



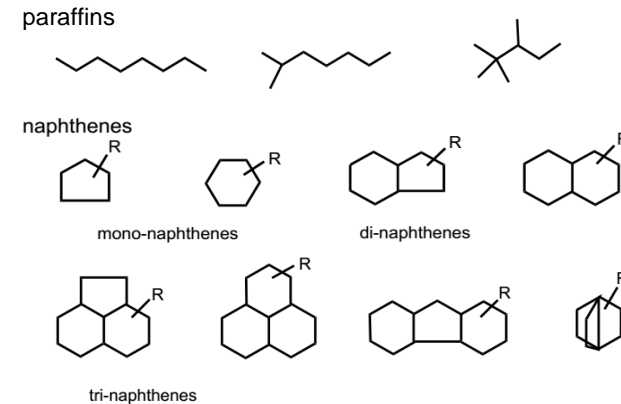
Application of the Migratox method for mineral oil risk assessment

Mineral Oil Hydrocarbons

...are a complex mixture of hydrocarbons originating from crude oil. They are divided into MOSH & MOAH.

MOSH (mineral oil saturated hydrocarbons)

- Paraffins (linear and branched alkenes)
- Naphthenes (alkyl-substituted cycloalkenes)

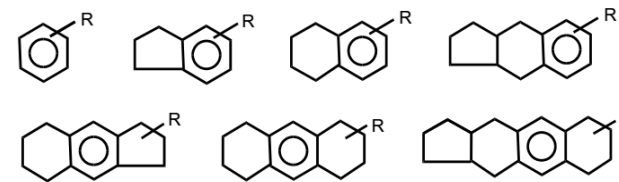


No indications for
DNA-reactive,
genotoxicity

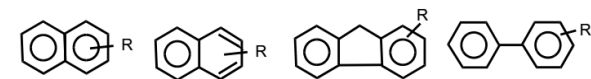
MOAH (mineral oil aromatic hydrocarbons)

- alkyl-substituted mono- and polyaromatic compounds
- non-alkylated PAHs in small amounts
- Non-alkylated PAHs (with 3-7 rings) are potentially mutagenic / carcinogenic

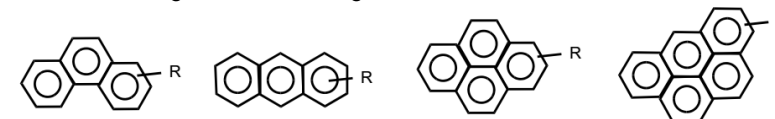
Monoaromatics (1 aromatic ring)



Diaromatics (2 aromatic rings)



Three-, and higher aromatic rings



Potential DNA-
reactive mutagens /
carcinogens

Extraction of MOH from food sample

Separation of MOSH & MOAH on HPLC

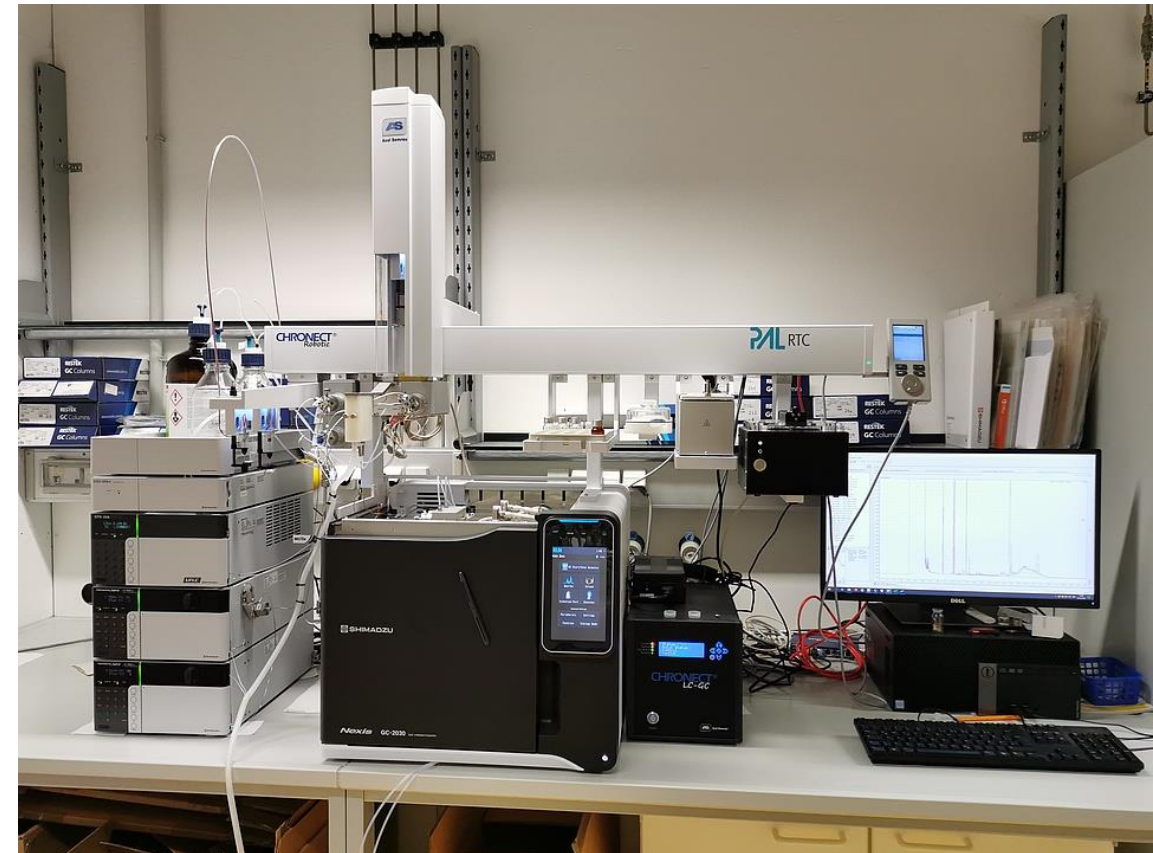
- fractionation is controlled by standards

Further “separation” with GC

- high volume injection (450µL)
- “separation” and identification of interferences

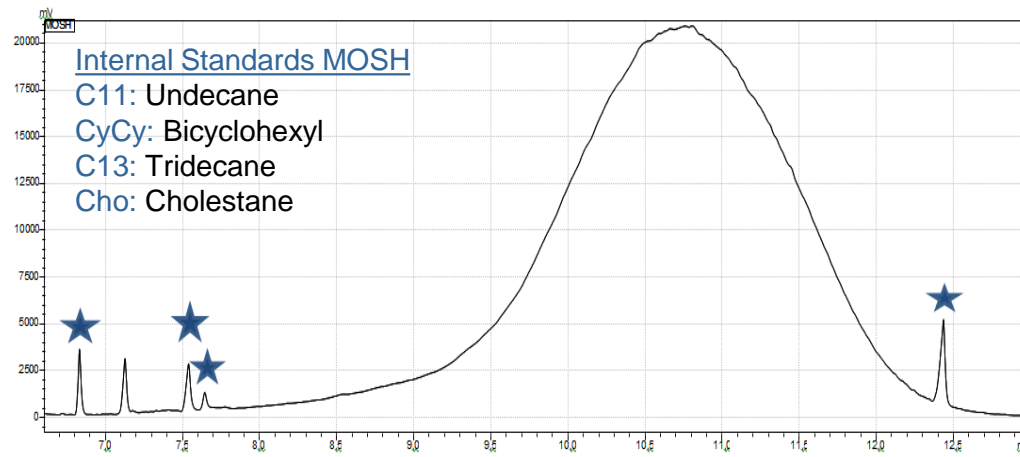
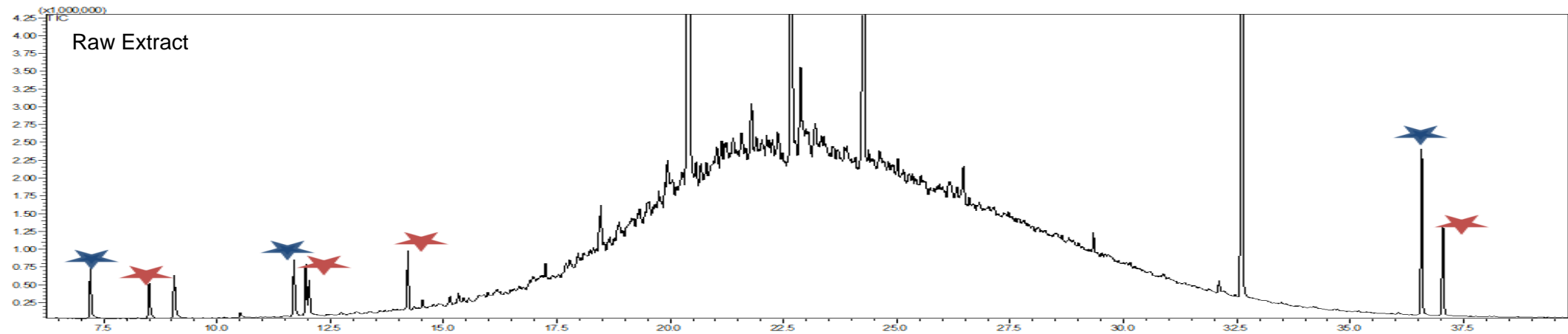
FID - detection

- without calculation of response factors
- wide linear range
- no change in sensitivity over a long period

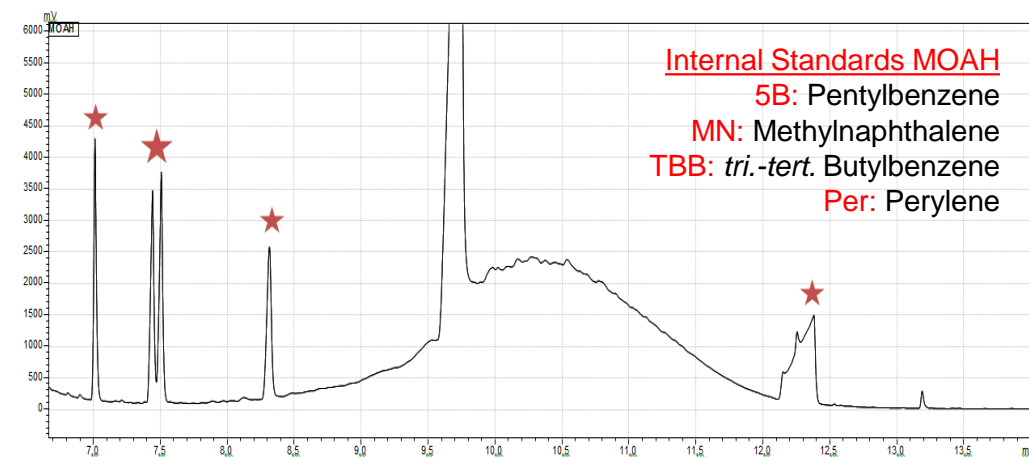


M. Biedermann, K. Grob. *Journal of Chromatography A*, 1255 (2012) 56-75.

Example Recycled Paper



MOSH Fraction



MOAH Fraction

Report defines **harmonised LOQs** for different food groups to have common levels **for enforcement in the EU**.
The JRC maximum LOQs are interpreted as „**limits**“ **for total MOAH**.

If MOAH is detected above this limit: **Recall of the products!**

Matrix	Fat content	MOAH Limit
Dry foods with a low fat content	$\leq 4\%$	0.5 mg/kg
Foods with a higher fat content	$> 4\%$ and $\leq 50\%$	1 mg/kg
Fats/oils and foods with $> 50\%$	$> 50\%$	2 mg/kg

Analysis of Austrian Food Samples (~200)

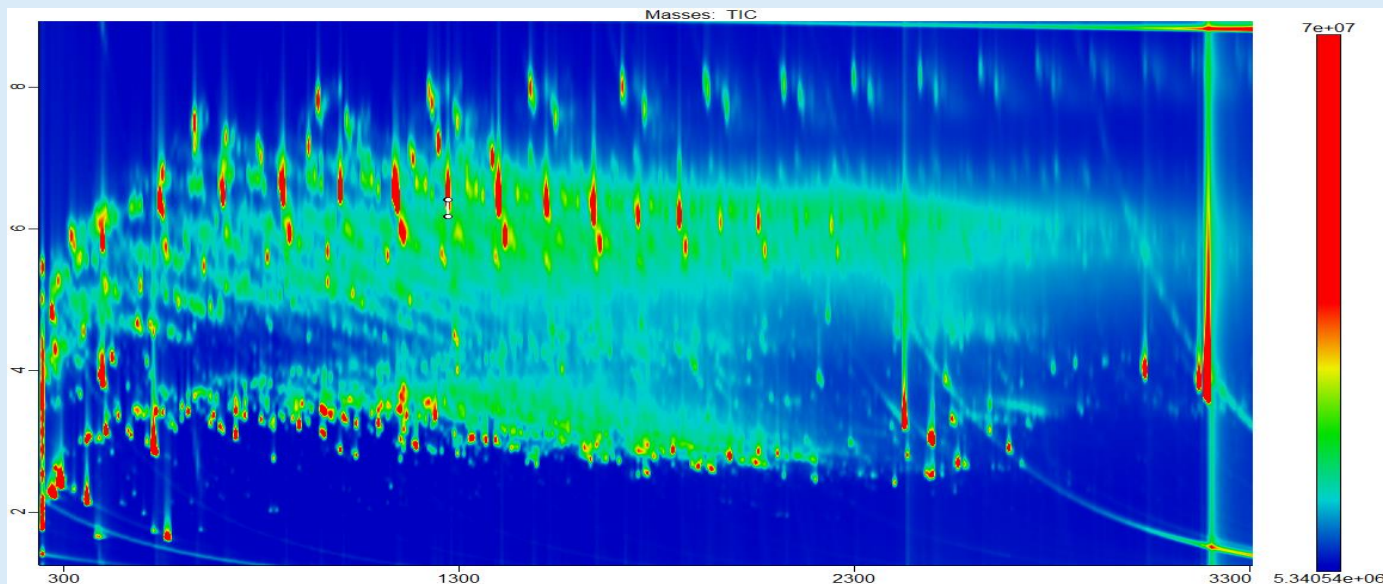
Sample description	#	MOSH (mg/kg)				MOAH (mg/kg)				>Limit
		from	to	Median	Mean	from	to	Median	Mean	
low fat content ($\leq 4\%$ fat/oil): 0.5 mg/kg	55	<0.5	89	1.1	5.33	<0.5	4.5	<0.5	0.65	4
higher fat content (4% - 50 % fat/oil): 1 mg/kg	78	<0.5	60	3.1	9.4	<0.5	17	<0.5	1.14	24
Fats/oils ($> 50\%$ fat/oil): 2 mg/kg	72	<0.5	55	8	11	<0.5	6	<0.5	0.75	7

A closer look: 2D comprehensive GC×GC

A typical mineral oil contamination contains about
~15.000 substances.

What does this mean for human health?

Comprehensive 2D chromatogramm



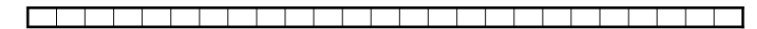
Methodology overview:

1D chromatography

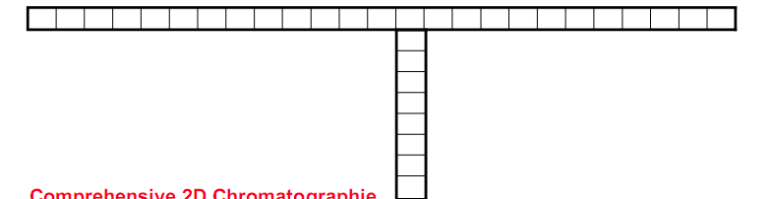
Heart-cut 2D chromatography

Comprehensive 2D chromatography

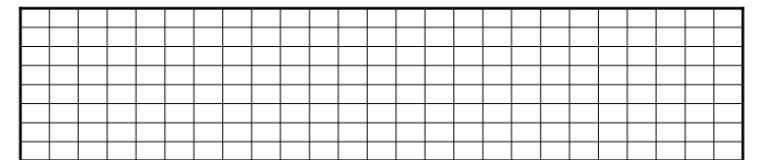
Normale 1D Chromatographie



"Heart-cut" 2D Chromatographie



Comprehensive 2D Chromatographie

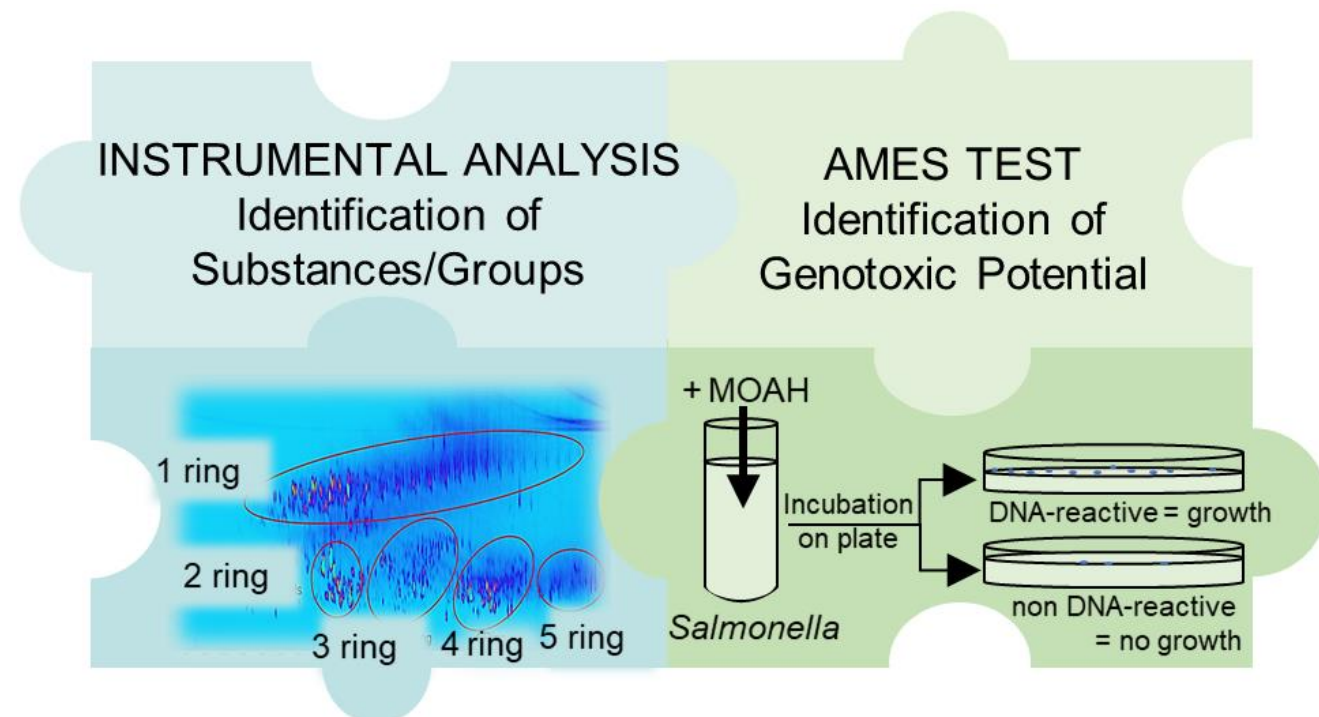


Certain sub-fractions of MOAH may be mutagenic and carcinogenic.

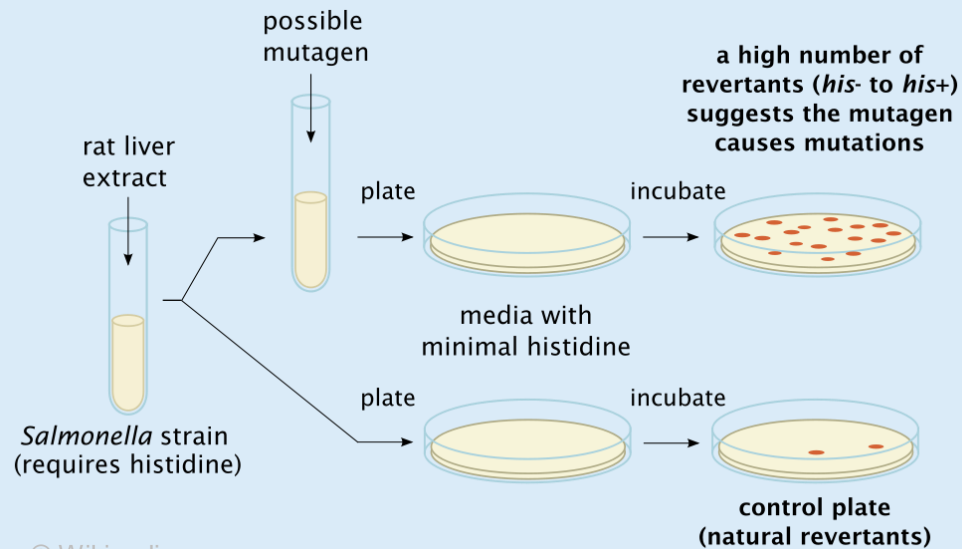
According to EFSA 2012 & 2019: 3-7 ring MOAH more harmful than 1-2 ring MOAH (so far no proof).

Aim:

- Pre-separation of the MOAH by ring number & identification / characterisation of individual substances (classes) by instrumental analysis
- Combination of information from instrumental analysis with bioassays to allow for a comprehensive hazard & risk assessment of MOAH found in food



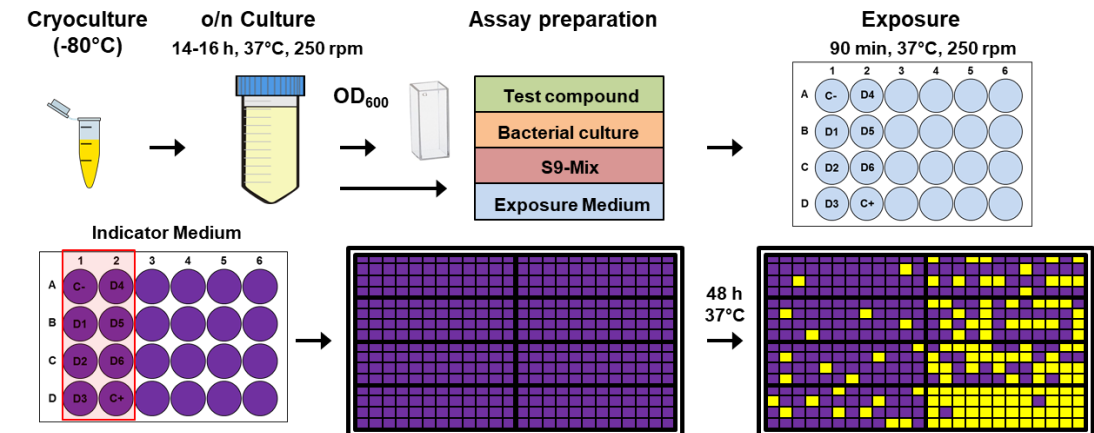
Ames Test



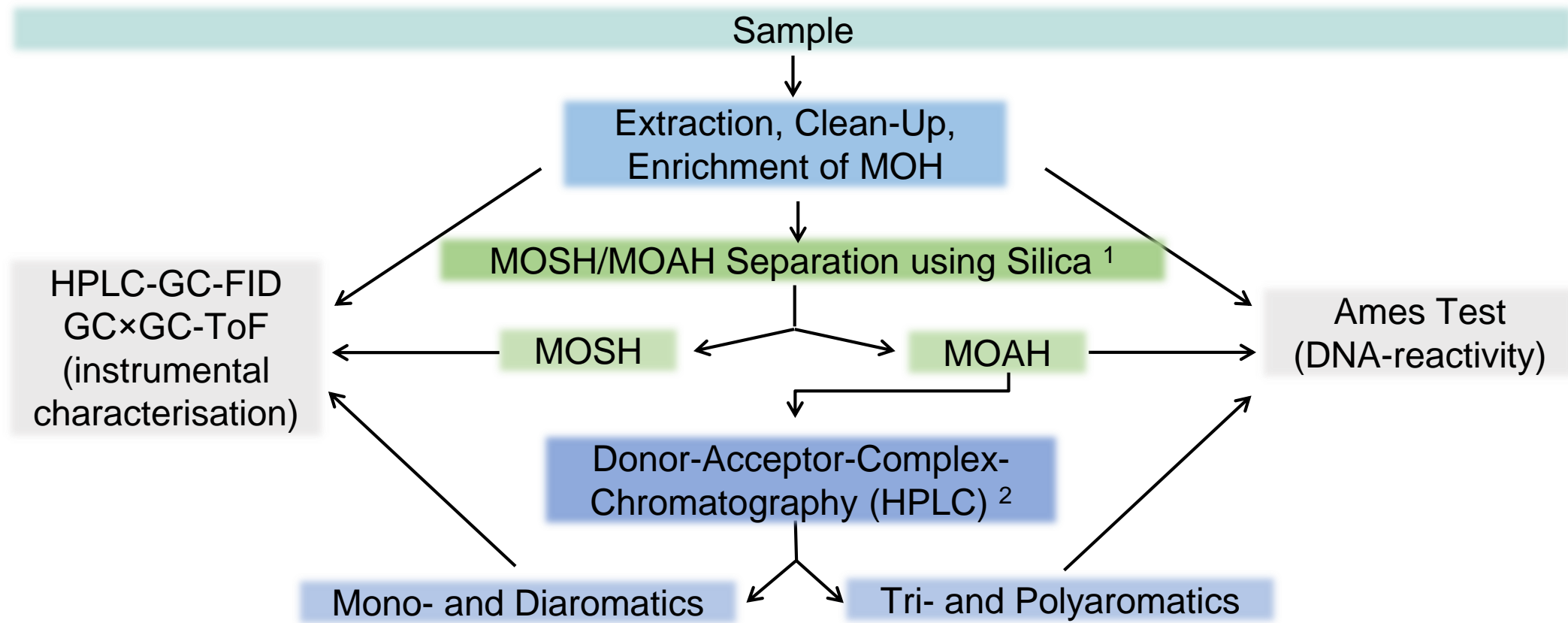
© Wikipedia

- + Sensitive and robust
- + Broad acceptance (OECD Guidelines)
- + Focus on direct DNA-reactive substances
- Labour intensive
- Space consuming

Miniaturized Ames Test (Ames MPF)



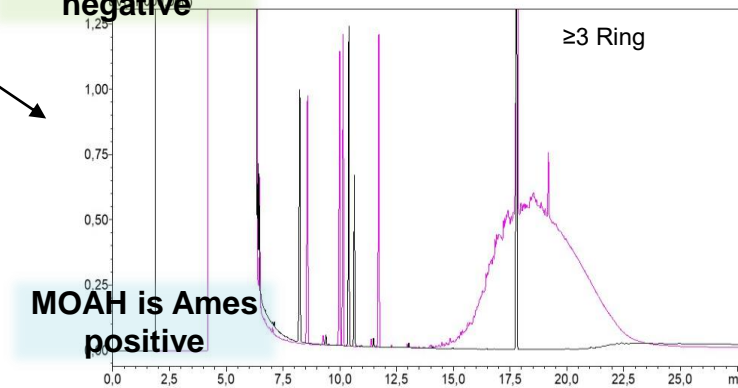
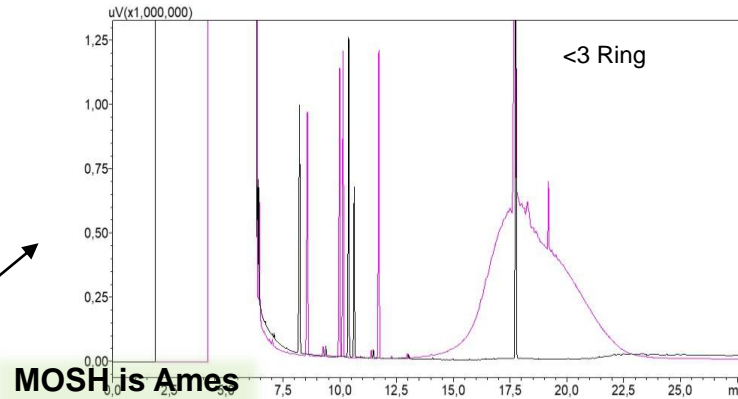
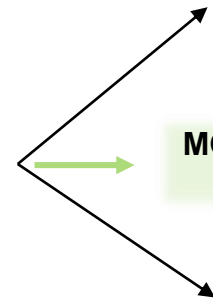
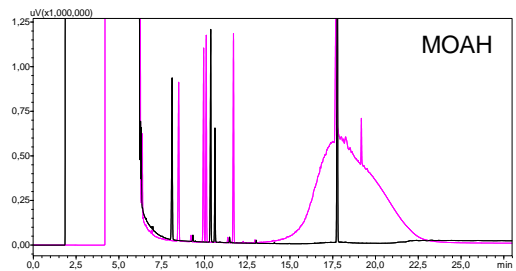
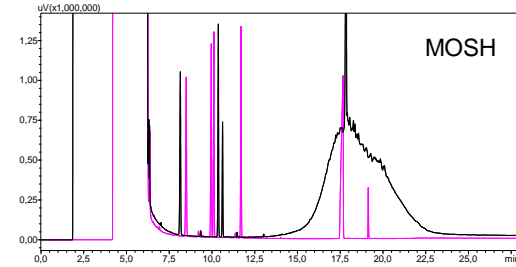
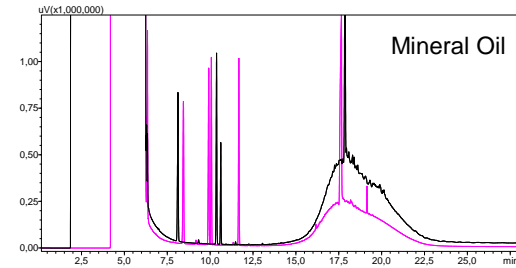
- Cultivation and exposure in liquid medium
- Colorimetric readout
- Higher throughput (microtiter plates)
- + Lower sample amounts required
- + Less incubator space required



1) Fiselier, et.al. (2013), DOI: 10.1016/j.chroma.2012.11.034

2) Koch, et.al. (2020), DOI: 10.1002/jssc.201900833

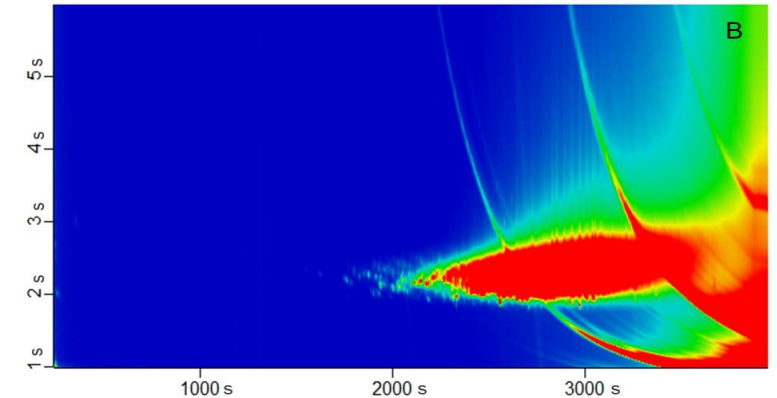
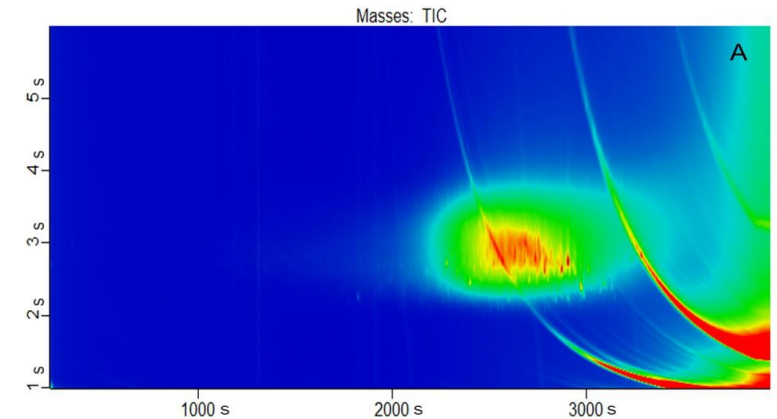
Proof of concept study



MOSH is Ames
negative

MOAH is Ames
positive

HPLC-GC-FID quantification



GCxGC characterisation

Proof of concept study – Ames Test Results

Mono- and diaromatic compounds are **non-mutagenic**.

Tri- and poly aromatic compounds are **mutagenic**.

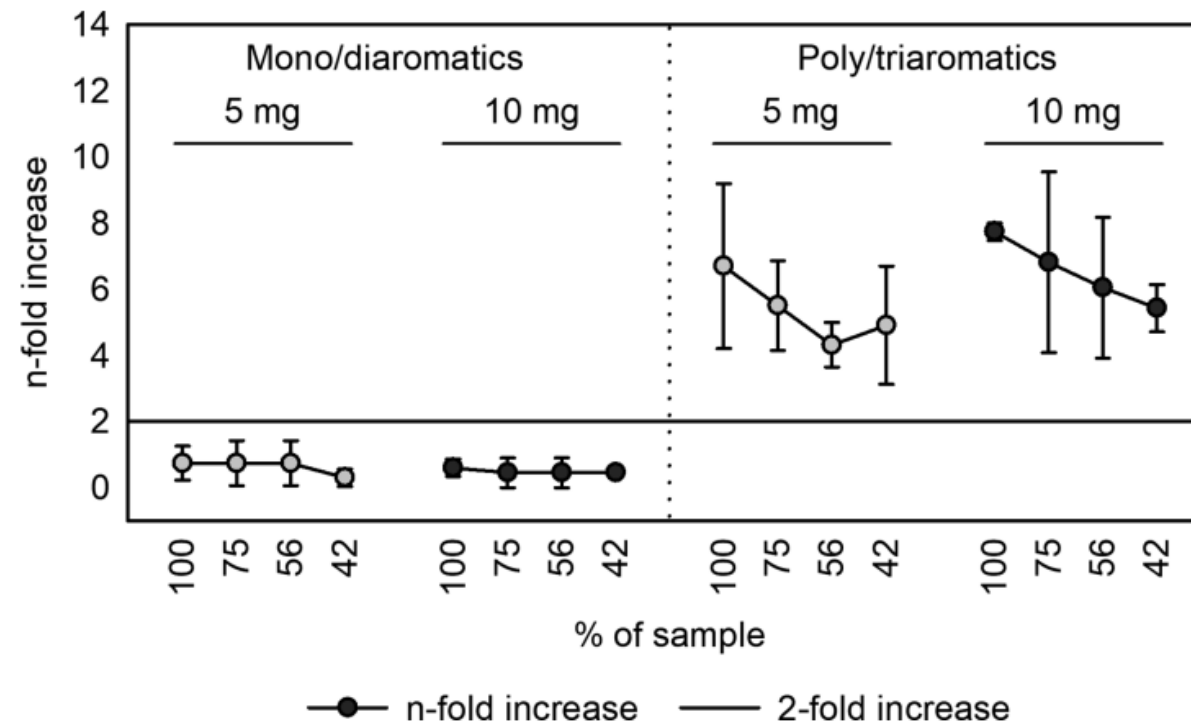
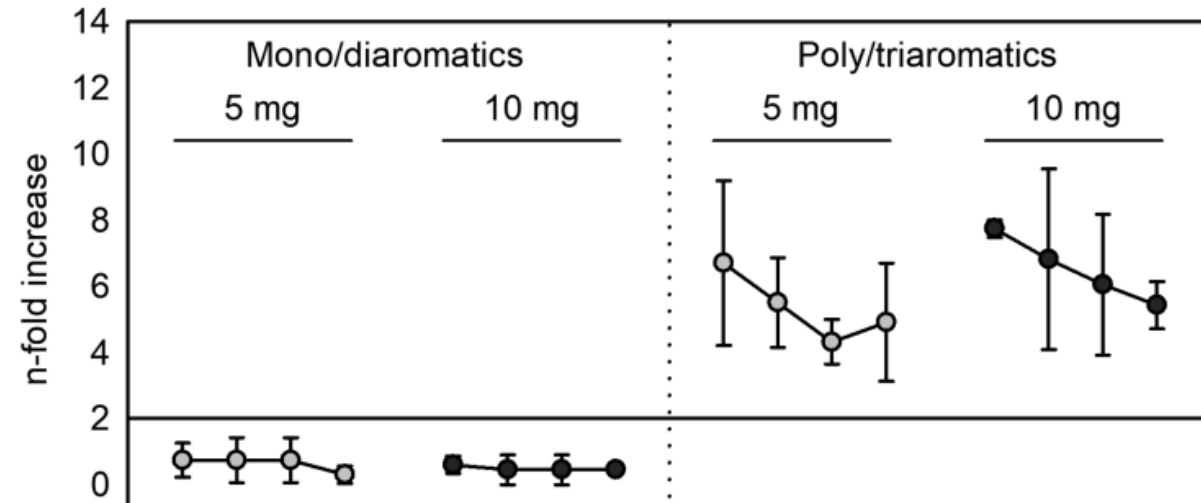


Figure 6. Miniaturized Ames analysis of DMSO extracts prepared from the mono- and diaromatic (left) and tri- and polyaromatic (right) subfraction of a mineral reference oil. Two extraction ratios, 5 mg/250 μ L and 10 mg/250 μ L DMSO, were compared.

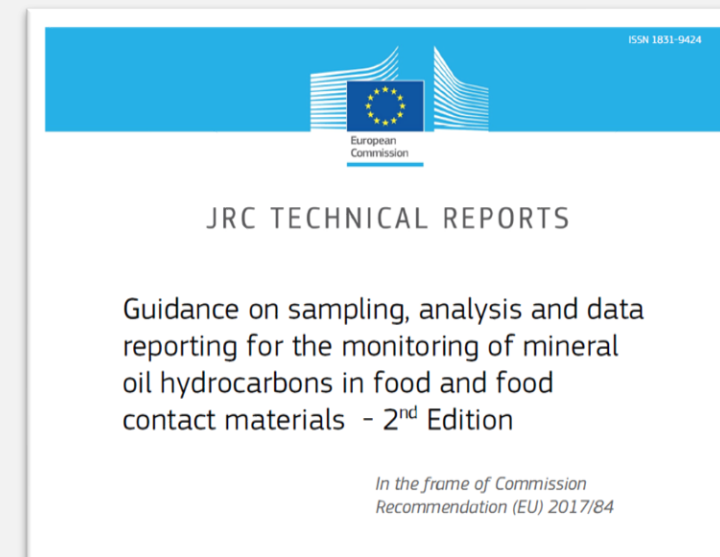
Proof of concept study – Ames Test Results

Mono- and diaromatic compounds are **non-mutagenic**.



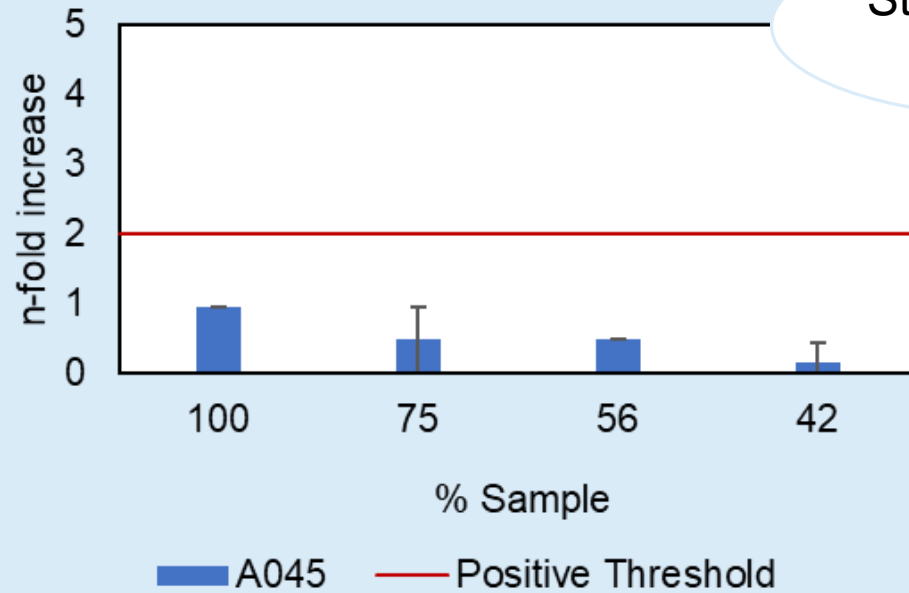
Data published by
OFI & TU Graz:

Included in
EFSA & JRC
documents!



Correlation of Ames MPF and GCxGC results

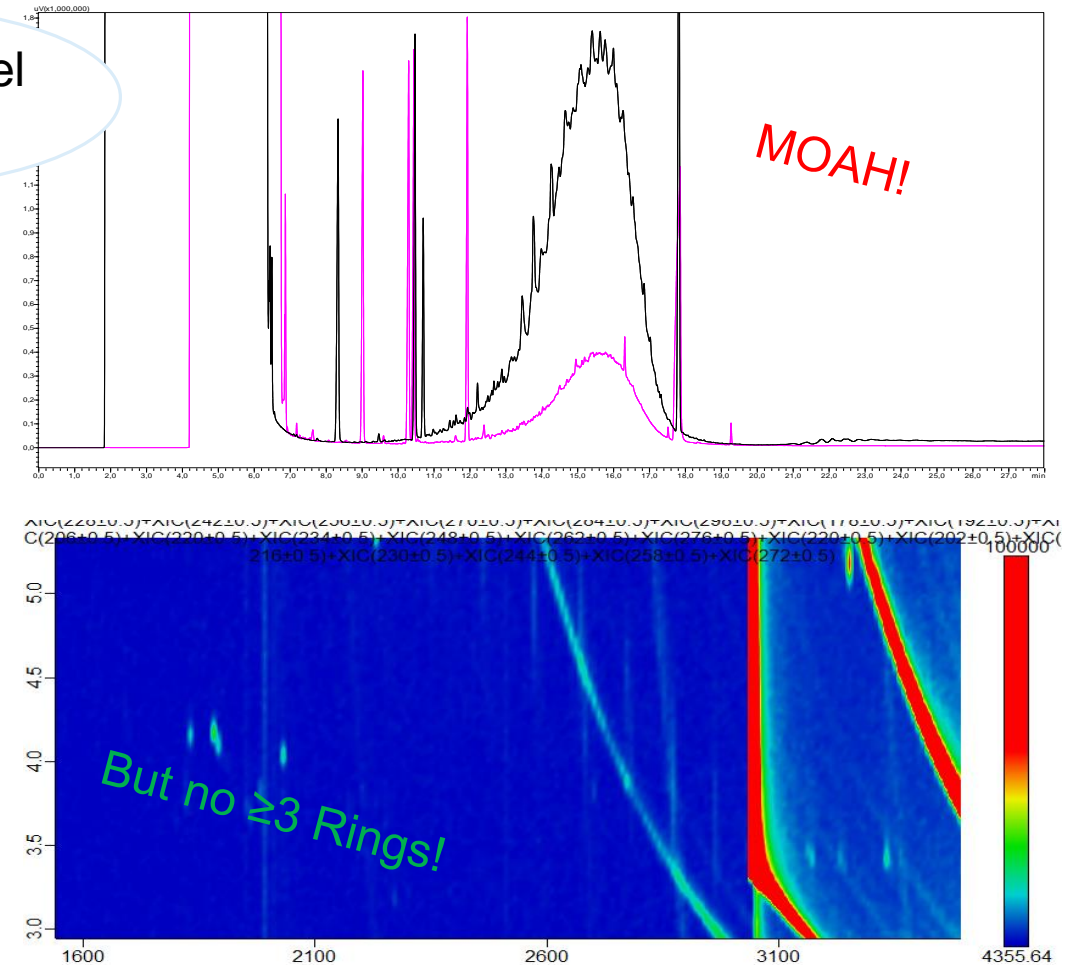
Ames MPF



Negative!

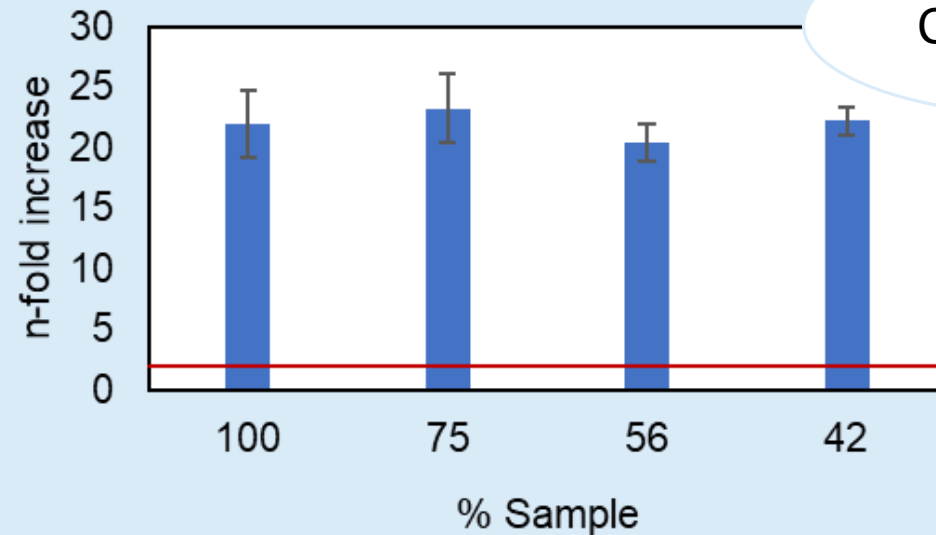
Stainless steel
care

GCxGC



Correlation of Ames MPF and GCxGC results

Ames MPF

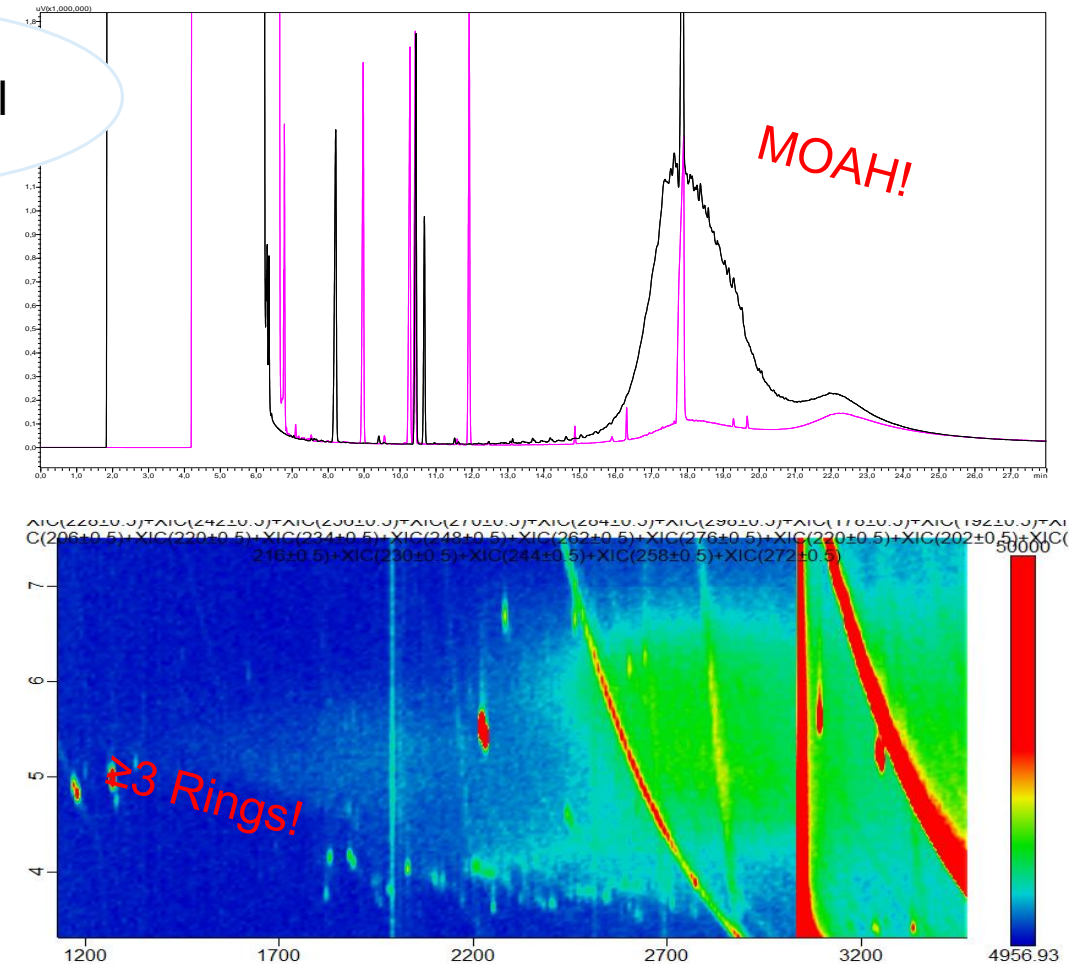


Chainsaw oil

■ A030 — Positive Threshold

Positive!

GCxGC

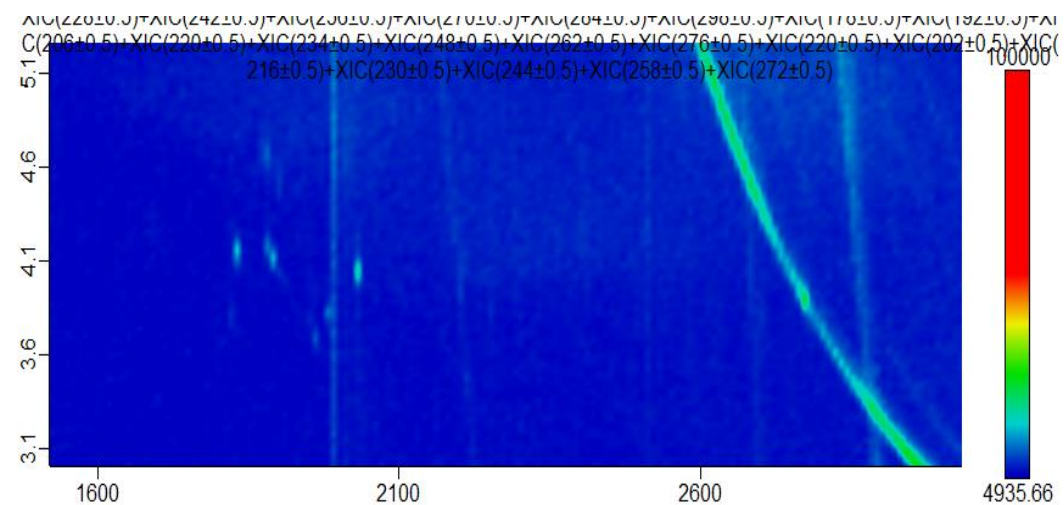
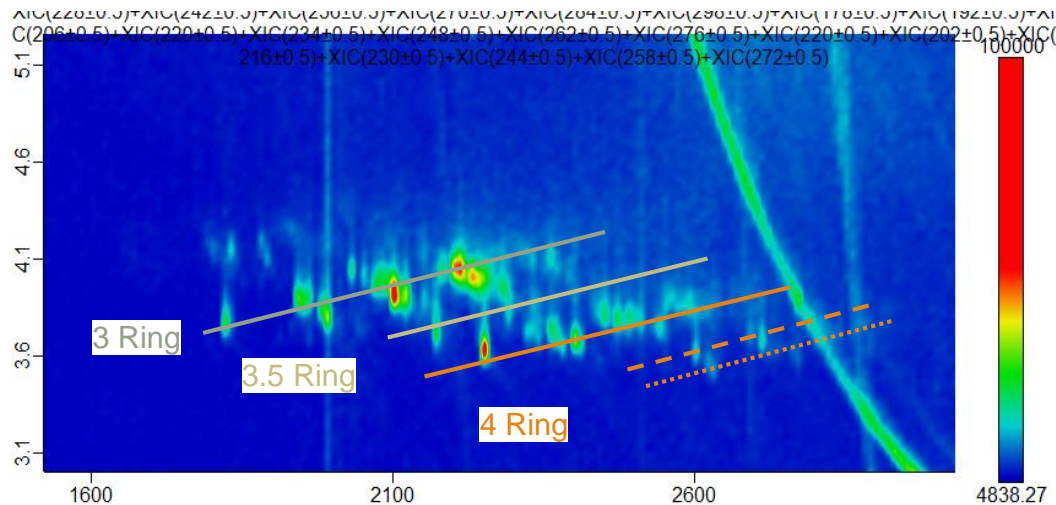


So far 20 products were evaluated:

- 16 „mineral oil“ products for different applications (also food grades ones)
- 5 packaging materials (recycled / fresh fiber paper and recycled plastics)

A clear correlation between the presence of ≥ 3 ring aromatics and Ames positive results was observed.

Prediction of Ames MPF results by GCxGC analysis seems possible!





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