Work Area 4

Implementation of novel rapid and quantitative bioassays for water quality monitoring

Ecotoxicological evaluations and examples for demonstration studies
• Mostly *in vivo* bioassays applied for regulatory purposes

• Short-term assays with luminescent bacteria, algae, and water flea most common, partly also fish applied

• Chronic assays mostly conducted with duckweed and/or water fleas

• Apart from the umuC assay for genotoxicity (e.g. applied in Germany), the majority of *in vitro* bioassays was/is lacking regulatory acceptance to date
Task 4.1.1: Establish selection criteria, e.g.:
- Sensitivity
- Reproducibility
- Robustness
- Ease of use
- Cost Effectiveness

Task 4.1.2: Select bioassays

Tasks 4.1.4 and 4.1.5: Develop bioassay guidelines and apply and validate selected *(in vitro)* bioassays
- Laboratory studies with complementary chemical analytical analysis
- Validation of bioassays
Reconstituted samples
Surface water: 0.13-0.43 ng EEQ/L
Effluent receiving water: 1.28-4.3 ng EEQ/L

**Intra-day and Inter-day Variability**

- Reconstituted extracts
- 5x
- 5x
- • Intra-day variability
- • Inter-day variability
- • Recovery
- Testing: ER-TA assays

**Overall Variability**

- Reconstituted water samples
- Solid Phase Extraction
- 5x
- 5x
- • Overall variability
- • Recovery
- Testing: ER-TA assays

*Kunz et al., Ecotox Centre, CH*
Are *in vitro* bioassays suitable for monitoring waste and surface waters?

**Yes, but…**

I. Suitable assays have to be standardised (ISO)

II. Clear, assay specific guidance on EEQ derivation has to be provided.

*Kunz et al., Ecotox Centre, CH*
WP 4.2 Implementation of bioassays for water quality monitoring

**Task 4.2.1: Promotion of regulatory acceptance**

ISO  OECD  DIN

**Task 4.2.2: Establishment of a Testing Framework**

Establish a concept on how to evaluate bioassay results for risk assessment.

**Task 4.2.3: Introduce bioassays at labs and water utilities**

- Great interest in bioassays in Switzerland
- A cost-effective and easy to use set of tests would be very much appreciated

**Task 4.2.4: Demonstration in pilot studies**

- ARA Neugut Dübendorf, Switzerland
- Assess samples from other pilot plants
Develop a routine concept for water quality assessment in Switzerland

Recommendation of promising methods to assess surface water quality

Estrogenic and photosystem II-inhibiting effects

Evaluation of surface water quality based on ecotoxicological bioassays
In vitro bioassays to screen for endocrine active pharmaceuticals in context of the EU Water framework directive
Change conventional monitoring practice

Smart monitoring

- Visual inspection
- Routine chemistry
- General toxicity
- Specific toxicity
- Passive and/or grab sampling

Hazards:
- Suspected?
- > Trigger value?

Potential Risk:
- Advanced chemistry &
  Toxicology (mechanism-based adverse outcome pathway biomarkers, TIE, EDA)

Risk:
- RISK
  - High Risk
  - Low Risk

Toxicity: ‘Traffic light’

Analytical chemistry alone might not cover the entire load of water pollution

Multidisciplinary quality assessment
Demonstration studies

Demonstration of promising technologies to address emerging pollutants in water and waste water

- MAR SAMPLING
- BIOASSAYS
  - MICROTOX ASSAY
  - PHOTOSYNTHESIS INHIBITION
  - CALUX SCREENING
- RESULTS INTERPRETATION
  - TRIGGER VALUES
  - CHEMICAL ANALYSIS
Example:
WWTP sample (CAS-01)

Chemical concentrations

Relative Potency Factor (REP)

Expected equivalent concentrations

Chemical concentrations

Anti-androgenic activity

Expected

Measured

42%

Estrogenic activity

Expected

Measured

3%

Glucocorticoid activity

Expected

Measured

0%
Demonstration of promising technologies to address emerging pollutants in water and waste water

**Estrogenic Activity**

**ER\(\alpha\) CALUX**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF01</td>
<td>0.0</td>
</tr>
<tr>
<td>INF02</td>
<td>0.1</td>
</tr>
<tr>
<td>BSV01</td>
<td>0.2</td>
</tr>
<tr>
<td>BSV05</td>
<td>0.3</td>
</tr>
<tr>
<td>BSV09</td>
<td>0.4</td>
</tr>
<tr>
<td>BSW10</td>
<td>0.5</td>
</tr>
<tr>
<td>BSV8.1</td>
<td>0.6</td>
</tr>
<tr>
<td>BSV8.3</td>
<td>0.7</td>
</tr>
<tr>
<td>CAS-01</td>
<td>0.8</td>
</tr>
<tr>
<td>CAS-05</td>
<td>0.9</td>
</tr>
<tr>
<td>CAS-06</td>
<td>1.0</td>
</tr>
<tr>
<td>CAS-07</td>
<td>1.1</td>
</tr>
<tr>
<td>CAS-08</td>
<td>1.2</td>
</tr>
<tr>
<td>CAS-09</td>
<td>1.3</td>
</tr>
<tr>
<td>CAS-11</td>
<td>1.4</td>
</tr>
<tr>
<td>CAS-12</td>
<td>1.5</td>
</tr>
<tr>
<td>Proc. Blank</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**EU EQS proposal for \(17\beta\)-Estradiol = 0.4 ng/L**

**LOQ**

**LOD**
Photosynthesis Inhibition

1st campaign

DEQ (ng/L)

INF01  INF02  BSV01  BSV05  BSV09  BSW10  BSV8.1  BSV8.3  CAS-01  CAS-05  CAS-06  CAS-07  CAS-08  CAS-09  CAS-11  CAS-12  Proc. Blank

EU EQS proposal for Diuron = 200 ng/L

CH EQS proposal for Diuron = 20 ng/L

DEQ = Diuron equivalent concentration

AGRICULTURAL ACTIVITIES

PESTICIDES
• In vitro bioassays can be applied for environmental monitoring of waste and surface water
• Monitoring concepts are developed
• The application of *in vitro* bioassays in the various demonstration studies has shown their added value.
• Further validation and promotion of regulatory acceptance is needed.
Thank you for your attention!

This project has received funding from the European Union’s Seventh Programme for Research, Technological Development and Demonstration under Grant Agreement no. 308339.