Chapter 12
Sampling, sample preparation and dosing

This presentation accompanies Chapter 12 of
“Bioanalytical Tools in Water Quality Assessment”
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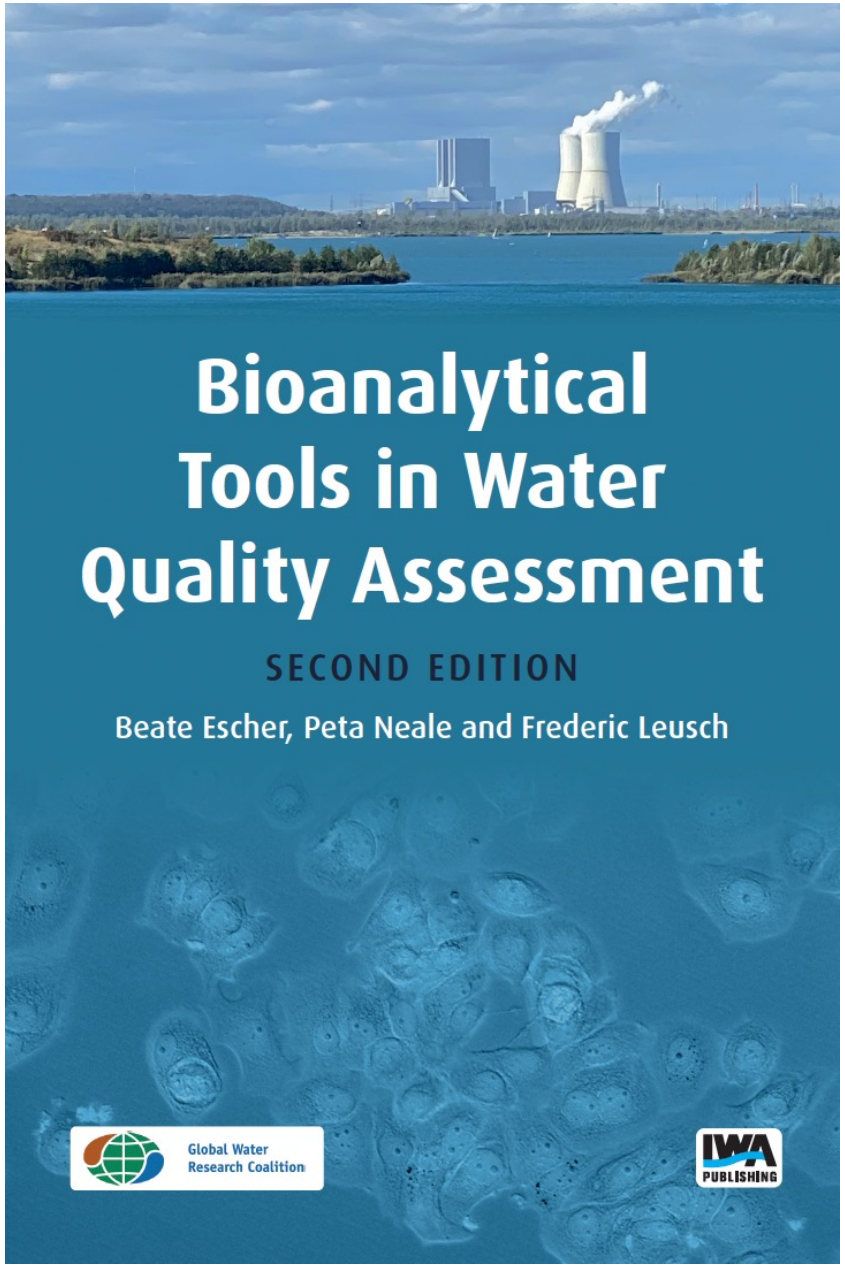


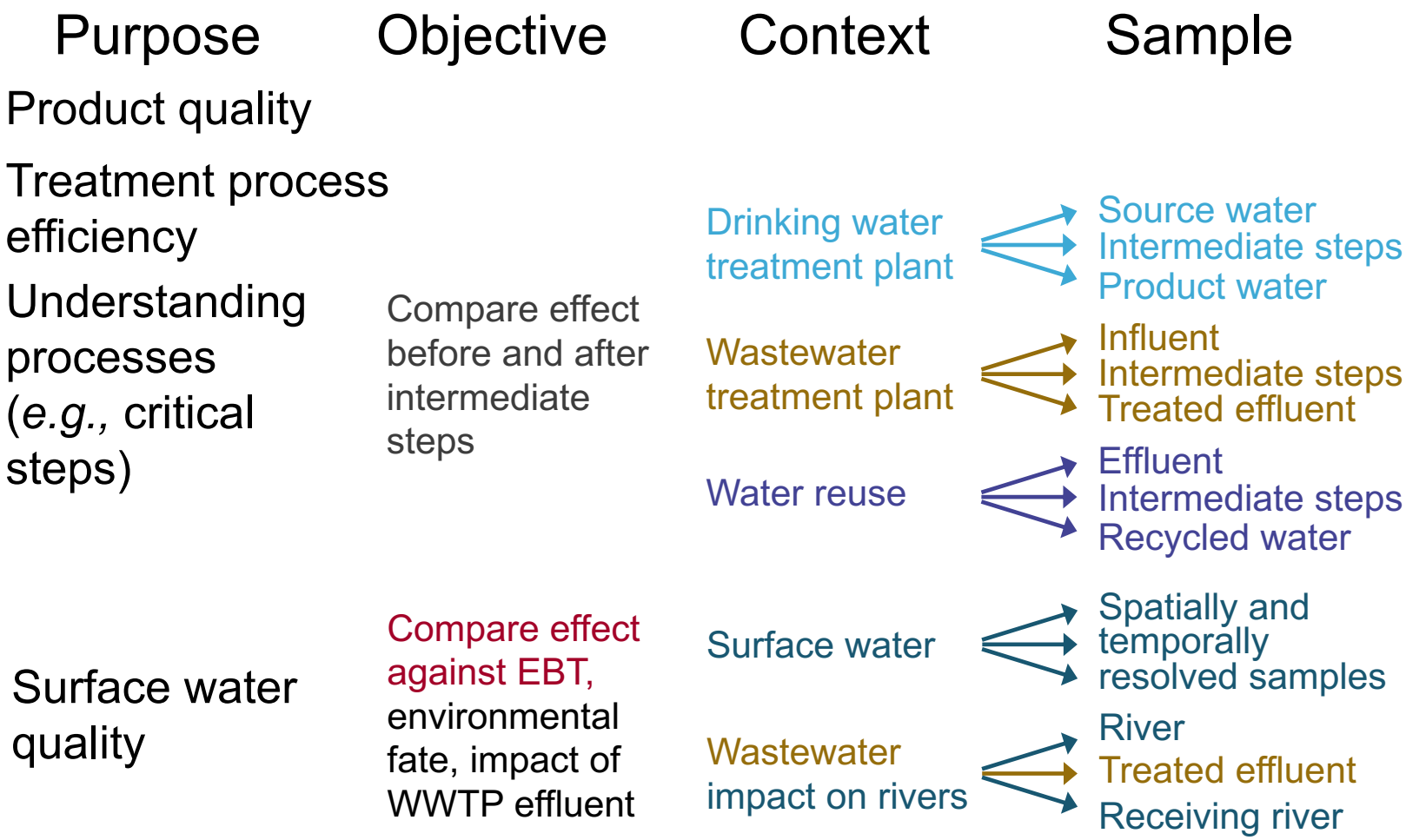
Learning goals

- Appreciate the importance of sampling and sample preparation
  Even the best bioassay cannot rectify problems created by poor sample collection and preparation
- Validation of extraction ideally not only with bioassay but also with chemical analysis
- The bioassay is not forgiving (contaminated solvents and equipment will show, no internal standards for recovery etc. possible)
- Sampling and dosing need to go hand in hand

Water sampling strategy

Purpose	Objective	Context	Sample
Product quality	Compare effect against EBT	Drinking water treatment plant	Product water
		Wastewater treatment plant	Treated effluent
		Water reuse	Recycled water
Treatment process efficiency	Compare the effect of inlet and outlet water	Drinking water treatment plant	Source water
			Product water
		Wastewater treatment plant	Influent
			Treated effluent
		Water reuse	Effluent
			Recycled water

Water sampling strategy



Time resolution of sampling

- Grab water samples: drinking water, recycled water
- 24h composite samples (diurnal variation): wastewater treatment plants
- 7 consecutive 24h composite samples (weekly variation)
- Seasonal sampling (4 times a year)
- Event-triggered sampling:
  - rain and storm events
  - night/day variation

Sampling

- Sample volume

Rule of thumb: 0.5 L wastewater or 1 L surface water or 2 L drinking water on one 200 mg HLB SPE cartridge

- Sample preservation

The best is to extract immediately (<48h). Lowering pH to 3 might help extraction of organic acids and preserve sample

Chlorinated drinking water: quench with Na thiosulphate

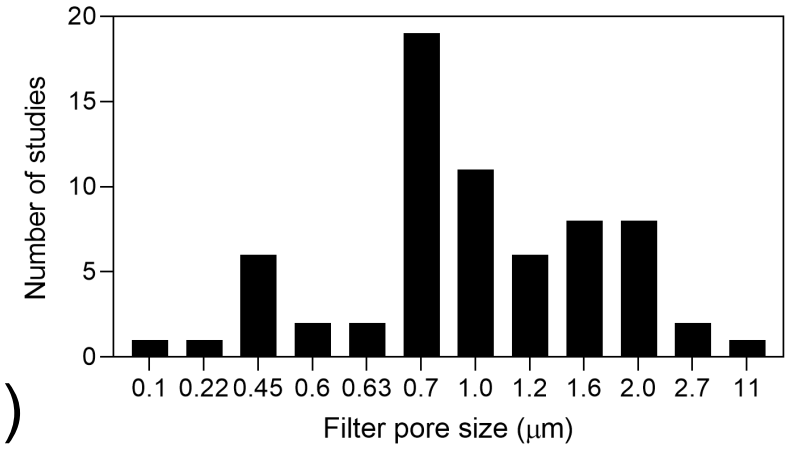
- Sample filtration

Glass fibre filter 0.7 to 2 µm if NTU > 5

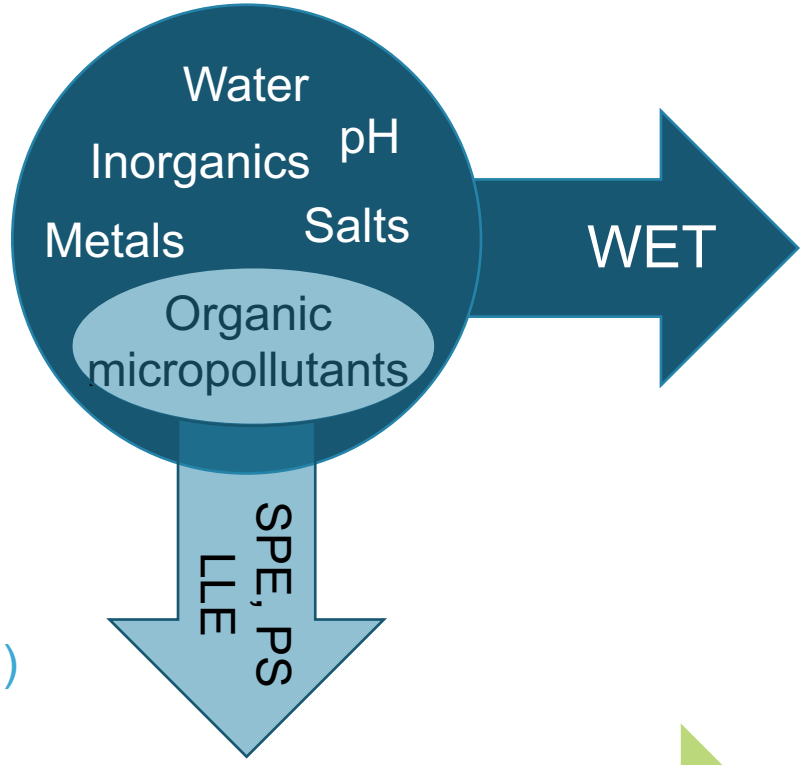
Caution: a lot of bioactivity on SPM!

- Sample extraction

Solvent extraction (LLE) or solid-phase extraction (SPE)



To extract or not to extract?



Whole-effluent toxicity

- *In vivo* assays
- Limited *in vitro* assays
  (sterile filtration < 0.22 µm)
- Biomarkers

Solvent extraction (LLE)
passive sampling (PS)
solid-phase extraction (SPE)

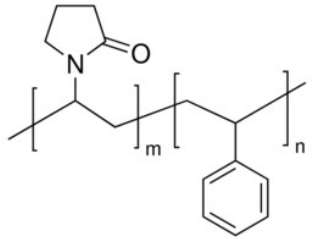
Extracts of organic micropollutants

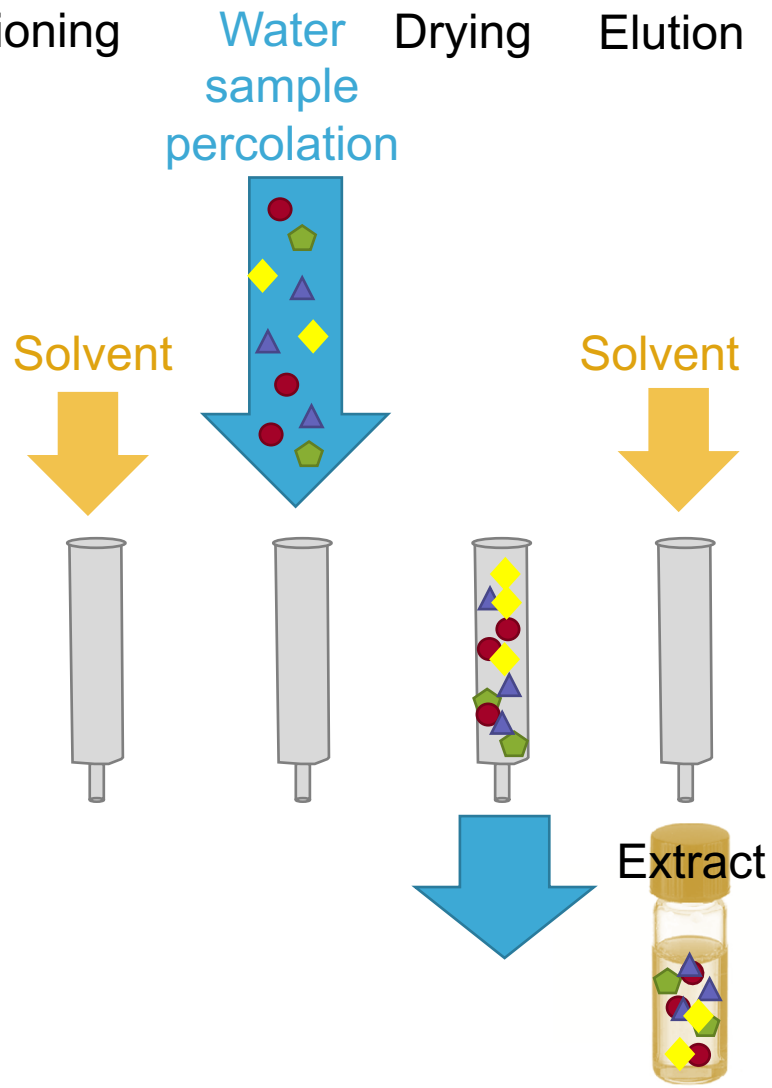
Bioanalytical methods

Battery of *in vitro* assays

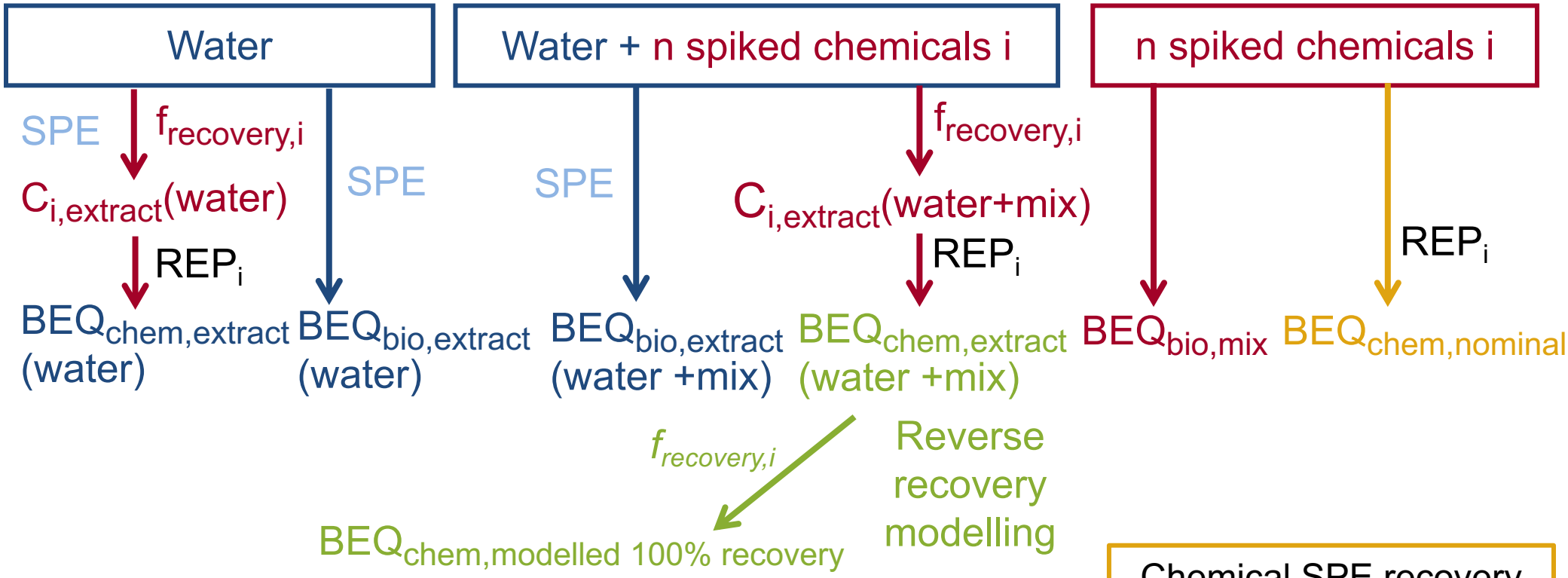
Solid-phase extraction

- Purpose
  - Chemical analysis: selective and specific for target analytes
  - Bioassays: as broad as possible
- Materials
  - Silica-based (C2, C8, C18)
  - Poly(divinylbenzene-co-N-vinylpyrrolidone) copolymers
  - Graphitised carbon black, coconut charcoal
  - Ion-exchange materials (WAX, WCX)
- Solvents:
  - Methanol, ethyl acetate
  - Elution: methanol, ethyl acetate, acetonitrile, dichloromethane , MTBE, hexane:acetone
  - Final solvent: methanol, DMSO
- Capacity: 1-5% of sorbent mass (2-10 mg/200mg$_{sorbent}$)





Effect recovery by SPE



Predicted loss of effect after SPE

$$= 1 - \frac{BEQ_{chem,extract}(water+mix)}{BEQ_{chem,\ modelled\ 100\%\ recovery}}$$

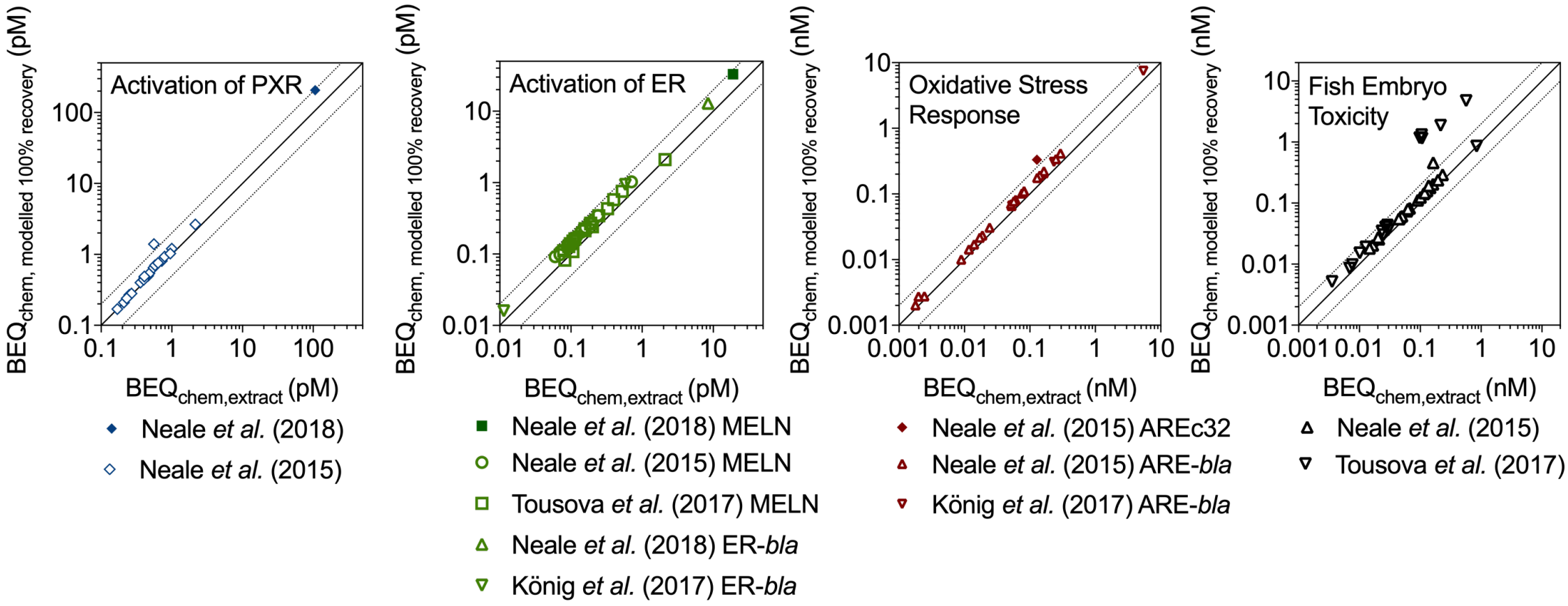
Chemical SPE recovery expressed as effect

$$= \frac{BEQ_{bio,mix}}{BEQ_{chem,nominal}}$$

Effect recovery by SPE

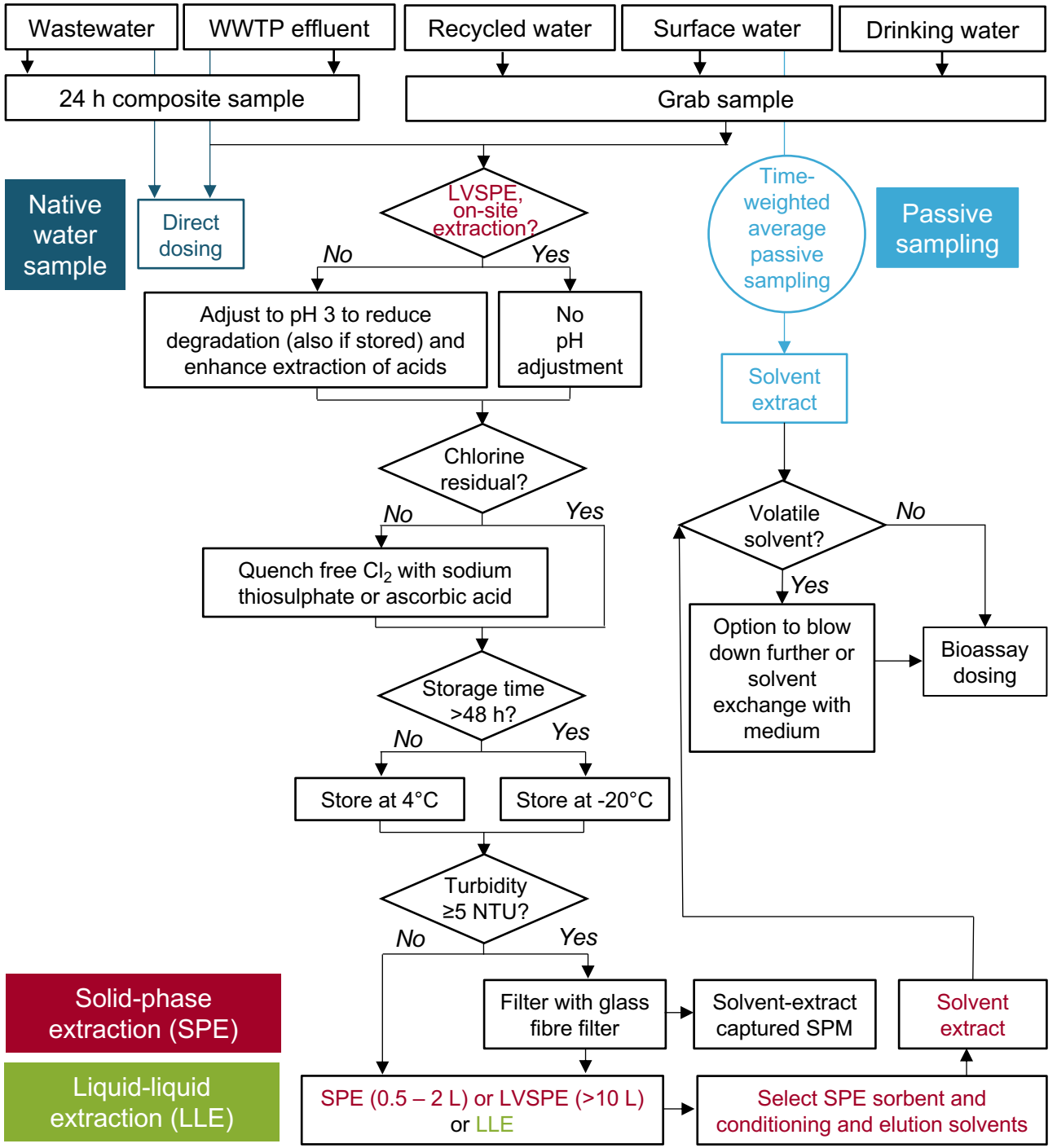
$$= 1 - \frac{BEQ_{bio,extract}(water+mix) - BEQ_{bio,extract}(water)}{BEQ_{bio}(mix)}$$

Effect recovery by SPE



Within a factor of 2!

What to consider…..



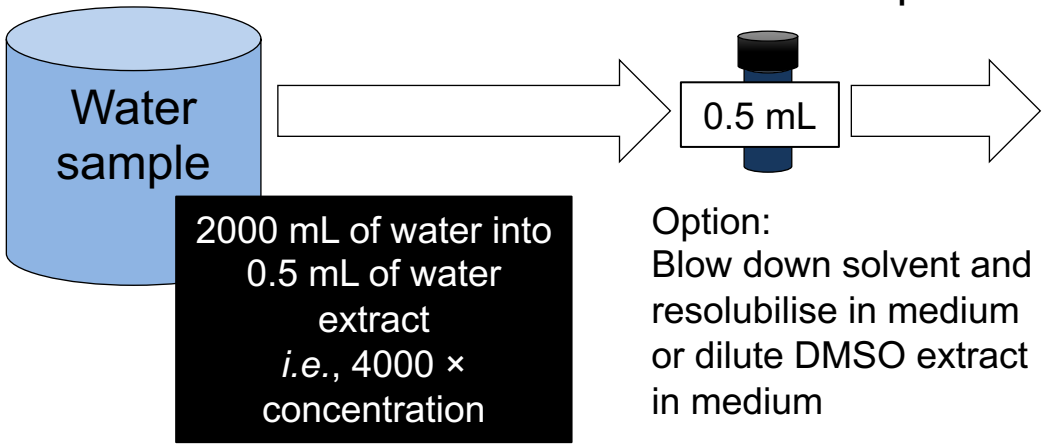
Dosing into bioassays

Step 1
Solid-phase extraction

Step 2
Transfer water extract to dilution plate

Step 3
Serial dilution

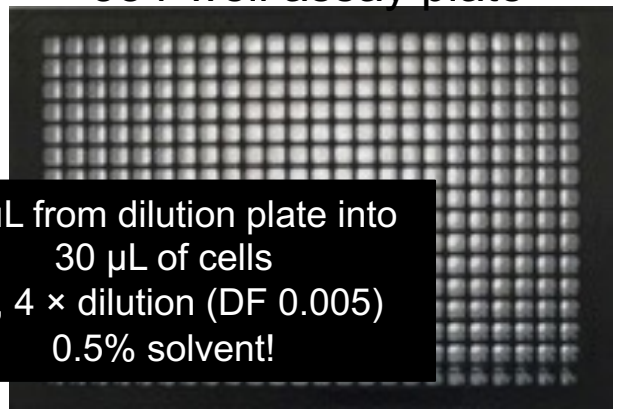
Step 4
Add dilution series to assay plate (in this case in triplicate)



Serial dilution in 96-well dosing plate

96-well assay plate
384-well assay plate

robot



$$\text{Extraction factor EF} = \frac{\text{volume water extracted}}{\text{final volume of extract}} = 4000$$

$$\text{Dosing factor DF} = \frac{\text{volume of extract dosed}}{\text{final volume in bioassay}} = 0.005$$

Relative extraction factor (or relative enrichment factor)

$$\text{REF} = \frac{\text{volume water extracted}}{\text{final volume in bioassay}} = \text{EF} \cdot \text{DF} = 20$$

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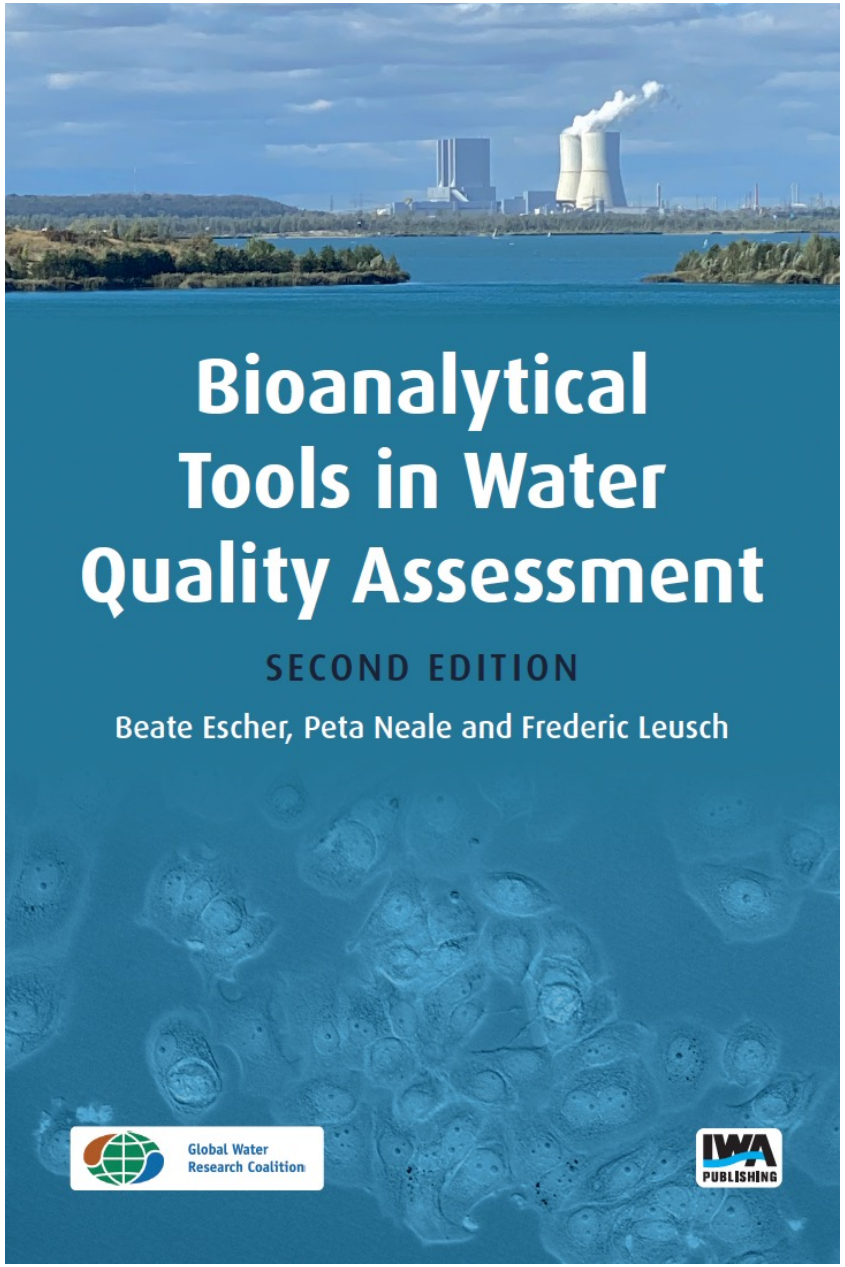

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