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RESEARCH REPORT No 30

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BY
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Academic Dissertation
for the Degree of
Doctor of Philosophy

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PREFACE

The present investigations were carried out in the Department of Chemistry, University of Jyväskylä, during the years 1979-1981 and 1985-1988.

I wish to express my gratitude to Professor Jaakko Paasivirta for his valuable advice, encouragement and support during these years.

I am grateful to the whole staff of the department of Chemistry at the University of Jyväskylä.

Especially I want to thank Professor Rainer Herzschuh from DDR for help in planning the mass spectrometric work.

The present study was partly financed by the Ministry of Interior, Department of Environmental Protection, Finland. Financial support was received from the Ellen and Artturi Nyyssönen Foundation.

To my husband, Jorma, and my children, Sirpa and Sanna, I owe the largest debt of gratitude for their understanding and unfailing support. Warm thanks must go also to my parents.

In Jyväskylä, March 1989

ABSTRACT

Polycyclic aromatic hydrocarbons and alkylated dibenzothiophenes were analysed from natural samples and different oils using GC/MS and HPLC. These compounds were identified from different samples by GC/MS. Quantitative determinations were made by GC/MS and HPLC.

Alkylated dibenzothiophenes were found to be quite persistent oil residues in nature.

A method to separate the alkylated dibenzothiophenes from the non-polar aromatic compound fraction was searched. TLC, ligand exchange chromatography and oxidation of dibenzothiophenes to sulfones were tried.

The oxidation of the dibenzothiophenes to the corresponding sulfones was found to be quantitative and the separation of the sulfones from other compounds was achieved by column chromatography.

Methyldibenzothiophenes were synthesized from methylbiphenyls and sulfur by Friedel Crafts reaction using aluminium chloride as catalyte. The structure of the synthesized compounds was verified by GC/MS and NMR.

LIST OF ORIGINAL PAPERS

This thesis are based on the following original papers which are referred to in the text by their roman numerals.

I. Jaakko Paasivirta, Rainer Herzshuh, Mirja Lahtiperä, Jukka Pellinen and Seija Sinkkonen, Oil Residues in Baltic Sediment, Mussel and Fish. I. Development of the Analysis methods, Chemosphere 10(1981), pp. 919-928
https://doi.org/10.1016/0045-6535(81)90091-6

II. Jaakko Paasivirta, Helena Kääriäinen, Mirja Lahtiperä, Jukka Pellinen and Seija Sinkkonen, Oil Residues in Baltic Sediment, Mussel and Fish. II. Study of the Finnish Archipelago 1979-1981. Chemosphere 11(1982), pp. 811-821
https://doi.org/10.1016/0045-6535(82)90110-2

III. Seija Sinkkonen, Appereance and Structure Analysis of Aromatic Oil Residues in Baltic Mussel and Fish, Toxicological and Environmental Chemistry 5(1982), pp. 217-225
https://doi.org/10.1080/02772248209356980

IV. Seija Sinkkonen, The Fate of Some Crude Oil Residues in Sediments, Chemosphere 1989, in print
https://www.scopus.com

V. Seija Sinkkonen, Liquid Chromatographic Determination of Planar Aromatic Sulfur Compounds in Crude Oil, Journal of Chromatography 1989, in print
https://doi.org/10.1016/S0021-9673(01)89698-9

CONTENTS

INTRODUCTION

Composition of the crude oil

Crude oil consists of many thousands of different compounds. Crudes differ greatly depending on the area of their origin. However, they consist mainly of hydrocarbons and their derivatives, which are more sulfur and nitrogen, less oxygen compounds. The crude oils contain also detectable amounts of nickel, vanadium, iron and other metals/1-10/.

All crudes can be divided to three main fractions: alkanes, cycloalkanes and aromatic compounds.

The most common aromatic hydrocarbons in crude oils are alkyl-substituted benzenes, naphthalenes and phenanthrenes /1,11/. Other polycyclic aromatic hydrocarbons (PAH), alkylated and nonalkylated, found are anthracene, pyrene, fluoranthene, benzo(a)anthracene, chrysene, triphenylene, benzo(g,h,i)fluoranthene, benzo(b)-fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(e)pyrene, perylene and benzo(g,h,i)perylene /12/. The amounts of alkylated derivatives in crude oils are significantly higher than those of the parent aromatic hydrocarbons /1,12,13/.

About half of the sulfur contents (1-10 %) of crude oils is organically bound /8/ and in great part in aromatic structures. These can be called polycyclic aromatic sulfur heterocycles (PASH). Most important parent structure is dibenzothiophene (DBT) and about 80 % of the aromatic sulfur compounds in crude oils may consist of DBT and its alkyl derivatives /14/.

Persistency of the crude oil components in nature

The crude oil components released to environmental surfaces, water and soil are susceptible to evaporation, oxidation and microbial degradation /1,13,15-38/. It has been estimated that 50 % of petroleum spilled to sea evaporates. The greatest changes in the oil composition occur during first 24-48 hours after the spill /16/.

Solubility of the crude oil into water is about 6-20 mg/l. Most of the dissolved components are alkylated, one to three ring aromatic hydrocarbons /1,13,20,39/.
An important weathering process is photo-oxidation in the presence of sunlight, atmospheric oxygen and/or other oxidants. The oxidation products of crude oil components at surface are dissolved in the seawater column and their toxicity is probably higher than that of the parent compounds. Such oxygenated compounds while dispersed in the aquatic environment become bioavailable to marine organisms. /40-42/.

Laboratory photo-oxidation of crude oils under simulated environmental conditions yielded alcohols, phenols, carboxylic acids, diacids, ketones, aldehydes, esters, peroxides and sulfoxides /42-44/.

Dibenzothiophene and its alkyl homologs and their sulfoxides were studied from environmental and laboratory photo-oxidation samples of crude oil. Nonoxidized congeners were eluted from silica column with benzene-hexane (2:3), sulfoxides with methanol /42,43/. Kinetic measurements on the surface photo-oxidation process showed that (first order) rate of the reaction decreases with increasing number of methyl groups at the DBT molecule. Fluorescence by exitation at 200-450 nm was applied to follow DBT sulfoxides and their methyl homologs in spilled crude oil. The three aromatic ring zone (330-390 nm) had maximum at 335 nm which was progressively shifted towards 375 nm during 70 days at surface indicating the formation of DBT sulfoxides rather than the dissolution of three- and tetra-ring PAH in the water column /40,45/

There are only limited data on the degradation of crude oil sulfur heterocycles /46,47/. Microbial cultures degradated 40 % of the benzothiophenes (BTs) and 50 % of the DBTs from South Louisiana crude oil according to mass spectrometric follow-up /47/. Losses of methyldibenzothiophenes (C1DBT) and dimethyldibenzothiophenes (C2DBT) were indicated in residual oil from the Amoco Cadiz spill and attributed to microbial degradation /48/.

20 Different strains of micro-organisms which utilize DBT as their sole source of carbon, energy and sulfur have been isolated /47,48/.

Capillary gas chromatography with FPD was used to show that a variety of alkyl BTs and DBTs in Prudhoe Bay crude oil was degradated by mixed microbial populations from marine environments /47/.
Alkyl substituents retard microbial degradation of DBTs. For example C2DBTs were degraded before C3DBTs. Order of the rates of degradation DBT>C1DBT>C2DBT has been experimentally observed. The only C3DBT which was monitored, degradated only after all C2DBTs were lost/46,47,50-52/.

Liver microsomes from Aroclor-induced rats transform DBT to DBT-5-oxide and subsequently to DBT-5,5-dioxide. As these compounds are more soluble in water than the parent substance, this sulfoxidation represents a mechanism of elimination. The same metabolic S-oxidation is a general pathway of drugs such as phenothiazines /40,53/.

Bioaccumulation of dibenzothiophenes

DBT and its alkyl derivatives have been found to accumulate in eels, clams, short-necked clams, oysters and mussels /50,55,57,58-65/.

Methyl derivatives of DBT in crude oil suspension are transferred from water to fish /54/. This was analytically confirmed with sulfur specific GC/FPD (394 nm flame emission) and with GC/MS, where the ratio 95:4 for the intensity of M^+ and $(M+2)^+$ suggested the presence of $^{32}S:^{34}S$.

Alkyldibenzothiophenes were considered to be mainly methyl derivatives of DBT because the presence of isomers appeared as several sharp and narrow peaks in alkyl-DBT peak group of the M^+ curves in mass chromatograms of oil and eel. The longer alkyl chains would cause broader peaks in GC /54/.

The ratios of the contents of phenanthrene (Ph), DBT and alkylated DBTs are used as an indicator of bioaccumulation. The ratios in sediment differ remarkably from those in biota. For example:

	Ph/DBT	DBT/C1DBT	DBT/C2DBT	Ref.
Ratios in sediment	19	11	44	55
Ratios in fish	9	1.6	4.0	55

Consequently, DBT bioaccumulates more than phenanthrene and alkylated DBTs more than the parent DBT /55/. DBTs showed increasing bioconcentration factor and partition coefficient octanol/water with increasing alkyl substitution /56/.

The present study

Purpose of the present investigation is to improve analysis methods of aromatic oil residues and especially their sulfur-containing congeners in environment, study their bioaccumulation and fate after oil spills in marine ecosystem and find other applications like identification of sources of oil pollutants.

ANALYTICAL METHODOLOGY

Oxidation of dibenzotiophenes to sulfones and their separation from hydrocarbons

No successfull method to separate polycyclic aromatic sulfur heterocycles (PASH) as original compounds from the non-polar aromatic compound fraction has been reported. However, chemical treatments to achieve the separation has been suggested. One of them is to shake with conc. sulfuric acid to remove the aromatic hydrocarbons as sulfonation products. Another method is to form adducts of sulfur compounds with mercury salts and the third method involves oxidation of sulfides to sulfoxides and sulfones which could be separated due to their increased polarity.

Sulfuric acid extraction will cause side reactions such as alkylation by alkenes which are also present in non-polar aromatic fraction. Mercury adduct method seemed to be nonpractical for chromatographic analysis. In contrary, sulfones could be readily determined by HPLC.

The sulfone method described in literature was applied for GC determination and required reduction of the isolated sulfones back to heterocyclic sulfides /66,74-79/. The aromatic fraction was treated in benzene:acetic acid (1:1) with H_2O_2 and then fractionated in silica gel column. The unoxidized aromatic components were eluted with benzene and then sulfone/quinone fraction was collected with benzene:methanol (1:1) as eluent. The latter fraction was reduced with $LiAlH_4$ in ether and the hydroquinones and sulfides were separated by elution with hexane in silica gel column.

Other separation procedures

Adsorption chromatography using silica gel and/or alumina has been generally used as the first step for isolating the PASHs. However, the isolation of the PASH from PAH has been difficult.

Organic sulfides are known to form adducts with metal cations and with many other substances /67/. Complexation of organic sulfur compounds by means of copper, mercury, palladium and other metal salts is a well known technique and has been applied to coal extracts and coal products /71/. Procedures using these salts have been effective for the isolation of aliphatic sulfides but are generally not applicable for the separation of thiophene compounds.

Thiophene compounds can be separated on silver nitrate coated silica column /72/. $CuCl_2$ is a good complexing agent for aliphatic sulfides and $PdCl_2$ for aromatic sulfides /67-70,73/. Sulfur compounds are not eluted with cyclohexane from palladium chloride - silica gel column but are eluted with cyclohexane-chloroform (1:1) according to their Lewis-basicity /68,70/.

Gas chromatography

The majority of PAH analyses by GC have been carried out on non-polar type OV-1 or moderately polar SE-52 or OV-73 stationary phases.

It has been shown that stationary phases of polarity greater than SE-52 have little effect on the resolution of isomeric polycyclic aromatic hydrocarbons. The presence of a sulfur heteroatom in the aromatic structure impacts a more polar character to the molecule. Therefore, polar phases exhibit some selectivity for various isomeric polycyclic aromatic sulfur heterocycles (PASH). Optimal resolution has been obtained by mixing high column efficiency of the non-polar and selectivity of polar phases. Complete resolution of the three-ring PASH isomers was obtained with the 50 % Superox 20 M and SE-52 mixed phase and with pure Superox 20 M. The selectivity (solvent efficiency) increased when going from pure SE-52 to pure Superox 20 M, while the column efficiency decreased and the retention of the solutes increased /50,80,81/.

Because of the complexities of PASH-rich fractions recent efforts focused on high resolution GC with sulfur-selective detection.

Several procedures for GC determination of DBTs have been published. One method involves clean up with LC on aminosilane column and GC on a glass capillary coated by IN SITU gummification with methylphenyl silicone. FPD with 394 nm filter (sulfur selective mode) was used. Sample was introduced via a cold on column injector at oven temperature of 66^{o} C. The oven was then ballistically heated to higher temperature and finally temperature programmed /40/.

Retention indices of some pure methylated DBTs are listed in Table 1./40/. They are given according to the linear retention index proposed by Lee et al /82/.

Table 1. Relative retention indices of methylated dibenzothiophenes on methylphenylsilicone (=RSL-500) with programmed temperature /40/

DBT	295.96	1,6-dimethyl-DBT	324.93
4-methyl-DBT	308.49	3,4-dimethyl-DBT	326.80
2-methyl-DBT	311.07	1,7-dimethyl-DBT	327.63
3-methyl-DBT	312.17	2,4,8-trimethyl-DBT	336.59
1-methyl-DBT	317.99	2,4,6,8-tetramethyl-DBT	341.81
4,6-dimethyl-DBT	318.12	2,4,7,8-tetramethyl-DBT	358.18
2,6-dimethyl-DBT	319.13	3,4,6,7-tetramethyl-DBT	365.25
3,6-dimethyl-DBT	319.65	3,4,6,8-tetramethyl-DBT	365.74
2,8-dimethyl-DBT	321.62	3,4,7,8-tetramethyl-DBT	367.05
1,8-dimethyl-DBT	324.54	3,4,8,9-tetramethyl-DBT	373.07

The retention indices of three-, four- and five-ring PASH isomers on SE-52 deviated only slightly when measured in temperature programming instead of constant temperature GC conditions /80/. Silica columns coated with LiCl, PMMS, and biphenyl/methyl polysiloxane (1:3) have been used in oil analyses /83-85/.

The use of FPD in GC analyses of sulfur compounds is widely reported /47,54,56,60,76,77,80,85-99/. Selectivity of FDP against sulfur is achieved by a proper optical filter. This detector type has a low dead volume and it is easy to fit to most gas chromatographs.

However, its response is a complex function of concentration and, regrettably, its responce to sulfur is reduced at the presence of significant amounts of co-eluting hydrocarbons or other sulfurless organic materials. The extent to which quenching occurs depends on the concentrations of both the sulfur compound and the co-eluting material and is further complicated by the square-law nature of the sulfur response.

Reasons for the non-equivalence in the FPD responses to sulfur compounds due to flame properties and molecular structure have been investigated widely /78,90,95/.

Negative signals of hydrocarbons on the FPD have been explained to be due to continuous elution of backround sulfur compounds from the column which has rised the GC baseline /89/.

The presence of too large an amount of hydrocarbons has been explained to cause a reduced sulfur (394 nm) emission in FPD by rising the temperature of the detector device /90/.

An average response ratio of FPD at 394 nm between S and C is 10^4 : 1 /92,95/.

Gas chromatography/mass spectrometry

Molecular ions of aromatic oil hydrocarbons are very abundant even in the electron impact (EI) mode of cationic mass spectrometry at normal ionization potential of 70 eV. Intensity ratios of their fragments vary greatly depending on structures /100,101).

The molecular ion (M^+) of DBT and its alkyl homologues is very intense, as well. The fragment ions from DBTs are primarily due to loss of HCS and CS /100,102/. Abundances of the fragments of DBTs are, however, small related to the intensity of the molecular ion /103/. All known sulfur-containing organic compounds produce upon EI a fragment ion m/e = 44.9799 (CHS^+) /103/. Other ions on nominal mass 45 are common and thus limit the use of ion 45 as indication of sulfur-organic substance to high resolution MS with resolution better than 3400 /103/. Unsubstituted PASH give a significant fragment M-32 due to loss of S in addition to M-45 (loss of CHS)

/100,101/.

Alkylated DBTs give a fragment ion M-1 due to loss of hydrogen from the molecular ion. Accordingly, C1DBTs, C2DBTs and C3DBTs have intense peaks m/e 197, 211 and 225, respectively, in their EI mass spectra /102/.

Mass chromatography or mass fragmentography (SIM) is useful in determination of DBTs in complex aromatic mixtures. The molecular ions m/e 184 (DBT), 198 (C1DBT), 212 (C2DBT), 226 (C3DBT), 240 (C4DBT) etc. are most useful /50,56,83/. If the mixture is very complex, interferences from other substances might occur. In such cases M+2 ions of the sulfur compounds (^{34}S abundant) might be used as an additional trace /50,83/. Intensity ratio of the M and M+2 ions of a compound containing one S atom must be 95:4 /50,57,81,83, 89/.

Liquid chromatography

Different LC procedures on normal and reversed phases have been used to separate DBTs in oils, oil fractions, coals and biological samples /86,105,106/.

Methylsubstituted and nonsubstituted PASHs having the same parent structure are not separated in normal phase LC, but different parent groups are well separated. In contrary, reversed phase LC separates homologues. This is the same phenomenon as in the case of PAHs. Selectivity of C_{18} columns in PASH separation varies between the types "monomeric" or "polymeric". A suitable eluent was acetonitrile/water 85/15. Retention indices were used for presentation of the data. Fractions from normal phase LC were analysed by reversed phase /104/.

Normal-phase LC retention data were obtained on an aminosilane column using hexane as the mobile phase /104/.

LC on aminosilane was used for clean up of aromatic fraction and isolation of the dibenzothiophene series. The LC analyses were performed on two columns of 25 cm * 4.6 mm coupled in series. They were packed with 5 µm aminosilane material. The mobile phase was nheptane at a flow rate of 1 ml/min. The fraction containing DBTs was eluted between the capacity factors determined with the methyl DBT standards, i.e. between the k' values of 0.9 and 1.6 /105/.

OIL RESIDUES IN NATURAL SAMPLES

Baltic oil spill 1979

Introduction

Five to six thousand tons of crude oil was released to the Baltic Sea when tanker Antonio Gramsky grounded on the coast of Latvia on 27 February 1979. The oil drifted in the northern Baltic for two

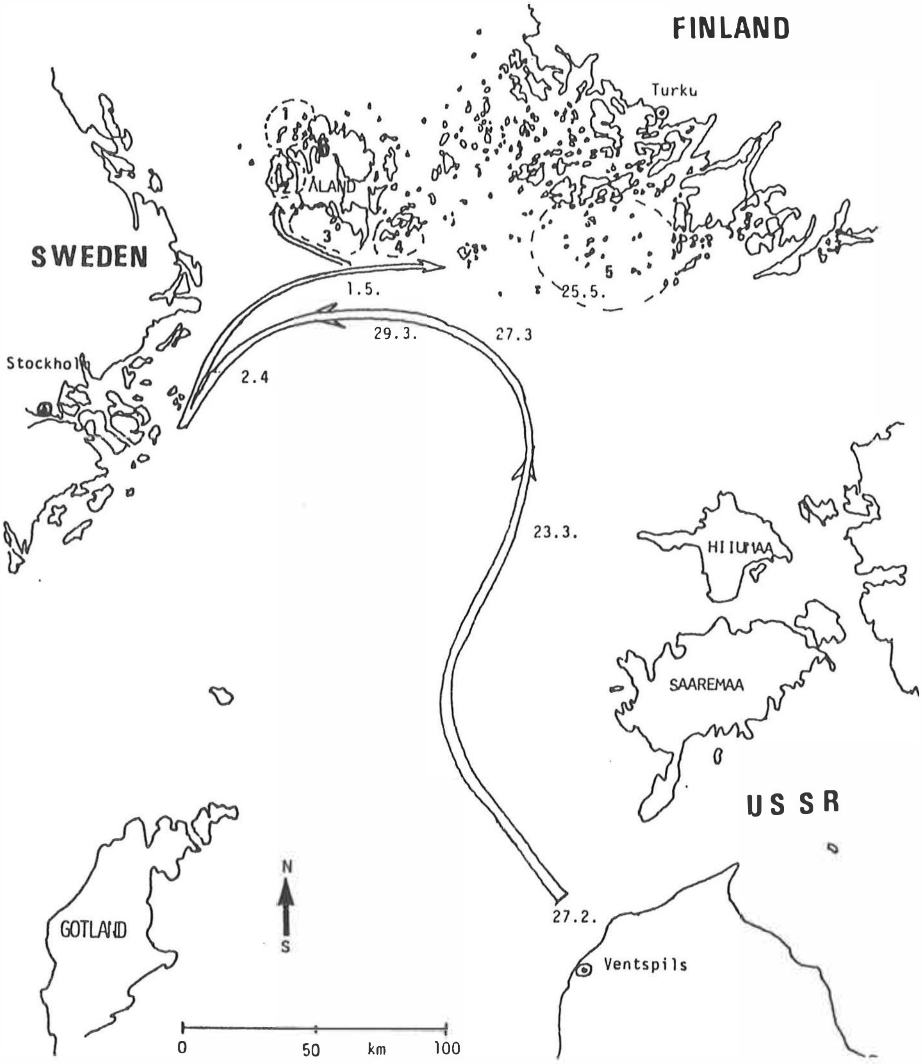

Figure 1. Main route of the spilled oil from Antonio Gramsky 27. 2.1979 including dates of the appearance of oil. Sampling areas: 1. Äppelö-Geta, 2. Kappalö-Borgö sund, 3. Askö-Gloskär, 4. Föglö and 5. Nötö-Jurmo-Vänö /107/.

to three months (Figure 1). Part of the oil landed in April to Swedish and in May-June to Finnish Archipelago. Finnish Ministry of the Interior's Department for Environmental Protection arranged a research project on the marine-biological effects of the oil spill. The chemistry part of the project was done in Jyväskylä. Oil concentrations in blue mussels (Mytilus edulis) and Baltic clams (Macoma Baltica), in sedimenting and sedimented material were taken to objects of analyses. The analyses were continued at following summers 1980 and 1981 in mussels, clams and, as an extension of the programme, in flounder. The analysis methods had to be developed and a method to the distinction between the oil residue and naturally occuring compounds.

Analysis of the oil contamination

A sample of the oil drifting on the sea surface 4.6.1979 near the shore of Askö-Stenvik (Area 3) named residue oil was investigated and compared with a sample of crude oil from USSR. Contents of the aliphatic hydrocarbons and ratios of certain GC peaks appeared to be very similar in both samples. Change of the aliphatic hydrocarbon ratios due to weathering seems to be smaller than generally expected. The non-polar aromatic compound fraction of the both oil samples were analyzed by GC/MS. The results showed more clear weathering effects. Evaporation of the dimethylnaphthalenes (C2N) had been almost complete and the less volatile components like TMBs and C2DBT had been enriched considerably in theresidue oil (Figure 2)

Figure 2. Contents of the non-polar aromatics in two oil samples.

Preparation of the analysis fractions

Weighed (1-7 g) samples of the tissues of mussels and clams were digested for four hours in 0.5 M KOH in methanol:water 1:3. The internal standards androstane and hexaethylbenzene had been added. After extraction the samples were fractionated to the aliphatic hydrocarbons fraction and the non-polar aromatic compound fraction. The sediments were treated in the same way. The flounder samples were before the digestion Soxhlet extracted 6 hours with hexane-acetone-diethylether-petroleum ether (40-60° C) 2.5:5.5:1:9 solution /108/.

GC/MS analyses

Contents of the non-polar aromatic fractions from mussel and fish samples were analyzed mainly by gas chromatography/mass spectrometry.

Gas chromatograph Varian 3 700 connected with a mass spectrometer system Finnigan MAT 212 was used. The column was 0.3 mm diameter 30 m long glass capillary coated with SE-30. Helium was used as carrier gas 1 ml/min. Temperature program applied started at 80° C and then increased 4 °/min up to 250° C. During the gas chromatography repeated scans of low resolution mass spectra were done and stored in the disc unit.

Total ion chromatograms obtained are very complex. Peaks of the oil residue compounds are partly overlapped by peaks of the compounds of natural origin. The possible oil residue compounds were analysed from the total ion chromatogram by selective ion mass chromatography

Alkyltrimethylbenzenes which are the major components in the non-polar aromatic fractions extracted from sediments and crude oils, were sought by their characteristic m/z -values 77, 91, 105, 133, 147, etc. Naphthalene and alkylnaphthalenes were sought by m/z -values of their molecular ions 128, 142, 156, 170, 184 and 198. Anthracene/phenanthrene and the corresponding methylated compounds could be detected by m/z -values of their molecular ions 178, 192, 206, 220 and 234. Biphenyl and alkylbiphenyls found with m/z -values 154, 168, 182, etc. and fluorenes and alkylfluorenes with m/z -values 166, 180, 194, 208, etc. are also possible oil pollutants.

Dibenzothiophene and methyl-, dimethyl- and trimethyldibenzothiophenes were detected by their molecular ions m/z 184, 198, 212 and 226. From the polycyclic aromatic hydrocarbons pyrene (m/z of the molecular ion 202), benzofluorene (216), chrysene (228), benzo(c)phenanthrene (228), perylene (252), benzo(a)pyrene (252) and coronene (300) sought only pyrene and chrysene were found in a few samples. It is possible that the compounds containing more than four aromatic rings did not move in the GC column used.

Total mass spectra of the peaks in the total ion chromatogram were run. Usually there were so many compounds that all of them were not completely resolved, so it was impossible to get mass spectra of pure compounds because there are interfering peaks from the background.

The following m/z -values were chosen for the quantitative GC/MS analyses: 133 (alkyltrimethylbenzenes TMB), 156 (dimethylnaphthalenes C2N), 184 (dibenzothiophene DBT), 178 (antracene/ phenanthrene A/Ph), 198 (methyldibenzothiophenes C1DBT), 212 (dimethyldibenzothiophenes C2DBT) and 192 (methylphenanthrenes C1Ph). Hexaethylbenzene (246) was used as an internal standard. Quantitation from the mass chromatograms was done by dividing the peak intensities of the indicated oil residue compounds with that of HEB and multiplying with added amount of standard per sample weight. The results are dependent on the responses of individual compounds against MS which mainly are not measured due to lack of model compounds. However, the contents obtained for different samples are comparable as the quantitation has the same base in each case.

High performance liquid chromatography

The method was first presented 1981 in Kiel in the "Workshop on the Analysis of Hydrocarbons in Seawater" for the analysis of aromatic hydrocarbons /108/ and has then been further developed.

Non-polar aromatic fractions of mussel and fish samples were analyzed by HPLC. A Pye Unicam LC-3 liquid chromatograph coupled with a variable wavelength UV-detector was used.

Different reversed phase columns were tested for the separation of the aromatic hydrocarbons with standard solutions. The following standard compounds were used: biphenyl, naphthalene, fluorene, anthracene, phenanthrene, pyrene, chrysene, benzo(c)phenanthrene, benzo(a)pyrene, perylene, coronene and some methylated forms of these. The Zorbax ODS-column (25 cm long, ID 4,6 mm, particle size 5.5 µm) proved to be the best to separation of these compounds.

Methanol:water and acetonitrile:water solutions were used as eluents. The latter eluent was found to have higher separating power for these aromatic hydrocarbons.

Sensitivity of the UV-detector for the polycyclic aromatic compounds is very good when compared to the FI-detector in gas chromatography. The wavelength 254 nm is best when there is a complex mixture of different hydrocarbons to be measured.

However, higher polyaromatic hydrocarbons do have absorption maxima at higher wavelengths and these can be used for their more selective analysis.

The complexity of the mixtures of the non-polar aromatic fractions extracted from mussel and fish samples is such that all the compounds cannot be resolved. Some compounds however were identified by comparing retention times and the UV-absorbances at different wavelengths with the corresponding data of standard compounds and by recording mass spectra of some fractions Figure 3.

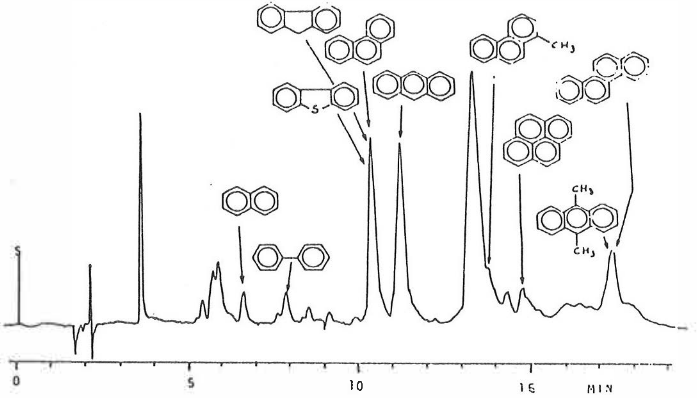

Figure 3. High pressure liquid chromatogram of non-polar aromatic fraction of a Mytilus sample. Isocratic run with acetonitrile-water 3:1 in a Zorbax ODS column (25 cm long, ID 4,6 mm) 1 ml/min. Detection with UV-detector at 254 nm. Chromatographic identity of the peaks with model compounds is indicated.

The quantitative determination was done by running standard mixture after each sample run and comparing the heights of the peaks in the sample and in the standard run.

Evaluation of the analysis results

Practical limit of detection in GC/MS analysis for aromatic residues for mussel samples proved to be better than 3 ng/g for individual components. Baltic water is polluted by oil everywhere and thus contents of the oil residues in nearly all cases exceeded this limit. However, large local and time differences were detected. The analysis results are presented in Paper I, Paper II and Paper III.

Quantitative results of ten different compound groups obtained from mussel samples 1979 were numerous enough to allow statistical treatment in purpose to differentiate oil components from the naturally in mussels occuring compounds. In Mytilus only DBT, C1DBT and C2DBT, in Macoma also anthracene/phenanthrene, CIPh and TMB showed correlation behavior assumed for dominantly oil residues.

Linear regression of UCM + RES with dibenzothiophenes gave coefficients r^2 0.81 for Macoma, 0.74 for Mytilus and 0.89 for sediment samples thus verifying the nature of DBT, C1DBT and C2DBT as residues of oil contamination.

Persistence of DBTs in sediments

Introduction

In the Finnish Archipelago there was planned an experiment, which was began on 16 October 1978, to follow up the natural degradation of crude oil in the sediment. In the experiment place there were choosen 80 spots on the shore which were treated with fresh Russian crude oil using 10 kg of oil/m^2. The experiment place was a shore which had not been previously contaminated by oil. The degradation of some oil residue compounds in the sediment was followed by analysing sediment samples taken from the same spot in the years 1979, 1980, 1981 and 1988.

In the sediments there are always polycyclic aromatic hydrocarbons such as perylene, benzo(a)pyrene and methylphenanthrenes. Sediments contain also aliphatic hydrocarbons, especially some longchained odd aliphatic hydrocarbons, pentacyclic triterpenes, tetra- and tricyclic terpanes, methylsteroids etc., but not such complete series of homologs of hydrocarbons as in the crude oils.

It is often difficult to deduce which hydrocarcons originate from nature and which from oil pollution.

Because of their low water solubility the polycyclic aromatic hydrocarbons tend to settle down to the bottom and sedimentate by adsorption on different particles. The 2-3 ring aromatic hydrocarbons are much more water soluble and they partly evaporate before solvation. That is why low weight aromatic compounds are not usually found in sediments. However the PAC-fraction extracted from sediments is very complicated. It contains a lot of alkyl- and cycloalkyl derivatives of polycyclic aromatic hydrocarbons, at least 11 different parent compounds, and all possible alkylsubstituted homologs (C_1-C_{15}). The natural concentration of PAH in sediments is however low.

The analysis of the non-polar aromatic fraction

The weight of sediment samples was 5-10 g. The internal standards androstane and hexaethylbenzene were added and the samples were extracted for 4 hours in 2N NaOH methanol-water solution. The non-polar organic compounds were extracted in hexane and fractionated using column chromatography to aliphatic hydrocarbons and non-polar aromatic compounds.

The non-polar aromatic fraction was analysed by GC/MS.

The results are dependent on the responses of individual compounds against MS which mainly are not measured due to lack of model compounds. However, relative concentration changes are obtained accurately for each compound.

The main components in GC/MS were alkyltrimethylbenzenes (TMB), which had 4-11 carbons in side chains. The molecular weights were 176, 190, 204, 218, 232, 246, 260 and 274.

Small aromatic compounds such as naphthalene, biphenyl and fluorene were not found. Anthracene and phenanthrene were found in small amounts. The concentrations of alkylsubstituted phenanthrenes were quite high. In the samples from the year 1988 there were not any more detectable amounts of alkyltrimethylbenzenes, anthracene and phenanthrene and alkyl substituted phenanthrenes.

Most aromatic crude oil components were biodegradable in sediment in few years to n.d. levels. Dibenzothiophenes were significantly more persistent than the other components studied. The analysis results are presented in Paper IV.

The present result strongly supports the previous suggestions that aromatic compounds like TMB, A/Ph, C1Ph, C2Ph, C3Ph, DBT, C1DBT, C2DBT and C3DBT in sediment are from crude oil pollution /109-113/. Age of the pollution incident can be seen from the ratios of the residues due to their different persistency in sediment.

SEPARATION OF THE PASH FRACTION (Paper V)

Thin layer chromatography

TLC experiments were performed to see if the polycyclic aromatic sulfur hetrocycles (PASH) could be separated from the polycyclic aromatic hydrocarbons (PAH) in the non-polar aromatic fraction of crude oil. Silica gel and alumina plates with a fluorescence indicator were used. Forty solvent systems were tried.

The components tested were biphenyl, dibenzothiophene and the non-polar aromatic fraction of crude oil. Biphenyl and dibenzothiophene were used to see, how the addition of a sulfur atom to an aromatic hydrocarbon changes the R_f values in different solvet systems.

It was found that the PASH could not be separated as a different fraction from the non-polar aromatic fraction with TLC on silica gel or alumina plates by any of the forty solvent systems tried.

The only measurable differences in R_f values for biphenyl and dibenzothiophene on silica gel TLC were detected when light petroleum-diethylether-acetic acid (80:15:5), light petroleum-ethylacetate-acetic acid (80:15:5) or light petroleum-acetone-acetic acid were used as eluent. Separation of biphenyl and DBT, however, was not complete on silica gel with any solvent tried. On alumina TLC biphenyl and DBT could be succesfully separated with light petroleum or hexane.

Ligand-exchange chromatography

Ligand-exchange chromatography in the form of silica gel /$PdCl_2$ column chromatography was applied to the separation of PASH from the non-polar aromatic fraction of crude oil. The fraction obtained was analysed by GC/MS.

Approximately 10 g of silica gel was mixed with 0,5 g palladium chloride in a aqueous solution and dried at 140° C in an oven overnight. 4 g of silica gel impregnated with $PdCl_2$ was introduced into a glass column. About 200 mg of the non-polar aromatic fraction of crude oil in 2 ml of hexane was added to the top of the column. Hexane, chloroform and benzene were experimented as eluents. Hexane or benzene alone, when chloroform or diethylamine was not added, eluted only the PAH. The PASH were eluted with hexane-chloroform or with hexane when little diethylamine was added.

The hexane fraction to which little diethylamine was added was analysed by GC/MS. Mass chromatograms using ions 184, 186, 198, 200, 212, 214, 226 and 228 were run to find the dibenzothiophenes. The M+2 ions were run with enchanged sensitivity to be sure that the compounds 184, 198, 212 and 226 really contained sulfur. The chromatograms are presented in Figure 4.

When the scan numbers of the dibenzothiophenes were found from the total ion chromatogram the corresponding mass spectra were run. Despite of disturbing peaks many dibenzothiophenes could be identified. Figure 5 presents the total ion chromatogram of the same fraction. Some peaks identified as dibenzothiophenes are marked on the chromatogram.

Oxidation of dibenzothiophenes to sulfones and analyses by HPLC

The non-polar aromatic fraction of Russian crude oil was about 28 % of the crude oil sample. The non-polar aromatic fraction was

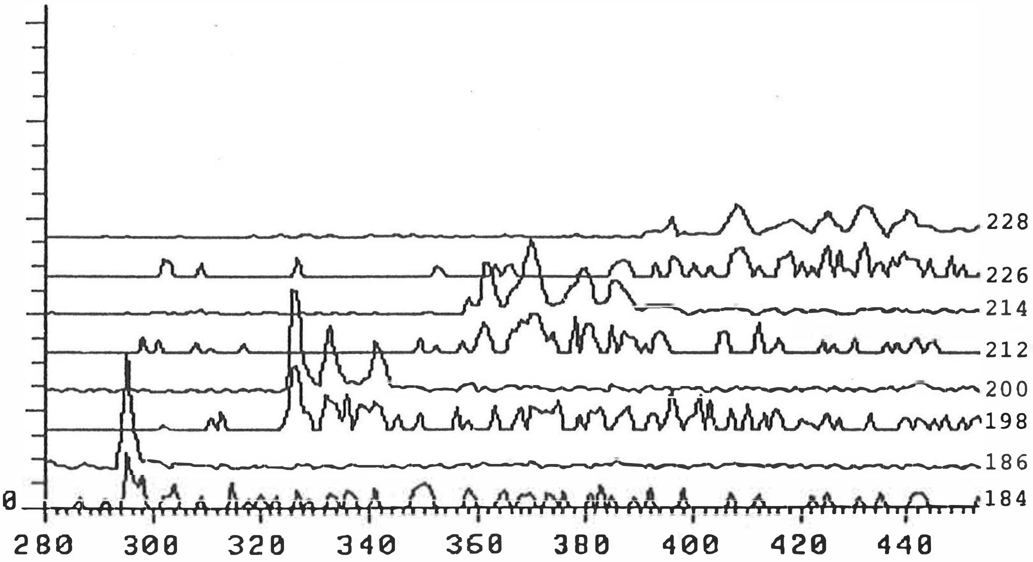

Figure 4. Mass chromatograms of ions 184, 186, 198, 200, 212, 214, 226 and 228 for the PASH-fraction.

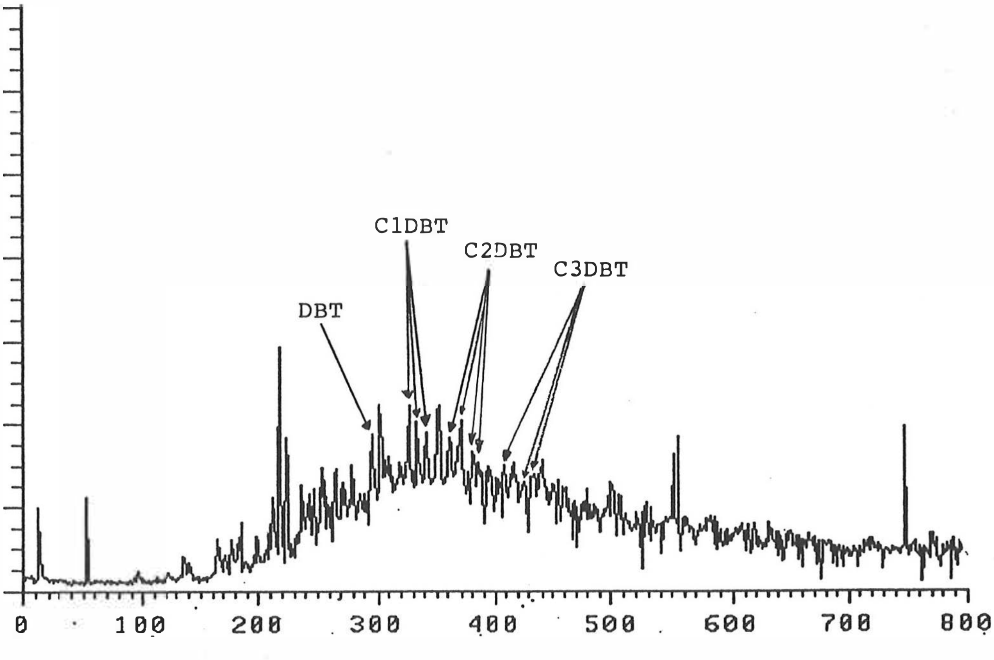

Figure 5. Dibenzothiophenes identified from the total ion chromatogram and from the corresponding full mass spectra.

oxidised by hydrogen peroxide in glacial acetic acid by refluxing the mixture for four hours. Then the mixture was let to stand overnight. Then it was extracted with dichloromethane and washed with water to remove acetic acid. Silica gel column chromatography was used to separate the sulfones from the dichloromethane extract. Non-oxidized compounds were eluted with hexane and sulfones with methanol.

The methanol fraction was analysed by GC/MS by a short SE-54 column. It contained mainly dibenzothiophene-5,5-dioxide and methylated dibenzothiophene-5,5- dioxides with molecular ions 216, 230, 244 and 258. The mass spectra are presented in Figure 6.

The procedure was tested with dibenzothiophene. It was oxidised completely to dibenzothiophene-5,5-dioxide and recovered in 90-100 % yield after silica gel column chromatography. The methyl- and dimethyldibenzothiophenes are expected to behave in the same manner.

The sulfones cannot be analysed by normal gas chromatography because they degradate at about 200° C in normal capillary GC-columns and do not move in usual non-polar columns. HPLC is a suitable method to analyse sulfones.

In reversed-phase HPLC sulfones elute in a relatively short time when the eluent contains 35-45 % water. An ODS column Spherisorb 5µm was used. The retention time of dibenzothiophene-5,5-dioxide was 5.60 min when acetonitrile:water (60:40) was used as eluent. The sulfones from crude oil eluted all before 15 min. The non-oxidized dibenzothiophenes and aromatic hydrocarbons have much longer retention times. When the eluent contains less than 35 % of water the sulfones do not move in the column. When the eluent contains more than 45 % of water the retention times are longer than with 40 % of water. The wavelengths used in the UV-detector were 225 nm, 231 nm, 240 nm and 254 nm. At shorter wavelengths the sensitivity increases for sulfones. For example at 231 nm it is tenfold compared to 254 nm, but there appears disturbing peaks. Therefore the wavelength of 254 nm was choosed for the analysis. In Figure 7 can be seen the HPLC chromatogram of the non-oxidized aromatic fraction of crude oil and the chromatogram of the same fraction after oxidation and column chromatography and in Figure 8 the HPLC chromatogram of DBT and $DBT-O_2$.

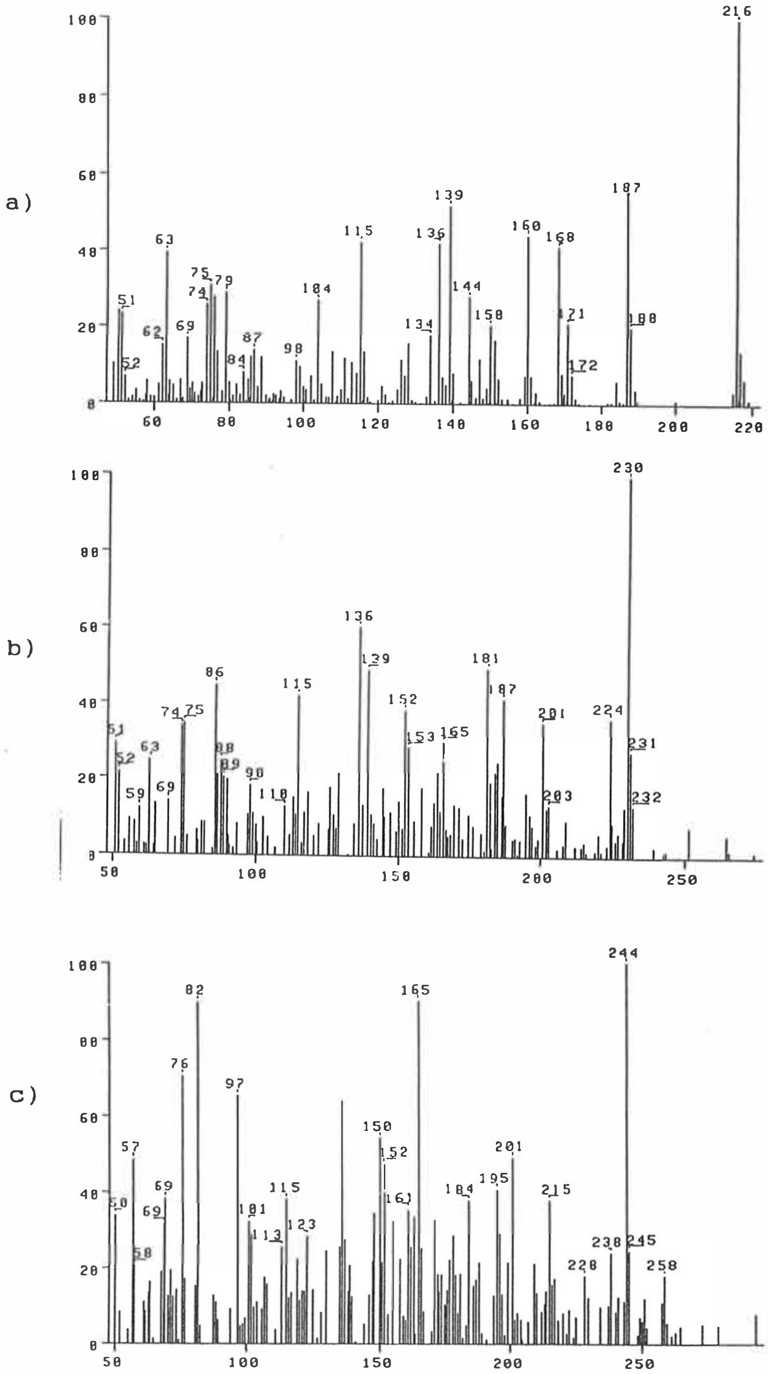

Figure 6. Mass spectra of the compounds identified from the methanol fraction. a) dibenzothiophene-5,5-dioxide, b) methyldibenzothiophene-5,5-dioxides, c) dimethyl- and trimethyldibenzothiophene-5,5-dioxides.

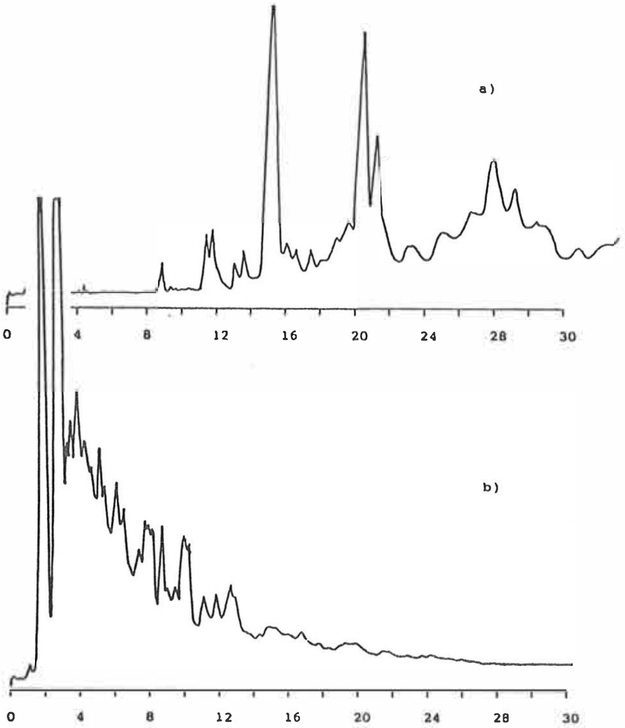

Figure 7. High-pressure liquid chromatograms of a) the non-polar aromatic fraction of crude oil (28,7 µg) and b) the sulfone fraction of the same oil (13,8 µg).Column Spherisorb 5 µm, acetonitrile:water 60:40 1 ml/min and UV=254.

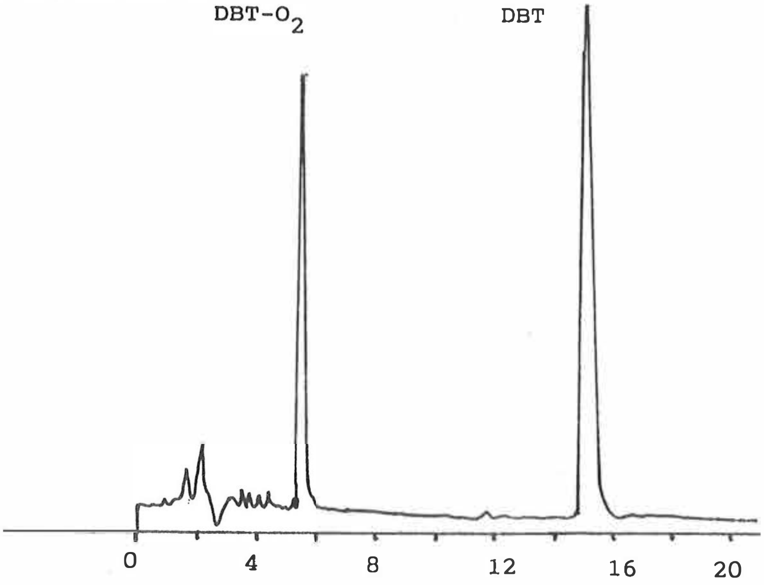

Figure 8. High-pressure liquid chromatogram of a mixture of DBT (0,14 µg) and DBT-O$_2$ (0,20 µg). Column Spherisorb 5 µm, acetonitrile:water 60:40 1 ml/min and UV 254 nm.

SYNTHESES

Dibenzothiophene and methyldibenzothiophenes were synthesized at Monsanto Chemicals Limited allready in 1951 /114/. However the synthesis methods are complicated and have not been used to synthezise model compounds for oil analysis. The starting compounds, thiophenols and thiocresols, cannot be handled in an ordinary laboratory due to their extremely intense smell.

The tetrahydrodibenzothiophene ring structure can be formed by a two stage process in which thiophenol is condensed with 2-chlorocyclohexanone to form a ketosulphide by elimination of hydrogen halide, and ring closure of the ketosulphide is then brought about by dehydration.

Dibenzothiophene is obtained on dehydrogenating the tetrahydrodibenzothiophene.

When thiocresols are used as starting compounds methyldibenzothiophenes are obtained.

In 1983 Marvin L. Tetjamulia et. al. wrote a paper about the synthesis of all the dimethyldibenzothiophenes and monoethyldibenzothiophenes /115/. The syntheses procedures were to a great part analogous to those used by Monsanto Chemicals.

Henry Gilman and Arthur L. Jacoby synthesized in the year 1939 dibenzothiophene from biphenyl and sulfur by Friedel Crafts reaction using aluminium chloride as catalyte /116/.

In this work dibenzothiophene and methyldibenzothiophenes were synthesized by Friedel Crafts reaction from biphenyl and sulfur and from methylbiphenyls and sulfur. The reaction is very slow and difficult to start. The purification of the reaction products is complicated and time consuming. The methylbiphenyls were synthesized from toluidines and benzene by diazotization /117/.

The corresponding sulfones can be prepared by oxidising dibenzothiophene and methyldibenzothiophenes with hydrogen peroxide in glacial acetic acid /118/.

Synthesis of methyldibenzothiophenes

500 mg of 1-, 2- or 3-methylbiphenyl and 200 mg of sulfur were heated to 100 ^{o} C. The reaction mixture was kept at this temperature for four hours during which time aluminium chloride was added very slowly. Then the temperature was gradually increased to 160 ° C and kept at this temperature for five hours. After cooling the mixture was extracted with hexane and ethanol. The extract still contained biphenyl, methylbiphenyl, dibenzothiophene, methyldibentzothiophene and sulfur.

From $PdCl_2$/silica gel column chromatography, RP-TLC and Al_2O_3 column chromatography with hexane tried the last was choosed for the purification of the methyldibenzothiophenes.

The structures of synthesized compounds were verified by MS and NMR. Mass spectra of 2-methyldibenzothiophene, 3-methyldibenzothiophene, dibenzothiophene-5,5-dioxide and 2,4-dimethylbiphenyl are presented in Figure 9-12.

The different isomers of methyldibenzothiophenes and methylbiphenyls cannot be identified by MS. NMR is needed to verify the structure of isomers.

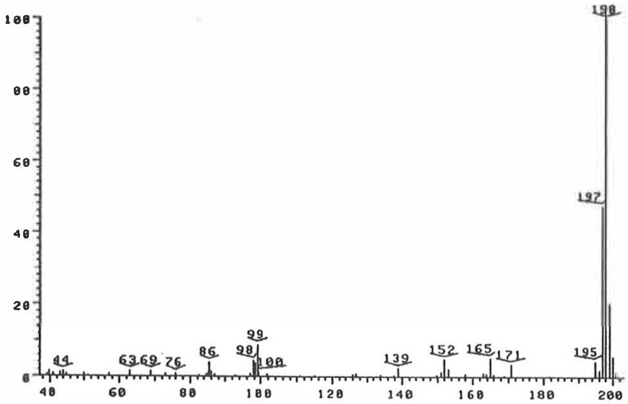

Figure 9. Mass spectrum of 2-methyldibenzothiophene

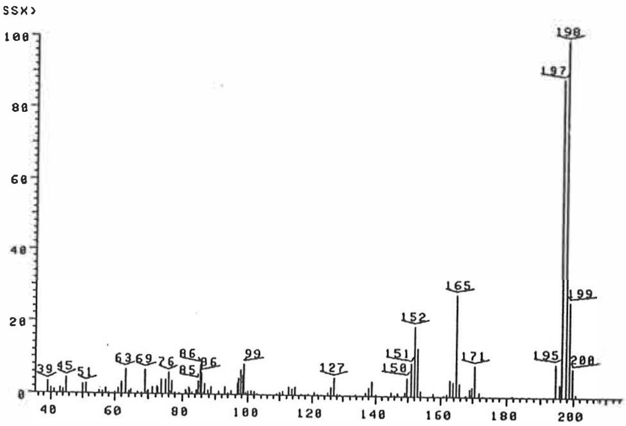

Figure 10. Mass spectrum of 3-methyldibenzothiophene

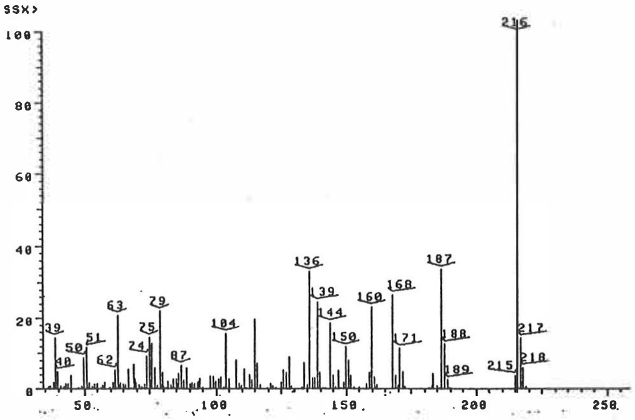

Figure 11. Mass spectrum of dibenzothiophene-5,5-dioxide

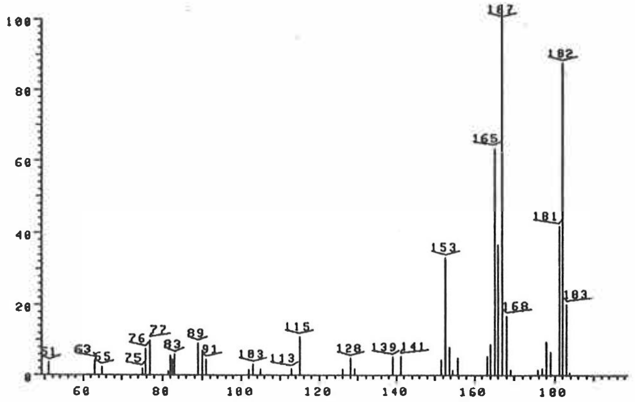

Figure 12. Mass spectrum of 2,4-dimethylbiphenyl

CONCLUSIONS

Mussels were shown to be good indicators of oil pollution. In this work fish and sediment samples were used in addition to mussels to examine the distribution of oil in the Baltic Sea. Alkylated dibenzothiophenes were found to be more persistent than other compounds studied in the environment and these compounds have not been found in unpolluted samples as natural compounds.

The greatest problem in the analysis of dibenzothiophenes from environmental samples is how to extract these compounds from other material. The non-polar aromatic fraction can be separated from a complex mixture of compounds, but the separation of the PASH fraction from this is complicated.

The oxidation of the dibenzothiophenes with hydrogen peroxide to the corresponding sulfones was found to be quantitative and the separation of the sulfones from oxidized and unoxidized polycyclic aromatic hydrocarbons was achieved by column chromatography. The oxidation time of four hours used was long enough to oxidate all of the dibenzothiophenes to the corresponding sulfones, which can be analyzed as such or reduced back to the original sulfur compounds.

Different adsorbents like silica gel, aluminium oxide and amino silane used in TLC experiments could not produce the separation of the dibenzothiophenes as a special group. Column chromatography with silica gel impregnated with palladium chloride produced a moderate separation of the PASH, but the compounds were partly eluted as PASH-$PdCl_2$ complexes which cannot be analyzed by conventional methods.

The lack of model compounds is a remarkable drawback in all analysis methods. Alkyldibenzothiophenes are not commercially available and have not been used as model compounds in DBT analysis methods. In this work the four methyldibenzothiophenes were synthesized by a new method but the compounds were not yet incorporated in the development of analysis methods.

GC alone is not selective enough to analyze separate polyaromatic hydrocarbons and dibenzothiophenes from a complex mixture although there exists good detectors like FID, FPD and PID. Pure DBTs can be analyzed by GC with different detectors.

When GC/MS is used it is not necessary to separate the PAH and PASH compounds to get quantitative determinations of the dibenzothiophenes. The selective ion monitoring method can be used to find the DBTs.

In HPLC reversed phase columns give a good separation and UV- and fluorescence detectors are sensitive for PAH and PASH including the sulfones.

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PAPER I

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PAPER II

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