REGIONAL SEAS

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Determination of petroleum hydrocarbons in sediments

Reference Methods For Marine Pollution Studies No. 20

Prepared in co-operation with

IOC IAEA

UNEP 1992

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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 and of its resources, and of the sources and trends of the pollution, and the impact of pollution 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 is 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 are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations 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 Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation 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
B.P. No. 800
MC-98012 MONACO Cedex

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

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(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 assessments. UNEP 1990.

This Reference Method was designed to update and replace the IOC Manuals and Guides No.11 which was originally released in 1982. The present document was prepared by Dr. Kathryn Burns with input from the IOC/UNEP Group of Experts on Methods, Standards and Intercalibration (GEMSI), particularly Drs. John Farrington, Manfred Ehrhardt and Karsten Palmork. The Method is based upon continued experience in environmental monitoring studies and the results of intercomparison exercices and training courses and workshops worldwide.

The format of this Method is slightly different from many of the other chemical Reference Methods in that it includes an extensive introduction and review of selected literature. The reference list is not exhaustive but has been chosen in order to give the readers the basic knowledge they require in order to understand the underlying principles of petroleum hydrocarbon environmental chemistry, to select appropriate techniques and equipment and to have an insight into data interpretation. The reader should be aware that although the full suite of techniques described in this document requires very sophisticated instruments, it is possible to generate valid and useful data with a UV-Fluorescence spectrophotometer in order to conduct preliminary pollution assessments. In all cases, adequate measures should be taken for quality assurance and the reader is encouraged to check his/her results by participating in the regular intercomparison exercises organized by IAEA/IOC/UNEP, ICES, and other organizations.

Contents

1. INTRODUCTION

Hydrocarbons in sediments can have several sources, as is the case for hydrocarbons in the marine environment in general. These can be grouped into the following categories :

1) Petroleum inputs into the ocean. For example, see Table 1 taken from NAS (1985).
2) Hydrocarbons, especially PAH (polycyclic aromatic hydrocarbons, also known as polynuclear aromatic hydrocarbons), released as the result of partial combustion of fuels (such as gas, coal, oil and wood) for transportation, heating and power generation.
3) PAHs originating in forest and grass fires, transported to the marine environment *via* aeolean and fluvial processes. This source is usually important to consider only if analyzing at the ppm ($\mu g.g^{-1}$ dry weight) or less concentration level.
4) Biosynthesis of selected (biogenic) hydrocarbons by marine or terrigenous organisms.
5) Early diagenesis transformations in sediments or in the overlying water column of non-hydrocarbon biosynthetic natural products to hydrocarbons. An example is the microbial, and possibly chemical, transformation of sterols and hopanols to partially aromatized hydrocarbons as described in Brassell *et al.* (1981). This source will be quantitatively important primarily in organic rich sediments of 1 to 2% or greater organic carbon content and in areas receiving sewage and sewage sludge inputs, and when attempting to analyze for pollutant hydrocarbons at the ppm ($\mu g.g^{-1}$ dry weight) concentration level or less.

The need to analyse for pollutant hydrocarbons in sediments may arise for different reasons. For example :

1) The need to establish background values before offshore drilling and oil production activities are started,
2) The need to assess the extent of contamination of a fishing ground that has been subjected to an oil spill,
3) The need to assess the condition of a harbour subject to chronic inputs of oil products from urban street runoff, shipping and refineries, or
4) The need to assess the impact of long range atmospheric transport of contaminants to remote ecosystems.

These and other pollution situations may have a negative impact on marine ecosystems and fisheries resources. However, before analytical methods for monitoring can be recommended it is necessary to present brief descriptions of the chemistry of oil in relation to environmental monitoring, of relevant biogeochemical processes affecting the oil once released into the marine environment, and of current strategies for monitoring hydrocarbons in marine ecosystems. Only in this context can the methods described herein be understood. The reader is encouraged to consult the references cited for more detailed discussions.

The term "oil" can often be confused as it has been used in the literature to mean natural oils in the sense of animal fats and plant oils. However, "oil" in the context of marine pollution monitoring studies refers to the complex mixtures of chemicals that have been produced as the result of a combination of biological and geological processes culminating in the production and accumulation of oil in ancient sediments. A general description of these processes is found in Philp (1986) and more detailed discussions are presented in the books by Tissot and Welte (1988) and Hunt (1979). Oil and natural gas together are referred to as petroleum. Thus "oil pollution" is synonymous with "petroleum pollution" within the context of the UNEP and IOC/UNESCO marine monitoring programmes. The oil, as it is produced from wells is termed "crude oil".

Table 1 : Input of petroleum hydrocarbons into the marine environment (million metric tonnes per annum) (From:N.A.S., 1985)

Source	Probable Range	Best Estimate[a]
Natural sources		
Marine seeps	0.02 - 2.0	0.2
Sediment erosion	0.005 - 0.5	0.05
Offshore production	0.04 - 0.06	0.05
Transportation		
Tanker operations	0.4 - 1.5	0.7
Dry-docking	0.02 - 0.05	0.03
Marine terminals	0.01 - 0.03	0.02
Bilge and fuel oils	0.2 - 0.6	0.3
Tanker accidents	0.3 - 0.4	0.4
Nontanker accidents	0.02 - 0.04	0.02
Atmosphere	0.05 - 0.5	0.3
Municipal and industrial wastes and runoff		
Municipal wastes	0.4 - 1.5	0.7
Refineries	0.06 - 0.6	0.1
Non-refining industrial waste	0.1 - 0.3	0.2
Urban runoff	0.01 - 0.2	0.12
River runoff	0.01 - 0.5	0.04
Ocean dumping	0.005 - 0.02	0.02
TOTAL	1.7 - 8.8	3.2

[a] : The best total estimate, 3.2 mta, is a sum of the individual best estimates. A value of 0.3 was used for the atmospheric inputs to obtain the total, although we realize that this best estimate is a centre point between the range limits and cannot be supported rigorously by the data and calculations used for estimation of this input.

Crude oils and most of their distillation products are extremely complex mixtures containing thousands of compounds, comprised mostly of hydrocarbons, but often containing some sulphur, nitrogen and oxygen heterocyclics, quinones, acids, phenols, thiols as well as metallo-organic complexes. The major classes of hydrocarbons as simplistically depicted in Figure 1 are :

1) *n*-ALKANES. Crudes often contain a homologous series of *n*-alkanes extending from C_2 to beyond C_{60} with a smooth distribution between odd and even numbered carbon chain lengths. Lube oils have had alkanes removed by "dewaxing". Other refined products are distillation cuts and are classified by boiling point distributions.

2) Branched ALKANES. Many parallel homologous series of isomers including series of isoprenoid alkanes.

3) Cyclo-ALKANES (naphthenes). Complex mixture including substituted and unsubstituted rings.

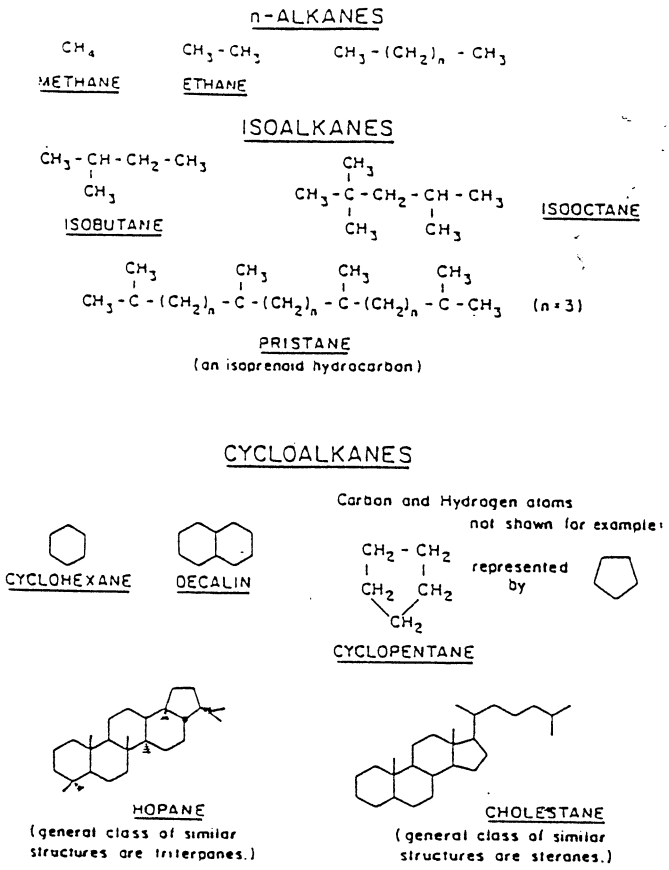
4) AROMATICS. Complex mixtures including mono and polyalkyl-benzenes, naphthalenes, and polynuclear aromatics with multiple alkyl-substitutions. Also naphthenoaromatics or mixed ring structures.

5) ALKENES (olefins). Often present in refined products but absent in crude oils.

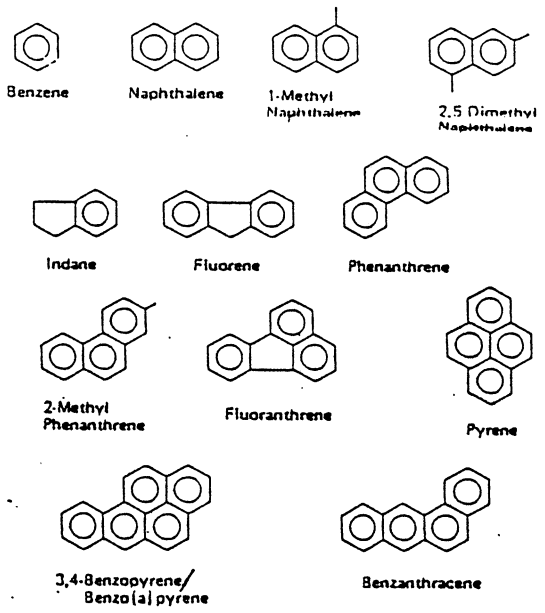
The drawings in Figure 1 show the general carbon skeletons on which are attached myriads of substitutions. Hydrocarbons contain only carbon and hydrogen and these account for 50 to 98 % of the total composition of most crude oils. Crude oil is a complex mixture of hydrocarbons and their derivatives, covering a wide range of molecular structures and weights. An "average" crude contains about 30% alkanes, 50% cycloalkanes, 15% aromatics and 5% nitrogen, sulfur and oxygenated derivatives. Crudes contain widely varying concentrations of V, Ni, Fe, Na, Ca, Cu and U. The composition of oils from different global regions or even within particular geological formations can vary tremendously, as can the composition of products refined from them or produced to satisfy specific uses.

Refined petroleum products are made by fractional distillation of crude oils and are thus discrete cuts or subsets of the boiling range of the starting crude. This is depicted in Fig. 2.

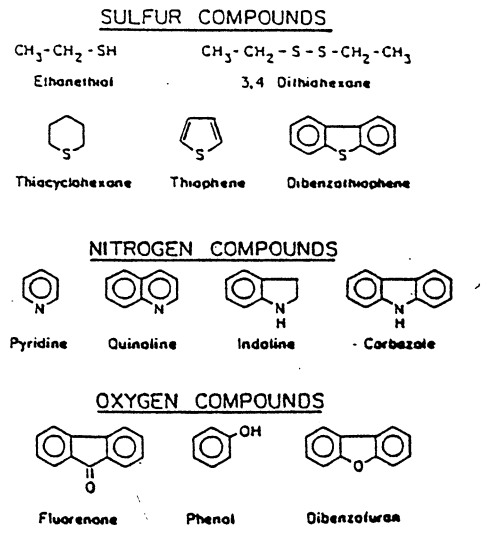
The extreme complexity of the mixture of chemicals found in crude oils and most of their distillation products is a major challenge to analytical chemists. When mixed with other sources of hydrocarbons in the marine environment, the challenges are greater. Even in situations where a known oil is discharged to the environment, natural biogeochemical processes start immediately to alter its composition. Consider the simplest case of an oil spilled on the surface of the sea (Fig. 3). The oil rapidly forms a slick and undergoes spreading, evaporation and dissolution. The most volatile fractions, including the light aromatics are rapidly lost, leaving a residue greatly changed in chemical composition. The picture is further complicated with the inclusion of phenomena such as bacterial decomposition, selective adsorption of specific compounds by organisms, fractionation of residues between particulate matter and sediments, and the action of sunlight. Clearly, no matter which component of the ecosystem is analysed, the oil seen in environmental samples is different from the source material. Figure 4 is a synopsis of the biogeochemical cycle of one group of hydrocarbons, the PAH, and illustrates the complexity of sources, processes and fates that must be understood by the environmental chemist in order to chose methods of analysis that will provide the required data for each specific monitoring project. **There is no single method that can be used to analyse all of the components of oil residues.** Furthermore, any method chosen is a compromise between the feasibility of the analysis as constrained by the equipment and other resources available to analysts and the degree of accuracy, precision and chemical detail necessary to answer the questions posed in specific programmes. The strategy generally adopted is to use a hierarchical approach and is briefly developed as follows :



(A). Saturated hydrocarbons



(B). Aromatic hydrocarbons



(C). NSO compounds

Fig 1 : Chemical Structures of Petroleum Hydrocarbons

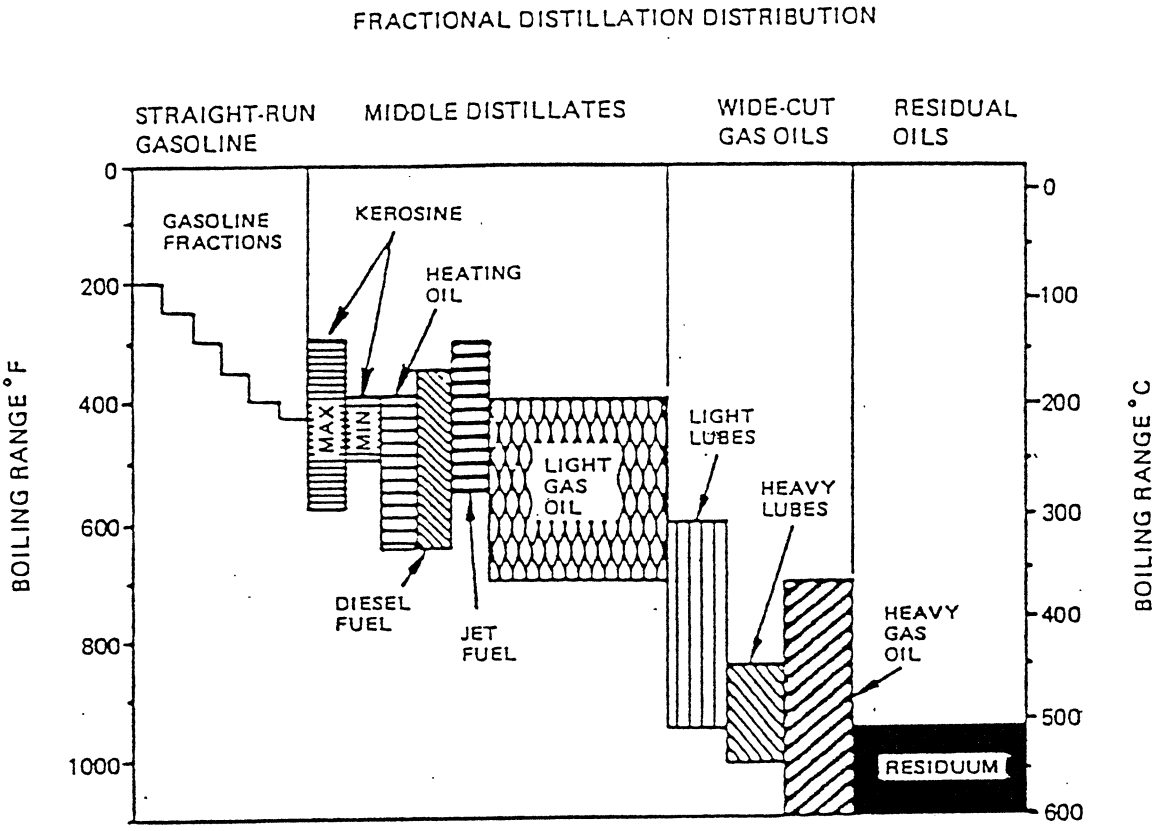
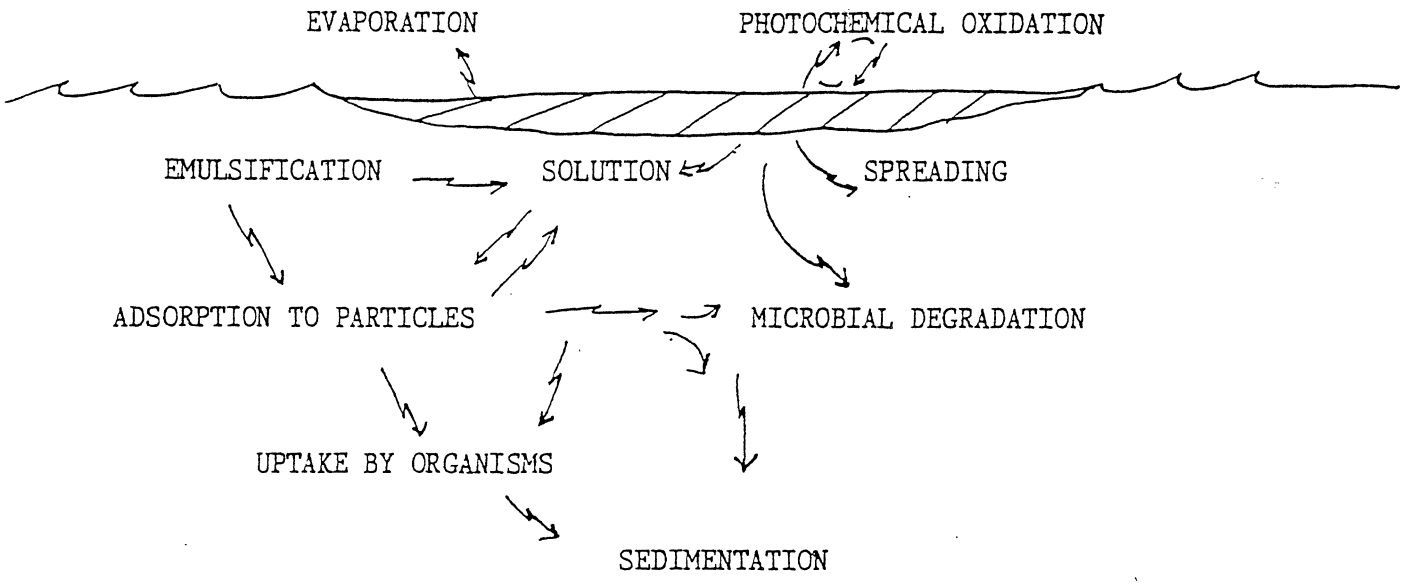


Fig 2 : Boiling point range of fractions of crude petroleum. (From : N.A.S. 1985).



SPREADING: Increases surface area, promotes dissipation

EVAPORATION: Removes volatiles, increases viscosity of slick

SOLUTION: Removes light weight saturates and aromatics

PHOTOCHEMICAL OXIDATION: Condensation reactions, formation of volatile and non-volatile polar reaction products

EMULSIFICATION: Mousse formation

PARTICLE ADSORPTION: Aggregation of least soluble components (alkanes, cycloalkanes, heavy aromatics)

SEDIMENTATION: Removal from the water column

MICROBIAL DEGRADATION: alkanes > alkenes > aromatics > cycloalkanes

UPTAKE BY ORGANISMS: Selective accumulation in tissues, aggregation of remainder into fecal materials

Fig 3 : Processes of oil slick dissipation.

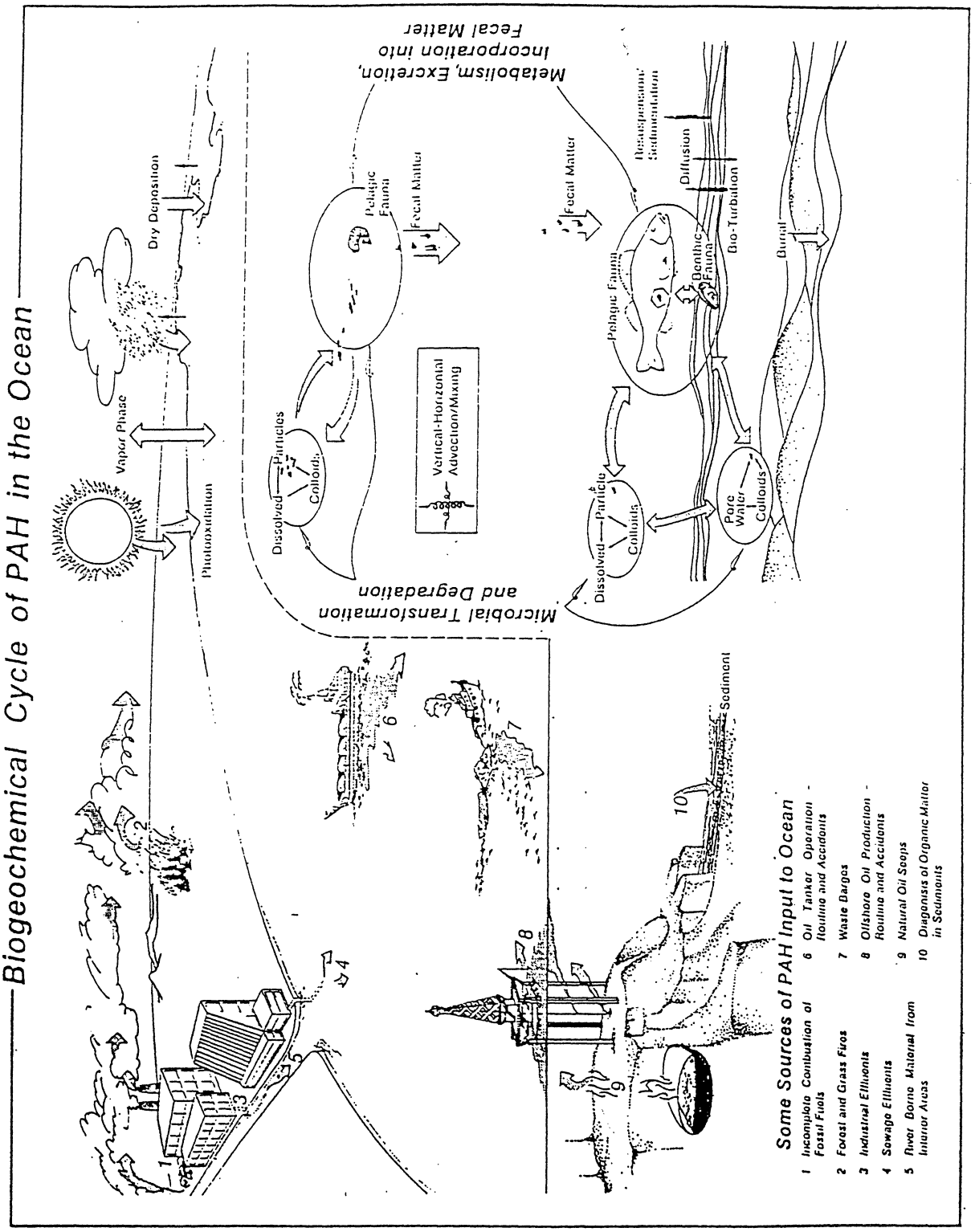


Fig 4 : (From : Farrington, *et al.* , 1986)

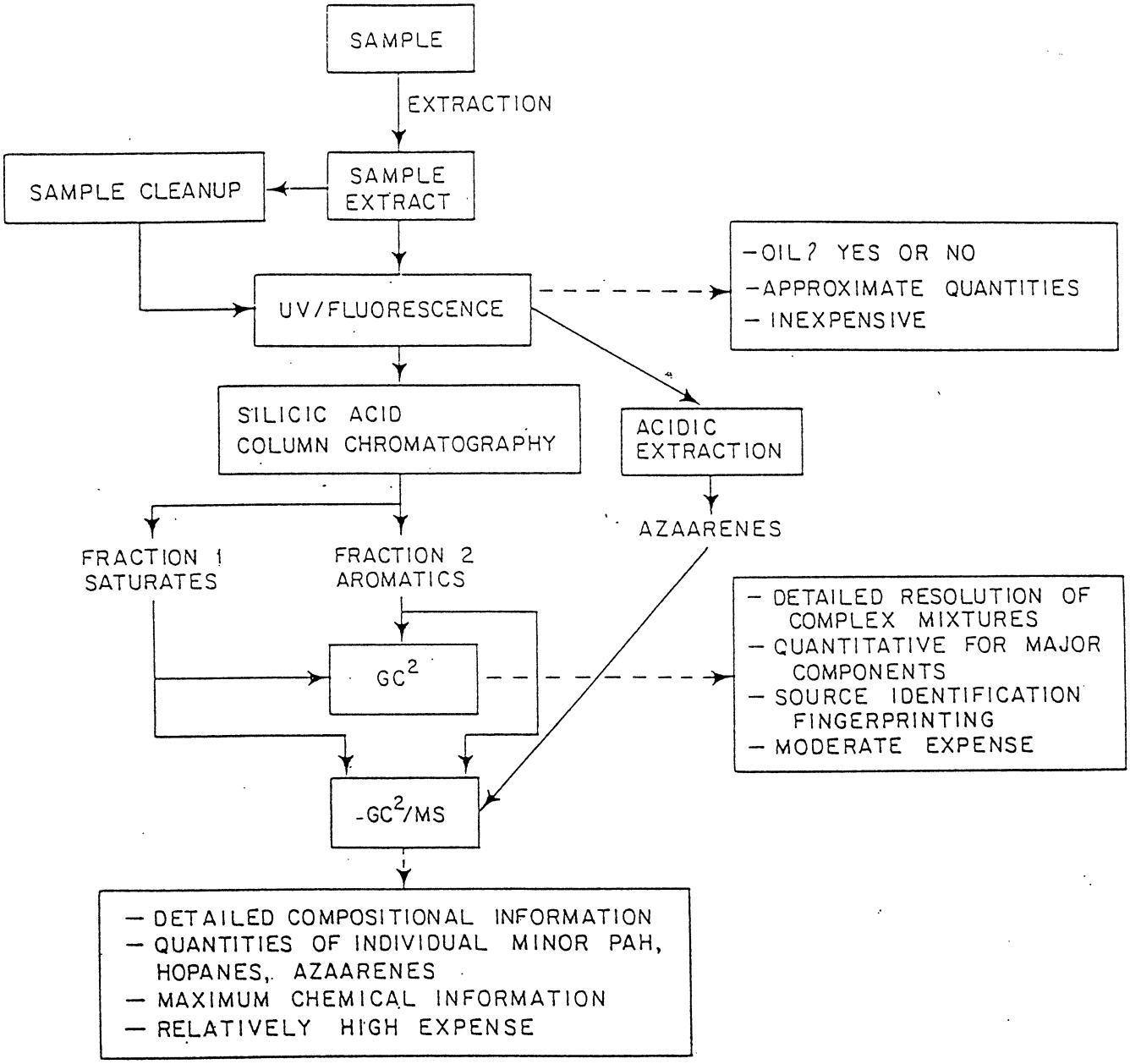


Fig 5 : Hierarchical scheme for analysis of petroleum hydrocarbons in marine sediments. (From : N.A.S. , 1985)

Figure 5 shows a stepped or hierarchical scheme for the analysis of petroleum hydrocarbons in environmental samples.

The simplest case would be the analysis of large amounts of oil mixed with sand. The oil would be dissolved in hexane and separated from any water present. If calibrated against an appropriate standard, ultra violet fluorescence (UVF) spectroscopy will yield a relatively inexpensive estimate of the amount of oil in samples. It is the aromatic components of oils that fluoresce and so the UVF signal is greatly affected by processes controlling the fate of the aromatic hydrocarbons. When the source oil is known the method can be used as a first screening method to trace the movement of oil through the system and to rank the relative degree of contamination in the environment. Aromatic hydrocarbons are relatively soluble compared to other hydrocarbon classes. Figure 6 illustrates the effect of dissolution processes on the UVF spectra of water samples collected near surface slicks. Note that compared to the original oil which exhibited a broad spectrum resulting from single through to five ring aromatics, the surrounding seawater contained mostly the more soluble benzene and naphthalene derivatives. Differences in the spectra of hydrocarbons between oil residues extracted from contaminated sediments and the contaminating oil will also be apparent as sediments tend to retain the heavier more condensed ring structures. Figure 6 also illustrates that results will vary dramatically depending on which wavelengths are used for quantification. In addition, the intensity of fluorescent emissions is dependent on molecular structure. Condensed multiple ring structures emit at higher wavelengths and with much higher intensities than the 1 through 3 ringed structures. Thus, although the technique is extremely sensitive ($\mu g.L^{-1}$ or $\mu g.g^{-1}$ range), it is only semi-quantitative.

Infrared spectrometry has also been used for analyses of bulk oil samples or oil slicks. It may be of use when there is a large amount of oil in sediments. However, most sediments contain lipid materials such as fatty acids, fatty alcohols and sterols in concentrations that would interfere with the infrared spectrum of trace amounts of oils extracted from sediments. The sensitivity of the method is generally in the $mg.g^{-1}$ range. Thus, it has not been used except in very limited cases to analyse for petroleum hydrocarbons in sediments.

Quantitative accuracy is increased if interfering lipid compounds are removed from sediment extracts by an adsorption chromatography procedure and the extracts then analysed by gas chromatography (GC). Preparative procedures employing silica and alumina adsorbants can also be used to fractionate the hydrocarbons into classes before analysis. Two procedures will be described in this manual : one based on column chromatography and the other based on high performance liquid chromatography (HPLC). Gas chromatography using packed columns was the common method of analysis up to the 1970s. With the development of glass and fused silica capillary columns, compound resolution was dramatically increased. High resolution gas chromatography coupled with mass spectrometry has now emerged as the method of choice for accurate identifications based on specific marker compounds and for the quantification of toxic aromatic hydrocarbons in the benzene through to six ring PAH series. All of these methods will be described in this manual.

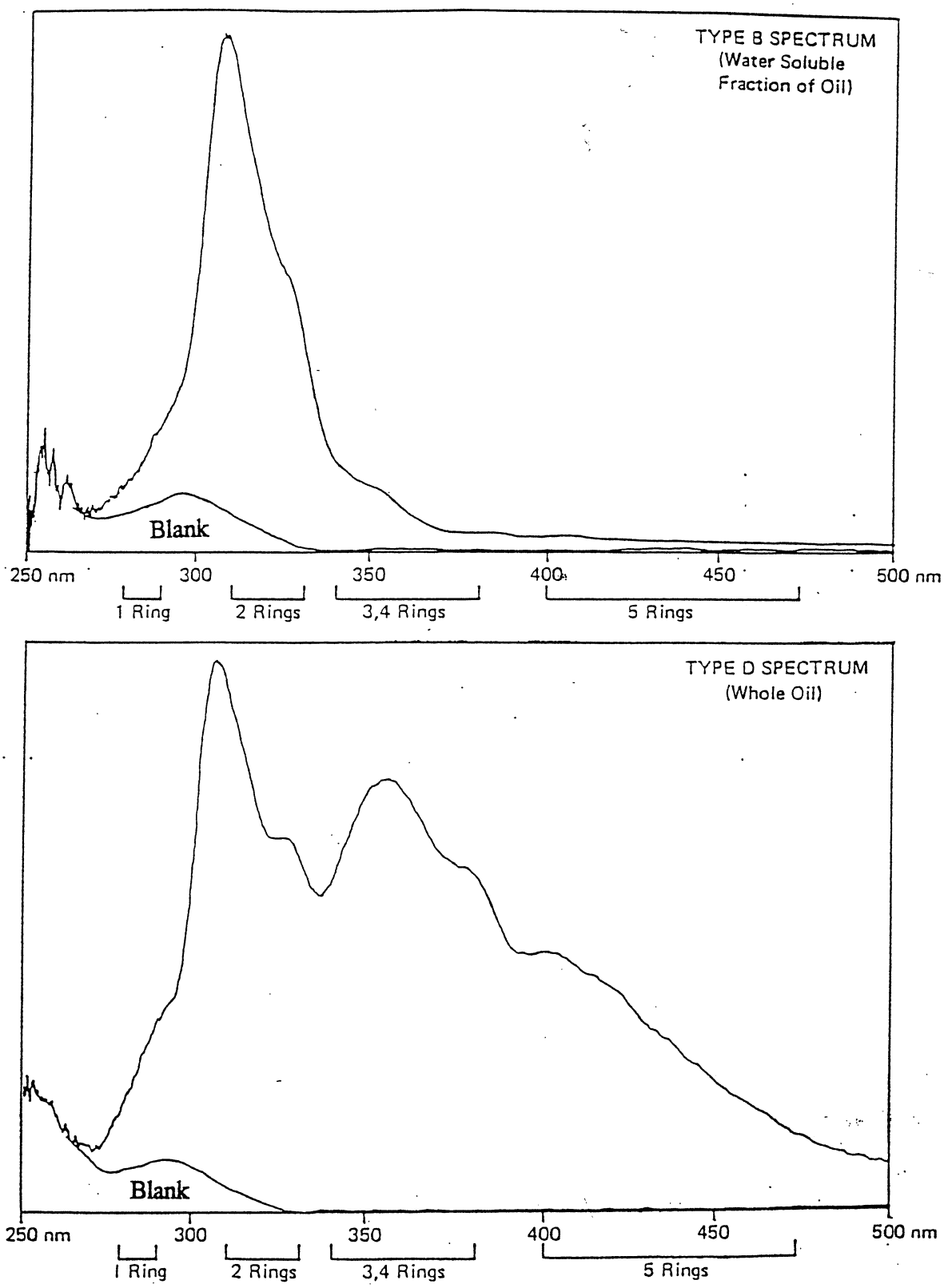


Fig 6 : Representative synchronous fluorescence spectra of seawater samples collected near the IXTOC 1 blowout. (From : N.A.S. , 1985).

As an introduction to the interpretation of gas chromatography data, Fig. 7 contrasts the chromatogram of saturated hydrocarbons extracted from a relatively uncontaminated sediment with that from the mousse of a medium range crude oil. The flame ionisation detector on the GC yields a response in proportion to the weight of material passing through the flame. When coupled to an efficient chromatographic system, it becomes a powerful analytical tool for source investigation. To locate the position of specific compounds, a series of reference compounds run under identical conditions is used to compute relative retention indices (RRI). The chromatograms shown in Fig. 7 are saturated hydrocarbon fractions of the extracts. The RRI system is based on the elution pattern of the straight chain alkanes. The position of elution of *n*-alkanes is marked in the figure with a number indicating the number of carbon atoms. Other compounds can then be identified by their position relative to these markers. Authors reporting the positions of specific hydrocarbons on modern capillary instruments using temperature programming should report them as four digit numbers. For example, pristane, an isoprenoid hydrocarbon which elutes just after heptadecane (C_{17} with RRI 1700) on an SE-52 column will be listed as approximately 1710. Authors must specify how they compute the RRIs because the traditional procedure known as a Kovats index was based on elution under isothermal conditions using a log scale interpolation. A RRI system for aromatic hydrocarbons based on the elution position of benzene, naphthalene, phenanthrene, chrysene and picene as the 1 to 5 ring markers was published by Lee *et al.*, (1979) and was based on a linear interpolation between markers. The use of RRI combined with structural identification based on mass spectrometry is the accepted standard for comfirmation of compounds.

The differences between the chromatograms from the uncontaminated sediment and the oiled sediment can be described using the following information as a guide. Organisms synthesize discrete hydrocarbons with specific biological function, whereas petroleum is a complex mixture. In general :

Biogenics :

1) Biogenic hydrocarbons of recent origin show a high predominance of the odd carbon *n*-alkanes. Some possible exceptions occur in the C_{22} to C_{30} range when low concentrations of even carbon *n*-alkanes can be formed by bacteria.

2) Terrestrial plant waxes contain the odd numbered alkanes in the C_{23}-C_{33} region. Phytoplankton synthesize *n*-C_{15}, C_{17}, C_{19} and often a suite of compounds around C_{21}. Usually these are split peaks resulting from one or more alkenes.

3) A predominance of a single isoprenoid, such as pristane, indicates a biogenic input. The ratio of pristane to phytane is usually much greater in biogenic samples than in oil.

4) One or more alkenes may occur, and are usually associated with one of the odd carbon alkanes.

5) Some specific polyolefins such as heneicosahexaene in algae and squalene in animals may be present. These will predominate in the unsaturated fraction from the preparative adsorption chromatography separation.

6) The concentrations of biogenic hydrocarbons are usually small in relation to contaminant concentrations in polluted samples. Levels of biogenic hydrocarbons average about 5 to 10 $\mu g.g^{-1}$ dry wt in coastal sediments; in oil spill sites it is not uncommon to find levels of petroleum hydrocarbons exceeding 3,000 $\mu g.g^{-1}$. Urban-industrial harbour sediments can have concentrations of petroleum hydrocarbons ranging from 10 to 1,000 $\mu g.g^{-1}$ dry wt.

7) Stable carbon isotope ratios ($\delta^{13}C$), and radiocarbon (^{14}C) content of the extracts can also be used to distinguish "new" biogenic carbon from fossil oil hydrocarbons in some situations.

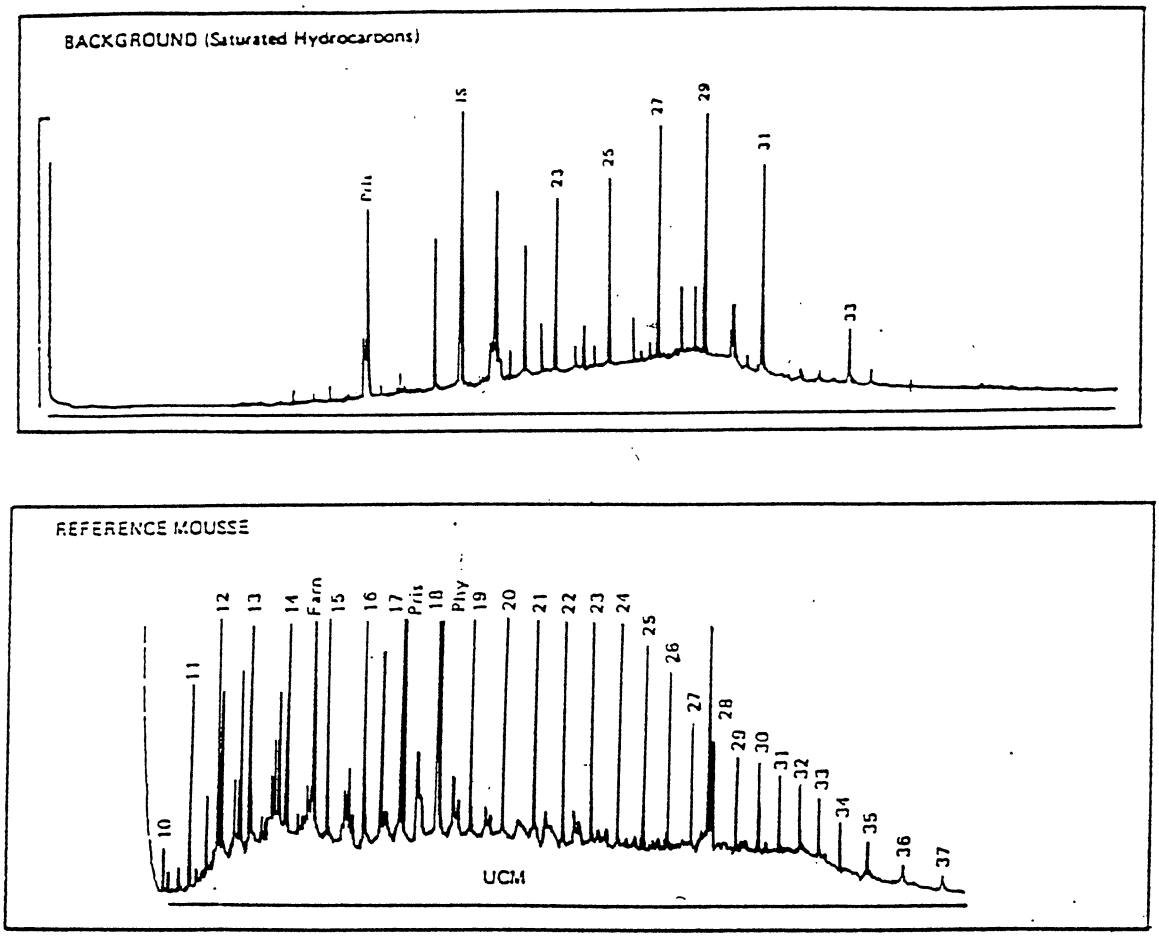


Fig. 7 : High resolution gas chromatography traces of saturated hydrocarbon fractions from marine sediment and petroleum mousse. (Adapted from N.A.S. , 1985).

Petroleum :

1) Petroleum contains the broad boiling range of a complex mixture of hydrocarbons which results from the molecular scrambling of diagenetic processes acting on biogenic components over geological time.

2) Crude oils do not usually contain olefin compounds.

3) The distribution of odd and even *n*-alkanes is uniform and if one computes the carbon preference index (CPI) which is a ratio of odd to even chain lengths, the CPI is close to 1.

4) Petroleum contains several homologous series of hydrocarbons, such as the *n*-alkanes, the branched alkanes, isoprenoid alkanes, cycloalkanes and the polycycloalkanes (steranes and hopanes).

5) Petroleum contains series of alkylated aromatics such as mono-, di-, tri-, tetra- etc-, methyl-benzenes, naphthalenes, and phenanthrenes. The percentage of alkyl substituted aromatics compared to the parent ring structures can sometimes be used to distinguish an oil residue from a combustion product pattern of aromatic hydrocarbons as will be illustrated later in this manual.

6) Petroleum contains naphthenic and naphtheno-aromatics and the heterocyclics such as the dibenzothiophenes.

7) Isotope studies show petroleum has little ^{14}C radioactivity since it is a fossil fuel and the stable isotope composition is usu ally heavier than biogenic inputs.

All these characteristics can be observed in crude oils and the refined products derived from petroleum, although as seen in Fig. 2, the distillate fractions cover restricted boiling ranges. These distinctions between natural and pollutant sources are obvious when confronted with fresh oil residues. However, the pattern is modified by biogeochemical processes (Figs. 3 and 4). This is termed "weathering".

Figure 8 shows some of the characteristics of weathered petroleum residues. The composition is altered on time scales of days to years by the following:

Weathered petroleum :

1) Loss of the low boiling ($<C_{20}$) aromatic and saturated hydrocarbons through evaporation.

2) Loss of the low boiling aromatics ($<C_{15}$) through dissolution. (Recall Fig. 6). The resulting residue is relatively enriched in the higher ring aromatics such as the alkylated phenanthrenes, dibenzothiophenes, benzanthracenes, etc.

3) Bacterial degradation of the *n*-alkanes is followed by the branched alkanes. This makes the unresolved signal on the GC appear enhanced. It also changes the ratios between the *n*-alkanes and the isoprenoids, and the *n*-alkanes and the branched alkanes. The complex mixture of polycyclic aliphatic compounds (UCM) becomes a dominant feature of the saturated hydrocarbon fraction. At this stage source identification would be very difficult unless GC/MS procedures are used to identify specific markers in the steroid and triterpenoid hydrocarbon series as discussed later.

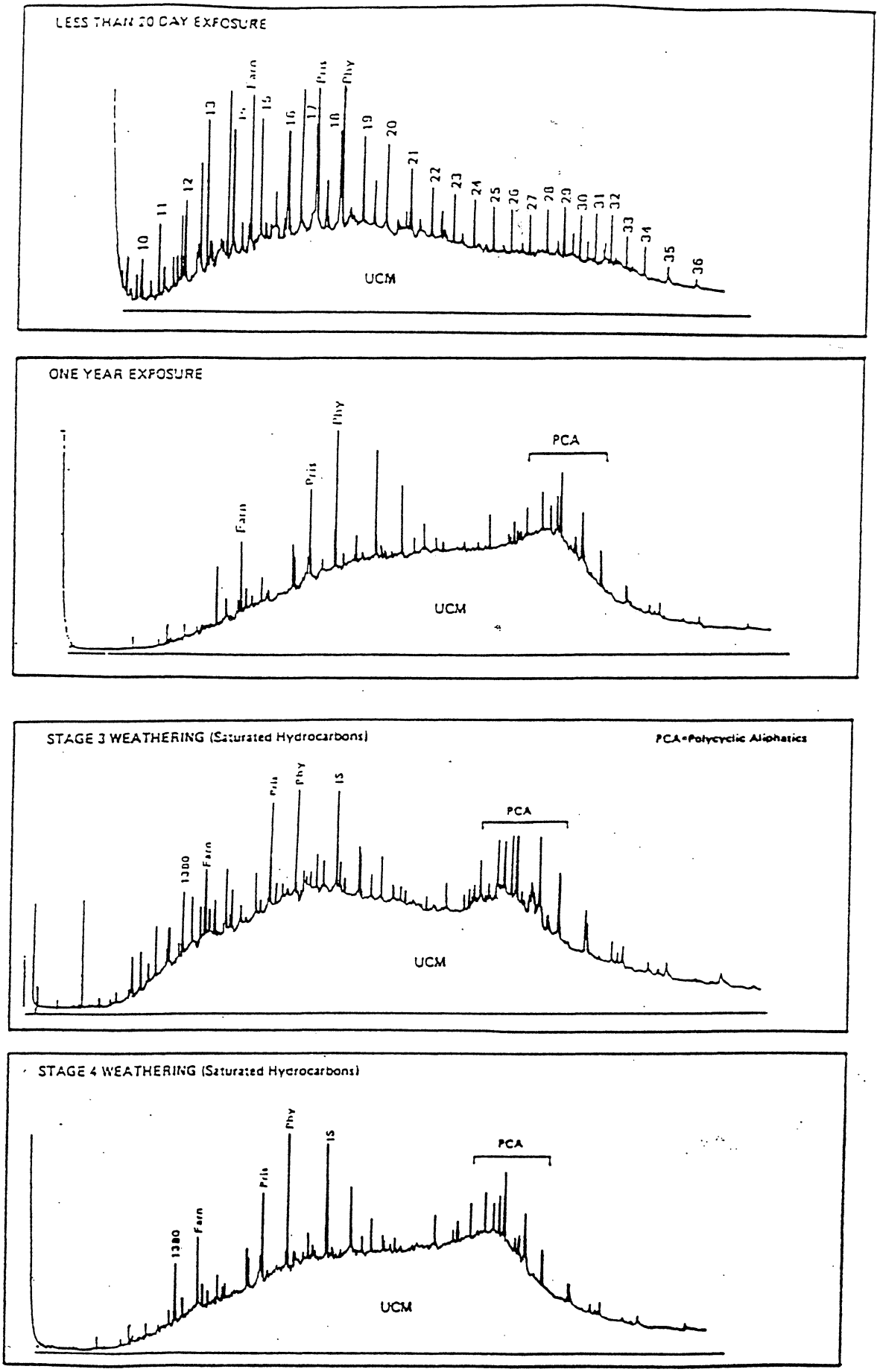


Fig 8 : Times series for fresh petroleum subjected to alteration by natural processes in sediments, "weathering". (From N.A.S. , 1985).

Figures 7 and 8 illustrate the power of gas chromatography in identifying petroleum residues and distinguishing them from biogenic hydrocarbons as well as in understanding the biogeochemical processes modifying petroleum residues over time. However, the techniques of modern chemistry allow even more details to be discerned from the fractions.

If the GC is coupled to a mass spectrometer (MS) with a sophisticated data system and a selective ion monitoring feature, the potential for identifying useful chemical markers is very great. For example, within the complex mixture of residual saturated hydrocarbons, the series of hopanes and triterpanes can be distinguished. Figure 9 shows how the characteristic pattern of these highly stable compounds is still present even after extensive weathering has altered the total ion and flame ionisation detector (FID) traces.

A flame photometric detector (FPD) used on the GC, can be tuned to selectively detect N and/or S compounds. Figure 10 shows an example of the FID and the FPD traces from a fuel oil. It shows a characteristic complex pattern which is not derived from biogenic sources.

By use of these selective techniques, extreme precision in source identification can be achieved. But to do this requires extensive investment in analytical time, equipment and most importantly, expertise which is not always available in many regional monitoring programmes. However, because of the increasing access to desk top model mass selective detectors and instrument in regional head laboratories, this manual summarizes relevant advances in GC/MS methods for characterization of petroleum oils in sediment extracts. The need for continued research in the biogeochemistry of organic contaminants and the use of the most sophisticated techniques available to verify the conclusions drawn from less specific methods in the hierarchical approach are obvious.

Biogeochemical studies of organic contaminants provide the information on inputs, pathways and rates of transfer and transformation, and the reservoirs for accumulation of these compounds in the marine environment. Such research is a necessary complement to studies on the lethal and sublethal effects of contaminants in order to understand the time scales of exposure and in what form the compounds are available to organisms (dissolved, colloidal, particulate, adsorbed on sediment). A few observations from recent research will illustrate these points.

During a study of the cycling of organic contaminants in the region of the New York Bight, sediments and bottom dwelling organisms were collected for analysis. Farrington *et al.*, (1986) reported that extracts of polychaetes showed UVF spectra similar to that produced by common oil products. But further analysis by GC and GC/MS showed the signal was due to a series of diaromatic-tetracyclic hydrocarbons of apparent biogenic or diagenetic origin. These authors concluded that microbiological processes in sewage sludge dumped in the area were the likely sources of these compounds. These presumed diagenesis products were more abundant in the polychaetes than were the fossil fuel and combustion-derived polycyclic aromatic hydrocarbons which were found in the sediment. If the assessment had stopped at the UVF screening method and/or used only sediment analysis, this important finding would have been missed.

Attempts to identify hydrocarbon residues in coastal and open ocean sediments have shown that the criteria described above are useful to distinguish biogenic from fossil fuel derived compounds. But once having established that the bulk of hydrocarbons in a sample are of fossil fuel and not of biogenic origin, the next question is: Are they from oil or from some other fossil source? Other sources of polynuclear aromatic hydrocarbons in sediments include coal and soot as well as diagenesis products.

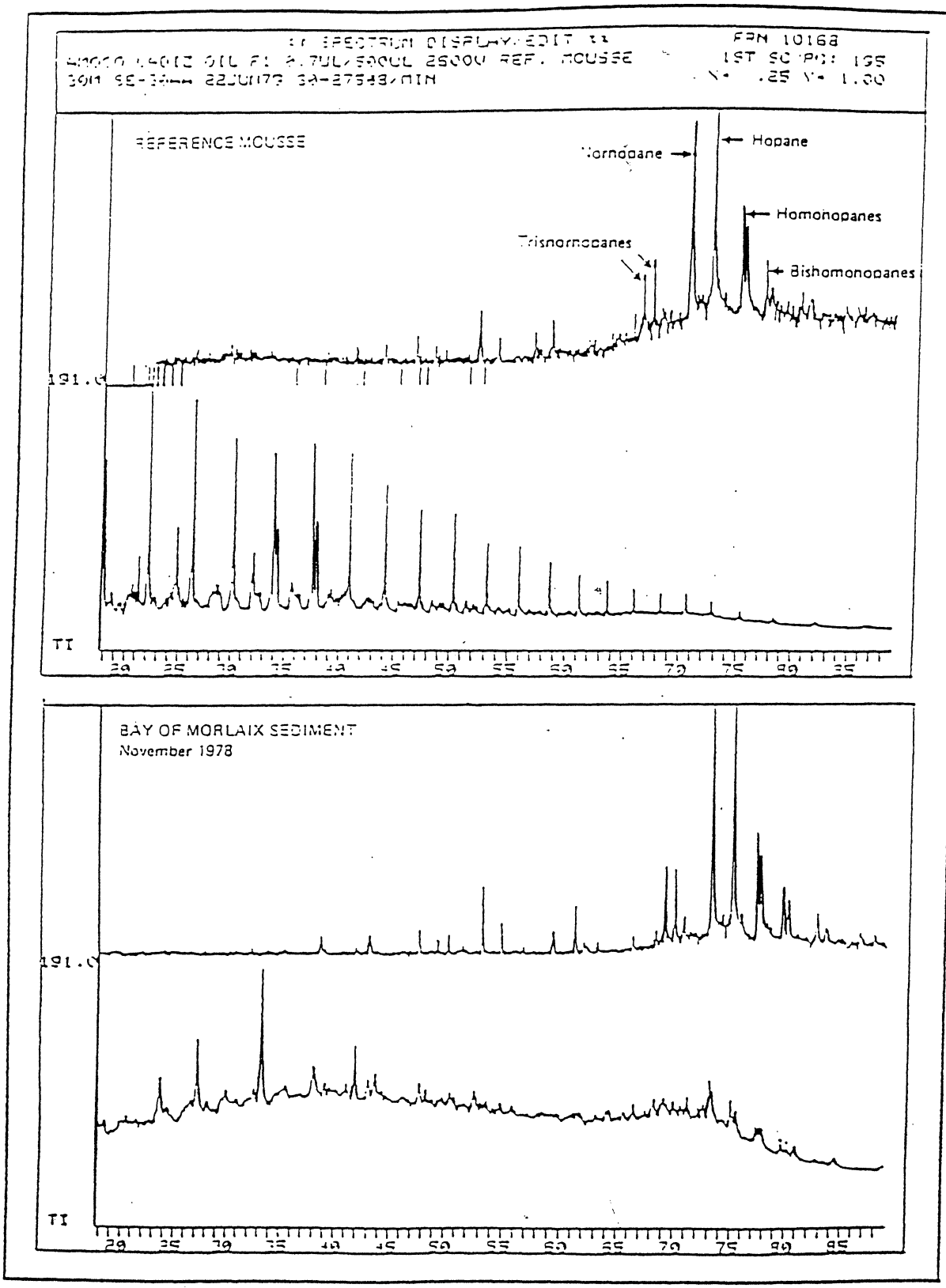


Fig 9 : Gas chromatographic mass spectometry selected ion searches for pentacyclic triterpanes (hopanes) in Amoco Cadiz reference oil and November 1978 weathered oil in sediments. (From : N.A.S. , 1985)

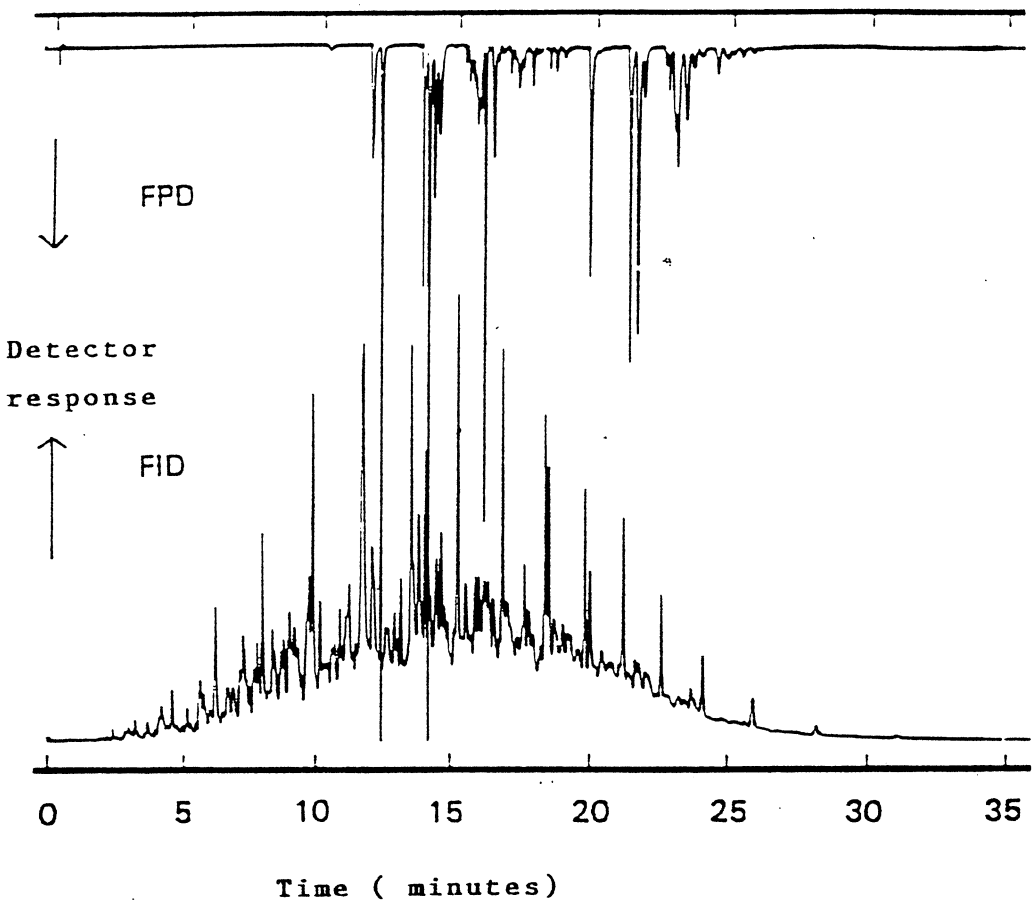
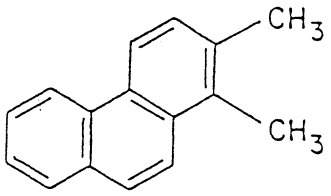


Fig 10 : Simultaneous flame photometric (FPD) and flame ionization (FID) detection of components of No. 2 fuel oil. (From A.P. Bentz, 1976, *Anal. Chem. : 48* (6), pp 454A - 472A. Reproduced with permission of the American Chemical Society).

EXAMPLE: TWO ALKYL CARBONS
ON PHENANTHRENE



(1, 10- DIMETHYLPHENANTHRENE)

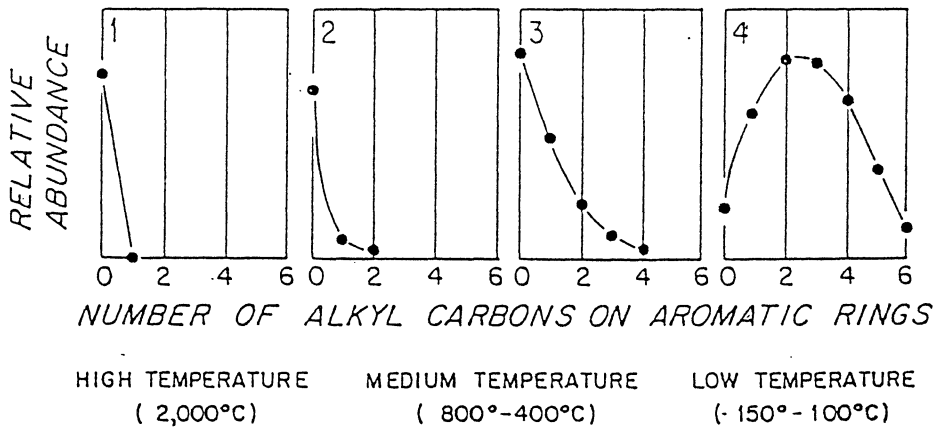


Fig 11 : Relative abundance of parent aromatic compound and alkyl substituents as influenced by temperature of formation. (From : N.A.S. , 1985).

When fossil fuels are burned, a characteristic assemblage of aromatic hydrocarbons is produced in the combustion process. These combustion products are transported atmospherically in vapour and aerosol form for long distances, deposited on the surface ocean and transported to sediments, or deposited on land and then washed by rainwater into land runoff and sewers to rivers and estuaries or directly to coastal waters. Their presence in remote atmospheres and deep sea sediments is evidence for long range transport and the stability of the pyrolytic or combustion product PAHs in the environment. Residues from combustion products can be distinguished from those of fresh oils by the ratio of alkyl-substituted PAH to parent compounds. Oils and coals display a complex mixture of alkyl substitutions while the PAHs formed during combustion are predominantly the parent hydrocarbons. This is illustrated in Fig. 11. Panel 4 is the situation encountered with crude oil and fuel oils while panels 2 and 3 represent the situation encountered with most combustion processes involving coal and oil. Panel 1 might be expected for very efficient combusion of natural gas. The PAHs from combustion processes exhaust or smoke stack emissions are not the PAHs of the original fuel but are formed by chemical reactions during the incomplete combustion process. Soot contains high concentrations of these PAH. Examples of the PAH parent compound and alkyl PAH plots similar to those shown in Fig. 11 but for actual sediments sampled in the New York Bight area of the United States are shown in Fig. 12. The top panel, A, shows plots of the PAH extracted from sediments contaminated mainly by petroleum inputs as indicated by greater relative abundance of the alkylated (methyl-; dimethyl- or ethyl-; trimethyl-, dimethylethyl, or propyl-, isopropyl-) substitutions (indicated as C_1, C_2, C_3) in the naphthalene, fluoranthene, phenanthrene or anthracene, and dibenzothiophene series. The appropriate molecular weights are plotted on the x axis. (e.g. for naphthalenes these are 128, 142, 156, etc..., for phenanthrenes and anthracenes these are 178, 192, 206, etc...). The alkyl-PAH plots for the fluoranthenes through the benzofluoranthenes show a combustion product source for these compounds. Panel B shows a predominance of the combustion source in the high molecular weight PAH with small contribution from a possible petroleum source in the low molecular weight range.

Figure 12 exemplifies the PAH patterns and concentrations found in sediments near urban harbours or petrochemical complexes. Sediments in these areas that are deposited over time often provide a historical record of PAH and other hydrocarbon inputs (Wakeham and Farrington, 1980). Gas chromatograms that illustrate the alkane, cycloalkane and aromatic hydrocarbon distributions before and after modern use of fossil fuels in the Seattle, Washington USA area are shown in Fig. 13 A, B and Fig. 14 A, B. The B panels are from the bottom of a sediment core (90-94 cm) deposited in a time period before modern use of fossil fuels. The A panels are from sediments deposited about the mid-1970s. The saturated hydrocarbons depicted in Fig. 13 show that the non-polluted bottom sediments contain typical biogenic hydrocarbons whereas the modern sediments receive chronic oil inputs with the biogenics superimposed on the unresolved petroleum derived hydrocarbons. The PAH distributions (Fig. 14) show combustion derived aromatics in surface sediments, but only low concentrations of the early diagenesis transformations of biogenic steroid and hopanoid compounds deposited in the deeper aquatic sediments.

In summary, many coastal sediments near urban-industrial areas or at the mouths of rivers draining such areas, can contain fossil fuel hydrocarbons from several sources. The analyst must be aware of these different sources when attempting to interpret data. High resolution GC analyses are necessary to differentiate between these various sources. Distinguishing oil from coal residues is still a research challenge although progress using nitrogen containing PAH as tracers has been reported (Furlong and Carpenter, 1982). In many cases, assignment of sources of specific proportions of total hydrocarbons and specific hydrocarbons to a single source of input is not possible given the current state of knowledge. The situation can be even more complex near sources of natural oil seeps. However, it is possible to establish the current situation and then proceed to monitor for changes associated with new sources of inputs or reductions of inputs resulting from regulatory actions.

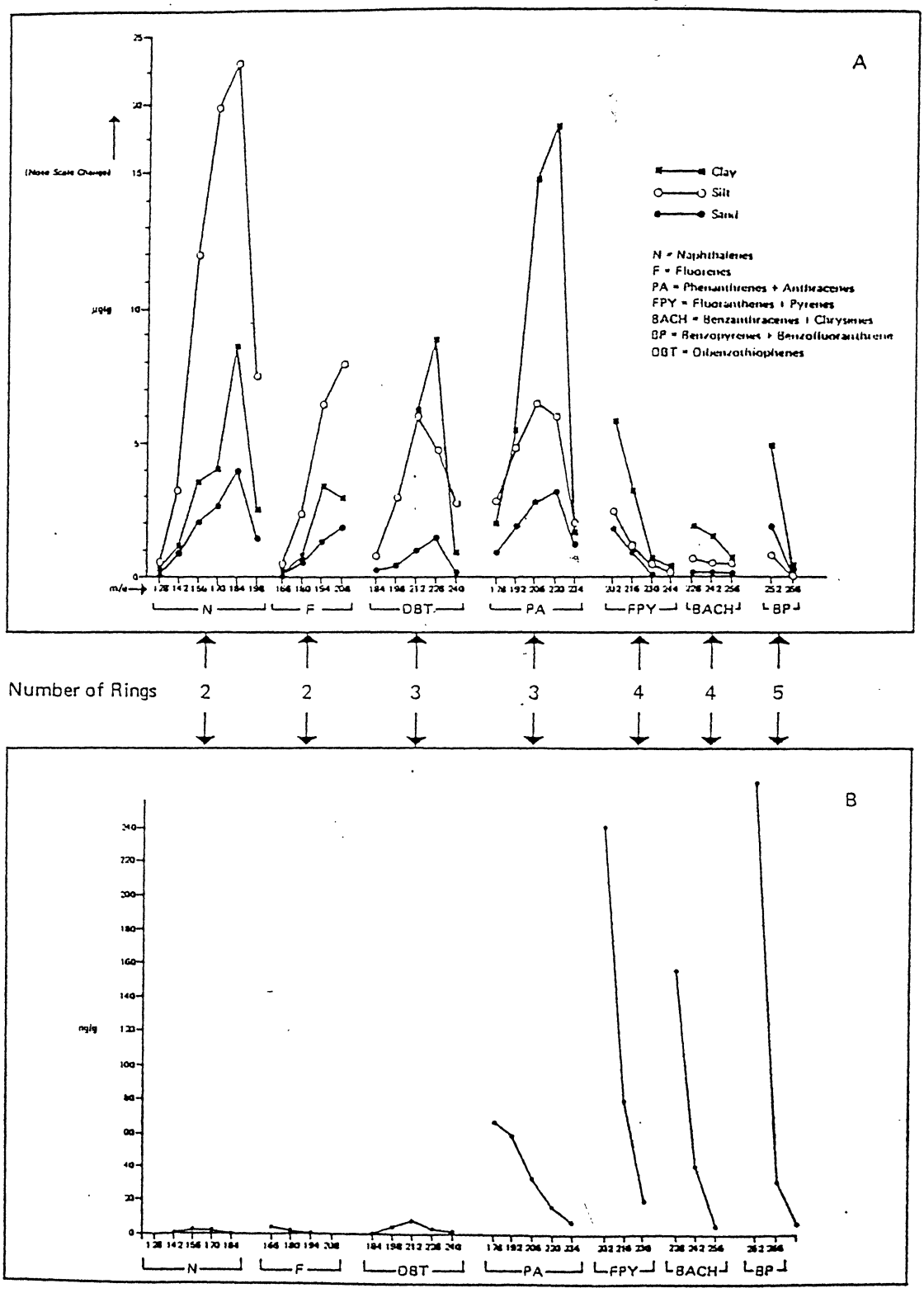


Fig 12 : PAH alkyl homologue distributions showing sewage sludge - mainly petroleum derived (A) and dredge spoil - mainly combustion derived (B); within each homologous series numbers represent molecular weight of parent (unsubstitued), methyl, dimethyl, etc...compounds
(e.g. 128 = N; 142 = C_1; 156 = C_2N; etc...)

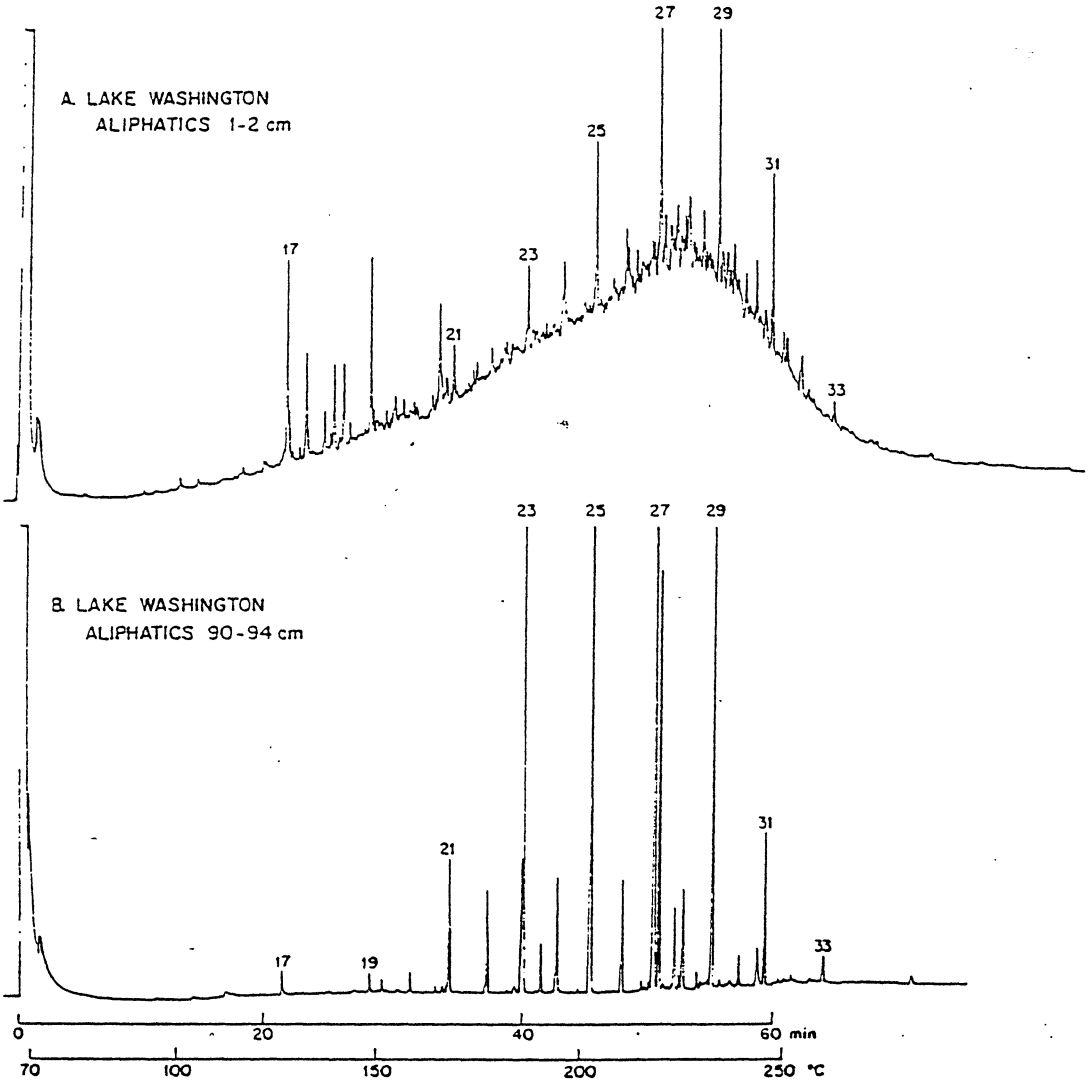


Fig 13 : Saturated hydrocarbons in sediments from a dated core.
(From : Wakeman and Farrington, 1980).

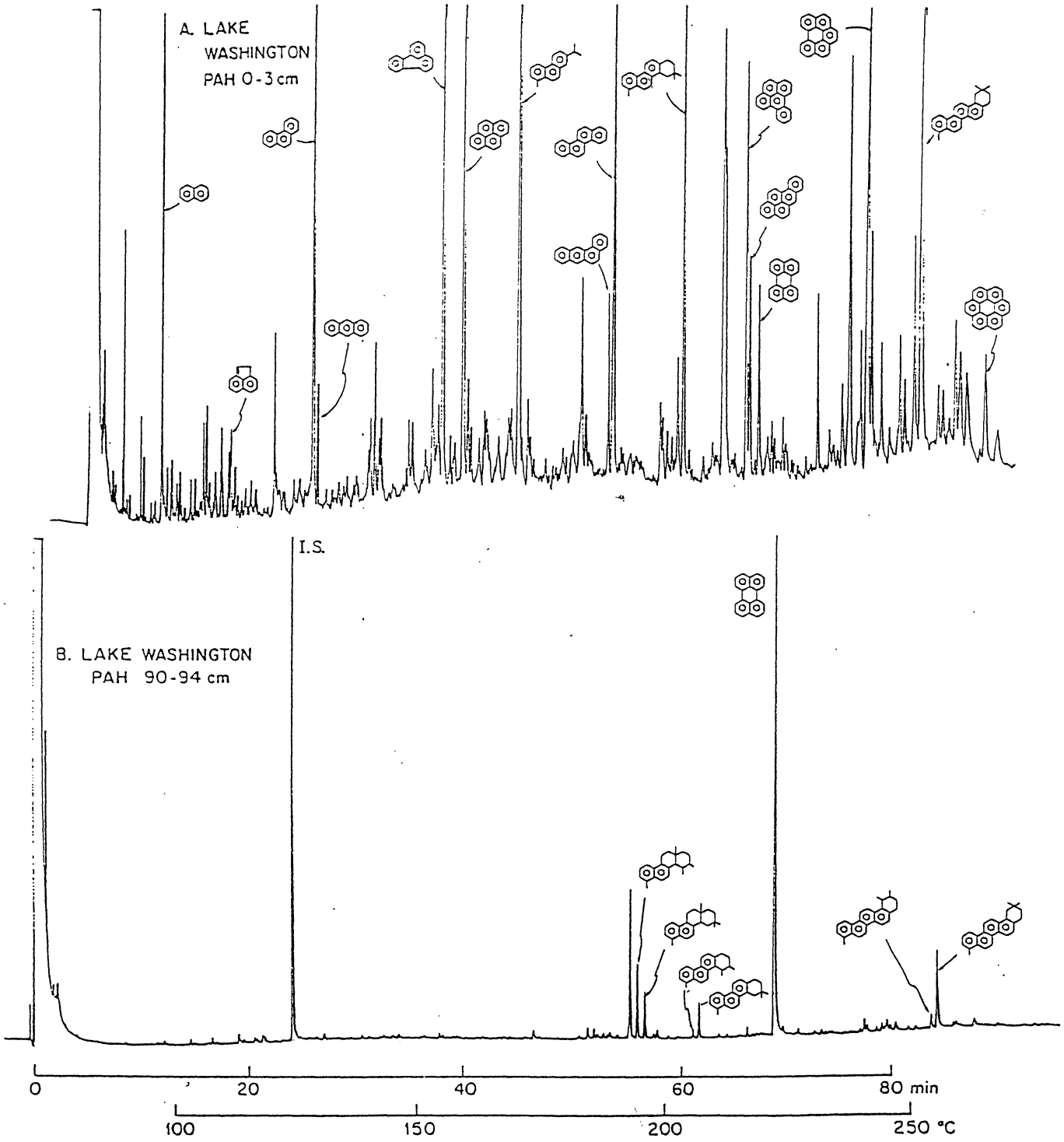


Fig 14 : Aromatic hydrocarbon fractions from sediments in a dated core.
(from Wakeman and Farrington, 1980)

In addition to the nitrogen containing PAH described in the previous paragraph, oxygenated products of hydrocarbons are also present in the environment. These are produced by processes of microbial and photo-degradation. Due to the analytical difficulty of separating polar contaminants from biogenic lipids and because of a lack of compounds to use as calibration standards, the analysis of reaction products has until recently been restricted to radiotracer experiments and the identification of only a few reaction products in environmental samples. Biogeochemical studies show that oxygenated products may have lifetimes of days to months in marine sediments and may represent a significant portion of the contaminant load in coastal sediments. Photo-oxidation is expected to be an important pathway for the removal of oil residues, and may eclipse the rate of microbial degradation, especially in tropical regions where low nutrient levels may limit the growth of microbes.

Organic contaminants escaping into estuaries, rivers and other coastal waters from urban street runoff, industrial and municipal discharges add to the loads in coastal waters derived from the deposition of atmospherically transported residues of combustion and the contaminant loads associated with spills and disposal operations. The organic residues distribute themselves between dissolved and particulate phases of surface seawater, generally in relation to their solubility characteristics. PAHs and the majority of petroleum derived hydrocarbons generally adsorb to suspended particles in surface waters. The lighter aromatic hydrocarbons often remain in solution. The dispersion processes which are most important on a short time scale are those that control the water and particle movement out of rivers, along coasts and over continental shelves. (Processes reviewed by Boehm; 1987).

Sorption of organic contaminants onto particles has been shown to depend not only on the particulate loading of estuaries and oceans, but also on the types and degree of aggregation of the particles available. Particles of high lipid content should more efficiently adsorb organic contaminants and the expression of analytical results normalized to extractable lipid or to organic carbon can be a useful measure of contaminant loadings. (Dixon *et al.*, 1987).

Vertical fluxes through the water column are significantly accelerated in coastal waters by packaging of both soluble and particulate organic residues into fast sinking fecal materials generated by the zooplankton communities. This phenomenon permits the rapid settling of organic contaminants out of the surface waters and into the sediments. The time scale for this process is estimated to result in a residence time of less than one year for petroleum hydrocarbons even in oligotrophic waters with small particle loadings. (Burns, *et al.*, 1985).

These few examples and figures illustrate the necessary interplay between research and monitoring activities.

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3. SCOPE AND FIELD OF APPLICATION

The reference methods described in this manual are intended for use in marine monitoring programmes. They consist of a UV-fluorescence screening method, a gas chromatographic-flame ionisation detector(GC-FID) and a gas chromatographic/mass spectrometric (GC/MS) method for the determination of total and individual petroleum hydrocarbons in sediment samples in the dodecane (C_{12}) to hexatriacontane (C_{36}) elution range. All three methods require the same careful planning, sampling and extraction procedures. Differences occur at the analytical steps, the use of instrumentation and the expression of results. In the UVF method the quantitative result is expressed as the number of "chrysene" and/or "oil" units in samples as determined by comparison with response curves generated by these standards. In the GC-FID method the result is expressed as total hydrocarbons (THC), unresolved complex mixture (UCM), and as the concentrations of individual resolved compounds. In the GC/MS method the results are expressed as the concentrations of individual marker compounds.

Three extraction procedures are described : 1) Soxhlet extraction after drying the sample with sodium sulphate, 2) extraction of sediment by sonication with organic solvent and 3) a wet hydrolysis procedure. Before analysis by GC-FID or GC/MS, elemental sulphur is adsorbed onto activated copper. Hydrocarbons are then separated from interfering lipids and divided into classes by means of an adsorption chromatography step. Traditional column chromatography using silica and alumina gels is described in addition to an alternative procedure using high performance liquid chromatography. The detection limit for THC is approximately 10 $\mu g.g^{-1}$ dry wt; and for individual hydrocarbons it is approximately 100 $ng.g^{-1}$ dry weight.

Many of the trace halogenated contaminants associated with our industrial society such as PCBs will also be recovered in these procedures. Since many organochlorine residues are not stable to saponification treatments, care must be exercised in extending the analyses to other classes of compounds if a hydrolysis extraction is used.

4. PRINCIPLE

After collecting sediments with a grab or coring device, appropriate layers or subsamples are stored in non-contaminating jars at -20°C until analysis. For analysis, the samples are defrosted and prepared for solvent extraction. For satisfactory recovery of hydrocarbons, sediments must either be dried by absorbing water with anhydrous sodium sulphate and then subjected to direct extraction with dichloromethane or the wet sediments must undergo a hydrolysis extraction using methanol and potassium hydroxide. The solvent is changed to hexane at which stage preliminary screening by UVF can be done. The result is expressed as chrysene or oil equivalents and is semi-quantitative. For further work the extract is concentrated by rotary evaporation. Elemental sulphur can interfere in the GC and especially the GC/MS analysis and thus is removed from the hexane extract by adsorption on activated copper. The extract is further concentrated and total extractable organic matter is determined gravimetrically. Interfering lipids are removed by a normal phase adsorption chromatography step. Fractions are collected which contain the saturated hydrocarbons in F1 and the aromatic hydrocarbons in F2 and F3. These fractions are again reduced in volume and analysed by UVF, GC-FID and/or GC/MS. Surrogate standards are added to extraction solvents to track recovery of the hydrocarbons through the analytical procedure. Hydrocarbons in the dodecane (C_{12}) through hexatriacontane (C_{36}) elution range are quantified. Subsamples of the wet sediment are dried at 105°C to determine the wet to dry weight ratio. This dried sediment is sieved through standard mesh screens and weighed to determine the approximate size composition through sands and silt-clay fractions. Results are expressed as total hydrocarbons (THC), unresolved hydrocarbons (UCM), and/or individual hydrocarbons per g dry weight and per g extractable organic matter (EOM).

5. REAGENTS, SOLVENTS, STANDARDS

All reagents, solvents, and standards must be of the best analytical quality, and tested for their suitability before attempting sample analysis. Solvents must of "distilled in glass" quality. If not regionally available, less pure grades could be used after redistillation in a glass still in the laboratory.

5.1 Ultra pure distilled water. Milli-Q organic grade or equivalent or distilled-in-glass with a few $KMnO_4$ crystals in the boiling flask.

5.2 Solvents : Distilled-in-glass quality: Hexane (or pentane), Dichloromethane, Acetone, Methanol, Iso-octane (trimethyl-pentane).

5.3 Concentrated acids: Hydrochloric (HCl), Sulphuric (H_2SO_4).

5.4 Powdered or crystalline reagents[1] : Sodium nitrate ($NaNO_3$), anhydrous Sodium sulphate (Na_2SO_4)*, Sodium chloride (NaCl)*, Copper sulphate ($CuSO_4.5H_2O$), Potassium hydroxide (KOH), Potassium permanganate ($KMnO_4$), powdered zinc (Zn)

5.5 Chromatography supports : Alumina (eg : Merck, aluminum oxide 90 Aktiv, 0.063-0.200 mm, 80-200 mesh), Silica Gel (eg : Merck, Kiesegel 60, 0.040-0.063 mm, 230-400 mesh) or equivalents.

5.6 Carbon or carborundum boiling chips*.

NOTE 1 :

Any reagent which comes in contact with the sample or its extracts must be thoroughly cleaned before use. For those indicated by an *, this will require precombustion in a muffle furnace at approximately 400°C (Section 8.1).

5.7 Ultra pure gases : Nitrogen (N_2), Hydrogen (H_2), Helium (He) for GC/MS, Air. Cylinders must be fitted with two-stage regulators and filter traps filled with molecular sieve 5A and indicating silica gel, to remove trace organics and water. An oxygen trap must be fitted to the carrier gas for the GC/MS.

5.8 Surrogate standards[2] : mg to 1 g quantities of: *n*-octadecene ($C_{18:1}$), *n*-eicosene ($C_{20:1}$), 9,10-dihydroanthracene, ortho-terphenyl (for GC-FID), deuterated polycyclic aromatic hydrocarbons: diphenyl-d_{10}, phenanthrene-d_{10}, and pyrene-d_{10} (for GC/MS analysis). Stock solutions should be prepared in iso-octane at concentrations sufficient to add the required amount to each sample extract to yield a final concentration in the reduced sample fractions of approximately 20 $ng.\mu L^{-1}$ for capillary GC analysis. If oil levels are high, this amount will need to be increased by a factor of 10 or 100. The amount of surrogate standard needed must be determined by experience with the samples being analysed.

NOTE 2 :

Other compounds could be used but some such as anthracene are unstable. All standard solutions must be stored cold (< 4°C) in the dark and concentrations must be confirmed on a regular basis. As refrigeration of solutions often causes some of the less soluble higher molecular weight compounds to come out of solution, adequate time must be allowed for solutions to come to room temperature before use or subsampling. Note that higher molecular weight aromatic hydrocarbons are not readily soluble at high concentration in iso-octane, thus intermediate solutions in methanol or other polar solvents may be necessary.

5.9 Calibration standards[3] for GC, GC/MS: mg quantities of $_n$-alkanes in the C_{10} to C_{35} elution range, aromatic hydrocarbons in the trimethyl-benzene to benzo(ghi)perylene elution range, pristane, phytane and other useful markers. Calibration solutions should be diluted to concentrations of approximately 200 $ng.\mu L^{-1}$ for use with packed column GC, and to 20 $ng.\mu L^{-1}$ for use with capillary column GC and scan mode GC/MS. For SIM mode GC/MS, PAH standards in the 40 to 400 $pg.\mu L^{-1}$ range are required. Calibration curves must be determined for the compounds to be quantified and the concentrations of standard solutions should be adjusted to yield responses within the linear calibration ranges for individual instruments.

NOTE 3 :

The availability of pure and accurate standard solutions for calibration is often a limiting factor in the analysis of organic contaminants in the environment. Thus IAEA/UNEP/IOC is endeavoring to prepare and distribute calibration solutions to laboratories participating in regional monitoring programmes. High quality mixtures are available from some commercial suppliers and some national government sources.

Carefully follow the suggestions for preparation and storage of standard solutions given in the analysis sections. It is good practice to make solutions up by weight on an accurately calibrated and checked balance. To reduce the problems of solvent loss, it is recommended to make stock and working solutions up in iso-octane (density = 0.692). Glass scintillation vials with aluminum foil-lined lids make convenient containers. After solutions are brought to volume on the balance, the final weights (gross weights) are written on the vial. Each vial should have its own useage log. When aliquots are removed for dilutions, the log is marked and the expected weight on the vial reduced accordingly. Before subsampling any stock solution, its weight should be compared with that expected. If any significant deviation is noted, it should be discarded and a new solution made. Laboratories equipped with glass blowing torches may find it effective to heat seal stock solutions in glass vials. This is another skill which takes practice.

CAUTION : Solvent solutions must be chilled to well below zero for the heat sealing procedure.

5.10 Standard oils and chrysene for UVF analysis. Milligram to gram quantities of chrysene, Arabian light crude oil, Ekofisk crude oil, Kuwait crude oil or any other oils relevant to the individual monitoring programme.

5.11 Standard Reference Materials of marine sediments for use in quality assurance procedures. Samples for hydrocarbon analysis are available through IAEA/UNEP/IOC and some national sources such as the National Research Council of Canada and the National Institute of Standards and Technology of the United States.

6. APPARATUS AND EQUIPMENT

The laboratory used for trace analysis must be a dedicated facility, isolated from other projects that are sources of contamination. It must be properly constructed with fume hoods and benches with electricity outlets that are safe for use with flammable solvents. The laboratory must have cooling water to run the stills, extractors and rotary evaporators. In tropical regions and in dry climates a refridgerated recirculating 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 solvent cleaned. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc... The lab should be equipped with an emergency cut-off switch for the electrical supply. This cutoff should be reachable from inside and outside the laboratory. Mount fire fighting equipment in obvious places and train laboratory personnel in their use.

6.1 A coring device with liners and plunger or a grab sampler. Thoroughly cleaned with detergents and solvents before use.

6.2 Glass jars and aluminum foil, stainless steel knives, scoops, forceps, labels, marking pens, log book.

6.3 Insulated plastic boxes for transporting samples. Ice or dry ice.

6.4 Deep freezer[4] (-18 to -20°C) for sample preservation.

NOTE 4 :
Frost free type freezers heat to > 0°C during frost removal cycles. These cannot be used for long term sample storage.

6.5 Muffle furnace for precombusting reagents (If none available, some can be cleaned by extraction in the Soxhlet apparatus with $MeCl_2$ followed by drying in a clean drying oven.).

6.6 Drying oven for glassware, reagents, and activating chromatography supports. This oven must be non-contaminating and must be controllable between ambient and 240°C. For drying sediments a small oven stable in the 60 to 105°C range is required.

6.7 Ungreased, clean glass desiccator for storing adsorbants.

6.8 Cotton wool and glass wool precleaned with solvent extraction in a Soxhlet apparatus. Store them in sealed jars with ground glass stoppers or aluminium foil or teflon-lined plastic screw lids.

6.9 Analytical balances : top loading electronic balance with 0.001 g accuracy, and an electronic microbalance with 0.1 µg accuracy. (If no microbalance is available, the procedures can be adapted to use a 4 or 5 place electronic or mechanical balance). All balances must be regularly cleaned and calibrated.

6.10 All glass, solvent distillation apparatus. This is required to bring new solvents to a grade suitable for trace analysis and to reclaim solvents after condensation in the rotary evaporator. The distillation column should be approximately 1 m tall and filled with glass ratchets. Flasks, ratchets, columns, condensers and adapters should all be cleaned with acid before assembling into a solvent still. Suitable heating mantles, thermometers, carbon boiling chips, etc. are required for use with the appararus. It is recommended that the hexane still be a dedicated still and other solvents be processed in a separate still. Carefully separate and label solvents to be distilled or to be disposed.

6.11 Magnetic stirrer and teflon coated stir bars.

6.12 Rotary evaporator equipped with a glass trap between sample and evaporator and traps to condense evaporated solvents, cooling water for condensers, heated water bath for sample flasks (never set to exceed 30°C) and a molecular sieve trap on the air inlet.

6.13 Heating mantles and variable electric controllers to power stills and extractors. Note that most Variacs have a 10 amp maximum. Multiple Variacs are required to power multiple heating mantles. Note the demand of each mantle and do not exceed the capacity of the Variacs (Because these apparatus are water cooled, it is good practice to mount the Variacs on the wall and to elevate the mantles off the bench for protection in case a hose springs a leak.).

6.14 Glassware : mortar and pestle; 50, 100, 250 etc mL beakers; 500 mL Erlenmeyer flasks; 500, 250, 100 mL round (flat bottomed) boiling flasks with ground glass necks; ground glass stoppers to fit flasks; 250 mL, 100 mL, 50 mL measuring cylinders; Soxhlet extractors (125 mL; complete with flasks, condensers and thimbles) + one large (1L) complete Soxhlet apparatus for precleaning adsorbants etc. ; 500 and 1,000 mL separating funnels with teflon keys; 50 mL burettes with teflon keys and sintered glass discs at bottom; 100 mL volumetric flasks; 20 mL vials with Al foil-lined lids; 2 and 4 mL vials with teflon-lined screw caps; Pasteur pipettes; graduated glass centrifuge tubes with ground glass stoppers; 500, 250, 100, 50 µL syringes for making up standards.

6.15 Fluorescence spectrophotometer capable of synchronous scanning of emission and excitation wavelengths, 10 mm quartz cells, recorder, chart paper, pens, etc...

6.16 Gas chromatograph (GC) for packed column work or preferably equipped with capillary split/split-less or on-column injectors. Either must have a flame ionisation detector and an electronic integrator. Columns are described under sections 8.6.1 and 8.6.2 respectively. The integrator can be a stand alone type or a personal computer equipped with an analogue to digital interface and chromatography software.

6.17 Capillary gas chromatograph interfaced to mass selective detector, mass spectrometer or ion trap spectrometer and a computerized data system.

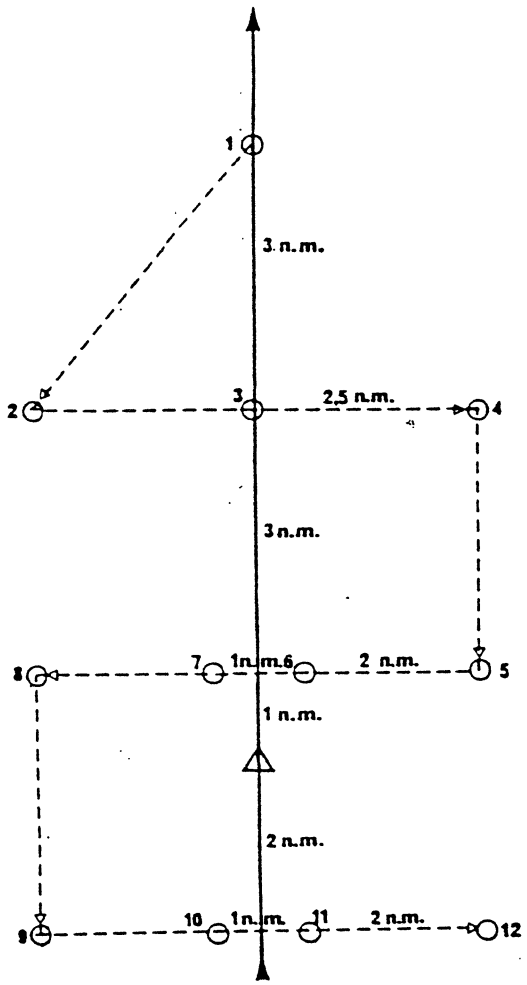


Fig 15 : Grid system for the sampling of sediments. O-sampling stations, Δ-drilling position and the arrow is pointing in the direction of the residual current. (distances in nautical miles).

6.18 Regulators and molecular sieve traps for the GC gases.

6.19 High performance liquid chromatograph with gradient solvent programmer, dual or ternary solvent delivery system, Rheodyne 7125 variable volume injector with 200 µL sample loop (or equivalent), six port back flush valve, in line filters, guard columns etc. as described in section 8.3.2.

6.20 Glass syringes 10 or 1 µL for injection into GC, GC/MS and 100 µL for injection into HPLC.

6.21 Power stabilizers for the electronic integrators, microbalance, computer, etc...

6.22 Sedimentological sieves, stainless steel: 63, 125 and 1000 µm.

6.23 Miscellaneous laboratory equipment such as safety glasses, gloves and laboratory coats for handling acids, pH paper, laboratory detergent, scrub brushes, test tube racks, light table for overlaying spectra and chromatograms, stopwatch, chart papers, print heads, GC septa, ferrules and other consumable parts, stainless steel tubing and Swagelok type connectors for gases, etc..., tubing cutters and files, spanners and other tools used for maintenance, etc...

7. SAMPLING PROCEDURE

The sampling scheme is the basis of the work on petroleum hydrocarbon pollution studies and must therefore be carefully planned and executed.

7.1 Planning

A sampling plan should be designed in accordance with the nature of the situation to be investigated. It must be a collaborative plan approved by the analysts who are familiar with how samples must be treated and what methods will be used in the analysis, the scientist who will use the data to answer specific project objectives and the statistician who will assist in the evaluation of the data.

From the descriptions of estuarine and coastal processes given in the introductory discussion, it is clear that the interaction of organic contaminants with biological systems is of prime importance with respect to the toxicity of the contaminants and in terms of the transport and retention of contaminants in coastal and oceanic ecosystems. Thus the sediment analysis programme should be an integral part of a larger assessment programme.

Before monitoring programmes are started, it is desirable to have a set of baseline measurements made on samples collected before the pollution event commenced. In acute spill situations and in heavily or chronically contaminated situations this is not always possible and reference sites of similar characteristics will have to suffice for comparisons.

7.2 Selection of sample sites.

Sample sites are normally chosen on a broad grid network which covers the geographical area that has been affected by an oil spill, which is chosen as a reference area, or where drilling or dumping activities are to be conducted in the future (e.g. Fig. 15).

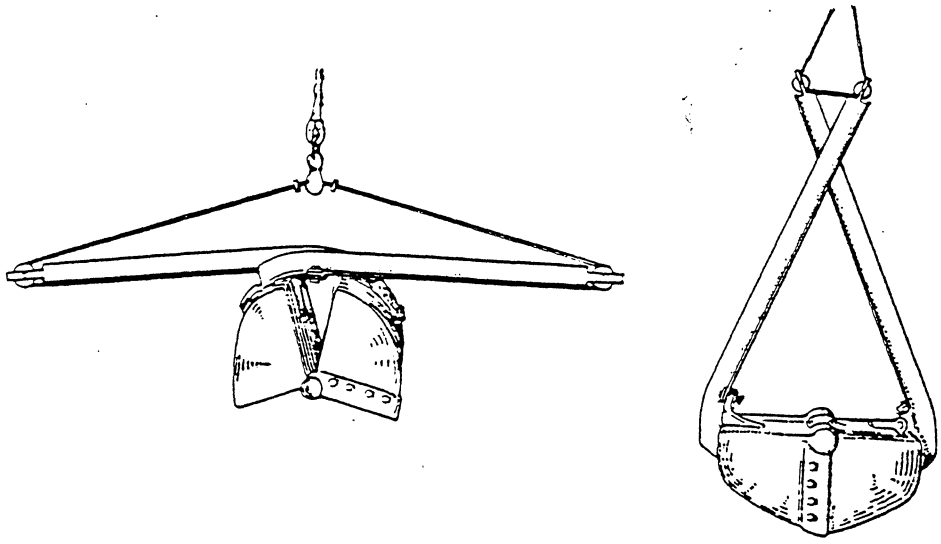


Fig 16 : van Veen grab for sediment sampling (after H. Friedrich).

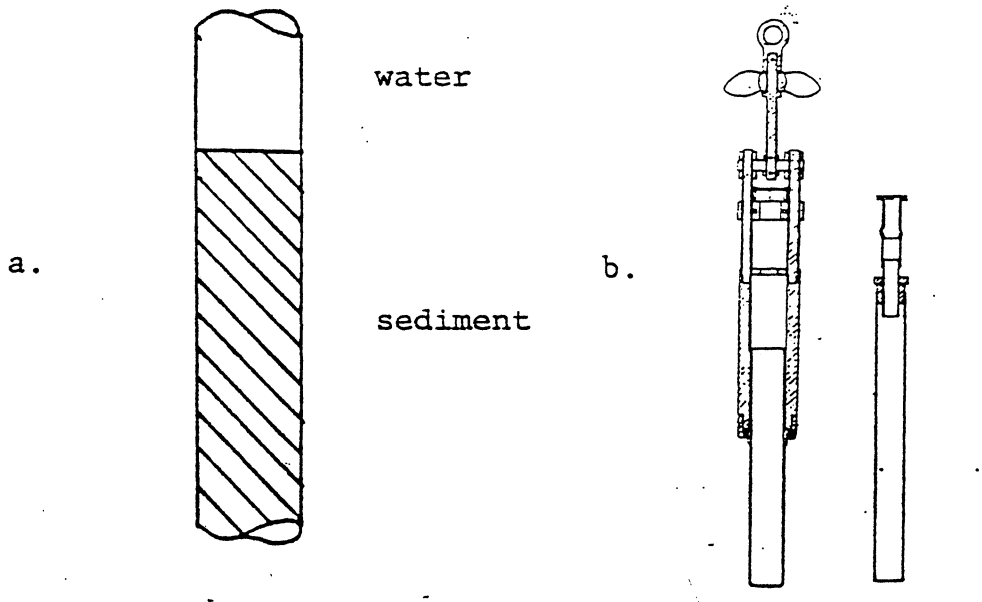


Fig 17 : a. Glass liner from a gravity corer. The sediment from the upper part may be used for trend-analyses. b. Total display of bottom corer.

Because the analysis of petroleum hydrocarbons in samples is expensive, care must be taken in the selection of the sample sites and the number of samples collected. Enough samples must be collected to achieve the project goals; this usually means over-sampling, so that only a selected number of samples will be analyzed as dialog between the analysts, scientists and statisticians proceed.

Replicate samples should be collected, whenever possible, at each grid location. Analysis of the replicates will allow for the variance in the measured parameters to be determined. This variance is then used to indicate the number of sample replicates required to detect a statistically significant change within specified confidence limits. The number of replicates calculated for statistical purposes assumes that values are "Gaussian" or "normally distributed". In an environmental situation this distribution of petroleum hydrocarbons is only found if the source of the hydrocarbons is atmospheric deposition over time. If the hydrocarbons originate from a recent oil spill, their distribution in sediments is generally extremely patchy and groups of replicates may not be normally distributed. In such cases the samples must be classified according to the situation causing the pollution and the processes acting to disperse it.

7.3 Sample collection and storage.

The device used for sample collection will vary according to the environment being sampled and the equipment available. Holme and McIntyre (1984) describe and discuss a selection of suitable devices. In coastal salt marshes, hand-held coring tubes can be used. Similarly in shallow water environments, SCUBA divers can collect sediments with hand coring devices. In sandy coastal sediments, coring devices will not work and a scoop or a grab sampler must be used (for example, the Van Veen grab shown in Fig. 16). The first few cm of sediment are spooned into the sample jars. The performance and bite profiles of a selection of grab samplers were discussed by Riddle (1989). In any situation in which a corer will work, this sampling device is preferable to a grab which tends to loose surface layers as it is brought up through the water column. Cores are cut into specific layers for analysis. An example of a corer which has traditionally been used is shown in Fig. 17. The initial arrival of petroleum hydrocarbons to the sediment may be in the form of fine flocculent material at the sediment/water interface. Careful sampling of this layer is required in order to avoid loss of the hydrocarbons. Most grab samplers and many coring devices do not efficiently retain this layer (Baxter, *et al.*, 1981). A few efficient coring devices have been developed which include modifications of the sphincter corer described by Burke (1968) and Soutar type and other box corers. Designs are continually being improved and anyone interested in purchasing such a device should consult the major oceanographic instrument companies. Cores should be allowed to settle resuspended surface material inside the liners before it is cut into layers. If water persists above compacted cores, it should be siphoned off though cleaned copper or stainless steel tubing, filtered through a precombusted glass fiber filter, and the filter and filtered water saved for analysis. For extrusion the plunger is fitted to the bottom of the core and the surface layer pushed up through the top of the core liner. The required thickness is sliced off with a stainless steel knife onto a solvent cleaned sheet of aluminum foil or glass dish. Edges of the core are then trimmed off and the remaining sediment spooned into sample jars. Successive layers can be sampled by pushing up the plunger and repeating the process. Surface layers in large box cores are usually subsampled with smaller hand held tubes.

All relevant data on the location and sampling of the core, including the water depth from which it was retrieved, the sampling method, environmental conditions, and a visual estimate of core condition should be logged. If possible, subsamples should be saved for complementary and ancillary analyses. Sample jars must be sealed with lids lined with solvent rinsed Al foil or sheet teflon. Clearly label all jars in a manner which matches the sample log. If necessary, seal the outside of the jars with "evidence" tape and attach a tag which will be used to indicate the chain of custody. Place the samples in insulated boxes on ice or dry ice until they can be moved to a deep freeze for storage. As labels tend to come off sample jars it is good practice to pack the jars in sequence in a compartmentalized box. The position of each sample in the box is logged in a notebook. If several boxes are loaded into storage, a freezer plan is also prepared. The sample inventory should be transcribed to a computerized spreadsheet with sample codes and specific details. As samples are removed from the freezers for analysis, the inventory must be updated.

8. ANALYTICAL PROCEDURES

8.1 Preparation of reagents and apparatus.

Clean glassware by scrubbing with brushes in hot water and detergent. Rinse five times with tap water and twice with distilled water. Drain. Rinse with acetone. Drain. Rinse with hexane. Drain. If a clean oven is available to bake the glass, it should be used after the water rinses. Only non-volumetric glassware should be heated to between 200 and 240°C. All glassware should be tightly sealed with solvent rinsed aluminium foil and stored in clean cabinets when not in use. For extremely trace work, the glassware is rinsed again with hexane just prior to use. If necessary to remove tough organic residues, dry glassware can be soaked for several hours in $NaNO_3$-sulphuric acid cleaning solution. This treatment should only be necessary for the boiling flasks on a routine basis. After acid soaking, the glassware is rinsed in tap water and run again through the normal cleaning procedure. Extreme care must be taken in handling and storing the acid[1]. Note that glassware used for trace analysis must be kept separate from other uses.

NOTE 1 :

The acid cleaning solution is made by adding solid sodium nitrate to concentrated sulfuric acid to achieve a saturated solution. Glassware should be cleaned of as much material as can be removed by normal procedures and then thoroughly dried before cleaning with the acid. This solution is recommended above chromate solutions because the latter may leave reactive ions in the glassware that can cause artifacts in the analysis. **As with all concentrated acid solutions extreme CAUTION must be used to prevent contact with skin, eyes, lungs and clothes by the use of gloves, glasses, fume hoods and protective clothing.** The acid can be stored in brown glass bottles and reused many times. Use glass containers for the acid bath and keep it covered with glass. This not only contains vapours but reduces the absorption of water into the acid. The acid solution looses efficiency if it is significantly contaminated with water.

All solvents used in the analysis must be distilled-in-glass quality and the purity carefully checked on a routine basis[2]. Solid reagents such as Na_2SO_4 used to dry samples must be precleaned by solvent extraction and/or precombusted by heating at 400°C in a muffle furnace for 6 hrs. Solid supports for column chromatography steps should be precleaned by solvent extraction and oven dried at low temperature (<50°C), preferably under vacuum. Other materials used in the analysis, such as cotton or glass wool must also be precleaned by solvent extraction and dried. All these items must be stored in air tight glass jars with ground glass or screw lids lined with Teflon or aluminum foil. Chromatography supports must undergo the additional activation procedures within a few days of use.

NOTE 2 :

Only commercially available brands of solvents that are specifically labelled "distilled in glass" can be used straight out of the bottle for trace hydrocarbon analysis. Even these must be routinely checked for purity. Less expensive grades of solvents can be used if they can be sufficiently redistilled. Hexane is often contaminated with fluorescent materials. These can usually be removed by soaking the hexane over sulphuric acid and redistilling it. For this procedure it is convenient to pour a few cm of concentrated sulphuric acid into the bottom of a clean solvent bottle. Add hexane up to about 2/3 full. Stir the mixture slowly overnight with a magnetic stirrer using a teflon coated stir bar. Take care that the bottle is only loosely capped and that it is isolated from any heat generated by the stir motor. Use a fume hood. Aromatic contaminants will be pulled into the acid, causing the acid to change colour. The cleaned hexane is carefully decanted into a large separatory funnel. (The acid can be reused for many batches of hexane). Rinse the cleaned hexane with approximately 100 mL of saturated NaCl made up in precleaned water. Allow phases to separate and drain the brine. Drain the hexane into another bottle and dry it with precombusted Na_2SO_4 or other clean drying agents. Decant the hexane into a clean distillation flask and redistill it, careful to collect only the fraction boiling at 68.9°C. **Solvent distillation is an extremely hazardous procedure.** It must only be done in facilities approved by fire experts and the analysts must be carefully trained in distillation procedures and adopt safe practices. **Also see caution note overleaf.**

CAUTIONS :

Only use acid soaking on saturated hydrocarbon solvents, because use with other solvents may cause explosions; make sure the hexane is totally dry before attempting redistillation. Take care with disposal of spent acids.

Preclean Soxhlet apparatus[3] by assembling them from clean glassware, adding 200 mL of $MeCl_2$ and cycling them through their extractions for 4 hrs. Cool. The volumes suggested are for an apparatus accommodating a 500 or 250 mL solvent flask and glass thimbles which will hold 30 to 100 g of a homogenized solid sample. Use a 250 mL flask for the first extraction.

NOTE 3 :

Soxhlet apparatus are especially convenient to a laboratory already equipped with the glassware. Alternative procedures using reflux, sonication , or ball-mill extractors could be substituted, providing they are shown to be efficient in extracting the compounds of interest from the particular matrix. Several review articles have compared the efficiency of various extraction procedures. Several points are relevant to choosing an extraction system. First, the presence of water in the sample matrix prohibits the effective extraction of hydrocarbons by non-polar solvents. This is why these reference methods suggest grinding samples with Na_2SO_4, although a wet hydrolysis procedure is effective for most oil spill assessment studies without this added manipulation (section 8.2.2). If Soxhlets are used a few notes are relevant. First the cellulose thimbles commercially sold are difficult to clean for trace analysis. The use of glass thimbles which can be re-cleaned for repeated use are recommended. A less expensive option is to make holes in bottoms of large glass test tubes using a gas torch and loosely plug the holes with glass wool. It is also convenient to hold samples in the thimbles with a layer of glass wool over the sediment. Small glass ratchets under the thimbles will facilitate draining. Alternatively, thimbles need not to be used and the siphon holes for the solvent in the extractor body can be plugged with precleaned glass wool and the sediment spooned directly into the extractor. Do not fill the extractor past the top of the siphon line. Precombusted carborundum chips must be added to the solvent flask to ensure smooth boiling. Cooling water must be maintained through the condensers at all times. Use stainless steel forceps and spatulas to handle samples and thimbles. The whole apparatus should be precleaned before use.

8.2 Sample preparation and extraction.

Three solvent extraction procedures are described below. All of these will quantitatively recover hydrocarbons in the approximate elution range of C_{10} through C_{34}. If volatiles are the most important fractions to quantify, then steam distillation techniques as described by Donkin and Evans (1984) would be useful. Recent advances in critical fluid extraction technology is expected to make CFE a method of choice for environmental analysis in the near future.

8.2.1 Direct solvent extraction.

The following procedure is for use with fresh samples. Freeze drying will remove some of the volatile components of petroleum residues and its use is not recommended for hydrocarbon analysis. If the sample is already dry, such as is often the case for intercomparison samples, then mixing with Na_2SO_4 can be eliminated.

Decant excess water from the sediment surface then homogenize it by stirring. Discard large pebbles, seaweed, animals, wood and other debris. Weigh 50 g wet weight of sample and place in a glass beaker. Thoroughly homogenize the wet sediment with a stainless steel spatula. Remove a 20 g subsample for dry weight determination and size fractionation. To the remaining sediment add three times the wet weight of precombusted Na_2SO_4 and mix into a homogeneous mixture.

8.2.1a Soxhlet extraction

Add the dried homogenized sample into the Soxhlet apparatus and cover it with a plug of pre-extracted glass wool. If the entire sample is not used, record the aliquot and compute the weight of the sample extracted. Change the $MeCl_2$ in the solvent flask and add surrogate standards[4] and 1 or 2 precombusted carbon boiling chips. Reassemble and extract the sample at a fill/empty rate of 4 to 5 cycles per hour for 6 hours. Cool. An equal weight of Na_2SO_4 should be extracted in one of the Soxhlets as a procedural blank. Remove the first extract and stopper. Replace these flasks on the extractors with a second 250 or 500 mL flask containing 200 mL fresh $MeCl_2$ and extract for another 6 hrs. Cool. Combine first and second extractions.

NOTE 4 :

In this context "surrogate standard" is meant to be a compound added to the sample which will behave in an identical manner to the compounds to be quantified and which is used to correct the results for recovery through all steps of the analytical procedure. Since the hydrocarbons are to be separated into fractions, it is necessary to add at least two standards. *N*-octadecene is suggested as a surrogate standard which will elute with the saturated hydrocarbons, and 9,10-dihydroanthracene for elution in the aromatic fractions. Neither compound occurs in petroleum and neither have been noted to be biogenic in marine samples. The 9,10-dihydroanthracene has a very weak fluorescence signal (maximum at 410 nm) if excited at 310 nm. Thus it will not interfere with UV fluorescence measurements. O-terphenyl is also a useful surrogate for the aromatic fractions. If samples are to be analyzed by GCMS, there is a variety of deuterated aromatic hydrocarbon standards that could be added. Deuterated biphenyl, phenanthrene and pyrene are suggested in section 8.7.

A further note about standards is warranted. Since the basis of all quantitative work is the analytical balance, control must be exercised over the care of the balances. They must be routinely checked for accuracy against a set of high quality calibration weights. Also since standards for hydrocarbon analysis are made up in volatile solvents, extreme care must be taken to ensure that concentrations are not altered by evaporation. Solutions should be made up by weight into tight sealing vials and the total weight recorded on each vial. Working solutions are then weighed before every use. The weight of every stock vial should be recorded for future evaluation of the usefulness of the solutions. Iso-octane is recommended as the dilution solvent for stock solutions, while hexane dilutions of these should be added to samples as surrogates.

Reduce the volume of the combined $MeCl_2$ extracts on a rotary evaporator to approximately 5 mL. Never allow the water bath to exceed 30°C and control vacuum to prevent bumping. Transfer reduced extracts to graduated tubes. The intention is to end up with extracts sufficiently concentrated to give readable lipid weights but not so concentrated that the lipids precipitate out of solution. Continue with section 8.2.3.

8.2.1b. Sonic or shaker extraction.

As an alternative to soxhlet extraction, labs equipped with a sonicator can follow US EPA SW846 Method 3550 or NSTP procedures described by Krahn et al., (1988). In this method, the sediment is mixed with Na_2SO_4 as above and spooned into 250 mL glass or Teflon jars. Add surrogate standards to each jar. Add 100 mL of 1:1 v/v dichloromethane/acetone. Sonicate for about 3 min at settings appropriate to yield efficient extraction. Alternatively, the extracts can be shaken several hours on a mechanical shaker. After extraction, decant the solvent into a clean flask. Repeat the extraction two more times, combining all extracts into the flask. Filter and dry the extract through glass wool and Na_2SO_4. Reduce the extract volume as described in section 8.2.1a.

8.2.2 Wet hydrolysis/saponification extraction.

Extraction efficiencies in sediments can be increased with a wet hydrolysis/saponification procedure. The wet hydrolysis is performed in the Soxhlet apparatus. 250 mL methanol is used for the initial extraction (4 or 5 hours). The apparatus is then allowed to cool, saponification reagents are added, and the extraction is continued for another two hours (Stronger solvents than MeOH are often required to obtain efficient recovery from sediments contaminated with highly degraded residues. Thus the extraction could be made more efficient for sediments by the addition of toluene to the extraction solvent, or by sequential extractions with more efficient solvents such as $MeCl_2$. This will impact the subsequent separation and evaporation procedures and the analyst must make these modifications knowledgeably.). **Note that $MeCl_2$ must not be boiled with added base due to explosion hazard.**

Saponification is a general term for converting neutral fats such as glycerol esters into free fatty acids which are water soluble and can be separated from the hydrocarbons by partitioning and chromatography. With any saponification procedure there is an equilibrium established between the fatty acids and their methyl esters. The aim of the hydrolysis extraction is to favor the production of the free fatty acid salts and minimize the production of their methyl esters which would partition with the hydrocarbons. This is achieved by ensuring that the amount of water in the methanol/KOH mixture is kept at about 20% (v/v). To calculate the amount of KOH required, a general estimate of 1 m equivalent (56 mg) per every 200 mg of biological lipid can be used.

For the weights and volumes suggested here a convenient ratio is to add 20 ml of 0.7 N KOH and 30 mL ultra pure water to the solvent flasks. At the end of the saponification a drop of the mixture should be tested on pH paper to insure it was basic. If not, more KOH should be added and the step repeated. The next step is a partitioning of the non-saponifiable lipids (NSL) which contain the hydrocarbons from the saponifiable lipids (SL).

Pour the KOH/MeOH extract into a glass separating funnel with a teflon stopcock. Rinse the extraction flask with aliquots of hexane totaling 50 mL and add these washings to the separating funnel. Tightly fit the glass stopper and shake vigorously. Carefully vent vapors out of the inverted funnel through the stopcock. Repeat vigorous shaking with frequent venting for several minutes. Place the separating funnel in a rack and allow phases to separate. Processing several samples sequentially will allow time for the phase separation. Four or five samples and a spiked blank are convenient to process at one time. Ideally, the methanol (bottom) and hexane (top) phases should separate with a clear interface. This is seldom achieved in working with environmental samples in which emulsions often form at the interface. Even for the experienced analyst, this is often a source of frustration. Addition of a few mL of pre-extracted water and/or NaCl brine solution will help break the emulsion in most cases for sediment samples. However, it will occasionally be necessary to drain the emulsion into clean glass tubes and centrifuge. The hexane can then be pipetted out of the top of the tube and the aqueous layer returned to the separator for the repeated partitioning.
CAUTION : Wear safety glasses and vent fumes into a fume hood.

After phases have separated in the funnel, drain the aqueous MeOH phase into a clean Erlenmeyer flask. Filter and dry the hexane phase by passing it through a small column of Na_2SO_4, collecting it in a 250 mL round flask. Return the aqueous phase to the separator and repeat the partitioning twice more with 25 mL aliquots of hexane. Combine all the dried and filtered hexane extracts and a further rinsing with a few mL of hexane of the drying column into the round flask. This is the non-saponifiable lipid (NSL) fraction and contains the non-volatile hydrocarbons[5].

If it is necessary to determine the total extractable lipid weight of the sample; the aqueous-MeOH phase should be returned to the separator and enough HCl added to acidify the extract. For the volumes suggested here approximately 20 mL of 0.7 N HCl should be sufficient. Test a drop on pH paper and add more acid if the extract is not acid. Re-extract this acidified solution with hexane. Filter, dry and collect the hexane extracts into another round flask. This is the saponifiable lipid (SL) fraction. Alternatively 1 g subsample from the original homogenous sediment could be mixed with Na_2SO_4 and directly extracted with $MeCl_2$ in a scintillation vial. This extract would be used to determine the total extractable organic matter (EOM).

NOTE 5 :

The hydrolysis/saponification procedure efficiently removes a significant percentage of the non-hydrocarbon lipids from the NSL extract. This procedure may be desirable for high fat samples. However many chlorinated hydrocarbons are unstable to this procedure. Thus it is often useful to substitute the $MeCl_2$ extraction procedure and then run the extract through a gel-permeation chromatography step. Gels stable to solvents include Sephadex LH-20 and Biobeads SX-3 for column chromatography and the μSpherogel A100 and Phenomenex Phenogel A100 HPLC packings.

8.2.3 Dry and concentrate extract.

The reduced extracts are filtered through a small column of Na_2SO_4 and into glass stoppered graduated tubes. The round flasks are then rinsed with minimum volumes of $MeCl_2$ or hexane which are also filtered into the tubes. Extracts can be further concentrated by evaporation with a gentle stream of pure nitrogen gas[6]. When extracts reach one mL they are transferred to small preweighed vials and one mL of hexane is added. The $MeCl_2$ is selectively removed using the stream of nitrogen gas displacing the sample into the remaining hexane. The final volume is adjusted to 1 mL by weight (density of hexane = 0.6603 g).

NOTE 6 :

High purity nitrogen gas should be used to evaporate samples. A molecular sieve trap should be placed in-line. Either a single line could be used or a manifold could be constructed. Flows must be adjustable and all materials in-line must be non-contaminating (glass, teflon, stainless steel). If necessary, high purity air could be substituted for the N_2. Prevent cross contamination of samples by solvent rinsing the evaporation tips between each sample.

8.2.4 Determination of lipid weights.

Determine the lipid weights by weighing small aliquots on a micro-balance[7]. If no microbalance is available, then a known fraction of the total extract can be transferred to a clean preweighed aluminum pan and evaporated to a constant weight with a stream of pure nitrogen. Calculate the amount of lipid in the total sample and note the amount carried through the subsequent analytical procedures.

NOTE 7 :

Microweighing is an art which requires practice to achieve accuracy and precision. A digital electrobalance with display reading to 0.1 μg is most desirable but with practice acceptable precision can be obtained on older balances. The balance should be calibrated and zeroed on the 0 to 1 mg range. The weighing chamber should contain a beaker of drying agent and the balance should be located in a humidity controlled room. A small hot plate regulated to minimum heat (@40°C, it should feel just warm) is used to warm forceps and weighing pans and eliminate water condensation after solvent cleaning. Solvent clean the surface of the hot plate (a piece of aluminum foil makes a good surface on the hot plate), forceps and balance pans. When all are dry carefully place pan on the stirrup in the chamber and zero the balance. Remove the pan, carefully transfer 10 μL of lipid extract directly onto the balance pan, allow the solvent to evaporate and weigh the residue. The pan is then solvent cleaned and dried and rezeroed for the next determination. The weight is divided by the volume evaporated onto the pan and multiplied by the total volume to yield total lipid weights. Total extractable organic matter (EOM in a hydrolysis extraction) is the sum of the SL plus the NSL extracts.

8.3 Adsorption chromatography clean-up.

An adsorption chromatography step is used to remove interfering lipids and to split the hydrocarbon extracts into saturated and aromatic fractions. For certain screening applications, total lipid extracts could be analyzed by UVF. However, a more accurate result will be achieved if interferences are first removed. Many variations of adsorption chromatography clean-up procedures have been published and could be substituted here. Whichever procedure is followed, it must be calibrated for the efficiency of the required separation by running through a series of standards and quantifying their recovery in the collected fractions. Two suggestions for adsorption procedures are offered here.

8.3.1 Normal phase column chromatography.

The following procedure is designed for a maximum column loading of 100 to 150 mg lipid. The volume of the extract used must be adjusted to give this amount in 1 mL of hexane. The reason for this strict limitation of lipid applied to the column is to ensure the column is not overloaded which would reduce its separation capability. One general rule for non-saponified samples is to keep the ratio of adsorbant to lipid weight greater than 100 to 1. Note the proportion of the lipid extract used in the clean-up procedure as this will be important for the final quantification of hydrocarbons. Note 5 describes additional methods to remove interfering lipids if necessary before the column chromatography procedure.

The precleaned adsorbants are activated by heating them in an oven at 200°C for 4 hrs and are then and partially deactivated by adding 5% (wt/wt) of precleaned water to the silica gel and 2% water to the alumina. Supports should be gently mixed to distribute the added water and allowed to equilibrate in air-tight jars overnight (Note that they must have been precleaned by solvent extraction.).

Glass burettes (1 cm internal diameter) with teflon stopcocks make convenient adsorption columns. If not equipped with sintered glass discs then the columns can be plugged with pre-extracted cotton or glass wool. Prepare separate columns for each sample and blank. A slurry method of layering the supports into the columns is recommended. The column is partially filled with hexane. Eight g of silica gel are mixed with hexane in a small beaker. With the aid of a glass funnel and stirring rod, the support is poured into the column. Several rinses of hexane will be required to get all the support into the column. The column is gently tapped to form a homogeneous bed of silica. A rubber stopper on the end of a pencil makes a good tapping aid. The stopcock can be opened and the draining solvent used to wash all remaining particles from beaker, funnel and column sides into the bed. Allow enough solvent to remain above the silica to permit the introduction of 8 g of alumina in the same manner. Finally, 1 g of Na_2SO_4 is layered on top of the adsorbant bed. The solvent is drained to the top of the bed and further hexane rinses used to ensure the column is clean. Drain the solvent to the top of the adsorbant, taking care to ensure the bed is **never allowed to run dry.**

One mL of the lipid extract is pipetted into the column and is drained into the adsorbant bed. The column is then carefully eluted by adding solvents (in small aliquots) and draining them into the adsorbant bed before adding further aliquots. The saturated hydrocarbons are eluted with a total of 20 mL (approximately 1 column volume) of hexane (F1). Unsaturated and aromatic hydrocarbons are eluted with 20 mL of 20% $MeCl_2$ in hexane (v/v) followed by 20 mL of 50% $MeCl_2$ in hexane (v/v). These two fractions can be collected separately or combined depending on the analytical detail required (F2 + F3).

The column chromatography procedure must be calibrated to achieve the desired elutions. The goal is to recover all of the *n*-alkanes and other saturates in F1 and all of the aromatics, without breakthrough of biogenic fats, by F3. Exact elution volumes and the necessary percentages of $MeCl_2$ must be determined experimentally. Benzene and its alkyl substituted derivatives usually elute with the saturated hydrocarbons.

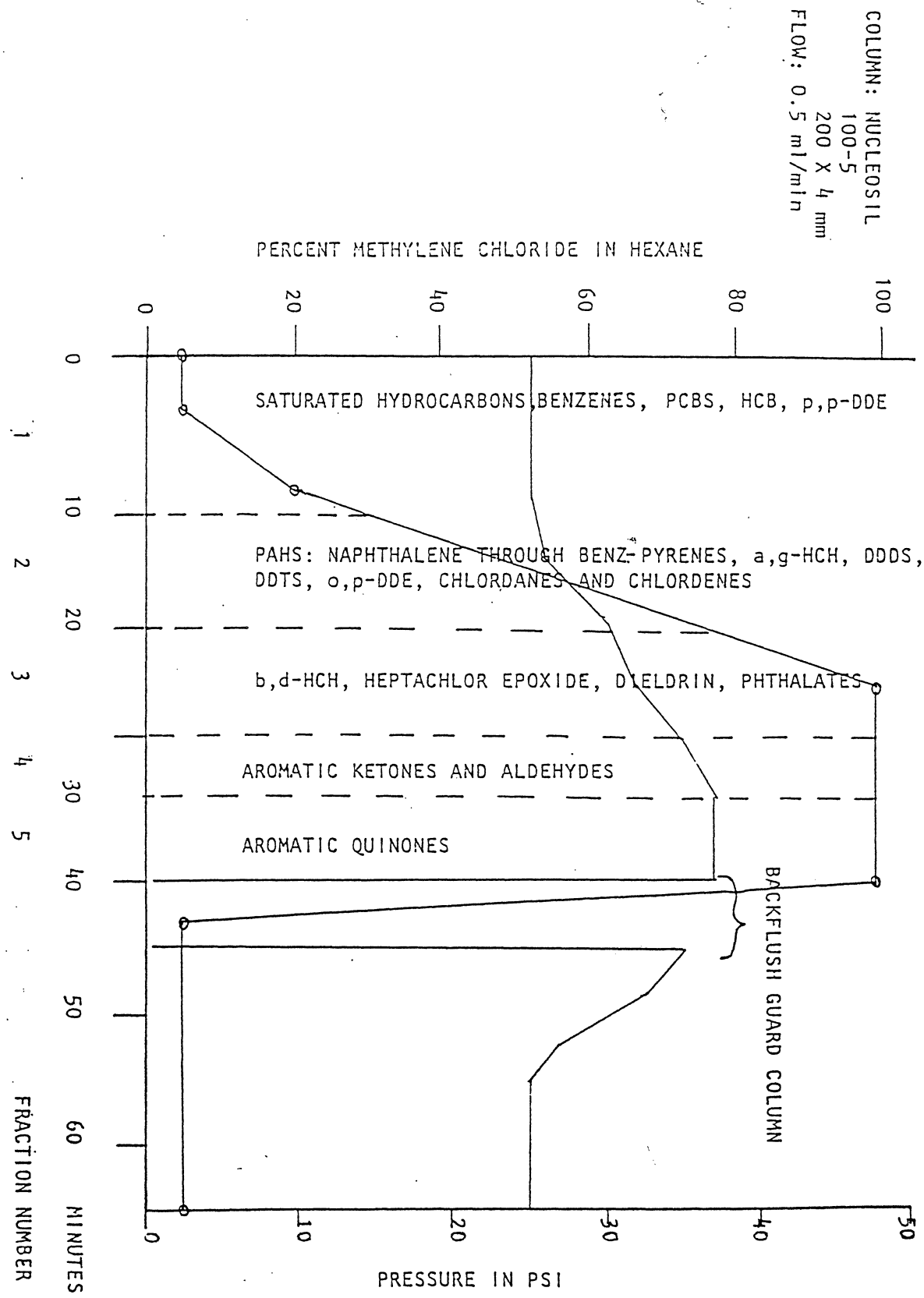


Fig 18 : HPLC method for class separation.

8.3.2 HPLC procedure.

Better separation efficiency can be achieved by using normal phase high performance liquid chromatography (HPLC). The procedure described here is adapted from Petrick *et al.* (1988) and is suitable for both petroleum and chlorinated hydrocarbon analysis. However before the lipid extract can be injected into the HPLC it must be passed through a small column of alumina to remove the majority of non-hydrocarbon lipids. This is done by plugging a pasteur pipette with pre-extracted cotton or glass wool and layering in a 4 cm bed-height of alumina prepared as described in section 8.3.1. The sample is added in 1 mL hexane and collected in a graduated tube. The sample is further rinsed through the mini column with an additional 2 mL of hexane followed by 2 mL $MeCl_2$ added in small aliquots. The final eluant is again concentrated to 1 mL using a gentle stream of ultra pure nitrogen. As it is necessary to reduce the extract to less than 100 µL for injection into the HPLC, 75 µL of iso-octane are added and the extract further reduced to 75 µL. Extreme care must be used in final evaporations to prevent the loss of volatile compounds.

The HPLC can be a simple isocratic instrument capable of programming an elution scheme of three sequential solvents. The injection valve should be variable volume with a 200 µL loop to permit quantitative injection of the sample extract including a 25 µL vial wash. A guard column is followed by a six port back flush valve. The column recommended is 20 cm X 0.4 cm i.d. packed with 5 µm Nucleosil. The sample is eluted at 0.5 mL per minute flow rate with 11 mL hexane followed by 4 mL of 20% $MeCl_2$ in hexane, then 10 mL pure $MeCl_2$. Finally the guard column is backflushed for 5 min with $MeCl_2$. If equipped with a ternary solvent delivery system, the guard column should be flushed with MeOH follwed by $MeCl_2$. The system is re-equilibrated with hexane or the initial solvent composition before the next sample.

Expected Fractions	**elution volume (mL)**
1. alkanes and alkenes	0.5 - 2.0
2. alkylbenzenes, naphthalenes, HCB,PCBs	2.0 - 4.5
3. PAHs and Toxaphene	4.5 - 11.0
4. Pesticides	11.0 - 15.0
5. acids and polar compounds (including aromatic aldehydes & ketones)	15.0 - 25.0

The exact elution volumes must be calibrated for any individual set up. It is essential to do this for a suite of mixed standards. For "petroleum" analysis the same split as the column chromatography procedure will be obtained by analysis of F1 (saturates) and F2 + F3 (aromatics). For chlorinated hydrocarbons and the hydrocarbon reaction products every compound to be quantified must be tested for recovery through the HPLC fractionation. The separation on silica is affected by water in samples. Thus care must be taken to insure all the hexane extracts are dry before attempting an adsorption chromatography separation.

Better reproducibility in separations can be achieved with an HPLC capable of gradient programming. A useful elution programme using the same column described above is shown diagrammatically in Fig. 18. The actual composition of solvents will undergo some time delay compared to the programme set on the pumps and the volumes for collections must be determined experimentally with a set of mixed standards. Substituted silica columns used in normal phase operation are more stable than unsubstituted silica and successful separations have been achieved with cyano- and amino-substituted silicas.

Now that a set of hydrocarbon fractions has been obtained for each sample and blank, the fractions are reduced in volume by rotary evaporation, and/or transferred to preweighed vials and further reduced with N_2 gas. They can then be analyzed by a number of relevant techniques including the gravimetric, UVF, GC and GC/MS procedures described below[8].

NOTE 8 :

Other techniques such as Infra-red spectrophotometry, gas chromatography with flame photometric detection, or reverse phase HPLC with UV or UVF detection could be used depending on the analytical detail required and the available equipment and expertise. The analyst is thus referred to the literature on the environmental biogeochemistry of hydrocarbons to obtain further information useful for data interpretation with these techniques.

8.3.3 Sulphur removal.

Marine sediments, especially those with high organic carbon content, often contain elemental sulphur resulting from chemical and microbial oxidation of sulphide ion. This latter compound is a product of anoxic remineralization of organic matter by microorganisms which, in the absence of free oxygen, use sulphate ion as an oxygen source.

This elemental sulphur, much of which appears to be in colloidal form dissolves in the organic solvents used to extract the sediments. Although alkaline saponification will convert most of the elemental sulphur to thiosulphate thus partially preventing the carry over of sulphur into the solvent extract, any acidification of the extract would reprecipitate the sulphur in such fine dispersion that a significant portion would partition into the hexane.

Most extracts of sediments obtained either by direct solvent extraction or after alkaline hydrolysis are likely to contain elemental sulphur. In the adsorption chromatography separations it elutes in the non-polar fraction and is easily mistaken for a hydrocarbon during GC analysis, unless it is identified by its mass spectrum (major m/z peaks at 32, 64, 96, 128, 160, 192, 224, 256). Because of this and other interferences, it is necessary to remove elemental sulphur by percolating the reduced extracts through a short column of activated copper[9]. Dibenzothiophenes, often used to fingerprint oil contamination, are not removed by this procedure. However mercaptans are likely to react with the copper. The usual order is to remove the sulphur from the saturated hydrocarbon fractions after the adsorption chromatography step. Occassionally extracts contain so much sulphur that yellow crystals may precipitate in the crude lipid extracts. In this case it will be necessary to run the extract through at the total lipid or NSL stage and again after fractionation. The copper column can be reused for several samples, but care must be used to clean it for each sample. This is done by rinsing the samples through with additional small volumes of $MeCl_2$ and then rinsing the column with further solvent aliquots before the next sample is passed through. Extracts must again be concentrated.

NOTE 9 :

Activated copper can be prepared as follows: In a 250 ml plastic 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 min (An ice bath over a magnetic stirrer is useful.). Make a slurry of 3 g powdered Zn, 5 mL distilled H_2O and a trace of detergent. Slowly add the slurry to the $CuSO_4$ mixture while it is spinning over the ice. Remove the plastic beaker and let it stand on the bench for 1 hr at room temperature. Then stir gently until all the blue of the $CuSO_4$ has disappeared from solution. The Cu forms a flocculent red 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 this will oxidize 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. When required, defrost the Cu slurry and pack it into a small glass column with teflon stopcock. If the column is not equipped with a glass frit, it can be plugged with cotton or glass wool. The bed of copper need only be a few centimeters long and should allow a flow rate of several mL per minute. Drain the water to the top of the bed and thoroughly rinse with several aliquots of acetone to remove the water. Replace the acetone with $MeCl_2$ or hexane and the column is ready for use. Pass the saturated hydrocarbon fractions of sediment extracts through the column taking care to clean the column with several bed volumes of dichloromethane between each sample. When the Cu adsorbs sulphur it turns black. The column can be used many times but replace the copper when about 50% of the bed turns black. The Cu column can be stored between use by filling it with 2 N HCl and keeping it cold. Always remove traces of acid, water, and acetone before passing samples through it. While other procedures for removing copper from lipid extracts have been published, such as adsorption onto Hg, adsorption onto copper has been found to be efficient and the least hazardous.

8.4 Gravimetric determinations using the microbalance.

For this determination the hydrocarbon fractions are reduced to measured minimum volume and 10 µL aliquots are treated according to the protocol described in section 8.2.5. The agreement between quantifications based on GC and gravimetric determinations should be quite good for medium boiling range oils. There may be significant deviations between these two methods if the oil residues are low boiling and thus lost during the weighing procedure or higher boiling than can be eluted from the GC system. Of course if contaminant levels are low, the yield of hydrocarbons will be below the detection limit of gravimetric methods. Also there is no means of distinguishing the source of hydrocarbons based on hydrocarbon composition.

8.5 UV-Fluorescence spectrophotometry.

A semi-quantitative estimate of the type of aromatic hydrocarbons contained in the fractions can be obtained with the UVF procedure but the analyst is reminded of the limitations expressed in the introductory discussion of this manual.

8.5.1 General UVF protocol.

The fluorometer must be capable of synchronous scanning of excitation and emission wavelengths and be connected to a recorder or data system. The fluorometer is first tested for its signal to noise ratio and the accuracy of the monochromators by following the instructions in the instrument manual. This procedure is briefly described below. The hexane used for extraction is then tested for its contribution to blank signals. Aliquots of sample fractions in hexane are then scanned. Emission scans and synchronous excitation / emission scans are recorded and evaluated for the type of aromatic hydrocarbons in the samples. Standard response curves of fluorescence intensity versus concentration are generated for appropriate oil standards and for the standard aromatic hydrocarbon, chrysene. The sample fractions are diluted to give a reading within the linearly calibrated range of the fluorometer. Aliquots of the hexane solutions of blanks, standards and samples are pipetted in turn into a quartz fluorometer cell. All blanks, standards and samples should be run at identical instrument settings and conditions and using the same batch of pre-tested hexane.

8.5.2 Fluorometer pretest.

The signal to noise ratio is tested as follows. Turn on the xenon lamp and allow instrument to warm up for about 30 minutes. Set controls :

mode :	ENERGY (electronic filtering mechanism turned off)
response :	NORM (2.0 Sec.)
GAIN switch :	NORM (750V)

Carefully fill the 10 mm quartz cell with distilled water ensuring that there are no bubbles, and position the cell in the sample compartment. Set the following conditions :

EMISSION SLIT :	10 nm
EXCITATION SLIT :	10 nm
SAMPLE SHUTTER :	open
EMISSION WAVELENGTH :	397 nm
EXCITATION WAVELENGTH :	350 nm
SCAN SPEED :	60 nm/min

By turning the course and fine SENSITIVITY knobs, deflect the recorder pen to about 80% of full scale at 397 nm (Range typically 3.0 or 1.0).

Excitation at 350 nm

Distilled H_2O

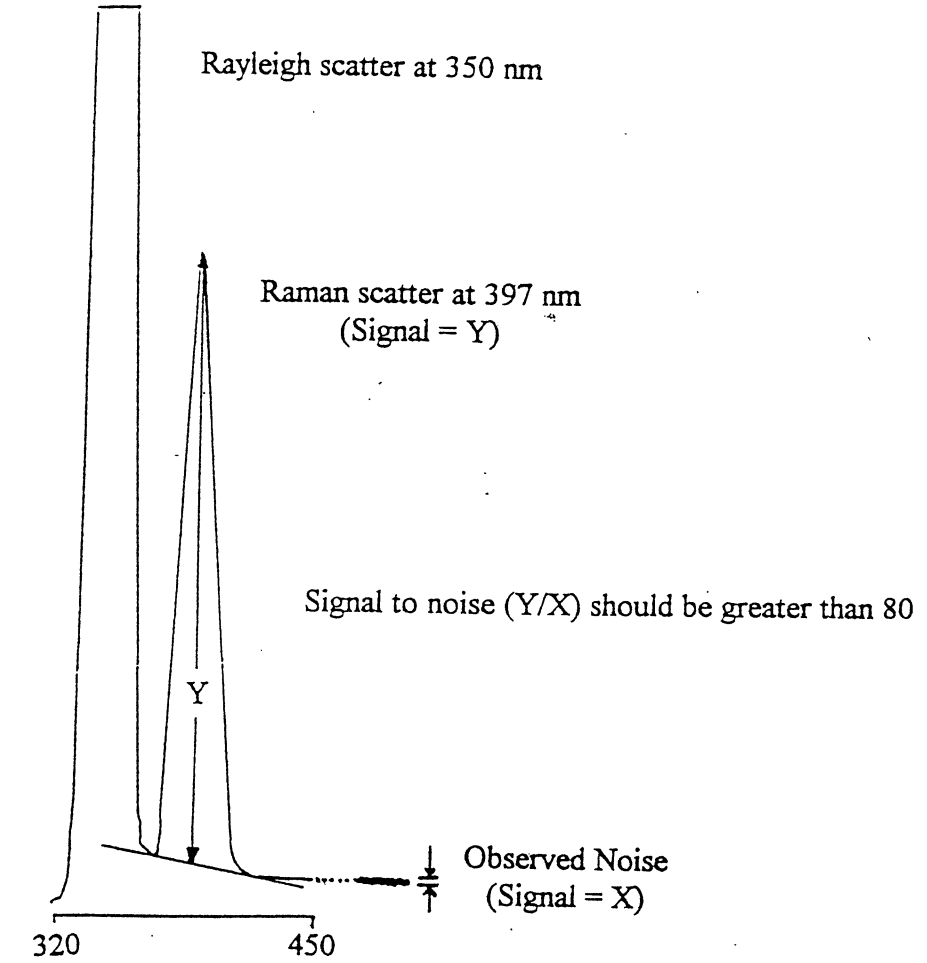


Fig 19 : Rayleigh & Raman scatter plus signal to noise ratio.

Set EMISSION WAVELENGTH dial at 330 nm. Record the emission spectrum of the distilled water from 330 nm to about 500 nm. Turn off the WAVELENGTH DRIVE and manually scan back to the emission maximum of the Raman band which should be at 397 nm. Record the noise level at this maximum. Measure the signal height of the Raman peak from the floating baseline to the peak height. Measure the height of the noise. The signal to noise ratio should be 80/1 or greater. (Fig. 19). Keep a running log of signal to noise ratio and hours of use on the xenon lamp. Replace the lamp when its signal to noise ratio is unsatisfactory. Note : Follow all precautions in the instrument manual for handling and aligning new lamps. Use gloves and never touch the lamps with your fingers. Wear protective cobalt eye glasses if necessary to view a lighted UV lamp.

The instrument manual will have more tests for stability and wavelength accuracy. Follow these instructions to become familiar with the operation and performance of the fluorometer.

8.5.3 Blanks.

Whatever extraction method is used, great care must be taken throughout the entire procedure to ensure that samples are not contaminated. For example, avoid exposing solvents, glassware or sample to the laboratory air. Blanks should accompany batches of samples and be run at the same time, under the same conditions and at the same dilutions as samples. When blanks show contamination, the source of contamination must be found and eliminated in preference to correcting the analytical data with high blank values. The quality of the extraction solvent is of crucial importance. For the UVF analysis samples are dissolved in hexane. The complete procedural blank value is acceptable for sample correction if its fluorescence reading does not exceed twice the fluorescence reading of the unconcentrated hexane (See NOTE 2 Section 8.1 for instructions on removing fluorescent contamination from hexane.).

8.5.4 Preparation of standards.

One set of standards should be chrysene in hexane because previous results from the MARPOLMON-P and some intercalibration programmes have reported results in terms of chrysene units. Construction of this calibration curve will thus aid in the intercomparison of data sets. However, before the standard chrysene can be used for calibration, its correct emission spectrum must be verified. This is done by exciting the chrysene solution at 310 nm and recording its emission spectrum. The correct spectrum will show an emission maximum near 362 nm and 381 nm and a smaller maximum near 408 nm.

A hexane stock solution is prepared by dissolving 1.0 mg chrysene per 100 mL of hexane. Note that although the chrysene is quite soluble at this concentration, the kinetics of dissolution are slow. Thus the stock solutions must stand at least 12 hrs before dilution. From the stock solution a range of dilutions is prepared. The required range will depend on the sensitivity of the fluorometer. Under the conditions specified for the analysis, modern instruments will give a linear response up to a chrysene concentration of approximately 3 $\mu g.mL^{-1}$. Thus a useful set of standards would be 0.1, 0.2, 0.5, 0.7, 1.0, 2.0, 3.0 $\mu g.mL^{-1}$. 20 mL glass scintillation vials with aluminum foil lined lids are useful vessels for the preparation of standard dilutions. The necessary volume of stock solution is pipetted into the preweighed vial which is partially filled with hexane. The volume is then brought up to 20 mL with hexane (1.0 mL of hexane weighs 0.6603 g.).

Standard solutions should also be prepared from refined or crude oils. Oil standards are prepared in much the same way as the chrysene standards. The density of the oil in $g.mL^{-1}$ is determined by weighing a measured volume of oil. Then enough oil is transferred to the 100 mL volumetric stock flask to yield a concentration of approximately 0.5 mg oil per mL of hexane. This stock solution is then used to prepare the standard dilutions in the linear calibration range. This is usually in the 0 to 10 $\mu g.mL^{-1}$ range for most oils, but will vary depending on the aromatic content of the oil. Some oils, such as Kuwait crude, are difficult to dissolve and will require that the primary stock solution is much less concentrated than the average values given here. The oil standards are run in the fluorometer at the same sensitivity settings as the chrysene standards.

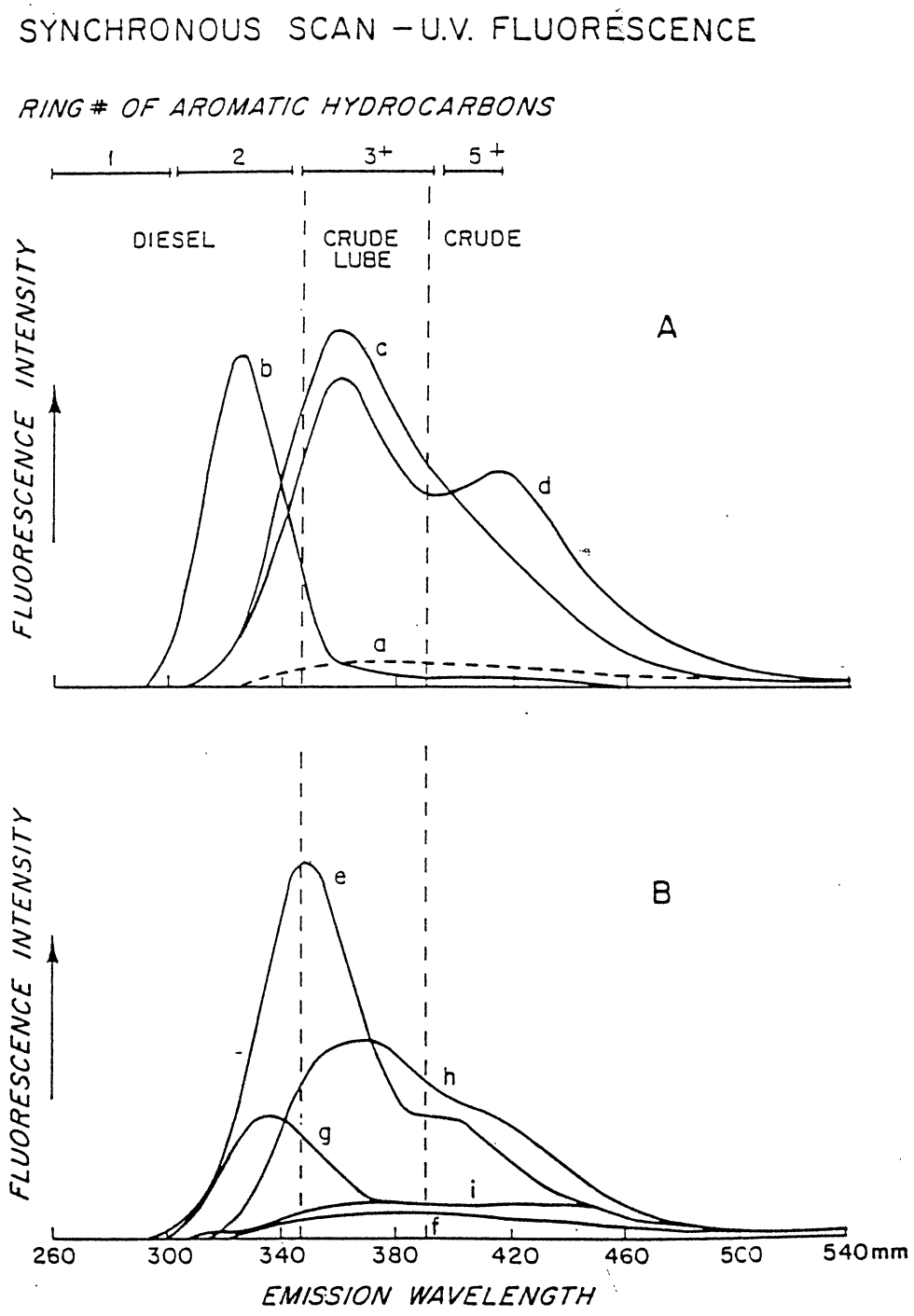


Fig 20 : Synchronous excitation/emission fluorescence spectra of aromatic hydrocarbon fractions of :
A. Source oils and B. Environmental samples.
Scan from 260 to 540 emission with excitation set 23 nm lower. Band pass 5 nm.
a. Hexane blank, b. Diesel oil, c. lube oil, d. Gippsland crude oil, e. mussels from refinery wharf, f. clean mussels, g. mussels from boat wharf, h. sediments near refinery, i. clean sediments.
(From Burns and Smith, 1977).

When reporting results in terms of oil units, an intercomparison ratio between the fluorescence intensity of the oil and that of chrysene should be calculated according to the formula :

$$R = \frac{\text{fluorescence intensity of chrysene standard}}{\text{fluorescence intensity of oil standard}} \times \frac{\text{concentration of oil standard in } \mu g.mL^{-1}}{\text{concentration of chrysene standard in } \mu g.mL^{-1}}$$

The choice of oils used for calibration and the wavelengths for the quantitative measurement will depend on the types of oil seen in samples from individual monitoring programmes. The analyst should compile a "library" of spectra and response curves generated from the particular instrument used.

8.5.5 Spectra

For qualitative identification of oil type based on the fluorescence of aromatic hydrocarbon components, a synchronous excitation/emission scan provides the best resolution with elimination of spectral interferences due to Rayleigh and Raman scattering. The excitation monochromator is set 23 nm below the emission wavelength and the two monochromators scanned synchronously (Start with excitation at 237 nm and emission at 260 nm). Set the excitation monochromator slits (or band pass widths) at 10 nm and emission monochromator slits at 3 to 5 nm depending on instrument sensitivity. Set scan speed at 60 $nm.min^{-1}$ and recorder speed at 3 or 6 $cm.min^{-1}$. Record synchronous spectra between 260 nm and about 525 nm emission. Figure 20 shows examples of synchronous excitation-emission spectra generated from standard oils and extracts from sediment contaminated with oil, using a Perkin Elmer 650 instrument. Notations for the emission region of single through five ringed compounds are taken from Popi *et al.*, (1975). Inspection of these spectra indicate that diesel oil contains mostly substituted naphthalenes and no signal due to the higher ringed aromatics. Crude oils show maxima in higher wavelength regions. Microbial degradation of the side chains on the aromatic rings will shift emission maxima to shorter wavelengths (as much as 15 nm), whereas solution processes will preferentially remove the more soluble single and double ring components. Thus for accurate interpretation the analyst must be familiar not only with the characteristic spectra generated by standard oils but also with the effects of weathering processes in altering the fluorescence spectra.

When the oil in samples has been matched as closely as possible to the available standards, then this standard can be used for constructing the calibration curve. First determine the optimum wavelengths for the quantitative measurement. This is achieved by fixing the emission wavelength and obtaining an excitation spectrum. The wavelength of optimum excitation is then chosen and fixed and an emission spectrum obtained. The most sensitive wavelengths would then be those empirically determined from the excitation and emission spectra. For average crude oils these optima are at 310 nm excitation and 360 nm emission. For diesel oil these optima are at 280 nm/ 327 nm.

8.5.6 Semi-quantitative UVF determinations.

Calibration curves are constructed by setting the excitation and emission monochromators at 310 nm and 360 nm respectively for chrysene and most crude oils. The standard dilutions or stock aliquots are pipetted into the 10 mm cell and the fluorescence intensity (FI) measured. A plot of FI vs $\mu g.mL^{-1}$ should be constructed and the regression equation calculated over the linear range as per Figure 21. Calibrations should be obtained daily and a running log kept. As aliquots are removed from the stock vials, the vials should be reweighed to ensure no solvent loss, and hence change in concentration. New dilutions should be made up on a routine basis.

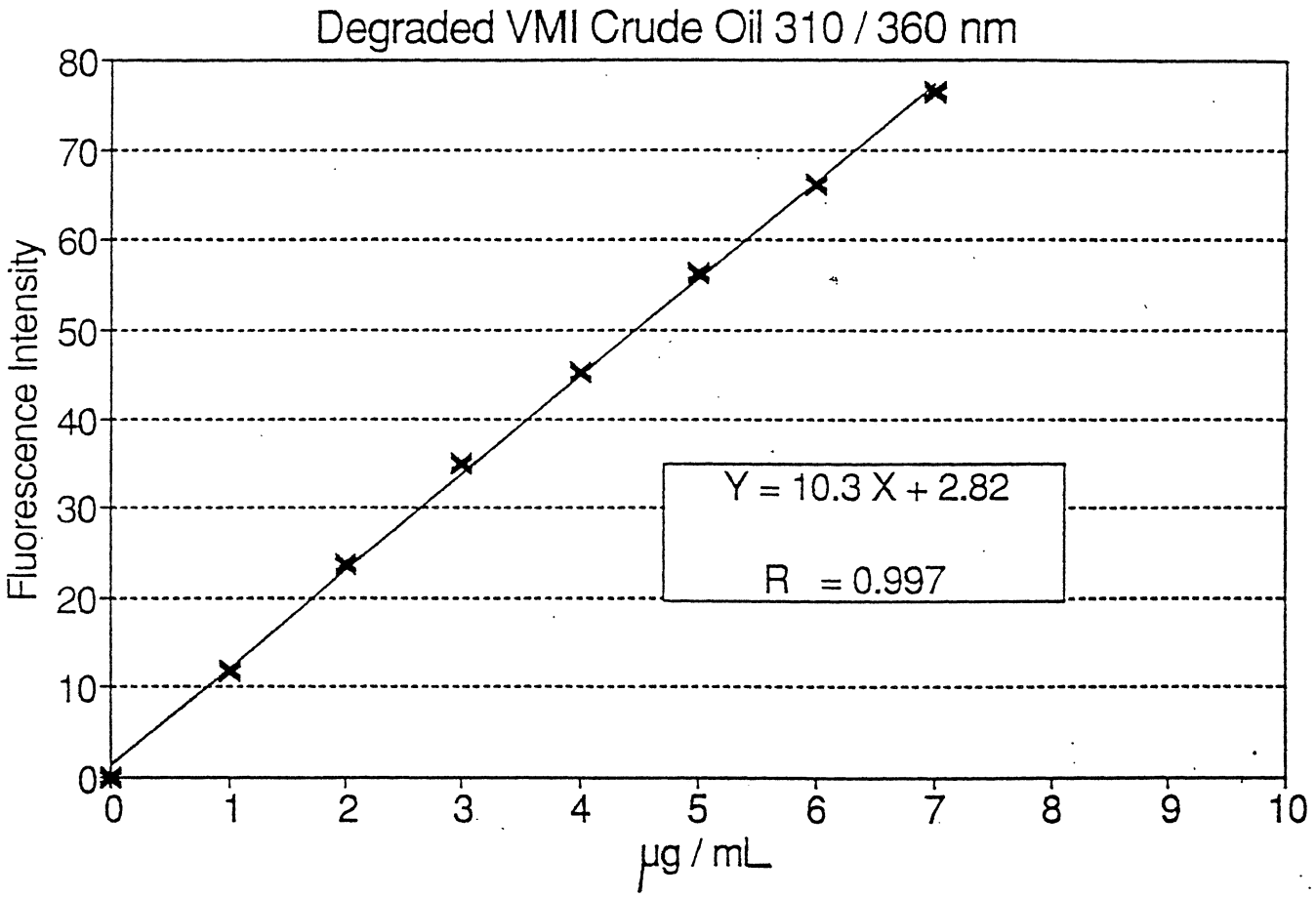


Fig 21 : Calibration graph : degraded VMI crude oil 310/360 nm

A quick alternative method for constructing calibration curves is to make up the standard oil solutions to a stock concentration of approximately 0.2 $mg.mL^{-1}$ for most medium weight crude oils or 1 $mg.mL^{-1}$ for less fluorescent oils such as diesel fuel. The calibration curve is then constructed by placing 2 mL of hexane into the cuvette and taking the blank reading at the appropriate wavelengths and instrument settings. Sequential 10 µL or 5 µL aliquots of the stock oil solutions are then added to the cuvette, the sample mixed and a reading taken after each addition.

The FI of each sample (total extractable lipids or the aromatic fractions of sample extracts) is determined and the concentration of oil determined from the standard curve. Samples must be diluted to produce readings in the linear region. This is easily done by the procedure of placing 2 mL hexane in the cell and adding sample extract in µL aliquots. The sample dilutions must be recorded (Dilution is total µL sample extract divided by the µL used for FI determination. If any correction must be applied for only a portion of the extract brought to this stage of the analysis, it must also be applied to the dilution.). The semi-quantitative estimate of oil content is calculated from the regression line and expressed as $\mu g.mL^{-1}$. Sample content is then calculated as follows :

$$\frac{\mu g \text{ oil (or chrysene)}}{g \text{ dry weight}} = \frac{\mu g}{mL} \times 2mL \times \text{dilution} \times \frac{1}{g \text{ dry weight}}$$

The results are reported in $\mu g.g^{-1}$ "chrysene or oil equivalents". If the sample extract has been fractionated into hydrocarbon classes, the intensity of fluorescence of each fraction will depend on the relative amounts of single through five and heavier ringed aromatics in the fractions. An example of the relative distribution of FI measured at 310nm/360nm between fractions from the column chromatography separation (section 8.3.1) of a few standards oils are listed below :

	(F1)	(F2)	(F3)
Venez/Mex Ismus Crude oil	4%	48%	20%
Gippsland Crude oil	5%	53%	42%
Marine diesel oil	0%	93%	7%
Lubricating oil	16%	44%	40%

8.5.7 Testing for inner filter effects.

The intensity of the emitted fluorescence is a function of the character of the fluorescent compounds in a sample, their concentrations, the dimension of the sample in the light path of the photometer, and also of the intensity of the excitation source.

There is a linear relationship between the sample concentration of fluorescing compounds and the intensity of the emitted light only within certain limits when the total absorbance of the sample is low. If the absorbance exceeds 0.05 absorbance units (less than 95% transmittance), FI will be reduced by the presence of any compound in the sample that can absorb either the excitation light or the emitted light. As this effect takes place in the sample cell, the disturbance is called an "inner filter effect". At high concentration this can be caused by the fluorescing compound itself. This is common in highly polluted sample extracts. Other compounds co-extracted with the oil may also cause a reduction in emitted light by "quenching". In this instance the energy is transferred to the co-extracted substances or "quenchers", rather than being emitted as a fluorescence emission.

The following tests can be used to test for inner filter effects. None of these experiments are fully conclusive and a good knowledge of fluorometry is necessary for their application and effective evaluation.

8.5.7a. Raman-scattering test.

This test can help to reveal inner filter effects but not quenching. The test will work only if the Raman scattering peak is separated from the emission band of the petroleum hydrocarbons. If the Raman peak is superimposed on the slope of any other emission peak, the test is not conclusive.

Use 270 nm as the excitation wavelength. Fill the cell with pure hexane. Scan, mechanically or manually, the emission spectrum to find the maximum of the Raman scattering which should occur at 297 nm (In order to resolve the Rayleigh from the Raman scatter, narrow slits and high sensitivity settings must be used.). Record the spectrum. Replace the hexane with the sample and scan in the same way to record the Raman scatter. It should be of the same intensity as that from the pure hexane. If it is significantly lower, an inner filter effect is present and the sample must be diluted and/or subject to an adsorption chromatography clean up procedure.

8.5.7b. Sample dilution.

Make a series of measurements of FI (310 nm/ 360 nm) of the sample at different dilutions. Plot FI vs the diluted concentrations. If a straight line is obtained there is no inner filter effect and any of the measurements can be used for quantification. If the relationship is not linear, dilute the sample until a linear relationship is obtained and use the FI at these dilutions for the quantification. For routine purposes, a single dilution can be used provided the results agree within 20%.

8.5.7c. Standard addition.

Measure the FI of the sample, then dilute it with an equal volume of a standard of chrysene or oil solution that has the same FI as the sample. Then measure the FI of the sample plus standard mixture. The FI of the mixture should be equal to :

$$\text{FI of mixture} = (\text{FI of standard}) / 2 + (\text{FI of sample}) / 2$$

If the FI of the mixture is less than that predicted by more than 20%, then significant inner filter effects exist and the FI of the sample must be multiplied by this correction factor for the quantification.

Note :
The synchronous excitation/emission scan is much more selective to aromatic hydrocarbons by showing less interference from biogenic pigments. It is feasible to quantify by constructing calibration curves based on synchronous scans. At the time of revision of this manual, the U.S. Coast Guard Oil Spills Laboratory is experimenting to determine optimum wavelength differences. The reader is advised to look for modifications to be published through the American Society of Testing Materials.

8.6 Gas chromatography with flame ionisation detection (GC-FID)

With proper calibration, this technique gives both quantitative and qualitative determinations of the amounts and types of hydrocarbons present in the fractions. Recovery of spiked surrogate standards is used to correct results for losses during the extraction and clean-up procedures. The qualitative composition of hydrocarbons is necessary to distinguish biogenic from contaminant hydrocarbons, to aid in source identification and to determine the degree of weathering undergone by the residues. Aspects of this can be achieved with excellent reproducibility by means of gas chromatography using packed columns. While sensitivity is generally not as great as that achieved with capillary work and resolution is obviously lower, if extremelypure carrier gases are not available there is some advantage in being able to run samples under less than state-of-the-art conditions. Both methods are described here. The discussion on quantification is relevant to both methods.

Note :

It is possbile to convert many packed column gas chromatographs to use fused silica open tubular columns. "Megabore" columns of approximately 0.5 mm i.d. are comparatively easy to fit into packed column instruments and offer intermediate performance between packed and capillary column separations.

8.6.1 Gas chromatography using packed columns.

Use thick walled 1/8 inch stainless steel tubing or equivalent. Tubing 1/8 inch (3.2 mm) o.d., 0.035 inch (0.9 mm) wall thickness and 15 feet (4.6 m) long is an optimum size. Clean tubing with solvents, dry and plug one end with pre-extracted glass wool. Pack the column with 3% SP 2100 on Chromosorb W HMDSO 80-100 mesh. Pack as tightly as possible and record amount of packing material used. Plug the fill end with glass wool and coil the column to fit the configuration of the GC oven. Connect inlet end to the injector and condition the column for several hours with nitrogen carrier gas and oven set at 340°C. Do not connect column outlet to the detector until the column is conditioned. Turn off the hydrogen in the detector and plug the detector inlet in the oven during this conditioning. Connect up the system with moisture traps and molecular sieve traps on all gases entering the GC. Set the zone temperatures and other parameters as shown in Table 2.

Table 2. Conditions for packed column gas chromatography.

Carrier gas :	Nitrogen at between 10 and 20 $mL.min^{-1}$
Column :	As above or equivalent
Injector temperature :	250°C
Detector temperature :	350°C
Initial oven temperature :	100°C
Initial time:	1 min.
Programme rate :	$5°C.min^{-1}$
Final Temperature :	325°C
Final Time :	15 min.
Injection volume :	1 to 2 µL

The GC should be run through its programme and the signal monitored until the column bleed stabilizes. The carrier gas flow rate should be optimized to resolve C_{17} from pristane and C_{18} from phytane. At this point the GC is ready for calibration. Mixtures of *n*-alkanes and aromatic hydrocarbons in the C_{10} - C_{34} and the trimethyl-benzene through benzo(ghi)perylene elution range should be prepared at concentrations in the order of 200 $ng.\mu L^{-1}$ each component in working calibration solutions. Response factors (RFs) for each peak should be calculated as the area generated per ng injected. When the baseline and RFs are stable, the system is ready for sample analysis. Reduce extracts to volumes appropriate to introduce approximately 200 ng of each resolved component in 1 to 2 µL. Addition of nitrogen through the detector as a make-up gas may improve sensitivity. The system should be stable for many samples but will gradually deteriorate as the liquid phase evaporates off the packing and contaminants build up in the column inlet. Thus records should be kept of the ability of the column to resolve specific marker compounds such as C_{17}/pristane as well as the individual and average response factors. If contaminants build up in the inlet, the column can often be regenerated by cutting off a few cm at the inlet end and replacing the glass wool plug. Resolution should be adequate to quantify individual *n*-alkanes, some other specific markers and the unresolved complex mixture (UCM) of hydrocarbons typical of oil residues as described in section 8.6.3 for GC quantification.

8.6.2 Gas chromatography using fused silica capillary columns.

Better resolution is achieved by using an instrument manufactured to accommodate fused silica capillary columns. In order to achieve stable response factors, all of the gases must be of ultra pure grade. Each must have accurate flow control based on multiple staged constant pressures. Extreme caution to eliminate oxygen and water in the lines is necessary. Columns useful for hydrocarbon analysis are 5% phenyl methyl silicone (SE 52 or equivalent) cross bonded onto fused silica. Optimum performance is achieved with a "Grob" type on-column injector which requires 0.32 mm i.d. columns. Alternatively a split/splitless injector using 0.2 mm i.d. columns can be used. Carrier gas can be hydrogen, helium or nitrogen depending on the availability of high quality grades. Safety cut offs **must** be included if hydrogen is used. The carrier gas should be plumbed independently of the detector gases. As flow rates are low, make up gas is essential. The flow rate of the carrier gas should be optimized. The following procedure is useful for 25 m columns and H_2 carrier gas. Set oven temperature to 150°C. If using splitless injection, time the valve opening for 60 sec. after the start. Set carrier flow rate to approximately 10 psi or 1 to 2 $mL.min^{-1}$. Inject 1 µL of a solution containing 20 $ng.\mu L^{-1}$ *n*-tetradecane (C_{14}). Time the emergence of the C_{14} peak from the leading edge of the solvent peak. Adjust the carrier flow rate to make this difference approximately 2.7 min. . Then adjust the flows of H_2, air and N_2 through the detector to give optimum response. With these optimum flows the system is now ready for calibration. Under the conditions given in Table 3, it will take approximately 30 min. to elute C_{17}. C_{17}/pristane and C_{18}/phytane should show baseline resolution and the entire run will take approximately 70 min. .

Table 3. Conditions for a capillary gas chromatograph.

Carrier gas :	approximately 8 psi or 60 kpa (1 to 2 $mL.min^{-1}$)
Make up gas :	N_2 at 40 $mL.min^{-1}$
Column :	Fused silica as above or equivalent
Injector temperature :	Splitless : 250°C;
	On-column : cooled to 30°C
Detector temperature :	325°C
Initial oven temperature :	50°C
Initial time :	1 min.
Programme rate :	$5°C.min^{-1}$
Final Temperature :	290°C
Final Time :	20 min.
Injection volume :	1 to 2 µL

Successful analyses using gas chromatography is a skill acquired by years of practice. The novice is referred to Freeman (1981) for further details.

8.6.3 Quantification by gas chromatography.

8.6.3.a. Calculations for resolved components.

First the chromatograph is calibrated by injecting an appropriate standard mixture which includes all the surrogate standards used in the analysis. Response factors (RFs) are calculated in terms of $area.ng^{-1}$. This is usually done for the peaks on the basis of area units calculated by an electronic integrator. As the response of the flame detector is quantitative over a large linear range and is relatively unaffected by the structure of individual hydrocarbons, the response factors for individual peaks should not vary by more than 20%. With vaporizing injectors, the high boiling peaks are not eluted as efficiently as the lower boiling compounds and so the response factors will generally decline from C_{26} through C_{36} (Under the conditions specified here, it is not generally possible to quantify compounds eluting after about C_{36}. If high boilers are of particular importance, the use of a 12 m fused silica column will improve recovery and still maintain reasonable resolution. New research instruments and columns are now available commercially for high temperature work, but this is beyond the purpose of this manual and routine monitoring needs).

Hydrocarbons in samples are determined by injecting a known aliquot of the fractionated extracts into the GC. Peak areas are integrated and amounts calculated from the RFs of the external standards. The areas of the surrogate standards are used to compute the recovery through the analytical procedure. Surrogate standards should show better than 70% recovery. If not, the procedure should be evaluated for losses and the problems corrected. The calculations are exemplified below :

Compute RFs from external standard run and tabulate as area ng^{-1}.

Compute XF (dilution factor) as total extract volume (µL) divided by µL injected. Correct this factor for whatever fraction of the total extract was carried through to this stage of the analysis.
(eg. X 2 if only one half of the total lipid extract was subject to the clean up procedures).

$$[C_{17}]\ (ng.g^{-1}\ \text{dry weight}) = \text{Peak Area on GC} \times \frac{1}{\text{RF of } C_{17}} \times XF \times \frac{1}{\text{g dry weight}}$$

If necessary this result can be corrected for the recovery of the surrogate standards through the procedures.

When it is necessary to reduce extracts to very small volumes for trace analysis, quantitative accuracy is increased by adding another standard to the extract just before the final concentration step for GC injection. This peak, for example $C_{20:1}$, is then used to compute a more accurate dilution factor which replaces XF in the formula above :

$$XF_{13} = \frac{\text{total ng } C_{20:1} \text{ added to extract}}{\text{area of } C_{20:1} \text{ from GC of sample} \times \dfrac{1}{\text{RF for } C_{20:1}}}$$

Peaks not included in the standard mixture can be quantified on the basis of the response factors of hydrocarbons eluting nearby for which standards are included, or by use of an average response factor calculated over an appropriate elution range. This procedure is unique to a flame ionization detector and cannot be used on other detectors such as an electron capture detector where molecular structure drastically affects response factors.
Individual compounds are reported as $ng.g^{-1}$ dry weight.

8.6.3b. Calculations for unresolved compounds.

As the majority of hydrocarbons in extracts of contaminated samples are often in the unresolved complex mixture (UCM) of the chromatogram, it is necessary to quantify this signal. Laboratories equipped with graphics interfaces for computers will be able to do this electronically. Presented below is a simple graphic method applicable to both packed and capillary column work.

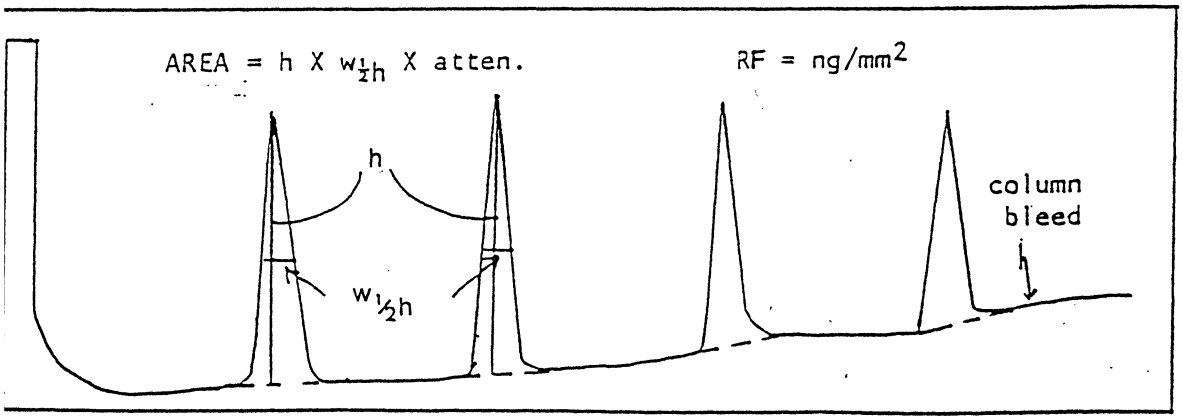


Fig 22 : Measuring peak areas and calculating Response Factors manually.

First run the calibration mixture at a concentration such that all peaks are on scale and can be measured with a ruler. With packed columns the peaks are wide enough that the standard chromatogram can be recorded at the same chart speed as used for recording samples. Note the integrator attenuation used to obtain the plots. Measure each peak in terms of square mm by measuring peak height times the width at half height (Fig. 22) (The peaks are so narrow with capillary columns that it is necessary to run the standard chromatogram at double the speed used for samples, measure peak widths with a micrometer scale under a dissecting type microscope and divide this in half to be comparable to sample runs.). Multiply each area by the attenuation and compute response factors as ng per mm^2. Compute an average response factor over the appropriate elution range. Next, cut several squares of the integrator/recorder paper and weigh them. Compute the average weight of the paper and express the result as mm2 per mg paper. Now compute a response factor for the UCM based on the average RF of the peaks over the appropriate elution range :

$$RF_{UCM} = \frac{mm^2}{mg\ paper} \times \frac{ng\ HC}{mm^2} \times Attenuation = \frac{ng\ UCM\ (atten.\ xl)}{mg\ paper}$$

To calculate the UCM in samples, trace the area underneath the peaks and above the column bleed signal. Cut the paper to this shape and weigh it to the nearest 0.1 mg. Multiply this weight by the attenuation used to plot the sample chromatogram. Compute the amount of UCM per g dry weight:

$$\frac{ng\ UCM}{g\ dry\ weight} = mg\ paper \times Attenuation \times RF_{UCM} \times XF \times \frac{1}{g\ dry\ weight}$$

XF is the dilution factor and is calculated as the aliquot injected or from the recovery of the GC quantification standard as demonstrated previously for the peak calculations.

Some computer programmes are available for acquisition of GC data and calculation of the results. For petroleum hydrocarbon analysis, the software must be capable of calculating the UCM independently of the peaks.

8.7 Computerized gas chromatography/mass spectrometry (GC/MS)

A computerized GC/MS system capable of selected ion monitoring (SIM) greatly extends the scope for determining the presence and concentration of specific marker compounds that may not be visible in the complex mixture of hydrocarbons seen by GC with FID. This technique requires extensive investment in analytical equipment and scientific expertise. The information below is intended as a starting point for applying this expertise to analysis of samples for petroleum pollution. Conditions useful for hydrocarbon analysis are given in Table 4.

Table 4. Conditions for the GC attached to the MS.

Carrier gas :	He at 1 $mL.min^{-1}$ flow rate at 200°C oven temperature.
Column :	SE 52 bonded fused silica or equivalent
Injector temperature :	280°C for split/splitless; 30 °C for on-column
Initial oven temperature :	50°C
Initial time :	1 min.
Program rate :	4 to 6°$C.min^{-1}$
Final Temperature :	280°C
Final Time :	20 min.
Injection volume :	0.3 to 1 µL

Table 5 : "Target" ions to be used for quantification and "Confirming" ions. The relative percentage of the confirming ions with ranges shown vary as a result of different isomer configurations within the alkyl group.

Compound	Target	Confirming	% Abundance
benzene	78		
C_1-benzene	92		
C_2-benzene	106		
C_3-benzene	120		
C_4-benzene	134		
naphthalene	128	127	10
C_1-naphthalene	142	141	80
C_2-naphthalene	156	141	47-95
C_3-naphthalene	170	155	61-300
C_4-naphthalene	184	169	189
d10-diphenyl	164	162	32
acenaphthylene	152	151	20
acenaphthene	154	153	86
fluorene	166	165	80
C_1-fluorene	180	165	95-144
C_2-fluorene	194	179	25
C_3-fluorene	208	193	
d_{10}-phenanthrene	188	187	98
phenanthrene	178	179	16
anthracene	178	176	20
C_1-phenanthrene/anthracene	192	191	39-66
C_2-phenanthrene/anthracene	206	191	16-150
C_3-phenanthrene/anthracene	220	205	
C_4-phenanthrene/anthracene	234	219,191	73-297
dibenzothiophene	184	185	14
C_1-dibenzothiophene	198	197	53
C_2-dibenzothiophene	212	211	
C_3-dibenzothiophene	226	211	
C_4-dibenzothiophene	240	211	
fluoranthene	202	200	17
pyrene	202	200	21
C_1-fluoranthene/pyrene	216	215	36-64
benz (a) anthracene	228	226	19
chrysene	228	226	21
C_1-benzanthracene/chrysene	242	243	20
C_2-benzanthracene/chrysene	256	241	75-131
C_3-benzanthracene/chrysene	270	255	
C_4-benzanthracene/chrysene	284	269,241	
d_{12}-perylene	264	260	21
perylene	252	253	22
benzo (b or k) fluoranthene	252	253	23
benzo (a or e) pyrene	252	253	22
indeno [1,2,3-c,d] pyrene	276	138	50
dibenz [a,h] anthracene	278	279	24
benzo [g,h,i] perylene	276	138	37

The mass spectrometer is operated at 70 eV electron energy in the electron impact ionization mode. It is tuned to optimum sensitivity according to manufacturer specifications. The column is extended all the way through to the ion source on most modern GC/MS instruments and on the mass selective detectors (MSDs). Mass to charge ratios (m/z) for the most abundant fragments or "target" ions of aromatic hydrocarbons found in light to medium weight oils are listed in Table 5. These are the major compounds of usual interest in the determination of petroleum hydrocarbons in marine sediments.

The range of hydrocarbons to be quantified will need to be established in each sample set. Benzenes are relatively soluble and volatile and may not be expected to persist in oil contaminated marine sediments unless the residues are isolated from oxidation, solution and volatilization processes. Thus, it will be important to determine the concentrations of the low molecular weight aromatics only in sediments contaminated with relatively fresh crude oil or light fuel oils. With weathering, these fractions are expected to evaporate or dissolve and the analytical strategy then emphasises the higher molecular weight compounds.

Prior to use as a quantitative instrument for low level analysis of PAH, the linear range of response must be determined by injecting a series of standard mixtures of PAH and constructing calibration curves. Quantitative analysis can only be conducted when the concentrations of analytes are within the linearly calibrated range.

Similarly to GC calibration data, continuous records of the response factors generated must be used to evaluate daily performance. Daily verification checks should generate RFs which deviate from initial average calibrations by no more than 40% for each analyte.

8.7.1 Scan mode GC/MS.

In samples of relatively fresh oil, the aromatics are likely to be present in concentrations suitable for analysis by scanning mode GC/MS. This is especially true if saturated and aromatic fractions have been separated using the preparative chemistry procedures. Quantification by GC/MS can be achieved in a similar manner to GC-FID when the mass spectrometer system is calibrated with standard solutions of known concentrations. However, response factors are determined from the ion current generated and will depend on the molecular structure of the compounds and their fragmentation patterns. Response factors are generated for each compound of interest from the integrated areas of specific parent ions acquired in scan mode.

The calibration solution containing the parent hydrocarbons and as many alkyl substituted compounds as available is injected into the GC/MS operated in the scan or total ion current (TIC) mode. This establishes the elution time of the PAH. Table 6 is an example of RT data for a standard PAH mixture. The positions of the alkyl substituted peaks for which pure standards are unavialable are determined by programming the computer to generate reconstructed ion chromatograms using m/z ratios specific to each class of compounds (128 for naphthalenes, 178 for phenanthrenes, etc). Peak confirmation is achieved by evaluating the retention times of the peaks on the reconstructed ion chromatograms and evaluation of the associated spectrum. The spectra are also used to determine if any coeluting substances are contributing to the areas of target ions to be used for the quantifications.

Table 6 : Example of retention time reproducibility achieved with manual injection of 1 µL aliquots of standard PAH solution into an HP mass selective detector system.

Peak	Target ion	Retention Time (min) X ± S.D. (n)
naphthalene	128	17.944 ± 0.031 (13)
2-m-naphthalene	142	22.010 ± 0.041 (8)
1-m-naphthalene	142	22.632 ± 0.041 (9)
acenaphthene	153	28.566 ± 0.034 (13)
fluorene	166	31.690 ± 0.039 (9)
phenanthrene	178	37.518 ± 0.034 (13)
fluoranthene	202	44.918 ± 0.034 (13)
pyrene	202	46.222 ± 0.035 (13)
1,2-benzanthracene	228	53.782 ± 0.034 (13)
chrysene	228	54.017 ± 0.036 (13)
perylene	252	62.242 ± 0.040 (13)
1,2,5,6-dibenzanthracene	278	69.537 ± 0.064 (12)

For the peaks that can be calibrated against pure standards in the calibration mixture, the response factors are calculated directly by dividing the area of the parent ion peak by the amount injected. Very few alkyl PAH compounds are available as pure standards. Thus to compute the amounts of the alkyl PAH, the response factor of the parent compound must be used. This is not rigorously accurate for quantification of the alkyl PAH and methods for estimating the response factors have been proposed. Some of these will be described in section 8.7.3.

Once a table of RFs has been constructed, the concentrations of PAH and alkyl PAH in samples can be calculated by acquiring data in the scan mode. Then reconstructed ion chromatograms are generated using each of the target ions. The integrated peak areas are then divided by the appropriate RF for each peak, similar to the method for quantification by GC-FID. This is the external standard method.

Coinjection of internal standards increases quantitative accuracy in a manner similar to GC-FID analysis. The internal standard must not be present in the sample, but it must be structurally similar to the compounds of interest. Deuterated analogues of PAH are useful internal standards. Structural similarity is important for the following reason : the justificaton for using substances other than the compound to be quantified rests on the assumption that the standard yields equal, or very similar, total ion current per amount injected. This assumption is valid only for related structures. For the internal standard method the external response factors are redefined in relation to the series of internal standards and a new table of RF_{IS} generated. The internal standards must be included in the calibration runs. The quantification of the naphthalene series is based on the internal standard, deuterated biphenyl (ion 164). Deuterated phenanthrene (ion 188) is used for quantification of the phenanthrene, dibenzothiophene, and fluorene series. Deuterated pyrene (ion 212) is used for quantification of the fluoranthene and pyrene series. Deuterated perylene (ion 264) is used for quantification of the chrysene, benzanthracene, perylene, and benzopyrene series.
For example :

$$RF_{IS}\ \text{naphthalene} = \frac{\text{Area ion 128} / \text{ng naphthalene}}{\text{Area ion 164} / \text{ng } d_{10} - \text{biphenyl}}$$

Sample calculations are then done as follows from areas based on reconstructed ion chromatograms from sample runs :

$$[C_{naph}] = \frac{\text{Area ion 128}}{RF_{IS}\text{naphthalene}} \times \frac{\text{ng } d_{10} - \text{biphenyl}}{\text{Area ion 164}}$$

The practical detection limit for GC/MS analysis in scan mode is generally in the range of 5 to 10 ng per compound injected.

8.7.2 Selected ion monitoring mode.

Sensitivity and signal to noise ratio is greatly increased by acquiring the data in the selected ion monitoring or SIM mode. In this procedure, the time windows for ion monitoring are established with scan data for pure compounds and standard oils. Then an acquisition program is written in which specific ions are scanned over the appropriate time windows in SIM mode. A sample SIM acquisition programme developed for oil spill studies is shown in Table 7 and is visually depicted in Fig. 23.

Figure 24 shows examples of ion chromatograms for selected aromatic hydrocarbons in a crude oil obtained using the SIM acquisition mode. As described above, compound identification is based on the relative retention indices and the full mass spectra of the compounds. The full mass spectra are required to avoid mistakenly integrating fragment ions of non-PAH compounds coeluting with the PAH as if they were actual PAH molecular ions. The mass spectra can only be obtained in a full scan run since SIM mode does not collect mass spectra. For routine SIM acquisition, Table 5 lists some confirmatory ions and the approximate percent relative abundance. These percentages can be used as a first level of confirmation of peak identity for SIM data.

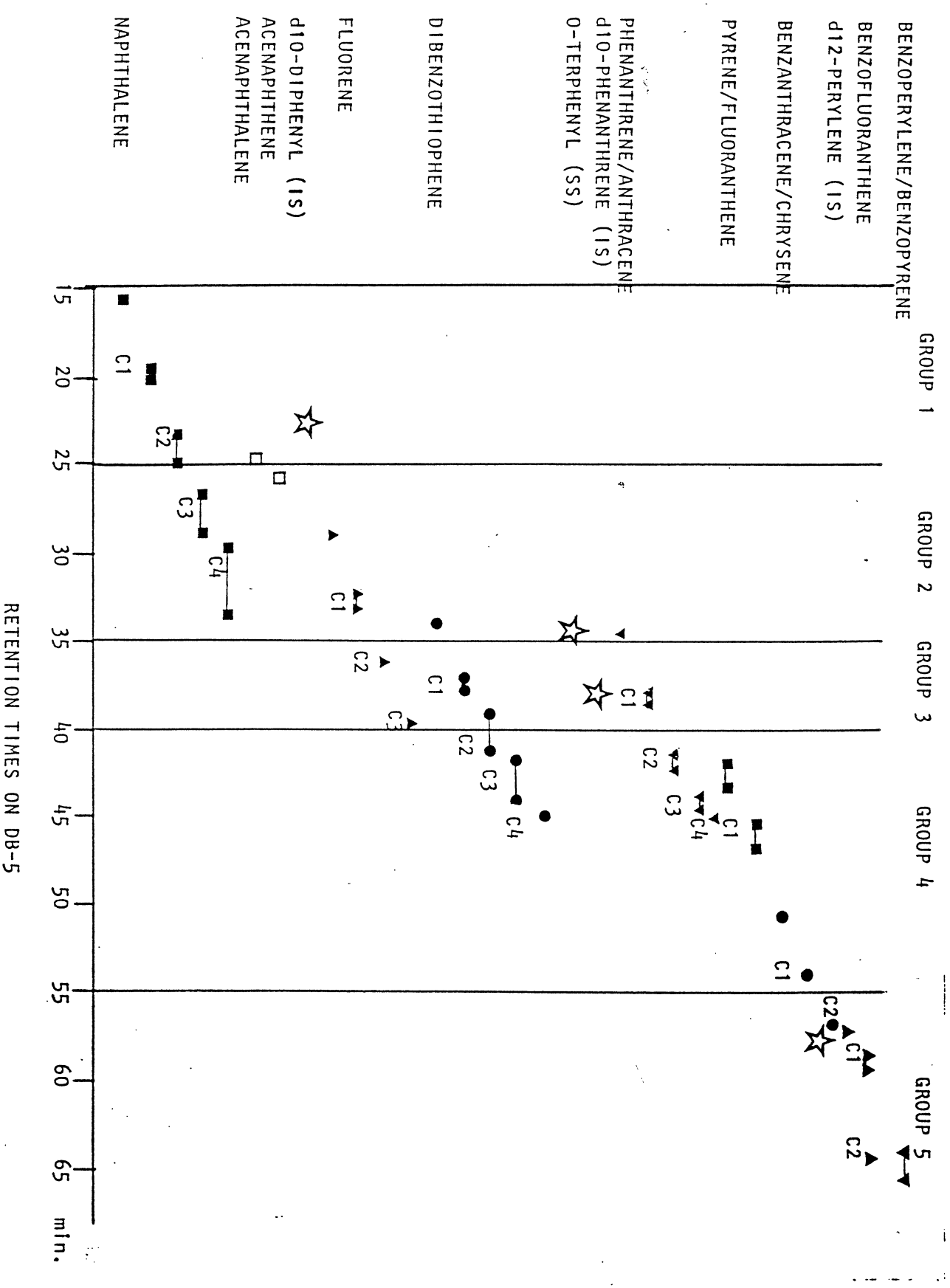


Fig 23 : SIM acquisition for PAHs.

Table 7 : Example of a selection ion monitoring programme useful for quantitative analyses of aromatic hydrocarbons in oil spill studies.

	Target ion	Confirmation ion	Retention Time
Group 1 (15 - 25 min)			
naphthalene	128	127	15.5
C_1-naphthalene	142	141	19.5 - 20.1
C_2-naphthalene	156	141	23.3 - 25.0
d_{10}-diphenyl	164	162	22.4
acenaphthylene	152	151	24.7
Group 2 (25-35 min)			
acenaphthene	154	153	25.7
C_3-benzothiophene	176	161	25.9 - 27.6
C_4-benzothiophene	190	175	27.0
C_3-naphthalene	170	155	26.6 -29.2
C_4-naphthalene	184	169	29.7 - 33.5
fluorene	166	165	28.9
C_1-fluorene	180	165	32.5 - 32.9
dibenzothiophene	184	185	33.9
d_{10}-phenanthrene	188	187	34.6
phenanthrene/anthracene	178	179	34.7
Group 3 (35-40 min)			
C_2-fluorene	194	179	36.0
C_3-fluorene	208	193	39.6
C_1-dibenzothiophene	198	197	36.7 - 37.3
C_2-dibenzothiophene	212	211	39.1 - 41.0
O-terphenyl	229	230	37.7
C_1-phenanthrene/anthracene	192	191	37.9 - 38.5
Group 4 (40 - 55 min)			
C_3-dibenzothiophene	226	211	41.6 - 44.2
C_4-dibenzothiophene	240	211	44.9
C_2-phenanthrene/anthracene	206	191	41.3 - 42.2
C_3-phenanthrene/anthracene	220	205	44.0 - 44.3
C_4-phenanthrene/anthracene	234	219/191	
fluoranthene/pyrene	202	200	41.9 - 43.2
C_1-fluoranthene/pyrene	216	215	45.5 - 46.8
benzanthracene/chrysene	228	226	50.7 - 50.9
C_1-benzanthracene/chrysene	242	243	54.0
Group 5 (55 - 70 min)			
C_2-benzanthracene/chrysene	256	241	55.0 - 5.07
benzofluoranthenes	252	241	56.9 - 57.1
benzopyrenes	252	253	58.8
d_{12}-perylene	264	260	58.8
perylene	252	253	58.9
indenopyrene	276	138	64.3
dibenzanthracene	278	279	64.5
benzoperylene	276	138	65.7

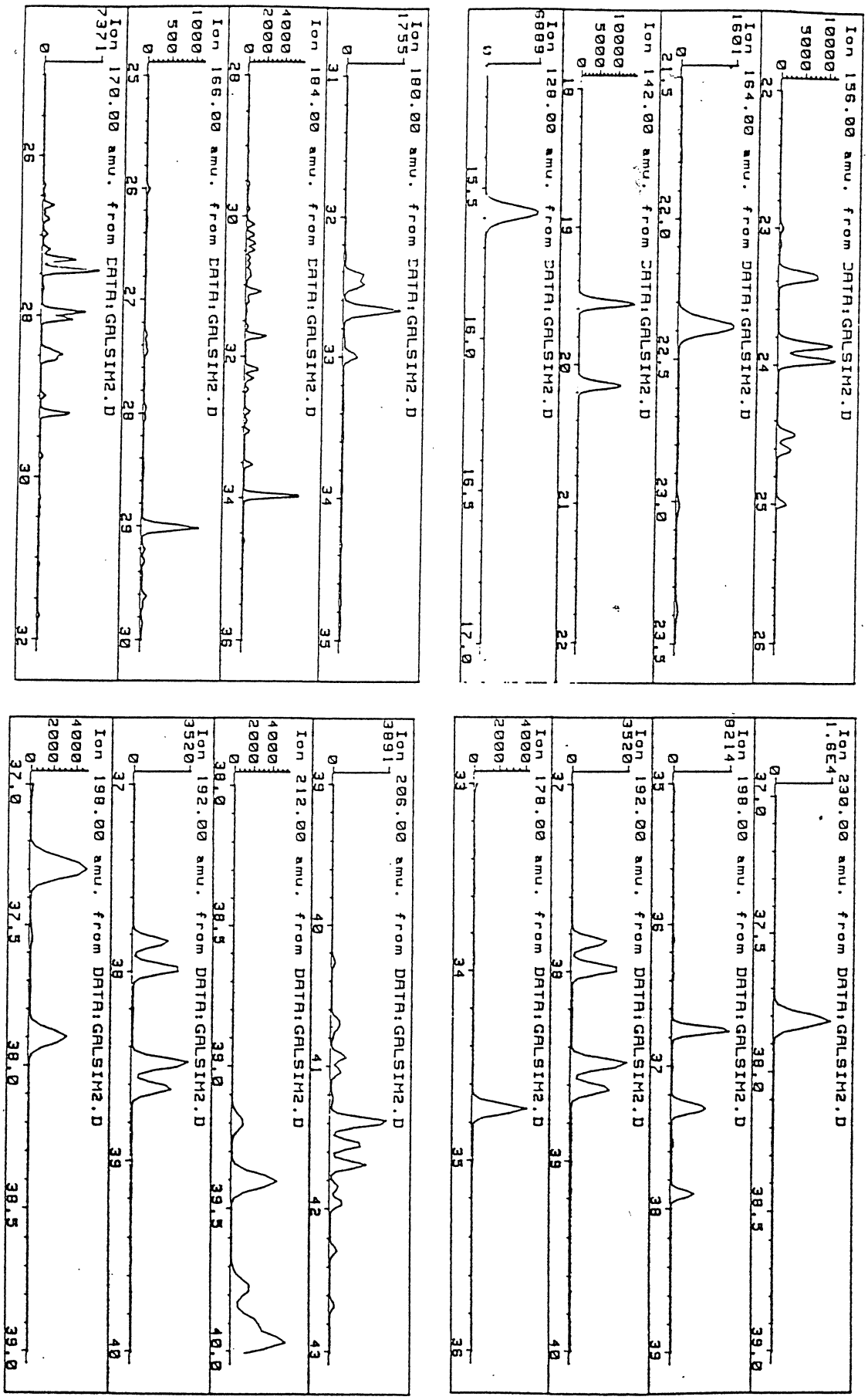


Fig 24 : Ion chromatograms of target ions as per Table 7 and Fig 23.
Venezuela / Mexican Ismus Crude Oil.

Quantification by SIM mode GC/MS is done by external or internal standard methods as described for scan mode (section 8.7.1). A calibration solution containing the PAH standards and the deuterated internal standards is injected and the data acquired using the SIM acquisition programme. The integrated peak areas of the specific ions within appropriate time windows are stored. The response factor table is then used to calculate the amount of each compound in a manner identical to that described for scan mode.

The detection limit for PAH in SIM mode is generally in the range of 40 pg per compound injected. The linear calibration range is generally up to approximately 5 ng. Samples should be concentrated or diluted to yield area counts within the linear range of instrument calibration.

8.7.3. Potential corrections for alkyl PAH response factors.

As stated above, the assumption that the response factors for the alkyl homologues are equal to that of the corresponding parent PAH is not rigorously accurate. It will be shown below that the use of RFs calculated from parent compounds may underestimate the content of alkyl PAH by as much as 50%. Several procedures for interpolating the response factors have been suggested and will be mentioned here. At the time of writing of this manual, there is no standard method for quantification of compounds by GC/MS for which pure standards are unavailable. It therefore remains the responsibility of the expert analyst to defend the calculation procedures used or to follow the guidelines suggested in individual monitoring programmes.

8.7.3.a. Calibration using standard oils.

This procedure was described in the original version of this manual and is reproduced here. It relies on an interplay between GC-FID for determining amounts of alkyl PAH in fractions obtained from a standard oil and the internal standard method on the GC/MS.

The response factors for the different alkyl PAH relative to the appropriate deuterated standards are determined by injecting known amounts of the aromatic components in a mixture of Ekofisk Crude oil (rich in naphthalenes and phenanthrenes) and Arabian Light Crude oil (rich in dibenzothiophenes). The standard oils (20 mg) are first separated into saturated and aromatic fractions by adsorption chromatography (Section 8.3). The aromatic fractions are then reduced to near dryness and retained in 100 µL hexane.

These two aromatic mixtures are then analyzed by high resolution GC-FID using the same conditions that will be used for the GC/MS analysis. The composition of the aromatic fractions (in percent) is calculated from the areas on the gas chromatograms. After the peak areas of the unspiked mixtures are calculated, exact amounts of naphthalene, 2,6-dimethyl naphthalene and 2,3,6- trimethyl naphthalene are summed so that the response factors of the naphthalene series can be determined. Phenanthrene is added to determine RFs for methyl-phenanthrenes and methyl-dibenzothiophenes. Fluoranthene is added for the determination of RFs for dimethyl-phenanthrenes, di- and trimethyl dibenzothiophenes. The new mixtures are then analyzed by GC and the peak areas integrated. The calibration information is now sufficient to determine the concentrations of naphthalenes, phenanthrenes, and dibenzothiophenes in the aromatic mixtures by GC/MS.

To calculate the RFs for these compounds on the GC/MS, known amounts of the deuterated standards are added to the aromatic mixtures and the samples are then analyzed by GC/MS in the SIM acquisition mode. Since both the amounts of the aromatic hydrocarbons and the deuterated standards are now known, the RFs can be calculated from the peak areas of target ions integrated by the computer. For example, the RF for naphthalene in relation to d_{10}-biphenyl is the number used to divide the area of the 128 fragment to obtain the true concentration.

$$RF_{IS\ naphthalene} = \frac{\text{Area 128 ion} / \text{pg naphthalene}}{\text{Area 164 ion} / \text{pg } d_{10} - \text{biphenyl}}$$

The average response factors for each class of PAH are then used to determine the concentrations of each compound within the class in sample extracts.

8.7.3.b Interpolation using relative ion abundances.

Another method that has been proposed is to calculate response factors for alkyl-PAH from the relative abundance of specific ions using scan data. The mass spectrum of the substance to be quantified is examined and the area (A) under the reconstructed ion chromatogram of its most intense fragment (ususally, but not always, the molecular ion for PAH) is integrated. Using the full mass spectrum of the substance, the abundance (R1) of this most intense fragment is calculated relative to the sum of abundances of the 10 most intense fragments including itself. This ratio is independent of the amount of the alky-PAH present in the sample. Very few alkyl substituted PAH are available to determine this ratio from pure standards. It can be computed from tabulated spectra from a library generated on a similar instrument. If the required spectra are not available in tabulated libraries and if the peaks of interest are well resolved from interfering compounds, the ratios can be computed from scan data obtained from the aromatic fraction of a standard oil run under the same conditions. Subtracting background interference should result in ratios of ion abundances similar to those obtained from pure standards. However, spectra obtained from complex mixtures cannot be used to compute the ion abundance ratios if the spectrum of the alkyl-PAH is contaminated with a coeluting substance that contributes any peaks with ion abundance greater than the least intense of the 10 most abundant ions in the mass spectrum of the pure alkyl-PAH. The ion abundance ratios for the alkyl-PAH are called R1 values and the ratios for the parent PAH are called R2 values (Table 8). The system is calibrated on a routine basis for quantification in SIM mode by injecting a calibration solution of the parent hydrocarbons and the alkyl-PAH for which pure standards are available. Areas for the major ions to be used in quantification are tabulated as are those of a few "confirming" ions (Table 7). Response factors (RFs) for the parent PAH are calculated as areas of the major ions per picogram. The response factor (RF_{alk}) for the alkyl-PAH is then equal to the response factor (RF) of the parent PAH multiplied by R1 / R2 :

$$\mathbf{RF_{alk} = RF \times R1 / R2}$$

A table of RF and RF_{alk} is constructed to include all of the "target" compounds (Table 9). The corrected response factor RF_{alk} is usually smaller than the response factor RF (in units of area.pg^{-1}), because the parent peak in the spectrum of an alkyl PAH in many cases, contributes less to the total ion intensity then the parent peak in the spectrum of the corresponding unsubstituted PAH.

The amount of alkyl-PAH in pg is then :

$$[PAH_{alk}] = \frac{Area}{RF_{alk}}$$

The accuracy of this interpolation method can be checked by direct determination of response factors for alkyl-PAH for which pure standards can be obtained. Table 8 shows the ion abundance ratios for the "target" compounds obtained from published libraries and confirmed where possible by scan data from direct injection of pure alkyl-PAH standards. Examples of the interpolated response factors for the alkyl-PAH are listed in Table 9. Also shown in Table 9 are the RFs for a few alkyl-PAH calculated from the direct injection of the standards. Agreement between the interpolated RF_{alk} and those calculated from direct injection of standards confirms that the method appears to work reasonably well.

This quantification procedure is further improved if internal standards are included in both the calibration solutions and in samples. In this case the internal standards are used to correct for the fraction of the total sample injected (dilution factor, XF) as per GC analysis (section 8.6.3.a). Including three standards which elute at different times allows the analyst to note and correct for any discrimination over the elution range which may be due to the injector or detector configurations.

Table 8 : Ion abundance ratios R1 or R2 (base ion / sum 10 ions) calculated from library spectra compared to spectra generated fromdirect injection of pure standards into an H.P. mass selective detector system.

Compounds	R1 or R2 NBS.L	R1 or R2 HCARB.L	R 1or R2 Std Inj.
naphthalene	57.3		57.3
2 methyl-naphthalene	37.7		38.5
1 methyl-naphthalene	38.3		36.7
2,3 di-methyl-naphthalene	37.3		33.9
acenaphthylene	52.4		51.1
acenaphthene	32.1		29.7
fluorene		40.0	37.2
dibenzothiophene	58.8		59.4
naphthalene	52.3	50.7	51.3
anthracene		53.2	49.0
o-terphenyl	35.1	33.6	33.9
3,6 di-methyl phenanthrene	43.4	43.4	41.5
fluoranthene	54.7	54.7	53.5
pyrene	44.8	50.4	51.0
1 methyl-pyrene	43.6	40.9	42.0
benz(a)anthracene	51.2	51.6	
chrysene	51.1	51.1	50.0
1 methyl-chrysene		49.0	49.0
benzo(k)fluoranthene		46.3	46.2
benzo(a)pyrene	48.2	55.9	48.4
perylene		47.1	51.8
indenopyrene	44.3		51.8
dibenzanthracene	49.4		57.6

8.7.3.c. Interpolation using assumed increments within classes.

It has been suggested that the response factors within a class of compounds can be assumed to vary in a predictable manner with alkyl substitution. This variation can be determined on individual GC/MS systems from a series of PAH ranging from parent through substituted compounds for which standards are available. For example, the series naphthalene; 1-methyl- and 2-methyl-; 2,3, and 2,6 dimethyl-naphthalenes; 2,3,6-trimethyl-naphthalene. The increment of variation within the series is then used to estimate the change in response factor with alkyl substitution. Data included in table 9 indicates that this method is not very accurate.

Table 9 : Table showing R1/R2 ion abundance corrections for alkyl-PAH and sample calculations of response factors. Compounds for which no published spectra or pure standards could be located are given the RF of the nearest alkyl-substituted or the parent PAH.

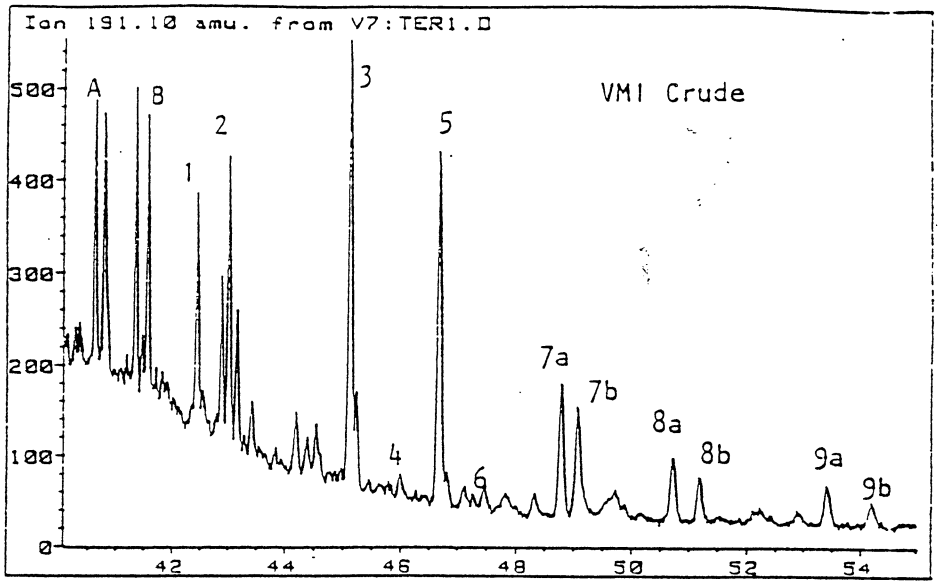
Compound	Target	R1/R2	Std RF	RFalk
benzene	78			
C_1-benzene	92			
C_2-benzene	106			
C_3-benzene	120			
C_4-benzene	134			
naphthalene	128		106	
C_1-naphthalene	142	0.67	75	71
C_2-naphthalene	156	0.59	58	63
C_3-naphthalene	170	0.71	71	75
C_4-naphthalene	184	0.69		73
d_{10}-diphenyl	164			119
acenaphthylene	152			
acenaphthene	154			
fluorene	166		75	
C_1-fluorene	180	0.85		63
C_2-fluorene	194			63
C_3-fluorene	208			63
d_{10}-phenanthrene	188		77	
phenanthrene	178		114	
anthracene	178		114	
C_1-phenanthrene/anthracene	192	0.80		92
C_2-phenanthrene/anthracene	206	0.81		92
C_3-phenanthrene/anthracene	220	0.67		77
C_4-phenanthrene/anthracene	234	0.61		70
dibenzothiophene	184		122	
C_1-dibenzothiophene	198	0.83		101
C_2-dibenzothiophene	212			101
C_3-dibenzothiophene	226			101
C_4-dibenzothiophene	240			101
fluoranthene	202		132	
pyrene	202		135	
C_1-fluoranthene/pyrene	216	0.78		105
Benz (a) anthracene	228		79	
chrysene	228		102	
C_1-benzanthracene/chrysene	242	0.89		70
C_2-benzanthracene/chrysene	256	0.61		48
C_3-benzanthracene/chrysene	270			48
C_4-benzanthracene/chrysene	284			48
d-$_{12}$-perylene	264		34	34
perylene	252			
benzo (b or k) fluoranthene	252			
benzo (a or e) pyrene	252			
indeno [1,2,3-c,d] pyrene	276			
dibenz [a,h] anthracene	278			
benzo [g,h,i] perylene	276			

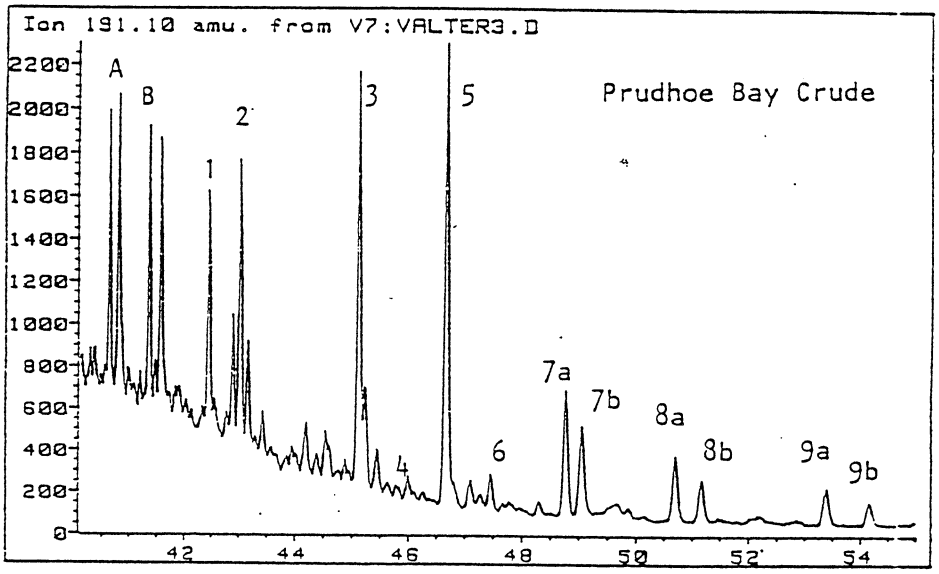
Table 10 : Triterpane peaks as identified in Figure 24.

Peak	Abbreviated Notation	Empirical Formula	Molecular Weight	Compound
A	Tricyclic A	Unknown		Tricyclic triterpanes
B	Tricyclic B	Unknown		Tricyclic triterpanes
1	C27α (Ts)	$C_{27}H_{46}$	370	18α (H)-22,29,30-trisnorneohopane
2	C27α (Tm)	$C_{27}H_{46}$	370	17α (H)-22,29,30-trisnorhopane
3	C29α ,β	$C_{29}H_{50}$	398	17α (H), 21β (H)-30-norhopane
4	C29β ,α	$C_{29}H_{50}$	398	17β (H), 21α (H)-30-normoretane
5	C30α ,β	$C_{30}H_{52}$	412	17α (H), 21β (H)-hopane
6	C30β ,α	$C_{30}H_{52}$	412	17β (H), 21α (H)-moretane
7	C31α ,β	$C_{32}H_{56}$	426	17α (H), 21β (H)-30-homohopanes (22S+22R)
8	C32α ,β	$C_{32}H_{56}$	440	17α (H), 21β (H)-30,31-bishomohopanes (22S+22R)
9	C33α ,β	$C_{33}H_{58}$	454	17α (H), 21β (H)-30,31,32-trishomohopanes (22S+22R)

8.7.4. Other marker compounds.

There are numerous other qualitative and quantitative GC/MS analyses that are possible for specific monitoring programmes. For example, it may be possible to identify specific steroid or triterpenoid hydrocarbons indicative of a particular oil input from an offshore production platform effluent to use as a tracer for that input. Figure 25 shows the triterpane pattern determined by selected monitoring of ion 191 of the saturated hydrocarbon fractions of three different source oils. Peak identifications are listed in Table 10. Ratios of specific compounds (such as Tm/Ts) may be useful for source identification. The obvious differences in patterns between the Venezuelan/Mexican Ismus Crude (VMIC) oil and the Sunniland crude oil from Florida demonstrates the usefulness of the technique in differentiating source oils. The visual similarity between the VMIC and the Prudhoe Bay Crude oil demonstrates that in some cases sophisticated pattern recognition methods would be required to distinguish oils which may be closely related or which may have undergone similar diagenesis histories. Many references in section 2 discuss the application of triterpane, steroid and isoprenoid hydrocarbon markers. Clearly these types of measurements require sophisticated instruments, elaborate calibration standards, and analytical experts with the ability to interpret the data within the context of the organic geochemistry literature. These may be made available to regional monitoring programmes *via* cooperative arrangements with advanced analytical facilities in a region or external to the region. Detailed description of these methods is beyond the scope of this manual.





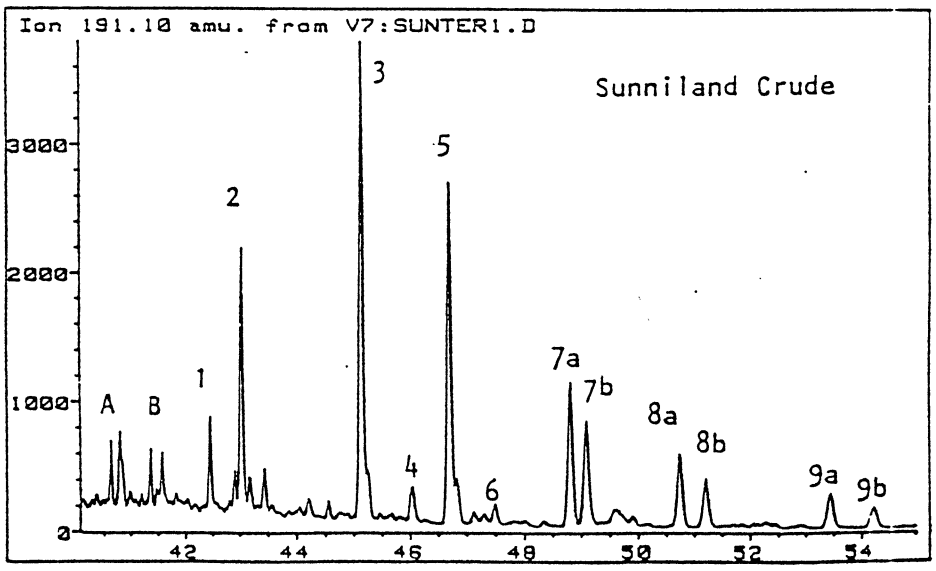


Fig 25 : Reconstructed ion chromatograms of ion 191 from the saturated hydrocarbon fraction of three crude oils.

8.8 Determination of sediment particle size composition.

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 sedimentological data.

The scheme is based on a factor-of-two scale. The categories for wet sieved sediment are summarized in Table 11.

Table 11 : Sedimentological classes.

Class		Size (μm)	Suggested sieves (μm)
		2,000	
	very coarse	1,000	1000
	coarse	500	
sand	medium	250	
	fine	125	125
	very fine	62	63
	very coarse	31	
	coarse	16	
silt	medium	8	
	fine	4	
	very fine	2	
clay		<2	

The homogenized sediment used for dry weight determination should be sieved to estimate size composition. This information and the total extractable organic matter will be useful in 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.

Reference materials are available from United Nations and national sources. These consist, among other items, of homogenized marine sediments which can be distributed to interested laboratories on a regular basis. These materials should be used by the laboratories to establish their precision and accuracy for the determination of petroleum hydrocarbons in marine sediments by performing the initial analysis on three aliquots of the reference material. Levels of hydrocarbons should be tabulated on both a dry weight and a lipid weight basis. Means and standard deviations should be computed for each compound quantified and any unresolved hydrocarbons present. This data will establish the precision of replicate analysis for each result tabulated. Intercomparison with the results of other reputable laboratories will aid in the assessment of accuracy.

9.3 Analytical Quality Control Charts (AQCCs).

9.3.1 Purpose of AQCCs

It has been recommended that a reference material should be analysed periodically to provide a check on the quality of analytical data. The simplist way to assess the results of these analyses is to examine them at the end of the analytical period and decide whether or not they are satisfactory, and thus whether or not the results for samples are acceptable. This approach is very subjective and a much better approach is to plot the results of the analysis of RMs on a simple chart, which contains guide lines that allow an objective decision to be made on the quality of the data. This chart is known as an analytical quality control chart (AQCC).

9.3.2 Construction of an AQCC

Analysts are reminded that before a method is used routinely for samples it must have been rigorously assessed to ensure that it will provide data of the required quality. Assuming that such a method is used the analyst should carry out the following procedure to construct an AQCC, along the lines of that given below in Fig. 26

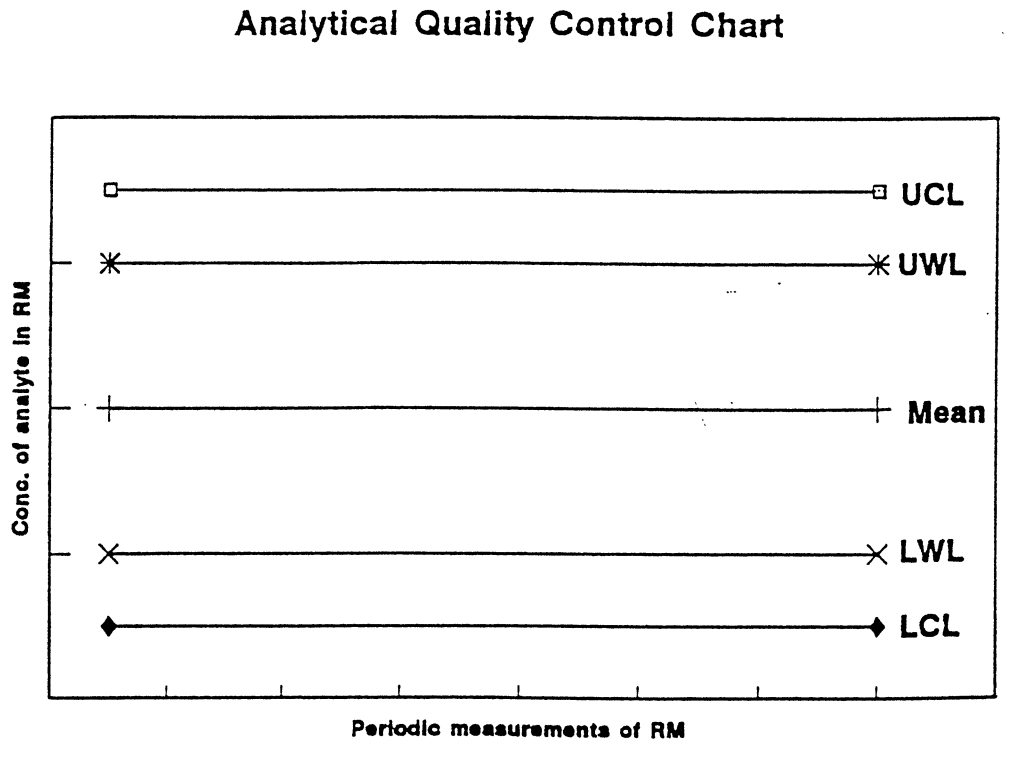
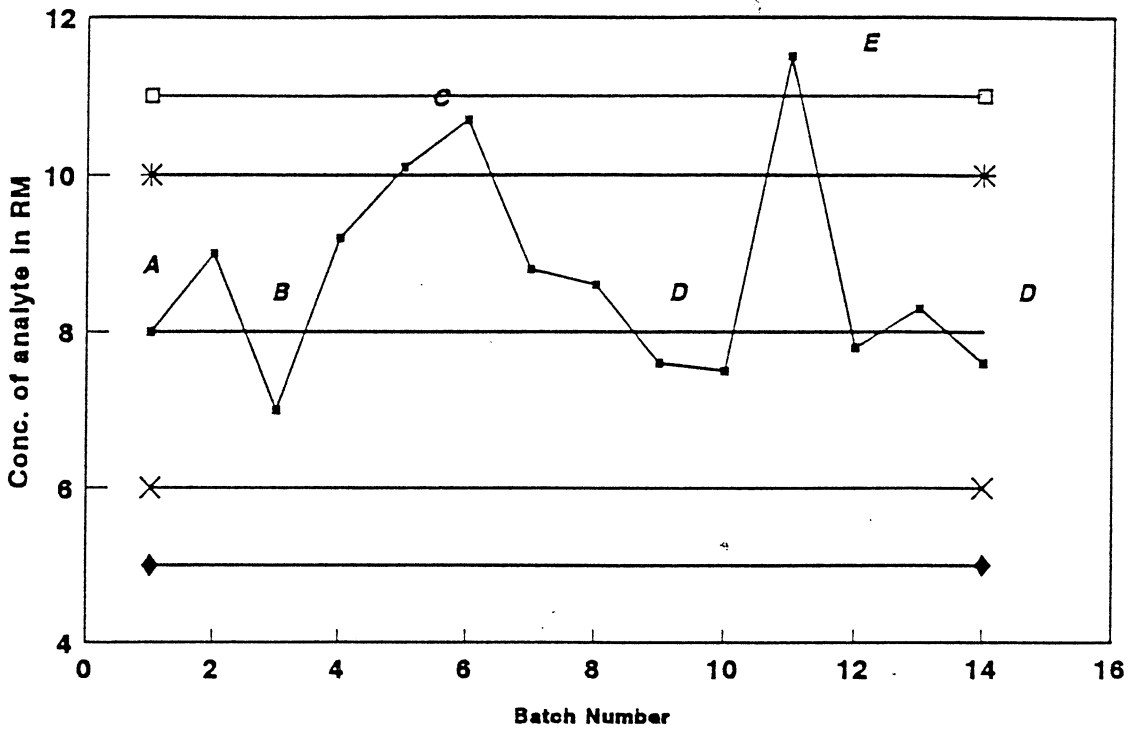


Figure 26. Blank control chart

QA
Quality Control chart



Units are arbitrary

ANNOTATIONS

A - A "consensus value" is established by repeated analyses of an IRM. Upper and lower warning and control limits are determined statistically from the standard deviation (s) of the n measurements made.

B - The routine measurements of the IRM are well within the warning limits. Measurements are under control.

C - Something appears to be contaminating the samples here. Reagents were investigated and a new batch of solvents was found to be at blame and was replaced immediately.

D - The process is back under control.

E - Here, a serious problem was discovered. The previous ten data were rejected and all analyses were discontinued until the fault (dirty glassware this time) was detected and corrected.

Figure 27 : An example of a Quality Control Chart.

(i) Select the RM to be analysed with samples on a regular basis.

(ii) Analyse the RM at least 10 times for the analyte(s) under examination. These analyses should not be done on the same day but spread out over a period of time in an attempt to ensure that the full range of random errors within and between batch analyses are covered.

(iii) Calculate the mean value ($\bar{X}$), and the standard deviation (s) and then plot the following values on a blank control chart :

$\bar{X}$, $\bar{X} + 2s$ (UWL), $\bar{X} + 3s$ (UCL), $\bar{X} - 2s$ (LWL) and $\bar{X} - 3s$ (LCL).

9.3.3 Using an AQCC

Assuming that the analytical measurements for RM(s) follow a normal distribution, 95% of them (19 in every 20) should fall within the area between UWL (upper warning limit) and LWL (lower warning limit). Similarly 99.7% of the results should fall within the area between UCL (upper control limit) and LCL (lower control limit).

The analyst should plot the results of the analysis of RM(s) after each batch of analyses to check where the data lies in relation to these limits. An example of such a plot is given in Fig. 27

The following guidelines can be used to assess whether the data for the RM(s) and consequently the data for the samples are of acceptable quality, i.e., are the analyses under control.

(a) The mere fact that one result falls outside the warning limits need not require the analyst to doubt the result or take any action provided that the next result falls within the warning limits.

(b) If the results fall outside the warning limits too frequently, particularly if the same warning limit has been crossed more than once on consecutive results, then the analyst needs to assess the source of this systematic error.

(c) If the results on more than 10 successive occasions fall on the same side of the X line (either between X and UWL or X and LWL) then the analyst needs to check the analytical procedure to determine the cause of this error.

(d) If the result fallsD outside the UCL or LCL lines then the analyst should check the analytical procedure to determine the cause of this source of error.

If any of the above cases occur the analyst should reject the results of the analysis of the particular batch of samples and should not carry out any further analysis of samples until the source(s) of the errors have been identified and he/she is satisfied that future analyses will be under control.

9.3.4 Use of Internal Reference Materials

The accuracy of a method can only be checked with an SRM or a CRM for which the mean values and standard deviations are well documented. Analysts who choose to use their own specially prepared RM (i.e., an internal RM, IRM) for quality control purposes should note that they are primarily checking the precision of measurements and not their accuracy. These IRMs are very convenient however, for analyses where large quantities of materials are required for each determination (e.g., analyses for organic contaminants) and where the cost of these materials for QC charts would be prohibitive. Full instructions on the preparation and calibration of IRMs will be given in another publication in the present series.

Sample : I.A.E.A. : SD-K-1 marine sediment

$\frac{\text{wet wt.}}{\text{dry wt.}}$ = _____ ; % water in freeze dried sample determined by drying at 105°C : _____ ;

_____ g freezedried wt. extracted with 50% $MeCl_2$/hexane in Soxhlet extractor for 8 hrs. _____ µg *n*-octadecene and _____ µg 9,10-dihydroanthracene were added as internal standard. The _____ mL extract was reduced by rotary evaporator to approximately _____ mL. This was passed over small column of sodium sulphate to filter and dry the extract.....Then over a small column of activated copper to remove sulphur.....This was further reduced to _____ mL for lipid determinations. Corrected dry wt. : _____ g.

Lipid determinations :

_____ mL total extract;

10 µL aliquots weighted on microbalance : _____ mg; _____ mg; _____ mg;

_____ mg; _____ mg;

E.O.M. = _____ $mg.g^{-1}$ dry wt. ;

_____ mg lipid subjected to column chromatography fractionation. 8g Silica gel under 8g Alumina.

F_1 _____ mL hexane;

$F_2 + F_3$ _____ mL 10% ether/hexane; plus _____ mL 20% $MeCl_2$.

GC determinations :

$C_{20:1}$: _____ ng (F_1) GC; _____ ng (F_2+F_3) GC; _____ ng total in each fraction.

Dilution factor _____ (F_1); _____(F_2+F_3).

_____ µg $C_{18:1}$ recovered (F_1) _____ %R;

_____ µg 9,10-dihydroanthracene recovered (F_2+F_3) _____%R.

Average UCM RF _____ $\frac{\mu g \text{ hydrocarbon}}{g \text{ paper}}$; paper wt. _____ g; _____ µg UCM total.

UCM $\mu g.g^{-1}$ dry wt. _____.

UVF determination : $F_2 + F_3$

_____ $\mu g.g^{-1}$ ALC equivalents _____ $\mu g.g^{-1}$ chrysene equivalents.

Attach tabulation of individual compounds, GCs and UVF spectra.

Fig 8 : Sample worksheet for analysis of petroleum hydrocarbons in marine sediments.

9.4 Definitions of some relevant statistical terms.

The **specificity** of an analytical chemical method is the degree to which the mean value of the measurements is due to the substance to be determined and not to other substances that may be present in the sample being analyzed.

The **sensitivity** of an analytical chemical method is the smallest change in the quantity to be measured which produces a detectable change in the output. In this case it is synonymous with the term minimum detectability.

The **precision** of an analytical chemical method is the degree to which one representative determination of a substance in a sample will yield a measurement that approaches the average measurement of an infinite number of determinations of the same sample (In other words, the precision is the reproducibility of the analytical results.).

The **accuracy** of an analytical chemical method is the degree to which the mean value of the measurements obtained by the method approaches the true value for the measured substance (the effects of other substances interfering being eliminated physically or mathematically).

10. ARCHIVING AND REPORTING OF RESULTS.

Every sample should have an associated worksheet which follows the samples and the extracts through the various stages of the procedure and upon which the analyst notes all relevant details. An example of such a worksheet is given in Fig. 28. Each laboratory should construct and follow such a worksheet. Relevant spectra and/or chromatograms should be attached to the worksheet. Analyses should be grouped and composite or summary analysis sheets archived with each group. Final disposal of the data will depend on the reasons for which it was collected but should follow the overall plan or model.

Notes for Fig. 28.

1 The horizontal line of lipid weights represents individual 10 µL determinations by cleaning pan and rezeroing every time. The vertical line represents an addition method where 10 µL aliquots were added and evaporated in sequence to the same pan and the weights determined by difference. There should be no difference in the standard deviation computed by either method on modern microbalances.

2 In this example $C_{18:1}$ and 9,10-dihydroanthracene were used as internal standards added to the extraction solvent. $C_{20:1}$ was a quantification standard added to extracts just before injection into the GC and was used to determine the GC dilution factors.

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