Bioanalytical Screening Tools for Recycled Water: Recommendations from the CEC Science Advisory Panel

Jörg E. Drewes, Paul Anderson, Nancy Denslow, Adam Olivieri, Walt Jakubowski, Dan Schlenk, Shane Snyder

Bioanalytical Screening of Recycled Water Workshop
Costa Mesa, California
June 11, 2018
Panel Recommendations

The results and recommendations are documented in a final report (SCCWRP TR1020), released to the public on 27 April 2018

https://www.waterboards.ca.gov/
Panel Charges

• What are the appropriate constituents to be monitored in recycled water, including analytical methods and method detection limits?

• What is the known toxicological information of the above constituents?

• Would the above list change based on level of treatment and uses as specified in Title 22 and for surface water augmentation (SWA)? If so, how?

• What indicators or surrogates can be used to represent a suite of CECs?

• What concentrations of CECs should trigger enhanced monitoring?
CEC Definition (2018)
‘Constituents of emerging concern’

- Personal care products
- Pharmaceuticals
- Industrial
- Agricultural
- Natural hormones
- Inorganic constituents (boron, chlorate)
- Food additives and constituents (phytoestrogens, caffeine, sweeteners)
- Transformation products
- Nanomaterials
- Microplastics
- Antibiotic resistance
Use of Bioanalytical Methods for CEC Screening

- Two bioassays:
  - ER
  - AhR

- Standardized methods available (USEPA, OECD)
- Commercial labs

- NTA
  - Only in support of positive bioassay results
Cell assays have a long history of use for screening “good from bad” chemicals

- Pharma use
- Endocrine Disruptor Screening Program (EPA)
- European application (ISO & OECD)
Cell assays screen by groups of chemicals with similar bioactivity (i.e. via common MOAs)

Effects are additive
Cell assays recommended by the Panel are

1) integrative exposure screens...

- Chemically estimated estrogen equivalence matches fairly well with bioassay results

(Bulloch et al., 2010).
...and are 2) linkable to deleterious effects on human health

Adapted from Toxicity Testing in the 21st Century, NRC
Cell assays considered by the Panel are calibrated and referenced to a known toxic chemical, resulting in a concentration (EEQ or BEQ).

Example of ER Bioassay Screening Trigger Level

- Human Health EE2 PNEC = 3.5 ng/L
Identifying monitoring trigger levels (MTLs) for bioanalytical assays

**ER MTL = 3.5 ng/L**
1. Health based trigger level → Predicted no effect concentration (PNEC) for human health = 3.5 ng/L
2. MTL in Europe for ER = 3.8 ng/L
3. Method reporting level for ER assay with E2 = 0.5 ng/L
4. This value is well within the current calibration range

**AhR**
1. Method reporting level for AhR assay with TCDD = 0.5 ng/L
2. As more data comes in we can develop a health-based trigger level
3. Similar/lower to MTLs determined in Europe
ER assay and evaluation of water

Standard curve

Water samples

Legend for samples
A = Effluent 2
B = Effluent 1
C = Ozonation
D = Storm water
E = Membrane
F = RO
G = River Water
H = AO
J = Blank
K = Drinking water
CA = SCCWRP proj
Reproducibility of assays - results from 4 different labs.

MTL (Ph I) = 3.5 ng E2/L

- WWTP effluent
- Membrane filtration
- Reverse osmosis
- Recycled water
- Ambient water

Estrogen Equivalency – EEQ (ng/L)
Recommendation: Phased Approach with Collaborative Guidance

• Phase I – data collection
• Phase II – pilot evaluation of interpretive framework (includes initial or interim MTLs)
• Phase III – full implementation with actionable thresholds
• A steering committee would guide selection of appropriate endpoints, measurement goals (QA/QC) qualified vendors/labs, SOPs, and interpretive guidelines (i.e. MTLs)
Phase 1: Collection of data

Unmonitored CECs

- PERFORM BIOASSAYS
  - Residual bioactivity?
    - No: STOP
    - Yes: Targeted analysis

Targeted analysis

- Yes: Exposure screening (MEC/MTL)
- No: PERFORM NTA

Chemicals identified?

- Yes: Develop method
- No: Risk Assessment
CEC Monitoring Only Makes Sense for Potable, not Non-Potable Practices

- 45 different applications instead of a single landscape irrigation practice
- Exposures associated with Title 22 non-potable uses were estimated to be at least 10x lower than exposures associated with the potable reuse applications for all CECs and likely to be 100x lower for most CECs (one exception: impoundment with fishing)
- Panel recommends deriving MTLs for non-potable reuse by multiplying the potable reuse MTLs by a factor of 10
- Surrogate measurements are best way to assess Title 22 recycled water quality
## Expansion of Bioanalytical Toolbox

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor– ER alpha (Agonist mode)</td>
<td>Impaired reproduction, feminization of males</td>
</tr>
<tr>
<td>Aryl hydrocarbon receptor-- Ahr</td>
<td>Dioxin-like toxicity, cancer, tissue damage</td>
</tr>
<tr>
<td>Glucocorticoid Activity – Glucocorticoid receptor--GR</td>
<td>Impaired development, immune diseases</td>
</tr>
<tr>
<td>Androgen receptor – AR (Antagonist mode)</td>
<td>Impaired reproduction, de-masculinization of males, hypospadias</td>
</tr>
<tr>
<td>Peroxisome proliferator activated receptor -- PPAR</td>
<td>Metabolic disorders, impaired immune function, cancer</td>
</tr>
<tr>
<td>Tumor suppressor protein response element – P53</td>
<td>DNA damage, mutagenicity, cancer</td>
</tr>
</tbody>
</table>
CEC Science Advisory Panel

Acknowledgments

[Image of group photo]