4 Characterization of Polycyclic Aromatic Sulfur Heterocycles for Source Identification

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4.1 Introduction

The fingerprinting of crude oil spills can be a very complex analytical problem. Not only is the crude oil a highly complex matrix and many of the components of interest are present in low amounts, but an oil in the environment is subject to several weathering factors, such as evaporation and dissolution (physical), biodegradation (biological), and photooxidation (chemical), that often have a major influence on the composition. Furthermore, some of these changes can be dependent on the place and time of the spill. In many cases it is true that the older the pollutant, the more its distinguishing characteristics tend to disappear, but situations are known in which petroleum residues appear after 30 years in concentrations "similar to those observed immediately after the spill" (Reddy et al., 2002), and many biomarkers have proven useful in hydrocarbon fingerprinting and oil spill source identification even in a wide variety of weathering conditions (Stout et al., 2002; Wang and Fingas, 2003).

The most frequent question legal investigators ask of the scientist is to establish the origin of a petroleum spill. Clearly, the chemical analyst can only provide information on the object under investigation based on chemical-analytical data. Linking this information to the source of the pollution and, by implication, the perpetrator, usually needs further evidence that is outside the responsibility of the analytical scientist.

It is reasonable to expect the analyst to state with a certain degree of certainty whether or not a crude oil, finished product, ship bilge, material from tanker washing, or natural seeps is involved. This subject has been studied by a large number of researchers, as is evident from many chapters in this book. To arrive at the most reasonable answer to this question, they have used a multitude of chemical compound classes as markers for different properties, reflecting the complexity of crude oil. Among these are also the polycyclic aromatic sulfur heterocycles (PASHs), which play a major role among the constituents of petroleum and its refined products.

In this chapter we provide recommendations that are directed particularly toward the characterization of PASHs for forensic purposes of any petroleum-derived pollutant that is likely to be encountered mainly in the marine environment and that arrived there through accident or oversight. Obviously, PASHs are only one of several classes of compounds that should be studied for such purposes, as there is no one class of compounds that will be sufficient for answering all the questions of the forensic investigator.

Sulfur compounds in crude oil and petroleum products will be discussed first with the view to showing how varied the compound classes can be in different petroleums and refined products. Next, some changes in PASH patterns when petroleum is treated in refineries are described since this will have an influence on the PASH pattern of petroleum products that may end up in the environment. To understand the importance of PASHs in oil spill source identification, we give an overview on the stability of these compounds in the environment and their reactions there. Analytical methods and techniques used for oil spill fingerprinting have made major advances in recent years, and this development continues. Therefore, in this chapter, we will review the current state of information on PASH analytical techniques. Finally, some case studies in which PASHs were investigated will be described. Our main objective is to clarify how PASHs can be used to help solving problems encountered in oil spill source identification.

4.2 Sulfur Compounds in Crude Oil and Petroleum Products

The petroleum industry has continually been troubled with various problems related to sulfur compounds in petroleum and its products, such as product odor and storage stability, catalyst poisoning, corrosion of processing equipment, and pollution emitted during usage. Thus, it is essential to characterize the structures of sulfur compounds in crude oils and petroleum products. The motivation for this may have technical grounds, for example, to optimize the desulfurization processes, but has the added benefit that the knowledge gained can also be used for other purposes. The discussion ahead will show which kinds of sulfur compounds can be expected and therefore may be targets of an analysis of oil spill residues.

Sulfur is usually the most abundant heteroelement in petroleum. Most of the sulfur present in crude oils is organically bound sulfur while elemental sulfur and hydrogen sulfide usually represent a very minor portion. The sulfur content is in the range 0.1–3.0% in most crudes (Morrison, 1999) but can reach 8% in the vacuum residue of heavy crudes (Severin and Glinzer, 1984). Organic sulfur compounds in crude oils are distributed over a wide range of molecular structures: aliphatic thiols, mono- and disulfides are sometimes present (Rygle et al., 1984) as well as alkyl phenyl disulfides (Nishioka, 1988), but a large amount occurs in aromatic structures, especially as alkylated thiophene benzologues (Arpino et al., 1987). After distillation, mercaptanes, sulfides, and thiophenes are concentrated in the gasoline-range products (Stumpf et al., 1998) while benzothiophenes (BTs), dibenzothiophene (DBT), and alkylated dibenzothiophenes (DBTs) are concentrated in the middle distillate fractions. They may represent up to 70% of the sulfur present in diesel fuel.

The PASH class is by far the most thoroughly investigated of them all, but possibly the sulfides may gain some potential as marker compounds of individual oils since there seems to be quite a diversity of different structures present in fossil material from different sources. A problem could be the ease of their oxidation to sulfoxides in photochemical reactions (Burwood and Speers, 1974) that might lead to a rapid change in their pattern and concentration.

The structures of 1,4,4-trimethyl-4,5,6,7-tetrahydroisothianaphthene and methyl 3,4,5-trimethyl-2-thienyl sulfide (Figure 4-1 and Table 4-1) were established in the kerosene boiling range of Middle East distillates (Birch et al., 1959). Moreover, the sulfides from the petroleum fraction b.p. 200–275° comprised mainly of thiamonocyclanes and included smaller amounts of polycyclic sulfides (Polyakova et al., 1978). The thiamonocyclanes were thiophane homologs. The sulfides from the fraction b.p. 350–450° contained thiamonocyclanes and thiapolycyclanes containing 2–8 rings. The thiapolycyclanes were derivates of thiophane condensed mainly with 5-membered rings. A series of novel, terpenoid-derived polycyclic sulfides were identified by Charrié-Duhaut et al. (2003) in highly desulfurized diesel oils and identified as

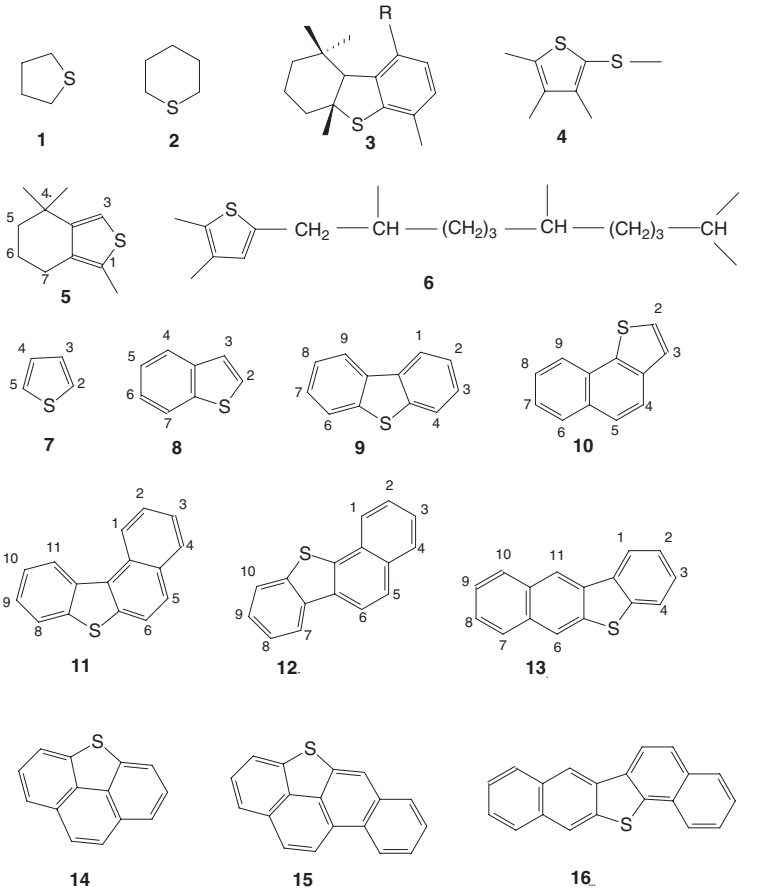

Figure 4-1 Structures of sulfur compounds in petroleum. For names, see Table 4-1.

Table 4-1 Names of Sulfur Compounds in Petroleum as Illustrated in Figure 4-1

Compound	*Name*
1	Thiophane
2	Thiacyclohexane
3	1,1,4a,6-Tetramethyl-9-alkyl-1,2,3,4,4a, 9b-hexahydrodibenzothiophenes
4	Methyl 3,4,5-trimethyl-2-thienyl sulfide
5	1,4,4-Trimethyl-4,5,6,7-tetrahydroisothianaphthene
6	2,3-Dimethyl-5-(2,6,10-trimethylundecyl) thiophene
7	Thiophene
8	Benzothiophene
9	Dibenzothiophene
10	Naphtho[1,2-*b*]thiophene
11	Benzo[*b*]naphtho[1,2-*d*]thiophene
12	Benzo[*b*]naphtho[2,1-*d*]thiophene
13	Benzo[*b*]naphtho[2,3-*d*]thiophene
14	Phenanthrothiophene
15	Chrysenothiophene
16	Dinaphthothiophene

1,1,4a,6-tetramethyl-9-alkyl-1,2,3,4,4a,9b-hexahydrodibenzothiophenes.

Organo-sulfur compounds may also be present in various other structures. Schmid et al. (1987) and Sinninghe Damsté et al. (1987) describe long-chain dialkylthiacyclopentanes in oils while Payzant et al. (1986) identified terpenoid sulfides in petroleum. Hopanes and steranes are routinely used for petroleum correlation studies but the sulfur-containing compounds do not seem to have been investigated for such purposes. Sinninghe Damsté et al. (1987) and Valisolalao et al. (1984) have tentatively identified steroid and hopanoid thiophenes, respectively, in petroleum. In a later article, Sinninghe Damsté et al. (1989) cited the presence of various highly branched isoprenoid thiophenes in sediments and immature oils. The identification of isoprenoid C20 and

C15 sulfur compounds in Rozel Point Oil has also been described (Sinninghe Damsté and de Leeuw, 1987). These authors reported the occurrence of isoprenoid thiophenes, thiolanes, benzothiophenes, bithiophenes, (thienyl)alkylthiophenes, and thienylthiolanes in this oil and in other oils and sediments. Since isoprenoids often show higher stability in environmental situations, such compounds may be promising for forensic research.

A vast amount of knowledge has accumulated on aromatic sulfur compounds and the following can only give a hint at what is known. Mössner and Wise (1999) identified and quantified naphtho[1,2-*b*]thiophene, dibenzothiophene (DBT), all four methyldibenzothiophenes (MDBTs), three of the four ethyldibenzothiophenes, 15 of the 16 possible dimethyldibenzothiophenes, and 6 of the 28 possible trimethyldibenzothiophenes in a standard reference crude oil sample (SRM 1582), which is a Wilmington crude. They also determined the three isomeric benzo[*b*] naphtho[1,2-*d*]thiophene, benzo[*b*]naphtho[2, 1-*d*]thiophene, and benzo-[*b*]naphtho[2,3-*d*] thiophene and 28 of the 30 possible methylbenzonaphthothiophenes (MBNTs) in the same sample. Recently, many of these PASHs were identified in Egyptian crude oils (Hegazi et al., 2003, 2004a). As an illustration of the complexity of the PASH pattern, a partial extended gas chromatogram with the atomic emission detector (AED) in the sulfur-selective mode of an Egyptian crude oil, showing the distribution pattern of BTs and DBTs, is reproduced in Figure 4-2 and an identification of the corresponding peaks is given in Table 4-2. This identification was considerably facilitated due to the availability of reference compounds (PASH, 2006).

In heavy distillates (225–500°C) of Saudi Arabian crude oils (Arabian heavy, light, medium, and extra light), BTs, DBTs, and BNTs are the major PASH types (Farhat et al., 1991). The polar petroleum resins contain many different compounds, such as sulfoxides, sulfides, sulfones, thiophenes, and mixed sulfur-nitrogen heterocycles (Rudzinski and Aminabhavi, 2000; Mitra-Kirtley et al., 1998).

Sulfur compounds in aviation fuels were found to occur mainly as thiols, sulfides, and disulfides while thiophenes and DBTs are minor constituents (Link et al., 2003). Somewhat similar classes of sulfur components in a fluid catalytic cracking (FCC) gasoline were thiols, sulfides, thiophene and alkylthiophenes, tetrahydrothiophene, thiophenols, and benzothiophene (Hatanaka et al., 1997; Cheng et al., 1998). Recent data by Yin and Xia (2004) confirm that thiophene sulfur represents a large fraction of the total sulfur content in FCC gasoline (60 wt. % and more). They detected more than 20 kinds of thiophenes among which some (di- and trimethyl-, ethyl-, ethylmethyl-, di- and triethyl-, *iso*-propyl-, *t*-butyl-) could be identified by GC-MS analysis. Also, they identified cyclo-sulfides in FCC and residue fluid catalytic cracking gasoline produced in China. In an earlier study (Nishioka et al., 1986) 3-ring, 4-ring, and 5-ring PASHs were investigated in a catalytically cracked petroleum vacuum residue. These PASHs are C_{1-5}-DBTs, C_{2-6}-phenanthrothiophenes, C_{1-5}-naphthobenzothiophenes, C_3- and C_4-bis(benzothiophene)s, C_{1-4}-chrysenothiophenes, and C_2-dinaphthothiophene.

4.3 Influence of Refinery Processes on PASH Patterns

Nitrogen and sulfur oxides are a subset of acid rain gases receiving attention throughout the world under agreements aimed at reducing their emission to protect the environment and human health. Current U.S. and E.U. specifications for diesel fuel mandate that the maximum sulfur concentration be 50 ppm, but future values may be as low as 10 ppm (U.S. Environmental Protection Agency, 1999). However, current U.S., Canada, and Europe gasoline sulfur is 30 ppm. Moreover, the introduction of "sulfur-free gasoline" (<10 ppm) has been proposed in Europe for the year 2007 (Nocca et al., 2000).

Those regulations have led to a change in the sulfur compound pattern in refined products that is necessary to recognize since it will be found in the environment in case of spills.

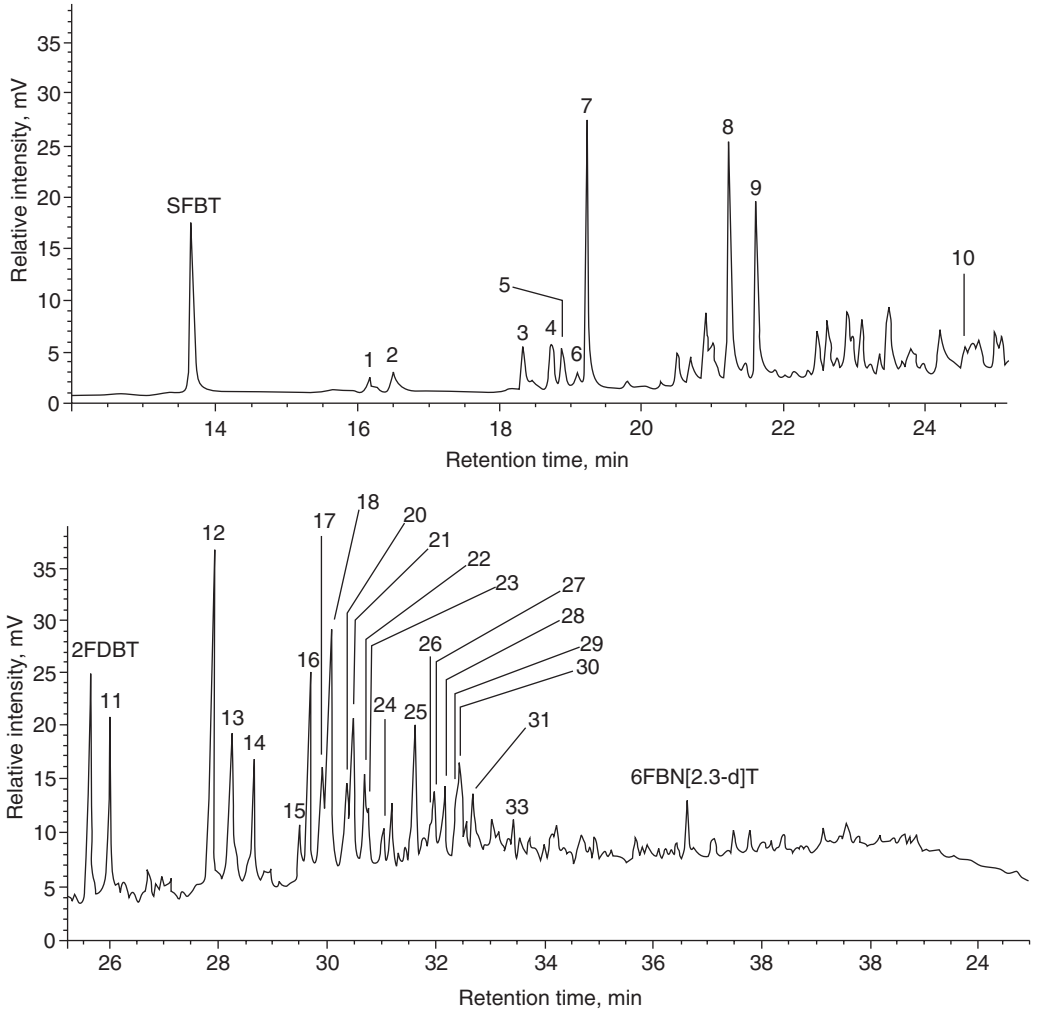

Figure 4-2 Partial extended AED chromatogram of polyaromatic fraction showing the distribution and relative retention times of PASH in an Egyptian crude oil. Peak identifications are listed in Table 4-2. 5FBT, 2FDBT, and 6FBN[2,3-d]T are fluorinated internal standards. (Reprinted from Hegazi et al., 2003, with permission from Elsevier Science.)

The easiest way to limit the amount of sulfur dioxide emitted into the atmosphere is to lower the amount of sulfur in fuels. Conventional physicochemical processes such as hydrodesulfurization (HDS) are effective for the treatment of petroleum. HDS is a catalytic process that uses hydrogen gas to reduce the sulfur in petroleum fractions to hydrogen sulfide, which is readily separated from the fuel.

Under normal conditions for HDS, the most reactive and easiest-to-remove classes of sulfur compounds are thiols, sulfides, and disulfides; compounds such as substituted BTs and DBTs react somewhat more sluggishly (Mössner and Wise, 1999; Lamure-Meille et al., 1995; Shafi and Hutchings, 2000; Knudsen et al., 1999; Stumpf et al., 1998).

Deep hydrodesulfurization of PASHs in light oil was carried out by using Co-Mo/Al_2O_3 under experimental conditions representative of industrial practice (Kabe et al., 1992). The PASHs were determined by GC-AED and GC-MS. It was found that alkyl-substituted DBTs were the most difficult compounds to desulfu-

Table 4-2 Polycyclic Aromatic Sulfur Heterocycles Identified in the Egyptian Crude Oil in Figure 4-2

Peak	*Compound*
1	2-Methylbenzothiophene
2	3-Methylbenzothiophene
3	2,7-Dimethylbenzothiophene
4	2,6+3,7+4,7-Dimethylbenzothiophenes
5	4,6-Dimethylbenzothiophene
6	3,5-Dimethylbenzothiophene
7	2,3-Dimethylbenzothiophene
8	2,3,7-Trimethylbenzothiophene
9	2,3,5-Trimethylbenzothiophene
10	2,3,4,7-Tetramethylbenzothiophene
11	Dibenzothiophene
12	4-Methyldibenzothiophene
13	2+3-Methyldibenzothiophenes
14	1-Methyldibenzothiophene
15	4-Ethyldibenzothiophene
16	4,6-Dimethyldibenzothiophene
17	2,4+2,6-Dimethyldibenzothiophenes+ 2-Ethyldibenzothiophene
18	3,6-Dimethyldibenzothiophene
19	2,7+2,8+3,7-Dimethyldibenzothiophenes
20	1,4+1,6+1,8-Dimethyldibenzothiophenes
21	1,3-Dimethyldibenzothiophene
22	3,4-Dimethyldibenzothiophene
23	2,3-Dimethyldibenzothiophene
24	2,4,6-Trimethyldibenzothiophene
25	2,4,8-Trimethyldibenzothiophene
26	2,4,7-Trimethyldibenzothiophene
27	1,4,8-Trimethyldibenzothiophene
28	1,4,7-Trimethyldibenzothiophene
29	2-Methyl-3-ethyldibenzothiophene
30	1,3,7-Trimethyldibenzothiophene
31	2,6-Diethyldibenzothiophene

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rize and 4,6-DMDBT, having substituents in both 4- and 6-positions, remained until the final stage of the reaction (390°C) while alkylbenzothiophenes were completely desulfurized at 350°C. The desulfurization of C_1-BTs results in C_3-benzenes (Depauw and Froment, 1997).

Diesel fuel is an important energy source for industry, a major fuel for ships and vehicles and for building heating. Resembling crude oil's PASH pattern, nondesulfurized diesel shows alkylated BTs and DBTs as the major sulfur-containing compounds (Figure 4-3) (Schade et al., 2002). HDS affects the PASH pattern strongly. Most of the alkylated BTs as well as a large number of the alkylated DBTs (e.g., 1-, 2-, and 3-MDBT) had vanished after a deeper HDS process as seen in Figure 4-3 (Schade et al., 2002), leading to an exceptional prominence of some of the remaining alkylated DBTs, particularly 4MDBT, 4,6DMDBT, and 4E6MDBT. These changes in the pattern of PASHs should help in source identification of spilled refinery products. The HDS reactivities of PASHs are determined by the strength of the C-S bonds, steric hindrance, and electron density on the sulfur atom (Ma et al., 1994). This imparts a reduced desulfurization kinetics to isomers alkylated in the 4-position and thus they are more slowly desulfurized than other alkylated DBTs. More research is needed to establish whether HDS fuels from different processes or different starting crudes retain a common pattern of PASHs, or if individual differences will still be discernible after the HDS. If the latter case turns out to prevail, a tool for source identification may be available.

Recently, high-molecular-weight sulfur containing aromatics were analyzed in a vacuum residue using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) (Müller et al., 2005). The authors reported that HDS affects the distribution patterns of these compounds (Figure 4-4). The amount, as well as class and type, of sulfur compounds is changed during the partial HDS process, which removed primarily compounds with one S atom, whereas those with two S atoms were largely unaffected. Such materials are used as ship fuels and are therefore likely to be found in spills. Although at the moment no restrictions on sulfur in such fuels are legally binding, it is expected that they will be introduced in the future so that again a changed pattern of PASHs can be expected with implications for forensic studies.

4.4 Stability of Polycyclic Aromatic Sulfur Heterocycles in the Environment

As soon as oil is spilled into the marine environment, the processes of spreading, evapora-

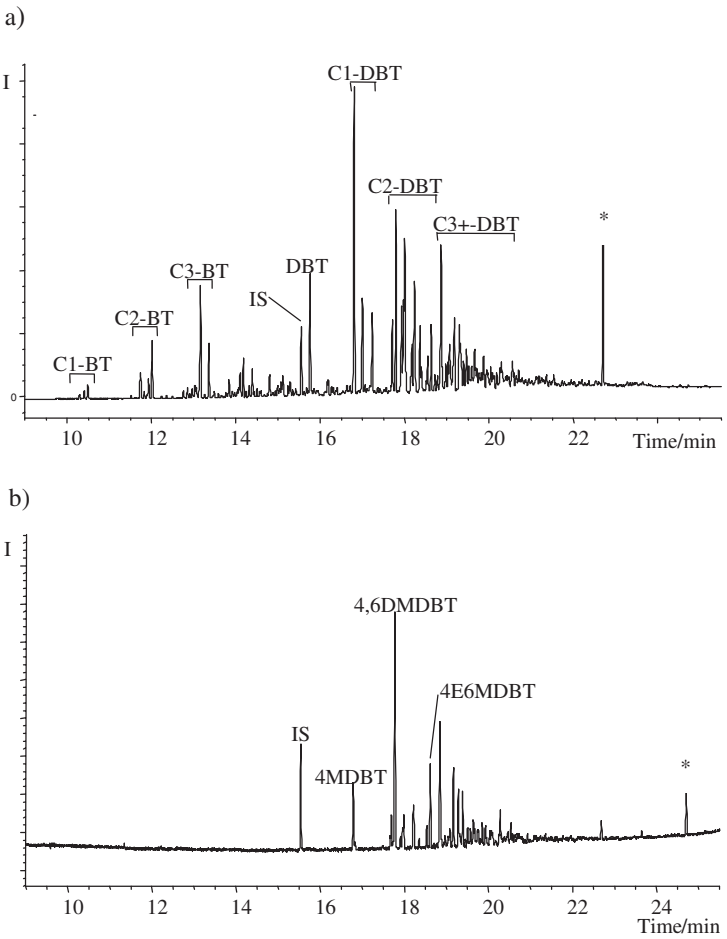

Figure 4-3 GC-FID chromatograms of (a) nondesulfurized diesel fuel and (b) deeply hydrodesulfurized diesel fuel. The asterisk indicates an impurity from the dichloromethane used in the sample workup. (Reprinted from Schade et al., 2002, with permission from Taylor & Francis.)

tion of light ends, emulsification, solubility losses, and microbial and photochemical degradation act to change its composition, making the unambiguous identification of the source of the release and the monitoring of its fate and effect in the environment something of a challenge.

Photooxidation and biodegradation are the two most important factors involved in the chemical transformation of crude oil and its products after a release into the marine environment. Photooxidation affects the aromatic compounds in crude oil and converts them to polar species of higher-aqueous solubility. Natural microbial populations in seawater can biodegrade 28% of crude oil and 36% of photooxidized crude oil within 8 weeks at 20°C (Dutta and Harayama, 2000). For the *Exxon Valdez* accident in Alaska in 1989, the mass balance showed that 50% of the oil was removed through aqueous biodegradation and photolysis and a further 20% through atmospheric photolysis. The remaining 30% were beached, recovered by man or deposited on sediments (Wolfe et al., 1994).

Although seemingly possessing a noticeable degree of stability under environmental conditions, like other organic compounds the PASHs are removed from the environment through different processes, such as microbial degradation (Saftic et al., 1992). Bacteria of the *Pseudomonas* type oxidize methylbenzothiophenes to the sulfoxide if an alkyl substituent is present on the thiophene ring and, if not,

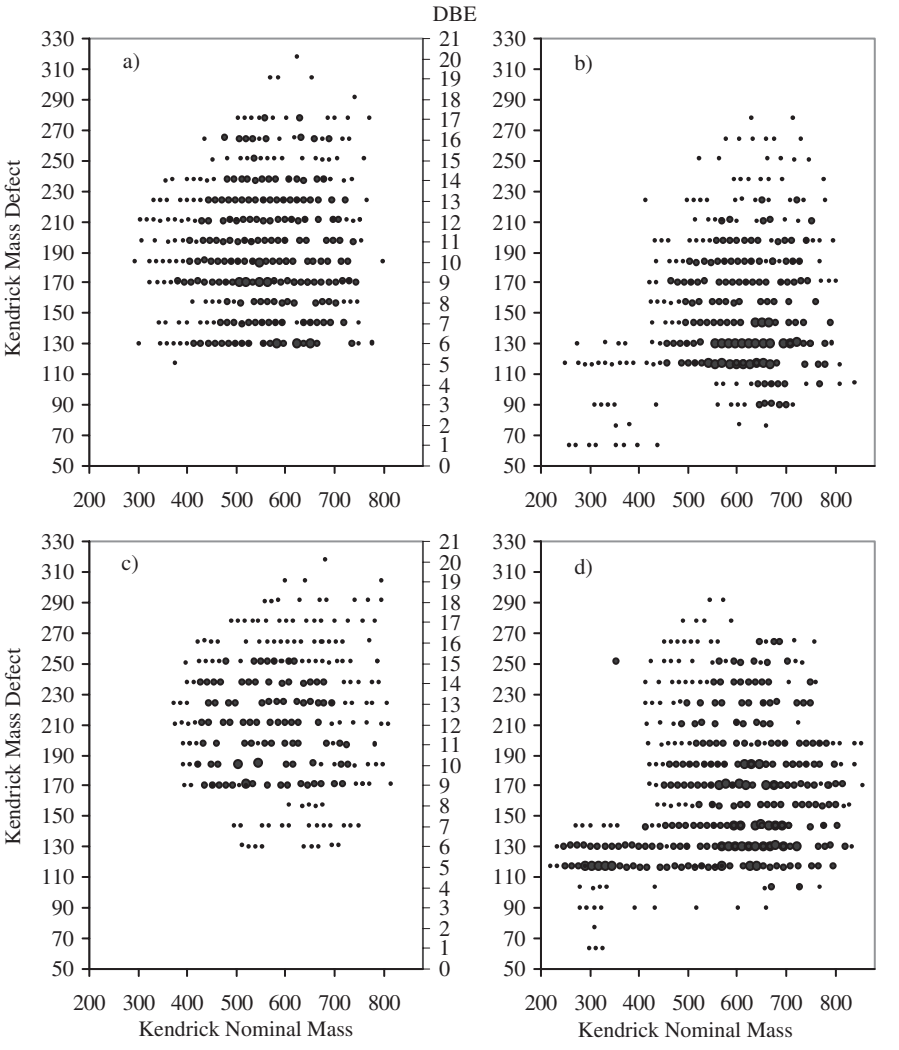

Figure 4-4 Kendrick mass defects versus Kendrick nominal mass of vacuum residues: (a) first ligand exchange chromatography (LEC) fraction, (b) second LEC fraction before HDS, (c) first LEC fraction, and (d) second LEC fraction after HDS. The double bond equivalent (DBE) scale given is valid for both panels. (Reprinted from Müller et al., 2005, with permission from the American Chemical Society.)

as in 7-methylbenzothiophene, to the 2,3-dione with the sulfone as a minor metabolite (Kropp et al., 1994a, b). In some instances, products of other types, namely carboxylic acids, ring cleavage products, and dimers, were noticed.

Dutta and Harayama (2000) noted that complete depletion of alkanes and naphthalene (N) derivatives and less extensive elimination of other components by biodegradation in the order *n*-alkane > naphthalenes > branched alkanes > fluorenes > phenanthrenes (P) > dibenzothiophenes was the rule. The rate of polycyclic aromatic compound degradation in the environment decreases with increasing ring size and, within a homologous series, decreases with increasing alkylation (Neff, 1979; Douglas et al., 1994; Elmendorf et al., 1994). A study of microbial degradation of organic sulfur compounds in Prudhoe Bay crude oil revealed that the order of suscepti-

bility of the sulfur heterocycles in homologous series was (1) C2-BTs > C3-BTs, (2) DBT > C1-DBTs > C2-DBTs > C3-DBTs (Fedorak and Westlake, 1983, 1984).

Photochemical processes are also effective in the environmental transformation of PASHs. Benzothiophene in aqueous solution is photochemically oxidized to 2-sulfobenzoic acid (Andersson and Bobinger, 1992) and DBT yields 2-sulfobenzoic acid, benzothiophene-2,3-dicarboxylic acid, two isomeric benzothiophenemonocarboxylic acids and an isomer of thiophenetricarboxylic acid (Traulsen et al., 1999). Moreover, monomethylbenzothiophenes are photochemically oxidized through two pathways. One principal reaction pathway involves oxidation of the methyl group(s) to the carboxylic acid(s) via aldehydes, and the other pathway leads, via oxidation of the thiophene ring, to the quinone and then, after ring opening, to 2-sulfobenzoic acids as the ultimate products for all compounds (Andersson and Bobinger, 1996; Bobinger and Andersson, 1998). Opening of the benzo ring is also seen, in which case thiophenealdehydes or -ketones are formed. In a photochemical experiment simulating an oil spill in water, Andersson (1993) found that the sulfur heterocycles are more stable toward photodegradation than the corresponding hydrocarbons.

Even after burning and subsequent weathering (up to 12 days) a petrol sample can be readily identified as such through a sulfur-selective gas chromatogram that also after such severely destructive treatment preserved the monomethylbenzothiophenes. This identification of petrol could not be made based on the universal flame ionization detector (Dynes and Burns, 1987). Diphenyl disulfides and homologues have been found by GC/MS to be indicators of (weathered) gasoline. Since they were not present in creosote or other petroleum derivatives, they can, if present, be used as markers for gasoline (Coulombe, 1995).

Recognition and subsequent correlation of biodegraded and/or water-washed oils with their unaltered counterparts have been long-recognized problems in petroleum geochemistry and oil spill source identification. Experimental data indicate that sulfur-containing aromatics such as thiophene and 2-ethylthiophene are approximately twice as soluble in water as aromatic hydrocarbons of similar molecular weight (Price, 1976). The data also show that dibenzothiophenes have a higher aqueous solubility compared to that of aromatic hydrocarbons (Palmer, 1984) as is evident from their lower k_{ow} (octanol-water partition coefficient) (Andersson and Schräder, 1999). However, there are indications that PASHs are resistant to water washing (Manowitz and Jeon, 1992) and therefore accumulate in water-washed oils. Although the mentioned results may seem contradictory, many reports in the literature indicate the usefulness of PASHs and their ratios to polycyclic aromatic hydrocarbons (PAHs) in oil spill source identification (see, for example, Wang and Fingas, 1995; Douglas et al., 1996) as discussed ahead.

All those natural effects on a spill mean that before any chemical data are used for a source correlation, a conscientious evaluation of the possibility of changes of the composition of a petroleum sample must be carried out, no matter what the molecular markers used are. If a spill is chemically changed in a selective fashion, for example, in that certain compound classes or isomers are lost at a higher rate than others, it may be that an identification relying heavily on those marker compounds may incorrectly assign the source of the spill. To avoid such misidentifications, several chemical compounds, both within a general group of compounds, such as PASHs, as well as different compound classes should always be used for a source correlation since it is much less likely that they are all affected in a similar way.

4.5 Petroleum PASH Analysis Techniques

Analytical methods for the efficient and unambiguous characterization of petroleum form the basis for any evaluation of the consequences associated with an oil spill. The analytical strategy must be formulated so that it leads to information that can answer questions

regarding (1) the identity of the pollutant source, (2) distinguishing spilled oil from background hydrocarbons, (3) evaluating quantifiably the extent of impacted ecosystems, (4) establishing the responsibilities associated with the environmental damage and cleanup operations, and (5) enforcing pollution control laws.

General sampling procedures, including the collection, storage, and transportation of the samples to the laboratory and detailed procedures for recovering oil from different environmental samples, are reviewed in detail by Butt et al. (1986a), and Barman et al. (2000) as well as Wang and Fingas (1997) have reviewed chromatographic techniques for petroleum analysis.

To identify the PASHs in environmental samples, the extract (or oil recovery) must not only be isolated from its particular matrix but requires separation into specific fractions. Numerous procedures for the fractionation of petroleum components have been reported in the literature. Procedures that lead to a fraction that contains the polycyclic aromatic compounds include open column chromatography on various stationary phases (silica, alumina, and silica-alumina combinations), thin layer chromatography, as well as normal-phase high performance liquid chromatography (Wang et al., 1994; Reddy and Quinn, 1999; Jaouen-Madoulet et al., 2000). Solid-phase extraction (SPE) has been used as an alternative technique, with high selectivity, a faster elution profile, and minimization of solvent consumption being among the advantages (Bennett and Larter, 2000; Sauvain et al., 2001; Alzaga et al., 2004).

A question often not addressed in forensic work is that of coelution in gas chromatography. A peak found to appear at the expected retention time is frequently assumed to be the pure compound and its peak height or area is evaluated. As is shown in Section 4.5.1.4, this is not necessarily true. If isomeric compounds are involved, neither sulfur-selective nor mass-selective detection can detect even a massive coelution. For instance, DBT coelutes with naphtho[1,2-*b*]thiophene on nonpolar stationary phases (Schmid and Andersson, 1997). In the standard reference material SRM 1580 Shale Oil the concentration of the naphthothiophene is 46% of that of DBT. If two coeluting compounds have different properties in the environment after a spill but all changes are thought to arise from only one of them, wrong conclusions might be drawn.

4.5.1 Selective Detection in Gas Chromatography

Generally speaking, the fraction containing the polycyclic aromatic compounds can be used for the analysis of both the PAHs and the PASHs if a chromatographic system of a high resolving power and a selective detection system is used. The selective detection is needed since most samples will be too complex for a direct analysis with, e.g., GC-flame ionization detection. Selective detectors include those that show a high selectivity for the element sulfur and mass selective detectors (GC-MS). Unlike in petroleum research, such sulfur-selective detectors have not found much prominence in forensic studies where the mass selective detector is preferentially used, probably because the PASHs can be determined together with many other classes of compounds in one chromatographic run. However, this may not always be advisable (see Section 4.5.1.4). In some cases, a class separation of PAHs and PASHs may present advantages as discussed in Section 4.5.2. It should be stressed that an analytical procedure with as low a detection limit as possible should be used; otherwise false conclusions may easily be drawn regarding the identity of the sample (Douglas et al., 2004).

4.5.1.1 Flame Photometric Detection (FPD)

The aromatic sulfur compounds and their alkylated isomers in crude oil are numerous so the identification and quantification of individual isomers require selective and sensitive methods of detection (Andersson, 2001). Much early research conducted on the identi-

fication and application of PASHs was performed using GC-FPD. Although showing a nonlinear response in dependence of the sulfur concentration, the FPD was useful for the identification of heavily biodegraded spill samples (Butt et al., 1986b). A biodegraded crude oil was unrecognizable from its flame ionization detector chromatogram but still readily recognizable from its FPD chromatogram of the sulfur heterocycles resistant to biodegradation. Arpino et al. (1987) determined PASHs in the aromatic fractions, from the cleanup of petroleum and its derived industrial products, by GC-FPD and GC-single ion monitoring (SIM)-MS. GC with dual FID/FPD was used for the detection of PAHs and PASHs in crude oils and sediment samples from North Sea reservoirs (Schou and Myhr, 1988). The same technique together with GC-SIM-MS shows the variation in distribution of PASHs in oils and condensates of different maturity levels (Chakhmakhchev and Suzuki, 1995).

4.5.1.2 Atomic Emission Detection (AED)

The American Society for Testing and Materials (ASTM) method 5623–94 (sulfur compounds in light petroleum liquids by GC and sulfur selective detection) recommends the use of either an AED or a sulfur chemiluminescence detector (SCD) but not the FPD (Quimby et al., 1998). Andersson and Schmid (1995) determined 22 PASHs in a shale oil with GC-AED. The photochemical degradation of monomethylbenzo[*b*]thiophenes and DBTs was studied by GC-AED and GC-MS to elucidate the possible fate of crude oil components after an oil spill (Bobinger and Andersson, 1998; Bobinger et al., 1999). Mössner and Wise (1999) used GC-SIM-MS and GC-AED to determine PASHs in fossil fuel-related samples. Moreover, Schmid and Andersson (1997) examined PASHs quantitatively in three standard reference materials from the National Institute of Standards and Technology (NIST), namely, SRM 1597 coal tar, 1582 crude oil, and 1580 shale oil using GC-AED in the carbon- and sulfur-selective modes. The same technique was used to determine the sulfur compounds in gasoline range petroleum products (Stumpf et al., 1998). Recently, Hegazi et al. (2003, 2004a, b) applied GC-AED to investigate PASHs in Egyptian crude oils and environmentally weathered tar balls.

4.5.1.3 Sulfur Chemiluminescence Detection (SCD)

Although possessing excellent properties for sulfur-selective detection, the SCD has seemingly not received quite the popularity of the other detectors. An example for its use in the determination of various types of sulfur compounds in a crude is given by Andari et al. (1996).

4.5.1.4 Mass-Selective Detection (MSD)

Low-resolution, mass-selective detectors are routinely used with GC for PASH determination but may exhibit an interference that, although known, is not often taken into account. Since DBT and C_4-naphthalenes share the mass 184, they appear together in the chromatogram if this ion is selected, as is illustrated in Figure 4-5. Furthermore, the peak at the correct retention time for DBT, 16.86 min in this case, is not pure but a result of coelution with other substances as is shown by the full spectrum taken at this retention time (Figure 4-6). Any quantification of DBT using single-ion monitoring MS is obviously likely to yield wrong results unless it is shown that the GC peak at the expected retention time is generated only by DBT. Likewise, C_{x+4}-naphthalenes may interfere with the determination of C_x-DBTs, having identical M^+ values. The same is true for the benzothiophenes and benzenes with four more carbon atoms, and benzonaphthothiophenes and phenanthrenes with four more alkyl carbon atoms.

4.5.2 Class Separation of PAH and PASH

If the level of sulfur is low, the selectivity of a sulfur-selective detector such as the AED

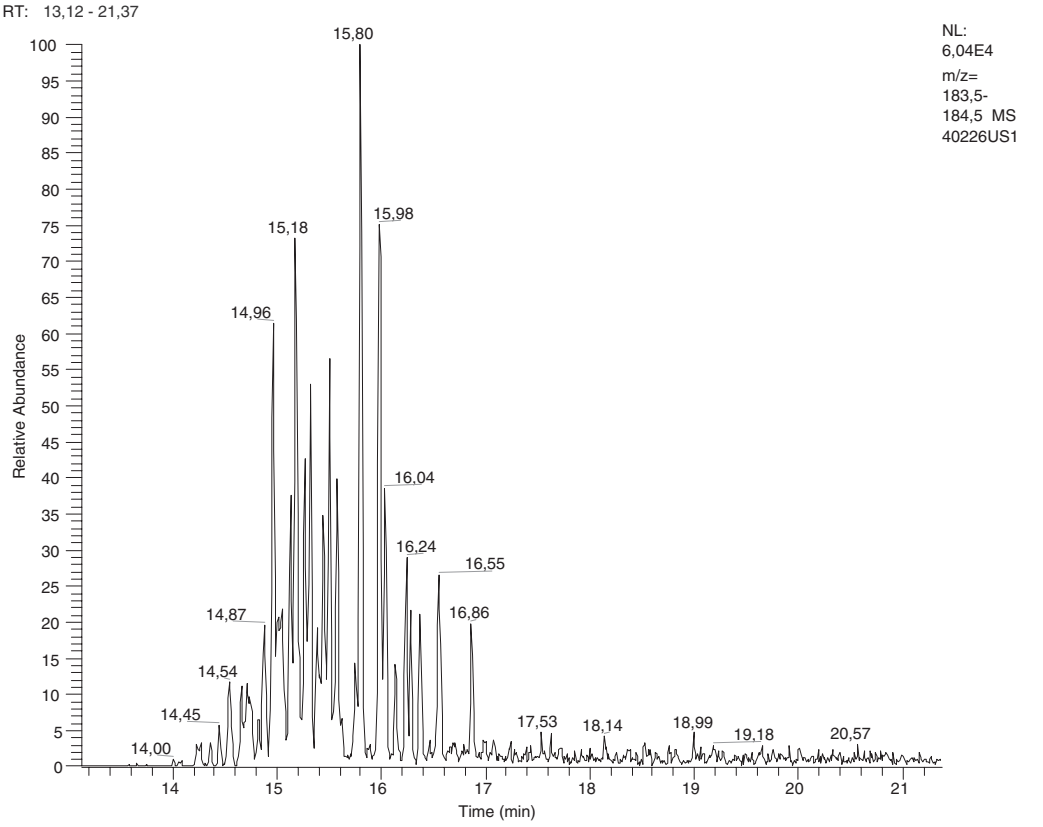

Figure 4-5 Gas chromatogram of a diesel (350 ppm sulfur) monitored at *m/z* 184, which is the mass for DBT. Coinjection with DBT reveals this compound at a retention time of 16.86 minutes.

(selectivity of sulfur vs. carbon: ca 37,000) may not be high enough to generate a sulfur-selective gas chromatogram without interference from PAHs. Also, as discussed above, the (low-resolution) mass selective detector may include a PAH interference with the PASH determination. In such situations it may be favorable to separate the interfering PAHs from the PASHs, which can then be analyzed using any detector. This procedure can also have the advantage of concentrating the analytes and thus make the analysis more reliable. The only workable method for such a separation is liquid chromatography on palladium(II)-containing stationary phases. A review is available (Andersson, 2001) and more recent work has confirmed the usefulness of the method (Schade et al., 2002; Müller et al., 2005).

4.5.3 Comprehensive Two-Dimensional Gas Chromatography

Fairly recently, comprehensive two-dimensional gas chromatography (GC × GC) with FID has been applied to oil spill source identification (Gaines et al., 1999). PASHs in crude oils have also been separated (Frysinger and Gaines, 2001). The very high chromatographic resolution possible with this technique makes it of interest in forensic studies. Since selective detectors like the AED and MS (van Stee et al., 2003) as well as the SCD (Blomberg et al., 2004) can be used with GC × GC, sulfur compounds may be targets for future applications.

4.5.4 Quantification of PASH

In much forensic work, the absolute quantities of sulfur compounds are not of as great an

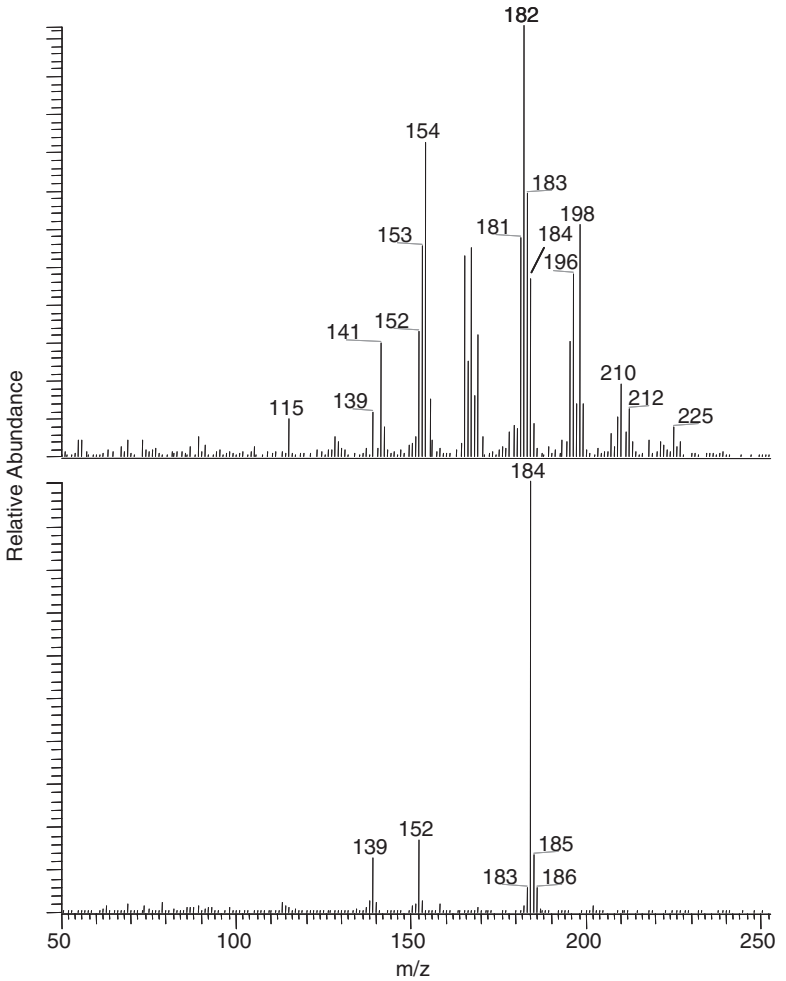

Figure 4-6 Mass spectrum of the peak at 16.86 minutes in Figure 4-5 (top) and a mass spectrum of pure DBT (bottom).

interest as ratios (usually peak heights or areas put in relation to each other) of various compounds. Such ratios can help in establishing the origin of an oil spill through correlation with suspected sources. They can also give information with respect to transformations of the oil in the environment since characteristic changes are known to occur as a result of biotransformation, for example. Ratios are experimentally convenient since a concentration determination presupposes knowledge of the response factor for each compound regardless of detector type (Schmid and Andersson, 1997). Only some detectors show the same molar response for all compounds; if not, a quantification may entail a lot of work and demand the use of pure reference compounds.

4.6 Petroleum PASH Markers in Environmental Forensic Investigations

Compounds used as markers for oil pollution should preferably meet several criteria, some of which demand that markers (1) be present in all crude oils, irrespective of source, (2) be present in large enough amounts that they can be analyzed also in cases of low-level pollution, (3) show a defined degree of stability in the environment, and (4) have no other significant inputs into the environment besides

petroleum. Polycyclic aromatic compounds (PACs) are relatively stable and diagnostic constituents of petroleum. Several studies have been carried out using the distribution of the alkylated polycyclic aromatic homologues as environmental fate indicators and source specific markers of oil spills. Among PACs, PASHs fulfill the mentioned criteria to a high degree and therefore their use as organic markers of oil pollution has been suggested. That they have been found in environmental samples many years after an oil spill testifies to their relatively high stability (Friocourt et al., 1982; Reddy et al., 2002).

It is important to keep in mind that PASHs are always used together with several other markers in order to establish forensic evidence. Their occurrence is thus one piece in a chain of chemical information and, as is true for all other markers, they must not be viewed alone. Different markers will provide different pieces of evidence that can be characteristic of the source or the environmental processes that the spill has been subjected to. Since the pattern of the sulfur compounds differs appreciably between different sources, it has a high informational content with respect to source identification. Nonaromatic sulfur compounds have not been used for such purposes, so in this section we will discuss only investigations that show how PASHs can be used to provide important information on an oil spill situation.

4.6.1 PASHs as Source Markers

Ratios of compounds that degrade at the same rates retain the initial oil signature until they can no longer be detected (Boehm et al., 1983). These ratios are called *source ratios* and can be contrasted to ratios that change substantially with weathering and biodegradation, termed *weathering ratios* (Douglas et al., 1996). Alkylated three-ring PAC compounds are quite useful for source identification because of their abundance in petroleum and many of its refined products, their relative concentrations vary among different oils making them source-specific, and they can be quantitatively measured using routine analytical methods (Douglas and Uhler, 1993; Douglas et al., 1994, 1996; Page et al., 1995).

It has been established that the ratio of the three GC peaks from the four methyldibenzothiophenes (2- and 3-MDBT coelute on nonpolar stationary phases) varies strongly with the source of a petroleum (Wang and Fingas, 1995). The ratio of 1-MDBT to 4-MDBT was plotted vs. the ratio of 2/3-MDBT to 4-MDBT for 25 oils and, as shown in Figure 4-7, a satisfactorily wide scattering was obtained, except for oils from the same fields (*ibid.*). This plot can therefore be used to support the suspected origin of a crude oil. An advantage of these compounds is that they have similar volatilities so that evaporative weathering affects them in a consistent manner.

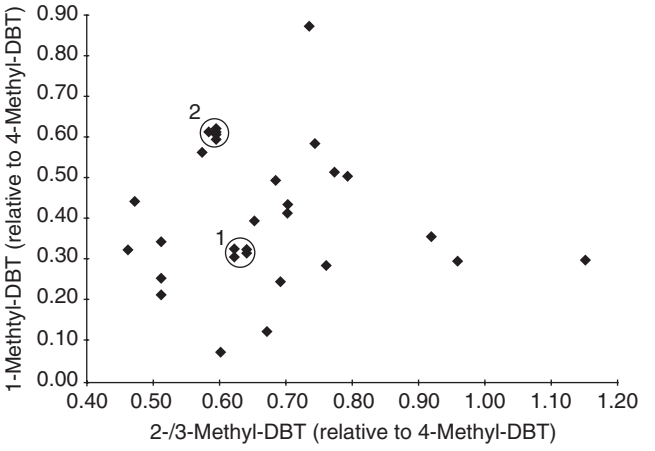

Figure 4-7 Plot of the relative ratios of 2-/3-methyl-DBT to 4-methyl-DBT versus the relative ratios of 1-methyl-DBT to 4-methyl-DBT for 25 different oils. The circles 1 and 2 indicate related samples from origins of North Slope and Terra Nova, respectively. (Reprinted from Wang and Fingas, 1995, with permission from the American Chemical Society.)

A study of composition changes of artificially weathered Alberta Sweet Mixed Blend (ASMB) crude oil indeed indicated that the isomeric distributions within C_1-DBTs, C_1-, C_2-, and C_3-naphthalenes, C_1-phenanthrenes, C_1-fluorenes exhibited great consistency in their relative ratios for ASMB weathered from 0 to 45% (Wang et al., 1998a). Excellent consistency of the relative distribution within the MDBT series was demonstrated not only for artificially weathered oils but also for many short-term field weathering and burned oil samples. It therefore can be used as a method for source identification and differentiation of crude and weathered oils (Wang and Fingas, 1995). The method has distinct advantages: (1) C_1-DBT isomers are found in all crude oils at high enough concentrations and their distribution fingerprints vary significantly; (2) C_1-DBTs are chromatographically well separated with little interference from other compounds (but see the warning in Section 4.5.1.4), so their ratios can be accurately determined; and (3) the relative distributions of C_1-DBTs are subject to little interference from not too severe evaporative weathering.

Wang and Fingas (1997) demonstrated that compared to most crude oils, the Bunker-type fuels have a relative ratio of 2-/3- to 4-MDBT around 1.0, which is unusually high. The relative concentrations of 4-, 2-/3-, and 1-MDBT were determined to be about 1.0 : 0.92 : 0.60 and 1.0 : 0.92 : 0.54 for tarballs from the coasts of Northern California and Vancouver Island of British Columbia, respectively (Wang et al., 1998b). The high 2-/3- to 4-MDBT ratios of these samples together with other source-specific markers indicate that Bunker C was the original source (Wang et al., 1998b).

The relative distribution of alkylated DBTs was used in a study of the PASHs in Egyptian crude oils that indicated a dependence of the PASH distribution patterns on the origin (Hegazi et al., 2003, 2004a). Some oils exhibit a methylbenzothiophene (MBT) pattern, where the ratio 3-MBT/2-MBT is less than unity, whereas it is >1 in other oils. Furthermore, Gulf of Suez oils show a higher relative abundance of 4,6-dimethylbenzothiophene than Western Desert oils. The presence of a V pattern (4-methyl >2-+3-methyl < 1-methyl) in the MDBTs is clear in some oils, while others have a stair-step pattern (4-methyl > 2-+3-methyl > 1-methyl). Moreover, some oils are characterized by their abundance of BTs and a fairly equal distribution of substituted DBTs. Others exhibit low concentrations of BTs and decreasing amounts of C_2- and C_3-DBTs relative to MDBTs. These differences in PASH pattern among crude oils provide a means of resolving multiple oil spill sources.

Groundwater is an important source of potable water. Refined petroleum products and combustion-related products, such as coal tar, are released into soil during spills at industrial facilities, chemical accidents, leakage from hazardous waste landfills, leaking storage and underground fuel tanks, etc. The contamination from these sources will eventually seep into the groundwater, resulting in PAC incorporation into the water. The DBTs are valuable for source correlation because their concentrations reflect the sulfur content of the source, which varies widely between different oils and coal tar (Havenga and Rohwer, 2002). We found that the C1-DBT/C1-P source ratio for groundwater samples is useful for source determination, but the isomers must be present in concentrations > 0.07 ng/cm^3. A double ratio plot of C1-DBT/C1-P source ratio versus C2-N/C1-P weathering ratio could be used as a means of resolving multiple oil spill sources as well as differences in the extent of weathering and degradation from a single source (*ibid.*). This plot could differentiate coal tar- from mineral oil-contaminated groundwater from South Africa.

The higher alkylated an aromatic compound is, the more stable to weathering processes, including biodegradation, it will be. Polycyclic aromatic ratios C_2-DBTs/C_2–phenanthrenes (C2-DBT/C2-P) and C3-DBT/C3-P, which vary among oils having different sulfur contents, have been shown to remain relatively constant as spilled oil weathers and can therefore be used for identification purposes when the residues are heavily degraded (Douglas et al., 1996; Pavlova and Dimov, 2001; Wang

and Fingas, 2003). This constancy of C2-DBT/C2-P and C3-DBT/C3-P despite degradation was confirmed in controlled soil biodegradation experiments (Douglas et al., 1996). At up to 70% total petroleum hydrocarbon and 98% total PAC depletion, the ratios remained stable. Therefore, these ratios can be used for moderately degraded oils (30–70%) in both marine and terrestrial environments. DBTs are somewhat more resistant to biodegradation than phenanthrenes so that at higher depletions a trend to slightly higher ratios could be observed. Dutta and Harayama (2000) used a natural seawater for the biodegradation of an Arabian light crude and found that C2-DBT was degraded to 95% while the C_2-phenanthrenes were lost to 100%. Similarly for the C_3-substituted compounds: DBTs were lost to 89%, phenanthrenes to 96%. The photooxidation produced comparative results, e.g., for the C3-derivatives: DBTs were diminished to 81.6%, the phenanthrenes to 100%.

Dibenzothiophenes have played a key role in the environmental assessments conducted following the *Exxon Valdez* oil spill. Their distribution has been used to distinguish *Exxon Valdez* oil and its weathered residues from background petrogenic, pyrogenic, and biogenic compounds in benthic sediments (Bence et al., 1996; see also Chapter 15 herein). The C2-DBT/C2-P and C3-DBT/C3-P ratios were of particular usefulness in conjunction with several other data sources.

Overton et al. (1981) also reported on the utility of C3-DBT/C3-P ratios to identify the source of petroleum residues, in this case Arabian light and Louisiana sweet crudes, after a fire and oil spill. The ratios were also utilized in assessments of the 1991 M/C *Haven* oil spill in Italy to distinguish high-sulfur heavy Iranian cargo crude from a low-sulfur, prespill background (Martinelli et al., 1995).

Stout et al. (2001) utilized a strategy, incorporating statistical analysis of the quantitative chemical data, in order to identify 19 chemical indices (out of 45 evaluated) based on PACs, including sulfur heterocycles, and biomarkers that were both unaffected by weathering and could be precisely measured. The strategy was used in a case study to correlate a spilled heavy fuel oil to 66 candidate sources. Among the diagnostic indices studied were 4MDBT/1MDBT, DBT/P, C2-DBT/C2-P, and C3-DBT/C3-P.

4.6.2 PASHs as Weathering Markers

Such source correlations as described above can only be employed if it is established that the parameters used have not been distorted by alterations in the environment. However, as indicated above, it is well known that aromatic ratios are affected by biodegradation. Not only are, for instance, phenanthrenes degraded faster than DBTs, but, as biodegradation proceeds, the three GC peaks for the MDBTs undergo pronounced changes in their relative ratios (Wang et al., 1998a). 2-/3-MDBTs biodegrade at the fastest rate as shown by the strong decrease of their relative ratio to 4-MDBT, while 1-MDBT is slightly more resistant to biodegradation than 4-MDBT, indicated by an increase of the ratio 1-MDBT : 4-MDBT. The same results were obtained for those oil components in clams after the *Aegean Sea* oil spill in Spain (Albaigés et al., 2000) as is evident in Figure 4-8. Hence, this method can be used to indicate the occurrence of biotic degradation of oils, but then, of course, the pattern in the source oil must be known.

A commonly used weathering ratio is defined by C3-DBT/C3-chrysenes (C3-DBT/C3-C) (Douglas et al., 1994). The four-ring chrysenes are less affected by weathering than the three-ring DBTs, and the three alkyl substituents make biodegradation less likely. Again, the corresponding ratios in the source oil must be known so that the change in these indexes can be established.

Plots of a source ratio C3-DBT/C3-P versus a weathering ratio C3-DBT/C3-C provide a means of resolving multiple sources as well as differences in the extent of degradation for samples from a single source (Douglas et al., 1996). These authors used such a plot to distinguish three oil spills; the *Exxon Valdez* (Alaskan North Slope crude oil), M/C *Haven* (heavy Iranian crude oil), and a North Sea

production leak (North Sea crude oil). This approach was useful to confirm the finding (Page et al., 1995) of a regional petroleum signature in the deep subtidal sediments that was derived from terrestrial sources of natural hydrocarbons along the northern Gulf of Alaska. The ratios were useful in freshwater deposits also where Athabasca Oil Sands contributed to the PACs (Akre et al., 2004).

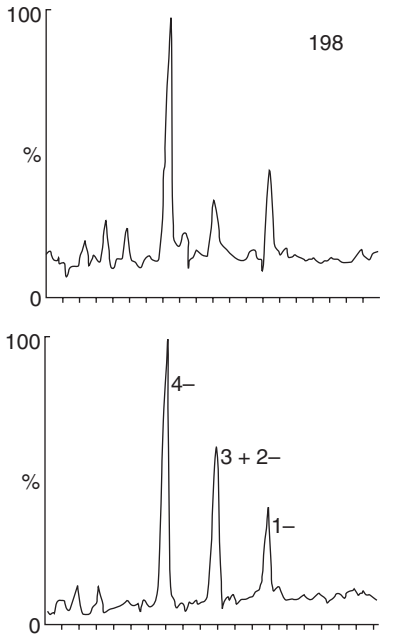

Figure 4-8 Mass fragmentograms of methyl-DBTs (*m/z* 198) from clams collected in April 1993 (lower trace) and December 1995 (upper trace). 2- and 3-MDBT are biodegraded appreciably faster than 1- and 4-MDBT. (Reprinted from Albaigés et al., 2000, with permission from WIT Press.)

Double-ratio plots of C2/C3-DBTs versus C2/C3-phenanthrenes classified 14 oil residues from Prince William Sound, Alaska, eight years after the *Exxon Valdez* spill, according to their weathering degrees (Figure 4-9) (Michel and Hayes, 1999). The oil residues ranged from moderately to extremely weathered. It was found in the same study that the benzonaphthothiophenes had increased in relative abundance and were the dominant PACs in advanced and extreme weathering stages.

The coasts of the Mediterranean city of Alexandria, Egypt, are a major tourist attraction and are vital for fisheries and marine activities. However, they are under constant threat of petroleum pollution in the form of heavy tar loads from several different sources. Furthermore, petroleum contamination from sources such as the oil fields and the Suez-Mediterranean pipeline terminal (SUMED) 27 km west of the city are of concern. A study representing a forensic chemical analysis was carried out to define the liability for the coastal bitumens polluting the beaches of Alexandria (Hegazi et al., 2004b). Tarballs from several locations along the coast were analyzed for their acyclic and polycyclic hydrocarbons as well as sulfur heterocycles using high-resolution GC-AED and GC-MS techniques.

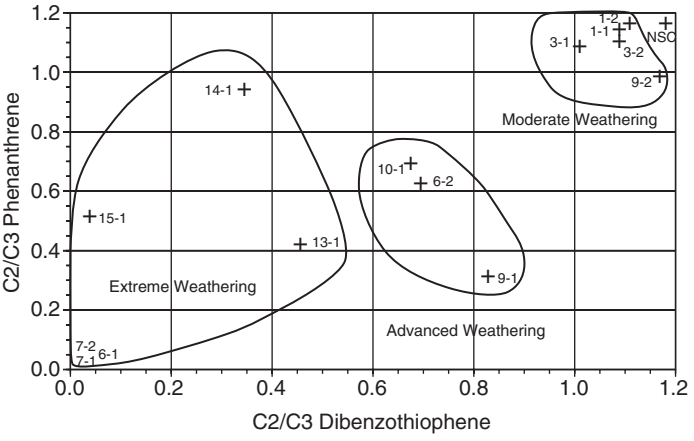

Figure 4-9 Double-ratio plot of C2/C3 dibenzothiophenes versus C2/C3 phenanthrenes from oil residues from Prince William Sound, Alaska, eight years after the *Exxon Valdez* spill. NSC is the source oil from the *Exxon Valdez*. (Reprinted from Michel and Hayes, 1999, with permission from Elsevier Science.)

Ratios involving DBT/P, C2-DBT/C2-P, and C3-DBT/C3-P were applied to differentiate between the tarballs according to their sources. The ratios varied from one sample to another, revealing a considerable amount of compositional heterogeneity and suggesting multiple sources of the tarballs.

4.7 Conclusions

The source identification of petroleum spills is a very important subject for both environmentalists and governments and consequently much research has been carried out to facilitate the fingerprinting of crude oil spills. Among the molecular markers finding widespread use, the PASHs, present in all crudes in a variety of derivatives, have been identified as very useful compounds for both source and weathering studies. Despite the fact that they are present in easily measured concentrations using routinely available techniques, some analytical quality considerations must not be overlooked, as stressed in this chapter. With the desulfurization now required in many countries, transportation fuels released into the environment may be too low in sulfur to let the PASHs be an easily measured marker class, but this does not affect crude oils.

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