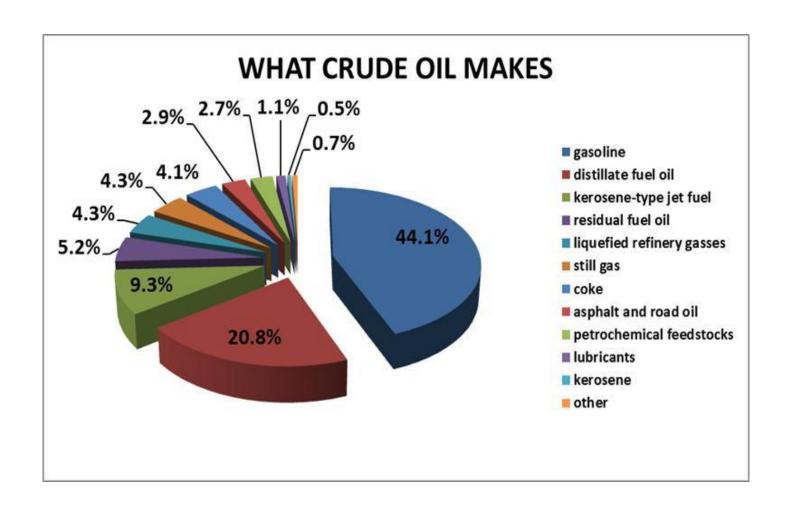
# PERSAMPELAN DAN ANALISA



Kursus Penguatkuasaam dan Kawalan Pencemaran Marin 21 March 2018

# CONTENT

- INTRODUCTION
- SAMPLING
- ANALYSIS



# Oil spill:

- Release of liquid petroleum hydrocarbons into the environment
- Human activity
- A form of pollution
- Marine oil spills released into the ocean or coastal waters

Oil spill can occur at any of these stages:

- Oil extracted from ground
- Oil is pumped to a pipeline
- Oil is transported to a refinery
- Oil is transported to final destination
- Illegal dumping

- Environmental effects
  - Smothering impact on physiological functions
  - Chemical toxicity
    - o lethal and sub-lethal
    - o impairment of cellular functions



- Ecological change
  - o loss of key organisms
  - o Invading of habitat by opportunistic species
- Indirect effects
  - Loss of habitat
  - Elimination of ecologically important species

- Economic effects
  - Tourism
    - short term swimming, boating, angling, diving
    - long term public perception (for prolonged, wide scale pollution)
  - Fisheries and mariculture
    - Fish stocks
    - Fouling gears, access to fishing sites
  - Shipyards, ports and harbours
    - Disrupted both spill and clean up
  - Heavy industry
    - Depends on seawater for operations

# **SAMPLING**

# SAMPLING FOR THE PURPOSE OF SOURCE IDENTIFICATION

- The most important stage of any chemical analysis is the sampling
- It does not matter how good your quality systems and test methods are, if you don't begin with a representative sample you cannot expect to produce accurate results
- Obtaining truly representative samples from oil spills can prove challenging:
  - because the composition of oil can vary widely before it is even removed from the ground.

#### SAMPLING METHODS/GUIDELINE

- ASTM 4489 standard practice for sampling of waterborne spills
- CEN/TR 1552-1:2006 oil spill identification. Waterborne petroleum and petroleum products. Sampling
- USCG marine safety lab: Oil spill handling and transmittal guide. 8<sup>th</sup> edition 2013
- Bonn agreement counter pollution manual: Volume 3, chapter 32, oil spill identification Part 1
- IMO manual on oil pollution: Section VI, IMO guideline for sampling and identification of oil spills. IMO 1578E
- ITOPF TIP14 sampling and monitoring of marine oil spills

#### Practical sampling guidelines

- Samples taken must be representative of the spill and suspected source
- Care must be taken to avoid contamination of the samples from other hydrocarbon sources, including cross contamination between samples
- Samples must be stored safely (neat oil may be highly flammable) and in a manner that preserves the sample integrity
- Collected samples must be clearly and uniquely labelled with as much information about the sample as possible
- Samples should be securely sealed in a manner to prevent tampering and maintain a robust chain-of-custody, particularly when samples may be used as evidence in court action

# GENERAL REQUIREMENTS

### Safety - first priority, for the sampler

- Minimize risk of toxic exposure from oil spilled
- Follow regulations
- Guided/accompanied of knowledgeable people/crew

#### Training

- All personal involved in sampling need to be trained
- Ensure correct sampling
- Asked by lawyers in case legal proceedings
- Sampling from ships (suspected polluters), the sampler must be working on board

# GENERAL REQUIREMENTS

# Sample planning

- Spilled sample, collect quickly avoid evaporation
- Source sample
- Reference (blank) Samples

# Sample Custody

- Sample handle as legal evidence and kept within "chain of custody"
- Make notes, take photos and videos.

## OIL SPILL SAMPLING RULES

- All spills encountered and potential sources of spill should be sampled
- Samples should be taken even when it is clear where the spill originates
- Scattered and/or only thin sheen remains, every effort should be made to take a least a small sample
- If any part of oil spill differs from other part, take extra samples to check if more than one oil spill occurred in that area

#### OIL SPILL SAMPLING RULES

- If spill response operation continues for more than one day, samples should be taken every day for determination of degree of weathering and possible contamination by other oils
- Blank samples if an oil suspected of contamination
- Samples and sampling equipment handled and stored properly
  - Reusable sampling equipment should be carefully cleaned and stored in plastic bags
  - NEVER reuse sample bottles even after careful washing

#### OIL SPILL SAMPLING RULES

- Sample handle as legal evidence and kept within "chain of custody" until identification and possible legal procedures have been completed
- Make notes of all relevant information about sample and sample sites
- Quick handling of samples
  - Sent as soon as possible to laboratory
  - Delayed, keep at +4°C (not frozen)

## Amount of oil required

Description	Minimum required quantity (per sample)
Pure oil source sample	30-50 mL
Contaminated oil (eg. emulsified oil, oil from sea or shore, tarball, etc.)	10-20 g
Debries with oil, oil stranded sand	Sufficient quantity that oil content is approx. 10 g
Oiled feather	5-10 feathers depending on oil quantity present
Fish, shellfish	Multiple individuals of same species totaling 30 g
Water sample with visible oil	1 liter
Water sample with no visible oil	3-5 liters

Source: ITOPF, 2011

# SAMPLING TECHNIQUES

# 1) Thick waterborne layers, viscous oil, tarball Aluminium beaker

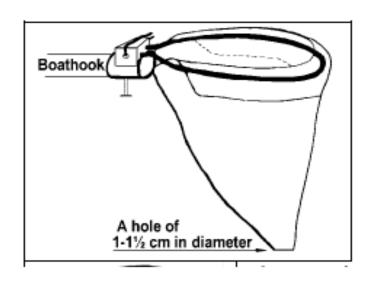


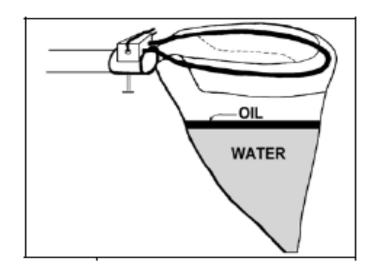


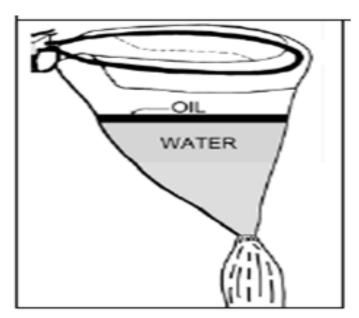




# 1) Thick waterborne layers, viscous oil, tarball









- Try to get 20-50 mL of oil, repeat the procedure to get the amount wanted
- Transfer the oil gather into 100 mL clean glass bottle, label. May use wooden spatula
- Place the bottle in the safety bag and seal the bag
- For large spill area take several positions within the spill

## 2) Thin oil layer on water (sheen)



## 2) Thin oil layer on water (sheen)



# 2) Thin oil layer on water (sheen) WATER

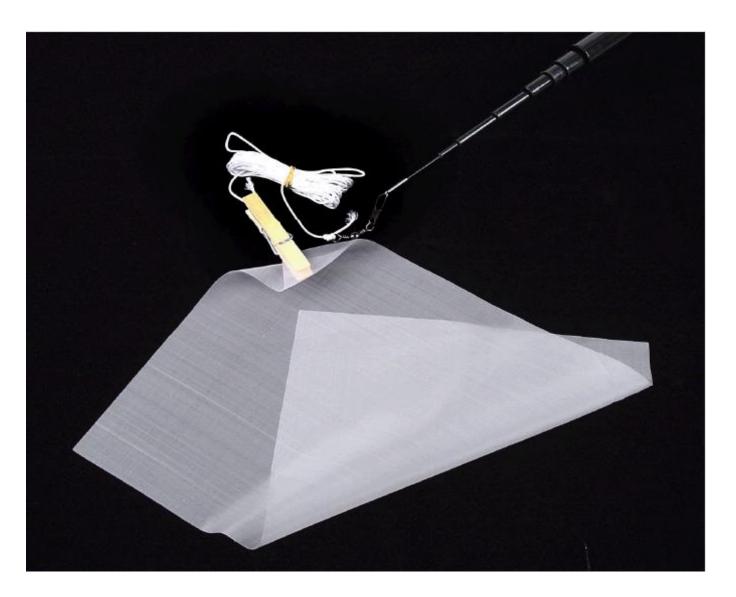




# 2) Thin oil layer on water (sheen) WATER



# 2) Thin oil layer on water (sheen) ETFE NET



# SAMPLING TECHNIQUES

ETFE is ethylene tetrafluoroethylene,

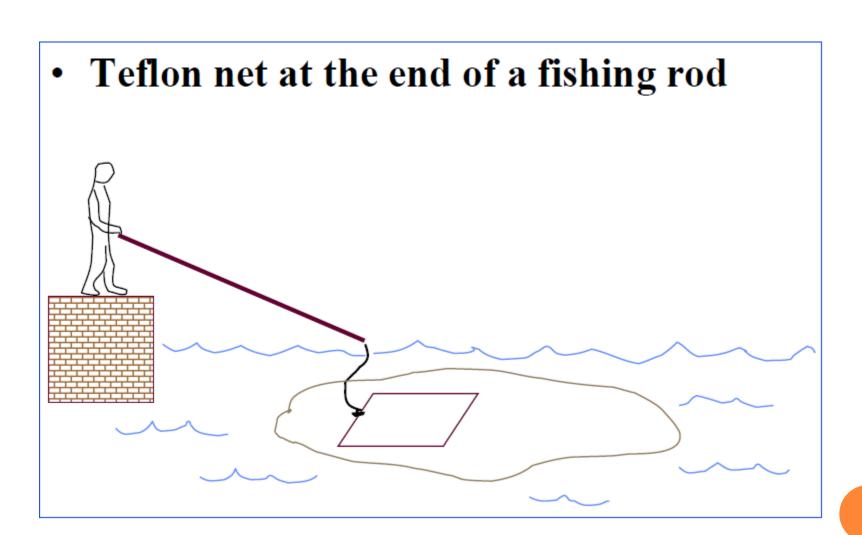
$$\begin{bmatrix}
H & H & F & F \\
- & - & - & - \\
C - C - C - C - C - \\
- & - & - & - \\
H & H & F & F
\end{bmatrix}_n$$

not teflon (polytetrafluoroethylene)

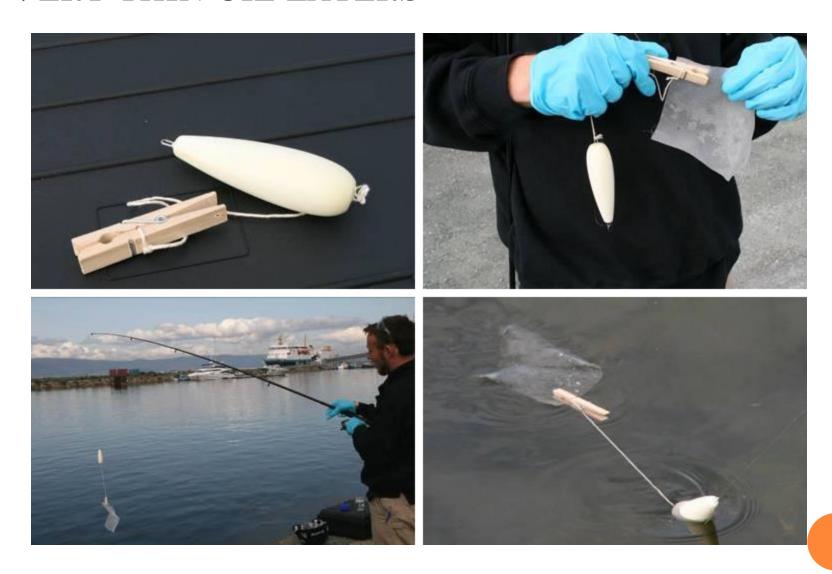
$$\left(\begin{array}{c|c} F & F \\ \hline F & F \end{array}\right)_n$$

 CEN/TF 120 N035 Oil spill identification, Technical Report 2006

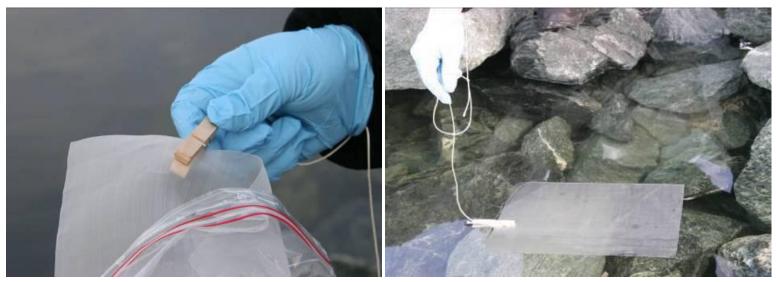
# VERY THIN OIL LAYERS



# VERY THIN OIL LAYERS



# VERY THIN OIL LAYERS





- rock/gravels/seaweeds/woodwork
  - Large amount of oil
    - •Scrap the oil with spatula and transfer directly into 100 mL bottle
  - Lesser amount of oil
    - wipe the oil with the ETFE net and transfer the whole ETFE net to the glass bottle



- If none of the above works
  - transfer the contaminated object (gravel/rocks/seaweed etc.) directly in the glass bottle
- Reference samples
  - Non-polluted material (rock, gravel, seaweed etc.)
  - As close to the "real" sampling area as possible.

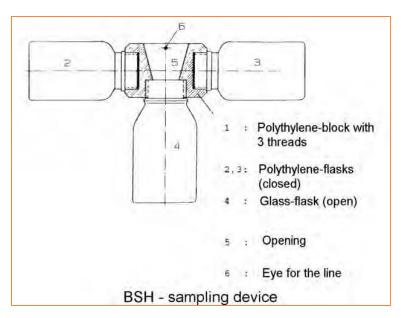
- Oil from sea birds and marine mammals
  - Heavy polluted animal
    - Wipe oil onto the Teflon net and transfer the net with oil to the 100 mL glass flask (glass).

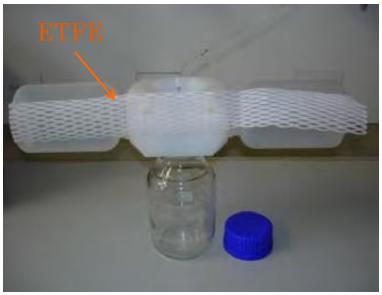


- Less polluted animal
  - Contaminated feather is picked off or contaminated fur is cut of (with the knife) and transferred to the 100 mL glass flask
- Never take whole animal samples, body tissues, etc. which may become rotten during shipment.



## USE OF SAMPLING BUOY FROM AIRPLANE

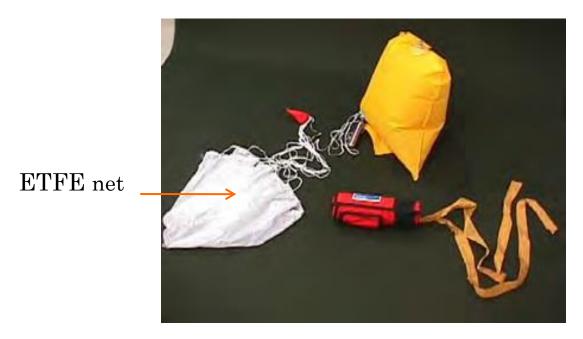




• Also can be used from bigger ships or from bridges in harbours

#### USE OF SAMPLING BUOY FROM AIRPLANE

• Drop a sampling buoy into an oil spill from an air craft



- Record the position, wind and sea current
- Lift the buoy from the water without touching the sample net
- Drain the excess water, insert the ETFE into glass bottle

## SOURCE SAMPLE

- Investigations and oil sampling on board vessels
  - Cargo oil
  - Machinery spaces
     (bilge water, waste oil etc.)
  - Follow safety regulations and protocols
  - Get assistants from ship by crew under control
    - Tanks/ships hazardous are

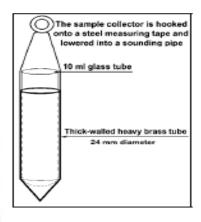
## SOURCE SAMPLE

- NEVER accept unknown samples handed over by ship representatives or shipping company
- Record observations using digital camera/video
- Use ships own sampling equipment if available
  - •Get approval for external equipment usage

# SOURCE SAMPLE

• Sample collector – tanker or difficult access places







Sample collector with steel measuring tape.

• ETFE net – very small amount of oil



# OIL SHEEN



# OIL ON GROUND/SOIL



# OIL FROM SHIP



# OIL FROM SHIP



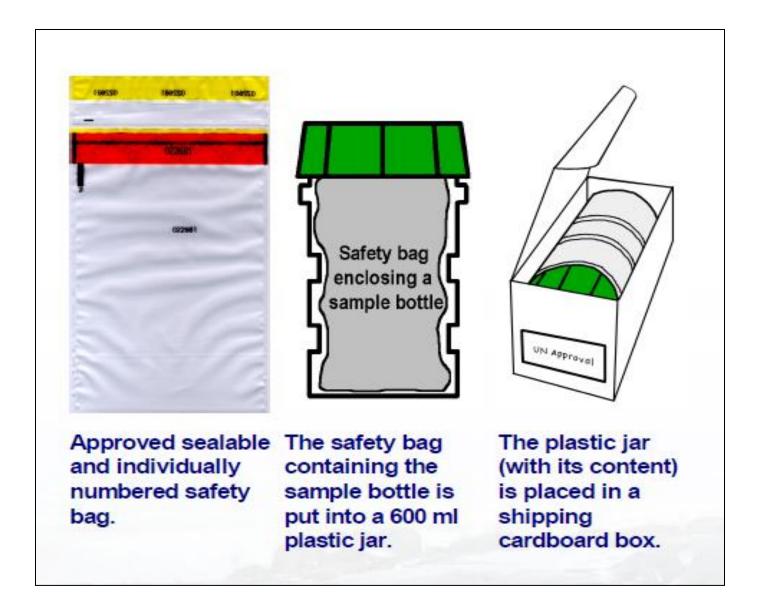
- Sample custody and documentation
  - Samples cannot be:
    - Manipulated
    - Mixed-up
    - Contamination
  - Chain of custody
  - How to maintain the 'chain of custody'
    - Approved sealable and individually numbered safety bags with adhesive number labels



- Filling and labeling of sample bottles
  - Sample bottles
    - Thick walled wide-mouth borosilicate glass bottles
    - $\circ 100 250 \text{ mL}$
    - Suitable Inner neck diameter 30mm
    - Lid tightening
    - New (unused) bottled for each sampling site
  - Avoid getting water into sample bottles
  - Do not fill to a higher level
  - Check the lid fits tightly
  - Wipe excess oil and water from the outside of the bottle



- Packing of sample
  - 3 forms of oil:
    - Free oil
    - ETFE net containing oil
    - Oiled items (feathers, wood pieces, stone etc)
  - Affix label onto the sample bottle
  - Insert the bottle into safety bag and seal
  - Place the bottle package into a plastic jar
  - Ship the sample immediately or keep below +4°C
  - Attach letter of request







# COMMERCIAL SAMPLING KITS

• Case with equipment for sampling of oil spills



Case with equipment for sampling of oil spills	No.
Metal ring (for polyethylene cornet)	1
Holder for metal ring	1
Polyethylene comet	20
Teflon® pad (20x30 cm) for thin oil films	15
Peg (for Teflon® pad)	20
Line (for rod and peg)	coil
Rod (for Teflon <sup>®</sup> pad) (perhaps not enough room in the case for this rod)	1
Sample bottle, 100 ml thick-walled borosilicate glass	10
Sample label	50
Safety bag (20x30 cm), approved, sealable, individually numbered	50
Wiping cloth (or paper)	package
Disposable gloves	package
Sampling peg (for scraping small samples of oil)	50
Plastic bag (for filled sample bottles, garbage etc.)	20
Laminated oil sampling flow diagram (cf. Annex3, Section 5)	1
Laminated instruction "Oil spill sampling" (cf. Appendix 1)	1

Laminated equipment list

# COMMERCIAL SAMPLING KITS

• Case with equipment for sampling on board ships



Case with equipment for sampling on board ships	No.
Sample collector of brass for sounding pipes (to be hooked on the steel measuring tape)	1
Steel measuring tape	1
Brass weight (to be hooked on the steel measuring tape)	1
Ground wire (to be hooked on the steel measuring tape)	1
Water finding paste	tube
Blackboard chalks (for chalking steel measuring tapes)	package
Glass tube 10 ml (with lid) for sample collector	10
Teflon® pad (20x30 cm) for thin oil films	15
Line	coil
Sample bottle, 100 ml thick-walled borosilicate glass	20
Sample label	50
Safety bag (20x30 cm), approved, sealable, individually numbered	50
Writing-pad (with cover and pen)	1
Wiping cloth (or paper)	package
Disposable gloves	package
Sampling peg (for scraping small samples of oil)	50
Plastic bag (for filled sample bottles, garbage etc.)	20
Earplugs	30x2
Laminated oil sampling flow diagram (cf. Annex3, Section 5)	1
Laminated equipment list	1

# DO AND DON'TS IN SAMPLING

- DO NOT composite any oil samples (oil spill, waste oil)
- DO NOT use plastic bottles/containers for samples
- DO Rinse all sampling equipments and bottles with solvents (dichloromethane) prior to use
- Use of EFTE net is HIGHLY RECOMMENDED for oil sheen instead of skimming technique

# EXAMPLE OF REQUEST FOR ANALYSIS FORM

#### Request for analysis

Request analysis of the samples listed below

Sampling date:		No. of samples:		No. of pages:	
General information (Occurred incident, weather, spill size, suspected source, judged oil type, etc.)	Numbe	Number label from safety bag		Information which is not given on the sample bottle *	
*Information	n: Sampler N	ame/field unit			
momato	*Information: Sampler: Name/field unit Spill: Latitude & longitude, and sampling site's geographical name Ship: Ship name and sample site on board the ship				
Signature	Nam	e in block letters		Rank	

# ANALYSIS

# WHAT HAPPENS IN THE LABORATORY?



#### Evaluation

- Chromatograms;
- Distribution profiles;
- Weathering check (WR and distribution profiles);
- Diagnostic ratios (DR);

#### Conclusion

- √ Positive match;
- ✓/x Probable match
- Negative match
- ? Inconclusive



Technical report



analysis

Sample preparation



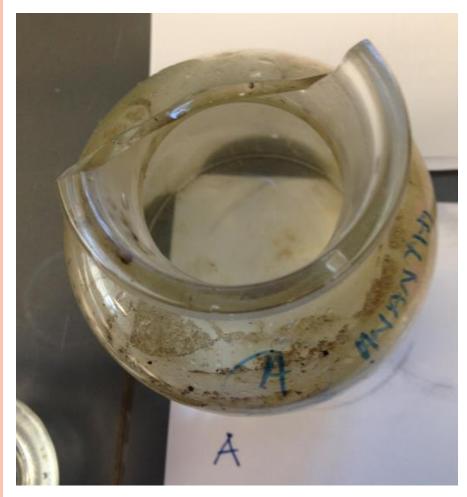
Data processing



Data assessment















# SAMPLE PRESERVATION

- Water/emulsions
  - Sample should be stored in refrigerator at 4°C or less
  - Undisturbed storage of sample may allow oil to separate for water facilitating the oil collection for preparation
  - Sample preparation within a week

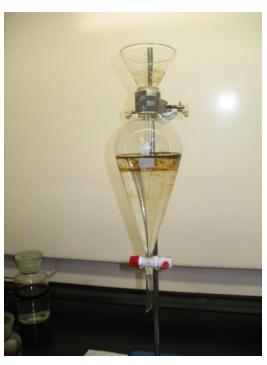
## SAMPLE PRESERVATION

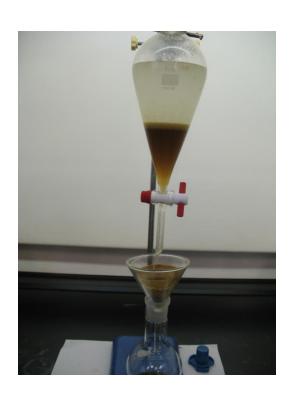
- Sample containing primarily water
  - Add dichloromethane (DCM) added immediately
    - example: 10ml to 1L water, shake
  - Store in refrigerator at 4°C or below
  - Sample preparation within a week
- Neat oil/oily sediment/oily soil/tarballs
  - Store in refrigerator at 4°C or below

- Visual examination and description of samples
  - Note and record:
    - Colour
    - Odour
    - Viscosity
    - Free water
    - Debris
    - Photography 'as-received' condition

- Water samples
  - Liquid-liquid extraction



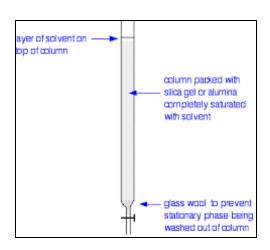




- Water samples
  - ETFE net



- Sample clean up
  - Minimize matrix interference
  - Remove asphaltenes
  - Soot particles
  - Silica or florisil column







# CEN METHOD

- CEN Method: European Committee for Standardization
- Developed by Bonn-OSINET \
  - Group of experts collaborating on oil spill identification
- Improvement of 1991 Nordtest method
- Published in 2006 and 2012

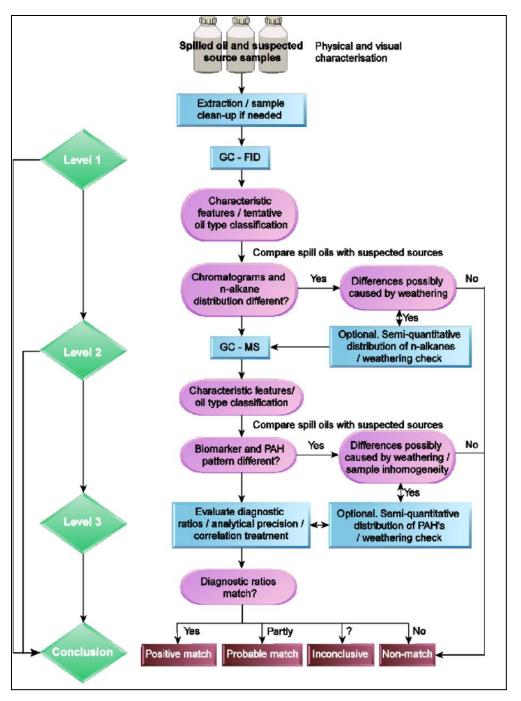
# **CEN METHOD**

- Nordtest used visual comparison
- CEN introduced quantitative compound ratios
- Three level tiered approached
  - Level 1 :GC-FID
  - Level 2 : GCMS
  - Level 3 : Diagnostic ratios

# **CEN METHOD**

# Four technically defensible conclusion:

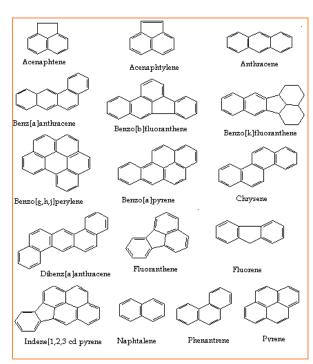
- Positive match
- Probable match
- Inconclusive
- Non-match

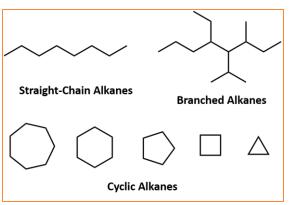


Source: SINTEF

# COMPOUNDS OF INTEREST

- ALKANES
- PAHs
- BIOMARKERS





# DIAGNOSTIC RATIO

### Diagnostic ratios

A suite of biomarkers (28) that are robust against weathering have been selected to cover genuine differences in oil samples and oil types

Diagnostic	c ratio	Light fuel oil	Lub oil	Bilge	HFO	Sludge	Crude oil
GC FID							
C17/pr		X		X		X	
C18/Ph		X		X		X	
Pr/ph		X		X		X	
GC MS							
m/z 85	C17/pr	X		X	X	X	X
	C18/ph	X		X	X	X	X
	Pr/ph	X		X	X	X	X
m/z 192	2MP/1MP	X		X	X	X	X
m/z 198	4MDBT/1MDBT	X		X	X	X	X
m/z 216	2MFL/4MPy	X		X	X	X	X
	B(a)F/4MPy	X		X	X	X	X
	B(b+c)F/4MPy	X		X	X	X	X
	2MPy/4MPy	X		X	X	X	X
	1MPy/4MPy	X		X	X	X	X
m/z 234	Retene/TMP	X		X	X	X	X
	BNT/TMP	X		X	X	X	X
m/z 191	27Ts/30ab		X	X	X	X	X
	27Tm/30ab		X	X	X	X	X
	28ab/30ab		X	X	X	X	X
	29ab/30ab		X	X	X	X	X
	30O/30ab		X	X	X	X	X
	31ab/30ab		X	X	X	X	X
	30G/30ab		X	X	X	X	X
m/z 217	27 dbR/27 dbS		X	X	X	X	X
m/z 218	27bb/29bb		X	X	X	X	X
m/z 231	SC26/RC26+SC27				X	X	X
	SC28/RC26+SC27				X	X	X
	RC27/RC26+SC27				X	X	X
	RC28/RC26+SC27				X	X	X

#### INSTRUMENT

- Flame ionization detection (GC-FID)
  - Gives indication of the sample type
  - Compares alkanes patterns
- Mass spectrometry (GC-MS)
  - Detailed information about compounds
  - Compares PAHs and biomarkers patterns

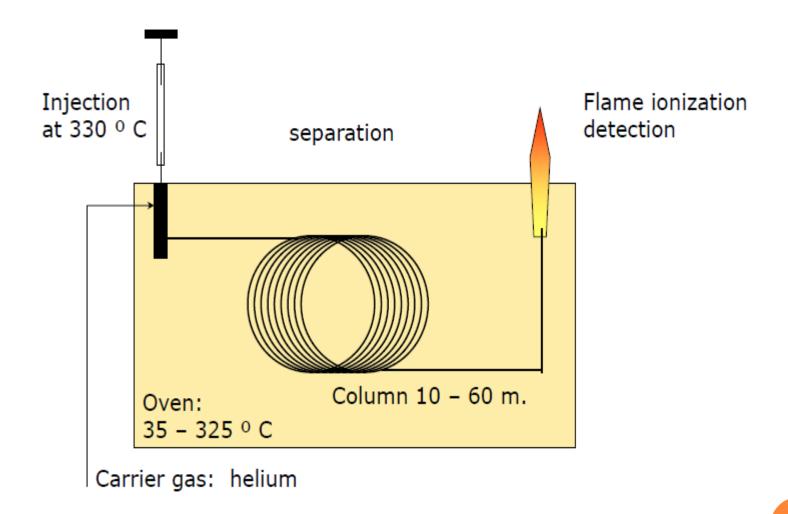


# GC-MS

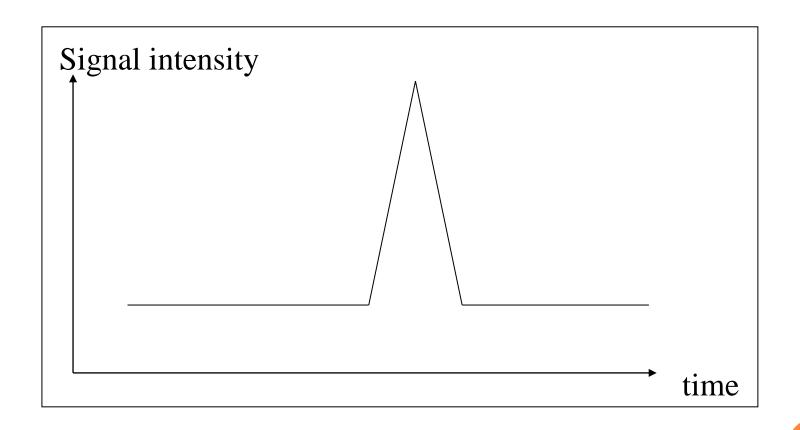
# GC-FID



# LEVEL 1 : GC-FID

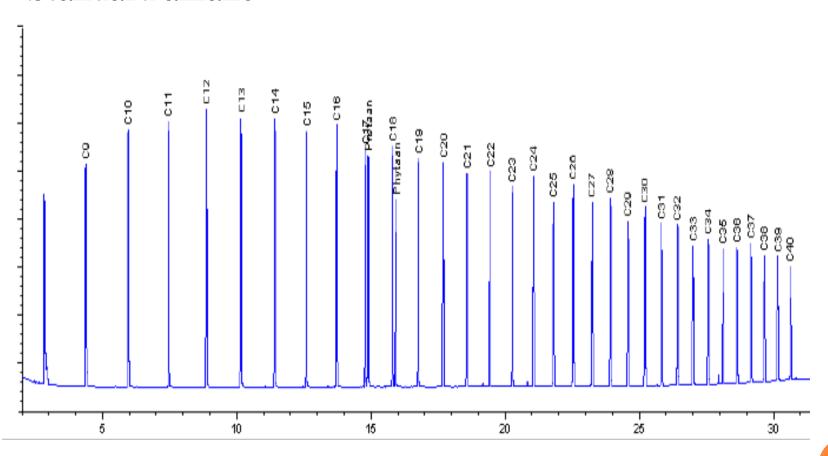


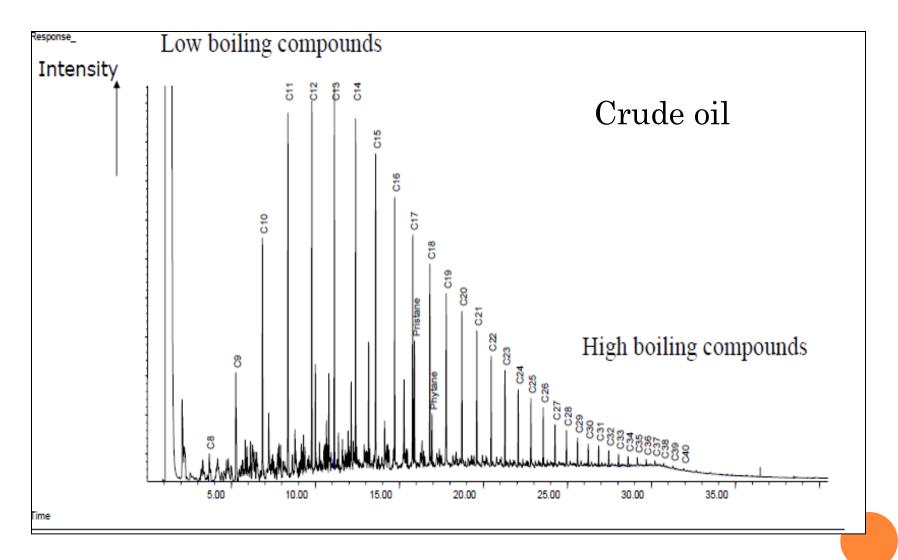
# SYSTEM OUTPUT

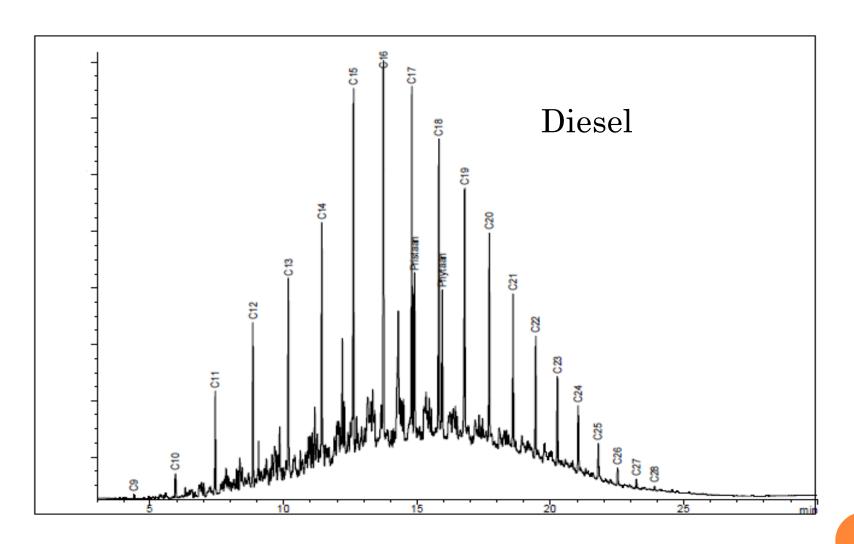


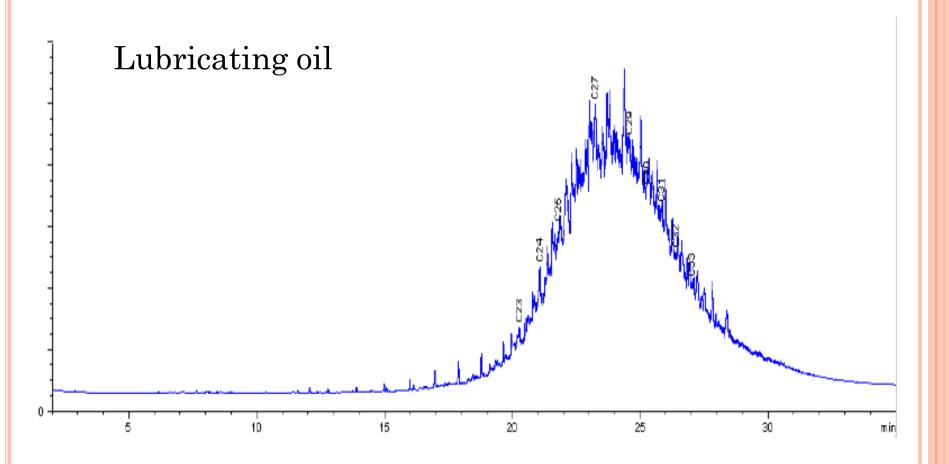
# CHROMATHOGRAM

#### Standard alkane

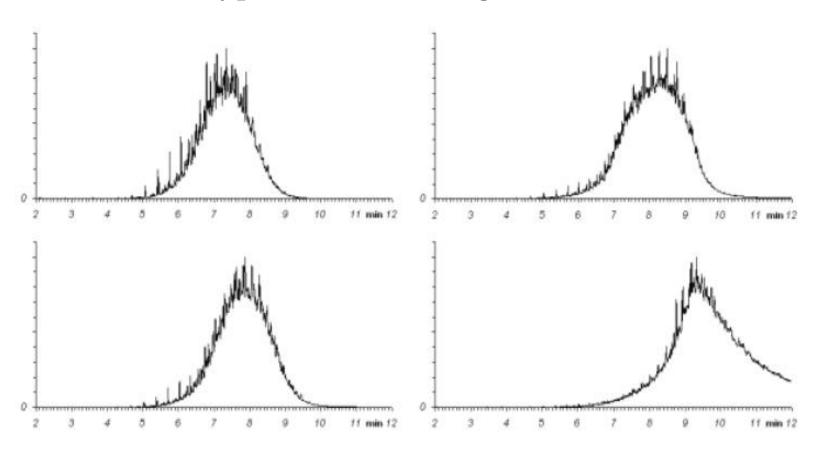


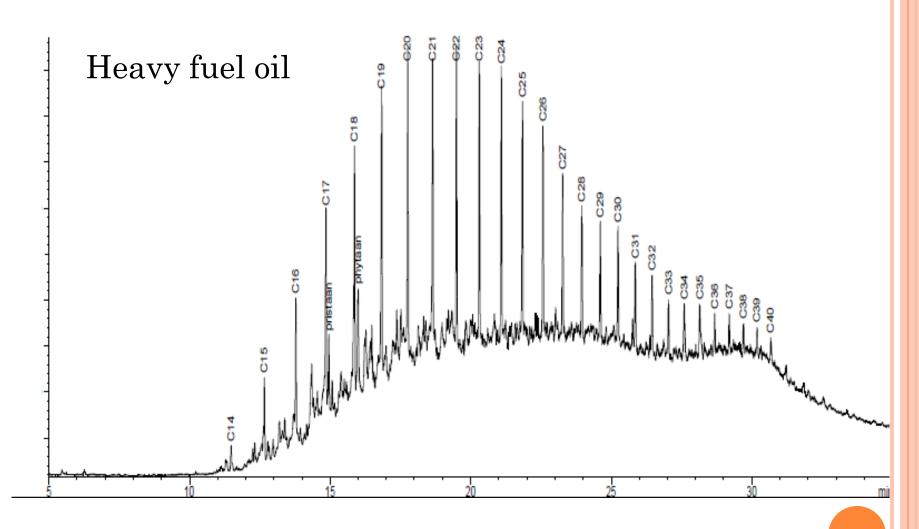


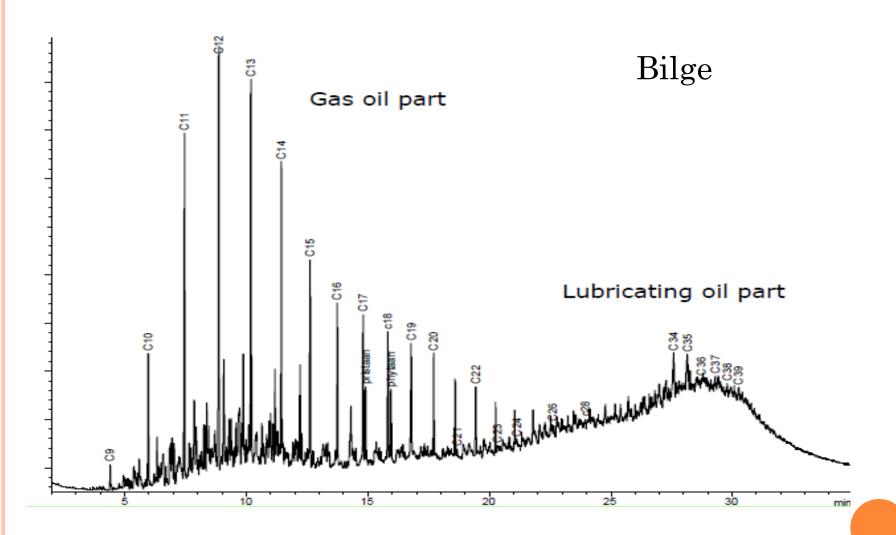




#### Different types of lubricating oil

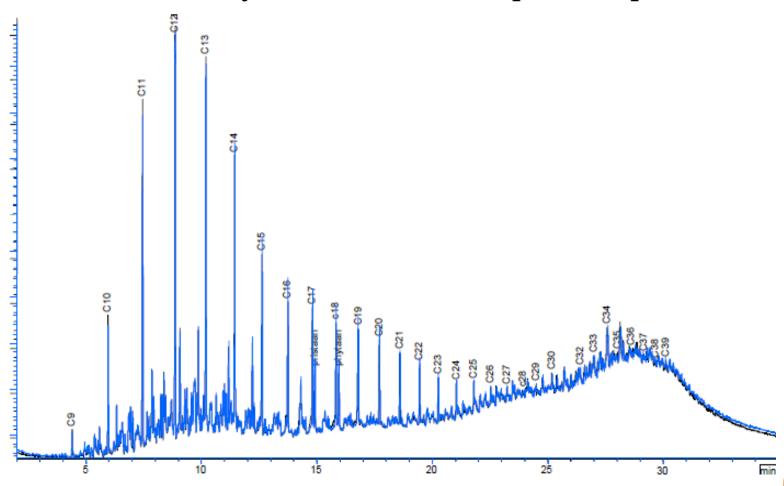




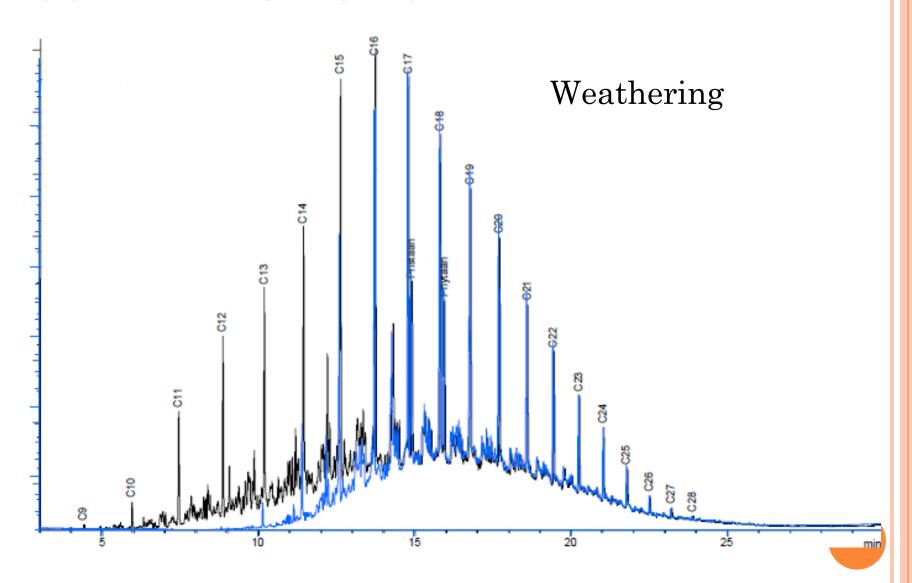


# COMPARISON OF OIL

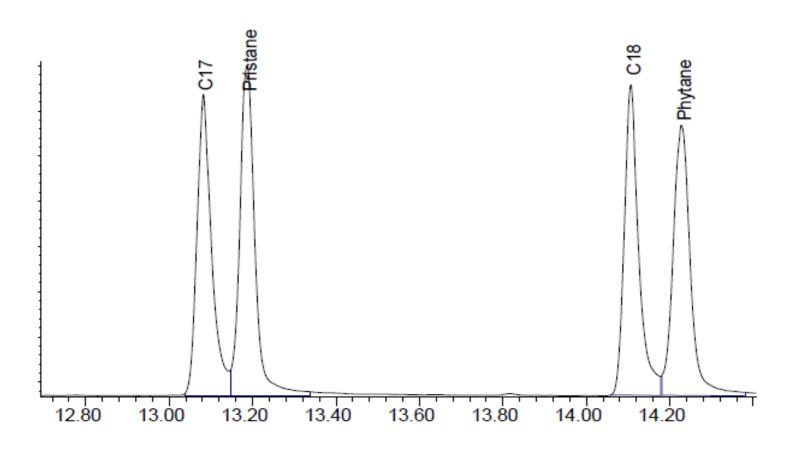
Overlay of a source and spill samples



# COMPARISON OF OIL



#### INTEGRATION OF PEAKS

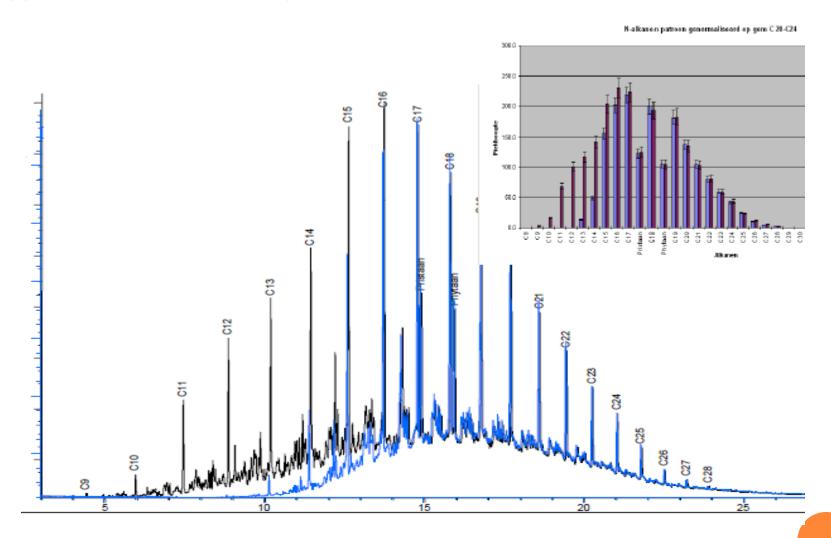


Calculation of peaks area or height

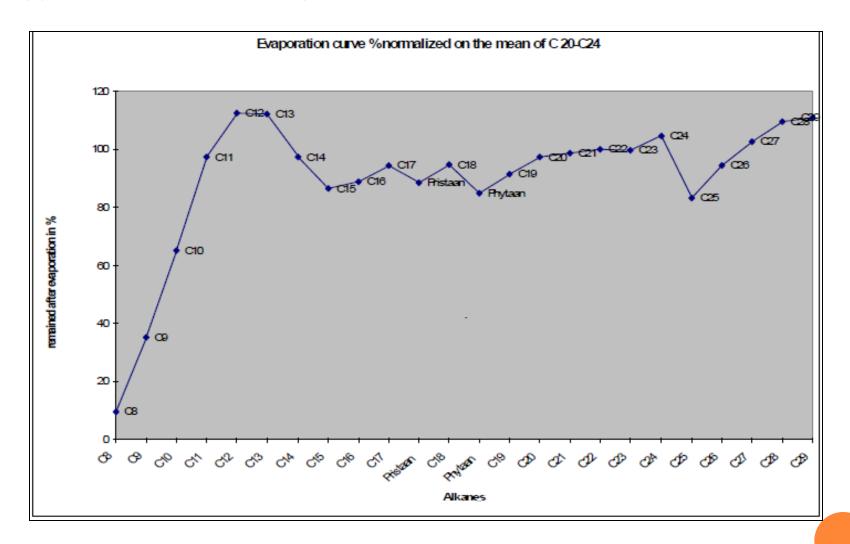
# DATA

	Source 2	Source 2 dup		Spill	source		PW-plot calculation	% Spill/Source
	161121-rr16	161121-rr16			normalised		(source-	after normalisation
Alkane	Source 2	source 2	Alkane	Source 2	source 2	Alkane	spill)/source * 100 %	
C8	7389	7120	C8	7389.0	7587.2	C8	3	97
C9	108642	106326	C9	108642.0	113303.2		4	96
C10	234165	220631	C10	234165.0	235108.9	C10	0	100
C11	341101	321418	C11	341101.0	342509.6		0	100
C12	373511	349510	C12	373511.0	372445.1	C12	0	100
C13	426133	397692	C13	426133.0	423788.8		-1	101
C14	455020	433448	C14	455020.0	461891.1	C14	1	99
C15	454633	435124	C15	454633.0	463677.1		2	98
C16	421128	404862	C16	421128.0	431429.3	C16	2	98
norpristan	102210	91287	npri	102210.0	97277.3	npri	-5	105
C17	409233	379533	C17	409233.0	404438.2		-1	101
Pristane	164180	154218	Pristane	164180.0	164337.9	pri	0	100
C18	349341	334827	C18	349341.0	356798.5	C18	2	98
Phytane	150249	140302	Phytane	150249.0	149508.7	phy	0	100
C19	343076	322468	C19	343076.0	343628.5		0	100
C20	323455	303586	C20	323455.0	323507.5	C20	0	100
C21	320115	302709	C21	320115.0	322572.9		1	99
C22	296034	274002	C22	296034.0	291982.2	C22	-1	101
C23	290954	278508	C23	290954.0	296783.9		2	98
C24	273636	253353	C24	273636.0	269978.2	C24	-1	101
C25	267566	251728	C25	267566.0	268246.5		0	100
C26	269302	250768	C26	269302.0	267223.5	C26	-1	101
C27	286101	271009	C27	286101.0	288792.8		1	99
C28	270049	254192	C28	270049.0	270872.2	C28	0	100

# WEATHERING



#### WEATHERING IN %



#### RATIOS

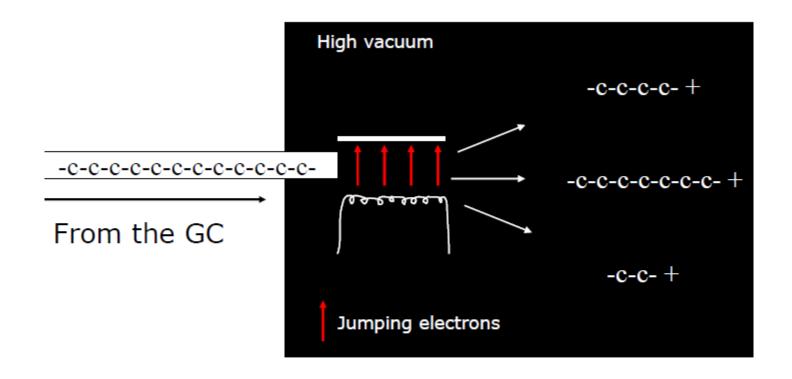
	absolute difference / mean difference*100				
sample comparison	C16/npri	C17/pri	C18/phy	pri/phy	
Source 1 - Source 2	34.4	1.2	7.6	35.7	
Source 1 - Source 3	18.1	44.5	28.3	61.4	
Source 2 - Source 3	16.6	43.4	20.8	27.2	
Source 1 - Spill	12.7	44.4	29.9	52.6	
Source 2 - Spill	22.0	43.2	22.5	17.7	
Source 3 - Spill	5.5	0.2	1.7	9.6	

Assumption: Differences < 14% is caused by analysis => no significant difference

#### **SUMMARY**

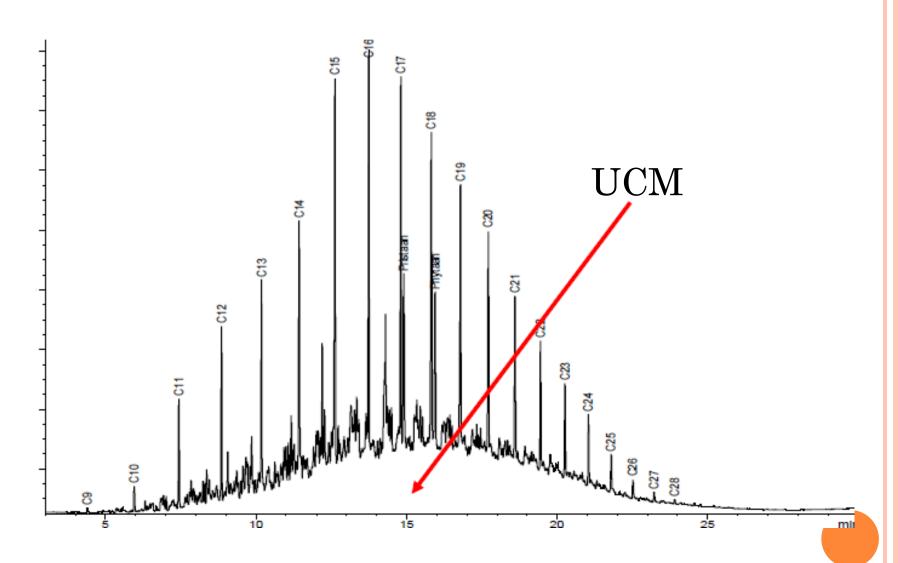
- Type of oil
- Comparison of chromatograms
- Weathering
- Comparison of ratios

#### LEVEL 2: GC-MS

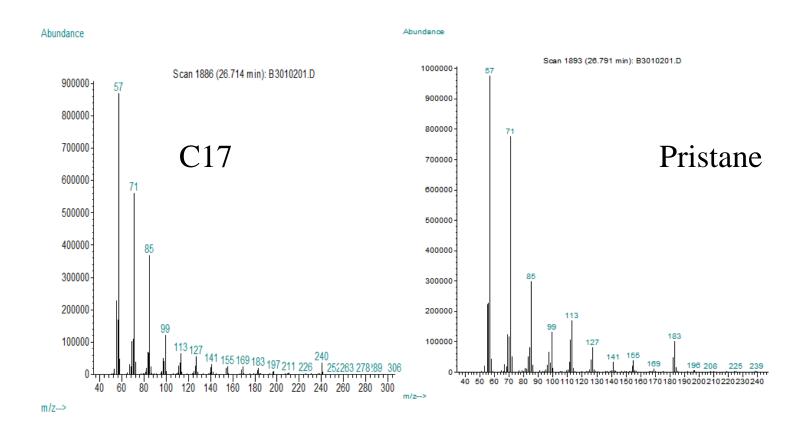


Analysis of charged fragments

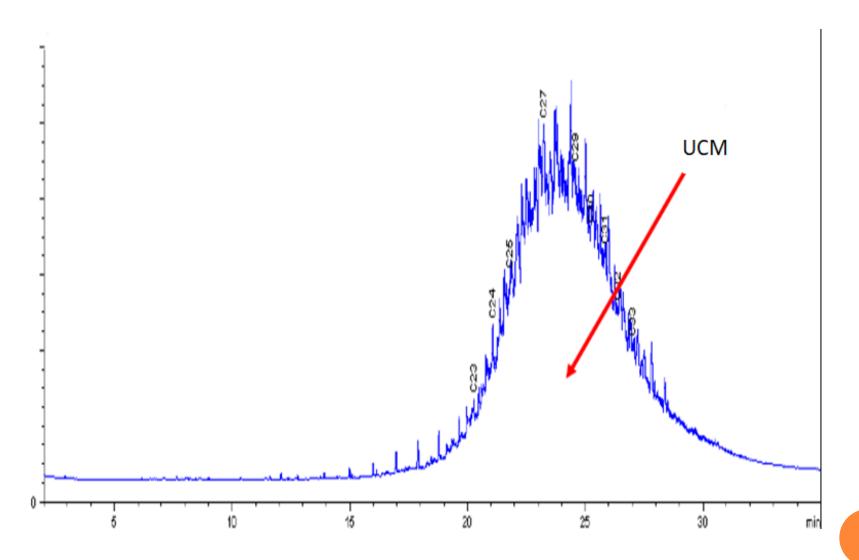
#### DIESEL



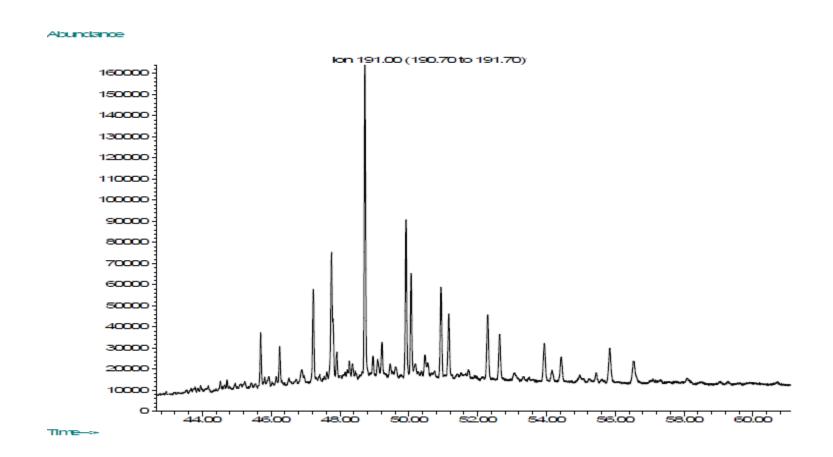
#### MASS SPECTRUM



# LUBRICATING OIL

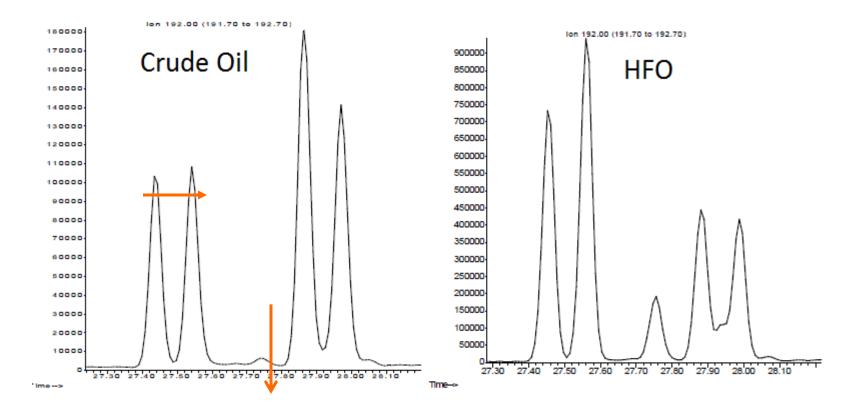


#### BIOMARKER ION M/Z 191



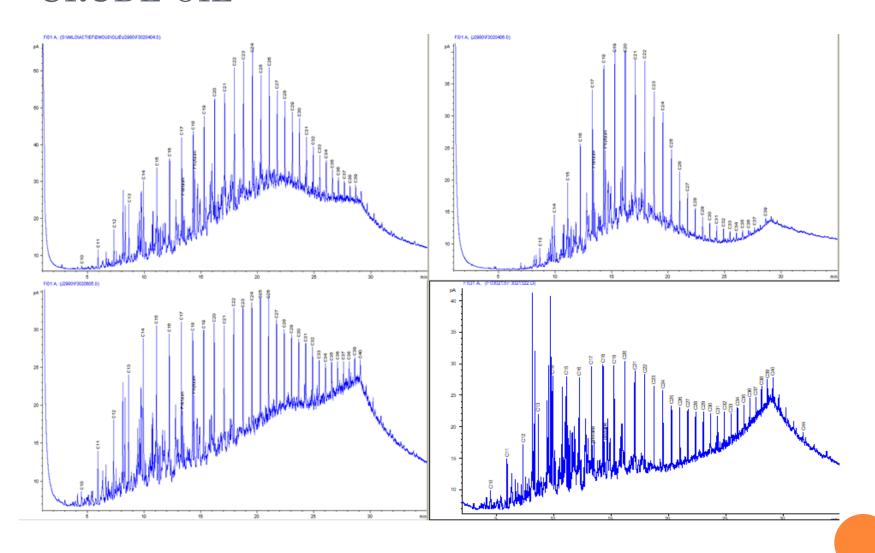
Plot of mass over time => chromatogram

#### **PAHS**

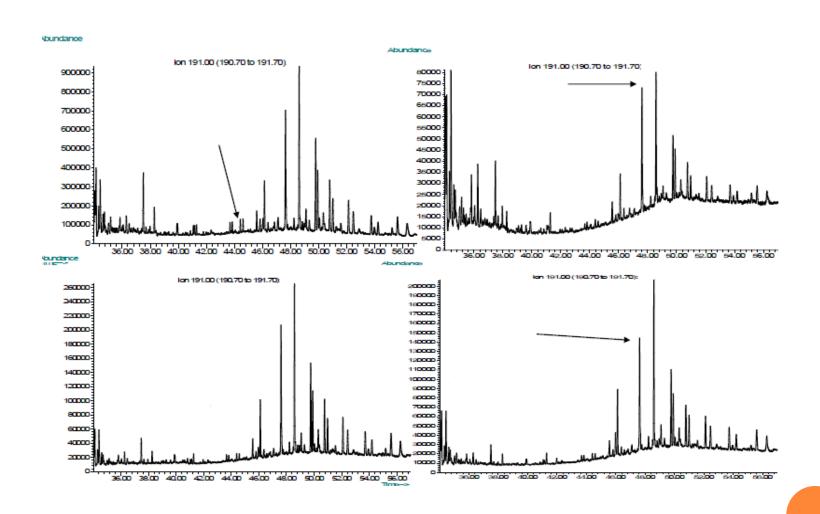


Methyl phenanthrene and methylantracene One of the differences between crude oil and HFO

# CRUDE OIL



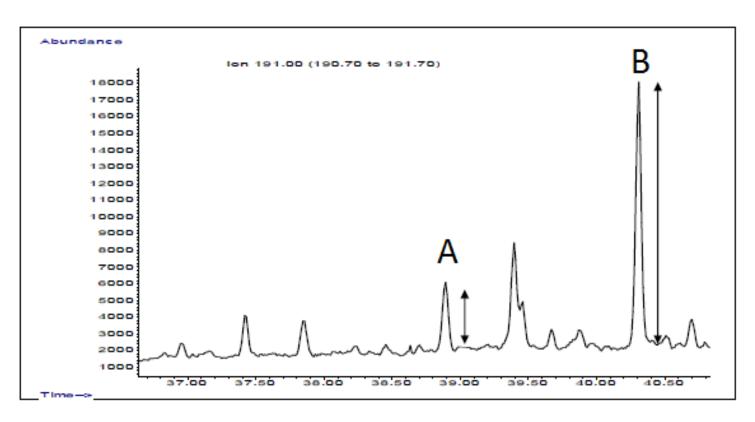
#### BIOMARKER ION M/Z 191



#### LEVEL 3: DIAGOSTIC RATIO

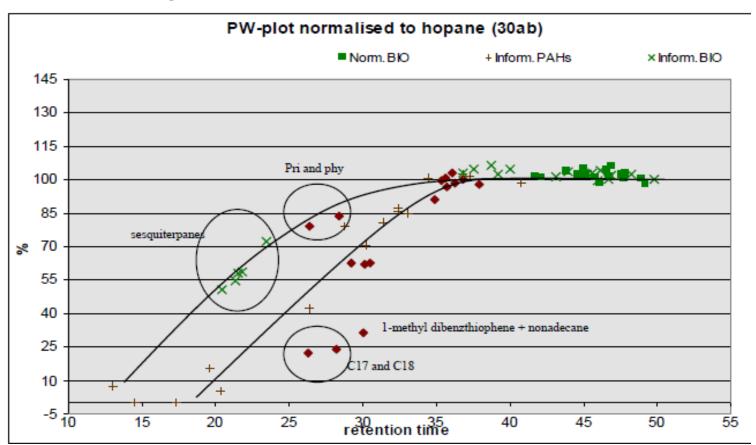
Minimum of 36 compounds are integrated Ratio calculated:

A/B \* 100%



#### WEATHERING PLOT

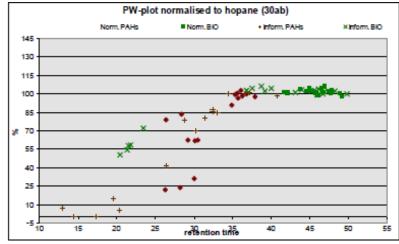
- Evaporation, dissolution and biodegradation have different effect
- No weathering effect after 35 min

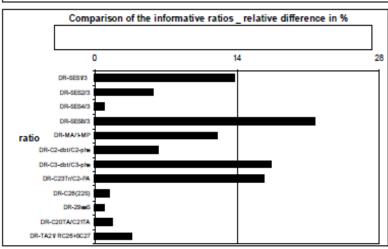


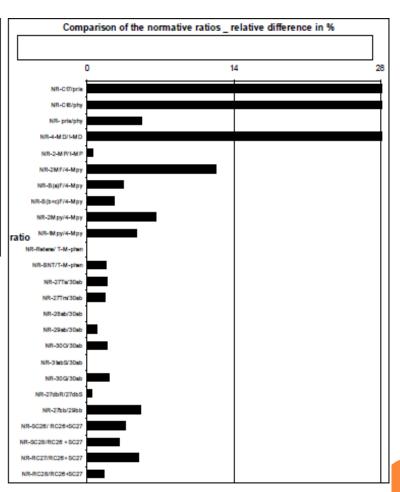
# PEAK RATIOS

				absolute	repeatability	flag
Diagnostic	mean	mean		difference	based on	
ratios	sample 1	sample 3	mean		RSD 5%	
					14	0
BaF-4MP%	45.75	45.96	45.86	0.21	6.42	
BbF-4MP%	34.56	37.33	35.94	2.77	5.03	
Retene/C4-phe%	0.00	0.00	0.00	0.00	0.00	
2-MP/1-MP%	66.91	65.48	66.19	1.43	9.27	
C2-dbt/C2-phe%	36.12	32.93	34.52	3.20	4.83	
C3-dbt/C3-phe%	41.42	39.49	40.45	1.93	5.66	
4-MD/1-MD%	80.20	77.83	79.02	2.38	11.06	
C3-dbt/C3-chr%	83.71	84.79	84.25	1.08	11.80	
C23tri-30ab%	46.02	45.18	45.60	0.84	6.38	
C24tri-30ab%	29.00	27.71	28.36	1.28	3.97	
C25tri-30ab%	25.34	24.04	24.69	1.30	3.46	
C24tetra-30ab%	0.00	0.00	0.00	0.00	0.00	
C28+C29 tri-30ab%	29.07	29.69	29.38	0.62	4.11	
C28tri-30ab%	18.40	18.55	18.47	0.16	2.59	
C29tri-30ab%	15.56	16.28	15.92	0.71	2.23	
27Ts/27Tm%	43.75	45.24	44.49	1.49	6.23	
28ab/30ab%	0.00	0.00	0.00	0.00	0.00	
25nor30ab/30ab%	0.00	0.00	0.00	0.00	0.00	
29ab/30ab%	43.78	44.44	44.11	0.67	6.18	
29Ts/30ab%	14.53	15.11	14.82	0.58	2.08	
30d/30ab%	6.77	6.00	6.38	0.77	0.89	
30O/30ab%	0.00	0.00	0.00	0.00	0.00	
30g/30ab%	13.32	12.51	12.91	0.81	1.81	

# COMBINATION OF INFORMATION - WEATHERING AND RATIO







#### **SUMMARY**

- Separate compounds that not separated by GC-FID
  - Visual comparison of ion patterns
  - Identification of oil type
  - Comparison of peak patterns
  - Weathering effects
    - Spill and source sample

#### **SUMMARY**

- Oil spill identification is a 'fingerprinting' technique
- Samples are exactly compared
- Comparison based on stable compounds
- Weathering can be estimated

#### PT OIL SPILL IDENTIFICATION



BONN AGREEMENT Oil Spill Identification Network of Experts

- JKM participated in Round Robin 2015, 2016 and 2017
- 31 laboratories form 22 countries participated.

# THANK YOU QUESTIONS?