Detection and Identification of Endocrine Active Substances in Food Packaging

OFI  Austrian Research Inst. for Chem. and Tech.
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!
Scientists Fear Chemical in Plastic Could Be Harmful

By JAIME J. HENNESSEY  
July 6, 2006

From food-storage containers to disposable silverware, plastics are such a part of our lives that it’s easy to think that they won’t harm us.

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.

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

The findings are reported in the Independent.

Are Plastic Baby Bottles Harmful?

By Laura Elise  
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 — in food and beverage containers.
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

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

Limit of detection: < 10 ppb

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
Scheme of analysis

- Concentration step
- Migration at 60°C
- Identification
- Chromatography
- Solving in 100 µl DMSO
- Comparison to Standard
- Bioassay
Comparison of the bioassays

YES and ER CALUX: good correlation of estrogenic activity
The majority of bottled water products showed antiestrogenic and antiandrogenic effects!

- Significant correlation between antiestrogenic and antiandrogenic samples
OFI – Antiestrogenic effects in the YES

![Graph showing recovery of estrogen activity vs. concentration factor for different substances and 4-OHT.](image-url)
OFI – Antiestrogenic effects in the ER-CALUX
OFI – Antiandrogenic effects in the YAS

Graph showing the recovery of androgen activity vs. concentration factor for different samples and a reference concentration of flutamide.
OFI – Antiandrogenic effects in the AR-CALUX

recovery of androgen activity

concentration factor

PS 2
PP 7
PE 1
FC 1
1 mg/l flutamide
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): \(0.05 \pm 0.02\) ng EEQ/L
- Estrogen activity in bottled water (Wagner 2009): 75 ng EEQ/L
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

Monomer: Styrene

Degradation products

2,4-dicumylphenol

1 2-diphenylcyclobutane

Byproduct of polymerization

No Estrogen Activity!

Low Estrogen Activity!

High Estrogen Activity!
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 !!!
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