ENVIRONMENT AGENCY

A guide to practices, procedures and methodologies following oil spill contamination incidents (2004)

Methods for the Examination of Waters and Associated Materials

A guide to practices, procedures and methodologies following oil spill contamination incidents (2004)

Methods for the Examination of Waters and Associated Materials

This booklet contains guidance and information on the practices, procedures and methodologies to be considered in the event of oil contamination incidents.

Chromatographic methods are very sensitive to small physical and chemical variations in the quality of materials and apparatus used. Whilst this booklet reports details of the materials actually used in some of the methods, this does not constitute an endorsement of these products. Equivalent products are available and it should be understood that the performance characteristics of the methods might differ when other materials are used. It is left to users to evaluate these methods in their own laboratories. Limited performance data are provided.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soil (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results encompassing at least ten degrees of freedom from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available. An indication of the status of the methods is shown at the front of this publication on whether the methods have undergone full performance testing.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical and physical methods
4 Metals and metalloids
5 General non-metallic substances
6 Organic impurities
7 Biological methods
8 Biodegradability and inhibition methods
9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back.

Publication of new or revised methods will be notified to the technical press. An index of methods and details on how to obtain documents published within this series are available on the Agency's internet web-page (www.environment-agency.gov.uk/nls) or from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

March 2003

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

Glossary

Alkylated PAH	PAH compound with alkyl substitution.
Angiosperm	Flowering plant.
Anthracene	PAH compound with 3 fused rings, $C_{14}H_{10}$, MW 178.
Asphalt	Fraction of crude oil boiling above 400 ^{o}C, comprising high molecular weight compounds usually containing 70 or more carbon atoms.
Asphaltenes	Compounds of high molecular weight (up to 20,000 Daltons). These compounds comprise PAHs joined together by alkylated substituent chains.
n-alkane	A straight chain (un-substituted) hydrocarbon with the molecular formula C_nH_{2n+2}; for example n-nonane, C_9H_{20}.
Benz[*a*]anthracene	PAH compound with 4 fused rings, $C_{18}H_{12}$, MW 228.
Benzene	A mono-aromatic compound with 1 fused ring C_6H_6, MW 78.
Benzo[*b*]fluoranthene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Benzo[*j*]fluoranthene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Benzo[*k*]fluoranthene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Benzo[*g,h,i*]perylene	PAH compound with 6 fused rings, $C_{22}H_{12}$, MW 276.
Benzo[*a*]pyrene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Benzo[*e*]pyrene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Bile	A bitter greenish-brown alkaline fluid which is secreted by the liver and aids digestion.
Biomarkers	Biochemical, physiological or histological indicators of exposure to, or effects of, chemicals, at either the whole organism or sub-organism level.
C_1-PAH	Methyl substituted PAH.
C_2-PAH	Dimethyl or ethyl substituted PAH.
C_3-PAH	Trimethyl, methylethyl or propyl substituted PAH.
C_4-PAH	Tetramethyl, methylpropyl, diethyl, ethyldimethyl or butyl substituted PAH.
Carbon isotope ratio	The relative amount of ^{13}C versus ^{12}C stable isotopes in organic matter, generally used to show relationships between oils or oils and source rocks.
Cata-condensed	Describes the structure of a PAH where the rings are fused in a particular way. An example is chrysene (see below and Figure 2).
Clastic rocks	Sedimentary rocks including shale, sandstone and mudstone.
Corer	A device used for taking a sample of bottom sediment, normally to a depth of 250 mm or more.
Chrysene	PAH compound with 4 fused rings, $C_{18}H_{12}$, MW 228.
Cytosolic enzymes	Enzymes contained within the soluble part of a cell's cytoplasm, i.e. that part that does not sediment during ultra-centrifugation.
Dalton	Unit of molecular mass.
Deltaic	Material deriving from a delta.
Demersal fish	Fish that live on the seabed, such as cod, haddock and halibut.
Dibenz[*a,h*]anthracene	PAH compound with 5 fused rings, $C_{22}H_{14}$, MW 278.
Dibenzothiophene	PAH compound with 3 fused rings, one of which contains a sulphur atom, $C_{12}H_8$ S, MW 184.
Diesel fuel	A distillate product of crude oil boiling between about 260 - 360 °C, known in the UK as "Derv" (fuel for diesel engine road vehicles).

Dispersant	Chemical oil dispersants are used in the treatment of spilled oil at sea and on beaches. Dispersants contain surface-active compounds which reduce interfacial surface tension between the oil and water and assist the formation of small oil droplets and their dispersion into the water column.
DNA	Deoxyribonucleic acid.
EI	Electron impact, an MS ionisation mode in which compounds eluting from a GC into an MS ion source are bombarded with energetic electrons, typically with an energy of 70 eV.
Electrophilic centres	A compound, functional group or other region of a compound that can attract an electron pair.
Endogenous compounds	Compounds arising from within an organism or cell.
EROD	Ethoxyresorufin-O-deethylase.
eV	Electron volt, a unit of energy.
Exogenous xenobiotics	Compounds with some biological activity arising from outside an organism or cell; often a compound that an organism must eliminate or neutralize by some detoxification strategy.
Fingerprint	A chromatographic feature used to characterise oil.
FID	Flame-ionisation detection.
Fluoranthene	PAH compound with 4 fused rings, $C_{16}H_{10}$, MW 202.
Fluorene	PAH compound with 3 fused rings, $C_{13}H_{10}$, MW 166.
Gas oil	A distillate product of crude oil which is similar to diesel fuel, but is not taxed and is dyed (red) and contains other chemical markers at low concentration.
Gasoline	A distillate product of crude oil boiling between about 40 - 180 °C, comprising low molecular weight compounds usually containing 12 carbon atoms or less.
Grab	Device used for taking samples of surface sediments.
GC	Gas chromatography or gas chromatograph.
Geochemical marker	An organic compound composed of carbon, hydrogen and possibly other elements which is found in crude oil and distillate products and shows little or no change in structure from its parent organic molecule in living organisms.
Glutathione	A tri-peptide.
GST	Glutathione-*S*-transferase.
GST-induction	An increase in the synthesis or activity of glutathione-*S*-transferase caused by the presence of a particular compound, i.e. the inducer.
Hopanes	Pentacyclic hydrocarbon compounds of the triterpane group believed to be derived primarily from bacterial membranes.
HPLC	High performance liquid chromatography.
Indeno[1,2,3-*cd*]pyrene	A PAH compound with 6 fused rings, $C_{22}H_{12}$, MW 276.
Inducible enzyme	An enzyme whose rate of synthesis can be increased by the presence of a particular compound, i.e. the inducer.
Iso-enzymes	One of two or more structural variants of an enzyme that can occur in the same individual species; they are often relatively specific for a particular tissue, each with unique kinetic characteristics.
Isomers	Compounds with the same molecular formulae, but different arrangement of atoms (for example, anthracene and phenanthrene, 1- and 2-methylnaphthalene).
Isoprene	Isopentadiene, $H_2C{=}CHC(CH_3){=}CH_2$, is the basic structural unit found in many geochemical marker compounds, including steranes and triterpanes.

Jet fuel	Aviation kerosene and "wide-cut" fuels. The latter are used exclusively in military aircraft. The boiling range of aviation kerosene is about 150 - 300 °C, whilst that of "wide-cut" fuels is about 50 - 270 °C.
Kerosene	A distillate product of crude oil boiling between about 190 - 260 °C, comprising hydrocarbon compounds ranging from C_8 to C_{14}. Originally known in the UK as paraffin.
Lacustrine	Material deriving from a lake or deposited in a lake.
Littoral zone	The section of the shore which is regularly covered and uncovered during each tidal cycle.
LOD	Limit of detection.
MS	Mass spectrometry, mass spectrometric.
MW	Molecular weight.
Naphthalene	PAH compound with 2 fused rings, $C_{10}H_8$, MW 128.
Odd-even predominance	The ratio of odd- to even-numbered n-alkanes in a given range. In oils this ratio is usually close to 1.
Oleanane	A geochemical marker compound derived from angiosperms.
PAH	Polycyclic aromatic hydrocarbon. PAHs constitute an extraordinarily large and diverse class of organic molecules. Over 350 parent PAH compounds with 4 - 7 fused rings have been described.
Parent PAH	An un-substituted PAH compound.
Pelagic fish	Fish that live in the water column, such as herring and mackerel.
Peri-condensed	Describes the structure of a PAH where the rings are fused in a particular way. An example is pyrene (see below and Figure 2)
Perylene	PAH compound with 5 fused rings, $C_{20}H_{12}$, MW 252.
Phytane	An isoprenoid alkane, 2,6,10,14-tetramethylhexadecane, $C_{20}H_{42}$.
Phytoplankton	Marine plants which form the base of the food chain.
Pristane	An isoprenoid alkane, 2,6,10,14-tetramethylpentadecane, $C_{19}H_{40}$.
Pyrene	PAH compound with 4 fused rings, $C_{16}H_{10}$, MW 202.
RSD	Relative standard deviation.
Steranes	A class of tetracyclic, saturated geochemical marker compounds based on 6 isoprene sub-units which contain about 30 carbon atoms.
Sub-littoral zone	The lowest level of the shore, which is usually uncovered only at the lowest level of the spring tides.
Terpanes	A broad class of complex branched, cyclic geochemical marker compounds including hopanes and tricyclic compounds commonly monitored via mass chromatograms at 191 Daltons.
Triterpanes	A class of tetracyclic, saturated geochemical marker compounds based on 6 isoprene sub-units which contain about 30 carbon atoms.
UCM	Unresolved complex mixture.
UK	United Kingdom.
US	United States of America.
UVF	Ultra-violet fluorescence.

A guide to practices, procedures and methodologies following oil spill contamination incidents

1 Introduction

Within UK waters in the 1990s there were two major oil spills[1-4]. These were among the twenty largest oil spills that have occurred in the world to date. In both cases, extensive programmes of research were undertaken following the oil spills. These programmes were intended to provide information to assist with the management of precautionary fishery closures and to aid with the overall impact assessments carried out on the surrounding areas and environments.

The Maritime and Coastguard Agency which has a national remit, is the organisation responsible for responding to marine oil and chemical spills, and for activating the UK's national contingency plan. Other organisations are involved, however, and have regional responsibilities. The Department for Environment Food and Rural Affairs regulates the use of dispersants in England and Wales, and the Scottish Executive Environment and Rural Affairs Department has similar responsibilities in Scotland. Nature conservation responsibility falls to English Nature in England, and the Countryside Council for Wales, the Scottish Natural Heritage, and the Environment and Heritage Service in Northern Ireland have similar responsibilities respectively. The responsibilities and functions of the Environment Agency (in England and Wales) are similar to those of the Scottish Environment Protection Agency. Thus, because of the number of different organisations and responsibilities potentially involved in oil spill management, it is difficult without continual liaison between these organisations for information and details of all of the lessons learned following one oil spill incident to be readily available following a subsequent incident.

As a result of the numerous UK national, regional and local agencies involved in responding to a major marine oil spill, there is often a period of confusion immediately following the spillage where conflicting roles and responsibilities are sorted and assigned. There is thus a need for continuous appraisal and training between oil spill incidents to minimise this confusion and help reduce the frustrations experienced during this initial period following a spill. One initiative being developed by the Maritime and Coastguard Agency is the formation of Standing Environment Groups around the UK coastline. These groups have a defined role within the national contingency plan[5] and provide advice to salvage control units and the marine and shoreline response centres.

One way in which the response can be improved (in terms of the scientific assessment of oil spill impacts) is to document, in advance of the oil spill, the technical methods, practices and procedures that may need to be used. This process would help reduce inappropriate sampling, analytical and data recording practices, and hence avoid loss of valuable time following an incident and the generation of data that may be flawed.

The documentation of technical methods, practices and procedures to be used following oil spill situations is of potential benefit to all concerned, in that

(i) staff can respond quickly and confidently;
(ii) managers can assess more speedily the requirements of impact studies; and
(iii) researchers can compare and correlate their own observations with parallel measurements being undertaken.

Whilst the harmonisation of methods should ensure the comparability of data between laboratories analysing similar samples, this would not be possible without undertaking extensive inter-calibration exercises or method comparability studies. Whilst these studies are time-consuming and expensive, they should not be discouraged. Similar techniques for biological and geo-morphological methodologies should also be developed, as many of the above-mentioned factors directly impact

on the conduct of work for beach reconnaissance surveys, clean-up feasibility studies and ecological impact assessments. The aim of this document is seen as a key part of the documentation for providing advice and guidance on the chemical aspects of sampling and analysis of water, sediments and biota in support of the technical response following an oil spill.

The use of methods, practices and procedures described in this booklet will contribute to a more coherent scientific and technical response following an oil spill. Staff of the main laboratories undertaking the analysis can be prepared and field and laboratory equipment made ready. In common with most major "clean-up" organisations, it may be appropriate for those concerned to be prepared for a much larger spillage and nothing should be overlooked. For example, in such complex operations, attention should be paid to details of equipment, the amount of consumable products to be used, the requirement for chain-of-custody statements, and the finalisation of reports. In addition, temporary staff may need to be recruited and/or work sub-contracted to other organisations. Full documentation of the methods, practices and procedures is therefore essential to provide guidance and continuity to such new recruits.

Details of the health and safety implications following an oil spill are not covered in this booklet. Guidance on, for example the safety of staff at sea, on sampling boats, ships and other vessels, gaining access to affected beach areas and understanding tidal movements and variations should be considered. In addition, the safety of the working site, and hazards posed by chemicals used on-site and in the laboratory should be considered and addressed. Advice may also need to be obtained when dealing with potentially large numbers of volunteer staff, especially members of the public, following an oil spill.

The aim of this booklet is to provide guidance and information on useful approaches and techniques for those involved in responding to oil spill incidents and subsequent environmental impact assessments. The approaches and techniques that have been used and shown to be successful are described and illustrative examples provided.

2 Crude oil and its constituents

Over considerable periods of time, crude oils were produced when plants and animals underwent decay processes and were deposited under layers of sand and mud etc. Crude oils vary in appearance, as their characteristics depend upon where the crude oil originates. Sometimes crude oil is almost colourless, or it can be black and viscous. Usually, however, crude oil has the appearance of thin, brown treacle. This variation in appearance, consistency and colour is a consequence of the different chemicals present in the crude oils. All crude oils (and other fossil fuels) contain similar molecular species amounting to thousands of compounds ranging from low molecular weight gases to high molecular weight residues that boil above 350 ^{o}C. In addition, crude oils vary markedly in composition, both within and between regions, even during the lifetime of a single regional source. Notwithstanding this, most of the compounds (usually greater than 75 %) are hydrocarbon compounds (i.e. contain hydrogen and carbon only). Other compounds present comprise organosulphur and organonitrogen compounds, acids, phenols, and highly complex asphaltenes containing ten or more fused aromatic rings (some of which are heterocyclic) with aliphatic and naphthenic side-chains. The presence and composition of these compounds in different crude oils markedly affect their properties. For example, Brent and Forties crude oils are regarded as light oils. Whilst Gullfaks crude oil is also a light oil, it originates from a reservoir in which the crude oil is highly biodegraded, i.e. contains small amounts of n-alkanes but large amounts of aromatic compounds. Beatrice crude oil is a heavy, waxy crude oil. Despite these differences, however, all these crude oils originate under the North Sea.

Hydrocarbon compounds have generally been divided into two broad classes of compounds, namely aliphatic and aromatic hydrocarbon compounds. The term aliphatic (see Figure 1) relates to open-chain saturated or unsaturated compounds and includes alicyclic hydrocarbon compounds (i.e. aliphatic cyclic hydrocarbon compounds) that contain at least one saturated fused ring. The term aromatic relates to hydrocarbon compounds that contain at least one unsaturated fused ring (benzene nucleus) see Figure 2. Aliphatic side-chains can be bonded to a fused ring (benzene nucleus) and such hydrocarbon compounds are collectively known as arenes.

The diverse nature of hydrocarbon compounds in crude oils has given rise to a complex system of nomenclature. This system contains a number of synonyms and subgroups for each component group (see Table 1). Several of the component groups comprise a series of compounds known as a homologous series, where for example each compound differs from its neighbour by a constant amount. For the homologous series of n-alkanes, C_nH_{2n+2}, the constant difference is CH_2. As the series progresses, the number of carbon atoms increases and can exceed 40 or more. As the number of carbon atoms increases, so does the number of isomers for each homologue. See Table 14 in Section 9.3.1. Also, the series may show little or no preference for compounds containing an odd or even number of carbon atoms. See Figure 3A. For the n-alkane homologous series in a non-biodegraded crude oil, each homologue is present at a greater individual concentration than its corresponding branched-isomer, although a decrease in the individual n-alkane concentration is generally observed with increasing carbon number. This effect can easily be seen in a typical chromatographic pattern (see Figure 3A) obtained when a non-biodegraded crude oil is analysed by gas chromatography with flame ionisation detection. Gas chromatographic resolution of the individual branched-isomers contained in a crude oil is generally not undertaken. In addition, such crude oils contain a large number of naphthenic hydrocarbon compounds (see Table 1). This unresolved complex mixture (UCM) of compounds appears as a very broad peak or "hump" in the GC-FID chromatogram, see Figure 3B.

Of the polycyclic aliphatic hydrocarbon compounds, pentacyclic triterpane and sterane compounds have proved useful in the identification of crude oils (see Section 9.3). Their determination is based on the fact that cyclic hydrocarbon compounds are more resistant to biodegradation than open-chain compounds.

The presence of polycyclic aromatic hydrocarbon (PAH) compounds are particularly important with respect to environmental assessments of oil spill incidents. As well as being toxic, PAH compounds also have the potential to taint food. The PAH composition of crude oils varies, but generally, lower molecular weight compounds, for example the two-ring naphthalene compounds (and associated alkylated isomers) dominate the profile, comprising approximately 70 % of the total PAH concentrations (see Figure 4). However, the PAH concentration in crude oil comprises only about 2 % of the total hydrocarbon concentration.

The lower boiling, more water-soluble compounds, which have an octanol-water partition coefficient of approximately 3 - 4, are considered (with others) to be responsible for the petrogenic taint in fish and shellfish following their exposure to crude oils and their distillate products. Naphthalene is slightly water-soluble and as such can be absorbed by finfish through their gills. This causes the fish to become tainted after only about 30 minutes exposure to crude oil. In addition, there tends to be selective absorption of the naphthalene compounds by finfish resulting in these particular PAH compounds constituting over 90 % of the total PAH concentration in the muscle tissue of exposed fish. The three-ring PAH compounds, especially the phenanthrene/anthracene and associated alkylated PAH compounds, are the second most abundant group of compounds present in crude oils (see Figure 4). The determination of both phenanthrene and anthracene concentrations is important as the phenanthrene/anthracene concentration ratio can help identify the possible source of the PAH compounds. In addition, the

dibenzothiophene/phenanthrene concentration ratio, which is greater than 1 in crude oils derived from high sulphur carbonates, can similarly be used.

The peri-condensed four-ring PAH compounds (for example pyrene, see Figure 2) are usually present in smaller quantities than the three-ring PAH compounds, and the alkylated substituted compounds dominate the profile. The cata-condensed four-ring PAH compounds (for example chrysene, see Figure 2) and, more importantly, the five- and six-ring PAH compounds, are regarded as being chronically toxic. Toxicity is usually attributable to the interactions of hydroxylated PAH metabolites with DNA. The most toxic of the parent PAH compounds include benzo[*a*]pyrene and dibenz[*a,h*]anthracene. However, these large PAH compounds represent relatively small proportions of the total PAH compounds present in crude oils. In view of their lower water solubilities and higher octanol-water partition coefficients, these and similar compounds tend to associate with particulate material and accumulate in lipid-rich tissues (for example scallop gonad and brown crab meat etc). In addition, the relative proportion of these compounds in such tissues tends to increase, such that in some exposed molluscs the proportion of these compounds can exceed 25 % of the total PAH concentration.

Crude oils form the basis of a large range of products produced by processes including refining and fractional distillation. These distillate products include, in order of increasing boiling range and number of carbon atoms per molecule, petrol (or gasoline in the US), paraffin (or kerosene in the US), diesel (gas oil), lubricating oils, fuel oils and asphalt. The composition of these distillate products is sufficiently different to facilitate interpretation of data. As the lower boiling compounds volatilise more quickly, evaporation should be taken into account when results are being interpreted.

The discharge of oil into the environment poses many challenges for analysts. These include the selection of the most appropriate compounds and group of compounds requiring analysis to provide protection to the public and relevant data to enable environmental assessments to be made of the impact of the spill, both for short- and long-term periods. Oil contains such a large range of compounds with different melting points, boiling points, vapour pressures and aqueous solubilities that the complexity of the analysis poses real problems that need to be addressed.

Table 1 Summary of hydrocarbon groups

Hydrocarbons	Synonyms	General formula/ abbreviation	Descriptions	Examples
Aliphatic				
Paraffinic	Paraffins Alkanes	C_nH_{2n+2}	Contains saturated open-chain molecules that may or may not contain branched side chains	pentadecane ($C_{15}H_{32}$) phytane ($C_{20}H_{42}$) squalane ($C_{30}H_{62}$)
Naphthenic	Naphthenes Cycloalkanes Cycloparaffins		Saturated but contains at least one ring	stigmastane ($C_{29}H_{52}$) hopane ($C_{30}H_{52}$)
Olefinic	Olefins Alkenes	C_nH_{2n}[a]	Contains at least one non-aromatic double bond	squalene ($C_{30}H_{50}$) 2,4-ethylcholesta-3,5-diene ($C_{29}H_{48}$) diploptene ($C_{30}H_{50}$)
Acetylenic	Acetylenes Alkynes	C_nH_{2n-2}[a]	Contains at least one triple bond	ethyne (C_2H_2) 1-decyne ($C_{10}H_{18}$)
Aromatic				
Monocyclic	Alkylbenzenes Alkenylbenzenes Alkynylbenzenes Arenes[b]	MAH	Contains one benzene ring	benzene (C_6H_6) toluene (C_7H_8) xylene (C_8H_{10})
Polycyclic	Polynuclear Polyaromatic	PAH	Contains a minimum of two benzene fused rings	naphthalene ($C_{10}H_8$, two fused rings) phenanthrene ($C_{14}H_{10}$, three fused rings) pyrene ($C_{16}H_{10}$, four fused rings) benz[*a*]anthracene ($C_{18}H_{12}$, four fused rings) benzo[*a*]pyrene ($C_{20}H_{12}$, five fused rings) 3-methylcholanthrene ($C_{21}H_{16}$)

[a] Where applicable, the general formula relates to an open-chain molecule containing only one double or triple bond respectively.
[b] The term arene generally relates to hydrocarbon compounds that contain both aliphatic and aromatic units.

Figure 1 Structure of aliphatic hydrocarbon compounds

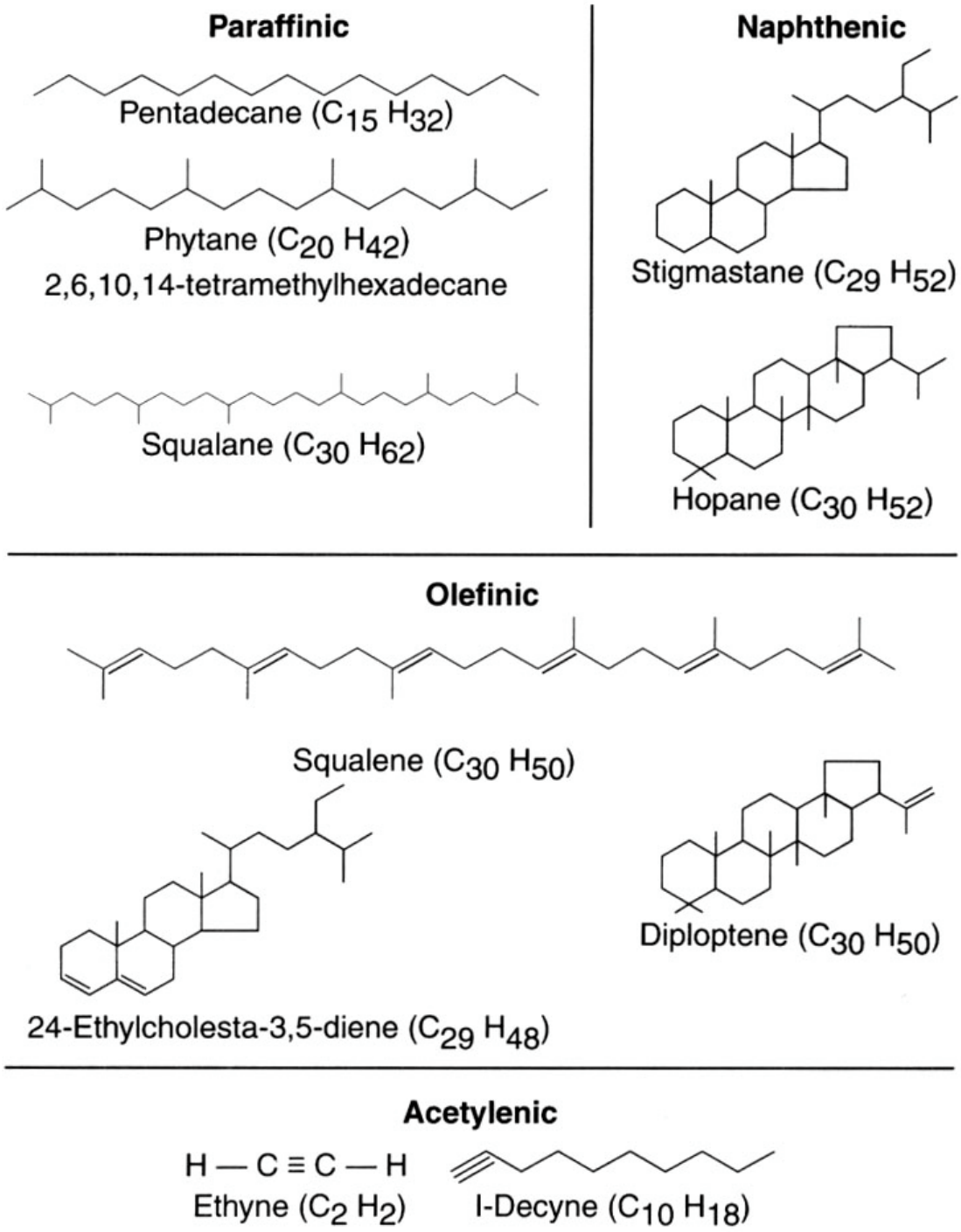
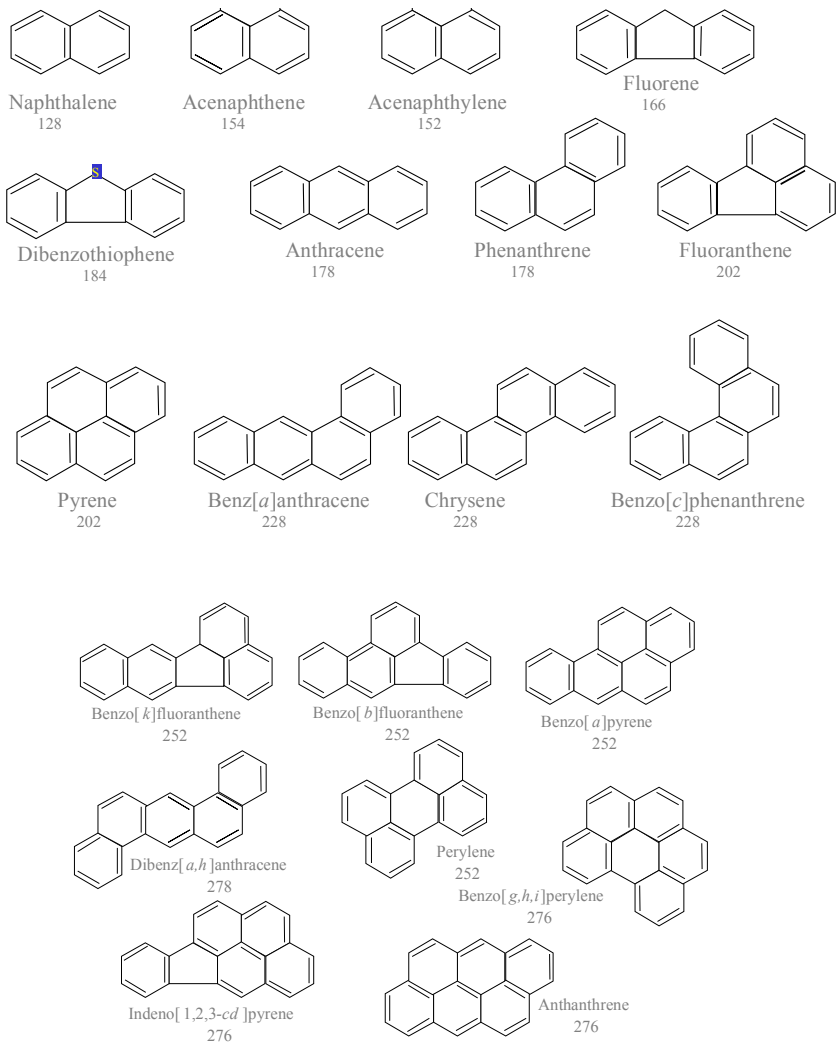
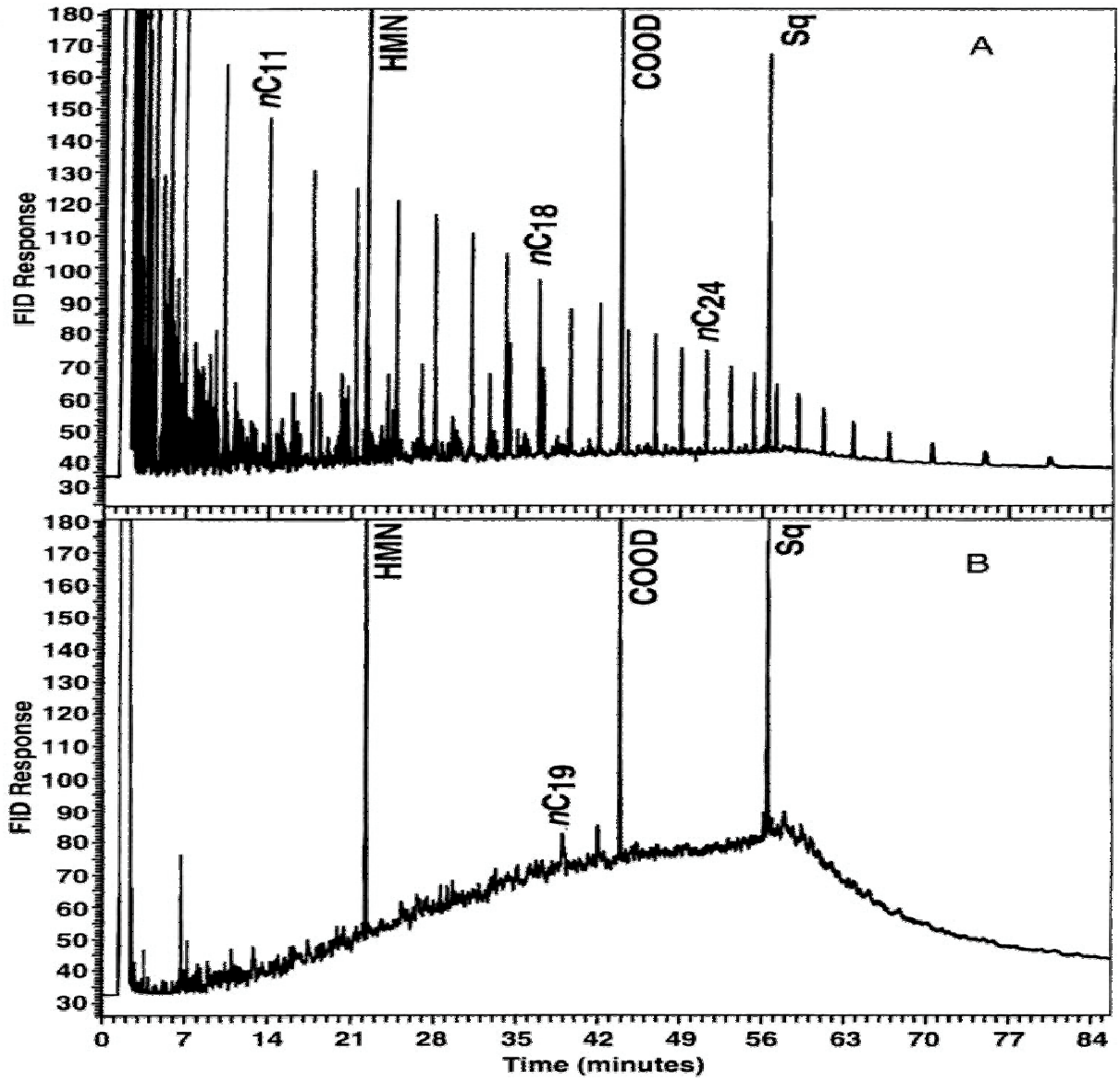


Figure 2 Structure of PAH compounds with associated molecular masses



Details of additional structures are given elsewhere[6].

Figure 3 GC-FID profiles

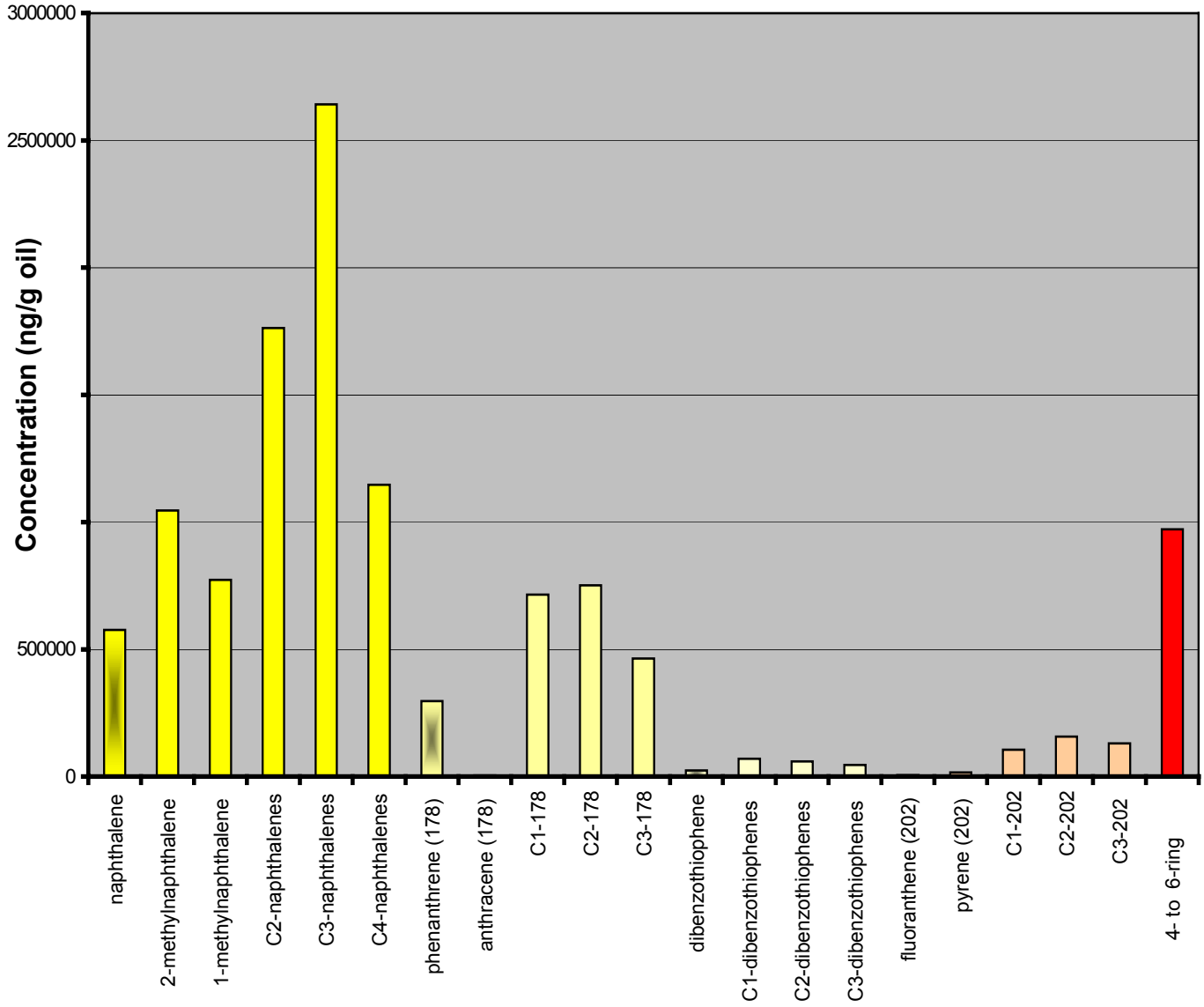


Aliphatic hydrocarbon compound profiles of two North Sea crude oils:

A Forties Field crude oil - This profile contains a typical n-alkane pattern for a non-biodegraded oil.
B Captain Field crude oil - In contrast, for this biodegraded oil, there is no definitive n-alkane profile. There is, however, an unresolved complex mixture (UCM).

Added internal standards are heptamethylnonane (HMN) chlorooctadecane (COOD) and squalane (Sq). The identification of specific n-alkanes is shown using n-C_{18} (octadecane) and n-C_{19} (nonadecane).

Figure 4 Typical PAH composition of Beatrice crude oil



3 Processes operating on spilled oil

When oil is spilt at sea, areas impacted include the sea surface and associated surface waters. Spilled crude oil is, however, relatively transient on the sea surface, as a number of processes occur to change the composition of the crude oil and/or even remove it from the original location[7]. Much of the lighter fraction (i.e. volatile components of the crude oil) is lost to the atmosphere by evaporation; in addition, some of the mono-aromatic hydrocarbon compounds for example the BTEX compounds (benzene, toluene, ethylbenzene and xylenes) are dissolved in the seawater. A portion of the bulk oil is naturally dispersed, as small droplets, into the water column due to turbulent mixing processes caused by wave action, and remaining oil may form a water-in-oil emulsion[8]. The dispersed and emulsified oil may adhere to suspended particulate material present in the upper water column, which eventually settles to the seabed as bottom sediments. The oil attached to suspended particulate material may build up in certain areas, some distance from the original source[3]. Where chemical dispersants are used, a large proportion of the oil may be dispersed into the water column. Chemically dispersed oil often forms very small droplets, which do not adhere to suspended particulate material and may undergo (due to their very large surface area) rapid degradation by marine bacteria.

Compared with oil in the majority of sediments, surface and water column oils persist for relatively short periods of time. Oil adhered to inter-tidal sediments may be stranded as surface slicks, where penetration into coarse sediments or burial by coarse-medium grained sediments then follows. Alternatively, oil may be incorporated (by deposition) into areas of fine sediment accretion. The importance of sediments in determining the fate and effects of petroleum spills lies in their ability to act both as a hydrocarbon "sink" and then as a long-term pollution source to biological communities.

Oil will adhere to sediments for considerable periods of time, which vary depending on a number of factors. These factors include oxidation-reduction conditions, the rate of microbial degradation, wave action and resultant washing processes. Also included are the rate at which infiltration of oil in the sediment occurs and further sediment accretion allowing the oil to be buried below the depth where mechanical disturbance and erosion takes place[9, 10].

Quantitative data on the composition of crude oils and hydrocarbon concentrations in water, sediment and biota, together with consequential changes caused by clean-up activities and natural processes etc, can facilitate the assessment, and possible prediction, of ecological damage after oil spills[11, 12]. The partitioning of hydrocarbon compounds in sediments is dependent on the particle size distribution of the sediment, and also its organic carbon content. In general terms, fine sediments will contain higher concentrations of hydrocarbon or PAH compounds than coarse sediments. This is partly due to the much greater surface area available for adsorption. The interpretation of results is therefore very difficult unless this consideration is taken into account. In some instances, the concentration of hydrocarbon or PAH compounds in sediments has been normalised on the basis of the percentage sediment present with a particle size diameter of less than 63 μm or the percentage organic carbon in the sediment. However, problems in the interpretation can still arise following normalisation as, for example, organic carbon in sediments is present as a variety of speciated forms, which can have different affinities for the hydrocarbon compounds determined[13]. In addition, if the quality of data is to be regarded as reliable, the degree of accuracy with which the normalising factor is determined needs to be better than, or equal to, that of the hydrocarbon concentration determined. This situation is, however, seldom the case. Similar caveats apply to other common normalising factors, for example determination of lipid concentrations in biota. Different tissues can contain different lipid materials (for example wax esters, triglycerides, phospholipids etc) which possess different accumulation behaviours towards hydrocarbon compounds. Similarly, different extraction techniques will extract different lipid material with

different efficiencies[14]. Whilst normalisation techniques offer a simple concept, they are much more difficult to apply in practice.

4 Factors to be considered when assessing the impact of oil spills

Much of the world's crude oil originates in countries such as those of the Middle East, former Soviet Union, South America and South East Asia, generally remote from the locations in which it is refined and used. The resulting large-scale and world-wide transport of crude oil, and the smaller-scale and usually more localised transport of refined distillate products, yields the potential for accidents and consequent oil spillages along shipping routes and close to ports and harbours. The production process itself can also lead to catastrophic releases of oil, as in the case of blow-outs[15]. Although tankers are sometimes lost in the open sea, most shipping incidents occur near in-shore areas in restricted waters where the potential for damage to fishery resources is greatest[16]. The majority of the world's fishing activity occurs near the highly productive waters overlying the continental shelves, relatively close to land. Thus, in the event of a large oil spill, contamination of fish and shellfish stocks is likely. The oil is most likely to affect fish and shellfish that cannot escape from the oil. This can be particularly serious in areas with extensive in-shore fisheries or aquaculture activities, as stocks can be killed, or rendered unmarketable due to the tainting of their flesh[17]. In some incidents, cultivated farmed fish and shellfish stocks have been heavily contaminated[18-24]. Damage to sea-food on a scale similar to that experienced after a major oil spill from a tanker[22] has also been recorded after smaller oil spills[23] as a result of their impact on extensive mariculture activities. Contingency planning should, therefore, include informing (without delay) those organisations undertaking mariculture activities, so that in the event of oil spills that threaten facilities, remedial action (such as closing seawater intakes) can be carried out, if possible.

Certain fishing activities only take place over a few days or weeks[25] and the rearing of larvae in on-shore tanks may be seasonal. These activities can be seriously affected by contaminated water. The time taken for the tainting of tissues from spilled oil to disappear once pre-spillage conditions have been restored is usually a matter of a few weeks. This period, however, can be highly variable depending on the fish and shellfish species and the level and type of petroleum-derived hydrocarbon contamination[23]. Although the tainting of sea-food is often short-lived, market confidence in these products can be affected for much longer periods[26].

Uncultivated or wild stocks of fish or shellfish may also become contaminated. Fish are generally affected to a lesser extent than crustaceans or molluscs. To date, there is no evidence that oil spills have had a significant impact on adult fish populations in the open sea, and even where larvae may have suffered extensive mortalities, evidence of pollution has seldom been detected subsequently in adult populations. However, exceptions to this have been reported following some oil spills[22] where species of plaice and sole disappeared for some time from the worst affected areas[16]. Also, oysters have remained contaminated and the affected areas closed for fishing for 7 years[22]. This has been reported as being due to buried oil in anaerobic sub-tidal sediments that were periodically disturbed by storms and high tides. Contamination of mussel tissues by PAH compounds has also produced sub-lethal effects following oil spills[27, 28].

Once fish and shellfish stocks are contaminated, action is usually taken to restrict fishing activities in the affected areas[29]. Within the UK, this is initiated by the Food Standards Agency under appropriate legislation[30]. The primary aim of these restrictions is to protect humans from risks of consuming contaminated food. Concern for human health is of primary importance, especially from the oil-derived carcinogen contaminants (for example the high-molecular weight PAH compounds) which may be accumulated from crude oil, albeit at low concentrations relative to the alkylated derivatives of the low molecular weight PAH compounds[31].

Epidemiological studies have not, however, demonstrated an increased incidence of risk of cancer, or other diseases, in humans following consumption of sea-food from areas where oil spills have occurred[32]. Following one such oil spill[27] the question of whether oil-exposed fish were safe for human consumption was considered and the conclusion of the study was that consumption of flesh from oil-exposed fish involved little risk, due to their extensive metabolism and excretion of PAH compounds. This finding was later supported by the US Food and Drug Administration[33]. It was concluded that the risk of contracting cancer following consumption of finfish (collected from the spill area) was so low that it could not be calculated, and was therefore, for practical purposes, considered to be zero[34]. It was similarly concluded that the risk of contracting cancer from a lifetime consumption of shellfish from the most heavily impacted areas was also extremely low.

Conditions imposed for the lifting of a closure order[30] following an oil spill often require that the species affected must be free of taint from petroleum-based sources and that PAH concentrations in tissues must be within the range observed for reference samples collected outside of the closure area[35]. These requirements are alternatives to those of re-establishing pre-existing background levels before the occurrence of the oil spill, as these data are rarely available for the full range of species and locations affected. From an ecological perspective, the greatest flaw in the damage assessment process following an oil spill is the reliance on baseline data. For many areas, adequate data are not available, and these circumstances are unlikely to change dramatically in future situations. The gathering of copious background data is a time-consuming and expensive undertaking. In addition, it is impossible to forecast locations and occurrence of oil spills and the rate at which ecosystems adapt and change (requiring baseline data to be continually updated) is probably similar to that at which baseline data might be collected. Hence, little overall progress would be made[36]. However, background data gathered within certain programmes can be useful in providing reference data on PAH concentrations in mussels and oysters. These data can be of use in the event of an oil spill, as in most cases, point sources of contamination are avoided in these programmes when sampling locations have been selected[37]. It has been reported that PAH concentrations in mussels and other bivalve shellfish vary seasonally with their reproductive cycle[38, 39]. The concentration of PAH compounds in molluscs (including filter feeders, detrital feeders, algal grazers, carnivores, etc) varies with habitat and feeding behaviour and also differs between species[40].

To date, the focus of attention has been on commercial species of fish and shellfish, but the overall assessment of the environmental impact of an oil spill incident will require that a much broader range of samples be taken and analysed. Following a particular oil spill[41], temporal monitoring of hydrocarbon concentrations in water and sediments was undertaken across the whole of the affected area. In addition, numerous different species, of nature conservation importance, were taken and analysed as well as a full range of species important from a commercial point of view. A large number of animal and fish deaths occurred and a full range of samples were taken and analysed, including periwinkles, dogwhelks, razorshells, sand gapers, pullet carpet shells, wedge shells, purple heart urchins, rayed trough shells, common otter-shells and striped venus. In addition, round fish and flat fish species, crabs and lobsters, cockles, mussels, oysters, whelks, shrimp, seaweed and samphire were sampled and analysed, as these were recognised as being of commercial interest[41].

Whilst this monitoring programme was in operation for 2 years, other programmes have continued for much longer periods[22, 26]. In many cases, this is due to the long-term contamination of sediments, as can occur in environments where long sea inlets, fine salt-marsh sediments and deep cobble beaches occur. Normally, oil spills which occur on flat sandy beaches are relatively easy to remove and clean, and oiled rocks which are exposed to winter storms are usually washed clean by wave action during the following winter period.

5 The determination of oil in waters using ultra-violet fluorescence detection

5.1 Introduction

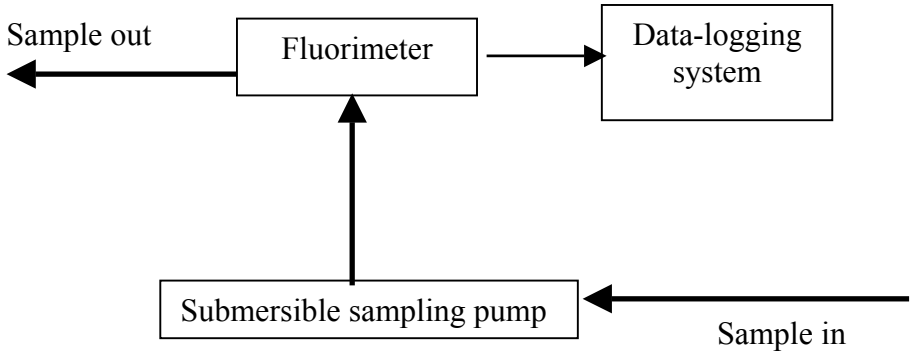
Following an oil spill the application of dispersants may be considered. Dispersants enhance the rate of oil dispersion into the water column, thereby removing oil from the sea surface. The effectiveness of chemical dispersants depends on a range of variables, including the oil composition, the extent of weathering and emulsification of the oil, the formulation of the dispersant and the prevailing weather conditions. During and after dispersant treatment, it can be difficult to assess the effectiveness of the dispersant application. This has led to concerns that dispersants may be used inappropriately and in response to these concerns, techniques have been developed to monitor dispersant spraying operations. One such technique for monitoring and assessing the effectiveness of dispersant spraying operations following oil spill incidents is based on ultra-violet fluorescence measurements. These data can be collected for water located under the oil surface, typically at depths of up to 5 metres, before and after dispersant has been applied. A comparison of the data, and hence the oil concentrations before and after dispersant has been used, enables the effectiveness of the dispersant operation to be assessed.

The determination of oil concentrations in water can be carried out using ultra-violet fluorescence detection. This technique may be set up in a continuous flow-monitoring mode. Water can be pumped through the fluorimeter at a constant rate and the fluorescence of the sample measured. If the time interval of the measurement is sufficiently small (for example every 2 seconds) then a "continuous" reading may be obtained. Based on the fluorescence measurements observed for different concentrations of reference oil[42], the amount of fluorescence detected for each sample can be related to the oil concentration in the sample. When the technique is used at sea, the equipment used should be robust and rugged, and if measurements are to be collected on a "continuous" basis, a data-logging device should be used.

5.2 Flow-through systems

Following an oil spill incident, the equipment should be set up according to the manufacturer's instructions. The equipment required usually comprises a flow-through fluorimeter, a submersible sampling pump and a data processing system. It may be appropriate to link the equipment to a global positioning system so that precise locations can be monitored and re-visited, if necessary. A schematic diagram is shown in Figure 5.

Figure 5 A schematic diagram for flow-through systems



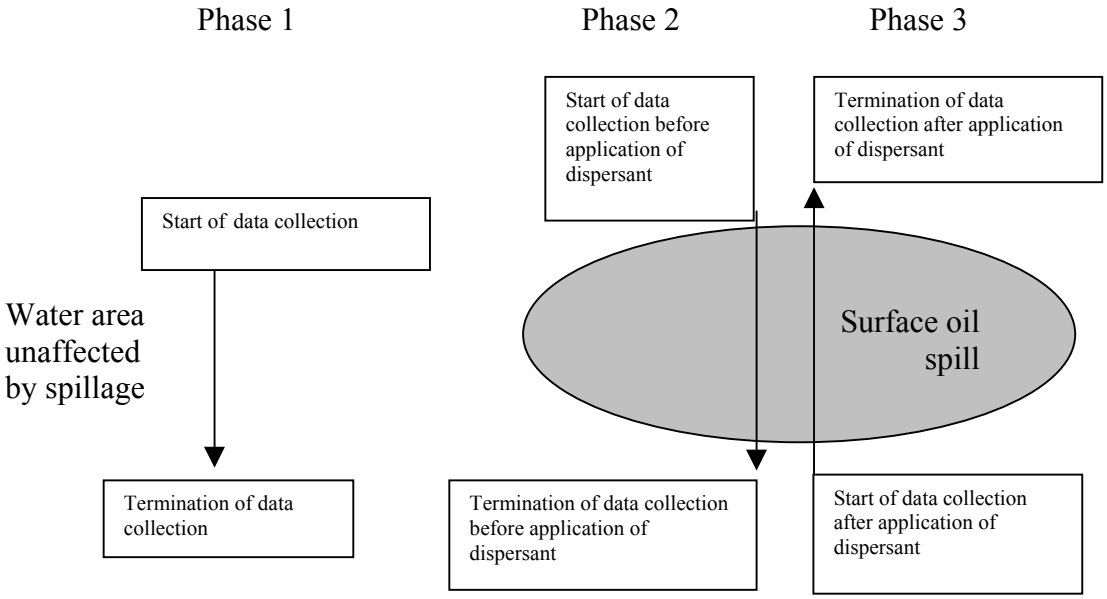
Before sampling and ultra-violet fluorescence measurement can begin, the fluorimeter should produce stable reliable measurements and be calibrated using suitable amounts of reference oil. Once the calibration process is complete**,** the fluorimeter should be connected to the submersible pump (capable of maintaining constant flow rates of up to approximately 20 litres per minute) located at the required depth (usually between 1 - 5 metres). This can be achieved by attaching the pump to a weighted chain or rope of the required length and submersing the pump. To prevent any contamination of the sample, it is important to position the sampling point, i.e. location of the pump, well away from any outlet located on the sampling vessel. A flexible tube or hose of suitable material is then securely attached to the outlet of the pump and to the inlet of the fluorimeter. Another length of tube or hose attached to the outlet of the fluorimeter and directed away from the sampling location ensures the sample (after measurement has been made) is appropriately discarded to cause minimum interference with subsequent samples. Discarded sample may be directed over the side of the sampling vessel some distance downstream of the sampling point. Instead of discarding the sample, it may be appropriate to retain some of the sample (stored under appropriate conditions) so that subsequent laboratory analysis[43, 44] can be carried out. The results of these laboratory analyses can then be used to verify the ultra-violet fluorescence measurements against actual hydrocarbon concentrations determined.

5.2.1 Operation

Before the assessment of the effectiveness of the dispersant application can be made, it is important to collect data on oil concentrations in the water column unaffected by the spillage prior to using the dispersant. If data are collected in areas not affected by the oil spillage, then this allows operators to establish background oil concentrations. If data are collected in areas affected by the oil spillage, then this allows operators to establish prevailing oil concentrations prior to using the dispersant. When these measurements have been recorded and the dispersant applied, measurements can then be taken in areas affected by the oil spillage following application of dispersant.

Initially, data should be collected in water areas unaffected by the oil spillage. Monitoring should then be undertaken prior to dispersant application in the area of the surface oil spillage where dispersant is to be applied. This is typically in the thickest part of the oil slick where the depth of oil on the sea surface is at its greatest. Data should be recorded while the sampling vessel is travelling in a straight line across the oil slick. Once dispersant has been applied, the sampling vessel should then return across the oil slick taking measurements at similar locations to where the pre-dispersant spraying data were taken. A comparison of the data obtained before and after dispersant spraying can then be undertaken. A schematic diagram of these operations is shown in Figure 6. Dispersant is usually applied between phase 2 and phase 3 of the monitoring cycle.

Figure 6 Schematic diagram of monitoring



5.2.2 Sub-surface water sampling

At the same time as the monitoring operations are carried out, discrete water samples should be taken. These samples (typically, 1 litre) may be taken at the outlet from the fluorimeter or from adjacent locations situated in proximity to the pump. These independent samples are used to give an immediate visual assessment of the oil dispersion and for retrospective verification of the ultra-violet fluorescence measurements[43, 44]. The sub-surface water samples should be collected in areas unaffected by the oil spillage (for background hydrocarbon concentration levels) and at locations where low and high hydrocarbon concentrations are indicated.

The samples should be collected in suitable sample bottles and immediately preserved or stabilised with 50 ml of solvent (for example chloroform or dichloromethane). Sufficient sample should be collected to ensure that all fluorimeters used are calibrated using various amounts of reference oil[42] and verified against actual hydrocarbon concentrations[43, 44] under investigation.

5.2.3 Calibration procedures

Different oils contain different proportions of aliphatic and aromatic constituents. Hence, different oils will possess different fluorescent properties. Thus, wherever possible, the calibration of the fluorimeter should be undertaken using the same oil being investigated. If this is not possible, then a reference oil possessing similar characteristics to the oil being investigated should be used.

Before calibration, the fluorimeter and filters should be checked according to manufacturer's instructions and the cell cleaned. For calibration purposes, a clean, suitable container, usually a 10-litre metal bucket, is required for the preparation of blank and calibration solutions. The blank solution, usually comprising water from an area unaffected by the oil spillage and containing negligible amounts of oil, is collected in the container. The submersible pump is placed in the container and flexible tubing or hose of suitable material is securely attached to the outlet of the pump and to the inlet of the fluorimeter. Another length of tubing or hose is attached to the outlet of the fluorimeter and directed back to the container. If several fluorimeters are used, these may be

connected in series, i.e. tubing or hose is attached to the outlet of one fluorimeter and to the inlet of another fluorimeter.

When the equipment is set up, the calibration procedures recommended by manufacturer's instructions should be followed and the ultra-violet fluorescence of the blank solution measured. These procedures are then repeated using a series of standard solutions containing suitable amounts of the reference oil (for example between 1-20 mg l^{-1}). It may be necessary to add dispersant to the water to facilitate dispersion of the oil in the water. This technique is not a definitive calibration technique, but does enable analysts to check the equipment is working correctly and provides information on the equipment's response in relation to oil concentrations. Once calibrated, the fluorimeter can be used to obtain ultra-violet fluorescence measurements of samples collected before and after dispersant has been applied to the affected area.

In order to correlate the ultra-violet fluorescence measurements against actual hydrocarbon concentrations in the water (as opposed to the reference oil used in the calibration procedures) sub-surface water samples should be collected during the monitoring operation and subsequently analysed in the laboratory[43, 44]. Appropriate calibration factors can then be determined and applied to the data collected.

5.2.4 Assessment of dispersant operation

Once the ultra-violet fluorescence measurements have been recorded they can be downloaded into a spreadsheet and used for further manipulation. An immediate initial assessment of the data can be made by comparing the ultra-violet fluorescence measurements (and hence, the oil concentrations) determined before and after the dispersant has been applied.

Alternatively, graphical representations of the data can be produced. For example, Figures 7 and 8 indicate the oil concentrations at 1 m below the oil spillage before, and after, dispersant has been applied. From the figures it can be observed that after the dispersant has been applied the oil concentration increases significantly (demonstrating that the application of dispersant has been effective). These oil concentrations will decrease over time as the oil is dispersed and becomes more diluted. Similar graphs can be produced for concentrations at different depths and over time.

In addition to correlating the ultra-violet fluorescence measurements with actual hydrocarbon concentrations, the sub-surface water samples collected during the monitoring operations also provide information for assessing the effectiveness of dispersion. A visual comparison of water samples taken at the same depth before and after application of the dispersant illustrates whether a change in colour is noticeable or has occurred. This can be much easier to assess after the solvent has been added to the water in the sample bottle. An increase in the depth of colour suggests a higher oil concentration and is indicative of greater dispersion.

Figure 7 Oil concentrations before dispersant application

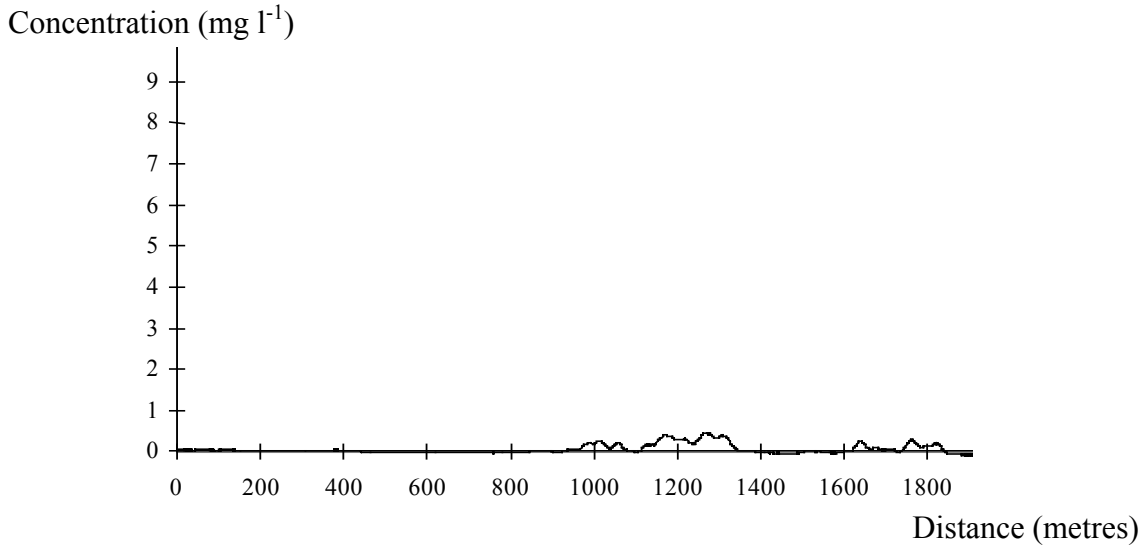
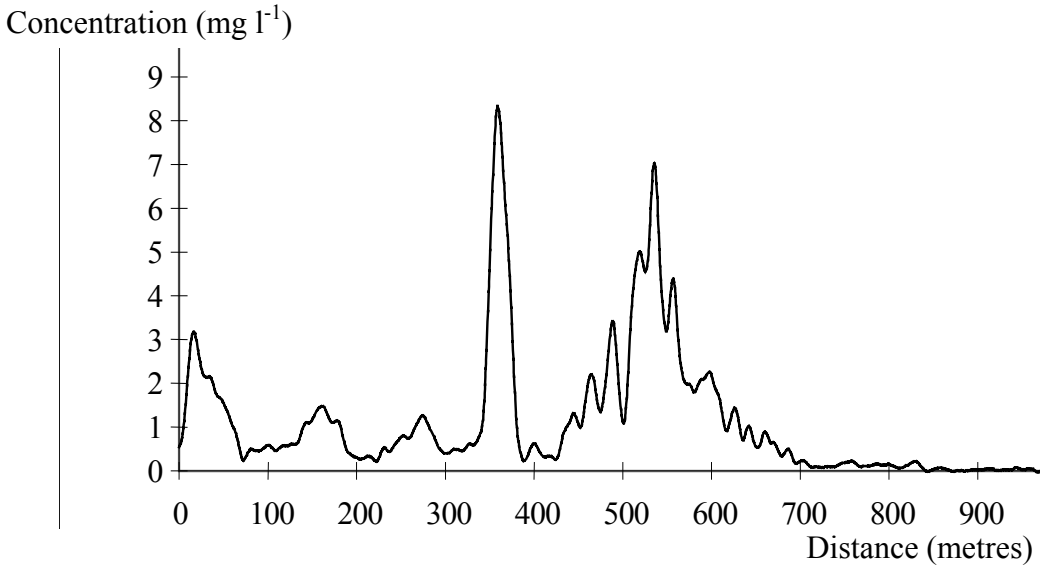


Figure 8 Oil concentrations after dispersant application



5.3 Towed submersible systems

Flow-through fluorimeters are generally not sufficiently sensitive to measure very low concentrations and towed submersible fluorimetry systems may need to be used. The deployment of continuous flow-through fluorimetry systems may be extremely useful during the early stages of an oil spill when dispersed oil concentrations in the high micro-grams per litre range need to be determined. Further dispersion and dilution of the oil (over extended periods of time) will lead to lower oil concentrations needing to be determined, until the concentrations return to pre-existing background concentrations for the affected area. Background concentrations are often variable, generally in the low micro-grams per litre range, and depend on the degree of urbanisation and industrialisation, as well as hydrographic characteristics and other factors.

Following oil spills in the UK[4, 45], a towed submersible fluorimeter was used to establish oil concentrations in affected and unaffected areas. The limit of detection of these filter instruments is

approximately 1 μg l^{-1} and they generally operate at an excitation wavelength of 310 nm and an emission wavelength of 360 nm. These wavelengths can be changed if the oil under investigation possesses fluorescent characteristics within a different range, for example diesel oil with corresponding wavelengths of emission and excitation of 270 and 330 nm respectively.

A submersible fluorimeter may be deployed by mounting the instrument in a suitable structure, for example a plankton sampling device, which is then towed behind a sampling vessel. Such devices usually contain a conductivity-temperature-depth unit (which measure conductivity (as a surrogate for salinity), temperature and pressure (as a surrogate for depth) and can be used to power the fluorimeter and data-logging system, and allow data transfer to the surface. With the data-logging system set to accept readings every 2 seconds and the speed of the sampling vessel at 5 knots, a concentration reading may be taken every 5 metres within a sampling area. On smaller vessels, the fluorimeter may be deployed alongside the boat and powered directly.

As for flow-through systems, discrete water samples should be collected at intervals and analysed[43, 44]. In addition, the instrument should be calibrated using known concentrations of solutions of the oil under investigation or of a reference oil[42]. For the crude oils tested[4, 45], the linear range of the oil concentrations extends to at least 50 μg l^{-1}, with a useful range of up to approximately 100 μg l^{-1}.

6 Sample strategy and design

Details of statistical aspects of experimental design are not covered in this booklet and expert advice on this matter should be obtained. Where comparisons are made between affected and unaffected areas, sufficient sampling and analytical effort should be directed towards the provision of adequate control reference data. It is essential that samples are collected by such means that enable significant changes in the determinand concentrations to be detected. Certain principles[46] may be used to facilitate the planning of a chemical response programme following an oil spill and these include:

(i) Questions that need to be asked should be stated clearly and concisely. The answers to these questions will be as coherent and as comprehensible as the initial perception of the problem.

(ii) Replicate samples should be taken for each sampling occasion with respect to time, location, or other controlled variable. Differences in results between sampling occasions can only be demonstrated by comparison to results within the replicated samples.

(iii) An equal number of randomly allocated samples should be taken for each sampling occasion with respect to time, location, or other controlled variable. Samples that are “representative” or “typical” of certain situations are not random samples.

(iv) A situation or condition should be tested to ascertain whether that situation or condition has an effect on the resultant value. This can only be achieved by collecting and analysing samples where the situation or condition is observed or known to be present and where the situation or condition is not observed or present, all other factors remaining constant. The effect of a situation or condition can only be demonstrated by comparing the results obtained with those of control samples.

(v) Preliminary sampling should be undertaken to provide a basic foundation for evaluating subsequent sampling designs and statistical analyses. It has been found that where this process has been omitted, time may be wasted in the long term.

(vi) Sampling techniques and devices should be verified to ensure the required population is in fact being sampled. Verification should be undertaken over the entire range of situations and conditions to be encountered. Variability in the efficiency of sampling within an area will bias the variability obtained between areas.

(vii) Where an area that is to be sampled exhibits a large-scale environmental pattern, the area should be sub-divided into smaller relatively homogeneous sub-areas. Samples should be taken from each sub-area relative to the size of the sub-area.

(viii) The unit size of the sample should be verified as being appropriate to the spatial distribution and variability of the sample being taken. The number of replicate samples needed to demonstrate the required precision can then be estimated.

(ix) Data should be tested to ascertain whether the error variability is homogeneous, distributed in a Gaussian manner, and independent of the mean result. If not (as may be the case with most field data) then

- the data should be appropriately transformed;
- a distribution-free (non-parametric) procedure should be used;
- an appropriate sequential sampling design should be used; or
- the data should be tested against simulated data.

(x) Having chosen the most appropriate statistical procedure, it should not be changed. An unexpected or unexplained result is not sufficient reason for rejecting the procedure and seeking an alternative one.

7 Sampling and sample storage

7.1 Introduction

The selection and collection of sample material (whether water, sediment or biota) are integral parts in assessing the impact of oil spills. They are crucial to the provision of the information required when considering the actions that need to be taken in response to oil spill incidents. A proper assessment of the environmental impact of an oil spill can only be achieved if relevant samples are collected and appropriately analysed. Sample selection and collection are the first of many stages that ultimately result in the production of scientific data and provision of information. If relevant samples are not collected or if inappropriate samples are collected, then the subsequent assessment process will be of little value. Similarly, the handling, processing and storage of samples immediately following collection should be strictly controlled.

Hydrocarbon compounds are ubiquitous within the environment and hence the potential for the adventitious contamination of samples is considerable. Contamination can occur at any stage, for example during the initial collection and sub-sampling, from the storage container and during transfer to the laboratory. Care should, therefore, be taken, especially when collecting material to provide background or reference data. In addition, care should be taken to ensure samples do not come in contact with plastic, rubber or similar materials (such as gloves, bags, etc) or solvents, cleaning fluids or oils. When collecting samples following an oil spill, it may not be possible to use familiar laboratory items such as blue paper roll, as such items may contain fluorescent dyes or other potentially interfering compounds that may contaminate samples.

Smoking should not be allowed in areas in proximity to those locations where samples are handled or analysed. Smoke contains PAH compounds that may contaminate samples being treated.

Exposed areas on boats, ships and other vessels are often, out of necessity, smeared with grease or other oils, and vehicles used for transport purposes may also contain areas with trace amounts of diesel or petroleum fuels. These areas pose a potential risk for the contamination of samples. Additional risks exist when samples are collected for sensory assessment and evaluation. The presence of flavours and odours originating from sources (for example disinfectant or cleaning materials) not associated with the oil spill, may adversely affect samples of biota, thus preventing staff making an objective sensory assessment of the sample under investigation.

During sampling, storage and analysis, appropriate precautions should be observed at all times. Although some of the procedures outlined may appear to be unnecessary and time-consuming, they are essential if sample integrity is to be maintained and assured. For example, sample containers should be rinsed with an appropriate organic solvent such as pentane, hexane or dichloromethane. Furthermore, solvents should be of an appropriate quality and should be tested to ensure their suitability.

Following an oil spill incident, many samples may be collected. However, it may be that not all samples are analysed. An important factor in the initial response to an incident is to ensure that samples are collected as soon as possible after the start of the incident. The critical samples are those that will give background or baseline concentrations. Such data, or stored samples, may also be available from organisations that may have undertaken prior monitoring in the impacted area. Once the samples have been transported to the laboratory, the requirements for storage and order of analysis can then be prioritised. Throughout the whole sampling process it is essential that reliable records be maintained for all samples. To facilitate this, pre-printed sample sheets may be used to ensure that all the required information is collected and recorded at the time of sampling. Such information should include for example, sample identification code, date and time sample collected, depth at which sample was collected (if pertinent) and location (grid reference) and type of sample and manner in which sample was collected etc. In addition, consideration should be given to the manner in which samples are tagged or labelled. For example, container surfaces may often be wet, making them unsuitable for permanent marking and alternative arrangements should be considered, for example “tie-on-tags”. If care is taken and attention to detail maintained during the early stages of sample collection, then the risks of errors occurring and samples becoming misidentified will be reduced.

7.2 Sample selection

7.2.1 Fish and shellfish for chemical and sensory assessment

The collection and analysis of fish and shellfish are generally undertaken to assess the risks to humans from the consumption of such foods following an oil spill incident. Where fish and shellfish (see Annex 4 for a list of common and scientific names) are sampled in order to characterise the oil, these should be representative of the affected area and be of commercial importance. Where appropriate, species of a round (demersal) fish (for example haddock) and a flat fish species (for example plaice or lemon sole) should be sampled. Whilst the results of analysis from the round fish should provide an assessment of the impact of pollution within the water column, flat fish are more closely associated with pollution of seabed sediments. Aquaculture activities form a significant part of coastal industries and where farmed or cultivated fish are confined, they cannot escape the oil following an oil spill incident. Such stocks of fish are likely to be impacted by the oil spill and may also need to be monitored[45, 47, 48].

Many varieties of shellfish (especially bivalve molluscs) and crustaceans tend to accumulate hydrocarbon compounds. These species, often found in coastal and off-shore waters, should therefore be sampled as required. Such species include Norway lobsters which inhabit muddy

sediment areas, the brown crab, molluscs, scallops and cockles. The blue mussel is relatively common and has been shown to be a good general indicator species of exposure to hydrocarbon compounds.

7.2.2 Surface oil slicks

The collection of oil when present as a surface slick, can often present severe practical problems. Thus, wherever possible, a sample of the oil should be obtained directly from the vessel involved in the incident. If a sample has to be taken from a surface slick then representative samples should be taken from that part of the oil slick where the oil thickness is at its greatest. Wide-necked jars, that have previously been solvent-cleaned, may be used to collect the oil from the surface. Where this is not practical, absorbent materials may be used. However, such materials need to be thoroughly solvent-cleaned before use, as the absorbed oil will need to be solvent extracted from the material prior to analysis as this process will also remove hydrocarbon compounds that are integral to the sampling material. Samples should be stored at a temperature of 4 ± 2 °C for short periods of time but should be stored at -20 °C (or below) if a longer storage period is required.

7.2.3 Deposited oil

Oil deposited on shorelines and sea defences may be scraped directly into solvent-cleaned sample containers. These containers should not be made of plastic or similar materials that may adversely affect the sample. Wooden spatulas provide a convenient means of sampling the oil without presenting a risk of contamination, and may be discarded after use. Where it is necessary to sample oil present on birds or shellfish, oil may be removed from the affected species using a wooden spatula and transferred to suitable, solvent-cleaned sample containers. Plumage may also be similarly treated, or transferred directly to appropriate, solvent-cleaned, sample containers. The plumage is then subsequently solvent-extracted in the laboratory. Samples should be stored at a temperature of 4 ± 2 °C for short periods of time (for example less than 3 days) but for longer storage, samples should be kept at -20 °C (or below).

7.2.4 Sediments

The sampling of sediments often takes place under a range of circumstances and samples should be collected after water samples have been taken if the same location is to be sampled for sediments and water. In addition, when fish need to be sampled, sediment samples should be collected at suitable intervals throughout the whole of the trawling process. In general, the distribution pattern of the oil spill will determine the type of sampling to be undertaken. The prevailing wind speeds and directions, tidal currents and the nature and type of dispersants used (if appropriate) will have an effect on the distribution pattern. Hydrocarbon compounds will associate with particulate material in the water column and be deposited with fine-grained sediments onto the seabed. In addition, within the littoral zone, oil may be transported onto the shore during high tides and affect underlying sediment material. Heavy oils may be re-worked from the shoreline by wave action, incorporating sand/shell particles and then sink in the near-shore zone to form tarballs, not necessarily associated with fine-grain sediments.

7.2.5 Seaweed and other aquatic flora and fauna

The sampling and analysis of aquatic flora and fauna may also need to be carried out to assess the environmental impact of an oil spill on the ecology of the affected area. This assessment may however be restricted to field studies. For example, a study of limpet density may be undertaken. Following one oil spill incident[41], samples of seaweed and marsh samphire were collected from affected areas to assess levels of contamination. Certain types of seaweed and other marine plants

are sometimes used locally in food preparation processes, for example the production of laver bread. In the study, whole fronds were collected and stored in glass jars prior to alkaline digestion and subsequent analysis. Most of the contamination was reported to be on the outside of the plants, and diminished as new growth occurred. Similar trends were identified in samples of grass collected on the mainland following the same incident[49].

7.2.6 Birds and sea mammals

Exposure to crude oil has long been known to affect many marine mammalian species. Animals (for example cetaceans and seals) that depend on a layer of blubber for insulation are affected primarily via ingestion of oil, or by inhalation of volatile organic compounds from the oil. Those animals (for example, fur seals and otters) with dense fur and little blubber are also affected by loss of thermal insulation. The impact of an oil spill on otters, common seals and grey seals has been summarised[50] recently. A sampling regime was described for otters and aerial surveillance used to assess changes in the numbers of seals observed. Short- and long-term effects were investigated and it was concluded that the impact from the oil spill showed very little effect on the otter and seal populations. The value of background data collected over a period of time before the incident was also demonstrated.

Marine birds, including shags, black guillemots, kittiwakes, great northern divers, eiders, Arctic terns, skuas, gulls and other terns can experience direct[51, 52] and sub-lethal[53] effects following an oil spill. Direct effects (oiling and mortality) are the most obvious and widely shown effects, but sub-lethal effects may have a greater long-term impact on bird populations. Sampling programme designs may involve long-term studies and require specialist advice for both the sampling programme design and the interpretation of data.

7.2.7 Crops, vegetation and livestock

Oil spills which occur at sea can also contaminate the terrestrial environment[54, 55] as crude oil may also be deposited on land. Severe winds can produce aerosols of oil droplets and sea-spray that are then driven towards the land. In addition to affecting grazing areas and arable land, agricultural livestock may also be at risk. Thus, an off-shore oil spill may have a considerable impact on local communities and businesses.

Following an oil spill incident, the primary aim of any study should be to assess potential risks to humans. Wherever possible, background information on contaminant levels, for example PAH concentrations, in soils and plants should be obtained to assess the relevant contribution of contaminants following such an incident. As such it may be necessary to monitor, for example grazing areas of livestock, local food crops and flora, and coastal vegetation and maritime grassland communities. The monitoring of livestock tissues, including milk, may also be required if the livestock have been in contact with contaminated grazing areas, as it is known that PAH compounds can be transferred from animal feeding stuffs to livestock tissues[56].

Much of the advice and guidance described for the sampling of marine species, especially in relation to minimising contamination of samples, applies also to the collection of crops, vegetation and livestock tissues. In addition, meteorological data should be collated and used to determine those areas most likely to be affected. Visual inspection of these areas may provide useful information prior to sampling.

Following an oil spill incident, an assessment of the likely affected terrestrial environmental areas may be undertaken by a variety of organisations and authorities responsible for managing different aspects of oil spill incidents. As pointed out in section 1, thorough liaison between these

organisations and authorities should be maintained. Details of possible organisations involved are given in Annex 1.

7.3 Collection of samples

Following an oil spill, primary concern will be directed towards protecting public health, for example establishing fishery exclusion zones around affected areas. Part of this programme will involve the rapid development of an integrated sampling programme. This should include the collection of samples to assess sub-lethal effects of various species and to determine local background concentrations of hydrocarbon compounds, some of which may be of historical petrogenic origin. The collection of such samples at the same time will ensure that all data can be generated and assessed relevant to a single reference point.

All samples should be appropriately preserved and the container clearly labelled in a manner which will ensure no risk of mis-identification; tie-on labels and marking of both the container and the top (if applicable) will minimise the risk of both cross-contamination and loss of the sample identification. During transportation to the laboratory, samples should, if possible, be kept cool and in the dark.

Fish and shellfish are dealt with separately in Section 7.5.

7.3.1 Water

Samples of water should be collected in clean, solvent-rinsed glass bottles that may be secured in a sampling device to enable samples to be taken at different depths. Plastic materials should be avoided when collecting samples for hydrocarbon analysis. Ideally, bottles should be deployed in a closed state and opened only at the appropriate depth. There are many sampling devices[57] available for the collection of water, for example a Winchester sampling device. In the case of a dual sampling device, five litres of water known or suspected of being contaminated may be collected. Such a device comprises a weighted basket containing two-2.5 litre bottles, each suspended from a line. At the required depth, a “messenger” or signal activates the opening sequence, and the sample is collected.

The sample container should be rigorously cleaned with a suitable solvent (for example, pentane, hexane or dichloromethane) before use. In addition, the sampling device (particularly the stopper) should be cleaned with solvent at the beginning of the sampling operation and after use in areas of known or suspected high concentrations of hydrocarbon compounds.

The volume required for water samples depends on the degree of contamination, but a minimum volume of 2.5 litres is generally sufficient for most analyses. A larger volume, for example 5 litres, may be required for samples collected in the open sea, especially if used to obtain background or reference data. The depth at which water is collected will depend on the total water depth, and the purpose for which the samples are taken. In order to avoid surface slicks, water should be sampled at a minimum depth of 1 m below the surface. In addition, water should be sampled at least 5 m above the seabed in order to avoid contamination from re-suspended sediment material. Samples taken between 1-5 m below the surface, 5 m above the seabed and at a depth mid-way between should provide sufficient information to adequately assess the contamination in the water column. Prior to analysis, samples should be stored at temperatures between 4 ± 2 °C. If commencement of analysis is not to begin within 12 hours of the water being taken, then a suitable amount of solvent, for example 50 ml of dichloromethane, may be added to the sample container. If the samples are to be extracted within 12 hours then they should be transported to the laboratory and stored safely to avoid breakage.

7.3.2 Sediment

The most suitable sampling device to be used will depend on the water depth and the type of sampling platform available. For example, when sampling in-shore, shallow waters, a hand held grab or corer may be suitable. For off-shore deeper waters, a winch deployed weighted single or multi corer grab sampling device may be required. The type of sediment to be collected will determine the amount of weight needed to be added. The harder the ground, the greater the amount of weight needed on the grab. Conversely, for soft sediments, the grab weight should be reduced. Ideally, the collection chamber on the sampling device should be filled with sediment until approximately two-thirds to three-quarters full. If the collection chamber is too full, losses may occur due to surface run-off; if less than half full, the sediment may not have been sampled correctly.

Before sampling the sediment from the sampling device, any overlying water should be carefully discarded. Once the water has been removed, the sediment should be sub-sampled by removing the top 10-20 mm of sediment to a metal beaker, for example stainless steel or aluminium. This should be done using a stainless steel scoop or equivalent. The sediment that has been sampled into the beaker constitutes the sample that will be analysed. This should be thoroughly mixed using a metal spatula and homogenised portions transferred to smaller, separate containers which are then sealed, appropriately labelled and stored at -20 ^{o}C prior to analysis. Both the scoop and the metal beaker should be water-washed and then solvent rinsed between samples to prevent sample carryover.

The amount of sediment available via cored sampling devices will depend on the length and diameter of the coring device. A suitably sized core tube for sediment samples for general purpose is about 50 mm diameter. When divided into 20 mm lengths, approximately 20 g of sediment should be available from each section of cored sample. Each quantity of sediment, comprising approximately 20 g should be transferred to separate metal (for example aluminium or stainless steel) containers that are then sealed, appropriately labelled and stored at -20 ^{o}C. Samples stored in this manner may be stable for up to six months. Where particle size, carbon or nitrogen analyses are required for possible normalisation of the contaminant data, the sediment may be further sub-sampled and freeze dried prior to analysis.

7.3.3 Soil

A suitable sampling device, which depends on the analysis required and the type of soil to be collected and which normally collects a cored sample, should be used. In addition, the type of analysis required on the sample may dictate the type of sampling device, for example if volatile hydrocarbon analysis is required. For hard mineral soils, a robust sampling device is required capable of cutting roots and similar items etc without compressing the soil or becoming trapped in the soil. Other devices, for example spades or trowels may be used if relatively shallow soils are sampled. The cored sample should be divided into sections, each section thoroughly mixed and then transferred to a metal beaker and appropriately labelled. It may be necessary to divide the soil into specific sections depending on the depth at which the soil was collected or by its organic and inorganic content. Before sampling, moss or lichen layers may need to be separated from the soil and plant material may also need to be removed.

The storage time of wet soils should be kept to a minimum, and frozen soils should be stored only for short periods of time. If soil samples are to be stored for any appreciable length of time, then the sample should be dried either by air-drying at ambient temperatures (less than 30 ^{o}C) or by drying at elevated temperatures, and possibly sieved to remove large stones and roots etc. Drying the sample

will depend on the analytical requirement as hydrocarbon compounds may be volatilised during the drying process or degrade during storage.

7.3.4 Spilt oil

It is important that samples of the oil associated with the spill are obtained at the earliest opportunity. When an oil spill has occurred, the composition of the oil will change due to weathering effects (see Section 9.3.1), for example due to evaporation, biodegradation, chemical degradation, and possibly phase partitioning. In addition, if the oil is sprayed with dispersant, this will influence the distribution and dispersal of the oil. Over periods of time, these effects will affect the concentrations of the more volatile hydrocarbon and PAH compounds. However, as described in Section 9, some compounds in oil are more recalcitrant than others and may be determined to identify particular types of oil. The PAH composition in fish or shellfish affected by the oil spillage will also depend on their exposure to and relative uptake[58] of the oil.

7.3.4.1 Sources of oil

Following an oil spill, it is essential to establish that contamination in for example fish, shellfish, or plant material results from the oil spilled in the incident. A sample of the oil should therefore be obtained from the suspected source so that suitable comparisons can be made. This sample should be collected in a suitable, leak-proof container (for example a solvent-washed metal can) and stored out of direct sunlight and away from sources of heat. The collection of the sample may involve liaison with a number of organisations. If the sample cannot be obtained directly from the vessel (or vehicle) involved in the incident then an alternative source should be sought, for example the relevant oil company. Where appropriate, samples of fuel oil, or other oils used on the vessel, should also be obtained. These oils may be a secondary, or indeed the primary, source of contamination. Where possible, appropriate details of the physical and chemical composition of the oils under investigation should be obtained from the relevant organisations.

Oil samples should be appropriately labelled, stored at 4 ± 2 °C and transported directly to the laboratory. In addition, they should be stored separately from other samples collected as part of the environmental monitoring assessment programme. Where practicable, the preparation of oil samples for analysis should be carried out in designated areas using glassware reserved for that purpose. At all times, care should be taken to avoid cross contamination.

Where oil adheres to the external surfaces of shellfish, birds and other species etc, the oil should be removed from the affected species within the laboratory. It may be necessary to store certain species, for example crabs and shellfish that have been affected by the oil, in a freezer prior to transportation to the laboratory. On arrival at the laboratory, the affected species should be wiped clean, for example using cotton wool that has been previously cleaned with solvent, such as dichloromethane or pentane. The cotton wool should then be extracted with solvent and the extract transferred to a suitable vial. Prior to analysis the extract may need to be concentrated or diluted, or analysed directly. Additional considerations should be given to those volatile hydrocarbon compounds that may be lost during these processes.

Following an oil spill, a surface sheen is often noticeable as a very thin layer of oil adhering to the area affected. This surface sheen can be quite marked, even when only a small quantity of oil is involved. A number of methods are available for sampling a surface sheen and further details are given in Annex 2.

The collection of oil from fishing gear and other equipment may also need to be considered especially where unusual hydrocarbon concentrations are reported for the samples examined, for example the hydrocarbon indices referred to in Section 9.

7.3.4.2 Beaches and rocky shores

Spilled oil that has been stranded on sandy beaches, large rocks or stones can be sampled by scraping it directly into suitable solvent-cleaned aluminium, stainless steel or glass storage containers. Wooden tongue depressors offer convenient means of providing "single-use" spatulas for field use, without presenting a contamination risk. When sampling oil on beaches and rocky shores some of the oil will penetrate around larger stones and affect underlying sediment. Thus, after taking oil samples from the beach, the stones, etc should be moved to allow sediment samples to be collected. Using a clean, stainless steel spoon, sediment material can be sampled and transferred to a clean suitable glass container, sealed and stored at -20 °C prior to analysis. Solvent-extracted aluminium foil may need to be placed over the mouth of the container to prevent contact between the sample and any waxed lid-liner, which may be present. Care should be taken not to overfill the container with sample, as sediment that contains significant amounts of water will expand when frozen, thus presenting a risk of breaking the container, resulting in leakage and possible contamination.

7.3.5 Birds

Shore birds are generally at much less risk following an oil spill. This contrasts with sea birds which often become seriously debilitated, or die due to the ingestion of oil or from the resultant stress caused by fouling of their plumage. However, since it is often necessary to establish that the oil on the birds originates from the spillage, collection of oiled birds from beaches may be required.

7.3.6 Seaweed, crops, vegetation, livestock

The effects of crude oil spillages on shoreline and terrestrial environments may be significant and areas affected may extend well beyond the high tidal water regions as the action of wind and sea breezes may cause oil to be blown far inland. This can impact on vegetation and livestock. Hence, there may be considerable variation in the type of samples required for analysis.

7.3.7 Comparison material

It is essential that samples of control material that are regarded as being unaffected by the oil spill be collected to enable a comparison of results to be made with samples collected from affected areas. When managing the response to an oil spill incident, consideration should be given to possible sources of control material. In addition, relevant background concentrations of determinands of interest may be obtained from various sources[59, 60]. Information gathered for the impact assessment may need to be compared with data collated as part of regulatory requirements for assessing the biological quality of certain waters[61]. For example, such determinations may include measurements of phytoplankton, seaweeds, angiosperms and benthic invertebrate fauna.

If available, background data obtained for the water quality of the area affected prior to the oil spill should be reviewed. If not available, samples should be collected from locations close to, but unaffected by, the oil spill. At all times the possibility of contamination from a source other than that responsible for the oil spillage should be considered.

Background data for sediment and soil quality should also be obtained, if possible, prior to the oil spillage. If this is not possible, sediment and soil samples should be collected from locations close to, but unaffected by the oil spill.

As part of the monitoring programme following an oil spill, samples of fish and shellfish species should be collected from areas close to but unaffected by the oil spill as well as samples from affected areas. This should enable sensory assessments to be compared using control or reference samples with typical flavours and odours characteristic of the species both before and after the spillage.

7.3.8 Sample size

During an oil spill incident, samples should be collected as early as possible. In some cases, oil may take several days to be deposited along a coastline and this affords an opportunity to collect samples for potential background information. In addition, it should be established whether other organisations have collected samples in the past and whether analytical information can be made available. It may often be impossible or inappropriate to resample and collect further samples later. The size of the sample, in terms of numbers and amount collected, should be sufficient to enable all analytical and sensory tests to be carried out, but will depend on the availability of the species under investigation and the need to demonstrate statistical confidence. Sampling wild populations in the open sea is often more difficult than sampling cultivated or farmed stocks confined by limitations of their cultivation.

7.4 Adventitious contamination and toxins

7.4.1 Soils, sediments and water

Metal containers (for samples of soils and sediments) and lids should be rinsed with solvent, and should then be allowed to drain. They should then be sealed and packaged ready for use. Suitable containers for water, for example glass Winchester bottles should be rinsed with solvent, such as dichloromethane, pentane or hexane, and inverted and then allowed to dry before being capped and packaged ready for use. If sediment samples are to be collected at the same location, then water samples should be collected first. This should avoid contaminating water with suspended sediment material. When the sampling device is recovered, care should be taken especially when the device passes through a surface sheen or oil slick. The outside of the sampling bottle should be wiped clean and dry and any surface sheen removed. Sources of possible contamination may include contact with oil on sampling equipment and gloves of personnel handling the samples. Clean gloves should be worn by personnel at all times, and any sign of oil on the sampling equipment should be washed off, for example with water and detergent, followed by a solvent rinse.

7.4.2 Fish and shellfish

Samples may become contaminated at various stages during handling and processing. These include contamination from contact with oil on fishing gear, on surfaces where fish are landed, on hands or gloves by personnel sorting the samples, on baskets or buckets used for moving samples around, from processing areas and with cleaning fluids.

Work surfaces and equipment should be visually inspected for signs of contamination and scrubbed clean, for example with detergent, followed by rinsing with water, for example uncontaminated seawater or potable water. Contamination from cleaning fluids and disinfectants or by contact with contaminated equipment should be avoided. Wherever appropriate, odourless detergents should be

used. In addition, personnel involved in the preparation of samples should avoid the use of perfumed toiletries and cosmetics.

Equipment and containers for transporting samples should be constructed from an odour-free material and be thoroughly clean prior to use. Samples should be stored separately from other material that may affect the sensory evaluation.

7.5 Sampling and processing of fish and shellfish for sensory and chemical analysis

7.5.1 Sample collection

For the collection of appropriate fish samples and depending on the extent of the impacted areas and the availability of vessels, fishing can be carried out aboard commercial fishing boats, or specific research vessels that may be better equipped for dealing with the sampling practices required. Suitable working environments and appropriate storage facilities should be made available in both cases. A summary of the species (and appropriate tissue material) that may need to be collected, and tests that may need to be carried out is given in Table 2, and Table 3 highlights the numbers normally required.

Table 2 Summary of species and tests

Species	**Tissue required for sensory assessment**	**Tissue required for chemical determination***	**Tissue required for biological effect measurements****
Round and flat fish			
	Fish muscle	Fish muscle Fish liver	Fish liver (EROD) Bile (PAH metabolites)
Shellfish			
Norway lobster	Tail muscle	Tail muscle	Hepatopancreas (GST)
Lobster	Claw and brown meat	Claw and brown meat	
Crab	Claw and brown meat	Claw and brown meat	
Other species			
	Whole animal		Digestive gland (GST)

* For example, hydrocarbon compounds, including PAH compounds.

** There are many techniques involving biological effect measurements. Often, several different measurements are undertaken to enable an environmental assessment to be made. The specific measurement required may be species dependent and should be considered at an early stage when planning the sampling strategy.

Table 3 Sample size and tissue requirement for sensory assessments

Biota	Number of individuals required	Amount of tissue or number of individuals required for each sample
Flat fish	5-10	35-50 g of fillet.
Round fish	5-10	35-50 g of fillet.
Farmed fish	5-10	35-50 g of fillet.
Mussels	25-100	5-15 individual mussels.
Norway lobster	20-60	5-15 individual pieces of tail meat.
Lobster	1-5	30-50 g of tail and claw meat.
Crab	1-5	20-40 g of claw meat. Brown meat to be tasted separately where sufficient sample is available.
Scallops	5-20	1-2 whole adductor muscle and gonads.
Other species	25-100 for smaller species, 10-50 for larger species	5-15 for smaller species, 2-5 for larger species.

Samples of shellfish associated with shore-affected areas may provide material for assessing the levels of oil contamination. It has been shown that mussels can be a good indicator species for monitoring exposure to hydrocarbon compounds in the water column. Following an oil spill, there may be large-scale mortalities of local fauna, especially the bivalve molluscs of sandy sub-littoral environments. These deaths may be more than those generally observed for, or associated with, the prevailing weather conditions at the time of the oil spill. Thus the cause of death needs to be attributed to either the oil spill or to the prevailing weather conditions[4, 62, 63, 64].

7.5.2 Aquaculture activities

Access to restricted areas for sampling purposes should be considered at all times, as should any risks resulting from such activities. Cultivated or farmed stocks may offer a useful source for providing large numbers of species for sampling, often from a single location and comprising species of the same size, gender and age. In most cases, sampling may require access to a boat or similar sampling vessel, but in some cases, it may be possible to obtain samples from the beach at low water. In all cases, it will be essential to liaise with the owners or managers concerned.

As sensory assessments may be carried out, precautions should be exercised and hygienic conditions used throughout.

7.5.3 On-board sampling

7.5.3.1 On-board holding tanks

Flat fish species should be kept alive in tanks with running water until they can be processed. The species should be separated by gender for subsequent sex-specific biological effect measurements. Round fish do not survive the trawling process and should be separated by species. The number of fish required (see Table 3) should be set aside quickly and identified as samples either for sensory evaluation, chemical analysis or biological effect measurement (see Table 2). Other species sampled should be separated by species.

7.5.3.2 Round fish species

Round fish should be collected for chemical analysis and sensory assessment, and if appropriate the determination of biological effect measurements. It may not always be possible to undertake

biological effect measurements, but samples should be available in case measurements can be made.

Whole fish should be transported to the processing area in for example, plastic buckets. For individual species, the sample size should be approximately the same. Where possible, two sets, each comprising five fish should be processed.

Using an appropriate knife, the ventral side of the fish should be opened and an incision made the full length of the stomach from the vent to the throat. The liver should then be removed and the bile duct exposed. An aliquot, typically up to 500 µl, of the bile should then be taken and transferred to a suitable, labelled container. The sample should be immediately frozen using liquid nitrogen.

The remainder of the gut should then be removed, including heart and liver, and the fish left for approximately 15 minutes to bleed. After this period the fish should be rinsed with cold water prior to filleting. A portion, typically, 500 mg of the liver should then be taken for biological effect measurements, and placed in a suitable, labelled container, for example a cryovial. The samples from the five fish should then be individually and immediately frozen using liquid nitrogen. For each fish, the portion of liver should be taken from approximately the same area, and the remainder of the liver should be wrapped, for example in aluminium foil, and stored at -20 ^{o}C. Each fish should be filleted and the fillets skinned. The fillets should then be divided into two groups, one from each side of the fish. One group of fillets should be used for the sensory assessment evaluations and the other group for chemical analysis. The fillets should be rinsed under running uncontaminated sea water or potable water as appropriate, suitably wrapped, for example in aluminium foil and frozen at -20 ^{o}C. After freezing, the fillets should be placed in plastic bags that are then sealed and appropriately labelled.

7.5.3.3 Flat fish species

If a sufficient number of male fish are available, this should constitute the sample. If sufficient numbers of male fish are not available, then female fish may be used. In any event, details of the gender of the fish processed should be recorded. Where possible, two sets, each comprising five fish should be processed. The fish should be humanely killed and the length and weight of each fish recorded. The gut cavity should then be opened and the liver and bile duct exposed. Care should be taken to avoid contaminating the liver with bile salts or damaging the bile duct. An aliquot, typically 500 µl, of bile should be collected and transferred to a suitable container and immediately frozen using liquid nitrogen. The liver should then be removed, weighed and a portion taken for biological effect measurements. For each fish, the portion of liver should be taken from approximately the same area and the remainder of the liver should be wrapped, for example in aluminium foil, and stored at -20 ^{o}C. The gonads should then be removed and placed in a suitably sized plastic bag, labelled and stored frozen. The weight of this sample should be recorded, generally in the laboratory, wherever small amounts of material can be weighed accurately. The remainder of the gut should then be removed and discarded.

Each fish should then be filleted and the fillets skinned. Fillets should be cut longitudinally to give 4 fillets per fish. From the 20 fillets, 10 should be used for the sensory assessment evaluations and 10 for chemical analysis. The fillets should be rinsed under running uncontaminated sea water or potable water as appropriate, suitably wrapped, for example in aluminium foil and frozen at -20 ^{o}C. After freezing, the fillets should be placed in plastic bags that are then sealed and appropriately labelled.

7.5.3.4 Norway lobster

Using a plastic bucket or basket, a whole Norway lobster should be transferred to the processing area. The head and carapace with the claw attached should then be removed. The gut should also be removed by twisting off the middle tail section and the gut extruded. The unshelled tail should be rinsed in uncontaminated seawater to remove external debris. The tail (as groups of twenty) should then be wrapped, for example in aluminium foil, and frozen at -20 ^{o}C. After freezing, the sample should be placed in a plastic bag that is then sealed and appropriately labelled.

7.5.3.5 King and queen scallops

Using plastic buckets or baskets, the scallops should be transferred to the processing area. The shells and the adductor muscle and gonad, if present, should then be removed. The tissues should then be rinsed in uncontaminated water, seawater or potable water as appropriate, to remove external debris. Muscle tissue and gonad material (as groups of an appropriate number) should then be wrapped, for example in aluminium foil, and frozen at -20 ^{o}C. After freezing, the sample should be placed in a plastic bag that is then sealed and appropriately labelled. The shells should then be discarded, and the remaining soft tissue (mantle, hepatopancreas etc) placed in a plastic bag, sealed, appropriately labelled and frozen. This material may be useful for the determination of shellfish toxins, if required.

7.5.3.6 Lobsters and crabs

Using a plastic bucket or basket, the whole lobster or crab should be transferred to the processing area. The lobster or crab should be humanely killed, for example by being placed in a freezer at less than -20 °C for 12 hours, whereupon it is removed and allowed to thaw to room temperature. For both species, the claws should be removed and the claw meat extracted and wrapped, for example in aluminium foil, and frozen at -20 ^{o}C. After freezing, the sample should be placed in a plastic bag that is then sealed and appropriately labelled. The body should then be longitudinally cut into two pieces. One portion should be submitted for chemical analysis, and the other for sensory assessment. Each piece should be individually wrapped, for example in aluminium foil, and frozen at -20 ^{o}C. For crabs, the white and brown flesh meat should be wrapped and frozen separately. After freezing, the samples should be placed in plastic bags that are then sealed and appropriately labelled.

7.5.3.7 Other species

Other species which may include periwinkles etc should be transferred to the processing area and washed under running uncontaminated water, for example seawater or potable water, as appropriate. The samples should then be wrapped, for example in aluminium foil, and frozen at -20 ^{o}C. After freezing, the samples should be placed in plastic bags that are then sealed and appropriately labelled.

7.5.4 Shore sampling

For mussels undergoing sensory assessment evaluations, the shells should be rinsed in potable water to remove any surface debris and packed in ice to keep them alive prior to testing. If mussels are only to undergo chemical analysis, the shells should be opened and the whole tissue removed. These tissues should then be stored in metal containers for example aluminium cans, and appropriately labelled and stored at -20 ^{o}C. The digestive gland should be excised and placed in a suitable container and immediately frozen in liquid nitrogen. After freezing the can should be appropriately labelled and stored at -70 ^{o}C.

7.6 Storage

7.6.1 On-board

When fish or shellfish cannot be processed on board the fishing vessel, the species should be stored appropriately[65], for example in cool boxes covered with wet paper or seaweed and ice packs placed on top. When stored under these conditions, it would not be appropriate to carry out certain biological effect measurements, for example EROD measurements. Round fish do not survive after being caught. However, flat fish and other species can be kept alive in suitable holding tanks supplied with a continuous flow of uncontaminated water until required for processing. After processing, samples should be frozen and stored using conditions appropriate to the sample. Factors for consideration include the delay between harvesting and testing of the samples, the nature of harvesting and the species. It cannot be over-emphasised that each sample should be appropriately and securely labelled. If correctly carried out, this will eliminate problems over misidentification of samples. A summary of details of treatment and storage conditions is given in Tables 4 and 5.

Table 4 Species and storage conditions

Type of sample	Treatment and storage
Fish flesh for sensory assessment.	Frozen and stored at -20 ^{o}C or below.
Fish flesh, shellfish tissue and liver tissue for chemical analysis, for example PAH determination.	Frozen and stored at -20 ^{o}C or below.
Shellfish tissue and fish liver for biological effect measurements.	Immediately frozen in liquid nitrogen and stored at -70 ^{o}C or below.
Shellfish tissue for sensory assessment (excluding mussels).*	Frozen and stored at -20 ^{o}C or below.

* Mussels should be kept alive prior to sensory assessment, as freezing prevents the muscle from being extracted whole.

Table 5 Storage and transportation of samples for sensory assessment evaluations

Species	**Storage and transportation to laboratory**		
	Frozen storage	***Iced storage***	***Refrigerated storage***
Round fish/flat fish	Frozen and stored at -20 ^{o}C for a maximum of 3 months.	If no freezing facilities are available, store whole gutted fish on ice. If the delay in testing is greater than 72 hours, samples should be frozen.	If testing within 72 hours, refrigerate at 4 ± 2 °C.
Shellfish (excluding mussels)	Frozen and stored at -20 ^{o}C for a maximum of 3 months.		
Mussels		Mussels should be kept alive on ice prior to sensory assessment. If the delay in testing is greater than 72 hours, samples should be cooked, the cooked flesh removed from the shells and frozen.	If testing within 72 hours, refrigerate at 4 ± 2 °C.
Norway lobsters, lobsters or crabs	Frozen and stored at -20 ^{o}C for a maximum of 3 months.	If no freezing facilities are available, for lobsters and crabs bind the claws and keep alive or store on ice, and for Norway lobster store tails on ice. If the delay in testing is greater than 72 hours, samples should be frozen.	If testing within 72 hours, refrigerate at 4 ± 2 °C.
Salmon or trout	Frozen and stored at -20 ^{o}C for a maximum of 3 months.	If no freezing facilities are available, store whole gutted salmon or trout on ice. If the delay in testing is greater than 72 hours, samples should be frozen.	If testing within 72 hours, refrigerate at 4 ± 2 °C.

8 Analysis of hydrocarbon compounds in sediment and biota

8.1 Introduction

This section provides guidance on the quantitative determination of hydrocarbon compounds in samples of sediment and biota. The methods are applicable to typical oil spill incidents but may not be suitable, without suitable modification, for determining hydrocarbon products which are highly volatile (for example jet fuel and petrol) or comprise high molecular weight compounds (for example heavy fuel oils, bitumen and waxes). Neither are they appropriate for vegetable-based trigylceride oils. The laboratory analysis of water samples for hydrocarbon compounds is described elsewhere in this series[42]. In-situ measurements of hydrocarbon compounds in water by UVF are described in Section 5.

In the case of petroleum hydrocarbon compounds, these may be present in their natural form (as crude oil) or as refined distillate products. Each crude oil of a particular source comprises varying proportions of numerous general classes of compounds, for example alkanes, alkenes, cycloalkanes, aromatic and polycyclic aromatic compounds etc. In addition, each class of compounds incorporates a vast number of individual compounds, each with slightly differing properties.

8.2 Method selection and terminology

A variety of different analytical techniques have been developed to determine hydrocarbon concentrations in environmental samples and many methods have been optimised to focus on specific compounds or group of compounds[43, 44, 66, 67]. There is no single analytical method that is suitable for all situations or circumstances. Analysts should, therefore, carefully select the method or group of methods that suit the intended purpose. This will ensure that the results generated are fit for the intended purpose. Unfortunately, the large number of methods for hydrocarbon analysis has led to an equally large number of associated terms to describe the results determined. These terms (many of which are poorly defined) are often meaningless and lack clarity. For example, the following terms (plus others, but not including the vast range of PAH compounds) have all been used to express hydrocarbon data;

- total petroleum hydrocarbons;
- total hydrocarbons;
- total oil;
- mineral oil;
- total oil and grease;
- hexane extractable material;
- hydrocarbon oil index;
- petroleum range organics; and
- diesel range organics

This ambiguity and lack of consistency has led to a significant amount of confusion over the expression of results and their consequential interpretation. The analytical method should, therefore, be clearly and unambiguously stated with the results, thus minimising the possibility that they will be used inappropriately or taken out of context.

8.3 PAH determinations

Polycyclic aromatic hydrocarbons (either as parent or alkylated compounds) are an environmentally important group of compounds. A relatively small number of the parent PAH compounds, often based on toxicity effects and relative environmental abundance, has been determined for routine

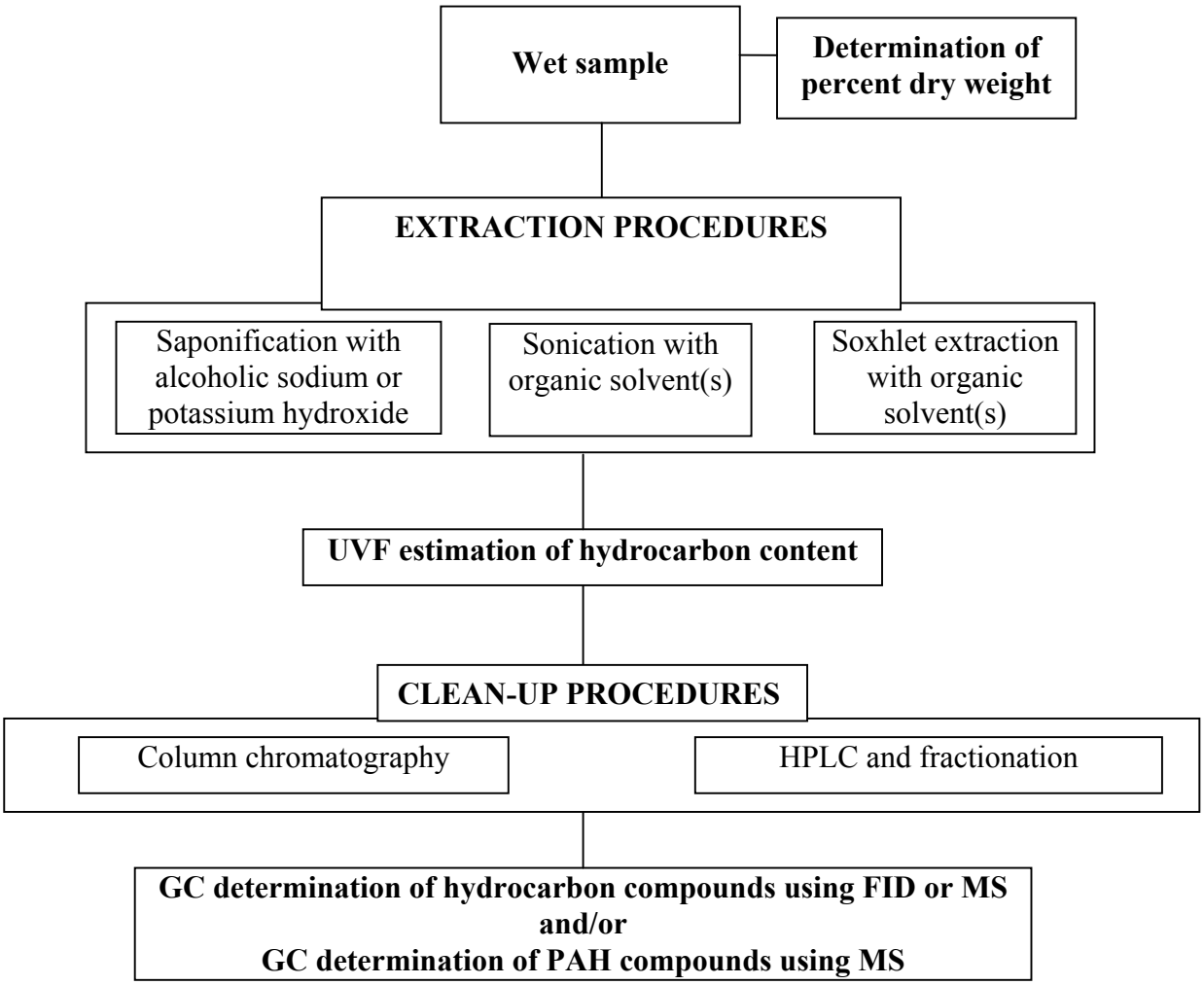
monitoring purposes[66]. These compounds however represent only a very small fraction of the total number of PAH compounds present in the environment. When investigating petroleum contamination it is important to include the analysis of alkyl substituted PAH compounds as well as parent PAHs and other aliphatic and aromatic hydrocarbon compounds, since these compounds may significantly contribute to the overall hydrocarbon content. The analyst should therefore ensure that the most appropriate hydrocarbon compounds are selected for analysis, in order to provide the necessary information required for each study.

When parent PAH compounds are determined, the analysis should be subjected to full quality assurance and quality control procedures using, for example certified reference materials. For the determination of alkylated PAH compounds, these reference materials are generally not so widely available and other procedures need to be employed, see also Section 9.2. Hence, in order to allow inter-comparison of data, the technique used and the manner in which results are expressed should be reported to ensure the opportunity for misinterpretation of data is reduced and results are compared on a like-for-like basis.

8.4 Selection of methods

Figure 9 illustrates an overall scheme of analysis (with options) that can be followed when the determination of hydrocarbon compounds in sediment and biota needs to be undertaken.

Figure 9 Analytical pathway for the determination of hydrocarbon compounds in sediments and biota



In addition, Tables 6 to 8 show summary comparisons of various techniques and procedures used in the determination of hydrocarbon compounds.

Table 6 Extraction procedures

	Saponification	**Sonication**	**Soxhlet extraction**
Time required	Approximately 2 hours.	Approximately 1 hour.	At least 6 hours.
Equipment required	Basic laboratory glassware and reagents, heating mantle, water supply for condensers.	Basic laboratory glassware and reagents, ultrasonic bath.	Soxhlet extraction equipment and basic reagents, heating mantle, water supply for condensers.
Description	Hydrocarbons extracted and break-down of lipids and complex isomers of sulphur leading to a cleaner overall extract.	Depending on solvent used yields an extract containing a wide range of organic compounds.	Depending on solvent used yields an extract containing a wide range of organic compounds.
Safety considerations	Hot alcoholic alkali used which is flammable and toxic.	Flammable and/or toxic solvents used.	Hot flammable and/or toxic solvents used.
Sample matrix	Sediment and biota.	Sediment and biota. The extract from biota often requires further clean-up.	Sediment and biota. The extract from biota often requires further clean-up.

Table 7 Clean-up procedures

	Column chromatography	**HPLC and fractionation**
Time required	Manual technique - relatively quick, column preparation required.	Automated procedure, relatively quick, regular maintenance required.
Equipment required	Basic laboratory glassware and reagents.	Dedicated HPLC instrumentation required - including analytical column.
Description	Sorbent retains polar organic compounds, allowing elution of the hydrocarbon fraction.	Stationary phase analytical column retains polar organic compounds, allowing elution of the hydrocarbon fraction.
Safety considerations	Flammable and/or toxic solvents. Absorbents may pose an inhalation risk.	Flammable and/or toxic solvents.
Sample matrix	Extracts from sediments and biota.	Extracts from sediments and biota.

Note: The saponification extraction procedure may be used effectively on a wide range of sample matrices. Relatively clean solvent extracts are produced that may not require further clean-up, either by traditional column chromatography or HPLC and fractionation. Other less frequently used extraction techniques, for example pressurised solvent extraction, and clean-up procedures, for example gel permeation chromatography may also be used.

Table 8 Hydrocarbon quantification

GC using FID or MS	UVF
Possible to quantify concentrations of individual aliphatic and aromatic hydrocarbon compounds. Relatively high LOD, low sensitivity.	Not possible to quantify individual hydrocarbon compounds, especially aliphatic hydrocarbons. Relatively low LOD, high sensitivity.
Requires a clean sample extract.	Analysis can be undertaken on sample extract with no clean-up step.
Significant losses of volatile hydrocarbons may occur due to the required evaporation of the sample extract prior to analysis. These losses may be less significant (in terms of detection, but not accuracy) if high hydrocarbon concentrations are present.	No evaporation stage required prior to analysis. Sample extracts may contain higher proportions of the more volatile hydrocarbon compounds.
Requires experienced operator, relatively high maintenance requirement for instrumentation. More detailed preparation required.	Less intricate measurement procedure, less maintenance for instrumentation. Relatively quick and easy sample preparation.
Enables more detailed investigations to be undertaken.	A good screening method that may be supplemented with GC determinations.

Note: The estimation of hydrocarbon compounds based on the determination by UVF is a good general screening method and is capable of detecting very small amounts of hydrocarbons in a wide range of matrices. However, the technique is based on the measurement of relatively small numbers of aromatic compounds and the correlation of these results with results obtained from suitable reference oils. In the case of an oil spill incident, this may normally be a sample of the spilled oil or reference oil from the same source. Following an oil spillage, natural weathering of the spilled oil may occur and the technique may lead to significant variations being observed between the results of the oil sample and the results obtained from the reference oil. This will lead to difficulties over the interpretation of data obtained via UVF measurements. The hydrocarbon concentrations obtained with GC using FID or MS detection are less susceptible to environmental and source oil variations. This type of analysis also provides information on the distribution patterns of hydrocarbon compounds present in the oil. In addition, typical GC-MS analysis of PAH compounds (in place of GC-FID methodologies) provides potentially more data for the alkylated PAH compounds as well as the parent PAH compounds.

8.5 Methods

See Annex 3 for details of methods for the extraction, clean-up and determination of hydrocarbon compounds in sediments and biota. Methods A, B and C give details of extraction techniques; methods D, E and F give details of clean-up procedures; and methods G, H and I give details for determining hydrocarbons in sediments and biota. Other methods in this series[42 - 44, 66, 67] may also be suitably adapted.

8.5.1 Reagents and apparatus

All reagents should be of sufficient purity so that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemicals and reagents and verified by running procedural blanks with each batch of samples analysed. Solvents suitable for high performance liquid chromatography, glass distilled or pesticide use and analytical

grade materials are normally suitable unless otherwise stated and details of preparation are given, where appropriate.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed containers or other suitable vessels and kept in the dark, if necessary.

To avoid excessive evaporation of solvent, all standard solutions should be stored in a refrigerator. However, prior to use, all solutions and solvents should be allowed to reach ambient room temperature before volumetric measurements are made. When a standard solution is required for use, the flask and its contents can be weighed, the stopper removed and a portion of the solution transferred to a clean, dry suitable container. The stopper is then replaced and the flask and its (reduced) contents weighed again. If, after appropriate storage, any significant difference occurs in the weight of the flask and its contents since it was last used, then this might indicate a possible change in the concentration of the standard solution. Standard solutions of hydrocarbon compounds including n-alkanes and PAH compounds are available from a number of suppliers and their purchase may be more appropriate than their in-house preparation.

In addition to normal laboratory glassware, specific items are identified within the methods. All glassware should be soaked in an appropriate detergent or dilute acid, for example 10 %v/v nitric acid. After cleaning, the glassware should be rinsed with water, allowed to dry, and then rinsed with an appropriate solvent. Glassware should then be thoroughly dried.

8.5.2 Sample collection and preparation

Procedures for the collection of representative samples of sediment and biota, both contaminated and uncontaminated, for hydrocarbon analysis are given in Section 7. Frozen samples should be defrosted, and wherever possible, representative or homogenised sub-samples taken for analysis.

8.5.3 Determination of percent dry weight

Whilst the concentration of hydrocarbons in sediments (and soils) is usually expressed on a dry weight basis, the concentration of hydrocarbons in biota may be expressed on a wet weight or dry weight basis. In either case, the dry weight content of the sample should be reported to enable comparisons of results to be undertaken.

A representative or homogenised sub-sample, typically 10-15 g of the wet sample (S_{ww} g) should be taken and accurately weighed into a pre-weighed metal dish (D g). This sample should, as far as is possible, be identical to the sample undergoing hydrocarbon analysis. The dish and its contents should then be placed in an oven at, for example 105 ± 2 ^{o}C for about 16 hours. The temperature and drying time will vary depending on the nature and composition of the sample. For example, if the sample contains significant amounts of volatile or semi-volatile organic compounds, these compounds together with water will be driven off from the sample during drying. This will affect the actual "moisture" content determined. After the appropriate temperature and drying time the dish should be removed from the oven and allowed to cool in a dessicator. The dish and dried contents are then weighed (D_{dw} g) and the percent dried weight (DW) calculated according to the following equation.

$$\text{percent dried weight, DW} = \frac{(D_{dw} - D)}{S_{ww}} \times 100$$

where

D_{dw} is the weight of the dish and dried sample;
D is the weight of the empty dish;
S_{ww} is the weight of the wet sample.

8.5.4 Extraction

Generally, extraction techniques involve intimate mixing of samples with a suitable solvent or solvent mixture. Different solvents and conditions may be used depending on the type of hydrocarbon to be extracted and the nature of the sample. The procedures described in Annex 3 should enable a solvent extract to be obtained that is suitable for

- the estimation of hydrocarbon compounds using a screening test based on UVF; and
- following suitable clean-up, for determination of hydrocarbon compounds, including specific PAH compounds, by GC using FID or MS.

Incomplete extraction of the sample may occur with samples that contain high levels of hydrocarbon compounds. Where a sample is known or suspected of containing high levels of hydrocarbons, the amount of sample used in the extraction should be reduced proportionately. Where it is not known, and subsequent extraction or analysis reveals high levels, the extract should be discarded. The sample extract should not be diluted. The analysis should then be repeated using a smaller amount of sample. At all times, and especially when the amounts of sample to be extracted are reduced, great care should be taken to ensure that the sample analysed remains representative of all the material submitted. Methods A, B and C in Annex 3 give details of suitable extraction techniques. It is sometimes the case that when saponification procedures are used no subsequent clean-up procedures are required. The saponification process includes a back-extraction procedure which often reduces the need for further clean-up.

8.5.5 Clean up procedure

Sample extracts to be analysed by UVF generally do not require clean-up. Sample extracts to be analysed by GC using FID or MS detection methods usually require a clean-up procedure to be used to remove polar, co-extractable material. For example, lipids and higher molecular weight acids may interfere with the GC determination. The extent of the clean-up depends upon the amount of co-extracted material present and the nature of the extraction technique. The saponification process breaks down lipids into fatty acid salts and destroys complex isomers of sulphur (for example S_8) and mercaptans. Subsequent clean-up requirements may therefore be minimal. Extractions based on sonication and Soxhlet techniques do not tend to break down lipids, and hence these extracts usually contain higher quantities of co-extracted material. Subsequent clean-up may therefore be required. Methods D, E and F give details of suitable clean-up procedures.

8.5.6 Checking the validity of analytical data

Before analytical methods are brought into routine use, they should be validated and performance tested to ensure confidence in the results reported. The performance of an analytical method is generally based on an assessment of precision, bias and limit of detection. The values established for these performance characteristics will vary with individual laboratories depending on the capability of the analyst, analytical technique used and the instrumentation available.

There may also be differences in the way laboratories determine and report performance characteristics. For example, laboratories may report analytical reproducibility for samples, spiked

samples or standards. The number of replicate samples analysed and replicate determinations carried out, and therefore, the number of degrees of freedom reported, may also differ.

Once a method is used routinely, many factors may subsequently adversely affect the reliability of the analytical result. Experimental tests should be carried out regularly to check sources of inaccuracy. Analytical quality control samples should be used to check the integrity of the standard solutions and the performance of the instrumentation. In addition, where appropriate, replicate analyses may need to be carried out at regular intervals.

The analysis of certified reference materials enables an estimate of bias to be determined. However, few appropriate or relevant certified reference materials are available. Where certified reference materials are not available, the use of spiking experiments enables an estimate of the recovery efficiency to be determined as an alternative to bias. In addition, the use of laboratory reference materials provides a useful means of providing information on the long-term precision of the analysis.

8.5.7 Typical method performances

The performance characteristics of analytical methods depend on many factors and unless established to known and identical criteria make direct comparisons difficult, if not impossible. Tables 9-12 illustrate typical performances achieved routinely in several laboratories using a variety of methods to determine hydrocarbon compounds in sediments and biota. Data were provided by laboratories from the Centre for Environment, Fisheries and Aquaculture Science, Environment Agency, ERT (Scotland) and Fisheries Research Services Marine Laboratory.

Table 9 Analysis of hydrocarbon compounds in sediments using UVF

Mass of sample extracted	30-40g (wet)
Extraction technique	Saponification
Clean up	not applicable
LOD	0.05 - 0.1 mg kg^{-1}
RSD	2.7 - 6.3% (with up to 9 degrees of freedom)

Table 10 Analysis of hydrocarbon compounds in sediments using GC-FID

Mass of sample extracted	<50g (wet)
Extraction technique	Sonication or soxhlet
Clean up	Column chromatography (large scale)
LOD	1 mg kg^{-1}
RSD	15%

Table 11 Analysis of PAH compounds in sediments using GC-MS

Mass of sample extracted	30-40g (wet)
Extraction technique	Saponification
Clean up	Column chromatography (small scale)
LOD	0.10 µg kg^{-1} (individual compounds)
RSD	<15% (depending on PAH, with 19 degrees of freedom)

Table 12 Analysis of PAH compounds in sediments using GC-MS

Mass of sample extracted	2-20.5g (wet)
Extraction technique	Sonication
Clean up	HPLC
LOD	0.20 µg kg^{-1} (individual compounds)
RSD	<15% (depending on PAH, with 7 degrees of freedom)

9 The use of fingerprinting techniques in relation to oil spills

9.1 Introduction

The concentration of hydrocarbon compounds, including aliphatic, monocyclic aromatic and polycyclic aromatic hydrocarbons (PAHs) are determined in a variety of matrices (for example water, sediments, biota etc) for a number of purposes. These include compliance monitoring, source identification, the assessment of pollution impacts (for example following oil spills) and for source fingerprinting purposes (i.e. the possible identification of the source of the oil). Crude oils are complex mixtures of highly variable compositions comprising many individual compounds. The composition of crude oils from different locations depends on the conditions under which the oils are originally formed. Hence, knowledge of the type of compounds present in crude oils (as individual compounds, groups of similar compounds or isomer distribution patterns) originating from specific sources, coupled with information on respective concentrations, enables a series of indices to be formulated. These indices can then be used to possibly predict or identify particular oil spills originating from specific types of oil from known sources[68, 69, 70].

Crude oils and the lighter refined products produced from crude oils undergo rapid changes following release into the environment from incidents such as oil spills. Processes such as evaporation, dissolution, biodegradation, and photo-oxidation all contribute to these changes[71]. The large surface area of an uncontrolled spreading oil slick on the surface of the sea makes evaporation of the volatile components of the oil and dissolution of the more polar components the dominant processes by which these changes occur to the composition of the spilled oil.

Whilst many of the compounds present in oil are potentially useful as fingerprinting markers, those which are rapidly lost from the oil after release into the environment are less useful than others which remain within the oil. Hence, the less volatile, less water-soluble compounds, which are also more resistant to degradation, are preferred for long-term monitoring comparison purposes[72].

9.2 Sampling and analysis

Crude oils contain thousands of different chemical compounds and, hence, the chemical composition of crude oils from different regions, and even within a particular region varies enormously. Hydrocarbon compounds are the most abundant type of compounds found in crude oils, accounting for as much as between 50 - 98 % of the total composition. Crude oils also contain compounds which contain heteroatoms such as nitrogen, oxygen or sulphur either as heterocyclic constituents, for example thiophene and pyrrole or within a functional group[73].

A sample of the spilled oil should be obtained for fingerprinting and reference purposes and diluted with a suitable solvent. A final concentration of approximately 1000 mg l^{-1} is typically appropriate. Suitable (less polar) solvents include pentane and n-hexane. Certain hydrocarbons, for example asphaltenes are, however, not soluble in these solvents and will precipitate out of solution.

Dichloromethane, a more polar solvent, dissolves asphaltenes. However, asphaltenes cannot adequately be separated by gas chromatography, and if injected into a gas chromatographic capillary column will eventually lead to degradation of the column and result in loss of chromatographic performance. Hence, care needs to be taken to ensure the correct solvent is used.

From a gas chromatographic point of view, the lower-boiling hydrocarbon components of crude oil are better resolved with on-column injection techniques than with split-less injection techniques. Traditionally, gas chromatography with flame ionisation detection was used to generate hydrocarbon profiles, especially in samples where the homologous n-alkane series of compounds can be readily seen. However, with the advent of bench-top mass spectrometers, in particular the ion-trap mass spectrometer which offers high sensitivity in scanning mode, gas chromatography with flame-ionisation detection is becoming less popular. The ability of mass spectrometers to collect data across a very wide mass range is a valuable technique for fingerprinting purposes, especially when multiple-ion detection techniques are used and ions (and hence, compounds of interest) can be detected. In addition, with full-scan data being collected routinely, a wide range of fingerprinting techniques can be applied to single sets of data and across a wide range of samples.

The chromatographic conditions used for the analysis of hydrocarbons are often dependent on the type of oil being analysed (for example, whether the sample comprises a crude oil, or a refined product such as petrol, diesel, or a light, medium or heavy fuel oil). Injection temperatures will need to be low (i.e. less than 50 °C) if low-boiling distillate fractions such as petrol, kerosene, or jet fuel are being analysed. Generally, fused-silica capillary columns, 25 - 50 m in length, 0.2 - 0.3 mm internal diameter, with non-polar or slightly polar stationary phases, will yield adequate resolution. The mass spectrometer is usually operated in the positive-ion electron impact mode with an ionisation energy of about 70 eV. Cycle times of about 1 second or less are required in order to obtain the desired resolution and peak shapes.

An example of a formalised protocol giving details of sampling procedures, transport, storage and analysis has been reported[74] recently. These procedures can be used following oil pollution incidents and help facilitate and establish the identification of oil samples, compared with information obtained from suspected or known sources.

Calibration procedures involving parent PAH compounds are relatively straightforward, as un-substituted PAH compounds are readily available. In addition, a wide range of deuterated parent PAH analogues (for example naphthalene-d_8, $C_{10}D_8$, MW 136) is also available for use as internal or surrogate standards. For alkyl-substituted PAH compounds, the situation is quite different. In most cases, only a limited number of the many alkyl-substituted PAH isomers are available. The determination of alkylated PAH compounds is therefore, generally, an approximation, being based on this limited number of isomers for which response factors may be determined. Within a single laboratory study, this may not be an issue. For inter-laboratory studies, especially where determinations are compared, and different calibration standards have been used, this may be of concern, since like-for-like comparisons may not be possible. If proper data comparisons are to be made, it may be necessary for laboratories to agree common issues and specify individual alkylated PAH compounds prior to commencement of analysis. To some extent, however, these issues are alleviated due to the very wide range of PAH concentrations encountered in oil spill monitoring studies. The range of concentrations encountered far outweighs the differences in calibration procedures.

9.3 Compounds used for fingerprinting purposes

For oil fingerprinting purposes, two main classes of compounds can be used. These are:

- classes of compounds which retain those characteristics of the original plant material from which the crude oil was formed; and

- classes of compounds which represent specific hydrocarbons, for example PAH compounds and heterocyclic aromatic compounds.

In general, mass chromatograms of the diagnostic ions (which might be molecular ions or characteristic fragmentation ions of the compounds of interest) are acquired or compiled from full-scan data. The chromatograms of oil samples taken following a spillage incident and oil samples from known or suspected sources, can be compared either visually or statistically, using various techniques.

9.3.1 *Alkanes*

Fingerprinting techniques for determining n-alkanes have been commonly used following oil spills. Fresh crude oils and distillate products (other than lubricating oils) generally contain compounds from the homologous n-alkane series of compounds, see Figure 3A. However, there are exceptions to this, and these include those crude oil samples and their derivative products where the crude oil has undergone biodegradation at source in the underground oil reservoir prior to extraction and subsequent fuel production, see Figure 3B. In chromatograms of fresh crude oil samples, the peaks due to n-alkanes are usually very prominent. Where the crude oil undergoes biodegradation or weathering, as in cases following oil spills to the environment, the chromatograms of these samples show n-alkane peaks where the relative proportions are reduced. A system for pattern matching of n-alkane profiles of oils and suspected sources has been developed recently[75].

A simple chromatographic index that can be used to follow the progress of biodegradation of n-alkanes relates the peak-height of the branched isoprenoid alkane, pristane (2, 6, 10, 14-tetramethylpentadecane, $C_{19}H_{40}$) to the peak-height of the adjacent straight chain n-alkane, n-heptadecane ($C_{17}H_{36}$). Following an oil spill[76], this index ratio (i.e. C_{17}/pristane) was initially shown to be 4.9 in fresh crude oil collected close to the wreck (see Table 13). This ratio reduced progressively as degradation proceeded (i.e. the C_{17}-alkane concentrations decreased). The slow progress of biodegradation in a sample (site 12) after 1 year was explained by reference to the fact that the oil was buried during a beach building phase and uncovered later. A similar index based on the ratio of the peak-height of the isoprenoid alkane phytane (2, 6, 10, 14-tetramethylhexadecane, $C_{20}H_{42}$) to that of the adjacent n-alkane n-octadecane ($C_{18}H_{38}$) can also be used in the same way.

Table 13 The use of the C_{17}/pristane ratio highlighting degradation process following an oil spill

Number of days after spill	Site number and location	C_{17}/pristane ratio
1	1 - Close to wreck	4.9
6	2 - Close to wreck	4.4
13	3 - 3 miles from wreck	3.7
13	4 - 30 miles from wreck	4.6
19	5 - Close to wreck	3.1
65	6 - 30 miles from wreck	3.2
65	7 - 30 miles from wreck	3.4
67	8 - 40 miles from wreck	2.3
82	9 - 40 miles from wreck	2.1
84	10 - 3 miles from wreck	3.3
85	11 - 30 miles from wreck	3.3
379	12 - 45 miles from wreck	4.5

These indices can provide an indication of biodegradation that takes place (or has taken place) in spilt oil relative to oil from the original or known source. Where the discrimination of the individual chromatographic peaks is difficult, mass chromatograms of common fragmentation ions can be used to improve detection of the straight-chain compounds (for example, 57 Daltons due to $C_4H_9^+$, 85 Daltons due to $C_6H_{13}^+$ and 113 Daltons due to $C_8H_{17}^+$).

Biogenic sources of recent origin show a high predominance of n-alkanes containing an odd number of carbon atoms. Terrestrial plants are characterised by n-alkanes containing odd numbers of carbon atoms in the range C_{23}-C_{33} (i.e. n-tricosane, $C_{23}H_{48}$ to n-tritriacontane, $C_{33}H_{68}$), and marine biogenic inputs (for example from phytoplankton) are often marked by the presence of n-pentadecane ($C_{15}H_{32}$) n-heptadecane ($C_{17}H_{32}$) and n-nonadecane ($C_{19}H_{40}$). Biogenic inputs may also show high concentrations of pristane, and the concentration ratio of pristane to phytane is usually much greater than one. Biogenic compounds also include polyolefins such as heneicosahexaene ($C_{21}H_{36}$) in algae and squalene (2, 6, 10, 15, 19, 23-hexamethyl-2, 6, 10, 14, 18, 22-tetracosahexaene, $C_{30}H_{50}$) in higher animals, such as sharks.

Other peaks (of which there are many) within the chromatograms are derived from cyclic and alkylated alkanes, and as the carbon length increases, the number of possible isomers increases dramatically. See Table 14. The chromatographic peaks from these alkylated alkanes generally appear as a fine structure, interspersed between and overlapping the n-alkane peaks. As the isomer patterns are too complex, the majority of individual compounds cannot readily be distinguished and generally appear as an unresolved complex mixture in the chromatogram. However, the recognition of selective patterns may be used as a simple aid for fingerprinting purposes.

Table 14 Alkylated isomers of n-alkanes

Alkane	Carbon length	Number of possible alkylated alkanes
methane	1 (CH_4)	1
ethane	2 (C_2H_6)	1
propane	3 (C_3H_8)	1
butane	4 (C_4H_{10})	2
pentane	5 (C_5H_{12})	3
hexane	6 (C_6H_{14})	5
heptane	7 (C_7H_{16})	9
octane	8 (C_8H_{18})	18
nonane	9 (C_9H_{20})	35
decane	10 ($C_{10}H_{22}$)	75
dodecane	12 ($C_{12}H_{26}$)	355
pentadecane	15 ($C_{15}H_{32}$)	4,347
eicosane	20 ($C_{20}H_{42}$)	366,319

In addition to the alkylated alkanes, chromatographic peaks of cyclic alkanes (including the aromatic compounds such as naphthenes etc) likewise cannot be adequately resolved and individual peaks distinguished. These peaks, again appearing as fine structure, contribute to an unresolved complex “hump” above the baseline in the chromatogram (see Figure 3B). As already stated, n-alkanes are readily biodegraded. Hence, the usefulness of these compounds for fingerprinting purposes is essentially restricted to fresh and the more lightly weathered oils immediately following an oil spill. When techniques such as gas chromatography with flame ionisation detection were the primary analytical techniques used in this area, the complexity of the chromatograms and the fact that many peaks could not be resolved was a major limitation to their use. With access to techniques

using gas chromatography with mass spectrometric detection, this situation has changed and there are now a number of alternative approaches that can be adopted.

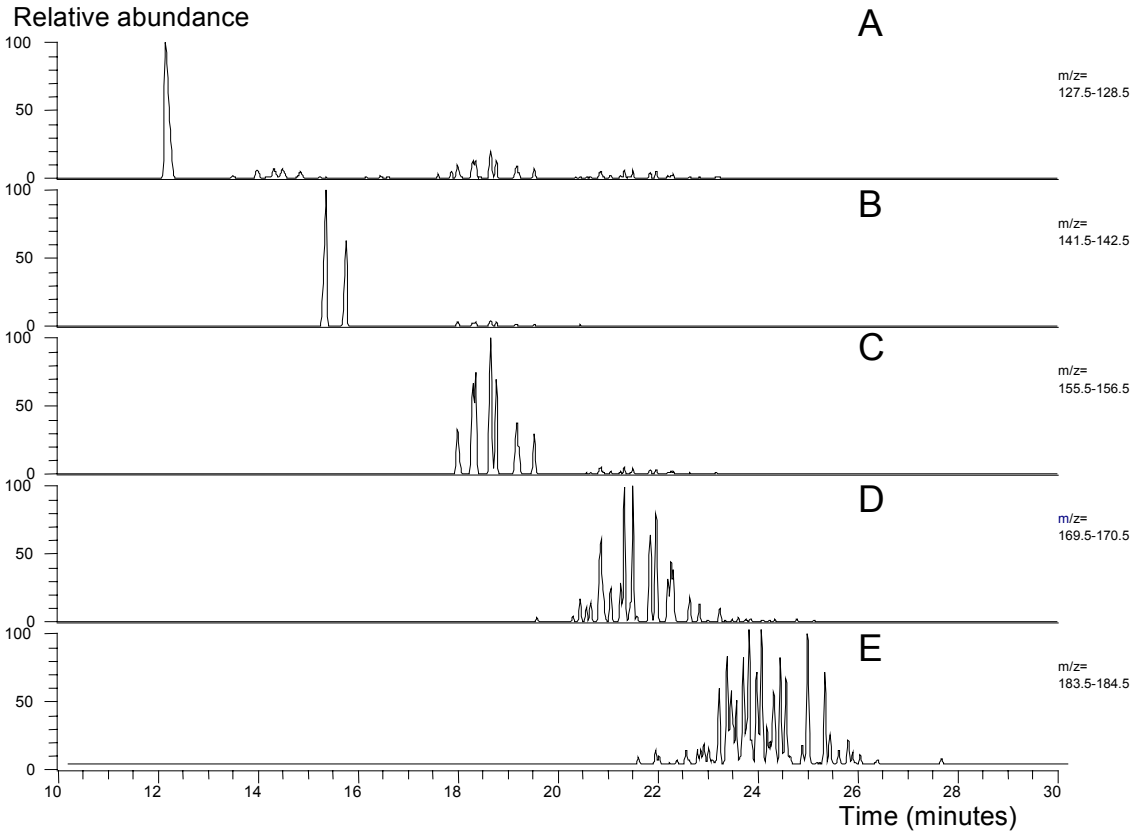
In recent years, spills of heavy fuel oil have become more frequent, both from oil product tankers and from the fuel tanks of large cargo vessels that use the fuel for propulsion. The density of heavy fuel oil is much closer to that of seawater than the majority of crude oils, which are usually lighter. In turbid waters, sediment material within the water column adheres to the heavy fuel oil, increasing the density of the oil, thus making it sink below the surface of the sea. This fuel oil can then be transported in the water column under the influence of prevailing tidal currents until either thrown up onto the beach, caught in fishing nets, or deposited onto the seabed[77, 78]. Heavy fuel oil transported in this way tends to remain as large "pancakes" which are not spread widely. This makes the oil less likely to undergo biodegradation as the "pancakes" possess low surface area to volume ratios. In contrast, crude oils are usually dispersed as fine droplets, whether by natural dispersion due to wave action or by the use of dispersant chemicals.

9.3.2 *Alkylated (alkyl-substituted) PAH compounds*

PAH compounds generated from combustion processes predominantly contain parent (un-alkylated or non-substituted) PAHs. In contrast, crude oils and distillate products contain a diverse range of PAHs and their alkyl-substituted PAHs. These alkylated PAH compounds are usually present at considerably higher concentrations than the parent PAH compounds (see Figure 10). Each parent PAH can be substituted at a number of different carbon atom positions, for example 1-naphthalene and 2-naphthalene substitution positions. In addition, the carbon length of the alkylated substituent group can vary. For naphthalene (the simplest 2-ring parent PAH compound) there are 2 mono-substituted isomers, 10 di-substituted isomers, and so on. For larger ring system PAH compounds[79] (there are over 350 different parent PAHs with 4 - 7 fused rings) when the substituent groups differ between the substituted positions, the potential number of possible compounds rises rapidly. The total number of possible isomers for each ring system PAH is dependent on the molecular symmetry of the parent PAH and the nature of the substituent groups. Hence, the potential number of different hydrocarbon compounds present in crude oil is enormous and it is common practice to group alkylated PAHs by their degree of alkylation.

From a fingerprinting viewpoint, this presents an ideal situation, as differences in the composition of crude oils will depend on the original conditions of formation when the crude oils were first formed. These conditions will result in different chemical transformations and hence different isomer patterns will emerge. Useful compounds for fingerprinting purposes are for example C_1-C_4-naphthalenes, C_1-C_3-fluorenes, C_1-C_4-dibenzothiophenes, C_1-C_4-phenanthrenes, and C_1-C_3-chrysenes. With the availability of full-scan data there are, therefore, many possible isomer groups that can be investigated. The alkylated dibenzothiophenes and phenanthrenes have been found to be useful for source identification purposes as have alkylated naphthalenes (see Figure 10), phenanthrenes and chrysenes. Indices based on these compounds can qualitatively assess the extent of weathering following an oil spill[80]. The distribution of isomeric methyldibenzothiophenes (C_1-dibenzothiophenes) has also been shown to provide an effective means of differentiating fresh crude oils and weathered oils[81].

Figure 10 Mass chromatogram of naphthalene and alkylated naphthalenes



A - naphthalene, $C_{10}H_8$, MW 128.
B - C_1-naphthalenes, $C_{11}H_{10}$, MW 142.
C - C_2-naphthalenes, $C_{12}H_{12}$, MW 156.
D - C_3-naphthalenes, $C_{13}H_{14}$, MW 170.
E - C_4-naphthalenes, $C_{14}H_{16}$, MW 184.

Similar chromatograms can be obtained for

- phenanthrene and anthracene and the alkylated C_1-, C_2- and C_3- phenanthrene and anthracene PAH compounds with mass fragmentation ions of 178, 192, 206 and 220 respectively.
- dibenzothiophene and the alkylated C_1-, C_2- and C_3- dibenzothiophene PAH compounds with mass fragmentation ions of 184, 212, 226 and 240 respectively.
- fluoranthene and pyrene and the alkylated C_1-, C_2- and C_3- fluoranthene and pyrene PAH compounds at mass fragmentation ions of 202, 216, 230 and 240 respectively.
- benz[*a*]anthracene, triphenlene and chrysene and the alkylated C_1- and C_2- benz[*a*]anthracene, triphenlene and chrysene PAH compounds with mass fragmentation ions of 228, 242 and 256 respectively.
- benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene and the alkylated C_1- and C_2- benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene and benzo[*a*]pyrene PAH compounds with mass fragmentation ions of 252, 266 and 280 respectively.
- indeno[1,2,3-*cd*]pyrene and benzo[*g,h,i*]perylene and the alkylated C_1- and C_2- indeno[1,2,3-*cd*]pyrene and benzo[*g,h,i*]perylene PAH compounds with mass fragmentation ions of 276, 290 and 304 respectively.

Following release of an unknown petroleum product into a river, the source of the oil was identified by determining and comparing the concentration ratios of a series of parent PAH compounds and

their alkylated derivatives[82]. Despite the fact that some of the oil samples had been heavily weathered and/or biodegraded, the identity and source of the oil was established. Data[83] for a wide range of PAH compounds (including parent PAHs and alkylated naphthalenes, fluorenes, phenanthrenes, anthracenes, dibenzothiophenes, fluoranthenes, pyrenes and chrysenes) were collected to identify oil in sediments to particular sources following an oil spill. The percentage contribution of each of four potential sources of oil was estimated. The suspected origins comprised pyrogenic (human habitation), natural petroleum background, the spilled oil and diagenetic sources (production of perylene in sediments). This information was then used to predict sedimentary PAH profiles, allowing contributions to be estimated from the four sources in each sample.

9.3.3 *Hopanes* (*Steranes and terpanes*)

Hydrocarbon compounds such as hopanes (also known as pentacyclic triterpanes) are very stable aliphatic compounds based on a 5-ring structure, see Figure 1. These compounds and other groups of compounds, such as the C_{27} to C_{30} steranes, can be used as fingerprint or geochemical marker compounds to differentiate between specific crude oils. These geochemical markers can be regarded as "molecular fossils" derived originally from living organisms or animals[70]. These compounds are produced during the very slow process by which sediment material is changed or transformed into rock, i.e. a process known as diagenesis. The compounds are highly refractory and do not change after being deposited in the environment until extreme weathering has occurred[84, 85].

The amount of individual steranes and triterpanes present in crude oils will vary and is dependent on the original matter from which the oil was formed and its geochemical history. Thus, the concentrations of these compounds in crude oils from different geographical areas often display significant variability, and these variations may be used to help differentiate between crude oils from different sources.

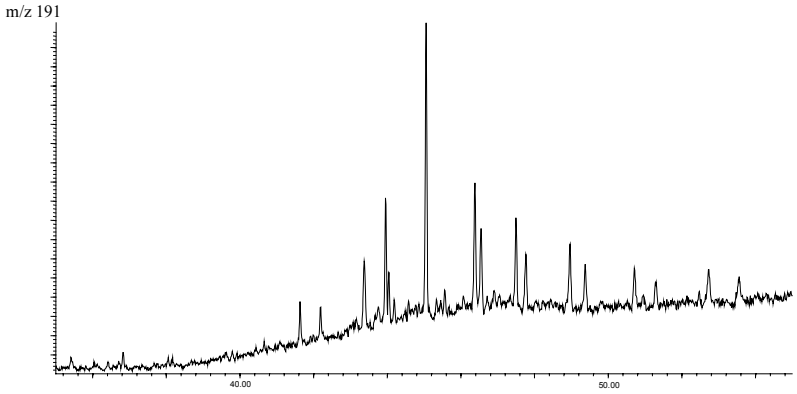
Hopanes, steranes and related compounds may be determined following gas chromatography with mass spectrometric detection by displaying the mass chromatograms detected, for instance, at 191 Daltons for the hopanes and at 217 Daltons for the steranes, see Figures 11-14. The most stable of the hopane compounds, 17α(H), 21β(H)-hopane can be used as an internal marker against which biodegradation can be assessed. The ratios of the concentrations of other compounds to the concentration of this hopane compound can then be compared to assess subsequent biodegradation over time following an oil spill[86].

Recently, triterpane and sterane geochemical marker compounds[87] were analysed using gas chromatography with mass spectrometric detection. The markers were selected because they are common in crude oils, and are more resistant to degradation and weathering than the n-alkanes, and the isoprenoid hydrocarbons such as pristane and phytane.

Hopane concentrations have been used[88] to distinguish different types of spilled oil from different sources. Crude oils tend to form in shales and can be derived from reservoirs in which the porosity is due to carbonate or clastic sediments which can be marine or non-marine in origin. Examples include carbonate (reefs) and lacustrine or deltaic sediments. Comparison of hopane concentrations would help to distinguish these sources.

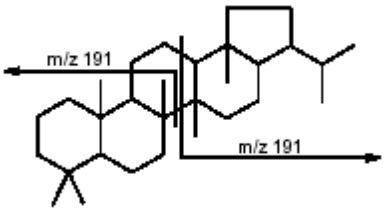
Twelve year old deposits of weathered crude oil were studied using a combination of techniques, including sterane and hopane determinations[85]. The changes in chemical composition of the oil were monitored via individual aliphatic, aromatic and geochemical marker compounds, and using "pattern recognition" techniques involving over 100 compounds and component groupings in order to assess the weathering process.

Figure 11 Hopane (triterpane) profile of a crude oil



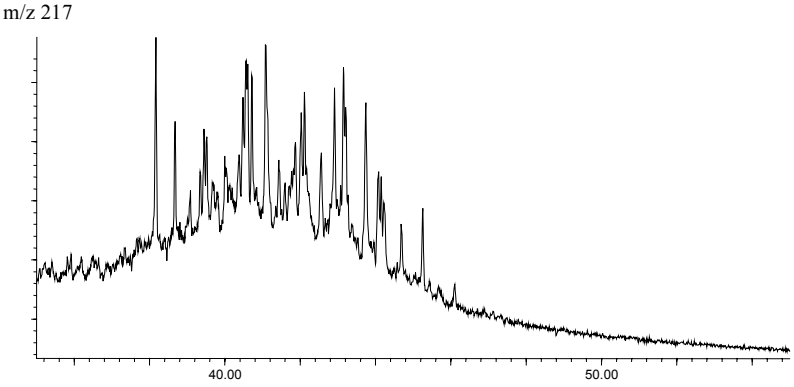
Time (minutes)

Figure 12 Hopane MS fragmentation



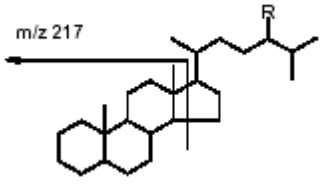
Basic hopane (triterpane) structure indicating electron-impact MS ionisation pathway to yield mass fragmentation ion of 191 Daltons, $C_{14}H_{23}^{+}$.

Figure 13 Sterane profile of a crude oil



Time (minutes)

Figure 14 Sterane MS fragmentation



Basic sterane structure indicating electron-impact MS ionisation pathway to yield mass fragmentation ion of 217 Daltons, $C_{16}H_{25}^{+}$.

9.3.4 *Other techniques*

1 Carbon isotope ratio analysis

As with the geochemical marker compounds discussed above, the carbon isotopic compositions of crude oils are related to the conditions under which the crude oils were originally formed. The isotopic composition will also depend on the refined products and the refining processes. The $^{13}C/^{12}C$ ratio can be used as an index to classify crude oils and petroleum products, and is particularly useful for the analysis of samples that have undergone extensive weathering. The methodology involves complete combustion at high temperatures of samples to form carbon dioxide and water. The isotopic composition of the isolated and purified carbon dioxide is then determined by isotope ratio mass spectrometry in which the two ions are counted in separate detectors.

The $^{13}C/^{12}C$ ratio is expressed in the usual delta-notation ($\delta^{13}C$) relative to a standard material, namely PeeDee Belemnite, which is defined as the zero point. The ratios are expressed in parts per thousand (‰).

The standard material is basically a carbonate mineral with a generally accepted absolute $^{13}C/^{12}C$ ratio of 0.0112372. By convention, a sample with a $^{13}C/^{12}C$ ratio greater than 0.00112372 will possess a positive δ -value and a sample with a $^{13}C/^{12}C$ ratio of less than 0.00112372 will possess a negative δ -value. Positive δ -values indicate relatively recent origins, whilst larger negative δ -values indicate crude oils of greater age.

Following the development of coupled instrumental techniques such as gas chromatography-isotope ratio mass spectrometric techniques, the isotopic composition of individual compounds can now also be determined[89]. The technique is particularly useful for correlating refined products containing significant concentrations of n-alkanes, especially in the C_{10}-C_{20} range. These include the kerosene (paraffin) and diesel fuel hydrocarbon compounds, which do not contain any sterane or hopane geochemical markers, as these compounds are not found within the distillation range of these products.

The determination of the carbon isotopic composition of tars and oils has been used to identify the sources of these samples. Shoreline tars with a ^{13}C composition ($\delta^{13}C$ of approximately -24‰, i.e. 2.4 % less than the reference value of 0.00112372) were found to be indicative of a particular source, in contrast to other samples derived elsewhere which yielded $\delta^{13}C$ values in the range -26% to -31‰. Information on sulphur, n-alkane, alkylated PAH, and sterane/triterpane fingerprint marker compositions were utilised alongside the carbon isotopic ratios to determine the source of the contamination in the samples[90].

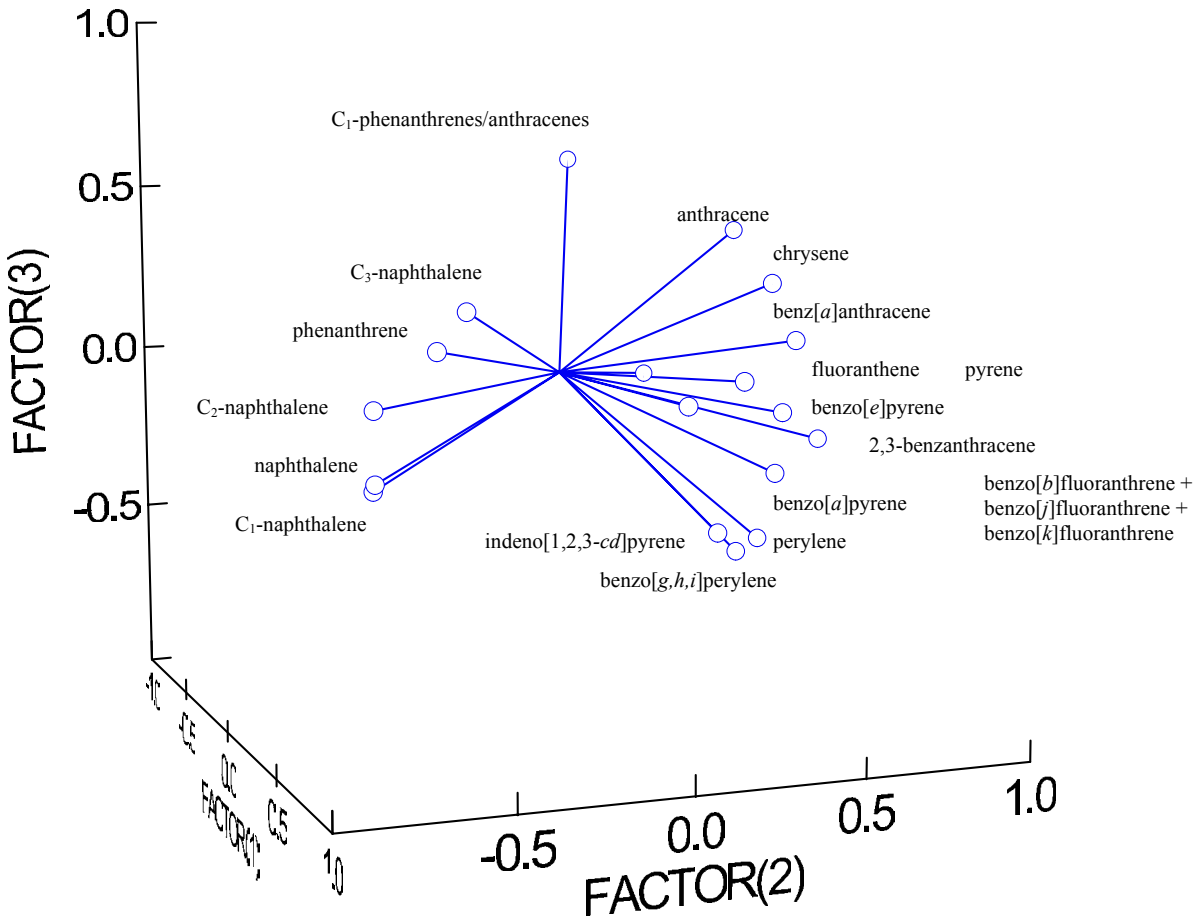
In a recent study[91, 92], the primary source inputs of PAHs to sediments were assessed using a combination of molecular abundance and carbon isotope measurements for individual 4- and 5-ring system PAHs using gas chromatography-isotope ratio mass spectrometric techniques. Mass balance calculations showed that 50 - 80% of the PAH input to the sediments was of combustion origin, probably from vehicle exhausts. The direct petroleum-related contribution, possibly dominated by lubricating oil, accounted for the remaining 20 - 50%. Few, if any, oil spills occur in entirely pristine environments, and the contribution of all sources of contamination is important if pre-existing background levels of contamination are to be acknowledged and the impact of the oil spill properly assessed[93].

2 *Statistical analyses*

Pattern recognition and various statistical techniques, including principal component analysis, cluster analysis and discriminant analysis, have been used in source identification of spilled oils[69]. Principal component analysis is one technique that has been widely used in this area, as it is particularly suited to exploring the correlation between multiple determinands, as exists for PAH data sets. Principal component analysis was applied[93] to PAH concentrations in bivalve shellfish (cockles, mussels and oysters) sampled during an 18 month period following an oil spill. Figure 15 shows the results of the principal component analysis, for a group of PAH and alkyl-substituted PAH compounds. In Figure 15, PAH compounds which are mainly oil-derived (naphthalene and alkylated naphthalenes, phenanthrene and alkylated phenanthrenes and anthracenes) correlated differently to those PAH compounds which are mainly combustion-derived (anthracene, fluoranthene, pyrene, chrysene, benzanthracenes, benzofluoranthenes, benzopyrenes, indeno[1,2,3-*cd*]pyrene and benzo[*g,h,i*]perylene). Further investigation showed that the concentrations of the oil-derived PAH compounds peaked after the release of oil ceased (about 8 days after the oil spill began) whilst the combustion-derived PAH compounds showed an annual cycle of concentrations. There were high concentrations in the shellfish during each winter-spring period prior to spawning and very low concentrations in mid-summer, see Figure 16. This probably relates to lipid storage within the bivalves as part of their spawning cycle.

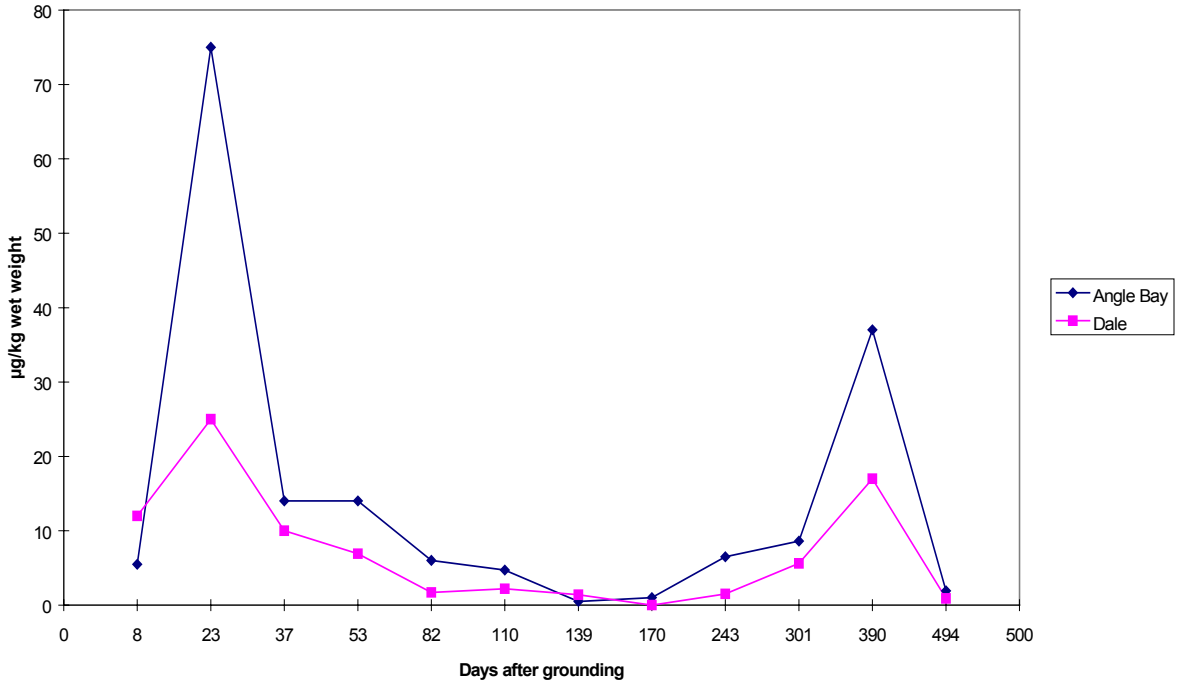
A more sophisticated approach[94] has been applied to PAH concentrations in sediments in order to allocate PAH concentrations to different sources. The method used principal component analysis to identify possible sources, and a least squares model to determine the possible source contributions that produced the best fit for the PAH concentrations determined in each sample. This technique identified 18 possible sources contributing to the contamination.

Figure 15 Principal component analysis – Factor loadings plot



PAH data derived from principal component analysis for bivalve molluscs analysed following an oil spill incident. This plot shows the vectors for oil-derived PAH compounds (naphthalene, C_1- to C_3-naphthalenes, phenanthrene and C_1-phenanthrenes/anthracenes) clustering away from PAH compounds derived from combustion sources.

Figure 16 Concentrations of benzo[*a*]pyrene in mussels



Concentrations were determined over approximately 500 days following the grounding of the *Sea Empress*. The concentration of benzo[*a*]pyrene reached a maximum at both sites in March 1996 and March 1997, reflecting an annual/seasonal cycle. This was related to sexual maturation and spawning. Similar concentrations were observed for all combustion-related PAH compounds, including the reference site at Oxwich (Gower peninsula).

9.4 Background levels of contamination

A recent oil spill[90] prompted a wide range of studies intended to establish the environmental impact of the spill and organic geochemistry played a major role. Rather than being a pristine location, the marine environment surrounding the spillage exhibited a complex background of petrogenic, pyrogenic and biogenic hydrocarbon concentrations arising from natural and anthropogenic sources. A proper evaluation of the effects of the spilled oil required that the contamination from the oil and its residues be distinguished from the pre-existing background contamination. Thus, a variety of molecular and isotopic techniques were employed to identify various hydrocarbon sources, and to assess individual contributions. Concentrations of selected PAH compounds and dibenzothiophenes in benthic sediments were used to distinguish the spilled oil and its weathered residues from background hydrocarbon concentrations. Concentration ratios of C_2-dibenzothiophenes to C_2-phenanthrenes, and C_3-dibenzothiophenes to C_3-phenanthrenes were found useful. Carbon isotope ratios and fingerprint marker concentrations were determined to distinguish the spilled oil from other sources of contamination. Diesel and diesel soot were identified as possible sources by the absence of alkylated chrysenes and a narrow distribution pattern of n-alkane concentrations. Pyrogenic products were distinguished by the dominance of non-alkylated homologues of selective PAH compounds compared to alkylated substituted PAHs. The presence of the terpane fingerprint maker compound, 18α(H)-oleanane in benthic sediments, coupled with its absence in the spilled oil, confirmed other petrogenic sources of contamination. Further details of this approach can be found elsewhere[95 - 101]. A summary of the indices that have been shown to be useful in the identification of oils is provided in Table 15.

Table 15 Summary of fingerprinting ratio or pattern index techniques

These and similar techniques[102] can be used following an oil spill incident to assist in the source evaluation of the hydrocarbon compounds detected in samples of biota, sediment and water.

Hydrocarbon	Specific determinands	Ratio or pattern index	Remarks
PAHs	2- to 6-ring system PAHs and substituted PAHs	Total parent PAH: total substituted PAH	Ratio value for petrogenic sources are characterised by a high proportion of alkylated substituted PAHs. Ratio value for pyrogenic sources are characterised by a low proportion of alkylated substituted PAHs.
	Phenanthrene and anthracene	phenanthrene:anthracene	Ratio value for petrogenic sources > 10; ratio value for pyrogenic sources <10.
	Fluoranthene and pyrene	fluoranthene:pyrene	Ratio value for petrogenic sources < 1; ratio value for pyrogenic sources >1.
	Methylphenanthrene and phenanthrene	methylphenanthrene: phenanthrene	Ratio value for petrogenic sources >2; ratio value for pyrogenic sources <1.
	Fluoranthene and pyrene, methyfluoranthene and methylpyrene	(fluoranthene + pyrene) : (methyfluoranthene + methylpyrene)	Ratio value for petrogenic sources < 1; ratio value for pyrogenic sources ~ 3.

Table 15 continued

Hydrocarbon	Specific determinands	Ratio or pattern indices	Remarks
Aliphatic hydrocarbons	n-alkanes	Carbon preference index	Terrestrial plant material is characterised by an odd-chain predominance in the range C_{23}-C_{33}. Biogenic alkanes of marine origin show a strong odd-chain preference in the range C_{15}-C_{21}. Petrogenic hydrocarbons show little or no odd-chain preference.
	n-alkanes	Distribution of n-C_{11} to n-C_{40} alkanes	Non-biodegraded/weathered crude oils – progressively decreasing concentration from n-C_{11} to n-C_{40}. Refined products, such as kerosene or diesel fuel, can be distinguished by the boiling range of n-alkanes present.
	n-alkanes and substituted alkanes	n-C_{17}/pristane ratio; and n-C_{18}/phytane ratio	In fresh, non-biodegraded oils, the ratios of n-C_{17}/pristane and n-C_{18}/phytane are greater than the corresponding ratios in weathered or degraded oils.
	Hopanes and steranes	Ratio respective compound to the hopane internal marker	Can be used to distinguish potential sources of oils and to follow the process of biodegradation.

10 Sub-lethal biological response measurements

10.1 Introduction

In the aftermath of major oil spills a number of environmental impacts become obvious. These include the observation of dead or moribund seabirds, shellfish and other organisms that have been exposed to or been in contact with oil. Some environmental impacts are, however, more subtle and difficult to assess, and, for this reason, a variety of sub-lethal biological response measurements have been developed. These measurements are based on techniques whereby the presence of contaminants in the oil induces a response within an organism or host. This response, or "biological effect", is then measured in some way and related back to the level of contaminant, either by establishing its presence or by quantifying its amount present.

In many cases, these response measurements are used as bio-markers (biological indicators) of the exposure of the organism or host to the oil. In other cases, the measurements can provide advance warning of the potential for long-term damage, either to the host or the environment. The available suite of techniques applicable to oil spill incidents continues to increase, and, in the aftermath of major oil spills, it is likely that all techniques that are available may need to be considered.

10.2 Biological response techniques

Validation and quality assurance issues are just as important in the application of biological response measurements (biological effect measurements) as they are in analytical chemistry, and initiatives are underway to promote the use of quality control protocols[103, 104]. In the following sections, a series of techniques are described with reference to their application in environmental situations.

10.2.1 *Whole-sediment bio-assays*

Techniques utilising whole-sediment bio-assays are intended to provide an overall assessment of the toxicity of contaminated sediments. These contaminants include PAH and other hydrocarbon compounds, which may be present in sediments following an oil spill. Two methods[105, 106] have been described, using the amphipod and the lugworm. Both possess acute end-points (based on mortality) and for the lugworm, there is also a sub-lethal end-point based upon feeding (casting) rates. Both methods have been used following oil spills where extended studies[41] on sediments showed no acute toxicity to either host. However, the studies revealed that the feeding rate of the lugworm was reduced, compared with feeding rates demonstrated earlier[107] on control sediments.

10.2.2 *Scope for growth*

The "scope for growth" technique gives a measure of the physiological fitness of an organism or host in response to changes in the environmental conditions in which the organism or host lives. In mussels the response has been shown quantitatively to reflect changes in environmental water quality. These changes are closely linked to the contaminant levels present in mussel tissues. This technique has provided a toxicological interpretation of PAH contamination in the environment[108, 109]. It has been shown that mussels respond rapidly to changes in levels of PAH contamination present in the environment, and pollution gradients can be identified using this technique over relatively short distances (less than 10 km).

After a particular oil spill, mussels were collected at regular intervals, and tissue material analysed for PAH and hydrocarbon contents. On two of these occasions, additional mussels were collected and analysed by the "scope for growth" technique. After the spillage, initial reports were of mussels

being heavily contaminated by oil at certain locations, and there was the expectation that the "scope for growth" technique might provide additional information into the physiological damage and stress caused to the mussels by the oil contamination. Within the mussel tissue, increased concentrations of selected hydrocarbon and PAH compounds depress the "scope for growth" of the mussel, an effect which has been demonstrated in laboratory and field studies. "Scope for growth" is a measure of the amount of energy available to an organism for growth. This value decreases in polluted organisms that have to devote more energy to detoxification and tissue repair. A concentration-response relationship, based on certain "toxic" low molecular weight PAH compounds in mussel tissue, has been derived. In addition, the measurement process has been shown to respond rapidly to increases and decreases in water soluble hydrocarbon concentrations. The interpretation of data is not so straightforward, however, as relatively high "scope for growth" measurements were observed in mussels at certain locations where tissue contaminant concentrations were still high. It is clear that mussels at a number of sites were severely stressed, four weeks after the oil spill, but that there had been some recovery, six months later[41].

10.2.3 *Ethoxyresorufin-O-deethylase determinations*

Cytochrome P450-dependent mono-oxygenases (CYP P450) are membrane-bound enzymes, known as Phase I enzymes, which metabolise a wide range of organic compounds. These enzymes catalyse the insertion of oxygen atoms into certain molecules so converting them to more polar compounds and hence, making them more water-soluble. This process facilitates further chemical reaction mechanisms, known as Phase II conjugation (for example conjugation with glucuronide or sulphate compounds) and subsequent elimination from the host. In fish, the enzymes are often found (at higher concentrations) in certain organs, for example, liver.

Although this whole process is, generally, a detoxification mechanism, certain specific compounds can become activated to form highly reactive and damaging intermediary compounds. In fish, one form of the CYP P450 enzyme, known as CYP1A, is induced into activity by environmental exposure to a range of planar molecules. These compounds include certain PAH and PCB compounds, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. Following an oil spill, exposure to the organic contaminants present in crude oil and its refined products causes induction of this enzyme, the activity of which can be determined as ethoxyresorufin O-deethylase activity, i.e. EROD activity.

The artificial substrate, 7-ethoxyresorufin, is used as an artificial substrate for the CYP1A enzyme, as the assay becomes more simplified and highly sensitive and specific. For this technique, suitable procedures, using a conventional fluorimeter with internal standardisation have been described[110] and although the method is written specifically for dab it is equally appropriate for use with other flat fish species such as flounder and plaice. An extensive review of the use of EROD activity as a bio-marker of chemical exposure has been published recently[111].

Determination of CYP1A activity using EROD measurements have been widely applied following oil spills and in other pollution studies. Following an oil spill[112], dab and plaice were collected within 2 weeks of the incident, and again after 3 months. Whilst elevated levels of EROD activity were initially found at locations close to the oil spill, relative to EROD activities observed in fish from other locations, there was no clear relationship between the observed activity and the concentration of PAH compounds. In view of this, an attempt was made to correlate EROD levels with other variables and it was showed that sexual maturity exerted the greatest influence in dab. A similar relationship was not observed with plaice, as the fish sampled were all immature. As the influence of sexual development and spawning on EROD levels is large, then for general monitoring purposes following an oil spill, fish should be sampled during their sexually inactive phase. Given that it is not possible to predict the time and location of oil spills, it should be

recognised that when interpreting results, close attention should be paid to other variables that might affect EROD activities. These variables include depth, temperature, body length, gonado-somatic index, and mobilisation of contaminants other than PAH compounds.

EROD activities were measured[113] in livers from a variety of fish species following an oil spill and levels of up to 7-9 times the background level were observed. Whilst the oil pollution was visible for about two weeks, the levels of PAHs in fish muscle were not above background levels, three months after the incident. Approximate six-fold increases in EROD activities were observed five months after the incident. However, after 7 months, activities had returned to those observed in fish from non-polluted areas.

In another study[114] of the same incident, immediate sub-lethal effects and long-term responses were studied in two fish species, one farm cultivated and the other, feral. If possible, feral fish will try and evade and swim away from the site of pollution. Exposure will therefore, generally, be lower than that observed for cultivated or farmed fish that are constrained by limits imposed due to their cultivation. Immediately following the oil spill there was observed a marked induction of the cytochrome P4501A enzymes in cultivated salmon. This is indicative of exposure to PAH compounds. Whilst in dab there was evidence of some induction at certain sites sampled immediately following the oil spill, there was no evidence, subsequently, to suggest contamination of fish species (despite very high concentrations of oil persisting in sediments). This was interpreted as indicating that PAH compounds in these sediments was not directly bio-available to the fish[29, 115].

10.2.4 *PAH metabolites in bile*

In contrast to shellfish, fish have a high capacity for metabolising PAH compounds, the conjugated metabolites of which are excreted in bile. The analysis of bile for these metabolites can therefore provide an indication of the degree of exposure experienced by fish[116, 117]. As fish can be exposed to a wide range of PAH compounds following an oil spill, a correspondingly wide range of metabolites can be produced. In general, however, two types of analyses are conducted. Fluorimetric techniques yield an overall non-specific indication of metabolite concentration, and hence an overall PAH concentration. The determination of 1-hydroxypyrene (a major PAH metabolite in fish exposed to PAH-polluted sediments) by HPLC with fluorescence detection yields more specific information on PAH concentrations.

Techniques involving fixed wavelength, and synchronous excitation and emission fluorescence spectrometry have been widely used[118-120] to determine PAH metabolites in bile. In both cases, the major determinand studied was the conjugated 1-hydroxypyrene metabolite[118, 120, 121, 122]. The specific determination of the metabolites of benzo[*a*]pyrene using HPLC with fluorescence detection has also been reported[123, 124].

10.2.5 *DNA-adduct determination*

PAH compounds are readily metabolised by many animals, including fish, to yield phase I metabolites. Prior to phase II conjugation, some of these metabolites become bound to DNA, forming DNA-adducts. These adducts are persistent in fish, and represent one of the initial stages of chemical carcinogenesis.

The isotopic $^{32}P_{15}$ labelling technique is a sensitive technique for the detection of a wide range of compounds bound to DNA[125]. DNA-adducts have been determined in both fish and mussels[126] and elevated levels detected in samples from sites following an oil spill. Later, these levels decreased to those found in uncontaminated reference areas. Similar results were also obtained for

dab and plaice, with higher levels being detected at sites close to the site of contamination. However, the detection of DNA-adducts in dab at other sites sampled much later suggested continued exposure. The presence of DNA-adducts in dab samples taken much later after the oil spill may reflect a difference in PAH-uptake between species, but might also be indicative of other local PAH contamination, unrelated to the original oil spill.

These studies indicate that exposure to potentially mutagenic and carcinogenic compounds have occurred in vertebrate and invertebrate species, and initial histopathological studies have subsequently demonstrated structural changes in flat fish livers. However, it is not clear whether these observations represent the normal frequency of liver changes in fish.

10.2.6 *Glutathione S-transferase determinations*

Glutathione *S*-transferases (GSTs) comprise cytosolic enzymes which metabolise a wide variety of endogenous compounds and exogenous xenobiotics. With respect to their primary role in detoxification, these enzymes catalyse the conjugation of glutathione (a tri-peptide) to electrophilic centres on organic xenobiotics (Phase II metabolism). This, generally, has the net result of decreasing the toxicity and increasing the solubility of xenobiotics, thereby aiding excretion of these metabolites from marine organisms.

Glutathione *S*-transferases are abundant in the cells of most marine organisms and have been demonstrated to be inducible (to a lesser extent than Phase I xenobiotic metabolising enzymes) on exposure to a wide variety of organic compounds such as hydrocarbon compounds. It has been reported that GST-induction occurs in fish livers and the digestive glands and gills of mussels, following exposure to compounds such as PAH and PCB compounds.

Although GST-induction is rarely a marked response, and can be affected by a number of compounds, this biological effect measurement has some potential for application in relation to acute hydrocarbon incidents. Notably, it has been used in monitoring for the effects of PAH compounds in the digestive glands of mussels and has been applied to monitoring the effects of oil spills.

Although GSTs comprise numerous iso-enzymes with different substrate specificity, the majority of iso-enzymes possess a common artificial substrate, namely, 1-chloro 2, 4-dinitrobenzene. GST activity can be measured using a spectrophotometric kinetic assay, which involves the measurement of the increase in absorbance (at 340 nm) from the reaction product, glutathione conjugated-chlorodinitrobenzene[127]. Results can be normalised (but see Section 3) based on the protein content of the sample (for example mussel gill, digestive glands or fish liver) and expressed as specific activity at a fixed temperature.

10.2.7 *Other techniques*

There are a number of other techniques that have been used in studies following oil spills. Whilst these techniques have not been properly validated by inter-laboratory studies, they could be useful in an integrated chemical and biological effects monitoring programme. Following an oil spill, studies[128, 129] of immune function in mussels have demonstrated effects due to the oil spill and also to local background levels of combustion PAH-derived contamination due to other sources. In addition, oxidative stress techniques have been determined[130] in mussels following an oil spill.

11 References

1. *The impact of an oil spill in turbulent waters: The Braer.* (Eds) J M Davies and G Topping, 1997, The Stationery Office, Edinburgh, ISBN 0114957983.

2. The *Sea Empress* oil spill, (Eds) R Edwards and H Sime, 1998, The Chartered Institute of Water and Environmental Management.

3. The environmental impact of the wreck of the *Braer*. W Ritchie and M O'Sullivan, The Scottish Office, 1994, pp207, ISBN 0748009000.

4. The environmental impact of the *Sea Empress* oil spill. Final report of the Sea Empress Environmental Evaluation Committee, February 1998, The Stationery Office, London, ISBN 0 11 702156 3, pp135.

5. National Contingency Plan for Marine Pollution from Shipping and Offshore Installations. Maritime and Coastguard Agency, Southampton.

6. Retention indices for programmed-temperature capillary-column gas chromatography of polycyclic aromatic hydrocarbons. *Analytical Chemistry*, M L Lee, D L Vassilaros, C M White and M V Novotny, 1979, **51**, pp768 -773.

7. *A Guide to Contingency Planning for Oil Spills on Water*. International Petroleum Industry Environmental Conservation Association, London, *Report Series, 2000, Volume 2.* Available to download from: http://www.ipieca.org/publications/oilspill.html.

8. *Response to Marine Oil Spills*. International Tanker Owner's Pollution Federation, London. ISBN 0948691514.

9. The fate and effects of dispersant-treated compared with untreated crude oil, with particular reference to sheltered intertidal sediments, D I Little, J M Baker, T P Abbiss, S J Rowland and P J C Tibbetts, in Proceedings of the 1981 Oil Spill Conference (Prevention, Behaviour, Control, Clean-up), American Petroleum Institute, Washington DC, 1981, pp283-293.

10. Some factors affecting the fate of estuarine sediment hydrocarbons and trace metals in Milford Haven, D I Little, S E Howells, T P Abbiss and D Rostron, in *Pollutant Transport and Fate in Ecosystems*. (Eds) P J Coughtrey, M H J Martin and M H Unsworth, Blackwell, 1987, pp55-87.

11. Comparison of the fate and ecological effects of dispersed and non-dispersed oil in a variety of intertidal habitats, J M Baker, J H Crothers, D I Little, J H Oldham and C M Wilson, in *Oil Spill Dispersants: Five Years of Research, Experience and Recommendations*, STP 840, American Society for Testing and Materials, Philadelphia, PA, 1984, pp239-279.

12. Tidal area dispersant experiment, Searsport Maine: an overview, E S Gilfillan, D S Page, S A Hanson, J C Foster, J Hotham, D Vallas, E Pendergast, S Hebert, S D Pratt and R Gerber, in *Proceedings of the 1985 Oil Spill Conference (Prevention, Behavior, Control, Cleanup)*. American Petroleum Institute, Washington D.C. 1985, pp 553-559.

13. Extractability of polycyclic aromatic hydrocarbons in sediments: a matter of association. *Australian Journal of Chemistry*, M J Ahrens and C W Hickey, 2003, **56**, pp193-199.

14. Revisiting the development of the Bligh and Dyer total lipid method, *Marine Pollution Bulletin*, F Smedes and T K Askland, 1999, **38**, pp193-201.

15. The *Ekofisk* Bravo incident in the North Sea and the *Ixtoc I* well in the Gulf of Mexico, 1977.

16. Biological impacts of oil pollution: Fisheries. International Petroleum Industry Environmental Conservation Association Report Series, 1997, Volume 8, London, pp28.

17. Guidelines on biological impacts of oil pollution. International Petroleum Industry Environmental Conservation Association Report Series, 1991, Volume 1, London, pp15.

18. The impact of the *Braer* oil on caged salmon, K J Whittle, D A Anderson, P R Mackie, C F Moffat, N J Shepherd and A H McVicar in "The impact of an oil spill in turbulent waters: The *Braer*", (Eds) J M Davies and G Topping, The Stationery Office, Edinburgh. 1997, pp144-160.

19. Classification of the coastal waters of Galicia (NW Spain) on the basis of total aliphatic hydrocarbon concentrations in mussels (*Mytilus galloprovincialis*). *Marine Pollution Bulletin*, A Hermida-Ameijeiras, J Simal-Gándara, J López-Hernández and J Simal-Lozano, 1994, **28**, pp396-398.

20. Aliphatic hydrocarbon levels in turbot and salmon farmed close to the site of the *Aegean Sea* oil spill. *Bulletin of Environmental Contamination and Toxicology***,** M E Alvarez-Piñeiro, M A Lage-Yusty, S T Carril-González-Barros and J Simal-Lozano, 1996, **57**, pp811-815.

21. Contamination of juvenile pink salmon and chum salmon by hydrocarbons in Prince William Sound after the *Exxon Valdez* oil spill, M G Carls, A C Wertheimer, J W Short, R M Smolowitz and J J Stegeman in Proceedings of the *Exxon Valdez* oil spill symposium, (Eds) S D Rice, R B Spies, D A Wolfe and B A Wright, American Fisheries Society Symposium, 1996, **18**, pp593-607.

22. The occurrence of hydrocarbons and histopathological abnormalities in oysters for seven years following the wreck of the *Amoco Cadiz* in Brittany (France). *Marine Environmental Research*, F Berthou, G Balouët, G Bodennec and M Marchand, 1987, **23**, pp103-133.

23. Fisheries and mariculture affected by oil spills. T H Moller, B Dicks and C N Goodman, International Oil Spill Conference: (Prevention, Behavior, Control, Cleanup). San Antonio, Texas, USA, American Petroleum Institute, Washington DC, USA, 13-16 February 1989, pp389-394.

24. The protection of mariculture facilities from spilled oil. International Tanker Owner's Pollution Federation, 1996.

25. Workshop on managing seafood problems during the response phase of an oil spill, A J Mearns and R Yender in Proceedings of the 20th Arctic and Marine Oil spill Program Technical Seminar. Coast Plaza Hotel, Vancouver BC, Canada. 11-13 June 1997, pp 203-214.

26. Effects of the *Braer* oil spill on the Shetland seafood industry. *Science of the Total Environment*, J Goodlad, 1996, **186**, pp127-133.

27. Evaluation of the condition of Prince William Sound shorelines following the *Exxon Valdez* oil spill and subsequent shoreline treatment, J P Houghton, A K Fukuyama, D C Lees, H Teas, H L Cumberland, P M Harper, T A Ebert and W B Driskell, Volume II, 1991, Biological Monitoring Survey. NOAA Technical Memorandum NOS ORCA 67. Pub. NOAA/National Ocean Service, Seattle, Washington, April, 1993.

28. Modulations in cell-mediated immunity of *Mytilus edulis* following the *Sea Empress* oil spill. *Journal of the Marine Biological Association of the United Kingdom,* E A Dyrynda, R J Law, P E J Dyrynda, C A Kelly, R K Pipe, K L Graham and N A Ratcliffe, 1997, **77**, pp281-284.

29. Contamination of fish and shellfish following oil spill incidents. *Environmental Geosciences,* R J Law and J Hellou, 1999, **6**, pp90-98.

30. Food and Environment Protection Act, 1985.

31. Toxic equivalency factors for PAH and their applicability in shellfish pollution monitoring studies. *Journal of Environmental Monitoring*, R J Law, C A Kelly, K Baker, J Jones, A D McIntosh and C F Moffat, 2002, **4**, pp383-388.

32. Impact of oil and related chemicals and wastes on the marine environment. Joint Group of Experts on the Scientific Aspects of Marine Pollution, (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP, Rep. Stud. GESAMP (50): 1993, pp180.

33. Petroleum exposure and associated biochemical effects in subtidal fish after the *Exxon Valdez* oil spill, T K Collier, C A Krone, M M Krahn, J E Stein, S-L Chan and U Varanasi in Proceedings of the *Exxon Valdez* oil spill symposium. (Eds) S D Rice, R B Spies, D A Wolfe and B A Wright, American Fisheries Society Symposium, 1996, **18**, pp671-683.

34. Hazard and risk assessment of crude oil contaminants in subsistence seafood samples from Prince William Sound. *American Fisheries Society Symposium*, M Bolger, S H Henry and C D Carrington, 1996, **18**, pp837-843.

35. The long term monitoring of polycyclic aromatic hydrocarbons (PAHs) in mussels (*Mytilus edulis*) following the *Braer* oil spill. *Analyst*, L Webster, L Angus, G Topping, E J Dalgarno and C F Moffat, 1997, **122**, pp1491-1495.

36. Environmental science in a legal context: the *Exxon Valdez* experience. *Ambio*, D G Shaw and H R Bader, 1996, **25**, pp430-434.

37. Trends in chemical concentrations in mussels and oysters collected along the US coast from 1986 to 1993. *Marine Environmental Research*, T P O'Connor, 1996, **41**, pp183-200.

38. Long-term decline of atmospheric and marine pollution by polycyclic aromatic hydrocarbons (PAHs) in Germany. *Chemosphere,* J Jacob, G Grimmer and A Hildebrandt, 1997, **34**, pp2099-2108.

39. Polycyclic aromatic hydrocarbons (PAH) in shellfish affected by the *Sea Empress* oil spill in Wales in 1996. *Polycyclic Aromatic Compounds,* R J Law, C A Kelly and M D Nicholson, 1999, **17**, pp229-239.

40. Total unsaturated compounds and polycyclic aromatic hydrocarbons in molluscs collected from waters around Newfoundland. *Archives of Environmental Contamination and Toxicology*, J Hellou, C Upshall, J F Payne, S Naidu and M A Paranjape, 1993, **24**, pp249-257.

41. The impact of the *Sea Empress* oil spill on fish and shellfish, R J Law, J E Thain, M F Kirby, Y T Allen, B P Lyons, C A Kelly, S Haworth, E A Dyrynda, P E J Dyrynda, J S Harvey, S Page, M D Nicholson and D R P Leonard in The *Sea Empress* Oil Spill: Proceedings of the International Conference held in Cardiff, 11-13 February 1998. (Eds) R Edwards and H Sime, Chartered Institute of Water and Environmental Management, London, pp109-136.

42. Standing Committee of Analysts, Analysis of hydrocarbons in waters – A review and an ultra-violet fluorescence spectrometric method 1988. *Methods for the Examination of Waters and Associated Materials*, in this series, ISBN 0117521701.

43. Standing Committee of Analysts, The determination of very low concentrations of hydrocarbons and halogenated hydrocarbons in water 1984-5. *Methods for the Examination of Waters and Associated Materials*, in this series, ISBN 0117520047.

44. Standing Committee of Analysts, The determination of volatile organic compounds in waters and complex matrices by purge and trap or by headspace techniques 1998. *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

45. The fate of the *Braer* oil in the marine and terrestrial environments, J M Davies, A D McIntosh, R Stagg, G Topping and J Rees in The impact of an oil spill in turbulent waters: The *Braer*, (Eds) J M Davies and G Topping, Stationery Office, Edinburgh, 1997, pp26-41, ISBN 0114957983.

46. Sampling Design and Statistical Methods for Environmental Biologists, R H Green, 1979, Wiley, Toronto, ISBN 0471039012.

47. Tainting of caged salmon, (*Salmo salar*) as a consequence of the *Braer* oil spill, K J Whittle, D A Anderson, H K Davis, J Lavety, P R Mackie, A S McGill, C F Moffat, N J Shepherd and A H McVicar in Seafood from producer to consumer, Integrated approach to quality, (Eds) J B Luten, T Børresen and J Oehlenschläger, *Developments in Food Science*, 1997, **38**, pp665-679.

48. Experimental tainting of marine fish by three chemically dispersed petroleum products, with comparisons to the *Braer* oil spill, H K Davis, C F Moffat and N J Shepherd, S*pill Science & Technology Bulletin*, 2002, **7**, pp257-278.

49. Polycyclic aromatic hydrocarbon (PAH) and n-alkane composition of herbage samples following the Sea Empress oil spill, L Webster, E Dalgarno and C F Moffat, Fisheries Research Services Report 07/00.

50. The effects of the *Braer* oil spill on otters and seals in Shetland, J W H Conroy, H Kruuk and A J Hall in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp106-120, ISBN 0114957983.

51. The direct effects of the Braer oil spill on seabird populations, and an assessment of the role of the wildlife response centre, M Heubeck in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp73-90, ISBN 0114957983.

52. Effects of the *Sea Empress* oil spill on breeding seabirds in South West Wales, R J Haycock, M E Baines and S J Earl in The *Sea Empress* Oil Spill: Proceedings of the International Conference held in Cardiff, (Eds) R Edwards and H Sime, 11-13 February 1998. Chartered Institute of Water and Environmental Management, London, pp227-246.

53. Sub-lethal effects of the *Braer* oil spill on seabirds, P Monaghan, P Walton, G Austin, M D Burns, C M Turner and P J Wright in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp91-105, ISBN 0114957983.

54. Impacts of oil pollution form the *Braer* on otters and semi-natural vegetation, N G Bayfield and J R Frankiss in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp52-62, ISBN 0114957983.

55. The effects of the *Braer* on crops and sheep, J A Milne, R W Mayes, A Smith and A H Sinclair in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp63-72, ISBN 0114957983.

56. Polycyclic aromatic hydrocarbons, petroleum and other hydrocarbon contaminants, C F Moffat and K J Whittle in Environmental Contaminants in Food (Eds) C F Moffat and K J Whittle, Sheffield Academic Press, 1999, pp364-429.

57. Standing Committee of Analysts, General Principles of Sampling Water and Associated Materials (second edition) 1996; Estimation of Flow and Load 1996, *Methods for the Examination of Waters and Associated Materials*, in this series, ISBN 011752364X.

58. The impact of the *Braer* spill on commercial fish and shellfish, G Topping, J M Davies, P R Mackie and C F Moffat in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp121-143, ISBN 0114957983.

59. OSPAR Commission 2000. Quality Status Report 2000, Region II-Greater North Sea, OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic, London, UK, 136 + xiii pp.

60. OSPAR Commission 2000. Quality Status Report 2000, Region III-Celtic Seas, OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic, London, UK, 116 + xiii pp.

61. The Water Framework Directive, Directive 2000/60/EC of the European Parliamnet and the Council 23 October 2000, establishing a framework for community action in the field of water policy. Official Journal of the European Community, L327, 72pp.

62. The effect on the marine benthos, G P Rutt, D Levell, G Hobbs, D M Rostron, B Bullimore, R J Law and A W Robinson in The *Sea Empress* Oil Spill: Proceedings of the International Conference held in Cardiff, (Eds) R Edwards and H Sime, 11-13 February 1998. Chartered Institute of Water and Environmental Management, London, pp189-206.

63. Studies on the response of intertidal and subtidal marine benthic communities to the *Braer* oil spill, P F Kingston, I M T Dixon, S Hamilton, C G Moore and D C Moore in The impact of an oil spill in turbulent waters: The *Braer* (Eds) J M Davies and G Topping, The Stationery Office, 1997, pp209-233, ISBN 0114957983.

64. Impact of the *Exxon Valdez* oil spill on intertidal biota, *American Fisheries Science Symposium*, R C Highsmith, T L Rucker, M S Stekoll, S M Saupe, M R Lindeberg, R N Jenne and W P Erickson, 1996, **18**, pp212-237.

65. Sea Fish Industry Authority Data Sheets: 1997/02/FT - Iced Storage of Fish on Inshore Vessels; 1997/01/FT - Fish Handling at Sea.

66. Standing Committee of Analysts, The determination of polycyclic aromatic hydrocarbons in soil by dichloromethane extraction using gas chromatography with mass spectrometric detection (2003), *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

67. Standing Committee of Analysts, The determination of polynuclear aromatic hydrocarbons in waters (additional methods) 1997, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

68. Pollutant source identification and allocation: advances in hydrocarbon fingerprinting. *Remediation*, T C Sauer and A D Uhler, 1994, pp25-50.

69. Oil spill identification. *Journal of Chromatography A*, Z Wang, M Fingas and D S Page, 1999, **843**, pp369-411.

70. *The Bio-marker Guide: Interpreting molecular fossils in petroleum and ancient sediments.* K E Peters and J M Moldowan, Prentice Hall, New Jersey USA, 1993, ISBN 0130867527, - expected to be reissued in 2004.

71. Response to Marine Oil Pollution - Review and Assessment, D Cormack, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1999, pp385, ISBN 0 7923 5674 8.

72. *Arrow* oil spill, 1970-90: persistence of 20-yr weathered bunker C fuel oil. *Canadian Journal of Fisheries and Aquatic Sciences*, J H Vandermeulen and J G Sing, 1994, **51**, pp845-855.

73. *Oil in the Sea: Inputs, Fates and Effects*. National Academy Press, Wahington D C, NRC, 1985.

74. Nordtest Method NT CHEM 001. Oil spill identification (Edition 2, approved 1991-02) pp24 ISSN 07831692. See also Improved and standardized methodology for oil spill fingerprinting. *Water Studies Series*, P S Daling, L-G Faksness, A B Hansen and S A Stout, 2002, **11** (Oil and Hydrocarbon Spills III), pp77-103.

75. Use of pattern recognition to identify the source of an oil spill on an inland water. Environment Agency R&D Technical Report E1-109/TR, 2003.

76. Changes in the composition of oil from the *Amoco Cadiz*. *Science of the Total Environment*, R J Law, 1981, **15**, pp37-49.

77. The *Eleni V* oil spill: fate and effects of the oil over the first twelve months. Part I. Oil in waters and sediments. *Marine Pollution Bulletin*, R A A Blackman and R J Law, 1980, **11**, pp199-204.

78. The *Eleni V* oil spill: return to normal conditions. *Marine Pollution Bulletin*, R A A Blackman and R J Law, 1981, **12**, pp126-130.

79. *Polycyclic Aromatic Hydrocarbons.* R G Harvey, 1997, Wiley-VCH Inc, New York, ISBN 0471186082.

80. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. *Environmental Science and Technology*, G S Douglas, A E Bence, R C Prince, S J McMillen and E L Butler, 1996, **30**, pp2332-2339.

81. Use of methyldibenzothiophenes as markers for differentiation and source identification of crude and weathered oils. *Environmental Science and Technology*, Z Wang and M Fingas, 1995, **29**, pp2842-2849.

82. Source identification of an unknown spilled oil from Quebec (1998) by unique bio-markers and diagnostic ratios of "source-specific marker" compounds. *Environmental Technology*, Z Wang, M Fingas, M Landriault, L Sigouin, S Grenon and D Zhang, 1999, **20**, pp851-862.

83. Study of the fates and effects of the Exxon Valdez oil spill on benthic sediments in two bays in Prince William Sound, Alaska. 1. Study design, chemistry and source fingerprinting. *Environmental Science and Technology*, P D Boehm, D S Page, E S Gilfillan, A E Bence, W A Burns and P J Mankiewicz, 1998, **32**, pp567-576.

84. The effect of biodegradation on steranes and terpanes in crude oils. *Geochimica Cosmochimica Acta*, W K Seifert and J M Moldowan, 1979, **43**, pp111-126.

85. Chemical characterization of crude oil residues from an Arctic beach by GC/MS and GC/FID. *Environmental Science and Technology*, Z Wang, M Fingas and G Sergy, 1995, **29**, pp2622-2631.

86. 17α(H), 21β(H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environmental Science and Technology*, R C Prince, D L Elmendorf, J R Lute, C S Hsu, C E Halth, J D Senius, G J Decvhert, G S Douglas and E L Butler, 1994, **28**, pp142-145.

87. Application of a multimolecular marker approach to fingerprint petroleum pollution in the marine environment. *Marine Pollution Bulletin*, A O Barakat, A R Mostafa, J Rullkötter and A R Hegazi, 1999, **38**, pp535-544.

88. Oil pollution in the Straits of Malacca, Malaysia: application of molecular markers for source identification. *Environmental Science and Technology***,** M P Zakaria, A Horinouchi, S Tsutsumi, H Takada, S Tanabe and A Ismail, 2000, **34**, pp1189-1196.

89. Source identification of oil spills based on the isotopic composition of individual components in weathered oil samples. *Environmental Science and Technology*, L Mansuy, R P Philp and J Allen, 1997, **31**, pp3417-3425.

90. Organic geochemistry applied to environmental assessments of Prince William Sound, Alaska, after the Exxon Valdez oil spill – a review. *Organic Geochemistry*, A E Bence, K A Kvenvolden and M C Kennicutt II, 1996, **24**, pp7-42.

91. Determination of the $^{13}C/^{12}C$ ratios of individual PAH from environmental samples: can PAH sources be apportioned? *Organic Geochemistry*, V P O'Malley, T A Abrajano and J Hellou, 1994, **21**, pp809-822.

92. Stable carbon isotopic apportionment of individual polycyclic aromatic hydrocarbons in St. John's Harbour, Newfoundland. *Environmental Science and Technology*, V P O'Malley, T A Abrajano and J Hellou, 1996, **30**, pp634-639.

93. Polycyclic aromatic hydrocarbons (PAH) in shellfish affected by the *Sea Empress* oil spill in Wales in 1996. *Polycyclic Aromatic Compounds*, R J Law, C A Kelly and M D Nicholson, 1999, **17**, pp229-239.

94. A principal-component and least-squares method for allocating polycyclic aromatic hydrocarbons in sediment to multiple sources. *Environmental Toxicology and Chemistry*, W A Burns, P J Mankiewicz, A E Bence, D S Page and K R Parker, 1997, **16**, pp1119-1131.

95. The natural petroleum hydrocarbon background in sub-tidal sediments of Prince William Sound, Alaska, USA. *Environmental Toxicology and Chemistry*, D S Page, P D Boehm, G S Douglas, A E Bence, W A Burns and P J Mankiewicz, 1996, **15**, pp1266-1281.

96. An estimate of the annual input of natural petroleum hydrocarbons to seafloor sediments in Prince William Sound, Alaska. *Marine Pollution Bulletin*, D S Page, P D Boehm, G S Douglas, A E Bence, W A Burns and P J Mankiewicz, 1997, **34**, pp744-749.

97. Petroleum sources in the western Gulf of Alaska/Shelikoff Strait area. *Marine Pollution Bulletin*, D S Page, P D Boehm, G S Douglas, A E Bence, W A Burns and P J Mankiewicz, 1998, **36**, pp1004-1012.

98. Pyrogenic polycyclic aromatic hydrocarbons in sediments record past human activity: a case study in Prince William Sound, Alaska. *Marine Pollution Bulletin*, D S Page, P D Boehm, G S Douglas, A E Bence, W A Burns and P J Mankiewicz, 1999, **38**, pp247-260.

99. Application of petroleum hydrocarbon fingerprinting and allocation techniques after the *Exxon Valdez* oil spill. *Marine Pollution Bulletin*, P D Boehm, G S Douglas, W A Burns, P J Mankiewicz, D S Page and A E Bence, 1997, **34**, pp599-613.

100. Resolving the origin of the petrogenic hydrocarbon background in Prince William Sound, Alaska. *Environmental Science and Technology*, P D Boehm, D S Page, W A Burns, A E Bence, P J Mankiewicz and J S Brown, 2001, **35**, pp471-479.

101. Natural hydrocarbon background in benthic sediments of Prince William Sound, Alaska: oil vs coal. *Environmental Science and Technology*, J W Short, K A Kvenvolden, P R Carlson, F D Hostettler, R J Rosenbauer and B A Wright, 1999, **33**, pp34-42.

102. Distribution of n-alkanes as a clue to recognition of source beds. *Geochim. Cosmochim. Acta*, E E Bray and E D Evans, 1961, **22**, pp2-15.

103. Within the UK National Marine Monitoring Programme, the biological AQC group has been working for a number of years to validate methods to be applied to coastal and off-shore monitoring. See "www.frs-scotland.gov.uk/FRS.Web/Delivery/display_standalone_with_menu.aspx?contentid=509".

104. In addition, the BEQUALM programme, originally funded under the EU Standards, Measurement and Testing programme, is currently developing a quality assurance scheme for biological effects measurements, similar to that undertaken by the QUASIMEME laboratory proficiency scheme for chemical measurements and sample handling. See also "www.cefas.co.uk/bequalm".

105. Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test. International Conference of the Environment of the Sea Techniques in *Marine Environmental Science*, B D Roddie and J E Thain, 2001, 28 pp19, ISSN 09032606.

106. Biological effects of contaminants: sediment bioassay using the polychaete *Arenicola marina*. International Conference of the Environment of the Sea Techniques in *Marine Environmental Science*, J E Thain and S Bifield, 2001, 29 pp16, ISSN 09032606.

107. The effect of Kuwait crude oil and the dispersant BP1100X on the lugworm *Arenicola marina*, D Levell in *Marine Ecology and Oil Pollution*, (Ed) J M Baker, Applied Science Publishers, 1976, pp131-185.

108. Quantitative structure-activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). *Aquatic Toxicology*, P Donkin, J Widdows, S V Evans, C M Worrall and M Carr, 1989, **14**, pp277-294.

109. QSARs for the sublethal responses of marine mussels (*Mytilus edulis*). *Science of the Total Environment*, P Donkin, J Widdows, S V Evans and M D Brinsley, 1991, **109/110**, pp461-476.

110. Biological effects of contaminants: Determination of CYP1A-dependent mono-oxygenase activity in dab by fluorimetric measurement of EROD activity. International Conference of the Environment of the Sea Techniques in *Marine Environmental Science*, R Stagg and A McIntosh, 1998, 23, pp16, ISSN 09032606. A report describing the method is available from the International Council for the Exploration of the Sea based in Copenhagen, Denmark.

111. Ethoxyresorufin-*O*-deethylase (EROD) activity in fish as a bio-marker of chemical exposure. *Critical Reviews in Toxicology,* J J Whyte, R E Jung, C J Schmit and D E Tillitt, 2000, **30**, pp347-570.

112. EROD activity measured in flat fish from the area of the *Sea Empress* oil spill. *Chemosphere*, M F Kirby, P Neall and T Tylor, 1999, **38**, pp2929-2949.

113. Temporal studies of the impact of the *Braer* oil spill on inshore feral fish from Shetland, Scotland. *Archives of Environmental Contamination and Toxicology*, S G George, J Wright and J Conroy, 1995, **29**, pp530-534.

114. The effects of the *Braer* oil spill, Shetland Islands, Scotland, on P4501A in farmed Atlantic salmon (*Salmo salar*) and the common dab (*Limanda limanda). Marine Environmental Research*, R Stagg, C Robinson, A M McIntosh, C F Moffat and D W Bruno, 1998, **46**, pp301-306.

115. The analysis of polycyclic aromatic hydrocarbons in marine samples. *International Journal of Environment and Pollution*, R J Law and J Klungsøyr, 2000, **13**, pp262-283.

116. Bio-markers of polycylic aromatic hydrocarbon (PAH) exposure in fish and their application in marine monitoring. CEFAS Science Series Technical Report No. 110, M F Kirby, B P Lyons, M J Waldock, R J Woodhead, F Goodsir, R J Law, P Matthiessen, P Neall, C Stewart, J E Thain, T Tylor and S W Feist, 2000, pp30.

117. Biomarkers for polyaromatic hydrocarbon exposure in fish. PhD thesis, University of Bergen, Norway, E Aas, 2000.

118. PAH in fish bile detected by fixed wavelength fluoresence. *Marine Environmental Research*, E Aas, J Beyer and A Goksøyr, 1998, **46**, pp225-228.

119. Synchronous fluorescence spectrometry of fish bile: a rapid screening method for the biomonitoring of PAH exposure. *Aquatic Toxicology*, F Ariese, S J Kok, M Verkaik, C Googier, N H Velthorst and J W Hofstraat, 1993, **26**, pp273-286.

120. Synchronous fluorimetric measurement of metabolites of polycyclic aromatic hydrocarbons in the bile of brown bullhead. *Environmental Toxicology and Chemistry*, E L C Lin, S M Cormier and R N Racine, 1994, **13**, pp707-715.

121. Bioaccumulation, biotransformation and DNA binding of PAHs in feral eel (*Anguilla anguilla*) exposed to polluted sediments: a field survey. *Environmental Toxicology and Chemistry*, R van der Oost, F-J van Scooten, F Ariese, H Heida, K Satumalay and N P E Vermeulen, 1994, **13**, pp859-870.

122. Bile metabolites of polycyclic aromatic hydrocarbons in three species of fish from the Severn estuary. *Ecotoxicology and Environmental Safety*, P J Ruddock, D J Bird and D V McCalley, 2002, **51**, pp97-105.

123. Dose- and time-dependent formation of biliary benzo[*a*]pyrene metabolites in the marine flat fish dab (*Limanda limanda*). *Environmental Toxicology and Chemistry*, A van Schanke, F Holtz, J van der Meer, J P Boon, F Ariese, G Stroomberg, M van den Berg and J M Everaarts, 2001, **20**, pp1641-1647.

124. Chemical derivatization and Shpol'skii spectrometric determination of benzo[*a*]pyrene metabolites in fish bile. *Analytical Chemistry*, F Ariese, S J Kok, M Verkaik, G P Hoornweg, C Gooijer, N H Velthorst and J W Hofstraat, 1993, **65**, pp1100-1106.

125. Biological effects of contaminants: Measurement of DNA adducts in fish by ^{32}P-postlabelling. ICES Techniques in Marine Environmental Science, W L Reichert, B L French and J E Stein, 1999, 25 pp45. ISSN 09032606.

126. An initial assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in the intertidal teleost *Lipophrys pholis*. *Mutation Research*, B P Lyons, J S Harvey and J M Parry, 1997, **390**, pp263-268.

127. Glutathione S-transferases, the first step in mercapturic acid formation. *Journal of Biological Chemistry*, W H Habig, M J Pabs and W B Jakoby, 1974, **249(22**), pp7130-7139.

128. Modulations in cell mediated immunity of *Mytilus edulis* following the "Sea Empress" oil spill. *Journal of the Marine Biological Association of the UK*, E A Dyrynda, R J Law, P E J Dyrynda, C A Kelly, R K Pipe, K L Graham and N A Ratcliffe, 1997, **77**, pp281-284.

129. Changes in immune parameters of natural mussel *Mytilus edulis* populations following a major oil spill (*Sea Empress*, Wales, UK). *Marine Ecology Progress Series*, E A Dyrynda, R J Law, P E J Dyrynda, C A Kelly, R K Pipe and N A Ratcliffe, 2000, **206**, pp155-170.

130. Effects of the *Aegean Sea* oil spill on biotransformation enzymes, oxidative stress and DNA-adducts in digestive gland of the mussel (*Mytilus edulis* L.). *Comparative Biochemistry and Physiology,* M Sole, C Porte, X Biosca, C L Mitchelmore, J K Chipman, D R Livingstone and J Albaiges, 1996, **113C**, pp257-265.

Annex 1 Organisations with responsibilities and experience with respect to the terrestrial environment

Centre for Ecology and Hydrology
Countryside Council for Wales
Department for Environment Food and Rural Affairs
Department of Agriculture and Rural Development
English Nature
Environment Agency
Environment and Heritage Service
Institute of Terrestrial Ecology
Joint Nature Conservation Committee
Macaulay Institute
Scottish Environment Protection Agency
Scottish Executive Environment and Rural Affairs Department
Scottish Natural Heritage

The above details are correct at the time of publication. However, changes to names and responsibilities of organisations may occur over time.

Annex 2 Procedure for collecting micro-layer samples for hydrocarbon analysis

Oil, whether released from the seabed or other sources can produce a surface sheen comprising a micro-layer of oil. This can occur even when small quantities of oil are released. In addition, the sea surface is an important source for natural biogenic surface films or micro-layers. The sea surface micro-layer, which is at its most stable during calm conditions, is of the order of hundreds of micrometres in thickness. Hydrophobic contaminants such as PAH compounds can be concentrated within the micro-layer to concentrations much higher than those normally observed in the underlying water column. Anthropogenic pollutants entering the sea by atmospheric deposition following aerial transport from land-based sources may also be trapped in the micro-layer. Pelagic fish eggs float at the surface of the sea due to the high lipid content of the egg, and these eggs and fish larvae are therefore exposed to elevated concentrations of PAH compounds and other contaminants. Hence, it is important to analyse samples of the micro-layer when assessing the effects posed by PAH compounds in the sea.

The collection of a micro-layer sample using, for example a Garrett screen (see Figure 17) depends on the weather. Only in good weather conditions can stable micro-layers be formed and samples taken. In poor weather, the micro-layer is broken up by wave and bubble action. In addition, it is essential that all containers, working and storage areas are free of greases, tars or oils.

Figure 17 Garrett screen

Annex 3 Methods for the extraction, clean-up and determination of hydrocarbon compounds in sediments and biota

A Saponification of samples

A1 Principle

Samples that are submitted to the laboratory on a wet weight basis are initially digested with alcoholic sodium or potassium hydroxide. Hydrocarbon compounds in the filtered digest are then extracted with an organic solvent, which is then dried prior to further clean-up or analysis. The method is not suitable for the determination of volatile hydrocarbon compounds.

A2 Reagents

A2.1 n-pentane.

A2.2 Methanol.

A2.3 Sodium or potassium hydroxide.

A2.4 Sodium sulphate (anhydrous). Anhydrous sodium sulphate is Soxhlet extracted for at least 3 hours with an appropriate solvent. The sodium sulphate is then dried and placed in a muffle furnace at 250 ± 20 ^{o}C for at least 2 hours and then allowed to cool prior to use.

A2.5 Internal standard solutions. To aid quantification, internal standard solutions may be added to the sample prior to extraction. The actual compounds added are dependent on the type of compounds being determined and method being used. See sections H5.1 and I5.3.

A2.6 Anti-bumping granules.

A3 Apparatus

A3.1 Reflux apparatus comprising 250 ml round bottom flask and condenser.

A3.2 Heating mantle.

A3.3 Phase separator paper (or appropriate filter paper) which should be solvent-washed prior to use.

A4 Analytical procedure

Step	Procedure	Notes
A4.1	Add an accurately weighed amount of the sample (on a wet weight basis) (note a) to a 250 ml round bottom flask. To the flask, add a suitable amount of internal standard solution (A2.5) (note b), and sodium or potassium hydroxide (A2.3) (note c) in 100 ± 5 ml of methanol (A2.2). Add a few anti-bumping granules to the flask and reflux on the	(a) Typically, this may comprise up to 50 g of wet sediment, or up to 20 g of wet biota. (b) For the analysis of PAH compounds by GC-MS or hydrocarbon compounds by GC using FID or MS, internal

heating mantle for 2.0 ± 0.5 hours. Allow the solution to cool to ambient temperature. Filter the digested solution through a filter paper (A3.3) into a 250 ml separating funnel.

standards should be added to the sample prior to extraction in order to assess recovery of the compounds of interest. See Sections H5.1 and I5.3.

(c) Typically, about 5 g of potassium hydroxide are added to sediment samples, and 20 g to samples of biota.

A4.2 Add 50 ± 1 ml of pentane (A2.1) to the 250 ml round bottomed flask and rinse the flask. Filter the solvent through the filter paper into the separating funnel.

A4.3 Shake the separating funnel vigorously for 2.0 ± 0.2 minutes. Vent the funnel regularly. Allow the layers to separate. Run the lower methanolic layer into a second 250 ml separating funnel.

A4.4 Run the upper pentane layer in a clean 100 ml conical flask.

A4.5 Add 50 ± 1 ml of pentane (A2.1) to the 250 ml round bottomed flask and rinse the flask. Filter the solvent through the filter paper into the first separating funnel. Rinse the funnel and transfer the solvent to the second separating funnel. Shake this funnel vigorously for 2.0 ± 0.2 minutes, venting the funnel regularly. Allow the layers to separate. Discard the lower methanolic layer, and combine the pentane extracts by running the pentane layer into the conical flask. Add sufficient (note d) sodium sulphate (A2.4) to the combined pentane extracts and dry the solvent. When dry, the solvent is filtered through a second (clean) filter paper (A3.3). Approximately 5-10 ml of pentane are added to the flask and the flask rinsed. The solvent is then filtered and the extracts combined. The volume of extract is then reduced (note e) to an appropriate volume. The solution is now ready to undergo clean-up or UVF determination.

(d) Typically, 1.0 ± 0.1 g.

(e) This may be carried out using for example rotary evaporation (less than 30 ^{o}C); or a stream of nitrogen (usually used for small scale evaporations). Potential losses of volatile hydrocarbon compounds may occur at this stage.

B Sonication of samples of sediment

B1 Principle

Hydrocarbon compounds are extracted from wet samples using a sonication technique with solvents of mixed polarity. The solvent extract is then partitioned with water and dried. The method is not usually applicable to biota or for the determination of volatile hydrocarbon compounds.

B2 Reagents

B2.1 Water. This should be solvent-extracted prior to use.

B2.2 Methanol.

B2.3 Dichloromethane.

B2.4 Sodium sulphate (anhydrous). Anhydrous sodium sulphate is Soxhlet extracted for at least 3 hours with an appropriate solvent. The sodium sulphate is then dried and placed in a muffle furnace at 250 ± 20 ^{o}C for at least 2 hours and then allowed to cool prior to use.

B2.5 Internal standard solution. To aid quantification, internal standard solutions may be added to the sample prior to extraction. The actual compounds added are dependent on the type of compounds being determined and method being used. See Sections H5.1 and I5.3.

B3 Apparatus

B3.1 Ultrasonic bath.

B3.2 Phase separator paper (or appropriate filter paper) which should be solvent-washed prior to use.

B3.3 Centrifuge (optional).

B4 Analytical procedure

Step Procedure	Notes
B4.1 To a 250 ml conical flask add an accurately weighed amount of the sample (on a wet weight basis) (note a). To the flask, add a suitable amount of internal standard solution (B2.5) (note b) and 50 ± 1 ml of methanol (B2.2). Swirl the contents, add 60 ± 1 ml of dichloromethane (B2.3) and stopper the flask. Place the flask in an ultrasonic bath and sonicate for 30 ± 1 minutes.	(a) Typically, up to 50 g of wet sediment are normally taken. (b) For the analysis of PAH compounds by GC-MS or hydrocarbon compounds by GC using FID or MS, internal standards should be added to the sample prior to extraction in order to assess recovery of the compounds of interest. See Sections H5.1 and I5.3.
B4.2 Remove the flask from the bath and allow the sample to settle. Carefully decant the supernatant solvent through a filter paper (B3.2) into a 1 litre	(c) Alternatively, the suspension can be centrifuged at 1800 rpm for 10 minutes at 5 ± 2 ^{o}C.

separating funnel containing 100 ml of water (B2.1) and stopper (note c).

B4.3 Shake the separating funnel for 1.0 ± 0.1 minute, and allow the layers to separate. Run the lower dichloromethane layer into a 500 ml round bottomed flask.

B4.4 Add 50 ± 1 ml of dichloromethane to the sediment and ultrasonicate for a further 15 ± 1 minutes. Remove the flask from the bath and allow the sample to settle. Carefully decant the supernatant solvent through the filter paper (note c) into the separating funnel containing the residual aqueous methanol. Stopper and shake the separating funnel for 1.0 ± 0.1 minute. Allow the layers to separate and combine the dichloromethane extracts by running the lower dichloromethane layer into the 500 ml round bottomed flask.

B4.5 Repeat step B4.4 and combine the dichloromethane extracts.

B4.6 Add sufficient (note d) sodium sulphate (B2.4) to the combined dichloromethane extracts and dry the solvent. When dry, the solvent is filtered through a second (clean) filter paper (B3.2). Approximately 5-10 ml of dichoromethane are added to the flask and the flask rinsed. The solvent is then filtered and the extracts combined. The volume of extract is then reduced (note e) to an appropriate volume. The solution is now ready to undergo clean-up or UVF determination.

(d) Typically, 1.0 ± 0.1 g.

(e) This may be carried out using for example rotary evaporation (less than 30 ^{o}C); or a stream of nitrogen (usually used for small scale evaporations). Potential losses of volatile hydrocarbon compounds may occur at this stage.

C Soxhlet extraction of samples

C1 Principle

Hydrocarbon compounds are Soxhlet extracted from chemically dried samples with an organic solvent mixture. The solvent extract is then filtered and dried. The method is not suitable for the determination of volatile organic compounds.

C2 Reagents

C2.1 Dichloromethane.

C2.2 Sodium sulphate (anhydrous). Anhydrous sodium sulphate is Soxhlet extracted for at least 3 hours with an appropriate solvent. The sodium sulphate is then dried and placed in a muffle furnace at 250 ± 20 ^{o}C for at least 2 hours and then allowed to cool prior to use.

C2.3 Anti-bumping granules.

C2.4 Internal standard solutions. **T**o aid quantification, internal standard solutions may be added to the sample prior to extraction. The actual compounds added are dependent on the type of compounds being determined and method being used. See Sections H5.1 and I5.3.

C3 Apparatus

C3.1 Soxhlet apparatus including extraction thimbles.

C3.2 Heating mantle.

C3.3 Phase separator paper (or appropriate filter paper) which should be solvent-washed prior to use.

C4 Analytical procedure

Step Procedure	Notes
C4.1 An accurately weighed portion of the sample (on a wet weight basis) (note a) should be thoroughly mixed with anhydrous sodium sulphate (C2.2) until a fine free-flowing powder is formed (note b). Quantitatively transfer the dried sample-sulphate mixture to a Soxhlet extraction thimble. To the thimble, add a suitable amount of internal standard solution (C2.4) (note c).	(a) The amount taken will depend on the hydrocarbon content of the sample. (b) Alternative drying agents may also be used. (c) For the analysis of PAH compounds by GC-MS or hydrocarbon compounds by GC using FID or MS, internal standards should be added to the sample prior to extraction in order to assess recovery of the compounds of interest. See Sections H5.1 and I5.3.
C4.2 To a 250 ml round-bottomed flask, add 120 ± 5 ml of dichloromethane (C2.1) (note d) and	(d) Alternative solvents may be used, including mixed solvents of different

several anti-bumping granules.	polarity, for example 1:1 v/v hexane and acetone.
C4.3 Set up the Soxhlet extraction apparatus and extract the dried sample for 6.0 ± 0.5 hours. After this time, remove the source of heat and allow the solvent to cool. Filter the extract through a filter paper (C3.3) and collect the extract in a suitable container.	
C4.4 Rinse the flask with about 20 ml of dichloromethane (note d) and filter through the filter paper, combining the solvent extracts.	
C4.5 Repeat step C4.4 two more times. The volume of extract is then reduced (note e) to an appropriate volume. The solution is now ready to undergo clean-up or UVF determination.	(e) This may be carried out using for example rotary evaporation (less than 30 ^{o}C); or a stream of nitrogen (usually used for small scale evaporations). Potential losses of volatile hydrocarbon compounds may occur at this stage.

D Clean-up procedure by column chromatography (small-scale)

D1 Principle

The sample extract is eluted through a column containing suitable sorbent material. During elution, interfering polar compounds are retained on the sorbent packing material whilst hydrocarbon compounds are not. This small-scale procedure is suitable for samples containing low levels of polar material.

D2 Reagents

D2.1 Water. This should be solvent-extracted prior to use.

D2.2 Methanol.

D2.3 Dichloromethane.

D2.4 n-pentane.

D2.5 Concentrated hydrochloric acid. SG 1.18

D2.6 Alumina. Heat alumina in a muffle furnace at a temperature of at least 200 °C for 2-4 hours. Allow the alumina to cool to ambient temperature and store in a desiccator.

D2.7 De-activated alumina. The amount of water to be added to the alumina in order to de-activate it will vary depending on the alumina and type of sample. For example, add 5.0 ± 0.2 %m/m of water (D2.1) to alumina (D2.6) and seal in a suitable container. Thoroughly agitate the container until a homogenised free-flowing powder is formed.

D2.8 Copper powder or turnings. Add approximately 50 g of copper powder or turnings (40 mesh) to 50 ml of concentrated hydrochloric acid (D2.5). Swirl the contents to remove any oxidised surface layer from the copper. Decant the supernatant acid from the copper and discard the acid. Repeatedly rinse the copper with water (D2.1) and discard the water until all the acid has been removed. The copper is then rinsed thoroughly, sequentially with methanol (D2.2) dichloromethane (D2.3) and finally pentane (D2.4) and the solvents discarded. The activated copper is then stored under pentane until required for use.

D2.9 Glass wool (or equivalent packing material, for example cotton wool). This should be solvent-cleaned prior to use.

D3 Apparatus

D3.1 Muffle furnace.

D4 Analytical procedure

Step	Procedure	Notes
D4.1	Prepare a chromatography column by plugging glass wool (D2.9) into a long bodied	(a) The addition of copper (which is used to remove sulphur) is not required for

Pasteur pipette. Add approximately 1 g of de-activated alumina (D2.7). If necessary (note a) add 0.5 g of activated copper (D2.8) to the column. Elute the column (note b) with 2 ml of a 50 %v/v mixture of pentane (D2.4) and dichloromethane (D2.3) (note c). Discard the solvent.

extracts obtained using the saponification process.

(b) Do not allow the meniscus of the solvent to fall below the surface of the column.

(c) An alternative solvent or solvent mixture can be used depending on the hydrocarbon compounds to be extracted.

D4.2 Place a suitable collection vessel under the column. Using a Pasteur pipette, transfer the sample extract from A4.5, B4.6 or C4.5 to the top of the column. Elute the column (note b) collecting the eluate.

D4.3 Rinse the sample extract container with 1-2 ml of a 50 %v/v mixture of pentane (D2.4) and dichloromethane (D2.3) (note c) and transfer the washings to the column. Elute the column (note b) collecting the eluate.

D4.4 Repeat step D4.3 collecting the eluate. The volume of the combined extracts is then reduced to approximately 1 ml (note d). This solution is now ready for GC determination.

(d) This may be carried out using for example rotary evaporation (less than 30 ^{o}C); or a stream of nitrogen (usually used for small scale evaporations). Potential losses of volatile hydrocarbon compounds may occur at this stage.

E Clean-up procedure by column chromatography (large-scale)

E1 Principle

The sample extract is eluted through a column containing suitable sorbent material. During elution, interfering polar compounds are retained on the sorbent packing material whilst hydrocarbon compounds are not. This large-scale procedure is suitable for samples containing high levels of polar material.

E2 Reagents

E2.1 Water. This should be solvent-extracted prior to use.

E2.2 Methanol.

E2.3 Dichloromethane.

E2.4 n-pentane.

E2.5 Concentrated hydrochloric acid. SG 1.18.

E2.6 Activated silica gel. Heat silica gel (70 - 230 mesh) in a muffle furnace at 400 ± 20 °C for 5 hours. Transfer to an oven at 200 °C for at least 1 hour. Allow the silica gel to cool to ambient temperature prior to use.

E2.7 Alumina. Heat alumina in a muffle furnace at a temperature of at least 200 °C for 2-4 hours. Allow the alumina to cool to ambient temperature and store in a desiccator.

E2.8 De-activated alumina. The amount of water to be added to the alumina in order to de-activate it will vary depending on the alumina and type of sample. For example, add 5.0 ± 0.2 %m/m of water (E2.1) to alumina (E2.6) and seal in a suitable container. Thoroughly agitate the container until a homogenised free-flowing powder is formed.

E2.9 Copper powder or turnings. Add approximately 50 g of copper powder or turnings (40 mesh) to 50 ml of concentrated hydrochloric acid (E2.5). Swirl the contents to remove any oxidised surface layer from the copper. Decant the supernatant acid from the copper and discard the acid. Repeatedly rinse the copper with water (E2.1) and discard the water until all the acid has been removed. The copper is then rinsed thoroughly sequentially with methanol (E2.2) dichloromethane (E2.3) then finally pentane (E2.4) and the solvent discarded. The activated copper is then stored under n-pentane until required for use.

E2.10 Glass wool (or equivalent packing material, for example cotton wool). This should be solvent-cleaned prior to use.

E3 Apparatus

E3.1 Muffle furnace.

E3.2 Oven.

E4 Analytical procedure

Step	Procedure	Notes
E4.1	To a chromatography column, add sufficient pentane (E2.4) to three-quarters fill the column. Insert a small piece of glass wool (E2.10) into the column and using a glass rod, push the wool to the bottom of the column. If necessary (note a) add about 0.5 g of activated copper (D2.9) to the column. Add 5 ± 1 g of silica gel (E2.6) (note b). Elute the column and discard the solvent (note c).	(a) The addition of copper (which is used to remove sulphur) is not required for extracts obtained using the saponification process. (b) Alternatively, about 5 g of de-activated alumina (E2.8) may be used. (c) Do not allow the meniscus of the solvent to fall below the surface of the column.
E4.2	Quantitatively transfer the sample extract from A4.5, B4.6 or C4.5 to the column. Place a suitable collection vessel under the column and elute the column with the sample extract (note c). Elute the column 20 ml of a 2:1 v/v pentane (E2.4) and dichloromethane (E2.3) mixture (note d). Rinse the sample extract container with 2-3 ml of a 2:1 v/v pentane (E2.4) and dichloromethane (E2.3) mixture (note d) and transfer the washings to the column. Elute the column. Add a further 20 ml quantity of the 2:1 v/v pentane (E2.4) and dichloromethane (E2.3) mixture to the column and continue eluting. The volume of the extract is then reduced to approximately 1 ml (note e). This solution is now ready for GC determination.	(d) An alternative solvent or solvent mixture can be used depending on the hydrocarbon compounds to be extracted. For example, a 50 %v/v pentane (E2.4) and dichloromethane (E2.3) mixture may be used. (e) This may be carried out using for example rotary evaporation (less than 30 ^{o}C); or a stream of nitrogen (usually used for small scale evaporations). Potential losses of volatile hydrocarbon compounds may occur at this stage.

F Clean-up procedure using HPLC

F1 Principle

This method is essentially an automated version of method E. The sample extract from A4.5 or B4.6 or possibly C4.5 is eluted through an HPLC column, which retains potentially interfering polar compounds but not hydrocarbon compounds. Aliphatic hydrocarbon compounds are eluted prior to aromatic and polycyclic aromatic hydrocarbon compounds, allowing fractionation of the sample extract. This method is suitable for samples containing higher levels of polar material.

F2 Reagents

F2.1 n-hexane.

F3 Apparatus

F3.1 The chromatographic conditions will vary depending on the equipment used. The following conditions have been found useful for routine purposes in the clean-up of sample extracts.

Mode:	isocratic normal phase.
Column:	genesis (metal-free) or equivalent, 250 mm x 4.6 mm.
Column temperature:	ambient temperature 20-25 °C.
Mobile phase:	n-hexane.
Flow rate:	approximately 2 ml min^{-1}.

F4 Analytical procedure

Step	Procedure	Note
F4.1	The HPLC system should be set up according to manufacturer's instructions using for example the conditions described in section F3.1 (note a).	(a) It may be necessary to clean the system at regular intervals, and especially if the performance deteriorates.
F4.2	Inject into the HPLC system an aliquot of the sample extract from A4.5 or B4.6 or if necessary from C4.5 (note b).	(b) The volume of the aliquot injected onto the column will depend on the sample extract, but typically ranges between 50 - 400 µl.
F4.3	Over a period of approximately 20 minutes, collect the eluant from the column. During this time, both aliphatic and aromatic hydrocarbon compounds will elute from the column. If separate aliphatic and aromatic fractions are required, separate containers should be used to collect the desired fractions (note c).	(c) If separate fractions of aliphatic and aromatic hydrocarbon compounds are required, the collection times should be determined. Prior to clean-up of a particular sample, a standard solution containing aliphatic, aromatic and polycyclic aromatic hydrocarbon compounds should be injected onto the column and then the eluate collected at appropriate time intervals. For example, this may require collection of eluate in separate containers within the first 2 minutes, then at 15-30 second intervals for up

to about 4 minutes. Finally, in one separate container the eluate should be collected over the following 20 minutes. The eluate in each container should then be analysed and each fraction identified in terms of the number and type of hydrocarbon compounds eluted. Once identified in this manner, the elution time periods for the sample extract can be determined and the appropriate fractions analysed.

G The determination of hydrocarbons in sediment and biota extracts using ultra-violet fluorescence spectroscopy

G1 Introduction

The determination of hydrocarbon compounds in waters using ultra-violet fluorescence spectroscopy has been described elsewhere[42] in this series. Aromatic and polycylic aromatic hydrocarbons (which are major components in crude oils and refined products) fluoresce under ultra-violet radiation. Fluorescence spectrometry can, therefore, be used both qualitatively and quantitatively for detecting oils in environmental samples. In general, the measurement of fluorescence emission at a single wavelength followed by measurement of fluorescence excitation at a different wavelength can be used quantitatively, whilst scanning techniques can be used for qualitative comparisons. Fluorescence spectrometry may be used to distinguish different types of oil, which may be present in a sample, as different oils contain different types and amounts of aromatic and polycyclic aromatic hydrocarbons. Thus, each crude oil has the potential to possess its own distinct fluorescence fingerprint spectrum. Since the fluorescence signal of many PAH compounds is strong, the technique can be used to detect very low concentrations of oils (for example, low $\mu g\ l^{-1}$ concentrations in water and low $mg\ kg^{-1}$ concentrations in sediments and biota). Fluorescence spectrometry is often used as a simple, quick screening technique where samples need to be prioritised, for example, when ascertaining to undertake GC or GC-MS analyses.

Since there is no absolute measure of fluorescence emission, the fluorimeter should first be calibrated with solutions (in a suitable solvent) of a reference or standard oil. These solutions should be prepared from the oil (or oils) most applicable to the incident under investigation. The fluorimeter should possess monochromators for selecting and scanning simultaneously both excitation and emission wavelengths. All measurements should be made within the linear range of the spectrometer so as to preclude quenching or self-absorption effects, and consequent distortion of the spectra. Examples of emission and excitation wavelengths generally used for crude oil and diesel fuel are :

Excitation wavelength λ nm	Emission wavelength λ nm	Oil	Slit width nm
270	330	diesel oils	5
310	360	crude oils	5

The fluorescence observed for a sample extract is compared with the fluorescence of standard solutions, and the result expressed as a concentration equivalent calculated with reference to the oil used in the calibration, which may be a reference oil from a known source or standard mixture of hydrocarbon compounds.

G2 Performance characteristics of the method

G2.1	Substances determined	Those aromatic compounds that fluoresce at the excitation and emission wavelengths selected.
G2.2	Type of sample	Sediments and biota.
G2.3	Basis of method	Hydrocarbon compounds are extracted from sediments and biota using Methods A, B or C. The extract is then analysed by ultra-violet fluorescence spectroscopy.

G2.4	Range of application	This should be within the linear range of the spectrofluorimeter and depends on the amount of sample analysed. Sample extract concentrations should, typically, be in the range of 0.1-10.0 mg l^{-1}. For highly contaminated samples, smaller amounts of sample should be taken and extracted.
G2.5	Limit of detection	Typically 0.10 mg kg^{-1}

G3 Principle

Hydrocarbon concentrations in sediments and biota are determined using ultra-violet fluorescence spectroscopy with an emission wavelength of 360 nm and an excitation wavelength of 310 nm. A comparison is made of the fluorescence observed for the sample extract against the fluorescence observed for standard solutions of a reference or standard oil made under the same conditions. The reference or standard oil may comprise the oil under investigation, or be a crude oil, kerosene, diesel oil or fuel oil, of known source.

G4 Interferences

Any non-hydrocarbon compound co-extracted by the solvent and which fluoresces at the excitation and emission wavelengths selected will interfere.

G5 Reagents

G5.1 n-pentane.

G5.2 Standard solutions of reference oil. A primary stock solution of oil at a concentration of 1000 mg l^{-1} may be prepared by accurately weighing 0.100 ± 0.005 g of well mixed reference oil into a 100 ml volumetric flask and diluting to volume with pentane (G5.1). This stock solution may be stored for up to 2 months if kept in a spark-proof refrigerator at 4 ± 2 °C.

An intermediate standard solution of 100 mg l^{-1} may be prepared by adding 10.00 ± 0.05 ml of the primary stock solution into a 100 ml volumetric flask and diluting to volume with pentane (G5.1). This solution should be prepared on the day of use, but may stored at 4 ± 2 °C in a spark-proof refrigerator prior to use.

Working standard solutions within the range 0-10 mg l^{-1} may be prepared by adding various volumes of the intermediate standard solution to separate 100 ml volumetric flasks and diluting to volume with pentane (G5.1). A blank solution should also be prepared.

G6 Apparatus

G6.1 A spectrofluorimeter capable of synchronously scanning both excitation and emission wavelengths whilst maintaining a fixed off-set between wavelengths selected.

G7 Analytical procedure

Step	Procedure	Notes
G7.1	Quantitatively transfer the sample extract from A4.5, B4.6 or C4.5 to a 100 ml volumetric flask. Rinse the sample extract container with 1-2 ml of pentane (G5.1) and transfer the washings to the flask. Dilute to volume with pentane (G5.1) and stopper (note a).	(a) Alternatively, another solvent or solvent mixture may be used. However, sample and standard solutions should be prepared using the same solvent or solvent mixture.
G7.2	Set up the spectrofluorimeter according to the manufacturer's instructions using excitation wavelength of 310 nm, emission wavelength of 360 nm and slit widths of 5 nm. Using the working standard solutions (G5.2) and the corresponding fluorescence measurements, construct a calibration graph of the reference oil, and measure the fluorescence emission of the sample extract. From the graph, determine the amount of reference oil equivalents (µg ml^{-1}) in the sample extract.	
G7.3	Dilute the sample extract two-fold and re-measure the fluorescence emission. Repeat the determination of the amount of reference oil equivalents (µg ml^{-1}) in the sample extract. Compare the two concentrations obtained (note b).	(b) Due to the re-adsorption of emitted light, quenching of fluorescence emission can occur in concentrated solutions. This results in a lower apparent concentration being determined for highly concentrated solutions. If the concentration in the diluted sample extract is significantly higher than the undiluted sample extract, the analysis should be repeated using a smaller amount of sample.
G7.4	A procedural blank solution should be included in each batch of analyses. Ideally, this should include the extraction procedures (note c).	(c) The limit of detection may be calculated from the results obtained for blank solutions.

G8 Calculation

The concentration (expressed as reference oil equivalents) of hydrocarbon compounds in the original sediment or biota (expressed on a wet weight basis) may be calculated using the following equation:

$$C_w = \frac{F \times 100}{Ms} - C_b$$

Where:

C_w is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a wet weight basis;
F is the reference oil equivalents (µg ml^{-1}) in the sample extract;
Ms is the mass of wet sample; and
C_b is the equivalent concentration in the blank solution.

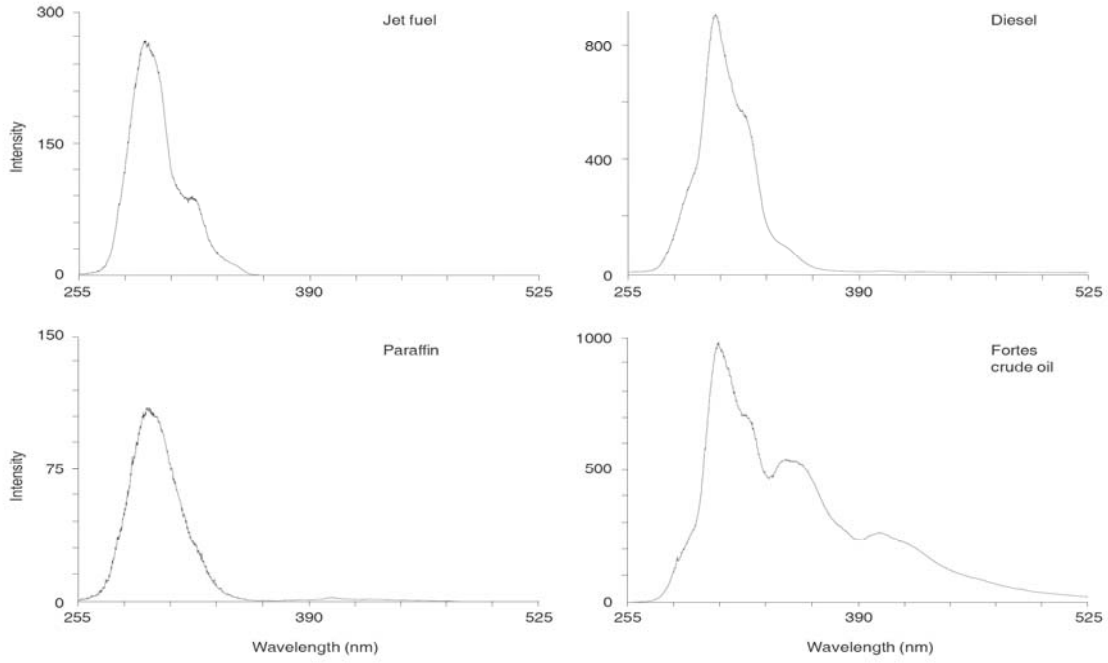
To convert the result to a dry weight basis, the following equation may be used:

$$C_d = C_w \times \frac{100}{DW}$$

C_w is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a wet weight basis;
C_d is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a dry weight basis;
DW is the percent dried weight of the sample (see Section 8.5.3).

See Figure 18 for typical UVF spectra.

Figure 18 Typical UVF spectra



H The determination polycyclic aromatic hydrocarbon compounds in sediment and biota using gas chromatography with mass spectrometric detection

H1 Introduction

This method is similar to that for determining PAH compounds in soils described elsewhere in this series[66] and includes additional guidance on alkylated PAH compounds.

Typical PAH compounds do not fragment to a great extent under typical election impact ionisation conditions. The molecular ion is, therefore, normally one of the most dominant ions in the mass spectrum. Extracting the molecular ion signal for each of the compounds of interest allows the response for each PAH compound or group of PAH compounds to be determined and when used with data from internal standards allows quantification of the PAH compounds present.

H2 Performance characteristics of the method

H2.1	Substances determined	See Table 16 for a selection of the PAH compounds that may be determined.
H2.2	Type of sample	Sediment and biota.
H2.3	Basis of method	Samples of sediment and biota that have undergone extraction and clean-up are determined by gas chromatography with mass spectrometric detection. Typically, the volume of extract should be reduced to between 0.1-1 ml.
H2.4	Range of application	This will depend on equipment used. Typically 0.01-5 ng of individual compounds injected on column.
H2.5	Limit of detection	This will depend on equipment used. Approximately 0.01 ng of individual compound injected on column.

H3 Principle

PAH compounds are analysed by gas chromatography with mass spectrometric detection. Deuterated PAH compounds (as internal standards) which are representative of the individual PAHs being determined are added to the sample prior to extraction and clean-up, see Sections A4.1, B4.1 and C4.1.

H4 Interferences

Any compound with a similar retention time to any of the PAHs of interest and with identical mass fragmentation ions in electron impact mode will interfere.

H5 Reagents

H5.1 Internal standard solution. The appropriate deuterated PAH compounds should be used as internal standards for individual PAH compounds of interest. A mixed internal standard solution containing approximately 10 mg l^{-1} of each deuterated PAH may be used in addition to individual standard solutions. These solutions should be stored at 4 ± 2 °C in a spark-proof refrigerator and may be stored for up to 6 months.

H5.2 PAH calibration solutions. A convenient mixed primary stock solution of individual PAH compounds each at a concentration of 100 mg l^{-1} in dichloromethane may be used. This solution may be suitably diluted to produce intermediate and working stock solutions. These solutions should contain the same amount of internal standard and should be stored in a spark-proof refrigerator at 4 ± 2 °C for up to 6 months.

The following table provides an example of typical calibration standard solutions. However, each laboratory should use standard solutions optimised for the instrument being used and the PAH concentrations in the samples being analysed.

Amount of each internal standard compound (µg)	**Amount of each PAH compound (µg)**	**Volume of dichoromethane (ml)**
1.0	2.0	1.0
1.0	1.0	1.0
1.0	0.5	1.0
1.0	0.2	1.0

H5.3 Dichloromethane.

H5.4 n-pentane.

H6 Apparatus

H6.1 A gas chromatograph equipped with mass spectrometric detection (quadrapole or ion trap based detector) fitted with a non-discriminating injection system. Suitable conditions are as follows:

Column:	95% dimethylpolysiloxane:5% diphenylpolysiloxane capillary column, 30-60 m, 0.25-0.32 mm internal diameter, 0.25-1.0 µm film thickness, or equivalent.
Injector:	On-column or splitless.
Injection volume:	1 µl.
Carrier gas:	Helium.
Carrier gas flow-rate:	20-40 ml per second.
Oven temperature programme:	60 ºC for 1 minute, 60-300 ºC at 5 ºC per minute, then hold at 300 ºC until all PAHs of interest have been eluted.
MS operating conditions:	Electron impact mode. Selected ion monitoring for quadrupole-based systems increases sensitivity; full scan monitoring is normally used for ion trap systems

H6.2 Data handling system for chromatographic control, integration and reporting.

H7 Analytical procedure

Step	**Procedure**	**Notes**
H7.1	Set up and optimise the gas chromatograph according to the manufacturer's instructions (note a). Construct a calibration graph of the ratio of the peak height or area of the PAH to be determined relative	(a) Suitable monitoring fragmentation ions are given in Table 16. (b) If the amount determined exceeds the

to the internal standard against the amount of PAH in the calibration solution. Using the sample extract from D4.4 or E4.2 determine the amount of PAH from the graph (notes b and c).	linear range of calibration, it may be necessary to repeat the analysis using a smaller quantity of sample. (c) Where certain PAH compounds are reported but no suitable calibration standards are available (see Section 9.2, last paragraph) response factors may be obtained from the most appropriate PAH-related compound.
H7.2 A procedural blank solution should be included in each batch of analyses. Ideally, this should include the extraction and clean-up procedures (note d).	(d) The limit of detection may be calculated from the results obtained for blank solutions.

H8 Calculation

The concentration of PAH compounds in the original wet sample, C_w, may be calculated from the following equation:

$$C_w = \frac{As \times Amount(IS)}{Ais \times RRF \times Ms}$$

where:

C_w is the concentration ($\mu g\ kg^{-1}$) of the PAH of interest in the sample, expressed on a wet weight basis;
As is the peak height or area of the PAH of interest;
Ais is the peak height or area of the corresponding internal standard;
Amount(IS) is the amount of internal standard added to the sample and standard solutions;
RRF is the relative response factor; and
Ms = mass of sample taken.

Relative response factors, RRF, are calculated from:

$$RRF = \frac{Astd \times Amount(IS)}{Ais \times Amount(S)}$$

where

Astd is the peak height or area of the standard PAH;
Amount(S) is the amount of standard PAH;
Amount (IS) is the amount of internal standard added to the sample and standard solutions; and
Ais is the peak height or area of the internal standard.

To convert the result to a dry weight basis, the following equation may be used:

$$C_d = C_w \times \frac{100}{DW}$$

where

C_w is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a wet weight basis;
C_d is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a dry weight basis;
DW is the percent dried weight of the sample (see Section 8.5.3).

See Figure 10 for typical GC-MS chromatograms of alkylated PAH compounds.

Table 16 PAH compounds and suitable mass fragmentation ions

Compound	m/z	Internal standard	m/z
naphthalene	128	naphthalene-d_8	136
C_1-naphthalenes	142		
C_2-naphthalenes	156	acenaphthylene-d_8	160
C_3-naphthalenes	170		
C_4-naphthalenes	184		
acenaphthylene	152		
acenaphthene	154		
fluorene	166		
phenanthrene	178	phenanthrene-d_{10}	188
anthracene	178		
C_1-phenanthenes / C_1-anthracenes	192		
C_2-phenanthenes / C_2-anthracenes	206		
C_3-phenanthenes / C_3-anthracenes	220		
dibenzothiophene	184		
C_1-dibenzothiophenes	198		
C_2-dibenzothiophenes	212		
C_3-dibenzothiophenes	226		
fluoranthene	202	pyrene-d_{10}	212
pyrene	202		
C_1-fluoranthenes / C_1-pyrenes	216		
C_2-fluoranthenes / C_2-pyrenes	230		
C_3-fluoranthenes / C_3-pyrenes	244		
benz[*a*]anthracene	228	chrysene-d_{12} (or benz[*a*]anthracene-d_{12})	240
chrysene	228		
Total molecular weight 228 PAHs	228		
C_1- 228 PAHs	242		
C_2- 228 PAHs	256		
benzo[*b*]fluoranthene	252	perylene-d_{12} (or benzo[*a*]pyrene-d_{12})	264
benzo[*k*]fluoranthene	252		
benzo[*a*]pyrene	252		
Total molecular weight 252 PAHs	252		
C_1- 252 PAHs	266		
C_2- 252 PAHs	280		
indeno[1,2,3-*cd*]pyrene	276		
benzo[*g,h,i*]perylene	276		
Total molecular weight 276 PAHs	276		
C_1- 276 PAHs	290		
C_2- 276 PAHs	304		
dibenz[*a,h*]anthracene	278		

Note: For the alkylated PAH compounds where calibration standards may not be available, other appropriate compounds should be used. For example, in the case of alkylated fluoranthenes and pyrenes, it may be appropriate to use 1-methylpyrene, see note c.

I The determination of hydrocarbon compounds in sediment and biota using gas chromatography with flame ionisation detection

I1 Introduction

All hydrocarbon compounds are readily ionised in a flame ionisation detector. In addition, the signal response obtained for different saturated, unsaturated and aromatic hydrocarbon compounds is relatively constant. This property makes FID suitable for the quantitative measurement of hydrocarbon compounds in environmental samples. Since different oils contain varying types and proportions of a wide range of different hydrocarbon compounds, a study of the chromatogram can provide information on the type of crude oil or distillate product present in a particular sample. The GC-FID technique is, therefore, a useful technique for providing qualitative and quantitative information. The method described is based on an internal standard quantification technique that enables the concentrations of hydrocarbon compounds to be determined.

I2 Performance characteristics of the method

I2.1	Substances determined	Those substances which give a signal response in a flame ionisation detector. The boiling point of the compounds determined should fall within the range 175-500 ºC, representing hydrocarbon compounds eluting between n-C_{10} and n-C_{40}.
I2.2	Type of sample	Sediments and biota.
I2.3	Basis of method	Samples of sediments and biota that have undergone extraction and clean-up are determined by gas chromatography with flame ionisation detection. Typically, the volume of extract should be reduced to between 0.1-1 ml.
I2.4	Range of application	This will depend on equipment used. Measurements should be made within the linear range of the detector. The range is dependent on the type of hydrocarbon compound and the quantity of sample extracted. A typical working range for an individual hydrocarbon compound is approximately 0.5-50 ng injected on column.
I2.5	Limit of detection	This will depend upon the equipment used, but typically, approximately 0.5 ng per hydrocarbon compound injected on column.

I3 Principle

The compounds present in the extracted sample are separated using gas chromatography with flame ionisation detection. Internal standards which are representative of the hydrocarbon compounds being determined are added to the sample prior to extraction and clean-up.

I4 Interferences

Any compound present in the extract which is not removed by the clean up procedure and which gives a significant detector response within the range of the retention time studied will interfere.

I5 Reagents

I5.1 Dichloromethane.

I5.2 n-pentane.

I5.3 Internal standard solutions. Compounds such as heptamethylnonane ($C_{16}H_{34}$) hexadecane-d_{34} ($C_{16}D_{34}$) squalane and 1-chlorooctadecane ($C_{18}H_{35}Cl$) have been found suitable for use as internal standards for hydrocarbon analysis by GC-FID. The internal standard should be chosen to have similar properties to the hydrocarbon compound being analysed and should not be present in the sample being analysed. More than one internal standard should be considered where a variety of hydrocarbon compounds are to be determined. A mixed internal standard solution containing approximately 100 mg l^{-1} of each standard in pentane or dichloromethane may be used for spiking purposes. In addition, individual standard solutions may be necessary where retention time data are to be determined. These standard solutions may be stored at 4 ± 2 °C in a spark-proof refrigerator and kept for up to 6 months.

I5.4 Standard calibration solutions. These may comprise solutions of a reference oil relevant to the oil under investigation or a series of standard solutions containing a mixture of known hydrocarbon compounds, for example a mixture of n-alkanes.

I5.4.1 Reference oil. A convenient primary stock solution of 5000 mg l^{-1} may be prepared by weighing 0.050 ± 0.001 g of a well mixed reference oil into a 10 ml volumetric flask. Dilute to volume with dichloromethane (I5.1) or pentane (I5.2). This solution may be stored in a spark-proof refrigerator at 4 ± 2 °C and kept for up to 2 months.

I5.4.2 Standard solution of n-alkanes. A convenient primary stock solution of n-alkanes, each at a concentration of 100 mg l^{-1} may be prepared by weighing 0.100 ± 0.005 g of each individual n-alkane into a 1 litre volumetric flask. Dilute to volume with dichloromethane (I5.1) or pentane (I5.2). A number of n-alkanes may be chosen to cover the n-C_{10} to n-C_{40} range. This solution may be stored in a spark-proof refrigerator at 4 ± 2 °C and kept for up to 6 months.

The following table provides an example of typical calibration standard solutions. However, each laboratory should use standard solutions optimised for the instrument being used and the PAH concentrations in the samples being analysed.

Amount of each internal standard compound (µg)	**Amount of each n-alkane (µg)**	**Volume of pentane or dichoromethane (ml)**
10.0	20.0	1.0
10.0	10.0	1.0
10.0	5.0	1.0
10.0	2.0	1.0

I6 Apparatus

I6.1 Gas chromatograph equipped with a non-discriminating injection system and flame ionisation detector. Suitable conditions are as follows:

GC column:	100% dimethylpolysiloxane capillary column 30-60 m, 0.25-0.32 mm internal diameter, 0.25-1.0 μm film thickness, or equivalent.
GC Injector:	On-column or splitless.
Injection volume:	1 μl.
Carrier gas:	Helium or hydrogen.
Carrier gas flow-rate:	20-40 ml per second for helium or 40-60 ml per second for hydrogen.
Oven temperature programme:	60 ºC for 1 minute, 60-300 ºC at 8 ºC per minute, hold at 300 ºC until all hydrocarbon compounds of interest have been eluted.
FID operating temperature:	300-320 ºC.

I7 Analytical procedure

Step	Procedure	Notes
I7.1	Set up and optimise the gas chromatograph according to the manufacturer's instructions. Using the calibration solutions of the reference oil (note a) construct a calibration graph of the ratio of the peak height or area of the reference oil (note a) relative to the internal standard against the amount of reference oil (note a). Using the sample extract from D4.4 or E4.2 determine the amounts of hydrocarbons from the graph. (notes b and c).	(a) Alternatively, the mixture of known hydrocarbon compounds may be used. (b) If the amount determined exceeds the linear range of calibration, it may be necessary to repeat the analysis using a smaller quantity of sample. (c) Where certain hydrocarbons are reported but no suitable calibration standards are available (see Section 9.2, last paragraph) response factors may be obtained from the most appropriate compound.
I7.2	A procedural blank solution should be included in each batch of analyses. Ideally, this should include the extraction and clean-up procedures (note d).	(d) The limit of detection may be calculated from the results obtained for blank solutions.

I8 Calculation

The concentration of hydrocarbon compounds in the original wet sample, C_w, may be calculated from the following equation:

$$C_w = \frac{As \times Amount(IS)}{Ais \times RRF \times Ms}$$

where:
C_w is the concentration ($\mu g\ kg^{-1}$) of the hydrocarbon compound of interest in the sample, expressed on a wet weight basis;
As is the peak height or area of the hydrocarbon compound of interest;

Ais is the peak height or area of the corresponding internal standard;
Amount(IS) is the amount of internal standard added to the sample and standard solutions;
RFF is the relative response factor; and
Ms = mass of sample taken.

Relative response factors, RRF, are calculated from:

$$RRF = \frac{Astd \times Amount(IS)}{Ais \times Amount(S)}$$

where

Astd is the peak height or area of the standard hydrocarbon;
Amount(S) is the amount of standard hydrocarbon;
Amount (IS) is the amount of internal standard added to the sample and standard solutions; and
Ais is the peak height or area of the internal standard.

To convert the result to a dry weight basis, the following equation may be used:

$$C_d = C_w \times \frac{100}{DW}$$

C_w is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a wet weight basis;
C_d is the concentration (expressed as mg kg^{-1} reference oil equivalents) of hydrocarbon compounds expressed on a dry weight basis;
DW is the percent dried weight of the sample (see Section 8.5.3).

Where individual hydrocarbon compounds are not determined it may be appropriate to express the result as some form of “total hydrocarbon content”. This can be expressed as reference oil equivalents or standard mixture of hydrocarbons. Under such circumstances, the total peak area under the chromatogram (less the peak area of the internal standards) may be used to determine the “total hydrocarbon content”. However, it should be clearly defined on the report exactly what has been determined, the calculation used in the determination and some interpretation of the expressed result.

See Figure 3 for typical GC-FID chromatograms.

Annex 4 List of common and scientific names

Amphipod	small, shrimp-like animal (for example *Corophium volutator*)
Arctic skua	*Stercorarius parasiticus*
Artic tern	*Sterna paradisaea*
Black guillemot	*Cepphus grylle*
Blue mussel	*Mytilus edulis*
Cockle	*Cerastoderma edule*
Common (harbour) seal	*Phoca vitulina*
Common lobster	*Homarus gammarus*
Common otter shell	*Lutraria lutraria*
Dab	*Limanda limanda*
Eider duck	*Somateria mollissima*
Edible (brown) crab	*Cancer pagurus*
Flounder	*Platichthys flesus*
Great northern diver	*Gavia immer*
Great skua	*Catharacta skua*
Grey seal	*Halichoerus grypus*
Guillemot	*Uria aalge*
Haddock	*Melanogrammus aeglefinus*
King scallop	*Pecten maximus*
Kittiwake	*Rissa tridactyla*
Lemon sole	*Microstomus kitt*
Limpet	*Patella vulgata*
Lugworm	*Arenicola marina*
Marsh samphire	*Crithmum maritimum*
Norway lobster	*Nephrops norvegicus*
Otter	*Lutra lutra*
Periwinkle	*Littorina littorea*
Plaice	*Pleuronectes platessa*
Puffin	*Fratercula arctica*
Pullet carpet shell	*Venerupis pullastra*
Purple heart urchin	*Spatangus purpureus*
Queen scallop	*Chlamys opercularis*
Rayed trough shell	*Mactra corallina*
Razorbill	*Alca torda*
Razorshell	*Ensis ensis*, *Ensis siliqua* or *Pharus legumen*
Salmon	*Salmo salar*
Sand gaper	*Mya arenaria*
Sea trout	*Salmo trutta*
Seaweed	*Chondrus crispus*, *Porphyra umbilicalis*, *Rhodymenia palmata*
Shag (European)	*Phalacrocorax aristotelis*
Sole	*Solea vulgaris*
Striped venus	*Venus striatula*
Wedge shell	*Donax vittatus*
Whelk (Common)	*Buccinum undatum*
Whelk (Dog)	*Nucella lapillus*

Analytical Quality Control

1 Routine control

Once a method has been selected for routine use, a system of analytical quality control should be adopted in order to confirm the analysis remains in control. At least one control sample should be analysed with each batch of samples and the results plotted on a control chart. Corrective action should be taken if one value falls outside of the action limit (set at three times the standard deviation of the mean) or two consecutive values exceed the warning limit (set at twice the standard deviation of the mean). As more data are acquired, the standard deviation should be updated and the control chart limits re-calculated.

2 Estimation of the accuracy of analytical results using these procedures

None of the procedures given in this booklet have been thoroughly investigated for all types of samples and before general use, the accuracy achievable should be known. It would be of great value if analysts using these procedures would estimate the accuracy of their own analytical results and report their findings to the Secretary of the Standing Committee of Analysts.

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

Standing Committee of Analysts
Environment Agency (National Laboratory Service)
56 Town Green Street
Rothley, Leicester
LE7 7NW
www.environment-agency.gov.uk/nls

Standing Committee of Analysts
Members assisting with this booklet

L Bristow
M Cohen
M Coyle
A Gravell
C Kelly
N King
D I Little
R J Law
C F Moffat
N Rowlands
D Runciman

CONTACTS:

ENVIRONMENT AGENCY HEAD OFFICE

Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol BS32 4UD
Tel: 01454 624 400 Fax: 01454 624 409

www.environment-agency.gov.uk
www.environment-agency.wales.gov.uk

ENVIRONMENT AGENCY REGIONAL OFFICES

ANGLIAN
Kingfisher House
Goldhay Way
Orton Goldhay
Peterborough PE2 5ZR
Tel: 01733 371 811
Fax: 01733 231 840

MIDLANDS
Sapphire East
550 Streetsbrook Road
Solihull B91 1QT
Tel: 0121 711 2324
Fax: 0121 711 5824

NORTH EAST
Rivers House
21 Park Square South
Leeds LS1 2QG
Tel: 0113 244 0191
Fax: 0113 246 1889

NORTH WEST
PO Box 12
Richard Fairclough House
Knutsford Road
Warrington WA4 1HG
Tel: 01925 653 999
Fax: 01925 415 961

SOUTHERN
Guildbourne House
Chatsworth Road
Worthing
West Sussex BN11 1LD
Tel: 01903 832 000
Fax: 01903 821 832

SOUTH WEST
Manley House
Kestrel Way
Exeter EX2 7LQ
Tel: 01392 444 000
Fax: 01392 444 238

THAMES
Kings Meadow House
Kings Meadow Road
Reading RG1 8DQ
Tel: 0118 953 5000
Fax: 0118 950 0388

WALES
Rivers House/Plas-yr-Afon
St Mellons Business Park
Fortran Road
St Mellons
Cardiff CF3 0EY
Tel: 029 2077 0088
Fax: 029 2079 8555

ENVIRONMENT AGENCY
GENERAL ENQUIRY LINE
0845 9 333 111

ENVIRONMENT AGENCY
FLOODLINE
0845 988 1188

ENVIRONMENT AGENCY
EMERGENCY HOTLINE
0800 80 70 60

ENVIRONMENT AGENCY