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**METHODS FOR ANALYSIS FOR  
HYDROCARBONS AND POLYCYCLIC AROMATIC  
HYDROCARBONS (PAH) IN MARINE SAMPLES**

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# 1. BACKGROUND

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Burnham-on-Crouch Laboratory is called upon to provide a comprehensive range of analytical services. A majority of the analyses undertaken stem from programmes which assess the impact, or potential impact, of many types of discharge (other than radioactive discharges) on marine and freshwater fisheries. Some of this work arises from the involvement of CEFAS in regulatory activities, such as the licensing of the disposal of dredged material. This is administered by the Ministry of Agriculture, Fisheries and Food (MAFF); of which CEFAS is an Executive Agency. The role of CEFAS is to provide advice during the licensing of these activities, and undertake effects-monitoring as required in order to validate the assessments made. In addition, there is a major programme of general environmental quality monitoring, which is used both to support pollution prevention and environmental protection activities. This programme also provides data for submission to both national and international monitoring programmes, such as the UK National Marine Monitoring Programme, the activities co-ordinated within the International Council for the Exploration of the Sea, and the Joint Assessment and Monitoring Programme of the Oslo and Paris Commissions. The CEFAS Burnham Laboratory also fulfils an emergency response advisory role for MAFF in relation to spills of oil and chemicals in marine waters, and develops monitoring programmes following major spills in support of fishery closure orders and environmental impact studies.

The methods for the analysis of hydrocarbons and polycyclic aromatic hydrocarbons (PAH) described here have been applied over a number of years to the study of general pollution, accidental oil spills, and studies of the effects of offshore oil and gas exploration and exploitation. These procedures have been thoroughly tested, and in most cases have also been applied in intercomparison and laboratory proficiency studies alongside those of other laboratories. Routine determinations are also applied within analytical quality control protocols which require the analysis of procedural blanks, standards and reference materials alongside the samples in order to provide quality assurance information with which to monitor analytical performance on a batch-to-batch basis.

This report is intended to replace an earlier publication of methods for hydrocarbon analysis (Law *et al.*, 1988). The level of detail provided, whilst not meeting the requirements of a standard operating procedure, is intended to be adequate to allow other analysts to assess the procedures, the apparatus and analytical instrumentation required, and the performance of the method in routine use. If difficulties are encountered when applying the methods, contact should be made with the lead author from whom further details can be obtained.

Details are provided of any special sampling techniques or sample preservation procedures, and although some information is given on the design of sampling programmes this is not exhaustive. Indeed, given the range and scope of collaborative monitoring programmes currently, this is impractical in a report of this type. This applies particularly to the analyses of fish and shellfish for the detection of temporal or spatial trends, or for human health risk assessment purposes. In most cases the organisations responsible for co-ordinating these programmes have laid down guidelines, and references to these are given where possible.

# 2. INTRODUCTION

Hydrocarbon and PAH analyses are carried out at the CEFAS Burnham Laboratory generally for three main reasons. They are conducted to investigate gradients of concentration around point sources (oil spills, oil production platform discharges, etc.), to investigate the distribution of these contaminants over a wider geographical area (baseline/benchmark studies), or in support of laboratory-based experiments. In many cases this necessitates the analysis of a relatively large number of samples. For this reason we have adopted a two-stage procedure, in which all samples are initially analysed ('screened') by ultra-violet fluorescence spectrometry (UVF). Samples are prioritised on the basis of these results for analysis using one of the more sophisticated techniques based upon either high-performance liquid chromatography with fluorescence detection (HPLC-UVF), or coupled gas chromatography - mass spectrometry (GC-MS). UVF analysis alone is inadequate for purposes other than screening as it provides little or no information on the sources of the aromatic hydrocarbons detected. However an indication of the range of PAH present in the sample can be obtained by the application of synchronous excitation-emission scanning UVF to the same raw sample extracts. This is also undertaken routinely alongside the simple quantitative determination at fixed fluorescence wavelengths. Further characterisation of the hydrocarbons and PAH present in the sample extracts requires the use of GC-MS or HPLC-UVF. Gas chromatography with flame-ionisation detection (GC-FID) could be used for the analysis of cleaned extracts or fractions in order to generate qualitative profiles, but for quantitative determinations the more selective detection achieved with GC-MS or HPLC-UVF is preferred. GC-FID of whole cleaned extracts or aliphatic fractions will yield chromatograms in which, in fresh crude oils for instance, peaks due to *n*-alkanes predominate. The alkane patterns found in samples can then indicate the presence of biogenic and isoprenoid hydrocarbons, and the degree to which oils have been affected by evaporation and degradation. The use of coupled techniques (HPLC-UVF and GC-MS) provides a further level of sophistication, and allows the use of

deuterated analogues of the compounds to be added and determined for purposes of quantification. HPLC-UVF allows the concentrations of individual parent (unsubstituted) PAH to be determined. GC-MS allows the concentrations of both parent PAH and groups of alkylated PAH to be determined, and techniques such as sterane/triterpane finger printing and other pattern techniques can, if required, provide discrimination between possible source oils, and between combustion and fossil fuel sources. These techniques are not covered in detail in this report, but are well covered in the literature – see for example, Burns and Bence, 1993; Page *et al.*, 1996 and 1997; Rowland and Robson, 1990; Sporstol *et al.*, 1983. Intercomparison studies concerning PAH are also underway within the QUASIMEME programme, and the data produced are periodically assessed and reported (Law and Klungsoyr, 1996; Law *et al.*, 1997(a); Law *et al.*, 2000). We no longer use GC-FID routinely as the use of an ion-trap mass spectrometer allows the collection of full-scan MS data with no limitation of instrumental sensitivity, and the total ion current chromatogram provides the same information as the GC-FID without the need for a separate analytical step. This mass spectral information may then be archived using writeable CD-ROMs and the data examined at a later date, e.g. for the investigation of novel compounds whose environmental presence has recently been demonstrated in historic samples.

### **3. GENERAL CONSIDERATIONS**

#### **3.1 Cleaning of glassware**

Glassware is soaked for at least 12 h in a dilute solution of Pyroneg, the detergent is then removed by rinsing under running tap water. The glassware is then placed for a similar period in 10% nitric acid. On removal it is rinsed well with running tap water, dried at 50°C, and stored in a cupboard until required. Immediately prior to use it is rinsed at least three times with *n*-pentane.

#### **3.2 Blank determinations**

For all analyses blank determinations are made. These are normally full procedural blanks, i.e. all the stages of an analysis are performed but in the absence of sample material. Blank values are subtracted from all results quoted.

#### **3.3 Internal standardisation**

GC-MS: Deuterated analogues of most of the parent aromatic compounds of interest are available for use as internal standards (both recovery and surrogate standards), and the alkylated derivatives of the parent PAH are also determined relative to the surrogate standard by application of experimentally determined response factors. Only a limited number of the

enormous range of possible alkylated PAH compounds are currently available as pure compounds for use in preparation of standard solutions. So the determination of these compounds is in reality an approximation based on those isomers for which response factors can be determined. Intercomparison studies are now progressing to the stage where they include the determination of alkylated compounds as well as parent PAH, and this will be an area for investigation if differences between laboratories are to be minimised. It may prove necessary to define individual isomers within each group on which calibration and quantification should be based in order to improve intercomparability.

Internal standards used for quantification purposes (surrogate standards) are added at the start of the extraction procedure and taken through all stages of the analysis for both HPLC-UVF and GC-MS techniques, whilst those added in order to estimate recovery on an individual sample basis are added prior to GC-MS analysis.

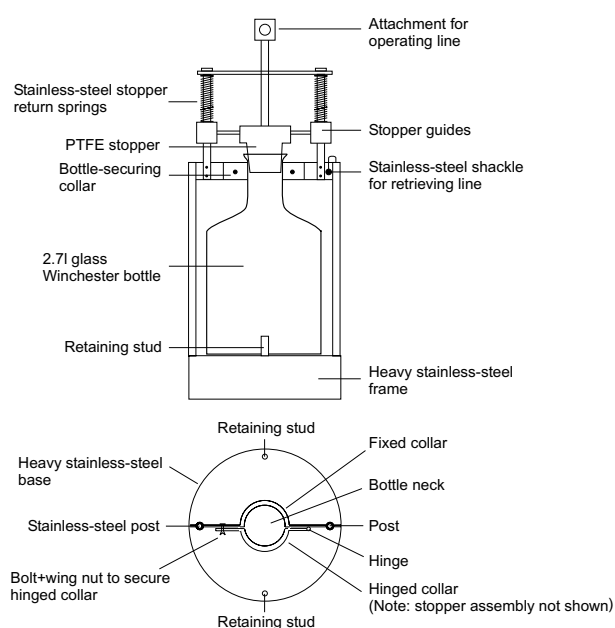
### **3.4 Shipboard contamination**

Ships use a variety of oil products and wastes containing small quantities of oil are routinely discharged into the sea. In addition, losses of oil occur during normal operation from leaks, spills and the use of lubricated machinery. In order that such contamination may be recognised, it is strongly recommended that, as a matter of routine, samples of fuel oil, bilge oil and any other oils and greases used on board a sampling vessel should be collected and returned to the laboratory for analysis. They should be analysed by GC-MS and the hydrocarbon profiles compared with those of the environmental samples to identify any samples which have been contaminated. In practice this is generally more of a problem with water samples than with sediments, as the concentrations found are usually so much lower and the opportunities for contamination during collection are greater. The potential for contamination should, however, never be either neglected or underestimated.

## **4. SAMPLING**

### **4.1 Water**

Subsurface water samples are collected in 2.7 l glass Winchester bottles mounted in a weighted stainless-steel frame which is deployed by means of a nylon rope (Figure 1). The bottle is sealed using a Teflon (PTFE) stopper which may be removed at the sampling depth by means of a second nylon rope. The stopper is spring-loaded so that the bottle may be resealed when full, being open therefore only during sample collection, and sealed during deployment and recovery. Sample bottles are rigorously cleaned with pentane before use.



**Figure 1. Diagram of a shallow water sampler used for hydrocarbon analysis**

The sampler (particularly the stopper) is cleaned with pentane at the start of each day's sampling, periodically during sampling, following a period of inactivity, or after use in areas of high hydrocarbon concentrations.

This shallow water sampler has been in routine use for more than 15 years, and has proved to be both robust and reliable. It has been shown to be capable of collecting uncontaminated samples for a variety of other trace organic contaminants as well as hydrocarbons, including *iso*-propyl benzene, tetrachlorethene and phthalate esters (esters of 1, 2-benzenedicarboxylic acid) in the low ng l<sup>-1</sup> range. When deployed from a hydrowire, the bottles can be used to a depth of at least 50 m without imploding; whilst for sampling at depths of less than 10 m, the sampler can be operated by hand.

In general, subsurface water samples are taken from 1 m depth and analysed without filtration. In areas of high particulate loading it may be desirable to carry out separate analyses of filtered water and suspended particulate material (SPM). The phases are separated using pressure filtration in systems in which the water sample contacts only surfaces made of stainless steel or PTFE.

## 4.2 Suspended particulate material

If an analysis of SPM is required, water samples collected as described above are transferred to a 10 l stainless-steel pressure vessel and filtered under nitrogen top pressure (0.3-3 bar) through a 142 mm

diameter filter unit of stainless-steel and PTFE construction (SM 165 40; Sartorius Ltd, Belmont, Surrey). A number of types of filter are available but as yet we have used only one type successfully, yielding adequately low blanks from analysis of the pre-cleaned filters alone. These are 0.45 µm membrane filters made from regenerated cellulose (Sartorius Ltd). They are cleaned in pentane and dried and weighed before use, then air-dried at ambient laboratory temperature and reweighed after use to determine the particulate loading of a sample. Before the filter is placed in the filter holder, 500 ml of pentane is used to rinse the inside of the pressure vessel and then forced by top pressure through the connecting hose (PTFE lined stainless-steel) and the filter holder to clean them. This yields an operationally-defined fractionation into dissolved and particulate phases. The true distribution of hydrocarbons in water samples seems to lie along a continuous axis from truly dissolved components, through associations with colloidal material (the quantity of which varies with sampling location) to adsorption onto biological or sedimentary particles. Other techniques such as continuous-flow centrifugation have also been applied with the aim of improving the information on phase-association (Smedes, 1994), but as there is in reality no clear functional division between dissolved and particulate hydrocarbons a simple separation is impractical.

## 4.3 Surface sediment

Surface sediments are usually sampled at sea by means of a 0.1 m<sup>2</sup> modified Day grab fitted with stainless-steel buckets. The grab has been described elsewhere (Eagle *et al.*, 1978). Other grabs such as the Hamon, Shipek, and van Veen have also been applied successfully - the choice of grab can often be influenced by the sediment type to be sampled. Whenever possible the grabs are deployed by means of a clean rope and allowed to free-fall to the seabed, and recovered using a whipping-drum. If it is necessary to deploy the grab on a wire from a winch, then a length of clean rope (~ 8 m in length) is used between the grab and the wire end to prevent lubricants used on the wire contaminating the collected samples. The jaws of the bucket are cleaned with *n*-pentane before the grab is used, and periodically thereafter. Samples of sediment collected by the grab are taken from the centre of the grab to a depth of ~1 cm by means of a stainless steel or PTFE-bladed spoon which has been previously cleaned with *n*-pentane. Sediment is transferred to clean 500 ml wide-mouthed glass Beatson jar, solvent extracted aluminium foil is placed over the mouth of the jar to prevent contact between the sample and the waxed lid liner, and the jar is sealed and stored at -20°C prior to analysis. Care should be taken not to overfill the jars, bearing in mind that the wet sediment expands on freezing and sufficient space must be left to allow for the expansion or the jars will break.

## 4.4 Biota

Collaborative monitoring programmes generally issue guidelines regarding the species, number and size range of animals to be collected, and the means by which tissues selected for analysis are to be homogenized and pooled for analysis. See for example, the guidelines for the UK National Marine Monitoring Programme (MPMMG, 1994 and 1999) and the OSPARCOM Joint Assessment and Monitoring programme (JAMP: Oslo and Paris Commissions, 1997). These are intended to allow the analytical and sample (individual to individual) variability to be estimated in order that the true environmental variability (spatial or temporal) can be assessed. Different strategies may be needed depending on the aims of an individual programme, for instance depending on the magnitude of changes which it is desired to detect. Sampling methods for biota are not critical although, in the case of spilled oil investigations, fish should not be hauled through visible surface oil or sheens. Samples of fish, etc, are wrapped whole in new aluminium foil, placed in plastic bags and stored at -20°C prior to analysis.

## 5. EXTRACTION FOR ANALYSIS USING FLUORESCENCE SPECTROMETRY AND GAS CHROMATOGRAPHY – MASS SPECTROMETRY

### 5.1 Water

Water samples (2.7 l) are transferred directly from the sample bottle to a 3 l cylindrical glass separating funnel fitted with a glass stopper and PTFE stopcock and extracted with *n*-pentane, shaking for five minutes (2 x 50 ml). The first aliquot of solvent is used to rinse the inside of the sampling bottle to remove any adhering hydrocarbons before it is added to the separating funnel. When the phases have separated, the water is returned to the sampling bottle and the *n*-pentane run into a 100 ml glass conical flask fitted with a glass stopper. The extraction procedure, including the rinse, is repeated with the second aliquot of *n*-pentane and this is also added to the conical flask. A small quantity of solvent-rinsed, anhydrous sodium sulphate is then added to the conical flask to dry the *n*-pentane extract. If, after swirling gently, free or emulsified water remains in the extract, more sodium sulphate is added. After drying, the *n*-pentane extract is transferred, with rinsing of the sodium sulphate residue, to a round-bottomed flask, and concentrated using a rotary evaporator whose water bath temperature does not exceed 28°C. The concentrated extract is finally quantitatively transferred to a 5 ml volumetric flask and made up to volume prior to UVF analysis. Under no circumstances must the extract be

taken to dryness, as serious losses of low-boiling hydrocarbons and PAH (such as naphthalene) will result.

After separation of SPM, as described above, the filtrate should be collected in a separating funnel and processed as above to yield an extract of filtered seawater.

### 5.1.1 Preparation of sodium sulphate

To avoid sample extract contamination the sodium sulphate must be cleaned before use. This procedure requires that the sodium sulphate is solvent-extracted using an all glass large Soxhlet apparatus for 3 hours. The sodium sulphate is transferred into quartz dishes and allowed to dry in an open oven for at least 16 hours. The dishes are then placed in a muffle furnace heated to 250°C for 4 hours, after which they are removed and allowed to cool in a desiccator. Once cool the sodium sulphate is transferred to a clean sealed glass jar and stored until required.

### 5.2 Suspended particulate material

The air-dried filters holding the SPM are extracted with *n*-pentane (100 ml) in a Soxhlet apparatus for 6 h. The extract is then concentrated using a rotary evaporator, as described above, but omitting the sodium sulphate drying stages, and made up to a suitable final volume (usually 5 or 10 ml) prior to UVF analysis.

### 5.3 Sediment and biota

The frozen sediment samples are allowed to defrost to room temperature and then well mixed with a stainless steel spatula. The normal weight of sediment taken for analysis is 40-50 g, but smaller quantities may be used where it is known that contamination levels are high; for instance, in fine mud from industrialised estuaries. Concentrations of hydrocarbons and PAH are expressed on a dry weight basis, as the water content is not an intrinsic property of sediments but is influenced by the means of collection and storage. The dry weight percentage is determined using a separate sub-sample (10-15 g) which is weighed into a pre-weighed foil dish, dried at 105°C in an oven for 16 h, allowed to cool and then reweighed. This procedure has been shown to be adequate to dry the sediment to a constant weight.

Biota samples, fish, shellfish and marine mammal tissues are allowed to defrost, and then up to 20 g of sample is taken.

Both biota and sediment samples are extracted using alkaline saponification. The samples are weighed into a 250 ml round-bottomed flask, and potassium hydroxide pellets added (5 g for sediment or 20 g for biota), along with 100 ml of methanol and a few Teflon anti-bumping



granules. Known amounts of the appropriate surrogate standards are added at this stage. The samples are digested under reflux using a heating mantle for 2 hours and allowed to cool. The digests are then filtered through solvent-rinsed filter papers (Whatman 113v or similar) into 250 ml separating funnels fitted with glass stoppers and PTFE stopcocks. These digests are twice extracted with *n*-pentane (2 x 50 ml), the pentane aliquots being used to rinse the round-bottomed flask before addition to the separating funnel. After shaking the separating funnel is placed in a rack and the extract allowed to separate, into two layers. The bottom layer is collected in a clean conical flask, and the upper *n*-pentane layer collected in a 100 ml conical flask containing solvent-rinsed anhydrous sodium sulphate. The bottom layer is then poured back into the separating funnel and the process repeated. The two pentane extracts are combined in the 100 ml conical flask and dried over anhydrous sodium sulphate. The extract is then quantitatively transferred to a 100 ml volumetric flask and made up to volume prior to UVF analysis.

A batch usually consists of 4 samples, a procedural blank, and an appropriate certified or laboratory reference material.

## 6. ANALYSIS BY FLUORESCENCE SPECTROMETRY

This is conducted in a similar way for all samples types, and is usually carried out prior to sample clean-up. As there is no absolute measure of fluorescence emission the spectrofluorimeter is first calibrated with solutions of reference standards: Ekofisk crude oil Forties crude oil, and diesel oil have all been used routinely within our own programmes. A suitable instrument should have monochromators for selection of both excitation

and emission wavelengths, and be capable of scanning both monochromators simultaneously (in this case an RF-5301PC spectrofluorimeter; Shimadzu Ltd, Milton Keynes). All measurements are made within the linear range of the spectrometer so as to preclude self-absorption effects and consequent distortion of the spectra; in practical terms this is taken as 0-10  $\mu\text{g ml}^{-1}$  of the oil standard used. Two sets of fixed analysis wavelengths are used routinely:

<u>Ex<math>\lambda</math></u>	<u>Em<math>\lambda</math></u>	<u>Standard</u>
270	330	diesel oil
310	360	crude oils

Excitation and emission slits are adjusted to 5 nm in each case. The former conditions have been used primarily in studies of drill cutting discharges from offshore oil and gas production platforms, and following diesel spills in rivers upstream of fish farms; the latter in oil spill investigations and general pollution studies. The calibration standard is prepared in a volumetric flask by dissolving 0.2 g of the required oil in 100 ml of *n*-pentane. This 2000  $\mu\text{g ml}^{-1}$  solution can then be diluted, to prepare a range of standards which are used to calibrate the fluorescence instrument. Dilutions are made using a range of volumetric flasks and a glass syringe. For example, in order to prepare a 10  $\mu\text{g ml}^{-1}$  standard, 100  $\mu\text{l}$  of the stock 2000  $\mu\text{g ml}^{-1}$  solution is transferred to a 20 ml volumetric flask and made up to volume with *n*-pentane. The fluorescence of the sample extracts is compared with that of the standard solutions, and the results calculated as equivalents of the oil standard, after performing the appropriate blank correction if necessary. Sample extracts exhibiting greater fluorescence than the 10  $\mu\text{g ml}^{-1}$  standard are diluted as required to bring them within the linear range of the calibration. Limits of detection and reproducibility for UVF analyses of water and sediments are given in Table 1, and a calibration graph is shown in Figure 2.

**Table 1. Limits of detection and reproducibility for analyses of water and sediment by fluorescence spectroscopy ( $\mu\text{g l}^{-1}$  and  $\mu\text{g g}^{-1}$  dry weight Ekofisk crude oil equivalents respectively)**

	Number of determinations	Mean	SD <sup>a</sup>
<b>Water</b>			
Blank	11	0.27	0.08
Seawater <sup>b</sup>	9	2.2	0.19
Spike recovery <sup>c</sup>	5	95.8%	4.4%
Limit of detection	(3 x SD of blank) = 0.24 $\mu\text{g l}^{-1}$		
<b>Sediment</b>			
Blank	11	0.10	0.033
Sediment <sup>d</sup>	4	38.5	2.3
Sediment <sup>e</sup>	4	284 <sup>f</sup>	7.7
Limit of detection	(3 x SD of blank) = 0.10 $\mu\text{g g}^{-1}$ dry weight		

<sup>a</sup> standard deviation of the sample.

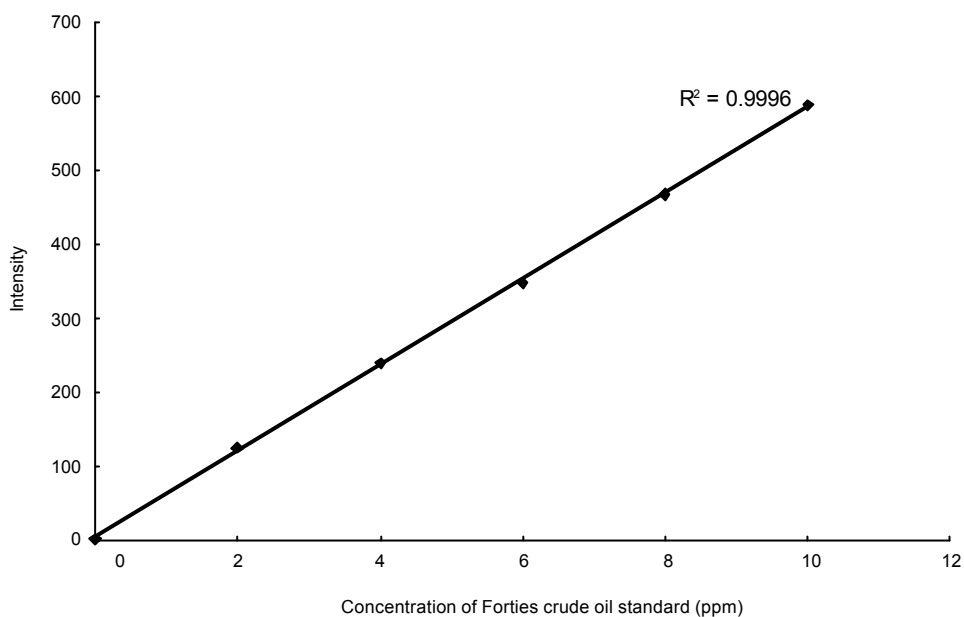
<sup>b</sup> coastal seawater from NE England.

<sup>c</sup> at 16.7  $\mu\text{g l}^{-1}$  Ekofisk crude oil.

<sup>d</sup> intercalibration sample (Law and Portmann, 1982).

<sup>e</sup> intercalibration sample (Howells, 1989).

<sup>f</sup> diesel oil equivalents.



**Figure 2. Spectrofluorimeter calibration plot showing a linear regression line**

Synchronous excitation-emission spectra are run for all samples, blanks and standard solutions, with a fixed offset of 25 nm ( $E_{m\lambda} > E_{x\lambda}$ ) in the range  $E_{x\lambda} = 230$  to 500 nm. Representative synchronous excitation-emission spectra for jet fuel, diesel oil, paraffin and Forties crude oil are shown in Figure 3. Comparison of the spectra obtained for the samples and the standard indicates whether or not the standard contains aromatic hydrocarbons within a similar boiling range to the samples, i.e. whether or not an appropriate standard was used. If this was not so, then a more suitable standard should be selected. The presence of larger molecular weight aromatics with a greater number of fused rings results in fluorescence emission at progressively higher wavelengths (Law, 1980). Although not used routinely, it is also possible to generate contour maps of fluorescence emission for use in source fingerprinting, and an example is given in Figure 4.

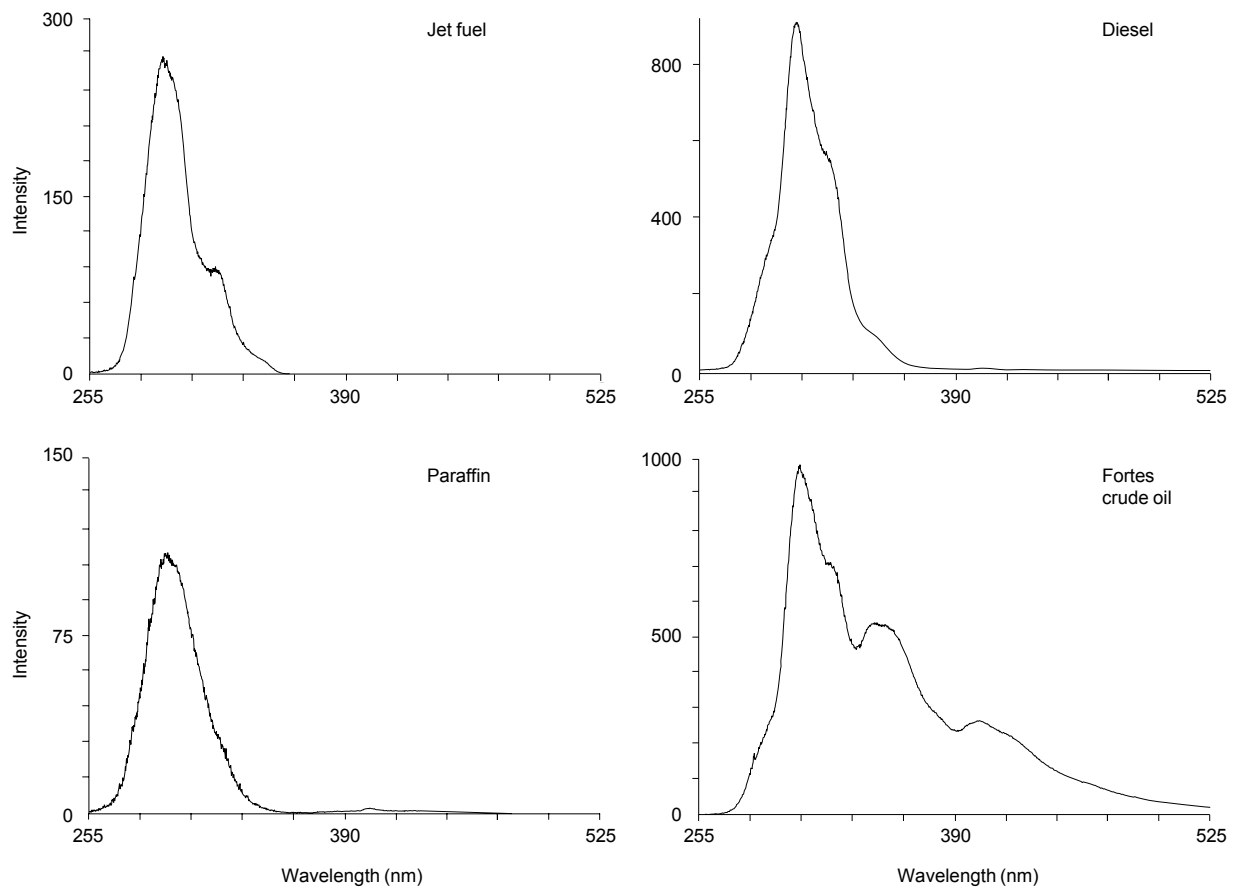
## 7. CLEAN-UP FOR FURTHER ANALYSIS

Although UVF analyses may be carried out without clean-up of sample extracts, the removal of lipids and other co-extracted material is necessary prior to HPLC-UVF or GC-MS analysis. As the samples have been subjected to alkaline digestion, which will break down lipids (and quantitatively destroy elemental sulphur and mercaptans in sediments), the quantities of

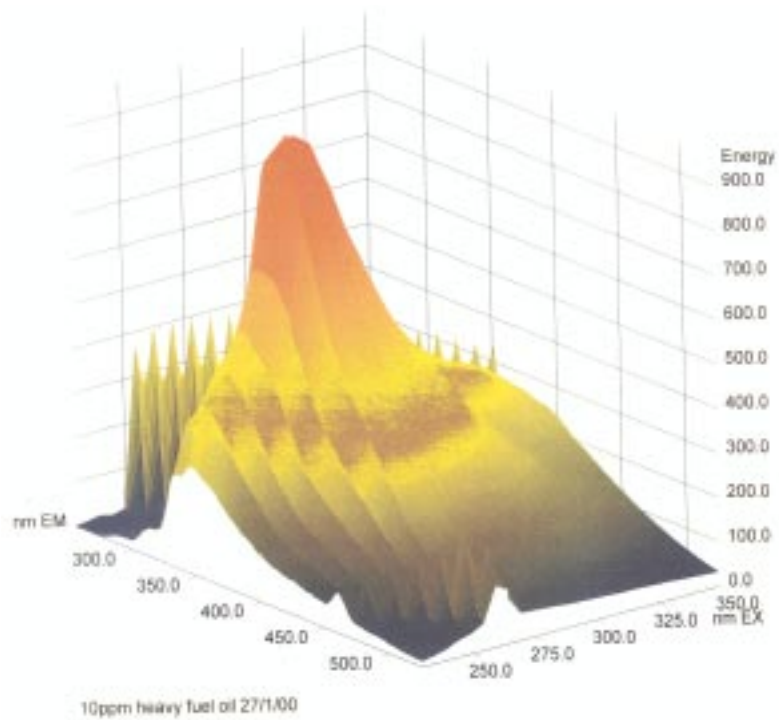
residual lipids in the extracts are very small, relative to those encountered following Soxhlet extraction for instance. However additional clean-up is required to remove these remaining traces of lipid and other co-extractives, such as pigments, from the pentane extract. This is carried out using column chromatography on alumina, and the procedure is the same for all sample types.

### 7.1 Preparation of alumina

The alumina is prepared for use by being placed in a quartz dish and heated to 800°C for 4 h in a muffle furnace, after which it is allowed to cool in a desiccator. The alumina is then deactivated by weighing out 95 g of the activated alumina into a clean 100 ml flat bottomed flask, and adding 5 g of pentane-extracted distilled water dropwise from a Pasteur pipette. The flask is placed on a wrist action shaker for 40 minutes, after which time it is removed and visually inspected for lumps. If it appears as a free flowing powder it is labelled to record the degree of deactivation (in this case 5%), the date and the weight, and is then stored in a desiccator until required. The weight is checked immediately prior to use and recorded on the flask after use, before being returned to the desiccator. This procedure checks that the alumina remains at the required deactivation level prior to use for column chromatography.



**Figure 3. Synchronous excitation/emission fluorescence spectra**



**Figure 4. A 3-D synchronous excitation/emission fluorescence spectra perspective plot**

## 7.2 Alumina column chromatography clean-up

For alumina clean-up the sample extracts are concentrated to 1 ml in volume, using rotary evaporation. The columns are prepared by using clean long-form Pasteur pipettes, plugged with cotton wool. Approximately 1 g of 5% deactivated alumina is poured into the column and the sides gently tapped to settle it. A beaker is placed under the column and 2 ml of eluent (50% *n*-pentane in dichloromethane) is passed through the column to clean it before use. Once the solvent has drained into the bed of the column, the beaker is replaced with a clean round/flat-bottomed flask, and the sample transferred from the rotary evaporator flask to the column using a Pasteur pipette. The sample is allowed to just soak into the alumina and then the hydrocarbons and PAH are eluted with 2 x 1 ml aliquots of the eluent as described above. When all of the eluent has passed through the column the flask is removed. The cleaned extracts are then reduced to a suitable volume (usually between 100 µl and 1 ml) prior to analysis by GC-MS and transferred to crimp-top autosampler vials.

## 8. ANALYSIS BY COUPLED GAS CHROMATOGRAPHY–MASS SPECTROMETRY

Aliquots of the extracts (1 µl) are analysed using a 30 m x 0.25 mm ID crosslinked fused silica capillary column coated with 5% phenyl methyl silicone fluid (0.25 µm film thickness). The carrier gas is helium at a linear flow rate of 40 cm s<sup>-1</sup>. Injection is via a split/splitless injector operated in the splitless mode, with the split valve being opened to purge the injector 1 minute after injection. The injector temperature is 270°C. The injection is made with the column at 60°C, and following injection the oven temperature is held at 60°C for 2 minutes, and subsequently raised at 5°C min<sup>-1</sup> to 300°C where it is held for 10 min, giving a standard run time of 60 min. The GC column is directly coupled, via a transfer line heated to 250°C, to the ion source of a GCQ ion trap mass spectrometer (ThermoQuest, Hemel Hempstead, Herts.). This is interfaced to and controlled by a data system using Xcalibur software running under Windows NT. The software controls the GC-MS instrument as well as performing data acquisition,

**Table 2. Compounds analysed by GC-MS and the relevant internal standards used for quantification**

Compound	MW	ISTD	MW
Naphthalene	128	Naphthalene-d <sub>8</sub>	136
C <sub>1</sub> -naphthalenes	142		
C <sub>2</sub> -naphthalenes	156	Acenaphthylene-d <sub>8</sub>	160
C <sub>3</sub> -naphthalenes	170		
Acenaphthylene	152		
Acenaphthene	154		
Fluorene	166		
Anthracene	178	Anthracene-d <sub>10</sub>	188
Phenanthrene	178		
C <sub>1</sub> -phenanthrenes	192		
Pyrene	202	Pyrene-d <sub>10</sub>	212
Fluoranthene	202		
C <sub>1</sub> -pyrenes	216		
Benz[ <i>a</i> ]anthracene	228	Benz[ <i>a</i> ]anthracene-d <sup>12</sup>	240
Chrysene/triphenylene †	228		
2,3-benzanthracene	228		
Benzo[ <i>b</i> ]fluoranthene †	252	Benzo[ <i>a</i> ]pyrene-d <sub>12</sub>	264
Benzo[ <i>j</i> ]fluoranthene †	252		
Benzo[ <i>k</i> ]fluoranthene †	252		
Benzo[ <i>e</i> ]pyrene	252		
Benzo[ <i>a</i> ]pyrene	252		
Perylene	252		
Benzo[ <i>ghi</i> ]perylene	276		
Indeno[123- <i>cd</i> ]pyrene	276		
Dibenz[ <i>a,h</i> ]anthracene	278		
Chrysene-d <sub>12</sub> (recovery standard) which is compared to Benz[ <i>a</i> ]anthracene-d <sub>12</sub>	240		

† *chrysene and triphenylene, and the three benzofluoranthene isomers are not fully resolved*

storage and reduction. The mass spectrometer is operated in full scan electron-impact (EI) ionisation mode, and scans from 35 to 325 Daltons. A cycle time of 1 s is used to ensure an adequate number of data points across fast-eluting capillary GC peaks. A list of compounds normally analysed and the relevant internal standards used are given in Table 2. In general, quantification of polycyclic aromatic hydrocarbons using EI is carried out from mass fragmentograms of the molecular ion for each compound or class of compounds. When using an ion-trap spectrometer there is little or no loss in sensitivity as a result of acquiring full-scan data, rather than using multiple-ion detection as is routine for quadrupole instruments. It is preferable therefore to use full-scan acquisition at all times, enabling the use of library search routine to confirm the identity of the compounds determined and to tentatively identify unknown compounds. Total ion current chromatograms for the same range of oils as used for Figure 3 are given in Figure 5.

For further detail on the use and application of the GC-MS methodology described here, see Kelly and Law (1998), Law *et al.* (1998), and Law and Kelly (1999).

## 9. EXTRACTION FOR ANALYSIS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

### 9.1 Seawater

Seawater samples are collected in the same manner as described in Section 4.1 above. A 2 l volume of seawater is required for extraction, and the excess is utilised to rinse the separating funnel before use. A known aliquot of an internal standard solution containing 100 ng of anthracene- $d_{10}$  is added to each 2 l sample prior to extraction. The extraction is then conducted as in Section 5.1 above, except that dichloromethane (2 x 50 ml) is used as the extraction solvent instead of *n*-pentane (note also that dichloromethane is heavier than water and forms the lower rather than the upper layer). After the addition of the deuterated internal standard, samples in which the 'dissolved' PAH are to be determined are filtered through a 0.45  $\mu\text{m}$  filter (Whatman GF/F filter heated in a muffle furnace at 300°C overnight); and the filtrate solvent extracted as for the unfiltered sample.

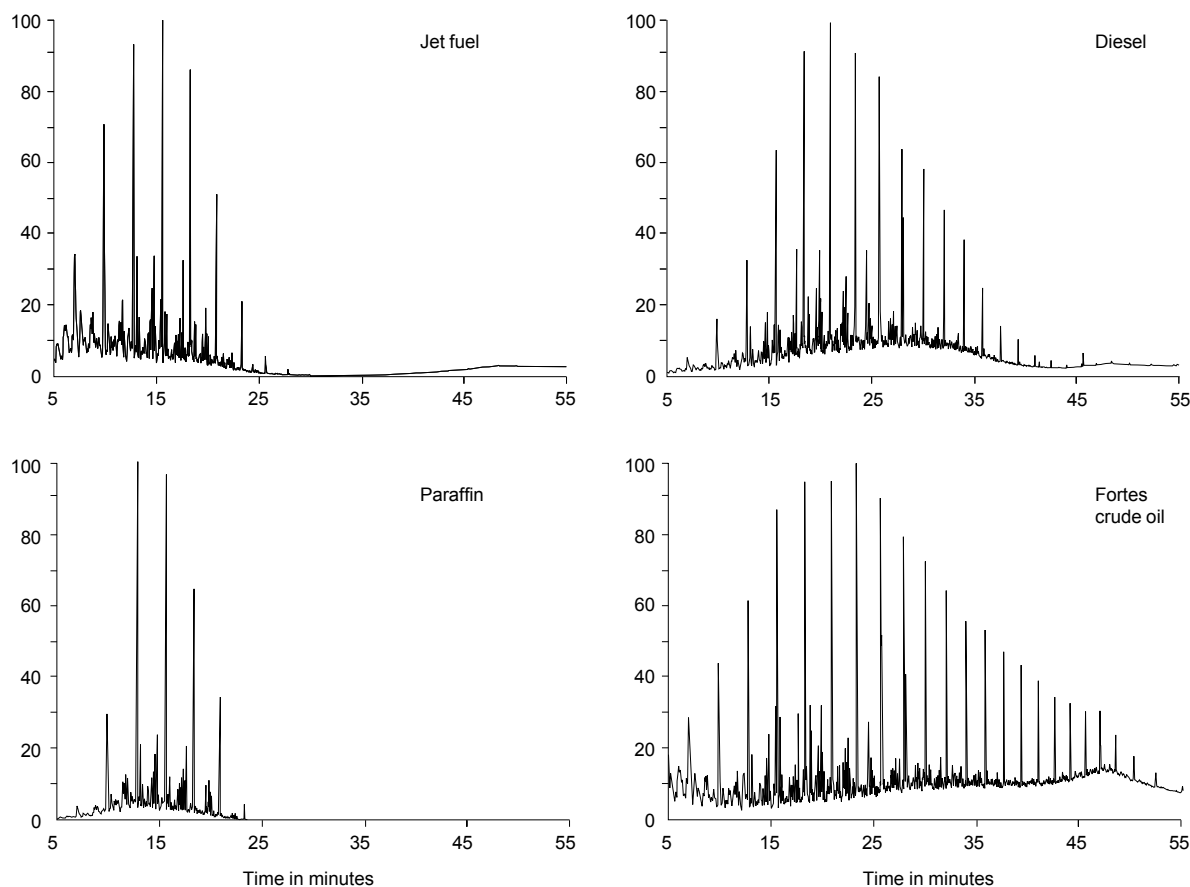


Figure 5. Total ion chromatograms

Subsequent manipulations are the same for both filtered and unfiltered samples.

After drying the extract over anhydrous sodium sulphate it is placed in a round-bottomed flask and concentrated using a rotary evaporator to a volume of approximately 2 ml. This is then quantitatively transferred to a 10 ml test tube, and reduced to 200  $\mu$ l volume using a flow of clean nitrogen gas. The volume is then made up to 1-2 ml with acetone, and the acetone extract is filtered using a 0.5  $\mu$ m filter attached to a syringe (Millex LCR4, Millipore (UK) Ltd., Watford, Hertfordshire; pre-rinsed with acetone) and transferred to a crimp-top autosampler vial. The vials are refrigerated until required.

## 9.2 Sediment and biota

Extraction and clean up procedure as for GC-MS (see Section 5).

The flask containing the eluent collected from the alumina column is removed and the sample reduced to between 100  $\mu$ l and 1 ml. This is then transferred to a 5 ml volumetric flask and made up to the mark with acetone. An aliquot of the acetone extract is filtered through a 0.5  $\mu$ m filter attached to a syringe (pre-rinsed with acetone) and transferred to a crimp-top auto-sampler vial. The vials are stored at 5°C until required. The dry weight percentage of sediment is determined as in Section 5.3 above, and PAH concentration expressed on a dry weight basis.

## 9.3 Analysis by High Performance Liquid Chromatography

The HPLC analysis is carried out in two stages. Firstly an aliquot of the crude extract (50  $\mu$ l) is transferred to

a pre-column (ChromSpher PI SPE column; 20 mm x 3.0 mm ID) and rinsed with 50% methanol/water (v/v) for 2 minutes at a flowrate of approximately 0.5 ml  $\text{minute}^{-1}$ . PAH are strongly retained on this column due to  $\pi$ - $\pi$  interactions, whilst mono-aromatic and aliphatic components are not retained and so can be discarded. After a pre-set time the PAH are desorbed from the SPE column by backflushing with the analytical eluent (acetonitrile/water) and transferred directly onto the analytical column (ChromSpher 5 PAH; 250 mm x 4.6 mm ID) via a guard column. The PAH are separated by HPLC using gradient elution, and detected by a fluorescence detector with programmable excitation and emission values appropriate to the individual PAH eluting at the time. The system in routine operation is fully automated and controlled using Turbochrom Workstation software (Perkin Elmer Instruments, Beaconsfield, Buckinghamshire) which also handles data acquisition and reduction.

The HPLC mobile phase gradient is formed using mixtures of water and acetonitrile. The flow rate is held at 1.5 ml  $\text{min}^{-1}$  and the analytical column temperature held at 35°C throughout the analysis. A typical gradient programme starts with an eluent composed of water/ acetonitrile (50:50 v/v), which is progressively changed to pure acetonitrile. During this period the majority of PAH (components 1 to 17) will be eluted. The final component (18) is eluted whilst the eluent composition is returning to the initial composition.

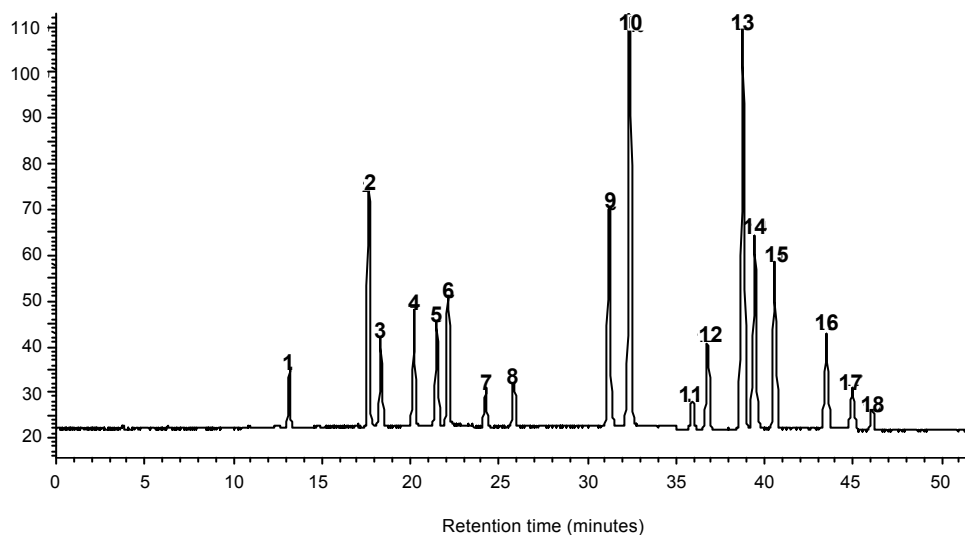
The fluorescence detector wavelength programme is changed during an analytical run so as to optimise detection of the eluting PAH. The wavelength pairs are listed in Table 3, with an indication of the PAH detected in each case. The identities of the PAH determined are given in Table 4 in order of their elution, and a standard chromatogram is shown in Figure 6.

**Table 3. Fluorescence detector wavelength programme**

Time (minutes)	Ex $\lambda$	Em $\lambda$	Components
0	280 nm	340 nm	1-3
19.5	257 nm	375 nm	4-6
23.5	275 nm	415 nm	7-8
30.5	265 nm	385 nm	9-10
35.0	280 nm	420 nm	11-17
45.2	305 nm	500 nm	18
48.3	280 nm	340 nm	Re-equilibration to initial composition

**Table 4. PAH determined using HPLC–UVF**

Component no.	Compound
1	Naphthalene
2	Acenaphthene
3	Fluorene
4	Phenanthrene
5	Anthracene-d <sub>10</sub> (internal standard)
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benz[ <i>a</i> ]anthracene
10	Chrysene
11	Benzo[ <i>e</i> ]pyrene
12	Benzo[ <i>b</i> ]fluoranthene
13	Benzo[ <i>k</i> ]fluoranthene
14	Benzo[ <i>a</i> ]pyrene-d <sub>12</sub> (internal standard)
15	Benzo[ <i>a</i> ]pyrene
16	Dibenz[ <i>a,h</i> ]anthracene
17	Benzo[ <i>ghi</i> ]perylene
18	Indeno[123- <i>cd</i> ]pyrene

**Figure 6. HPLC chromatogram of a 100 ng ml<sup>-1</sup> external standard. For peak identities see Table 4**

The quantification of PAH in water samples is carried out relative to the responses obtained during analysis of a solvent extracted spiked nanopure water sample, to which a mixed PAH standard has been added at a concentration of 100 ng ml<sup>-1</sup> per component. A response factor is then calculated from this for each of the individual PAH components relative to the surrogate standard (anthracene-d<sub>10</sub>) and blank sample values subtracted. The final result is expressed in ng l<sup>-1</sup>. A UK survey using this technique has been reported by Law *et al.* (1997(b)).

The quantification of PAH in sediment samples is by direct external standardisation following subtraction of blank values. The results are expressed in µg kg<sup>-1</sup> dry weight of sediment. A UK survey using this technique has been reported by Woodhead *et al.* (1999).

Limits of detection (3 x blank concentration using a batch mean dry/wet ratio for sediments) typically range from 15 µg kg<sup>-1</sup> for low molecular weight PAH, to 1 µg kg<sup>-1</sup> for some of the higher molecular weight PAH compounds in sediments, and from 1 to 50 ng l<sup>-1</sup> in seawater.

## 10. QUALITY CONTROL

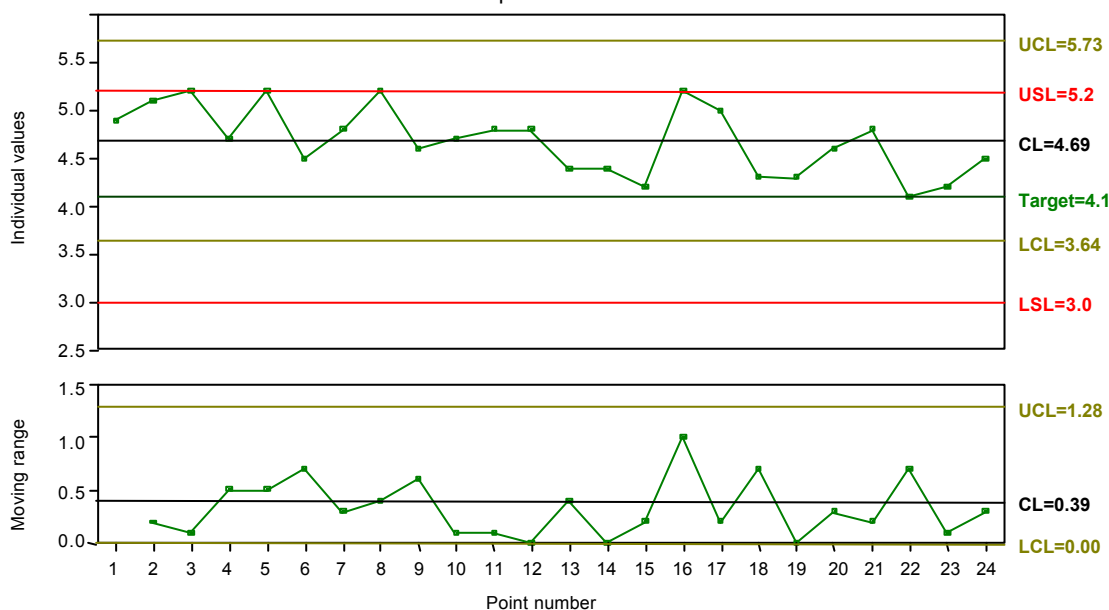
To maintain data quality it is very important to analyse either certified or laboratory reference materials routinely alongside samples. All samples are run under an analytical quality control (AQC) protocol, which involves analysing blanks and reference materials within each sample batch. There is currently a wide range of sediment reference materials available, including a series of harbour sediments produced and

certified by the National Research Council, Canada, within its marine analytical chemistry standards programme. As these samples cover a range of contamination levels from light to heavy, it is therefore possible to match the reference material to the samples being analysed. For general pollution studies in estuaries and coastal areas around England and Wales we have routinely used the marine sediment certified reference material (CRM) HS-6, and have built up a history of method performance using this material for both HPLC-UVF and GC-MS techniques. For biota however, only a single biological tissue reference material certified for PAH is currently available, and it is very expensive. For these reasons we have utilised spare stored homogenates from our own programmes to prepare a laboratory reference material (LRM) containing PAH at background concentrations. The results obtained from analyses of reference materials are used in the preparation of Shewart charts (example in Figure 7), which are used for monitoring analytical performance. Any batches that fail the AQC procedure are investigated so that the problems can be eliminated, and the analyses repeated.

We also participate in the QUASIMEME laboratory proficiency studies which runs two PAH rounds annually, and allows an external check on accuracy and comparison of performance with that of other laboratories engaged in marine monitoring studies (Law *et al.*, 2000).

## 11. DETECTION LIMITS

The limit of detection is calculated as an operational limit of quantification, which is set above the maximum expected real blank level. This limit of quantification is based upon replicate analysis of standard solutions, procedural blanks, spiked samples containing low levels of the analytes, and subsequent calculation of the associated variability. A detection limit of  $0.1 \mu\text{g kg}^{-1}$  wet weight in biota and  $0.1 \mu\text{g kg}^{-1}$  dry weight in sediment for each PAH compound or class is one which we have found to be readily achievable in all samples, and that also provides an adequate detection capability. If the individual limits of detection were determined for each analyte then for the most part these would yield somewhat lower values than those generally applied.



**Figure 7.** An example of a Shewart chart showing the variation in naphthalene concentration found in HS-6 when analysed using GC-MS



## 12. CHEMICALS REQUIRED AND THEIR SUPPLIERS

### 12.1 Solvents

Pentane	HPLC grade	Rathburn Chemicals Ltd Walkerburn, Scotland
Dichloromethane	"	"
Acetone	"	"
Methanol	HPLC grade	Romil Ltd Waterbeach Cambridge

### 12.2 Reagents

Potassium hydroxide	Analar	Merck Ltd Poole, Dorset
Sodium sulphate (anhydrous)	Analar	"
Alumina	(Merck no. 1077)	"

### 12.3 Standards

Naphthalene-d <sub>8</sub>		Aldrich Gillingham, Dorset
Anthracene-d <sub>10</sub>		"
Acenaphthylene-d <sub>8</sub>		Promochem Welwyn Garden City, Herts.
Phenanthrene-d <sub>10</sub>		"
Pyrene-d <sub>10</sub>		"
Benzo[a]pyrene-d <sub>12</sub>		"
Benz[a]anthracene-d <sub>12</sub>		"

PAH listed in Tables 2 and 5 are also supplied by Aldrich, Gillingham, Dorset.

### 12.4 Reference material

Marine sediment reference materials	National Research Council, Institute for Marine Biosciences, Marine Analytical Chemistry Standards Programme, 1411 Oxford Street, Halifax, Nova Scotia, Canada, B3H 3Z1
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*Disclaimer: The reference to proprietary products in this report should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.*

## 13. REFERENCES

- BURNS, W.A. AND BENICE, A.E., 1993. Fingerprinting polycyclic aromatic hydrocarbons (PAH) in subtidal sediments. Abstracts, 14th Annual Meeting, Society of Environmental Toxicology and Chemistry, November 14-18, Houston, TX, USA. p.16.
- EAGLE, R.A., NORTON, M.G., NUNNY, R.S. AND ROLFE, M.S., 1978. The field assessment of effects of dumping wastes at sea. 2: Methods. Fish. Res. Tech. Rep., MAFF Direct. Fish. Res., Lowestoft, 47: 1-24.
- HOWELLS, S.E., 1989. Laboratory intercalibration and intercomparison exercise for analysis of hydrocarbons in marine sediments: summary report. Report no. FSC/RC/1/89. Oil Pollution Research Unit, Field Studies Council, Wales.
- KELLY, C.A. AND LAW, R.J., 1998. Monitoring of PAH in fish and shellfish following the Sea Empress incident. pp. 467-473, *In*: (Edwards, R. and Sime, H. (Eds)). The Sea Empress Oil Spill: Proceedings of the International Conference held in Cardiff, 11-13 February 1998. Chartered Institute of Water and Environmental Management, London.
- LAW, R.J., 1980. Analytical methods and their problems in the analysis of oil in water. pp. 167-176, *In*: (Johnston, C.S. and Morris, R.J. (Eds.)) Oily Water Discharges - Regulatory, Technical and Scientific Considerations. Applied Science Publishers, London.
- LAW, R.J. AND KELLY, C.A., 1999. The Sea Empress oil spill: fisheries closure and criteria for removal of restrictions. pp. 975-979, *In*: Proceedings of the 1999 International Oil Spill Conference, Seattle, USA, 8 - 11 March 1999. Beyond 2000 - Balancing Perspectives. American Petroleum Institute, Washington DC. Publication no. 4686A/B. ISSN 75-4161.
- LAW, R. J. AND KLUNGSØYR, J., 1996. The 1994 QUASIMEME laboratory-performance studies: Polycyclic aromatic hydrocarbons (PAH) in standard solutions. Mar. Pollut. Bull., 32: 667-673.
- LAW, R. J. AND PORTMANN, J. E., 1982. Report on the first ICES intercomparison exercise on petroleum hydrocarbon analyses in marine samples. ICES Co-op. Res. Rep., 117: 55pp.
- LAW, R.J., KLUNGSØYR, J. AND FRERIKS, I.L., 1997(a). The QUASIMEME laboratory performance study of polycyclic aromatic hydrocarbons (PAH): assessment of the first three rounds, 1994-1995. Mar. Pollut. Bull., 35: 64-77.

- LAW, R.J., DAWES, V.J., WOODHEAD, R.J. AND MATTHIESSEN, P., 1997(b). Polycyclic aromatic hydrocarbons (PAH) in seawater around England and Wales. *Mar. Pollut. Bull.*, **34**: 306-322.
- LAW, R.J., FILEMAN, T.W. AND PORTMANN, J.E., 1988. Methods of analysis for hydrocarbons in marine samples. *Aquat. Environ. Prot.: Anal. Meth.*, MAFF Direct. Fish. Res., Lowestoft, **2**: 25pp.
- LAW, R.J., THAIN, J.E., KIRBY, M.F., ALLEN, Y.T., LYONS, B.P., KELLY, C.A., HAWORTH, S., DRYNDA, E.A., DRYNDA, P.E.J., HARVEY, J.S., PAGE, S., NICHOLSON, M.D. AND LEONARD, D.R.P., 1998. The impact of the *Sea Empress* oil spill on fish and shellfish. pp. 109-136, *In*: (Edwards, R. and Sime, H. (Eds)) *The Sea Empress Oil Spill: Proceedings of the International Conference held in Cardiff, 11-13 February 1998*. Chartered Institute of Water and Environmental Management, London.
- LAW, R.J., KELLY, C.A. AND NICHOLSON, M.D., 2000. The QUASIMEME laboratory performance study of polycyclic aromatic hydrocarbons (PAH): assessment for the period 1996-1999. *J. Environ. Monit.*, **2**: 517-523.
- MARINE POLLUTION MONITORING MANAGEMENT GROUP, 1994. UK National Monitoring Plan. Unpublished manuscript, Her Majesty's Inspectorate of Pollution, London. 39 pp.
- MARINE POLLUTION MONITORING MANAGEMENT GROUP, 1999. UK National Marine Monitoring programme. The Green Book. Version 2, 12th March 1999. Unpublished manuscript.
- OSLO AND PARIS COMMISSIONS, 1997. JAMP guidelines for monitoring contaminants in biota. Oslo and Paris Commissions, London. 40 pp.
- PAGE, D.S., BOEHM, P.D., DOUGLAS, G.S., BENICE, A.E., BURNS, W.A. AND MANKIEWICZ, P.J., 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska. *Environ. Toxicol. Chem.*, **15**: 1266-1281.
- PAGE, D.S., BOEHM, P.D., DOUGLAS, G.S., BENICE, A.E., BURNS, W.A. AND MANKIEWICZ, P.J., 1997. An estimate of the annual input of natural petroleum hydrocarbons to seafloor sediments in Prince William Sound, Alaska. *Mar. Pollut. Bull.*, **34**: 744-749.
- ROWLAND, S.J. AND ROBSON, J.N., 1990. The widespread occurrence of highly branched acyclic C<sub>20</sub>, C<sub>25</sub>, and C<sub>30</sub> hydrocarbons in recent sediments and biota – a review. *Mar. Environ. Res.*, **30**: 191-216.
- SMEDES, F., 1994. Sampling and partition of neutral organic contaminants in surface waters with regard to legislation, environmental quality and flux estimations. *Int. J. Environ. Anal. Chem.*, **57**: 215-229.
- SPORSTOL, S., GIOS, N., LICHTENTHALER, R.G., GUSTAVSON, K.O., URDALL, K. AND ORELD, F., 1983. Source identification of aromatic hydrocarbons in sediments using GC/MS. *Environ. Sci. Technol.*, **17**: 282-286.
- WOODHEAD, R.J., LAW, R.J., AND MATTHIESSEN, P., 1999. Polycyclic aromatic hydrocarbons (PAH) in surface sediments around England and Wales, and their possible biological significance. *Mar. Pollut. Bull.*, **38**: 773-790.