Detection and Identification of Endocrine Active Substances in Food Packaging

OFI Austrian Research Inst. for Chem. and Tech.

How did it all start ?

Endocrine activity in mineral water!

- Several independent studies
- Endocrine activity was detected in various mineral water brands
- Source remained unclear (source water, contaminations during filling process, PET bottle, PE screw caps)
- PET bottles suspected

=> Direct analysis of PET bottles necessary!

Media Headlines

Scientists Fear Chemical in Plastic Could Be Harmful

ABC NEWS

By JAIME J. HENNESSEY
July 6, 2006

From food-storage containers to disposable silverware, plast
are such a part of our lives that it's
that could harm u

What's in YOUR blood?

First Run Features presents A DOCUMENTARY BY WERNER BOOTE
PLASTIC PLANET

BBC NEWS

Plastic chemicals 'feminise boys'

Chemicals in plastics alter the brains of baby boys, making them "more feminine", say US researchers.

Males exposed to high doses in the womb went on to be less likely to play with boys' toys like cars or to join in rough and tumble games, they found.

Male hormones drive boyish play

The University of Rochester team's latest work adds to concerns about the safety of phthalates, found in vinyl flooring and PVC sho

The findings are reported in the In

derStandard.at › Gesundheit › Leben › Umweltmedizin

Inland Wirtschaft Web Sport Panorama Etat

Weichmacher

Österreich verhält
Bisphenol A in Ba
stecken aber noc

foto: ernst rose/pixelio.de

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News > Science

Study shows dangers of BPA chemical used in plastic packaging

Bisphenol A is used to line drinks cans and in tests affected the way genes work in the brains of laboratory rats

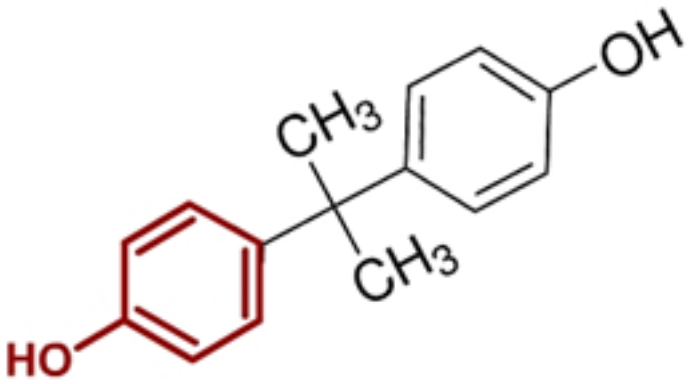
Are Plastic Baby Bottles Harmful?

By Laura Blue | Friday, Feb. 08, 2008

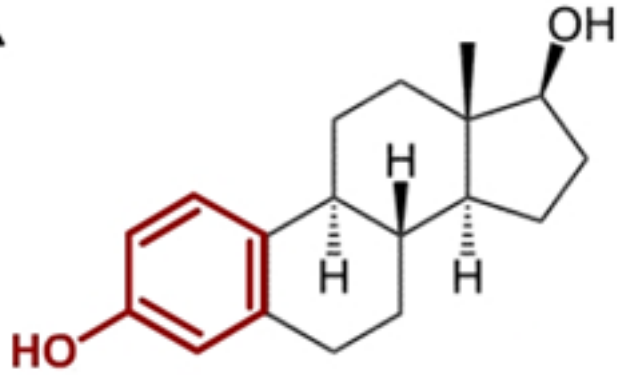
If a new report is to be believed, an entire generation of children has grown up drinking a toxic chemical from their earliest months: bisphenol A. A consortium of North American environmental and health groups released a paper Thursday showing that many major-brand baby bottles leach bisphenol A, and is now calling for a moratorium on the use of the compound — used to make polycarbonate plastic

Consumers are concerned…

Structural Similarities



Bisphenol A
(Monomer of polycarbonate)



Estrogen (17β-Estradiol)
(Natural female sex hormone)

How can food packaging be endocrine active?

Endocrine active substances!

- Exogenous man-made substances:
  - Plasticisers
  - Stabilizers
  - Antioxidants
  - Monomers
  - Print colours
  - Contaminants
  - Degradation products

Established Bioassays

Yeast assays:

- Yeast Estrogen Screen
- Yeast Androgen Screen

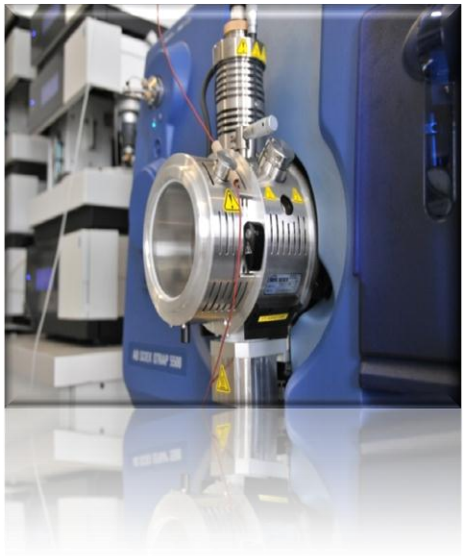
Human cell assays: (CALUX)

- Estrogens (female sex hormone)
- Androgens (male sex hormone)
- Thyroid hormones
- Substances interfering with PPAR-receptors

Chemical Analysis

HPLC-UV/VIS-MS/MS^n

Dionex U3000
Qtrap 5500, Triple-Quad with linear Ion trap
Source: ESI and APCI

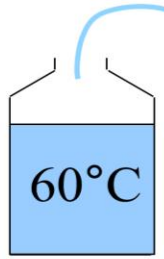


GC MS

- TDU-GC/MS: 7890A (GC) + 5975C inert (MS) + FID with multipurpose Sampler: TDU/HS/FI
- Screening of unknown substances + Semi-quantification with FID

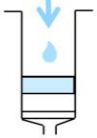
Limit of detection: < 10 ppb

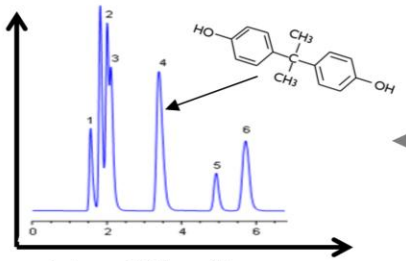
Scheme of analysis



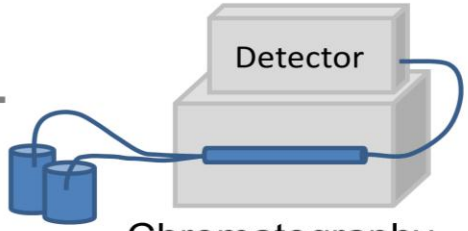
Migration

Concentration step

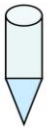




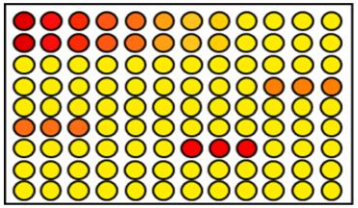
Identification



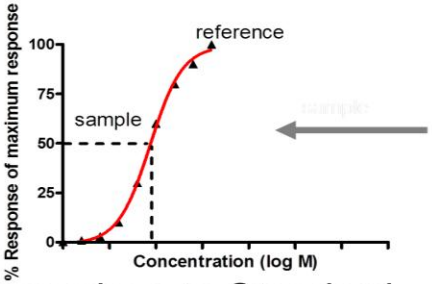
Chromatography



Solving in 100 µl DMSO



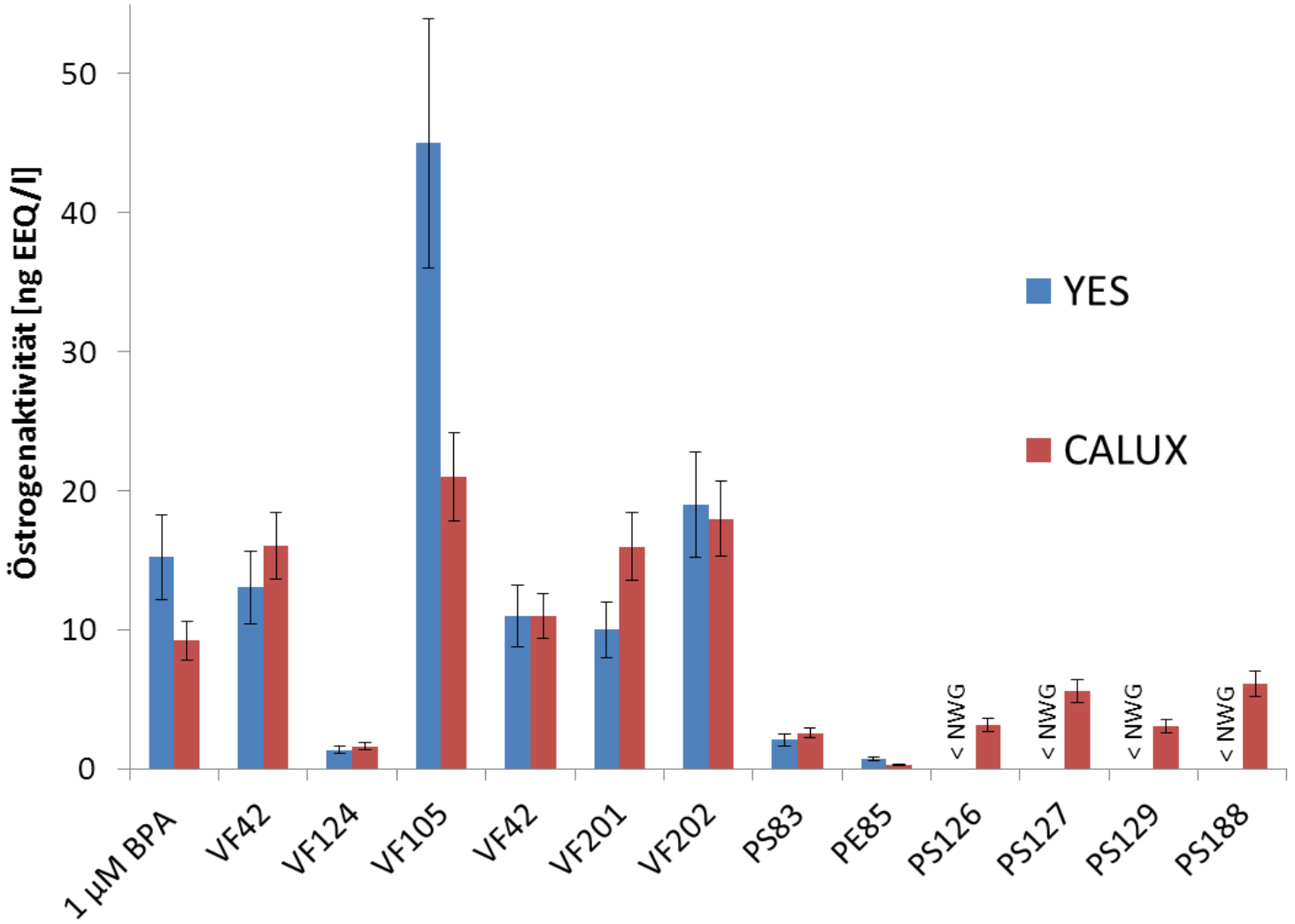
Bioassay



Comparison to Standard

Comparison of the bioassays

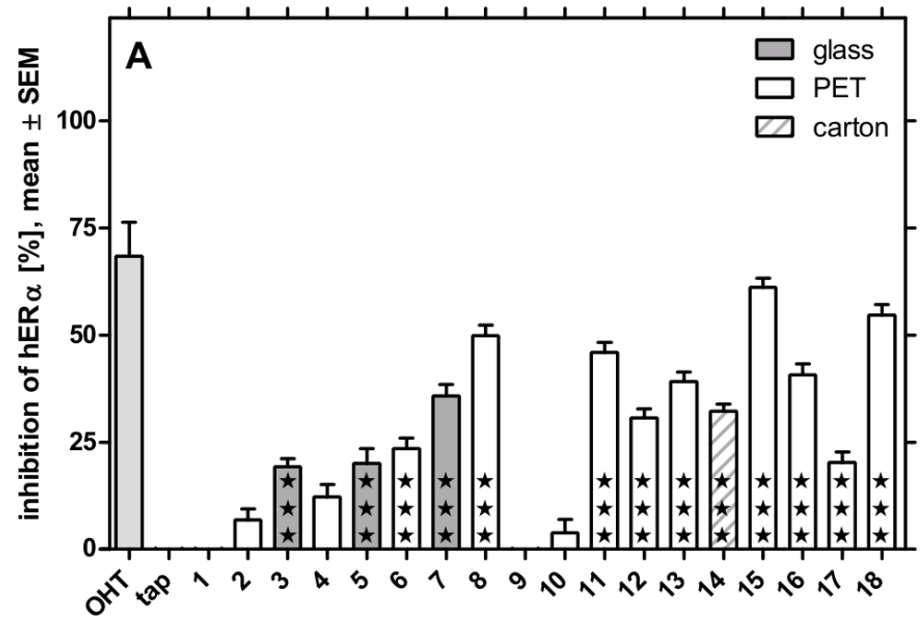
YES and ER CALUX: good correlation of estrogenic activity

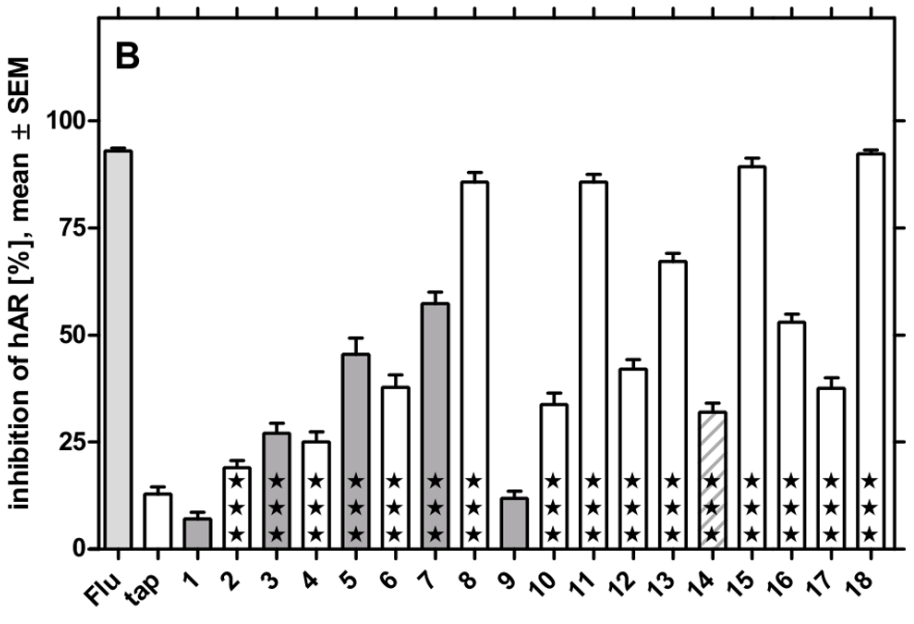


Wagner 2013 – Antagonistic effects in mineral water

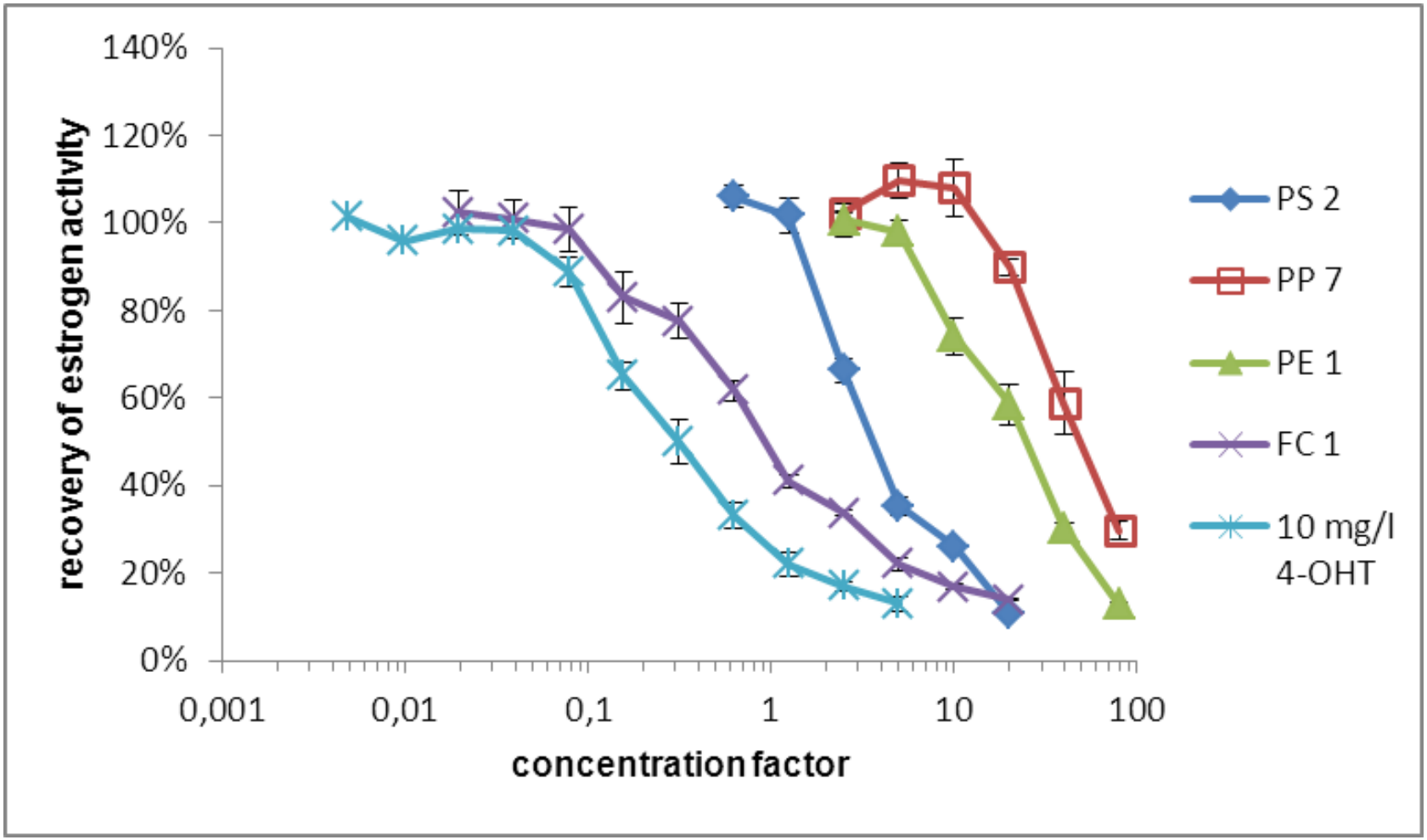
The majority of bottled water products showed antiestrogenic and antiandrogenic effects!

- Significant correlation between antiestrogenic and antiandrogenic samples

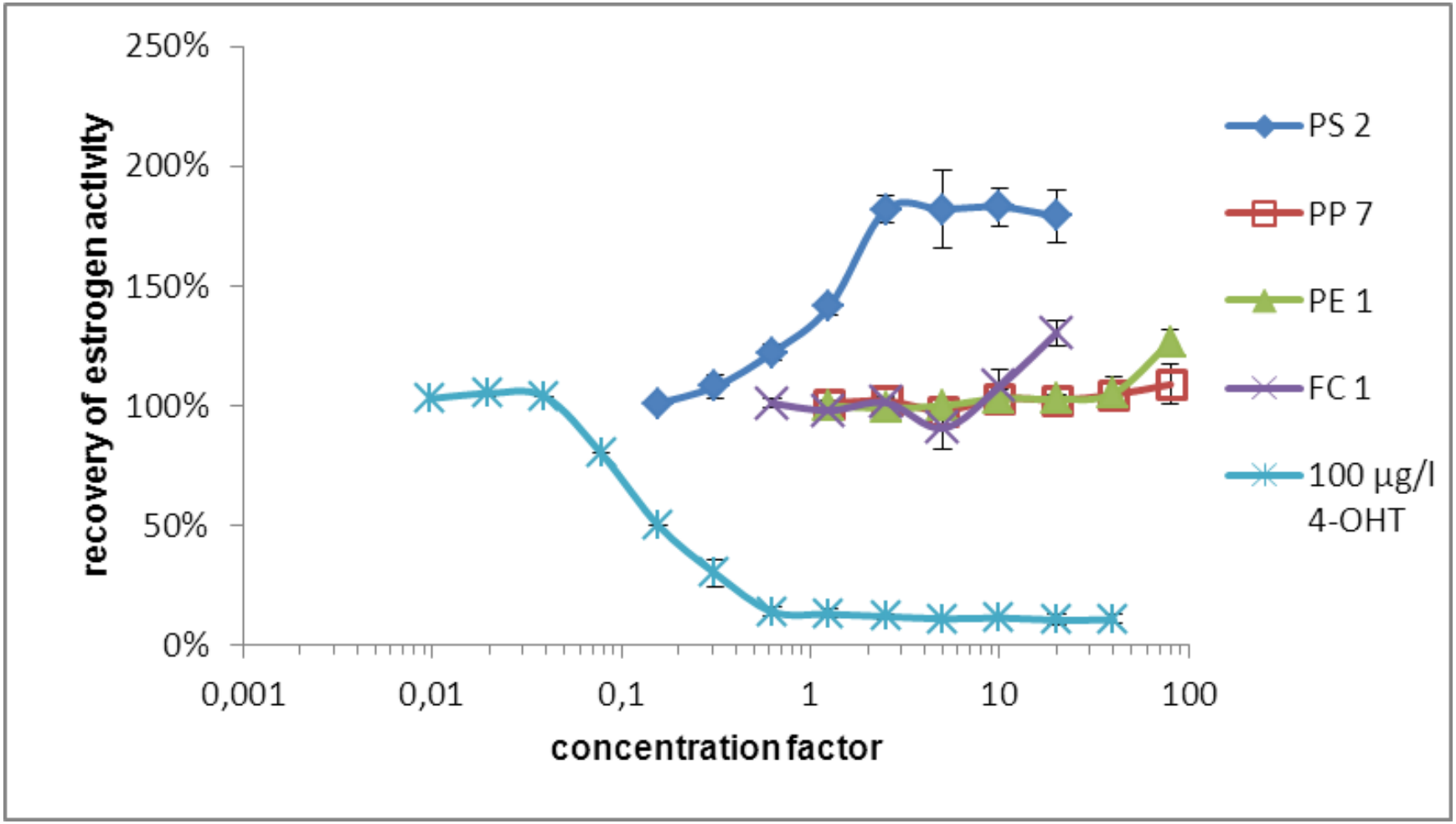




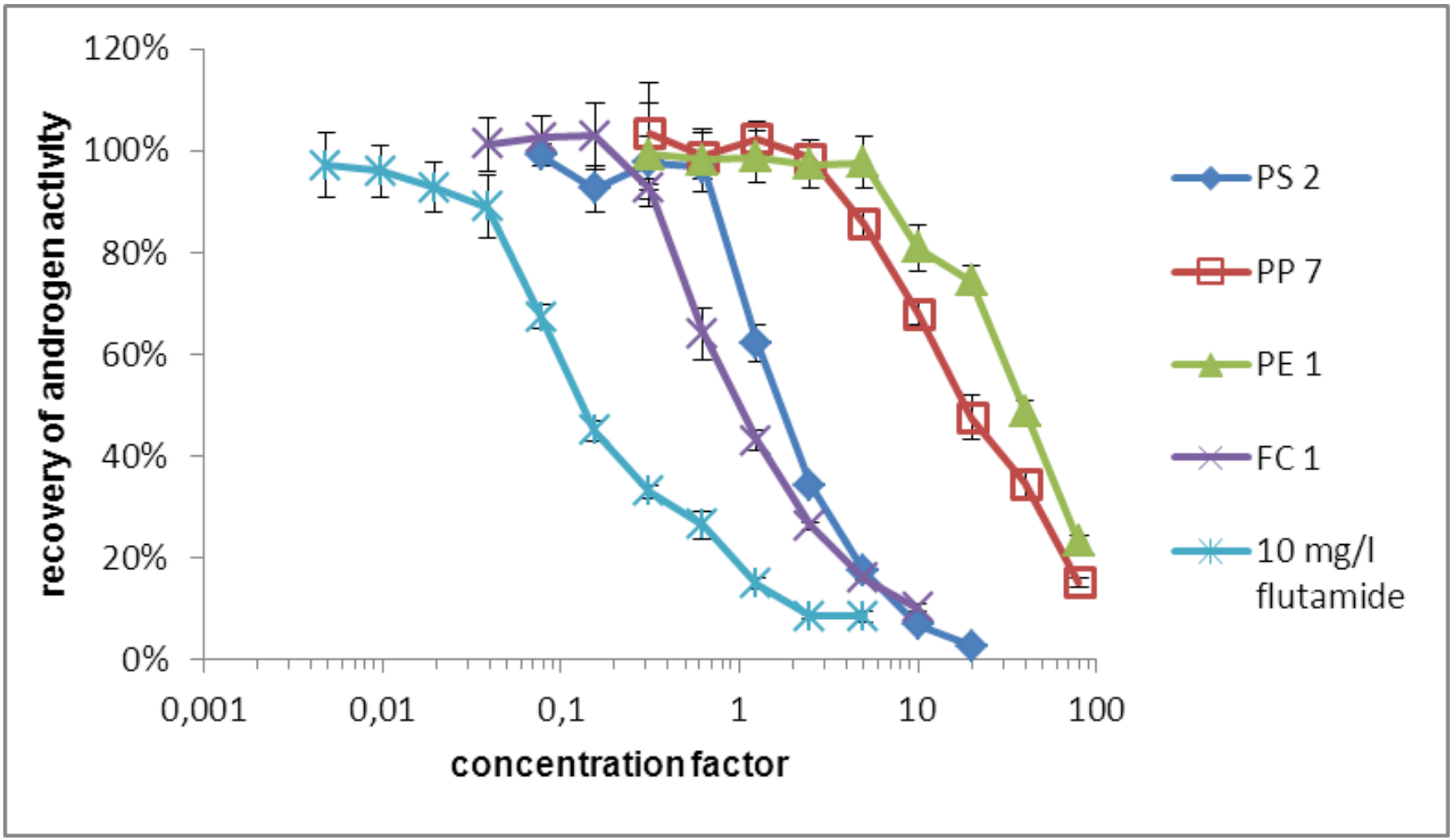
OFI – Antiestrogenic effects in the YES



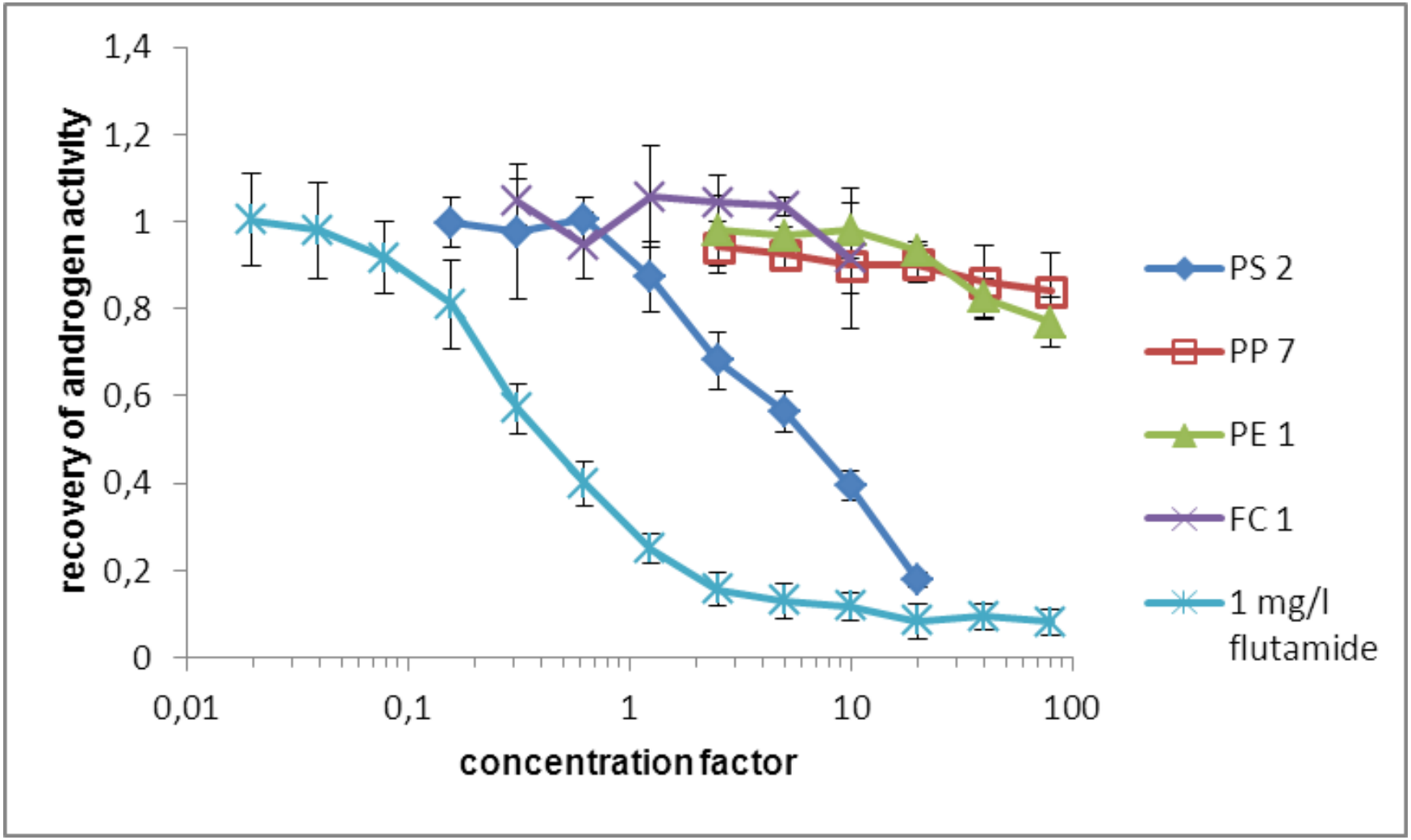
OFI – Antiestrogenic effects in the ER-CALUX



OFI – Antiandrogenic effects in the YAS



OFI – Antiandrogenic effects in the AR-CALUX



Interpretation of results

Significant correlation between results of yeast and human bioassays when testing for agonism, but not for antagonism

- Samples that showed antiestrogenic effects in the YES also showed antiandrogenic effects in the YAS.
- Indicates that the observed effect is not specific to the respective hormone receptor

- => YES and YAS are not suitable for an analysis of food packaging for antagonistic effects.

- CALUX – Bioassays for screening of food packaging

Screening of samples

250 samples of different food packaging

empty packages (bottles, cups, microwave packaging, caps), granulates, films,…)

Samples included various materials:

- Composite films
- Polystyrene
- Polyolefins
- Food cartons
- Cans

Results of bioassays

- >80% of all tested samples: no endocrine activity
- About 15% of all tested sample: estrogen activity
  - Detected activity is mostly much lower than the activities previously found in mineral water (<7 ng/L EEQ)
  - < 2% (4 samples): activities between 50 – 100 ng/L EEQ
- About 10% of all tested samples: anti-androgen activity
- No thyroid activity detected

Screening of PET samples

35 PET samples from different European Producers

Virgin and recycling-material:

- Unprocessed PET: granulates
- Recycling Flakes
- Preforms
- Bottles with HDPE screw caps

Only 1 out of 35 samples showed a very low estrogen activity:

- Estrogen active sample (Recycling flakes): **0,05 ± 0,02 ng EEQ/L**
- Estrogen activity in bottled water (Wagner 2009): **75 ng EEQ/L**

Chemical Analysis

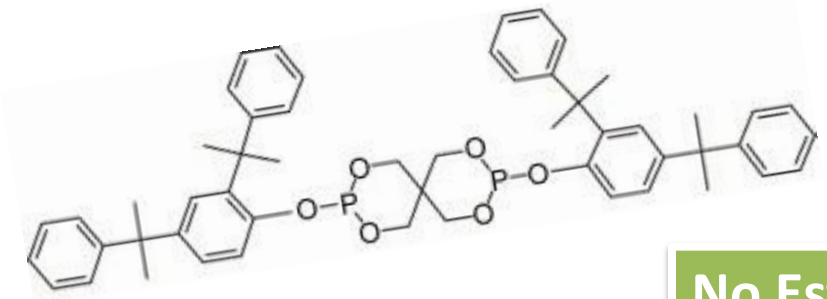
Other endocrine active substances identified in positive testes samples:

- Bisphenol A
- Styrene dimers and trimers
- Phthalates: BBP, DBP,…
- Components of printing colours (photo initiators)
- Degradation products of antioxidants

- BUT: in many sample - no explanation for endocrine activity

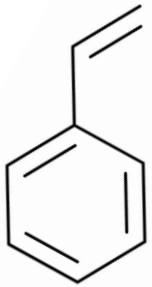
Development of endocrine activity

Antioxidant: Alkanox 28



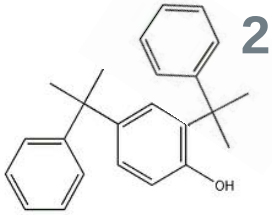
No Estrogen Activity!

Monomer: Styrene



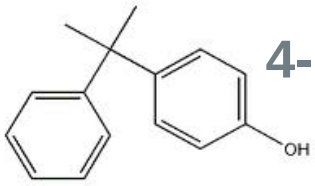
Degradation products

2,4-dicumylphenol



Low Estrogen Activity!

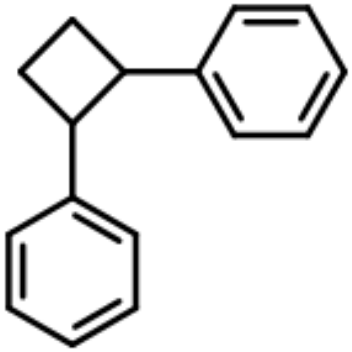
4-cumylphenol



High Estrogen Activity!

Byproduct of polymerization

1 2-diphenylcyclobutane



Summary of our screening of food packaging

Majority of food packaging is not endocrine active (> 80 %)

- Activities are mostly much lower than the activities previously detected in mineral water
- Some samples show significant higher activity
- PET results clearly indicate that the activities found in mineral water were not caused by PET
- Cell assays only show binding to the hormone receptor => No direct conclusion on the activity in an organism possible !!!

Contact: Johannes Mertl

t: +43 1 7981601 633

johannes.mertl@ofi.at

www.ofi.at