Chemical substance solubilities in mixtures: not so simple

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Introduction

Mixtures and mixture toxicity is of current environmental concern as actual aqueous concentrations of each constituent and therefore the toxicity of the mixture cannot be readily predicted. Today, the current OECD 23 Guideline[1] advocates use of the water-accommodated fraction (WAF) method (i.e. testing the constituents together at a specific loading concentration) in order to determine toxicity for multi-component substances. In the absence of experimental data it is possible to use an additivity approach to predict toxicity as described in the CLP Regulation[2]. However, the EC_{50}s from WAF experimental values and those determined by the CLP additivity method have shown large differences (1 to 2 magnitude orders). **The aim of this poster is to present recent work on *in silico* methods to predict aqueous concentrations of complex mixtures and their subsequent toxicity to aquatic organisms which may ultimately allow us to replace both WAF and CLP methods.**

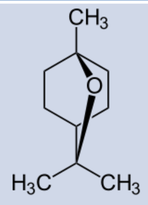
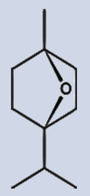
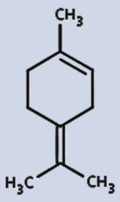
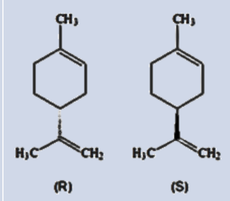
Materials and Methods

For a specific mixture with four constituents, water solubility and Daphnid acute EC_{50} values of the individual constituents are provided from our own QSAR[3]. Some solubility values are obtained from studies following improved slow-stir method of OECD 105 Guideline (Thomas and Burosse, 2012)[4].

The mixture composition is for a hypothetical compound in which all constituents are present at the same mass fraction (25%).

We used an algorithm based on the thermodynamic activity (i.e. the effective concentration for non-ideal solution) to calculate water solubility of each constituent in mixture.

Table 1: Constituents within mixture together respective structures, water solubilities, EC_{50} and mass fractions.

Name	Cineol 1,8	Cineol 1,4	Terpinolene	Limonenes
Chemical structure				
Water solubility (mg/L)	>2000	ca. 466	ca. 7	ca. 4
Daphnid Acute EC_{50} (mg/L)	29,85	9,65	0,53	0,36
Mass fraction in mixture (%)	25	25	25	25

Results and Discussion

For a hypothetical mixture in which the relative concentration of all components (mass fraction) is equal, the following results were determined. The graph below presents mass fraction (in percent) for individual constituents within a mixture at a series of aqueous concentrations (1, 10 and 100 mg/L mixture in water). The calculated toxicity following the additivity approach in the CLP Guidance has been employed to classify this mixture at different WAF concentrations and is outlined in Table 2.

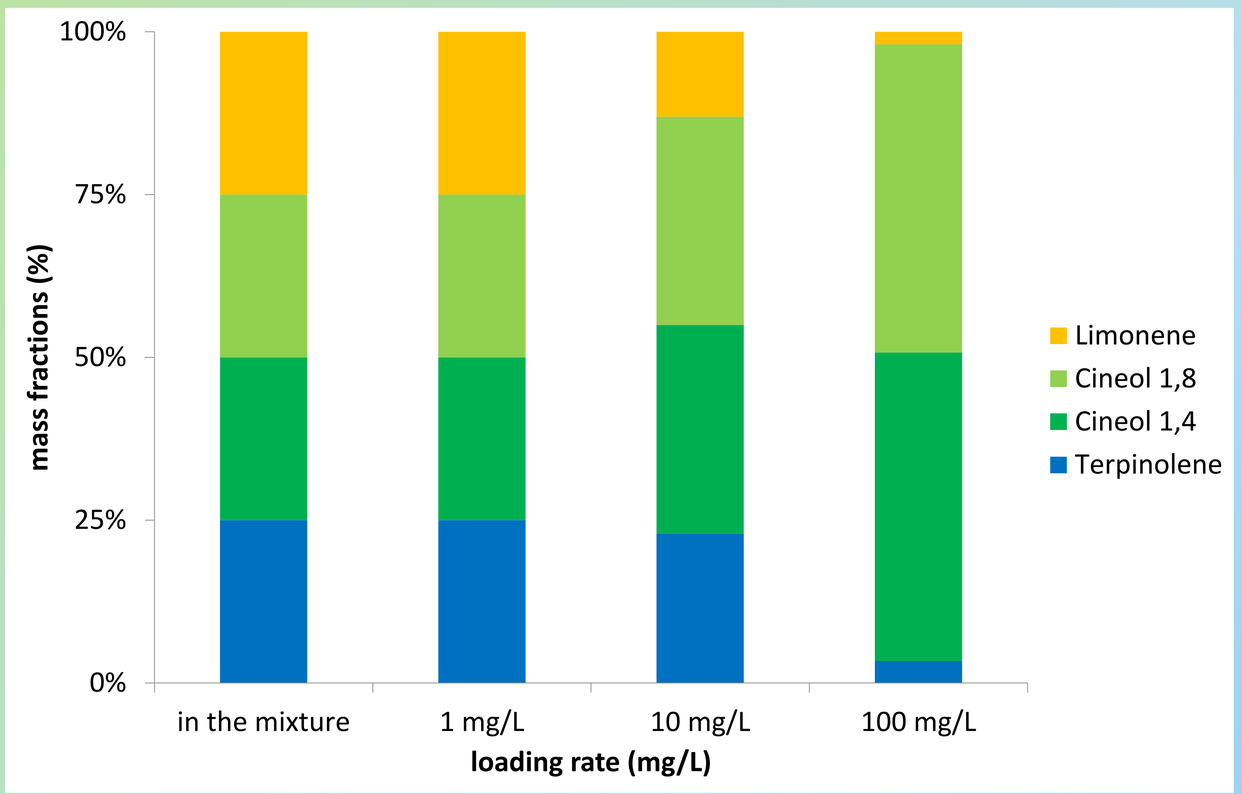


Figure 1: iSAFERAT®[5] prediction of mass fraction at a series of WAF loading rates.

Table 2: Limonene and Cineol 1,8 concentrations and CLP calculation of toxicity at a series of WAF loading rates.

Loading rate	1 mg/L	10 mg/L	100 mg/L
Limonene concentration (mg/L)	0.25	1.02	1.02
Cineol 1,8 concentration (mg/L)	0.25	2.50	25.00
EC_{50} mixture acute daphnid (mg/L)	0.84	1.19	5.48
Aquatic Acute Classification of the mixture	**Acute 1**	**No Acute C&L**	**No Acute C&L**

Within the mixture, the concentration of Cineol 1,8 is not limited by its solubility at any aqueous concentration as illustrated in Figure 1. However, the concentration of Limonene (or Terpinolene) depends not only upon its solubility but also on the maximum aqueous concentration it can attain within the specific mixture. So the higher the loading rate, the lower the proportion of the most toxic substances (i.e. Limonene and Terpinolene) in the aqueous solution. Therefore, according to the CLP additivity method based on percent of each constituent in mixture, the higher the loading rate, the less the EC_{50} fraction contributes to the toxicity of the mixture (Table 2). This is of course in contradiction with the reality. With a 1 mg/L loading rate, the mixture is thus classified Aquatic Acute 1 while at 10 mg/L (or *a fortiori* 100 mg/L), the mixture is not classified as an acute hazard by the CLP Regulation.

Conclusions

- These results confirm **the constituents of a mixture tend to suppress each other's solubility** (Banerjee, 1984)[6]. Thus, the aqueous solubility of the constituents in a mixture is less than the pure substance.
- **Substance solubility in mixtures depends on constituent mass fraction** in the mixture and interactions between substances. Consequently, the concentration of a particular constituent in the mixture cannot be easily measured.
- **The CLP prediction may be wrong**: in certain cases, a toxic fraction may be predicted to be present by CLP but in reality toxic concentration in a toxicity test may never be reached as its maximum mass fraction is limited by other constituents in the mixture.
- **The CLP additivity method is not adapted for WAFs with higher loading rates** because we obtain a paradoxical result: the higher the loading rate, the less the mixture toxicity.

For multi-component substances, the CLP calculation should be refined by normalizing with loading rate.

Alternative method: Today, it is possible to calculate the true concentration of individual constituents within a known mixture using *in silico* methods. One of the aims of iSAFERAT® is to determine the toxicity of individual constituent and using the activity based algorithm, accurately calculate a WAF based toxicity without recourse to animal experimentation.

References

[1]OECD guideline 23 [2000] Guidance document on aquatic toxicity testing of difficult substances and mixtures
[2]Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures
[3]CEHTRA internal tool 2013
[4]Thomas and Burosse, Improved OECD 105 Water solubility test design, presented at 6th SETAC World Congress, Berlin, 20-24 May 2012.
[5]iSAFERAT® Version 1.1 in Silico Algorythm For Environmental Risk Assessment And Toxicity
[6]Banerjee, S. Solubility of organic mixtures in water. *Environ. Sci. Technol.* 1984, 18, 587-591

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