Cell Bioassays to Assess Chemical Mixtures in Waters

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Novel Approach for Chemical Monitoring

• Targeted analytical chemistry
  - Measure of contaminants prioritized by the State or EPA

• Non-targeted analytical chemistry
  - Broad chemical screening of all detectable chemicals

• Cell bioassays (or bioanalytical tools)
  - Integrated measure of known and unexpected bioactive chemicals
Cell Bioassays

- High-throughput method, with rapid turnaround
- Mammalian cells engineered to track cellular effects of chemicals
- Combined measure of all chemicals with same biological activity
- Results expressed relative to a reference chemical - bioanalytical equivalent concentration (BEQ), ng/L
Cell Bioassay Mechanism

Bioactive CECs

Cell

nuclear receptor

Reporter gene activated

Control cells

Activated cells
# Relevant Cell Bioassays

<table>
<thead>
<tr>
<th>Assay endpoint</th>
<th>Chemicals screened</th>
<th>Associated risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor (ER)</td>
<td>Estrogens, alkylphenols</td>
<td>Impaired reproduction</td>
</tr>
<tr>
<td>Aryl hydrocarbon receptor (AhR)</td>
<td>Polychlorinated biphenyls, polycyclic aromatic hydrocarbons</td>
<td>Developmental anomalies and tumors</td>
</tr>
<tr>
<td>Glucocorticoid receptor (GR)</td>
<td>Anti-inflammatory steroids</td>
<td>Immune-related diseases</td>
</tr>
<tr>
<td>Thyroid receptor (TR)</td>
<td>Pesticides, bisphenols</td>
<td>Altered neurodevelopment</td>
</tr>
<tr>
<td>Peroxisome proliferator activ. receptor (PPAR)</td>
<td>Pharmaceuticals, phthalates</td>
<td>Metabolic disorders</td>
</tr>
</tbody>
</table>
Commercial Availability

• Several manufacturers with proprietary cell lines

• Full or partial kits:
  - Cells for culture (immortal) or ready to be plated (division-arrested)
  - Fluorescent/luminescence substrate
  - Recommended assay media and reference chemical
  - Instructions for cell handling, substrate addition and bioassay reading parameters
Cell Bioassays to Assess Water Quality

• Technology currently used for pharmacology, food industry and chemical registration

• Must be adapted for unknown chemical mixtures

What is the sensitivity of these assays?

Are the methods reproducible and transferable?

Do the patterns of responses make sense?
Evaluating Bioassay Sensitivity

• Bioassay responses should reflect level of treatment and/or amounts of chemicals in a sample

• Bioscreening analyses conducted on various sample types
  - Influent
  - Secondary and tertiary treated effluents
  - Advanced treated water (microfiltration, reverse osmosis, UV...)
  - Ambient water (stream, river, stormwater...)
Benchmarking Water Quality Is Possible

- 20 laboratories tested over 100 different bioassays
Standardizing Assay Protocols

- Performance-based criteria developed to ensure robustness and comparability of data
- Reproducibility of protocols demonstrated through inter-laboratory exercises

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance criteria</th>
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<tbody>
<tr>
<td>Cell viability</td>
<td>≥80% viability compared to control wells</td>
</tr>
<tr>
<td>Calibration</td>
<td>Hill slope, EC50, Z’ within expected range, R²&gt;0.95</td>
</tr>
<tr>
<td>Matrix spike</td>
<td>Recovery of spiked chemicals between 70 - 130%</td>
</tr>
<tr>
<td>Precision</td>
<td>RSD/CV of triplicate measurements ≤30%</td>
</tr>
</tbody>
</table>
Explaining Measured Bioactivity

- Targeted analyses to measure known chemicals
  - Mass balance based on relative potency of individual chemicals
    - estradiol > estrone > bisphenol A > nonylphenol

<table>
<thead>
<tr>
<th></th>
<th>Santa Rosa</th>
<th>Mirabel</th>
<th>Piner Crk</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER- BEQ (ng E2/L)</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Targeted chemical analyses (ng/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-Estradiol (E2)</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>11</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>16</td>
<td>&lt;10</td>
<td>55</td>
<td>12</td>
</tr>
<tr>
<td>4-Nonylphenol</td>
<td>63</td>
<td>25</td>
<td>53</td>
<td>247</td>
</tr>
</tbody>
</table>
Explaining Measured Bioactivity

- Non-targeted MS analyses to measure unexpected chemicals
  - Promote discovery of emerging chemicals
Transitioning Cell Bioassay to Water Quality Agencies

• Results are encouraging
  - Bioscreening patterns are indicative of water quality
  - Standardized bioassay protocols exist for a handful of endpoints

• Next steps
  - Develop better testing guidelines (from sample collection to data analyses)
  - Conduct interlaboratory exercises to assess lab proficiency and bioassay comparability
  - Establish relevant bioscreening thresholds for data interpretation
Bioanalytical Implementation Advisory Group

• Convened by CA WateReuse, led by NWRI

• Members include cell assay experts and stakeholders

• Goal is to produce a guidance document with detailed recommendations for:
  - Collection (incl. QA), preservation, storage
  - Extraction procedure
  - Samples plating instructions
  - Data acceptability criteria
  - BEQ calculation and data interpretation
Questions?

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Back up slides
Developing Bioscreening Thresholds

• We envision **four thresholds** that could inform management actions

• This is achieved through lab and field-based studies to quantify the relationship between cell assay response and animal response
Developing Bioscreening Thresholds

- We envision **four thresholds** that could inform management actions

- **High** concern — in depth toxicity identification, control (all controllable) sources
- **Elevated** concern — confirm levels using targeted and non-targeted methods; expand monitoring
- **Moderate** concern — continue monitoring to ensure bioactivity levels are not increasing
- **Little/No** concern — Reduce frequency of monitoring