Application of GCxGC to Develop Biodegradation data on Petroleum UVCBs: whole substance biodegradation with peak tracking

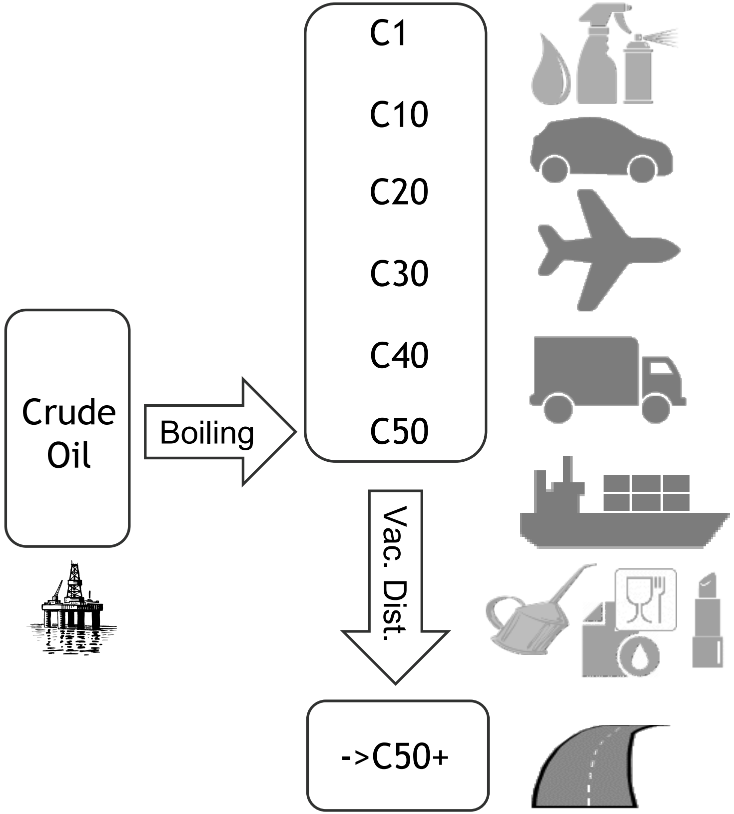
Delina Lyon
Sintef: Andy Booth, Lisbet Sørensen, Mari Creese, Odd G Brakstad
Oleolytics: J Samuel Arey
Jonas Gros
BP: Kat Colvin, S. Alex Villalobos
ExxonMobil: Aaron Redman, Louise Camenzuli
Shell: Dave Saunders
TotalEnergies: Sandrine Sourisseau
Concawe: Alberto Martin-Aparicio, Leslie Saunders, Neil Wang,

HESI UVCB workshop – September 2023

Disclaimer

This slide presentation is for discussion purposes only. The information, methods and any results shared herein are preliminary and subject to revision and should not be treated as final or comprehensive and shall not be used to draw conclusions or as the basis for any assessment, decision or other action.

Petroleum Substances (PS) are archetypical UVCBs



- **UVCB**
  - **U**nknown or
  - **V**ariable composition,
  - **C**omplex reaction products,
  - **B**iological origin
- **Hundreds to millions of HydroCarbon molecules** (isomers) per PS
  - Not all individually identified
  - PS constituents collectively characterized
- PS are variable in nature
  - Variability is limited to meet product specification
  - Petroleum substances form a continuum whereby physical-chemical properties overlap in the hydrocarbon space

C number	Boiling point °C (n-alkanes) (*)	Number of isomers (alkanes only!)
3	-42	1
4	-1	2
5	36	3
6	69	5
7	98	9
8	126	18
10	174	75
15	269	4 347
20	343	366 231
25	402	36 777 419
30	450	4 108 221 447
35	490	493 054 243 760
40	525	62 353 826 654 563

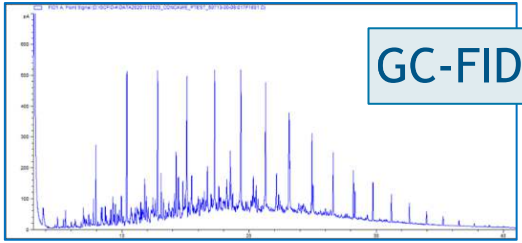
https://www.fuelseurope.eu/knowledge/how-refining-works
https://www.concawe.eu/publication/hazard-classification-and-labelling-of-petroleum-substances-in-the-european-economic-area-2020/

Concawe

How could you assess biodegradability of a PS?

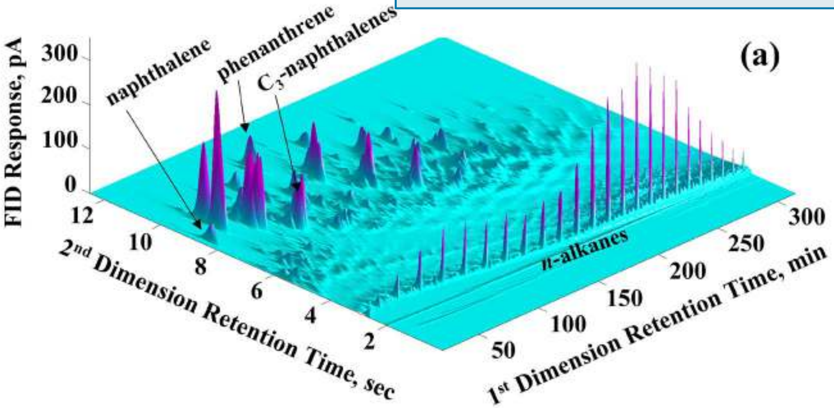
- **Constituent approach?**
  - ➢ Test data cannot be obtained for all constituents
    - Too many possible constituents
    - Most not available as pure substances
    - Many PS constituents not compatible with guideline test methods
- **Whole substance approach?**
  - ➢ Testing the whole substance gives a weighted average biodegradation.
  - ➢ Potential to overlook persistent constituents
- **Combine the two?**
  - ➢ Can we track constituents in the whole substance during a biodegradation test?

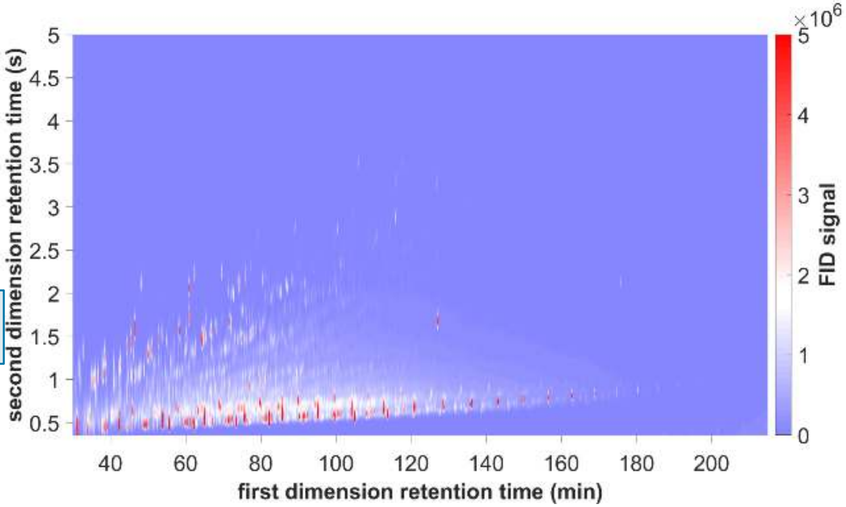
Two-dimensional gas chromatography separates PS UVCB constituents by polarity and volatility



GC-FID example

GCxGC-FID examples



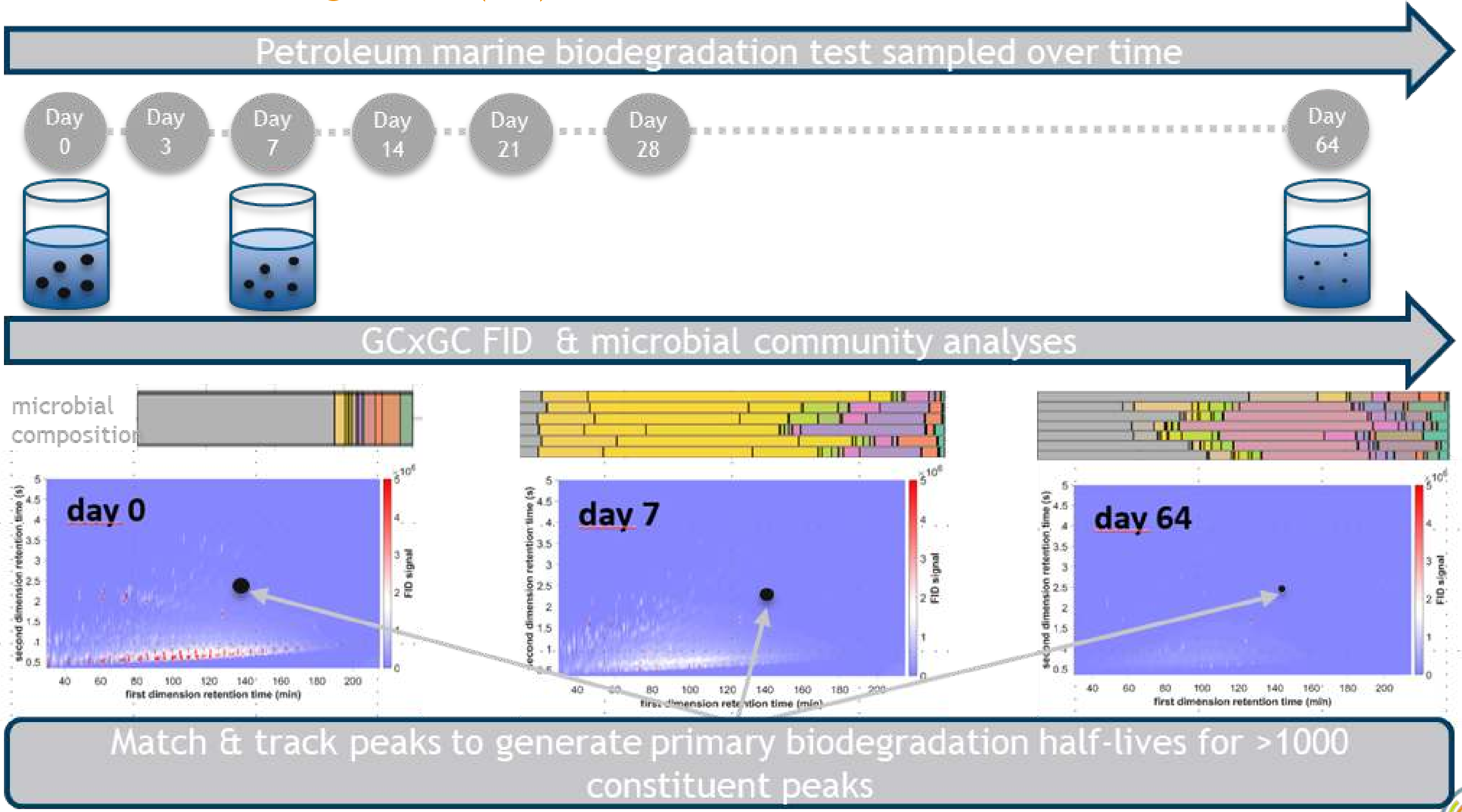


Existing literature shows it is possible to use GCxGC to track peaks (representing constituents) over the course of a biodegradation test

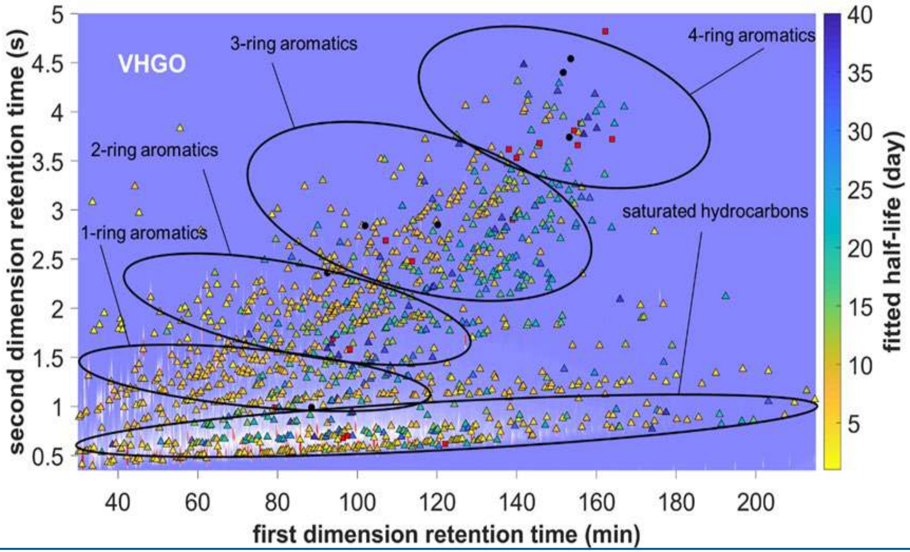
Disentangling Oil Weathering Using GC×GC. 2. Mass Transfer Calculations. J. Samuel Arey, Robert K. Nelson, Desiree L. Plata, and Christopher M. Reddy. Environmental Science & Technology 2007 41 (16), 5747-5755. DOI: 10.1021/es070006p

Concawe

Booth, et al. Comprehensive Two-Dimensional Gas Chromatography with Peak Tracking for Screening of Constituent Biodegradation in Petroleum UVCB Substances. Environ Sci Technol. 2023 Aug 29;57(34):12583-12593. doi: 10.1021/acs.est.3c01624.



~1100 peaks tracked with GCxGC-FID represent ~50% of the mass



For regulatory application, we need better peak identification, so some of the samples were analyzed using GCxGC-qTOF-MS

200 peaks with relatively long half-lives were evaluated

It was not feasible to assign structure without a comparable MS spectrum

Many peaks showed some overlapping constituents, though usually with one majority constituent

Example of mass spec identification for tracked peaks

BlobID FID	Identity	Formula (CnHn)	Carbon number	Class
385	**C6 Decalin**	**C16H30**	16	dN
385	C5 Decalin	C15H28	15	dN
389	**C7 Decalin**	**C17H32**	17	dN
389	C8 Decalin	C18H34	18	dN
459	**C7 Decalin**	**C17H32**	17	dN
459	C10 Cyclohexane	C16H32	16	mN
459	C8 Decalin	C18H34	18	dN
463	**Isoprenoid**	**C20H42**	20	iP
558	**C9 Benzene**	**C15H24**	15	mAr
558	C1 Perhydrophenanthrene	C16H14	16	PolyN
558	C10 Benzene	C16H26	16	mAr

Concawe

Example of potential data interpretation

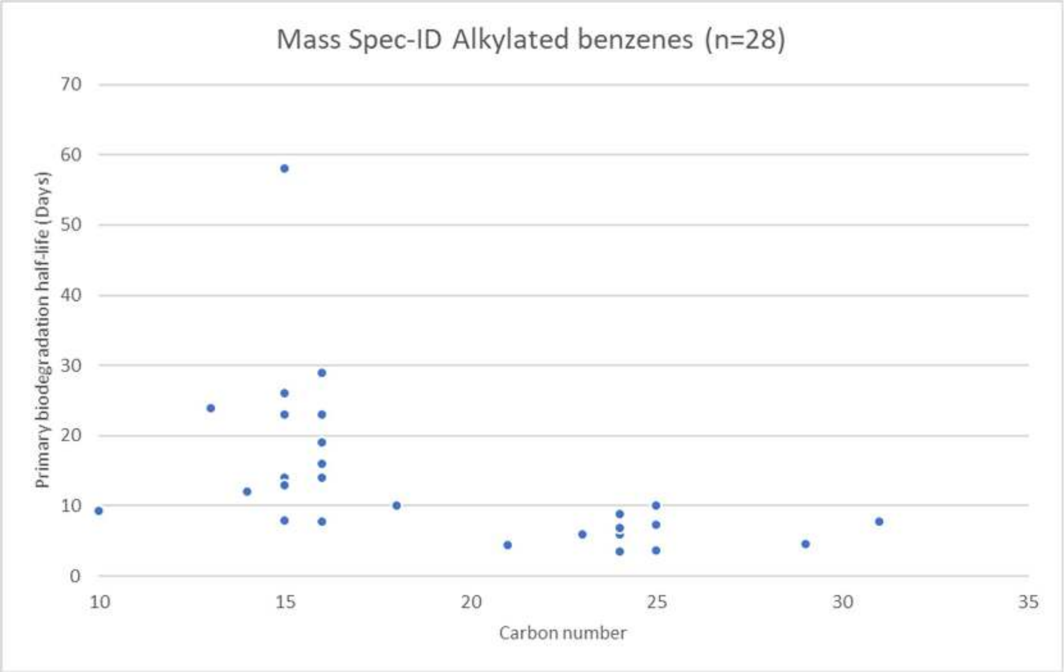
Contingent on more accurate half-life data and constituent ID

The rough peak identities provided by GCxGC-qToF-MS allowed us to look at families of chemicals, like the alkylated benzenes.

Tentative primary biodegradation half-lives for alkylated benzenes seem to be highest around C15-C16.

Considering the natural variability of biodegradation, there appears to be a limited impact of carbon number on biodegradation.

Allows us to look at structural features affecting biodegradability



Can this data fit into a regulatory application?

Currently proposed as a screening approach

For the screening of possible Persistent constituents:

- Using the MS identification with tentative half-life information to identify constituents for further testing

For efficient identification of groups of chemicals of interest:

- Focus on half-life data for groups of interest (as shown)
- Biodegradation testing of groups of related chemicals with the peak-tracking method

But what is the regulatory acceptability of whole substance UVCB biodegradation testing?

Next steps?

See related poster from Sam Arey

To avoid co-elution of constituents in one peak and increase confidence in the assignment of half-lives to constituents:

- Pre-fractionate the sample during analysis
- Use mass spec instead of FID

To increase the percentage of mass tracked:

- Try other analytical methods to track more constituents
- Try other data analysis approaches (see Sam Arey's poster)

To improve realism:

- Avoid high loading rates (oil droplets)
- Increase microbial load

Try this approach with other substances & with other endpoints (bioaccumulation)

Concawe

Concawe
Environmental Science for European Refining

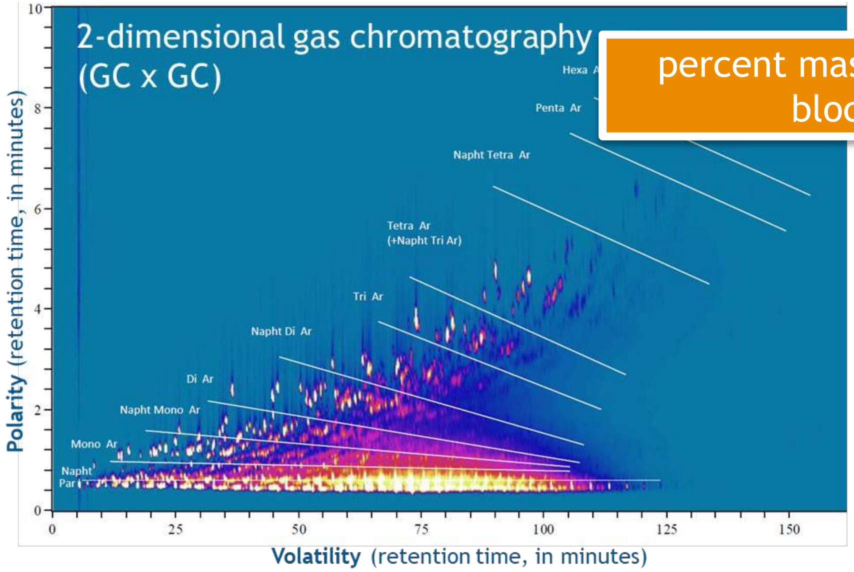
www.concawe.eu

Thank you for your attention

Delina Lyon
Delina.lyon@concawe.eu

What is Concawe’s Hydrocarbon Block Method?

- Hydrocarbon block method (HCBM)* - based on a combination of polarity and volatility, yielding information on chemical class and C#
- Assumes constituents in a block will have similar fate, hazard & risk
- Most Concawe substances are resolved into hydrocarbon blocks with GCxGC analysis (not bitumens)
- HCBM with experimental or QSAR data for each block is the basis for environmental hazard and risk assessment, with supporting whole substance test data



percent mass of each block

C#	n-Paraffins	i-Paraffins	Mono- Napthenics	Di-naphtenics	Poly-napthenics	Mono-aromatics	Napthenic mono-aromatics	Di-aromatics	Naphtenic di-aromatics	Poly-aromatics	Napthenic poly-aromatics
10				`							
11											
12											
13											
14											
15											
…											
30											

* King DJ, Lyne RL, Girling A, Peterson DR, Stephenson R, Short D. 1996. Environmental risk assessment of petroleum substances: the hydrocarbon block method. Concawe report No. 96/52. https://www.concawe.eu/wp-content/uploads/2017/01/rpt_96-52-2004-01719-01-e.pdf