

Shaping the toxicity profile of chemicals to avoid animal testing

Franklin Bauer, QSAR modeller and chemist at Kreatis, introduces the MechoA method*

TO DETERMINE THE toxicity of a substance to the environment or human health, standardised toxicity tests using animals can be performed. However, there is increasing international, societal pressure on industry to replace, reduce and refine animal experimentation according to the principles of the '3Rs', first described by Russell and Burch in 1959.¹

In some regulatory spheres, such as cosmetics, vertebrate animal testing has been banned altogether and the regulatory pressure to move to alternative testing methods is increasing in others, like REACH. Among the alternatives, the use of *in silico* methods such as (quantitative) structure-activity relationships ((Q)SARs) and appropriately chosen read-across strategies are powerful tools to predict the toxicity of chemicals without the need for further animal testing.

QSARs are mathematical models relating a toxicological activity to molecular or physico-chemical

parameters, or 'descriptors', for a group of compounds. Read-across strategies use one or several analogues with available toxicological data and consider that these data are also applicable to the target substance.

These approaches all employ the first of the '3Rs': replacement. The other two, reduction and refinement, do not completely remove the use of vertebrate animals in the experimentation procedure and will not be further discussed in this article. To ensure the reliability of predictions from *in silico* methods, strict recommendations, the five OECD principles for the validation of QSARs, should be followed²:

1. A defined endpoint
2. An unambiguous algorithm
3. A defined domain of applicability
4. Appropriate measures of goodness-of-fit, robustness and predictability
5. A mechanistic interpretation, if possible

Classification schemes

While QSARs are becoming an increasingly recognised source of information in hazard assessment, the risk of using an inappropriate QSAR or read-across to determine toxicity and getting the answer wrong has been relatively high until recently. What has been missing is a comprehensive classification system to fit the right QSAR or read-across to the chemical structure of interest.

Several classification methods have been described in the literature. The most famous one in the ecotoxicology field is the Verhaar scheme.^{3,4} This classifies substances into four very general modes of action, based partly on molecular mechanisms and partly on toxic outcomes. However, this method was developed from fish toxicity data alone and many substances cannot be classified, due to a lack of rules to classify them.

Another method has been published and made freely available in the OECD QSAR Toolbox.^{5,6} This is a more elaborate

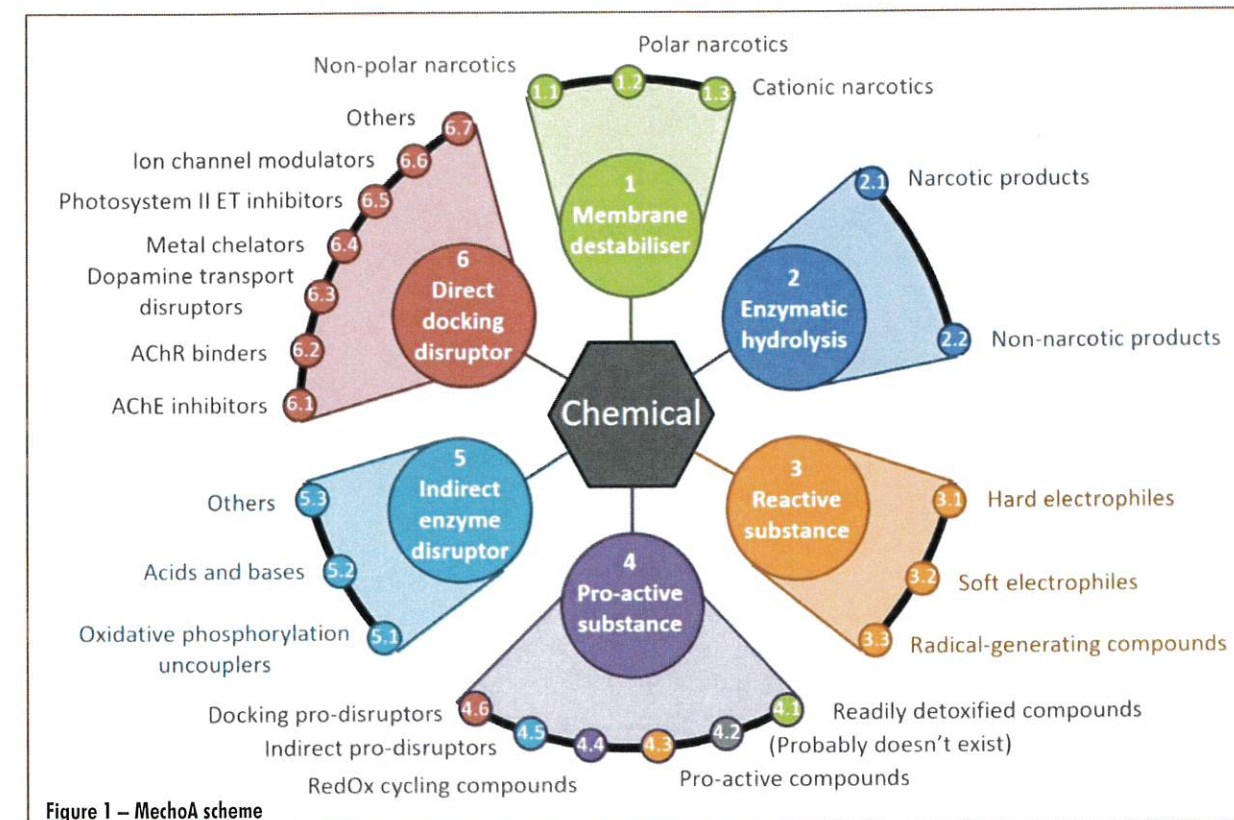


Figure 1 – MechoA scheme

classification method but is also based only on fish toxicity data. Like the Verhaar scheme, it has limited applicability in terms of chemical structures, mechanisms of action and species.

Other classifications used in mammalian toxicity assessment exist but they do not cover all mammalian endpoints and use arbitrary classification bins. For example, Cramer *et al.* proposed a decision tree approach for classifying chemicals according to toxicological threshold concerns, based on chemical structure, metabolism, chronic and sub-chronic oral toxicity and natural occurrence.⁷

MechoAs: A new scheme

The Mechanism of Action (MechoA) scheme classifies organic chemical structures according to their capacity to induce toxic effects in whole organisms.^{8,9} MechoAs strongly contribute to points 3 and 5 of the OECD principles. Substances are classified based on their molecular behaviour (reactivity, docking in receptors, etc.), which can be deduced

from their structure – that is their degree and type of interaction with living material, also known as the molecular initiating event.

All organic substances can be classified under one of just six different main classes (Figure 1) although there are numerous sub-classes. Currently 23 are proposed, but at least 50 will probably be recognised once all of the types of structural reactivity have been unravelled.

We can relate the MechoA of a chemical structural group to experimental data on toxicity. Each MechoA sub-class corresponds to a specific adverse outcome pathway (AOP) with specific study outcomes (termed 'endpoints' in modern hazard assessment). Therefore, we can build MechoA-specific QSARs using appropriate descriptors.

We can quantitatively predict study results with great accuracy by linking each MechoA sub-class to an endpoint-specific QSAR. As we are talking about a chemical-specific interaction with living organic matter, the scheme is relevant to ecotoxicity, mammalian toxicity and even human health.

Because each specific endpoint is unique, we need to create one QSAR for each endpoint and for each MechoA. For example, fish and rodent acute toxicities are conducted in totally different ways, with fish exposed to test substances through the water and rats by gavage or in their diet. Each type of test will therefore have its own specific QSAR.

The model has been developed mainly with fish and rodent toxicity data, but information on other species, especially daphnids and algae, is also included. Therefore, the differences found in toxicity between these species are included in the classification outcome, uniting, for the first time, toxicology and eco-toxicology under the same classification system.

In our experience, grouping chemicals by their MechoA leads to more accurate local QSAR models with a clearly defined mechanistic applicability domain and a strong mechanistic interpretation. This provides higher certainty and greater assurance that the predictions are accurate.

Classification result (%)	MechoA scheme	Verhaar scheme	Russom method
Correctly classified	92.2	54.0	82.5
Slightly different	3.5	NA	6.3
Misclassified	4.3	13.4	11.2
Unclassified	0.0	32.6	0.0

Table 1 – MechoA classification method v. other methods

Note: Results given as percentage of the dataset. Correctly Classified = classification result in accordance with literature; Slightly different = classification result has small deviations from the literature (e.g. general MechoA correctly predicted but MechoA subclass not same as literature); Misclassified = classification result not in accordance with literature; Unclassified = mechanism of mode of action cannot be predicted by this method.

How it works

The MechoA scheme uses the molecular structure of a substance to determine the MechoA of the parent substance and its major metabolites, if identified, i.e. the first key events in the AOP. The scheme is composed of more than 60 rules, organised as a linear decision tree based on 2D and 3D structural

alerts. The model classifies the substances into six major classes (Figure 1): the output is the MechoA class and sub-class, together with a short description.

In the simplest case (MechoA 1), there is a defined relationship between the toxicity and the hydrophobicity of the test substance, due to reversible membrane-disruption effects. Examples of compounds acting with this MechoA are the alkanes and the alcohols inducing narcotic effects.

As we move up the classes, the reactions between structure and living material become more complex. In Class 2, the toxicity is triggered by hydrolysis, a simple but mostly enzyme-initiated reaction occurring between the substance and the water inside the organisms, as we observe for the esters.

In Class 3, we find groups with high intrinsic reactivity, acting



with non-enzyme-controlled mechanisms – for example acrylates, which fall under MechoA 3.2. Thus, they are generally more toxic than the previous classes and their toxicity is not highly dependent on hydrophobicity or related to metabolic transformation.

The remaining classes treat substances that are transformed into biologically active compounds by enzymes i.e. metabolism (Class 4); that modify the environment of an enzyme, preventing its normal activity (Class 5); and that bind to a docking site of a key protein (Class 6). In this last class we find the endocrine disruptors (MechoA 6.7), an area of ongoing research at Kreatis.

The MechoA classification method has been compared to other available methods and the results are shown in Table 1.



Given the higher rate of correct classifications and lower rate of misclassifications, in addition to more detailed and clearly defined classes, MechoAs provide a more complete characterisation of toxicity mechanisms than previous classifications.

MechoAs can thus shape the toxicity profile of a new chemical by supporting category-based read-across and (Q)SAR predictions. The decision tree has been implemented in a fully automated tool and is freely available online. ●

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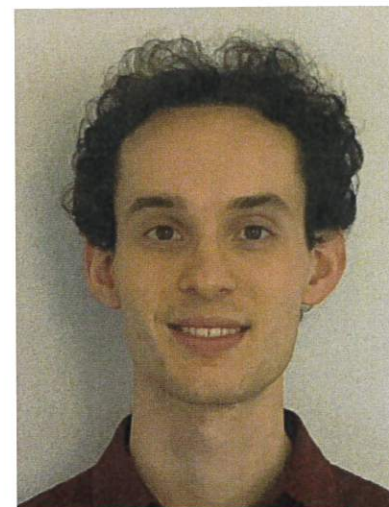
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