



Good food, Good life

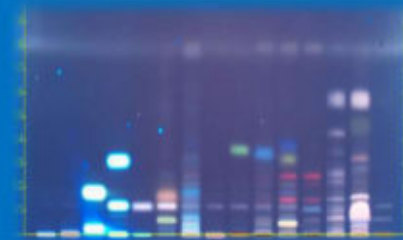
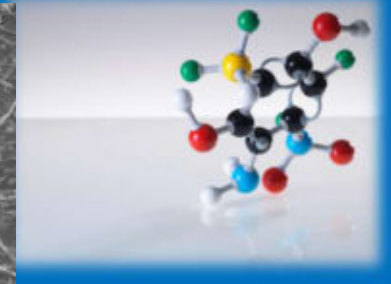
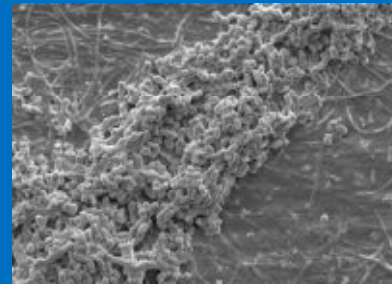
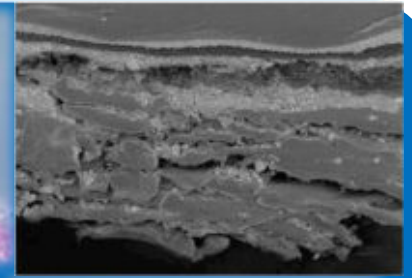
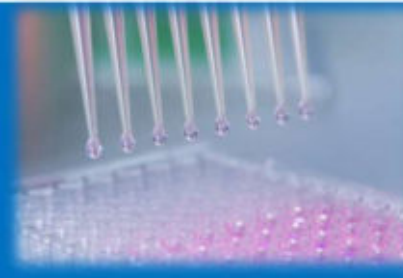
## Industry board meeting Project "MIGRATOX"



# "Lessons learned from the contribution of bioassays to address packaging safety"

Maricel Marin-Kuan  
Institute of Food Safety & Analytical Sciences  
Nestlé Research

24<sup>th</sup> January 2024



# Challenges to establish a framework to assess the safety of FCM

Novel material

Samples  
Preparation

Bioassays & Chemical  
Analysis

Data  
Processing

Prioritization

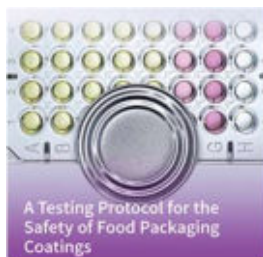


**Safety-by-design**  
Composition



**Assessment of  
Migration(s)**

**Assessment of  
Extraction**

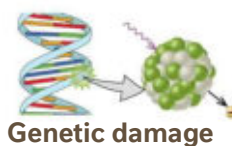


Suitable bioassays?  
LOD?  
Matrix effect?  
Exposure dose?



Chemical analysis  
limitations

**Recommended biological targets**



Endocrine activity

Identification ?  
Semi-quantification



Target / Suspected / Non-Target  
Screening



**Lack of guidelines for  
harmonization?**



# Limitations in the usage of bioassays to exclude mutagenicity

## Past -Today and preparing for the future

### KEY LIMITATIONS:

- Regulatory guidelines constraints
- Selection of test/s
- In-site performance validation
- **SENSITIVITY** (Food exposure context)
- Fit-for-purpose methods

### PAST

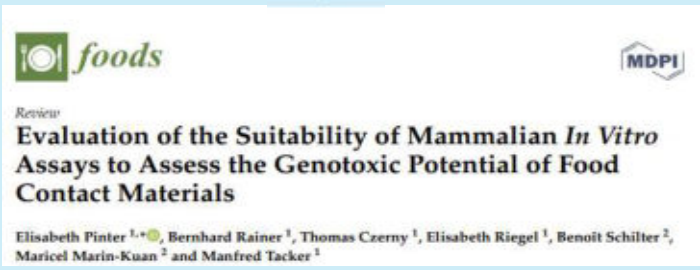
#### Requirement

Implementation of *in vitro* genotoxicity testing based on mammalian cells for FCM (migration and extraction) sample testing

#### Deliverable

Bluescreen test (Gentronix) was improved and implemented for FCM

Approach was **not suitable** according to the requirements for genotoxicity assessment of FCM  
**(genotoxicity vs mutagenicity)**



E. Pinter et al. Evaluation of the Suitability of Mammalian In Vitro Assays to Assess the Genotoxic Potential of Food Contact Materials. *Foods* 2020, 9, 237

### TODAY

#### ILSI packaging WG (Schilter et al 2019):

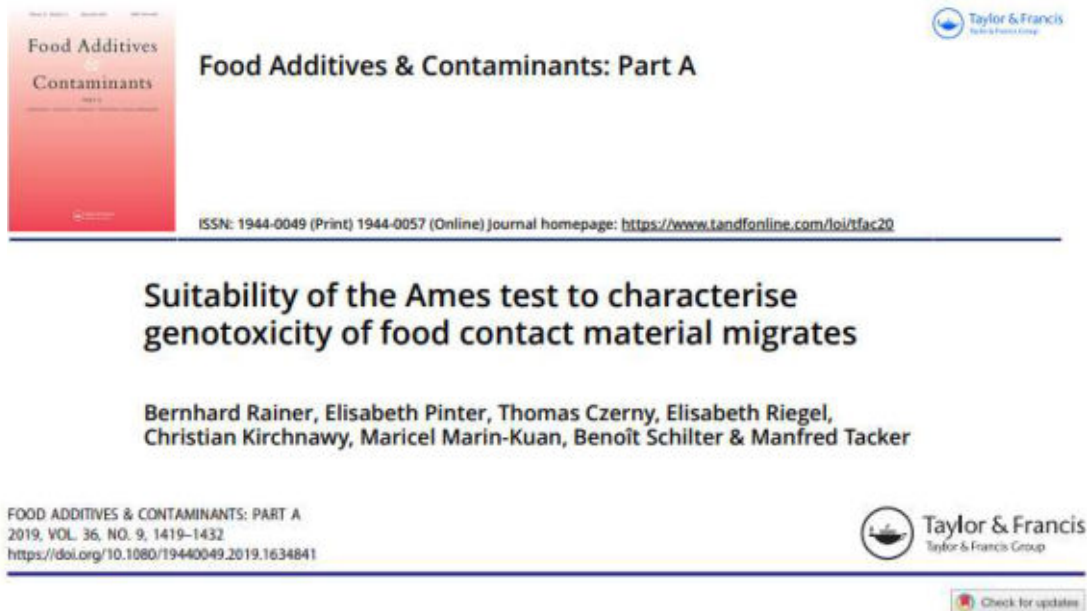


**Recommendation:** Exclusion of mutagenicity to apply **TTC Cramer Class III**

**AMES assay**  
limit of detection is not suitable for TTC threshold?

**Future? Need breakthrough strategy?**

# Suitability of mutagenicity Ames assays to assess FCM (Migratox project contribution)

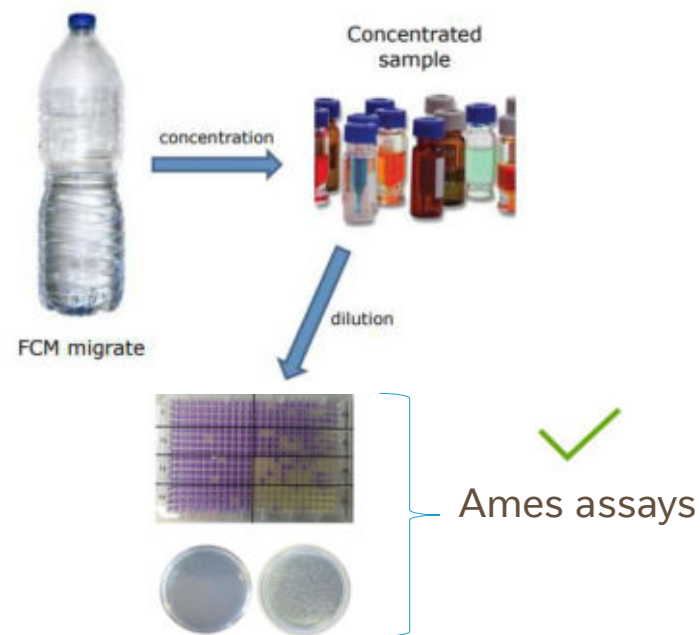


## Mutagenicity assessment of food contact material migrates with the Ames MPF assay

Bernhard Rainer<sup>a,\*</sup>, Elisa Mayrhofer<sup>a,b</sup>, Miriam Redl<sup>a</sup>, Irene Dolak<sup>a</sup>, Daniela Mislivecek<sup>b</sup>, Thomas Czerny<sup>a</sup>, Christian Kirchnawy<sup>b</sup>, Maricel Marin-Kuan<sup>c</sup>, Benoît Schilter<sup>c</sup> and Manfred Tacker<sup>a</sup>

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*Adapted from Rainer et al, 2019*



Limit of Detection (TTC)?  
Compounds responsible of mutagenic effect?  
Other solutions needed?

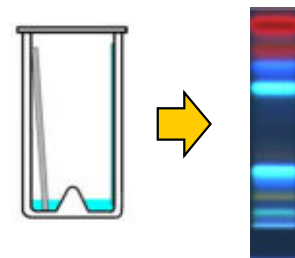
# Proposed strategy to achieve ILSI recommendations on mutagenicity

## REQUIREMENTS NEEDED

- Limit of detection
- Metabolic activation
- Solvent used prepare samples (e.g.; DMSO toxicity)
- Whole sample vs fractionation
- Matrix effect
- Identification of compounds responsible biological activity (e.g; mutagenicity)
- Exclusion of mutagenicity vs genotoxicity
- ...

Develop new approach combining three technologies:

(1) The High-Performance Thin Layer Chromatography (HPTLC) coupled to a genotoxicity test



Separation of complex mixtures possible

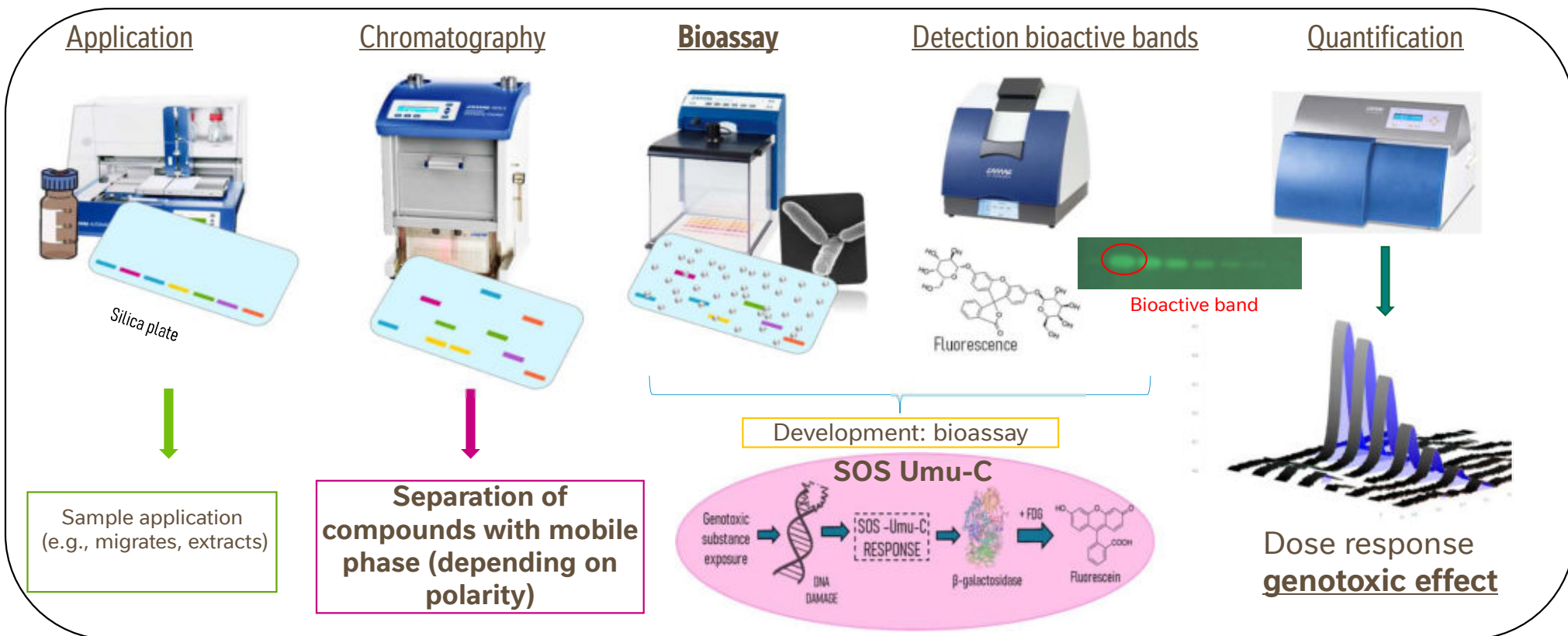
(2) **AMES assay** (Miniaturized liquid AMES-MPF to Ames agar to assess mutagenicity)

(3) LC-HRMS for chemical identification of bioactives

**Effect-Directed Analysis Approach as solution**



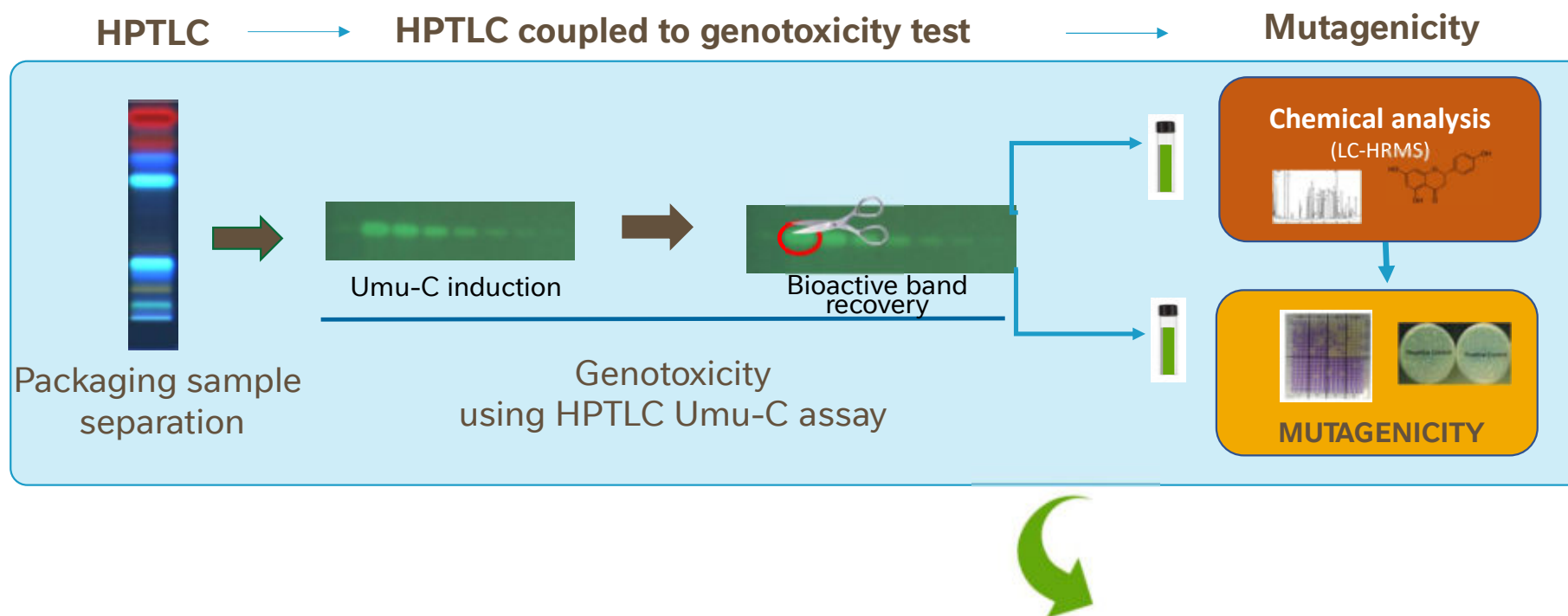
# New HPTLC methodology coupled to genotoxicity assay



Meyer, D., et al (2021) "ALTEX -2021 Alternatives to animal experimentation, 38(3), pp. 387–397. doi: 10.14573/altex.2006201.

Debon E, et al . (2022) *Toxics*. 2022 Aug 27;10(9):501. doi: 10.3390/toxics10090501. PMID: 36136466; PMCID: PMC9500983.

# Identification of substance(s) with genotoxic potential and /or exclusion of MUTAGENICITY



Facilitate TTC application & risk assessment

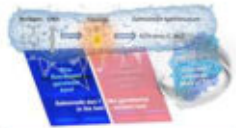
# PoC of capability to detect low levels of a wide range of DNA-damaging substances in accordance with TTC requirements

✓ Limit of detection suitable according to ILSI recommendations



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## Detection of low levels of genotoxic compounds in food contact materials using an alternative HPTLC-SOS-Umu-C assay



pdf

sup

Published: Jul 19, 2021

DOI:  
<https://doi.org/10.14573/altex.2006201>

**Keywords:**  
genotoxicity assay, genotoxins, Salmonella typhimurium, Ames MPF assay, planar assay, RP-HPTLC-UV/Vis/FLD-SOS-Umu-C assay

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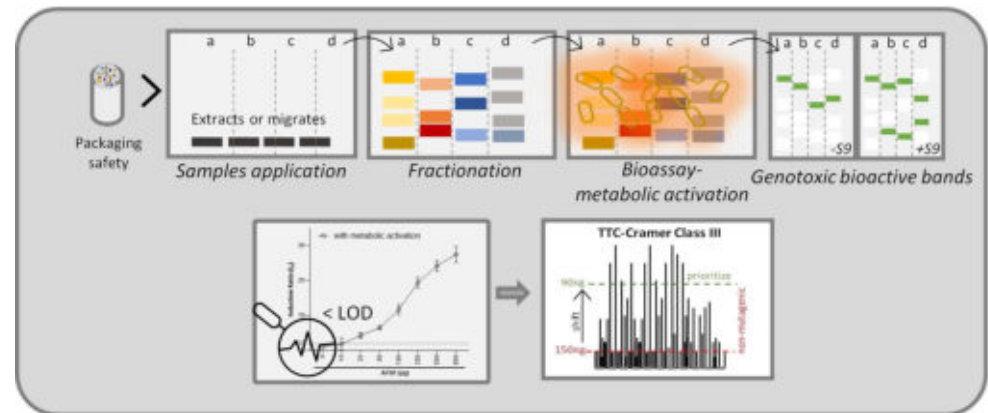
Chair of Food Science, Institute of Nutritional Science, and TransMIT Center of Effect-Directed Analysis, Justus Liebig University Gießen, Gießen, Germany



Article

## Incorporation of Metabolic Activation in the HPTLC-SOS-Umu-C Bioassay to Detect Low Levels of Genotoxic Chemicals in Food Contact Materials

Emma Debon <sup>1</sup>, Paul Rogebox <sup>1</sup>, Hélia Latado <sup>1</sup>, Gertrud E. Morlock <sup>2</sup>, Daniel Meyer <sup>2</sup>, Claudine Cottet-Fontannaz <sup>1</sup>, Gabriele Scholz <sup>1</sup>, Benoît Schiller <sup>1</sup> and Maricel Marin-Kuan <sup>1,\*</sup>



Meyer, D., et al (2021) "ALTEX -2021 Alternatives to animal experimentation, 38(3), pp. 387–397. doi: 10.14573/altex.2006201.

Debon E, et al . (2022) Toxics. 2022 Aug 27;10(9):501. doi: 10.3390/toxics10090501. PMID: 36136466; PMCID: PMC9500983.

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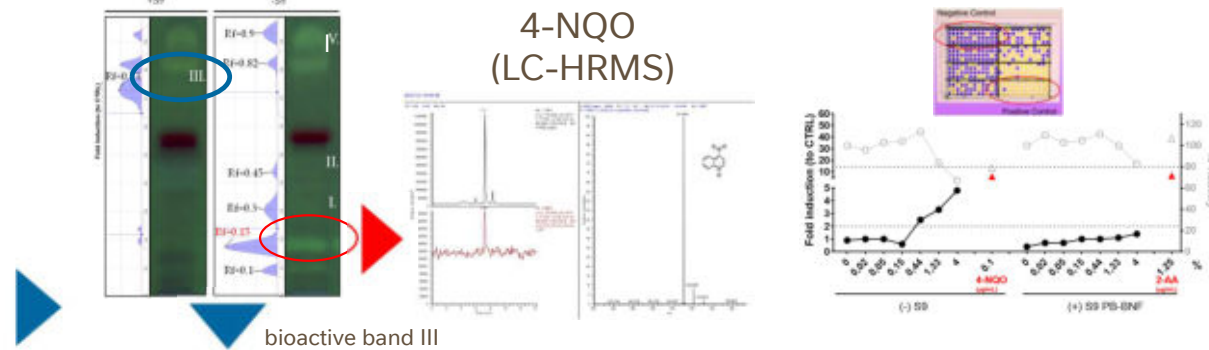
Lessons learned from the contribution of bioassays to address packaging safety



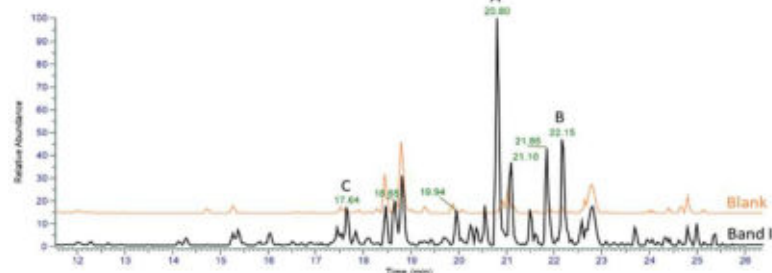


# From packaging extract/migrate to the identification of potential genotoxicants/mutagens using paper as case study

- ✓ Spiked mutagenic compound was confirmed with Ames-MPF assay and LC-HRMS



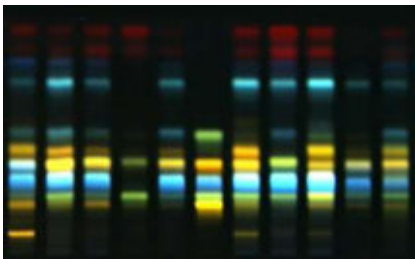
- ✓ No mutagenic compounds were identified in bioactive band III
- LC-HRMS-number of picks ↓



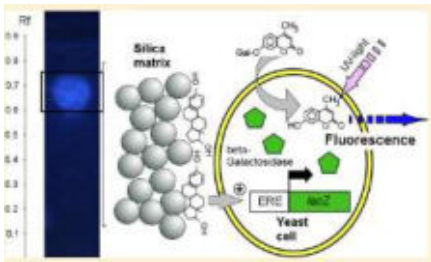
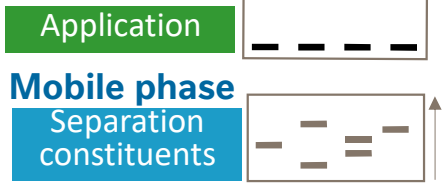
- HPTLC approach facilitate detection and identification of potential genotoxicity/mutagenicity hazards
- Chemical identification still challenging
- P. Rogeboz et al (in preparation)

# Progress on other biological endpoints?

## High Performance Thin-Layer Chromatography coupled to estrogen and androgen receptors



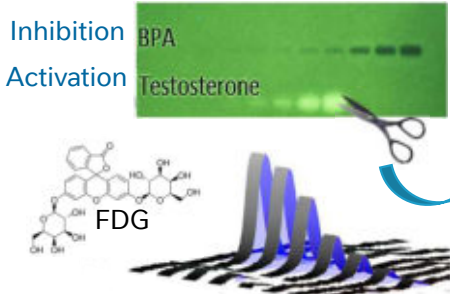
Stationary phase (silica plate)



p-YES assay

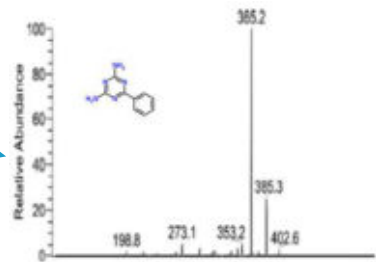
Spraying of yeast cells on the silica plate

Incubation 3h / 37°C



Dose-response

Bioassay method development (fluorescence) and identification of bioactive bands



LC-HRMS

Chemical identification of compound(s) present in bioactive band responsible of biological effect (e.g; estrogenic activity)

# Endocrine activity assessment using HPTLC application has been demonstrated with FCM

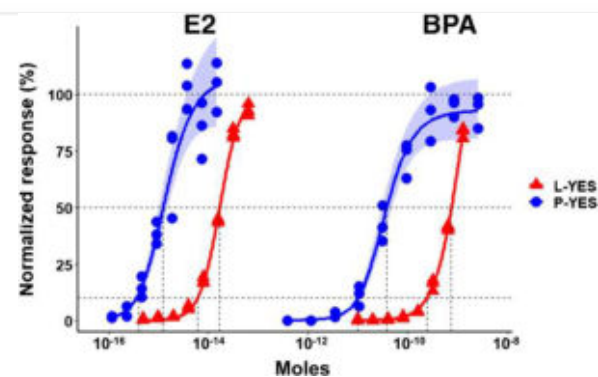
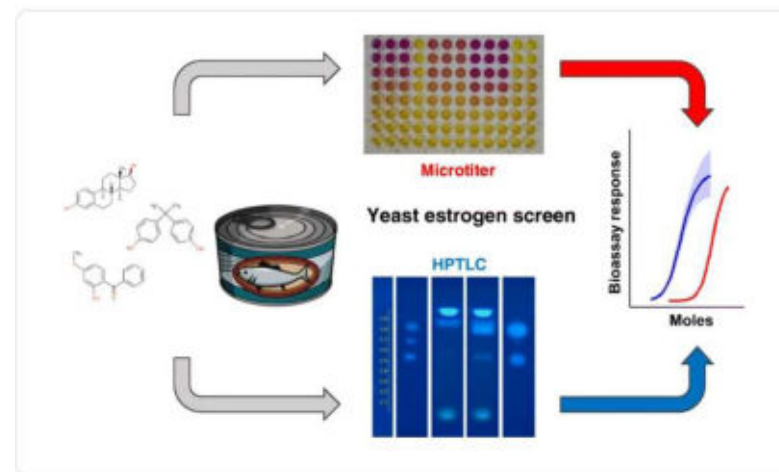
Analytical and Bioanalytical Chemistry (2020) 412:4527–4536  
<https://doi.org/10.1007/s00216-020-02701-w>

## RESEARCH PAPER

### Estrogenic activity of food contact materials—evaluation of 20 chemicals using a yeast estrogen screen on HPTLC or 96-well plates

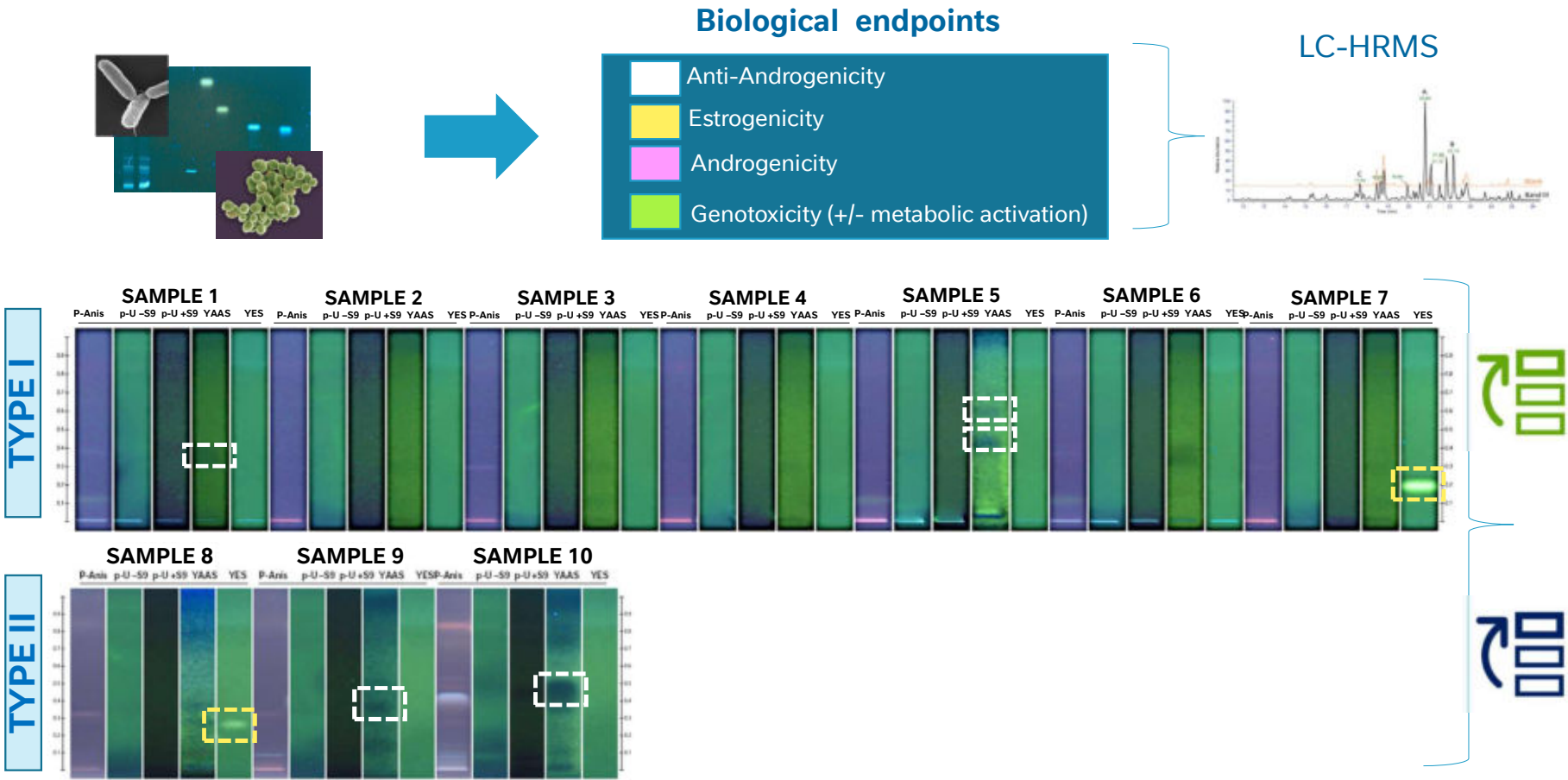
Alan J. Bergmann<sup>1</sup>  · Eszter Simon<sup>1</sup> · Andrea Schifferli<sup>1</sup> · Andreas Schönborn<sup>2</sup> · Etienne L. M. Vermeirssen<sup>1</sup>

Received: 11 February 2020 / Revised: 8 April 2020 / Accepted: 11 May 2020 / Published online: 26 May 2020  
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Bergman et al; 2020

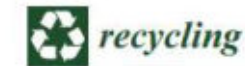
# Packaging materials prioritization tool using effect-direct analysis approach (genotoxic & endocrine activity)



24th January 2024

Lessons learned from the contribution of bioassays to address packaging safety

# BUT...Chemical identification for concordance analysis is still key limiting factor?



Article

## Safety Assessment of Recycled Plastics from Post-Consumer Waste with a Combination of a Miniaturized Ames Test and Chromatographic Analysis

Elisa Mayrhofer <sup>1,\*</sup>, Lukas Prielinger <sup>2,†</sup>, Victor Sharp <sup>1</sup>, Bernhard Rainer <sup>2</sup>, Christian Kirchnawy <sup>1</sup>, Christian Rung <sup>3</sup>, Anita Gruner <sup>3</sup>, Mladen Juric <sup>3</sup> and Arielle Springer <sup>3,\*</sup>

Food Control 147 (2023) 109546



Contents lists available at ScienceDirect

Food Control

journal homepage: [www.elsevier.com/locate/foodcont](http://www.elsevier.com/locate/foodcont)



Effect-detection by planar SOS-Umu-C genotoxicity bioassay and chemical identification of genotoxins in packaging migrates, proven by microtiter plate assays SOS-Umu-C and Ames-MPF

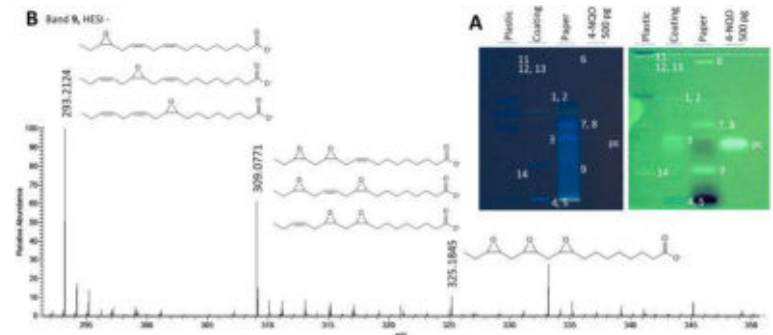
Daniel Meyer <sup>a</sup>, Maricel Marin-Kuan <sup>b</sup>, Elisa Mayrhofer <sup>c</sup>, Christian Kirchnawy <sup>c</sup>, Emma Debon <sup>b</sup>, Helia Latado <sup>b</sup>, Amaury Patin <sup>b</sup>, Benoît Schilter <sup>b</sup>, Gertrud Morlock <sup>a,\*</sup>

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<sup>c</sup> Department for Microbiology and Cell Culture, Austrian Research Institute for Chemistry and Technology, Franz-Greif-Straße 5, 1030, Vienna, Austria

- DNA-reactive contaminants are not randomly introduced through the misuse of recycled packaging by consumers but are systematically formed during the **recycling process** from precursors in the input.
- This publication highlights the **need to identify the source for this critical contaminant** to enable the future use of polyethylene, polypropylene and polystyrene in sensitive applications



Step forward using HPTLC coupled to bioassays, but still work needed

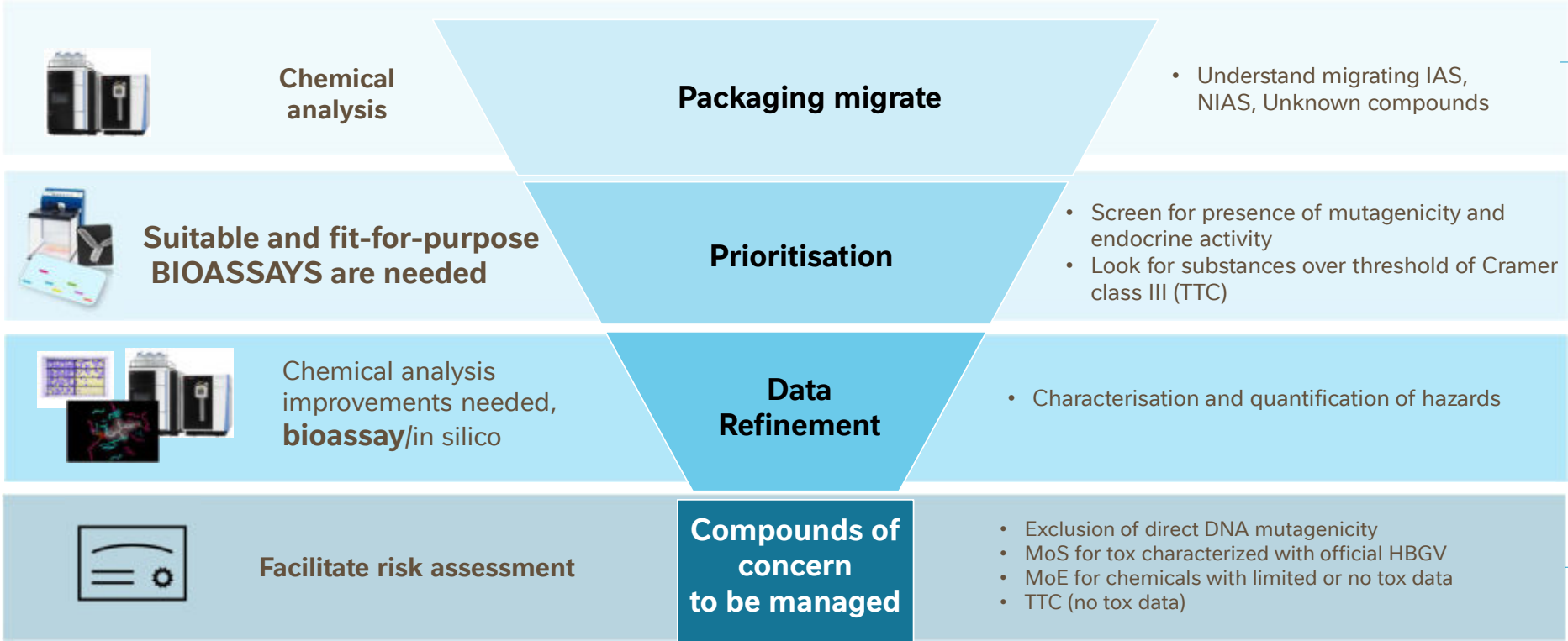
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Lessons learned from the contribution of bioassays to address packaging safety





# The combination of different technologies is required to conclude on which migrating chemicals are of concern



MoS: Margin of Safety  
MoE: Margin of Exposure  
HBGV: Health Based Guidance Value  
TTC: Threshold of toxicological concern

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