Ecotoxicity Testing of UVCBs

Paul Thomas[1], Floriane Larras[1], Pascal Bicherel[1], Nicolas Delpit[2], Lise Mata[2], Mélanie Persillon[2], Marina Canton[3], Marina Thierry[3], Sylvain Antoniotti[3], Aurelia Lapczynski[4]

[1] KREATiS, 38080 L'Isle d'Abeau – France
[2] Laboratoires des Pyrénées et des Landes, 64150 Lagor, France
[3] Université Côte d'Azur, 06103 Nice Cedex 2
[4] RIFM, Woodcliff Lake NJ, USA

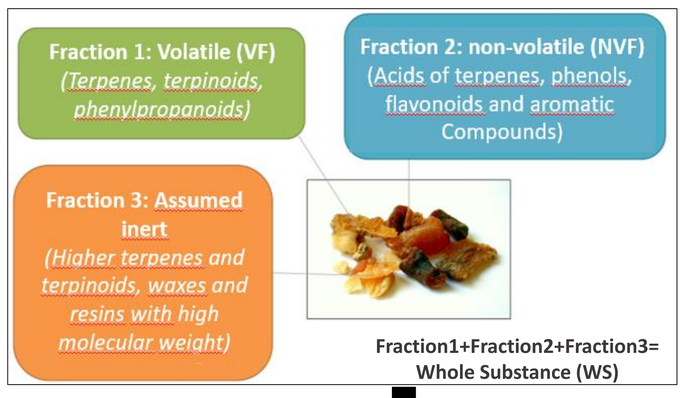
Contact us :
www.kreatis.eu / floriane.larras@kreatis.eu

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 extracts. 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.



QUESTION

Can we characterise, mechanistically understand and finally predict the ecotoxicity *in silico* of natural complex mixtures that are <50% analysable (and include 100s of constituents and resinous compounds)?

HYPOTHESES

The resinoid can be separated into 3 fractions then reconstituted and compared to the whole substance:

1) **Fraction 1 (VF)** known constituents: toxicity precisely **modelled *in silico*** using the iSafeRat® WAF model;
2) **Fraction 2 (NVF)** **toxicity** to organisms can be **measured** and then **modelled** (= not non-polar narcotics);
3) **Fraction 3 (inert)** is expected to be **inert** (= not to be toxic in a chronic algae & chronic daphnid limit test);
4) The results of the analytical and toxicity tests of these **three fractions** can be summed (using an *in silico* chemical activity approach) to obtain the same toxicity result as found for the whole substance.

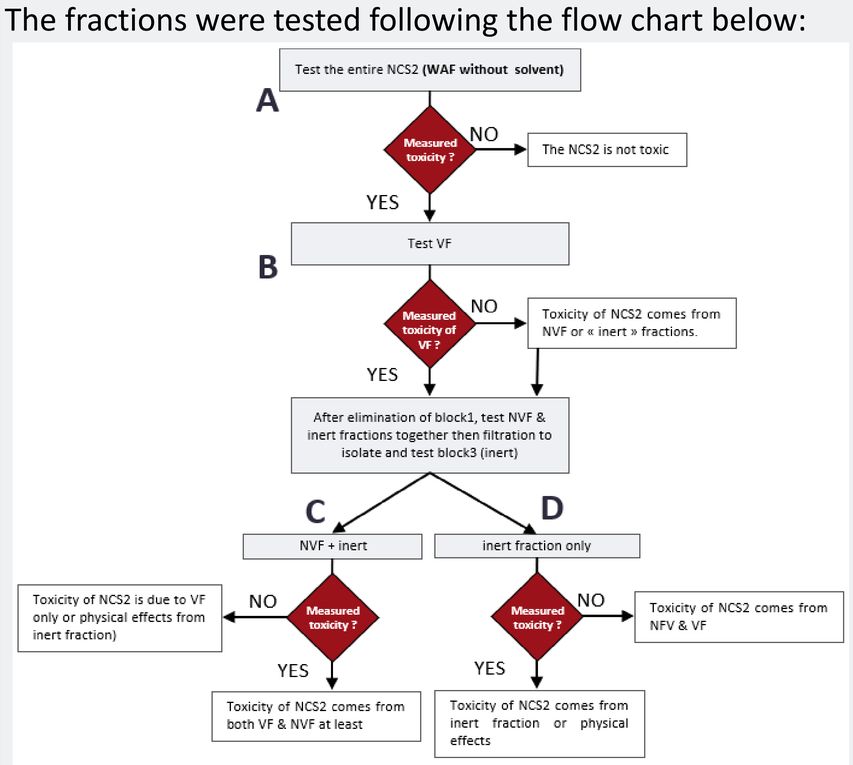
METHODS

1/ CHEMICAL ANALYSIS

Substances are tested whole and are split into three fractions as designated above and extensively analysed.

2/ TOXICITY TESTING

The fractions were tested following the flow chart below:



Each fraction was tested, applying the **Water Accommodated Fraction (WAF)** approach to:

- OECD 201 tests (algal 72h-EC50 and NOEC)
- OECD 202 test (daphnids 48h-EC50)
- OECD 211 test (daphnids 21d-EC10)

3/ TOXICITY MODELLING & PREDICTION

Four different approaches were tested, based on expected and adjusted fraction composition, and using WAF *in silico* approach for fractions with similar mechanisms of action (MechoA) like VF or additivity for diverging MechoAs within the same fraction, like NVF.

Predictions 1 and 3 assume that NVF et WS toxicity can be explained using non-polar narcotic MechoA, uniquely.

Predictions 2 and 4 assume that NVF et WS toxicity can be explained using additivity of different MechoAs.

Fractions	Prediction 1*	Prediction 2*	Prediction 3**	Prediction 4**
VF	WAF	WAF	WAF	WAF
NVF	WAF	Additivity	WAF	Additivity
WS	WAF	Additivity	WAF	Additivity

** According to expected composition of each fraction*

*** According to analytical monitoring during experiments*

RESULTS

1/ CHEMICAL CHARACTERISATION

NCS2 successfully separated into three fractions, analysed and toxicity tested

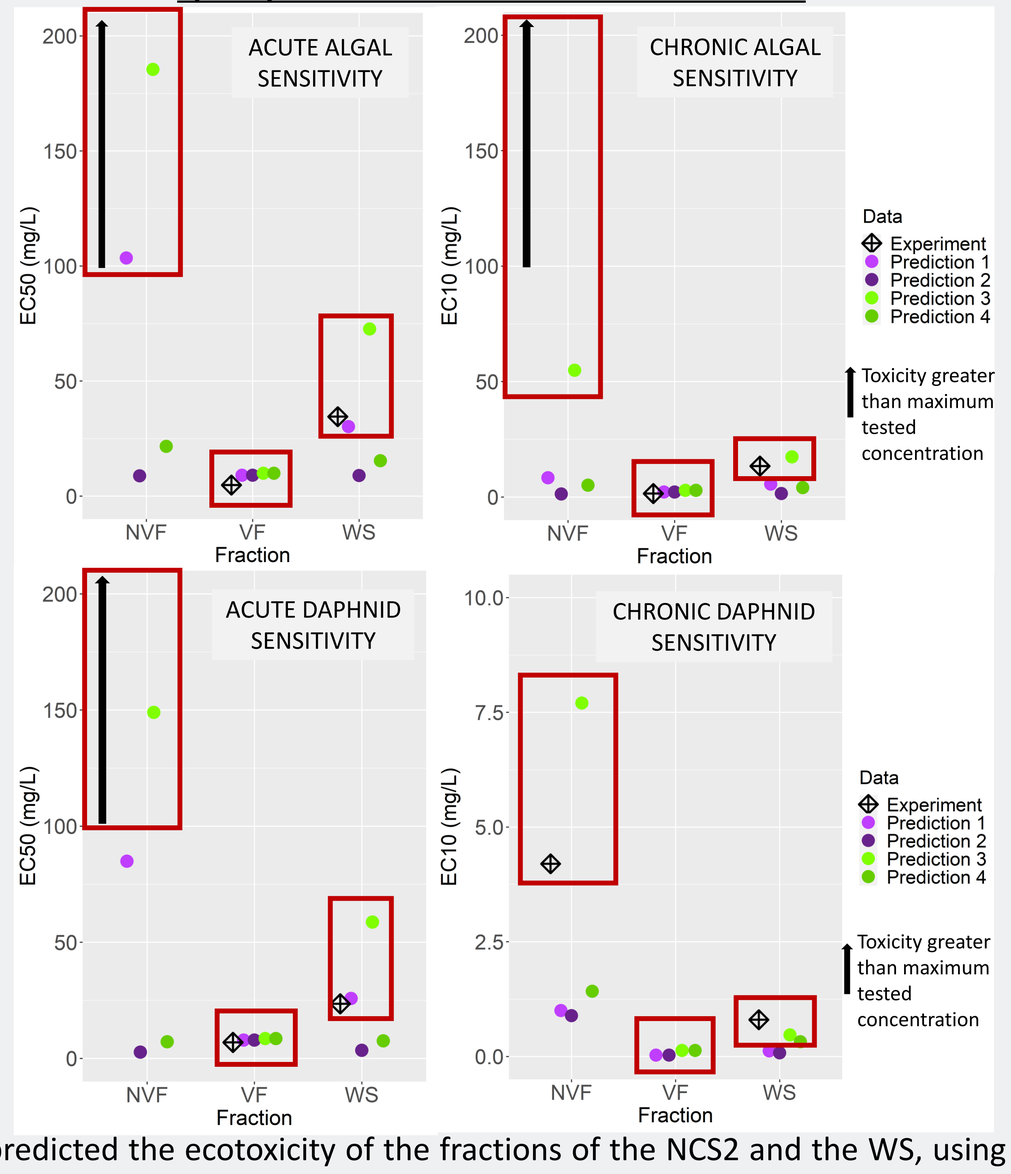
➢ **Volatile fraction** (VF)
- 34.5% of the whole substance (WS)
- 80% of the VF identified
- **sesquiterpenes** & **monoterpenes**
- Non-polar narcotic compounds

➢ **Non-Volatile Fraction** (NVF)
- 65.5% of the WS
- 25% of the NVF identified
- 5 major constituents identified as acids of terpenes and alcohols
- Acids and reactive substances

➢ **Inert Fraction**

Not analysed: no ecotoxicity observed in chronic studies on daphnids and algae

2/ & 3/ TOXICITY TESTING AND PREDICTION



➢ The WAF *in silico* model accurately predicted the ecotoxicity of the fractions of the NCS2 and the WS, using non-polar narcosis MechoA (Prediction 1).

➢ In experiments, analysis revealed significant differences between expected and observed concentrations (e.g., beta-pinene in WS, loss of germacrene-B, galbanic acid and farnesiferol A in NVF) -> inclusion of actual measured concentrations into the model enhanced prediction accuracy to within a factor of 2 of the experimental values using the MechoA 1.1 WAF *in silico* method (Prediction 3 vs. experimental in red boxes).

➢ Coupling the WAF MechoA 1.1 with other MechoAs in an additivity approach (Predictions 2 & 4) also resulted in solid predictions of toxicity especially in chronic studies but less precise than Prediction 3.

CONCLUSION

The 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 WS and validate the outcome of the *in silico* model; 2/ the *in silico* model provides a mechanistic explanation of the observed toxicity. The model can be used to predict toxicity of different constituent blends.

A key result was the accurate prediction of the chronic daphnid study which could not have been anticipated from the experimental acute daphnid study results.

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