A thermodynamically-based step by step calculation method to assess aquatic toxicity of mixtures.

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Replacing Experimentation

Introduction

In the aquatic environment, chemicals are always mixtures. However the action of chemical mixtures is not often understood since complex molecular interactions are involved. Backhaus *et al.* (2003) reproposed that the toxicity of chemical mixtures could be anticipated if they follow the principle of Concentration Addition (CA) or Independent Action (IA), a concept first hypothised by Loewe and Muischnek (1926). In the first case chemicals would be expected to exert the same mode of toxic action. Provided this assumption is valid, the toxicity of the mixture should be equal to the sum of the toxic units of the constituents as follows:

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1$$

c_i is the concentration of the constituent i within the mixture,
ECx_i is the effective concentration of the constituent i (i.e. needed to lead to x% of effect).

This methodology appears to be scientifically justified even if it has not passed into the global legislation on chemicals or for classification of mixtures in Europe. However we observed this assumption does not hold true at least for **Natural Complex Substances (NCS),** mixtures composed of hydrophobic organic liquids. This work emphasises the need to consider further parameters to predict the aquatic toxicity of NCS.

1. Application of CA principle to NCS

Case study: The acute toxicity of the NCS, CYPRESS OIL, has been experimentally assessed on *Daphnia magna* following the OECD 202 guideline and using the Water Accommodated Fraction methodology.
The concentrations of seven main constituents (covering 95% of the mixture) have been analytically followed during the study for several loading rates. Thereby the sum of toxic units can be calculated for each loading rate (Table 1).
According to the CA principle, the 48h-EL50 is expected slightly below 1.5 mg/L as highlighted in Table 1 and extrapolated to be even below the CLP threshold of 1 mg/L.

The lab test result was an effective loading rate (48h-EL50) of **3.0 mg/L.** However at this loading rate, the sum of toxic units is around 2 (based on the % immobilisation). Thus the use of CA principle would overestimate the toxicity of this mixture.

Loading rate of the NCS (mg/L)	% of effect	Sum of toxic units
1.5	0	1.16
1.9	0	1.63
2.4	0	1.63
3.1	75	2.05
3.9	100	2.51
5.0	100	3.17

Table 1: Toxicity results (as % of daphnid immobility) for increasing loading rate of TERPINOLENE MULTICONSITUENT and the analysis of the sum of toxic units.

2. Reduction of bioavailability

We assumed that the high hydrophobic moiety of the NCS constituents may explain the observed reduction of the toxicity. The hypothesis is that constituents are able to partition between themselves thus forming a pseudo-hydrophobic phase smaller than micro-emulsions but sizable enough in quantity to reduce their bioavailability to aquatic organisms (Figure 1) and not readily separable from the WAF analytically. There is a need to take into account this fraction.

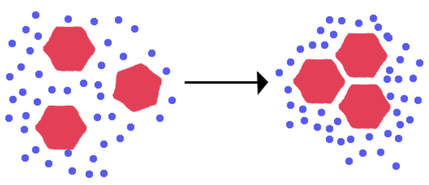

Figure 1: Hydrophobic interactions tend to reduce bioavailability of mixture constituents like terpene. Once gathered the total surface of hydrophobic chemicals is reduced.

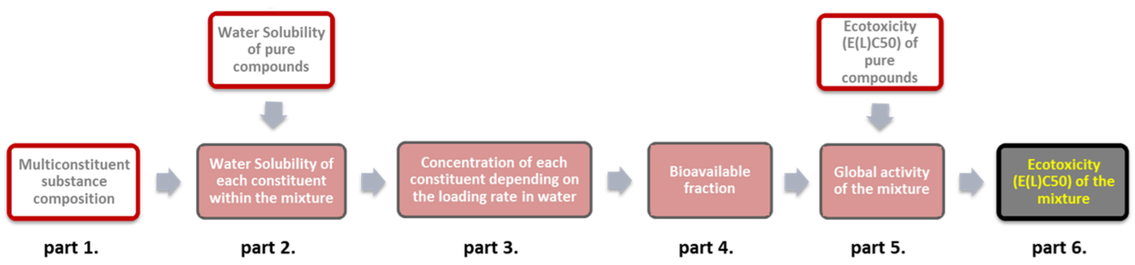

Figure2: Flow-chart of the iSafeRat® calculation method to assess mixture ecotoxicity.

Intermediate conclusions

1. CA alone is not enough to accurately predict aquatic toxicity of NCS,
2. CA leads to an antagonistic effect between the different constituents for mixtures like NCS.

The second interpretation can be excluded because we observed CA systematically overestimates toxicity for another dozen NCS tested regardless of their composition. Moreover, all the constituents of NCS act via non-polar narcosis (except a small percentage of ester compounds, no specific or reactive compounds were identified within these NCS).

3. Calculation method to predict NCS toxicity

We developed a calculation method (Figure 2) which takes into account a correction factor to consider the bioavailability issue. The principle of this method is detailed in the poster of Bicherel *et al.,* 2014. With this new methodology we managed to accurately anticipate the aquatic acute toxicity to *Daphnia magna* of the NCS, CYPRESS OIL.
Using iSafeRat® calculation we predicted a **48h-EL50 at 3.0 mg/L** consistent with **experimental result of 3.0 mg/L** whereas the CA principle suggests a higher toxicity with a **48-EL50 around 1 mg/L**, and **CLP methodology at 0.48 mg/L.**

Moreover we demonstrated this method could be applied to dozen other cases of NCS where the CA principle ailed to accurately determine the aquatic toxicity value (Bicherel *et al.*, 2014).

Conclusions

Hydrophobic moiety of NCS constituents make CA principle insufficient to anticipate aquatic toxicity correctly. Therefore a correction factor is needed to take into account the non-bioavailable fraction as part of the WAF of NCS.
Combining the additivity principle and the non-bioavailable fraction, acute aquatic toxicity can be accurately determined.

References

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