Ecotoxicity Testing of UVCBs

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INTRODUCTION

Testing of UVCBs poses a regulatory challenge and especially so when the accent is on the "U" for Unknown as well as "V" for Variable as is often the case for botanical extractions. Certain categories of **Natural Complex Substances (NCS2) present multiple complexities for regulatory dossier submission**:

- Only a small fraction of the mixture has been identified and may contain hundreds of constituents;
- Constituent concentrations may vary according to batch and quality;
- They may have high viscosity, high volatility, low solubility and/or hydrophobicity;
- They may be toxic, but it is unknown which constituents are responsible.

The **CHANCES2 project** started in October 2020 and aims to **provide an experimental methodology supported by *in silico* modelling to test, mechanistically explain and predict aquatic toxicity of NCS2**. We sought the optimal strategy between ecotoxicity laboratory testing using chemical analysis and *in silico* modelling laboratory results.

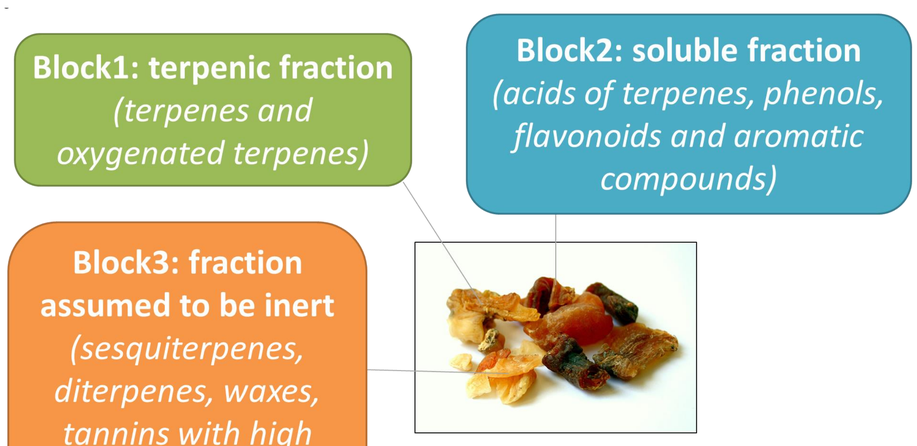


Figure 1: Decomposition of a NCS2 into 3 blocks.

QUESTION

Can we mechanistically understand, determine and predict the toxicity of a natural complex mixture that is <50% analysable?

HYPOTHESES

1) **Fraction 1** (fully known constituents) toxicity precisely **modelled *in silico*** using the iSafeRat® WAF model;
2) **Fraction 2 c**ritical constituents can be **analytically elucidated**;
3) **Fraction 2 toxicity** to daphnids and algae can be **measured** and then **modelled**;
4) **Fraction 3** is expected to be **inert** (= not to be toxic in a chronic algae & daphnid limit test);
5) The results of the analytical and toxicity results of these **three fractions** can be summed (using an in silico chemical activity approach) to obtain the same toxicity result as that found for the whole NCS2 substance.

METHODS

Substances are tested whole and are split into three fractions.
The blocks are defined as follows:

- Terpenic/volatile: high % of profile analysable
- Soluble/non-volatile: moderate % analysable
- Inert: negligible % analysable

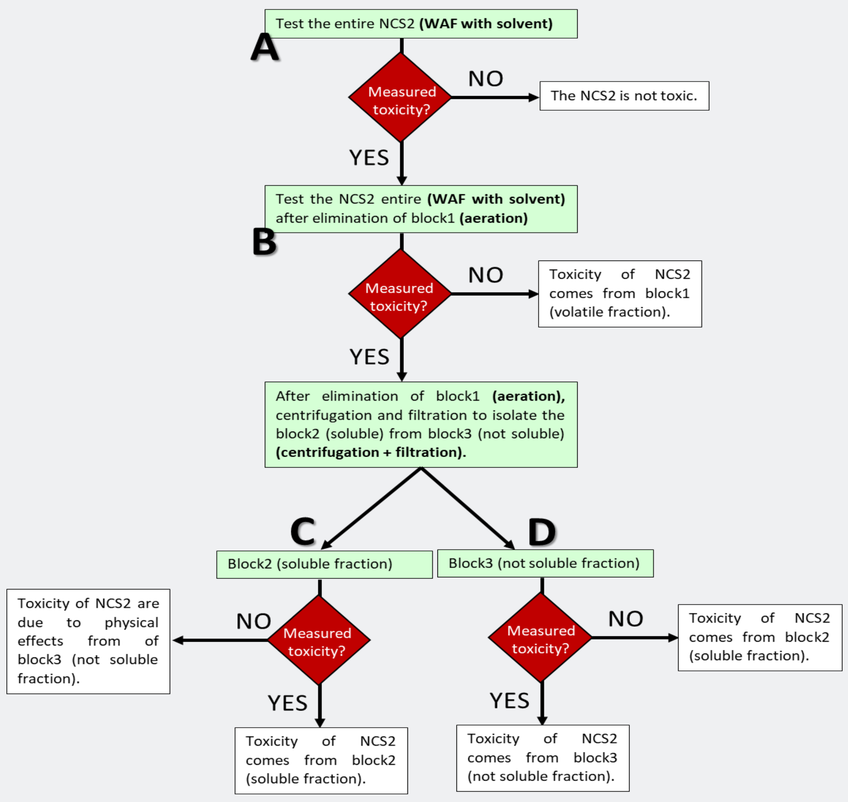
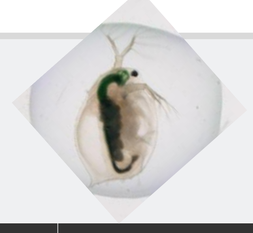


Figure 2: Scheme outlining the testing methodology used to determine relative toxicity of whole substance and the 3 fractions.

RESULTS



	In vivo (mg/L)		*In silico* prediction (mg/L)	
	Algae	Daphnids	Algae	Daphnids
ACUTE studies				
Volatile fraction:	6.3	[1;10]	8.5	7.6
Non-volatile bioavailable fraction:	>80	>80	105	85
"inert" fraction:	>100	>80	-	-
Whole substance	51	[10;25]	33	26
CHRONIC studies				
Volatile fraction:	[1;3.2]	-	2.9	0.027*
Non-volatile bioavailable fraction:	[10;80]	*not yet*	29	1.5**
"inert" fraction:	>100	≥10	-	-
Whole substance	[1;10]	*NYC*	9.6	0.11*/**

Majority of **acute** ecotoxicity of the whole substance attributed to **β-pinene**

Inert fraction not toxic

*if long-term toxicity of **germacrene B** (or sesquiterpenes) to daphnids is confirmed to be around 1-5 µg/L
if long-term toxicity of **galbanic acid to daphnids is confirmed to be 20-30 µg/L; NYC: not yet conducted

- NCS2 successfully separated into three fractions, analysed and toxicity tested
- Majority of volatile substance = 1 terpene (β-pinene) & monoterpene and sesquiterpene derivatives
- Majority of non-volatile bioavailable substances = sesquiterpene coumarin derivatives

- Acute toxicity of Galbanum resinoid mostly explained by **β-pinene**
- **Galbanic acid & Farnesiferol** not indicators of acute toxicity in the NCS2
- The **inert fraction** did not exhibit ecotoxicity in chronic studies on daphnids and algae
- The *in silico* model accurately modelled the laboratory ecotoxicity results

CONCLUSION

Despite the constraints of testing partially known UVCB substances, application of a fractionation approach in combination with an *in silico* model for mixture toxicity testing reduces uncertainty in aquatic hazard testing.

The 2 approaches are highly complementary: 1/ the laboratory tests provide empirical values of acute and chronic toxicity for each fraction and the whole substance and validate the outcome of the *in silico* model; 2/ the *in silico* model provides a mechanistic explanation of the observed toxicity of the whole substance via the components and the model can be used to predict toxicity of different constituent blends. We are awaiting results of the chronic daphnid test on the whole substance to confirm the hypothesis for the *in silico* approach

Part 2 of the project is ongoing to demonstrate reproducibility of the method on a second NCS2.

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