Effect based evaluation of the safety of recycled paper food contact materials

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Overview

1. Introduction
2. Use of food simulants
3. In vitro toxicological assessment
4. Correlation of responses
5. Conclusions

Introduction

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01

Recycled food contact materials (FCMs) contain both intentionally (IAS) and non-intentionally added substances (NIAS).

02

11,000 intentionally added substances and 40,000-100,000 NIAS can potentially migrate to food.

DIBP	DEP	BP
DEHP	BHT	PFAS

03

The vast majority of those substances have not been assessed from toxicological perspective. Many NIAS not yet even been identified.

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The EU's Paper and Cardboard Risk Assessment: Current State

Major challenges are currently encountered regarding the **commercial use** of paper and board food contact materials (FCMs) due to the:

- **Lack** of harmonized EU regulatory framework.
- **Huge** amounts of **unassessed** food contact chemicals (**FCCs**).
- Significant **concerns** regarding the chemical safety.
- **Wide variability** in sample preparation and assessment protocols.

Typical FCMs Chromatograms: Challenges of FCCs Analysis

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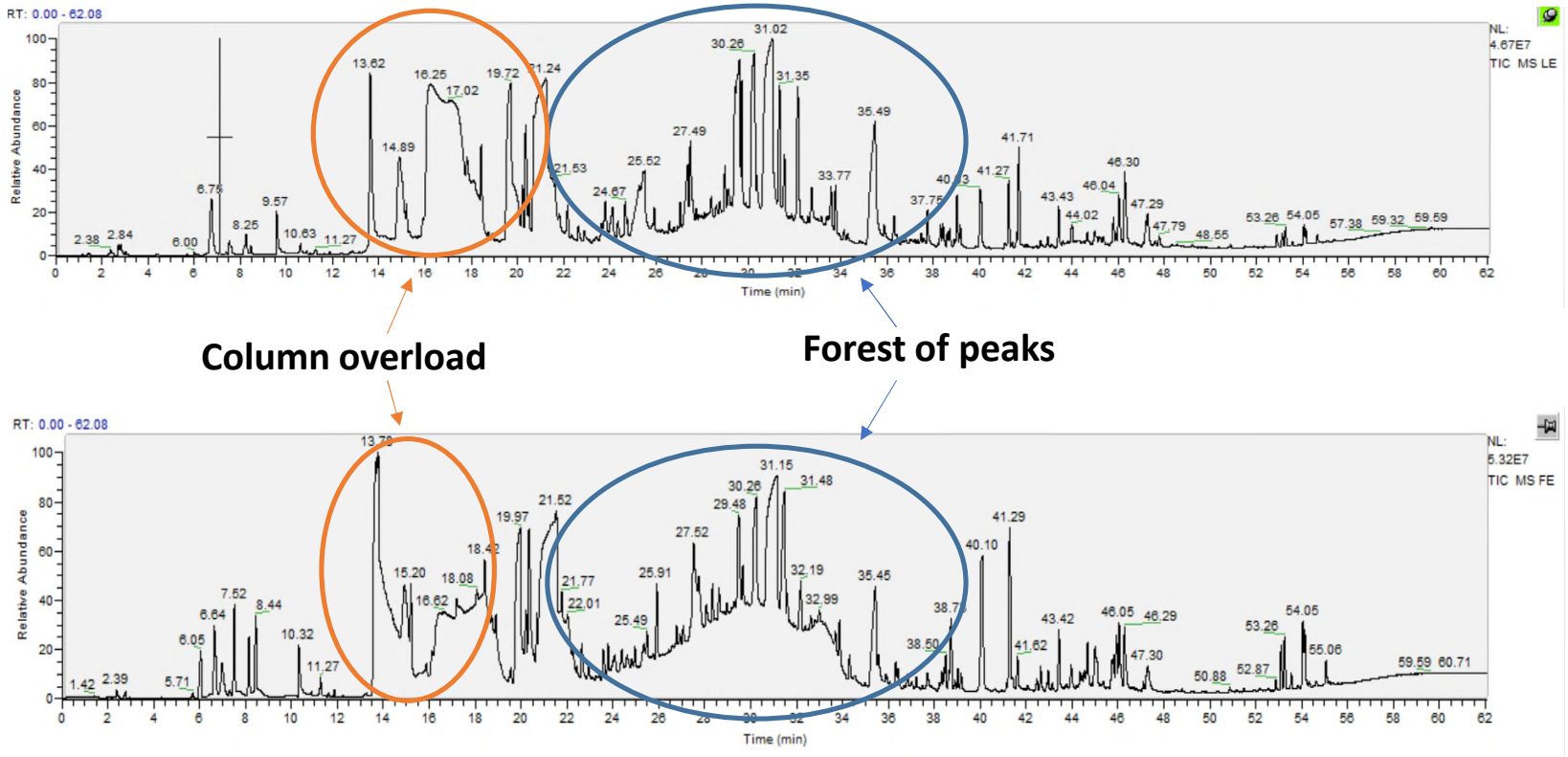
FCM sample extraction with acidic food simulant



FCM sample extraction with THF-Meth-Water

Proposed Approach: Effect based evaluation

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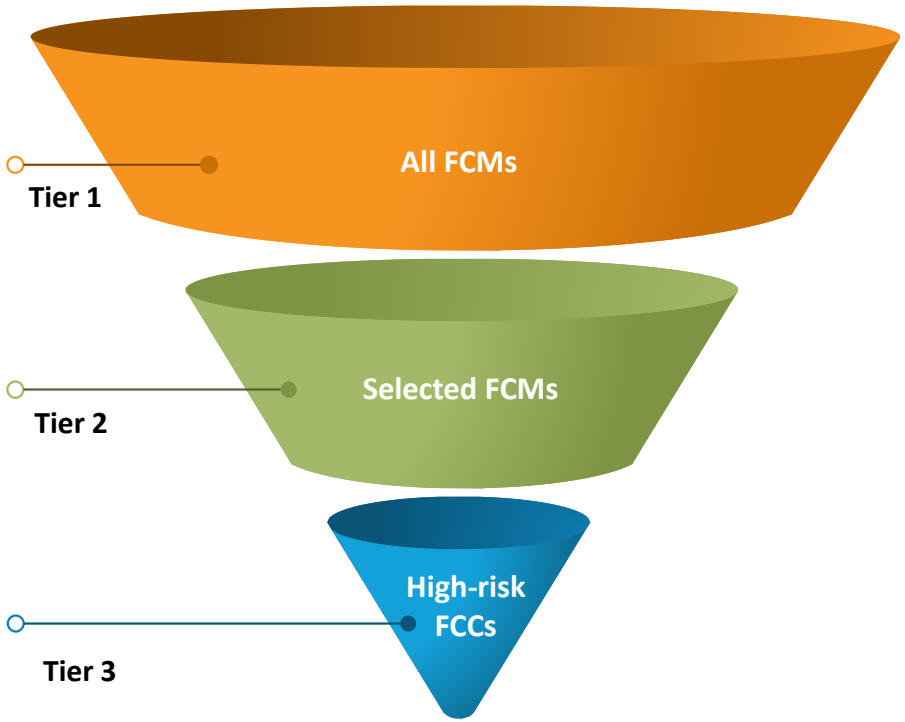
From recycled FCMs to high-risk FCCs: A top-down approach



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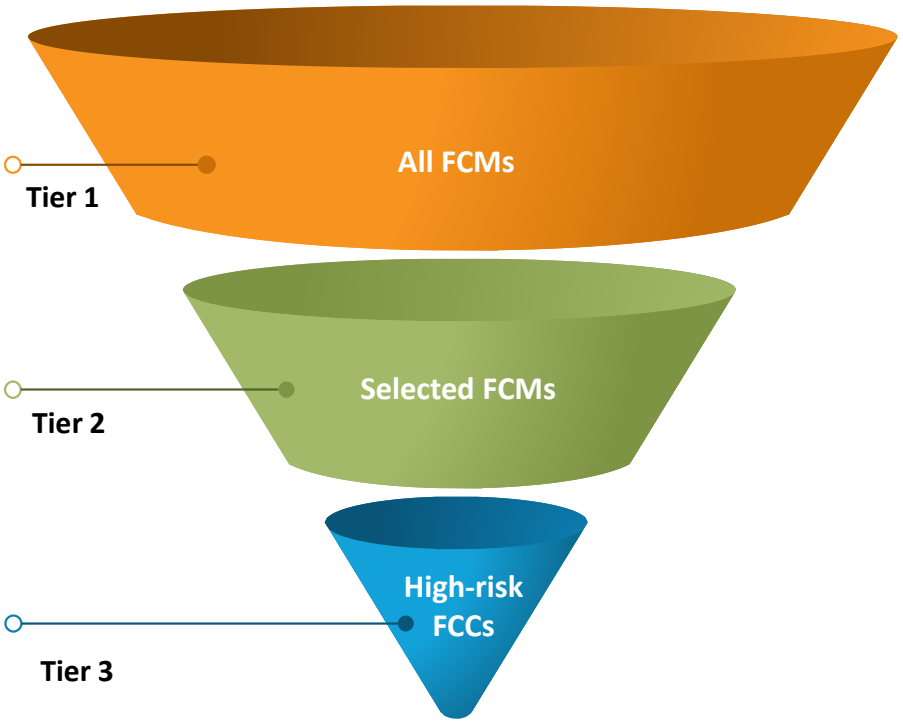

Effect based evaluation, which is a **combined** in vitro bioassays/chemical analysis, provides:

- An **integrated picture** of **total toxicity** of FCMs as affected by all FCCs (including **NIAS** and **mixtures**).
- **High throughput** and **cost-effective.**
- **Many toxicological endpoints** through battery of bioassays
- **Prioritization** of FCCs of higher toxicological potency.

Sample Preparation & Toxicity Testing Workflow

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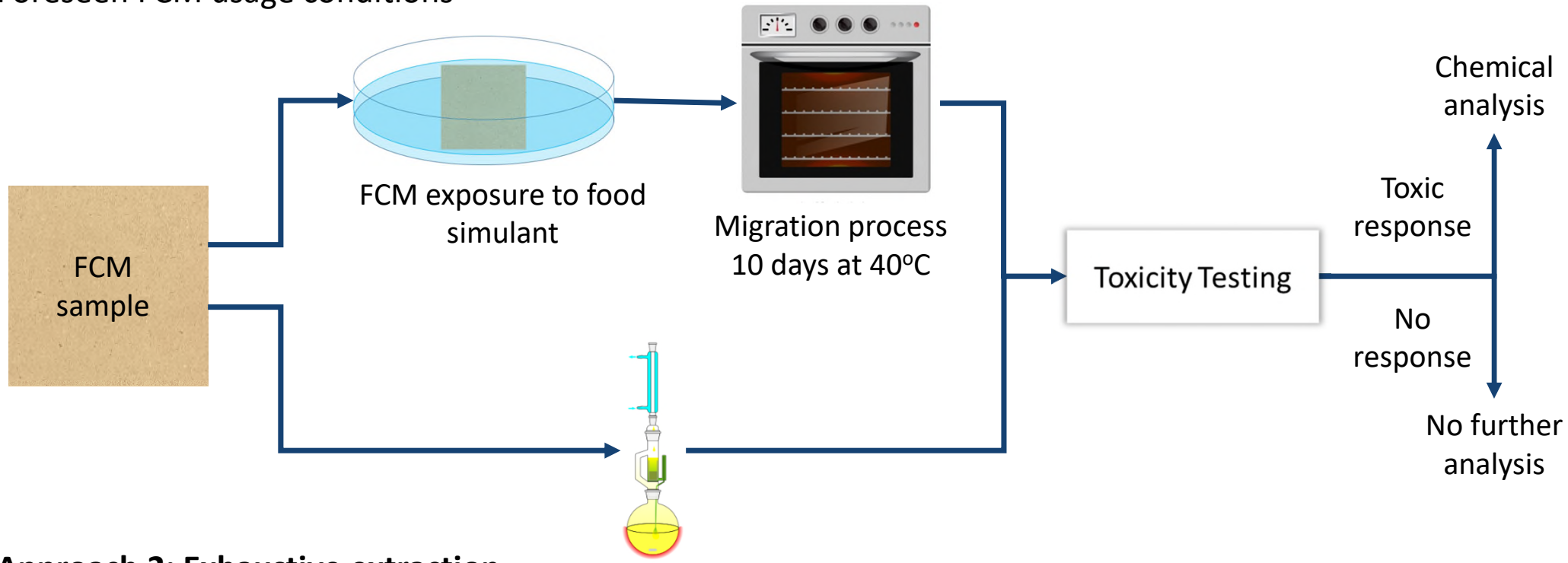
Approach 1: Migration
Foreseen FCM usage conditions



Approach 2: Exhaustive extraction
Unforeseen usage conditions

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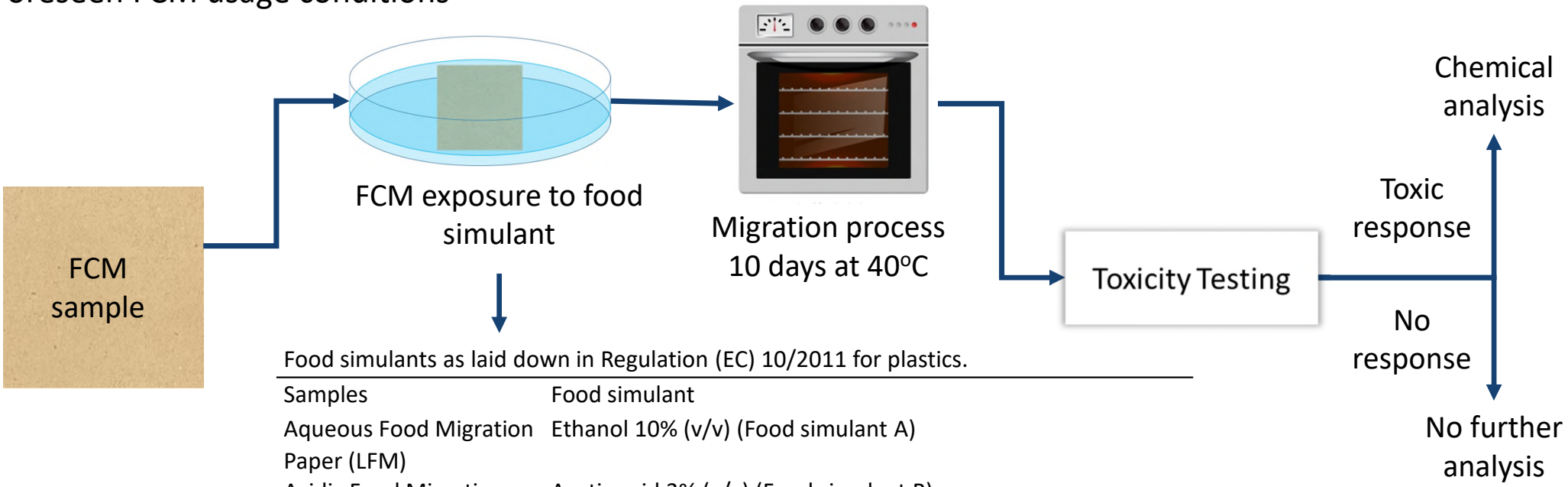
Approach 1: Migration

Foreseen FCM usage conditions



Food simulants as laid down in Regulation (EC) 10/2011 for plastics.

Samples	Food simulant
Aqueous Food Migration Paper (LFM)	Ethanol 10% (v/v) (Food simulant A)
Acidic Food Migration Paper (AFM)	Acetic acid 3% (v/v) (Food simulant B)
Fatty Food Migration Paper (FFM)	Ethanol 50% (v/v) (Food simulant D1)
Dry Foods Food Migration Paper (DFM)	Poly (2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm (Commercially known as Tenax) (Food simulant E)

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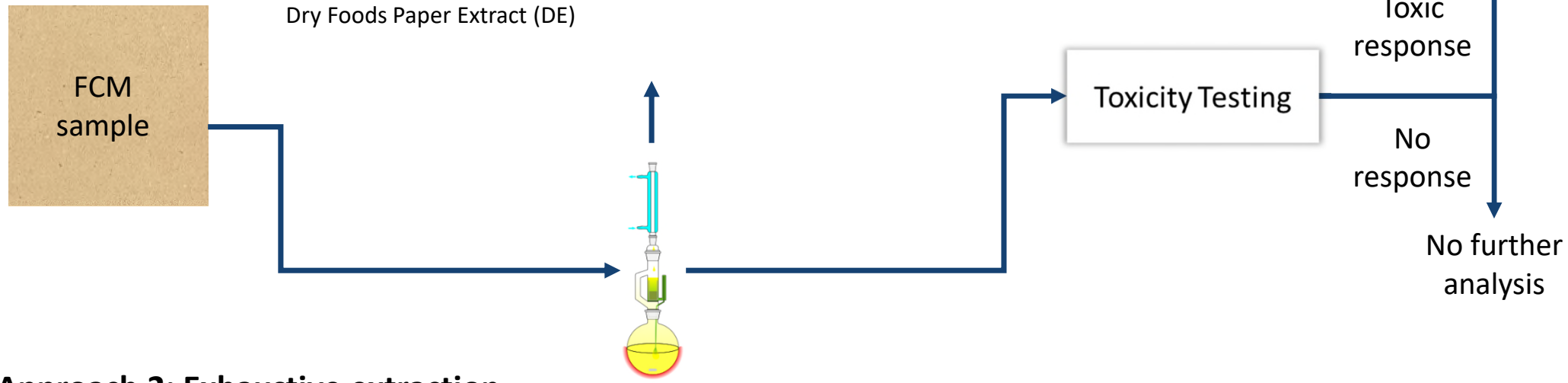
Food simulants as laid down in Regulation (EC) 10/2011 for plastics.

Samples	Exhaustive Extraction Solvent
Acidic-Aqueous Foods Paper Extract (LAE) Fatty Foods Paper Extract (FE) Dry Foods Paper Extract (DE)	50%/50% v/v methanol/acetone



Approach 2: Exhaustive extraction
Unforeseen usage conditions

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Approach 1: Migration
Foreseen FCM usage conditions

FCM sample

In vitro bioassays involved in the battery.

Cytotoxicity	Genotoxicity	Endocrine disruption	Dioxin like activity
MTT	Ames assay	AR-/Anti-AR-Calux	AhR-/Anti- AhR Calux
LDH	Micronucleus assay	ERα-/Anti-ERa-Calux	
	Comet assay		

Toxicity Testing

Chemical analysis

Toxic response

No response

No further analysis

Approach 2: Exhaustive extraction
Unforeseen usage conditions

Sample Preparation & Toxicity Testing Workflow

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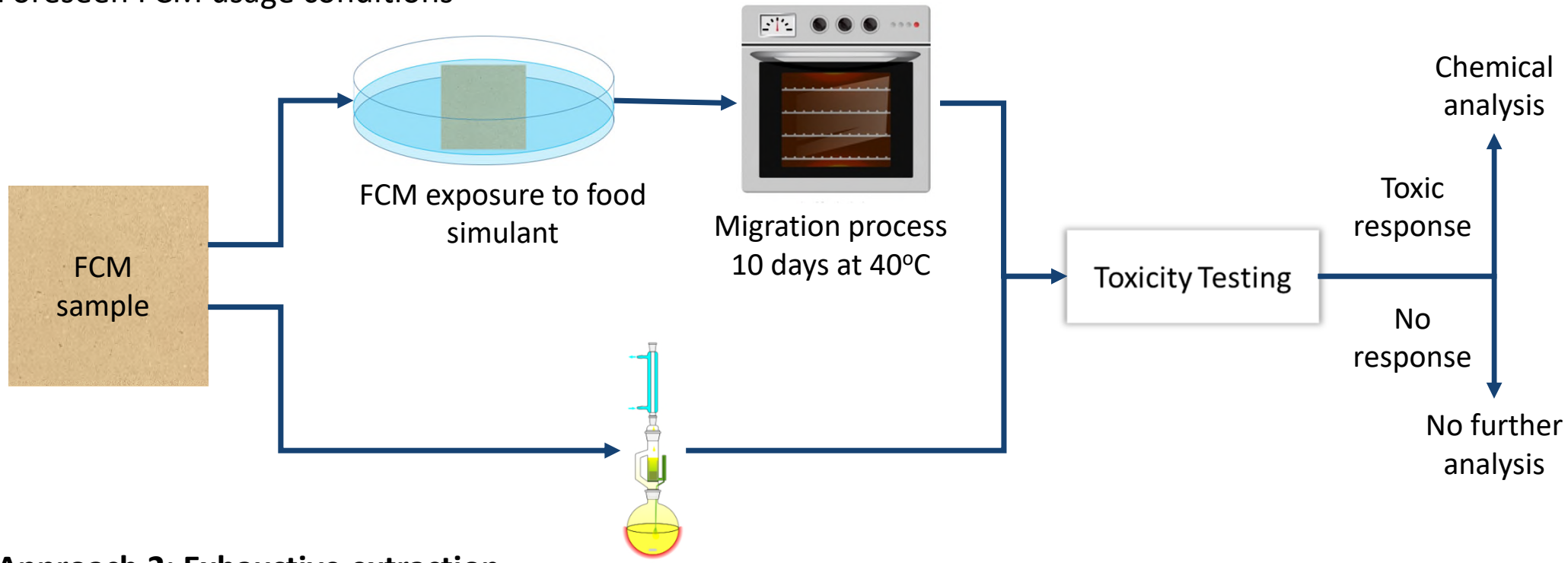
Approach 1: Migration
Foreseen FCM usage conditions



Approach 2: Exhaustive extraction
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Screening of FCM samples

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Toxicity Testing Workflow

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Battery of in vitro bioassays: Testing Procedure

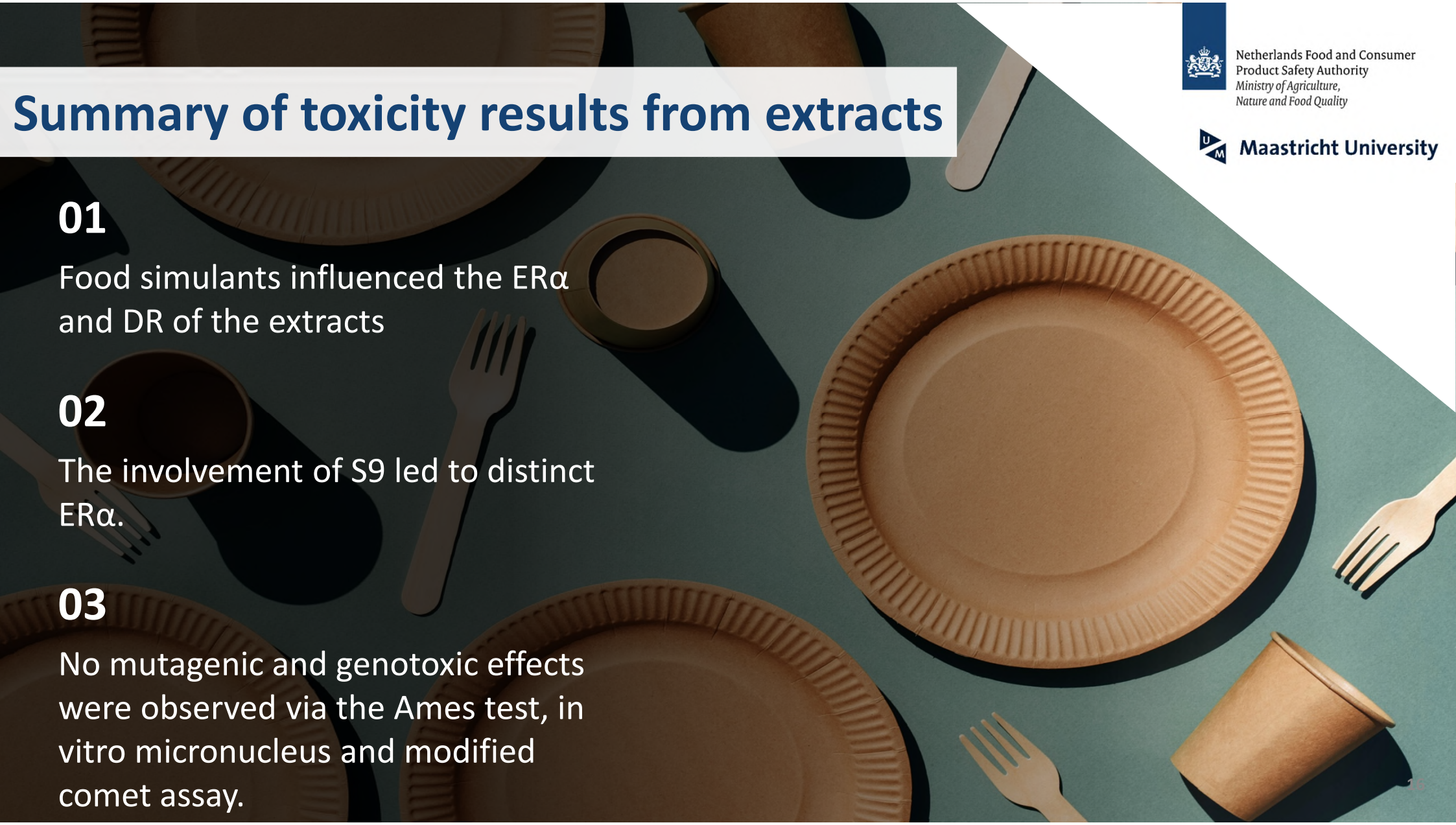
Pre-screening of extracts cytotoxicity → Identification of **cytotoxicity thresholds** for extracts (NOAECs) → Selection of **testing concentration range** for extracts

Mutagenicity and **chromosomal aberration** testing of extracts:
- Ames test
- In vitro Micronucleus Assay

Androgen receptor (AR), **estrogen receptor alpha** (ERα) & **aryl hydrocarbon receptor** (AhR) **disrupting** testing of extracts:
- AR, ERα, AhR Calux

The extracts were tested both in the **absence** and presence of **external metabolizing system (S9) in all the bioassays.**

Summary of toxicity results from extracts



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01

Food simulants influenced the ERα and DR of the extracts

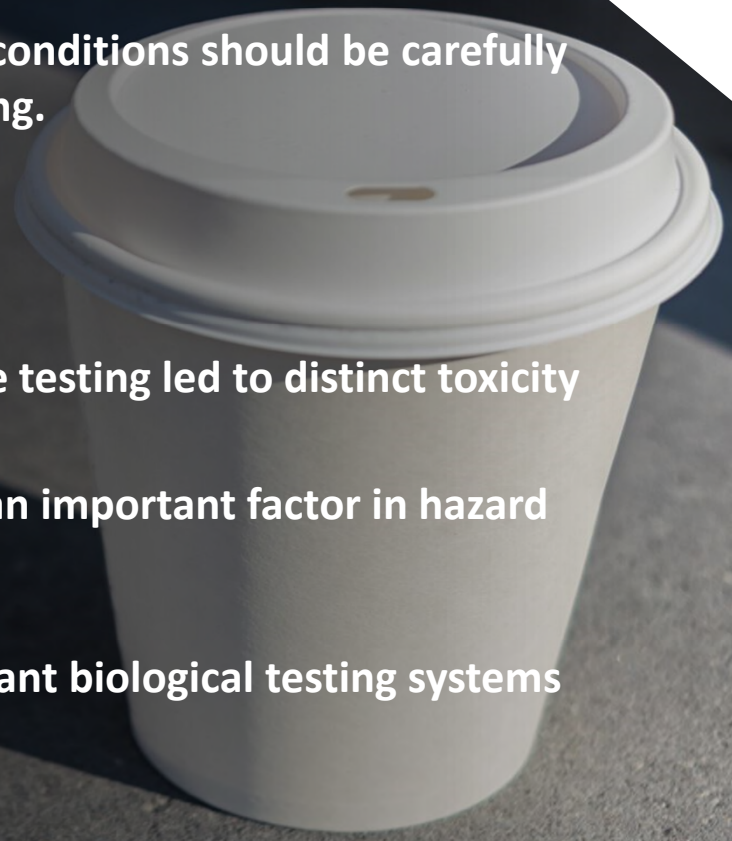
02

The involvement of S9 led to distinct ERα.

03

No mutagenic and genotoxic effects were observed via the Ames test, in vitro micronucleus and modified comet assay.

Conclusions



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01

Sample preparation method, in vitro bioassays and testing conditions should be carefully selected to not interfere with the in vitro toxicological testing.

02

DR shows distinct toxicity compared to ERα and AR

03

The involvement of external metabolizing system (S9) in the testing led to distinct toxicity profiles of the extracts.

- **Xenobiotic metabolism should be taken into account as an important factor in hazard identification.**

04

Predicted site of toxicity should guide the selection of relevant biological testing systems (preferably more than one)

05

The current framework does not assess for the presence of EDC

- **While genotoxic effects were not identified, EDCs were present in the majority of samples.**