Rijkswaterstaat

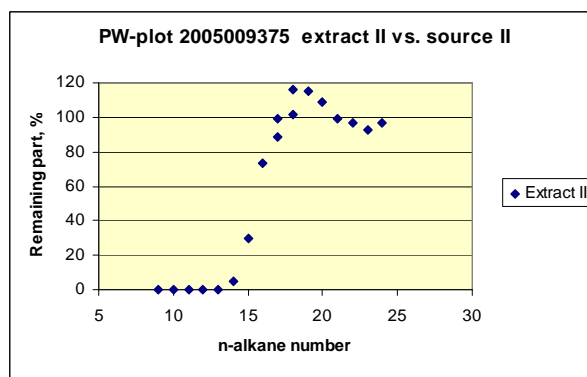# Oil Spill Identification - Round Robin 2005

The comparison of four bilge samples

3 May 2006

PW-plot 2005009375 extract I vs. source I

PW-plot 2005009375 extract II vs. source I

PW-plot 2005009375 extract I vs. source II

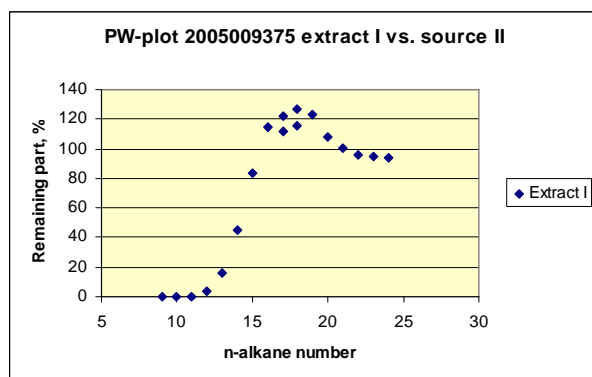PW-plot 2005009375 extract II vs. source II

Rijkswaterstaat

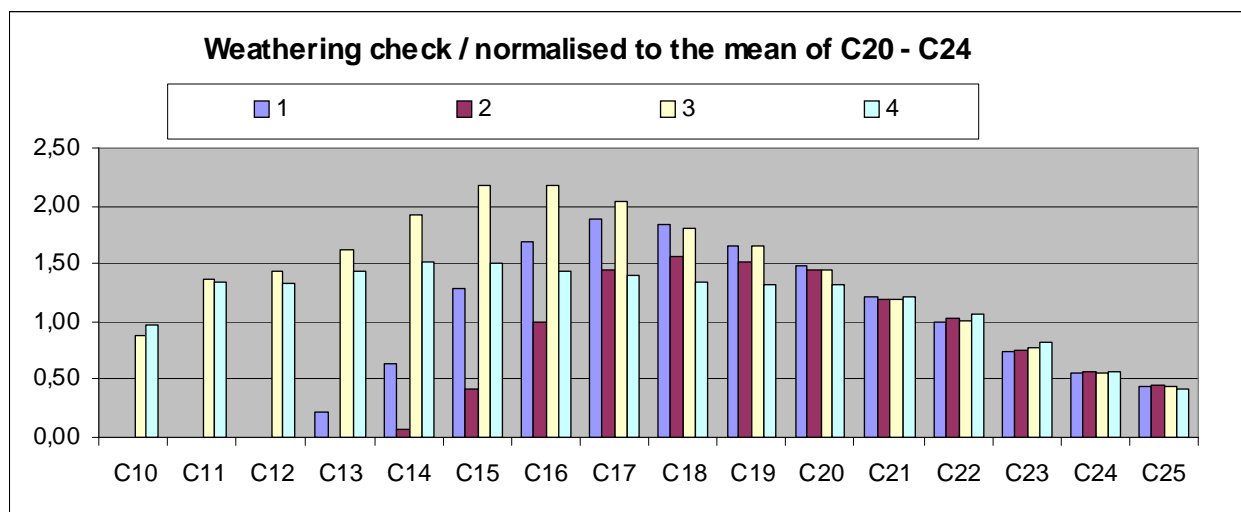# Oil Spill Identification - Round Robin 2005

The comparison of four bilge samples

3 May 2006

The results of round robin 2005 - Oil Spill Identification between twelve international laboratories.

Weathering check / normalised to the mean of C20 - C24

Front page:
Percentage weathering (PW) plots from the report of Sweden.

Second page:
Weathering check from the report of Finland.
1 = Extract I;   2 = Extract 2,   3 = Source I and   4 = Source II

Contents

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Abstract

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RIZA and BSH have organized a round robin concerning oil spill identification between 12 international laboratories
Composed and artificially weathered bilge samples have been sent to the participants with the following information:

Extract I and II are from two samples from the water (dichloromethane extracts).
Source I and II are bilges samples from two boats (pure oil).
Samples from the surface water were taken in a canal in the Netherlands. An oil spill was located and a sample (Extract I) taken. The next day a second spill was found further on in the canal. Here also a sample was obtained (Extract II). Sailing records revealed that two boats could have caused the spills. From each boat a bilge sample was obtained (Source I and II).

Question: Do samples match? (Extract I and/or Extract II with Source I and/or Source II).

The spill samples (E I and E II) had to be compared with both possible source samples (S I and S II). The conclusions are summarized in the table.

Participants	Methods		Results			
	FID	MS	S I – E I	S I – E II	S II – E I	S II – E II
BMM	X	X	+	+	--	--
Cedre		X	--	--	--	--
LASEM		X	--	--	--	--
LVA	X	X	++	++	--	--
NBI	X	X	++	+	--	--
NERI	X	X	++	+	--	--
NFI		X	++	++	--	--
Petrobas	X	X	++	++	++	++
RIZA	X	X	++	++	--	--
Sintef	X	X	++	++	--	--
SKL	X	X	++	+	--	--
WRD	X		++	++	-	-
--	Significant differences between the samples have been found.					
++	No significant differences between the samples have been found.					
- and +	Conclusions are indicated to be less certain.					

The two spill samples were prepared from Source sample I by evaporation (with extract II to a larger extent). Most participants have found correctly a match between Source I and Extract I and Extract II and a non-match for Source II.

In chapter 2 of the report the individual participants are mentioned and their original conclusions shown. The complete original reports are published on CD, because the information (> 15Mb) is too large to be published on paper.

In chapter 3 the results are discussed in detail.

A goal of this round robin is to check the own laboratory's method and to learn from the methods of other labs. Therefore all original reports are provided and it is strongly advised to the participants to read the reports.

It can be concluded that the round robin has resulted in a very useful set of reports dealing with the comparison of bilge samples. The individual participants can check and verify their conclusions against a large number of laboratories.

1. Introduction

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1.1   Invitation

The round robin started with this invitation by email with enclosed letter on 19-5-2005:

Dear all,

RIZA (Institute for inland water management and waste water treatment) is a governmental institute responsible for the environmental quality of the surface water in the Netherlands.
One of our tasks is the comparison of oil samples from spills with possible sources.
For many years we have exchanged spill samples each year with the Dutch Forensic Institute (NFI) for quality assurance. The results of each exchange were evaluated in a report with the original reports as appendix.
Due to questions of other laboratories we started together with BSH (Bundesambt für Seeschifffahrt und Hydrographie) last year to organize an (inter)national round robin. The round robin of 2004 (RR2004) was dealing with gas oil (diesel) samples. Fifteen laboratories of nine countries participated, resulting in a general report and 15 original reports as appendixes. The results are available on request.

Samples and analysis method
It is intended to use each year a different kind of sample. This year our study deals with three to four bilge samples (diesel-lubricating oil), which must be compared.
In most Round Robins a method is prescribed. Oil Spill Identification however is a typical "expert" method. Analytical methods can be given, but at the end an expert has to take the decision based on all information. For us the RR is a means to inform each other how to deal with cases.
From the results of RR 2004, it can be seen that most of the laboratories have an own method, although a lot of them follow more or less the Nordtest method.
The CEN working group CEN/BT/TF 120 intents to finish in June an European guideline for sampling and oil spill identification of waterborne oil, based on the Nordtest method.
So for next year we can advice to follow this guideline and discuss and compare the results of the laboratories that have followed it, in more detail. But in general each laboratory has to follow its own standard procedure for oil comparison.
The resulting report should not only consist of a simple yes or no, but also a summary of the procedure should be given, together with detailed results and reasons why a decision has been taken.

The original reports will be combined in a final report, together with an evaluation of the results.

Time schedule
May: Request for participation
June: Delivery of the samples.
July/August: Reports have been returned.
September: Final report will be sent to the participants.

We hope it will be possible for everybody to analyze the samples and make a report within two months, although we know that most of us will have a vacation in that period.

Costs
Last year RIZA hasn't asked a contribution for the costs of organization and reporting. Due to the number of laboratories participating and the time needed, which was quite more than expected, we ask this year a contribution of € 500.

Coded results.
It's the intention to mention participants and their results. It has the big advantage, that information can be shared more easily. If this is a reason for you, not to join the round robin please inform me. You can participate anonymously.

Participants
This request is sent to Dutch inland water labs, participants of CEN/BT/TF 120 and participants of RR2004 and some other labs working in this field. If you know other labs that like to join the round robin, please inform me.

If you are willing to participate in this round robin, please return the register form by post or fax.

Reactions of one local authority (Dutch inland water lab), the Netherlands Forensic Institute (NFI), one Brazilian and eight European laboratories were received.
None of them wanted to participate anonymously. In chapter 2 the participants will be mentioned.

1.2   Samples

At the beginning of August the samples were sent to the laboratories with the following letter enclosed:

Dear all,

Enclosed you will find 4 samples

Extract I and II are from two samples from the water (dichloromethane extracts).
Source I and II are bilges samples from two boats (pure oil).
Samples from the surface water were taken in a canal in the Netherlands. An oil spill was located and a sample (Extract I) taken. The next day a second spill was found further on in the canal. Here also a sample was obtained (Extract II). Sailing records revealed that two boats could have caused the spills. From each boat a bilge sample was obtained (Source I and II).

Question: Do samples match? (Extract I and/or Extract II with Source I and/or Source II).

For convenience and to be sure that each participant becomes the same samples, the two spill samples have been extracted with dichloromethane and dried with Na_2SO_4
As indication: A dilution of the extract with a factor 8 will give a good signal on the GC-FID.
It could be necessary to homogenize the bilge samples by stirring (or ultrasonic dispersion) prior to the analysis and to make a clean-up of these samples.

The problems associated with the identification of bilge oil samples are especially well described in http://www.bsh.de/de/Produkte/Buecher/Berichte/Bericht31/Bericht31.pdf. Hints, tips and precautions are given here concerning their analysis and result interpretation.

Because the method of analysis is free, I would like to have a short description of the method, followed by a discussion about the results and where the conclusions are based on. Chromatograms are very welcome. If numbers are used for comparison (absolute concentrations, peak ratios), error handling should be included (see also RR 2004, Final Report).

In the final report your original contribution will be present as annex.
Therefore I have to ask you to send the results in a digital format by email.
The main part of the report will be small and give a summary of the results.
I will send you a concept and make it final after receiving your reactions.

Time schedule:
Start of August: Delivery of the samples.
August/September: Reports have been returned.
October: Final report will be sent to the participants.
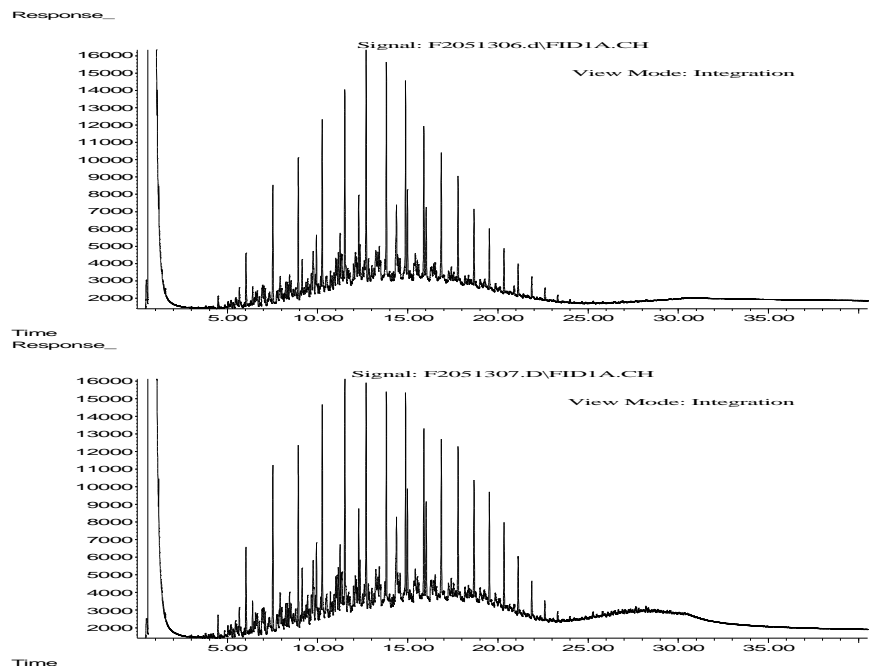

1.3   Sample preparation

In RR2004 three gas oil samples from a real case were used. The "solution" to this case was not known, which was regarded as a disadvantage by some of the participants.
This year a different type of oil was taken and to be able to know the answer, the samples were artificial prepared.

Signal: F2082706.D\FID1A.CH

View Mode: Integration

Signal: F2082707.D\FID1A.CH

View Mode: Integration
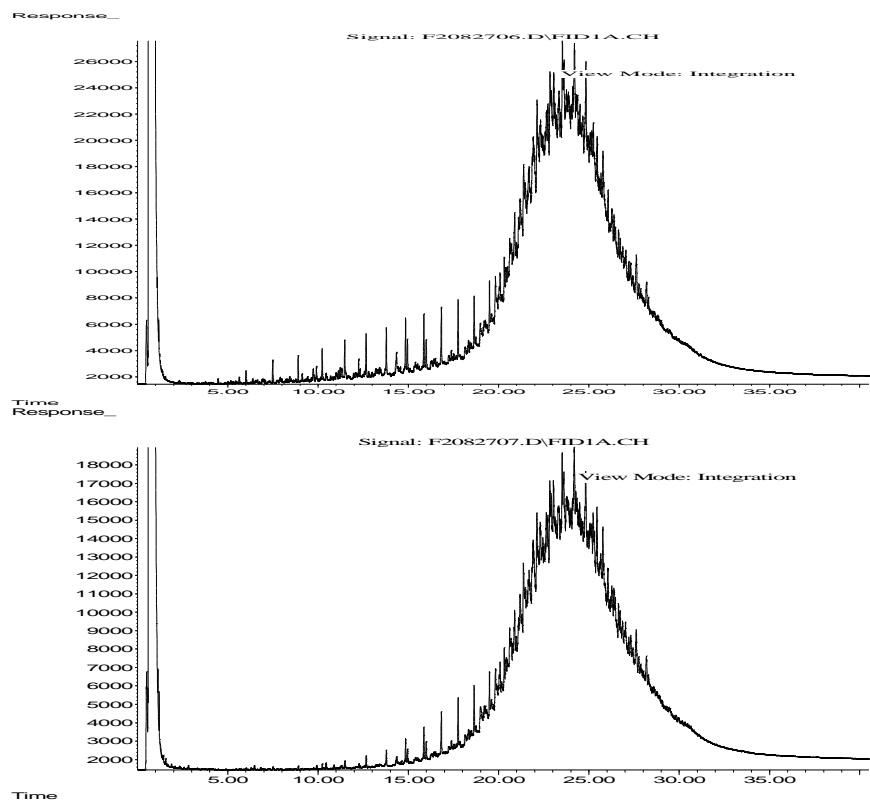
Fig 1 Bilge samples used to prepare the source samples.

Two bilge samples (Fig 1) from a ship with three engines and a bilge tank for each engine were used. The amount of gas oil was small, but different for the samples. The biomarker patterns were quite similar. To increase the amount of gas oil the two bilge samples were mixed with the bilge samples of two different ships from another case, containing mainly gas oil(Fig 2). These bilge samples are different in the amount of lubricating oil and also show a different alkane pattern.

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View Mode: Integration

Signal: F2051307.D\FID1A.CH

View Mode: Integration

Fig 2 Bilge samples used to prepare the source samples.

One of the first two bilge samples was mixed 1:1 with one of the second two bilge samples and the remaining samples were also mixed 1:1. The resulting mixtures represent the two source samples.

To create the spill samples the mixture called Source I was weathered artificially. Two glass Petri dishes containing 2 g of the Source I mixture each stayed in a ventilated oven (Heraeus UT12) for 1,5 and 4 hours at a temperature of 75°C. The remaining oil was dissolved in dichloromethane and filtered over a glass fibre filter to remove small particles visible and, to be sure, dried with Na_2SO_4.

The resulting "case" contains two source samples with a quite similar lubricating oil part and a different gas oil part. This is similar to the real situation on inland ships. The variation in base oil to produce lubricating oil is limited and the variation in lubricating products used on board is lower than the variation in gas oil used as fuel.

1.4   Report

Most results were received by email. One result had to be scanned.
A combination of all data would result in a huge report. Therefore the report is published on CD. The individual results can be found on the CD, whereas in this final report of the Round Robin the information is summarized and commented in chapter 2 and discussed in chapter 3. On the CD also Part I and II of the concept of the European Guideline, as send to CEN for comments on 13-3-06, can be found.

The results were not discussed in great detail with the intention that all individual reports are available and all participants can compare their own method with others.
Reading and comparing the results of the participants was an instructive and valuable job. We hope that our comments and advices are appreciated and can help to improve also the knowledge about oil spill identification.

2.Individual results

. . . .

In this chapter a summary of the results of each participant will be given. All original results, separated in directories, can be found on the CD.
Each participant is introduced shortly and the method(s) used to analyze the samples are summarized. The conclusion, indicated with the => sign, is an exact copy of the conclusion found in the original report. Finally for some of the participants additional notes/remarks can be found. In annex 6.2 a summary of the analytical protocols is shown.

2.1 Beheerseenheid Mathematisch Model van de Noordzee (BMM)

Contact: Patrick Roose

The institute uses three abbreviations depending on the language. See http://www.mumm.ac.be/
In English: The Management Unit of the North Sea Mathematical Models and the Scheldt estuary, abbreviated to MUMM, is a department of the Royal Belgian Institute of Natural Sciences (RBINS), a federal scientific establishment that comes under the Federal Science Policy (previously known as OSTC).

The samples were analyzed with GC-FID and GC-MS according to the standard procedure that is largely based on the revised Nordtest Methodology as described in Faksness et al. (2002). Normally, approximately 50 mg of oil is dissolved in 5ml dichloromethane (DCM) and the removal of co-extracted material prior to GC-MS analysis is carried out by column chromatography on a silicagel column (pretreated at 150°C for 24h).

=> The FID patterns of source 1 and 2 (Figure 1) show, in both cases, a mixture of a heavy fuel oil and lubricants, as do the patterns of the spill samples (Figure 2). Source 2 was omitted from further investigation based on the FID chromatogram and the MS Total ion chromatogram.
Although neither of the samples could be unequivocally related to source 1 based on this comparison, the patterns are very similar. This is illustrated in Figure 10. This would make source 1 the likely candidate for the spill if the presence of other potential sources can be excluded. The observed differences can be related to weathering and sample inhomogeneity.

Notes/remarks:
A mixture of a heavy fuel oil and lubricants   should be a mixture of gas oil and lubricants
The GC-FID chromatograms of the source samples have a strange pattern. Only the beginning of the chromatograms shows normal

peaks. This might be concentration related: in combination with a small (ID 0.18 mm) column overloading might be a reason for this behavior. The MS chromatogram of Source II is also strange. Resolution is far less compared to the other chromatograms. As a result Source II has been excluded. Comparing Fig 3 and Fig 4 of the BMM report indicates that the injection concentration between source and spill samples is a factor of 10 or more. It is advised to optimize the injection concentration.
The evaluation of the chromatograms is laborious and susceptible to failures. Patrick has indicated that he intends to improve data handling Differences between the ratios of small peaks can be larger than normal because of the difference in the injection concentration. This is e.g. visible in the m/z 191 and m/z 216 chromatograms.
GC-screening may be used to adjust the concentration of samples, as it is done, for example, by SKL:

After a first preliminary GC-FID run we eventually dilute or concentrate the extracts so they all will have approximately the same concentration. Most of our samples that are collected from the water are taken with a teflon cloth, so the concentration can vary a lot.

2.2   Centre de Documentation de Recherche et d'Experimentation sur les pollutions accidentelles des eaux (Cedre)

Contact: Julien Guyomarch

Cedre (http://www.le-cedre.fr/) was created in 1978 within measures taken after the wreckage of the "Amoco Cadiz", to improve preparedness against accidental water pollution and strengthen the national response organization. Its expertise encompasses both marine and inland waters.

The RR-samples have been analyzed with GC-MS in SIM mode on alkanes, PAH's and biomarkers.

=> The comparison of the samples Source I and II, and Extract I and II led to the following conclusions:
- n-alkanes show differences in the region n-C11 to n-C19, but these variations are not significant due to possible evaporation processes affecting these distributions. On the other hand, the relative abundances of compounds ranking from n-C20 to n-C30 did not show differences.
- The biomarkers (m/z=191, 217, 218, 231) did not allow to differentiate the 4 samples due to high variability.
- Finally, the diagnostic ratios calculated from PAHs analyses present variations that could explain differences of origins for Source I and II. These significant differences showed for fragment 216 and 234.

=> The conclusion of this oil spill identification is that the samples collected at the water surface do not seem to come from the two boats. Moreover, the two bilge samples and the two surface samples have different origins.

Notes/remarks:

Cedre remarks that the biomarkers have a low abundance and a high variance. This is shown on page 12 of their report between Source I and Extract I for e.g. %32abS and %30g.

Several other participants use the same instrument (see 6.2) and were able to analyze the biomarkers with a good sensitivity and a low variance. In the report of Cedre no information about the injection concentration could be found.

The method applied is quite normal although the injection temperature is lower than most participants use.

RIZA has tested the injection temperature for the GC-FID analysis with a split/splitless injector at 300°C, 325°C and 350°C and found a better performance and a lower variance of the alkanes in the range of C30-C44. Using the split/splitless injector in a series at a temperature of 350°C however resulted in a fast decrease of the performance of the rubber sealing around the liner, although a high temperature ring was used.

Based on that a temperature of 325°C is used for the split/splitless injector (GC-FID) and 350°C for the PTV injector (GC-MS).

2.3   LASEM

Contact: Pauline Pierre

LASEM is a laboratory of the ministry of Defense and is located in Brest.

The samples have been analyzed with GC-MS in the full scan mode (m/z 50 – 300) with an ion trap as detector.

=> In first approximation, the chromatogram of the four samples have the same shape: mixture of light and heavier oils.

The comparison between the n-alkanes repartition of the four samples shows differences in low boiling rang, but it could come from volatility of these compounds: it does not allow to differentiate them.

The usually used biomarkers do not permit to conclude, because of their too low abundance. Diagnostic ratios coming from the PAH's, present more or less significant differences, depending on the considered ratio.

As a conclusion,
- Oil spills at day 1 seem to be different from source I and II,
- Oil spills at day 2 seem to be different from source I and II,
- Source I and source II are different,
- Oil spill at day 1 seems to have the same source as the day 2's.

Notes/remarks:
LASEM reports:
The usually used biomarkers do not permit to conclude, because of their too low abundance.

RIZA has diluted the extract 8 times and diluted 3 ul of the source samples in 1 ml DCM. Injections of 1 ul resulted in a good sensitivity with about the same peak height for all samples (see fig 11 of the RIZA report)

LASEM has diluted the extracts a factor 2 and diluted 100µl of the source samples in 1 ml DCM. The injection volume is 1 µl. As a result the source samples should give about a 10 times higher signal than the spill samples. In annex IV the areas of m/z 216 are shown. From the table the mean and RSD has been calculated:

m/z=216	extract I mean	RSD in %	extract II mean	RSD in %	Source I mean	RSD in %	Source II mean	RSD in %
2M-fluoranthene	23055	8.4	23079	9.6	67871	84.8	83779	23.8
benzo(a)fluorene	60827	7.4	58517	2.9	233463	64.0	277480	21.1
benzo(b)fluorene	24226	11.4	23276	4.5	71067	53.6	69110	42.8
2M-pyrene	14071	21.5	14846	4.7	135941	77.2	177571	19.8
4M-pyrene	29785	11.6	30783	3.6	216228	52.9	306144	9.5
1M-pyrene	18138	8.2	17829	1.6	106854	49.6	150598	8.4

. . . .
Table 1.
Mean and RDS calculated from annex IV of the LASEM report

The source results show much higher RSD's than the extract results.

From the table with the analytical methods (see 7.2 of this report) it can be seen that LASEM uses the lowest injection temperature of all participants. This subject is discussed in 2.3 Cedre.

It can also be see that only LASEM uses an ion trap for the biomarker analysis. Petrobas uses the same instrument for the PAH analysis, but uses a quadrupole instrument for the biomarkers.
An ion trap has to reduce the total number of ions in the source to prevent chemical ionization reactions and to be able to filter the ions properly. Therefore the number of ions is measured before each scan and only a limited number is entering the source. It is therefore possible that for the samples of RR2005 the number of biomarker ions compared to the total number of ions is so small that it is difficult to analyze them correctly with an ion trap.
Note: An email to Petrobas with a request for the reason why the ion trap is not used for biomarkers has not been answered before finishing the report.

Advise:
- Optimize the injection concentration for bilge samples by means of a calibration line
- Validate the instrument by means of 7 to 8 injections and calculate the relative st dev of (some of the) the ratios. They should not be much higher that 5%.
- Analyze the same samples on a GC-MS with a quadrupole detector when possible and compare the results.

2.4 LVA

Contact: Rita Skolmeistere

LVA (http://www.lva.gov.lv/lea) is a governmental institution subordinated to the Ministry of Environment of the Republic of Latvia. Their aim is to establish a seamless environmental information system to improve the environment and move towards sustainability.

The samples have been analyzed by GC-FID and GC-MS.

=> There is match between extract I and extract II. Extract I and extract II match to source I.

2.5   The National Bureau of Investigation (NBI)

Contact: Niina Viitala

The National Bureau of Investigation http://www.poliisi.fi/nbi is one of the national units of the Finnish Police. The NBI is specialized in investigating and preventing serious, organized and international crime. At the national level, the NBI is responsible for criminal intelligence and identification of new types of crime. The NBI also develops techniques for criminal investigation and provides training in the mentioned subjects.

The samples have been analyzed with GC-FID and GC-MS in SIM mode. The chemical analytical methodology is based on the CEN/TC BT WI CSS27002.4 Oil spill identification – Waterborne petroleum and petroleum products- Part 1, Date: 2003-11-31.

=> Based on the visual inspection, the samples 1, 2 and 3 are the same kind of oil. There are some differences between the samples 1 and 2, but they are quite similar oils. The sample 4 is different from the samples 1 and 2.

The conclusion is:
- Samples 1 and 2 are possible matches.
- Samples 1 and 3 are a positive match.
- Samples 1 and 4 are no match.
- Samples 2 and 3 are a possible match.
- Samples 2 and 4 are no match.

Notes/remarks:
The report starts with a triplicate analysis of the Sintef mixture (mixture of 4 oils produced for finding the right peaks). It is concluded that ratios are different, when calculated based on peak height or peak area. A calibration line based on height or area can be straight but the slope is different. Besides the height also the peak width increases slowly when increasing the concentration. So ratios to be compared can be based on height or area but should not be mixed.
The st. dev should theoretically be the same but is in practice different. A difference in baseline drawing influences the st. dev of the area calculation to a greater extent, because the broadest part of a peak is at baseline level. On the other hand is the peak height calculation to a larger extent influenced by the scan rate and the stability of the

detector, because only one scan or data point is used. E.g. in Fig 1 of the report of NBI the peak of BaF is slightly cut off. As a result the top of the peak is slightly higher than analyzed. This is caused by the limited scan speed of a mass spectrometer compared to the peak width.

It is stated that the CEN-method is used to compare the samples, but actually mass chromatograms were only visually compared.

2.6   The National Environmental Research Institute (NERI)

Contacts: Asger Hansen

NERI http://www.dmu.dk/International/ is an independent research institute under the Danish Ministry of the Environment. NERI undertakes scientific consultancy work, monitoring of nature and the environment as well as applied and strategic research. NERI's task is to establish a scientific foundation for environmental policy decisions.

The RR-samples were analyzed in duplicate with GC-FID and GC-MS. A VG 70S-250 double-sector instrument operated in single-ion-recording (SIR) mode was used for the GC-MS analyses.

=> Based on visual comparison of GC-FID chromatograms and GC-MS ion fragmentograms of spill and PRP source samples, the generation and direct and statistical comparison of 45 diagnostic compound ratios for each sample based on the repeatability limit, the following conclusions can be made:

Spill-I (Extract-I) contains a mixture of fuel and lubricating (motor) oil, that has been weathered to some degree affecting compounds below nC20. This sample match the PRP Source-I sample almost perfectly, and hence these two sample are identical beyond reasonable doubt. The spill sample does not match the PRP Source-II sample, and the two samples are non-identical.

Spill-II (extract-II) also contains a mixture of fuel and lubricating (motor) oil like Spill-I. It has been weathered and degraded to higher degree than Spill-I, but these two spill samples are probably identical, although this has not been directly tested. Spill-II sample match the PRP source-I sample closely but not perfectly, and the two samples are therefore probably identical. Spill-II does not match Source-II, and the two samples are non-identical.

2.7   Netherlands Forensic Institute (NFI)

Contact: Rene de Bruyn

The Netherlands Forensic Institute www.forensischinstituut.nl analyses oil samples in cases of arson (e.g. gasoline and kerosene) and environmental contaminations.
The RR-samples were analyzed in duplicate with GC-MS. In full scan mode, at a concentration of 1 g/l dissolved in petroleum ether for screening and at the same concentration for SIM analysis of PAH's and terpanes. The method uses 14 specifically formulated PAH ratios and 7

terpane ratios, which are separated in two graphs based on stability and weathering behavior according to Venosa et al.

=> There is no match between Source II and Extract I or Extract II.
- There is a match between Source I and Extract I.
- There is a match between Source I and Extract II.

2.8   Petrobas

Contacts: Maria de Fatima Guadalupe Meniconi

The founding of Petrobras www2.petrobras.com.br/ingles/index.asp was authorized in October 1953, by Law 2004, with the objective of executing, on behalf of the Federal Government, the activities of the oil sector in Brazil. Over more than four decades the company has become the Country's leader in the distribution of oil products, an activity not covered by the Government monopoly, and is internationally acknowledged as one of the fifteenth largest oil companies in the world today.
The Environmental Assessment & Monitoring Department and the Geochemistry Division from PETROBRAS Research and Development Center (CENPES), carry out environmental analyses from all around Brazil, giving support to the Company in forensic litigations issues. These Divisions analysed the samples from RIZA Round Robin Oil Spill Identification.

The Round Robin 2005 samples have been fractioned into aliphatic and aromatic fractions. The aliphatic fraction was analyzed to determine fingerprint (GC-FID) and saturated biomarkers (sesquiterpanes, terpanes and steranes) (GC-MS), and the aromatic fraction was analysed by GC-MS to determine polycyclic aromatic hydrocarbons (PAH) and aromatic biomarkers.

=> According to the results of GC fingerprints, PAH distribution and aliphatic biomarkes, no significant differences could be observed among the samples Extract I, Extract II, Source I and Source II for this round robin. Thus, Extract I and Extract II match with both Sources I and II.

2.9   Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling (RIZA)

Contact: P. Kienhuis

The acronym RIZA stands for Institute for Inland Water Management and Waste Water Treatment. RIZA www.riza.nl is the research and advisory body for the Rijkswaterstaat (the Directorate-General for Public Works and Water Management) for inland water in the Netherlands and a leading international centre of knowledge for integrated water management.

Oil spill identification samples from inland waters and the North Sea are analyzed according the concept guideline Part 2 of CEN BT/TF 120.

In general: Samples are analyzed in duplicate with GC-FID. After assessment of the results the (probably) matching samples are also analyzed with GC-MS in SIM mode.
After a visual assessment of the chromatograms (probably) matching samples are integrated for a large range of compounds (depending on the type of oil and compounds available). Ratios between spill and source samples are compared by using the repeatability limit (ISO 5725) as a match criterion.

=>

	Extract I	Extract II
Source I	match	match
Source II	non-match	non-match

Notes/remarks:
RIZA has diluted the Sintef mixture for peak recognition a factor of 3 with DCM and has analyzed the diluted mixture 8 times in order to calculate the RSD for the mixture at the given concentration.
The results can be found on the CD in file **St Sintef ratio validation.xls.**
In the guideline it is stated that the RSD should be equal or lower than 5% during validation of the method. This is valid for all ratios with the ratio DR-30d/30ab at limit.

2.10 Sintef

Contacts: K.R. Sørheim, L-G. Faksness and P.S. Daling.

Sintef http://www.sintef.no/ is a large Norwegian engineering institute and is among others active in the petroleum industry and petroleum research. Sintef analyzes all oil spill samples for the Norwegian government.

As a basis for this oil spill fingerprinting case, SINTEF has used the presently draft version, August 2005, of the "CEN/BT/TaskForce 120 Oil Spill Identification": Part 2 Analytical methodology and interpretation of results"
Sintef analyzed the RR-samples with GC-FID and with GC-MS in SIM mode on PAH's and biomarkers. The resulting chromatograms are first evaluated visually and when a possible match is assumed a series of ratios are statistically compared.

=> Extract I and II: Positive match between the spill samples.
The spill samples are from the same source

- Source I: Positive match to the spills (Extract I and Extract II)
- Source II: Non-match to the spills (Extract I and Extract II)

2.11 Swedish National Laboratory of Forensic Science (SKL)

Contact: M. Källberg

SKL is the only forensic laboratory in Sweden and thus all kinds of investigations are performed, mainly to assist the police. The total number of persons employed is about 240. SKL has four departments:
  biology+morphology
  documents+IT
  drugs
  chemistry+technique+weapon.
One group of nine persons is dealing with investigations related to fire, oil and environment. A big part of this is the analyses of oil samples in cases of fire-raising (e.g. gasoline and kerosene) and environmental contaminations.

SKL does not (yet) have a home page on the Internet in English, they only have information in Swedish: http://www.skl.polisen.se/.
The samples were analysed according to the standard procedure for oil investigation at the Swedish National Laboratory of Forensic Science. The method used is based on the "Nordtest method NT CHEM 001 Edition 2" which may be found also in the Internet:
http://www.nordicinnovation.net/nordtestfiler/chem001.pdf

The oil samples were diluted with dichloromethane and analysed with gas chromatography with flame ionisation detection.
If there are differences between the samples that cannot be explained by weathering effects we conclude that the samples are not identical (have not been identical at the moment of discharge) and make no further investigations.
If the results can be explained by weathering effects we continue by running the extracts on GC-MS in SIM mode

=> According to the result of our analysis there is a positive match between spill samples (Extract I and II) and Source I. Between the spilled samples (Extract I and II) and the Source II there is a non-match. We express this in the same way as with other forensic comparisons, please see the last page of the SKL report.

The findings show with certainty that the oil spill sample Extract I was identical with the bilge oil sample Source I at the time of discharge (Level +4).

The findings strongly indicate that the oil spill sample Extract II was identical with the bilge oil sample Source I at the time of discharge (Level +3).

The findings show with certainty that none of the oil spill samples Extract I or II were identical with the bilge oil sample Source II (Level -4).

2.12   Waterschap Regge en Dinkel (WRD)

Contact: A Kroeskamp

Waterschap Regge en Dinkel www.wrd.nl is a local authority responsible for the water quality of an eastern part of the Netherlands. The analysis of the total amount of mineral oil in water samples (ISO 9377) is a routine method, which is also applied to identify the type of oil in samples and sometimes to compare samples in case of an oil spill. For the analysis GC-FID is used. The analysis with MS, mentioned in the conclusions, is beyond the scope of their lab.
Resulting chromatograms can be found in the original report.
The results were received by letter in Dutch. Page 1 and 2 contains the most relevant information within the framework of this report and has therefore been translated, see document Results page 1 and 2 english.doc on the CD. The original document, including the chromatograms, has been scanned into document WRD RR2005.pdf.

=>
Comparison of extract 1 and extract 2:
- The fractions C10-C12 and C12-C16 of the extracts 1 and 2 are different and are also different from the same fractions of source 1 and source 2, so the contamination of the canal is not caused by one of the sources and are also not related to each other.
- If the difference in percentages of the more volatile fractions is caused by evaporation and biological degradation, then the two extract samples are comparable and from the same source.

Contamination in the canal is from source I or source II:
- The source 1 mineral oil percentages in the "more heavier" fractions after C20 match very well with the same fractions of extract 1 and extract 2. (see shading in table 2)
- Similar properties of the oil chromatograms of extract 1 and 2 en source 1; the alkanes of fraction C18-C26 show an irregular reduction in peak height. The peak heights of the alkanes of fraction C18-C26 of source 2 show a hyperbolic pattern.

Based on the above-mentioned observations and results (GC/FID) it is concluded that source 1 is responsible for the contamination.
Because the properties of the 4 samples don't show clear differences confirmation with MS will be necessary.

3. Discussion

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3.1 Introduction

In contrast to Round Robin tests, where the concentrations of single compounds have to be measured (part of the QM in environmental monitoring) it is merely asked for a conclusion (mainly "yes" or "no") in the RR-tests for oil spill identification. But the participants put much work into analysis and presentation. Thus, in addition to simply summarizing the mere "overall results" in a final RR-report, emphasis should also be put here on how results are achieved and how these results are presented.

This RR offers a variety of possibilities to check the applied method and to make improvements towards a better effectiveness of the analysis and a better clarity of the report:

1.   The samples source I and source II contained different light fuel oils.
2.   Weathering was effective in the samples in the order: source I (no weathering) < spill I < spill II.
3.   In order to make the comparison not too easy, samples with very similar lubricating oil were chosen as possible source samples. The "true" biomarkers, i.e. hopanes and steranes, which originate from the lub oil, were thus hardly different in all 4 samples.
4.   The aromatic steranes, except maybe the two lightest boiling ones, i.e. C20, C21, were not present.

The results of this Round Robin were jointly evaluated by RIZA and BSH.

Although RIZA has prepared the samples and thus knows the result from the beginning, RIZA "participated" in this Round Robin even in a twofold way:
Firstly, samples were analyzed and results (raw data) were produced ready for being included into the COSI database of the BSH. BSH has included them afterwards. (see for a presentation of COSI on the CD for Oeldatenbank- COSI.ppt or use http://www.bsh.de/de/Meeresdaten/Umweltschutz/Oelidentifizierung/Oeldatenbank.ppt)
Secondly RIZA has produced results according to the draft CEN-guidelines.
On the other hand, BSH has received 4 samples without knowing anything about them before the main part of this discussion was already written. There was merely the information that there were two

· ·
Remark
Similar lubricating oil in bilge samples is often found by RIZA. This may be due to the fact that RIZA mainly receives cases from inland waters, where smaller ships with diesel driven engines are involved (in a limited area). BSH`s experience is different: receiving mainly open sea and coastal cases quite different lubricating oils are found, even with regard to viscosity.

spill samples and two source samples and the question would be asked, "which samples match".

3.2   GC-screening

Nine of the twelve participants have started with GC-FID to screen the samples. In oil spill identification a fast GC-FID analysis is often used as a quick method to analyze all samples and to see which type of oil is involved and whether some of the samples can be eliminated from the final comparison by means of GC-MS. Additionally the method can be used to adjust the injection concentration for the GC-MS analysis.

Differences in the fuel oil part between source I and source II could have been found already by GC-screening:
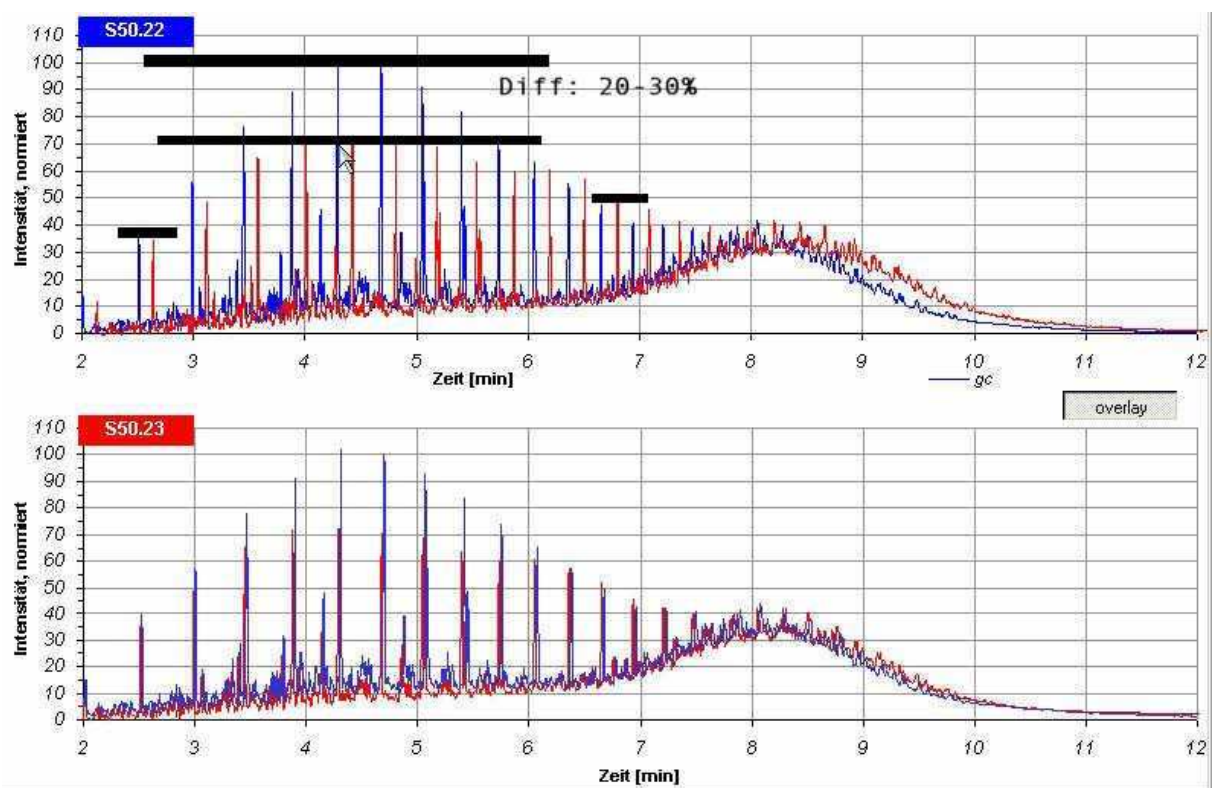
Fig. 3: Gas-chromatograms of samples source I and source II (source II slightly shifted to the right above) (BSH)
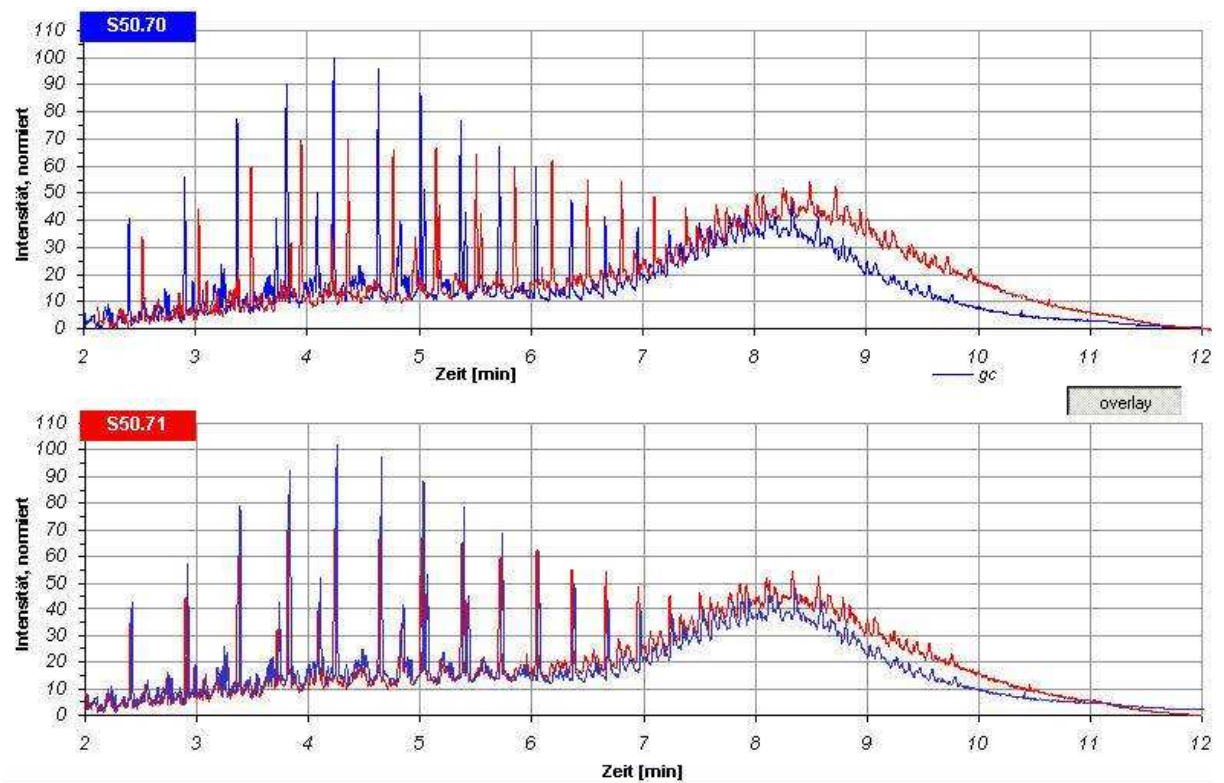
Fig. 4: Gas-chromatograms of samples source I and source II (RIZA)

The distribution of n-alkanes in source I (blue) and source II (red) is totally different:
If, for example, the peak heights of nC14, nC15, n-C16 and n-C17 are divided by either the peak height of the lower boiling n-C11 or the higher boiling n-C22, then differences between 20 and 30% are found in the ratios of these peaks. These differences -found in the main constituents of the oils- are beyond analytical error.

The relation of the two spill samples to the two possible source samples is perfectly visualized by the percentage weathering (PW)-plots produced by SKL: no differences except those caused by evaporation are recognized, when the spill samples are compared with source I. But compared with source II, alkanes of the spill samples are 20 to 30% too high in the region C16 to C19.
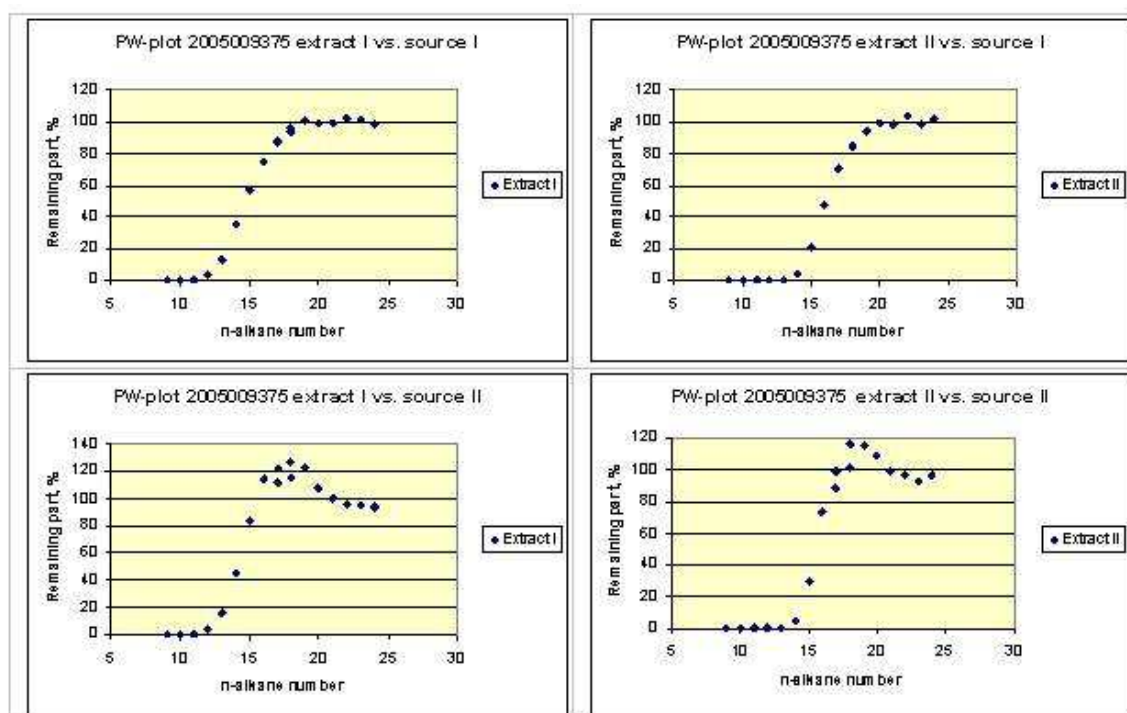
Fig 5
Percentage weathering (PW)-plots
(SKL)

Corresponding big differences in the n-alkane distribution between source I and source II are obvious also in the weathering checks produced by NERI:
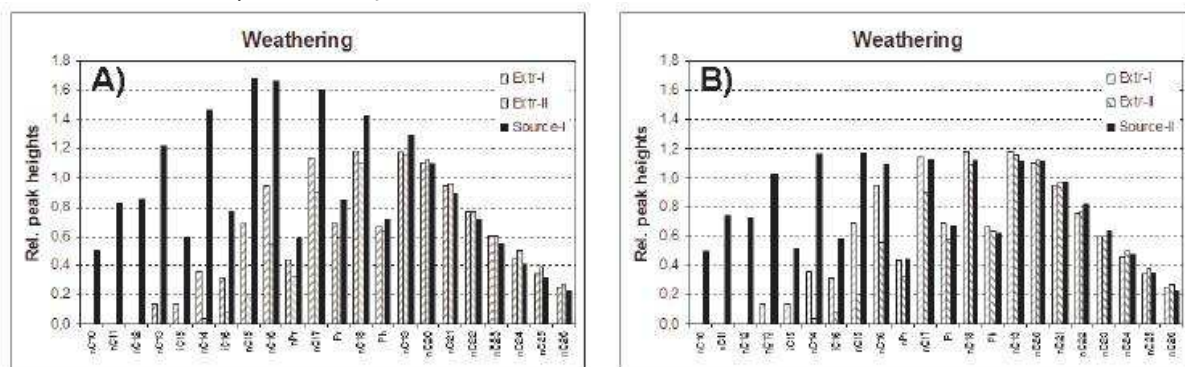
Fig 6
Weathering check (NERI)

But these differences cannot be attributed to the fact that source II had a relatively lower fuel oil content then source I, as suggested here: n-alkanes are not present in the lub oil. So, these differences have nothing to do with the relation of fuel oil to lub oil.

Differences between the spill samples and source II were not so obvious here: C19 is only slightly higher, whereas C22 and C23 are slightly lower in the spill samples. This might be due to the fact that the peaks are normalized to the mean of C19 to C22 here, where C19 could already have been affected by weathering.

Results look obviously already much clearer, when the alkanes are based on the mean of C20 to C24, as done by NBI:
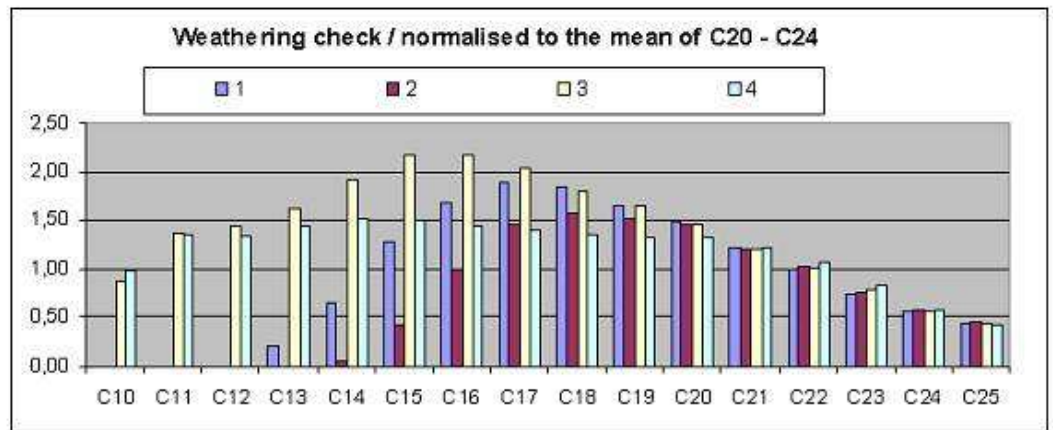
Figure 6. Weathering check of the samples 1 – 4.

Here, not only the big difference between source I and source II is perfectly visualized, but also the difference between the two extracts and source II (where the difference of extract I is more obvious). NBI`s conclusion "sample 4 (source II) cannot come from the same source as sample 1 (extract I) and sample 2 (extract II)" (and, of course sample 3 (source I) is meant here, too) as the differences cannot be explained by the weathering effects, seems to be justified.

The same differences in the n-alkane distribution between source I and source II can be observed in the chromatograms shown by SINTEF.
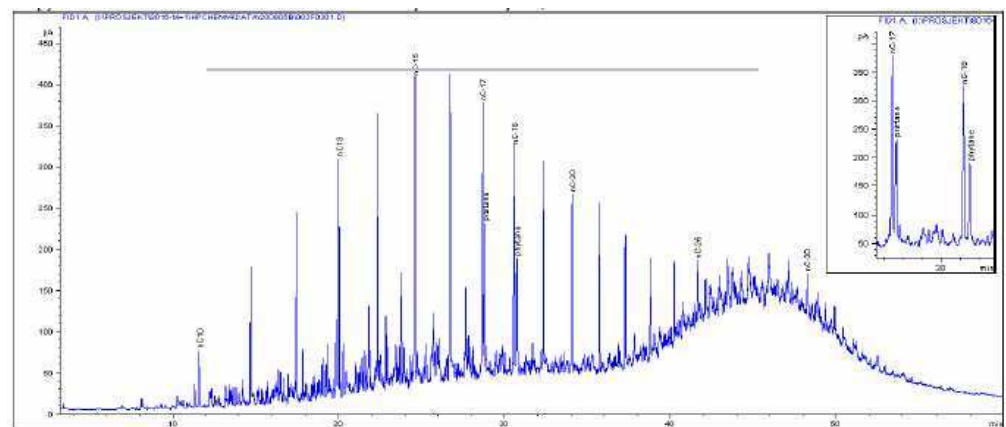
Figure 3.1C   Source I: Suspected source, SINTEF ID 20054-0841
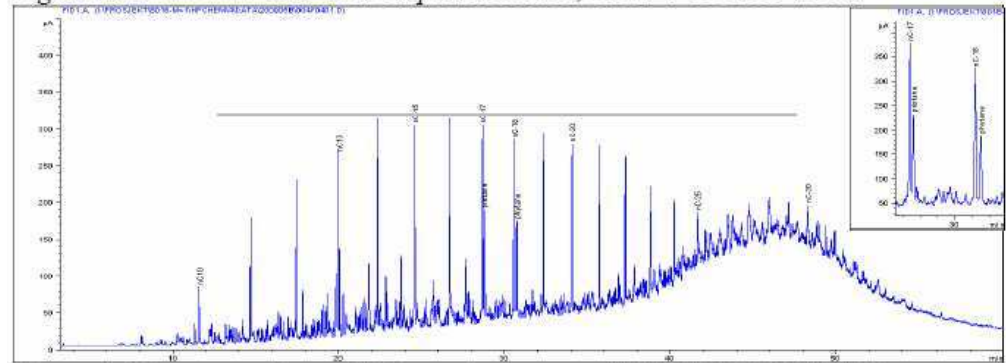
Figure 3.1D   Source II: Suspected source, SINTEF ID 20054-0842

Drawing a horizontal line at the peak maximum of C15 (as added here to the figures) visualizes that C15 is not an outstanding maximum in source II (as it is in source I).

As a result of the GC-FID analyses Sintef decided not to evaluate the GC-FID results further and to go directly to the GC-MS analysis, because all samples show the same type of oil and because the C17/pristane, C18/phytan and pristane/phytane ratios are weathered. Sintef discusses in their report the usefulness of the C17/pristane, C18/phytan and pristane/phytane ratios, which leads to the recommendation that those ratios should only be used carefully, because "generally the compounds in the C17 to C20 area are often affected by evaporative loss".

The corresponding difference in the n-alkane distribution of source I and source II has clearly been found also by LVA (source II middle, source I below):
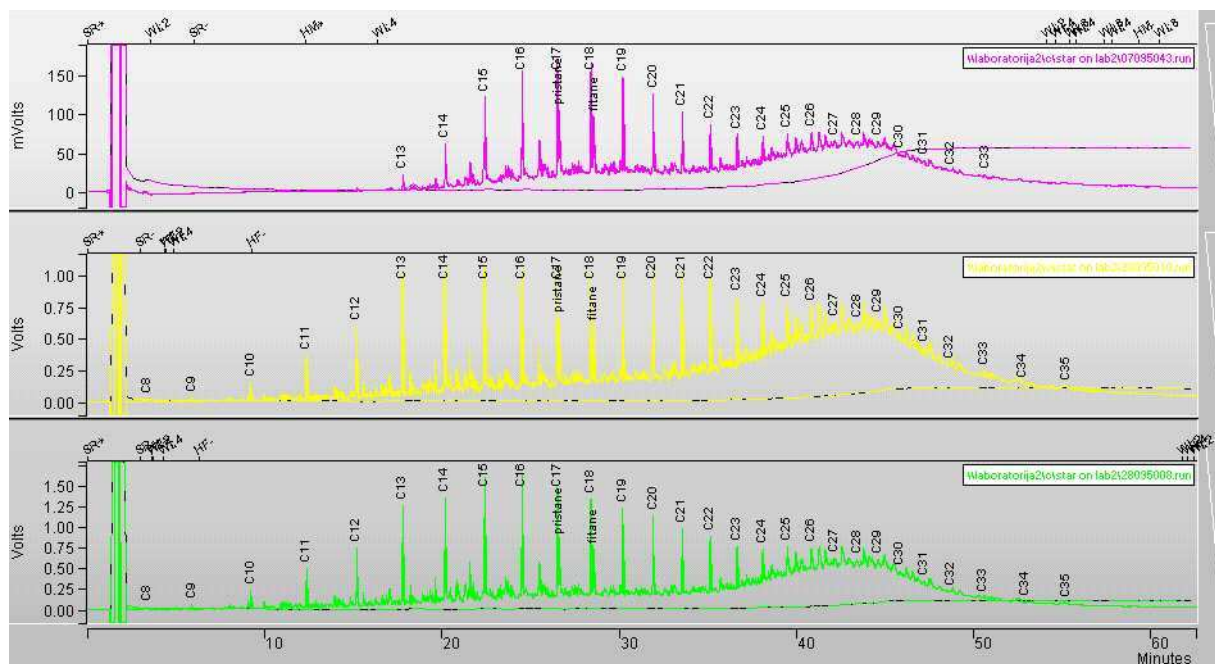

Fig 9
Gas Chromatograms (LVA)

Based on the GC-FID analysis five of the nine labs have noticed that Source II shows differences compared to both spill samples. BMM decided, based on the GC-FID results, to eliminate Source II from the GC-MS analysis.

3.3   Obvious differences in the mass-chromatograms

In order to be able to exclude possible source samples, which are obviously different, mass-chromatograms should be carefully checked visually before peak ratios are produced.

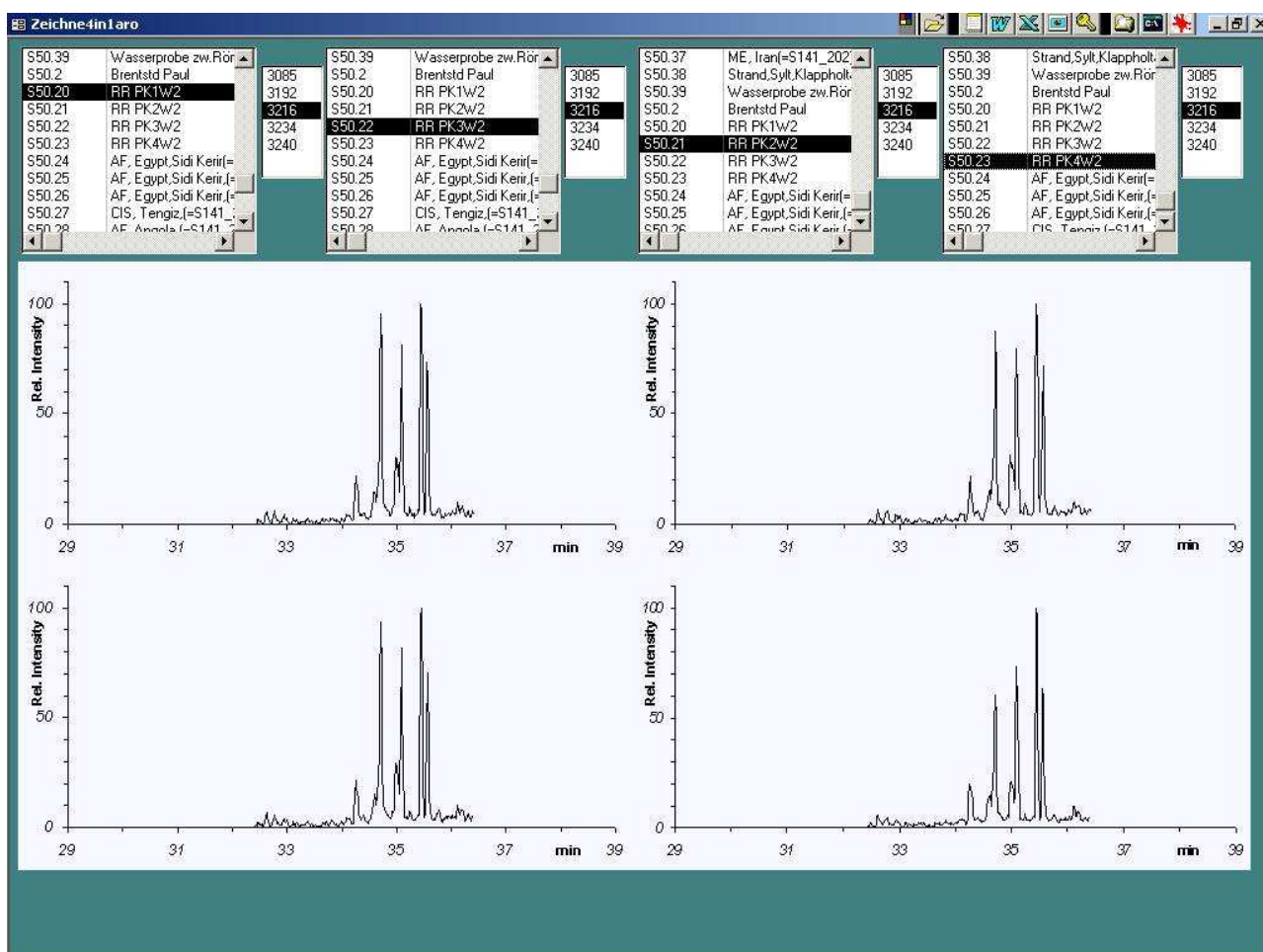This saves time and resources and improves the clarity of a report.

. . . .
Fig 10:
Mass 216 (BSH)

An obvious difference in the relation of benzo(a)fluorene (B(a)F to 4-methylpyrene (mass 216, see Fig 11 for identification) compared to all other samples could have been found in source II (lower right). This difference cannot be caused by weathering, because B(a)F is reduced in the source sample and not in the spill sample, as would have been when caused by weathering.

Expressed in numbers, this difference reaches 40 to 46% (repeatability, both, BSH and RIZA results). Further differences of 18 to 20% are found in masses 192 (M-phenantrenes) and 234 (retene-methylated naphthalenes).

Differences in the same order of magnitude in the compounds of mass 216 (and 192) of source II compared to all other samples are also presented by SINTEF and pronounced by arrows.
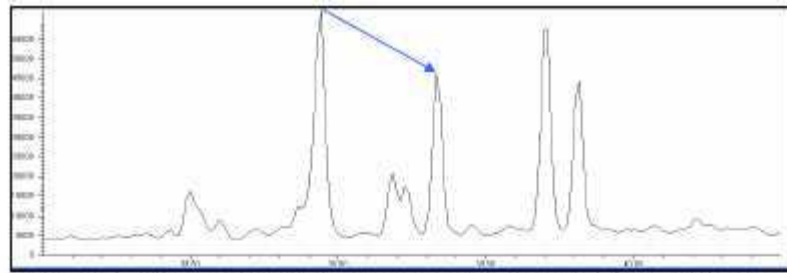

Figure 3.5.a   Extract I: C1-fluoranthenes/pyrenes/benzofluorenes (m/z 216) SINTEF ID 2005-0839
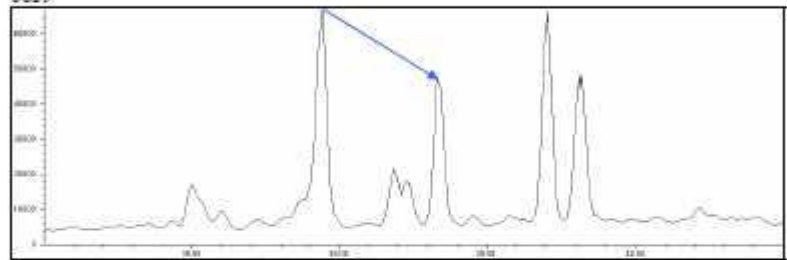

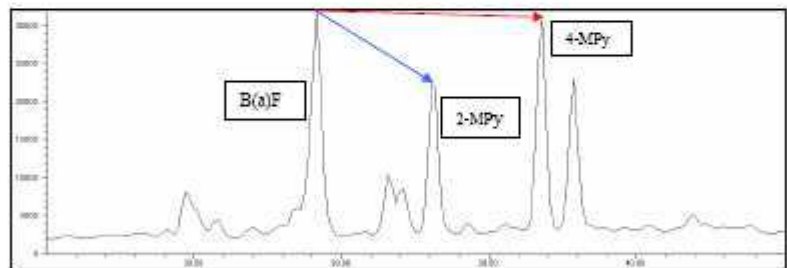Figure 3.5.b   Extract II: C1-fluoranthenes/pyrene/benzofluoreness (m/z 216) SINTEF ID 2005-0840

Figure 3.5.c   Source I (oil I): C1-fluoranthenes/pyrenes/benzofluorenes (m/z 216) SINTEF ID 2005-0841
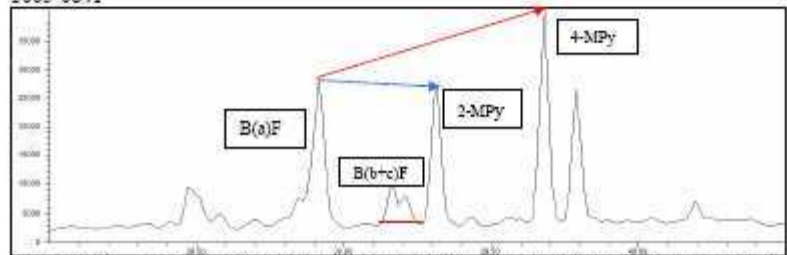

Figure 3.5.d   Source II (oil II): C1-fluoranthenes/pyrenes/benzofluorenes (m/z 216) SINTEF ID

. . . .
Fig 11:
Mass 216 (SINTEF)

3.4   Comparison of compound ratios

Besides the visual comparison of chromatograms and plots of peak heights or area's of ranges of compounds (e.g. see Fig 6 and 7), all labs used, to a certain extend, ratio's to improve the foundation of the final conclusions. A summary of the methods reported is given in Table 2.

Labs	Methods		GC-FID				GC-MS				
	FID	MS	visual inspect.	height or area plots	PW plots	ratio calc.	visual inspect.	height or area plots	PW plots	ratio calc.	numerical limitations
BMM	X	X	X				X	X		X	X
Cedre		X					X	X		X	X
LASEM		X					X	X		X	X
LVA	X	X	X	X		X	X			X	
NBI	X	X	X	X		X	X				
NERI	X	X	X	X		X	X			X	X
NFI		X					X	X		X	X
Petrobas	X	X	X			X	X	X		X	
RIZA	X	X	X	X	X	X	X	X	X	X	X
Sintef	X	X	X			X	X			X	X
SKL	X	X	X		X	X	X				
WRD	X		X	X		X					

. . . .

Table 2
Methods to compare samples

A type of ratio calculation is the percentage-weathering (PW) plot. After normalization on compounds not weathered, the percentage of a compound in the spill sample compared to the source sample is calculated for a range of compounds and shown in a sequence based on retention time. These plots were produced by RIZA and SKL: Weathering is investigated with help of the PW plot, where the area of each of the n-alkanes is compared with the average of the area of five unweathered n-alkanes. For slightly weathered oil samples we use the average area of $C17$ - $C21$ and for more highly weathered samples we use $C20$ - $C24$.

These plots are very helpful to estimate evaporation and to find differences in patterns. For examples see Fig. 5 and 19 in this report and Fig. 19 and 20 in the RIZA report.

Very common is also the calculation of ratios between compounds. Assuming that a spill and source sample are coming from the same source, it can be assumed that ratio's between compounds are also identical, although it must be keep in mind that weathering, contamination and homogeneity can cause differences.
An example is the calculation of the C17/pristane, C18/phytane and pristane/phytane ratios from the GC-FID and/or GCMS results.
In this case however the value of these ratio's was limited. First of all the differences were very small and additionally the ratio's were influenced by weathering. SKL has applied a calculation to compensate for weathering, but no conclusions based on the results are mentioned.

Another example is the ratio calculation of LVA shown in Table 3. The table highlights the difference in ratio between B(a)F/ 4-methylpyrene for Source II.

Ratio	EXTR I	EXTR II	Source I	Source II
a/e	0.23	0.23	0.25	0.16
b/e	**1.0**	**0.94**	**0.93**	0.36
c/e	0.19	0.16	0.16	0.1
d/e	0.79	0.76	0.8	0.72
f/e	0.73	0.70	0.75	0.60

Such tables show differences, but what is acceptable is still a personal decision.
In the CEN guideline it is defined that differences caused by the analytical method may not lead to the conclusion that samples are different:
positive match
differences in chromatographic patterns and diagnostic ratios of the samples submitted for comparison are lower than the analytical variance of the method or can clearly be explained by weathering. The samples are identical beyond reasonable doubt

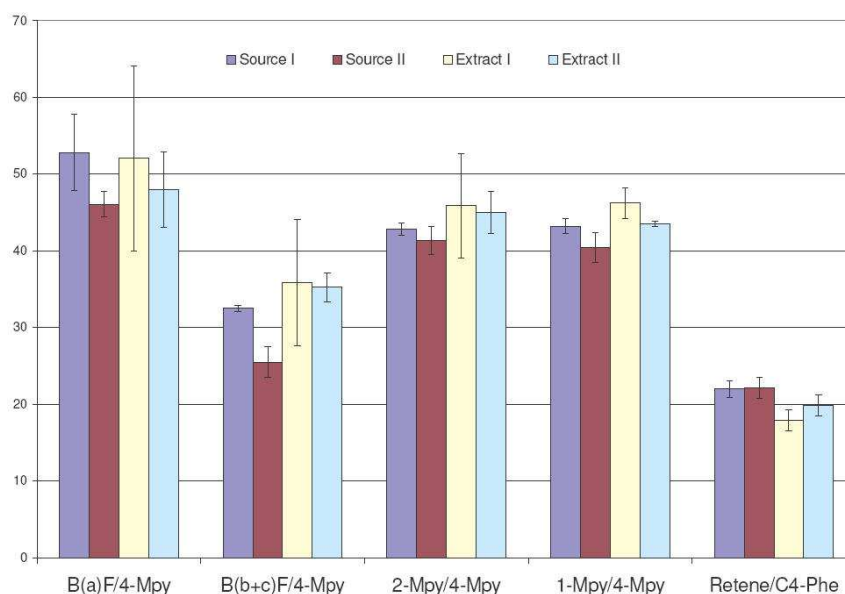Several labs have used numerical limitations to estimate the acceptable difference.

Figure 5   Aromatics distribution of 4 samples (m/z = 216 and 234) (error bars represent 95% confidence level intervals, N=3)

Fig 12 shows a bar graph of Cedre with error bars:
The analyses were run in triplicates for each sample, and error bars considering 95% confidence level intervals were plotted on the histogram.

In the report of Cedre also graphs with a 95% and 98% confidence are shown and used to estimate, whether differences are significant or not.

Note: Cedre's Fig 5 indicates a difference higher than the 95% confidence level for the ratio B(b+c)F/4-Mpy instead of the ratio B(a)F/4-Mpy shown and discussed above. The report of Cedre doesn't show chromatograms of these peaks, so it is difficult to find a reason for this difference.

NERI used an excessive suite of compound ratios for sample comparison:
From the fragmentograms, an initial suite of 57 diagnostic compound ratios (DRs) were calculated (most based on individual peak heights, some on areas of whole isomer groups). Both the number of recorded ions and selected ratios are higher than and somewhat different from those suggested in the CEN Guideline.

Generally, ratios were derived from measured peak heights and calculated as A/B, but in a few cases calculated as A/(B+C). Some of the selected peaks did not represent specifically identified compounds but were chosen as being well-resolved and of good intensity, while other peaks represented identified compounds (primarily biomarkers).

Asger Hansen indicated that they have used the round robin samples not only for the round robin, but also to test and validate their instrument (Personal information not mentioned in the NERI report) Neri uses a double sector instrument :
Following the GC-FID screening, all four samples were analyzed by GC-MS fingerprinting using a VG 70S-250 double-sector instrument operated in single-ion-recording (SIR) mode at a resolution of 2000 and by using a J&W 30 m x 0.25 mm x 0.25 µm DB-5 capillary column and manual cold on column injection with He as carrier gas. 35 ion masses (+ two additional lock-masses) were recorded in two separate runs (runtime: 75 min.) for each sample. All samples were analyzed in duplicates.

From the duplicate analysis of each sample a pooled st. dev. for each ratio has been calculated. Nine ratios had a $RSD_{pool} > 10\%$ and were excluded. Furthermore, three sesquiterpane (the nC13-nC16 range) ratios were affected by weathering and hence excluded as well. For the remaining 45 ratios, the absolute difference between a spill sample and a source sample was compared with the critical difference, CrD as defined in the report. A result of this evaluation is shown in the figure below (Fig. 13)

The results of NERI and Cedre show the limited value of the numerical limitations, when calculated from a limited data set. CEDRE has analyzed all samples in triplicate and from the triplicate results for each sample the 95% confidence level is calculated for each ratio. Fig 12 shows the variation in limits for one ratio calculated from different samples(see e.g the large difference in error bars for the ratio B(a)F/4Mpy. NERI worked with a pooled st. dev. to increase the dataset, but still a lot of variation is visible. Low RSD's can lead to false negative results (e.g. 1.2% for the ratio Ts/Tm in the example given in Fig 13, which has led to several differences above the critical difference)
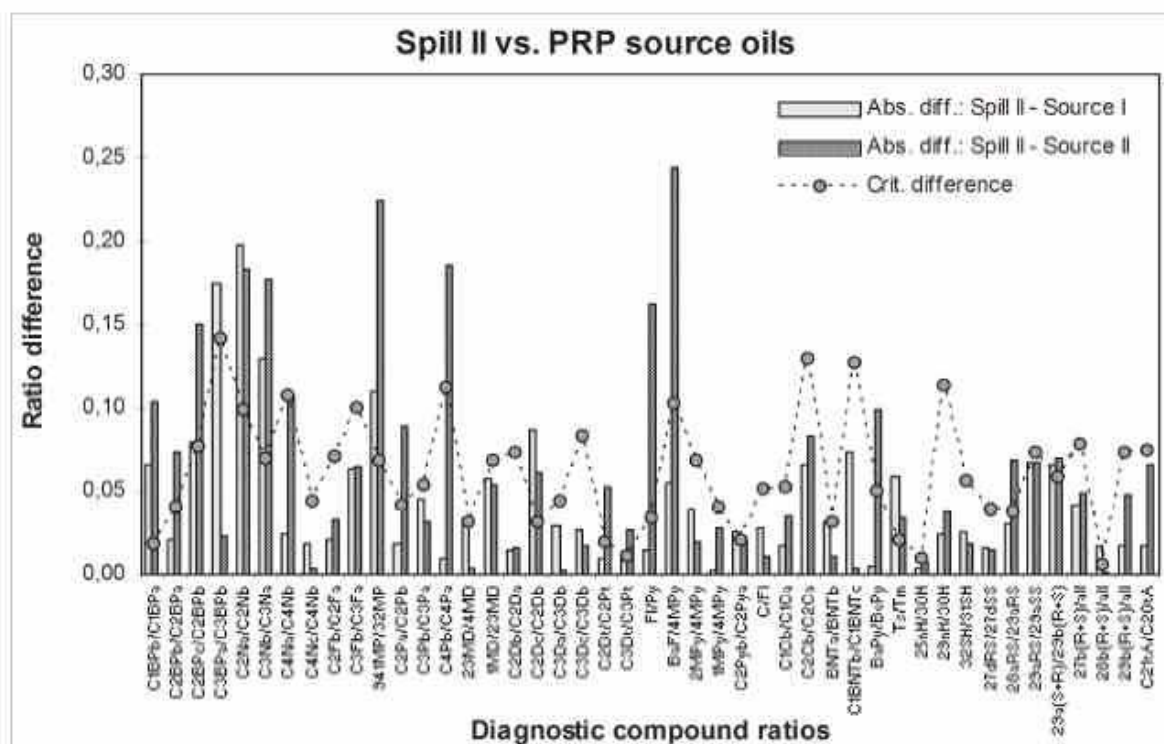
Figure 5. Comparison of absolute differences between DRs of Spill-II and Source-I and Source-II with the corresponding CrDs based on the r$_{95\%}$ repeatability limit.

The use of a fix RSD overcomes this situation.
NFI calculates ratios from a series of PAH's and biomarkers. The ratios shown in fig 14 have been normalized on the ratio's of Source I.
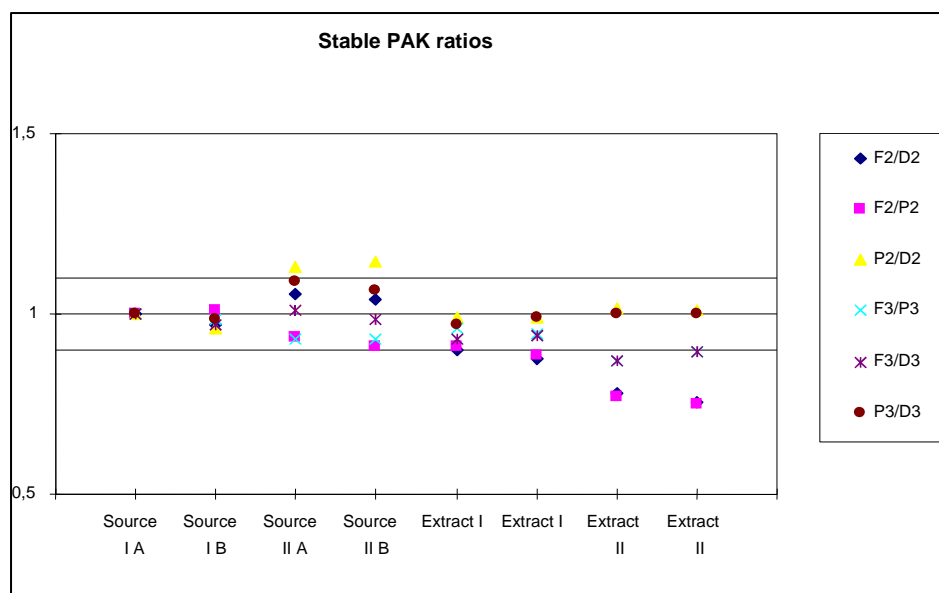
Because Extract I and II are prepared from Source I all ratios theoretically should have the value of 1. In practice the analytical method causes small differences. As a numerical limit 10% lines are

used in the figure. The ratios have been chosen such that weathering causes a reduction of the value of a ratio. This has been found for e.g. F2/P2 of Extract II. The plot also shows that the ratio P2/D2 of Source II is above the upper limit for both duplicate analyses.

For the CEN guideline it has been decided to work with a fixed RSD of 5% for all ratio's to overcome the variation in critical differences. To test this value a method must be validated by analyzing a sample at least 7 times and to calculate the RSD's of the ratios. RIZA has done this recently for the peak identification mixture prepared by SINTEF. The results can be found on the CD in the RIZA map.
The RSD of 5% can be seen as a quality criterion, because methods producing higher values may not be used to analyze/compare oil samples. E.g. fast methods with a low number of scans over a peak will produce higher RSD's and are therefore not acceptable.
A difficulty with this approach is the higher RSD for small peaks. Therefore it is advised to analyze in each case some of the samples in duplicate and to compare the ratio's of the duplicates. When small peaks show differences above the critical limit these peaks should only be used for a visual comparison. Additional a S/N criterion is added to be able to decide not to integrate small peaks.

As a result it is possible that labs will use and eliminate different ratios: SINTEF comes to a full match of source I with spill I and spill II (all parameters match). But compared to the draft CEN-guidelines, several ratios are left out (marked as "Not Quantified"). It is indicated here, for example, that the retene/4-phen ratio is not "considered to be significant" since the retene peak is very small compared to the area of the 4-phenantrenes and hence "give a large uncertainty".
The retene/4-phen ratio was determined by RIZA without any problem. Even a higher value than in the peak identification mixture produced by SINTEF was found.
Furthermore, neither RIZA nor MUMM nor BSH had any problem with oleanane, which according to SINTEF revealed a too low signal to noise ratio (lower than 10) and also bisnorhopane (28ab) could be determined without any problem by these labs (SINTEF has also signed bisnorhopane as "Not Quantified").

This may seem a problem, but it reflects the difficulty to treat samples in the same way in different labs with different instrumentation. The problem can be solved to demand, that eliminated peaks must be used in the (final) visual comparison. This is done in the guideline.

Also serious is the remark of e.g. Cedre and LASEM (see also 2.3) that the hopanes are low and difficult to analyze.
LASEM: The calculated ratios with 191 fragment do not permit significant conclusions, because of their low abundance and the high variability of ratios values.
Cedre: Biomarkers could be analyzed in regular conditions but they proved to be present in oils in low abundance. Consequently, Hopanes, steranes/Diasteranes and Triaromatic Steranes distributions

illustrated figures 2, 3 and 4 have to be considered with care. However, the great variability represented by the error bars did not allow to make differences between samples.

NERI had no problems with the RSD's of the hopanes, while the sensitivity at RIZA was good.
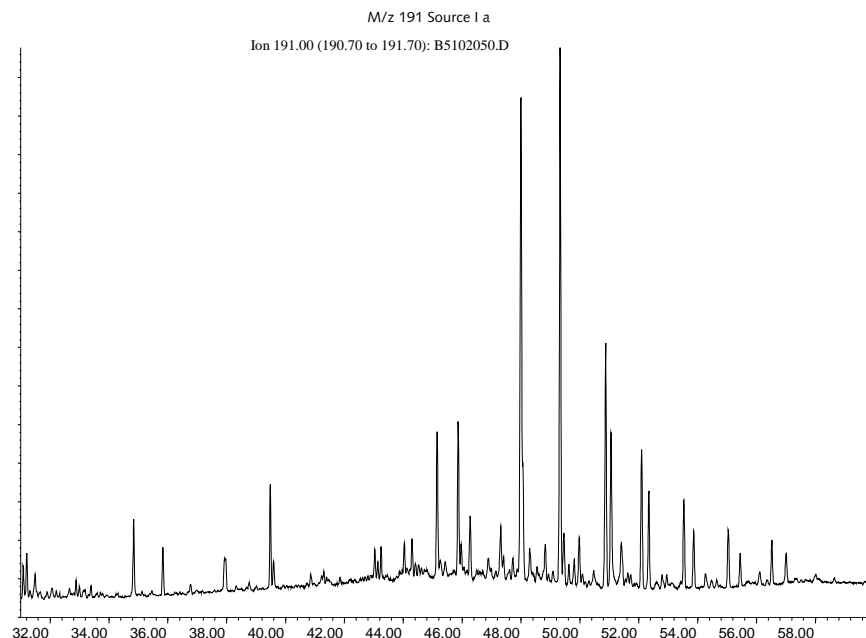

M/z 191 Source I a

Ion 191.00 (190.70 to 191.70): B5102050.D

Fig 15
Hopanes of Source I (RIZA)

Different is the low abundance of the triaromatic steranes (m/z 231)
By means of GC-screening, it was easy to find out that a mixture of light fuel oil and lubricating oil was involved.
The "true" biomarkers", such as the hopanes and steranes thus originated from the lubricating oil. But aromatic steranes are not present in this type of oil. Instead unresolved peaks on a hump appear. Very probably these are the 4-alpha-M-steranes.
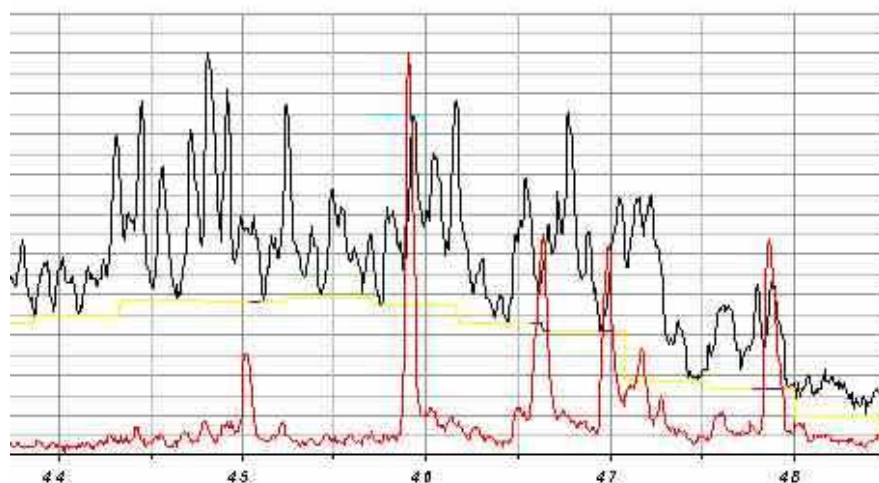

Fig 16.
Mass 231 (RIZA)

The black line shows mass 231 of sample source1 together with the aromatic steranes of a crude oil (Brent-crude, red line). Obviously, aromatic steranes were definitely not present in the samples.

If in this case the oiltype found by GC-screening (mixture of light fuel with lubricating oil) is not considered and a fixed set of parameters is used for all oiltypes, then the analyst will suddenly be in a situation, where highly overlapping peaks on a hump are found instead of 4 to 5 normally very well resolved peaks.
He may, nevertheless, find peaks at the proper retention-times and even integrate them. This might not be a bigger failure, if he does the same with all 4 samples, since all peak relations must match, if samples are regarded as identical. But the integration of highly overlapping peaks on a hump is very crucial and should be avoided because those peaks cannot be measured precisely.
It may even have been tested, whether peaks can be measured precisely or not by determining the corresponding peak ratios in two or three consecutive injections of the same sample.
But such a test cannot be regarded as sufficient for excluding ratios because it is possible that those ratios can be measured precisely in this case by chance (because the peaks are measured here definitely in the same sample). But there would be the high risk of a non-math, when a comparison is made afterwards between the spill sample and the possible source sample although these samples actually originate from the same source because these samples may have a totally different "history" (The spill sample may even have been extracted, whereas the possible source sample may have been weighted out. The two samples may thus even have a quite different oil concentration).

Generally, the analyst must use the knowledge here, that aromatic steranes are not present in lubricating oil. He should not be astonished, when he finds overlapping peaks on a hump instead of 4 to 5 well resolved peaks.
In addition, he must avoid to integrate peaks in a highly overlapping cluster (bad chromatographic practice).

This means that the analyst must be able to decide, which ratios are useful in a distinct oil spill situation and which are not.
Using a fixed, broad set of parameters (compound ratios) for all oiltypes and in all oil spill situations from the beginning, and letting the system "decide", may thus easily lead to wrong conclusions.

In the CEN guideline therefore a contribution of Gerhard Dahlmann can be found (annex H) describing the peculiarities of the different oil types. It has been finished already a few years ago and has been advised to all participants of the Round Robin last two years:
The problems associated with the identification of bilge oil samples are especially well described in
http://www.bsh.de/de/Produkte/Buecher/Berichte/Bericht31/Bericht31.pdf.
Hints, tips and precautions are given here concerning their analysis and result interpretation.

Here also the absence of the triaromatic steranes in lubrication oil is mentioned.

3.5 Number of ratios

The labs using ratios apply a varying number of ratios. E.g. LVA has used 5 ratios from m/z 216 to show the difference between source II and the extract samples, NFI has used 21 ratios and Petrobas 22 ratios to test weathering and identity and NERI has calculated the highest number of 57 ratios.

Ratios can be used to show differences, but also to "proof " identity. Gerhard Dahlmann has build a database containing the chromatograms of more than 1000 cases (see Oeldatenbank-COSI.ppt on the CD for a presentation). The database is able to sort on identity and makes it possible to test how many ratios are needed to specify a sample. Gerhard has learned that for most samples 5 to 6 ratios are sufficient to eliminate non-matching samples.

Without a database it becomes more difficult to "proof" identity on a limited number of ratios. Therefore 29 ratios are mentioned in the CEN guideline to be used for comparison. The ratios should be used in a flexible way, based on presence and stability of the compounds involved.

Finally it must be stated that a comparison without using ratios to proof identity is highly susceptible for (mis)interpretation, while a comparison only based on ratio comparison can easily lead to false matches, because the differences could be present elsewhere. Therefore ratios must be seen as a tool,

- to force analysts to compare a large range of compounds,
- to be able to set a quality criterion for the comparison and
- to compare samples unaffected by personal interpretation.

3.6 Sesquiterpanes.

Comments of Gerhard Dahlmann on the use of the sesquiterpanes by some of the participants:

RIZA indicates:
Changes compared to the round robin of last year:
The sesquiterpanes are added as compound group to be analysed with GCMS (m/z 123).

Thus, although it is found in the weathering check that alkanes are evaporated up to C20 in the spill samples, sesquiterpenes, which are
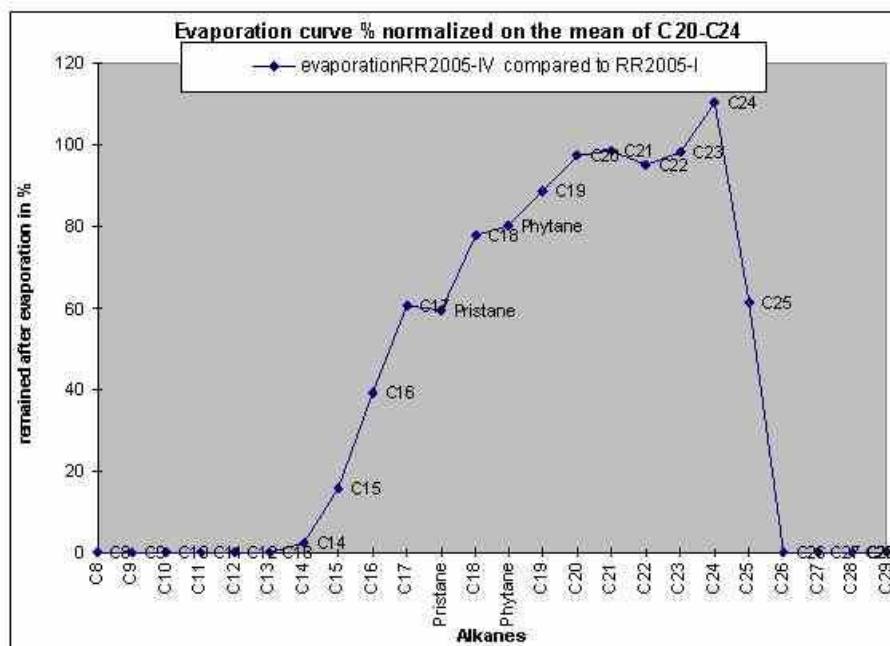
Fig 6: Source I with extract II. The graph shows a consequent weathering pattern. The spill

boiling between C13 and C16, are determined and compared.
Consequently, firstly a "big alert" is produced in the diagrams showing that the corresponding error bars highly exceed the critical difference, and afterwards an "all clear" is produced by showing in a second diagram that those big differences are caused by evaporation:
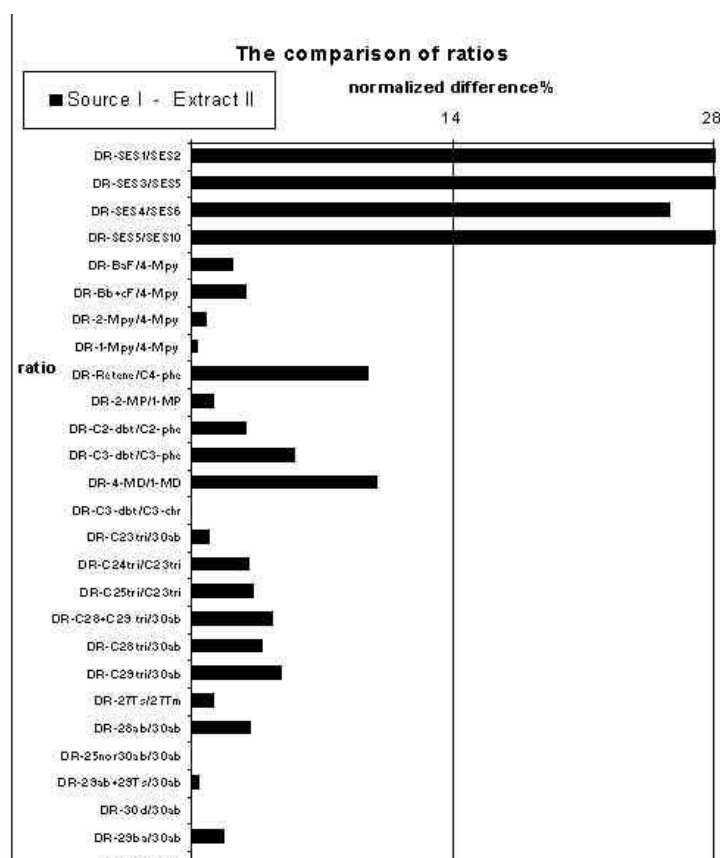
Fig 18. % area for each compound of Source I and Extract II.

Fig 19.
RIZA additional weathering check

This must be regarded as a highly uneconomical practice.
What cannot be said about the C17/pristane, C18/phytane and pristane/phytane ratios is much more likely for compounds boiling from C13: in spill samples, these might nearly always be affected by evaporation. Generally, it is given in the literature that sesquiterpanes may be useful in distinct cases for source correlation (i.e. for finding "general sources") because of their resistance to biodegradation. A case such as the one, which is given in this Round Robin here, is, of course, neither mentioned nor meant, when the usefulness of sesquiterpanes is discussed in literature.

It is indeed true that the sesquiterpanes should not have been used in this case . The CEN working group has recently decided to add some ratios of the sesquiterpanes, because this group of biomarkers is well known and often mentioned in source specifications. See the contribution of Wang in RR2004, the contribution of Petrobas in RR2005 and CEN Guideline Part II 6.3.5.4. In the guideline the sesquiterpanes are mentioned optionally with the restriction:
As any other low-boiling compounds, the sesquiterpanes are subject to evaporative weathering, a fact which has to be taken into account before using them for diagnostic purposes.

It is nice to see that all labs working on the guideline have individually decided to analyze and integrate the sesquiterpanes although it is mentioned in their reports, that they are weathered.

Remarkable is the difference of 22% in one of the ratios found by Sintef (DM3/DM5). But RIZA and NERI, having also given the DM3/DM5 ratio, find only differences of this ratio in the two source samples of well below the repeatability limit of 14%.

It would be useful to compare the ratios of all labs to be able to estimate whether such differences occur more often, but due to the difference in method and integration we decided not to do so this year.

4. Conclusions

Table 4 gives a summary of the results of all labs. Because the source of the spill samples is known, it can be concluded, that almost all labs have drawn the right conclusion. Cedre and LASEM have both mentioned problems in detecting the biomarkers and a higher variability of the ratios. Petrobas joins the round robin for the first time and reports good analyses. But interpretation seems to be focused on similarities instead of differences.

Participants	Methods		Results			
	FID	MS	S I – E I	S I – E II	S II – E I	S II – E II
BMM	X	X	+	+	--	--
Cedre		X	--	--	--	--
LASEM		X	--	--	--	--
LVA	X	X	++	++	--	--
NBI	X	X	++	+	--	--
NERI	X	X	++	+	--	--
NFI		X	++	++	--	--
Petrobas	X	X	++	++	++	++
RIZA	X	X	++	++	--	--
Sintef	X	X	++	++	--	--
SKL	X	X	++	+	--	--
WRD	X		++	++	-	-
--	Significant differences between the samples have been found.					
++	No significant differences between the samples have been found.					
- and +	Conclusions are indicated to be less certain.					

The table does not show the large differences found in the reports how to analyze and assess the results. It would be good to come to a more common approach in dealing with oil spill samples.

On the CD versions of Part I (Sampling) and Part II (analytic and interpretation), as send to CEN for comments, are available for the participants. The guidelines are the result of several years of discussion how to handle oil spill samples. Hopefully this effort will lead a more common method for all labs.

5. Future aspects

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5.1 The Bonnagreement expert group.

According to the Bonnagreement meeting in September 2005:

A forum of BONN experts on oil-spill identification should be created, with Dr Gerhard Dahlmann (Germany) as convenor. As recommended by the workshop, the forum should aim to provide mutual assistance in difficult cases, to promote quality assurance in oil-spill identification (especially through ring-tests, development of common reference materials (CRMs), and sample exchanges) and consider the possibility of a common database of oil sources.

This forum/network of experts will be established in February 2006. Each BA member country will nominate a national representative. The future activities (e.g. Round Robins) will thus be conducted in the framework of the Bonn Agreement.

Nevertheless, since oil spill identification is a very special area and scientists working in this field are rare, colleagues from outside the Bonn Agreement area are always very welcome to join the BA network.

5.2 Countries cooperating in Bonn agreement.

Agreement for cooperation in dealing with pollution of the North Sea by oil and other harmful substances, 1983, by the Governments of
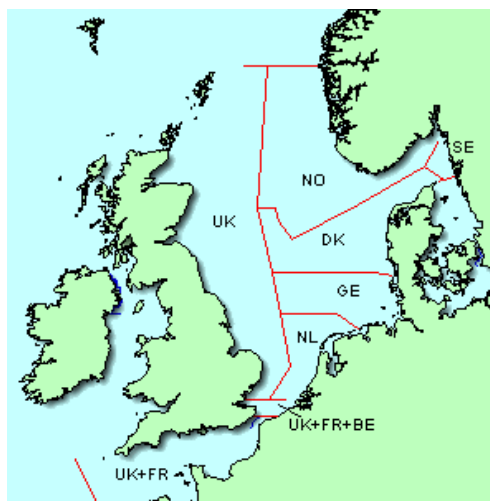

Fig. 20.
Countries cooperating in
Bonnagreement

the Kingdom of Belgium,
the Kingdom of Denmark,
the French Republic,
the Federal Republic of Germany,
 the Kingdom of the Netherlands,
the Kingdom of Norway,
the Kingdom of Sweden,
the United Kingdom of Great Britain and Northern Ireland

recognizing that pollution of the sea by oil and other harmful substances in the North Sea area may threaten the marine environment and the interests of coastal States.

6.References

CEN Guideline Part 2 (can be found on the CD)
CEN/BT/Task Force 120 Oil Spill Identification: Part 2 Analytical methodology and interpretation of results" (TC BT WI 27002, 2005-9).

Faksness, L.G., H. Weiss, P. S. Daling (2002). Revision of the Nordtest Methodology for Oil Spill Identification – Technical report. Nordtest Technical Report no. 498. (SINTEF report STF66 A01028, 2002).

Faksness, L.-G., Daling, P.S., Hansen, A.B., Kienhuis, P. & Duus, R. 2005: New Guidelines for Oil Spill Identification of Waterborne Petroleum and Petroleum Products. Proceedings of the Twenty-eighth Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Calgary, Canada, June 7-9, 2005. Ottawa: Environment Canada. Vol. 1, pp. 183-202.

Venosa, A.D.; Suidan, M.T.; Wrenn, B.A.; Strohmeier, K.L.; Haines, J.R.; Eberhart, B.L.; King, D.; Holder, E.
Environ. Sci. Technol. 1996, 30, 1764-1775.

7.Annexes

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7.1   Directory information of the CD

This report is published on CD. The individual reports can be found on the CD in directories.

|		
BMM_Roose_B	File Folder	7-2-2006 10:41
CEDRE_guyomarch-Fr	File Folder	8-12-2005 10:13
LASEM_Pierre_Fr	File Folder	6-2-2006 16:03
LVA_Skolmeistere_Lv	File Folder	23-3-2006 16:56
NBI_Viitala_Fin	File Folder	31-1-2006 14:19
NERI-Hansen_De	File Folder	6-2-2006 16:45
NFI_Bruyn_Nl	File Folder	23-3-2006 17:12
Petrobas_Meniconi_Br	File Folder	31-1-2006 14:22
RIZA_Kienhuis_NL	File Folder	27-3-2006 15:41
Sintef_Sorheim_No	File Folder	8-11-2005 14:16
SKL_Turesson_S	File Folder	22-3-2006 18:09
WRD_kroeskamp_-NL	File Folder	7-2-2006 10:09
CEN guideline Part1_2006-02-08.pdf	564 kB	Adobe Acrobat Doc...	27-2-2006 14:45
CEN guideline Part2_TC_13_march.pdf	6,539 kB	Adobe Acrobat Doc...	13-3-2006 14:27
Oeldatenbank- COSI.ppt	1,846 kB	Microsoft PowerPoi...	2-2-2005 13:59
Report RR2005.doc	1,225 kB	Microsoft Word-doc...	22-3-2006 12:32

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Fig 21
Directory information of the CD

7.2 Summary of the methods

Summary of the GC-FID protocols

Participants	FID		Column				Carrier	Oven	Injection		Detector
	instrument		type	L in m	ID in mm	Film in um		temp in C	type	(max) temp	
BMM	no information										
BSH	Agilent 6890N		J&W DB-5	10	0.1	0.1	H	300	PTV splitless		320
Cedre	Not used										
LASEM	Not used										
LVA	Varian Star		Rtx-5:	30m	0.32mm	0.5µm	He 1.1ml/min	100->300	split	280	280
NBI	HP5890		NB-1	25	0.32	0.25	He 1.4 ml/min	60->300	split	280	300
NERI	HP5890		J&W DB-1	30 m	0.25 mm	0.25µm	H	no inf	cold on-column		no inf
NFI	Not used										
Petrobas	agilent 6890/5973 MS										
RIZA	agilent 6890/5973 MS		J&W DB-5-MS	10 m	0.25 mm	0.25µm	He 1.1 ml/min	35->325	splitless	325	375
Sintef	agilent 6890/5973 MS		HP-5 MS	60	0.25	0.25	He 1 ml/min	40->300	split/splitless	325	280
SKL	HP5890		HP Ultra 1	25 m	0.2 mm	0.33 um	He 0.8 ml/min	50->300	splitless 2 min	300	300
WRD	Varian Star		no information								

Summary of the GC-MS protocols

Participants	MS		Column				Carrier	Oven	Injection		MS
	instrument	type	type	L in m	ID in mm	Film in um		temp in C	type	(max) temp	transferline
BMM	Thermoquest Trace	quadrupole	Restek RTX-1	40	0.18	0.2	He 1.5 ml/min	60->300	PTV splitless	380	310
BSH	HP 6890N/5973 MS	quadrupole	Varian CP-Sil 8 CB	30	0.25	0.25	He 1 ml/min	50->320	PTV splitless	320	280
Cedre	HP 6890N/5973 MS	quadrupole	Varian VF-1 ms	60	0.25	0.25	He 1 ml/min	50->300	splitless	270	300
LASEM	Finnigan Polaris-Q	ion trap	Varian CP-Sil 5 CB	30	0.25	no inf	He 48 kPa	50->290	splitless	250	no inf
LVA	Perkin-ElmerQMass910	quadrupole	PTE-5	30	0.32	0.32	He 48 kPa	50->300	splitless	300	280
NBI	HP6890/HP 5973	quadrupole	HP-5 MS	30	0.25	0.25	He 1 ml/min	40->300	split 1:5	300	300
NERI	VG 70S-250	double sector	J&W DB-5	30	0.25 mm	0.25	He	no inf	cold on-column	no inf	no inf
NFI	agilent 6890/5973 MS	quadrupole SIM	HP-5 MS	30	0.25 mm	0.25	He 1 ml/min	50->320	PTV splitless	320	320
NFI	agilent 6890/5973 MS	quadrupole Full scan	HP-5 MS	30	0.25 mm	0.25	He 1.5 ml/min	50->320	PTV splitless	320	320
Petrobas Biomarkers	agilent 6890/5972 MS	quadrupole	J&W DB-5	30	0.25 mm	0.25	no inf	no inf	split/splitless	no inf	no inf
Petrobas PAH's	Finnigan Polaris-Q	ion trap	J&W DB-5	30	0.25 mm	0.25	He 1.2 ml/min	50->280	splitless	no inf	no inf
RIZA	agilent 6890/5973 MS	quadrupole	J&W DB-5-MS	30	0.25 mm	0.25	He 1.1 ml/min	50->325	PTV splitless	350	280
Sintef	agilent 6890/5973 MS	quadrupole SIM	HP-5 MS	60	0.25	0.25	He 1 ml/min	40->300	split/splitless	325	280
SKL	HP5890/5972A MS	quadrupole	HP Ultra 1	25	0.2 mm	0.33	He 0.7 ml/min	60->300	splitless 2 min	300	300
WRD	Not used										