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Mechanistic *in silico* NAMs: An overview of iSafeRat® ecotoxicity QSARs

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Introduction

The Green Deal incentivises the chemical industry to transition towards a more safe and sustainable portfolio. NAMs are an essential element of this paradigm.
Unlike *in vitro* NAMs, *in silico* NAMs directly predict the endpoint that their training set is based upon. Mechanistic QSARs (i.e. quantitative relationships between molecular initiating events of specific chemical moieties and effects on biological organisms) can accurately substitute for experimental studies when designed and created correctly. After 10 years research, the iSafeRat® platform boasts over 40 aquatic ecotoxicity high accuracy mechanistic QSARs (HA-QSAR) covering acute and chronic toxicity to algae, invertebrate and fish.
This poster explores our vision of how to construct reliable and regulatory compliant Mechanistic QSARs. But first we must define "Mechanistic" in the context of *in silico* (eco)toxicology and then explore how we can use it to quantify study outcomes.

Concept of our mechanistic approach

As *in silico* expert-knowledge-based models, mechanistic QSARs are linked to two keystones: on one side structural features and on the other quantified biological matrix interactions via a mechanism of toxic action (MechoA) scheme[1,2] which are akin to Molecular Initiating Events (MIE) only more complete as narcotic ("baseline") effects are also included. The inclusion of a mechanistic interpretation which is underlined by the principles of (bio)chemistry, increases confidence in the outcome by providing a scientific justification for the predicted toxicity. MechoA are inextricably linked to two keystones: on one side, **structural features** and on the other, **quantifiable biological matrix interactions**. Unlike read-across methods, more than one structural group can have the same mechanism of action (thus, the same (eco)toxicological impact). The primary toxophore within a structural group defines the MechoA, but not all the structures in a MechoA will have the same (or even any) toxicity. The iSafeRat® platform uses MechoA Premium[2] to identify an appropriate QSAR to predict toxicity.

MechoA

MechoA SAR (Figure 1) is used in both ecotoxicity and human health modelling: Mechanistic insight (in the form of Structural Alerts or SAR) is a necessary first step in our process to produce a reliable QSAR with a well-defined applicability domain encompassing chemicals via their shared MIE. It prioritises the key to Identified MechoA sub-classes, provides an understanding of the interactions between the test substance and the biological matrix and explains observed toxicity (as extracted from the literature and incorporated in the model via >160 structural alerts). It links toxicity to structurally similar moieties (or toxiphores) within a structure that will impact the biota at different trophic levels. MechoAs may be different for different organisms (e.g., a substance which is a neurotoxic to fish may be a "baseline" toxicant to algae). Any endpoint can ultimately be related to a MechoA, by combining with a QSAR related to the endpoint: species, apical effect of interest, timeframe of the study and compartment of concern (e.g. 35d EC10 chronic toxicity to fish following OECD 210 Guideline) for a quantitative prediction.

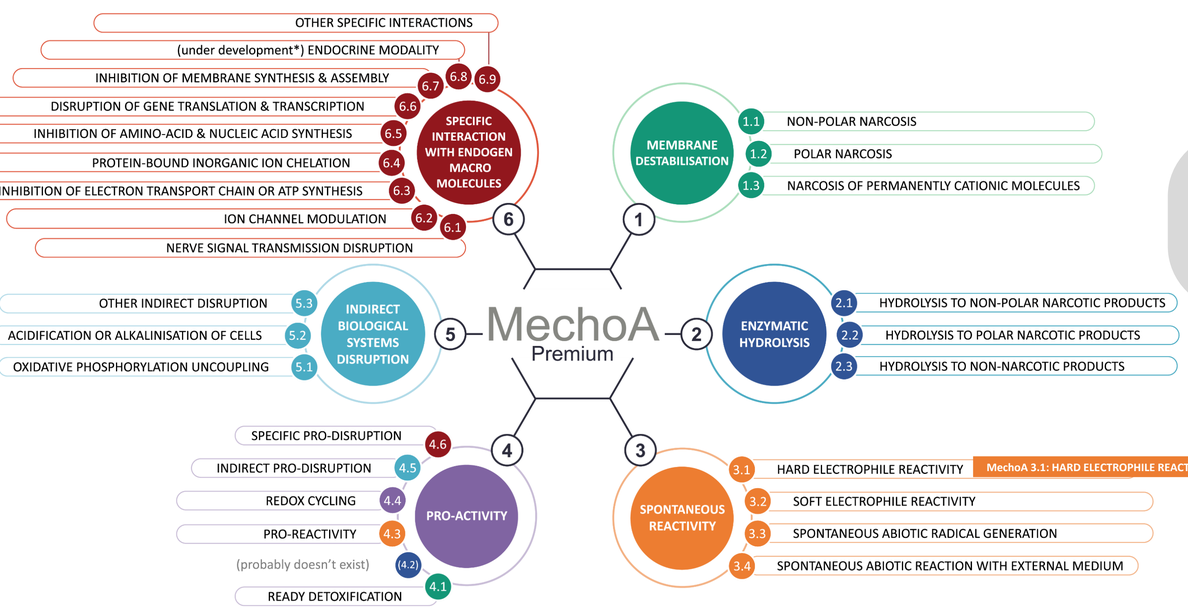


Figure 1. *The MechoA scheme includes 6 classes and 27 sub-classes based on MIE. The adverse outcome of the pathway is quantitatively predicted in the downstream HA-QSAR where one endpoint is linked to one sub-class but may include several structural groups*

Ecotoxicity HA-QSAR

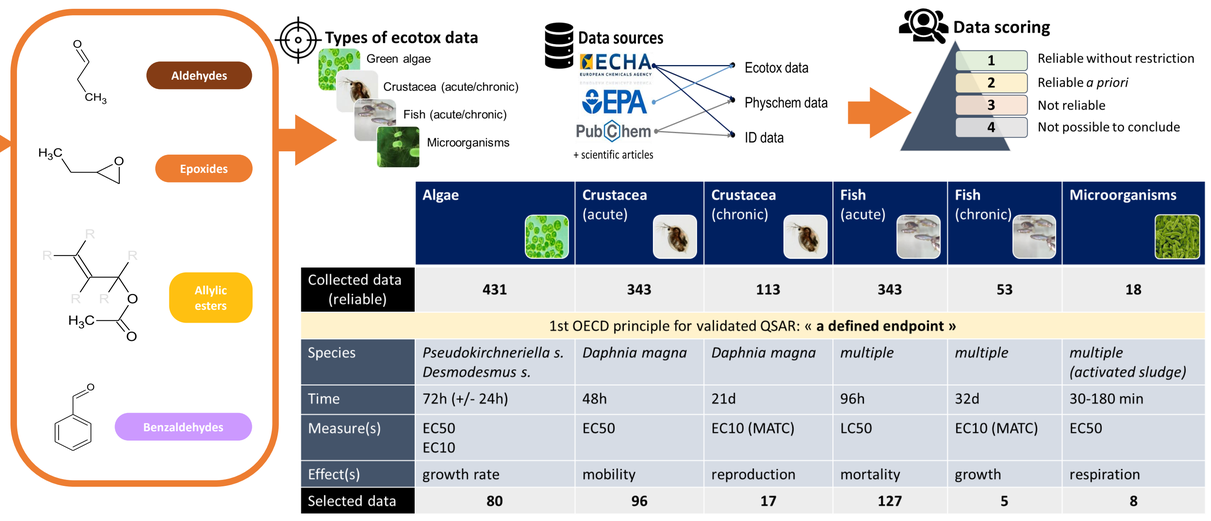
Combining MechoA with internally validated descriptor and toxicity data, we create what we term "High Accuracy QSARs" (or HA-QSARs). We define these as models which are considered accurate enough within their applicability domain, based on their goodness of fit, to be able to substitute for experimental studies (figure 2). For MechoA 3.1, in Figure 3, an acute daphnid toxicity QSAR is shown as an example containing multiple structural groups. These groups are not at all structural similar but mechanistically they all share the same MechoA and the data fall on the same toxicity regression lines for both acute and chronic fish, daphnid and algae toxicity.

Mechanistic model advantages

- MechoA-based QSARs allow the development of more robust models with greater chemical space coverage compared to structural based QSARs limited to one structural group.
- Mechanistic models provide better understanding of the underlying toxic molecular initiating event and limits the need for a validation set (for regulatory purposes).
- Mechanistic understanding increases transparency and the confidence in the prediction and thereby regulatory acceptability.
- MechoA coupled with simple linear regression using water solubility (= hydrophobic toxicity impact) provides an excellent predictive tool capable of high accuracy when plotted using top quality experimental data based on measured concentrations.
- The descriptor (e.g. water solubility) will quantify the prediction between 2 boundaries either:
  1. Min and max values between 2 extreme descriptor values on a regression line or
  2. Until the regression meets a boundary (e.g. toxicity-water solubility cut-off limit)

Data collection & validation

To quantify the impact of the MechoA alert, we need a robust QSAR. All the data used in iSafeRat® QSARs are validated internally by trained (eco)toxicologists. All studies are included in our internal DB but, surprisingly, about 50% of data reviewed are considered unfit for purpose for use in QSARs due to technical flaws in the study and are excluded from the model.



	Algae	Crustacea (acute)	Crustacea (chronic)	Fish (acute)	Fish (chronic)	Microorganisms
Collected data (reliable)	431	343	113	343	53	18
1st OECD principle for validated QSAR: « **a defined endpoint** »						
Species	*Pseudokirchneriella s. Desmodesmus s.*	*Daphnia magna*	*Daphnia magna*	*multiple*	*multiple*	*multiple (activated sludge)*
Time	72h (+/- 24h)	48h	21d	96h	32d	30-180 min
Measure(s)	EC50 EC10	EC50	EC10 (MATC)	LC50	EC10 (MATC)	EC50
Effect(s)	growth rate	mobility	reproduction	mortality	growth	respiration
Selected data	80	96	17	127	5	8

Figure 2*. Initial to final data in MechoA 3.1 (hard electrophile reactivity) having passed through the rigorous data quality check for each endpoint. Full checklist for validation not shown*

The valid data (only), which are specific to the MechoA sub-Class for one specific endpoint are selected, plotted and analysed statistically for goodness of fit[3]. All HA-QSARs are fully QAF compliant, meet the OECD 5 principles and can be accompanied by the regulatory documentation to demonstrate fitness for purpose (QMRF/QPRF).

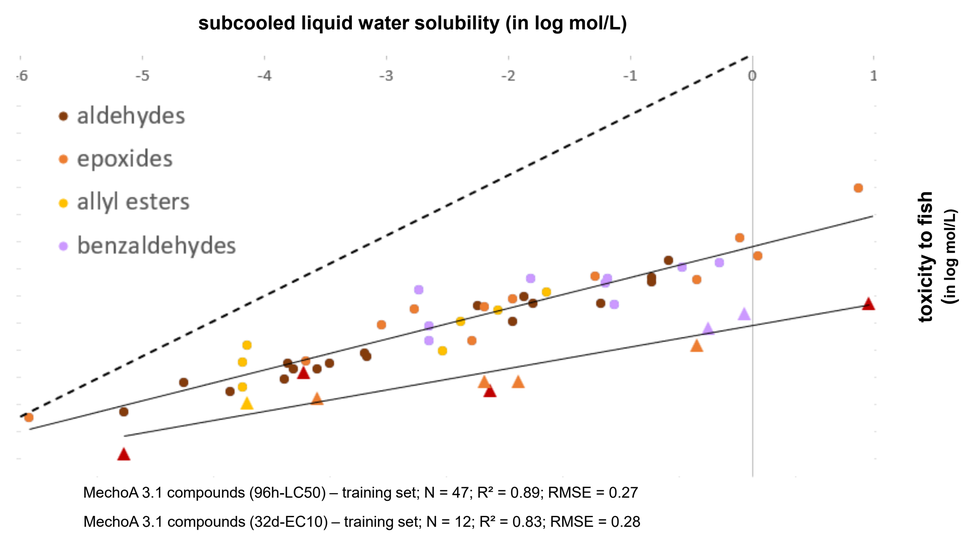


Figure 3*. MechoA 3.1 regression for the fish 96h-LC50 (circles) and the fish 32d-EC10 (triangles). Dotted line = water solubility limit. Structurally different but mechanistically similar substances fall on the same regression lines. Goodness-of-fit statistics are shown for both acute and chronic toxicity QSARs.*

Conclusion

Pros: Mechanistic based QSARs can be used to quantitatively and directly predict apical endpoints across a diverse set of methods providing that:
1) The specific structure-matrix interaction has been elucidated for each organism tested
2) The data used in the HA-QSAR are sufficient and provide a robust, statistically valid result of sufficient quality to replace an empirical study.

Overall, the individual regressions provide far more detail and mechanistic understanding than an empirical study.

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References
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