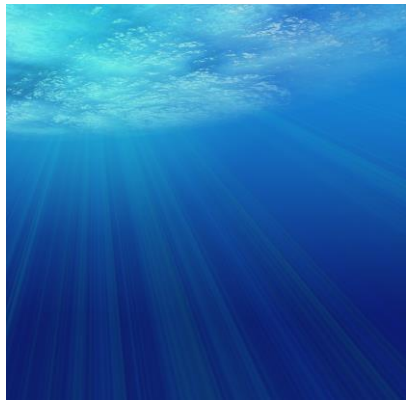


Bioassay Science and Policy Forum

BLUE WATER 2022

Program

16:00 to 16:05 - Introduction about "**Bioassay Science and Policy Forum"**

16:05 to 16:10 - Dutch Bioassay BLUE Water Strategy (Dr. Tessa Pronk, KWR)

16:10 to 16:15 – Australian bioassay experiences (Dr. Anu Kumar, CSIRO)

16:15 to 16:20 – Toxicity profiling of wastewater (Dr. Heidi Schaar, TU Vienna)

16:20 to 16:25 – PFAS water monitoring (Harrie Besselink, BDS)

16:25 to 16:30 – Antibiotic water monitoring (Dr. Tjalf de Boer, MLS)

Bioassay Science & Policy Development: Mission & Milestones

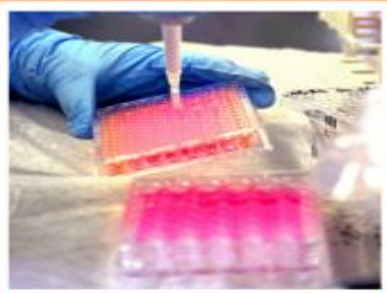
Assay [W = Water]; [F= Food] [C= Chemicals]	Assay ready [year]	1st Paper [year]	EU Project [year]	Crisis Intern. [year]	National Guideline [year]	Intern. Guideline [year]	Trigger value (WFD)	Regulatory Testing [year]	Time [years]
Dioxins/PCB (Food)	1993	1995	2005	1995		2001	2001	2001	8
Dioxins (Water)	1993	2017	2012		2005-NL	2023-ISO			**29**
Estrogens (W)	1998	2000	2006		2001-NL	2018-ISO			**24**
Anti-Androgens (W)	2005	2008	2006			2020-OECD			**17**
Glucocorticoids (W)	2005	2008	2006						**17**
Genotoxicity p53 (C)	2010	2014	2012						**12**
Ox. Stress Nrf2 (C)	2010	2014	2012						**12**
PXR (W)	2014	2019	2016						**8**
PAH (C)	2013	2014	2007						**9**
PFAS (W)	2020	2021	2021	2000					**2**

Others: **[ECVAM DB-ALM Nr. 197 (2013; rev. 2019): Automated CALUX bioassay]; [Japan IS 463 & US-EPA 4435: Dioxins soil-sediment]**

EU application projects: Difference 2005; Techneau 2006; FaceIt 2007; Demeau 2012; AquaNes 2016; Promisces 2021

13TH BIODETECTORS CONFERENCE 2022

PRAHA - CZECH REPUBLIC
13-14 SEPTEMBER 2022



You are invited to attend and actively participate (Presentation or Poster).

The meeting will bring together scientists involved in the development and application of in vitro effect-based bioanalysis technologies

Venue location:
Czech Association of Scientific Societies
Novotného lávka 5, 110 00 Praha 1

Registration and Participation
For registration and further info please contact either:
info@bds.nl or ***info@arnika.org***

Registration fee per person:
100 € / day professionals
75 € / day for students and post-docs
50 € for the dinner

How to get there:
By train: Prague Main Station
By plane: Prague International Airport

Recommended Hotels:
Hotel 1: Motel One Prague
Hotel 2: Hotel Mercure Prague
Hotel 3: Hotel Adler

BDS BioDetection Systems

BERTHOLD LC Tech ARNIKA

A Bioassay-track to determine waterquality in five classes

Sleutelfactor toxiciteit 2

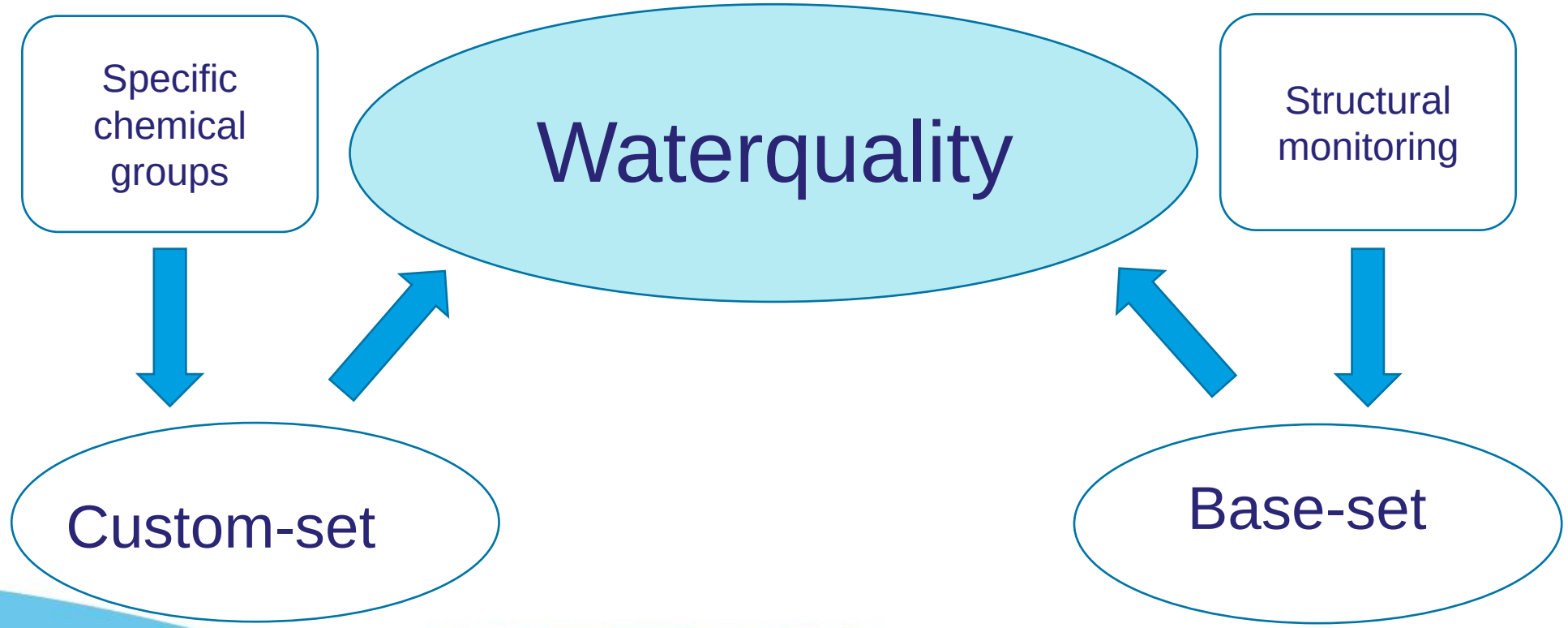
Tessa Pronk (KWR water)

With work of oa Milo de Baat (KWR), Sanne van den Berg (WenR), Leo Posthuma (RIVM), Ron van der Oost (Waternet), Milou Dingemans (KWR)

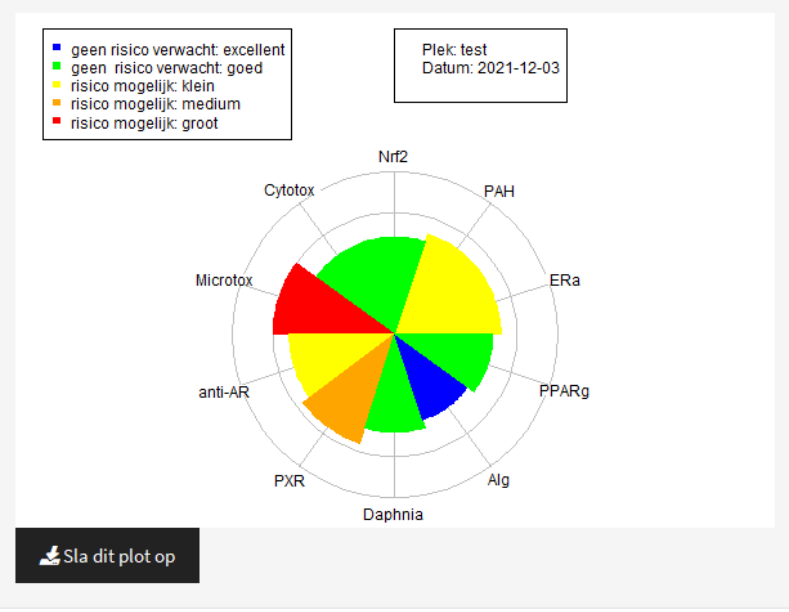
The ‘Bioassay-track’ is embedded in a framework; from drivers of pollution to abatement measures

De sleutelfactor Toxiciteit biedt waterbeheerders hulpmiddelen om problemen met chemische verontreinigingen te signaleren, vervolgens systematisch te onderzoeken, en tenslotte goede maatregelen te nemen. Het eindresultaat is een genuanceerd inzicht in de locaties en stofgroepen die de waterkwaliteit het meest bedreigen, samengevat in een vijfklassen-systeem.”

Bioassay-track (incl. background providers and protocols)



Base-set: Waterquality in five colours



Colours based on distance from ‘Trigger value’

- Per chemical
- Provides most sensitive mode of action and CALUX Analogue

Custom-set: selection

Bioassay Selectie Tool | Visualisatie | Informatie

Bioassay Selectie Tool

Voor de Bioassay Selectie Tool is het casnummer van de stof een vereiste input. Aan de hand daarvan worden de bioassays van de ToxCast database getoont welke een meetbaar response hebben laten zien voor deze stof. Kijk bijvoorbeeld eens naar de stoffen tolazoline (casnummer 59-97-2), rotenone (83-79-4) of chlorpyrifos (2921-88-2)

casnummer stof

59-97-2

Werkingsmechanisme beschrijft het werkingsmechanisme van de bioassay, en kan gebruikt worden om geschikte bioassays te vinden in de Bioassay Database

AC50 geeft de concentratie (in ug/L) aan die 50% van de maximale response veroorzaakt, dus hoe lager de AC50 waarde, hoe gevoeliger de bioassay

ToxCast_Bioassay geeft de naam van de bioassay weer, en kan gebruikt worden om achtergrondinformatie en links naar werkprotocollen over de assay te vinden op deze website

CALUX_analoog geeft aan welke CALUX assay waarschijnlijk een analoog is voor de ToxCast bioassay. **Let op,** de concentratie waarbij een response wordt gevonden kan afwijken!

Simpele uitkomst

Het meest gevoelige ToxCast werkingsmechanisme is **Horm_ER_Rec**
Voor dit werkingsmechanisme is **ER.CALUX als CALUX analoog beschikbaar**
Het meest gevoelige ToxCast werkingsmechanisme met beschikbare CALUX analoog is **Horm_ER_Rec**
Hiervoor is de **ER.CALUX** als CALUX analoog beschikbaar

Uitgebreid resultaat

Werkingsmechanisme	AC50	ToxCast_Bioassay	CALUX_Analoog
Horm_ER_Rec	3.07e-06 1.47e-05 1.07e-04	TOX21_ERa_LUC_VM7_Agonist TOX21_ERa_BLA_Agonist_ch2 TOX21_ERa_BLA_Agonist_ratio	ER.CALUX NA ER.CALUX
Cell_viab	0.0551 0.0817 0.0836	TOX21_RT_HEPG2_FLO_00hr_ctrl_viability TOX21_RT_HEPG2_FLO_16hr_ctrl_viability TOX21_RT_HEPG2_FLO_08hr_viability	NA NA NA
Met_PPAR_Rec	33.3	TOX21_PPARd_BLA_Antagonist_ch2	NA

Conclusion

- Base-set for monitoring
- Custom-set for specific chemical groups
- Interpretation / selection: application
- Embedded in framework (‘Status’ and ‘Impact’ part)

- Perfect? Improve via ‘Community of Practice’
- Further experience is needed



Australian bioassay experiences

Dr. Anu Kumar

Leader Environmental Toxicology and Chemistry Team

Land and Water Business Unit

AUSTRALIA'S NATIONAL SCIENCE AGENCY CSIRO

Adelaide

LAND AND WATER FLAGSHIP

www.csiro.au

Australian Government

Government of South Australia

Department of Environment, Water and Natural Resources

CSIRO

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 9999, No. 9999, pp. 1–11, 2014

Printed in the USA

Environmental Toxicology

ASSESSMENT OF MULTIPLE HORMONAL ACTIVITIES IN WASTEWATER AT DIFFERENT STAGES OF TREATMENT

PETER A. BAIN,* MIKE WILLIAMS, and ANU KUMAR
Commonwealth Scientific and Industrial Research Organisation Land and Water, Glen Osmond, South Australia, Australia

(Submitted 12 February 2014; Returned for Revision 15 March 2014; Accepted 24 June 2014)

Abstract: Changes in the endocrine potency of municipal wastewater at 3 wastewater treatment plants (WWTPs) in Australia were investigated using a panel of in vitro receptor-driven transactivation assays. The assays were based on human estrogen receptor α, androgen receptor, progesterone receptor, glucocorticoid receptor, and peroxisome proliferator-activated receptor γ2. Total removal efficiencies for estrogenic activity in the dissolved phase were 79.8% to 99.4%. Chemical analysis of 17β-estradiol, estrone, and 17α-ethinylestradiol levels showed that they accounted for the majority of the observed in vitro estrogenic activity in the final effluents but only 18% to 70% of estrogenic activity in the influents. Removal efficiency for androgenic activity was 97.5% to 100%. Endocrine activity levels were low in the final effluent of the WWTP with the lowest catchment population, with only estrogenic activity detected. In the final effluent of the WWTP with an intermediate catchment population, estrogenic, glucocorticoid, and PPARγ2 activities were detected. Estrogenic, antiandrogenic, progestagenic, glucocorticoid, and peroxisome proliferator activities were detected in the final effluent of the WWTP with the highest catchment population. The present study confirms the efficacy of secondary and tertiary treatment in reducing the concentrations of endocrine-active compounds in municipal wastewater. Further work is required to determine the possible health risks to aquatic biota posed by multiple hormonal activities present at low levels. *Environ Toxicol Chem* 2014;9999:1–11. © 2014 SETAC

Keywords: Endocrine-disrupting compounds Effects-based monitoring Emerging pollutants In vitro bioassays

Chemosphere 198 (2018) 565–573

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

In vitro nuclear receptor inhibition and cytotoxicity of hydraulic fracturing chemicals and their binary mixtures

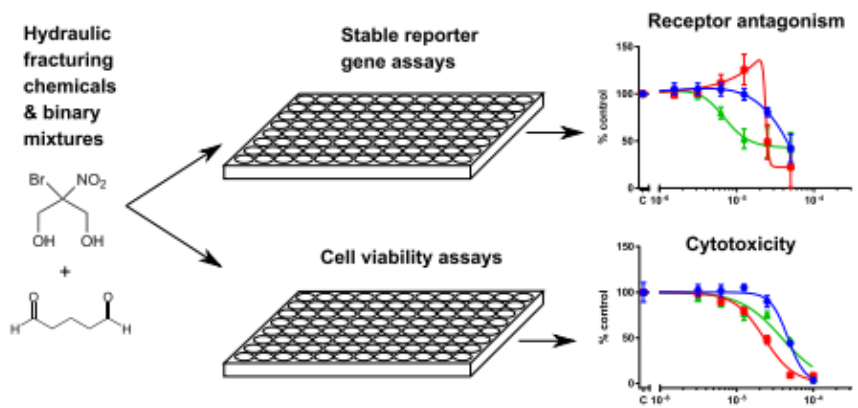
Peter A. Bain*, Anu Kumar*

CSIRO Land & Water, Locked bag 2, Glen Osmond, SA 5064, Australia

HIGHLIGHTS

- Six hydraulic fracturing chemicals were screened for nuclear receptor activity.
- Cytotoxicity was assessed in parallel in order to validate antagonism.
- No receptor agonism was observed for the chemicals tested.
- Receptor antagonism by three chemicals was mostly due to cytotoxic effects.
- Once chemical, THPS, inhibited receptor signalling at sub-cytotoxic concentrations.

GRAPHICAL ABSTRACT



Journal of Hazardous Materials 404 (2021) 124135

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Using bioanalytical tools to detect and track organic micropollutants in the Ganga River near two major cities

Peter A. Bain [a], Adrienne Gregg [a], Alok K. Pandey [b], Mohana Krishna Reddy Mudiam [c], Peta A. Neale [d], Anu Kumar [a,*]

[a] CSIRO Land and Water, Locked Bag 2, Glen Osmond 5062, South Australia, Australia
[b] Nanomaterial Toxicology Group, CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhavan, 31, Mahatma Gandhi Marg, Lucknow, Uttar Pradesh 226001, India
[c] CSIR-Indian Institute of Chemical Technology, Analytical & Structural Chemistry Department, Uppal Road, Tarnaka, Hyderabad, Telangana 500007, India
[d] Australian Rivers Institute, School of Environment and Science, Griffith University, Southport 4222, Queensland, Australia

ABSTRACT

Major rivers in India are subject to ongoing impacts from urban drain discharges, most of which contain high levels of domestic and industrial wastewater and stormwater. The aim of the present study was to determine the levels of bioactive organic micropollutants at the discharge points of major urban drains in comparison to upstream and downstream sites. To achieve this, we employed a panel of *in vitro* bioanalytical tools to quantify estrogenic, androgenic, progestogenic, glucocorticoid and peroxisome proliferator-like activity in water extracts collected from two Indian cities in the Ganga Basin. Cytotoxicity of the water extracts in a human-derived cell line and the potential to cause oxidative stress in a fish cell line were also investigated. We found high levels of activity for all endpoints in samples directly receiving urban drain discharge and low levels at sites upstream from drain discharges. Estrogenicity was detected at levels equivalent to 10 ng/L 17β-estradiol, representing a high likelihood of biomarker effects in fish. Sites located downstream from drain discharges exhibited low to intermediate activity in all assays. This study demonstrates the importance of managing urban drain discharges and the utility of applying bioanalytical tools to assess water quality.

TECHNISCHE UNIVERSITÄT WIEN

Toxicity profiling of wastewater treatment

Bioassay Science und Policy Forum“ 7 April 2022

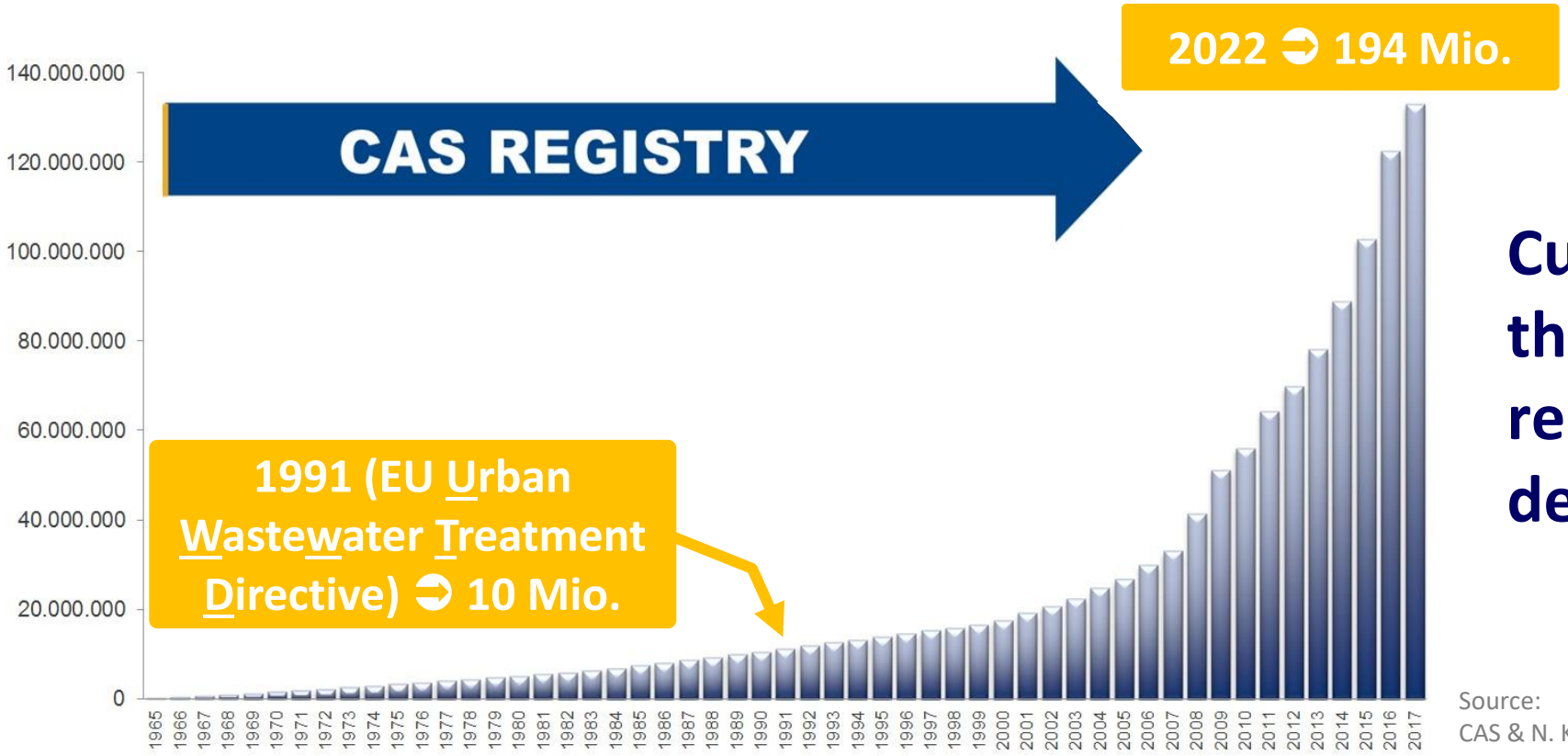
➲ BLUE WATER

Heidi Schaar, Norbert Kreuzinger

TU Wien

Why bioassays in the field of wastewater treatment?

Wastewater is such a complex environmental sample that chemical analysis alone cannot reveal its chemical burden…



Current revision of the UWWTD recognizes that development

Source:
CAS & N. Kreuzinger

Our reasons for the application of in vitro bioassays

- **assessment of treatment technology ("product"/effluent quality)**
  - conventional (= biological) treatment
  - ➲ toxicity integrated in a currently discussed UWWTD revision
  - advanced treatment implemented for micropollutant abatement
- **assessment of process performance (treatment efficiency)**

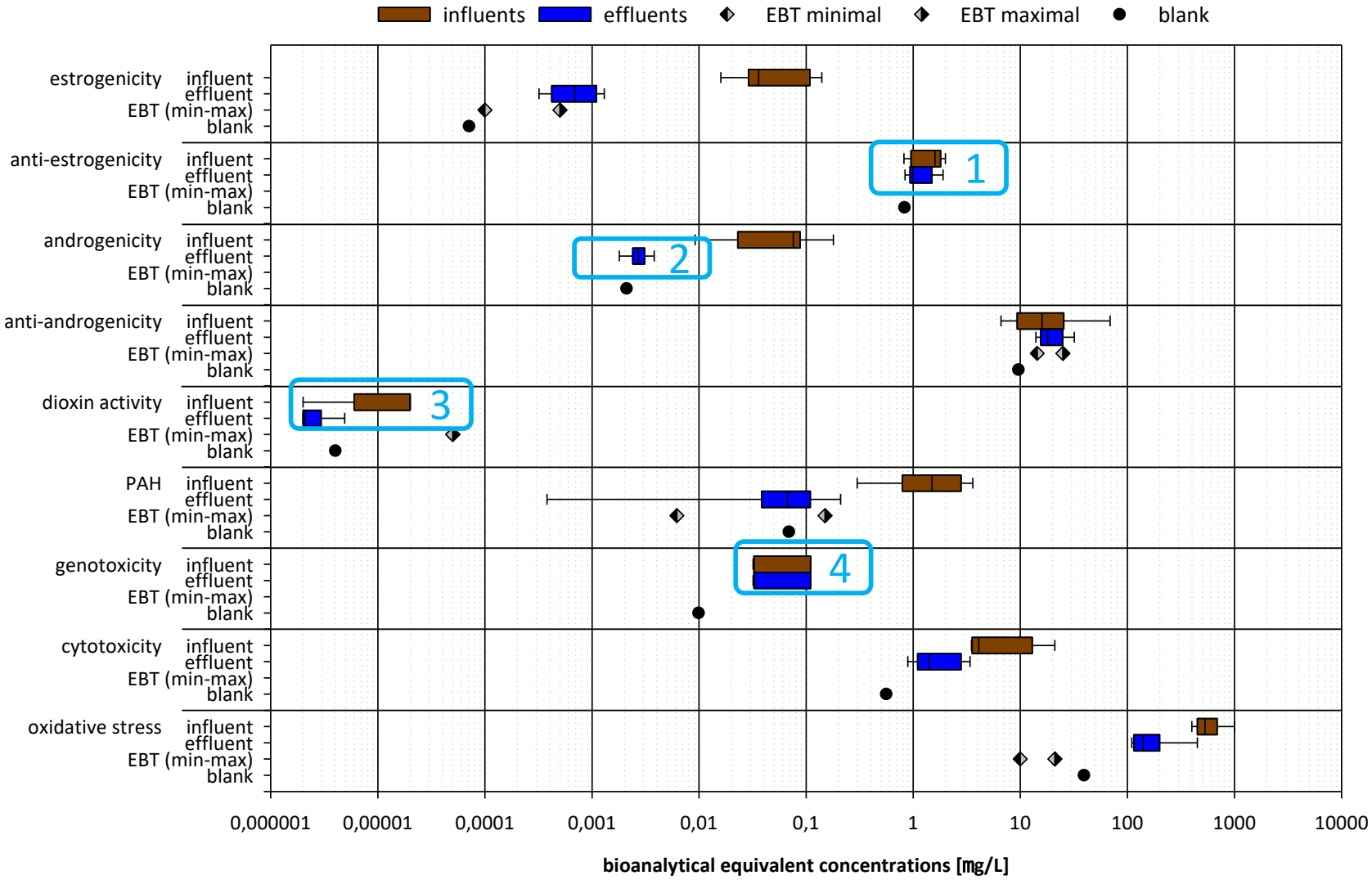
➲ two approaches

  - decrease in toxicity
  - comparison with environmental *effect-based trigger values* (EBT)
- **future compliance assessment**
  - EBTs as treatment goals ?

Our experience with in vitro bioassays

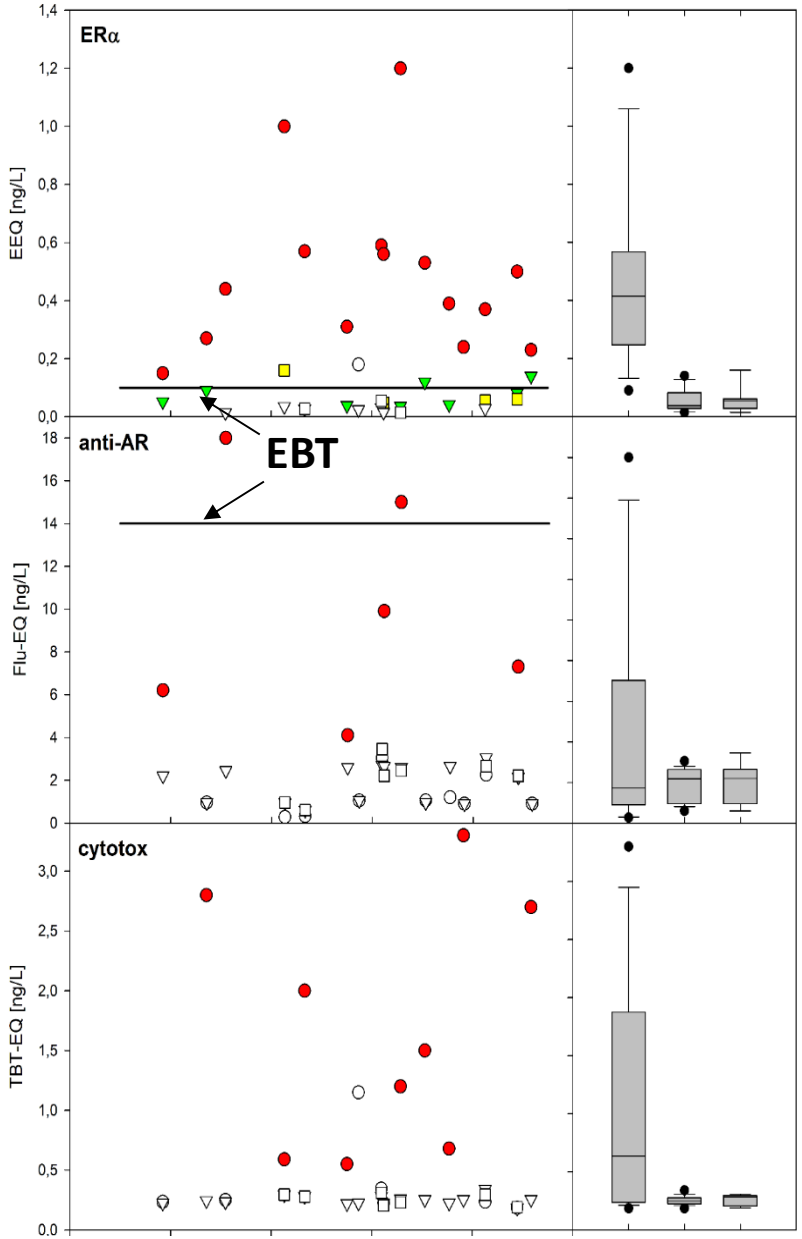
- investigated treatment technologies
  - **conventional** wastewater treatment according to BAT (best available technology in Austria: C / N / P removal)
  - **advanced** treatment
    - ozonation / ozonation & activated carbon treatment
    - new powdered activated carbon-based product
- (surface water)
- future plan ➲ wastewater of industrial park

Conventional wastewater treatment ➲ CALUX RESULTS



For results below LOQ:
LOQ was taken as „result“

1. **anti-estrogenicity:**
   all results < LOQ
2. **androgenicity:**
   all effluent results < LOQ
3. **dioxins:**
   13/18 samples < LOQ
4. **genotoxicity:**
   all samples < LOQ



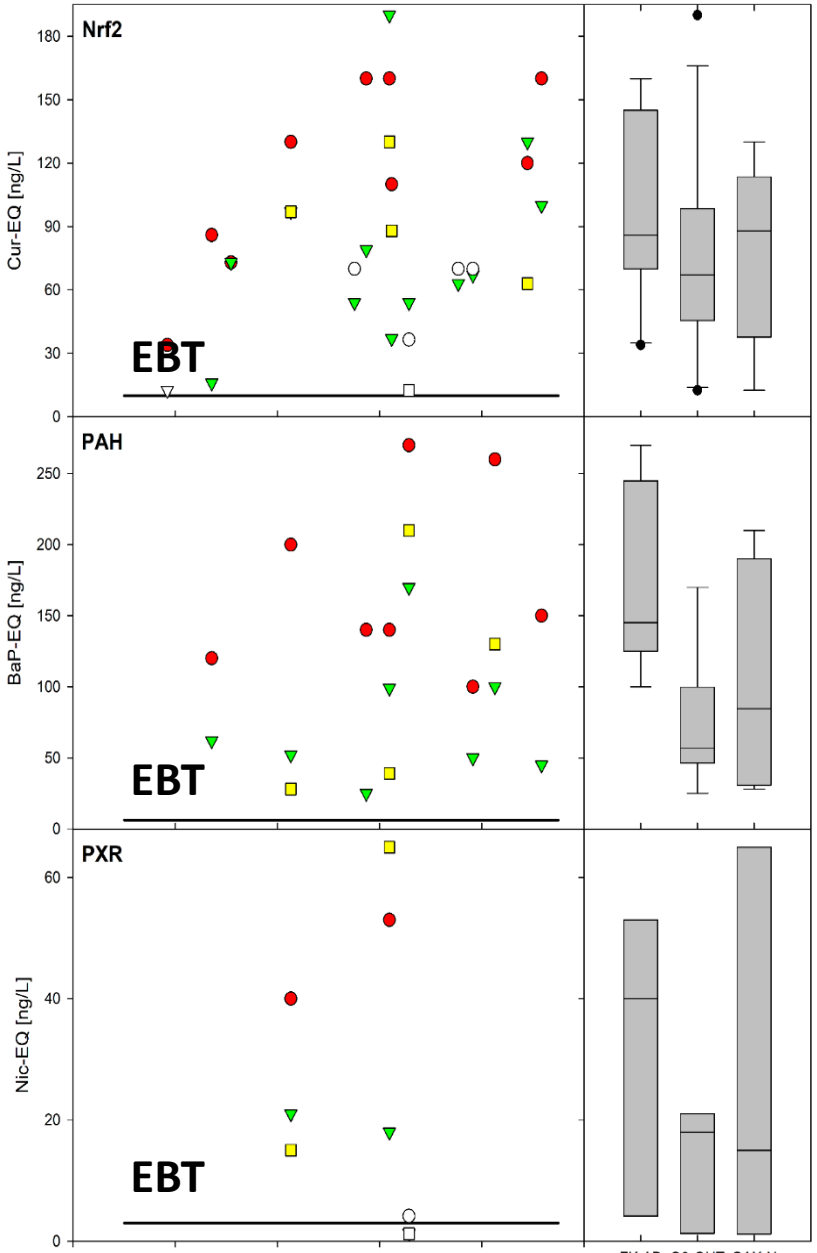
Advanced treatment ➲ CALUX RESULTS

multibarrier system (one-year sampling campaign)

- CAS... effluent conventional treatment > LOQ
- O_3 ... effluent ozonation > LOQ
- GAC... effluent activated carbon > LOQ
- ○ ▽ □ **< LOQ**: no fill colours

Results after advanced treatment

- ERα ➲ mostly < LOQ & EBT
- anti-AR ➲ < LOQ & EBT
- cytotox ➲ < LOQ
- major decrease by O_3 ➲ beneficial multibarrier effect hard to demonstrate

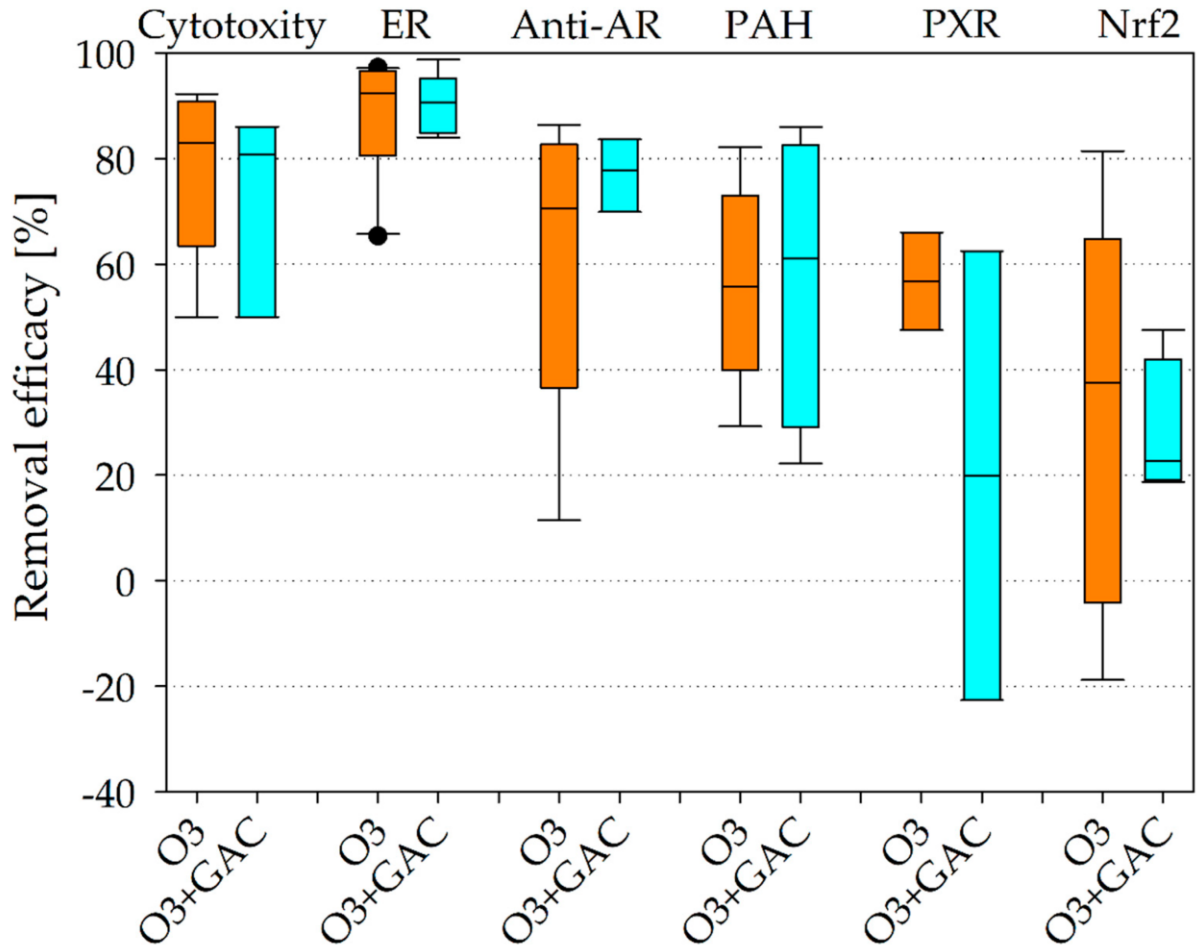


Advanced treatment ➲ RESULTS ctd.

Results for oxidative stress, PAH and PXR

➲ (slight) decrease, but EBT still exceeded

OVERVIEW ➲ removal during advanced treatment



Literature

Conventional treatment

➲ **Projekt report (in German)**

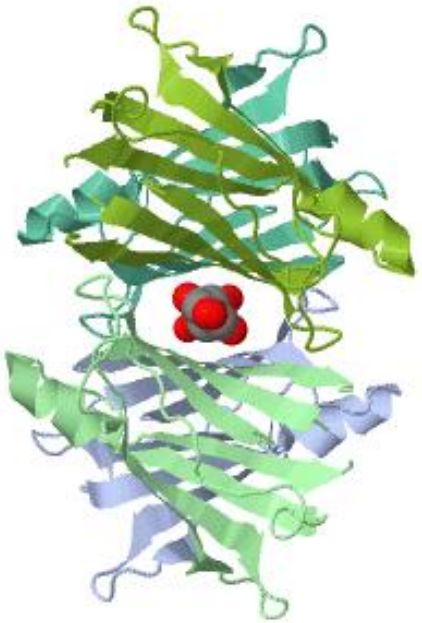
https://info.bmlrt.gv.at/dam/jcr:e813d086-8b30-4d9d-bb03-2a1996aeb669/Projektbericht_Biologische_Wirktests.pdf

Advanced treatment (ozonation + activated carbon)

➲ **publication (open access)**

https://doi.org/10.3390/w13223245

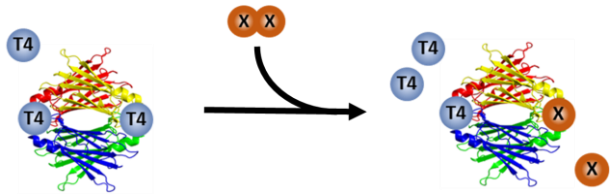
PFAS Water Monitoring using PFAS CALUX



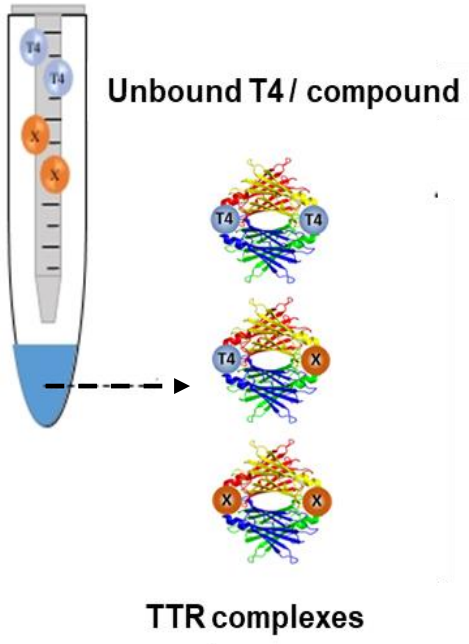
Dr.Ir. Harrie Besselink
Director Product and Application Unit

PFAS CALUX analyses

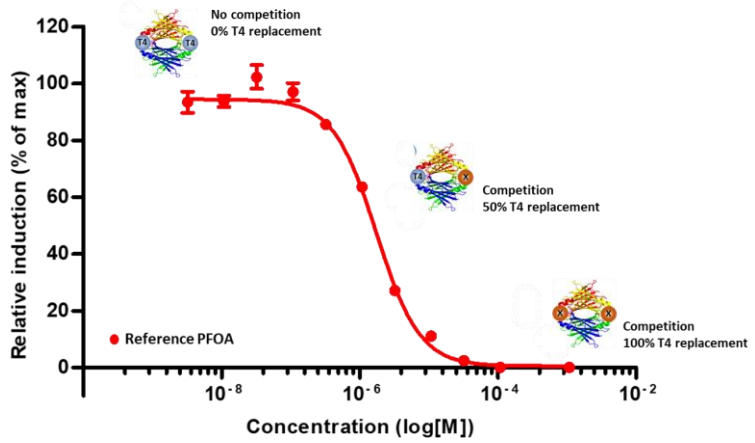
Step 1



Step 2



Step 3

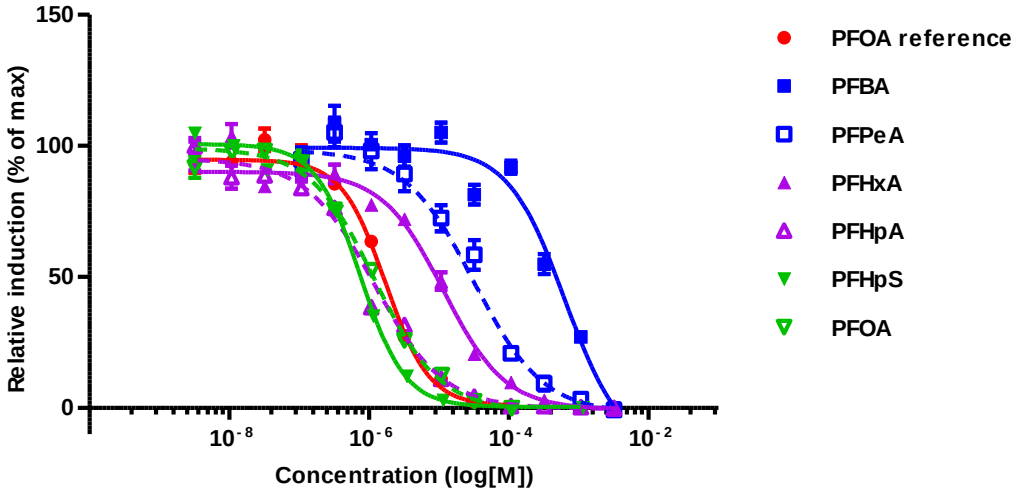


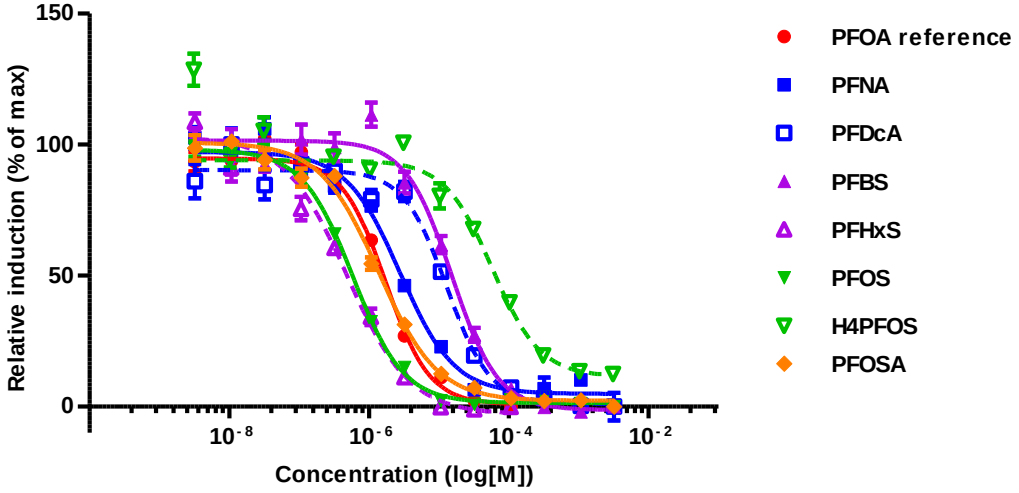
Step 1: Incubation (TTR competition)

Step 2: Separation TTR-bound and free T4 / compound (Biogel P6DG)

Step 3: TRβ CALUX analysis

PFAS CALUX REPs





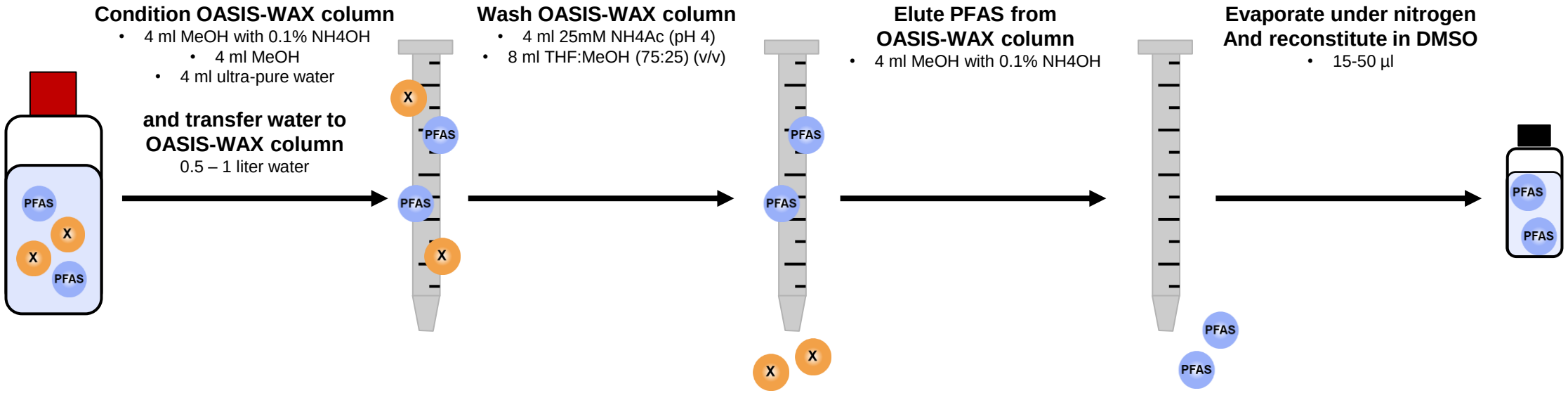
Potency factors of tested PFASs compounds in the TTR-TRβ CALUX® bioassay.

Compound	Potency factor IC_{50}-based (PFOA = 1)	Potency factor PC_{80}-based (PFOA = 1)
PFBA	0.0012	**0.0018**
PFPeA	0.048	**0.080**
PFHxA	0.16	**0.19**
PFHpA	1.3	**1.4**
PFOA	1.0	**1.0**
PFNA	0.48	**0.32**
PFDcA	0.12	**0.12**
PFBS	0.10	**0.052**
PFHxS	2.5	**1.6**
PFHpS	2.0	**1.0**
PFOS	3.0	**2.0**
H4PFOS (6:2 FTS)	0.033	**0.019**
PFOSA	1.2	**0.72**

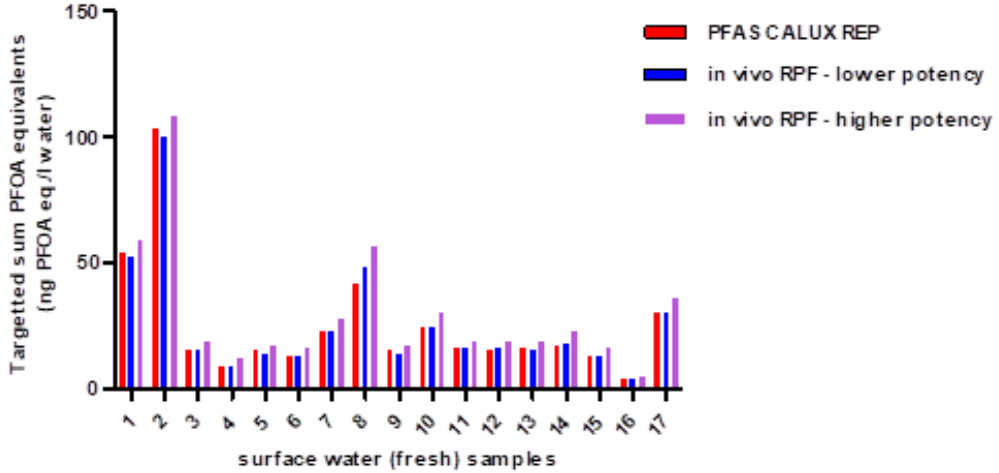
Behnisch et al. 2021

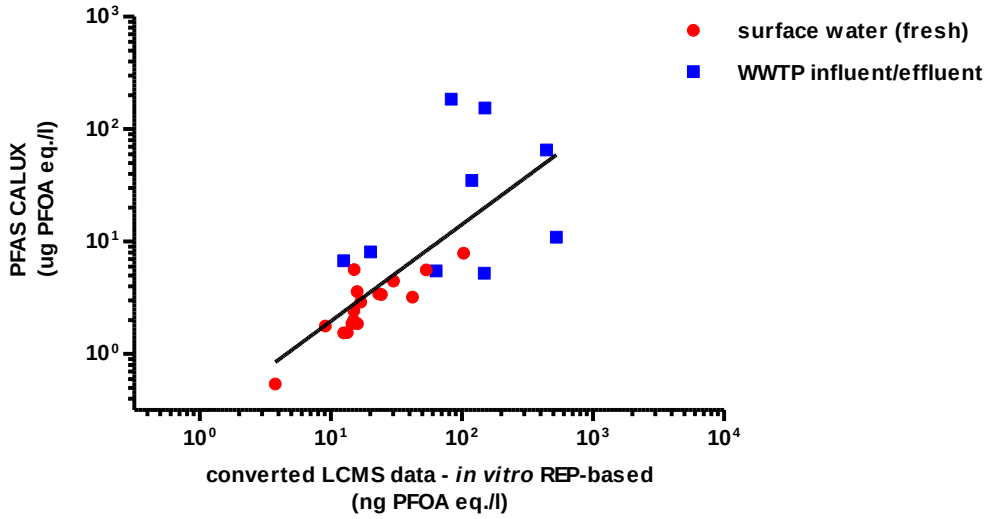
Extraction of PFAS

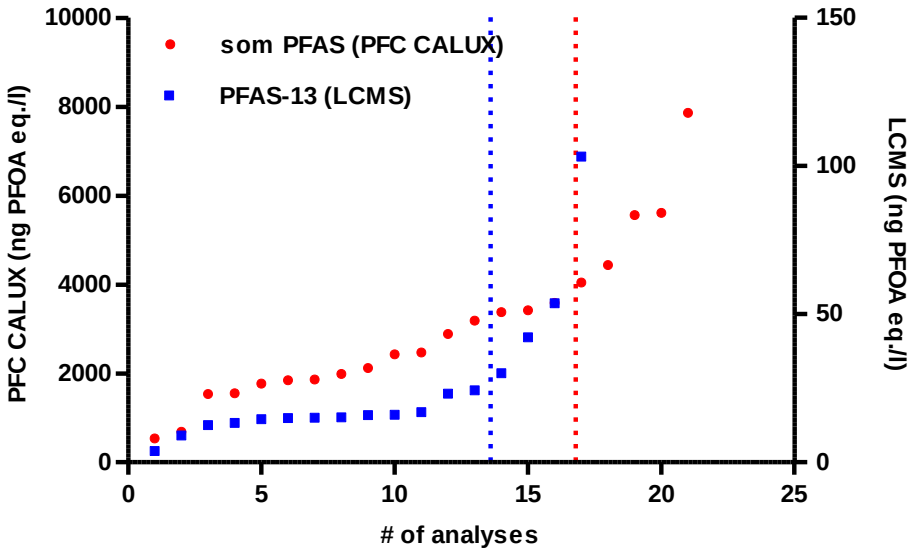
Separation on WAX-SPE column



Comparison PFAS CALUX vs LCMS







Proposed EBT (80% perc.)

som PFAS (PFC CALUX): **4000 ng PFOA eq./l water**
PFAS-13 (LCMS): **30 ng PFOA eq./l water**

Green Deal Zero Pollution

The PROMISCES project, coordinated by BRGM and financed by the European "Green Deal" programme, aims to contribute to the deployment of the circular economy by reducing the risks associated with certain industrial pollutants, in particular perfluoroalkylated and polyfluoroalkylated substances (PFAS).

European Commission

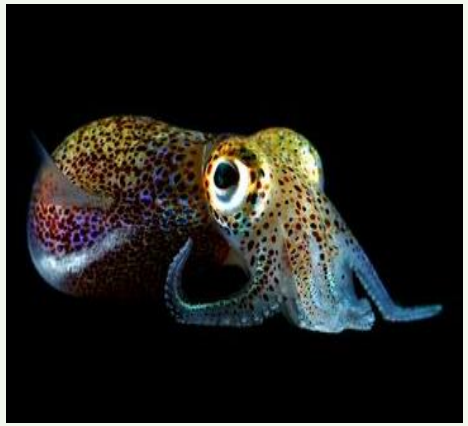
MicroLife Solutions

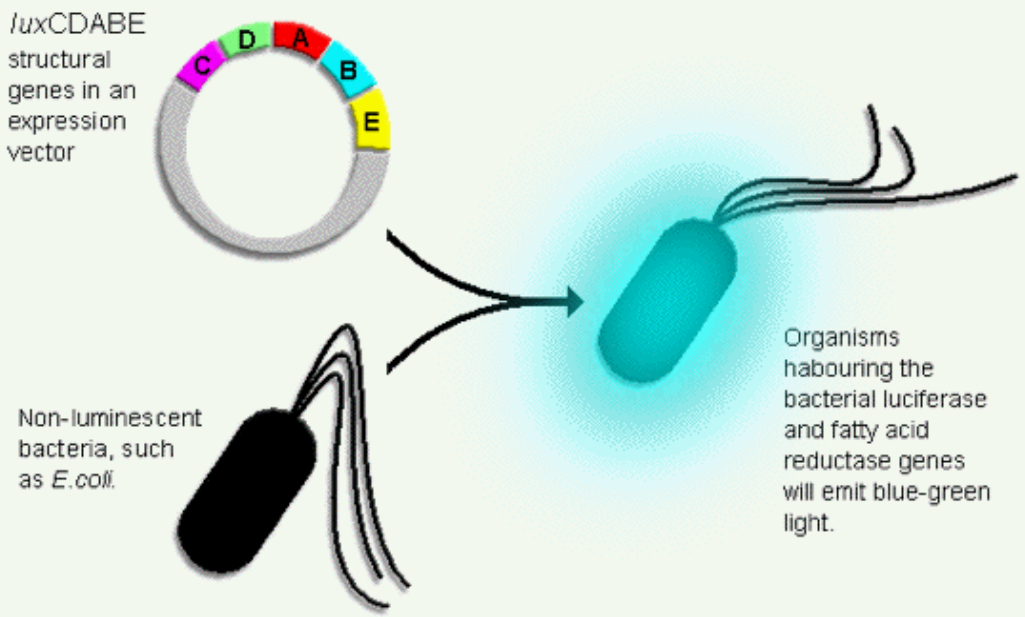
MicroGLO: Antibiotic Bioassays

Tjalf.deBoer@microlifesolutions.nl

MicroLife Solutions BV
Science Park 406
1098 XH Amsterdam
The Netherlands

MicroGLO Bioassay make use of bacterial bio-luminescence

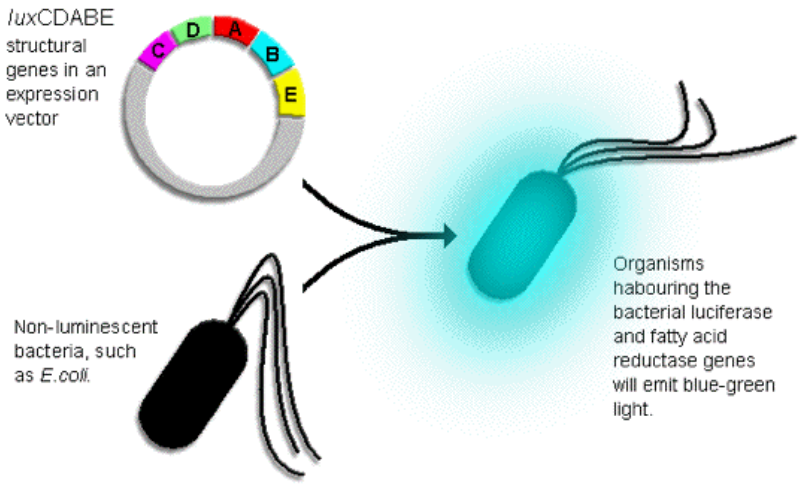




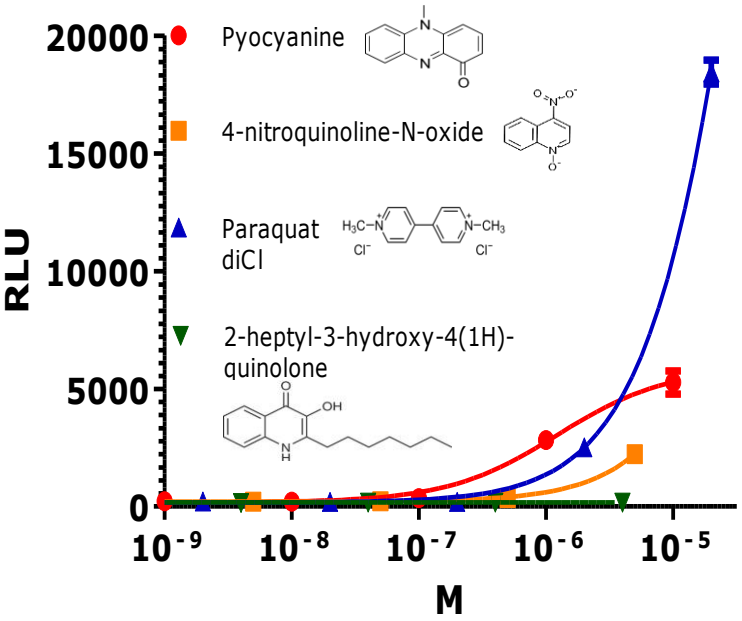
microlife solutions

MicroGLO reporter assays

Principle



Measured Results



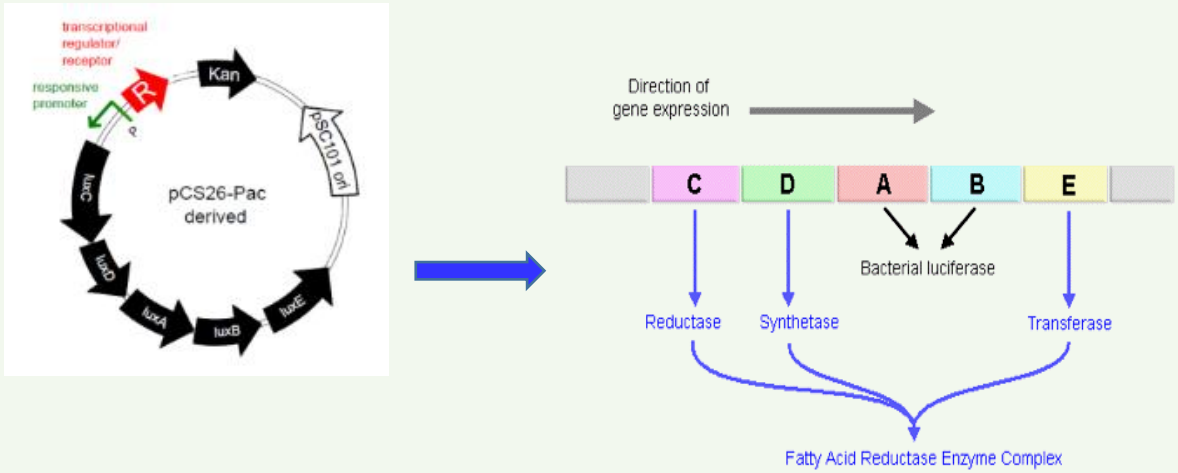
Antibiotics bioassays

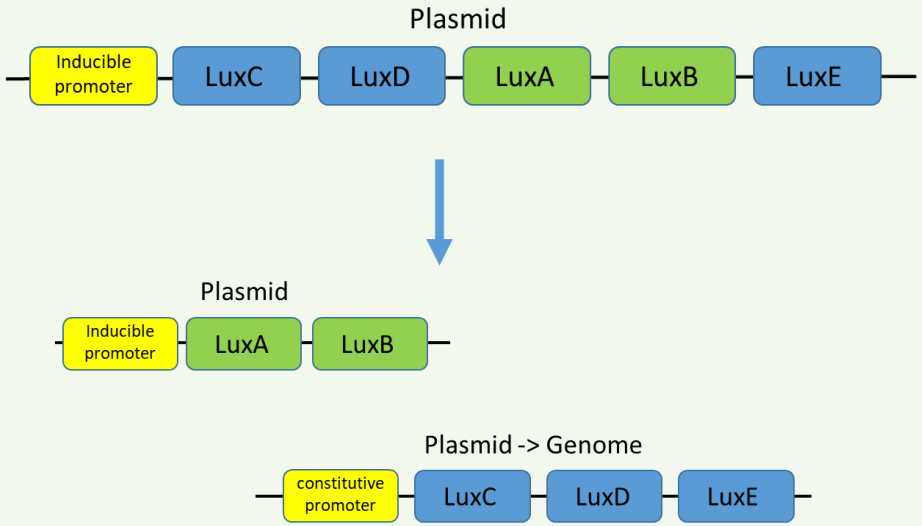
reporter	Measured effects
TETlux	Protein synthesis inhibition (detects **Tetracyclines**)
SOSlux	Reporter that detects DNA damage (detects **Quinolones**)
BLAlux	Cell wall synthesis inhibition (detects **Beta-lactams**)
MAClux	Protein synthesis inhibition (detects **Macrolides**)
SULFlux	Inhibition of folate synthesis (detects **Sulfonamides**)
CYTOlux	**General toxicity and growth inhibition**

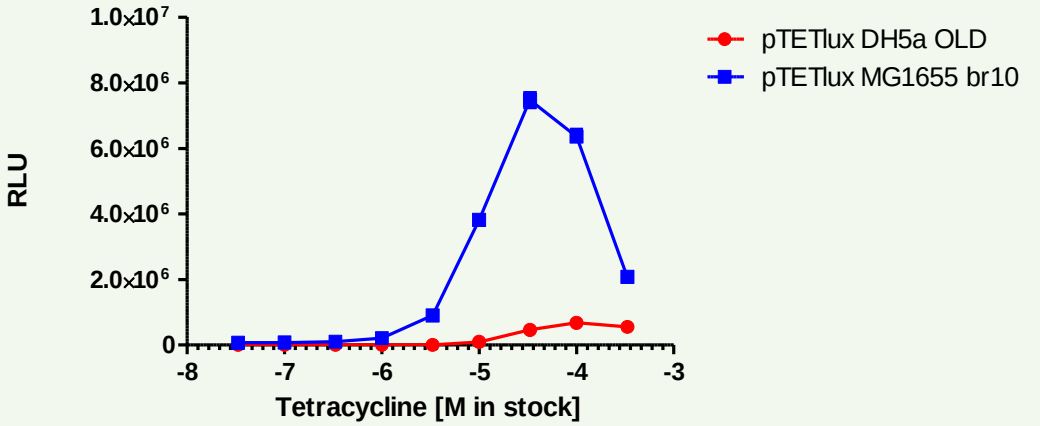
- **Reproducible results**
- **Fast (2 hrs. exposure)**
- **On-site potential**

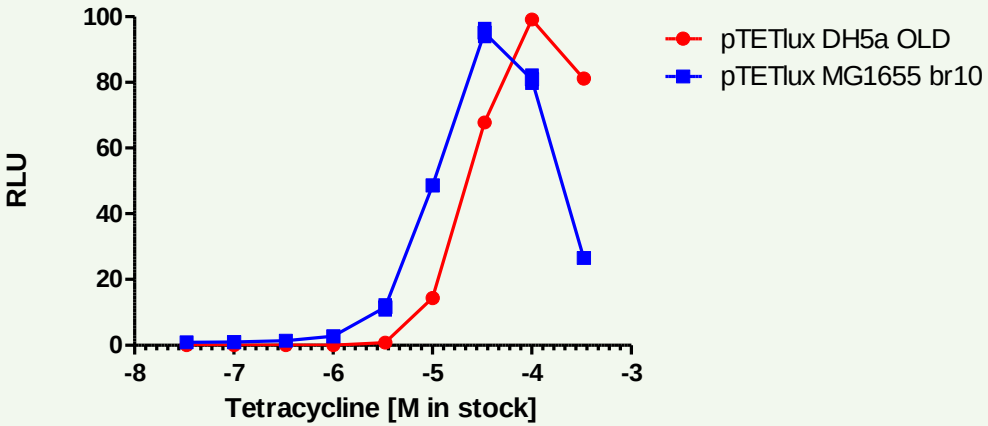
microlife solutions

MicroGLO unique properties









Antibiotic compound screening

BLAlux (beta-lactams)

Compound	Assay	Active?
Penicilline-G	BLAlux	yes
amoxicilline	BLAlux	yes
Ampicilline	BLAlux	yes
Cefazolin	BLAlux	no
Cefquinome	BLAlux	no
Cefoperazone	BLAlux	yes
Ceftiofur	BLAlux	no
Dicloxacillin	BLAlux	yes
Nafcillin	BLAlux	maybe
Oxacillin	BLAlux	yes
Cefalexin	BLAlux	yes
Penicilline-V	BLAlux	yes
Cefalonium	BLAlux	yes

SULFlux (sulfonamides)

Compound	Assay	Active?
Sulfamethoxazole	SULFlux	yes
Sulfadiazine	SULFlux	yes
Sulfademethoxine	SULFlux	yes
Sulfadimidini	SULFlux	yes
Sulfamethiozole	SULFlux	yes
Sulfamerazine	SULFlux	maybe
Sulfamethoxypyridazine	SULFlux	yes
Sulfapyridine	SULFlux	yes
Sulfisoxazole	SULFlux	yes
Sulfaquinoxaline	SULFlux	yes
Sulfatihazole	SULFlux	yes
Trimethoprim	SULFlux	yes

SOSlux (quinolones)

Compound	Assay	Active?
Ciprofloxacine	SOSlux	yes
Danofloxacin	SOSlux	yes
Difloxacin	SOSlux	yes
Enrofloxacine	SOSlux	yes
Norfloxacine	SOSlux	yes
Flumequin	SOSlux	yes
Nalidic acid	SOSlux	yes
Marbofloxacin	SOSlux	yes
Oxolonic acid	SOSlux	yes
Sarafloxacine	SOSlux	yes

MAClux (macrolides)

Compound	Assay	Active?
Erythomycine	MAClux	yes
Lincomicin	MAClux	yes
Spiramycin	MAClux	maybe
Tylosin	MAClux	yes
Valnemulin	MAClux	maybe

TETlux (tetracyclines)

Compound	Assay	Active?
Tetracycline	TETlux	yes
Doxycycline	TETlux	yes
Oxytetracycline	TETlux	yes
Chlorotetracycline	TETlux	yes

Next:

- Redo “maybe’s”
- Calculate potency

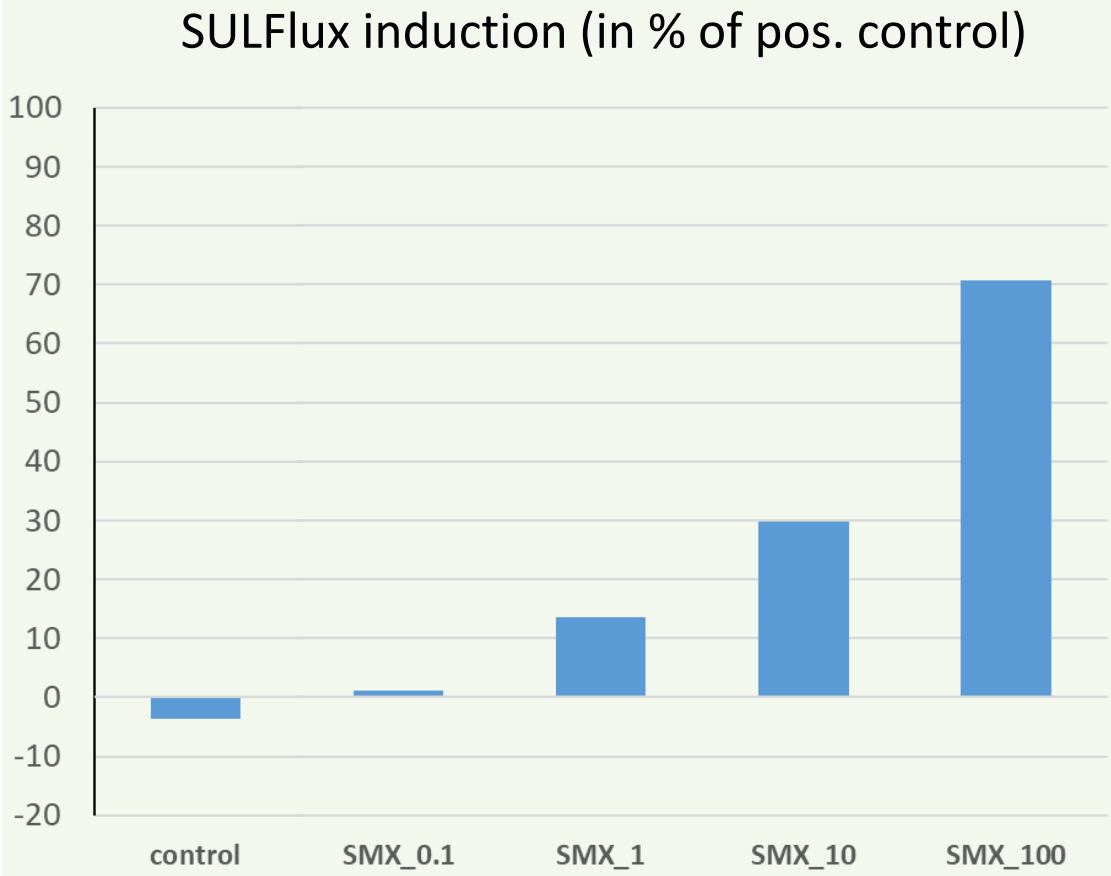
microlife solutions

Case study EMERCHE project

- Experimental containers (~500L) were spiked with sulfamethoxazole (among others)
- 4 sulfamethoxazole (SMX) concentrations

condition	SMX (ug/L)
control	0
SMX_0.1	0.2
SMX_1	2.0
SMX_10	20.0
SMX_100	200.0

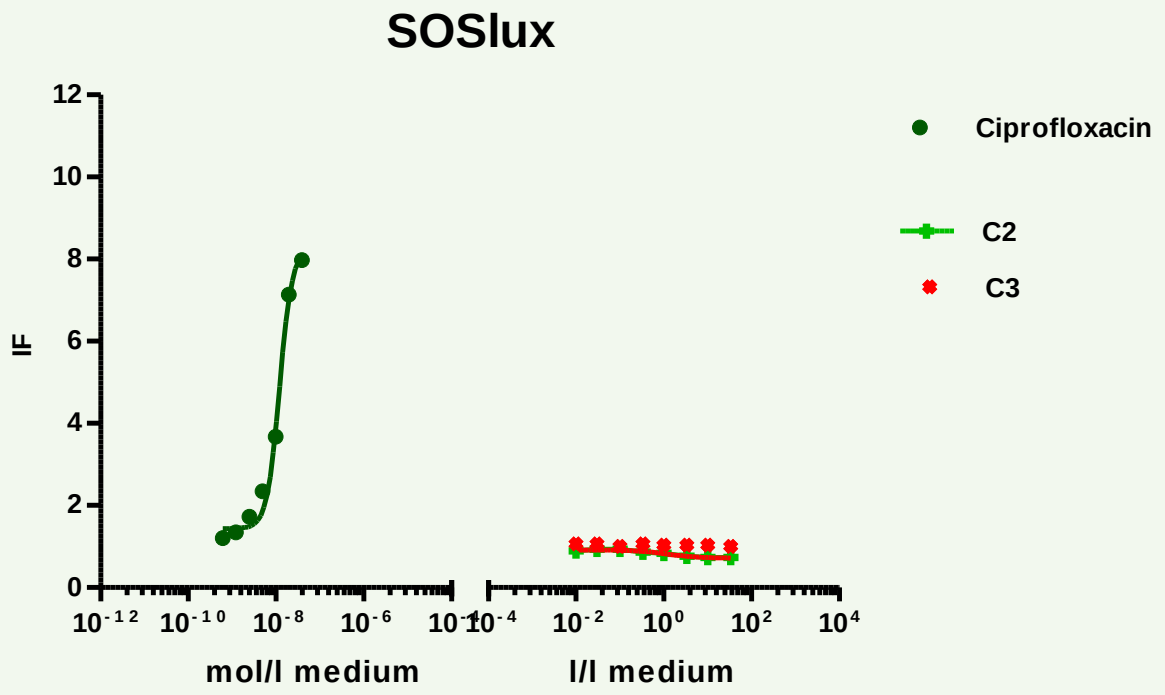
- Sampling after two weeks (1L.) and extracted using SPE HLB-OASIS



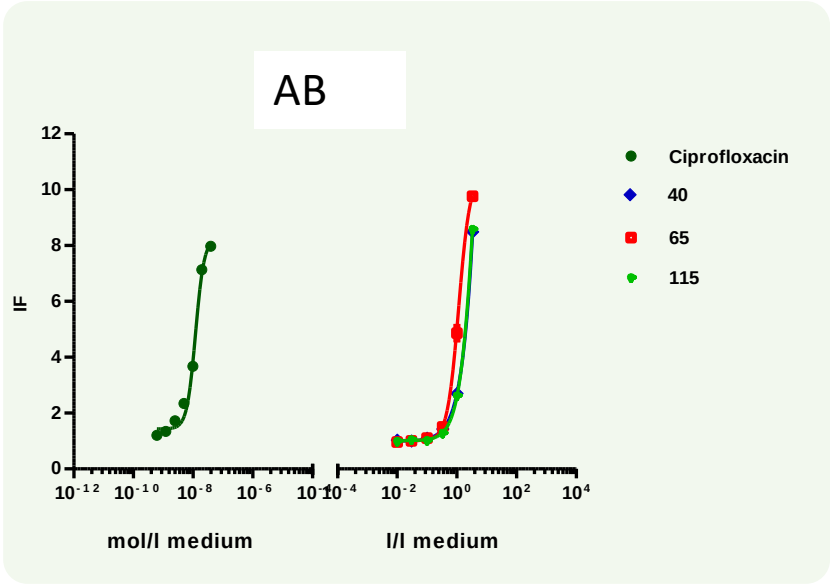
Influence of suspended particulate matter (SPM) on MicroGLO Testcase for SOSlux:

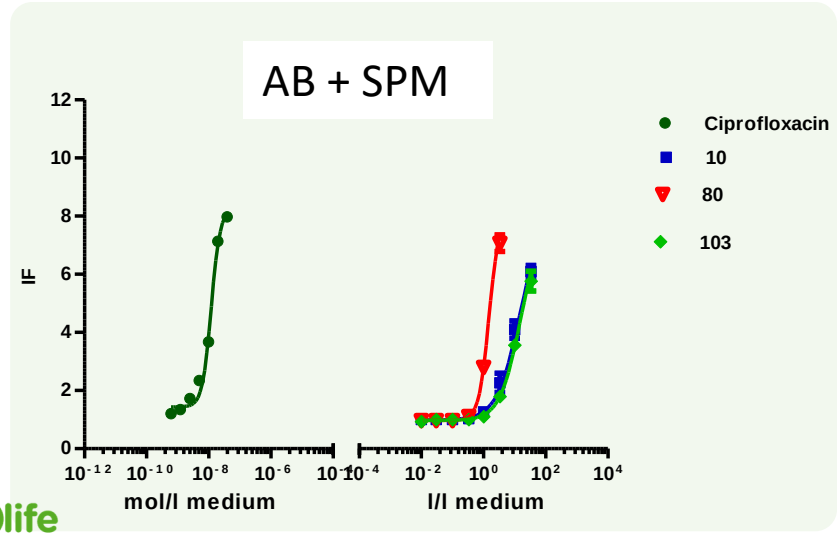
- Influence of SPM material on extraction and subsequent MicroGLO analysis of antibiotics
- Quinolone antibiotics:
  - Levofloxacin (20 ug/L)
- Water spiked with antibiotics, SPM and a combination of both
- SPE HLB oasis and SOSlux analysis

SPM control samples



Influence of suspended particulate matter (SPM) on MicroGLO Testcase for SOSlux:





Final results

Condition	Result	LOQ	Unit
AB	17.51	0.07	µg Ciprofloxacin eq./L processed water
AB	20.90	0.07	µg Ciprofloxacin eq./L processed water
AB	14.61	0.07	µg Ciprofloxacin eq./L processed water
AP + SPM	4.28	0.07	µg Ciprofloxacin eq./L processed water
AP + SPM	11.61	0.07	µg Ciprofloxacin eq./L processed water
AP + SPM	2.97	0.07	µg Ciprofloxacin eq./L processed water
SPM	<LOQ	0.07	µg Ciprofloxacin eq./L processed water
SPM	<LOQ	0.07	µg Ciprofloxacin eq./L processed water
SPM	<LOQ	0.07	µg Ciprofloxacin eq./L processed water

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