Chapter 13
Design of test batteries and interpretation of bioassay results

This presentation accompanies Chapter 13
(and a bit of chapter 14) of
“Bioanalytical Tools in Water Quality Assessment”
https://www.iwapublishing.com/books/9781789061970/bioanalytical-tools-water-quality-assessment-2nd-edition

Exercises and more material can be found at
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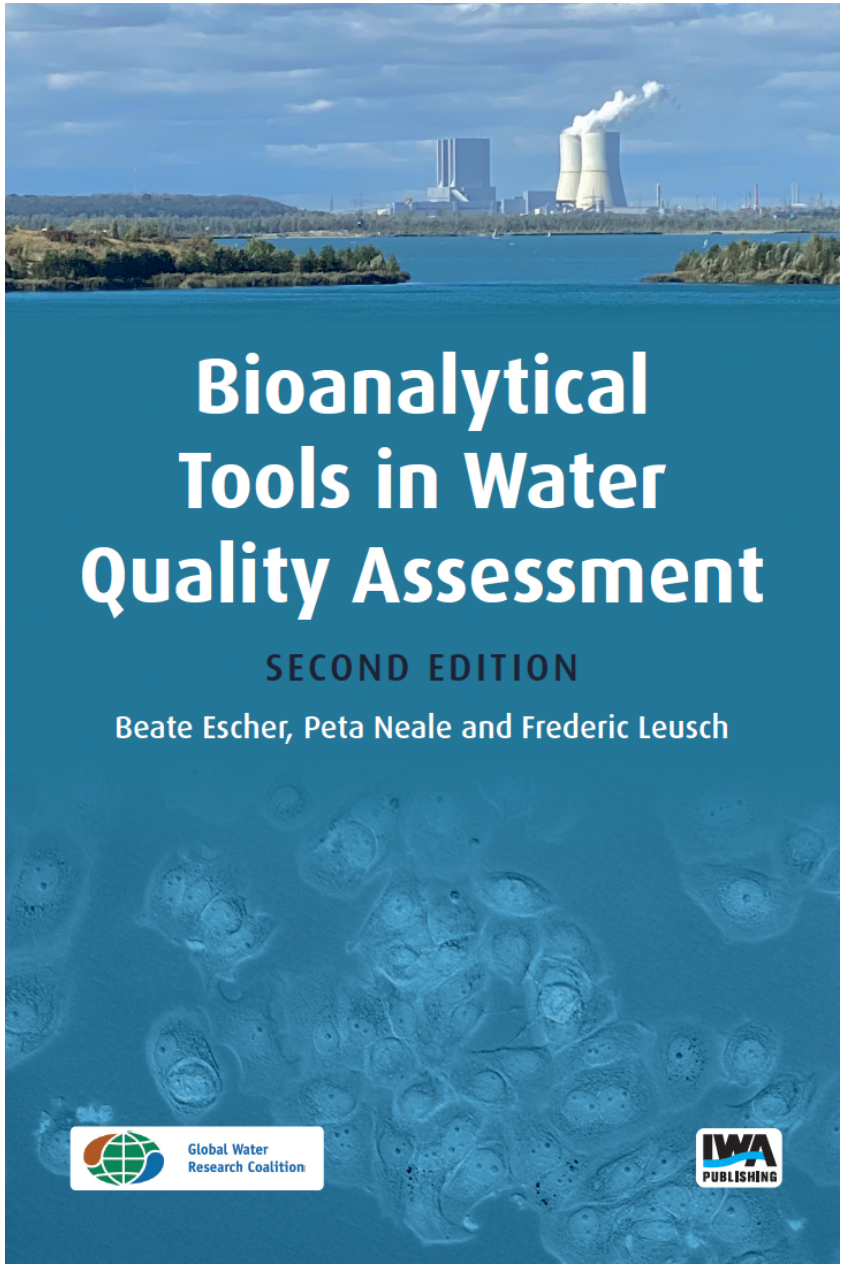
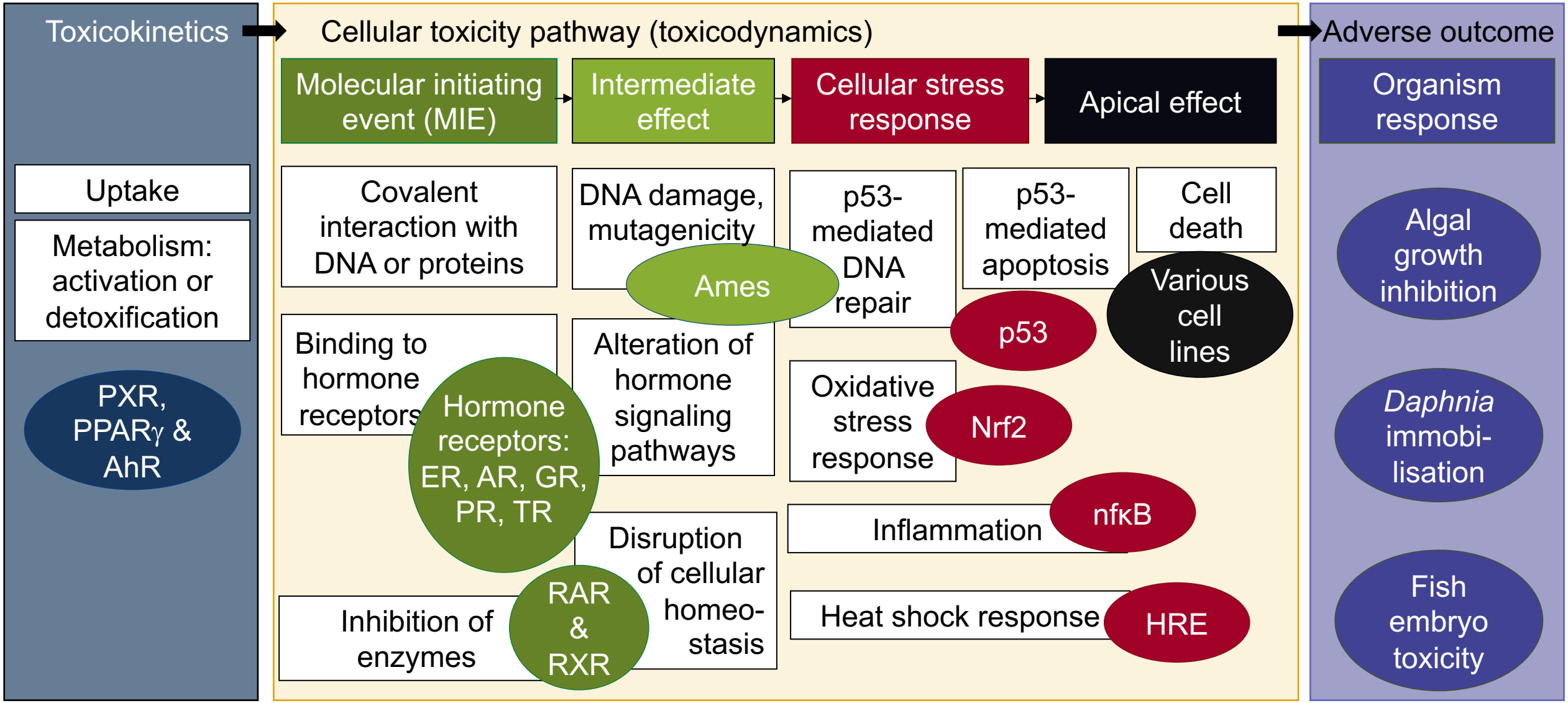


Learning goals

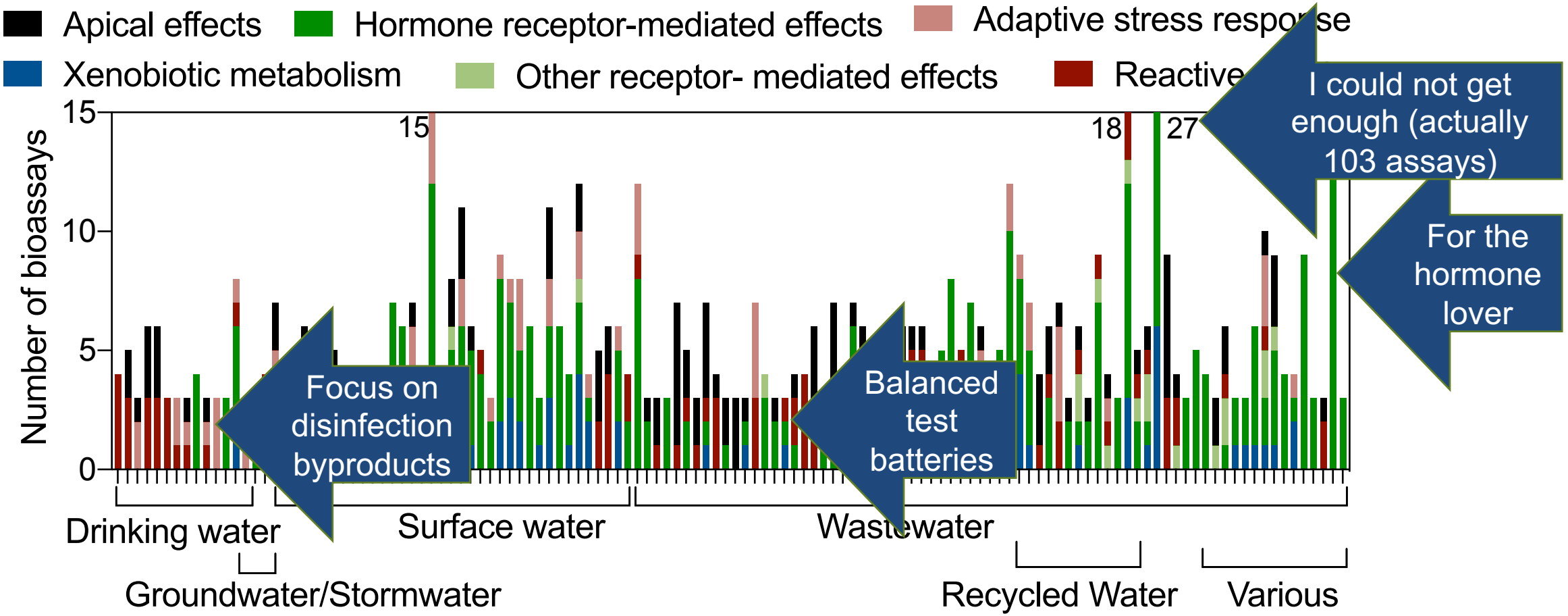
- To design test batteries for water quality assessment under consideration of the toxicity pathway concept
- To interpret the results of the bioassay testing by several approaches
  - To link the results from bioassays with those from chemical analysis
    - Through effect-directed analysis
    - Through mixture modelling (also called ”iceberg modelling”)
  - To compare measured effect in water against so-called effect based trigger values

Note: the concepts presented in Chapter 13 will be illustrated in Chapter 14 “Case studies”. There is no video for chapter 14 but we will illustrate the concepts of chapter 13 with material from Chapter 14.

Design of a test battery of *in vitro* bioassays



Application of test batteries of *in vitro* bioassays



Case study 1
in Chapter 14

Evaluation of treatment efficacy

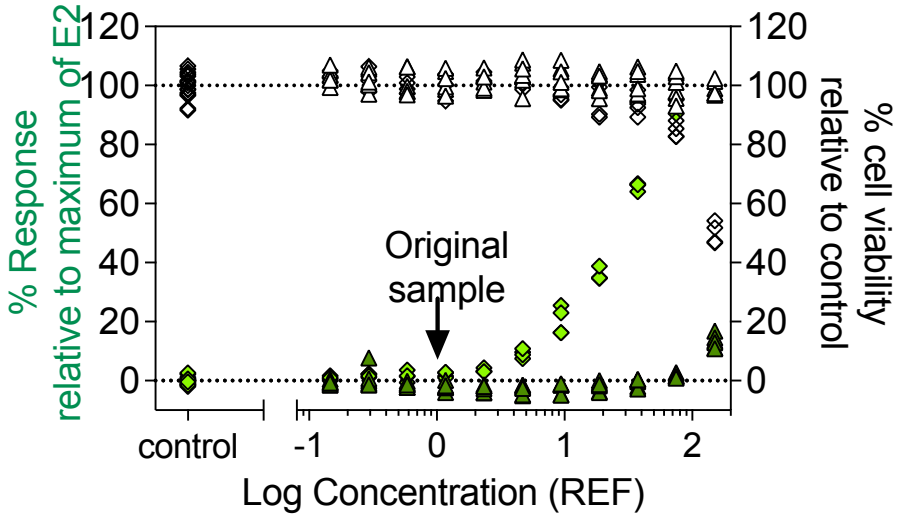
- Example: removal of estrogenic activity during drinking water treatment

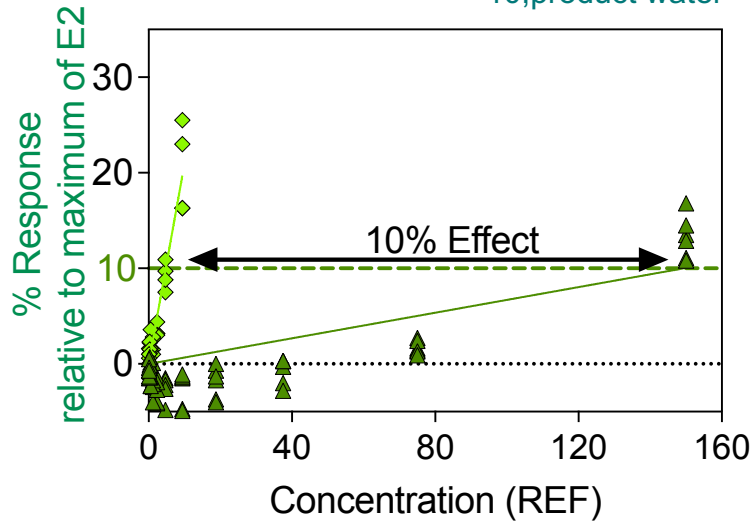
$$\text{Effect removal efficacy} = 1 - \frac{EU_{\text{product water}}}{EU_{\text{source water}}} = 1 - \frac{EC_{\text{source water}}}{EC_{\text{product water}}}$$

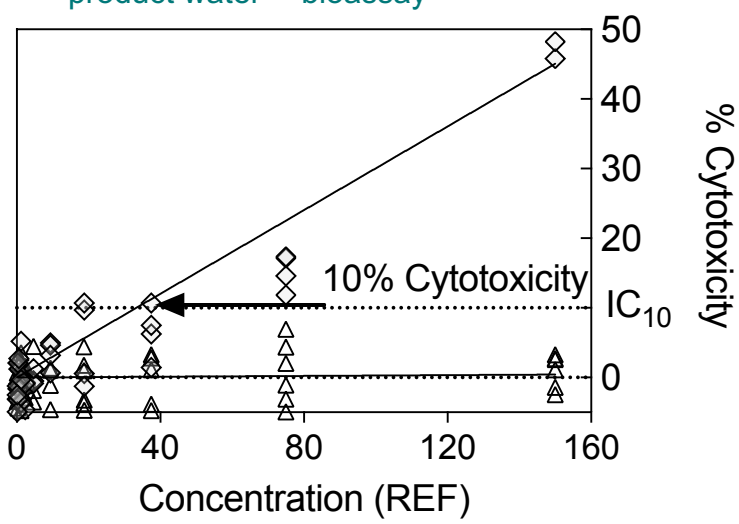
Effect removal efficacy = 96.7%

$EC_{10,\text{source water}} = 5\ L_{\text{source water}}/L_{\text{bioassay}}$

$EC_{10,\text{product water}} = 149\ L_{\text{product water}}/L_{\text{bioassay}}$







Source water - activation of ER ◇ Source water - cell viability

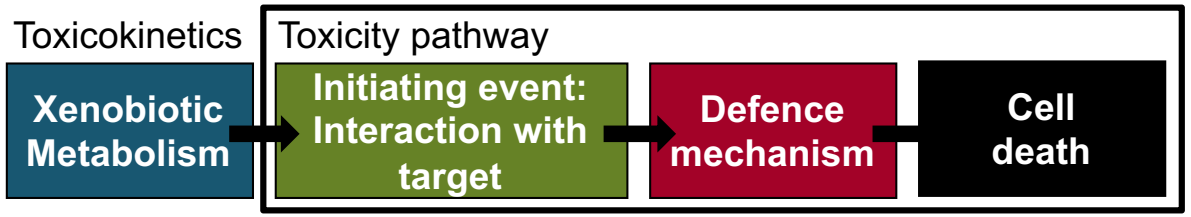
Product water - activation of ER △ Product water - cell viability

Case study 3 in Chapter 14

Evaluation of treatment efficacy

Constructed wetlands in comparison to a conventional wastewater treatment plant

$$\text{Effect removal efficacy} = 1 - \frac{EU_{\text{product water}}}{EU_{\text{source water}}} = 1 - \frac{EC_{\text{source water}}}{EC_{\text{product water}}}$$

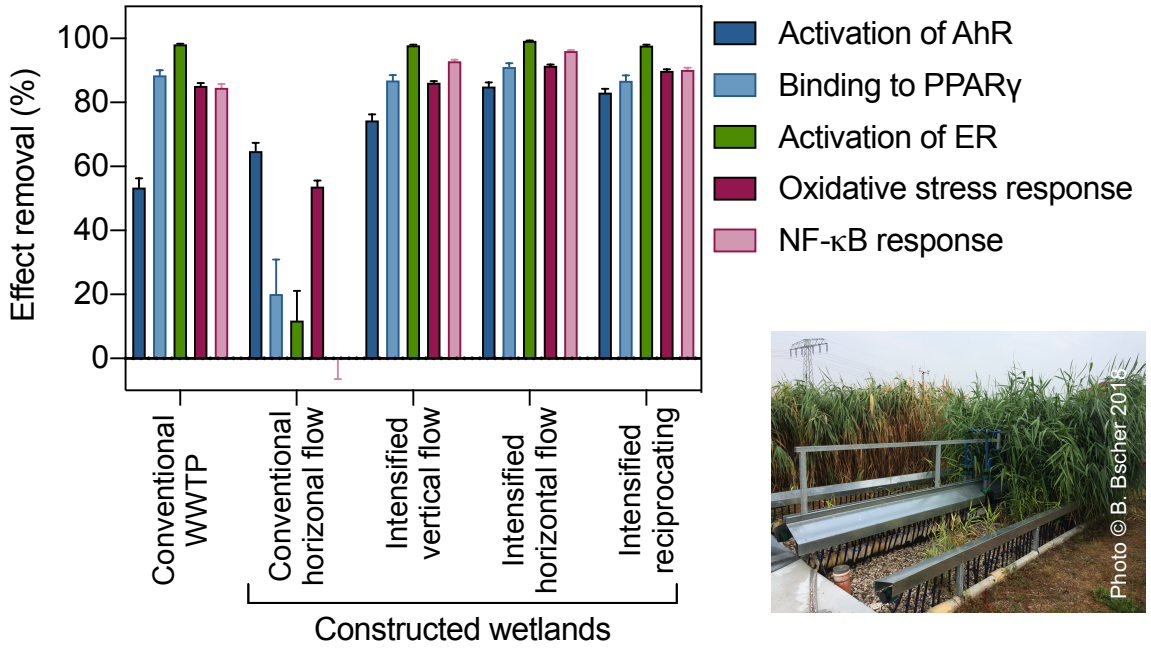


- Activation of aryl hydrocarbon receptor (AhR)

Specific MOA
- Activation of estrogen receptor (ER)

Adaptive stress responses
- Oxidative stress response (Nrf2)
- Inflammation (NF-κB)

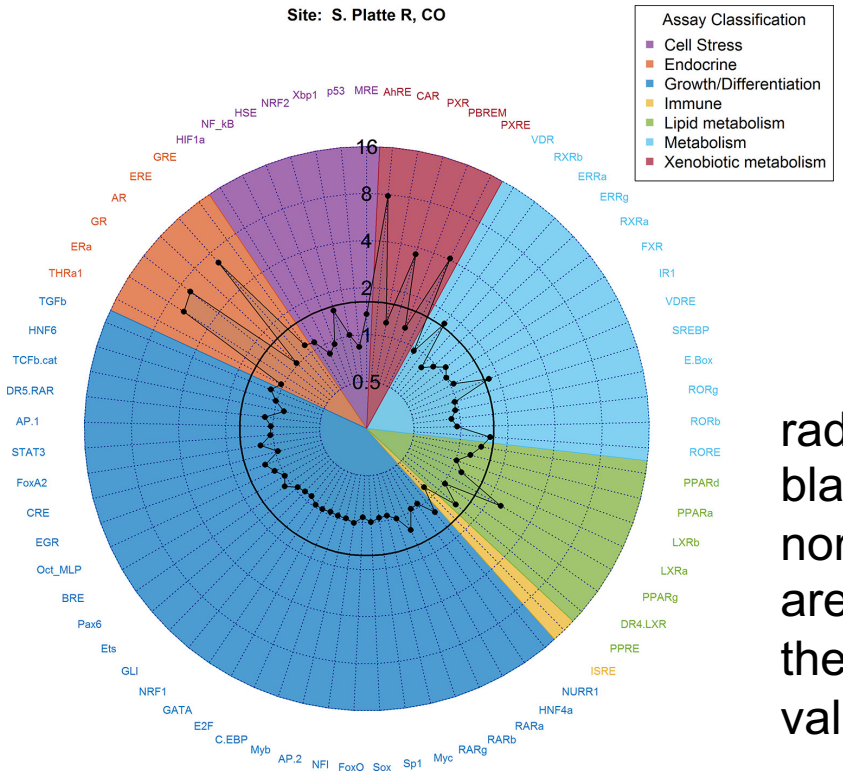
- Cell viability



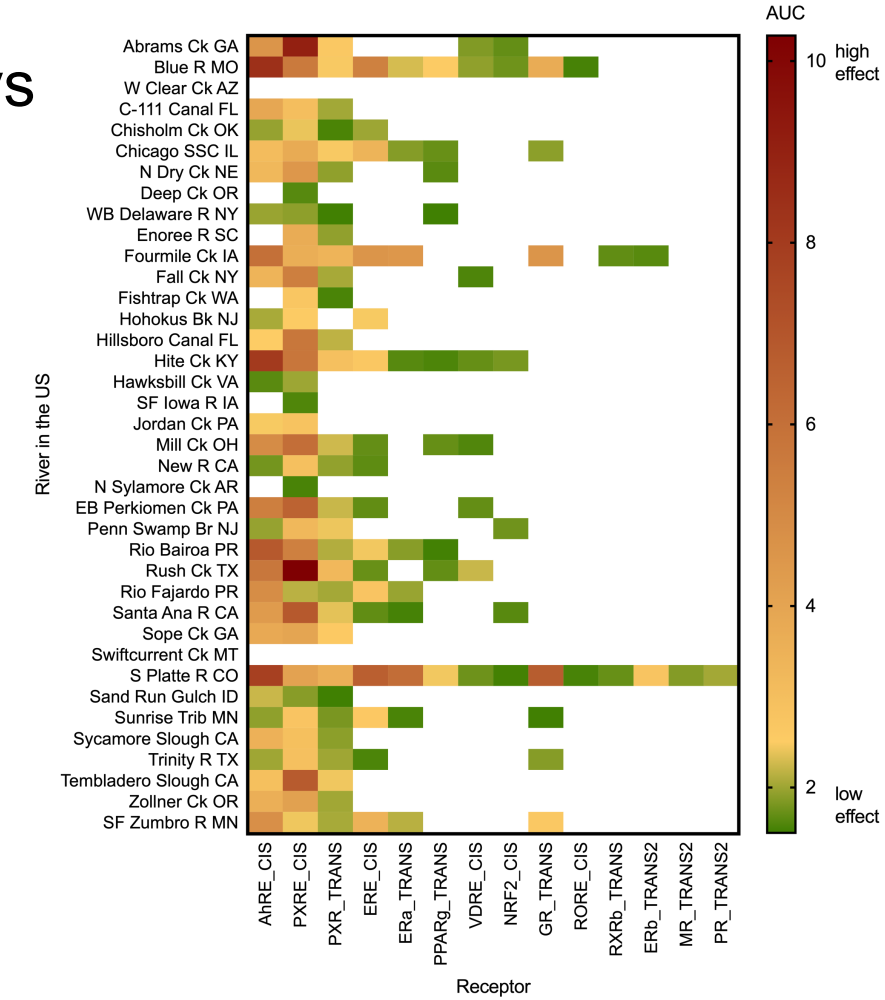
Multiplex high-throughput screening assays such as Attagene cis- and trans-FACTORIAL™

Case study 5 in Chapter 14

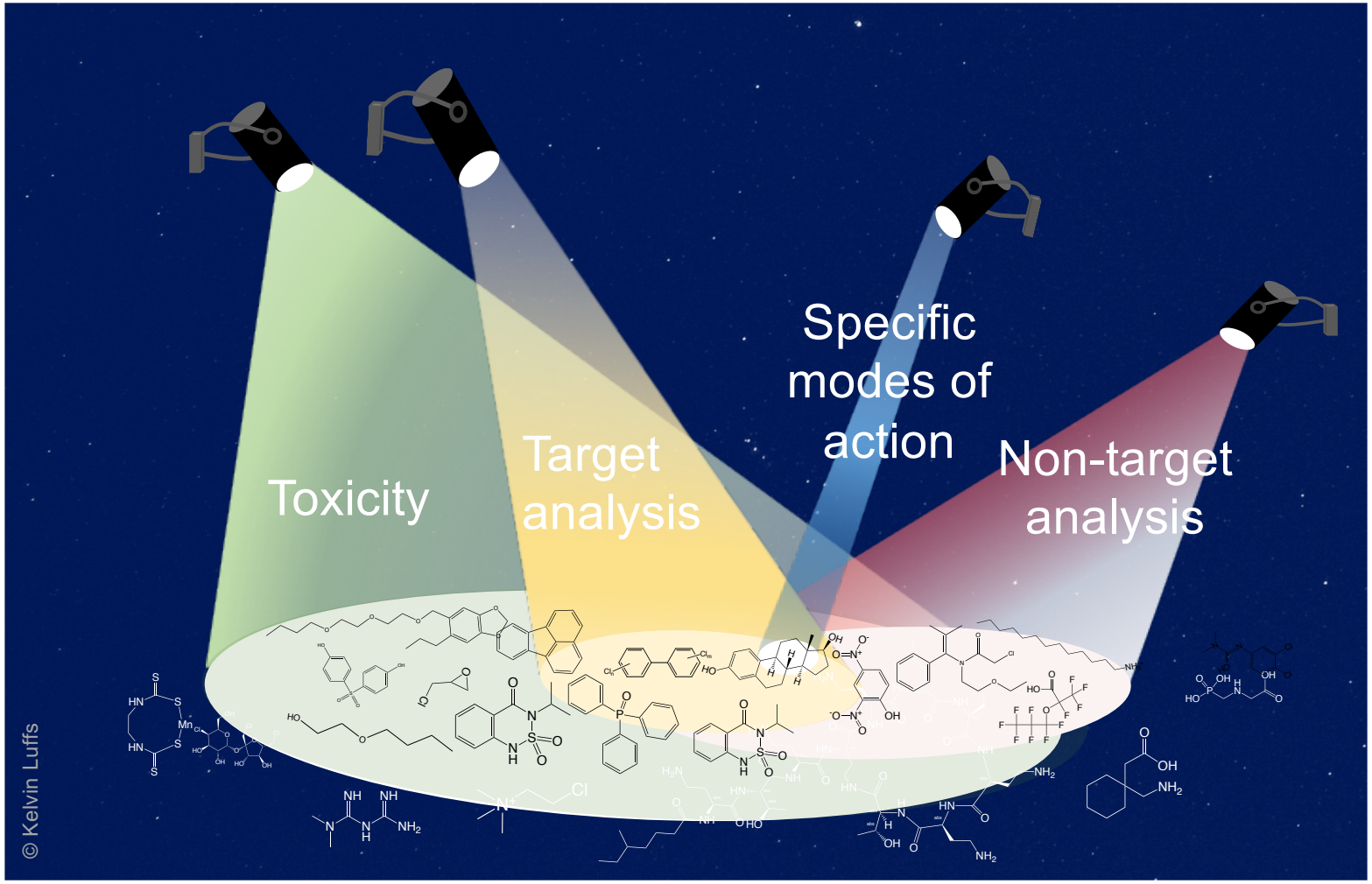
- 69 endpoints in two Attagene FACTORIAL™ assays
- Few strong responses across 38 surface water samples in the US: PXR, AhR, ERα, GR, PPAR

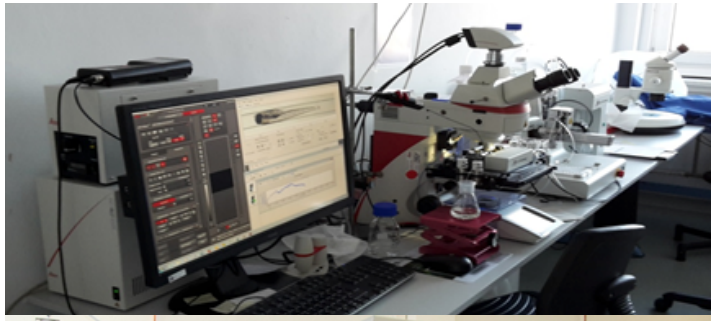


radar plot of blank-normalised area-under-the-curve values



Combining chemical analysis and bioassays





Iceberg modelling

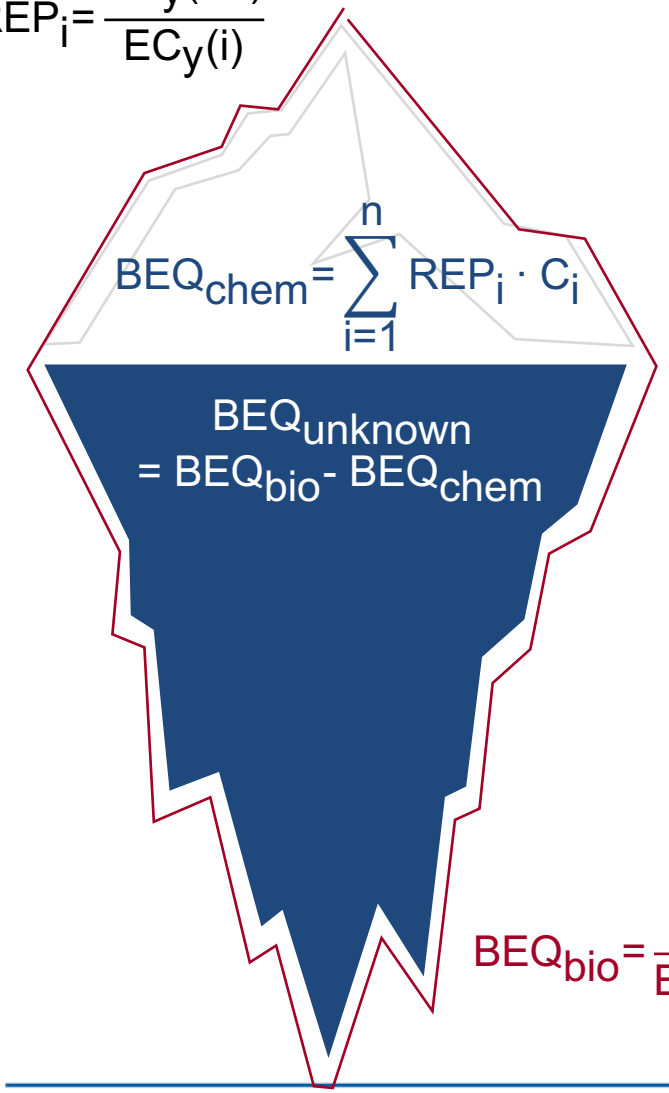
- Chemicals detected in the environment, biota and humans
- Chemicals with known effects
- Mixture effects

- Chemicals below detection limits
- Unknown chemicals
- Chemicals without analytical methods
- Transformation products
- Mixture effects

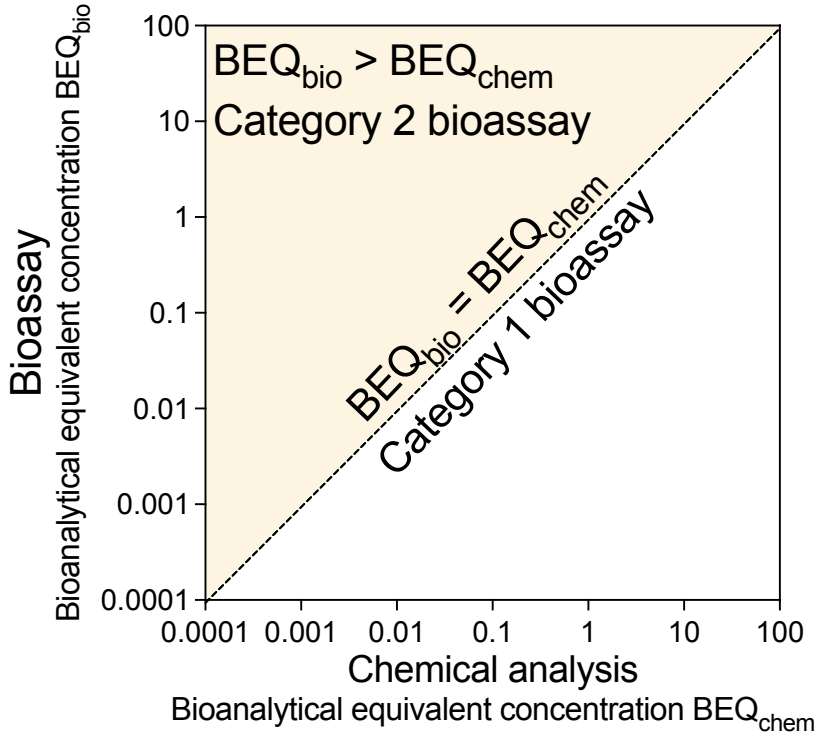
Bioanalytical tools can capture
and characterise the entire iceberg

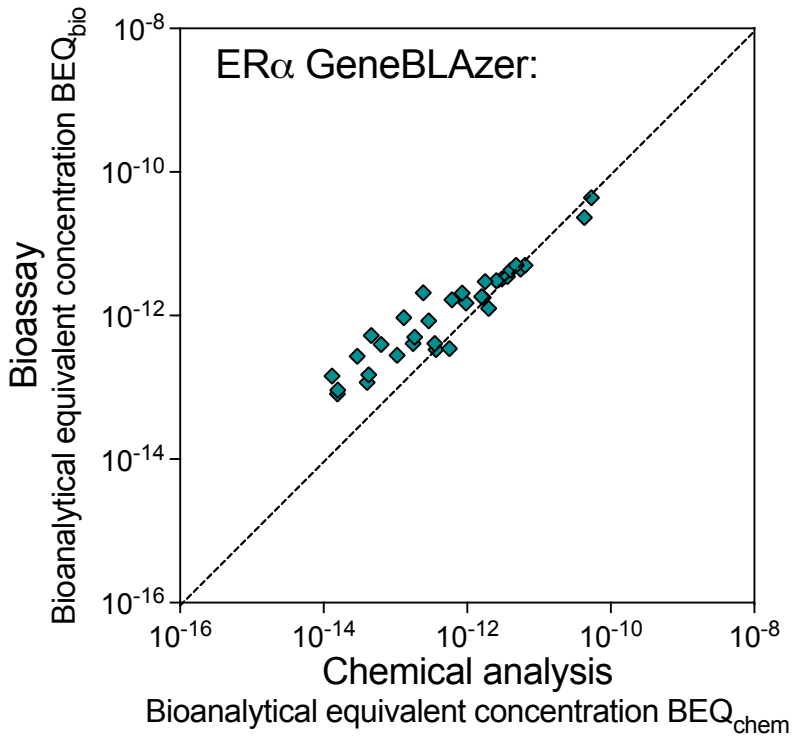
Iceberg modelling

$$REP_i = \frac{EC_y(ref)}{EC_y(i)}$$

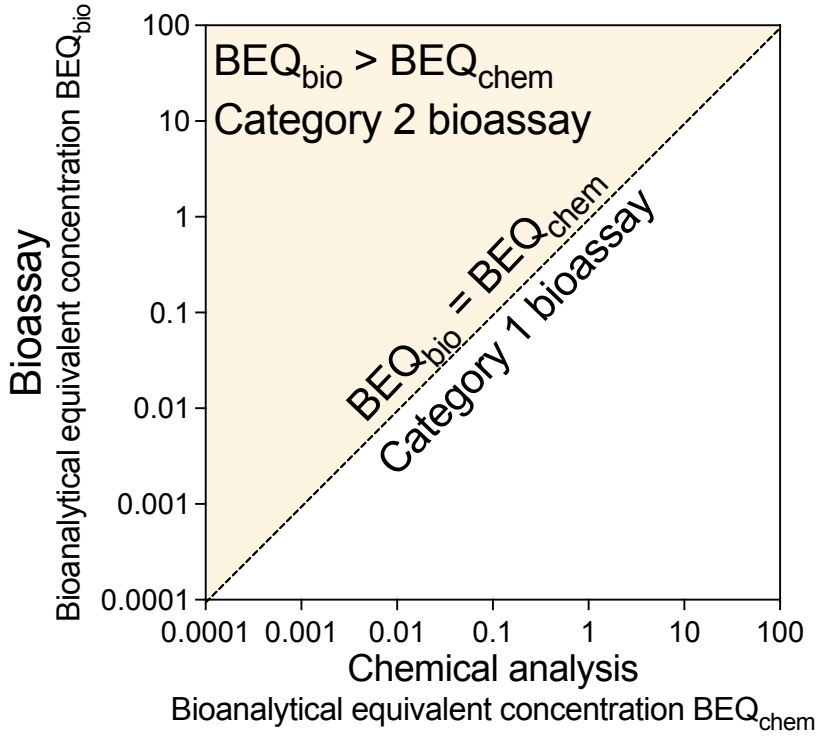


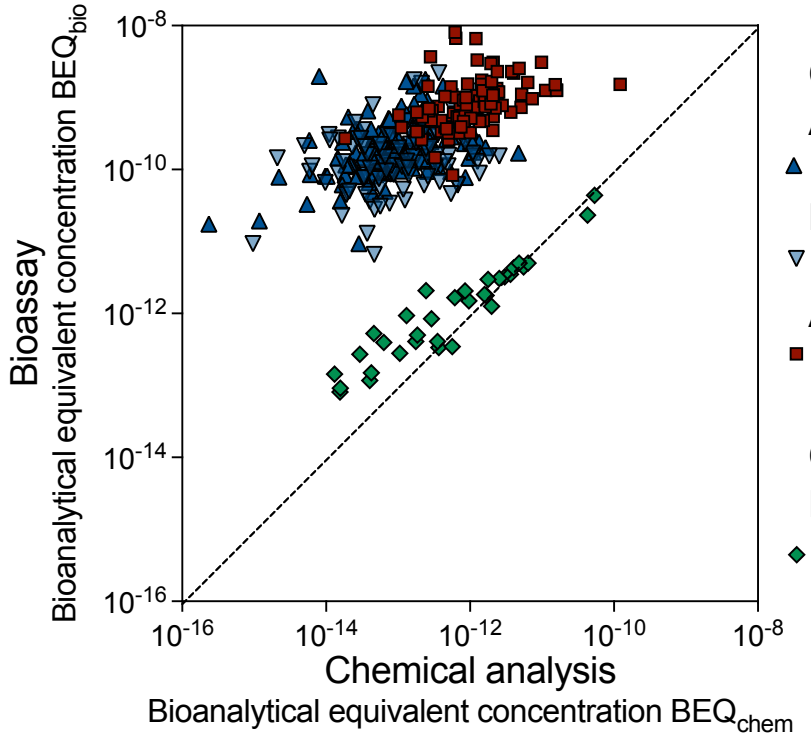
$$BEQ_{bio} = \frac{EC_y\ (ref)}{EC_y\ (sample)}$$





Iceberg modelling





Category 2 bioassays:

AhR CALUX:

▲ B[a]P EQ (M)

PPARγ GeneBLAzer:

▽ Rosglitazone EQ_{chem} (M)

AREc32:

■ Dichlorvos EQ (10^3 M)

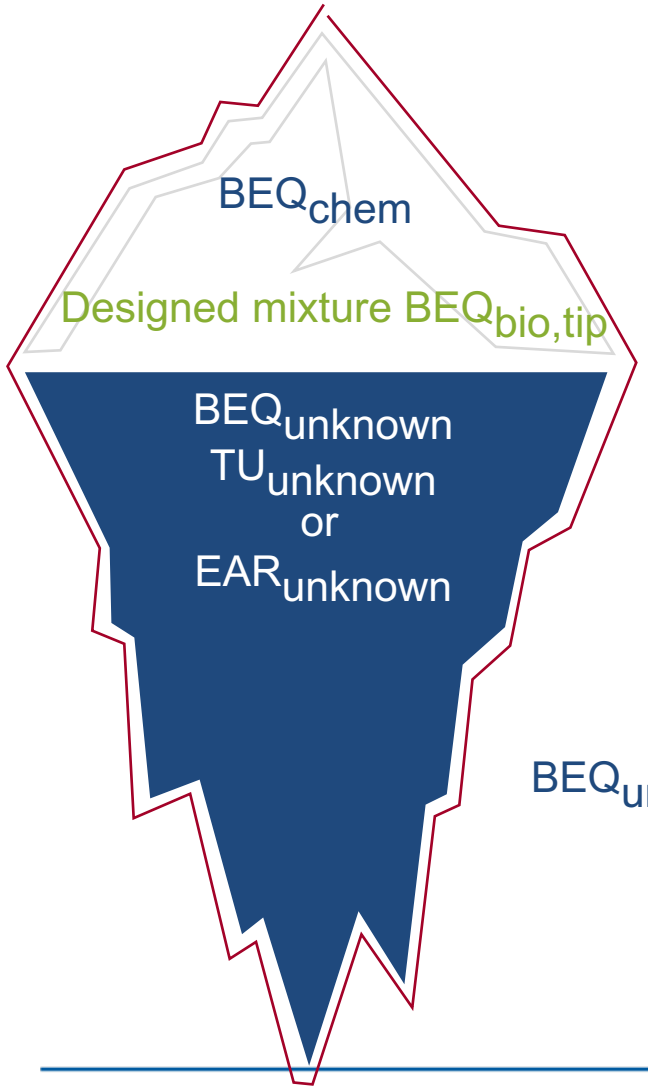
Category 1 bioassay:

ERα GeneBLAzer:

◆ EEQ (M)

Iceberg modelling combined with designed mixtures experiments

Case study 6 in Chapter 14



$$BEQ_{chem} = \sum_{i=1}^{n} REP_i \cdot C_i$$

CA?

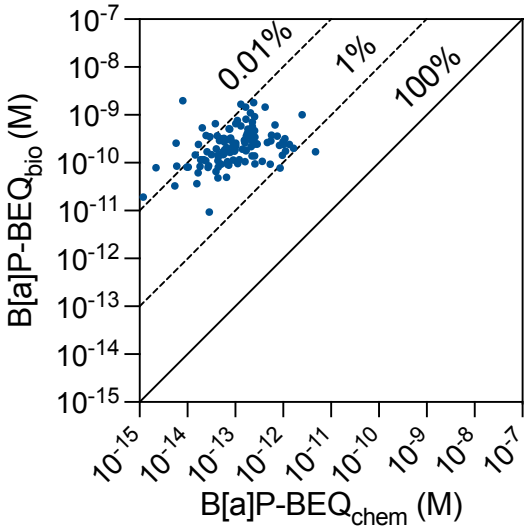
$$BEQ_{bio,tip} = \frac{EC_y\ (ref)}{EC_y\ (mixture)}$$

$$BEQ_{bio} = \frac{EC_y\ (ref)}{EC_y\ (sample)}$$

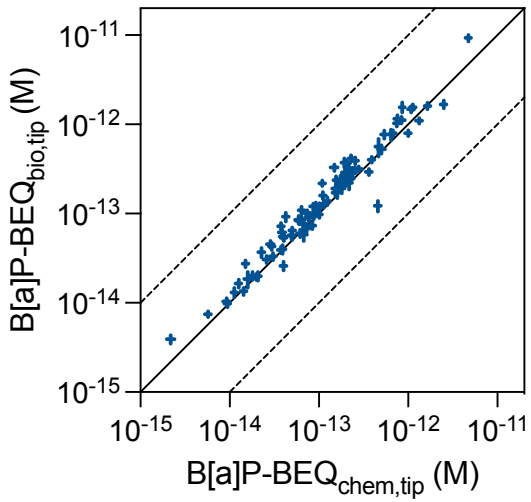
$$BEQ_{unknown} = BEQ_{bio} - BEQ_{chem}$$

- Less than 1% effect explained by chemical analysis (400 analytes)
- Detected chemicals mixed in concentration as detected acted concentration-additive

AhR CALUX: all chemicals

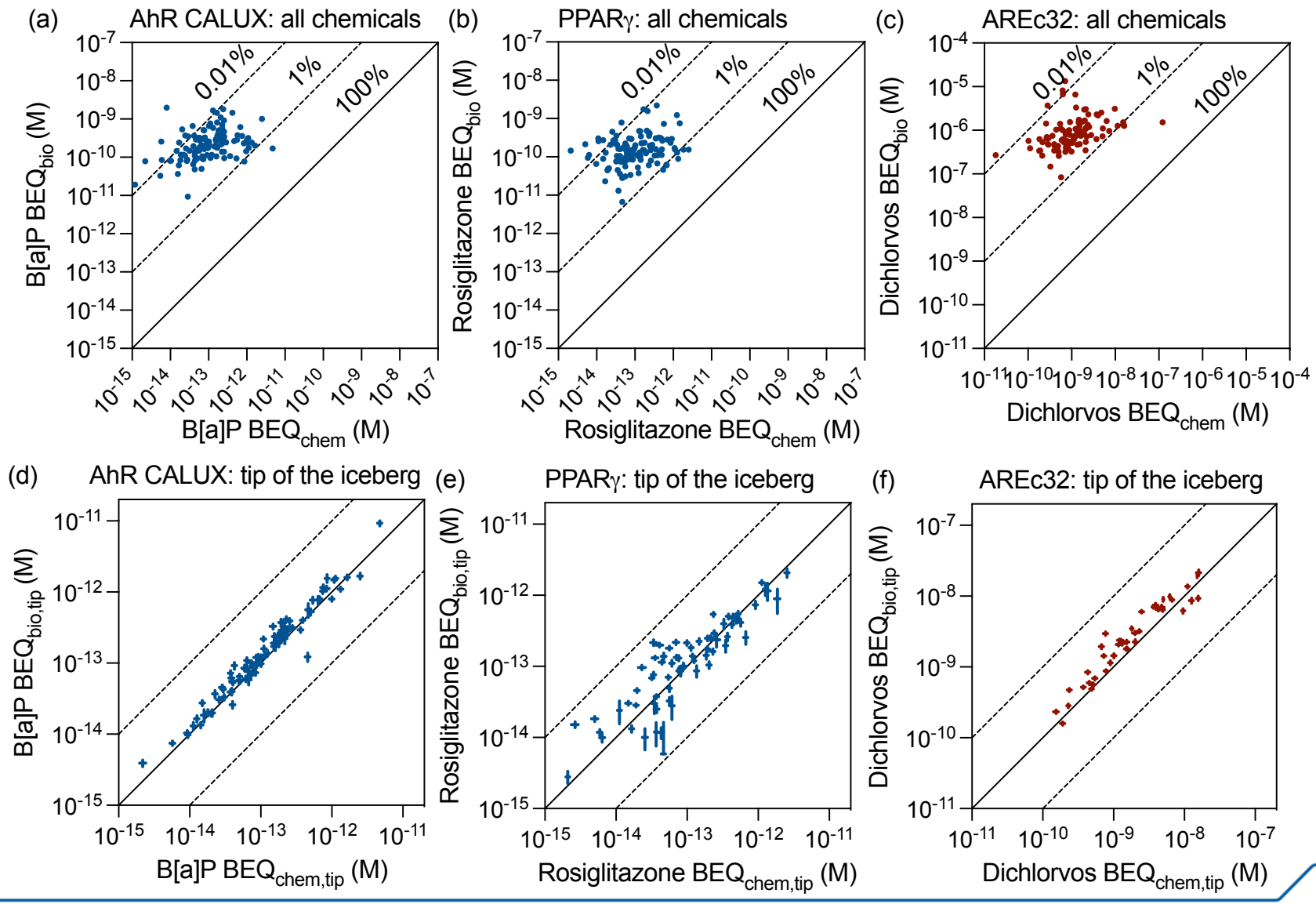


AhR CALUX: tip of the iceberg



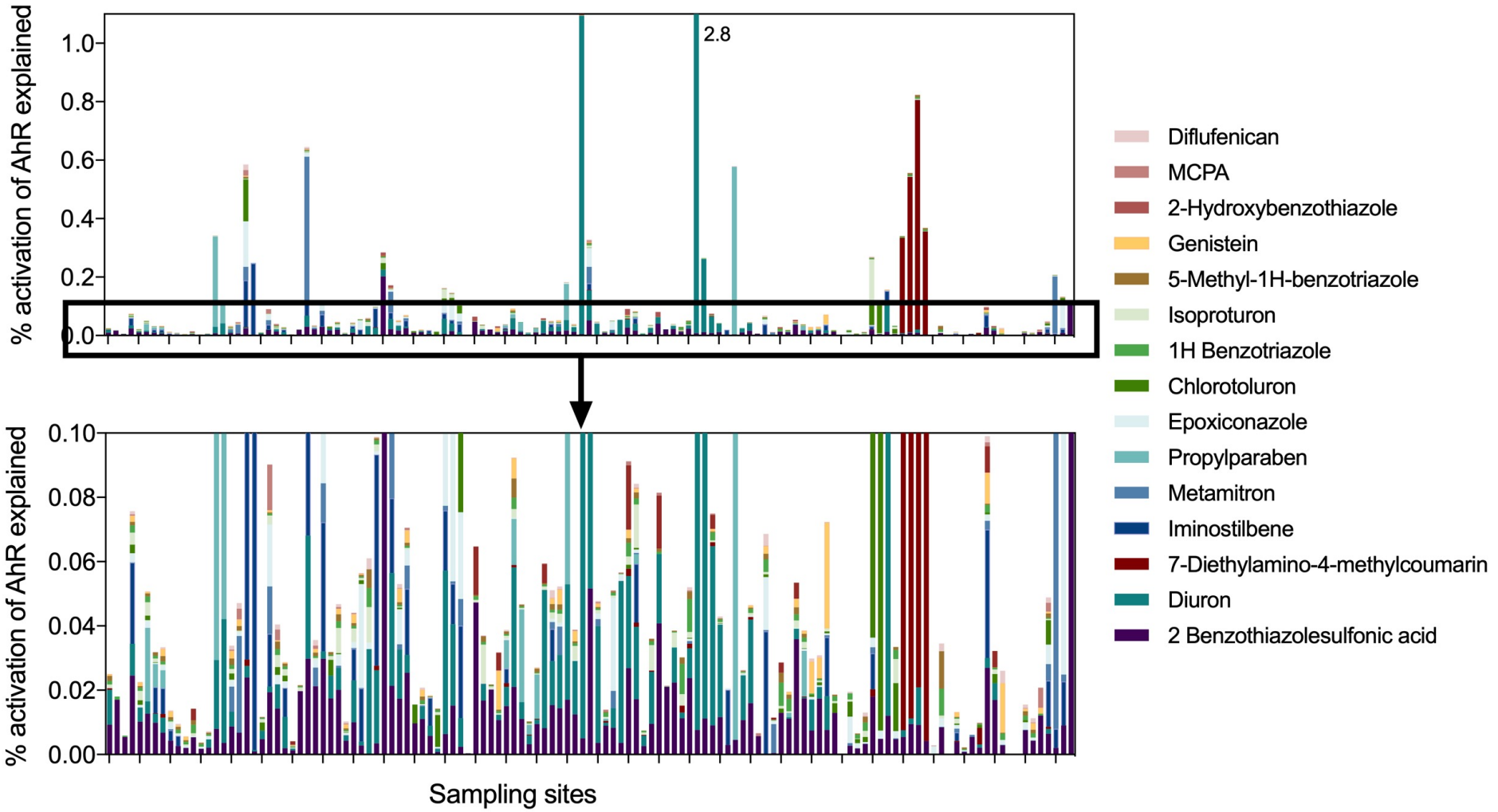
Iceberg modelling vs. designed mixtures experiments

Case study 6 in Chapter 14



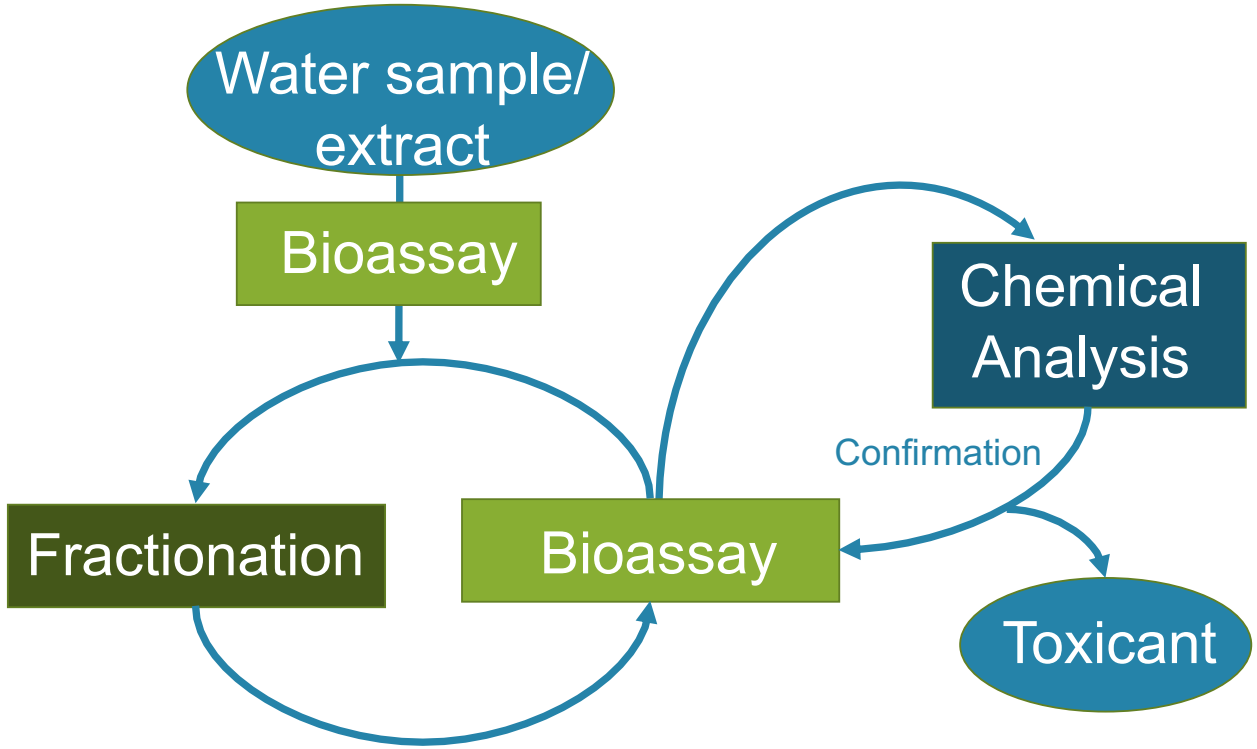
Case study 6
in Chapter 14

Which chemicals contribute to the BEQ_{chem}?



Effect-directed analysis for category 1 bioassays

- Only for category 1 bioassays
  - Hormone receptors ER, AR, PR, GR
  - Low number of highly bioactive chemicals
  - Distinct physicochemical properties
  - Masking by cytotoxicity avoided by fractionation
- Compare: Cat 2 bioassays in many fractions -> activity lost/diluted

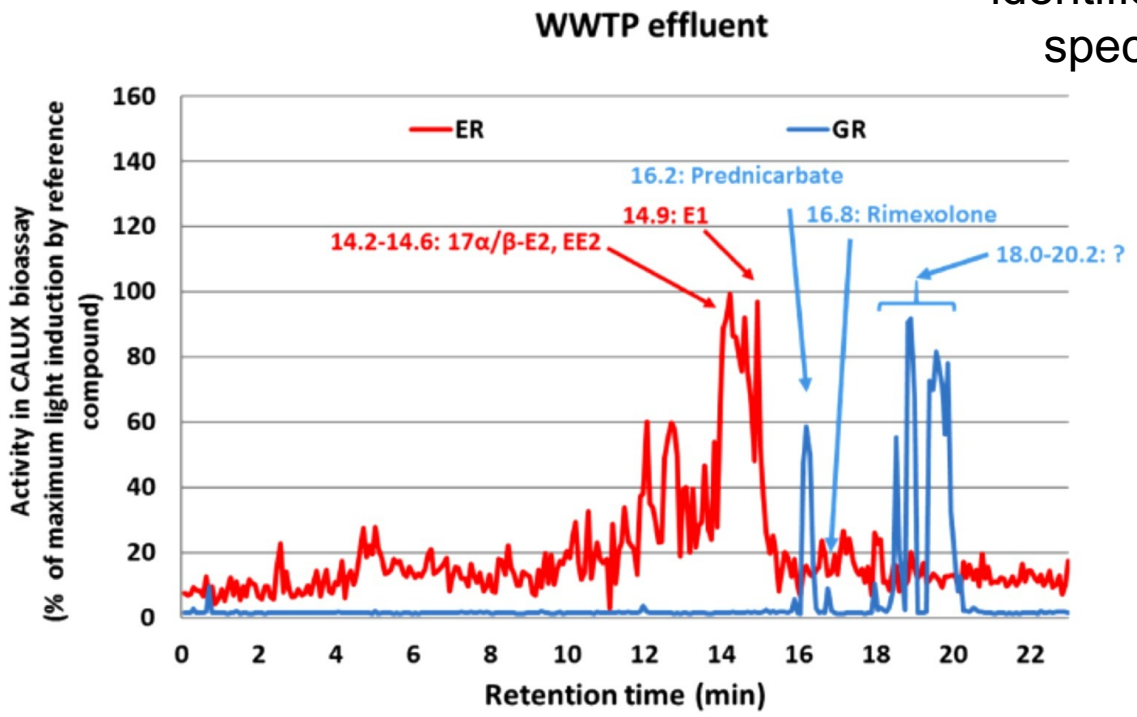


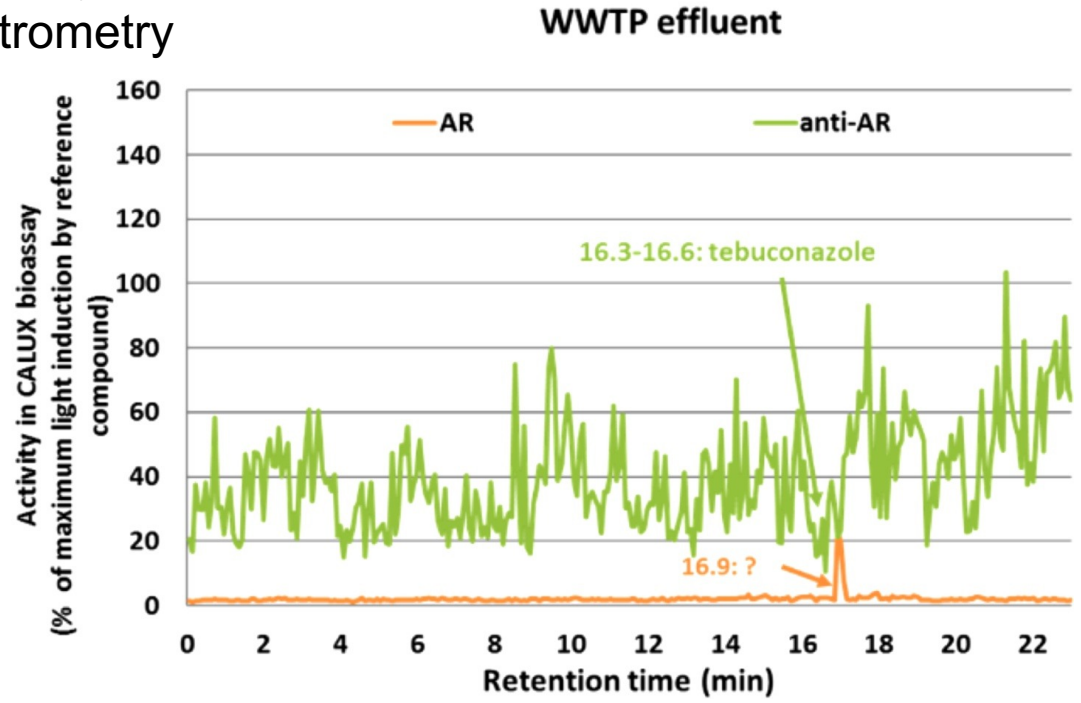
High-throughput effect-directed analysis

Bioassay chromatogram for ER and GR CALUX

AR CALUX run in agonistic and antagonistic mode

bioactive components identified by mass spectrometry





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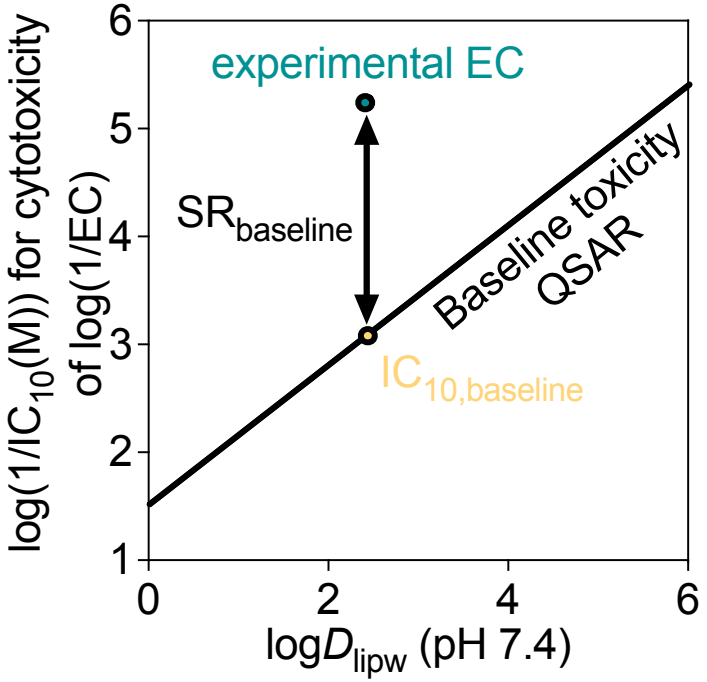
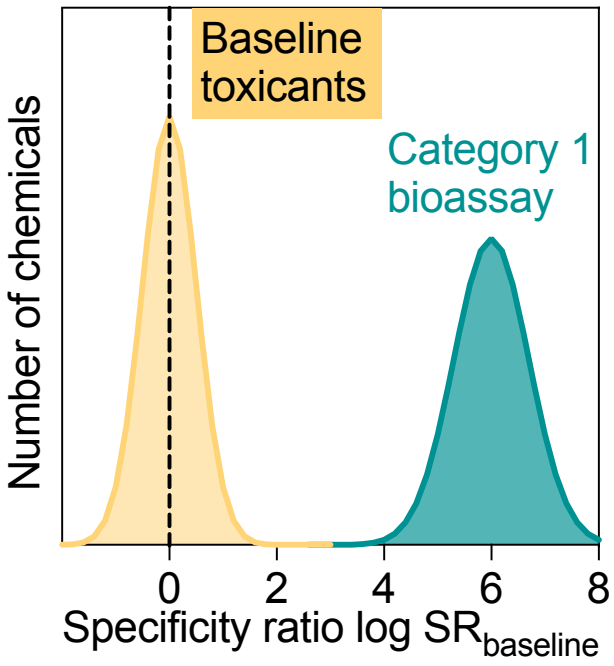
Identification of category 1 bioassay

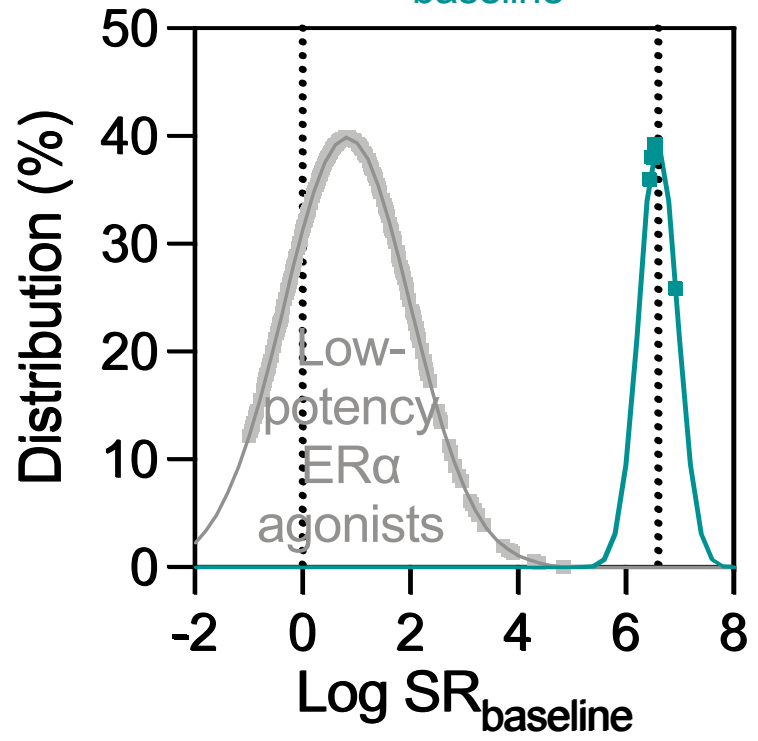
Specificity ratio against baseline toxicity: $SR_{baseline}$





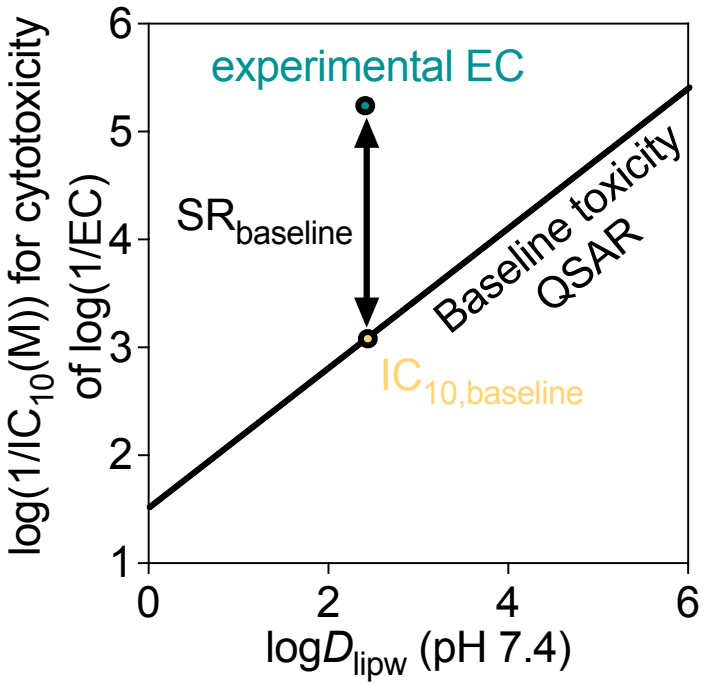
Example: Estrogenic chemicals

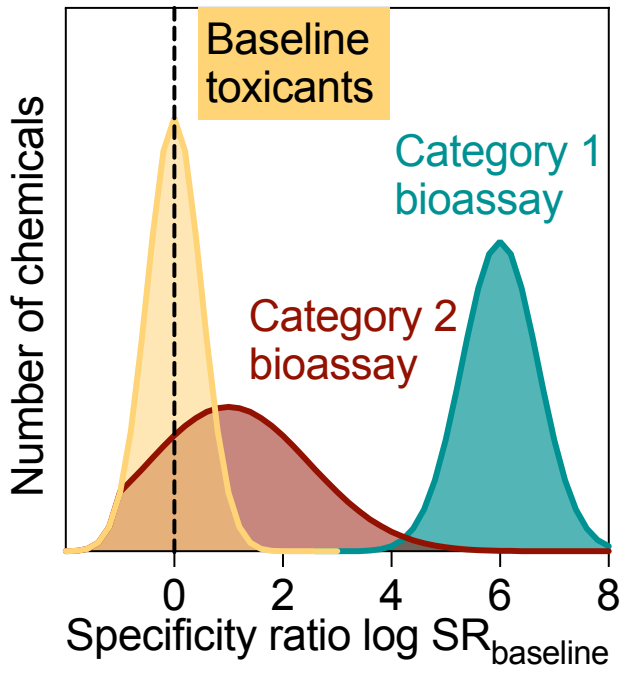
$SR_{baseline} = 4x10^6$



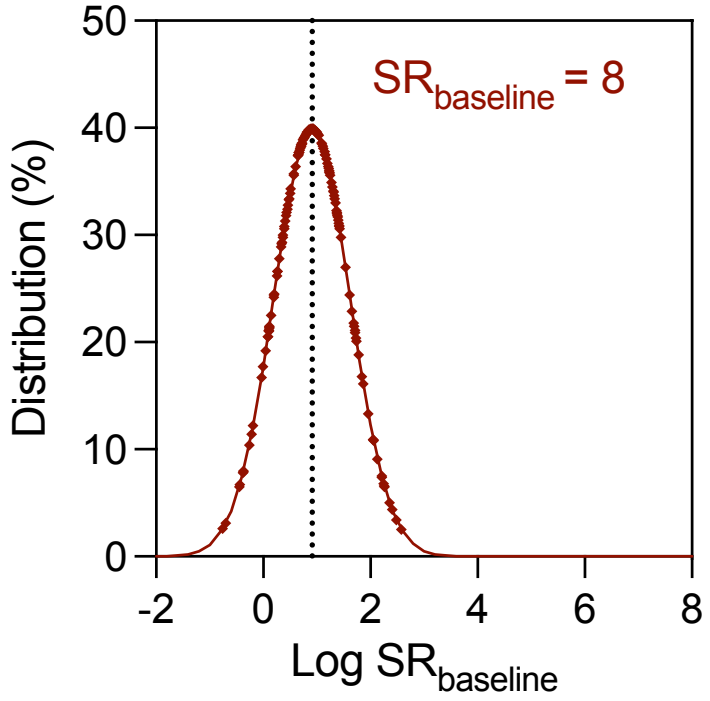
Identification of category 1 bioassay

Specificity ratio against baseline toxicity: $SR_{baseline}$



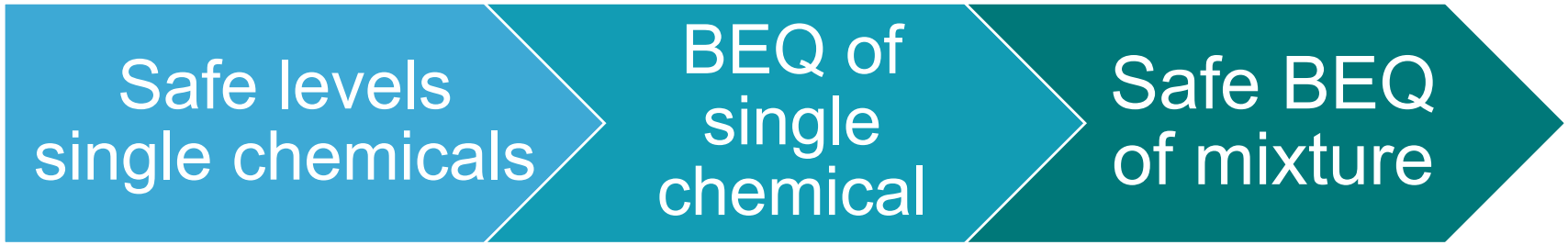


Example: chemicals that activate oxidative stress response



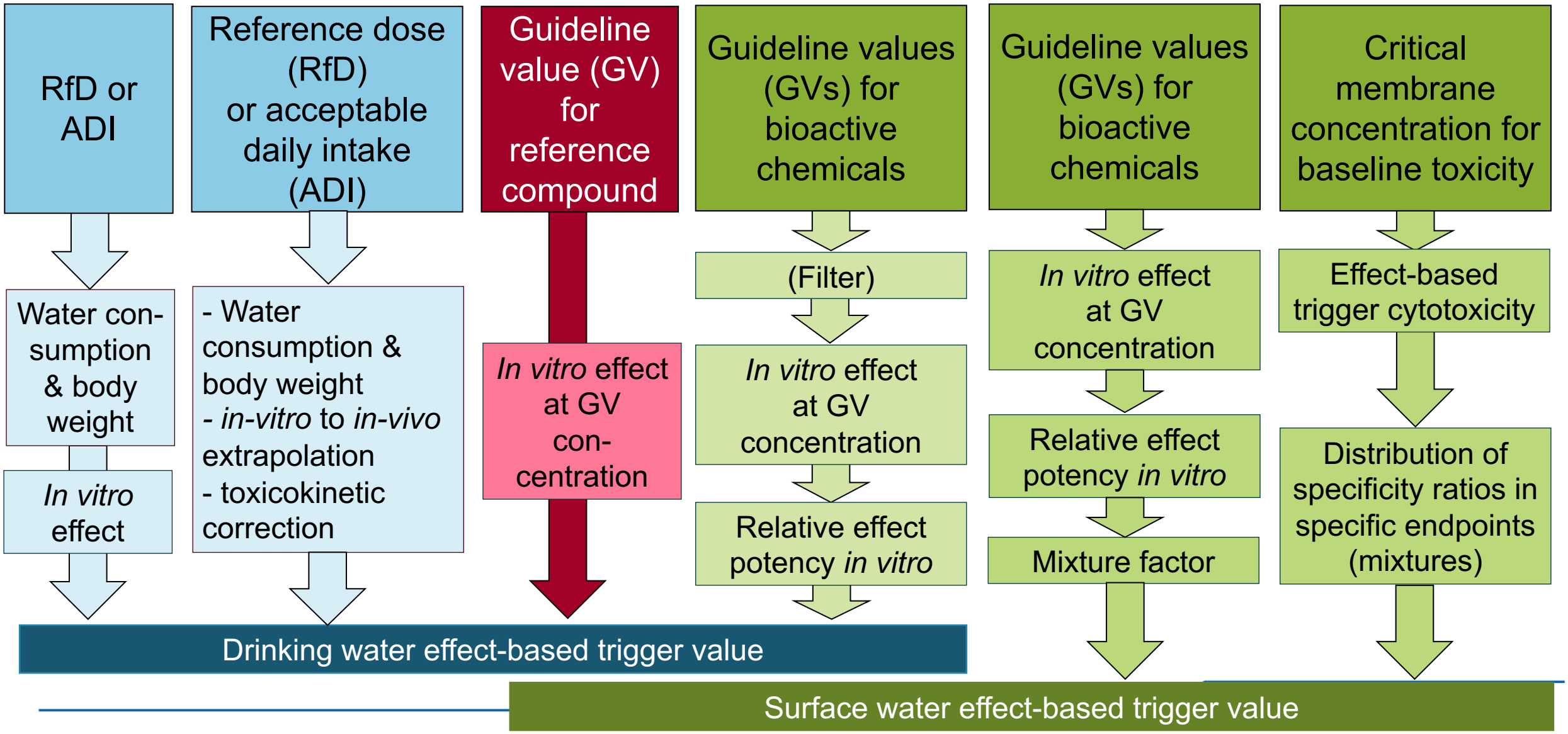
Implications for effect-based trigger values

- Effect-based trigger (EBT) values are BEQs or effects of water samples that are considered safe
- They can be directly translated from chemical guideline values if only a small number of chemicals can explain the entire observed effect in a water sample (i.e. for category 1 bioassays)
- It is more complex for category 2 bioassays, mixtures need to be accounted for



Effect-based trigger values for category 1 bioassays

for category 2 bioassays

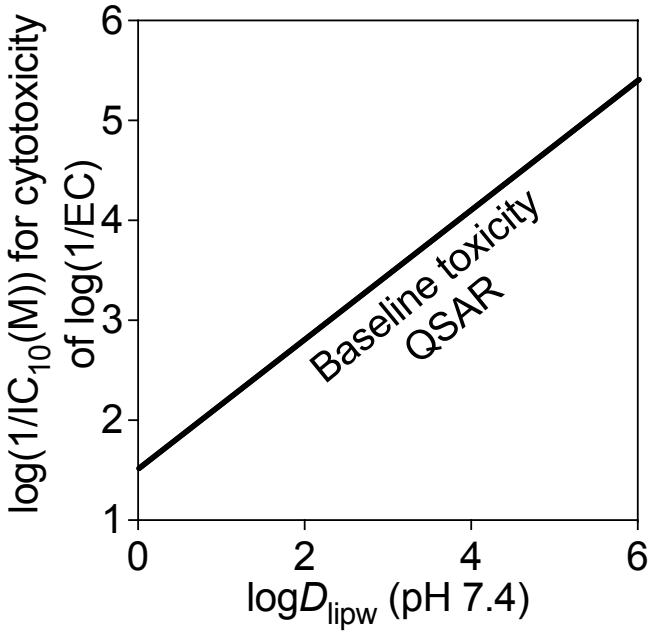


Cytotoxicity effect-based trigger value

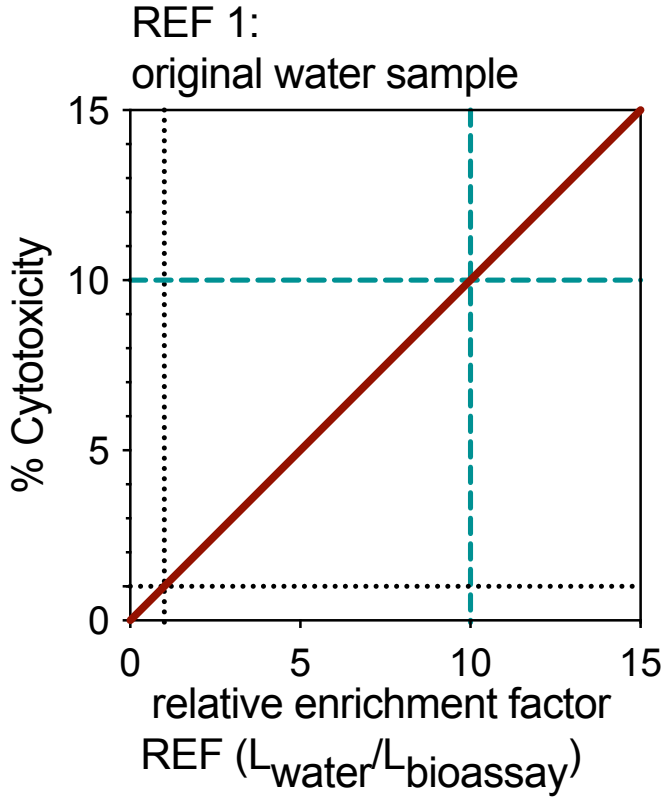
Baseline toxicity QSARs for cell lines are very similar with critical membrane concentration of 69 mmol/L_{lip}

If we accept 1% cytotoxicity as safe

→ Cytotoxicity EBT-IC10 = REF 10



How much baseline toxicity is safe?



Escher, B.; Glauch, L.; Konig, M.; Mayer, P.; Schlichting, R., Baseline Toxicity and Volatility Cutoff in Reporter Gene Assays Used for High-Throughput Screening. *Chem. Res. Toxicol.* **2019,** *32*, (8), 1646-1655.

Effect based triggers for mixtures of low-potency chemicals

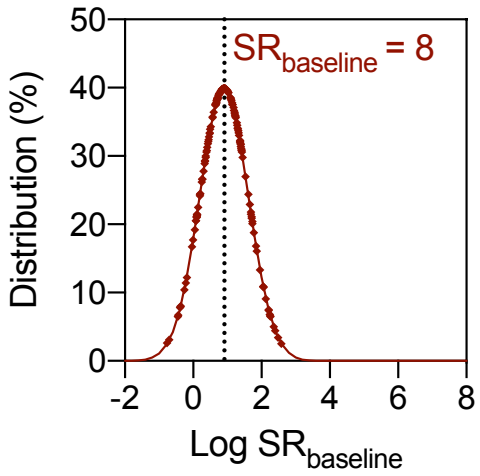
Cytotoxicity EBT @REF10

$SR_{baseline}$

$$EBT\text{-}EC = \frac{\text{Cytotoxicity EBT}}{SR_{baseline}}$$

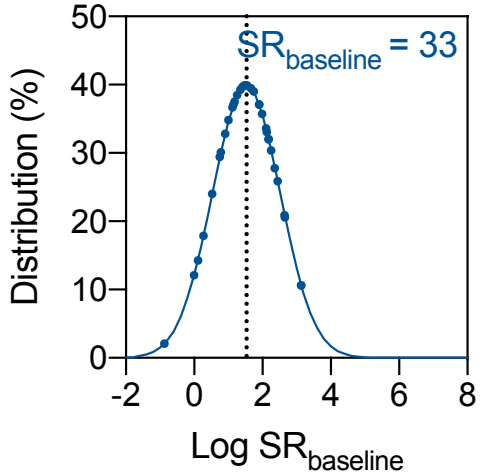
$$EBT\text{-}BEQ = \frac{EC_{reference}}{EBT\text{-}EC}$$

ARE



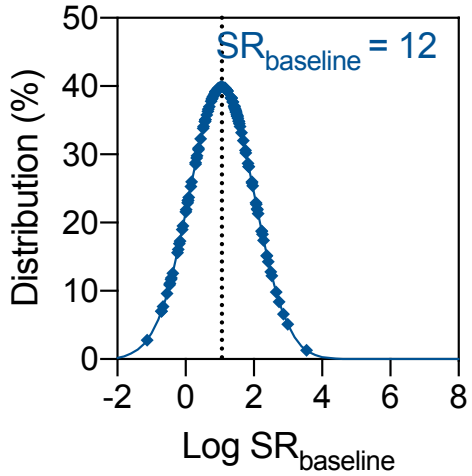
EBT-Dichlorvos-EQ
= 1.4 $mg_{Dichlorvos}/L$

PPARγ



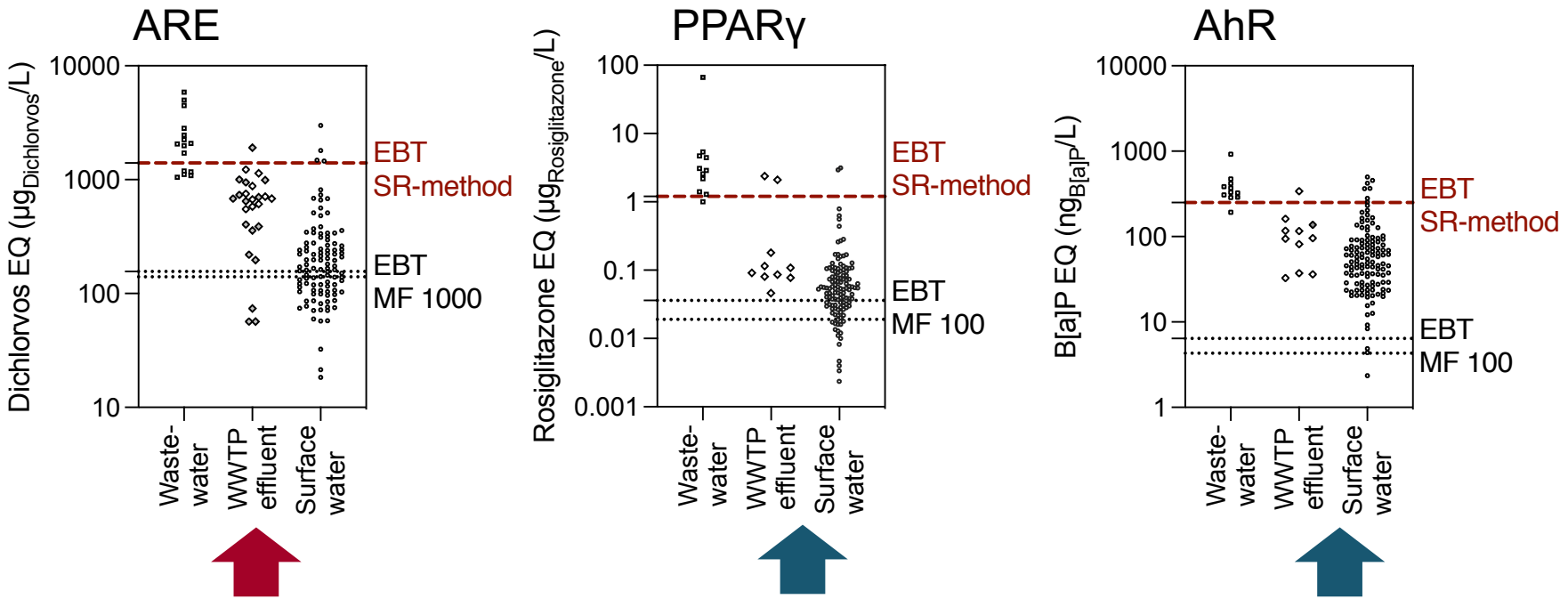
EBT-Rosiglitazone-EQ
= 1.2 $\mu g_{Rosiglitazone}/L$

AhR



EBT-B[a]P-EQ
= 250 $ng_{B[a]P}/L$

Effect based triggers for mixtures of low-potency chemicals



$$EBT\text{-}BEQ = \frac{EC_{reference}}{EBT\text{-}EC}$$

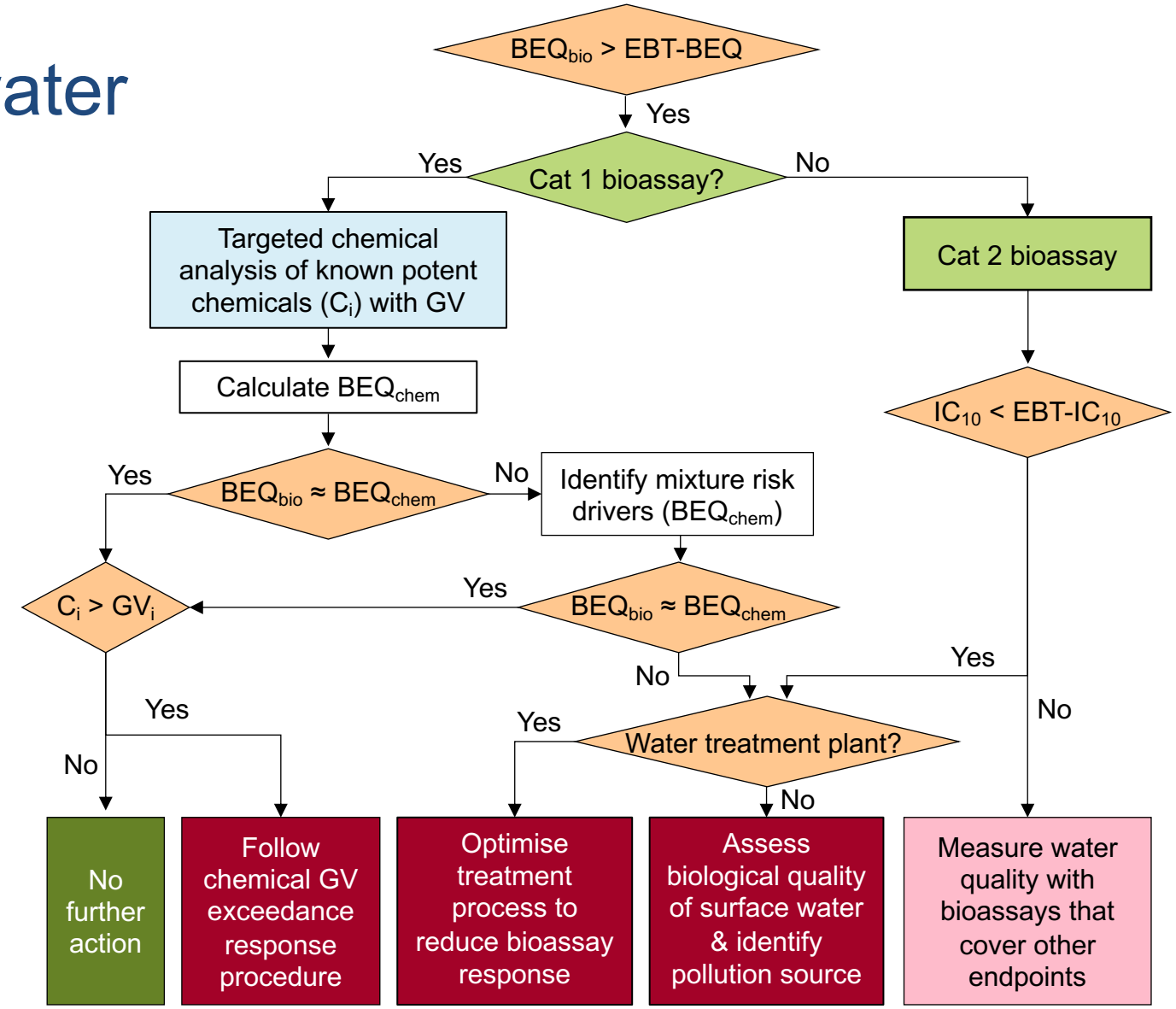
EBT-Dichlorvos-EQ
= 1.4 mg$_{Dichlorvos}$/L

EBT-Rosiglitazone-EQ
= 1.2 µg$_{Rosiglitazone}$/L

EBT-B[a]P-EQ
= 250 ng$_{B[a]P}$/L

Framework for the application of EBTs in water quality assessment

- For category 1 chemicals tiered strategy that asks for chemical analysis after EBT has been exceeded
- For category 2 chemicals direct action after EBT has been exceeded



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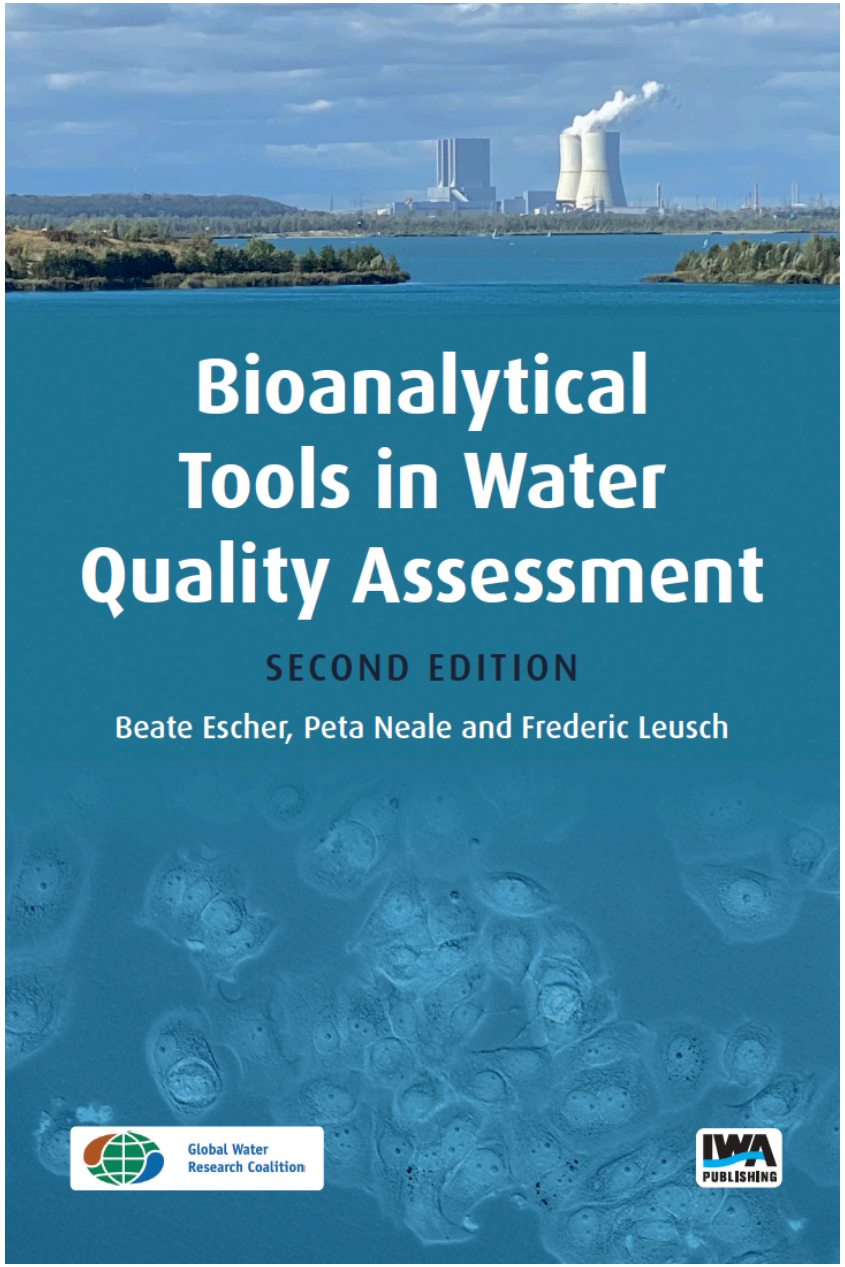

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