Bioanalytical tools in water

Review of the past and present and a vision for the future

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Bioanalytical tools

- Bioanalytical tools = *in vitro* bioassays
- Effects testing at the cellular level rather than at the organism level (*in vivo*)
Bioanalytical tools

“Bioanalytical” → emphasize use as analytical method in environmental science
Adverse outcome pathway

- Link between initiating event at molecular/cellular level and whole organism response

[Diagram of Adverse Outcome Pathway]

- Toxicant
  - Chemical Properties
    - Receptor/Ligand Interaction
    - DNA Binding
    - Protein Oxidation
  - Macro-Molecular Interactions
    - Gene Activation
    - Protein Production
    - Altered Signaling
    - Protein Depletion
  - Cellular Responses
    - Altered Physiology
    - Disrupted Homeostasis
    - Altered Tissue Development or Function
  - Organ Responses
    - Lethality
    - Impaired Development
    - Impaired Reproduction
    - Cancer
  - Population Responses
    - Structure
    - Recruitment
    - Extinction

Continuum of toxicity

Percentage of population under study responding

Increasing dose or exposure

Biomolecule
Cell
Organ
Organism
BATs and adverse outcome pathway

Adverse outcome pathway

Uptake of chemical into the organism and cell

Bioanalytical tools can be used as early indicators of hazard potential of chemical mixtures and are indicative of modes of toxic action

Ultimate protection goal

Cellular toxicity pathway

Metabolism (toxification/detoxification) → Interaction with target (initiating event) → Defense mechanisms → Cell death/damage

# Advantages and limitations of BATs

<table>
<thead>
<tr>
<th></th>
<th>In vivo</th>
<th>In vitro</th>
<th>Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxicological information</strong></td>
<td>YES</td>
<td>yes but …</td>
<td>no</td>
</tr>
<tr>
<td><strong>Detect mixture interactions</strong></td>
<td>YES</td>
<td>yes but …</td>
<td>no</td>
</tr>
<tr>
<td><strong>Online monitoring</strong></td>
<td>YES</td>
<td>yes but …</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Detect presence of “unknowns”</strong></td>
<td>YES</td>
<td>YES</td>
<td>yes but …</td>
</tr>
<tr>
<td><strong>Rapid</strong></td>
<td>no</td>
<td>YES</td>
<td>yes but …</td>
</tr>
<tr>
<td><strong>Cost-effective</strong></td>
<td>no</td>
<td>YES</td>
<td>yes but …</td>
</tr>
<tr>
<td><strong>Ethical</strong></td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>High throughput</strong></td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>High sensitivity</strong></td>
<td>no</td>
<td>YES</td>
<td>yes but …</td>
</tr>
<tr>
<td><strong>Identify causative chemical</strong></td>
<td>no</td>
<td>yes but …</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Fits within current regulation</strong></td>
<td>YES</td>
<td>no</td>
<td>YES</td>
</tr>
</tbody>
</table>
Why do we need BATs?

- Environmental monitoring relies mostly on chemical analysis
- Chemical analysis can have significant limitations
  - Too many chemicals to measure them all
  - Requires *a priori* selection of contaminants to monitor → Matthew Effect
  - Cannot detect non-target compounds
  - Cannot detect transformation products
  - Does not account for mixture effects
- Need additional tools ...
Toxicity testing

- Toxicity testing can overcome some limitations
- Detect chemicals by their effect, not structure
  - Non-target compounds
  - Transformation products
  - Mixtures (good agreement with mixture predictions*)
- Measures an effect, i.e., can be directly relevant to protection goal
- “Is there a needle in the haystack?”

Application of BATs to WQ testing

Application of BATs to recycled water

- 1960-1998: mutagenicity and genotoxicity at 7 schemes (Israel, CA, VA, FL, AZ)
- 1998-2007: a slow decade (3 studies: Namibia, Qld, USA), but renewed interest in \textit{in vitro} methods because of concerns about endocrine disrupting compounds
- 2007-2014: Renewed dynamism due to severe and widespread water scarcity (7 large studies, up to 103 bioassays)

How much more can BATs detect?

- Depends on bioassay category
  - **Specific toxicity** (driven by comparatively limited number of compounds) often agrees well with chemical analysis (although sometimes issues with chemical limit of detection)
  - For **other types**, even extensive chemical analysis usually explains <5% of bioassay response
    - *E.g.*, in recycled water:
      - 100% of photosynthesis inhibition
      - <3% of cytotoxicity
      - <1% oxidative stress

How do we use BATs today?

- BATs are used as measures of water quality
  - Benchmarking of different water sources
  - Assessment and monitoring of treatment efficacy
  - Detection of bioactive transformation products
  - Surrogate measure for wide range of compound
How much is too much?

- Development of effect based trigger values
  - EBT-BEQ for “specific toxicity”:
    - From ADI and reverse PK
    - Reading across from current guideline values
  - EBT-EC for other mechanisms:
    - Oxidative stress
    - Baseline toxicity
How do we use bioassay results?

A BRAVE future?

- **Bioanalytical tools in Risk Assessment – Validation and Experimentation**
  - Think-tank formed in Feb 2015
  - Supported by AWRCoeE, WE&RF and PUB
  - Q: Can *in vitro* bioassays assess safety of DW?

Huntington Beach (Feb 2016)

Leura (Feb 2015)
Can BATs be used to assess safety?

- No … (not yet?) …
- Can we envision the steps needed to reach this goal?
- Leura workshop identified four major knowledge gaps:
  1. Identify adverse effects and endpoints relevant to water
  2. Translate *in vitro* response to *in vivo* adverse effect (AOP)
  3. Convert *in vitro* concentration to *in vivo* exposure dose (IVIVE)
  4. Adapt all of above to mixtures
In summary …

- Bioanalytical tools today can be used to measure water quality (measure of exposure) but not safety (measure of effect)
- A good test battery should include assays covering different stages of the cellular toxicity pathway
- Indicator bioassays representative of crucial toxicity pathways provide good monitoring tools to:
  - Benchmark water quality
  - Assess treatment efficacy
  - Expand our analytical universe a little further into the unknown
- Future work in developing IVIVE and AOP and adapting those from single chemicals to mixture concepts will greatly expand the usefulness of bioanalytical tools
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