Effect-based analysis in water/biota

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Why use bioanalytical tools for monitoring?

- There are too many chemicals out there to quantify them one-by-one
- In addition: transformation products formed during treatment and in environment
- Any mixture effects?

Bioassays can be used as sum parameters indicating the overall toxic potential of an unknown chemical cocktail

The "world of organic micropollutants"

The "view" of an analytical chemist

The "view" of an environmental toxicologist

Ah receptor

oxidative stress

geno-toxicity

The world of chemical toxins

biocides

pesticides

pharmaceuticals

natural hormones

consumer and personal care products

industrial chemicals

combustion by-products

water treatment by-products

human metabolites and environmental transformation products

consumer and personal care products

industrial chemicals

combustion by-products

water treatment by-products

natural hormones

cytotoxicity
What is our protection goal?

There is more to health than cellular effects

BUT:
For chemical-induced effects, the initial interaction with the cells is a necessary but not a sufficient precondition

Conceptual framework: Adverse outcome pathway (AOP)

Adverse outcome pathway (AOP)

target concentration → initiating event: interaction with target → cellular response

toxicodynamics (toxicity pathway)

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

Toxicity pathway


Toxicodynamics (toxicity pathway)

- Target concentration
- Initiating event: interaction with target
- Cellular response

- Non-specific toxicity
- Specific, receptor-mediated toxicity
- Reactive toxicity
- Induction of xenobiotic metabolism pathways
- Induction of general stress response pathways

Inhibition and damage on cellular level
Bioanalytical tools

- Simulate the toxicokinetics (including metabolism)
- Indicative of the primary interactions with the biological target
  - three main classes of modes of toxic action
    - or indicative of adaptive stress response/defense mechanisms
- Low-complexity or in-vitro bioassays—ideally based on cell lines
- Cost-efficient and high-throughput
  - 96 well plate format
  - reporter gene assays
### Bioanalytical test battery

<table>
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<tr>
<th>Mode of action</th>
<th>Assay</th>
<th>Targeted chemicals</th>
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<tr>
<td><strong>Non specific toxicity</strong></td>
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<tr>
<td>Baseline toxicity</td>
<td>Bioluminescence inhibition assay</td>
<td>All chemicals</td>
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<tr>
<td>General cytotoxicity</td>
<td>Mammalian cell lines, MTS and NRU</td>
<td>All chemicals</td>
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<tr>
<td>Acetylcholinesterase AChE inhibition</td>
<td>AChE (neurotox)</td>
<td>Organophosphates, carbamates</td>
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<td>Photosynthesis inhibition</td>
<td>I-PAM (phytotox)</td>
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<td>Estrogenic effects</td>
<td>E-SCREEN</td>
<td>Estrogens, estrogenic industrial chemicals</td>
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<td>Genotoxicity</td>
<td><em>umuC</em> (genotox)</td>
<td>Aromatic amines, PAH, hard electrophiles (e.g., MMS)</td>
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<td>Protein damage</td>
<td><em>E.coli</em> GSH±</td>
<td>soft electrophiles (e.g., Seanine)</td>
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<tr>
<td>Oxidative stress</td>
<td>Induction of Nrf2 in AREc32</td>
<td>quinones, reactive oxygen species</td>
</tr>
</tbody>
</table>

**Specific toxicity**

**Reactive toxicity**
What an experiment looks like

- **solid phase extraction**
- **enrichment**
- **dilution**

\[
\frac{V_{\text{water}}}{V_{\text{extract}}} \quad \frac{\text{volume of extract added to assay}}{\text{total volume of assay}}
\]

**relative enrichment factor** \(\text{REF} = \text{enrichment factor}_{\text{SPE}} \times \text{dilution factor}_{\text{assay}}\)

\(\text{REF} = 1 \text{ “original sample”}\)

- Oxley Ck WWTP Inlet
- Oxley Ck WWTP Activated Sludge
- Oxley Ck WWTP post Clarifiers
- Oxley Ck WWTP post UV
- Field blank
- Lab blank

What an experiment looks like

solid phase extraction

enrichment

\[
\frac{V_{\text{water}}}{V_{\text{extract}}}
\]

dilution

volume of extract added to assay

total volume of assay

relative enrichment factor \( \text{REF} = \text{enrichment factor}_{\text{SPE}} \times \text{dilution factor}_{\text{assay}} \)

\[
\text{REF} = 1 \text{ “original sample”}
\]

From Sewage to Drinking Water: The Seven Barriers of Water Recycling

Barrier 1: Residential/Industrial Source Control, including hospital waste
Removes: suspended solids, organics (BOD & COD), nitrogen, phosphorus

Barrier 2: Wastewater treatment plant
Removes: organics

Barrier 3: Microfiltration
Removes: turbidity, particles, organics

Barrier 4: Reverse Osmosis
Removes: turbidity, inorganics, viruses, bacteria, protozoa, organics

Barrier 5: Advanced Oxidation
Removes: organics, viruses, bacteria, protozoa

Barrier 6: Natural Environment

Barrier 7: Water Treatment Plant, disinfection, distribution and quality management

Purified Recycled Water Process

Source: Queensland Water Commission
Microtox assay:

bioluminescence inhibition w/ Vibrio fischeri

Disinfection

Specific (receptor-mediated) toxicity

Secondary treatment does not remove herbicides sufficiently

Reverse osmosis almost completely removes herbicides and estrogens

Genotoxicity – *umuC* assay

Toxicity reduced across the seven treatment barriers in all bioassays

- Micropollutant burden was reduced by two order of magnitude or more, but to a different extent, in Barriers 2 to 5

- Effects in Barrier 6 and 7 and in drinking water were very low for most endpoints, typically falling below the detection limit or not significantly different from the blank

- Detection limits of the bioassays comparable or lower than the quantification limits of the routine chemical analysis

- Application for
  - benchmarking of different water sources (stormwater, bore water, coal seam gas water)
  - benchmarking of different treatment technologies:

Bioanalytical tools for assessing drinking water treatment

TOC: Total organic carbon

bioanalytical tools applied after solid phase extraction

all non-volatile micropollutants

non-volatile

DBP analysis

disinfection by-products

TOX: total organic halogen

volatile

disinfection by-products
Bioanalytical assessment of the formation of DBPs during drinking water treatment

- Full-scale metropolitan drinking water treatment plant
- Nonspecific toxicity and reactive toxicity increased with increase in total absorbable organic halogens (and individual DBPs) during drinking water treatment
- Overall levels are low, none of the drinking water standards are exceeded

Graph showing AOX (µg/L) and log (1/EC50 or 1/EC1.5) for nonspecific toxicity and reactive toxicity across different treatment stages (Inlet, Coagulation, Sand filtration, Chlorination, Chemical addition, Storage, Chloramination).

- Spin-off from industry and regulator’s workshops to communicate the scientific basis of bioanalytical tools
- Prepared as part of the development of a risk communication strategy for the Urban Water Security Research Alliance

Conclusion

Where we are

- Bioanalytical tools are recognized as valuable research tool
- Bioassays complement chemical analysis
- Information on the mixture effects of chemicals
- Wide applicability across the water cycle

The future?

- Evaluate the pollutant burden in biota
- Evaluate the role of transformation products (incl. volatile DBPs?)
- Accepted monitoring tool? International harmonisation?
- Bioassay based water quality criteria?