A QAF compliant regulatory *in silico* model for predicting the acute oral toxicity of esters

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Introduction

In a regulatory context, the assessment of **acute oral toxicity** (AOT) is **required to classify** the substances according to the CLP criteria. OECD guideline studies in line with the 3R principles **decreasing the animal suffering and the number of animals are used**[1] (OECD 420, 423, 425). For instance, using the OECD 420 guideline, a substance may be classified based on acute toxicity estimates (ATE) related to «evident toxicity» instead of 50% mortality. Although ethically defensible, these refinements have resulted in ATE triggering more conservative CLP classification. Furthermore, **currently no *in vitro* study or set of studies can really be considered to replace this *in vivo* endpoint**. In this context, a **QAF compliant *in silico* model used as a stand-alone model**, was developed to predict the ATE for AOT according to the CLP classification for specific mono-constituent esters. This model is based on a decision tree identifying specific esters. The model performance is **illustrated using a fragrance, Patchoulyl-acetate**, as a case-study.

Figure 1. *Knowledge-based expert model for the AOT of esters with the example of Patchoulyl acetate. Whenever a substance does not validate one of the first two steps, the test item is defined as out of the applicability domain. If the statistical analysis do not reject H_0 hypothesis, the prediction is assessed unreliable.*

Methods

All AOT studies on esters were extracted from ECHA dossiers and were validated internally in agreement with the OECD guidelines. The applicability domain was defined based on a decision tree taking into account both **physicochemical parameters** (molecular weight, log Kow, *etc*.) and **structural fragments** (inculsion or exclusion rules based on structural alert) relevant for the acute toxicity.

The applicability domain was first defined by the **mechanism of toxic action (MechoA based on structural alert) and toxicological profile of ester**. Chemical families (*e.g.* phthalates, α,β-unsaturated esters, lactones, *etc*.) were excluded from the applicability domain as they may lead to endocrine activity or may be reactive with biological molecules, prior or after metabolism. Furthermore, esters which were classified under CLP (*e.g.* oxalate-like esters, C-aromatic esters, O-benzyl esters, etc.), were also excluded. Whenever a substance fell inside the structural domain, the **Tanimoto index** was calculated and a structural similarity acceptance threshold for the identified nearest neighbours (analogues) was set at ≥ 70%.

Finally, **statistical analysis** was performed on the AOT prediction. The null hypothesis H_0 of this study is defined as follows: the structural similarity between the analogues and the target substance as well as the absence of CLP classification for the analogues, occurred by chance. If the p-value < 5%, the H_0 is rejected. Therefore, the test item is unlikely to be classified for the AOT according to CLP by chance. Otherwise, if the p-value > 5%, H_0 is not rejected and, therefore, the prediction is assessed unreliable.

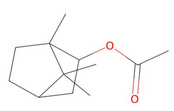
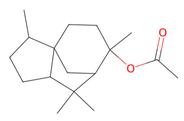
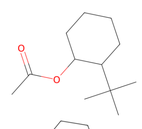
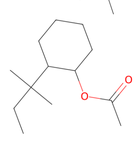
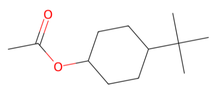
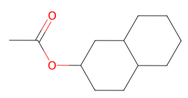
Analogues substances names	CAS	Structure	Experimental CLP classification for AOT	Similarity index (%)
Bornyl acetate	5655-61-8		Not classified	**90**
Cedryl acetate	77-54-3		Not classified	**87**
2-tert-butylcyclohexyl acetate	20298-69-5		Not classified	**86**
2-tert-pentylcyclohexyl acetate	67874-72-0		Not classified	**84**
4-tert-butylcyclohexyl acetate	32210-23-4		Not classified	**83**
Decahydro-2-naphtyl acetate	10519-11-6		Not classified	**72**

Table 1. *Analogues with a significant threshold similarity index identified within the database for Patchoulyl-acetate.*

Results

The fragrance, **Patchoulyl-acetate was inside the applicability domain of the model**, as it fell entirely inside the descriptor space, the structural space, and the response space of the model (*i.e.* "not classified" based on the CLP classification).

The model identified 6 structural analogues with an ATE > 2000 mg/kg bw (no CLP classification for the AOT). For those analogues, a Tanimoto similarity index ≥ 72%, and with a mean of 84%, was retrieved (Table 1).

Following statistical analysis, a p-value of 6.4×10^{-5} was calculated, which is below the 5% significance level. Therefore, hypothesis H_0 was rejected, meaning that the **structural similarity** between the 6 analogues and Patchoulyl-acetate, as well as the **absence of CLP classification** of the analogues is not due to chance.

Therefore, Patchoulyl-acetate was predicted to be not classified for AOT according to CLP. This prediction was assessed reliable.

Conclusion

This knowledge-based expert model was developed to assess specific AOT based on both **physicochemical, structural and mechanistic features** of the substances. As illustrated by the fragrance, Patchoulyl-acetate, the restricted applicability domain of the model, in addition to the statistical analysis, allows **high confidence in the prediction**. Furthermore, statistical analysis and analogues identified by the model **determine correctly the AOT prediction with regards to the QSAR Assessment Framework** (QAF)[2] . This model is the **keystone in a larger accurate and reliable AOT model currently under development** for the prediction of a large domain of chemical families. This model can be used as a stand-alone *in silico* method to fulfill the AOT endpoint required in the Annex VII of REACH.

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[1] Zwickl CM *et al*. *Principles and Procedures for Assessment of Acute Toxicity Incorporating In Silico Methods*. Comput Toxicol. 2022;24:100237.

[2] OECD. *(Q)SAR assessment framework: Guidance for the regulatory assessment of (quantitative) structure activity relationship models and predictions*. Paris: OECD Publishing. 2023;386.