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Schweizerische Eidgenossenschaft  
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**Food Safety and  
Veterinary Office**

# Sensitive detection of toxic chemicals in food packaging with HPTLC-bioassays

**Alan Bergmann**

**With:**

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Andreas Schönborn, Inge Werner, Etienne Vermeirssen

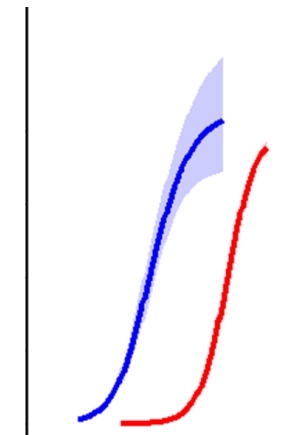
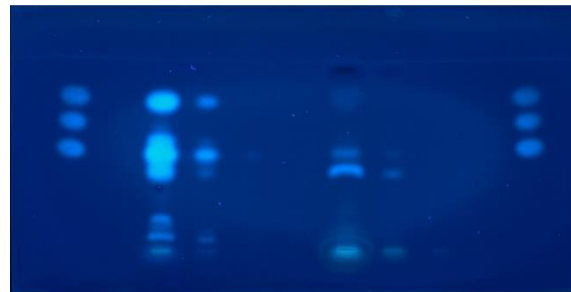
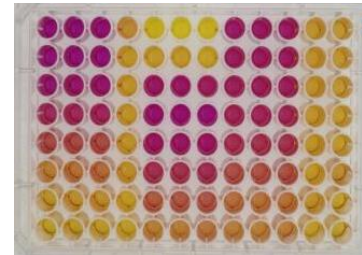
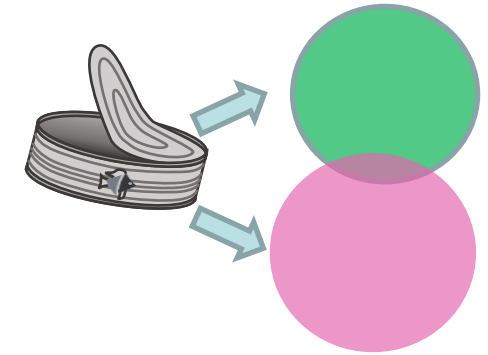
30/06/2020

# Outline



## Agenda

1. Detecting hazardous chemicals in food packaging
2. Introduction to analysis with HPTLC bioassays
3. Comparison of HPTLC bioassays to microtiter plates
4. Outlook





# Chemicals can migrate to food

There are complex, potentially toxic, mixtures in food contact materials

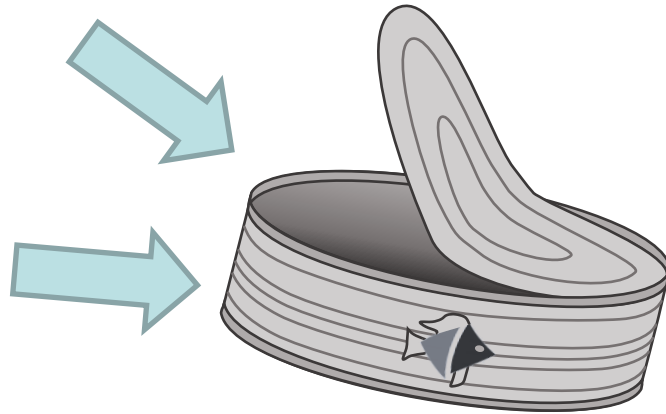
- undefined number of unknown chemicals

## Ingredients

monomers

plasticizers

inks



Migration to food

## Migrants

**IAS**

plasticizers

oligomers

**NIAS**

Unintended reaction products

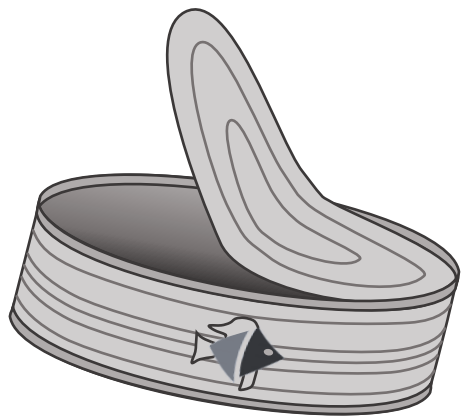
impurities ??

## Q's

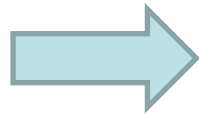
What are these chemicals?  
Do they pose a risk?



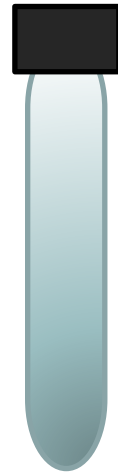
# Simulating migration from coated metal cans



95% Ethanol  
10 days  
60°C



Simulating contact  
with fatty foods  
(think: fish in oil)



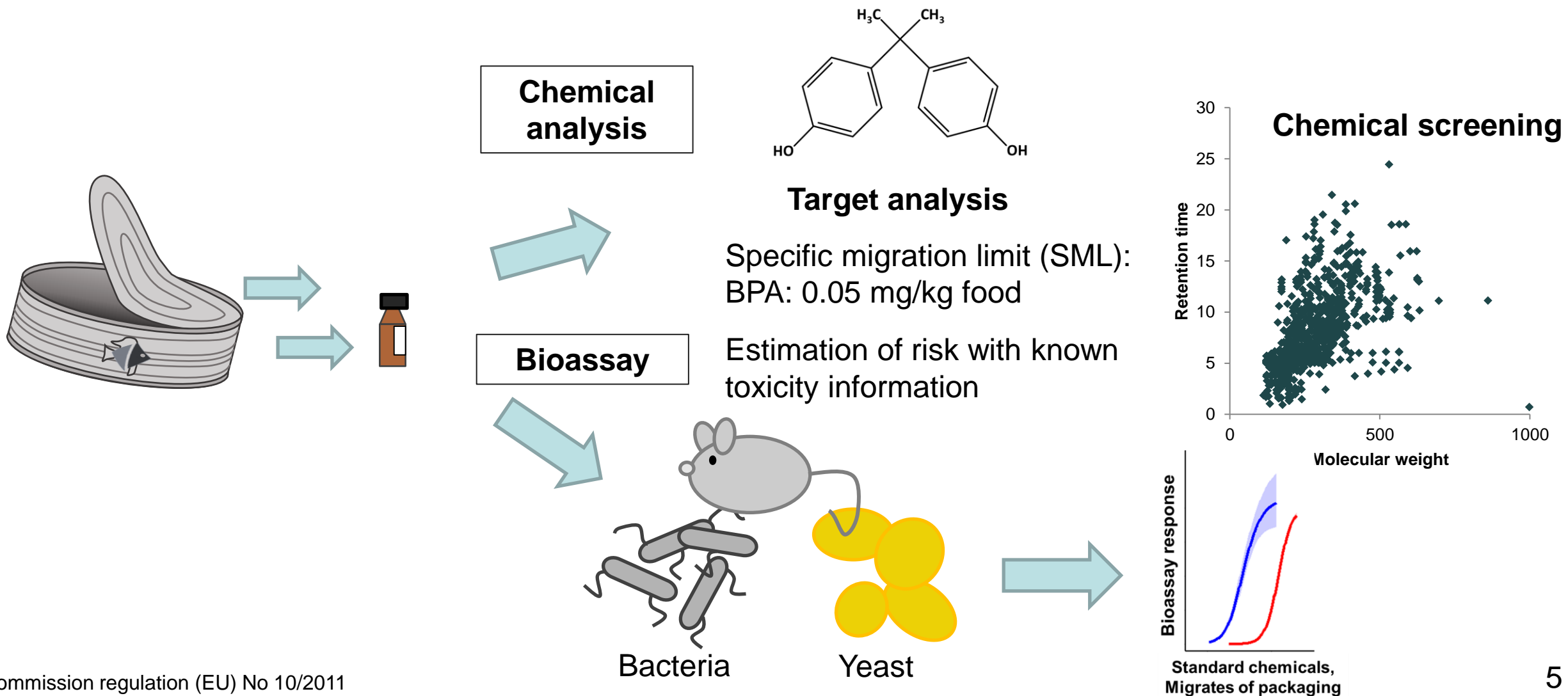
Nitrogen evaporation  
20x concentration



Analysis!

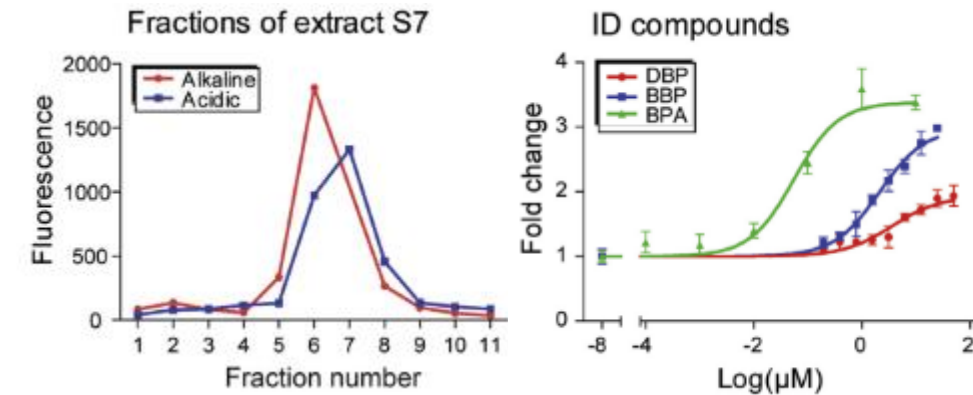
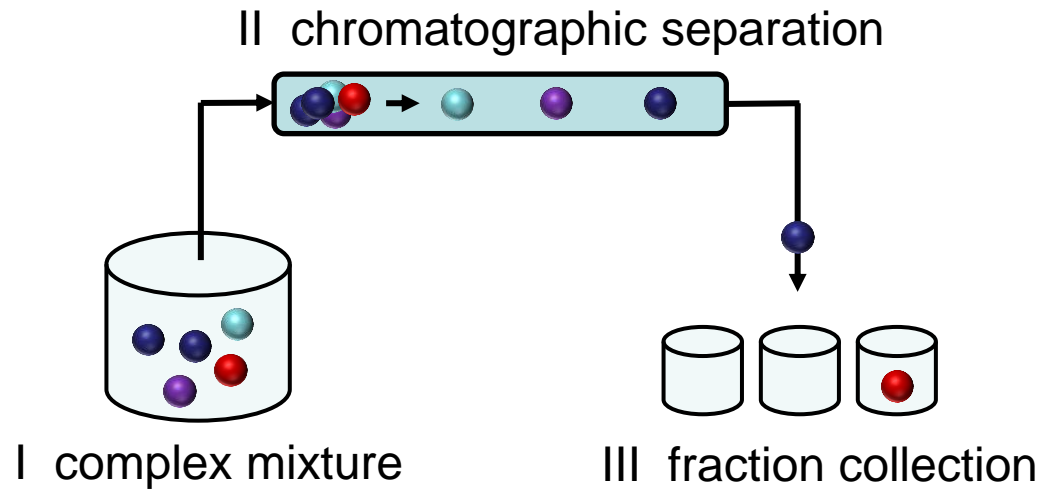
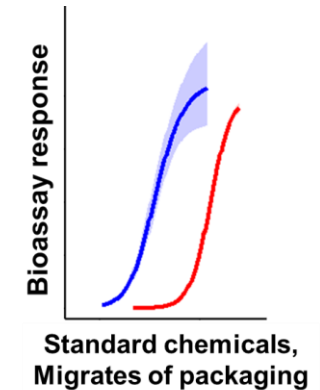
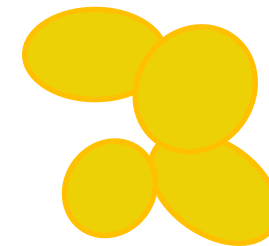
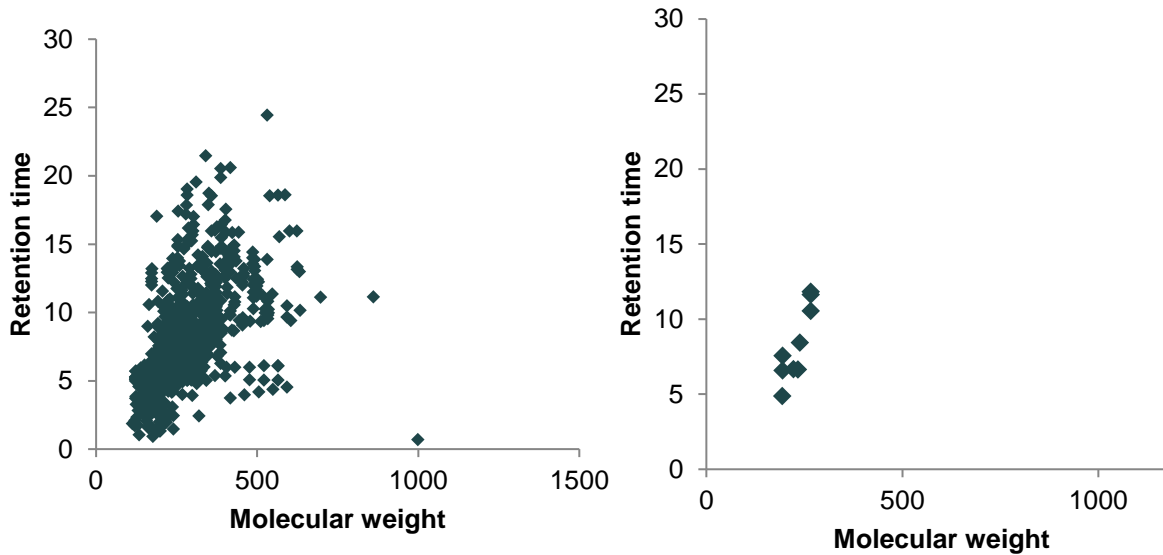


# Analysis: Many chemicals in migrates





# Simplifying the mixture

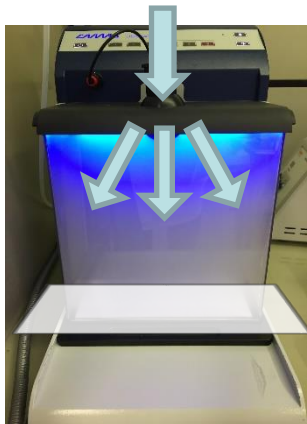
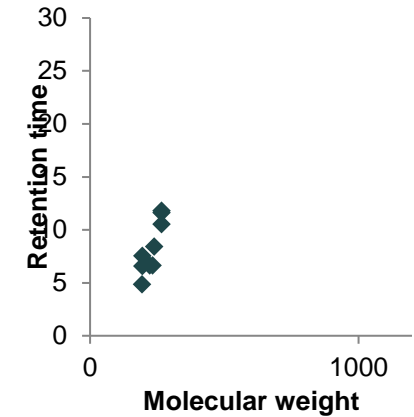
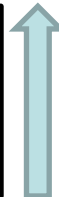
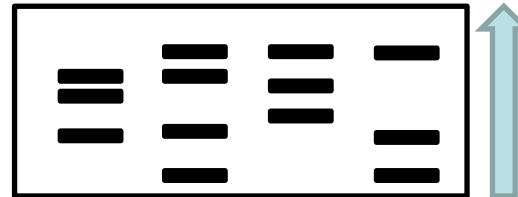
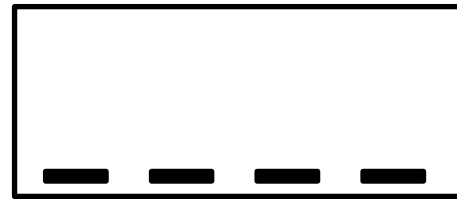




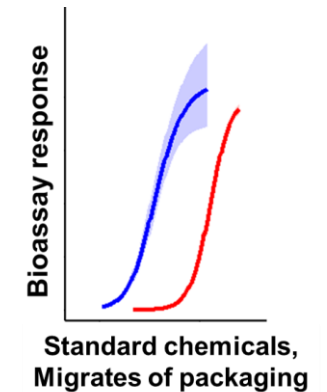
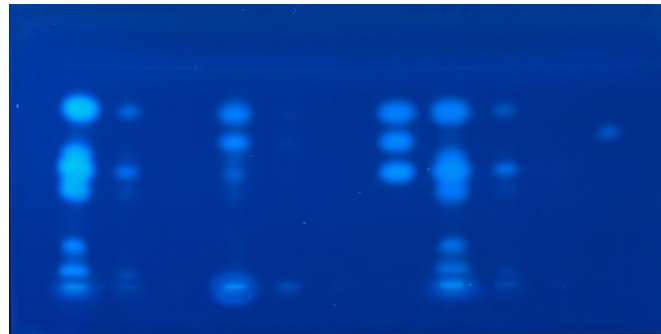
# HPTLC- bioassay

## High performance thin-layer chromatography

- Silica gel HPTLC plates
- Sample application
- Chromatography
- Yeast spray
- Indicator spray

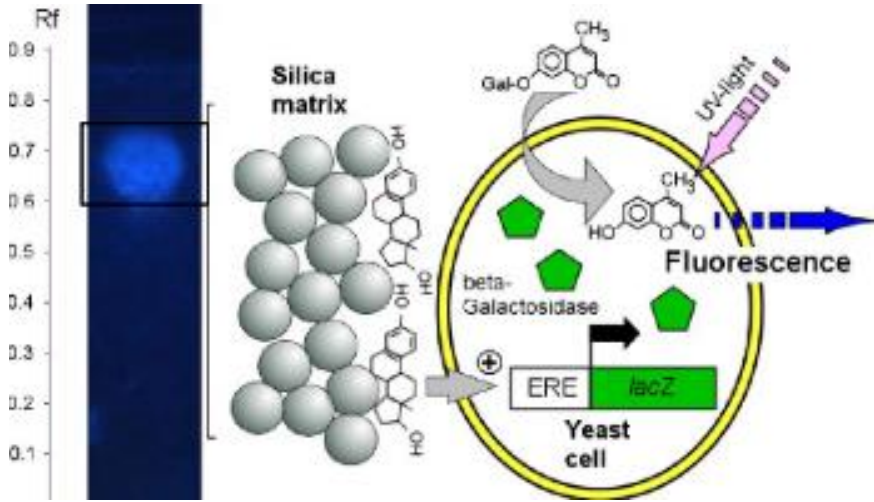


CAMAG  
Derivatizer

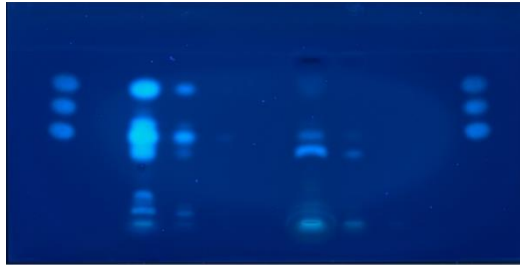




# Yeast estrogen screen

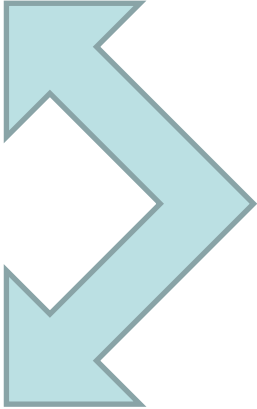


Buchinger et al. 2013. Anal Chem.



### P-YES (planar-YES)

Müller et al. 2004. Chromatographia  
 Klingelhofer and Morlock. 2014. J. Chrom. A  
 Klingelhofer and Morlock. 2015. Anal. Chem.  
 Schönborn et al. 2017. J. Chrom. A

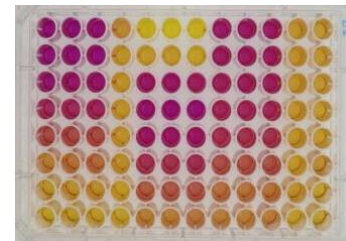
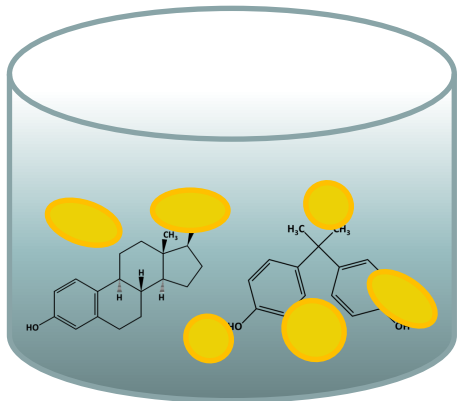


Few comparisons

### Q's

Is P-YES more sensitive than L-YES for food contact chemicals?

How do they perform for example FCM migrates?

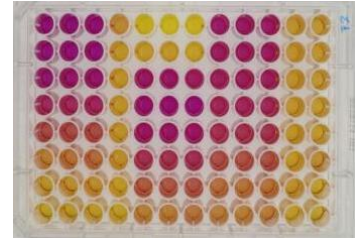
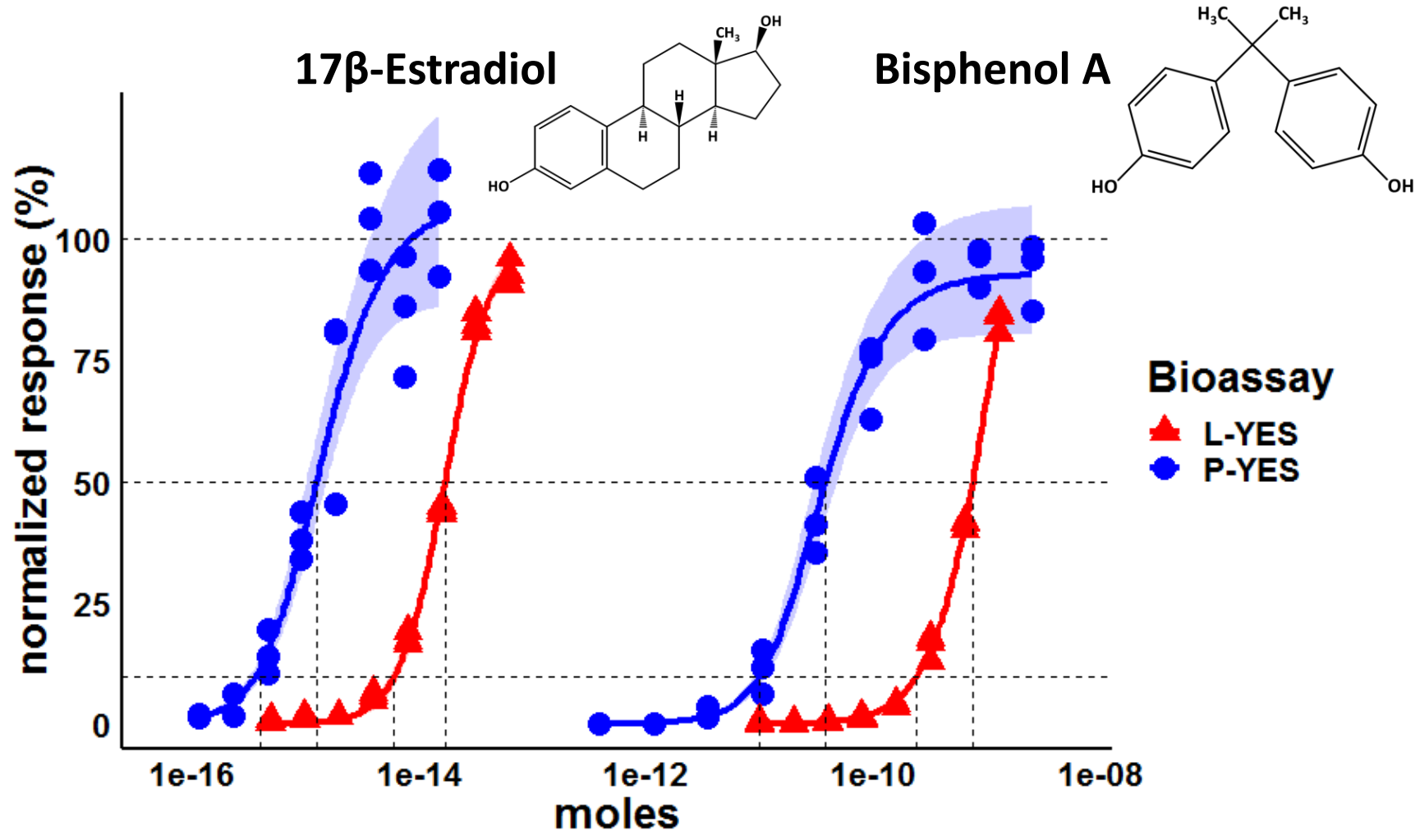


### L-YES (lyticase-YES)

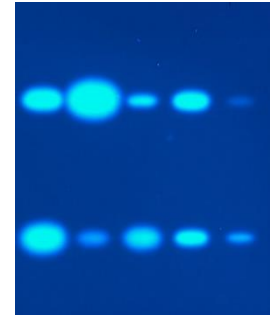
ISO 19040-1. 2018. Water quality — Determination of the estrogenic potential of water and waste water — Part 1: Yeast estrogen screen (*Saccharomyces cerevisiae*)



# L-YES vs P-YES



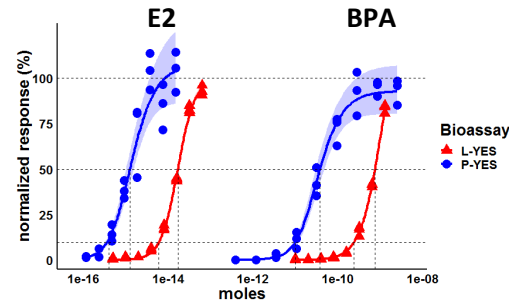
L-YES  
OD<sub>540</sub>



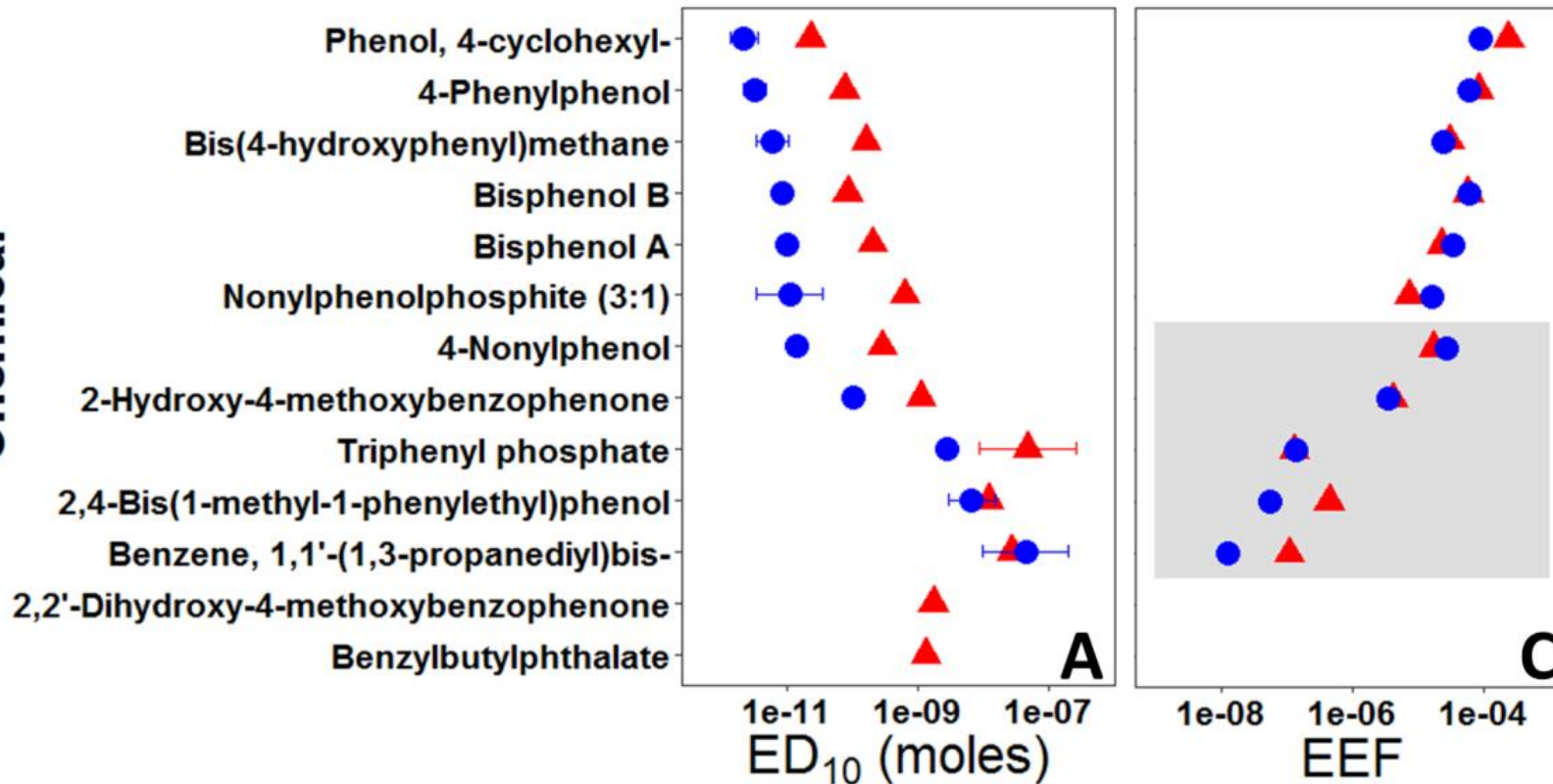
P-YES  
Fluorescence peak height



# L-YES vs P-YES

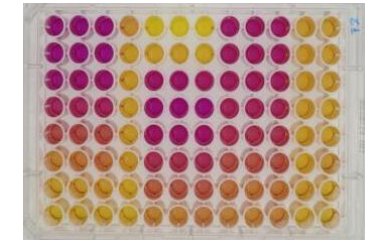


Chemical

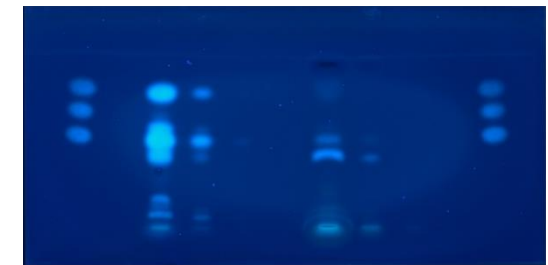


Bioassay

▲ L-YES  
● P-YES



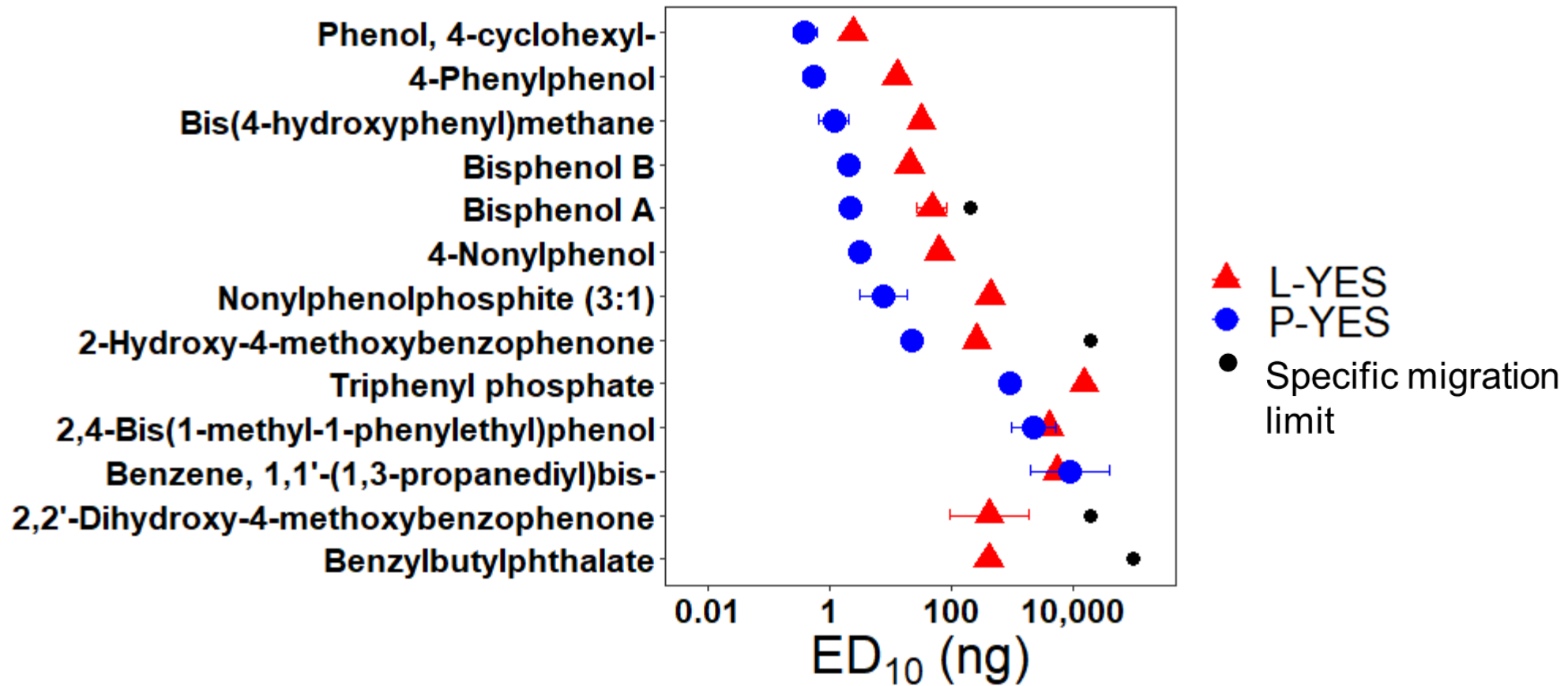
L-YES



P-YES

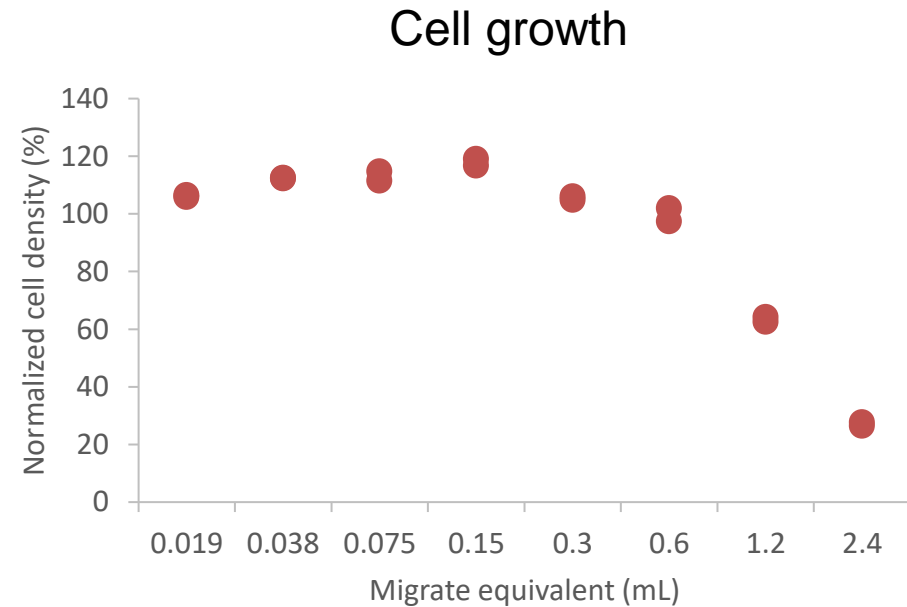
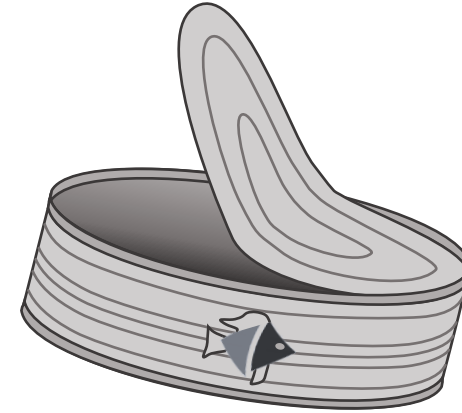
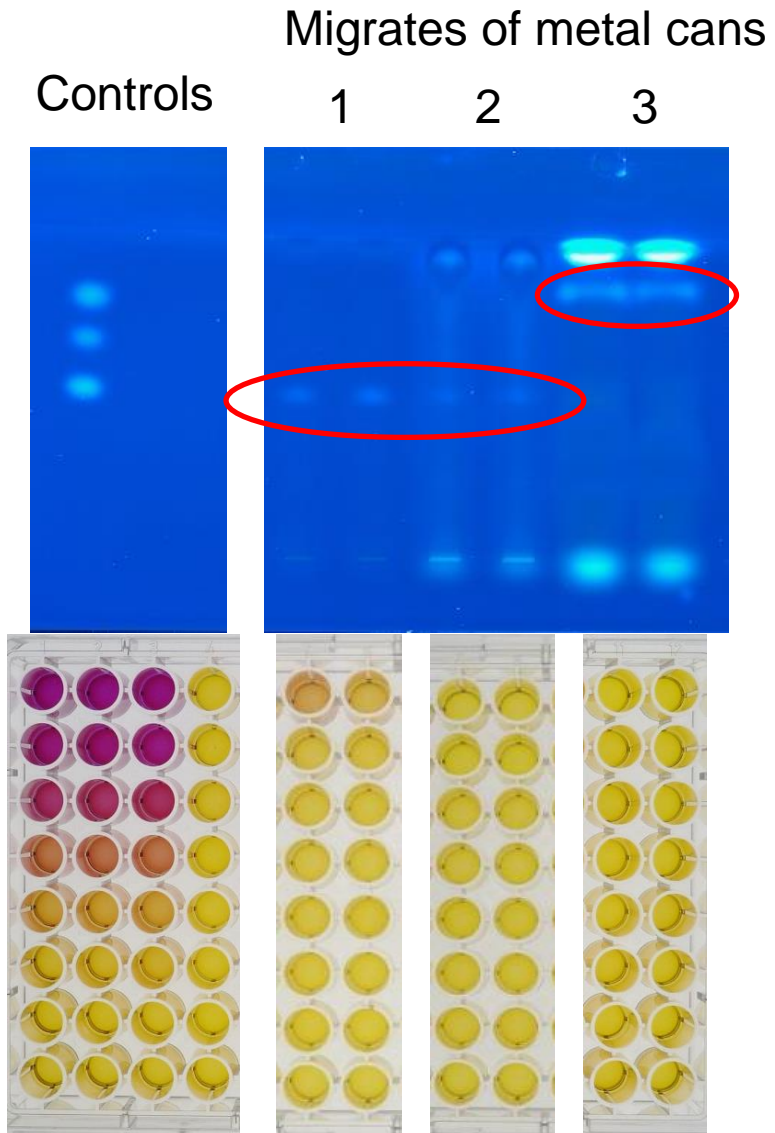


## Comparison to regulatory guidelines





# Estrogenicity of coated metal cans





## L-YES vs P-YES conclusions

P-YES is more sensitive than L-YES, but yields similar relative potencies

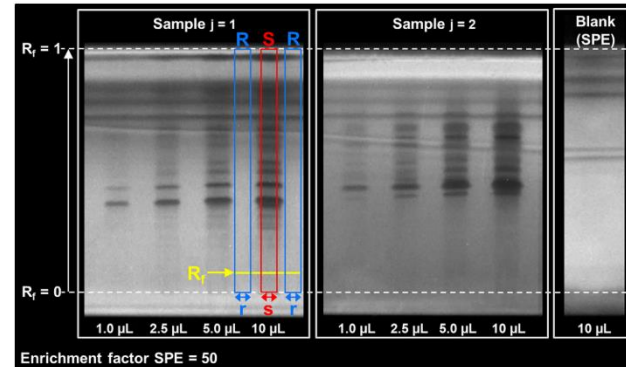
Estrogenic chemicals detectable below specific migration limits

P-YES reveals effects hidden in the whole mixture

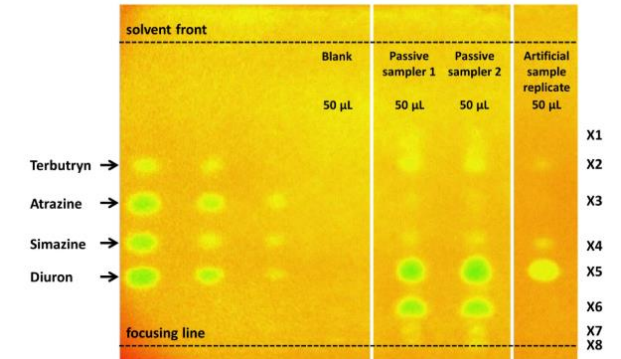


# Other bioassay endpoints on HPTLC plates

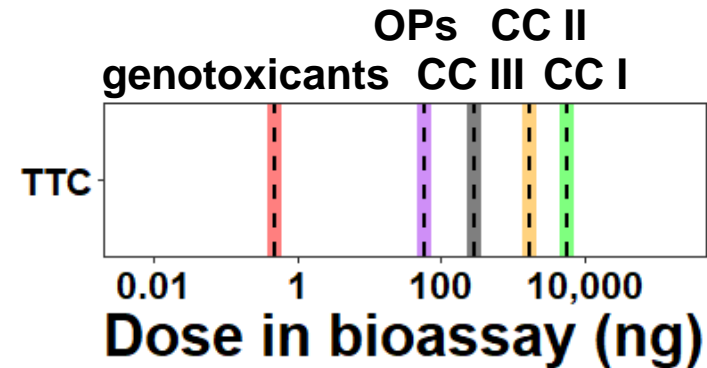
- Bacteria luminescence inhibition
  - Schulz et al. 2017
- Photosystem II inhibition
  - Riegraf et al. 2019
- Androgenicity
  - Riegraf et al. 2019
- Thyroid hormone receptor binding
  - Riegraf et al. 2019
- Acetylcholinesterase inhibition
- **Genotoxicity**
  - Egenteinmeyer and Weiss 2017
  - Shakibai et al. 2019



Schulz et al. 2017. J. Chrom. A



Riegraf et al. 2019. Environ. Sci. Technol.



Thresholds of toxicological concern

Egenteinmeyer and Weiss 2017. J. Liquid Chrom. Rel. Technol.  
 Shakibai et al. 2019. Environ. Sci. Technol.  
 Schilter et al. 2019. Food Additives Contam.: Part A



# Genotoxicity testing - umuC

## SOS-umuC test

- Primary DNA damage:
  - multiple mechanisms of action
- Reporter gene response
- ISO standard for microtiter plates
  - ISO 13829:2000(E)
- Good agreement with Ames

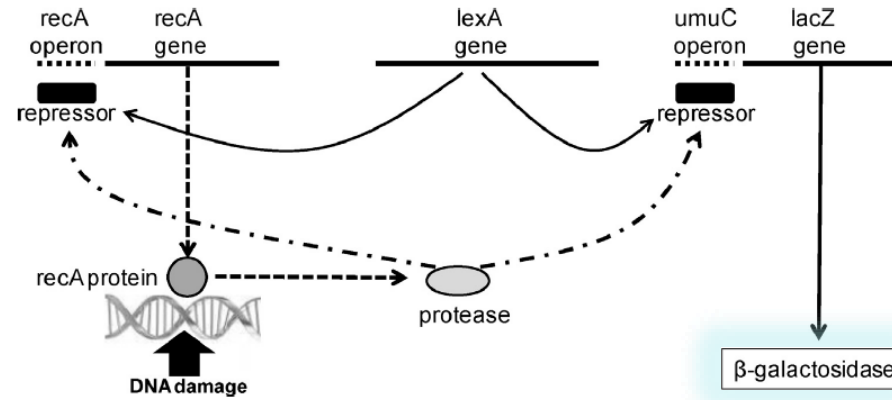
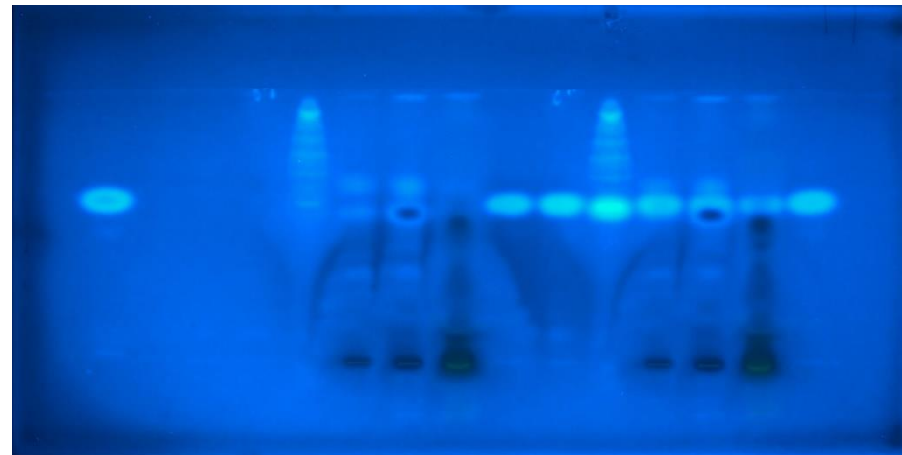


Fig. 3. Illustration of the fused *umuC-lacZ* gene reporting DNA damage detected by *recA*.



Planar-umuC with fluorescence detection



## Genotoxicity testing: Aims

Evaluate HPTLC umuC for chemicals relevant for food contact materials

Evaluate diverse samples for genotoxicity profiles

Persue identity of genotoxicants for case-study FCMs using HPTLC coupled to high resolution mass spectrometry



# Acknowledgements

## Funding

- Federal Food Safety and Veterinary Office - Switzerland
- Ectotox Centre
- Federal Office for the Environment - Switzerland
- Eawag – ANACOM

## Cantonal Laboratory Zürich

- Gregor McCombie

## Swiss Quality Testing Services

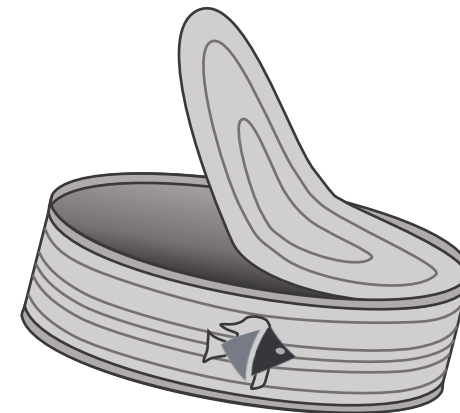
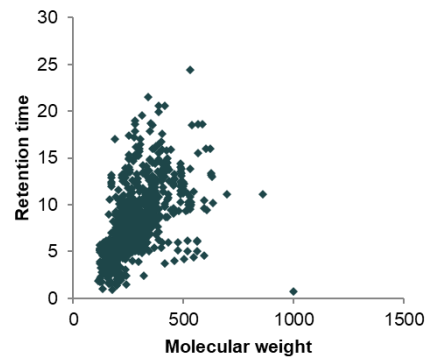
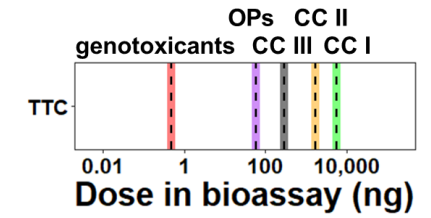
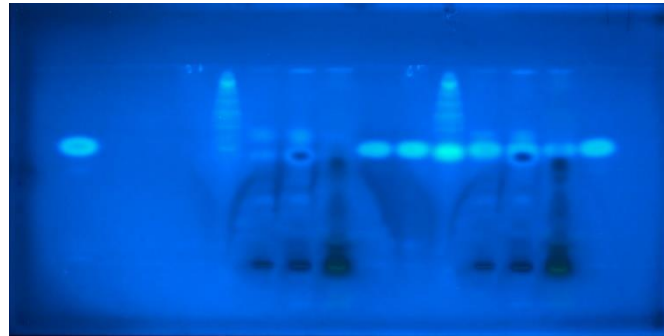
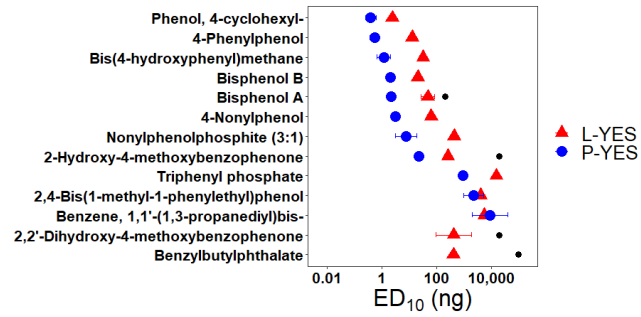
- Vera Baumgartner

## Cantonal Laboratory St. Gallen





# Thank you





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# Supplemental

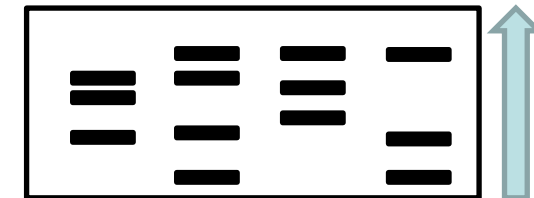
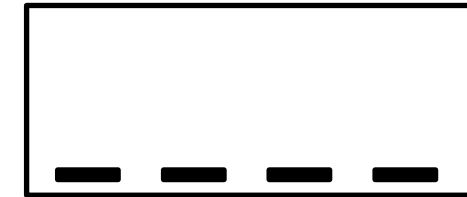


# Planar yeast estrogen screen

We started with planar-YES (P-YES):

- YES relevant to CMR chemicals
- One of the more established bioassays on HPTLC
- ISO standardized in microtiter plates (ISO 19040-1)

- Silica gel 60 (Merck), cleaned by development with methanol
- Sample application in 6 mm bands, 40  $\mu$ L sample in ethanol
- Chromatography based on Cimpoiou et al. 2005
  - Isocratic: chloroform, acetone, petroleum ether
- Yeast sprayed onto plate with CAMAG Derivatizer
- MUG as fluorescent indicator





## Native fluorescence of FCM

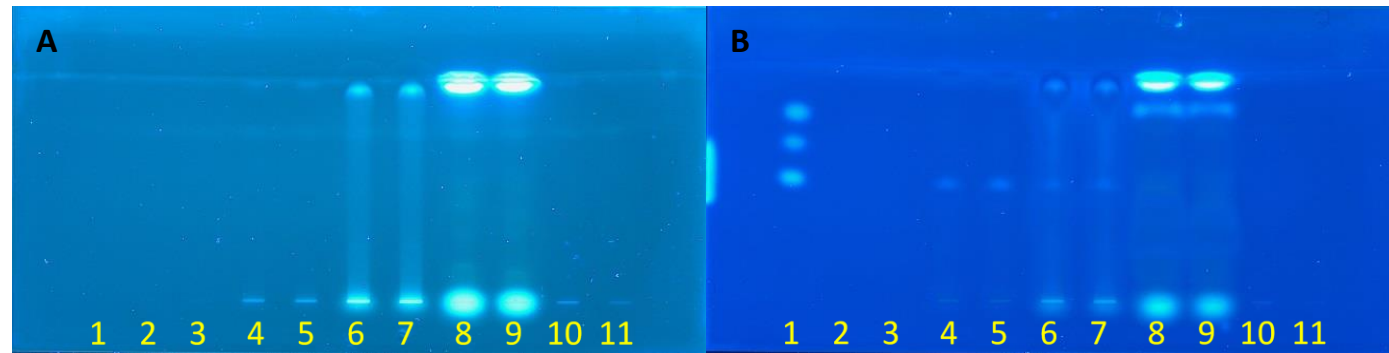
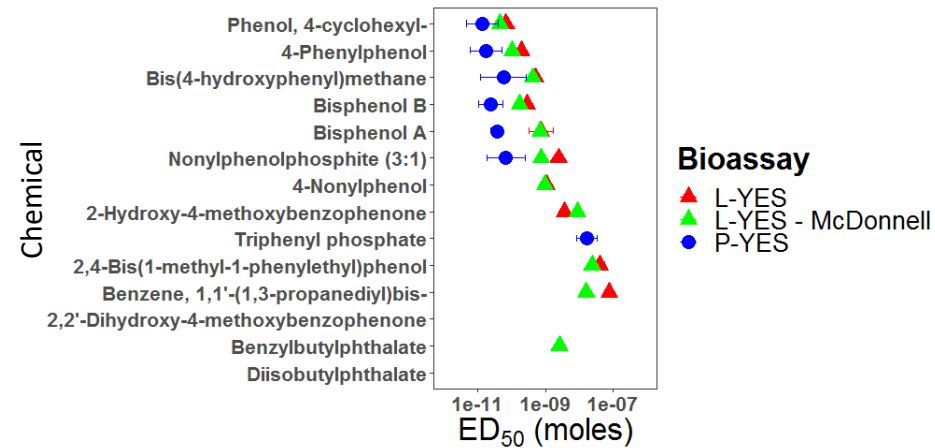
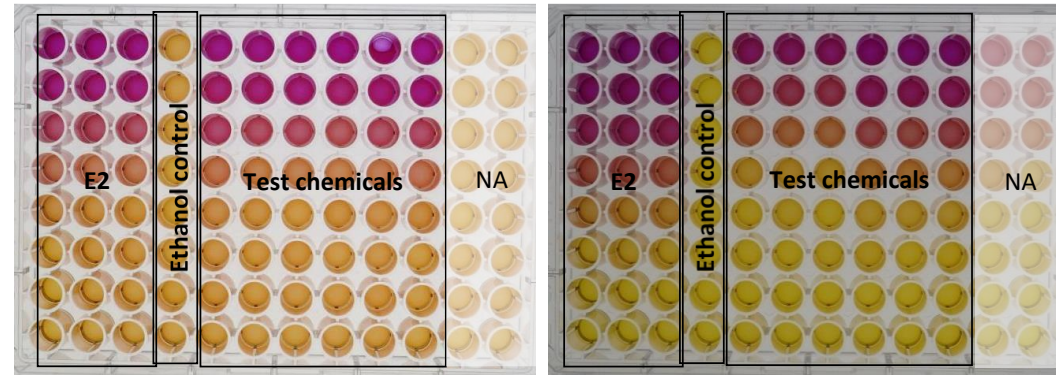
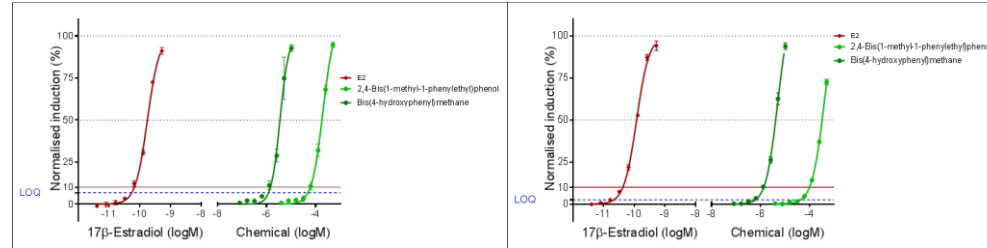


Figure S12. Estrogen screening of food contact materials with P-YES . Fluorescence (A) after incubation with yeast but before detection of  $\beta$ -galactosidase with MUG (*i.e.* native fluorescence) and (B) after addition of MUG in *lacZ* buffer. From left to right the samples on the plate are (1) positive control estrogen mix, (2) solvent control, (3) sample preparation control, and duplicate migrates of (4-5) big metal cans, (6-7) small metal cans, (8-9) fish cans, and (10-11) migration negative controls. Images were collected with CAMAG Visualizer II, with illumination at 366 nm and camera duration 550 ms.

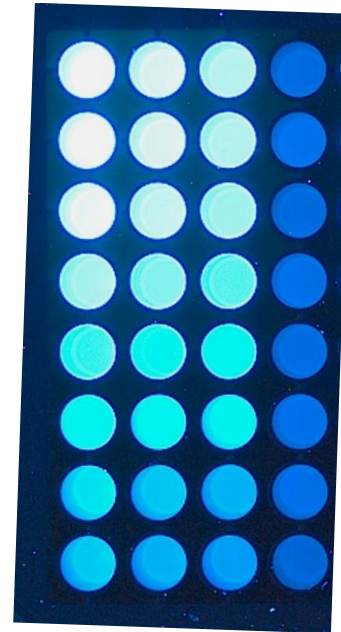


# Effect of Yeast Strain





# Effect of indicator solution

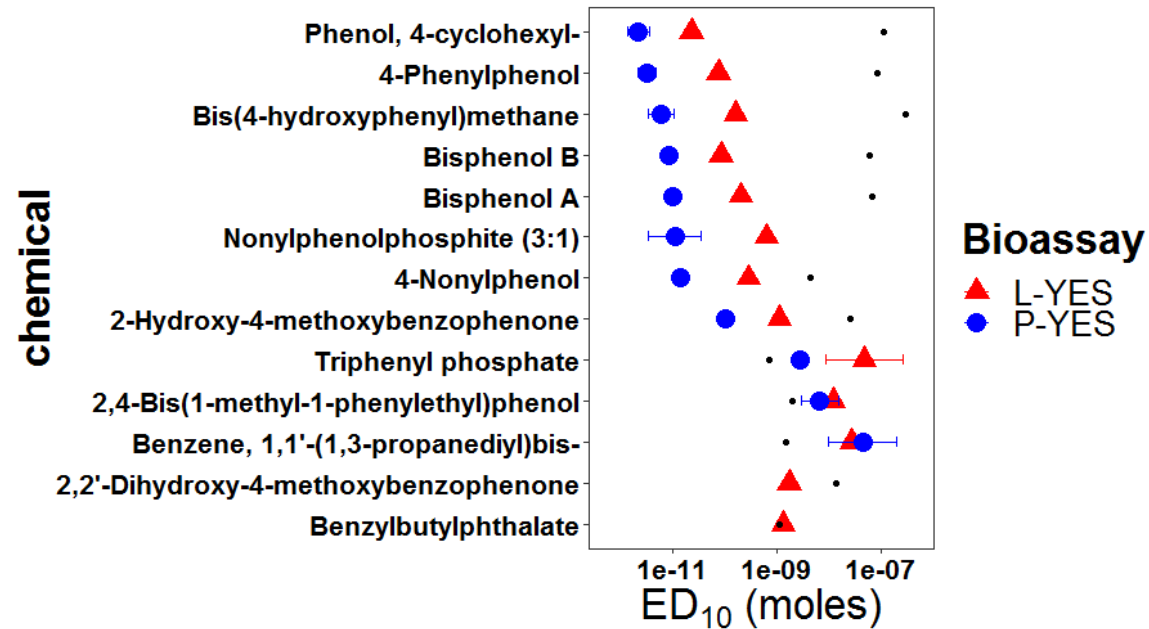


ED<sub>50</sub> of L-YES with CPRG or MUG

plate	CPRG	MUG	ratio (CPRG/MUG)
1	6.07E-11	5.33E-11	1.1
2	5.37E-11	5.01E-11	1.1
3	9.12E-11	5.35E-11	1.7
summary			
average	6.85E-11	5.23E-11	1.3
standard deviation	2.00E-11	1.93E-12	0.35



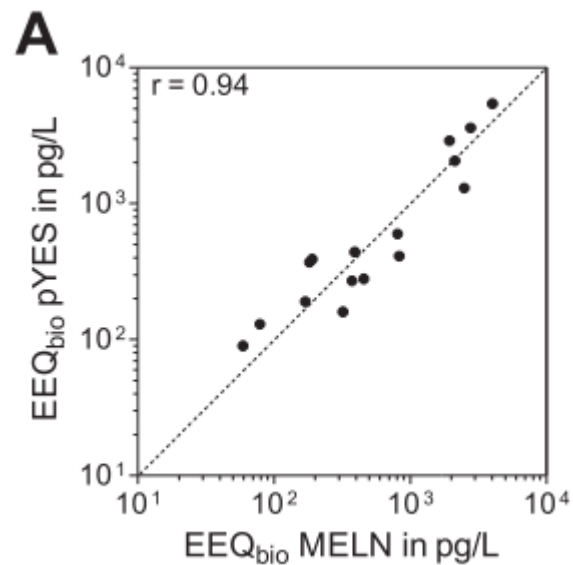
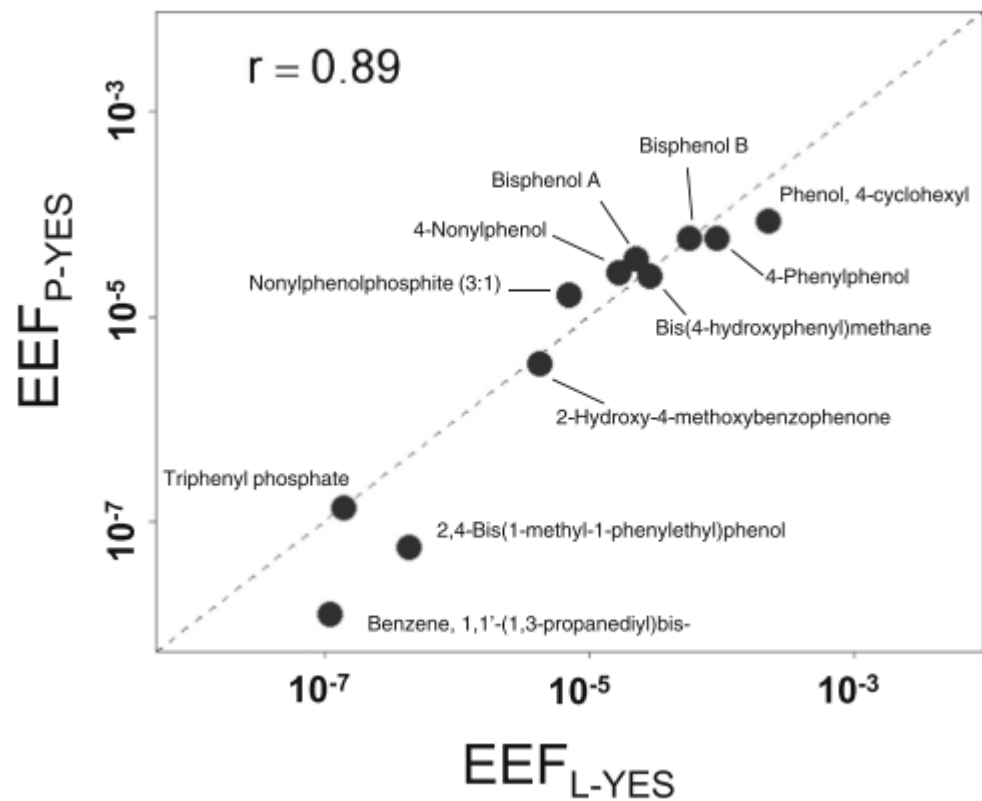
# Water solubility...



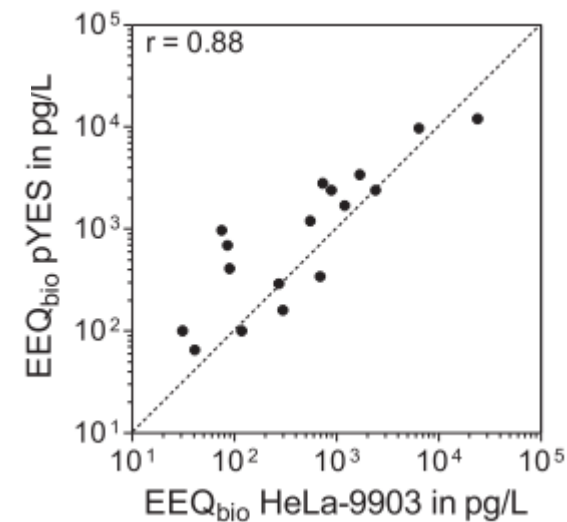
Dose at 10% effect compared to water solubility. Water solubility (black dots) of test chemicals is shown as moles at saturation in 120  $\mu$ L (volume of L-YES). Specifically calculated as: water solubility (M, according to U.S.EPA Chemistry Dashboard in Table 1) times  $1.2 \times 10^{-6}$  L. Water solubility is not calculable for P-YES because the assay has no defined volume. Water solubility was not available for nonylphenylphosphite (3:1).



# L-YES vs P-YES



Könneman et al. 2018. Trends Anal. Chem.





## Comparison to regulatory guidelines

