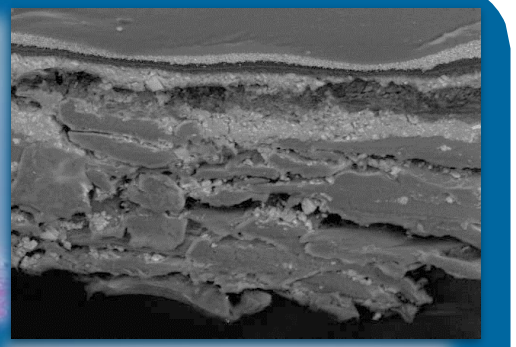
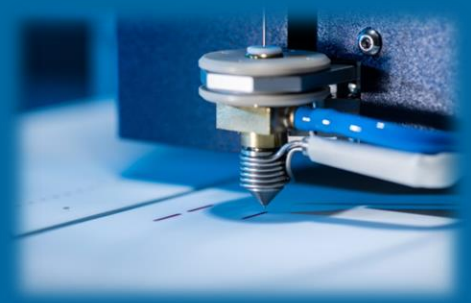
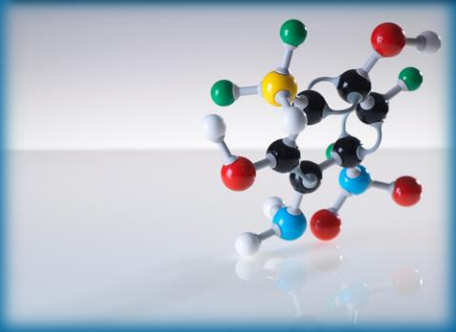
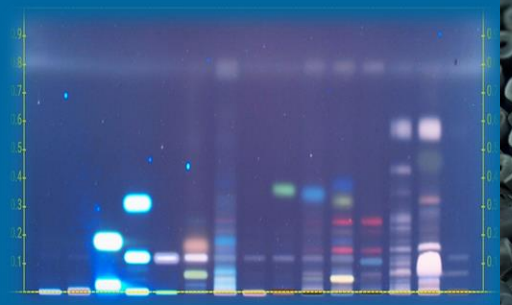
Nestlé Good food, Good life

Food Packaging Forum

# Assessing Non-Intentionally Added Substances migrating (or extracted) from food packaging - combining bioassays with chemical analysis

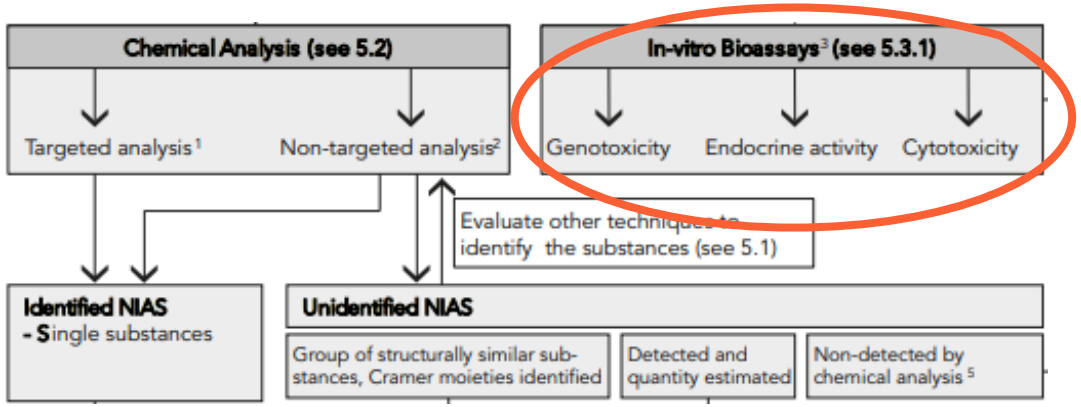

**Maricel Marin-Kuan Ph. D**
**Institute of Food Safety & Analytical Sciences**
**Nestlé Research-Lausanne**

Zurich, 28th September 2023

# Role of Bioassays in packaging safety?
# In absence of chemical identification of packaging substances, Biotesting is encouraged to facilitate safety assessment

ILSI Europe
International Life Sciences Institute

Best Practices
RA NIAS in FCM




European Parliament
2014-2019

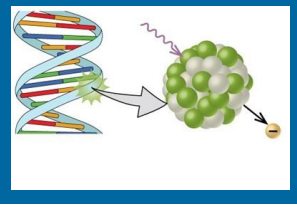
*Plenary sitting*

18.7.2016 **A8-0237/2016**

**REPORT**

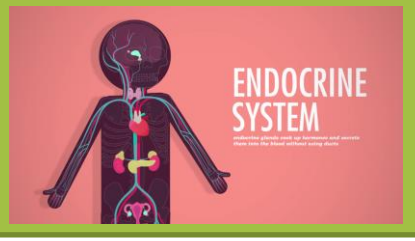
on the implementation of the Food Contact Materials Regulation ((EC) No 1935/2004)
(2015/2259(INI))

## Genetic damage



**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.

## Endocrine active substances (EAS)




- EAS are substances that can interact or interfere with normal hormonal action but may or not cause harm.
- This topic is of increasing concern in regulations and have poor public perception.

## Battery of tests to assess effects on DNA-damage & on the endocrine system need to be taken into consideration

*ILSI 2015: Best Practices on the Risk Assessment (RA) of Non-Intentionally Added Substances (NIAS) in Food Contact Materials and Articles*

Research and Development

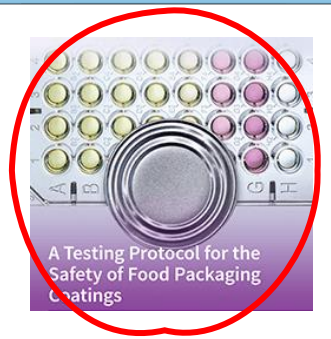
# Workflow of Approach to Assess Food Contact Materials Safety

**Novel material** → **Samples Preparation** → **Bioassays & GC/LC Analysis** → **Data Processing** → **Prioritization**


**Full assessment**
Composition

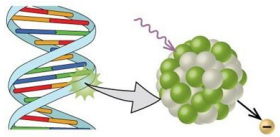
**Assessment of Migration**
from FCM to EU food simulants

**Assessment of Extraction**

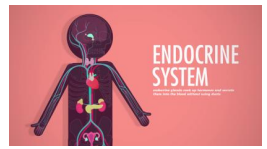



LOD?
Matrix effect?
Exposure dose (DMSO)?

Chromatography



**Genetic damage**




**Endocrine activity**

Identification
Semi-quantification

Target / Suspected / Non-Target Screening

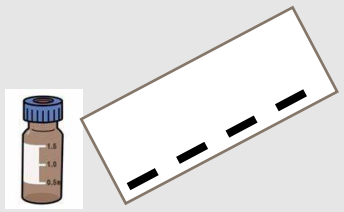


Research and Development

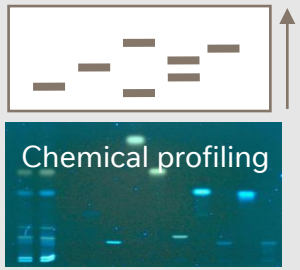
# Implementation of new *in vitro* competence to analyze packaging samples: High Performance Thin Layer Chromatography (HPTLC)

## Chemical profiling, coupling to effect-directed analysis (EDA)(genotoxicity and endocrine activity)

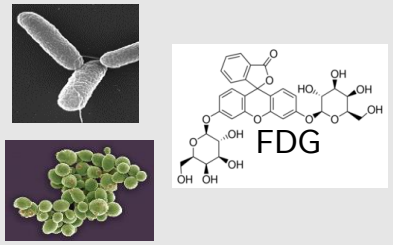
**Stationary phase**



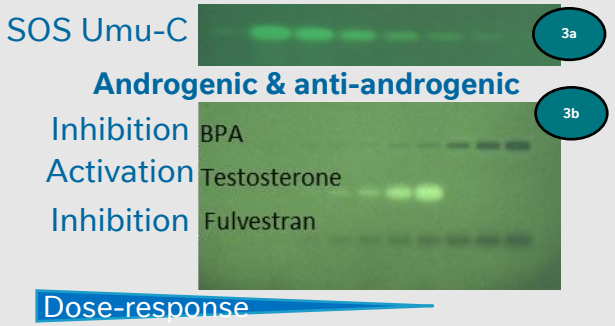
**Mobile phase**



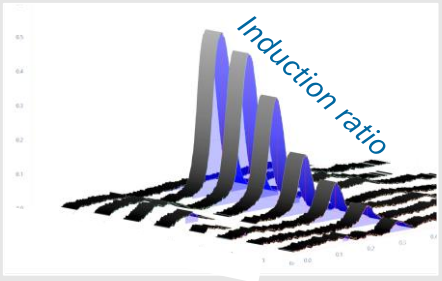

**Coupling bioassay**




**Detection EDA**




**Quantification**




1. Application
2. Separation constituents
3. Spraying of Bacterial or yeast cells
4. Detection
5. Visualisation
6. Scanner

Sample (Migration product or total extract or compound and reference compounds)

Mobile Phase: adapted for each mixture or compound analyzed

**3a SOS UmuC assay +/- metabolic activation) (Genotoxicity)**

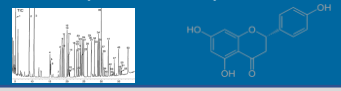
**3b Yeast estrogen/androgen activity screening (Endocrine activity)**

*Confirmation: chemical identification/bioassay*

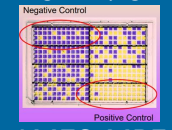
Genotoxicity

Estrogenic activity

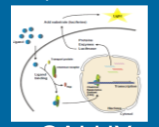
**Chemical analysis**
(LC-HRMS)



**Bioassays**
(mutagenicity, genotoxicity, endocrine)



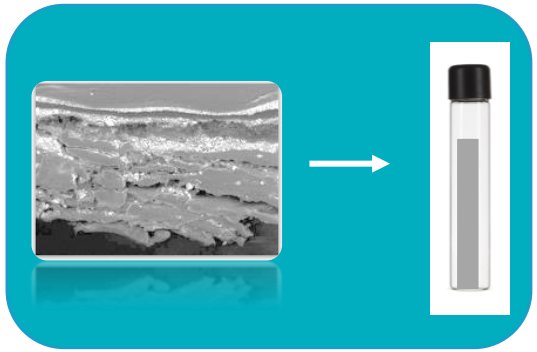

AMES-MPF



CALUX

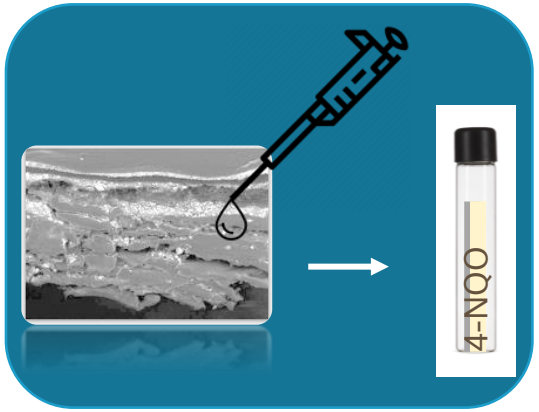
# From packaging extract/migrate to identification of genotoxicants/mutagens using paper as case study

EXTRACTION (H/A)



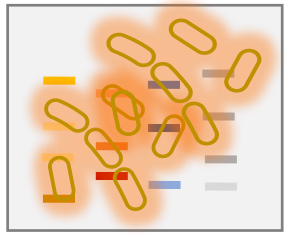
*Commercial colored paper material (non-Nestlé product)*

SPIKING EXTRACTION



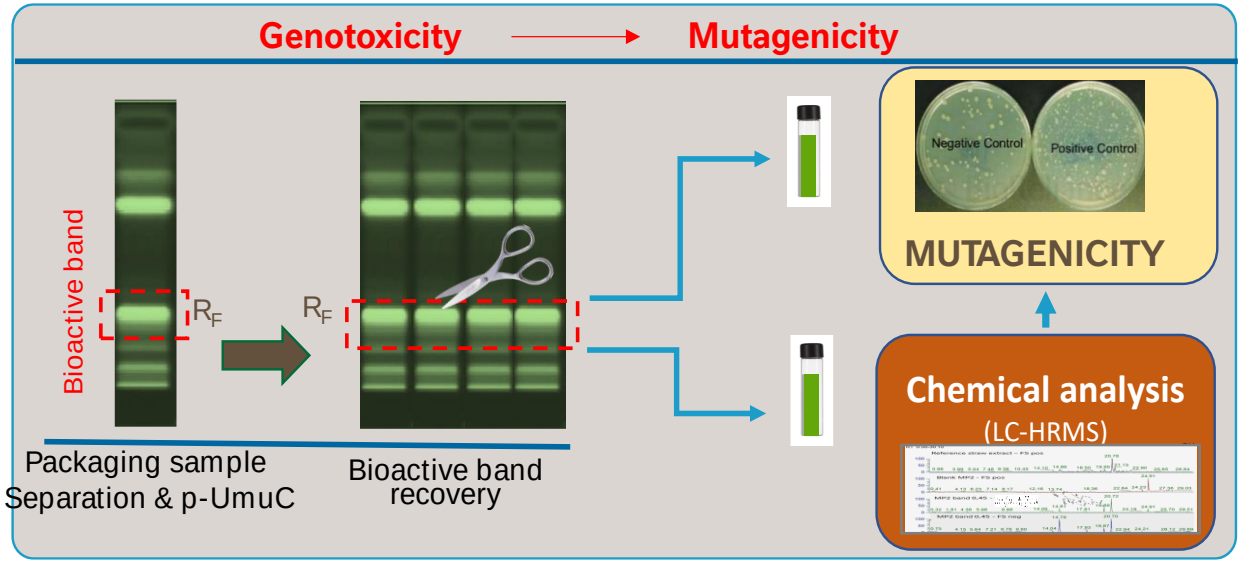

*Mutagenic compound*

*Salmonella SOS Umu-C assay*



***+/- S9 metabolic activation***

## HPTLC coupled to SOS Umu-C assay




***Genotoxic bioactive bands***



Research and Development

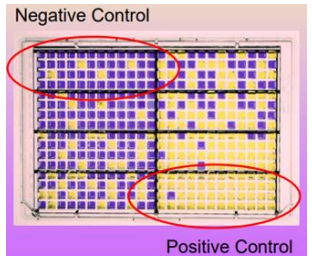
# HPTLC-Umu-C: recovery of mutagenic compounds (4-NQO)

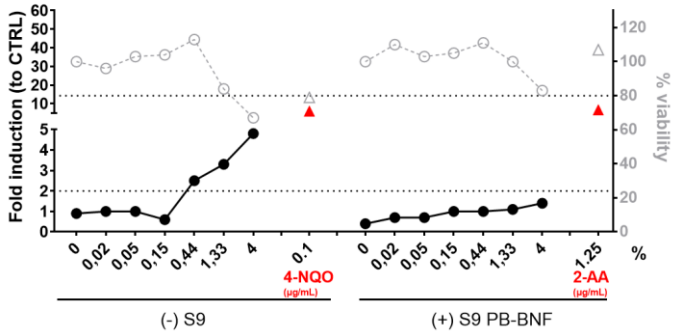
Signal of 4-NQO spiked in paper extract

→ Recovered band extraction →

mutagenicity confirmation + chemical identification

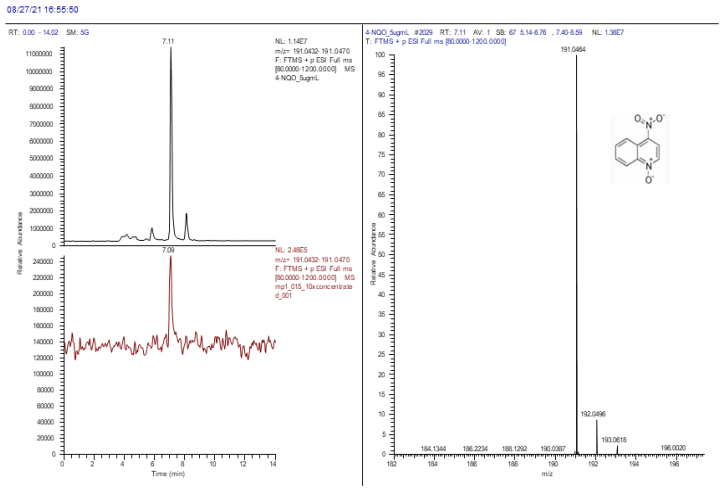
## Ames-MPF




## Chemical analysis

4-NQO* (LC-HRMS)



*4-Nitroquinoline-1-oxide (4-NQO) (CAS 56-57-5)

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



Research and Development

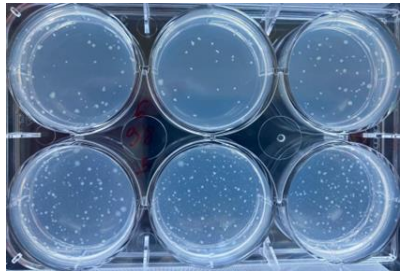
# HPTLC-Umu-C: recovery of mutagenic compounds (glycidol)

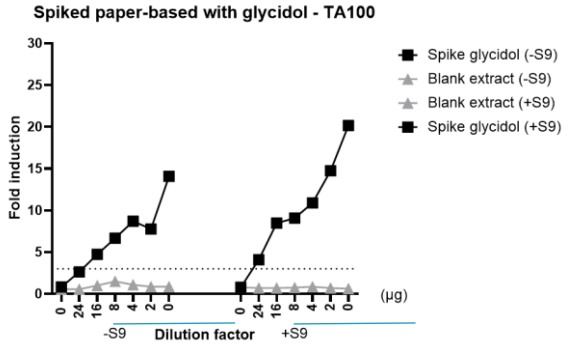
Signal of glycidol spiked in paper extract

Recovered band extraction

mutagenicity confirmation + chemical identification

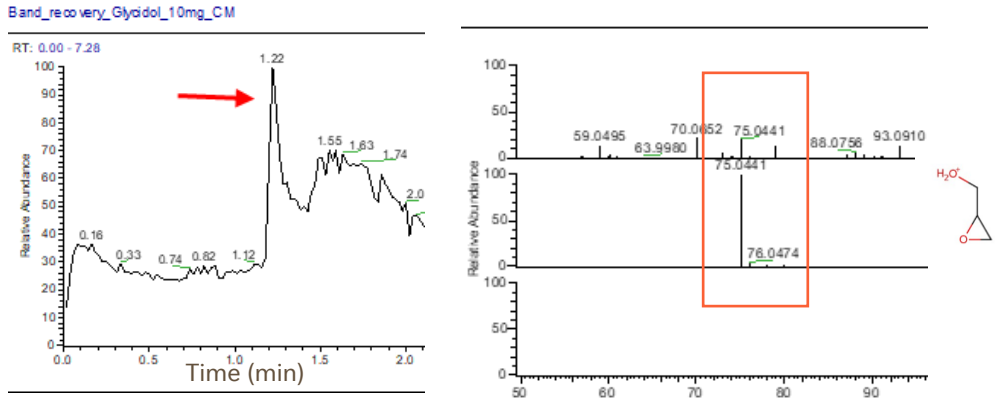
## MicroAmes6






## Chemical analysis

Glycidol*
(LC-HRMS)

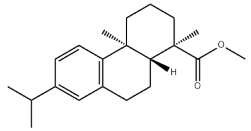

*Reactive diluent in epoxy resin systems

- ✓ The recovered bioactive band was confirmed as mutagenic with MicroAmes6 assay
- ✓ The glycidol was detected using LC-HRMS



Research and Development

# Chemical identification using LC-HRMS and concordance with genetic damage



## Dehydroabietic acid, metyl ester

### LC-HRMS identification

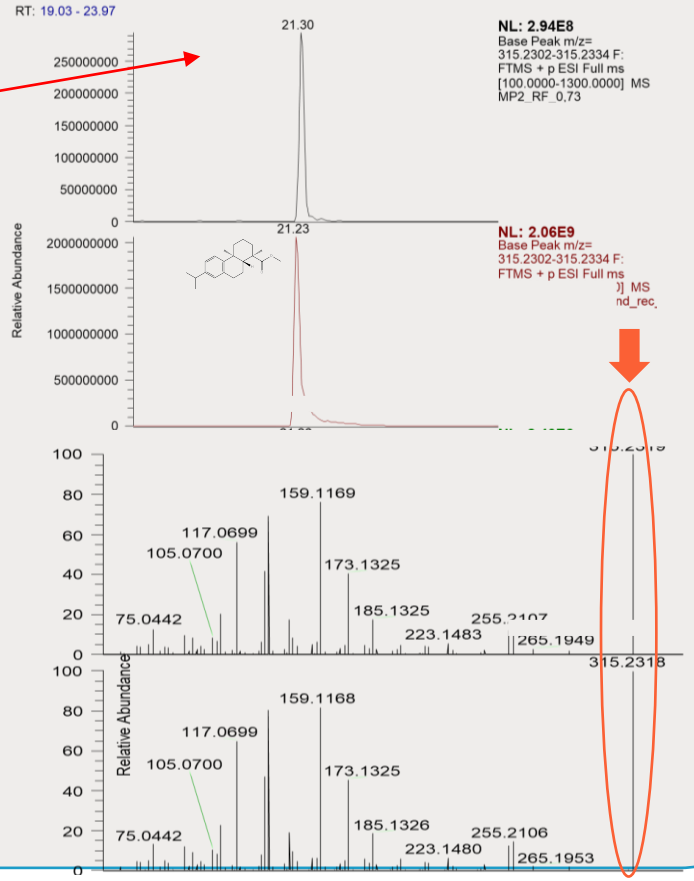
Bioactive band 2

Recovered bioactive band 2 RT

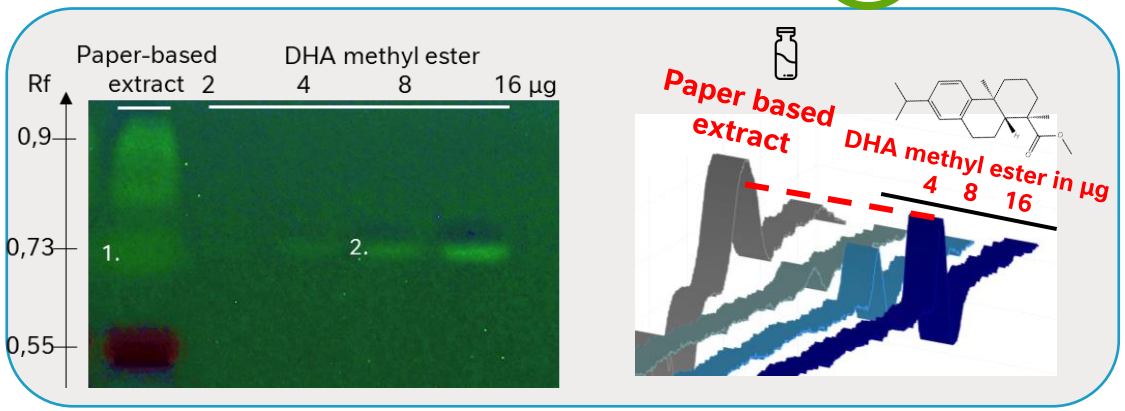
Recovered standard RT

Recovered bioactive band 2 fragmentation

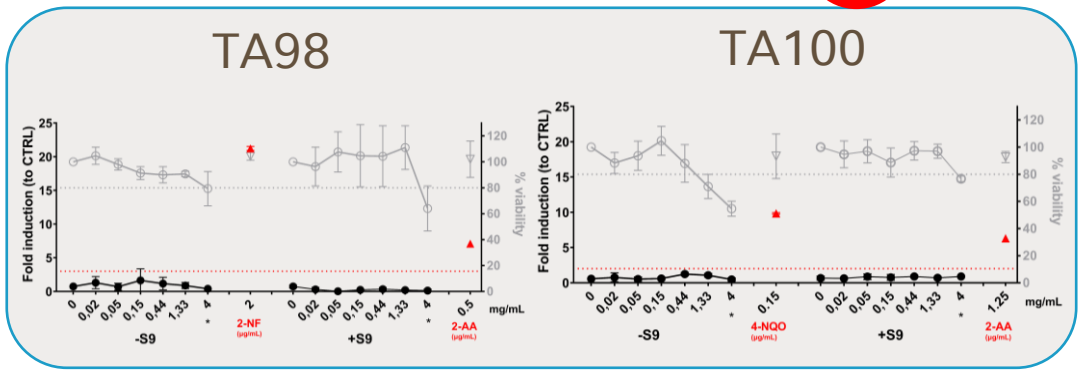
Recovered standard fragmentation




### HPTLC-Umu-C confirmation




### Mutagenicity (AMES-MPF)

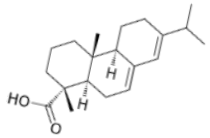



- ✓ High confidence that dehydroabietic acid, metyl ester is present in the paper-extract.
- ✓ No **mutagenic** effect was observed for dehydroabietic acid, metyl ester

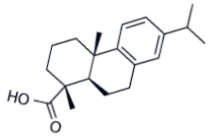


Research and Development

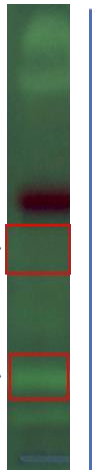
# Chemical identification using LC-HRMS and concordance with genetic damage



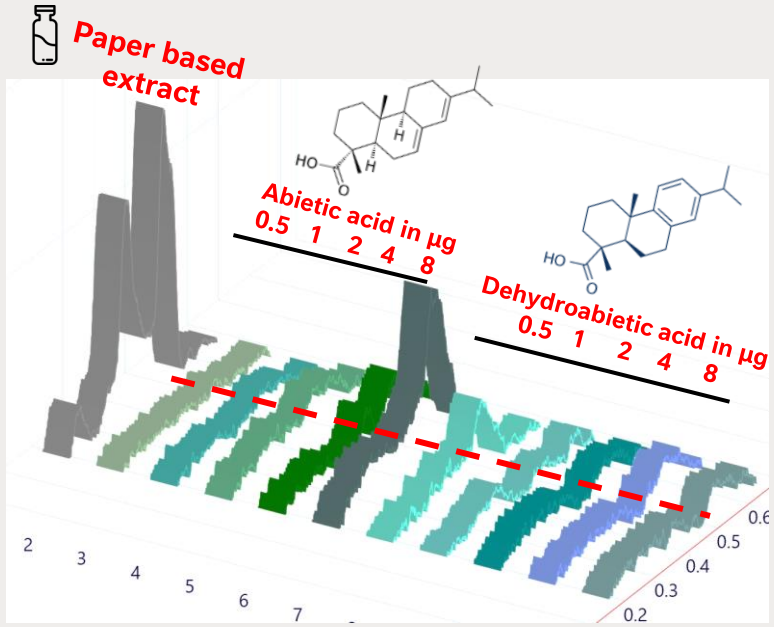
## Abietic and dehydroabietic acids



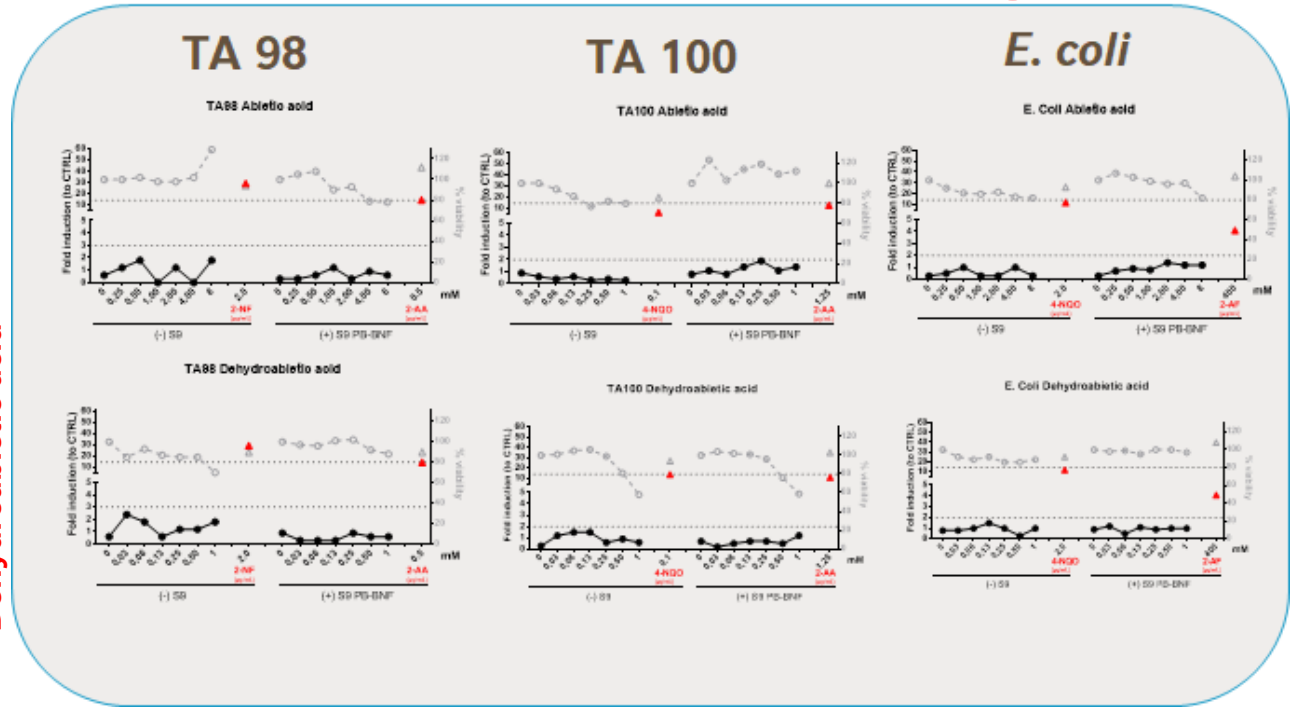
Bioactive band 1



## HPTLC-Umu-C genotoxicity




## Mutagenicity (AMES-MPF)



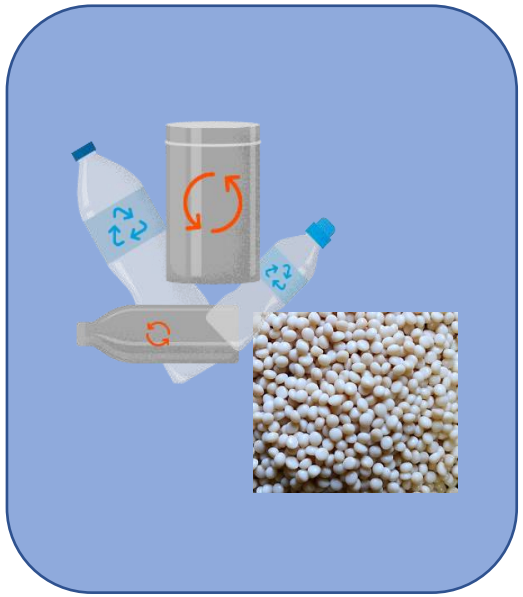

- ✓ High confidence that abietic and dehydroabietic acids are present in the paper-extract
- ✓ No **mutagenic** effect was observed for abietic and dehydroabietic acids
- ✓ PoC confirming performance of the approach

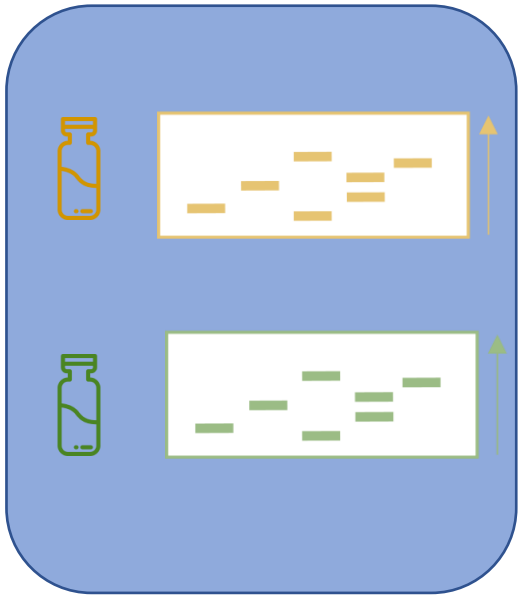


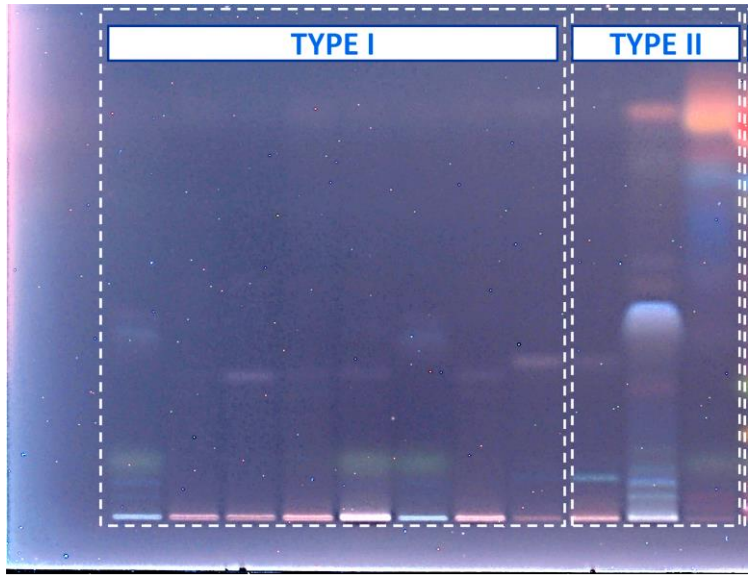
Research and Development

# Screening tool to characterize batch of packaging materials using chemical and effect-based approach

**Packaging sample preparation** → **Sample separation** → **Chemical and biological profiling** → **Prioritization**

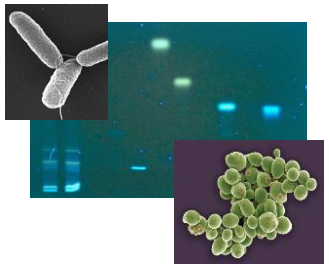






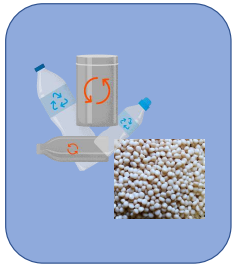

Chemical profiling (derivatization with p-anisaldehyde)
Batches of different types of packaging materials

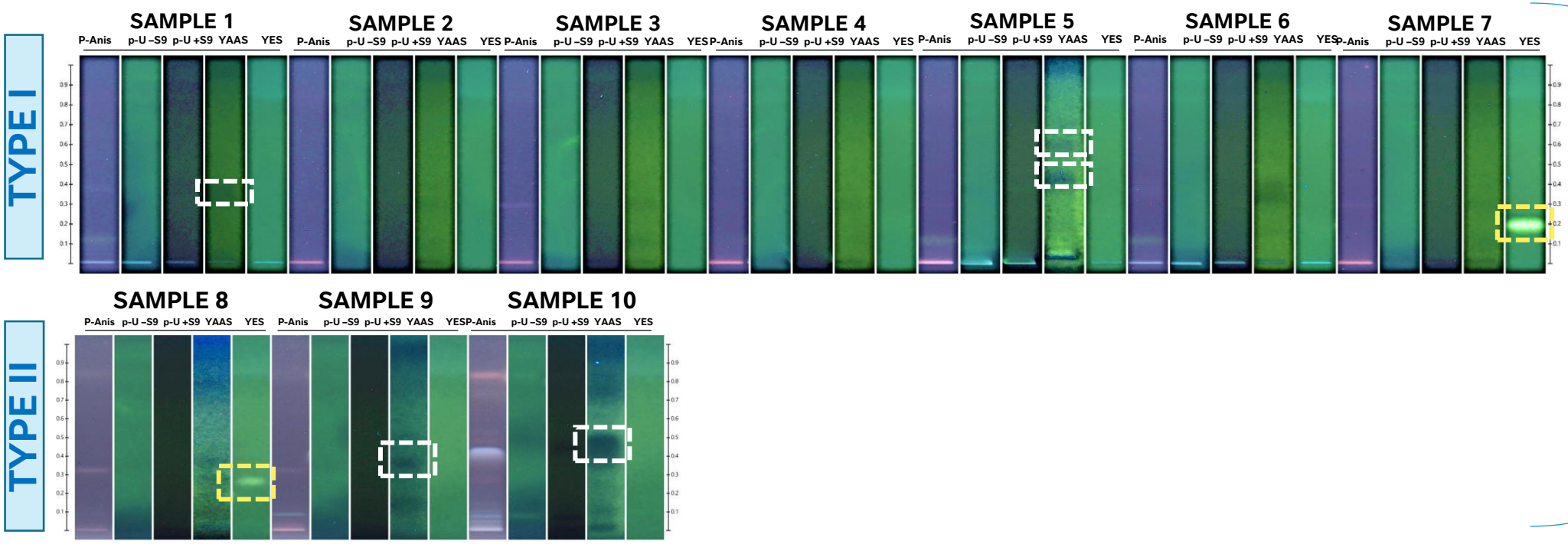
# Packaging materials prioritization tool using effect-based approach (genotoxic & endocrine activity)



## Biological effect-based endpoints

- Anti-Androgenicity
- Estrogenicity
- Androgenicity
- Genotoxicity (+/- metabolic activation)





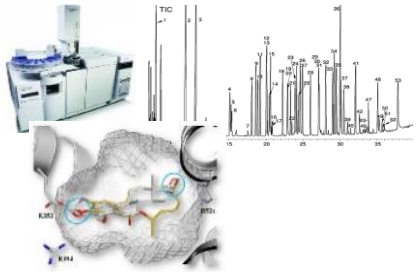



Research and Development

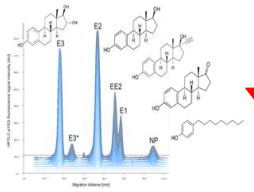
# Suitability of current approach to address packaging safety

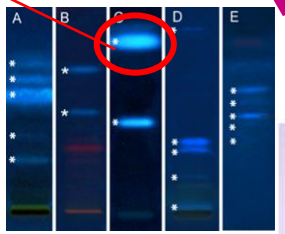
- ✓ Adequate LODs to exclude mutagenicity
- ✓ Facilitate identification of responsible factor(s)
- ✓ Aligned with ILSI recommendations on application of bioassays
- ✓ Promising additional tool for packaging safety evaluation

LC-MS/MS – LC-HR/MS

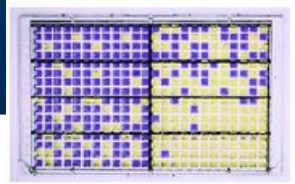


In silico

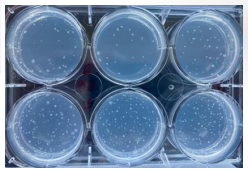


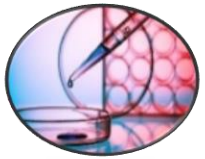


HPTLC
Genotoxicity &
endocrine activity

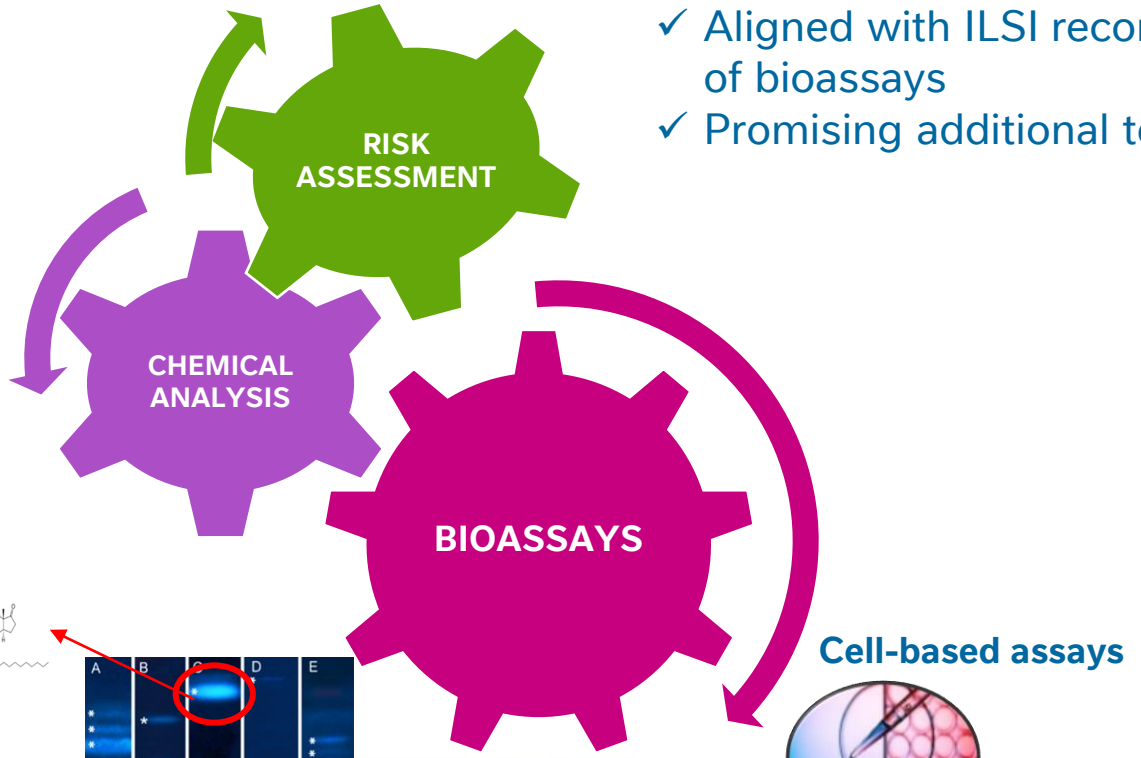


Mutagenicity





Cell-based assays




Research Article

**Detection of Low Levels of Genotoxic Compounds in Food Contact Materials Using an Alternative HPTLC-SOS-Umu-C Assay**

*Daniel Meyer[1], Maricel Marin-Kuan[2], Emma Debon[2], Patrick Serrant[2], Claudine Cottet-Fontannaz[2], Benoît Schilter[2] and Gertrud E. Morlock[1]*

[1]Chair of Food Science, Institute of Nutritional Science, and TransMIT Center of Effect-Directed Analysis, Justus Liebig University Giessen, Giessen, Germany; [2]Chemical Food Safety Group, Société des Produits Nestlé SA – Nestlé Research, Vers-chez-les-Blanc, Switzerland

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**New incorporation of the S9 metabolizing system into methods for detecting acetylcholinesterase inhibition**

Ebrahim Azadniya [a, 1], Julie Mollergues [b, 1], Thomas Stroheker [b], Kathrin Billerbeck [a], Gertrud E. Morlock [a, *]

[a] Chair of Food Science, Institute of Nutritional Science, and TransMIT Center of Effect-Directed Analysis, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392, Giessen, Germany
[b] Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

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**Deciphering the origin of total estrogenic activity of complex mixtures**

Emma Debon[1,2], Bastien Gentili[1], Hélia Latado[1], Patrick Serrant[1], Flavia Badoud[1], Marion Ernest[1], Nicolas Christinat[1], Thomas Bessaire[1], Benoit Schilter[1,3] and Maricel Marin-Kuan[1]*

[1]Nestlé Research, Société des Produits Nestlé SA, Lausanne, Switzerland, [2]Independent Researcher, Bouc Bel Air, France, [3]Retired, Lausanne, Switzerland

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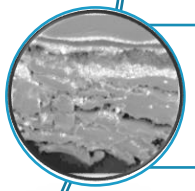
# Conclusions

- Anchoring HPTLC to bioassays (genotoxicity & endocrine activity) is a promising approach to characterize packaging materials
- Facilitates the identification of candidate(s) compound(s) responsible of genotoxic and endocrine activity
- Enable confirmation/exclusion of mutagenic activity in recovered bioactive band (*as shown with 4-NQO*)
- 
  No mutagenicity concern detected for the paper materials used as case study
- Allows application of Cramer class III-TTC and prioritization

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Walburga Seefelder (DH)


Maricel Marin-Kuan
maricel.marin-kuan@rdls.nestle.com



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