A new model to perform high accuracy calculations of aquatic toxicity for mixtures.

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Replacing Experimentation

Introduction

Toxicity of multi-constituent substances can be experimentally assessed following the OECD Guidance 23[1] describing adaptations of the standard methods to test water-accommodated fractions (WAFs), where the different constituents of a mixture are tested together at a specific loading rate. The problem with the method is that, unlike studies on single substances, it is difficult to validate the results based on measured concentrations. There is a need for a recognized calculation method to determine the acute ecotoxicity of mixture using the values of E(L)C50 of each constituent anticipating the analytical variability of each loading rate based on thermodynamic principles. One such method is a calculation proposed by the CLP Regulation[2]. However this method is too conservative and can lead to misclassification of the substance. The iSafeRat®[3] method is a mechanistically derived, accurate calculation for WAF toxicity and its validation is described below.

Materials and Methods

The acute aquatic toxicity of multi-constituent MoA 1 (non-polar narcotics) substances (in liquid and solid state at ambient temperature) can be assessed by a multi-step calculation method (Figure 1). The first step (part 1) is to determine the composition of the mixture as accurate as possible (>90%). The water solubility of each constituent must be known or estimated. In an aqueous mixture the water solubility of each constituent is not the same as that of the pure constituents (part 2). Complex and significant modifications in solubility can occur because of molecular interactions between the constituents in the mixture. Therefore the aqueous solubility of each individual constituent within the mixture can be determined using a phase-equilibrium thermodynamic approach (De Hemptinne *et al.*, 1998)[4]. This provides values for the maximum solubility level of each constituent that can be achieved at their saturation point for that specific mixture. Then the concentrations of each constituents can be calculated depending a predetermined loading rate (part 3).

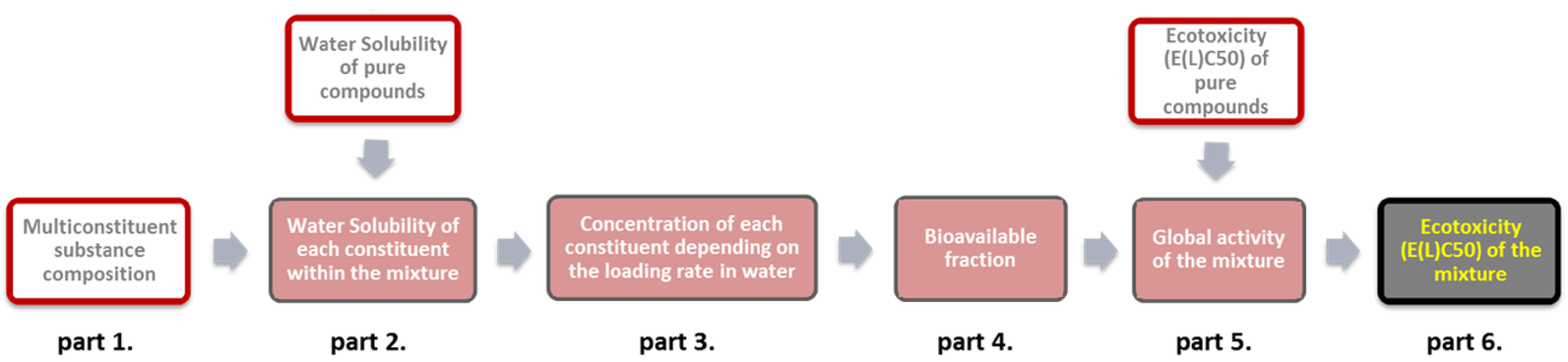

Figure 1: Flow-chart of the iSafeRat® calculation method to assess mixture ecotoxicity.

The next step (part 4) involves removing the non-bioavailable fraction from the calculation.

From this stage each constituent concentration is converted from concentration (mol/L) into activity (unitless) and the activities of each constituent are added up (part 5). The loading rate is adjusted to reach the global Ea50 of the mixture (i.e. the mean weighted-molar fraction of the activity of each constituent) (part 6).

This calculation method has been tested and validated for the three ecological trophic levels (fish, daphnid and algae) using a set of ten Natural Complex Substances (NCS), which are MoA 1 multiconsituent substances, tested following OECD Guideline 23 methods under GLP with extensive analysis of individual constituents (Table 1).

For comparative purposes, the method for determining mixture toxicity for Classification & Labelling as set down by CLP was used to determine the E(L)C50 for the same mixtures and the same species. Details on the calculation method can be found in the CLP text (Guidance on the Application of the CLP Criteria, 2013) but the overall calculation recommended is as follows:

$$\frac{\sum C_i}{E(L)C_{50m}} = \sum_n \frac{C_i}{E(L)C_{50i}}$$

C_i = concentration of component I (weight percentage)
$E(L)C50_i$ = (mg/l) LC50 or EC50 for component i
n = number of components
$E(L)C50_m$ = $E(L)C50$ of the part of the mixture with test data

Results and Discussion

Table 1: Experimental acute ecotoxicity of NCS comparing the iSafeRat® calculation method and CLP calculation method. [a – b] 95% confidence intervals.

endpoint	mixtures (NCS/UVCB)	Experimental (mg/L)	iSafeRat® v1.1 (mg/L)	CLP (mg/L)
96h-LL50 WAF fish	A1	42 [32 - 56]	33.6	4.11
	A2	18 [10 - 32]	10.2	1.22
	A3	6.104 [5.500 - 6.817]	6.6	1.21
	A4	5.07 [3.10 - 10.0]	6.0	0.88
48h-EL50 WAF daphnid	B1	5.184 [4.664 - 5.766]	2.9	0.81
	B2	2.7 [2.1 - 3.4]	3.0	0.76
	B3	3.004 [2.4 – 3.9]	3.0	0.48
72h-ErL50 WAF algae	C1	6.864 [n.d. – n.d.]	3.5	0.89
	C2	4.779 [4.599 – 4.957]	4.3	0.81
	C3	9.129* [6.871 – 14.778]	6.5	0.50

Ecotoxicity values for the NCS have been determined using the iSafeRat® calculation method as explained above (see also Platform presentation by Thomas *et al*. (ET07) for more details on the calculation method). The predictions systematically fall within a factor of two for results from all 10 WAF studies. This figure is akin to the level of repeatability reasonably expected from two GLP laboratory studies. The Mean Absolute Error is 21 %.

Based on this validation study, the iSafeRat® calculation may be considered to be a valid method for the estimation of acute WAF test results for MoA 1 multiconstituent substances (and can even be used to validate analytical results from laboratory WAF studies) and is considered acceptable for risk assessment and C&L purposes.

On the other hand, the CLP calculation method consistently overestimates the toxicity of these NCS by at least a factor of 3 and in certain cases, by over an order of magnitude, wrongly leading to the classification of at least seven of them.

While it is recognized that the CLP calculation has been designed to be conservative, systematic over-classification of mixtures will lead to unnecessary expense to industry for containment and handling of these substances and potentially even wrongly influence consumer choice decisions.

Conclusions

The CLP calculation is too conservative by at least a factor of 3 and up to an order of magnitude in some cases (leading to the misclassification of 7 out 10 substances in this case) when compared to high quality experimental values from ten non-polar narcotic multiconstituent mixtures.
The iSafeRat® calculation is extremely accurate in all cases, predicting experimental results within a factor of 2 with no misclassifications. The calculation may be considered an acceptable alternative to the CLP calculation for MoA 1 multiconstituent substances and for use in risk assessment.

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]iSafeRat® – in Silico Algorithms For Environmental Risk And Toxicity version 1.1
[4]De Hemptinne JC, Delepine H, Jose C, Jose J (1998) Aqueous Solubility of Hydrocarbon Mixtures. *Oil & Gas Science and Technology – Rev. IFP* 53 (4), 409-419.

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