Monitoring contaminants of emerging concern (CECs)

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SCCWRP Commission
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Chemical methods are limited in scope

There are thousands of chemicals to consider

Chemical usage will change over time
new drugs
replacement chemicals

We have methods for ~500 chemicals
~200 priority pollutants
~300 “CECs”
Current toxicity testing has low relevance

Complex chemical mixtures are present in scenarios of interest

Current toxicity tests target lethality, growth

For CECs (e.g. synthetic hormones), latent effects at sub-lethal exposure levels are of concern
A new CEC monitoring approach is needed

Tools that can screen for various chemicals with high sensitivity

Analytical methods that identify problematic CECs

Biological tests that target plausible “CEC outcomes”
intersex, reproduction immunosuppression

<table>
<thead>
<tr>
<th>CEC</th>
<th>Class</th>
<th>Reporting Limit (ng/L)</th>
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<tbody>
<tr>
<td><strong>17-β estradiol</strong></td>
<td>Natural hormone</td>
<td><em><em>0.09 (1.0</em>)</em>*</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Food product</td>
<td><em><em>35 (50</em>)</em>*</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Pers care prod</td>
<td><strong>50</strong></td>
</tr>
<tr>
<td><strong>NDMA</strong></td>
<td>Rxn by-product</td>
<td><em><em>0.1 (2.0</em>)</em>*</td>
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from Anderson et al (2010).
Monitoring strategies for recycled water applications in California.
Our strategy

• Define chemicals we should and can monitor now
  – Pervasiveness
  – Potency
  – Availability of robust methods

• Develop an approach to screen for the larger universe of CECs
  – Bioanalytical tools

• Identify methods to interpret screening (bioassay) results
  – Non-targeted chemical analysis
SCCWRP convened a panel of experts to give us a process to identify chemicals for pilot monitoring:

• Step 1: measure or predict occurrence (i.e. concentration)
  – investigative monitoring (e.g. regional, special studies)

• Step 2: establish threshold that is protective of resource
  – toxicity tests (no/low observable effects concentrations)

• Step 3: does occurrence exceed toxicity threshold?
  – If no, do not monitor at this time
  – If yes, add to monitoring list
We are collaborating to fill data gaps

- CECs are prevalent in urban waterways
  - monitor effluent, in stream

- Few CECs accumulate in coastal bivalves
  - monitor to ensure levels aren’t increasing

- Marine fish are exposed to CECs, but show little evidence of impact (so far)
  - We will see changes in effluent quality?
The long term solution features bioanalytical screening...

Cellular ("in vitro") bioassays
Efficient screening for ALL chemicals of interest, including CECs

Whole animal ("in vivo") toxicity testing
Linking screening bioassays response to higher order effects

Field ("in situ") monitoring
Assessing health of resident/sentinel species
Adapting cell assays for water quality monitoring

- Collaborated with international consortium of investigators
- Perform literature review to identify endpoints of concern
- Evaluate and optimize performance of off-the-shelf products
- Establish a protocol for water samples
- Analyze samples representing a range of water quality
- Compare accuracy, precision across laboratories
Products that screen for CECs are *commercially* available

<table>
<thead>
<tr>
<th>ENDPOINT</th>
<th>SIGNIFICANCE</th>
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<tbody>
<tr>
<td>Estrogen Receptor (ER)</td>
<td>Feminization of males</td>
</tr>
<tr>
<td>Androgen Receptor (AR)</td>
<td>Masculinization of females</td>
</tr>
<tr>
<td>Glucocorticoid Receptor (GR)</td>
<td>Impaired development, <em>immunosuppression</em></td>
</tr>
<tr>
<td>Progesterone Receptor (PR)</td>
<td><em>Cancer</em>, hormone resistance syndrome</td>
</tr>
<tr>
<td>Aryl hydrocarbon Receptor (AhR)</td>
<td>Dioxin-like toxicity, <em>tissue damage</em>, cancer</td>
</tr>
<tr>
<td>Umu or p53</td>
<td>Genotoxicity, <em>DNA damage</em>, cancer</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>Cell → tissue damage → <em>death</em></td>
</tr>
</tbody>
</table>
We established a water testing protocol

Day 1
- Thaw frozen cells
- Cell count
- Plate cells @ specific density
- Overnight incubation (~16 hrs) at 37°C, 5% CO₂

Day 2
- Add substrate
- Incubation (~2 hrs) at room temperature
- Add diluted extracts
- Measure fluorescence
- Extract water sample

Thaw frozen cells
Plate cells @ specific density
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Overnight incubation (~16 hrs) at 37°C, 5% CO₂
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Incubation (~2 hrs) at room temperature
Measure fluorescence
Extract water sample
Our tests screened by water quality...

- blind analysis of samples from water recycling treatment train
- 20 labs measuring > 100 bioassay endpoints
- *Test for estrogenic chemicals ranked samples by water quality*
  - WWTP effluent > filtered effluent > drinking, surface water
...and were reproducible across labs

Five CA team labs used standardized protocol for estrogenicity test kit

Some variability in absolute response observed

*Test response across samples was similar*
These tests are ready for trial

- State-of-the-art cell assays can screen for a wide variety of relevant environmental contaminants, including CECs

- Off the shelf product that tests for *estrogenic chemicals* was
  - adapted for analysis of recycled water samples
  - successful in ranking samples according to quality
  - precise in the hands of capable (research) labs
  - vetted by international scientific community

- Other cell-based test products (e.g. genotoxicity) were less successful
Better chemical diagnostics will explain bioassay results

- If screening bioassay response is below threshold, keep monitoring
- If screening bioassay response is above threshold
  - confirm response, isolate affected area or unit process

  \[ \downarrow \]

- Initiate whole organism testing (does exposure result in a real effect?)
- Perform targeted monitoring for likely candidate stressors

  \[ \downarrow \]

- **What do we do when targeted monitoring is inconclusive?**
  
  *Non-targeted analysis (in toxicity identification mode)*
What is non-targeted chemical analysis?

- Integrated method that investigates all compounds in a sample
- Current mass spectrometric technology allows for
  - excellent resolution (separation) of co-eluting peaks (“2-D” chromatography)
  - precise chemical identification using “full scan” mass spectrum
  - Low detection limits for chemicals of interest
- Requires development of data handling and analysis tools (custom libraries)
Non-targeted chemical analysis can identify bioactive CECs

- There are hundreds of chemicals that can cause toxicity.
- Targeted chemical methods are limited as diagnostic tools (i.e., for identifying causative chemicals).
- Non-targeted analysis broadens the scope of CECs that can be identified in toxic samples.
- We are testing the “linkage” between non-targeted data and toxicity results:
  - bioanalytical screening
  - whole organism (CEC) toxicity tests
Are we missing relevant contaminants?

- CECs are often discovered after they have become pervasive
  - flame retardants (PBDEs)
  - perfluorinated compounds

- Non-targeted analysis can identify CECs \textit{BEFORE} they become an issue

- We are testing this concept by cataloguing ALL chemicals in marine top predators
Non-targeted analysis identifies problematic CECs...

Targeted chemical analysis
  Develop & implement methods for individual, high priority CECs

Non-targeted chemical analysis
  Broadens window to include unexpected, bioactive contaminants

Field ("in situ") monitoring
  Assessing CEC exposure in resident/sentinel species
New tools are critical pieces of our CEC monitoring strategy

- **Sample (water, sediment, tissue)**
  - Tier I: many samples, higher frequency
  - Tier II: selected samples, lower frequency

  - **Targeted Analytical Chemistry**
    - In vitro response refines target list

  - **Non-Targeted Analysis (NTA)**
    - Targeted analysis inconclusive?

  - **Cell bioassay (mode of action)**
    - If (+) in vivo

  - **Animal testing (invertebrates, fish)**
    - Population level effects (in situ)?
We need your help…

- continued participation in CEC occurrence studies
- pilot testing of bioanalytical screening tools
- proof of concept for non-targeted analysis in diagnosing toxicity

Current mechanisms for testing of our CEC strategy
- Statewide CEC Pilot Monitoring Study
- Stormwater Monitoring Coalition
- Regional special studies
- Bight activities

Please contact us
- Framework/Strategy: keithm@sccwrp.org
- Bioanalytical screening: alvinam@sccwrp.org
- Non-targeted analysis: nathand@sccwrp.org;
Three questions

• Do you see merit in moving from a chemically-based monitoring approach to one based on bioassays?

• What do you see as the biggest impediments to adoption of a bioassay approach?

• Are we ready to start a pilot implementation?
  • If so, where would be the best place to do so?