HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 33, 257–271 (1980)

Analysis for petroleum products in marine environments

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ABSTRACT: Petroleum is composed of a complex mixture of hydrocarbons that readily undergo chemical and biological conversions on entering aquatic environments. These conversions lead to the formation of a host of oxygenated products, some of which are potentially toxic to marine life and to the consumer of fishery products. State-of-the-art analytical methods, as employed in our laboratories, utilize glass-capillary gas chromatography in conjunction with mass spectrometry to analyze environmental samples containing trace amounts of aliphatic and aromatic petroleum hydrocarbons. These procedures are applied on a routine basis to the analysis of seawater, sediments and tissues of marine organisms. Despite this analytical proficiency, a need exists for analyzing oxygenated and other polar petroleum products in environmental samples. For example, techniques such as high-performance liquid chromatography (HPLC), in conjunction with on-line fluorometric assay techniques and mass spectrometry, make possible the analysis of polar oxygenated compounds resulting from both chemical and biological conversions. These methodologies are first steps toward the development of routine assay procedures for environmental samples. Current techniques for hydrocarbon analyses and new methods for analyzing polar aromatic compounds are discussed.

INTRODUCTION

Each year an estimated four million metric tons of petroleum enter the marine environment through sea and land-base discharges, tanker mishaps, atmospheric fallout, and various other human activities (Clark & MacLeod, 1977). The petroleum is a complex mixture of compounds such as aliphatic, alicyclic and aromatic hydrocarbons, and a variety of polar aromatic compounds and high molecular weight polymers (Clark & Brown, 1977). Extracts of seawater, sediment and tissues are often routinely analyzed by gas chromatography for aliphatic hydrocarbons as well as for aromatic hydrocarbons containing as many as six benzene rings (Brown et al., 1980). Yet, certain components, notably polar aromatic compounds and high molecular weight polymers, are not well suited to gas chromatography. Analyses of these compounds, because of their low volatility or instability at elevated temperatures, are a continuing challenge to the ingenuity of the analytical chemist (Malins, 1980).

Other factors add tremendously to the problem. Some of the petroleum components such as aromatic hydrocarbons readily undergo structural alterations in aquatic environments. Oxygenated products are formed through photochemical reactions, as well as by

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enzymatic and other chemical conversions that occur in living systems as diverse as microorganisms and fish (Karrick, 1977; Varanasi & Malins, 1977). The transformations of petroleum hydrocarbons generally yield pollutants of enhanced water solubility, and their presence adds greatly to problems in studying the chemical composition of petroleum-derived products in marine environments.

Because of these difficulties, it is obvious that conventional gas chromatographic analyses for a narrow range of hydrocarbons in seawater, sediment and tissues may provide only a limited, perhaps myopic, perspective of petroleum contamination.

Responding to the need for a detailed perspective of petroleum pollution, we have developed sophisticated high-resolution gas chromatographic techniques for hydrocarbons (Ramos et al., 1979). We are also exploring ways to analyze the polar aromatic compounds (e. g. oxygenated products) arising from chemical and biological processes.

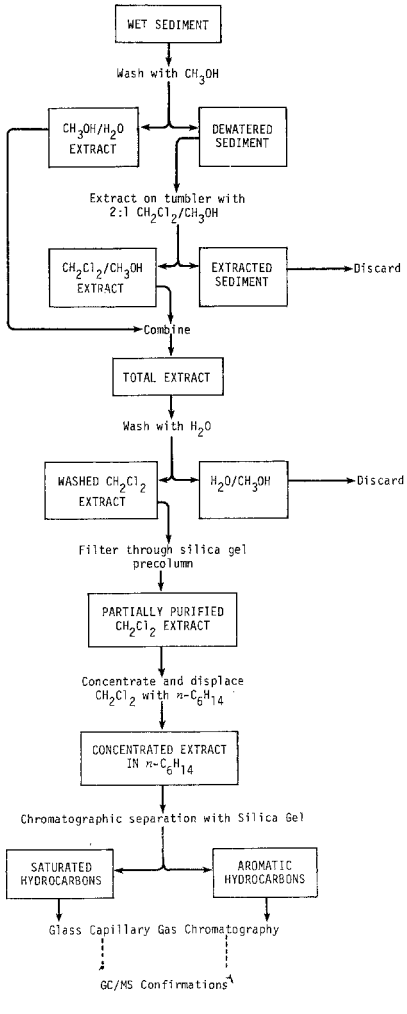

Fig. 1. Analytical scheme for hydrocarbons in sediment

In this paper, we describe our gas chromatographic procedures as applied to hydrocarbons in water, sediment, and tissues. In addition, we discuss our advances in developing new methods (e. g., applications of high-performance liquid chromatography) for the resolution and quantitation of complex mixtures of the polar aromatic compounds present in environmental samples.

METHODOLOGY

Hydrocarbons and dibenzothiophenes

Sample collection

Special precautions were taken in collecting and storing marine environmental samples for trace analyses of petroleum hydrocarbons (MacLeod et al., 1976). Sampling devices (corers, bottles, etc.) were scrupulously cleaned to avoid extraneous organic components that could invalidate the analyses. This was accomplished by usual laboratory washing procedures, followed by sequential rinsing with contaminant-free acetone and dichloromethane.

Samples of sediments or mussels *(Mytilus edulis)* were stored in pre-cleaned glass jars tightly sealed with Teflon-lined lids. These samples were refrigerated immediately and frozen as soon as possible to inhibit evaporation and chemical and biological oxidative changes.

Water samples were collected in pre-cleaned glass bottles containing 5 % v/v contaminant-free dichloromethane, sealed with Teflon-lined lids, and refrigerated. The dichloromethane served both as an extraction solvent and a biocide.

Sample preparation

Samples of mussels (at least 15 individuals) were thawed and homogenized. A 10-g portion of the homogenate (wet wt) was digested with 4 N aqueous sodium hydroxide

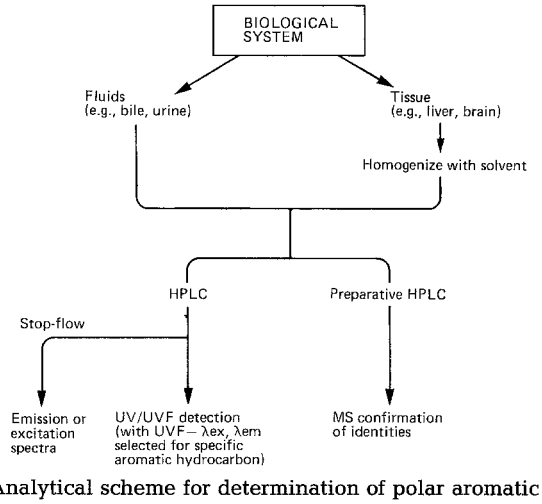

Fig. 2. Analytical scheme for determination of polar aromatic compounds

overnight at 30 °C (MacLeod et al., 1976). The digested sample was extracted with two portions of diethyl ether (ethanol- and peroxide-free). The ether extract was concentrated and filtered through a silica gel bed prepared in diethyl ether. The ether eluate was concentrated by fractional distillation and the ether was displaced with hexane. The resulting hexane solution was then chromatographed on silica gel to isolate the saturated and aromatic hydrocarbons and other weakly polar compounds (e. g. dibenzothiophenes). These fractions were analyzed by gas chromatography (GC).

Sediment samples (100 g wet wt) were extracted first with methanol and then with dichloromethane/methanol as shown in Figure 1 (Brown et al., 1979, 1980). One-liter water samples were acidified and extracted with dichloromethane. The extracts were concentrated, exchanged into hexane, and then analyzed by GC. If complex patterns of petroleum-related compounds were found, the extract was chromatographed on silica gel, as described above, and analyzed by GC.

Sample analysis

Gas chromatography (GC). Analyses were performed using a microprocessor-controlled GC equipped with (1) an automatic sample injector, (2) a wall-coated, open tubular (WCOT) glass-capillary column (20–30 m long and 0.25 mm i. d.), and (3) a hydrogen flame-ionization detector (FID). The packed column GC sample injection port was modified for splitless sample injection as described by Ramos et al. (1979). Column conditions and operating parameters are shown in the figure captions.

Gas chromatography/mass spectrometry (GC/MS). The identities of compounds detected and measured by GC were confirmed by GC/MS analysis. A microprocessor-controlled GC, similar to that above, was interfaced directly to a quadrupole type mass spectrometer (Ramos et al., 1979).

Polar aromatic compounds

Sample collection and preparation

Exposure studies. Rainbow trout were acclimated to experimental conditions for two weeks prior to exposure. The test animals were anesthetized with tricaine-methanesulfonate (50 μg/l) and force-fed a gelatin capsule containing the test compound dissolved in ca. 50 μl of ethanol (see figure captions for details of amount fed and time elapsed before sampling). Tissue and fluid samples were frozen at − 60 °C prior to analysis. Bile samples were injected directly into the HPLC columns.

Mussel field studies and "Amoco Cadiz" oil spill. As part of a study by Wolfe et al. (unpublished), mussels were collected from essentially petroleum-free waters and placed in cages 1 m below the surface at two sites: (1) directly in the pathway of the spilled petroleum, and (2) removed from the direct pathway of the spilled petroleum, but in an area that was slightly polluted. Samples were taken for chemical analyses and microscopy at the time they were placed in the cages and after 5, 8, 12, and 25 days. Mussel tissues, with 1.5 ml methanol added per gram wet tissue, were homogenized, centrifuged, and the supernatant was decanted. The tissue pellet was resuspended and reextracted with 1.0 ml methanol per gram original wet tissue and recentrifuged. The supernatant was decanted, and the combined supernatants were

concentrated and used for HPLC injections. The mussel samples were separately extracted for GC and GC/MS analyses as described above. Tissues were also examined by electron microscopy for evidence of abnormalities (Wolfe et al., unpublished).

Sample analysis

High-performance liquid chromatography (HPLC). We use HPLC to analyze for nonvolatile or thermally unstable oxygenated compounds which cannot be determined by GC (Krahn et al., 1980). The analytical procedures used to separate and identify these polar aromatic compounds are shown in Figure 2. Separations were carried out with a dual-pump liquid chromatograph equipped with a reverse phase (10 μm C-18 hydrocarbon coated packing) analytical column and a guard column. The detector was a dual-monochromator fluorescence spectrometer fitted with a 20-μl square flow cell.

Gradient elutions were performed with 0.5 % v/v acetic acid in water (Solvent A) and methanol (Solvent B) in a 15-min linear gradient from 5 to 95 % Solvent B, followed by 5 min at final conditions.

Fluorescence emission or excitation spectra were recorded on the fluorescence spectrometer after the effluent components were trapped in the detector flow cell.

Mass spectrometry. The chemical ionization instrument was used for plasma desorption chemical ionization mass spectrometry (PD/CI MS) (Krahn et al., 1980). Spectra of commercial 1-naphthyl-β-D-glucuronic acid (naphthyl glucuronide) and naphthyl glucuronide from fish bile were obtained by PD/CI MS. A 2-mm o. d. × 2-cm long glass tube sealed at one end and filled with 0.25-mm o. d. silver wire was inserted into the solid-sample probe. This insert served as the support for the compound to be analyzed and was placed directly into the CI plasma (ionized ammonia). Heat was conducted by the silver wire to the sample as the probe temperature was raised from 50° to 400 °C in about 90 s. A multiple-on detection computer program was used to obtain a spectrum of 100 ng of naphthyl glucuronide standard.

RESULTS AND DISCUSSION

In the last few years, significant advances have been made in our ability to analyze complex mixtures of hydrocarbons in seawater, sediment, and biological samples (MacLeod et al., 1976; Brown et al., 1979, 1980). Much of the progress was made through the use of glass-capillary gas chromatography (Ramos et al., 1979). Using this technique one

Table 1. Phenols extracted from seawater-soluble fraction of Prudhoe Bay crude oil and identified by GC/MS (adapted from Ramos et al., 1979)

No. of isomers present	Compounds	No. of isomers present	Compounds
1	Phenol	2	C_5-Phenols
2	Cresols	1	Methylphenetole
5	C_2-Phenols	2	C_2-Bromophenols
7	C_3-Phenols	1	C_3-Bromophenol
4	C_4-Phenols	1	Cyclohexylphenol

Table 2. Aromatic petroleum hydrocarbon concentrations in sediments experimentally oiled with Prudhoe Bay crude oil. Samples were taken from the top 2 cm of a 5-cm sediment layer (adapted from McCain et al., 1980)

Hydrocarbons	% composition at day 0	Days 0	2	7	16	30	% remaining on day 30
		concentration (ng/g dry wt.)					
1, 2, 3, 4-Tetramethylbenzene	4	105	74	50	64	61	58
Naphthalene	8	210	200	61	89	82	39
1-Methylnaphthalene	16	408	736	400	362	557	137
2-Methylnaphthalene	29	753	742	601	723	519	69
2,6-Dimethylnaphthalene	14	375	185	411	344	218	58
2,3,5-Trimethylnaphthalene	15	379	338	318	336	187	49
Fluorene	3	87	75	67	78	102	117
Phenanthrene	11	275	155	150	156	112	41

is able to separate hundreds of individual compounds in less than one hour. Using our sample-preparation procedures, the approximate levels of sensitivity are 0.1 ng/g for seawater and dry sediment, and 4.0 ng/g for dry tissue. GC/MS is employed in instances where elucidation of structure is required. Although the GC and GC/MS procedures are usually applied to the analysis of petroleum hydrocarbons, and weakly polar compounds, such as benzothiophenes, it is also possible to resolve a number of the more volatile oxygenated organics by these techniques. For example, we have analysed a wide range of phenols present in the water-soluble fraction (WSF) of Prudhoe Bay crude oil (Table 1) (Ramos et al., 1979).

The following example serves to illustrate the use of gas chromatographic analyses of hydrocarbons in laboratory studies relating to the fate and biological effects of

Table 3. Petroleum hydrocarbons in the tissues of English sole exposed to oil-contaminated sediment (test, T) and to nonoiled sediment (control, C) for two months continuously (adapted from McCain et al., 1978)

Polycyclic aromatic hydrocarbons*	Skin		Muscle		Liver		Skin		Muscle		Liver		Skin		Muscle		Liver	
	11 days						27 days						51 days					
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
	concentration (ng/g dry weight)**																	
1,2,3,4-Tetramethylbenzene	–	33	–	44	–	922	–	–	–	–	–	863	–	–	–	–	–	124
Biphenyl	–	156	–	–	–	307	–	–	–	–	–	278	–	–	–	–	–	–
Naphtalene	–	82	–	20	–	100	–	–	–	–	–	–	–	–	–	–	–	–
1-Methylnaphthalene	–	1189	–	369	–	1940	–	–	–	–	–	1325	–	–	–	–	–	–
2-Methylnaphthalene	–	888	–	279	–	3070	–	–	–	–	–	1500	–	–	–	–	–	60
2,6-Dimethylnaphthalene	–	130	–	–	–	69	–	–	–	–	–	–	–	–	–	–	–	–

* Arenes with aromatic rings ranging from one to six were determined; (–) refers to values below limits of detection

** Skin and liver samples were pooled from three fish at each analysis; muscle samples were analyzed individually

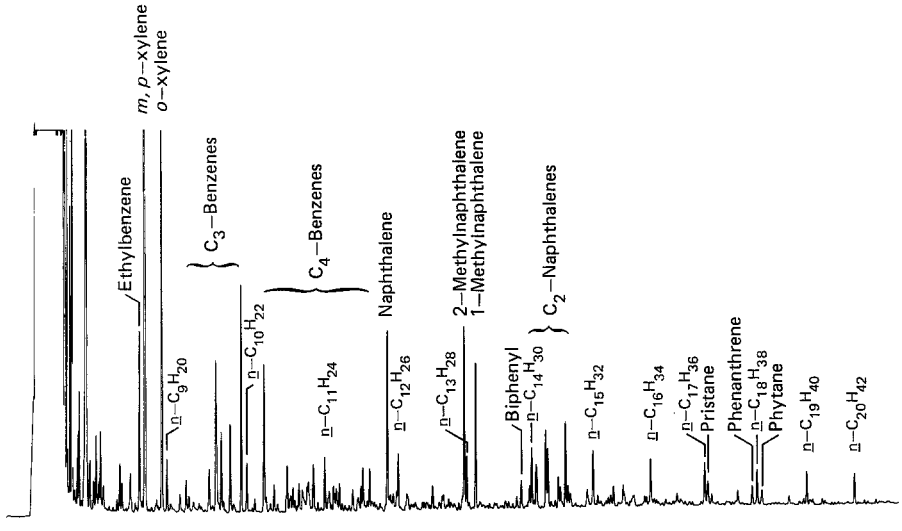

Fig. 3. Gas chromatogram of the seawater-soluble fraction of Prudhoe Bay crude oil. Hewlett-Packard 5840A GC with FID. J & W Scientific 30 m × 0.25 mm SE-54 WCOT glass column. Helium carrier pressure 20 psi. Three μl splitless injection, with split valve opened after 18 s to a 20 : 1 split. Isothermal for first 5 min at 50 °C, then programmed to 270 °C at 4 °C/min

petroleum. Studies were conducted on the bioavailability to English sole *(Parophrys vetulus)* of petroleum from experimentally-oiled sediments (McCain et al., 1978). Gas chromatographic analyses of sediment hydrocarbons and hydrocarbons accumulated in skin, muscle, and liver revealed that these tissues concentrated compounds such as 1- and 2-methylnaphthalene and 1,2,3,4-tetramethylbenzene from the sediment, but did not accumulate higher molecular weight compounds, such as fluorene and phenanthrene (Tables 2, 3) (McCain et al., 1978). As exposure periods increased beyond about two weeks, hydrocarbon concentrations in tissues decreased. These changes were attributed to the induction of mixed function oxygenase enzymes that promote the conversion of hydrocarbons to metabolites. The chemical analyses were of critical importance in relating the exposures of petroleum to morphological changes (e. g., hepatocellular lipid vacuolization) in the English sole and in determining which hydrocarbons had entered the organisms. When McCain et al. (1978) conducted their study, methods had not yet been developed for analyzing nonradioactive metabolites, although there were indications from our biochemical studies that these conversion products were readily formed and accumulated in tissues and body fluids of fish exposed to hydrocarbons (Roubal et al., 1977; Collier et al., 1978; Malins et al., 1979; Varanasi et al., 1979).

When we analyzed the WSF of Prudhoe Bay crude oil, a number of alkylated, low-molecular weight aromatic hydrocarbons were found to be major components (Fig. 3). Both the selective deposition of certain hydrocarbons and extensive bioconversion may lead, in effect, to a "scrambling" of the relative proportions of petroleum hydrocarbons in fish exposed to the WSF (Roubal et al., 1978). Thus, sometimes it may be difficult to relate the hydrocarbon accumulations in organisms to a specific type of petroleum in the environment.

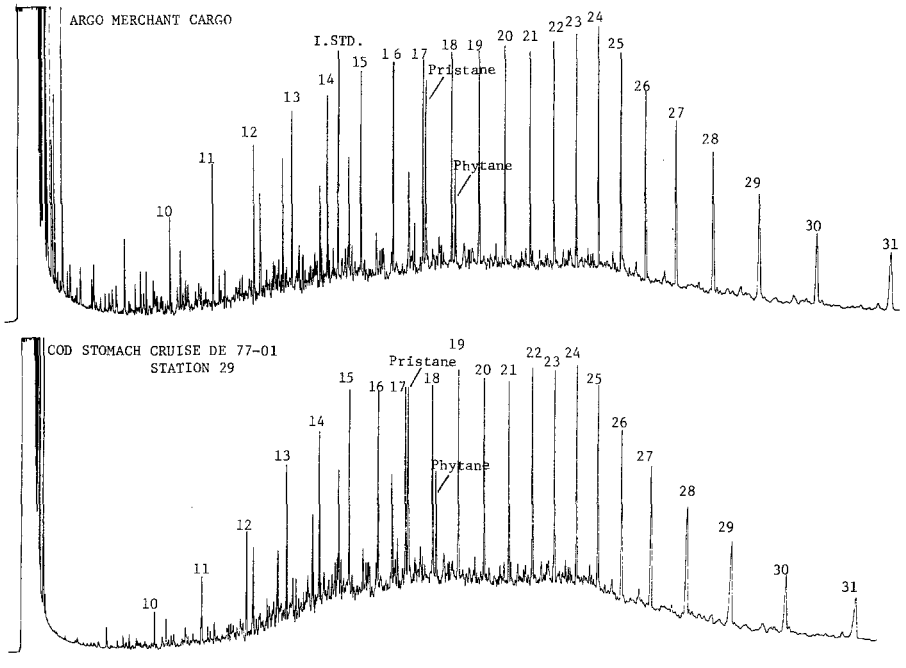

Fig. 4. Gas chromatograms of saturated hydrocarbons from a sample of Argo Merchant cargo (upper) and saturated hydrocarbons extracted from the stomach contents of cod collected in the region of the spilled Argo Merchant oil (lower). Numbers denote *n*-alkane carbon chain lengths. 20 m × 0.25 mm SE-30 WCOT glass column. Other conditions same as Figure 3

Notwithstanding the effects of scrambling, it is sometimes possible to establish relationships between petroleum contamination and accumulations of hydrocarbons in organisms. It was possible, for example, to show a probable cause and effect relationship between spilled Argo Merchant oil and hydrocarbons present in the stomachs of cod caught near the impact zone (MacLeod et al., 1978). Using high resolution gas chromatography, matching compositions were obtained between both the aliphatic and aromatic hydrocarbons comprising the cargo oil and the corresponding hydrocarbons in the stomach (Fig. 4). Fingerprinting of this type is useful in identifying contamination of marine organisms in petroleum-impacted areas.

We have applied similar approaches in studying the impact on sediments and mussels of a small oil spill in Port Angeles harbor, Washington. The relative proportions of aromatic structures were not exactly the same in sediment and organisms (Fig. 5); however, the high resolution chromatograms of the mussel and sediment extracts left little doubt that the accumulated hydrocarbons arose from petroleum similar to that present in the sediment.

Hydrocarbon analyses of water, sediment, and tissues are useful ways of assessing the possible impact of petroleum pollution on marine life. Where petroleum hydrocarbons are definitely found in organisms, petroleum from the environment is implicated; however, the absence of petroleum hydrocarbons in tissues does not necessarily preclude the possibility that polar compounds (e. g., oxygenated products) from petroleum

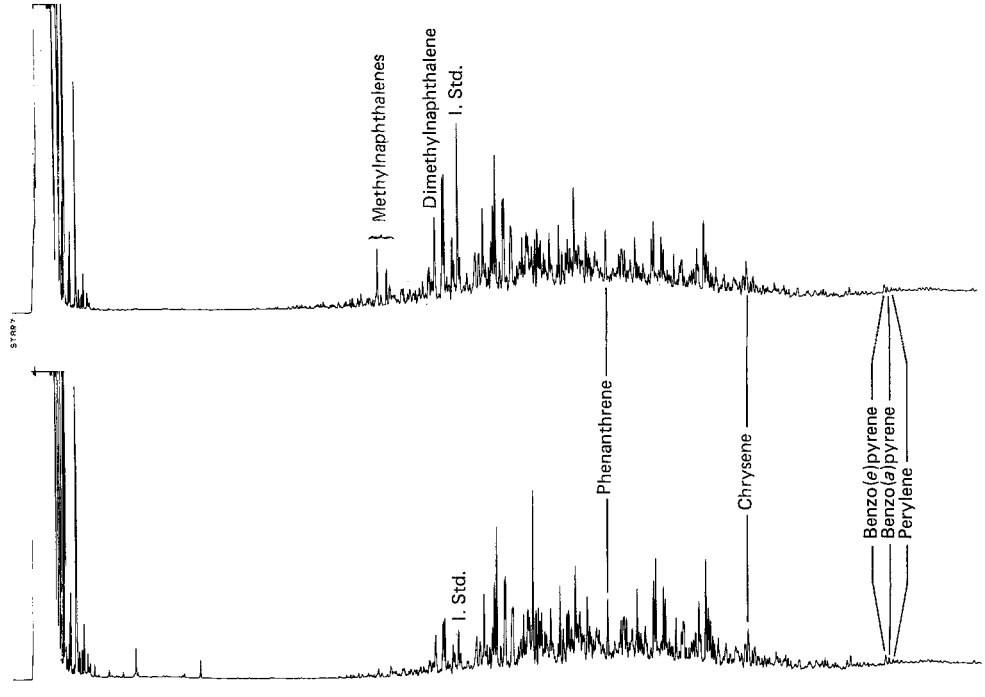

Fig. 5. Gas chromatograms of the aromatic hydrocarbons from sediment (upper) and mussels (lower) collected in the vicinity of a small oil spill in the harbor at Port Angeles, Washington, 1979. Parameters and conditions as in Figure 3

may be present in significant concentrations. Certain of the oxygenated compounds have been implicated as toxicants, some as mutagens and carcinogens (Sims & Grover, 1974). Examples are the products of the bioconversion of benzo[a]pyrene and benz[a]anthracene. Laboratory studies have shown that aromatic hydrocarbons readily undergo extensive conversion in fish tissues (Varanasi & Malins, 1977), so it is reasonable to conclude that potentially damaging metabolites are formed in fish after environmental exposure to petroleum products.

The tendency for aromatic hydrocarbons to oxidize and form polar aromatic compounds in marine systems is of concern. However, because of the limitations of conventional gas chromatography in analyzing most of these products, alternatives must be explored. Efforts to widen our perspectives of petroleum pollution to encompass the analysis of the essentially non-volatile polar compounds have resulted in a two-phase approach to the problem: (1) a study of ways to analyze metabolic products of single aromatic hydrocarbons by HPLC in conjunction with UVF, UV, or UV-UVF techniques, and (2) a study of ways to analyze total aromatic polar compounds in field samples by HPLC in combination with UVF detectors operating at wavelengths characteristic of representative petroleum derived components.

In the first approach, Krahn et al. (1980) report a HPLC/UVF method of analyzing fish and mouse bile samples for nanogram amounts of low-fluorescent naphthalene and its metabolites. In the current study, two groups of rainbow trout were force-fed

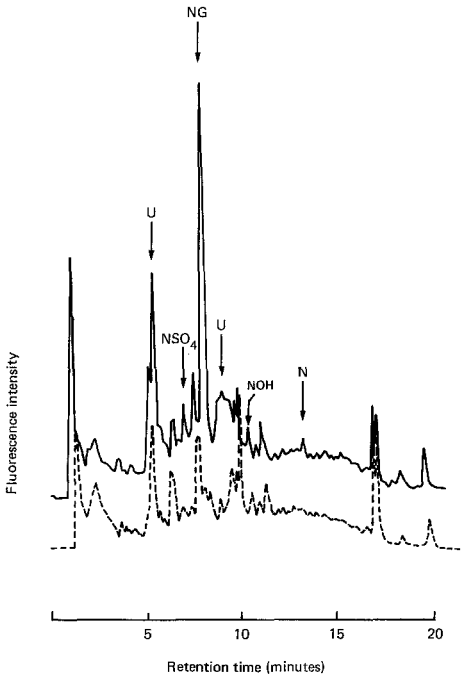

Fig. 6. HPLC profiles of naphthalene and its metabolites in the bile of rainbow trout. Upper: Chromatogram of bile from fish which received 2.7 mg of naphthalene (11 mg/kg) in each of 3 force-feedings 12 h apart. Bile was collected 24 h after the last feeding. Lower: Chromatogram of the control bile. Compounds were detected at UVF wavelengths λ_{ex} 305 nm and λ_{em} 340 nm. Identification of the compounds: N, naphthalene; NOH, 1-naphthol; NG, 1-naphthyl-β-D-glucuronic acid (naphthyl glucuronide); NSO_4, 1-naphthyl sulfate; and U, unknown

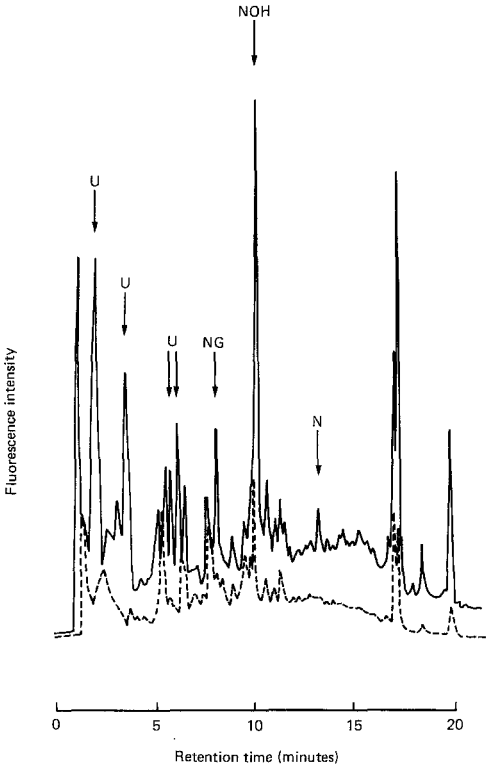

Fig. 7. HPLC profiles of naphthalene and its metabolites in the bile of rainbow trout. Upper: Chromatogram of bile from fish which received 2.7 mg (11 mg/kg) in one force-feeding. Bile was collected 24 h later. Lower: Chromatogram of the control bile. Compounds were detected at UVF wavelengths λ_{ex} 305 nm and λ_{em} 340 nm. Identification of the compounds: N, naphthalene; NOH, 1-naphthol; NG, 1-naphthyl-β-D-glucuronic acid (naphthyl glucuronide); NSO_4, 1-nyphthyl sulfate; and U, unknown

naphthalene (11 mg/kg) in our laboratories. One group received the dose in each of three feedings, and the other in one feeding. The HPLC/UVF chromatogram resulting from the direct injection of bile from fish fed naphthalene three times is shown in Figure 6 and that from fish fed naphthalene once is shown in Figure 7. In each chromatogram the parent compound, naphthalene (N), and two metabolites, 1-naphthylglucuronide (NG) and 1-naphthol (NOH), were detected. In addition, 1-naphthyl sulfate (NSO_4) was detected in the chromatogram from the multiple-feeding study. Unknown polar aromatic compounds (U) were found in both chromatograms. In the multiple-feeding study, the main metabolite was 1-naphthyl glucuronide; however, 1-naphthol was the main metabolite in the single-feeding experiment.

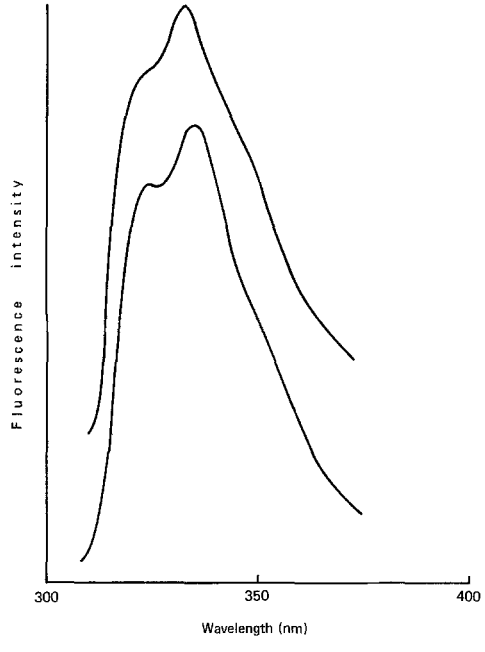

Fig. 8. Emission spectrum of naphthyl glucuronide (UVF λ_{ex} 294 nm). Upper spectrum: Naphthyl glucuronide obtained from bile of rainbow trout force-fed naphthalene (see Fig. 6). Lower spectrum: The commercially available standard of the sodium salt of naphthyl glucuronide

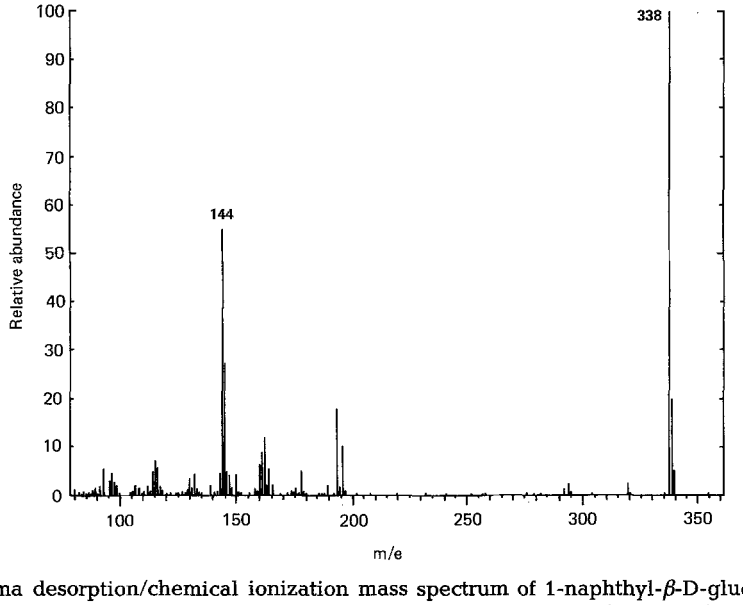

Fig. 9. Plasma desorption/chemical ionization mass spectrum of 1-naphthyl-β-D-glucuronic acid using ammonia reagent gas at 0.9 torr. The probe was heated from 50 °C to 400 °C in ca. 90 s. A multiple-ion-detection computer program was used to emphasize the 144, 194, and 338 m/e ions

Structural determinations of metabolites were reported by Krahn et al. (1980). Structural information was obtained by a stop-flow HPLC technique leading to the fluorescence emission spectrum of 1-naphthyl glucuronide (Fig. 8). Confirmation of the naphthyl glucuronide structure was obtained by PD/CI MS; a spectrum of the commercial standard is shown in Figure 9.

These techniques for the analysis of oxygenated compounds in biological tissues and fluids using HPLC/UVF and MS should have significant impact in several areas of research. For instance, the metabolism and disposition of petroleum hydrocarbons in biological systems can be studied in the laboratory without the use of isotopically-labeled compounds. In addition, organisms subjected to xenobiotic contamination of their natural environment can be investigated. We are presently conducting studies of

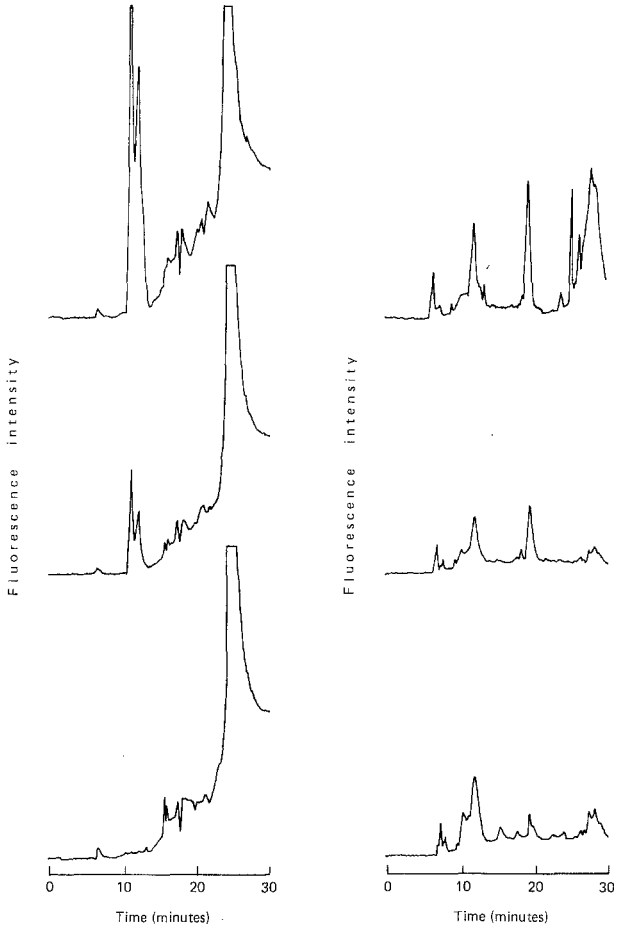

Fig. 10. HPLC profiles of methanol extracts of mussel samples: Upper – directly in the path of the "Amoco Cadiz" spilled oil; middle – out of the direct path of the "Amoco Cadiz" spilled oil; and lower – mussels before exposure to "Amoco Cadiz" oil. The profiles on the left were detected at λ_{ex} 270 nm and λ_{em} 305 nm (characteristic of phenol); the profiles on the right side were detected at λ_{ex} 305 nm and λ_{em} 340 nm (characteristic of naphthalene metabolites)

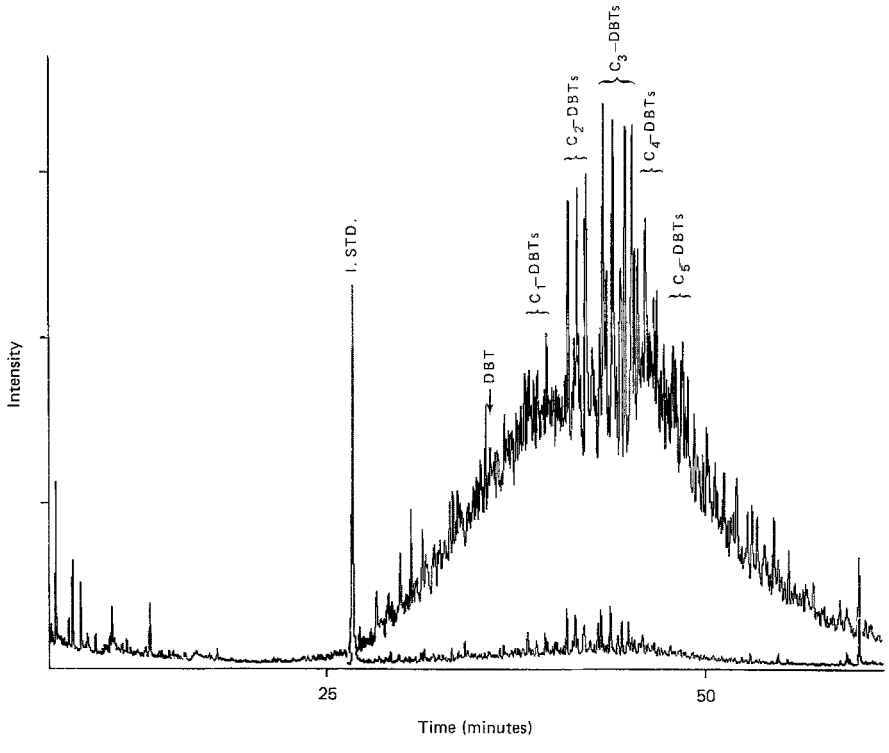

Fig. 11. GC/MS total ion chromatogram of aromatic hydrocarbon fraction from mussels: upper – directly in path of the "Amoco Cadiz" spilled oil; lower – out of direct path of the "Amoco Cadiz" spilled oil. GC conditions same as Fig. 3, except that the capillary GC effluent was admitted to a Finnigan 200 mass spectrometer. Intensities of both chromatograms adjusted to give same peak height above base line for internal standard (I. STD.). Alkylated dibenzothiophenes (C_n-DBTs) denoted according to carbon substitution, where n = number of carbons

this type. For example, an analysis for polar aromatic compounds in field samples is presented: Wavelengths characteristic of naphthalene metabolites and phenols (Schabron et al., 1979; Krahn et al., 1980) were used to analyze samples from a study of mussels placed in cages in the path of the "Amoco Cadiz" oil spill off the Brittany coast. Differences in HPLC/UVF profiles (Fig. 10) were found in extracts of mussels (1) deployed 1 m below the water surface directly in the path of the spill, (2) from mussels similarly deployed, but away from the path of the spill, and (3) from extracts of the original batch of mussels before deployment. Differences in HPLC/UVF responses correlate well with the proximity of the mussels to the pollution source. Gas chromatographic analyses of the weakly polar aromatic compounds also distinguished between mussels from impact sites and reference sites, particularly with respect to the dibenzothiophenes (Fig. 11). Thus, two types of analytical procedures, in a complementary way, demonstrated the apparent accumulation of "Amoco Cadiz" petroleum products in mussels from the impact zones. Wolfe et al. (unpublished) have shown that cellular changes (e. g., increases in lysosomal granules) occurred in mussels from impact sites in relation to mussels from reference areas. Accordingly, the chemical data and the

observed histological alterations appear to be associated with exposure to "Amoco Cadiz" oil.

To summarize, large quantities of petroleum-based pollutants are entering the marine environment each year, creating a need for accurate analytical determinations of these compounds in water, sediment, tissues, and other samples. Presently, many of the petroleum hydrocarbons are routinely determined by GC or GC/MS. However, a vast array of polar petroleum products – many of which are known to be generated by chemical and biological processes – have not previously been determined. Our methods for analysing these polar aromatic compounds show promise. Metabolites for aromatic hydrocarbons can now be determined in connection with laboratory exposure studies. In addition, research on the polar compounds can be conducted on field samples taken from polluted areas. We hope to expand our ability to detect and quantitate individual polar petroleum derived compounds in complex mixtures from both the laboratory and field.

Acknowledgment. This study was supported in part by the Bureau of Land Management through interagency agreement with the National Oceanic and Atmospheric Administration, which is managed by the Outer Continental Shelf Environmental Assessment Program (OCSEAP) Office.

LITERATURE CITED

Brown, D. W., Ramos, L. S., Friedman, A. J. & MacLeod, W. D., Jr., 1979. Analysis of trace levels of petroleum hydrocarbons in marine sediments using a solvent/slurry extraction procedure. In: Trace organic analysis: A new frontier in analytical chemistry. Ed. by S. N. Chesler & H. S. Hertz. – Spec. Publs natl Bur. Stand. *519*, 161–167.

Brown, D. W., Ramos, L. S., Uyeda, M. Y., Friedman, A. J. & MacLeod, W. D., Jr., 1980. Ambient-temperature extraction of hydrocarbons from marine sediments-comparison with boiling-solvent extractions. (Advances in Chemistry Series, "Petroleum in the Marine Environment".) American Chemical Society, Washington, D. C. (In press.)

Clark, R. C., Jr. & Brown, D. W., 1977. Petroleum properties and analyses in biotic and abiotic systems. In: Effects of petroleum on arctic and subarctic marine environments and organisms. Ed. by D. C. Malins. Acad. Press, New York *1*, 1–89.

Clark, R. C., Jr. & MacLeod, W. D. Jr., 1977. Inputs, transport mechanisms, and observed concentrations of petroleum in the marine environment. In: Effects of petroleum on arctic and subarctic marine environments and organisms. Ed. by D. C. Malins. Acad. Press, New York *1*, 91–223.

Collier, T. K., Thomas, L. & Malins, D. C., 1978. Influence of environmental temperature on disposition of dietary naphthalene in coho salmon *(Oncorhynchus kisutch)*: Isolation and identification of individual metabolites. – Comp. Biochem. Physiol. *61C*, 23–28.

Karrick, N. L., 1977. Alterations in petroleum resulting from physico-chemical and microbiological factors. In: Effects of petroleum on arctic and subarctic marine environments and organisms. Ed. by D. C. Malins. Acad. Press, New York *1*, 225–299.

Krahn, M. M., Brown, D. W., Collier, T. K., Friedman, A. J., Jenkins, R. G. & Malins, D. C., 1980. Rapid analysis of naphthalene and its metabolites in biological systems: Determination by high-performance liquid chromatography/fluorescence detection and by plasma desorption/chemical ionization mass spectrometry. – J. biochem. biophys. Meth. *2* (in press).

MacLeod, W. D., Jr., Brown, D. W., Jenkins, R. G., Ramos, L. S. & Henry, V. D., 1976. Petroleum hydrocarbons in the northern Puget Sound area – a pilot study. – NOAA tech. Mem. *ERL MESA-8*, 1–53.

MacLeod, W. D., Jr., Uyeda, M. Y., Thomas, L. C. & Brown, D. W., 1978. Hydrocarbon patterns in some marine biota and sediments following the Argo Merchant spill. In: Proceedings of the symposium "In the Wake of the Argo Merchant". Center for Ocean Management Studies, University of Rhode Island, Kingston, 72–79.

Malins, D. C., 1980. Pollution of the marine environment. – Environ. Sci. Technol. *14*, 32–37.

Malins, D. C., Collier, T. K., Thomas, L. C. & Roubal, W. T., 1979. Metabolic fate of aromatic hydrocarbons in aquatic organisms: Analysis of metabolites by thin-layer chromatography and high-pressure liquid chromatography. – Int. J. environ. analyt. Chem. *6*, 55–66.

McCain, B. B., Hodgins, H. O., Gronlund, W. D., Hawkes, J. W., Brown, D. W., Myers, M. S. & Vandermeulen, J. H., 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole *(Parophrys vetulus)* and pathological consequences. – J. Fish. Res. Bd Can. *35*, 657–664.

Ramos, L. S., Brown, D. W., Jenkins, R. G. & MacLeod, W. D., Jr., 1979. Modification of conventional gas chromatographic inlets for the use of glass capillary columns. In: Trace organic analysis: A new frontier in analytical chemistry. Ed. by S. N. Chesler & H. S. Hertz. – Spec. Publs natl Bur. Stand. *519*, 713–718.

Roubal, W. T., Collier, T. K. & Malins, D. C., 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon *(Oncorhynchus kisutch)*. – Archs environ. Contam. Toxicol. *5*, 513–529.

Roubal, W. T., Stranahan, S. I. & Malins, D. C., 1978. The accumulation of low molecular weight hydrocarbons of crude oil by coho salmon *(Oncorhynchus kisutch)* and starry flounder *(Platichthys stellatus)*. – Archs environ. Contam. Toxicol. *7*, 237–244.

Schabron, J. F., Hurtubise, R. J. & Silver, H. F., 1979. Chromatographic and spectrometric methods for the separation, characterization and identification of alkyl phenols in coal-derived solvents. – Analyt. Chem., *51*, 1426–1433.

Sims, P. & Grover, P. L., 1974. Polycyclic aromatic hydrocarbons. – Adv. Cancer Res. *20*, 165–274.

Varanasi, U. & Malins, D. C., 1977. Metabolism of petroleum hydrocarbons: Accumulation and biotransformation in marine organisms. In: Effects of petroleum on arctic and subarctic marine environments and organisms. Ed. by D. C. Malins. Acad. Press, New York *2*, 175–270.

Varanasi, U., Gmur, D. J. & Treseler, P. A., 1979. Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder *(Platichthys stellatus)* and rock sole *(Lepidopsetta bilineata)*. – Archs environ. Contam. Toxicol. *8*, 673–692.