Application of bioassays for packaging safety evaluation

Benoît Schilter

Head of Food Safety Research Department,

Nestlé Research Center, Lausanne Switzerland

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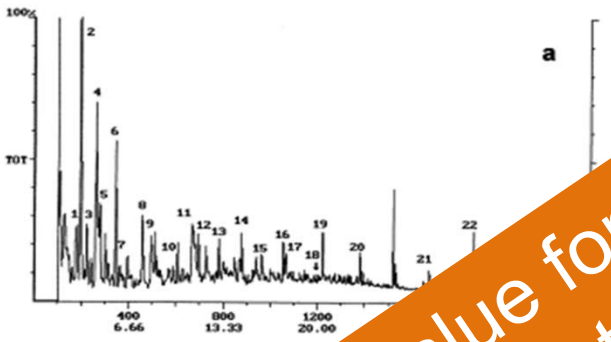
What is the problem?



GC-MS

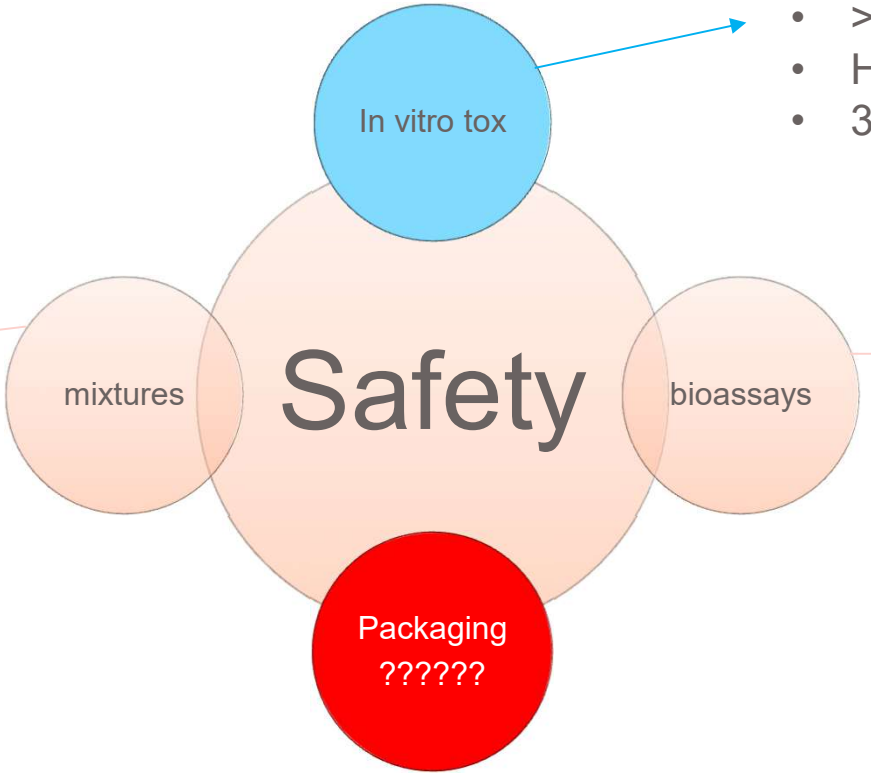
- …nized mixture of
- …turally characterized (identified)
- …artially characterized
- Not characterized/identified

- Id…
- …itions
- …pplications
- …ology

Is there a role/value for bioassays in safety assessment of such mixtures?

- Not practical
- Not desirable

Why not?



- > 1000 (Tox21)
- Human stem cells
- 3-D

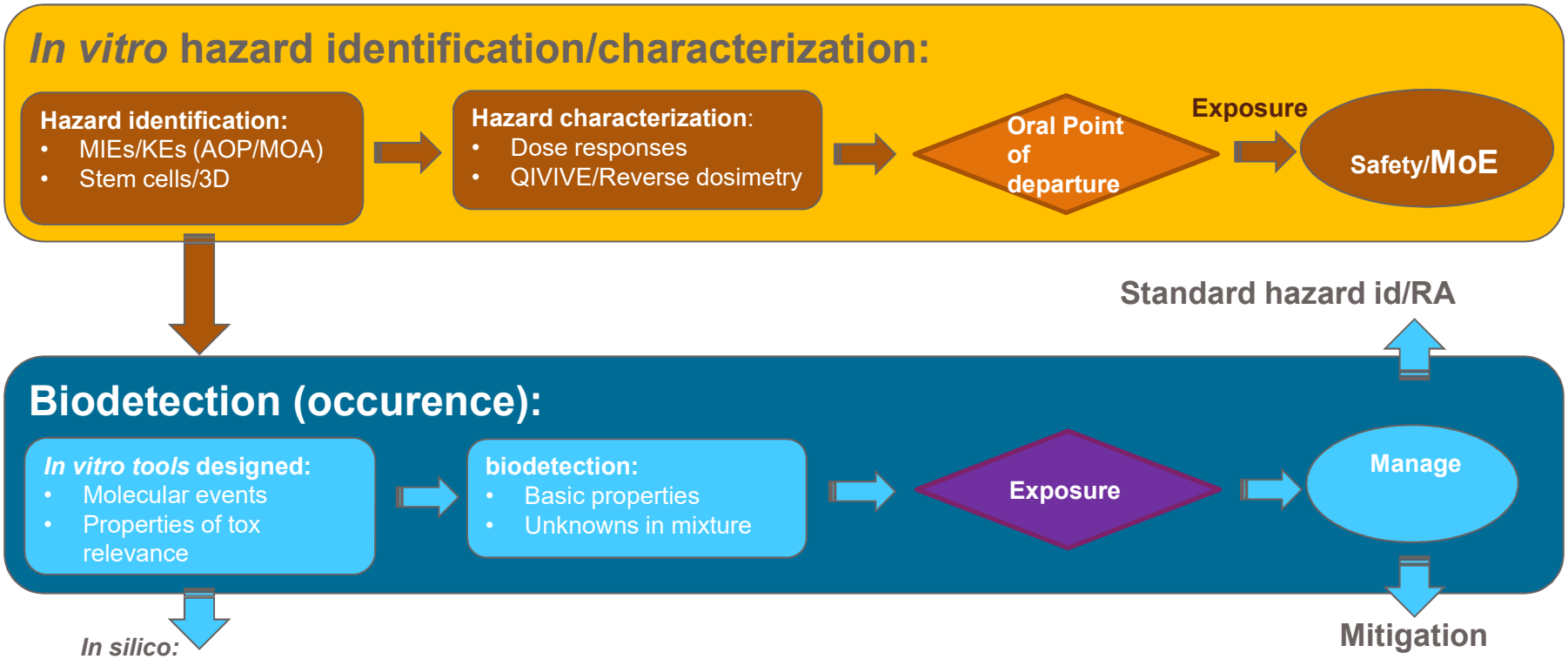
- Novel foods
- Plant extracts
- Medical devices
- Water quality

- Genotoxicity
- Endocrine act.

The topic is increasingly discussed in context of packaging safety

- Quality/validation
- Limitations
- Data interpretation

What are bioassays?



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Example of nuclear receptor activation

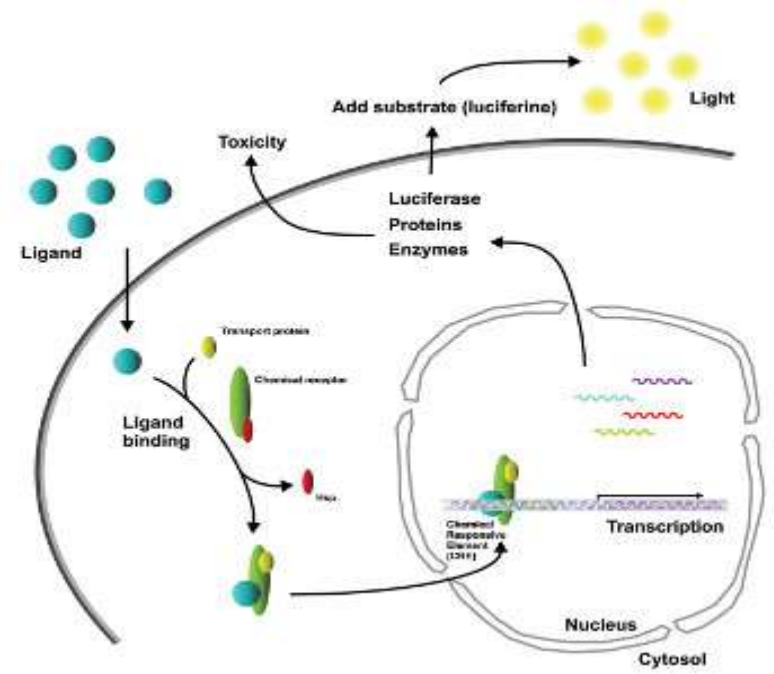
transcriptional activation assay

CALUX = **C**hemical-**A**ctivated L**u**ciferase Reporter Gene-E**x**pression Assay

- ✓ Several hormone receptors available
- ✓ Agonist and antagonist modes

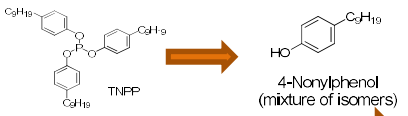
ERα
AR
AhR
GR
TR
PPARγ
…..

–/+ S9
cytotox

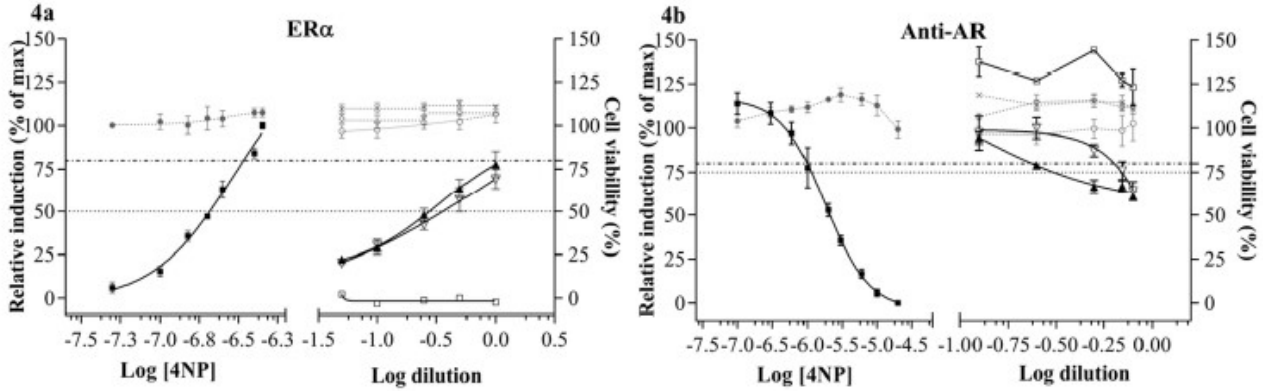


Application of the Calux assay

Migration study:
- ***Analytical chemistry***
- ***Bioassay***



156 µg/L NP (LC-MS)



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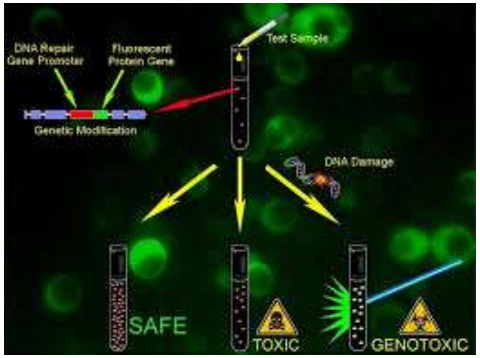
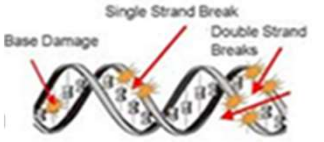
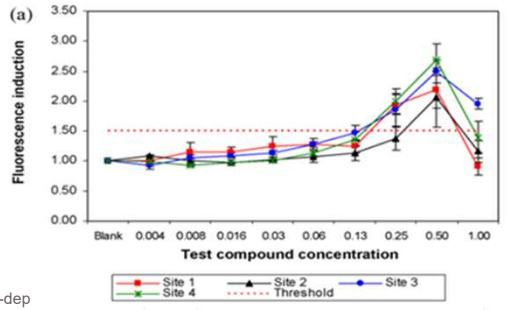
Example of genotoxicity assay

Gadd45α induction (Bluescreen)







- ✓ Cell's genotoxic stress response
- ✓ Identifies diverse genotoxic agents:
  - Direct acting
  - Others (with threshold)
- ✓ Possibility to apply metabolic system (S9)
- ✓ Cytotoxicity test included
- ✓ High sensitivity: little false negatives
- ✓ High specificity: little false positives
- ✓ Good within/between lab reproducibility
- ✓ Commercially available
- ✓ Getting increasing acceptance for screening
- ✓ Potential for improvement/optimization

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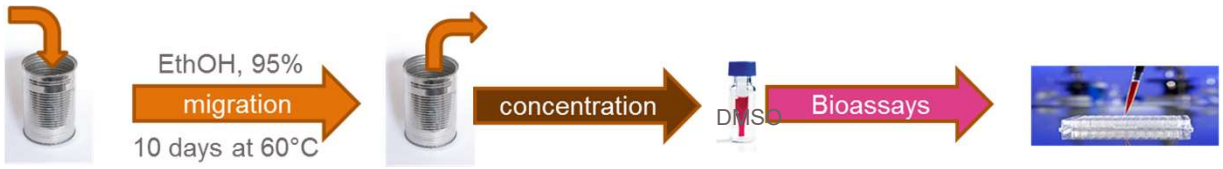
Gadd45α induction in FCM-migrates of experimental material



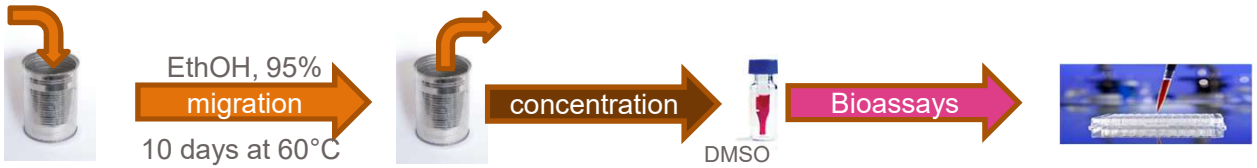
Gaps and limitations need to be adressed:

- Relevance of migration studies (stability of the materials?)
- Identify causative agent(s)
  - Current analytical data did not reveal chemicals with alert for genotox (DNA-reactivity)
  - Test of identified NIAS ongoing
  - Fractionation planned
- Address mechanisms of genotoxicity
  - Mutagenic
  - No positive samples in Ames (no DNA reactive? Threshold?)
- …..

Why and When?

1. Safety by design
2. Application of the TTC

Safety by design: bioassay data on R&D materials.



Biological activity	Coating 1	Coating 2	Coating 3	Coating 4	Coating 5
Anti-estrogenic (ERα)					
PPARγ					
Anti-androgenic					
AhR					
Gadd45α					
Cytotoxicity					
AMES	*	*	N/A	N/A	N/A

Biological activity: + → -

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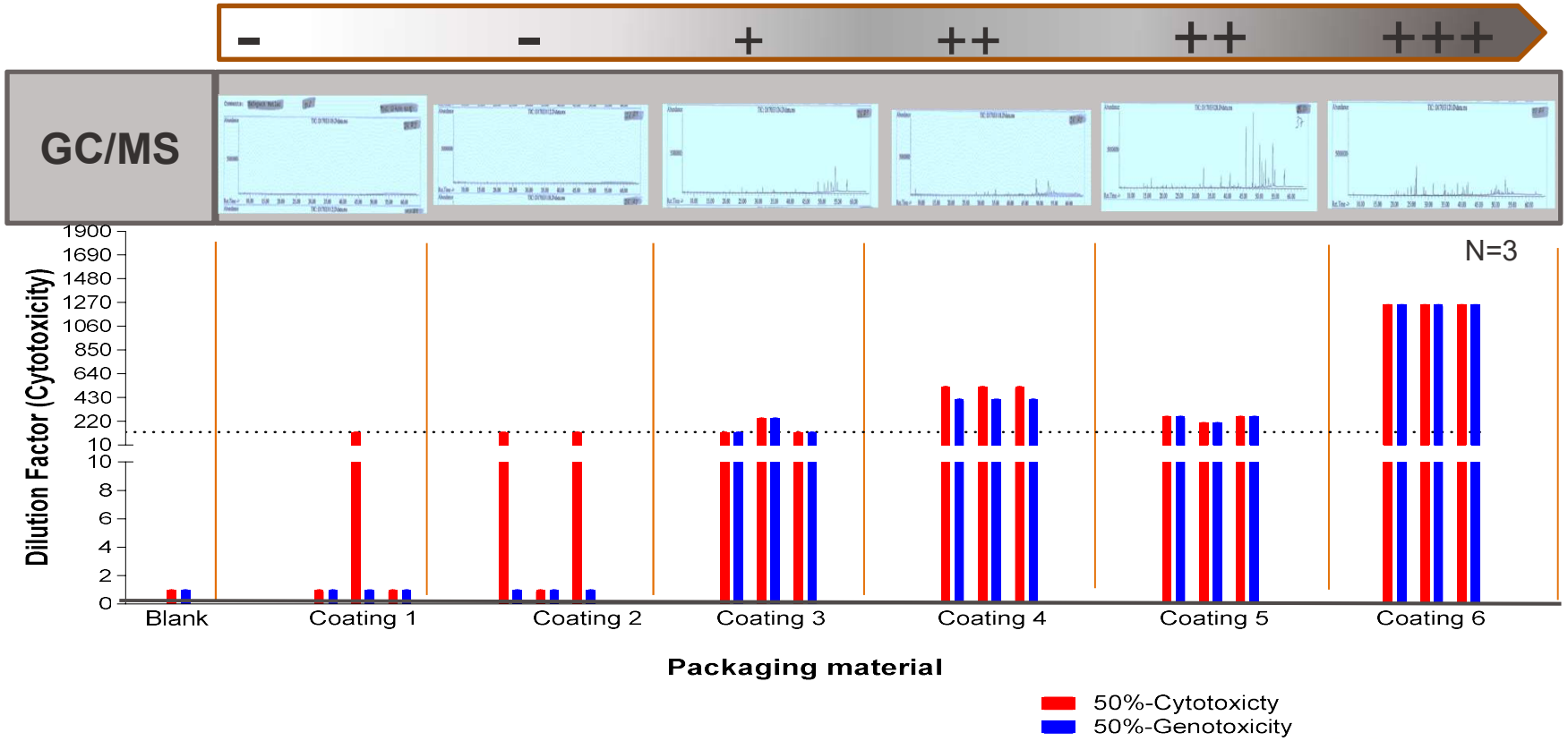
Good correlation analytical vs biological profile



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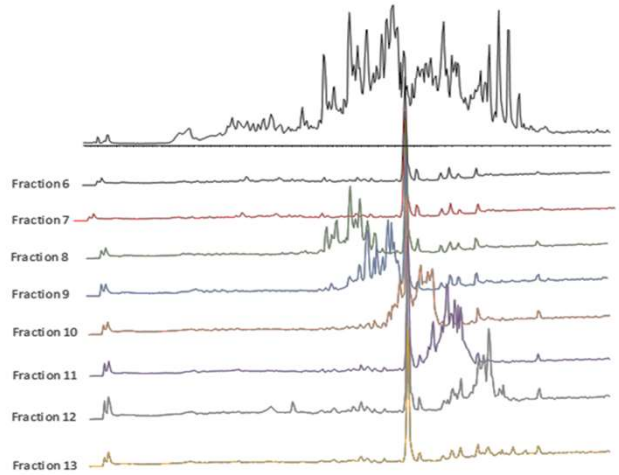
A way forward (effect directed analysis)



Fractionation:
LC-HRMS platform coupled
with a Waters fraction collector

Fraction 6	genotox	endocrine
Fraction 7	--	--
Fraction 8	--	--
Fraction 9	--	--
Fraction 10	--	✓
Fraction 11	✓	✓
Fraction 12	✓	--
Fraction 13	--	--

- *Monomers*
- *Additives*
- *Synthetic oligomers*

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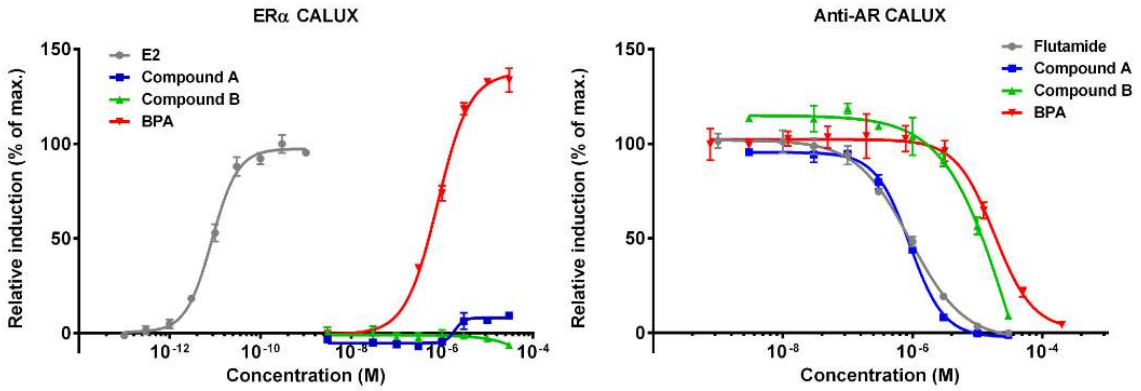
Safety by design:
evaluate raw materials early (e.g. monomers)



Compound A:

- PPARγ antagonist effect
- No antagonistic effect on ERα
- No agonistic effect on AR

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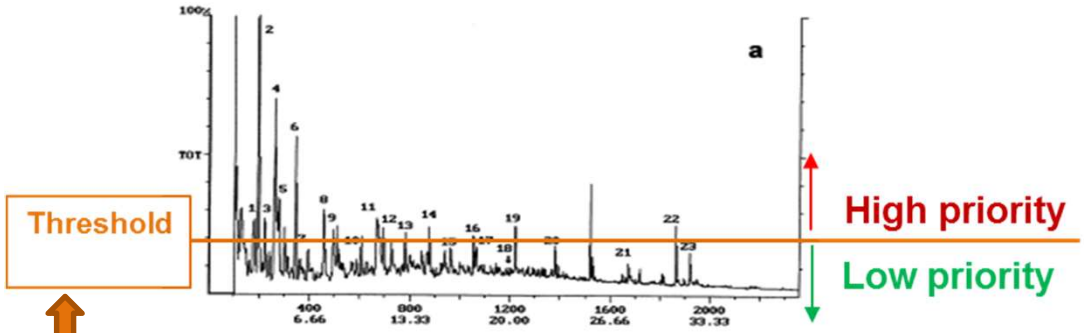
Why and When?

1. Safety by design
2. Application of the TTC

Application of the Cramer class III-TTC to unknow NIAS (safety assessment)?



Exclude member of cohort of concern.
Exclude chemicals not covered by TTC.
TTC = 0.15 μg, if alert of genotox.
TTC = 18 μg, inh. AChE.

AhR; DR-Calux.
ER, AR assays
Ach-esterase inhibition
Genotoxicity assays

Cramer class III TTC

Proposed steps in assessment of unknowns

Steps	What?	Why?
1. Material characterization	Composition, manufacturing, processing, degradation, …	**Exclude chemicals of high concern (cohort of concern)**
2. Analytical methods	- Sample preparation - Chromatographic techniques - Detection methods - Partial identification	
3. Targeted analysis	- Methods for specific chemicals	
4. Food intake	Material application, population, dietary habits	**Estimate exposure**
5. Quantification	Quantification of unknowns	

Adapted from Koster et al., Fd Chem Tox 49 (2011) 1643-60; Rennen et al., Fd Chem. Tox 49 (2011) 933-940

«Sensitivity?»

Genotox tests are sensitive:

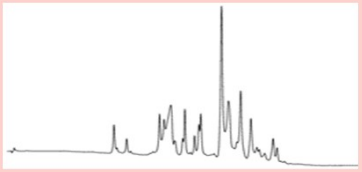
$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

TP: true positive
FN: false negative

Genotox tests are not sensitive:

Limits of detection (LoD) in mixture are currently poor:

- 0.15 µg/person
- risk (10^{-6})
- 10 ppb
- 90 µg/person



LoD for genotoxicity can be improved

			LEC Standard protocol	LEC optimized protocol	Improvement
Direct D co					
Indirect DN compounds					
oxidative st					
Chromoson					
Cytotoxic n					
Negative genotoxic, non-cytotoxic		D-mannitol (-S9)		-ve	

Genotox:

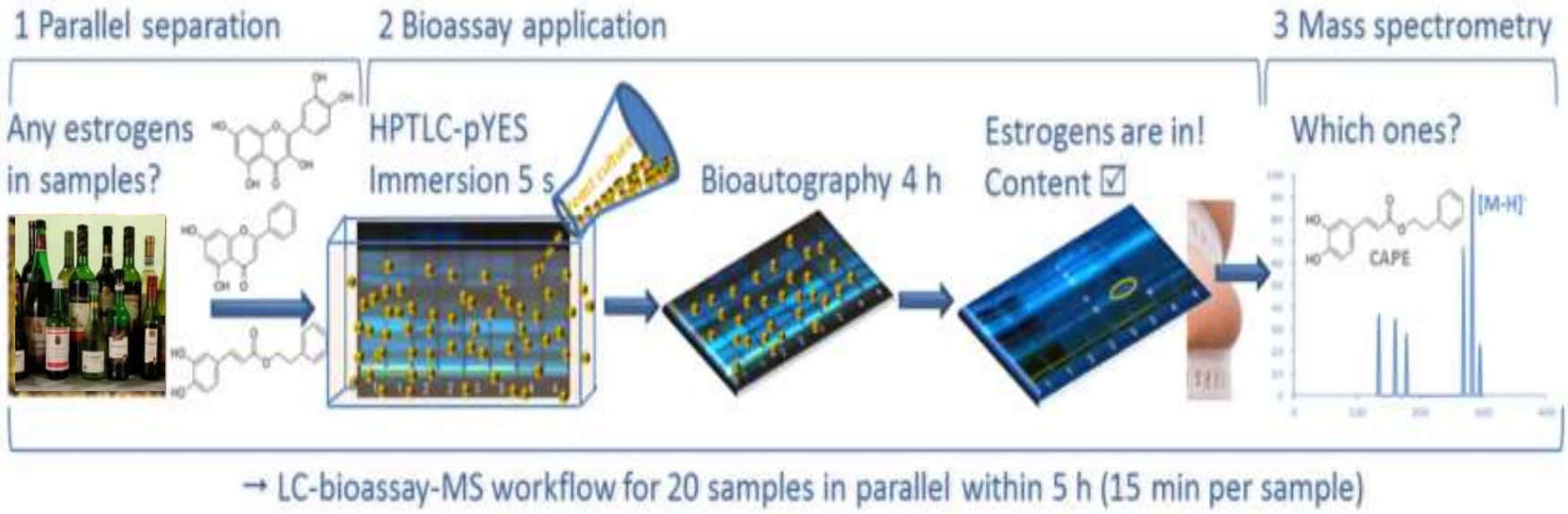
- LoD can be improved, observed for key mechanisms
- Important for mixture testing
- Important to discriminate genotoxicity from cytotoxicity
- Depends upon:
  - *Type of assay*
  - *Culture conditions*
  - *Substances*
  - *….*
- Clearly insufficient (compared with LoD requirements)
- **Need a breakthrough**

Other endpoints:

- Similar conclusion for Ach-esterase inhibition
- Less an issue for receptor mediated activation

The way forward?

High performance thin layer chromatography (HPTLC)-bioassay



I. Klingelhöfer, G. Morlock, *Anal Chem* 87 (2015) 11098−11104

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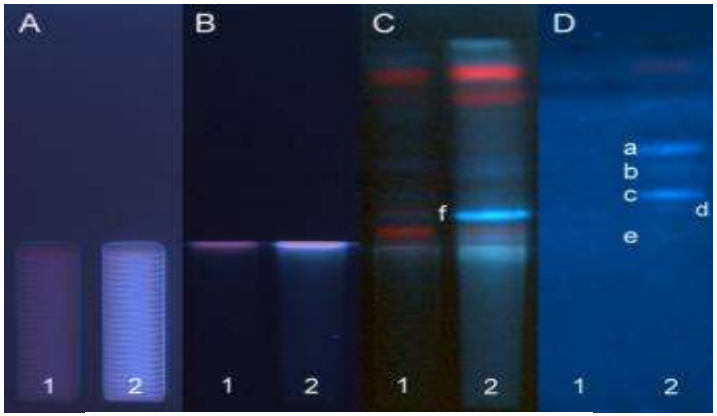
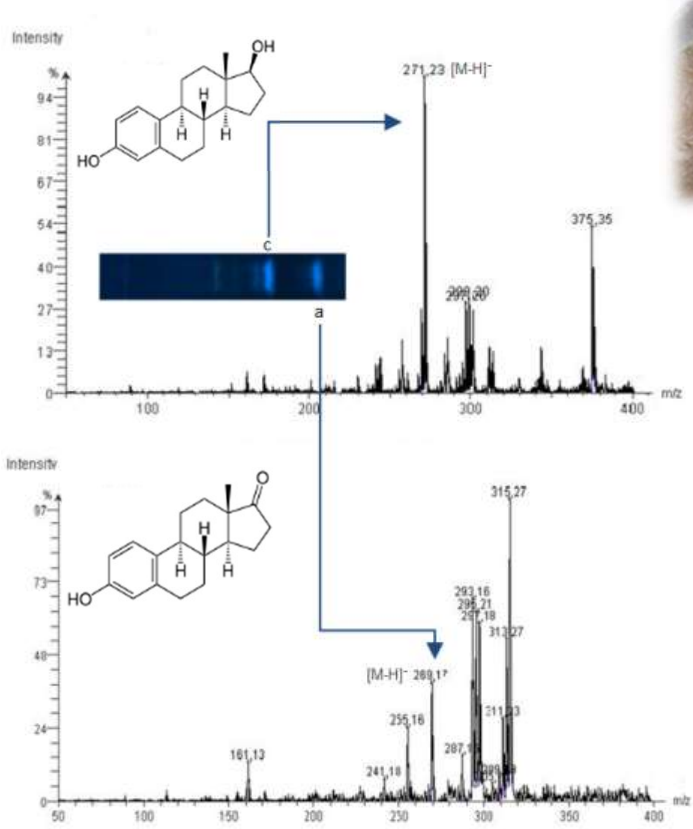
Discovery in surface/waste water



Substance	LOD [ng/L]	LOQ [ng/L]
E2	1.0	2.5
EE2	2.5	5.0
E1	4.3	15.0
E3	75.0	250.0
BPA	1.6 x 10^3	5.0 x 10^3
NP	15.0 x 10^3	65.0 x 10^3



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Bioassays: roles in packaging safety

To prioritize structurally uncharacterized chemicals with TTC-class III:

- *To contribute to exclusion of chemicals of the cohort of concern*
- *To exclude ACHE-inhibitors and chemicals with genotoxic alert*
- *In combination with other parameters*
- *More sensitive methods required*

To test for the presence of chemicals:

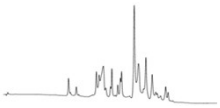
- *Endocrine activity*
- *High toxic potency*
- *To be assessed/managed (early)*

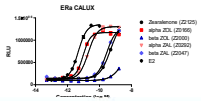
HPTLC-bioassay is likely to significantly improve the situation:

- May increase LoDs by orders of magnitude (e.g. genotox, AChE-inh, receptor med, …).

Chemical screening vs biodetection

final thoughts





	Chem. screening	Biodetection
Observation	Peaks	Response
experience *acceptance,* *clarity*	+++ +++ ++	++ + +
Perception	Ah, well…	**Oouuuhhh!!!**
Risk assessm.	Concern?	Concern?
Action	Identify/quantify	Identify/quantify

Together with analytical chemistry, bioassays have a role to play
In safety assessment of FCMs

Acknowledgements:

Packaging Safety Group

Early warning Group

Chemical Food Safety Group

OFI (Vienna)

Thanks for your attention