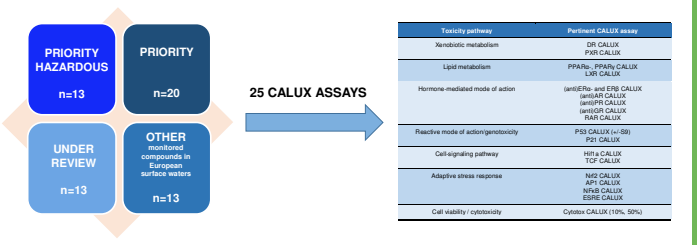
Comprehensive safety screening of substances in European waste and surface waterbodies using a large panel of 25 CALUX bioassyas

Harrie Besselink[1], Barbara van Vugt[1], M. Naderman[1], E. Simon[2], Bram Brouwer[1, 3], Bart van der Burg[1]
[1]BioDetection Systems bv, Amsterdam, the Netherlands; [3]Oekotoxzentrum/Centre Ecotox, Zürich, Switzerland;
[3]Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

Introduction

- Surface and (bio-based) waste waters might contain various unknown toxicants, metabolites, degradation products, which are not included in current regulations.
- Direct measurement of possible effects of chemical contaminants in extracts of water samples using bioassays is expected to deliver a better approach for water quality assessment compared to measurement of a limited number of individual chemicals.
- To demonstrate bioassays as complementary tools to chemical analytics we aimed at a comprehensive toxicity screening of the currently monitored substances in European waterbodies and compared the identified toxic endpoints to case studies on effect-based water quality screening.

Approach



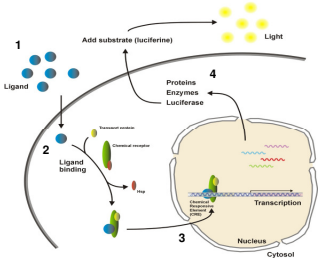
Toxicity pathway	Pertinent CALUX assay
Xenobiotic metabolism	DR CALUX PXR CALUX
Lipid metabolism	PPARα- , PPARγ CALUX LXR CALUX
Hormone-mediated mode of action	(anti)ERα- and ERβ CALUX (anti)AR CALUX (anti)PR CALUX (anti)GR CALUX RAR CALUX
Reactive mode of action/genotoxicity	P53 CALUX (+/-S9) P21 CALUX
Cell-signaling pathway	Hif1α CALUX TGF CALUX
Adaptive stress response	Nrf2 CALUX AP1 CALUX NFκB CALUX ESRE CALUX
Cell viability / cytotoxicity	Cytotox CALUX (10%, 50%)

1. **Toxicity profile** of the routinely/occasionally monitored substances in European surface waters (n=66)
2. Identification of the **toxic pathways** picked up by these substances
3. **Comparison** of the toxicity **pathways** induced by the routinely monitored substances with **relevant toxicity pathways** for water quality assessment (suggested by various case studies [1-3])

Materials and Methods

In vitro reporter gene assay panel (25 CALUX) was first compiled covering a wide range of toxic endpoints, that are assumed to be indicative for water samples.

Figure 1 – The CALUX assay principle



1 Chemicals enter cell

2 Active chemicals bind to a nuclear receptor or activate a signaling pathway

3 Activated complexes are translocated to the nucleus and bind to responsive elements

4 Endogenous situation:
various proteins and/or enzymes are produced;
a biological effect occurs.
CALUX sutuation:
luciferase is expressed (luminescence)

The assays have been automated using a compact liquid handling system. Dilution series of 16 concentrations in triplicate are tested for each chemical to derive PC_{10} (agonism; 10% induction relative to reference) and PC_{80} (antagonism; 80% inducution relative to reference) values.

Figure 2 – Workflow of automated compound screening

66 compounds → HTP screening 25 CALUX assays → PC_{10}/PC_{80} based data set

Results

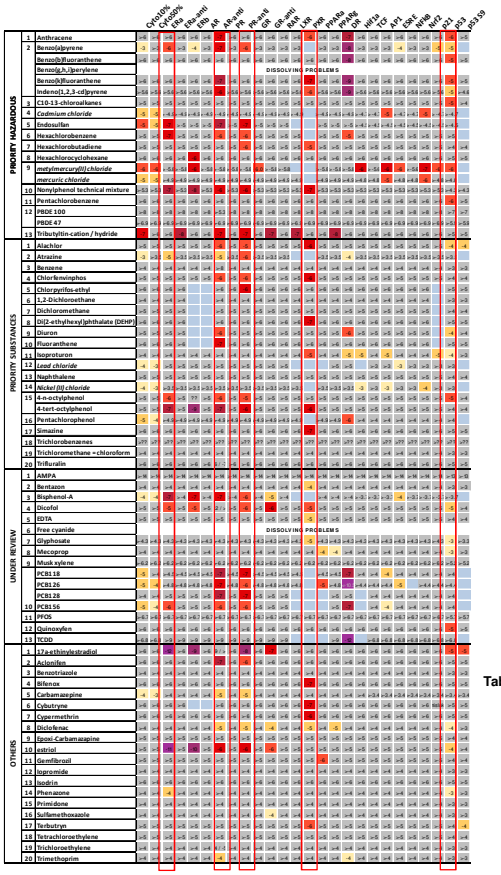


Table 1: Toxicity screening of the set of substances routinely/occasionally monitored in European water bodies. Values represent logarithmic PC_{10} (agonistic assays) and PC_{20} (antagonistic assays) concentrations.

Conclusions

- Compound screening revealed the importance of ENDOCRINE - (particularly the activation of the **ERα-**, **anti-AR**, **anti-PR** receptors), GENOTOXICITY (**p53-CALUX**) and XENOBIOTIC METABOLISM–related (**PXR-CALUX**) pathways of the routinely monitored substances (*Table 1*) .
- This profile of pathway effects is consistent with only part of the profile observed in water samples (*Figure 3*) indicating that **not all substances** are **picked up** by the compound panel of the monitoring programs .

Activated by monitoring compounds		Activated by water samples [1,2,3]
DR CALUX PXR CALUX	✔	DR CALUX PXR CALUX
- -	⊘	PPARγ CALUX -
ERα CALUX Anti-AR CALUX Anti-PR CALUX	✔	ERα CALUX (anti-)AR CALUX -
-	⊘	GR CALUX -
P53 (-S9) CALUX -	✔	P53 (+/-S9) CALUX -
- -	✔	- -
- - - - - -	⊘	Nrf2 CALUX - - - - -
Cytotox CALUX	✔	Cytotox CALUX

Figure 3 – Identified toxic pathways of the chemically monitored substances and compared with the pathways indicated by Dutch and Australian case studies [1-3] on effect-based screening of water samples

This study demonstrates that **effect-based methods** could complement conventional chemical analysis in water quality monitoring as **prescreening** techniques by

- ✓ identifying toxic "**hotspots**" for further investigation,
- ✓ assessing the **effect** of the entire **mixture** of compounds present in waters and therefore,
- ✓ reduce uncertainty in **safety evaluation**.

References

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BioDetection Systems bv
Email: info@bds.nl
Internet: www.bds.nl

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