AquaNES

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

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

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

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BioDetection Systems BV (BDS)

BDS BioDetection Systems

Facts and figures

Funding
Horizon 2020 (EU), 7.8 M€ + SERI (CH) 0.87 M€

Call:
H2020-WATER-2015-two-stage, Topic WATER-1b-2015

30 partners:
industry & SMEs, utilities, research

Duration:
36 months (Juni 2016 – Mai 2019)

Demonstrators:
13 sites in Europe, Israel and India

SMEs & Industry

AKUT Partner · AUTARCON · imaGeau · géo-hyd membre d'Antea Group · HydroBusiness · BDS BioDetection Systems · microLAN · WatStech Water Science & Technology Group · PENTAIR · xylem Let's Solve Water · WADIS Water Disinfection · VERTECH GROUP

Water Utilities

IWB · BUDAPEST WATERWORKS · ΔΕΥΑΘ · ΔΗΜΟΣ ΑΝΤΙΠΑΡΟΥ · MEKOROT · Erft Verband · Berliner Wasserbetriebe · drewag NETZ

Universities & Research Institutions

n|w Fachhochschule Nordwestschweiz Hochschule für Life Sciences · KOMPETENZZENTRUM WasserBerlin · brgm · KWR Watercycle Research Institute · Cranfield University · NATIONAL UNIVERSITY OF PUBLIC SERVICE · UAM ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ · HTW HOCHSCHULE FÜR TECHNIK UND WIRTSCHAFT DRESDEN UNIVERSITY OF APPLIED SCIENCES

The AquaNES Project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 689450

Challenges and objectives

Increasing the understanding of the capacity of natural treatment steps

– Enhance and maintain performance through adequate pre-treatment
– Make up for variation through post-treatment, complement

Assuring water quality

– Micropollutants, pathogens and indicators, antibiotic ARG, nutrients

Developing adapted operating and monitoring concepts

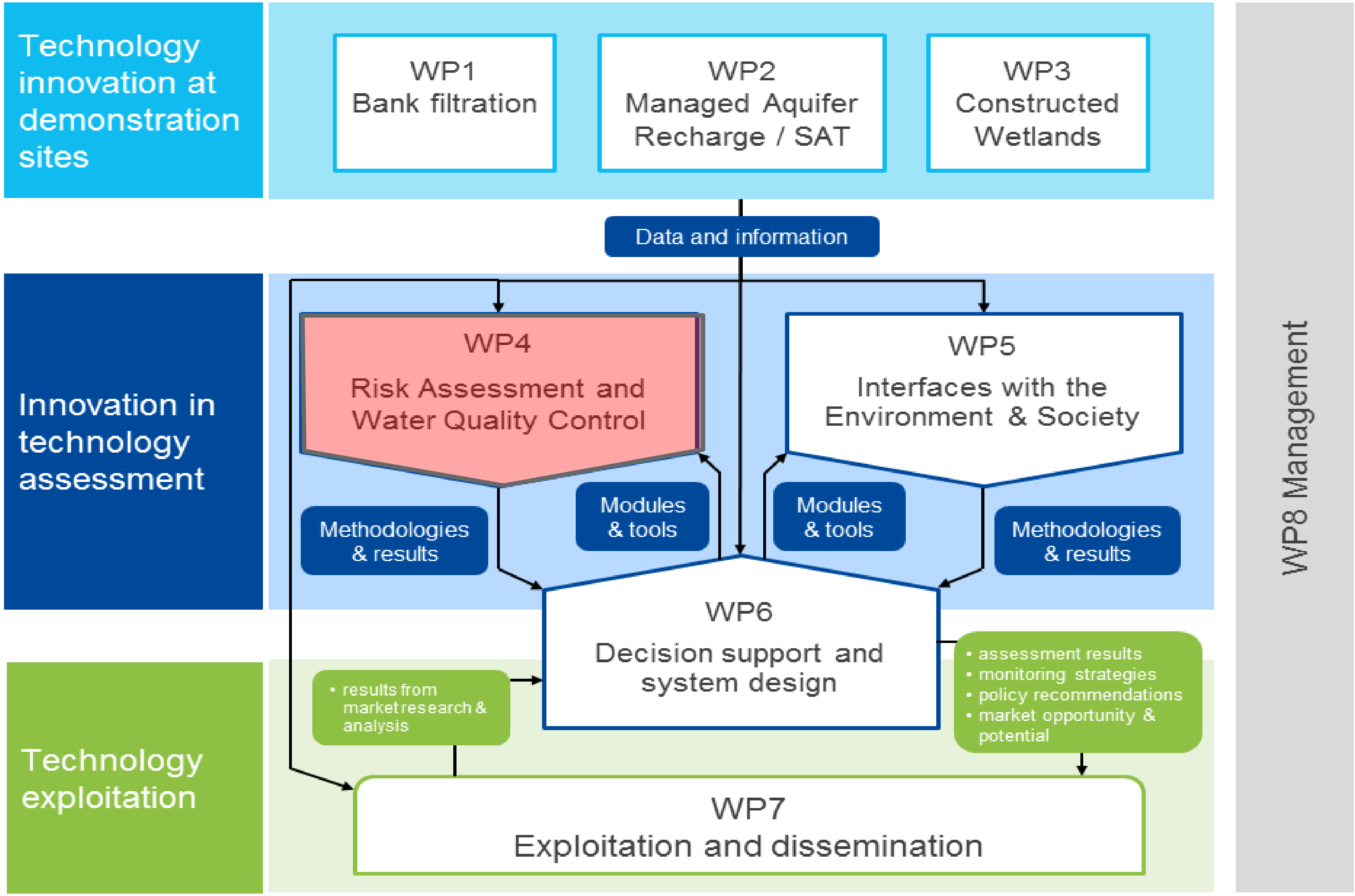
Assessing the environmental impact (& benefits) and costs

– Energy demand, use of chemicals …
– Land requirements and use

Providing decision support

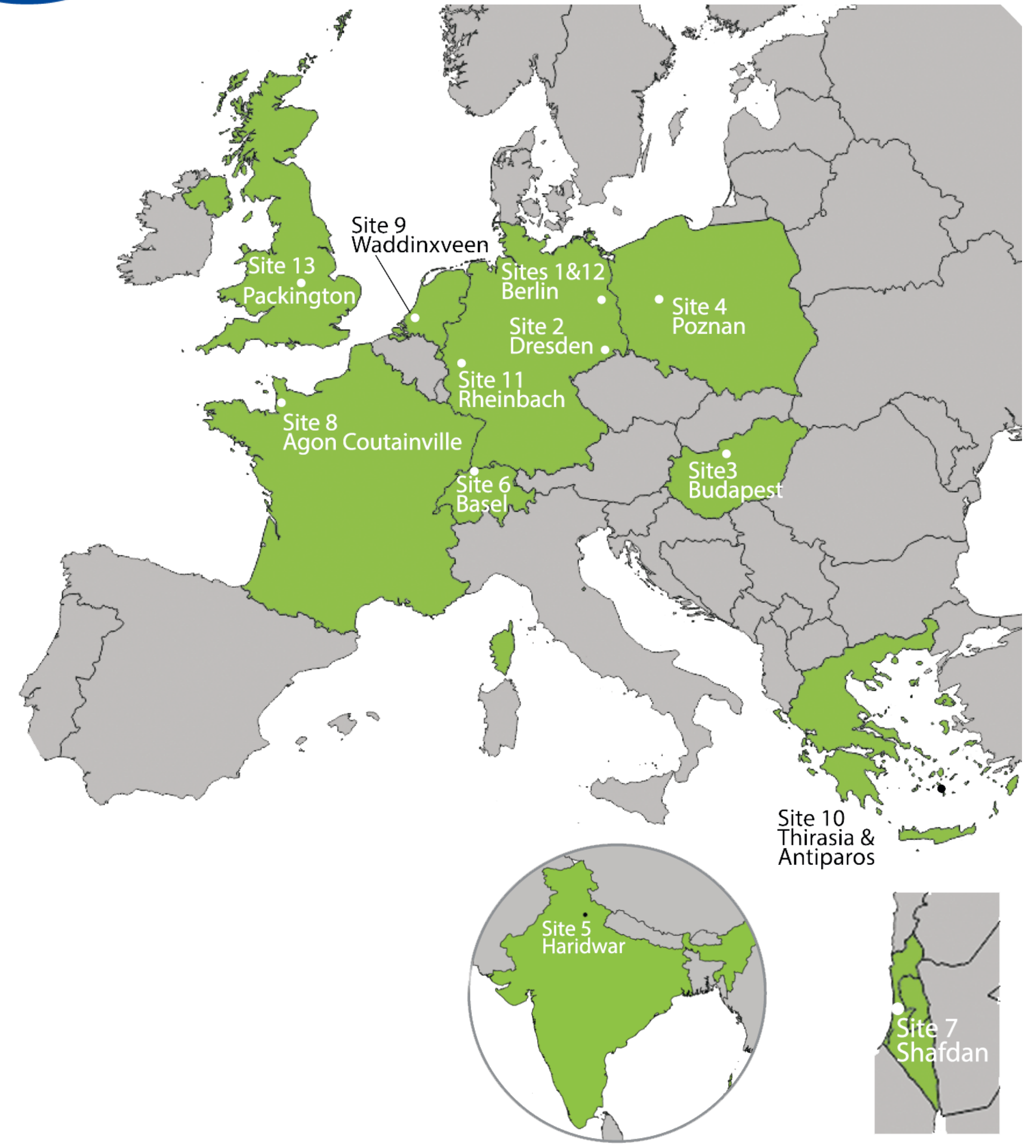
BDS BioDetection Systems

Project structure



BDS BioDetection Systems

Demonstration sites locations



River Bank Filtration schemes for the production of drinking water		
Demonstration	Site No.1	Havel River, Berlin, Germany
Demonstration	Site No.2	Elbe River, Dresden, Germany
Demonstration	Site No.3	Danube River, Budapest, Hungary
Demonstration	Site No.4	Warta River, Poznan, Poland
Demonstration	Site No.5	Ganga River, Haridwar, India

Managed Aquifer Recharge & Soil Aquifer Treatment schemes for water storage & quality improvement		
Demonstration	Site No.6	Lange Erlen, Basel, Switzerland
Demonstration	Site No.7	Shafdan WWTP, Tel Aviv, Israel
Demonstration	Site No.8	Agon-Coutainville, France
Demonstration	Site No.9	Waddinxveen, Rotterdam, the Netherlands

Constructed wetlands and other natural systems for improved wastewater treatment		
Demonstration	Site No.10	Thirasia and Antiparos Islands, Greece
Demonstration	Site No.11	Erftverband, Germany
Demonstration	Site No.12	Berlin, Germany
Demonstration	Site No.13	Packington, UK

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Demonstration sites: technology combinations

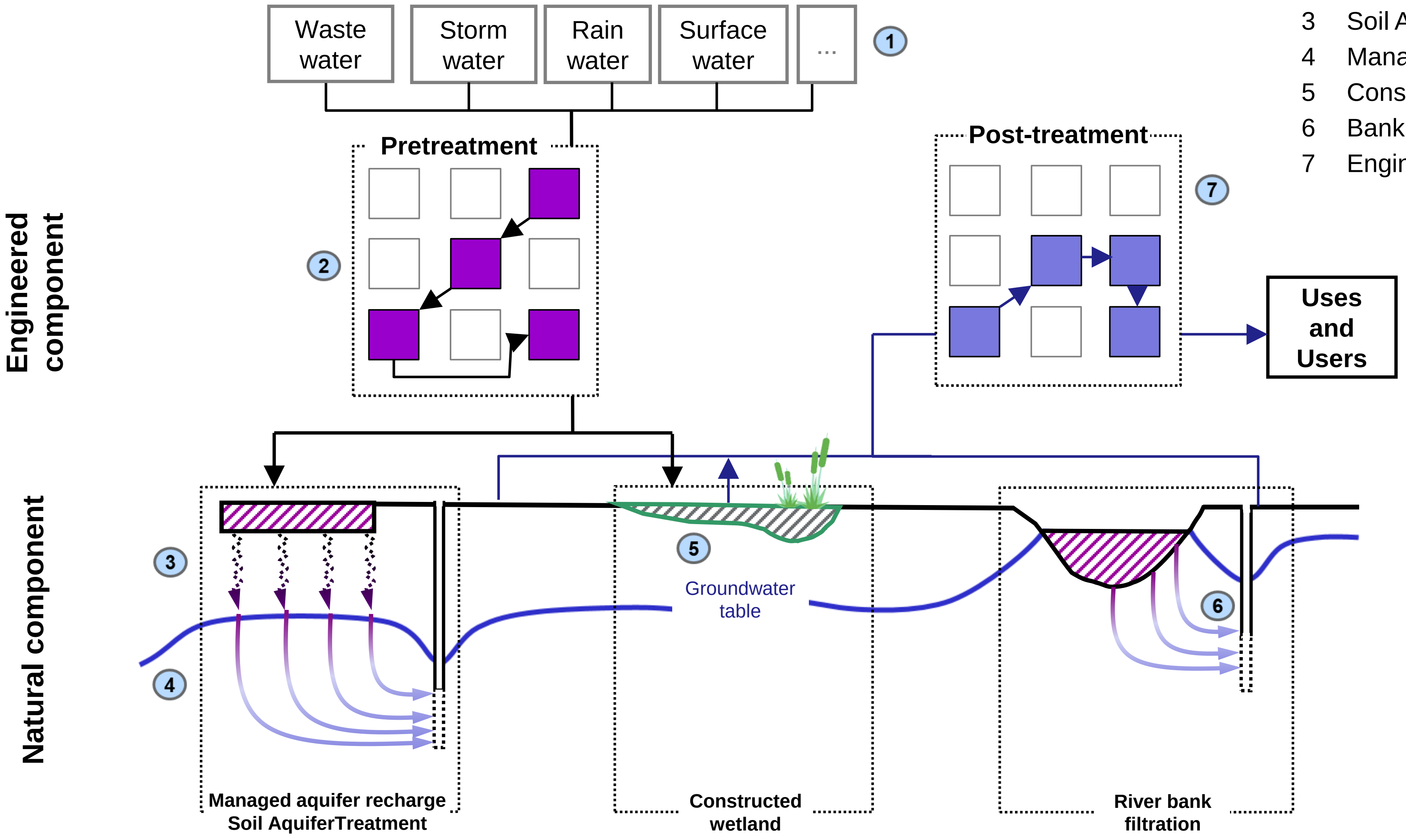
- **Oxidative pre-treatment**
  (O_3, H_2O_2+UV, electropulse, solar photocatalysis – TiO_2)
- **Post-treatment with membranes**
  (UF, NF, RO) or ozone
- **Biofiltration and biological activated carbon filtration (BAC)**
- **Disinfection processes**
  (electrochlorination, UV)
- **Sorptive and biological P-removal**
  (algae reactor)

WP1 bank filtration	WP2 MAR / SAT	WP3 Constructed wetland

Project concept

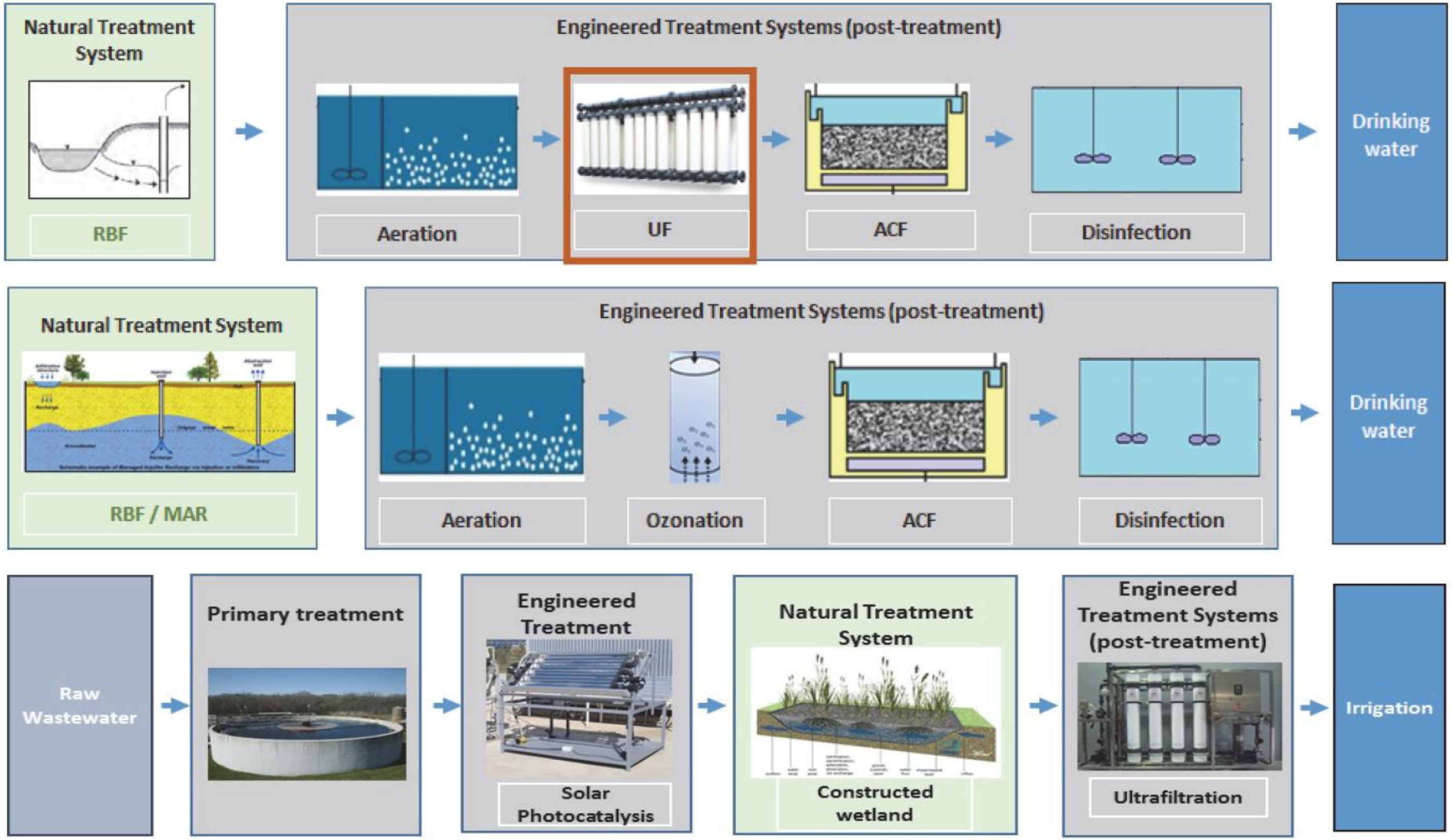
Overview of cNES treatment technologies

1 Sources
2 Engineered pre-treatment (Site 2, 6-13)
3 Soil Aquifer Treatment (Site 6-9)
4 Managed Aquifer Recharge (Site 6-9)
5 Constructed Wetland (Site 10-13)
6 Bank filtration (1-5)
7 Engineered post-treatment (all sites)



BioDetection Systems

Example demonstration sites



RBF - River Bank Filtration
CNF - Capillary Nanofiltration
UF - UltraFiltration
ACF - Activated Carbon Filtration
MAR - Managed Aquifer Recharge
AC - Activated Carbon Filtration

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Effect-based CALUX bioassays in cNES

- Assess the remediation potential of the natural component (e.g. bankfiltration)
- Describe the impact of engineered treatment steps (e.g. ozonation) or adsorption on generation or removal of toxicity effects
- Identify points of attention (intake water quality, operational parameter, dosage, retention time …)
- Development water quality assessment framework

BDS BioDetection Systems

Effect-based CALUX bioassays in cNES

Water Quality Monitoring using effect-based CALUX bioassays

1 – Optimalisation of SOPs for (waste)water analysis

2 – pilot study

- selection most informative bioassays for analysis of cNES efficiency
- selection of 6 WTS to enter the comprehensive study

1 sampling campaign

Number of demonstration sites:	13
Number of samples per site:	2
Total number of samples to be analysed:	26
Number of assays per sample:	18

3 – comprehensive study

- assess remediation potential natural component
- describe the impact of engineered treatment steps
- evaluate the efficiency/effectivness cNES

- 3 sampling campaigns (temporal sampling)
- Multiple sampling points (spatial sampling)

Number of demonstration sites:	6
Number of samples per site:	18
Total number of samples to be analysed:	108
Number of assays per sample:	10

BioDetection Systems

Selection of bioassay panel

Assessment of water quality

Broad range screening

Full panel of bioassays

- ERα, Erβ, anti-Erα, anti-Erβ
- AR, anti-AR
- PR, anti-PR
- GR, anti-GR

TRβ, TTR-TRβ, RAR

- DR, PAH, PXR
- P53 (-S9), P53 (+S9)
- Nrf2, ESRE
- PPARγ, PPARα, PPARδ, LXR
- Cytotox

Relevant range screening

Panel of bioassays

- ERα
- anti-AR
- anti-PR
- anti-GR

DR, PAH, PXR

- P53 (-S9), P53 (+S9)
- Nrf2
- Cytotox

Regulatory screening (application of trigger values)

Regulatory panel of bioassays

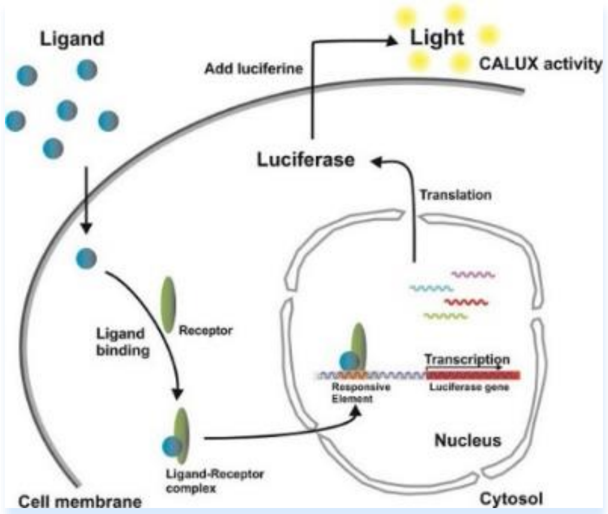
- ERα, anti-AR, anti-PR etc.

BioDetection Systems

1 – pilot study

- selection most informative bioassays for analysis of cNES efficiency
- selection of 6 WTS to enter the comprehensive study

Toxicity pathway	Pertinent CALUX assay	Health effects
Xenobiotic metabolism	DR CALUX PAH CALUX PXR CALUX	Reproductive and developmental probl Interfere with hormone action Cancer
Hormone-mediated mode of action	ERα CALUX and anti-ERα CALUX AR CALUX and anti-AR CALUX GR CALUX and anti-GR CALUX PR CALUX and anti-PR CALUX	Tumor development Birth defects (Sexual) developmental disorders
Lipid metabolism	PPARα CALUX PPARδ CALUX PPARγ CALUX	Peroxisome proliferation Obesity Inflammatory diseases
Reactive mode of action Genotoxicity	P53 (-S9) CALUX P53 (+S9) CALUX	Tumor development
Adaptive stress response	Nrf2 CALUX	Oxidative stress Inflammation, organ toxicity, sensitisati and neurodegenerative diseases
Cell viability/cytotoxicity	Cytotox CALUX	General toxicity



BDS
BioDetection Systems

1 – pilot study

- selection most informative bioassays for analysis of cNES efficiency
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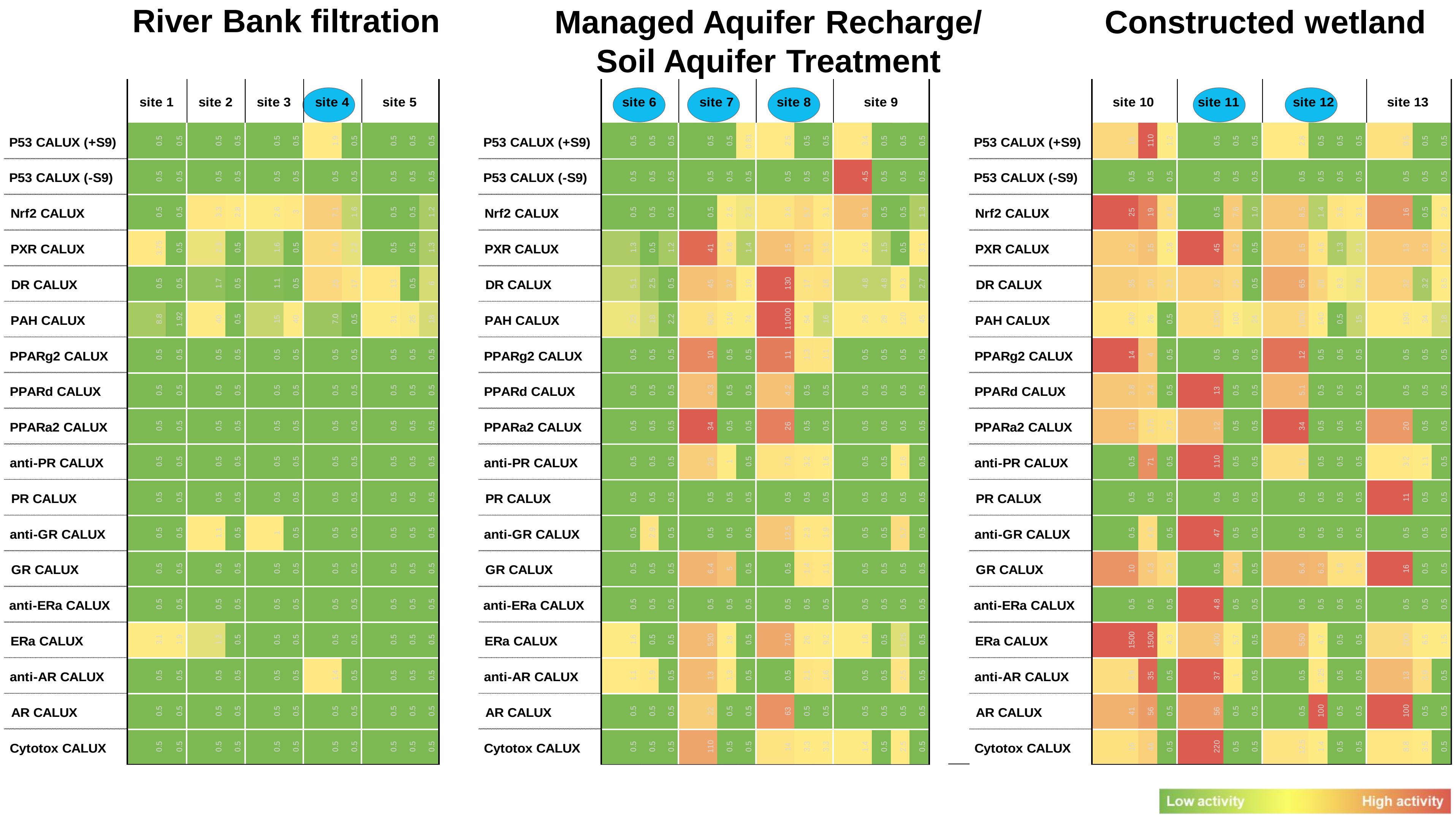

1 – pilot study

- selection most informative bioassays for analysis of cNES efficiency
- selection of 6 WTS to enter the comprehensive study

Toxicity pathway	Pertinent CALUX assay	Health effects
Xenobiotic metabolism	PXR CALUX	Reproductive and developmental probl Interfere with hormone action Cancer
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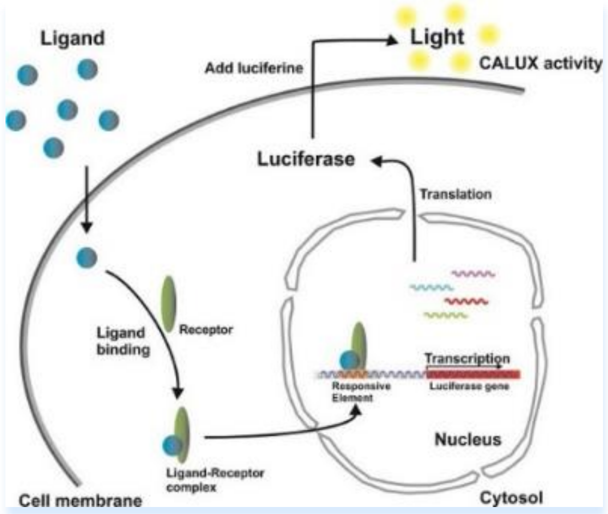

2 – Comprehensive study

- temporal/spatial sampling
- cNES efficiency

Cytotox CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	1.8	ug Tributyltin acetate eq./l
	28971	site12, ozonation effluent	LOQ (<0.47)	ug Tributyltin acetate eq./l
	28972	site12, sand/anthracite filter	LOQ (<0.47)	ug Tributyltin acetate eq./l
	28973	site12, sand/BAC filter	LOQ (<0.46)	ug Tributyltin acetate eq./l
	28974	site12, constructed wetland 1	LOQ (<0.44)	ug Tributyltin acetate eq./l
	28975	site12, primary sedimentation effluent	LOQ (<9.2)	ug Tributyltin acetate eq./l

AR CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	LOQ (<0.81)	ng Dihydrotestosterone (DHT) eq./l
	28971	site12, ozonation effluent	LOQ (<0.79)	ng Dihydrotestosterone (DHT) eq./l
	28972	site12, sand/anthracite filter	LOQ (<0.52)	ng Dihydrotestosterone (DHT) eq./l
	28973	site12, sand/BAC filter	LOQ (<0.51)	ng Dihydrotestosterone (DHT) eq./l
	28974	site12, constructed wetland 1	LOQ (<0.62)	ng Dihydrotestosterone (DHT) eq./l
	28975	site12, primary sedimentation effluent	155	ng Dihydrotestosterone (DHT) eq./l

anti-AR CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	4.6	ug Flutamide eq./l
	28971	site12, ozonation effluent	LOQ (<3.8)	ug Flutamide eq./l
	28972	site12, sand/anthracite filter	LOQ (<3.8)	ug Flutamide eq./l
	28973	site12, sand/BAC filter	LOQ (<3.7)	ug Flutamide eq./l
	28974	site12, constructed wetland 1	LOQ (<4.5)	ug Flutamide eq./l
	28975	site12, primary sedimentation effluent	LOQ (<28)	ug Flutamide eq./l

ERa CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	1.8	ng 17b Estradiol eq./l
	28971	site12, ozonation effluent	0.13	ng 17b Estradiol eq./l
	28972	site12, sand/anthracite filter	0.44	ng 17b Estradiol eq./l
	28973	site12, sand/BAC filter	0.32	ng 17b Estradiol eq./l
	28974	site12, constructed wetland 1	0.052	ng 17b Estradiol eq./l
	28975	site12, primary sedimentation effluent	0.36	ng 17b Estradiol eq./l

GR CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	210	ng Dexamethasone eq./l
	28971	site12, ozonation effluent	71	ng Dexamethasone eq./l
	28972	site12, sand/anthracite filter	87	ng Dexamethasone eq./l
	28973	site12, sand/BAC filter	21	ng Dexamethasone eq./l
	28974	site12, constructed wetland 1	25	ng Dexamethasone eq./l
	28975	site12, primary sedimentation effluent	110	ng Dexamethasone eq./l

anti-PR CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	LOQ (<0.0019)	ug Ru486 eq./l
	28971	site12, ozonation effluent	LOQ (<0.0019)	ug Ru486 eq./l
	28972	site12, sand/anthracite filter	LOQ (<0.0020)	ug Ru486 eq./l
	28973	site12, sand/BAC filter	LOQ (<0.0020)	ug Ru486 eq./l
	28974	site12, constructed wetland 1	LOQ (<0.0012)	ug Ru486 eq./l
	28975	site12, primary sedimentation effluent	0.04	ug Ru486 eq./l

PPARa CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	LOQ (<8.2)	ng GW7647 eq./l
	28971	site12, ozonation effluent	LOQ (<8.0)	ng GW7647 eq./l
	28972	site12, sand/anthracite filter	LOQ (<29)	ng GW7647 eq./l
	28973	site12, sand/BAC filter	LOQ (<28)	ng GW7647 eq./l
	28974	site12, constructed wetland 1	LOQ (<4.9)	ng GW7647 eq./l
	28975	site12, primary sedimentation effluent	400	ng GW7647 eq./l

PPARg CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	LOQ (<120)	ng Rosiglitazone eq./l
	28971	site12, ozonation effluent	LOQ (<120)	ng Rosiglitazone eq./l
	28972	site12, sand/anthracite filter	LOQ (<130)	ng Rosiglitazone eq./l
	28973	site12, sand/BAC filter	LOQ (<120)	ng Rosiglitazone eq./l
	28974	site12, constructed wetland 1	LOQ (<150)	ng Rosiglitazone eq./l
	28975	site12, primary sedimentation effluent	1300	ng Rosiglitazone eq./l

Nrf2 CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	180	ug Curcumine eq./liter
	28971	site12, ozonation effluent	110	ug Curcumine eq./liter
	28972	site12, sand/anthracite filter	77	ug Curcumine eq./liter
	28973	site12, sand/BAC filter	79	ug Curcumine eq./liter
	28974	site12, constructed wetland 1	51	ug Curcumine eq./liter
	28975	site12, primary sedimentation effluent	760	ug Curcumine eq./liter

P53 (+S9) CALUX	BDS number	Client sample code	Result	Unit
	28970	site12, secondary sedimentation effluent	10000	ug Cyclophosphamide/liter
	28971	site12, ozonation effluent	LOQ (<450)	ug Cyclophosphamide/liter
	28972	site12, sand/anthracite filter	LOQ (<460)	ug Cyclophosphamide/liter
	28973	site12, sand/BAC filter	LOQ (<440)	ug Cyclophosphamide/liter
	28974	site12, constructed wetland 1	LOQ (<450)	ug Cyclophosphamide/liter
	28975	site12, primary sedimentation effluent	LOQ (<9400)	ug Cyclophosphamide/liter

Site 12 - Berlin, Germany

	Cytotox CALUX	AR CALUX	anti-AR CALUX	ERa CALUX	GR CALUX	anti-PR CALUX	PPARa2 CALUX	PPARg2 CALUX	PXR CALUX	Nrf2 CALUX	P53 CALUX (+S9)
prim. sedimentation effl. (infl. biol. treatment of WWTP)	0.5	120	0.5	5	8.5	16	40	4.3	0.5	19	0.5
sec. sedimentation effl. (ozonation influent)	3.9	0.5	1.2	50	18	0.5	0.5	0.5	4.2	9	22
ozonation effluent	0.5	0.5	0.5	3.5	6.5	0.5	0.5	0.5	2.2	5.8	0.5
sand/anthracite filter	0.5	0.5	0.5	16	9.3	0.5	0.5	0.5	3.7	4.1	0.5
sand/BAC filter	0.5	0.5	0.5	12	2.3	0.5	0.5	0.5	0.5	4.2	0.5
constructed wetland 1	0.5	0.5	0.5	1.5	4.2	0.5	0.5	0.5	1.2	2.7	0.5
Primary sedimentation effluent 18/4/18	87	215	0.5	1200	1.4	19	13	0.5	14	21	4.3
Ozonation influent 17/4/18	0.5	0.5	1	3.8	7.6	0.5	0.5	1.2	5.9	8.6	1.2
Ozonation effluent 17/4/18	0.5	0.5	1.9	0.5	0.5	0.5	0.5	0.5	4	2.5	0.5
Sand/BAC filter 17/4/18	0.5	0.5	1.1	0.5	0.5	0.5	0.5	0.5	4	2.1	0.5
Sand/anthracite filter 17/4/18	0.5	0.5	0.5	0.5	1.6	1.5	0.5	0.5	4.9	4.3	0.5
Post-GAC filter 17/4/18	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
constructed wetland 1 18/4/18	0.5	0.5	0.5	0.5	1.4	0.5	0.5	0.5	2.3	3.8	0.5

Low activity — High activity

Interpretation of data – assessment of water quality

Implementation of effect-based bioassays in regulatory frameworks for safety/quality evaluation

selection bioassays (application specific)

↓

development of standardized protocols

↓

formal validation

↓

establishment of effect-based trigger values ←

↓

implementation in regulatory frameworks for risk assessment

BioDetection Systems

Interpretation of data – assessment of water quality

Development of criteria for water quality assessment

- Effect-based trigger values

	Brand	Van der Oost	BDS	Escher
Estrogens (ng 17β-estradiol eq./l water)	3.8	1.0	1.1	3.1
Androgens (ng DHT eq./l water)	11	---	32	14
Glucocorticoids (ng Dex. Eq./l water)	21	30	56	150

Brand et al. (2013) Environmental International, 55:109-118
Van der Oost et al. (2017) Environmental Toxicology and Chemistry, submitted
Escher et al (2015) Water Research 81:137-148

ELSEVI

Effect-b
surface
(EQS) of the European Water Framework Directive

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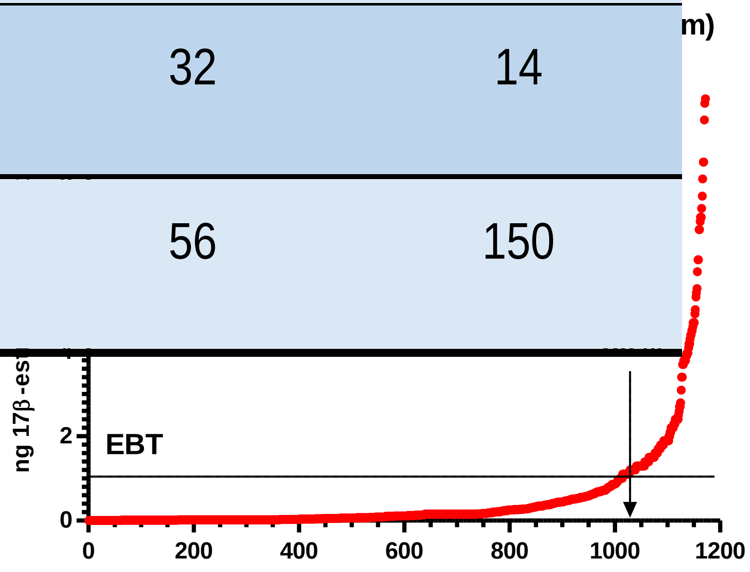

Interpretation of data – assessment of water quality

Development of criteria for water quality assessment

- Effect-based trigger values
- Action criteria

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	0.3	1	10
GR CALUX	ng Dexamethasone eq./l	56	Besselink	56	168	560	5600
anti-PR CALUX	ug Ru486 eq./l	0.0012	Escher et al 2018	0	0.0036	0.012	0.12
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	54	Escher et al 2018	54	162	540	5400
Nrf2 CALUX	ug Curcumine eq./l	21	Escher et al 2018	21	63	210	2100
P53 (+S9) CALUX	ug Cyclophosphamide eq./l	170	Besselink	170	510	1700	17000

Interpretation of data – assessment of water quality

Exceedance of proposed trigger values should initiate the following actions:

TO BE DISCUSSED

- Bioassay response < EBT or LOQ of bioassay
  - no further action required
- EBT < bioassay response < 3*EBT
  - quality data check.
  - continued monitoring (1 year; every 3 months) until bioassay response < 1*EBT
- 3*EBT< bioassay response < 10*EBT
  - quality data check
  - re-sampling and re-analysis to confirm EBT
  - quantify specific target compounds known to cause effects observed in bioassay.
  - continued monitoring (1 year; every 3 months) until bioassay response < 1*EBT
- 10*EBT< bioassay response < 100*EBT:
  - all actions indicated above
  - enhance source identification program
  - monitor distribution system close to point of exposure (to confirm attenuation of CEC and to confirm magnitude of safety factors associated with removal efficiency, dilution and post-treatment)
- bioassay response >100*EBT:
  - all actions indicated above
  - confer with the local environmental authority's to determine the required response action.
  - confirm plant corrective actions through additional monitoring

BioDetection Systems

Application of EBTs and action plan

Fold induction

	prim. sedimentation effluent	sec. sedimentation effluent	ozonation effluent	sand/anthracite filter	sand/BAC filter	constructed wetland 1
Cytotox CALUX	0.5	3.8	0.5	0.5	0.5	0.5
AR CALUX	120	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	1.2	0.5	0.5	0.5	0.5
ERa CALUX	5.0	50	3.5	16	11.9	1.5
GR CALUX	8.5	18	6.5	9.3	2.3	4.2
anti-PR CALUX	16	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	40	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	4.3	0.5	0.5	0.5	0.5	0.5
PXR CALUX	0.5	4.2	2.2	3.7	0.5	1.2
Nrf2 CALUX	19	9.0	5.8	4.1	4.2	2.7
P53 CALUX (+S9)	0.5	21	0.5	0.5	0.5	0.5

Low activity — High activity

EBTs

	prim. sedimentation effluent	sec. sedimentation effluent	ozonation effluent	sand/anthracite filter	sand/BAC filter	constructed wetland 1
Cytotox CALUX	LOQ	1.8	LOQ	LOQ	LOQ	LOQ
AR CALUX	155	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	4.8	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	0.040	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	400	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	1300	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	LOQ	25	12.0	19.0	LOQ	8.1
Nrf2 CALUX	760	180	110	77	79	51
P53 CALUX (+S9)	LOQ	10000	LOQ	LOQ	LOQ	LOQ

= CALUX eq. < EBT

= EBT<CALUX eq.<3*EBT

= 3*EBT<CALUX eq.<10*EBT

= 10*EBT<CALUX eq.<100*EBT

= CALUX eq.>100*EBT

Conclusions

- Application driven selection of relevant bioassays

- Optimisation of procedures for sample processing/analysis required

- Use of bioassay for monitoring efficiency and effectiveness of innovative, natural and engineered water treatment systems very promising

- In order to develop a regulatory excepted framework for water quality monitoring, the establishment of widely accepted trigger/threshold levels is required.

BioDetection Systems

Thank you for your attention.

AquaNES

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Advanced oxidation processes in AquaNES

		Site no. 6 Lange Erlen, CH	Site no. 7 Shafdan, IL	Site no. 12 Berlin-Schönerlinde, DE
	Water use	Drinking water production	Wastewater reuse	Wastewater discharge
Process	Engineered	UV+H_2O_2	O_3	O_3
	Natural	Managed aquifer recharge	Soil-Aquifer treatment (SAT)	constructed wetland & discharge
	Purpose	Organic micropollutant removal		
	Further aspects	bioassays to observe eco-toxicological effects		
			Improved process operation and control for optimisation of energy demand	
				Fate of antibiotic resistance gene

Trigger values

Parameter	BDS	Brand et al.	v.d. Oost et al	B. Escher
estrogens	1.1	3.8	1	3.1
androgens	31.8	11	-	14
glucocorticoids	56	21	30	150
progestins	0.462	333	-	-

2 – Comprehensive study

- temporal/spatial sampling
- cNES efficiency