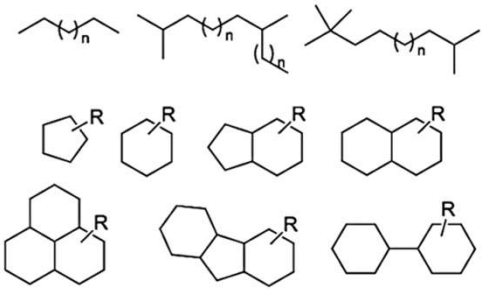
Update on MOSH&MOAH characterization

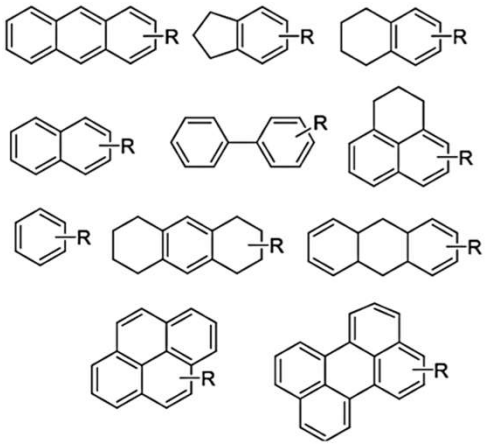
Aleksandra Gorska, Carlo Bellinghieri, Damien Pierret, Andrea Hochegger, Marco Beccaria,
Giorgia Purcaro

AOCS - **ANNUAL MEETING & EXPO** Hyatt Regency New Orleans, New Orleans, Louisiana, USA
May 3-6, 2026

Definition and context

MOSH	MOAH
Mineral oil **SATURATED** hydrocarbons	Mineral oil **AROMATIC** hydrocarbons





Ubiquitous **food contaminants** of petrogenic origin

MOAH **≥3 aromatic rings** associated with **GENOTOXICITY**

2022: control of **MOAH content in food** in EU was established and soon (2027) a **regulation**

Recent Updates – AOCS Meeting 2024

MOSH

MOAH

Updates – EFSA requirements

For **MOSH** :

- **Improvement of analytical methodology for better characterisation of MOSH** .....
- Better investigation of the **sources of the hydrocarbons** in food, ...
- Data are needed on ... **accumulation potential**.
- Investigation is recommended on the **structural features of MOSH** that ... result **in accumulation**.
- Additional toxicity data are needed ... in particular on bioaccumulating MOSH following their characterisation.
- More data on human MOSH tissue concentrations.
- .... needs further investigation with regard to occurrence of **MOSH and potential compositional modification and bioaccumulation**.
- ...

For **MOAH**:

- **More selective and sensitive analytical method should be implemented**.
- More data on **MOAH composition by aromatic ring number** in food are needed, in particular with respect to the levels of ≥ 3 ring MOAH.
- Sources of food contamination should be investigated when MOAH are detected.
- More data are needed on the **influence of ring alkylation on genotoxicity and carcinogenic** potency of ≥ 3 ring MOAH.
- **Oral toxicity data** in particular of **1–2 ring MOAH**.
- Technical specifications of white mineral oils and waxes used as **food additives and food packaging materials** should be updated, with **detailed information about the MOAH content and composition**.

Updates – EFSA requirements

efsa

SCIENTIFIC OPINION

efsa JOURNAL

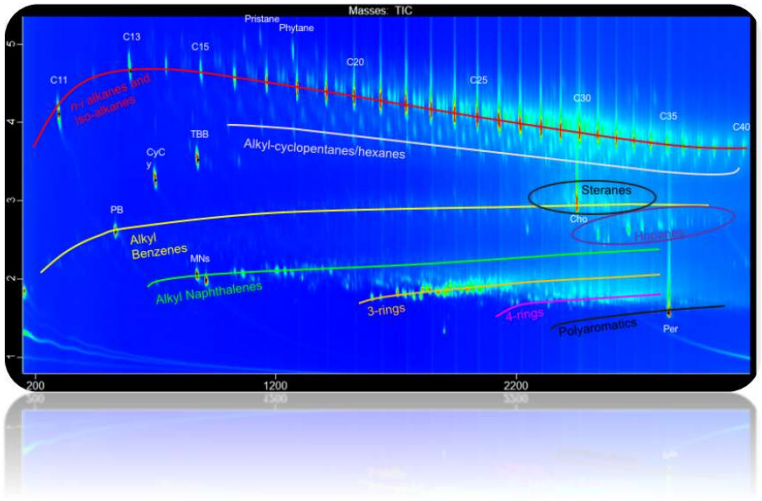
ADOPTED: 12 July 2023

doi: 10.2903/j.efsa.2023.8215

2023

Update of the risk assessment of mineral oil hydrocarbons in food

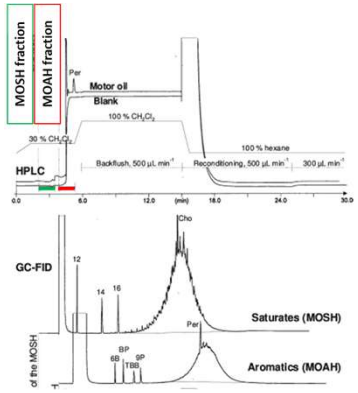
EFSA Panel on Contaminants in the Food Chain (CONTAM),



Recommendations:

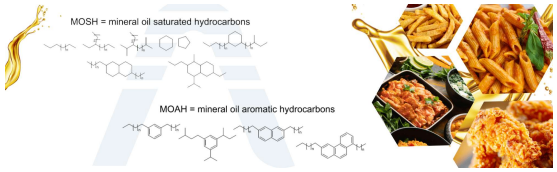
ANALYTICS

Improvement of the methods



EXPOSITION

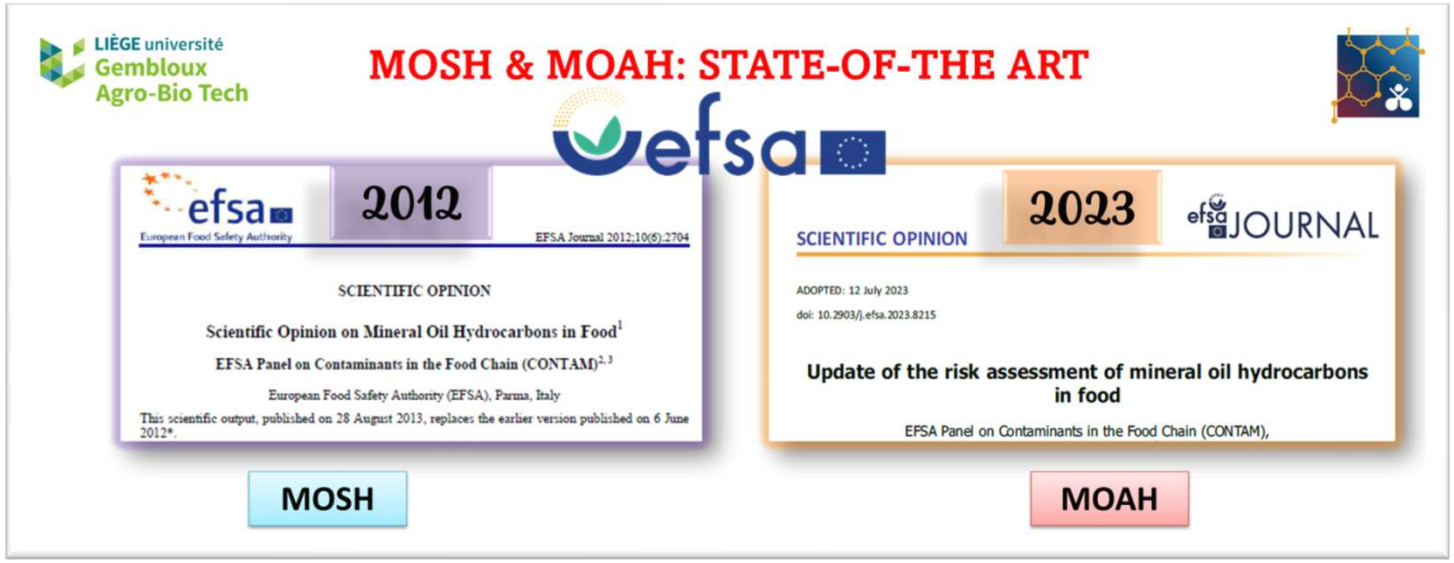
More detailed characterization

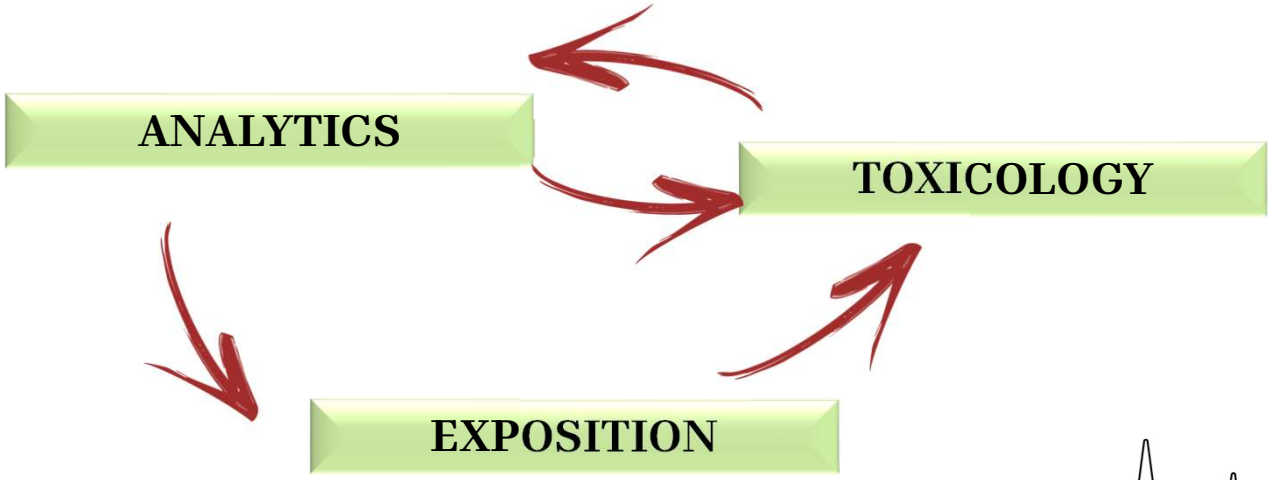


TOXICOLOGY

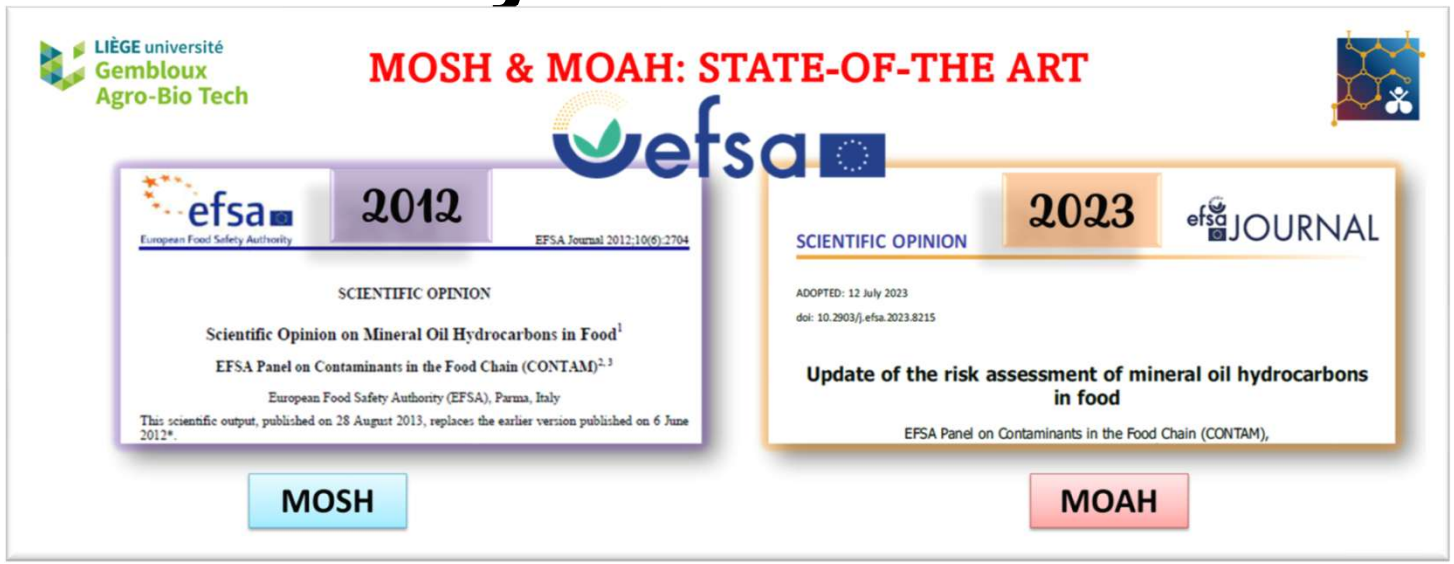
Additional data on the sub-groups

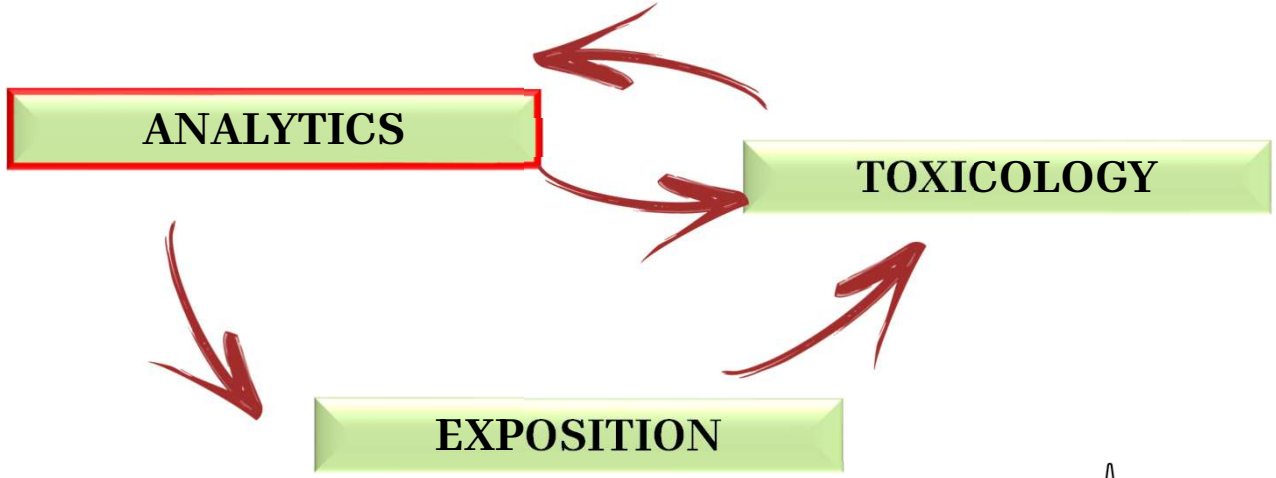
Updates – Today





Updates – Today





Updates - Analytics

Conventional Analytical method

Sample dilution (e.g., fats and oil) → **LC-GC-FID**

INTERNATIONAL STANDARD **ISO 17780**

First edition 2015-08-15

Animal and vegetable fats and oils — Determination of aliphatic hydrocarbons in vegetable oils

Corps gras d'origines animale et végétale — Détermination des hydrocarbures aliphatiques en corps gras d'origines végétale

SIST EN 16995:2017

EUROPEAN STANDARD **EN 16995**

NORME EUROPÉENNE

EUROPÄISCHE NORM June 2017

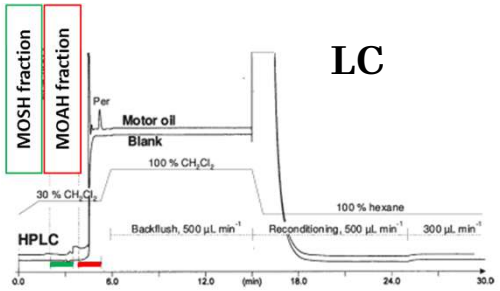
ICS 67.200.10

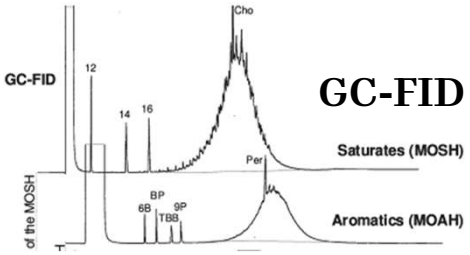
English Version

Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

Produits alimentaires - Huiles végétales et produits alimentaires à base d'huiles végétales - Dosage des hydrocarbures saturés d'huile minérale (MOSH) et des hydrocarbures aromatiques d'huile minérale (MOAH) par analyse par CLHP-CG-FID en ligne

Lebensmittel - Pflanzliche Öle und Lebensmittel auf Basis pflanzlicher Öle - Bestimmung von gesättigten Mineralöl-Kohlenwasserstoffen (MOSH) und aromatischen Mineralöl-Kohlenwasserstoffen (MOAH) mit on-line HPLC-GC-FID





Sample fractionation

(= sample preparation)

- LOQ **10 mg/kg**
- Max LC loading : **20 mg** of fat

↓

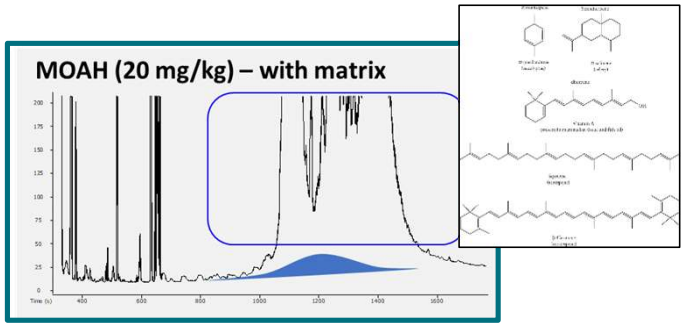
SENSITIVITY

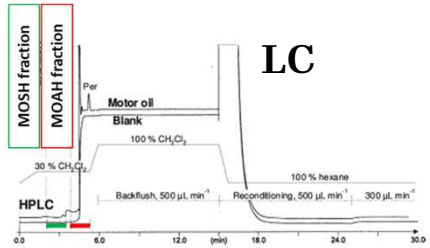
Isolation of MOSH and MOAH from part of the matrix

Updates - Analytics

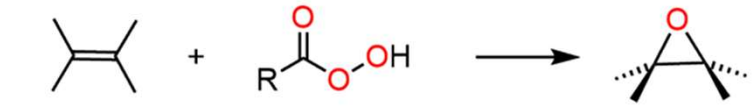
Conventional Analytical method

EXTRACTION/SAPONIFICATION → **PURIFICATION** → **LC-GC-FID**





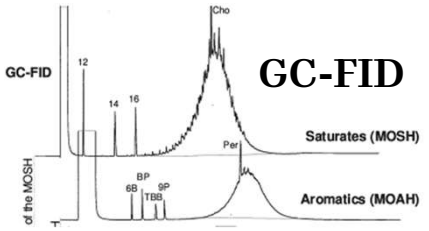
EPOXIDATION

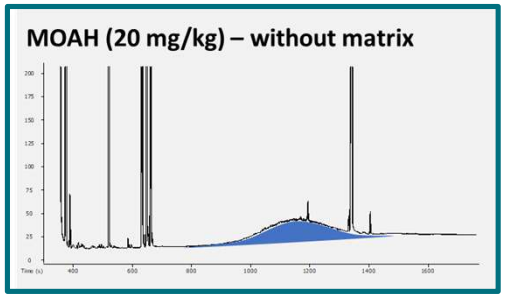


Double bond of the olefin

Peroxide

Formation of an epoxy bond ----> **Olefin becomes more polar**





↑ Sensitivity (2 mg/kg*)

*Depends on the initial content of fat

ISO 20122:2024

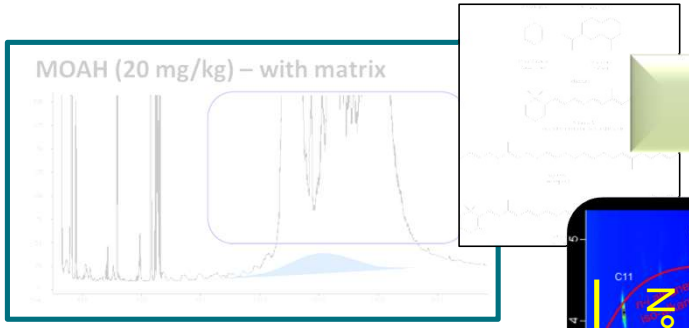
Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis — Method for low limit of quantification

Updates - Analytics

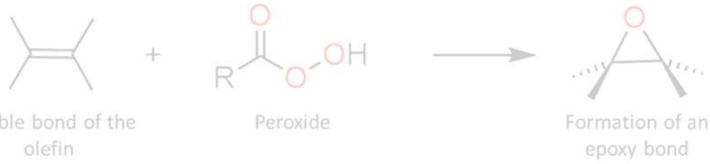
Conventional Analytical method

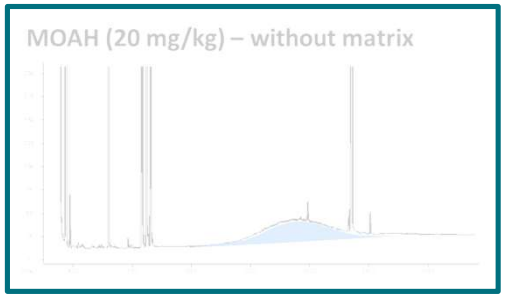
EXTRACTION/SAPONIFICATION → **PURIFICATION** → **LC-GC×GC-FID**

Microwave-assisted saponification and extraction (*MASE*) method



EPOXIDATION

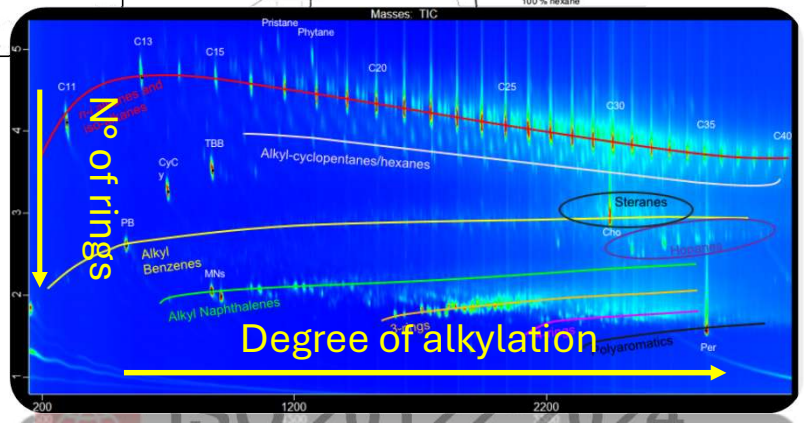




↑ Sensitivity (2 mg/kg*)

*Depends on the initial content of fat

GC×GC

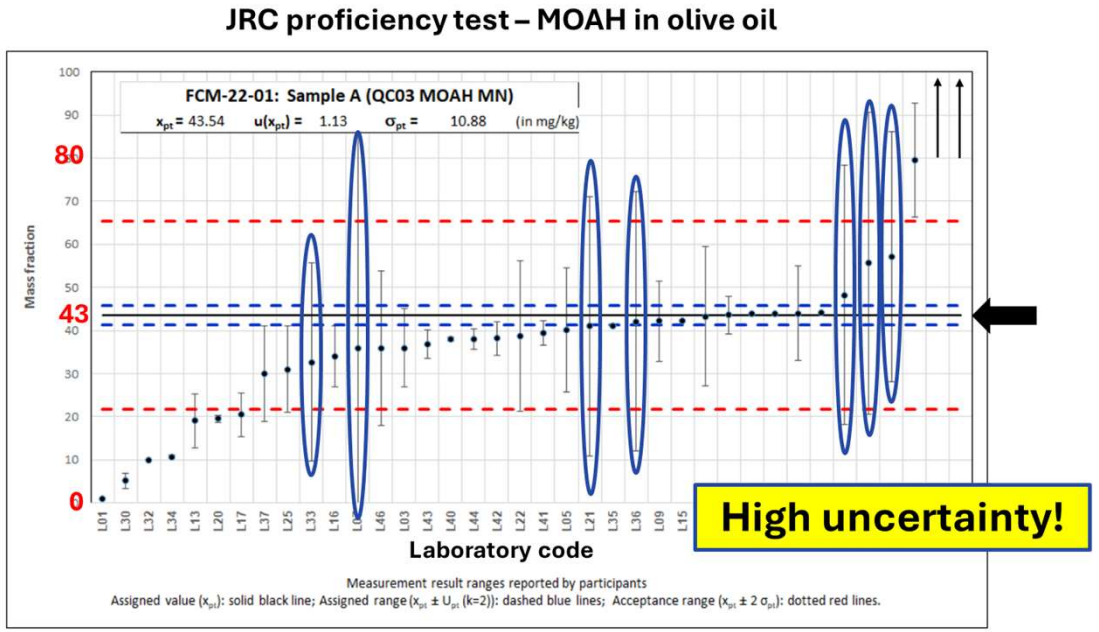


Vegetable oils — Determination of mineral oil

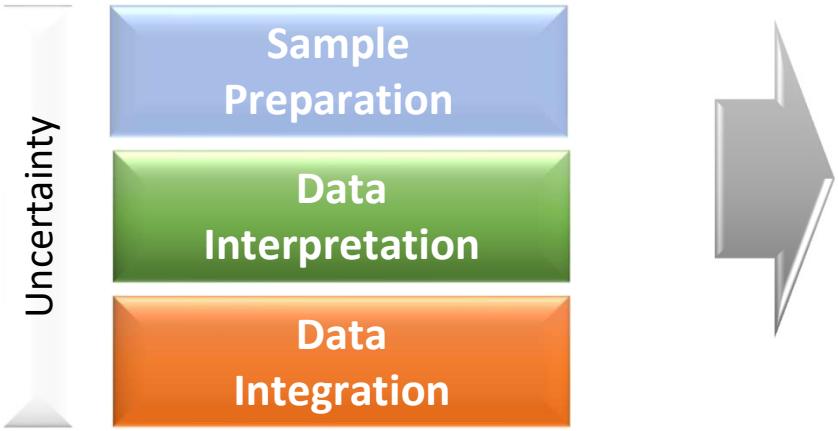
↑ Sensitivity
↑ Accuracy
↑ information

Updates - Analytics

1. **Uncertainty in measurement at low regulatory limits –** Conventional methods may not reliably quantify MOAH at 0.5–2 mg/kg, creating a risk of inconsistent compliance



Updates - Analytics



- ✓ Improved sample preparation
- ✓ Increased knowledge/understanding of the interferences
- ✓ Standardization needed
- ✓ Harmonization

EXTRACTION/SAPONIFICATION → PURIFICATION → LC-GC(×GC)-FID

Updates - Analytics

Uncertainty

Sample Preparation

Data Interpretation

Data Integration

- ✓ Improved sample preparation
- ✓ Increased knowledge/understanding of the interferences
- ✓ Standardization needed
- ✓ Harmonization


Journal of Chromatography A, 1255 (2012) 76–90

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

On-line coupled high performance liquid chromatography–gas chromatography for the analysis of contamination by mineral oil. Part 2: Migration from paperboard into dry foods: Interpretation of chromatograms

Maurus Biedermann, Koni Grob*


JRC TECHNICAL REPORT

Guidance on sampling, analysis and d reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials

JRC TECHNICAL REPORT

Mineral oil in infant formulas - guidelines for integrating chromatograms

JRC IF 2021-04: a virtual inter-laboratory comparison


JRC TECHNICAL REPORT

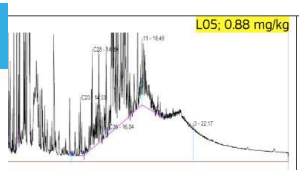
Determination of MOAH in Infant Formula

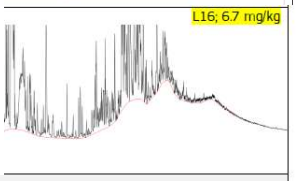
JRC IF 2020-01 – an exploratory interlaboratory comparison


JRC TECHNICAL REPORT

Mineral oil in infant formulas - guidelines for integrating chromatograms

JRC IF 2021-04: a virtual inter-laboratory comparison






JRC TECHNICAL REPORT

Determination of MOSH and MOAH in r and paper

Determination of MOSH/MOAH in Shell SN500* mineral oil

Proficiency Te JRC FC

JRC IF 2021-03 - The third interlaboratory comparison


JRC TECHNICAL REPORT

Determination of MOSH and MOAH in edible oil

Proficiency Test Report JRC FCM-22/01

JRC TECHNICAL REPORT

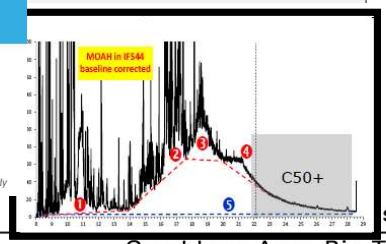
Determination of MOAH in Infant Formula

JRC IF 2020-02 - The second interlaboratory comparison


JRC TECHNICAL REPORT

Determination of MOAH in Infant formula

JRC IF 2022-05 – the ring trial validation study



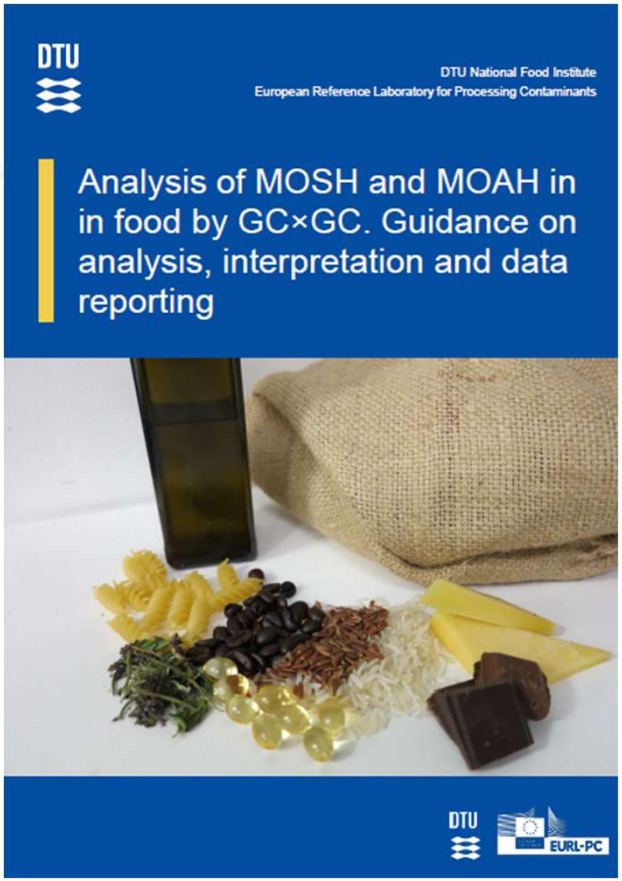
stry

Gembloux Agro-Bio Tech

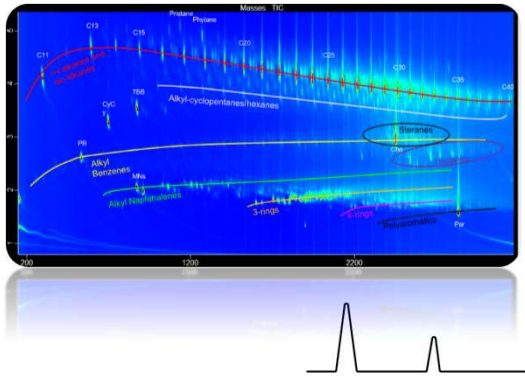
Updates - Analytics

Conventional Analytical method

EXTRACTION/SAPONIFICATION → PURIFICATION → LC-GC×GC-FID



- ✓ **Qualitative interpretation:** the EURL-PC has just published a Guidance for supporting interpretation
- ✓ **Quantitative characterization:** higher accuracy but inter-laboratory standardization still missing.



LIÈGE université
Gembloux
Agro-Bio Tech

Outline of the Analytical approach

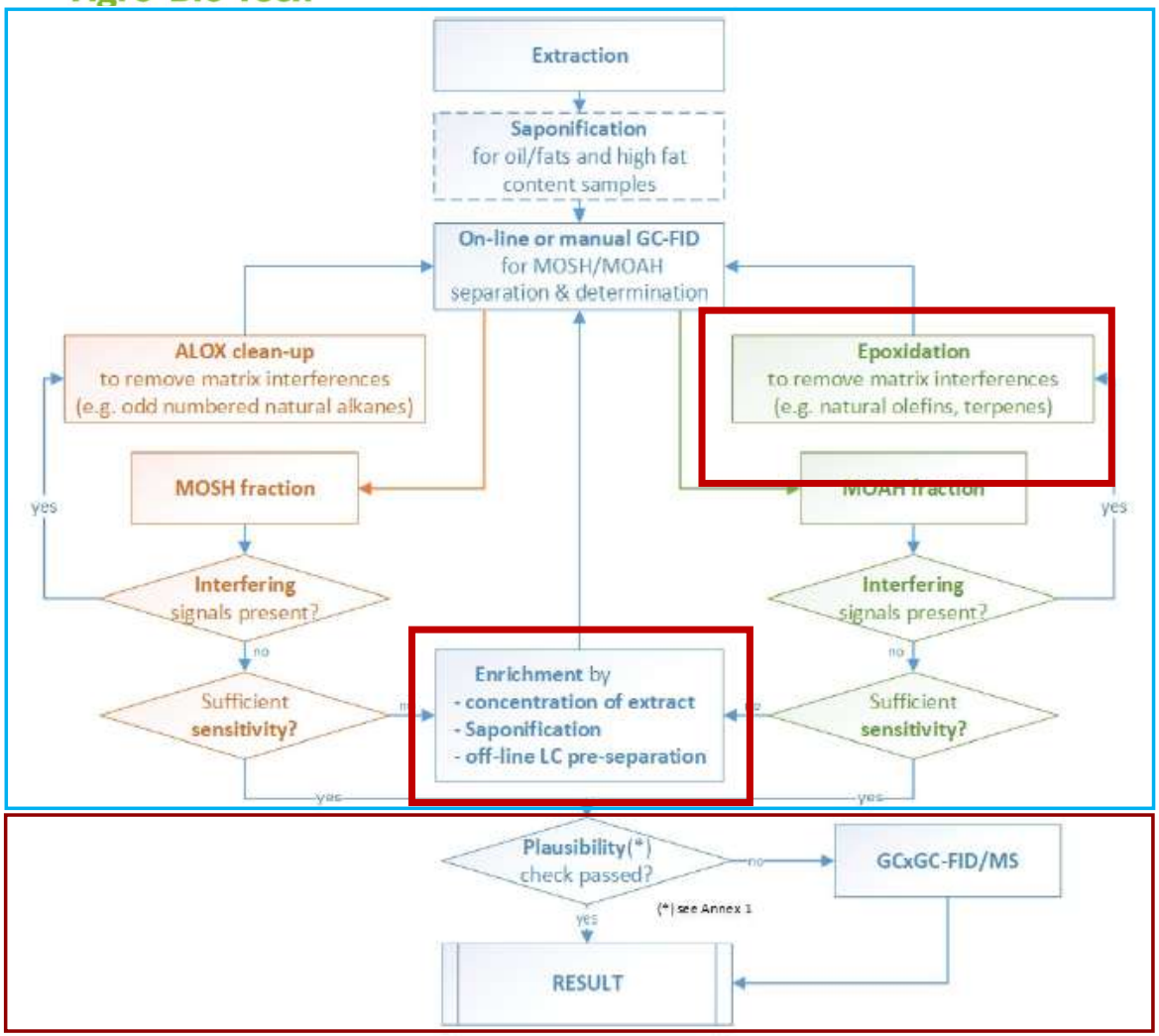


Figure 5 Decision tree on the use of auxiliary methods.

European Commission

JRC TECHNICAL REPORTS

Updates - Analytics

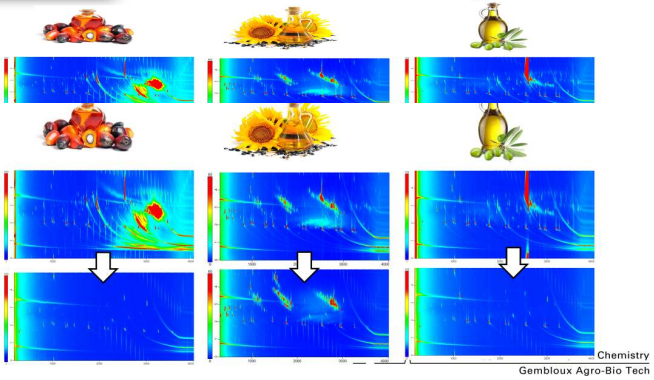
Purification of mineral oil aromatic hydrocarbons and separation based on the number of aromatic rings using a liquid chromatography silica column. An alternative to epoxidation

Purification/Alternative

LC-GC×GC-FID/MS

Very good removal of carotenoids and squalene

Other terpenoids are less well removed



MOAH Recovery LC Purification:
94% ± 2%

MOAH Recovery LC Purification:
94% ± 2%

Epoxidation

mCPBA	performic acid
82%± 10%	71 ± 16%

Across 4 matrices

Chemistry
Gembloux Agro-Bio Tech

> 20% of uncertainty!

Data Interpretation

Data Integration

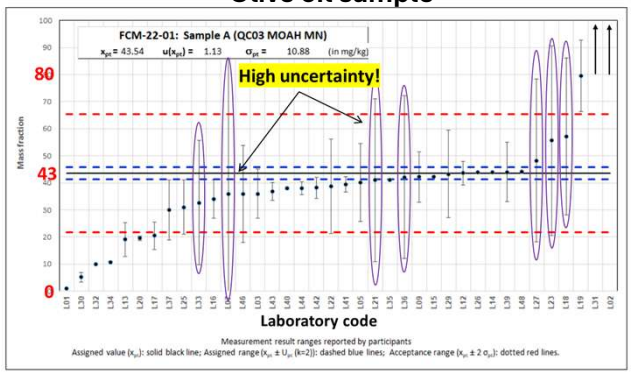
Updates - Analytics

JRC Edible oil PT

JRC TECHNICAL REPORT

Determination of MOSH and MOAH in edible oil

Olive oil sample



Not specifically discussed, but discrepancies TBB/2MN observed from multiple labs

ISO 20122:2024

In order to ensure correct signal evaluation, especially at the level of the LOQ, the offset of the baseline in the area of integration of MOSH and MOAH must not exceed one quarter of the height of the signal to be integrated (see Figure 18, Annex A). In such cases the chromatographic separation performance of the system must be improved or the LOQ must be increased.

In summary, the following conditions should be examined:

- appropriate straight baseline, blank level ≤ 1/3 of the LOQ,
- peaks are symmetrical and do not show a significant tailing,
- complete separation of solvent and C10,
- discrimination C10/C20 and C50/C20 between 80 % ≤ x ≤ 120 %,
- peak ratio between TBB and 2MN ≤1,25,
- Check the LOQ of a matrix by adding a suitable mineral oil product before sample preparation. The signal of the hump at the LOQ should have a relative standard deviation ≤ 20 % and a signal to noise ratio ≥ 10. In addition, the LOQ should be tested with a mineral oil product, whose signal width from start to end is comparable to or higher than the boiling range of the MOH in the sample to be evaluated.

peak ratio between TBB and 2MN **≤1.25**

Infant Formula

Even if statistically negligible, the mass fraction of total MOAH referred to TBB was constantly 13 % lower than the one obtained using 2MN. It is therefore recommended, in order to ensure proper comparability of results, to report the MOAH in IF using TBB as the internal standard.

Still, users should be aware that this experimental protocol does not fully remove all the interfering compounds present in some challenging matrices, which may require a cautious interpretation and integration of the recorded chromatograms. Further characterisation with two-dimensional GC-GC techniques may be required.

total MOAH referred to **TBB** was constantly **13 %** lower than the one obtained using **2MN**.

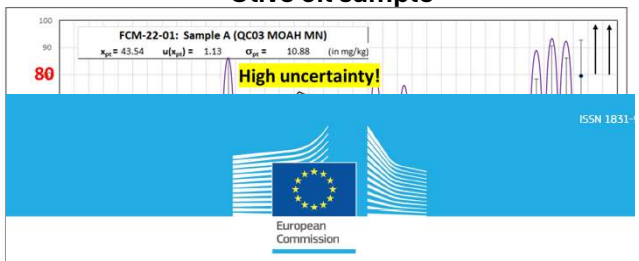
peak ratio between TBB and 2MN **= 1.00**

Updates - Analytics

Extraction/saponification

JRC Edible oil PT

Olive oil sample



Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition

2023

In the frame of Commission Recommendation (EU) 2017/84

S. Bratinova, P. Robouch, E. Hoekstra

2023

ISO 20122:2024

When complete saponification is applied and the extraction of mineral oils is performed in hexane in the absence of fat layers, the ratio TBB vs 1- or 2-MN is above 1 with a mean value around 1.15, even after a second hexane extraction of the aqueous saponification solution. This was confirmed during the two ring trial validation studies for the determination of MOSH/MOAH in edible oil and fats by CEN and in infant formula by the JRC organised in 2021/22. It might be attributed to the difference in distribution between the aqueous and hexane phases of the two IS *belonging to two different classes* of substances (2-ring non-branched and single ring branched aromatic [illegible] the quantification is performed *vs* TBB as the efficiency of the extra[illegible] hexane is lower. Consequently, when saponification is applied, the results reported *vs* TBB should be reported with uncertainty reflecting the contribution of the saponification step.

TBB/2MN =**1.15**

peak ratio between TBB and 2MN **≤1.25**

peak ratio between TBB and 2MN **= 1.00**

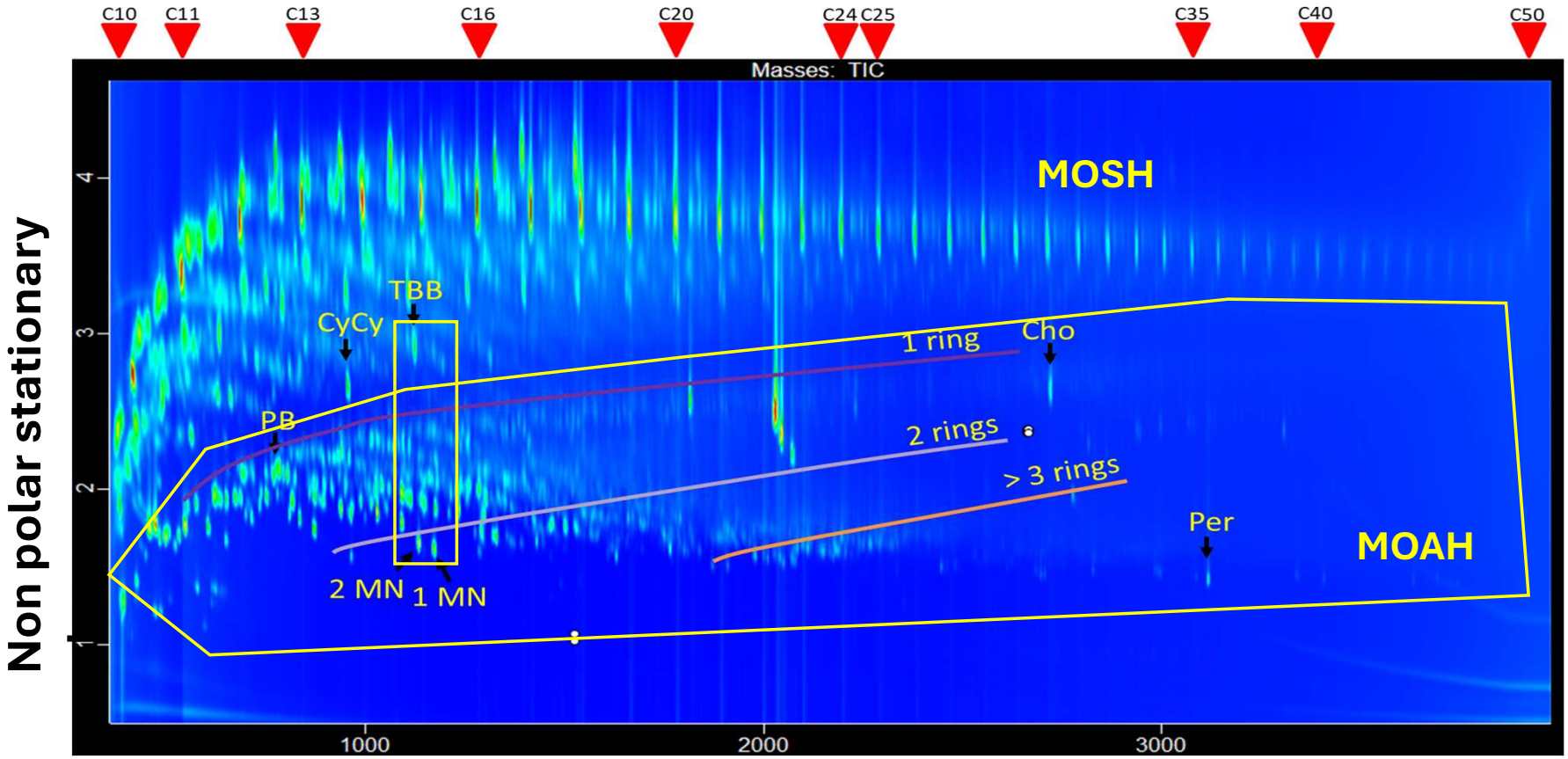
Infant Formula

total MOAH referred to **TBB** was constantly **13 %** lower than the one obtained using **2MN**.

LIÈGE université
Gembloux
Agro-Bio Tech

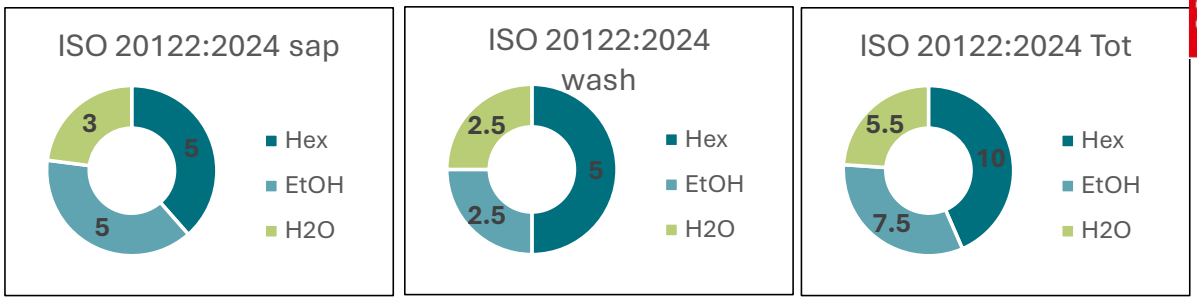
GC: a partition process

An internal standard should be representative!



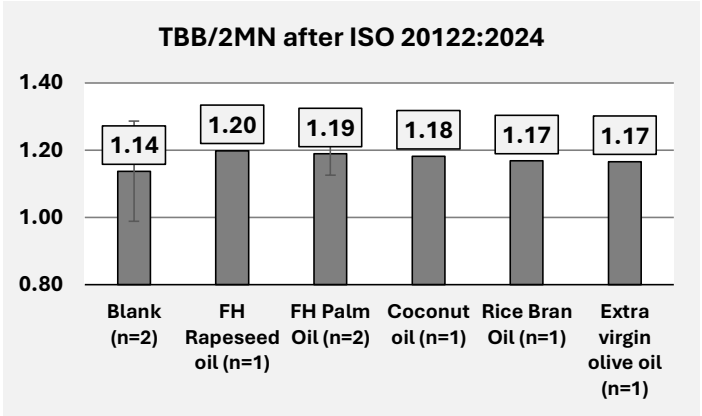
Updates - Analytics

Extraction/saponification



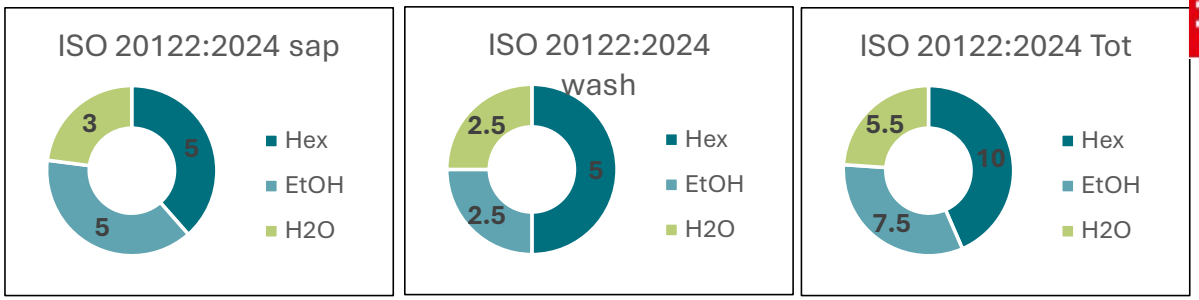
KOH: 3.4 M

ISO 20122:2024

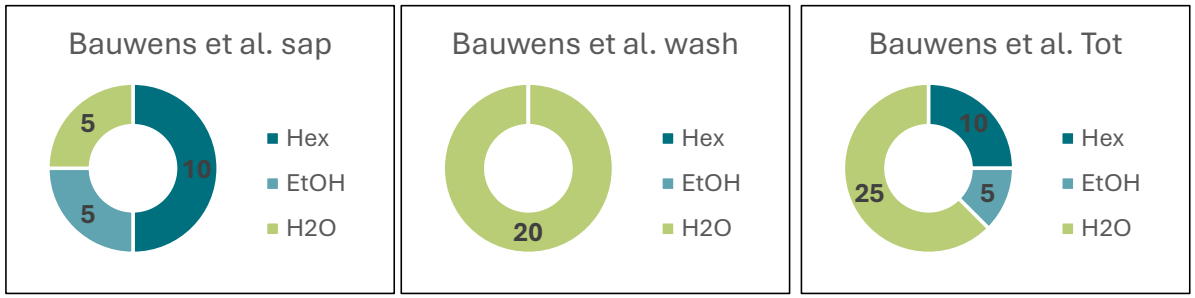


Updates - Analytics

Extraction/saponification

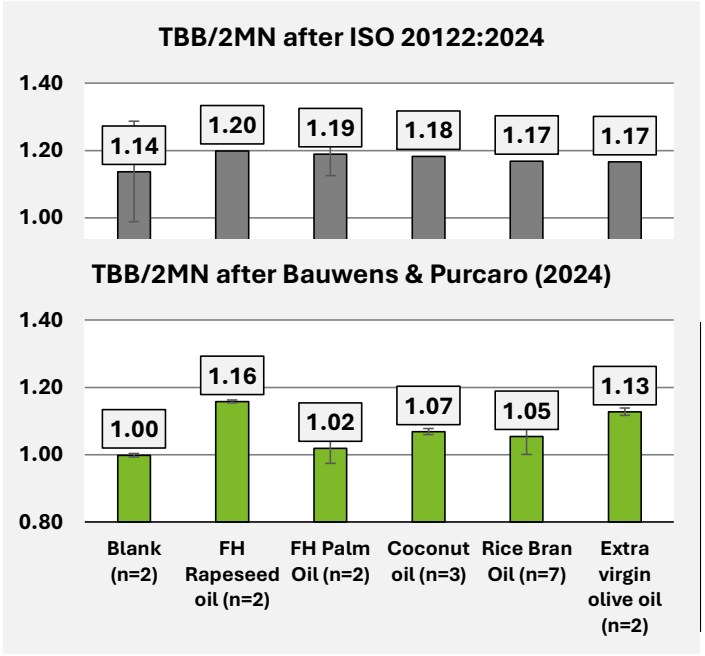


KOH: 3.4 M



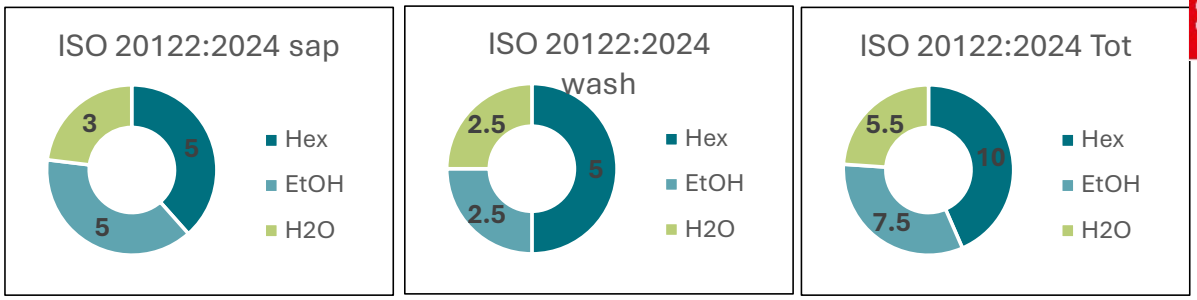
KOH: 2 M

ISO 20122:2024

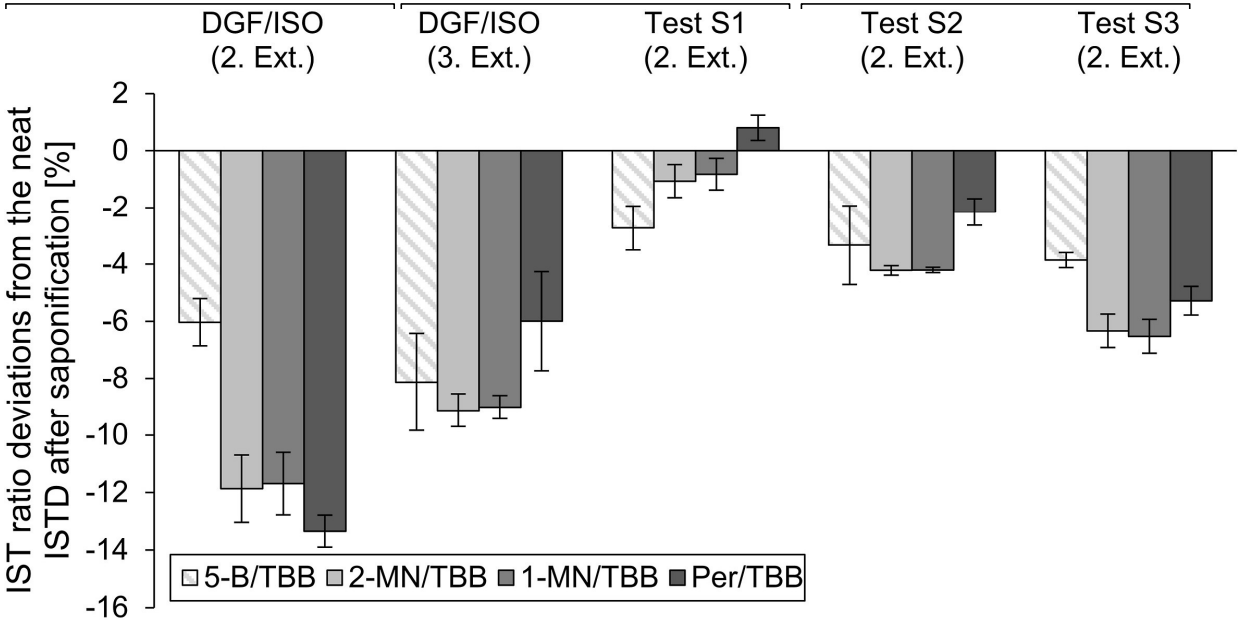


Updates - Analytics

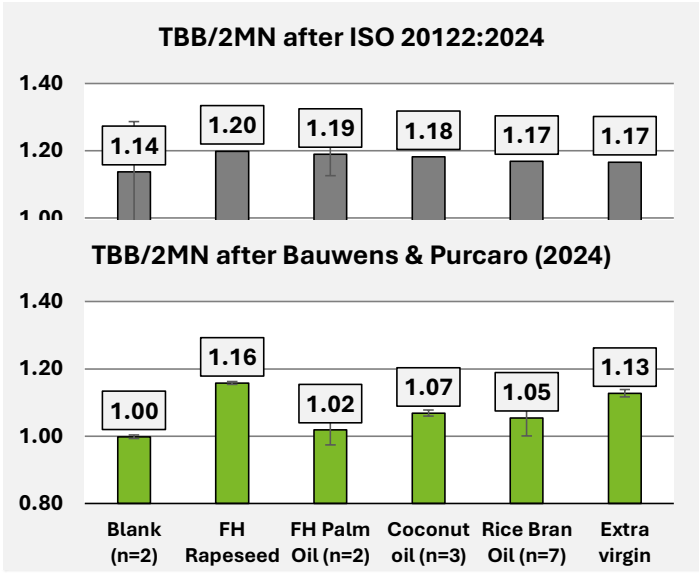
Extraction/saponification



KOH: 3.4 M



ISO 20122:2024



Analytica Chimica Acta 1376 (2025) 344591
Contents lists available at ScienceDirect
Analytica Chimica Acta
journal homepage: www.elsevier.com/locate/aca

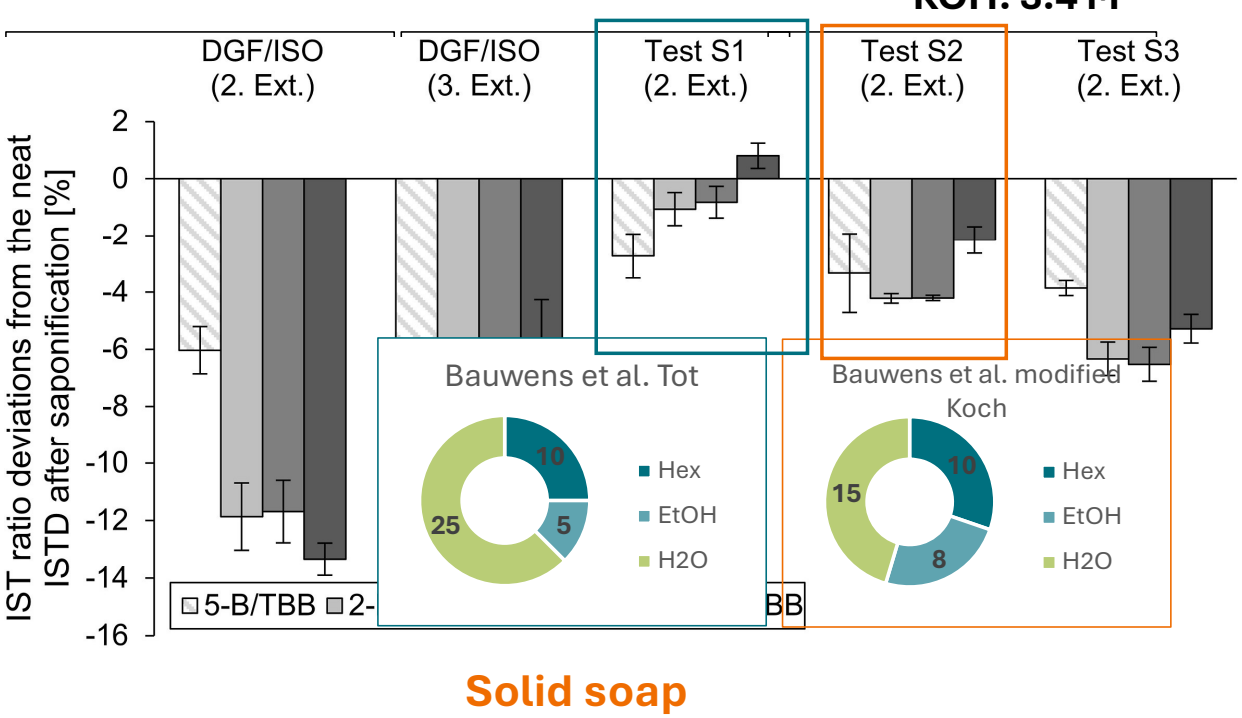
Towards a better understanding of saponification for the determination of mineral oil hydrocarbons (MOH) in edible oils and fats – Evaluation and optimization of current protocols

Maximilian Koch [a,b,*], Gerd Hamscher [b], Ludger Brühl [a,*]

[a] Department of Safety and Quality of Cereals, Max Rubner-Institut, Federal Research Institute for Nutrition and Food, Schützenberg 12, 32756, Detmold, Germany
[b] Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Heinrich-Buff-Ring 17, 35392, Giessen, Germany

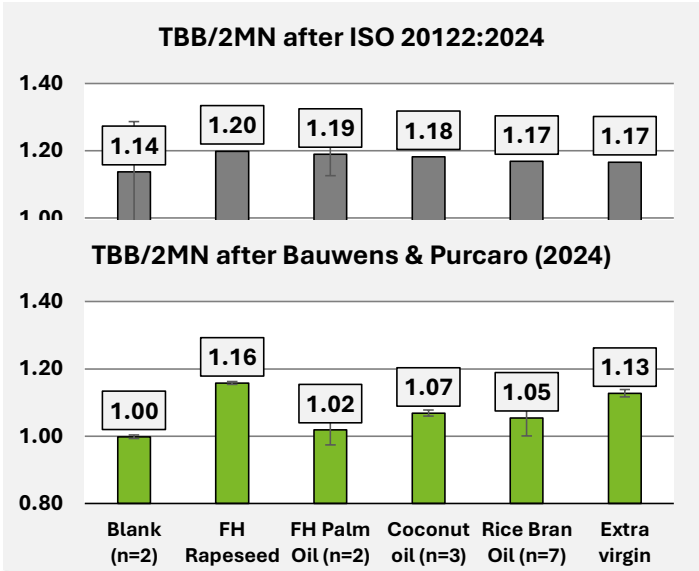
Updates - Analytics

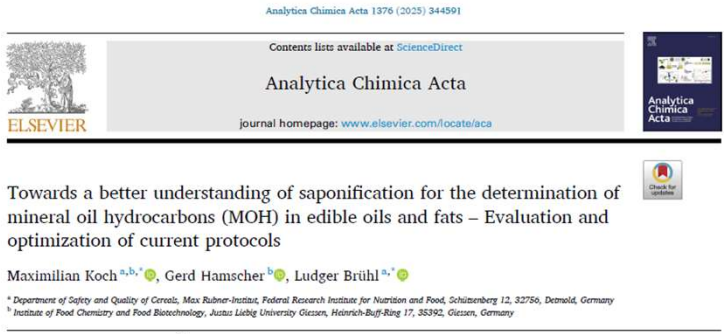
Extraction/saponification



Solid soap

ISO 20122:2024




Analytica Chimica Acta 1376 (2025) 344591
Contents lists available at ScienceDirect
Analytica Chimica Acta
journal homepage: www.elsevier.com/locate/aca
ELSEVIER

Towards a better understanding of saponification for the determination of mineral oil hydrocarbons (MOH) in edible oils and fats – Evaluation and optimization of current protocols

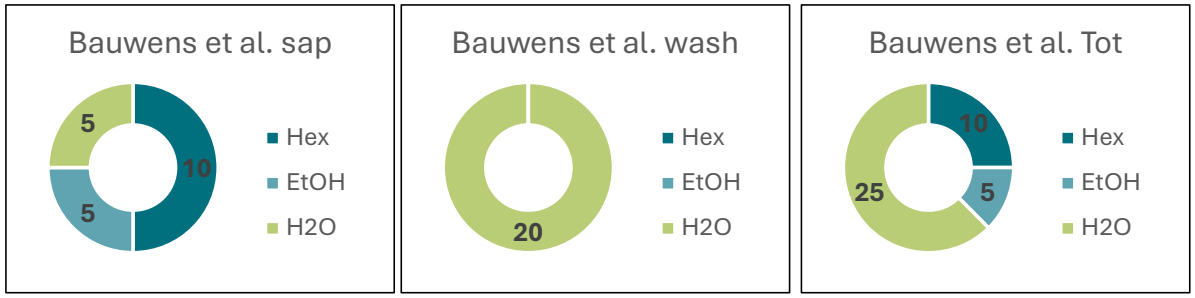
Maximilian Koch [a,b,*], Gerd Hamscher [b], Ludger Brühl [a,*]

[a] Department of Safety and Quality of Cereals, Max Rubner-Institut, Federal Research Institute for Nutrition and Food, Schützenberg 12, 32756, Detmold, Germany
[b] Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Heinrich-Buff-Ring 17, 35392, Giessen, Germany

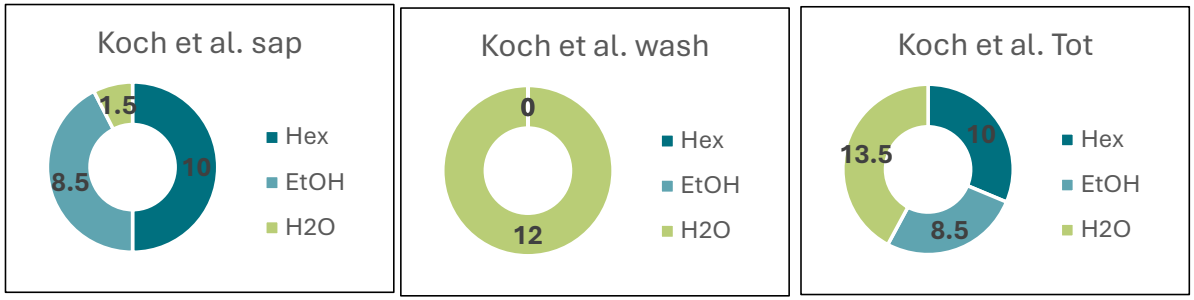
Updates - Analytics

Extraction/saponification

KOH: 3.4 M

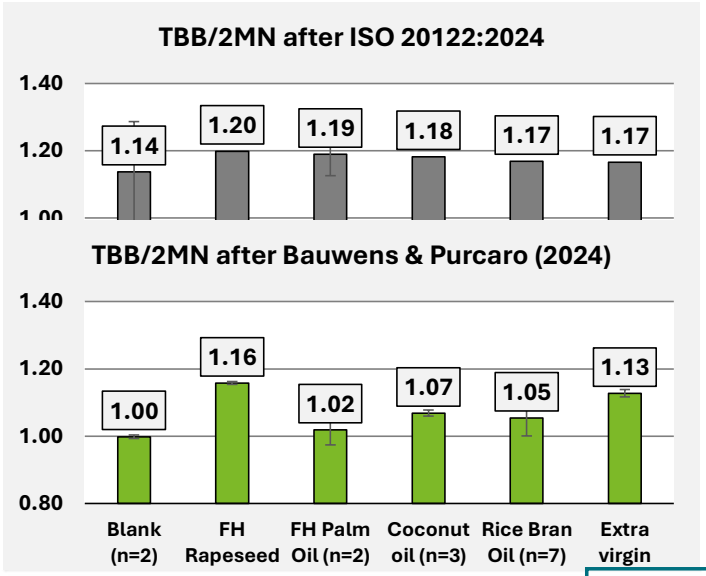


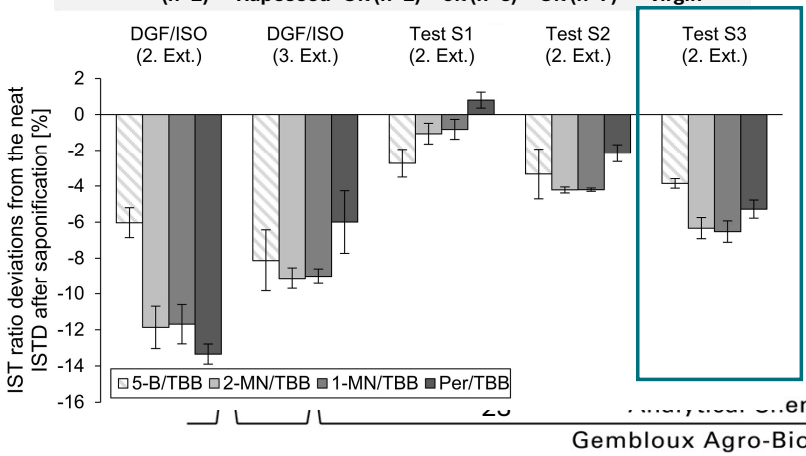
KOH: 2 M



KOH: 2.01 M

ISO 20122:2024

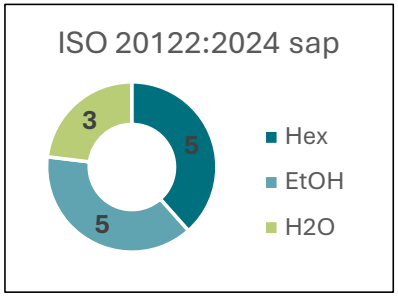


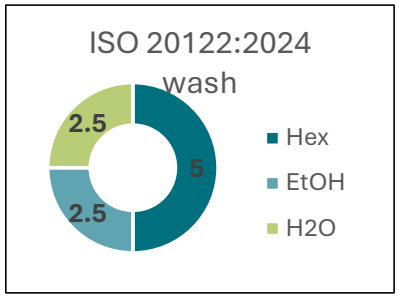


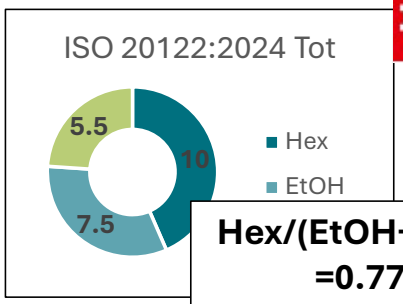
Updates - Analytics

Extraction/saponification

ISO 20122:2024

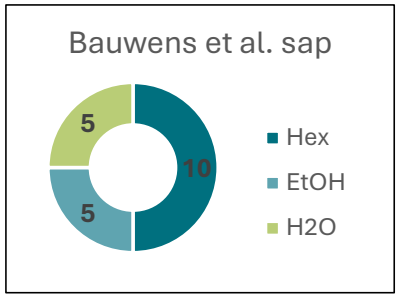


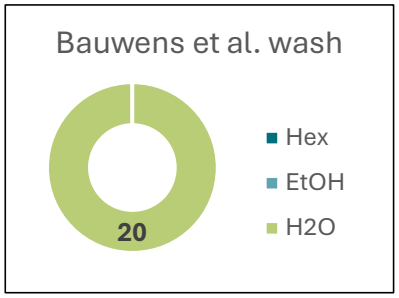


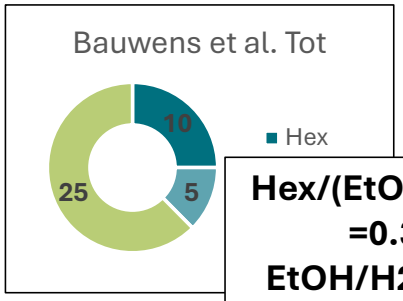


Hex/(EtOH+H2O) =0.77
EtOH/H2O =1.4

KOH

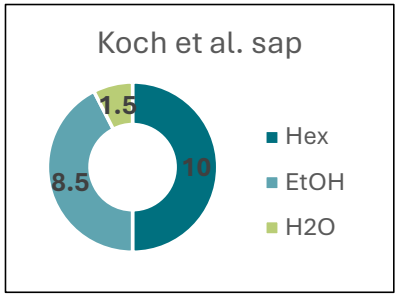


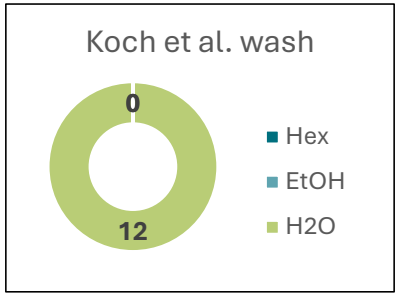


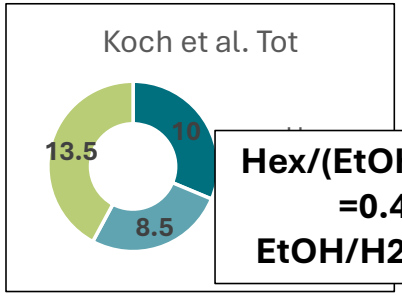


Hex/(EtOH+H2O) =0.33
EtOH/H2O =0.2

KOH: 2 M

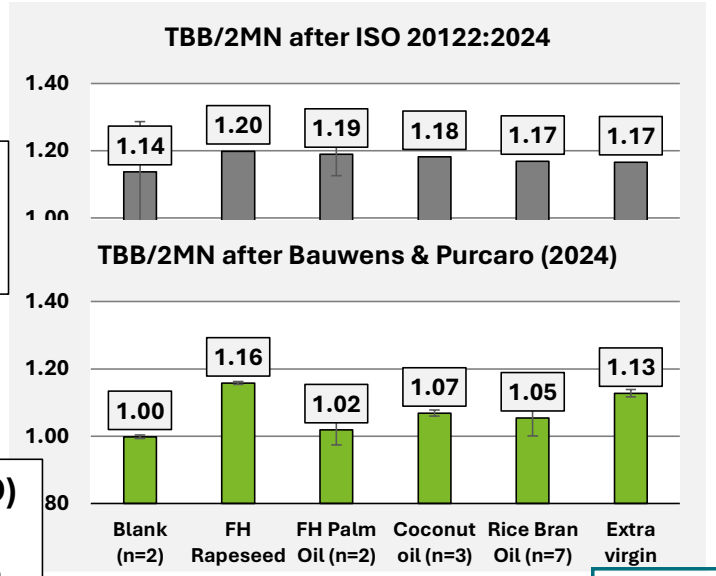


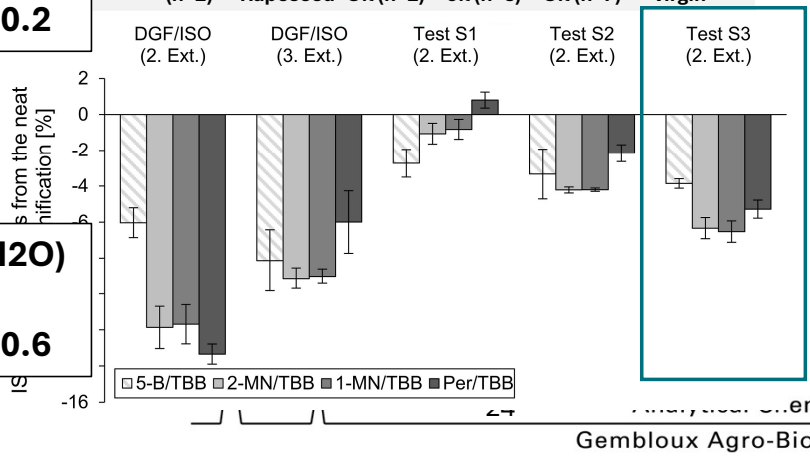




Hex/(EtOH+H2O) =0.45
EtOH/H2O =0.6

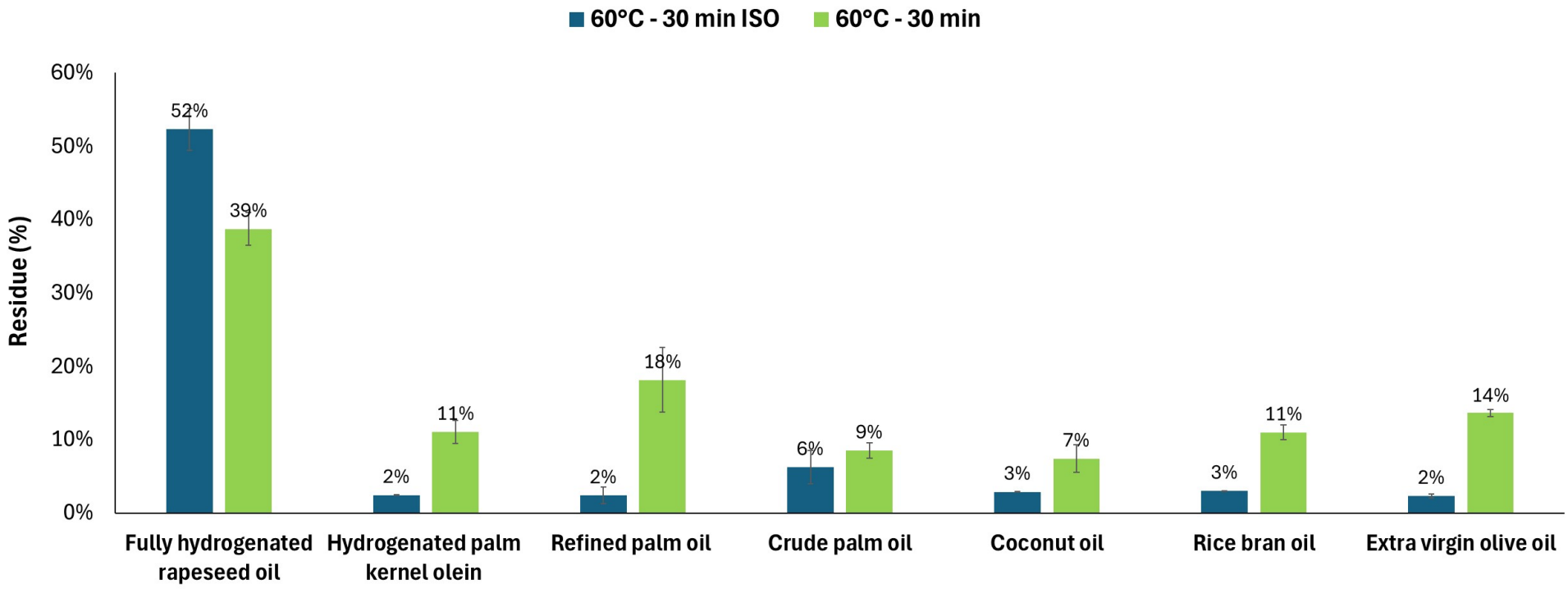
KOH: 2.01 M





Updates - Analytics

Extraction/saponification

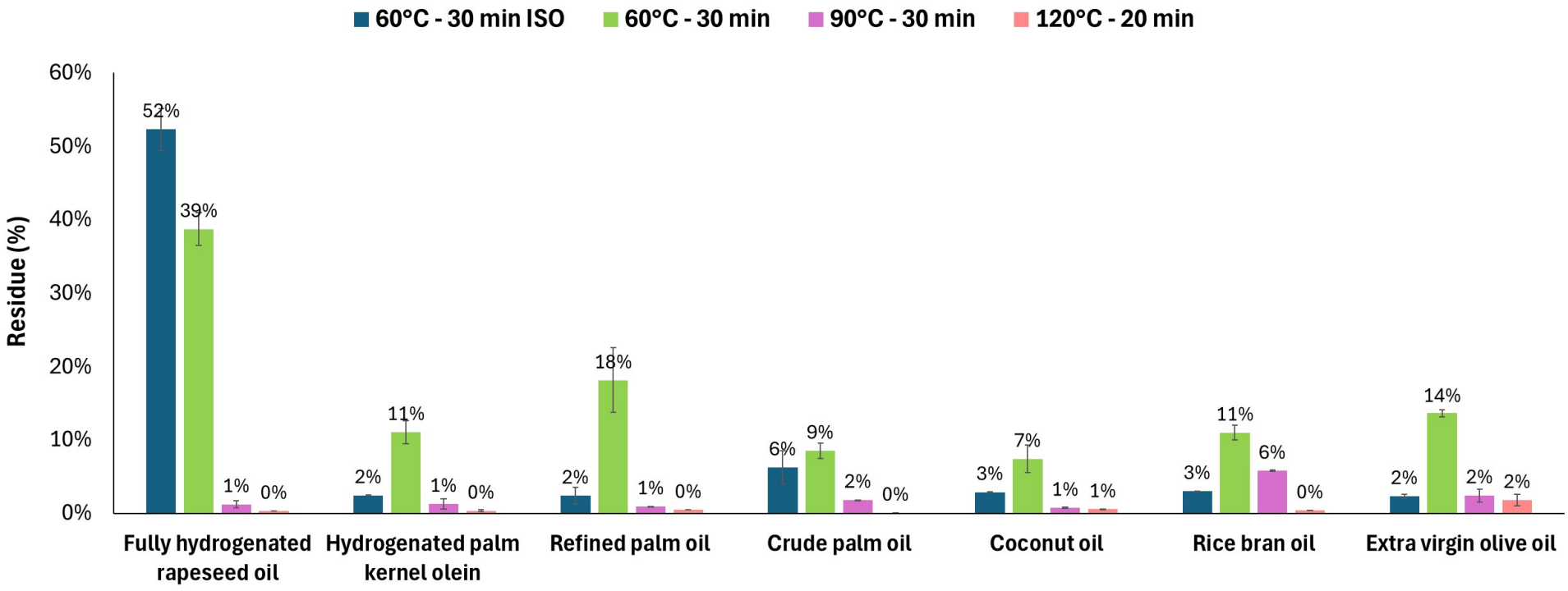


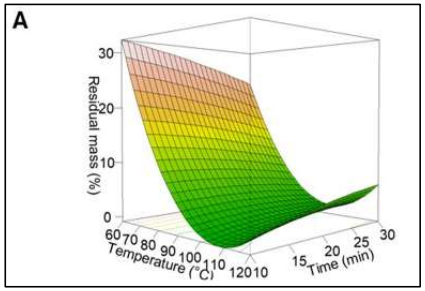
Gorska, A., Ferrara, D., Albendea, P., Cordero, C. E., Purcaro, G., Update on the microwave-assisted saponification conditions for mineral oil hydrocarbons determination in fats and oils, Analyst, 2025,150, 5190-5200

Sample Preparation

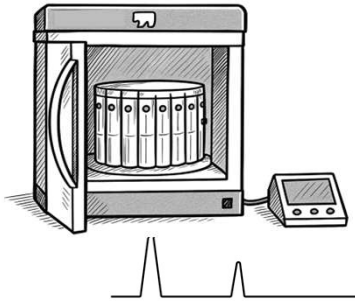
Updates - Analytics

Extraction/saponification





120 °C × 20 min



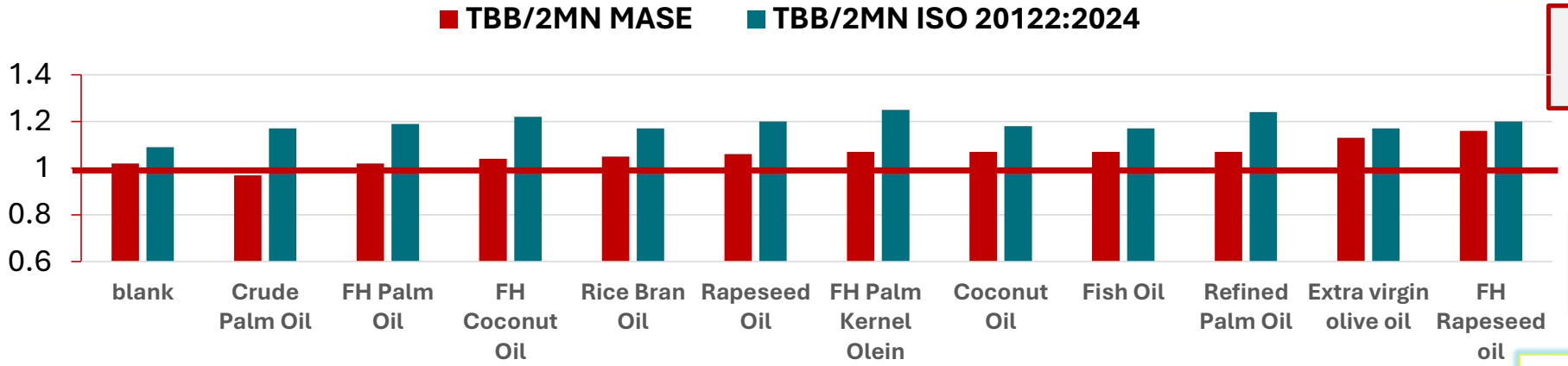
Sample Preparation

Gorska, A., Ferrara, D., Albendea, P., Cordero, C. E., Purcaro, G., Update on the microwave-assisted saponification conditions for mineral oil hydrocarbons determination in fats and oils, Analyst, 2025,150, 5190-5200

Updates - Analytics

Extraction/saponification

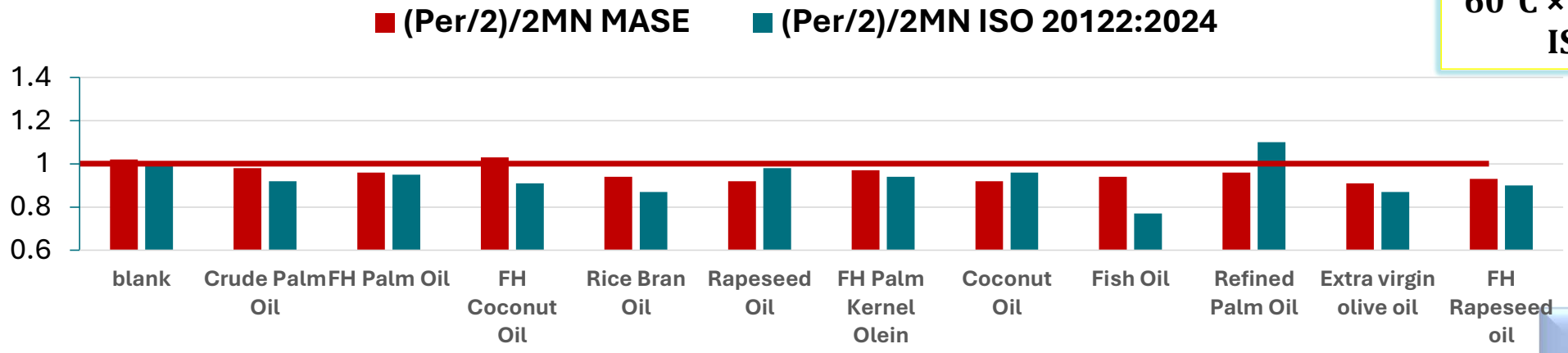
120 °C × 20 min



TBB/2MN:
1.05 ± 0.02

TBB/2MN:
1.19 ± 0.09

60°C × 30 min
ISO



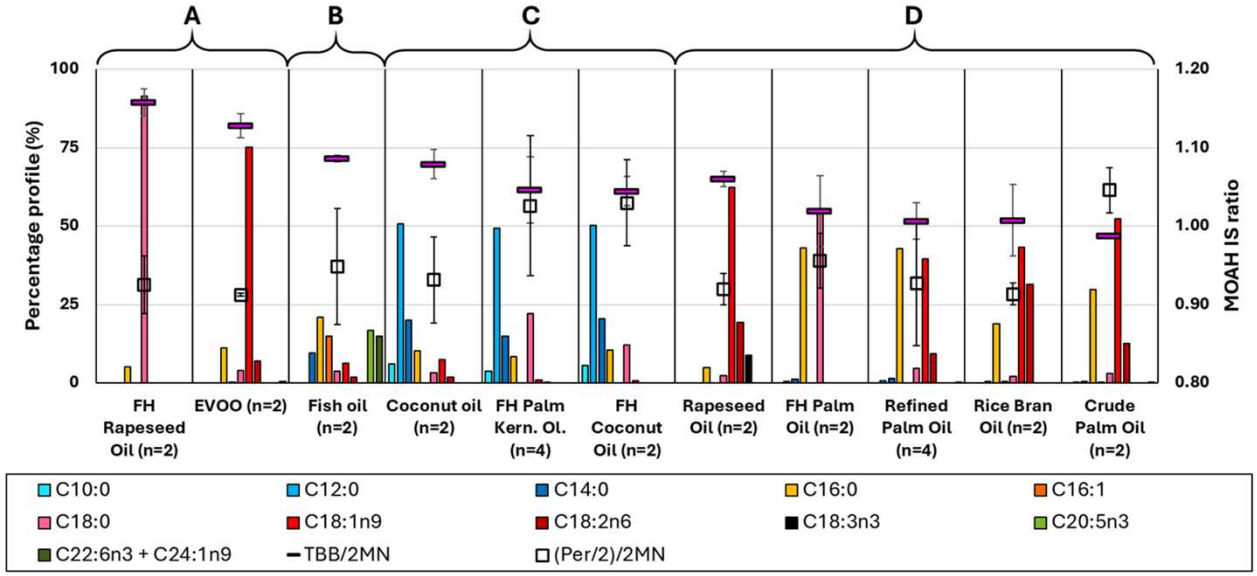
Sample Preparation

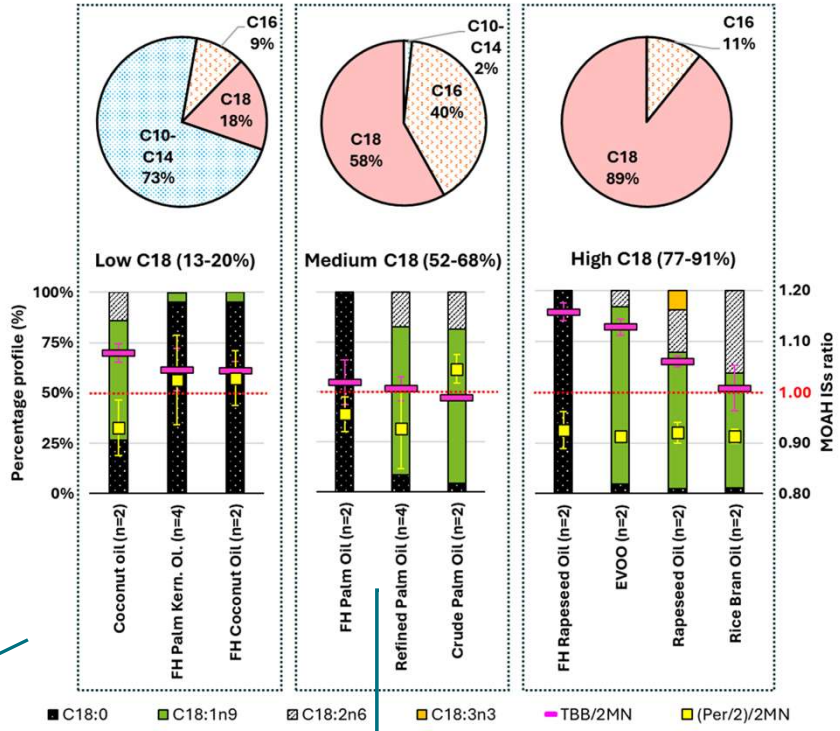
Gorska, A., Ferrara, D., Albendea, P., Cordero, C. E., Purcaro, G., Update on the microwave-assisted saponification conditions for mineral oil hydrocarbons determination in fats and oils, Analyst, 2025,150, 5190-5200

Updates - Analytics

Extraction/saponification

Correlation with FAMEs





Low C18:

- ❖ Increased saturation reduced discrimination of 2MN and Per

Medium C18:

- ❖ the best TBB/MN ratio
- ❖ Unsaturation variations → minor impact

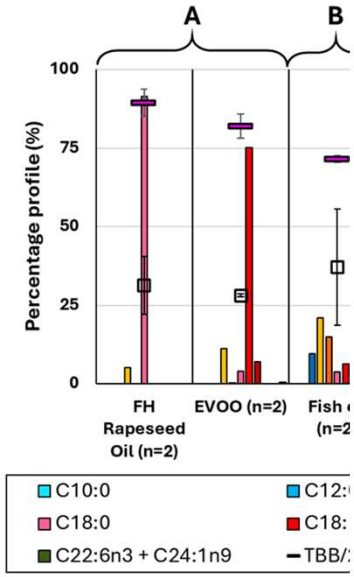
High C18:

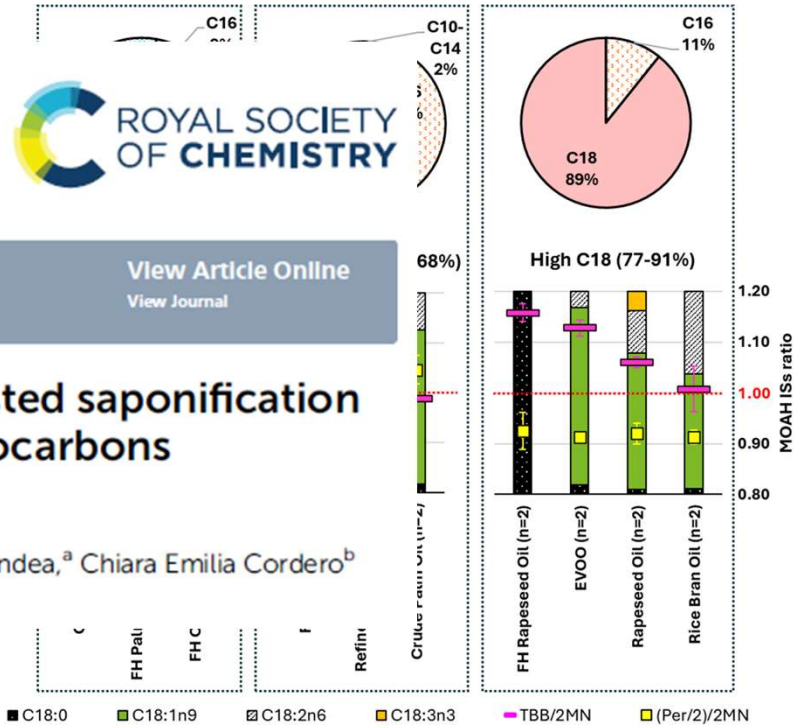
- ❖ ↓ TBB/2MN ratio with ↑ unsaturation
- ❖ Per increase proportionally to 2MN

Updates - Analytics

Extraction/saponification

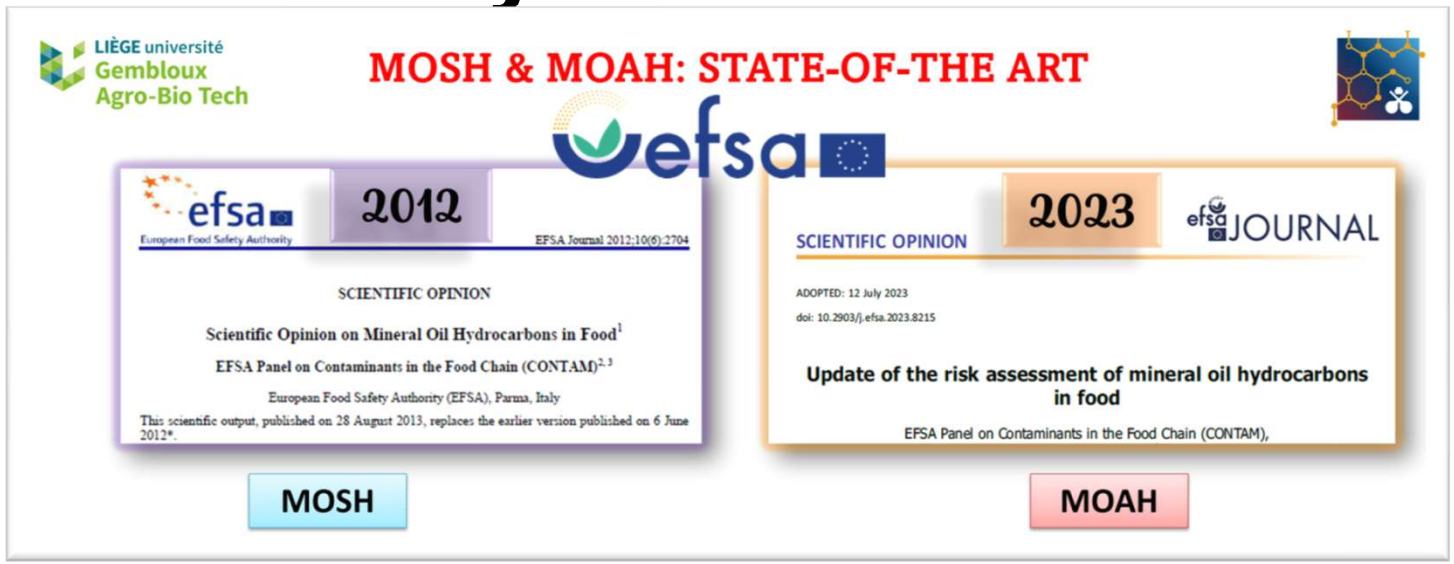
Correlation with FAMEs

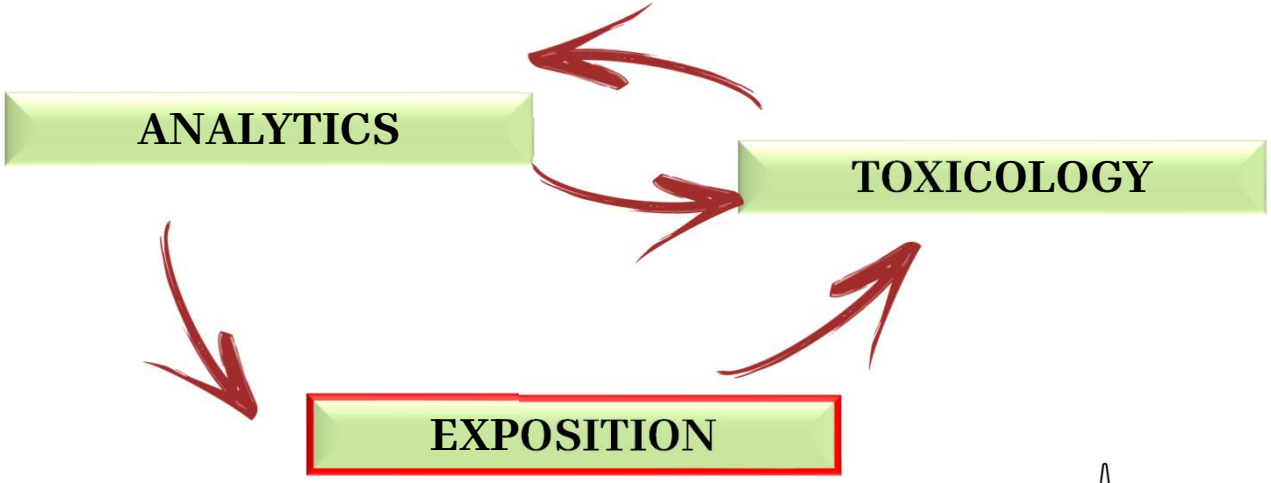




FAME profiling further revealed that fatty acid **chain length**, **degree of unsaturation**, and **profile distribution** influence soap properties, which in turn affect MOAH IS partitioning. However, this slight matrix effect does not compromise the method's applicability.

Updates – Today





Updates – EFSA

2023


SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 July 2023

doi: 10.2903/j.efsa.2023.8215

Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

For **MOSH** :

- **Improvement of analytical methodology for better characterisation of MOSH** .....
- Better investigation of the **sources of the hydrocarbons** in food, ...
- Data are needed on ... **accumulation potential**.
- Investigation is recommended on the **structural features of MOSH** that ... result **in accumulation**.
- Additional toxicity data are needed ... in particular on bioaccumulating MOSH following their characterisation.
- More data on human MOSH tissue concentrations.
- .... needs further investigation with regard to occurrence of **MOSH and potential compositional modification and bioaccumulation**.
- ...

For **MOAH**:

- **More selective and sensitive analytical method should be implemented**.
- More data on **MOAH composition by aromatic ring number** in food are needed, in particular with respect to the levels of ≥ 3 ring MOAH.
- Sources of food contamination should be investigated when MOAH are detected.
- More data are needed on the **influence of ring alkylation on genotoxicity and carcinogenic** potency of ≥ 3 ring MOAH.
- **Oral toxicity data** in particular of **1–2 ring MOAH**.
- Technical specifications of white mineral oils and waxes used as **food additives and food packaging materials** should be updated, with **detailed information about the MOAH content and composition**.

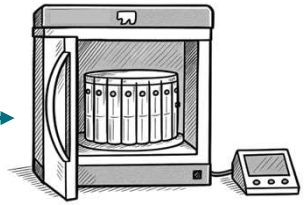
What about bioaccumulation from animal to human?

Experimental design

Preliminary tests:

Homogenized meat samples

Subjected to



Microwave assisted saponification and extraction (*MASE*) method

Using

Optimization of sample preparation

Saturated KOH in MeOH [1]
Abbreviated as Sap MeOH

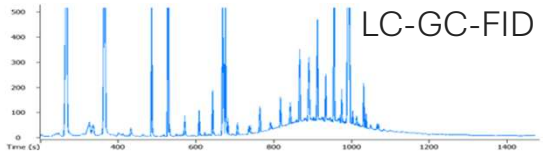
or

2M KOH in EtOH:H_2O (1:1,v:v)
(adapted from [2])
Abbreviated as Sap EtOH: H_2O

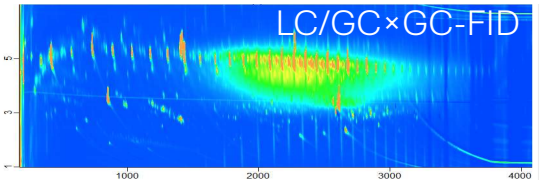
Evaluated in terms of:
ISs distribution (n =3 per type of meat)
Focused on TBB/2-MN ratio, which should be 1
&
MOSH and MOAH recovery
(n =4 per level of MOH studied)

Validation of LC/GC× GC-FID

Analyzed by



&



Comparison between the results obtained by LC-GC-FID (*abbreviated as 1D*) and LC-GC×GC-FID (*abbreviated as 2D*)

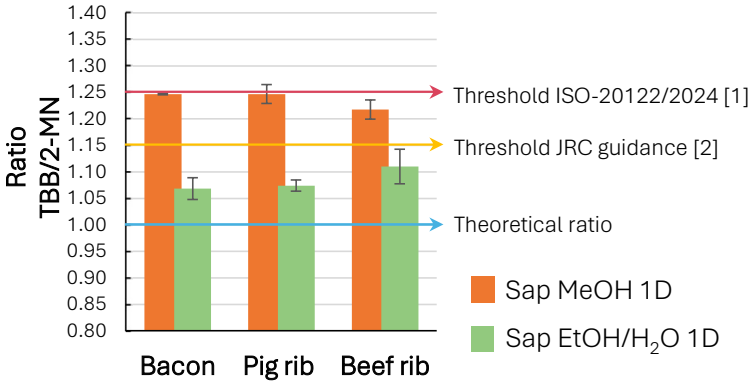
Samples: 30 samples of unprocessed meat (bacon, pig rib, beef rib, chicken and turkey) purchased from different local supermarkets

[1] P. Albendea, et al., Animals 14 (2024) 1450
[2] G. Bauwens, G. Purcaro Anal. Chim. Acta 1312 (2024) 34278

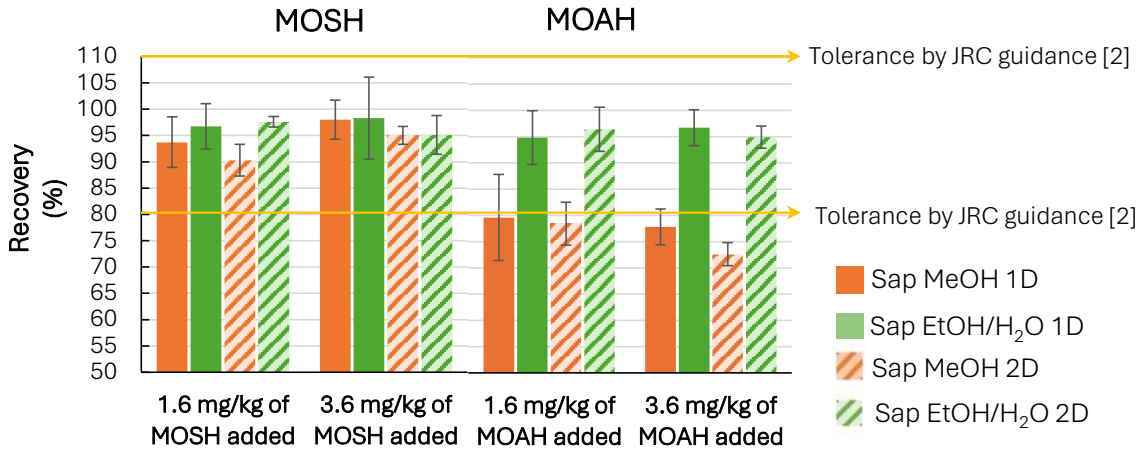
Results and discussion

Comparison between MASE methods

ISs distribution



MOH recovery *(beef rib sample spiked with two different MOH levels)*

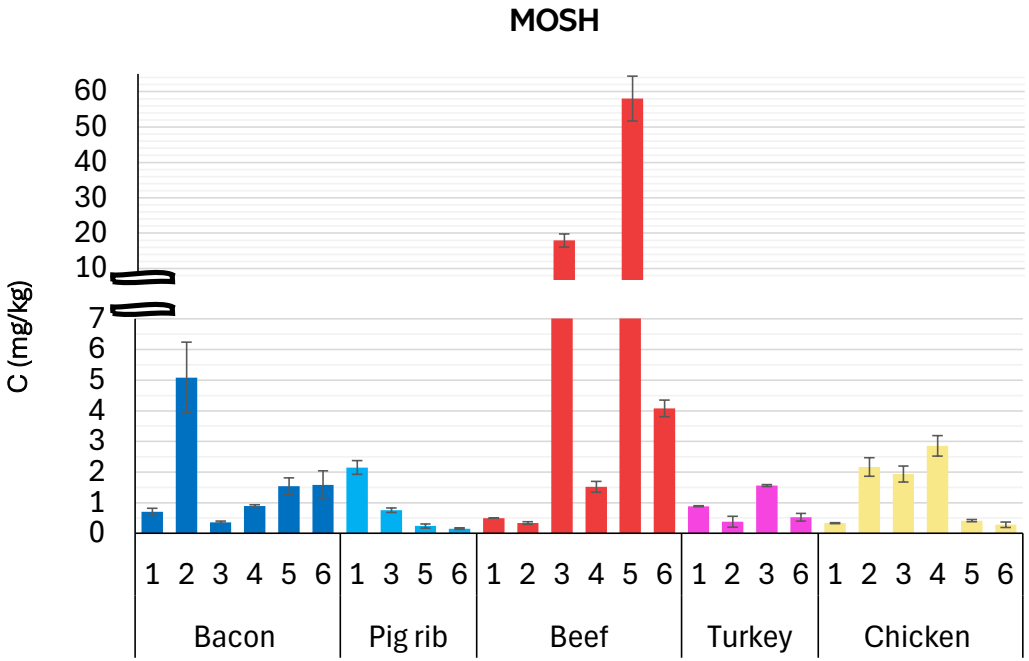


- ✓ 2M KOH in EtOH/H_2O (1:1,v:v) MASE method was selected for the analysis of MOH in meat samples
- ✓ Similar results were obtained with LC/GC×GC-FID and LC-GC-FID

[1] ISO/DIS 20122. https://www.iso.org/obp/ui/en/#iso:std:iso:20122:dis:ed-1:v1:en
[2] S. Bratinova, et al, 2nd ed., Publications Office of the European Union, Luxembourg, 2023

Results and discussion

Total MOSH content in meat

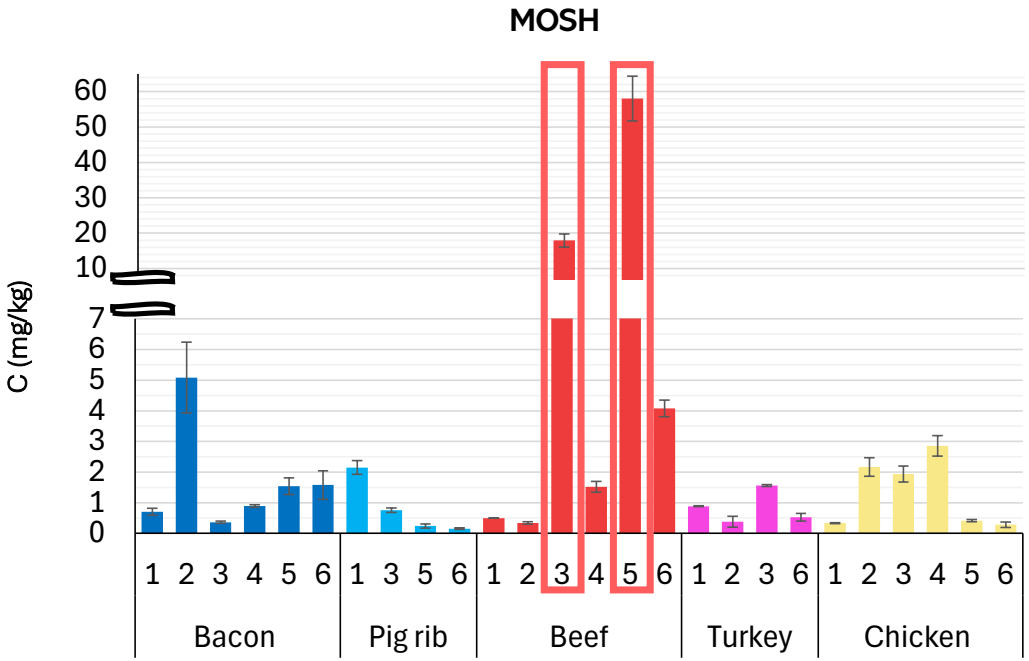


Variable MOSH levels in meat samples

- 4 samples < LOQ (2 pig rib and 2 turkey)
- 2 samples highly contaminated (beef rib)

Results and discussion

Total MOSH content in meat

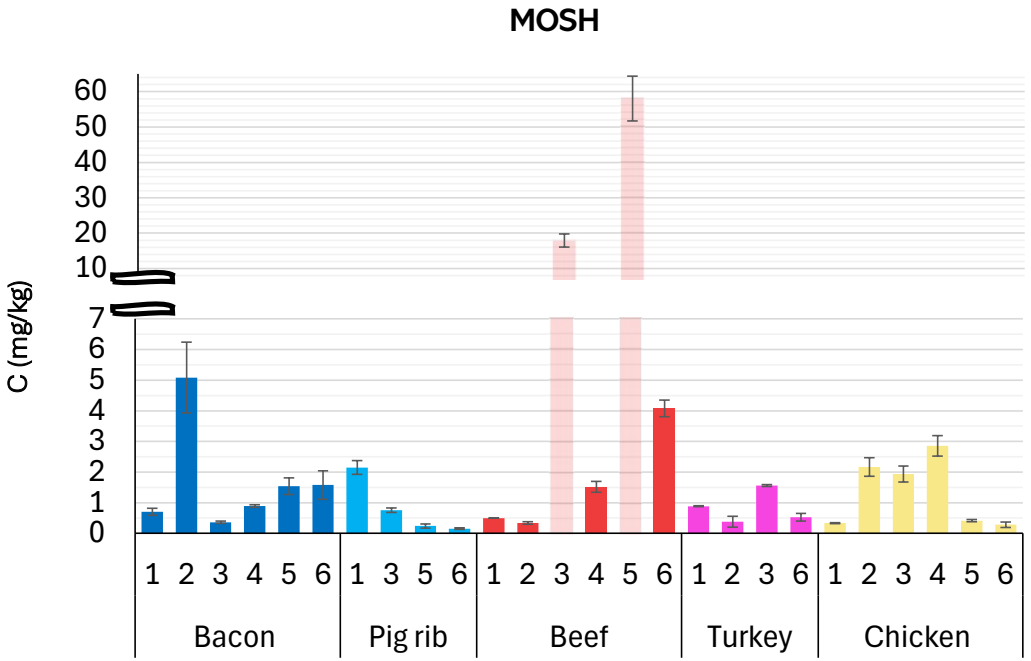


Variable MOSH levels in meat samples

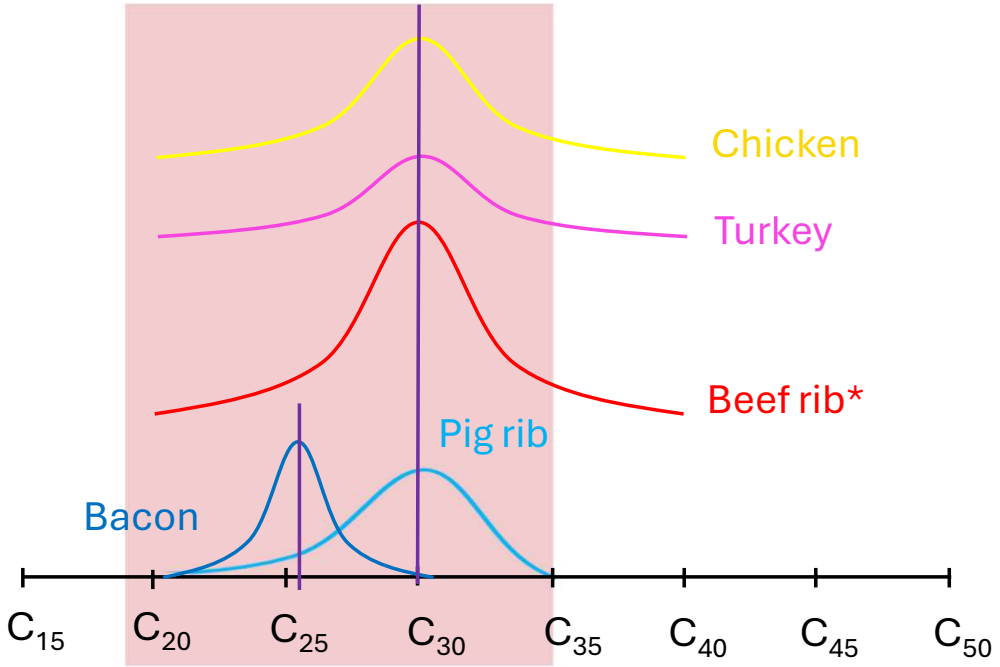
- 4 samples < LOQ (2 pig rib and 2 turkey)
- 2 samples highly contaminated (beef rib)

Results and discussion

MOSH profile: Carbon chain length



Schematic representation of the MOSH profile



Carbon chain length

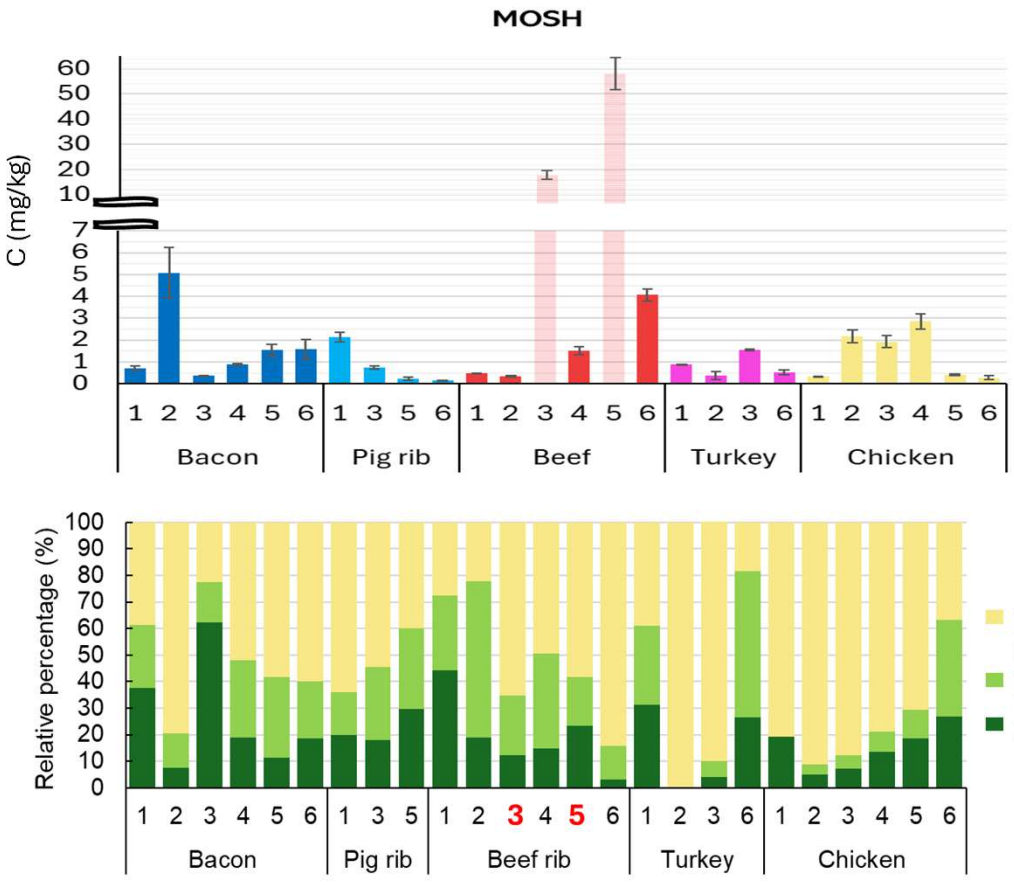
Range that can be accumulated by animals

**Beef rib: samples 3 and 5 are not considered in this representation*

EFSA Journal 2023,21 8215
K. Grob 2018, J. Agric. Food Chem., 66, 6968
Cravedi et al. 2016, J. EFSA 2017, 1090
P. Albendea, et al. 2024, Animals 14, 1450

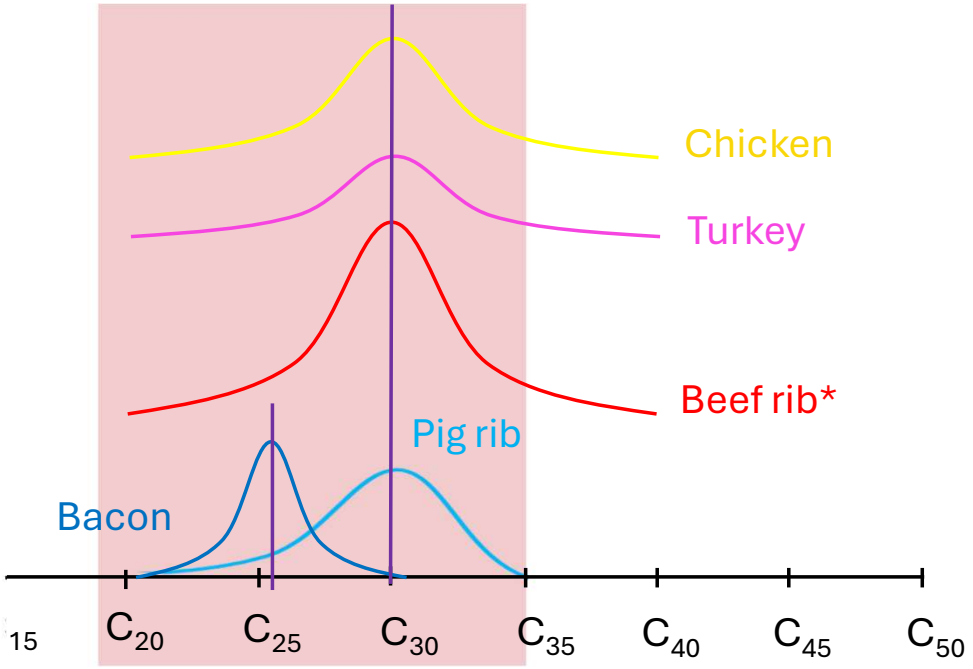
Results and discussion

MOSH profile: Carbon chain length



No relationship between the subclasses and the type of meat evaluated

Schematic representation of the MOSH profile

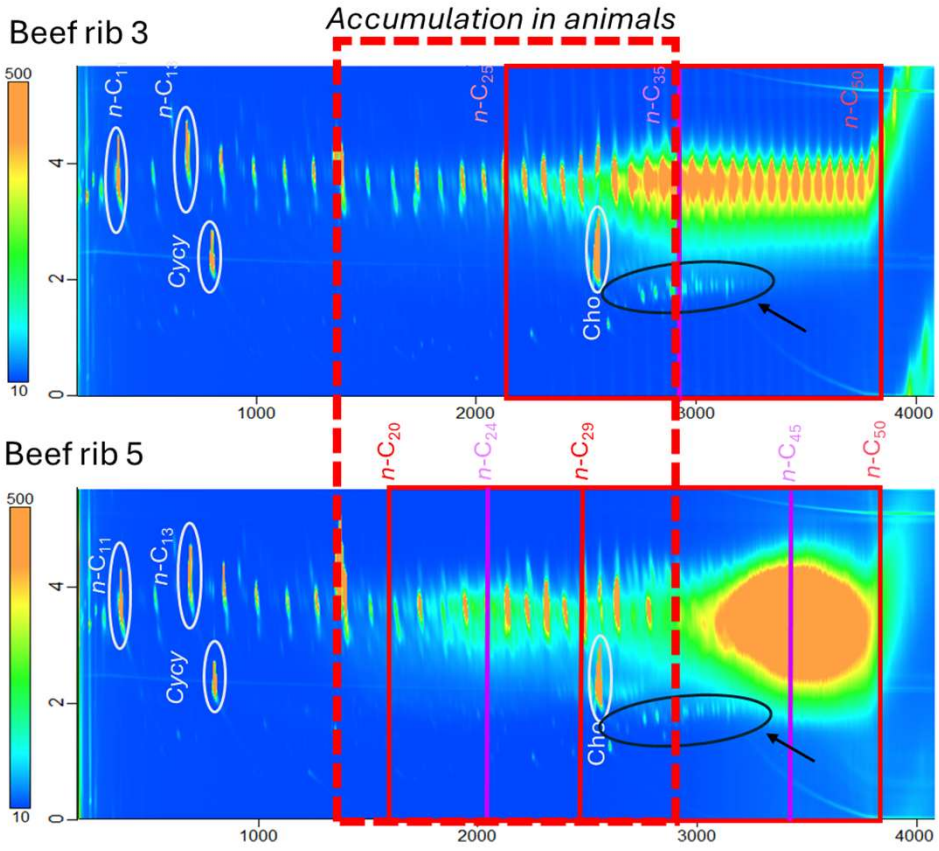


Carbon chain length

Range that can be accumulated by animals

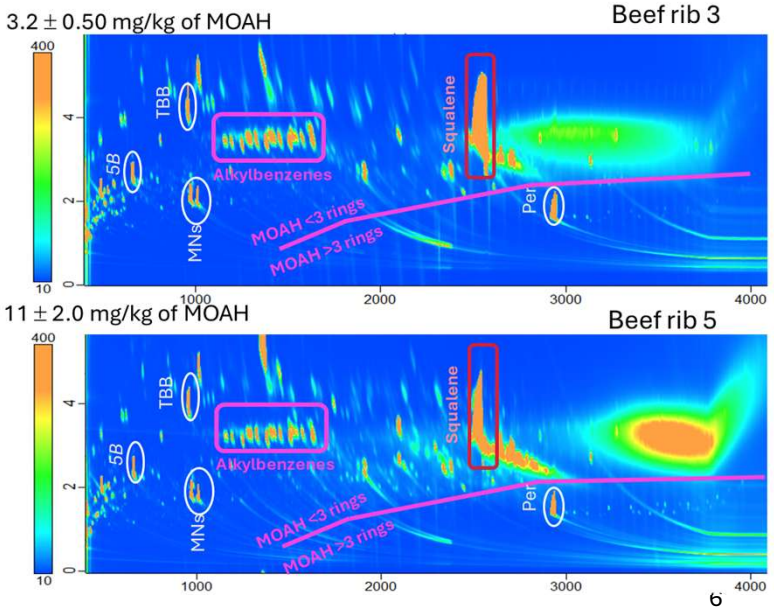
**Beef rib: samples 3 and 5 are not considered in this representation*

Results and discussion



Hopanes → No evidence of being retain in human or animal tissues

The contamination occurred entirely (Beef rib 3) or partially (Beef rib 5) after the slaughter of the animals



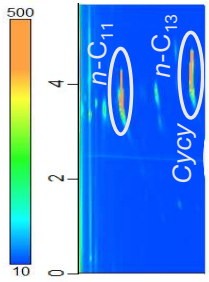
Results and discussion

Packaging of beef rib 3 and 5

MOSH

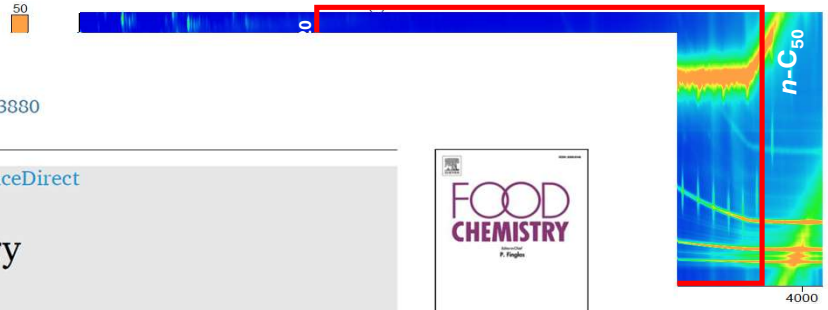
Beef rib 3

Beef rib 5

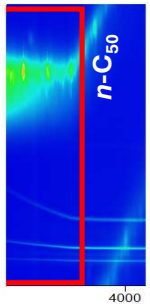


Packaging beef rib 3

79 mg/kg of MOSH



445 mg/kg of MOSH



Food Chemistry 480 (2025) 143880

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Study on the content and profile of MOSH and MOAH in unprocessed meat by LC/GC × GC-FID/MS

Paula Albendea, Giorgia Purcaro *

Analytical Chemistry Lab, Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium

- ✓ Low similarities between the profiles found in the meat and in the correspondent packaging
- ✓ MOAH were present in the meat but virtually absent in the packaging

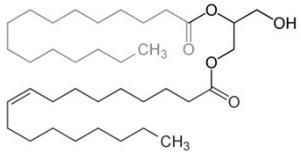
- ✓ Contamination of beef rib 3 and 5 during the manipulation of the meat (e.g. when cutting it into the commercial portions)
- ✓ The presence of alkylbenzenes could be due to rests of sulfonated surfactants on cutting tools

Updates – Occurrence – Additives

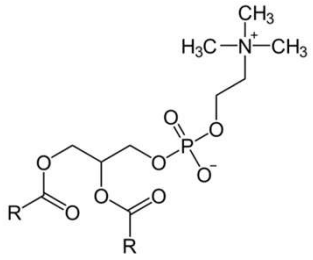
- Are substance added to food for different reasons, such as to help preserve them, improve taste, texture, or appearance
- Can have animal and vegetal origin
- Classified in many class

Safe approved by Europe Union

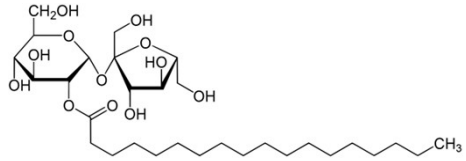
→ E300 → Function of additives



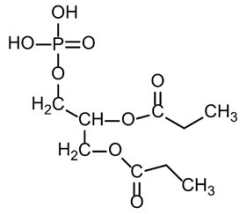
E471
Mono- and diaciglycerides of FA



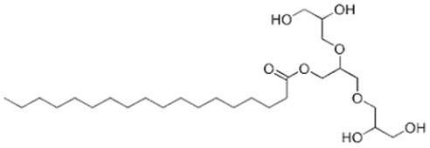
E322
Lecithine



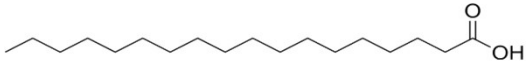
E473
Sucrose esters of FA



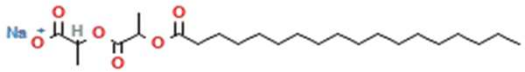
E442
Ammonium Phosfatides



E475
Polyglycerol esters of FA

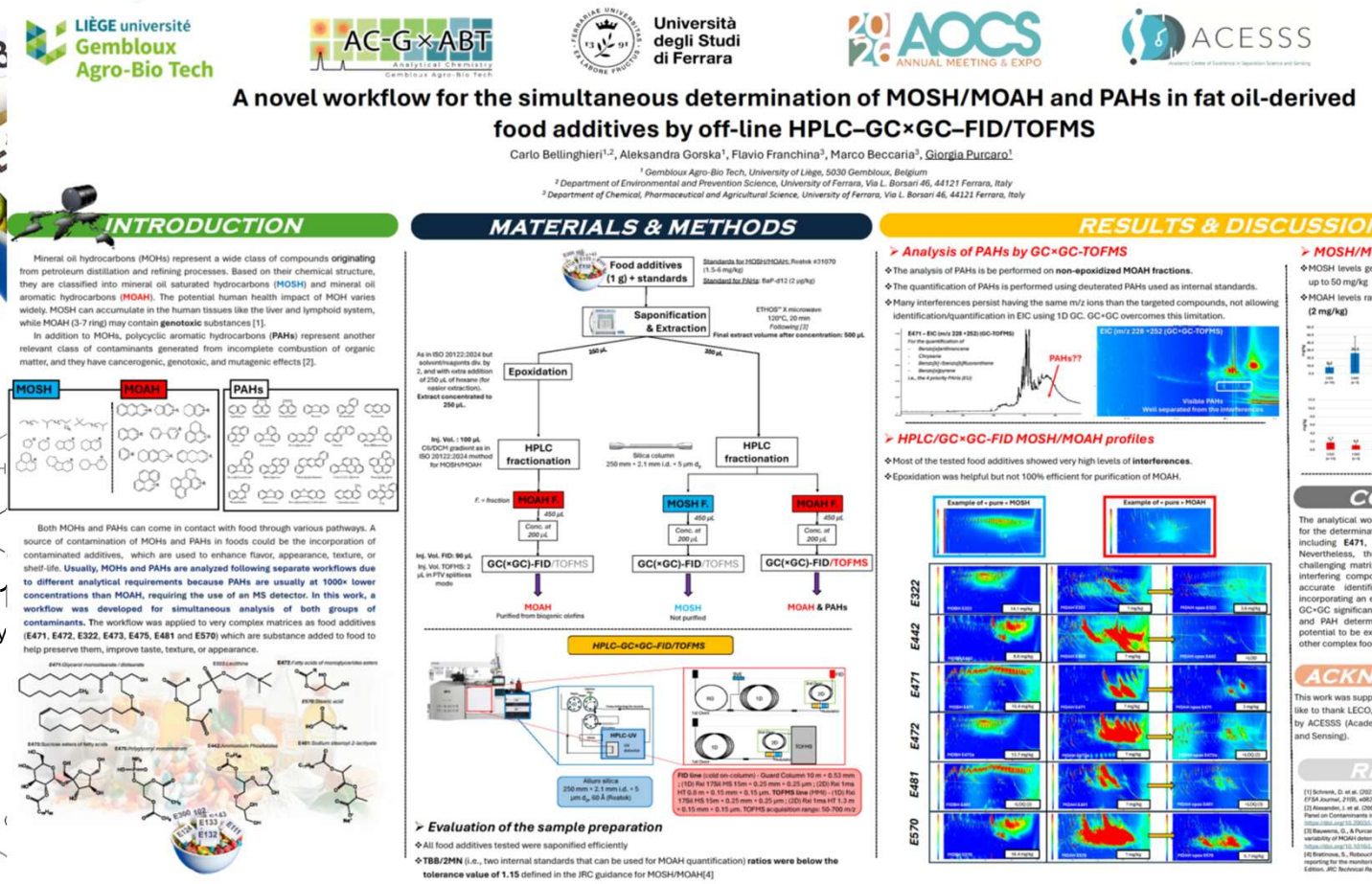


E570
Stearic acid



E481
Sodium stearoyl-2-lactiyate

Updates – Occurrence – Additives



LIÈGE université Gembloux Agro-Bio Tech · AC-G×ABT Analytical Chemistry Gembloux Agro-Bio Tech · Università degli Studi di Ferrara · AOCS 2024 Annual Meeting & Expo · ACESSS · WiFST

A novel workflow for the simultaneous determination of MOSH/MOAH and PAHs in fat oil-derived food additives by off-line HPLC–GC×GC–FID/TOFMS

Carlo Bellinghieri[1,2], Aleksandra Gorska[1], Flavio Franchina[3], Marco Beccaria[3], Giorgia Purcaro[1]

[1] Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium
[2] Department of Environmental and Prevention Science, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy
[3] Department of Chemical, Pharmaceutical and Agricultural Science, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy

INTRODUCTION

Mineral oil hydrocarbons (MOHs) represent a wide class of compounds originating from petroleum distillation and refining processes. Based on their chemical structure, they are classified into mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). The potential human health impact of MOH varies widely. MOSH can accumulate in the human tissues like the liver and lymphoid system, while MOAH (3-7 ring) may contain **genotoxic** substances [1].

In addition to MOHs, polycyclic aromatic hydrocarbons (**PAHs**) represent another relevant class of contaminants generated from incomplete combustion of organic matter, and they have cancerogenic, genotoxic, and mutagenic effects [2].

MOSH · MOAH · PAHs

Both MOHs and PAHs can come in contact with food through various pathways. A source of contamination of MOHs and PAHs in foods could be the incorporation of contaminated additives, which are used to enhance flavor, appearance, texture, or shelf-life. **Usually, MOHs and PAHs are analyzed following separate workflows due to different analytical requirements because PAHs are usually at 1000× lower concentrations than MOAH, requiring the use of an MS detector. In this work, a workflow was developed for simultaneous analysis of both groups of contaminants.** The workflow was applied to very complex matrices as food additives (**E471**, **E472**, **E322**, **E473**, **E475**, **E481** and **E570**) which are substance added to food to help preserve them, improve taste, texture, or appearance.

MATERIALS & METHODS

Food additives (1 g) + standards → Saponification & Extraction → Epoxidation → HPLC fractionation → GC(×GC)-FID/TOFMS → MOAH (Purified from biogenic olefins) / MOSH (Not purified) / MOAH & PAHs

HPLC-GC×GC-FID/TOFMS

➢ *Evaluation of the sample preparation*

❖ All food additives tested were saponified efficiently

❖ **TBB/2MN** (i.e., two internal standards that can be used for MOAH quantification) **ratios were below the tolerance value of 1.15** defined in the JRC guidance for MOSH/MOAH[4]

RESULTS & DISCUSSION

➢ *Analysis of PAHs by GC×GC-TOFMS*

❖ The analysis of PAHs is be performed on **non-epoxidized MOAH fractions.**

❖ The quantification of PAHs is performed using deuterated PAHs used as internal standards.

❖ Many interferences persist having the same m/z ions than the targeted compounds, not allowing identification/quantification in EIC using 1D GC. GC×GC overcomes this limitation.

PAHs??

➢ *HPLC/GC×GC-FID MOSH/MOAH profiles*

❖ Most of the tested food additives showed very high levels of **interferences.**

❖ Epoxidation was helpful but not 100% efficient for purification of MOAH.

E322 · E442 · E471 · E472 · E481 · E570

➢ *MOSH/MOAH results*

❖ MOSH levels generally below 15 mg/kg, with exceptions going up to 50 mg/kg

❖ MOAH levels rather low. **In most samples, MOAH were <LOQ (2 mg/kg)**

CONCLUSION

The analytical workflow described was successfully implemented for the determination of MOSH/MOAH and PAHs in food additives including **E471**, **E322**, **E472**, **E473**, **E475**, **E481**, and **E570**. Nevertheless, these food additives represent a particularly challenging matrix owing to the high concentration of co-eluting interfering compounds, which pose a notable obstacle to the accurate identification of MOAH. To address this issue, incorporating an epoxidation step proved essential. In conclusion, GC×GC significantly improved the reliability of both MOSH/MOAH and PAH determination, and the proposed workflow has the potential to be extended to the analysis of these contaminants in other complex food matrices.

ACKNOWLEDGMENTS

This work was supported by the IMPOFAD project. The authors would like to thank LECO, Restek and Milestone. This work was supported by ACESSS (Academic Center of Exellence in Separation Science and Sensing).

REFERENCES

(1) Schrenk, D. et al. (2023). Update of the risk assessment of mineral oil hydrocarbons in food. *EFSA Journal*, *21*(9), e08191. https://doi.org/10.2903/j.efsa.2023.8191

(2) Alexander, J. et al. (2008). Polycyclic Aromatic Hydrocarbons in Food - Scientific Opinion of the Panel on Contaminants in the Food Chain. *EFSA Journal*, *6*(8), 724. https://doi.org/10.2903/j.efsa.2008.724

(3) Bauwens, G., & Purcaro, G. (2024). Improved microwave-assisted saponification to reduce the variability of MOAH determination in edible oils. *Analytica Chimica Acta*, *1312*, 342788. https://doi.org/10.1016/j.aca.2024.342788

(4) Bratinova, S., Robouch, P., & Hoekstra, E. (2023). Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition. *JRC Technical Reports*. https://doi.org/10.2760/867728

Europe Union

→ Function of additives

E471
Mono- and diacigly

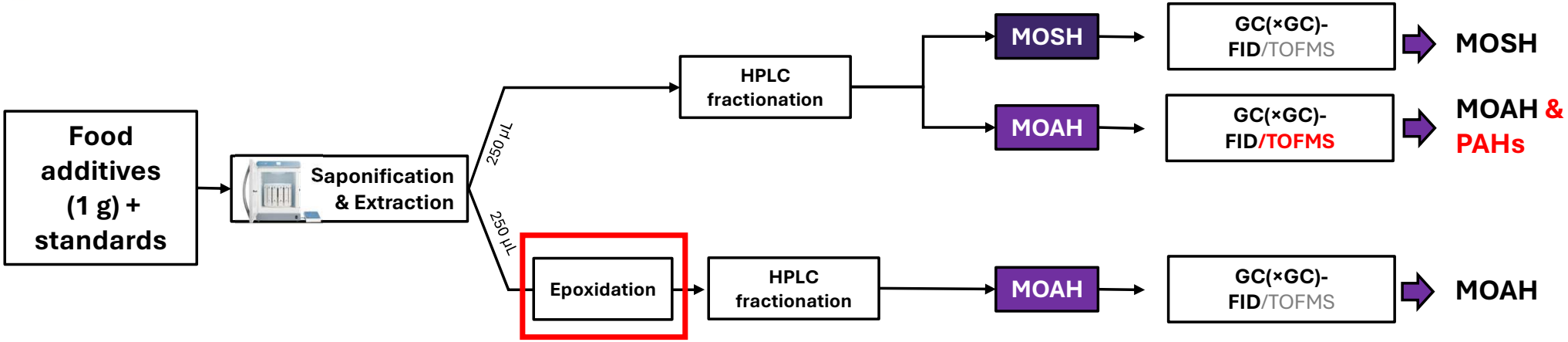
E475
Polyglycerol esters of FA

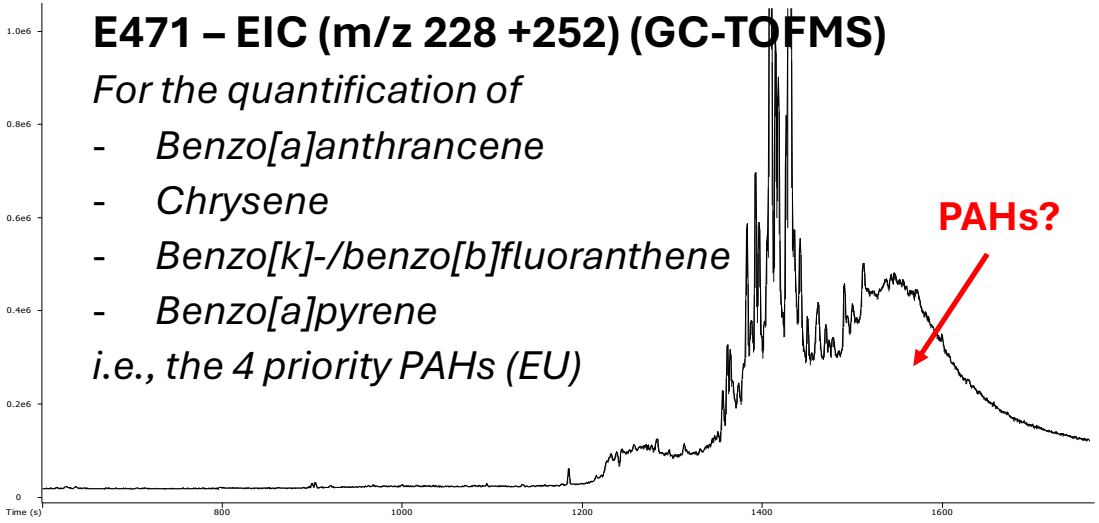
E570
Stearic acid

E481
Sodium stearoyl-2-lactiyate

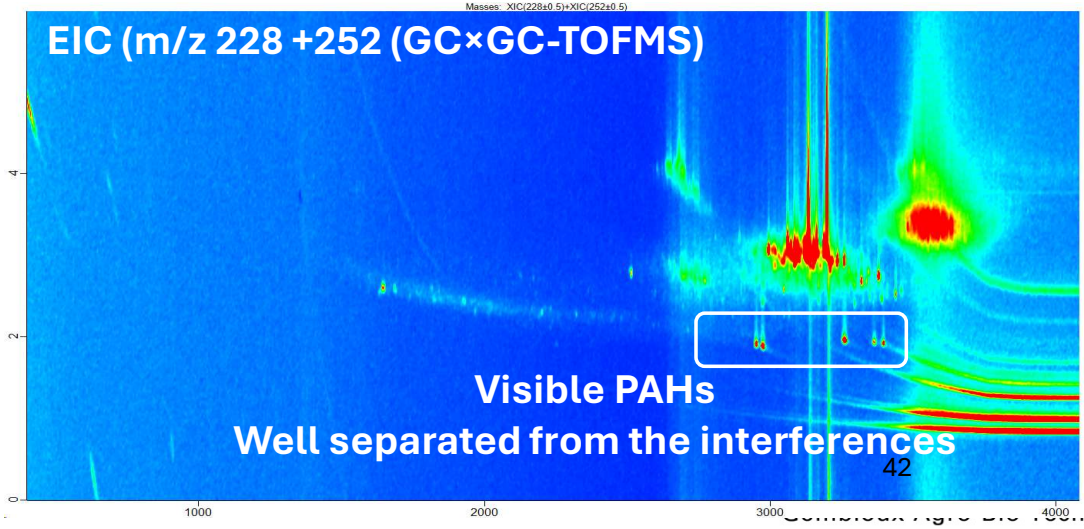
2
hosfatides

Updates – Occurrence – Additives

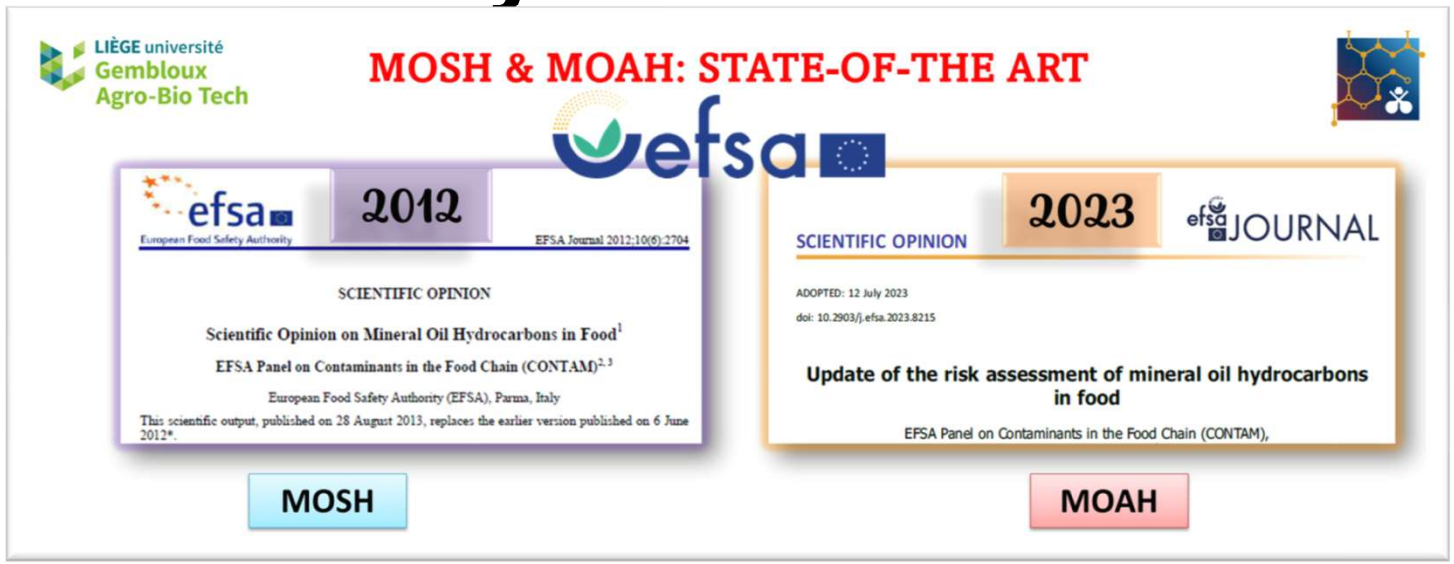


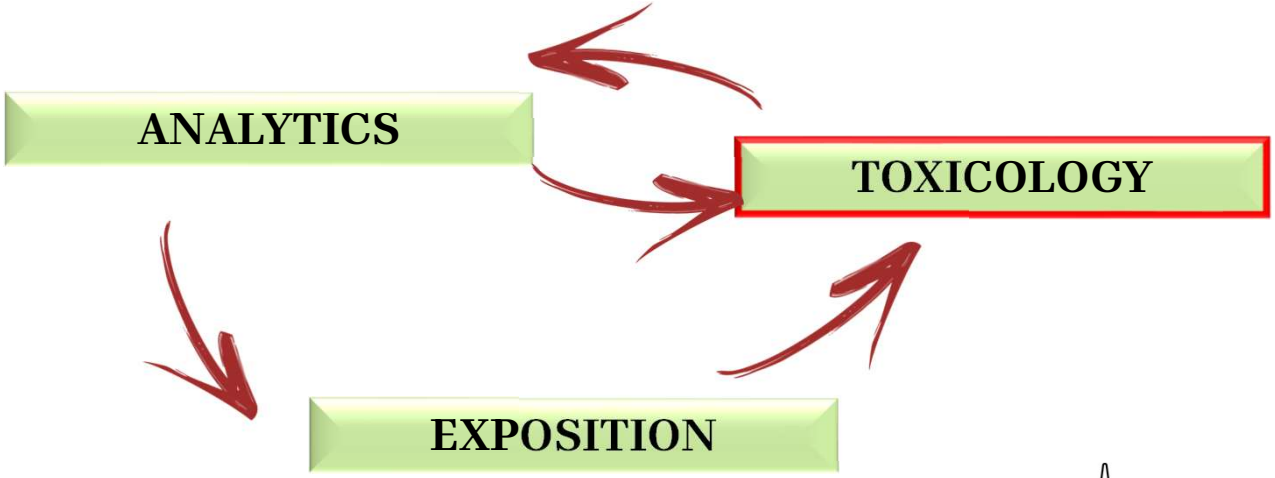


GC×GC is needed



Updates – Today





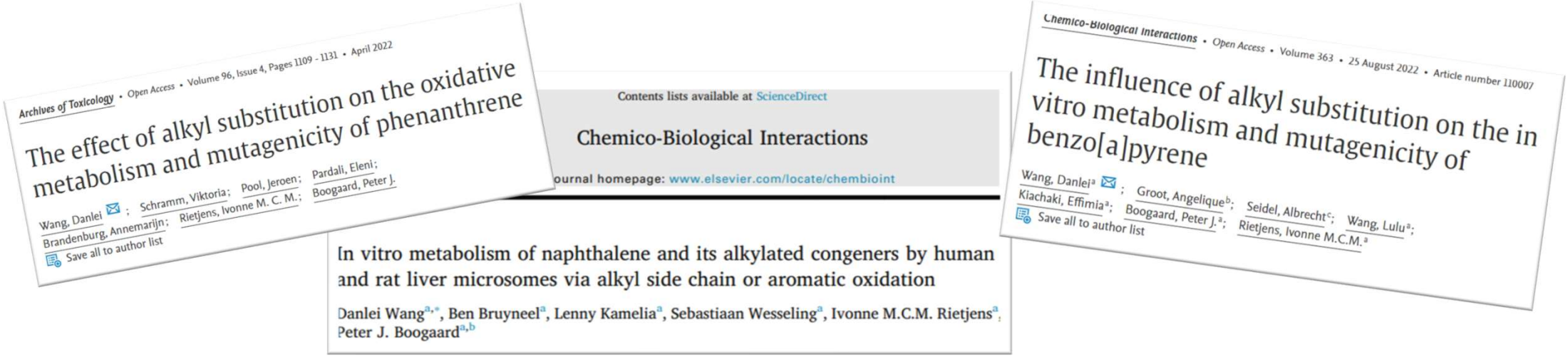
Updates - Toxicology

Open gaps:

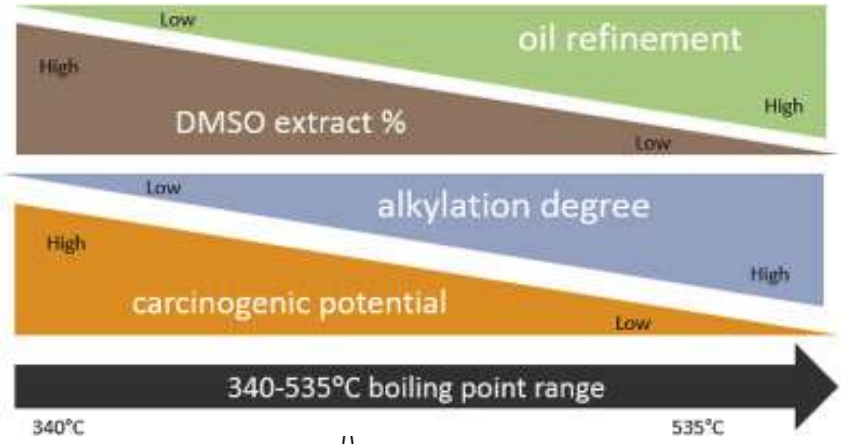
- **Limited link between composition and toxicity**: High variability in MOSH/MOAH composition, but insufficient data to associate specific fractions with defined toxicological effects
- ***In vitro* testing biased toward more polar fractions**: Reliance on dissolution in aqueous media means mainly DMSO-extractable (more polar) compounds are assessed, potentially overlooking less polar fractions
- **Lack of oral toxicity data for specific MOAH fractions** – Particularly for 1–2 ring MOAH, limited data complicates risk assessment, while regulation is still based on total MOAH

Updates – Toxicology - MOAH

Long alkyl chain substitution decreases carcinogenic potential



- Experiments with 2,3 and 5 ring PAH
- Alkyl substitution shifts the oxidative metabolism from the aromatic ring to the alkyl side chain
- Long alkylation decreases carcinogenic potential of MOAH
- MOAH in refined oils is mostly 1 and 2 rings
- These can only be present at 300°C if they are highly alkylated
  → Alkylation rises the bp



Updates – EFSA

2023


SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 July 2023

doi: 10.2903/j.efsa.2023.8215

Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

For **MOSH** :

- **Improvement of analytical methodology for better characterisation of MOSH** .....
- Better investigation of the **sources of the hydrocarbons** in food, ...
- Data are needed on ... **accumulation potential**.
- Investigation is recommended on the **structural features of MOSH** that ... result **in accumulation**.
- Additional toxicity data are needed ... in particular on bioaccumulating MOSH following their characterisation.
- More data on human MOSH tissue concentrations.
- .... needs further investigation with regard to occurrence of **MOSH and potential compositional modification and bioaccumulation**.
- ...

For **MOAH**:

- **More selective and sensitive analytical method should be implemented**.
- More data on **MOAH composition by aromatic ring number** in food are needed, in particular with respect to the levels of ≥ 3 ring MOAH.
- Sources of food contamination should be investigated when MOAH are detected.
- More data are needed on the **influence of ring alkylation on genotoxicity and carcinogenic** potency of ≥ 3 ring MOAH.
- **Oral toxicity data** in particular of **1–2 ring MOAH**.
- Technical specifications of white mineral oils and waxes used as **food additives and food packaging materials** should be updated, with **detailed information about the MOAH content and composition**.

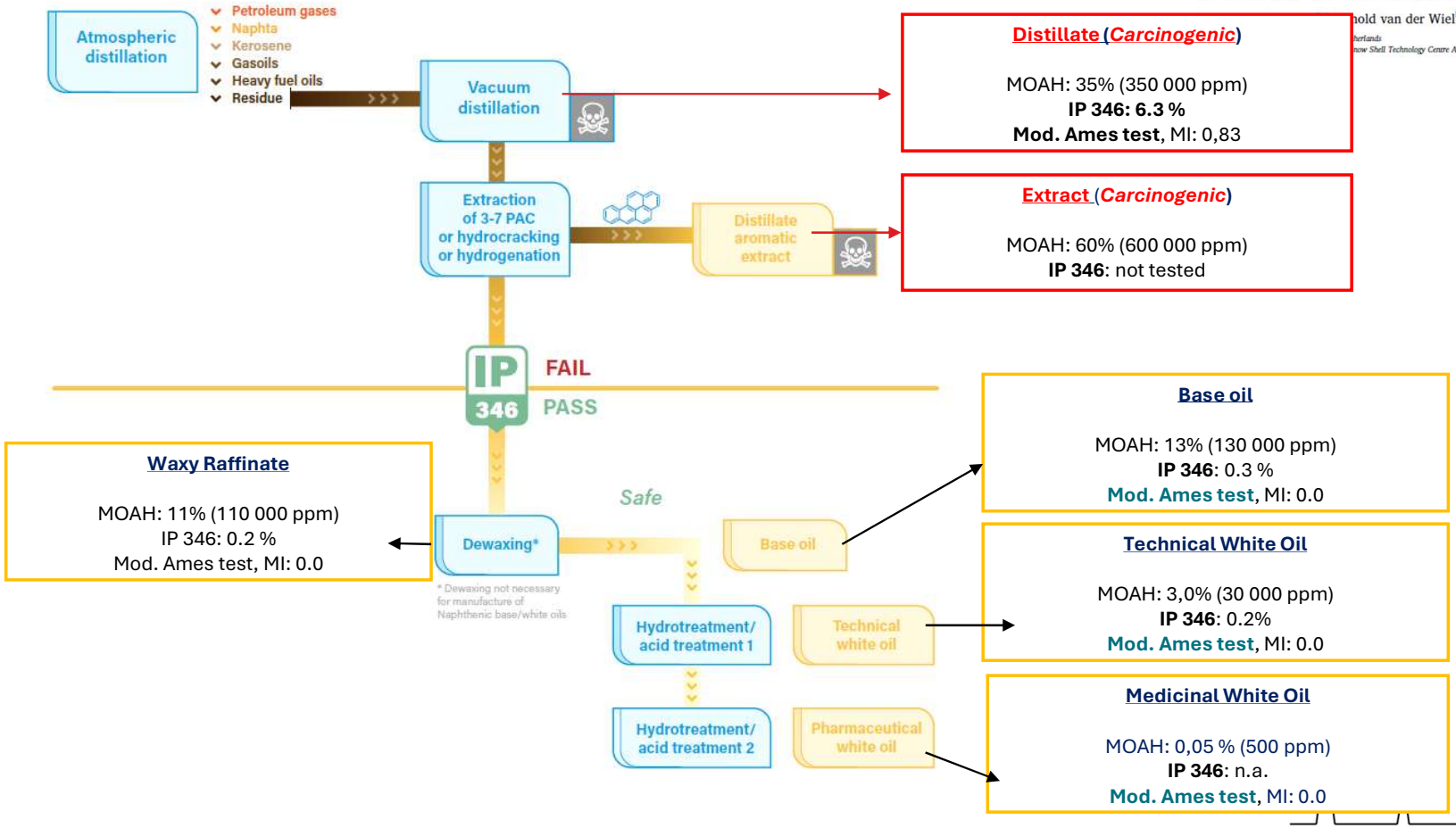
What about Toxicology?

Updates - Toxicology

The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

hold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]

Pharmaceutical white oil manufacture

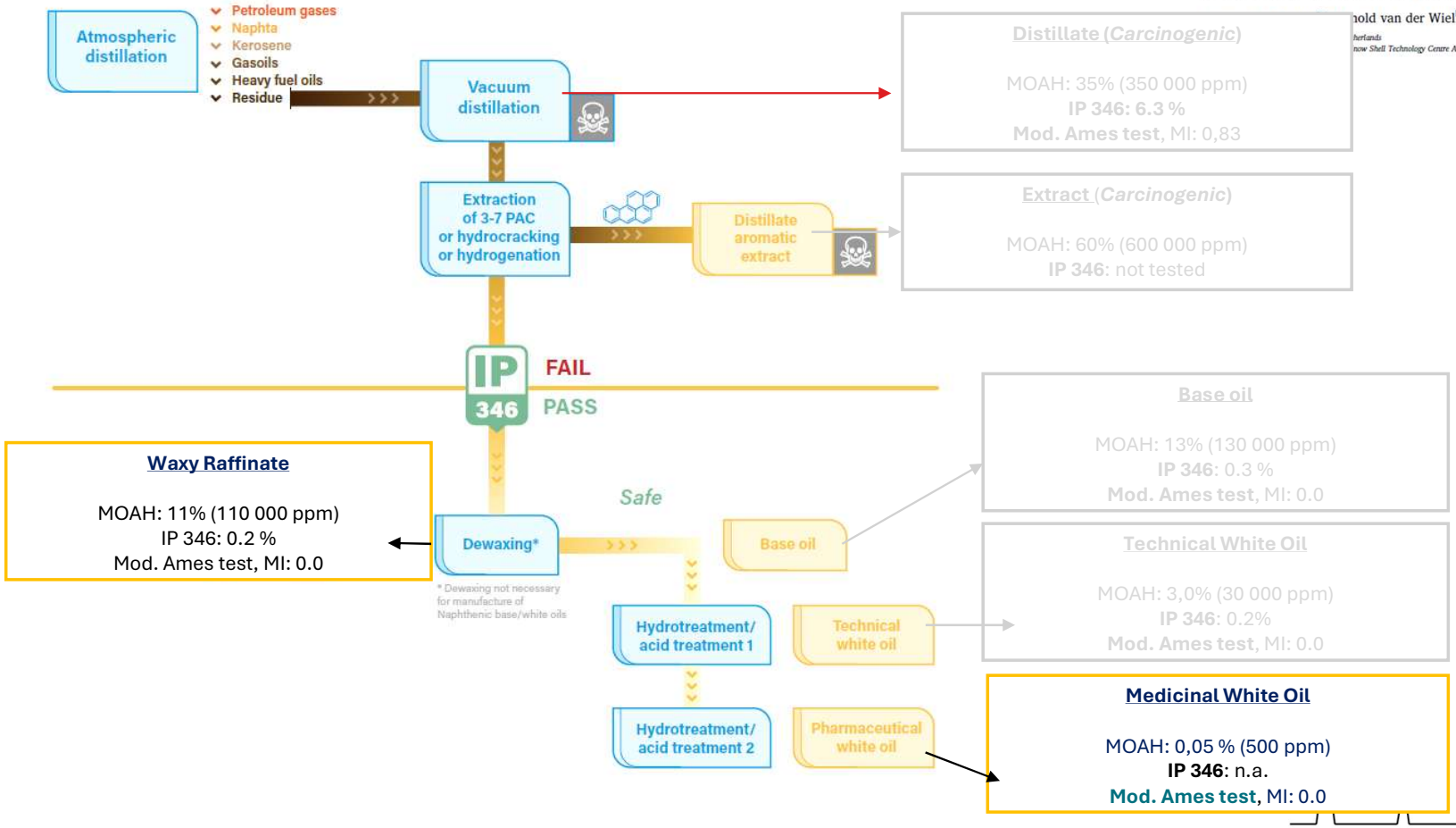


Carcinogenic potential:

- IP346
- Modified Ames Test (mutagenicity)

Updates - Toxicology

European Pharmacopea –DMSO UV/Vis




Regulatory Toxicology and Pharmacology 106 (2019) 316–333

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

nold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]

herlands

now Shell Technology Centre Amsterdam), the Netherlands

Carcinogenic potential:

- IP346
- Modified Ames Test (mutagenicity)
- **DMSO-UV/Vis (European Pharm)**

Updates - Toxicology

European Pharmacopea –DMSO UV/Vis


Regulatory Toxicology and Pharmacology 106 (2019) 316–333

Contents lists available at ScienceDirect

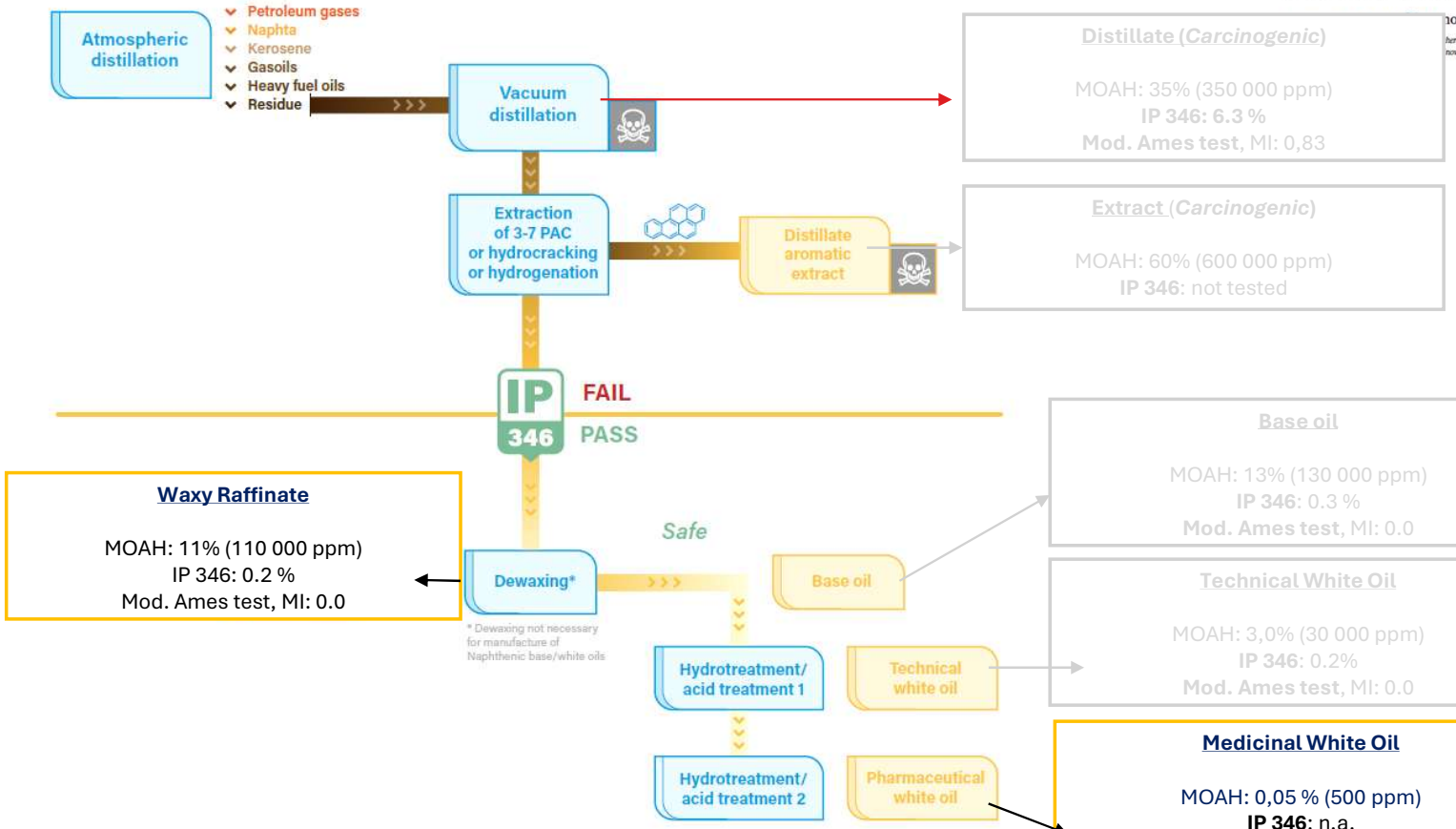
Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

nold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]

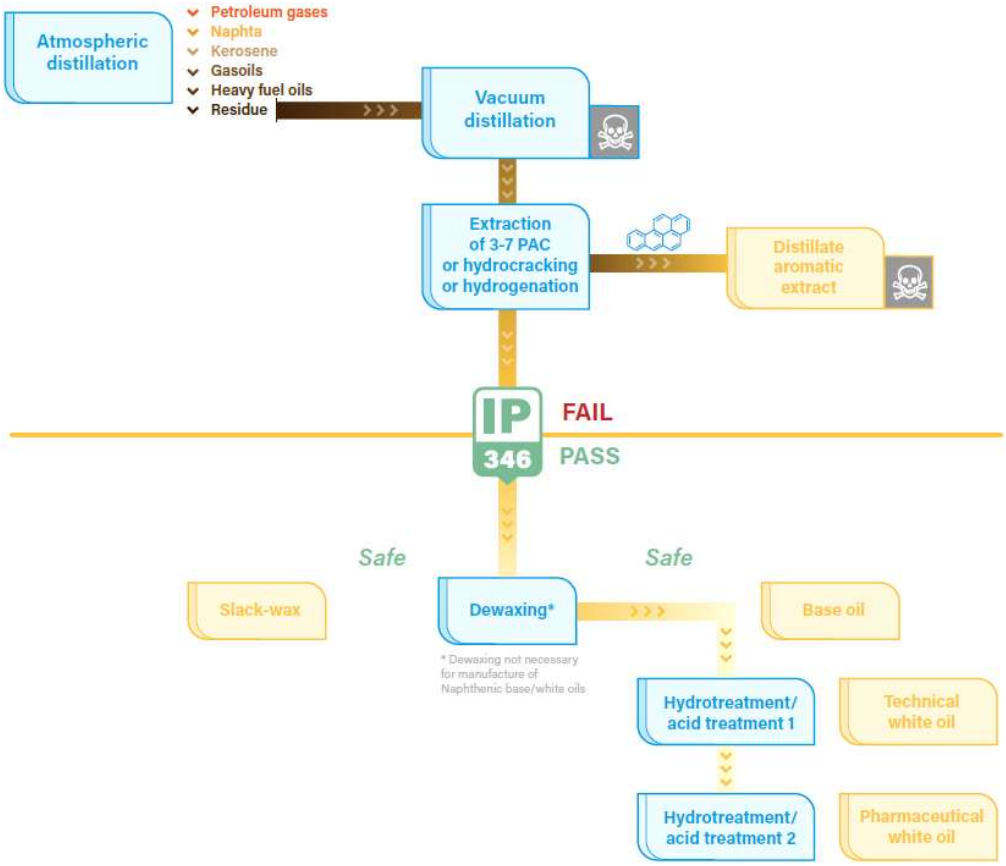
herlands
now Shell Technology Centre Amsterdam), the Netherlands



Carcinogenic potential:

- IP346
- Modified Ames Test (mutagenicity)
- **DMSO-UV/Vis (European Pharm)**

Updates - Toxicology



Regulatory Toxicology and Pharmacology 106 (2019) 316–333

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

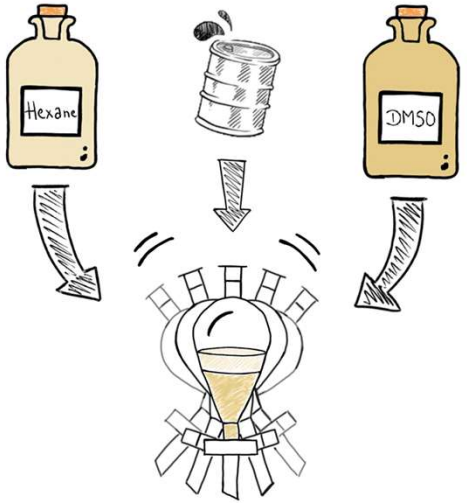
journal homepage: www.elsevier.com/locate/yrtph

The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

Juan-Carlos Carrillo[a,*], Arnold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]

[a] Shell Health, Shell International B.V, the Netherlands
[b] Kontinklijke Shell Laboratorium Amsterdam (now Shell Technology Centre Amsterdam), the Netherlands
[c] European Wax Federation atsbl, Belgium
[d] Shell Deutschland Oil GmbH, Germany

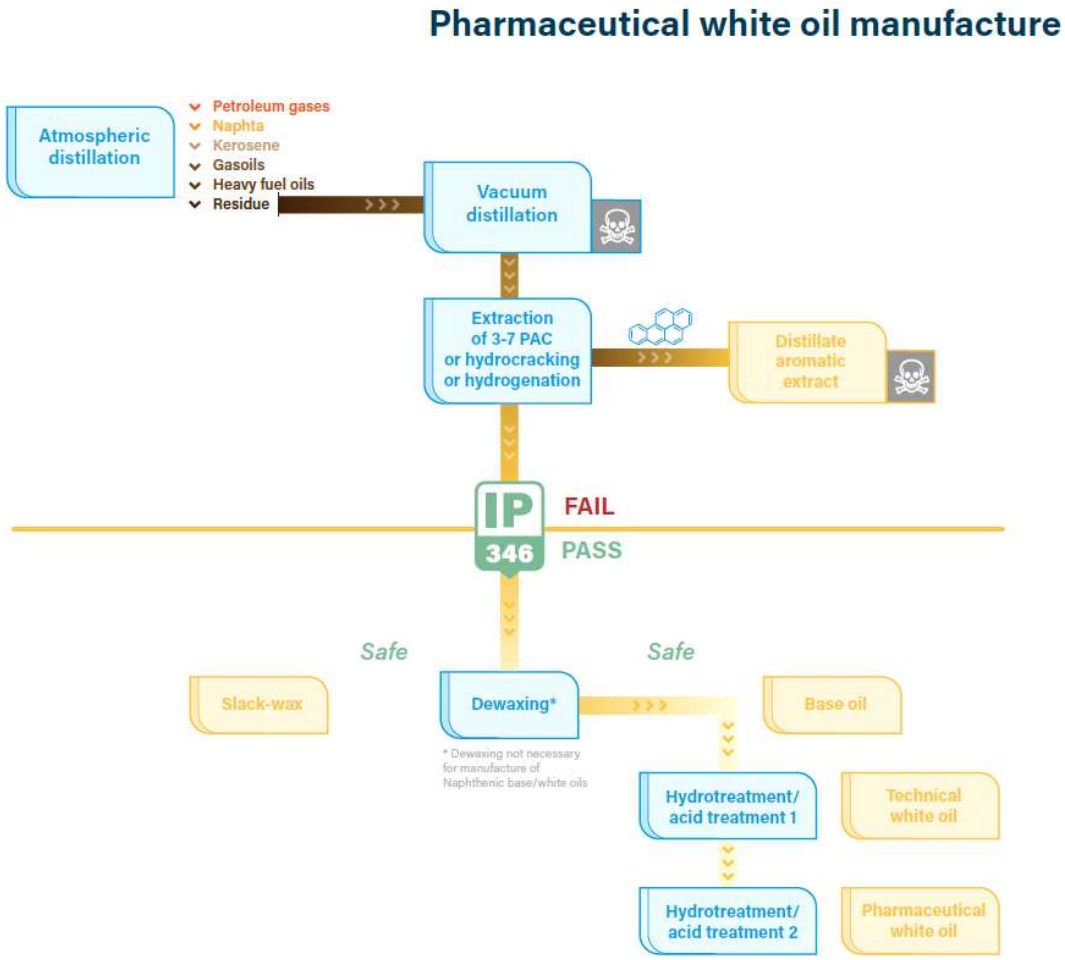
IP346 – protocol



1. LLE
2. Back extraction of DMSO in C6
3. Drying of C6
4. Weighing of the residues

IP, 1996. Determination of Polycyclic Aromatics in Unused Lubricating Base Oils and Asphaltene Free Petroleum Fractions - Dimethyl Sulfoxide Extraction Refractive Index Method IP346/92 Vol BS 2000 Part 346. BSI Standards.

Updates - Toxicology



Regulatory Toxicology and Pharmacology 106 (2019) 316–333

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

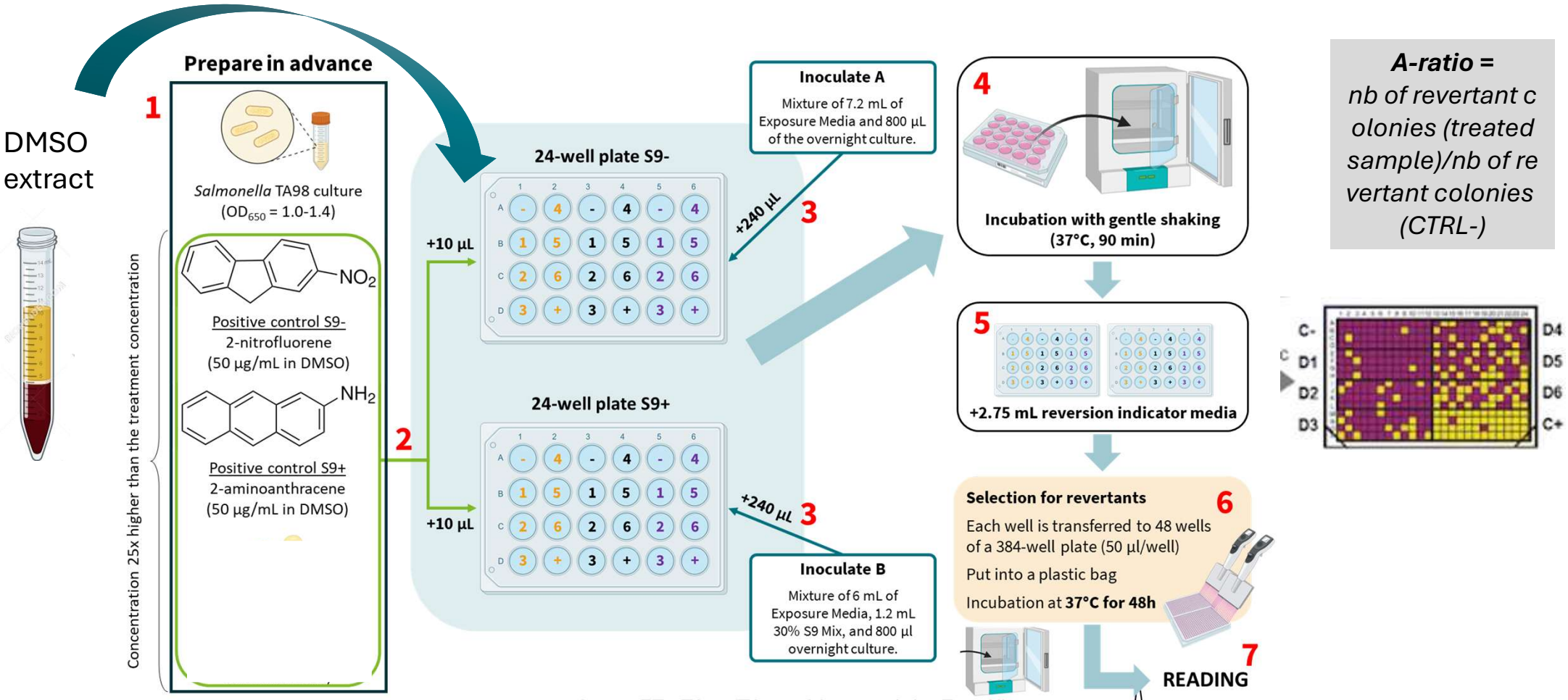
Juan-Carlos Carrillo[a,*], Arnold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]

[a] Shell Health, Shell International B.V, the Netherlands
[b] Koninklijke Shell Laboratorium Amsterdam (now Shell Technology Centre Amsterdam), the Netherlands
[c] European Wax Federation aisbl, Belgium
[d] Shell Deutschland Oil GmbH, Germany

Correlation between **IP346** and **mouse skin painting assays** showed the predictability of the carcinogenic potential of a mineral oil (with 94% accuracy, using a 3% cutoff on DMSO extract in the IP346)

Updates - Toxicology

The miniaturized Ames test – protocol



A-ratio =
nb of revertant colonies (treated sample)/nb of revertant colonies (CTRL-)

Ames FT™ TA98/TA100 Mutagenicity Test Kit
Instruction Manual 31-300

Updates - Toxicology

European Pharmacopea –DMSO UV/Vis


Regulatory Toxicology and Pharmacology 106 (2019) 316–333

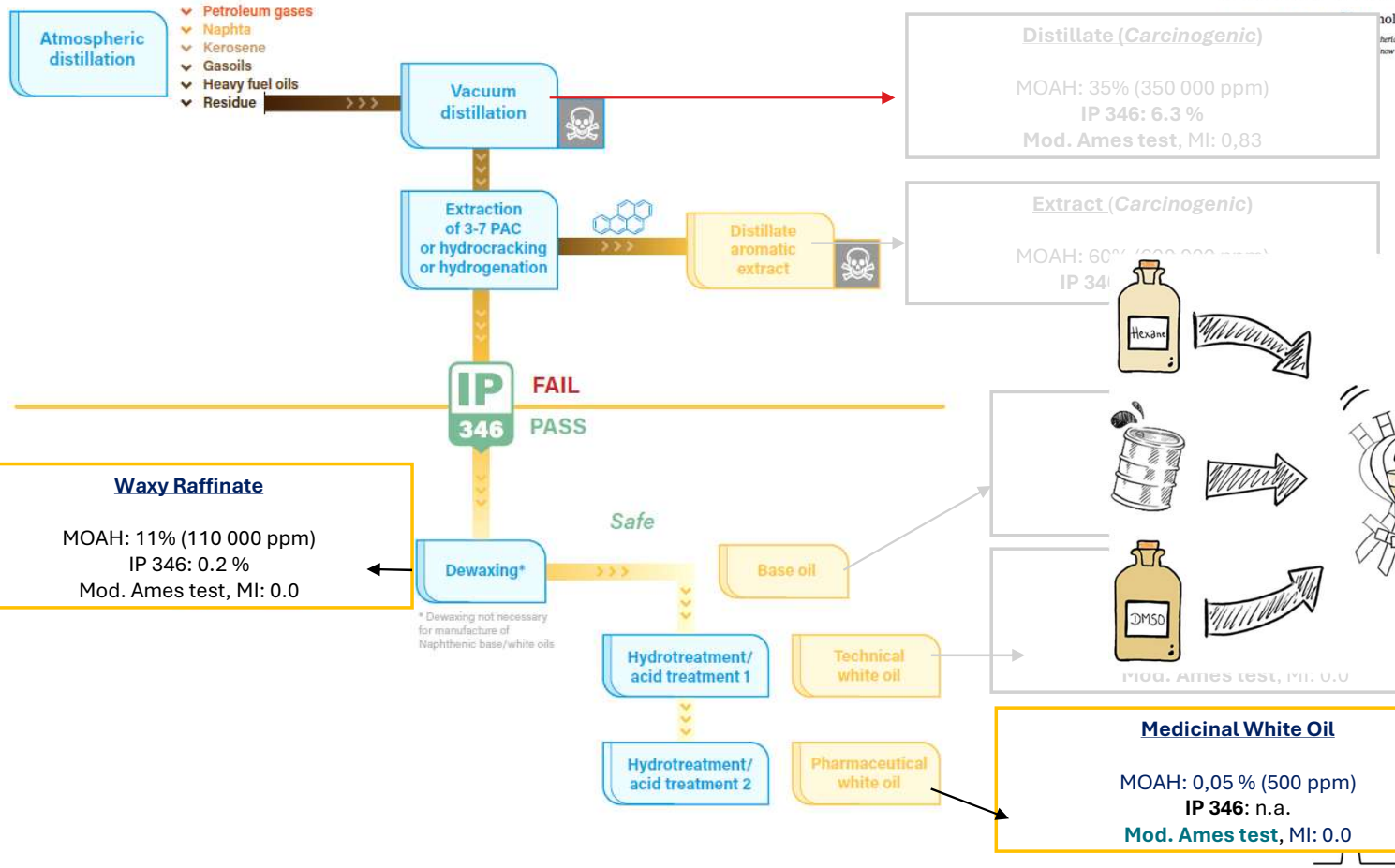
Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

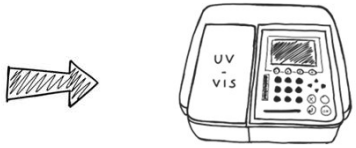
The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays

...nold van der Wiel[b], Dirk Danneels[c], Olaf Kral[d], Peter J. Boogaard[a]



Carcinogenic potential:

- IP346
- Modified Ames Test (mutagenicity)
- **DMSO-UV/Vis (European Pharm)**

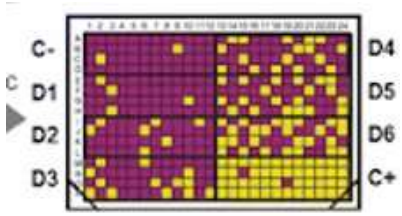


DMSO Extr

Updates - Toxicology

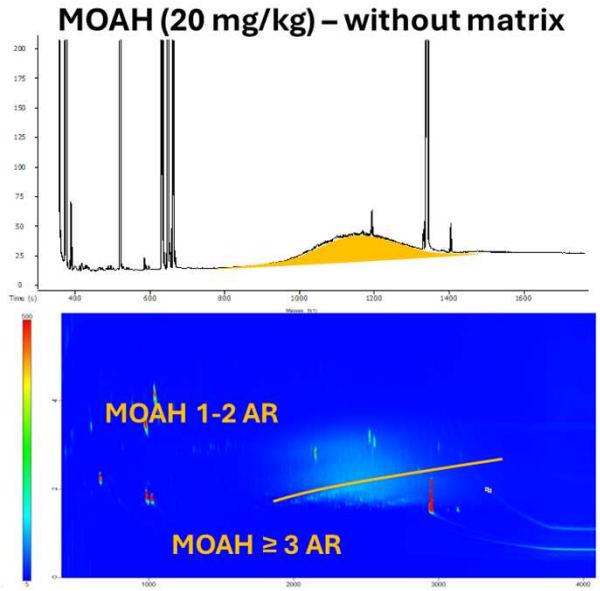
Toxicological prediction

IP 346



VS

Total MOAH quantification



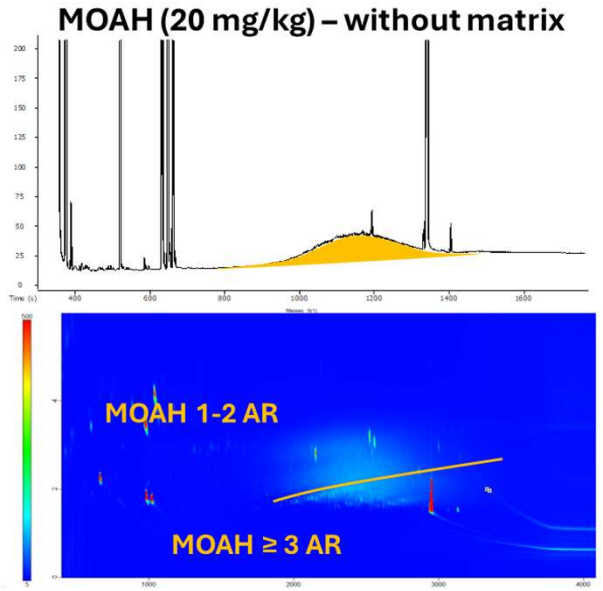
Updates - Toxicology

Toxicological prediction

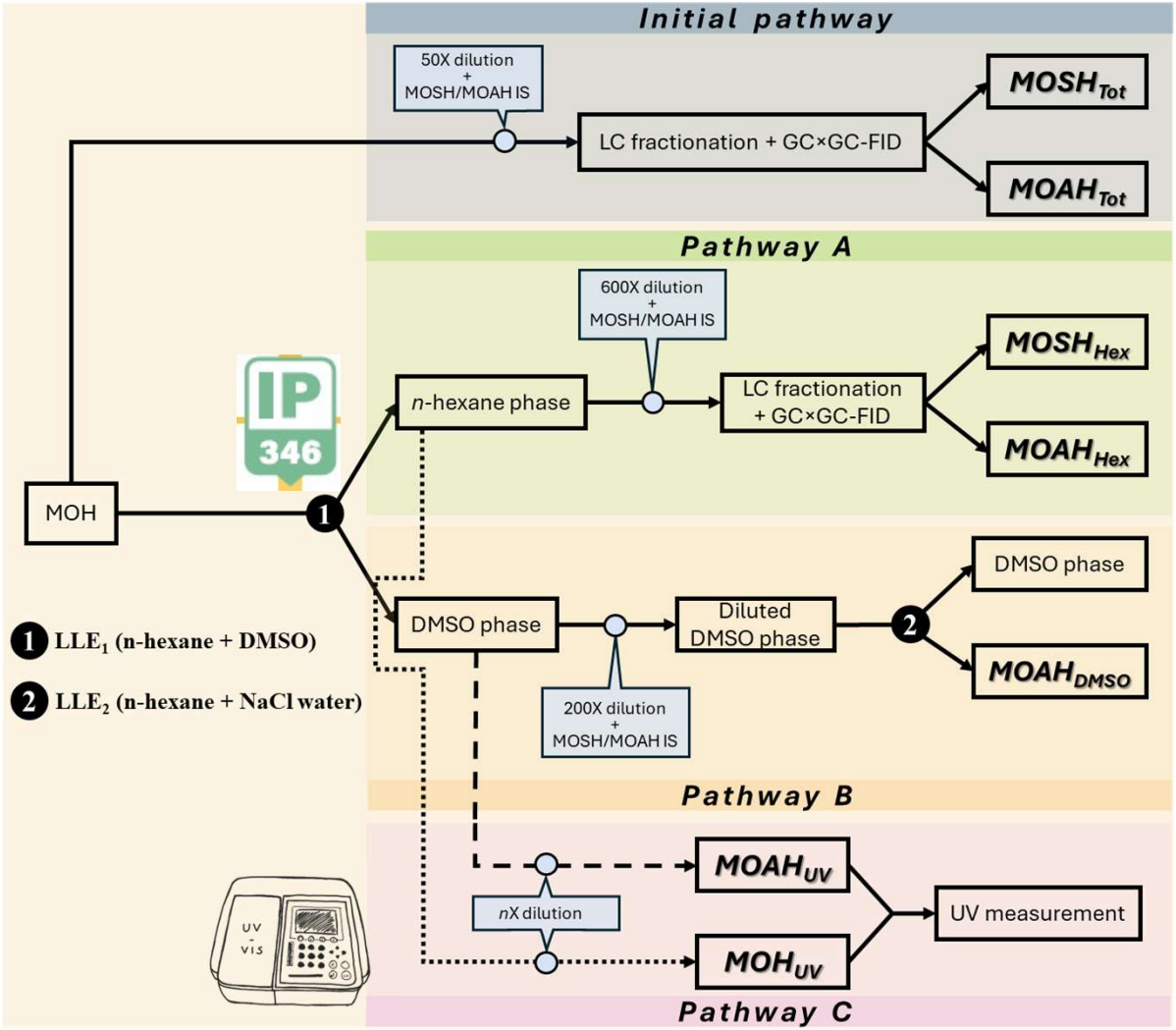
IP 346

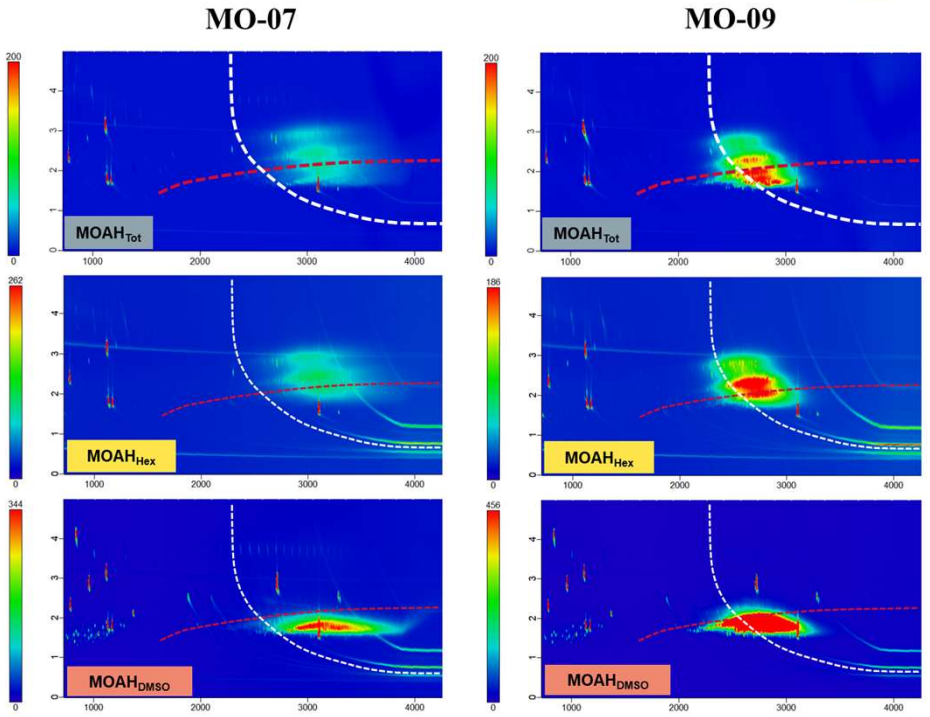
VS

Total MOAH quantification

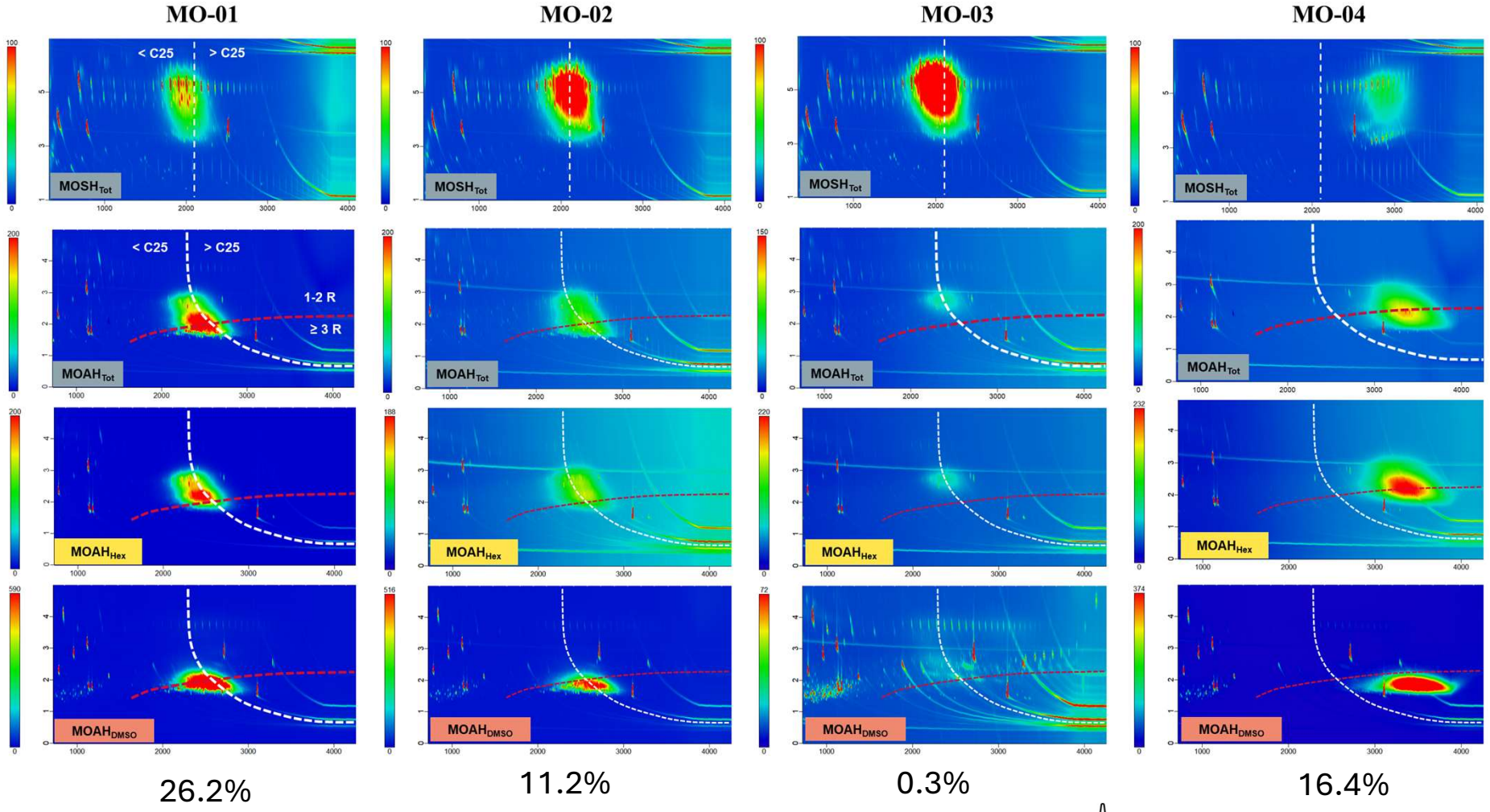


Updates - Toxicology

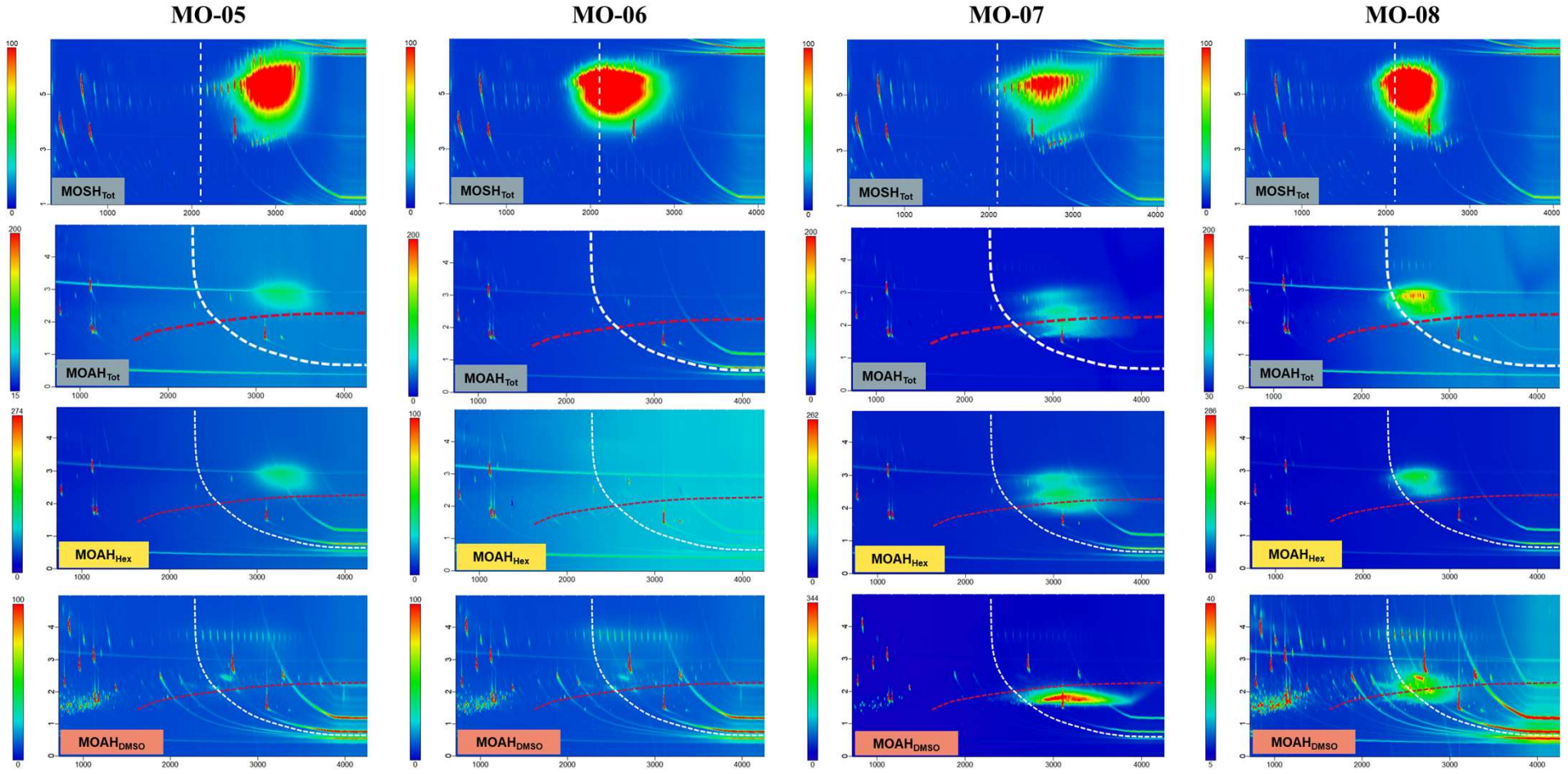




Updates - Toxicology



Updates - Toxicology



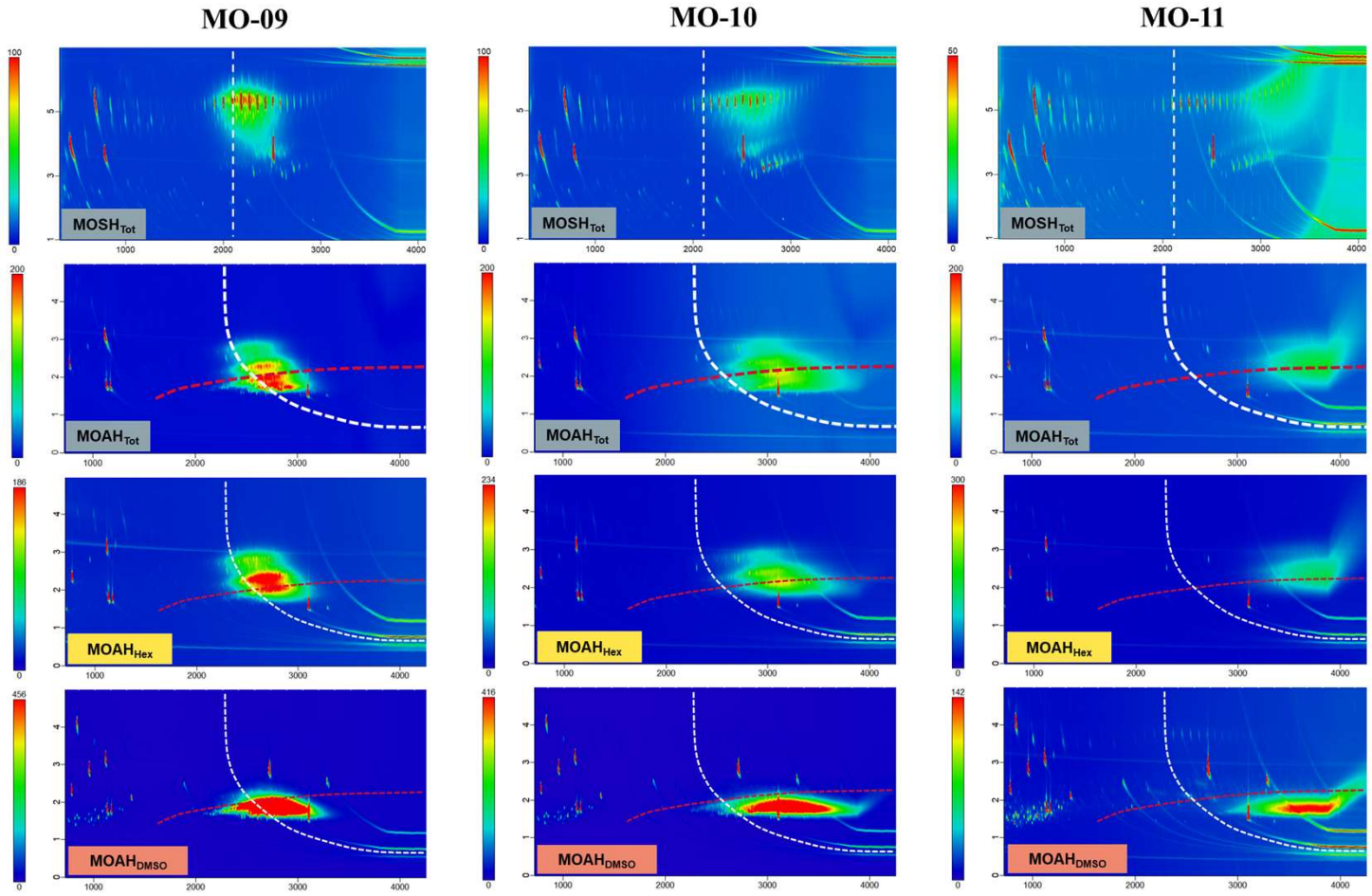
Updates - Toxicology

MOSH
Tot

MOAH
Tot

MOAH
Hex

MOAH
DMSO

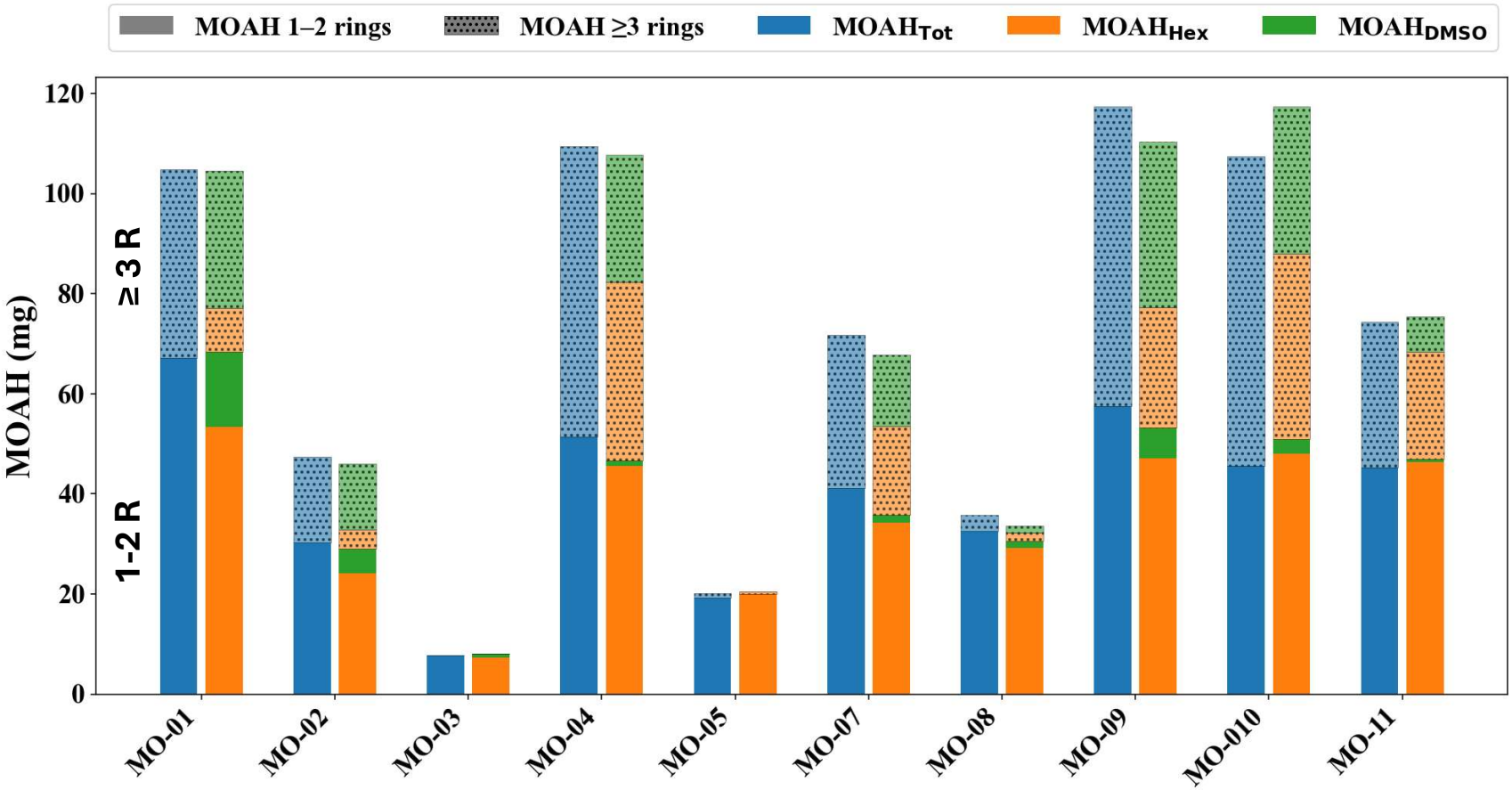


25 %

17.5 %

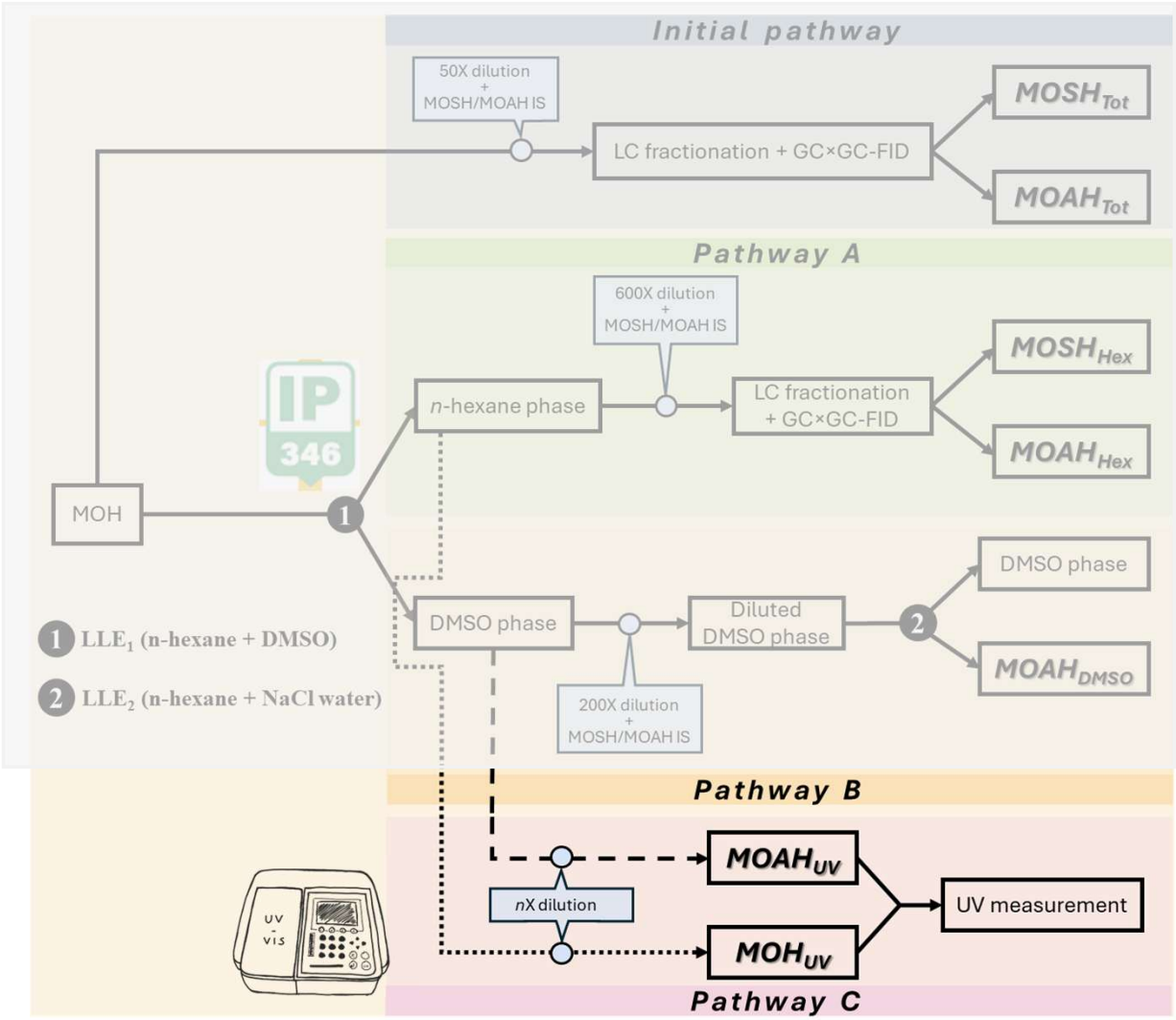
4 %

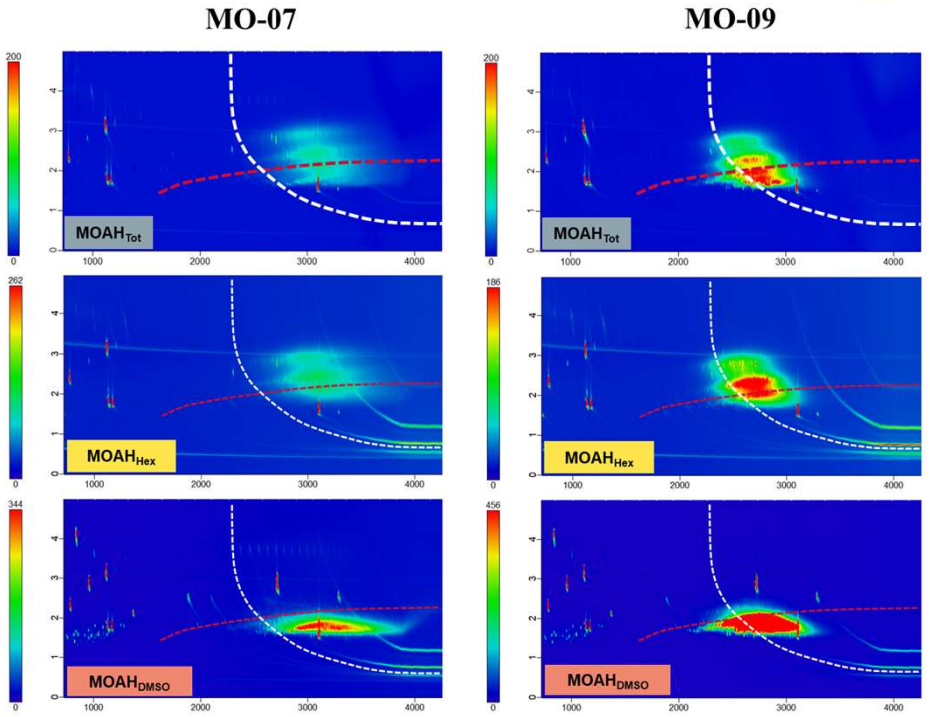
Updates - Toxicology



- ≥3 MOAH enriched in DMSO
- Very low residual ≥3 MOAH

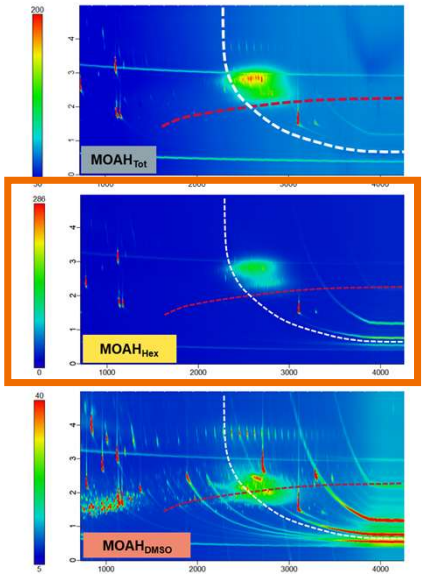
Updates - Toxicology





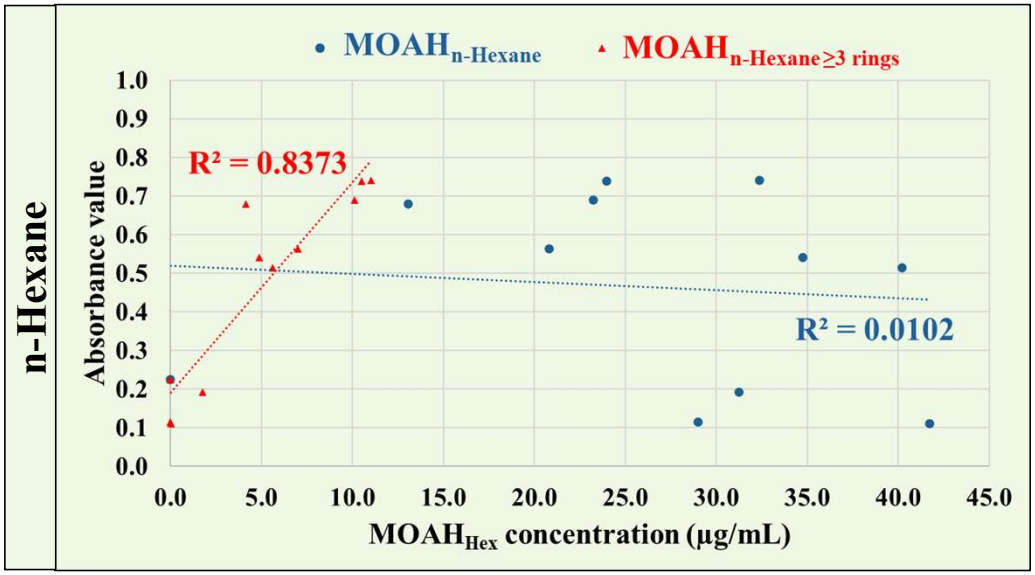
Updates - Toxicology

Absorption at 270 nm



MOAH

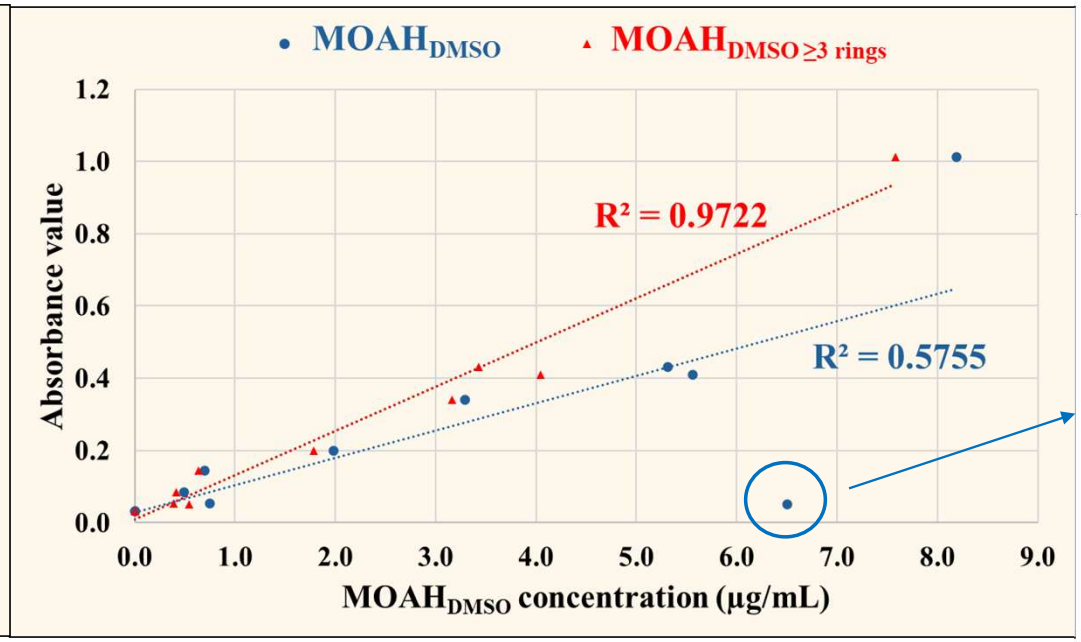
Hex



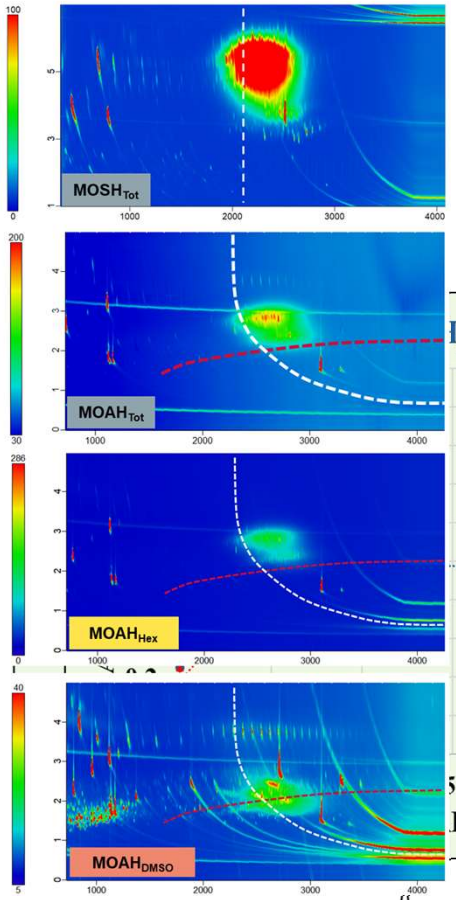
Updates - Toxicology

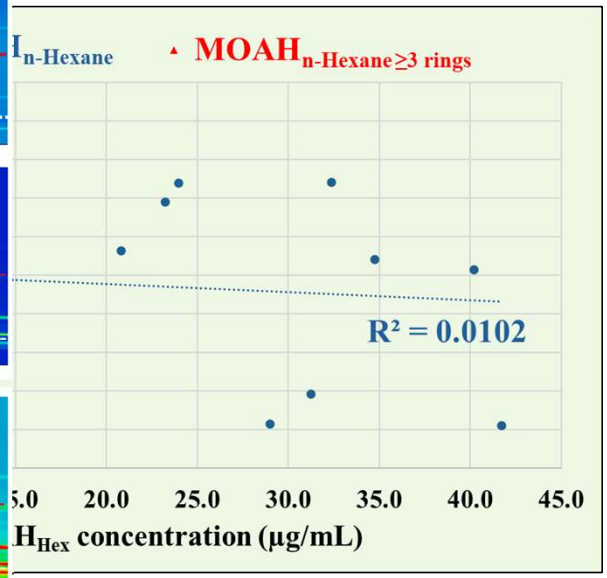
Absorption at 270 nm

DMSO



MO-08

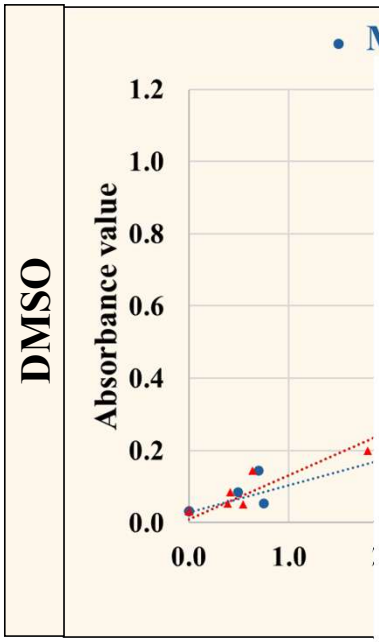


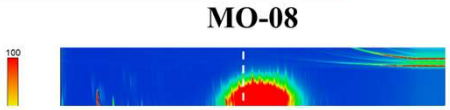


0.4%

Updates - Toxicology

Absorption at 270 nm



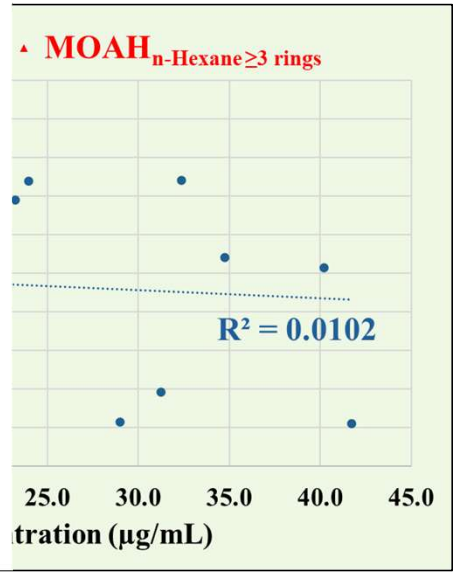


Food Research International

INSIGHT INTO WHAT IP346 MEASURES THROUGH CHARACTERISATION OF MOAH DISTRIBUTION IN DMSO AND ORGANIC PHASE BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

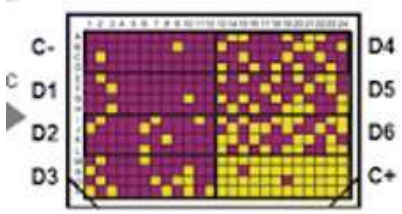
--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Food Toxicology
Keywords:	Mineral oil aromatic hydrocarbons (MOAH); IP346; Dimethyl sulfoxide (DMSO); GC×GC, UV-Vis.
Corresponding Author:	Giorgia Purcaro, PhD University of Liege Faculty of Gembloux Agro-Bio Tech Gembloux, BELGIUM
First Author:	Damien Pierret
Order of Authors:	Damien Pierret
	Aleksandra Gorska
	Juan-Carlos Carrillo
	Giorgia Purcaro, PhD
Abstract:	Mineral oil hydrocarbons are complex mixture used in a wide range of applications



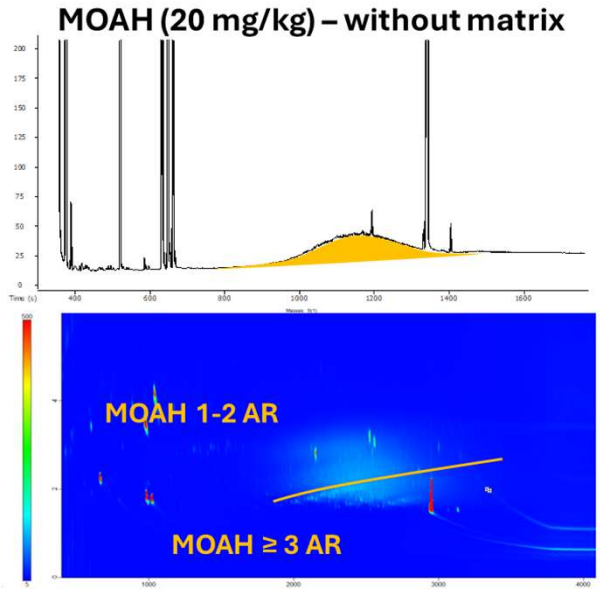
Updates - Toxicology

Toxicological prediction

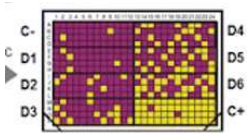


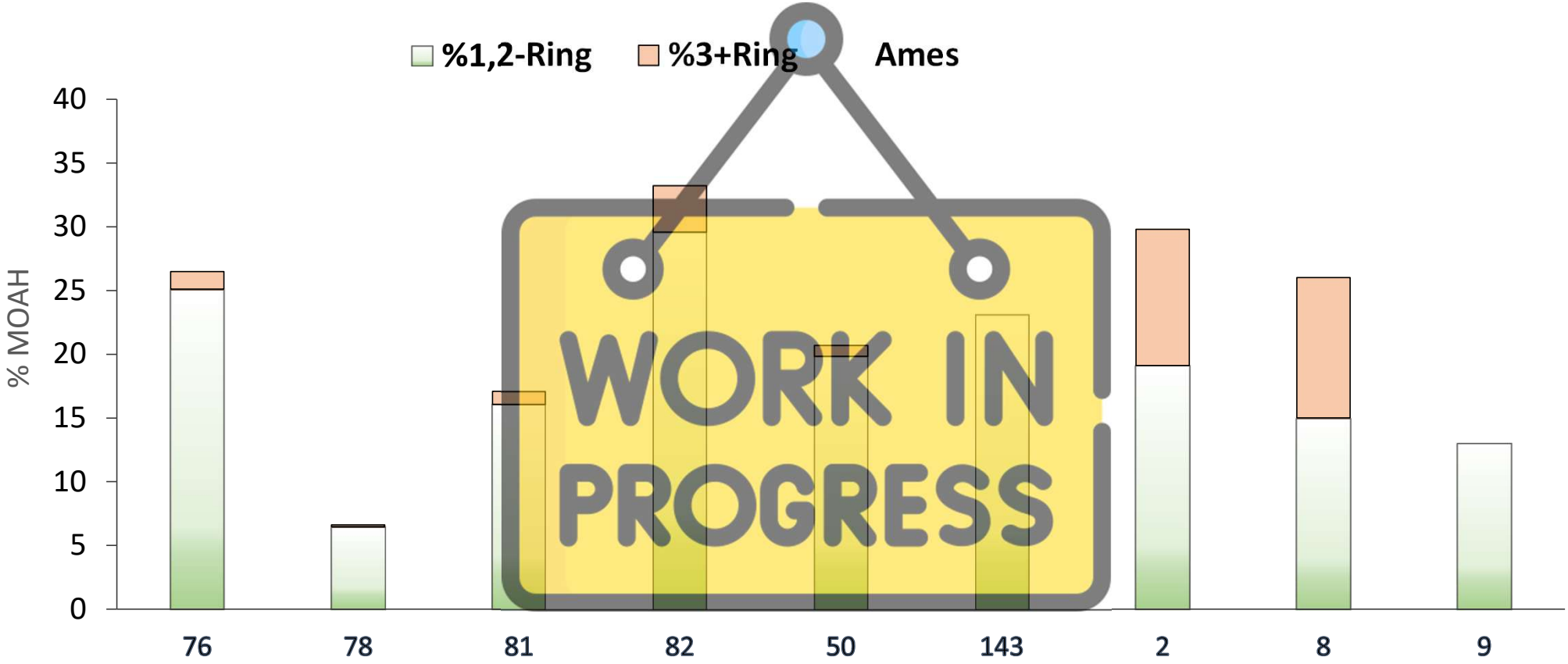
VS

Total MOAH quantification

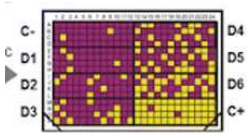


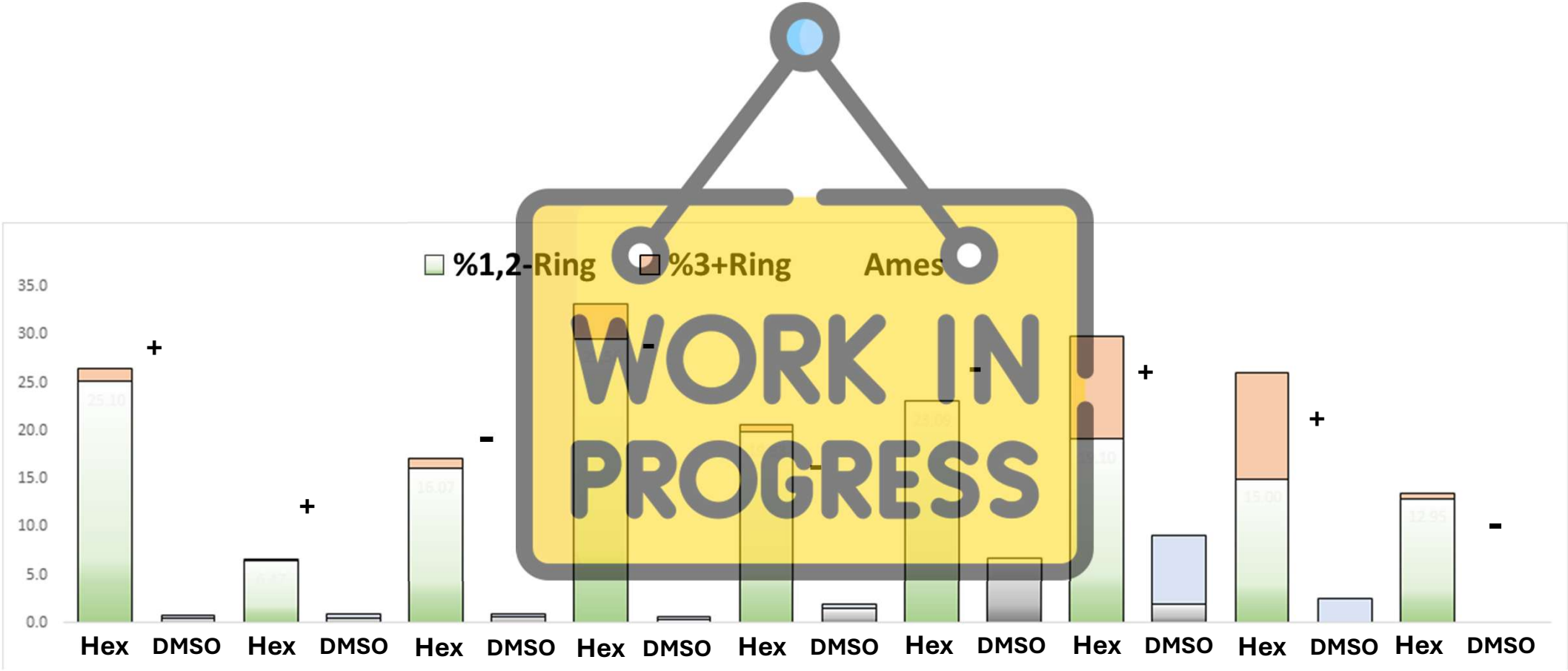
Updates - Toxicology





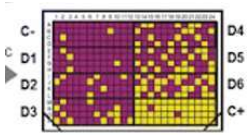
Updates - Toxicology





WORK IN PROGRESS

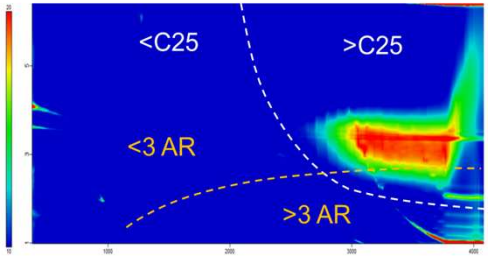
Updates - Toxicology



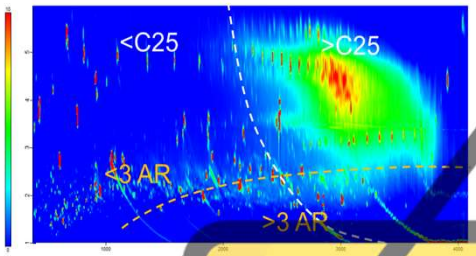
Med Uni Graz

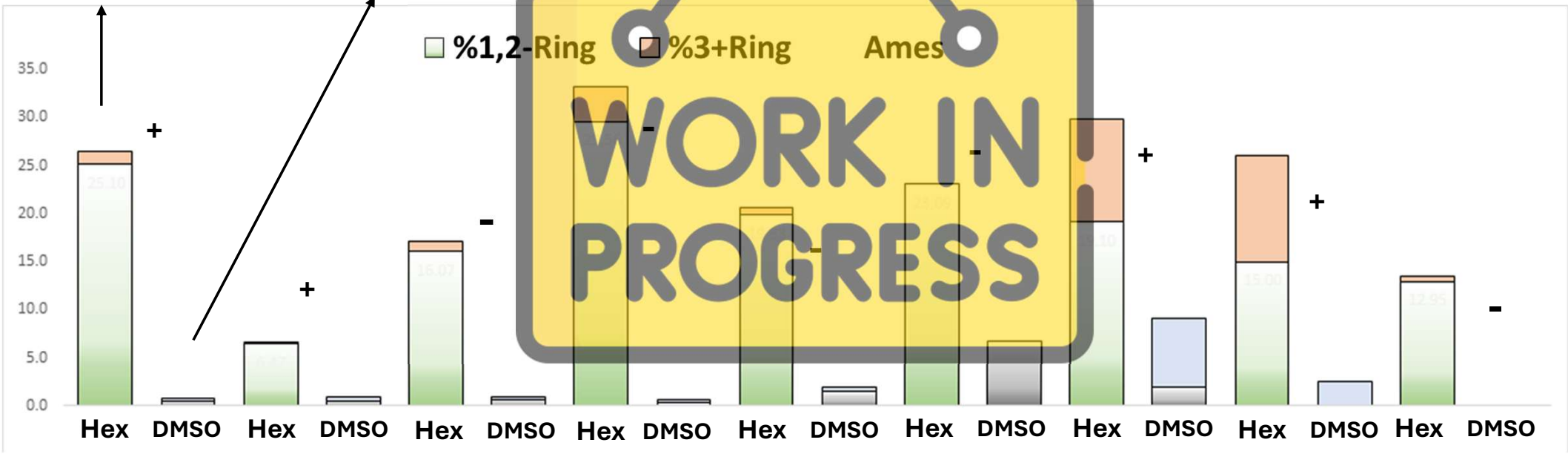
TU Graz

„Sample 76



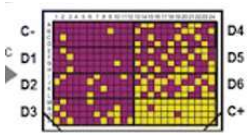
DMSO - 76



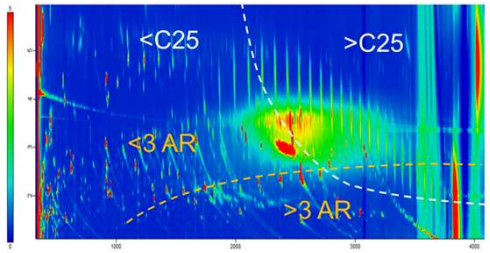


WORK IN PROGRESS

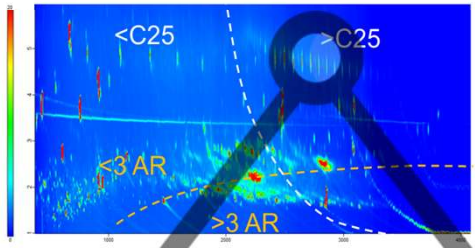
Updates - Toxicology

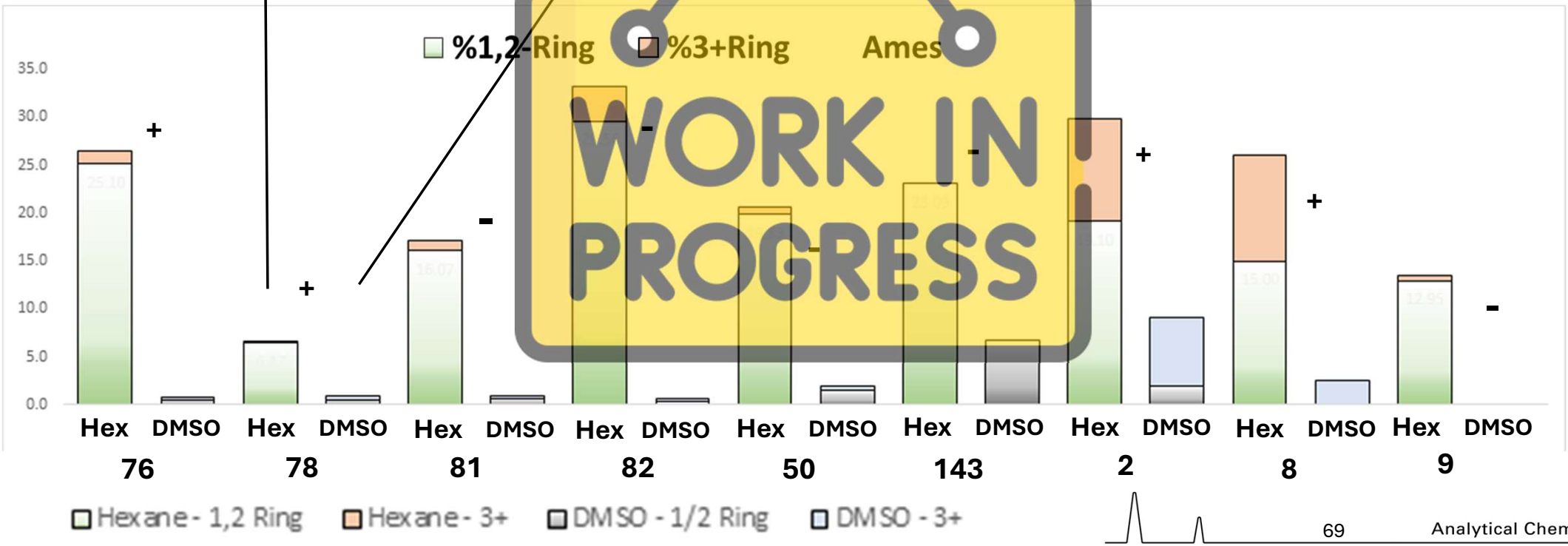


Engine Oil (78)



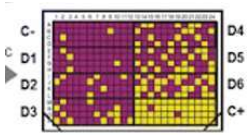
DMSO - 78





WORK IN PROGRESS

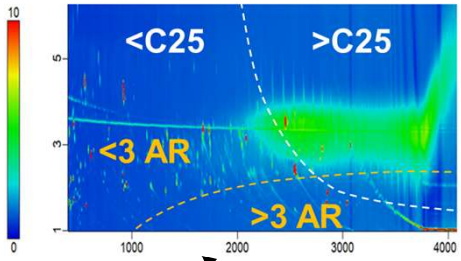
Updates - Toxicology



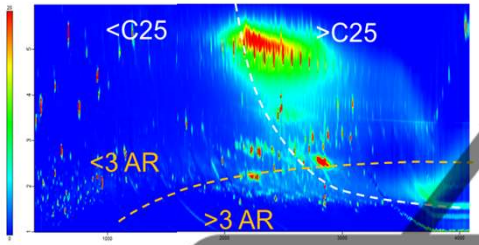
Med Uni Graz

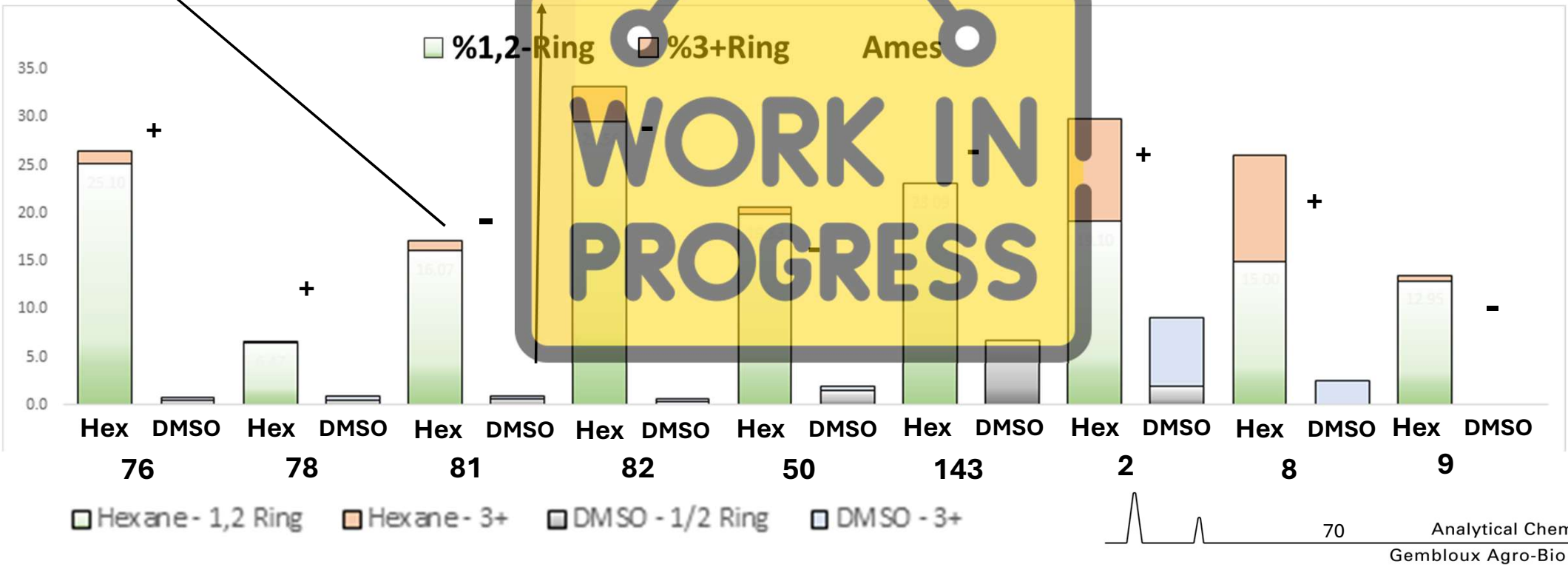
TU Graz

Chainsaw Oil (No 81)



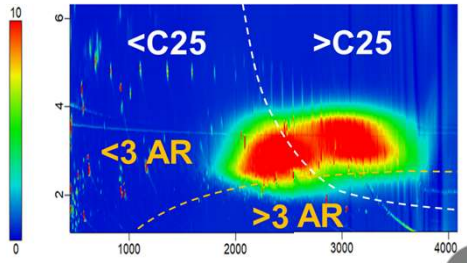
DMSO - 81



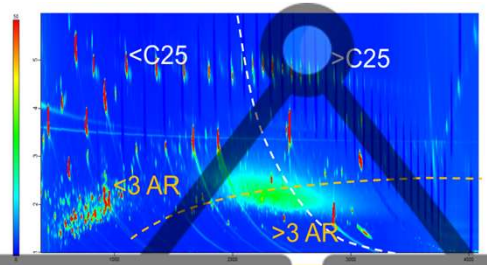


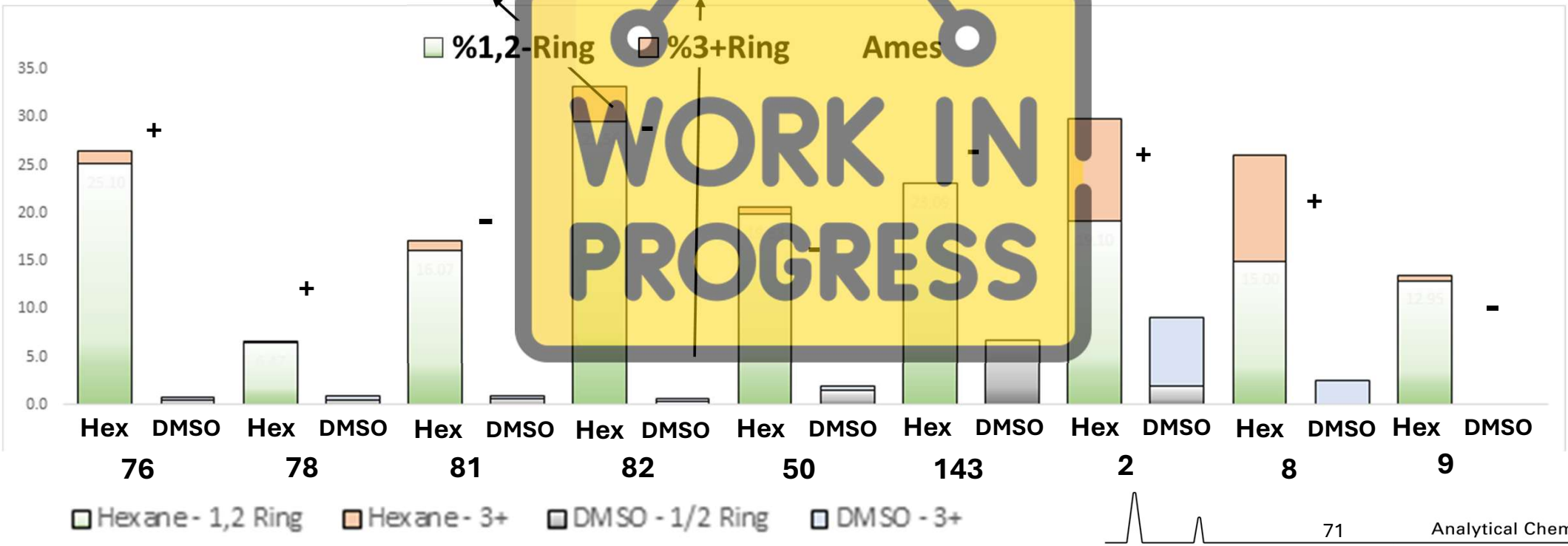
Updates - Toxicology

Multi Purpose Grease (No 82)



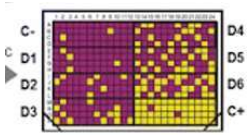
DMSO - 82



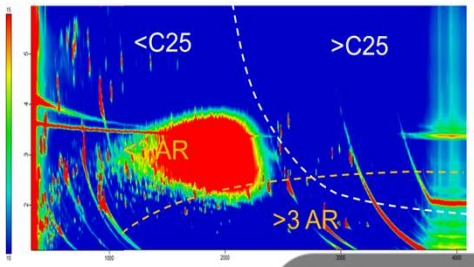


WORK IN PROGRESS

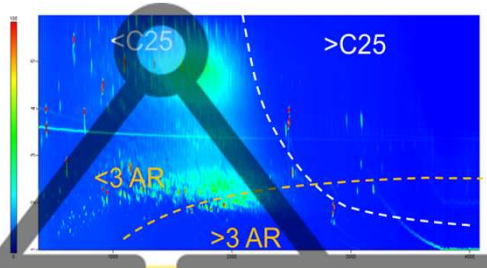
Updates - Toxicology

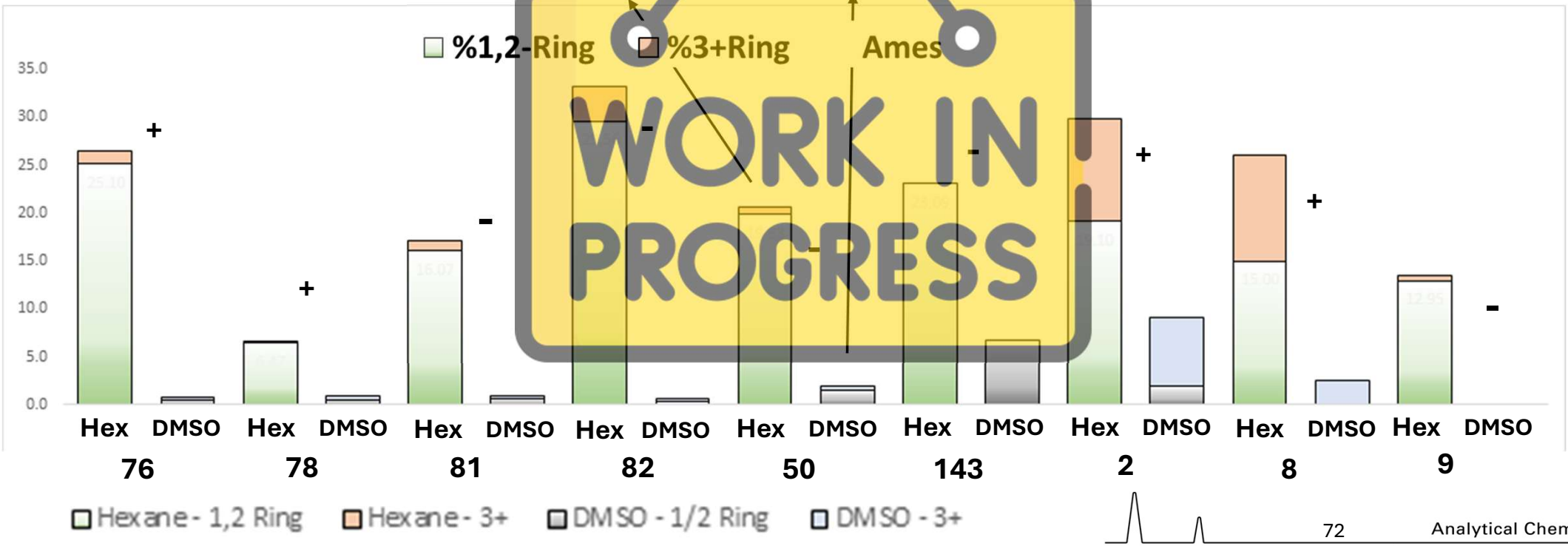


50



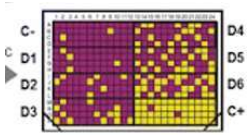
DMSO - 50



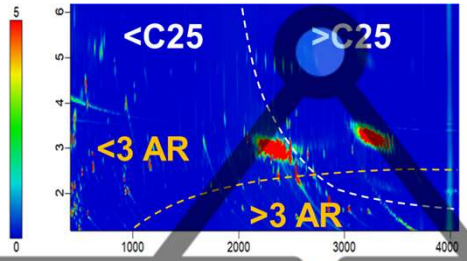


WORK IN PROGRESS

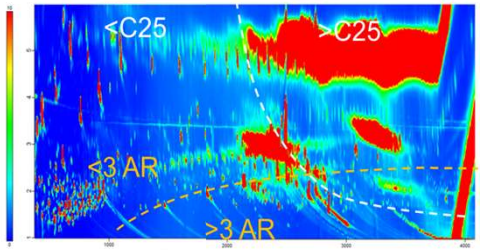
Updates - Toxicology

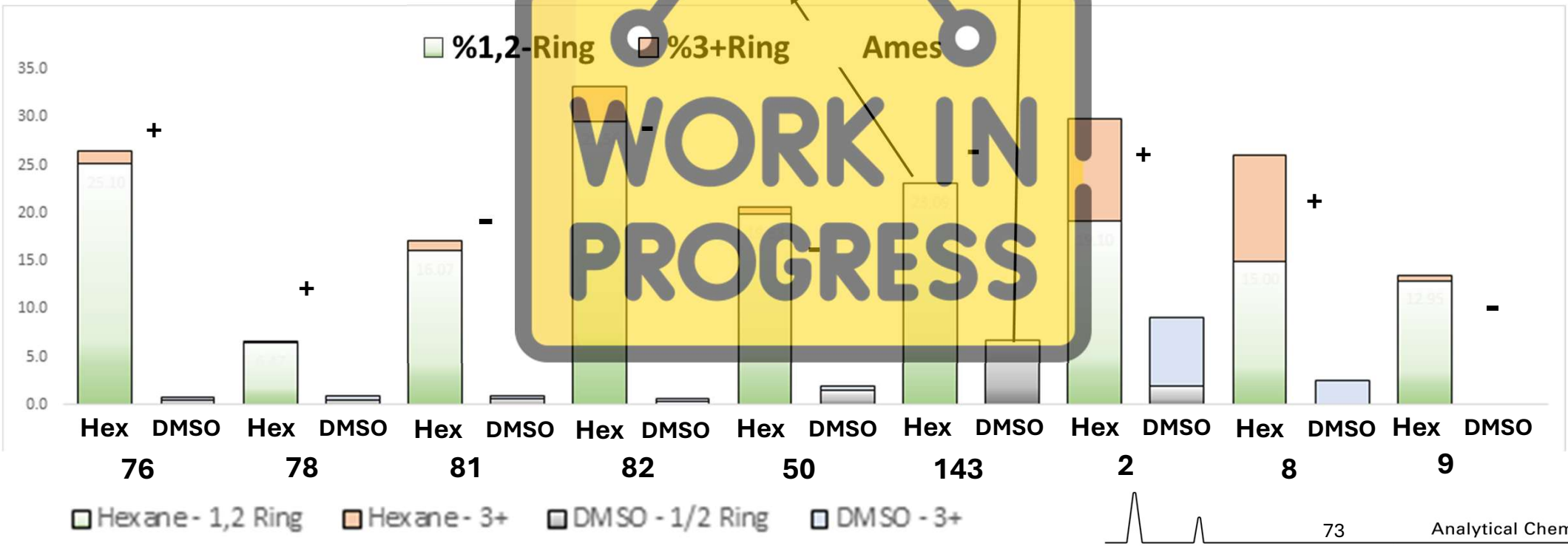


143



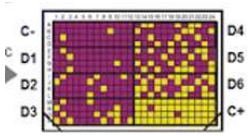
DMSO - 143



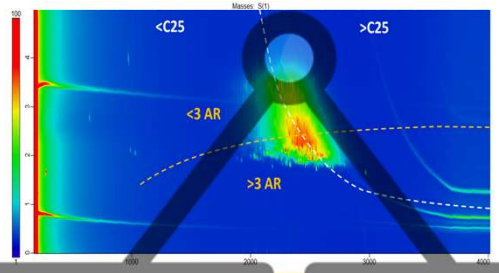


WORK IN PROGRESS

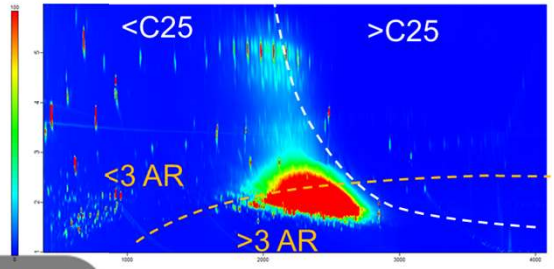
Updates - Toxicology

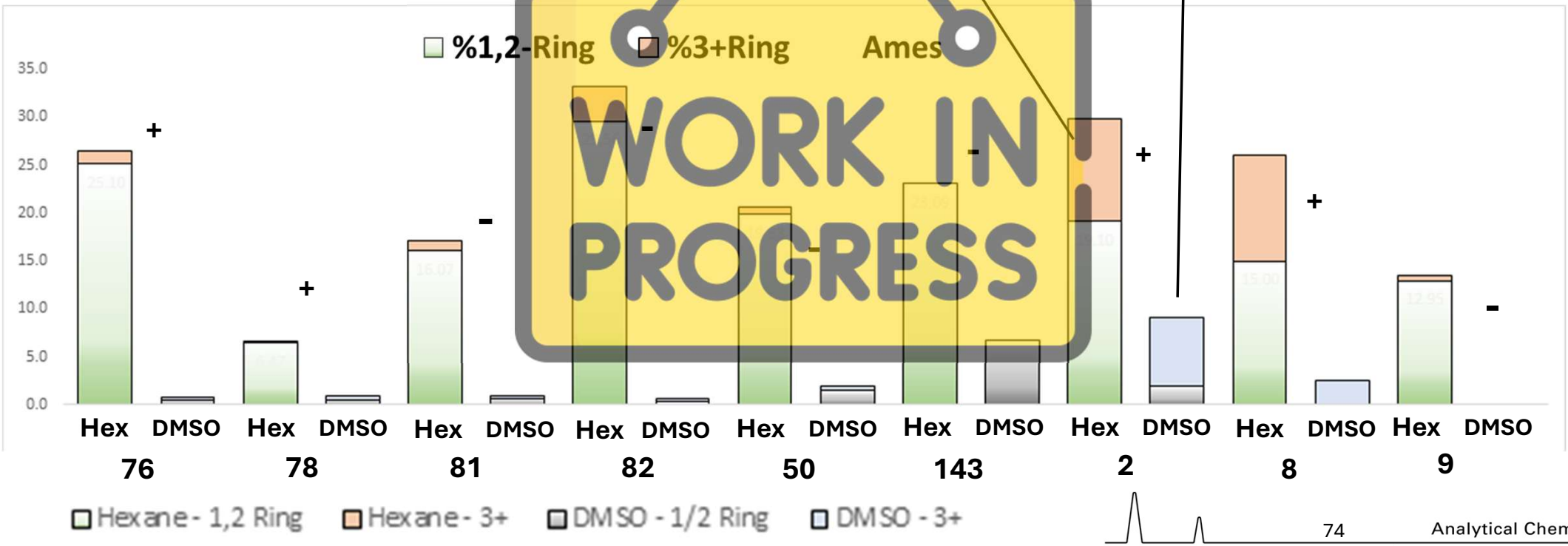


SN100 Distillate (2)



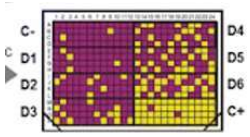
DMSO - 2



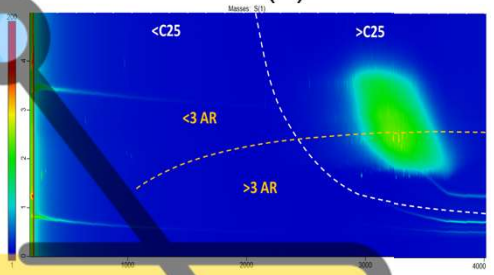


WORK IN PROGRESS

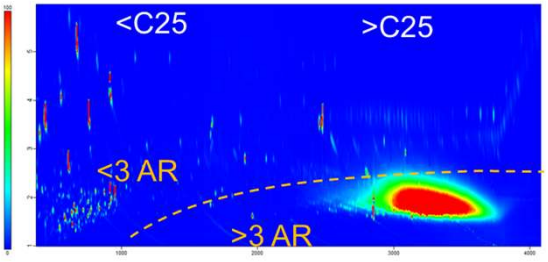
Updates - Toxicology

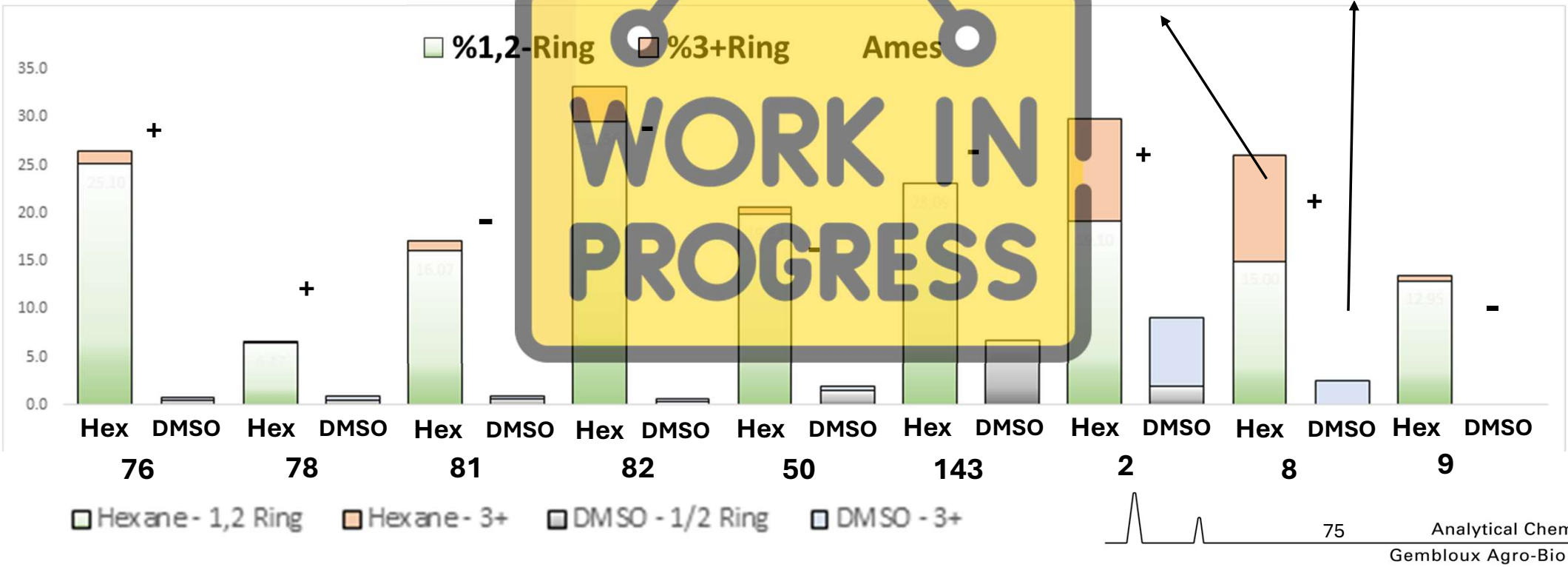


SN500 Distillate (8)



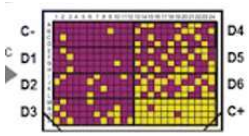
DMSO - 8



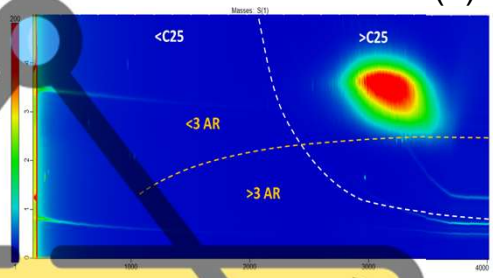


WORK IN PROGRESS

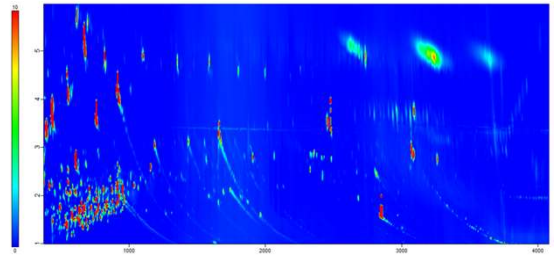
Updates - Toxicology

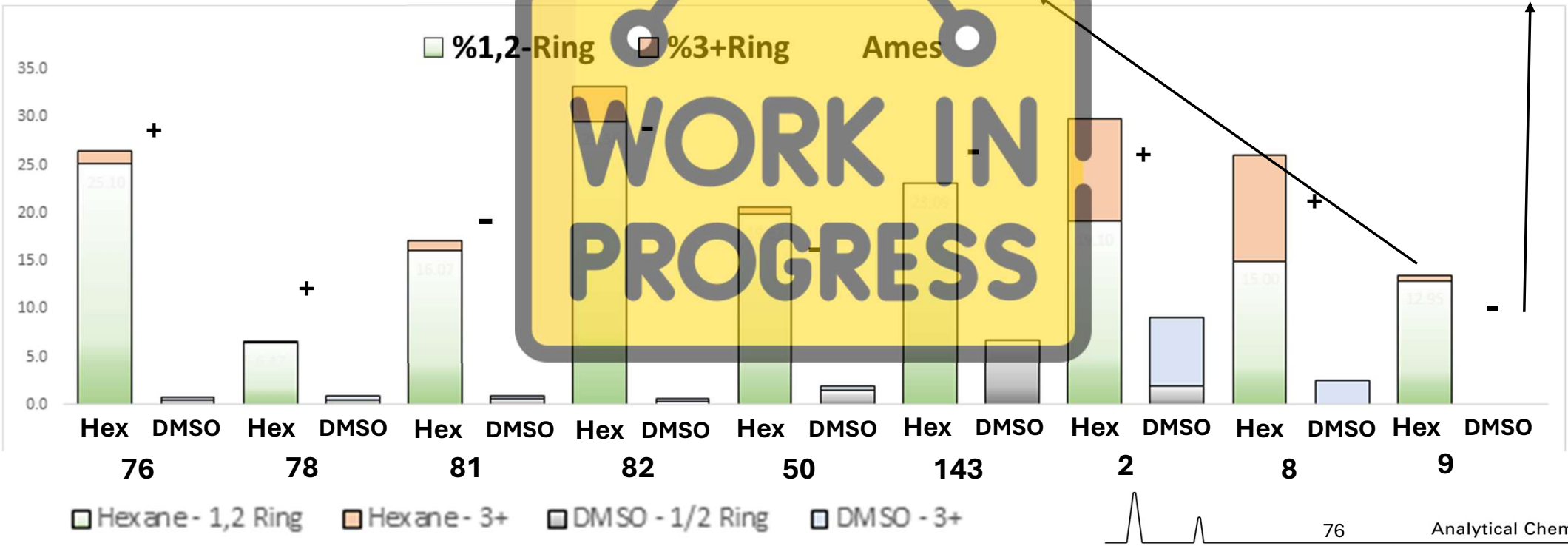


SN500 Medicinal White Oil (9)



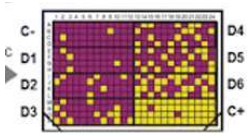
DMSO - 9

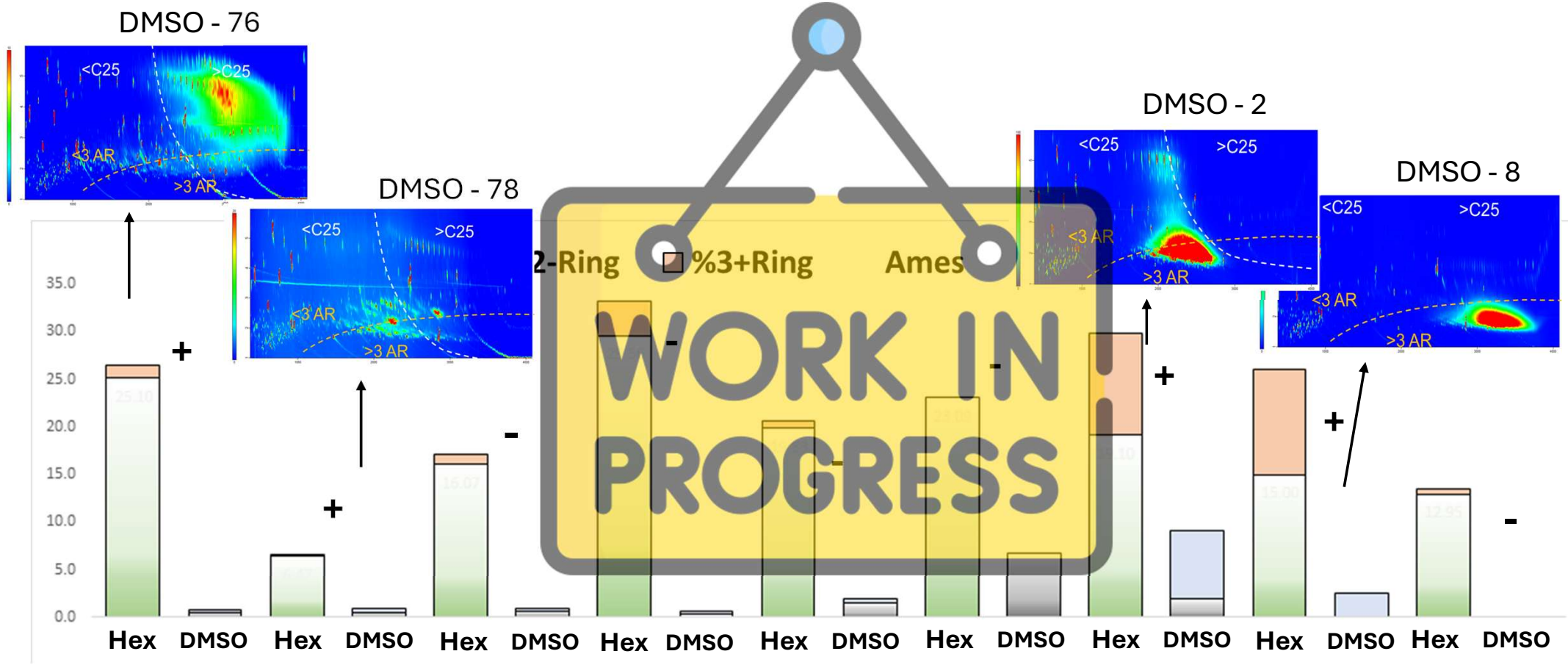




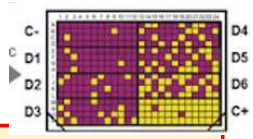
WORK IN PROGRESS

Updates - Toxicology





Updates - Toxicology



DMSO - 82 - DMSO - 50

DMSO - 76

DMSO - 78

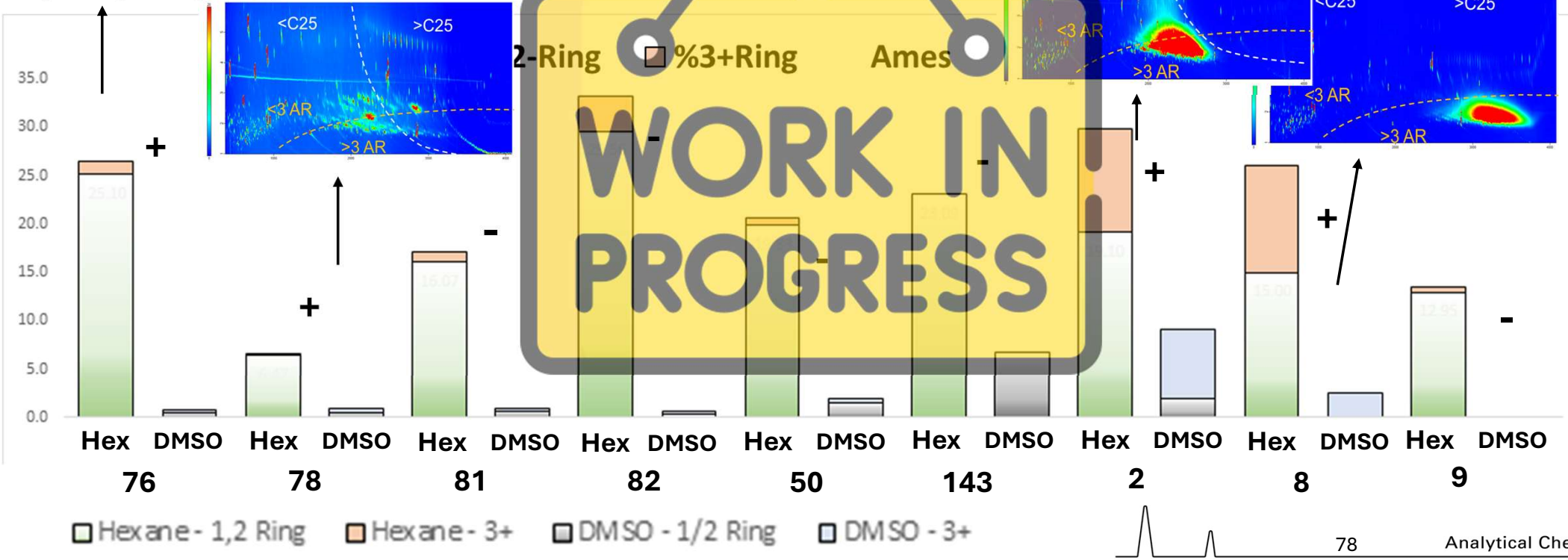
DMSO - 2

DMSO - 8

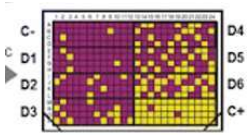
<C25 >C25 <3 AR >3 AR

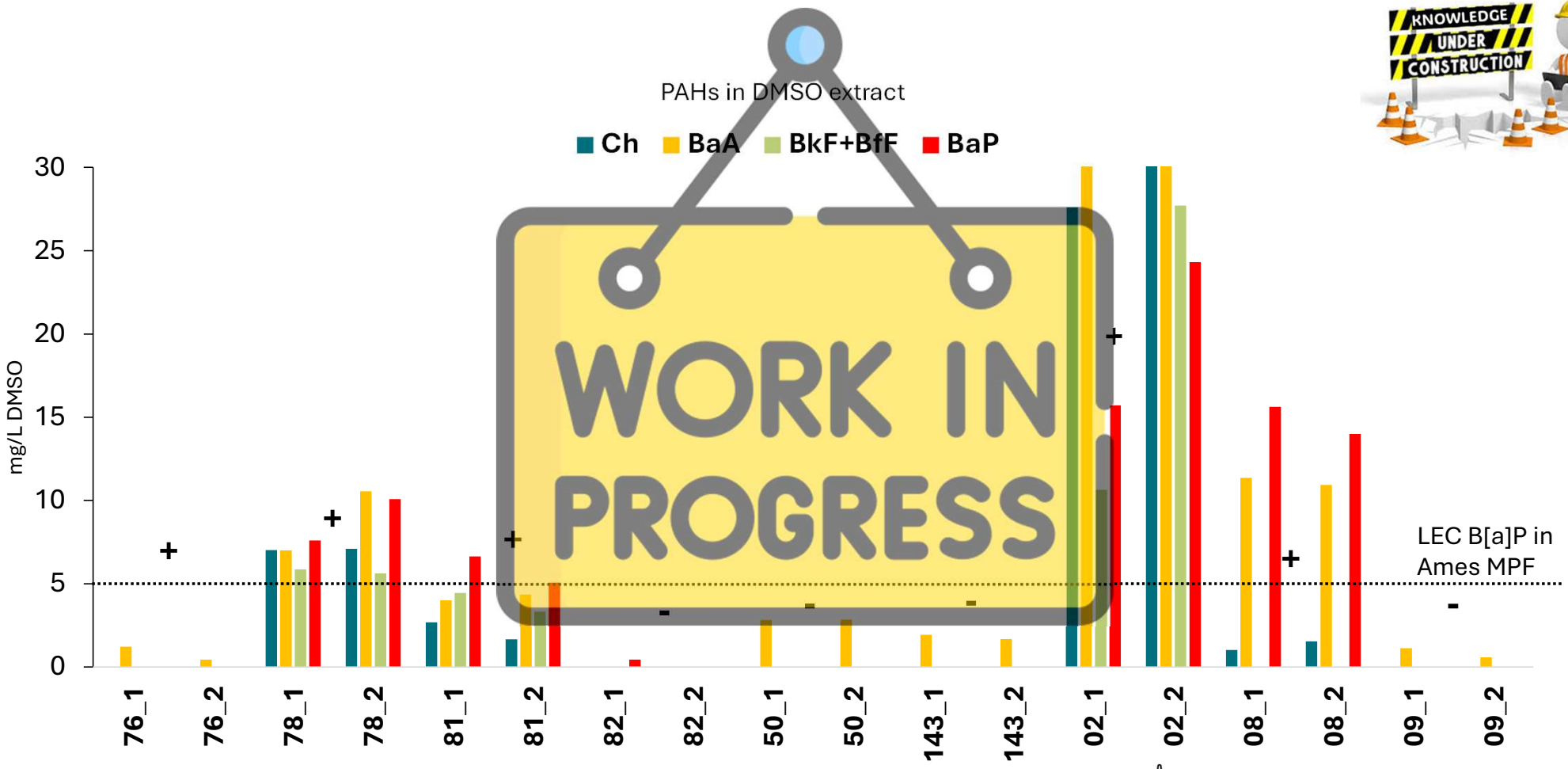
2-Ring %3+Ring Ames

WORK IN PROGRESS

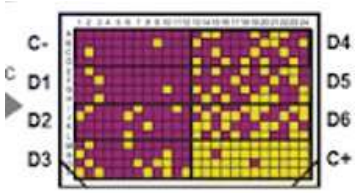


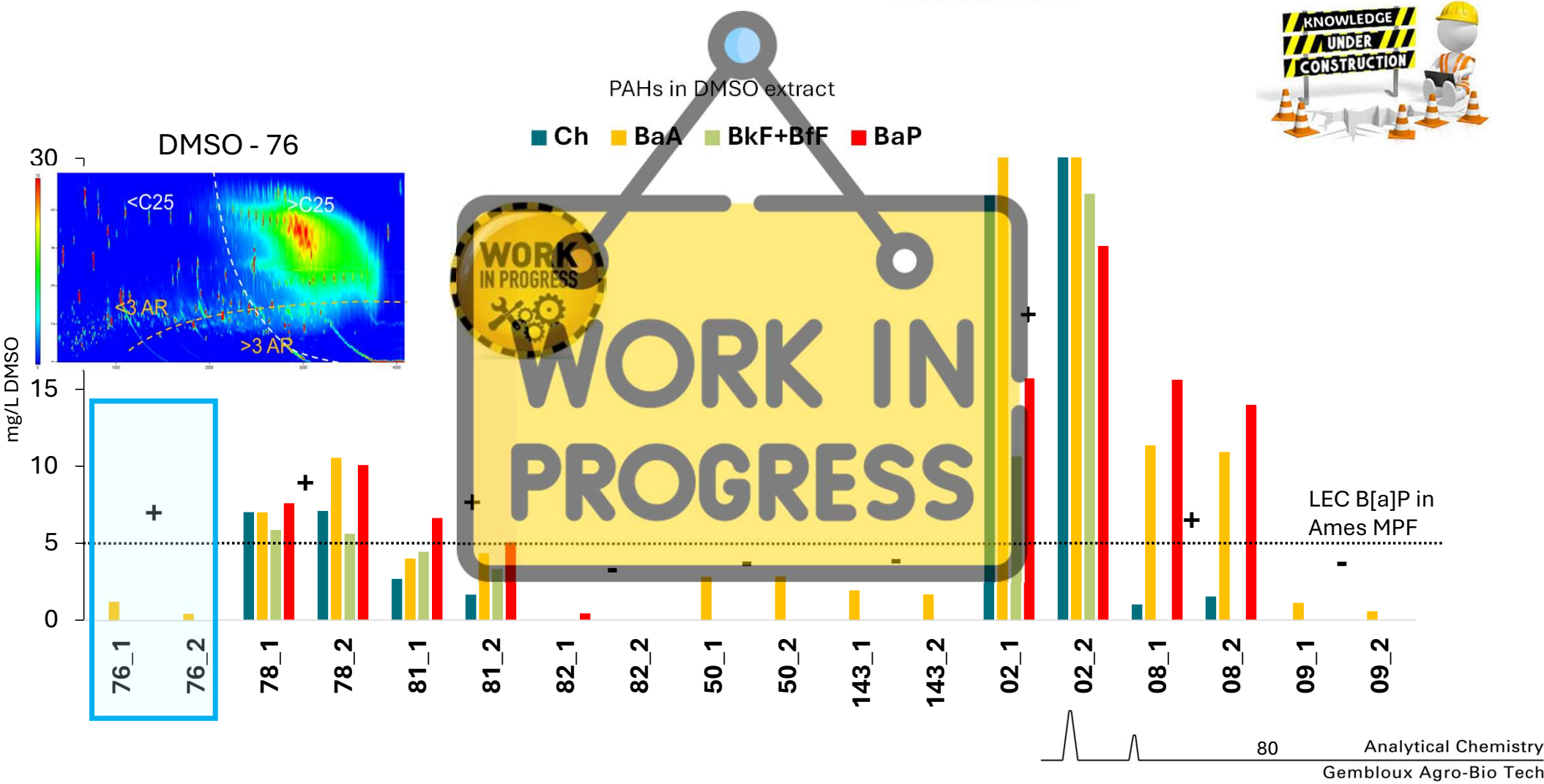
Updates - Toxicology





Updates - Toxicology



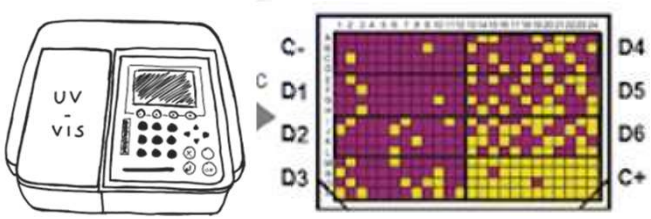


Conclusion

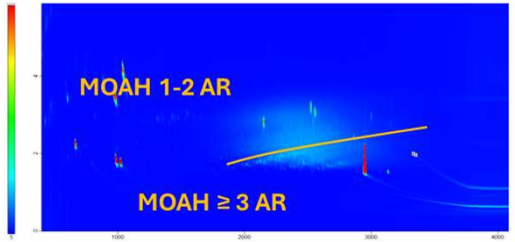
➢ Efforts are still needed to **improve** **SAMPLE PREPARATION** and thus **reducing** **UNCERTAINTY**

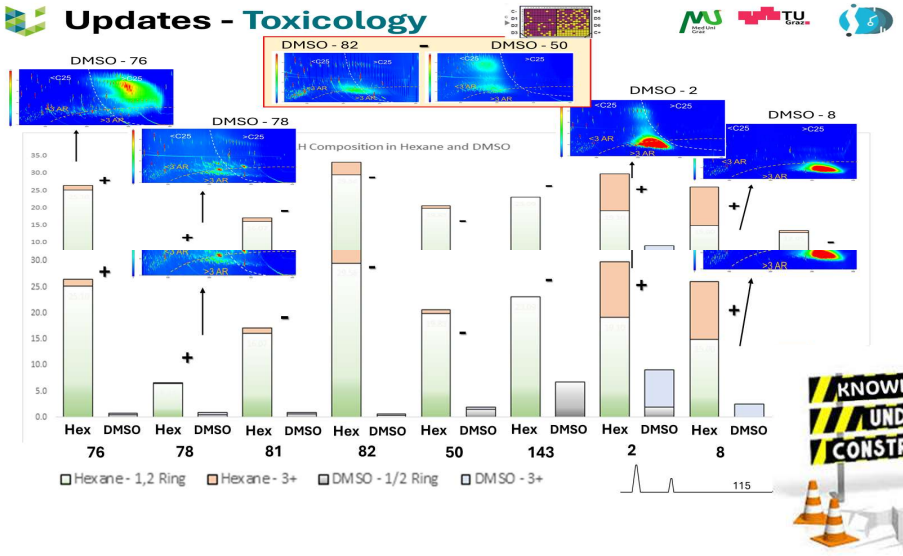
Conclusion - Toxicology

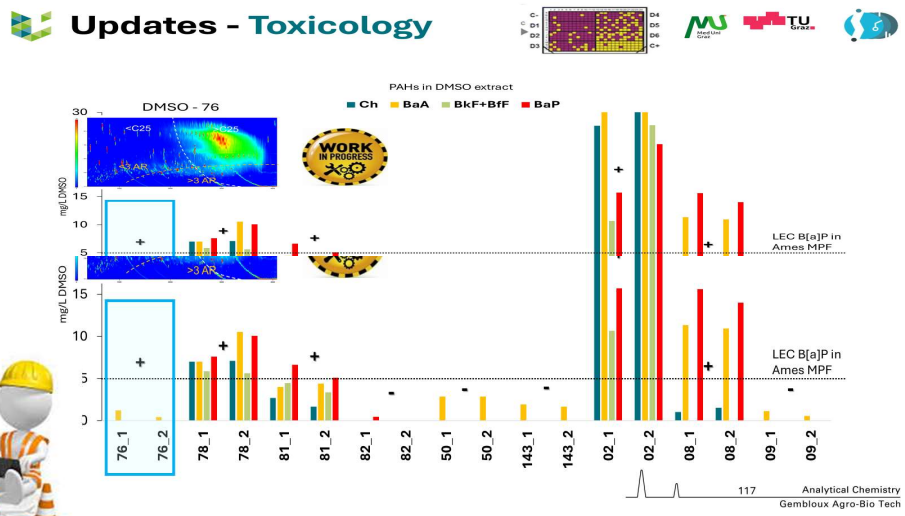
Toxicological prediction



Total MOAH quantification







Conclusion

2023


SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 July 2023

doi: 10.2903/j.efsa.2023.8215

Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

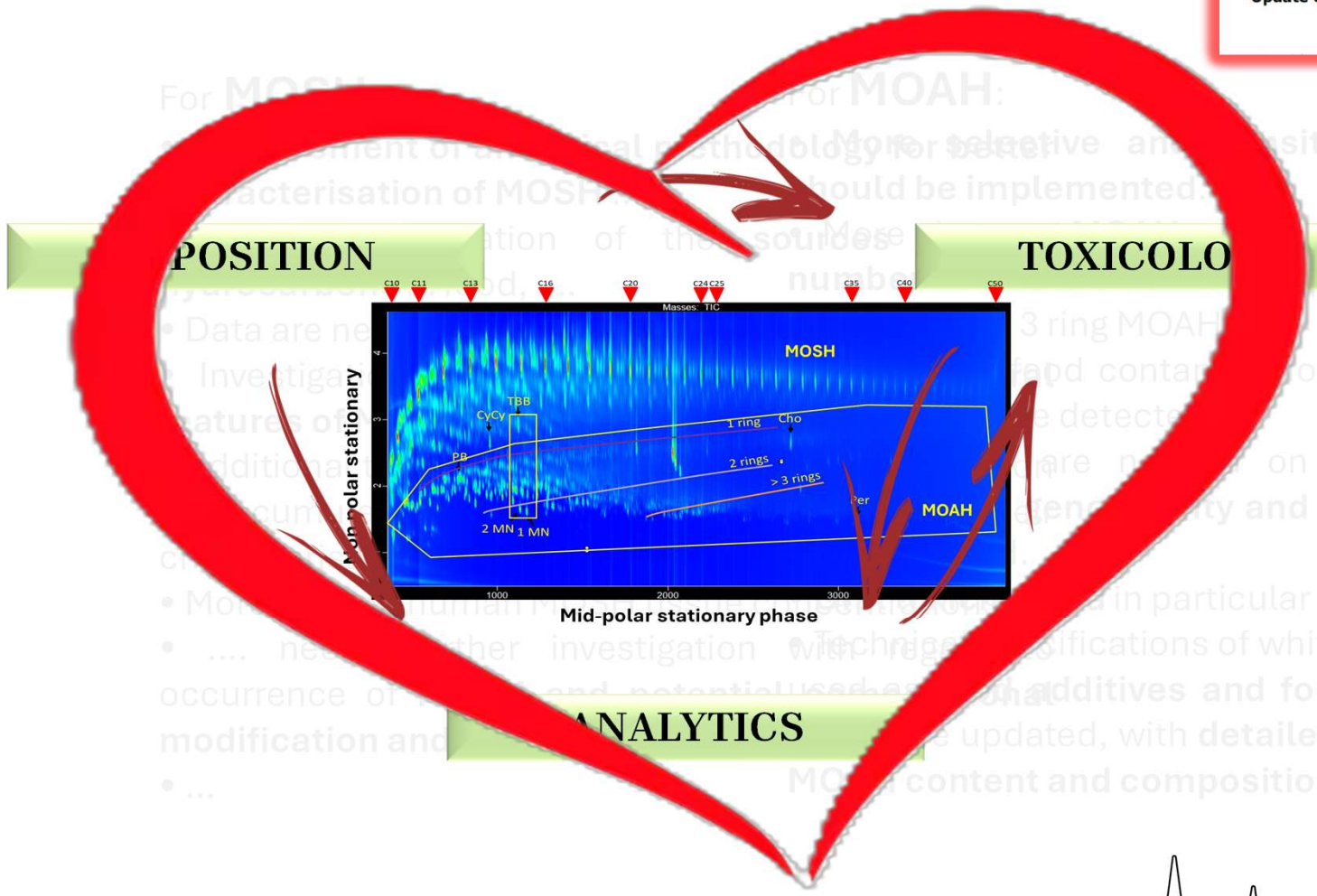
For **MOSH** :

- **Improvement of analytical methodology for better characterisation of MOSH** .....
- Better investigation of the **sources of the hydrocarbons** in food, ...
- Data are needed on ... **accumulation potential**.
- Investigation is recommended on the **structural features of MOSH** that ... result **in accumulation**.
- Additional toxicity data are needed ... in particular on bioaccumulating MOSH following their characterisation.
- More data on human MOSH tissue concentrations.
- .... needs further investigation with regard to occurrence of **MOSH and potential compositional modification and bioaccumulation**.
- ...

For **MOAH**:

- **More selective and sensitive analytical method should be implemented**.
- More data on **MOAH composition by aromatic ring number** in food are needed, in particular with respect to the levels of ≥ 3 ring MOAH.
- Sources of food contamination should be investigated when MOAH are detected.
- More data are needed on the **influence of ring alkylation on genotoxicity and carcinogenic** potency of ≥ 3 ring MOAH.
- **Oral toxicity data** in particular of **1–2 ring MOAH**.
- Technical specifications of white mineral oils and waxes used as **food additives and food packaging materials** should be updated, with **detailed information about the MOAH content and composition**.

Conclusion



SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 July 2023

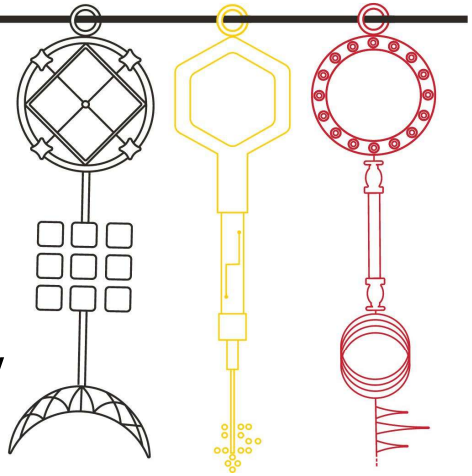
doi: 10.2903/j.efsa.2023.8215

Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

JOIN

ExTech 2026



6-9 JULY 2026
GEMBLOUX AGRO-BIO TECH

BECAUSE SCIENCE IS THE KEY

LIÈGE université Gembloux Agro-Bio Tech

https://www.extech2026.uliege.be

ACESSS
Academic Center of Excellence in Separation Science and Sensing

Thank you for the attention

Research team

<u>Giorgia Purcaro</u>
Sophie Vancraenenbroeck
<u>Aleksandra Gorska</u>
<u>Damien Pierret</u>
Damien Eggermont
<u>Carlo Bellinghieri</u>
Donatella Ferrara
Nicola Ruin
Chaimae Chaibi

- AOCS - **ANNUAL MEETING & EXPO** Hyatt Regency New Orleans, New Orleans, Louisiana, USA
May 3-6, 2026