



# Update on Sample Screening

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Mitglied bei:



AUSTRIAN COOPERATIVE RESEARCH

> **400 Samples** analysed for  
genotoxicity and endocrine effects

Different material types were  
analysed:

- Composite materials
- Coatings
- Polyolefins (incl. PS)
- Paper & Board
- Others

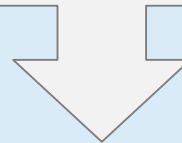
> **400 Samples** analysed for genotoxicity and endocrine effects

Different material types were analysed:

- Composite materials
- Coatings
- Polyolefins
- Polystyrene
- Recycled Plastic
- Paper & Board
- Others

## Material sources

1. Provided by the Industry partners
2. Empty packaging provided by retailer
3. Medical device grade materials
4. Research samples with known composition

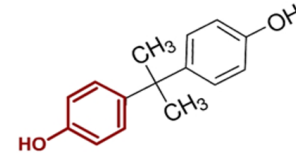
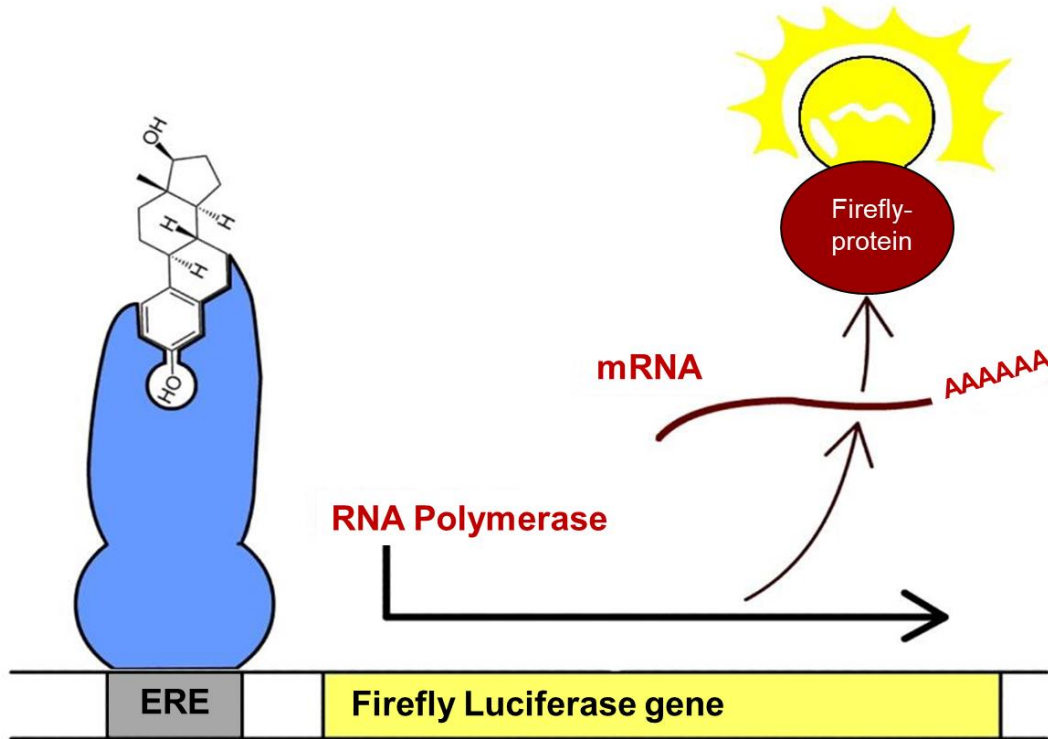


**Wide range of materials / samples**

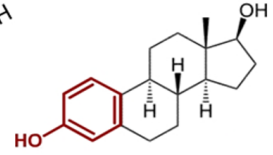
**Ensures anonymization!**

(Not possible to guess who provided samples!)

# CALUX: Human Cell based Test for Endocrine Activity



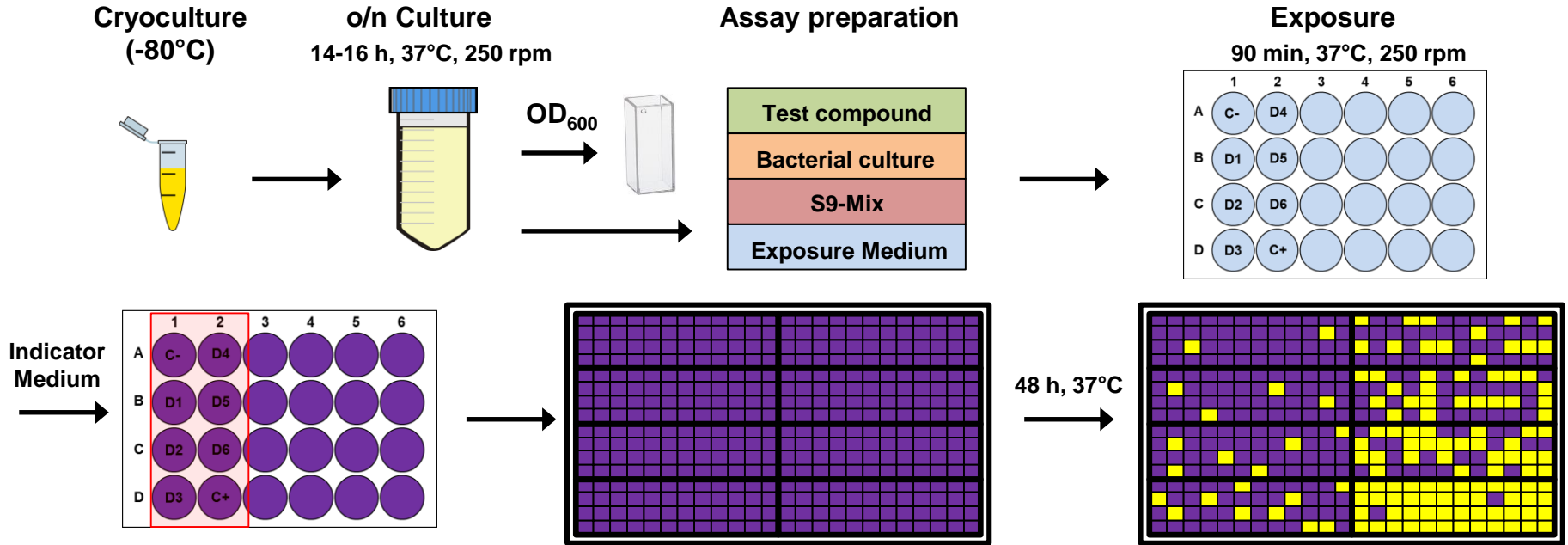
**Bisphenol A**  
(Monomer of polycarbonate)



**Estrogen (17 $\beta$ -Estradiol)**  
(Natural female sex hormone)



# Main Focus of the MIGRATOX Project - Ames MPF Assay

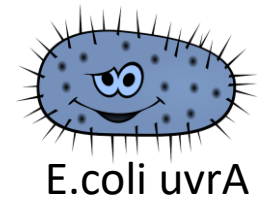
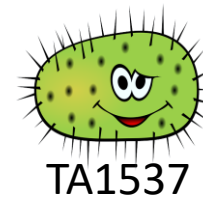
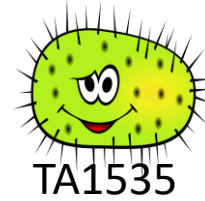
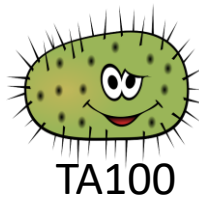
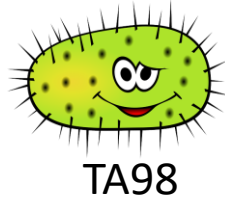


- Cultivation and exposure in liquid medium
- In microtiter plates (24-well, 384-well)
- Colorimetric readout

- Higher throughput
- Lower sample amounts required
- Less incubator space required

# One Ames Test analysis consists of several single tests!

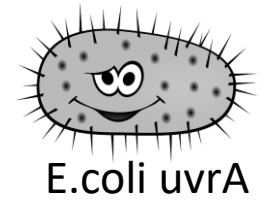
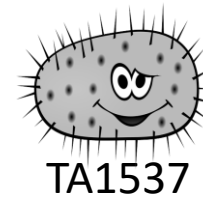
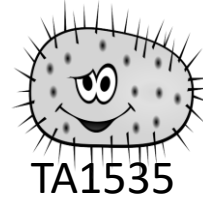
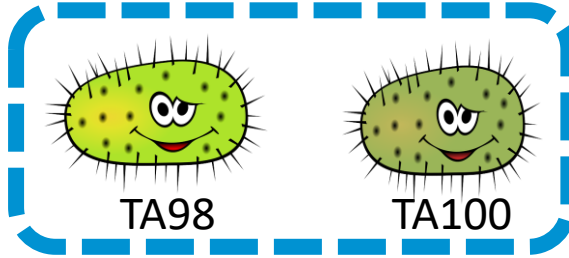
**Five different  
bacteria strains**



**Different Bacteria Strains detect different types of mutation!**

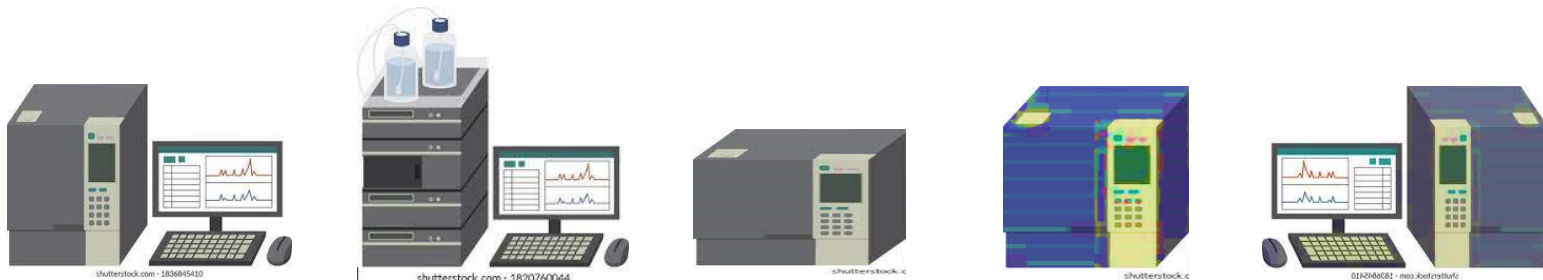
# One Ames Test analysis consists of several single tests!

**Five different  
bacteria strains**



- **Two of the five strains cover 93% (3822/4101) of a big set of mutagenic substances (Williams 2019).**
- **No additional positive results found with 5 strains when screening 88 samples with all five instead of just these two strains.**

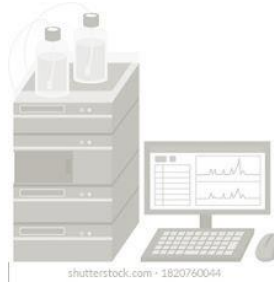
# Analogy: Selection of Chemical Analysis Methods for NIAS



**Different Analytical Non-Target Methods detect different types NIAS**  
(e.g. HS-GC-MS, GC-MS, HPLC-HRMS, HPLC-UV/VIS, HPLC-ELSD, GC-FID, ICP-MS, SPME-GC-MS, 2D-GC-MS, HPTLC,...)



# Analogy: Selection of Chemical Analysis Methods

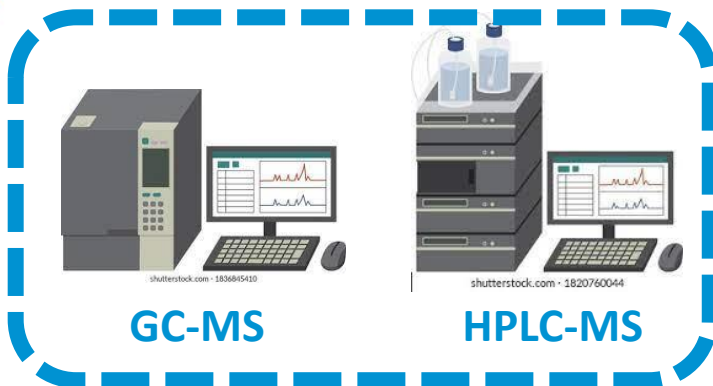


## NIAS-Screening:

Typically just based on a single analysis technique/method  
(GC-MS 10ppb Screening)

How many % does this cover? No reliable data, but << 93%!

# Analogy: Selection of Chemical Analysis Methods



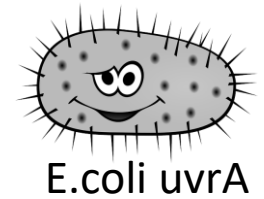
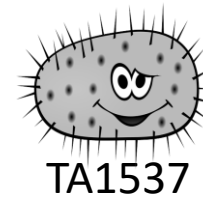
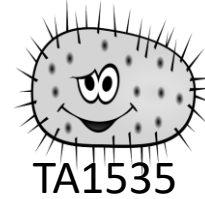
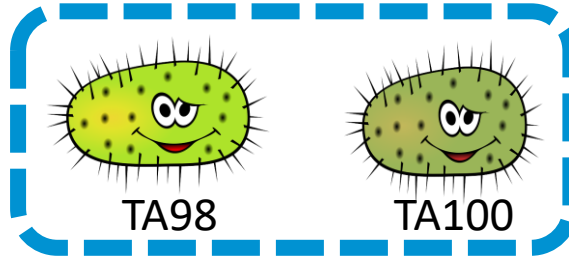
## Extensive NIAS-Screening:

The most extensive NIAS screenings are based on a combination of two methods: GC-MS & HPLC-MS

Further detectors/methods are very rarely applied!

# One Ames Test analysis consists of several single tests!

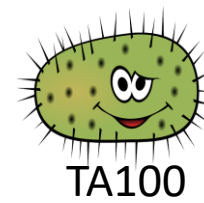
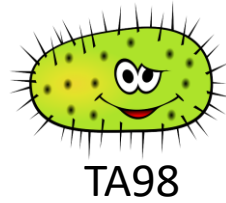
**Five different  
bacteria strains**



- **Two of the five strains cover 93%** (3822/4101) of a big set of mutagenic substances (Williams 2019).
  - No additional positive results found when screening 88 samples with all five instead of just these two strains.
- ➔ **For Sample Screening: pragmatic focus on the two strains: TA98 & TA100!**

# One Ames Test analysis still consists of several single tests!

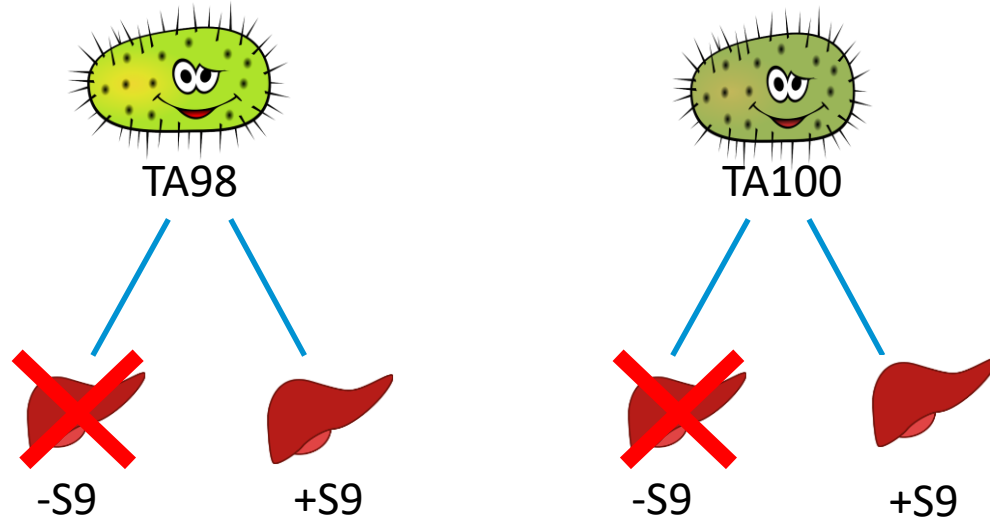
**Two different  
bacteria strains**



# One Ames Test analysis still consists of several single tests!

**Two different  
bacteria strains**

**For each Strain:  
with & without S9  
Rat-Liver Enzymes**

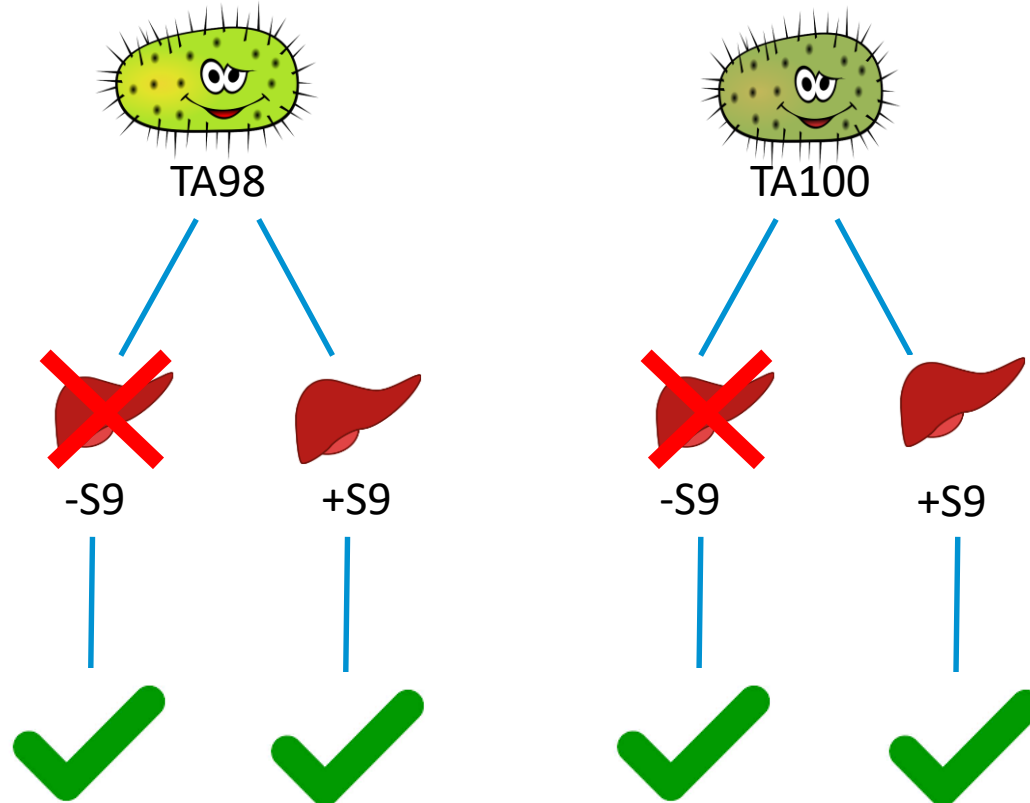


# One Ames Test analysis still consists of several single tests!

**Two different  
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**For each Strain:  
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**Four independent  
Results**

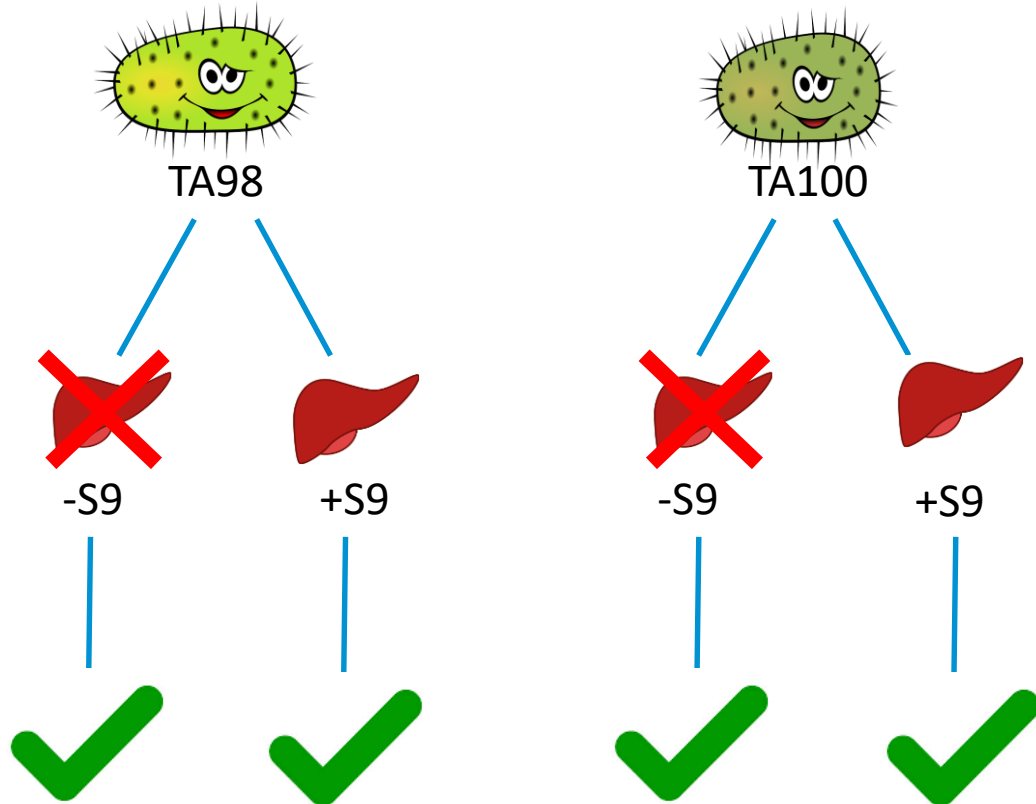


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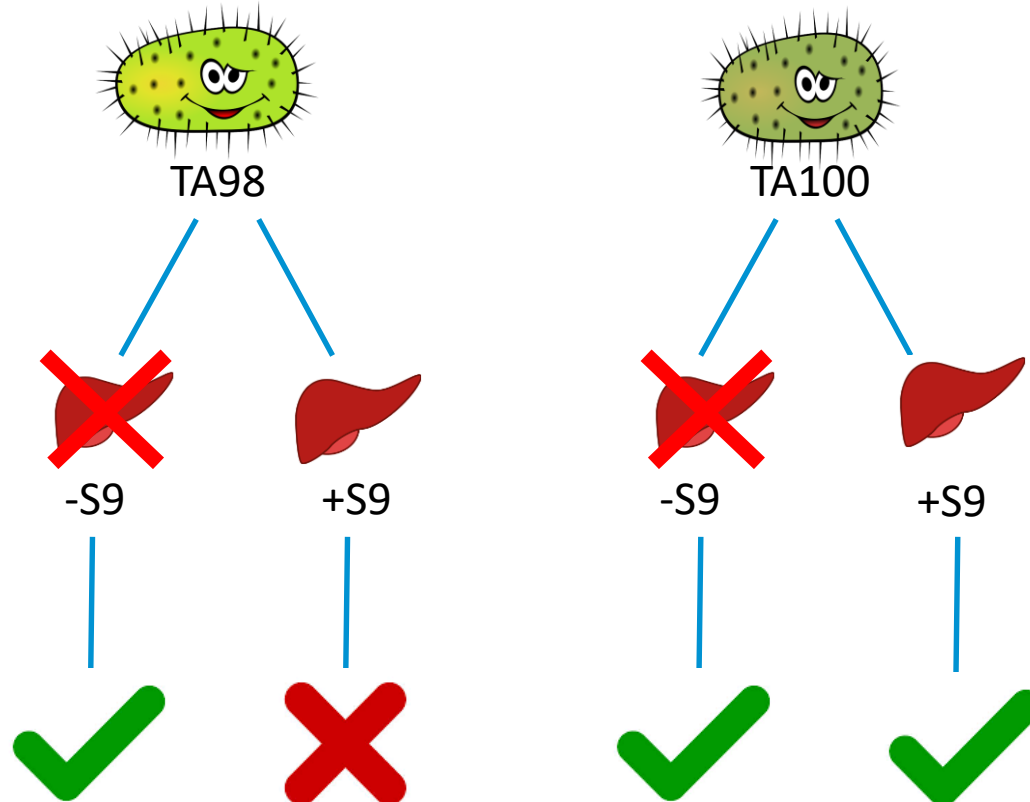


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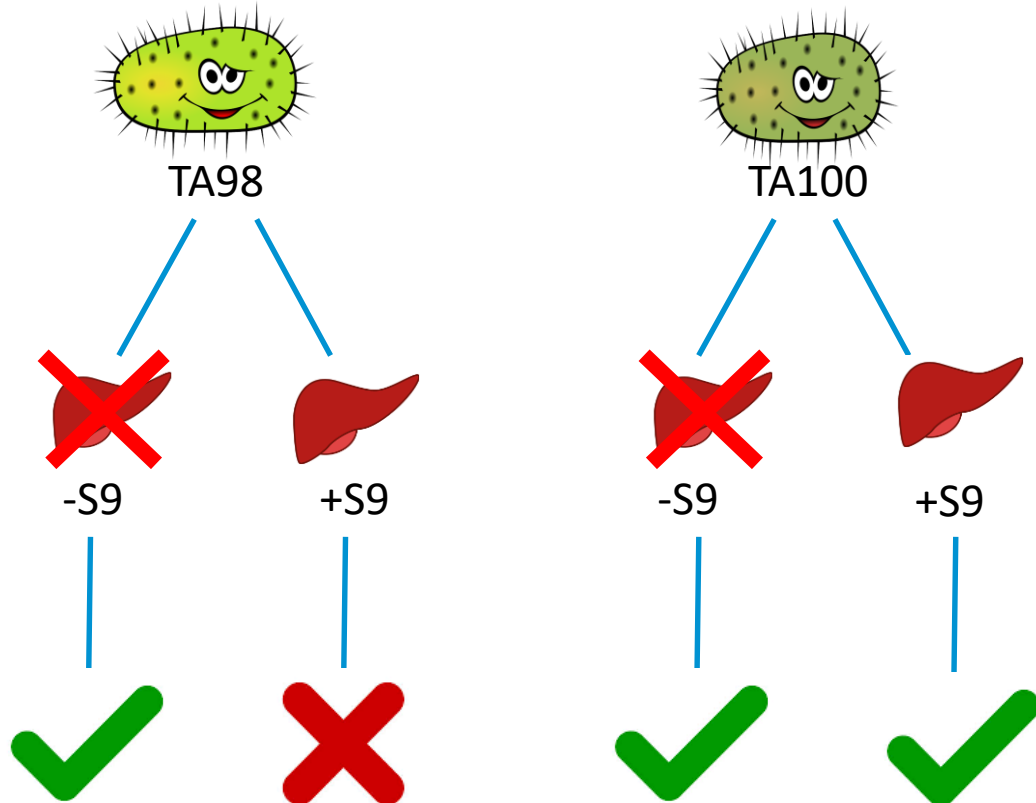


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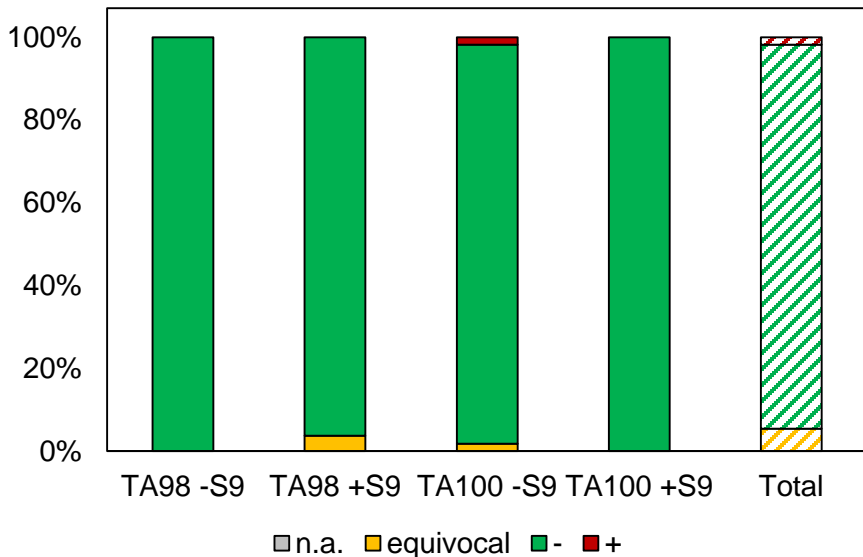
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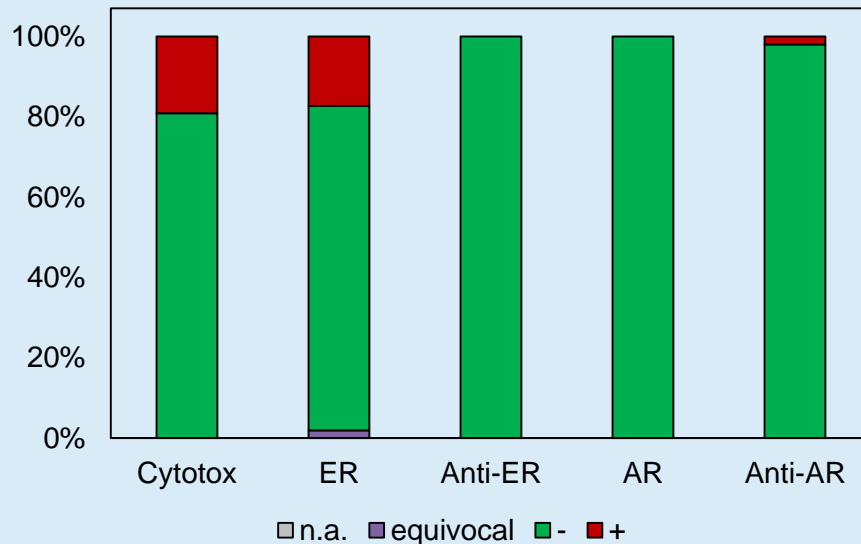


# Virgin Polyolefines (LDPE, HDPE, PP)

## Mutagenicity



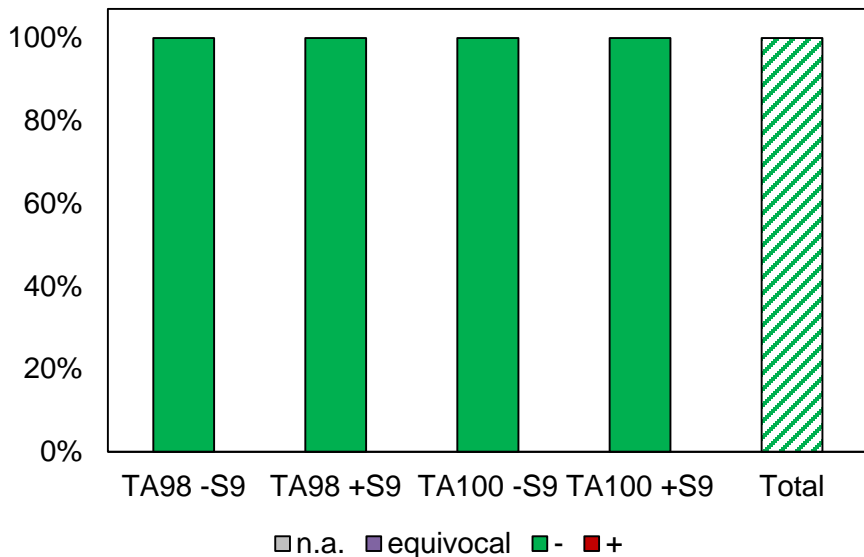
## Cytotoxicity & Endocrine Activity



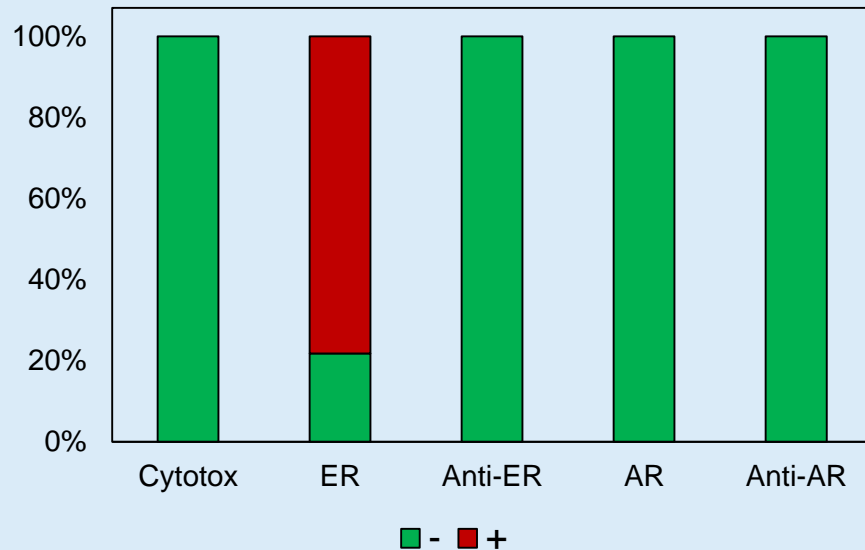
Most virgin Polyolefins do not show any Bioassay Activity  
Some Antioxidants (e.g. Doverphos 9228) can lead to estrogen activity

# Virgin Polystyrene (PS & HIPS)

## Mutagenicity



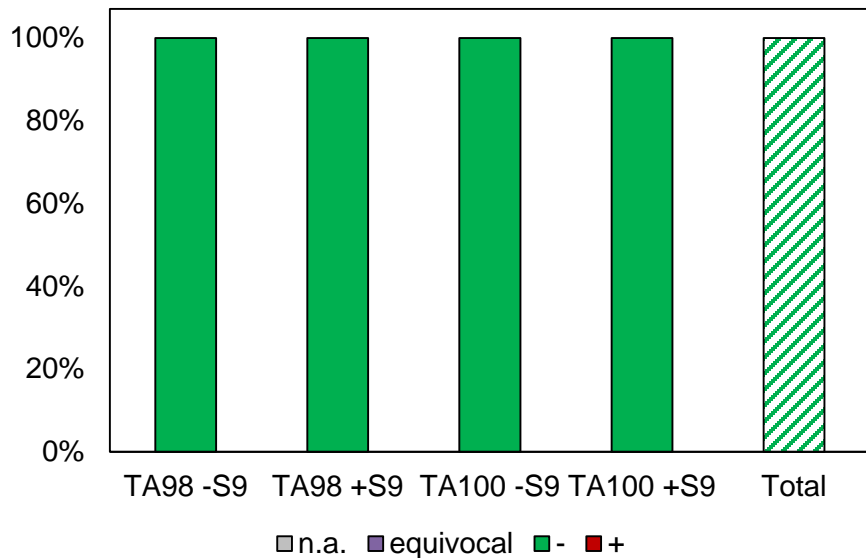
## Cytotoxicity & Endocrine Activity



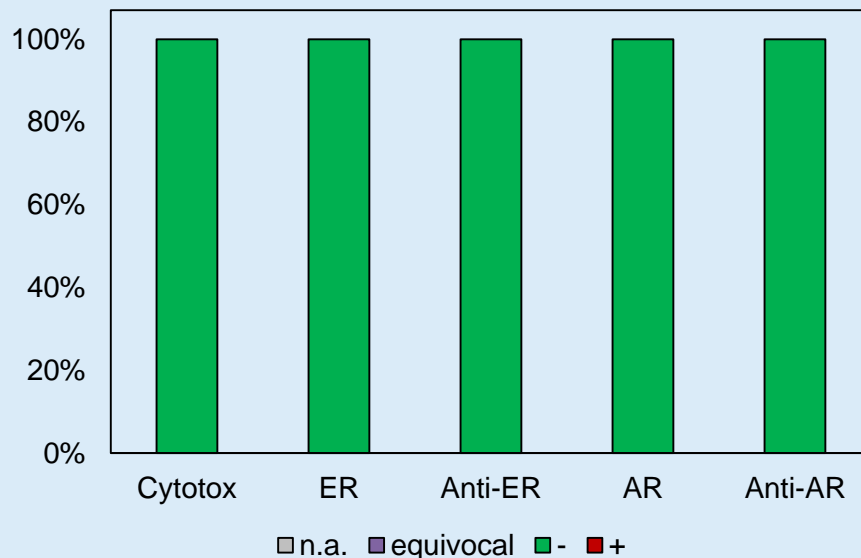
Most Polystyrene sample show estrogen activity when harsh migration conditions (60°C) are applied! (probably caused by oligomers)

# Virgin PET

## Mutagenicity



## Cytotoxicity & Endocrine Activity



In Contrast to Warnings in Newspapers and some Scientific Studies:  
No Bioassay Activity in Virgin PET!

## Mutagenicity



What's in YOUR blood?



ORIGINAL MUSIC BY THE ORB

## Unser tägliches Östrogen

...en, jetzt Hormone - die Qualität von Mineralwasser kann mehr appetitlich sein. Wasser aus PET-Flaschen ist häufig mit dem hormon Östrogen belastet.

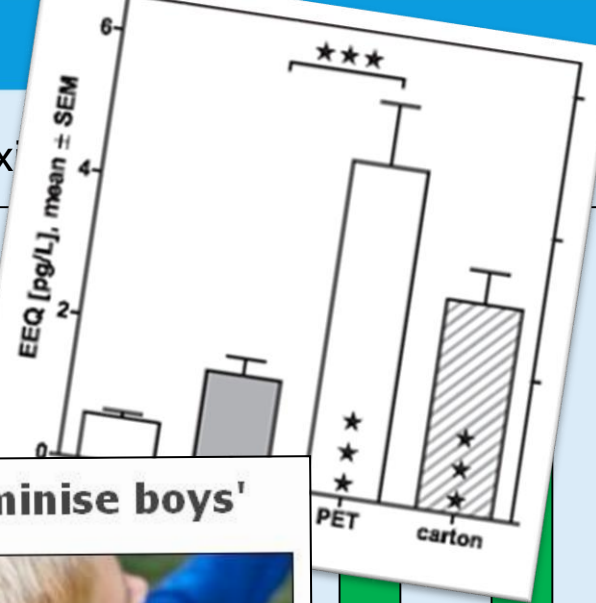
Mineralwasser in Flaschen ist nach einer Studie der Universität Frankfurt am Main häufig mit Umwelthormonen belastet. Wie die Studenten Jörg Oehlman und Martin Wagner

**The Guardian**  
Are we poisoning our children with plastic?



## Plastic chemicals 'feminise boys'

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

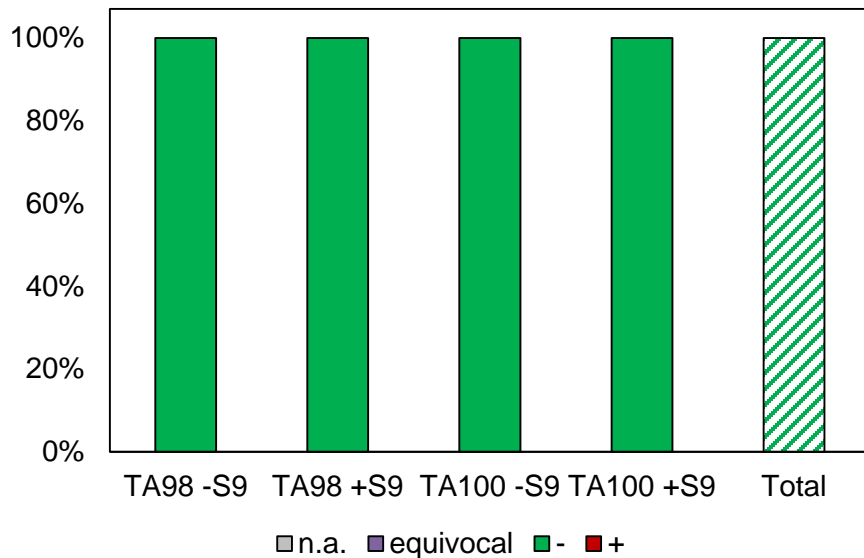


## Hormone in Mineralwasser aus PET-Flaschen

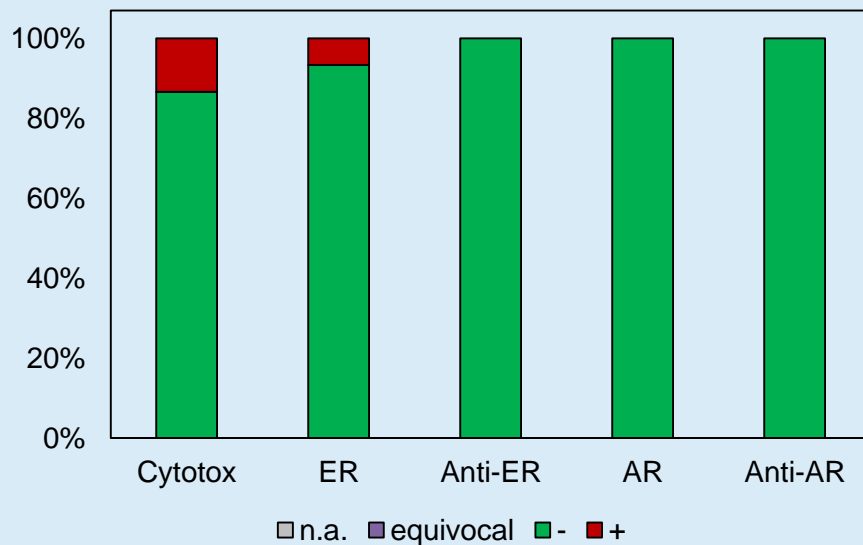


# Recycled PET

## Mutagenicity



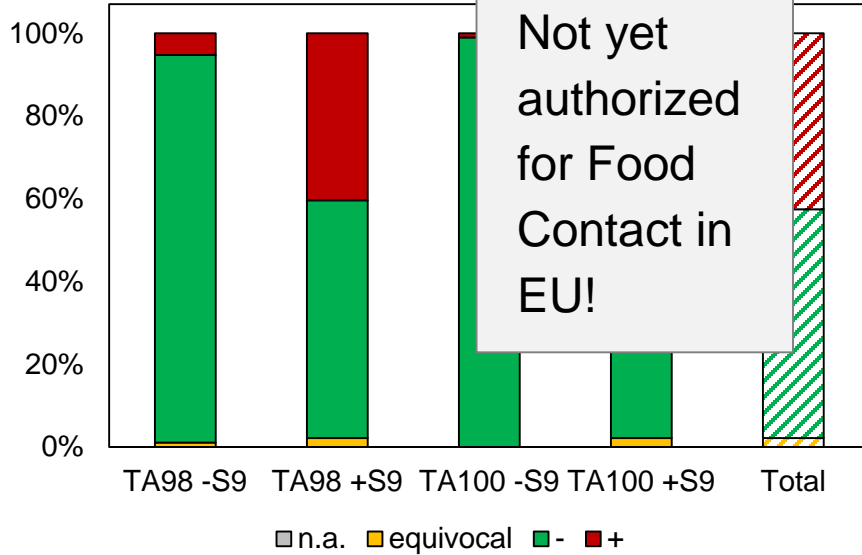
## Cytotoxicity & Endocrine Activity



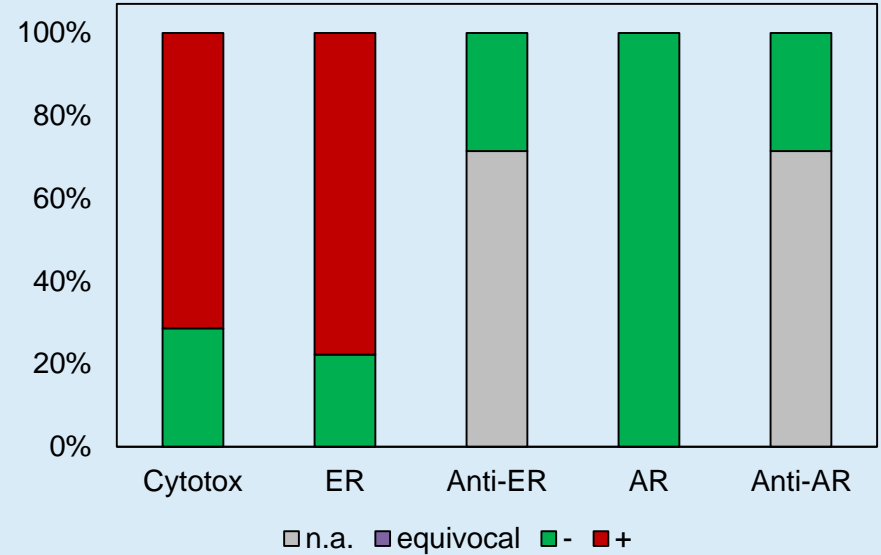
Bioassay activity also very rare for recycled PET,  
only one 100% recycled PET sample showed minor estrogen activity

# Recycled Polyolefins (rHDPE, rLDPE, rPP)

## Mutagenicity

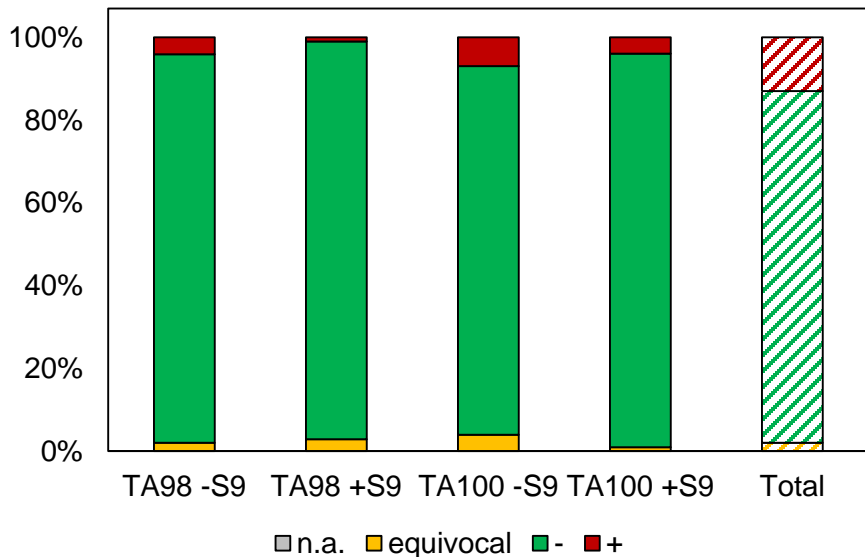


## Cytotoxicity & Endocrine Activity

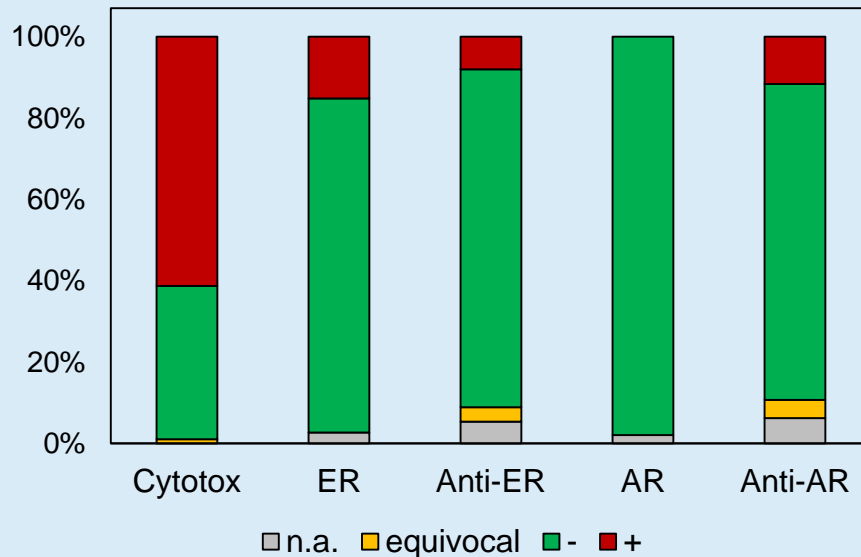


Potential mutagenicity in many recycled polyolefins: challenge for FCM applications!  
Very strong Ames-Test Activity in Teststrain TA98 with metabolic activation!

## Mutagenicity



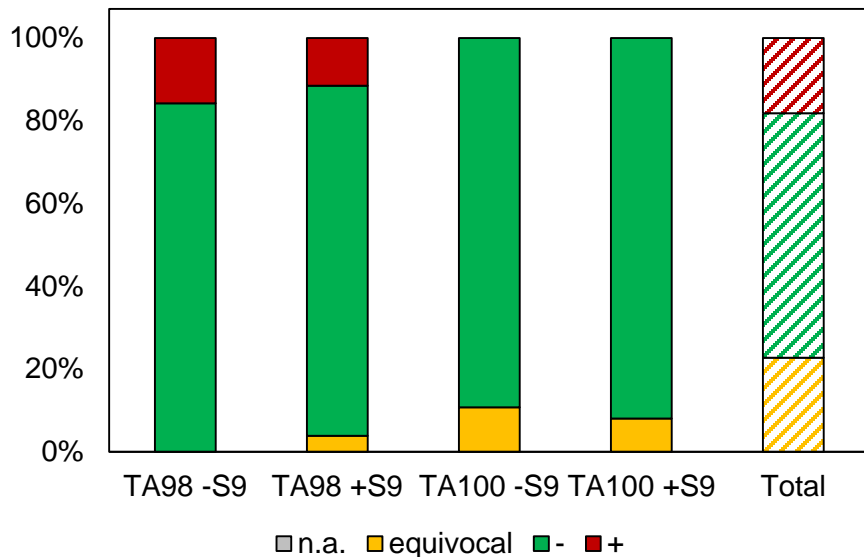
## Cytotoxicity & Endocrine Activity



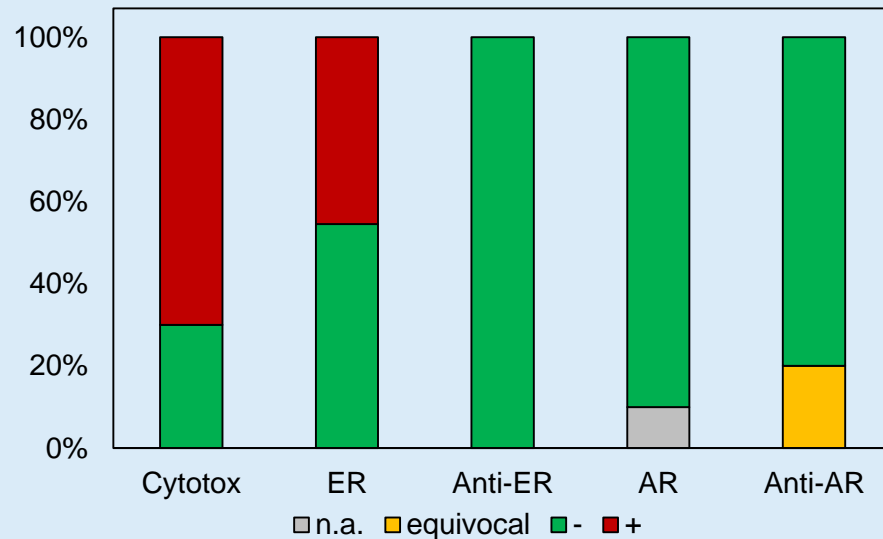
Many coatings are cytotoxic and also toxic to bacteria (→ inhibition of bioassay)  
Epoxy monomer can lead to mutagenic effect (especially in tests without metabolism)



## Mutagenicity

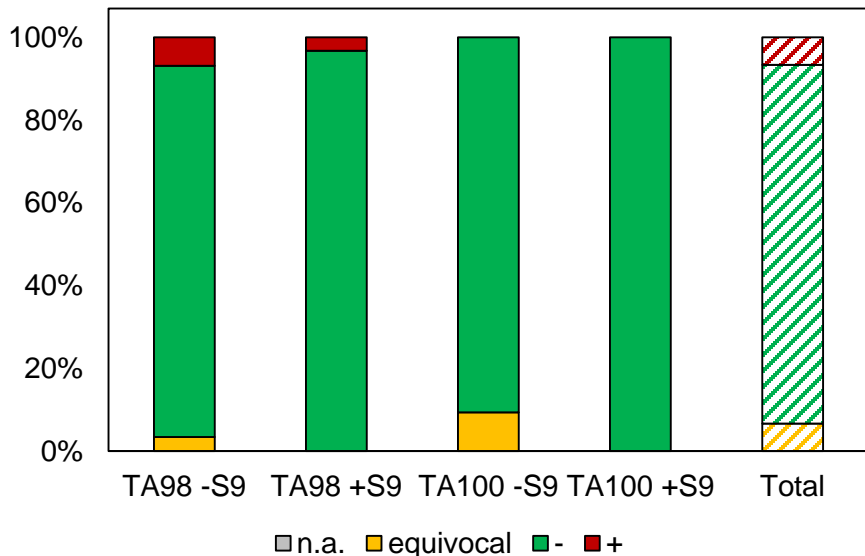


## Cytotoxicity & Endocrine Activity

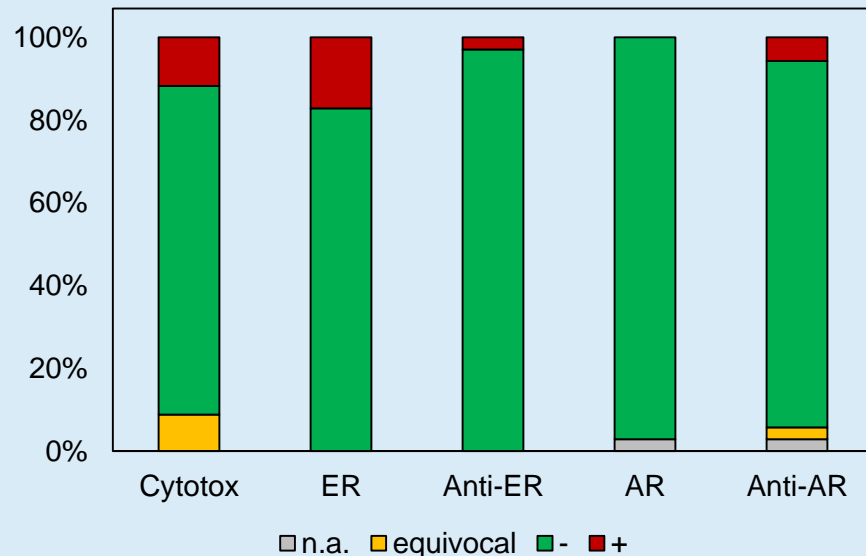


Results from extraction with ethanol → no realistic migration tests possible!  
Paper & Board often cytotoxic and also toxic to bacteria (→ inhibition of bioassay)

## Mutagenicity



## Cytotoxicity & Endocrine Activity



Product facing material mostly Polyethylene. Mutagenic effects could be related to set-off effects or migration through thin, diffusive PE layer.

## Summary: Conclusions from testing >400 samples

### Ames-Test: Results in bacteria-based assay for DNA-reactive genotoxicity

- Mutagenic activity very rare in virgin plastics and food grade recycled PET
- Ames Test uncovers DNA-reactive contaminants in recycled Polyolefins
- Coatings: DNA-reactivity detected at conditions that are sensitive for epoxides

### Cell Culture Tests: Results for Cytotoxicity and Endocrine Activity

- Approx. 20% of Samples estrogen active, but activity in general lower than in Food
- Alarming Studies on Estrogen Activity of PET cannot be confirmed
- Cytotoxicity regularly detected in Coatings, Paper&Board and recycled Plastic
  - But: Cytotoxicity not associated with effects at low concentrations!



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