A new *in silico* NAM to discriminate classified/not-classified chemical substances for acute oral toxicity

Etienne Bourgart[1], Carole Charmeau-Genevois[1], Franklin J. Bauer[1], Emel Ay-Albrecht[1], Paul C. Thomas[1]

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

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

INTRODUCTION

In a regulatory context, acute oral toxicity (AOT) studies are required for classification of substances according to the CLP/GHS criteria. The original guideline (OECD 401) was designed to determine the median lethal dose (LD_{50}) re-named acute toxicity estimates (ATE) in the CLP. The recent OECD guidelines in line with the 3R principles allow to decrease the animal suffering and the number of animals used[1] (OECD 420, 423, 426). Moreover, using the OECD 420 guideline, a substance may be classified based on an ATE related to «evident toxicity» instead of 50% mortality. Although ethically defensible, these refinements have resulted in ATE triggering more conservative CLP classification This is exacerbated by premature sacrifice at the first signs of serious suffering, which also artificially inflates the death count as animal recovery was often observed in older studies. Furthermore, the precise cause of animal death was often unclear in past studies as acute adverse effects can arise from either local or systemic toxicity and results were not always well described in reports. Furthermore, no *in vitro* study or set of studies can really be considered to replace this *in vivo* endpoint today.

In contrast, *in silico* methods are ethical, more rapid and of lower cost compared to *in vivo* testing. And importantly, they provide a scientifically based alternative to the implied AOT. During this work, we developed a pilot QSAR for AOT classification for rat LD50 using the chemical family and the water solubility as descriptors.

METHODS

A fundamental and time-consuming part of (Q)SAR development was spent building a curated & validated training set of experimental ATE values before data analysis, model structuring & preliminary testing.

AOT studies with ATE gathering from ECHA registered substances
input of ~2000 CAS

↓ ~4500 lines

Database building & data curation
database of ~840 CAS

Descriptors (*e.g.* water solubility) prediction using iSafeRat®
database of ~700 CAS

Sorting CAS by chemical family using an in-house version of FP4 fingerprint (OpenBabel)[3]

Data validation: mono-constituents, guidelines, specie (rat), ATE values
database of ~540 CAS

↓

Data analysis, pilot model structuring & testing
alcohols, aldehydes & carboxylic esters

Chemical class	Training set (n)	Test set (n)
Alcohols	56	8
Aldehydes	23	5
Carboxylic esters	19	5

Table 1: Global accuracy and specificity of iSafeRat® AOT pilot model relative to the training set using internal validated studies.

*** N = number of substances from the training set within the applicability domain of the pilot model**

Inside AD*	Accuracy (%)	Specificity (%)
17	94,1	93,8

A PROMISING ALTERNATIVE TO *IN VIVO* AOT STUDIES

for CLP/GHS classification & ATE prediction

Reduce, Refine, Replace with high-accuracy (Q)SAR models

PILOT STUDY RESULTS

Following data selection and validation for the training set, we analysed the data and compared the toxicity of the different chemical classes. Within the total training set, the AOT values of substances followed a gaussian distribution (data not shown). Surprisingly, AOT values were very similar between chemical classes tested here as only slight statistical differences were observed between reactive substances (aldehydes) and carboxylic esters after ANOVA analysis (**Figure 1**).

Then, we studied the relationship between ATE and WS for each chemical family. For alcohols, a correlation (R^2 = 0,64) was observed under the toxicity-water solubility limit (**Figure 2**), while no correlation was obtained between WS and the AOT of aldehydes and carboxylic esters (data not shown). For those two latter chemical classes, the worst-cases ATE delimiting AOT categories were graphically determined and used for category prediction.

The performances of our pilot model were subsequently tested. Global results are detailed in **Table 1**. In total, 17 predictions were produced as 1 alcohol was outside of the applicability domain of the model (*i.e.* above the toxicity-water solubility limit). The preliminary results are very encouraging with an overall accuracy of 94.1% and a specificity of 93.8%.

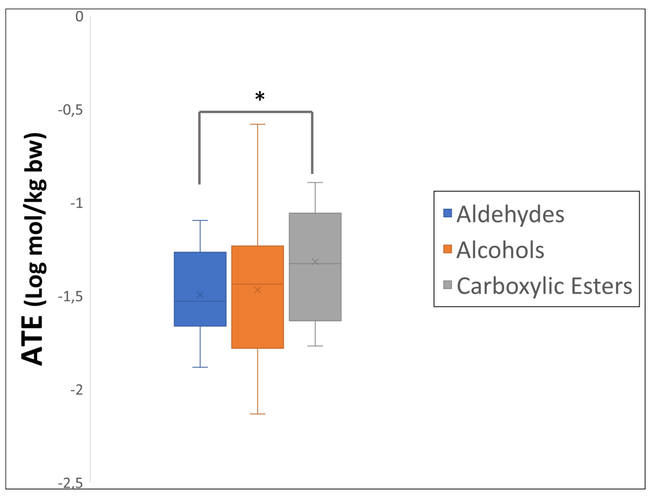

Figure 1: Comparison of experimental ATE values distribution for alcohols, aldehydes & carboxylic esters.

*** Slight statistical difference (p value = 0,041)**

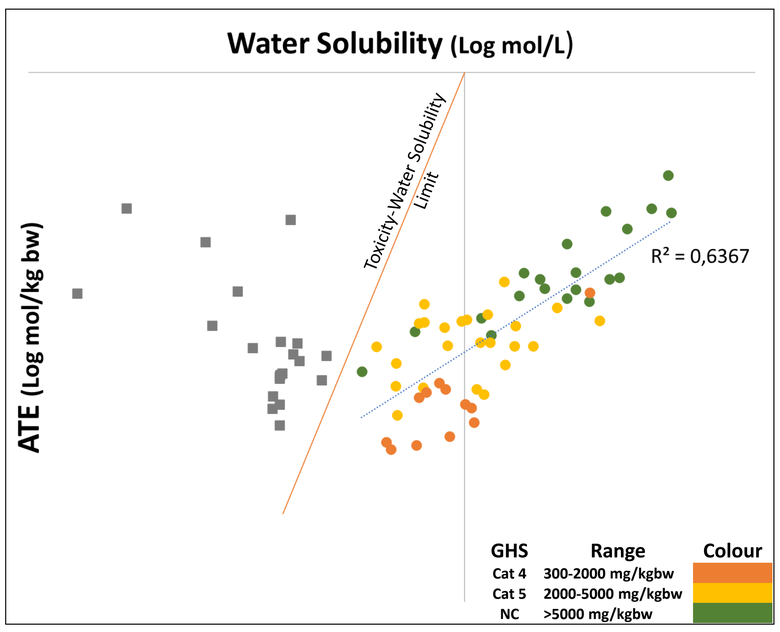

Figure 2: Relationship between water solubility and ATE values in rats for alcohols.

PERSPECTIVES

At this stage, the results from the pilot study are very promising given that very accurate predictions were produced with preliminary models. Further work will rapidly extend the applicability domain of our model by considerably increasing the size training set. In addition, supplementary descriptors based on mechanisms of action are currently under consideration in order to take into account severe local effects leading to mortality (*e.g.* irritation), while systemic adverse effects are usually expected to lead to animal death in AOT studies. Further, the model will be tuned to predict ATE values, the current golden standard in regulatory toxicology. To this purpose, we are currently developing models using more complex statistical approaches such as Support Vector Machine (SVM) models associated with in-house fingerprints adapted to AOT requirements.

Altogether, we are confident that these model refinements will largely extend the scope and improve the predictivity of our model, thus, helping to replace AOT studies in regulatory dossiers by thoroughly validated *in silico* alternatives.

WE CAPTURE WHAT MOVES MANE

With thanks to MANE for sponsoring this project

REFERENCES

1. Craig M. Zwickl et al. Principles and procedures for assessment of acute toxicity incorporating in silico methods. Computational Toxicology. Volume 24, 2022, 100237, ISSN 2468-1113. https://doi.org/10.1016/j.comtox.2022.100237.
2. ECHA guidance on the application of the CLP criteria (2017)
3. O'Boyle, N.M., Banck, M., James, C.A. et al. Open Babel: An open chemical toolbox. J Cheminform 3, 33 (2011). https://doi.org/10.1186/1758-2946-3-33

Poster ID LP-89