High Throughput Screening tools for water quality monitoring

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Introduction

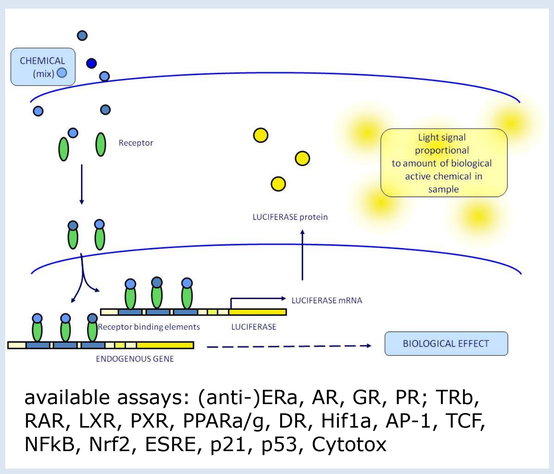
For water quality monitoring, chemical analysis is widely used and well-grounded in regulatory frameworks. However, the practical use of chemical analysis is severely hampered by the need for *a priori* selection of compounds.

As an alternative, several effect-based bioanalytical tools for water quality evaluation have been developed in FS8.1.1 and 8.1.4. Effect-based assays have the advantage that they provide information on the biological effect of compounds, irrespective of their chemical nature.

Aim

Recently, two case-studies [1] have been performed where a representative set of water samples was analysed using a broad panel of CALUX reporter gene assays (figure 1).

The aim of the current study was to demonstrate the added value of such an effect-based analysis. To this end, 81 prioritized compounds were screened on the CALUX panel, and the resulting activity profile was compared to the CALUX profile of the water samples (figure 2).



available assays: (anti-)ERa, AR, GR, PR; TRb, RAR, LXR, PXR, PPARa/g, DR, Hif1a, AP-1, TCF, NFkB, Nrf2, ESRE, p21, p53, Cytotox

Figure 1. Principle of the CALUX assay

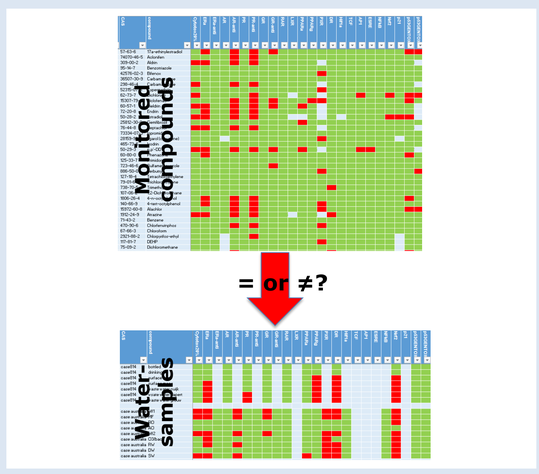


Figure 2. compare CALUX profiles

Approach

The water samples and a set of 81 routinely monitored substances (figure 3) were measured on the human reporter gene CALUX panel (figure 4), thus generating a toxicity profile.

PRIORITY HAZARDOUS		PRIORITY		UNDER REVIEW		OTHERS
1	Anthracene	1	Alachlor	1	AMPA	Pharmaceuticals, list of compounds provided by Swiss and Italian water companies
2	Benzo(a)pyrene	2	Atrazine	2	Bentazon	
	Benzo(b)fluoranthene	3	Benzene	3	Bisphenol-A	
	Benzo(g,h,i)perylene	4	Chlorfenvinphos	4	Dicofol	
	Benzo(k)fluoranthene	5	Chlorpyrifos-ethyl	5	EDTA	
	Indeno(1,2,3-cd)pyrene	6	1,2-Dichloroethane	6	Free cyanide	
3	C10-13-chloroalkanes	7	Dichloromethane	7	Glyphosate	
4	Cadmium and its compounds	8	Di(2-ethylhexyl)phthalate (DEHP)	8	Mecoprop	
	cadmium chloride	9	Diuron	9	Musk xylene	
5	Endosulfan	10	Fluoranthene	10	PCB118	
6	Hexachlorobenzene	11	Isoproturon		PCB126	
7	Hexachlorobutadiene	12	Lead and its compounds		PCB128	
8	Hexachlorocyclohexane		lead chloride		PCB156	
9	Mercury and its compounds	13	Naphthalene	11	PFOS	
	metylmercury(II) chloride	14	Nickel and its compounds	12	Quinoxyfen	
	mercuric chloride		nickel (II) chloride	13	TCDD	
10	Nonylphenol technical mixture	15	4-n-octylphenol			
11	Pentachlorobenzene		4-tert-octylphenol			
12	PBDE 100	16	Pentachlorophenol			
	PBDE 47	17	Simazine			
13	Tributyltin-cation / hydride	18	Trichlorobenzenes			
		19	Trichloromethane = chloroform			
		20	Trifluralin			

Figure 3. Selected compounds: monitored or under review

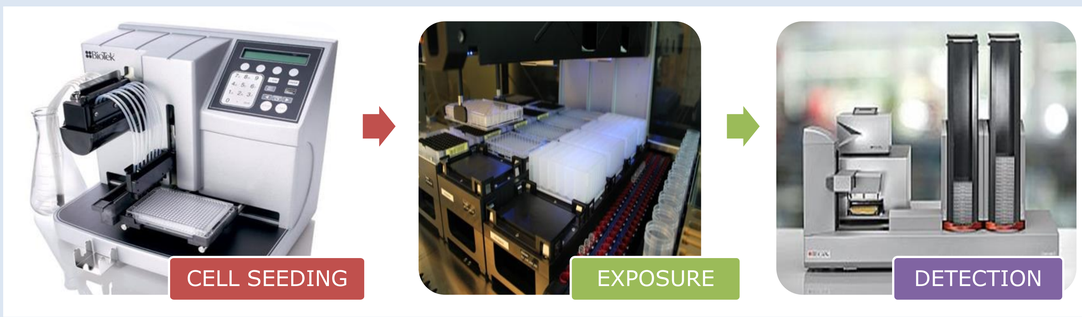


Figure 4. Automated high-throughput screening procedure

Screening results

The compounds and the samples activated similar molecular pathways: induction of xenobiotic metabolism, endocrine pathways, and reactive mode-of-action. However, the particular assays activated within these categories were different (table 1). For example, the water samples consistently showed activity on pathways related to oxidative stress (Nrf2) and lipid metabolism (PPAR), while these pathways were hardly activated by the routinely monitored compounds.

Molecular pathways	Activated by monitored compounds:	Activated by Water Samples case studies:
Xenobiotic Metabolism:	DR CALUX PXR CALUX	DR CALUX PXR CALUX PPAR CALUX
Endocrine (nuclear hormone receptors):	ERa CALUX antiAR CALUX antiPR CALUX	ERa CALUX antiAR CALUX GR CALUX
Reactive MoA:	p53 CALUX	 Nrf2 CALUX
Cell Viability:	Cytotox CALUX	Cytotox CALUX

Table 1. Activated molecular pathways and corresponding CALUX assays for both datasets

Activity profiles

Table 2 shows CALUX panel profiles of the compounds (left) and samples (right). The black boxes indicate matches between the two datasets (ERa, antiAR, PXR, DR, Cytotox), while the yellow boxes indicate mismatches (PPARs, Nrf2).

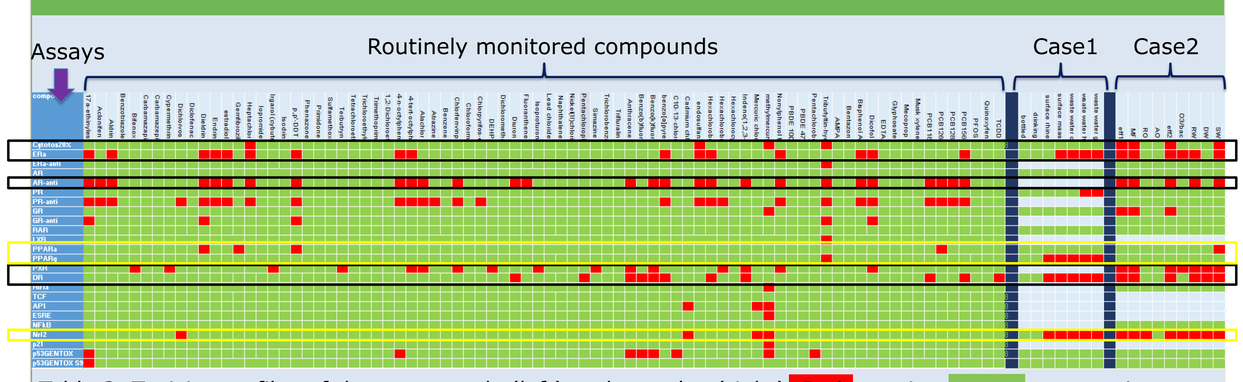


Table 2. Toxicity profiles of the compounds (left) and samples (right). Red = active, green = not active.

Conclusions

The CALUX profile of a set of water samples was compared to the profile of 81 priority pollutants. Although there was considerable overlap between the activated pathways in both datasets, some pathways activated by the water samples were clearly absent in the monitored compounds dataset. This suggests that these compounds cannot fully account for all activities observed in water samples.

The majority of the 81 selected compounds showed activity in at least one CALUX assay. A select panel of CALUX assays could be used to replace laborious chemical analysis; this would enable the detection of the priority pollutants, as well as other compounds that might be present in water samples. The present study clearly shows the added value of effect-based monitoring compared to standard chemical analysis.

References

[1] Escher BI et al, Environ Sci Technol, 48,1940-56. 2014.

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BE-Basic Foundation