TECHNIQUES IN MARINE ENVIRONMENTAL SCIENCES

No. 12

Hydrocarbons: Review of methods for analysis in sea water, biota, and sediments

M. EHRHARDT

Institute of Marine Research
Department of Marine Chemistry
Düsternbrooker Weg 20
D-2300 Kiel
Federal Republic of Germany

J. KLUNGSØYR

Institute of Marine Research
P.O. Box 1870
N-5024 Bergen-Nordnes
Norway

R.J. LAW

Ministry of Agriculture, Fisheries and Food
Directorate of Fisheries Research
Fisheries Laboratory
Remembrance Avenue
Burnham-on-Crouch
Essex CM0 8HA
United Kingdom

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

Palægade 2-4, DK-1261 Copenhagen K, Denmark

February 1991

ISSN [redacted] 2707-6997
ISBN 978-87-7482-267-7
https://doi.org/10.17895/ices.pub.5038

HYDROCARBONS: REVIEW OF METHODS FOR ANALYSIS IN SEA WATER, BIOTA, AND SEDIMENTS

1 INTRODUCTION

The overview presented in the following paragraphs is not a collection of analytical procedures in the sense of a 'cookbook'. It is intended as an introduction to the subject and a collection of references from which a judicious choice has to be made based upon the specific objectives of an investigation. Scientists active in the field of environmental trace analysis are envisaged as potential users, as well as persons on the decision-making level who wish to familiarize themselves with the complexity, range of application, and limitations of contaminant hydrocarbon analyses in the marine environment.

Hydrocarbons, a class of chemical substances consisting exclusively of the elements carbon and hydrogen, are trace constituents of all compartments of the marine environment, i.e., water, suspended solids, organisms, and sediments. The sources of hydrocarbons are both natural in the sense that they occur irrespective of man's interference, and artificial as their occurrence is linked to a multitude of human activities. Interest, from a water management point of view, in the analysis of hydrocarbons in the marine environment stems from the often-repeated observation that elevated concentrations of non-biosynthetic hydrocarbons have detrimental effects on many marine life forms. A voluminous literature exists on the subject; reviews and collated papers may be found in GESAMP (1977), Connell and Miller (1980a, b), Gundlach and Marchand (1982), and Kuiper and van den Brink (1987).

Owing to the ability of carbon atoms to form chemical bonds not only with other elements, but also among themselves, an almost limitless number of different structures can be conceived of, containing chains of practically any length, branched chains, rings, and any combination of these structural elements. It is the characteristics of the sources which, to a certain extent, determine the number of related molecular structures and the range of different structures. Thus, fossil hydrocarbons span a very wide range of molecular weights and structure types, whereas recent biogenic hydrocarbons contain either saturated or olefinic carbon-carbon bonds in straight or branched chains or rings with five or six carbon atoms. In contrast to the multitude of individual compounds in fossil hydrocarbon mixtures, their number in any given source organism is limited owing to specific pathways for their biosynthesis either de novo or by conversion of dietary precursors (Blumer, 1967; Blumer et al., 1971; Connell and Miller, 1980a).

Aromatic structures are frequent among fossil hydrocarbons and among those generated by combustion processes. To a certain extent, it is possible to use the degree of alkyl substitution of aromatic hydrocarbons for differentiating between these sources (Blumer and

Youngblood, 1975; Youngblood and Blumer, 1975; Sporstøl et al., 1983.) In petroleum, alkyl-substituted derivatives usually predominate, whereas combustion-generated hydrocarbon mixtures are richer in the unsubstituted parent compounds. It is not quite clear, however, whether the predominance of unsubstituted aromatic hydrocarbons in environmental samples necessarily indicates the presence of combustion products. Recent analytical results (Davies and Tibbetts, 1987; Ehrhardt and Burns, 1990) suggest that alkyl-substituted aromatic hydrocarbons may be less refractory under environmental conditions than many unsubstituted nuclei, whose slower rate of decomposition would eventually lead to their preponderance.

Aromatic structures, frequent as they are in fossil hydrocarbons, are rare in hydrocarbons biosynthesized by marine organisms. As examples may be cited: the tetralene derivative, calamene, in gorgonians (Weinheimer et al., 1968); the substituted benzene, laurene, in Laurencia species (Irie et al., 1965); carotenes with benzoid terminal groups in the sponge Reniera japonica (Yamaguchi, 1957a,b; 1958a,b); an alkyl-substituted octahydrochrysene in a polychaete (Farrington et al., 1986).

These aromatic hydrocarbons occurring in marine organisms have not yet been characterized as components of dissolved organic material in sea water. Since some saturated hydrocarbons (e.g. n-pentadecane, n-heptadecane, n-nonadecane) which are synthesized by marine phytoplankton may also be detected in uncontaminated sea water, the assumption is plausible, however, that these biosynthetic aromatic hydrocarbons eventually find their way into the aqueous phase. In proportion to non-biosynthetic sources of aromatic hydrocarbons the contribution may be insignificant, but their possible presence should be kept in mind when aromatic hydrocarbons indiscriminately are labelled non-biosynthetic and, hence, contaminants.

For the various objectives of surveillance and monitoring programmes as well as activities related to basic research, it has been found expedient to analyse and quantitate hydrocarbons separately in different matrices. Thus, water as the principal agent for transport and dispersal as well as the medium in which marine organisms live is analysed to gather information on the sources, inputs, distribution, and concentrations of hydrocarbons to which its inhabitants are exposed.

Marine organisms are analysed to investigate the chemical nature of biosynthetic hydrocarbons, the accumulation of contaminant hydrocarbons from the surrounding water owing to the higher lipophilicity of living tissue as compared with sea water, and the associated stress to organisms. A global ocean monitoring programme consisting of many regional components, but relying on common strategies, is based on the use of the sedentary filter-feeding blue mussels and oysters as biological concentrators. Assessment of contamination by hydrocarbons is one component of this programme (Farrington et al., 1982; Goldberg, 1986; Murray and Law, 1980; Reynolds et al., 1981; Risebrough et al., 1983).

The analysis of sediments for hydrocarbons is a component of many

investigations and monitoring programmes because concentrations are generally higher, and thus easier to measure, than in water and also less variable (less patchy) in the short term. Although similar concentrations may be found in organisms, the biological lipid matrix from which they have to be separated is far more complex. Nevertheless, analyses of hydrocarbons in sediments have often been found to be just as challenging as in any other matrix.

Around point sources, such as offshore oil production platforms or refinery outfalls, gradients of concentration may be established which help to determine the maximum area of effect. Hydrocarbons deposited in sediments may persist for a long period of time, particularly under anoxic conditions. The hydrocarbon composition within the sediments can be altered both by degradation, which leads to the loss of some components, and by early diagenetic reactions in shallow sediments which lead to the in situ production of particular compounds, such as perylene and retene. Biogenic precursor molecules may be altered by chemical and microbiological processes to yield a variety of compounds, such as steranes and pentacyclic triterpanes (Aizenshtat, 1973; Hites et al., 1980; NRC, 1985; Venkatesan, 1988).

The range of hydrocarbon concentrations found in sediments is very wide, total hydrocarbon concentrations varying from approximately 1 µg/g dry weight in clean offshore sand deposits to >10% in areas impacted by oil spills or close to platforms discharging cuttings resulting from the use of oil-based drilling muds. In addition, different particle sizes and types within a given sediment may have different hydrocarbon compositions (Thompson and Eglinton, 1978; Prahl and Carpenter, 1983). The wide range of boiling points and polarities of hydrocarbon compounds found in sediments also complicate the analysis, as no one method can efficiently extract and concentrate all hydrocarbons present. To some extent, therefore, the analytical method chosen will determine the types of hydrocarbons found.

The compilation of methodologies which follows does not include analysis of the sea-surface microlayer, nor does it specifically address analysis of suspended particles. This may appear as a serious shortcoming, because hydrocarbon concentrations in the microlayer tend to surpass those in bulk water by at least an order of magnitude (Burns, 1986; Marty and Saliot, 1976). Particles as principal carriers for vertical transport also claim the attention of environmental analysts, but in both cases methodological differences with respect to the procedures described rest in the proper collection of samples. This is straightforward for particles which are collected by filtration on suitable filters, usually made of glass fibre. The extraction of hydrocarbons then parallels sediment extraction. Procedures for their analysis may be selected from the methods given for other matrices. Sampling of the sea-surface microlayer is more difficult. A useful procedure is delineated in IOC Manuals and Guides No.15 (UNESCO, 1985); Knap et al. (1986) describe its application. A detailed study on the composition of petroleum hydrocarbons in the microlayer is given by Butler and Sibbald (1987) who use a Teflon disk for collecting samples practically free of a separate aqueous phase. Carlson et al. (1988) present a new micro-

layer sampling device based on the rotating drum principle. The collected material, of course, is appropriate for analysis by any method selected for a specific purpose.

2 HYDROCARBONS DISSOLVED AND/OR FINELY DISPERSED IN SEA WATER

2.1 Sampling

2.1.1 Collection of discrete water samples for hydrocarbon analysis

A basic consideration to be kept in mind in the collection of water samples for hydrocarbon analysis is that the water volume is an important determinant for the detection limit of any given analytical method. Sensitive methods that measure bulk properties of hydrocarbon mixtures, such as UV fluorescence spectrophotometry, require smaller sample volumes to achieve a given detection limit than those which determine properties of individual hydrocarbons after separation. In this latter case, because specific separate compounds, which may constitute minute portions of the sample, are the source of the analytical signal rather than their sum, it can almost be stated as a general rule that the more information about sample composition to be acquired, the greater the amount of material needed. For instance, it was found that the sum of dissolved fossil fuel residues can be determined spectrofluorimetrically with currently available instrumentation down to concentrations of approximately 50 ng per liter in sample volumes of 3 to 4 liters (Ehrhardt and Petrick, 1989). If single compound analysis is the objective, however, sample volumes have to be 10 to 100 times larger to reach the same detection limit.

2.1.1.1 Water samplers

In cases of gross contamination of water with oil, the type of sampler is not very critical. Boehm and Fiest (1982), for instance, successfully used a 10 liter Teflon-lined "GO-FLO" sampler, a 30 liter glass Bodman bottle, and a 90 liter aluminium Bodega Bodman bottle for studies of the IXTOC I blow-out in the Bay of Campeche, Gulf of Mexico.

For hydrocarbon trace analyses, however, great care has to be exercised if conventional water samplers are used, because they often have greased mechanical parts leaching hydrocarbons or adsorbing surfaces removing them from the sample water. To eliminate these shortcomings, several different types of samplers have been developed specifically for collecting water samples for hydrocarbon analyses. If samples are to be taken from surface water only, the use of weighted solvent bottles is a simple, inexpensive, and reliable method for collecting seawater samples uncontaminated by secondary hydrocarbon sources (UNESCO, 1976, 1984; Knap *et al.*, 1986; Ehrhardt and Knap, 1989). More elaborate versions of this type of sampler, which can be lowered with the intake orifice closed, are described

by Gump et al. (1975) and Law et al. (1988). The latter sampler has been employed successfully on a Kevlar hydrowire at depths down to 50 meters.

For clean sample collection at greater depths, Clark et al. (1967) described a pressure-resistant casing holding water bottles with variable capacities of up to 15 liters. Calibrated rupture discs permit sampling at predetermined depths down to 4500 meters. While the bottle slowly fills with water through a restrictor capillary, particulate material is collected on a suitable filter. The sampler is not actuated from the surface and, therefore, does not need to be attached to a hydrographic wire as guide for a messenger, but can be operated on any clean line. For hydrocarbon trace analyses this is a decided advantage, since hydrographic wires are notorious sources of hydrocarbons from greases used to protect them against corrosion. Farrington et al. (1976) caution against the use of the sampler, popularly known as the Blumer bottle, at depths exceeding 2000 meters because of the risk of overfilling and subsequent contamination of the sample. Levy (1979) compared a Blumer bottle and a Niskin sampler and found both equally satisfactory for hydrocarbon trace analyses, although concentrations of hydrocarbons measured spectrofluorimetrically were less variable in samples taken with the Blumer sampler.

Sampling without simultaneous filtration at depths down to 500 meters is possible with a spherical glass vessel described by Stadler and Schomaker (1977) which, unlike the Blumer bottle, is not pressure protected. The sampler has a capacity of 10 liters. The inlet is a piece of glass tubing flame-sealed at one end which is broken at the desired depth by the impact of a hydrographic messenger. When in operation, the sampler has to be attached to a hydrographic wire, with all the consequent risks of contaminating the sample. Application of the sampler is described by Gassmann and Pocklington (1984).

Tokar et al. (1981) describe a gas lift system capable of supplying large volumes of water from depths down to approximately 100 meters. The system, which is based on the injection of a clean gas (air or nitrogen) into the sampling conduit near the intake orifice, has been used successfully to collect near-surface water for intercalibrating UV fluorescence determinations of dissolved/dispersed petroleum residues (Bermuda Biological Station for Research, 1986 Summer Course: Analysis of Marine Pollution).

The monitoring of hydrocarbon concentrations in sea water by means of discrete samples has certain disadvantages, particularly where steep gradients are encountered or when concentrations are variable over time. In situ towed fluorimeters provide the capability of continuous measurement of low concentrations of total hydrocarbons (ca. 1 µg per liter), and commercially produced examples are now available. They have been used in experimental oil spill studies (Bocard et al., 1982), in coastal waters (Law, 1984), and in studies of oily water discharge plumes from North Sea oil production platforms (Law and Hudson, 1986).

2.1.2 Separation of hydrocarbons and other dissolved lipophilic material from the aqueous phase

Fossil hydrocarbons as a group of compounds occur in sea water at concentrations around a few hundred micrograms per liter in cases of extreme contamination (El Samra et al., 1986) and between a few tens to a few hundred nanograms per liter in little-affected open ocean sea areas (Ehrhardt and Knap, 1989; Ehrhardt and Petrick, 1989; Fogelqvist et al., 1982). Concentrations of individual hydrocarbons are correspondingly lower. Because no analytical instruments are available to detect, characterize, and measure individual hydrocarbons directly in sea water at such low concentration levels, methods have been developed for their separation from sea water and concentration prior to analysis. These methods are liquid-liquid extraction, liquid-solid adsorption, and gas stripping.

2.1.2.1 Liquid-liquid extraction

Hydrocarbons are lipophilic and, therefore, can be separated from sea water by extraction with a water-immiscible organic solvent. Not all such solvents that might be applicable in principle are equally well suited. Important criteria for selection are:

- low water solubility,
- low boiling point,
- small hazard to human health,
- low cost, and
- universal availability.

With these criteria in mind, carbon tetrachloride was recommended in the initial phase of the IOC/WMO Marine Pollution (Petroleum) Monitoring Pilot Project (MAPMOPP) as the solvent to extract fossil fuel residues from sea water (UNESCO, 1976). Except for posing a certain health hazard, it combines all the desirable properties. A relatively low boiling point (76.7 ^{0}C at 760 mm) permits evaporation of extracts with moderate losses of low boiling components.

Disadvantageous for its use is its relatively high human toxicity. Carbon tetrachloride was found to cause symptoms of illness at concentrations in air of 3200 mg/m^{3} and severe toxic effects at 12,800 mg/m^{3} (Verschueren, 1977). Another disadvantage for the selective extraction of hydrocarbons is that, in addition to hydrocarbons, carbon tetrachloride also dissolves other, more polar, organic compounds from sea water, especially in highly productive or inshore sea areas, and they may interfere with hydrocarbon analysis.

Trichloromethane (chloroform) has also been used for extracting lipophilic organics from sea water (Barbier et al., 1973; Boehm, 1980; Goutx and Saliot, 1980). It is, however, more toxic than carbon tetrachloride, causing symptoms of illness at concentrations exceeding 2400 mg/m^{3} (Verschueren, 1977).

Dichloromethane has properties similar to those of carbon tetrachloride and trichloromethane. It has the lowest boiling point of the polychloromethane solvents (40 ^{0}C at 760 mm), which makes it the

most suitable solvent for low boiling analytes. Its toxicity, however, is even higher than that of trichloromethane (disagreeable and toxic at concentrations in air of more than 1400 mg/m^3 (Verschueren, 1977)). For dissolved organic material more polar than hydrocarbons, both trichloromethane and dichloromethane are better solvents than carbon tetrachloride, which aggravates the problem of co-extraction of polar materials.

Mainly because of human health considerations, the use of carbon tetrachloride as an extractant was abandoned in favour of n-hexane at a later stage in the development of large-scale marine pollution monitoring schemes (UNESCO, 1984). Hexane is less toxic, the no-effects level for humans being 7200 mg/m^3 in air at 10 minutes exposure time (Verschueren, 1977). It is a less powerful solvent for more polar lipophilic materials, discriminating to a certain extent against their extraction from the aqueous phase.

Gjavotchanoff (1972) described an extraction procedure with liquid n-butane under pressure. Although the low boiling point of n-butane (-0.6 ^{0}C at 760 mm) would permit enrichment of volatile material otherwise hardly accessible, the pressure extraction method has never found application in studies of organic compounds dissolved in sea water.

Water samples can be extracted in separatory funnels, but because their use entails exposure of the sample to additional large surfaces and, thus, the hazard of contamination, some samplers have been designed so that the sample water can be extracted in them (UNESCO, 1976, 1984; Stadler and Schomaker, 1977). The collection of extractants heavier than water (carbon tetrachloride, trichloromethane, dichloromethane) is easy after extraction in a sample bottle, but less convenient for solvents lighter than water (n-hexane). Stadler and Schomaker (1977), UNESCO (1976, 1984), and Ehrhardt and Petrick (1989) describe adapters suitable for that purpose.

2.1.2.2 Liquid-solid adsorption

The advantages offered by a solid material which selectively adsorbs dissolved lipophilic compounds from sea water have been recognized early. In principle, it is possible to pass very large water volumes through beds of adsorbent material and to collect amounts of dissolved organics in theory limited only by the capacity of the sorbent and its mass. However, solid sorbents should not be charged with unfiltered water, because they also tend to retain particles. Subsequent elution of the sorbent is not completely effective in removing the particles which, with repeated use of the sorbent, is tantamount to cumulative deposition of an uncontrollable source of extractable material. The water to be passed through a sorbent, therefore, has to be freed of particles by filtration. Because the flow rate through a filter drops as particles accumulate on it, filters have to be changed at intervals determined by the flow rate, the concentration and size of the particles, as well as the porosity and size of the filter. This limits the amount of material which can be collected on a sorbent before filters have to be changed either manually or automatically. On the other hand, filtration enables analyses

of particulate material, which can be important, e.g., for studies of removal mechanisms for hydrocarbons because of their propensity to cling to particles.

A variety of sorbents has been used to concentrate lipophilic organic trace constituents from sea water. The sorbent employed most frequently is a porous copolymer of styrene and divinylbenzene which is available commercially under its trade name Amberlite XAD-2. Applied initially for concentrating chlorinated hydrocarbons (Harvey, 1972; Harvey et al., 1973; Dawson et al., 1976; Dawson and Riley, 1977), its applicability for collecting hydrocarbons from sea water was explored by Osterroht (1974) and Derenbach et al., 1978). It was found that aromatic hydrocarbons are recovered almost quantitatively, but that the recovery of saturated hydrocarbons is rather poor. In order to overcome this problem, Burns and Smith (1980) added an equal amount of Teflon particles (Chromosorb T) to the XAD-2 resin bed.

Josefson et al. (1984) investigated the use of Chromosorb T and Fluoropak 80 (two different brands of poly(tetrafluoroethylene) adsorbents) for concentrating lipophilic organic compounds from water and compared their efficiencies with those of Amberlite XAD-2 and Amberlite XAD-8. Quantitative recoveries were obtained with Chromosorb T for a variety of solutes at a concentration of 50 µg per liter in water containing 2 mg humic acids per liter.

Porous polyurethane foam is another sorbent material frequently used in investigations of lipophilic organic trace contaminants of sea water. Originally adopted for chlorinated hydrocarbons (Uthe et al., 1972), its application for hydrocarbon trace analyses has been described by de Lappe et al. (1983) and Albaigés et al. (1984). De Lappe et al. (1983) compared extraction efficiencies of polyurethane foam and cyclohexane and found, for the foam, generally lower recoveries of saturated hydrocarbons. The same applies for total unresolved aromatics, whereas total resolved aromatics were concentrated with more or less equal efficiencies.

Gómez-Belinchón et al. (1988) tested recoveries of hydrocarbons, among other dissolved materials, from natural sea water by parallel extraction with XAD-2 resin, polyurethane foam, and cyclohexane. Significantly different extraction efficiencies were found for higher molecular weight aliphatic and aromatic hydrocarbons, with cyclohexane liquid-liquid extraction being most effective. The incomplete recoveries on solid adsorbents are attributed to modifications of lipophilicity by association of adsorbates with natural marine fulvic and humic acids.

Contaminants in synthetic adsorbents (Amberlite XAD-2, XAD-4, Ambersorb XE-340), originating from their manufacture and decomposition, were detected by Hunt and Pangaro (1982). They include alkylbenzenes, styrenes, indenes, naphthalene, alkylnaphthalenes, biphenyls, and diphenylalkanes (XAD-2 and XAD-4) as well as a number of non-alkylated PAHs (Ambersorb XE-340). A cleaning procedure for the resins is described in a literature reference quoted in the publication. Ehrhardt (1987) also describes an apparatus and a procedure

for cleaning adsorbent resins and for eluting sorbed sample materials from them.

In recent years, octadecyl (C-18) groups bonded chemically to porous silica has found increasing use for contaminant studies in fresh water. Junk and Richard (1988) evaluated this sorbent material and found it to yield excellent recoveries of >85% for pesticides and polycyclic aromatics in water samples spiked at the nanogram per milliliter concentration range. Flow rates of up to 250 bed volumes per minute are possible.

Concern has occasionally been expressed regarding the stability of hydrocarbons sorbed onto solid materials. The results published by Green and le Pape (1987) are reassuring, therefore. They found that hydrocarbons sorbed onto Amberlite XAD-2 and octadecane bonded on silica gel were stabilized to the degree that they remained unchanged for up to 100 days, even in the presence of oleophilic bacteria.

2.1.2.3 Gas stripping

In the early 1970s, Grob (1973), Grob and Grob (1974), and Grob et al. (1975) developed an ingenious method of selectively concentrating volatile organic substances by gas stripping from aqueous solution and deposition on a tiny bed of activated charcoal from a relatively small gas volume circulated in a closed loop by a non-contaminating membrane pump. Grob and Zürcher (1976) describe in detail an application of this method for fresh water. Gschwend et al. (1982) applied it for analysing volatile organics in sea water and Marchand and Caprais (1983) published a systematic study on recoveries of alkanes, aromatic hydrocarbons, halogenated aliphatics, and halogenated aromatic hydrocarbons. Recoveries of benzene and alkylated benzenes (methyl- to tetramethyl-) were between 70 and 92%, for naphthalene and methylnaphthalenes between 22 and 27%, and for n-alkanes (n-heptane to n-nonadecane) they decreased from 107 to 48%. In a variant of Grob's method described by Wasik (1974), the extracting gas (hydrogen) is generated electrolytically in the sea water sample to be investigated.

2.1.3 Large-volume water extractors

Except in the vicinity of massive sources of spilled fossil fuels, concentrations of hydrocarbons in sea water usually are so low that large volumes of water have to be processed for detailed qualitative and quantitative hydrocarbon analyses on the single compound level. This situation was seen by a number of marine and freshwater chemists as an incentive to develop automatic systems for unattended extraction of lipophilic organic material from its aqueous matrix. These systems may either depend on a ship or another structure as operating platform, or they can be self-contained units for deployment in situ.

An extractor of the first type is described by Werner and Waldichuk (1962). The design of a multistage continuous liquid-liquid extractor based on the same principle was published by Kahn and Wayman

(1964). Goldberg and DeLong (1973) developed continuous liquid-liquid extractors for solvents both lighter and heavier than water.

While the liquid-liquid extractors mentioned above use refluxing solvents and thus consume more power than is usually available in small independent units, the extractant is circulated with a pump in the continuous liquid-liquid extractor designed by Ahnoff and Josefsson (1974, 1976). The battery-operated apparatus can be deployed *in situ* at depths between 0 and 50 meters.

For use in a ship laboratory, Osterroht (1974) described a liquid-solid extractor containing Amberlite XAD-2 resin. The ship-borne large-volume sampling assembly of de Lappe *et al.* (1983) features polyurethane foam plug liquid-solid adsorption cartridges in combination with Ahnoff and Josefsson extractors. Ehrhardt (1978) adopted XAD-2 adsorption cartridges for use in a moored surface buoy and, in a modification, extended its depth range to 300 meters (Ehrhardt *et al.*, 1982). Battery-operated *in situ* adsorption units with depth ranges of 0 to 300 meters and 0 to 3000 meters are available commercially (Seastar Instruments Ltd., Sidney, B.C., Canada). Their use is described by Green *et al.* (1986) and Ehrhardt and Burns (1990).

2.2 Sample work-up

The expression "sample work-up" is used here as a generic term for all manipulations required to prepare a seawater extract, be it a solution in an organic solvent or a charged solid adsorbent, for the actual measurement. Depending upon which analytical method is to be used to obtain information on quantities of analytes and sample composition, different work-up procedures may be necessary.

The UV fluorescence method for quantifying dissolved and/or finely dispersed residues of fossil fuels in sea water (UNESCO, 1976, 1984), which is highly selective in being sensitive only to lipophilic aromatic compounds, was found in most cases not to require removal of non-hydrocarbon material. A simple separation of hydrocarbons from more polar organic material by column chromatography over silica gel is described in UNESCO (1976).

Solvents which interfere with the UV fluorescence analysis by either absorbing radiation energy at the wavelengths used for sample excitation or by quenching the fluorescence have to be evaporated and the sample redissolved in a UV-transparent solvent. This procedure has been found necessary when carbon tetrachloride is used for extracting the seawater sample. If for no other reason, extraction into a non-interfering solvent such as n-hexane or cyclohexane is, therefore, to be preferred.

2.2.1 Elution from solid adsorbents

Hydrocarbons and other lipophilic organic material concentrated from sea water on a solid adsorbent have to be eluted for subsequent analysis. Acetone and hexane have been used by de Lappe *et al.* (1983) and Albaigés *et al.* (1984) for eluting polyurethane plugs. For elution from Amberlite XAD-2 resin, various solvents have been

tested by one of the authors (Ehrhardt), and acetone followed by acetonitrile has been found to lead to the most satisfactory results. Acetone is an excellent solvent for the compounds of interest: it has a low boiling point (56.5 °C at 760 mm); it mixes with water in all proportions, which is advantageous for elution of water-saturated adsorbents; it is inexpensive; and it can be purified to a degree commensurate with the task by careful distillation even of technical products. Neither self-condensation nor the formation of artifacts was observed with elution under reflux.

However, some more polar adsorbed material clings to XAD-2 resin so tenaciously that it is not completely eluted with acetone. In order to prevent a build-up of this material on the resin, which might change its adsorbent and elution characteristics, an occasional thorough cleaning with acetonitrile is indicated. A method for purification and elution of Amberlite XAD-2 resin, which was found to be adequate even for trace analyses of chlorinated hydrocarbons in open ocean waters, is given by Ehrhardt (1987).

2.2.2 Compound group separation

Other than with concentrates obtained by gas stripping, which normally do not require further purification, the number and variety of organic compounds concentrated from sea water by solvent extraction or liquid-solid adsorption usually is so large, especially in coastal and highly productive sea water, that a separation into compound groups must precede any gas chromatographic analysis.

Sometimes the bulk of organic material adsorbed from sea water even at its natural pH is acidic material. Even the neutral fraction containing the hydrocarbons is often composed of so many different compounds that a column chromatographic pre-separation is necessary, e.g., for gas chromatographic quantification of individual polycyclic aromatic hydrocarbons in combination with mass spectrometric compound identification. Because the proportion of hydrocarbons in the material to be charged to a silica gel column should be as high as possible in order to keep the column dimensions and the solvent volumes at a minimum, it is often advantageous to separate the acidic components by aqueous base extraction from the organic solution. The method is described by Ehrhardt et al. (1980, 1982). Albaigés et al. (1984) effected separation into acid and neutral fractions by chromatography on 10%-KOH-impregnated silica gel.

Because of the minute quantities of material normally concentrated even from hundreds of liters of sea water, column chromatographic separation of the neutral fraction is best performed on a column of very small dimensions. Organic phases have to be dried, usually with anhydrous sodium sulfate, prior to column chromatographic separation because the residual water content would deactivate the solid phase and thus change its separation characteristics. Anhydrous sodium sulfate may be a source of organic trace impurities, if used as purchased. In order to safeguard against sample contamination, it has to be cleaned, usually by heating to 350 °C in a muffel furnace, followed by cooling in a clean desiccator.

Columns used routinely by one of the authors (Ehrhardt) for separating neutral fractions concentrated from several hundred liters of Baltic Sea water have an inner diameter of 3 mm and are slurry filled with a Pasteur pipette to a height of 10 cm. The column outlet is a platinum capillary. Individual fractions can thus be collected in glass vials made of drawn-out glass tubing, as described by Ehrhardt and Derenbach (1980) and Ehrhardt (1983). Individual fractions are evaporated in the vials (resting in a bore of a brass cube cooled to -10 ^{0}C by a refrigerated and recirculated water/methanol mixture), with very little loss even of low-boiling components, by blowing a gentle stream of high-purity dry nitrogen over the solvent surface with a capillary inserted through the neck of the vial. A molecular sieve, periodically baked out at elevated temperatures, is routinely used to remove the last traces of organic impurities from the nitrogen. Samples stored in the dark and under nitrogen in flame-sealed vials of this type have been found not to change in composition over several years.

Fully activated silica gel (120 ^{0}C under vacuum for several hours) has routinely been used as a solid phase in column chromatographic separations of neutral lipophilic material extracted from sea water. Even benzylalcohols, notoriously prone to dehydration, survived the treatment unchanged. The first fraction containing aliphatic hydrocarbons is eluted with n-hexane; aromatic hydrocarbons are eluted with n-hexane plus 50% dichloromethane. Other authors (e.g., Burns and Smith, 1980; de Lappe et al., 1983) used deactivated silica gel covered with a thin layer of deactivated alumina and eluted with n-hexane followed by n-hexane with increasing additions of benzene.

2.3 Analysis

2.3.1 UV spectrofluorimetry

The UV spectrofluorimetric method for quantifying fossil fuel residues in organic seawater extracts was first applied by Levy (1971) to study spatial and temporal variations in the oil plume released from the tanker "Arrow", which sank in Chedabucto Bay, Nova Scotia, in February 1970. Because of its sensitivity and simplicity, the method has later been adopted as the standard analytical procedure for the IOC/WMO Marine Pollution (Petroleum) Monitoring Pilot Project (MAPMOPP) and its successor, the IOC Marine Pollution Monitoring System (MARPOLMON-P). Because all technical details have been described meticulously elsewhere (UNESCO, 1976, 1984), the following short discussion will focus on its limitations.

In its simplest form, the method generates a fluorescence intensity signal at a specific wavelength of a lipophilic organic seawater extract excited with UV light of another, also fixed, wavelength. The use of non-polar solvents guards against the extraction of natural, polar, fluorescent seawater constituents, the material causing the "himmelblaue Fluoreszenz" (cerulean fluorescence) first investigated by Kalle (1938).

The measurements are not absolute, i.e., in order to relate the signal strength to a concentration, solutions with known concentrations

of a known substance have to be used as quantitative standards. Østgaard and Jensen (1983) discussed difficulties encountered with quantification and the spectral positions of fluorescence maxima of several petroleum components. The standard reference substance stipulated within the monitoring programmes is chrysene, a tetracyclic aromatic hydrocarbon. Because crude oils, more or less regardless of their origin, were found to be excited most strongly around 310 nm and to emit the most intense fluorescence light around 360 nm, these wavelengths are used for fluorimetric determinations. None of the fluorescence maxima in the spectrum of chrysene, however, peak at 360 nm which renders quantification difficult, since small variations in the emission wavelength result in considerable changes of the fluorescence intensity. Even disregarding this difficulty, concentrations expressed in chrysene equivalents have little meaning other than being comparable; but this applies only if the composition of the material extracted from sea water is uniform in time and space, which is debatable.

Since most crude oils have very similar concentration-specific fluorescence intensities at 360 nm (with 310 nm excitation wavelength), any crude oil can also serve as a standard. However, a crude oil, if released into the sea, will undergo changes in its composition, the initial changes probably being the most rapid. This means that the composition of any but a very recently spilled oil differs from that of a whole crude oil, which renders quantification based on the latter unreliable, until the rate of change eventually becomes so small that it ceases to have an influence on weight-specific fluorescence intensities. But the composition of this material which, as was shown by gas chromatographic and mass spectrometric analyses, together with fluorescent compounds from other sources, causes the baseline fluorescence of non-polar organic seawater extracts, differs considerably from that of any crude oil. Thus concentrations, even if given in crude oil equivalents, are mere approximations. The method is useful, however, to screen large areas for unusual events, because many data can be generated at little expense within a short period of time.

The application and results of spectrofluorimetric analyses of sea water extracts for quantifying fossil fuel residues dissolved or finely dispersed may be found in, e.g., Andrulewicz (1983); Corredor et al. (1983); Ehrhardt and Knap (1989); Ehrhardt and Petrick (1989); El Samra et al. (1986); Fogelqvist et al. (1982); Law and Knap et al. (1986); Keizer et al. (1977); Levy et al. (1981); Levy and Walton (1973); Marchand (1980).

With fixed excitation and emission wavelengths, UV fluorescence analyses provide very little information on sample composition. Scanning the emission wavelengths between 320 and, e.g., 500 nm, with excitation locked at 310 nm, is an effective way of detecting secondary sample contamination because co-extracted substances other than oil distort the characteristic shape of seawater extracts containing fossil fuel residues only. Simultaneous scanning of excitation and emission wavelengths with a fixed wavelength difference (usually 25 nm) yields more information on sample composition, because the wavelength of maximal excitation increases with increasing

number of condensed aromatic rings (Boehm and Fiest, 1982). It is thus possible to differentiate between light distillates, whole crudes, and residual fuels. If, finally, the emission intensities are plotted as a function of both excitation and emission wavelength in a contour plot, an even better source correlation is possible (Hargrave and Phillips, 1975; Vandermeulen and Gordon, 1976).

2.3.2 Gas chromatography

Gas chromatographic separations of complex mixtures of organic compounds are routine operations in so many laboratories and the literature on the subject is so voluminous that technical aspects of the method will not be discussed here nor will the literature be reviewed. The principal task in the application of gas chromatographic separation for organic seawater analyses is the collection and appropriate purification of samples containing a sufficient amount of material to result in an acceptable signal-to-noise ratio. These topics have been discussed in the preceding paragraphs. The additional gain of information which gas chromatographic analyses are capable of providing is described below.

Although gas chromatography on packed columns continues to be used for analyses of environmental samples, the considerably higher resolving power and sensitivity of capillary gas chromatography first made it possible to study the complex composition of non-polar organic seawater extracts on the single compound level. However, even high-resolution capillary gas chromatography is no match for the enormous multitude of different hydrocarbons in a crude oil so that, in a seawater sample severely contaminated with oil, an unknown and obviously large number of individual compounds remain hidden in an unresolved detector signal. On the other hand, the resolution of a considerable number of major components enables:

a) differentiation between recent biosynthetic and fossil hydrocarbons,

b) investigations of compositional changes that result from weathering, and

c) source identification of oil spills.

The differentiation between recently biosynthesized and fossil hydrocarbons is based on the compositional differences, mentioned above, which the high resolution of gas chromatographic separation is able to detect. A detailed study of the composition is also the basis for investigating the effects of degradation reactions whose rate constants are structure dependent (see, e.g., Blumer et al., 1973). The source identification of oil spills is possible because oils from different production areas and, within these, even different production horizons are characterized by their own unique composition for the description of which gas chromatography plays a significant role (Albaigés and Albrecht, 1979; Ehrhardt and Blumer, 1972; U.S.EPA, 1973; Bentz, 1976; Gough and Rowland, 1990; Gouygou and Michel, 1981; Urdal et al., 1986; Lundbom, 1987; Van Vleet, 1984).

2.3.3 Gas chromatography - mass spectrometry (GC/MS)

The combination of a mass spectrometer with a high resolution gas chromatograph adds another dimension to the analysis of complex organic seawater extracts. Not only is it possible to use a mass spectrometer, in the selected ion monitoring mode, as a highly selective and, because of a very high signal-to-noise ratio, extremely sensitive detector, but also, if the full mass range is scanned, individual compounds can be identified and their structures determined on the basis of their mass spectra. In many cases, this is possible by computer search of a digitized spectral library, but frequently mass spectra have to be interpreted according to documented fragmentation rules. A wealth of information on various technicalities and applications of GC/MS methodology for environmental analyses may be found in Karasek et al. (1984).

3 THE ANALYSIS OF HYDROCARBONS IN BIOTA

3.1 Introduction

Marine biota include thousands of different species ranging in size from one-celled planktonic organisms to whales. Both biosynthetic and contaminant hydrocarbons can be found in marine organisms; the composition may be complex, with highly varying concentrations of individual components. Several methods have been developed for the analysis of hydrocarbons in biota, and no single method alone can be used for all kinds of tissues. Results from intercalibration exercises on the determination of hydrocarbons in biological tissues have shown that great discrepancies still exist among the results from different laboratories (Farrington et al., 1986, and references cited therein). Improvements in methodology and continuing method development should therefore be encouraged. This section summarizes the currently applied techniques for the analysis of hydrocarbons in biota.

The general analytical procedure for hydrocarbon analysis in organisms involves the same main steps as hydrocarbon analysis in sediments and water: the collection and preservation of samples, extraction of the hydrocarbons from the sample matrix, separation from naturally occurring interfering compounds, and final analysis to identify and quantify the compounds of interest. These steps are, of course, interrelated. Analytical procedures and expected concentrations place certain restrictions and requirements on sample size. The selectivity and sensitivity of the final analytical step determine how extensive the clean-up procedures have to be. Although the basic principles for hydrocarbon analysis in biota are relatively simple, the analytical procedures currently applied are numerous. Fortunately, however, many of the methodological differences are in the details.

3.2 Sampling

The analysis of hydrocarbons in biota may have different purposes or aims, and this affects sampling and sampling strategies. The acquisition of reliable data is not solely dependent on the accuracy of the analytical measurements. Representative sampling programmes and suitable storage and pre-treatment procedures of samples following collection are equally important. A sampling strategy has to be devised for each programme individually. It is difficult to devise general rules on where to obtain representative samples within a given area, what sample sizes are needed, and when and how frequently samples should be collected. The nature and aims of each programme and the characteristics of the sea area to be investigated have to be taken into account for each specific case.

The International Council for the Exploration of the Sea (ICES) has provided detailed guidelines for the sampling and preservation of samples for monitoring contaminants in biota. Such programmes often fall into the following categories: monitoring for compliance verification, monitoring patterns and trends, or monitoring for research purposes. Some general factors should always be considered when analyses of hydrocarbons in biota are to be performed. Seasonal variations in food supply may influence total body weight, as well as lipid concentration and composition. Spawning cycles and other differences in life stage have similar effects. Hydrocarbons are concentrated in the lipid-rich tissues of organisms, and changes in lipid concentration may, therefore, influence the amounts of hydrocarbons in tissues. In order to minimize these variations, it is often suggested that sampling be undertaken during the pre-spawning period or as synoptically as possible to ensure that the organisms under investigation are in the same physiological state. For the analysis of organic contaminants, it is always important to measure the lipid content of the tissues in order to ensure that data can be compared on an equal-lipid basis.

The concentration of non-biogenic hydrocarbons in biota is dependent on the concentration levels the organisms have been exposed to, for how long, and how fast they are able to depurate themselves either by excretion or metabolism. Hydrocarbons can be taken up through the gut, gills, and skin. The enzymatic system responsible for metabolizing hydrocarbons in organisms may differ as a function of species, age, and sex. Environmental stress factors may also play a role. For the interpretation of results, it is, therefore, important to note all relevant information about each organism, such as length, weight, sex, age, etc., and the history of the sample.

Traditional equipment is used for sampling marine biota. The almost ubiquitous occurrence of traces of fossil and/or combustion-generated hydrocarbons necessitates taking extreme precautions during sampling to avoid secondary contamination of the organisms to be investigated. If a choice can be made between different sampling methods, the one offering the least chance of contamination should be selected. On board a ship, there are many sources of hydrocarbons that may contaminate samples. Grice *et al.* (1972) described precautions to be taken to avoid such contamination. Therefore, samples of

the different hydrocarbon sources on board a ship should always be taken for comparison with the hydrocarbons found in biota samples.

Samples can be wrapped in clean aluminum foil and placed in suitable plastic bags; they can be put into glass or metal jars and should be frozen immediately at -20 ^{0}C. The ICES guidelines for sample collection, preparation, and analysis of fish and shellfish in the conduct of the Cooperative ICES Monitoring Studies Programme (ICES, 1990) states a possible exception to this rule: generally, mussels should be held alive in clean (settled) sea water from the area of collection for 10 to 15 hours, to allow discharge of unassimilated particles in the mantle cavity or gut that might contaminate the sample, before freezing. However, as this procedure may be inappropriate when hydrocarbons are to be determined, the guidelines state that the decision on whether or not to depurate the mussels before freezing should be taken according to local conditions and requirements. Freezing is important to avoid losses of volatile compounds and to avert enzymatic and other oxidative processes which may change the hydrocarbon composition within the tissue. Cell membranes and cellular structures may be ruptured by the freezing process. Care must be exercised to avoid losses of intracellular fluids by thawing samples before extraction.

3.3 Extraction

Hydrocarbons are concentrated in the lipoid substance of the organisms, and a number of methods have been reported for removing the lipid fraction containing the hydrocarbons from the rest of the cellular matrix. These methods generally employ Soxhlet extraction, alcoholic or aqueous caustic digestion followed by organic solvent extraction, or homogenization and/or sonication in the presence of organic solvents. For the more volatile hydrocarbons, which are lost or will have low recoveries in the methods mentioned above, steam distillation and head space sampling have been used.

Soxhlet extraction has been performed on lyophilized or dried tissues (e.g., Ehrhardt and Heinemann, 1975) and wet tissues (Blumer et al., 1970; Farrington and Medeiros, 1975). Tissues are often minced or homogenized before extraction. Dried tissue may be extracted with solvents such as pentane, hexane, hexane and benzene, or different combinations of these. Benzene is fairly toxic; inhalation of its vapours must be avoided. Precautions have to be taken during lyophilization to avoid contamination from pump oil. Losses of volatile hydrocarbons may also occur. Homogenization or grinding of wet tissue to a powder in the presence of anhydrous sodium sulfate is another way to remove water before Soxhlet extraction (Schantz et al., 1988).

Wet tissues are usually Soxhlet extracted with methanol or methanol-benzene combinations, or other solvents of similar polarity (Blumer et al., 1970; Farrington and Madeiros, 1975). Successful extraction of lipids from moist biological tissues requires solvation of the water in the organic phase of the organic extracting system, and optimal solubility of both neutral and polar lipids. Smith et al.

(1973) found that a binary solvent system using an aromatic hydrocarbon and a low molecular weight alcohol are efficient.

The lipid extracts obtained by Soxhlet extraction are often saponified to remove fatty acid triglycerides which may interfere with subsequent chromatographic separations (Gritz and Shaw, 1977; Burns and Smith, 1982). This is followed by the partitioning of hydrocarbons and non-saponifiable lipids into organic solvents such as pentane or hexane. Everything that will come into contact with the sample (glassware, solvents, drying material, reagents) must be clean and carefully checked for blanks. A disadvantage of the Soxhlet extraction procedure is the time- and solvent-consuming cleaning of the thimble. The extraction of samples is also a rather lengthy procedure. Nevertheless, Soxhlet extraction provides a suitable method for many types of tissue, both with low and high fat or protein content. Internal standards (i.e., hydrocarbons not occurring in the sample) are added to enable an eventual check on extraction efficiency and losses during work-up.

One of the most commonly used methods for the extraction of hydrocarbons from tissues is direct alkaline hydrolysis or saponification followed by extraction into an organic solvent. This is shown both by the published literature and by scrutinizing methods used by laboratories participating in intercalibration exercises (Wise et al., 1980; Uthe et al., 1986). The combined procedure disrupts the cellular matrix, extracts the lipids, and saponifies the lipoid material all in one step, thereby reducing sample handling. Saponification is normally carried out on wet tissue, but freeze-dried samples may be treated in a similar manner. Farrington et al. (1986) reported, however, that freeze-drying a mussel homogenate before saponification not unexpectedly led to some losses of hydrocarbons. Another less expected result of their study was a significantly lower recovery of internal standards. They suggested that freeze-drying of the tissue homogenate produces some active sites of adsorption which retain a portion of the hydrocarbons present in the extraction solution.

Vassilaros et al. (1982) have discussed the many variations of the direct saponification method described in the literature. Tissues can be extracted by digestion either with aqueous KOH followed by partitioning of the hydrocarbons into an organic solvent such as diethyl ether, or with KOH in ethanol or methanol followed by addition of water and partitioning of hydrocarbons into suitable solvents, e.g., pentane, hexane, or iso-octane. The disadvantages reported for the method are that stable emulsions may be formed, lowering the extraction efficiency of the hydrocarbons into the organic phase. Fats can react with methanol in the presence of an alkaline catalyst such as KOH and form fatty acid methyl esters, which may interfere with the subsequent chromatographic analysis. These problems, however, are not unsurmountable. Emulsions formed by extraction of the aqueous caustic digestate will most often be eliminated by acidification of the digestate and thus removal of soaps (Vassilaros et al., 1982). In alcoholic solutions, saponification must be complete to avoid emulsions (Grimmer and Böhnke, 1975). The formation of fatty acid methyl esters in methanolic saponification is normally not a problem as long as some water is present (ca. 10%). If necessary,

water can be added before saponification. Saponification can alter other contaminants of interest in tissue samples, e.g., chlorinated compounds such as DDT, which is converted into DDE by removal of HCl.

Several well-documented methods for the analysis of PAHs in biological material employ the direct saponification method. They are reviewed by Howard and Fazio (1980). In a general screening, Grimmer and Böhnke (1975) compared the extraction efficiency for PAHs in protein-rich fish tissue by saponification/extraction with extraction by methanol alone. They found that only 30% of the PAH components were extractable by methanol alone, whereas an additional alkaline hydrolysis of the fish protein yielded another 60% of PAHs. Based on these results, it was claimed that hydrolysis was an absolute necessity to isolate PAHs quantitatively from insoluble samples such as fish muscle tissue. The saponification step was not found necessary for more easily soluble biological products such as fish oil. The method of Grimmer and Böhnke was adopted in 1976 by the Commission of Food Additives, IUPAC.

Grinding, homogenization or sonication of samples in the presence of organic solvents are also common for hydrocarbon extractions. The method of Bligh and Dyer (1959) for lipid extraction of wet tissues was used by Mackie et al. (1980). Berthou and Friocourt (1982) used an acetone/pentane solution on freeze-dried oyster homogenate in their analysis for aromatic hydrocarbons. Ultrasonication of wet mussel tissue in a mixture of propanol and light petroleum ether was used by Rowland and Volkman (1982). In the U.S. National Oceanic and Atmospheric Administration (NOAA) Status and Trends Mussel Watch Program for the analysis of PAHs and other organic contaminants, tissues are extracted by maceration in dichloromethane and anhydrous sodium sulfate (Wade et al., 1988). The method is described in detail by MacLeod et al. (1985).

Farrington and Medeiros (1975) compared some of the above-mentioned extraction methods and found that the extraction of a clam homogenate with anhydrous sodium sulfate and pentane in a Virtis homogenizer was equally or slightly less efficient than digestion in alcoholic alkali. The best results were obtained with Soxhlet extraction using benzene/methanol. It was concluded, however, that the differences between the methods were small. The results were based on gravimetric determinations of total aliphatic and aromatic fractions. Gritz and Shaw (1977) compared the efficiency of Soxhlet extraction followed by saponification with direct alkaline digestion and concluded that the recovery of single aliphatic compounds was essentially the same. The recovery of aromatic hydrocarbons could not be determined, because their peaks in the gas chromatographic analysis were obscured by superimposing peaks originating from other compounds in the clam tissue.

Volatile hydrocarbons can be found in organisms exposed to petroleum or petroleum products. Compounds boiling at a lower temperature than n-decane (b.p. 174.1 ^{0}C at 760 mm) cannot be determined in tissue by the above-mentioned solvent extraction methods because they are largely or entirely lost during the drying and concentration steps.

Steam distillation (Donkin and Evans, 1984, and references cited therein) and dynamic headspace sampling (Chesler et al., 1978; Warner et al., 1980) are more suitable. Donkin and Evans (1984) reported recoveries by steam distillation in excess of 80% for aromatic hydrocarbons in the volatility range bracketed by toluene and pyrene for mussels exposed to crude oil. The saponification procedure was shown to increase the recovery from mussel tissue. This observation is consistent with other evidence showing that the presence of lipids in biota can adversely affect the recovery of aromatic hydrocarbons by vapour phase procedures (Chesler et al., 1978). Steam distillation gives relatively clean extracts, but recoveries are low for the aliphatic and higher boiling aromatic hydrocarbons.

Chesler et al. (1978) used dynamic headspace sampling on saponified tissue homogenates to extract and collect volatile hydrocarbons. Tenax, a porous polymer resin, was used as the trapping material. Interfering polar biogenic components were removed by normal-phase high-performance liquid chromatography (HPLC), and final analysis was performed by gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS). Recoveries from mussel tissue homogenates approached 80-100% for 2- and 3-ring aromatic hydrocarbons, but were only 30% for aliphatic components. They assumed that the aliphatic hydrocarbons were retained in the lipophilic portion of the tissue homogenate, and that the partition coefficients for these hydrocarbons between the headspace sampling gas and the lipophilic fraction were unfavorable. Saponification was shown to improve the recovery of aromatic hydrocarbons somewhat, but had no appreciable effect on the recovery of aliphatic hydrocarbons. Warner et al. (1980) injected components retained on Tenax after headspace sampling directly onto a GC column and found this technique very useful for determining low levels of volatile aromatic hydrocarbons from benzene to dimethyl naphthalenes.

3.4 Isolation and separation

Tissue extracts will always contain many compounds other than hydrocarbons. A suitable chemical clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Tissue extracts are complex; therefore, the methods used for isolation should also be suitable to differentiate between hydrocarbon classes such as alkanes and aromatics. Many techniques have been used to perform this, e.g., column chromatography, thin-layer chromatography (TLC), gel permeation chromatography, and high-performance liquid chromatography (HPLC). Different techniques may be used singly or in combination, depending on such factors as the compounds or class of compounds to be analysed, their concentrations, and the selectivity and sensitivity of the final analytical tool.

Silica gel column chromatography has been used to isolate hydrocarbons from non-hydrocarbons and to further separate the hydrocarbons into aliphatic and aromatic fractions (Warner, 1976; Galloway et al., 1983). Alumina (Vassilaros et al., 1982) or alumina on top of silica gel (Farrington et al., 1973) have been applied for the same purpose. The aromatic fraction will also contain biogenic olefins. The aromatic fraction may be further separated into sub-fractions

based on similar molecular weights or similar numbers of aromatic rings (Cahnman and Kuratsune, 1957). The latter is more readily achieved using alumina rather than silica gel. Alumina retains high molecular weight polar material and unsaturated hydrocarbons more effectively than silica gel. Isolation and fractionation are usually performed on saponified tissue extracts, but the methods have also been applied to unsaponified extracts (Blumer et al., 1970; Zitko, 1975).

Florisil, a synthetic magnesium silicate, is another adsorption material commonly employed for the isolation of hydrocarbons from tissue extracts (Howard et al., 1968; Dunn and Armour, 1980), and for further separation into hydrocarbon fractions (Dunn and Stick, 1976). Berthou et al. (1981) used Florisil for the isolation of hydrocarbons and silica gel for their fractionation. Mackie et al. (1974) isolated and separated hydrocarbons using silicic acid. TLC, with classical adsorbent-covered plates, has also been used (Lee et al., 1972; Rowland and Volkman, 1982). Photo-oxidative and evaporative losses can, however, be a problem with the use of TLC. In adsorption chromatography, high affinity of the sorbate to the adsorbent material can result in irreversible adsorption and peak tailing with less efficient separation. Separation characteristics depend on the activity grade, which is dependent upon the water content of the adsorbent. If not carefully controlled, variations can lead to poor reproducibility of separations. Fully active columns may lead to the formation of hydrocarbon artifacts, such as phytadienes from phytol (Blumer et al., 1970).

Separations by HPLC are similar to those obtained with classical adsorbents such as silica gel (Wise et al., 1977). This technique has also been used to fractionate aromatic hydrocarbons according to number of aromatic rings (Wise et al., 1977; Berthou et al., 1981). Problems encountered with classical adsorption chromatography can be avoided by the use of normal phase columns.

Aliphatic fractions obtained by the above-mentioned methods are usually analysed directly by gravimetry, GC, or GC/MS. Urea clathration may be used before final analysis to separate alkanes from cycloalkanes (Wade and Quinn, 1980). The aromatic fractions are analysed by the same methods. In addition, methods for detection and quantification by UV absorption or UV fluorescence are available for aromatic hydrocarbons.

Biogenic compounds in the aromatic fractions may interfere with aromatic hydrocarbon analysis. Additional clean-up is often needed, especially if relatively non-selective methods are used, such as GC with FID detection. Various procedures have been developed to accomplish this. Lipophilic gels, such as Sephadex LH-20 or the BioBeads series, in adsorption/partition mode have been used by several authors. Mackie et al. (1980) separated the aromatic hydrocarbons from residual lipids by fractionation on a Sephadex LH-20 column after removing the saturated hydrocarbons by silicic acid chromatography. The gel chromatography was performed following a method developed for sediments (Giger and Schaffner, 1978). Another silicic acid column was applied before analysis by GC/MS. Ramos and Prohaska (1981)

used silica gel and then Sephadex LH-20 fractionation to obtain an aromatic fraction pure enough for GC analysis. Gel permeation chromatography on BioBeads S-X12 was used by Vassilaros et al. (1982), after alumina chromatography, in analyses of polycyclic aromatic hydrocarbons (PAHs) in fish tissue. Heit et al. (1980) omitted the adsorption chromatography step and fractionated saponified mussel extracts for PAH analysis by GC/MS directly on BioBeads S-X8 gel, but poor precision was reported. Warner et al. (1980) have used μ-Styragel and BioBeads S-X8 in their methods for determination of aromatic hydrocarbons in zooplankton and macrofauna. Hanus et al. (1979) passed saponified tissue extracts through silica gel to isolate the aromatic hydrocarbons, and purified and fractionated the aromatics by μ-Styragel chromatography. They determined individual aromatic hydrocarbons by reverse phase HPLC and fluorescence detection. The lipophilic gels separate the aromatic hydrocarbons from biogenic interfering material, and they also yield fractions containing compounds in sequence of increasing ring number. PAHs are eluted with isopropanol from Sephadex LH-20 in sequence of increasing ring number (Grimmer and Böhnke, 1975).

An alternative method for the clean-up of tissue extracts for the analysis of aromatic hydrocarbons has been described by Krahn et al. (1988). After initial removal of some of the lipids on a silica gel-alumina column, the tissue extracts were chromatographed on a Phenogel 100A size exclusion HPLC column. Comparison was made between their method and the Sephadex LH-20 method described by MacLeod et al. (1985). In general, the analytical results showed good comparability. The HPLC method was reported to have several advantages over the more commonly used older methods: improved precision, the ability to monitor chromatographic conditions, the potential for automatic analyses, and reduced consumption of solvents and other materials.

Another approach to purification is selective partitioning of aromatic hydrocarbons into dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF). Numerous methods exist, but most of them follow the general procedures outlined by Howard et al. (1966) for DMSO-partitioning, and by Grimmer and Hildebrandt (1972) for DMF-partitioning. After partitioning into DMSO or DMF, water is added, and the aromatic hydrocarbons are back-extracted into an alkane solvent. High recoveries have been reported for unsubstituted aromatic hydrocarbons (Howard et al., 1966, 1968; Grimmer and Hildebrandt, 1972; Natusch and Tomkins, 1978). Partition coefficients may, however, be less favorable for alkylated aromatic hydrocarbons (Natusch and Tomkins, 1978). This may cause losses and, therefore, the recovery of different aromatic hydrocarbons may vary. DMSO-partitioning and Sephadex LH-20 chromatography have been used in combination. Dunn and Armour (1980) suggest that the two techniques are complementary in that they remove different interfering compounds.

3.5 Analysis

A multitude of hydrocarbons can be found in living organisms covering a wide range of molecular weights and an enormous number of molecular structures, often at highly variable concentrations. No

single method is available to measure them all. Since often the hydrocarbons of interest are present at low concentrations in complex matrices, separation techniques with high sensitivity and/or selectivity have to be employed to obtain the required information. Gas chromatography, gas chromatography in combination with mass spectrometry, and high performance liquid chromatography are, therefore, the most widely used methods for the analysis of hydrocarbons in biota. Technical details of these methods are given elsewhere in this review. For analyses of total hydrocarbon fractions, additional techniques have been used, such as gravimetry and UV fluorescence spectrophotometry. An overview of common methods for the analysis of hydrocarbons in biota has been given by NAS (1980). The analytical chemistry of PAHs has been described by Lee et al. (1981).

4 THE ANALYSIS OF HYDROCARBONS IN MARINE SEDIMENTS

4.1 Sampling

4.1.1 Surface sediments

A variety of sampling devices, including divers in shallow water, has been used to collect samples of surface sediment. Grab samplers are commonly used and can be effective in most types of sediment, though grabs with buckets which close at the bottom (e.g., Day, Smith-McIntyre, Van Veen) may suffer the loss of sediment or fines during recovery if stones or shells prevent proper closure of the jaws. In gravelly or shelly sediments, therefore, the use of a rotating bucket type of sampler (e.g., Shipek) may be more successful, as water cannot wash through the bucket during recovery even if it is not completely closed. Problems may also be encountered in fluid muds, where there may be insufficient resistance on impact to trigger the grab's closure.

Coring devices, such as gravity corers, are used to collect cylindrical sediment cores which may be sub-divided for analysis. They are most useful in soft sediments; penetration of the corer into the seabed may be restricted in harder substrates. The analysis of sediment cores allows the determination of concentration gradients with depth below the sediment surface and, if combined with radioactive dating techniques, may allow inferences to be made of changing concentrations with time. Hydrostatically damped corers in multiple arrays have also been used to collect multiple short cores for surface sediment analysis (e.g., Wakeham and Carpenter, 1976; Barnett et al., 1984), the damped rate of penetration minimizing disturbance of the sediment surface.

The floc present at the sediment surface represents the most recently deposited material, and is important as a site for the initial accumulation of petroleum compounds deposited to the sediment-water interface. It is, therefore, of great interest (Gearing et al., 1980; Boehm et al., 1982). The floc is difficult to sample as it is as yet unconsolidated and is very easily disturbed by the sampler; it may be pushed away altogether by the 'bow wave' of a grab sampler falling to the seabed. Manual collection is probably the preferred

method for the collection of floc samples, if it is feasible, though special sampling devices have been constructed (e.g., Bryant et al., 1980).

Sampling devices used for the collection of sediments for hydrocarbon analysis should, whenever possible, be constructed of materials which may be solvent-cleaned prior to use, such as stainless steel and PTFE, and sub-samples should be taken from the centre of the sample collected so as to minimize the chance of contamination from the sampling device. Where these are deployed by wire, there is the possibility of contamination from lubricants, oils, and greases used both on the wire itself and associated winches. Whenever possible, the wire and the sampler should not be connected together directly, but by means of a clean rope (5-10 m). Grab samplers may be deployed entirely using rope, free-falling to the seabed and then raised using a whipping-drum, rather than a main winch drum, as these are generally unlubricated. Samples of the ship's fuel, bilge contents, and any lubricants used should be taken for comparison with the hydrocarbons found in the samples so as to rule out adventitious shipboard contamination. Sediment samples are best stored at -20 ^{0}C prior to analysis and should be frozen as quickly as possible after collection to minimize degradation.

4.1.2 Sedimenting material

In studies of the flux of organics (including hydrocarbons) to the seabed, the deployment of sediment trap arrays (e.g., Payne and Davies, 1977; Wakeham et al., 1980) to collect sedimenting material for analysis has played a major role. Care must be taken to prevent degradation of the organic material collected during long deployments and to identify resuspended bottom sediments in near-bottom traps. In addition, the design of sediment traps which can be used to make quantitative estimates of transport is complex (Gardner, 1980a, 1980b). Even relatively simple traps have, however, yielded important information on the sedimentation of oil spilled at sea (Boehm et al., 1982).

4.2 Extraction and clean-up

Hydrocarbons in the range C_1 to C_{10} are not amenable to routine solvent extraction techniques because of their high volatility. Methods for their isolation and analysis are mainly limited to sea water, although dynamic headspace techniques using inert gas purge and trap systems have also been used on sediments (Bernard et al., 1978).

Although the determination of volatile hydrocarbons (C_1 to C_8) in sediments is not a general feature of oil pollution investigations, it is relevant in studies of biological and low-temperature (less than 50 ^{0}C) processes in recent sediments. Such analyses have been conducted using a headspace sampling method followed by gas chromatographic analysis (Whelan, 1983; Whelan and Hunt, 1983). Sediment cores are sub-sampled and frozen prior to analysis. Aliquots of the frozen wet sediment, distilled water, and a helium headspace of known volume are sealed in a stainless steel vessel equipped with a silicone rubber septum and two stainless steel balls. The sediment

is allowed to thaw, and the vessel is shaken vigorously and then heated for 30 minutes in a water bath at 95 ^{0}C, after which the headspace is sampled for analysis.

A number of different solvent extraction methods are commonly used for the extraction of hydrocarbons (>C_{10}) from sediments. No standard method exists, but most methods involve the combined use of polar and non-polar solvents to obtain an efficient extraction (NRC, 1985). Fine sediments (mud, silt, clay) are much more difficult for solvents to penetrate so as to release the hydrocarbons bound to sediment particles, and may require a more careful choice of method and solvents to extract effectively than coarser and relatively open sediments (sand, gravel). Very high concentrations of hydrocarbons (>50,000 µg/g dry weight) may also require smaller sample sizes than normal if the capacity of the solvent to dissolve a mass of hydrocarbons is not to be exceeded. Solvent extractions may be carried out at ambient temperature with mixing of the sediment and solvent phase by shaking, ball-mill tumbling, or the use of ultrasonic probes. Refluxing with solvent, or with methanolic KOH to perform an alkaline digestion prior to solvent extraction, and the use of Soxhlet extraction with either methanol alone or a mixed solvent, such as dichloromethane/methanol, complete the suite of most commonly used extraction techniques. Although historically benzene was very commonly used as an extraction solvent or as one component of a solvent mixture, it has now generally been discarded on health grounds and replaced by toluene or dichloromethane. The use of carbon tetrachloride as a solvent in studies using infra-red spectrophotometry has also now begun to decline for similar reasons, trichlorotrifluoroethane being a suitable alternative.

Comparisons of the performance of a number of these extraction methods have been reported. Farrington and Tripp (1975) compared by gas chromatography:

a) Soxhlet extraction (benzene/methanol),
b) alkaline hydrolysis (methanolic KOH; benzene), and
c) Soxhlet extraction (acid wash, benzene/methanol).

The sediment used was a wet sandy silt containing about 250 µg/g dry weight of hydrocarbons. No significant differences were found in the efficiency of the three techniques.

Hilpert et al. (1978) carried out an intercomparison exercise among eight laboratories using two intertidal sediment samples of fine- to medium-grain sand. Analysis was by gas chromatography for both aliphatic and aromatic hydrocarbons, and the methods used by the laboratories were:

- Soxhlet extraction and headspace (dichloromethane/diethyl ether);
- ball-mill tumbler (diethyl ether);
- reflux (benzene/methanol);
- cold solvent extraction (benzene/methanol);
- ball-mill tumbler (heptane);

- reflux (toluene/methanol);
- alkaline digestion (methanolic KOH); and
- reflux (toluene/methanol).

Concentrations of total extractable hydrocarbons were from 9 to 500 µg/g and 49 to 6625 µg/g dry weight for the two sediments, respectively, with results for aliphatic and aromatic hydrocarbons showing a similar variation. Pristane/phytane ratios were more consistent, but there was little agreement over which was the most abundant n-alkane or aromatic hydrocarbon. Little interpretation could be made of the results obtained against extraction method employed.

Wong and Williams (1980) compared three extraction methods:

1) alkaline digestion (methanolic KOH),
2) Soxhlet extraction (chloroform), and
3) Soxhlet extraction (carbon tetrachloride),

by analysis of a heavily polluted estuarine sediment of fine particle size. With wet sediments, method 1 gave results which both indicated greater extraction efficiency and more reproducibility than methods 2 and 3, and method 2 was somewhat more efficient than method 3. When dried sediments were used, all three methods yielded broadly comparable results, although about 16% of the hydrocarbons determined by the wet extraction procedures were lost as a consequence of the drying process.

Lake et al. (1980) compared the analysis of PAHs in sediments by GC/MS, using three extraction techniques:

1) Soxhlet extraction (benzene/methanol),
2) ball-mill tumbler (methanol/dichloromethane), and
3) reflux (dichloromethane).

Methods 1 and 3 were found to be equally efficient, but method 2 was only about 72% as efficient as the other two methods. The relative proportions of different compounds and compound types was, however, very similar for all three methods.

Brown et al. (1980) also compared a ball-mill tumbler method with other methods for an intertidal sediment (fine to medium sand), using gas chromatography. The methods used were:

1) ball-mill tumbler (dichloromethane/methanol),
2) alkaline digestion (methanolic KOH),
3) Soxhlet extraction (benzene/methanol), and
4) Soxhlet extraction (dichloromethane/methanol).

In this study, method 1 was found to be as efficient as the other three methods for the extraction of aliphatic hydrocarbons in the range C_{13} to C_{26}, although Soxhlet extraction was more efficient in the range C_{27} to C_{31}. Method 2 was generally the least efficient but the most reproducible for both aliphatic and aromatic hydrocarbons, and method 4 was the least reproducible in both cases. The better performance of the ball-mill tumbler method in this study may be due

to particle size variations between this sediment and that used by Lake et al. (1980), but unfortunately their paper gives few details on the sediment used.

MacLeod et al. (1982) compared the results of 13 laboratories, each using its own methodology in the first phase of an intercomparison exercise, using an intertidal harbour sediment and gas chromatography. Concentrations of 18 alkanes in the range 5-300 ng/g dry weight generally agreed within a factor of three. Less precise results were obtained for 10 aromatic compounds present at concentrations up to 10 times greater. The second phase of the exercise involved eight laboratories in the analysis of a subtidal river sediment. Two methods: 1) Soxhlet extraction, and 2) ball-mill tumbler, were used with various solvent systems, followed by capillary gas chromatography. The results from two laboratories were significantly different from the other six, whose results (within the range 10-2000 ng/g dry weight) generally agreed within a factor of two. Again, the choice of solvents seemed more important than the choice of extraction method. The recommendations made to promote further improvement of comparability were:

1) to rechromatograph the aromatic hydrocarbon fraction from silica gel or Sephadex LH-20;

2) to specify the capillary GC column and mode of integration; and

3) to use internal standards.

In a later study, MacLeod et al. (1988) demonstrated a great improvement in comparability among six laboratories following their adoption of a common, improved methodology for the analysis of PAHs in marine sediments (MacLeod et al., 1985). The procedure involved drying the sample (under the extraction solvent) with anhydrous sodium sulfate, extraction by tumbling with dichloromethane, chromatography on a) silica gel/alumina and b) Sephadex LH-20, and analysis by GC/FID using a common range of internal and calibration standards. Confirmatory analyses were conducted by GC/MS. These methods were applied to two naturally contaminated sediments for the determination of 18 compounds, ranging from naphthalene to dibenzo-[a,h]anthracene. Overall relative standard deviations (RSDs) varied from 15 to 36% of the grand mean of results from the six laboratories, against 14 to 81% in the immediately preceding exercise in which detailed analytical protocols were not laid down. MacLeod et al. (1988) concluded that these measures and the demonstrated improvements in comparability provided a basis for the implementation of statistically valid quality control and quality assurance programmes for analyses in marine sediments.

The first ICES intercomparison exercise on petroleum hydrocarbon analyses (Law and Portmann, 1982) used a fine sandy, intertidal sediment from an industrial estuary, oven-dried, with a total hydrocarbon concentration of about 35 µg/g dry weight. No methods were specified and various cold solvent, alkaline digestion, and Soxhlet extraction techniques were employed. The largest set of data reported was for total hydrocarbons by UV fluorescence using excita-

tion and emission wavelengths of 310 and 360 nm, respectively (UNESCO, 1976). The mean value obtained (30 laboratories) was 33.2 µg/g Ekofisk oil equivalents, with a relative standard deviation (RSD) of 18.4%. Four laboratories making four or more measurements achieved intralaboratory RSDs of 1.9 to 3.0%. The means obtained by infra-red spectrophotometry and gas chromatography were 28.1 µg/g (RSD 79.4%, 15 laboratories) and 26.2 µg/g (RSD 27.5%, 9 laboratories), respectively. The results for specific aliphatic and aromatic hydrocarbons were fewer and much more variable. For total hydrocarbon analyses, at least, most extraction techniques seemed to be capable of yielding good results, although alkaline digestion methods seemed to be the most reproducible.

The extraction of sediments using ultrasonication has been compared with saponification and Soxhlet methods by Lichtenthaler et al. (1986). Tests were conducted with diesel oil and a low-aromatic oil of the type used in the formulation of drilling fluids. Average recoveries were 79-85% for samples spiked with diesel oil, and 78 to 101% for those spiked with the low-aromatic oil, with both ultrasonication and Soxhlet extraction yielding lower recoveries than saponification methods. The reproducibility of all methods was good, with relative standard deviations generally below 10%.

Clean-up techniques seem to be fairly standard. TLC methods have now been largely replaced by column chromatography on alumina and silica gel, and treatment with activated copper is commonly used to remove elemental sulfur (Blumer, 1957). The aromatic fraction obtained from chromatography on silica gel will also contain olefins, which can be removed by chromatography using Sephadex LH-20. Preparative HPLC is also now beginning to be used as a routine clean-up technique for hydrocarbon extracts, particularly as it can easily be automated.

4.3 Analytical techniques

A number of methods are in routine use for hydrocarbon analysis, and a brief summary of their advantages and disadvantages is included in the following list.

4.3.1 Gravimetry

This is a relatively cheap and simple method with a sensitivity in the low µg/g range for total hydrocarbons, although errors are much greater at low concentrations. It cannot be used for volatile oils, as evaporation to dryness entails heavy losses of low-boiling materials. No discrimination can be made between hydrocarbon and non-hydrocarbon material.

4.3.2 Infra-red spectrophotometry

This technique is largely unaffected by calibration errors for different oils, but is very insensitive to aromatics. Lipid-type materials will also be detected, so quantitative measurements are badly affected by inefficient clean-up. It has been commonly used for fingerprinting, either in the absence of, or in addition to, chromatographic techniques.

4.3.3 Ultra-violet fluorescence spectroscopy

This technique is very sensitive to aromatic hydrocarbons, but aliphatic hydrocarbons and lipids are not detected. Calibration of the technique is affected by the choice of oil, to a small extent for different crude oils for example, but to a large degree for the new low-aromatic base oils used in drilling fluids, in which the aromatic content may be <1%. Synchronous excitation spectra provide some qualitative information on the range of aromatics present, and contour diagrams may be used for fingerprinting. Care must be taken to make measurements and run spectra only in a dilution range where quenching and self-absorption effects are absent. Simple ways to check this are:

- monitor the size of the Raman absorption peak for both pure solvent and samples;

- dilute the extracts by a known amount and remeasure the fluorescence. The fluorescence signal should decrease by the same factor. If the fluorescence yield decreases by a lesser amount, then quenching or internal reabsorption of emitted light were occurring (and may still be doing so).

4.3.4 Capillary gas chromatography

This technique is useful both quantitatively and qualitatively. Chromatograms of aromatic hydrocarbon fractions may be very complex if large quantities of alkylated PAHs are present, but are much simpler when combustion PAHs are the major source. It is excellent for fingerprinting. New column materials and more thermostable stationary phases may extend the useful analytical range for n-alkanes above n-C_{50}, possibly up to n-C_{100} (Lipsky and Duffy, 1986).

4.3.5 High pressure liquid chromatography

This is a useful technique for the analysis of aromatics when coupled with UV absorption or, particularly, fluorescence detectors. It lacks resolution compared to capillary gas chromatography unless microbore systems are used, in which case the pumping system must be superior because of the very low flow rates required; analysis times are very long (1-2 days). Resolution very similar to that of capillary GC may then be obtained, but this technique is of little use as a routine tool.

4.3.6 Computerized gas chromatography/mass spectrometry

This is currently the most powerful technique available for the analysis of specific aromatic and aliphatic hydrocarbons. Mass spectra provide confirmation of a compound's identity and the ability to identify unknowns. The use of multiple internal standards allows correction for extraction efficiences to be made at various points in the boiling range and for different compound types. The use of

multiple ion detection (mass fragmentography) gives maximum sensitivity for predetermined compounds with a high degree of specificity.

Fingerprinting techniques possible using GC/MS include:

- sterane/triterpane distribution patterns, and
- alkylated aromatic isomer distributions.

In addition, combustion PAHs, geochemically produced PAHs, biogenic and petroleum inputs can all be recognized from GC/MS information, although unequivocal estimates of the relative contribution from each source may be difficult as the same compounds may be derived from one or more sources.

4.4 Comparison of some techniques

A recent comparison (Howells, 1986) has investigated the effect of applying different analytical methods (gravimetry, infrared (IR) spectrophotometry, ultra-violet fluorescence (UVF), gas chromatography) to a range of sediment samples. These were obtained along known concentration gradients at several North Sea oil fields with contrasting contaminant types (i.e., at which different types of oil-based drilling fluids have been used), in order to compare the absolute concentrations, concentration trends, and interpretation produced by each. A total of 31 sediment samples was analysed, and concentrations of total hydrocarbons were in the range 4 to 100,000 µg/g dry weight. Results obtained by gravimetry, IR and UVF generally showed good agreement, provided that the correct choice of calibrant oil had been made for UVF. A specified extraction method (alkaline digestion) and clean-up was used. This had previously been shown to give good precision when applied to a variety of sediments and relatively good accuracy as determined from spiked samples, and this contributed greatly to the good agreement found between analytical techniques. It is still necessary, however, to exercise extreme caution when comparing results from different laboratories who may be utilizing different methods and solvent systems.

ACKNOWLEDGEMENT

The authors thank Dr John Farrington, University of Massachusetts, Boston, for his valuable comments. This review has been prepared following a request by the ICES Advisory Committee on Marine Pollution (ACMP) to the ICES Marine Chemistry Working Group.

5 REFERENCES

Ahnoff, M., and Josefsson, B. 1974. Simple apparatus for on-site continuous liquid-liquid extraction of organic compounds from natural waters. Anal. Chem., 46: 658-663.

Ahnoff, M., and Josefsson, B. 1976. Apparatuses for in situ solvent extraction of non-polar organic compounds in sea and river water. Anal. Chem., 48: 1268-1270.

Aizenshtat, A. 1973. Perylene and its geochemical significance. Geochim. Cosmochim. Acta, 37: 559-567.

Albaigés, J., and Albrecht, P. 1979. Fingerprinting marine pollutant hydrocarbons by computerized gas chromatography-mass spectrometry. Intern. J. environ. Anal. Chem., 6: 171-190.

Albaigés, J., Grimalt, J., Bayana, J.M., Risebrough, R., de Lappe, B., and Walker II, W. 1984. Dissolved, particulate and sedimentary hydrocarbons in a deltaic environment. Org. Geochem., 6: 237-248.

Barbier, M., Joly, D., Saliot, A., and Tourres, D. 1973. Hydrocarbons from sea water. Deep-Sea Res., 20: 305-314.

Barnett, P.R.O., Watson, J., and Connelly, D. 1984. A multiple corer for taking virtually undisturbed samples from shelf, bathyal and abyssal sediments. Oceanol. Acta., 7: 399-408.

Bentz, A.P. 1976. Oil spill identification. Anal. Chem., 48: 454A-472A.

Bernard, B.B., Brooks, J.M., and Sackett, W.M. 1978. Light hydrocarbons in recent Texas continental shelf and slope sediments. J. Geophys. Res., 83: 4053-4061.

Berthou, F., and Friocourt, M.P. 1982. Combination of high-performance chromatographic methods for the analysis of aromatic hydrocarbon pollutants in marine biota. In Analytical techniques in environmental chemistry. 2, pp. 221-230. Ed. by J. Albaigés. Pergamon Press, Oxford and New York. 473 pp.

Berthou, F., Gourmelun, Y., Dreano, Y., and Friocourt, M.P. 1981. Application of gas chromatography on glass capillary columns to the analysis of hydrocarbon pollutants from the Amoco Cadiz oil spill. J. Chromatogr., 203: 279-292.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Blumer, M. 1957. Removal of elemental sulfur from hydrocarbon fractions. Anal. Chem., 29: 1039-1041.

Blumer, M. 1967. Hydrocarbons in the digestive tract and liver of a basking shark. Science, 156: 390-391.

Blumer, M., Ehrhardt, M., and Jones, J.H. 1973. The environmental fate of stranded crude oil. Deep-Sea Res., 20: 239-259.

Blumer, M., Guillard, R.R.L., and Chase, T. 1971. Hydrocarbons of marine phytoplankton. Mar. Biol., 8: 183-189.

Blumer, M., Souza, G., and Sass, J. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Mar. Biol., 5: 195-202.

Blumer, M., and Youngblood, W.W. 1975. Polycyclic aromatic hydrocarbons in soils and recent sediments. Science, 188: 53-55.

Bocard, C., Costaing, G., and Gatellier, C. 1982. Chemical oil dispersion in trials at sea and laboratory tests: The key role of dilution processes. Paper given at ASTM Symposium on Oil Spill Dispersants, West Palm Beach, Florida, USA, 12-13 October 1982. ASTM, Philadelphia.

Boehm, P.D. 1980. Evidence for the decoupling of dissolved, particulate and surface microlayer hydrocarbons in northwestern Atlantic continental shelf waters. Mar. Chem., 9: 255-281.

Boehm, P.D., Barak, J.E., Fiest, D.L., and Elskus, A.A. 1982. A chemical investigation of the transport and fate of petroleum hydrocarbons in littoral and benthic environments: The TSESIS oil spill. Mar. environ. Res., 6: 157-188.

Boehm, P.D., and Fiest, D.L. 1982. Sub-surface distributions of petroleum from an offshore well blowout. The IXTOX I blowout, Bay of Compeche. Environ. Sci. Technol., 16: 67-74.

Brown, D.W., Ramos, L.S., Uyeda, M.Y., Friedman, A.J., and MacLeod, W.D.jr. 1980. Ambient-temperature extraction of hydrocarbons from marine sediment - comparison with boiling-solvent extractions. Adv. Chem. Ser., 185: 313-326.

Bryant, R.A., Williams, D.J.A., and James, A.E. 1980. A sampler for cohesive sediment in the benthic boundary layer. Limnol. Oceanogr., 25: 572-576.

Burns, K.A. 1986. Evidence for rapid in situ oxidation rate of pollutant hydrocarbons in the open Mediterranean. Rapp. P.-v. Réun. Cons. int. Explor. Mer., 186: 432-441.

Burns, K.A., and Smith, J.L. 1980. Hydrocarbons in Victorian coastal waters. Aust. J. Mar. Freshwater Res., 31: 251-256.

Burns, K.A., and Smith, J.L. 1982. Hydrocarbons in Victorian coastal ecosystems (Australia): Chronic petroleum inputs to Western Port and Port Phillips Bays. Arch. environm. Contam. Toxicol., 11: 129-140.

Butler, A.C., and Sibbald, R.R. 1987. Sampling and GC-FID, GC/MS analysis of petroleum hydrocarbons in the ocean surface microlayer off Richards Bay, South Africa. Est. coastal Shelf Sci., 25: 27-42.

Cahnman, H.J., and Kuratsune, M. 1957. Determination of polycyclic aromatic hydrocarbons in oysters collected in polluted water. Anal. Chem., 29: 1312-1317.

Carlson, D.J., Cantey, J.L., and Cullen, J.J. 1988. Description of and results from a new surface microlayer sampling device. Deep-Sea Res., 35: 1205-1213.

Chesler, S.N., Gump, B.H., Hertz, H.S., May, W.E., and Wise, S.A. 1978. Determination of trace level hydrocarbons in marine biota. Anal. Chem., 50: 805-810.

Clark, R.C., Blumer, M., and Raymond, S.O. 1967. A large water sampler, rupture-disc triggered, for studies of dissolved organic compounds. Deep-Sea Res., 14: 125-128.

Connell, D.W., and Miller, G.J. 1980a. Petroleum hydrocarbons in aquatic ecosystems - behaviour and effects of sublethal concentrations: Part 1. CRC Critical Reviews in Environmental Control, 11: 37-104.

Connell, W.D., and Miller, G.J. 1980b. Petroleum hydrocarbons in aquatic ecosystems - behaviour and effects of sublethal concentrations: Part 2. CRC Critical Reviews in Environmental Control, 11: 105-162.

Corredor, J.E., Morelli, J., and Méndéz, A. 1983. Pelagic petroleum pollution off the south-west coast of Puerto Rico. Mar. Pollut. Bull., 14: 166-168.

CRC. 1981. Handbook of chemistry and physics. CRC Press, Inc. Baton Rouge, Florida 33431. pp. E63-E65.

Davies, J.M., and Tibbetts, P.J.C. 1987. The use of in situ benthic chambers to study the fate of oil in sublittoral sediments. Estuar. coast. Shelf Sci., 24: 205-223.

Dawson, R., and Riley, J.P. 1977. Chlorine-containing pesticides and chlorinated biphenyls in British coastal waters. Estuar. coast. mar. Sci., 5: 55-69.

Dawson, R., Riley, J.P., and Tennant, R.H. 1976. Two samplers for large-volume collection of chlorinated hydrocarbons. Mar. Chem., 4: 83-88.

de Lappe, B.W., Risebrough, R.W., and Walker II, W. 1983. A large volume sampling assembly for the determination of synthetic organic and petroleum compounds in the dissolved and particulate phases of sea water. Can. J. Fish. Aquat. Sci., 40 (Suppl. 2): 322-336.

Derenbach, J.B., Ehrhardt, M., Osterroht, C., and Petrick, G. 1978. Sampling of dissolved organic material from sea water with reversed phase techniques. Mar. Chem., 6: 351-364.

Donkin, P., and Evans, S.V. 1984. Application of steam distillation in the determination of petroleum hydrocarbons in water and mussels (Mytilus edulis) from dosing experiments with crude oil. Anal. Chim. Acta, 156: 207-219.

Dunn, B.P. and Armour, R.J. 1980. Sample extraction and purification for determination of polycyclic aromatic hydrocarbons by reversed-phase chromatography. Anal. Chem., 52: 2027-2031.

Dunn, B.P., and Stick, H.F. 1976. Monitoring procedures for chemical carcinogens in coastal waters. J. Fish. Res. Bd Can., 33: 2040-2046.

Ehrhardt, M. 1978. An automatic sampling buoy for the accumulation of dissolved and particulate organic material from sea wate Deep-Sea Res., 25: 119-126.

Ehrhardt, M. 1983. Preparation of lipophilic organic seawater concentrates. In Methods of seawater analysis, pp. 276-281. Ed. by K. Grasshoff, M. Ehrhardt, K. Kremling. Verlag Chemie, Weinheim. 419 pp.

Ehrhardt, M. 1987. Lipophilic organic material: An apparatus for extracting solids used for their concentration from sea water. ICES Techn. Mar. Environ. Sci., No. 4, 14 pp.

Ehrhardt, M., and Blumer, M. 1972. The source identification of marine hydrocarbons by gas chromatography. Environ. Pollut., 3: 179-194.

Ehrhardt, M., Bouchertall, F., and Hopf, H.-P. 1982. Aromatic ketones concentrated from Baltic sea water. Mar. Chem., 15: 47-58.

Ehrhardt, M.G., and Burns, K.A. 1990. Petroleum derived dissolved organic compounds concentrated from inshore waters during the 1988 GEEP Workshop in Bermuda. J. Exp. Mar. Biol. Ecol., 138: 35-47.

Ehrhardt, M., and Derenbach, J. 1980. Phthalate esters in the Kiel Bight. Mar. Chem., 8: 339-346.

Ehrhardt, M., and Heinemann, J. 1975. Hydrocarbons in blue mussels from the Kiel Bight. Environ. Pollut., 9: 263-282.

Ehrhardt, M., and Knap, A. 1989. A direct comparison of UV fluorescence and GC/MS data of lipophilic open-ocean seawater extracts. Mar. Chem., 26: 178-188.

Ehrhardt, M., Osterroht, C., and Petrick, G. 1980. Fatty-acid methyl esters dissolved in sea water and associated with suspended particulate material. Mar. Chem., 10: 67-76.

Ehrhardt, M., and Petrick, G. 1989. Relative concentrations of dissolved/dispersed fossil fuel residues in the Mediterranean measured by UV fluorescence. Mar. Pollut. Bull., 20(11): 560-564.

El Samra, M.I., Emara, H.I., and Shunbo, F. 1986. Dissolved petroleum hydrocarbons in the northwestern Arabian Gulf. Mar. Pollut. Bull., 11: 65-68.

Farrington, J.W., Davis, A.C., Frew, N.M., and Knap, A. 1986. ICES/IOC intercomparison exercise on the determination of petroleum hydrocarbons in biological tissues (mussel homogenate). Mar. Pollut. Bull., 19(8): 372-380.

Farrington, J.W. and Medeiros, G.C. 1975. Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms. Proc. 1975 Conference on Prevention and Control of Oil Pollution, pp. 115-121. American Petroleum Institute, Washington, D.C.

Farrington, J.W., Risebrough, R.W., Parker, P.L., Davis, A.C., and de Lappe, B. 1982. Hydrocarbons, polychlorinated biphenyls, and DDE in mussels and oysters from the US coast - 1976-1978 - the Mussel Watch. Scripps Institution of Oceanography, La Jolla, CA, USA. NTIS Order No. PB83-133371. 111 pp.

Farrington, J.W., Teal, J.M., and Parker, P.L. 1976. Petroleum hydrocarbons. In Strategies for marine pollution monitoring, pp. 3-34. Ed. by E.D. Goldberg. Wiley Interscience, New York, London, Sydney, Toronto. 310 pp.

Farrington, J.W., Teal, J.M., Quinn, J.G., Wade, T., and Burns, K. 1973. Intercalibration of analyses of recently biosynthesized hydrocarbons and petroleum hydrocarbons in marine lipids. Bull. environ. Contam. Toxicol., 10: 129-136.

Farrington, J.W., and Tripp, B.W. 1975. A comparison of analysis methods for hydrocarbons in surface sediments. In Marine chemistry in the coastal environment, pp. 267-289. Ed. by T.M. Church. American Chemical Society, Washington, D.C. 710 pp.

Farrington, J.W., Wakeham, St.G., Livramento, J.B., Tripp, B.W., and Teal, J.M. 1986. Aromatic hydrocarbons in New York Bight polychaetes: Ultraviolet fluorescence analyses and gas chromatography/gas chromatography-mass spectrometry analyses. Environ. Sci. Technol., 20: 69-72.

Fogelqvist, E., Lagerkvist, S., and Lindroth, P. 1982. Petroleum hydrocarbons in Arctic ocean surface water. Mar. Pollut. Bull., 13: 211-213.

Galloway, W.B., Lake, J.L., Phelps, D.K., and Rogerson, P.F. 1983. The Mussel Watch: Intercomparison of trace level constituent determinations. Environ. Toxicol. Chem., 2: 395-410.

Gardner, W.D. 1980a. Sediment trap dynamics and calibration: a laboratory evaluation. J. Mar. Res., 38: 17-39.

Gardner, W.D. 1980b. Field assessment of sediment traps. J. Mar. Res., 38: 41-52.

Gassmann, G., and Pocklington, R. 1984. Hydrocarbons in waters adjacent to an oil exploratory site in the western North Atlantic Ocean. Environ. Sci. Technol., 18: 869-872.

Gearing, P.J., Gearing, J.N., Pruel, R.J., Wade, T.L., and Quinn, J.G. 1980. Partitioning on No. 2 fuel oil in controlled estuarine ecosystems: sediments and suspended particulate matter. Environ. Sci. Technol., 14: 1129-1136.

GESAMP. 1977. Impact of oil on the marine environment. IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP). Reports and Studies No. 6, 250 pp.

Giger, W., and Schaffner, C. 1978. Determination of polycyclic aromatic hydrocarbons in the environment by glass capillary gas chromatography. Anal. Chem., 50: 243-249.

Gjavotchanoff, St. 1972. Druckextraktion als neues Verfahren zur Anreicherung von organischen Stoffen aus wässrigen Systemen. Wasser und Boden, 5: 140-141.

Goldberg, E.D. 1986. The mussel watch concept. Proc. First Intl. Symp. on Integrated Global Ocean Monitoring. Tallinn, USSR, 2-10 October 1983. Vol. 1, pp. 71-82.

Goldberg, M.C., and DeLong, L. 1973. Extraction and concentration of organic solutes from water. Anal. Chem., 45: 89-93.

Gómez-Belinchón, J.I., Grimalt, J.O., and Albaigés, J. 1988. Intercomparison study of liquid-liquid extraction and adsorption on polyurethane and Amberlite XAD-2 for the analysis of hydrocarbons, polychlorobiphenyls, and fatty acids dissolved in sea water. Environ. Sci. Technol., 22: 677-685.

Gough, M.A., and Rowland, S.J. 1990. Characterization of unresolved complex mixtures of hydrocarbons in petroleum. Nature, 344: 648-650.

Goutx, M., and Saliot, A. 1980. Relationship between dissolved and particulate fatty acids and hydrocarbons, chlorophyll-a and zooplankton biomass in Villefranche Bay, Mediterranean Sea. Mar. Chem., 8: 299-318.

Gouygou, J.P., and Michel, P. 1981. Trois ans après l'Amoco Cadiz: Identification des hydrocarbures persistants. ICES CM 1981/E:53. 5 pp.

Green, D.R., and le Pape, D. 1987. Stability of hydrocarbon samples on solid phase extraction columns. Anal. Chem., 59: 699-703.

Green, D.R., Stull, J.K., and Heesen, T.C. 1986. Determination of chlorinated hydrocarbons in coastal waters using a moored *in situ* sampler and transplanted live mussels. Mar. Pollut. Bull., 17: 324-329.

Grice, G.D., Harvey, G.R., Bowen, V.T., and Backus, R.H. 1972. The collection and preservation of open ocean marine organisms for pollutant analysis. Bull. environ. Contam. Toxicol., 7: 125-132.

Grimmer, G., and Böhnke, H. 1975. Polycyclic aromatic hydrocarbon profile analysis of high-protein foods, oils, and fats by gas chromatography. J. Assoc. Off. Anal. Chem., 58: 725-733.

Grimmer, G., and Hildebrandt, A. 1972. Concentration and estimation of 14 polycyclic aromatic hydrocarbons at low levels in high-protein foods, oils, and fats. J. Assoc. Off. Anal. Chem., 55: 631-635.

Gritz, R.L., and Shaw, D.G. 1977. A comparison of methods for hydrocarbon analysis of marine biota. Bull. environ. Contam. Toxicol., 17: 408-414.

Grob, K. 1973. Organic substances in potable water and its precursor. Part I. Methods for their determination by gas-liquid chromatography. J. Chromatogr., 84: 255-273.

Grob, K., and Grob, G. 1974. Organic substances in potable water and its precursor. Part II. Applications in the area of Zürich. J. Chromatogr., 90: 303-313.

Grob, K., Grob, K. jr., and Grob, G. 1975. Organic substances in potable water and in its precursor. Part III. The closed loop stripping procedure compared with rapid liquid extraction. J. Chromatogr., 106: 299-315.

Grob, K., and Zürcher, F. 1976. Stripping of trace organic substances from water: equipment and procedure. J. Chromatogr., 117: 285-294.

Gschwend, P.M., Zafiriou, O.C., Mantoura, R.F.C., Schwarzenbach, R.P., and Gagosian, R.B. 1982. Volatile organic compounds at a coastal site. 1. Seasonal variations. Environ. Sci. Technol., 16: 31-38.

Gump, B.H., Hertz, H.S., May, W.E., Chesler, S.N., Dyszel, S.M., and Eugenio, D.P. 1975. Drop sampler for obtaining fresh and sea water samples for organic compound analysis. Anal. Chem., 47: 1223-1224.

Gundlach, E.R., and Marchand, M. (eds.) 1982. Ecological study of the Amoco Cadiz oil spill: Report of the NOAA-CNEXO Joint Scientific Commission. 479 pp.

Hanus, J.P., Guerrero, H., Biehl, E.R., and Kenner, C.T. 1979. High pressure liquid chromatographic determination of polynuclear aromatic hydrocarbons in oysters. J. Assoc. Off. Anal. Chem., 62: 29-35.

Hargrave, B.T., and Phillips, G.A. 1975. Estimates of oil in aquatic sediments by fluorescence spectroscopy. Environ. Pollut., 8: 193-215.

Harvey, G.R. 1972. Adsorption of chlorinated hydrocarbons from sea water by a crosslinked polymer. Woods Hole Oceanographic Institution Technical Report, WHOI-72-86. 19 pp.

Harvey, G.R., Steinhauer, W.G., and Teal, J.M. 1973. Polychlorobiphenyls in North Atlantic Ocean water. Science, 182: 643-644.

Heit, M., Klusek, C.S., and Miller, K.M. 1980. Trace element, radionuclide, and polynuclear aromatic hydrocarbon concentrations in Unionidae mussels from Northern Lake George. Environ. Sci. Technol., 14: 465-468.

Hilpert, L.R., May, W.E., Wise, S.A., Chesler, S.N., and Hertz, H.S. 1978. Interlaboratory comparison of determinations of trace level petroleum hydrocarbons in marine sediment. Anal. Chem., 50: 458-463.

Hites, R.A., LaFlamme, R.E., and Windsor, J.G. jr., 1980. Polycyclic aromatic hydrocarbons in marine/aquatic sediments. In Petroleum in the marine environment, pp. 289-311. Ed. by L. Petrakis and F.T. Wiess. Advances in Chemistry Series 185, American Chemical Society, Washington, D.C.

Howard, J.W. and Fazio, T. 1980. Analytical methodology and reported findings of polycyclic aromatic hydrocarbons in food. J. Assoc. Off. Anal. Chem., 63: 1077-1104.

Howard, J.W., Fazio, T., White, R.H., and Klimeck, B.A. 1968. Extraction and estimation of polycyclic aromatic hydrocarbons in total diet composites. J. Assoc. Off. Anal. Chem., 51: 122-129.

Howard, J.W., Teague, R.T., White, R.H., and Fry, B.E. jr. 1966. Extraction and estimation of polycyclic aromatic hydrocarbons in smoked foods. I. General method. J. Assoc. Off. Agr. Chem., 49: 595-611.

Howells, S.E. 1986. A comparison of gravimetric, infrared, ultraviolet fluorescence, and gas chromatographic techniques for determining the concentration and nature of hydrocarbons in marine sediments. Field Studies Council Report no. FSC/OPRU/12/86 (Unpublished manuscript).

Hunt, G., and Pangaro, N. 1982. Potential contamination from the use of synthetic adsorbents in air sampling procedures. Anal. Chem., 54: 369-372.

ICES. 1990. Report of the ICES Advisory Committee on Marine Pollution, 1990. ICES Coop. Res. Rep. No. 172, p. 118. 153 pp.

Irie, T., Yasunati, Y., Suzuki, T., Imai, N., Kurosawa, E., and Masamune, T. 1965. A new sequiterpene hydrocarbon from Laurencia glandulifera. Tetrah. L.: 3619-3624.

Josefson, C.M., Josefson, J.B., and Trubey, R. 1984. Adsorption of organic compounds from water with porous poly(tetrafluoroethylene). Anal. Chem., 56: 764-768.

Junk, G.A., and Richard, J.J. 1988. Organics in water: solid phase extraction on a small scale. Anal. Chem., 60: 451-454.

Kahn, L., and Wayman, C.H. 1964. Apparatus for continuous extraction of non-polar compounds from water applied to determination of pesticides and intermediates. Anal. Chem., 36: 1340-1343.

Kalle, K. 1938. Zum Problem der Meerwasserfarbe. Ann. d. Hydr., 65: 1-13.

Karasek, F.W., Hutzinger, O., and Safe, S., eds. 1984. Mass spectrometry in environmental sciences. Plenum Press, New York and London. ISBN 0-306-41552-6. 578 pp.

Keizer, P.D., Gordon, D.C. jr., and Dale, J. 1977. Hydrocarbons in eastern Canadian waters determined by fluorescence spectroscopy and gas-liquid chromatography. J. Fish. Res. Bd Can., 34: 347-353.

Knap, A.H., Burns, K.A., Dawson, R., Ehrhardt, M., and Palmork, K. 1986. Dissolved/dispersed hydrocarbons, tar balls, and the surface microlayer: experiences from an IOC/UNEP workshop in Bermuda, December 1984. Mar. Pollut. Bull., 11: 313-319.

Krahn, M.M., Moore, L.K., Bogar, R.G., Wigren, C.A., Chan, S.L., and Brown, D.W. 1988. High performance liquid chromatographic method for isolating organic contaminants from tissue and sediment extracts. J. Chromatogr., 437: 161-175.

Kuiper, J., and van den Brink, W.J. (eds.) 1987. Fate and Effects of Oil in the Marine Ecosystem. Proceedings of the Conference on Oil Pollution organized under the International Association on Water Pollution Research and Control (IAWPRC) by the Netherlands Organization for Applied Scientific Research. Amsterdam, The Netherlands. ISBN 90-247-3489-4, 289 pp.

Lake, J.L., Dimock, C.W., and Norwood, C.B. 1980. A comparison of methods for the analysis of hydrocarbons in marine sediments. Adv. Chem. Ser., 185: 343-360.

Law, R.J. 1984. The continuous measurement of low concentrations of hydrocarbons in water using a towed fluorimeter. ICES CM 1984/E:8. 10 pp.

Law, R., and Andrulewicz, E. 1983. Hydrocarbons in water, sediments and mussels from the southern Baltic Sea. Mar. Pollut. Bull., 14: 289-293.

Law, R.J., Fileman, T.W., and Portmann, J.E. 1988. Methods of analysis of hydrocarbons in marine and other samples. Aquat. Environ. analyt. Meth.: MAFF Direct. Fish. Res., Lowestoft, (2).

Law, R.J., and Hudson, P.M. 1986. Preliminary studies of the dispersion of oily water discharges from North Sea oil production platforms. ICES CM 1986/E:15. 11 pp.

Law, R.J., and Portmann, J.E. 1982. Report on the first ICES intercomparison exercise on petroleum hydrocarbon analyses in marine samples. ICES Coop. Res. Rep. no. 117. 55 pp.

Lee, M.L., Novotny, M.V., and Bartle, K.D. 1981. Analytical Chemistry of Polycyclic Aromatic Compounds. Academic Press, New York, ISBN 0-12-440840-0, 462 pp.

Lee, R.R., Sauerheber, R., and Benson, A.A. 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel Mytilus edulis. Science, 177: 344-346.

Levy, E.M. 1971. Presence of petroleum residues off the east coast of Nova Scotia, in the Gulf of St. Lawrence and in the St. Lawrence River. Water Res., 5: 723-733.

Levy, E.M. 1979. Intercomparison of Niskin and Blumer samplers for the study of dissolved and dispersed petroleum residues in seawater. J. Fish. Res. Bd Can., 36: 1513-1516.

Levy, E.M., Ehrhardt, M., Kohnke, D., Sobchenko, E., Suzuoki, T., and Tokuhiro, A. 1981. Global oil pollution. Results of MAPPMOP, the IGOSS Pilot Project on Marine Pollution (Petroleum) Monitoring. UNESCO/IOC. 35 pp.

Levy, E.M., and Walton, A. 1973. Dispersed and particulate petroleum residues in the Gulf of St. Lawrence. J. Fish. Res. Bd Can., 30: 261-267.

Lichtenthaler, R.G., Oreld, F., Sporstøl, S., and Vogt, N.B. 1986. Comparison of extraction methods for the analysis of oily drill cuttings. Proc. Conf. on Oil Based Drilling Fluids: Cleaning and Environmental Effects of Oil Contaminated Drill Cuttings. Trondheim, Norway, 24-26 February 1986, pp. 100-102.

Lipsky, S.R., and Duffy, M.L. 1986. High temperature gas chromatography: The development of new aluminium clad flexible fused silica glass capillary columns coated with thermostable non-polar phases: Part 1. J. High Resol. Chromatog. and Chromatog. Comm., 9: 376-382.

Lundbom, O. 1987. Identification of oil spills in the Baltic countries. A state-of-the-art report. Helsinki Comm. Baltic Sea Environ. Proc., 22: 136-148.

Mackie, P.R., Hardy, R., Whittle, K.J., Bruce, C., and McGill, A.S. 1980. The tissue hydrocarbons burden of mussels from various sites around the Scottish coast. In Polynuclear aromatic hydrocarbons: chemistry and biological effects, pp. 379-393. Ed. by A. Bjørseth and A.J. Dennis. Battelle Press, Ohio. 1097 pp.

Mackie, P.R., Whittle, K.J., and Hardy, R. 1974. Hydrocarbons in the marine environment. I. n-Alkanes in the Firth of Clyde. Estuar. coast. mar. Sci., 2: 359-374.

MacLeod, W.D. jr., Brown, D.W., Friedman, A.J., Burrows, D.G., Maynes, O., Pearce, R.W., Wigren, C.A., and Bogar, R.G. 1985. Standard analytical procedures of the NOAA National Analytical Facility 1985-1986. Extractable toxic organic compounds, 2nd Ed. U.S. Department of Commerce, NOAA/NMFS. NOAA Tech. Memo. NMFS/NWC-92. 121 pp.

MacLeod, W.D. jr., Friedman, A.J., and Brown, D.W. 1988. Improved interlaboratory comparisons of polycyclic aromatic hydrocarbons in marine sediments. Mar. Environ. Res., 26: 209-221.

MacLeod, W.D. jr., Prohaska, P.G., Gennero, D.D., and Brown, D.W. 1982. Interlaboratory comparisons of selected trace hydrocarbons from marine sediments. Anal. Chem., 54: 386-392.

Marchand, M. 1980. The Amoco Cadiz oil spill. Distribution and evolution of hydrocarbon concentrations in sea water and marine sediments. Environ. Internat., 4: 421-429.

Marchand, M., and Caprais, J.-Cl. 1983. Analyse des hydrocarbures volatils dans l'eau. Application de la technique de Grob. Analysis, 11: 216-224.

Marty, J.C., and Saliot, A. 1976. Hydrocarbons (normal alkanes) in the surface microlayer of seawater. Deep-Sea Res., 23: 863-873.

Murray, A.J., and Law, R.J. 1980. Results of a mussel watch programme in England and Wales in 1977 and 1978. ICES CM 1980/E:15. 16 pp.

NAS. 1980. The International Mussel Watch. National Academy of Sciences, Washington, D.C. pp. 48-77.

National Research Council. 1985. Oil in the sea. Inputs, fates and effects. National Academy Press, Washington, D.C. 601 pp.

Natusch, D.F.S., and Tomkins, B.A. 1978. Isolation of polycyclic organic components by solvent extraction with dimethyl sulfoxide. Anal. Chem., 50: 1429-1434.

Osterroht, Chr. 1974. Development of a method for the extraction and determination of non-polar, dissolved organic substances in sea water. J. Chromatogr., 101: 289-298.

Østgaard, K., and Jensen, A. 1983. Evaluation of direct fluorescence spectroscopy for monitoring aqueous petroleum solutions. Intern. J. environ. Anal. Chem., 14: 55-72.

Payne, R., and Davies, J.M 1977. The Aberdeen sedimentation trap and its moorings. Scott. Fish. Res. Rep., No. 8. 11 pp.

Prahl, F.G., and Carpenter, R. 1983. Polycyclic aromatic hydrocarbon (PAH)-phase associations in Washington coastal sediments. Geochim. Cosmochim. Acta, 47: 1013-1023.

Ramos, L.S., and Prohaska, P.G. 1981. Sephadex LH-20 chromatography of extracts of marine sediment and biological samples for the isolation of polynuclear aromatic hydrocarbons. J. Chromatogr., 211: 284-289.

Reynolds, B.H., Barszcz, C.A., Phelps, D.K., and Heltshe, J. 1981. Mussel watch - correlation of histopathology and chemical bioaccumulation in mussels (Mytilus edulis and M. californianus) and oysters (Crassostrea virginica). ICES CM 1981/E:25. 22 pp.

Risebrough, R.W., de Lappe, B.W., Walker, W. II, Simoneit, B.T., Grimalt, J., and Albaigs, J. 1983. Application of the Mussel Watch concept in studies of the distribution of hydrocarbons in the coastal zone of the Ebre delta. Environ. Pollut. Bull., 14(5): 181-187.

Rowland, S.J., and Volkman, J.K. 1982. Biogenic and pollutant aliphatic hydrocarbons in Mytilus edulis from the North Sea. Mar. Environ. Res., 7: 117-130.

Schantz, M.M., Chesler, S.N., Koster, B.J., and Wise, S.A. 1988. Analytical methods for the determination of organic contaminants in sediments and tissues. In Progress in environmental specimen banking, pp. 40-52. Ed. by S.A. Wise, R. Zeisler, and G.M. Goldstein. U.S. National Bureau of Standards (NBS) Spec. Publ. No. 740. 202 pp.

Smith, P., Calvert, J., and Steiner, R. 1973. Extraction and purification of lipids: 4. Alternative binary solvent systems to replace chloroform/methanol in studies on biological membranes. Physiol. Chem. and Physics, 5: 157-166.

Sporstøl, S., Gjøs, N., Lichtenthaler, R.G., Gustavsen, K.O., Urdall, K., Oreld, F., and Skei, J. 1983. Source identification of aromatic hydrocarbons in sediments using GC/MS. Environ. Sci. Technol., 17: 282-286.

Stadler, D., and Schomaker, K. 1977. Ein Glaskugelschöpfer zur kontaminationsfreien Entnahme von Seewasser unter der Oberfläche für die Analyse von Kohlenwasserstoffen und halogenierten Kohlenwasserstoffen. Dt. Hydrogr. Z., 30: 20-25.

Thompson, S., and Eglinton, G. 1978. The fractionation of a recent sediment for organic geochemical analysis. Geochim. Cosmochim. Acta, 42: 199-207.

Tokar, J.M., Harvey, G.R., and Chesal, L.A. 1981. A gas lift system for large volume water sampling [on board research vessels]. Construction, use, performance. Deep-Sea Res., 28(11A): 1395-1399.

UNESCO. 1976. Guide to operational procedures for the IGOSS Pilot Project on Marine Pollution (Petroleum) Monitoring. UNESCO, Intergovernmental Oceanographic Commission, Manuals and Guides No. 7. 50 pp.

UNESCO. 1984. Manual for monitoring oil and dissolved/dispersed hydrocarbons in marine waters and on beaches. Intergovernmental Oceanographic Commission, Manuals and Guides No. 13. 35 pp.

UNESCO. 1985. Procedure for sampling the sea-surface microlayer. Intergovernmental Oceanographic Commission, Manuals and Guides No. 15, 12 pp.

Urdal, K., Vogt, N.B., Sporstøl, S.P., Lichtenthaler, R.G., Mostad, H., Kolset, K., Nordenson, S., and Esbensen, K. 1986. Classification of weathered crude oils using multimethod chemical analysis, statistical methods and SIMCA pattern recognition. Mar. Pollut. Bull., 17(8): 366-373.

U.S.EPA. 1973. Oil pollution source identification. US Environmental Protection Agency, EPA-R2-73-102. 175 pp.

Uthe, J.F., Musial, C.J., and Sirota, G.R. 1986. Report on the intercomparative study 03/HC/BT on the determination of polycyclic aromatic hydrocarbons in biological tissue. ICES Coop. Res. Rep., 141: 76-85.

Uthe, J.F., Reinke, J., and Gesser, H.D. 1972. Extraction of organochlorine pesticides from water by porous polyurethane coated with selective adsorbent. Environ. Lett., 3: 117-135.

Vandermeulen, J.H., and Gordon, D.C. jr. 1976. Reentry of 5-year-old stranded bunker C fuel oil from a low energy beach into the water, sediments, and biota of Chedabucto Bay, Nova Scotia. J. Fish. Res. Bd Can., 33: 2002-2010.

Van Vleet, E.S. 1984. Fingerprinting oil spills in the marine environment. Mar. Technol. Sci. J., 18(3): 11-23.

Vassilaros, D.L., Stoker, P.W., Booth, G.M., and Lee, M.L. 1982. Capillary gas chromatographic determination of polycyclic aromatic compounds in vertebrate fish tissue. Anal. Chem., 54: 106-112.

Venkatesan, M.I. 1988. Occurrence and possible sources of perylene in marine sediments - a review. Mar. Chem., 25: 1-27.

Verschueren, K. 1977. Handbook of environmental data on organic chemicals. Van Nostrand Reinhold Company, New York, Cincinnati, Atlanta, Dallas, San Francisco, London, Toronto, Melbourne. 659 pp.

Wade, T.L., Atlas, E.L., Brooks, J.M., Kennicutt, M.C., Fox, R.G., Sericano, J., Garcia-Romero, B., and DeFreitas, D. 1988. NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediments and oysters. Estuar., 11(3): 171-179.

Wade, T.L., and Quinn, J.G. 1980. Incorporation, distribution, and fate of saturated petroleum hydrocarbons in sediments from a controlled marine ecosystem. Mar. Environ. Res., 3: 15-33.

Wakeham, S.G., and Carpenter, R. 1976. Aliphatic hydrocarbons in sediments of Lake Washington. Limnol. Oceanogr., 21: 711-723.

Wakeham, S.G., Farrington, J.W., Gogosian, R.B., Lee, C., DeBaar, H., Nigrelli, G.E., Tripp, B.W., Smith, S.O., and Frew, N.M. 1980. Organic matter fluxes from sediment traps in the equatorial Atlantic Ocean. Nature, 286: 798-800.

Warner, J.S. 1976. Determination of aliphatic and aromatic hydrocarbons in marine organisms. Anal. Chem., 48: 578-583.

Warner, J.S., Riggin, R.M., and Engel, T.M. 1980. Recent advances in the determination of aromatic hydrocarbons in zooplankton and macrofauna. In Petroleum in the marine environment, pp. 87-102. Ed. by L. Petrakis and R.T. Weiss. Advances in Chemistry Series, American Chemical Society. Washington, D.C. 371 pp.

Wasik, St.P. 1974. Determination of hydrocarbons in sea water using an electrolytic stripping cell. J. Chromatogr. Sci., 12: 845-848.

Weinheimer, A.J., Washechek, P.H., v.d. Melm, D., Bilayet Hossain, M. 1968. The sesquiterpene hydrocarbon of the Gorgonian, Pseudopterogorgia americana, the norisoprenoid β-gorgonene. Chem. Comm.: 1070-1071.

Werner, A.E., and Waldichuk, M. 1962. A continuous liquid-liquid extractor. Anal. Chem., 34: 1674-1676.

Whelan, J.K. 1983. Volatile C_1-C_8 compounds in marine sediments. In Gas chromatography/mass spectrometry applications in microbiology. Ed. by L. Larson and P.A. Mardh. Plenum Press.

Whelan, J.K., and Hunt, J.M. 1983. Volatile C_1-C_8 organic compounds in sediments from the Peru Upwelling Region. Org. Geochem., 5: 13-28.

Wise, S.A., Chesler, S.N., Guenther, F.R., Hertz, H.S., Hilpert, L.R., May, W.E., and Parris, R.M. 1980. Interlaboratory comparison of determinations of trace level hydrocarbons in mussels. Anal. Chem., 52(12): 1828-1833.

Wise, S.A., Chesler, S.N., Hertz, H.S., Hilpert, L.R. and May, W.E. 1977. Chemically-bonded aminosilane stationary phase for the high-performance liquid chromatographic separation of polynuclear aromatic compounds. Anal. Chem., 49: 2306-2310.

Wong, M.K., and Williams, P.J. le B. 1980. A study of three extraction methods for hydrocarbons in marine sediment. Mar. Chem., 9: 183-190.

Yamaguchi, M. 1957a. Carotenoids of the sponge Reniera japonica. Bull. Chem. Soc. Japan, 30: 111-114.

Yamaguchi, M. 1957b. Chemical constitution of renieratene. Bull. Chem. Soc. Japan, 30: 979-983.

Yamaguchi, M. 1958a. Chemical constitution of renieratene. Bull. Chem. Soc. Japan, 31: 51-55.

Yamaguchi, M. 1958b. Renieratene, a new carotenoid containing benzene rings, isolated from a sea sponge. Bull. Chem. Soc. Japan, 31: 739-742.

Youngblood, W.W., and Blumer, M. 1975. Polycyclic aromatic hydrocarbons in the environment: homologous series in soil and recent marine sediments. Geochim. Cosmochim. Acta, 39(9): 1303-1314.

Zitko, V. 1975. Aromatic hydrocarbons in aquatic fauna. Bull. environ. Contam. Toxicol., 14: 621-631.