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Aalto University

# Wastewater Treatment and Bioassays

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# Introduction





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## Estrogenic activity in Finnish municipal wastewater effluents



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## “Effect-based assessment of toxicity removal during wastewater treatment processes”

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# Background

## Aims of this study:



To determine the estrogenic potencies of effluents in eight municipal WWTPs in Finland

Use a combination of analytical approaches (LC-MS/MS) and bioassays (ER-CALUX, ELISA, *D.magna*)

Test if ELISA-E2 is suitable for analysis of wastewater effluents



To evaluate the contribution of the target compounds to the observed effects.

# Samples and extraction

- 8 WWTP effluents (24h composite samples)
- Solid-phase extraction (SPE) with Oasis HLB extraction discs

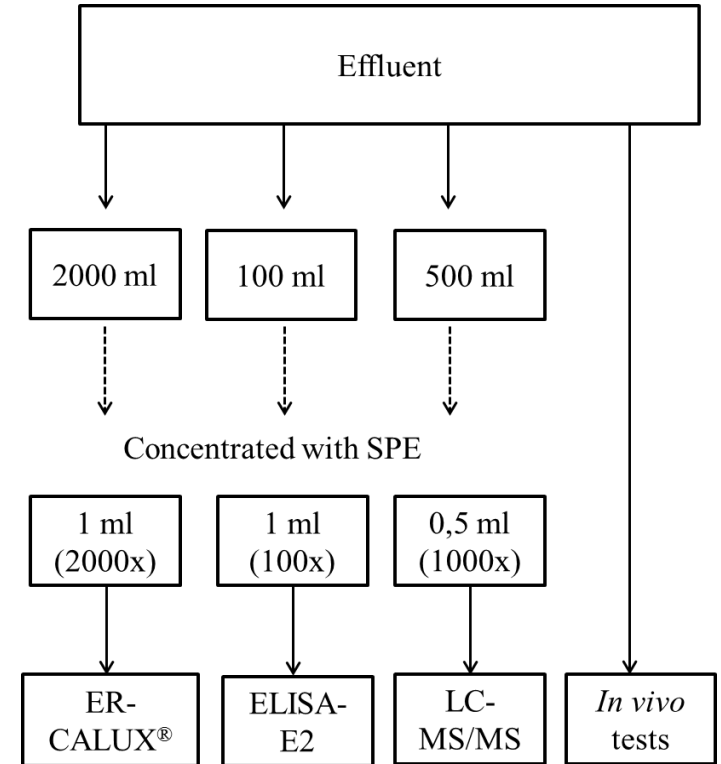


Figure 1. A scheme of the sample treatment process.

# Results

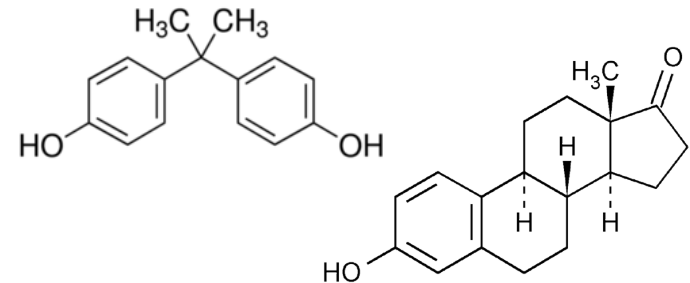
## Chemical analysis (LC-MS/MS)

### Compounds selected for analysis:

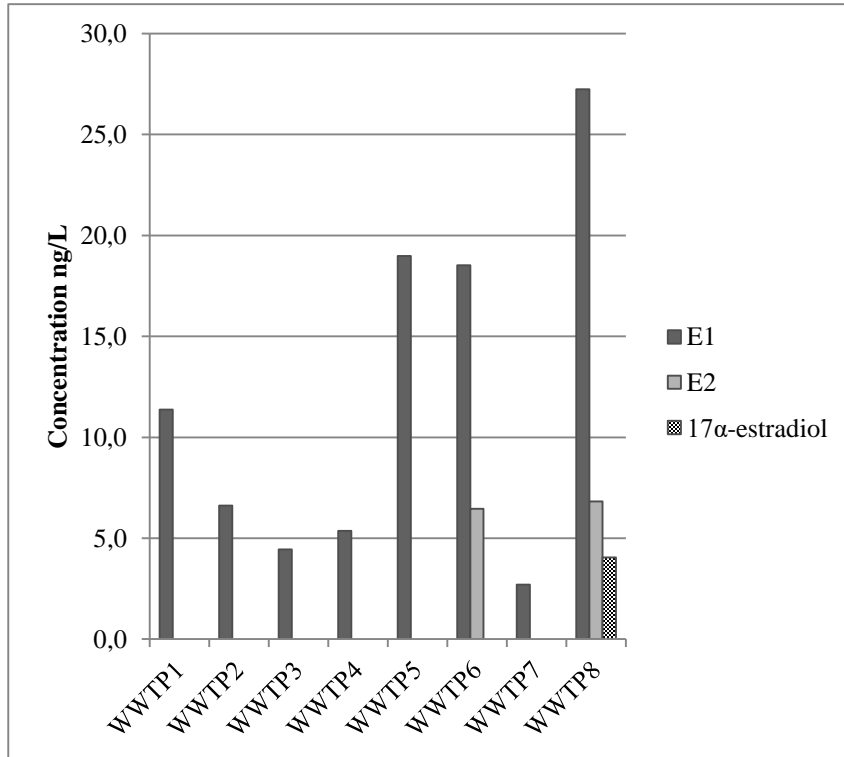
- Natural and synthetic hormones
  - Estrone (E1)
  - 17 $\beta$ -estradiol (E2)
  - Estriol (E3)
  - 17 $\beta$ -ethinylestradiol (EE2)
  - 17 $\alpha$ -estradiol
  - Progesterone

In addition...

Bisphenol A (BPA)

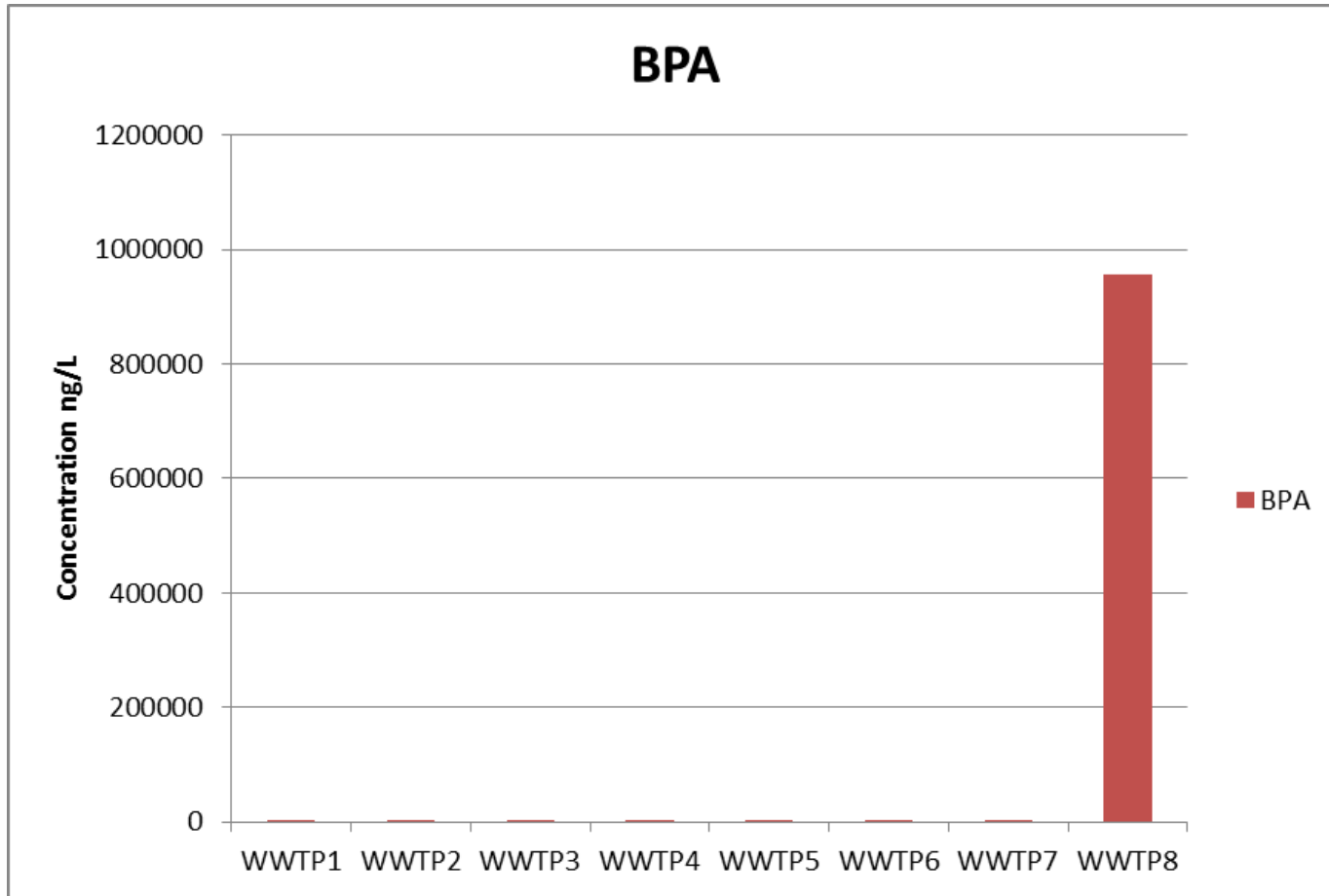


# Which compounds were detected? And how much?



Compound	LOQ ng/L	Concentration ng/L
E1	1	2.7 – 27.2
E2	5	<5 – 6.8
E3	3	<3
EE2	10	<10
17α-estradiol	4	<4 - 4
Progesterone	n.d.	n.d.
BPA	0.7	131 – 956 000

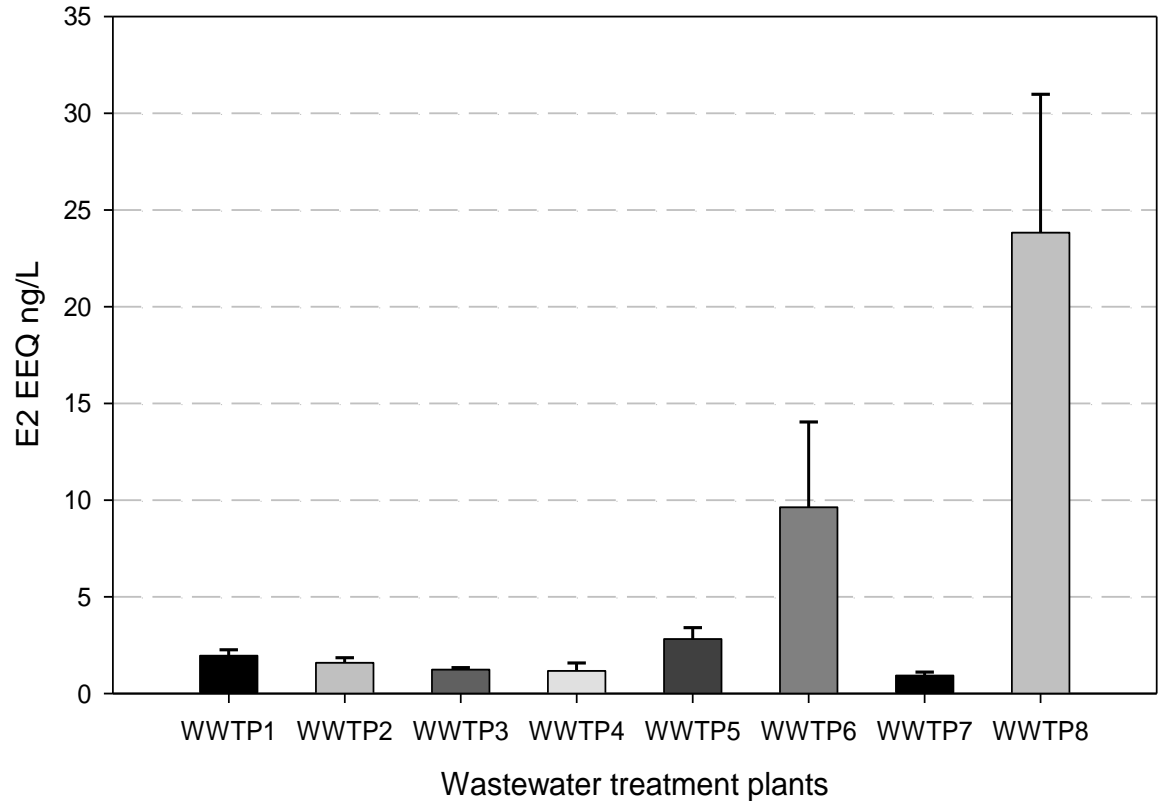
# BPA





# ER $\alpha$ -CALUX

Estrogenic potential of effluents from eight WWTPs tested with ER $\alpha$ -CALUX. The error bars represent standard deviation of three replicates. The estrogenic potency of samples is calculated as equivalence values for E2.



# Contribution of analyzed compounds to the observed effects

Treatment plant	E1	E2	E3	EE2	17 $\alpha$ -estradiol	BPA	Total	ER-CALUX <sup>®</sup> (ng/L)	% explained <sup>b</sup>
WWTP1	0,18	n.d.	n.d.	n.d.	n.d.	0,05	0,23	1,95	12
WWTP2	0,11	n.d.	n.d.	n.d.	n.d.	0,01	0,11	1,58	7
WWTP3	0,07	n.d.	n.d.	n.d.	n.d.	0,01	0,08	1,24	6
WWTP4	0,09	n.d.	n.d.	n.d.	n.d.	0,00	0,09	1,17	8
WWTP5	0,30	n.d.	n.d.	n.d.	n.d.	0,02	0,33	2,82	12
WWTP6	0,30	6,46	n.d.	n.d.	n.d.	0,01	6,77	9,63	70
WWTP7	0,04	n.d.	n.d.	n.d.	n.d.	0,00	0,05	0,92	5
WWTP8	0,44	6,83	0,03	n.d.	n.d.	4,21	11,50	23,83	48

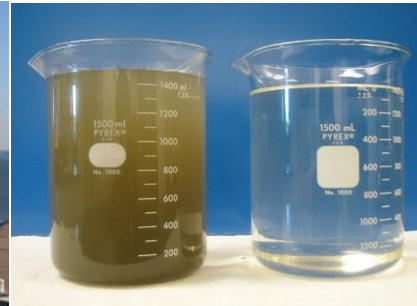
a) The calculations were done according to Maletz et al. (2013); Calculated concentrations of EEQ = Relative estrogenic potency x concentration (ng/L).

b) “% Explained” = (Total/ER CALUX<sup>®</sup> EEQ (ng/L))\*100

n.d.= no data available

LOQs are still too high

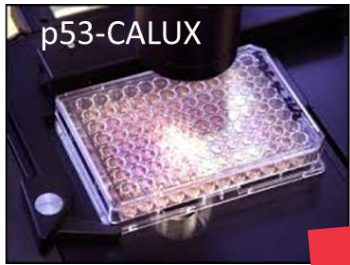
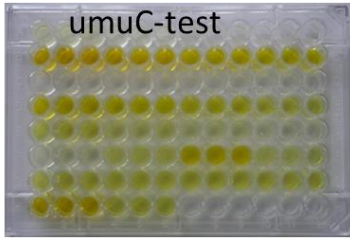
# “Effect-based assessment of toxicity removal during wastewater treatment processes”



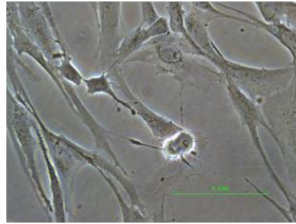
- How efficiently toxic effects are removed?
  - Multiple toxicological endpoints!
- Application of a new sampling and extraction method

# Selected bioassays

Genotoxicity

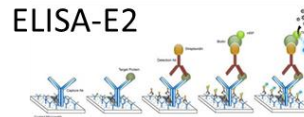
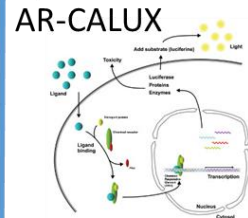
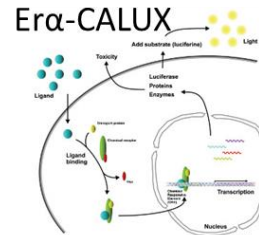


Cytotoxicity

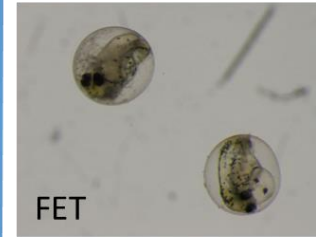


NRR with fish cells

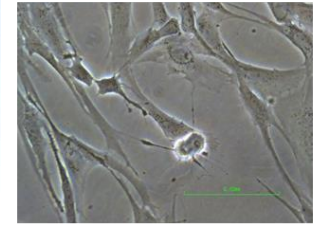
Endocrine disruption



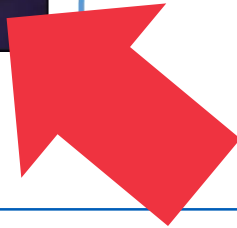
Embryotoxicity



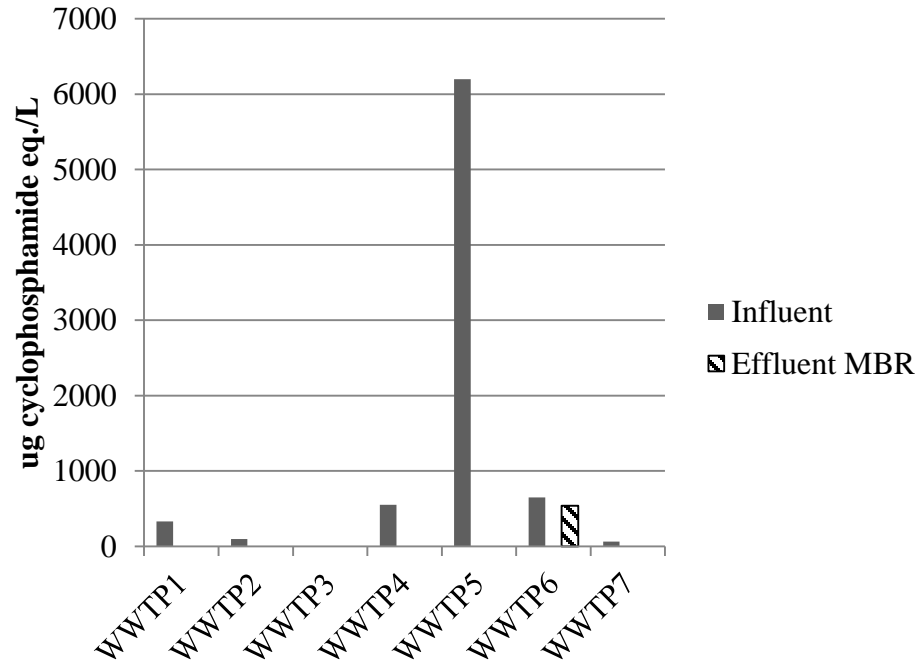
cytochrome P4501A (CYP1A) induction



EROD with fish cells



# Genotoxic effects



**Figure 4.** The genotoxic potency of influent samples collected from seven WWTPs and one MBR effluent sample analyzed with the p53-CALUX<sup>®</sup> assay. The genotoxic activity was below the LOD (53  $\mu$ g cyclophosphamide eq./L) in all of the effluent samples.

# Genotoxic effects

Genotoxic potency of wastewater influent and effluent samples at different sample concentrations measured with the umuC assay

WWTP	Influent								Effluent							
	Induction factor +S9				Induction factor -S9				Induction factor +S9				Induction factor -S9			
	REF40	REF20	REF10	REF5	REF40	REF20	REF10	REF5	REF40	REF20	REF10	REF5	REF40	REF20	REF10	REF5
WWTP1	1,59	1,32	1,21	1,11	1,56	1,33	1,22	1,14	1,47	1,29	1,17	1,08	1,97	1,60	1,42	1,29
WWTP2	1,52	1,29	1,20	1,17	1,97	1,52	1,23	1,13	1,43	1,33	1,19	1,09	1,98	1,44	1,28	1,16
WWTP3	1,49	1,28	1,17	1,09	1,58	1,35	1,22	1,10	1,34	1,23	1,17	1,06	1,82	1,45	1,28	1,11
WWTP4	1,78	1,39	1,24	1,14	2,98	1,82	1,37	1,16	1,56	1,22	1,17	1,06	1,99	1,60	1,35	1,23
WWTP5	1,45	1,19	1,16	1,09	2,14	1,47	1,18	1,24	1,32	1,16	0,97	0,99	1,94	1,48	1,27	1,24
WWTP7	1,63	1,38	1,06	1,10	1,89	1,40	1,31	1,09	1,31	1,08	0,97	0,97	1,68	1,45	1,17	1,06

# Conclusions

- ❑ **The CALUX assays were sensitive and quick and suitable for wastewater analysis.**
  - ❑ Estrogenic activity was detected in all of the analyzed wastewater samples
- ❑ **The p53-CALUX was more sensitive than the umuC-assay in detecting genotoxic effects in influent samples.**
- ❑ **A combination of biological tools covering multiple endpoints and organisms provide a more comprehensive insight on the potential harmful effects and how they are removed during the wastewater treatment processes.**