Nestlé Good food, Good life

# Contribution of the HPTLC to the food safety research area

Maricel Marin-Kuan
Biodetection group
Food Safety and Research Department

Lyon, 20th September 2022

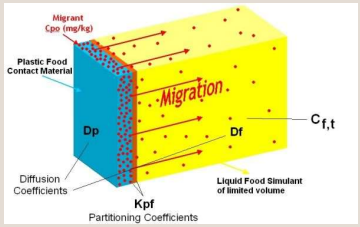
FORUM LABO LYON

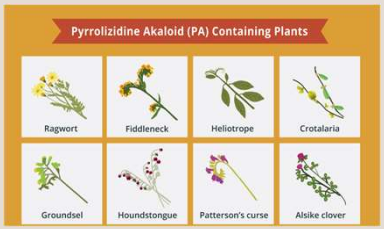
# Chemical Food Safety

- **Toxicology:**
  Hazard Identification & Hazard Characterization
- **Exposure:**
  Food intake &
  Occurrence of chemicals
- **Risk Characterization:**
  Informing decision making


BUT…in absence of toxicological data?

# Food and food-related samples are chemicals

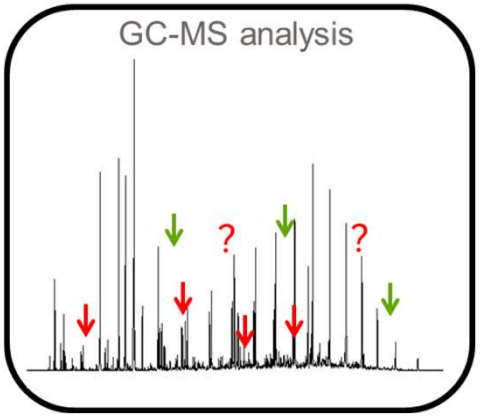



**Packaging material**

**Many chemicals implicated in many packagings potentially migrating into food**

How to assess the safety???




Migrate contains:
known/characterized (authorized?)
unknown/unexpected

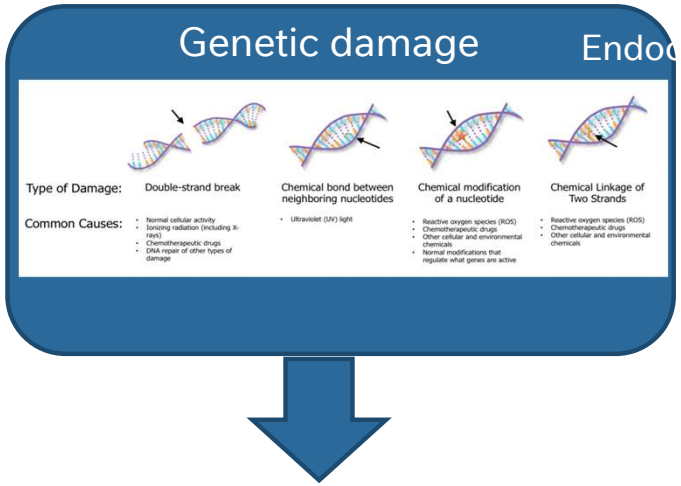
Full identification & characterization?

Bioassays can play important role to exclude endpoints of toxicological concern(s)



Research and Development

# Assessment of Toxicological Endpoint




- **European Food Safety Agency (EFSA)** Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (2011):
- "due to the adverse consequences of genetic damage to human health, the assessment of mutagenic potential is a basic component of chemical risk assessment.

**Bioassay to assess effects on DNA-damage need to be taken into consideration**



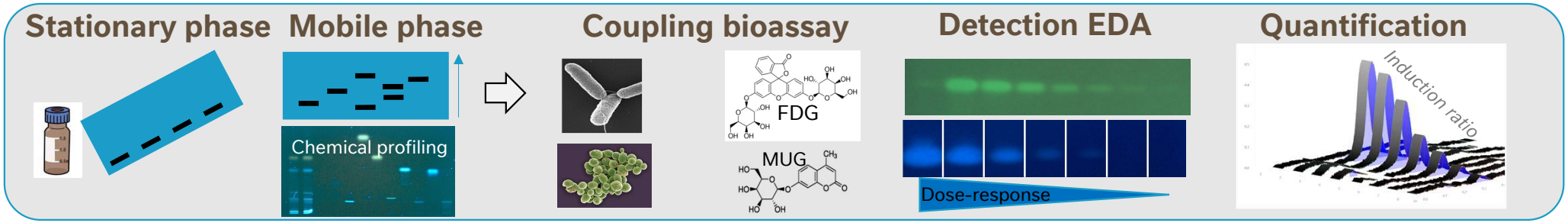
Research and Development

# Limitations encountered with regular cell-based methodologies to analyse complex mixtures

**Cell-based assays have several limitation:**

1. Limit of detection (LOD) multi-well tests is not suitable
2. Cells are sensitive to solvents (extraction  solvents)
3. Whole extract exposure
4. Matrix effect
5. No clue molecule responsible biological activity
6. Time consuming
7. Lab consumables spent

# Implementation of new *in vitro* competence to analyse complex mixtures: GENOTOXICITY & ENDOCRINE ACTIVITIES




1 Application Normal (Si60)

2 Sample separation

3 Spraying of Bacterial (37°C) or yeast (31°C) cells

4 Fluorescence

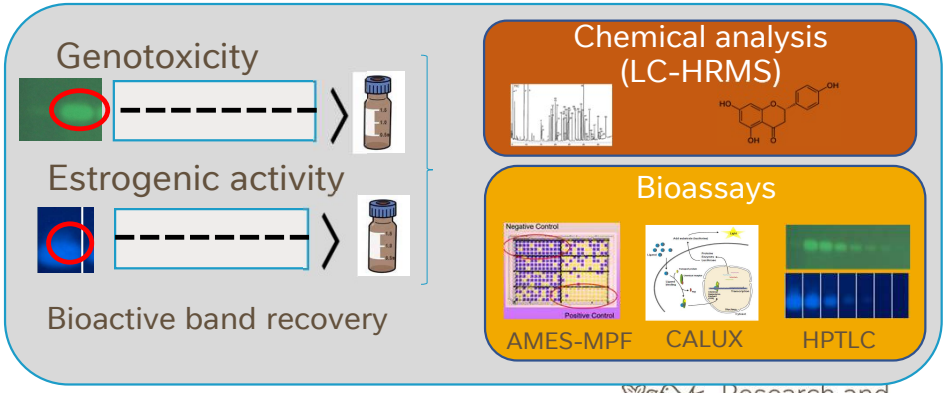
5 Visualisation

6 Scanner (peak height)

*Confirmation: chemical identification/bioassay*

3a SOS UmuC assay (Genotoxicity) in presence and absence metabolic activation (+/-S9)

3b Yeast estrogen activity (ERα) screening (pYES assay)

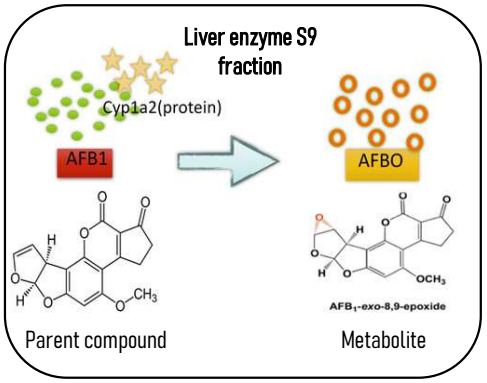



FDG: fluorescein-di-β-D-galactopyranoside (485/>500)
MUG: 4-methylumbelliferyl-β-D-galactopyranoside (320/>400 nm )

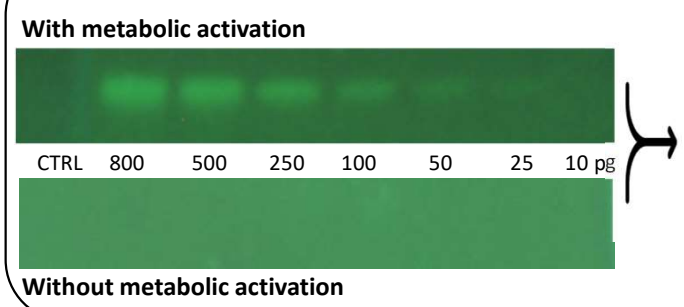


Research and Development

# HPTLC genotoxicity assay with the integration of metabolic activation condition

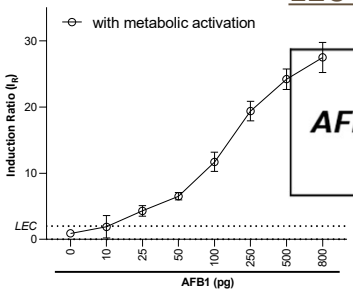



## Aflatoxin B1




LEC

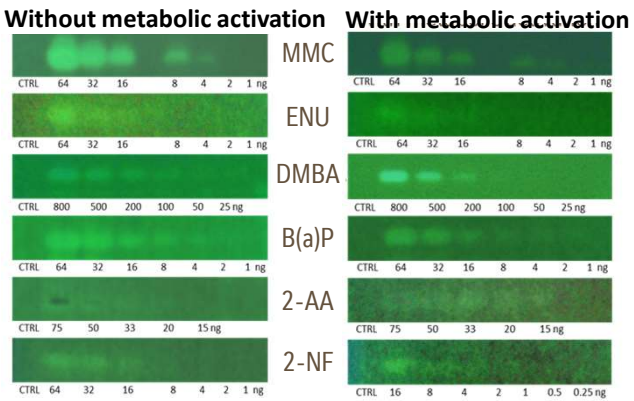
LEC in ng (HPTLC vs AMES )

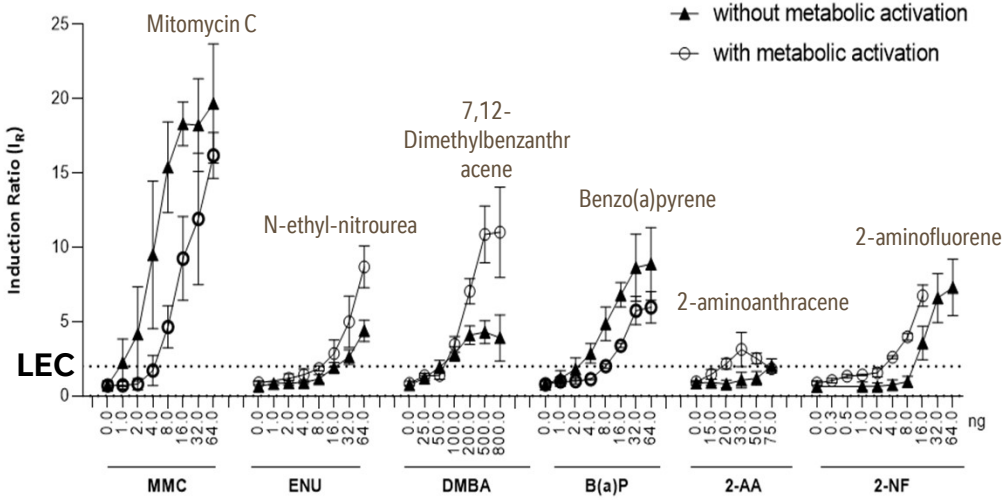



| | HPTLC-Umu-C | AMES MPF | |
|---|---|---|---|
| | | TA98 | TA100 |
| AFB1 | 0.025 | 0.06 | 0.24 |

Lower LECs for HPTLC

## Validation using reference genotoxic compounds with different mechanisms of actions




| LEC (ng) | -S9 | +S9 |
|---|---|---|
| ***MMC*** | 1 | 4 |
| ***ENU*** | 16 | 16 |
| ***DMBA*** | 50 | 100 |
| ***B(a)P*** | 4 | 8 |
| ***2-AA*** | 75 | 20 |
| ***2-NF*** | 16 | 4 |

*Debon et al. (2022) Special Issue **TOXICS 2022***

Toxics 2022, 10, 501.
https://doi.org/10.3390/toxics10090501



Research and Development

# FOOD CASE STUDY APPLICATION

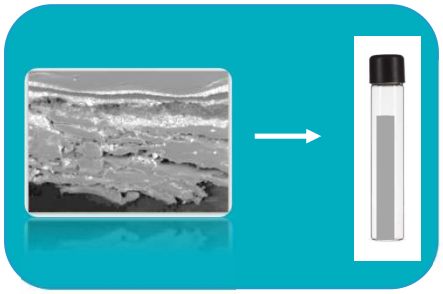
Paper material



Research and Development

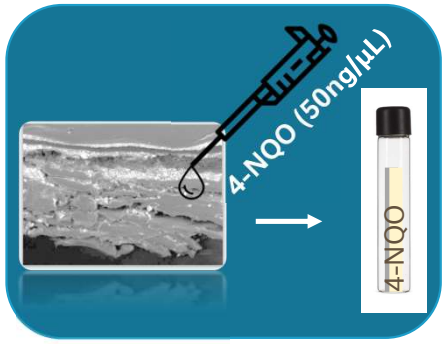
# From packaging extract/migrate to identification of genotoxicants/mutagens using paper as case study

## EXTRACTION (H/A)



*Commercial colored paper material (non-Nestlé product)*

## SPIKING EXTRACTION

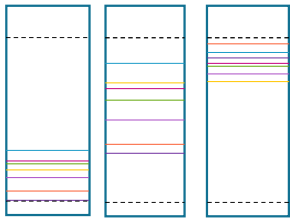



*4-Nitroquinoline 1-oxide (4-NQO) reference genotoxic compound*

**Normal Si60 plates**

## HPTLC coupled to SOS Umu-C assay

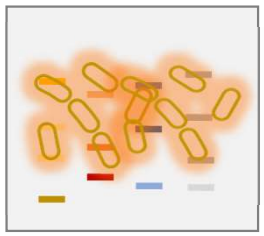
Three Mobile Phases Concept



MP1 MP2 MP3

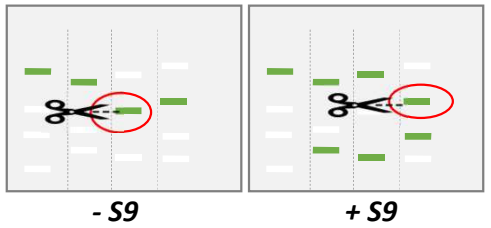
Selectivity based on mobile phase polarity

*Salmonella* SOS Umu-C assay



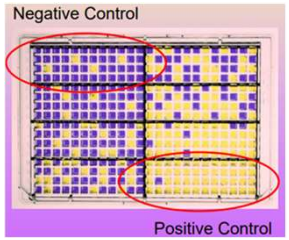
Presence and absence metabolic activation (+/-S9)

Genotoxicity bioactive bands

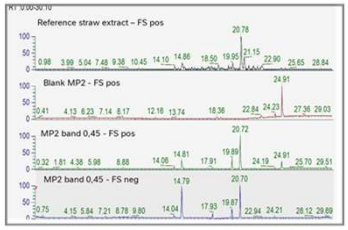



**Recovery**

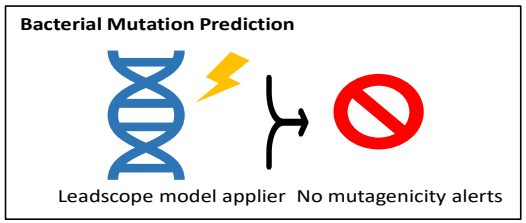
Extracted bands testing




**MUTAGENICITY (AMES-MPF ASSAY)**




**Chemical analysis (LC/HRMS)**




**Mutagenicity In-silico prediction**

Research and Development

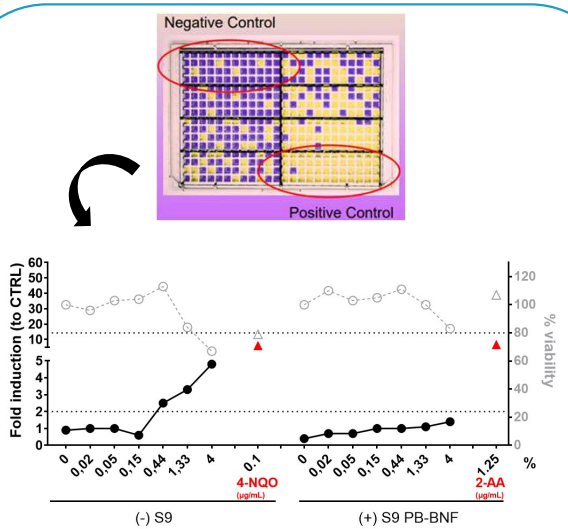
# Proof of feasibility HPTLC-Umu-C: recovery of genotoxic compound

Signal of 4-NQO spiked in paper extract

Recovered band extraction

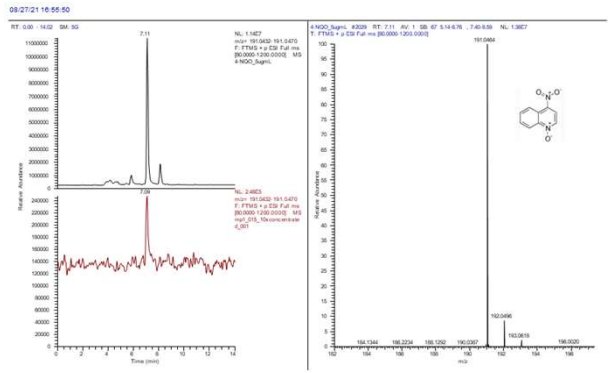
mutagenicity confirmation + chemical identification

## Ames-MPF




## Chemical analysis

4-NQO (LC-HRMS)

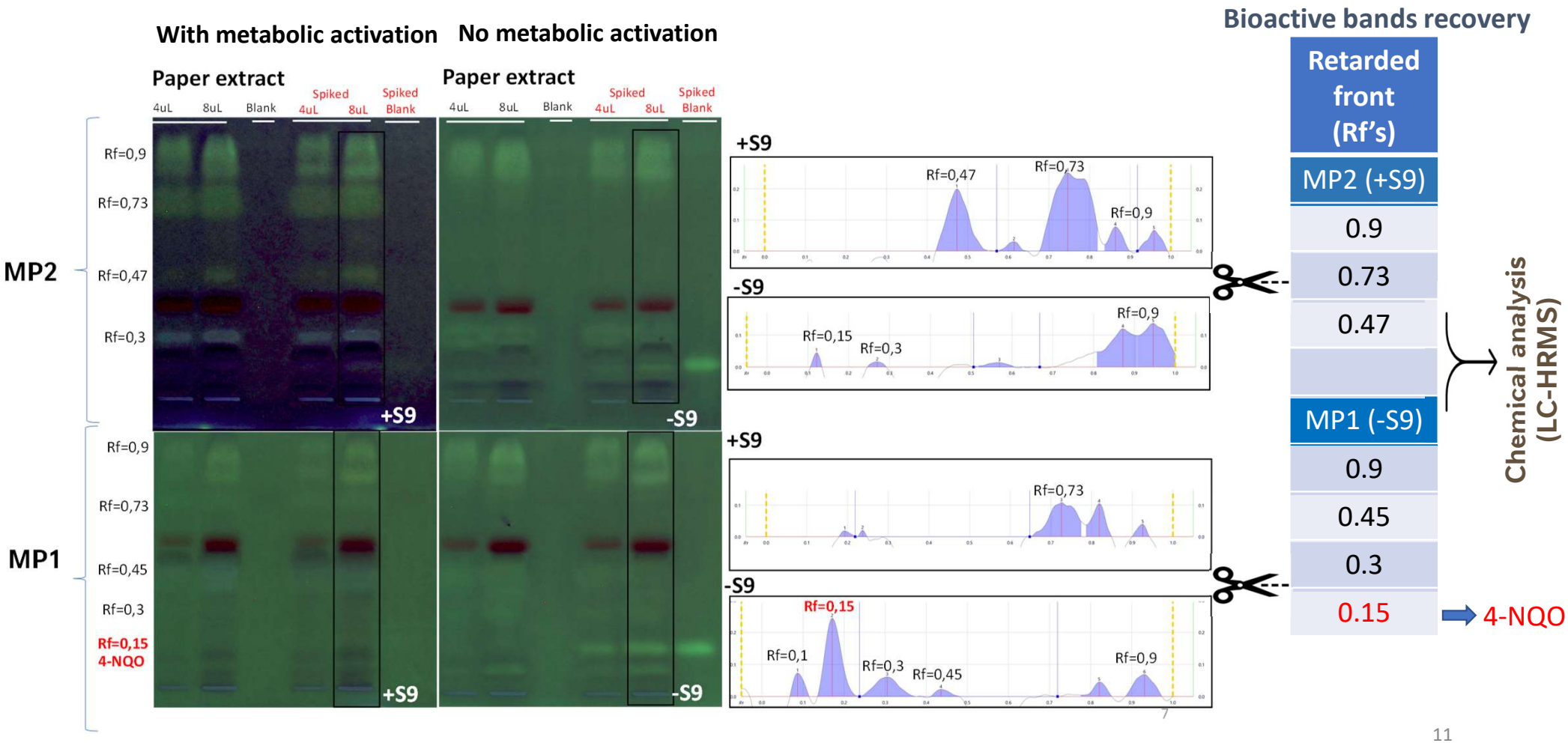


- ✓ The recovered bioactive band was confirmed as mutagenic with Ames-MPF assay
- ✓ The 4-NQO was detected using the LC-HRMS



Research and Development

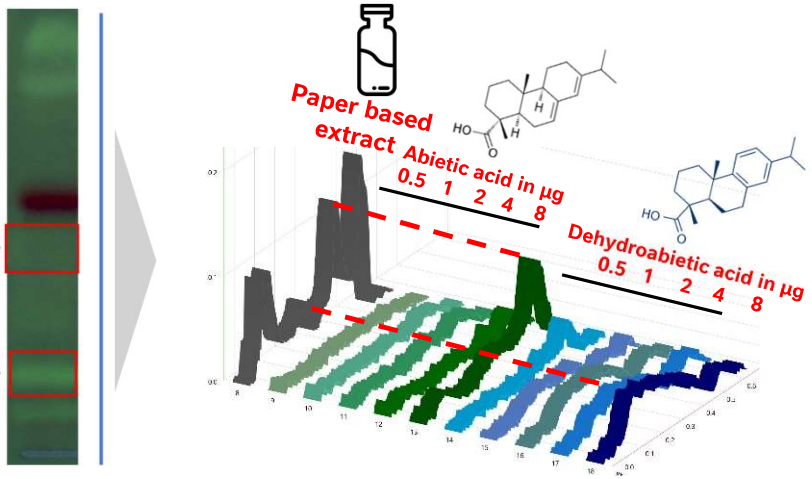
# Screening of HPTLC-Umu-C induction by paper extract samples



# Genotoxic activity of abietic and dehydroabietic acids

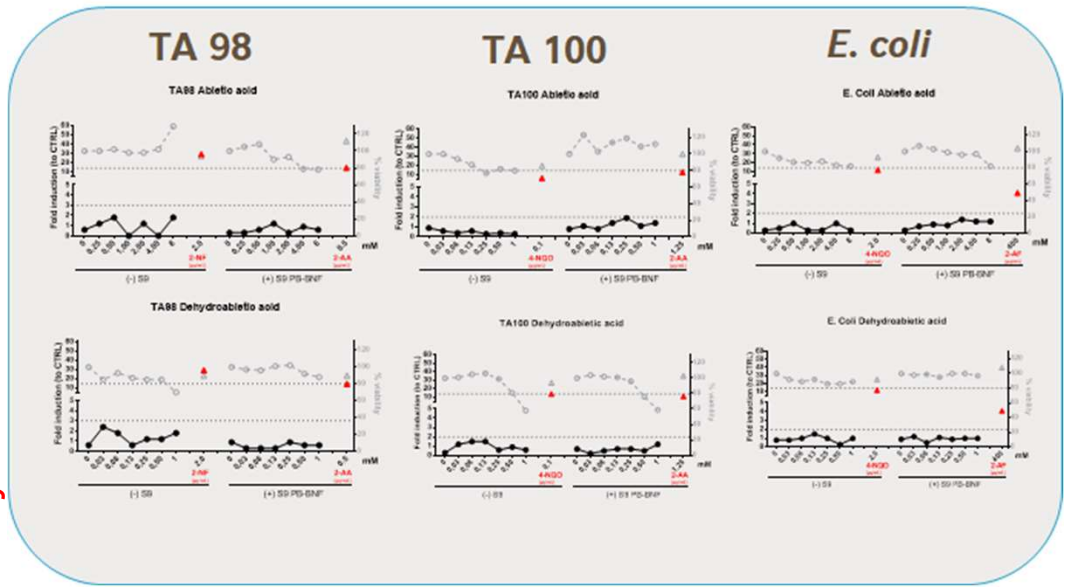
## HPTLC-Umu-C Confirmation

Bioactive band 1




## MUTAGENICITY ASSESSMENT

### AMES-MPF confirmation

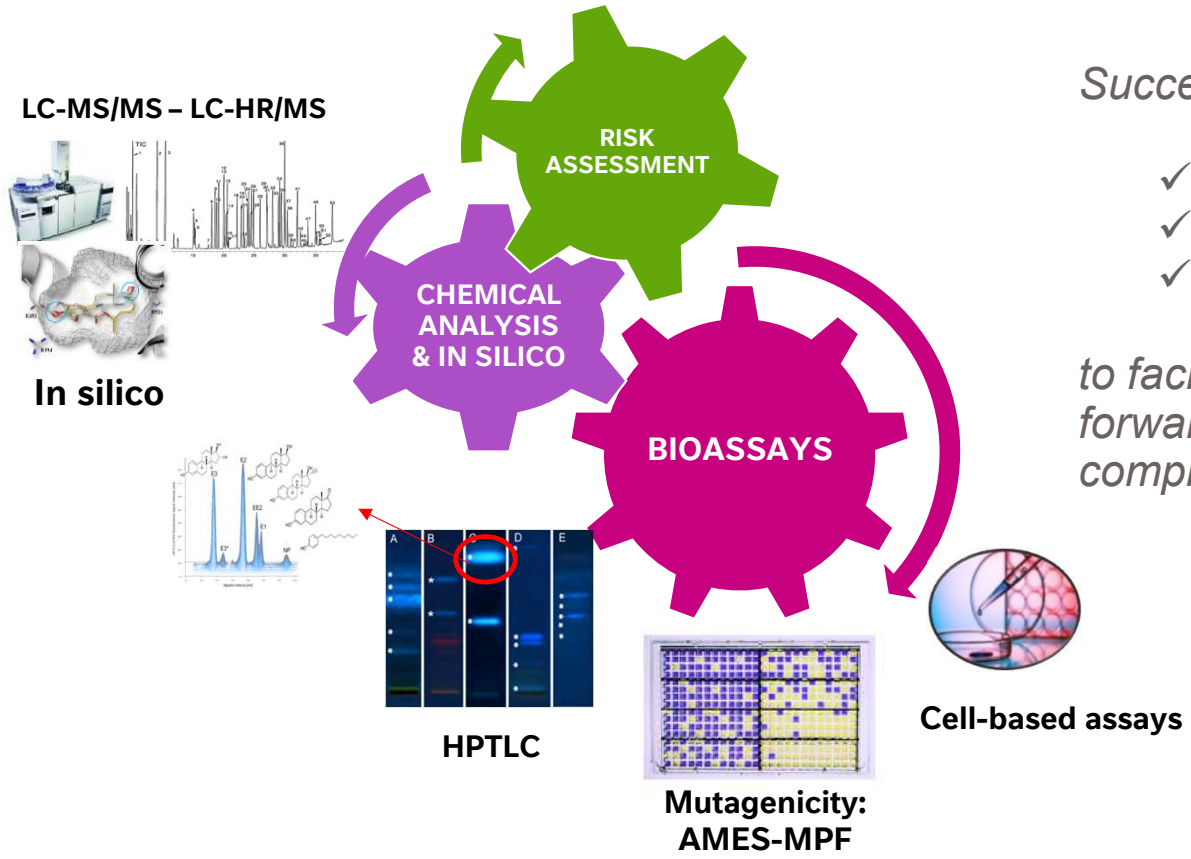



- ✓ No **mutagenic** effect was observed for abietic and dehydroabietic acids
- ✓ Confirmation of feasibility and reliability of HPTLC genotoxicity approach to assess safety of FCMs



Research and Development

# Key contribution of HPTLC biodetection approach to characterize complex mixtures




*Succesfull integration of:*

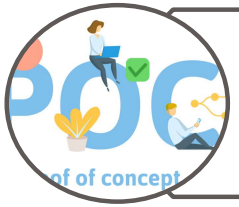
- ✓ *highly sensitive bioassays,*
- ✓ *chemical analysis*
- ✓ *in silico techniques*

*to facilitate risk assessment as a way forward for the safety evalaution of complex mixtures.*

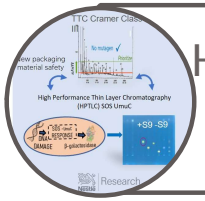


Research and Development

# Conclusions and take home-messages

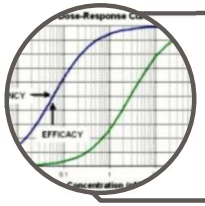



Proof-of-concept of an approach bridging **effect-directed bioassays** with **chemical analytics** to qualitatively and quantitatively characterize genotoxic compounds present in food (e.g., packaging material, food ingredients)




HPTLC overcomes some limitations of the multi-well plates with good **detection limit**, reliability, versatility and efficiency (semi-automation (HPTLC-PRO))


Important contribution for the characterization of food and food-related samples → **facilitating safety assessment**




Expansion of the HPTLC applications with implementation of new biological and chemical derivatization methods is ongoing (e.g. adulerations)

# Acknowledgements

**Nestlé Teams:**

**Food Safety Research Department**
Walburga Seefelder
Benoît Schilter

**Biodetection group**
Helia Latado
Paul Rogeboz
Emma Debon
Karma Fussell
Patrick Serrant
Julie Mollergues
Claudine Cottet
Bastien Gentili
Lucrecia Pons (apprentice)
Amaury Patin (GL)

**Packaging Food Safety**
Yves-Alexis Hammel
Elsa Omer (PM)
Sander Koster (GL)

**Untargeted Screening group**
Flavia Nagy
Christinat,Nicolas

**Prof. Gertrude Morlock**
JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

TransMIT

CAMAG®

CHROMACIM

biostep
SARSTEDT-GROUP

sigma
CLERMONT

BDS BioDetection Systems
CALUX®

XENOMETRIX
Swiss Commitment for Bioassays

# Thank You!

**Contact: maricel.marin-kuan@rdls.nestle.com**