

Urban Water Security Research Alliance



Monitoring the Biological Activity of Micropollutants during Enhanced Wastewater Treatment Process

Miroslava Macova

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Jochen Mueller, EnTox, UQ
Juerg Keller, Julien Reungoat, AWMC, UQ**

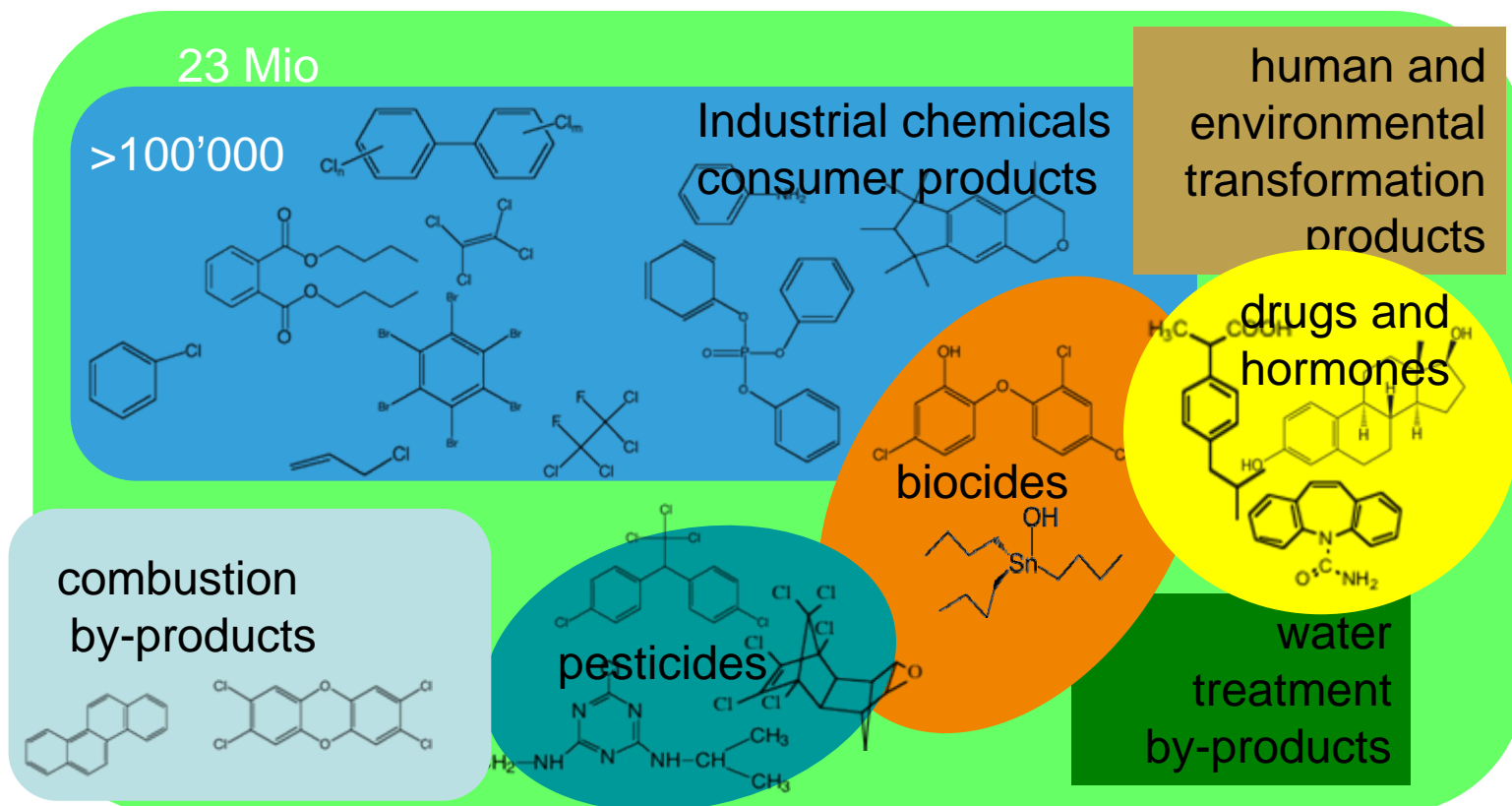
Enhanced Treatment Project

17 - 18 August 2009



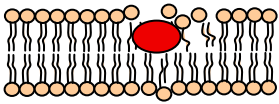
Why Bioanalytical Tools?

- There are too many chemicals out there to quantify them one-by-one
- Bioassays can be used as sum parameters indicating the overall toxic potential of an unknown chemical cocktail



Bioanalytical Test Battery

Non specific toxicity



Mode of action

baseline toxicity

Bioassay

Bioluminescence inhibition assay

Targeted chemicals

all chemicals

Phytotoxicity
photosynthesis inhibition

Imaging-PAM

Triazine and phenyl-
urea herbicides

estrogenic effects

E-SCREEN

Estrogens, estrogenic
industrial chemicals

binding to Ah receptor

AhR-CAFLUX

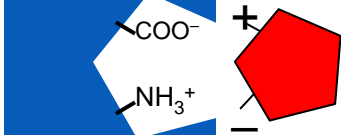
Polychlorinated
dibenzidioxins/furans,
PCB, PAH

acetylcholinesterase
(AChE) inhibition

AChE

Organophosphates,
carbamate insecticides

Receptor



Reactive toxicity



genotoxicity

umuC

Chlorinated byproducts,
Aromatic amines, PAH

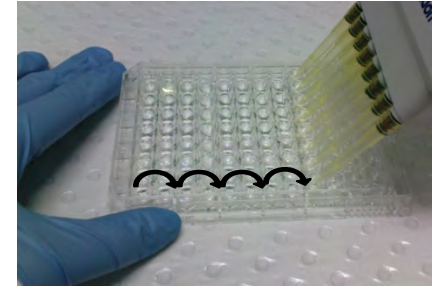
Relative Enrichment Factor - REF



SPE
enrichment →

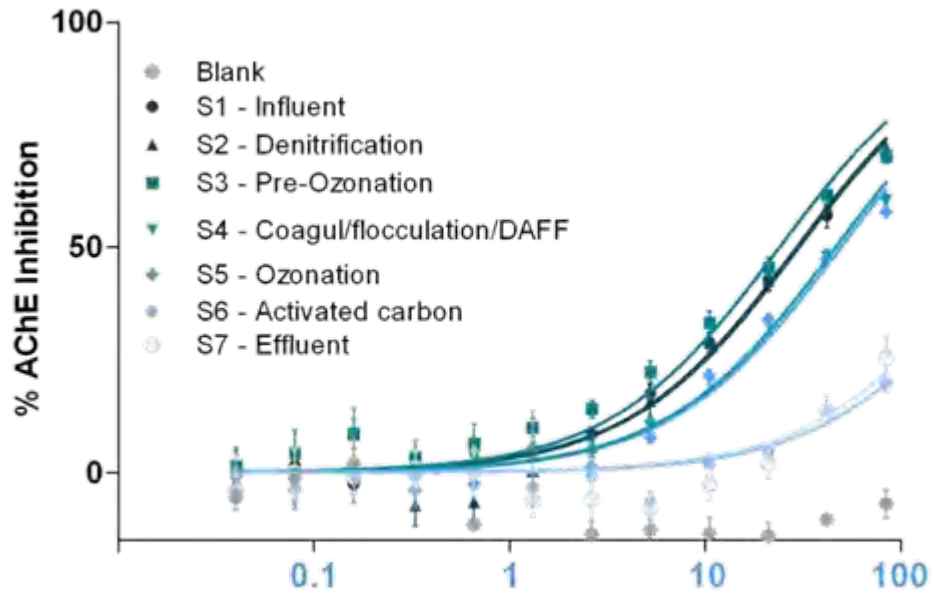


dilution →



$$\text{enrichment factor}_{\text{SPE}} = \frac{V_{\text{water}}}{V_{\text{extract}}}$$

$$\text{dilution factor}_{\text{assay}} = \frac{\text{volume of extract added to assay}}{\text{total volume of assay}}$$



Relative Enrichment Factor - REF



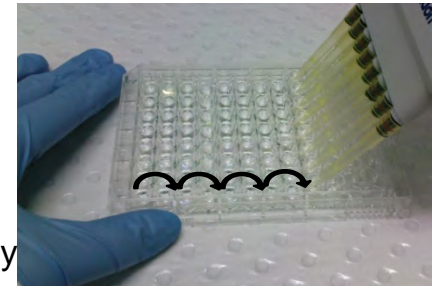
SPE
enrichment →

$$\frac{V_{\text{water}}}{V_{\text{extract}}}$$

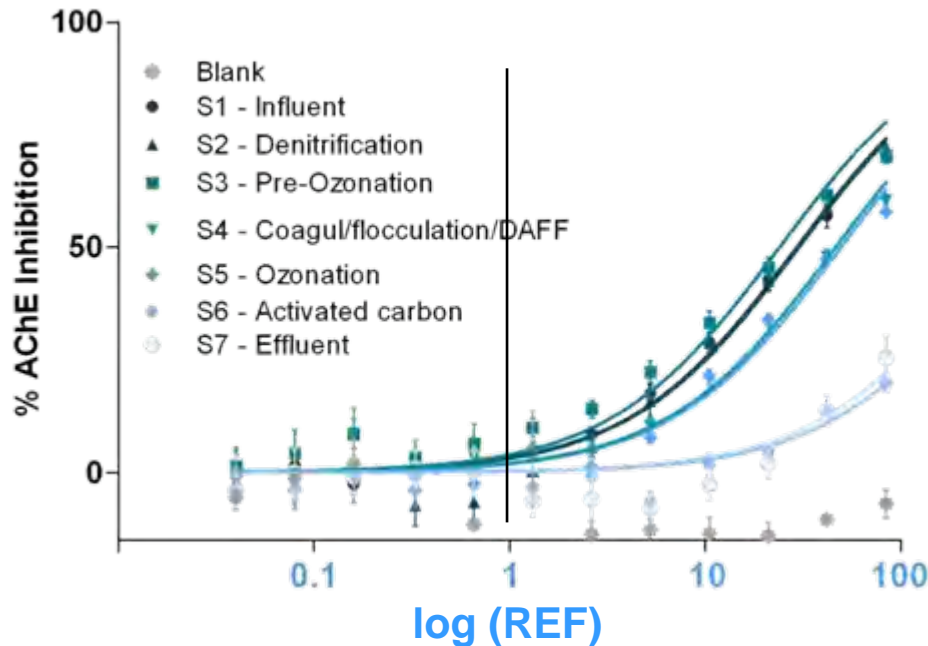


dilution →

$$\frac{\text{volume of extract added to assay}}{\text{total volume of assay}}$$



$$\text{REF} = \text{enrichment factor}_{\text{SPE}} \times \text{dilution factor}_{\text{assay}}$$



dilution ←

enrichment →

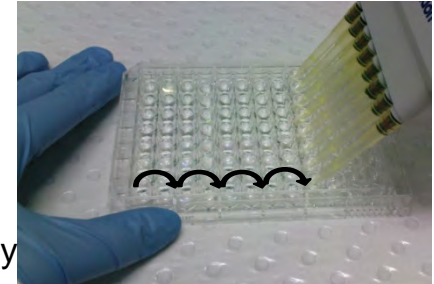
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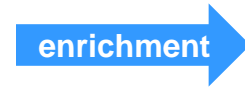
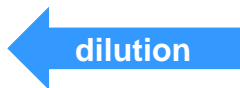
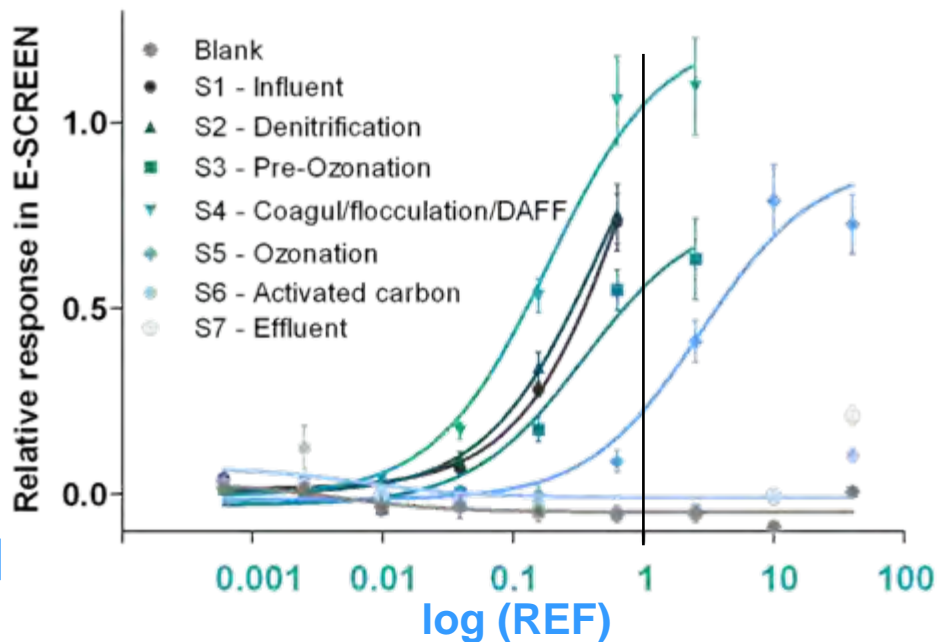
$$\frac{V_{\text{water}}}{V_{\text{extract}}}$$



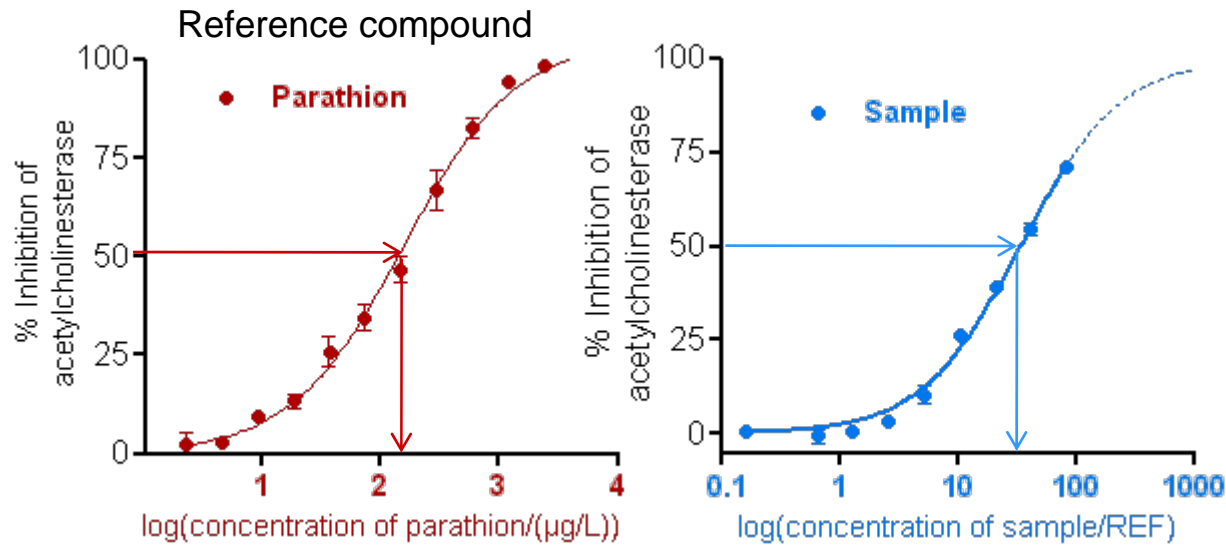
$$\frac{\text{volume of extract added to assay}}{\text{total volume of assay}}$$



$$\text{REF} = \text{enrichment factor}_{\text{SPE}} \times \text{dilution factor}_{\text{assay}}$$



Toxic Equivalent Concentration



$$\text{TEQ} = \frac{\text{EC}_{50} (\text{reference compound})}{\text{EC}_{50} (\text{sample})} = \frac{154.9 \mu\text{g/L}}{33.9 (\text{REF})} = 4.6 \mu\text{g/L}$$

Assay	Reference compound	Results expression
Baseline toxicity	Baseline toxicant	Baseline toxic equivalent concentration
E-SCREEN	17 β-Estradiol	Estradiol equivalent concentration
AhR CAFLUX	TCDD	TCDD equivalent concentration
I-PAM (phytotox)	diuron	Diuron equivalent concentration
AChE (neurotox)	parathion	Parathion equivalent concentration
umuC (genotox)	-	Concentration required to elicit genotoxic effect

Baseline-TEQ

EEQ

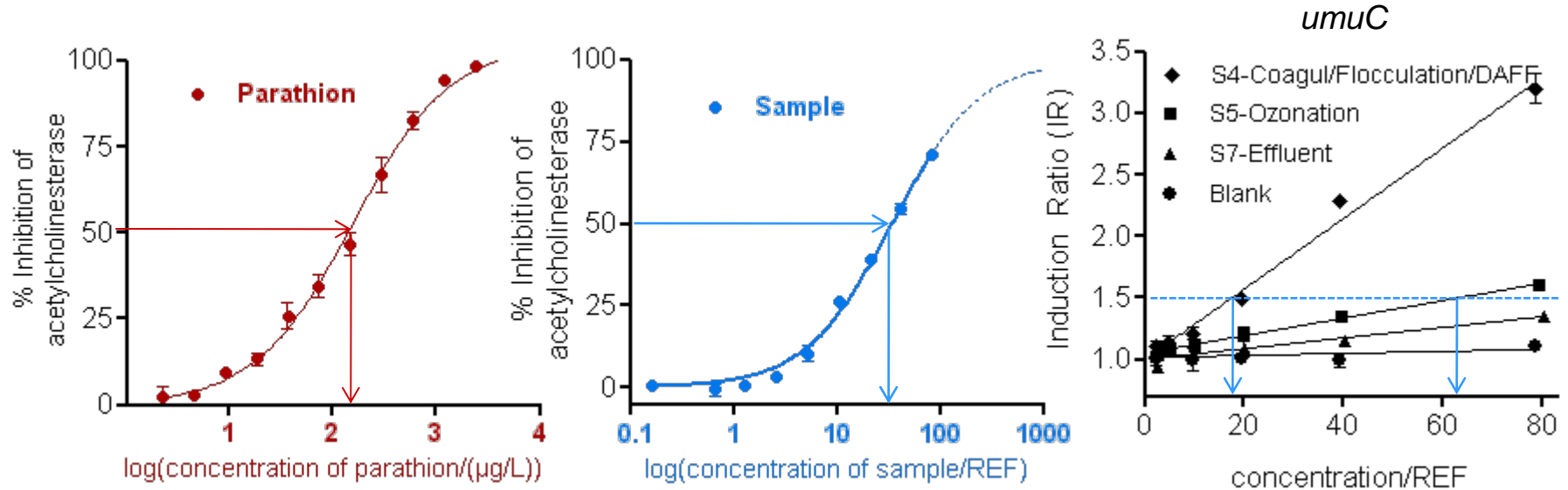
TCDDDEQ

DEQ

PTEQ

EC_{IR1.5}

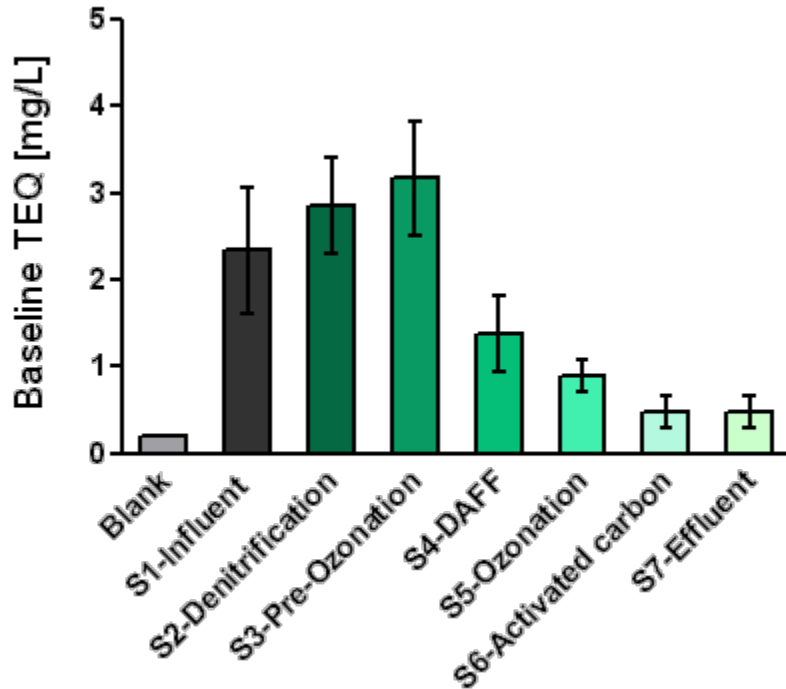
Toxic Equivalent Concentration



Effective concentration - $EC_{IR1.5}$

Assay	Reference compound	Results expression	
Baseline toxicity	Baseline toxicant	Baseline toxic equivalent concentration	Baseline-TEQ
E-SCREEN	17 β -Estradiol	Estradiol equivalent concentration	EEQ
AhR CAFLUX	TCDD	TCDD equivalent concentration	TCDD-TEQ
I-PAM (phytotox)	diuron	Diuron equivalent concentration	DEQ
AChE (neurotox)	parathion	Parathion equivalent concentration	PTEQ
<i>umuC</i> (genotox)	-	Concentration required to elicit genotoxic effect	$EC_{IR1.5}$

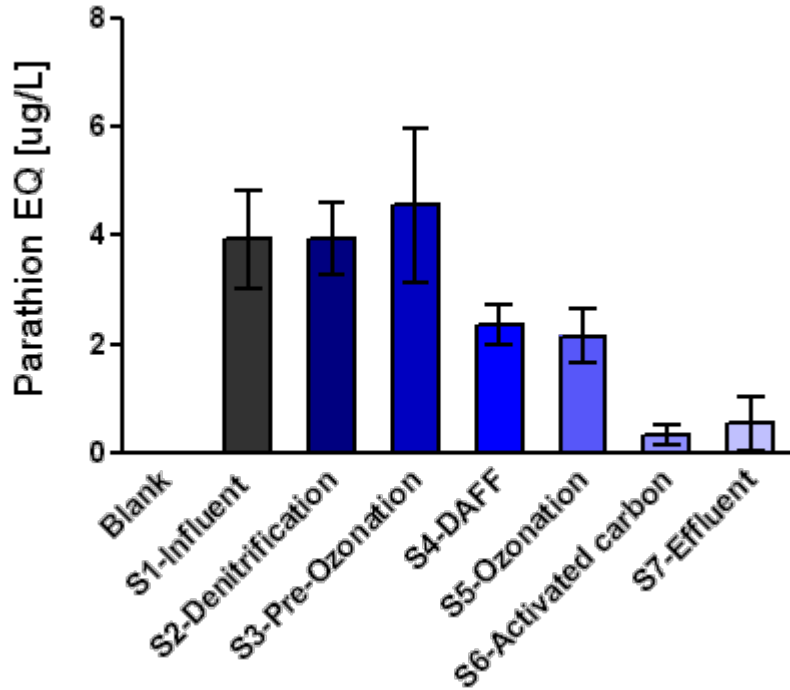
Baseline Toxicity



- Reduction in luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri*
- All chemicals

Site description	Baseline toxicity	Neuro-toxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
	Baseline-TEQ (mg/L)	PTEQ (µg/L)	DEQ (µg/L)	EEQ (ng/L)	TCDDEQ (ng/L)	1/EC _{IR1.5} (1/REF)
Blank	0.21 ± 0.01	<0.3	<0.01	<0.02	0.06 ± 0.01	<0.01
Influent (WWTP Effluent)	2.3 ± 0.4	3.2 ± 0.5	0.12 ± 0.1	6.0 ± 2.1	0.09 ± 0.03 ^b	0.21 ± 0.1
Denitrification	2.9 ± 0.5	3.3 ± 0.4	0.30 ± 0.1	8.7 ± 2.7	0.10 ± 0.01 ^b	0.16 ± 0.02
Pre-ozonation	3.2 ± 0.7	4.2 ± 1.2	0.34 ± 0.2 ^a	3.5 ± 1.3	0.11 ± 0.01 ^b	0.17 ± 0.06
Coagulation/ Flocculation/DAFF	1.4 ± 0.5	2.1 ± 0.4	0.05 ± 0.04	9.8 ± 1.3 ^a	0.11 ± 0.04 ^b	0.08 ± 0.06^a
Main ozonation	0.9 ± 0.2^{ab}	1.9 ± 0.5^a	0.03 ± 0.03	0.4 ± 0.2^a	0.09 ± 0.02 ^b	0.02 ± 0.01 ^a
Activated carbon	0.5 ± 0.2 ^{ab}	<0.3	0.02 ± 0.02	<0.02	0.10 ± 0.06 ^b	<0.01
Effluent	0.5 ± 0.2 ^{ab}	<0.3 ^d	0.02 ± 0.01	<0.06	0.10 ± 0.01 ^b	<0.01

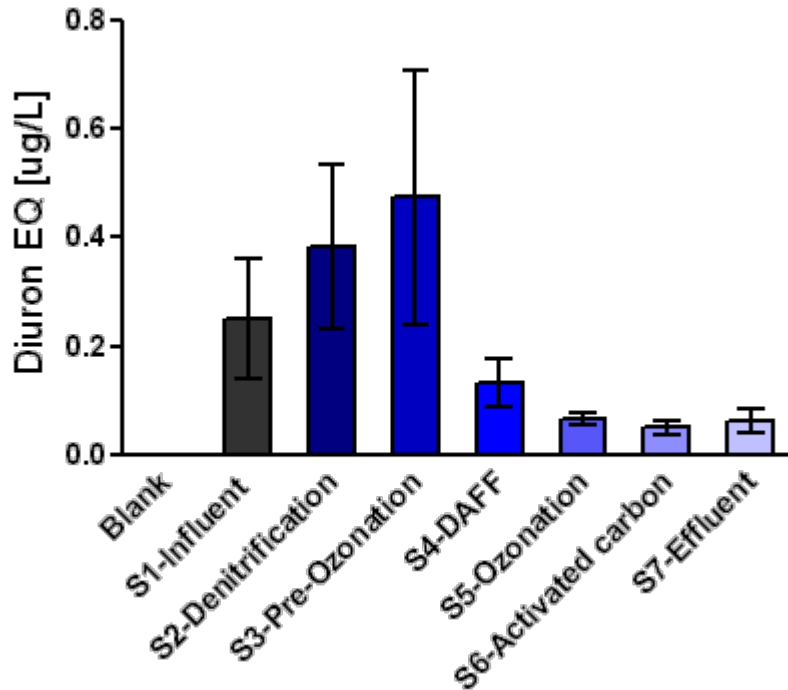
Neurotoxicity



- Acetylcholinesterase inhibition assay
- organophosphates and carbamate insecticides
- Detection limit
AChE: 0.3 ug/L
GCMS: 0.1 ug/L

Site description	Baseline toxicity	Neurotoxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
	Baseline-TEQ (mg/L)	PTEQ (μg/L)	DEQ (μg/L)	EEQ (ng/L)	TCDDEQ (ng/L)	1/EC _{IR1.5} (1/REF)
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Activated carbon	0.5 ± 0.2 ^{ab}	<0.3	0.02 ± 0.02	<0.02	0.10 ± 0.06 ^b	<0.01
Effluent	0.5 ± 0.2 ^{ab}	<0.3 ^d	0.02 ± 0.01	<0.06	0.10 ± 0.01 ^b	<0.01

Phytotoxicity



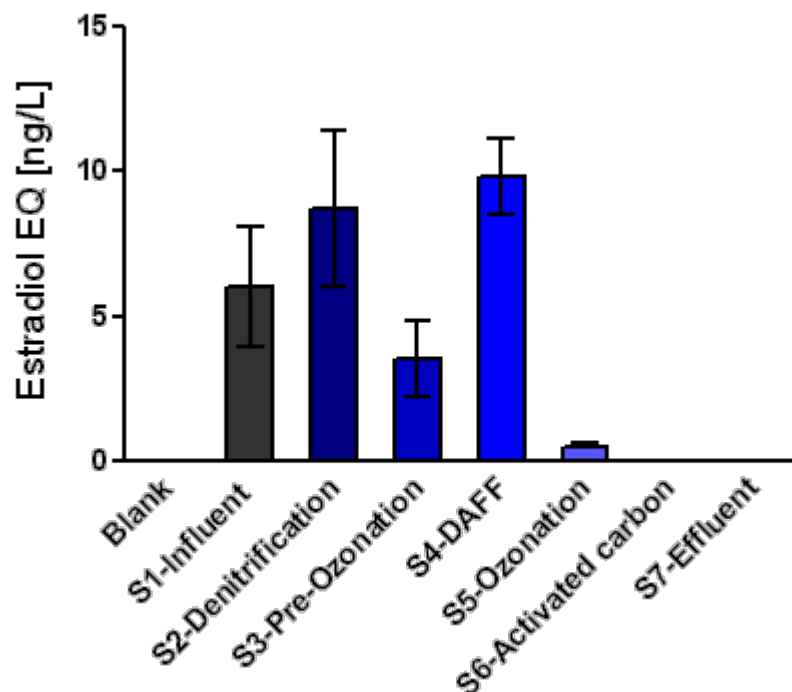
- I-PAM
- PSII derived photosynthesis inhibition
- PSII herbicides
- Detection limit

I-PAM: 0.01 µg/L

LCMS: 0.01 µg/L

Site description	Baseline toxicity	Neuro-toxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
	Baseline-TEQ (mg/L)	PTEQ (µg/L)	DEQ (µg/L)	EEQ (ng/L)	TCDDEQ (ng/L)	1/EC _{IR1.5} (1/REF)
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Activated carbon	0.5 ± 0.2 ^{ab}	<0.3	0.02 ± 0.02	<0.02	0.10 ± 0.06 ^b	<0.01
Effluent	0.5 ± 0.2 ^{ab}	<0.3 ^d	0.02 ± 0.01	<0.06	0.10 ± 0.01 ^b	<0.01

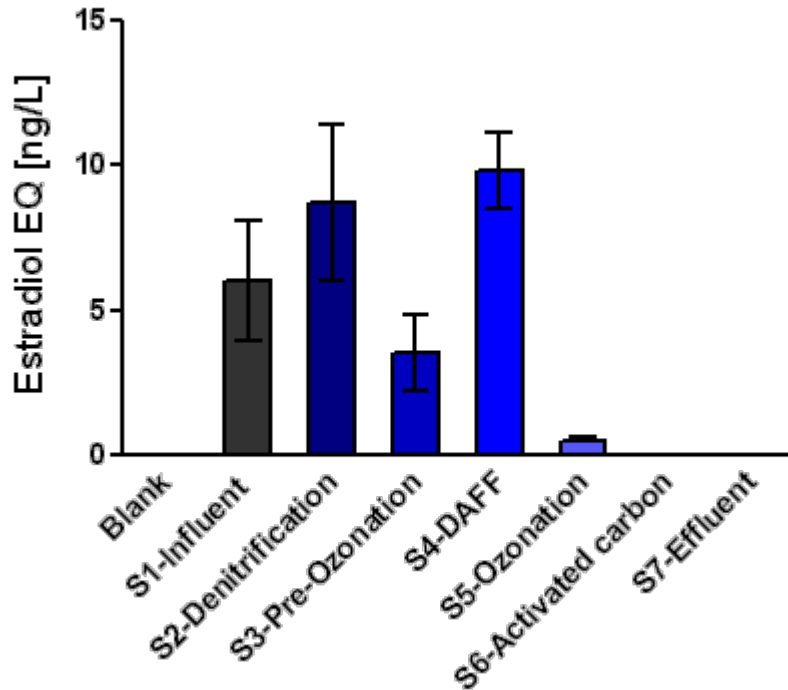
E-SCREEN



- Cellular proliferation in estrogenic dependent cell line
- Estrogens and estrogenic industrial chemicals
- Detection limit
E-SCREEN: 0.02 ng/L
GCMS: 1 ng/L

Site description	Baseline toxicity	Neurotoxicity	Phytotoxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
	Baseline-TEQ (mg/L)	PTEQ ($\mu\text{g/L}$)	DEQ ($\mu\text{g/L}$)	EEQ (ng/L)	TCDDEQ (ng/L)	1/EC _{IR1.5} (1/REF)
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Activated carbon	0.5 \pm 0.2 ^{ab}	<0.3	0.02 \pm 0.02	<0.02	0.10 \pm 0.06 ^b	<0.01
Effluent	0.5 \pm 0.2 ^{ab}	<0.3 ^d	0.02 \pm 0.01	<0.06	0.10 \pm 0.01 ^b	<0.01

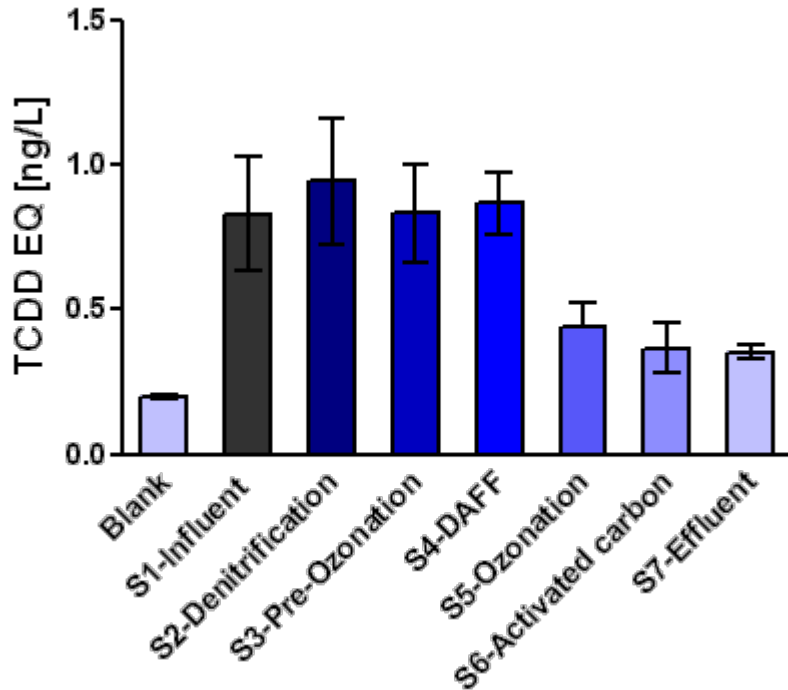
E-SCREEN



- <1 - 7.8 ng/L Germany, **E-SCREEN** (Körner et al., 2001)
- 0.8 - 5.9 ng/L Switzerland, **YES** (Escher et al., 2009)
- <1 - 4.2 ng/L Australia and NZ, **receptor binding assay** (Leusch et al., 2006)
- <1 - 16 ng/L the Netherlands, **ER-CALUX** (Murk et al., 2002)
- 1 – 67 ng/L Australia, **E-SCREEN** assay (Tan et al., 2007)

Site description	Baseline toxicity	Neurotoxicity	Phytotoxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
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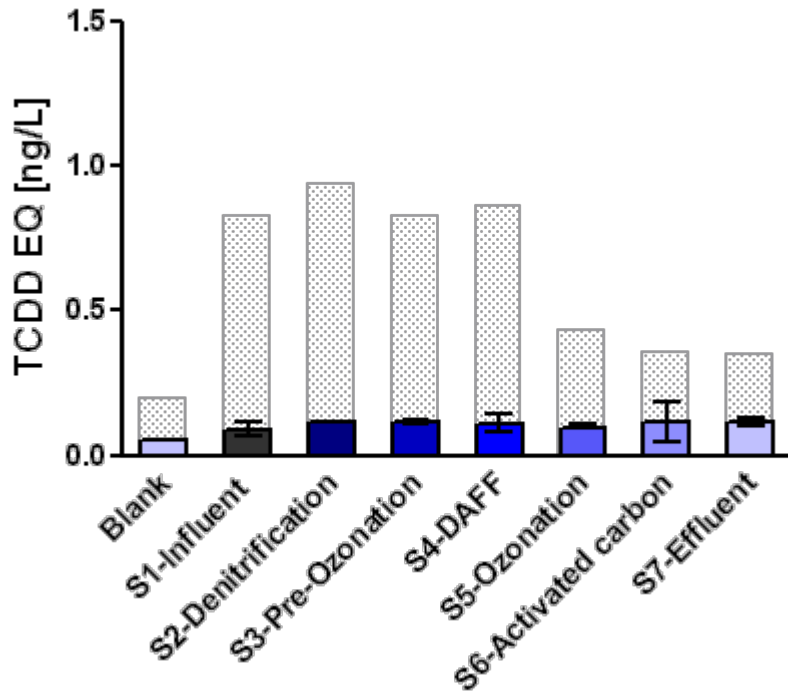
AhR-CAFLUX



- Induction of green fluorescent protein under the control of aryl hydrocarbon receptor (AhR)
- polychlorinated dibenzodioxins
polychlorinated dibenzofurans
Polychlorinated biphenyls
polycyclic aromatic hydrocarbons

Site description	Baseline toxicity	Neuro-toxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
	Baseline-TEQ (mg/L)	PTEQ ($\mu\text{g/L}$)	DEQ ($\mu\text{g/L}$)	EEQ (ng/L)	TCDDEQ (ng/L)	1/EC _{IR1.5} (1/REF)
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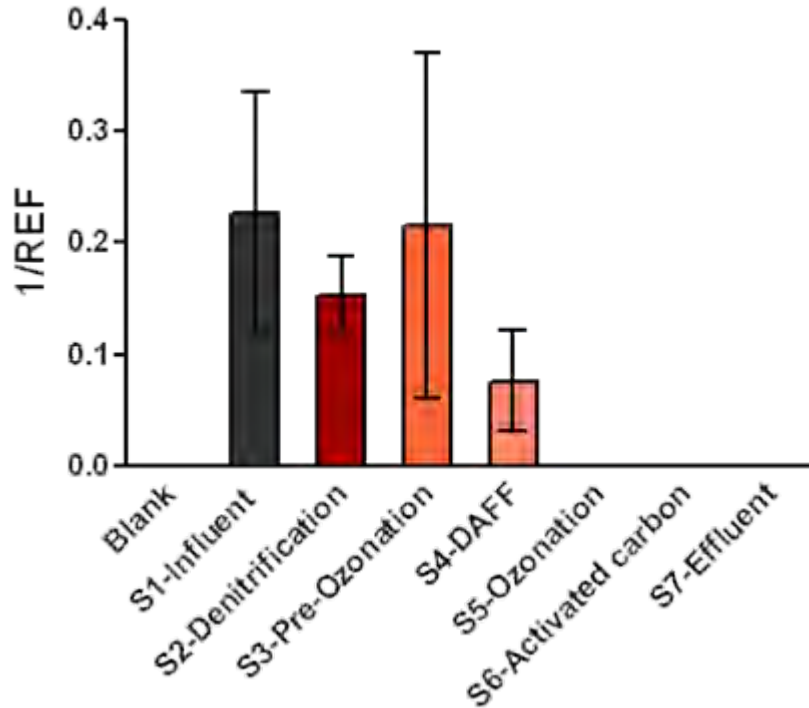
AhR-CAFLUX



- **Sulphuric acid silica gel clean-up:** most organic chemicals (e.g. PAH) except persistent chemicals have been removed

Site description	Baseline toxicity	Neuro-toxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
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Genotoxicity



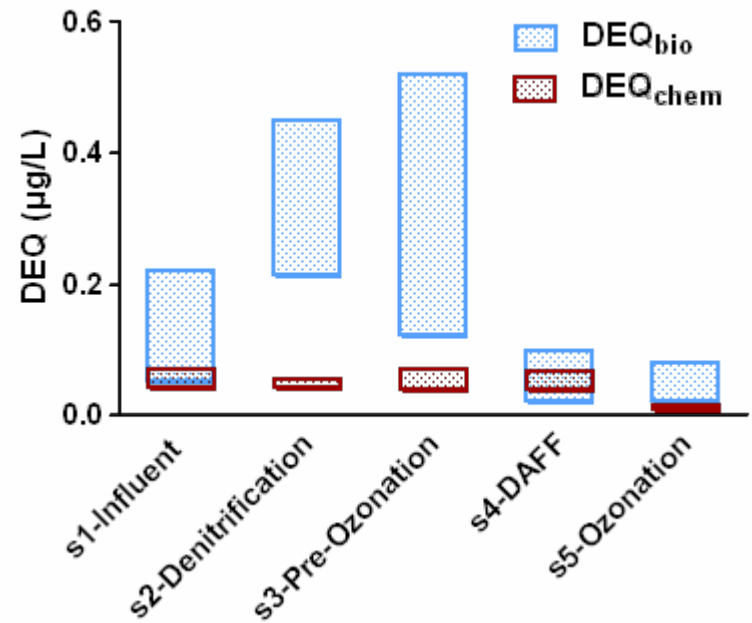
- Activation of the repair genes after DNA damage
- Chlorinated byproducts
aromatic amines
polycyclic aromatic hydrocarbons

Site description	Baseline toxicity	Neuro-toxicity	Phyto-toxicity	E-SCREEN	AhR-CAFLUX	Genotoxicity
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How much toxicity can we explain?

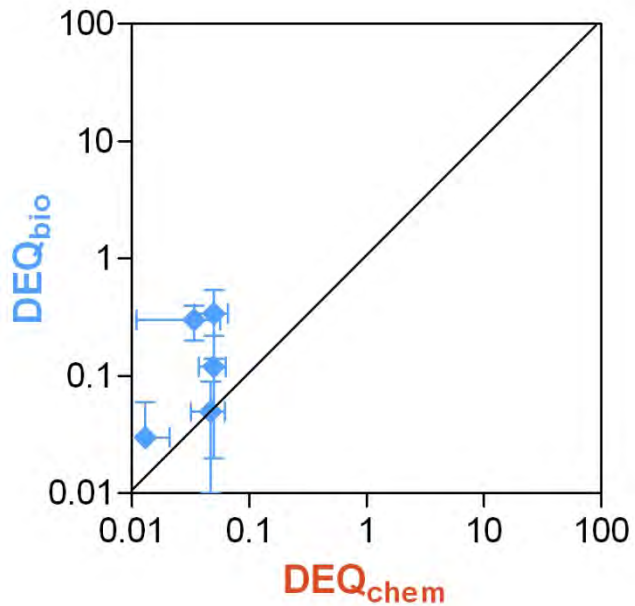
I-PAM assay/herbicides

	Relative potency
diuron	1
simazine	0.15
atrazine	0.15
Desisopropyl atrazine	0.003

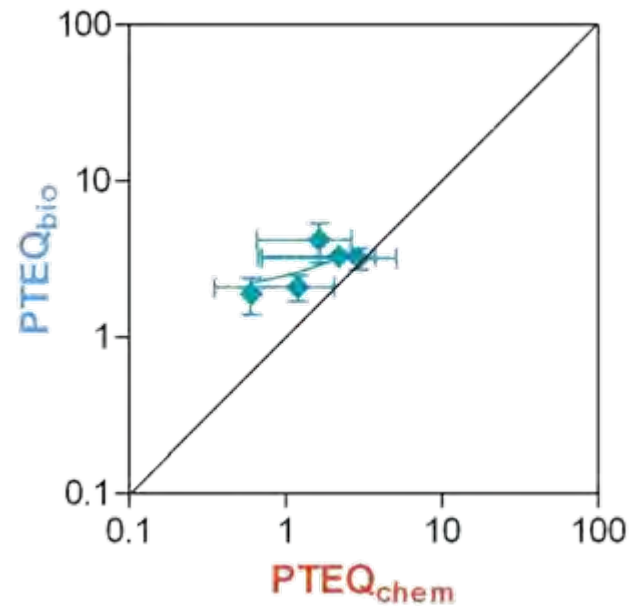


How much toxicity can we explain?

I-PAM assay/herbicides



AChE assay/
organophosphates



based on Chlorpyrifos
quantified by chemical
analysis

Conclusions

- Combination of nonspecific and selective bioassays provide information about **mixture effects of micropollutants** in WWTP
- Test battery is **low-cost, high throughput and robust**
- **Sensitivity high enough** to allow measurements in influent and effluent
- Limitations
 - A. not all chemicals extracted by **SPE**
 - B. Contribution of **organic matter** to toxicity
- Bioanalytical tools provide **complementary information to analytical chemistry** about water quality and water treatment process

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Thank you

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