Rijkswaterstaat
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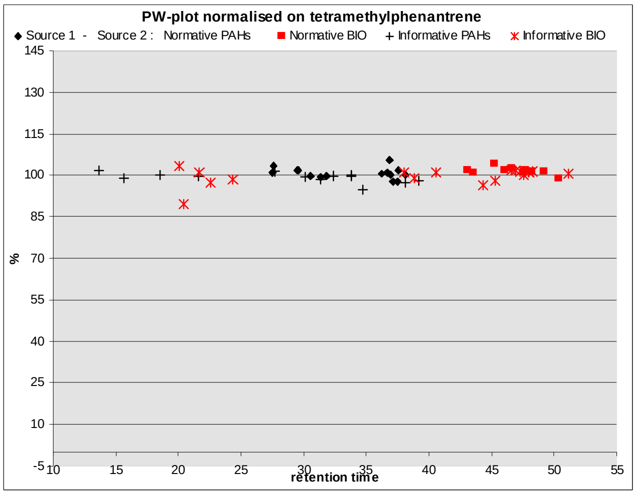
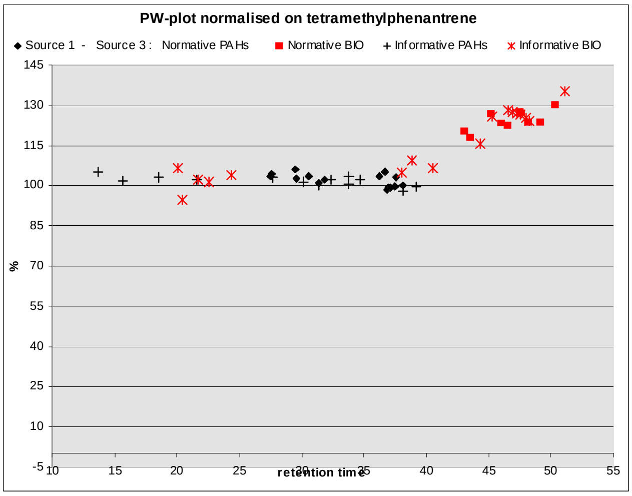
BONN AGREEMENT
Oil Spill Identification
Network of Experts

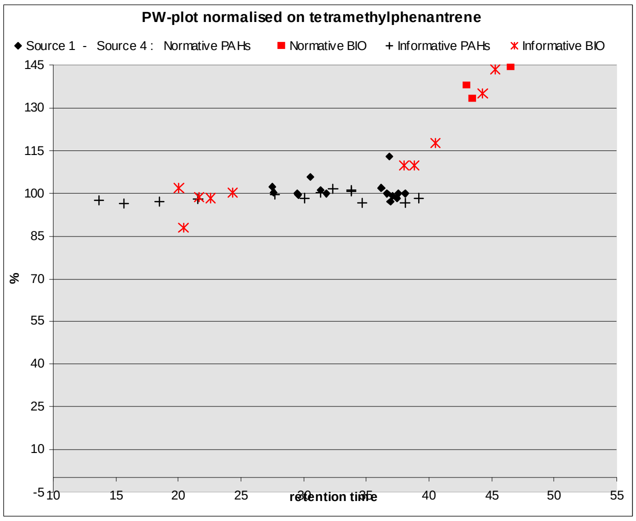
RR2009 - The comparison of 6 bilge samples

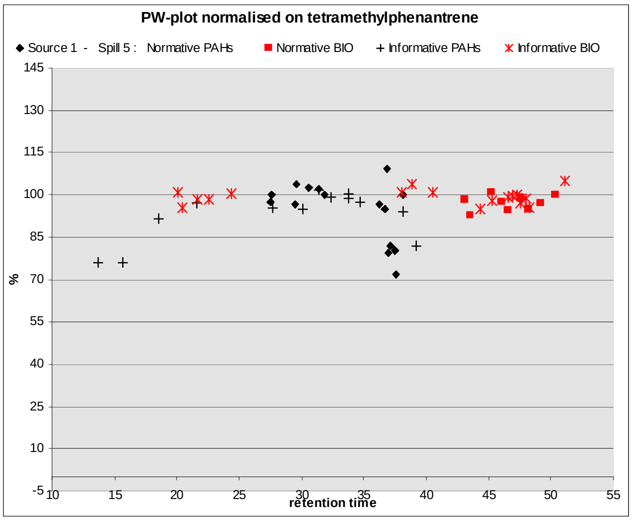
Fourth intercalibration in the framework of Bonn-OSINET

The results of twenty-one international laboratories









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On the front page:
MS-PW-plots of the RR2009 sample combinations 1-2, 1-3, 1-4 and 1-5
(Petrobas, Br)

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The results of twenty-one international laboratories

Colophon

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Contents

Executive summary

Round Robin 2009 was the fourth world-wide ring test of the expert group on oil spill identification of the Bonn-Agreement (Bonn-OSINET), in which 21 laboratories from 16 countries participated.
Six oil samples, which contained gas oil - lubricating oil mixtures, i.e. typical bilge oils of smaller ships, were sent to the participants. It was requested to work (if possible) according to the draft version 3 of CEN/Tr 2, published in September 2009 on the OSPAR web-server. A technical report should be returned and a well-prepared spreadsheet file, which was sent to the participants before, should be filled with the measured data. The spreadsheet file could already be used to evaluate the results by means of ratio comparisons and MS-PW-plots.

Results from all spreadsheets files were used to calculate an error range for the MS-PW-plot statistically. The result is that it is very well possible to keep the standard deviation of the single data points below 7% to 8%. This results in an error range of about +/- 15% from the 100% line.

Many “problems” were included into this RR-exercise, such as mixing, low sulphur content, addition of biodiesel, photo oxidation and biodegradation. But based on the information of draft version 3 of CEN/Tr 2 version 2 still a match could have been concluded for all sample comparisons.

Summary of the conclusions (only a comparison of sample 1 with the other 5 samples was requested):

Participant	**Conclusion of the comparison of RR2009-1 with**				
	RR2009-2	**RR2009-3**	**RR2009-4**	**RR2009-5**	**RR2009-6**
AU_EC_CES	M	M	M	M	M
AU_NSWDECC	M	PM	PM	M	M
BE_BMM	NM	NM	NM	NM	NM
BR_Petrobas	M	M	M	PM	M
BG_SGS	NM	PM	NM	NM	NM
CA_EC_ALET	PM	PM	PM	PM	NM
CA_ECTD	M	M	M	M	NM
CA_PESC_EC	M	M	M	M	NM
CN_NCSEMC	M	M	M	PM	M
DE_BSH	M	M	M	M	M
EE_EERC	no result	no result	no result	I	M
ES_CEDEX	PM	PM	M	M	NM
ES_CSIC	M	M	PM	NM	PM
FI_NBI	M	M	M	PM	PM
FR_CEDRE	PM	PM	NM	NM	NM
LV_LVGMC	M	M	M	M	PM
NL_NFI	M	NM	NM	NM	NM
NL_RWS-WD	M	M	M	M	M
NO_Sintef	M	M	M	PM	M
SE_SKE	M	M	M	M	PM
UK-ERT	PM	M	M	M	PM

Table
Conclusions of the participants.
M=match, PM= probably match, I= inconclusive and NM = non-match

Since samples 1 and 2 are definitely the same (produced from the same solution), a match must have been concluded here. Any measured deviations between these samples can only originate from the analytical variation.
Since it was given in the "Instructions" that samples 1 to 4 originate from the same ship's bilge, this exercise was rather a test of the method in these cases: does the method lead to a match, despite of the bigger differences (caused by mixing here).

Not all participants came to the conclusion of a match in all cases. This may be related to the fact that many of the participants do not work normally with light fuel oil and small degrees of mixing ánd don't have knowledge about the specific weathering behaviour of biodiesel and some aromatic compounds.

- Based on the match conclusion definition of CEN/TR 2v2 a match can defensibly be concluded also for the combinations 1-3 and 1-4, because a inhomogeneous mixture of lube oil and gasoil in a bilge is often found.
- For the comparison of sample 1 with sample 5, knowledge about the weathering behaviour of the C1 to C3 Pyrenes is needed. But the differences are not too big, and even those smaller differences must be seen in the light that everything else fully matches. So, only a slight uncertainty could have remained here.
- For the comparison of sample 1 with sample 6, knowledge about the weathering behaviour of the FAMEs is needed.

By going through the Analytical Scheme of the CEN-TR step by step, all the difficulties included/produced here in RR2009 should have been recognized.

- The mixing of lube oil with gas oil is discussed in Annex I.5 of CEN/TR 2v2. Generally, the PW-plot reveals to be very useful, because concentration differences between compound groups are tested here.
- The possible absence of sulphur containing compounds and compound groups is not directly mentioned in the CEN/TR, but by following the general "rules" for the exclusion of ratios and the determination of very small peaks or peaks, which otherwise cannot be measured precisely, the scheme can be followed as usual. In contrast to earlier versions of the CEN_TR the normative ratios for sulphur containing compounds are now defined in such a way that values go to zero instead to infinity, if the sulphur content goes to zero.
- The reduction of the C1 to C3 pyrenes is mentioned in Annex I.4 of the CEN/TR. Photo oxidation as the cause for the change is only mentioned as a possible reason for this reduction in draft version 3. After this RR2009, and the tests described in Annex B here, we are surer, and we could change the text of the CEN-TR accordingly.
- Mixing of FAMEs with diesel is not mentioned in the CEN/Tr 2v2. But the presence and concentration of the individual FAMEs may even give useful information in oil sample comparison. But this phenomenon is limited to a few cases -and diesel-oil-, so far, and the tendency in the future cannot be assessed. May be, FAMEs should be treated in the same way as other additions sometimes found in oil samples, such as additives, or cleaning agents in case of waste oil (bilge oil, sludge). In the CEN-TR the general advice is given that the analyst should look for peculiarities in the samples. But, depending on the case, such additions may make identification easier in one case but more difficult in the other. General rules cannot be given, so far.

1 Introduction

This report is the summary report of Round Robin 2009. The individual reports of the participants are available on the Bonn-OSInet forum (password protected), while the summary report will be publicly available on the Bonn-OSInet section of the Bonnagreement website (www.bonnagreement.org).

The method advised is CEN/Technical Report 15522-2. The first version has been published in 2006. In the meanwhile the Bonn-OSInet expert group has been worked on a second version, abbreviated in this report as CEN/TR 2v2. The third draft version of CEN/TR 2v2 is used as method for the intercalibration of 2009.

The summary report starts with the invitation letter send to 24 laboratories (see Chapter 2). The letter contains an introduction to the annual round robins in the framework of Bonn-OSInet, describes the scenario of the case and gives instructions how to handle the samples and send in the analysis report.

Note
Gerhard Dahlmann, as co-author, was not informed about the real scenario until Paul Kienhuis had received the BSH report.

For different reasons three labs were not able to sent in a report.
Results were received from the laboratories listed in Table 1.

Laboratory	Location	Contact
EPA-CES	Victoria (AU)	Syed Hasnain
NSWDECC	Lidcombe (AU)	Steve Fuller
MUMM	Oostende (BE)	Patrick Roose/Marijke Neyts
SGS	Varna (BG)	Aleksandar Panayotov/ Veselka Pashova
Petrobas	Rio de Janeiro (BR)	Fabiana D. C. Gallotta
EC-ALET	Moncton (CA)	Rene Losier
ECTD	Ottawa (CA)	Chun Yang
PESC-EC	Vancouver (CA)	Dayue Shang
NCSEMC	Qingdao (CN)	Zhou Qing/Wang Xinping
BSH	Hamburg (DE)	Gerhard Dahlmann
EERC	Tallinn (EE)	Juhan Tamm/Krista Mötz
CEDEX	Madrid (ES)	Maria Plaza
CSIC	Barcelona (ES)	Joan Albaiges
NBI	Helsinki (FI)	Ninna Viitala
CEDRE	Brest (FR)	Julien Guyomarch
LVA	Riga (LV)	Rita Skolmeistere
NFI	Den Haag (NL)	Rene de Bruyn
RWS-WD	Lelystad (NL)	Paul Kienhuis
Sintef	Trondheim (NO)	Kristin Sorheim/Per Daling
SKL	Linköping (SE)	Helen Turesson/Magnus Kallberg
ERT	Edinburgh (UK)	Gordon Todd

Table 1
Received results

The laboratories received 6 extracts from an artificially composed case based on a real case. In chapter 3 the real scenario is described.

In chapter 4 the RR2009 case is evaluated according to the evaluation scheme given in Fig 1 of CEN/TR 2v2. Mainly figures and citations from some of the individual reports of the participants are used for illustration.

Based on the sample composition of the artificially prepared oil spill identification case the following issues will be addressed in the chapters 5 to 10:

1.1 The MS-PW-plot: a powerful tool in oil sample comparison

PW-plots were a major issue at the Bonn-OSInet meeting in Tallinn (April 2009) and will be an essential part of CEN/TR 2v2. It is discussed here, how to use and tread these graphs. A spreadsheet file that can be use to generate the MS-PW-plots, has been provided to the participants.
To test the basic variation of the PW plot two samples from the same extract (sample 1 and 2) have been send to the participants to be sure to receive at least one "duplicate" analysis from each participant.

1.2 Mixing

In the working groups and meetings mixing has been an important point of discussion. What is mixing and how do we have to deal with it. According to CEN/TR 2v2 differences caused by mixing, can in some cases result in a positive match.

1.3 Low sulphur content

There is a general wish to reduce the amount of sulphur in oil. Refineries are able now to reduce the amount of sulphur in fuel to very low concentrations. More and more cases show samples with reduced or absent sulphur containing compounds and compound groups like the benz(di)thiophenes. These compounds however are part of the normative ratios. Does the method still work on this kind of samples?

1.4 Biodiesel.

5 to 10 % mixing of biodiesel in mineral oil based diesel is quite common nowadays in many countries. Can the addition of biodiesel be recognized with the analytical method applied and if so can it be used for comparison.

1.5 Reduction of the C1-pyrenes in spill samples

Many of us have observed this effect (presentations of Niina, Magnus and Joan at the meeting in Tallinn). Consequences will be discussed here.

1.6 Biodegradation

In all our round robins since 2004 weathering has been mainly based on evaporation. So it becomes time to test the effect of biodegradation on spill samples in a Round Robin. We realize that the simulation of the biodegradation process as performed by Joan Albaiges can be different from real situations, but nevertheless led to interesting results.

2 Instructions

On 23-9-09 the instructions for RR2009 were published on the Bonn-OSINET forum:

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

Subject
Oil Spill Identification Round Robin 2009

Dear Colleagues,

This is the fourth oil spill identification intercalibration round within the Bonn-OSINET expert group.
We have prepared six samples for comparison.

Scenario and sample information:
The samples are from an oil spill in a harbour in the south of the Netherlands.
Samples were taken from the bilge of an inland transport ship with three engines above a large bilge. To be sure to have a representative sample, 4 samples have been taken from the bilge on different spots and levels on 23 April 2009 at about 21:10.
These samples are indicated with RR2009-1 to RR2009-4.
RR2009-5 (Spill) is from around the ship taken on 23 April 2009 at about 15:30.
RR2009-6 (Spill) was taken after 10 days. Some oil was found under a floating scaffolding and the sample taker wanted to know whether the oil is related to the same case or from another spill.
You will receive extracts of the samples dissolved in DCM at a concentration of 30 mg/ml.

Method
At the meeting in Tallinn we have discussed the second draft version of the new version of CEN Technical Report 15522-2. I have implemented the comments and Gerhard and I are working on finishing the third draft version. It will be ready and copied to the Bonn OSInet forum at around 28 September.
We would like to ask you (if possible) to use the method including the proposed analytical parameters described in the second and third draft version. (There are no essential differences between the two versions on this point). One of the tasks of Bonn-OSInet is to cooperate and give mutual assistance, and the use of the same analytical parameters would make it easier.

I have adapted the spreadsheet file we used already in 2007 according to the new CEN/TR, and would like to ask you to make use of it. We will use it to test the reproducibility of the data and maybe it can help you to evaluate the samples.

If you have activated “macro warnings”, a warning will pop-up. Simply click on “deactivate macros” (I use a macro to read in the quantification reports from an AGILENT MSD, but here it is not needed).

In the spreadsheet file you will find a sheet with “instructions” on how to use it.

Results have to be entered on the sheet “data sheet”.

From row A6 until A39 you will see the list of compounds needed to calculate the normative ratios (See Table 2 of the new CEN/TR). Below cell A39 you will find additional compounds and compound groups.

These informative compounds are not requested. I use the worksheet for my own evaluation and make a selection of the informative ratio's (or don't use them) depending on the case. I developed it as a flexible tool for comparison. It is a personal selection of informative ratios. You are free to make (partly) use of it or not.

The spreadsheet file I send to you contains data of the case I have used as an example in annex J of the new CEN/Tr. Just to show you how it looks when data have been entered.

To make use of the spreadsheet select the Table “data sheet” and then select the range D6: O75.

Delete the values and start to enter your own results.

Replace the sample information in the range E2:O4. E.g. sample information for sample 1 can be entered in the range E2 to E4: E2 for a short description, E3 for the file name, E4 for e.g. a case/ sample number. The information is used in the evaluation sheets.

Note: Do not draw results with your mouse from one cell to another cell. Then a calculation error messages will be seen in the evaluation sheets.

Parameters

In the Excel- file you will also find a “parameters” sheet. Here you can enter the parameters of the MS analysis. An example with the parameters of RWS-WD(NL) is given.

Reporting

We would like to ask you to compare the two spill samples with the four samples from the ship. Information achieved by comparing the 4 samples of the ship with each other can be used to interpret the comparison of the spill samples with the ship's samples.

Generally, any two combinations out of a set of six samples would result in 15 sample comparisons. But this seems to be too much, with regard to the time needed, the length of the reports and the overall result evaluation. So we like to ask you to report only the comparison of sample 1 with the other samples (So only sample combinations 1-2, 1-3, 1-4, 1-5, 1-6) and send in a report by email. The resulting report should not only consist of a simple non-match, inconclusive, probable match or match, but also reasons why a decision has been taken, should be given. The original reports will be combined in a final report, together with an evaluation of the overall results.

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

Time schedule

14-9-09: The samples have been sending out.

28-9-09: The third draft version of the CEN/TR will be copied to the Bonn OSInet forum. You will be informed.

First week of November: Reports have been returned.

Medium December: Final report will be sent to the participants.

Next Meeting: Linkoping, Sweden 2010, April 13-15th, dealing with the results of RR2009 and other issues.

3 Real scenario

3.1 Samples

A case with a diesel spill, showing the reduction of the C1-pyrenes (see chapter 8), has been used as base for the samples. RWS-WD receives one or two cases each year in which the reduction of the C1-pyrenes is clearly visible. The amount of spill sample of this case was sufficient to be able to prepare samples for this intercalibration. The diesel also contained an amount of fatty acid esters (biodiesel) and the sulphur content was extremely low.

In order to test the influence of mixing, lubricating oil (unused carter oil) was added to both the source and spill sample. To realize some variation 5, 6 and 7% was added to the source diesel and 5% to the spill diesel (Fig. 1)

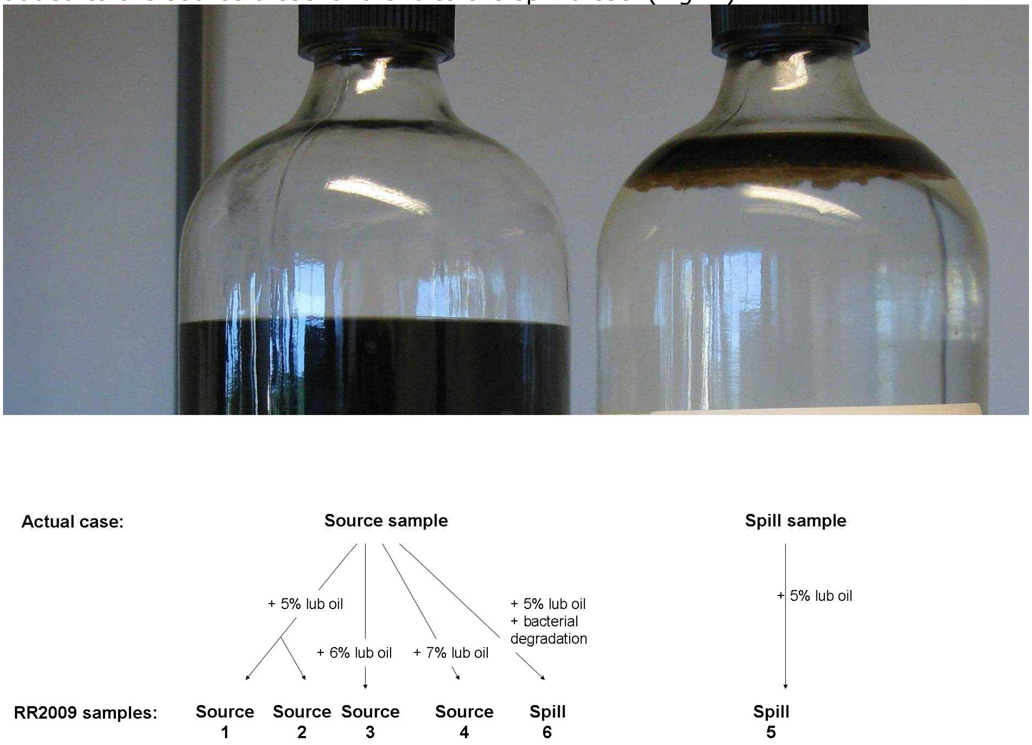


Fig. 1
Photo of the samples of the original case and a scheme that shows the lubricating oil addition.

The mixtures of diesel and carter oil simulate typical bilge samples.

Joan Albaiges (CSIC) applies a method to simulate biodegradation on oil samples. For RR2009 sample 6 he has used the following method on 4 ml of the source diesel + 5% carter oil mixture:

The sample was inoculated with a marine autochthonous hydrocarbon-degrading consortium growing in diesel oil as a sole carbon source.

50 mL of the growing culture were pelleted in order to separate the biomass from the diesel to avoid hydrocarbon contamination.

The pellet was resuspended in 10 mL of sterile artificial seawater.

The culture medium was prepared as follows:

- 250 mL open flasks containing 50 mL of artificial seawater (Scharlau), 5mM NH_4NO_3, 0,35 mM K_2HPO_4, 0,5 mL of the inoculum and 250 µL of the oily product.
- Flasks were incubated for 10 days at 150 rpm and 25ºC.
- After this period all flasks were liquid-liquid extracted with CH_2Cl_2 (3 x 15 ml), the extracts combined, dehydrated through anh. Na_2SO_4 (2 g) and rotatory evaporated.
- The final extract was gently evaporated under nitrogen.

3.2 Composition of the source and spill samples

In Table 2 the sample compositions are shown.

Source samples	Source diesel case	Carter oil	Dissolved in DCM	% Carter oil
1 and 2	8005 mg	406 mg	200 ml	5.07
3	4011 mg	240 mg	100 ml	5.99
4	4023 mg	284 mg	100 ml	7.05
Spill samples	Spill diesel case	Carter oil		
5	4011 mg	201 mg	100 ml	5.01
6	Is solution 1-2 treated by Joan Albaiges			

Table 2
The compositions of the RR 2009 samples

Sample 1, 2 and 6 are from the same mixture solution.

4 Evaluation of the case following CEN/TR 15522-2 draft 3

In this chapter the RR2009 case is evaluated following the flowchart shown in Fig 1 of the CEN/TR 2v2. Figures and quotes from some of the individual reports of the participants are used for illustration.

4.1 GC-FID

GC-FID-screening revealed that all 6 samples consisted of light fuel (Diesel-) oil: the boiling ranges ended at C27, i.e. at about 420°C.

4.1.1 FAME

But when an n-alkane distribution such as in Fig. 2 is seen in an oil sample, the alarm-bell must ring immediately:

> “In GC FID chromatograms, source samples presented nC21 in a different pattern from that expected for a modal distribution of light fuel oil. The differences are probably due to a co-elution at nC21 since this behaviour was not observed for GC MS results” (Petrobras, Br).

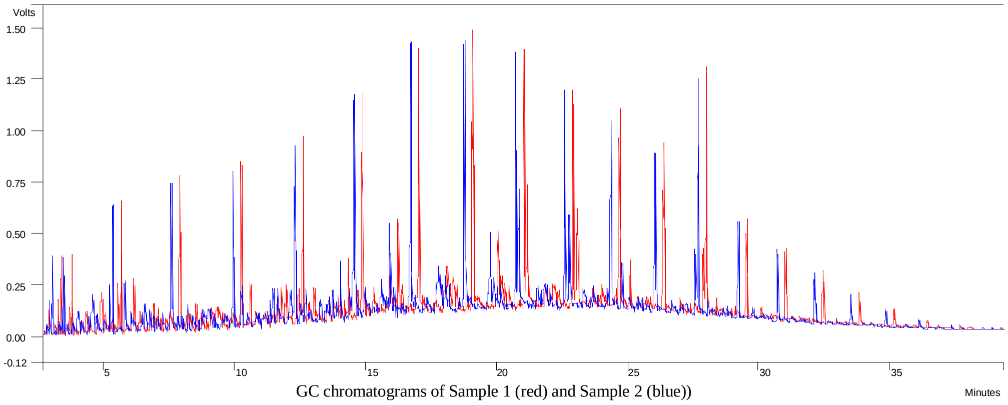


Fig 2: Overlay of sample 1 with sample 2
LVGMC, LV

But, depending on the separation characteristic of the GC-system, this additional peak is also separated from C21:

In the figure below (Fig. 3) only Sample 1 is shown. The small insert in the figure shows a standard Swedish diesel oil with the addition of FAME (for vehicle use). (SKL, SE: HP Ultra 1 column))

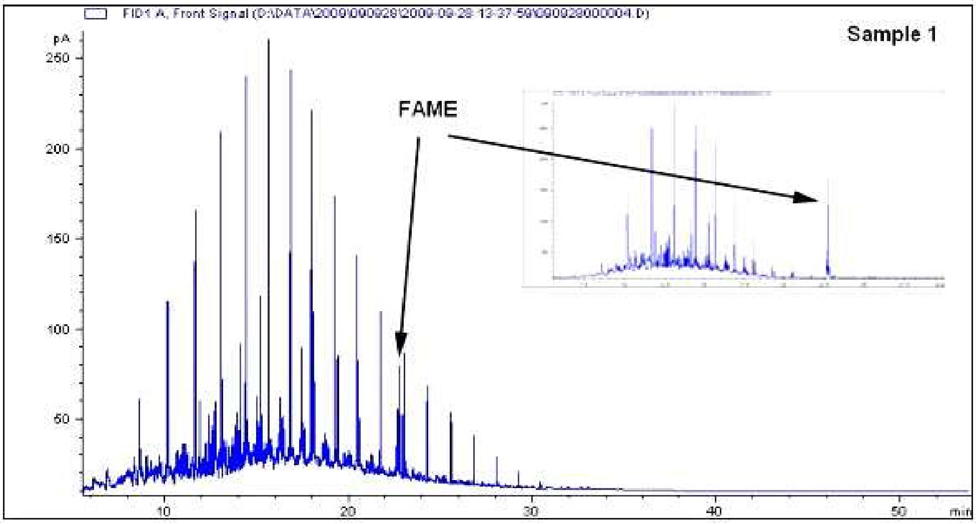


Fig 3
GC-FID Sample1 (SKL, SE)

Further, uncommon peaks were found:

“Some “Ghost” peaks appeared in sample 1 ~ sample 5. And there’s only slightly abnormality on sample 6. The area of exceptional peak (C21) of sample 5 is less than sample 1~sample 4, and greater than sample 6” (NCSEMC, Ch).

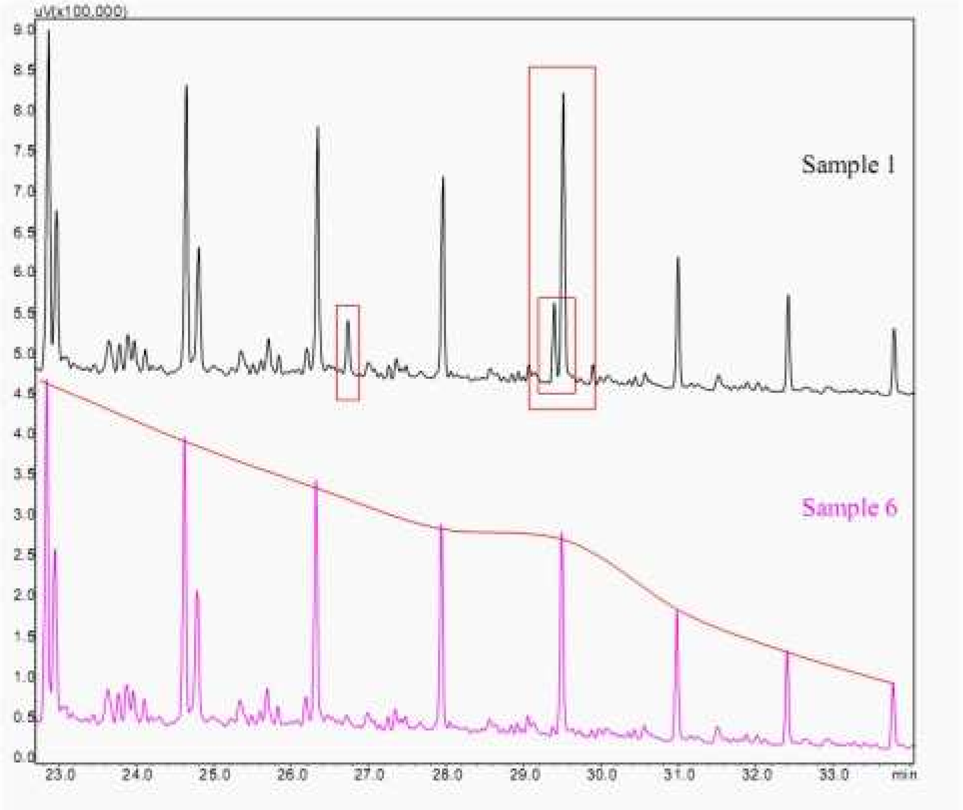
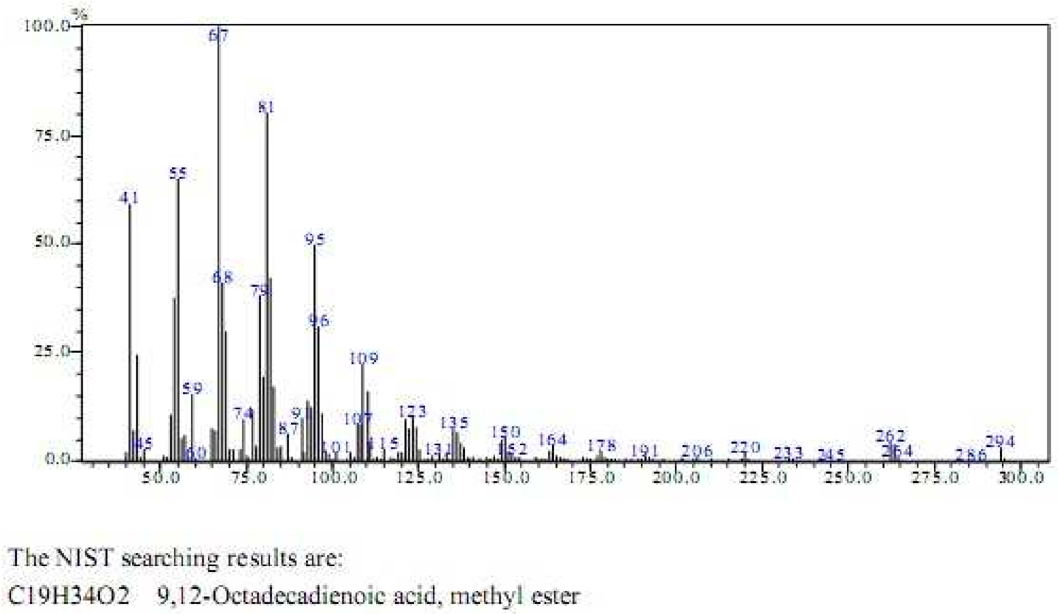


Fig 4
GC-FID samples 1 and 6
(NCSEMC, Ch)

Thus, those additional peaks turned out to be important, because there was a reduction from the ship samples 1 to 4 over spill 5 up to spill 6. This means that a difference appeared between the ship- and the spill samples –a difference, which had to be discussed.

A full-scan run -together with a library search- is needed to determine the nature of the additional peaks:



The NIST searching results are:
$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid, methyl ester

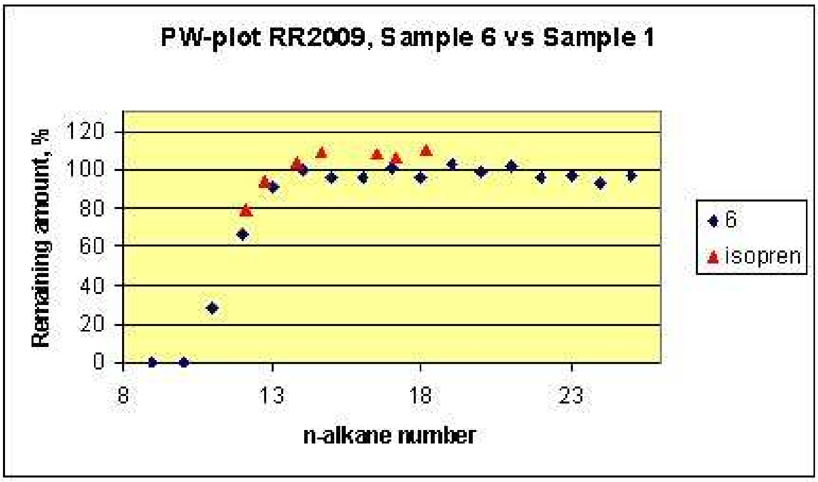
Fig 5
Mass-spectrum of the peak near C21 and library search result (NCSEMC, Ch)

“All the searching results turn out to be unsaturated or saturated fatty acid esters” (NCSEMC, Ch).

Thus, the additional peaks were FAME (see Fig. 3), known also as “Biodiesel”.

4.1.2 Bacterial degradation

The effect of artificial biodegradation of sample 6 was low, and only a slight decrease of the n-alkanes could be observed –if at all:



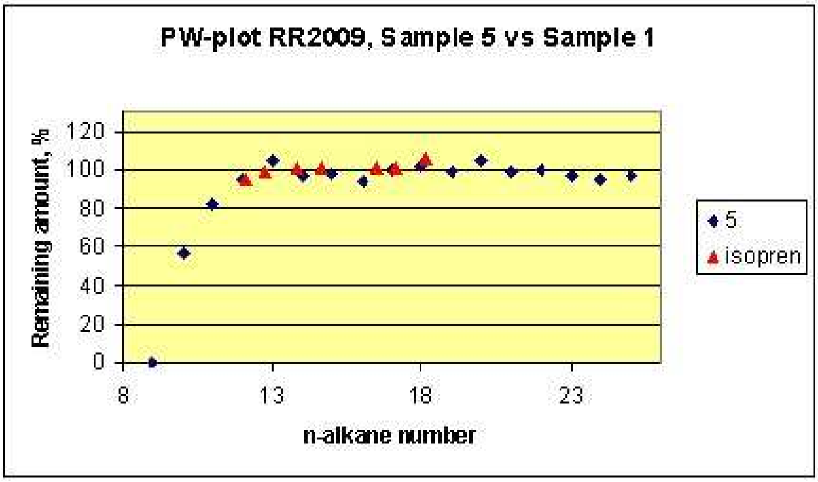


Fig 6
GC-PW-plots (SKL, Se)

But the handling of sample 6 rather led to increased evaporation and a strong reduction of the FAMES (see Fig. 4).

4.1.3 GC-FID screening, Preliminary conclusion

The samples consist of Diesel, which contains a (small) proportion of Biodiesel:

"The PW plots and the GC FID chromatograms indicated the following assumptions:
- All the sources are possible matches.
- Source 1 may be the source of Spills 5 and 6, considering weathering processes." (Petrobras, Br).

4.2 GC-MS

4.2.1 Mixing

Astonishingly, the initial assumption that merely compounds in the Diesel-oil range were present must be revised: biomarkers are present in very well measurable, but different concentrations in the samples:

"The PW plots based on Source 1 (reference), suggested mixing of 2 different oil types in different concentrations for samples Source 1 and Source 3 (Figure 7). The compounds eluting after 40 min are close to 130%. This could be explained by a higher concentration of lubricating oil in LFO for Source 3 when compared to Source 1. In this case, the lubricating oil fraction present in the samples was not high enough to be visualized in GC FID chromatograms. The same behaviour was observed for source 4 (Source 1 as reference), where compounds eluting after 40 min are close to 150 % (Figure 9). Therefore, it is plausible that Source 4 contains a higher amount of lubricating oil than Source 3" (Petrobras, Br).

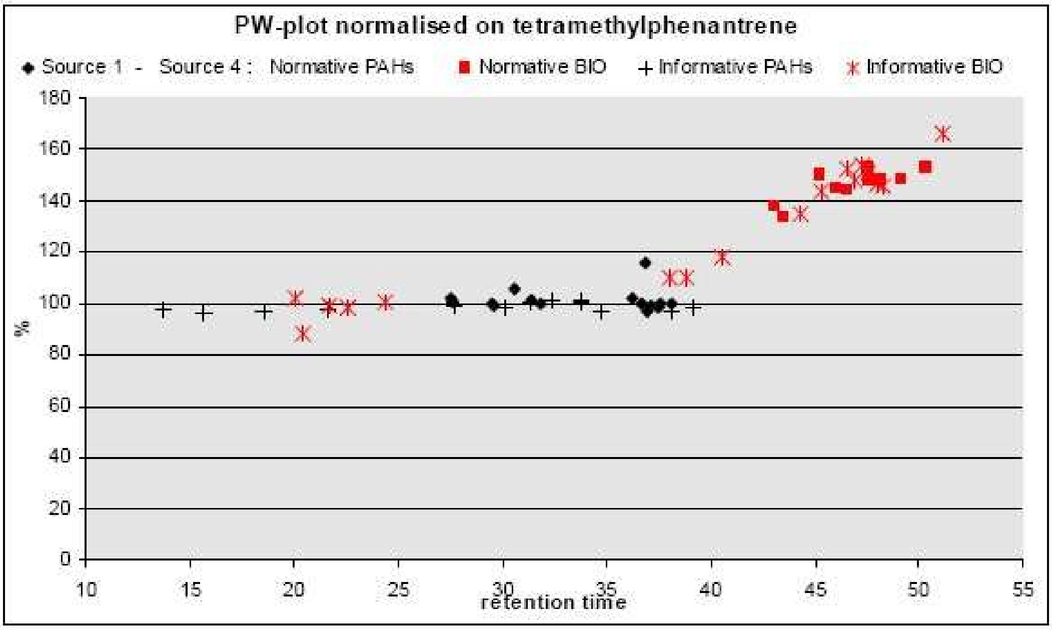


Fig 7
Source 4 vs source 1
(Fig.9 ; Petrobras, Br)

In fact, the samples definitely contain lubricating oil, additionally, although there is not the slightest sign of an additional unresolved hump in the GC-FID chromatograms.
The biomarkers cannot originate from the fuel: Lubricating oil is present in all 6 samples:

- There is a break in the PW-plots of samples 3 and 4 (based on sample 1): all alkanes and aromatics are at 100%, whereas all biomarkers are considerably higher. The situation is the same as described in the CEN-TR 2v2; Annex I.5.
- There is an increase of the biomarkers from sample 3 to sample 4 (sample 1 as reference)
- Despite, ratios and clusters of the biomarkers are the same in all 6 samples
- Aromatic steranes are missing.

The DR's comparison does not indicate the differences caused by mixing (Figure 8).

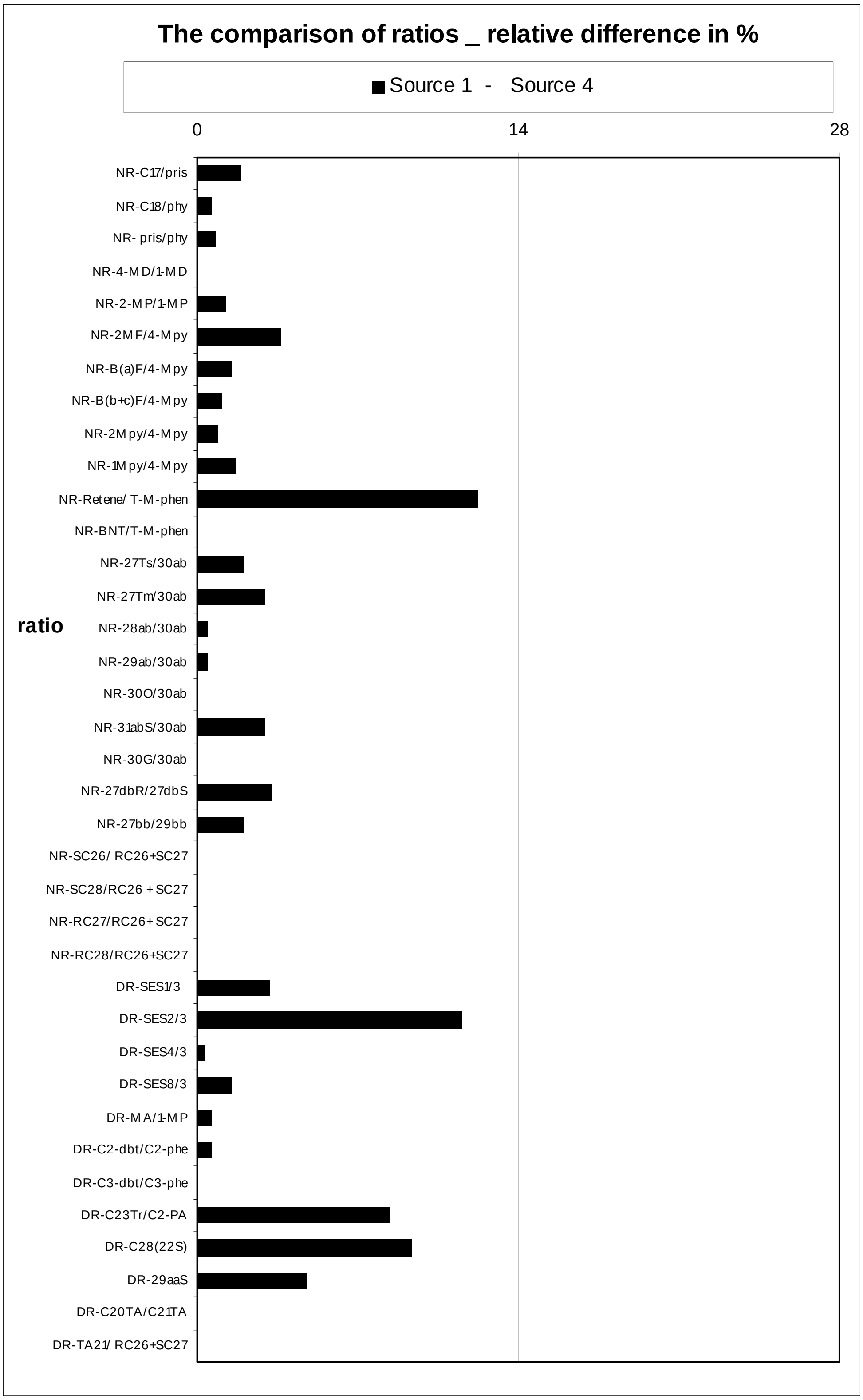


Fig 8

Ratio comparison of source 1 vs source 4 (Petrobras, Br)

4.2.2 Low sulfur content

BNT is not present:

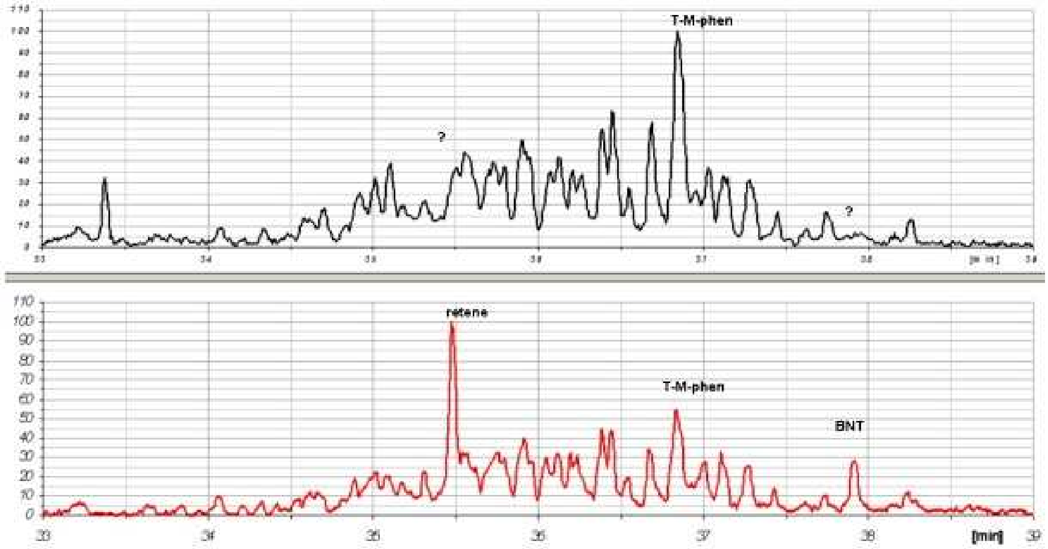


Fig 9
m/z 234, sample 1 above, typical pattern of m/z 234 below

"Unknown" peaks appear in m/z 198 originating from fragments of other compounds, e.g. alkanes:

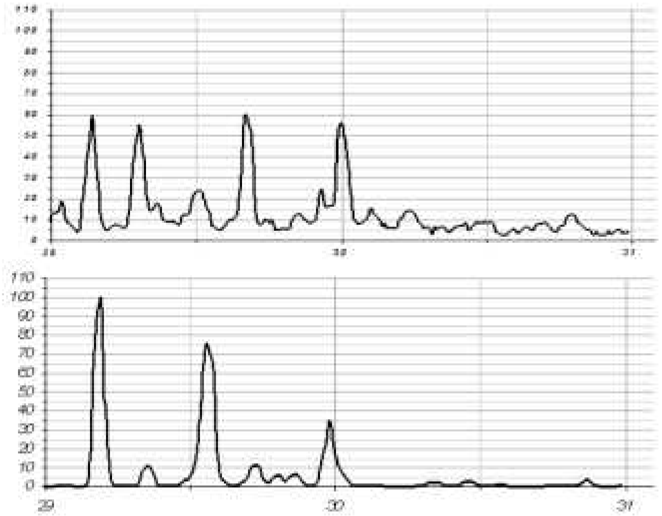


Fig 10
m/z 198, sample 1 above, typical cluster of DBTs below

4.2.3 Reduction of the C1-pyrenes

As given in the description of the actual case, differences appeared between spill sample and comparison sample: a reduction of the C1-pyrenes was observed in the spill sample. The differences can be seen in the chromatograms, but can also easily be detected in the MS-PW-plot of sample 1 versus sample 5:

“Figure 4.4: Spill sample 5 compared with source sample 1: The variability of the analysis is mainly between 85-118 %, and there is not observed any evaporation of the lightest components except for the slight decrease of the C1-decaline and naphthalene. However, there is observed a significant difference in m/z 216 between these samples with a critical difference higher that 14 % (refers to Figure 4.10). A significant relative reduction in the content of 1-MPy and 4-Mpy in sample 5 is observed. It is difficult to have an explanation of this, but it may due to lack of robustness of m/z 216 caused by photo-oxidation. However, this assumption has to be further documented”. (Sintef, NO)

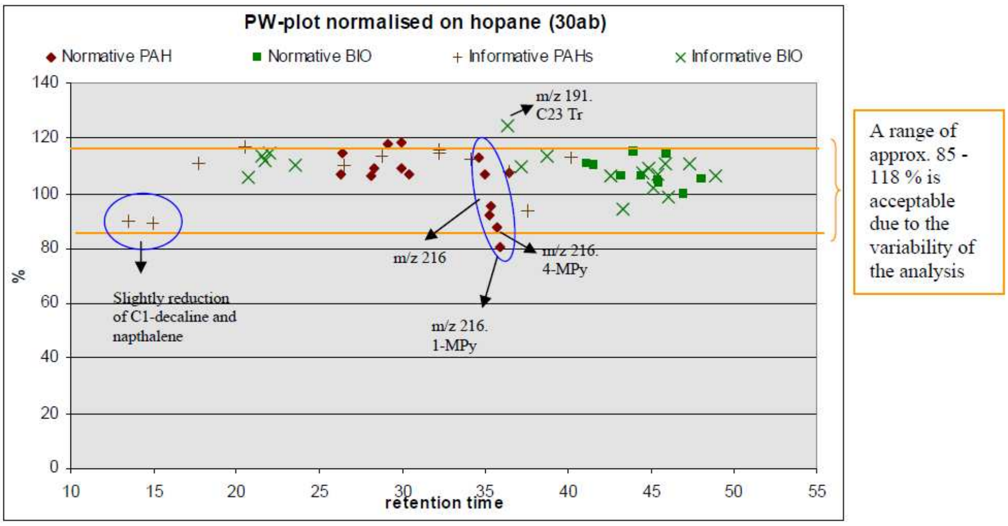


Figure 4.4 MS-PW plot. Relative intensity in % of peak height of PAH and biomarkers of the spill sample 5 (SINTEF ID: 2009-0733) compared to ship sample 1 (SINTEF ID: 2009-0729), after normalization relative to hopane (30 ab), m/z 191.

Fig 11
MS-PW-plot of Sintef, NO comparing source sample 1 with spill sample 5.

Steve Fuller (AU_NSWDECC) remarks:

Two diagnostic ratios exceeded the critical difference; both of these ratios relate to the methyl pyrenes. Methyl pyrenes can undergo degradation, possibly due to photo-oxidative processes. In laboratory tests we have found that this degradation occurs more rapidly with biodiesel blends. The greatest change in relative abundance among the pyrenes was observed with the 1-MPy isomer; advice in method CEN/TR 15522-2 was that this isomer degrades the fastest. There was no age estimate provided for Spill Sample 5 to assist with the evaluation. The m/z 230 profiles also exhibited some differences, which are possibly related to photo-oxidative weathering. The remaining diagnostic ratios are all less than 6%.

4.3 Conclusion

By going through the Analytical Scheme of the CEN-TR step by step, all the difficulties included/produced here in RR2009 should have been recognized –except, may be, the a little bit too low effect of the artificial bacterial degradation.
The “truth” could have been found out, i.e. a match at least between samples 1,2,3,4 and 6. A small uncertainty could have remained, as to whether a match or a probable match is the right conclusion, when sample 1 is compared with sample 5.

5 MS-PW-plot evaluation

5.1 General remarks

In CEN/TR 2v2, the MS-PW-plot is introduced as an additional, powerful tool for result evaluation. In the earlier version, published in 2006, the advice was given, for example, to use only the non-weathered compounds in ratio comparison.
But now, even biggest differences caused by weathering may be used and useful, and may contribute to the proof, that the samples have been identical before weathering occurred, i.e. at the time of the discharge (see Figure 12).

Note
Annex I of CEN/TR 2v2 gives basic information about the MS-PW-plot

In chapter 5 the results of the participants are discussed anonymously

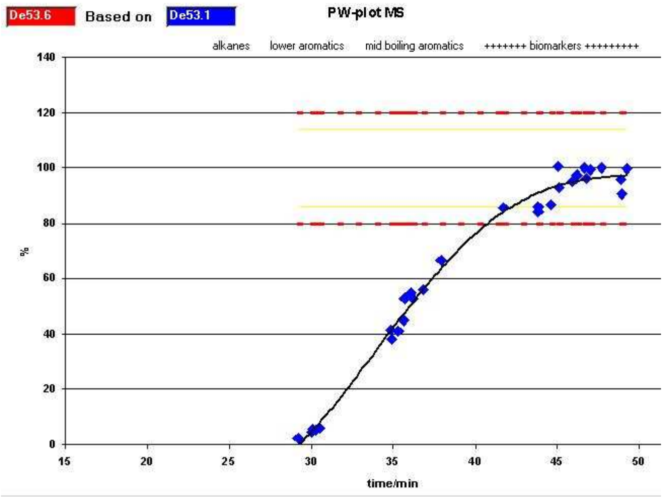


Fig. 12
RR2007-results: MS-PW-plot of strongly weathered (evaporated) sample 6 compared with spill sample 1 (BSH, De).

The second big advantage of these plots is the recognition (and proof) of mixing.

In these PW-plots, all peak measurements from the different mass-chromatograms are related to the higher boiling hopane (m/z 191), or, in case hopane is not present, to TM-Phen (m/z 234).
But this is also problematic, both from the chromatographic side as well as from the spectrometric side, and it has to be ensured that the whole system is stable. Of course, the system should be checked regularly for mass-discrimination, and mass-spectrometric tuning between samples to be compared should not be done. Generally, it is advised to run a standard oil before and after –or may be also in between- a series of measurements, and rely only on those results, where the standard has shown that the system is stable.

5.2 Samples 1-2

Since samples 1 and 2 of this Round Robin are definitely the same (taken from the same solution), the variation of the data-points in the plots of samples 1-2 merely shows the influence of the analytical method, i.e. the analytical variation.

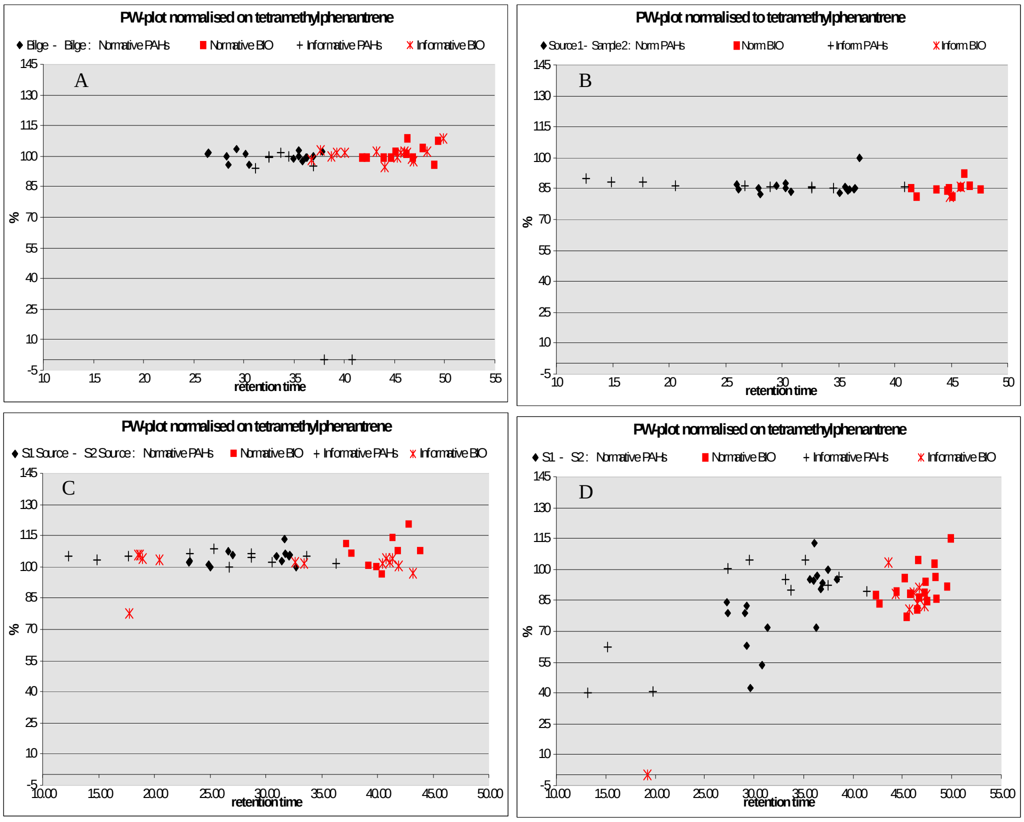


Fig 13

Examples of MS-PW-plots for the comparison of samples 1-2.

Fig 13 shows the results of four participants.
A very low variation around 100% can be seen in MS-PW-plot A.
Also Plot B shows very low deviations from a straight line. But this line is at 85%. Only the compound that is used for normalization (here TMP) is at 100%. Of course, in such a situation, the first idea must be to check the outlier. It can definitely not be expected that there is a true difference in the two samples in TMP, if –as in this case- everything else matches (including the results from the three previous analytical steps).
Checking outliers is also necessary in C. This plot shows some more variation of the data points, while most of the data points are in the range of 100 to 115%. Only a few points are out of this range.

Plot D is definitely not acceptable. The high variation of the data points of D indicates that the system is not in control –a fact, which should have been found out by the quality management of the laboratory BEFORE these samples were analyzed.

It must be pointed out here that the MS-PW-plot is a new tool for most participants. Some of them even did not have a look at their plots and did not spend any word about these plots although they have spent much time for filling in their data into the Excel-spreadsheet, whereby these plots were automatically produced.

Generally, the evaluation of the MS-PW-plot is the same as the evaluation of the compound ratios: deviations from the 100%-line must be interpreted in the same way as the deviations of the compound ratios (analytical errors or true differences?).

But in addition, this test is sensitive for weathering of any kind and for differences between spill and source samples, which are caused by inhomogeneous distributions (mixing) -these are even the reasons, why this test is conducted.

Thus the data points of compounds not influenced by weathering and/or mixing should be at the 100%-line. Since the MS-PW-plot is the last step/test in the analytical procedure, bigger differences may not be expected. As with the compound ratios, outliers must be checked (possible reasons: small and/or overlapped peaks, and different positions of the baseline during peak measurements).

Note

Some participants have indicated that they had bigger laboratory restrictions, e.g. there was not enough time, and the GC/MS-instrument was mainly used for other purposes. But one has to bear in mind here the possibly high consequences of forensic investigations. It is simply not done with the fast analysis of 3 samples in case, 3 samples are sent in the framework of a criminal proceeding.

In order to find out the error limits of the MS-PW-plots, and to define an acceptable range, into which the data-points of identical samples should fall, the results of the comparisons of sample 1 with sample 2 are used in the following discussion.

A table has been added to each spreadsheet file that we received, on sheet “1-2” at cell U2, in which mean values and standard deviations where calculated.

Table 3 shows the resulting table of participant B based on TMP as normalizing compound. To be able to fit the table on one page about half of the compounds have been removed. The mean and standard deviation however are based on the original table.

Table 3 shows a range of compounds in the left column. The compounds above the central line are the normative compounds shown in Table 2 of CEN/TR 2v2. The compounds below the central line are informative compounds given there. The second column of Table 3 indicates whether the measurement is based on area (**A**) or height (**H**)
RWS-WD applies this spreadsheet file for all oil cases in which samples have to be compared. The normative compounds are measured according to the sample type mentioned in Table 2 of CEN/TR 2v2 and according to the rules for excluding non-existing or small peaks. Depending on the samples to be compared in an oil case, some to all of the informative compounds are also integrated.

Table 3 shows as first criterion **all>1**
In this column measured values are shown; the integration result should be more than 1. E.g. 11)NR-retene has not been integrated by participant B.
At the bottom of the column the mean and standard deviation of this selection is calculated.
Based on the standard deviation of the PW-plot(s_{PW}) the 95% borders are calculated relative to a theoretical mean of 100% (100 +/- 2 s_{PW}). So theoretically 95% of the data points are between 93 and 107%. In practice however the mean is 86% and the data points are between 79 and 93%. Based on all information available this difference might have mainly been caused by a different way TMP was measured in the samples.

PW plot evaluation based on normalisation compound **16) tetramethylphenantrene**									
compounds	**A**rea **H**eight						max height 1310699 13107	= 1% of max	
		all > 1	norm PAHs	Norm Bio	inform PAHs	inform Bio	H small <1%	H high >1%	area
1) NR-C17	H	87	87					87	
4) NR-phytane	H	82	82					82	
5) NR-4-Methyl Dibenzothi...	H	87	87				87		
8) NR-1-methyl phenanthrene	H	83	83					83	
9) NR-2-Methylfluoranthene	H	83	83				83		
10) NR-benzo(a)-fluorene	H	86	86					86	
11) NR-retene	H								
12) NR-benzo(b+c)fluorene	H	84	84				84		
15) NR-1-methylpyrene	H	85	85					85	
16) NR-tetramethyl-phenant...	H	100	100					100	
17) NR-BNT	H								
18) NR-27dbS	H	85		85			85		
23) NR-27-TM	H	81		81			81		
28) NR-29ab	H	86		86			86		
29) NR-RC27TA	H								
30) NR-30O	H								
31) NR-30ab	H	85		85			85		
34) NR-30G	H								
35) C1-dekalin	**A**	90			90				90
36) Naphthalene	H	88			88			88	
37) C1-Naphthalenes	**A**	88			88				88
39) C2 benzothiophenes	**A**								
40) C2-Naphthalenes	**A**	87			87				87
41) SES2	H								
45) C1-Fluorenes	**A**	86			86				86
48) C2-dibenzothiophenes	**A**								
49) C2-highest peak phe. a...	H	86			86			86	
50) C2 phenanthrenes anthr...	**A**	85			85				85
51) C3 dibenzothiophenes	**A**								
52) C3 phenanthrenes anthr...	**A**	85			85				85
53) C4 phenanthrenes anthr...	**A**								
59) C21 TA	H								
60) C1 chrysenes	**A**	86			86				86
62) C29 (22S)	H								
63) 28bbR+S	H	81				81	81		
65) 29aaS	H								
66) 29bbR+S	H	86				86	86		
70) 32abS	H								
		all > 1	norm PAHs	norm Bio	inform PAHs	inform Bio	H small <1%	H high >1%	area
Mean		86	86	85	87	84	85	87	87
st dev (s_{PW})		3.25	4.15	3.08	1.58	3.31	2.69	4.34	1.66
upper 95% line		107	108	106	103	107	105	109	103
lower 95 % line		93	92	94	97	93	95	91	97

Table 3
Table of participant B to evaluate the variance of the MS-PW-plot for samples 1-2

The next 4 sub-selections (normative PAHs, normative biomarkers, informative PAH's and informative biomarkers) have been made from the **all>1** criterion.

The standard deviation of these subsections are about the same as for the **all>1** criterion with an exception of the informative PAH's. This group, mainly cluster integrations based on area, shows a remarkable low value of 1.58.

The following criterion is used to differentiate between high and low peaks with an integration based on height. The maximum height value is the highest response value for all integrated compounds based on height. For these two samples (1 and 2) it is the peak height of C17. 1% of this maximum value is used as criterion to separate low and high peaks. E.g. the peak height of "5)NR-4-methyl dibenzthiophene" is lower than 1% of the peak height of C17, while the peak height of "8)NR-1-methyl phenantrene" is higher than the peak height of C17.
In general it is expected that small peaks are more difficult to integrate than high peaks, but the results of Participant B shows the opposite with an s_{PW} of 2.69 for the small peaks.

As last criterion the compounds integrated based on area are selected. The result shows an s_{PW} comparable with the s_{PW} of the informative PAH's, which are mainly based on area integration.

In the same way in each spreadsheet file, next to the table for normalization compound tetramethylphenantrene a second table is present for normalization compound hopane (30ab)

5.3 Assessment of an error range of the MS-PW-plot range by means of a statistical evaluation of the data.

For an assessment of the error range of the MS-PW-plot the s_{PW} is calculated from the combined data points.
To be able to eliminate outliers, the data points have been sorted on participant starting with the results with the lowest variance (See Table 4).
The standard deviation shown for participant 1 is the st. dev. of the data points of participant 1 for **all>1**.
The st. dev. shown for participant 2 is calculated from the combined results of participant 1 and participant 2.
The st. dev. shown for participant 3 is calculated from the combined results of participant 1, 2 and 3.
The st. dev. shown for participant 20 is calculated from the combined results of all participants.

Participants	**all>1** TMP st dev combined	 Mean combined	**all>1** hopane st dev combined	 Mean combined
1	2.5	99.9	2.6	101.2
2	2.8	100.1	3.7	99.0
3	4.3	102.1	4.9	96.5
4	5.1	100.2	6.1	99.0
5	5.9	101.7	5.8	98.5
6	7.7	99.7	5.7	98.9
7	7.2	99.8	5.7	99.6
8	7.2	100.1	5.6	99.5
9	6.9	100.3	6.5	98.3
10	6.9	100.6	7.3	97.1
11	6.9	100.3	7.2	96.7
12	6.9	99.7	8.2	98.2
13	6.9	99.7	8.1	98.2
14	7.0	99.4	8.4	98.9
15	7.5	98.9	9.2	99.7
16	10.3	100.5	9.5	99.6
17	13.3	101.4	11.4	99.4
18	15.0	101.0	17.2	101.3
19	21.4	100.9	23.4	101.4
20	24.4	100.9	26.3	101.4

Table 4
Cumulative standard deviation of the PW-plot

Table 4 shows an increasing standard deviation with a flat part in the middle. This is visualized in Fig 14.

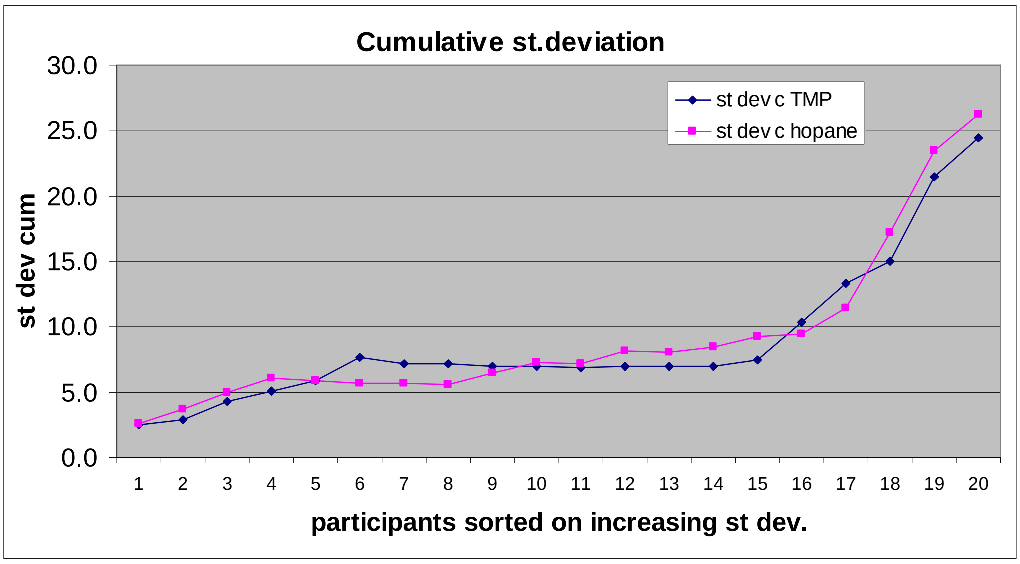


Fig. 14
Cumulative standard deviation of the PW-plot based on the **all>1** results

In the same way the results of the sub-selections with TMP as normalizing compound have been evaluated (See Fig. 15).

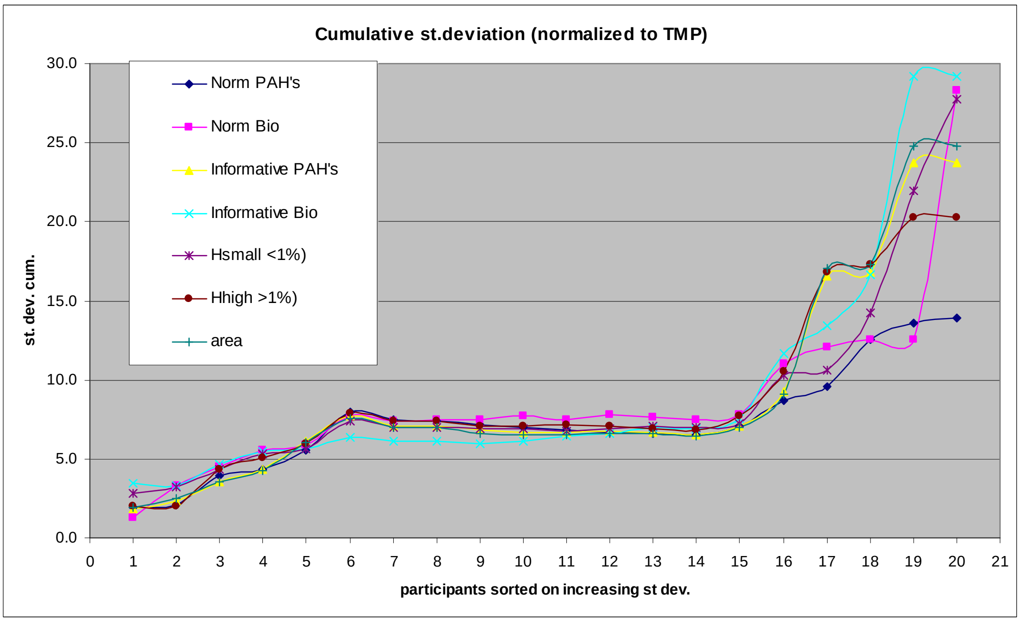


Fig. 15
Cumulative standard deviation of the PW-plot for the sub-selections with TMP as normalizing compound.

Fig 15 shows that the cumulative st. dev of the PW-plot is almost independent for the type of sub-selection. The integration on peak height of high peaks and low peaks results in the same cumulative st. dev profile. Also group integrations of PAHs, based on area, result in about the same cumulative st. dev. profile.

Using the flat part of the graphs as acceptable s_{PW}, results in a value of 7 to 8. The mean of the combined results is close to 100%. So the 95% error range will be 100+/- 2*7.5 = 100 +/- 15%.

Note
Paul Blomstedt received his MSc in statistics from the University of Helsinki in 2007 and is currently working toward the PhD degree at Åbo Akademi University. For his thesis he is at the moment working at the Finnish forensic institute (NBI) in order to implement statistical methods in forensic science.

The statistical evaluation of the PW-plot results for samples 1-2 have been discussed with Paul Blomstedt. His comment on section 5.3:

As for the approach in section 5.3, I see no problem with that. If I understood correctly, it simply sets the accepted range to 100% +- 2*(combined std. excluding outliers). It makes direct use of an empirical assessment of the combined standard deviation.

From a mathematical point of view the error range should be 85 to 118%. Choosing one of the samples as 100% and calculating the results of the other sample relative to it has effect on the difference to the mean of 100%. The mathematical effect is shown in Table 5.

Sample 1 peak height	100	100	100	100	100	100	100
Sample 2 peak height	100	95	90	85	80	75	50
Sample 2/Sample 1 in %	100	95	90	85	80	75	50
Sample 1/Sample 2 in %	100	105	111	118	125	133	200

Table 5
MS-PW-plot data point calculation. Influence on the error range by choosing one of the samples as reference to the other.

For practical reasons still a range of 100% +/- 15% has been choosen. Also because this range is just an upper range for what is acceptable when comparing an in duplicate analysed sample extract.

5.4 Conclusions

The assessment to estimate the variance of the MS-PW-plot results in a range of 85 to 115%. This range is assigned as a maximum acceptable range when comparing in duplicate analysed sample extracts. When such a comparison leads to outliers the integration and maybe even the method should be checked and improved.

In translating this range into a range to be used in real cases, it should be emphasised that:

- The range is calculated/estimated based on duplicate analyses in which the variations are only caused by the method. For real cases differences in sample treatment, sample composition and injection concentration will increase the variation.
- The calculations are based on the results of participants that for a large part are not used to apply MS-PW-plots. The PW-plot itself informs about outliers based on mistakes in the integration. So it may be expected that the variance will reduce, when users gain experience with the plot.

In general the 85-115% range should be used as guidance for an acceptable range for the comparison of oil spill identification samples.

6 Lubricating oil and mixing: Samples 1-3-4.

6.1 Lubricating oil.

Most ships build for inland waters have diesel engines. Related spills can be pure diesel, but also mixtures of diesel and lubricating oil.
Spills of diesel fuel are mainly related with ships bunkering (and guessed to be non-intended discharges as fuel is expensive), spills of mixtures of diesel and lubricating oil are related to the discharge of bilge.
Older ships use diesel engines with an open carter and can use several hundreds of litres of lubricating oil a week. The used lubricating oil is collected in a bilge, a large open space below the engines, together with other oily waste products and water leaking along the propeller shaft(s). The combined mixture is also called "bilge". Normally the bilge is cleaned when the ship is serviced, but in case of engine problems or more than normal leakage of water, the bilge is partly emptied by pumping the water layer overboard. It sometimes happens that also oil is discharged.

6.2 Mixing

Typical for mixtures of diesel and lubrication oil is that many of the compounds that are used for comparison are only present at a higher level in one of the products. Pure diesel contains high concentrations of aromatics and low to very low concentrations of biomarkers.
In Fig. 16 the m/z 191 ion chromatograms of the diesel and carter oil used to prepare the mixtures for RR2009 are shown.

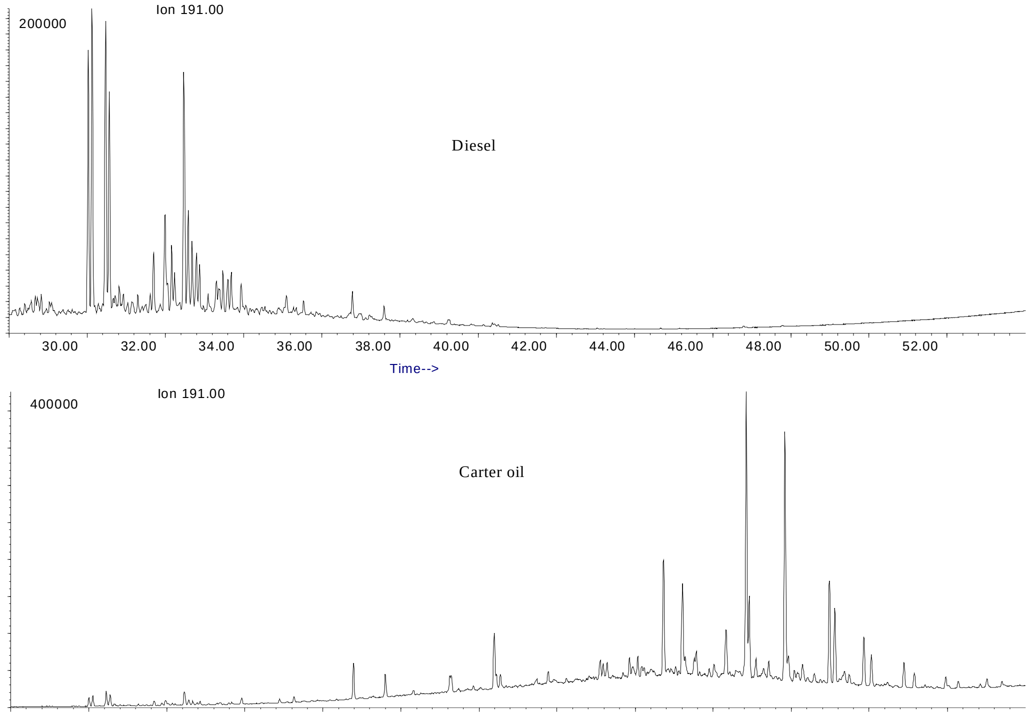


Fig 16
M/z 191 chromatograms. Original Diesel and Carter oil used to prepare the mixtures of RR2009

In the production process of lubricating the refinery removes the alkanes, while the aromatic compounds, including the aromatic steranes, are changed to statured compounds.

Fig 17 shows the m/z 231 chromatograms of Brent crude oil, the original source diesel and carter oil and the 5% mixture of diesel and carter oil (sample 1).

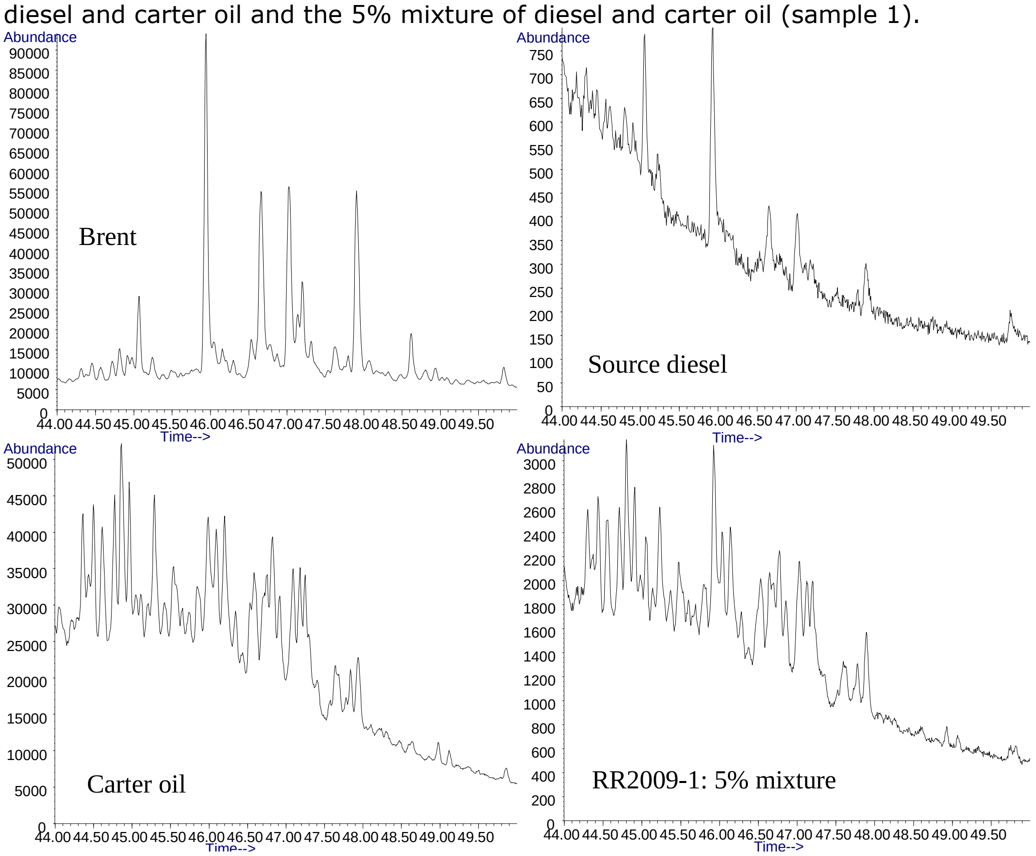


Fig. 17
M/z 231 chromatograms showing the aromatic steranes concentration.

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 4α-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.

In CEN/Tr 2v2 Table 2 the aromatic steranes are excluded from the normative ratios for lubricating oil and bilge, while it can be found in annex H:

> One of the most significant findings for characterizing oil products is that aromatic steranes (mass fragment 231) are not present in commonly used lubricating oils (Figures H.11 and H.12, see note).

Many of the participants are not acquainted with bilge samples. Who has recognised the low amount of lubricating oil, increasing from sample 1=2 over sample 3 to sample 4?

Table 6 shows the identification of the participants for the RR2009 samples.

Participant	**presence of lub oil** mentioned	**TAS** Not measured	**MS-PW-plot** conc. diff
AU_EC_CES		1	1
AU_NSWDECC	1	1	1
BE_BMM		1	
BR_Petrobas	1	1	1
BG_SGS		1	
CA_EC_ESC		1	
CA_ECTD			
CA_PESC_EC		1	
CN_NCSEMC	1		1
DE_BSH	1	1	1
EE_EERC		1	
ES_CEDEX	1	1	1
ES_CSIC		1	
FI_NBI			
FR_CEDRE			
LV_LVGMC			
NL_NFI	1	1	1
NL_RWS-WD	1	1	1
NO_Sintef	1	1	1
SE_SKE	1	1	1
UK-ERT		1	

Table 6
Report results of the participants related to the addition of lubricating oil.

About half of the participants have mentioned the presences of lubrication oil in their individual reports, while most of the participants did not measure the aromatic steranes.

6.3 MS-PW-plots

Comparing sample 1 with samples 3 and 4 means comparing mixtures of two petroleum products in slightly different concentrations.
The effect on the MS-PW-plot can be seen in the figure on the front page and in Fig. 7.

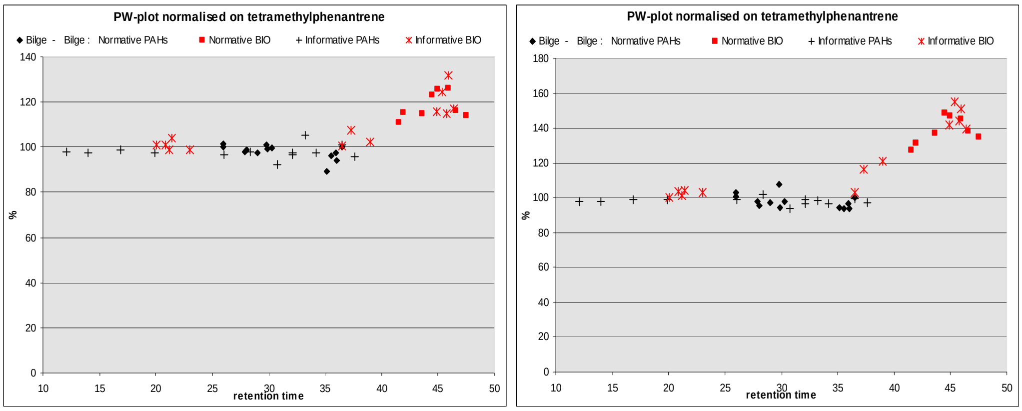


Fig 18
MS-PW-plots of AU -EC_CES for sample comparisons 1-3 (left) and 1-4 showing the difference in concentration of the lubricating oil

Fig 18 shows the results of AU_EC_CES for the sample comparisons **1-3** and **1-4** normalized to TMP. Sample 1 contains 5% lubricating oil while sample 3 contains 6%. So sample 3 contains 120% of lubricating oil compared to sample 1. In the same way the 7% of lubricating oil in sample 4 leads to a relative concentration of 140%. Both values are very well visible in the PW-plots in which the biomarkers vary around 120 and 140%. In the right plot some of the first eluting informative biomarkers (indicated by the red *) show an intermediate value of about 120%. These results are related to the triterpanes C23 Tr (103%), C24 Tr (117%) and C25 Tr (121%). These compounds are eluting around the end of the distillation range of the diesel. Fig 16 shows C23 and C24 Tr in a measurable concentration (retention time between 36 and 38 min.) and also a small peak at 39 min. for C25 Tr in the original diesel sample. As a result the addition of a low amount of lubrication oil has a lower impact on the relative concentration here compared to the later eluting hopanes.

The ratio comparison table (see e.g. Fig 8) doesn’t show differences of more than 14% in the ratios, and the different amounts of lubricating oil are not recognised here. This is due to the fact that only ratios within compound groups and between close eluting compounds are produced here.

Also in the GC-FID-chromatograms, lubricating oil could not be detected: an additional “unresolved hump” could not be found.

But the MS-PW-plots do not only show the difference in –and thus presence of -lubricating in the samples. There is even an exact quantitative correlation.

Table 6 shows that 50% of the participants have mentioned the differences in the lubricating oil concentration in their individual report.

6.4 Conclusions

The MS-PW-plot is a powerful tool for the detection of mixing. But it’s use leads to a problem here because a difference is found, which would not have been detected otherwise.- How can differences in oil samples of such kind become the proof of a match?

It is a well known fact that such different products like diesel oil and lubricating oil do not mix very well, and differences in their relations are often found in a ship's bilge. If this Round Robin was handled as a real case –and, according to the "Instructions", participants could not do anything else- those differences in the 4 bilge samples should have been found, and it must have been realised that the sample combinations 1-2, and especially the questionable combinations 1-5 and 1-6, have only "by chance" the same diesel/lub oil relationships.
So, mixing is a fact here, and a match could have been justified, even if the amount of lubricating oil in the spill samples would not be the same as in one of the bilge samples.
In addition, oil spill identification is based on the complexity of oil, and, undoubtedly, a mixture of two oil products is much more complex than one of these products alone. Thus, a full match of two products (this is the case, when these products are compared separately in the samples) should lead to a higher certainty of the conclusion than in case, only one of these products was present, even though the relations of these two products in the samples do not fully match.

7 Sulphur content

To protect the environment communities and countries around the world strive to reduce the sulphur content in petroleum products.

DIRECTIVE 2009/30/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 23 April 2009:

2.Member States shall ensure that, no later than from1 January 2008, gas oils intended for use by non-road mobile machinery (including inland waterway vessels), agricultural and forestry tractors and recreational craft may be placed on the market within their territory only if the sulphur content of those gas oils does not exceed 1 000 mg/kg. From 1 January 2011, the maximum permissible sulphur content of those gas oils shall be 10 mg/kg. Member States shall ensure that liquid fuels other than those gas oils may be used in inland waterway vessels and recreational craft only if the sulphur content of those liquid fuels does not exceed the maximum permissible content of those gas oils.

Refineries can reduce the sulphur content by a hydrogen treatment of the products.

Hydrogen has three important roles in the reactions – it can remove sulphide by forming hydrogen sulphide gas, which is removed to recover the sulphur; it can also hydrogenate unsaturated compounds in the stream to produce better quality naphtha; and it can also carry out hydrogenolysis of certain undesirable compounds such as thiophenes.
http://www.hydrocarbons-technology.com/projects/shellultralow/

The results are e.g. gas oils with a very low amount of sulphur. But the process also modifies the oil composition. So it is important to realize that these changes will have effect on the oil spill identification method.

Remark of Scott Stout (Newfield-US):

Low sulphur light oils will certainly become more frequently encountered with the new stringent sulphur maximums that you mention. We have seen the same in the US with the 15 ppm maximum for on-road diesel fuels. As such, evaluating the individual C1- and C2-dibenzothiophene isomers has become increasingly useful since those with an alkyl group in the C-4 position are resistant to hydrodesulphurization and will be preserved relative to other isomers in deeply desulphurized fuels. Thus, there may be value in comparing the m/z 198 and 212 patterns in comparing light oils.
I have used ratios of 1MDT/4MDT and 4,6-DMDBT/total C2-DBT with some success.

The gas oil tested in RR2009 is from a RWS-WD oil case dated April 2009 and has a very low sulphur content. The reduction of the sulphur resulted in the absence of the normative compounds BNT and the methyl-dibenzthiophenes 1-MD and 4-MD (See Fig. 9 and 10). Of the list of informative compound groups in the spreadsheet file, the C2 benzthiophenes and the C2 and C3 dibenzthiophenes are very low or absent.

Participant	**low sulfur** mentioned	**Participant**	**low sulfur** mentioned
AU_EC_CES	1	ES_CEDEX	1
AU_NSWDECC	1	ES_CSIC	1
BE_BMM		FI_NBI	
BR_Petrobas		FR_CEDRE	
BG_SGS		LV_LVGMC	
CA_EC_ESC	1	NL_NFI	
CA_ECTD	1	NL_RWS-WD	1
CA_PESC_EC		NO_Sintef	
CN_NCSEMC	1	SE_SKE	
DE_BSH	1	UK-ERT	
EE_EERC			

Table 7
Participants that mentioned the low sulphur content of the samples in their report

About half of the participants mentioned the low sulphur content.

Both normal and low sulphur gas oil is on the market, so far. So, looking for the sulphur containing compounds is useful to differentiate between those products. When all gas oil available on the market has a sulphur content < 10 ppm it might be a good idea to look for differences such as mentioned by Scott above.
On the other hand, gas oil is only one of the products, and the main product involved in the cases, RWS-WD is dealing with. This is different in different laboratories.

8 Photo oxidation: Samples 1-5

8.1 History

Sample 5 is a mixture of the gas oil spill sample of the original case with 5% of carter oil. So in the MS-PW-plot of samples 1-5 the biomarker level should be close to 100%.
The original case has been chosen because the spill sample showed the reduction of the methyl pyrenes (m/z 216; See Fig 20). This reduction was first reported by Niinna Vittala (NBI, FI) in spring 2009. She showed a presentation about the case at the meeting in Tallinn. She was absolutely sure that the spill samples were related to the source samples, but could not explain the differences.
Other participants have also found the reduction. In Tallinn, Magnus Kallberg(SKL-SE) showed chromatograms of a diesel case and Joan Albaiges(CSIC-ES) presented a heavy fuel oil case in which one of the spill sample showed a reduced methyl pyrenes pattern.
RWS-WD had a similar case in 2008. Although the sample taker was very sure about the case, a non-match had to be concluded, because the differences in the C1- and C2-pyrene patterns could not be explained by weathering.

8.2 Sample comparison

As example the MS-PW-plot of Sintef-NO (Fig. 11) is shown in section 4.2.3. The reduction has influenced some of the normative ratios (See Fig 19).

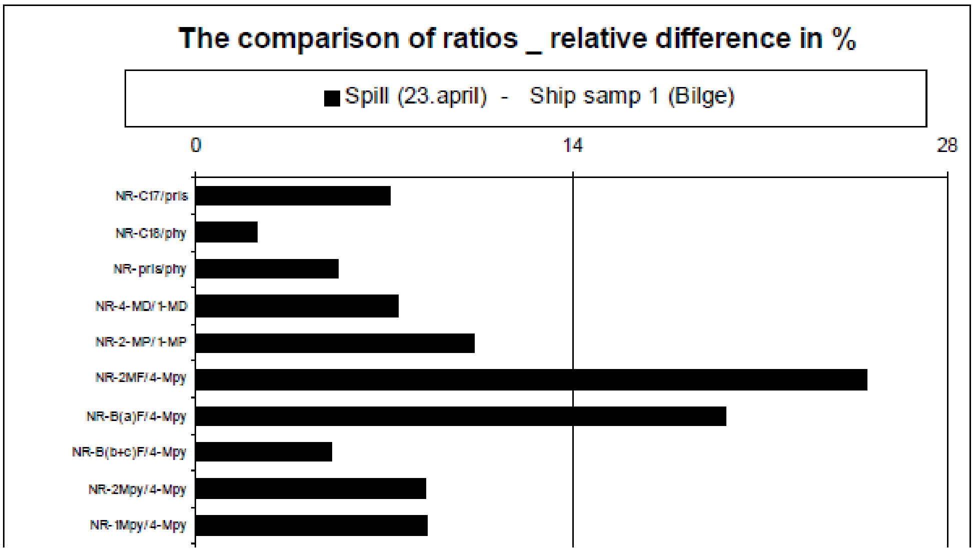


Fig 19
Part of the ratio graph of Sintef-NO for the sample comparison 1-5

The reduction has an effect on the ratios of 4-MPy with 2-MF and B(a)F, but this effect is for this case not strong enough to show a difference of more than 14% for the ratios between 1-MPy and 2-MPy with 4-MPy.

The corresponding mass-chromatograms are shown in Fig 20.

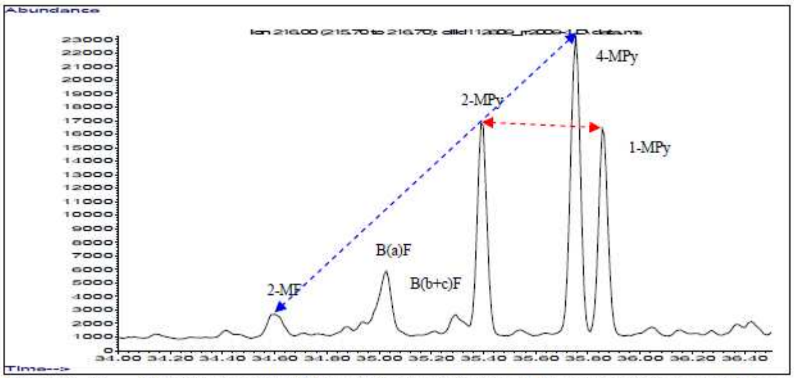


Figure A.10 *SINTEF ID: 2009-0732. Ship sample 4 (Bilge) Ion chromatogram of C1-fluoranthenes /pyrenes/benzofluorenes (m/z 216)*

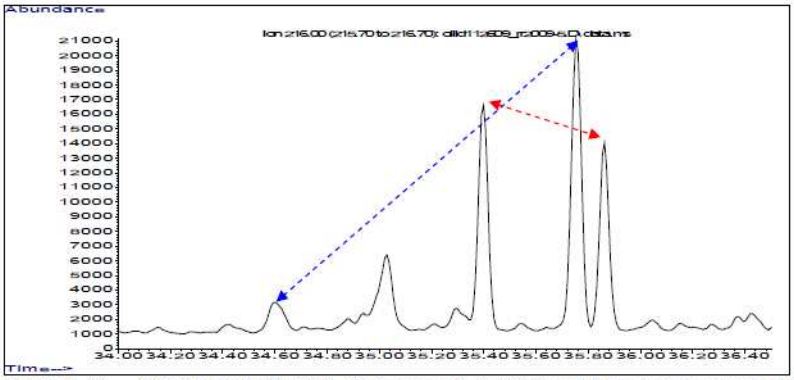


Figure A.11 *SINTEF Id: 2009-0733. Spill sample 5, Spill (23.april) Ion chromatogram of C1-fluoranthenes /pyrenes/benzofluorenes (m/z 216)*

Fig. 20

Reduction of the C1-pyrenes. Fig A.10 and A.11 from the report of Sintef-NO.

Table 8

Participants that mentioned the reduction of the C1-pyrenes in their report

Participant	**C1-Py reduction** mentioned	**Participant**	**C1-Py reduction** mentioned
AU_EC_CES	1	ES_CEDEX	1
AU_NSWDECC	1	ES_CSIC	1
BE_BMM		FI_NBI	
BR_Petrobas	1	FR_CEDRE	
BG_SGS		LV_LVGMC	1
CA_EC_ESC		NL_NFI	
CA_ECTD		NL_RWS-WD	1
CA_PESC_EC		NO_Sintef	1
CN_NCSEMC	1	SE_SKE	
DE_BSH	1	UK-ERT	
EE_EERC	1		

Table 8 shows that about half of the participants have reported the reduction.

8.3 Experimental

RWS-WD has performed two tests to find out whether the reduction of the C1 and C2-pyrenes is related to photo oxidation.

In the first test 10 ml of gas oil was transferred into a Petri glass with a diameter of 11 cm. This resulted in a layer thickness of 1 mm of oil.
The glass was placed in direct sunlight at summer time for 40 min.
No differences could be detected.

In the second test 100 µl of gas oil was dissolved in 5 ml DCM and then transferred to a Petri glass. After evaporation of the DCM this resulted in a layer thickness of 10 µm of oil, i.e. a thickness, which might be more realistic in more open waters.
Two glasses were placed in direct sunlight in January 2010 (winter time, low sun, -2 °C) for one and three hours. After exposure the Petri glasses were "extracted" quantitatively with DCM to a final amount of 5 ml.

In this second test the reduction of the C1-pyrenes is obvious. But also the C2- and C3-pyrenes are involved. For oil cases the common pattern in reduction is 1-MPy> 4-MPY> 2-MPY and the results of the sun exposure experiment is in good agreement with that pattern.
A detailed description of the second experiment can be found in Annex A.

Based on the information from both experiments it is quite reasonable that the effect of the sunlight is limited to the upper layer of the oil. Thus, photo-oxidation might explain the reduction of the M-pyrenes, but only:

- for thin layers of oil
- on sunny days
- the reduction of the 3 MPy's should be strongest for 1-MPY and lowest for 2-MPy.
- some peaks of the C2 and C3 fluoranthenes-pyrenes should/can be reduced.

Sintef has performed a test with crude oil in the sun on rocks. The results are shown in annex B.
CSIC has performed UV radiation tests with different crude oils and the HFO from the Prestige spill. The results are shown in annex C.

The experiments of Sintef and CSIC show that the effect of sunlight can be different for different types of oil products. For the oils tested the whole group of m/z 216 ions reduces while in the diesel experiment of RWS-WD only the methyl pyrenes are affected.

In 2010 RWS-WD, Sintef and CSIC intend to perform extra tests for the behaviour of petroleum products in sunlight.

8.4 Conclusion

The reduction of PAH standards mixtures by light is well known. As a result the standards are stored in brown bottles and the samples in the dark.
In literature however the effect of sunlight on methylated PAH is not well described. This can be related to the fact that:

- The use of the methyl pyrenes for oil spill identification has been introduced around the implementation of the CEN/Tr.
- The study of these compounds is related to the possibility to sample from very thin layers of oil with the Teflon net, which has been introduced around 2000.

From the experiments it can be concluded that the reduction of some of the methylated PAHs compound groups can be related to the effect of sunlight on the upper layer of an oil spill.

9 Biodiesel

To reduce the amount of mineral oil needed, it might be more and more common nowadays to add biodiesel to petroleum products.

[Prince, 2010]
Biodiesel is the term commonly reserved for the methyl esters of the fatty acids in plant or animal triglycerides. The principal biodiesels in commerce today are methyl esters from soybean, oilseed rape and oil palm, and they are typically blended at low levels (usually 2 or 5%, but up to 20%) with petroleum diesel. The fatty acid methyl esters are readily degraded under aerobic and at least some anaerobic conditions.

Remark of Steve Fuller (AU_NSWDECC):
Oil sample 1 was also found to contain a biodiesel fraction, readily seen as a complex peak cluster of saturated and unsaturated fatty acid methyl esters (FAMEs) around the C21 n-alkane and the presence of biogenic marker compounds. Similarly derived biodiesel fractions were found in all of the other oil samples. The amount of biodiesel present in the bilge samples (1 to 4) was estimated to be 5%.

Table 9 shows that most of the participants have recognised that, beside the normal diesel pattern, other peaks are present in the chromatograms. Eight participants identified the extra peaks as coming from biodiesel addition.

Presence of biodiesel					
Participant	Mentioned	Identified	Participant	Mentioned	Identified
AU_EC_CES	1	1	ES_CEDEX		
AU_NSWDECC	1	1	ES_CSIC		
BE_BMM			FI_NBI		
BR_Petrobas	1		FR_CEDRE		
BG_SGS			LV_LVGMC	1	1
CA_EC_ESC			NL_NFI	1	1
CA_ECTD	1		NL_RWS-WD	1	1
CA_PESC_EC	1		NO_Sintef		
CN_NCSEMC	1	1	SE_SKE	1	1
DE_BSH	1	1	UK-ERT		
EE_EERC					

Table 9
Biodiesel related information from the reports of the participants

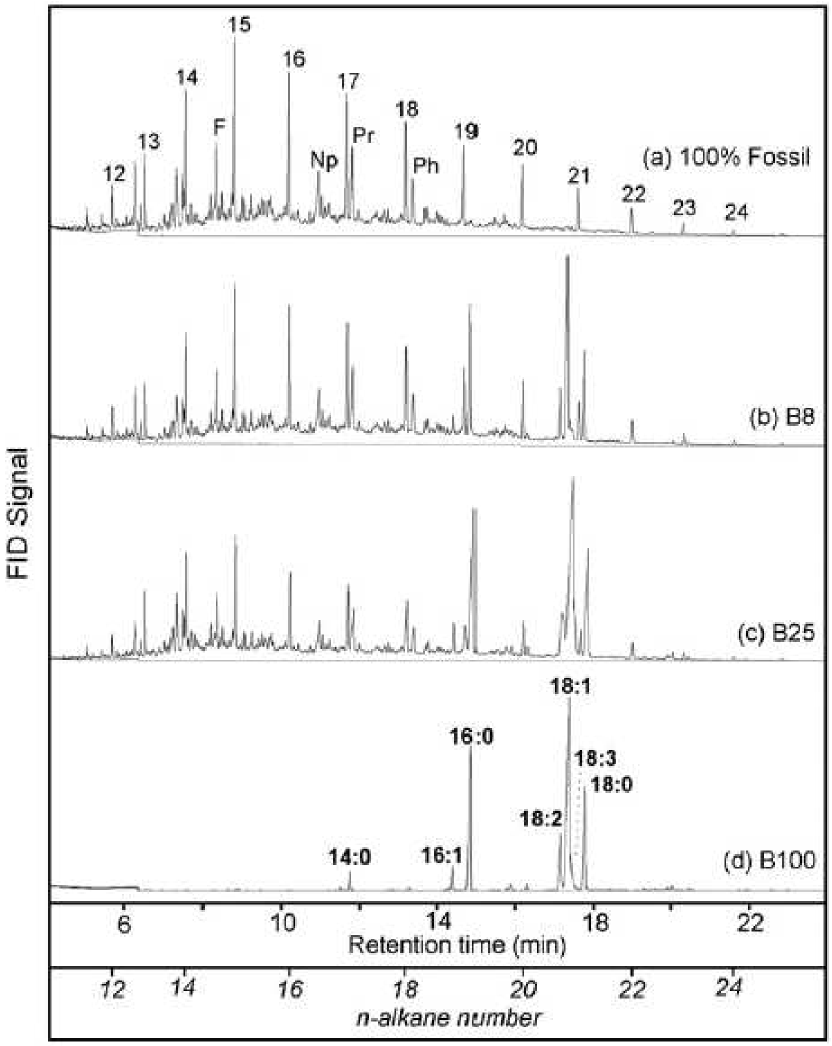


Fig. 1. GC-FID chromatograms of the four neat starting solutions used in the microbial experiments: (a) 100% fossil diesel, (b) B8, (c) B25, and (d) B100. In (a) peaks representing the *n*-alkanes 12–24 are numbered, and F, Np, Pr, and Ph are the isoprenoids farnesane, norpristane, pristane and phytane, respectively. In (d) the FAMEs are labeled in bold. For reference, the hexane baseline is included with all chromatograms.

Fig 21
Fig 1 of the paper of [deMello, 2007] with permission of the author and publisher (Elsevier)

The paper of [DeMello, 2007] deals with the composition and fate of (bio)diesel mixtures at sea. Fig 21 (Fig 1 of the paper) shows the retention times and composition of biodiesel mixtures:

The chain length and degree of unsaturation can vary in animal fats and vegetable oils for numerous reasons, but transesterification of either yields primarily a mixture of methyl hexadecanoate (C16 FAME), methyl octadecanoate (C18 FAME), and C18 FAME isomers with one, two, or three double bonds referred to as C18:1, C18:2, or C18:3 FAMEs, respectively.

Steve Fuller has quantified and reported the intensities of the FAMEs in a separated spreadsheet file.

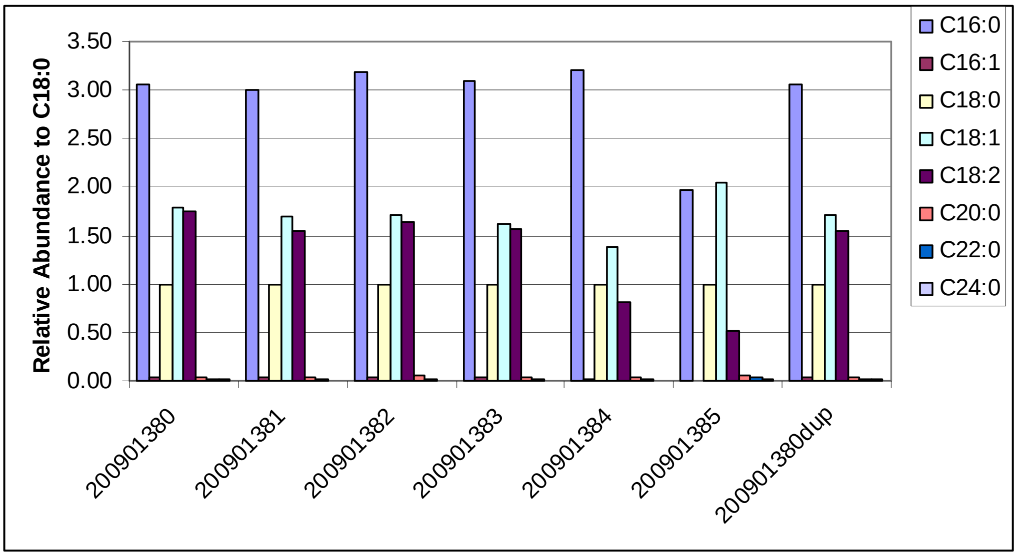


Fig 22
Response of FAME compounds relative to C18:0. (AU_NSWDECC)

Fig 22 shows the results relative to the response of C18:0. At the end of the sequence Sample 1 is injected again. The graph shows that four of the FAMEs are dominating the biodiesel composition of the RR2009 samples.

The retention times of the FAMEs relative to the alkanes are depending on the column phase. On a non-polar column (e.g. DB-1) the peaks around C18:1 elute before the retention time of C21(see Fig. 3 and 21). On a more polar column (e.g. DB-5) the more polar properties of the FAMEs relative to the alkanes cause that the FAMEs are more retarded than the alkanes with the result that the peaks around C18:1 elute at the retention time of C21 (see Fig. 2).

10 Biodegradation: Samples 1-6

The extract of sample 1 has been biodegraded by Joan Albaiges (ES_CSIC). The protocol is given in section 3.1 and the result appeared to be very realistic for RR2009.
The sample was suggested to come from an oil spill under a floating scaffolding. In such a situation there will be a layer of oil on water that evaporates slowly, shows some dissolution effects, will not show photo-oxidation effects and will biodegrade slowly.
All of this can be found in sample 6.
In the "Invitation letter" it was indicated that the concentration of all samples was 30 mg/ml. The oil concentration of sample 6 however turned out to be only about 20 mg/ml, due to losses in the biodegradation process. The final concentration was not checked before sending the samples to the participants.

10.1 Mineral oil results

The GC-PW-plot (Fig 6, SE-SKL) shows that the alkanes are slightly reduced compared to the isoprenoids. Due to the open biodegradation process the first eluting alkanes are slightly reduced by evaporation.

The MS-PW-plot (Fig 23, CA-ECTD) shows no photo oxidation and no concentration difference in the diesel and lub oil. The mean is around 100% while the late eluting compounds show some higher variability. The highest value of 144% comes from 30ba. This may be related to the low concentration of the biomarkers combined with the injection concentration difference.
The corresponding ratio comparison graph of Chun Yang shows that none of the ratio comparisons exceed a 10% difference.

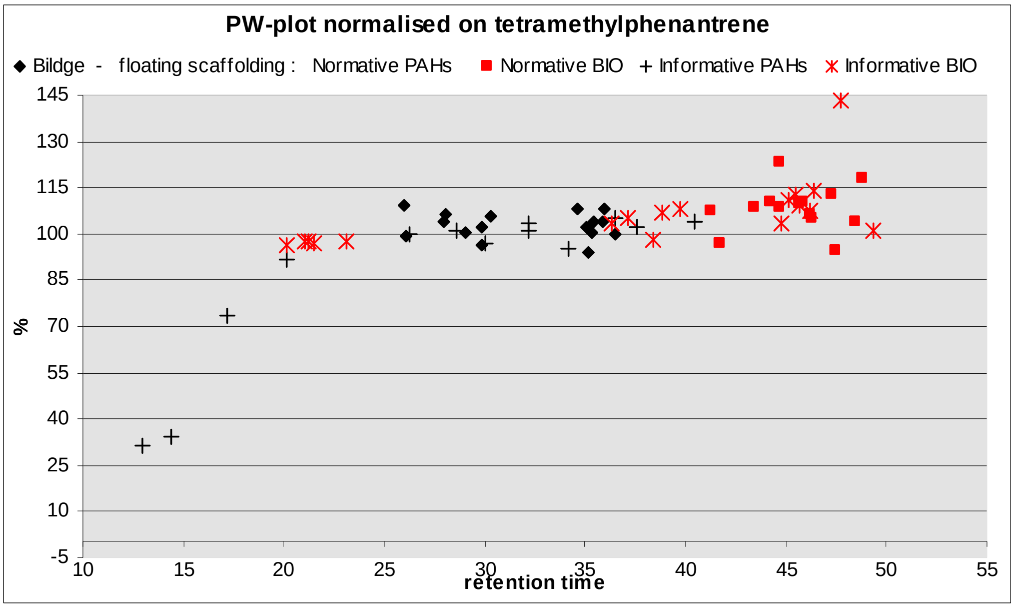


Fig 23
MS-PW-plot of CA_ECTD comparing sample 1 and 6

Thus a match can be concluded.

10.2 Visual comparison and biodiesel evaluation

The visual comparison of the chromatograms shows however that the biodiesel peaks of sample 6 are strongly reduced. This was mentioned in the reports of 9 of the participants (see Table 10).

Reduction of biodiesel compounds					
Participant	Mentioned	Participant	Mentioned	Participant	Mentioned
AU_EC_CES	1	CA_PESC_EC		FR_CEDRE	
AU_NSWDECC	1	CN_NCSEMC	1	LV_LVGMC	
BE_BMM		DE_BSH	1	NL_NFI	
BR_Petrobas		EE_EERC	1	NL_RWS-WD	1
BG_SGS		ES_CEDEX	1	NO_Sintef	
CA_EC_ESC		ES_CSIC		SE_SKE	1
CA_ECTD	1	FI_NBI		UK-ERT	

Table 10
Biodiesel related information from the reports of the participants

The results of AU_NSWDECC (see Fig 22) have been used to prepare Fig 24. To level out the intensities of the different compounds, each value was divided by the mean of all values of a compound. Additionally, the results were normalized to the tetramethylphenantrene results of AU_NSWDECC to compensate for the lower concentration of sample 6.

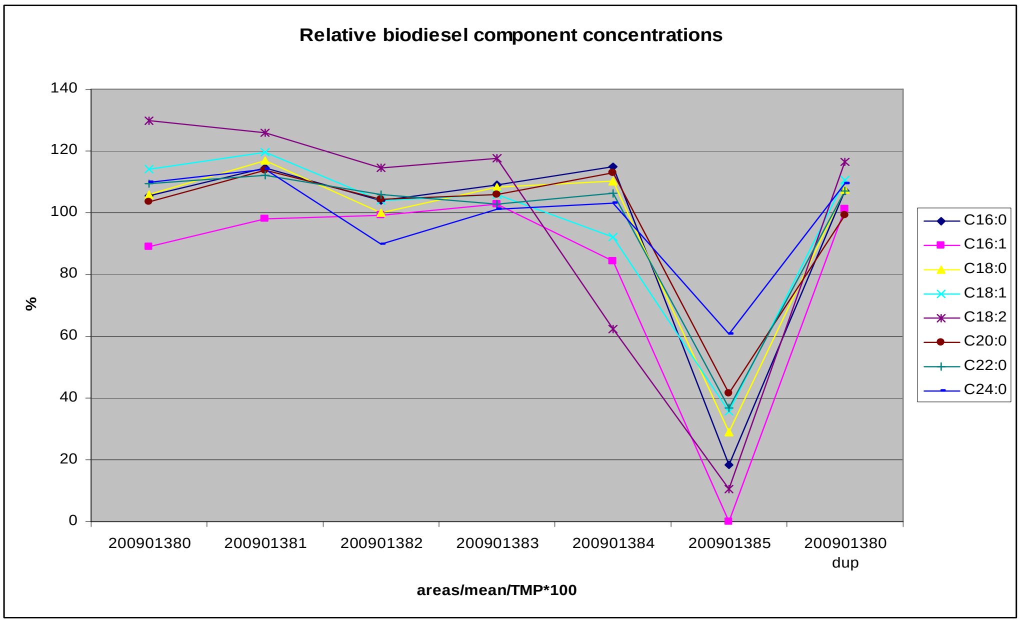


Fig. 24
FAME concentrations relative to the mean of each compound and normalized to TMP. Based on the results of AU-NSWDECC

Fig 24 shows that the FAMEs of sample 1 to 4 (200901380 to 200901383) have a similar concentration. For sample 5, of which the diesel part is from a real spill, three compounds are reduced, i.e. C18:2 > C16:1> C18:1. All are unsaturated FAMEs with the strongest reduction for the compound with two double bonds. Due to the more polar properties these compounds can more easily dissolve in the water and are more sensitive for biodegradation.

SE-SKL
The only major deviation in this case was indeed seen in the FAME peaks in Sample 6. FAME is known to oxidise. The order of oxidation is C18:3 > C18:2 > C18:1 whereas the C16:0 and C18:0 are not oxidised, at least not in a short time perspective and in cases we have seen before. In this case we see principally oxidation of the kind just described. The picture is however shadowed by what may be interference from other compounds in the very complicated mixture of compounds that mineral oil constitutes. The uncertainty of the FAME peaks of Sample 6 compared to the other samples led us to a lowering of the match conclusion.

AU_NSWDECC
Sample 5 (200901384) exhibited some notable difference in the profiles of the FAMEs compared to Sample 1; the unsaturated FAMEs were lower in abundance compared to the saturated FAMEs. The loss of relative abundance of the FAMEs was found to correspond to the levels of unsaturation in the species; the more unsaturated species degraded at a faster rate. These observed trends are consistent with differences we have observed in experiments on the weathering of diesel–biodiesel blends. The process of FAME weathering was more advanced in Sample 6; virtually no unsaturated FAMEs were present, compared to sample 1.

Fig 24 shows that all FAME’s are reduced, partly very strongly, by Joan’s treatment of this sample. The long chained FAMEs are more stable while the short chained and unsaturated compounds are mostly affected.

10.3 Conclusions

Based on the mineral oil comparison a match can be concluded between sample 1 and 6. The strong reduction of the biodiesel components can be explained by Joan’s treatment of the sample and mainly the rapid hydrolysis of these compounds.

The presence of biodiesel in gas oil could have consequences for the oil spill identification method.
For the GC-FID analysis the compounds must be recognized and the weathering behaviour known.
For the GC-MS analysis no specific ion is present in the method to recognize the FAME’s. In the current method FAME related peaks show up in the PAH chromatograms. So in a visual comparison of the chromatograms it is important to know the retention times of the FAMEs in order to separate the FAMEs from the mineral oil compounds.

In the draft version of the second version of the CEN/TR biodiesel is not mentioned. Futher experience must be gained with the analysis of the FAMEs

11 Conclusions

It should be emphasized, that many of the participants do not work normally with light fuel oil, small degrees of mixing and have knowledge of the specific weathering behaviour of biodiesel and some aromatic compounds.
Some of the participants have used an own method or a mixture of the own method with the CEN method as provided by CEN/TR 2v2 draft 3. E.g. the three labs from Canada have developed a procedure based on a separation of the aromatic and aliphatic compounds and specific methods to analyse and evaluate the samples. The procedure was kindly offered by CA_PESC_EC and can be found in their directory of the individual results. The three labs have in their own way combined their own procedure with the CEN/TR procedure to be able to enter the data in the spreadsheet file provided.

11.1 Participants conclusions

Table 11 gives an overview of the conclusions of the participants.

Participant	Conclusion of the comparison of RR2009-1 with				
	RR2009-2	RR2009-3	RR2009-4	RR2009-5	RR2009-6
AU_EC_CES	M	M	M	M	M
AU_NSWDECC	M	PM	PM	M	M
BE_BMM	NM	NM	NM	NM	NM
BR_Petrobas	M	M	M	PM	M
BG_SGS	NM	PM	NM	NM	NM
CA_EC_ALET	PM	PM	PM	PM	NM
CA_ECTD	M	M	M	M	NM
CA_PESC_EC	M	M	M	M	NM
CN_NCSEMC	M	M	M	PM	M
DE_BSH	M	M	M	M	M
EE_EERC	no result	no result	no result	I	M
ES_CEDEX	PM	PM	M	M	NM
ES_CSIC	M	M	PM	NM	PM
FI_NBI	M	M	M	PM	PM
FR_CEDRE	PM	PM	NM	NM	NM
LV_LVGMC	M	M	M	M	PM
NL_NFI	M	NM	NM	NM	NM
NL_RWS-WD	M	M	M	M	M
NO_Sintef	M	M	M	PM	M
SE_SKE	M	M	M	M	PM
UK-ERT	PM	M	M	M	PM

Table 11
Conclusions of the participants.
M=match, PM= probably match, I= inconclusive and NM = non-match

A match can defensible be concluded for all comparisons of sample 1 with the other samples. Still many other conclusions can be seen in Table 11.

The comparison with sample 2 can only result in a match because the samples are from the same extract.
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".

The CEN/TR 2v1 definition:

3.8
positive match
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

The CEN/TR 2v2 definition:

3.3.1 positive match
when 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 be identical beyond any reasonable doubt

Here, a match can defensibly concluded for the combinations 1-3 and 1-4 based on the conclusion definition of CEN/TR 2v2.

For the comparison of sample 1 with sample 5 knowledge about the weathering behaviour of the C1 to C3 Pyrenes is needed. But the differences are not too big, and even those smaller differences must be seen in the light that everything else fully matches. So, only a slight uncertainty could have remained here.

For the comparison of sample 1 with sample 6, knowledge about the weathering behaviour of the FAMEs is needed.

11.2 CEN/TR 2v2 - RR2009 Conclusions

In RR2009 draft version 3 of CEN/TR 2v2 has been tested. By going through the Analytical Scheme of the CEN-TR step by step, all the difficulties included/produced here in RR2009 should have been recognized.

Based on the results of the participants a 95% error range of 100 +/- 15% has been calculated/defined from all available data for the data points of the MS-PW-plot in case of matching samples at about the same injection concentration.

The mixing of lube oil with gas oil is discussed in Annex I.5 of the CEN-TR. Based on the information available here a match can defensively be concluded despite differences in the diesel-lub oil relations. It can be concluded that the PW-plot reveals different, additional information compared to the ratio comparison. Concentration differences between the compound groups are tested here.

The possible absence of sulphur containing compounds and compound groups is not mentioned in the CEN/TR, but by following the general “rules” for the exclusion of ratios, the scheme can be followed as usual. The normative ratios for sulphur containing compounds are defined in such a way that values go to zero instead of infinity in case the sulfur concentration goes to zero.

The change of the C1 to C3 pyrenes is mentioned in Annex I.4 of the CEN/TR. Photo oxidation as the cause for the change is only mentioned as option. After the sunlight test described in Annex B of the RR2009 report this option can be changed into a weathering effect.

The change of the C1 to C3 pyrenes is mentioned in Annex I.4 of the CEN/TR. Photo oxidation as the cause for this change is only mentioned as option. Results achieved here make this statement more sounded.

Mixing of FAMEs with diesel is not mentioned in the CEN/Tr 2v2. But the presence and concentration of the individual FAMEs may even give useful information in oil sample comparison. But this phenomenon is limited to a few cases -and diesel-oil-, so far, and the tendency in the future cannot be assessed. May be, FAMEs should be treated in the same way as other additions sometimes found in oil samples, such as additives, or cleaning agents in case of waste oil (bilge oil, sludge). In the CEN-TR the general advice is given that the analyst should look for peculiarities in the samples. But, depending on the case, such additions may make identification easier in one case but more difficult in the other.

12 References

DeMello J.A., Carmichael C.A., Peacock E.E., Nelson R.K., Arey J.S. and Reddy C. M. Biodegradation and environmental behavior of biodiesel mixtures in the sea: An initial study. Marine Pollution Bulletin.Vol 54, Issue 7, (2007) 894-904.

Prince C.. Biodiesel. Handbook of Hydrocarbon and Lipid Microbiology. ISBN 978-3-540-77584-3, Springer Berlin Heidelberg. (2010) 2271-2275

Annex A RWS-WD photo-oxidation experiment

A.1 Experimental

100 ul gas oil (the same source oil as used to prepare the mixtures for RR2009) is dissolved in 5 ml DCM to be able to spread the oil over the glass surface. This is done three times. The content of two of the solutions is spread over two Petri glasses with a diameter of 11 cm. After evaporation of the DCM the Petri glasses are exposed to direct sunlight on 6-1-2010. The gas oil layer thickness is 10.5 µm.

According to the layer thickness table of the Bonn Agreement Oil Appearance Code (BAOAC) for oil on water 10.5 µm is a layer thickness in the metallic range, what is realistic for a diesel spill.

Code	Description - appearance	Layer thickness interval (µm)	Litres per Km²
1	Sheen	0.04 to 0.30	40 – 300
2	Rainbow	0.3 to 5.0	300 – 5000
3	Metallic	5.0 to 50	5000 – 50.000
4	Discontinuous True Oil Colour	50 to 200	50 000 – 200.000
5	Continuous True Oil Colour	More than 200	More than 200.000

Table A.1
The Bonn Agreement Oil Appearance Code (BAOAC) for oil on water

6-1-10 is a sunny day with a low solar zenith angle (the Netherlands is at the 52nd degree of latitude), no clouds and a temperature of about -2°C. The experiment started at 13:00.
After 1 hour and after 3 hours of exposure the Petri glasses were “extracted” quantitatively with DCM to a final amount of 5 ml DCM. The solutions are specified as sun 0h, sun 1h and sun 3h according to the exposure time of the samples.

The samples were analysed with GC-FID in sequence F00106.s
Sample sun 0h is diluted 10 times to get a normal injection concentration. Because it is unknown what has been left/extracted of the exposed samples the solutions were analyzed without dilution with GC-FID.

```
 Instrument Name: MS8
   Sequence File: C:\MSDChem\1\sequence\F00106.s
         Comment: sun experiment
        Operator: Paul
       Data Path: D:\DATA 2010\F00106\
     Method Path: C:\MSDCHEM\1\METHODS\1OLIE\

   Line Type       Vial  DataFile  Method    Sample Name
   -----------------------------------------------------------------
     1) Sample        1  F0010601  OLIEFIDP  st alkanes
     2) Sample        2  F0010602  OLIEFIDP  st alkanes
     3) Sample        3  F0010603  OLIEFIDP  sun 0  10vv
     4) Sample        4  F0010604  OLIEFIDP  sun 1 hour
     5) Sample        5  F0010605  OLIEFIDP  sun 3 hours
```

```
   6) Sample         6  F0010606  OLIEFIDP  bl chem
   7) Sample         7  F0010607  OLIEFIDP  st alkanes

  Sequence completed Wed Jan 06 22:46:22 2010
```

The signals for the sun 1h and sun 3h samples are much higher than for the sun 0h sample. So the sun 1h and sun 3h samples were diluted 5 times with DCM for the GC-MS analysis
The samples were analysed with in sequence C00107.s

```
 Instrument Name: MS8
  Sequence File: C:\MSDChem\1\sequence\C00107.s
        Comment: sun experiment MS analyse
       Operator: Paul
      Data Path: D:\DATA 2010\C00107\
    Method Path: C:\MSDCHEM\1\METHODS\1OLIE\

  Line Type        Vial  DataFile  Method     Sample Name
  ----------------------------------------------------------------------
   1) Sample         1  C0010701  CENSIM    st Brent
   2) Sample         2  C0010702  CENSIM    st Brent
   3) Sample         3  C0010703  CENSIM    0 hour 10vv
   4) Sample         4  C0010704  CENSIM    1 hour 5 vv
   5) Sample         5  C0010705  CENSIM    3 hour 5vv
   6) Sample         6  C0010706  CENSIM    Blanc
Sequence completed Thu Jan 07 18:24:32 2010
```

A.2 Results FID analysis

The chromatograms have been integrated and the results were used to compare the isoprenoids and to generate GC-PW-plots.

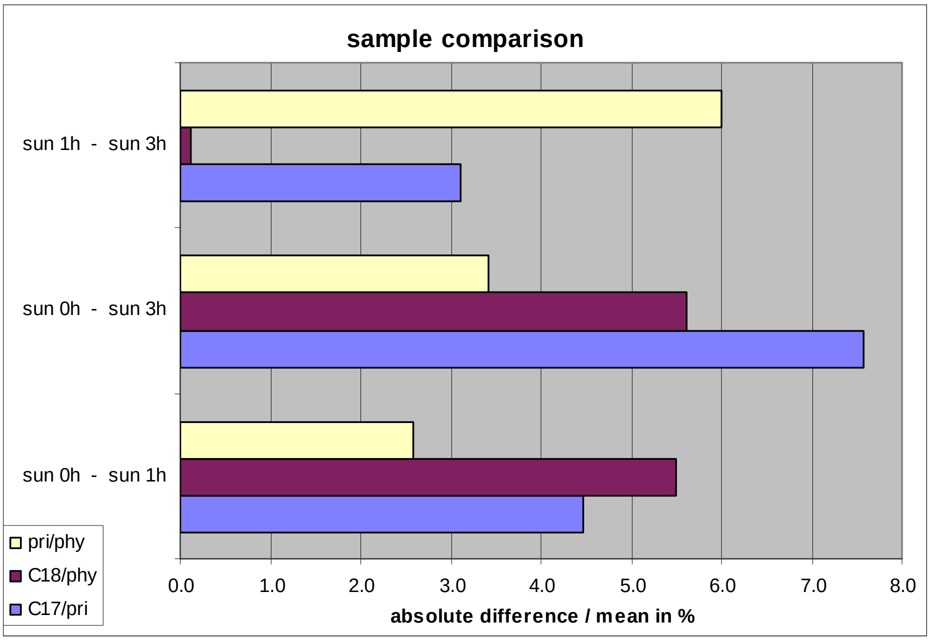


Fig. A.1
Isoprenoids comparison of the three samples. The samples are indicated as sun 0h, sun 1h and sun 3h.

The isoprenoid ratios differ far less than 14% relative.

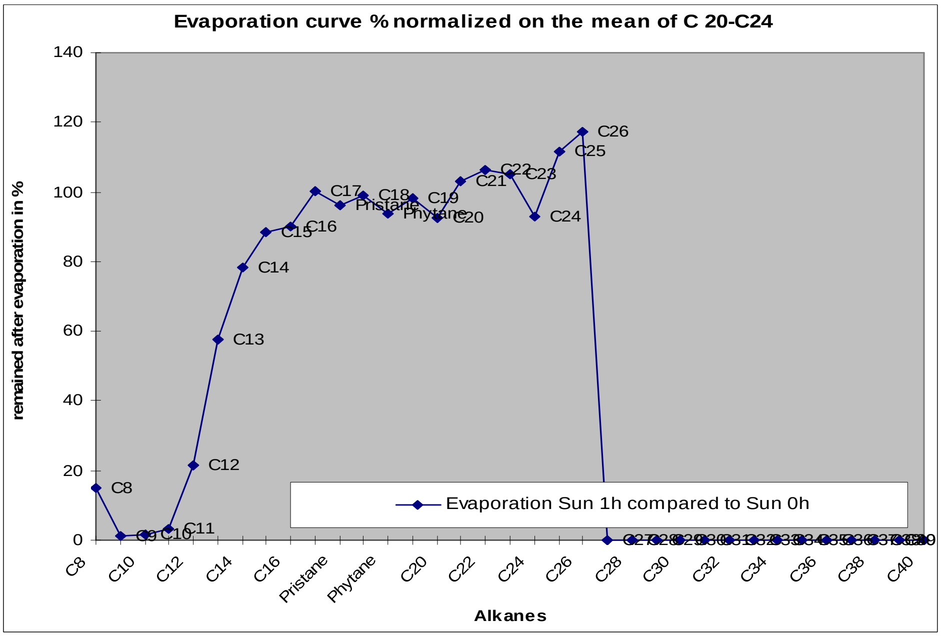


Fig. A.2
GC-PW-plot of sun 0h with sun 1h

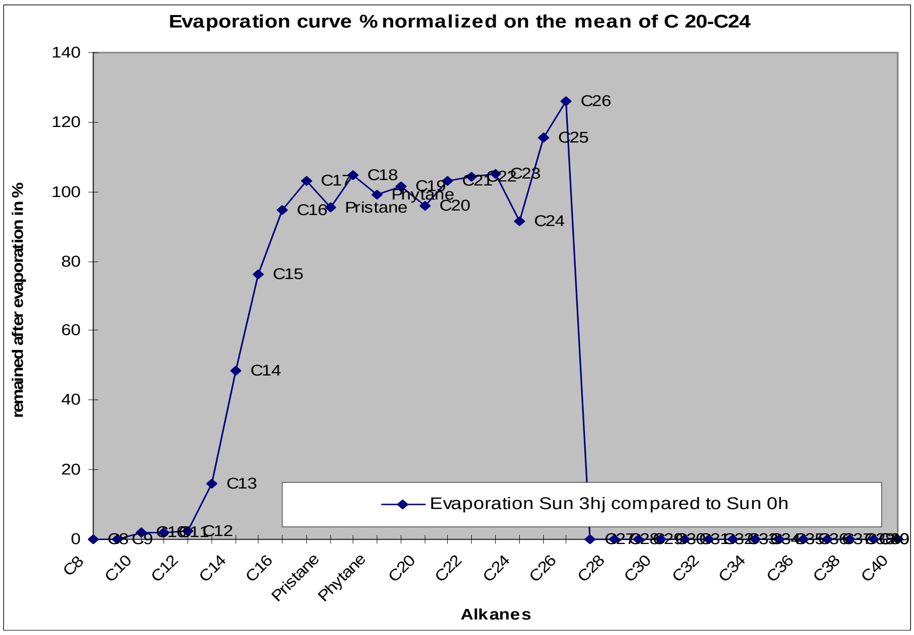


Fig. A.3
GC-PW-plot of sun 0h with sun 3h

The PW-plots show a normal pattern with evaporation up to C15 for sun 1h and C16 for sun 3h.

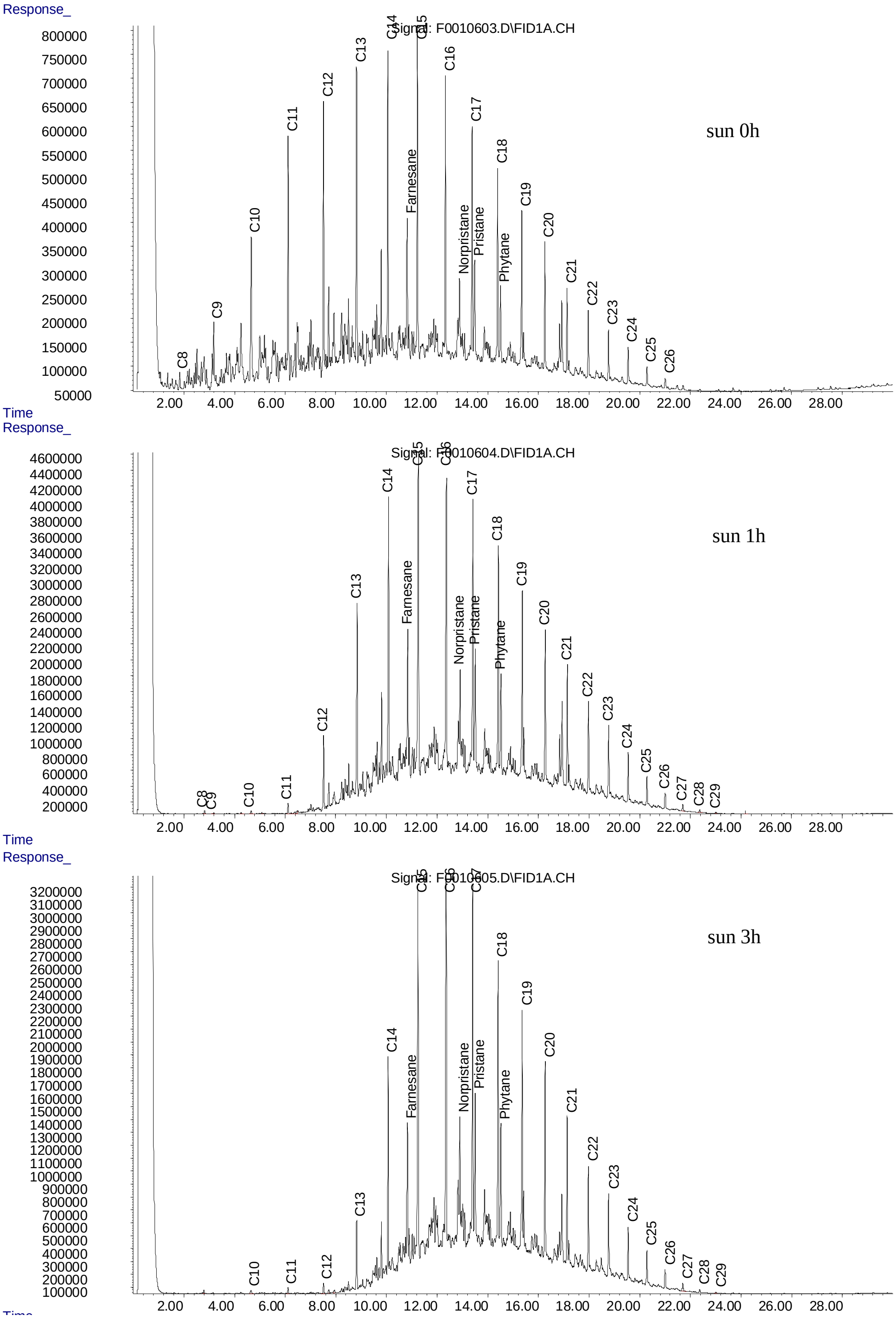


Fig. A.4
FID chromatograms of the gas oil sample exposed to sunlight for 0, 1 and 3 hours

The FID chromatograms show also the slight evaporation of the sample up to C15 for the 1 hours exposure and to C16 for the 3 hours exposure.
This is according to a normal weathering behaviour.

It is also visible that the two biodiesel peaks just before C21 are reduced. It indicates that these compounds are sensitive for sunlight. It would be good to test this with a standard of biodiesel components separately.
No further differences are visible.

A.3 GC-MS results.

A.3.1 Comparison of sun 0h with sun 1h.

The samples have been integrated. Due to the composition of gas oil almost no biomarkers have been integrated based on the S/N criterion.

The MS-PW-plot shows a normal evaporation pattern and a slight reduction of the C1-pyrenes.

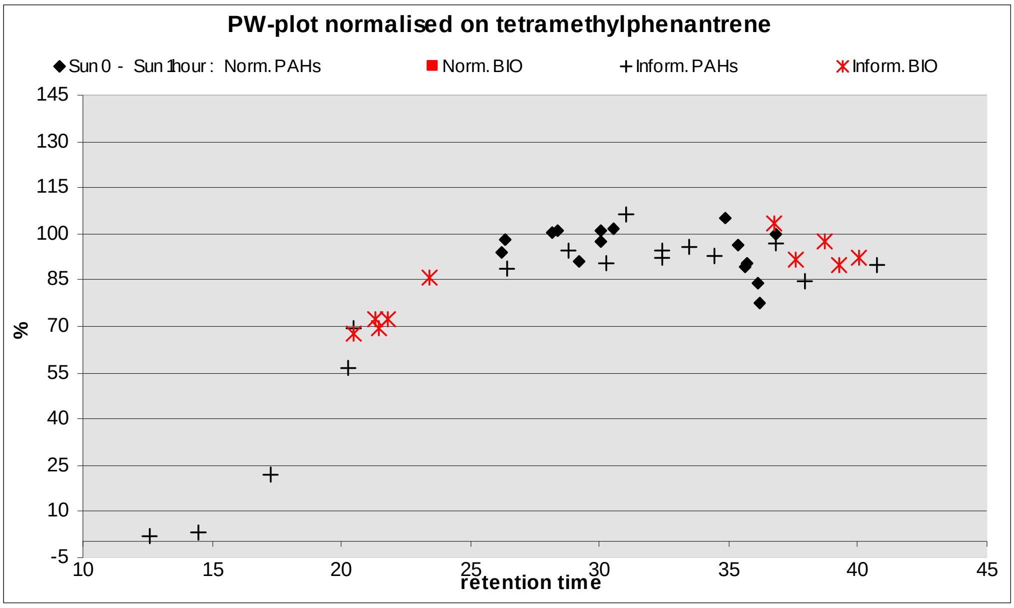


Fig. A5
MS-PW-plot Sun 0h and Sun 1h

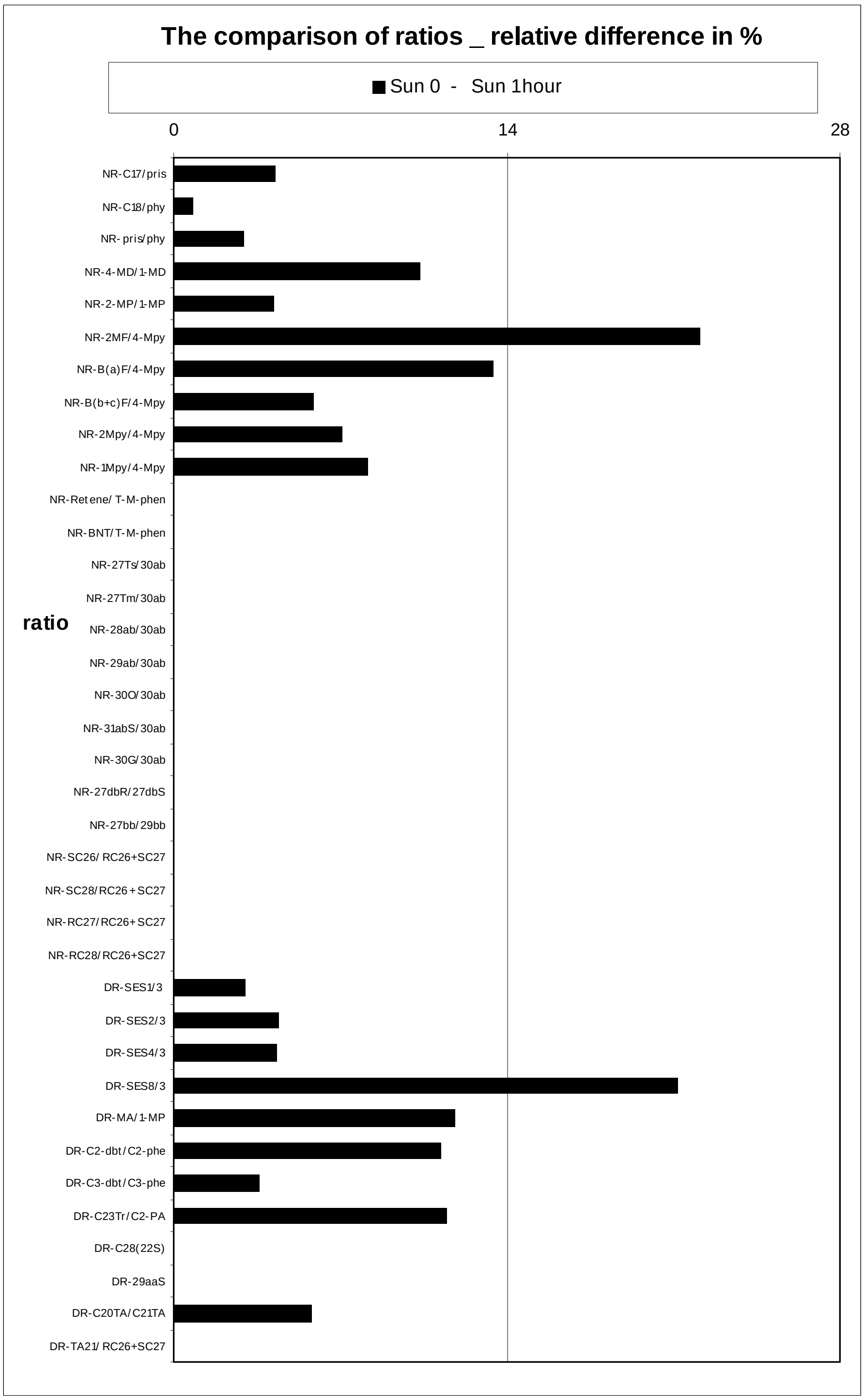


Fig. A.6

Ratio comparison between samples Sun 0h and Sun 1h

Fig B.6 shows a relative difference of 22 % for the normative ratio NR-2MF/4-Mpy. It shows that 4-MPy has been reduced relative to 2-MF.
The informative ratio DR-SES8/3 shows a difference of 21%, which is caused by evaporation.

A.3.2 Comparison of sun 0h with sun 3h.

A stronger effect is shown in sample sun 3h.

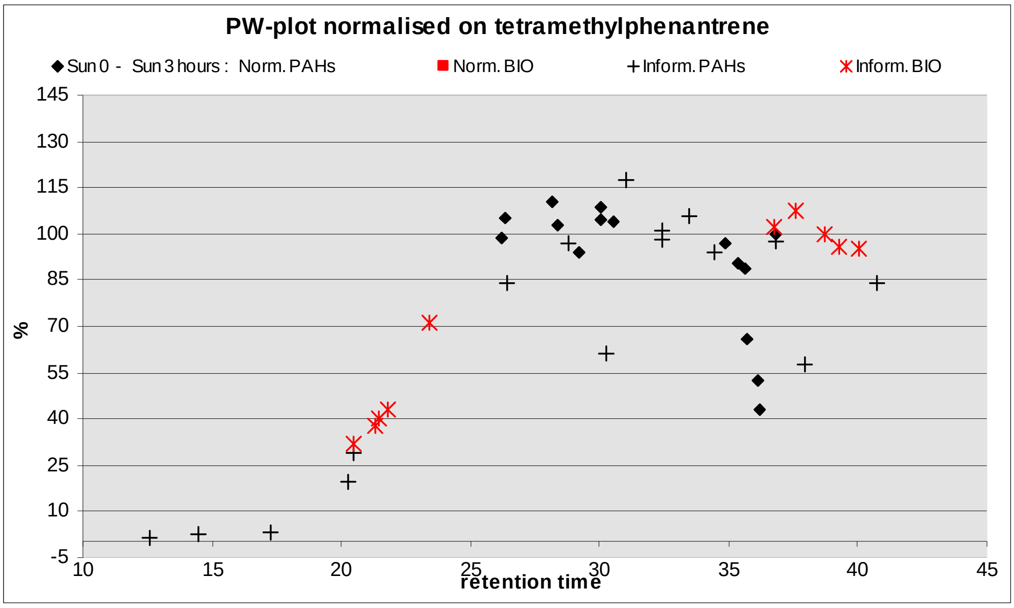


Fig. A.7
MS-PW-plot of sun 0h with sun 3h

Here not only the methyl pyrenes show a strong reduction in intensity but also methyl antracene and the summed area of the C2-fluoranthenes pyrenes (m/z 230). The strongest reduction effect is shown for 1-MPy. For oil cases the common pattern in reduction is 1-MPy> 4-MPY> 2-MPY and the results of the sun exposure experiment is in agreement with that pattern.

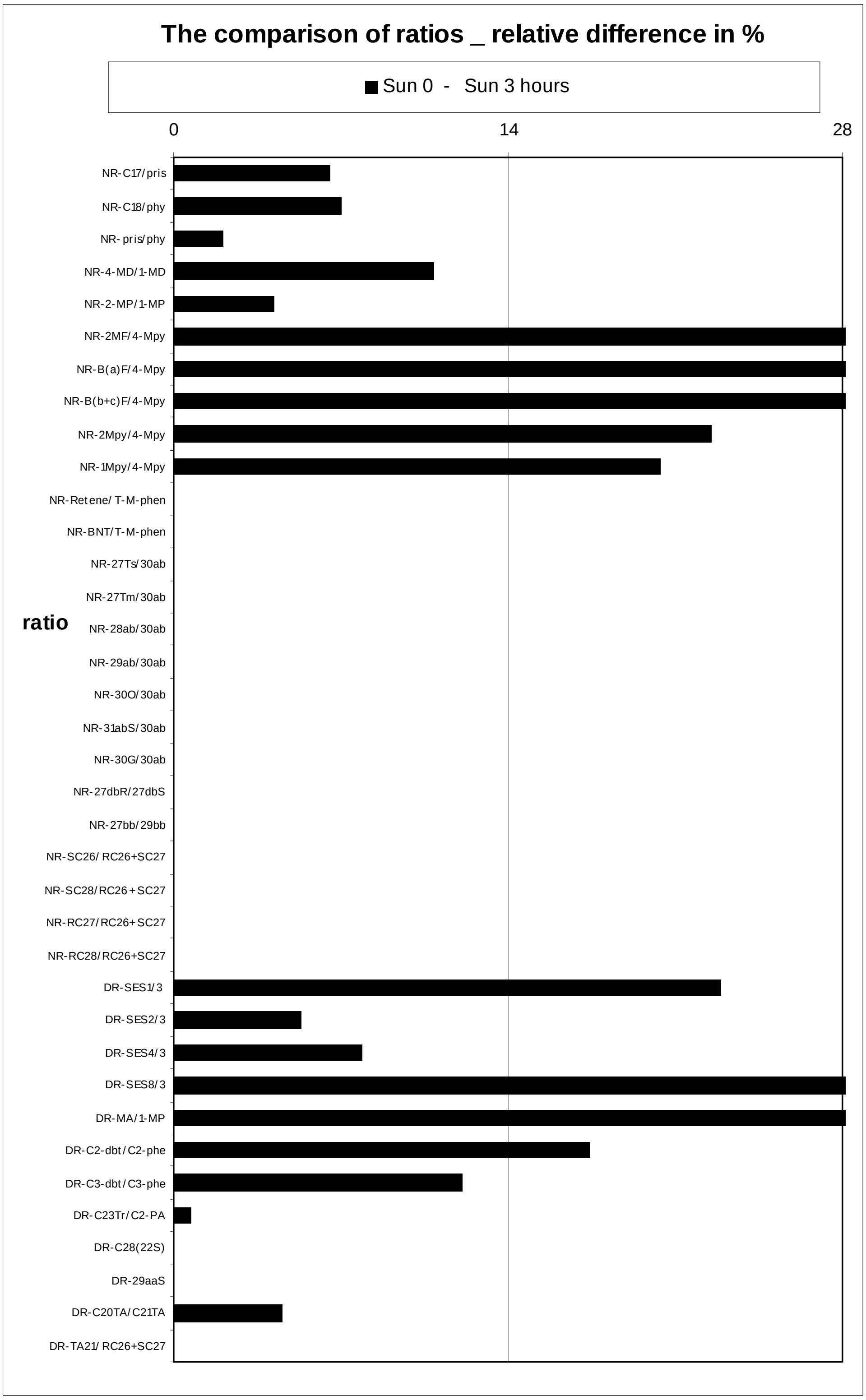


Fig. A.8
Ratio comparison between
samples Sun 0h and Sun 1h

The normative ratios involved are shown in Table B.2

Diagnostic **ratios** normative in bold	sun 0 C9091519 sun 0	sun 3 hours C9091521 sun 3 hours	**mean**	absolute difference	relative difference %	14% flag 0
NR-2MF/4-Mpy	8.07	14.93	11.50	6.86	59.69	1
NR-B(a)F/4-Mpy	20.53	35.47	28.00	14.94	53.37	1
NR-B(b+c)F/4-Mpy	6.11	10.31	8.21	4.20	51.19	1
NR-2Mpy/4-Mpy	66.31	83.14	74.73	16.84	22.53	1
NR-1Mpy/4-Mpy	66.81	54.45	60.63	12.35	20.38	1

Table A.2
Ratio calculation for the normative ratios of the C1 fluoranthenes-pyrenes.

The differences are very high for the ratios based on the compounds shown in the m/z 216 ion-chromatogram.

The informative ratios show also some differences.

Diagnostic **ratios** normative in bold	sun 0 C9091519 sun 0	sun 3 hours C9091521 sun 3 hours	**mean**	absolute difference	relative difference %	14% flag 0
DR-SES1/3	85.52	67.94	76.73	17.58	22.91	1
DR-SES2/3	62.08	58.86	60.47	3.22	5.32	
DR-SES4/3	49.37	53.41	51.39	4.04	7.86	
DR-SES8/3	132.07	235.96	184.01	103.89	56.46	1
DR-MA/1-MP	4.95	2.90	3.93	2.05	52.23	1
DR-C2-dbt/C2-phe	6.81	8.11	7.46	1.30	17.41	1

Table A.3
Ratio calculation for some informative ratios

The differences for the sesquiterpanes are caused by evaporation. The ratio difference for methyl anthracene however is not related to evaporation, but is probably caused by the exposure to sunlight.
The relative difference for the ratio **DR-C2-dbt/C2-phe** is somewhat higher than 14%. This can be caused by the very low concentration of sulphur containing compounds in the sample.

The effects are very well visible in the chromatograms.

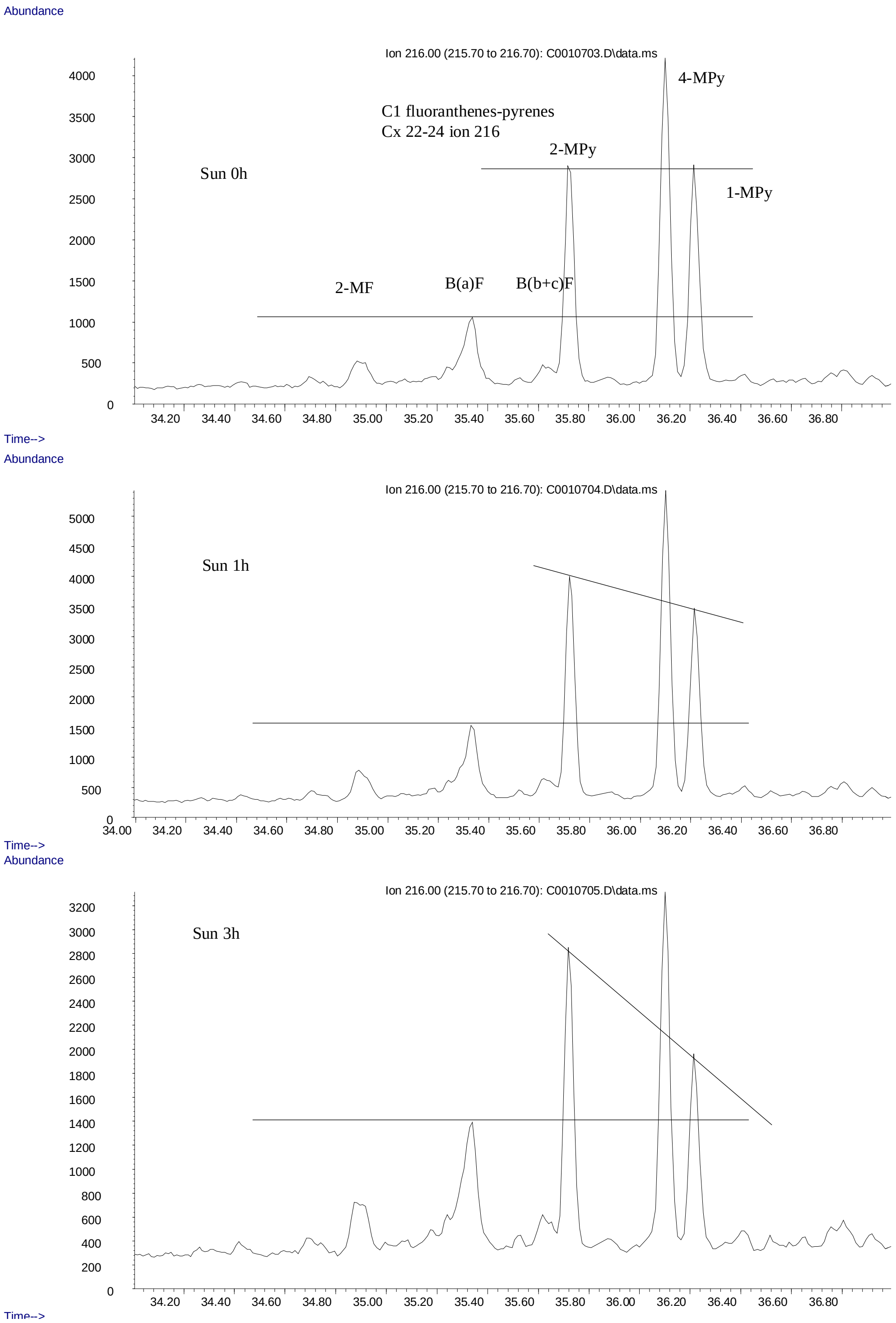


Fig. A.9

The ion chromatogram of m/z 216. A line is drawn between 2-MPy and 1-Mpy and at the level of B(a)F for each chromatogram.

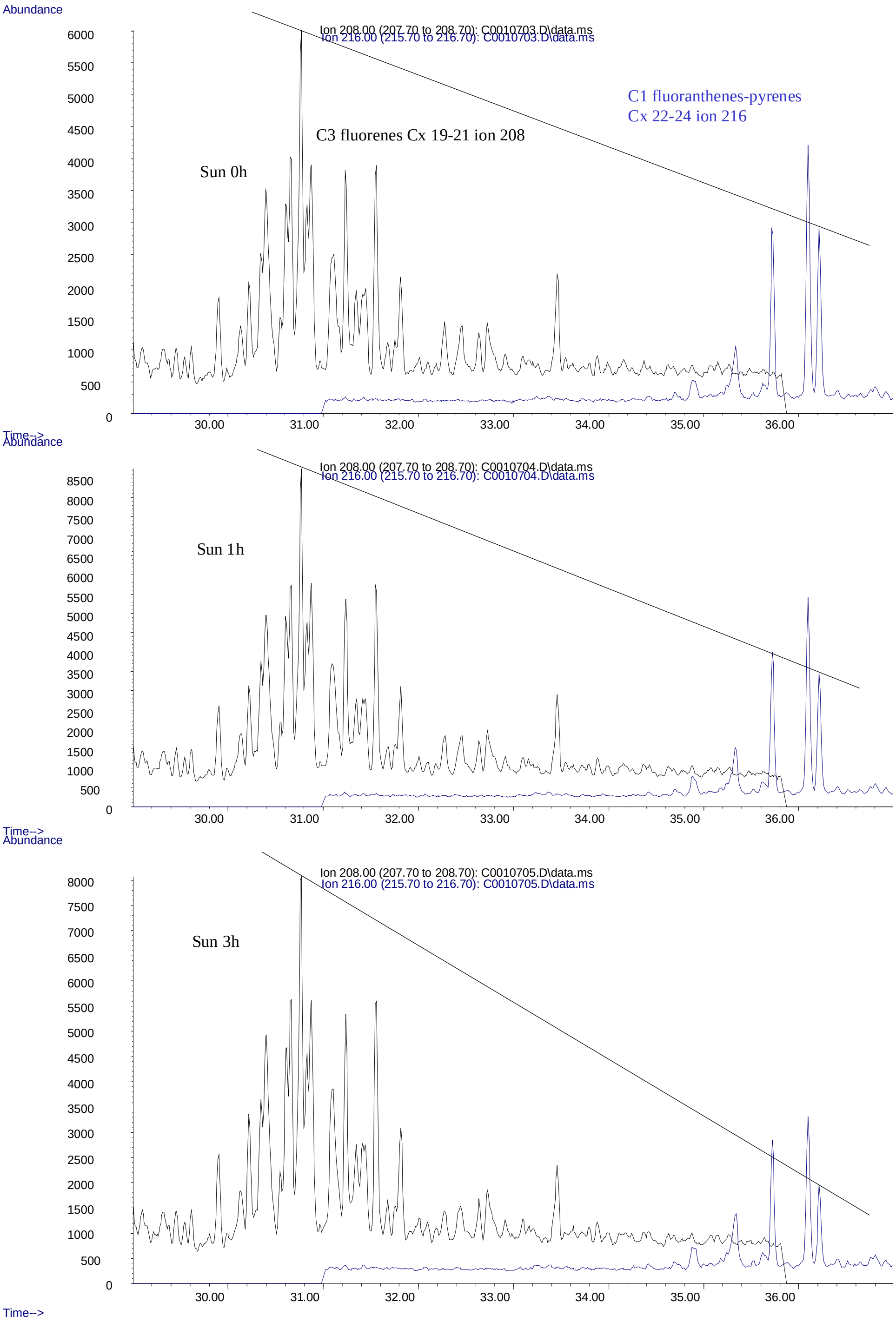


Fig. A.10

The ion chromatograms of m/z 208 and 216. By making a reference to the C3 fluorenes the concentration reduction of the C1-pyrenes is better visible. A line is drawn between the highest peak of the C3 fluorenes and 1-Mpy.

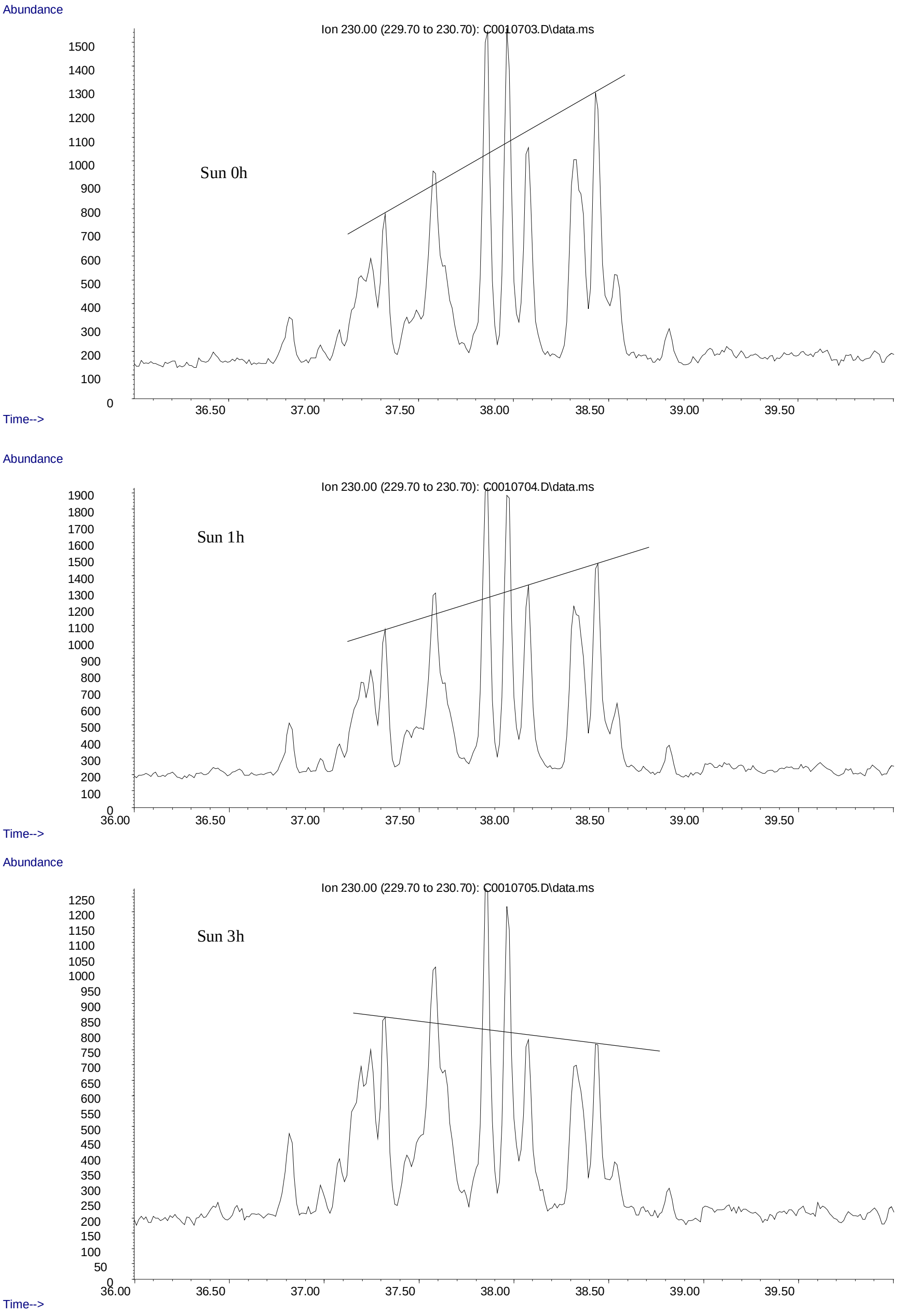


Fig. A.11

The ion chromatograms of m/z 230. A part of the compounds of the C2-fluoranthenes-pyrenes group are also reduced.

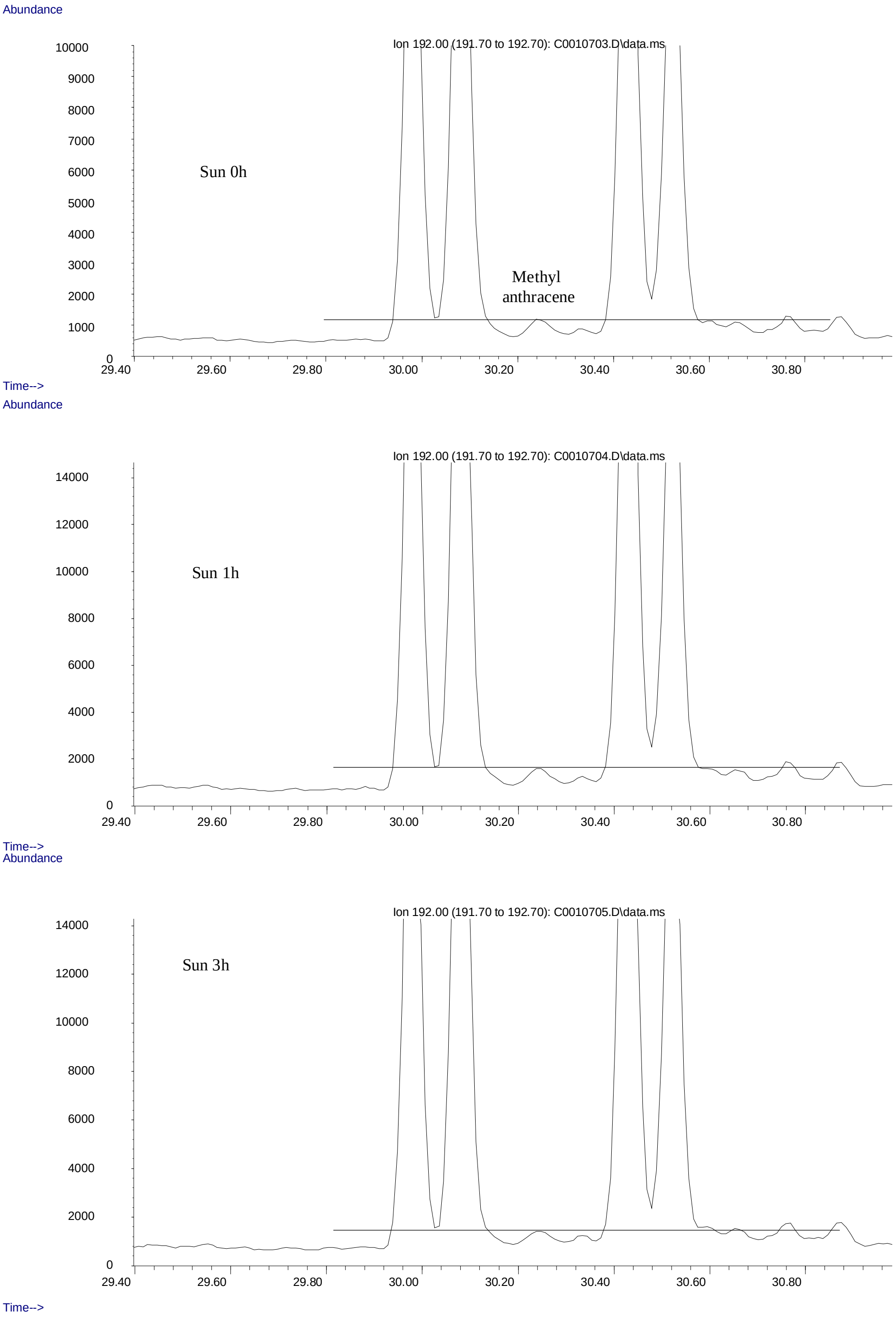


Fig. A.12
Ion chromatograms of m/z 192. The chromatograms are shown on 50% of the intensity of the highest peak

Methyl anthracene is reduced but because of the low concentration the difference is barely visible. It would be useful to repeat the experiment with an HFO sample with a higher concentration of methyl anthracene.

A.3.3 Visible comparison.

The chromatograms of sun 0h and sun 3h were printed for all ions monitored (see CEN 15522-2 table B2).
The visual comparison showed that some of the peaks of the C3-fluoranthenes-pyrenes also are reduced.
Some other peaks were slightly reduced. It is assumed that these peaks are related to biodiesel based on the retention time and the information of the GC-FID chromatograms.

The visual comparison revealed no other significant differences.

A.4 Conclusions

An experiment with the exposure to sunlight of a layer of 10 µm of diesel on glass revealed that, besides some evaporation, the C1 pyrenes and some peaks in the C2 and C3 fluoranthenes pyrenes groups are strongly reduced in concentration. The pattern is similar to what is seen in real spill cases.

Based on the information from both experiments it is quite reasonable that the effect of the sunlight is limited to the upper layer of the oil. So when in an oil case this behaviour is visible it can be explained as a weathering effect, but only:

- for thin layers of oil
- on sunny days
- the reduction of the 3 MPy's should be strongest for 1-MPY and lowest for 2-MPy.

Also some peaks of the C2 and C3 fluoranthenes-pyrenes should/can be reduced.

It is suggested to repeat the test with

-diesel without biodiesel
-pure biodiesel
-HFO to study the effect on black oil and methyl anthracene.

Annex B Sintef photo-oxidation experiment

Liv-Guri Faksness and Kjersti Almås (Sintef, NO) have performed a test with crude oil.

Oil on rocks exposed to sun (lab exp)

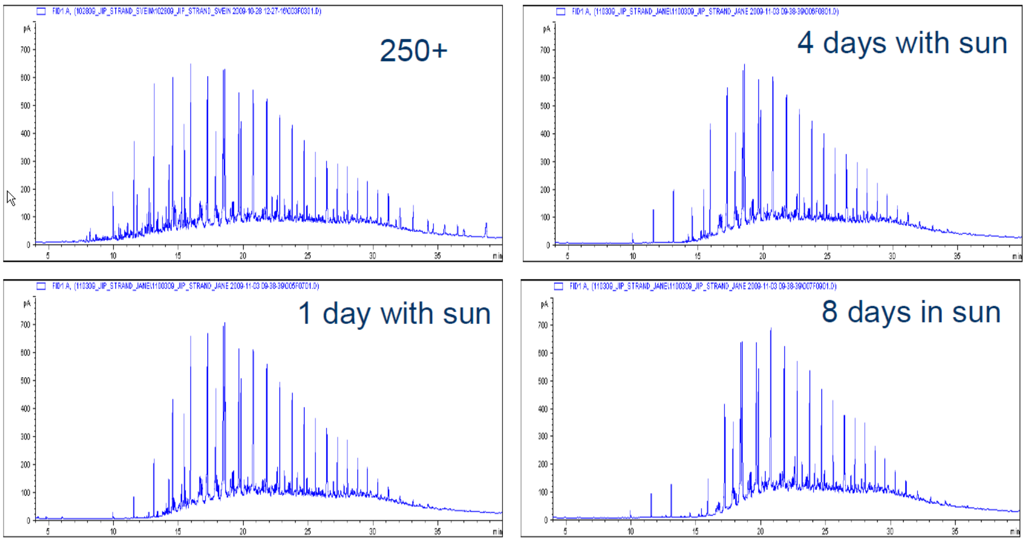


Fig B.1
GC-FID chromatograms of the crude oil

Hopanes - m/z 191

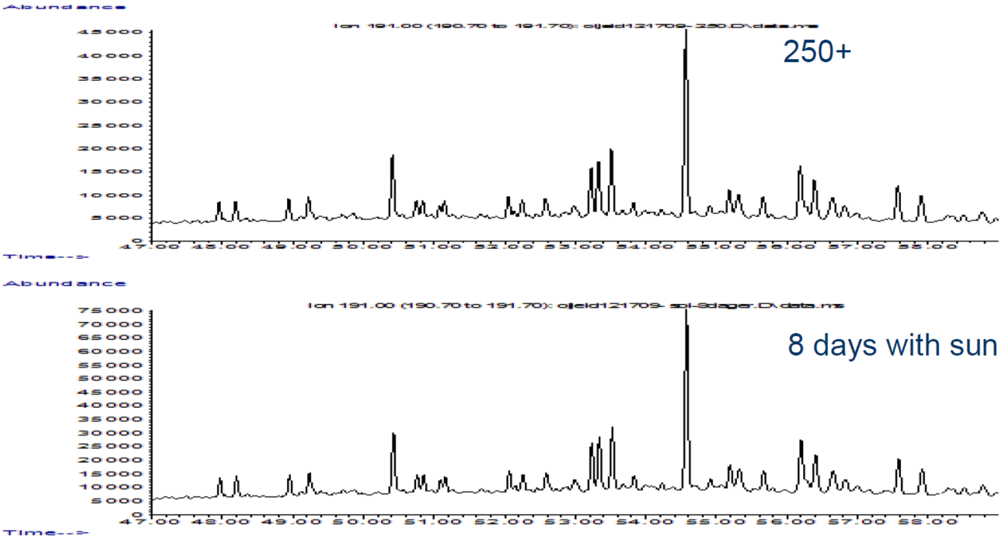


Fig. B.2
GC-MS ion chomatograms of ion m/z 191

No effect is found for the Hopanes after 8 days.

C1-fluoranthenes /pyrenes/benzofluorenes (m/z 216)

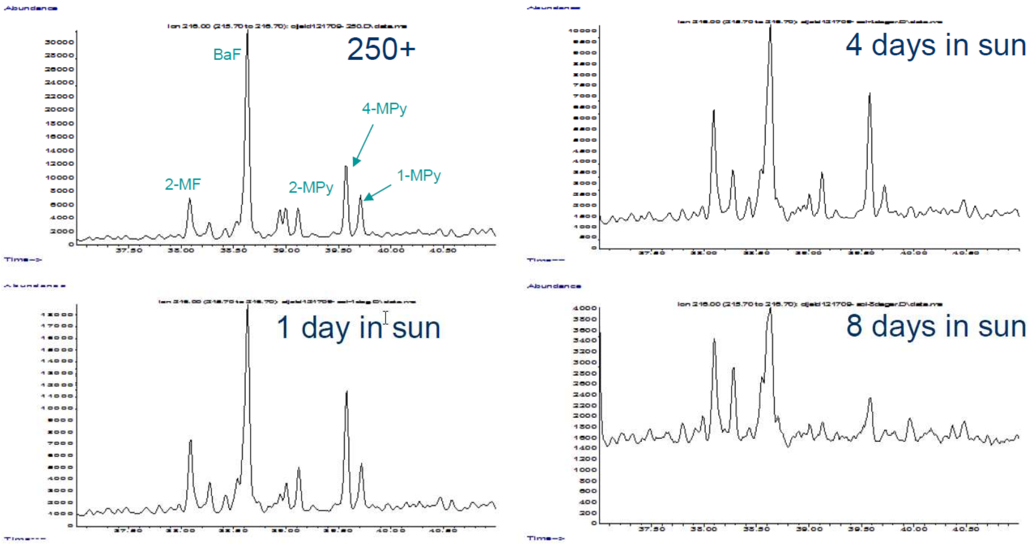


Fig. B.3
M.z 216 ion chromatograms

A general reduction of the intensities of the compounds which is not caused by evaporation based on the GC-FID chromnatgrams of Fig. B.1

C2-fluoranthenes /pyrenes/benzofluorenes (m/z 230)

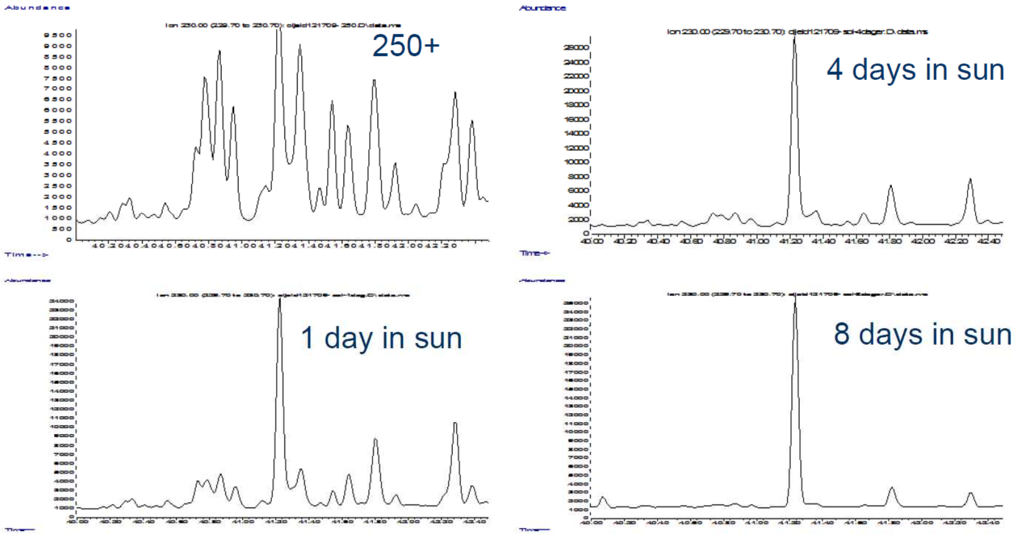


Fig. B.4
M/z 230 ion chromatograms

Here a strong relative effect on the compounds is visible. The identity of the three peaks in the chromatogram of “8 days in sun” are unkown.

C1-phenanthrenes (m/z 192)

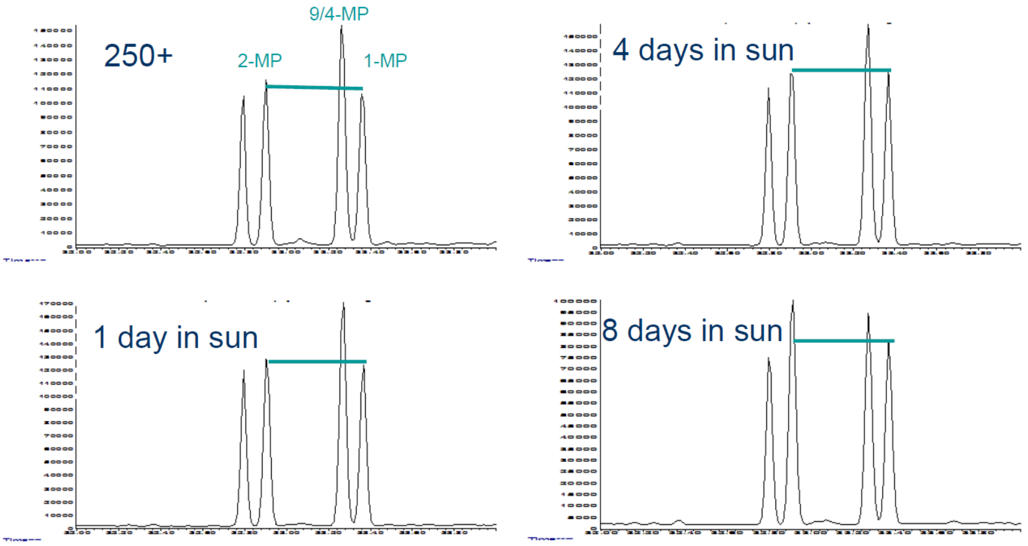


Fig. B.5
M/z 192 ion chromatograms

The two last eluting peaks of the phenantrenes are more reduced.

Summary – lab study: A crude oil on rock exposed to sun for 8 days

- Hopanes (m/z 191) are not influenced by photooxidation
- C1-phenanthrenes (m/z 192) changes when exposed to sun
- C2-fluoranthenes/pyrenes (m/z 230) are heavily photooxidized
- Several of the ratios for C1-fluoranthenes/pyrenes (m/z 216) are influenced by photooxidation:

DR	250+	1 day	4 days	8 days
2-MF/4-MPy	0.6	0.6	0.9	2.6
B(a)F/4-MPy	2.9	1.8	1.6	3.3
2-MPy/4-MPy	0.4	0.4	0.4	0.4
1-Mpy/4-MPy	0.6	0.4	0.3	ND

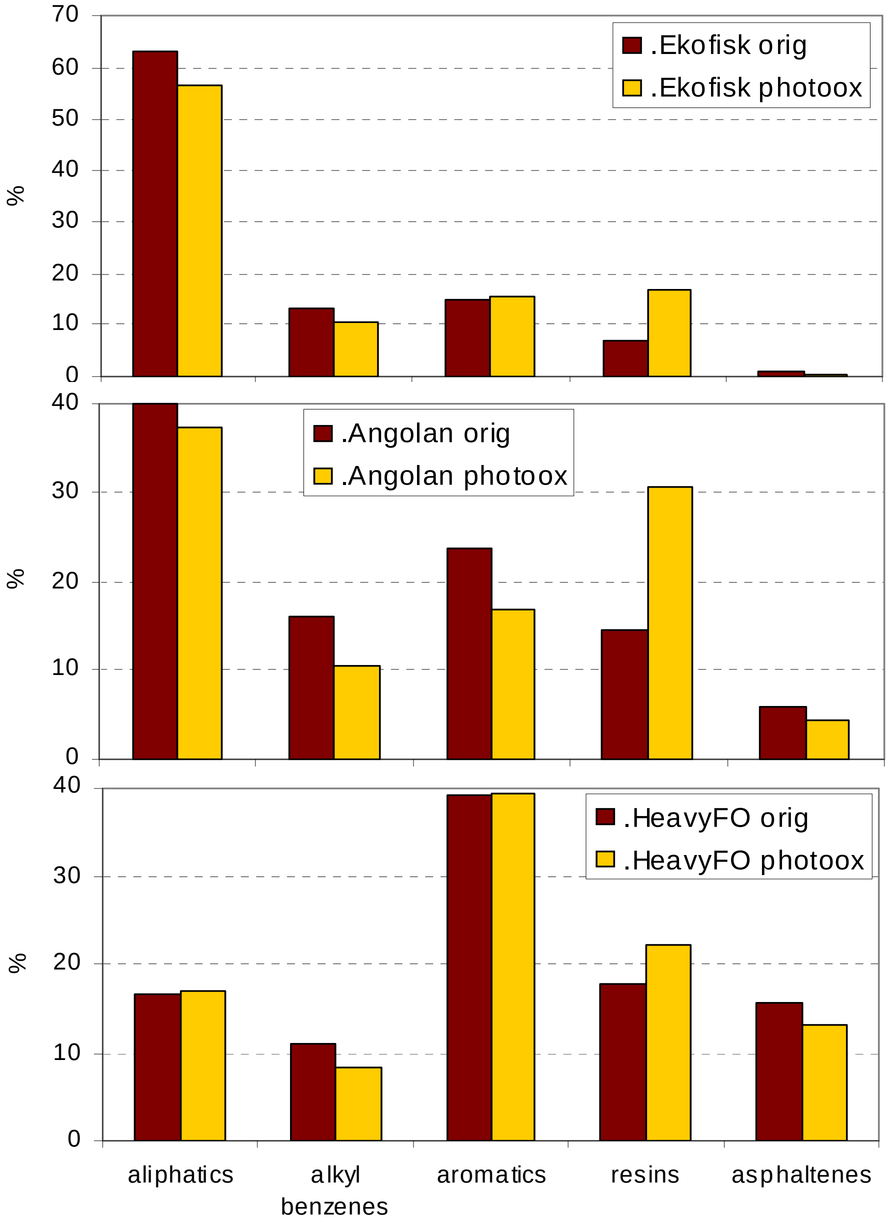
Fig B.6
Conclusions of Sintef

Annex C CSIC photo oxidation experiment

Joan Albaiges (CSIC, ES) has performed a photo oxidation experiment with the heavy fuel oil of the Prestige case.

Fig C.1
Instrumentation

Suntest® CPS, ATLAS (Chicago, USA) equipped with a 1500B NrB4 Xenon lamp (507.5 W/m2). The light emitted by this equipment has a UV spectrum similar to natural radiation. 1hour irradiation corresponds to 4 hours at natural sunny conditions.



Effect of photo oxidation on compound groups analysed by means of IR.

Fig C.2
IR analyses of compound group of two crude oils and one HFO

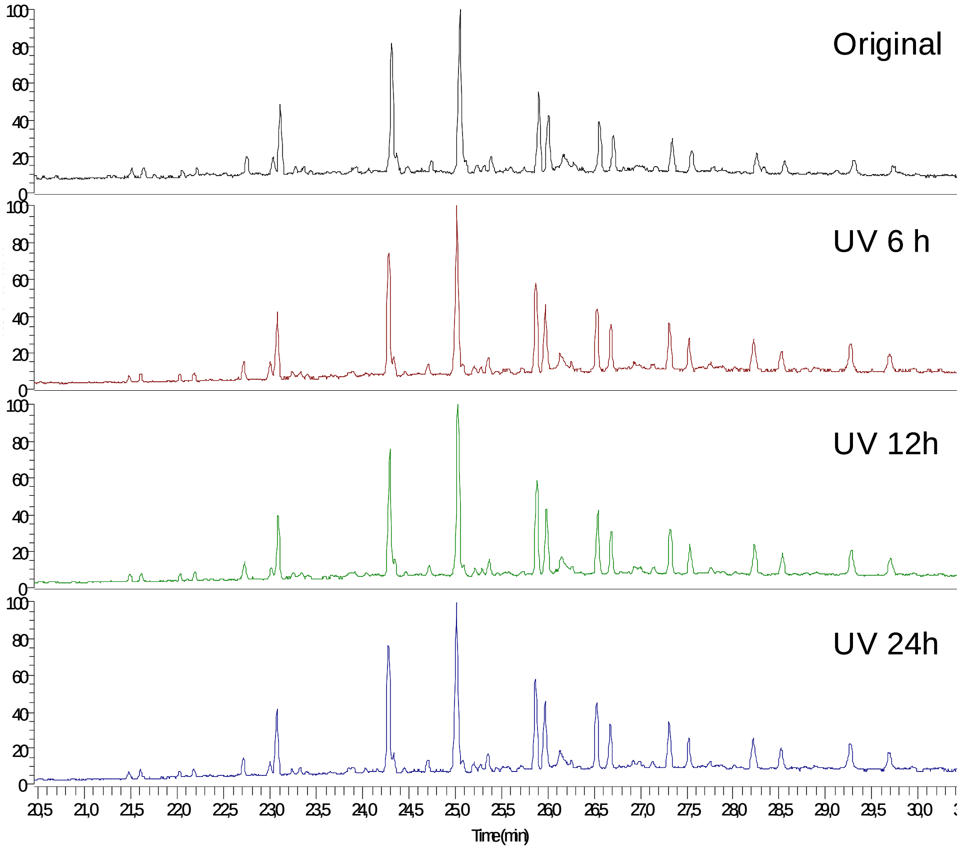


Fig C.3

Effect of photo oxidation on the triterpanes m/z 191 HFO prestige

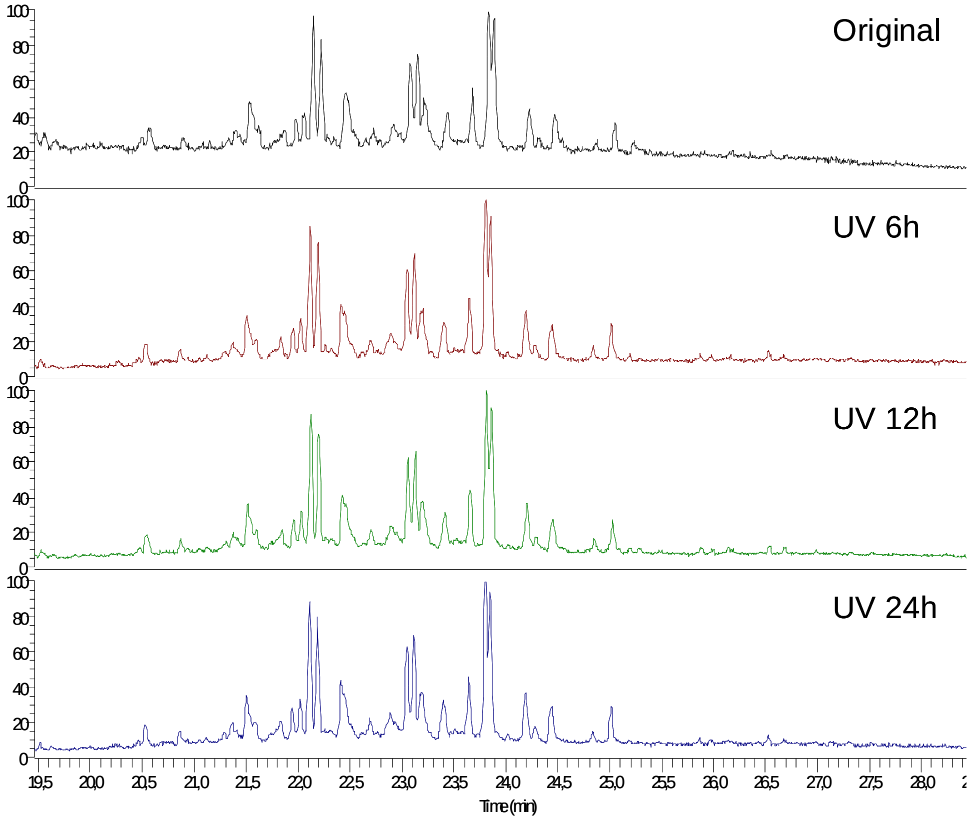


Fig C.4

Effect of photo oxidation on the steranes m/z 218 HFO prestige

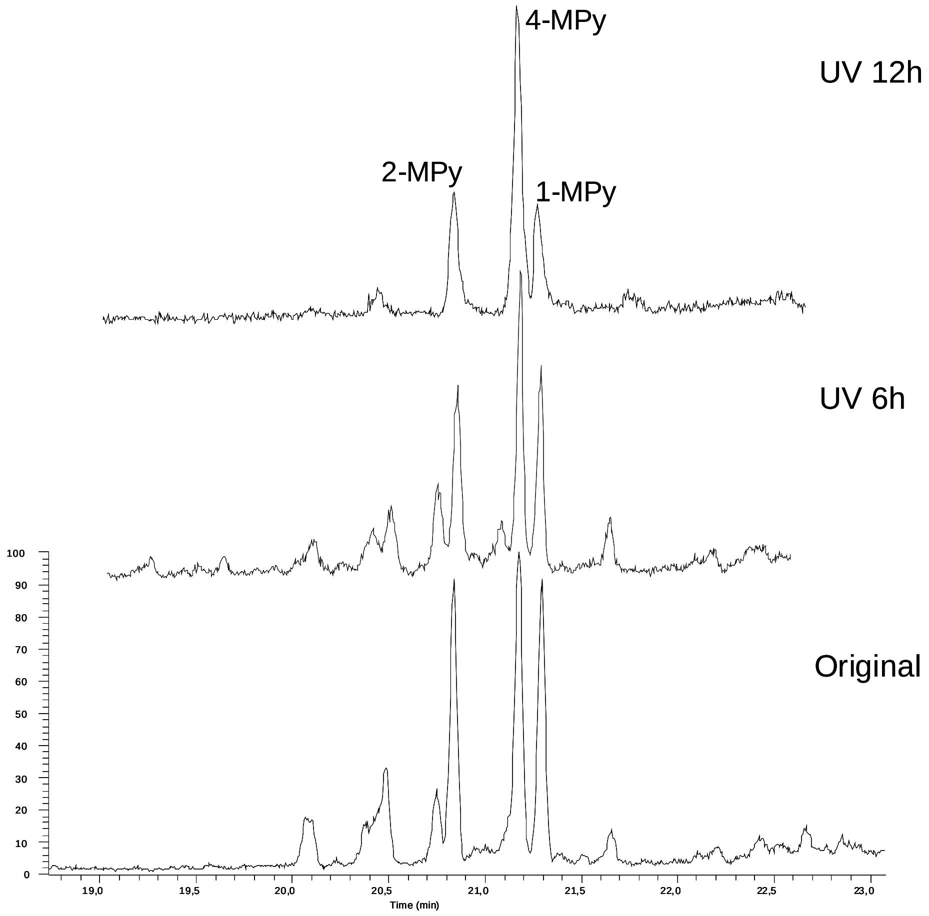


Fig C.5
Effect of photo oxidation on the m/z 216 compounds. HFO prestige