
# Bioanalytical Tools for the Assessment of Mixtures of Organic Micropollutants in the Aquatic Environment

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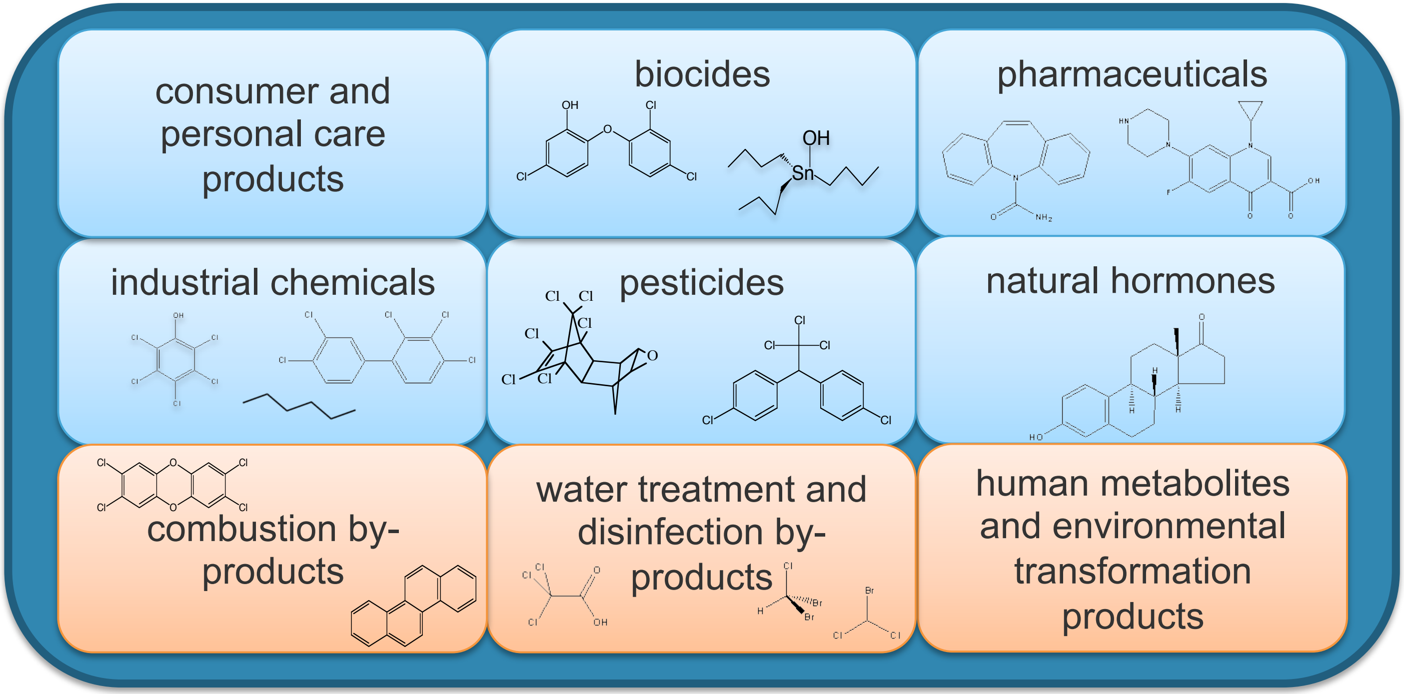
# Chemicals in the environment

Anthropogenic organic chemicals and transformation products put pressure on ecosystems and drinking water resources




*Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, 2012*

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# Chemicals in the environment

- Many chemicals

90,488,665 ORGANIC AND INORGANIC SUBSTANCES TO DATE

A global team of scientists is continually adding substance information from the world's disclosed chemistry to the CAS REGISTRY[SM], the gold standard for chemical substance information.

  - >100,000 in the environment
  - transformation products
  - low concentrations

- Mixture effects
  „Something from nothing“


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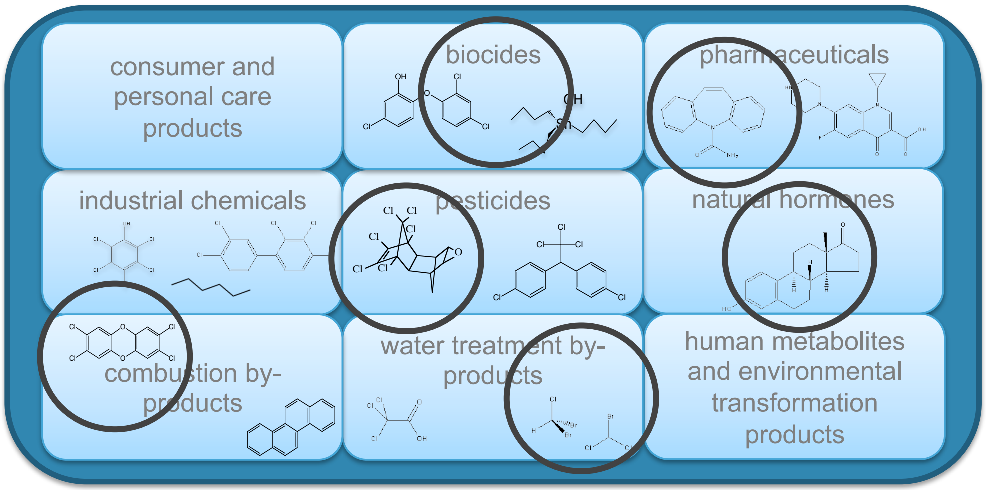
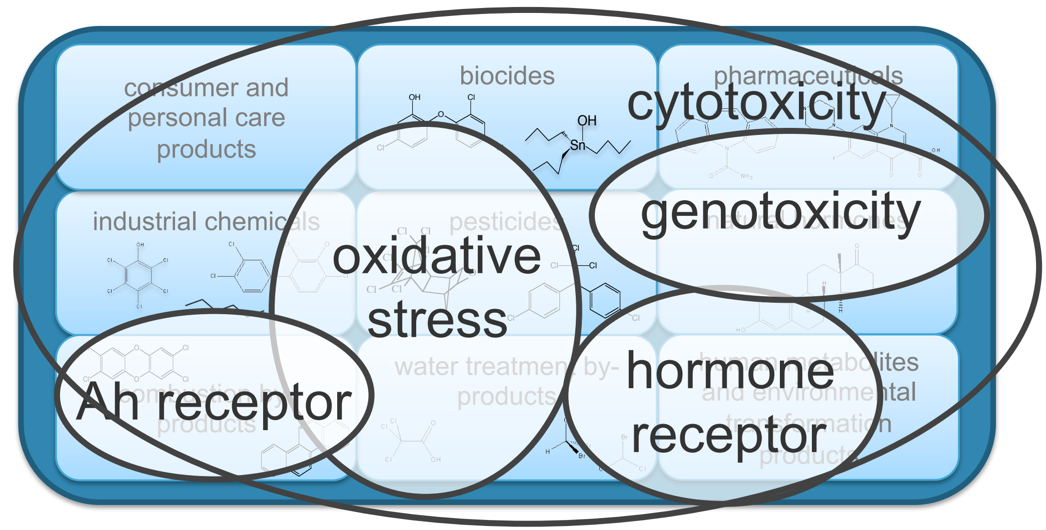
# Chemical and bio-analysis are complementary and deliver pieces of the puzzle

## Chemical analysis:




- **Quantitative for key target chemicals**
- Unknowns difficult and work-intensive to identify (non-target analysis)

## Bioanalytical tools:




- From **fully integrative** to summation of groups of chemicals with **common mode of action**
- **Single chemicals** cannot be resolved

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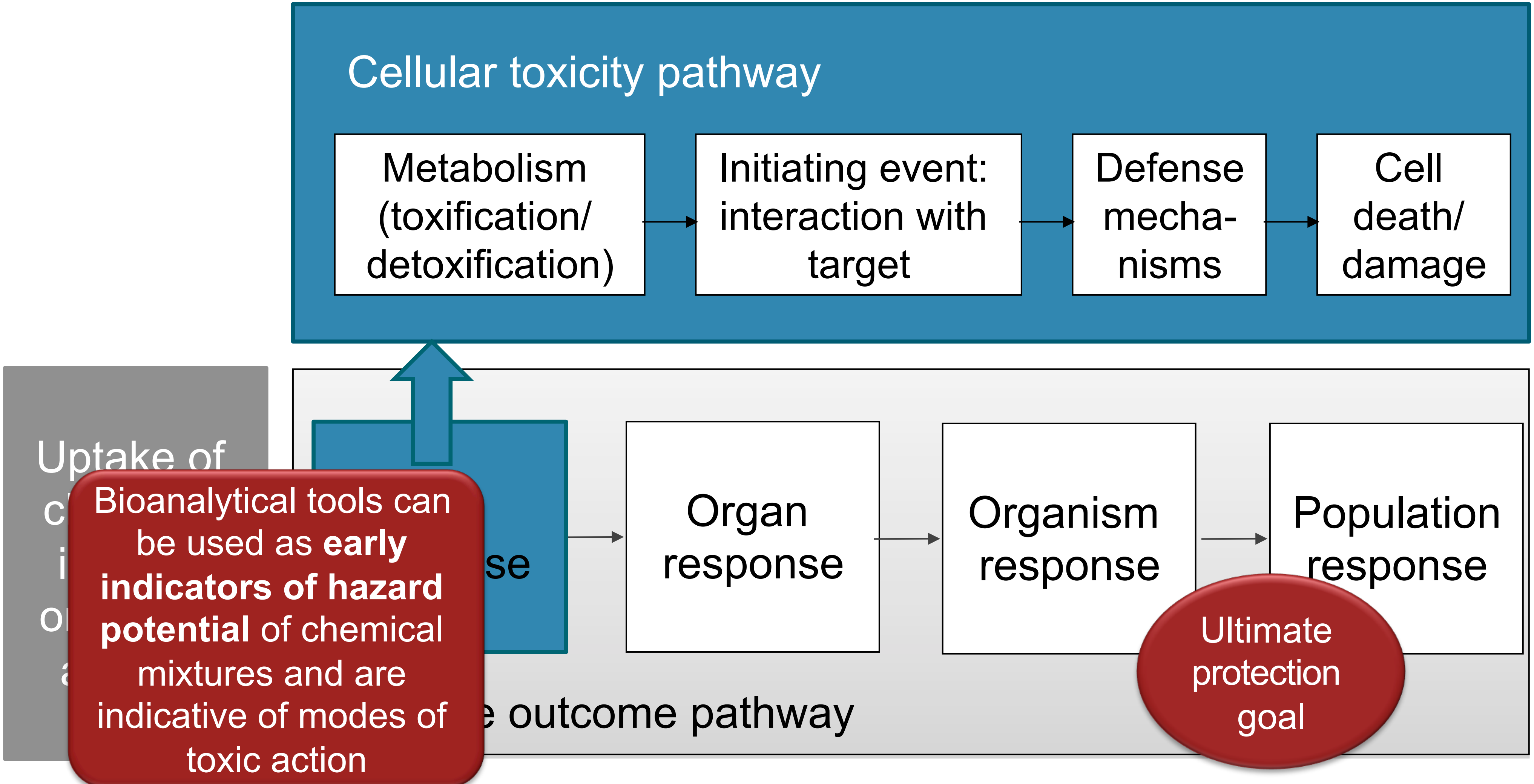
*Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, 2012*

# Conceptual framework: Toxicity and adverse outcome pathways (AOP)




*Adapted from Collin et al. (2008) and Ankley, et al. (2010) in Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, 2012*

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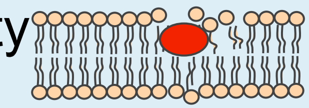
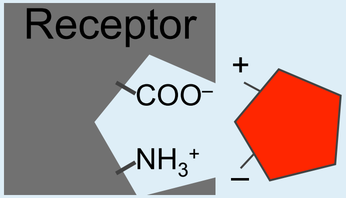
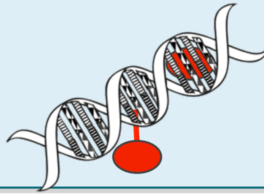
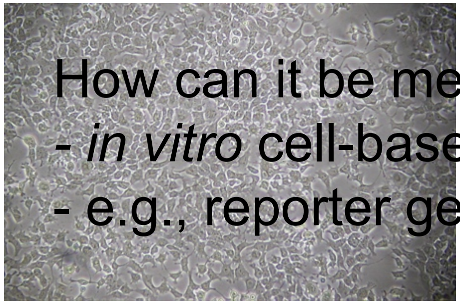
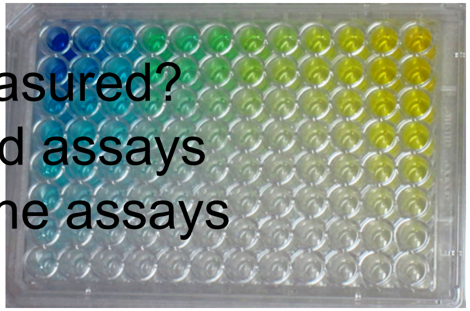
# Bioanalytical tools to assess cellular effects

## Cellular toxicity pathway

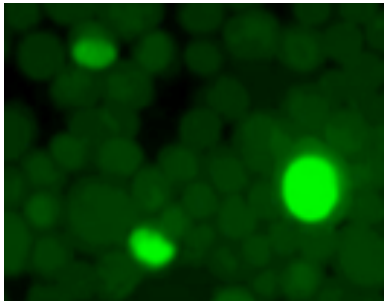
What can be measured?

| Metabolism → | Interaction with target → | Defense → | Cell death |
|---|---|---|---|
| Induction of xenobiotic metabolism pathways | Non-specific toxicity  specific, receptor-mediated toxicity   reactive toxicity  | Induction of general stress response pathways | Cell viability (cells repre-sentative for system response) |





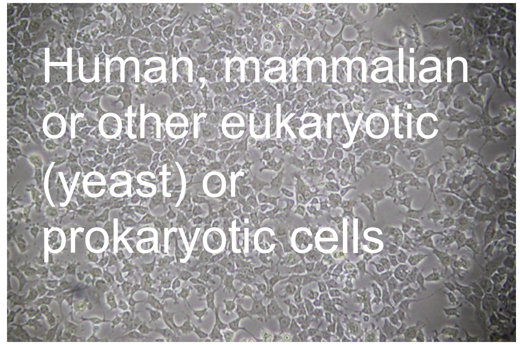
How can it be measured?
- *in vitro* cell-based assays
- e.g., reporter gene assays



Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, 2012

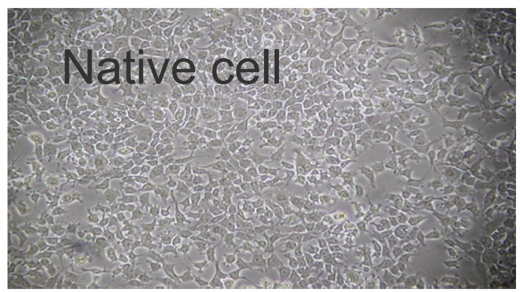
# Cell-based bioassays

Cell toxicity (Cytotoxicity)



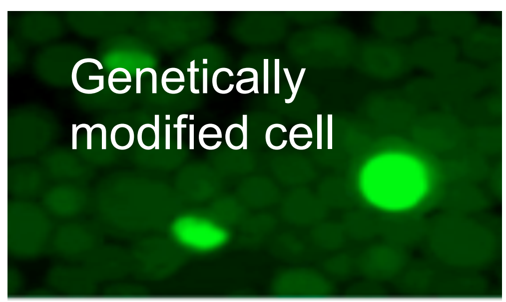

Growth rate
Cell viability

Biomarker




natural activity
(e.g. enzyme activity)

Reporter gene assay




“Foreign” gene added to amplify and visualize activity

*Escher and Leusch, Bioanalytical Tools in Water Quality Assessment, IWA, London, 2012*

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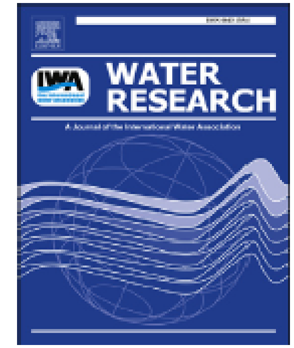


# Which chemicals drive biological effects in wastewater and recycled water?

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ABSTRACT

Removal of organic micropollutants from wastewater during secondary treatment followed by reverse osmosis and UV disinfection was evaluated by a combination of four *in-vitro* cell-

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*Tang, Busetti, Charrois, Escher (2014) Which chemicals drive biological effects in wastewater and recycled water? Water Res.* ***60****: 289-299.*

# Water samples

## Bioanalytical assessment

Biological effects in water samples

**Comparison**

Mixture toxicity of the designed, artificial “iceberg” mixture

The “iceberg” was also subdivided into six groups


## Chemical analysis

Target chemicals: Australian Guidelines for drinking water & water recycling

Mix chemicals in ratios of detected concentrations “iceberg mixtures”

*Tang, Busetti, Charrois, Escher (2014) Water Res.* ***60****: 289-299.*

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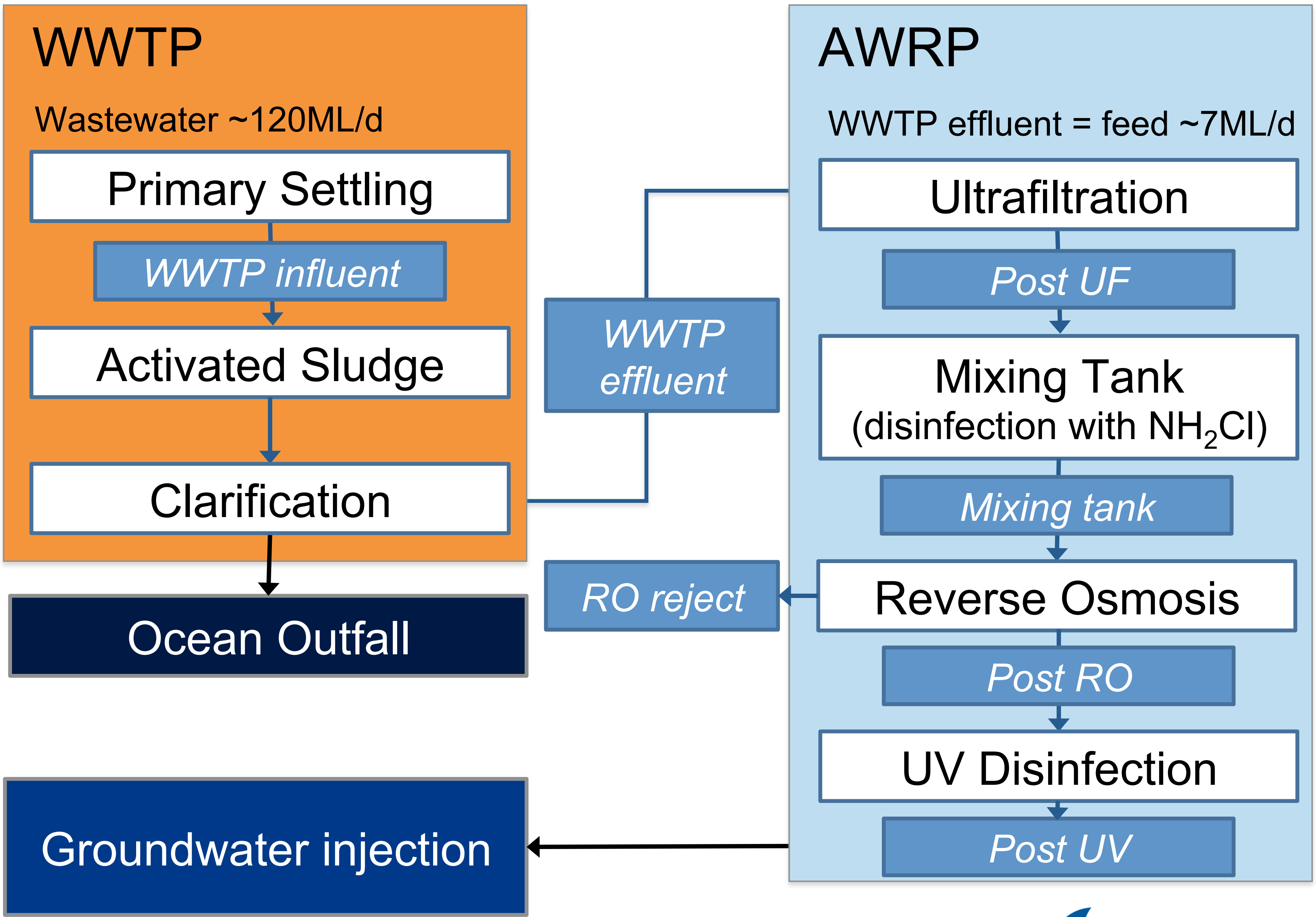
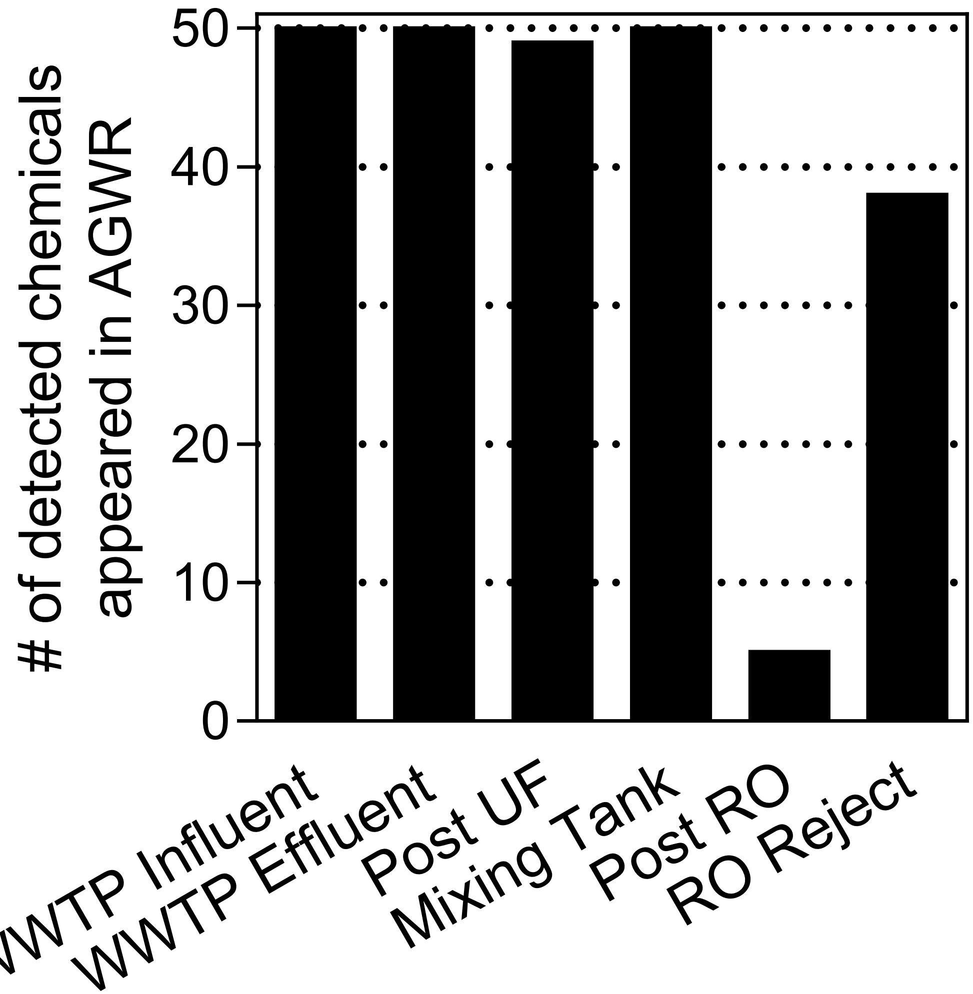



*Tang, Busetti, Charrois, Escher (2014) Which chemicals drive biological effects in wastewater and recycled water? Water Res.* ***60****: 289-299.*

# Chemical analysis

- **299 organic chemicals** analysed (172 of which listed in the Australian Guidelines for Water Recycling)
- **RO effective in removing organic chemicals** (only 5 chemicals in Post RO samples, all below AGWR guideline values)
- Complete removal of all organic chemicals by **UV disinfection**

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# "Iceberg" mixtures

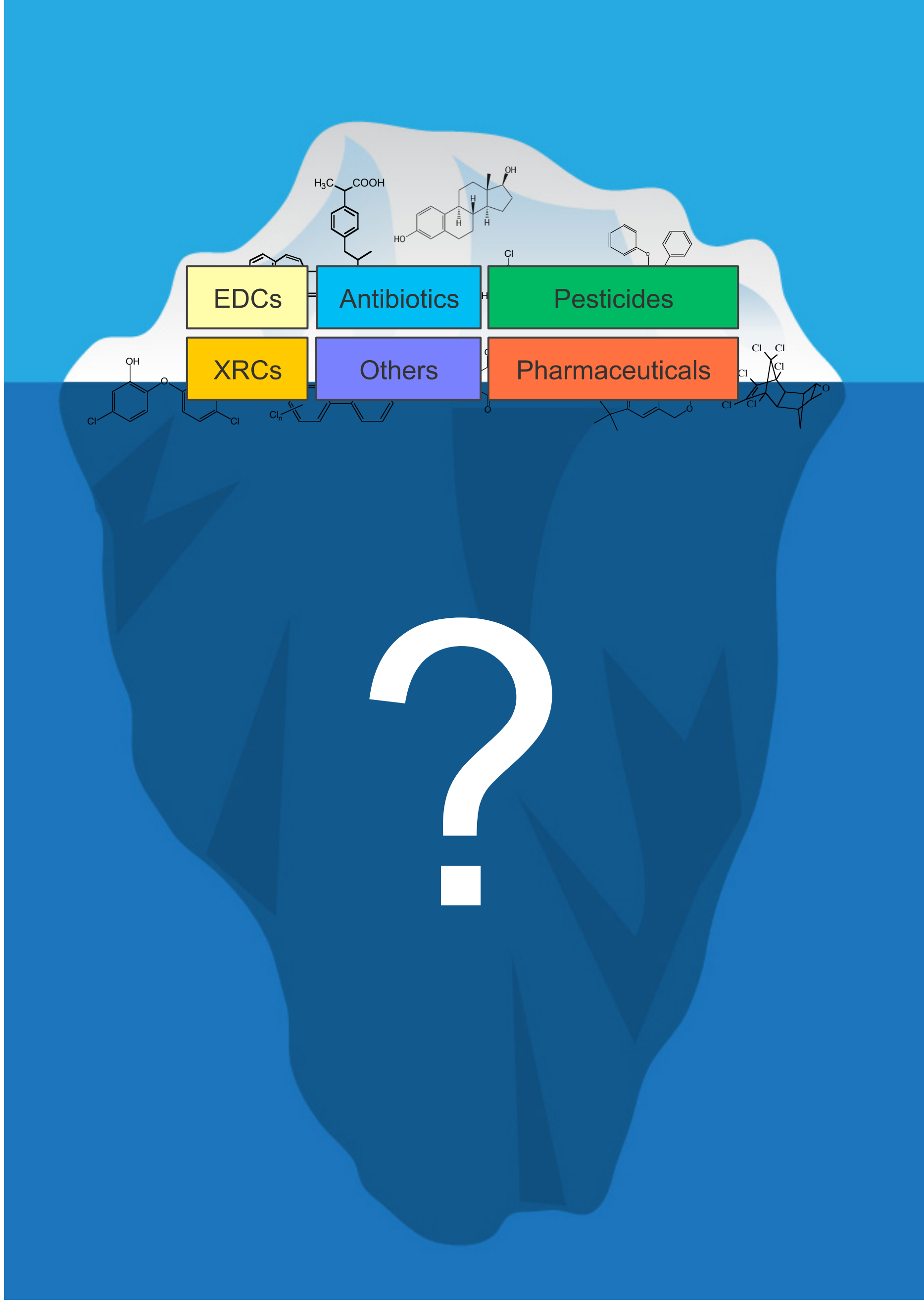
Mix chemicals in groups **according to their concentrations** from chemical target analysis

- To which degree do the known chemicals explain the observed biological effect?

**Water sample**

| | | |
|---|---|---|
| EDCs | Antibiotics | Pesticides |
| XRCs | Others | Pharmaceuticals |

- Which types of chemicals contribute to the response?

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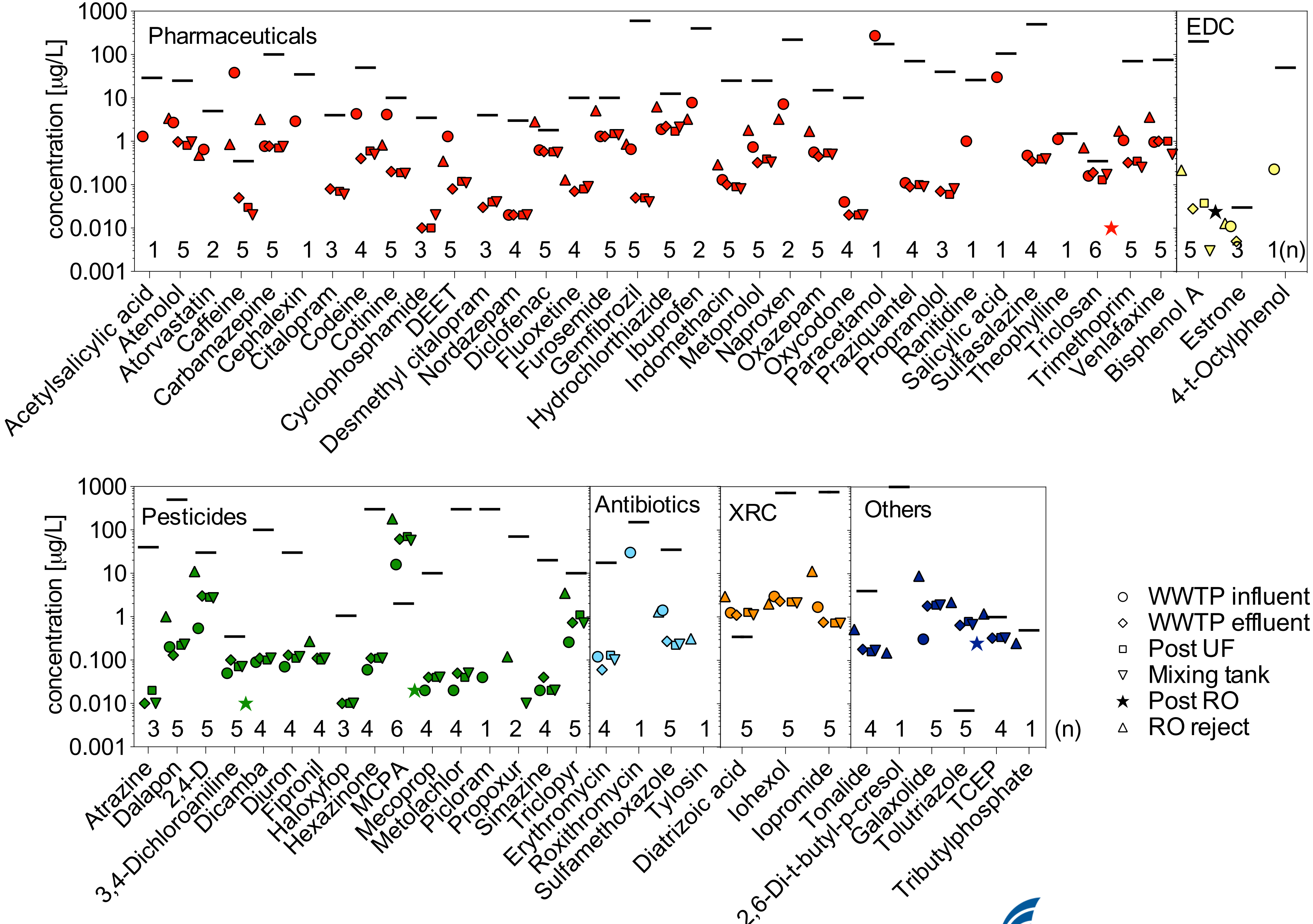


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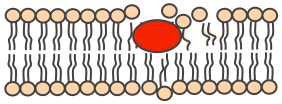
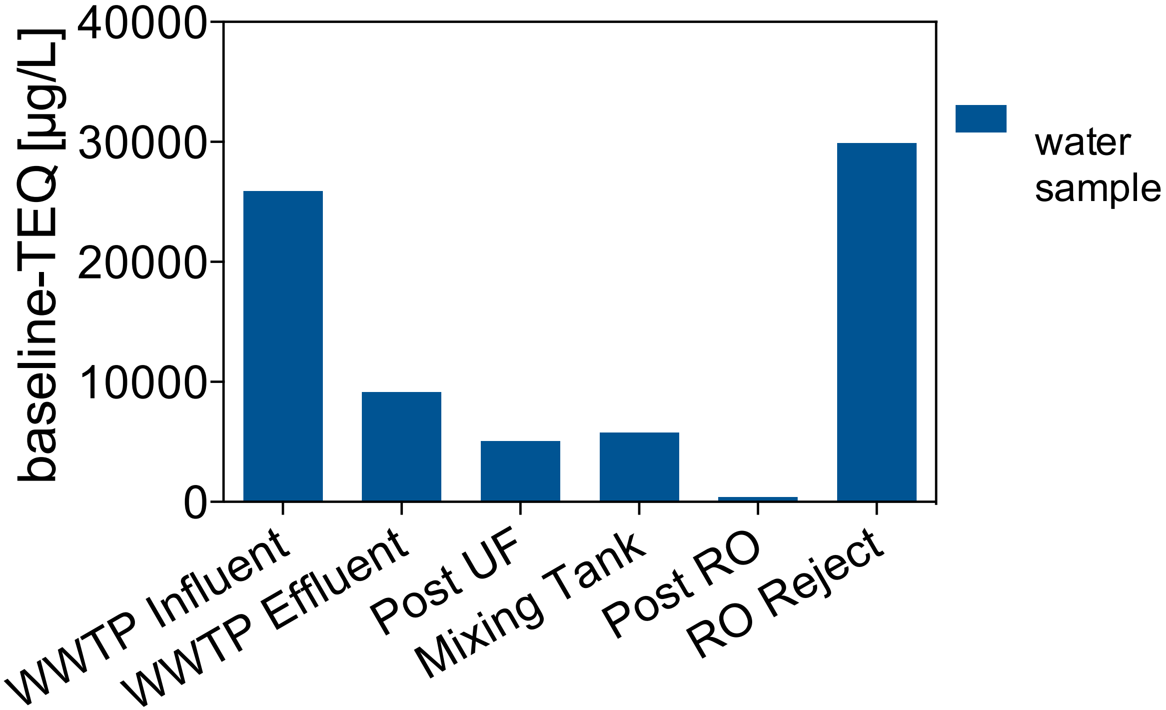
Non specific toxicity



# Baseline Toxicity

- Reduction in luminescence of naturally bioluminescent marine bacteria (Microtox, *Vibrio fischeri*)
- Effects expressed as baseline toxicity equivalents (baseline-TEQ)




- Highest baseline toxicity: WWTP influent and RO Reject
- **Decrease of toxicity** along the treatment train
- Effects **completely removed** post RO

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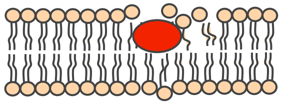
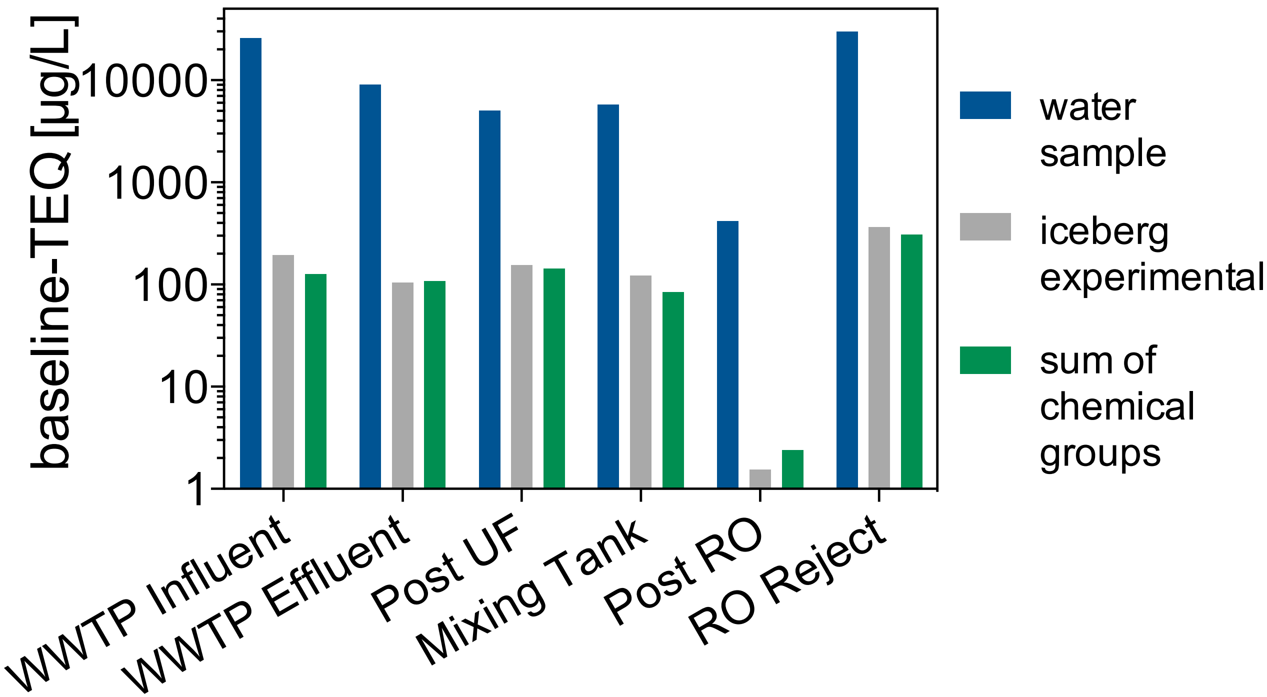
Non specific toxicity



# To which degree do the known chemicals explain the observed baseline toxicity?





Iceberg mixtures explain less than 3% of the effect

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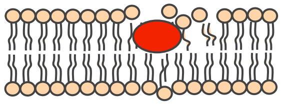
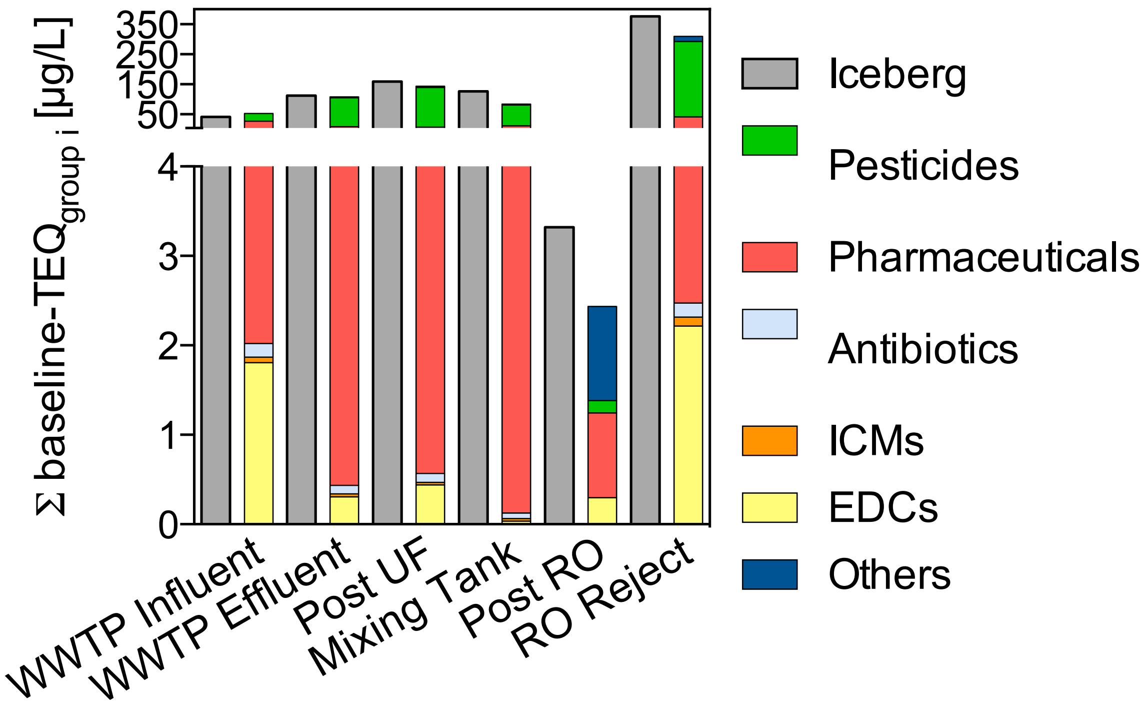
**Non specific toxicity**



# Which chemicals explain the observed baseline toxicity?




- **Pesticides and pharmaceuticals contributed similarly** to the baseline toxicity equivalents in WWTP influent
- **Pesticides dominated** in all other samples (preferential removal of pharmaceuticals during treatment?)

**Good agreement** between the entire icebergs (grey bars) and the sum of individual chemical groups (colored bars)

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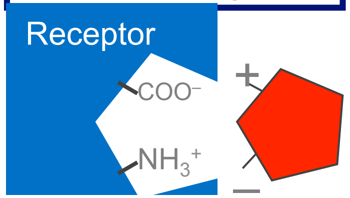
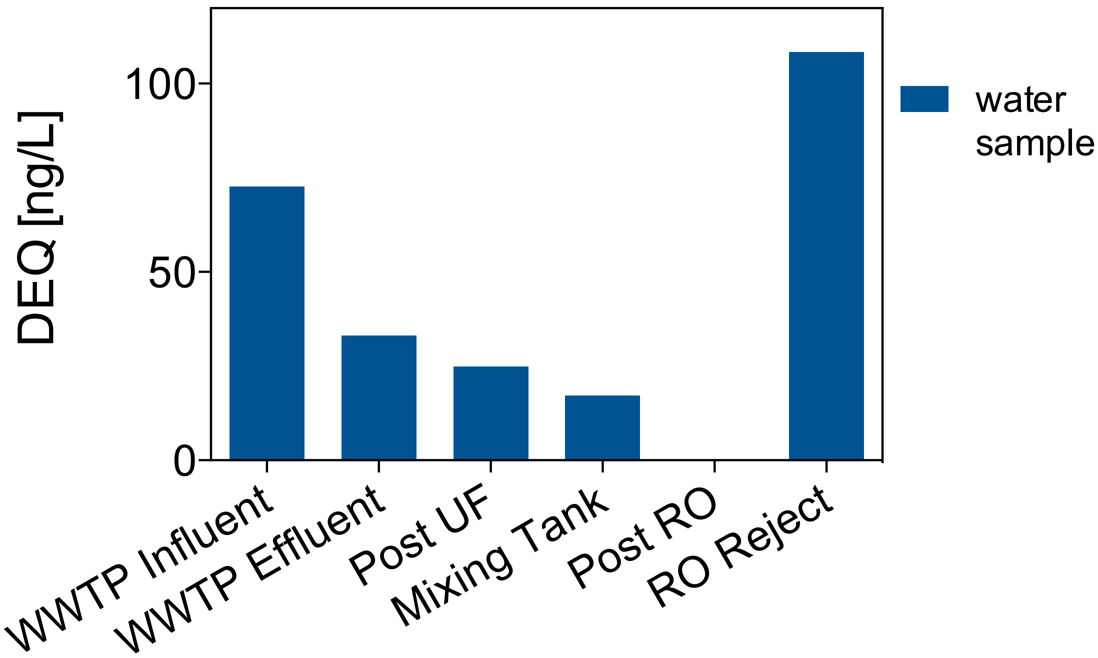
Specific toxicity




# Photosynthesis inhibition assay

- Triazine and phenylurea herbicides
- Diuron equivalent concentrations (DEQ)




- Highest photosynthesis inhibition: WWTP influent and RO Reject
- **Decrease of toxicity** along the treatment train
- Effects **completely removed** post RO

*Tang, Busetti, Charrois, Escher (2014) Which chemicals drive biological effects in wastewater and recycled water? Water Res.* ***60****: 289-299.*

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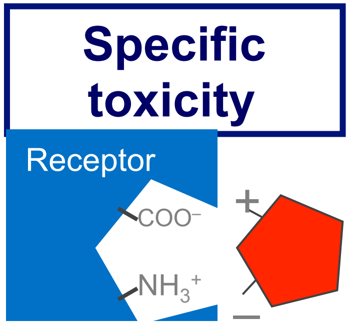
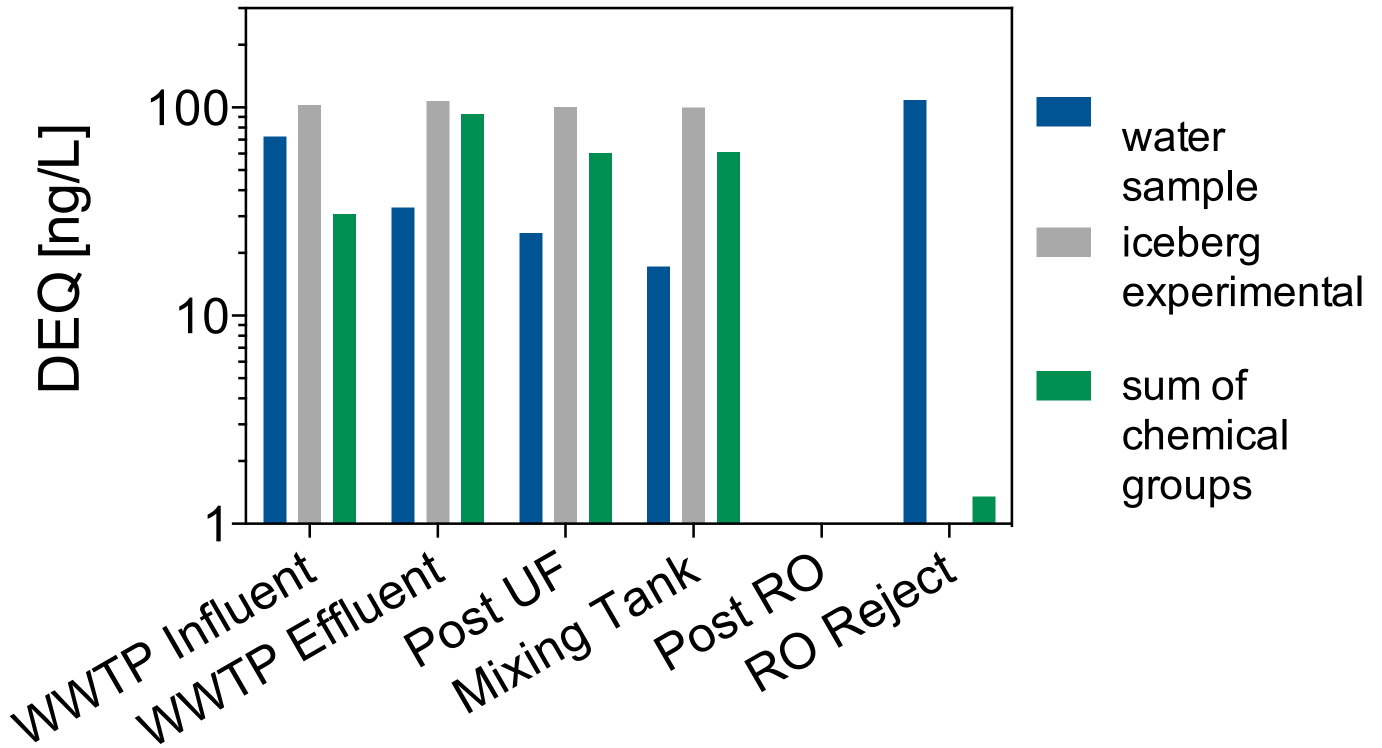
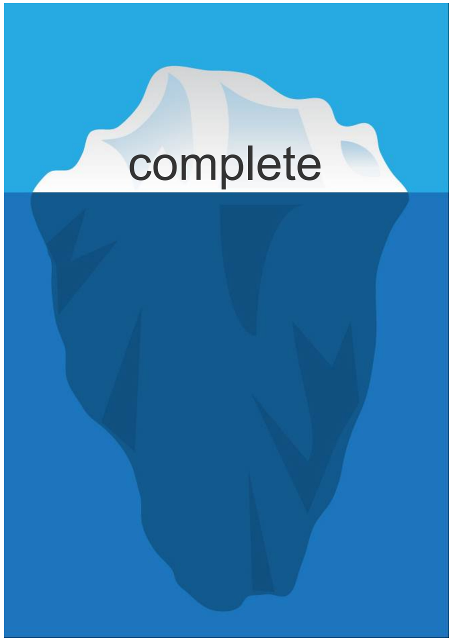



# To which degree do the known chemicals explain the photosynthesis inhibition?

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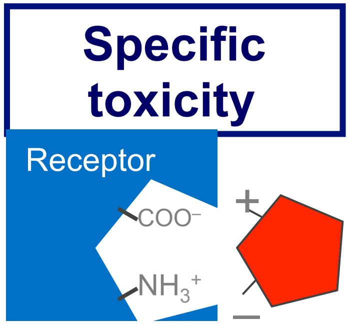
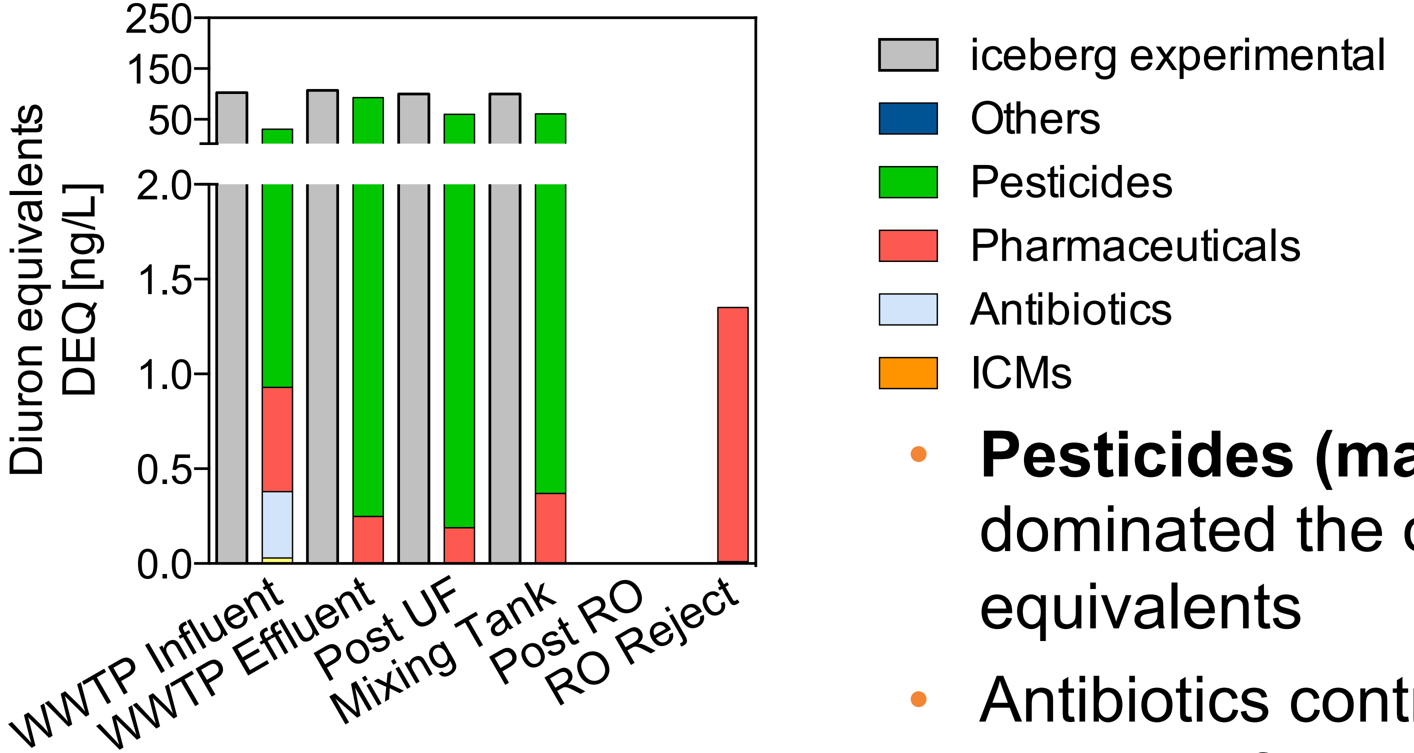



# Which chemicals explain the observed photosynthesis inhibition?




- **Pesticides (mainly herbicides)** dominated the overall diuron equivalents
- Antibiotics contributed 1% in WWTP influent; pharmaceuticals <2% in RO reject

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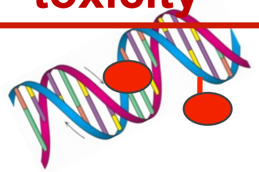
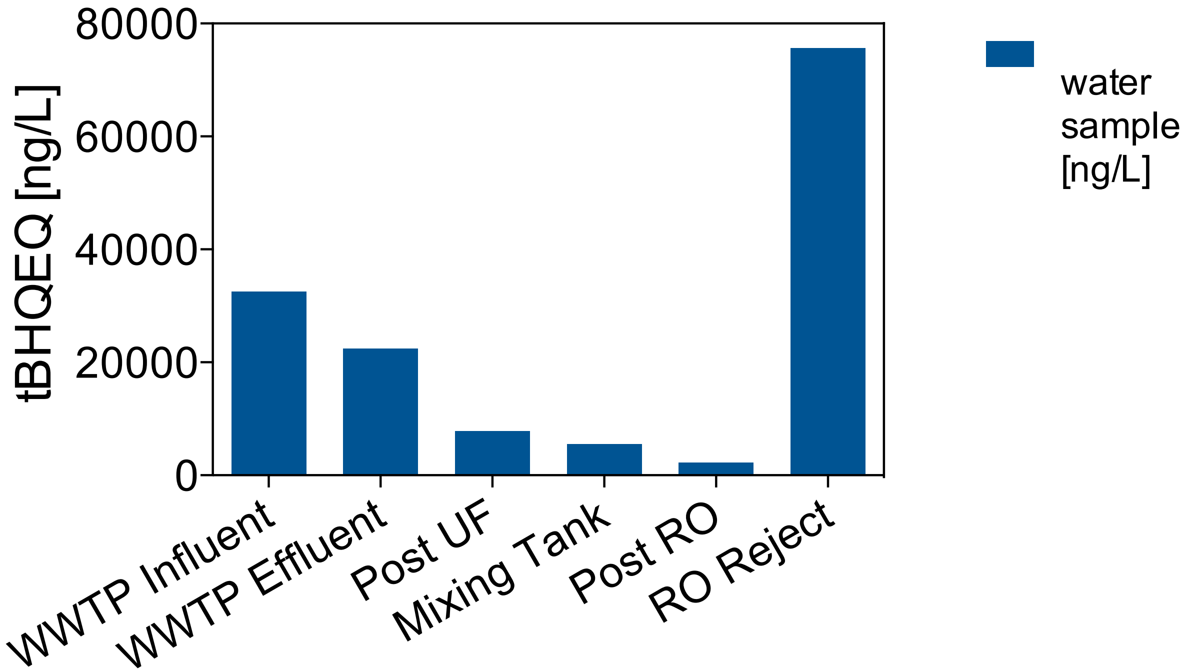
**Reactive toxicity**



# Oxidative stress response

- AREc32 bioassay
- Effects expressed as oxidative stress response equivalents (t-butylhydroquinone (tBHQ)-EQ)




- Highest effects were found in RO reject
- **Decrease of toxicity** along the treatment train
- Effects in post RO and post UV are **similar to the blanks**

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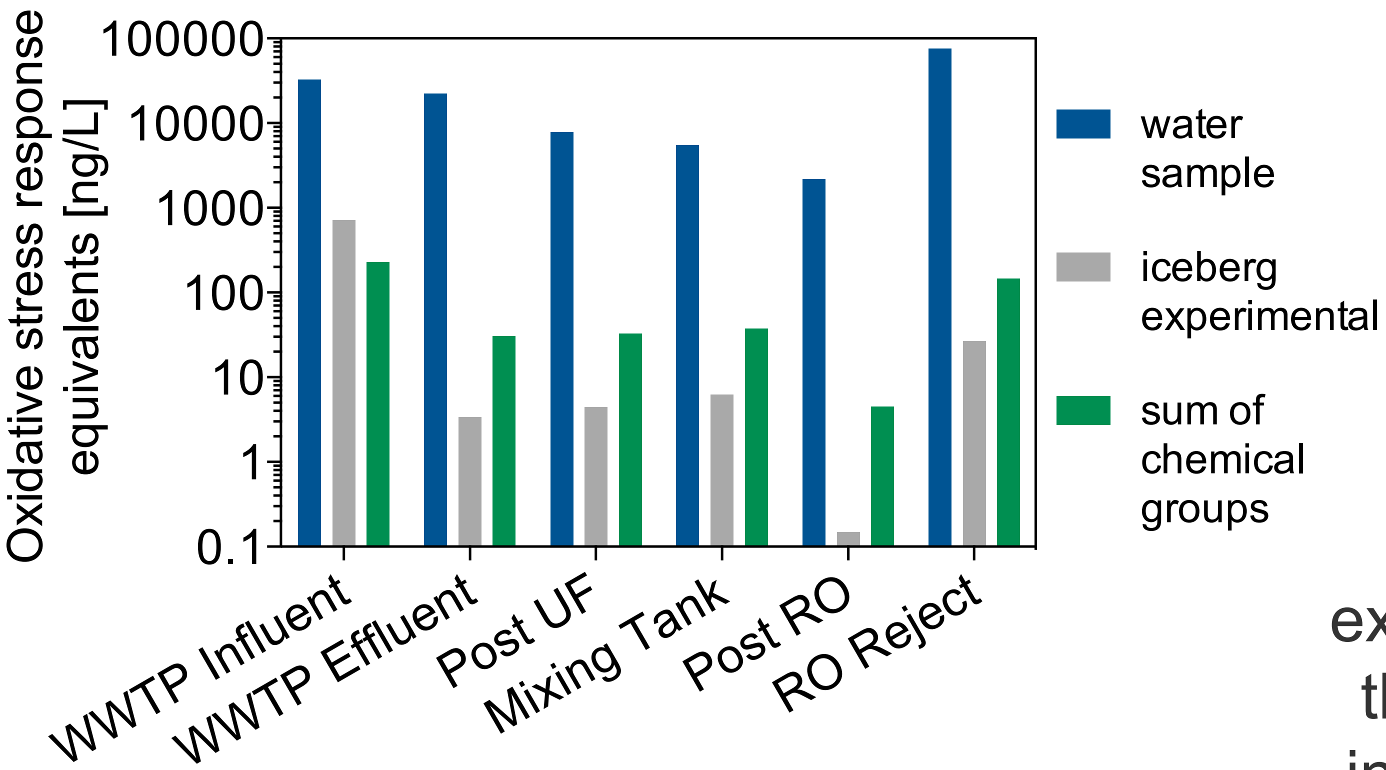
**Reactive toxicity**

# To which degree do the known chemicals explain the oxidative stress response?





Iceberg mixtures explain around 10% of the effect for WWTP influent and less than 1% for other samples

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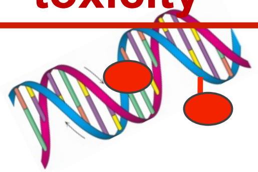
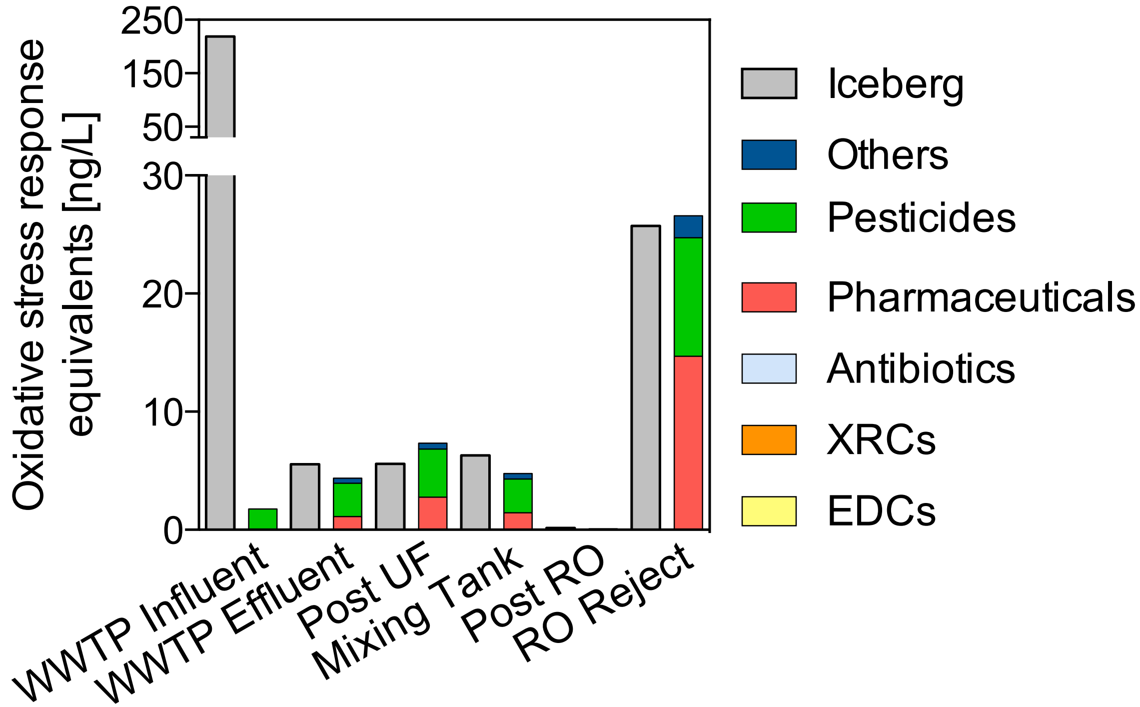
Reactive toxicity



# Which chemicals explain the observed oxidative stress response?




- **Pesticides** caused approximately 60% of oxidative stress response; **pharmaceuticals** contributed about 30%
- The proportions of the chemical groups did not vary much between different treatment steps, indicating comparable removal for all groups

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# Summary and conclusions

349 Chemicals in AGWR

299 chemicals targeted by chemical analysis

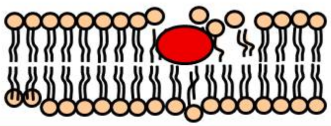
up to 50 chemicals detected

<3%

100%

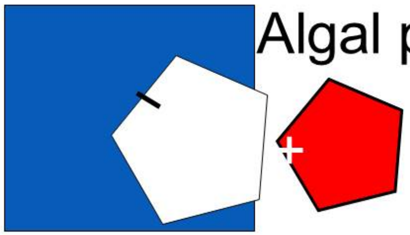
<1%

**Non-specific Toxicity (Cytotoxicity)**



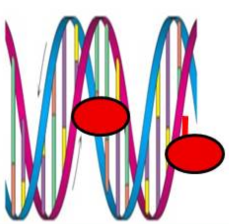
Microtox: baseline-toxicity equivalents

**Specific Toxicity**



Algal photosynthesis inhibition: Diuron equivalents (DEQ)

**Reactive Toxicity**



Oxidative stress response (OxStress-EQ)

## Cell-based bioassays are **sensitive monitoring tools to complement chemical analysis**

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