



Nestlé Good food, Good life

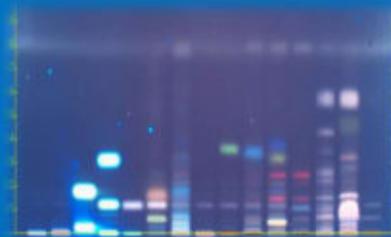
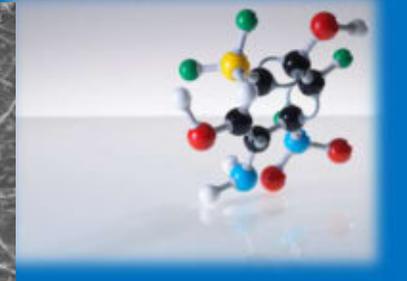
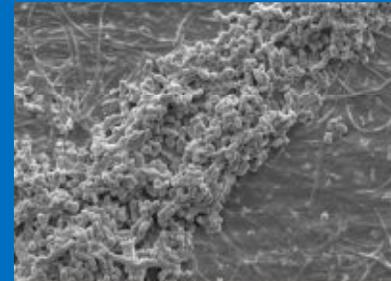
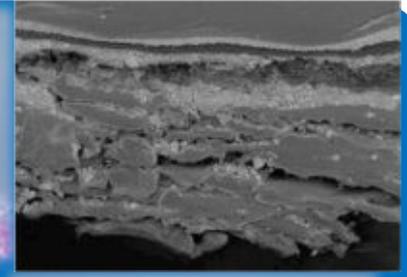
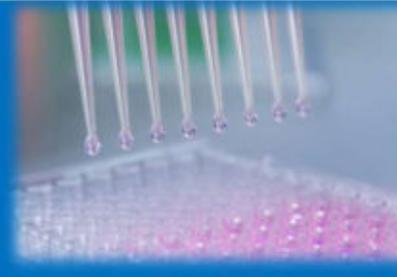
Industry board meeting Project "MIGRATOX"



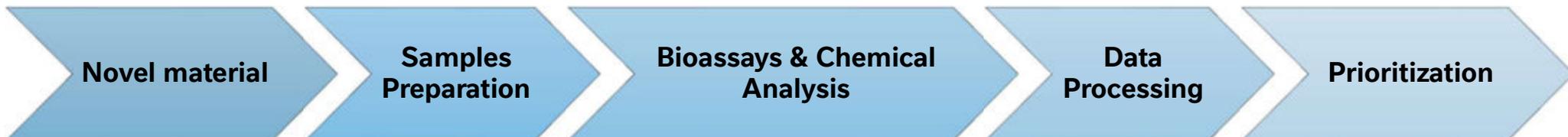
"Lessons learned from the contribution of bioassays to address packaging safety"

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

24th January 2024



Challenges to establish a framework to assess the safety of FCM

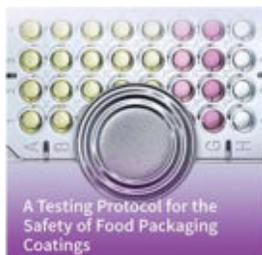


Safety-by-design Composition



Assessment of Migration(s)

Assessment of Extraction



A Testing Protocol for the Safety of Food Packaging Coatings

Suitable bioassays?
LOD?
Matrix effect?
Exposure dose?



Chemical analysis limitations

Recommended biological targets



Genetic damage



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:

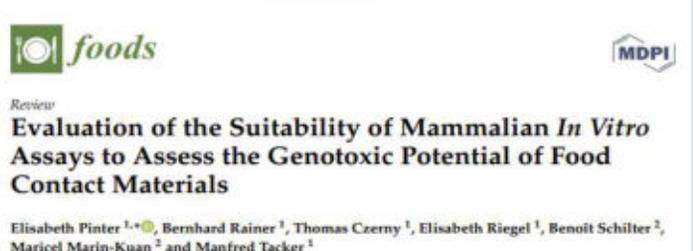
- Regulatory guidelines constraints
- Selection of test/s
- In-site performance validation
- **SENSITIVITY** (Food exposure context)
- Fit-for-purpose methods

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):

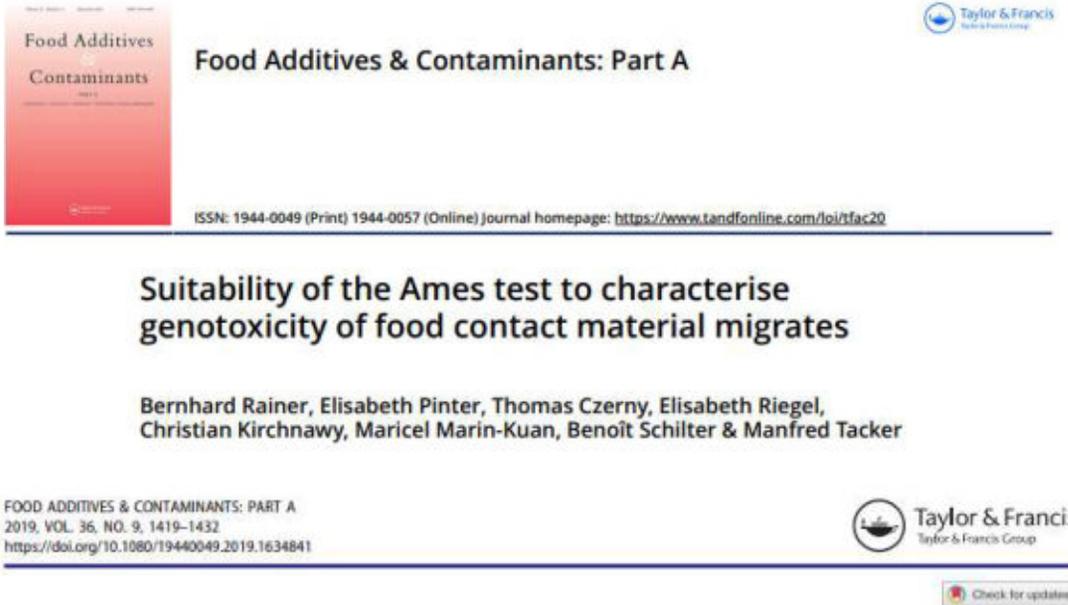


Recommendation: Exclusion of mutagenicity to apply TTC Cramer Class III

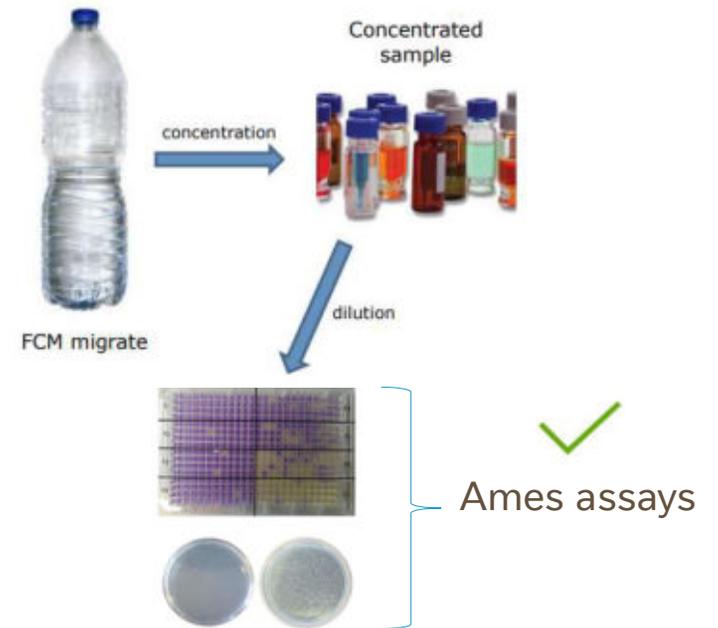
AMES assay
limit of detection is not suitable for TTC threshold?

Future? Need breakthrough strategy?

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



Adapted from Rainer et al, 2019



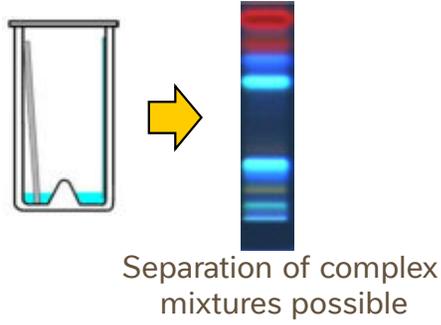
Limit of Detection (TTC)?
Compounds responsible of mutagenic effect?
Other solutions needed?

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



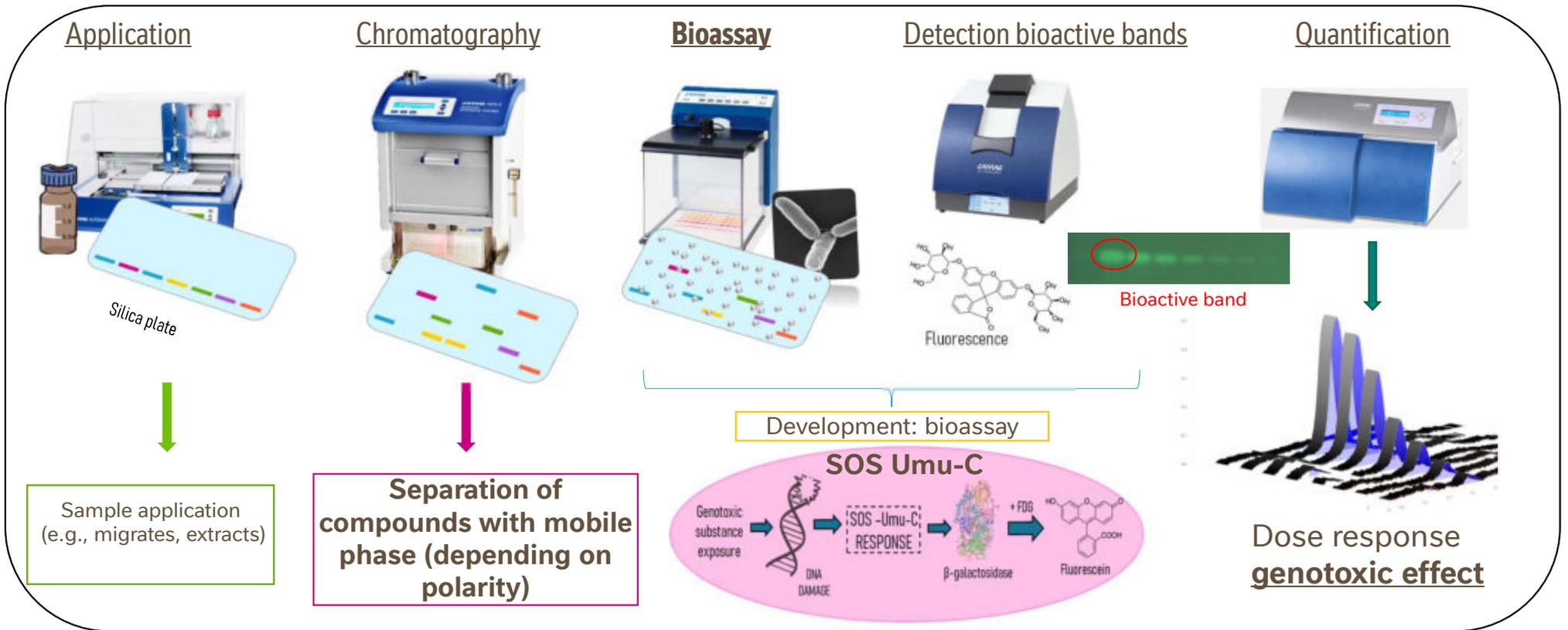
(2) **AMES assay** (Miniaturized liquid AMES-MPF to Ames agar to assess mutagenicity)

(3) LC-HRMS for chemical identification of bioactives



Effect-Directed Analysis Approach as solution

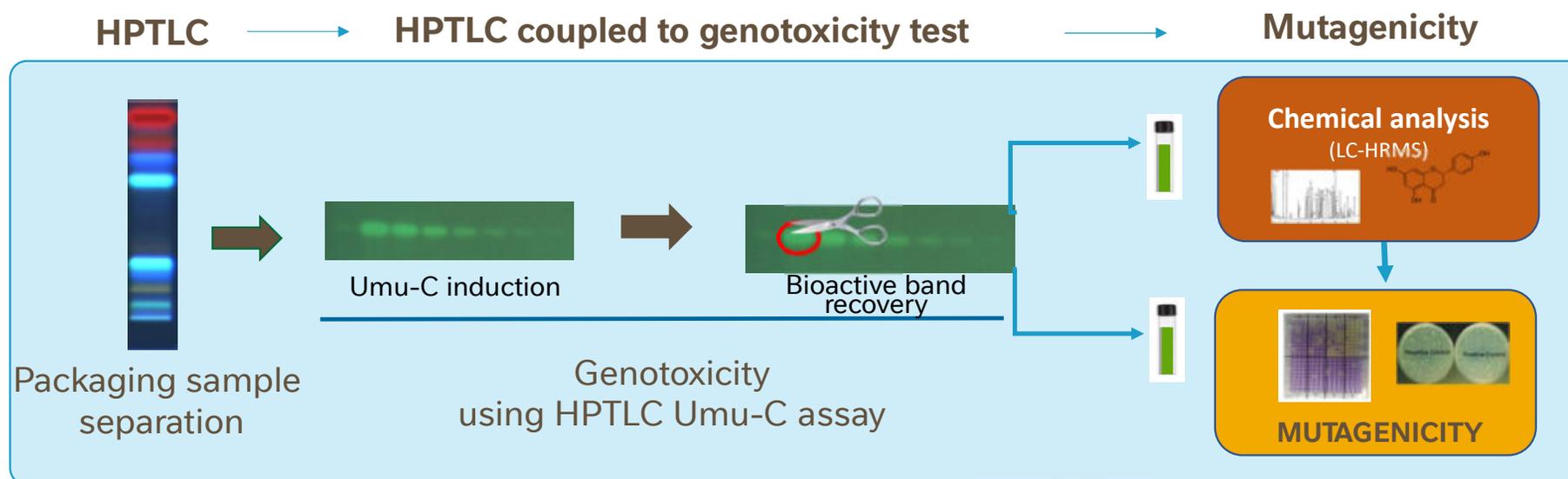
New HPTLC methodology coupled to genotoxicity assay



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



Home Latest Articles Current Issue News & Events - Archives Special Issues Resources - About -

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-UUV/Vis/FLD-SOS-Umu-C assay

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

Maricel Marin-Kuan
Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

Emma Debon
Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

Patrick Serrant
Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

Claudine Cottet-Fontannaz
Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

Benoît Schiller
Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Blanc, Switzerland

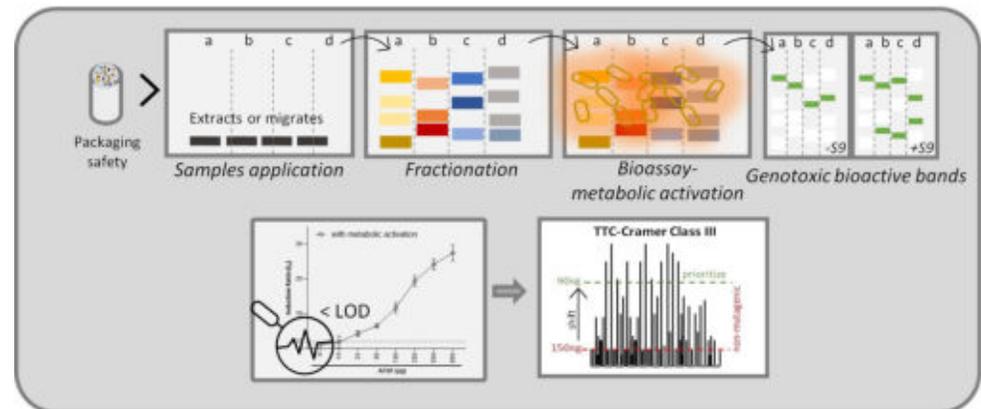
Gertrud E. Morlock
Chair of Food Science, Institute of Nutritional Science, and TransMIT Center of Effect-Directed Analysis, Justus Liebig University Giessen, Giessen, Germany



Article

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

Emma Debon¹, Paul Rogeboz¹, Hélia Latado¹, Gertrud E. Morlock², Daniel Meyer², Claudine Cottet-Fontannaz¹, Gabriele Scholz¹, Benoît Schiller¹ and Maricel Marin-Kuan^{1,*}



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.

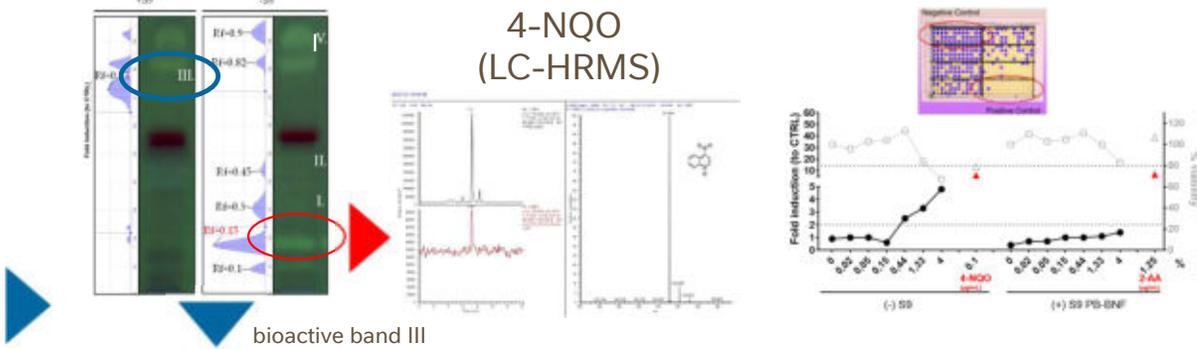
24th January 2024

Lessons learned from the contribution of bioassays to address packaging safety



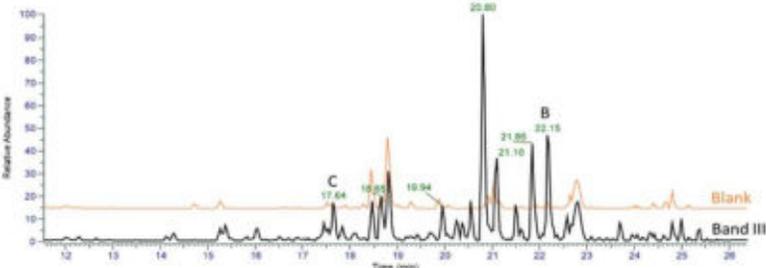
From packaging extract/migrate to the identification of potential genotoxicants/mutagens using paper as case study

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



✓ No mutagenic compounds were identified in bioactive band III

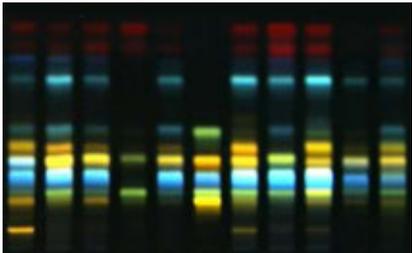
LC-HRMS-number of picks ↓



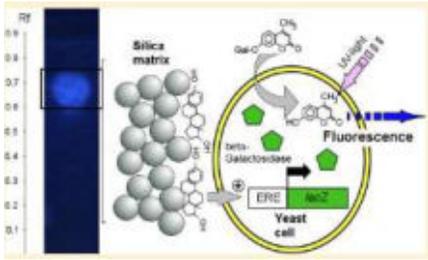
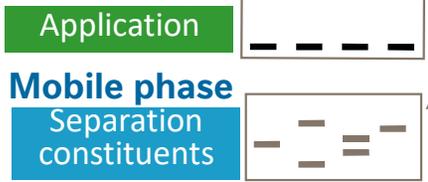
- ✓ • HPTLC approach facilitate detection and identification of potential genotoxicity/mutagenicity hazards
- Chemical identification still challenging
- P. Rogeboz et al (in preparation)

Progress on other biological endpoints?

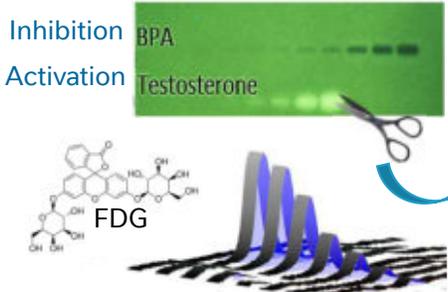
High Performance Thin-Layer Chromatography coupled to estrogen and androgen receptors



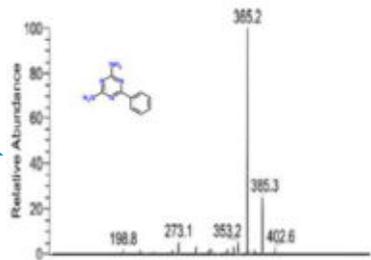
Stationary phase (silica plate)



p-YES assay



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

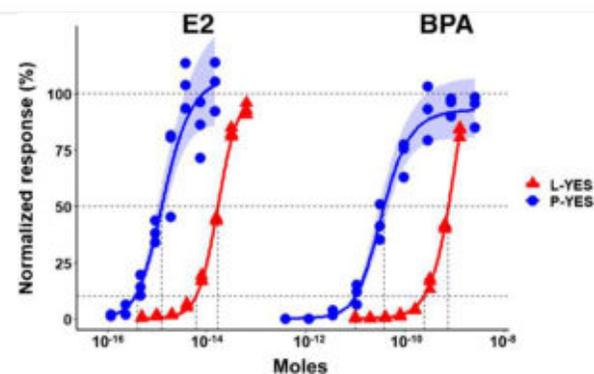
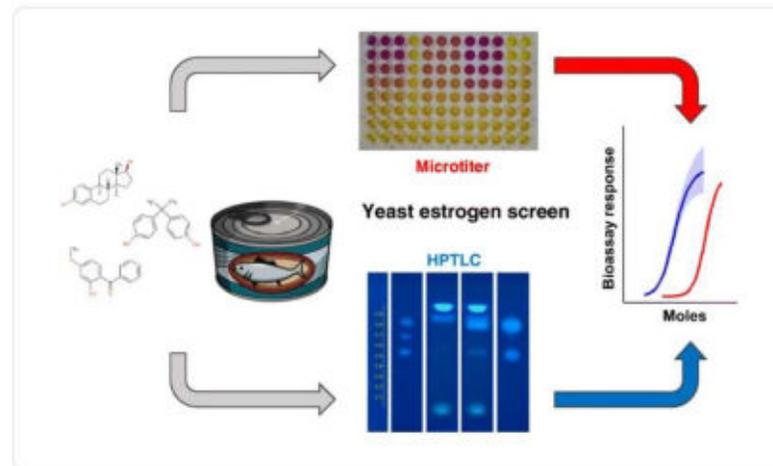
Analytical and Bioanalytical Chemistry (2020) 412:4527–4536
<https://doi.org/10.1007/s00216-020-02701-w>

RESEARCH PAPER

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

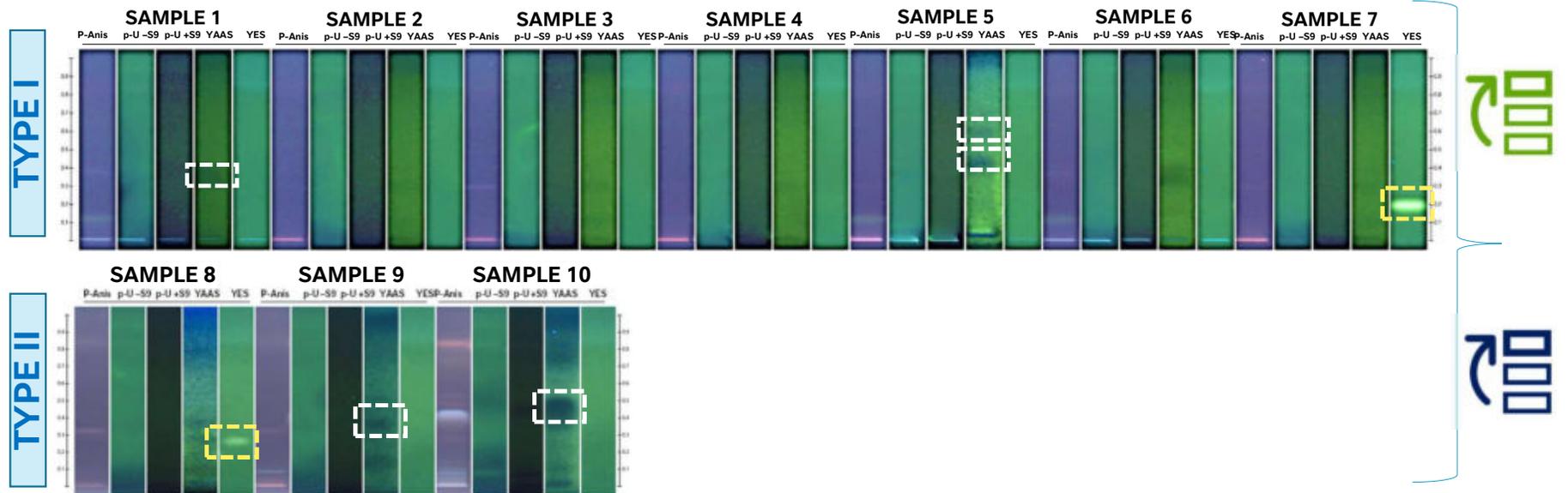
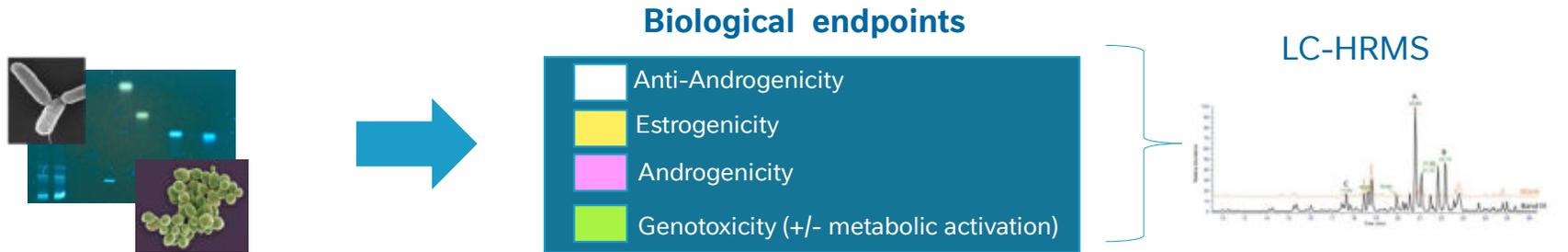
Alan J. Bergmann¹  · Eszter Simon¹ · Andrea Schifferli¹ · Andreas Schönborn² · Etiënne L. M. Vermeirssen¹

Received: 11 February 2020 / Revised: 8 April 2020 / Accepted: 11 May 2020 / Published online: 26 May 2020
© The Author(s) 2020



Bergman et al; 2020

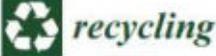
Packaging materials prioritization tool using effect-direct analysis approach (genotoxic & endocrine activity)



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 ^{1,*}, Lukas Prielinger ^{2,†}, Victor Sharp ¹, Bernhard Rainer ², Christian Kirchnawy ¹, Christian Rung ³, Anita Gruner ³, Mladen Juric ³ and Arielle Springer ^{3,*}

Food Control 147 (2023) 109546

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

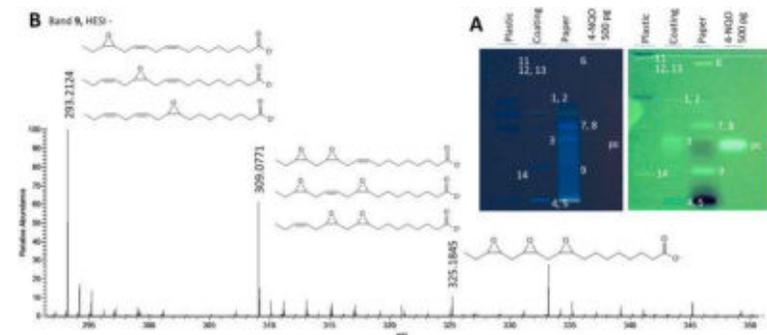



- DNA-reactive contaminants are not randomly introduced through the misuse of recycled packaging by consumers but are systematically formed during the **recycling process** from precursors in the input.
- This publication highlights the **need to identify the source for this critical contaminant** to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

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

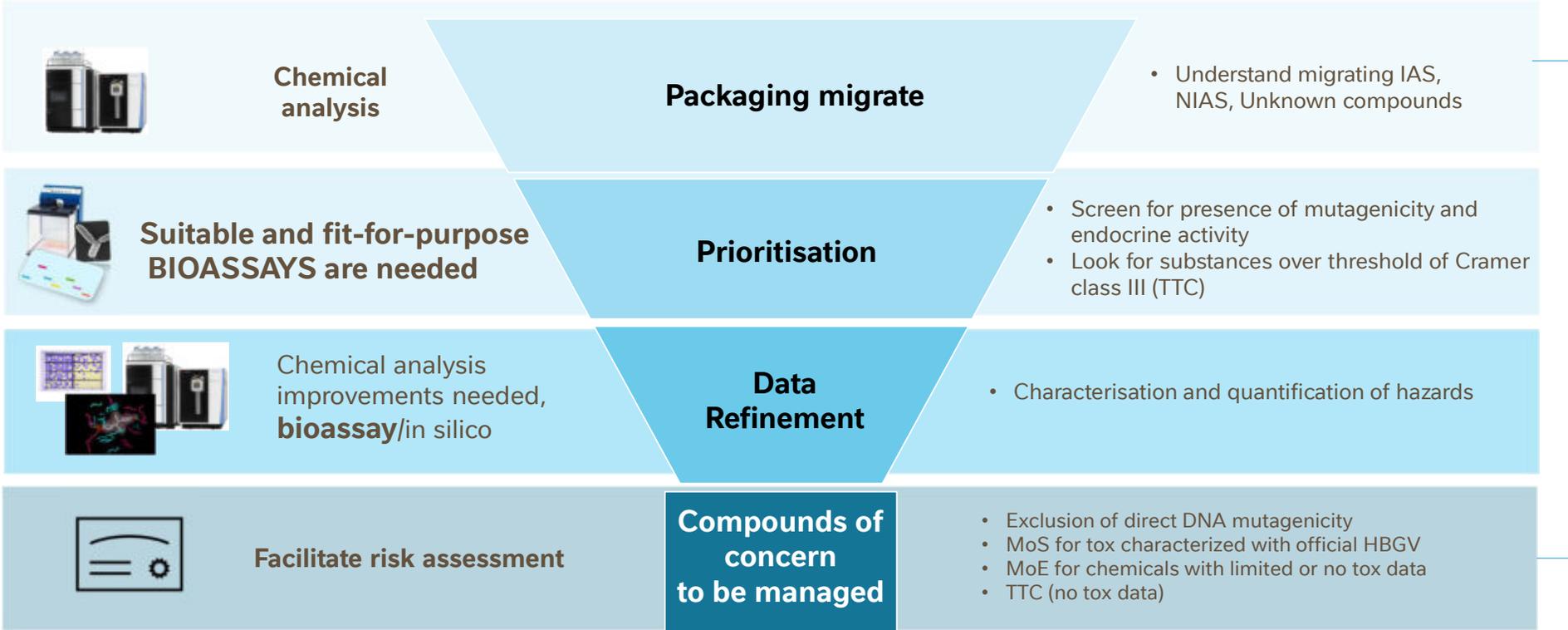
Daniel Meyer ^a, Maricel Marin-Kuan ^b, Elisa Mayrhofer ^c, Christian Kirchnawy ^c, Emma Debon ^d, Helia Latado ^b, Amaury Patin ^b, Benoît Schilter ^b, Gertrud Morlock ^{a,*}

^a Institute of Nutritional Science, Chair of Food Science, Justus Liebig University Gießen, Heinrich-Ruff-Ring 26-32, 35392, Gießen, Germany
^b Chemical Food Safety Group, Société des Produits Nestlé SA - Nestlé Research, Vers-chez-les-Allans, Switzerland
^c Department for Microbiology and Cell Culture, Austrian Research Institute for Chemistry and Technology, Franz Grill Straße 5, 1030, Vienna, Austria



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

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



Acknowledgements

Biodetection Group

Paul Rogeboz

Helia Latado

Bastien Gentili

Claudine Cottet

Amaury Patin (GL)

Maricel Marin-Kuan

Packaging Food Safety

Yves-Alexis Hammel

Elsa Omer (PM)

Sander Koster (GL)

Walburga Seefelder (DH)

