A mechanistic approach to regulatory compliant chronic fish Quantitative Structure-Activity Relationship modelling

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Introduction

Of all the studies in environmental toxicology, the **chronic fish** test is probably the hardest to replace and in doing so, would save the highest number of vertebrate animals. **QSARs** have long been seen as a "screening" method with limited capacity to directly replace experimental studies let alone chronic ones. Nevertheless, stricter approaches combining different *in silico* methodologies are allowing the production of far more accurate models that can serve as stand-alone replacements to experimental guideline studies. Moreover, it provides ecotoxicological values required by regulations faster and in a more ethical and economic way than experiments.

Concept of our mechanistic approach

High accuracy QSARs have been created combining two approaches:

- **Mechanisms of toxic action** (MechoA [1][2]), similar to Molecular Initiating Events (MIE) are inextricably linked to two keystones: on one side, structural features and on the other, biological matrix interactions. More than one structural group can have the same mechanism of action (thus, the same (eco)toxicological impact).
- The use of the **chemical activity approach** relating the external metric "water solubility" to the toxicity endpoint (EC10) using only data that was rigorously validated internally to create "High Accuracy QSARs". The first step resides in the use of a structural alert based scheme predicting molecular initiating events.

Mechanisms as alerts

MechoA (figure 1) are inextricably linked to two keystones: on one side **structural features** and on the other **biological matrix interactions.**
Unlike read-across, more than one structural group can have the same MechoA (thus, the same impact at the same molar concentration for a species and endpoint). 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.

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 database 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.

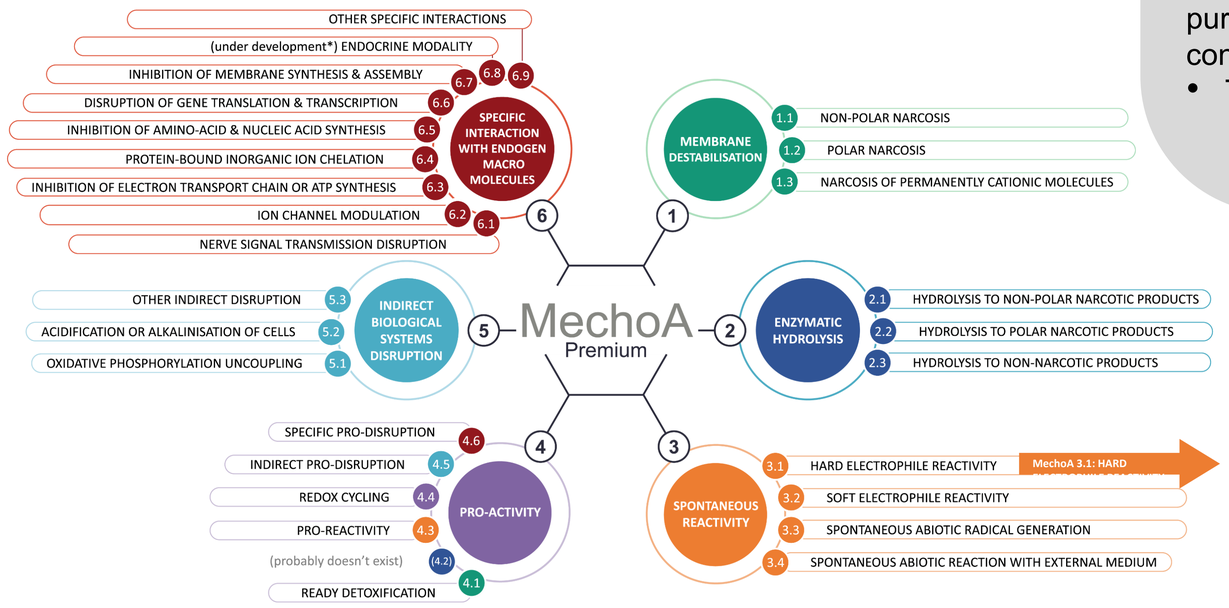


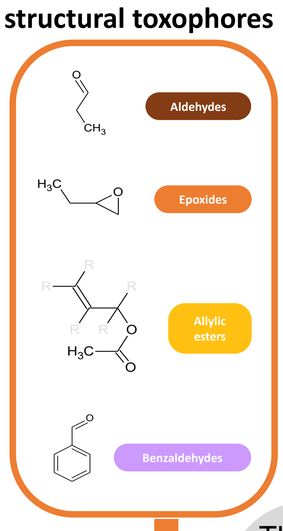
Figure 1. *The MechoA scheme includes 6 classes and 27 sub-classes based on MIE. The adverse outcome of the pathway is quantitatively predicted in the downstream HA-QSAR where one endpoint is linked to one sub-class but may include several structural groups*

Ecotoxicity QMARs

Mechanistic insight (in the form of Structural Alerts or SAR) is a necessary first step for us to produce a reliable QSAR with a well-defined applicability domain (AD).
MechoA was developed to explain the observed toxicity and link it to chemicals with the same mechanism of toxic action be they structurally similar or different. But as a mechanism provides better justification than just a structure, perhaps a better term for these models is "**QMAR**s"!
The descriptor (e.g. water solubility) quantifies the prediction between 2 boundaries either:
Min and max values between 2 extreme descriptor values on a regression line until one of the ends of the regression meets a boundary (e.g., water solubility cut-off value). Fig. 2 highlights an example for the MechoA 1.1 72hErC50 algae endpoint which we have divided into zones.
Zones 1, 2 and 3 are observed for both acute and chronic algae, daphnid and fish studies for MechoA 1.1 but for many other MechoAs Zone 2 is absent.
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.
Mechanistic understanding also **removes the need for a validation set** (for regulatory purposes) compared to statistical based QSARs (prone to overfitting) so more data can be consecrated to the training set.

- 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-solubility cut-off limit at Zone 3)

Example of MechoA sub-class 3.1 with diverse structural toxophores



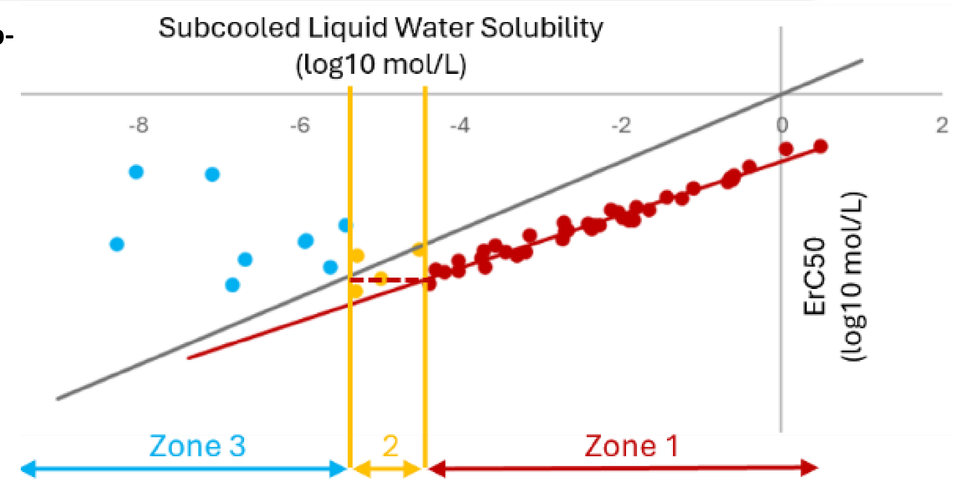


Figure 2. MechoA 1.1 72h ErC50 algae QSAR divided into three zones by 2 boundaries (regression zone, plateau zone and toxicity>solubility zone)[3]

Chronic fish QMARs

Any endpoint can ultimately be related to a MechoA (SAR) but the QSAR produced will also be related to the species, biology, timeframe of the study, compartment of concern and specific apical endpoint (e.g., MechoA 3.1, 96h LC50 acute fish study predictions for OECD 203 and 32 d EC10 chronic fish study predictions for OECD 210 Guideline, see fig. 3). **Using the QMAR approach, chronic fish toxicity can be accurately predicted**.

- Furthermore, due to multiple chemical structures with the same MechoA within the same QMAR, more robust models with **greater chemical space coverage (i.e.,** not limited to one structural group) can be produced. In fig 3 the inclusion of (structurally very different) epoxide, allyl ester and aldehyde data broaden the scope and improve statistics of the chronic fish QMAR
- Greater perspicacity of toxic impacts can be obtained, e.g., elucidation of accurate **Acute to Chronic Ratios** per MechoA sub-Class (eliminating uninformed "uncertainty factors" typically used in risk assessment). See fig. 3.

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).

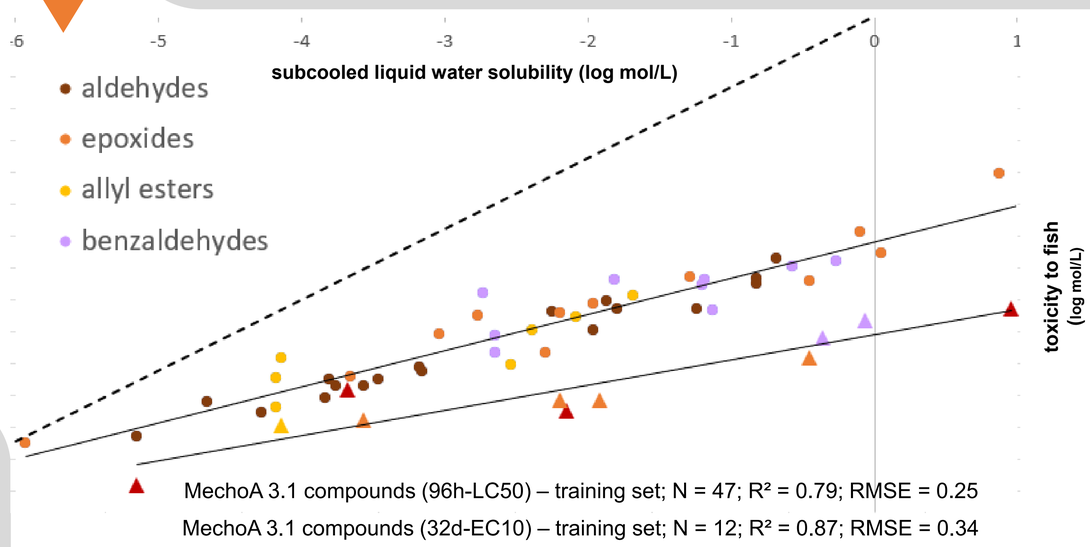


Figure 3*. MechoA 3.1 regression for the fish 96h-LC50 (circles) and the fish 32d-EC10 (triangles). Dotted line = water solubility limit. Structurally different but mechanistically similar substances fall on the same regression lines. Goodness-of-fit statistics are shown for both acute and chronic toxicity QSARs.*
Understanding of acute to chronic ratio, potential for metabolism and hydrophobicity is enhanced by considering the relationships between acute and chonic regressions

Conclusion

QMARs can be used to quantitatively and directly predict apical endpoints across a diverse set of Guideline based studies, replacing *in vivo* studies of the same endpoint, even for chronic aquatic studies including fish. Nevertheless:

1) The specific structure-matrix interaction has been elucidated for each organism tested;
2) The data used in the QMAR are sufficient and provide a robust, statistically valid result of sufficient quality to replace an empirical study.

Overall, the QMAR regressions provide far more detailed and mechanistic understanding than even a large group of empirical studies. Much of their potential remains to be explored.
Only QMARs can deliver this level of insight as to the relative toxicity of chemicals, the degree of influence of hydrophobicity and the boundaries of toxicity. For substances falling within the AD, they constitute a meaningful replacement of laboratory studies for regulatory ecotoxicity.

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References
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