



A Bioassay-assisted Testing Strategy: *Risk assessment of Food Contact Materials*

OFI - Austrian Research Inst. for Chemistry and Technology

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1030 Vienna, Austria

Mitglied bei:



AUSTRIAN COOPERATIVE RESEARCH

September 18

Safety Assessment of Food Contact Materials (FCMs)

Focus on Genotoxicity/Mutagenicity of Material Migrates

Based on *in-vitro* Bioassays



MIGRATOX



Industry Partners

NIAS: Non-Intentionally Added Substances

Raw materials:

**Monomers
Additives
Catalysers
Pigments,...**



Packaging materials



Medical devices



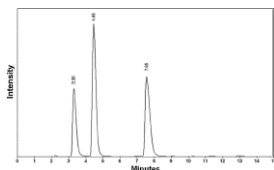
IAS:
Intentionally Added
Substances

Additives, Monomers,...

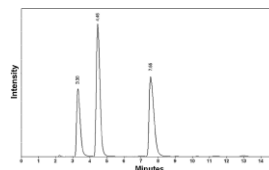


NIAS:
Non-Intentionally Added
Substances

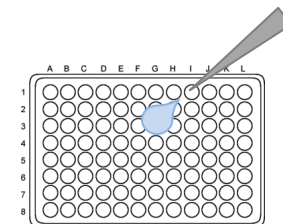
**Degradation products,
Contaminants, Side products**



Chemical Analysis



Chemical Analysis + Bioassays

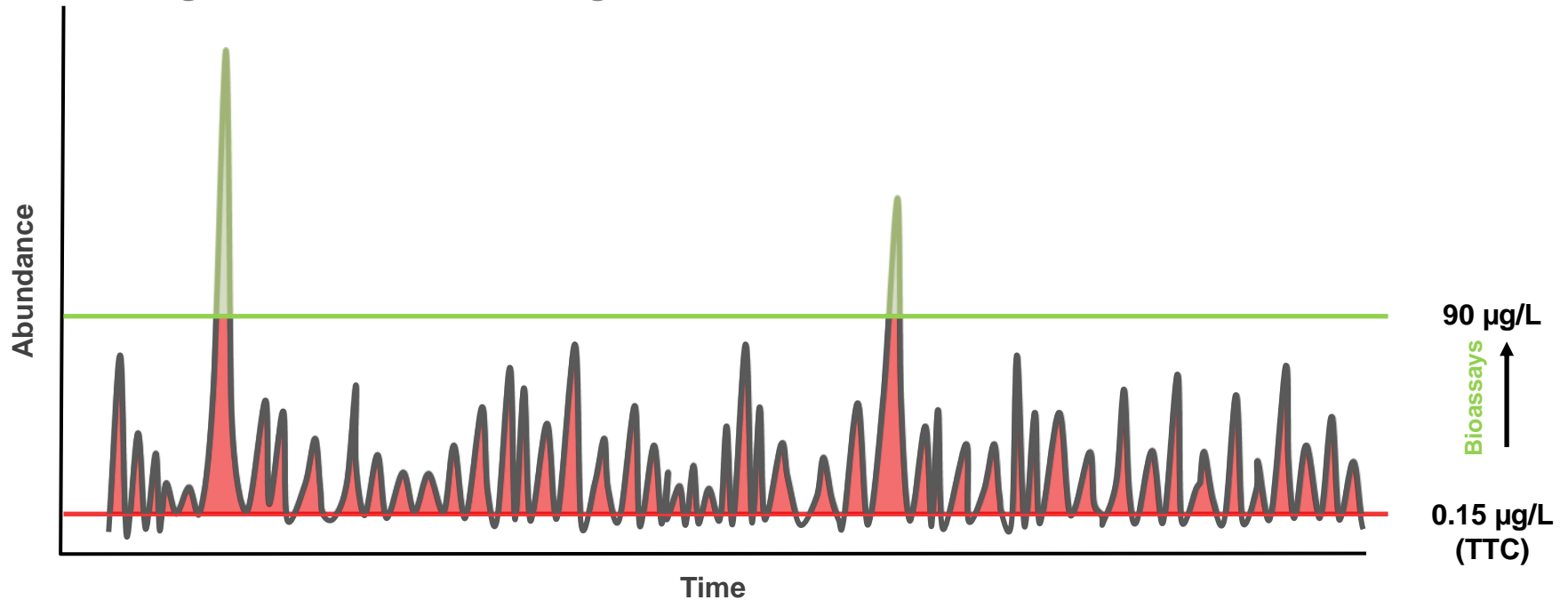


Threshold of toxicological concern (TTC):

LOD for a **chemically unknown** substance (60 kg person)

0.15 µg/d → 0.15 µg/L

Untargeted GC-MS Screening

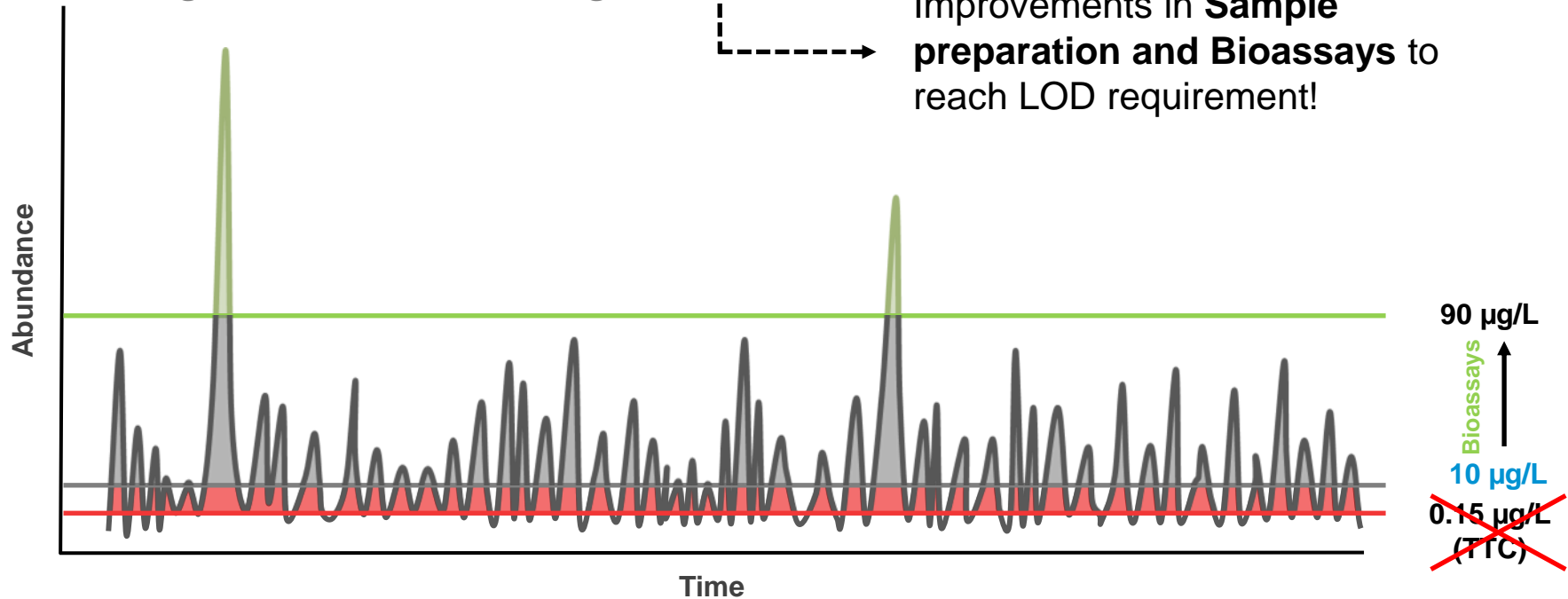


Detection limits of Bioassays

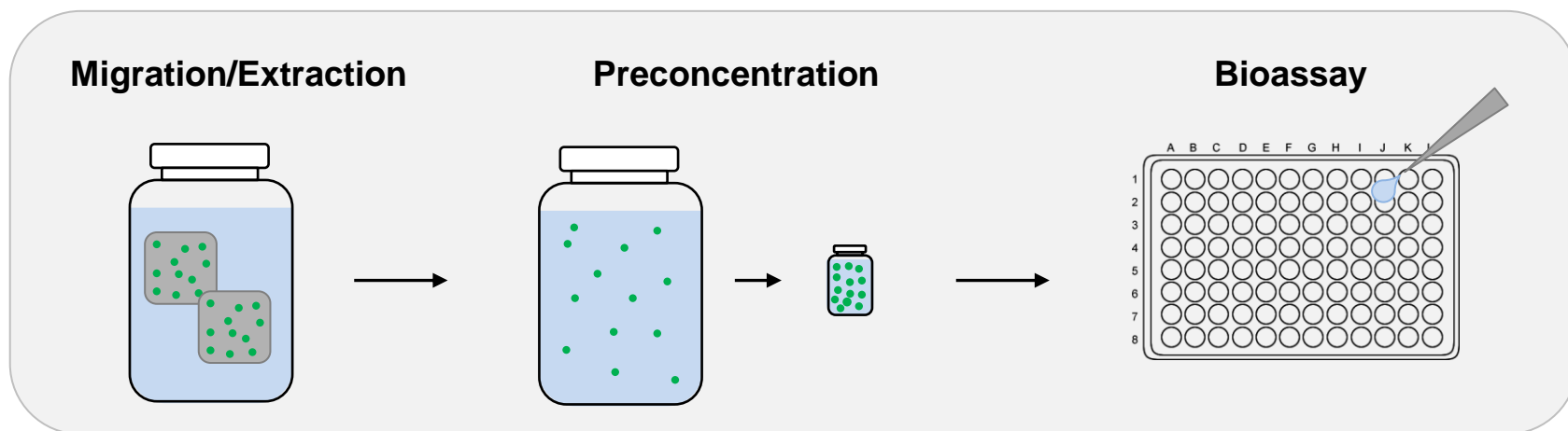
According to EU 10/2011:

Minimum requirement for limit of detection (LOD): **10 µg/L**

Untargeted GC-MS Screening



Bioassay-assisted Testing Strategy



Simulant

- 95% Ethanol

Simulant Volume

- 300 mL

S/V Ratio

- 1 dm²/100 mL (EN1186-1) or
- given by intended use (EU 10/2011)

Methods

- Evaporation
- (Solid Phase extraction)

Concentration factor

- 300

Assays

- MTT-Assay → **Cytotoxicity**
- (Anti-) ER-CALUX → **Endocrine Disruptors**
- (Anti-) AR-CALUX → **AhR-active substances**
- PAH-CALUX → **AhR-active substances**
- p53 –CALUX → **Genotoxic Substances**
- Ames MPF → **Genotoxic Substances**

(Bernhard Rainer,
FH Campus Vienna)

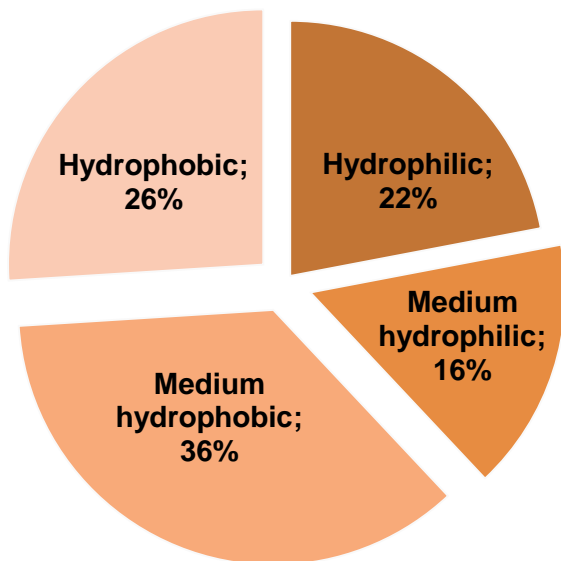
Major challenges:

- **Sample preparation:** false negatives (e.g. loss of volatiles), false positives (contaminants)
- **Bioassays:** Sensitivity - many genotox assays are not sensitive enough to detect low concentrations of genotoxins (0.15 µg/L, 10 µg/L)
- **Validation:** e.g. Influence sample matrix, Reproducibility
- **Standardization**

Genotoxic Substances

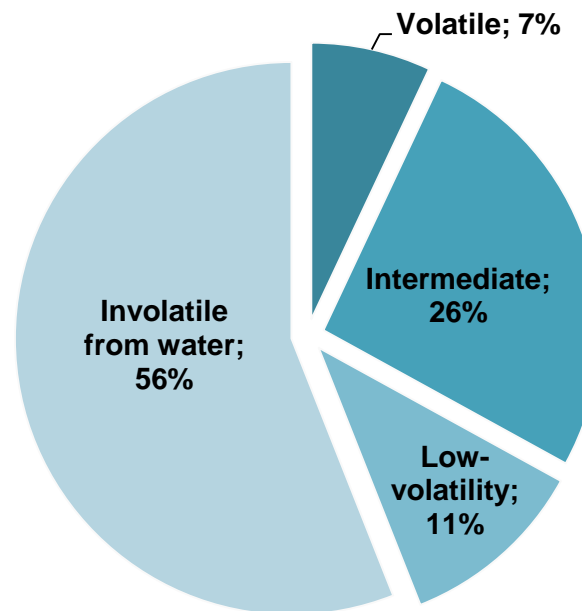
A broad spectrum of physico-chemical properties

Hydrophilic/hydrophobic Properties



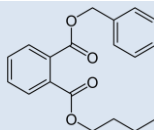
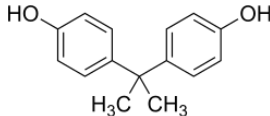
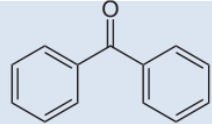
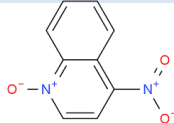
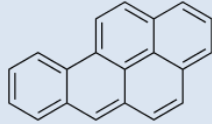
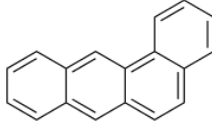
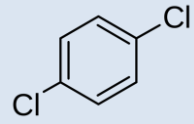
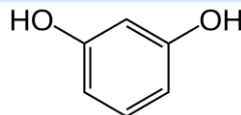
Category	Log K _{ow}
Hydrophilic	<0
Medium hydrophilic	0 to 1
Medium hydrophobic	1 to 3
Hydrophobic	>3

Volatility



Category	LogH [Pa.m ³ /mol]
Volatile	>1
Intermediate	-2 to 1
Low-volatility	-3 to -2
Involatile from water	< -3

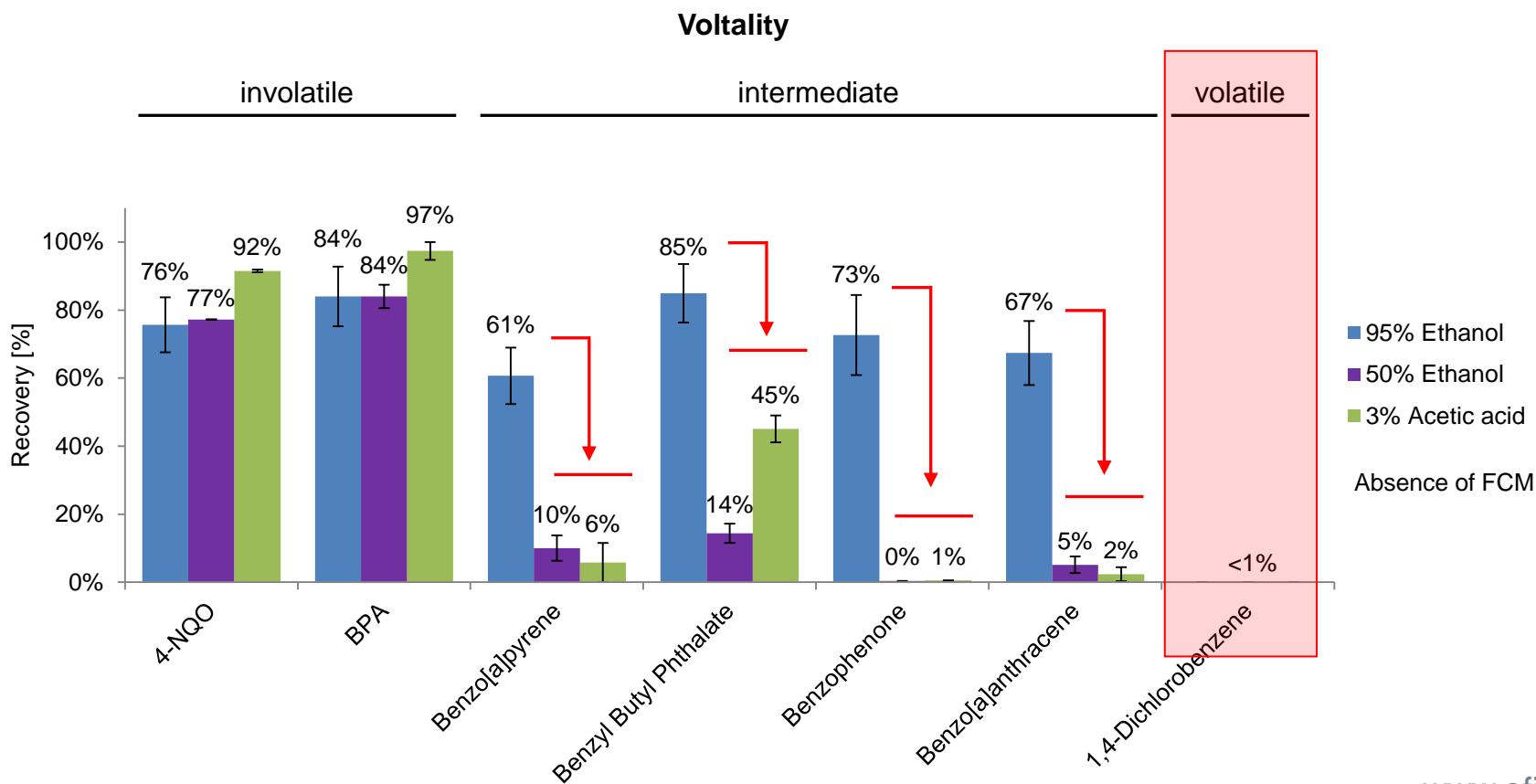
Model Substances

Substance		Hydrophilic/Hydrophobic (logK _{ow})	Volatility (logH [Pa.m ³ /mol])
Benzyl Butyl Phthalate endocrine		Hydrophobic (4,73)	Intermediate (-0,89)
Bisphenol A endocrine		hydrophobic (3,32)	Involatile (-5,40)
Benzophenone endocrine		hydrophobic (3,18)	Intermediate (-0,72)
4-Nitroquinoline N-Oxide genotoxic		Medium hydrophobic (1,09)	Involatile (-8,75)
Benzo[a]pyrene (+S9) genotoxic, AhR		hydrophobic (6,13)	Intermediate (-1,34)
Benzo[a]anthracene (+S9) genotoxic, AhR		hydrophobic (5,76)	Intermediate (0,07)
1,4-Dichlorobenzene carcinogenic		hydrophobic (3,44)	Volatile (2,43)
Resorcinol toxic		Medium Hydrophilic (0,80)	Involatile (-5,00)

Validation of Rotary Evaporation

95% Ethanol recovers **involatile** and **intermediate volatile** substances.

With **increasing boiling point** of the simulant, the **recovery** of **intermediate volatile** substances **decreases**.



114 samples analysed

Sample types:

- Paper & Board
- Food Carton
- Can Coating
(Epoxy, Polyester, Acryl,...)
- Polyolefins (PP, HDPE, LDPE, LLDPE)
- Polystyrene

Samples sources:

- Provided by the Industry Board (MIGRATOX)
- Empty packaging provided by retailer (market brand)
- Research samples
- Medical device grade materials

Extraction/Migration: 95% Ethanol

- **Sample preparation:**
 - Comparison of sample preparation methods
 - Validation: loss of volatiles, contaminations
- **Bioassays:**
 - Comparison of different *in-vitro* methods
 - Improve sensitivity, new test designs
- **Sample screenings:**
 - How many positives? → Avoiding false-negatives or false-positives?
- **Validation:**
 - Ensure that methods are suitable for FCM migrates/extracts
 - Ensure that methods are reproducible
- **Standardization:**
 - Defined protocols, specific guidelines
 - Acceptance by cooperation with authorities



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