Contrasting, Characterizing and Predicting the Toxicity and Risk of Natural Complex Substances

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INTRODUCTION

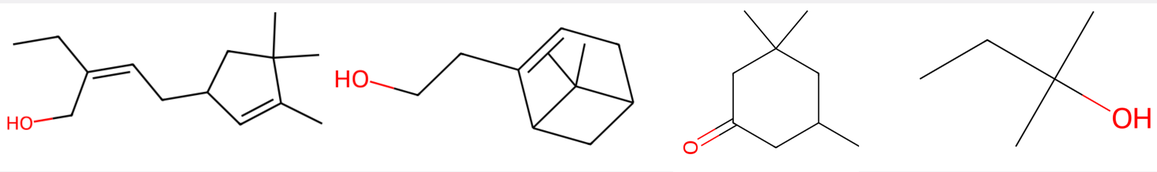
Testing of Unknown or Variable Composition, Complex Reaction Products or Biological Materials (UVCB) poses a regulatory challenge and classical Water Accommodated Fraction (WAF) tests are often criticized as uninterpretable for risk assessment purposes. **Natural Complex Substances** (NCS1) are characterized at >90% while NCS2 are UVCBs of which often <50% of the composition is known. NCS of all kinds are generally considered difficult to risk assess as Predicted Environmental Concentration (PEC) and Predicted No Effect Concentration (PNEC) between compartments cannot easily be calculated. Generally, it is considered that an **additivity approach** to toxicity can be used to assess aquatic toxicity of NCS. **But this assumption is not valid for non-polar narcotic substances, and notably for NCS1&2** (even though some of these contain non-narcotics). Based on two projects combining toxicity testing of complex mixture and toxicity predictive approaches, this presentation will discuss the potential of *in silico* approaches to predict the toxicity of NCS1&2 as well as to perform a meaningful environmental risk assessment.

SUBSTANCES

Artificial Mixture: risk assessment

Can WAF *in silico* approach derive PECs and PNECs for multi-constituents ?

- **Artificial equimolar mixture of 4 constituents** with logP values ranging from 1 to 4, increasing by one log unit from logP =1
- Constituents stable and easily monitored in water
- All constituents are non-polar narcotics compounds



Sanderol	**Nopol**	**3,3,5-trimethylcyclohexan-1-one**	**2-methylbutan-2-ol**
Log P: 4	logP : 3	(logP: 2)	logP: 1

Natural Complex Substance 2: hazard assessment

Can WAF *in silico* approach accurately predict NIbanum resinoid, *Ferula galbaniflua* Boiss.) acute and chronic ecotoxicity ?

NCS2 fragmented in 3 fractions

1) Volatile fraction (34%):
18 substances > 1%
(sesqui and mono-terpenes: all baseline toxicity)

2) Non-volatile and bio-available fraction (32%):
6 substances > 1% (e.g. galbanic acid, fernesiferol A, coladonin: 2 mechanisms of toxic action)

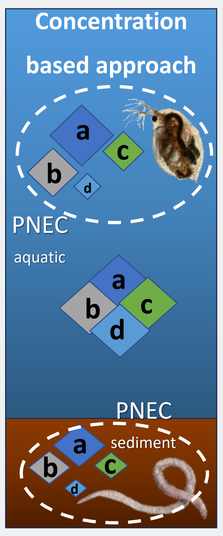
3) Non-volatile and non-bioavailable fraction (34%):
No identification of peaks was undertaken, all bioavailable parts of the substance having been leached out by extensive washing

EXPERIMENTAL SETTINGS

Equilibrium Partitioning (EP) theory from ECHA ERA

Pelagic tests were fully compliant with OECD 201, 202, 211 and 225 Guidelines. Daphnid acute and chronic studies were rerun after mixing with sediment (to indicate pore water concentration) and led to higher ECx values as more toxic hydrophobic components had adsorbed to the sediment organic matter.

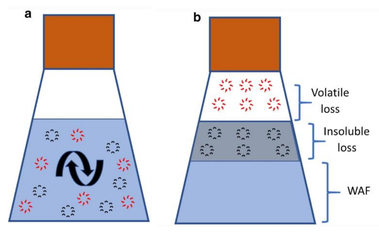
The PEC fraction in water and sediment was measured based on the relative fraction of each constituent within the mixture, which was, as expected, very different from the original mixture (due to the diverse logP values of the constituents).



OECD Guideline 202 (Immobilization)
OECD Guideline 211 (Reproduction)
Daphnia magna

OECD Guideline 225 (reproduction/biomass)
Lumbriculus variegatus

WATER ACCOMMODATED FRACTION APPROACH



Modified from Wheeler et al., 2020

WAF
Bio-available and dissolved fraction used for ecotoxicological testing

Acute toxicity to daphnids and algae was observed only for the volatile fraction although this was not surprising as the major component of this fraction, **beta pinene**, is known to be toxic even though the toxicity for this substance in an NCS mixture is lower than that when the beta pinene is tested pure.

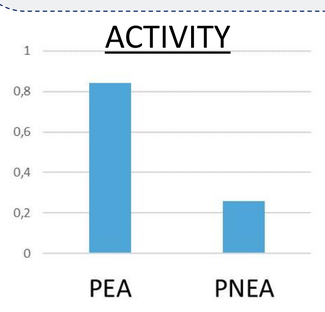
Chronic toxicity to **algae was observed for the volatile fraction** and **chronic toxicity of non-volatile and bioavailable fraction was observed for daphnids**.

No toxicity was observed for the "inert" fraction although it was tested complete with particles of undissolved material in the WAF. Not physical effects were found despite the risk that ingestion of the resinoid particle may have reduced the potential for adequate nutrition of the adults.

IN SILICO PREDICTION

The *in silico* methodologies comprising a chemical activity based method accurately predicted the acute and chronic ecotoxicity results of the pelagic fraction of the CS. The *in silico* method modified to include the EP calculation was used to predict the sediment toxicity. Strong similarities were found with the laboratory test although testing mixtures in laboratory sediment studies are technically too complicated.

The PEC fractions were estimated **using the chemical activity method** and the PEC/PNEC ratio characterised as activities (**PEA/PNEA**) allowing risk assessment to be performed in water and sediment compartments.



$$RCR = \frac{PEA}{PNEA}$$

Fundamentally no change from standard PEC/PNEC methodology

RCR > 1 in this example

CHEMICAL ACTIVITY APPROACH

Unifies the potential toxicity and exposure of constituent s within a mixture.

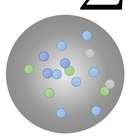
Activity of a compou<nd *i*

$$a_i = x_i \times \gamma_i$$

x_i: molar fraction of compound i
γ_i: activity coefficient of compound i

Activity of a mixture

$$a_m = \sum a_i$$



Mix of a set of chemicals in different proportions = different activities

2 main strategies were tested to predict the ecotoxicity of the whole substance:

1/ the *in silico* WAF method for each fraction and the whole substance

2/ using a combination of the *in silico* WAF method and the additivity approach when different mechanisms of action were identified.

For the volatile fraction, all constituents were non-polar narcotics, and all predictions were similar and within a factor 2 of the experimental data.

Non-volatile fraction: Predicted daphnids and algal acute ecotoxicity was overestimated because analytical monitoring demonstrated a strong loss of some compounds during ecotoxicological testing. Concentration adjustment *in silico* solved this issue. The additivity approach with different mechanisms of action led to a less accurate result with or without analytical result correction.

The *in silico* WAF predictions were very close to lab testing results for the whole substance and especially so when based on the measured composition for the chronic toxicity.

CONCLUSION

The WAF *in silico* modelling approach is relevant to predict the acute and the chronic aquatic ecotoxicity and risk of mixtures. The Artificial Mixture project demonstrated that environmental risk assessment of mixtures in multiple compartments is possible using a chemical activity approach.

The NCS2 project illustrated that fractionation and characterisation of the whole substance provides meaningful insight of the ecotoxicity mechanism of complex substances and could be amenable to risk assessment of the characterizable fractions as above, the inert fraction being considered in the same way as the organic fraction of sediment (i.e. not essential to be characterised to provide a meaningful result).

Modelling and experimental approaches are complementary. Experiments validate the in silico and the in silico mechanistically explains the experimental. The reproducibility of the approach in other NCS2 is currently undergoing.

Thomas, P.C., Larras, F., Bicherel, P., Delpit, N., Antoniotti, S., Thierry, M., Lapczynski, A. (2023) Compartmentalised Hazard Assessment for Natural Complex Extracted Substances of type 2 (CHANCES2). Internal report.

[Bicherel, P., and Thomas, P.C. (2021). Aquatic Toxicity Calculation of Mixtures: A Chemical Activity Approach Incorporating a Bioavailability Reduction Concept. Environ. Sci. Technol. 55, 11183–11191

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