

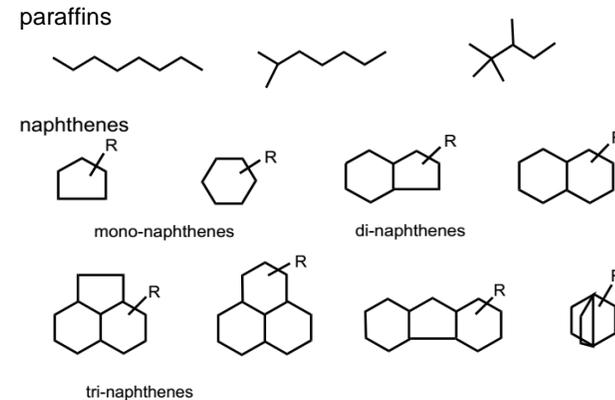


Application of the Migratox method for mineral oil
risk assessment

...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)

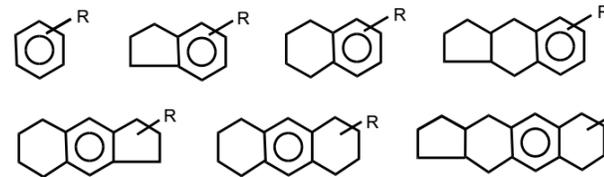


No indications for
DNA-reactive,
genotoxicity

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**

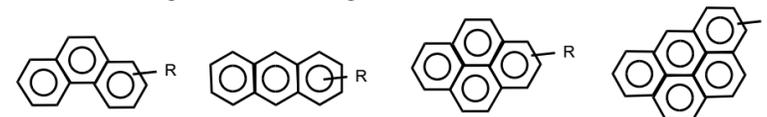
Monoaromatics (1 aromatic ring)



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

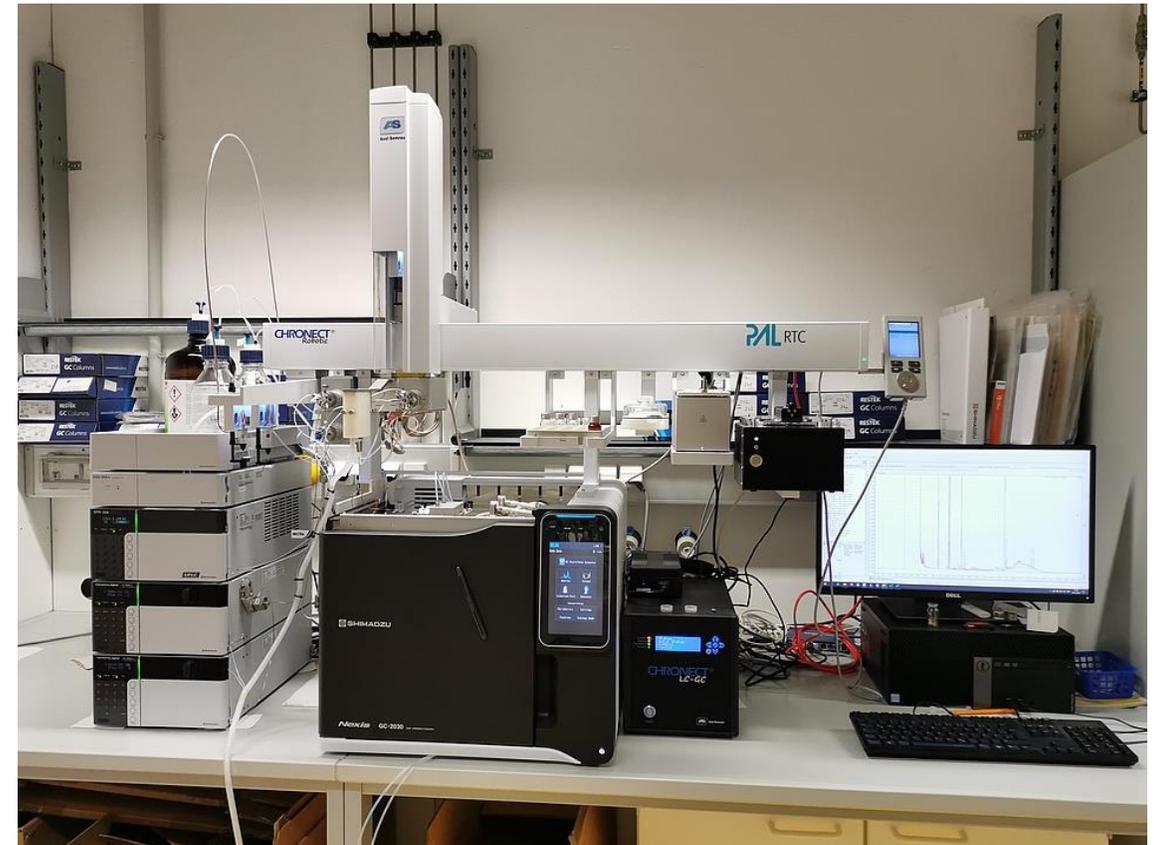
- fractionation is controlled by standards

Further “separation” with GC

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

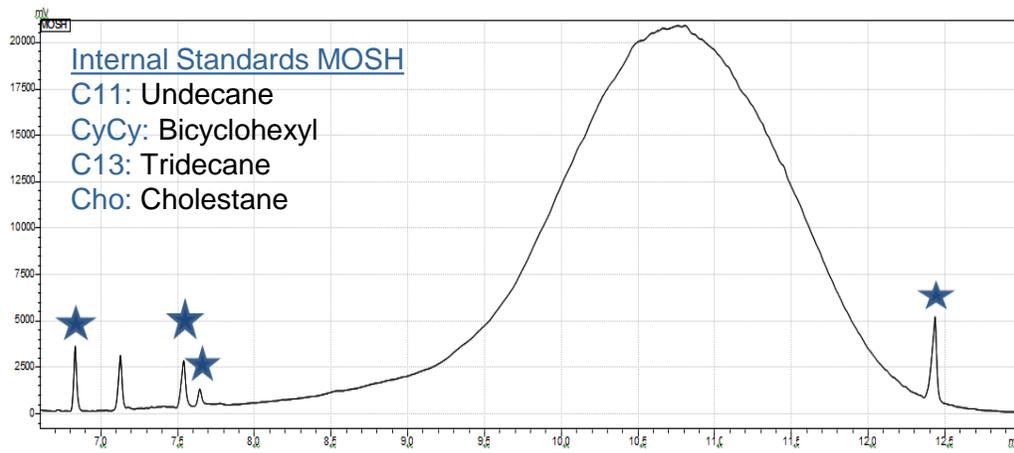
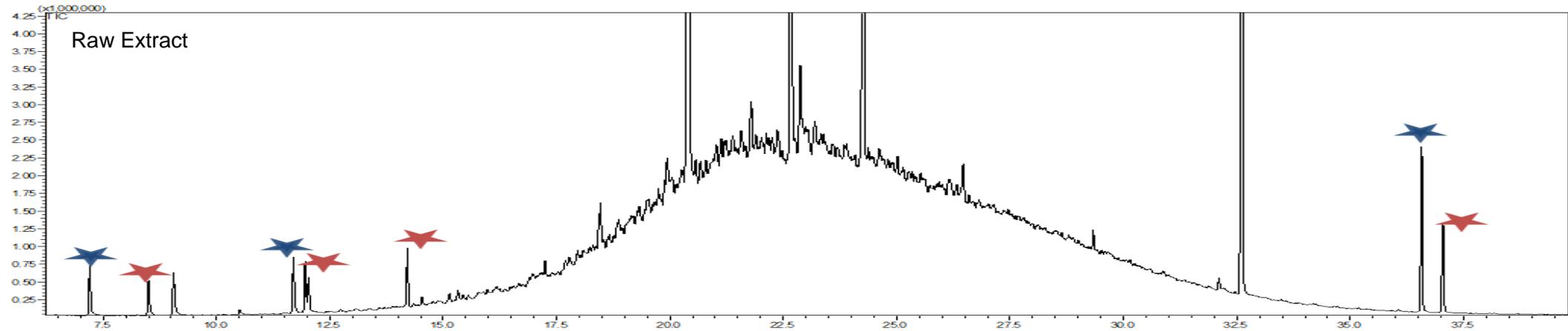
FID - detection

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

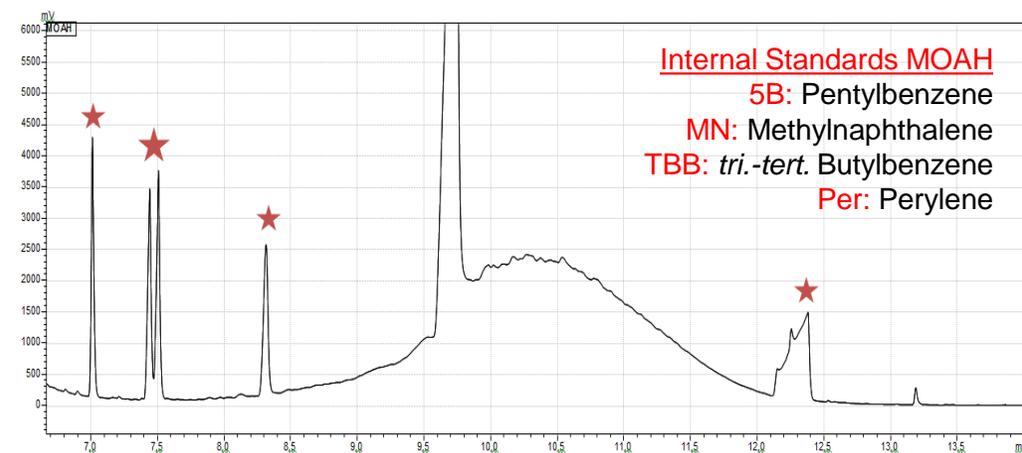


M. Biedermann, K. Grob. *Journal of Chromatography A*, 1255 (2012) 56-75.

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

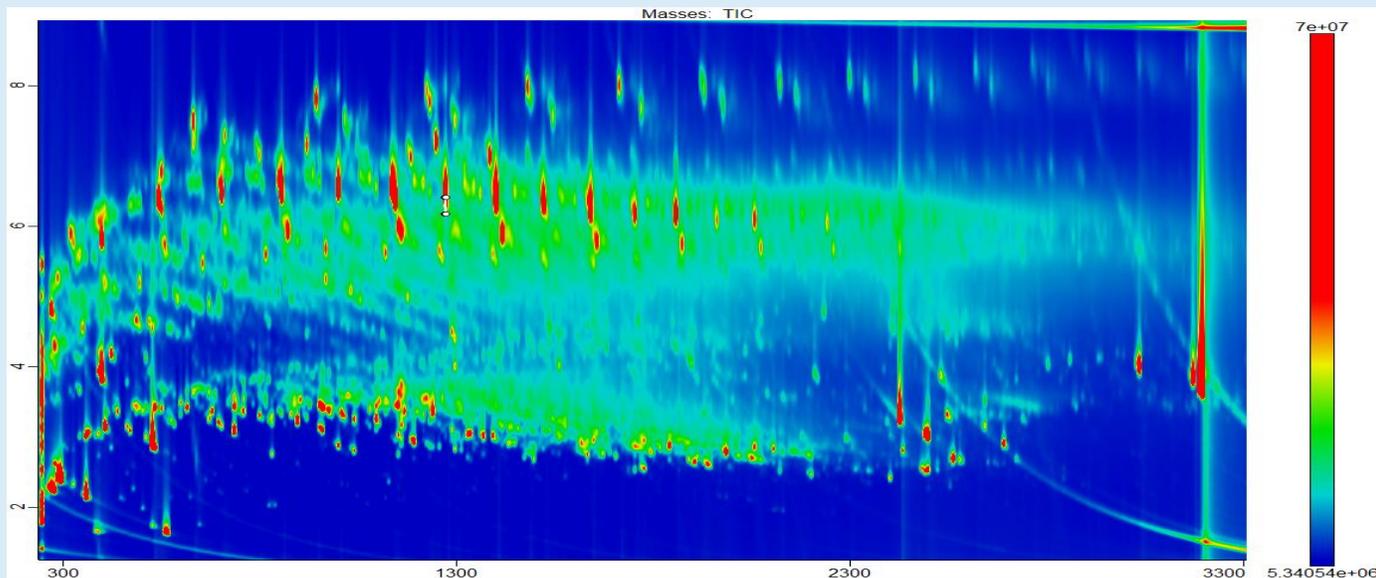
Sample description	#	MOSH (mg/kg)				MOAH (mg/kg)				
		from	to	Median	Mean	from	to	Median	Mean	>Limit
low fat content ($\leq 4\%$ fat/oil): 0.5 mg/kg	55	<0.5	89	1.1	5.33	<0.5	4.5	<0.5	0.65	4
higher fat content (4% - 50 % fat/oil): 1 mg/kg	78	<0.5	60	3.1	9.4	<0.5	17	<0.5	1.14	24
Fats/oils ($> 50\%$ fat/oil):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

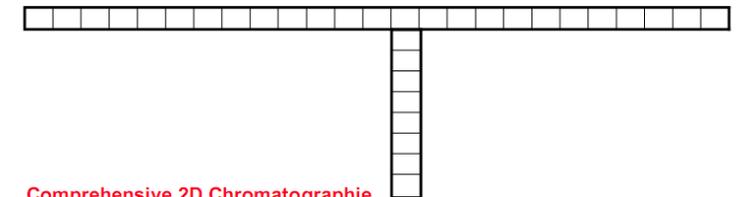
Heart-cut 2D chromatography

Comprehensive 2D chromatography

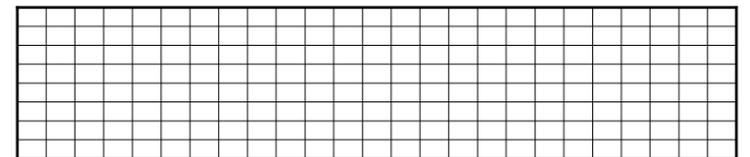
Normale 1D Chromatographie



"Heart-cut" 2D Chromatographie



Comprehensive 2D Chromatographie

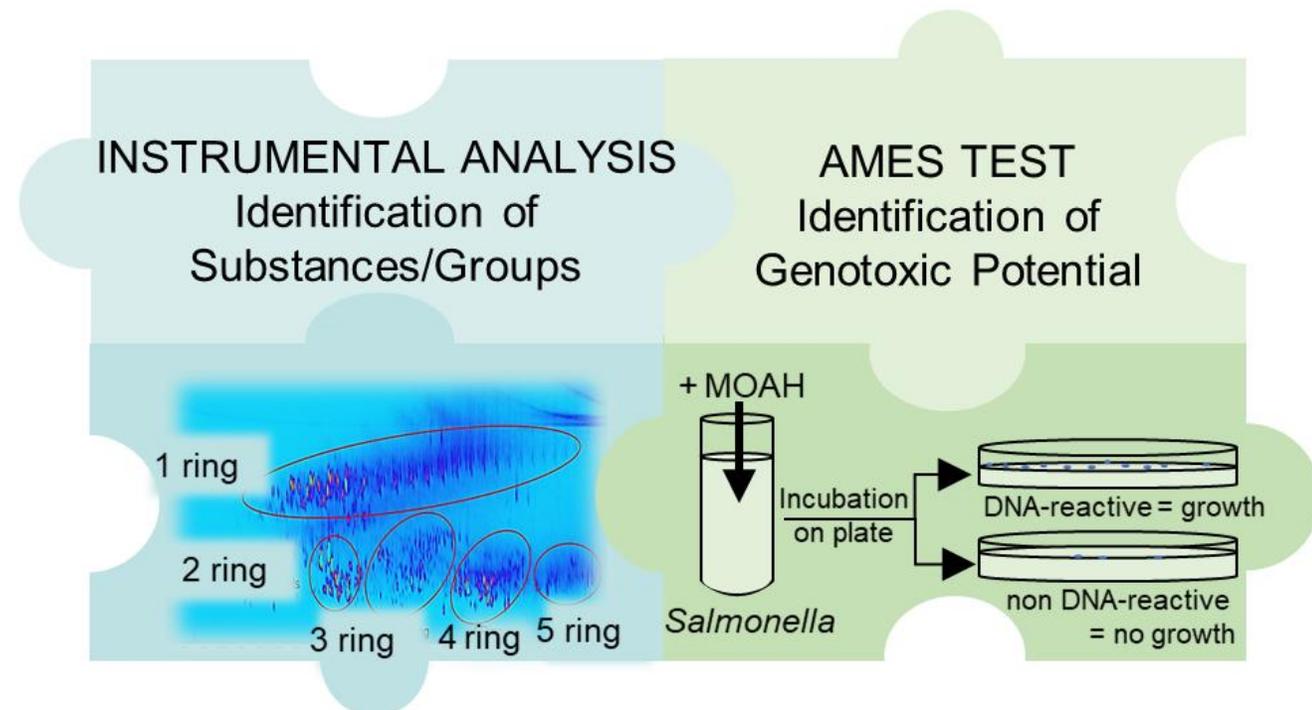


Certain sub-fractions of MOAH may be mutagenic and carcinogenic.

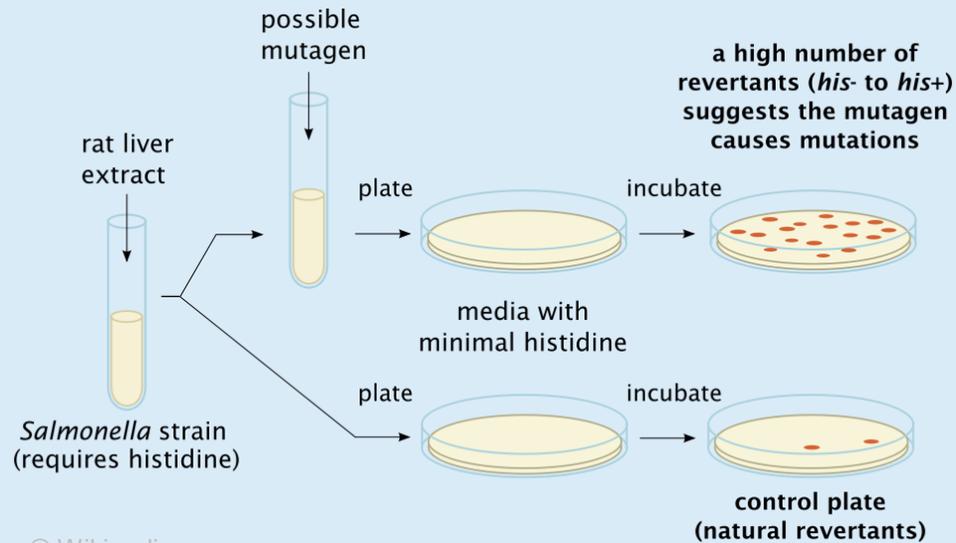
According to EFSA 2012 & 2019: 3-7 ring MOAH more harmful than 1-2 ring MOAH (so far no proof).

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



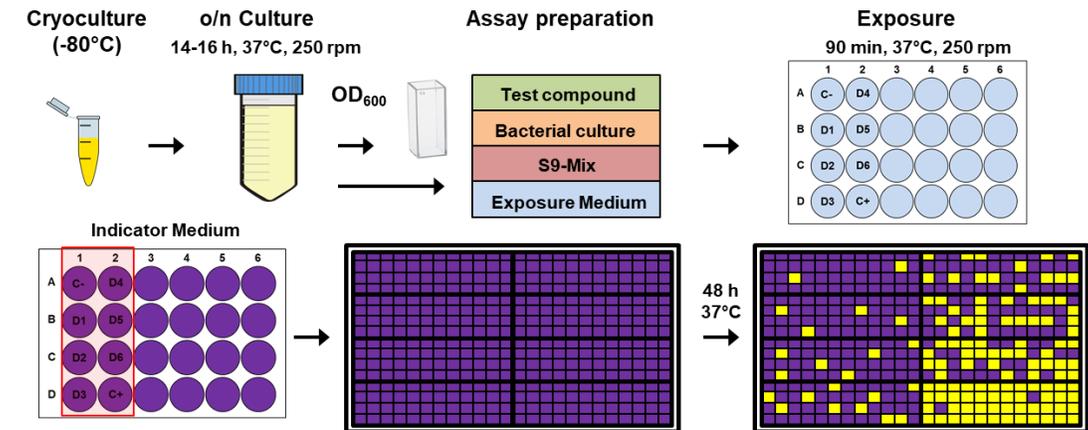
Ames Test



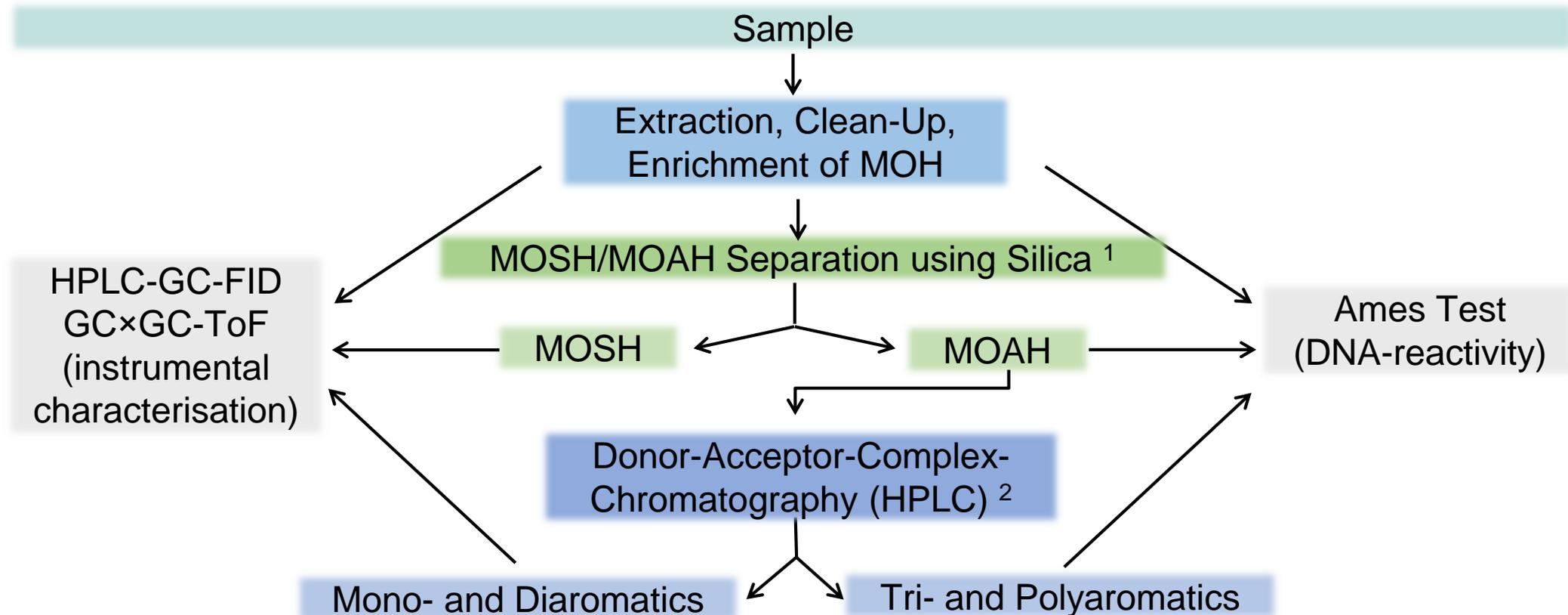
© Wikipedia

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

Miniaturized Ames Test (Ames MPF)



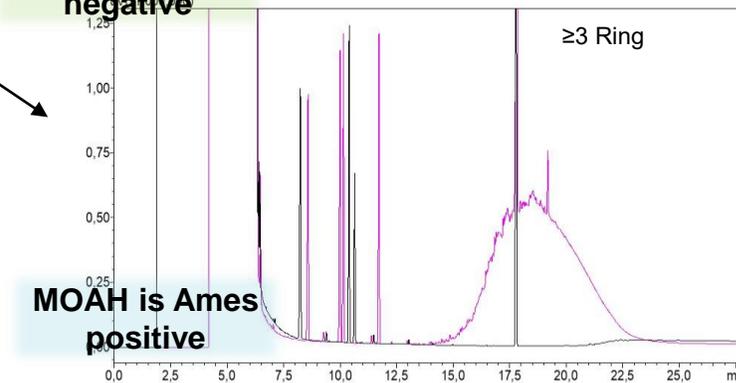
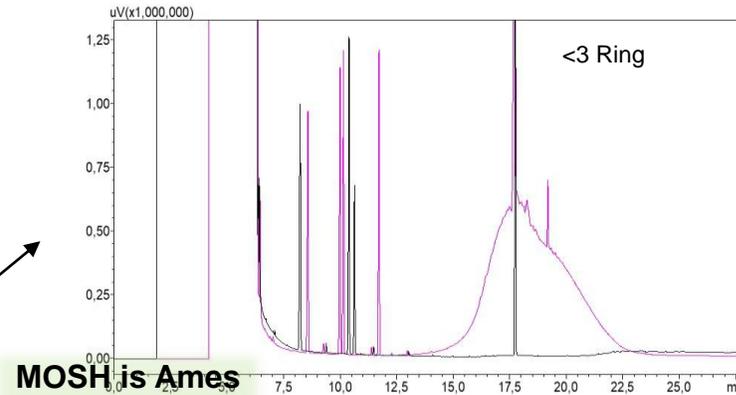
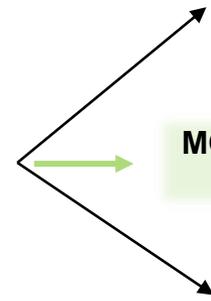
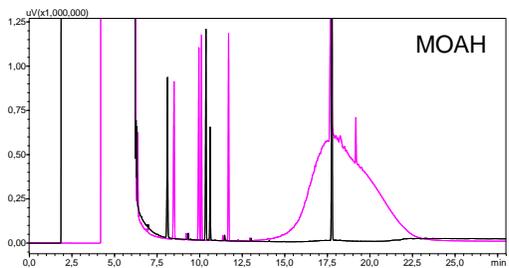
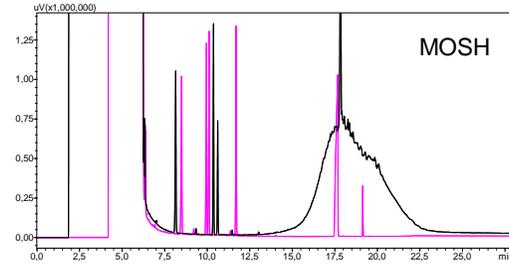
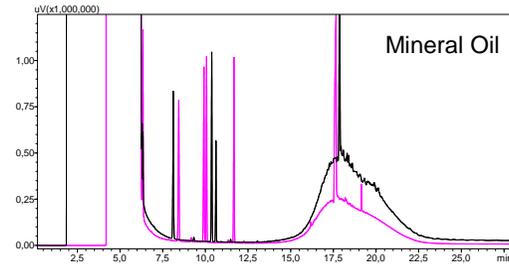
- Cultivation and exposure in liquid medium
- Colorimetric readout
- Higher throughput (microtiter plates)
- + Lower sample amounts required
- + Less incubator space required



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

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

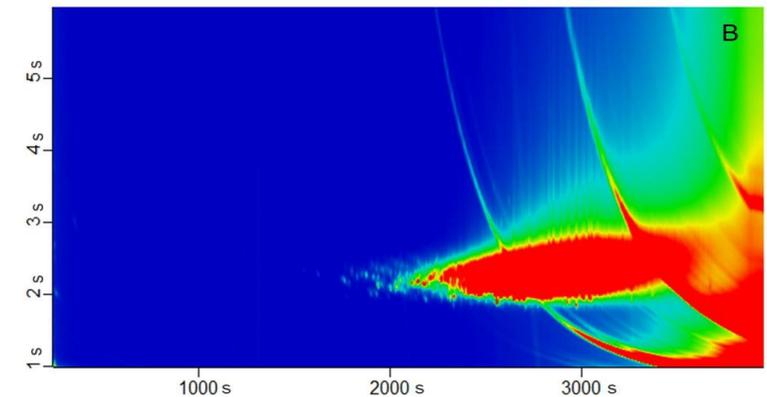
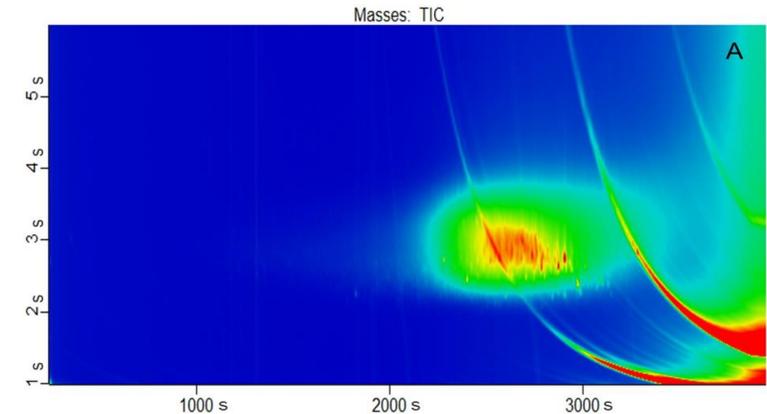
Proof of concept study



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**.

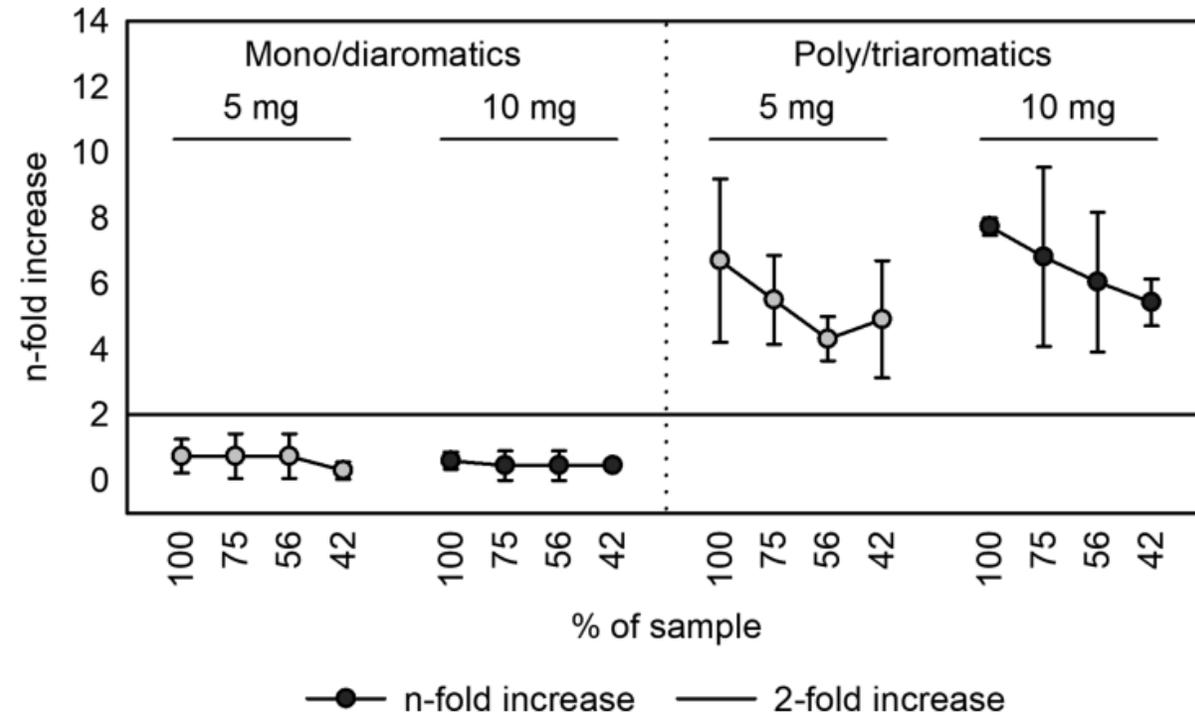
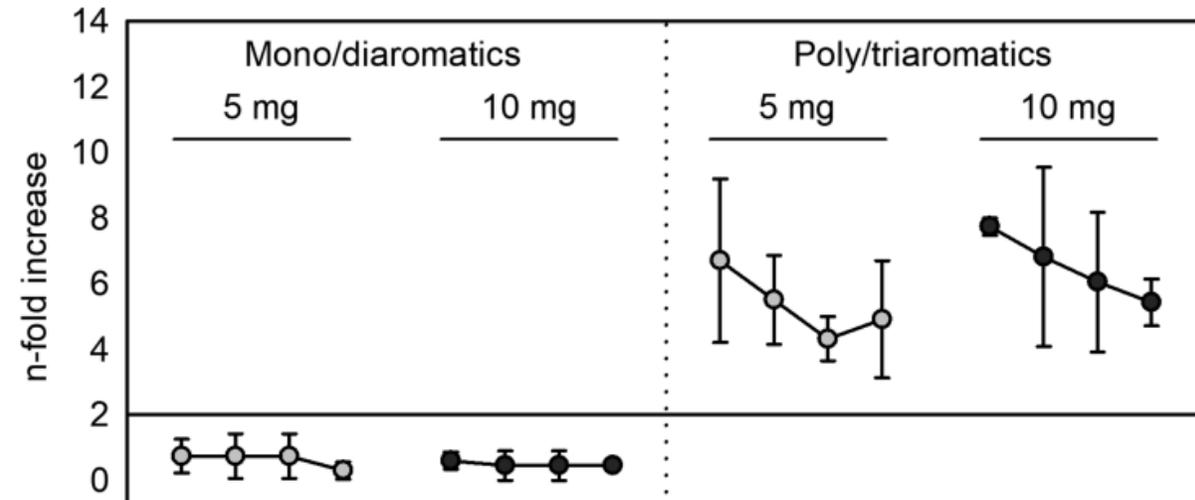


Figure 6. Miniaturized Ames analysis of DMSO extracts prepared from the mono- and diaromatic (left) and tri- and polyaromatic (right) subfraction of a mineral reference oil. Two extraction ratios, 5 mg/250 μ L and 10 mg/250 μ L DMSO, were compared.

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!**



SCIENTIFIC OPINION

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),
Dieter Schrenk, Margherita Bignami, Laurent Bodin, Jesús del Mazo, Bettina Grasl-Kraupp,
Christer Hogstrand, Laurentius (Ron) Hoogenboom, Jean-Charles Leblanc,
Carlo Stefano Nebbia, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand,
Tanja Schwerdtle, Christiane Vleminckx, Heather Wallace, Jan Alexander,
Christophe Goldbeck, Konrad Grob, Jose Ángel Gómez Ruiz, Olaf Mosbach-Schulz,
Marco Binaqlia and James Kevin Chipman



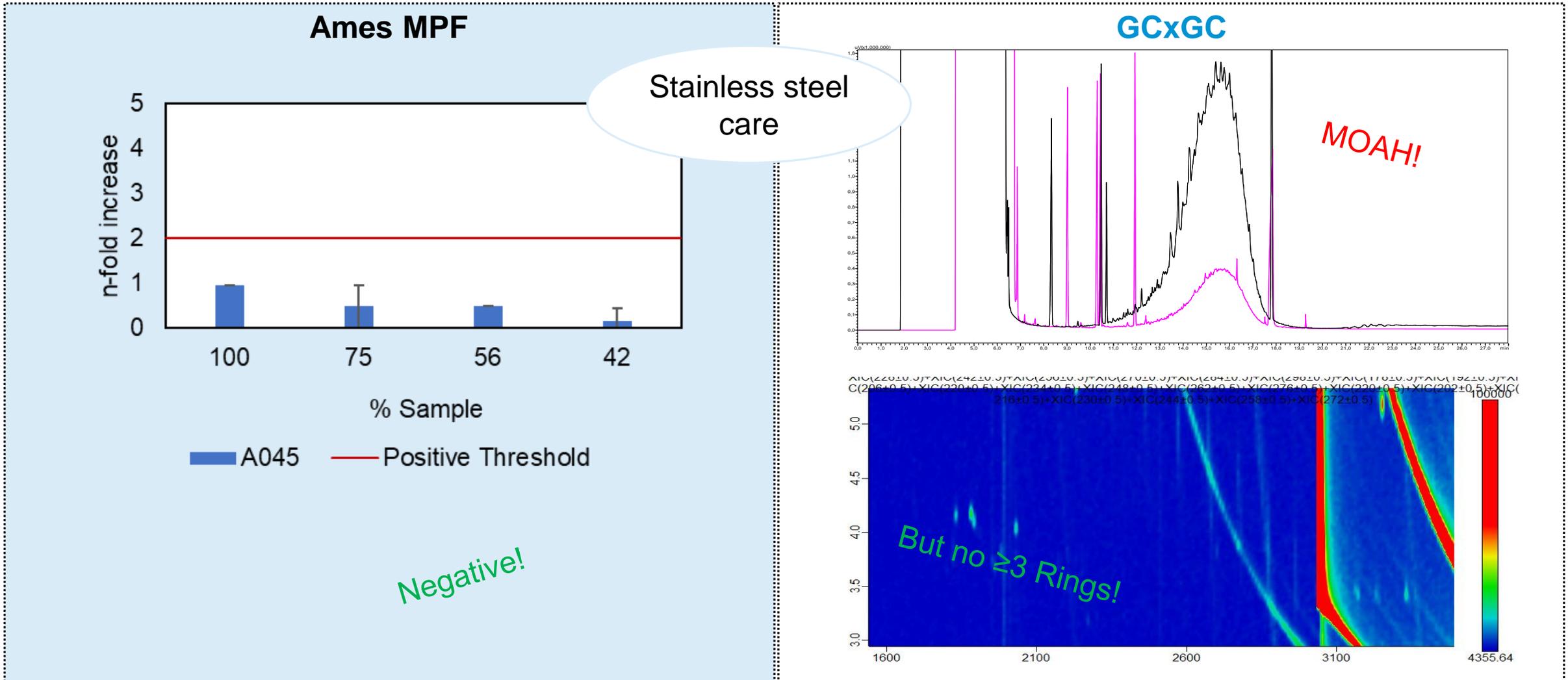
ISSN 1831-9424

JRC TECHNICAL REPORTS

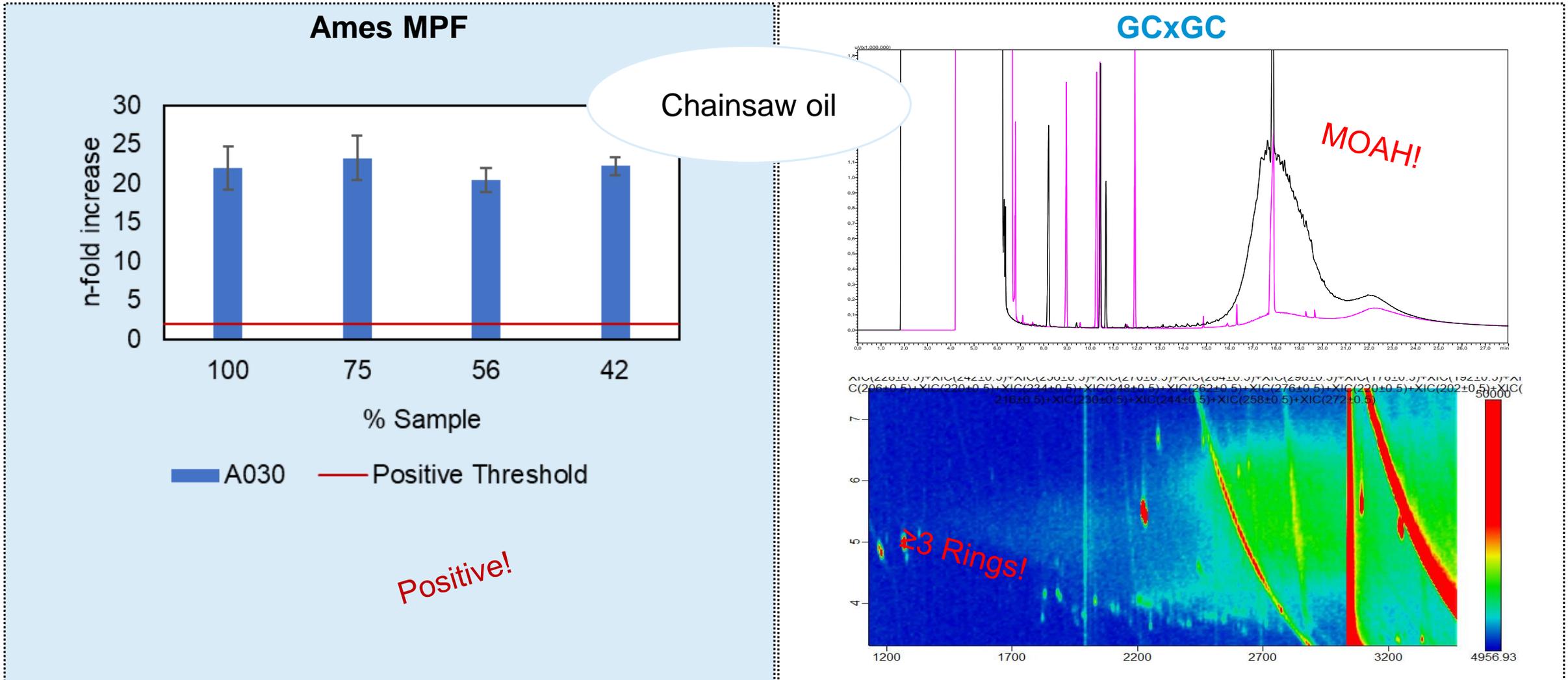
Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition

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

Correlation of Ames MPF and GCxGC results



Correlation of Ames MPF and GCxGC results

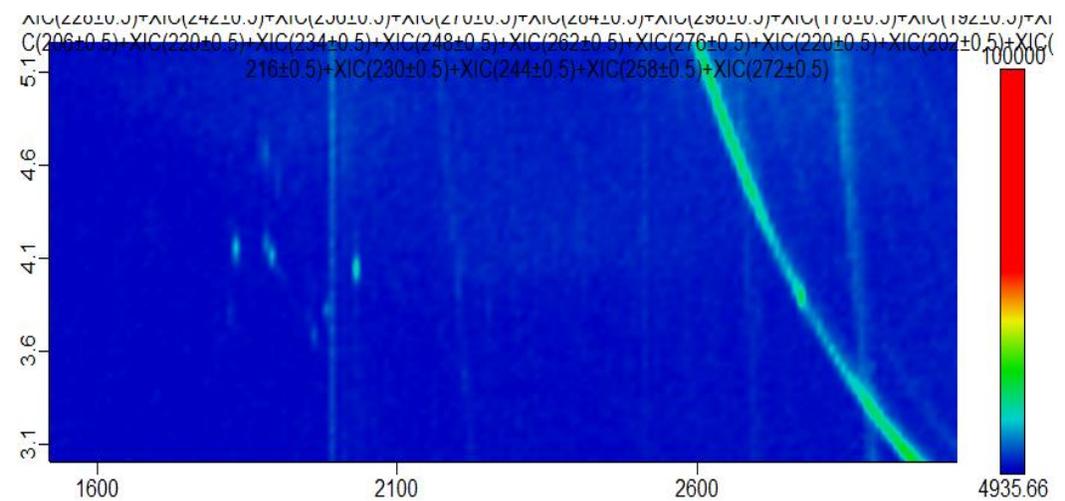
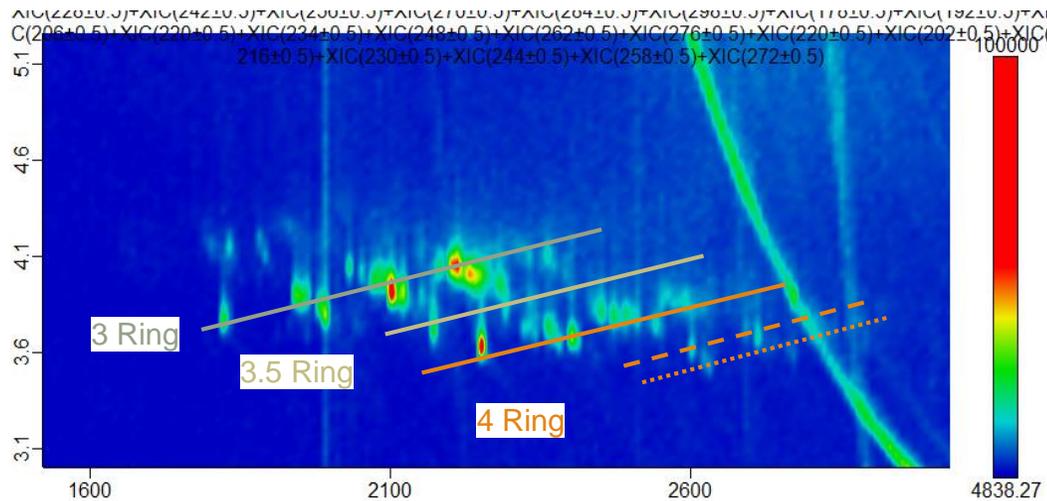


So far 20 products were evaluated:

- 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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