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

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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 cases on effect-based water quality screening.

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 translated to the nucleus and bind to responsive elements
4. Endogenous situation: various proteins and/or enzymes are produced; a biological effect occurs.

CALUX activation: 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 PC₁₀ (agonism; 10% induction relative to reference) and PC₉₀ (antagonism; 80% induction relative to reference) values.

Figure 2 – Workflow of automated compound screening

66 compounds

HTP screening 25 CALUX assays

PC₉₀/PC₁₀ data set

Approach

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

References


Results

Table 1: Toxicity screening of the set of substances routinely/occasionally monitored in European water bodies. Values represent logarithmic PC₉₀ (agonistic assays) and PC₉₀ (antagonistic assays) concentrations.

Conclusions

- Compound screening revealed the importance of ENDOCRINE - (particularly the activation of the ERE- anti-AR, anti-PR receptors, GENOTOXICITY ip/3-CALUX) and XENOBIOTIC METABOLISM-related (PXR-CALUX) pathways of the routinely monitored substances (Table 7).
- 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.

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