DETERMINATION OF SULFUR-CONTAINING PETROLEUM COMPONENTS IN MARINE SAMPLES

J. S. Warner
Battelle-Columbus Laboratories
Columbus, Ohio

ABSTRACT

Sulfur-containing petroleum components such as benzothiophene, dibenzothiophene, naphthobenzothiophene, and their alkyl derivatives are obtained along with aromatic hydrocarbons during the determination of hydrocarbons in marine samples. By using a sulfur-specific flame photometric detector in gas chromatographic analysis, the sulfur-containing components are determined separately from the aromatic hydrocarbons. Individual sulfur-containing components are identified by retention times and mass spectrometry.

It is shown that the fingerprint of sulfur-containing components can be more definitive of an oil source than the corresponding hydrocarbon fingerprint. It is also shown that sulfur-containing components can be preferentially concentrated in the marine environment. The techniques described have been used to identify and quantitate individual sulfur-containing compounds in marine tissue and sediment samples.

INTRODUCTION

The fingerprinting of petroleum components by gas chromatographic analysis using a flame ionization detector has been widely used during the past ten years for characterizing petroleum pollution present in the marine environment [1,2,3,4,5]. A saturated hydrocarbon fraction has been studied in most cases to determine the presence of individual normal paraffins and various isoprenoid hydrocarbons, especially pristane and phytane. To a lesser extent aromatic hydrocarbon fractions have been studied. Blaylock and coworkers [6] have studied the presence of certain naphthalenes, and Warner has studied biphenyls, fluorenes, and phenanthrenes as well as the naphthalenes [7]. Studies by Boylan and Tripp [8] and Anderson et al [9], and others showing the greater toxicity of aromatic hydrocarbons have led to a greater emphasis on aromatic hydrocarbons in more recent marine pollution studies.

Sulfur-containing petroleum components are usually present in aromatic hydrocarbon fractions. In most cases the sulfur compounds are overlooked simply because they are present at relatively low levels and cannot be resolved from the predominating hydrocarbons by gas chromatography using a flame ionization detector. However, the sulfur compounds can be quite important in characterizing an oil. A sulfur-specific flame photometric detector has been used by Adlard and coworkers [10], Miller [11], and Garza and Muth [12] for the fingerprinting of the sulfur components of oils. In our work different fractions of oil and marine sample extracts were obtained by silica gel column chromatography prior to gas chromatographic analysis using a flame photometric detector. The fingerprints resulting from the sulfur compounds present in the individual fractions were found to be very useful to characterizing the samples. Some of the sulfur-containing compounds involved were identified by retention times and mass spectrometry.

Experimental

The sulfur-containing petroleum components were extracted from tissue, sediment, and water samples along with the hydrocarbon components. An outline of the procedures used for tissue extraction, silica gel chromatographic separation, and gas chromatographic analysis is given here. A more detailed presentation including validation results will be reported elsewhere [13].

For tissue analysis, 10 g of a homogenized sample was mixed with 4 g of 4 *N* aqueous NaOH in a 50-ml screw-cap centrifuge tube having a Teflon cap liner. After heating at 90°C for 2 hours, the digest was extracted in the centrifuge tube with 25 ml of peroxide-free ethyl ether in two portions. Separation of the layers was facilitated by centrifugation. The combined ether layers were concentrated to 1 ml using a 25-ml evaporator tube and a Kontes tube heater. The ether was replaced with hexane by adding 2 ml of hexane and reconcentrating to 1 ml. The hexane solution was cleaned up and fractionated by column chromatography using a 0.9 × 25-cm column containing 10 g of silica gel activated at 150°C. Three fractions were collected: fraction 1 eluted with 25 ml of petroleum ether and fractions 2 and 3 each eluted with 25 ml of 20% methylene chloride in petroleum ether. Each fraction was concentrated to 200 μl for gas chromatographic analysis. For quantitative studies, 200 μg of phenyl sulfide or dodecyl sulfide was added as an internal standard.

Gas chromatography was carried out using a Varian Model 1740 gas chromatograph equipped with a flame ionization detector and a Tracor flame photometric detector operated in the sulfur mode. A 3.5 m N 2 mm ID glass column packed with 3% OV-17 on 100-120 mesh Gas Chrom Q was used with the column temperature programmed from 120 to 280°C at 6 degrees per minute. A column effluent splitter and a dual pen recorder were used to give simultaneous trace of the responses from the two detectors. Standard curves (figure 1) were prepared by analyzing various known concentrations of benzothiophene and dibenzothiophene containing phenylsulfide or dodecyl sulfide as an internal standard.

For sediment analysis a 150-ml sample was mixed with 100 ml of 0.1*N* H_2SO_4 and 150 ml of petroleum ether in a 1-quart narrow-mouth bottle having a Teflon-lined screw cap. The mixture was tumbled on rolls for 24 hours and then centrifuged in screw-capped centrifuge bottles. A 100-ml portion of the petroleum ether extract was dried with 1 g of anhydrous magnesium sulfate and concentrated to 1 ml. The concentrate was subjected to silica gel chromatography followed by gas chromatographic analysis as described above for tissue analysis.

For water analysis, a 500-ml sample was treated with 10 ml of 4*N* H_2SO_4 and extracted in a separatory funnel with three 25-ml portions of methylene chloride. The combined methylene chloride extracts were dried over 1 g of anhydrous magnesium sulfate and concentrated to 1 ml. The methylene chloride was replaced with hexane by adding 2 ml of hexane and reconcentrating to 1 ml. The

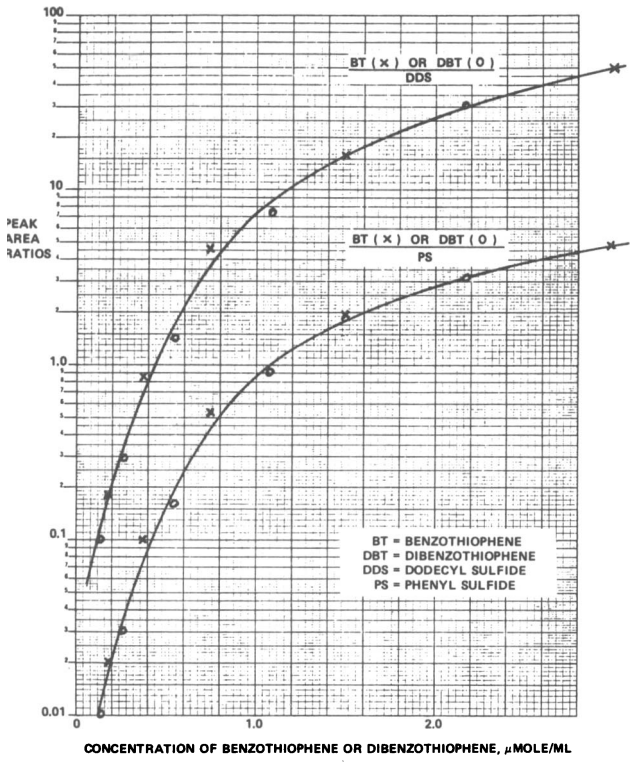

Figure 1. Standard curves (mole basis) for quantitative determination of sulfur compounds

hexane concentrate was subjected to silica gel chromatography followed by gas chromatographic analysis as described above for tissue analysis.

Mass spectrometric analysis was performed with a Finnigan Model 1015 quadrupole mass spectrometer equipped with a chemical ionization source and interfaced with a Varian Model 1740 gas chromatograph. Methane was used as the carrier and ionizing gas. Control and data acquisition were performed by a Finnigan System 150 interactive computer system. Unit mass scans were made over an m/e range of 100 to 350. A reconstructed gas chromatogram was obtained based on the total ion intensity of each individual spectrum. In printing out a mass spectrum from an individual spectrum number, the background represented by the baseline or valley preceding the peak was usually subtracted out.

Discussion

Sulfur-containing compounds were found along with aromatic hydrocarbons in fractions 2 and 3, but as expected, none were present with the saturated hydrocarbons in fraction 1. The usefulness of the flame photometric detector for the fingerprinting of oil fractions was demonstrated by the characterization of a sample of oil collected from the beach at Coal Oil Point in Santa Barbara,[1] California. This is an area where there are natural oil seeps offshore as well as offshore oil wells. The aromatic fractions from the beach sample were compared to those of reference samples of crude oil produced from a nearby offshore well and those of natural seep oil from the area collected by a diver.[2] The gas chromatograms of fraction 2 obtained using a flame ionization detector (FID) are shown in figure 2a, and those using a flame photometric detector (FPD) are shown in figure 2b. The resemblance of the beach sample to the seep oil rather than crude oil is particularly striking in the FPD chromatograms.

[1]The beach sample was collected in 1972 when the offshore oil wells were capped.

[2]The crude oil and seep oil reference samples were kindly provided by Dr. T. Myers of the University of Southern California.

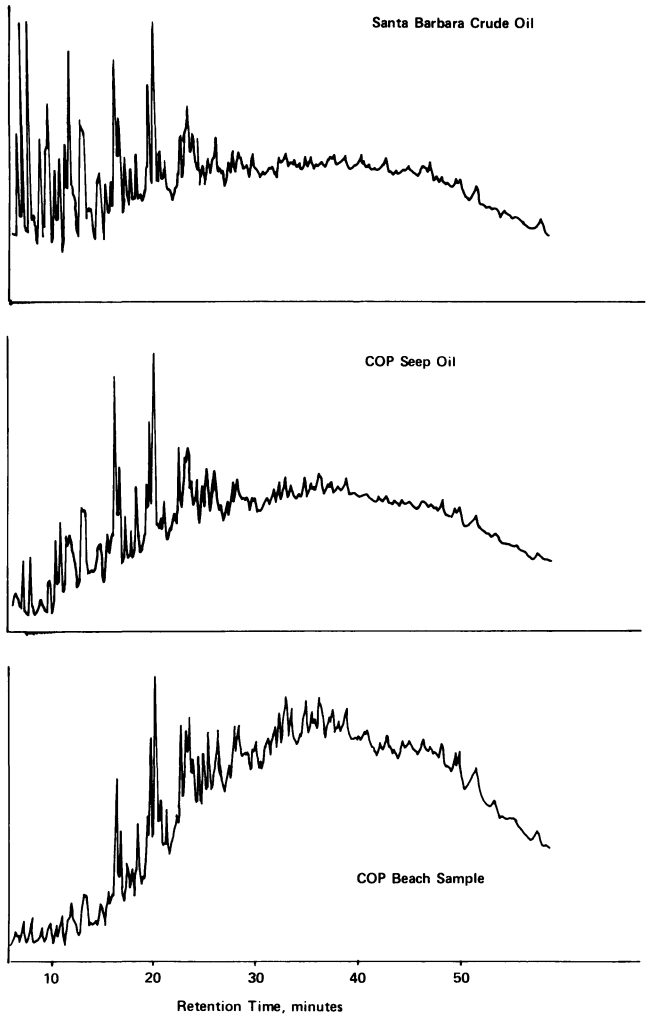

Figure 2. Gas chromatograms of aromatic hydrocarbon fractions using a flame ionization detector

API reference oil II, a highly aromatic No. 2 fuel oil, and API reference oil IV, a Bunker C fuel oil, were used to determine some of the types of sulfur-containing components that might result from petroleum pollution. In general, most of the benzothiophenes were found along with the benzenes and naphthalenes in fraction 2, and most of the dibenzothiophenes were found along with the phenanthrenes, biphenyls, and fluorenes in fraction 3.

The identification of the various sulfur-containing compounds is based partly upon retention time data and the chromatographic pattern. Reference samples of benzothiophene and dibenzothiophene were used to verify the retention times for those two materials. The gas chromatographic pattern of the sulfur-containing compounds in the alkylbenzothiophene range was very similar to that of the naphthalenes present in fuel oil. This is shown very clearly in figure 3, the gas chromatrogram of fraction 2 from the No. 2 fuel oil. The benzothiophene (BT) has a retention time slightly greater than that of naphthalene (N). There are two major sulfur-containing peaks with retention times similar to those of the two methyl naphthalenes (MN), three with retention times similar to those of C_2-naphthalenes (C_2-N), and three with retention times similar to those of C_3-naphthalenes (C_3-N) (The naphthalenes were identified by combined gas chromatography mass spectrometry.) The sulfur-containing compounds are, therefore, considered to be the corresponding benzothiophene (BT), methylbenzothiophenes (MBT), C_2-benzothiophenes (C_2-BT), and C_3 benzothiophenes (C_3-BT). Similarly, the gas chromatographic pattern of the sulfur-containing compounds in the alkyldibenzothiophene range was very similar to that

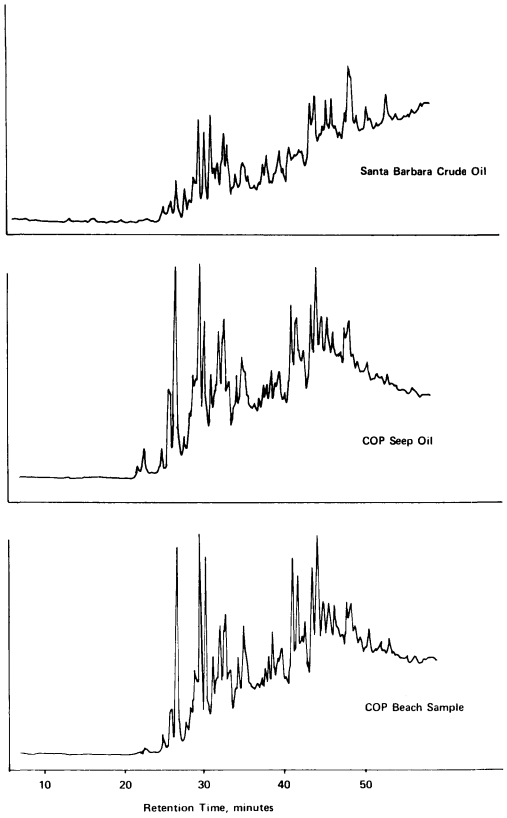

Figure 2b. Gas chromatograms of aromatic hydrocarbon fractions using a flame photometric detector

of the phenanthrenes present. This is shown in figure 4, the gas chromatogram of fraction 3 from the No. 2 fuel oil. The dibenzothiophene (DBT) has a retention time slightly less than that of phenanthrene (P). There are two major sulfur-containing peaks with retention times slightly less than those of methylphenanthrenes (MP). The sulfur-containing compounds were, therefore, considered to be the corresponding dibenzothiophene (DBT), methyldibenzothiophenes (MDBT), etc.

The identification of alkyl dibenzothiophenes in oil fractions by chemical ionization mass spectrometry, which primarily determines molecular weight, is subject to interference by naphthalene compounds. Dibenzothiophene, for example, has the same molecular weight as the tetramethylnaphthalenes and a similar retention time.

A fraction obtained from a field sample in one case was particularly useful for mass spectrometric analysis because nearly all of the components present were sulfur-containing compounds. This was a fraction 2 obtained from a sample of *Lygia* (an isopod commonly referred to as "roach of the rocks") collected after a spill of fuel oils, including a Bunker C fuel oil.[3] The gas chromatogram is shown in figure 5. Although much of the dibenzothiophenes were present in fraction 3, a significant amount appeared in fraction 2 along with any possible naphthalenes. Unlike most oil fractions, each major peak detected by the flame ionization detector in this fraction was also a major sulfur-containing peak as detected by the flame photometric detector. A reconstructed gas chromatogram of a GC-MS

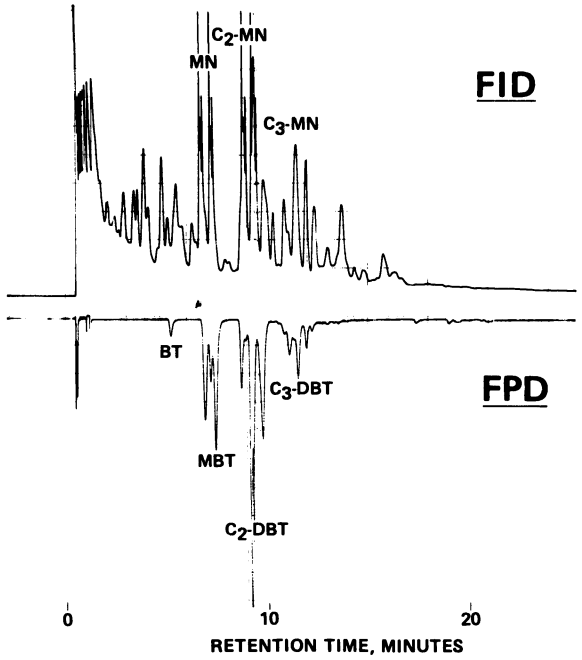

Figure 3. Gas chromatogram of fraction 2 from a No. 2 fuel oil

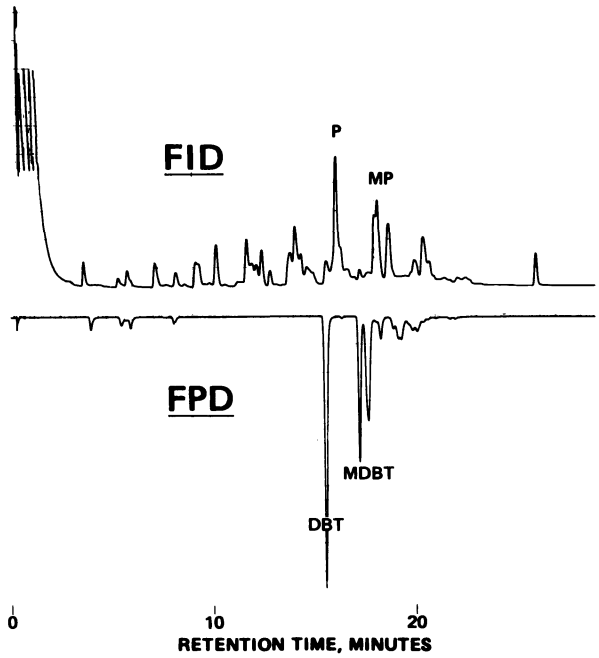

Figure 4. Gas chromatogram of fraction 3 from a No. 2 fuel oil

run on this fraction is shown in figure 6. The molecular weights found for the major peaks are given in table 1. In no case was there any large contribution from a possible alkylphenanthrene except in spectrum No. 154. However, since sulfur-containing compounds were found in that portion of the gas chromatogram, it is very likely that spectrum No. 154 actually represents a naphthobenzothiophene (NBT). The molecular weights of all of the major peaks between spectrum No. 105 and spectrum No. 144 correspond to C_1 to C_4 alkyldibenzothiophenes. The amounts of the various dibenzothiophenes found, using GC and the standard curves of figure 1, were 10, 50, 60, 25, and 15 μg/g, respectively, for DBT, MDBT, C_2-DBT, C_3-DBT, and C_4-DBT.

[3]The *Lygia* sample was supplied by Professor Jack W. Anderson of Texas A&M University. The sample was collected in the Houston Yacht Basin in Upper West Galveston Bay on March 13, 1973, four days after a collision in the Houston Ship Channel that resulted in the loss of nearly 400,000 gallons of oil from a barge carrying Bunker C and fuel oil. The final containment and cleanup operations took place at the Houston Yacht Basin. At the time of collection the water surface and nearby land in the area were covered with a 1/2" to 2" layer of heavy oil. The *Lygia* specimens had crawled up on the rocks and died.

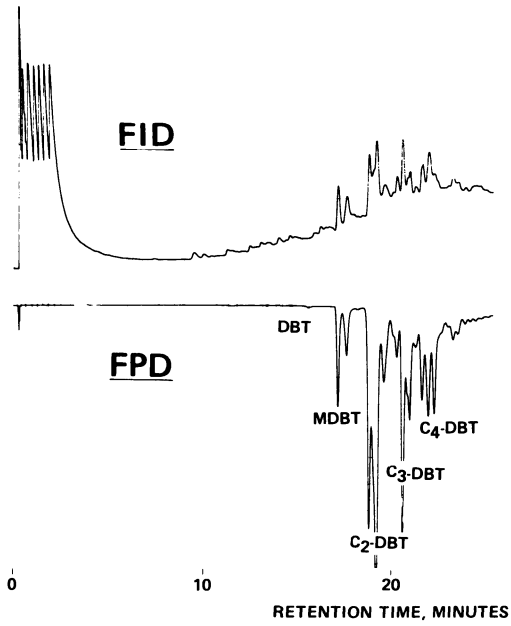

Figure 5. Gas chromatogram of fraction 2 from a contaminated *Lygia* sample

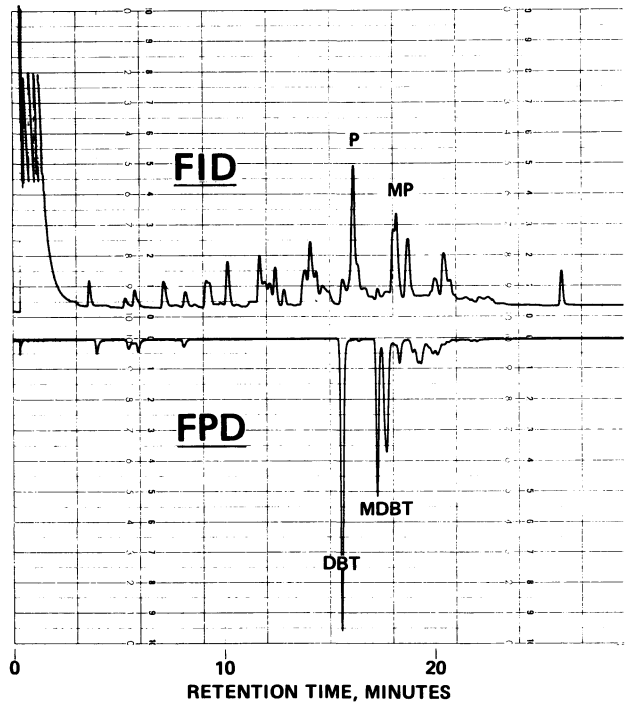

Figure 6. Reconstructed gas chromatogram of fraction 2 from a contaminated *Lygia* sample

In addition to being useful for mass spectrometric analysis, the field sample of *Lygia* was even more important in indicating that alkyldibenzothiophenes may be concentrated in the marine environment to an even greater extent than the aromatic hydrocarbons. This possibility is suggested by a comparison of the gas chromatogram of fraction 3 from a Bunker C fuel oil (figure 7) with that of fraction 3 from the field sample (figure 8). The ratios of the FID responses of the sulfur containing components to the FID responses of the nonsulfur-containing components are clearly much greater for the *Lygia* fraction than for the Bunker C fraction. If it is assumed that

Sulfur-Containing Components

TABLE 1. MASS SPECTRAL IDENTIFICATION OF GAS CHROMATOGRAPHIC PEAKS OF FRACTION 2 FROM A CONTAMINATED LYGIA SAMPLE

Spectrum No.[a]	Major Component M.W.	Tentative Identification
105	198	Methyldibenzothiophene or C_5-Naphthalene
110	198	Ditto
119	212	C_2-Dibenzothiophene or C_6-Naphthalene
122	212	Ditto
126[b]	212	"
131	226	C_3-Dibenzothiophene or C_7-Naphthalene
133	226	Ditto
136	226	"
141	240	C_4-Dibenzothiophene or C_8-Naphthalene
144	240	Ditto
154	234	Naphthobenzothiophene or C_4-Phenanthrene

a. See Figure 6.

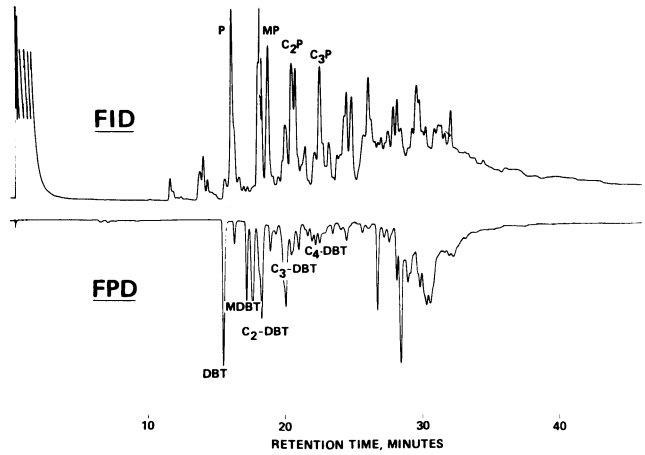

Figure 7. Gas chromatogram of fraction 3 from a Bunker C oil

the spilled oil that contaminated the *Lygia* was similar to the Bunker C oil used as a reference, the sulfur-containing components must have been concentrated relative to the nonsulfur-containing components by about a factor of five. If no such concentration occurred, the spilled oil would have had to have an alkyldibenzothiophene content five times greater than that of the reference oil. If sulfur content is used as an indicator of alkyldibenzothiophene content, the spilled oil would have had to have a sulfur content of about 8%, which is very high. It is considered more likely that selective concentration of the sulfur-containing components did indeed occur in the marine environment. Admittedly, this conclusion is based on several assumptions, and additional information would be required to substantiate the conclusion.

The *Lygia* sample considered here was not washed thoroughly with an organic solvent to remove surface contamination. Consequently much of the contamination found was very likely present on the outer surfaces of the animals rather than incorporated into the tissue structure. The selective concentration of alkyldibenzothiophenes may have resulted primarily from a physical weathering effect rather than from a biological effect. Regardless of the cause of the selective concentration, it seems reasonable to conclude that

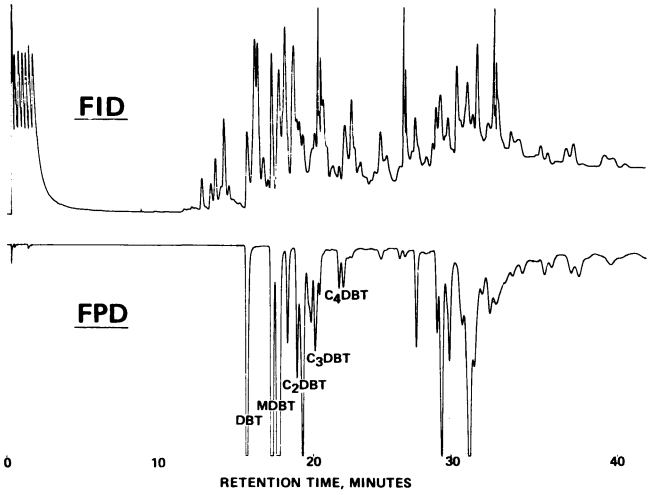

Figure 8. Gas chromatogram of fraction 3 from a contaminated *Lygia* sample

further consideration should be given to the analysis and possible biological effects of these sulfur-containing petroleum components.

Acknowledgments

This work was sponsored by the American Petroleum Institute under Contract No. OS-20-N. The author wishes to acknowledge the assistance in the area of mass spectrometry provided by Dr. Rodger L. Foltz and Dr. Pauline A. Clarke.

REFERENCES

1. 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. Envir. Contam. Toxicol.* 10:129.
2. Blummer, M.; Souza, G.; and Sass, J. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. *Marine Biology*. 5:195.
3. Clark, R.C., Jr., and Finley, J.S. 1973. Techniques for analysis of paraffin hydrocarbons and for interpretation of data to assess oil spill effects in aquatic organisms. *Proceedings of Joint Conference on Prevention and Control of Oil Spills*, p. 161. 1967. Washington, D.C.: American Petroleum Institute.
4. Clark, R.C., Jr., and Blumer, M. 1967. Distribution of n-parrafins in marine organisms and sediment. *Limnol. Oceanogr.* 12:79.
5. Oró, J.; Tornabene, T.G.; Nooner, D.W.; and Gelpi, E. 1967. Aliphatic hydrocarbons and fatty acids of some marine and freshwater microorganisms. *J. Bact.* 93:1811.
6. Blaylock, J.W.; O'Keefe, P.W.; Roehm, J.N.; and Wildung, R.E. 1973. Determination of n-alkane and methylnaphthalene compounds in shellfish. *Proceedings of Joint Conference on Prevention and Control of Oil Spills*, p. 173. Washington, D.C.: American Petroleum Institute.
7. Warner, J.S. 1974. Quantitative determination of hydrocarbons in marine organisms. *Proc. of Marine Pollution Monitoring Symposium and Workshop*, May 13-17, 1974. National Bureau of Standards, Gaithersburg, Maryland.
8. Boylan, D.B., and Tripp, B.W. 1971. Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. *Nature*, 230:44.
9. Anderson, J.W.; Neff, J.M.; Cox, B.A.; Tatem, H.E.; and Hightower, G.M. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity ot estuarine crustaceans and fish. *Marine Biology* (in press).
10. Adlard, E.R.; Creaser, L.F.; and Matthews, P.H.D. 1972. Identification of hydrocarbon pollutants on seas and beaches by gas chromatography. *Anal. Chem.* 44:64.
11. Miller, J.W. 1973. A multiparameter oil pollution source identification system. *Proceedings of Joint Conference on Prevention and Control of Oil Spills*, p. 195. Washington, D.C.: American Petroleum Institute.
12. Garza, M.E., Jr. and Muth, J. 1974. *Environ. Sci. Technol.* 8:249.
13. Warner, J.S. Semiquantitative determination of C_{10} to C_{30} aliphatic and aromatic hydrocarbons in marine organisms. (In preparation.)