



DEMEAU project highlights

- In vitro toxicity profiling
of water pollutants -

Eszter Simon et al.



Project



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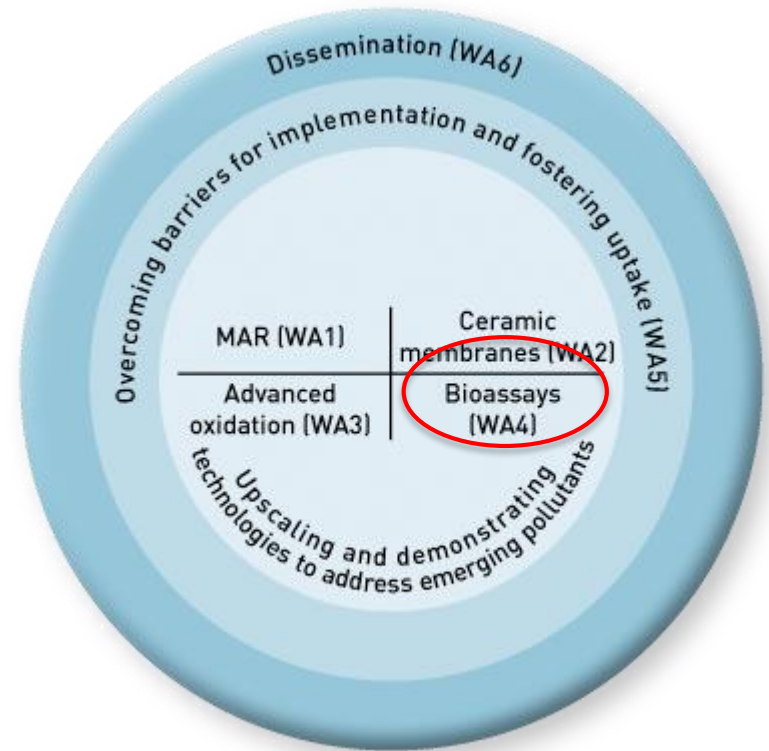
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Implementation of novel, rapid and quantitative bioassays for water quality monitoring

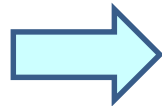


Key activities

WP1

Selection and validation

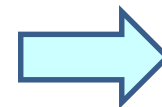
- ✓ Selection criteria
- ✓ Bioassay selection
- ✓ Automation
- ✓ Trigger values
- ✓ Validation



WP2

Implementation

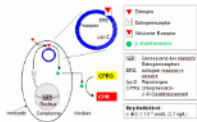
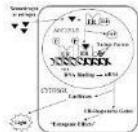
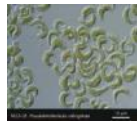
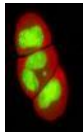
- ✓ Regulatory acceptance
- ✓ Testing framework
- ✓ Introduction to water utilities
- ✓ Demonstration



Market application



<http://cache.eb.com>



ARA Neuland

Selection and validation

WP1

- ✓ Selection criteria
- ✓ Bioassay selection
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- ✓ Validation



		Gene mutations (bacteria/yeast)				Mutations (mammals)		DNA replication		
		Ames test	Ames II/fluctuation test	Vibrio harveyi	MutaGen	Mitotic gene conversion assay	Mammalian gene mutation assays	Mouse Lymphoma Assay	Polymerase inhibition assay	ToxTracker
Assay applicability	Applied to environmental samples	3	3	3	1	3	2	2	1	1
	Validated to water samples	3	3	1	1	1	1	1	1	1
	Standardized protocol available/maturity	3	3	2	1	2	3	3	1	2
	Service and support available	1	3	1	1	1	1	2	1	3
	Costs	2	2	1	2	2	2	2	3	1
	Ease of use TOTAL	2	5	2	2	2	3	5	5	3
	Non-GMO	0	0	0	0	0	1	1	1	0
	No specialised skills/equipment required	1	1	1	1	1	1	1	1	0
	Automation possible	0	1	0	0	0	0	1	1	1
	Non-licensed (cell) in vitro model	1	1	1	1	1	1	1	1	0
	Kit available	0	1	0	0	0	0	1	1	1
Training availabilities	0	1	0	0	0	0	0	0	1	
Score		14	19	10	8	11	12	15	12	11
Assay performance	Selectivity	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Accuracy	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Reproducibility	1	2	NA	NA	NA	NA	NA	NA	3
	Robustness	3	3	NA	NA	NA	NA	NA	NA	3
	Sensitivity	2	2	2	2	NA	NA	3	NA	NA
	Specificity	1	1	NA	2	NA	1	1	NA	3
	LOD	1	2	1	NA	1	NA	2	NA	NA
	Cytotoxicity control	3	3	1	1	2	2	3	3	2
	Quick	2	2	2	3	2	1	1	3	2
	Clear/Straightforward read-out	2	3	2	3	2	2	1	3	3
High-throughput capacity	1	3	1	3	1	1	2	3	3	
Score		16	21	9	14	8	7	13	12	19
Total NA		2	2	5	5	6	6	4	7	4
Total score		32	42	24	27	25	25	32	31	34

- ✓ Selection criteria
- ✓ **Bioassay selection**
- ✓ Automation
- ✓ Trigger values
- ✓ Validation

Recommended assay panel

Toxic endpoints	Specific pathway	Promising in vitro bioassay(s)
Xenobiotic metabolism	PXR receptor agonists	HG5LN PXR assay, PXR HepG2 assay
	AhR receptor agonists	DR CALUX, AhR geneblazer
Hormone-mediated mode of action	(anti)estrogenic activity	ER α CALUX, YES assay
	(anti)androgenic activity	AR CALUX, AR-MDA-kb2
	(anti)glucocorticoid activity	GR CALUX, GR-MDA-kb2
Reactive mode of action/genotoxicity	Gene mutations	Ames fluctuation assay, ToxTracker
	Chromosomal mutations	Micronucleus assay, ToxTracker
	DNA damage response	UMUc assay, Vitotox, p53 CALUX (+/1S9), BlueScreen
Adaptive stress response	Oxidative stress pathway	Nrf2 CALUX, AREc32 assay
Developmental toxicity	Focus point endocrine disruption	Various nuclear receptor activation assays, H295R assay

<http://www.ecotoxcentre.ch/news-publications/reports>

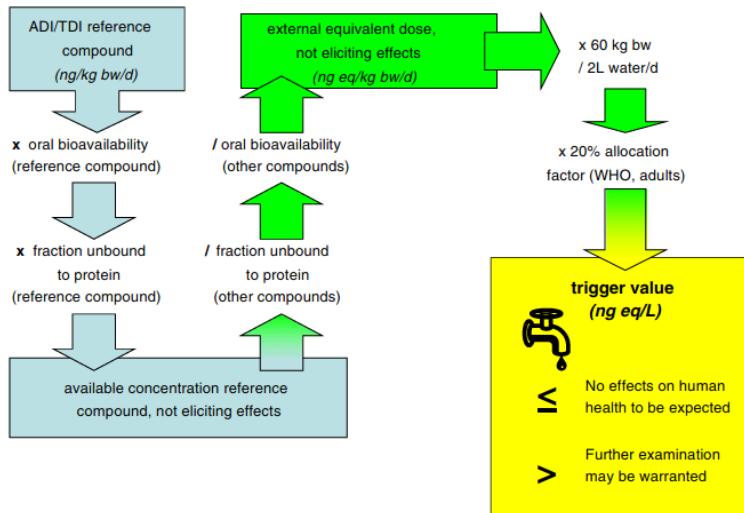


Selection and validation

WP1

- ✓ Selection criteria
- ✓ Bioassay selection
- ✓ Automation
- ✓ **Trigger values**
- ✓ Validation

Low / High risk



Assay	Human health relevant trigger values
ER α -CALUX	3.8 ng E2-eq / L
AR-CALUX	11 ng DHT-eq / L
GR-CALUX	21 ng DEX-eq / L
PR-CALUX	333 ng Org2058-eq / L



water^{net}

BDS
BioDetection Systems

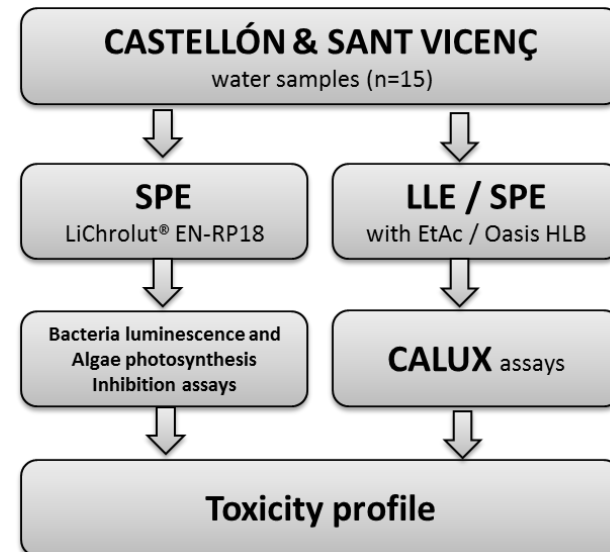
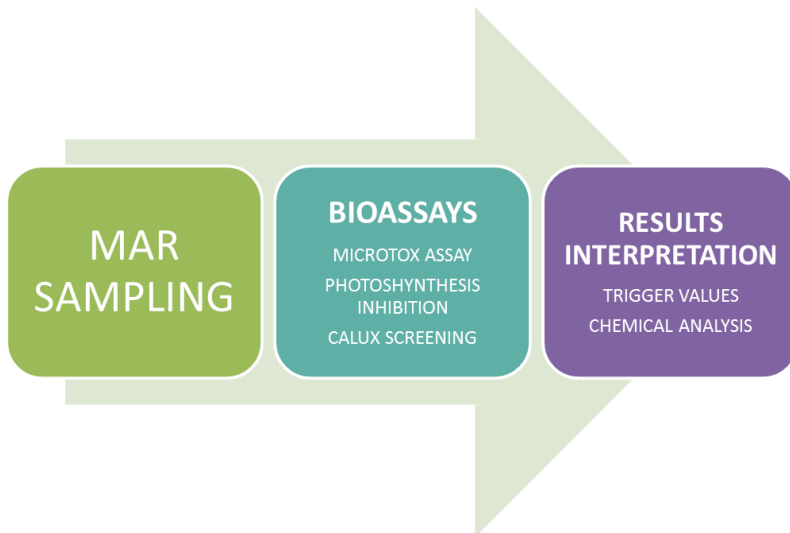
oekotoxzentrum
centre ecotox

Schweizerisches Zentrum für angewandte Ökotoxikologie
Centre Suisse d'écotoxicologie appliquée
Eawag EPFL

Assay	Ecosystem health relevant trigger values
Estrogenicity	0.4 ng E2-eq / L
Photosynthesis inhibition	20 ng Diuron-eq / L

- ✓ Regulatory acceptance
- ✓ Testing framework
- ✓ Introduction to water utilities
- ✓ **Demonstration**

I. Managed Aquifer Recharge (MAR)



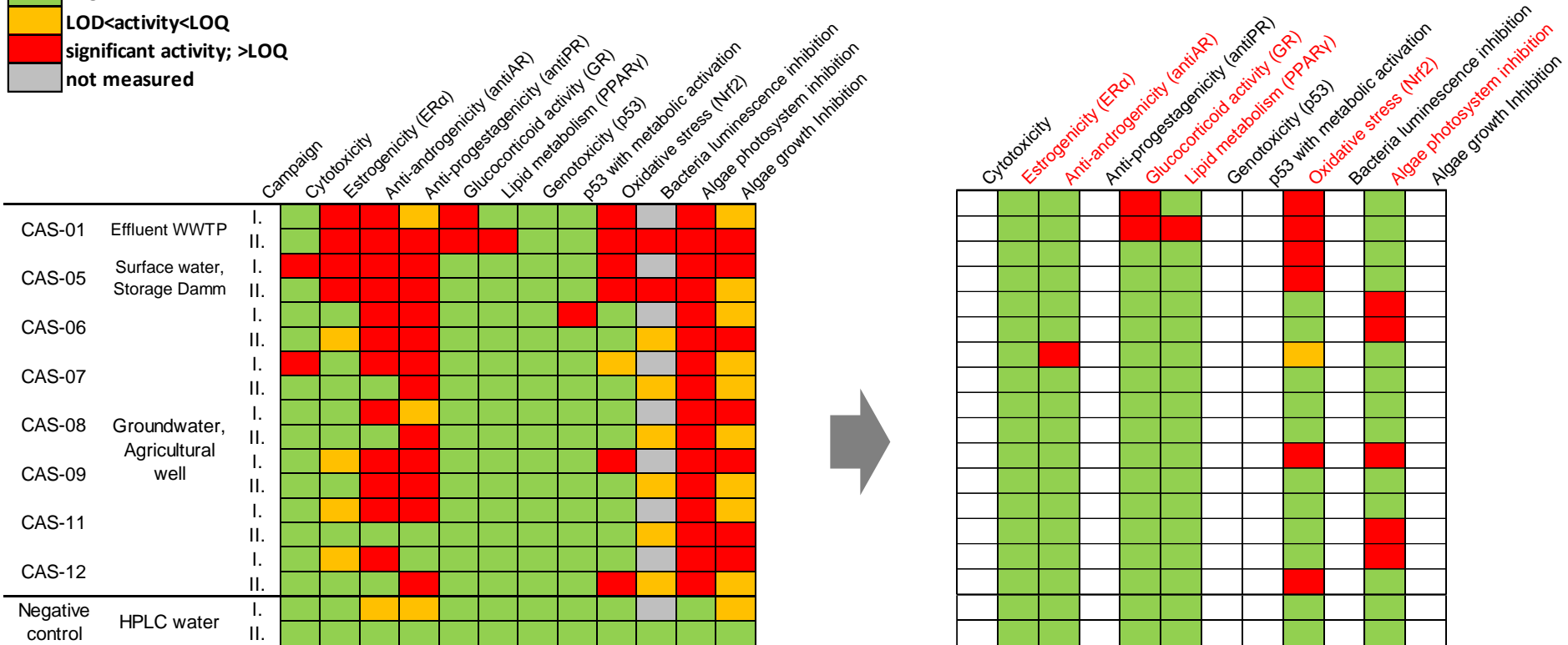
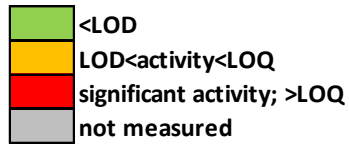
Implementation

WP2

- ✓ Regulatory acceptance
- ✓ Testing framework
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- ✓ Demonstration

Bioassay	Trigger value	Unit
ER α -CALUX:	1	ng 17 β -Estradiol-Eq / L
Anti-AR-CALUX:	40	μ g Flutamide-Eq / L
GR-CALUX	30	ng Dexamethasone-Eq / L
PPAR γ -CALUX	20	ng Rosiglitazone-Eq / L
Nrf2-CALUX	10	μ g Curcumin-Eq / L
Combined Algae Test (Photosystem II Inhibition)*	20 (EQS proposal CH), 200 (EQS EU)	ng Diuron-Eq / L

* For the "high/low risk evaluation" of the measured activities in the Combined algae assay the trigger value based on the EU EQS proposal was used and not based on the Swiss value.



- ✓ Regulatory acceptance
- ✓ Testing framework
- ✓ Introduction to water utilities
- ✓ **Demonstration**



II. WWTP Neugut

✓ 1st WWTP with full-scale ozonation in Switzerland



Ecotoxicological Evaluation



Pathway	<i>In vitro</i> assays		<i>In vivo</i> assays	
	Tool	Organisms	Observations	
Mutagenicity	Ames fluctuation assay	Primary producers (algae)	photosynthesis growth	
Genotoxicity	CALUX assays	Primary consumers (water flea)	reproduction	
Oxidative stress		Secondary consumers (fish)	hatching, swim-up, length, weight, changes in tissues and organs, expression of various pollutant-relevant genes	
Disturbance of lipid metabolism		Decomposers (luminescent bacteria)	general toxicity	
Various hormonal effects		Detritus feeders (oligochate worms)	reproduction	
Estrogenic effects	Yeast Estrogen Screen			





II. WWTP Neugut

Bioassay	Substance group (effect parameter)	Effect Biological treatment	Effect Ozonation	Effect Ozonation + Sand filtration	Effect Ozonation + GAC
YES	Estrogens (Estradiol equivalents, ng/L)	↓	↓	↓	↓
ER CALUX	Estrogens (Estradiol equivalents, ng/L)	↓	↓ (<LOD)	↓ (<LOD)	↓ (<LOD)
Anti-AR CALUX	Anti-Androgens (Flutamide equivalents, ng/L)	↓	var.	~	~
PR CALUX	Progesterons (Org-2058 equivalents, ng/L)	↓	↓ (<LOD)	↓ (<LOD)	↓ (<LOD)
Anti-PR CALUX	Anti-Progesterons (Ru486 equivalents, ng/L)	↓	~	↓	var.
PPARg1 CALUX	Peroxisome proliferator like acting substances (Rosiglitazone equivalents, ng/L)	↓	<LOD	<LOD	<LOD
Nrf2 CALUX	Substances inducing the adaptive stress response (Curcumin equivalents, µg/L)	↓	↓	↓	↓
PXR CALUX	Substances inducing the xenobiotic metabolism (Nocardipine equivalents, µg/L)	↓	~	~	↓ (<LOD)
Ames fluctuation assay	Mutagenic substances (increase in number of mutated bacteria colonies) (20fold concentrated sample)		Var.	Var.	↓
Bacteria luminescence inhibition	General toxicity (Baseline toxic equivalent concentrations, mg/L)	↓	↓	↓	↓
Green algae	Herbicides (Diuron equivalents, µg/L) (Photosynthesis inhibition)	↓	↓	↓	↓
	General Toxicity (baseline toxic equivalent conc., mg/L) (Growth inhibition)	↓	↓	↓	↓



Monitoring prioritized compounds

CAS numbers!

1	Alachlor
2	Atrazine
3	Benzene
4	Chlorfenvinphos
5	Chlorpyrifos-ethyl
6	1,2-Dichloroethane
7	Dichloromethane
8	Di(2-ethylhexyl)phthalate (DEHP)
9	Diuron
10	Fluoranthene
11	Isoproturon
12	Lead and its compounds lead chloride
13	Naphthalene
14	Nickel and its compounds nickel (II) chloride
15	4-n-octylphenol 4-tert-octylphenol
16	Pentachlorophenol
17	Simazine
18	Trichlorobenzenes
19	Trichloromethane = chloroform
20	Trifluralin

1	Anthracene
2	Benzo(a)pyrene
	Benzo(b)fluoranthene
	Benzo(g,h,i)perylene
	Benzo(k)fluoranthene
	Indeno(1,2,3-cd)pyrene
3	C10-13-chloroalkanes
4	Cadmium and its compounds <i>cadmium chloride</i>
	Endosulfan
6	Hexachlorobenzene
7	Hexachlorobutadiene
8	Hexachlorocyclohexane
10	<i>methylmercury(II) chloride</i>
	<i>mercuric chloride</i>
10	Nonylphenol technical mixture
11	Pentachlorobenzene
12	PBDE 100
	PBDE 47
13	Tributyltin-cation / hydride

1	AMPA
2	Bentazon
3	Bisphenol-A
4	Dicofol
5	EDTA
6	Free cyanide
7	Glyphosate
8	Mecoprop
9	Musk xylene
10	PCB118
	PCB126
	PCB128
	PCB156
11	PFOS
12	Quinoxyfen
13	TCDD

PRIORITY HAZARDOUS
n=13

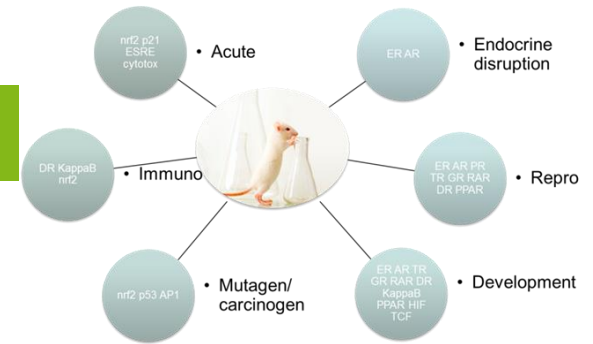
PRIORITY
n=20

UNDER REVIEW
n=13

DEMEAU + OTHERS

Pharmaceuticals, list of compounds provided by Swiss and Italian water companies

High-throughput screening (HTP)



AUTOMATED STEPS



SEEDING

Ready-to-seed frozen cells
384-well format



EXPOSURE

Dilution series
(13/compounds)



DETECTION

Luminescence
detection

25 CALUX ASSAYS

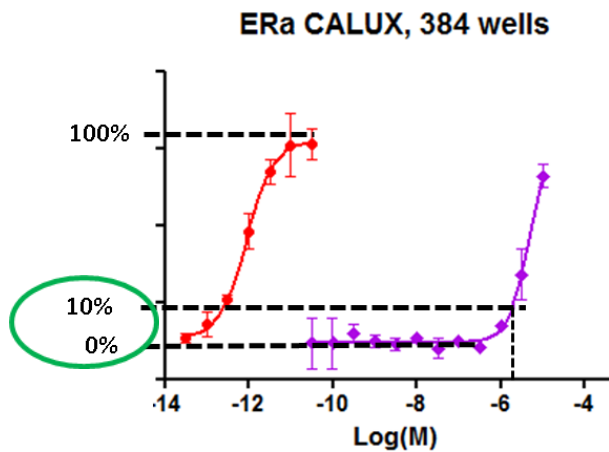
Duplicate measurement



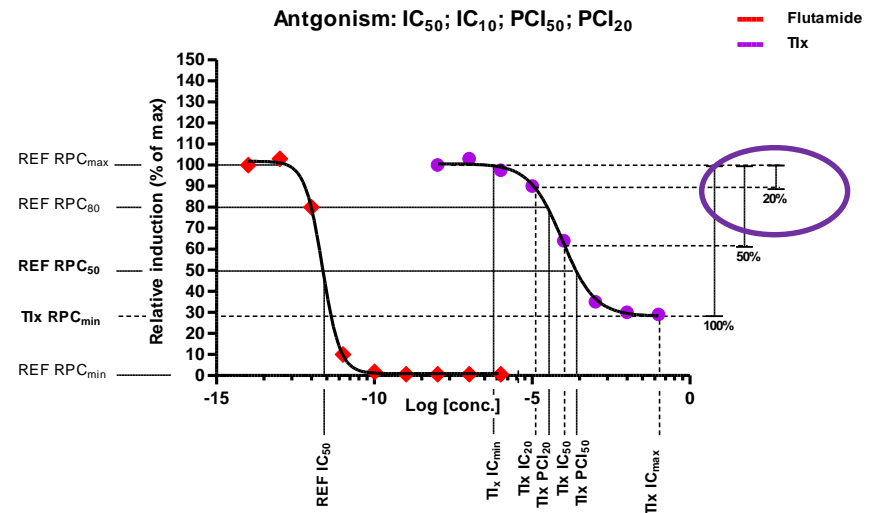
Data analysis

- ✓ TRIPLICATE MEASUREMENT → SD<20%
- ✓ REFERENCE CURVE
- ✓ CYTOTOXICITY

CURVE FIT → PC10 or PC20 (antagonist)



AGONIST



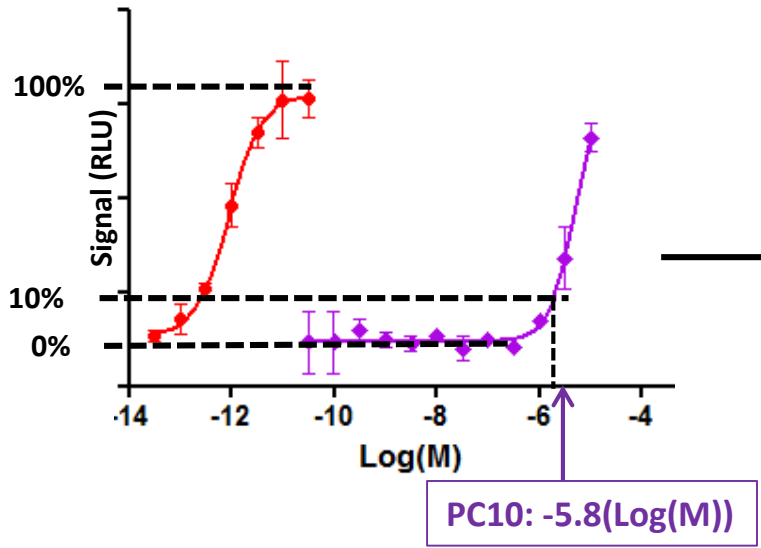
ANTAGONIST



Scoring example

reference compound (estradiol)
test compound

ERa CALUX, 384 wells

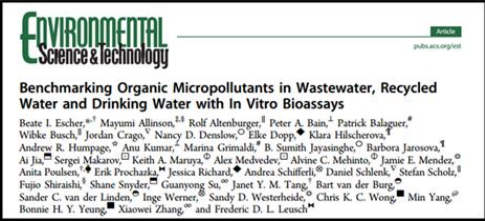


compound	Cytotox10%	Cytotox50%	ERa	ERa-anti	ERb	ERb-anti	AR	AR-anti	PR	PR-anti
cisplatin	-3.4	-3.2								
Clomiphene citrate	-5.5	-5.3		-9						
Clopyralid - 1			-3.2							
Clothianidin										-5.5
Test Compound	-3.9	-3.4	-5.8							
Copper chloride										
copper sulfate	-3.4	-3.2								
Corticosterone	-4	-3.4						-6.6		

$$REP = PC10_{ref. comp.} / PC10_{comp.}$$

✓ $PC10/20 < 1E-5M \rightarrow$ "Active"

Relevant toxic endpoints



Compound screening
DR-CALUX PXR-CALUX
ER α -CALUX antiAR-CALUX antiPR-CALUX
P53-CALUX
-
-
-
Cytotoxicity

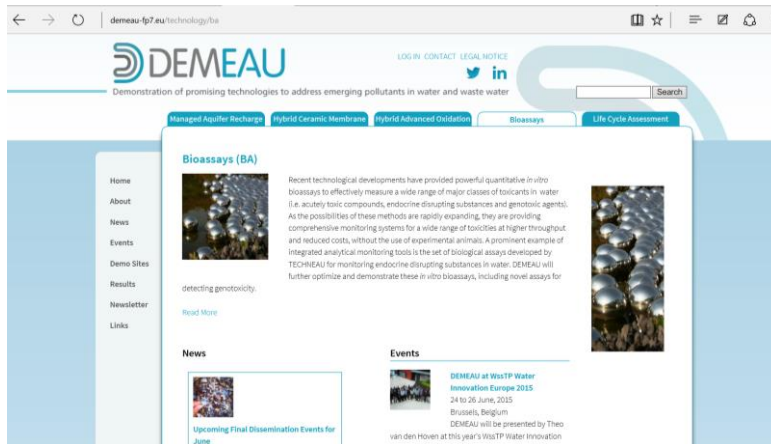


Case studies
Xenobiotic metabolism
Hormone-mediated MoA Era, GR, anti AR
Reactive MoA
Adaptive stress response
Developmental toxicity
Lipid metabolism
General response

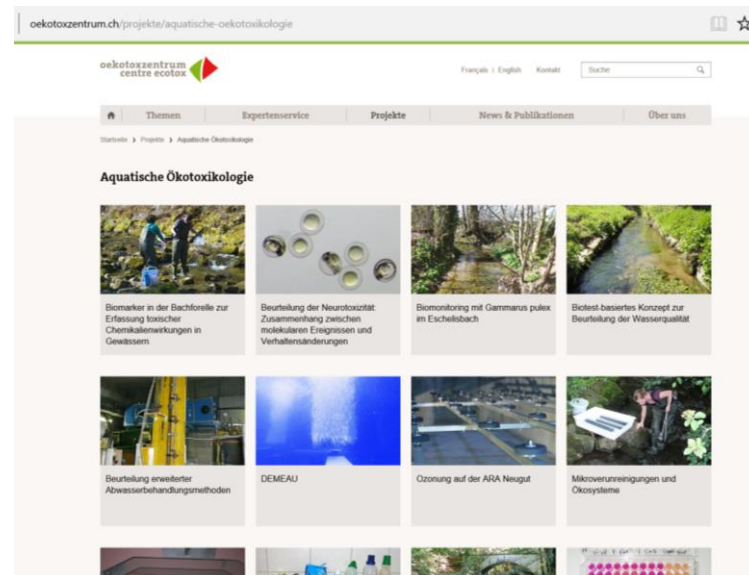




Thank you for your attention!



<http://demeau-fp7.eu/>



<http://www.oekotoxzentrum.ch/>