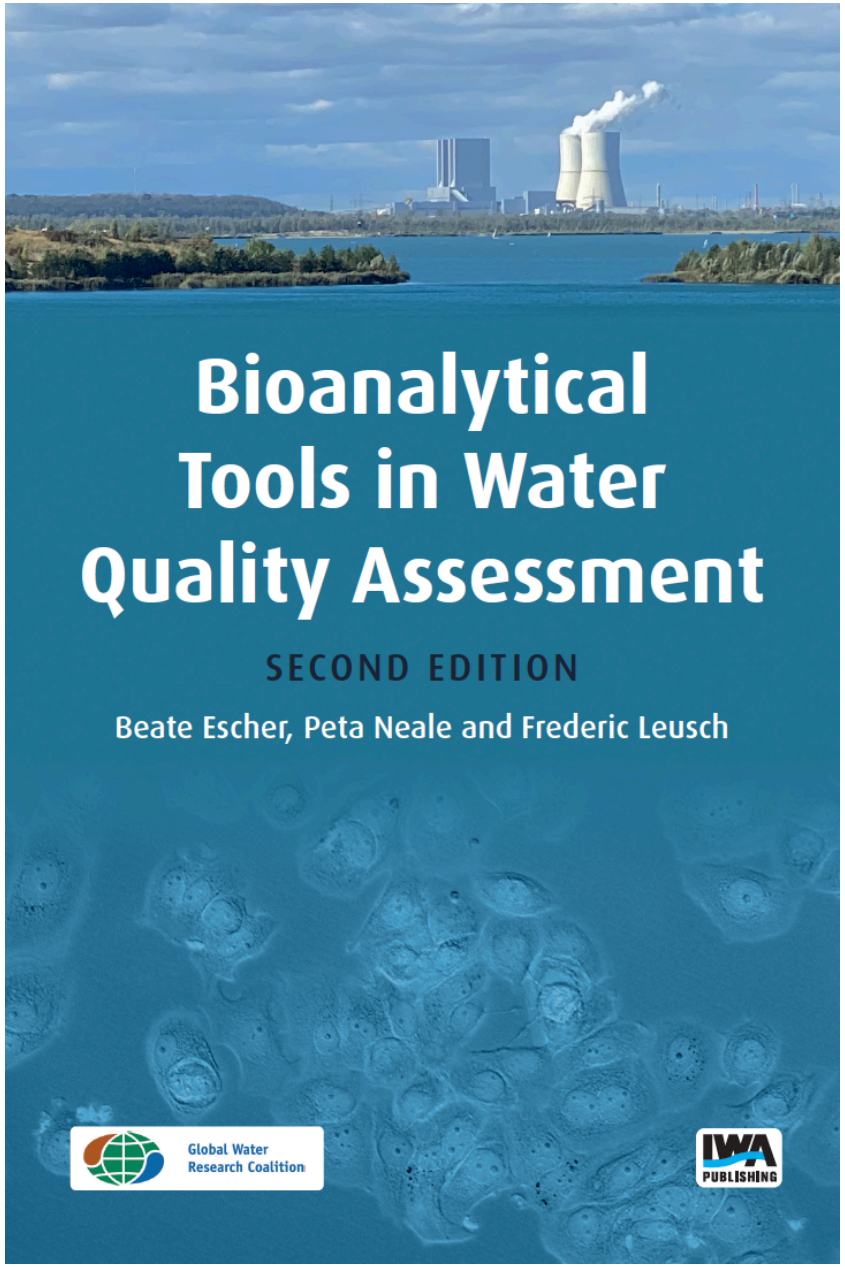
Chapter 8
Mixtures



This presentation accompanies Chapter 8 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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For questions please send e-mail to bioanalytical-tools@ufz.de

Learning goals

- You know the theoretical concepts behind the effect of mixtures
- You can apply practical methods for the evaluation of mixtures in risk assessment
- You can perform simple mixture modelling in *in vitro* bioassays

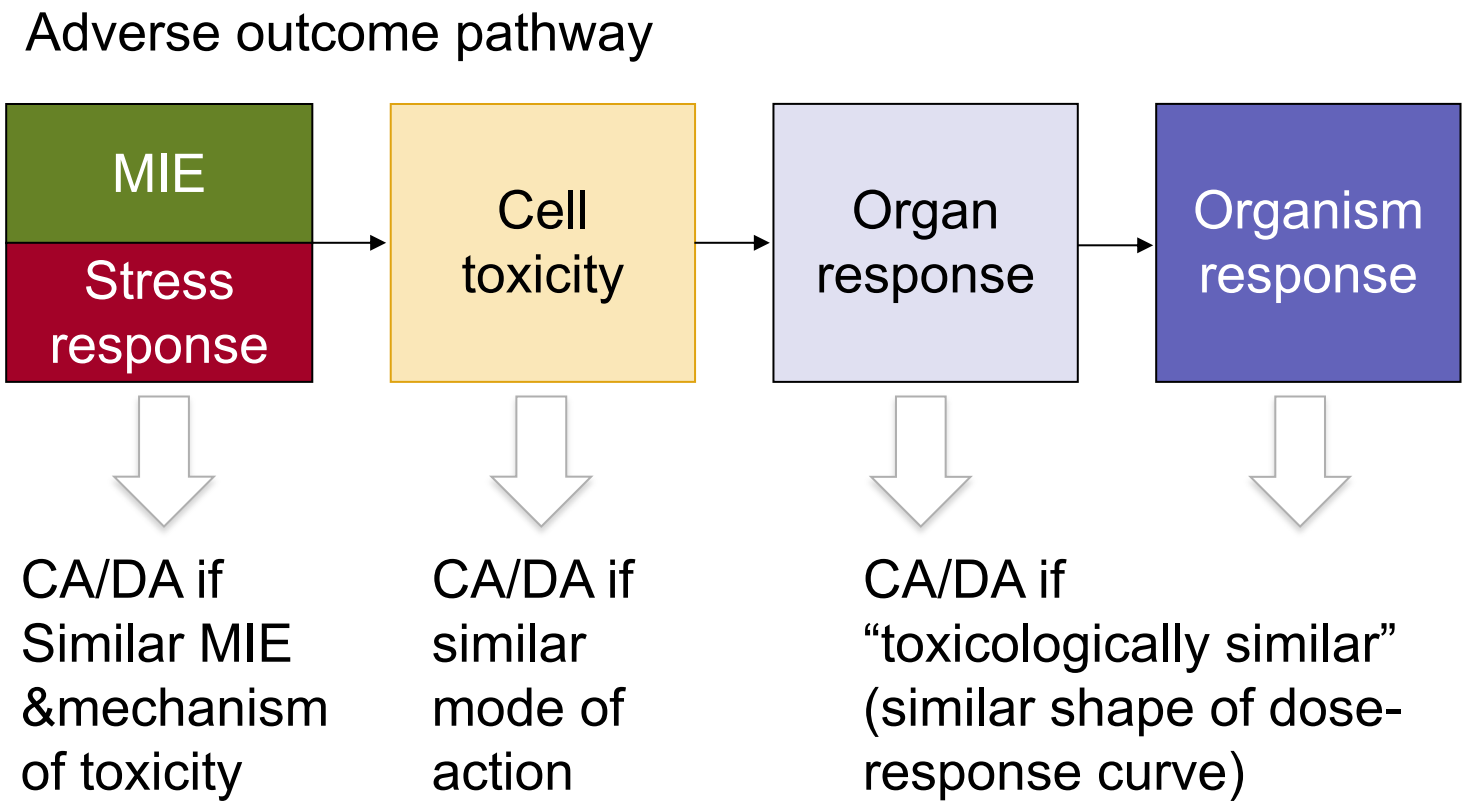
*Smith, K.E.C., Schmidt, S.N., Dom, N., Blust, R., Holmstrup, M. and Mayer, P. (2013). Baseline Toxic Mixtures of Non-Toxic Chemicals: "Solubility Addition" Increases Exposure for Solid Hydrophobic Chemicals. Environmental Science & Technology, **47**(4): 2026-2033.*

Concepts of mixture toxicity

Site of action (target site) and mode of action determine the joint action of chemicals

	Same target site Similar joint action	Different target sites Dissimilar joint action
Interaction absent	Simple similar action Concentration/dose Addition (CA/DA)	Independent action (IA) Response addition
Interaction present	Complex similar action	Dependent action

What is “similar” within an AOP framework



Toxicity of binary mixtures (= 2-component mixture)

Toxic Unit $TU_1 = C_1/EC_{y1}$

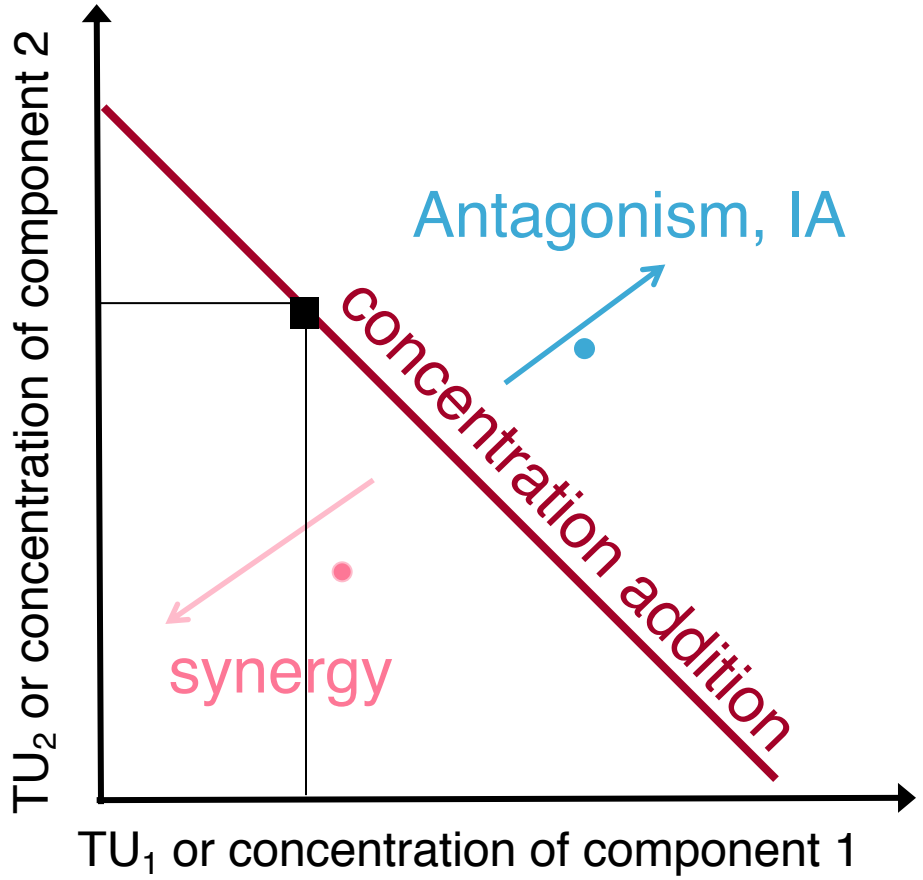
$TU_2 = C_2/EC_{y2}$

→ Isobole Diagramm

Σ TU = 1 concentration addition

Σ TU > 1 IA or Antagonism (less than additivity)

Σ TU < 1 Synergy (more than additivity)



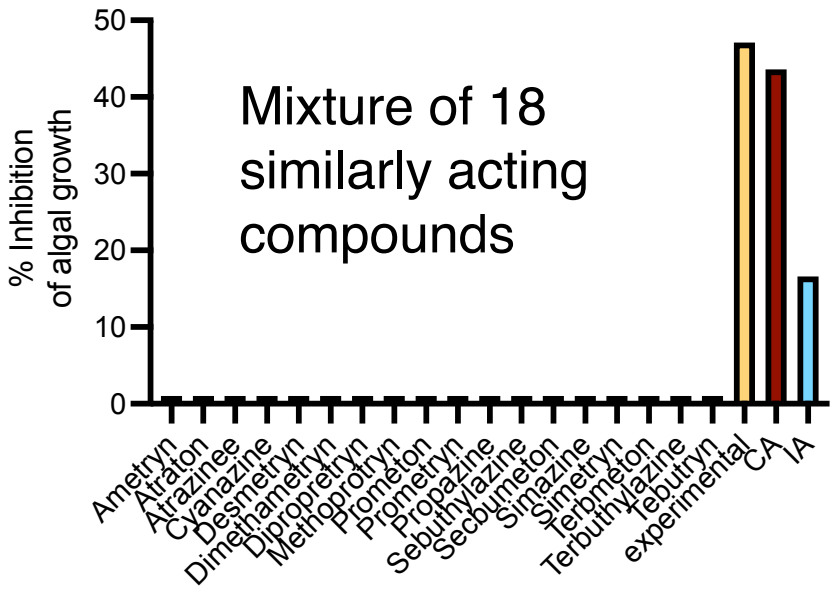
$$\frac{0.3*EC_{y1}}{EC_{y1}} + \frac{0.7*EC_{y2}}{EC_{y2}} = 1$$

Concentration addition vs. independent action

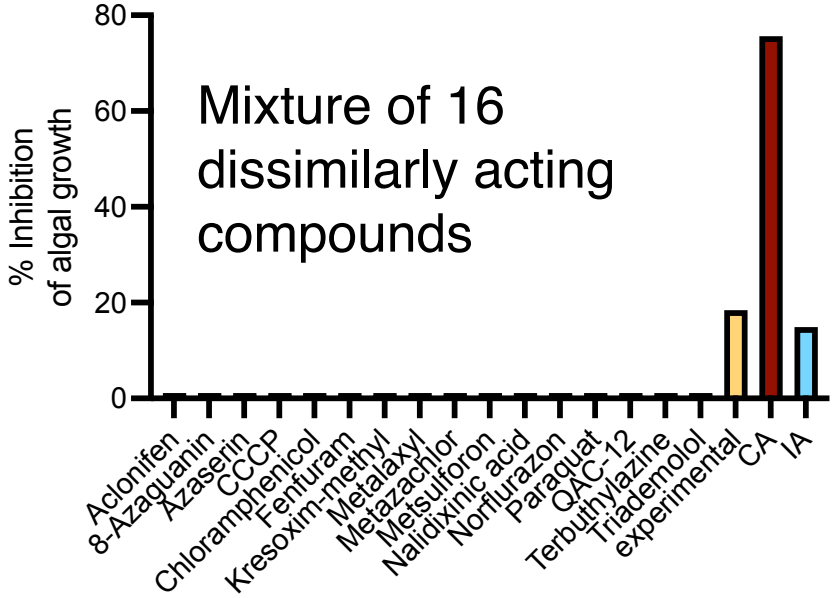
multicomponent mixtures with n components i, each in a fraction (p_i) of the total concentration

$$EC_y(\text{mixture}) = \frac{1}{\sum_{i=1}^{n} \frac{p_i}{ECy_i}}$$

$$\text{Effect(mixture)} = 1 - \prod_{i=1}^{n} (1 - \text{effect(i)})$$



Something from nothing!



Search for synergy

Synergy if low number of components at high concentrations, rare in complex mixtures, deviation max. factor 3-4

Mechanisms of synergy

- Bioavailability
  - pyrithione antifoulants: ZnPT and Cu^{2+} formation of more toxic CuPT complex
- Uptake and excretion
  - Increase of ventilation rate in fish
- internal transportation
- Metabolisation (dominant)
  - Activation/inhibition of metabolic enzymes
- Binding at the target site

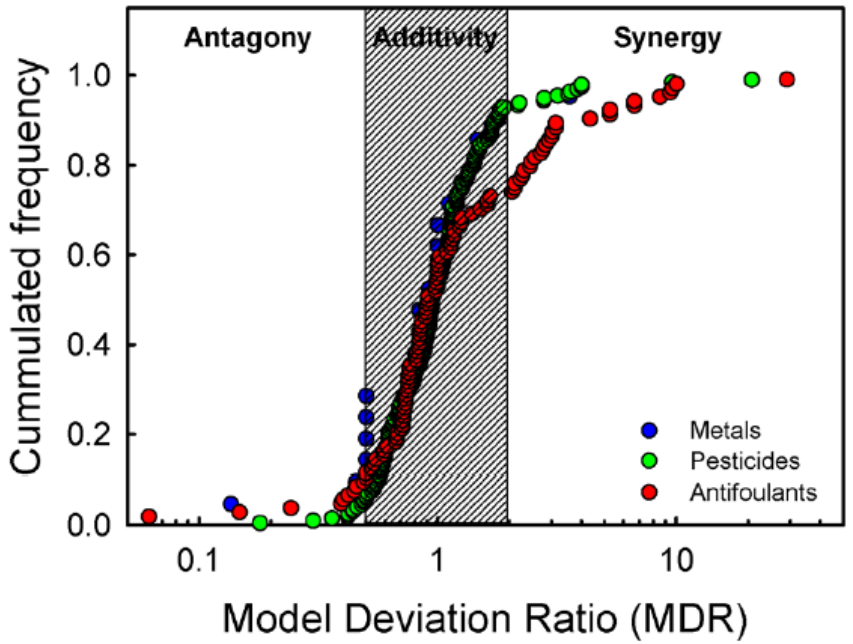
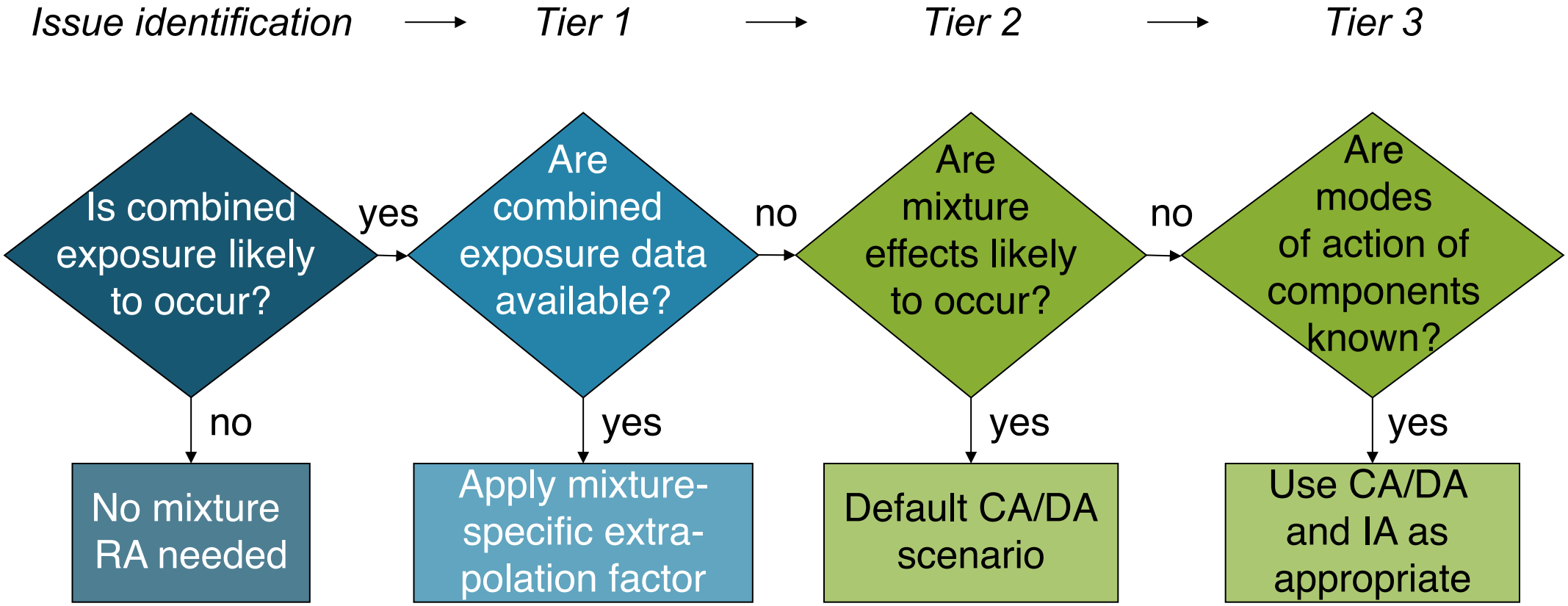


Figure 2. Cummulated frequency of Model Deviation Ratios. Cummulated frequency of Model Deviation Ratios. (MDR) of binary mixtures of pesticides ($n = 195$), metals ($n = 20$), and antifoulants ($n = 103$). The hatched interval where $0.5 \leq MDR \leq 2$ defines the mixtures that deviates less than two-fold from a Concentration Addition predictions. Mixtures having MDR values < 0.5 are termed antagonistic, while mixtures with MDR values > 2 are synergistic.
doi:10.1371/journal.pone.0096580.g002

Mixtures in practise and risk assessment (RA)

The International Programme on Chemical Safety (IPCS) of the WHO has developed a framework for assessing cumulative risk



Toxic equivalency concept

- Method for evaluation of concentration-additive mixtures of compounds with a common mode of action
- Initially developed for studies on receptor binding
  - Dioxins and dioxin-type compounds (reference compound 2,3,7,8-TCDD)
  - later extended to further receptor-mediated mechanisms
- later extended to integral endpoints (EC_x) and assessment endpoint (PNEC)

Toxic equivalent concentrations

$$TEQ = \sum_{i=1}^{n} TEQ_i = \sum_{i=1}^{n} C_i \times TEF_i$$

Toxic equivalent factor

$$TEF_i = \frac{(PN)EC_{ref}}{(PN)EC_i}$$

Similar to REP but consensus value

Application of TEQ to assess environmental risk of mixtures

Toxic equivalent concentrations

$$TEQ = \sum_{i=1}^{n} TEQ_i = \sum_{i=1}^{n} C_i \times TEF_i$$

Risk index = sum of individual risk quotients

Risk quotient of the mixture is the sum of the risk quotients of the single components *i*

Environmental risk assessment

$$RI = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{PEC_i}{PNEC_i} \quad \text{or:} \quad RI = \frac{TEQ}{PNEC_{reference\ chemical}}$$

Human health risk assessment

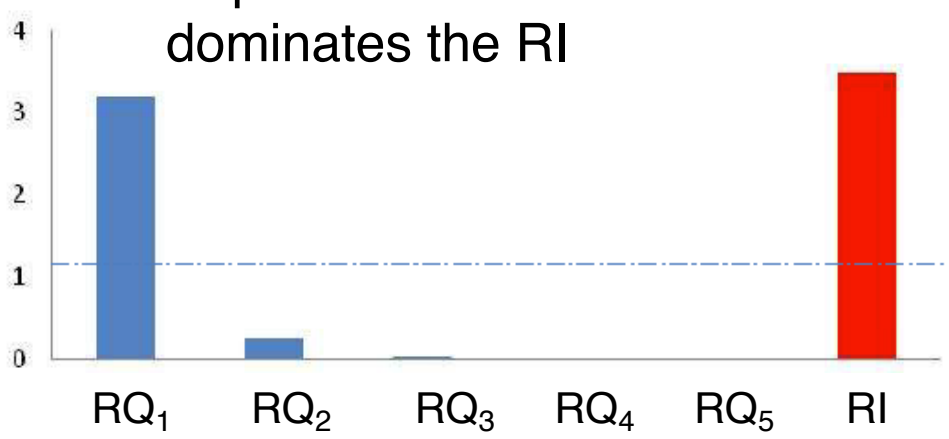
$$RI = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{exposure\ level_i}{DNEL_i} \quad \text{or:} \quad RI = \frac{TEQ}{DNEL_{reference\ chemical}}$$

Is cumulative risk assessment necessary?

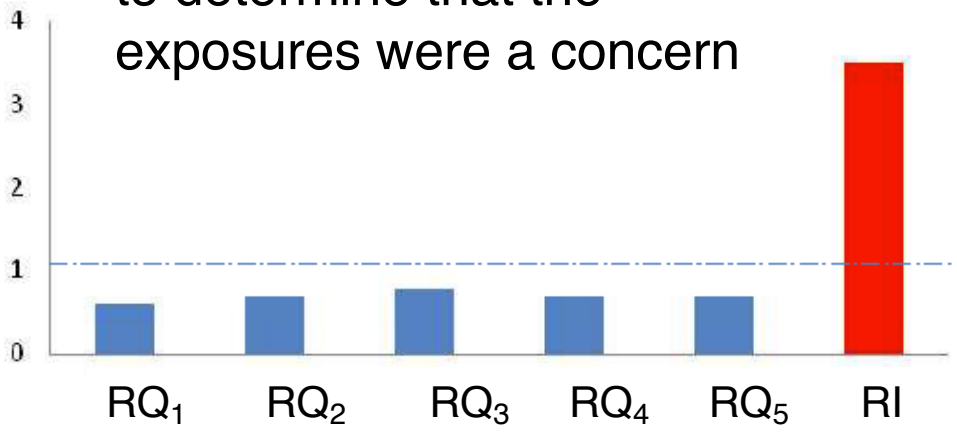
$$RI = \sum_{i=1}^{n} RQ_i$$

- Consider two scenarios, in both the environment is exposed to 5 chemicals
  - Each mixture has a risk index of 3.5 (unacceptable)

Scenario 1: A cumulative assessment was not required. Chemical 1 dominates the RI



Scenario 2: A cumulative assessment was required to determine that the exposures were a concern



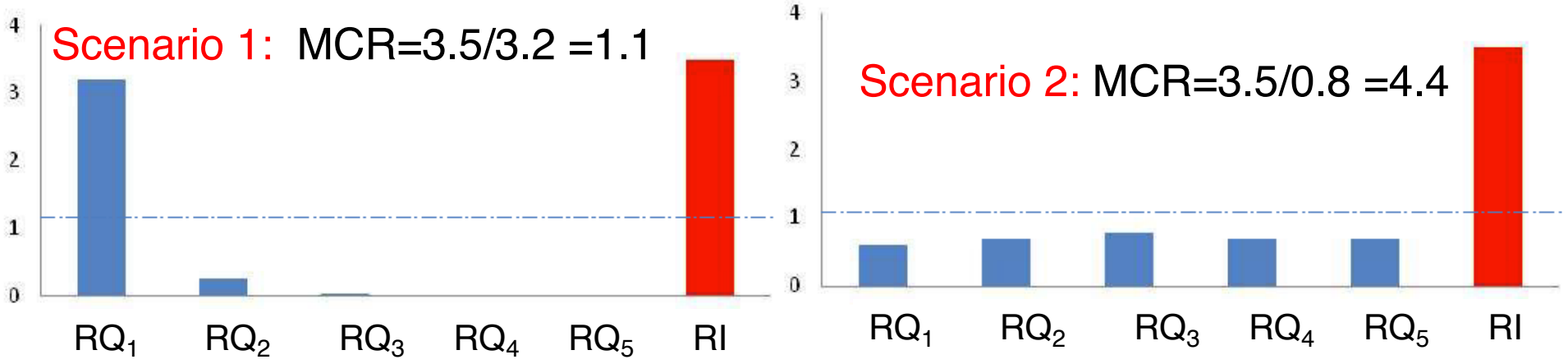
Price, P.S.; Han, X. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. International Journal of Environmental Research and Public Health. 8:2212-2225; 2011

Maximum Cumulative Ratio MCR

- the ratio between the observed cumulative toxicity and the maximum toxicity caused by one chemical

$$MCR = \frac{\text{cumulative toxicity}}{\text{maximum toxicity from one chemical}} = \frac{RI}{RQ_{max}}$$

  - If MCR = 1, mixture toxicity is caused solely by one component ➔ no cumulative RA required
  - If MCR >> 1, mixtures need to be accounted for
  - If all n mixture components contribute equally to the cumulative toxicity, the MCR will reach n ➔ cumulative risk assessment imperative



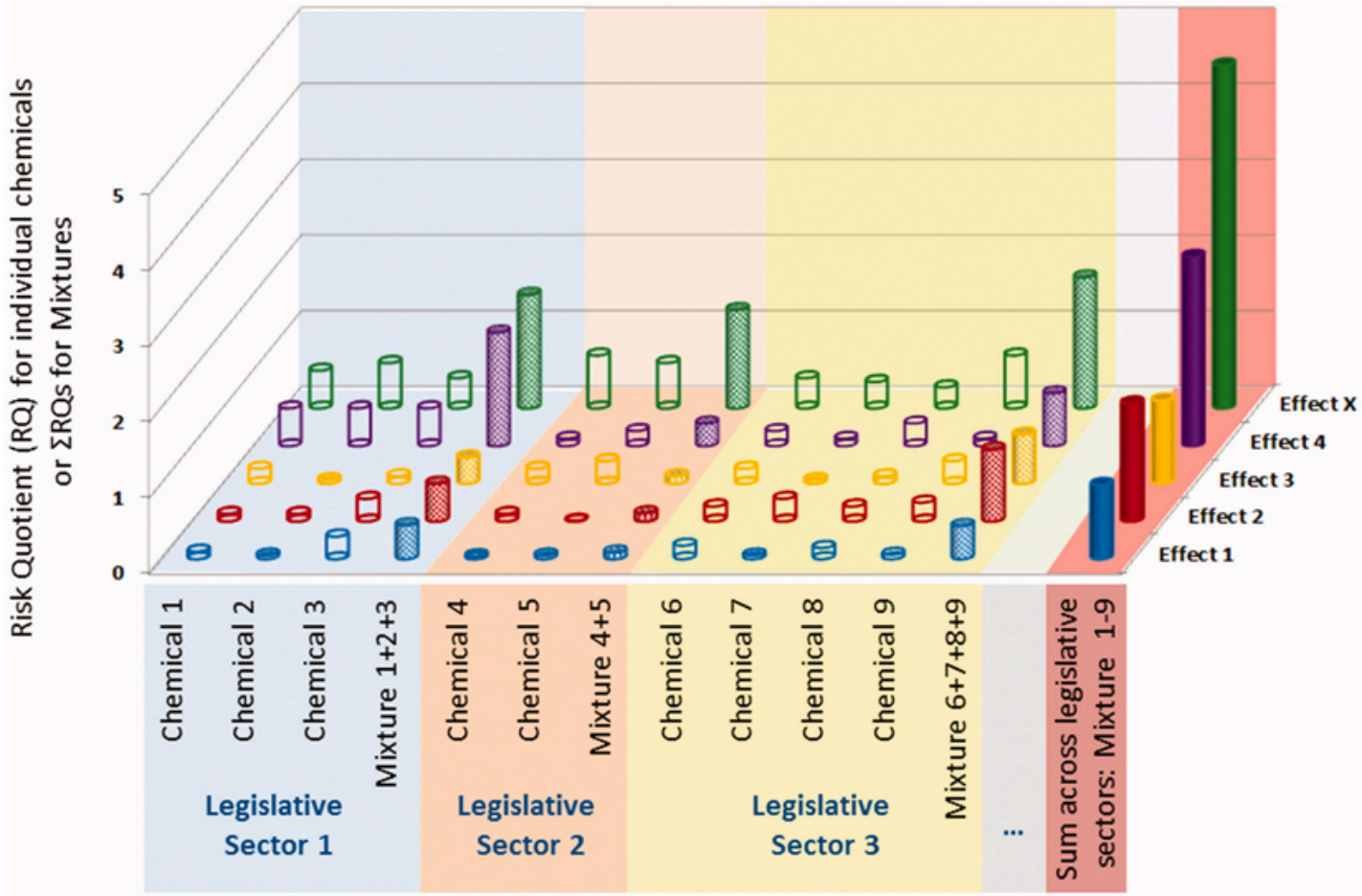
Price, P.S.; Han, X. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. International Journal of Environmental Research and Public Health. 8:2212-2225; 2011

Mixtures in risk assessment

$$RI = \sum_{i=1}^{n} RQ_i$$

Mapping of chemicals and their mixtures to the risks they pose for various toxicological effects. For each nine chemicals the individual risk quotients RQ_i are presented for different types of effect (e.g., hepatotoxicity, neurotoxicity, etc.). Chemicals are grouped according to the legislative sector they are regulated under (e.g., REACH, pesticides, cosmetics, food contaminants, etc.). The risk index RI, i.e., the sum of RQ_i is illustrated for mixtures within each sector and in the last column for the cross-sectorial mixture.

Bopp et al. (2019). Regulatory assessment and risk management of chemical mixtures: challenges and ways forward. Critical Reviews in Toxicology, 49(2): 174-189. BY-NC-ND licence © 2019 European Union.



Mixtures and water quality

~~Mixtures are complex, challenging and unpredictable~~

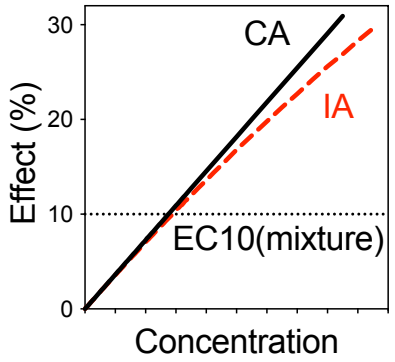
Complex mixtures at low effect level are predictable

Environment

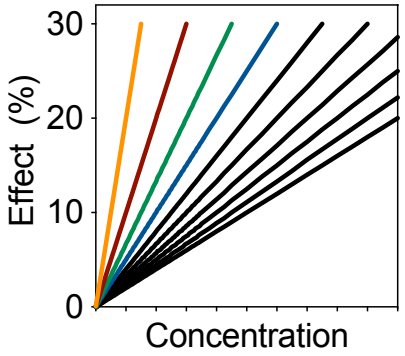
Mixtures
- of thousands of chemicals
- at low concentrations
- low effect levels

Concentration-response curves are linear < 30% effect

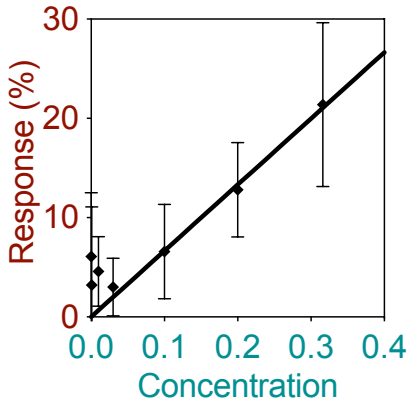
< 10 %
Concentration addition
= independent action



Linear up to 10%

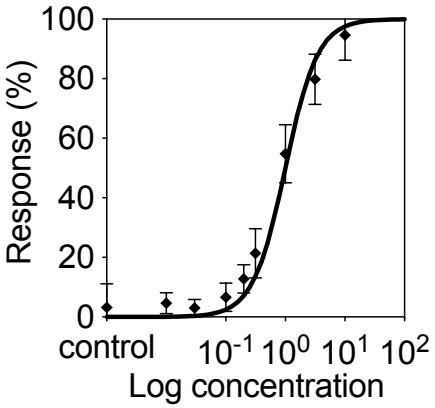


10-component mixture

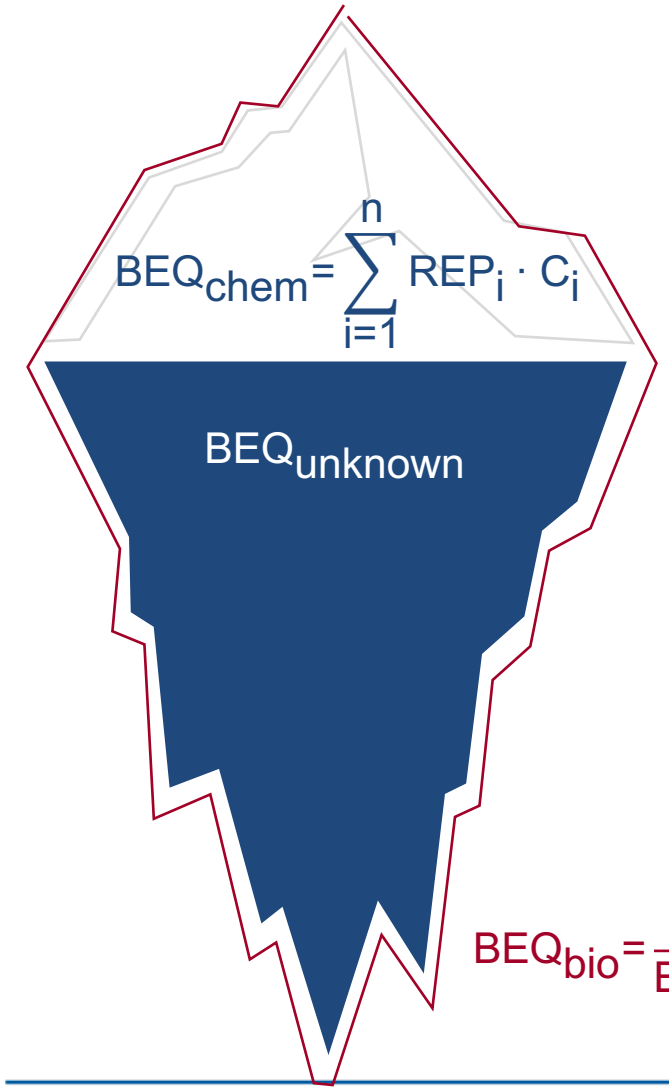


Linear response range up to 30%

Linear dose



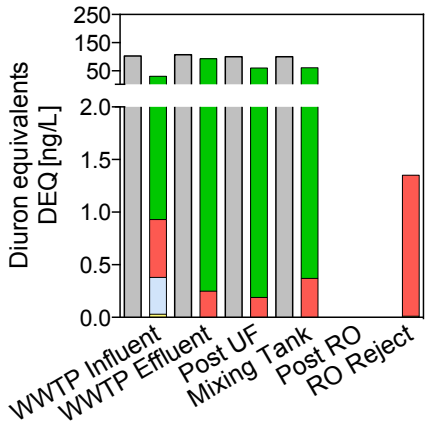
Mixtures and water quality: component-based approach



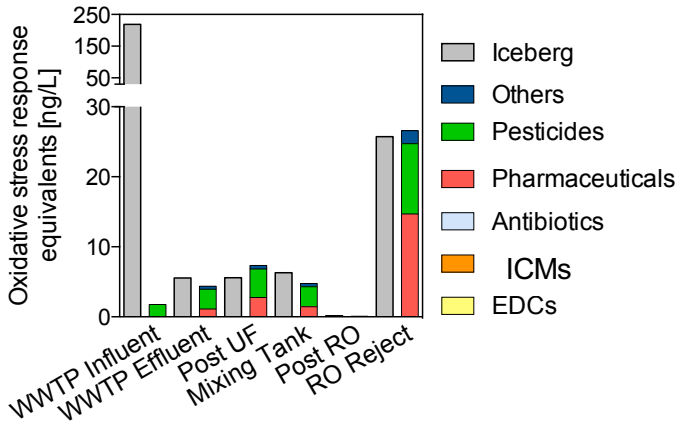
$$REP_i = \frac{EC_y(ref)}{EC_y(i)}$$

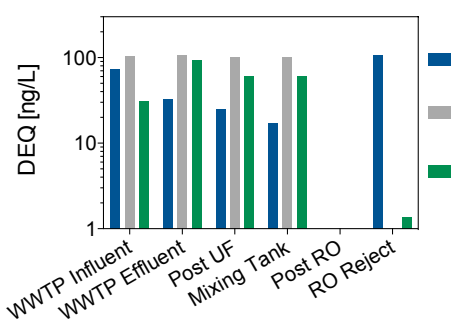
$$BEQ_{bio} = \frac{EC_y\ (ref)}{EC_y\ (sample)}$$

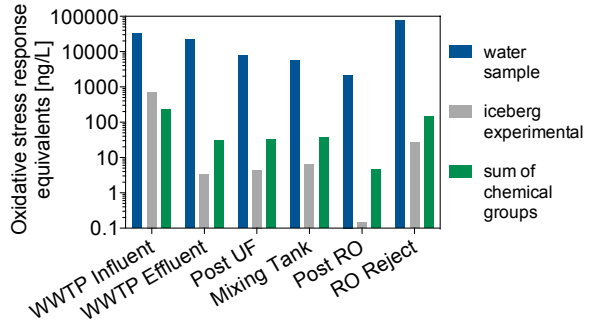
Photosynthesis inhibition (algae)



Oxidative stress response



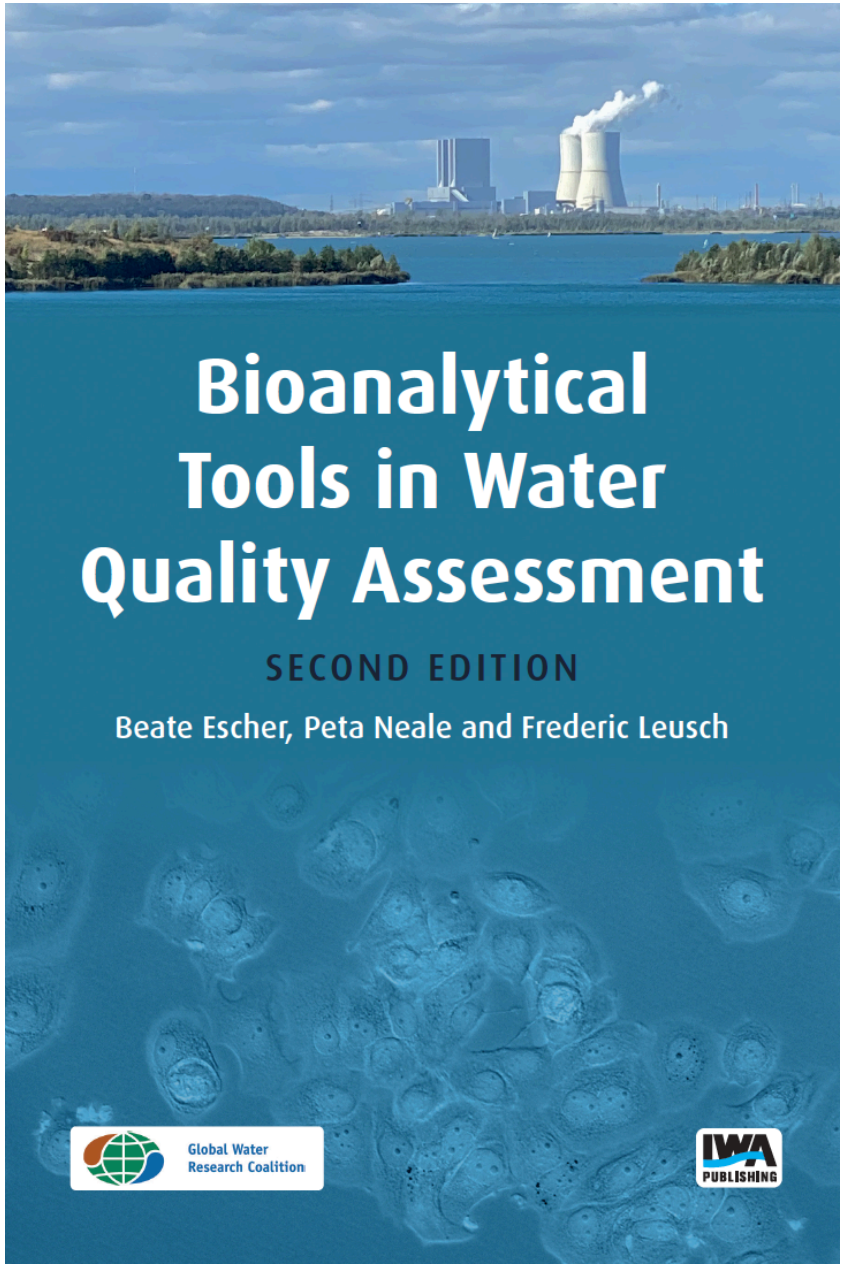




Iceberg mixture explained total effect in water sample

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

Chapter 8
Mixtures



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