136	0.91	1.12
56	0.44	0.58	137	0.25	0.25
60	0.34	0.54	138	3.61	3.2
63	0.15	0.05	141	0.98	1.04
64	0.71	0.45	146	0.8	0.83
66	0.5	0.59	149	4.5	2.21
67	0.13	0.09	151	1.22	1.17
70	3.85	3.21	153	4.17	4.26
74	1.35	0.78	156	1.43	1.62
82	1.05	0.95	157	0.31	0
83	0.53	0.45	158	0.98	0.77
84	2.08	1.95	167	0.35	0.21
85	1.85	1.66	170	0.65	0.31
87	4.22	3.78	171	0.5	0.5
90	0.85	0.93	172	0.09	0.05
91	0.92	0.83	173	0.09	0.09
92	1.53	1.58	174	0.37	0.34
95	6	6.02	175	0.11	0.05
96	0.05	0.08	176	0.43	0.32
97	2.8	2.55	177	0.21	0.21
99	4.06	3.6	178	0.19	1.35
100	0.15	0.1	179	0.2	0.21
101	7.72	7.94	180	0.53	0.38
105	1.9	3.83	183	0.21	0.17
107	0.94	0.72	187	0.3	0.32
110	6.27	5.85	190	0.05	0.08
			201	0.6	0.68

12.1. Precision

The precision of the method should be established by replicate analysis of samples of the appropriate matrix. Estimate the precision of the entire analytical procedure by extracting five sub-samples from the same sample after homogenisation. Alternatively, perform replicate analysis of an appropriate certified reference material (RM ; see below) containing the analytes of interest. The

principal advantage of using a RM is that the material permits the simultaneous evaluatic accuracy while offering a well homogenised sample. Precision should be evaluated as a matt course during the initial implementation procedure just before initiation of sample analysis.

12.2. Accuracy

The accuracy of the methods described here must be confirmed by analysis of a suitabl (i.e. appropriate matrix, analytes) prior to initiation of sample analysis. Agreement between mea and certified concentrations for any individual analyte should be within 35 % and on average w 25 %. It is advisable to introduce RM s on a regular basis (e.g. every 10-20 samples) as a meth checking the procedure. Further description of the preparation of control charts and criteria for acceptance are discussed in Reference Method No 57.

12.3. Blanks

Blanks represent an opportunity to evaluate and monitor the potential introductio contaminants into samples during processing. Contributions to the analyte signal can arise contaminants in the reagents, those arising from passive contact between the sample and environment (e.g. the atmosphere) and those introduced during sample handling by h implements or glassware. It is essential to establish a consistently low (i.e. with respect to anal blank prior to initiating analysis or even the determination of the method detection limit. In addi it is necessary to perform blank determinations on a regular basis (e.g. every batch of samples).

12.4. Recovery

Recovery reflects the ability of the analyst to fully recover surrogate compounds introduc the sample matrix or blank at the beginning of the procedure. The primary criteria for sele of compounds to be used for testing recovery are that they: 1) have physical chromatographic/partitioning) properties similar to and if necessary spanning those of the analyt interest, 2) do not suffer from interferences during gas chromatographic analysis, 3) are bas resolved from the analytes of interest.

Recovery should be tested on all samples and blanks as a routine matter of course. Recov below 70 % are to be considered unacceptable. Recoveries in excess of 100 % may indicate presence of an interference.

12.5. Archiving and reporting of results

Every sample should have an associated worksheet which follows the samples and extracts through the various stages of the procedure and upon which the analyst notes all rele details. An example of such a worksheet is given below. Each laboratory should construct complete such a worksheet. Relevant chromatograms should be attached to the worksheet. Anal should be grouped and composite or summary analysis sheets archived with each group. F disposal of the data will depend on the reasons for which it was collected but should follow the ov plan model.

All processed samples should be archived at all steps of the procedure :

- deep frozen (in the deep-freezer as it was received).
- freeze-dried (in sealed glass container kept in a dark place).
- extracted (after injection on the GC, sample extracts should be concentrated down to and transferred into sealed glass vials, a Pasteur pipette sealed with a butane burner is adequate cheap).

Sample : IAEA-357 : Marine Sediment

wet wt.
——— = ..., % water in freeze dried sample determined by drying at 105 °C : .....
dry wt.

.......g freeze-dried wt. extracted with hexane in Soxhlet extractor for 8 hours.

.......pg 2,4,5 Trichlorobiphenyl and ....pg ε HCH were added as internal standard.

The ....ml extract was reduced by rotary evaporator to approximately ......ml.

This was treated with sodium sulfate to dry the extract . Then treated with mercury to remove sulphur. This was further reduced to ....ml for lipid determinations. Corrected dry wt. : ....g.

Lipid determinations :

..............ml total extract;

10 μl aliquots weighed on micro-balance : ....mg; ....mg; ....mg.

HEOM = ...mg/g dry weight.

...........mg lipid subjected to column chromatography fractionation on Florisil.

F1 : ....ml hexane

F2 : ....ml hexane/dichloromethane (70:30)

F3 : ....ml dichloromethane

GC determinations :

2,4,5 TCB : ....ng recovered in F1 : ....% Recovery.

ε HCH : ....ng recovered in F2 : ....% Recovery.

Attach tabulation of individual compounds quantified in sample.

Sample worksheet for analysis of chlorinated compounds in marine sediments.

ANNEX

Preparation of the internal standards solution:
2,4,5 Trichlorobiphenyl and ε HCH:

Stock solution of 2,4,5 trichlorobiphenyl :

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains :

2.5 ng/ µl of 2,4,5 TCB

Working internal standards solution:

0.5 ml from the stock solution of 2,4,5 TCB should be transferred into a 50 ml volumetric flask, then 1 ml from the original vial of ε HCH should be transferred into that volumetric flask and the volume adjusted to 50 ml with hexane. This working solution contains :

25 pg/µl of 2,4,5 trichlorobiphenyl
20 pg/µl of ε HCH

<u>CAUTION : VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING</u>

Preparation of the Aroclor 1254 solution

Preparation of the stock solution :

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then, the volume is adjusted to 100 ml with hexane. This stock solution contains :

6.5 ng/µl of Aroclor 1254

Preparation of the working solution :

1 ml from this stock solution should be transferred into a 50 ml volumetric flask and the volume adjusted to 50 ml with hexane. This working solution contains :

0.13 ng/µl of Aroclor 1254

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the Aroclor 1260 solution

Preparation of the stock solution :

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5.44 ng/µl of Aroclor 1260

Preparation of the working solution :

1 ml from the stock solution should be transferred into a 50 ml volumetric flask, then the volume is adjusted to 50 ml with hexane. This working solution contains

0.1088 ng/µl of Aroclor 1260

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the pp' DDE, pp' DDD and pp' DDT solution

pp' DDE :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains :

5 ng/µl of pp' DDE

pp' DDD :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of pp' DDD

pp' DDT :

Stock solution : 1 ml of the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains :

5 ng/µl of pp' DDT

Working solution : pp' DDE, pp' DDD and pp' DDT together.

1 ml from the stock solution of pp' DDE, 2 ml of the stock solution of pp' DDD and 3 ml of the stock solution of pp' DDT should be transferred into a 100 ml volumetric flask and the volume adjusted to 100 ml with hexane. This solution contains

- pp' DDE : 50 pg/µl
- pp' DDD : 100 pg/µl
- pp' DDT : 150 pg/µl

NOTE : Further dilution may be necessary depending on the sensitivity of the EC Detector.

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of Aldrin, Diedrin and Endrin standard solutions :

Aldrin :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of Aldrin

Dieldrin :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of Dieldrin

Endrin :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of Endrin

Working solution : Aldrin, Dieldrin and Endrin together.

1 ml from the stock solution of Aldrin, 1 ml from the stock solution of Dieldrin and 1 ml from the stock solution of Endrin are transferred into a 100 ml volumetric flask and the volume is adjusted to 100 ml with hexane. This working solution contains :

Aldrin : 50 pg/µl
Dieldrin : 50 pg/µl
Endrin : 50 pg/µl

NOTE : Further dilution may be necessary depending on the sensitivity of the detector.

<u>CAUTION : VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING</u>

Preparation of the HCB and Lindane standard solutions :

HCB :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of HCB

Lindane :

Stock solution : 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

5 ng/µl of lindane

Working solution :

1 ml from the stock solution of HCB and 1 ml from the stock solution of Lindane are transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains :

HCB : 50 pg/µl
Lindane : 50 pg/µl

NOTE : further dilution may be necessary depending on the sensitivity of the EC Detector.

<u>**CAUTION : VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING**</u>

Preparation of the PCB congeners solution

In a 100 ml volumetric flask, transfer 1 ml from the original vial. Adjust to 100 ml with hexane in order to obtain the working solution with the following concentrations :

CB No:	Compounds :	Concentrations (pg/µl)
8	2,4'	17.50
18	2,2',5	12
31	2,4',5	10.6
28	2,4,4'	4.6
52	2,2',5,5'	8.6
49	2,2',4,5'	12.1
44	2,2',3,5'	10.7
66	2,3',4,4'	5.5
95	2,2',3,5',6	5.7
101	2,2',4,5,5'	9.3
110	2,3,3',4',6	11.1
149	2,2',3,4',5',6	12.1
118	2,3',4,4',5	8.5
153	2,2',4,4',5,5'	8.4
138	2,2',3,4,4',5'	13.8
183	2,2',3,4,4',5',6	10.3
174	2,2',3,3',4',5,6'	9.4
177	2,2',3,3',4',5,6	9.5
180	2,2',3,4,4',5,5'	16.3
170	2,2',3,3',4,4',5	13.4
199	2,2',3,3',4,5,5',6'	9.3
194	2,2',3,3',4,4',5,5'	12.6

Separate into 10 volumetric flasks of 10 ml, seal with Teflon tape and keep in refrigerated place in order not to evaporate them.

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

EUROF 224-02037-01, E/05 SED SHIMADZU

15.223
15.961
17.259
18.88
37.665
48.812
48.673
44.546
45.661

Retention time	Peak
50.523	8
	18
	31
57.198	28
	52
	49
	44
62.227	
62.877	
	66 + 95
65.511	
66.326	
	101
	110
	149
71.267	118
72.791	153
	138
74.333	
	183
	174
	177
78.194	
	180
80.298	
	170
83.178	199
84.751	
86.664	
	194

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