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Report

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Proceedings of the Concawe Workshop for an Analytical Technology Exchange to meet Health & Environmental Regulatory Challenges for UVCBs

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Proceedings of the Concawe Workshop for an Analytical Technology Exchange to meet Health & Environmental Regulatory Challenges for UVCBs

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ABSTRACT

These proceedings are a record of the Concawe workshop held in Helsinki, Finland at the European Chemicals Agency (ECHA) on the 8th November 2023.

The workshop aimed to increase the understanding of regulators, industry scientists, and academic scientists about the challenges in analysis of petroleum UVCB substance composition to support EU REACH human health & environment hazard assessments, particularly with regard to high boiling point and high-complexity petroleum substances. Posters from ten academic and independent testing laboratories were presented to demonstrate the current analytical capabilities with these substances.

The 130 attendees from ECHA, Member State competent authorities, the oil and chemical industries and analytical laboratories discussed both the chemical analysis requirements of the REACH regulations with presentations from ECHA and Concawe and the potential to provide information to meet these requirements with the current and evolving analytical technologies.

Discussions at the workshop highlighted the need for further work to clarify the analytical data required to comply with regulations. A number of limitations in the currently available analytical technologies are to be addressed including the extension of comprehensive and quantitative analysis of constituent groups with carbon numbers above C30 and the development of mass spectrometric output and data interpretation to allow both quantitative and structural information to be determined.

KEYWORDS

Workshop, UVCB, petroleum substance, chemical analysis, REACH, constituent, structural similarity, read across

INTERNET

This report is available as an Adobe pdf file on the Concawe website (www.concawe.eu).

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SUMMARY

These proceedings are a non-verbatim record of the Concawe workshop held in Helsinki, Finland at the European Chemicals Agency (ECHA) on the 8th November 2023.

The workshop aimed to increase the understanding of regulators, industry and academia about the challenges in analysis of petroleum UVCB substance composition to support EU REACH human health and environmental hazard assessment, particularly with regard to higher boiling point and more complex substances.

Participants included 130 attendees from ECHA, Member State competent authorities, the oil and chemical industries and analytical laboratories.

The meeting opened with presentations from ECHA and Concawe summarizing the chemical analysis requirements of the REACH regulations. A poster session followed with presentations from ten academic and independent analytical testing laboratories to demonstrate the current capabilities in petroleum substance characterisation across a range of technologies.

Breakout groups were formed to discuss the challenges laid out in the ECHA and Concawe presentations and the output of these discussions was summarised. A Concawe project (termed the “All-Constituent Challenge” (ACC)) was launched in a session for analytical laboratories immediately following the workshop. The ACC project aims to allow analytical laboratories to demonstrate their capabilities in providing as much compositional information as possible for a set of heavy (>C30) petroleum substances.

Discussions at the workshop highlighted the need to for further work to clarify the analytical data required to comply with EU REACH regulations. A number of limitations in the currently available analytical technologies are to be addressed including the extension of comprehensive and quantitative analysis of constituent groups above C30 and the development of mass spectrometric output and data interpretation to allow both quantitative and structural information to be determined in the absence of analytical standard reference compounds for most constituents.

1. INTRODUCTION

Concawe manages the EU REACH registration dossiers of over 140 registered petroleum UVCB (2) substances grouped into 20 categories ranging from light (*e.g.*, naphtha) to heavy (*e.g.* bitumen) substances. The EU REACH Regulation (1) requires registrants of UVCB substances to provide detailed and comprehensive, qualitative and quantitative information on the constituents present in these substances. This detailed information is required both to assess the potential environmental and human health impacts of these substances in normal use and, owing to the variation in their composition, to assess the degree of similarity between substances such that results obtained from toxicity tests can be applied from one substance to another ("read-across").

Naphthas (carbon chain length ~C4-C12), which contain a few hundred constituents, are relatively straightforward to characterise by gas chromatography (GC). Kerosines (~C9-C16), which contain several thousand constituents, and middle distillate substances such as diesel fuels (~C10-C26), which contain hundreds of thousands of constituents, can be characterised with sufficient granularity by comprehensive two-dimensional gas chromatography (GC x GC). However, it becomes increasingly challenging to characterise the heavier (>C30) petroleum substances such as lubricating oils, heavy fuel oils and bitumens which can contain many millions of constituents. The complexity of such substances not only arises from the exponential increase in isomers with carbon number but also from the more varied chemical functional groups (and multi-functionality) within the constituents present.

ECHA accepts that, for read-across purposes, it might not be possible (or practical) to identify all the constituents present in some UVCB substances and in these cases other approaches for demonstrating similarity between substances can be applied (3). One suggested approach is "fingerprinting" the UVCB substance by providing a relative quantitative overview of the constituents present using an appropriate analytical technique, although a condition of such an approach is that the measurement should cover >95% of the individual constituents present.

There are many publications describing analytical techniques which provide information on the identity of constituents present in the heavier petroleum substances. However, from our perspective, these papers do not provide both qualitative and quantitative information and/or only provide such information for a small fraction of the total substance.

In addition to the need for comprehensive substance-level analysis, targeted analysis of specific constituents is also important and might provide further toxicological insights. Further, regulatory obligations for the analysis of degradation products of UVCB substances require a larger suite of analytical approaches to capture the evolving structures.

The main challenge, therefore, is how to provide detailed and comprehensive, qualitative and quantitative information on petroleum substances constituents, which can then be used for regulatory assessment.

The Concawe All-Constituent Challenge (ACC) project aims to:

- Determine specific analytical approaches that can most fully quantify constituents/constituent groups of hydrocarbon substance streams with carbon ranges >C30
- Determine methods for identifying constituents, in particular those of potential regulatory interest
- Provide information to regulators regarding the limits of analysis of hydrocarbon substance constituents.

Analytical laboratories will be invited to analyse the same set of hydrocarbon substance samples (*i.e.*, gas oil, residual aromatic extract, lubricant base oil, bitumen, heavy vacuum oil and paraffin wax) with the analytical approach of their choice to provide as much quantitative information as possible for the constituents within the samples. Data from each analytical approach will be collated and analysed by Concawe to determine the most appropriate analytical approaches for each of these petroleum substance types.

2. WORKSHOP PROGRAMME AND PRESENTATIONS

Details of the workshop programme and presentations are available in Appendices 2-10.

Regulatory Challenge Session

- Objectives and practicalities of the day. A scene setting presentation by Carol Banner (Science Executive for Substance Identification at Concawe), and the workshop facilitator Glen Carty (REACH & Regulatory Compliance Manager at Shell and chair of the Concawe REACH Management Group).

- What are the requirements for analytical data in REACH substance hazard assessment and the challenges faced with hydrocarbon UVCB substances? A joint presentation from Michał Skowron (Senior Scientific Officer at ECHA) and Carol Banner.

- Regulatory challenges. This panel discussion presented by Delina Lyon (Science Executive for Environment at Concawe) and Nicholas Synhaeve (Science Executive for Human Health at Concawe), engaged workshop participants with two questions:

    - What analytical information do we need to support health and environmental hazard assessments?

    - What criteria does the analytical information need to meet?

  The Regulatory challenges session was concluded by a presentation of Michał Skowron "What is ECHA not seeing/not seeing clearly enough as input from Industry?"

All-Constituent Challenge Session

- All-Constituent Challenge: Overview of objectives, approach and participating analytical labs presented by Carol Banner. This was followed by a brief introduction to the workshop posters and analytical capabilities by each of the analytical laboratory representatives.

- Analytical laboratories presented posters describing their analytical capabilities in compositional analysis of petroleum substances. Details of posters are found in Appendix 10.

Laboratory	Technology
Biochemical Institute for Environmental Carcinogens (BIU)	Polycyclic Aromatic Hydrocarbons (PAH) by GC/MS
Intertek (ITS) UK	Field desorption time-of-flight mass spectrometry (FD-TOFMS)
JPEC Japan	Fourier-transform ion cyclotron resonance (FTICR) – mass spectrometry
Lommatzsch and Säger	GCxGC and HPLC-GC
SGS Germany	Derivatisation QTOF-MS, GCxGC FID
SINTEF	Fourier-transform ion cyclotron resonance (FTICR) – mass spectrometry
Texas A&M	Ion Mobility Mass Spectrometry (summary only)
University of Glasgow	2-dimensional gas chromatography (GCxGC)-TOF-MS
University of Plymouth	Range of chromatography/MS
University of Southampton	Supercritical fluid chromatography (SFC)-MS, HPLC-MS, GCxGC-MS
University of Warwick	Fourier-transform ion cyclotron resonance (FTICR) – mass spectrometry

- An introduction to the breakout sessions was then presented by Glen Carty. Workshop participants in Helsinki were split into 4 groups and online participants into 2 groups to discuss the following questions:
    - What was your key learning from today?
    - Which current technologies can deliver the required data? (Short term delivery)
    - What are the priorities for development of analytical approaches? (Medium to long term delivery)
    - Are there potential synergies between laboratories and complementary approaches?
    - Which requirements still appear unattainable?
- Feedback was provided by moderators of each of the six breakout groups and summarised by Concawe and ECHA

3. FEEDBACK FROM BREAKOUT GROUP DISCUSSIONS

3.1. REGULATORY CHALLENGES SESSION

There were 130 registered attendees to the meeting, both online and in person. Based on self-reporting during a Mentimeter survey, 6 identified as academia, 16 as regulators, 4 as CRO's, 48 as industry, and 1 with no stated affiliation. Two guiding questions were posed to the attendees:

Q: "What analytical information do we need to support human health and/or environmental hazard assessments?"

A: Workshop participants raised that the analytical information should be:

- Fit for purpose according to the end point being assessed (human health and environmental hazard assessment) and whether read across or direct substance assessment is being applied.
- Comprehensive, *i.e.*, describing the entire substance composition
- Provided for all registered petroleum substances, including heavier substances > C30.
- Describing constituent/constituent group structure (including molecular mass) and concentration.
- Describing the variability in concentration of constituents and/or constituent groups across samples of the same substance.
- Able to distinguish between a variety of structural isomers & presence of functional groups (*e.g.*, double bond equivalent and heteroatoms), hydrocarbon chain position and degree of branching of constituents. This is particularly relevant in support of environmental hazard assessment.
- Obtained with the application of marker substances (including synthetic standards) to provide a means to quantify petroleum substance constituents.
- Able to support an assessment of potential for exposure through constituent bioavailability (lipophilicity/hydrophobicity partitioning).
- Able to support the identification of metabolites and breakdown products of petroleum substances.
- Able to verify the doses of test substances in hazard assessment studies.

Q: "What criteria does the analytical information need to meet?"

A: Workshop participants considered the following important:

- The analytical information requires sufficient granularity (detailed compositional information), although a definition of "sufficient" was not determined

- The interpretation of analytical data needs to take into consideration matrix effects which may impact the ability to identify / quantify individual or groups of constituents because they are poorly resolved or their ability to ionise is compromised by high concentrations of other constituents. For example, a constituent group may not be identified in a whole petroleum substance sample, but may be detected in a fraction of that same sample.

- The importance of analytical method repeatability and reproducibility was highlighted by participants. In cases where there are analyses of multiple samples per substance for the purposes of read across at a single laboratory in a single experiment, method repeatability and reproducibility may be less important than in cases where studies are done for the purposes of substance identification.

- Appropriate analytical methodology needs to be accompanied by expertise in analytical data interpretation in order to make sense of instrument output (*e.g.*, statistical analyses, multivariate data interpretation).

- A measurement of precision in quantitative analyses. No guidance had been provided on the requirements for precision.

- Speed of analysis was also considered to be important. With such complex substances, detailed analysis and data interpretation can take considerable time. A pragmatic approach is required in order to meet regulatory timelines.

3.2. BREAKOUT SESSION

Feedback from the six breakout groups for each of the five steering questions is summarised as follows:

3.2.1. What was your key learning from today?

The range of analytical capabilities presented by the laboratories at the workshop was acknowledged to be impressive and an open exchange between participants gave good insight into the strengths and weaknesses of each analytical approach. This valuable interaction between analytical laboratories, academia, industry and regulators was a good starting point to understand how such technologies may be applied to support hazard assessments. However, there are limitations in the current technologies such that regulatory needs for comprehensive quantitative analysis of all constituents / constituent groups are not met for the full range of petroleum substances, particularly those with a carbon number range beyond C30. Furthermore, targeted analysis to identify specific hazardous constituents or constituent groups still requires alignment of regulatory requirements with analytical capabilities.

Participants expressed a need to continue this discussion across sectors to further clarify the regulatory requirements in the context of what is practically feasible and to maintain awareness of evolving analytical capabilities.

The approach to be applied in the analysis of petroleum substance constituents needs to be fit-for-purpose. This relates to the specifics of the substance itself including the carbon number range which impacts the choice of analytical methods

(separation and detection). The specific hazard end point and whether read-across in hazard assessment is applied will also determine the required analytical characterisation, for example qualitative analysis of isomeric constituents may support Persistence and Bioaccumulation assessment, while quantitative information on constituent group concentration variability is required to support structural similarity as a basis for read across in hazard assessment.

With this diversity of requirements, further clarity was requested by participants as to what should be characterised (whole substance, groups of constituents, or individual constituents) and the basis for grouping of constituents.

The workshop helped highlight the differences in the analytical requirements to support Annex VI substance identification and sameness for registration within a joint submission and Annex XI Section 15 requirements for supporting substance structural similarity for read-across in hazard assessment. While Annex VI requires standard industry analytical methods applied by registrants to deliver basic quantitative information about carbon number and hydrocarbon class, Annex XI requires more granular information on the concentration of constituent groups and the variability in concentration of these constituent groups across multiple samples of the substance.

Participants at the workshop expressed the need for method standardisation for the methods applied to structural similarity assessment. This is currently possible for existing analytical approaches for substances <C30, *e.g.*, Detailed Hydrocarbon Analysis (DHA) of naphthas (C5-C12), but is not currently the case for analytical techniques such as two-dimensional gas chromatography (GC x GC) applied to gas oils (C12-C30). Analytical methods to support structural similarity of substances above C30 still need to be developed.

Given the complexity of petroleum substances, many with hundreds of thousands of constituents, the impact of matrix effects on analytical characterisation was highlighted at the workshop. Such effects mean that the ability to detect and measure a single constituent or constituent group (*i.e.*, analyte of interest) may be impacted by the presence and concentrations of other constituents. This may prevent effective separation of the analyte of interest from other constituents and/or impact its degree of ionisation and therefore detection by mass spectrometry. Such matrix effects may mean that an inaccurately lower concentration of a constituent or constituent group is measured in a sample of the full substance compared with a fraction of the same sample with fewer constituents.

3.2.2. Which current technologies can deliver the required data? (Short-term delivery)

Participants expressed that the achievement of short-term goals requires, from the outset, a structured approach together with an understanding of the data required by regulators, including key parameters of granularity of constituent group information and precision in quantification and the role of all parties involved (regulators, industry (eco)toxicologists and analytical chemists/data analysts).

The early development of a structured database for the collection, sharing and comparison of data across analytical technologies and sample types (full sample, constituent groups and individual constituents) was noted as a valuable tool in attaining the required characterisation of petroleum substance constituents.

Currently, comprehensive two-dimensional gas chromatography (GC x GC) for substances in the carbon range C6-C30 coupled with hydrocarbon space mapping (visual map of relative concentrations of constituent groups (hydrocarbon class per carbon number) across multiple samples per substance) can deliver what is understood to be the required data to assess substance similarity and compositional variability across samples to support read across and selection of "representative" or "worst-case" substances.

The main priority is on quantitation of petroleum substances constituents and constituent groups in the range >C30. There were diverse opinions expressed by participants on whether current technologies including Orbitrap-MS, ion mobility MS and Fourier Transform Ion Cyclotron Resonance MS can deliver the required data for these heavier substances. It was also considered that one technology alone is unlikely to provide the required data for all petroleum substances.

While whole substance fingerprinting with a range of mass spectrometric technologies can currently provide detailed molecular formulae for many thousands of constituents, in the absence of calibration standards (*e.g.*, synthetic reference compounds), quantitation of these detected molecules is not possible. Participants questioned whether the provision of molecular formulae is sufficient to meet regulatory requirements

The pre-fractionation of petroleum substance samples can help reduce the potential for matrix effects. However, concern was raised around the precision of the fractionation (does a saturate fraction only contain saturates?) and the integrity of fractions impacted by the potential for oxidation/photodegradation during the process and therefore the loss or modification of constituents. Some examples of short-term delivery based on pre-fractionation and coupling of analytical technologies are:

- GC x GC coupled with pre-fractionation by High Performance Liquid Chromatography (HPLC) and detection by mass spectrometry may be able to extend characterisation to the C50 range for alkane constituents such as in paraffin waxes. Aromatic constituents will have a more limited scope, possibly to C40.

- Field Ionisation Mass Spectrometry (FIMS) can be used to characterise saturate and aromatic fractions of Lubricant Base Oils (LBO) in the carbon range up to C40.

3.2.3. What are the priorities for development of analytical approaches? (Medium to long-term delivery)

Workshop participants expressed the following priorities in the medium to long term:

- A need to maintain dialogue across the respective technical disciplines and stakeholders in order to share approaches and understand method practicalities and limitations.

- A recognition that multiple analytical technologies will be required to address any regulatory question. Such developments could include:

  - The application of Super Critical Fluid Chromatography with Flame Ionisation Detection and Mass Spectrometry (SFC FID/MS). This approach should allow

the identification and quantitation of constituents up to carbon number C138.

- The application of a Vacuum Ultraviolet (VUV) detection coupled to GC x GC could improve identification of functional groups and heteroatoms (sulphur and nitrogen containing molecules) within hydrocarbon blocks. Currently the ability to determine structural isomers and functional groups is limited by molecular size.

- The development of alternative ionisation sources for mass spectrometry such as **S**oft **I**onization by **C**hemical **R**eaction **I**n-**T**ransfer (SICRIT) cold plasma ionisation and two-step laser ionisation (also known as post ablation ionisation) will also improve ionisation efficiencies of difficult-to-ionise hydrocarbon classes and substances.

- A recognition that high resolution analytical methods may only be available in a few specialised analytical laboratories and not necessarily available in the facilities where hazard testing is performed. In cases where a high-resolution analytical method is required for valid hazard testing, it may be challenging to perform the test under Good Laboratory Practice (GLP) since this generally requires in-house analytical methods and not the use of external laboratory results.

- Develop understanding of alternative approaches to compare data from different samples in assessment of structural similarity and variability.

- Develop better procedures for selection of “representative” and “worst-case” substances.

- Combine and integrate chemical analysis with in vitro and/or in vivo hazard assessment studies.

- Identify key constituents driving hazard effects and an understanding of their mechanism of action.

- A strong recognition of concern regarding the volumes of data that will be generated and to ensure this is aligned with regulatory requirements

3.2.4. Are there potential synergies between laboratories / complementary approaches?

Workshop participants expressed the following potential for synergies between laboratories and / or complimentary approaches

- Participants expressed interest in the range of analytical approaches presented and agreed the workshop and the subsequent All-Constituent Challenge provide an opportunity for groups to learn about the other methodologies which could be a catalyst for future collaborative work including a comparison of data generated using different analytical techniques.

- Further work is needed to understand how to transition in-house methodologies performed by few laboratories worldwide to industry standard methods available to meet the analytical needs to support hazard assessment

- A comparison of whole petroleum substance chemistry and hazard with that of specific hydrocarbon blocks (chemical functionality per carbon number) and that of individual constituents of concern needs to be fit for purpose.

3.2.5. Which requirements still appear unattainable?

The regulatory requirements that workshop participants considered to be currently unattainable were:

- Complete constituent and constituent group analysis in most petroleum substances, with the exception of low boiling point naphthas. The question was raised about the level of granularity required by regulators for comprehensive and quantitative analysis.

- Although possible to generate hundreds of thousands of molecular formulae for constituents present in a UVCB substance, we cannot confidently assign structures to the vast majority of these constituents

- The analytical ability to determine structural isomers and branching structure of individual constituents

- However, participants expressed that once a full understanding of the regulatory requirements is attained, adequate resources and time to address them will be required. Regardless, uncertainties will remain given the challenges highlighted at the workshop, including the lack of synthetic standards for all constituents and the impact of matric effects on constituent separation, identification and quantitation.

3.3. PANEL SUMMARY OF DISCUSSIONS

Anna Steneholm (of TKT Consulting for the Concawe Health Management Group) summarized that substance chemical analysis supports the ultimate goal to achieve effective hazard evaluation by grouping similar petroleum substances and thereby minimising of animal testing. She highlighted that the regulatory expectations were quite different in the contexts of substance similarity to justify read-across (requiring quantitative data on constituent groups for >95 weight % of substance) and of Substances of Very High Concern (SVHC) where as little as 0.02 weight % of a petroleum substance can be of interest.

Delina Lyon reflected that a more structured approach to analytical data generation is required based on the regulatory requirements and environmental hazard assessment needs. Further interdisciplinary and inter-sector discussion are needed to advance in this space. With regard to the analytical technology and hazard assessment output, better data analysis and integration is needed to maximise the value of this work and build a comprehensive understanding of the hazard profile of petroleum substance constituents.

Carol Banner saw potential in the analytical capabilities presented at the workshop to advance beyond C30 in terms of comprehensive and quantitative analysis of petroleum substance constituents or constituent groups. For example, GC x GC may be extended as far as C50 for non-aromatic constituents when coupled with HPLC, and SFC/FID MS may in the future permit the analysis up to C138.

While mass spectrometric detection can yield enormous amounts of molecular formulae (many hundreds of thousands of formulae as possible output from a single

petroleum substance sample), the regulatory requirements for this information are unclear and the challenges to extrapolate these formulae to definitive structures remain.

Quantitation of constituent and constituent group concentration to support structural similarity in read-across justification also remains a challenge. The role of fractionation in sample preparation may be an approach to help reduce matrix effects and improve the potential for identification of structures and possibly quantification. However, sample fractionation in itself impacts the sample integrity and therefore introduce uncertainties into the analysis.

The workshop has started the critical discussion between regulators (who own the information requirements), (eco)toxicologists (who aim to comply with those information requirements) and the analytical laboratories (who have the capabilities to provide the data to support those information requirements). Going forward there is a clear need to better define the requirements, particularly with regard to the granularity of quantitative information on constituent group concentrations to support read across and on the precision of that quantitation. In the longer term, a degree of standardisation in data output and reporting will aid the comparison of data obtained from different technologies to the benefit of all parties.

Michał Skowron of ECHA concluded that ECHA appreciated the exchange of information between analysts, industry and regulators which improves transparency and explains why the regulatory legal requirements exist. The workshop showed analytical developments that could push the current limitations such as the boiling point limitations of GC x GC and the ability to identify alkylated polyaromatic hydrocarbons (PAHs).

With regard to the question on the granularity of quantitative information required on constituent groups to support structural similarity in read-across, ECHA indicated that this required a step-by-step approach to understand what is achievable and then determine whether more resolution is required. Michal acknowledged the different analytical data requirements to support human health and environmental hazard assessment.

In the development of new analytical approaches, ECHA has identified the need to standardise certain methods such as GCxGC & FDMS, to provide a wider pool of industry standard methods (ASTM/IP/EN) for the purpose for generating data for human health & environmental risk assessment in the future. ECHA will continue to push for this but asks industry for their support.

Michał concluded by noting the need to communicate to the wider public in the appropriate terms that ECHA works towards safer use of everyday chemicals using the data discussed today.

4. CONCLUSIONS AND FURTHER WORK

The workshop brought together key stakeholders in the provision and use of analytical data to support the regulatory requirements for human health and environmental hazard assessment of petroleum substances. Discussions at the workshop highlighted the following key areas for further work:

1. A need to clarify the granularity of analytical data required to comply with regulations. The response of ECHA to the following questions will help clarify the regulatory requirements in regard to analytical data to support hazard assessment:

   a. **What endpoint specificities are there for analytical data to support hazard assessment?**

   b. **What granularity in structural composition data is required for read-across?**

   c. **What precision in quantification is required for substance constituents and constituent groups?**

   d. **Are standardised methods required for analytical methods supporting hazard assessment?**

2. Providing the required granular level of compositional information for UVCB substances is very complex. The analytical laboratories and capability of techniques available today may not fully address the requirements of the REACH regulation update from 2022 in this regard. The current limitations in analytical technologies that need to be addressed to maintain alignment with the regulatory framework include:

   a. The extension of comprehensive and quantitative analysis of constituent groups above C30.

   b. The development of mass spectrometric output and data interpretation to allow both quantitative and structural information to be determined in the absence of synthetic reference compounds for most constituents.

3. Further work in comparing the capabilities of a range of analytical approaches applied to the same set of petroleum substance samples could be supportive in such developments.

   The Concawe All-Constituent Challenge project over the course of 2024 will ask analytical laboratories with a range of analytical capabilities in petroleum substance characterisation to provide as much compositional information as possible for the same set of heavy petroleum substance samples. Data provided by each of the laboratories will be compared by Concawe to identify analytical solutions for each substance type to meet the requirements for substance similarity assessment and the identification of hazardous individual constituents.

5. GLOSSARY

Term	Definition
Category	A Concawe system of grouping similar substances together based on process history and boiling point/carbon range
CLP	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures
CMR	Carcinogenic, mutagenic or reprotoxic.
Constituent	Discrete chemical structure, which is separable from its stereo-, regio- and constitutional isomers
Concawe ACC project	Concawe All-Constituent Challenge project
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Commercial chemical Substances
Good Laboratory Practice (GLP)	The OECD Principles of Good Laboratory Practice (GLP) ensure the generation of high quality and reliable test data related to the safety of industrial chemical substances and preparations. The principles have been created in the context of harmonising testing procedures for the Mutual Acceptance of Data (MAD)
Hydrocarbon Block	A group of compounds linked by carbon number and/or hydrocarbon class
Mutual Acceptance of Data (MAD)	OECD has developed the Mutual Acceptance of Data (MAD) system, a multilateral agreement which allows participating countries (including non-members) to share the results of various non- clinical tests done on chemicals using OECD methods and principles.
PBT	Persistent, Bioaccumulative and Toxic.
REACH	Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.
Read-across hypothesis	Hypothesis on the basis of which property(ies) of target substance(s) may be predicted from source substance(s). This hypothesis must be based on a relationship between structural similarity and the predicted property(ies) and needs to be supported by read-across justification.
Read-across justification	The reasoning and associated supporting evidence that are provided to verify the scientific validity and robustness of the read-across hypothesis.
Soft Ionization by Chemical Reaction In Transfer (SICRIT)	SICRIT® (Soft Ionization by Chemical Reaction in Transfer) is the first real flow-through soft ionization technique in mass spectrometry. In conventional methods the analyte gets ionized before being introduced into the MS.

Substances of Very High Concern (SVHC)	Substance included in the Candidate List established in accordance with REACH Article 59(1).
UVCB	Substance of Unknown or Variable composition, Complex reaction products or Biological materials. Most petroleum substances are UVCBs.
vPvB	Very persistent and very bioaccumulative.

Terms not listed here should be taken as defined in the REACH and/or CLP Regulations.

6. ACKNOWLEDGEMENTS

Concawe thanks ECHA for kindly hosting the workshop and support in its preparation. Thank you to members of the Concawe Substance Identity Management Group and Concawe staff for their review of this report.

7. REFERENCES

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3. Annex VI substance identification and sameness for registration within a joint submission and Annex XI Section 15

APPENDIX 1: LIST OF PARTICIPATING COMPANIES

AG-HERA CONSULTING
ANSES - FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH & SAFETY
BASF
BAUA - GERMAN FEDERAL INSTITUTE OF OCCUPATIONAL SAFETY AND HEALTH
BE FPS HEALTH
BIOCHEMICAL INSTITUTE FOR ENVIRONMENTAL CARCINOGENS
BOOTMAN CHEMICAL SAFETY
BOREALIS
BP
BUREAU FOR CHEMICAL SUBSTANCES
CEFAS
CEFIC
CEPSA
CONCAWE
ECHA - EUROPEAN CHEMICALS AGENCY
ENI
ENVIPROADVICE
EQUINOR
ERA-CONSULT
EUROFINS AGROSCIENCES
EUROPEAN COMMISSION DG ENV
EUROPEAN COPPER INSTITUTE
EUROPEAN WAX ASSOCIATION
EVONIK
EXXONMOBIL
GUNVOR
HELLENIQ ENERGY
HSPA - HYDROCARBON SOLVENT PRODUCERS ASSOCIATION
HUNTSMAN
IBERCERAS SPECIALTIES
IBN NAFEES MEDICAL CENTER
IMARA MEDCARE
INA (MOL GROUP)
INTERTEK UK
JAPAN PETROLEUM ENERGY CENTER
JRF GLOBAL
KC PETRO-ANALYTICS LTD
KPR&T
LABORATORY LOMMATZSCH & SÄGER (LLS)
LUKOIL NEFTOHIM BURGAS
MOL
NESTE
NYNAS
OMV
PCK RAFFINERIE
PENMAN CONSULTING

PETROCHINA
PLYMOUTH UNIVERSITY
RAIN CARBON GERMANY GMBH
REACH CENTRUM
RICARDO
ROSNEFT
SASOL
SF ANALYTICON LIMITED
SGS GERMANY
SHELL
SINTEF OCEAN
TECHNICAL UNIVERSITY OF DENMARK
TEXAS A&M UNIVERSITY
TOTALENERGIES
TOTSA
TOXCEL
TOXICOLOGY KNOWLEDGE TEAM
UBA
UNIVERSITY OF COPENHAGEN
UNIVERSITY OF GLASGOW
UNIVERSITY OF SOUTHAMPTON
UNIVERSITY OF WARWICK
US EPA
ZSCHIMMER & SCHWARZ

APPENDIX 2: CONCAWE WORKSHOP PROGRAM

Concawe Workshop for an Analytical Technology Exchange to Meet Health & Environmental Regulatory Challenges for UVCBs

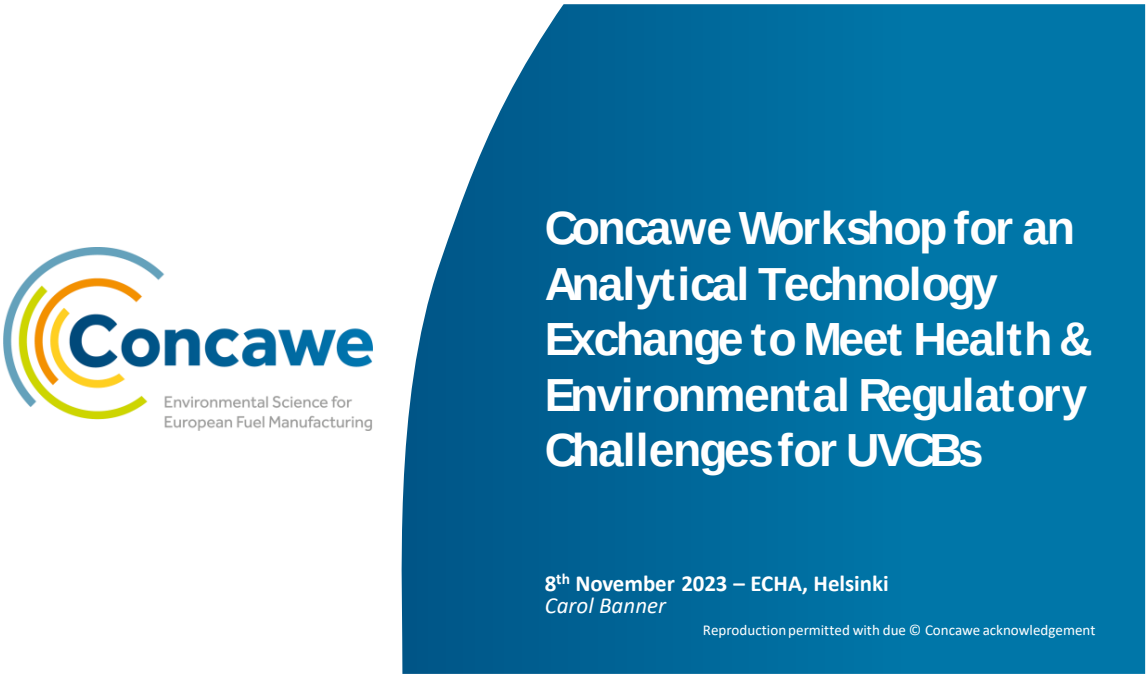
8 November 2023-ECHA offices, Helsinki & Online
08:30 - 17:30 EET

PROGRAMME

08:30-09:00	Registration	Coffee	
09:00-09:15	Welcome & Introduction	Objectives and practicalities for the day	Concawe/ Facilitator
09:15-09:45	Regulatory expectations	What are the requirements for analytical data in REACH substance hazard assessment and the challenges faced with hydrocarbon UVCB substances?	ECHA/ Concawe
09:45-10:45	Regulatory challenges	• What kind of analytical data may be needed and why? • What validation is required for an analytical method?	Panel Discussions
10:45-11:00		Coffee Break	
11:00-11:20	Regulatory challenges	What is ECHA not seeing/not seeing clearly enough as input from Industry?	ECHA

11:20-11:45	Exploring new technologies – introduction to the Concawe All Constituent Challenge	Overview of objectives, approach and participating analytical labs	Concawe
11:45-12:45		Networking Lunch	
12:45-13:45	Poster Session	Analytical labs present their capabilities to stakeholders, who build understanding of how analytical capabilities can address the above-identified requirements.	Analytical laboratories
13:45-13:55	Meeting the challenges	Introduction to breakout sessions	Facilitator
13:55-14:45	Meeting the challenges: Breakout session	Breakout groups discuss how the analytical capabilities may meet the hazard assessment requirements	All
14:45-15:00		Coffee Break	
15:00-16:15		Breakout group feedback and summary of workshop learning	Facilitator
16:15-16:30	Break	End of workshop for all except analytical labs	
16:30-17:30	Plenary with analytical labs/Concawe	Kick-off and practical considerations for the Concawe All Constituent Challenge	Concawe/ Analytical laboratories

APPENDIX 3: OBJECTIVES AND PRACTICALITIES OF THE DAY



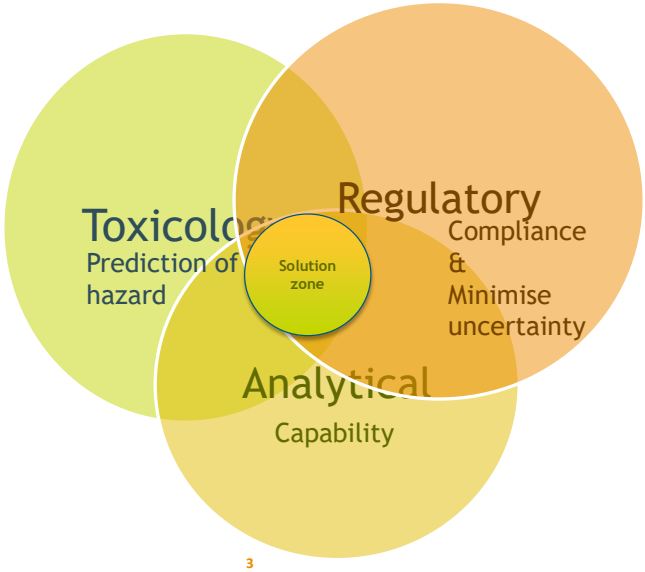
Objectives

- Clearly identify the analytical challenges to support REACH requirements for hazard assessment of petroleum UVCB substances
  - Structural similarity for UVCB read across
  - PBT assessment
  - Review the latest capabilities of analytical labs to meet those requirements

Deliverable Today → What can be achieved now, in the medium to long term with method development and what is not feasible

- Kick off the Concawe project "All-Constituent Challenge"
  - Analytical labs will demonstrate their capabilities to identify and quantify as many constituents and constituent groups as possible in the same set of samples
  - Widen our understanding of which technologies can be applied for different substances

Requirements for composition data?



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Scope of our discussions today

kyllä kiitos	ei kiitos
Requirements for analytical data to support hazard assessment	Substance sameness (Annex VI section 2 requirements)
Analytical capabilities and potential	Data modelling
Quantitation of constituent groups in C30+ petroleum UVCB substances	Classification / thresholds for classification
Identification of individual constituents of interest in PBT assessment	Regulatory policy / strategy for testing

4

Workshop practicalities

- Emergency evacuation procedure

Workshop practicalities

Hybrid meeting

- Mobile microphones in room for participants in the meeting room
- Online participants enter questions and comments in chat, managed in the meeting room

Concawe Workshop Morning Programme

09:00-09:15	Welcome & Introduction	Objectives and practicalities for the day	Concawe/ Facilitator
09:15-09:45	Regulatory expectations	What are the requirements for analytical data in REACH substance hazard assessment and the challenges faced with hydrocarbon UVCB substances?	ECHA/Concawe
09:45-10:45	Regulatory challenges	• What kind of analytical data may be needed and why? • What validation is required for an analytical method?	Panel Discussions
10:45-11:00	Coffee Break		
11:00-11:20	Regulatory challenges	What is ECHA not seeing/not seeing clearly enough as input from Industry?	ECHA
11:20-11:45	Exploring new technologies – introduction to the Concawe All Constituent Challenge	Overview of objectives, approach and participating analytical labs	Concawe
11:45-12:45	Networking Lunch		

To review Posters online

Go to https://www.concawe.eu/event/concawe-workshop-for-an-analytical-technology-exchange-to-meet-health-environmental-regulatory-challenges-for-uvcbs/

or

Scan the QR code and click on Analytical laboratories to review the posters

SCAN ME

Concawe Workshop Afternoon Programme

12:45-13:45	Poster Session	Analytical labs present their capabilities to stakeholders, who build understanding of how analytical capabilities can address the above-identified requirements	Analytical laboratories
13:45-13:55	Meeting the challenges	Introduction to breakout sessions	Facilitator
13:55-14:45	Meeting the challenges: Breakout session	Breakout groups discuss how the analytical capabilities may meet the hazard assessment requirements	All
14:45-15:00	Coffee Break		
15:00-16:15		Breakout group feedback and summary of workshop learning	Facilitator
16:15-16:30	Break	End of workshop for all except analytical labs	Concawe
16:30-17:30	Plenary with analytical labs/Concawe	Kick-off and practical considerations for the Concawe All Constituent Challenge	Concawe/ Analytical laboratories

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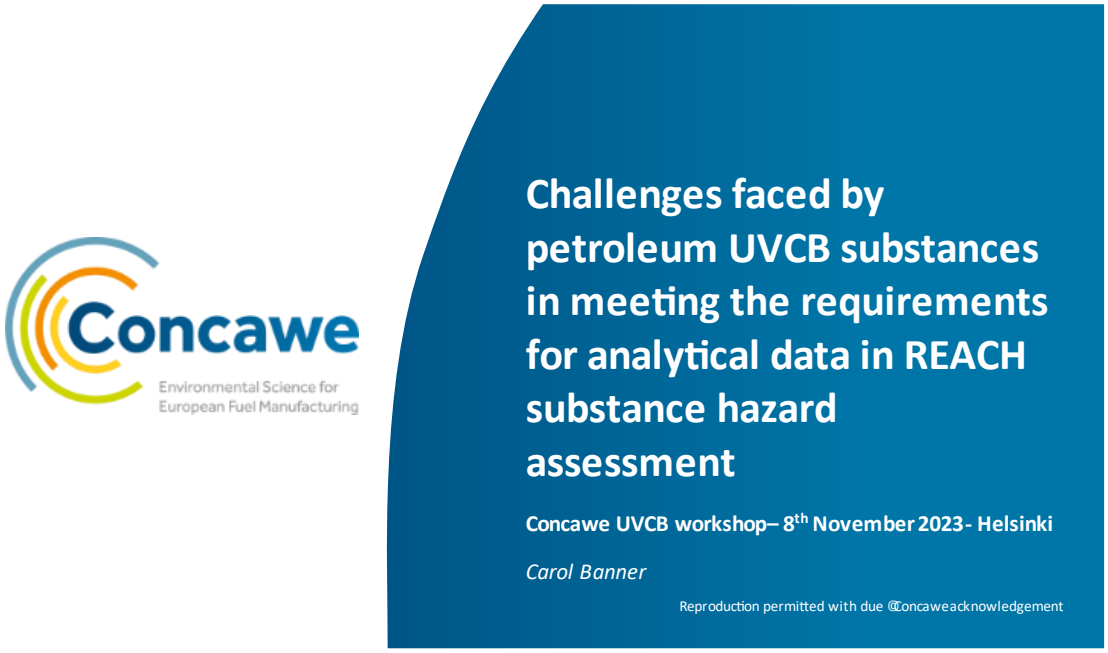
Thank you for your attention

APPENDIX 4: CHALLENGES FACED WITH HYDROCARBON UVCB SUBSTANCES



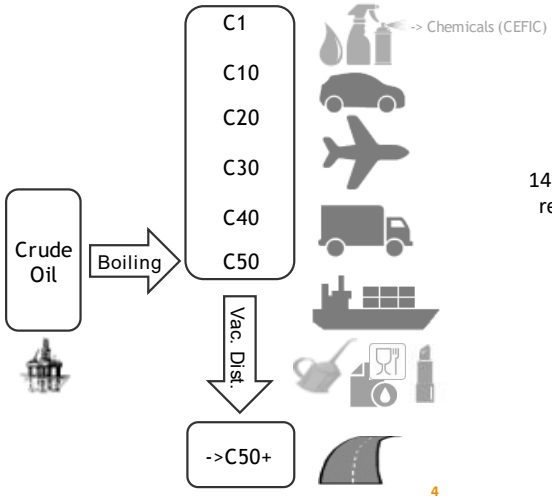
Agenda

01 Petroleum UVCB substance characteristics

02 Progress to date and challenges ahead

01 Petroleum UVCB substance characteristics

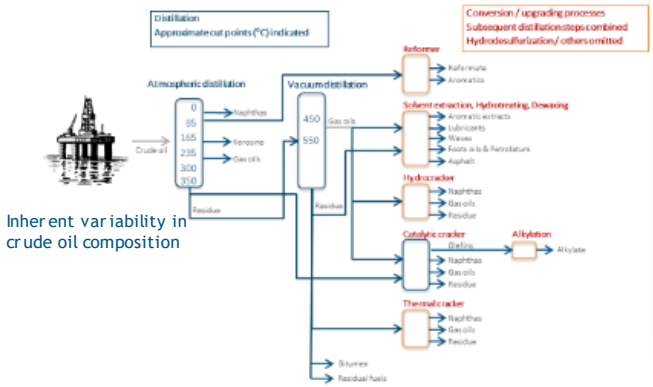
Petroleum UVCB Substances



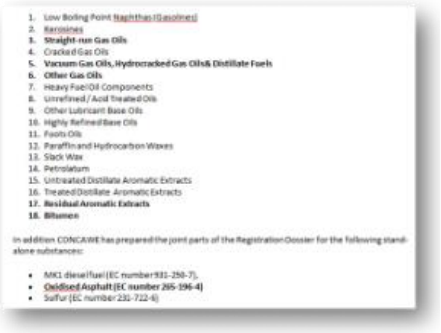
142 Petroleum Substances (PS) actively registered and managed by Concawe (>4000 registrations)

Substance grouping based on boiling/carbon range and manufacturing process

Examples of processes in refineries



Inherent variability in crude oil composition



Petroleum substances are manufactured to meet performance characteristics rather than specific chemical composition

Petroleum UVCB Substances characteristics

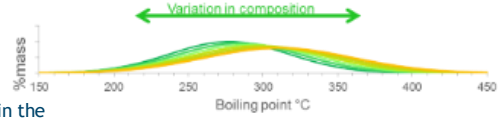
- Petroleum substances are UVCB
  - Unknown or
  - Variable composition,
  - Complex reaction products,
  - Biological materials

- Due to the high number of molecular hydrocarbon constituents,
  - Not all individually identified
  - However constituents are collectively characterized

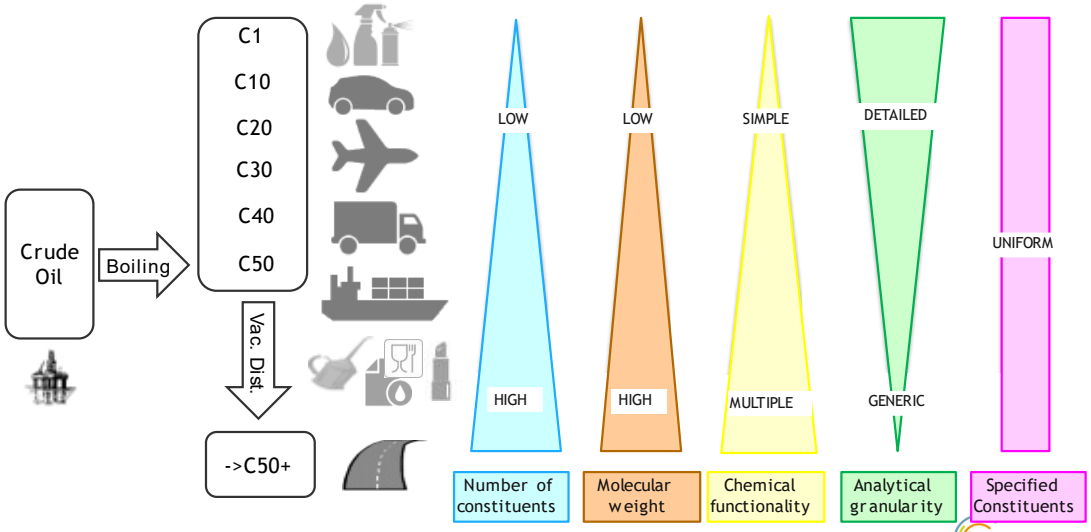
C number	Boiling point °C (n-alkanes) (*)	Number of isomers (alkanes only!)
3	-42	1
4	-1	2
5	36	3
6	69	5
7	98	9
8	126	18
10	174	75
15	269	4 347
20	343	366 231
25	402	36 777 419
30	450	4 108 221 447
35	490	493 054 243 760
40	525	62 353 826 654 563

- Petroleum substances are UVCB
  - Unknown or
  - Variable composition,
  - Complex reaction products,
  - Biological materials

- Petroleum Substances are variable by nature
  - Variability is limited to meet product specification
  - Petroleum substances form a continuum whereby physical-chemical properties overlap in the hydrocarbon space



Petroleum substance characterization



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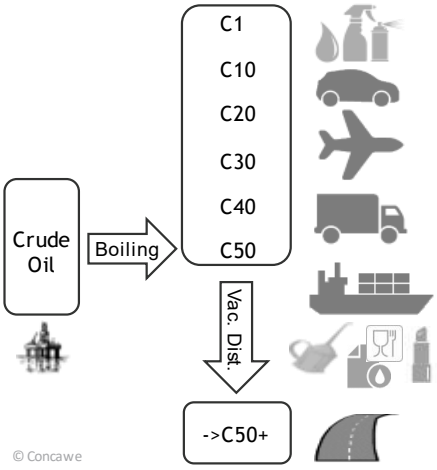
Concawe

02 Progress to date & challenges ahead

8

Concawe

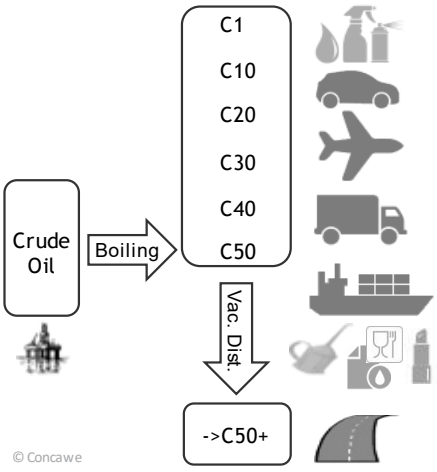
Demonstrating petroleum substance structural similarity to support read across



- Justify why identification of all individual constituents is not technically possible or impractical
- Measure concentration of constituent groups
- Characterise the variability in constituent groups across5 samples
- Characterise >95% of constituent/groups of constituents

9

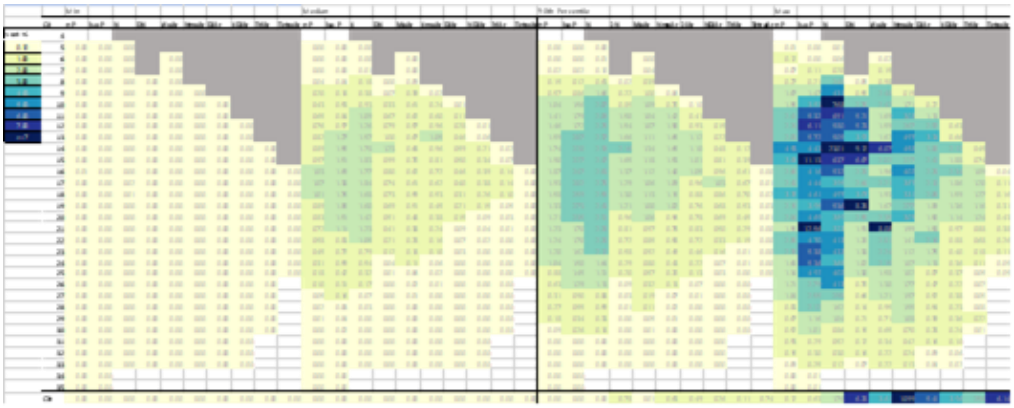
Demonstrating petroleum substance structural similarity to support read across



Category	Predominant carbon number range	Technique / Method to demonstrate structural similarity
Naphtha	5-12	PIONA DHA
Kerosine	9-16	GC x GC
Gas Oils	12-30	GC x GC
Paraffin Waxes	20-40	?
Heavy Fuel Oils	20-50	?
Lubricant Base Oils	20-40	?
Bitumen	30->100	?

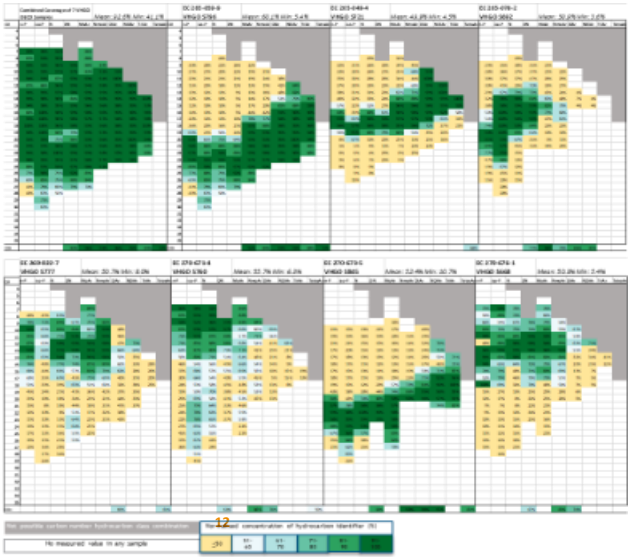
Example: Hydrocarbon space map of gas oils

Mapping of applicability domain – all samples



Example: Hydrocarbon space map of gas oils

Selection of representative samples for biological similarity assessment (human health)

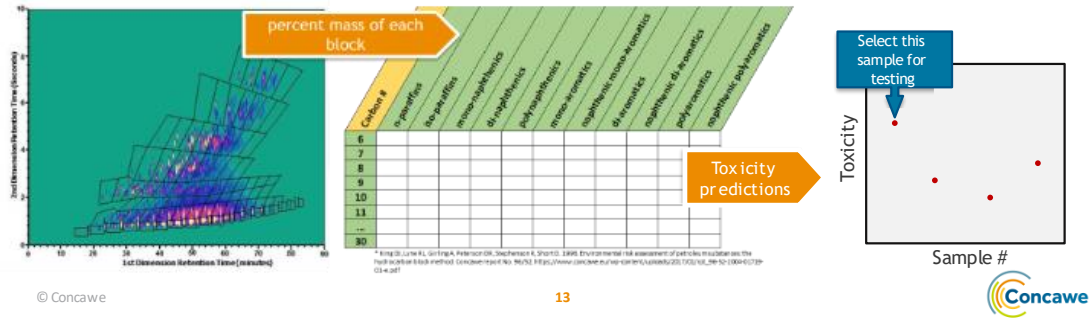


Example: Predictions of environmental toxicity

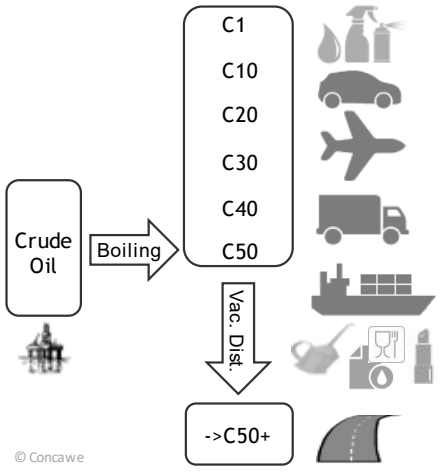
Selecting samples for testing by using analytical data to predict the most toxic

GCxGC analysis provides compositional information on a substance by dividing up the chemical space into hydrocarbon blocks, and then allocating percent concentration to each block

Using the target lipid model to predict the toxicity of each hydrocarbon block, it is possible to sum up the toxic units per block to predict the toxicity of the whole substance



Comprehensive and quantitative compositional data on heavier petroleum substances remains a challenge



Category	Predominant carbon number range	Technique / Method
Paraffin Waxes	20-40	?
Heavy Fuel Oils	20-50	?
Lubricant Base Oils	20-40	?
Bitumen	30->100	?

~~Structural similarity as basis for read across~~

~~Predicted environmental toxicity~~

Identification of specific constituent (sub)structures

REACH requires identification of constituents which are Persistent, Bioaccumulative & Toxic (PBT) or very Persistent & very Bioaccumulative (vPvB)

This requires an understanding of the constituents and the types of (sub)structures present in the petroleum UVCB substances

Improved understanding of the diversity of available substructural features can aid in linking properties, like biodegradability, to specific constituents / hydrocarbon blocks

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Thank you for your attention

Carol Banner
carol.banner@concawe.eu

APPENDIX 5: REQUIREMENTS FOR ANALYTICAL DATA IN REACH SUBSTANCE HAZARD ASSESSMENT

Annex VI Section 2 – Analytical information

- → 2.3.5. All necessary qualitative analytical data specific for the identification of the substance, such as ultra-violet, infra-red, nuclear magnetic resonance, mass spectrum or diffraction data
- → 2.3.6. All necessary quantitative analytical data specific for the identification of the substance, such as chromatographic, titrimetric, elemental analysis or diffraction data

3 ECHA

Annex VI Section 2 - Composition of a substance

→ 2.3.2. Names of constituents and impurities

→ In the case of a substance of unknown or variable composition, complex reaction products or biological materials (UVCB):

- names of constituents present at a concentration of ≥ 10 %;
- names of known constituents present at a concentration of < 10 %;
- for constituents that cannot be identified individually, description of groups of constituents based on chemical nature;
- description of the origin or source and the manufacturing process

→ 2.3.3. Typical concentration and concentration range (in percentage) of constituents, groups of constituents that cannot be identified individually and impurities as specified in point 2.3.2

2

Annex VI Section 2 – Analytical information

→ 2.3.7. Description of the analytical methods or the appropriate bibliographical references that are necessary for the identification of the substance (including the identification and quantification of its constituents and, where appropriate, its impurities and additives). The description shall consist of the experimental protocols followed and the relevant interpretation of the results reported under points 2.3.1 to 2.3.6. This information shall be sufficient to allow the methods to be reproduced

4

Annex XI Section 1.5 – Structural similarity for UVCB substances

→ *Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances.*

5 ECHA

Annex XI - Structural similarity for UVCB substances

→ **Structural similarity is a prerequisite for read-across**

→ Establishing structural similarity for UVCB substances
- Identification of constituents and their concentrations
- Variability in concentration of constituents

6 ECHA

Annex XI - Structural similarity for UVCB substances

→ By understanding dis-/similarities in structures of constituents
- Structures are the same/similar/not
- Concentrations are similar vs. different
- Variation of concentrations is similar vs. not

7

Annex XIII - PBT assessment

→ **Annex XIII:** The identification shall also take account of the PBT/vPvB-properties of relevant constituents of a substance

→ R11 guidance – *Regardless of whether full substance identification is possible or not for the whole composition, the registrant should make efforts for carrying out a PBT/vPvB assessment for all constituents, impurities and additives present in concentrations above 0.1% (w/w). Section R.11.4.2.2 provides further insight into how to carry out PBT/vPvB assessment for fractions of the substance that cannot be fully identified by the registrant*

8

Composition – detail and context

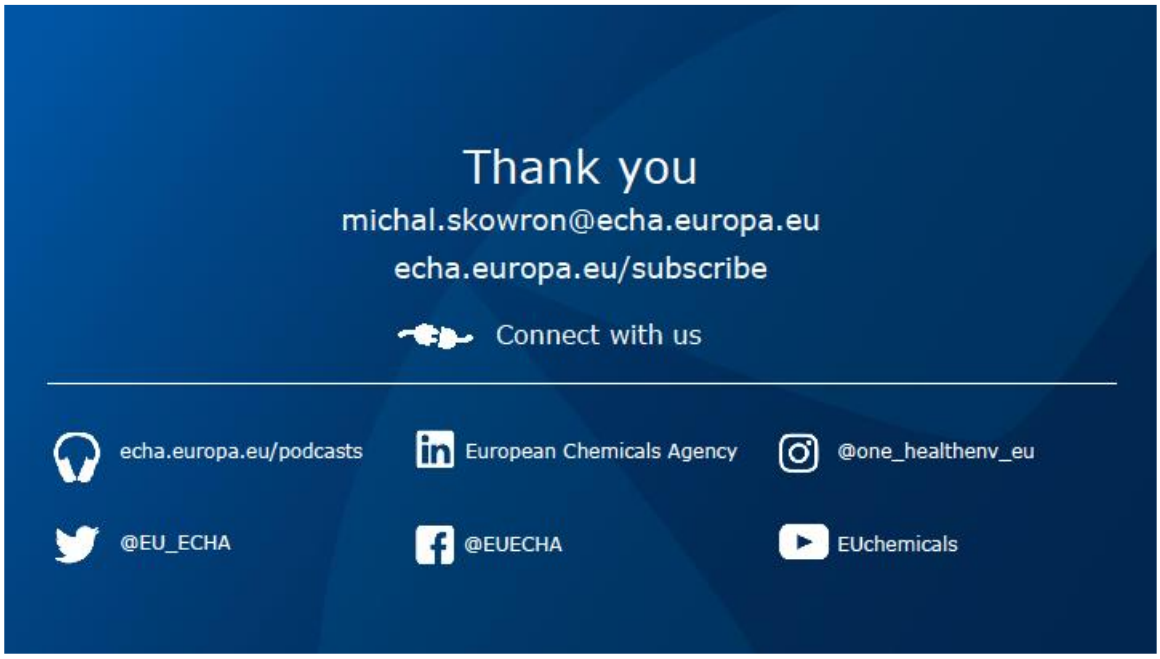
- Linear alkanes (n-alkanes)
- Branched alkanes (iso-alkanes)
- Cyclic alkanes (Naphthenics)
  - Mono-cyclic
  - Di-cyclic
  - Tri-cyclic
  - Tetra-cyclic
- Aromatics
  - Mono-aromatics
  - Di-Aromatics
  - Tri-aromatics
  - Tetra-aromatics
- Aromatics-Naphthenics
  - Mono-aromatics-Naphtenics
  - Di-aromatics-Naphthenics

Information on carbon number range for each class. Concentration (typical) and concentration ranges for each reported constituent (group of constituents).

Additional information, e.g. PAHs

9

ECHA



APPENDIX 6: REGULATORY CHALLENGES

Concawe
Environmental Science for European Refining

Analytical technology exchange to meet health & environmental regulatory challenges for UVCBs

Regulatory challenges from Environment & Human Health Perspectives

November 8th, 2023 - Helsinki

Delina Lyon & Nicholas Synhaeve

Mentimeter

Instructions

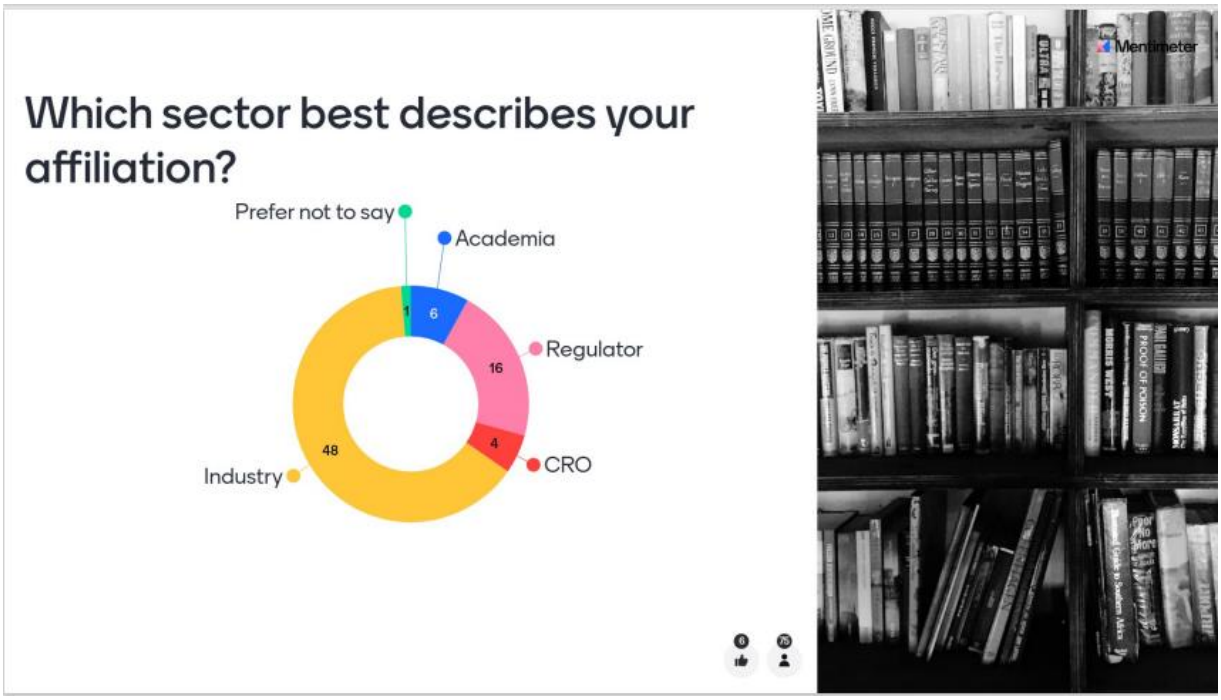
Go to

www.menti.com

Enter the code

3278 9100

Or use QR code



Hazard assessment for UVCBs

There are 3 basic health & environmental hazard endpoints:

Toxicity

Biodegradability/Persistence

Bioaccumulation

Due to the complexity of hydrocarbon UVCBs, it is not always possible to rely on whole substance hazard data. Based on regulatory guidance, we think about hydrocarbon UVCBs in 3 ways:

whole substance

hydrocarbon blocks

constituents

2

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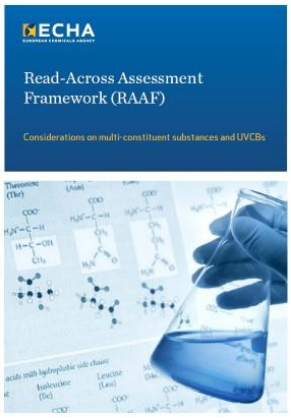
Hazard assessment for UVCBs

	Toxicity (T)	Biodegradation/ Persistence (P)	Bioaccumulation (B)
Whole substance	- Overall composition for read-across	(same as for T)	(same as for T)
Hydrocarbon block (HCB)/Constituent group	- Aromatic content (esp. PAHs) for Human Health - Relative concentration of hydrocarbon blocks	- Relative concentration of HCBs - Structures of constituents within a HCB	(same as for P)
Constituent	- Identify specific constituents of concern	(same as for T) - Substructural features driving P	(same as for T)

REACH and read-across

- REACH regulation allows for grouping of substances and use of read-across of data between similar substances to fill data gaps
  - REACH Annex XI
    - General **rules for adaptation** of the standard testing regime set out in Annex VII to X
  - Grouping: ECHA RAAF for UVCBs, 2017:
    - "For **UVCBs**, grouping on the basis of structural similarity may become even more **complex**, e.g. due to the presence of more constituents in the substances, potentially higher variations in the concentrations of the constituents and sometimes unknown constituents. Such grouping proposals also clearly **require extensive explanations and justified criteria** for group membership." (p30)



REACH and read-across

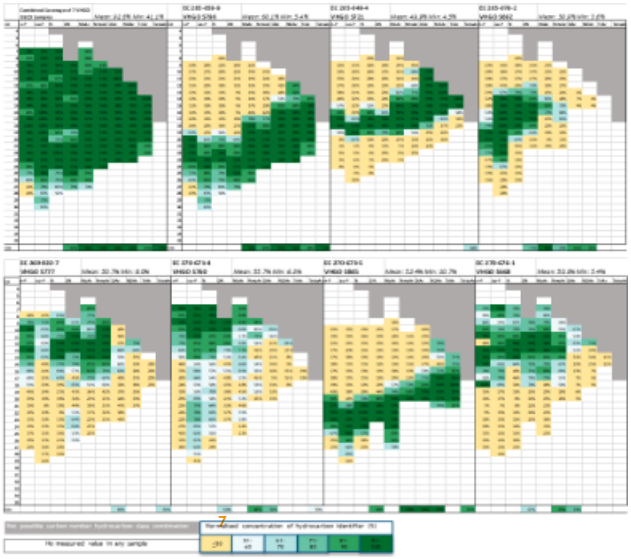
- REACH regulation allows for grouping of substances and use of read-across of data between similar substances to fill data gaps (cont'd)
  - Read-across: ECHA Advice on Using Read-Across for UVCB substances, 2022:
    - "If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity must be demonstrated **by other means**. Therefore, the registrant must provide a justification explaining why the other means enable **quantitative and qualitative comparison** of the actual composition between substances." (p10)
    - "An example of demonstrating structural similarity by other means could be **"fingerprinting"** of constituents and their concentrations in compositions using chromatographic methods to provide an overview (fingerprint) of the constituents, particularly where there are common constituents." (p10)
    - "Key issues in evaluating the acceptability of the fingerprinting method will be: … the provision of information on a **sufficient proportion of constituents** in a substance (i.e. covering >95 % of the constituents of a substance)…" (p10)
    - " …the concentration of constituents in **at least five independent samples of the substance** must be measured. The independent measurements must be from different production batches of the substance as produced by all the registrants." (p7)

What analytical information would support structural (compositional) similarity for UVCBs?

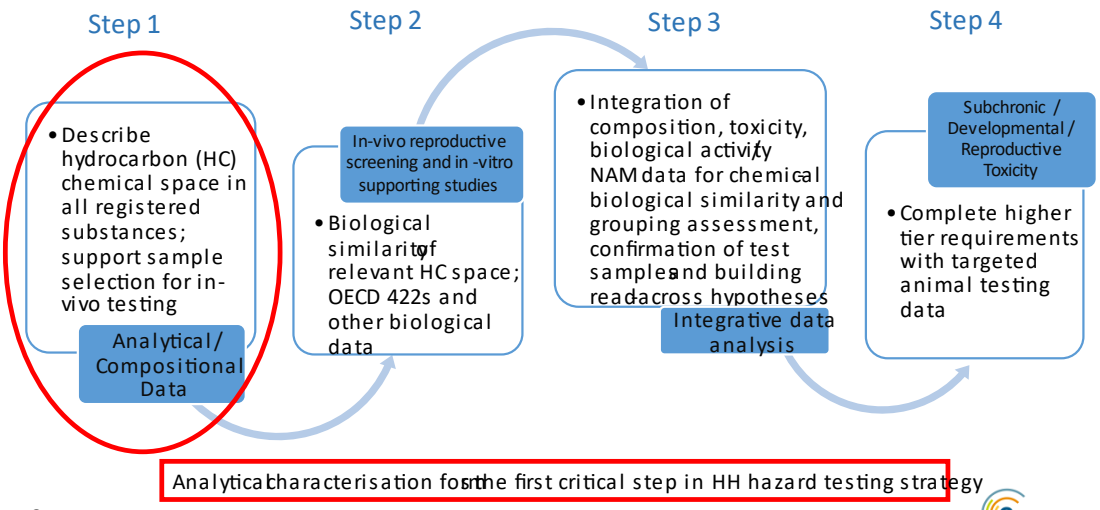
- Whole substance composition for development of the hydrocarbon space map
- Comparison of whole substance compositions for selecting (worst-case) representative sample (s)
- Groups of relevant constituents
- Specific constituents of concern
- Other?

Example: Hydrocarbon space map of gas oils

Selection of representative samples for biological similarity assessment (human health)



Use of read-across for HH toxicity



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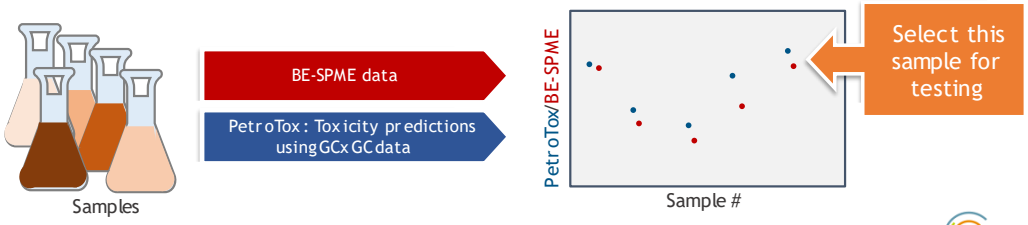
Read-across justification for ENV toxicity

Tying chemistry to mechanism of toxicity

Have GCxGC-FID data (hydrocarbonblock) and biomimetic extraction-Solid Phase Microextraction (BE-SPME) to measure bioavailability

- Use the hydrocarbonblock data for PetroTox predictions of aquatic and sediment/soil toxicity of available samples
- Use BE-SPME data to identify most bioavailable and therefore toxic samples
- To allow a conservative assessment, the most toxic sample based on BE SPME and PetroTox will be selected for aquatic, sediment and terrestrial toxicity testing and read-across to the other category members



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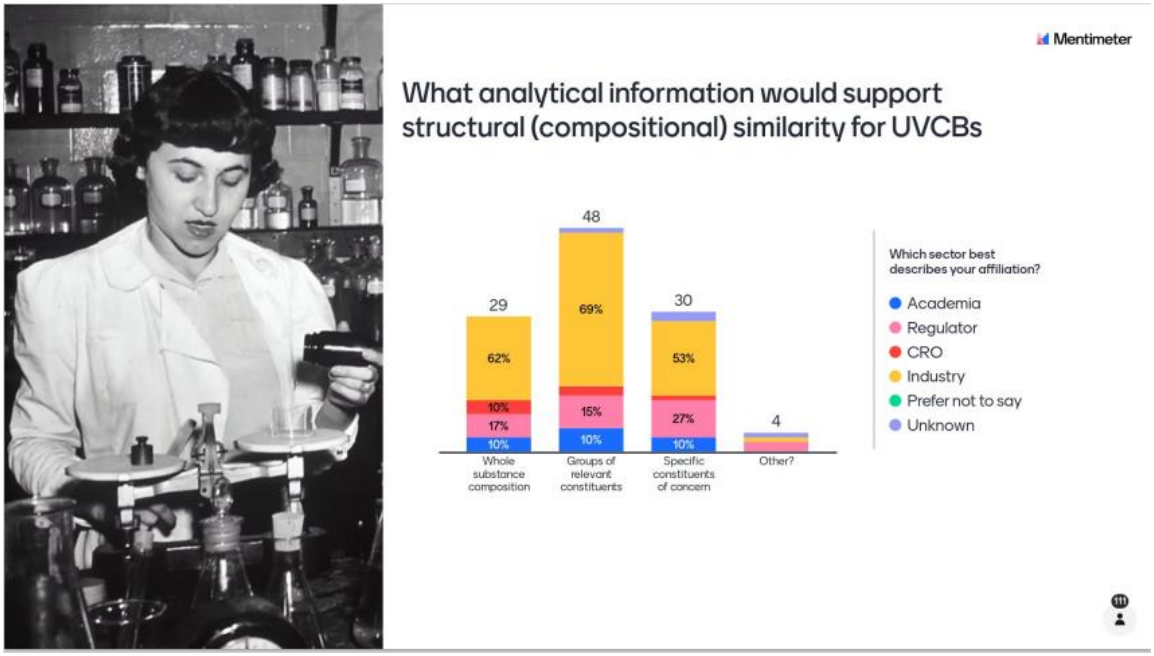
Without an analytical solution... no read-across

- Huge increase in number of (eco)toxicology tests needed
  - I.e. on each substance within a category
- Significant increase of experimental animals needed
- Significant impact on timing and delivery of testing results
- Even in context of correct sample selection for toxicology testing (i.e. without underestimating the hazard), better understanding of (specific) constituents might be required

WordPress.com



What kind of (additional to Annex VI) analytical data may be needed for hydrocarbon UVCB hazard assessment?

- e.g., GCxGC data to enable predictions of aquatic toxicity
- e.g., PAH content as key driver of toxicity
- Other?

Hazard assessment for UVCBs

	Toxicity (T)	Biodegradation/ Persistence (P)	Bioaccumulation (B)
Whole substance	- Overall composition for read-across	(same as for T)	(same as for T)
Hydrocarbon block (HCB)/Constituent group	- Aromatic content (esp. PAHs) for Human Health - Relative concentration of hydrocarbon blocks	- Relative concentration of hydrocarbon blocks - Structures of constituents within a HCB	(same as for P)
Constituent	- Identify specific constituents of concern	(same as for T) - Substructural features driving P	(same as for T)

Additional analytical data needs

Whole Substance

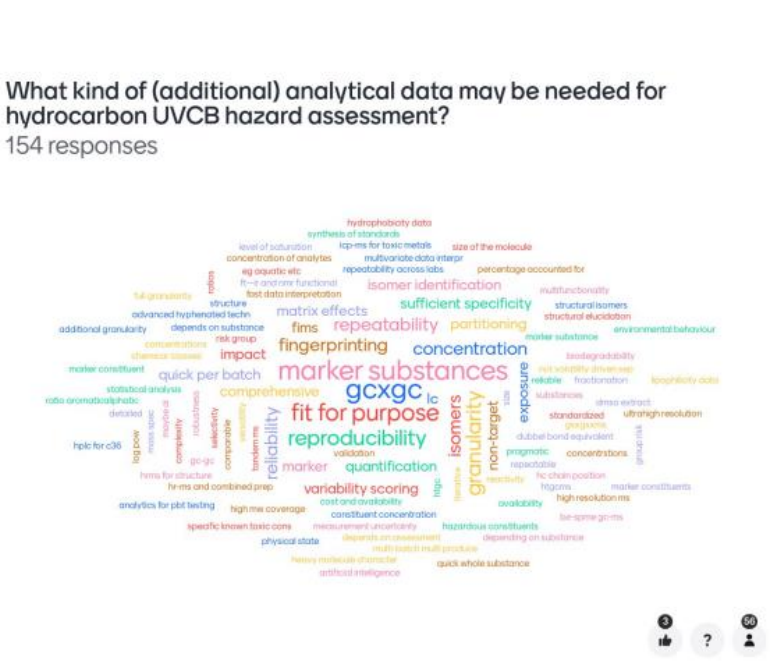
- Alternatives to GCxGC to get greater coverage of the whole hydrocarbon space to support read-across

Blocks

- Improve the justification for hydrocarbon blocking/grouping (e.g., identify chemical classes)

Constituents

- More information on (sub)structural features (e.g., branching)
- Detect and quantify specific constituents (e.g.,PAHs, C&L marker substances, etc.)



What criteria does an analytical approach need to fulfil to support hazard assessment?

- Coverage
- Granularity
- Repeatability
- Others?

Information of interest to Concawe

➢ Coverage & granularity of the method

- General (sub)structures of constituents is normally sufficient
- Justify assessment of similarity, especially for use in read-across arguments

EJ1046374.pdf (ed.gov)

➢ Associated benefits & limitations

- Strengths vs weaknesses of each approach, e.g., mass spec is poorer at quantification
- Reliability /repeatability of a method
- How to confirm identity if standards are not available for (sub)structures

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Thank you for your attention

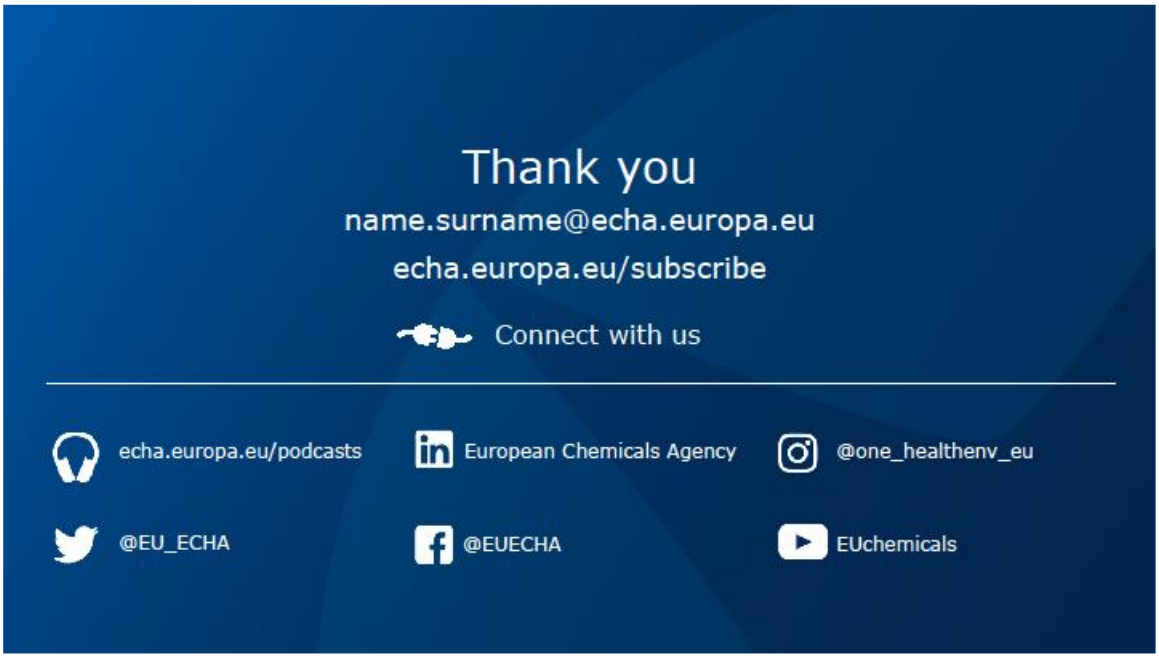
APPENDIX 7: COMMENTS ON DATA SUBMITTED TO ECHA

Composition data

- → Where data is included (ideal section 1.2 of IUCLID)
- → Format (e.g. name of a group)
- → Grouping - useful
- → Analytics – format, interpretation, description of a method
- → Unknown constituents
- → What is measured (analyte)

- → Representative constituents of a group (possibility to derive SMILES)

2 ECHA


Thank you
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@EU_ECHA
@EUECHA
EUchemicals

APPENDIX 8: INTRODUCTION TO BREAKOUT SESSION

Concawe
Environmental Science for European Fuel Manufacturing

Introduction to Breakout session

8th November 2023 – ECHA, Helsinki

Breakout Session Questions

1. What was your key learning from today?
2. Which current technologies can deliver the required data? (short term delivery)
3. What are the priorities for development of analytical approaches? (medium to long term delivery)
4. Are there potential synergies between laboratories / complementary approaches?
5. Which requirements still appear unattainable?

Concawe

Breakout groups

Participants in the meeting room to scan the QR code provided

Breakout groups F1, F2, F3 and F4 will meet in the four corners on this room

Online participants will be automatically placed in a breakout group

Make a note of your breakout group name (V5 or V6)

You will need this in case you become disconnected

Concawe Workshop Afternoon Programme

12:45-13:45	Poster Session	Analytical labs present their capabilities to stakeholders, who build understanding of how analytical capabilities can address the above-identified requirements	Analytical laboratories
13:45-13:55	Meeting the challenges	Introduction to breakout sessions	Facilitator
13:55-14:45	Meeting the challenges: Breakout session	Breakout groups discuss how the analytical capabilities may meet the hazard assessment requirements	All
14:45-15:00	Coffee Break		
15:00-16:15		Breakout group feedback and summary of workshop learning	Facilitator
16:15-16:30	Break	End of workshop for all except analytical labs	Concawe
16:30-17:30	Plenary with analytical labs/Concawe	Kick-off and practical considerations for the Concawe All Constituent Challenge	Concawe/ Analytical laboratories

Scope of our discussions today

kyllä kiitos	ei kiitos
Requirements for analytical data to support hazard assessment	Substance sameness (Annex VI section 2 requirements)
Analytical capabilities and potential	Data modelling
Quantitation of constituent groups in C30+ petroleum UVCB substances	Classification / thresholds for classification
Identification of individual constituents of interest in PBT assessment	Regulatory policy / strategy for testing

Breakout Session Questions

1. What was your key learning from today?
2. Which current technologies can deliver the required data? (short term delivery)
3. What are the priorities for development of analytical approaches? (medium to long term delivery)
4. Are there potential synergies between laboratories / complementary approaches?
5. Which requirements still appear unattainable?


Concawe
Environmental Science for
European Fuel Manufacturing
www.concawe.eu
Thank you for your attention

APPENDIX 9: CONCAWE ALL-CONSTITUENT CHALLENGE

Concawe All-Constituent Challenge

Concawe UVCB workshop – 8th November 2023 - Helsinki

Carol Banner

Objectives

- Determine specific analytical approaches that can most fully quantify constituents/constituent groups of petroleum UVCB substances with carbon range >C30
- Identify constituents that fill data gaps of potential biodegradation and bioaccumulation and human toxicology interest
- Provide information regarding the limits of analysis of petroleum UVCB substance constituents

Reference box for additional comments

Concawe

Method

Analytical laboratories will be invited to analyse using technology of their choice the same set of hydrocarbon **petroleum** substance samples

- Gas oil
- Residual aromatic extract
- Lubricant base oil
- Paraffin wax
- Heavy vacuum oil
- Bitumen

And provide as much qualitative and quantitative information about constituents in these samples as possible.

Data from each analytical laboratory will be collated by Concawe to assess how well current technologies meet the requirements to support hazard assessment.

Analytical capabilities at the workshop

Laboratory	Presenter	Technology
Biochemical Institute for Environmental Carcinogens (BIU)	Albrecht Seidel	Polycyclic Aromatic Hydrocarbons (PAH) by GC/MS
Glasgow University	CarolineGauchotteLindsay	2-dimensional gas chromatography (GCxGC)-TOF MS
Intertek (ITS) UK	Liam Mills	Field desorption time-of-flight mass spectrometry (FD-TOFMS)
JPEC Japan	Jun Akimoto	Fourier-transform ion cyclotron resonance (FTICR)- mass spectrometry
Lommatzsch and Säger	Martin Lommatzsch	GCxGC and HPLC-GC
Plymouth University	Paul Sutton	Range of chromatography/MS
SGS Germany	ThomasKüttler	Derivatisation QTOF-MS, GCxGC FID
SINTEF	Lisbet Sørensen	Fourier-transform ion cyclotron resonance (FTICR)- mass spectrometry
Texas A&M University	Ivan Rusyn	Ion Mobility Mass Spectrometry
University of Southampton	John Langley	Supercritical fluid chromatography (SFC)-MS, HPLC-MS, GCxGC-MS
University of Warwick	Mark Barrow	Fourier-transform ion cyclotron resonance (FTICR)- mass spectrometry

To review Posters online

Go to
https://www.concawe.eu/event/concawe-workshop-for-an-analytical-technology-exchange-to-meet-health-environmental-regulatory-challenges-for-uvcbs/

or

Scan the QR code and click on Analytical laboratories to review the posters

SCAN ME

5

Regulatory challenge session output

To be considered when reviewing posters

6

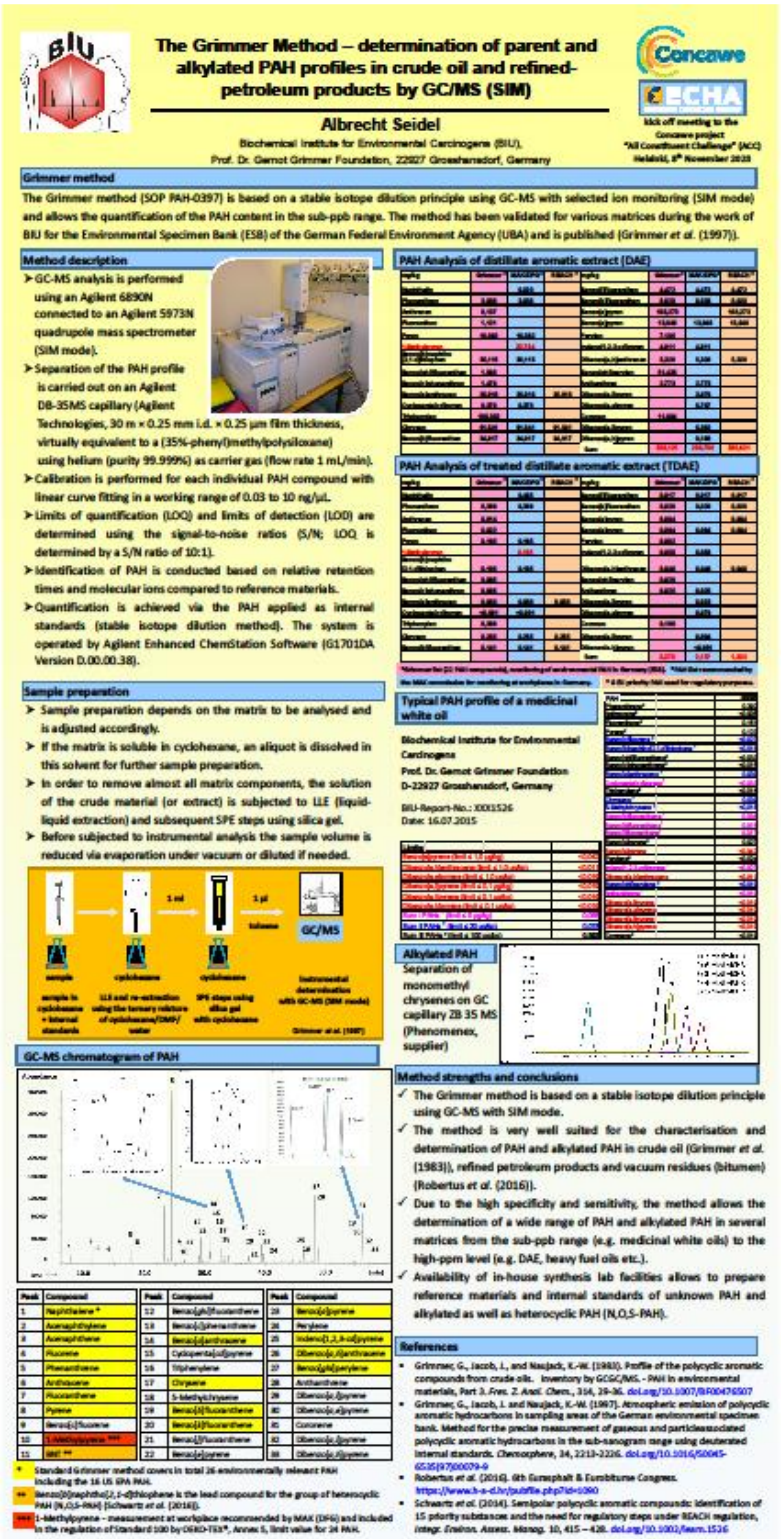
www.concawe.eu

Thank you for your attention

Carol Banner
carol.banner@concawe.eu

APPENDIX 10: ANALYTICAL LABS POSTERS

Analytical-Labs-documents (5).zip



The Grimmer Method – determination of parent and alkylated PAH profiles in crude oil and refined-petroleum products by GC/MS (SIM)

Albrecht Seidel

Biochemical Institute for Environmental Carcinogens (BIU),
Prof. Dr. Gernot Grimmer Foundation, 22927 Grosshansdorf, Germany

kick off meeting to the Concawe project "All Constituent Challenge" (ACC) Helsinki, 8th November 2023

Grimmer method

The Grimmer method (SOP PAH-0397) is based on a stable isotope dilution principle using GC-MS with selected ion monitoring (SIM mode) and allows the quantification of the PAH content in the sub-ppb range. The method has been validated for various matrices during the work of BIU for the Environmental Specimen Bank (ESB) of the German Federal Environment Agency (UBA) and is published (Grimmer *et al.* (1997)).

Method description

- GC-MS analysis is performed using an Agilent 6890N connected to an Agilent 5973N quadrupole mass spectrometer (SIM mode).
- Separation of the PAH profile is carried out on an Agilent DB-35MS capillary (Agilent Technologies, 30 m × 0.25 mm i.d. × 0.25 µm film thickness, virtually equivalent to a (35%-phenyl)methylpolysiloxane) using helium (purity 99.999%) as carrier gas (flow rate 1 mL/min).
- Calibration is performed for each individual PAH compound with linear curve fitting in a working range of 0.03 to 10 ng/µL.
- Limits of quantification (LOQ) and limits of detection (LOD) are determined using the signal-to-noise ratios (S/N; LOQ is determined by a S/N ratio of 10:1).
- Identification of PAH is conducted based on relative retention times and molecular ions compared to reference materials.
- Quantification is achieved via the PAH applied as internal standards (stable isotope dilution method). The system is operated by Agilent Enhanced ChemStation Software (G1701DA Version D.00.00.38).

PAH Analysis of distillate aromatic extract (DAE)

PAH Analysis of treated distillate aromatic extract (TDAE)

Sample preparation

- Sample preparation depends on the matrix to be analysed and is adjusted accordingly.
- If the matrix is soluble in cyclohexane, an aliquot is dissolved in this solvent for further sample preparation.
- In order to remove almost all matrix components, the solution of the crude material (or extract) is subjected to LLE (liquid-liquid extraction) and subsequent SPE steps using silica gel.
- Before subjected to instrumental analysis the sample volume is reduced via evaporation under vacuum or diluted if needed.

Typical PAH profile of a medicinal white oil

Biochemical Institute for Environmental Carcinogens
Prof. Dr. Gernot Grimmer Foundation
D-22927 Grosshansdorf, Germany

BIU-Report-No.: XXX1526
Date: 16.07.2015

Alkylated PAH

Separation of monomethyl chrysenes on GC capillary ZB 35 MS (Phenomenex, supplier)

GC-MS chromatogram of PAH

Method strengths and conclusions

- ✓ The Grimmer method is based on a stable isotope dilution principle using GC-MS with SIM mode.
- ✓ The method is very well suited for the characterisation and determination of PAH and alkylated PAH in crude oil (Grimmer *et al.* (1983)), refined petroleum products and vacuum residues (bitumen) (Robertus *et al.* (2016)).
- ✓ Due to the high specificity and sensitivity, the method allows the determination of a wide range of PAH and alkylated PAH in several matrices from the sub-ppb range (e.g. medicinal white oils) to the high-ppm level (e.g. DAE, heavy fuel oils etc.).
- ✓ Availability of in-house synthesis lab facilities allows to prepare reference materials and internal standards of unknown PAH and alkylated as well as heterocyclic PAH (N,O,S-PAH).

References

- Grimmer, G., Jacob, J., and Naujack, K.-W. (1983). Profile of the polycyclic aromatic compounds from crude oils. Inventory by GC/GC/MS. - PAH in environmental materials, Part 3. *Fres. Z. Anal. Chem.*, 314, 29–36. doi.org/10.1007/BF00476507
- Grimmer, G., Jacob, J. and Naujack, K.-W. (1997). Atmospheric emission of polycyclic aromatic hydrocarbons in sampling areas of the German environmental specimen bank. Method for the precise measurement of gaseous and particleassociated polycyclic aromatic hydrocarbons in the sub-nanogram range using deuterated internal standards. *Chemosphere*, 34, 2213–2226. doi.org/10.1016/S0045-6535(97)00079-9
- Robertus *et al.* (2016). 6th Eurasphalt & Eurobitume Congress. https://www.h-a-d.hr/publfile.php?id=1060
- Schwartz *et al.* (2014). Semipolar polycyclic aromatic compounds: identification of 15 priority substances and the need for regulatory steps under REACH regulation, *Integr. Environ. Assess. Manag.* 10, 415 – 428. doi.org/10.1002/ieam.1536

FIELD IONISATION / DESORPTION
TIME OF FLIGHT MASS SPECTROMETRY

intertek caleb brett

HIGHLIGHTS
High Mass Resolution: R=30,000
Wide Mass Range: 6000 M/Z
High Mass Accuracy
Soft Ionisation Ideal for MW Determination
C10-C120 Analysis

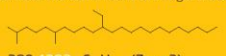
DEGREE OF SATURATION

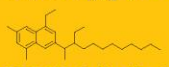
All organic compounds have non-integer molecular weights, including the mono-isotopic ions seen in mass spectra:

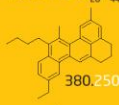
For example, the following compounds all have the same nominal integer mass of 380:

But accurate masses would be:

380.4382 $C_{27}H_{56}$ (Z = +2)
380.3443 $C_{28}H_{44}$ (Z = -12)
380.2504 $C_{29}H_{32}$ (Z = -26)
380.2538 $C_{26}H_{36}S$ (Z = -16S)
380.1565 $C_{30}H_{20}$ (Z = -40)


380.4382 $C_{27}H_{56}$ (Z = +2)


380.3443 $C_{28}H_{44}$ (Z = -12)


380.2504 $C_{29}H_{32}$ (Z = -26)


380.2538 $C_{26}H_{36}S$ (Z = -16S)

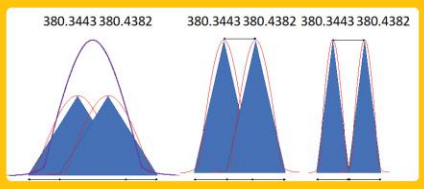

380.1565 $C_{30}H_{20}$ (Z = -40)

The general formula for hydrocarbons is presented as: C_nH_{2n+Z}

Z represents the degree of unsaturation i.e. the number of rings and double bonds in a structure

Z = 2 – (2*RDBE)
Z = 2 – (2*7) = 2-14 = -12

Mass Resolution

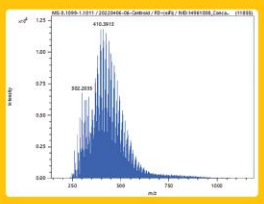


RESULTS

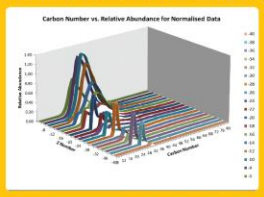
SATURATES	
HYDROCARBON TYPE	**Z NUMBER**
Total Alkanes	2
Cycloalkanes	0
Bicycloalkanes	-2
Tricycloalkanes	-4
Tetracycloalkanes	-6
Pentacycloalkanes	-8
Hexacycloalkanes	-10
Heptacycloalkanes	-12
Octacycloalkanes	-14
Nonacycloalkanes	-16
Decacycloalkanes	-18
Undecacycloalkanes	-20
Dodecacycloalkanes	-22
Tridecacycloalkanes	-24

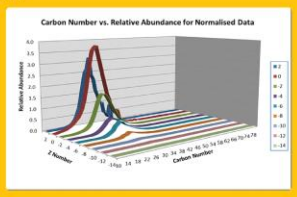
Sample data is tabulated into a matrix of Hydrocarbon type/z number versus carbon number with a limit of detection of 100 ppm. Yield data from the initial separation can be used to factor up the analysis into the whole sample.

AROMATICS	
HYDROCARBON TYPE	**Z NUMBER**
Alkyl Benzenes	-6
Indanes	-8
Indenes	-10
Naphthalenes	-12
Acenaphthenes	-14
Acenaphthylenes/ Fluorenes	-16
Phenanthrenes	-18
C_nH_{2n-20}	-20 (-10S)
C_nH_{2n-22}	-22
C_nH_{2n-24}	-24
C_nH_{2n-26}	-26 (-16S)
C_nH_{2n-28}	-28
C_nH_{2n-30}	-30
C_nH_{2n-32}	-32 (-22S)
C_nH_{2n-34}	-34
C_nH_{2n-36}	-36
C_nH_{2n-38}	-38
C_nH_{2n-40}	-40
C_nH_{2n-42}	-42
C_nH_{2n-44}	-44
C_nH_{2n-46}	-46



Alternatively, the data can be presented graphically



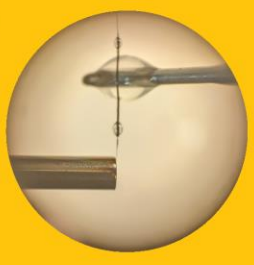


SAMPLE PREPARATION

In order to analyse samples should be separated into Saturates and Aromatics. This is typically conducted using Liquid Chromatography. A modified version of the method IP 368 allows good separation of most sample types in the analysis range.

Importantly the methodology uses Heptane as a mobile phase which needs removal prior to introduction to the FDMS. This is usually conducted by blowing down with a stream of nitrogen, therefore the sample should have a carbon distribution significantly away from C7, hence the analysis starts at C10.

Other separation techniques can be used and is dependent on the sample undertest. Trials are underway using Chromarods as a medium of separation of Bitumen and heavy residues.



INSTRUMENT

Field Desorption

As per the figure above the sample fraction is applied directly to the filament, a current is passed across this filament. Molecules in the condensed phase are ionised to produce molecular ions largely M+. and [M+H]+ ions

Field Ionisation

The field ionization source uses the same architecture but allows a GC column to be terminated in the proximity of the filament, Molecules in the gas phase are ionised to produce molecular ions largely M+. and [M+H]+ ions

After ionisation the ions pass into the time of flight mass spectrometer.

TARGETED ANALYSIS

GCMS/MS

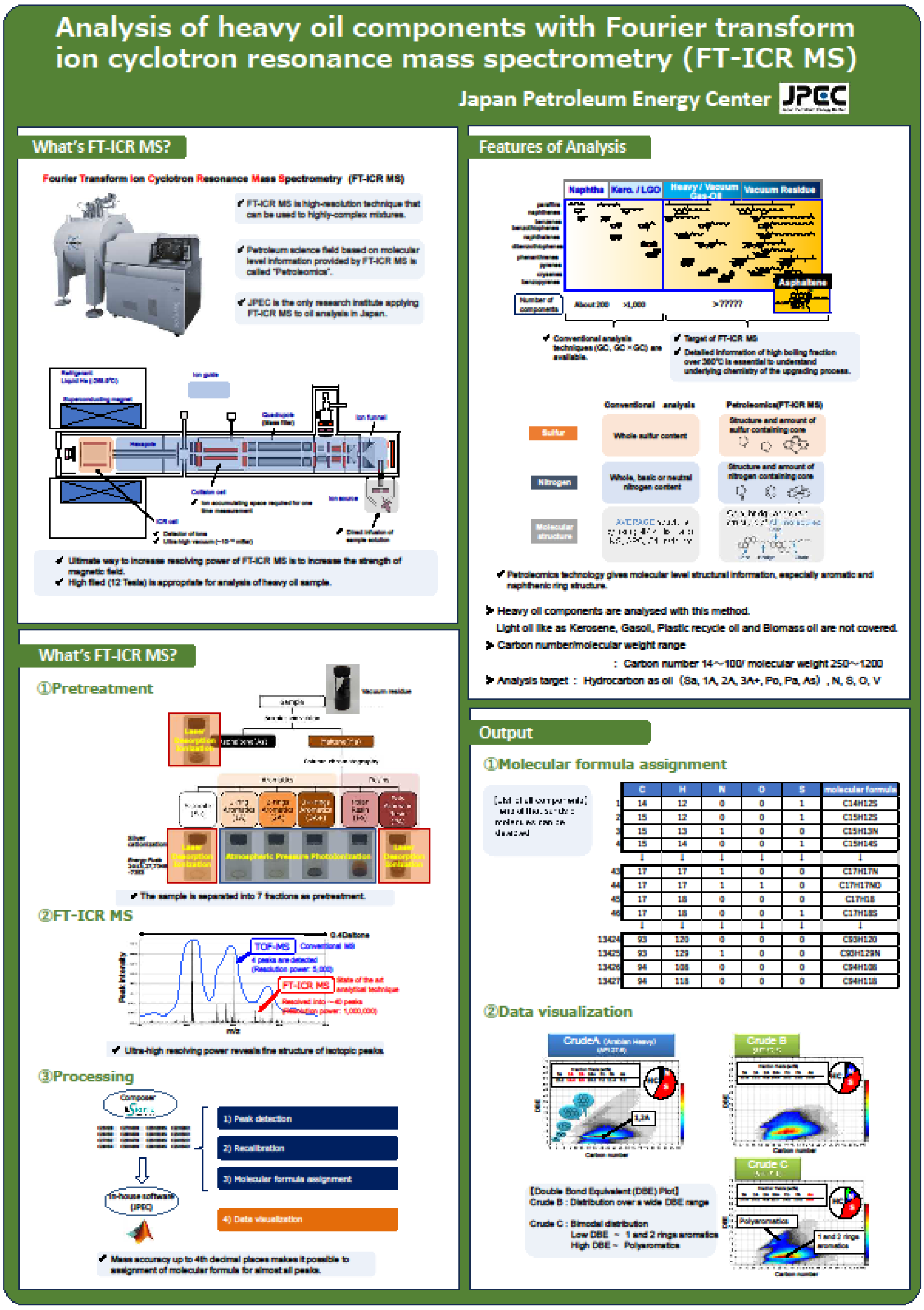
We have the capability of performing targeted analysis for analytes such as PAHs to low levels with a GC-MS/MS. The instrument allows for multiple reaction monitoring (MRM) which leads to increased sensitivity for low level compounds in complex mixutures. If the sample is ameanable to direct injection via a split/splitless GC injector the sample preparation can be minimal, but the power of the MS/MS allows for potential sub-ppm level detection limits for targeted compounds.

FUTURE DEVELOPMENTS

The instrument is also capable of coupling to a GC, however Field Desorption cannot be used, Instead the instrument operates in Field Ionisation mode. Work is underway to produce a GCxGC-FI-TOF-MS methodology. Initial data using Kerosene type samples compares well with data taken from GCxGC-FID methods using internationally recognized standards.

The eventual goal is produce a method in which a sample can be tested from C10-C120 without separation

SCAN ME
FOR MORE INFORMATION


Analysis of heavy oil components with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)
Japan Petroleum Energy Center JPEC
What's FT-ICR MS?
Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS)
FT-ICR MS is high-resolution technique that can be used to highly-complex mixtures.
Petroleum science field based on molecular level information provided by FT-ICR MS is called "Petroleomics".
JPEC is the only research institute applying FT-ICR MS to oil analysis in Japan.
Ultimate way to increase resolving power of FT-ICR MS is to increase the strength of magnetic field.
High filed (12 Tesla) is appropriate for analysis of heavy oil sample.
Features of Analysis
Naphtha
Kero. / LGO
Heavy / Vacuum Gas-Oil
Vacuum Residue
Asphaltene
Conventional analysis
Petroleomics(FT-ICR MS)
Sulfur
Nitrogen
Molecular structure
Petroleomics technology gives molecular level structural information, especially aromatic and naphthenic ring structure.
Heavy oil components are analysed with this method.
Light oil like as Kerosene, Gasoil, Plastic recycle oil and Biomass oil are not covered.
Carbon number/molecular weight range
: Carbon number 14~100/ molecular weight 250~1200
Analysis target : Hydrocarbon as oil (Sa, 1A, 2A, 3A+, Po, Pa, As) , N, S, O, V
What's FT-ICR MS?
①Pretreatment
The sample is separated into 7 fractions as pretreatment.
②FT-ICR MS
TOF-MS
FT-ICR MS
Ultra-high resolving power reveals fine structure of isotopic peaks.
③Processing
1) Peak detection
2) Recalibration
3) Molecular formula assignment
4) Data visualization
Mass accuracy up to 4th decimal places makes it possible to assignment of molecular formula for almost all peaks.
Output
①Molecular formula assignment
②Data visualization
CrudeA
Crude B
Crude C
[Double Bond Equivalent (DBE) Plot]
Crude B : Distribution over a wide DBE range
Crude C : Bimodal distribution
Low DBE ~ 1 and 2 rings aromatics
High DBE ~ Polyaromatics

Laboratory Lommatzsch & Säger

ACC Workshop

Comprehensive gas chromatography (GCxGC) with optional pre-fractionation into saturated & aromatic hydrocarbons via HPLC

M. Lommatzsch, S. Säger
Laboratory Lommatzsch & Säger; Gottfried-Hagen Str. 62; D-51105 Cologne

www.mosh-moah.de

Method description in brief

The sample extracts are analysed via comprehensive gas chromatography (GCxGC).

Injection: PTV on-column
Columns: Reversed setup
(1st dimension: mid-polar, 2nd dim.: apolar)
Modulation: Cryogenic
Detection: FID or MS

The GCxGC is increasing the separation power by a second dimension. This enables a chromatographic separation of hydrocarbon subgroups. A quantification can be performed via FID with internal standards (one point calibration for target and non-target screenings) instead of conventional calibration. An identification of separated single substances and subgroups can be performed via TOF-MS.

Previous to GCxGC, a HPLC fractionation can be performed to separate saturated and aromatic hydrocarbons (MOSH/MOAH methodology). Additionally, the HPLC fractionation enables a clean-up for matrix components (e.g. triglycerides) in environmental and food samples.

Applicability of method

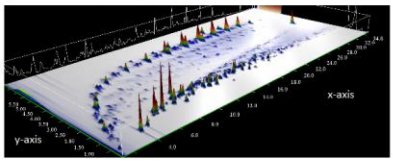
Carbon number: C8 – C40 *(nC8 – nC50)*

Internal Standards (IS) *with negligible coelution*

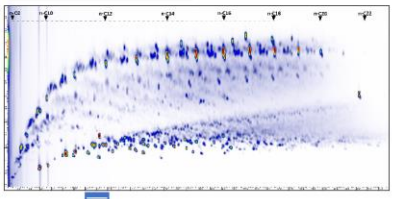
Saturated hydrocarbons:
iso-Alkanes & n-alkanes (**n-/iso-P**)
Monocyclo-alkanes (N)
Dicyclo-alkanes (DN)
Tricyclo-alkanes (TN)

Aromatic hydrocarbons:
Monoaromatics (MoAr)
Naphthenic Monoaromatics (NMoAr)
Diaromatics (DiAr)
Naphthenic Diaromatics (NDiAr)
Triaromatics (TriAr)
Tetraaromatics (TetAr)

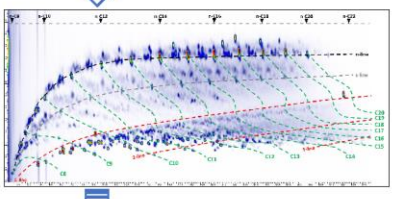
3D GCxGC plot:



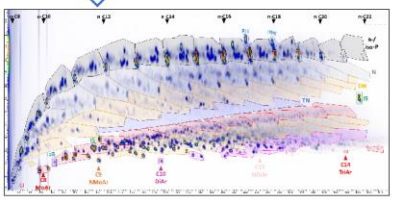
2D GCxGC plot:



2D Orientation



FID Quantification

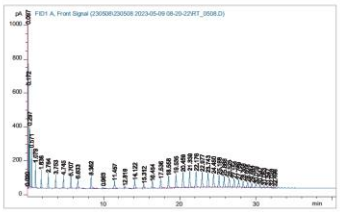


#	n-P	Iso-P	N	DN	MoAr	NMoAr	DiAr	NDiAr	TriAr	TN	Total
C8	0.178	0.367	0.391	0.020	1.174						2.13
C9	0.352	1.249	0.879	0.274	3.279	0.253					6.29
C10	0.407	1.657	1.268	0.487	2.983	1.280	0.041				8.12
C11	0.509	1.543	1.230	0.485	1.840	2.318	0.220				8.15
C12	0.616	1.405	1.087	0.754	1.168	2.493	0.498	0.003			8.02
C13	0.880	1.593	1.445	1.007	0.886	1.938	1.074	0.028			8.85
C14	1.411	1.788	1.925	1.836	0.945	1.590	0.717	0.315	0.014		10.54
C15	2.098	2.685	2.444	1.529	0.911	1.441	0.647	0.341	0.045		12.14
C16	2.147	2.494	2.305	1.166	0.802	1.300	0.525	0.226			10.96
C17	1.844	2.387	2.243	0.935	0.673	0.794	0.413				9.29
C18	1.405	1.745	1.788	0.693	0.551	0.545					6.73
C19	0.966	1.910	1.129	0.315	0.359	0.231					4.91
C20	0.334	1.262	0.408	0.061							2.06
C21	0.041	0.185	0.081	0.005							0.31
C22	0.003	0.027									0.03
C23											0.00
Total	13.19	22.30	18.62	9.57	15.57	14.18	4.14	0.91	0.06	1.46	100.00

[%]

SGS Germany – Oil analysis

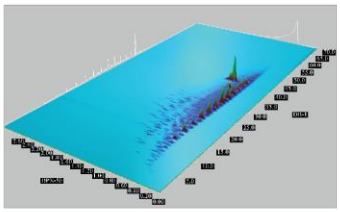
HIGH TEMPERATURE GC

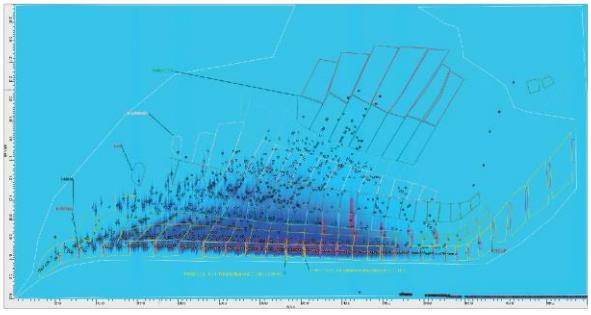


- Using this method, molecules up to 120 carbons, 750 centigrade can be found
- Relative amounts can be given by integration in sections of boiling point

GCXGC

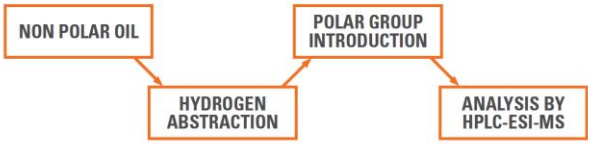
- Alkenes up to 36 carbons can be detected with this method, focused on Diesel
- Alkenes can be seperated from mono-, di- and tri-aromatics





DERIVATISATION AND HPLC

- Circumventing the problem of thermical evaporation the hydrocarbons can be derivatized to introduce polar groups.



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Quantification of bitumen constituents by FT-ICR-MS – is it achievable?

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2) Concawe, Brussels, Belgium
3) Nynas, Antwerp, Belgium
4) SF Analyticon, Cheshire, England

The challenge

- Bitumen, or asphalt is a highly viscous petroleum-derived substance. While it occurs naturally, it is commonly produced as a residue after distillation of crude oils. In essence, bitumen is a very complex mixture of (mostly) unresolved chemical constituents. A large fraction of bitumen is expected to be hydrocarbons (aliphatic and aromatic), but a fraction also contain nitrogen (N), sulfur (S) and oxygen (O) compounds.
- Due to the production method, it is expected that most chemical consitutents are 'heavy' (large, complex molecules with high boiling points).
- Most commonly used analytical techniques such as GC-MS and LC-MS are hardly applicable to bitumen due to either the high boling point (GC-MS) or the expected high content of apolar compounds (LC-MS).

Analytical strategy

- The primary aim of the presented work was to identify a suitable analytical strategy to unravel the chemical composition of bitumen. A secondary objective was to evaluate the potential for FT-ICRMS analysis to be used for *quantification* of relative compound or compound group concentrations within a sample.
- Two different approaches to SARA fractionation was applied:, 1) Methodology from ASTM D4124 and 2) *n*-hexane precipitation of asphaltenes followed by LC-separation of the maltene (SAR) fractions. The mass yield of each fraction was determined gravimatrically.
- Whole substance bitumen and all resulting fractions were analyzed by FT-ICRMS in both positive atmospheric pressure photoionization (APPI+) and negative electrospray (ESI-) modes. Accurate mass data was converted to molecular formulae and exported for further manipulation in R software.

Repeatability of fractionation and analysis

- Fractionation and analysis was performed in triplicate with acceptable repeatibility.
- Relative standard deviations of gravimetric mass yield of fractions was in the ranges 1-5 and 15-40% for the LC- and ASTM-method, respectively.
- For the APPI FT-ICRMS data, the mean standard deviation in determination of total abundance per chemical class was 7%, and the same for number of masses detected within each sample was 15%.

SARA who?

- The mass yield of each fraction by each method was compared to the data obtained using the TLC-fractionation (IP469(.
- The three methods gave different results, with the largest discrepancy in distribution between the aromatics and resins fractions between the two methods applied in this work and the standard method.

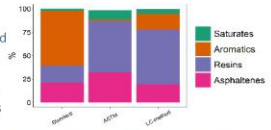


Figure 1 SARA composition of the bitumen sample from three different methods. The 'standard' here refers to IP469.

Compositional data of bitumen and fractions

- In the current study, only APPI(+) mode analysis provided reasonable compositional data. ESI(-) had a low number of detected masses for all samples. This indicates low presence of complex organic acids. In the following, only APPI data will be discussed.

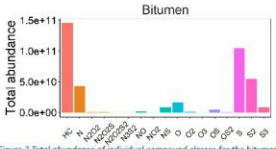


Figure 2 Total abundance of individual compound classes for the bitumen sample.

- As expected, the bitumen sample contained a range of hydrocarbons functionalized compounds ('NSO') over a large molecular size range.

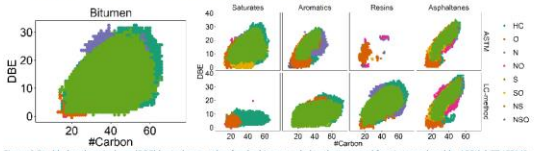


Figure 3 Double-bond equivalents (DBE) by carbon number for the bitumen whole substance and fractions analysed by APPI(+)-FT-ICRMS..

- With the exception of the asphaltenes, the fractions resulting from the two different fractionation methods were very different in composition. It appears that the LC-method more 'evenly' distributed the masses between the fractions (supported by the gravimetric data above).

The toolkit

- Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (**FT-ICRMS**) with its to date not challenged mass resolution (>100,000), allows accurate mass determination and elemental composition identification. Depending on the selected ionization technique, the method is applicable to a wide array of analyte sizes and polarities. As such, it lends itself to the ***characterization*** of very complex samples – and to compare variation in composition of such samples. It does not rely on compounds being both amenable to and stabile throughout an online chemical separation, overcoming challenges observed in e.g., gas chromatography-based techniques.
- When targeting detection of the highest number of compounds in a very complex sample – fractionation of the sample *may* be useful to simplify the matrix of each fraction – thus revealing the identity of the maximum number of analytes.
- **SARA** (saturates, aromatics, resins, asphaltenes) fractionation is a relatively simple industry standard method that has been applied to petrogenic samples for decades, and in combination with FT-ICRMS for years. Currently, SARA fractionation remains a standard method to characterise the composition of bitumen samples.

Matrix suppression?

- The number of detected masses resulting from the bitumen was overall higher after fractionation than when analysing the whole substance. The LC-method provided the highest number of masses, and also a higher total abundance of measured masses.
- A possible explanation is that the LC-method fractionation most evenly distributed bitumen mass between four fractions, meaning each fraction was less complex than bitumen and several of the ASTM fractions.

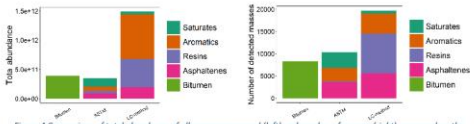


Figure 4 Comparison of total abundance of all masses measured (left) and number of masses (right) measured on the bitumen whole substance compared to the various fractions

Elemental composition

- The elemental composition of the bitumen sample was determined by standard methods including ASTM D5291 (Carbon, Hydrogen); ASTM D5768 (Nitrogen); MT/ELE/17 (Oxygen) and MT/ELE/05 (Sulfur). In addition, composition of each FT-ICR-MS identified constituents in combination with their relative abundance was used to calculate an approximated elemental composition of each fraction after analysis

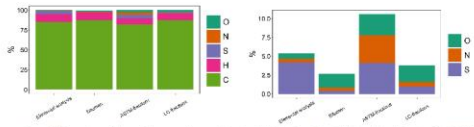


Figure 5 Comparison of elemental composition as determined by the standard methods, and as determined by FT-ICRMS analysis of bitumen full substance or after summing relative contributions of four SARA fractions by the two methods

Observations:

- N-compounds suppressed in full substance analysis, more 'visible' in fractions
- ASTM method "exaggerates" presence of nitrogen compared to other methods
- Sulfur least comparable between FT-ICRMS data and elemental analysis
- More oxygenated compounds in all APPI analysis than elemental analysis

Preliminary conclusions

- The methodology demonstrated good repeatability for both fractionation and analysis.
- Fractionation leads to higher total abundances and a different compositional picture compared to analysing the bitumen sample directly.
- Different fractionation techniques gives different results.
- So far this work has focused on proof-of-concept to one sample, and there is a lack of statistical power to draw conclusions.

Further work to tackle the challenge of quantification

- In an ideal (unrealistic) scenario there would standards and response factors for 'all' compounds or at least all 'representative' structures, but this is not achievable in the foreseeable term due to the number of potential chemicals and chemical groups in bitumen. To overcome this, whether or not a defendable semi-quantitative approach is possible to achieve needs to be investigated.
- Further challenges that will to be addressed are 1) the extent of ion suppression and matrix effects by analysis of further bitumen samples, 2) dealing with ionization and response discrimination based on chemical structure with a selected subset of representative compounds spiked into varying matrix complexity.

IP 469, Determination of saturated, aromatic and polar compounds in petroleum products by thin layer chromatography and flame ionization detection. 2006
ASTM D4124-09 Standard Test Method for Separation of Asphalt into Four Fractions
Aske, N., Kallevik, H., Sjöblom, J. Determination of Saturate, Aromatic, Resin, and Asphaltenic (SARA) Components in Crude Oils by Means of Infrared and Near-Infrared Spectroscopy. Energy Fuels 2001, 15 (5), 1304–1312.
Fan, T.; Buckley, J. S. Rapid and Accurate SARA Analysis of Medium Gravity Crude Oils. Energy Fuels 2002, 16 (6), 1571–1575.

Technology for a better society

University of Glasgow

hiRACE
High Resolution Analytical Chemistry for Environmental Engineering

Workflows for Signature Analysis by Gas Chromatography Coupled with Mass Spectrometry.

Examples for Description of Engineering Processes.

Caroline Gauchotte-Lindsay, Ioannis Sampsonidis, Kate Fell-
hiRACE, James Watt School of Engineering, University of Glasgow, Glasgow, UK.

With acknowledgments to Alan Griffiths and Nick Jones ate LECO, UK.

Signature Analysis: Non-Targeted Approaches to Complex Samples

Non-targeted analysis (NTA) is changing chromatography and mass spectrometry. In contrast to conventional analytical methods that focus on the targeted identification of specific compounds in samples, NTA offers a holistic view of complex samples, exploring the entire "chemical space." Enabled by cutting-edge hardware and data science and initiated by biological applications, the rapid growth of this technique is also significantly shaped by its applications in environmental sciences.

Just as an individual's signature evolves with time and stressors, so do the chemical profiles in environmental samples. Instead of a "chemical fingerprint," we should call it a "chemical signature." Think of NTA as signature analysis – the path to extracting unique insights from samples that targeted analysis can't uncover. NTA has the potential to elucidate the mechanisms of the transformation of complex environmental systems and to isolate markers for these reactions.

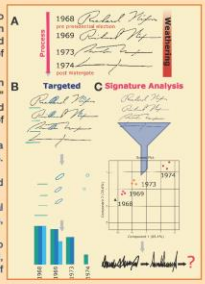


Figure 1- To grasp the value of signature analysis in the context of environmental samples, let's draw a parallel with handwritten signatures. In this illustration, we trace the evolution of signatures from two U.S. presidents, namely R. Nixon and D. Trump.
A- Nixon's signature seems to bear the marks of his time in office, marked by the Watergate scandal and the subsequent impeachment proceedings leading to his resignation.
B- However, if we restrict our analysis to specific graphological elements, like the shape of the capital 'R,' the loop on the 'h,' and the dots on the 'I,' we inadvertently overlook other significant characteristics, rendering the data unusable for further extrapolation.
C- Conversely, when we undertake a holistic examination of the entire signatures, we open the door to the identification of new statistical markers and the development of transformation models. The model, statistically constructed from R. Nixon's signatures, offers the potential to predict the impact of impeachment on D. Trump's signature.

Signature Analysis to Describe the Gasification of Coal and its By-Products

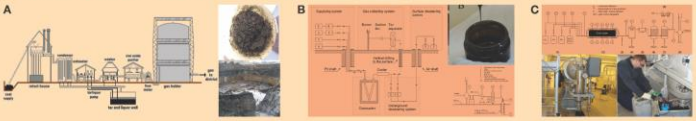
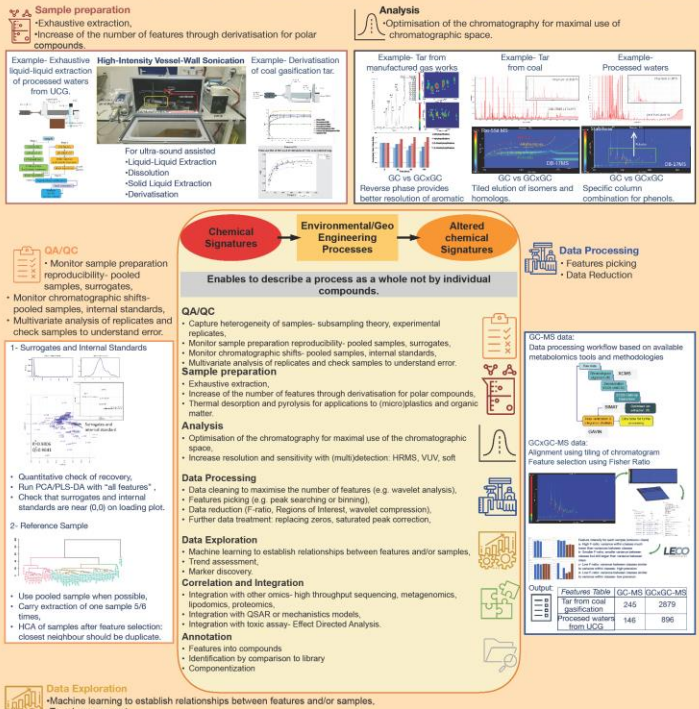


Figure 2- Processes of coal transformation investigated and the types of samples analysed.
A- Manufactured Gas Works and coal tar and tar contaminated soils.
B- Coal Gasification in Barbara Mine, Poland and coal tar. Diagrams from Wiatowski *et al.* (2019).
C- Ex-situ high pressure underground coal gasification (UCG) reactor and processed waters. Diagrama and photos from Pankiewicz-Sperka *et al.*



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McGregor, L.A. et al. (2012) *Multivariate statistical methods for the environmental forensic classification of coal tars from former manufactured gas plants*. Environmental Science and Technology, 46(7), pp. 3744-3752. (doi:10.1021/es203078w)
Sampsonidis, I. (2018) *Semi-volatile organic compounds in underground coal gasification effluents: from sample preparation to data analysis*. PhD thesis, University of Glasgow.
Wiatowski, M. et al. (2019) *Technological aspects of underground coal gasification in the Experimental "Barbara" Mine*. Fuel, 159,pp. 454-462. (doi:10.1016/j.fuel.2015.07.001)
Pankiewicz-Sperka et al. (2021) *Characteristics of Water Contaminants from Underground Coal Gasification (UCG) Process – Effect of Coal Properties and Gasification Pressure*. Energies, 14(20), pp.6533 (doi:10.3390/en14206533)

Chemical class separation of petroleum and wax analysis

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Proposal

A. Class-type separation of petroleum using ion exchange (IEX) solid phase extraction (SPE) and analysis of amenable fractions using high temperature gas chromatography with flame ionisation detection (HTGC-FID)

B. Wax analysis using (HTGC-FID)

A. *Class-type separation of petroleum using ion exchange (IEX) solid phase extraction (SPE) and analysis of amenable fractions using high temperature gas chromatography with flame ionisation detection (HTGC-FID)*

- *Sequential strong cation exchange (SCX), strong anion exchange (SAX) and adsorption on silica (Si) fractionation of crude oil (Figure A1)*
- *Variant used commercially for $C_{80\text{-}82:0\text{-}8}$ ('Arn') tetraacids (12 years; Sutton & Rowland, 2014)*
- *API gravity 12.1-38.3 °, TAN 0.1-3.6 mg KOH, S 0.25-2.7%, asphaltene 0-11%*
- *Immature to late maturity oils, biodegradation 0.9-8.2 and absent pristane/nC_{17} ratio*
- *Gravimetric determination of fractions (Table A1; quantitatively important)*
- *Procedure lends itself to 'read-across'*
- *Can be readily implemented in any laboratory*
- *Mass balance when evaporative losses accounted for*
- *Relatively quick sample preparation time, low solvent use*
- *Non-destructive*
- *Fractions available for toxicity testing (toxicity identification evaluation; rule in/out)*
- *Fractions available for molecular identification*
- *Most fractions amenable to GC, use HTGC for >C_{30}*

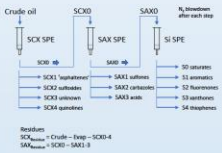


Figure A1. Flow diagram for gravimetric class type IEX SPE separation of crude oil

Table A1. Chemical class fractions obtained from crude oil using IEX SPE and the analytical techniques used to characterise them (Robson et al 2017, Robson 2018)

Fraction			Eluent	'Class'	Analysis
SCX1			Toluene	"Asphaltenes"	??
SCX2			THF	Sulfoxides	GC
SCX3			THF/H_2O/2% ammonia	No model compounds	??
SCX4			THF/H_2O/5% ammonia	Quinolines	LC-MS
SCX0 (unretained)	SAX1		Toluene	Sulfones	GC
	SAX2		THF	Carbazoles	GC
	SAX3		THF/H_2O/FA	Naphthenic acids	GC (TMS)
	SAX0 (unretained)	S0	*N*-hexane	"Saturates"	GC
		S1	DCM/*n*-hexane 1:4	"Aromatics"	GC
		S2	DCM/*n*-hexane 1:1	Fluorenones	GC
		S3	DCM	Xanthones	GC
		S4	THF	Thiophenes	GC

B. *Wax analysis*

- *Requires special precautions due to wax deposition*
- *T-SEP® 'Topping' procedure improves 'high-end' sensitivity (Figure B1)*
- *Gravimetric determination of 'total' wax*
- *Condensates, liquid and waxy oils, oily waxes and wax deposits*
- *Merge WHOLE and TOPPED n-alkane integrals, plot weight % (Figure B2)*
- *External semi-quantitation based on sensitivity of in-house n-alkane ($nC_{10\text{-}30,40,50,60}$) standard*
- *Used commercially for 14 years*

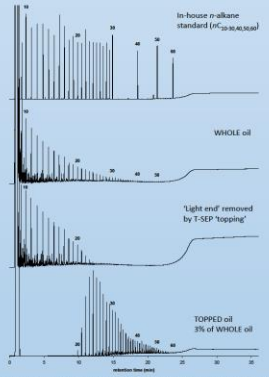


Figure B1. HTGC-FID chromatograms of *n*-alkanes in-house standard (top), Gulf of Mexico WHOLE crude oil (NIST 2779; upper), fraction removed by 'TOPPING' (lower), and the T-SEP® 'TOPPED' fraction (bottom). Numbers represent carbon number of *n*-alkane.

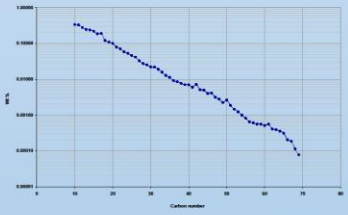
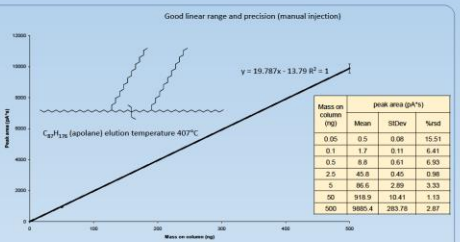


Figure B2. Weight percentage n-alkanes in Gulf of Mexico crude oil (NIST 2779)

HTGC-FID – Highlights

- *Good separation efficiency*
- *Robust using steel coated columns*
- *'Hot' injection of heated waxy samples*
- *Analysis up to ca. C_{100} with capillary column*
- *Four orders magnitude linearity (Figure 1)*
- *Good precision (%rsd)*
- *FID optimised LoD ca. 20 pg on column $C_{87}H_{176}$*



Mass on column (ng)	peak area (pA*s)		
	Mean	StDev	%rsd
0.05	0.5	0.08	15.51
0.1	1.7	0.11	6.41
0.5	8.8	0.61	6.93
2.5	45.8	0.45	0.98
5	86.6	2.89	3.33
50	918.9	10.41	1.13
500	9880.4	283.78	2.87

Figure 1. Calibration chart for $C_{87}H_{176}$ hydrocarbon apolane (n = 3 at each point)

References

Robson, W.J., Sutton, P.A., McCormack, P., Chilcott, N.P. and Rowland, S.J. (2017) Class type separation of the polar and apolar components of petroleum. *Analytical Chemistry* **89**, 2919-2927. [doi: 10.1021/acs.analchem.6b04202].

Robson, W.J. (2018) The separation of the polar constituents of petroleum. PhD. Thesis. University of Plymouth.

Sutton, P.A. and Rowland, S.J. (2014) Determination of the content of C_{80} tetraacids in petroleum. *Energy & Fuels* **28**, 5657-5669. [doi: 10.1021/ef5012337].

Supercritical Fluid Chromatography – Mass Spectrometry

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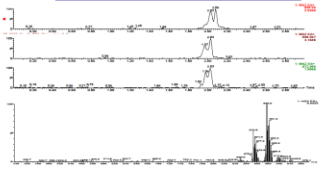
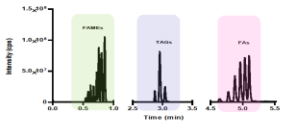
University of Southampton

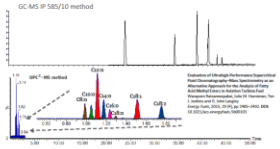
High Molecular Mass > C50 (O6)



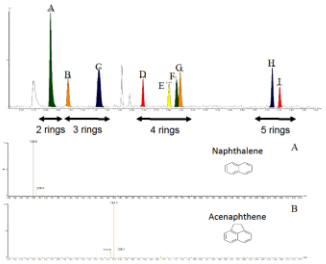
Biodiesel and Impurities

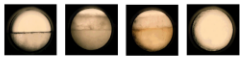


FAME in JET



PAHs



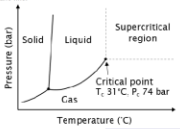


Fast solvent evaporation for preparative scale
CO_2 is safe and inexpensive
Achieve chiral separations
Green
Can use polar and apolar stationary phases
Reduced solvent consumption
Fast, efficient methods
Easily coupled to MS

Advantages of SFC

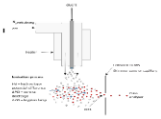
CO_2 Phase Diagram

- $scCO_2$ is intermediate between a liquid and a gas, with the density of liquids with the diffusivity and viscosity of gases.
- $scCO_2$ is non-toxic, non-flammable, chemically inert and inexpensive, and hexane like.

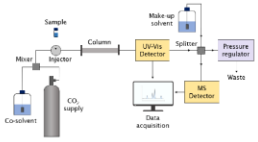


Selective Ionisation

Atmospheric Pressure Ionisation

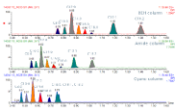


SFC-MS configuration

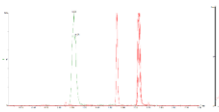


Chromatographic Selectivity

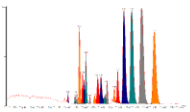
Column Selectivity



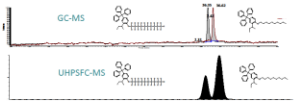
Class Separation (Corrosion Inhibitors)



Component Separation (CIs)



Larger molecules

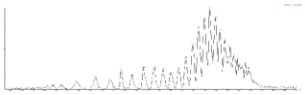


energy&fuels

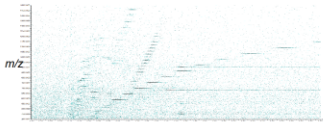
Detection and Quantitation of ACCUTRACE S10, a New Fiscal Marker Used in Low-Duty Fuels, Using a Novel Ultrahigh-Performance Supercritical Fluid Chromatography–Mass Spectrometry Approach

Polymerics

Simple Ethoxylate



Polymerics in Gasoline



SFC-FID (Selerity)

- No organic modifier used
- Compatible with FID, giving uniform detector response across compound types
- ASTM methods already in place, e.g.,
  - ASTM D5186-Total aromatics and polyaromatics in Diesel Fuels
  - ASTM D6650-Total Olefins in Gasoline
  - ASTM D8305-Total Aromatics and polyaromatics (naphthalenes) in aviation fuels
- Analysis of compounds > C30 routine/trivial
- Largest compound detected so far C138

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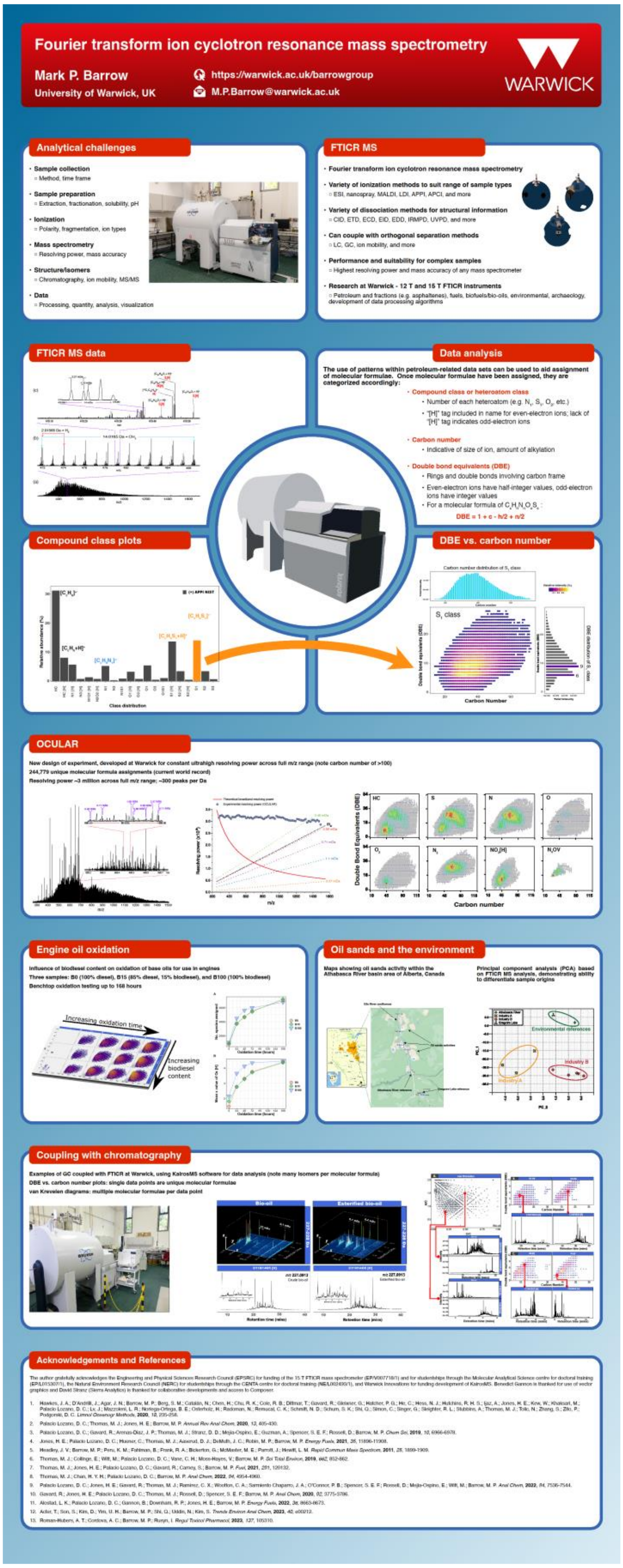

Fourier transform ion cyclotron resonance mass spectrometry
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WARWICK
Analytical challenges
Sample collection
Method, time frame
Sample preparation
Extraction, fractionation, solubility, pH
Ionization
Polarity, fragmentation, ion types
Mass spectrometry
Resolving power, mass accuracy
Structure/isomers
Chromatography, ion mobility, MS/MS
Data
Processing, quantity, analysis, visualization
FTICR MS
Fourier transform ion cyclotron resonance mass spectrometry
Variety of ionization methods to suit range of sample types
ESI, nanospray, MALDI, LDI, APPI, APCI, and more
Variety of dissociation methods for structural information
CID, ETD, ECD, EID, EDD, IRMPD, UVPD, and more
Can couple with orthogonal separation methods
LC, GC, ion mobility, and more
Performance and suitability for complex samples
Highest resolving power and mass accuracy of any mass spectrometer
Research at Warwick - 12 T and 15 T FTICR instruments
Petroleum and fractions (e.g. asphaltenes), fuels, biofuels/bio-oils, environmental, archaeology, development of data processing algorithms
FTICR MS data
Data analysis
The use of patterns within petroleum-related data sets can be used to aid assignment of molecular formulae. Once molecular formulae have been assigned, they are categorized accordingly:
Compound class or heteroatom class
Number of each heteroatom (e.g. N1, S1, O2, etc.)
"[H]" tag included in name for even-electron ions; lack of "[H]" tag indicates odd-electron ions
Carbon number
Indicative of size of ion, amount of alkylation
Double bond equivalents (DBE)
Rings and double bonds involving carbon frame
Even-electron ions have half-integer values, odd-electron ions have integer values
For a molecular formula of CcHhNnOoSs :
DBE = 1 + c - h/2 + n/2
Compound class plots
Class distribution
DBE vs. carbon number
Carbon number distribution of S1 class
S1 class
Carbon Number
OCULAR
New design of experiment, developed at Warwick for constant ultrahigh resolving power across full m/z range (note carbon number of >100)
244,779 unique molecular formula assignments (current world record)
Resolving power ~3 million across full m/z range; ~300 peaks per Da
Theoretical broadband resolving power
Experimental resolving power (OCULAR)
Resolving power (x10⁶)
m/z
Double Bond Equivalents (DBE)
Carbon number
Engine oil oxidation
Influence of biodiesel content on oxidation of base oils for use in engines
Three samples: B0 (100% diesel), B15 (85% diesel, 15% biodiesel), and B100 (100% biodiesel)
Benchtop oxidation testing up to 168 hours
Increasing oxidation time
Increasing biodiesel content
Oxidation time (hours)
Oil sands and the environment
Maps showing oil sands activity within the Athabasca River basin area of Alberta, Canada
Principal component analysis (PCA) based on FTICR MS analysis, demonstrating ability to differentiate sample origins
Environmental references
Industry A
Industry B
Coupling with chromatography
Examples of GC coupled with FTICR at Warwick, using KairosMS software for data analysis (note many isomers per molecular formula)
DBE vs. carbon number plots: single data points are unique molecular formulae
van Krevelen diagrams: multiple molecular formulae per data point
Bio-oil
Esterified bio-oil
227-228 Da
m/z 227.0913
Crude bio-oil
Esterified bio-oil
Retention time (mins)
Carbon Number
Acknowledgements and References
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