OECD QSAR Assessment Framework (QAF): Advantages and disadvantages of a tool designed to assess and ultimately strengthen predictive NAMs

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INTRODUCTION

New Approach Methodologies (NAMs) are an essential piece of the upcoming Green Deal if Europe is to move to a "toxic free environment" and safe and sustainable by design products. *In silico* NAMs up to now have obtained less attention than their *in vitro* cousins in this sprint towards faster and more animal free methodologies. And yet, *in silico* NAMs are ultimately the fastest, cheapest alternative to laboratory methods and the most elucidating, particularly so, when they are based on mechanistic interpretation.

The ambition of the fairly recent **QSAR Assessment Framework (QAF)** (OECD, 2023) is to help regulators across the OECD member states to better assess the likely prediction capacity of QSARs by using a reflective check list scheme to arrive at a consensus result. While the 5 OECD principles set criteria for model validity, **the QAF sets 4 principles for prediction validity.** An important fact to consider being that **using a valid model does not imply that the prediction will be valid** too. There is no doubt that the QAF will both increase recognition and use of these methods by the regulatory community. On the other hand, the QAF further limits the wider applicability and potential of QSARs, restricting their capacity to reduce animal testing and their use as tools that can further the science in a way that empirical studies alone cannot. In this poster we provide demonstrations of the advantages and some disadvantages of QAF.

EXAMPLES OF ADVANTAGES

1/ MORE CLARITY & HOMOGENEITY IN QMRF/QPRF

In many respects the QAF requests respect of the OECD 5 principles and the Guidance R6. There is no significant progress to QSAR development rules laid down by the OECD in 2004. The main advantages are:

- Clarification of the QPRF content: **4 principles to validate a prediction** (e.g : does the substance fall within the model applicability domain?)
- The addition of the **prediction checklist** (for single models) or the result checklist (for multiple predictions).
- The principles include **"Assessment Elements" (AE)** which are checklist points, each of which needs to be addressed so that an overall performance score is calculated at the end.

Not only does this provide clarity for assessors of QSARs and transparency and traceability on how a QSAR was assessed, but it also clarifies targets for QSAR producers to aim at when preparing the QMRF/QPRF documents for regulatory submissions. Furthermore, the document indicates the sincerity of the OECD MS to take *in silico* NAMs more seriously than previously and this should be a welcome message to QSAR modelers and users of the tools alike. Furthermore, in a world where QSARs can be found on every street corner, it should sift out the wheat from the chaff as **many of the weaker tools or publications will not be able to meet the obligations** that have been clarified in the QAF document

2/ GAIN OF CONFIDENCE IN PREDICTIONS: Weighting & Uncertainty

The QAF document considers 2 aspects: **Weight and Uncertainty**. The weight of an AE determines its relative importance in the overall score while to reduce uncertainty in prediction outcomes, the use of weighting (semi-quantitatively as **low, medium or high uncertainty**) is used. So, **an ideal QSAR prediction has the lowest uncertainty in the AEs with the highest weight**. This is good news for QSAR predictions which may not perfectly fulfill all OECD principles but are weaker in areas that are unlikely to influence the outcome (e.g., The structural domain only goes to C15 alkyl chain but the target substance is a C16 = high weight & low uncertainty).

EXAMPLES OF DISADVANTAGES

1/ MORE TIME REQUIRED TO PROVIDE DOCUMENTATION

As upbeat as it seems to have tools to better assess the viability of QSARs, the downside is that **the possibility of automating QPRFs has now shifted further away**. Certain tools provide automated QPRFs which may have been acceptable pre-QAF but it is doubtful that any of these would pass now without major updates. KREATiS was at 80% automation and has now abandoned the idea and moved back to internal expert production as the effort to produce compliant QPRFs has ballooned.

2/ STRUCTURAL DOMAIN CONSIDERED MORE IMPORTANT THAN MECHANISTIC DOMAIN

As QAFs deal with QSARs and the origin of QSARs is structural relationships, there is more emphasis on structural domain than mechanistic domain. Recent work on Adverse Outcome pathways (AOP) and specifically Molecular Initiating Events (MIE) puts more weight on mechanistic interaction with biological matrices than structural relationship (although obviously these are related). The KREATiS Mechanisms of toxic Action (MechoA) structural alert scheme is mechanistically based and therefore our QSARs are too. And yet **the greater influence of mechanism than structure is not sufficiently recognized by QAF** (or Principle 5 of the OECD Principles)

EXAMPLES OF TECHNICAL ISSUES NEEDING ATTENTION

1/ OECD 5 PRINCIPLES & QAF LACK COVERAGE

Due to the 2nd principle "unambiguous algorithm", certain well known ecotoxicological phenomena are difficult to transform into QSARs:

- **Ecotoxicological value > solubility limit:** substances no longer toxic for a specific endpoint as equilibrium has not been reached within the timeframe of the study. The point where this occurs can be identified by simple linear regression but beyond this point it is not possible to create a regression as all toxicity is found at values higher than solubility and if effects do occur they are physical not toxic.
- **Ecotoxicological value > highest tested concentration**: The experimental test provides a value that is not usable in quantitative models or easily as analogue, but we know that it does not have an effect (e.g. regulatory cut-off for acute aquatic ecotoxicological endpoint)

In both cases, there is **no "unambiguous algorithm" and the QPRF may be deemed unacceptable and yet experimental studies finding empirically the same result would be considered acceptable.**

2/ MISCIBLE SUBSTANCE TOXICITY PREDICTIONS

Another such case is when the descriptor used in the QSAR to determine ecotoxicity is water solubility. In some cases, measured data on water solubility are of poor quality and the substance analysed as "miscible" (typically a value of 1000 g/L is stated). In such cases **determining theoretical water solubility may be more appropriate than using the term "miscible" as this result is due to an analytical artifact**. The last value in the descriptor domain would therefore be considered to be the highest measurable value of water solubility. Thus to determine ecotoxicity of a substance deemed miscible implies using an *in silico* prediction of solubility and the ecotoxicity value would be **considered "out of descriptor domain"** by the QAF.

3) R^2 FOR NON-SIGNIFICANT LINEAR REGRESSION

R^2 is not always a relevant statistical indicator of the goodness-of-fit of a model, especially in rare cases where there is no significant link between the dependent and the independent variable and the Root Mean Square Error (RMSE) is low. For carboxylic acids, the hydrophobicity plays only a small role in defining aquatic toxicity and the R^2 is very low. Nevertheless, the model is excellent as attested to by the very low RMSE in this case. **It is therefore very important to recognize which statistical parameters are relevant to each case (OECD, 2007) and not to interpret a single statistical metric without considering all the characteristics of a model.**

CONCLUSION

- The **advent of QAF is a huge step forward** that will contribute to the reemergence of QSARs as a major force in the world of NAMs.
- Nevertheless, attention still needs to be paid to details which are at the periphery of QSAR applicability domains such as areas where regressions no longer exist (toxicity > water solubility) or where performance of experimental methods are technically not possible.
- These are areas of increasing concern as chemistry becomes more complex and laboratories increasingly struggle to provide valid, meaningful studies for regulatory dossiers
- *In silico* methods are able to provide solutions (even though these are increasingly theoretical as measurements become impossible) by extrapolation, but the QAF needs to increase in flexibility and recognize these solutions

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