

ENDOCRINE ACTIVE SUBSTANCES IN WASTEWATERS

COMBINATION EFFECTS

10. BioDetectors Conference
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H. Bielak A. Simon E. Dopp

EAS in the environment

Endocrine active substances (EAS):

Interaction with the biochemical mode of action of hormones.



WWTP effluents



EAS $\mu\text{g/L}$



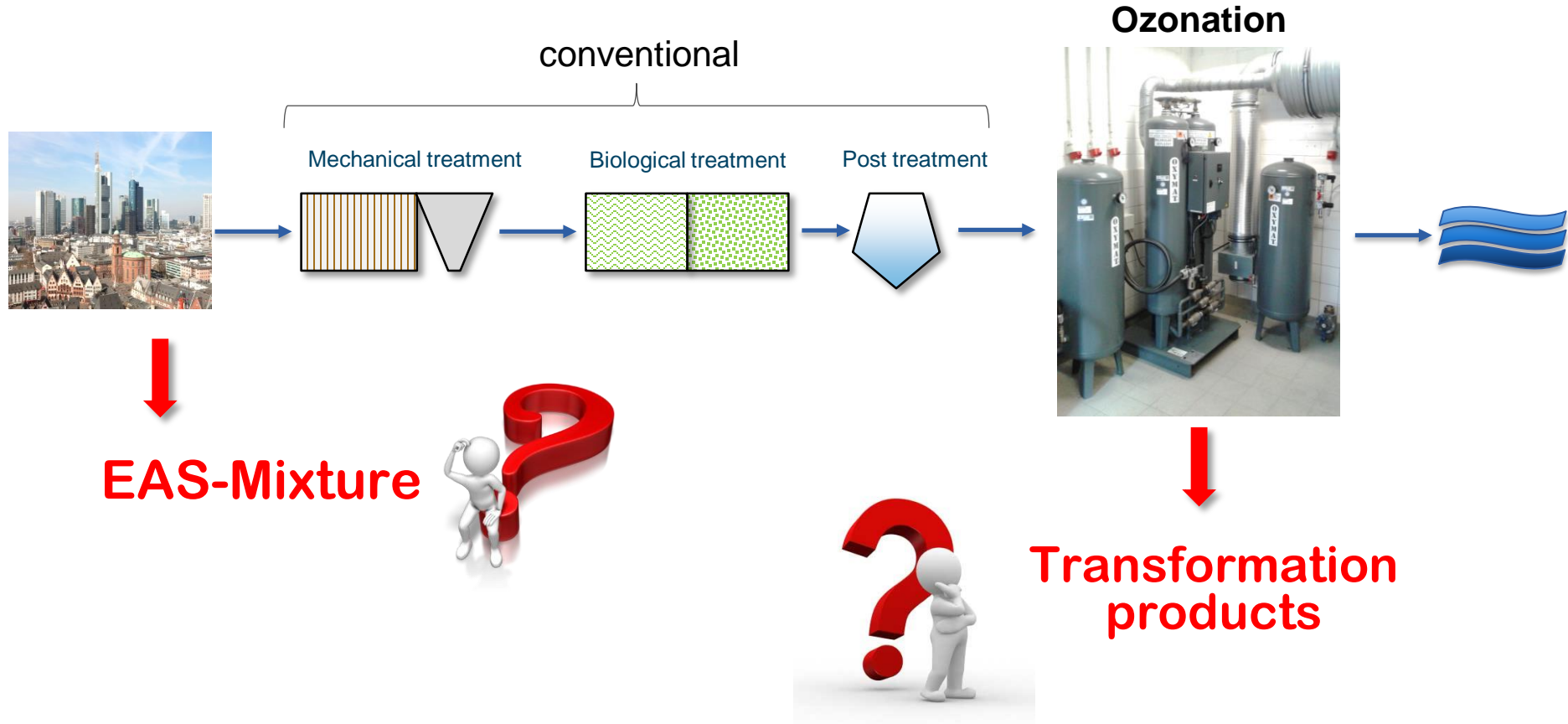
Adverse effects:

e.g. feminization or masculinization of wildlife (snails, reptiles, fish, amphibians), reproduction disorder of birds

- ▶ Pharmaceuticals:
e.g. cancer therapy,
biological activity ng/L

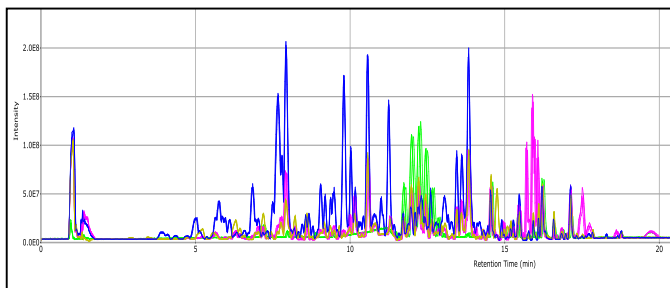
Wastewater treatment

Reduction of micropollutants by oxidative treatment



Chemical Analyses

- ▶ Substance identification
- ▶ Mostly target substances



What is in the water?

- **Relevance of combination effects for the assessment of complex water samples**

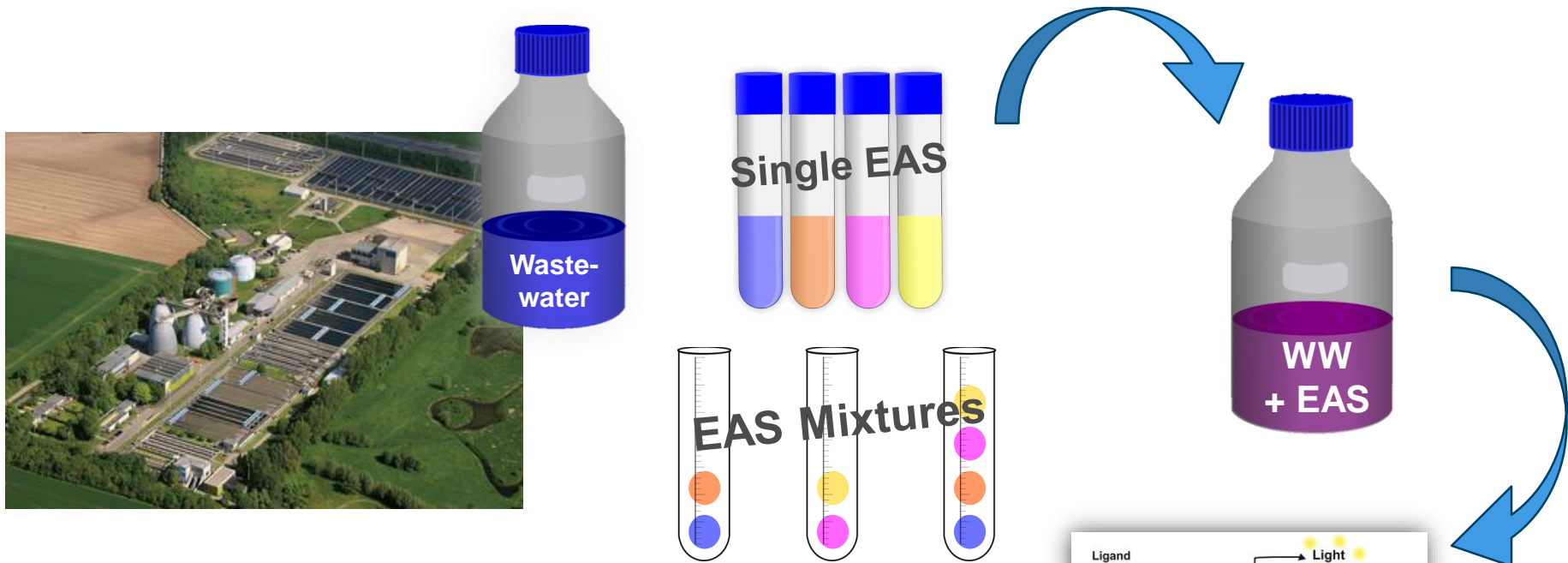
Bioassays

- ▶ Effect based
- ▶ Combination effects
- ▶ Toxicity of total sample



How does it act?

Methodical approach

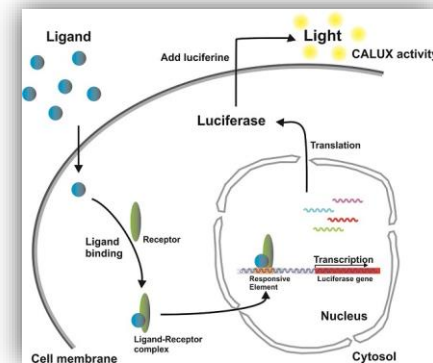


Wastewater matrix:

- municipal
- hospital

Test substances – pharmaceuticals:

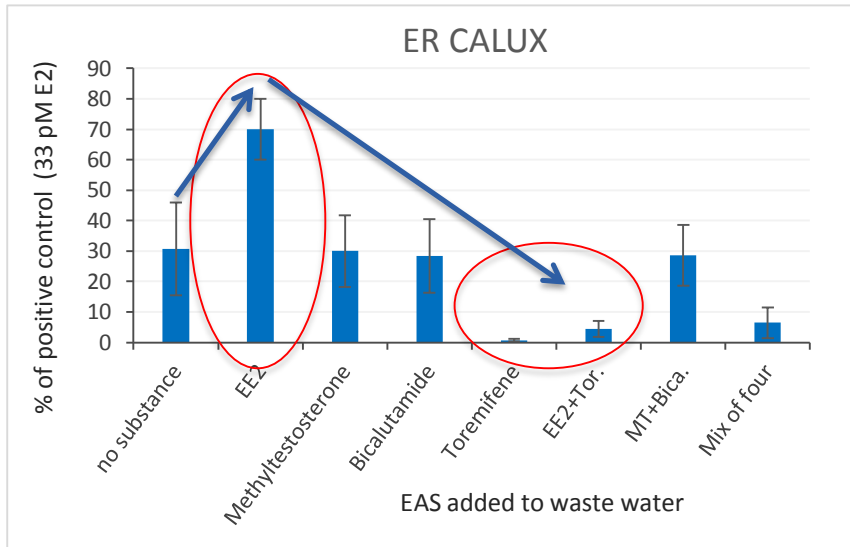
- 17 α -Ethinylestradiol (estrogen)
 - Toremifene (anti-estrogen)
 - Methyltestosteron (androgen)
 - Bicalutamid (anti-androgen)
- EC50 concentrations



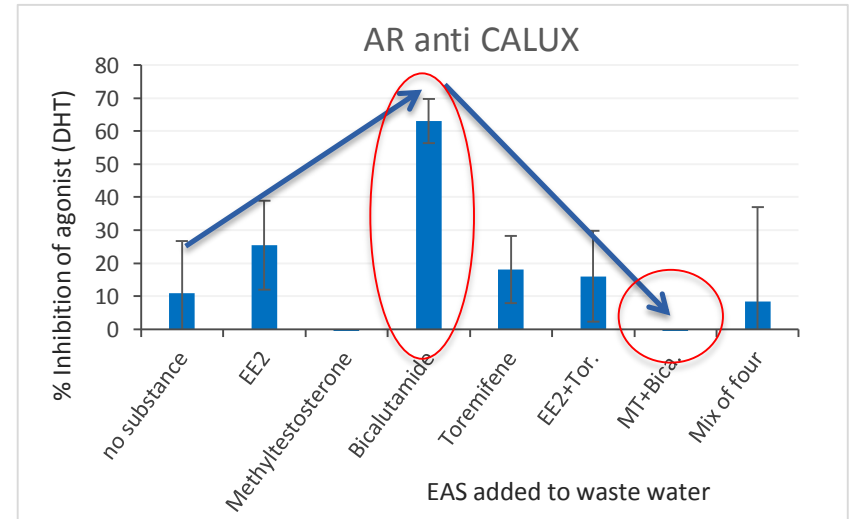
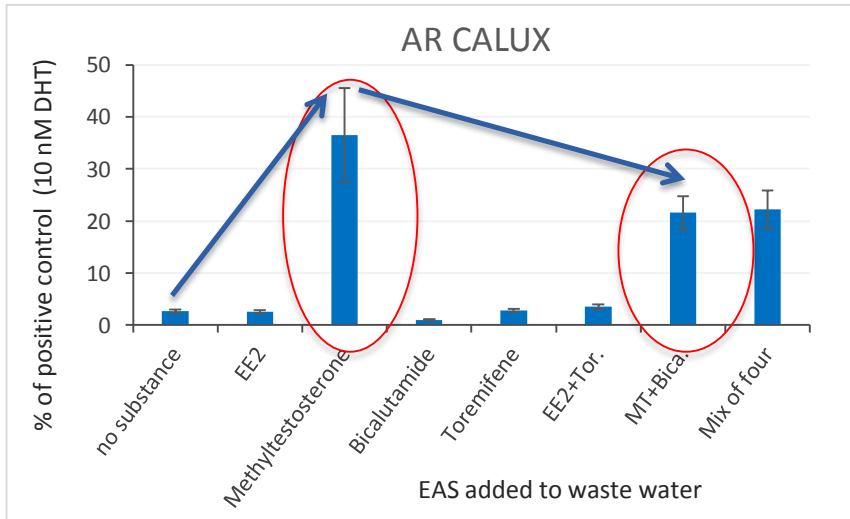
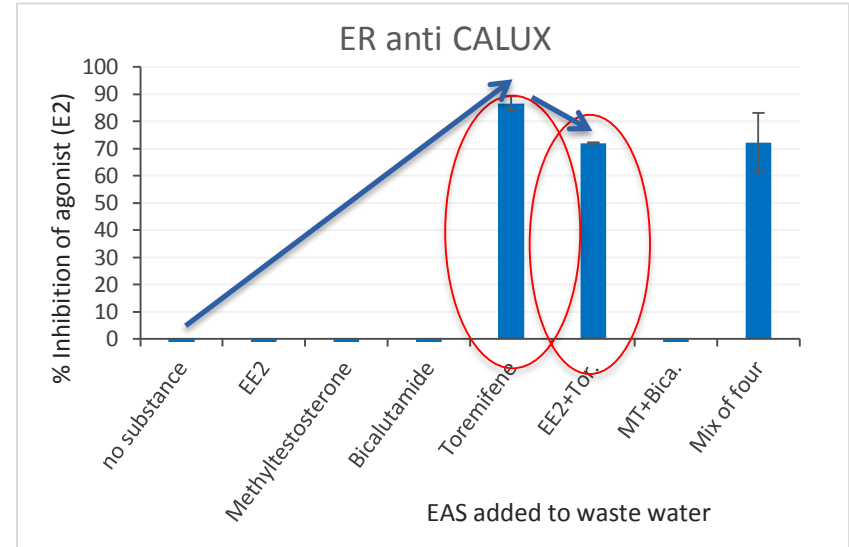
AR (anti) Calux
ER (anti) Calux

Results

Activation



Inhibition



Single substances vs. mixture:

- intensity of effects reduced in mixtures
- different magnitudes of reduction
 - anti-estrogenic cancels estrogenic, androgenic cancels anti-androgenic

Municipal vs. hospital wastewater:

- all wastewaters estrogenic, 3 of 4 anti-androgenic
- Similar trends, but intensities of effects different



Conclusions

- Significant importance of **masking effects** in the assessment of complex water samples
- Detection of a **specific EAS** in a substance mixture does not necessarily allow conclusions about the **final biological effect**
- Quantified changes on a specific endocrine activity are not transferable **from one wastewater to another**
- **Combination** of chemical analyses and effect-based tools is recommended



www.iww-online.de
info@iww-online.de



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Thank you for your attention



Helena Bielak, MSc

h.bielak@iww-online.de

Phone: +49 (0) 208 4 03 03-224

An-Institut der

UNIVERSITÄT
DUISBURG
ESSEN

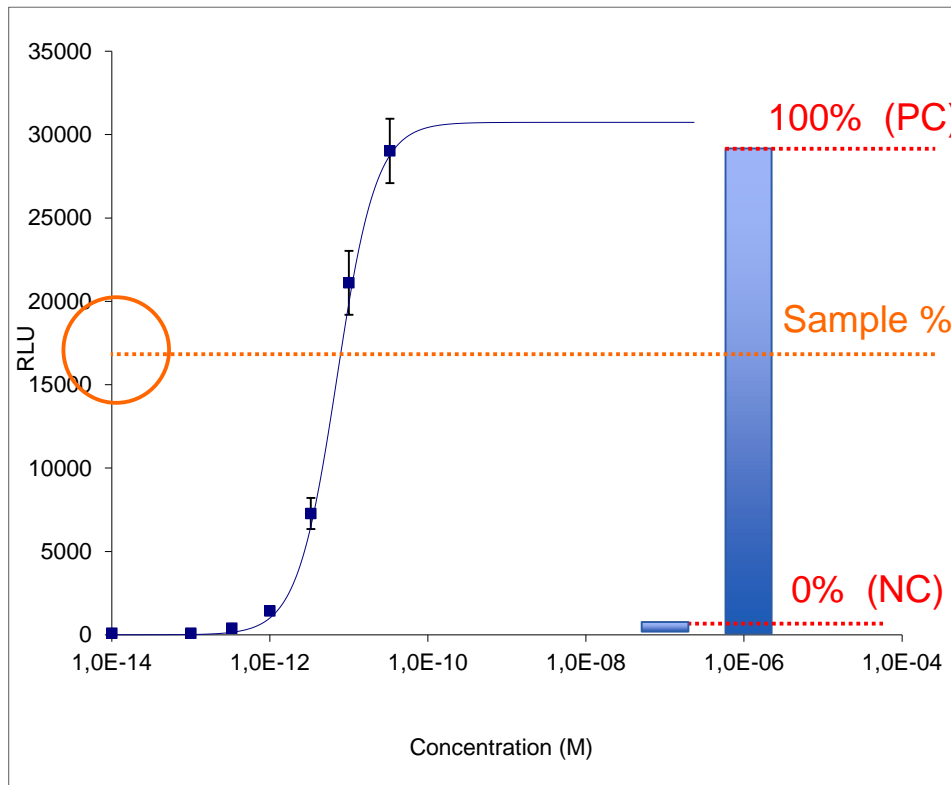
Offen im Denken



Substance addition (final exposure concentration)

Substance	Concentration added	
EE2	1,8 ng/L	6×10^{-12} M
Methyltestosterone	390 ng/L	1×10^{-09} M
Bicalutamide	40 μ g/L	9×10^{-08} M
Toremifene	50 μ g/L	1×10^{-07} M

Dose-response-curve



RLU = measured light units

Determine sample activity in relation to a positive control (activation) or negative control (inhibition)

NC: no reference substance
PC: high effect concentration of reference

ISO/CD 19040-3 (draft, 2016)
OECD Test No. 455 (2016)