COOPERATIVE RESEARCH REPORT

No. 117

REPORT ON THE FIRST ICES INTERCOMPARISON EXERCISE ON PETROLEUM HYDROCARBON ANALYSES IN MARINE SAMPLES

https://doi.org/10.17895/ices.pub.7907

ISBN 978-87-7482-594-4

ISSN 2707-7144

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Palægade 2-4, 1261 Copenhagen K
Denmark

November 1982

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REPORT ON THE FIRST ICES INTERCOMPARISON EXERCISE ON PETROLEUM HYDROCARBON ANALYSES IN MARINE SAMPLES

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ABSTRACT

An intercomparison of petroleum hydrocarbon analyses has been conducted for samples of crude oil, marine sediment and 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.

INTRODUCTION

At the meeting of ICES Marine Chemistry Working Group in May 1979, it was proposed that an intercomparison of methods for the analysis of petroleum hydrocarbons in marine samples should be conducted under the auspices of ICES. This proposal was approved by the Council at the 67th Statutory Meeting in October 1979, and its form was agreed. It was decided that the exercise should be in three parts, consisting of the examination of samples of crude oil and oil fractions, tissue samples and sediment samples.

AIMS

The aim of the intercomparison was two-fold:

1. to discover the range of methods in general use for the analysis of petroleum hydrocarbons in marine samples;
2. to compare the analytical results obtained both between laboratories and between methods.

For this first exercise, it was not thought possible to stipulate any particular methods; indeed, early proposals that this should be done (Grahl-Nielsen *et al.*, 1978) were strongly opposed on the grounds that it would restrict participation.

Accordingly, participants were encouraged to analyse the samples by a number of techniques, from broad fraction analysis to the analysis of individual hydrocarbons, if possible. Results were to be reported relative to a standard oil so as to facilitate comparison of the data.

Samples were distributed to the first participants in December 1979, and the deadline for submission of results was set at 30 June 1980.

To assist in fulfilling the first aim, a questionnaire was also prepared, and was circulated during late August 1980. Among the persons receiving copies of this questionnaire were all participants in the intercomparison exercise. Completed questionnaires were due for return to the Coordinator by 30 November 1980, and a summary of the information received was available at the 1981 meeting of the Marine Chemistry Working Group.

PREPARATION OF SAMPLES

In all, four samples were made available to participants. These were: (1) a crude oil, (2) an aliphatic fraction of the same oil, (3) a naturally contaminated marine sediment, and (4) a mussel homogenate. The second and fourth samples were supplied only to those who specially requested them.

Sample No.1: Crude oil standard

Ekofisk crude oil supplied by the Warren Spring Laboratory (Stevenage, United Kingdom) was lightly air-weathered to remove the most volatile fractions. The oil was cooled in liquid nitrogen and heat-sealed under nitrogen into 2 ml glass ampoules.

Sample No.2: Aliphatic fraction

This sample was in two parts, each sealed into an ampoule, consisting of:

a. a standard comprising the normal alkanes from n-C_{12} to n-C_{32}, pristane and phytane, all at known concentrations;

b. the aliphatic fraction of the standard crude oil.

As noted above, this sample was not distributed to all participants, but was available on request.

Sample No.3: Marine sediment

A fine sandy sediment was collected from the intertidal flats of the Isle of Grain (Thames Estuary), close to shipping routes and oil refineries. It was oven-dried at 105°C and passed through a 1.4 mm sieve. Aliquots (ca. 200 g) of that fraction which passed through the sieve were placed in glass jars. Analyses of several replicates (by fluorescence spectroscopy) from both a single aliquot and several different aliquots suggested that homogeneity was good to better than ± 10%.

Sample No.4: Mussel homogenate

This was prepared from mussels collected in Narragansett Bay, Rhode Island, USA, and was originally prepared for an intercalibration between participants in the EPA*mussel watch programme. Aliquots of ca 20 g were sealed in teflon containers. Homogeneity of the sample was assured by the EPA source laboratory, and a summary of the results are given in Table 1. These suggest that the sample homogeneity is good, although the results are considerably better for measurements of trace metals than for hydrocarbon determinations. This sample was supplied by the EPA laboratory in Narragansett (Dr Phelps). Requests for the samples were, however, routed via the Coordinator (Dr Portmann).

All samples were stored in a freezer at -20°C prior to distribution.

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* United States Environmental Protection Agency.

DISTRIBUTION OF SAMPLES

Samples were distributed by British Rail (Express) parcels service wherever possible within the United Kingdom, and by air to Europe or North America. Mussel samples were shipped by air packed in dry ice to prevent spoilage. Strict regulations govern the transport by air of crude oil because of its extreme flammability, and apply to 2 ml quantities as well as to larger quantities. For this reason, samples of oil and sediment were professionally packed to meet the regulations. All of the oil and sediment samples, and approximately one-third of the mussel samples, were dispatched from the Coordinator's laboratory at a cost of ca. £1 800 (packing £400, transport £1 400). In addition, the preparation of samples and organisation of dispatch took about 1 man month (UK costs ca £2 500).

RESULTS

The original estimate of the number of participants was 15-20, in fact 36 sets of samples were distributed. 25 sets of results were received by the 30 June deadline and one further set arrived in August, a total of 26 (a list of participants is appended to this report). Although the exercise received some critical comments, generally either expressing concern over the likely homogeneity of samples or the feeling that the use of widely differing methods may make comparison of results difficult, the general level of interest and commitment was high. One set of results from an overseas laboratory was even delivered in person to the Coordinator's laboratory, in order that the analyst could discuss the results of his analyses. Of the 10 samples issued for which as yet no response has been received, two were known initially to have gone to analysts who were doubtful of completing the work on time, and four analysts have written apologising for their failure to complete the work.

Analytical Methods

The methods used in the intercomparison covered almost the full range of methods available, from gravimetric determination following solvent extraction to GC/MS. Quantitative results were reported by gravimetry, ultraviolet and infrared spectrophotometry, ultraviolet spectroscopy, packed-column and capillary gas chromatography, high-performance liquid chromatography and gas chromatography/mass spectrometry. A list of the methods used by each laboratory for extraction and analysis of samples is given in Table 2. In addition, one laboratory (No.20) reported qualitative analyses by ultraviolet spectrophotometry and gas chromatography/mass spectrometry.

The standard of reporting the results was quite varied. All the analysts reported their results with respect to the Ekofisk crude oil supplied as a standard and, although it was stated that their own standards could be used in addition, few of the analysts actually took up this option. In most cases the minimum specifications for reporting results were observed. One error was detected and put right by correspondence and follow-up enquiries of several participants produced more relevant information to assist in the interpretation of the results.

The fundamental principles of the quantitative methods used are described in Appendix 1 (p. 11).

Extraction and Clean-up

Soxhlet extraction was the most commonly used extraction method, being used by thirteen laboratories. A wide range of solvent systems were used, and extraction times varied from 5 to 24 h for sediment samples and 5 to 36 h for mussels. Also commonly used were digestion in methanolic (or ethanolic) potassium hydroxide (six laboratories) and chloroform/methanol extraction after the method of Bligh and Dyer (1959) (three laboratories). Although there was this wide variety of extraction systems used, there is little to suggest that differences in total hydrocarbon results could be attributed either to the extraction system used or the time for extraction. The greatest variations in extraction efficiency would be expected to occur with the polycyclic aromatic hydrocarbons, but insufficient results were submitted for this to be assessed.

Interestingly, in view of the concern often expressed over the use of toxic solvents such as carbon tetrachloride and benzene, all laboratories employing IR used carbon tetrachloride when running the actual IR spectra and all but three of these laboratories also used it as an extracting solvent. No laboratory reported using any of the suggested alternative solvents, such as trichlorotrifluoroethane. Benzene was used by at least two laboratories as an extraction solvent with methanol.

Total Hydrocarbon Analyses

The results of total hydrocarbon analyses of the sediment sample are given in Table 3, and of the mussel homogenate in Table 4. All results are quoted as equivalents of the Ekofisk oil supplied as a standard.

a) Fluorescence spectroscopy

Eleven laboratories reported results for the sediment, and seven for the mussel homogenate. The overall mean value reported for the sediment sample was 33.5 $\mu g\ g^{-1}$, with an overall coefficient of variation of 31%. Most of the laboratories used the IGOSS wavelengths (excitation 310 nm, emission 360 nm) (IOC/WMO, 1976), and the overall mean value reported by these laboratories was similar, 32.3 $\mu g\ g^{-1}$, but the coefficient of variation was smaller at 24%. Of the laboratories using this method of analysis, laboratories 6 and 20 produced much lower results than the remainder. There is no obvious explanation for this difference, particularly as laboratory 6 has since carried out five additional replicate analyses, which have yielded results between 23.1 and 36.3 $\mu g\ g^{-1}$, with a mean of 30.4 $\mu g\ g^{-1}$. They are themselves unable to account for their earlier low results. Sample homogeneity both within and between samples appears to have been good. If these two sets of results are excluded, then the mean by the IGOSS method is 34.4 $\mu g\ g^{-1}$ with a CV of only 11%. One laboratory (No.10) reported values at 310/360 nm, 340/360 nm and 420/450 nm, the respective values obtained being 38, 56 and 70.8 $\mu g\ g^{-1}$. Longer excitation wavelengths cause peak fluorescence emission by larger molecules, and this rise in concentration reflects the larger proportion of higher fused-ring aromatics in the sediment relative to the standard Ekofisk oil. This was not seen, however, in the case of laboratory No.16, whose results appear low even by comparison with those obtained by the IGOSS method.

By considering only those results measured at the IGOSS wavelengths, the range of concentrations determined in the sediment sample is also reduced, from a range of 13.6 to 70.8 μg g^{-1} using various wavelengths down to a range of 13.6 to 42 μg g^{-1} using IGOSS wavelengths.

Although few problems were reported in analysing the sediment sample by UVF, a number of laboratories using this method reported quenching of the mussel extracts, necessitating dilution to constant fluoroscence. However, although not all laboratories mentioned this problem, it was observed by laboratories 1, 3 and 16. The relatively wide range of reported concentrations was from 17.4 to 130 μg g^{-1} wet weight, with a mean value of 67.7 μg g^{-1} and a coefficient of variation of 51%, which cannot be attributed solely to this cause. Very few laboratories reported replicate analyses, and none reported more than two. No doubt this was due to the fact that the total amount of mussel homogenate available to each analyst was small (~30 g) and the replicate analyses in at least one (laboratory 11) and possibly all three cases were probably not total process replicates. In comparison, a sample of ≮200 g of the sediment was supplied to each analyst. For the sediment analyses, coefficients of variation between 5.4 and 8.3% were reported for four replicate analyses by three laboratories (Nos. 1, 7 and 13), giving an intra-laboratory variation no worse than that observed between laboratories for the same sample.

b) Infrared spectrophotometry

Nine laboratories reported results for the sediment sample, and three for the mussel homogenate. Infrared spectrophotometry is not a particularly good technique for use with these types of sample as even only a small amount of residual lipid remaining after clean-up will, unless very high concentrations of oil are present, have drastic effects on the result of the analysis. Consequently, many analysts prefer to use infrared spectrophotometry only for the analysis of water extracts. Nevertheless, IR analysis of the sediment sample produced a result similar to that obtained by fluorescence analysis, 41.0 μg g^{-1}, although the overall coefficient of variation was much higher, 61%. The results obtained with the mussel homogenate were, however, both higher and very variable (Table 3), suggesting that differing amounts of lipid remained in the extracts. Discarding the results for sediment analyses without prior clean-up lowers the mean value to 28.1 μg g^{-1}, but does not improve the variability (Table 3).

c) Gas chromatography

Gas chromatography was widely used for qualitative analysis, but only four laboratories using a mixture of packed and capillary column techniques reported quantitative data for the sediment sample, and two for the mussel homogenate. No laboratory used only packed columns for analysis, and 14 used capillary GC to analyse samples. All GC/MS data reported were obtained using capillary columns. A full list of GC columns and conditions is given in Table 5.

The mean concentration for the sediment sample by GC was 26.2 μg g^{-1}, again comparable to that obtained by UVF. The overall coefficient of variation was quite low (28%), about double that achieved by one laboratory (No.17) for six replicate analyses. The overall mean

value obtained for the mussel homogenate was 40 $\mu g\ g^{-1}$ wet weight and, although only three separate results were reported, the apparent degree of replication for this sample was poorer than that for the sediment.

One laboratory (No. 22) drew attention to the different GC profiles exhibited by the standard oil and the sediment and mussel extracts, and the difficulties which this presented for quantification. Unfortunately, all the laboratories carrying out total hydrocarbon analyses by GC did not supply details of the procedures used, and comparison of the results is therefore difficult. Laboratories 22 and 24 both estimated the total oil content of the samples by calculation of the amounts of the aliphatic hydrocarbons present in the samples and in the crude oil standard. Laboratory No.14 used the total area of the packed column GC trace of the crude oil between n-C_{15} and n-C_{26} alkanes, and calculated an area units per microgram oil response factor.

d) Gravimetry

The two laboratories reporting results of analyses by gravimetry for the sediment samples gave results an order of magnitude apart. The higher results (mean value 165 $\mu g\ g^{-1}$) were obtained after soxhlet extraction with no clean-up, while the lower figure (15.6 $\mu g\ g^{-1}$) was obtained following a clean-up step. Only one result (256 $\mu g\ g^{-1}$ wet weight) was reported for the mussel homogenate. A clean-up step had been used in this analysis, but no information is available on the extract purity achieved.

Analyses for Specific Hydrocarbons

a) Ekofisk oil

Quoted concentrations of n-alkanes, pristane and phytane generally vary by up to a factor of 5 (Table 6), although above n-C_{26} this rapidly widens to more than an order of magnitude. One laboratory (No. 2) consistently reported lower than average concentrations. Peak height ratios of straight chain to branched isomers, often used as indices of degradation, also showed great variation, e.g., 17/Pr varied from 1.86 to 3.01, and 18/Ph from 2.08 to 8.14.

Aromatic hydrocarbons were measured by only four laboratories (Table 7). Apart from excellent agreement of the four results for C_2-naphthalenes (3686 to 4100 $\mu g\ g^{-1}$), the remaining results generally fall within a factor of 5, with the concentration of phenanthrene ranging from 84 to 1690 $\mu g\ g^{-1}$, a factor of 20. The highest figure was determined by HPLC, the other four values being from GC/MS analyses. The range of these latter four values was 84 to 334 $\mu g\ g^{-1}$, within a factor of 5. High values of HPLC relative to GC/MS have been noted by the authors in other contexts. The cause of the very substantial difference needs to be investigated.

b) Marine sediment and mussel homogenate

The degree of variability between results obtained by different laboratories for these samples was generally higher than that for the oil (Tables 8a and 8b). Most aliphatic hydrocarbon concentrations showed a range of greater than an order of magnitude, up to around 50 times. Peak height factors also vary considerably, 18/Ph for example has a range of 0.61 to 1.49 in the sediment and 0.83 to 4.21

in the mussel homogenate. Aromatic hydrocarbon concentrations (Table 9), however, show much closer agreement, particularly in sediment analyses conducted using GC/MS.

Ring type analysis

Only one laboratory (No. 9) submitted results for the ring type analysis, and these are presented in Table 10. Two laboratories analysed samples by HPLC, but the other laboratory (No. 18) determined the concentrations of individual aromatic hydrocarbons. Both used dual detection by UV absorption and fluoroscence detectors.

DISCUSSION

The response to this intercalibration was very good, 36 sets of samples were dispatched and results were received from 26 laboratories. Most reported analyses by more than one method. The most common approach seems to be to generate total hydrocarbon figures by UVF or IR and then to carry out GC analysis to give qualitative information on aliphatics present, and later to use GC/MS where available to obtain specific hydrocarbon data. Hydrocarbon analysts seem to have taken up capillary GC much more rapidly than workers in other fields, presumably because of the extremely complex mixtures of similar compounds encountered in their samples. The quality of chromatograms submitted was uniformly high. Examples of typical packed and capillary column chromatograms are included in Figures 1, 2 and 3.

For this first intercomparison exercise, no methods were specified for either extraction or analysis. Analysts were merely asked to report results relative to the standard oil, wherever possible. Total hydrocarbon analyses showed a reasonable measure of agreement, both within and between techniques, for the marine sediment even though extraction methods were often very different. The level of agreement obtained, when the same UVF excitation and emission wavelengths were used, was even better, even though this same qualification of extraction systems applied. Overall mean total hydrocarbon concentrations for the sediment were 33.5, 41.0 and 26.2 $\mu g\ g^{-1}$ by UVF, IR and GC, respectively.

Most laboratories also showed good repeatability with their own techniques; and those using UVF measurements at the IGOSS wavelengths showed the lowest interlaboratory variation (CV = 9.4% excluding two outliers). As fluorescence spectroscopy does not suffer from problems associated with interference from lipids, this seems to be the most useful broad fraction technique for laboratories analysing samples of water, sediments and biota. It has been stated that the UVF method is subject to interference by other naturally occurring fluorescing compounds as well as those present in oil. There is little evidence from this study that this was a problem, although laboratory 6 has reported observing a correlation between values for hydrocarbon concentrations and chlorophyll measurements in estuarine and coastal sediments. Work at the authors' laboratory using a variety of natural samples, including extracts of algal cultures, suggests that at the wavelengths selected for the IGOSS method the degree of interference which would be encountered in water or sediment samples from naturally occurring compounds is very small, i.e., the observed fluorescence is caused by oil-derived compounds. The same cannot be said about biota samples and, in the case of animal extracts at concentrations of 10 $\mu g\ g^{-1}$ wet weight and below, considerable interferences may be encountered. It

must be recognised, however, that the results are equivalents, i.e., that the standard chosen is arbitrary and almost certainly does not reflect the qualitative distribution of aromatic hydrocarbons associated with the samples. Coupling this technique with synchronous excitation/emission fluoroscence scanning and/or GC and GC/MS will supply the necessary qualitative information.

With regard to the determination of concentrations of specific hydrocarbons, one problem which was identified by a number of participants in this exercise was the availability of standards. As previously mentioned, hydrocarbon oils are extremely complex mixtures of compounds, particularly of isomers of aromatic compound types. When analysed by GC/MS in the mass fragmentographic mode, response factors are determined for those compounds which it has been possible to obtain, but often only one isomer, and sometimes none at all, can be obtained in each compound class. This problem was investigated during a previous bilateral exercise (Grahl-Nielsen et al., 1978) and a variation of 50% in response factors was found for three isomeric C_2-naphthalenes. While this will not explain fully the variations found in, e.g., the determination of aromatic hydrocarbon concentrations in the standard oil (up to a factor of 4), it must be a contributing factor. Comparing results from laboratories who were using similar methods and identical instrumentation (Nos. 1 and 14), it can be seen that for the naphthalene and phenanthrene type compounds, variations are always <50%, although the agreement between dibenzothiophene derivatives is poor.

In the few cases where parallel determinations of specific aromatic hydrocarbons were determined by both GC/MS and HPLC (Table 9, Laboratories 7 and 18, respectively; Table 7, Laboratories 1, 2, 14 and 18), the HPLC determinations always yield higher results, often around an order of magnitude. This may reflect the lower resolution available with liquid chromatographic columns and consequently lesser degree of specificity in the assignment of peak identities in very complex mixtures.

This exercise has provided useful information on the range of methods in use both for extraction and analysis, and of the spread of results obtained from them.

An international intercomparison exercise of this size obviously requires a huge input of time and effort, by all the participants as well as the organising laboratories. At least one analyst reported devoting 1 man month to the analyses. Thus, the dispatch costs of ca. £50 per participant are only a small proportion of the total costs involved in the conduct of such an exercise and such an input of resources requires that some progress be made towards greater comparability between results generated at different laboratories.

It is to be hoped that the participating laboratories will consider their techniques in the light of experience gained from this exercise, and continue to strive for improvements in technique wherever possible. However, experience with other exercises suggests that in many cases, unless positive steps are taken to encourage this, little further action will be taken by the analysts concerned. Accordingly, the following conclusions and recommendations are made.

CONCLUSIONS

1. Where laboratories use a single, comparable, relatively simple method, the level of agreement achievable is good for total hydrocarbons.

2. The use of a contaminated sediment sample for intercomparison studies has proved realistic and viable in this particular case. It should, however, be noted that the presence of large amounts of atmospheric particulates in sediments may cause sample inhomogeneity especially with regard to their content of polycyclic aromatic hydrocarbons. Depending on the particle-size of the sediment, sieving to a lower mesh size may be necessary, but 1.4 mm appeared adequate for the sample used in this study. For this exercise, the sediment was oven-dried to make it easier to homogenise, and in order to minimise changes during storage. In a future study, it would be more realistic to circulate wet sediments if possible.

3. Biological materials appear to pose more problems. Possibly this is due to sample variability, but greater analytical problems may also be the cause.

4. Analysts appear, at least on a one off basis, to be prepared to operate to a single reference standard. Although permitted, few analysts also reported results with reference to their personal choice of standard.

RECOMMENDATIONS

The results of this exercise point the way to a number of possible improvements and a further exercise on more restrictive lines would be worthwhile. Among other items, the exercise could usefully contain the following features:

1. Using a sediment sample, total quantitative estimation could be carried out based on both a common standard oil and (if different) the analyst's normal choice, using UVF at specified excitation and emission wavelengths plus, if desired, any other wavelengths used by the analyst. This should allow an assessment of the degree of comparability for 'total' oil and a study of the degree of variation likely to be introduced by the use of different oils.

2. Analysis could be done of a sediment sample and an oil sample and, if possible, a water sample or extract, for specific compounds using capillary GC and/or GC/MS. The compounds to be analysed should be agreed by the Marine Chemistry Working Group. It would be a useful aid to assessing GC and GC/MS results if a standard solution were circulated and analysed in addition to the samples, so as to facilitate the interpretation of interlaboratory differences. To overcome the different isomer response problem and the difficulty of obtaining standard materials, the necessary standards should be made available centrally, e.g., by volunteer supply of one or more standards from a number of laboratories. It is suggested that at least 10 compounds and one or more classes of compounds be specified. Suitable examples might be:

n-pentadecane	naphthalene	C_1-naphthalenes
n-heptadecane	phenanthrene	C_2-naphthalenes
pristane	anthracene	C_1-phenanthrenes
n-octadecane	fluoranthene	
phytane	pyrene	
n-pentacosane		

3. Provided adequate assurance can be made about the homogeneity of a biological sample, it would be useful to include such a material. Analysis for total oil should be by UVF at specified wavelengths after extraction by a specified method with quantitation relative to a common standard oil and, if different, the analysts' normal choice. Analysis for specified compounds should be conducted by capillary GC and/or GC/MS according to the same conditions as specified in (2) above.

4. If some means can be identified of investigating the differences in order of magnitude results obtained for aromatic hydrocarbons by HPLC and GC/MS methods, such a study should be incorporated for the few laboratories which could participate (few, because of the limited availability of equipment needed).

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APPENDIX 1

PRINCIPLES UNDERLYING THE ANALYTICAL METHODS

Oil is a complex and variable mixture of compounds. Although the majority are hydrocarbons, heterocyclic compounds and metal complexes are also present. These compounds exhibit a wide variety of chemical and physical properties, and for this reason there is no method available which will determine 'Total Hydrocarbon Content' by detection of all the materials present. Most analytical techniques applied to estimations of total oil measure either classes of compounds or specific functions of a wide range of compounds, and this measurement is extrapolated to give a THC, or Total Hydrocarbon Concentration. If a crude oil, e.g., Ekofisk crude oil, is used as a standard, then this value may be expressed as 'Ekofisk crude oil equivalents', i.e., 'X $\mu g\ g^{-1}$ Ekofisk crude oil equivalents' means that, given the analytical method used, the sample yielded a signal equivalent to a concentration of X $\mu g\ g^{-1}$ of Ekofisk crude oil. Similarly, if a single aromatic compound, e.g., chrysene is used, the THC value is expressed in terms of chrysene equivalents. Neither method of quantification reveals anything about the qualitative content of either sample or standard, both are purely arbitrary definitions of the quantity of oil present. If the standard chosen and oil present are very different in composition, significant under or over estimates will result.

The major analytical methods used in this study were as follows:

1. Infrared spectrophotometry

This method involves the measurement of light absorption at one or more wavelengths within the 3-4 μm region, corresponding to the C-H stretching frequencies of $\geq CH$, $> CH_2$ and $-CH_3$ groups within molecules. Solvents used for IR determinations must not absorb light within this region so as not to mask sample absorption. Consequently, solvents which do not contain any C-H functions (i.e., carbon tetrachloride, various Freons) are usually used. Most of the CH, CH_2 and CH_3 groups present will occur in aliphatic compounds, and aromatic compounds are not normally determined by this method. Lipids are predominantly aliphatic and, when sediment and biota samples are analysed, any lipid remaining after sample clean-up will enhance the apparent oil concentration.

2. Ultraviolet spectrophotometry

Oils absorb strongly in the ultraviolet region, owing to their aromatic content. The hydrocarbon content is calculated by measuring the absorption of light at a wavelength within the range 225 to 325 nm (usually 254 nm); this response is sensitive only to aromatic hydrocarbons. The amount of qualitative data generated by this technique is small (beyond an indication of the mere presence of aromatic hydrocarbons of some sort).

3. Fluorescence spectroscopy

Fluoroscence differs from the two methods detailed above in that it does not involve merely the absorption of light. When a compound fluoresces, it absorbs light at a particular wavelength and then immediately emits light at a longer wavelength. The fluorescence exhibited by an oil is due to its content of aromatic hydrocarbons. Since for the different oils this is variable, the accuracy of

quantification is very dependent on the standard used. Fluorescence is, however, more sensitive than IR and UV spectrophotometry, and is often the method of choice for low-level determinations of oil in seawater. This technique is also insensitive to the presence of lipids, and samples may be analysed prior to clean-up.

Synchronous scanning of excitation and emission (with a fixed wavelength offset) yields spectra in which the position of the fluorescence bands corresponds to the number of fused rings in the fluorescing compounds. This method may be used as a qualitative extension to the quantitative use of fluorescence emission.

4. Gravimetry

Gravimetric determinations involve a solvent extraction of the sample, followed by evaporation and weighing of the sample extract usually following a clean-up step. Losses of volatile hydrocarbons are heavy during the solvent evaporation stage, and this method is therefore only suitable for heavier oils and hydrocarbons. Similar problems may be encountered with any technique which involves sample concentration unless precautions are taken.

5. Gas-liquid chromatography

GLC with a flame-ionisation detector is generally used as a qualitative method for investigating the major component composition of samples, and for observing the presence of biogenic hydrocarbons. Packed column GLC is sufficient for those methods involving quantitation of total hydrocarbons by the integration of whole chromatograms, although the superior resolution of capillary columns is required for the quantitation of specific n-alkanes, pristane and phytane.

6. High-performance liquid chromatography

HPLC as a separation technique is similar in performance to packed column GLC, although it has the advantage of a wider range of useful detectors.

7. Gas chromatography - mass spectrometry

This is at present the most powerful technique for the analysis of hydrocarbons, particularly when capillary GLC columns are used. Its major use is in the unequivocal identification of specific hydrocarbons and their quantification, even in the presence of much larger quantities of other compounds (e.g., aromatic hydrocarbons in oil in the presence of large quantities of aliphatic hydrocarbons). The use of deuterated hydrocarbon standards permits compensation for extraction efficiencies within each sample.

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23	Netherlands	Dr P. Hoogweg, Governmental Institute for Sewage Purification, Maerlant 4-6, 8224 AC Lelystad.
24	Finland	Dr F. Koroleff, Institute of Marine Research, POB 166, 00141 Helsinki 14.
25	Finland	Dr K. Haapala, National Board of Waters, POB 250, 00101 Helsinki 10.
26	USA	Dr R.W. Risebrough, Bodega Marine Laboratory, University of California, POB 247, Bodega Bay, California 94923.
27	USA	Dr J.W. Farrington, Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543.
28	USA	Dr G.C. Lawler, Centre for Bio-Organic Studies, University of New Orleans, Lake Front, New Orleans, Louisiana 70122.
29	Ireland	Dr C. Murphy, The State Laboratory, Upper Merrion Street, Dublin 2.
30	Norway	Dr T. Haegh, Continental Shelf Institute, Hakon Magnussonsgt 1B, POB 1883, 7001 Trondheim.
31	Sweden	Dr S.R. Carlberg, Fisheries Board of Sweden, Laboratory of Hydrographic Research, Box 2566, 403-17 Göteborg.

Laboratory No.	Country	
32	Canada	Dr D. Cossa, INRS, Oceanologie Université du Quebec, 310 Ave des Ursulines, Rimouski, Quebec G5L 3A1.
33	Netherlands	Dr J. Duinker, Netherlands Institute for Sea Research, POB 59, Den Burg, 1790 Texel.
34	Bermuda	Dr A.H. Knap, Bermuda Biological Station, St Georges.
35	Denmark	Dr I. Andresen, National Institute for Testing and Verification, Amager Boulevard 115, DK-2300 Copenhagen S.
36	Portugal	Dr J.D. Calejo Monteiro, Dept. of Environmental Control, Trav. Legua da Povoa n°1-1°Dr°, 1200 Lisbon.

Table 1 Results of the characterisation of the mussel homogenate

a) Trace elements ($\mu g\ g^{-1}$ dry weight). Based on 50 replicate analyses.

ELEMENT	MEAN	SD	CV (%)	MINIMUM	MAXIMUM
Aluminium	270	52	19	173	374
Cadmium	2.08	0.04	1.9	1.99	2.18
Chromium	2.15	0.08	3.8	1.91	2.36
Copper	12.8	0.3	2.1	12.2	13.8
Iron	450	18	4.1	403	481
Manganese	26.8	0.7	2.6	24.8	28.2
Nickel	6.84	0.17	2.4	6.37	7.24
Lead	9.11	0.55	6.1	7.94	10.2
Zinc	135	3	2.0	126	142

b) Total hydrocarbons ($\mu g\ g^{-1}$ dry weight) and polychlorinated biphenyls ($ng\ g^{-1}$ dry weight)

LABORATORY	A	B	C	D
Saturates	61±17 (6)	87± 5 (10)	111±13 (4)	87± 27 (3)
Aromatics	21± 4 (6)	67± 13 (10)	38± 9 (4)	41± 6 (3)
PCBs	470±45 (12)	895±273 (10)	412±23 (4)	510±140 (3)

c) Polycyclic aromatic hydrocarbons ($ng\ g^{-1}$ dry weight)

LABORATORY	A	B	C
Naphthalene	4.8± 1.9	96 ±118	2.8± 0.8
C_1N	3.0± 1.0	20 ± 15	4.0± 2.6
C_1^1N	10 ± 5	6.8± 8.2	6.5± 2.0
C_3^2N	14 ± 3	0.7± 0.7	3.0± 2.5
Phenanthrene / Anthracene	13 ± 5	32 ± 40	7.9± 1.6
C_1P	15 ± 4	3.4± 7.5	15 ± 1.4
C_2^1P	38 ±15	68 ± 60	58 ±14
Dibenzothiophene	1.3± 0.4	ND	ND
C_1D	6.4± 2.4	3.4± 7.5	ND
C_1^1D	21 ± 9	68 ± 60	ND
C_3^2D	32 ±14	6.2± 6.1	NR
Fluoranthene	56 ±18	42 ± 37	80 ±12
Pyrene	46 ±13	34 ± 31	92 ±14
Benz(a)anthracene / Chrysene	29 ± 6	28 ± 32	47 ± 6

ND - not detected
N - naphthalene
P - phenanthrene
D - dibenzothiophene

NR - not reported
C_1 - methyl derivatives
C_2^1 - dimethyl and ethyl derivatives
C_3^2 - trimethyl, methyl ethyl, and propyl derivatives

In 1b, the number in parenthesis represents the number of determinations made.

Table 2 Methods used by each participating laboratory

LABORATORY NUMBER	ANALYTICAL METHODS USED	EXTRACTION METHOD	CLEAN-UP OR FRACTIONATION METHODS USED	
1	UVF, GC, GC/MS	Methanolic KOH digestion	Column chromatography, alumina over silica gel	
2	UVF, GC, GC/MS	Chloroform/methanol extraction	Column chromatography, silicic acid and sephadex LH-20	
3	UVF, GC	Dichloromethane/methanol extraction	Column chromatography, silicic acid	
4	GRAVIMETRY, GC	Soxhlet extraction (hexane)	None	*
5	IR	Soxhlet extraction (dichloromethane, 24h)	Column chromatography, florisil	*
6	UVF	Chloroform/methanol extraction	Column chromatography, spongy copper then silica gel	*
7	UVF, GC, GC/MS	Soxhlet extraction (acetone, 6h) " " (methanol/10% benzene, 36h)	Column chromatography, copper powder then silica gel Column chromatography, silica gel	* ✓
8	UVF, GC	Soxhlet extraction (hexane, 5h)	Column chromatography, freshly prepared copper	*
9	HPLC	Soxhlet extraction (pentane, 5h)	Column chromatography, florisil	
10	GRAVIMETRY, GC Methanolic KOH digestion, carbon tetrachloride extraction IR Carbon tetrachloride extraction UVF Cyclohexane extraction	Chloroform extraction	Column chromatography on copper, thin layer chromatography Thin layer chromatography Column chromatography, florisil None	* ✓
11	IR UVF	Chloroform extraction Homogenised with acetone/pentane after freeze-drying	None Column chromatography, florisil	* ✓
12	GC	Soxhlet extraction (methanol/benzene, 24h) Extracted with acetone/pentane after freeze-drying	Column chromatography, silica gel Column chromatography, florisil and silica gel	* ✓
13	UVF IR	Methanolic KOH digestion Soxhlet extraction (carbon tetrachloride)	None Column chromatography, alumina	* *
14	GC, GC/MS	Methanolic KOH digestion	Column chromatography, silica gel	
15	IR GC Methanolic KOH digestion	Soxhlet extraction (carbon tetrachloride) Soxhlet extraction (cyclohexane)	Column chromatography, florisil Column chromatography, silica gel; HPLC Column chromatography, silica gel; HPLC	* * ✓
16	IR, UVF	Chloroform/methanol extraction	Methanolic KOH hydrolysis. Column chromatography, alumina and silica gel	
17	GC	Methanolic KOH digestion, pentane extraction	Column chromatography, alumina and silica gel	
18	HPLC	Ethanolic KOH digestion, iso-octane extraction	Column chromatography, florisil	
19	UVF	Dichloromethane extraction	Column chromatography, alumina	*
20	UVF	Hexane extraction	Column chromatography, silica gel; copper	
21	IR	Soxhlet extraction (carbon tetrachloride, 16h)	None	
22	GC	Soxhlet extraction (pentane/diethyl ether, 8h)	Column chromatography, alumina and silica gel	

Cont'd.

Table 2 contd

LABORATORY NUMBER	ANALYTICAL METHODS USED	EXTRACTION METHOD	CLEAN-UP OR FRACTIONATION METHODS USED
23	IR	Soxhlet extraction (carbon tetrachloride)	Column chromatography, florisil
24	GC	Ultrasonic extraction (hexane, 5h)	Column chromatography, alumina and silica gel
25	IR	Soxhlet extraction (carbon tetrachloride, 24h)	Column chromatography, alumina
26	GC	Hexane extraction	Column chromatography, florisil

* Sediment only

/ Mussel homogenate only

UVF Ultra-violet fluorescence spectroscopy

GC Gas chromatography

GC/MS Gas chromatography/mass spectrometry

HPLC High performance liquid chromatography

KOH Potassium hydroxide

Table 3 Results of total hydrocarbon analyses of sediment samples (/ug g^{-1} Ekofisk oil equivalents)

a) Fluorescence spectroscopy

LABORATORY NUMBER	Ex (nm)	Em (nm)	RESULTS	MEAN	SD[1]
1	310	360	36, 37, 39, 42	38.5	2.3
2	310	360	36, 37	36.5	
3	310	360	35.8, 36.4	36.1	
6	310	360	13.6, 14.8	14.2	
7	310	360	33.8, 33.9, 34.5, 38.4	35.2	1.9
8	310	360	29.5, 32.8	31.2	
10	310	360	38.0	38.0	
	340	360	56.0	56.0	
	420	450	70.8	70.8	
13	310	360	30, 30, 30, 36	31.5	2.6
	310	360	33, 35, 35, 41	36.0	3.0
16	340	460	22.0	22.0	
19				29.5	4.5 *
20	310	360	16.3, 16.8	16.5	
Overall mean	33.5 (SD = 10.5, CV = 31.3%, n = 34) /All above values/				
Overall mean	32.3 (SD = 7.7, CV = 23.8%, n = 27) /IGOSS method only/				
6	310	360	23.1, 24.7, 30.3, 37.7, 36.3	30.4	5.9
Overall mean	33.2 (SD = 6.1, CV = 18.4%, n = 30) /IGOSS method only, substituting later results for laboratory 6/				

b) Infrared spectrophotometry

LABORATORY NUMBER	ABSORBANCES MEASURED (cm^{-1})	RESULTS	MEAN	SD[1]
5		15.0	15.0	
10	2925	16.2	16.2	
11	2925	63.0, 73.0	68.0	
13	2925	22, 22, 23, 24	22.8	0.8
15		23	23.0	
16	2850, 2925, 2960	93.6	93.6	
21	2925, 2960	39, 44, 53, 54, 56, 57, 79, 85	58.0	16
23	2925	54, 59	56.5	
25	2925, 2960	11, 11, 12, 16, 20	14.0	4.0
Overall mean	41.0 (SD = 25.0, CV = 61.0%, n = 25)			
Overall mean	28.1 (SD = 22.3, CV = 79.4%, n = 15) /After removal of results of laboratories 11 and 21, who performed no clean-up/			

Cont'd.

Table 3 contd

c) Gas chromatography

LABORATORY NUMBER	RESULTS	MEAN	SD[1]
14	30	30	
15	19	19	
17	26, 27, 27, 28, 33, 36	29.5	3.7
24	10.1	10.1	
Overall mean	26.2 (SD = 7.2, CV = 27.5%, n = 9)		

d) Gravimetry

LABORATORY NUMBER	RESULTS	MEAN
4	130, 170, 190	165
10	15.6	15.6
Overall mean	126 (SD = 68, CV = 54.0%, n = 4)	

SD - standard deviation (σ_n)

CV - coefficient of variation

1 - SD quoted only for four or more replicate measurements

Ex - excitation wavelength

Em - emission wavelength

* - individual values not reported

Table 4 Results of total hydrocarbon analyses of mussel homogenate ($\mu g\ g^{-1}$ wet flesh weight Ekofisk oil equivalents)

a) Fluorescence spectroscopy

LABORATORY NUMBER	Ex (nm)	Em	RESULTS	MEAN
1	310	360	55	55
3	310	360	17.4, 19.5	18.4
7	310	360	98	98
10	310	360	82	82
	340	360	96	96
	420	450	74	74
11	310	360	32, 32	32
16	340	460	130	130
20	310	360	86, 90	88
Overall mean	67.7 (SD = 34.4, CV = 50.8%, n = 12)			

b) Infrared spectrophotometry

LABORATORY NUMBER	RESULTS
5	<620
10	270
16	6011

c) Gas chromatography

LABORATORY NUMBER	RESULTS	MEAN
15	28	28
17	39, 53	46
Overall mean	40	

d) Gravimetry

LABORATORY NUMBER	RESULTS
10	256

Table 5 Conditions used in gas chromatographic analysis by the participating laboratories

LABORATORY NUMBER	ANALYSIS	COLUMN	INJECTION MODE	TEMPERATURE (°C) INITIAL	TEMPERATURE (°C) FINAL	PROGRAMME RATE (°C per minute)	CARRIER GAS	FLOW RATE (mls per minute)
1	GC	20m x 0.3mm ID SE-54	On-column	60	250	5	Hydrogen	1.7
	GC/MS	20m x 0.2mm ID SE-54 (Quartz)	Splitless	100	250	6	Helium	1.3 *
3	GC	15m (SE-52	Split (5:1))	70	80	5	Helium	
		(OV-101	Split (27:1))		270	2.5		
4	GC	25m OV-1		40	270	5	Nitrogen	
6	GC	20m x 0.2mm ID OV-101	Split (5:1)	50	250	5	Nitrogen	
7	GC and GC/MS	30m x 0.3mm ID OV-101		70	260	2	Hydrogen	2.5
8	GC	25m x 0.2mm ID OV-101		100	260	4	Nitrogen	
10	GC		Splitless	60	320	10	Helium	
12	GC	50m x 0.3mm ID OV-101	Splitless	115	285	4	Hydrogen	2
14	CC	2m glass SP2100		80	300	8	Nitrogen	17
	GC/MS	25m x 0.22mm ID SP2100 (Quartz)	Splitless	100	230	6	Helium	2 *
15	GC	1m 3% Dexsil 400		50	350	24	Nitrogen	15
	GC	20m x 0.31mm ID OV-1	Splitless	50	250	2	Hydrogen	
17	GC	1.8m glass 3% OV-1		85	275	4	Nitrogen	30
	GC	62m SP2100	Splitless	85	275	4	Nitrogen	1.5
20	GC	6ft stainless steel. 3% Dexsil 300		75	320	6		
	GC and GC/MS	30m OV-101	Splitless	50	220	4		
22	GC	Packed column. 1% OV-1		100	330	8	Nitrogen	30
	GC	25m x 0.3mm ID SE-30	Split (10:1)	100	285	5	Nitrogen	1.3
24	GC	25m (Quartz)		80	260	12		
26	GC	30m SP2100 or SE-54	Splitless	50	110	10		
					270	3.5		

* Direct GC/MS connection with no interface. GC column terminates in ion source of MS.

Table 6 Aliphatic hydrocarbons in Ekofisk oil ($\mu g\ g^{-1}$)

LABORATORY NUMBER	1	2		3		8	10*	15	17	22	24	26
nC 7											22 900	
nC 8							8 800				19 200	
nC 9							8 130				14 200	
nC10							8 310				11 700	
nC11	7 540			11 778			7 290				10 200	
nC12	7 190			10 638		7 070	7 590				10 200	7 600
nC13	6 740			9 118		6 260	6 320				8 100	8 200
nC14	6 070			7 789		9 290	8 430				7 500	8 000
nC15	5 790	2 139	2 313	6 776	6 500	7 680	6 020				7 100	8 600
nC16	4 920	2 062	2 188	5 857	5 741	6 870	5 360			4 700	6 200	7 500
nC17	5 330	1 867	1 964	4 837	4 906	6 570	4 820				5 300	7 300
Pristane	1 770	949	961	2 090	1 827	2 830	2 590				1 920	3 100
nC18	4 530	1 453	1 516	4 503	4 357	5 450	4 150			3 500	4 800	5 800
Phytane	1 560	656	668	1 805	1 472	2 420	1 810				590	2 200
nC19	3 300	1 323	1 371	3 910	3 868	5 350	4 220				4 100	5 900
nC20	2 950	1 199	1 251	3 673	2 742	5 050	3 370			2 400	3 400	4 400
nC21	2 630	1 089	1 135	3 261	2 550	3 540	3 010				3 000	3 800
nC22	2 570	1 008	1 189	2 776	2 334	3 540	2 350			1 600	2 200	3 300
nC23	2 330	924	998	2 659	2 213	3 130	2 220				1 400	3 200
nC24	2 090	747	774	2 327	2 021	2 530	1 990			1 400	1 000	2 700
nC25	1 950	680	667	1 931	1 732	2 530	1 860				720	2 300
nC26		482	448	1 504	1 395	2 220	1 750				510	2 000
nC27		318	326	1 393	982	1 720	1 260				300	1 600
nC28		256	250	1 251	808	1 620	1 020			700	200	1 400
nC29		224	217	1 243	751	1 620	960				120	1 200
nC30		179	171	1 140	616	1 520	660				70	1 000
nC31		173	168	1 029	494	1 210	580					820
nC32		169	164	776	388	1 920	310			500		
nC33		147	140	633	388							

Cont'd.

Table 6 contd

LABORATORY NUMBER	1	2		3		8	10*	15	17	22	24	26
17/Pr	3.01	1.97	2.04	2.31	2.68	2.32	1.86	2.37	2.17		2.76	2.37
18/Ph	2.90	2.21	2.27	2.49	2.96	2.25	2.29	2.08	2.57		8.14	2.68
Pr/Ph	1.13	1.45	1.44	1.16	1.24	1.17	1.43	1.05	1.45	1.48	3.25	1.41

17/Pr Ratio of the peak heights of n-heptadecane and pristane

18/Ph Ratio of the peak heights of n-octadecane and phytane

Pr/Ph Ratio of the peak heights of pristane and phytane

* Results submitted following the circulation of the draft report

Table 7 Aromatic hydrocarbons in Ekofisk oil ($\mu g\ g^{-1}$)

LABORATORY NUMBER	1	2		14	18
Naphthalene	970	247	251	811	
C_1N	3 200	928	853	3 585	
C_2N	4 100	3 686	3 897	3 959	
C_3N	3 000			1 985	
Phenanthrene	240	84*)	94*)	334	1 690
C_1P	700	227	244	868	
C_2P	400			270	
Anthracene	ND				
Dibenzothiophene	32				
C_1D	52			149	
C_2D	42			312	
C_3D	ND			163	
Fluoranthene					250
Pyrene					
Benz(a)anthracene					600
Chrysene					1 700
Triphenylene					4 000
Benzo(b)fluoranthene					70
Benzo(k)fluoranthene					30

*) Include anthracene

Table 8 (a) Aliphatic hydrocarbons in sediment (ng g^{-1})

LABORATORY NUMBER	1	2		3		6	8	12	15	17	22	24
nC12							8					11
nC13	4.7						3					16
nC14	7.9						12					26
nC15	16	35	40	9	26	17	10	1.9				33
nC16	17	38	38	14	34	22	15	7.4			10	35
nC17	46	68	73	48	84	)147	20	18.3				35
Pristane	32	100	106	24	39	)	15	10.4				41
nC18	23	42	41	35	61	) 79	25	15.5			80	31
Phytane	38	57	59	34	41	)	31	13.2				
nC19	19	42	44	36	55	34	18	16.5				52
nC20		51	47	44	46	29	15	18.5			140	50
nC21		45	43	59	58	17	15	21.5				68
nC22		42	36	102	124	18	15	17.7			110	107
nC23		41	41	241	304	36	13	21.6				151
nC24		49	46	458	623	16	15	20.1			70	192
nC25		59	65	605	752	54	15	32.4				214
nC26		66	71	718	896	50	14	38.2				190
nC27		84	93	611	819	30	20	74.3				131
nC28		62	69	501	738	25	14	44.9			40	92
nC29		98	111	471	576	59	16	74.5				41
nC30		59	66	235	367	29	15	48.8				26
nC31		68	80	232	260	ND		32.9				9
nC32		30	35	98	142	38					30	4
nC33		29	35	82	104	ND						
17/Pr	1.44	0.68	0.69	2.00	2.15		1.33	1.76	0.62	1.13		0.85
18/Ph	0.61	0.74	0.69	1.03	1.49		0.81	1.17	0.83	0.79		
Pr/Ph	0.84	1.75	1.80	0.71	0.95		0.48	0.79	0.90	0.44		

Table 8 (b) Aliphatic hydrocarbons in mussels (ng g^{-1} wet weight)

LABORATORY NUMBER	2		3		12	22
nC12						
nC13						
nC14						
nc15	71	48	49	67	40	
nC16	38	24	42	54	120	30
nC17	37	28	51	73	290	
Pristane	36	26	14	32	145	
nC18	16	14	32	39	242	150
Phytane	16	10	7.6	11	210	
nC19	16	12	19	31	100	
nC20	14	9	45	61	45	430
nC21	26	19	147	218		
nC22	29	28	410	356		430
nC23	29	26	723	515		
nC24	27	26	928	1330		180
nC25	32	30	962	1360		
nC26	36	32	976	1270		
nC27	38	37	911	1190		
nC28	38	46	687	972		<10
nC29	53	63	532	755		
nC30	33	34	357	501		
nC31	38	41	214	302		
nC32	26	25	116	202		<10
nC33	24	23	64	97		
17/Pr	1.03	1.08	3.64	2.28	2.00	
18/Ph	1.00	0.83	4.21	3.55	1.15	
Pr/Ph	2.25	2.60	1.84	2.91	0.69	

Table 9 (a) Aromatic hydrocarbons in sediment (ng g^{-1})

LABORATORY NUMBER	1	2	7	12	14	18			
Naphthalene	3.3	2			3.4				
C_1N	3.3	3			8.2				
C_2N	8.6	4			14.6				
C_3N	13				14.7				
Phenanthrene	19	19	4		16.2				
Anthracene	3.0				3.4				
C_1P	20	21			18.4				
C_2P	11				12.7				
Dibenzothiophene	1.8				ND				
C_1D	1.9				4.1				
C_2D	4.1				15.9				
C_3D	2.7				18.8				
Fluoranthene			7	20		72	73	88	129
Pyrene			6	20		ND	ND	ND	ND
Chrysene			16			165	162	121	206
Benzofluoranthene			34						
Benzo(a)pyrene			31			15.8	15.5	10.5	17
Benzo(e)pyrene						155	144	132	178
Triphenylene						ND	ND	ND	ND
Benz(a)anthracene						232	217	211	331
Benzo(b)fluoranthene						36.2	36.5	31	37
Benzo(k)fluoranthene						10.5	11	9	11
Benzo(ghi)perylene						83	78	70	74
o-phenylene pyrene						131	127	110	100

ND - not detected
N - naphthalene
P - phenanthrene
D - dibenzothiophene
C_1 - methyl derivatives
C_2 - dimethyl and ethyl derivatives
C_3 - trimethyl, methyl ethyl, and propyl derivatives

Table 9 (b) Aromatic hydrocarbons in mussel homogenate (ng g^{-1} wet weight)

LABORATORY NUMBER	1	12	14	18		
Naphthalene	3.4		1.8			
C_1N	8.3		1.7			
C_2N	21		3.6			
C_3N	51		3.2			
Phenanthrene	1.6		1.7	35	55	33
Anthracene	0.16					
C_1P	3.3		4.6			
C_2P	5.5		7.6			
Dibenzothiophene	0.29					
C_1D	0.71		0.8			
C_2D	1.7		6.8			
C_3D	1.0		7.6			
Fluoranthene				17	26	13
Pyrene		100		TR	TR	TR
Chrysene				36	40	34
Benzofluoranthene						
Benzo(a)pyrene				0.4	0.4	0.3
Benzo(e)pyrene				26	26	25
Triphenylene				21	19	19
Benz(a)anthracene				31	33	32
Benzo(b)fluoranthene				4.3	3.9	4
Benzo(k)fluoranthene				1.1	1.1	1.1
Benzo(ghi)perylene				6	5.8	5
o-phenylene pyrene				7.7	9.6	7.1

TR - trace
N - naphthalene
P - phenanthrene
D - dibenzothiophene
C_1 - methyl derivatives
C_2 - dimethyl and ethyl derivatives
C_3 - trimethyl, methyl ethyl and propyl derivatives

Table 10 Ring type analysis of aromatic hydrocarbons in oil, sediment and mussels ($\mu g\ g^{-1}$ chrysene equivalents)

LABORATORY NUMBER 9									
Ring number	Ekofisk oil			Sediment			Mussels		
1	1 900	2 000	2 000	0.01	0.01	0.01	0.13	0.09	0.10
2	11 600	11 600	11 800	0.12	0.14	0.13	0.65	0.51	0.66
3	13 000	13 000	13 400	0.18	0.23	0.25	1.05	0.76	1.03
4	6 100	6 200	6 000	0.12	0.19	0.22	0.87	0.58	0.75
5	1 915	2 200	1 800	0.16	0.25	0.33	0.31	0.21	0.34
Total	34 800	35 000	35 000	0.59	0.82	0.92	3.00	2.16	2.88

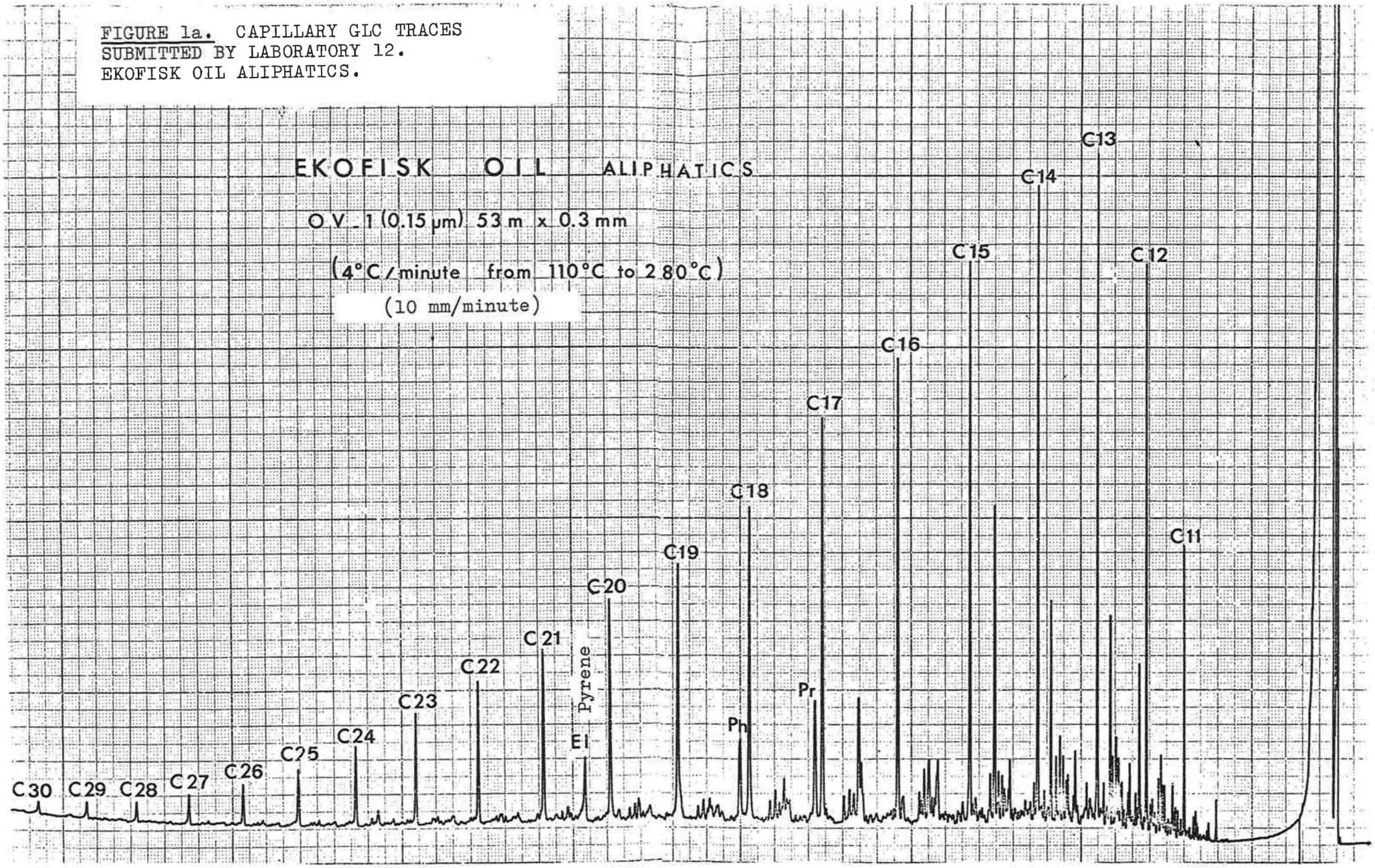


FIGURE 1a. CAPILLARY GLC TRACES SUBMITTED BY LABORATORY 12. EKOFISK OIL ALIPHATICS.

FIGURE 1b. CAPILLARY GLC TRACES
SUBMITTED BY LABORATORY 12.

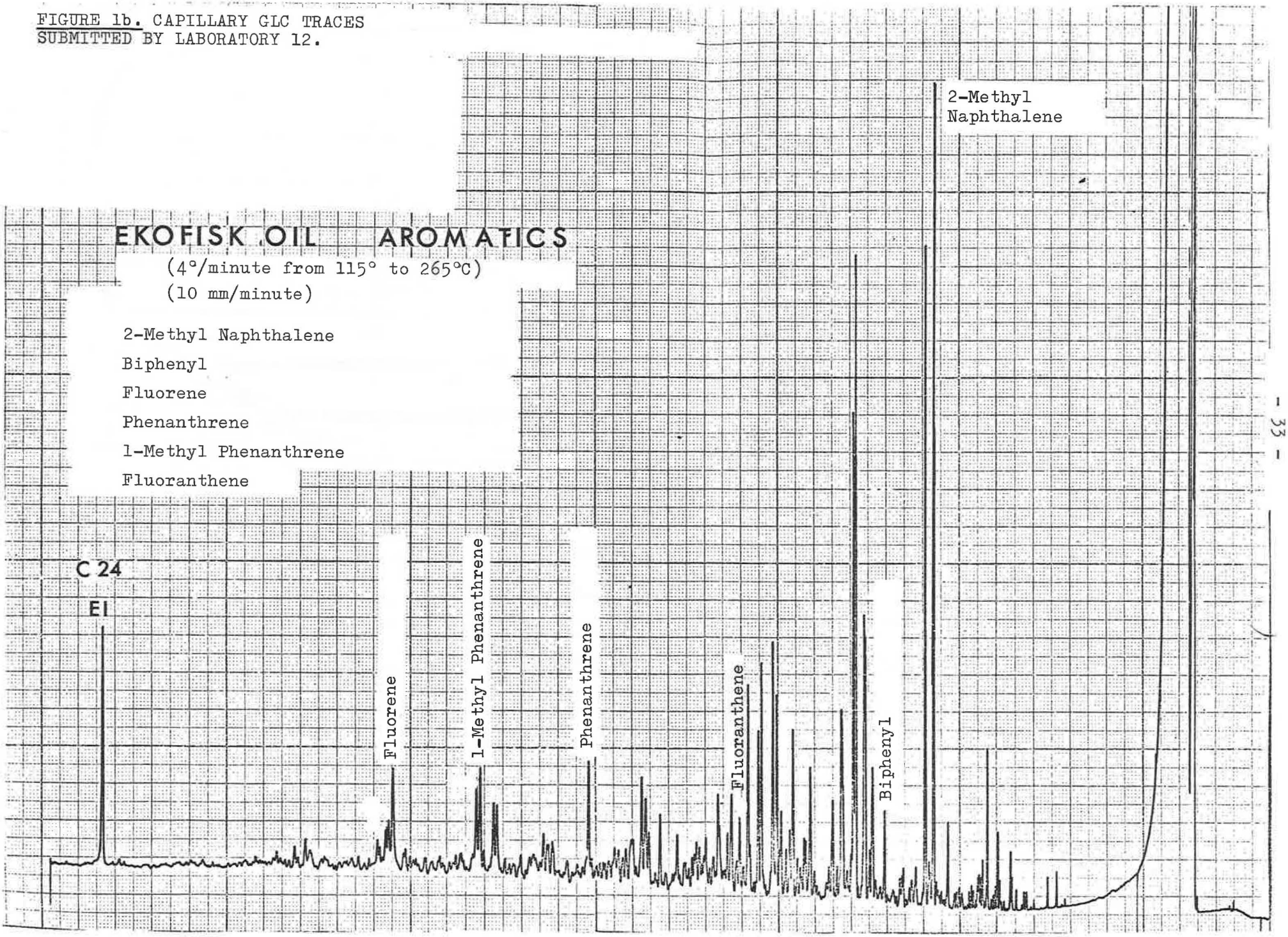


FIGURE 1c. CAPILLARY GLC TRACES SUBMITTED BY LABORATORY 12.

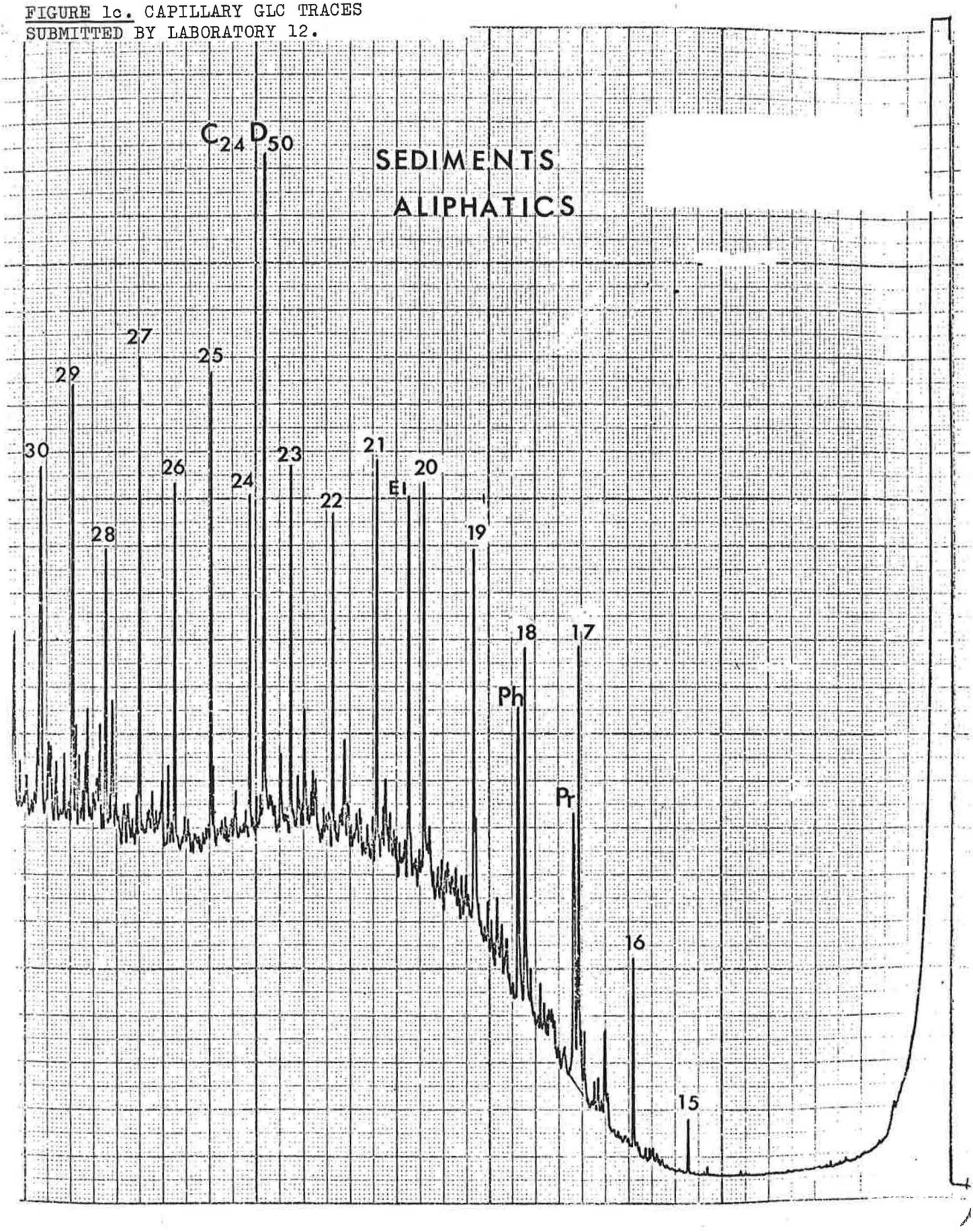
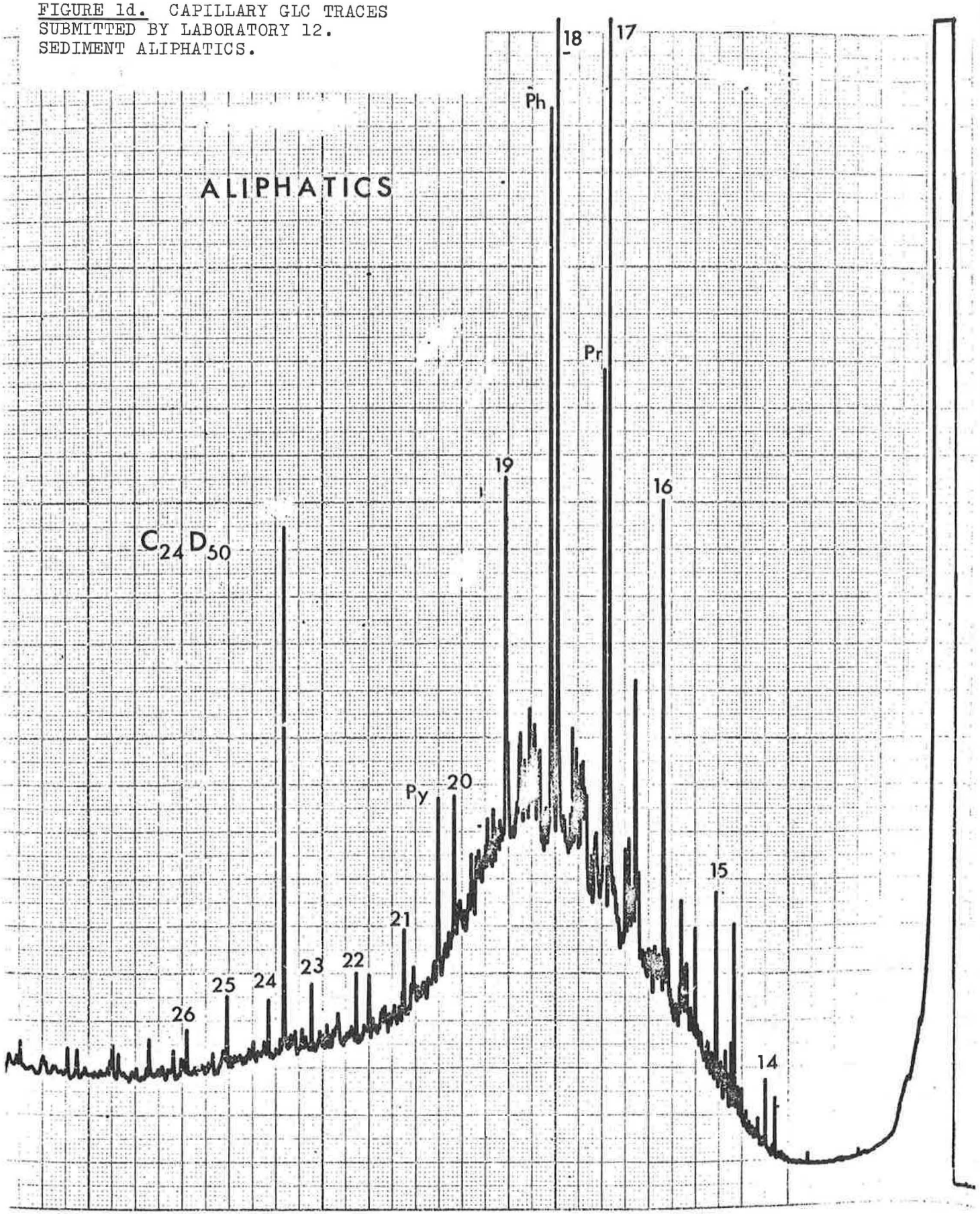


FIGURE 1d. CAPILLARY GLC TRACES SUBMITTED BY LABORATORY 12. SEDIMENT ALIPHATICS.



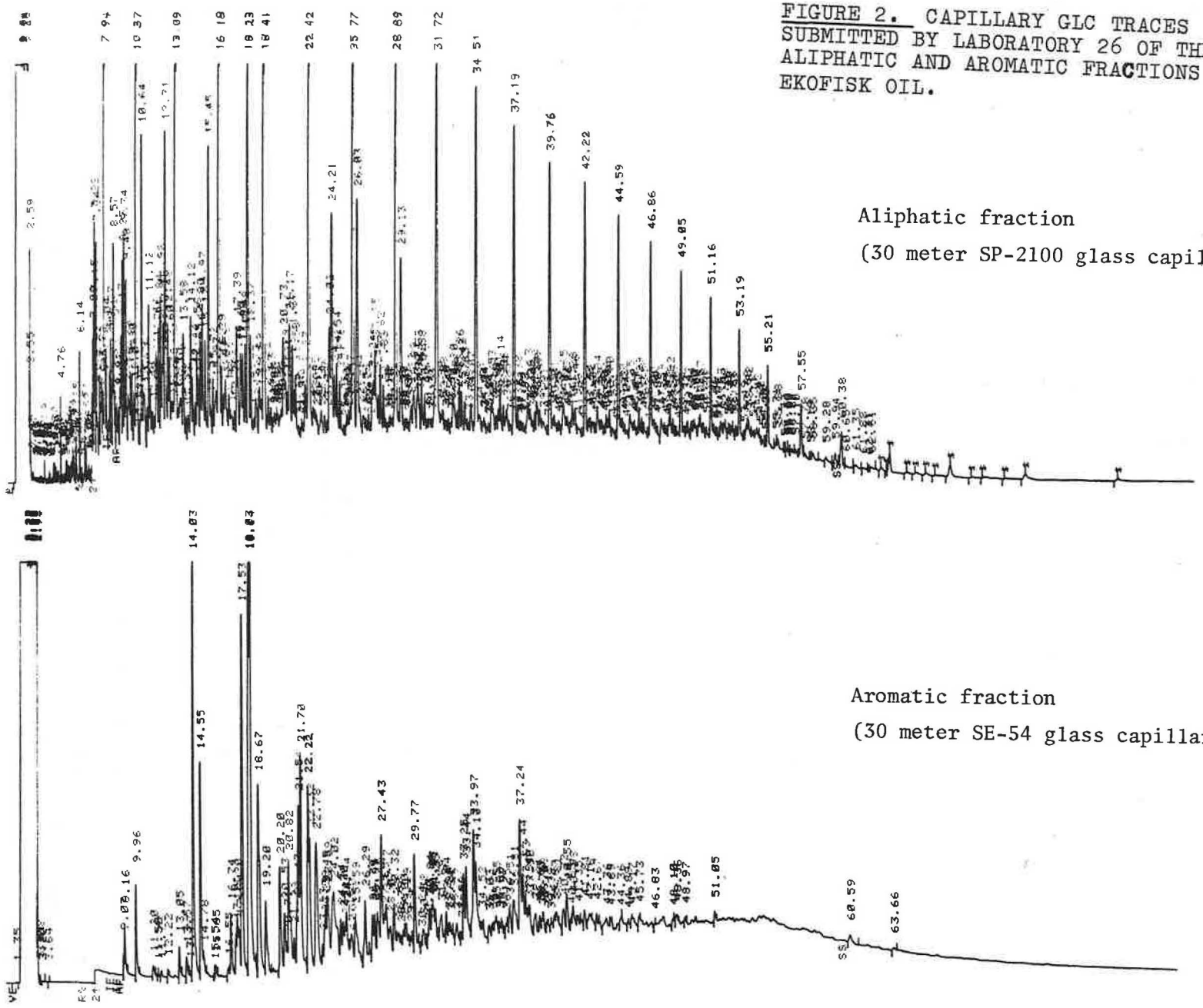
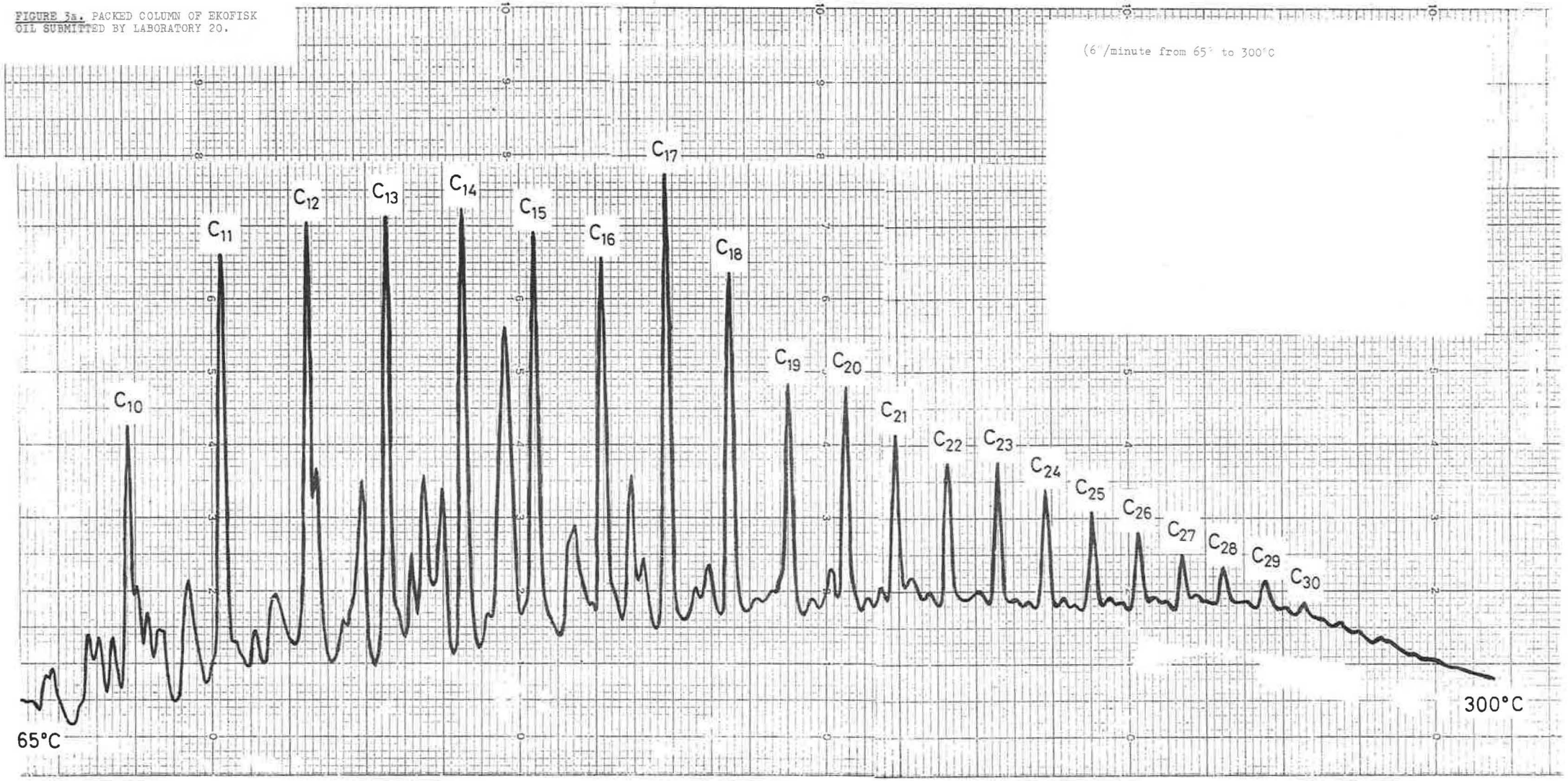


FIGURE 2. CAPILLARY GLC TRACES SUBMITTED BY LABORATORY 26 OF THE ALIPHATIC AND AROMATIC FRACTIONS OF EKOFISK OIL.

FID chromatograms of ICES weathered Ekofisk crude oil sample.

FIGURE 3a. PACKED COLUMN OF EKOFISK OIL SUBMITTED BY LABORATORY 20.



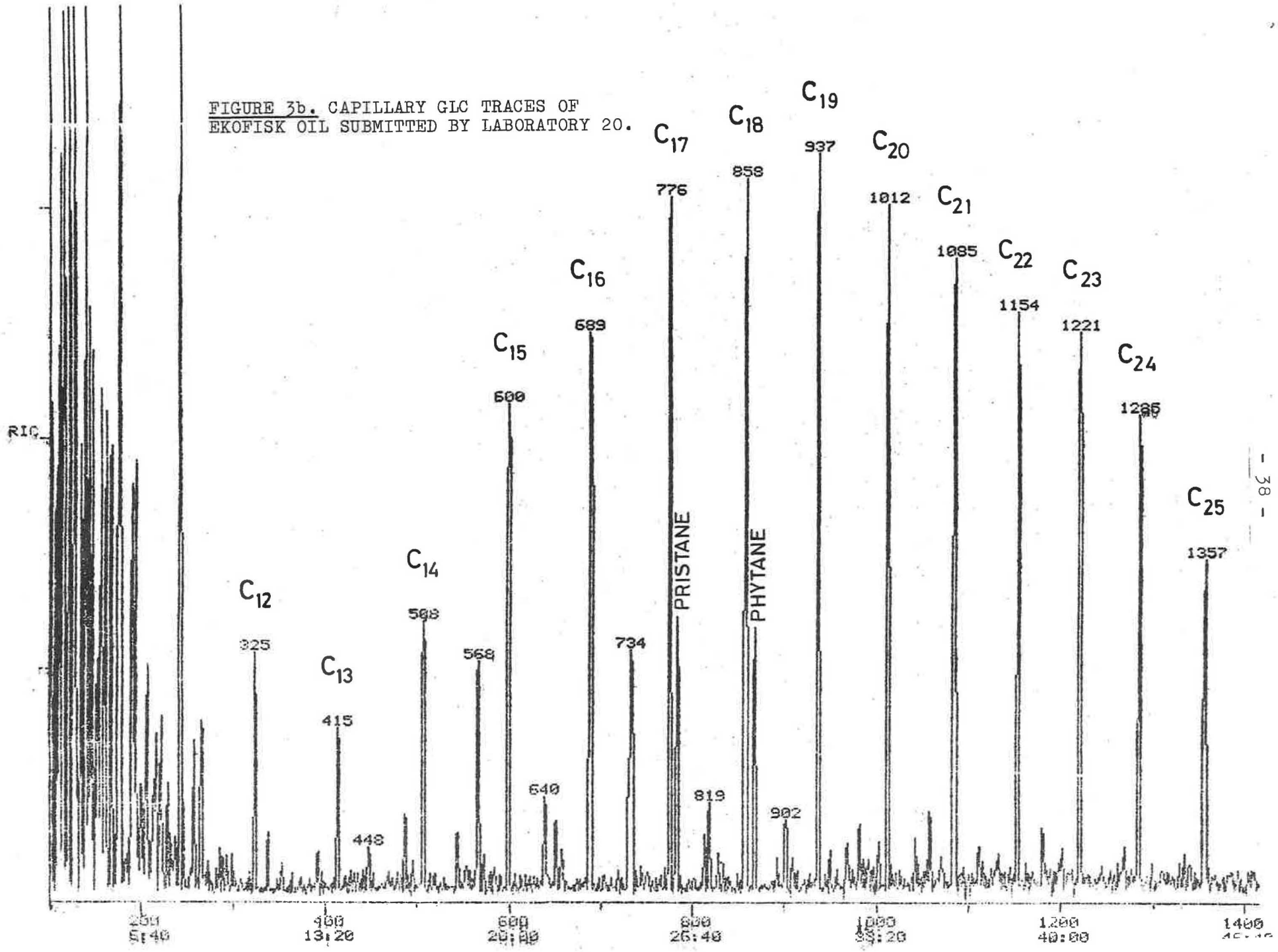


FIGURE 3b. CAPILLARY GLC TRACES OF EKOFISK OIL SUBMITTED BY LABORATORY 20.

ICES QUESTIONNAIRE ON SAMPLING AND ANALYSIS OF SEAWATER, SEDIMENTS AND BIOTA FOR TRACE ORGANIC COMPOUNDS

This questionnaire is intended to supplement the ICES petroleum hydrocarbon intercalibration exercise, for which samples have now been circulated. Samples of a marine sediment, a tissue sample, and a crude oil standard have been supplied to each participating analyst, who is free to choose his own method(s) of analysis, but is requested to relate his results to the standard oil if possible. By this means we hope to discover both the range of methods currently being applied by workers analysing oil in marine samples, and to investigate the comparability of results obtained both within and between the various methods used.

We are well aware that nobody likes completing questionnaires, but this has been designed to allow a fairly rapid method of answering to be used. As it should, if completed by all recipients, add substantially to the amount of information obtained from the inter-calibration programme, it is the sincere hope of the coordinator that all recipients will be able to find the 15-30 spare minutes it should take to complete.

GENERAL INFORMATION (please tick where applicable)

A Has your experience in the analysis of trace organic compounds been for samples collected in:

estuarine/nearshore/coastal

continental shelf

or oceanic areas?

B Please mark with a tick those compounds or classes for which you (or your laboratory) have analysed, and with a double tick (✓✓) those for which you routinely analyse.

chlorinated hydrocarbons:
- DDT and other insecticides
- PCB
- biogenic halogenated compounds

phthalates

petroleum hydrocarbons:
- total hydrocarbons
- n-alkanes
- biogenic hydrocarbons
- aliphatic/aromatic fractions
- polycyclic aromatic hydrocarbons

other (please specify)

THE REMAINING QUESTIONS RELATE SOLELY TO PETROLEUM HYDROCARBON ANALYSIS

I. GENERAL INFORMATION

(i) For what reason or reasons do you analyse petroleum hydrocarbons e.g., basic research, investigation of spills.

(ii) Please state type(s) of analysis used and usual determinands, include non-hydrocarbons if applicable, e.g., Ni, V, S etc.

(iii) Indicate with a tick which phase samples you normally examine for
Particulate
Dissolved/Dispersed
Surface film
Sediment incorporated
Other (state which)

II. SAMPLING

A Sample Size

What is your usual sample size in the analysis of
(i) Seawater
(ii) Sediment
(iii) Biota

B Physical Measurements

When sampling seawater, what general physical data do you collect at stations (e.g., sea state, temperature, salinity, etc.)

C Seawater Sampling

What sampling devices have you found satisfactory?
Please describe construction, materials and method of operation of novel devices, and include any relevant information (e.g., depth limitations).

D Sediment Sampling

If novel devices are used, please describe construction and method of operation. What sampling devices have you found satisfactory for collection of

(i) Surface sediments

(ii) Deep sediments or cores

E Contamination

What precautions are taken to avoid contamination of samples by:

(i) The sampling device

(ii) The sampling platform

F Blanks

How do you determine background or system blanks for your sampling procedures?

G Deficiencies

What do you consider to be the major deficiencies of the sampling procedures you have used? What would be desirable features of an improved system?

III. SAMPLE PREPARATION

A Seawater

Do you filter or centrifuge your seawater samples?

If so, is it

in situ

onboard ship/in the field

in the laboratory

If you use a Filter

What type of filter do you use?

Do you prefer vacuum

or pressure filtration?

Why?

How are filters cleaned before use?
How are filters stored until analysed?
How are filters extracted?
If you use centrifugation, please provide details of method and equipment used (speed, precautions, etc.).

Do you store seawater samples before or after* extraction?

---

* Delete as appropriate and state: Yes or No

If so, in what containers?
How are they cleaned?
How are the samples preserved?
What solvents do you use for extraction of samples (including purity)?
By what technique is sample extracted?
Do you use adsorbents to extract samples?
If so, what adsorbents?
How are they precleaned?
What volume is used per sample?
What flow rate is used?
How is the adsorbent extracted?
What is the extraction capacity of the adsorbent?

B Sediment and Biota

Are your samples dried before analysis?
If so, by what method?

Do you calculate dry weight for samples?
If so, by what method?

How are samples stored prior to analysis?
Is this before or after extraction?
How are samples extracted?
What solvents/reagents are used, including purity?
In the case of sediment samples, do you analyse particle size fractions?
If so, which?
In the case of biota, do you analyse individuals?
If bulked, what is usual number of individuals?

IV. ANALYSIS

A Clean-up

What clean-up methods do you use?
These may differ according to different methods of analysis you use; if so, state which clean-up is used for which method of analysis.

B How are Samples concentrated prior to Analysis?

C Instrumentation

Please tick analytical methods used, and supply details of columns, detectors, carrier gases, mobile phases etc. wherever applicable. Methods are grouped in approximate level of sophistication.

Gravimetric
Columetric
Colourimetric
IR
UV
UV fluorescence
(emission scanning)
(synchronous scanning)

HPLC
GLC - packed columns
- capillary columns
GC/MS
LC/MS
other (please specify)

If your standard technique involves the sequential application of a number of techniques, please indicate which and in what order.

D Detection Limit

What are your detection limits for "total hydrocarbon" measurements in:

(i) Seawater
(ii) Sediment
(iii) Biota

How do you define your "detection limit"?

V. OTHER COMMENTS

How do you deal with the problem of "patchiness" in the distribution of hydrocarbons in seawater?
Have you measured sampling variance?
Do you routinely determine analytical variance?
How do you estimate detection limits?
How do you determine extraction efficiencies and recoveries?
How do you determine blanks?
What standards do you use in your analytical work?
Do you have a standard reference oil?
If so, which?
If a single reference oil could be made available, would you be prepared to switch to that?

VI. ANY OTHER COMMENTS

If there is anything else you would like to add or comments you would like to make, please use the space below.

RESPONSES TO QUESTIONNAIRE ON SAMPLING AND ANALYSIS OF SEAWATER, SEDIMENTS AND BIOTA FOR TRACE ORGANIC COMPOUNDS

Following discussion at the 1979 meeting of the Marine Chemistry Working Group (MCWG), a questionnaire was developed with a view to establishing the range of experience and techniques available to analysts engaged in organics analysis and especially petroleum hydrocarbons. The design of the questionnaire was eventually completed following the 1980 meeting of the MCWG and was distributed late in August 1980 to all those who had participated in the petroleum intercomparison exercise, members of the MCWG and Marine Environmental Quality Committee (MEQC) of ICES and the ICES/SCOR Working Group on the Study of the Pollution of the Baltic. The number of copies which were eventually issued is unknown, but at least forty named individuals were sent copies. Responses were supposed to reach the Coordinator directly by 30 November 1980, but as late as mid-January 1981 completed questionnaires were still being received, some of them via the ICES Secretariat.

This summary of the responses received is derived from thirty completed questionnaires. It is, of course, impossible to include all the information obtained but the following summary provides the bulk of the information and gives a fair picture of experience and available techniques. The summary broadly follows the sequence of questions as asked in the original questionnaire, a copy of which is given on pages 39-44. Summary tables of some of the responses to the more important questions relating to experience and methods are given on pages 54-55 , but as the subject of anonymity was not mentioned in the questionnaire, no names are given.

General Experience

With only one exception, who worked mainly on problems related to the North Sea oil industry, all the respondents claimed to analyse samples from estuaries and coastal waters. Only nine claimed any experience on oceanic samples, but 2/3 had experience on samples from continental shelf areas.

About 1/3 of the respondents claimed to be involved only with compounds of petroleum origin. Only one person indicated that his laboratory had an interest in compounds of biogenic origin; but of the twenty or so who were interested in other pollutants, most had experience with DDT and PCBs and frequently they indicated that this was more extensive than their involvement with petroleum. Nine people indicated an interest in phthalates, two of whom indicated they had considerable experience with such analyses.

Almost all the analysts were able to analyse for total hydrocarbons and n-alkanes, although one did indicate his sole interest was in polycyclic aromatics. Most of the analysts (20) indicated that a proportion of their effort went into aliphatic and aromatic fraction analysis and a similar number (19 analysts) expressed interest in polycyclic aromatics, although in a few cases they indicated that they were only able to analyse qualitatively, not quantitatively. Seven analysts mentioned other constituents of oil for which they analyse: Ni, V and S were common to several of these analysts; other compounds mentioned were steranes, triterpanes, cycloalkanes, porphyrins, phenols and carbonyl compounds.

General Information in relation to Petroleum Hydrocarbon Analyses

Almost all of the analysts responded that their work was at least partly conducted in response to spill situations and in seven cases (eight if one includes chronic exposure sites), this was their sole involvement. The remainder claimed an involvement in basic research into behaviour and processes governing oil distribution and at least seven analysts were involved either in routine monitoring studies or in establishing baseline data against which future measurements can be assessed.

Although a full response was not obtained on equipment used, it is clear that a wide range of analytical techniques are employed by the analysts who replied and in many cases they are extremely well equipped. Most analysts use at least gas chromatography (either packed or capillary or both) and usually (all but two) this is supported by either IR or UVF analysis. UV absorption is used by two analysts, one of them also using UVF. About 1/3 of the analysts indicated that they routinely use capillary column chromatography but it is possible that several others do as well. Ten of the analysts indicated that they use GC/MS for at least a proportion of their sample analysis and in most cases these people also use UVF and GC^2 and several employ HPLC as routine.

All the analysts normally examine for dissolved/dispersed hydrocarbons in the water column or sediments and in most cases (seven exceptions) both substrates are analysed. Only six persons said they normally expect to look at surface films of oil, but eleven analyse for particulate oil. Twelve of the analysts also indicated that they analyse various organisms for petroleum, eleven subsequently provided details on how they perform the analysis. Several species of organisms were mentioned and mussels appear to be fairly popular, probably due to their wide availability and the interest in "mussel-watch". Three analysts mentioned sediment cores and two mentioned drill cuttings.

Sampling

The details of sample sizes, etc., employed suggested that the analysts operated in extremely different circumstances. Sample sizes for water ranged from a few hundred ml to 1 000 litres, although those using very small sample sizes were only involved either in very polluted situations or in experimental toxicological investigations. The majority of the analysts collect water samples of between 1 and 3 litres and in most cases seem to use devices similar to those recommended for the IGOSS pilot study on marine pollution monitoring. These devices are reported to be usable down to 35 metres and some of the modifications seem to be marked improvements. Those analysts who take samples at deeper depths usually use modified versions of such devices as Niskin, Menzel-Dazzler or Go-Flo bottles; two mention using, at least experimentally, plastic bag samplers. A few, especially those using very large samples, have developed their own devices but even they have reservations about the size of the equipment involved. Those analysts taking very large samples all expressed interest in continental shelf or oceanic samples but not all analysts with such an interest seemed to consider that such large samples were necessary, in many cases 1 to 3 litres were apparently thought to be adequate. Several of the analysts indicated that they would like a single sampler which operated well at all depths. This may, however, simply

be because large samplers are expensive, difficult to operate and not readily available; i.e., most analysts do the best they can with what they have.

A much greater uniformity of view existed in relation to the size of sediment sample needed, typically 25-100 g, and for the size of sample for biota analysis, most analysts speaking of 10-50 individuals with sub-samples of 10-20 g.

At least 9 analysts made no mention of taking any other observations at the time of sampling, although 3 of these were not involved in water analysis at all or only in special situations. Most analysts said that they take note of sea state, direction of wind and water movement, salinity and temperature and a few also take extensive water chemistry observations.

The methods used for collecting sediment samples ranged from sediment traps or divers using stainless steel spatulas or small glass corers, to various grabs and box corers. For deep water there seems to be only limited experience but gravity corers, vibro and piston corers, sometimes with modified liners, were all reported to have been used.

Most of the analysts obviously recognised the danger of contamination, especially of water samples, although the response of one or two suggested that they did not. Mention was also made of the possible loss of surface layers of sediment samples. In most cases, the view was that careful washing of the sampling device was essential but the measures taken ranged from extensive successive washing procedures to use simply of a solvent, although in at least one case the solvent used was said to be checked after use for the presence of oil. The need to avoid ship-source contamination is obvious and, as one analyst said, largely means using "common sense". A few analysts appear to prefer to carry out their sampling from a drifting rubber boat, which obviously limits sampling depth. Two analysts indicated that they were very wary of using hydrowires and preferred to use special nylon or polypropylene lines or traces. Several analysts identified as a problem the need to have good comparative data on the influence of sampling systems on the sample collected. Several also indicated their concern over their inability to collect reproducible samples and/or to detect patchiness both in water and in sediments. A few analysts who use grab samplers mentioned that they always try to take sediment samples from the centre of the grab.

Methods used for checking for contamination ranged from none at all to full procedural blanks, including in some cases use of the unopened sampler and container. A few analysts attempted to avoid the problem by preferring the use of control samples from clean areas. One analyst reported using repeated analysis of the same sample until the levels detected reached an asymptotic level, which was regarded as the blank.

As mentioned earlier, few analysts reported that they were entirely happy with the state of the art of sampling, although a few indicated that they were just as concerned about analytical aspects of sample treatment.

Preparation of Water Samples

The response to this section of the questionnaire involved only twenty-five analysts. None of them use centrifugation as a means of separating suspended material, indeed only eight use any method of

separation at all. Two of these latter use a filter, a stainless steel mesh or a glass wool plug simply to remove coarse suspended matter which they then discard. The remainder all used glass fibre filters of the Whatman GF/C type or similar, the filter having been cleaned prior to use by solvent washing and/or heating at 450°C for between 4 and 24 hours; for subsequent storage prior to use, the filters are kept in aluminium foil or a sealed Petri-dish. The preferred method of filtration was in all but two cases vacuum filtration, as this is simpler and less likely to cause contamination. One analyst indicated that he would use either system, provided the pressure was less than 15 psi. Subsequent extraction of filters was in all but two cases by soxhlet. The exceptions used pulping and solvent washing by shaking.

A few analysts said that they never stored water samples even after extraction, but the remainder indicated that they found it necessary to store samples and did so usually after carrying out an extraction phase. Methods of storage of whole water samples included (a) refrigeration for up to 1 month, after addition of mercuric chloride solution (a method said to have been tested and found safe), (b) acidification to pH2 plus chloroform, (c) dichloromethane, (d) acid with sodium nitride, and (e) no special measures. Methods of storage for the extracted sample differed less markedly, the most common method being use of a glass vial with an aluminium foil cap or teflon-lined screw cap in a deep freeze. Most analysts seem to use normal deep freezing at -20°C, but one specified -40°C and another -70°C.

A wide range of solvents appear to be used for extraction purposes. The most popular one was probably dichloromethane (8 analysts), but chloroform (3 analysts), carbon tetrachloride (3 analysts) and trichloro-trifluoroethane (1 analyst) were also mentioned. Two analysts prefer mixtures of solvents, one indicating benzene:methanol and another pentane:ether. Hexane was also fairly commonly mentioned. About half the analysts indicated that they bought their solvents as special high purity reagents, the remainder for one or other reason apparently purifying their own solvents by repeated distillation or by washing through silica columns. A few analysts mentioned that they carry out checks on reagent quality before using any new batch.

Almost all the analysts use simple separating funnel extraction methods for their water samples, but the shaking time varied from a few minutes to one hour; some use only a single extraction but most appear to use three. Two analysts preferred to use vortex stirring.

Only four analysts use adsorbents and then only for some samples. The only adsorbent mentioned was XAD-2 and its extraction capacity was reckoned to be several grams of material, with an efficiency of 50-100%. All four users precleaned their resin before use but only two specified how - removal of fines and soxhlet. The solvent used to desorb the extracted petroleum compounds was different in the three responses given: methanol, pentane and ethyl ether.

Preparation of Sediment and Biota Samples

Not all of the respondents analyse either biota or sediment samples and the responses obtained did not always make this clear. Ten analysts indicated that they dried their biota samples prior to analysis. Two analysts indicated that they used temperatures of around 105-110°C, although one of these clearly indicated that he was only interested in C_{15} - C_{33} alkanes or polycyclic aromatics. Four analysts used freeze drying and three used air or nitrogen at

room temperature or slightly above. Most analysts (about 75%) determined the dry weight of their samples when doing quantitative analysis, with most using simple loss on heating to constant weight. The usual drying temperature was 105°C but two analysts used 150°C and one used 70°C.

With only two exceptions, all the analysts stored samples prior to extraction, usually in a deep-freezer (-20°C), although some used only refrigerators and two used -70°C and another three used -40°C. The method of extraction employed was fairly standard: alcoholic potash under reflux for biota samples, followed by solvent extraction using one of a variety of solvents or mixtures, although a few analysts simply homogenised or soaked and stirred with a solvent. For sediments, most analysts used one of the latter techniques.

Regarding sediment samples, only five analysts reported analysing different size fractions on at least some of their samples, but there was no uniformity of approach. One analysed seven fractions using all of the sediment; another analysed three fractions using only the fractions sand and below; one analysed only the <63 micron fraction; another analysed both this fraction and that from 63 - 1 000 micron; finally, another analyst used the > and <45 micron fractions. One analyst reported analysing whole sediment but always performed a particle size analysis, too.

Eighteen analysts provided information on how they treat biota samples, and there was an almost 50:50 split on whether they analysed individual animals or bulked tissues. Some analysts indicated that the choice depended on the purpose and sample type. Preferences for sample numbers ranged up to 50, but 5-10 individuals seemed to meet the expectations of most analysts. Practical considerations can on occasions restrict the analysts' options and in some cases it is a matter of using whatever is available.

Extraction, Clean-Up and Analysis

Full details of methods of extraction, clean-up and analysis are in most cases to be found in the report of the intercomparison exercise which was conducted in parallel with the questionnaire survey and is reported in detail elsewhere in this report. For this reason, details are not provided in the survey tables to this section of the report and only a brief summary is provided here.

Clean-up was usually by column chromatography where this was felt necessary. Several analysts said it was either not necesssary at all or only rarely for seawater, especially if using UVF. For biota and sediment samples, the usual method was to use either silicic acid columns or alumina columns or both, but a few analysts indicated that they used Sephadex LH20 and silicic acid or Florisil columns. Those analysts who answered the question on the concentration step mainly favoured vacuum or rotary evaporation. Some used a stream of nitrogen instead of or in addition to a rotary evaporator. A few analysts mentioned special devices for vacuum evaporation and many specified using either room temperature or 35-40°C water baths. Two analysts used cold water baths but did not say what cold meant.

Ten analysts used gravimetric techniques for at least some samples. Twelve used IR and three used UV absorption. UVF was more widely used (17 analysts) and nine of these analysts used both excitation/emission and synchronous scanning, usually at 25 nm. Nine analysts used HPLC for at least a proportion of their samples. Thirteen

analysts used GLC and twenty-five used GC^2 methods. There was a fair degree of uniformity in the liquid phase used, with those mentioned in rough order of popularity being: OV101, SP2100, SE52, SE30, SE54, SF96, OV1, OV17. Several analysts indicated that they use two or more columns for some samples. Twelve analysts indicated here (ten to an earlier question) that they use GC/MS and one more added that he expected to use it by 1981. Five analysts said they normally used several techniques for each sample. Although the pattern in some cases varied according to sample type, the usual sequence of techniques used was UVF, GC^2 and GC/MS. Two analysts mentioned that they used other techniques in addition to those specified on the questionnaire, but only one said what it was - micro adsorption detector.

Detection Limits

Most analysts reported that their detection limits for seawater were within the range 0.1 to 1 µg/l. A few quoted a lower sensitivity, but they were using IR methods. Two indicated that they only analysed for specific compounds for which they reckoned to be able to achieve a sensitivity of 1 in 10^{12}. Three analysts claimed detection limits of the same sort of order for total oil, but these were not necessarily analysts who said they took large samples of water; the detection limits indicated were: 3 in 10^{12} (20 l), 50 in 10^{12} (up to 1 000 l), and 10 in 10^{12} (1 l). At least one of the other analysts taking large water samples quoted a detection limit of 1 in 10^9 (200 l). Agreement on what constitutes "background" concentrations, which some mentioned, was reasonably good, with ca 0.1 µg/l being mentioned in most cases.

A fairly comparable response was obtained for the detection limits claimed on sediment and biota samples: most analysts reporting between 0.1 and 1 in 10^6, usually with higher values quoted for biota samples.

Clearly, the level of detection cited depends to some extent on the method by which it is estimated or determined. Not all the analysts answered this question (eight did not), but there was a considerable divergence in view among those who did. Six analysts said they reckoned that their detection limit was roughly double the blank value, but two of these specified that this meant only the solvent blank and not the full procedural blank. One analyst used 10 x the blank value and another said anything above the blank value was detectable. One analyst used a value of 2 x SD of determinations close to the blank level and another 4.65 x the within-batch standard deviation of blank values. Only one analyst appeared to consider the matter in true statistical terms and quoted the formula:

Detection limit is significant "t" for

$$\frac{\bar{x}_s - \bar{x}_b}{\sqrt{\frac{N_s(N_s-1)\ \hat{\sigma}^2_{\bar{x}_s} + N_b(N_b-1)\ \hat{\sigma}^2_{\bar{x}_b}}{N_s + N_b - 2}}\ x\sqrt{\frac{1}{N_s} + \frac{1}{N_b}}}$$

where $\bar{x}_s$ = mean of sample

N_s = number of sample items

$\hat{\sigma}_{\bar{x}_s}$ = estimated standard error of sample,

and the subscript "b" refers to the blank.

The remaining analysts gave rather vague descriptive terms such as "1 cm deflection above the base line", "the trace detectable above the bleed line", "the lowest level above which we have confidence in the result", and "the smallest distinguishable peak from a standard run".

Other Questions

In relation to the question of patchiness, most analysts appeared either not to have understood the question or not to have considered the problem. Most of those who did respond, acknowledged that they had done little about it. A few analysts indicated that they do occasionally take replicate samples, but could not usually do so because of the work involved. One said that an integrating biological species should be used such as a mussel, which not all concerned would regard as a good choice. Only two analysts appeared to have considered and investigated the problem by taking repeated samples at intervals over a period, and at different depths at the same station.

Sixteen of the twenty-seven analysts who replied indicated that they had, at least sometimes, conducted an estimate of sample variance, although most of these simply referred to analysing duplicate samples, which as one admitted is not really sample variance. Eleven of the twenty-seven replies indicated that at least occasionally they carried out estimates of analytical variance; of the remainder, at least one said he did not consider it necessary.

Most analysts estimated their percentage recoveries on at least some samples, usually by means of a spike of one or more internal standards. Only one analyst admitted that the use of a single internal standard or spike was based on a number of possibly invalid assumptions. Several analysts responded that they use repeated extractions and then presumably hope they are achieving 100% recoveries or thereabouts. Almost all analysts use a full procedural blank.

The range of standards used was almost as large as the number of responses received. Three respondents use an "artificial oil" distributed by CONCAWE. Four use deuterated standards; the others use a variety of n-alkanes and aromatic compounds, the number depending on what they have available, which typically seems to be between 10 and 30. Most of the analysts (17) indicated that they use a standard oil for at least some of their work, although of these at least five indicated that this might vary depending on the problem in hand. One analyst expressed strong disagreement with the whole concept of using an oil as a reference base and said that the only meaningful results were those which referred to specific compounds. Six analysts use Ekofisk oil as a routine standard, four use API oils, three use 'Amoco Cadiz' oil, other analysts use Kuwait, topped Iranian, Arabian Light, Bunker oil, Russian Crude, etc. No doubt in most cases the selection is made, at least initially, on the basis of a problem on which they are working.

Of the analysts who responded (23) to the question on their willingness to adopt a common standard reference oil, seven indicated that they would be prepared to do so unconditionally, six more said they would seriously consider doing so at least to calibrate a more stable standard such as chrysene, or at least to the extent of always quoting a conversion factor. One analyst pointed out that this would be both easy and useful. Seven analysts stated they would not do so, one said probably not and another said only if he could be convinced that the merits of doing so were sufficient to persuade him to invalidate ten years of earlier effort.

Very few analysts found it either necessary, or had the time, to add any extra comments of their own, other than to elaborate on their responses to earlier questions. Two comments which may be worth recording were: a concern over possible confusion between biogenically derived hydrocarbons and those of petroleum origin and the importance of using internal standards. One analyst indicated that he saw future emphasis being placed on the identification of single specified compounds and would be interested in collaborative and cooperative effort in this area of work.

LIST OF PERSONS RESPONDING TO THE QUESTIONNAIRE

Dr T.P. Abbiss	Orielton F S, Wales
Dr J.W. Anderson	Batelle, USA
Dr F. Berthou	Faculty of Medicine, Brest, France
Mr G.A. Best	Clyde RPB, Scotland
Dr G. Bodennec	CNEXO, Brest, France
Mr D. Buchanan	Highland RPB, Scotland
Dr K. Burns	IAEA, Monaco
Dr S.R. Carlberg	Fishery Bd., Gothenburg, Sweden
Dr G. Dahlmann	DHI, Hamburg, Federal Republic of Germany
Dr M. Ehrhardt	University of Kiel, Federal Republic of Germany
Dr J.W. Farrington	WHOI, USA
Dr K. Haapala	National Board of Waters, Finland
Dr T. Haegh	Trondheim, Norway
Dr N. Hansen	Water Quality Institute, Hørsholm, Denmark
Dr M.A.J. Kerkhoff	RIVO, Netherlands
Dr J. Kiceniuk	St John's, Newfoundland, Canada
Dr A.H. Knap	Bermuda BS, Bermuda
Mr R.J. Law	MAFF, Burnham-on-Crouch, England
Dr E. Levy	AOL BIO, NS, Canada
Dr R.G. Lichtenthaler	Central Institute, Oslo, Norway
Dr P. Mackie	TRS, Aberdeen, Scotland
Dr L. Massie	DAFS, Aberdeen, Scotland
Dr K.H. Palmork	Institute of Marine Research, Bergen, Norway
Dr E.-L. Poutanen	Institute of Marine Research, Finland
Prof. J.G. Quinn	University of Rhode Island, USA
Mr J.C. Roussel	Inst. Fr. Pétrole, Paris, France
Dr G.B. Sirota	Halifax Laboratory, NS, Canada
Dr W. Tonkelaar	TNO, Netherlands
Dr T.L. Wade	Old Dominion University, Virginia, USA
Dr A. Zsolnay	NSTL, Mississippi, USA

SUMMARY TABLE

Analyst	General Questions		Questions specific to oil									
	Area of Experience	Residues Examined	Analytical Facilities Used	Type of Samples Analysed	Typical Sample Size			Other Observations taken at time of sampling	Details of Procedure for Blank Data	Method of Sample Preservation		Extraction System Used	
					Water litres	Sediment Dry g	Biota Dry in g or No.			Water	Sediment/Biota	Water	Sediment/Biota
1	C, S, O	C, P, Ph, O	Gr, UF, G, GC, GM	P, D, S, B	3-60	10-40	2-10g	Yes	Rinse gear & full proc.	B. DCM Dark	B. -20° Dark	DCM	Me/KOH or sox. DCM
2	C, S	C, P	G, GC	P, D, S	2.5	50-100	25-50	No	Full procedure	B. Cool/Frozen	Freeze	C or P & E	Sox. P/E
3	C	C, P	GC, GM	P, D, S	20	50	1-10g	Yes	Clean area	B. -70°/A. 4°	B. -70°	B & Me or XAD-2	Sox. B/Me
4	C, S, O	P, Ph, B	GC, GM	S	1-20	1-100	5-10g	Yes	Full procedure	B. CH	B. -20°	CH	Me
5	C	C, P, Ph, O	-	S	1-2	-	-	No	Clean area	B. max 24h. pH2/CH	N/A	H & C	-
6	S	P, O	UF, G, GM, HP, AA	D, S, C	3	500	-	Yes	-	B. DCM/H + HCl	B. Cans + Bact.	DCM & H	Sox. or US. DCM or H
7	C, S, O	C, P, Ph	UA, G, GM, HP	D, S, B	1-30	10	10g	Yes	Full procedure	No store	B. -70° Glass	TCTF	Sox. or Me/KOH
8	C	P, Ph	G	B	-	-	5-10	-	Full procedure	N/A	B. 40° Dessic.	-	Homog. A/P
9	C	C, P	UF, G	D, S, B	1	10	-	Yes	Solvent blank	No store	B. Frozen Al.	H	US. B/Me
10	C, S	C, P, O	-	S, B	2	1-200	10g	Yes	-	4°	B. Dessic.	H	Homog. A/P or Sox CH
11	C, S	P, O	I, N	P, D, S	2	100	-	No	-	A. Acid or Na_3N	B. -35°	P & C or XAD-2	Me/KOH Sox DCM
12	C, S	P	UF, G, GM	SF, D, S, B	5	1-20	100g	Yes	Full procedure	A. Frozen	B. Frozen	CH	Homog. CH/Me
13	C,S	C, P, Ph, O	UF, GC, GM, HP	-	400-1000	-	-	Yes	Full procedure	No store	-	A & E or XAD-2	-
14	C, S, O	C, P	UF, G	D, S	10	-	-	Yes	Full procedure or repeat extract	A. -	B. -40°	H	-
15	C	C, P, Ph, O	-	D	-	-	20g	-	Full procedure	N/A	B. -40°	A	Et/KOH Sox
16	C, O	C, P	-	SF, P, D, S	200	10	10	Yes	Full procedure	A. -40°	B. -20°	-	Sox CH/Me
17	C, S	P	G	S	-	50-80	20-30	-	Internal standard	N/A	B. -20°	H	Soak
18	C, S	P	-	D, S	0.3	20	-	N/A	Control tanks	N/A	B. live or in glass	-	DCM
19	C	P	UF, G, I, AA	D, S	2.5	30	10-20g	Yes	Rinse gear & full procedure	B. No Pres.	B. Frozen	DCM	Homog. CH/Me
20	C, S, O	C, P, B	UA, UF, I, G, GC, GM	P, D, S, SF, B	1	5	-	Yes	Use clean solvents	No store	B. Frozen Al/Gl	DCM	Sox or shake C
21	C, S, O	C, P, Ph	-	P, D, S, B	100	50	20-30g	Yes	Full procedure	B. 40°/A - 20°	A. Fridge C	-	Sox. Me/B
22	C	P	I, G	SF, S	2	100	-	-	None	B or A 4°	A or B. Frozen DCM	Me & H or XAD-2	Sox. C
23	C, S	P	UF, G, GC	P, D, S	2	-	-	Yes	Clean area	A. DCM/Fr, CH	-	-	Shake DCM
24	C	C, P	I, G, AA	D, S	-	-	-	-	-	N/A	B. -20°	DCM or CH	-
25	C, S	C, P	-	D, SF, S, B	3	20	10-30	Yes	-	No store	B. Frozen	-	Sox. Hy. or Homog. P
26	C, S, O	C, P	-	P, D, SF, S, B	16	15-100	1	Yes	Full procedure	A. DCM	B. Frozen	DCM	Me/KOH Sox Me/T
27	C, S, O	C, P	-	-	2.8-3	80	20	Yes	None	A. DCM, Freeze	B. -20°	DCM	Me/NaOH P
28	C, S	C, P, Ph	UF, I, GC, GM	P, D, S, B	2.7	70	20	Yes	Rinse gear & full procedure	A. DCM, Freeze	B. -20°	DCM	Me/KOH P
29	C	P	G, HP	S, B	-	100	40	No	-	-	B. -20°	-	Me/KOH P
30	C, S	P	UF, I, G	S	1-2	25-30	-	Yes	Full procedure	B. DCM, Fridge	B. -20°	H	Sox. C

Key:-

Note Order of Analysts above is not as in list of respondents

Coastal
Shelf
Oceanic

Chlorinated compounds
Petroleum
Phthalates
Others
Biogenic halogenated compounds

Gravimetric
UF = UV Fluorescence
UA = UV Absorbance
G = GLC
GC = Capillary GC
GM = GC/MS
I = Infrared
HP = HPLC
AA = AAS
N = NMR of oxygenated compounds

Particulate
Dissolved/Dispersed
Surface Film
Sediment
Biota
Cores/cuttings

N/A Not Applicable

B. = Before extract
A. = After extract
DCM = CH_2Cl_2
H = $nC_{12}H_{26}$
CH = $CHCl_3$
Fr = Frozen
All temps are in °C

B. = Before extract
A. = After extract
Bact = Bactericide
Al = Aluminium
Gl = Glass
DCM = CH_2Cl_2
C = CCl_4
All temps are in °C

C = CCl_4
P = Pentane
E = Ether
B = Benzene
Me = Methanol
CH = $CHCl_3$
A = Acetone
H = Hexane
DCM = CH_2Cl_2
TCTF = trichlorotrifluoroethane

Sox = Soxhlet
Homog = Homogenised
Me = CH_3OH
DCM = CH_2Cl_2
P = Pentane
E = Ether
H = n Hexane
Hy = cyclohexane
C = CCl_4
CH = $CHCl_3$
T = Toluene
B = Benzene
US = Ultrasonic

SUMMARY TABLE

Analyst	How deal with Patchiness	Sample Variance Measured	Routine Measure Analytical Variance	How define Detection Limit	How define Extraction Recovery	Main Standards	Normal Reference Oil
1	Sample Design	3/4 Repeats	Yes	?	Spikes	Wide Variety	API and No 2 Fuel
2	-	No	No	Spill - Not Imp.	-	Oil in Spill or n-Alkanes	None
3	-	Yes	Yes	2PA Rel Noise	Stands. Recov.	Several	None
4	-	Occasional 2 Rep.	No	Defl. >1cm	Stand. Addition	Raw Oil B or Iso O	None
5	N/A	Yes	No	-	St. Add or Int/Ext Stand.	n-Alk and PAH	4 API oils
6	-	-	-	-	-	-	Ekofisk
7	Many samples	No	Yes	Formula	Spike	$C_{22}H_{46}$ Int. St.	None
8	N/A	No	Yes	Very difficult	Int. Stand.	Deuterated HCs	Amoco Cadiz
9	-	Yes	No	2SD of levels = Bl.	Stands.	Oil, Chrysene, Aromatics	Russian Crude
10	-	Yes	No	Dfl. >1cm	Stands.	Pyrene, API Oil	API or Amoco Cadiz
11	-	No	No	-	Repeat Extracts	API oils and Gas oil	Gas oil
12	Impossible effort	Yes	Yes	Detectable Peak	Int. Stand.	4 Alks C_{15}-C_{30} & 30 PAHs	None
13	4 Replicates or 2 Days	Yes	No	Diluted Samples	Int/Ext Stand.	Ekofisk and Pure compounds	Ekofisk
14	Replicates	Yes	Yes	= Blank value	Spike	Ekofisk, C_{12}-C_{30}	Ekofisk and Topped Iranian
15	N/A	Yes	Yes	Detectable Peak	Spike	13 PAH	N/A
16	Impossible	Yes	No	10 SD of Blanks	Int. Stand.	Mixture	None
17	N/A	Yes	Yes	Detectable Peak	Int. Stand.	Squalene, MeNa and An.	Forties/Ekofisk/Arabian Light
18	Don't	Duplicates	Not Necessary	-	Spike	Crude Oil	None
19	Not a Problem	Yes	Yes	4.65 x SD of blanks	Spikes	Chrysene C_{12-28} Alkanes	None
20	Enough Replicates	No	-	-	-	API and Chrysene	Bunker Fuel
21	Use Biol. Indic.	-	No	2 x Blank	Spike	C_{12-34} Alkanes or App. oil	None
22	-	Replicates	Yes	-	2 Extracts	-	-
23	Grid	No	No	2 x Solvent Blank	Int. Stand.	Auk or App. Oil	Auk
24	-	No	No	0.2 Absorb. Units	Spike	App. Oil	None
25	N/A	Yes	Yes	'Determined' ND	Spike & Int. Stand.	CONCAWE & Deuterated HCs	Ekofisk/CONCAWE
26	Repeat Samples	-	Yes	2 x Blank	Int/Ext Stands	Phenanthrene and C_{22}	None
27	Haven't tried	No	No	Peak >Blank	Spikes	Deuterated HCs	Ekofisk, Arabian Light
28	Repeat Samples	No	Yes	2 x Blank	Spike low level	Deuterated HCs & Aromatics	Ekofisk
29	-	-	-	-	Spike	-	4 API oils
30	Haven't tried	Yes	No	2 x Blank	Several Extracts	CONCAWE	Kuwait/CONCAWE

Key:-

Note
Order of Analysts above is not as in list of respondents

Deflection
PA = Peak Area
Bl = Blank
SD = Standard Deviation
ND = No details given
Not Important
PA = Peak Area

Int = Internal
Ext = External
Stand = Standard
Stands = Standards

B = Benzene
Iso O = Iso Octane
n-Alk = Alkanes
HCs = Hydrocarbons
PAH = Polynuclear aromatic hydrocarbons
MeN = Methyl naphthalenes
An = Anthracene
App = Appropriate