entox

Effect-based analysis in water/biota

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The University Of Queensland AUSTRALIA

Queensland Government

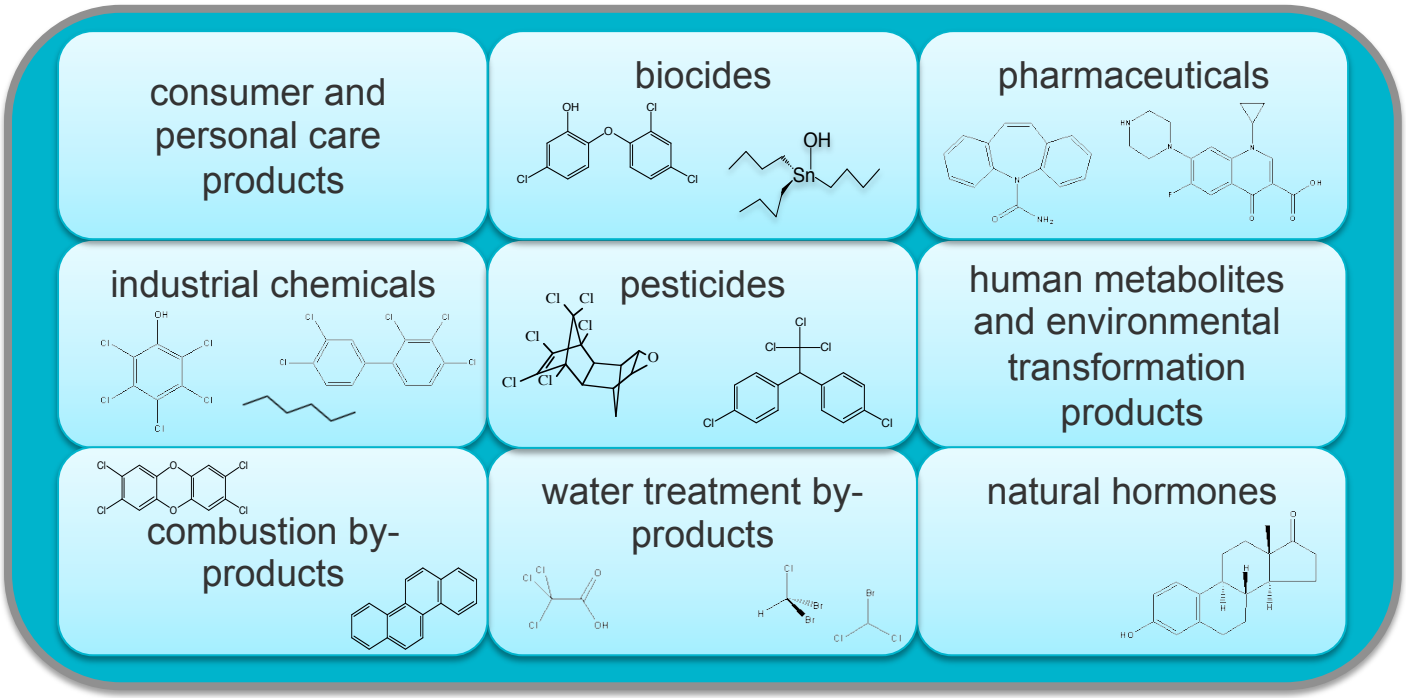
national research centre for environmental toxicology

Entox is a joint venture between The University of Queensland and Queensland Health

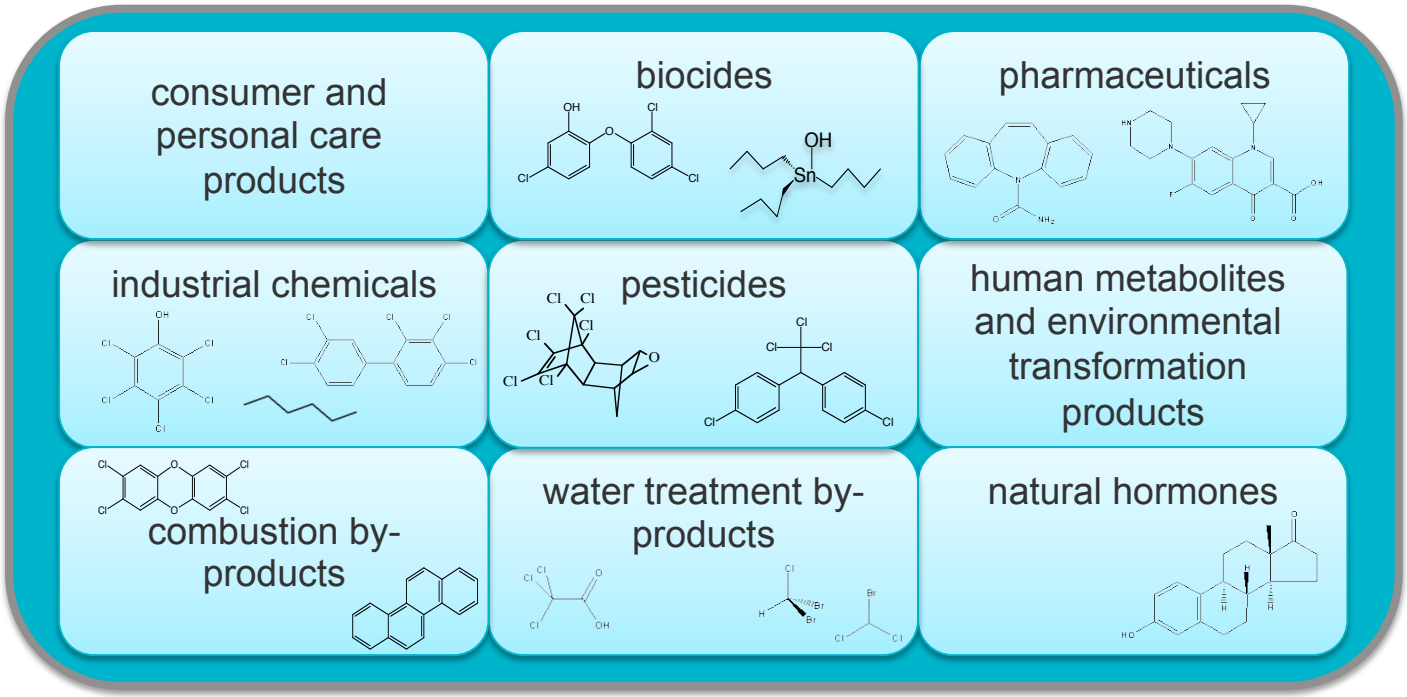
Why use bioanalytical tools for monitoring?

- 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?

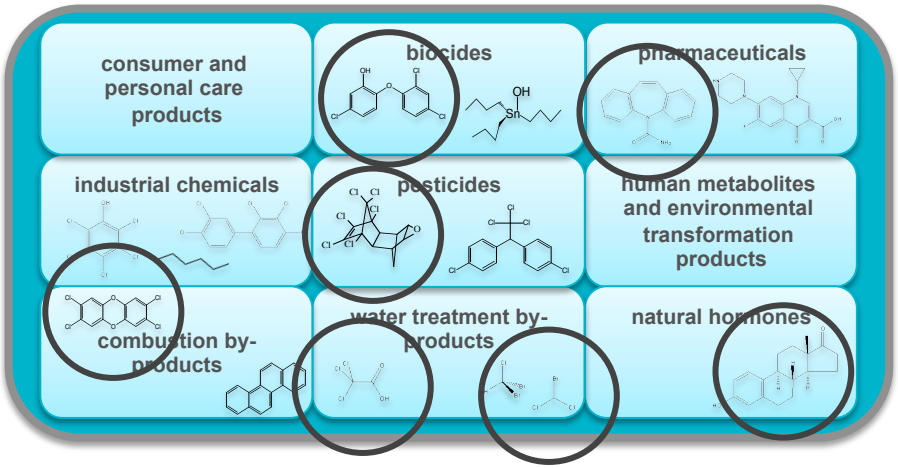
Bioassays can be used as sum parameters indicating the overall toxic potential of an unknown chemical cocktail



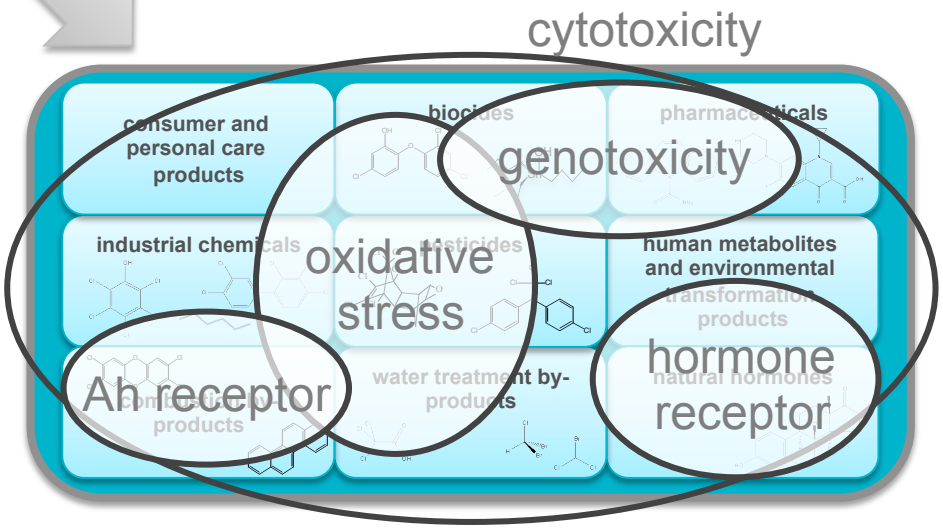
Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U. and Wehrli, B. (2006). The challenge of micropollutants in aquatic systems. Science, ***313(5790): 1072-1077.***



"the world of organic micropollutants"

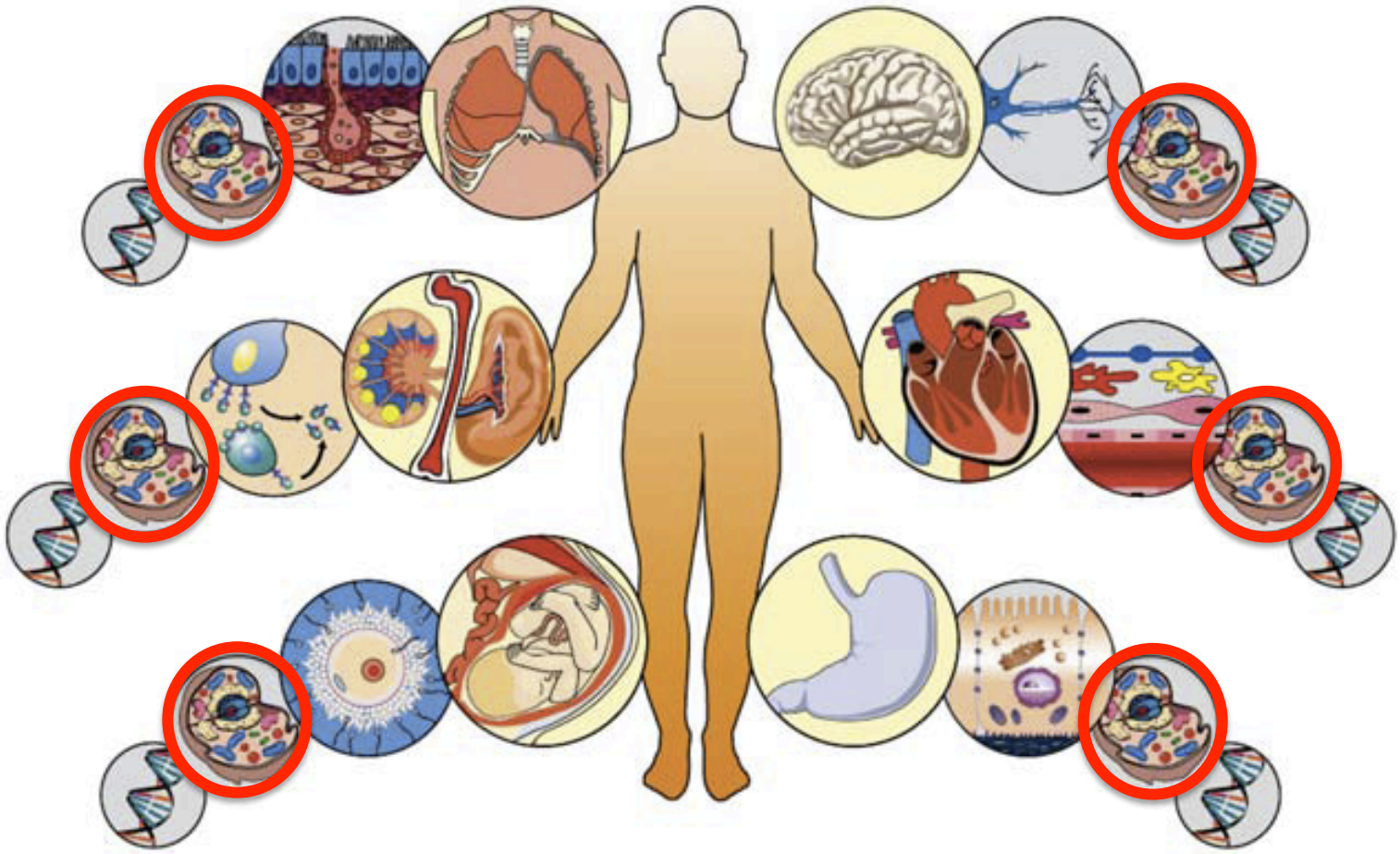


The "view" of an analytical chemist



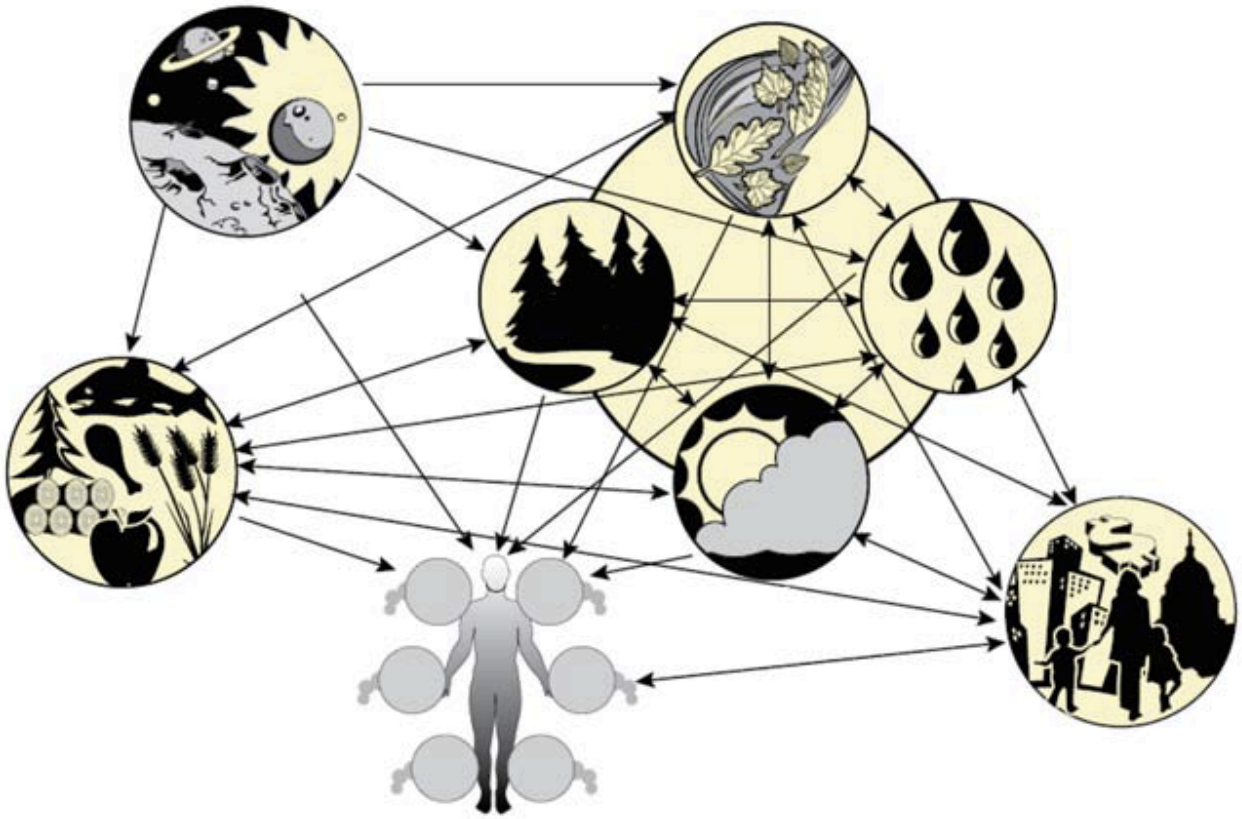
The "view" of an environmental toxicologist

What is our protection goal?



Gohlke and Portier, Environ Health Perspect 115:1261–1263 (2007)

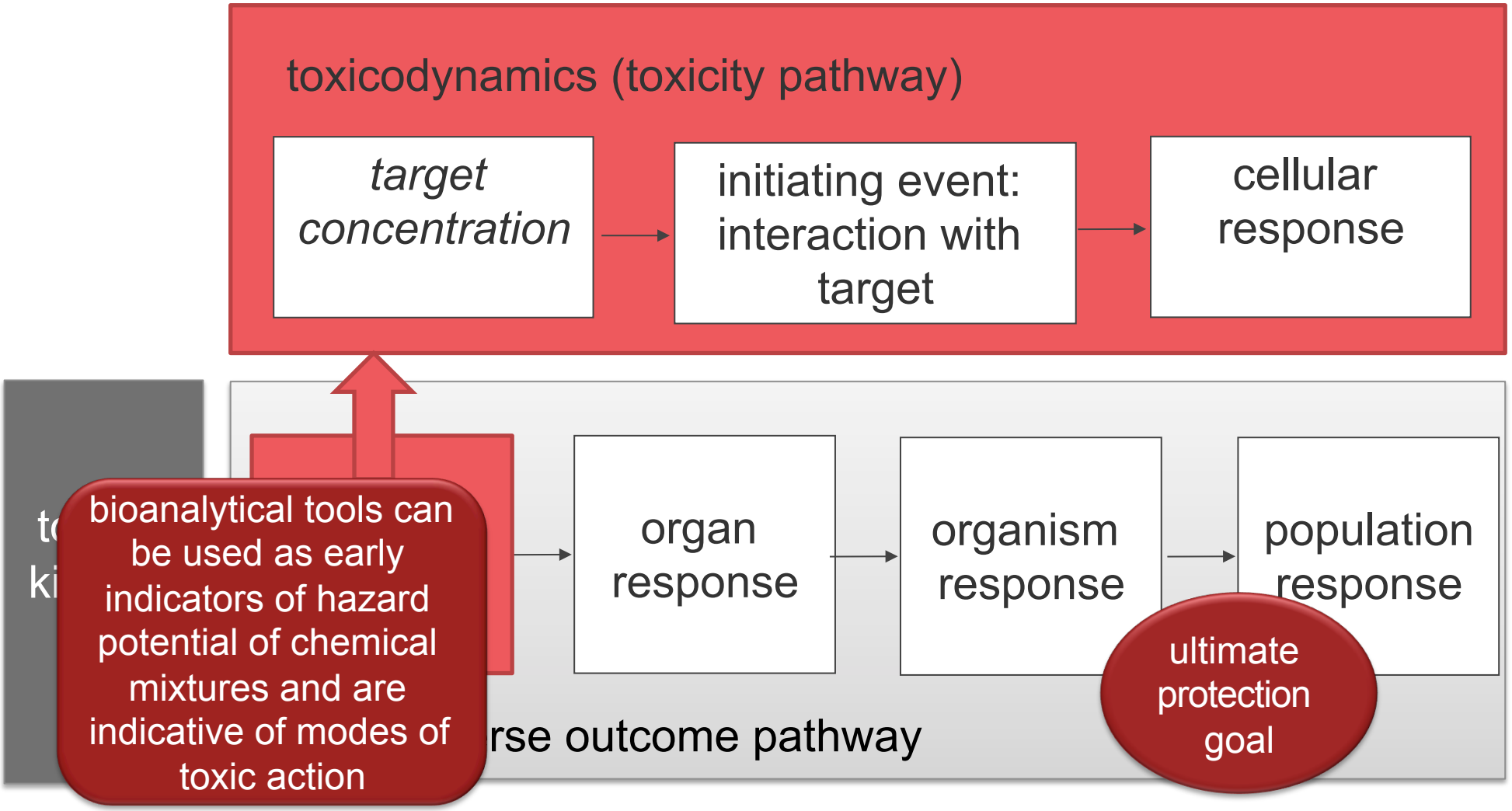
There is more to health than cellular effects



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

Gohlke and Portier, Environ Health Perspect 115:1261–1263 (2007)

Conceptual framework: Adverse outcome pathway (AOP)



Adapted from Collin et al. (2008) and Ankley, et al. (2010) in Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, December 2011

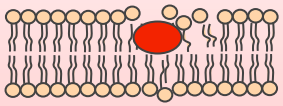
Toxicity pathway

toxico-kinetics

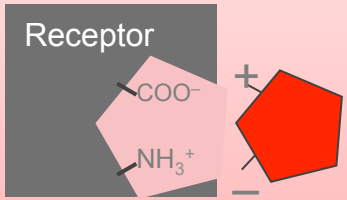
toxicodynamics (toxicity pathway)

target concen-tration → initiating event: interaction with target → cellular response

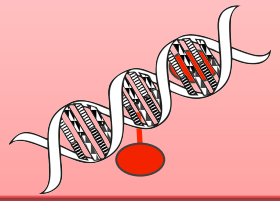
- non-specific toxicity



- specific, receptor-mediated toxicity



- reactive toxicity



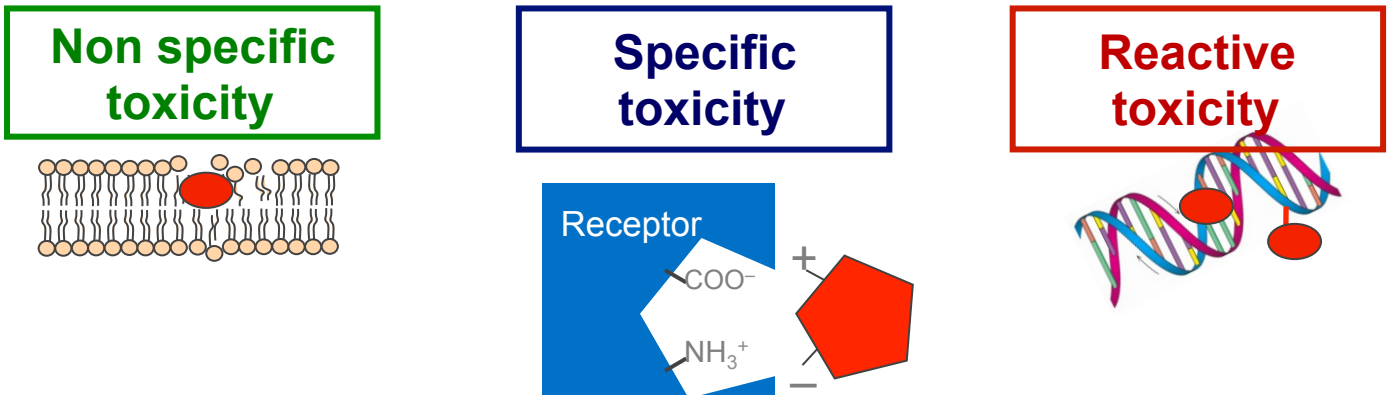
- induction of xenobiotic metabolism pathways
- induction of general stress response pathways

inhibition and damage on cellular level

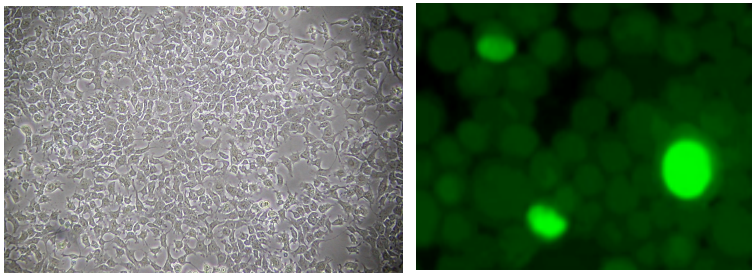
Adapted from Collin et al. (2008) and Ankley, et al. (2010) in Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, December 2011

Bioanalytical tools

- Simulate the toxicokinetics (including metabolism)
- Indicative of the primary interactions with the biological target
  - three main classes of modes of toxic action

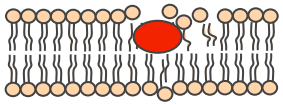


  - or indicative of adaptive stress response/defense mechanisms
- Low-complexity or in-vitro bioassays– ideally based on cell lines
- Cost-efficient and high-throughput
  - 96 well plate format
  - reporter gene assays

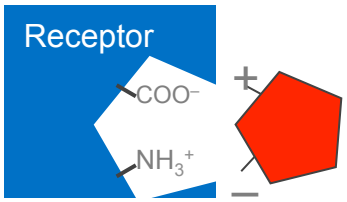


Bioanalytical test battery

Non specific toxicity



Specific toxicity



Reactive toxicity

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

What an experiment looks like

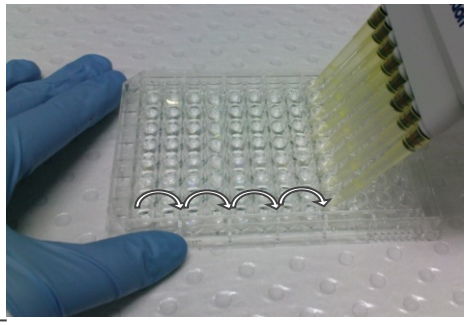
solid phase extraction

enrichment

$\frac{V_{water}}{V_{extract}}$

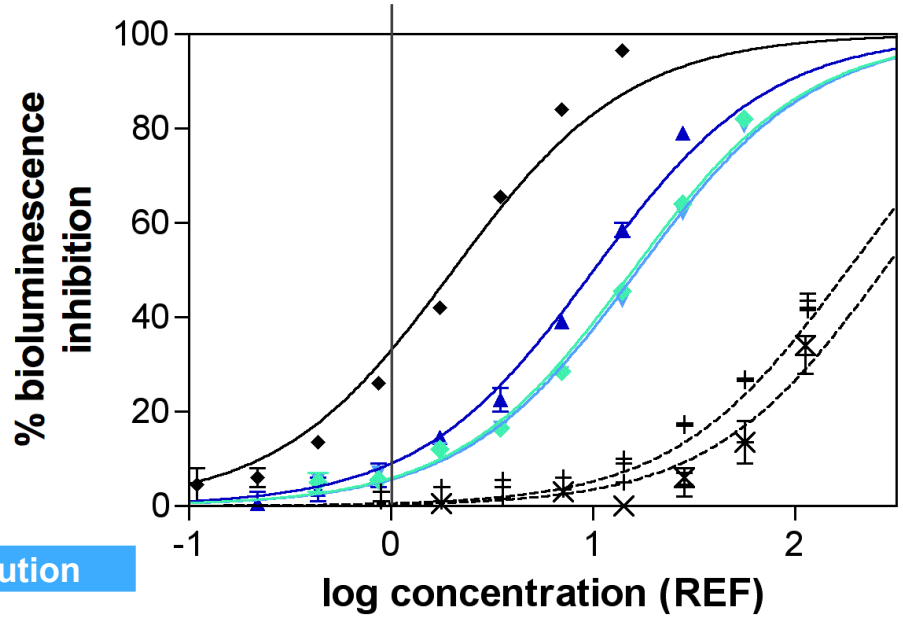
dilution

$\frac{\text{volume of extract added to assay}}{\text{total volume of assay}}$



relative enrichment factor REF = enrichment factor$_{SPE}$ x dilution factor$_{assay}$

REF = 1 "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

dilution

enrichment

*Macova, Toze, Hodgers, Mueller, Bartkow, Escher (2011). Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. Water Research, **45: 4238-4247.***

What an experiment looks like

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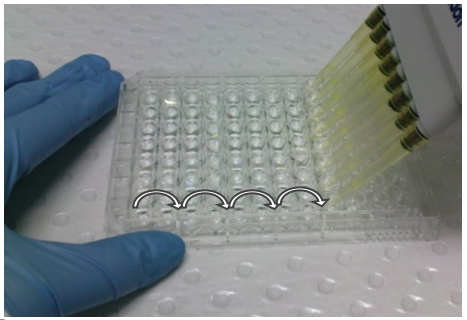

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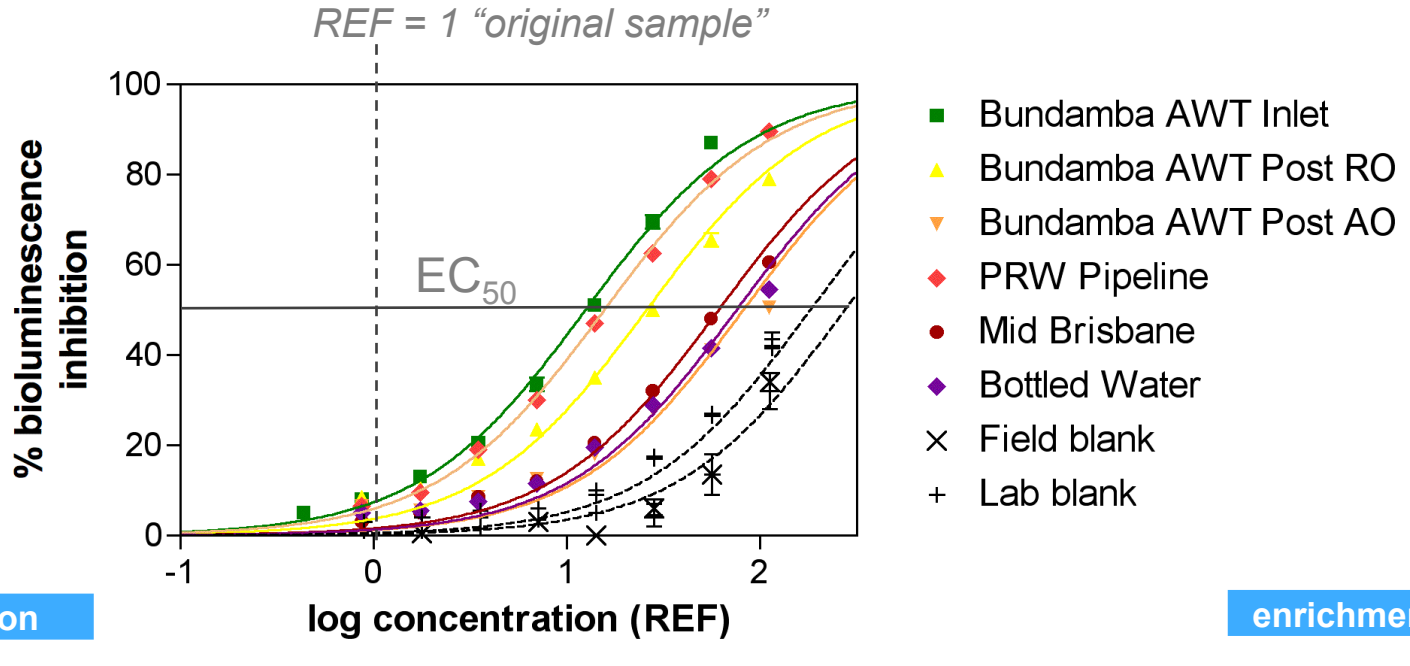
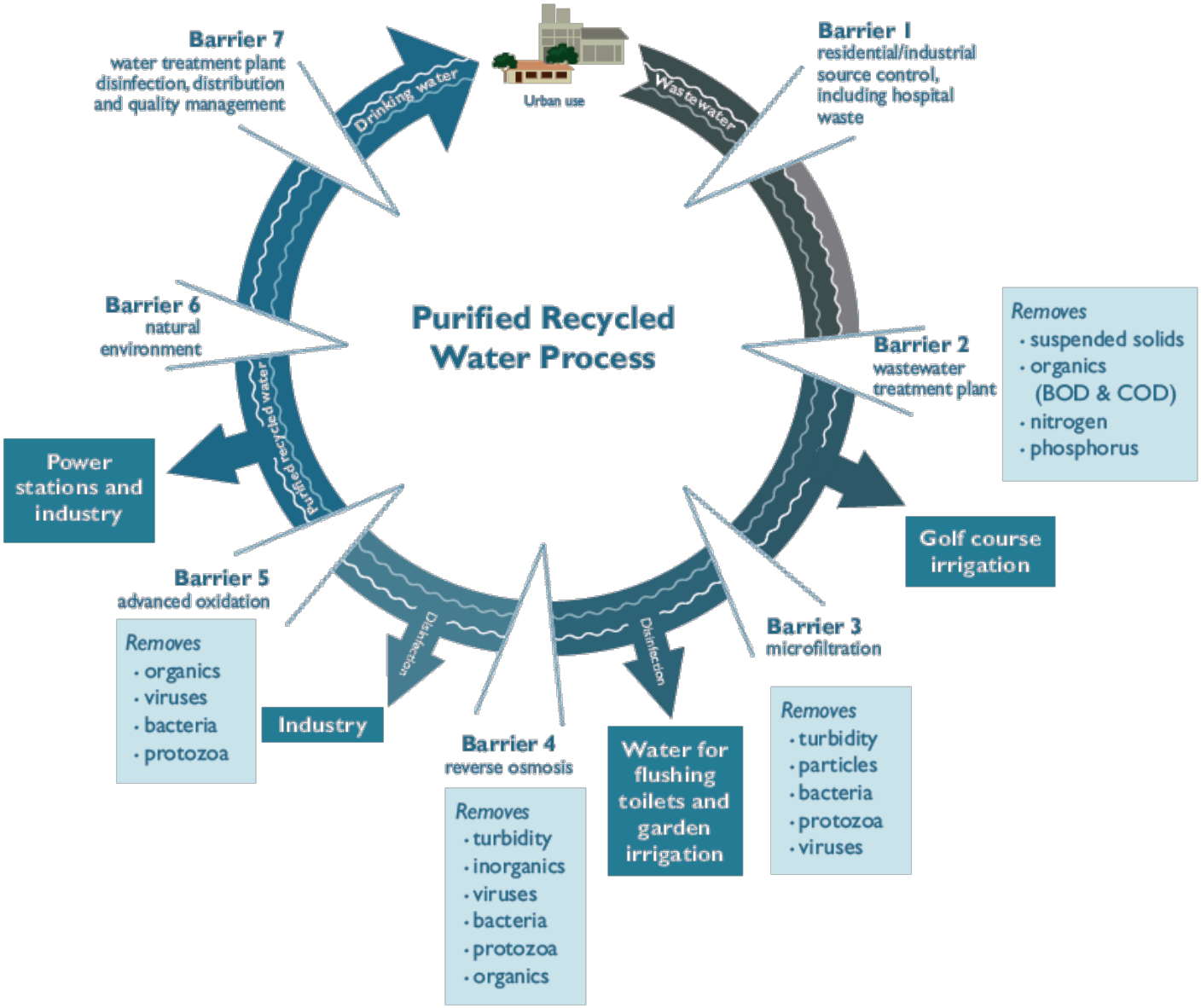


dilution **enrichment**

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



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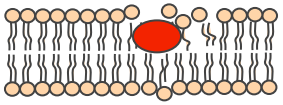
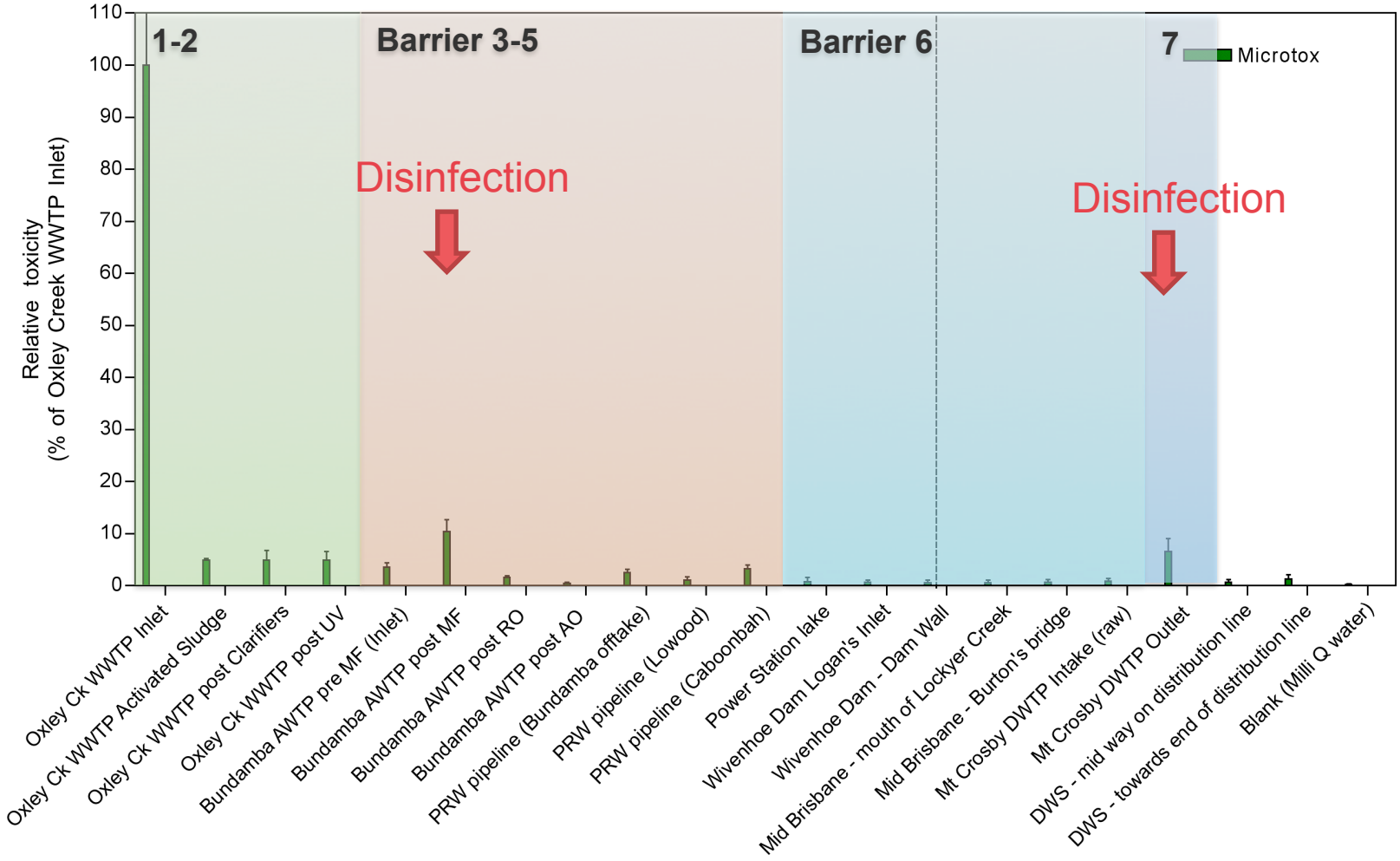
Source: Queensland water Commission

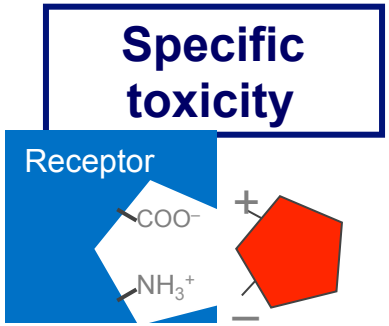
Non specific toxicity



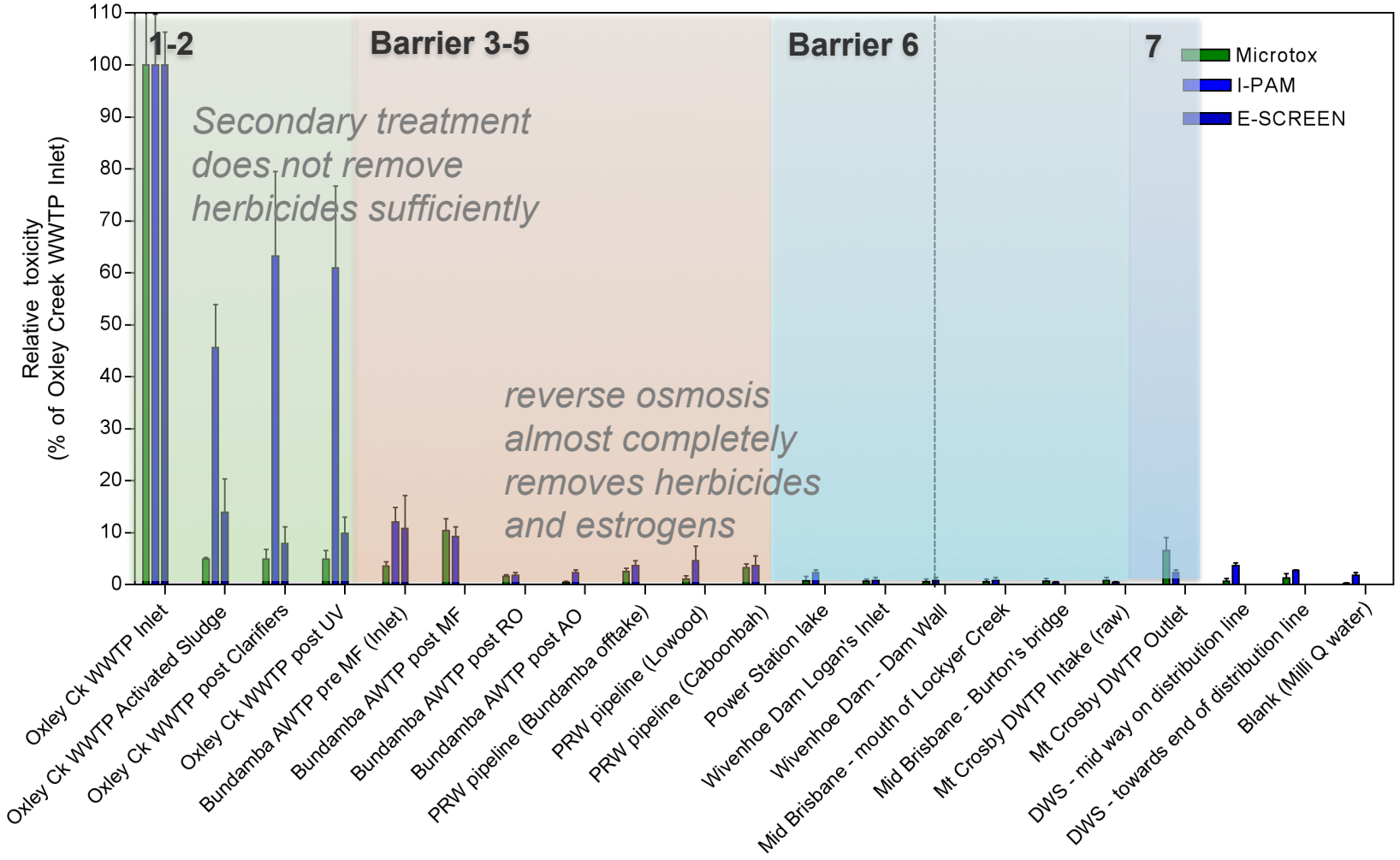
Microtox assay: bioluminescence inhibition w/ *Vibrio fischeri*



Macova, Toze, Hodgers, Mueller, Bartkow, Escher (2011). Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. Water Research, ***45: 4238-4247.***



Specific (receptor-mediated) toxicity



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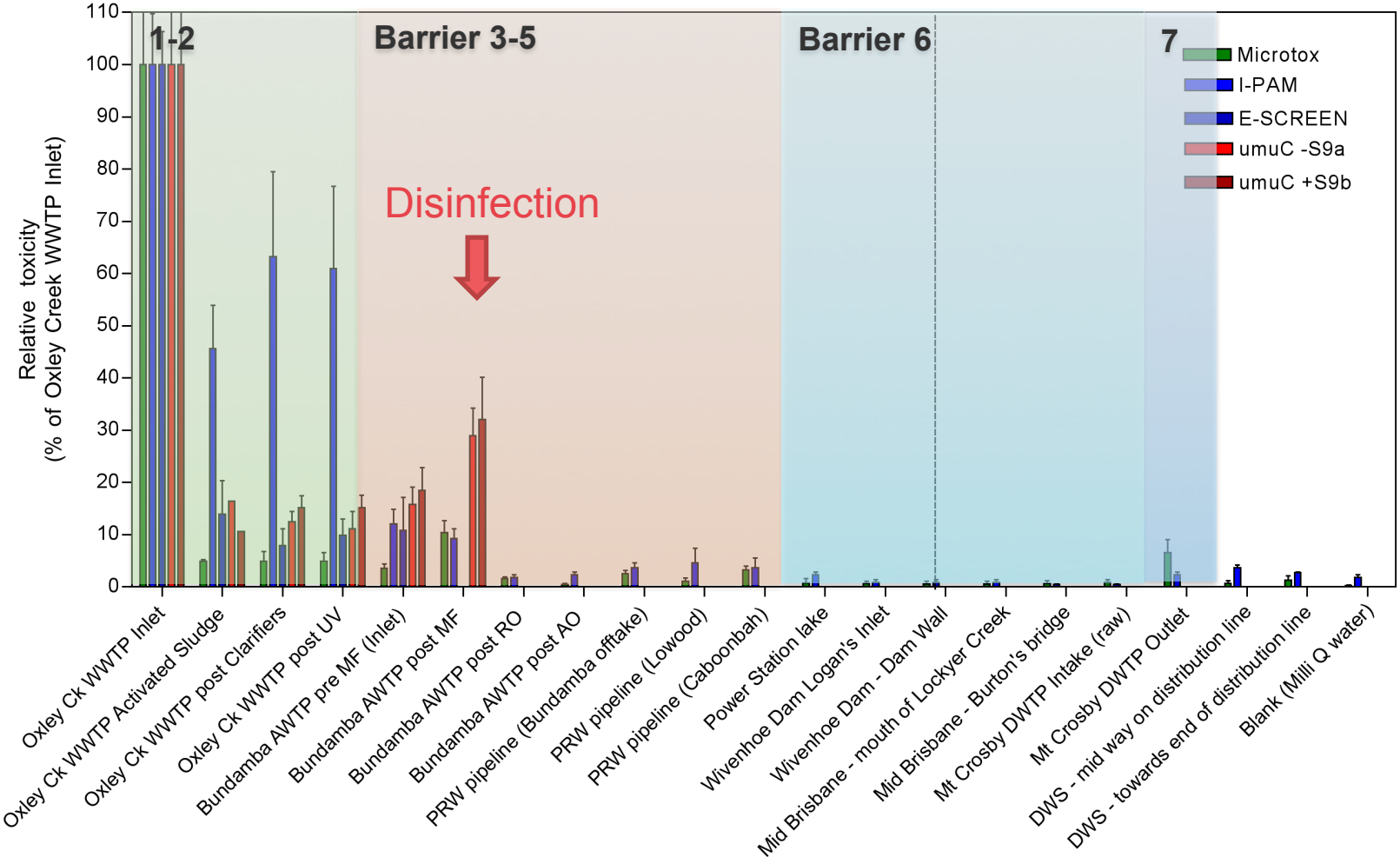
Reactive toxicity

Genotoxicity – *umuC* assay



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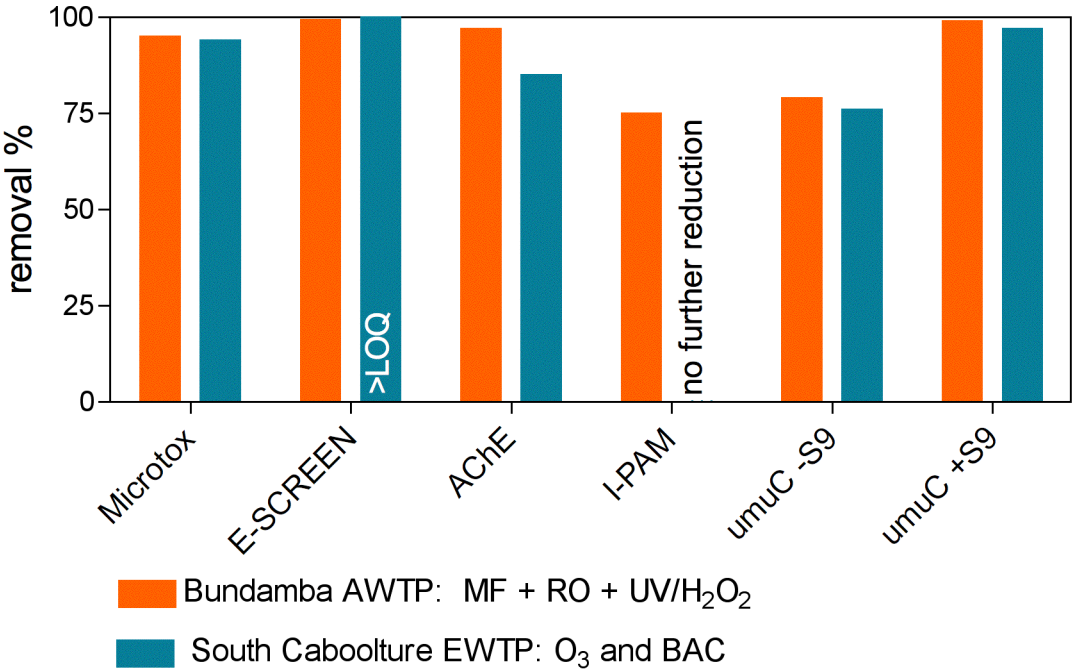
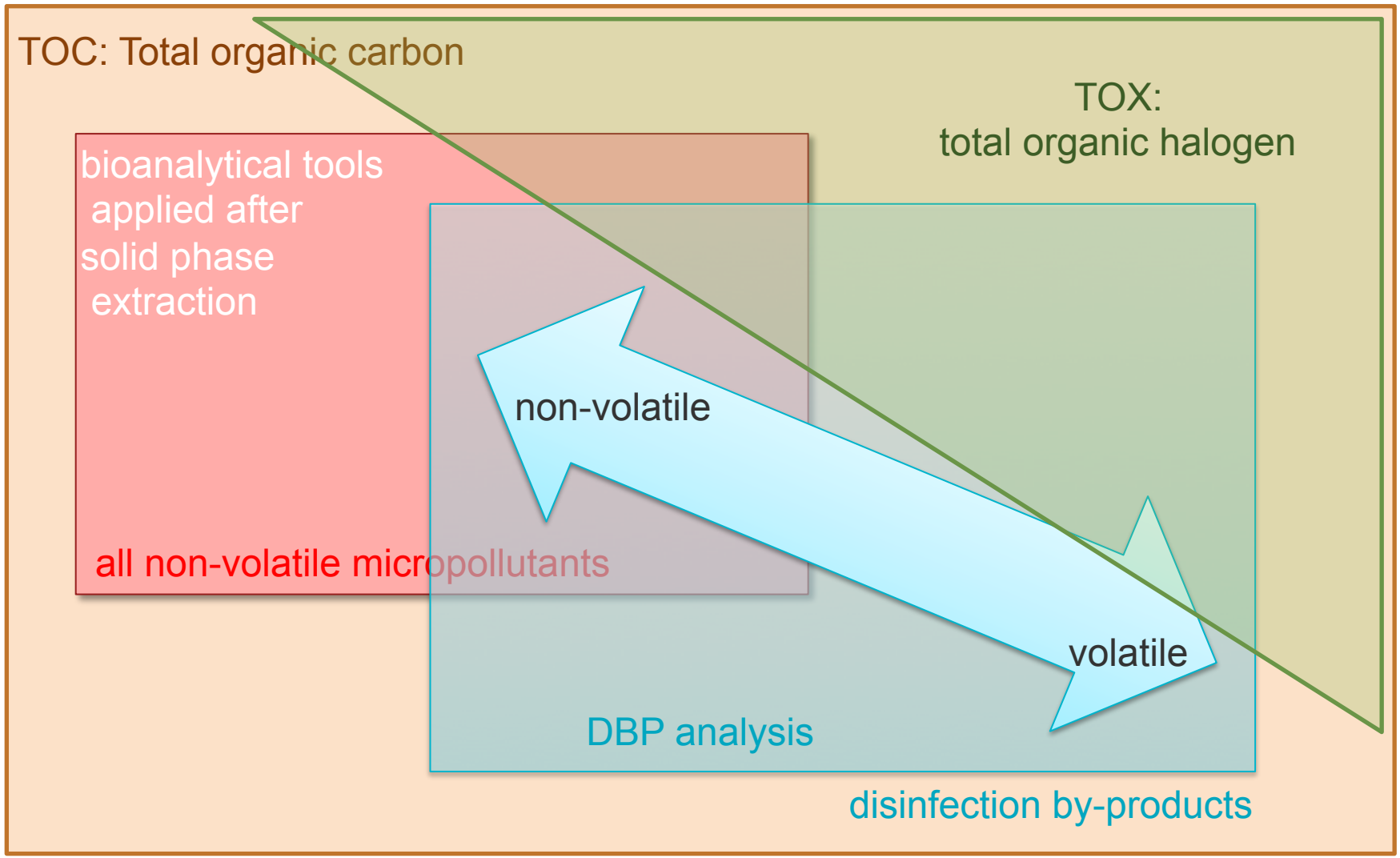
Toxicity reduced across the seven treatment barriers in all bioassays

- 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:

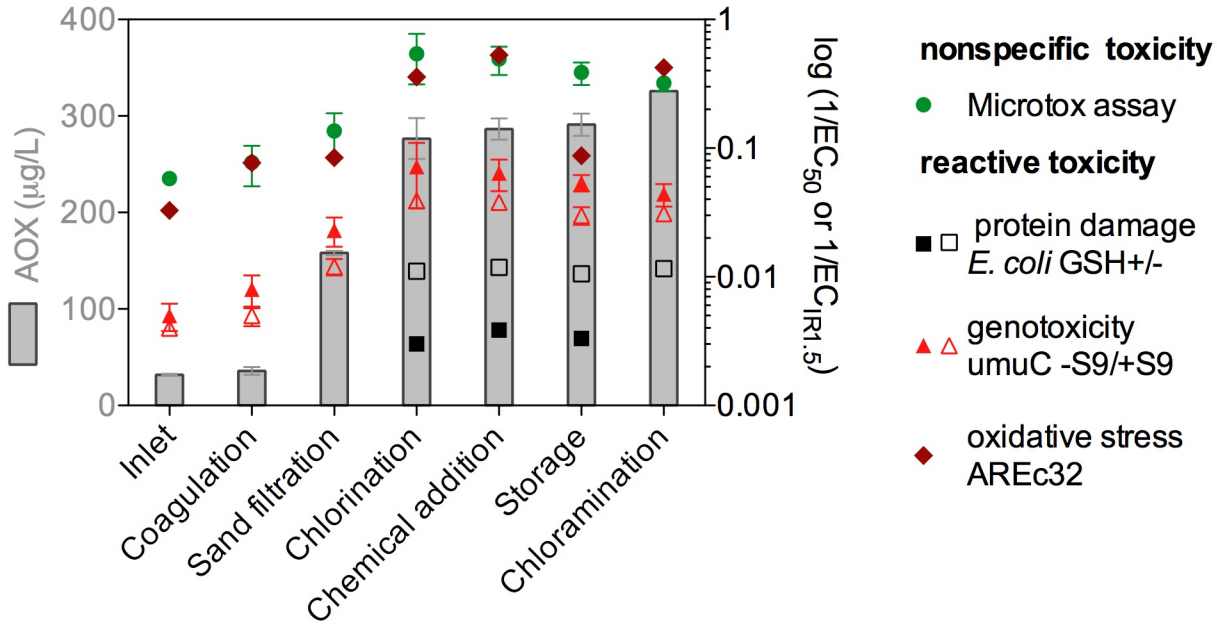


Bioanalytical tools for assessing drinking water treatment



Bioanalytical assessment of the formation of DBPs during drinking water treatment

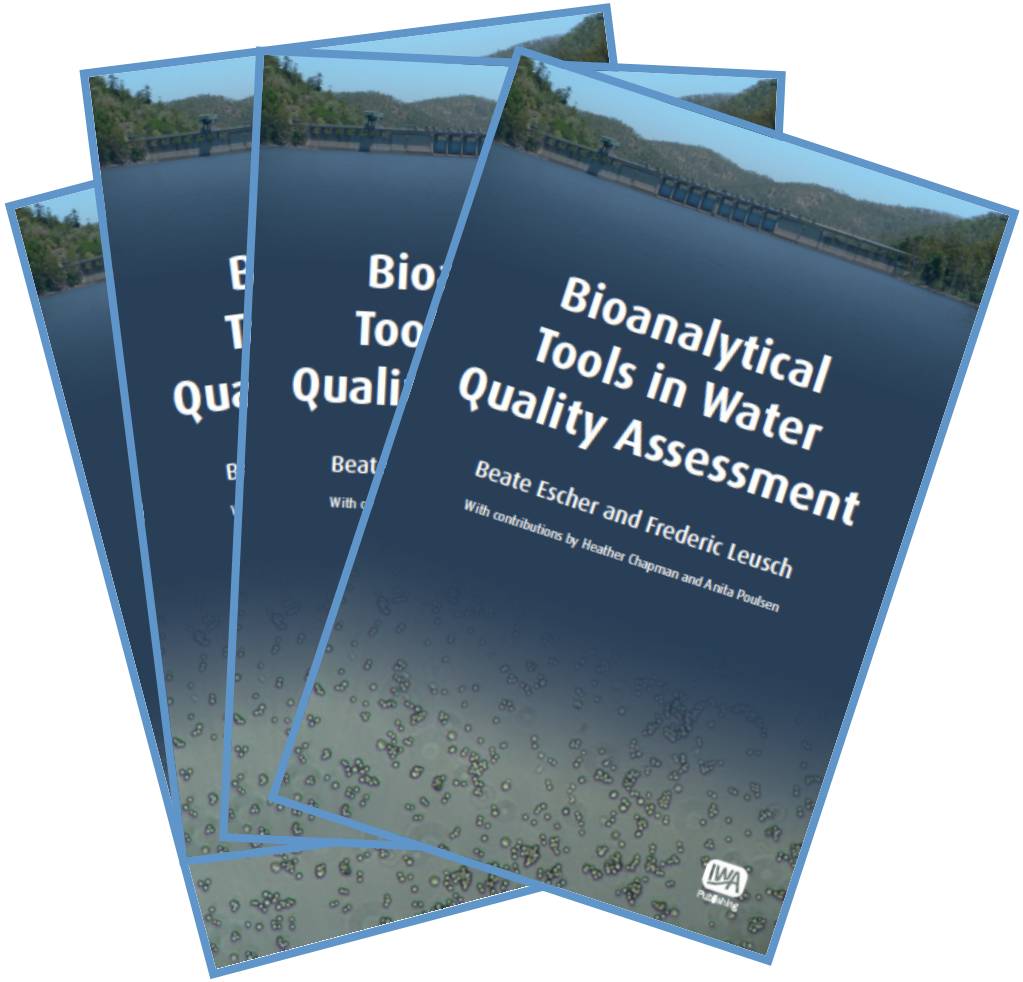
- Full-scale metropolitan drinking water treatment plant
- Nonspecific toxicity and reactive toxicity increased with increase in total absorbable organic halogens (and individual DBPs) during drinking water treatment
- Overall levels are low, none of the drinking water standards are exceeded



Neale, Antony, Bartkow, Farre, Heitz, Kristiana, Tang, Escher, in preparation.

More information: the book “Bioanalytical Tools in Water Quality Assessment”

- 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



Escher, B.I. and Leusch, F.D.L., with contributions by CHapman, H and Poulsen, A (2011). Bioanalytical tools in water quality assessment. IWA Publishing, London, UK.

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?