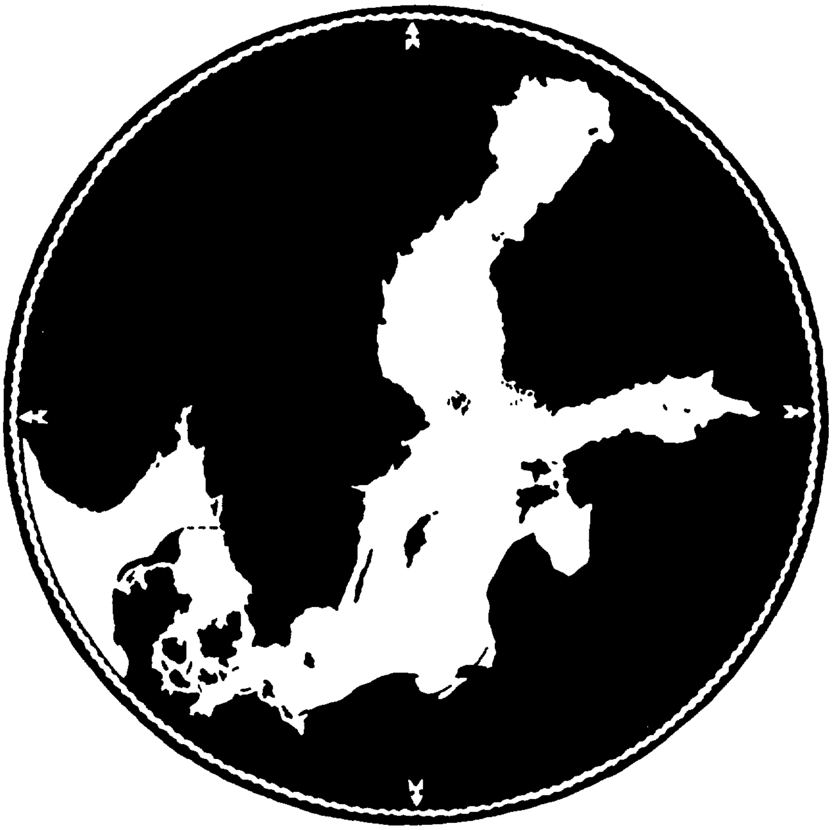
BALTIC SEA ENVIRONMENT PROCEEDINGS

No. 6

WORKSHOP ON THE ANALYSIS OF HYDROCARBONS IN SEAWATER

Institut für Meereskunde an der Universität Kiel
Department of Marine Chemistry

March 23 – April 3, 1981



BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION
— HELSINKI COMMISSION —

BALTIC SEA ENVIRONMENT PROCEEDINGS

No. 6

WORKSHOP ON THE ANALYSIS OF HYDROCARBONS IN SEAWATER

Institut für Meereskunde an der Universität Kiel
Department of Marine Chemistry

March 23 – April 3, 1981

BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION
– HELSINKI COMMISSION –

ISSN 0357-2994

Helsinki 1982. Government Printing Centre

TABLE OF CONTENTS

REPORT ON THE WORKSHOP ON THE ANALYSIS OF HYDROCARBONS IN SEAWATER

INTRODUCTION

The determination of hydrocarbons and their concentrations in seawater are activities of considerable concern both from the points of view of environmental monitoring of marine pollution by fossil fuels and/or research aiming at the elucidation of compositional features, persistence, pathways and final elimination of these compounds from the aqueous phase. Many methods exist for dealing with the different aspects of hydrocarbon analysis in seawater, the results of whose application form the basis of our understanding of the implications of marine oil pollution.

During the first part of the workshop invited lectures, therefore, provided an overview of the most recent developments in marine oil pollution related research. The ensueing practical work was thus based on a firm empirical and theoretical foundation. Practical work during the second part comprised collection of a uniform set of water samples which were analysed at the Kiel laboratory by a common method (UV-spectrofluorimetry).

Simultaneously but at a different sampling station lipophilic organic material including fossil hydrocarbons was concentrated on Amberlite ^{R} XAD-2 resin from more than 700 dm^3 of seawater. Aliquots of this concentrate were distributed to be analysed at the workshop participants' own laboratories. The results are incorporated in this report.

This approach, i.e. the joint collection of a uniform set of samples, work-up and analysis at the Kiel laboratory by a common method on the one hand and comparison of truely identical samples by individual methods on the other hand was believed to be more cost-effective and leading to more realistic results than having workshop participants bring their own analytical equipment to Kiel.

1. OPENING OF THE MEETING

The workshop was opened Monday, March 23, by the convener, Dr. Manfred Ehrhardt of the Department of Marine Chemistry, Institut für Meereskunde an der Universität Kiel.

In his welcoming address he explained briefly the reasons for holding a Workshop on the Analysis of Hydrocarbons in Seawater. He stressed the importance of supporting surveillance and monitoring exercises by adequate basic research providing a more detailed understanding of chemical processes in the water than can be obtained from the quantification of a few arbitrarily selected pollutants.

The participants of the workshop were then welcomed by Prof. Dr. Bernt Zeitzchel, acting director of the Institut für Meereskunde. He gave an introduction to the history of the institute and explained its internal administrative structure, one characteristic feature of which is the existence of ten different departments evenly distributed into biological and physical/ chemical disciplines covering, with the exception of marine geology, all aspects of marine sciences.

2. ADOPTION OF THE AGENDA

The convenor then took the chair again and introduced the workshop schedule which was accepted without change. It is appended as Annex I. Annex II contains the list of participants.

3. ELECTION OF CHAIRMAN

M. Ehrhardt was unanimously elected chairman of the workshop.

4. ELECTION OF RAPPORTEUR

S. Carlberg acted as rapporteur.

5. LECTURES AND PRELIMINARY DISCUSSIONS

In the afternoon of the first day, March 23, three lectures were presented, all dealing with problems associated with calibration, intercalibration and identification in petroleum hydrocarbon analysis. Abstracts of the lectures are appended to this report as Annex III.

Robin Law started by introducing the results from the first ICES Intercomparison Exercise on Petroleum Hydrocarbons. The samples used in the exercise were a reference oil (which was to be analysed by GC and, if possible, by GC-MS), a sediment, and a mussel tissue homogenate. Despite the very wide variety of methods applied the results were in good and in some cases in very good agreement.

In the discussion that followed it was pointed out that one should not draw too general conclusions from the results as they represented the first international intercomparison of this kind. It was also noted that a second similar exercise is being planned for 1982 with Dr. John Farrington (Woods Hole Oceanographic Institution, USA) and Dr. Anthony Knap (Bermuda Biological Station) as coordinators.

The discussion then turned to analytical details. It was noted that several of the participants used an excitation wavelength of 310 nm in the fluorescence analysis and measured the emission at 360 nm as prescribed in the UNESCO Manuals and Guides No. 7 x). It was pointed out that this wavelength combination was good for crude oils but very insensitive for analysis of light oils i.e. oils dominated by aromatics with one or two benzene rings in their structures. For measurements of these oils a combination of 270 nm excitation and 330 nm emission wavelength is more suitable.

The discussion turned over to solvents and extraction techniques. It was noted that extractions of petroleum hydrocarbons from seawater could be done efficiently at the natural pH of seawater. Only when other organic material is to be extracted it is necessary to change the pH of the water sample. It was also mentioned that carbon tetrachloride seems to be more efficient than hexane, especially for extracting high concentrations of petroleum. For low background concentrations this opinion was not supported by data obtained during the

---

x) Guide to Operational Procedures for the IGOSS Pilot Project on Marine Pollution (Petroleum) monitoring. Intergovernmental Oceanographic Commission, World Meteorological Organization. Manuals and Guides, 7. 1976 Unesco, 50 pp.

Workshop. n-Hexane also seemed to cause fewer problems with formation of emulsions. Law mentioned that dichloromethane seems not to cause quenching of fluorescence as other chlorinated solvents do. In fact samples could be measured in the fluorimeter as solutions in dichloromethane.

A discussion on GC analysis followed. The ICES intercomparison exercise did not contain a compilation of the various methods used for quantification. Such a compilation, based on a questionnaire, will be made for the final report which will appear in the ICES Cooperative Research Report Series. It was also concluded that although GC is a wonderful tool for identification and other qualitative purposes it is very difficult to do proper quantification of a hydrocarbon mixture from a gas chromatogram containing more than a few peaks.

In the paper introduced by Stig Carlberg the merits and problems were discussed associated with using either a single compound as standard or an oil as reference for the calibration. From the paper and also from the discussion that followed it was concluded that there was a need for a common standard. It should be an oil of some kind and not a single component such as chrysene which had been used in an international intercomparison exercise.

Carlberg also presented spectra obtained by the technique of simultaneous scanning of excitation and emission wavelengths,with a fixed wavelength difference. In each one of such spectra information is contained that would otherwise require a great number of emission spectra run at fixed excitation wavelengths. Thus, a synchronous scanning spectrum provides some information on the composition of sample (or reference oil) and can, therefore, be used for choosing a suitable reference oil for a sample.

The paper also stressed the importance of checking that extracts do not contain substances in amounts where they may influence the fluorescence measurements by quenching or self-absoprtion. Self-absorption can be identified easily by comparing the intensities of the Raman scattering peaks, which should be the same for the pure solvent and for the samples unless there are overlapping emission peaks. The group recommended that this procedure be included as a routine operation in the analysis.

In her paper "Development of an Oil Identification System" Inger Andresen described the work carried out by a NORDTEST working group with the aim of producing a system of unified techniques. The system for identification uses a stepwise approach involving determination of the boiling range by gas chromatography followed by UV-absorption and UV-fluorescence spectrophotometry as well as high resolution gas chromatography (GC), infrared spectrophotometry and determination of individual elements e.g. nickel, vanadium and sulphur, in the oil. The system is published in a report which soon will become available.

Andresen also described the successful applications of a UV-absorption technique published by Levy x). By studying the ratio between measurements made on peaks at ca. 228 and 254 nm the type of oil can be identified although a source identification cannot be done in that way. Andresen also mentioned that in emulsifiers,

---

x) E.M. Levy (1972) The identification of petroleum products in the marine environment by absorption spectrophotometry. Water Res., 6, 57-69 which are used for oil combatting operations, some stabilizers are often used. These stabilizers usually interfere with the UV-fluorescence of petroleum hydrocarbons.

In the discussion Law mentioned that samples of oil which were collected off the Dutch coast, had been characterized and identified successfully 10 months after the spill by GC techniques. The presentation of papers and also discussions continued in the morning of March 24.

Seija Sinkkonen presented in her paper a large amount of data concerning the analysis of hydrocarbons in sediments, mussels and flatfish. The analyses were part of a study of the biological effects of the "Antonio Gramsky" accident when oil was released into the Baltic in early 1979. The analyses were done by applying GC, mass spectrometry, mass chromatography and high performance liquid chromatography (HPLC). The results of the analyses, as well as certain analytical details, attracted a considerable interest from the group. The very impressing high resolution in the HPLC chromatograms was noted especially.

It was noted that the analyses showed a high incidence of anthracene in the samples. **This** compound is usually scarce in oils but was abundant in the Soviet crude oil which was spilled in this case. Anthracene is also relatively abundant in pyrolysis products of fuel oils and other carbonaceous materials.

The following two papers reported of studies conducted to investigate the relevance of the UV-fluorescence method for monitoring petroleum hydrocarbons.

In his paper Christoph Osterroht described a study of the possible influence of biogenic hydrocarbons on the fluorescence measurements. It was realised that biogenic hydrocarbons would dominate on a water body carrying a plankton production. If this material fluorescences, it would disturb the fluorescence analysis. Because particulate material acts as preconcentrator for lipophilic dissolved organic substances, the study focussed on the particulate fraction. Detailed analyses showed that in the hydrocarbon fraction there was a high content of all-cis **A3** 3,6,9,12,15,18,-heneicosahexaene and squalene and a strong predominance of aliphatics with an odd number of carbon atoms. Taking into consideration results of other works published in the literature it was concluded that the majority of the identified components were produced by marine flora and fauna. It was especially noted that no aromatic compounds were found. Particles were accumulated from appr. 400 dm^3 portions of seawater.

The other study relevant to fluorescence analysis was presented in a paper by Manfred Ehrhardt and Fathallah Bouchertall. The study referred to the background fluorescence in non-polluted waters. The investigators had chosen the approach to study what compounds fluoresce of those which can be extracted from seawater with the aid of a nonpolar solvent. There is no conclusive evidence that such fluorescing compounds are produced biosynthetically. It is concluded that if polyaromatic hydrocarbons (PAH's) can be produced by e.g. bacteria, the product should consists of a few but rather dominating components.

Large water volumes were solvent-extracted with solvent or lipophilic compound accumulated by liquid-solid sorption (Amberlite^R XAD-2). The mixture of substances thus obtained was very rich in components. In the extracts of Baltic seawater concentrations of non-substituted PAH's were found to be high (5 – 20 times) relative to the alkylated compounds. As the former compounds are not abundant in oils but in combustion products of oils, the results suggest a considerable input of air-borne PAH pollution rather than PAH's from petroleum oils. This matter is now being studied further. The extracted material also contained a number of aromatic ketones, which may have been formed by e.g. UV-irradiation of aromatics in the surface film on the water.

From the study it was concluded that as no fluorescent hydrocarbons were found in the fraction that can safely be attributed to biological sources the UV-fluorescence method is not sensitive to disturbance by such material. The results also showed, however, that the method can at present not distinguish between PAH's from petroleum and from incineration products.

In the discussion that followed it was noted that Law and Andrulewicz in 1978 found similar high concentrations of non-substituted PAH's in particulate matter in their study of the Gdansk Deep.

The last paper was presented by Jens Derenbach as a demonstration of the biological relevance of measuring low levels of petroleum hydrocarbons in the marine environment. In laboratory experiments it was shown e.g. how very low concentrations of crude oil, 15 $\mu g \cdot dm^{-3}$ down to as low as 0.2 $\mu g \cdot dm^{-3}$, interfered with the attraction of Fucus gametes thus disturbing the natural communication via sex pheromones. It was also noted that Fucus often grows extensively in areas which have been recently subjected to oil pollution. It is not known, however, whether this is an effect of growth stimulation or of reduced grazing by herbivorous organisms. Results reported from ongoing studies at

Woods Hole Oceanographic Institution on phytoplankton pheromone systems would indicate that in analogy with results from benthic algae components from oil pollution could have considerable effects on many forms of chemically mediated communication.

The discussions in the afternoon of March 24 concentrated on two main areas: the relevance of the UV-fluorescence method for monitoring of oil pollution and then on various analytical details to be tested in the following practical parts of the Workshop.

From some of the presentations above it was concluded that results measured with the UV-fluorescence method are not likely to be influenced by recently biosynthesized hydrocarbons. On the other hand it was also shown that the method cannot distinguish between aromatic compounds of different origins.

From the viewpoint of biological effects of pollution there is obviously no necessity to know whether a certain substance or mixture of substances originates from an oil spill or from combustion of an oil or other fossil fuels.

If Ehrhardt and Bouchertall's findings that a significant part of the PAH's found in waters of the Kiel Bight originate from atmospheric inputs are representative for the Baltic area, UV-fluorescence measurements would offer little help to monitor low level background values of oil contamination as a means of supporting administrative countermeasures against such pollution. From the latter viewpoint it would, at least theoretically, be more profitable to monitor single components or groups of components which may be more specific for oil pollution. This latter aspect is a direct parallel with the current debate on PCB monitoring. The argument was raised again, however, that as compared with PCB an oil contains many more toxic substances. Thus, monitoring a few of them would still leave too many without surveillance. In any case such more detailed monitoring would require more elaborate methods.

It was clearly realised that institutes involved in monitoring, e.g. within the Baltic Monitoring Programme, cannot on a routine basis process large numbers of samples if elaborate and time-consuming methods are required.

As a result of the discussions the group concluded that the UV-fluorescence method is comparatively selective, very sensitive, simple, inexpensive and fast. The method can produce good data in short time and thus provides an effective tool for screening large areas e.g. in the form of regional monitoring programmes. The group, therefore, recommended the application of the UVF method for monitoring purposes. Where clear cases of polluted areas are thus identified they need to be studied with more elaborate methods in order to obtain more detailed information on the extent and nature of such pollution. Such methods are GC with capillary columns and capillary GC coupled to a mass spectrometer.

The group also recommended that synchronous scanning of excitation and emission wavelength should be applied whenever possible using a wavelength difference of 25 nm and a band width of $\leq$ 10 nm, because this procedure produces more qualitative information on the composition of the extracts.

As for the analytical details it was concluded that some different solvents should be compared for use as extractants. Dichloromethane is a good solvent for hydrocarbons and PAH's, it is non-flammable and less toxic than carbon tetrachloride, and it separates readily from the water phase. It was, therefore, decided that this solvent should be tested together with carbon tetrachloride and hexane which are used frequently.

Concerning possible interferences in the fluorescence measurements it was pointed out that neither diphenylsulphones nor aromatic ketones or phthalates which have been identified in extracts of Baltic Sea water fluoresce under the experimental conditions of oil analyses.

Different oils may have different fluorescence intensities at any given wavelength. Quantitative measurements preferably should be made at a wavelength where the difference between different oils are at a minimum. To illustrate this subject Carlberg introduced a paper by two Hungarian scientists×).

They showed that the differences could be minimized e.g. by using an excitation wavelength of 270 nm and 375 nm for the emission measurements.

It was noted, however, that although oils from various areas have different composition differences in their fluorescence properties may be surprisingly small. A difference of only 15 % between the emission intensities between equal concentrations of an Ekofisk crude oil and a Kuwait crude was mentioned.

×) Kdsa, I. and Bajnóczy, G. (1977): Spectrofluorimetric study of petroleum fractions and crudes of different origin. A method for the determinations of petroleum in surface waters. Periodica Polytechnica, Chemical Engineering, 21, 199-209.

Law supplied the results of a study conducted at MAFF in Burnham-on-Crouch, UK, in which relative fluorescence intensities of crude oils from 21 different production areas were compared. Excited at 310 nm the fluorescence at 360 nm showed rather small differences (Annex IV). It was stressed, however, that for other crude oils differences may be greater.

6. PRACTICAL WORK

On Wednesday, March 25, all workshop participants embarked on R.V. "Alkor" for a short cruise into Kiel Bight in order to collect water samples near Kiel lighthouse (DHI station No 709, $54^{o}30,8'$ N, $10^{o}16,5'$ E).

In order to provide all participants of the workshop with identical samples of lipophilic material to be analysed for dissolved/dispersed hydrocarbon concentrations by individual methods in the participants' home laboratories a collection device was anchored en route to the sampling station approximately 2 miles off the north shore near the entrance to Eckernförde Bight ($54^{o}32'$ N, $10^{o}1,5'$ E). The collection device is described in the Proceedings of the XII Conference of the Baltic Oceanographers, Leningrad, April 1980. Annex V describes briefly how the sample was collected and processed.

At the anchor station near Kiel lighthouse water samples were taken by various samplers lowered both open and closed and ranging in volume from 1 to 10 dm^3. Immediately after retrieval the samples were extracted with a first portion of water-immiscible solvents (CCl_4, CH_2Cl_2, n-hexane) and set aside. Extractions were completed after return to the laboratory.

Analyses during the following two days revealed a comparatively good agreement among concentrations of dissolved/dispersed hydrocarbons determined by spectrofluorimetry according to the procedure outlined in UNESCO Manuals and Guides No 7, although different methods of sampling, extraction, and work-up were employed. All individual analytical techniques including sampling are described in detail in Annex VI.

7. DISCUSSION ON THE FIRST ROUND OF SAMPLES

For sampling of water from 1 m depth two different methods had been used: drop bottles which are open during their descent in the water and closed bottles which are opened after reaching the selected depth. All samples taken at greater depths were collected with samplers of the closed bottle type.

The group felt it was of interest to compare the results of the two modes of surface water sampling. The results (see Table 1, Annex VI) do not support the view that samples in the drop bottles become contaminated because the bottles are opened before they are cast into the water. Contrary to expectation the mean value of "oil" concentrations obtained from samples taken with closed bottles was slightly higher than the mean value from samples taken with drop bottles (see also discussion of the second round of the samples, page 15). It should also be noted, however, that the use of closed bottles resulted in smaller variations of the analytical results.

Results were reported of a sampling exercise carried out in Mediterranean surface water. Samples were taken both from the vessel and from a rubber dinghy which was rowed away upwind from the vessel. Results from two sets of samples were not statistically different which suggests that with the selected sampling method the ship itself normally does not cause contamination of the samples. One reason for this may be that, as was found when testing the samples in a hydrographic test tank, the open drop bottle is actually closed by an air bubble during its descent to 1 m depth. These observations had also been confirmed by SCUBA divers at actual sampling.

It was agreed to use the second cruise on Monday, March 30, to compare the extraction properties of carbon tetrachloride with those of dichloromethane. The use of the latter solvent which does not interfere with fluorescence analyses and thus does not have to be exchanged for n-hexane would simplify the method considerably.

The second sampling exercise took place on board the Deutsche Hydrographische Institut's R.V. "Gauss" which had generously been made available for this purpose.

8. DISCUSSION OF THE SECOND ROUND OF SAMPLES

When comparising results from extracts obtained with different solvents some differences became evident. Methylenechloride gave significantly higher concentrations (see Table 2, Annex VI). Not only were the concentrations of extracted materials higher but also gave the solvent rather deeply coloured extracts as compared with hexane and carbon tetrachloride solutions, and the resulting spectra were partly different.

As it is well established in earlier work, by the participants and others, that both carbon tetrachloride and hexane are good solvents capable of extracting from the water the petroleum hydrocarbons of interest, the additional extracting capability of methylene chloride was not regarded as a benefit for the analysis. The extra material which was extracted may, at least in some samples, cause self-absorption. Thus the analyst would have to check this frequently and possibly also apply a clean-up procedure before the photometric measurements could be made'. That would mean not only a requirement for more manpower for the analysis, but also some additional risk for introducing contaminants. The higher volatility of dichloromethane as compared with carbon tetrachloride was regarded as a definite advantage which, however, cannot compensate for the difficulties mentioned above.

Carbon tetrachloride and hexane again gave similar results on parallel analyses. The mean concentration found in n-hexane extracts again was slightly higher (see Table 2, Annex VI). The reason for this phenomenon has not been established with certainty, but it is assumed that some fluorescent material is lost during the evaporation of carbon tetrachloride. Since this solvent also has serious disadvantages, mainly from the viewpoint of occupational health and safety, the group decided to recommend that hexane (n-hexane or cyclohexane) be used as extractant and solvent for fluorescence measurements. This constitutes a substantial deviation from the original procedure (MAPMOPP), but the results of this intercalibration suggest that data will remain comparable with those obtained with the original procedure. Suitable ways to perform the extraction etc. are described in Annex VI.

It was noted that, especially when a great deal of biogenic compounds are present in the water, a significant part of the hexane will dissolve in the water. It was, therefore, advised that if extractions are carried out with a small volume of hexane (e.g. 10 $cm^3 \cdot dm^{-3}$) the separated solvent phase and possible emulsion should be collected and adjusted to volume after the extract has been dried with sodium sulphate. A second way to overcome the problem could be to increase the volume of hexane (to 20 - 30 $cm^3 \cdot dm^{-3}$) as to make the losses by dissolution insignificant.

9. SELECTION OF REFERENCE OIL

Synchronous excitation spectra of some crude oils were obtained (Fig. 1). The oils were the topped Iranian crude used in the intercalibration and a Soviet crude used by Finnish and Polish participants as standard as well as an Ekofisk crude. As the spectra showed the oils to be of similar composition up to the region of about 390 nm the Workshop recommended that a preparation of the Ekofisk crude should be used as a common standard for monitoring purposes in the Baltic. An additional advantage of this approach is that the same oil can be used for both the North Sea and the Baltic. From the analytical point of view the choice of reference oil is not so very critical as can be understood from the comparison of crude oils in Annex IV.

Gerhard Dahlmann of the German Hydrographic Institute undertook the responsibility to prepare such a common standard Ekofisk crude oil preparation and have it available for distribution. This offer was received with great appreciation by the Workshop participants.

10. COMPARISON OF SPECTROFLUORIMETERS

When comparing the results from measurements made on the two fluorimeters available to the participants it became evident that the Aminco SPF 500 instrument gave

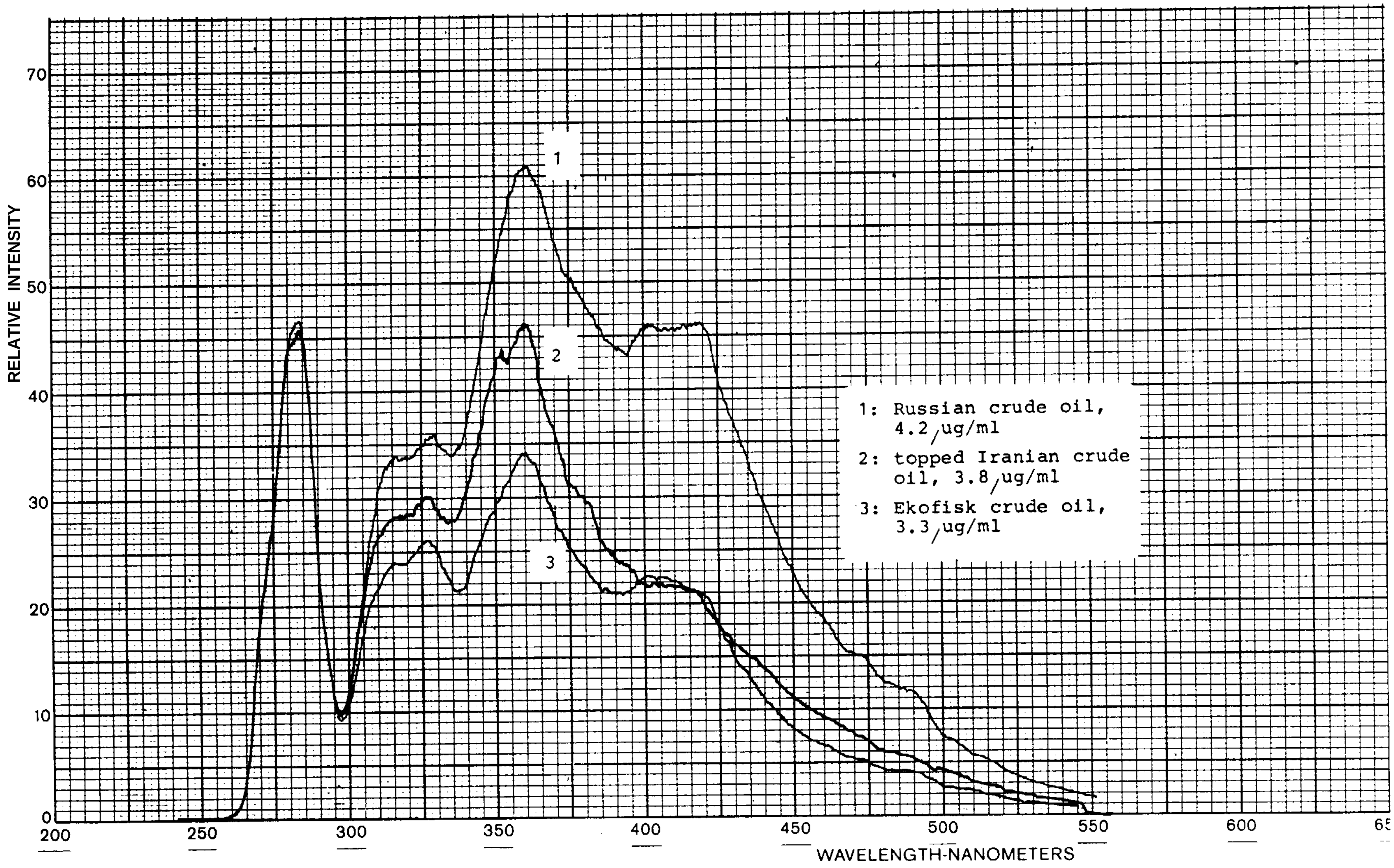


Fig. 1: Synchronous excitation spectra of three different crude oils.
$\Delta\lambda$ = 23 nm; Instrument: Aminco SPF 500

slightly higher concentration figures. This was found to be an effect of the different bandpass settings (5 nm on the Aminco and 10 nm on the Farrand MK1 instrument). When the same bandpass was used on both instruments the results were not significantly different. The reason for the higher concentration figures obtained at narrow bandpass was identified to be an effect of the better resolution in these spectra.

In view of the small differences it was not felt to be necessary to make a recommendation for the use of a 5 nm bandpass as this would make it impossble for several institutes to participate in monitoring activities. It was decided that measurements could be performed with either 10 or 5 nm bandpass. It was recognised, however, that measurements at 5 nm bandpass were more precise. It was, therefore, advise that, when possible, the monitoring institutes should start using instruments with narrow bandpass.

11. CLOSING OF THE MEETING

After a final discussion of the Workshop report the chairman closed the meeting on Friday, April 3, by thanking all participants for their keen interest in the subject matter of the Workshop and their many useful and constructive contributions.

12. RESULTS OF THE WORKSHOP

It should be noted that

1) the UV-fluorescence measurement of lipophilic seawater extracts at the exctitation and emission wavelengths specified in UNESCO Manuals and Guide

No 7 appears not to be influenced by products of recent biosynthesis and produces meaningful results at concentrations Of ≥ 1 µg dissolved/dispersed petroleum residues per dm^3.

2) the above method cannot differentiate between polycyclic aromatic hydrocarbons of different origin some of which are probably introduced into seawater by aeolian transport and precipitation from the atmosphere.

3) regardless of sampling and work-up procedures the results of the analyses were in fairly good agreement.

4) the solvent used to extract seawater samples appears to contribute most to the variability of results (see Table 2, Annex VI).

5) hexane is a suitable solvent leading to results which are approximately 30 % higher than those obtained with carbon tetrachloride as extractant. Its use simplifies the analytical procedure and reduces health hazards.

6) an artificially weathered (topped) crude oil is a suitable reference substance.

7) using different crude oils as standards will not have a dramatic influence on analytical results although their quality can be improved by comparison of unknowns with a common reference oil.

13. RECOMMENDATIONS

Concluding that the UV-fluorescence method for assessing concentrations of petroleum residues dissolved and/or dispersed in seawater is comparatively selective, very sensitive, simple and relatively inexpensive,

considering also that the application of this method is likely to produce good data in short time and thus provides an effective tool for screening large areas, e.g. in the form of regional monitoring programmes,

the Group recommends the use of the UV fluorescence method for monitoring purposes.

Recognizing the need to establish the absence in solutions to be analysed fluorimetrically of substances which cause self-absorption or quenching,

the Group recommends that the intensities of the peak caused by Raman scattering of the pure solvent should be compared with the intensity of the Raman peak of the solution. In the absence of interfering substances and overlapping emission bands both peaks have the same intensities.

Recognizing that additional qualitative information is thus obtained the Group recommends that synchronous scanning of excitation and emission wavelength should be applied whenever possible using a wavelength difference of 25 nm and a band width of $\leq$ 10 nm.

Based on a systematic comparison of the extraction efficiencies of n-hexane and carbon tetrachloride for fluorescent compounds from seawater,

recognizing the lower toxicity of n-hexane, and

appreciating the reduced complexity of the work-up procedure,

the Group recommends to use either n-hexane or cyclohexane as extractant and solvent for fluorescence measurements of dissolved/dispersed petroleum residues in seawater.

Taking into account the fact that the use of standards prepared from different crude oils does not introduce substantial errors, but recognizing the improved quality and credibility of data based on a common reference substance the Group recommends to use a topped Ekofisk crude oil as the standard for oil contamination monitoring purposes in both the North Sea and the Baltic Sea Area.

***

This study has been supported financially by the Federal Ministry for Research and Technology (Bundesministerium für Forschung und Technologie) of the Federal Republic of Germany (MFU 0511-l). The results of the study are published under the sole responsibility of the Workshop participants.

LIST OF ANNEXES

Annex I	Workshop schedule
Annex II	List of participants
Annex III	Abstracts of lectures held during the Workshop in the order of their presentation
Annex IV	Comparison of relative fluorescence intensities of crude oils from different production areas
Annex V	Preparation of an integrated sample for distribution to all Workshop participants
Annex VI	Description of analytical methods for the assessment of concentrations of dissolved/dispersed oil residues as employed by participants during the Workshop and results
Annex VII	Description of analytical methods for the assessment of concentrations of dissolved/dispersed oil residues as employed by participants in their home laboratories and results obtained from aliquots of a common sample (see Annex V)

ANNEX 1

WORKSHOP TIME SCHEDULE

Monday, March 23, 1981

morning:	Registration, opening of the meeting, welcoming address, election of chairman and rapporteur.
afternoon:	Seminar on recent developments in sampling, analysis, interpretation of results and their comparability with regards to hydrocarbons in seawater (lectures by invited speakers).

Tuesday, March 24

morning:	Seminar continued.
afternoon:	Discussion on lessons to be learned from recent analytical developments (sampling, storage, work-up, analysis, interpretation of results, reference material).

Wednesday, March 25

morning and afternoon:	Excursion on board ship. Collecting of samples for determination of dissolved/dispersed hydrocarbons by sampling gear to be brought along by workshop attendants. Aliquots to be analysed at Kiel laboratory by common method (spectrofluorimetry) and standard. Deployment of sampling

buoy at a location near the entrance to Eckernförde Bight (54°32'N, 10°1,5'E) for collection of integrated sample. Aliquots to be distributed to all workshop participants for analyses in home laboratories by own methods and common as well as own standards.

<u>Thursday, March 26</u>

morning and afternoon:	Sample preparation and measurement (spectrofluorimetry, common standard).

<u>Friday, March 27</u>

morning and afternoon:	Measurements continued, comparison of results. Demonstration of sea-water extraction devices and computerized GC-MS equipment.

<u>Saturday, March 28</u>

morning and afternoon:	Day off, sight-seeing, social activities.

<u>Monday, March 30</u>

morning and afternoon:	Sample collection from ship for a second round of analyses possibly incorporating improvements derived from comparison of first results.

Tuesday, March 31

morning and afternoon:	Sample preparation and measurement (spectrofluorimetry, common standard).

Wednesday, April 1

morning and afternoon:	Measurements continued.

Thursday, April 2

morning:	Discussion on recommendations to be derived from lectures and practical experience during the workshop and on preliminary workshop report.
afternoon:	Drafting of preliminary report and recommendations.

Friday, April 3

morning:	Acceptance of recommendations and preliminary workshop report. Distribution of common standards.
afternoon:	Closing of the meeting.

It was agreed that workshop attendants report the results of analyses performed at their own laboratories by individually developed methods of samples taken during the workshop prior to May 31. These results are reflected in this Report.

ANNEX II

LIST OF PARTICIPANTS

No

Andresen, Inger 1
Dantest
Amager Boulevard 114
DK 2300 Copenhagen, Denmark

Andrulewicz, Eugeniusz 2
Institute of Meteorology and Water
Management
ul. Waszyngtona 42
Pl 81-342 Gdynia, Poland

Berend, Peter 3
Wasserwirtschaftsdirektion Küste
Badenstr. 18
DDR 2300 Stralsund, GDR

Bouchertall, Fatallah 4
Institut für Meereskunde an der
Universitat Kiel
Dtisternbrooker Weg 20
D 2300 Kiel, FRG

Carlberg, Stig 5
National Board of Fisheries
Institute of Hydrographic Research
P.O.Box 2566
S 40317 Göteborg, Sweden

Dahlmann, Gerhard 6
Deutsches Hydrographisches Institut
Labor Sülldorf
Wüstland 2
D 2000 Hamburg 55, FRG

Derenbach, Jens 7
Institut fiir Meereskunde an der
Universitat Kiel
Diisternbooker Weg 20
D 2300 Kiel, FRG

Ehrhardt, Manfred 8
Institut für Meereskunde an der
Universitat Kiel
Diisternbrooker Weg 20
D 2300 Kiel, FRG

Erkomaa, Kirsti
National Board of Waters, Finland
Kyläsaarenkatu 10
SF 00550 Helsinki 55, Finland

Lange, Wilfried 10
Deutsches Hydrographisches Institut
Labor Sülldorf
Wüstland 2
D 2000 Hamburg 55, FRG

Law, Robin 11
Ministry of Agriculture, Fisheries and Food,
Fisheries Laboratory
Remembrance Avenue
GB Burnham-on-Crouch
Essex CMO 8HA, U.K.

Osterroht, Christoph 12
Institut für Meereskunde an der
Universität Kiel
Düsternbrooker Weg 20
D 2300 Kiel, FRG

Sinkkonen, Seija Inkeri 13
Jyväskylän Yliopisto
Kemian Laitos
Kyllikinkatu 1-3
SF 40100 Jyväskylä 10, Finland

Tervo, Vappu 14
Institute of Marine Research
P.O.Box 166
SF 00141 Helsinki 14, Finland

Tronczynski, Jacek 15
Academy of Agriculture
Institute of Fisheries
Oceanography and Sea Protection
Dept. of Hydrochemistry and Water
Protection
Kazimierza Krolewicza 4
Pl 71-505 Szczecin, Poland

ANNEX III

ABSTRACTS OF LECTURES HELD DURING THE WORKSHOP IN ORDER OF THEIR PRESENTATION.

Report on the first ICES Intercomparison Exercise on Petroleum Hydrocarbons

R.J. Law and J.E. Portmann

Abstract

An intercomparison of petroleum hydrocarbon analyses has been conducted for samples of crude oil, marine sediment and a mussel homogenate. Thirty-six sets of samples were distributed and results have been submitted by twenty-six laboratories in eleven countries. No analytical techniques were specified for the exercise, and analyses by gravimetry, infrared and ultraviolet spectrophotometry, fluorescence spectroscopy, gas and liquid chromatography, and combined gas chromatography/mass spectrometry were reported. A reasonable agreement was obtained between laboratories for broad fraction analyses, but the determined concentrations of specific hydrocarbons showed greater variation. Proposals are made for the general lines of a follow-up exercise.

Calibration problems related with analysis of petroleum hydrocarbons using UV-fluorescence photometry

Stig R. Carlberg

Abstract

Based upon experience from an international inter-

calibration exercise conducted within the framework of the IOC-WMO Marine Pollution (Petroleum) Monitoring Pilot Project (MAPMOPP) problems are discussed such as

- selection of reference material (petroleum oil or single component)
- spectral properties and requirements concerning the reference material
- quantitative aspects with reference to the selected reference material
- the use of filter fluorimeters and spectrofluorimeters
- selection of wavelengths for quantitative measurements
- practical hints how to avoid errors in quantitative analysis

as conclusions from the discussion the author proposes that:

- a suitable pretreated crude oil is used as a common reference material for calibration
- analysis is made with spectrofluorimeters rather than filter fluorimeters and that, preferably, emission spectra should be scanned and recorded
- the Raman peak should be used as internal calibration standard in quantitative work to avoid errors due to quenching or self-absorption of emitted light.

Development of an oil identification system

Inger Andresen

Abstract

During a long period (since 1960) the National Institute

for Testing and Verification (Dantest) in Denmark has been consultative to the Danish authorities with respect to oil pollution. The institute deals with oil combatting procedures as well as identification of the origin of spills.

During the period the analytical system has been revised several times. In the latest 2-3 years a standard system has been developed in collaboration with 4 other Scandinavian laboratories under the auspices of the Nordtest organization.

The standard system consists of a two-step analytical procedure, the first step (UV or UV-fluorescence and GC on conventional columns) is used as a screening procedure, the second step (GC capillary, IR, AAS, S-determination and HPLC) is used as identification procedure.

Monitoring of marine oil pollution by analysis of mussel and fish samples

Seija Sinkkonen and Jaakko Paasivirta

Abstract

Extraction, clean-up and determination methods for oil residues in mussels and fish of the Baltic Sea have been developed in order to find improved means of monitoring localized incidents of oil pollution and their time trends and to study the possible enrichment of individual oil components in food chains.

Aliphatic hydrocarbon residues were readily separated from other mussel and fish components and analysed by glass capillary gas chromatography using dry needle

injection and FI detector.

Liquid chromatography methods were tried in clean-up and group separation of aromatic oil residues. However, interference of natural compounds in GC/FID prevented the use of the latter method in final determinations. GC/mass spectrometry was applied to determine indididual aromatic compounds or compound groups. High pressure liquid chromatography was also applied as a final determination method for aromatic components.

Oil residue compounds could be differentiated from compounds occuring naturally in mussels and fish by statistical treatment of the analysis results.

Particulate hydrocarbons in the Baltic with respect to MAPMOPP method for determining dissolved and particulate hydrocarbons in seawater

C. Osterroht

Abstract

Particulate matter, collected by two different methods from 1 m and 5 m depth at a station east of Gotland ($57^{O}05'N$, $19^{O}26'E$) in May 1980 was extracted in a specially designed rotating extractor. The raw extract is cleaned and separated into different fractions in order of increasing polarities by liquid chromatography on silica gel. The hexane-fraction containing hydrocarbons is again chromatographed to separate highly unsaturated hydrocarbons from aliphatics and mono- and diolefins. The fractions finally resulting are subjected to GC-MS analysis and, if necessary and possible, to other analytical techniques. Only aliphatic and olefinic hydrocarbons were detected.

comparing our results with data from the literature, it appears most likely that the majority of hydrocarbons extracted from particulate matter at the time and location indicated above, were released by marine organisms, mainly planktonic and benthic algae. No aromatic or alicyclic hydrocarbons were found. Considering the MAPMOPP method and the compounds to which it is sensitive, we conclude that UVF indeed does not measure biogenic hydrocarbons but rather PAH's of anthropogenic origin. This does not necessarily mean that petroleum derived hydrocarbons are determined very precisely by this method.

Literature.reference:

Osterroht, Chr. and G. Petrick (1982):Aliphatic hydrocarbons in particulate matter from the Baltic. Accepted for publication in Marine Chemistry.

Compositional characteristics of lipophilic seawater extracts

Fathallah Bouchertall and Manfred Ehrhardt

Abstract

In order to ascertain the origin of lipophilic fluorescent compounds extractable from seawater with carbon tetrachloride or accessible by sorption onto Amberlite^{R}XAD-2 resin, such concentrates were analysed by GC and GC-MS techniques. The composition of concentrates differs from that of fresh crude oils in that low boiling alkanes up to twenty carbon atoms are usually missing. Among aromatic hydrocarbons unsubstituted PAH's such as phenanthene, pyrene, fluoranthene, chrysene, are substantially more abundant than in crude oils suggesting a pyrolytic origin of

a portion, as yet undetermined, of marine PAH's. Some aromatic ketones were discovered in the extracts which, however, hardly contribute to the fluorescence.

Literature reference:

Bouchertall, F. (1980): Anreicherung und Charakterisierung von im Meerwasser gelösten fluoreszierenden organischen Substanzen. Diss. Kiel, 1980.

Ehrhardt, M (1980): Preliminary results of a novel sampling technique for dissolved and particulate organic material in deeper water layers. Paper presented at the 12th Conference of Baltic Oceanographers, Leningrad, USSR, April 1980.

Interference of low concentrations of petroleum hydrocarbons with sex pheromone reactions of marine algae

Jens B. Derenbach

Abstract

Gametes of different brown algae are known to be attracted by their specific sex pheromone at concentration levels of some ng. dm^{-3}. This chemotaxis is not very specific. A range of similar olefinic compounds mimic pheromone reactions. In addition, petroleum hydrocarbons were discovered to interfere with the pheromone reaction of gametes. Different authors reported inhibition of zygote formation or reduced gamete attraction with Fucus, Cutleria, and Laminaria species at sublethal concentrations between 0.2 and 50 µg of crude oil or diesel oil per dm^3 Of seawater.

This interference of petroleum hydrocarbons with pheromone reaction systems may not **be** restricted to coastal areas. There is some indication that compounds similar to known pheromones of benthic algae also act as pheromones in plankton algae. By analogy one is tempted to assume that low concentrations of petroleum hydrocarbons thus possibly affect a large portion of organisms in the ocean.

Literature reference:

Derenbach, J.B. and M.V. Gereck (1980): Interference of petroleum hydrocarbons with the sex pheromone reaction of Fucus vesiculosus. J. exp. mar. Biol. Ecol., 44, 61-65.

Derenbach, J.B., W. Boland, E. Fölster, D.G. Müller (1980): Interference tests with the pheromone system of the brown alga Cutleria multifida. Mar. Ecol. Prog. Ser. 3, 357-361.

ANNEX IV

COMPARISON OF RELATIVE FLUORESCENCE INTENSITIES OF CRUDE OILS FROM DIFFERENT PRODUCTION AREAS

Equally concentrated solutions (IO micrograms per cm^3 of dichloromethane) of crude oils from different production areas were related to the Ekofisk crude. (Instrument: Baird-Atomic SFR-100, bandpass: 10 nm, excitation: 310 nm, emission: 360 nm). This work was carried out at the Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Fisheries Laboratory, Burnham-on-Crouch, Essex CMO 8HA, U.K.

Ekofisk	crude oil	1.00
Auk	" "	0.65
Beryl	" "	1.10
Kuwait	" "	0.80
Montrose	" "	0.80
Dunlin	" "	1.05
Forties	" "	0.85
Argyll	" "	0.60
Thristle	" "	0.85
Piper	" "	0.90
Claymore	" "	1.00
Murchison	" "	0.80
Ninian	" "	0.85
Brent	" "	0.75
Light Arabian	" "	0.78
Light Iranian	" "	1.00
Light Saudi Arabian crude oil		0.82
Libyan (Brega)	crude oil	0.86
Nigerian	" "	1.10
Abu Dhabi (Murban)	" "	0.63
Beatrice	" "	0.77

mean value = 0.856'0.145 (± 17,94 %)

ANNEX V

PREPARATION OF AN INTEGRATED SAMPLE FOR DISTRIBUTION TO ALL WORKSHOP PARTICIPANTS

Water is drawn through a 14 cm diameter kiln-fired glass fibre filter. The filtered water then passes through a glass column (20 x 200 mm) filled with 100 cm3 of pre-cleaned Amberlite^R XAD-2 resin. The volume of water pumped through the system is measured by counting the number of revolutions of the metering pump.

The system was operated for 48 hours at a depth of 5 m below the surface (water depth 20 m). Breaking of a tube at elevated pressure resulting from a high concentration of particulate material suspended in the water which finally clogged the filter prevented a precise determination of the volume of water which had passed through the XAD-2 cartridge. It should, however, be close to 760 dm^3 indicated by the meter.

After retrieval the XAD-2 cartridge was eluted for six hours with refluxing aqueous acetone. The acetone was evaporated under reduced pressure in a rotary evaporator. Lipophilic material was then extracted from the aqueous residue into spectrograde carbon tetrachloride (3 x 25 cm^3). The carbon tetrachloride solution was washed once with 25 cm^3 of high purity water (Millipore Super Q System) saturated with kiln-fired sodium chloride and dried over anhydrous sodium sulphate (solvent extracted and reactivated at 130^o, vacuum).

The solution was then made up to 200 cm^3 in a volumetric flask. 10 cm^3 portions were pipetted into glass ampoules which were flamesealed under a stream of ultrapure nitrogen.

Each ampoule contains lipophilic material accumulated on XAD-2 resin from $\frac{760}{20} = 38\ dm^3$ of filtered seawater and extracted by carbon tetrachloride from the acetone eluate of the XAD-2 column.

These samples were analysed by all Workshop participants in their home laboratories in order to determine the influence of individual analytical techniques on the variability of results obtained from a uniform set of samples. It was agreed that results should be reported to the Workshop convenor not later than May 31, 1981, for inclusion in the Final Report.

ANNEX VI

DESCRIPTION OF ANALYTICAL METHODS FOR THE ASSESSMENT OF CONCENTRATIONS OF DISSOLVED/DISPERSED OIL RESIDUES AS EMPLOYED BY PARTICIPANTS DURING THE WORKSHOP AND RESULTS

1) Andresen, Inger

MAPMOPP method as described in UNESCO Manuals and Guides No 7, without modifications.

2) Andrulewicz, Eugene

For the first round of samples the MAPMOPP procedure was employed without modifications.

The second round of samples was extracted with 2 x 40 cm^3 of dichloromethane each. Extraction was effected by rigorous shaking for 10 minutes. Dichloromethane extracts were used directly for fluorescence measurements.

3) Berend, Peter

MAPMOPP method without modifications.

4) Bouchertall, Fathallah

MAPMOPP method without modifications.

5) Carlberg, Stig

Sampling. Standard IGOSS sampler for 1 m depth. 2,8 cm^3 or 1 dm^3 thoroughly cleaned reagent bottle which is open during its decent in the water.

Extraction. Carbon tetrachloride 25 cm^3, or dichloromethane 50 cm^3 was used. The sample bottle is manually shaken for about 10 minutes and then the phases are left to separate. The solvent is withdrawn with a pipette and transferred into a flask. A second extraction is carried out with the same (25 cm^3 or 50 cm^3) volume of solvent.

Pretreatment. The extracts-containing emulsion were dried by adding pre-extracted sodium sulphate. The dried extracts were then transferred into clean flasks and evaporated to dryness using a rotary evaporator and a water bath at 30 - 40°C. The evaporation process was interrupted immediately before the last traces of solvent disappeared. It was made sure that all traces of carbon tetrachloride was removed from the flask before the residues were redissolved in 10 cm3 of hexane in a volumetric flask.

Calibration. 2,0 cm3 of the solvent was measured into the photometric cell and background emission was recorded using an excitation wavelength of 310 nm and an emission wavelength of 360 nm. Known amounts of reference oil (topped Iranian Crude dissolved in hexane) were added by means of a microsyringe (roughly 1 mm^3 in each portion). A calibration graph was constructed from the emission readings at 360 nm after substraction of the emission of the solvent at the same wavelength.

Analysis. The sample extracts were measured in the same way as described above. In the calculations account was made for the differencies in calibration volume (2 cm^3) and final extracts volume (10 cm^3).

6, 10) Dahlmann, Gerhard and Wilfried Lange

The sampling device consists of a 10 dm^3 round bottom flask described by Stadler and Schomaker, Dt. hydrogr. Z., 30, 20-25 (1977).

Advantages: Easy to clean glass surface, opens only at the desired depth, can be used for deep-water samples up to 2000 m. The sample is extracted within the sampler. The hexane-phase can be separated 'easily by a special device to be attached to the sampler after extraction rendering unnecessary the use of an additional separatory funnel.

Disadvantage: Somewhat unwieldy equipment. Before each sampling, the sampler is washed with water (and detergents) and rinsed with 500 cm^3 of acetone and 500 cm^3 of hexane. The sample is shaken on a mechanical shaker for 30 min. with 300 cm^3 hexane (nanograde) aboard the ship. In the laboratory the extracts are dried with Na_2SO_4 and concentrated in a rotary evaporator to 10 cm3. An aliquot of this solution is measured with an Aminco SPF 500 spectro fluorometer (IGOSS-wavelength).

Calibration of the instrument: The fluorescence intensity of a known concentration of Ekofisk-crude oil is dialled in with the "range vernier"-setting. The fluorescence of pure hexane is adjusted to 0.000 by means of the "blank substract vernier".

We normally produce two spectra of a sample: an emission spectrum at 310 nm excitation wavelength and a synchronous spectrum at a wavelength difference between excitation and emission of Ah= 23 nm.

7) Derenbach, Jens

For the first round of analysis the MAPMOPP method was used without modification.

Instead of carbon tetrachloride 2 x 20 cm^3 of dichloromethane were used for the second round of analyses.

Fluorescence signals of water-saturated standards and extracts were found to be low by 5 - 20 % relative to dry samples.

8, 12) Ehrhardt, Manfred and Christoph Osterroht

MAPMOPP method without modification.

9) Erkomaa, Kirsti

For the first and second round of analyses the MAPMOPP method was used. For the second round of analyses dichloromethane and carbon tetrachloride were used as solvents. In addition to the MAPMOPP procedure the following method was used for both rounds of analyses.

Sampling: Water samples are taken with 1 dm^3 Merck reagent bottles which are closed with a teflon foil while being lowered to the sampling depth. The teflon foil is then pierced with a messenger-activated device.

Extraction: 10 cm^3 of n-hexane are added to the sample bottle and its content is stirred with a magnetic stirrer for 45 min. Pre-extracted seawater is then added to force the hexane layer into the neck of the bottle from which it is withdrawn with a pipette.

Fluorescence spectra are obtained with 310 nm excitation wavelength. The fluorescence intensity of 360 nm is used for quantification.

11) Law, Robin

No water analyses during the Workshop.

13) Sinkkonen, Seija Inkeri

For the first round of samples the MAPMOPP method was used without modification.

In the second round the sample was extracted with dichloromethane (1 x 50, 2 x 30 cm^3) which was dried with Na_2SO_4, evaporated and replaced by n-hexane for the fluorescence measurement.

14) Tervo, Vappu

In both the first and the second sample collection the MAPMOPP method was used without modification for one sample, and for another sample the same method was employed as described under 9) above.

15) Tronczynski, Jacek

For samples taken during the first round the MAPMOPP method was used without modification.

Samples taken during the second round were analyzed both according to the MAPMOPP method and, alternatively, by using dichloromethane as extractant and subsequently as solvent for fluorescence measurement.

TABLE 1

Results of the first round of sample collection. Numbers refer to the group or individual who analysed the sample (see list of participants, Annex II). Particulars in parentheses denote the type of fluorimeter: Mk 1 = Farrand Mk 1, SPF 500 = Aminco SPF 500. Methods are described in detail in the beginning of the Annex.

No	Method	Result (µg "oil"·dm^{-3})
1	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	1.2 (Mk 1)
2	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.5 (SPF 500)
	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	1.2 (SPF 500)
3	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.5 (Mk 1)
	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.1 (SPF 500)
4	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.6 (Mk 1)
	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.9 (Mk 1)
5	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.6 (SPF 500)
6,10	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.6 (SPF 500)
	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.3 (Mk 1)
8,12	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.9 (Mk 1)
13	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.4 (Mk 1)
	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.4 (SPF 500)
14	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.9 (Mk 1)
15	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	1.4 (SPF 500)
	all samplers were lowered open	
	mean value =	1.10±0.37(±33.6 %)
6,10	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.7 (SPF 500)
9	1.0 dm^3 closed bottle, 10 cm^3 n-C_6	1.1 (Mk 1)
	1.0 dm^3 closed bottle, 10 cm^3 n-C_6	1.5 (Mk 1)
14	1.0 dm^3 closed bottle, 10 cm^3 n-C6	1.7 (Mk 1)
	all samplers were lowered closed	
	mean value =	1.5±0.28(±18.7 %)
	overall mean values of all surface samples=	1.24±0.37(±29.8%)

Table 1, continued

No	Method	Result (ug "oil"·dm^{-3})
5	1.0 dm^3 closed bottle, 10 cm^3 n-C6 10 m	2.1 (SPF 500)
	1.0 dm^3 closed bottle, 10 cm^3 n-C6 10 m	2.2 (SPF 500)
6,10	10 dm^3 spherical glass sampler, 300 cm^3 8 m	1.9 (SPF 500)
9	1.0 dm^3 closed bottle, 10 cn^3-C_6 10 m samples taken at greater depth, sampler closed during decent	1.4 (Mk 1)

mean value = 1.90±0.36(±18.9 %)

TABLE 2

Results of the second round of sample collection. Numbers refer to the group or individual who analysed the sample (see list of participants,Annex II). Particulars in parentheses denote the type of fluorimeter: Mk 1 = Farrand Mk 1, SPF 500 = Aminco SPF 500. Methods are described in detail in the beginning of this Annex.

No	Method	Result (µg "oil residue"·dm^{-3})
1		
2	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.6 (SPF 500)
3	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	1.3 (Mk 1)
4	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	0.8 (Mk 1)
5	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	1.7 (SPF 500)
6,10	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.1 (SPF 500)
8,12	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.7 (Mk 1)
	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.8 (Mk 1)
9	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	0.8 (Mk 1)
13	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	6.3 (Mk 1)ˣ
14	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C6	0.7 (Mk 1)
15	2.8 dm^3 drop bottle, 2 x 25 cm^3 CCl_4, n-C_6	1.3 (SPF 500)
	solvent: carbon tetrachloride	
	mean value =	0.98 ± 0.36 ($\pm$37,0 %)
6,10	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.4 (SPF 500)
	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.4 (SPF 500)
	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.3 (SPF 500)
	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.3 (SPF 500)
	10 dm^3 spherical glass sampler, 300 cm^3 n-C_6	1.4 (SPF 500)
9	1.0 dm^3 closed bottle, 1 x 10 cm^3 n-C_6	1.1 (Mk 1)
	1.0 dm^3 closed bottle, 1 x 10 cm^3 n-C_6	1.5 (Mk 1)
	1.0 dm^3 closed bottle, 1 x 10 cm^3 n-C_6 10 m	1.1 (Mk 1)ˣ
14	1.0 dm^3 closed bottle, 1 x 10 cm^3 n-C_6	1.7 (Mk 1)
	1.0 dm^3 closed bottle, 1 x 10 cm^3 n-C6	1.0 (Mk 1)
	solvent: n-hexane mean value =	1.35 ± 0.20 ($\pm$14.8 %)

ˣ Not used in calculation of mean value

Table 2, continued

No	Method	Result (µg "oil residue"·dm^{-3})
2	2.8 dm^3 drop bottle, 50, 25 cm^3 CH_2Cl_2	2.4 (SPF 500)
3	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	3.7 (Mk 1)
4	2.8 dm^3 drop bottle, 50, 25 cm^3 CH_2Cl_2	6.1 (Mk 1)*
	2.8 dm^3 drop bottle, 50, 25 cm^3 CH_2Cl_2,n-C_6	3.4 (Mk 1)
5	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	3.7 (SPF 500)
6,10	10 dm^3 spherical glass sampler, 3.3 dm^3 2 x 50 cm^3 CH_2Cl_2	1.3 (SPF 500)
7	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	2.3 (Mk 1)
8,12	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	30.0 (Mk 1)*
	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	2.1 (Mk 1)
9	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	2.0 (Mk 1)
13	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	9.6 (Mk 1)*
14	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2,n-C_6	1.9 (Mk 1)
15	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	3.0 (SPF 500)
	2.8 dm^3 drop bottle, 2 x 50 cm^3 CH_2Cl_2	3.0 (SPF 500)
	solvent: dichlormethane	
	mean value =	2.62±0.79(±30.2 %)

* Not used in calculation of mean value

ANNEX VII

DESCRIPTION OF ANALYTICAL METHODS FOR THE ASSESSMENT OF DISSOLVED/DISPERSED OIL RESIDUES AS EMPLOYED BY PARTICIPANTS IN THEIR HOME LABORATORIES AND RESULTS OBTAINED FROM ALIQUOTS OF A COMMON SAMPLE (SEE ANNEX V).

2) Andrulewicz, Eugene

The aliquot of the common sample distributed during the Workshop was evaporated to dryness on a rotary evaporator. The residue was taken up in spectrograde n-hexane and made up to volume in a 10 cm^3 volumetric flask. It was analysed with an Aminco SPF 500 spectrofluorimeter using 310 nm excitation and 360 nm emission wavelength and Iranian crude oil as standard.

3) Berend, Peter

The carbon tetrachloride solvent was removed from the extract by evaporating it to dryness in a rotary evaporator under reduced pressure at room temperature. The residue was dissolved in 10,0 cm^3 n-hexane. The fluorescence intensity was measured at 310 nm excitation and 360 nm emission wavelength using a JOBIN IVON SPECTROFLUO JY 3 D "Clair". A topped Iranian crude and Ekofisk crude were used as standard.

4,7, Bouchertall, Fathallah, Jens Derenbach;
8,12) Manfred Ehrhardt; Christoph Osterroht

The content of the ampoule containing the carbon tetrachloride solution of the extract was transferred quantitatively into a 100 cm^3 conical flask. The solvent was evaporated in a rotary evaporator under reduced pressure and a bath temperature of 40°C.

Remaining traces of CC14 were blown away with ultrapure nitrogen. The residue was dissolved in spectrograde n-hexane and made up to 10 cm^3 in a volumetric flask. Fluorescence spectra were recorded between 320 and 450 nm with an excitation wavelength of 310 nm both of the sample and different concentrations of the standard (Ekofisk crude, slightly weathered).

5) Carlberg, Stig

The aliquot of the extract from XAD-2 resin distributed during the Workshop was treated in the following way: the ampoule was opened and its content transferred quantitatively with hexane washings into a clean flask. The flask was, immersed into a water bath at 30°C, and the solvents were evaporated with a gentle stream of nitrogen. When no traces of carbon tetrachloride could be detected by smelling, the residue was redissolved in 10.0 cm^3 of n-hexane. The emission spectrum was recorded as for other extracts. A topped Iranian crude was used as standard.

6,10) Dahlmann, Gerhard, and Wilfried Lange

Two aliquots of the common sample were analysed.

Aliquot No 1: The carbon tetrachloride solvent was evaporated with a rotary evaporator. The residue was taken up in spectrograde n-hexane which was evaporated again. The residue thus obtained was dissolved in spectrograde n-hexane and made up to volume in a 10 cm^3 volumetric flask.

Aliquot No 2: The carbon tetrachloride was evaporated with a rotary evaporator except for a residue of appr. 0.2 cm^3. This small volume of remaining solvent was blown away with purified nitrogen. The residue was taken up in hexane and made up to volume in a 10 cm^3 volumetric flask.

Both aliquots were analysed with an Aminco SPF 500 spectrofluorimeter using a band width setting of 5 nm and operating the instrument in the "RATIO" mode. A topped Iranian crude was used as standard.

7) Erkomaa, Kirsti

Four 2 cm3 portions of the solution in the ampoule were taken of which two were evaporated to dryness with a flow of nitrogen and the other two with a rotary evaporator, the residues were dissolved in 10 cm^3 of n-hexane before the analysis. A topped Iranian crude was used as standard at 310 nm excitation. The fluorescence intensities of the samples and standard were compared at 360 nm.

11) Law, Robin

The carbon tetrachloride solvent of the aliquot was removed on a rotary evaporator. The residue was taker up in dichloromethane and made up to volume in a 10 cm^3 volumetric flask. The sample was analysed using 310 nm excitation and 360 nm emission wavelength.

14) Tervo, Vappu

The solvent was evaporated to dryness in a rotary evaporator at room temperature and the residue dissolved in 10 cm^3 Of n-hexane (Ratburn, HPLC). The fluorescence intensity was measured at 360 nm (excitation at 310 nm) using a Perkin Elmer 204 double-grating monochromator fluorescence spectrophotometer.

The reference solutions were made of Russian and Iranian crude oils which did not show remarkable differences.

15) Tronczynski, Jacek

The integrated sample was taken to dryness under reduced pressure in a rotary evaporator. The residue was dissolved in 10 cm^3 of n-hexane and the fluorescence intensity recorded at 360 nm with an excitation wavelength of 310 nm and band width settings of 5 and 10 nm.

TABLE 1

Results obtained by Workshop participants from analyses of aliquots of a common sample (see Annex V)

No	Results ($\mu g \cdot dm^{-3}$)	Comments
2	0.6	topped Iranian crude as standard
3	0.6	Ekofisk crude as standard
	0.45	topped Iranian crude as standard
4, 7, 8, 12	0.42	slightly weathered Ekofisk crude as standard
5	0.41	topped Iranian crude as reference
6, 10	0.48	Ekofisk crude as standard
	0.46	
	0.36	topped Iranian crude as standard.
	0.32	For differences see detailed description above.
	0.3	nitrogen flaw evaporation, topped
	0.3	Iranian crude as standard
	0.2	rotary evaporation, topped Iranian
	0.2	crude as standard
11	0.6	solvent CH_2Cl_2, Ekofisk crude as standard
14	0.5	Russian and Iranian crudes as standards

Table 1, continued

No	Results ($\mu g / dm^3$)	Comments
15	0.45	5 nm slit width
	0.41	10 nm **slit width**

Overall mean value:

$$\bar{x} = 0.42 \pm 0.12 \ \mu g.dm^{-3} \ (\pm 28.6 \%)$$

$$(n = 17)$$

It might be worth noting that the absolute standard deviation from the mean values of "oil" concentrations obtained from analyses of aliquots of an integrated sample is considerably smaller than standard deviations of "oil" concentrations measured in individual samples from - it was hoped - a uniform body of water. This may be taken to reflect the influence of sample variability, extraction efficiency, and individual work-up procedures on the analytical results.

BALTIC SEA ENVIRONMENT PROCEEDINGS

No. 1	JOINT ACTIVITIES OF THE BALTIC SEA STATES WITHIN THE FRAMEWORK OF THE CONVENTION ON THE PROTECTION OF THE MARINE ENVIRONMENT OF THE BALTIC SEA AREA 1974-1978 (1979) *
No. 2	REPORT OF THE INTERIM COMMISSION (IC) TO THE BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION (1981)
No. 3	ACTIVITIES OF THE COMMISSION 1980 (1981) - Report on the activities of the Baltic Marine Environment Protection Commission during 1980 - HELCOM Recommendations passed during 1980
No. 4	BALTIC MARINE ENVIRONMENT BIBLIOGRAPHY 1970-1979 (1981)
No. 5A	ASSESSMENT OF THE EFFECTS OF POLLUTION ON THE NATURAL RESOURCES OF THE BALTIC SEA, 1980 (1981) PART A-l: OVERALL CONCLUSIONS
No. 5B	ASSESSMENT OF THE EFFECTS OF POLLUTION ON THE NATURAL RESOURCES OF THE BALTIC SEA, 1980 (1981) PART A-l: OVERALL CONCLUSIONS PART A-2: SUMMARY OF RESULTS PART B: SCIENTIFIC MATERIAL

---

* out of print

Baltic Marine Environment
Protection Commission
Eteläesplanadi 22 C 43
SF-00 130 Helsinki 13

ISSN 0357-2994

Helsinki 1982. Government Printing Centre