

## Objectives

- I Investigation of variability of Acute-to-Chronic ratio(ACR) of mixtures in the combined toxicity
- II Derivation of PNEC of mixtures by the probabilistic approach

### Previous study: Funnel hypothesis

This hypothesis states that as the number of components in mixtures increases there is an increased tendency for the toxicity to be additive. Conversely, as the number of components decreases the tendency is for the toxicity of mixtures to increasingly deviate from additivity.

Warne M.S.J., Hawker D.W.(1995)

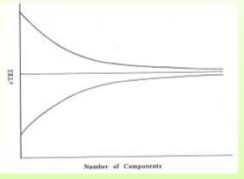


Fig 1. Funnel hypothesis

## I Investigation of variability of ACR of mixture in the combined toxicity

### Mixtures and Methods

**Mixtures:** Virtual mixtures were created randomly.

Number of chemicals: 2,5,10 and 20 components

Composition ratio:

- Uniform
- One component is 90% and the remaining components are uniform

Number of chemicals: Each 1,000 sets

Samples:

- Chemicals with both acute toxicity values(E(L)C50) and chronic toxicity values(NOEC)
- Algae: 443 chemicals Daphnia: 349 chemicals Fish: 71 chemicals

**Model:** Concentration Addition(CA)

**Assumption:** All virtual mixtures are additive.

**Derivation of PNEC<sub>mixture</sub>:**

$$RQ_{PEC/PNEC} = \sum_{i=1}^n \frac{PEC_i}{PNEC_i} \Rightarrow PNEC_{mix} = 1 / \sum_{i=1}^n \frac{P_i}{NOEC_i \times (\frac{1}{UF_f})}$$

**Derivation of ACR<sub>mixture</sub>:**

$$ACR_{mixture} = \frac{E(L)C50_{mixture}}{NOEC_{mixture}} = (1 / \sum_{i=1}^n \frac{P_i}{E(L)C50_i}) / (1 / \sum_{i=1}^n \frac{P_i}{NOEC_i})$$

RQ: Risk Quotient PEC: Predicted Environmental Concentration PNEC: Predicted No Effect Concentration P: Proportion of the components in the mixture UF: Uncertainty factor for the laboratory-to-field extrapolation(10)

## Results

### Composition ratio: Uniform

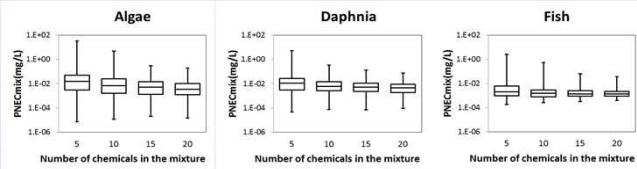
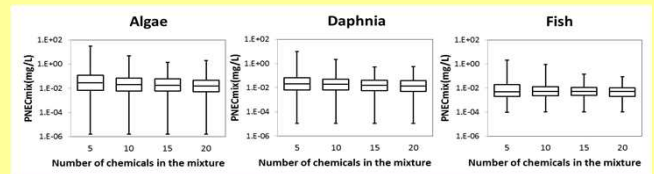


Fig.2 Boxplots of the distribution of PNEC<sub>mixture</sub> for the virtual mixtures.

The boxes, lines dividing the box, and whiskers show the quartile values, median values, and min-max values, respectively.

### Composition ratio: One component is 90%



Variance were not converged

Variance was converged

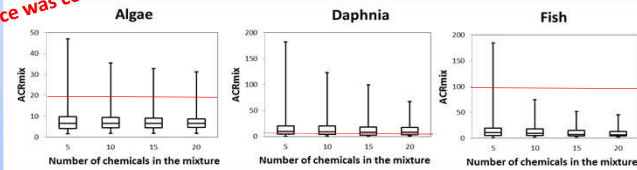
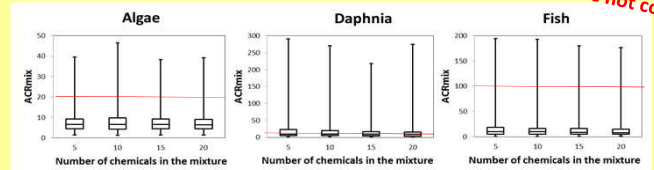


Fig.3 Boxplots of the distribution of ACR<sub>mixture</sub> for the virtual mixtures.

The boxes, lines dividing the box, and whiskers show the quartile values, median values, and min-max values, respectively.



Red lines are ACR of CSCL in Japan

## II Derivation of PNEC of mixtures by the probabilistic approach

### Mixtures and Methods

**Virtual mixtures:** Same as I (Composition ratio is uniform.)

**Derivation of distribution of ACR:**

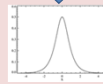
- Calculating the ACR by dividing E(L)C50 by NOEC of samples.
- Deriving the median and the standard deviation assuming a type as logarithmic normal distribution
- Normality was tested by the Kolmogorov-Smirnov test.

**Model:** Concentration Addition(CA)

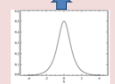
**Assumption:** All virtual mixtures are additive.

**Derivation of PNEC<sub>mixture</sub>:**

$$PNEC_{mixture} = 1 / \sum_{i=1}^n \frac{P_i}{PNEC_i} = 1 / \sum_{i=1}^n \frac{P_i}{E(L)C50_i \times (1 / (ACR \times 10))}$$



10,000 times



**Comparison of PNEC<sub>mixture</sub>:**

PNEC<sub>mixture</sub> 50%ile, 10%ile, 5%ile, 1%ile VS I Fig.2 PNEC<sub>mixture</sub> Derivation of the percentage of underestimation to the 1,000 mixtures

## Results

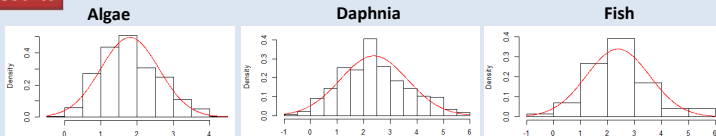


Fig 4. Distribution of acute to chronic ratio (logACR)

TABLE1 Mean and SD of distribution of acute to chronic ratio of chemicals

	Algae	Daphnia	Fish
Type of Distribution	Log-normal	Log-normal	Log-normal
Mean of logACR	1.80	2.38	2.39
SD of logACR	0.80	1.27	1.18

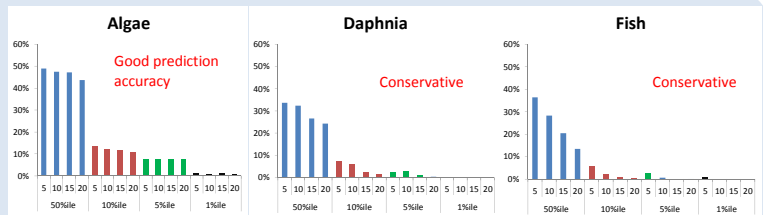


Fig 5. Percentage of underestimation of the PNEC using the variable ACR factor

## Conclusions

- If it is assumed to be additive, as the components in the mixture increased, the variance of the ACR of the same ratio mixture was converged. On the other hand, the variance of the ACR of the main component 90% mixtures was not converged.
- PNEC<sub>mixture</sub> could be derived by the probabilistic approach. Algae was a good prediction accuracy. Daphnia and fish were conservative.
- When used in combination with the uncertainty of interspecies extrapolation, the variability of the assessment factor would be greater. We consider the probabilistic approach to the assessment of the combined toxicity is effective.