AquaNES

Demonstrating Synergies in Combined Natural and Engineered Processes for Water Treatment Systems

Evaluation of water treatment technologies using a panel of effect-based CALUX bioassays

Challenge

- Water systems worldwide are confronted with a complex mixture of thousands of known and unknown (unregulated) emerging compounds. Furthermore, water systems and treatment technologies face a major challenge and are under great pressure to deliver safe and affordable water services to a growing population.
- Water quality and treatment performance is generally assessed for a limited set of individual parameters, possibly resulting in an incomplete assessment. Room is now given in the Drinking Water Directive to develop a risk based monitoring program.

New approach

- panel of cost- and capacity-efficient effect-based CALUX bioassays for evaluation of the efficiency of novel and innovative water treatment technologies and assessment of twater quality.

Case study

- 6 participating WWTP; multiple sampling points per participant (temporal/spatial); multiple sampling campaigns; 1 liter water processed
- 1 liter water processed (SPE); high-throughput analysis on a panel of CALUX bioassays (table 1)
- Development of effect-based trigger values and action plan for assessment of water quality and efficiency of innovative treatment technologies (table 2 and 3)
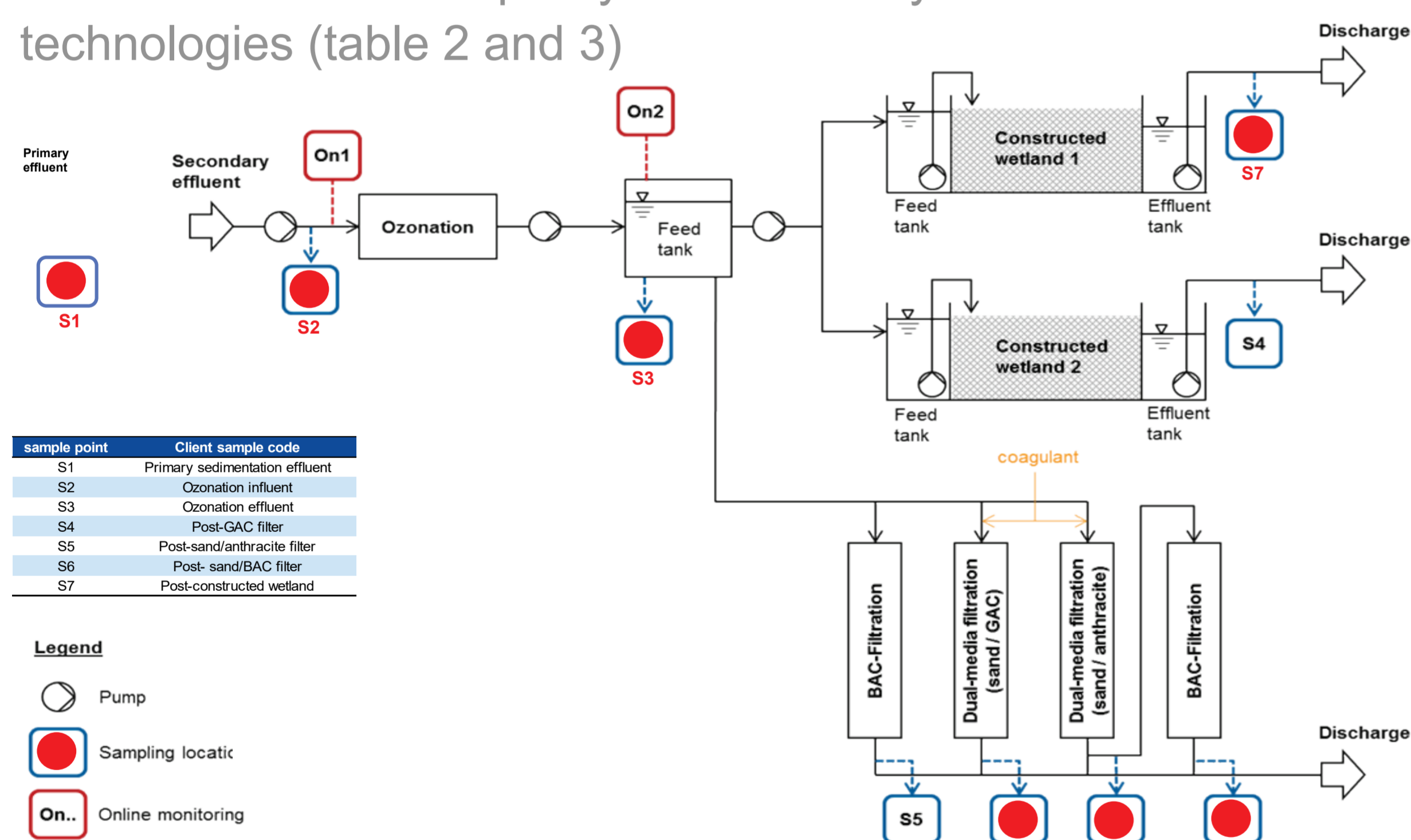

Figure 1 Schematic representation of WWTP (pilot plant) indicating sampling locations (red dots) used for CALUX bioanalyses.

Table 1 Selection of effect-based CALUX bioassays considered to be relevant for the evaluation and monitoring of innovative (waste)water treatment technologies

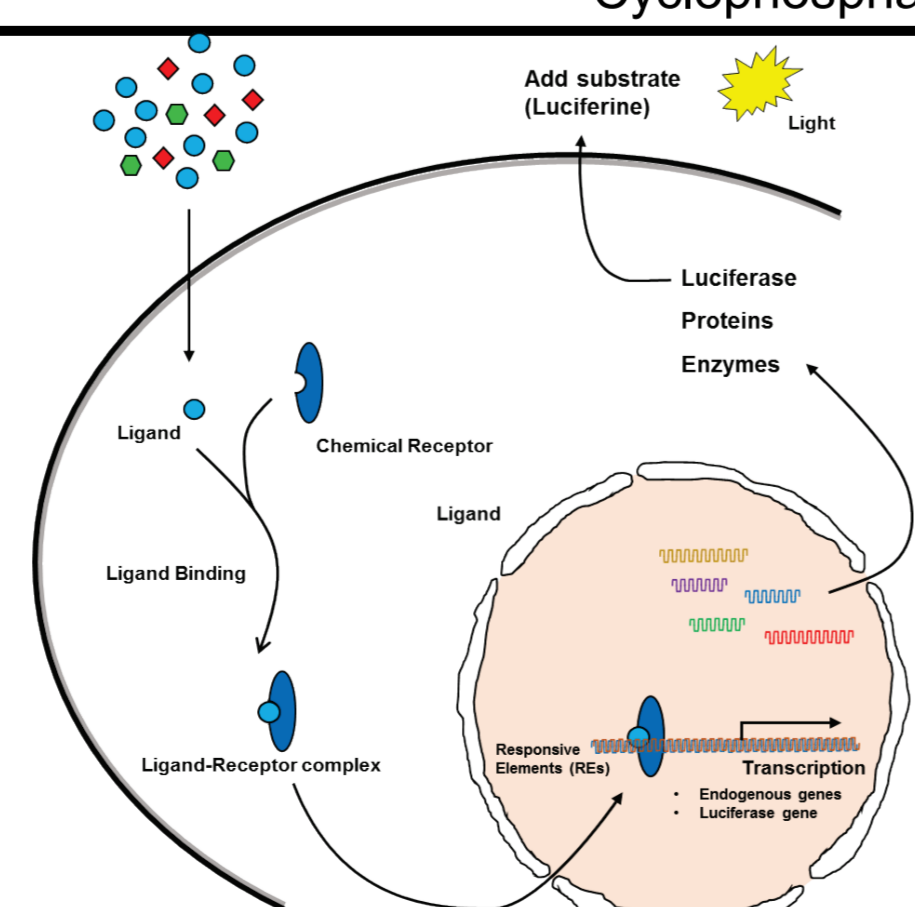| Assay	Responsive towards	Reference
Cytotox CALUX	cytotoxicity	TBT
ERα CALUX	hormone-mediated MoA (estrogen activity (ERα receptor))	17β-estradiol
AR CALUX	hormone-mediated MoA (androgen activity)	DHT
anti-AR CALUX	hormone-mediated MoA (anti-androgen activity)	Flutamide
GR CALUX	hormone-mediated MoA (glucocorticoid activity)	Dexamethasone
anti-PR CALUX	hormone-mediated MoA (anti-progestin activity)	Ru486
PPARα CALUX	peroxisome proliferators	GW7647
PPARγ CALUX	peroxisome proliferators	Rosiglitazone
PXR CALUX	xenobiotic metabolism	Nicardipine
Nrf2 CALUX	oxidative stress inducers	Curcumine
P53 CALUX (+S9)	genotoxicity (with metabolic activation)	Cyclophosphamide

Contact

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Figure 2 Principle of the CALUX reporter-gene assay

Table 2 Effect-based trigger values (EBTs) for a panel of selected CALUX bioassays

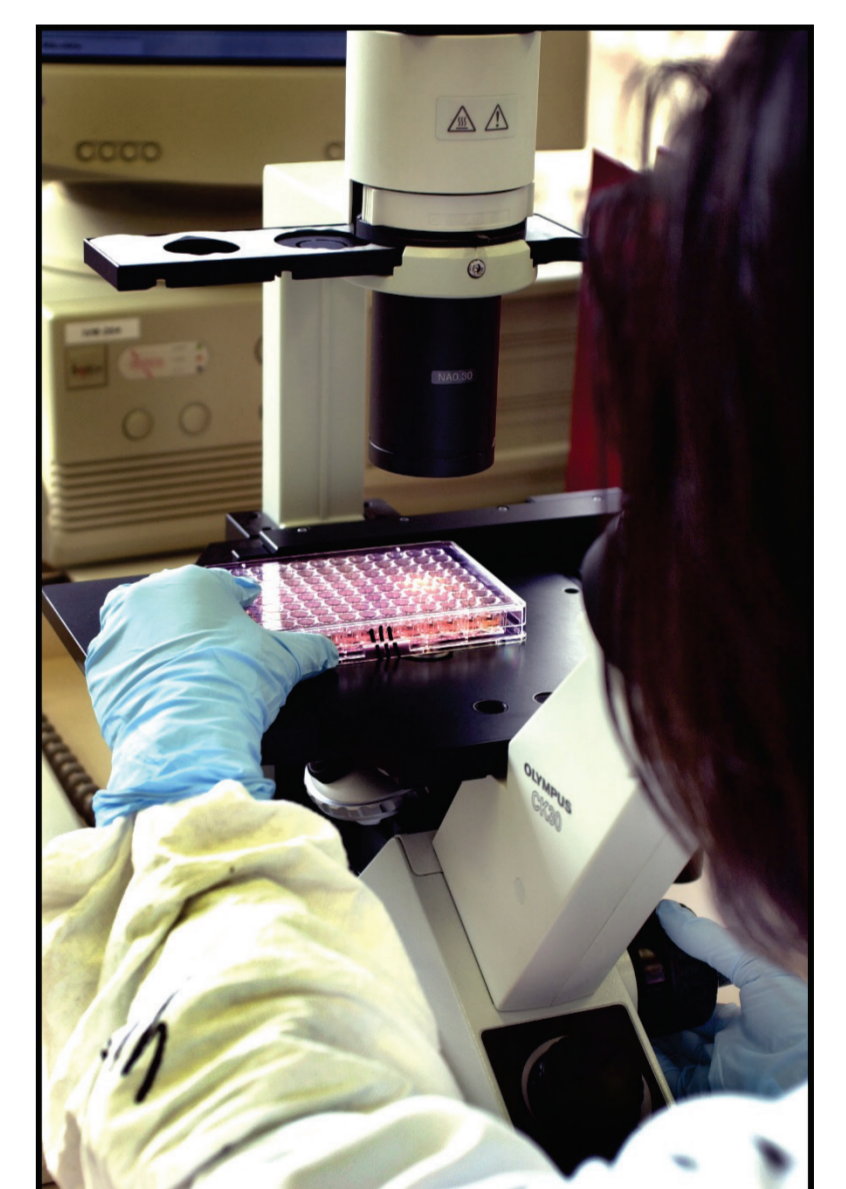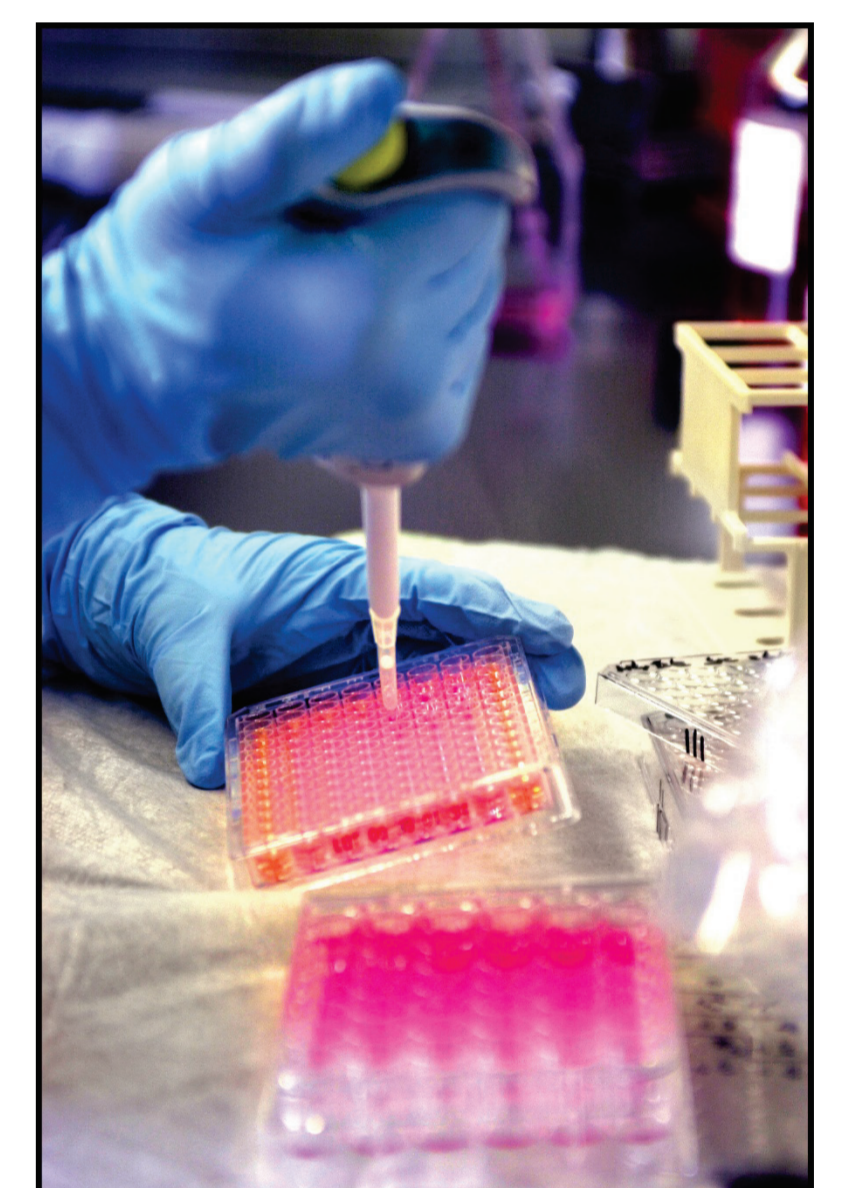| Assay	Unit	EBT	Reference	1*EBT	3*EBT	10*EBT	100*EBT
Cytotox CALUX							
AR CALUX	ng DHT eq./l	32	Besselink	32	96	320	3200
anti-AR CALUX	ug Flutamide eq./l	14	Escher et al. (2018)	14	42	140	1400
ERa CALUX	ng 17b-Estradiol eq./l	0.1	Escher et al. (2018)	0.1	0.3	1	10
GR CALUX	ng Dexamethasone eq./l	56	Besselink	56	168	560	5600
anti-PR CALUX	ng Ru486 eq./l	1.2	Escher et al. (2018)	1.2	3.6	12	120
PPARa CALUX	ng GW7647 eq./l	22	Besselink	22	66	220	2200
PPARg2 CALUX	ng Roziglitazone eq./l	91	Besselink	91	273	910	9100
PXR CALUX	ug Nicardipine eq./l	43	Escher et al. (2018)	43	129	430	4300
Nrf2 CALUX	ug Curcumine eq./l	20	Escher et al. (2018)	20	60	200	2000
P53 (+S9) CALUX	ug Cyclophosphamide eq./l	1100	Besselink	1100	3300	11000	110000

Table 3 Proposed action plan for water quality assessment, based on CALUX bioassays EBTs

Bio-analysis result	Action
CALUX result < EBT or LOQ of bioassay	- no further action required
1 x EBT < CALUX result EBT < 3 x EBT	- quality check data, continue monitoring every three months
3 xEBT < CALUX result EBT < 10 x EBT	- data check; re-sampling and re-analysis; quantify specific target compounds known to cause the effects observed in bioassay; continue monitoring every three months
10 x EBT < CALUX result < 100 x EBT	- monitoring in the distribution system closer to the point of exposure.
CALUX result > 100 x EBT	- all of the above; confer with the local environmental authority's to determine the required response action. Confirm plant corrective actions through additional monitoring.

23/01/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	LOQ	1.8	LOQ		LOQ	LOQ	LOQ
AR CALUX	155	LOQ	LOQ		LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	4.6	LOQ		LOQ	LOQ	LOQ
ERa CALUX	0.4	1.8	0.1		0.4	0.3	0.1
GR CALUX	110	210	71		87	21	25
anti-PR CALUX	40.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARa2 CALUX	400.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARg2 CALUX	1300	LOQ	LOQ		LOQ	LOQ	LOQ
PXR CALUX	LOQ	25	12		19	LOQ	8
Nrf2 CALUX	760	180	110		77	79	51
P53 CALUX (+S9)	LOQ	10000	LOQ		LOQ	LOQ	LOQ

17/04/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	53.0	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	430	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	1.1	2.6	LOQ	LOQ	1.8	LOQ
ERa CALUX	1.0	45	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	160	15.0	LOQ	LOQ	42.0	LOQ	50.0
anti-PR CALUX	54	LOQ	LOQ	LOQ	4.1	LOQ	LOQ
PPARa2 CALUX	140	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	81	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	80	100	93	LOQ	37	35	11
Nrf2 CALUX	810	320	130	LOQ	190	110	140
P53 CALUX (+S9)	570	2200	LOQ	LOQ	LOQ	LOQ	LOQ

16/07/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	25	2.8	0.7	LOQ	LOQ	LOQ	LOQ
AR CALUX	130	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	21	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	51	1.0	0.1	LOQ	LOQ	LOQ	LOQ
GR CALUX	130	110	48	LOQ	41	24	22
anti-PR CALUX	40	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	420	LOQ	20	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	1100	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	100	72	48	8.3	30.0	33.0	31.0
Nrf2 CALUX	740	200	170	LOQ	190	82	110
P53 CALUX (+S9)	25000	LOQ	LOQ	LOQ	1500	LOQ	LOQ

- CALUX result < EBT or LOQ of bioassay
- If 1xEBT < CALUX result EBT < 3xEBT
- 3 x EBT < CALUX result EBT < 10x EBT
- 10 x EBT < CALUX result < 100 x EBT
- CALUX result > 100xEBT

Figure 3 Quantified CALUX analysis results and application of proposed EBT action plan

Conlusions / lessons learned

- Effect-based bioanalysis allows for detection of known / unregulated / unknown chemical and assessment of total toxicity of water samples, including mixture effects
- Applied innovative treatment technologies improve water quality significantly
- Results from effect-based CALUX bioassays can be used as for evaluation of efficiency of treatment technologies to remove bioactive substances.
- Development of effect-based trigger values (EBTs) is required for the assessment of water quality and implementation of effect-based bioassays in regulatory water frameworks for risk assessment.
- The development of an action plan for water treatment plant operators based on EBT, enhances the applicability of effect-based bioassay for assessment of water quality and regulatory acceptance.

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