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Oil Spill Identification
Network of Experts

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RR2011 - The comparison of 7 HFO samples

Sixth intercalibration in the framework of Bonn-OSINET

The results of nineteen international laboratories

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Heavy Fuel Oil on the French coast

RR2011 - The comparison of 7 HFO samples

Sixth intercalibration in the framework of Bonn-OSINET

The results of nineteen international laboratories

Colophon

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Contents

Executive summary

Round Robin 2011 (RR2011) was the sixth world-wide ring test of the expert group on oil spill identification of the Bonn-Agreement (Bonn-OSINET), in which 19 laboratories from 14 countries participated.

Laboratory	**Location**	**Contact**
EPA-CES	Victoria (AU)	Syed Hasnain
NSWDECC	Lidcombe (AU)	Steve Fuller
MUMM	Oostende (BE)	Marijke Neyts
Petrobras	Rio de Janeiro (BR)	Fabiana D. C. Gallotta
EC-ALET	Moncton (CA)	Josee Losier
ESTS	Ottawa (CA)	Chun Yang
ALS	Edmonton (CA)	Deib Birkholz
NCSEMC	Qingdao (CN)	Sun Peiyan
BSH	Hamburg (DE)	Gerhard Dahlmann
EERC	Tallinn (EE)	Krista Mötz
CSIC	Barcelona (ES)	Joan Albaiges
NBI	Helsinki (FI)	Niina Viitala
CEDRE	Brest (FR)	Julien Guyomarch
LASEM	Toulon (FR)	Francois Davids
Total	Harfleur (Fr)	Pierre Giusti
LVA	Riga (LV)	Irina Dzene
RWS-WD	Lelystad (NL)	Paul Kienhuis
Sintef	Trondheim (NO)	Liv-Guri Faksness/ Kjersti Almås
SKL	Linköping (SE)	Helen Turesson/Magnus Kallberg

Table 1
Participants of RR2011

The laboratories received seven heavy fuel oil (HFO) samples related to a large oil spill that occurred in 2001 for the French coast. According to the proposed scenario two of the samples were collected in the year of the spill and four of the samples were collected 10 years after the spill.
It was requested to work (if possible) according to draft version 51 of CEN/Tr 15522-2, published in September 2011 on the BonnOSInet - OSPAR web-server. It is the same version that has been submitted to CEN as an update for CEN/Tr 15522-2 (2006). A technical report should be returned and two spreadsheet files, which were provided to the participants, should be filled with the measured data. The spreadsheet files should / could already be used to evaluate the analytical results by means of ratio comparisons and MS-PW-plots.

The reason to select these samples is to test whether the last update of the CEN/Tr is suitable to use for oil spill identification of very weathered oil samples.
In the real scenario spill samples 1 and 2 were prepared from artificially-weathered oil from the source sample. Spill samples 3 and 4 were collected on a contaminated beach. Spill sample 5 was also from a beach but on a place that was related to a different spill. Spill 6 was prepared from an artificial weathered different HFO.

On request of several participants Paul Kienhuis and Gerhard Dahlmann judged the reports of the participants this year for the first time. Points were given to aspects like analytical quality of the data, assessment of the case and conclusions of oil type and match conclusions. The final judgement was calculated as a percentage of the maximum number of points that could be achieved.

In the summary report of RR2011, 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 1.

Lab code	Spill 1	Spill 2	Spill 3	Spill 4	Spill 5	Spill 6
Scenario	M	M	PM	PM	NM	NM
Lab1	M	**NM**	I	I	no results	no results
Lab2	M	M	PM/I	PM/I	I	I
Lab3	M	M	**NM**	PM	I	I
Lab4	M	PM	PM	PM	NM	NM
Lab5	M	M	NM / PM	NM / PM	NM	NM
Lab6	M	PM	PM	PM	**PM**	**PM**
Lab7	M	PM	**NM**	I	NM	NM
Lab8	M	M	I	PM	NM	NM
Lab9	M	M	**NM**	**NM**	NM	NM
Lab10	M	M	I	I	I	I
Lab11	PM	PM	PM	**NM**	NM	I
Lab12	PM	PM	I	PM	I	I
Lab13	M	**NM**	**NM**	**NM**	NM	NM
Lab14	PM	**NM**	**NM**	**NM**	NM	NM
Lab15	M	M	PM	PM	NM	NM
Lab16	M	**NM**	I	I	I	I
Lab17	M	M	PM	PM	NM	NM
Lab18	no results	no results	no results	no results	no results	no results
Lab19	PM	PM	**NM**	PM	NM	NM

Table 2
Match conclusions of the labs for the spill samples in relation with the provided source sample:
M = match;
PM=probable match;
I = inconclusive;
NM = non-match.
Differences of "more than one" match conclusion apart from the true conclusion are indicated in **bold**

In this Round Robin all participants had problems to evaluate the severely weathered spill samples. Most labs have given a clear conclusion for the artificial weathered spill samples 1, 2 and 6, but were much more unsure regard to the real spill samples. Insufficient knowledge about the fate of oil in severely weathered oil samples was given as a reason. Several participants indicated that in a real case one would take much more samples and follow the process of weathering much closer.
For a Round Robin this is however not possible.

For the judgement of the reports two methods were used. Gerhard and Paul made both an own judgement method and evaluated the reports each with both methods.

The results of the judgement of the reports are given in Table 2.

Lab code	Method Paul	Method Gerhard	mean
Lab1	81	79	80
Lab2	100	100	100
Lab3	88	83	85
Lab4	100	100	100
Lab5	38	39	38
Lab6	56	52	54
Lab7	94	97	95
Lab8	100	100	100
Lab9	69	76	72
Lab10	88	82	85
Lab11	88	88	88
Lab12	75	82	78
Lab13	75	73	74
Lab14	81	74	78
Lab15	81	77	79
Lab16	81	88	85
Lab17	100	100	100
Lab18			
Lab19	94	97	95

Table 3

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

After combining the results it can be concluded that the differences between the two methods are small.

No points were given to the contribution of Lab 18. This lab participated for the first time and had problems with entering the analytical data into the provided spreadsheet files. Therefore no judgement was given.

Table 3 shows that many labs were able to write a good report, but also that some labs have problems. This is often related to the fact, that they have a very limited number of actual cases and are therefore less skilled in oil spill identification.

With regard to the test of the CEN-method, RR2011 was a very special and problematic case. The participants got only a very small insight in post spill investigations, and it has to be discussed, whether information of this kind can or should be included in a next update of the method.

1 Introduction

At the OSINET meeting in Barcelona 2011, Pierre Giusti and Julien Guyomarch gave a short presentation about their investigations in the Erika-case, which happened at the end of 2001, and which led to a massive oil pollution at the French coast. Even 11 years after this accident, pollution is still present at some places. Identification of the oil is difficult because this oil is extremely heavily weathered. Pierre and Julien stated that the CEN-guideline was used, but this method seemed not to be appropriate here: the compounds to calculate the DRs were often so heavily weathered that barely ratios were left for comparison.
Other ratios were mentioned, which mainly are used in geochemical investigations, and which obviously were more stable over the long time of weathering. It was proposed to improve the DRs of the CEN-guideline with these ratios.

To get experience with this kind of samples and to test the new ratios, it was decided that Pierre and Julien select relevant samples and arrange a scenario for the round robin of 2011. They have also built a spreadsheet file that contains the intended ratios and that should be used by the participants as an additional tool for sample comparison.

2 Instructions

On 14-9-11 the instructions for RR2011 were published on the Bonn-OSINET forum:

Subject: Oil Spill Identification Round Robin 2011
From: Julien Guyomarch, Ronan Jezequel, Paul Kienhuis and Gerhard Dahlmann

Dear Colleagues,

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

For the sixth oil spill identification intercalibration round within the Bonn-OSINET expert group you have received 7 samples.

You may regard 7 samples as too many for an inter-calibration as normally only up to 5 samples were sent. But we want to make this RR more interesting. Two of these samples were sent for special purposes (see below), and may be analyzed or not depending on your time and interest. But please notice: „real“ and rare samples are included!

Scenario and samples information:

In 2001, a tanker ran into a heavy storm and broke in two and sank, releasing thousands of tons of oil into the sea. The accident occurred 200 miles off the coast of Brittany, and thousands of kilometres of the shoreline were impacted. Few days after the accident, a sample representative of the cargo was received and was considered as the reference oil (**source 1**). In the next few months, many samples were collected to ensure the origin of the oil when cleaning beaches and rocks covered with oil. Ten years later, several samples were collected for analyses. Reference of samples collected either in 2001 or in 2011 is as follows:

2001	Reference oil (source 1)
2001 2001	Oil sampled on a rock (spill 1) Oil from a contaminated sediment (spill 2)
2011, Tregana Beach	Oil from a contaminated sediment (spill 3), Oil sampled on a rock (spill 4)
2011, Portez Beach	Oil sampled on a rock (spill 5), Oil from a contaminated sediment (spill 6),

Table 2.1
RR2011 sample composition

To be able to send all participants the same samples, the oil spill samples have been dissolved in DCM at a concentration of 100 mg/ml.

Work to be performed, additional samples and reporting

Based on the decisions taken at the meeting in Barcelona the final report of the Round Robin will be written anonymously from now on, and the contributions of the participants will be judged on quality. Several participants want / need this for their accreditation.

This means that you should follow version 51 of the CEN/Tr 2v2 (published on the BonnOSInet OSPAR server on 22-03-2011) and that we look in how far you have done that and give "points" for the presented results.

Since of course also your analytical precision will be a part of this judgement, you can easily improve your " ranking" by double measurements and by filling in Paul's spreadsheet (see below). Beside that all the means for coming to a conclusion are automatically produced here, you will see directly, whether your analytical precision is good enough in case of double measurements. If limits are exceeded, the CEN/TR gives advice on what to do.

Some of you need to follow an own protocol that is different from the CEN/Tr.

For our information and "judgement" please inform us in your internal documentation report whether you have analysed the samples conform draft version 51 of the CEN/Tr or not.

The round robin of this year is divided into two parts:

Part 1 deals with the comparison of the source sample with the spill samples 1 to 4 according to CEN/Tr 2v2. To be able to judge the analytical part we like to ask you to send us your results entered in Excel spreadsheet version 43 published on the BonnOSInet OSPAR server on 18-4-2011. According to the CEN/Tr you only need to enter the product related normative ratios mentioned in Table 3, but you are free to use additional informative compounds/ratios.

The results should be reported according to the internal documentation mentioned in the CEN/Tr in section 7.2 and annex I.

Part 2 is optional. We like to invite you to discuss the handling of heavily degraded oil samples a little bit further here. For that reason, two additional samples are included: spill 5 and spill 6, which should also be analyzed according to the CEN/TR. But in addition a second spreadsheet file (Table2_RR2011.xls) built by TOTAL (Pierre Giusti) and Cedre is provided here with specific ratios to be studied.

Here, Biomarker-ratios are produced mainly according to their geochemical meaning, and it seems to be interesting to compare those ratios with the ratios produced according to the CEN/TR.

We like to ask you to enter the results of all samples for the compounds mentioned on the sheet **Ratio** in the yellow part of the sheet. The values shown in the yellow part can be removed. They have been entered to test the sheet.

The remaining tables and the graphs on the sheet **Figures** are based on the data entered in the yellow part. The sheet **Chromato** shows chromatograms with the peaks intended.
All the analyses will have to be run in duplicates.
The results of the comparisons can directly be seen in the column diagrams.
Are these “geochemical” ratios better suitable in case of heavily degraded samples?
Or are they even better suitable in all cases?

Time schedule

19-09-11:	The samples have been sent out.
Before 1st February 2012:	Reports have to be returned.
March:	Final report will be sent to the participants.
Meeting:	24-26 April 2011 in France.

On request of some of the participants the final date to send the reports has been moved from the 1st of November to the 1st of February. When you have finished your results earlier, don’t hesitate to send us the results. It gives us more time for the evaluation.
In previous round robins we were quite liberal in accepting reports that were sent in too late. But we cannot do that with a final date of the 1st of February, so it is a real deadline.

The results should be sent by email to:
Julien.Guyomarch, Ronan.Jezequel; Gerhard.Dahlmann and Paul.Kienhuis.

Yours sincerely,

Julien Guyomarch & Ronan Jezequel, CEDRE.
Pierre Giusti, TOTAL
Paul Kienhuis, RWS-WD
Gerhard Dahlmann, BSH

3 Real scenario

Table 3.1 shows the real scenario of the samples of RR2011.

Sample	Sample name	RR2011 description	Sample nature
1	source	Reference oil	HFO #1 reference oil
2	Spill 1	Oil sediment	HFO #1 biodegraded in the laboratory for 2 months
3	Spill 2	Oil rock	HFO #1 exposed outside for 3 months (5 grams on a 20x20 cm granite tile)
4	Spill 3	Tregana sediment	HFO #1 sampled in the Portez site in 2011 (in the upper part of the beach, in the middle of vegetation)
5	Spill 4	Tregana rock	HFO #1 sampled in the Portez site in 2011 (on rocky shore exposed to tides)
6	Spill 5	Portez rock	HFO #2 (or HFO #1??) sampled in the Kerliguit site in 2011 (on rocky shore exposed to tides)
7	Spill 6	Portez sediment	HFO #3 biodegraded in the laboratory for 2 month

Table 3.1
Sample information

Spill 1 and 2 are artificial weathered aliquots of the source oil. Spill 1 is affected by biodegradation, while spill 2 is influenced by all kinds of weathering effects that can happen with a thin layer of oil on a rock.

Assuming that the specific gravity of an evaporated HFO is close to 1, the volume of oil of spill 2 was 5 ml. Spread over an area of 20 by 20 cm it results in a layer thickness of 0.125 mm. So some evaporation and photo-oxidation effects can be expected.

Unfortunately, a mistake has been made in the scenario information of the instructions. It was stated that spill 1 was from a rock and spill 2 from sediment. This may have led to wrong interpretations of single details. But even if the description was correct, the history, layer thickness, sampling method of each sample was not known. So, the influence of this error might have been limited.

4 Evaluation of the case following CEN/TR 15522-2v2 v51.

4.1 Preliminary remark

RR2011 was the most difficult inter-calibration we had so far. Generally, the question arose: how should an analyst decide, whether differences even among the most stable biomarkers are caused by weathering over ten years or are true differences? This is not possible by means of the few samples, included in this Round Robin. As indicated also by several participants, an investigation like this requires a much higher number of samples, taken continuously over the time of ten years. Only then, sound conclusions may be possible: depending on the specific environmental conditions (e.g. oil on rocks or oil in sediments) a homogeneous distribution cannot be expected as different parts of the spill might alter differently. Thus, the general trend has to be worked out over the years, and those inhomogeneous distributions have to be taken into account.
CEDRE has indeed monitored the spill over more than ten years, and from the big number of analyzed spill samples, only very few were chosen for this Round Robin.

It is indeed interesting to be confronted with highest degrees of weathering of oil spills.
But with regard to the very limited number of samples, which can be used in our Round Robins, „the scenario hinders a robust conclusion and it is a matter of definition (choice?) to conclude a "probable match" or a "non-match".
Therefore, in this exercise, the way to come to a conclusion must be regarded as much more important than the conclusion itself. This is especially true because even the conclusion of a "match" between source and spill samples might have only a very limited value in a court proceeding, because of many other possibly matching sources.

Diagrams from the individual reports, which best showed the single steps of the CEN/TR, were combined in this chapter, together with corresponding interpretations.

In Round Robins like this, a "story" has to be invented because participants should not know the results, i.e. the actual origin of the samples, from the beginning. On the other hand, the truth must be known because participants must know, whether they were right or not with regard to their conclusions.
The story as given here in the "Instructions" seems to be suitable: the two samples described as "taken one year after the accident", i.e. spill 1 and spill 2, were actually samples taken from the spill, but they were artificially weathered. The right conclusion is thus a match with the source sample 1. The next two samples described as "taken 10 years after the accident", where indeed taken after 10 years, and they indeed originate from source 1. This was ensured by CEDRE by continuous sample taking on the same location over this long time. Spill 6 originates from another accident, where also long time investigations have been carried out. Merely the origin of spill 5 is not clear, and CEDRE has used the opportunity of this Round Robin to get the opinions about this sample from different laboratories from all over the world.

4.2 GC-FID

Source 1 is easily identified as a typical HFO by its boiling range and especially the presence of high amounts of aromatics from cracking processes.

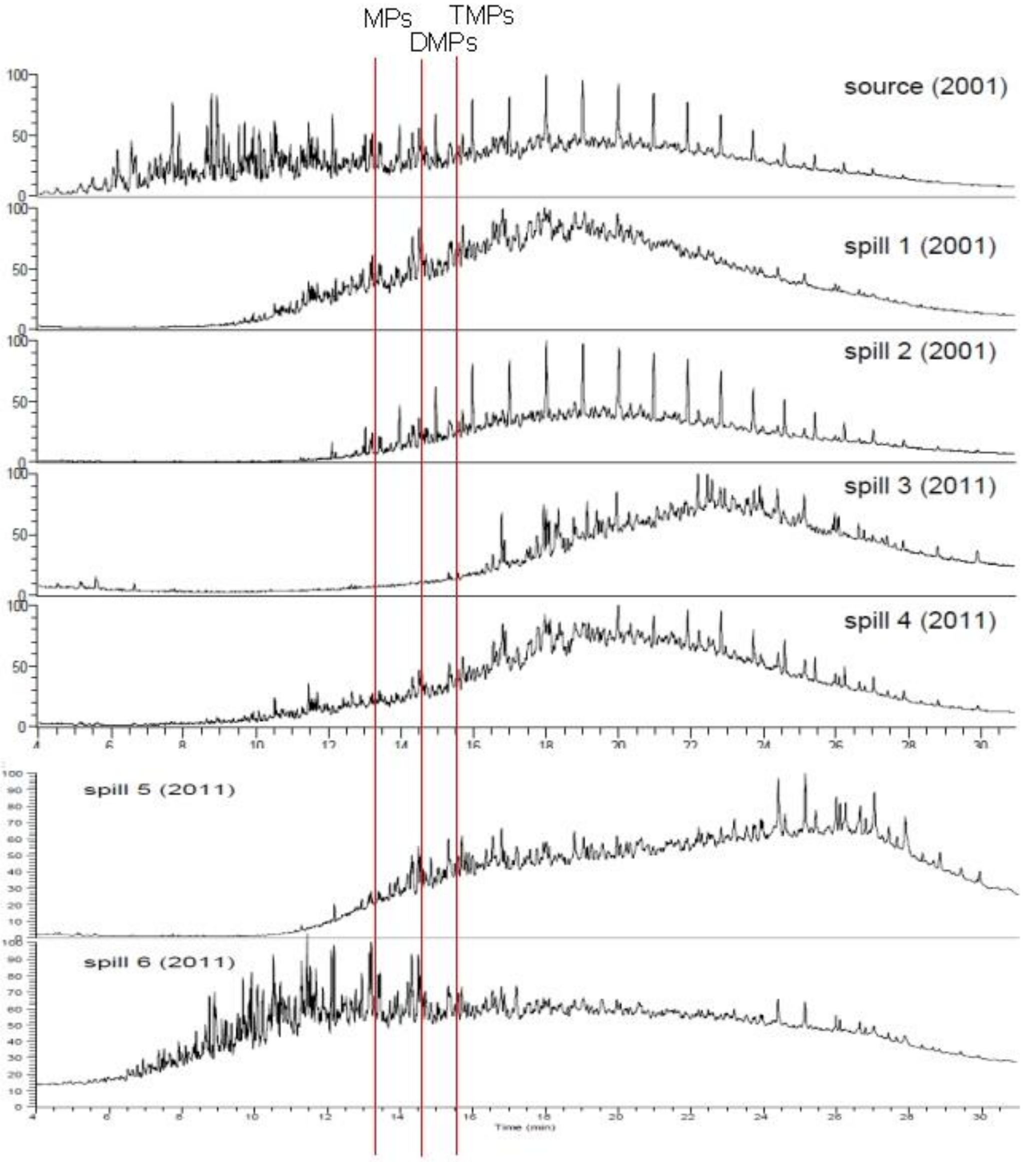


Fig 4.1 (ES, CSIC): Gas-chromatograms of all samples. Added red lines: methylated phenantrenes

Although all spill samples are weathered, partly highly, remaining aromatics, e.g. methylated phenantrenes, can still be found in all samples in relatively high concentrations, except in spill 3, which is the most weathered sample.
Whereas the UCM's of spill 1 to spill 4 are similar to source 1, and merely a shift of the apex of the hump to higher retention times in samples 3 and 4 can be observed, the UCM's of samples 5 and 6 are different. They show a bimodal distribution.

Overlays of the chromatograms show that spill 5 and especially spill 6 very probably do not originate from source 1. In contrast to spill 1 to spill 4, "something must have been added" in the mid boiling region in spill 5 and especially spill 6, if it is assumed that these samples originate from source 1.

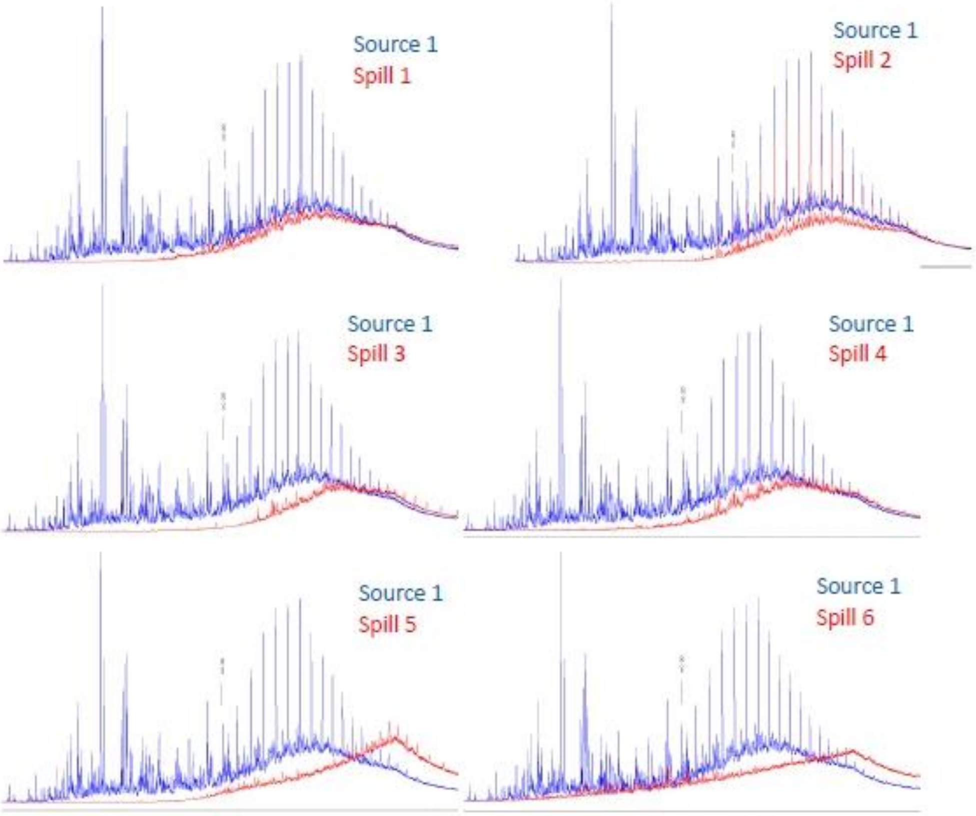


Fig 4.2 (NO, Sintef) Overlays of the chromatograms of the spill samples with source 1

The absence of n-alkanes in the spill samples indicates high bacterial degradation, except in sample 2, where the main weathering process might have been evaporation. Thus, a GC-PW plot makes sense only in this latter case.

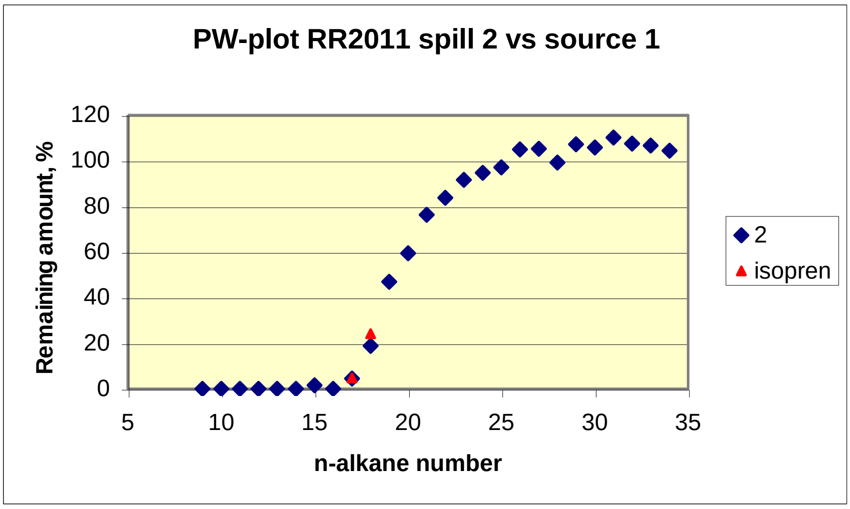


Fig 4.3 (SE, SKL)
GC-PW plot spill 2 versus source 1

The GC-PW plot of spill 2 versus source 1 points to a possible match: a typical S-shaped evaporation curve can be found. Pristane and phytane are in line with this curve. There is thus no indication of biodegradation.

4.3 GC-MS

4.3.1 First visual inspection

All samples consist of HFO: They show the typical mass-chromatograms of HFO including the typical pattern of the methylphenanthrenes and the absence of retene. Methylanthracene is present in the typical higher concentration except for spill 3 and 6, which can have been caused by weathering here.
Besides retene, 30O is not present, except may be in spill 6 in very low amount. SC26TA shows a too low S/N-ratio, except in spill 6 and spill 7.

4.3.2 Spill 1- source 1

As found already by GC-FID, also the MS-PW-plot of Fig. 4 shows that sample Spill 1 was affected by weathering when compared to sample Source 1. The complex pattern combines the effects of evaporation, biodegradation, and dissolution. The presence of pristane and phytane and the absence of C17 and C18 and of all further n-alkanes indicates that biodegradation has occurred.
The C1-fluoranthenes/pyrenes/benzofluorenes (m/z 216) pattern has been affected by dissolution and/or biodegradation. In this case, the compounds 2-methylfluoranthene and the two benzofluorenes were more reduced than the methyl pyrenes.
The compounds eluting after a retention time of 42 min are above 85% and have not been affected by weathering. These include both normative and informative biomarkers. The similar relative concentration of biomarkers is an indication of same source.

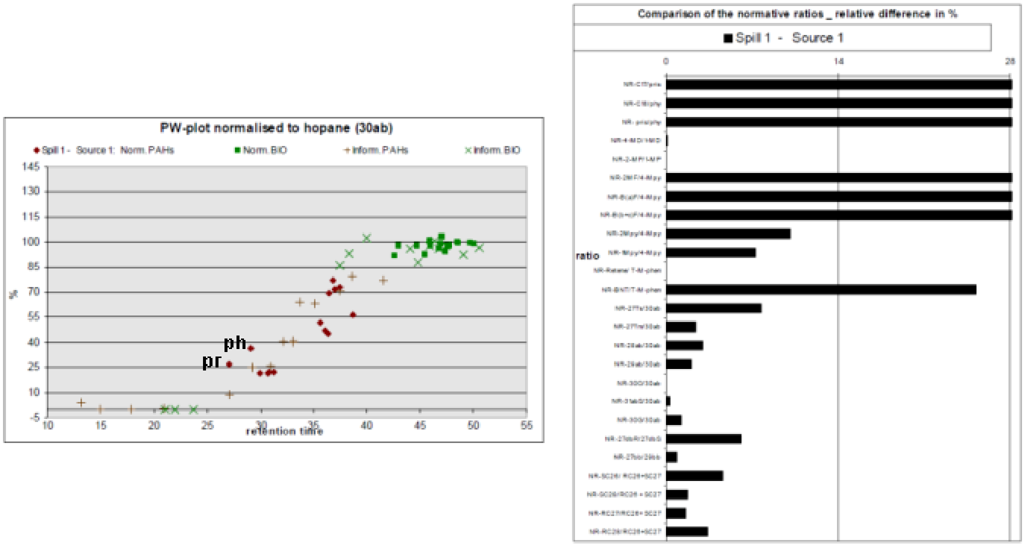


Fig 4.4
MS-PW-plot and DR comparison graph spill 1 - source 1.

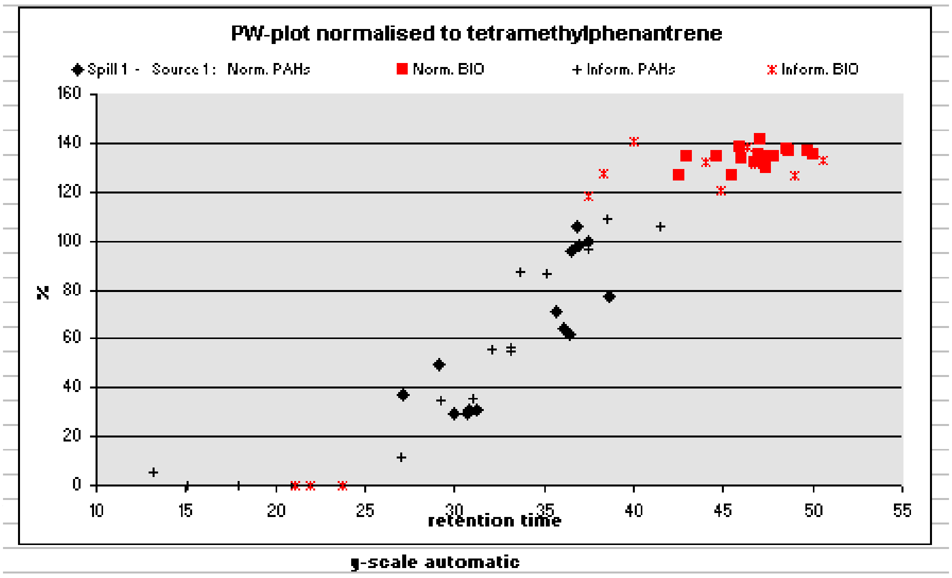


Fig 4.5
MS-PW-plot spill 1 - source 1, based on TMPhen.

As TMPhen is also reduced in spill 1 by dissolution and/or biodegradation, the stable biomarkers are shifted to the 130-140% line, when the PW-plot is based on TMPhen (Fig. 5).

A final visual inspection of all mass-chromatograms shows a high similarity between source 1 and spill 1.

Conclusion:
There is no significant difference between **spill 1** and **source 1**: **positive match**.

4.3.3 Spill 2 – source 1

As already was found in GC-FID and in the GC-PW-plot, spill 2 shows high evaporative loss of compounds up to about 40 minutes. But n-alkanes are still present, and the GC-PW plot shows a match situation (Fig. 3).

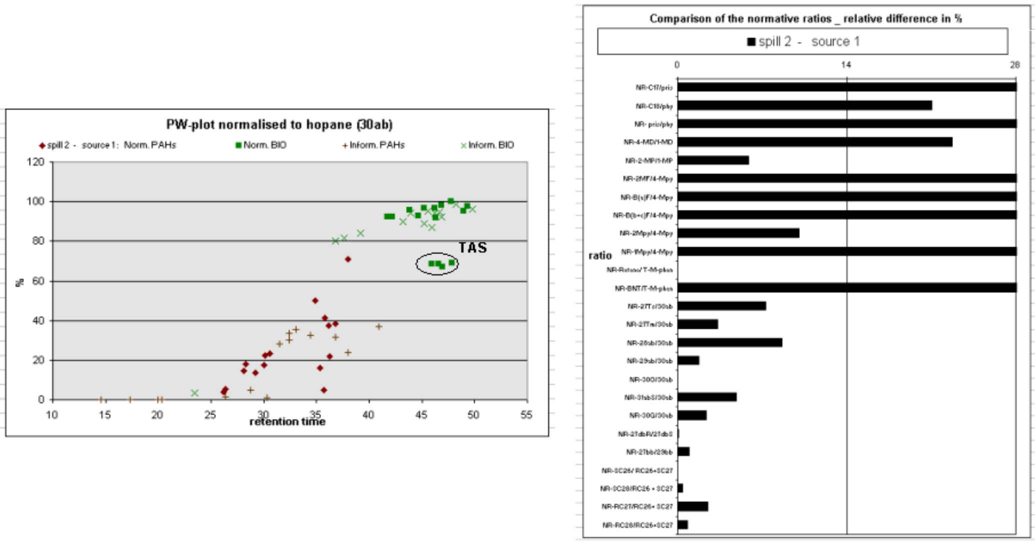


Fig 4.6

MS-PW-plot and DR comparison graph spill 2 - source 1.

The C1-fluoranthenes/pyrenes/benzofluorenes (m/z 216) show a strong reduction in intensity. For C1-pyrenes, the degradation pattern was 1-MPy (PW: 23%) > 4-MPy (PW: 40%) >2-MPy (PW: 44%). Benzo(b+c)fluorene and benzo(a)fluorene have been remarkably reduced. These effects must especially be attributed to photo oxidation.

But the most remarkable feature of spill 2 is the reduction of the triaromatic steranes to about 70%.
TAS are among the compounds most resistant to biodegradation.
But biodegradation has hardly occurred in spill 2 because high n-alkanes are still present, and pristane and phytane are in line with the S-shaped evaporation curve of the n-alkanes.

As belonging to the PAHs, TAS might have been affected in spill 2 in the same way as the other aromatics, i.e. by dissolution and especially by photo oxidation. This is in accordance with the strong effect of photo oxidation on the compounds of m/z 216 in this sample (see above).
It is worth to notice here that the TAS are effected nearly equally. This means that the differences of the DRs produced from the TAS are very well below the repeatability limit of 14%, when spill 2 is compared to source 1.

A final visual inspection of all mass-chromatograms shows a very high similarity between spill 2 and source 1.

Taking all this information into account, it can be concluded:

Conclusion:
There is no significant difference between **spill 2** and **source 1**: **positive match**.

4.3.4 Spill 3 and spill 4 – source 1

It is not astonishing that highest weathering effects occur in 10 year old samples, and the interpretation of the analytical results would be much easier, if samples taken continuously over this long time were present. In this Round Robin, we can only compare the samples we have. But it might be a good idea also to compare the spill samples among each other.

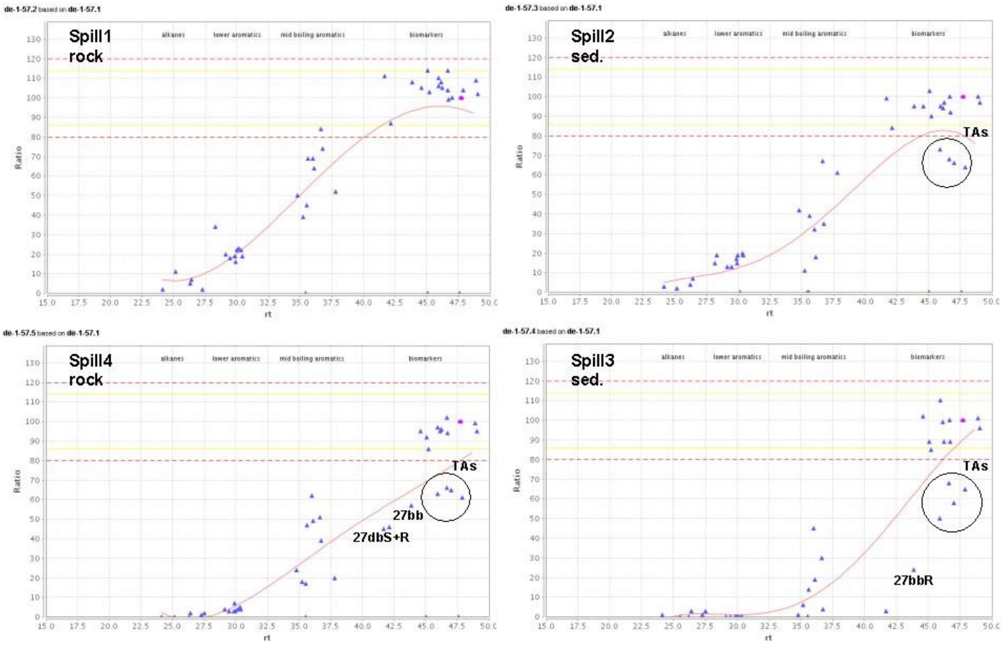


Fig 4.7 (DE, BSH)
MS-PW-plots spills 1 to 4 based on source 1

As found already in the comparison of the GCs (Fig. 1 and 2) the MS-PW-plots show increased weathering in spills 3 and 4. With regard to these weathering effects, discussed already, when spills 1 and 2 are compared to source 1, the following range can be observed:
spill 1 < spill 2 < spill 4 < spill 3. These include evaporation and biodegradation in spill 1, evaporation in spill 2, and the reduction of the aromatics by dissolution and photo oxidation.

The same reduction of the TAS as in spill 2 can be observed in spills 4 and 3, whereas these compounds are more spread in spill 3. Spill 3 is the most weathered sample, and if a match is assumed with source 1, this can only be explained by additional biodegradation. Correspondingly, the differences of the DRs produced from these TAS are above the repeatability limit, when spill 3 is compared to source 1.

Fig 4.8

DR comparison graphs spill 3 and 4 - source 1

But in addition, 27dbS and 27dbR (m/z 217) are reduced to about 45% in spill 4. These compounds are vanished in spill 3. Also 27bb (m/z 218) is reduced in spill 4 to about 55%. The doublet peak of 27bb (R+S) is vanished in spill 3, and 27bbR remained partly.

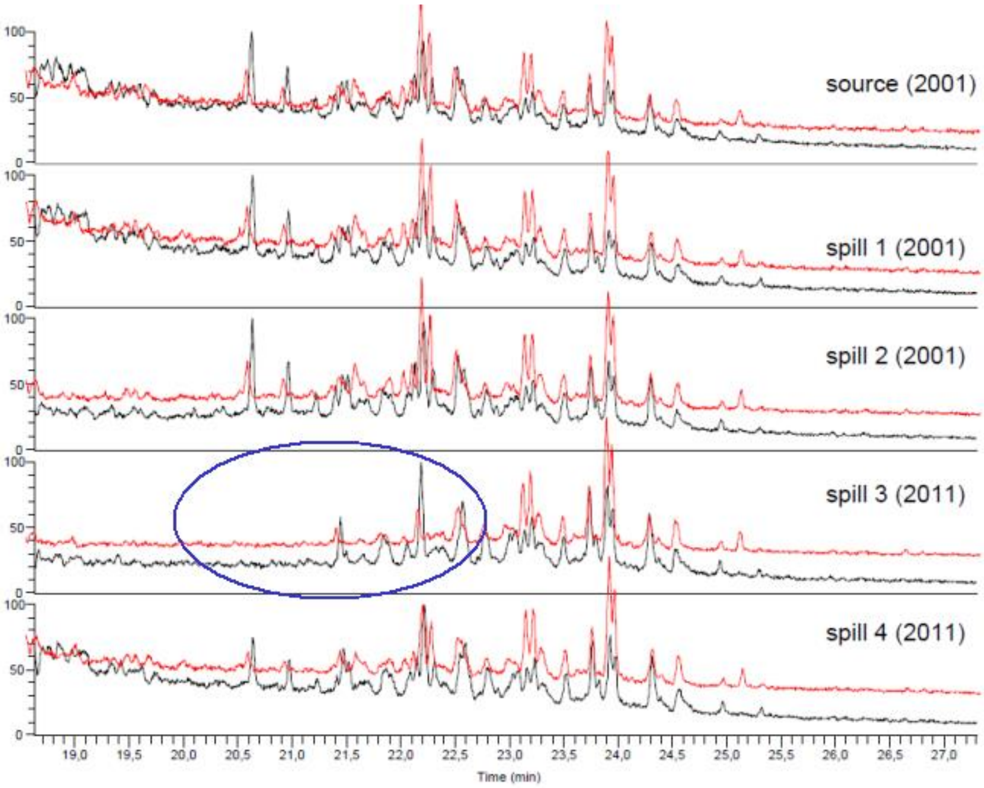


Fig 4.9 (ES, CSIC)

M/z 217 and m/z 218 of source 1 and spills 1 to 4

Beside those differences, the visual inspection of the mass-chromatograms still shows a high similarity between source 1 and spills 4 and 3.

Some differences are the same as found between spills 1, 2 and source 1 and could be explained here uniquely. The fact that there is a chain from source 1 over spill 1 and 2 and especially spill 4 to spill 3 makes it highly probable that spill 4 and also spill 3 originate from source 1.

Conclusion:
Spill 3 and source 1: **probable match**
Spill 4 and source 1: **probable match**

4.3.5 Spill 5 and spill 6 – source 1

As already observed in the GCs, spills 5 and 6 can hardly match with source 1 (Figures 1 and 2). The UCM's are different, and they show a bimodal distribution –or something must have been added in the mid- or even lower boiling region.

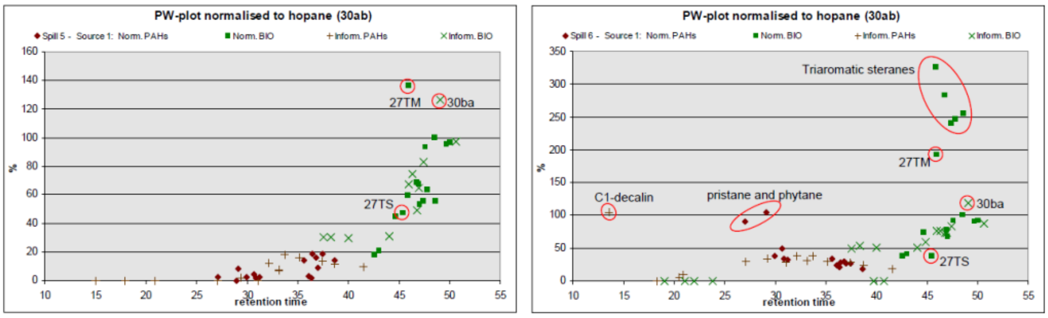


Fig 4.10 (BR, Petrobras)
MS-PW-plots spills 5 and 6

This is confirmed by the MS-PW-plots: many compounds have different relative concentrations when spills 5 and 6 are compared with Source 1, especially 27Ts, 27Tm and 30ba. 27Ts and 27Tm are generally not affected by even severe biodegradation. The differences within m/z 191 cannot be explained by any external factors such as weathering, contamination or heterogeneity.

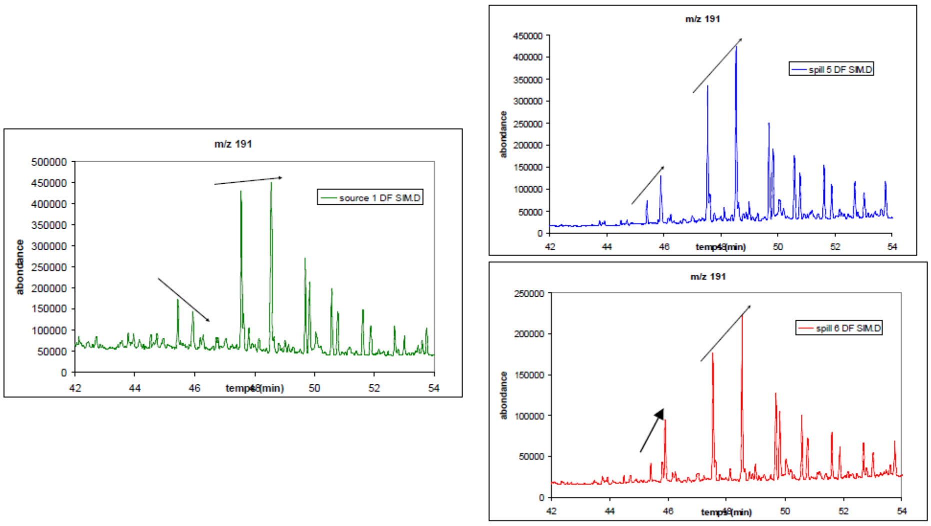


Fig 4.11 (FR, LASEM)
Differences in hopanes, source 1 compared to spills 5 and 6

Conclusion:
Spill 5 and source 1: **non match**
Spill 6 and source 1: **non match**

5 Common aspects of the reports

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

5.1 Sample treatment

In the CEN/Tr section 5.4.1 it is strongly advised to clean “black” samples over silica or florisil before analyses. From the scenario it can be concluded that the samples contain a high boiling fraction that is resistant against weathering for more than 10 years. The clean-up is used to remove the high amount of asphaltenes and/ or particles that do not leave the column and influence the column performance.

Table 5.1 shows that not all participants have cleaned the samples before injection.

Lab code	Clean-up	injection concentration mg/ml	injection volume µl
Lab1	no	10	1
Lab2	no	5	1
Lab3	no	2 FID_20 MS	n.a.
Lab4	yes	1	n.a.
Lab5	n.a.	n.a.	n.a.
Lab6	n.a.	5	1
Lab7	no	5 or 10	n.a.
Lab8	yes	5	1
Lab9	yes	n.a.	n.a.
Lab10	no	20	n.a.
Lab11	yes	20	1
Lab12	no	10	1
Lab13	n.a.	n.a.	n.a.
Lab14	no	2.5	1
Lab15	n.a.	n.a.	n.a.
Lab16	yes	5	1
Lab17	yes	5	3
Lab18	n.a.	n.a.	n.a.
Lab19	yes	3	1

Table 5.1
Sample clean-up and injection volume and concentration.
n.a. = not available.

A “yes” for the clean-up has been indicated 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 clean-up step. Some reports did not describe the sample treatment at all. The information could also not been found in the spreadsheet file. This is indicated with n.a.

The injection concentration varies from 1 to 20 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.

Specific for the heavily weathered samples of the ring test is that the alkanes are not present. For such samples it is advised to inject such an amount that the peak height of the hopanes is at the level of the hopanes in the source sample.

5.2 Variance of the duplicate analyses of the source sample

Table 5.2 shows the variance of the duplicate analyses of the source sample. The data are retrieved from two tables in the spreadsheet file v43 available on the BonnOSInet web server.
The MS-PW-plot variance can be found in cell AE82 of each comparison sheet. This table has 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 comparison 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 and informative ratios. So Table 5.2 shows also how many of the ratios have been used by each lab.

Lab code	MS-PW-plot Normalized to hopane		Number and st. dev. of the paired ratios					
			Normative ratios		Informative ratios		all ratios	
	mean	St. dev.	Number	St dev	Number	St dev	Number	St dev
Lab1	105	5.3	24	**5.5**	12	3.8	36	**5.0**
Lab2	97	2.7	17	2.7	6	0.9	23	2.3
Lab3	107	**7.4**	20	3.1	11	2.9	31	3.0
Lab4	104	3.4	23	3.2	6	3.4	29	3.2
Lab5	121	**19.0**	24	3.1			24	3.1
Lab6	101	**9.5**	22	**5.8**	5	3.3	27	**5.4**
Lab7	96	4.0	22	3.4	4	2.1	26	3.3
Lab8	96	4.0	22	**4.7**	6	2.9	28	4.3
Lab9	95	**7.3**	8	3.1	3	1.2	11	2.7
Lab10	108	**14.3**	24	4.2			24	4.2
Lab11	103	4.6	24	3.9	12	2.8	36	3.6
Lab12	106	**11.6**	24	**5.6**	6	2.9	30	**5.2**
Lab13	109	**6.5**	24	4.0	12	3.4	36	3.8
Lab14	101	4.8	25	3.1	12	4.4	37	3.6
Lab15	100	**12.1**	25	**6.3**			25	**6.3**
Lab16	99	4.3	21	3.3			21	3.3
Lab17	101	5.5	22	3.5			22	3.5
Lab18	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Lab19	93	3.0	22	3.2	6	4.1	28	3.4

Table 5.2
Variance of the MS-PW-plot and the combined ratios by means of a paired calculation of the st. dev of the ratios.
Pw-plot >7 in **bold**
Ratios > 4.5 in **bold**

In the summary report of RR2009 it was concluded that a reasonably value for the st. dev of the MS-PW-plot would be 7 to 8%. Table 5.2 shows that most of the participants were able to stay below that value.

The highest value was found for lab 5, while the st. dev of their ratio comparison is 3.1, much lower than the accepted limit of 5%. The higher value is related to a “drifting” of the data points of the MS-PW-plot.

Lab 5	MS-PW-plot		Number and st. dev of the paired ratios					
	Normalized to hopane		Normative		Informative		all ratios	
	mean	St. dev	Number	St dev	Number	St dev	Number	St dev
source	121	19.0	24	3.1				
spill 1	99	2.7	24	2.2				
spill 2	124	15.0	24	4.1				

Table 5.3
St dev of the MS-PW-plot and ratio comparison of Lab 5 for also spill 1 and spill 2

Table 5.3.shows that the results for spill 1 are much better but for spill 2 again very high for the MS-PW-plot. The reason is unknown.

Lab 10 has also a higher variance for the MS-PW-plot. Their report indicated that the duplicate sequence was analyzed one week later, which has probably caused the high variance of the PW plot. The variance of the ratios however is less sensible for the difference of one week in analysis.

The duplicate analyses of some to all of the samples is used to check the quality of the data and also to eliminate ratios with a higher variance (see sections 6.4.3 and 6.5.6.3 of the CEN/Tr). It is strongly advised to perform these steps to check the data quality and to be able to eliminate ratios with a higher variance.

5.3 Oil type recognition.

Table 5.4 shows the oil type indication found in the reports.

	source	spill 1	spill 2	spill 3	spill 4	spill 5	spill 6
Lab1	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Lab2	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab3	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Lab4	HFO	HFO	HFO	HFO	HFO	HFO/crude	HFO
Lab5	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Lab6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Lab7	HFO	HFO	HFO	HFO	HFO	HFO/crude	HFO
Lab8	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab9	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.
Lab10	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Lab11	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab12	HFO	HFO	HFO	n.m.	HFO	n.m.	n.m.
Lab13	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab14	HFO	HFO	HFO	HFO	HFO	n.m.	n.m.
Lab15	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab16	HFO	HFO	HFO/crude	n.p.	HFO	HFO/crude	HFO
Lab17	HFO	HFO	HFO	HFO	HFO	HFO	HFO
Lab18	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Lab19	HFO	HFO	HFO	HFO	HFO	HFO/crude	HFO

Table 5.4
Oil type recognition:
n.m. = not mentioned
n.p. = not possible
n.r. = no report received

Most of the participants reported that the samples contained heavy fuel oil (HFO). Lab 9 reported that an indication was not possible because of the severe weathering. Several labs reported the oil type only for those samples that were not too weathered (indicated with n.p. = not possible) or indicated some samples with an HFO/crude because the C1-phenantrenes pattern was changed too much.

Some labs just started with the sample comparison without mentioning the oil type. This is indicated with n.m. (not mentioned)

Lasem has analyzed the samples with High Temperature GC-FID. In the summary report of RR2010 is already discussed that the HT-GC-FID analysis can be used to recognize different fractions in an oil mixture.

HT-GC-FID method information of Lab 12:

Column SIMDIST Varian CP7542 CB 10mx0,53mm df 0,17µm

Helium Flow : 18ml/min

Injection splitless on column 0,5µL

Oven program : 40°C(0,5min) - 5°/min 150°(0min) – 10°C/min 300°C (0min) – 15°C/min 420°C (1min).

The results are shown in Fig 5.1 and Fig. 5.2, representing Fig 5 and 6 of the report of Lasem.

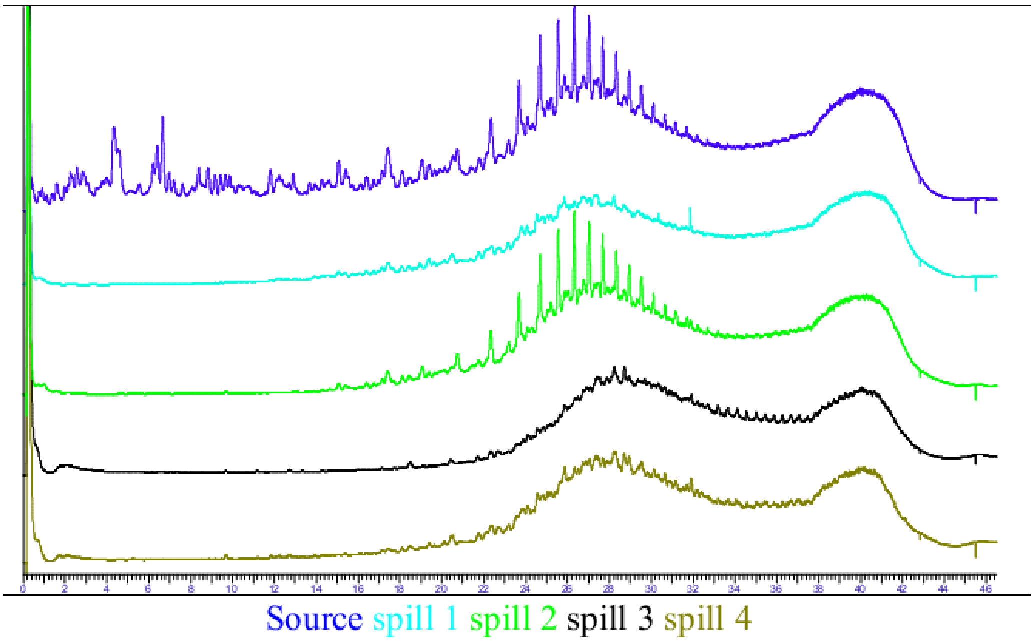


Figure 5 *: overlays SIMDIS chromatograms*

Fig 5.1 (FR, LASEM)
Fig 5 of LASEM showing a HT-GC-FID analysis of a part of the samples

Several humps can be seen in the chromatograms. This is partially caused by the three slope rates of 5 10 and 15 °C /min that have been used. But still it can be seen that the samples consist of different fractions of oil products.

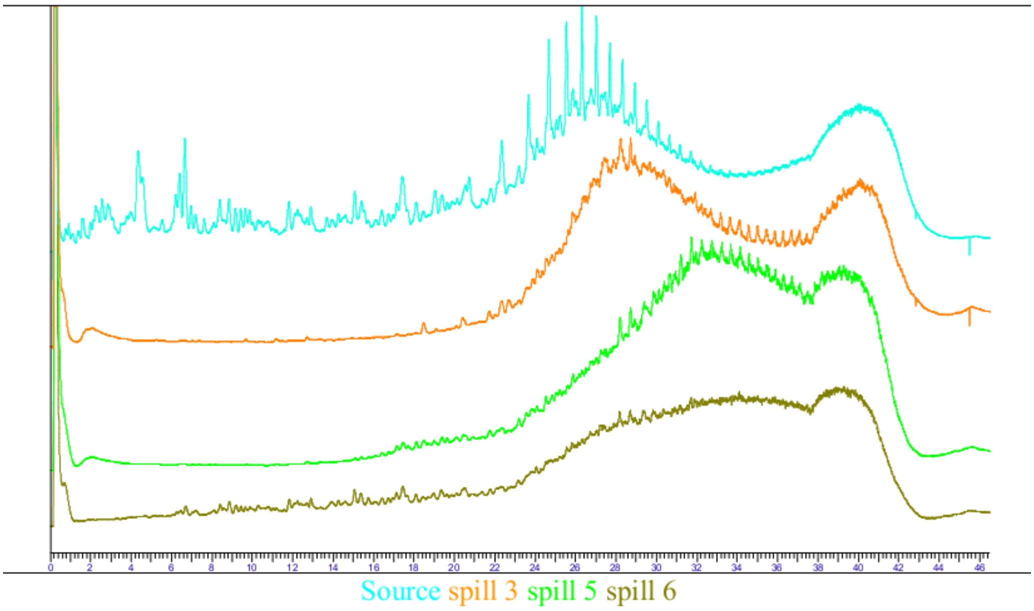


Figure 6 *: overlays SIMDIS chromatograms*

Fig 5.2 (FR, LASEM)
Fig 6 of LASEM showing a HT-GC-FID analysis of a part of the samples

Fig 5.2 shows that spill 5 and spill 6 are very different from the source sample but also different between themselves. Weathering might have had an effect but the difference in the HT part is very likely caused by a difference in composition.

The chromatogram of Spill 3 is remarkable. The sample comparison, see section 4.3.4 showed that the sample is more weathered than sample 4 which can explain the difference in UCM of the first hump compared with the source sample. The retention time section from 32 to 38 min shows however differences in shape and an n-alkane pattern that is not present in the source sample.

This extra range of alkanes is also shown in Fig 5.3.

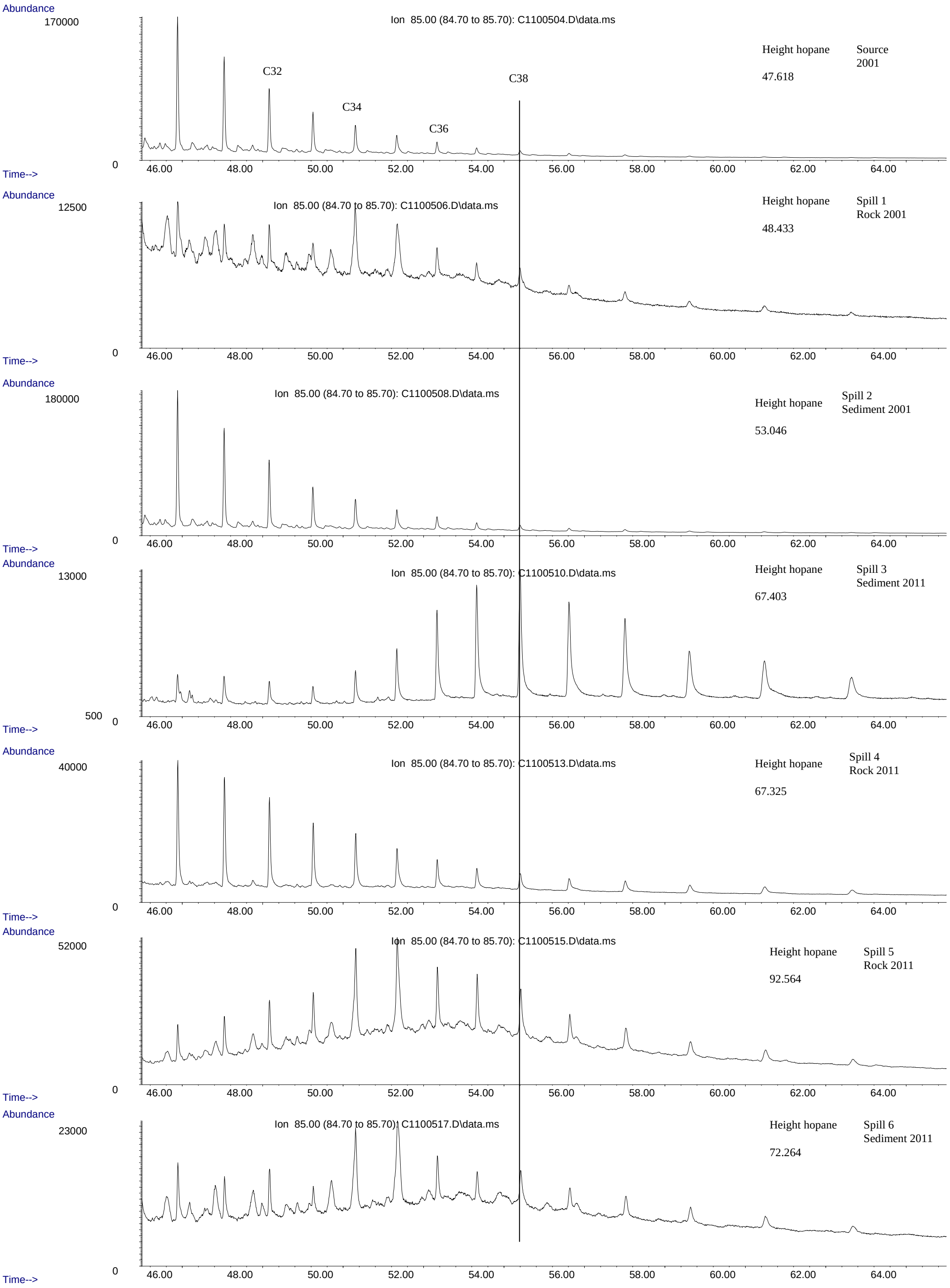


Fig 5.3 (NL, RWS-WD)
Comparison of the samples for m/z 85 representing the alkanes

It suggests oil from a different source or a contamination of the weathered source 1. Redistribution of the alkanes as weathering process is unlikely because the source barely contains alkanes in this range.

5.4 Normative compound/ratio exclusion

Table 5.5 shows that participants have excluded normative ratios differently. In section 6.5.3 of the CEN/TR one can find that the exclusion of normative ratios should be conducted carefully, and reasons should be mentioned in the technical report. Not all participants followed this advice. Information about this point was found partly in the reports and partly in the spreadsheet files. It is possible that a lab integrates all normative compounds and eliminates ratios for comparison based on the results of the duplicate comparison while the integration results can still be found in the spreadsheet file. We found however no indication of this procedure in the reports.

Retene is normally not present in HFO. This can be found in Table 3 of the CEN/Tr. Retene elutes together with the C4-phenantrenes (m/z 234) and it is sometimes difficult to see whether it is present or not. Checking the presence of fragment ion m/z 219 helps to recognize it properly, see Fig E.2 of the CEN/Tr.

The other peaks SC26TA, 30O and 28ab are on a very low level or absent in the samples. 30O is only present at a little bit higher level in spill sample 5.

	NR-retene	NR-SC26TA	NR-30O	28ab
Lab1	no	yes	yes	yes
Lab2	no	no	no	no
Lab3	no	no	no	no
Lab4	no	yes	no	yes
Lab5	yes	yes	no	yes
Lab6	no	no	no	yes
Lab7	no	no	no	yes
Lab8	no	no	no	yes
Lab9	no	no	no	no
Lab10	yes	yes	no	yes
Lab11	yes	yes	yes	yes
Lab12	no	no	no	no
Lab13	yes	yes	no	yes
Lab14	yes	yes	yes	yes
Lab15	yes	yes	yes	yes
Lab16	yes	yes	no	yes
Lab17	no	no	no	no
Lab18	yes	yes	no	yes
Lab19	no	yes	no	no

Table 5.5
Normative ratios excluded / included by the participants.

5.5 Informative compound integration.

The comparison spreadsheet used to generate the PW-plots and to compare the ratios, also contains a range of informative ratios that can be used, depending on the type of samples, to generate extra information about the weathering of compounds and the similarity of samples. Table 5.2 shows that 13 labs have used a part to most of these ratios.

Among these compounds are the sesquiterpanes, see Fig 5.4. These compounds have a low boiling point and thus easily evaporate but are very stable against other weathering effects like biodegradation and photo-oxidation.
According to the real scenario spill 1 is an aliquote of the source sample that is biodegraded for 3 months at the lab, spill 2 an aliquote of the source that has been weathered on a tile for three months.

Both spill samples show a clear pattern of the sesquiterpanes. The pattern of the source sample is however disturbed by a high background. The baseline of the source sample is at a level of 1500, while the baseline of the blanc is at a level of 140. This background is caused by the high number of lighter compounds in this section of the chromatogram, which generate m/z 123 as fragment ion.

In spill samples 1 and 2 this background is strongly reduced.
Together with the partly evaporation of the sesquiterpanes it is difficult to use this information for comparing the samples. Spill sample 6 is an exception because it can be seen that SES4 is higher than SES 8 in spill 6 while it is lower than SES 8 in spill samples 1 and 2. This cannot be caused by evaporation and is a real difference.

The blanc that underwent a cleanup shows besides a low baseline also some peaks of which two coelute with resp SES 4 and SES 8. SES 8 is used in the PW-plots shown in section 7 and the data are reliable because the peak height of the pek in the blanc is neglectable compared with the peak heights in the source and spill samples.

Besides the sesquiterpanes also decalin and the C1-dekalins are present in the samples. These compounds have the same weathering behaviour as the sesquiterpanes and can be used as weathering markers in the MS-PW-plots.

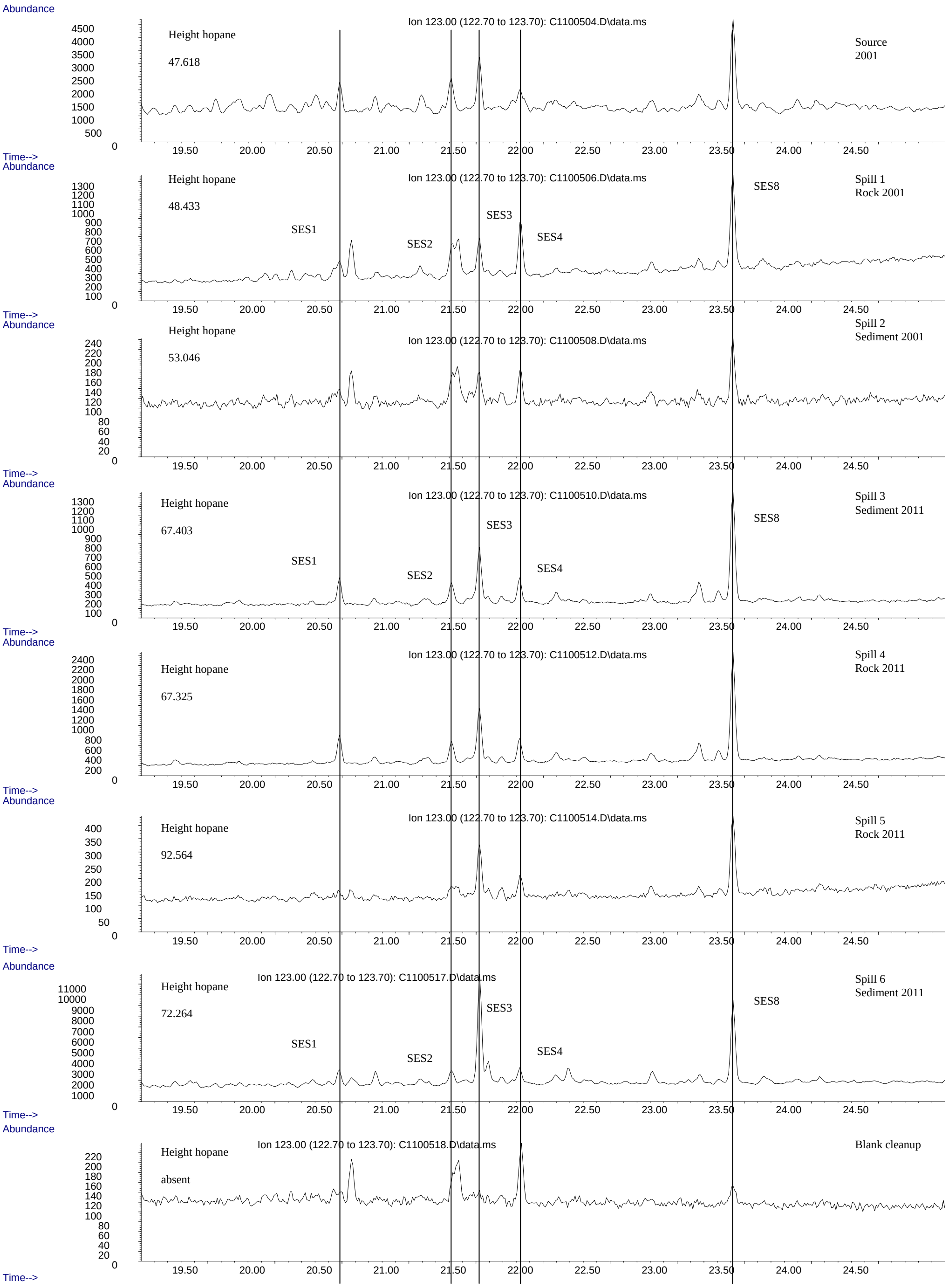


Fig. 5.4 (NL, RWS-WD)
The presence of the sesquiterpanes in the samples.

Fig 5.5 shows the retention window of the aromatic steranes C20TA and C21TA. Several participants have integrated these compounds but one should wonder whether they are present in the ion chromatograms of the samples at all.

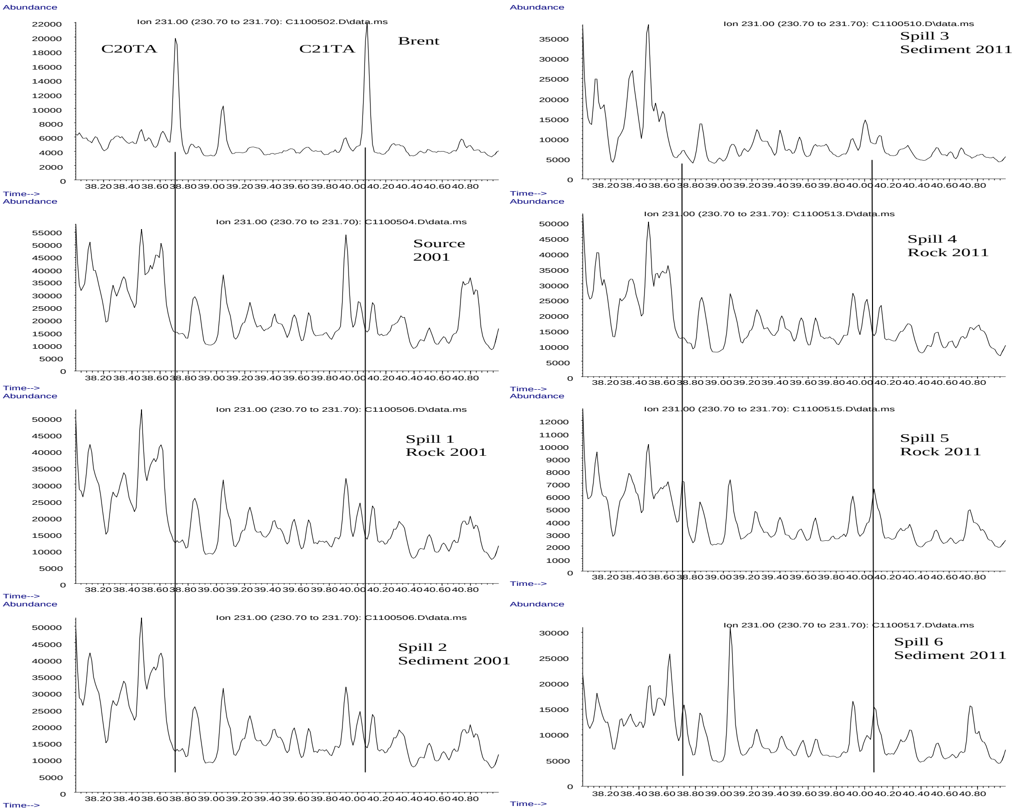


Fig 5.5 (NL, RWS-WD)
Comparison of Brent crude oil (as reference) and the RR2011 samples for m/z 231 ion chromatograms showing the elution windows of 20TA and 21TA.

Normally these compounds are not present or strongly disturbed in HFO samples. So it can be used as an additional marker for the identification of an oil product that has been treated in a refinery. This is however not based on a scientific explanation and might be not valid in all cases.
Comparing the ion chromatograms shows that samples source, spill 1, spill 2 and spill 4 show the same pattern, while at least spill 5 and spill 6 have a different pattern.

6 Table 2 evaluation

The participants were asked to enter results in a second spreadsheet file called "Table 2". The table makes use of ratios based on biomarkers.

R1	27Ts/27Tm (DR-27)
R2	27Ts/30ab
R3	29ab/30ab (DR-29ab)
R4	30d/30ab
R5	31abS/31abR
R6	31abS/30ab
R7	(27dbS + 27dbR)/28aaR
R8	29aaS/29aaR (DR-29aaS)
R9	27bb(R+S)/[28bb(R+S)+29bb(R+S)] (DR-27bbSTER)
R10	28bb(R+S)/[27bb(R+S)+29bb(R+S)] (DR-28bbSTER)
R11	29bb(R+S)/[27bb(R+S)+28bb(R+S)] (DR-29bbSTER)
R12	[(RC26TA+SC27TA) + SC28 TA] / RC28TA

Table 2 was made by Cedre and Total to test whether it is useful to add (some) of these ratios to the normative/informative ratios listed in the CEN/Tr.

The report of Total discusses and compares both methods:

Evaluation according the CEN/Tr
When using the guidelines and comparative tables described in the technical report CEN15522, it can be stated that:

- Extraction of specific hydrocarbons ions such as m/z=85, 191, 192, 198, 216, 217 and 218 show that the 4 samples (SPILL 1 to SPILL 4) are in the same boiling range (hydrocarbon cut) than the source.

- The height of the normal-paraffin C17 and C18 versus the height of pristane ($C_{19}H_{40}$) and phytane ($C_{20}H_{42}$) proved that the 4 spill samples are biodegraded.

Therefore, in order to correctly compare source and spill sample, only the biomarkers ratios should and will be used. The comparison of the different biomarkers ratios, shows that spill 1 and spill 2 have an common origin with source 1 (no ratio is over 14%) and that spill 3 and spill 4 have a different origin than source 1 (several ratio are over 28 %)

Conclusion: In accordance with the technical report CEN15522, it can be stated that the samples Spill 1 and Spill 2 are very biodegraded spills, which have the same origin than the source 1. On the other hand, Spill 3 and Spill 4 have a different origin than source 1.

Evaluation according Table 2
The second part of the RR2011 takes into account more specific diagnostics ratios of biomarkers in order to provide some information about the biodegradability level.

Ratios R7, R9, R10 and R11 (pointed in table 2) are not recommended ones in the Technical Report CEN15522, because these ratios are known to be affected by the biodegradation. For this reason, associated values from these 4 ratios can be used to estimate the biodegradability level.

After having checked and proved that the 12 ratios are stable by duplicated measurements in each sample (delta <14%), the comparison of the 6 spills samples 12 ratios with the source ones lead to the conclusion that:

- Spill 1 and Spill 2 have a common origin with Source 1
- Spill 3 and Spill 4 have a nearly common origin with Source 1 (without taking into account the 4 “biodegradation-level-ratios”). Solely a few ratios are between 14 and 28 %.
- Spill 3 seems to be a sample with a very advanced level of the biodegradation (absence of the following steranes : « 27dbS : 13(H),17(H),20S-cholestane » et « 27dbR : 13(H),17(H),20R-cholestane »),
- Spill 5 and Spill 6 have a clear different origin than the source 1 : 3 ratios other that R7, R9, R10 and R11 overrun the 28% barrier)

Conclusion: when using comparative ratios adapted to old spills, it can be stated that Spills 1 and 2 are clearly the same origin than Source1. Spills 3 and 4 have probably the same origin that source 1 but are very biodegraded and Spills 5 and 6 are coming from a different source.

Table 2 however didn’t help the participants very much in finding a conclusion for the comparison of spill 3 and spill 4 with Source 1. Many participants where “confused” by the severe weathering of the 10 years old samples and concluded an inconclusive or non-match. But still six reported a probable match for the relation between spill 3-source 1 and nine for the relation between spill 4 – source 1 based on the CEN/Tr.
This can be explained by the Cen/Tr approach that all sample information should be compared and not only the biomarkers. See chapter 9 for a discussion about this issue.

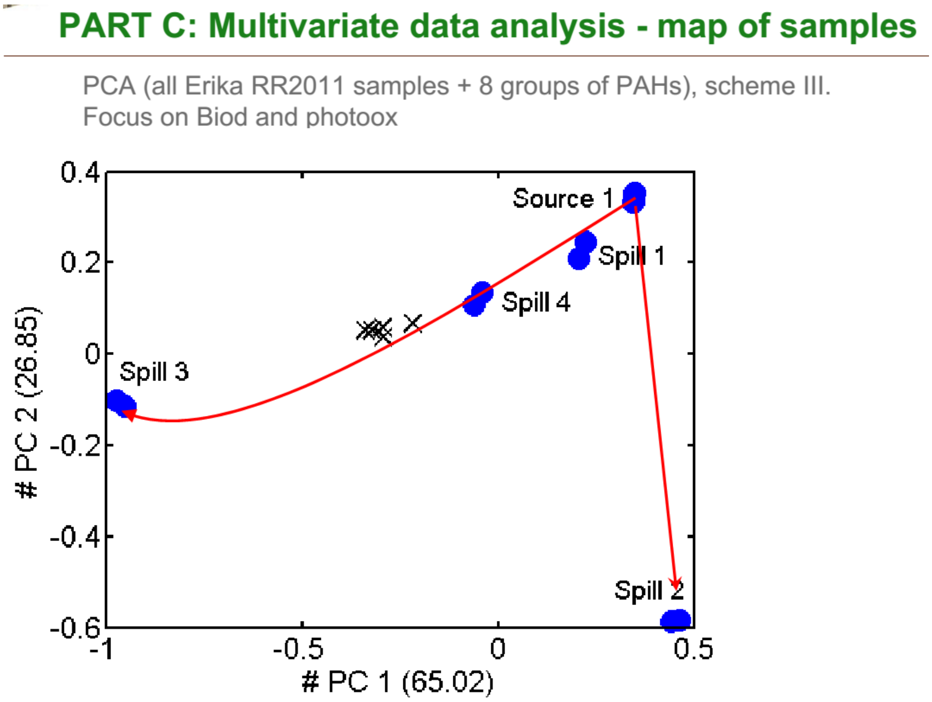
The samples of RR2011 and Table 2 however indicates that information about the effects of severe weathering, reducing even some of the biomarkers, is missing in the current version of the CEN/Tr.

7 Evaluation of the weathering of oil by IMOF.

7.1 IMOF

Jan Christensen presented at the meeting in Brest the IMOF method based on Principle Component Analyses (PCA) [10.3]. The method uses whole ion chromatograms as base for evaluation.
The presentations of Jan and Fabiana Gallota (BR, Petrobras) dealing with IMOF can be found on the BonnOSInet server for the participants of the round robin and on request for others. Some highligths from the presentation of Jan dealing with the weathering of the PAH's of the RR2011 samples will be discussed here.

The PCA plot of the RR2011 samples based on 8 ion chromatograms representing the PAH's is shown in Fig 7.1



PART C: Multivariate data analysis - map of samples

PCA (all Erika RR2011 samples + 8 groups of PAHs), scheme III.
Focus on Biod and photoox

Fig 7.1
PCA plot, based on the IMOF method, showing the RR2011 samples relative to each other.

The crosses in the middle of the plot are the result of 16 analyses of a Brent standard analysed in a period of two years. Also each RR2011 sample is analysed in duplicate. Both types of data sets indicate the variance caused by the GC-MS analyses. It indicates that the differences seen between the samples in the plot are significant. The PCA plot shows that the PC 1 axis differentiates in the rate of biodegradation and that the PC 2 axis differentiates in the rate of photo oxidation. Spill 1 is biodegraded in the lab and Spill 2 on a tile in the sun. Spill 3 and Spill 4 are real samples of which Spill 3 has been weathered most by both photo oxidation and biodegradation.

7.2 Photo oxidation

The effects of photo oxidation on the PAH's can be studied in the loading plot of PC2 showing eight PAH ion chromatograms. See Fig 7.2

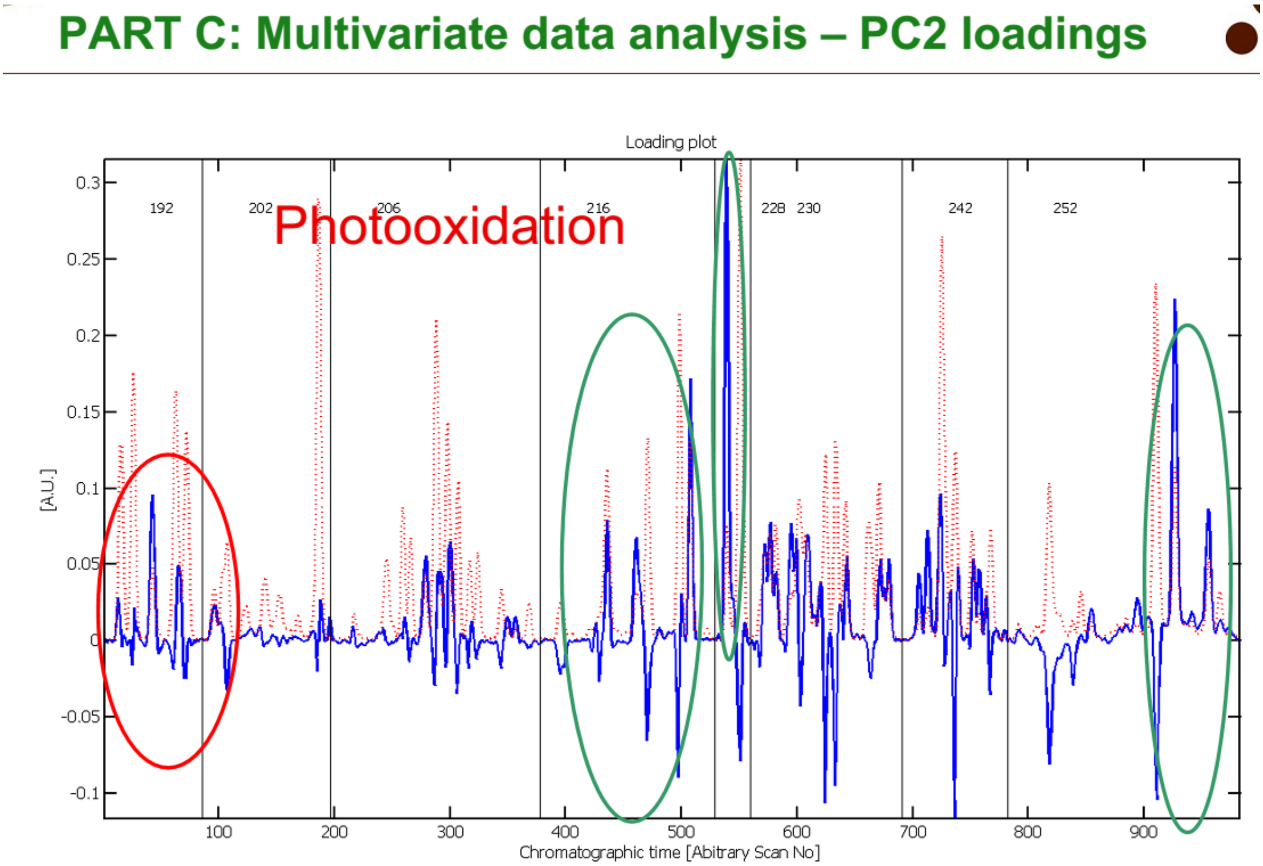

PART C: Multivariate data analysis – PC2 loadings

Fig 7.2 (IMOF method). PC2 loading plot of 8 PAH ion chromatograms behind each other. In red the normal chromatogram. In blue the relative differences related to photo xidation.

Blue peaks above zero indicate compounds strongly influenced by photo oxidation, while blue peaks below zero indicate compounds not sensitive for photo oxidation. M/z 192 shows the C1-phenantrenes and methyl anthracene and indicates that the C1-phenantrenes (MP) have not been changed.

N.B. The small peaks around the first doublet of 3-MP and 2-MP are caused by small differences of the peak shape between samples.

The two blue peaks visible at m/z 192 indicate 2-methyl anthracene and 1- methyl anthracene. 2-methyl anthracene can be found between the two MP clusters, but 1-methyl anthracene elutes in the 9/4- and 1-MP cluster. Both are very sensitive for photo oxidation.

M/z 252 of Fig 7.2 shows a reduction of two peaks. Fig 7.3 shows the ions traces of m/z 252 for the source sample and spill 2.
The reduction of perylene and benzo (a) pyrene are obvious, while benzo(e) pyrene and benzo (b) fluoranthene are less sensitive for photooxidation.

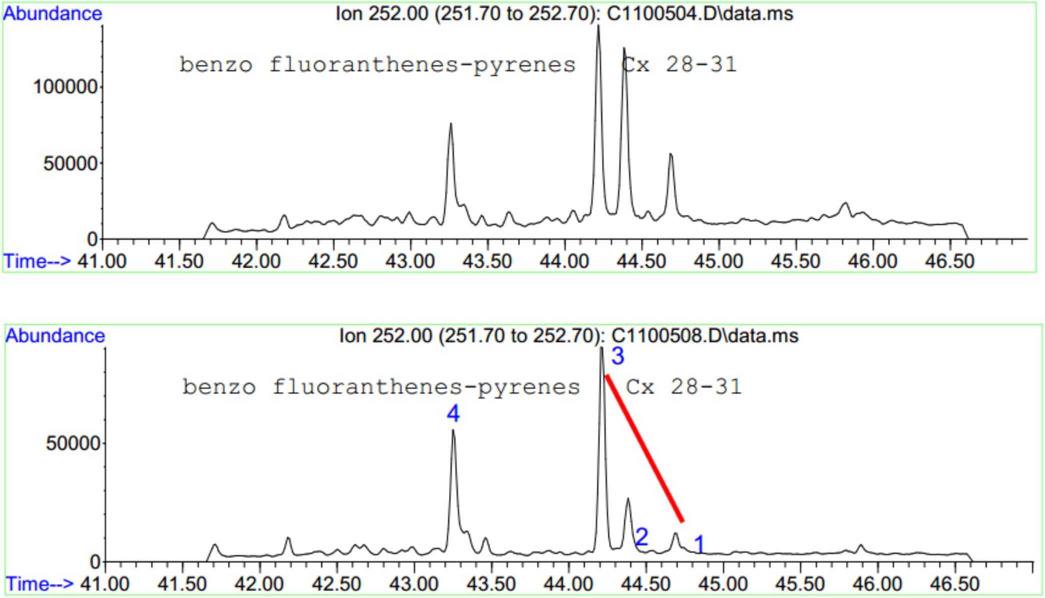


Fig 7.3. (NL, RWS-WD)
m/z 252 ion chromatogram of source 1 (above) and spill 2.
1 Perylene.
2 Benzo(a)pyrene
3 benzo(e) pyrene
4 benzo(b) fluoranthene

Fig 7.2 and Fig 7.4 show a preferential reduction of benz (a) anthracene compared to chrysene by photooxidation.

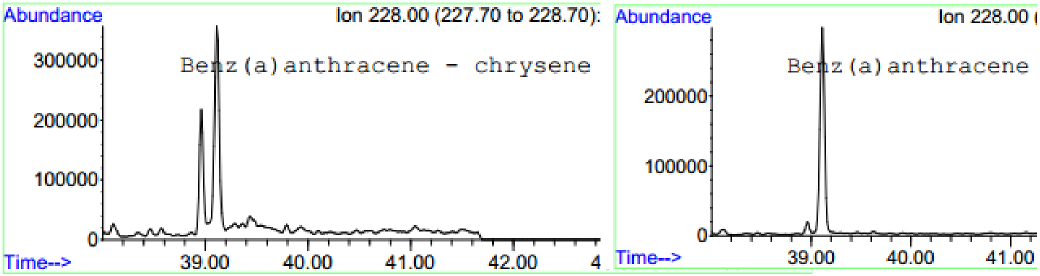


Fig 7.4. (NL, RWS-WD)
m/z 228 ion chromatogram of source 1 (left) and spill 2.
Left Peak is benz (a) anthracene.
Right peak is chrysene

In the same way it can also be concluded for

- m/z 216 Low relative concentration of 1-MPy and the benzofluorenes compared to 4-MPy and methylfluoranthene
- m/z 230 Very distinct changes in the isomer patterns of C2-pyrenes . Correlates nicely with the changes seen in the C1- pyrenes of m/z 216.
- m/z 242 Low relative concentration of C1-benzoanthracenes compared to C1-chrysenes

7.3 Biodegradation

Biodegradation has been simulated with spill 1. The PW/plot of spill 1 relative to source 1 is shown in Fig 7.5

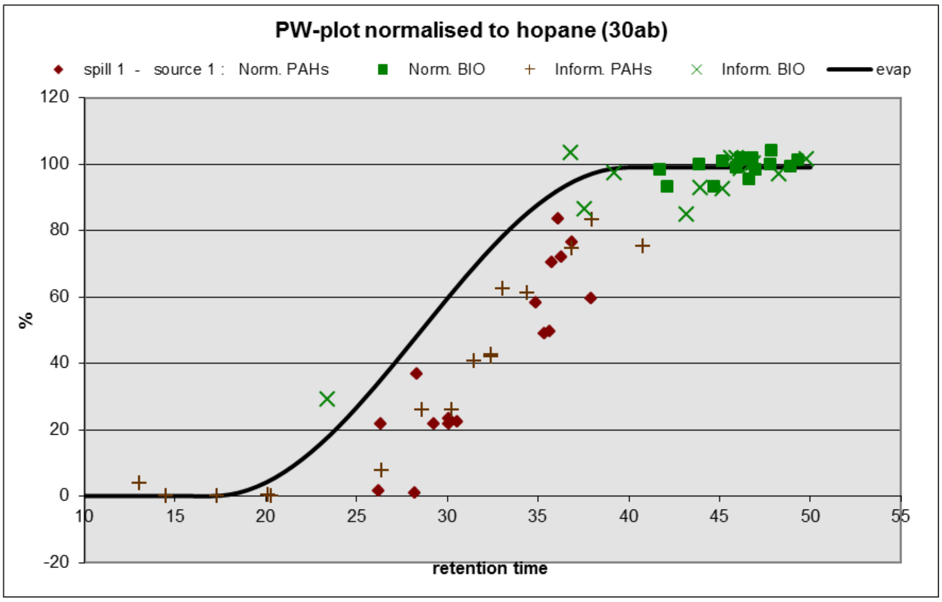


Fig 7.5. (NL, RWS-WD)
MS-PW-plot of spill 1 with source 1

The evaporation line is drawn below the data point of SES 8 because the peak height of SES 8 is relative low in source 1 because of the higher baseline. See Fig 5.4. The plot shows a reduction to zero of the alkanes C17 and C18 (two data points at zero between a retention time of 25 and 30 min.)

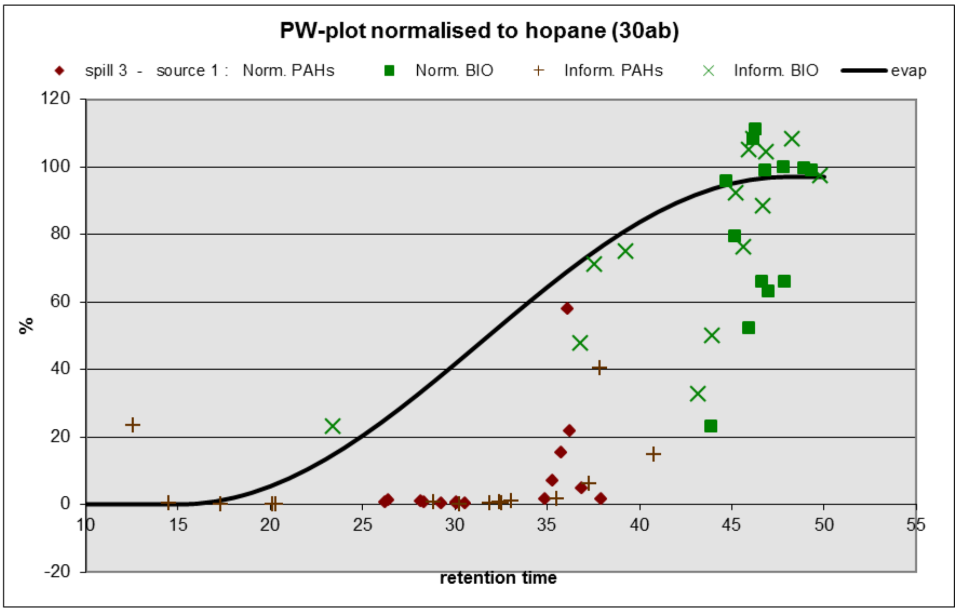
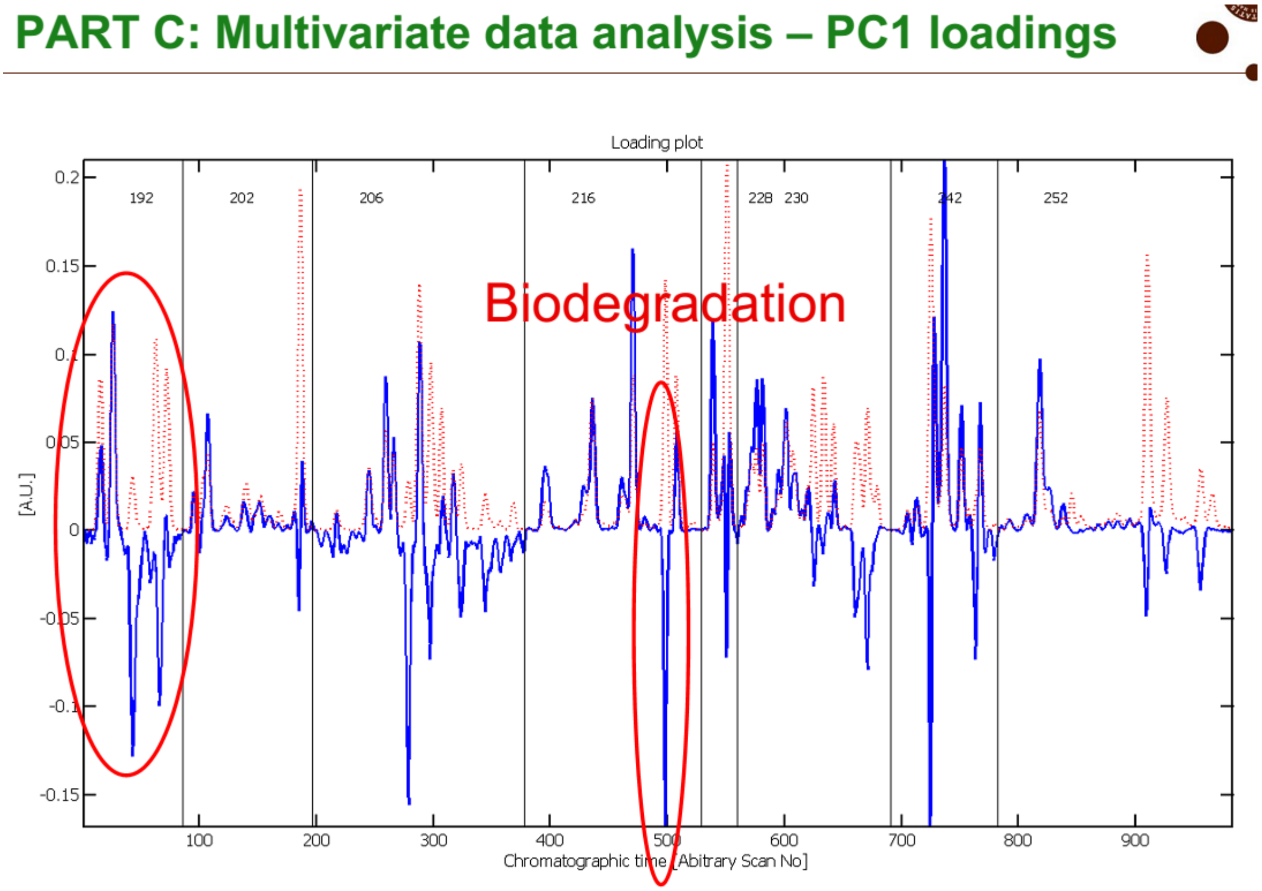


Fig 7.6. (NL, RWS-WD)
MS-PW-plot of spill 3 with source 1

The PW/plot of spill 3 relative to source 1 is shown in Fig 7.6. The evaporation line neglects the C1 decalins data point, is drawn again below the data point of SES 8 and uses the triterpanes C24 and C25 at 80% and the hopanes as references.

N.B. The data points of the C1-decalins and SES 8 are on the same level of about 23%. The evaporation line of spreadsheet file v51 is designed to start always from zero. Spill sample 3 is from a 10 years old patch of oil on a beach in between the vegetation. It is very well possible that the lighter compounds in the upper layer of the patch are evaporated, but in a lower layer still exist. For this PW-plot it would be useful to add the option to start the evaporation line at a higher choosen level.

The plot shows not alone a reduction to zero for the alkanes C17 and C18, but also for pristane, phytane and many PAH's and some of the biomarkers.



PART C: Multivariate data analysis – PC1 loadings

Fig 7.7 (IMOF)
PC1 loading plot of 8 PAH ion chromatograms behind each other. In red the normal chromatogram. In blue the relative differences specific for biodegradation.

The effects of biodegradation on the eight PAH ion chromatograms can be studied in the loading plot of PC1. See Fig 7.7

- m/z 192 shows a reduction order of 2 M-phenantrene > 3M-P > 1M-P > 9/4 M-P> 1 and 2 M-anthracene.
- m/z 202 reduction of fluoranthene and a better resistance of pyrene.
- m/z 206 evident changes in the C2-phenantrene isomers.

- m/z 216 4 M-Py is the most resistant m/z 216 compound to "biodegradation" followed by 1 M-Py and the other fluoranthene and benzofluorene isomers. The same is the case for the C2-isomers (m/z 230)
- m/z 228 reduction of benz (a) anthracene
- m/z 242 evident changes in the C1 chrysene isomers.
- m/z 252 reduction of benzo (b) fluoranthene

8 Judgement of the individual reports.

8.1 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 of then made a proposal and optimized the method in discussions.

8.2 Evaluation method of Gerhard

Gerhard has prepared Table 6.1 for report judgement:

Item	**Criteria**	**1 with 2**	**1 with 3**	**1 with 4**	**1 with 5**	**1 with 6**	**1 with 7**	**Sum**
1	presence of means	2	2	2	2	2	2	12
2	oil type correct?	1	1	1	1	1	1	6
3	ratios correctly chosen/excluded	2	2	2	2	2	2	12
4	QM	2	2	2	2	2	2	12
5	interpretation of ratios and weathering	2	2	2	2	2	2	12
6	Right conclusion?	2	2	2	2	2	2	12
	Max. reachable points for every comparison	11	11	11	11	11	11	66

Item	Criteria	Comments	
1	presence of means	0 or 2	Presence of all necessary means for tracing back a conclusion
2	oil type 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 6.1
Report evaluation by Gerhard with regard to correct and traceable conclusions

8.3 Evaluation method of Paul

Paul has prepared Table 6.2 for report judgement:

Item	Main groups	aspects	points	remarks
1	FID level 1.1	analysis		analytical method and data quality
2	FID level 1.2	data evaluation		which conclusions are drawn from the results, e.g. concentration adjustment, oil type, elimination of samples.
3	MS level 2.1	analysis - visual inspection		analytical method and data quality
4	MS level 2.2	PW-plots - ratios comp.		integration, elimination of ratios, variance.
5	Result evaluation	conclusions from chromatograms conclusions from PW-plots conclusions from the ratio comparison		e.g. oil type, elimination of samples. similarity and weathering aspects similarity and weathering aspects.
6	Match conclusion	final match conclusions.		Conclusions related to the scenario.
7	Reporting	internal documentation		results that are important for the final conclusions; description of the reasons for conclusions.
8	Reporting	external - summery report.		Completeness according to CEN/Tr chapter 7.3
9	Overall impression	Personal judgement of the whole report		To be able to give an additional personal opinion. Useful? I don't know We will see.
		Total (ranks from 0 to 18 points)	0	For each item: 0 = bad 1 = fair 2 = good

Table 6.2
Report evaluation by Paul with regard to correct and traceable conclusions

Item 8 “External report” is for this year not used, because it was not requested in the instructions. It should however be a part of the judgement of the next Round Robins.

8.4 Judgement of the results

The individual reports of the participants are available for members of the BonnOSInet expert group, but are treated as confidential for the 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 7 of the summary report while the results of the judgement of the labs is shown in Table 6.3 (Identical to Table 3 of the executive summary

Lab code	Method Paul	Method Gerhard	mean
Lab1	81	79	80
Lab2	100	100	100
Lab3	88	83	85
Lab4	100	100	100
Lab5	38	39	38
Lab6	56	52	54
Lab7	94	97	95
Lab8	100	100	100
Lab9	69	76	72
Lab10	88	82	85
Lab11	88	88	88
Lab12	75	82	78
Lab13	75	73	74
Lab14	81	74	78
Lab15	81	77	79
Lab16	81	88	85
Lab17	100	100	100
Lab18			
Lab19	94	97	95

Table 6.3

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

The results show that the difference between both methods is small.

A typical aspect of the method of Gerhard is the judgement of each source – spill sample relation separately. It gives possibilities to give points to match conclusions and oil type recognition. For e.g. QM it is less relevant because such an aspect is more general.

A typical aspect of the method of Paul is that the analytical scheme of the CEN/Tr is followed. It gives possibilities to judge the different steps of the procedure but lacks the possibility to judge source to spill sample relations.

It can be concluded that the combined methods might be a good compromise.

The results show that experience and skills of the different labs vary a lot. Labs, that have a long history in oil spill identification and that are participating in the expert group from the beginning, in general received highest points. Labs, that have a very limited number of cases in oil spill identification and/or that are more or less new in the expert group, in general received lower points.

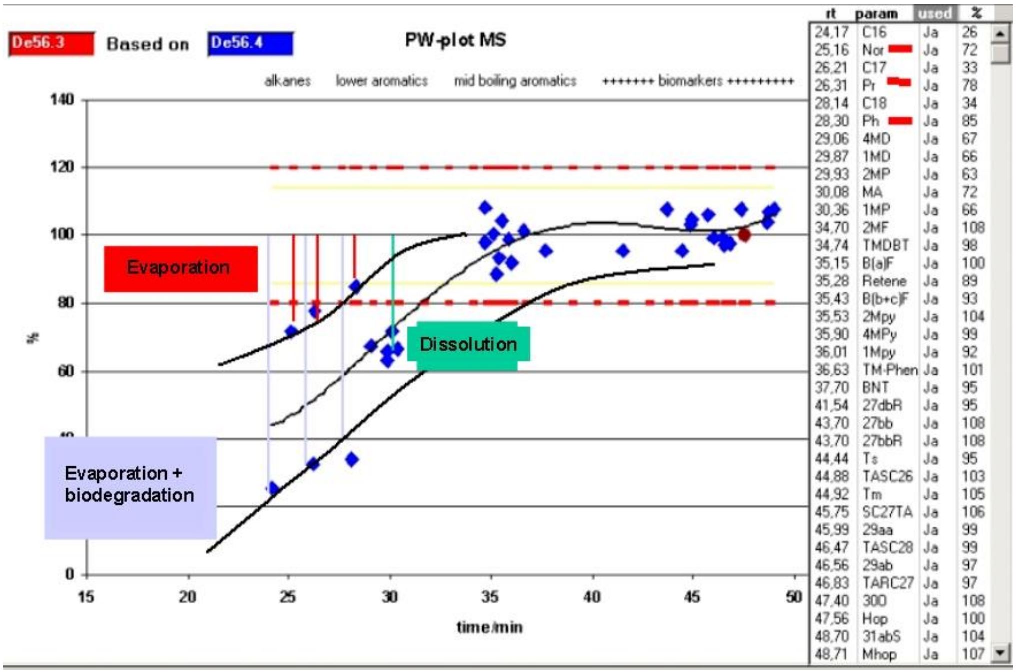
No judgement was given for Lab 18. The method was completely new for them and they had difficulties to enter the data in the spreadsheets files properly.

9 Conclusions

The demand to use more robust ratios, i.e. ratios being more resistant against weathering, is derived from the obvious mistake that the final conclusion in the CEN/TR is reached merely by comparing diagnostic ratios.
In the result evaluation of TOTAL the key passage in the text is:

" ..... the 4 spill samples are biodegraded. Therefore, in order to correctly compare source and spill sample, only the biomarkers ratios should and will be used".

It is given in the CEN/TR, in contrast, that the final conclusion should be reached by taking into account all available information.
This means that information achieved by GC-screening as well as by the visual comparison of all mass-chromatograms should also be taken into account.
But, in addition: the measurements of compounds affected by weathering processes, such as biodegradation here, are not simply ignored. In contrast, by means of the PW-plots, those differences are worked out.
Weathering of compounds follows distinct rules, and, if it is demonstrated that these rules are kept, corresponding differences even serve as a proof that samples had been identical at the time of the discharge, i.e. the proof that samples match.
In the CEN/TR, the influences of the different weathering process, such as evaporation, bacterial degradation, dissolution and photo oxidation, on the compounds used for producing the DRs are described.



rt	param	used	%
24,17	C16	Ja	26
25,16	Nor 🟥	Ja	72
26,21	C17	Ja	33
26,31	Pr 🟥	Ja	78
28,14	C18	Ja	34
28,30	Ph 🟥	Ja	85
29,06	4MD	Ja	67
29,87	1MD	Ja	66
29,93	2MP	Ja	63
30,08	MA	Ja	72
30,36	1MP	Ja	66
34,70	2MF	Ja	108
34,74	TMDBT	Ja	98
35,15	B(a)F	Ja	100
35,28	Retene	Ja	89
35,43	B(b+c)F	Ja	93
35,53	2Mpy	Ja	104
35,90	4MPy	Ja	99
36,01	1Mpy	Ja	92
36,63	TM-Phen	Ja	101
37,70	BNT	Ja	95
41,54	27dbR	Ja	95
43,70	27bb	Ja	108
43,70	27bbR	Ja	108
44,44	Ts	Ja	95
44,88	TASC26	Ja	103
44,92	Tm	Ja	105
45,75	SC27TA	Ja	106
45,99	29aa	Ja	99
46,47	TASC28	Ja	99
46,56	29ab	Ja	97
46,83	TARC27	Ja	97
47,40	300	Ja	108
47,56	Hop	Ja	100
48,70	31abS	Ja	104
48,71	Mhop	Ja	107

Fig 9.1
RR2010: PW-Plot of spill 3 based on source sample 4

But the PW-plots also have a second function here: error limits for the compounds unaffected by weathering are given in the CEN/TR. PW-plots thus serve as an additional means for coming to an objective conclusion based on comparisons of relative compound concentrations.

In summary: to reduce the role of the PW-plots to their mere function for deleting diagnostic ratios, which are affected by weathering, must therefore be regarded as an obvious mistake.

Admittedly, not all situations and circumstances are covered by the CEN/TR, and nothing is said about the bacterial degradation of biomarkers, which are the most stable compounds here. But the task to identify 10 year old oil samples must be regarded as a very special one, which cannot be solved my means of only 3 or 4 samples, taken at the beginning and at the end of this time.
Nevertheless, the analyst is not left alone here: in RR2011 additional information is present by comparing the two 10 year old samples among each other, and their relation to the samples, which were taken one year after the accident.

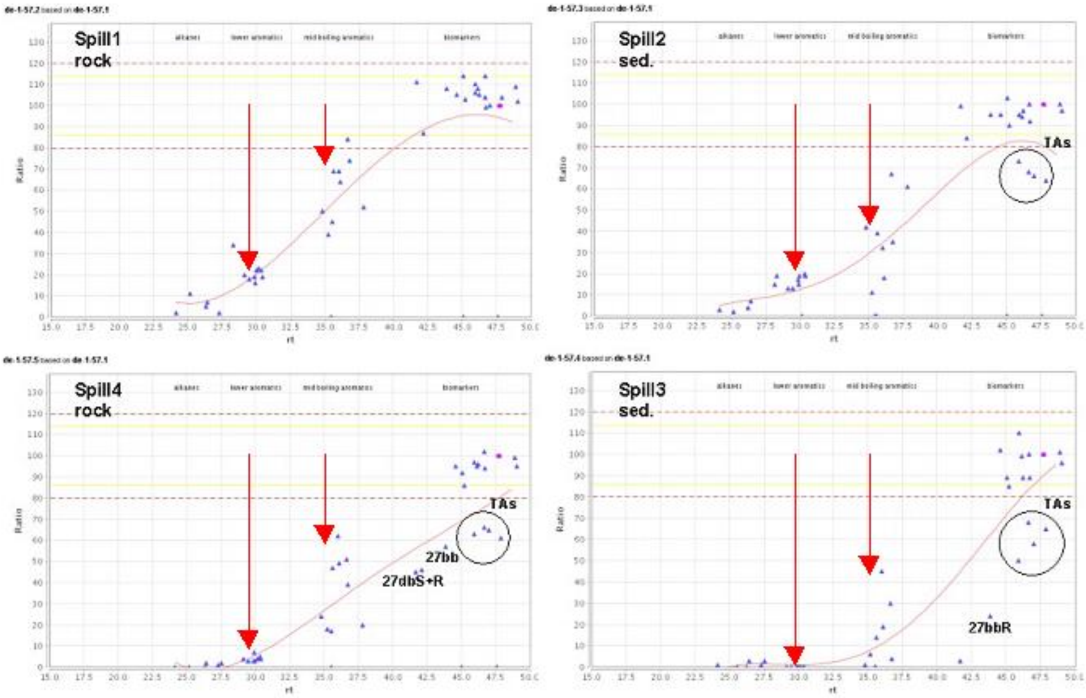
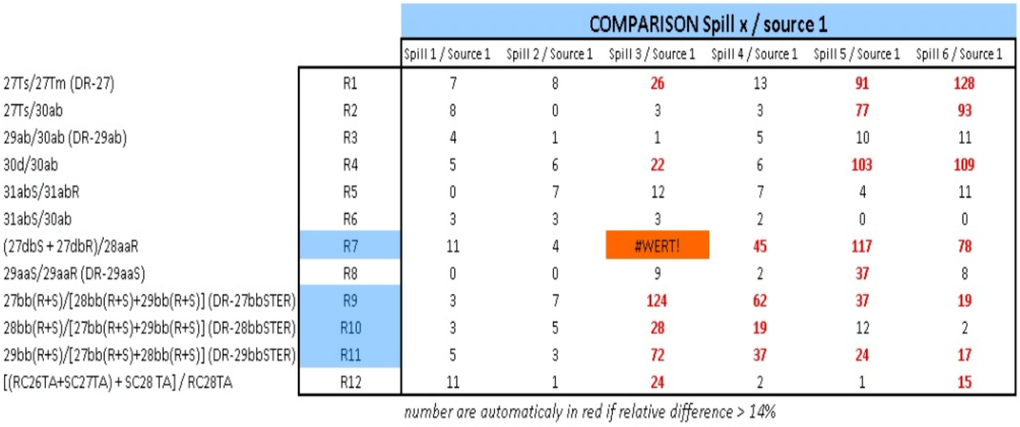


Fig 9.2
PW-Plots of spills 1 to 4, which show the increasing reduction of the lower boiling (M-Phens, M-DBTs) and mid boiling aromatics (pyrenes) from spills 1and 2 over spill 4 to 3, the reduction of the TAS in spills 2 to 4, and the stepwise reduction of 27dbS+27dbR and 27bb from spills 1 and 2 over spill 4 to spill 3.

Of course, it would be much better, if much more samples, taken also over the years, were present. Thus, in this Round Robin, the participants got only a short insight into the problems, which appear during post-spill investigations, conducted over a longer time.
But PW-plots may also lead to additional “problems”: the PW-plots here show differences, which are not found, when only the diagnostic ratios are compared: the reduction of the triaromatic steranes. These compounds are found to be very stable against biodegradation.
Spill 2 gives an answer here: as spill 2 is rarely affected by biodegradation, biodegradation cannot be responsible for the reduction. The reduction must have been caused by dissolution and photo oxidation. It could be found out that especially photo oxidation led to strong reductions of other aromatics in this sample.



		COMPARISON Spill x / source 1					
		Spill 1 / Source 1	Spill 2 / Source 1	Spill 3 / Source 1	Spill 4 / Source 1	Spill 5 / Source 1	Spill 6 / Source 1
27Ts/27Tm (DR-27)	R1	7	8	26	13	91	128
27Ts/30ab	R2	8	0	3	3	77	93
29ab/30ab (DR-29ab)	R3	4	1	1	5	10	11
30d/30ab	R4	5	6	22	6	103	109
31abS/31abR	R5	0	7	12	7	4	11
31abS/30ab	R6	3	3	3	2	0	0
(27dbS + 27dbR)/28aaR	R7	11	4	#WERT!	45	117	78
29aaS/29aaR (DR-29aaS)	R8	0	0	9	2	37	8
27bb(R+S)/[28bb(R+S)+29bb(R+S)] (DR-27bbSTER)	R9	3	7	124	62	37	19
28bb(R+S)/[27bb(R+S)+29bb(R+S)] (DR-28bbSTER)	R10	3	5	28	19	12	2
29bb(R+S)/[27bb(R+S)+28bb(R+S)] (DR-29bbSTER)	R11	5	3	72	37	24	17
[(RC26TA+SC27TA) + SC28 TA] / RC28TA	R12	11	1	24	2	1	15

number are automaticaly in red if relative difference > 14%

Fig 9.3
Table 2 developed by Total/Cedre

Generally it could be demonstrated that the same conclusions could be reached, when the CEN-guideline is used, and the DRs given here, and when the "special" ratios are used proposed by Pierre.
But when the CEN-guideline is used correctly, much more information is achieved, and the connection between the samples becomes much clearer.
In contrast to the PW-plots, nothing is said here as to whether compounds are lower or higher in concentration, when merely differences of ratios are compared. Thus a difference in a ratio may be a reduction due to weathering or it may be a difference between actually different samples.
It seems to be a little bit vague here, to base conclusions on only 12 ratios. 4 of them, marked blue, are "not further taken into account", when the 10 year old samples of spill 3 and 4 are compared with source 1.
On the other hand 3 ratios are obviously identical in all six spill samples. This means that those ratios obviously are not very suitable to differentiate between actually different oils. It does not make sense to choose ratios simply because of their stability or robustness against weathering. In oil spill identification ratios must be chosen, which differ in oils, or in other words, which have the necessary discrimination power to distinguish between oils.
With regard to spill 3, eventually only 2 ratios remain, which are identical in source 1 and spill 3. Concluding a "probable match" here thus seems to be very vague.

9.1 Summarizing conclusion

In this Round Robin, participants got a short insight into the problems and difficulties of post-spill investigations. Of course, much more samples, taken also continuously over the time, are needed in order follow strong weathering processes, when very old oil spills should be unequivocally connect with the originally discharged oil. Parts of the oil may have been exposed to the sun or to the waves, whereas other parts within thicker layers might have been more protected against weathering.
The CEN/TR is not correctly understood, if the final comparison is based merely on the comparison of the diagnostic ratios. All available information is used to come to a conclusion here, and especially the PW-plot is a powerful tool for result evaluation. The same results are achieved by using the CEN-guideline and by comparing the "specific" ratios proposed by Pierre. But because much more information is

achieved, when the CEN-guideline is used, conclusions achieved here are much more substantiated.
There is definitely no need to change the diagnostic ratios as given in the CEN-guideline

10 References

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