Current Status of Bioanalytical Methods

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A 3-step process similar to targeted analysis

1. Extract water samples using solid phase extraction (SPE)

2. Perform bioanalytical (cell) assay

3. Analyze and report results: convert light intensity into a bioassay equivalent concentration (BEQ, ng/L)
Standardized sample extraction methods are available

- Consensus method is SPE using Oasis HLB (C-18)

- Standard protocols for targeted chemistry are sufficient
  - e.g. EPA Methods 1694 (PPCPs) (539 for hormones)

- Slight modifications include:
  - selected fortification (e.g. QA/QC matrix spike samples only)
  - final carrier solvent exchange to DMSO
Standardized bioscreening methods for water quality

- Commercially available technology have standard operating procedures (SOPs)

- Some assays have been validated in Europe (e.g. OECD, ISO)

- SOPs include detailed recommendations for
  - reference chemicals
  - vehicle solvent
  - plating instructions
  - incubation conditions, etc...
Candidate ER-α transactivation assays

- ERα-CALUX, BDS (Besselink 2015)
- ERα GeneBLAzer, LifeTechnologies (Mehinto 2016)
- BG1Luc ER TA assay (OECD TG455)
- HERα transactivation assay (ISO 19040-3)
- HERα assay, INDIGO Biosciences
Candidate AhR transactivation assays

- DR-CALUX, BDS (Besselink 2004)
- AhR CALUX, M. Denison (EPA Method 4435)
- AhR assay, INDIGO Biosciences
Quality controls mirror that for targeted methods

- Living cells require an additional criterion (viability)

<table>
<thead>
<tr>
<th>QA/QC parameter</th>
<th>Frequency of analysis</th>
<th>Acceptance Limits</th>
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</thead>
<tbody>
<tr>
<td>Calibration</td>
<td>per batch</td>
<td>slope and EC50 within historical range; $R^2$ of sigmoidal curve $&gt; 0.95$</td>
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<tr>
<td>Vehicle blank</td>
<td>per batch</td>
<td>vehicle-induced response within 25% RSD of response without vehicle</td>
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<tr>
<td>Precision</td>
<td>per sample</td>
<td>&lt;30% RSD for triplicate measurements</td>
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<tr>
<td>Matrix spike</td>
<td>per batch</td>
<td>within 25% RSD of expected response</td>
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<tr>
<td>Cytotoxicity</td>
<td>per sample</td>
<td>&gt;80% cell viability</td>
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Validation of ER-α and AhR for water quality

- Independent round-robin exercises
  - e.g. Besselink 2004, Escher 2014, Mehinto 2015, Kunz et al. 2017, Altenburger 2018

- Application - WWTP effluent, product water (RO, MF), surface water, drinking water

- ER-α and AhR results indicated adequate sensitivity and precision for benchmarking

- Comparability among different commercial cell lines/labs still needed
Cell bioactivity reflects water quality / level of treatment

ER screening threshold: 3.5 ng/L
Cell bioactivity reflects water quality / level of treatment

AhR screening threshold: 0.5 ng/L
Implication / usage of bioscreening data

- Bioscreening thresholds should be interpreted the same as MTLs for targeted CECs
- Full interpretive framework for bioscreening results is not ready for regulatory application
- Future development of bioanalytical monitoring should include rigorous evaluation of bioscreening thresholds
Commercial services for bioanalytical monitoring

- Limited for full service (sample extraction + analysis) – e.g. Biodetection System (BDS)

- More options using sequential (“2-lab”) approach
  1. Competent analytical lab for SPE extraction using modified EPA method
  2. Sample extracts shipped to cell assay lab – e.g. Life Technologies, INDIGOBiosciences, IonTox, BDS, etc.
Guidance from technical experts

- Advisory group recommended by the CEC Expert Panel to guide phased bioanalytical monitoring

- Can assist with:
  - selection of methods
  - identification of qualified service labs
  - validation and analysis of data
Questions?

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