IWW

Water Centre

Consulting – Research – Training

Detection of estrogens in waste water treatment plant effluents

7th BioDetectors Conference

Istanbul (08. November 2013)

Dr. Jessica Richard, Prof. Dr. Elke Dopp

Endocrine disruption

- Chemicals of natural and synthetic origin found in surface waters might exhibit endocrine disruptive functions → structural similarity to hormones

- US EPA: Endocrine disruption is a mode of action which potentially might lead to adverse effects

- Hormonal effects at very low concentrations → test systems which are able to detect those effects

Research Project

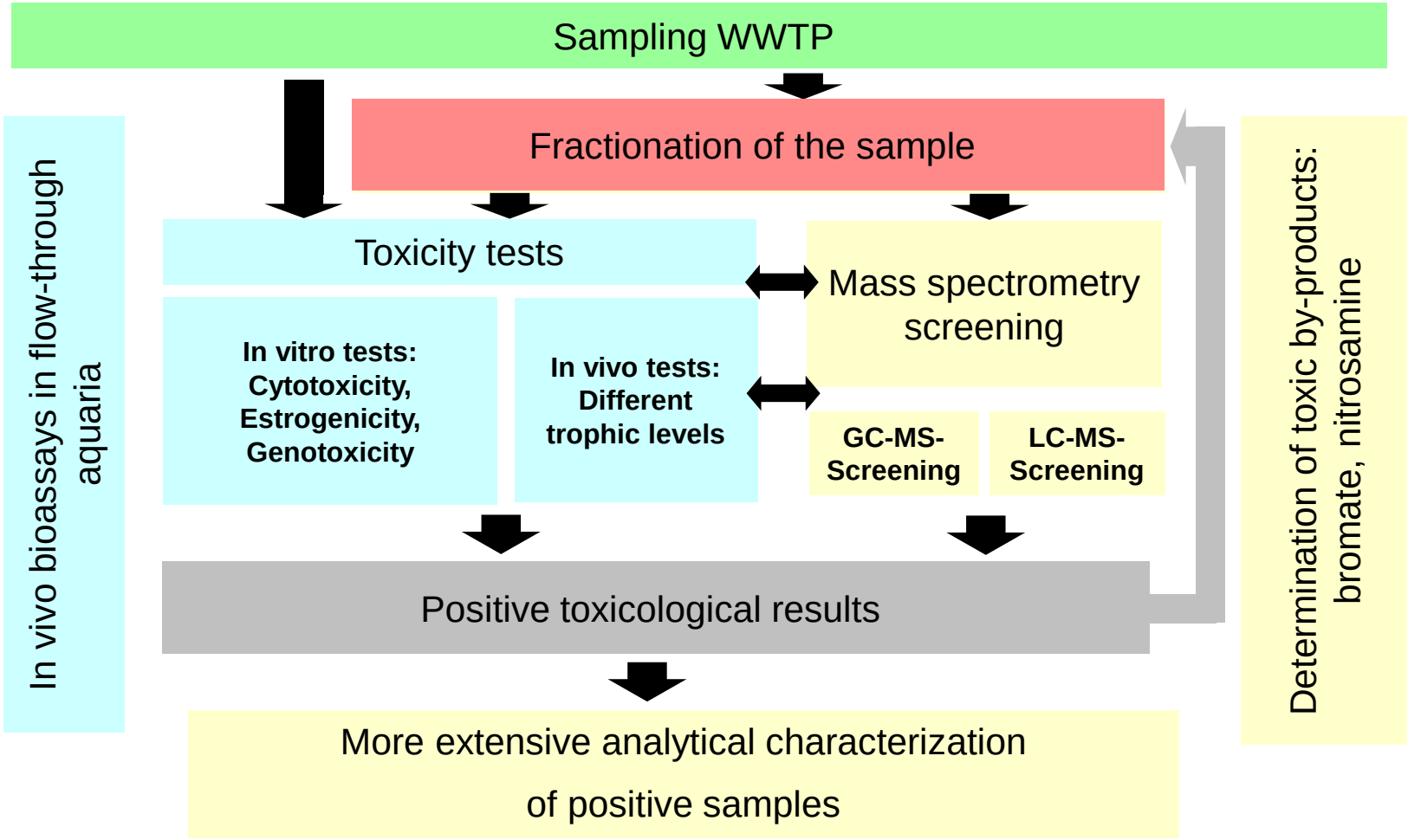
Study of metabolite formation during the use of ozone in municipal waste water treatment plants

Project management : IWW, Mülheim an der Ruhr

On behalf of the:

Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the German State of North Rhine-Westphalia (MKULNV) for the financial support of the project.

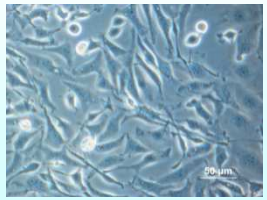
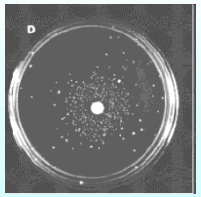
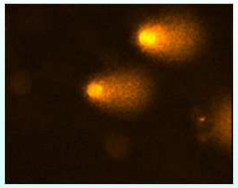
Toxicity based identification scheme



Combination of various bioanalytical tests

In vitro test systems

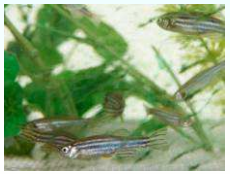
- Cytotoxicity
- Estrogenicity
- Genotoxicity
- Mutagenicity

In vivo test systems

- Growth inhibition
- Mortality
- Embryotoxicity



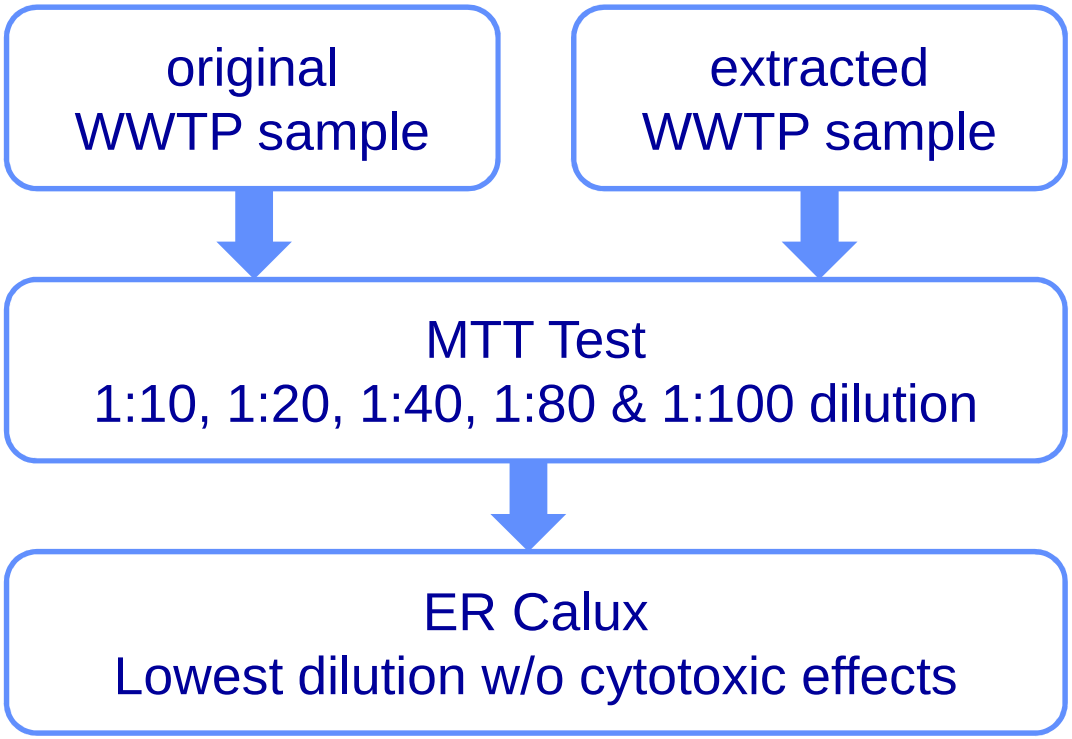
Mass spectrometric detection and characterization

- LC-MS
- GC-MS
- LC-(HR)MS^n

- structural characterization
- detection method

Methods

- **T47D cells; exposure for 24 h**
- **Cytotoxicity (MTT Test) & Estrogenicity (ER Calux)**
- **WWTP samples: 3 municipal WWTPs, different O_3 conc.**



Investigated municipal WWTP

WWTP Bad Sassendorf (Lippeverband)

- 12,000 PE.
- Post treatment dosing of ozone to the effluent of conventional biological treatment. Polishing pond.

WWTP Schwerte (Ruhrverband)

- 50,000 PE.
- Consists of two separated lines. Ozone and/or powdered activated carbon are applied. Recirculation process can be operated.

WWTP Duisburg-Vierlinden (Wirtschaftsbetriebe Duisburg AöR)

- 30,000 PE.
- Two parallel lines have been installed to compare ozone dosage by diffusor or by injector. The wastewater outline is fed to an additional biological stage (fluidised bed reactor).

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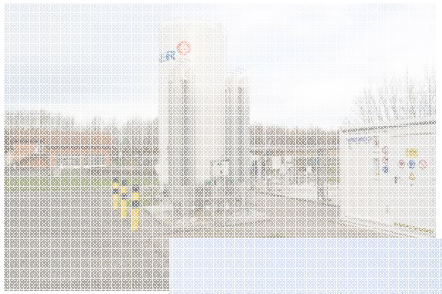
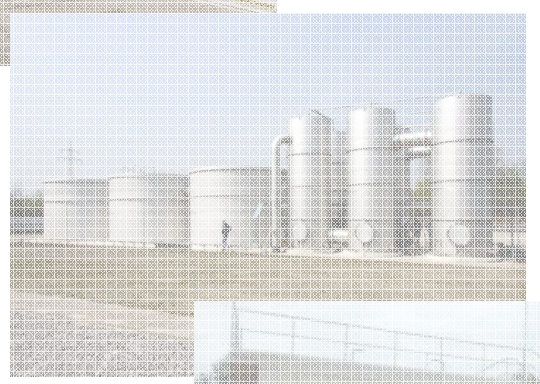
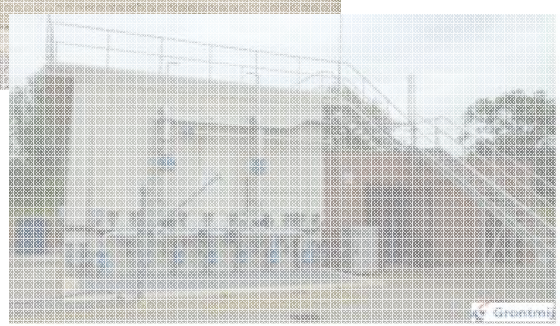
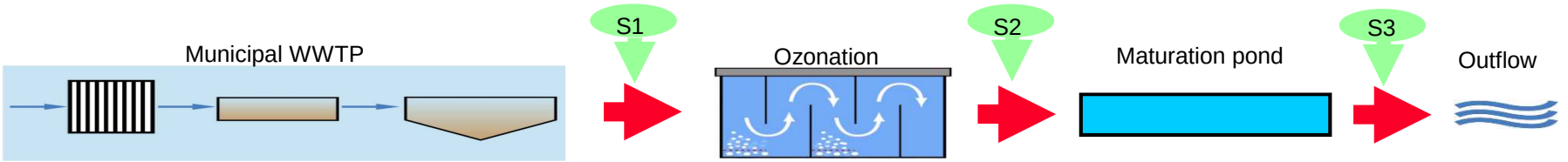


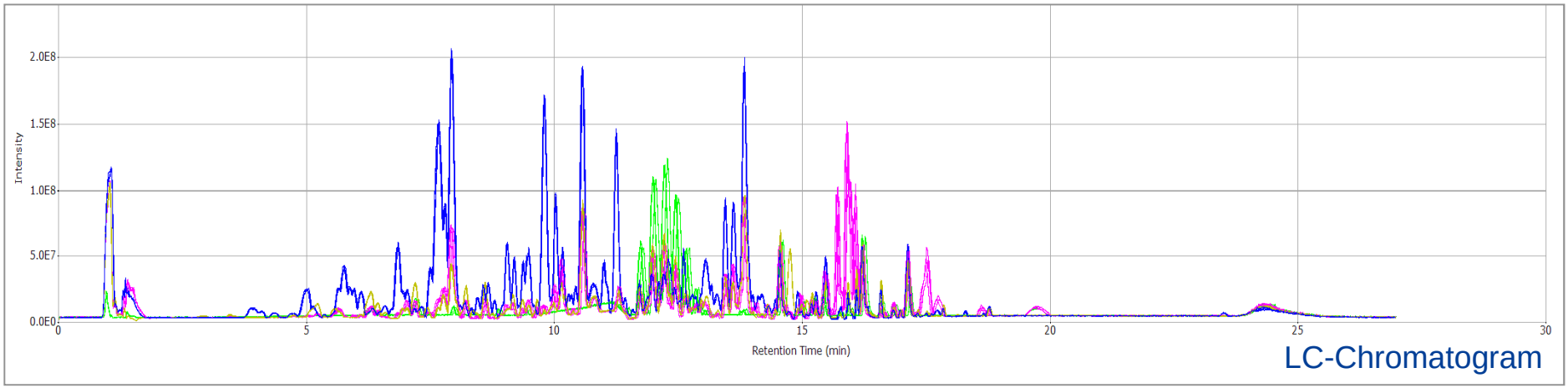






GC- and LC-MS Screening

WWTP Bad Sassendorf
5 mg/L Ozone



LC-Chromatogram

Control sample*
Before Ozonation
After Ozonation
After Maturation pond

* Internal Standard: ~ 120 substances

Estrogenicity WWTP Bad Sassendorf

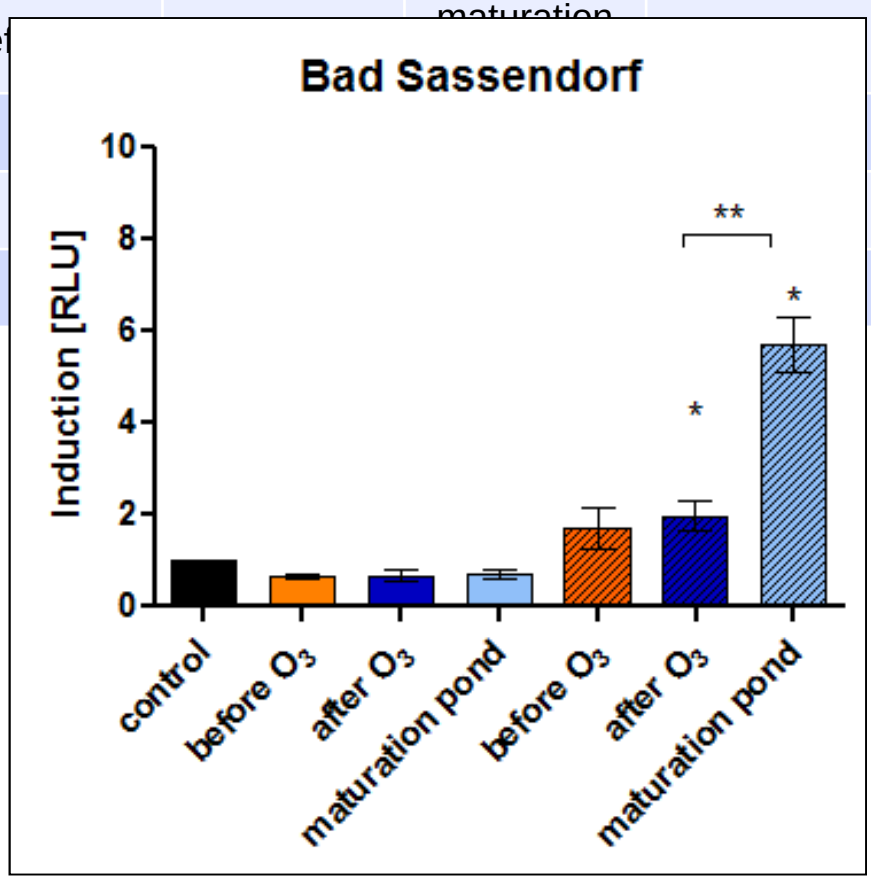
Date of sampling	O_3 z-spec.	Sample					
		original			*extract*		
		before O_3	after O_3	maturation pond	before O_3	after O_3	maturation pond
02.08.2013	0.7	n.d.	n.d.	n.d.	0.5 ng/L	1.1 ng/L	cytotoxic
16.08.2013	0.7	n.d.	n.d.	n.d.	0.3 ng/L	n.d.	9.9 ng/L
30.08.2013	0.9	n.d.	n.d.	n.d.	cytotoxic	1.2 ng/L	5.8 ng/L

n.d. = not detected

* statistically significant compared to neg. control
** statistically significant compared to previous treatment step

Estrogenicity WWTP Bad Sassendorf

Date of sampling	O_3 z-spec.	Sample					
		original			*extract*		
		bef		maturation		after O_3	maturation pond
02.08.2013	0.7					1.1 ng/L	cytotoxic
16.08.2013	0.7					n.d.	9.9 ng/L
30.08.2013	0.9					1.2 ng/L	5.8 ng/L



n.d. = not detected

* statistically significant compared to neg. control
** statistically significant compared to previous treatment step

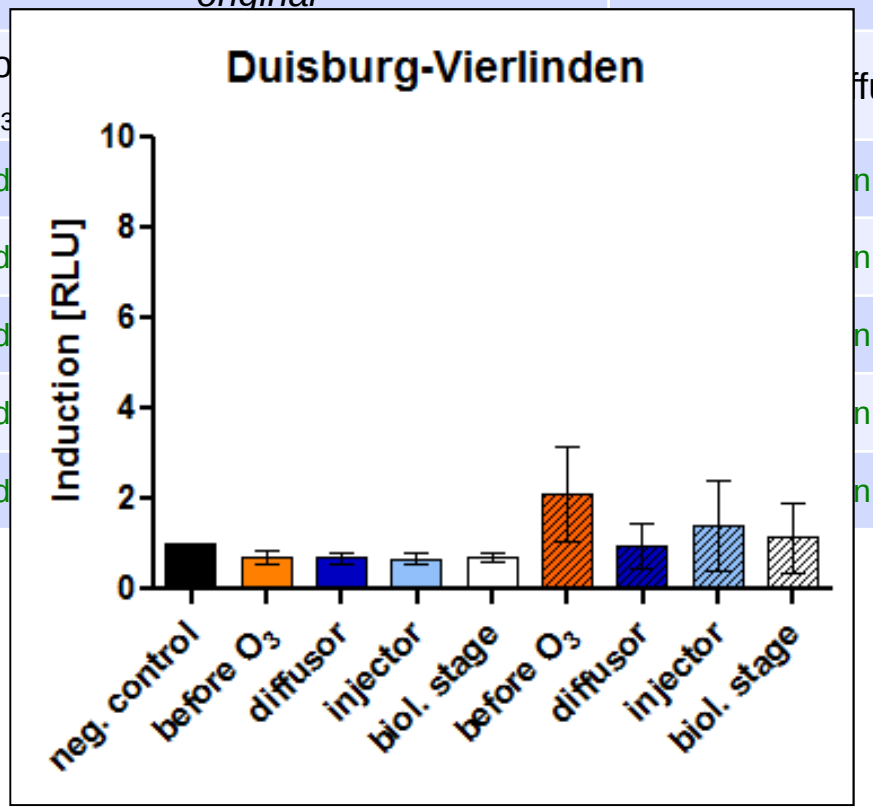
Estrogenitcity WWTP Duisburg-Vierlinden

Date of sampling	O_3 z-spec.	Sample							
		original				*extract*			
		before O_3	diffusor	injector	biol. stage	before O_3	diffusor	injector	biol. stage
13.09.2012	0.5	n.d.	n.d.	n.d.	n.d.	1.3 ng/L	n.d.	-	n.d.
20.09.2012	0.5	n.d.	n.d.	n.d.	n.d.	0.5 ng/L	n.d.	0.7 ng/L	0.3 ng/L
25.10.2012	0.7	n.d.	n.d.	n.d.	n.d.	7.1 ng/L	n.d.	n.d.	0.3 ng/L
31.10.2012	0.7	n.d.	n.d.	n.d.	n.d.	34.3 ng/L	n.d.	n.d.	n.d.
16.11.2012	0.9	n.d.	n.d.	n.d.	n.d.	15.3 ng/L	n.d.	n.d.	n.d.

n.d. = not detected;
- = not tested

Estrogenitcity WWTP Duisburg-Vierlinden

Date of sampling	O_3 z-spec.	Sample					
		original			*extract*		
		befo O_3			ffusor	injector	biol. stage
13.09.2012	0.5	n.d			n.d.	-	n.d.
20.09.2012	0.5	n.d			n.d.	0.7 ng/L	0.3 ng/L
25.10.2012	0.7	n.d			n.d.	n.d.	0.3 ng/L
31.10.2012	0.7	n.d			n.d.	n.d.	n.d.
16.11.2012	0.9	n.d			n.d.	n.d.	n.d.



n.d. = not detected;
- = not tested

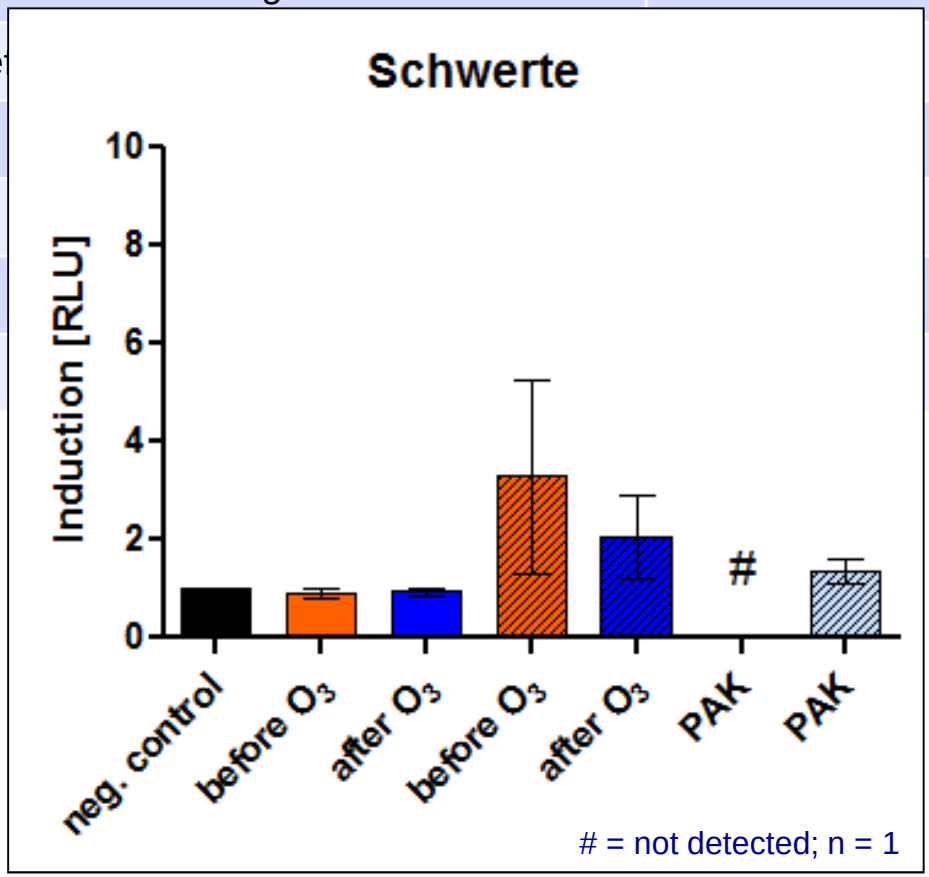
Estrogenicity WWTP Schwerte

Date of sampling	O_3 z-spec.	Sample					
		original			*extract*		
		before O_3	after O_3	PAK	before O_3	after O_3	PAK
29.11.2012	0.9	n.d.	n.d.	-	n.d.	1.5 ng/L	-
07.12.2012	0.9	n.d.	n.d.	-	16.4 ng/L	3.0 ng/L	-
12.12.2012	0.5	n.d.	n.d.	-	23.4 ng/L	1.6 ng/L	-
12.03.2013	0.9	n.d.	n.d.	n.d.	19.8 ng/L	21.1 ng/L	1.4 ng/L

n.d. = not detected;
- = not tested

Estrogenicity WWTP Schwerte

Date of sampling	O_3 z-spec.	Sample			
		original		*extract*	
		bef		after O_3	PAK
29.11.2012	0.9			1.5 ng/L	-
07.12.2012	0.9			3.0 ng/L	-
12.12.2012	0.5			1.6 ng/L	-
12.03.2013	0.9			21.1 ng/L	1.4 ng/L



n.d. = not detected;
- = not tested

Conclusions

- **Estrogenicity only detected in extracts**
  - Bad Sassendorf → increase in estrogenicity after ozonation (e.g. through phytoestrogens, matrix effects)
  - Duisburg Vierlinden → varying results, partial loss of estrogenicity
  - Schwerte → slight decrease in estrogenicity, but not statistically significant
- **High variation of effluent composition**
  - General statement on estrogenicity for one WWTP not possible
- **Efficiency of ozonation is dependent on WWTP effluent composition**

Acknowledgements

Project partners:

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Thank you for your attention