



**BONN AGREEMENT**  
Oil Spill Identification  
Network of Experts



Statens kriminaltekniska  
laboratorium - SKL

**Round Robin 2013 -  
The comparison of four samples from  
an oil spill in a Swedish Harbor.**

Eighth intercalibration in the framework of Bonn-OSINET

The results of 27 international laboratories



Date

27 January 2015

On the front page:

Vasa (or Wasa) is a Swedish warship built 1626-1628. The ship foundered and sank after sailing about 1,300 meters (1,400 yd) into her maiden voyage on 10 August 1628 in the harbour of Stockholm.

[http://en.wikipedia.org/wiki/Vasa\\_\(ship\)](http://en.wikipedia.org/wiki/Vasa_(ship))

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The results of 27 international laboratories

**Colophon**

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## Executive summary

Round Robin 2013 (RR2013) was the eighth world-wide ring test of the expert group on oil spill identification of the Bonn-Agreement (Bonn-OSINET), in which 27 laboratories from 18 countries participated.

Laboratory	Location	Contact
EPA-CES NSWDECC	Victoria (AU) Lidcombe (AU)	Syed Hasnain Steve Fuller
MUMM	Oostende (BE)	Marijke Neyts
Petrobras	Rio de Janeiro (BR)	Fabiana D. C. Gallotta
SGS	Varna (BU)	Daniela Georgieva
ALS EC-PESC EC-ALET ESTS PNLET	Edmonton (CA) North Vancouver (BC, CA) Moncton (CA) Ottawa (CA) Edmonton (CA)	Deib Birkholz Dayue Shang Josee Losier Chun Yang Chen Yang
NCSEMC	Qingdao (CN)	Sun Peiyan
BSH	Hamburg (DE)	Gerhard Dahlmann
EERC	Tallinn (EE)	Krista Mötz
CSIC	Barcelona (ES)	Joan Albaiges
NBI	Helsinki (FI)	Ninna Viitala
CEDRE LASEM Total	Brest (FR) Toulon (FR) Harfleur (Fr)	Julien Guyomarch David Francois Sabrina Marceau
CLS	Rosmuc, Galway (IE)	Darragh Hearne
KORDI	Jangmok-Myon (KR)	Un Hyuk Yim
LVGMC	Riga (LV)	Irina Dzene
Aqualysis NFI RWS	Zwolle (NL) Den Haag (NL) Lelystad (NL)	Klaas de Haan René de Bruyn Paul Kienhuis
Sintef	Trondheim (NO)	Liv-Guri Faksness
SKL	Linköping (SE)	Magnus Kallberg
Fugro EMU Ltd	Edinburgh (UK)	Gordon Todd

**Table 1**  
Participants of RR2013

Laboratories received four oil samples from an oil spill case, that occurred in winter 2007-2008 in a Swedish harbour. According to the instructions three source samples were collected from two ships and one spill sample from the water. The samples were provided by Helen Turesson and Magnus Kälberg from SKL Sweden and were from a real case, that occurred in winter time when ice was on the water. It was requested to work according to the CEN/Tr 15522-2: 2012 and to return a technical report.

Together with these 4 samples an oil product called Preem ACP Evolution Diesel has been added in order to inform participants about new products entering the market. This diesel fuel is processed from a mixture of fossil oil and renewable raw material, "tall diesel", made from a residual product from pulp mills.

The laboratories could choose between several options to participate:

- 1 Analyze the samples, evaluate the results and send a technical report.
- 2 Evaluate SKL's analytical results, which can be found in COSIweb and send a technical report.
- 3 Combine 1 and 2.
- 4 Add your own samples to COSIweb and send a technical report.

COSIweb is an online database developed by BSH. Chromatograms of samples can be added and are integrated automatically by the software. After a check of the integration, the added samples can be compared within a spill case, but also with the results of other cases and from other laboratories.

Gerhard Dahlmann(BSH, DE) and Paul Kienhuis(RWS, NL) have individually judged the reports of the participants. Points were given to aspects like analytical quality of the data, assessment of the case and conclusions about oil type and match conclusions. The final judgement is calculated as a percentage of the maximum number of points, that could be achieved.

In the summary report of RR2013, that is made available in public on the Bonn-agreement website, the results of the participants are presented anonymously by means of a code for each lab.

The match conclusions of the laboratories are presented in Table 2.

Lab code	s1-sp	s2-sp	s3-sp	Lab code	s1-sp	s2-sp	s3-sp
Scenario	NM	NM	M	Scenario	NM	NM	M
lab 1	NM	NM	M	lab 15	NM	NM	M
lab 2	NM	NM	M	lab 16	NM	NM	M
lab 3	NM	NM	M	lab 17	NM	NM	M
lab 4	NM	NM	M	lab 18	NM	NM	M
lab 5	NM	NM	M	lab 19	NM	NM	<b>PM</b>
lab 6	NM	NM	M	lab 20	NM	NM	<b>PM</b>
lab 7	NM	NM	M	lab 21	n.a.	n.a.	n.a.
lab 8	NM	NM	M	lab 22	NM	NM	M
lab 9	NM	NM	<b>PM</b>	lab 23	NM	NM	M
lab 10	NM	NM	M	lab 24	NM	NM	M
lab 11	NM	NM	M	lab 25	NM	NM	M
lab 12	NM	NM	M	lab 26	NM	NM	M
lab 13	NM	NM	M	lab 27	NM	NM	M
lab 14	NM	NM	<b>PM</b>				

**Table 2**

Match conclusions of the labs for the spill sample (sp) in relation with the provided source samples(s1, s2 and s3):

M = match; PM = Probable match; NM = non-match.

Differences from the scenario conclusions are indicated in **bold**

All participants concluded a non-match between the spill sample and the source samples 1 and 2 taken from the fuel tanks of two ships, while a correlation was found between the spill sample and the sample taken from a ballast tank a few weeks after the spill was detected.

In this Round Robin the changes due to weathering were the most challenging: Special for this spill case is that the source sample, "stored" in the ballast tank on water for several weeks, was more weathered than the spill sample taken from water at a temperature of -2°C shortly after the spill was detected.

Most participants that applied GC-FID or full scan MS reported additional compounds in both the spill and source 3 samples, which made the relation between them stronger.

Similar to RR2012 Gerhard and Paul used both their own judgement method and evaluated the reports individually. In this way different aspects of the reports are evaluated.

The results of the judgement of the reports is shown in Table 3.

labcode	Paul	Gerhard	Mean	labcode	Paul	Gerhard	Mean
lab 1	88	94	91	lab 15	88	94	91
lab 2	19	15	17	lab 16	94	94	94
lab 3	88	94	91	lab 17	94	94	94
lab 4	94	94	94	lab 18	88	94	91
lab 5	56	85	71	lab 19	88	91	89
lab 6	88	91	89	lab 20	81	94	88
lab 7	94	91	92	lab 21	0	0	0
lab 8	100	100	100	lab 22	100	97	98
lab 9	88	91	89	lab 23	94	94	94
lab 10	88	91	89	lab 24	44	76	60
lab 11	94	97	95	lab 25	94	97	95
lab 12	94	94	94	lab 26	94	91	92
lab 13	94	91	92	lab 27	94	97	95
lab 14	75	91	83				

**Table 3**

Results of the judgement of the reports as % of the maximum reachable number of points.

The differences between the judgements of Paul and Gerhard for some of the participants are a bit large. This is related with the assessment methods in which Gerhard has looked to all sample combinations and Paul has applied a more general assessment. But the trend is the same.

Low points were given to the contributions of Lab 2 and lab 24. Both labs reported to have no time for a careful assessment of the data and provided us mainly with the final conclusions.

No points were given to the results of Lab 21. Only a spreadsheet file with unusable data was provided. The lab indicated to have problems with the method and would like to have assistance in order to improve their skills.

Table 3 shows that 23 of the 27 labs had mean scores above 80%.

Fifteen labs have entered their data into COSIweb. Six more than last year. These new labs have changed their analytical parameters to the required, common settings and have sent the data to Gerhard Dahlmann. After a check, the data could be entered into the database and used for comparison.

RR2013 turned out to be an interesting case for Bonn-OSINet. The samples were selected by SKL, because the source sample of the ballast water tank was more weathered than the spill sample. The case showed however many details which were detected by different participants, e.g. the presence of lubricating oil and retene in the spill and source 3 samples and additional peaks. None of the participants has reported all the peculiarities of the samples of this case (as described in chapter 4). Even the organizers hadn't seen all of them, before they had received the individual reports.

An important aspect of this RR was the recognition of an additional amount of lubrication oil in the sample from the ballast water tank compared to the spill sample. The hopanes and steranes were increased here, but the tri aromatic steranes not, because these compounds are not present in lubrication oil.

## 1 Introduction

This summery report describes the invitation, real scenario, evaluation and assessment of a Round Robin test of the Bonn-OSINET group, where a real oil spill case, which happened in a Swedish harbor, was used.

In the history of the round robins organized by Bonn-OSINet real, artificial and a combination of them have been used.

The advantage of a case with artificially produced samples, is that the truth is known.

The advantage of real samples is that the case is realistic but may be more tricky than expected.

In RR2013 a real oil spill case has been used. The case was selected, because the related source sample was more weathered than the spill sample. The results of the participants are combined and such as in each real case a conclusion is made. Next the individual reports are assessed in relation to the combined result.

Chapter 2 shows the instructions, the participants have received together with the samples. It describes a short scenario and information how to handle the samples and how to report.

Chapter 3 gives additional background information about the case, the samples, and the result of the investigations.

Chapter 4 describes how the samples should have been analyzed and evaluated according the CEN/Tr. Special in this case is that different participants found different peculiarities of the samples, and none of the reports, including those of the organizers (SKL, BSH and RWS), contained all the information together. The contributions of the different participants are shown and discussed.

Chapter 5 gives information on how the participants have handled the case, and contains some tables showing the variance in their data.

Chapter 6 shows the comments of some of the participants on this round robin, suggestions for improvement, and a reaction from the organizers.

Chapter 7 gives information about the method and aspects that were applied by Gerhard Dahlmann and Paul Kienhuis to judge the reports.

Chapter 8 describes the analysis of the sample of Preem diesel, that has been send to all the participants to inform them about this relatively new product. The sample was not a part of the round robin and an analytical result was not requested. Background information about the Preem diesel can be found in Chapter 2, while chapter 8 shows the analytical results of RWS (NL).

## 2 Instructions

On 23-09-13 the instructions for RR2013 were published on the Bonn-OSINET server:

Subject: Oil Spill Identification Round Robin 2013, Instructions.  
From: Gerhard Dahlmann, Paul Kienhuis and Magnus Källberg

### NOTE

For literal quotations of parts of reports, publications and letters, in this report the typesetting of the text to the right is used

Dear Colleagues,

This is the eighth oil spill identification inter-calibration round within the Bonn-OSINET expert group. This year Helén Turesson and Magnus Källberg have prepared the samples and will send them to you.

### 2.1 Scenario and sample information

Oil under ice conditions

A big oil spill (Spill 1) occurred in a Swedish harbor in the winter season.

The air temperature was about -2 degrees, and there was ice on the water. Two ships were considered to be possible sources and oil was collected from each of these ships (Source 1 and Source 2). Some weeks later, a layer of oil floating on water was detected in the ballast tanks of one of these ships (Source 3). Spill 1 and source 3 were both taken by means of a teflon net.

Note: This case is in good contrast to RoundRobin2012, which dealt with a case, which had happened near the equator in the Niger Delta. Also in this case here a true story is given, and nothing is invented. (merely an arbitrary sampling date of 1-1-2008 was given in COSIweb to all samples).

Together with these 4 samples a diesel sample called Preem ACP Evolution Diesel is added. This diesel fuel is processed from a mixture of fossil oil and renewable raw material, “tall diesel”, made from a residual product from pulp mill. It seems to be useful to inform you about this product. It is however not a part of the Round Robin so you don’t need to report on it. The diesel is dissolved in DCM at a concentration of 10 mg/ml.

Easy to read information about the production of Evolution Diesel and also technical data can be found on the Internet:

[http://evolution.preem.se/assets/upload/documents/From\\_tall-oil\\_to\\_Diesel.pdf](http://evolution.preem.se/assets/upload/documents/From_tall-oil_to_Diesel.pdf)

<http://ipreem.preem.se/sm/prod3NySite.nsf/vProductsByLinkID/834?OpenDocument>

[http://www.preem.se/templates/ProductInformation\\_9971.aspx](http://www.preem.se/templates/ProductInformation_9971.aspx)

## 2.2 Tasks and method

CEN/Tr 15522-2:2012 should be used.

COSIweb will again be a part of this Round Robin. It can be found now on the BSH server.

<http://cosi.bsh.de:8080/CosiWeb/>

Note:

It is not actually understood, why not all laboratories made use of this online evaluation system, so far:

- those, which indicated that they cannot participate due to other tasks, would not have needed much time, and using COSIWeb is a very convenient way to hang in there, as cases can be evaluated according to CEN/TR 15522-2:2012 “as if” samples are analyzed in the own laboratory.
- all participants get information about variations in the data, measured in different laboratories (although the same analytical parameters are used), and possible shortcomings/problems in their own data can be found.

SKL has prepared and analysed the samples and uploaded the chromatograms already into COSIweb (case number Se-1-7007).

Several participants have already been added as “normal user” based on their analytical results of last year.

All others can enter COSI as guests by entering two times the word guest (username and password). For guests a limited number of samples and the results of other labs, that have uploaded their data of RR2013, will be available.

Different ways can be followed in this Round Robin:

- 1 Analyze the samples, evaluate the results and send us a technical report.
- 2 Evaluate Magnus’s analytical results by means of COSIweb and send us a technical report.

3 Combine 1 and 2

4 Make use of COSIweb to evaluate your own samples and send us a technical report (.Analyze the samples according to B.3.1 of CEN/Tr 15522-2:2012),

**Ad 1: Analyze the samples, evaluate the results and send us a technical report.**

This way is relevant for the participants that use/need the Round Robins for their accreditation of the analytical part of the method.

Compare the spill sample with the source samples by means of the CEN/TR 15522-2:2012. Integrate the relevant compounds and enter the results in Excel spreadsheet file v51 (needed for a statistically evaluation of the analytical results), produced by Paul. (Uploaded to the OSPAR server on 23-9-2013 ). You will notice that extra evaluation sheets are present. They are explained on the sheet “instructions”.

Write a technical report as described in annex I of the CEN/Tr. A summary report for clients is not necessary. Make use of the questionnaire table Checklist for RR2013 uploaded to the Ospar server on 23-9-2013) for each Spill-Source sample comparison. Send Gerhard and Paul the technical report, the questionnaire table and the spreadsheet file.

For the Checklist for RR2013 see **Table 2.1**

Bear in mind that the technical report should be written in such a way, that you can reproduce what you have done and concluded a long time ago.

**Ad 2: Evaluate Magnus’s analytical results by means of COSIweb and send us a technical report.**

Since this is a mere theoretical work, everybody can participate here.

Magnus has already imported the RR2013 samples into the database (case number Se-1-7007.x). Participants are asked to evaluate this case. In COSIweb, all means for coming to a conclusion according to the CEN-guideline can be produced, and a resulting report can be downloaded, eventually. Tested here are the possibilities to interpret data, produced by one of the group members, by others -in a manner that will stand up at court.

Under the “Results”-screen (See COSIweb manual) you will find a questionnaire. Fill in this questionnaire for each Spill-Source sample comparison. Some keywords should be sufficient.

But –as it is always easier to prove, what it is not, than what it is- some more text may be needed in case a match is concluded.

Send us a technical report and the PDF-files you have produced. Because the PDF files give a lot of information the technical report can be short and mainly consist of a summary of the results.

**Ad 3: Combine 1 and 2.**

Combining **ad 1:** and **ad 2:** will give you the ability to compare your own analytical results with the results of Magnus (and those of other participants, which will upload their results).

Send us a technical report, the spreadsheet file and the PDF files of COSIweb.

**Ad 4: Make use of COSIweb**

**“Normal users”:** upload the files into COSIWeb

**“Guest”:** send the chromatograms as CDF files to Gerhard or Paul.

For users that like to become normal users of COSIweb:

Your raw GC and GC/MS data can be imported into the COSIWEB system, if the analytical parameters as proposed in the CEN/Tr are used (e.g. a 30 m DB-5 column and a retention time of 30.00 min for 3-MP and 47.80 min. for hopane).

Note: Joint analytical parameters are not needed for the GC-FID data. Merely the retention times of pristane and phytane must be given.

Raw data files (together with the pr and phy retention times in GC-FID) should be sent to Paul and Gerhard, who will check and import the data. You will be informed when the results are uploaded into COSIweb. We will make an account for you, so you can start to upload your own cases in the future.

Write your report, as you have done in former exercises. Here, you may simply point to the figures of the PDF-files, which should be attached. But copy those figures, you regard as important, and which require further interpretation, into your report.

But compare also your samples with the results of others 's. Moreover: during this RR you will notice that data from more and more participants are present.

### 2.3 Latest development of COSIWEB

COSIweb is now working on the server of BSH.

<http://cosi.bsh.de:8080/CosiWeb/>

Data can be entered as CDF data:

The CDF file type is associated with 'ANDI/netCDF Mass Spectrometry Data Interchange format' (formerly known as "AIA Format"). The ANDI format is supported by many Mass Spectrometer software packages. Most commercial Mass Spectrometry software products allow export to the ANDI format for interchange of data files between different data processing systems.

As a result the database-system is now no longer dependent on the use of an Agilent-system and Agilent(Chemstation)-software.

The use of joint MS-parameters is thus the only requirement for the use of this system. This new development may bring those, who still use their own MS parameters, to switch to the parameters as proposed in the CEN-guideline.

Note: Co-operation and mutual assistance is much easier, when joint analytical parameters are used. It is thus highly recommended that the MS-parameters as given in the CEN/Tr 15522-2:2012 are used by OSINET-members. All mass-chromatograms in this guideline, showing the retention times of peaks, and also their positions in sometimes more complex clusters, are based on these parameters.

Recent COSIweb improvements:

- The MS-PW-plot is normalized to TMP, if hopane is not available.
- A search and sort function is added to the "compare" tab.

You are kindly asked to comment on the system. Any proposals for further improvements are very welcome. There is still the possibility to improve COSIWEB correspondingly.

## **2.4 How to use COSIWEB**

Participants not already working with COSIWEB are treated as “guests”. Guests don’t have a special username and password. Access to the system is limited to result evaluation. Only a limited set of samples can be seen. These are some crude oils from all over the world and the RR2013 samples here.

### **Sample naming**

Samples are named by

(country code)(case number)\_(sample number in that case).

Example:

**Uk101\_1**

When samples are imported, the program adds a laboratory number, and converts this sample name into

**Uk-1-101.1** (RR2012 sample 1 of Gordon’s samples).

### **Sending raw data**

Use the export function of your GC and GC/MS-software to produce .cdf-files (see above).

Since raw GC and GC/MS-files of a sample must have the same names, these must be put into different directories.

Create a main directory (e.g. RR2013), which includes the two subdirectories GC and MS, and export the GC and GC/MS-CDF-files into the corresponding subdirectories.

When all samples are present, pack the main directory, e.g. by using WINZIP, and send the packed file by e-mail to Gerhard or Paul

### **Handling doublets**

Double measurements for testing instrument performance should be handled by giving them consecutive numbers:

Sample numbers are 1 to 5 here; the numbers of the duplicates are thus 6 to 10.

According to the CEN/Tr 15522-2:2012 a minimum of two samples should be analysed in duplicate (See CEN/Tr section 6.1). Use for example one of the spill and one of the source samples.

Evaluation of these double measurements is done in the same way as different samples are compared (DRs and ratios used for the PW-plots below repeatability?).

### **COSIWeb manual**

A draft COSIWeb manual can be found on the OSPAR server (uploaded to the OSPAR server on 2-10-2012; Files tab; category RR2012).

Improvements of this manual might still be needed. So please give your comments.

### **2.5 Time schedule**

23 September 2013:	The samples have been sent out.
Before 1 February 2014:	Reports have been returned.
March 2014:	Final report will be sent to the participants.
Meeting:	Hamburg, 22-24 April 2014

Send the results by email to: paul.kienhuis@rws.nl and Gerhard.dahlmann@bsh.de

Yours sincerely,  
Gerhard Dahlmann,  
Paul Kienhuis,  
Magnus Källberg

## 1 GC

	Source 1	Source 2	Source 3	Spill 1
1.1 Visual inspection, overlays				
1.1.1 Oiltype (tentative)?				
1.1.2 Obvious differences with spill 1?				
1.2 Isoprenoid ratios, GC-PW plots				
1.2.1 Significant differences with spill 1?				

## 2 MS

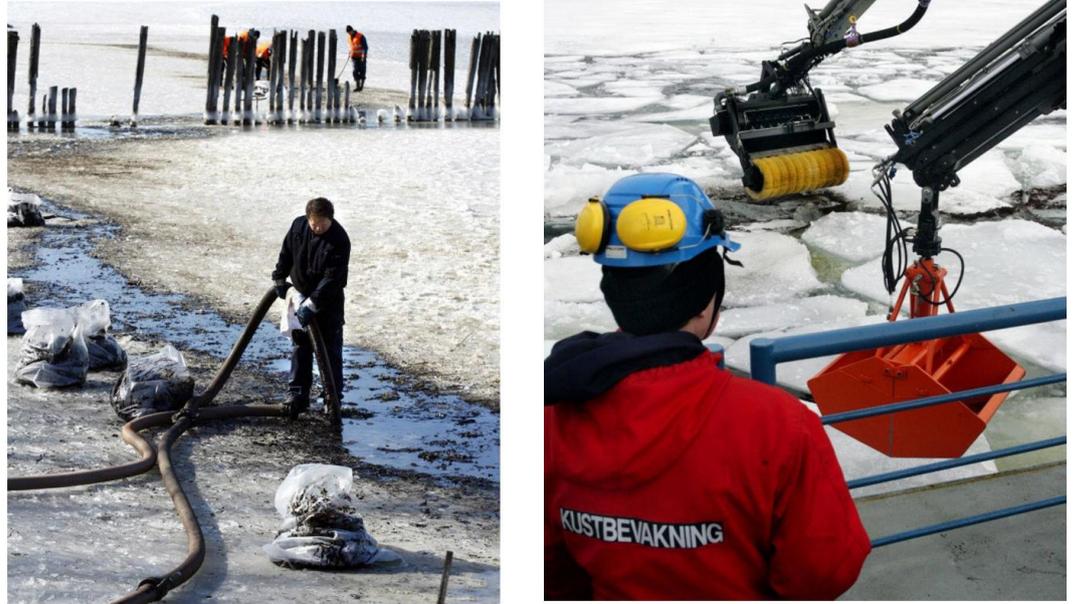
	Source 1	Source 2	Source 3	Spill 1
2.1 Visual inspection of the ion chromatograms (MS-tab)				
2.1.1 Oiltype?				
2.1.2 Obvious differences with spill 1?				
2.2 Peaks measurements				
2.2.1 Reasons for selecting/deselecting DRs and data points in the PW-plots?				
2.2.2 MS-PW plot -significant differences?				
2.2.3 Diagnostic Ratios - significant differences?				
3. Overall Result				
3.1 Uniqueness of the samples relative to the other samples in the database.				
3.2 Match conclusion (Match, Possible match, Inconclusive or Non-match)				

**Table 2.1:**

The Checklist for RR2013.  
Mention in section 2.2 ad 1



### 3 Scenario information



**Fig 3.1: (SKL,SE)**  
Pictures from the spill.

#### 3.1 Sampling

In winter time an oil spill occurred in a Swedish harbor.

In the beginning it was suspected, that the oil came from sources on land. Four samples from land were analyzed, but these samples, some with very heavy fuel oils, were completely different.

From the two ships in the harbor four samples were taken. At least three of them were from fuel tanks. One of these samples was a much lighter fuel oil (not included in the RR) and two were the "same" oil (one of these oils is not included in the RR). The other two samples from those ships are Source 1 and Source 2.

A little more than two months later three more samples from one of the ships were sent to the lab, two were bunker oils and one was from the ballast tank (Source 3) where oil had been detected. Source 1 and Source 3 are from the same ship.

#### 3.2 Investigation result of SKL

All of the first samples, including Source 1 and Source 2, were clearly non matches.

The sample from the ballast tank (Source 3) was a rather good match with the spill sample with respect to weathering with some biodegradation. Source 3 was more weathered than the spill sample, but this was attributed to a long contact time with water in the ballast water tank.

Most often a suspected source sample is less weathered than a spill sample.

In some cases, e.g. when the oil from the source has been leaking over a surface like the side of the ship down into the water and sampled by being wiped up with a sampling cloth, it can be considerably more evaporated than the spill sample.

In this case the spill sample was collected in cold water under ice conditions and the suspected source was found two months later inside a ship in the ballast water tank. Under these circumstances it seems reasonable that the suspected source is more weathered than the spill sample.

### 3.3 Law enforcement

The ship's owner claimed that the oil in the ballast water tank has been pumped into the ship from the water in the harbor.

This is strange because ballast water tanks are used to stabilize a ship. When a ship is empty or partly empty, water is pumped in the ballast water tanks to stabilize the ship.

This should be possible even when a ship is empty and the hull is at the highest level about the water level. The place where the water is sucked into the ship should even then be below the water level. As oil floats on water the chance, that a large amount of oil is pumped into the ship, is low.

The same ship caused later a second spill in another harbor. This time the inspectors found a leaking valve in a pipeline from the bottom of the ship to the ballast tank. The pipeline was intended to pump out water in case of emergency. Because of the leaking valve oil from the bottom of the ship could come into the ballast tank. It is believed, that the same has happened in the case that has been used for the round robin samples.

The ship had already paid a pollution fee of €15.000 for the second spill. The cleanup of the first spill has cost much more, but because the ship has already paid the fee, it could not be prosecuted again.

### 3.4 Assumed scenario

Based on the information available it is very likely that the ship, where the samples source 1 and source 3 have been taken, has caused the spill. It is also very likely that the spill occurred at the moment that the ballast tank of Source 3 was emptied. It doesn't mean however, that the samples should match.

- Sample Source 1 is from the fuel tank. It might be that fuel has been spilled in the ship ending up in the bottom of the ship. Here a mixture can be expected of fuel, lubricating oil from the engines, water leaking along the propeller shaft, remaining of cargo and other waste. By the pipe with the leaking valve it has been transported to the ballast water tank.

- Sample source 3 has been taken 2 months after the spill. In that time probably oil from the bottom of the ship has been added to the oil in the ballast tank.

Besides the addition of extra oil, it can also be expected that the oil in the ballast tank has weathered. The oil is floating on water and will be mixed with the water when the ship is sailing. Biodegradation and dissolution are very likely, while evaporation and photo-oxidation are unlikely.

Fig. 3.1 shows two pictures of the spill. Based on the amount of oil, the temperature and the time of the year it may be expected that the effects of evaporation, photo-oxidation, solubility and biodegradation on the spill sample are minimal.

## 4 Evaluation of the case following CEN/TR 15522-2:2012.

In this chapter the case will be evaluated according to the CEN/Tr, combined with suggestions and useful information from the reports of the participants.

### 4.1 Scenario remarks

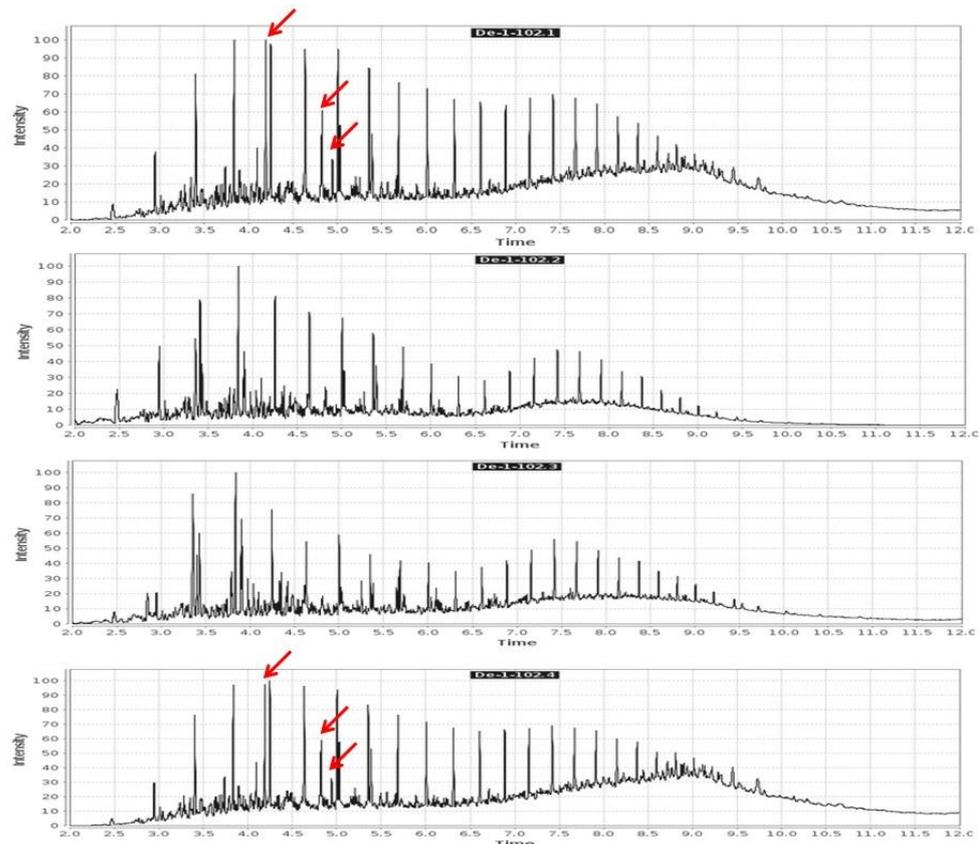
Some thoughts about the scenario as given in the instructions:

Although it is stated in the instructions that the truth was given, i.e. the case had happened as indicated, not all information was given in order not to make this RR too easy: it is not known from which tanks source 1 and source 2 were taken, for example. Moreover, there is the suspicion that the first comparison of spill 1 and source 1 and source 2 conducted by the Swedish lab yielded a "non-match".

Otherwise there should be no reason to investigate one of the ships again after two months and to take a sample again. It is also not given from which of the two ships a second sample was taken. But this might also mean that investigations on board of the two ships were not satisfactorily conducted: the investigating authorities should have known that there is more than one tank/compartment on board a ship, where the oil could have come from.

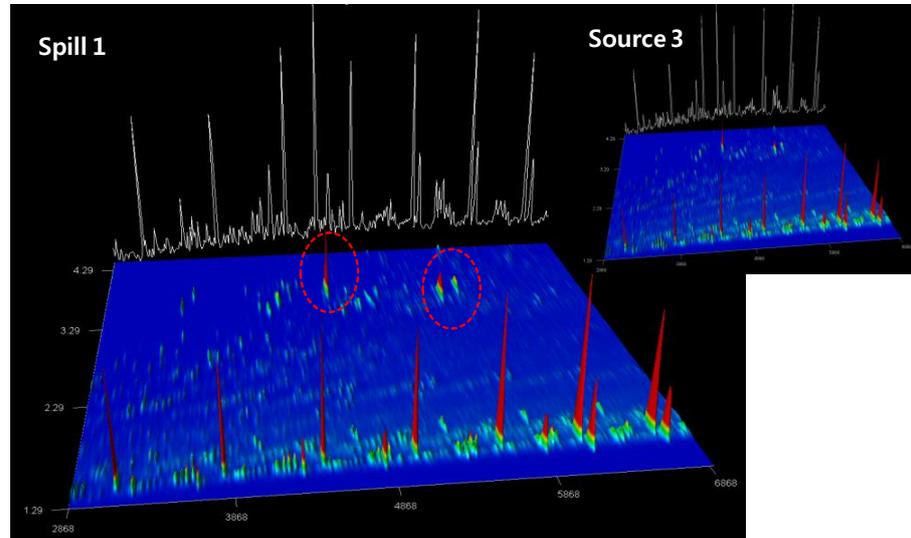
### 4.2 GC-FID

Whereas source 1 and source 2 showed the typical aromatics from cracking processes in the lower and mid-boiling region (alkylated naphthalenes and phenanthrenes) in relatively high concentrations, spill 1 and source 3 showed 3 peculiar peaks, which appeared just before C15 and between C16 and C17 (Fig. 4.1, 4.2 and 4.3).

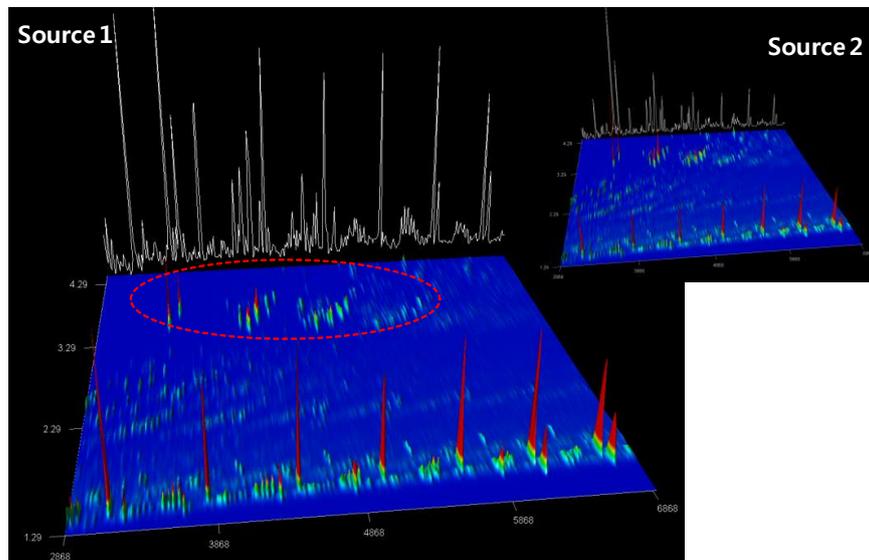


**Fig 4.1: (BSH, DE)**  
Gas-chromatograms of resp.  
spill 1, source 1, source 2  
and source 3.

Arrows show additional  
peaks of spill1 and source 3.



**Fig 4.2: (Kordi, KR)**  
GC-GC-FID: Spill 1 and Source 3 contain three distinct peaks. The presence of these peaks could serve as a strong evidence for a match between these two samples.

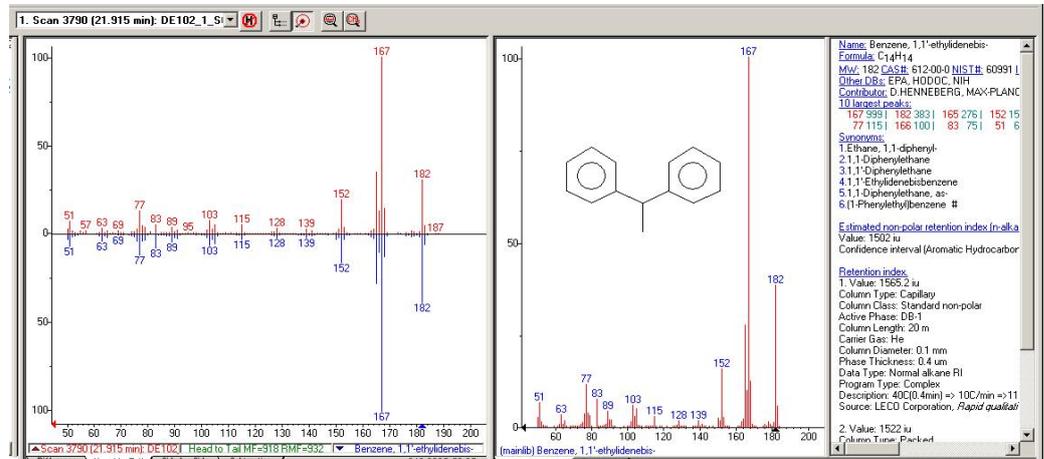


**Fig 4.3: (KORDI, KR)**  
GC-GC-FID: In case of Source 1 and 2 alkylated naphthalenes are enriched in that position.

An MS full-scan run revealed a very good correlation with Diphenylethane (Fig. 4.4) and further alkylated Biphenyls.

**Fig 4.4: (BSH, DE)**

Nist-search of the compound, which appeared just before n-C15.



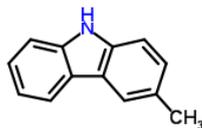
Besides these three high peaks, NFI (NL) reported also methylcarbazoles:

### NFI, NL

With this more detailed comparison it became clear that the pattern of the C1-fluorenes contained two peaks more than it should. Especially in Spill1 and Source 3 and to a lesser extend in Source 1. Only Source 2 didn't have those extra peaks. These peaks are the two compounds we think are 1,1'-ethyldienebistoluene isomers.

Although not directly disrupting the patterns that are used for the comparison, we also found extra peaks in the m/z 208 and m/z 220 traces in Source 3 and Spill 1, and to a lesser extend in Source 1. We think these peaks are methylcarbazoles based on GCxGC-TOFMS results.

### 3-METHYLCARBAZOLE

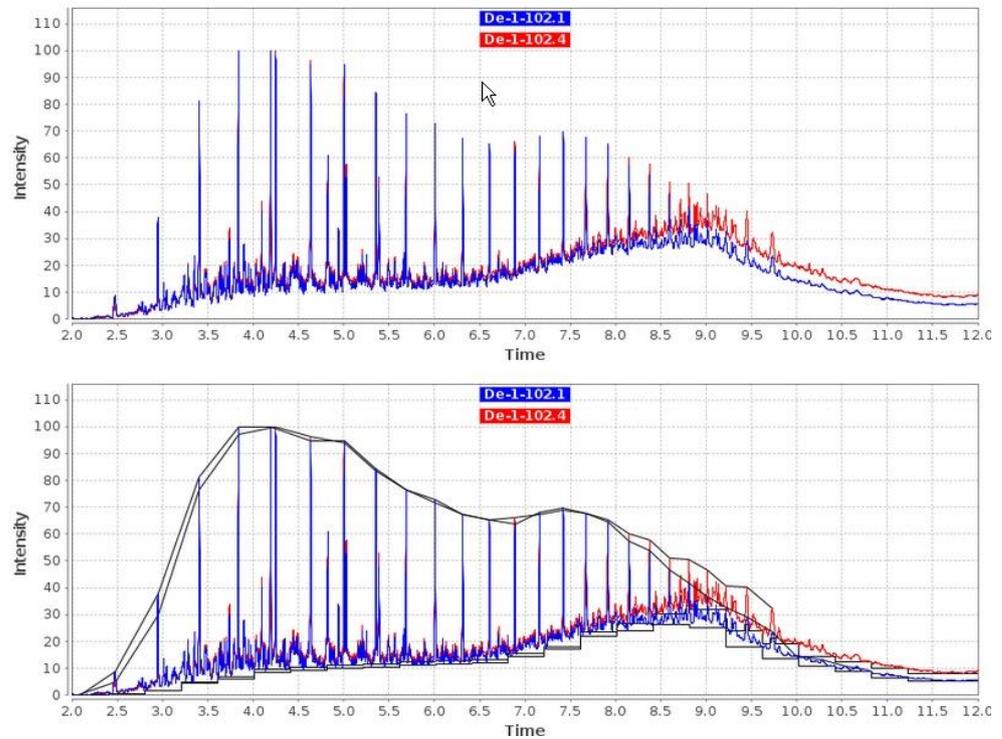


ChemSpider ID: **19534**  
 Molecular Formula: C<sub>13</sub>H<sub>11</sub>N  
 Average mass: 181.233093 Da  
 Monoisotopic mass: 181.089142 Da  
 Systematic name  
 3-Methyl-9H-carbazole

Source 1 and source 2 both showed a bimodal distribution, additionally, with apexes at about 4 minutes and at about 7.5 minutes. Spill 1 and source 3 showed a third unresolved hump at about 9 minutes.

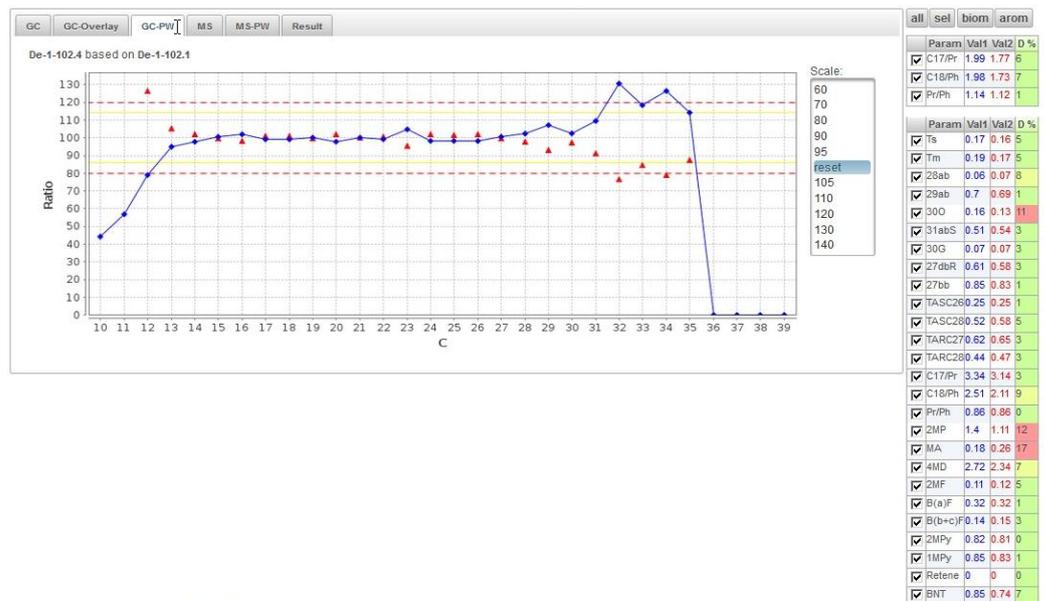
These results already show that source 1 and source 2 cannot match with spill 1, whereas the probability of a match between source 3 and spill 1 is high.

The GC-overlay of spill 1 and source 3 (Fig. 4. 5) shows an a little bit higher hump at about 9 minutes of source 3.



**Fig 4.5 : (BSH,DE)**  
 Overlay of the GCs of spill1 and source 3.

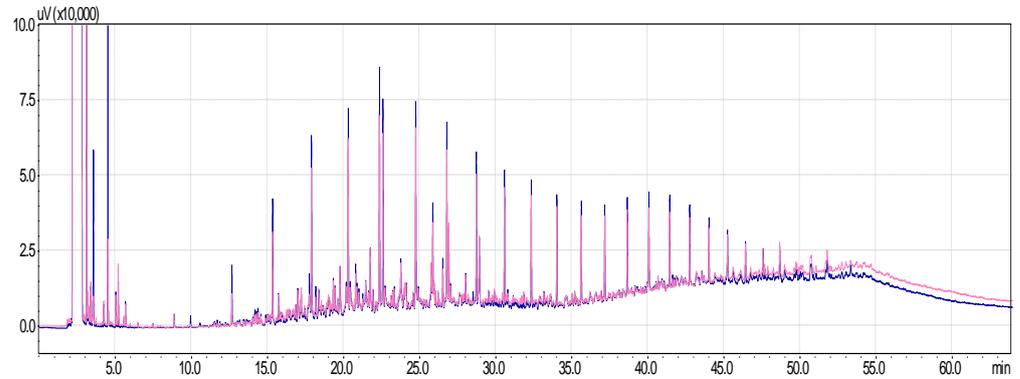
The GC/PW-plot (Fig. 4. 6) shows a match situation. The highest n-alkanes deviate, but these are small and cannot be measured sufficiently precisely. Small evaporation can be observed for source 3 (note: the blue line of the GC/PW-plot here is based on spill1, meaning that the ship's sample source 3 is more weathered).



**Fig 4.6 : (BSH, DE)**  
 Left: GC/PW-plots of source 3 based on spill 1 (blue line) and spill 1 based on source 3 (red line).  
 Right: ratio's and ratio comparison between spill 1 and source 3.

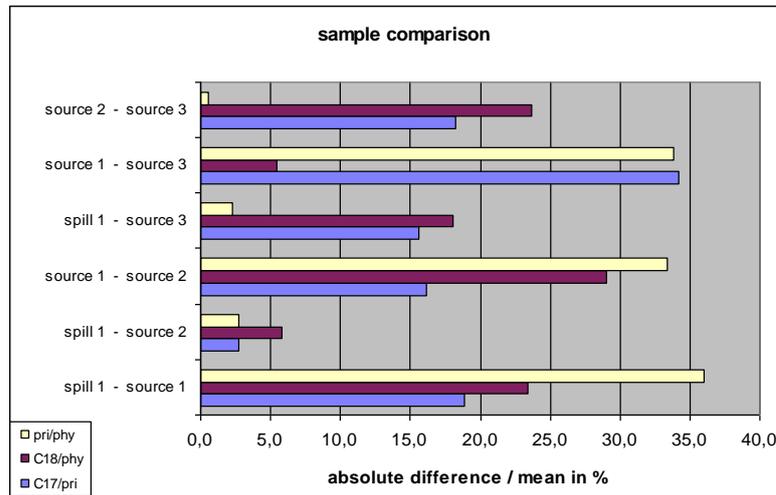
Download the report of the comparison: [Download](#)

Depending on how the baseline is drawn it is also visible that the alkanes of source 3 are slightly biodegraded over the whole range.( See Fig 4.7).



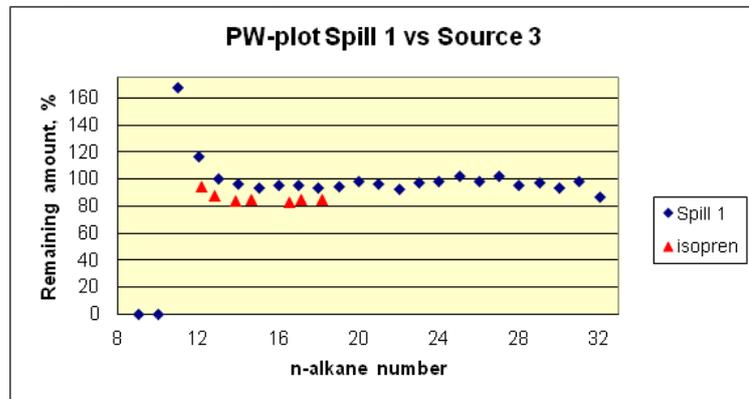
**Fig 4.7 : (LV, LVGMC)**  
 Overlay of the GC chromatograms of samples Spill 1 (blue) - Source 3 (pink), visually normalized to pristane and phytane.

This is reflected in the isoprenoid ratios between spill 1 and source 3. The ratio pr/phy shows a small difference, while the ratios C17/pr and C18/phy show a difference between 15 and 20%. (See Fig 4.8)



**Fig 4.8 : (LV, LVGMC)**  
 Comparison of isoprenoid ratios of samples Spill 1 and Source 1-3.

The biodegradation of the alkanes over the whole range is confirmed by the GC-PW-plot of SKL. Isoprenoids are more stable against biodegradation and the "isoprenoid line" is clearly separated from the "alkane line". It would have been unlikely, if no biodegradation had happened of oil on water in a ballast tank for several weeks.



**Fig 4.9 : (SE, SKL)**  
 GC-FID plot of spill 1 with Source 3 as reference.

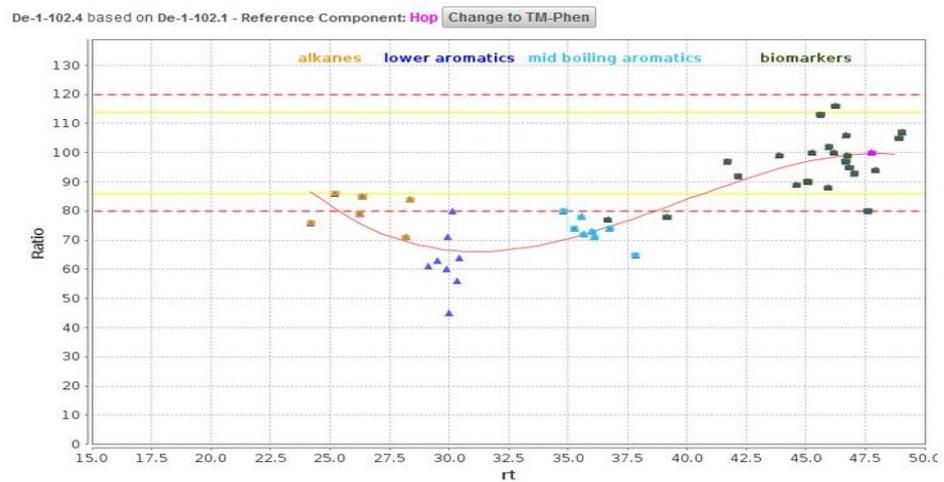
### 4.3 GC/MS

The diagnostic ratios of spill 1 compared with Source 3 (Figure 4.6) mainly show deviations above repeatability of 300, C18/phy, 2MP and MA.

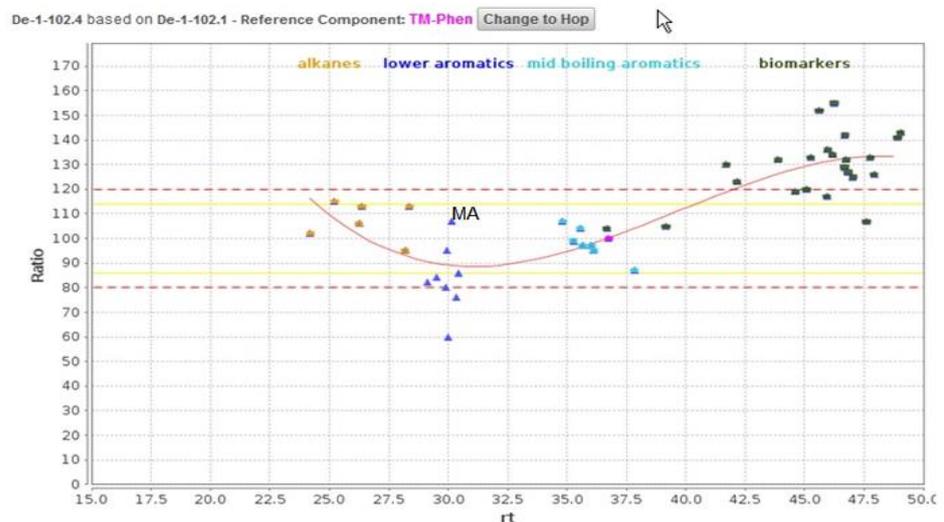
The MS/PW-plot based on hopane (Figure 4.10) shows a relatively high scattering of the biomarkers at around 100%. But most of them are still within the repeatability limits, whereas alkanes and most aromatics are on a level of about 80%. This indicates a relative higher amount of biomarkers in the HFO of Source 3. Because the PAH's and alkanes are low in concentration in lubricating oil, a larger amount of lubricating oil in source 3 is very likely.

Source 3 contains a higher amount of lubricating oil (see also Figure 4.5), and the higher scattering of the biomarkers is explained by the fact that these biomarkers partly originate from the fuel oil and partly from the lub oil.

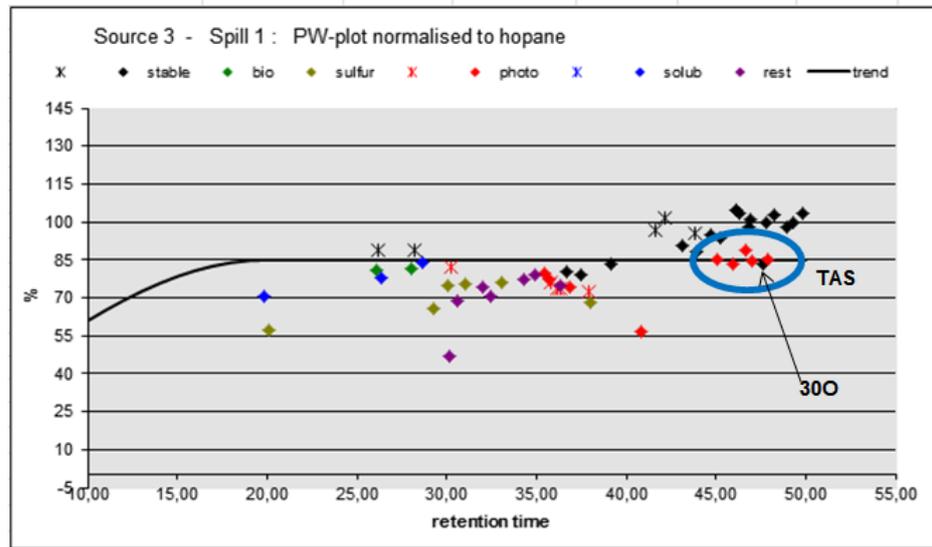
When the PW-plot is based on TMPHEn (Figure 4.11), most of the fuel components are within the repeatability-limits, whereas most of the biomarkers are between 120 and 150%, showing of course also a high scattering.



**Fig 4.10 : (BSH, DE)**  
MS/PW-plot (based on hopane) of source 3 based on spill 1.



**Fig 4.11 : (BSH, DE)**  
MS/PW-plot (based on TMPHEn) of source 3 based on spill 1.



**Fig 4.12: (Au, NSW)**

Source 3 based on spill 1.

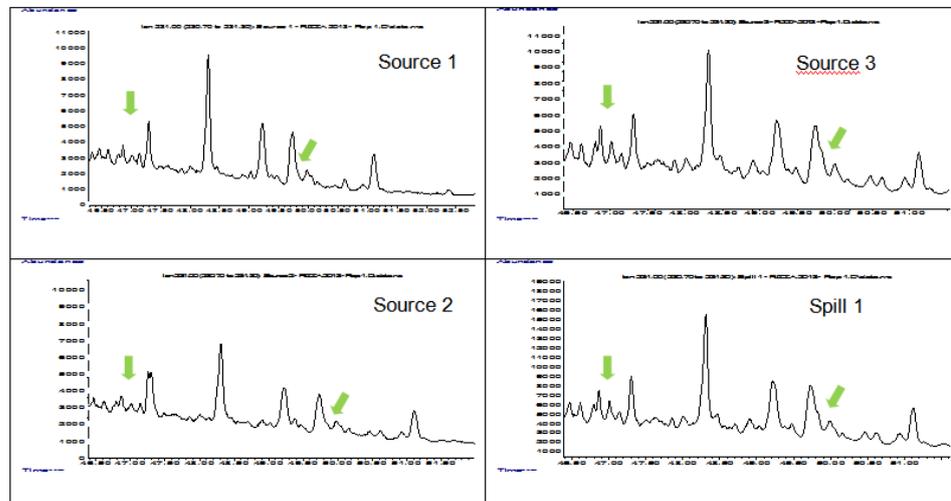
As given in Fig. 4.12 all the tri-aromatic steranes (TAS) are at a level of about 85%, and thus on the level of the fuel oil. Since lubricating oil does not contain TAS, and most hopanes are at the 100% level, this again strongly indicates the presence of a larger amount of lub oil in Source 3.

Furthermore, all the tricyclic hopanes (♦ between 35 and 40 min) originate mainly from the fuel. This is also true for oleanane( 300). Thus the difference of the DR-oleanane value between source 3 and spill 1 (see Fig. 4.6) originates from the fact that source 3 contains a larger amount of lub oil.

Several participants have checked the m/z 231 ion chromatogram for an indication of lubricating oil:

**PETROBRAS(BR)**

The presence of lubricating oil has also been investigated. Mass chromatograms of m/z 231 show a bit different profiles for samples Source 3 and Spill 1, with extra or broader peaks (Figure A.4.2.2). However these evidences are hardly discernible. If present in samples Source 3 and Spill 1, lubricating oil content is low.

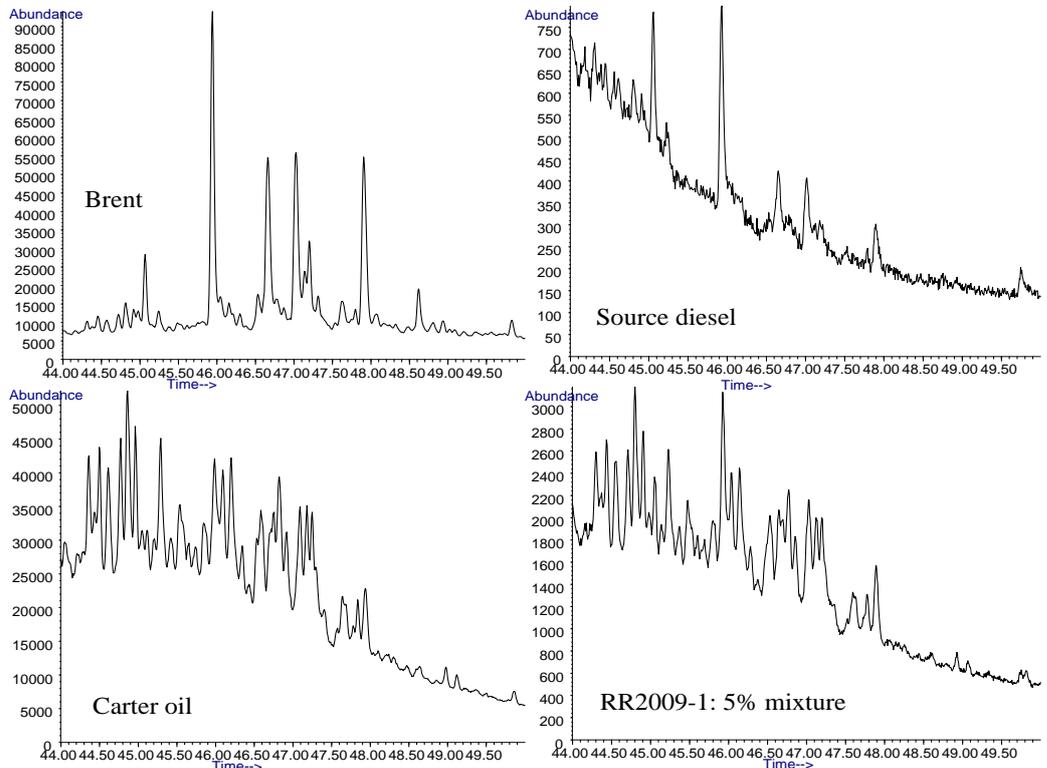


**Fig 4.13: (Petrobras, BR)**

Aromatic steranes.

**Figure A.4.2.2 Aromatic steranes (m/z 231) of all samples**

This topic is related to the mixing experiment of diesel with 5% lub oil in RR2009. See Fig. 4.14 and the text from the summary report of 2009 below.



**Fig 4.14: (RWS, NL)**  
**Fig. 17 (RR2009)**  
 M/z 231 chromatograms showing the aromatic steranes concentration.

In the diesel chromatogram the typical pattern of the aromatic steranes is clearly visible, while this pattern is missing in the carter oil. The compounds shown are assumed to be 4 $\alpha$ -M-steranes (see CEN/Tr 2v2 Fig H.12)

Interesting to see is that already in the 5% mixture the aromatic steranes pattern is vanished. Only RC26TA+SC27TA is visible in RR2009-1, co-eluting with a compound present in the carter oil.

Fig 4.14 shows that the addition of 5% carter oil is sufficient to disturb the TAS pattern. The tri-aromatic steranes in a diesel are at the end of the distillation range and their concentration is low ( abundance is 750 here). Adding the 5% lub oil results in an abundance of 3000.

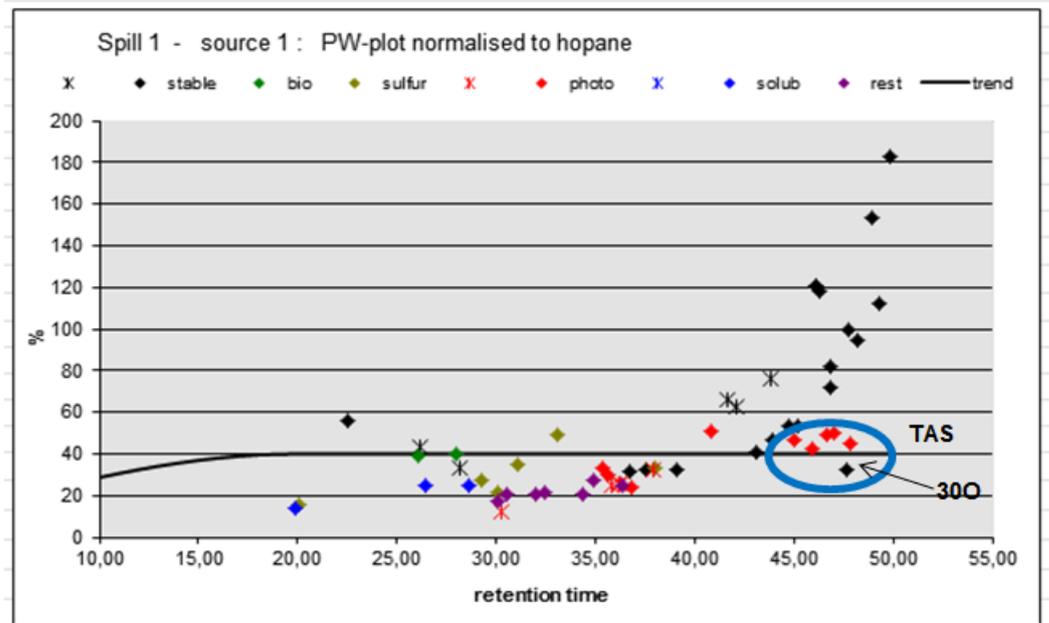
In HFO however the TAS are in the distillation range of the fuel, and have therefore, a much higher concentration. The abundances in Fig 4.13 range from 10,000 to 19,000. So the chance that a small portion of lub oil is visible in the aromatic sterane pattern of HFO is small.

For those who have access to the Bonn-OSINet section of the OSPAR server, see also:

[https://ospar.basecamphq.com/projects/2994028/file/51488469/Dahlmann\\_PWplot\\_sludgemixtures.ppt](https://ospar.basecamphq.com/projects/2994028/file/51488469/Dahlmann_PWplot_sludgemixtures.ppt)

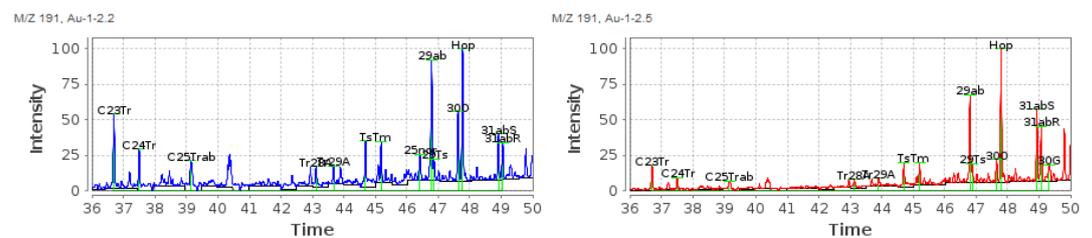
For others, the slideshow is available on request.

The presence of lubricating oil in the samples spill 1 and source 3, becomes very obvious, when spill 1 is compared with source 1.



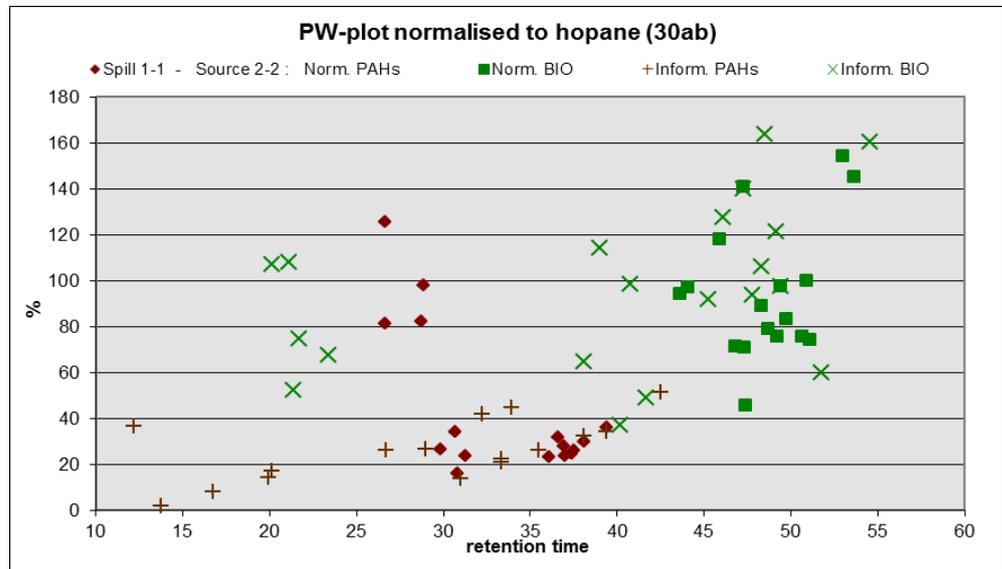
**Fig 4.15 : (AU, NSW)**  
MS-PW plot spill 1 based on source 1

According to the additional scenario information (See section 3.1) source 1 is a mere HFO, and if a product, which is poor in alkanes and PAH's and rich in hopanes and steranes is added, i.e. lub oil, a PW-plot such as given in Fig. 4.15 is found. Consequently, the TAS, originating only from the HFO, remain at the low level of 40% here. This corresponds with the fact, that from the relatively high level of about 50% of oleanane in relation to hopane in source 1, only about 15 percent remained in the HFO/LUB oil mixture.



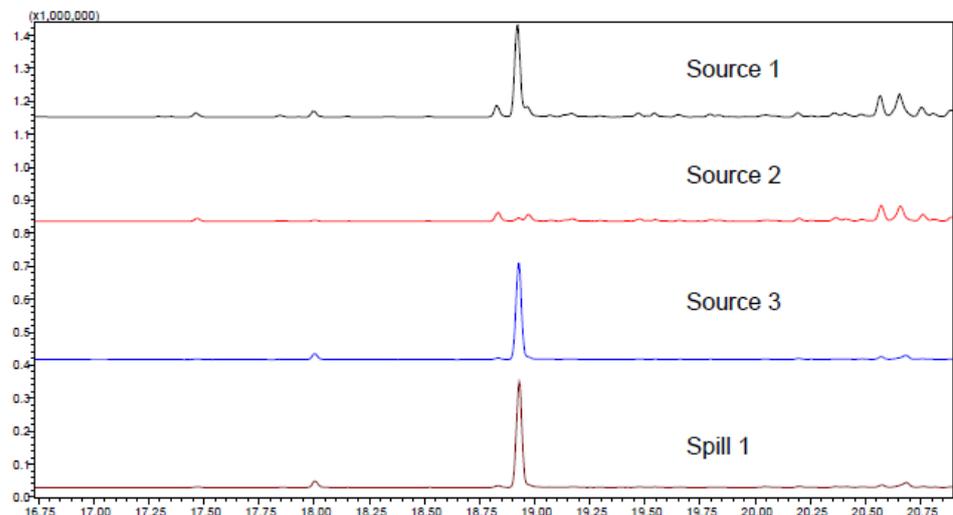
**Fig 4.16 : (BSH, DE)**  
Mass-chromatograms (m/z 191) of source 1, left, and spill 1 right. Oleanane is indicated as 300.

The PW-plot of spill1 based on source 2, in contrast, shows much more scattering and a "true" non-match situation.



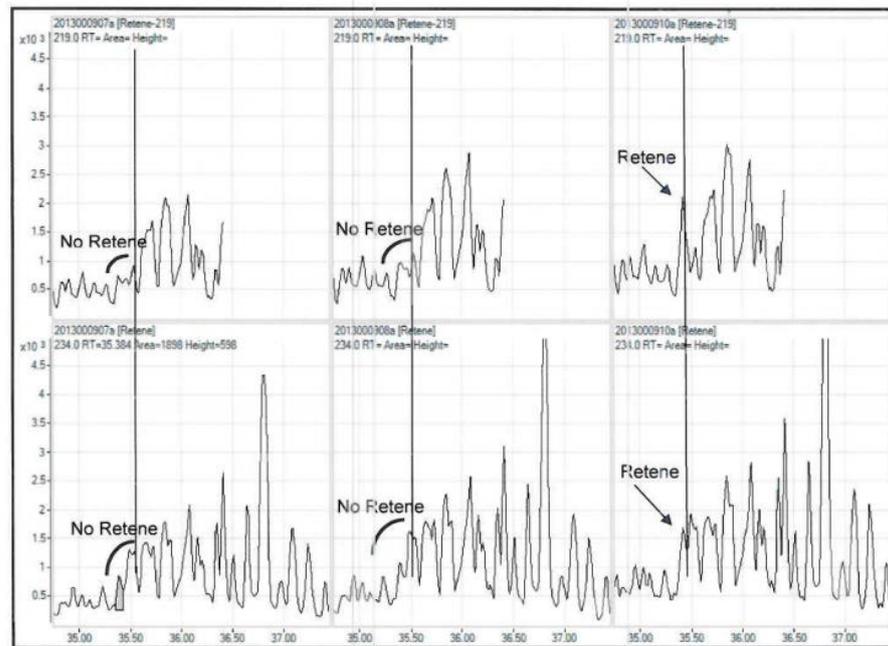
**Fig 4.17 : (Br, Petrobras)**  
 MS\_PW plot spill 1 based on source 2

Although the proof cannot be given that the fuel oil part of spill 1 originates from source 1, a further common characteristic feature could have been found: Fig. 4.18 reveals that the biphenyls found in source 3 and spill 1 (Figures 4.1 to 4.4) are also present in source 1 (and not in source 2).



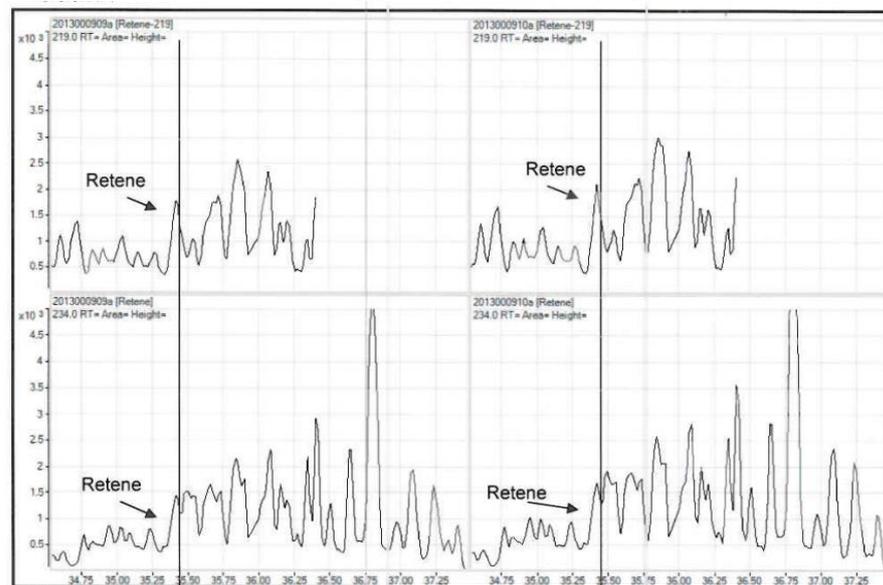
**Fig 4.18: (AU, NSW)**  
 Mass-chromatograms of ion fragment 167, which is characteristic for the biphenyls (see Fig. 2).

Another peculiarity of source 3 and spill 1 is the presence of small amounts of retene in these two samples (found by EC-ALET(CA)).



**Fig 4.19: (EC-ALET, CA)**  
Ion chromatograms of samples source 1, source 2 and spill 1 from left to right.

Figure 5: Ion chromatograms of m/z 219 top and 234 bottom from left to right samples 2013000907, 2013000908 and 2013000910



**Fig 4.20: (EC-ALET, CA)**  
Ion chromatograms of samples source 3 and spill 1 from left to right.

Figure 6: Ion chromatograms of m/z 219 top and 234 bottom from left to right samples 2013000909 and 2013000910

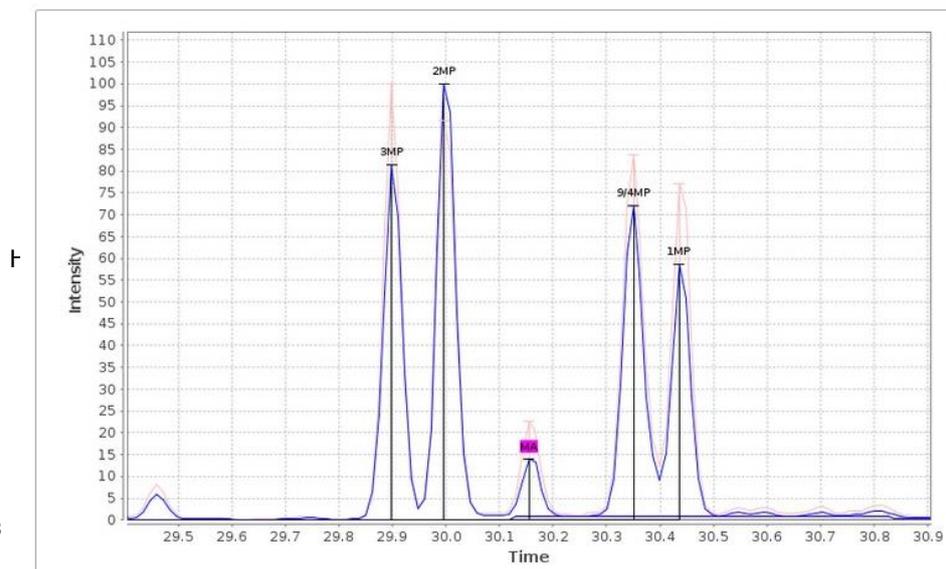
Retene can be found by the combination of m/z 219 (base peak) and m/z 234. See CEN/TR Fig. E2.

Fig. 4.19 and 4.20 strongly suggests, that retene is present in samples source 3 and spill 1, but not in source 1 and 2.

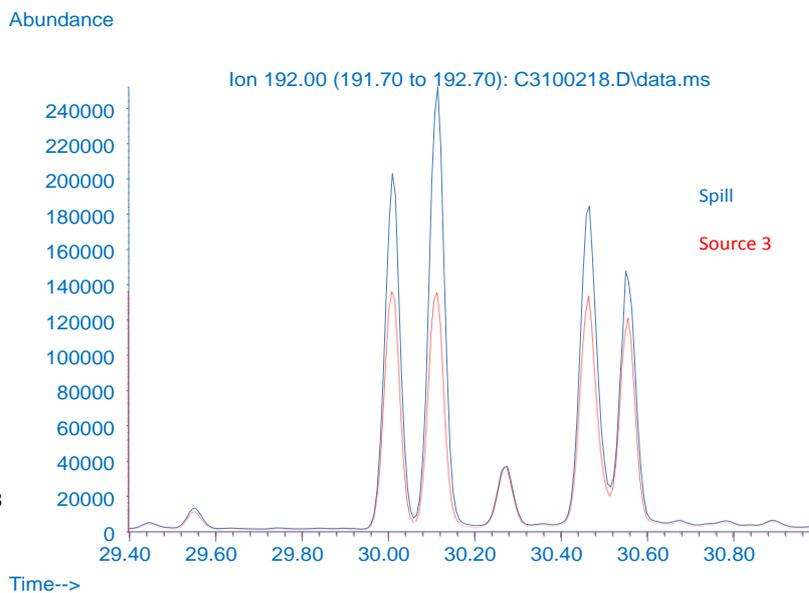
The HFO-compounds of source 3 are influenced by weathering. Figures 4.9 to 4.12 show that this must have happened within the ballast tank of the ship:

- The alternation of n-alkanes and isoprenoids shows the influence of bacterial degradation of source 3.
- The reduction of the methyl-phenantrenes is caused by dissolution.

The diagnostic ratio of MA is increased in source 3 (from 0,18 to 0,26, see Figure 4.6 and Fig. 4.21). But DR-MA is based on 1-MP, and there was a higher reduction of 1MP by dissolution. Thus, if MA is based on TM-PHen (Figure 4.11), it can be seen that MA was not at all influenced by weathering.



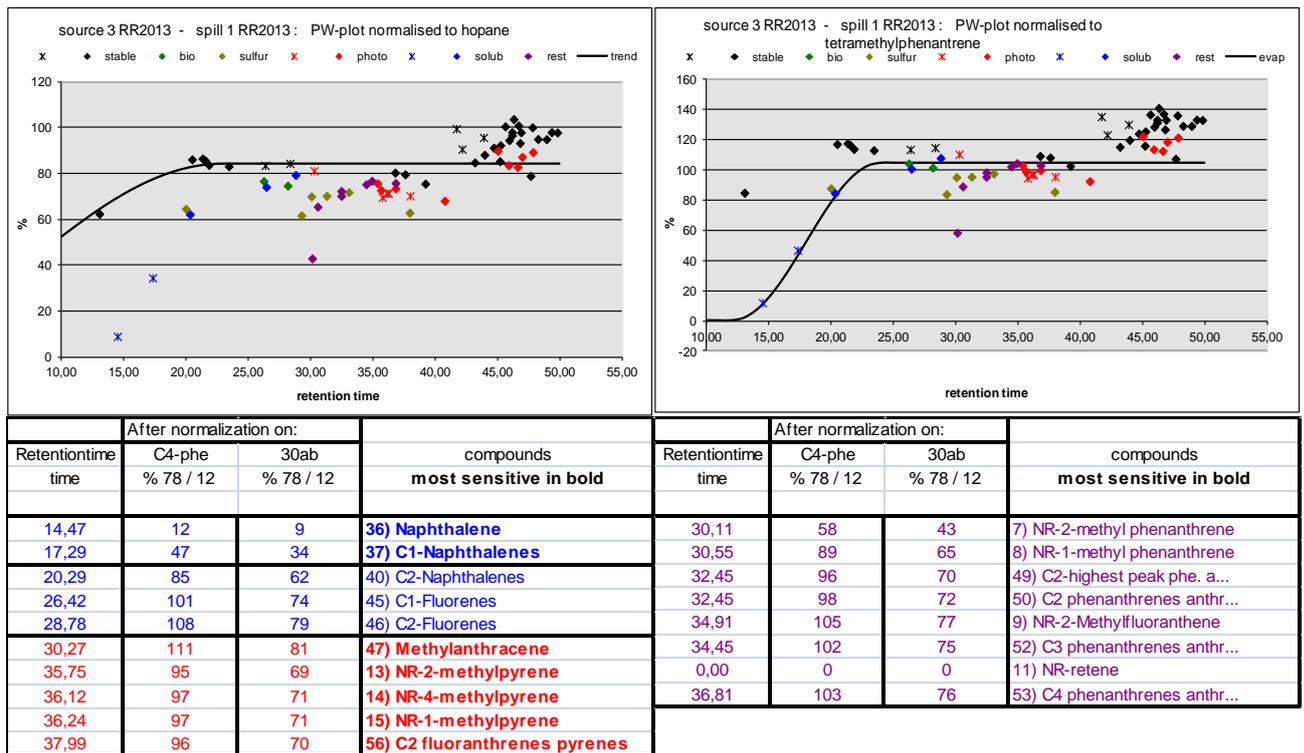
**Fig. 4.21 : (BSH, DE)**  
 Overlay of the 192-clusters of spill 1 (blue) and source 3 (red) normalized on 2MP.



**Fig 4.22 : (RWS, NL)**  
 Overlay of the 192-clusters of spill 1 (blue) and source 3 (red) normalized on MA.

In Fig. 4.21 the methyl-phenantrenes and methylanthracene are visually normalized to 2-MP. In Fig. 4.22 methylanthracene is used for normalization. It is obvious that the methyl- phenantrenes have been reduced in the sequence 2MP > 3-MP > 9/4-MP > 1-MP. This is according to the information shown in CEN/Tr annex G.3.

Interesting is the dissolution behavior of some other compounds of source 3 normalized to spill 1. See Fig 4.23.



**Fig 4.23 : (RWS, NL)**

MS-PW-plot of source 3 based on spill 1.

In the left MS-PW-plot normalized to hopane the evaporation line is drawn through the C-1 decalines, followed by the sesquiterpanes, pristane and phytane. These compounds have a high resistance against dissolution, photo oxidation and biodegradation.

In the right MS-pw-plot normalized to TMP the evaporation line is (mis)used to show the informative compounds known to be markers of solubility. The compounds are indicated in blue and the data points are shown in the left table of Fig 4.23.

The compounds naphthalene and C1-naphthalene's are marked with a \* to indicate that these compounds are most sensitive for the indicated weathering effect. The C2-naphthalene's and C1 and C2 fluorene's are marked with a blue dot to indicate that they also are sensitive for the weathering effect, but less than the other two.

Interesting is that 2-MP and even 1 MP are lower than the fluorene's. The same is valid for several other PAH's. This indicates that the solubility behavior of these compounds is not covered by the spreadsheet file v51. V51 should thus be improved.

This cannot be done based on the information of Fig. 4.22 alone, because it is the result of a complex combination of mixing and weathering here.

At the Bonn-OSINet meeting in Hamburg 2014 the solubility of mineral oil components in water has been discussed. Based on weathering tests and real cases of the participants, the solubility indication of the compounds in the spreadsheet file v51 will be improved according to Table 4.1. For the complete updated table see Annex 1.

Current assignments high = ж medium = ♦		New assignments high = ж medium = ♦	
compounds	solubility	compounds	solubility
7) NR-2-methyl phenanthrene		7) NR-2-methyl phenanthrene	ж
8) NR-1-methyl phenanthrene		8) NR-1-methyl phenanthrene	♦
36) Naphthalene	ж	36) Naphthalene	ж
37) C1-Naphthalenes	ж	37) C1-Naphthalenes	ж
40) C2-Naphthalenes	♦	40) C2-Naphthalenes	♦
45) C1-Fluorenes	♦	45) C1-Fluorenes	
46) C2-Fluorenes	♦	46) C2-Fluorenes	

**Table 4.1 :**

Assignment change for dissolution. ж = high sensitivity, ♦ medium sensitivity.

#### 4.4 Conclusions

##### 4.4.1 Spill 1 compared with Source 1

Reasons to conclude a match.

- Presence of the biphenyls.

Reasons not to conclude a match.

- The FID and MS chromatograms show distinct differences.
- Assuming that spill 1 is composed of source 1 and lubricating oil the first part of the chromatograms up to Tr-C23 should show no differences. Here however also differences are found, that cannot be explained by weathering.

Conclusion: **non-match.**

##### 4.4.2 Spill 1 compared with Source 2

Reasons to conclude a match.

- No reasons

Reasons not to conclude a match.

- The FID and MS chromatograms show distinct differences.
- The MS-PW-plot of Fig. 4.17 shows a scattering of data points of the whole retention time region
- Many ratios show a difference of > 14%

Conclusion: **non-match**

#### 4.4.3 Spill 1 compared with Source 3

Reasons to conclude a match.

- A visual comparison of the GC-FID chromatograms shows no differences except for a slightly higher concentration of lubricating oil in source 3.
- The GC-PW-plot shows no difference, beside some evaporation.
- The MS-PW-plot shows that source 3 has been influenced by biodegradation and dissolution. The differences can be explained according CEN/Tr 15522-2.
- The MS-PW-plot shows that source 3 contains an extra, but small, amount of lubricating oil. The triaromatic steranes can be used as extra evidence that the underlying oil doesn't show significant differences.
- The ratio comparison shows no differences except of what can be explained by weathering or mixing.
- The MS ion chromatograms show no differences except of what can be explained by weathering or mixing.
- Special and identical characteristics are also the biphenyls, methylcarbazoles and retene in both samples.

Reasons not to conclude a match.

- Source 3 contains more lubricating oil.

Conclusion:

According to the positive match definition of the old version of the CEN/Tr (CEN/Tr 15522-2:2006) the difference in concentration of the lubricating oil is not acceptable and a probably match should be concluded.

The positive match definition of CEN/TR 15522-2: 2006:

When 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.

This case is, however, a good example, why the definition of the CEN/Tr of 2006 has been changed into the definition of the CEN/Tr of 2012.

The positive match definition of CEN/TR 15522-2: 2012:

Differences in chromatographic patterns and diagnostic ratios of the samples submitted for comparison are lower than the variability of the method or can be explained unequivocally, for example by weathering.  
The samples are considered to match to a high degree of scientific certainty.

Participants have had cases in which some mixing has occurred, while the underlying oil is matching. There was a strong feeling that in certain cases a probably match does not reflect the similarity of the samples and that a positive match would be the right conclusion.

Therefore the definition of 2006 has been changed. It was decided not to add the word "mixing" to the definition, because then almost every case can be concluded as a positive match. The term "can be explained unequivocally" gives only a little bit more space to conclude a positive match for cases in which some mixing has occurred.

Based on the definition of CEN/Tr 15522-2:2012 a positive match can be concluded between spill 1 and source 3, as all observed differences can be explained by mixing of a small amount of lubricating oil and by weathering of source 3.

Conclusion: **positive match.**

## 5 The reports of the participants: Common aspects, remarks and suggestions

In this chapter some general issues are discussed. For the judgement of the individual reports see chapter 7.

### 5.1 Sample treatment

In CEN/Tr section 5.4.1 it is strongly advised to clean "black" samples over silica or florisil before analysis, to protect the column and improve the repeatability of the analysis. The type of oil was not given, but from the scenario and the color of the samples, it could be concluded that the samples contain HFO. Table 5.1 shows that only nine of the labs have indicated, that they have cleaned the samples.

Labcode	Cleanup	injection conc. mg/ml	injection volume ul	analytical system	column	
					FID	MS
lab 1	yes	3.5	1	Shimadzu	used MS	Restek RTX-5
lab 2	n.a.	n.a.	n.a.	Thermo	n.a.	n.a.
lab 3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
lab 4	yes	5	1	Agilent	DB-1	DB-5
lab 5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
lab 6	no	5	1	Trace MS	used MS	J&W DB 5
lab 7	no	4	1	Agilent	DB-5HT	DB-5 ms
lab 8	yes	4	n.a.	Agilent	n.a.	DB-5
lab 9	no	1,6	1	Agilent	HP-5ms	HP-5ms
lab 10	no	4	n.a.	n.a.	n.a.	n.a.
lab 11	no	8	n.a.	n.a.	n.a.	n.a.
lab 12	no	4-8	n.a.	Agilent	n.a.	J&W DB5
lab 13	yes	2	n.a.	Agilent	n.a.	J&W DB5
lab 14	yes	0.8	n.a.	Agilent	n.a.	Zebron ZB-5
lab 15	no	2	1	Agilent	DB-1	DB-5
lab 16	no	8	1	Agilent	n.a.	ZB-SemiVolatiles
lab 17	yes	5	1	Thermo	n.a.	TRB-5MS
lab 18	no	4	n.a.	n.a.	n.a.	n.a.
lab 19	yes	4	n.a.	n.a.	n.a.	n.a.
lab 20	no	5	1	n.a.	Elite 5MS id 0.32	HP 5MS
lab 21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
lab 22	yes	4	1	Agilent	RTXi - 5HT	RTXi - 5HT
lab 23	problem	n.a.	1	Agilent	HP-Ultra 1	HP-5ms
lab 24	no	2,5	1	Agilent TripleQ	n.a.	HP-5ms
lab 25	yes	n.a	n.a.	Agilent	n.a.	n.a.
lab 26	no	5-7	1	Agilent	n.a.	HP-5 MS
lab 27	n.a.	10	1	Agilent	ZB-5 HT	HP-5ms

**Table 5.1**

Sample cleanup and analytical specifications

n.a. = not available.

A "yes" for the cleanup is given, when it was mentioned in the report. A "no" was concluded when e.g. in the report it was mentioned that the samples were diluted before injection without mentioning the cleanup step. Some reports did not describe the sample treatment at all. The information could also not be found in the spreadsheet file. This is indicated with n.a.

Lab 23 indicated to have problems with the silica columns used for cleanup and decided to analyze the samples without cleanup.

Lab 1 indicated to have compared a sample without and with cleanup:

All samples underwent a silica clean-up step as described in the method.

A comparison of 2 duplicate oils (Source 1 vs Source 1 without SPE cleanup) is presented in Appendix 2 to verify that the SPE step did not introduce differences. The sample comparison yielded a positive match result.

The injection concentration varies from 0.8 to 10 mg/ml. Basically the amount of oil should not be more than needed to receive reliable data. Small peaks should be seen, while the higher peaks should not be overloaded. The variance of the data of the spreadsheet file of lab 14 is quite high (See Table 5.2) and might be related to the injection concentration of 0.8 mg/ml, what seems to be too low for a HFO. On the other hand the variance of the COSIweb data for lab 14 is much lower (See table 5.3). So the higher variance might also be related to the integration. The optimum injection concentration is not only related to the detector, but also to the column. Most participants have used a 0.25 mm ID column. A good load for such a column diameter should be 1 to 5 mg/ml for crude. If a column of 0.18 mm ID is used, a lower amount should be injected, because the total amount of phase is lower.

When indicated in the reports of the participants the columns used can be found in Table 5.1. In the report of RR2012 section 5.2, the effects of different column phases on the separation of compounds is described. Therefore this is not repeated in the report of RR2013.

## **5.2 Variance of the duplicate analyses of spill 1.**

Table 5.2 shows the variance of the data from the excel spreadsheet files provided by the participants. Some labs are missing in the list, because they have only worked with COSIweb and did not provide manually integrated results.

Basically the duplicate results of spill 1 have been taken for assessment. Some labs analysed only a limited number of samples in duplicate. In these cases another sample has been used, as indicated in the table.

The data are retrieved from two tables in the spreadsheet file version 51 available on the BonnOSInet web server.

Labcode	sample	MS-PW-plot		Number and st dev of the paired ratios					
		Normalized to hopane		Normative ratios		Inform. ratios		All ratios	
		mean	st dev	Number	St dev	Number	St dev	Number	St dev
lab 1	spill 1	105	3,0	24	2,8	5	3,8	29	3,0
lab 4	spill 1	98	4	24	4,5	10	2,7	34	4,1
lab 6	spill 1	112	7,5	24	3,1	8	2,9	32	2,2
lab 7	spill 1	101	3	25	2,6	12	2,8	37	2,6
lab 9	spill 1	97	3,7	24	4,3	10	3,3	34	4,1
lab 10	spill 1	105	<b>7,6</b>	25	<b>7,2</b>	12	3,4	37	<b>6,2</b>
lab 11	spill 1	103	5,3	24	3,5	12	<b>5,4</b>	36	4,2
lab 12	spill 1	98	3,5	24	3,1	10	2,4	34	2,9
lab 13	spill 1	97	2,9	23	2,2	10	1,9	33	2,1
lab 14	spill 1	92	<b>15,4</b>	23	<b>10,3</b>	12	<b>8,2</b>	35	<b>9,6</b>
lab 15	spill 1	100	4,5	24	3,8	12	3,5	36	3,7
lab 16	spill 1	95	3,9	24	4,1	12	5,2	36	4,5
lab 17	spill 1	101	3,2	24	2,4	10	1,9	34	2,2
lab 18	spill 1	104	4,5	25	2,4	12	3,2	37	2,7
lab 20	spill 1	107	<b>14,2</b>	23	<b>5,8</b>	10	4,6	33	<b>5,4</b>
lab 22	spill 1	105	3,8	25	3,2	12	2,6	27	3
lab 23	spill 1	96	3,1	25	4,6	12	1,5	37	3,9
lab 24	source 1	94	<b>16,2</b>	25	<b>6,3</b>	12	<b>6,6</b>	37	<b>6,4</b>
lab 25	spill 1	92	5,5	24	<b>6,4</b>	10	4,0	34	<b>5,8</b>
lab 26	spill 1	102	4,0	24	3,7	10	3,6	34	3,7
lab 27	spill 1	95	3,9	23	3,6	12	4,4	35	3,9

**Table 5.2 Variance data manual integration****In bold:**

PW plot: if the st. dev. is larger than the 95% limit of 7.5%)

Ratios: if the st. dev. is larger than 5% for the normative and/or all ratios.

The MS-PW-plot variance can be found in cell AE82 of each comparison sheet. This table has already been used for the evaluation of the PW-plot of RR2009 and more information can be found in section 5.2 of the summary report of RR2009.

The st. deviation of the paired ratios [1] can be found in the table belonging to cell A104 of each evaluation sheet. First the ratios, that have been analyzed should be selected in column G (**1** = taken into account and **empty** = not taken into account). The st. dev. can be calculated separately for the normative, informative and all ratios. So Table 5.2 shows also how many of the ratios have been used by each lab.

To indicate a higher variance than indicated in the CEN/Tr, values are shown in bold:

- For the PW-plot a value above 7.5% for the st.dev. is given in bold. In the summary report of RR2009, it was concluded, that a reasonable value for the st. dev. of the MS-PW-plot would be 7-8%. So a value below 7.5% can be indicated as a good value. For RR2013 4 of the 21 labs with results available, had a st. dev above 7.5%.

- For the combined paired ratios [1] a value above 5% st.dev is given in bold. In the CEN/Tr (see section 6.5.5 of the CEN/Tr) a st.dev of 5% is chosen as upper limit. Higher values are indicated in bold in Table 5.2 for the normative and all ratio columns. Six labs have not met the criterion of 5%.

A similar table has been made for the data entered in COSIweb. See Table 5.3. The data have been integrated automatically and could be checked after integration.

Labcode	sample	MS-PW-plot Normalized to hopane		Paired ratios All ratios	
		mean	st dev	Number	St dev
lab 1	spill 1	103,0	2,8	27	3,1
lab 3	spill 1	103,9	7,4	28	<b>5,6</b>
lab 4	spill 1	102,6	3,9	28	<b>5,1</b>
lab 5	no duplicates				
lab 6	spill 1	90,3	6,5	26	3,2
lab 8	spill 1	103,0	4,4	28	3,0
lab 12	spill 1	102,8	4,5	28	4,1
lab 14	spill 1	101,7	4,7	26	3,7
lab 15	no duplicates				
lab 16	spill 1 dup 1-2	106,4	4,1	27	4,8
lab 18	spill 1	103,8	4,0	27	3,4
lab 19	spill 1	92,0	4,2	27	<b>5,1</b>
lab 22	Spill 1	95,3	3,6	28	3,4
lab 23	spill 1	103,6	3,2	28	4,0
lab 26	spill 1	102,5	4,2	27	2,8

**Table 5.3 Variance data COSIweb**

**bold:**

PW plot: if the st. dev. Is larger than 7.5%

Ratios: if the st. dev is larger than 5% for all ratios.

If compared with Table 5.2, Table 5.3 shows that the automatic peak measurement by COSIweb works very well.

### 5.3 Oil type recognition

All samples mainly consists of HFO. This is recognized by all participants who reported the oil type.

Only 3 labs correctly identified source 3 and spill 1 as sludge (mixture of HFO and lubricating oil)

Sample	source 1	source 2	source 3	spill 1
Scenario	HFO	HFO	sludge	sludge
lab 1	HFO	HFO	HFO	HFO
lab 2	n.a.	n.a.	n.a.	n.a.
lab 3	HFO	HFO	HFO	HFO
lab 4	HFO	HFO	HFO	HFO
lab 5	n.a.	n.a.	n.a.	n.a.
lab 6	HFO	HFO	HFO	HFO
lab 7	HFO	HFO	HFO	HFO
lab 8	HFO	HFO	Sludge	Sludge
lab 9	HFO	HFO	HFO	HFO
lab 10	HFO	HFO	HFO	HFO
lab 11	HFO	HFO	HFO	HFO
lab 12	HFO	HFO	HFO	HFO
lab 13	HFO	HFO	HFO	HFO
lab 14	HFO	HFO	HFO	HFO
lab 15	HFO	HFO	HFO	HFO
lab 16	HFO	HFO	Sludge	Sludge
lab 17	HFO	HFO	HFO	HFO
lab 18	HFO	HFO	HFO	HFO
lab 19	HFO	HFO	HFO	HFO
lab 20	HFO	HFO	HFO/crude	HFO/crude
lab 21	n.a.	n.a.	n.a.	n.a.
lab 22	HFO	HFO	HFO	HFO
lab 23	HFO	HFO	HFO	HFO
lab 24	HFO	HFO	HFO	HFO
lab 25	HFO	HFO	Sludge	Sludge
lab 26	HFO	HFO	HFO	HFO
lab 27	HFO	HFO	HFO	HFO

**Table 5.4 Oil type.**

In the comparison of spill 1 with source 3 it was recognized that source 3 contains more lubrication oil than spill 1. So source 3 is a mixture of HFO and lubrication oil. It indicates a waste oil mixture, that can be called a sludge.

Whether spill 1 contains lubricating oil is more difficult to find. The method used for a combination of diesel and lubricating oil cannot be used. See Fig. 4.14. The FID chromatogram of spill 1 shows just like source 3 an unresolved hump at about 9 min, but this can also be a pattern of pure HFO. See Fig. 4.1. The comparison of source 1 with spill 1 shows however a higher concentration of the hopanes and steranes, but not of the aromatic steranes. Although it is concluded that also the comparison of source 1 and spill 1, before the retention time of Tr-C23, is a non-match, still it is strange that the aromatic steranes are at the PAH's level. See Fig. 4.15. So spill 1 is very likely also a mixture of HFO and lubricating oil.

#### 5.4 Visual inspection

A visual inspection of the chromatograms to find peculiarities is an important part of oil spill identification.

Source 1, source 3 and spill 1 contain extra peaks, which were identified by library search and GCxGC-TOFMS analyses as alkylated biphenyls. Table 5.5. shows that not all participants have mentioned these extra peaks in their report.

Note: For the assessment of the participants it was sufficient to mention the peaks.

One of the reasons to select this case for RR2013 was the fact that source 3 is more weathered than spill 1 (See 3.2). Table 5.5 shows that not all participants have mentioned this in their report.

	mentioned extra peaks	weathering source 3 > spill 1
lab 1	1,1-diphenyl ethane	no
lab 2	no	n.a.
lab 3	yes	no
lab 4	no	yes
lab 5	no	no
lab 6	no	no
lab 7	alkylated biphenyls	yes
lab 8	Diphenylethane	yes
lab 9	yes	yes
lab 10	yes	no
lab 11	benzene, 1,1'ethylidenebis	yes
lab 12	in m/z 231 trace	yes
lab 13	no	yes
lab 14	no	no
lab 15	no	yes
lab 16	yes	yes
lab 17	C2 and C4 biphenyls	no
lab 18	no	yes
lab 19	no	no
lab 20	alkylated biphenyls	yes
lab 21	no	n.a.
lab 22	yes	yes
lab 23	no	yes
lab 24	no	no
lab 25	no	yes
lab 26	yes	yes
lab 27	no	yes

**Table 5.5 Visual inspection**

Indication in the reports whether extra peaks are present and whether source 3 is more weathered than spill 1.

It was also possible to find retene and extra peaks in the m/z 208 and 220 traces, but due to the low concentration this has not been taken into account in the assessment.

#### 5.5 Normative compound/ratio integration

In the assessment we always look whether at least the normative compounds, that have been excluded from the evaluation of a case, have been mentioned (with reason). For this year however it was not very relevant because even retene could have been found in two of the samples. So for this year's round robin no table about this issue.

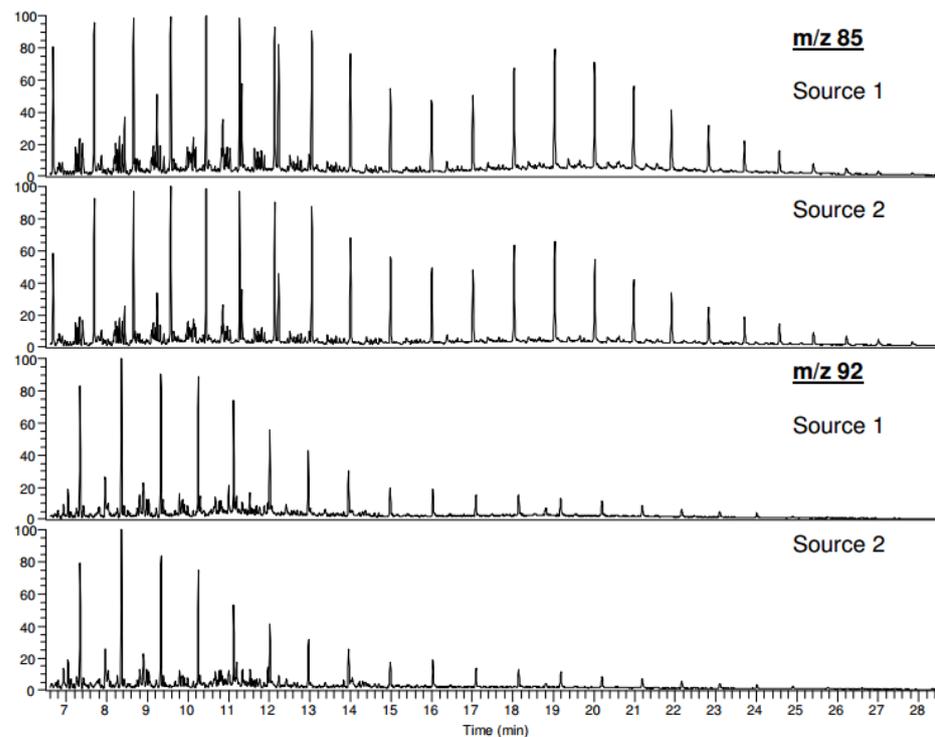
## 6 Comments and suggestions of the participants

The reports of several participants contained questions or remarks about the round robin or the method in general. In this chapter these remarks will be shown and discussed.

### 6.1 Joan Albaiges, CSIC

CSIC has studied the *n*-alkylbenzenes and alkyltoluenes [2] and has used these compounds for comparison in their report.

Joan suggests to add *m/z* 92 to the method.



**Fig. 6.1 : (CSIC, ES)**

Fig. 3 of the report of CSIC showing the difference in information derived from *m/z* 85 and *m/z* 92

**Figure 3.- Distributions of *n*-alkanes (*m/z* 85) and *n*-alkylbenzenes (*m/z* 92) in source 1 and 2 samples.**

Fig. 6.1 is an example showing the difference in information received from the *n*-alkanes and the *n*-alkylbenzenes. The first part up to a retention time of 16 min gives comparable information, but after 16 min the *n*-alkane concentrations increase, while the *n*-alkylbenzenes stay at the same level. Joan indicates in his report:

Moreover, the profiles of sources 1 and 2 exhibited a bimodal distribution of *n*-alkanes, illustrated in Figure 3, which is neither present in the other samples and could be the consequence of wax enrichment in the fuel oil residue by the low temperature. The mixture of two products can be discarded

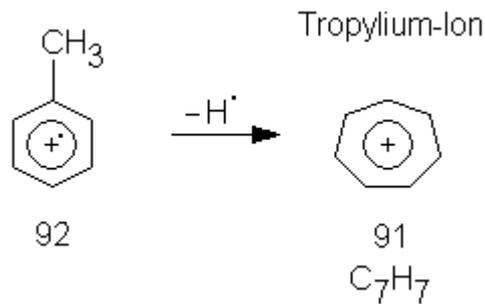
by the fact that this bimodality is not reflected in the profiles of n-alkylbenzenes (m/z 92).

Besides wax enrichment, another explanation can be that the HFO of source 1 and 2 are mixtures of a heavy fraction dissolved in a light fraction. HFO is a rest product of a refinery and has as such a very high viscosity. In order to be used by a ships engine it has to be diluted in a light fraction (diesel or light cycle oil (LCO) ) to reduce the viscosity. HFO is normally composed of two or three fractions. Examples can be found in the summary reports of RR 2010 and 2011.

Fig 6.1 suggest that source 1 and source 2 are composed of two fractions with a different n-alkanes/ n-alkylbenzenes ratio.

At the Bonn-OSINet meeting in Hamburg 2014 Joan Albaiges has presented his results.

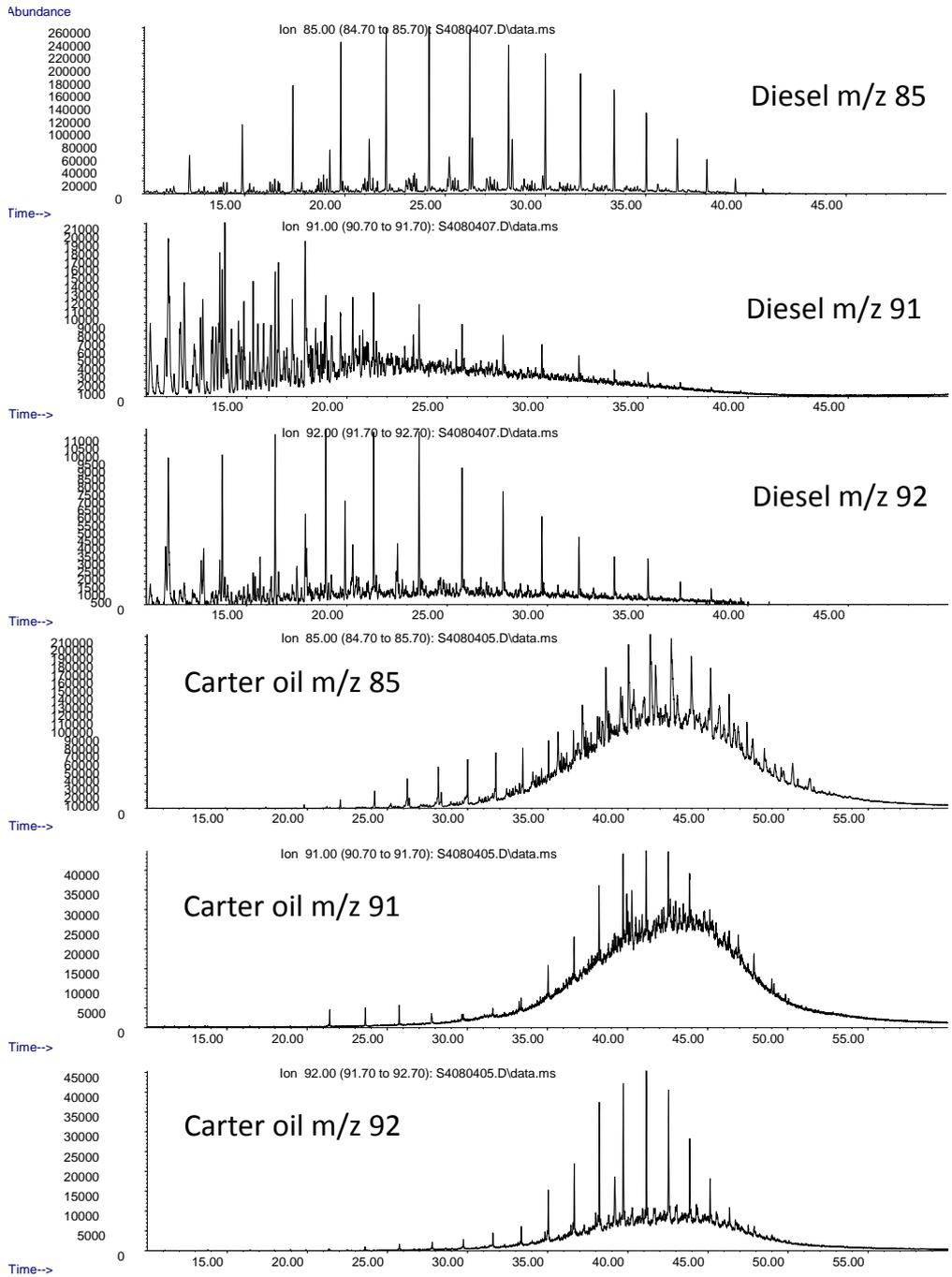
In the following discussion Gerhard Dahlmann and Norbert Theobald (BSH) suggested to use m/z 91. It represents the resonance stabilized tropylium ion.



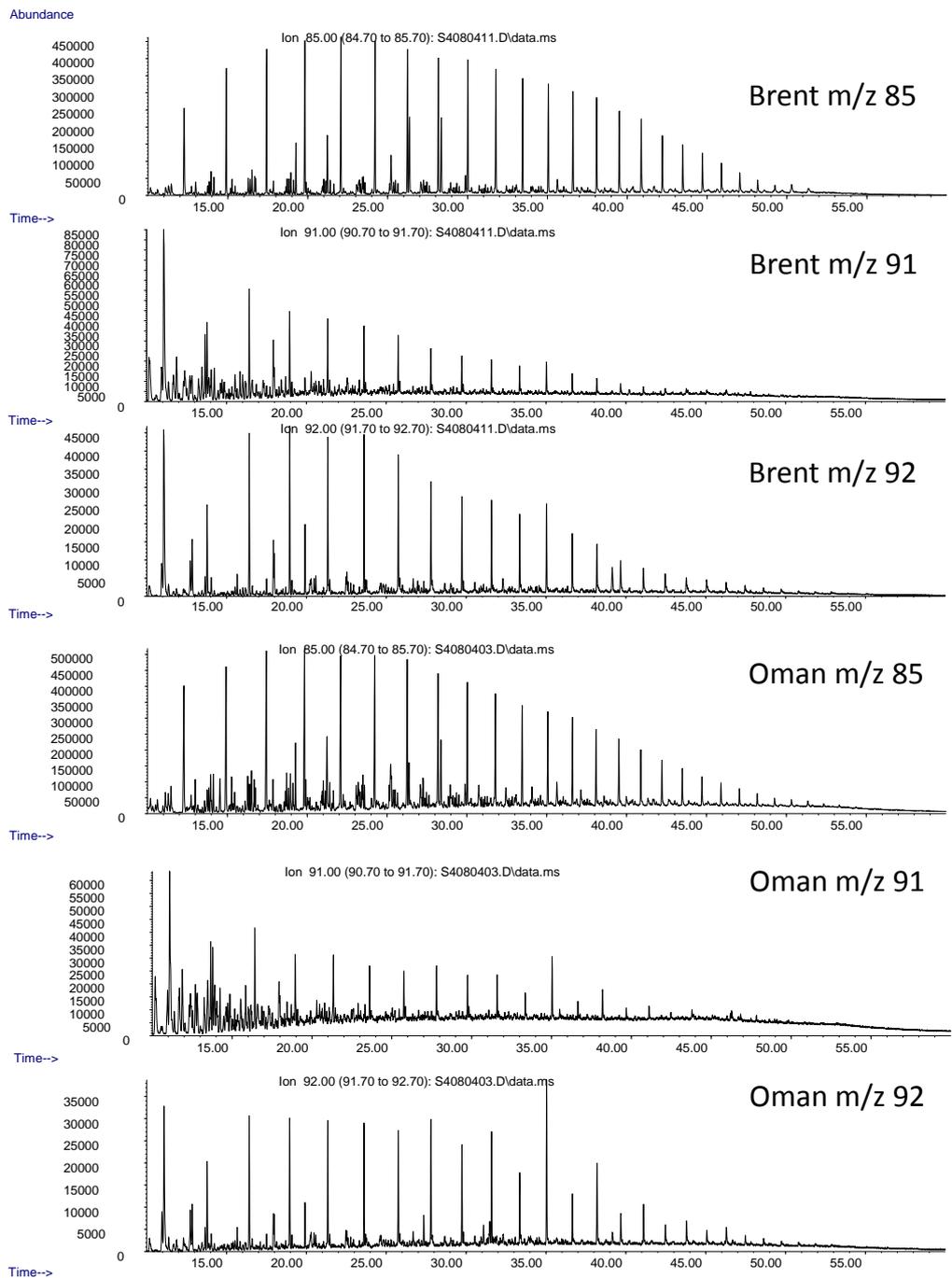
**Fig. 6.2 : Wikipedia**  
Tropylium ion

The tropylium ion is frequently encountered in mass spectrometry in the form of a signal at m/z = 91 and is used in mass spectrum analysis. This fragment is often found for aromatic compounds containing a benzyl unit. Upon ionization, the benzyl fragment is cleaved off as a cation ( $PhCH_2^+$ ), which rearranges to the highly stable tropylium cation ( $C_7H_7^+$ ). (Source: Wikipedia)

Fig. 6.3 and 6.4 show the information derived from m/z 85, 91 and 92 for diesel, lubricating oil (carter oil) and two crude oils ( Brent and Oman).



**Fig. 6.3 : (RWS, NL)**  
Ion chromatograms of diesel  
and lubricating oil.



**Fig. 6.4 : (RWS, NL)**  
Ion chromatogram of crude oils.

Both m/z values give a range of peaks and different information compared with m/z 85. The abundance of m/z 91 is in general higher, compared with m/z 92. While m/z 91 is formed by many small aromatic compounds (See e.g. Fig. 6.3 Diesel m/z 91), m/z 92 gives information about the n-alkyl benzenes.

## 6.2 Deib Birkholz, ALS

Figure 12 shows a GC/MS EICP for m/z 231, which is characteristic of triaromatic steranes. Spill 1 is compared with source 1, source 2 and source 3.

From this figure it appears that Spill 1 and source 3 are similar. Interestingly, the C20 and C21 triaromatic steranes are difficult to identify in the RR 2013 samples due to a cluster of peaks likely attributed to PAHs and/or naphthenoaromatic hydrocarbons.

Their presence can be used as evidence of cracked heavy components such as heavy fuel oil.

Deib refers here to an interesting paper of Chun Yang (EC-ESTD, Ca) [3]

## 6.3 Deib Birkholz, ALS

Several questions are posed, so the reactions are inserted in the text of Deib:

In reviewing the COSI-WEB results I noticed some similarities (i.e. common failing ratios) and many differences. Some labs reported very few failing ratios (e.g. Au). CEN/TR 15522-2:2012 does recommend clean-up of samples containing heavy fuel oil. I noticed when I pulled out the splitless glass insert from the GC/MS that it was very black and wonder if clean-up (which we normally do except this year) might have resulted in different data, specifically the PAH data.

### Reaction 6.3.1:

The RR2013 samples should indeed have been cleaned before injection. The clean-up methods described in the CEN/Tr, remove particles and high boiling compounds (e.g. asphaltenes) not used in the assessment. These particles and compounds will be collected in the liner and first part of the column and change the properties of the analytical system. HFO contains a lot of asphaltenes while used lub oil can contain particles. After a few injections of HFO the alkane peaks become smaller and higher, but after some extra injections and/or in the next sequence, the high boiling alkanes show broad peaks and low abundances. Oil spill identification is based on a stable analytical system, so these effects should be avoided.

In terms of COSI-WEB I do not use it because we are located in-land and most of the spill work we do relates to spills in rivers and creeks and on land. Most of those spills relate to middle distillates, heavy oil operations or coal mining operations. We just had a spill of over 1 billion liters of coal washings into a major river. It is doubtful that such information would be of interest to coastal labs investigating oil spills. It would be useful to have a COSI-WEB program which we could enter the CDF files ourselves in order to save time.

### Reaction 6.3.2:

Samples to be added to COSI-WEB must contain n-alkanes and/ or biomarkers. If both are not available the system cannot integrate the sample. The samples described by Deib might however contain these compounds. If so there is no reason not to enter them in COSI.

COSI doesn't contain at the moment samples from coal mining, so it seems not very useful to add them, but:

- Cosi can be used to compare the samples of a case as such.
- Coal mining takes place on many places on the world so the information can be very useful for other users dealing with this kind of samples.

All participants of Bonn-OSINet can enter their own samples in COSIweb when they use the correct GCMS settings (column type and length and the retention times of 3-MP and Hopane)

We are using CEN/TR 15522-2:2012 in relation to contaminated sites. We have many sites in North America that are highly contaminated. The cost with such clean-up is usually very large. Usually no liquid product is available. The question on everyone's mind (especially insurance underwriters) is whether samples taken from various sites are similar, dissimilar, or from a common source. To compound the situation the samples collected may represent samples that were spilled many years apart and so there really is no "source sample".

Without any concept of reproducibility from contaminated soil samples (EPA suggests a coefficient of variance of 25% for PAHs in contaminated soil), the best we can do is come up with probable matches. Considering the huge observed variability in the biomarker data this is a great achievement. I will be applying chemometrics to the GC/MS data (principle component analysis, linear discriminant analysis, and clustering) to determine if there is an improvement. Although the procedures developed by OSINET are largely in response to coastal oil spills, the process and the round robins are much appreciated by labs such as ours which respond to aquatic spills, terrestrial spills and contaminated sites. The procedures are legally defensible which is very important in this age of litigation.

I would appreciate some feedback on whether it is appropriate to use CEN/TR 15522-2:2012 for contaminated site samples.

#### Reaction 6.3.3:

Scott Stout has asked this question already several years ago. Therefore the following text has been added to the scope of Cen/Tr 15522-2:2012:

This method is not directly intended for oil spills impacting groundwater, vegetation, wildlife/tissues, soils, or sediments, and although its application in these matrices is not precluded, it requires caution. The reason for caution is that the extractable compounds in these matrices may alter and/or contribute additional compounds compared to the source sample, which if left unrecognised, can lead to "false non-matches". Including these "non-oil" matrices in this oil spill identification method may require additional sample preparation (e.g. cleanup) in the laboratory prior to analysis and consideration of the extent to which the matrix may affect the correlation achieved. Evaluating the possible effects in these matrices is beyond the scope of this guideline.

Whether the method can be used for this kind of "non-oil" matrices may depend on the oil concentration compared to the "matrix concentration" of the samples. In "non-oil" matrices containing a relative high concentration of oil, a positive match can still be concluded. In "non-oil" matrices containing a relative low concentration of spilled oil, a (false) non-match or an inconclusive match could be achieved due to matrix effects

#### **6.4 Liv-Guri Faksness, Sintef**

Several questions are posed so the reactions are inserted in the text of Liv-Guri:

##### 7 Comments to Round Robin 2013

It was an interesting and challenging case with the oils from a Swedish harbour, and the workload by comparing one spill samples with three suspected sources was appropriate. SINTEF has used both COSI and the Excel spreadsheets developed by Paul Kienhuis to evaluate the samples in the present Round Robin test, and would like to share of our experience with combining these two methods:

- COSI seems to be a useful tool when comparing the samples and gives a good overview of the GC/FID results with overlaying chromatograms and GC-PW plots. However, the PW plots are different than the PW plots presented in CEN (2012) which is more in accordance with the original plots developed by Per Wrang (Nordtest, 1991) displaying one line indicating the difference in evaporative loss between two samples. The GC-PW plots in COSI displaying both samples in the same figure are not so easy to interpret for an untrained user of the database. SINTEF has used COSI here, but would later prefer to use the PW-plots shown in CEN (2012).

#### Reaction 6.4.1:

The GC-PW-plot of COSI shows two lines (See e.g. Fig. 4.6). A PW-plot is a comparison of two samples. Normally the source is used as reference and the peak heights of the compounds of the spill sample are shown in % relative to the peak height of the same compound in the source sample.

With COSI it is possible to search on comparable samples with both source and spill samples. For the GC-MS-plot Gerhard has chosen to show a plot based on the search sample (left table) and a plot based on the sample selected from the search results. So the GC-PW-plot shows two graphs assigned in resp. red and blue.

The MS-PW-plot however shows only one graph. This has been done because the MS-PW-plot shows:

- much more data points.
- much more variation in the data points compared with the alkanes of the GC-PW-plot.

As a result for the MS-PW-plot one has to switch between search and result sample, if the low boiling compounds are larger than 100%.

The difference is also related to the development of COSI. In the stand-alone version a GC-PW-plot (with two plots) was available for the GC-FID data and a ratio comparison for the GC-MS data. In the web-based version the MS-PW-plot has been added.

An option for the web-based version could be to remove the double plot and to add a range of isoprenoids. The GC-PW-plots of SKL, SE (See e.g. Fig. 4.9) show that the use of isoprenoids, indicated in a different colour than the n-alkanes, is very useful.

- COSI calculates diagnostic ratios, which is time-saving. It would be excellent if there was an even better correlation between the target list in COSI and Paul's spreadsheet. So far, SINTEF think the spreadsheet is more informative and easier to interpret than COSI, but this could reflect that we are more familiar with the spreadsheet.

#### Reaction 6.4.2:

The development of the CEN/Tr started with a strong focus on the use of stable and high boiling compounds. This is reflected in the choice of the normative ratios for the CEN/Tr (See Table 3 of the CEN/Tr). Even the use of pristane and phytane was under discussion, because these compounds are often partly evaporated. COSI is mainly based on these normative compounds.

At the same time the focus was on crude oil and HFO, while (at sea) diesel was seen as a fast spreading and evaporating product.

Paul has however to deal with a lot of diesel cases from inland spills. To be able to test the CEN/Tr method under development, he and Gerhard started the first round

robin (RR2004) with a diesel case. To be able to receive analytical results, Paul developed a spreadsheet file to compare the normative compounds, but he added a range of informative compounds to make the spreadsheet file more flexible. The normative compounds are based on the need to assign a minimum range of ratio's, that must be calculated to prevent that analysts only select the compounds that suits them. The informative compounds were selected based on the following criteria:

- The Cen/Tr stimulates to use extra ratios depending on the properties of the samples involved. This may range from specific high boiling biomarkers like 29Ts and 30ba to low boiling biomarkers like the sesquiterpanes.
- The selection of a range of alkylated PAH's, because many lab were used to compare these compounds.
- The selection of some compounds and compound groups that are markers for different types of weathering like the C1-naphthalenes.
- The selection of compounds suitable in diesel cases like the tricyclic diterpanes.

After the assessment of a range of round robins, Gerhard and Paul learned to value the MS-PW-plot including the low boiling compounds. Even partly weathered these compounds are valuable for the sample comparison. So it would be good to add these compounds to COSIweb.

The problem however is that for most of the samples the ion chromatograms are not available in the database. To build the offline version of COSI Gerhard had to select a small dataset for each sample, because the software (Access-97) was limited to 1 GB in database size. Therefore the data were entered as csv files containing only the sections of the ion chromatograms needed to assess the normative compounds.

The same data were later-on used for COSIweb. COSIweb is however not limited in database size so it is possible to add all data of an analysis. Expanding the number of compounds for comparison has however as consequence that at least all the crude oil samples (about 450) have to be reanalysed.

So expanding COSIweb must be done carefully. First it must be decided carefully, which compounds should be added to the assessment. Next it must be decided whether and which samples have to be reanalysed.

The cdf files contain all the ion chromatograms analyzed. So in the meanwhile we strongly advise to analyse also the ion chromatograms for the informative ratios and to add your GC-MS data as cdf files to be sure that we can use the information in future.

- COSI is producing a pdf-report for comparing two oils, including most of the results, except the ion chromatograms from GC/MS. It would be very nice if the ion chromatograms were in the report too.

#### Reaction 6.4.3:

This has been implemented in the meanwhile.

- It might be useful if the oil type was editable after the sample has been uploaded into the database, as there may be necessary to evaluate the results before oil type is decided.

Reaction 6.4.4:

This is now also possible.

SINTEF has now used COSI in a realistic case with oil contaminated birds found three places along the Norwegian coast with and unknown source. In this specific case no match to the oil contamination could be found, but the database was excellent when comparing the spill samples and concluded that they were from different sources.

Reaction 6.4.5:

Good to hear.

### 6.5 David Francois, LASEM

Page 7 of the report of LASEM: These compounds have been detected only in sample spill1 and sample source 3 with a big abundance.

Contrary to samples source 1 and source 2, samples spill1 & source 3 have been sampling using a teflon net. It will be important to do an extract with DCM on the teflon net. To conclude, it will be important not detected these compounds on “the blank teflon net”

Reaction 6.5.1:

Correct remark for somebody who doesn't use these nets.

Many labs use the EFTE net on a regularly base and have never seen these peaks. So it may be expected that the compounds are a part of the samples.

### 6.6 René de Bruyn, NFI

René has posted a comment on the OSPAR server 18 April 2014. The issue has been discussed at the meeting on 24 April 2014, but did not lead to a discussion. Maybe also because at the meeting enough extra information was provided to conclude that the issue was not very relevant for RR2014.

Still the core of his comments are valid, and will be discussed in this section.

Several questions are posed so the reactions are inserted in the text of René:

I have some comments about the report and I want to start a discussion about mixing which I should have started years ago.

I will try to make it as clear as possible but find it difficult because the comments and the discussion are interconnected which makes it complex and expressing it in English makes it even harder than its already in my mother language.

The report implies that the round robin can be used to assess the trueness of the conclusion. I don't agree with that. It's always impossible when case samples are used because one never knows with certainty what the real scenario is or what really happened.

That becomes clear when some differences are found in the comparison of samples. You can explain them but it's not always possible to prove that an explanation is valid for the case at hand. And that's the case in this round robin with the comparison of Source 3 (S3) and Spill 1 (Sp). Some of the differences could be explained by mixing, i.e. some heavy (lubricating?) oil would have been added to the ballast tank, but nobody knows if that really happened or nobody can say how likely heterogeneity of the oil in the ballast tank is. In contrast to the differences that can be explained by weathering because it's a fact that S3 is taken 2 months later than Sp, comes from a ballast tank which contains water and (almost certainly) has a headspace. So if S3

#### NOTE

In his comment René refers to the draft reportv11 as discussed at the meeting. Based on his comments however the summery report has been changed, so sometimes the text will not be present anymore in the final report. Therefore the text of his comment will not be changed in an attempt to fit his references with the final report.

is the same oil as Sp one can be certain to expect weathering effects but one cannot be certain to expect differences due to mixing. Therefore it's impossible to conclude that a match should be the right answer.

It would be different if there is a statement that some lubricating oil was put in the ballast tank and that oil was available for analysis. Then one could analyse some mixtures of the spill and that oil to see if that could explain the differences.

One thing that came to mind was that heterogeneity of the oil in the ballast tank might be considered as something that is always the case with mixtures of light and heavy oil, due to the difference in viscosity of the oils. If that is what one would think of with mixing then I could understand that one would come to the conclusion 'match' for S3-Sp. But I would argue that due to the movements of the ship, a mixture of a light and heavy oil would become homogeneous and you would expect to get results which leads to the conclusion 'match' if S3 and Sp are the same oil. And I have not seen any literature or results that would support the idea that such a mixture is always heterogeneous.

One other reason why one would come to a match might be that one would think that because we know what effects in the results could be due to mixing (see CEN/TR 15522-2:2012 - 6.4.5 Evaluation of mixing), that mixing is the (only) explanation if one sees those effects. That is something I would consider a "prosecutor's fallacy": because an elephant is an animal, grey and has four legs, doesn't mean that a grey animal with four legs is an elephant.

I think that is enough stuff for the discussion at the meeting as mentioned on page 35 of the draft summary report.

So it will not come as a surprise that I don't agree with the sentence "There is a match between spill 1 and source 3, as all observed differences can be explained by mixing and weathering of the source 3 sample." under 4.4 on page 36.

I would also like to refer to the following text on page 58 of the RR2009 summary report:

"For the comparison with sample 3 and 4 the difference of the lube oil concentration in the gas oil was for some participants a reason to conclude a probable match or even a non-match. For this and some other situations the match conclusion of CEN/TR 2v1 has been changed into a definition more open to conclude a match also if mixing occurs. The key-term is: "can be explained unequivocally"."

For me, that last sentence says it all.

#### Reaction 6.6.1:

René is combining two issues:

- Can the conclusion of a real case be part of the assessment.
- Can a match be concluded in case of mixing.

Based on both issues draft report v11 has been improved.

The first issue has been shortly addressed in the assessment (Chapter 7).

Based on the second issue the conclusion section of Chapter 4 has been improved and the reason to change the match conclusion of CEN/Tr 15522-2:2006 is explained. This case is in fact a good example, why the text of CEN/Tr 15522-2:2006 has been changed.

A more general remark about the assessment is made at the end of this section.

In the next part under 4.4 it's not clear to me why the presence of those compounds in S3 and Sp would point to the fact that S3 was taken from the ballast tank of ship 1. We don't know where

the other sample of ship 1 (S1) was taken from. The information that was given only states "...oil was collected from each of these ships (Source 1 and Source 2). Some weeks later, a layer of oil floating on water was detected in the ballast tanks of one of these ships (Source 3)."

Under 4.5 "The real scenario confirms the conclusion, that source 1 is from the same ship as source 3. This extra information makes the discussion above stronger." I suspect that Spill 1 is meant instead of Source 1 but regardless it is not certain what has really happened so one cannot say that the real scenario confirms the conclusion.

Reaction 6.6.2:

The text was indeed not clear and has been modified.

I have also some difficulty with the part "- The oil spill identification gives a strong indication that source 1 and the spill are related. There is strong evidence that the spill consists of a mixture of source 1 and lubricating oil." For me the differences between Source 1 and Spill 1 are greater than can only be explained by an addition of lubricating oil. So I would remove the second sentence and remove the word strong.

Reaction 6.6.3:

The text has been improved.

The last comment I have is about the note of Gerhard under 5.6 on page 43. I agree with him that it might not sound logical (or is) when one expresses some doubts about a match when evaluating the results for the comparison between Source 3 and Spill 1, and conclude there is a match. But in general I can see where it might come from. The results make it difficult to reach a non-ambiguous conclusion. One may feel that a 'probable match' is too weak to express the relation between the two samples and 'match' might feel too strong but because there is no other option than 'probable match' or 'match' one chooses 'match'.

Reaction 6.6.4:

What Gerhard means is that the text of an internal report should be consequent (See also section 7.6). When all differences have been explained by e.g. weathering and when the whole report points to a match conclusion, the final conclusion cannot be different without an explanation why the final conclusion has been chosen. One has to realize that such a report has to be handed to the defender in a court case. So the report should be clear and at the end reasons should be given to explain the final conclusion. I have tried to do that at the end of Chapter 4. The explanation method is based on the hypothesis model that the forensic institutes SKL and NFI have introduced into the method. (See also CEN/Tr 15522-2:2012 Annex I 7)

We will never use 'match' in our reports because there is always uncertainty, if only because there are situations where you can't prove or have proof that there is no other scenario that would be possible or might even be the real scenario. We only conform to the terminology of the conclusion in CEN/TR 15522-2:2012 for these round robins. Normally we use an equivalent system that was tried in RR2007 and say only something about the probability of the results given two hypotheses.

Reaction 6.6.5:

René mentions the probability method with a scale from -7 to +7 applied at SKL and NFI. In RR2007 the participants were asked to describe their conclusion based on

the method described in CEN/TR 15522-2:2-012 and with the probability method. The result was that the probability method showed a wide variance. This is of course related to the fact that most participants were not used to it. But that is also valid for most clients, so it was decided to choose the method with the match definitions. Another aspect is that the probability method assumes knowledge of the total collection. Eg. For DNA comparison more information is available about the uniqueness of a certain combination of amino acids relative to all possible combinations. COSIweb helps to increase the knowledge about the uniqueness of samples.

General reaction on 6.6:

Until and including RR2013 we have judged the results of the participants based on the truth in an artificial case and on a general assumed match conclusion in a real case. In some cases however this is questionable.

Artificial cases have been used more often to test or introduce new aspects of the method. Following the CEN/Tr a participant might come to a conclusion different from the "truth".

Real cases can lead to the discussion above.

In all cases however it is important how the participant has described the case and the conclusion.

- For artificial cases we have to judge the report based on what the participant should know and/ or actual knows.
- For a real case specific information and assumptions must be described along with the conclusion.

For e.g. RR2013 it should have been remarked that a match is concluded if the presence of extra lubricating oil can be explained by the scenario. Else a possible match is the alternative, because of the similarity of all other parameters to compare.

If only a match is concluded based on a general impression without mentioning special aspects of the case along with the conclusion, it is even questionable whether this should lead to full points for the final conclusion.

It is practically not possible to judge all the reports again on the conclusion description. Therefore we will start with this approach with RR 2014.

## 7 Judgment of the individual reports.

### 7.1 Assessment based on a real case.

In RR2013 a real oil spill case has been used. The case was selected, because the related source sample was more weathered than the spill sample. The results of the participants will be combined and like in each real case a conclusion will be made. Next the individual reports will be assessed in relation with the combined result.

### 7.2 Evaluation methods.

Gerhard and Paul have discussed how to judge the reports. Just reading them and giving a final judgement in a description or figure is not sufficiently objective and informative. So both have made an own method to judge the reports. It has not been taken into account whether participants have delivered data for COSIweb. The use of COSIweb is optional and is not essential for the method. So whether participants have not used or mentioned COSIweb has no consequences for the assessment of the reports.

### 7.3 Evaluation method of Gerhard

Gerhard has prepared Table 7.1 for report judgement:

Item	Criteria	Source 1	Source 2	Source 3	Sum
1	presence of means	2	2	2	6
2	oiltype correct?	1	1	1	3
3	ratios correctly chosen/excluded	2	2	2	6
4	QM	2	2	2	6
5	interpretation of ratios and weathering	2	2	2	6
6	Right conclusion?	2	2	2	6
	Max. reachable points for every comparison	11	11	11	33
Item	Criteria	Comments			
1	presence of means	0 or 2	Presence of all necessary means for tracing back a conclusion		
2	oiltype correct?	0 or 1			
3	ratios correctly chosen/excluded	0 or 1 or 2			
4	QM	0 or 1 or 2	Double measurements sd<5%, quality of chromatograms		
5	interpretation of ratios and weathering	0 or 1 or 2	Explanations for differences >14%		
6	Right conclusion?	0 or 2			
0= not present/wrong/bad 1= fair 2= present/right/good					

**Table 7.1**

Report evaluation by Gerhard with regard to correct and traceable conclusions

### 7.4 Evaluation method of Paul

Paul has prepared Table 7.2 for report judgement:

Item	Main groups	aspects	points	remarks
1	FID level 1.1	analysis	2	analytical method and data quality
2	FID level 1.2	data evaluation	2	which conclusions are drawn from the results, e.g. concentration adjustment, oil type, elimination of samples.
3	MS level 2.1	analysis - visual inspection	2	analytical method and data quality
4	MS level 2.2	PW-plots - ratios comp.	2	integration, elimination of ratios, variance.
5	Result evaluation	conclusions from chromatograms conclusions from PW-plots conclusions from the ratio comparison	2	e.g. oil type, elimination of samples. similarity and weathering aspects similarity and weathering aspects.
6	Match conclusion	final match conclusions.	2	Conclusions related to the scenario.
7	Reporting	internal documentation	2	results that are important for the final conclusions; description of the reasons for conclusions.
8	Overall impression	Personal judgement of the whole report	2	To be able to give an additional personal opinion. Useful? I don't know We will see.
		Total (ranks from 0 to 18 points)	16	For each item: 0 = bad 1 = fair 2 = good

**Table 7.2**

Report evaluation by Paul with regard to correct and traceable conclusions

### 7.5 Judgement of the results.

The individual reports of the participants are available for members of the Bonn-OSInet expert group, but are treated as confidential for the general public. Therefore it does not make sense to discuss here the results of the judgement of the individual reports even by using a code for each participant. But certain aspects and the overall results can be shown and discussed. Therefore they are reported in chapter 5 of the summary report, while the results of the judgement of the labs is shown in Table 7.3 (Identical to Table 3 of the executive summary)

labcode	Paul	Gerhard	Mean	labcode	Paul	Gerhard	Mean
lab 1	88	94	91	lab 15	88	94	91
lab 2	19	15	17	lab 16	94	94	94
lab 3	88	94	91	lab 17	94	94	94
lab 4	94	94	94	lab 18	88	94	91
lab 5	56	85	71	lab 19	88	91	89
lab 6	88	91	89	lab 20	81	94	88
lab 7	94	91	92	lab 21	0	0	0
lab 8	100	100	100	lab 22	100	97	98
lab 9	88	91	89	lab 23	94	94	94
lab 10	88	91	89	lab 24	44	76	60
lab 11	94	97	95	lab 25	94	97	95
lab 12	94	94	94	lab 26	94	91	92
lab 13	94	91	92	lab 27	94	97	95
lab 14	75	91	83				

**Table 7.3**

Results of the judgement of the reports as % of the maximum reachable number of points.

This year the differences between the judgements of Paul and Gerhard are mainly small.

Gerhard has paid special attention to the presence of all means, the oil type recognition and the right conclusion, while Paul has reduced his points based on the issues: clean-up, quality assurance, extra peaks, variance of the data, the weathering of source 3 versus spill 1 and the general impression of the reports.

Low points were given to the contribution of Lab 2. This lab indicated to have no time for the assessment and has sent in 4 MS chromatograms and have only based their conclusions on a visual comparison. So a lot of information was missing resulting in a low score.

No points were given to the results of Lab 21. Only a spreadsheets file with wrong data was provided. At the moment there is a discussion with the lab how to improve their skills.

Basically 70-80 % should be reachable if the analytical part is OK. When a participants mentions all details and interprets the results correctly the judgement will be in the range of 90-100%.

When however all details have been indicated and correctly assessed, but the analytical part is weak, this can easily result in low points, because we see this as the base upon which an analyst has to draw his/her conclusions. That base has to be solid.

## 7.6 Differences between an match and a probable match.

Note of Gerhard

I am also thinking about a discussion about the differences of the definitions of "match" and "probable match". Because most participants used words like "probably" or even "possibly", when they explained the differences in the PW-plots between spill1 and source 3 –but despite came to the conclusion of a "match".

This note has been discussed in section 6.6 reaction 6.6.4.



## 8 New product: Preem diesel.

### 8.1 Sample handling

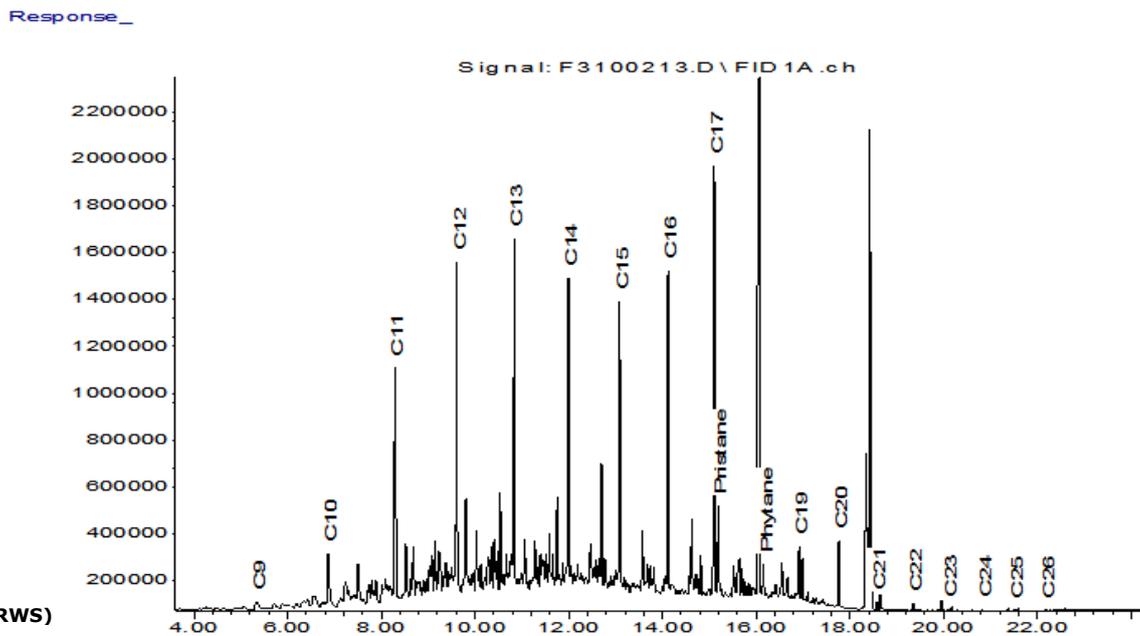


**Fig.: 8.1**  
Sample vials of RR2013 .

Fig 8.1 shows that the solution of 10 mg/ml Evolution diesel in DCM is a clear fluid. The sample has been tested with an injection concentration of 2,5 mg/ml, but is based on the results, finally used at a concentration of 0.8 mg/ml for GC-FID and GC-MS injection.

### 8.2 GC-FID

Fig 8.2 shows the relevant part of the GC-FID chromatogram.



**Fig. 8.2: (NL, RWS)**  
Detail of the FID chromatogram of the Preem Evolution diesel.

Integration on the time windows of the alkanes indicates the presence of alkanes in a regular pattern including pristane and phytane.

### 8.3 GC-MS with the CENSIM method

The SIM method mentioned in Annex B of the CEN/Tr (CENSIM) is used to analyze the sample on mineral oil components. The chromatogram has been printed to pdf in document **Chromprint CENSIM Evolution Diesel RR2013.pdf** with the macro **censim\_pdf.mac**.

The ion chromatograms show a lot of peaks, but with unusual patterns. Integration of the data file on the compounds analyzed with the CEN/TR reveals that the sesquiterpanes are present, but that the PAH's are absent or strongly reduced. It seems that a light mineral oil product is hydrolyzed to remove the aromatic compounds.

Biomarkers like hopanes and steranes cannot be found. Probably because of the light mineral oil fraction.

### 8.4 GC-MS results with the FATSISC1 method

The FATSISC1 method is used to analyze the sample on vegetable oil and biodiesel components .

The method is based on a combined SIM and full scan analysis with the temperature program of the CENSIM method (used for COSI).

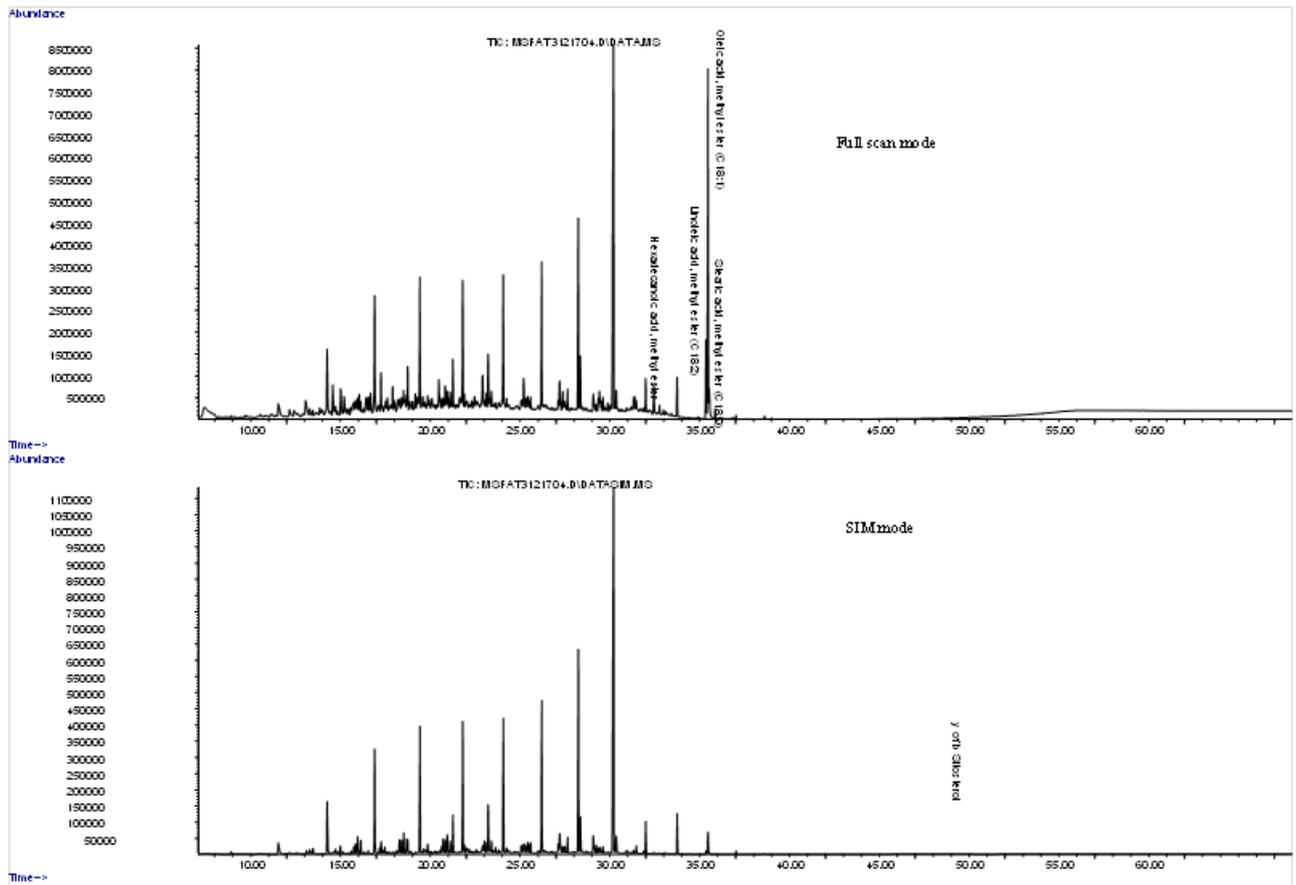
The chromatogram has been printed to pdf in document **Chromprint\_fatsisc\_Evolution diesel RR2013.pdf** with the macro **Fat\_simscan\_pdf.mac**.

The ion chromatograms show fatty acid methyl esters (See Fig 8.3). Abietic acid (to indicate tall oil) and sterols cannot be found at the concentration level injected, besides Sitosterol at noise level. The quantification report is shown as Table 8.1.

Target Compound	Ret time	Q ion	Respons
1) Dodecanoic acid, methylester	0.000	0	N.D.
2) dodecanoic acid	0.000	0	N.D.
3) Tetradecanoic acid, methylester	0.000	0	N.D.
4) Hexadecanoic acid, methylester	30.446	143	745910
5) n-hexadecanoic acid	0.000	0	N.D.
6) Linoleic acid, methylester	33.351	294	556828
7) Oleic acid, methylester	33.461	264	5550892
8) Stearic acid, methylester	33.865	298	204582
9) linoleic acid (C18:2)	0.000	0	N.D.
10) a-linolenic acid (C18:3)	0.000	0	N.D.
11) Oleic acid 18:1	0.000	0	N.D.
12) Octadecanoic acid 18:0	0.000	0	N.D.
13) cryptopinon	0.000	0	N.D.
14) 2-mono linolein	0.000	0	N.D.
15) Butyl 9,12,15-octadecatrienoate	0.000	0	N.D.
16) Dehydroabietic acid	0.000	0	N.D.
17) Butyl 9,12-octadecadienoate	0.000	0	N.D.
18) Abietic acid	0.000	0	N.D.
19) B-Tocopherol	0.000	0	N.D.
20) y-Tocopherol	0.000	0	N.D.

21) Stigmastan-3,5-diene	0.000	0	N.D.
22) cholesterol	0.000	0	N.D.
23) a-Tocopherol (vit e)	0.000	0	N.D.
24) Brassicasterol	0.000	0	N.D.
25) Campesterol	0.000	0	N.D.
26) Stigmasterol	0.000	0	N.D.
27) $\gamma$ of $\beta$ Sitosterol	49.681	414	419
28) Cycloartenol	0.000	0	N.D.

**Table 8.1: (NL, RWS)**  
Quantitation report of the  
FATSISC method.

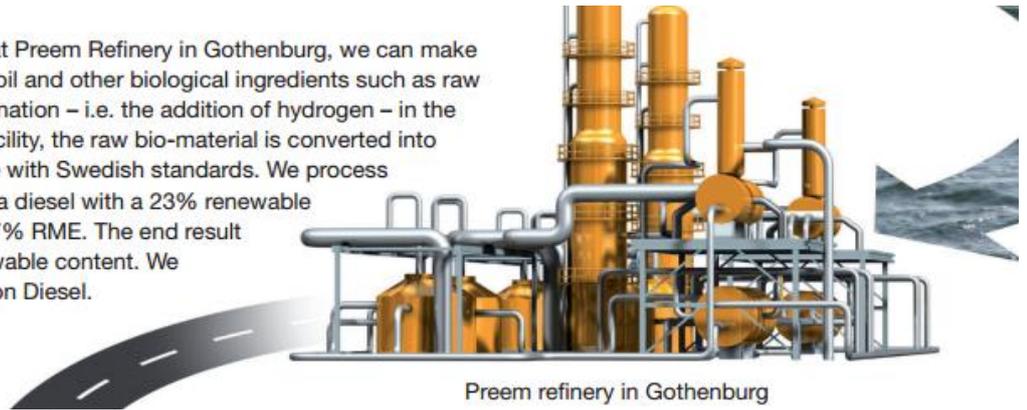


**Fig 8.3: (NL, RWS)**  
Scan and SIM chromatogram of  
the Preem Evolution diesel.

### 8.5 Conclusions

The sample of Preem evolution diesel shows a mixture of a light fraction of a mineral oil together with FAME's and compounds normally found in vegetable oil. Aromatic compounds are absent, indicating that a hydrogenation step is used in the production process. This is in accordance with the description of Evolution diesel:

4. In our biorefinery facility at Preem Refinery in Gothenburg, we can make standard diesel using plant oil and other biological ingredients such as raw tall diesel. Through hydrogenation – i.e. the addition of hydrogen – in the new Green Hydro Treater facility, the raw bio-material is converted into normal diesel in accordance with Swedish standards. We process this with raw oil to produce a diesel with a 23% renewable proportion. We then mix in 7% RME. The end result is a diesel with a 30% renewable content. We call this Preem ACP Evolution Diesel.



## 9 Conclusions

RR2013 turned out to be an interesting case for Bonn-OSINet. The samples were selected by SKL, because the source sample of the ballast water tank was more weathered than the spill sample. The case showed however many details which were detected by different participants. None of the participants has reported all details. Even the organizers hadn't seen them, before they had received the individual reports.

One aspect that has been learned is the recognition of an additional amount of lubrication oil in the ballast water tank sample compare with the spill sample. The hopanes and steranes were increased in %, but the tri aromatic steranes not, because these compounds are not present in lubrication oil. The difference can easily be detected in the MS-PW-plot.

Most participants were able to handle the samples and the analytical results in a correct way leading to judgement scores of above 80%.



## 10 References

- [1] Currie, L.A., Svehla, G.. Nomenclature for the presentation of results of chemical data. *Pure & Appl. Chem.* , Vol 66, No 3, pp 595-608, 1994.
- [2] Joan Albaigés, Núria Jiminez, Altamira Arcos, Carmen Dominguez, Josep Bayona. The use of long-chain alkydbenzenes and alkyltoluenes for fingerprinting marine oil wastes. *Chemosphere* 91 (2013) 336-343.
- [3] Yang, C. Z. Wang. Y. Liu, Z. Yang, Y. Li, K. Shah, G. Zhang. M. Landriault, B. Hollebhone, C. Brown, P. Lambert, Z. Liu and S. Tian (2013). Aromatic Steroids in Crude Oils and Petroleum Products and Their Applications in Forensic Oil Spill Identification. *Environmental Forensics*, 14: 278-293



## Annex A Weathering table

The updated weathering table applied in the MS-PW-plots of the spreadsheet file used for the Bonn-OSINet round robins.

Sensitivity of compounds for weathering effects	Evaporation is in relation with the retention time.			Properties of compounds		
	Markers for weathering effects.					
	high stability	photo oxidation	biodegradation	solubility	aromatic rings	sulfur
<b>Normative compounds</b>						
1) NR-C17			□		0	
2) NR-Pristane	□				0	
3) NR-C18			□		0	
4) NR-phytane	□				0	
5) NR-4-Methyl Dibenzothi...			◆		3	S
6) NR-1-Methyl Dibenzothi...					3	S
7) NR-2-methyl phenanthrene				□	3	
8) NR-1-methyl phenanthrene				◆	3	
9) NR-2-Methylfluoranthene					3	
10) NR-benzo(a)-fluorene		◆			3	
11) NR-retene					3	
12) NR-benzo(b+c)fluorene		◆			3	
13) NR-2-methylpyrene		□			4	
14) NR-4-methylpyrene		□			4	
15) NR-1-methylpyrene		□			4	
16) NR-tetramethyl-phenant...		◆			3	
17) NR-BNT					3	S
18) NR-27dbS	□				0	
19) NR-27dbR	□				0	
20) NR-27bbR+S	□				0	
21) NR-27-TS	▪				0	
22) NR-SC26TA		◆			3	
23) NR-27-TM	▪				0	
24) NR-RC26TA+SC27TA		◆			3	
25) NR-29bbR+S	▪				0	
26) NR-28ab	▪				0	
27) NR-SC28TA		◆			3	
28) NR-29ab	▪				0	
29) NR-RC27TA		◆			3	
30) NR-30O	▪				0	
31) NR-30ab	▪				0	
32) NR-RC28TA		◆			3	
33) NR-31abS	▪				0	
34) NR-30G	▪				0	

Informative compounds				
35) C1-dekalin	▪			0
36) Naphthalene		□		2
37) C1-Naphthalenes		□		2
38) SES1	▪			0
39) C2 benzothiophenes				2
40) C2-Naphthalenes		◆		2
41) SES2	▪			0
42) SES3	▪			0
43) SES4	▪			0
44) SES8	▪			0
45) C1-Fluorenes				2
46) C2-Fluorenes				2
47) Methylantracene		□		3
48) C2-dibenzothiophenes				2
49) C2-highest peak phe. a...				3
50) C2 phenanthrenes anthr...				3
51) C3 dibenzothiophenes				3
52) C3 phenanthrenes anthr...				3
53) C4 phenanthrenes anthr...				3
54) C23 Tr	▪			0
55) C24 Tr	▪			0
56) C2 fluoranthrenes pyrenes		□		3-4
57) C20TA		◆		3
58) C25 Tr	▪			0
59) C21 TA	▪	◆		3
60) C1 chrysenes		◆		4
61) C28 (22S)	▪			0
62) C29 (22S)	▪			0
63) 28bbR+S	▪			0
64) 28aaR	▪			0
65) 29aaS	▪			0
66) 29bbR+S	▪			0
67) 29aaR	▪			0
68) 29Ts	▪			0
69) 30ba	▪			0
70) 32abS	▪			0

**Marker code**

Compounds firstly reduced by the indicated weathering effect = □

Compounds sensitive for the indicated weathering effect = ◆

**Color code**

black: compounds stable up to a high degree of weathering except evaporation

The □ indicated compounds are markers for severe weathering.

green: markers for compounds sensitive for biodegradation.

ochre: sulfur containing compounds

red: markers for compounds sensitive for photo oxidation.

blue: markers for compounds sensitive for dissolution.

violet: remaining compounds not belonging to the above categories.