The Many Pros and a Few Cons of Mechanistic in silico NAMs

P.C. Thomas[1], F. Larras[1], M. Edelblout[1], F.J. Bauer[1], G.Levet[1]

[1] KREATiS, 38080 L'Isle d'Abeau – France

Contact us : www.kreatis.eu / contact@kreatis.eu

Introduction

NAMS, "New Approach Methodologies" to some, "Non-Animal Methods" to others, are neither new nor specifically related to vertebrate animals. NAMs are an essential collective pan-technological paradigm response to traditional long winded experimental techniques: Maybe NAMs are more specifically "Necessary Alternative Methods".

And it is critical that such approaches rapidly obtain the same recognition and support by regulatory authorities as empirical methods if the Green Deal is ever to attain the speed and precision necessary to meet the demands of eco-design*: i.e.,* compare and contrast long term (eco)toxicological effects of dozens of chemical structures simultaneously to rapidly stage-gate the most promising candidates. Mechanistic methods directly attack the root cause of their predictive outcome.

Mechanistic QSARs (i.e. any quantitative relationships observed between chemical structures and effects on biological organisms) can help us to achieve the necessary paradigm shift. But first we must define "Mechanistic" in the context of *in silico* (eco)toxicology and then we can see how we can use it to quantify study outcomes.

Concept of our mechanistic approach

In the context of (Q)SARs, mechanisms of toxic action (MechoA[1]), are akin to Molecular Initiating Events (MIE) only more complete as narcotic 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, 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). While it is the primary toxophore within a structural group that defines a mechanism, not all the structures in a group will have the same (or even any) toxicity. The iSafeRat® platform uses MechoA Premium[2], the SAR predicting Molecular Initiating Events (MIE), to identify an appropriate QSAR to predict toxicity.

MechoA

Use of the MechoA Premium model (Figure 1) in 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. MechoA was developed to do this on the click of a button. Identification of a MechoA class and sub-class provides an understanding of the interactions between the test substance and the biological matrix to explain the observed toxicity (as extracted from the literature and incorporated in the model via >160 structural alerts) and link it to structurally similar (or different) chemicals via their shared MIE. It prioritises the key toxophore within a structure that will impact the biota at different trophic levels (e.g., a substance which is a neurotoxic to fish may be a baseline toxic to algae). The relative toxicity of these MechoA can be quantified downstream in the QSARs.

Any endpoint can ultimately be related to a MechoA, by combining with a QSAR related to the endpoint: species, apical endpoint 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 of (eco)toxicity.

Ecotoxicity HA-QSAR

Combining MechoA with internally validated data to create our models results in 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, there is no zone 2. In Figure 3 acute daphnid toxicity shown as an example which contains multiple structural groups. No structural read-across is possible for these groups but mechanistically they all share the same MechoA and fall on the same toxicity regression lines for acute and chronic fish, daphnid and algae toxicity.

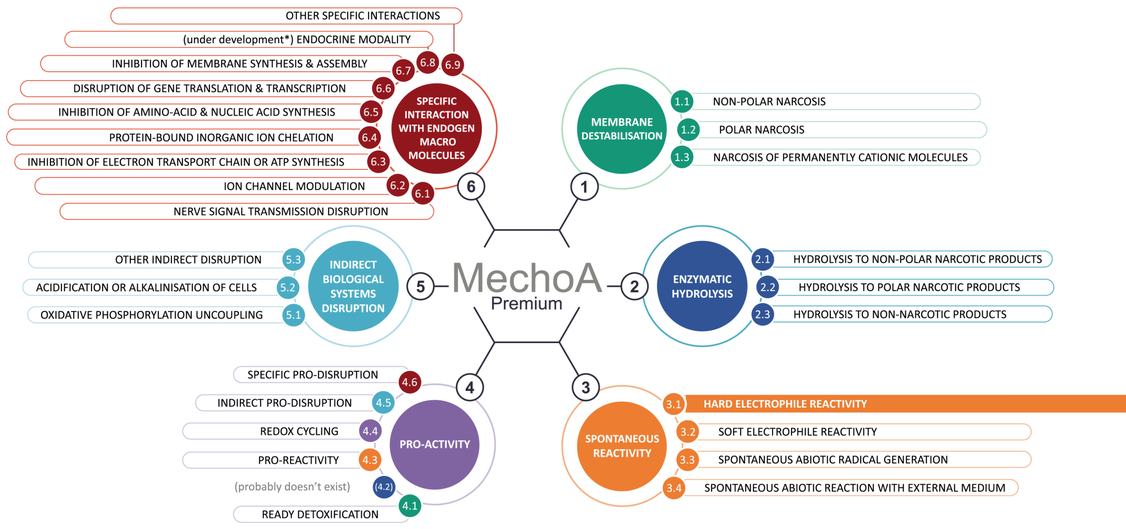

Figure 1. *The MechoA scheme include 6 classes and 27 sub-classes based on MIE. The outcome of the adverse pathway is quantitatively predicted in the downstream HA-QSAR where one endpoint is linked to one sub-class but may include several structural groups*

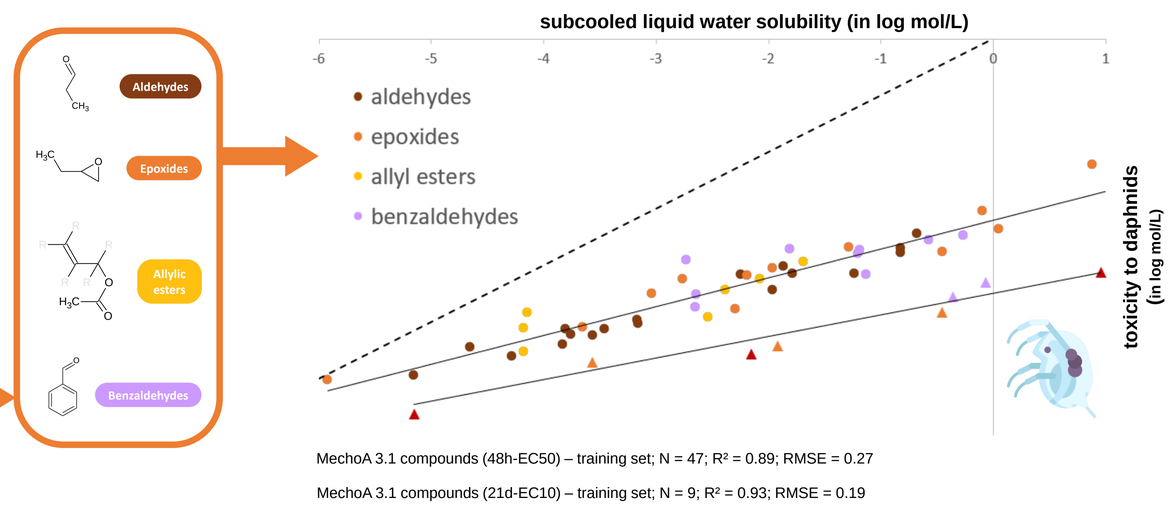

Figure 3*. regression of MechoA 3.1 (hard electrophile reactivity) QSAR for the daphnid 48h-EC50 (circle) and the daphnid 21d-EC10 (triangle). Dotted line is the water solubility limit. Goodness-of-fit statistics are provided for both acute and chronic daphnid toxicity QSARs*

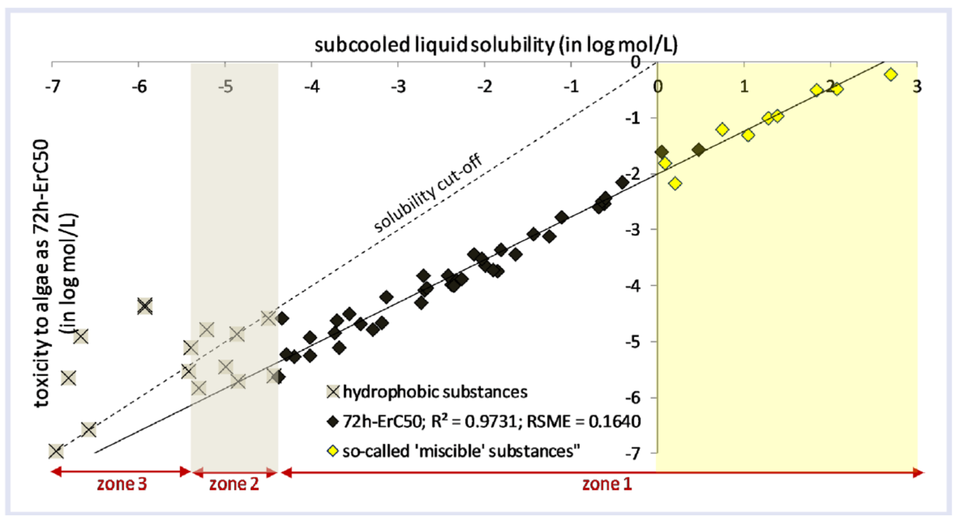

Figure 2. *Descriptor domains of the MechoA 1.1 non-polar narcosis model[3]. Example of algae but the 3 zones are observed in all organisms for both acute and chronic toxicity. Zone 1: regression line for ecotoxicity versus water solubility (yellow zone toxicity for "miscible substances") ; Zone 2: where experimental studies measure toxicity below the solubility limit but experience increasing difficulty to reach equilibrium within the duration of the study; Zone 3 where substance toxicity is > its solubility limit.*

Pros of the Mechanistic approach

- 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 provides understanding of the toxic action and limits the need for a validation set (for regulatory purposes).
- Better comprehension cut-off values and vision of Acute to Chronic Ratios per MechoA.
- 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
  2. Or until one of the ends of the regression meets a boundary (e.g. toxicity-water solubility)

Cons of the Mechanistic approach

- Mechanisms only work when we know them. Thus, in case of doubt, it is useful to use complementary NAMs (e.g. *in vitro*) to validate the predicted outcomes of the HA-QSAR.
- There is a need for vigilance to update QSARs based on newly discovered mechanisms.
- Figure 2 is an example showing where some data cannot readily fall under OECD 5 Principles (poor goodness-of-fit statistics in Zone 2, no unambiguous algorithm possible in zone 3).

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

Cons: What we don't know already we cannot predict, hence, in case of doubt, it makes sense to multiply the lines of evidence

*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)

Scan QR code to download the poster
Available on https://kreatis.eu

References

[1] Franklin Bauer, Paul Thomas, Samuel Fouchard, Serge Neunlist (2018). High-accuracy prediction of Mechanisms of Action using structural alerts. Computational Toxicology 7, 36-45 DOI: 10.1016/j.comtox.2018.06.004

2 Gaspard Levet, Franklin Bauer, Paul Thomas, James Firman,, Mark. Cronin, Jayne Roberts, Steve Gutsell, Bruno Campos,, Geoff Hodges, Leveling up mechanistic understanding with MechoA+ scheme, a SAR model developed for mammalian & environmental toxicologists (in Press)

[3] Paul Thomas, Pascal Bicherel, Franklin Bauer (Nov 2018). How in silico and QSAR approaches can increase confidence in environmental hazard and risk assessment. Integrated Environmental Assessment and Management 15, 1, 40–50