iSafeRat® CLASS: an innovative modular *in silico* model to predict skin sensitisation potential

E. Bourgart[1], C. Charmeau-Genevois[1], G. Levet[1], Z. Todorov[1], F. J. Bauer[1], E. Ay-Albrecht[1], P. C. Thomas[1]

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

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

Introduction

iSafeRat® CLASS (Classification & Labelling Assessment for Skin Sensitisation) is an innovative model mostly based on the first key events of the skin sensitisation (SS) adverse outcome pathway (AOP) leading to protein adduct formation. This model is designed to predict SS with high accuracy using a combination of four independent modules organised in an optimised decision tree to generate the final consensus (*i.e.*, skin sensitiser/not skin sensitiser). First, iSafeRat® Mechanism of toxic Action Premium (MechoA Premium)[1] is a robust and efficient Structure Activity Relationship (SAR) model which predicts Molecular Initiating Events (MIE)[2]. Among others, it efficiently detects reactive parent compounds (haptens) and pro-reactive substances (pro-haptens). Then, an autoxidation SAR model predicts whether substances encompass chemical features susceptible to become reactive after reaction with oxygen (pre-haptens). Thirdly, another SAR informs if positivity is to be expected in an LLNA study and believed inconsistent by comparison with results from other *in vivo* tests (*e.g.*, GPMT or Buehler). Finally, a skin penetration module estimates if a substance can sufficiently cross the stratum corneum to trigger skin sensitisation.

In this study, we present the validation of iSafeRat® CLASS using an in-house database constituted of LLNA studies assessed for their quality beforehand. Further, the ability of iSafeRat® CLASS and the Defined Approach for Skin Sensitisation (DASS) automated workflow of the OECD QSAR Toolbox (QSTB) to predict LLNA outcomes were compared based on the official LLNA dataset gathered by the OECD working group.

Methods

Database: LLNA data from the NICEATM dataset, the CosUE dataset[3], the ECHA dossiers and proprietary data were evaluated for their reliability according to the OECD 429 guideline (LLNA). Inorganic substances, organic salts and multi-constituents were removed from the database. The final database was composed of 647 substances (360 sensitisers and 287 non-sensitisers). Reliable experimental LogP were used when possible. Several models were investigated for logP predictions for missing values. iSafeRat® KOW v2.0 gave reliable results, but several substances were found to be out of domain, thus KOWWIN v1.68 was selected to predict all values to have a more readily traceable dataset.

Data splitting: Chemical structures were characterised using the Pubchem fingerprint, and a similarity matrix was generated based on Tanimoto coefficient. A similarity cut-off of 0.605 was used to regroup structurally similar substances into clusters. Next, the random method was applied to assign compounds to a training set and an external validation set (80:20 ratio). Clusters containing only one substance were automatically allocated to the training set.

Model building: The training set (506 substances) and scientific literature were used to develop and/or train the different modules of iSafeRat® CLASS. MechoA Premium v1.1 module was refined starting from a previously published MechoA version[1]. The three other modules were developed *de novo*. For instance, physico-chemical cut-off limits for skin penetration were based on the R.7C chapter of REACH guidance[4] and the autoxidation module was based on most likely C=C bonds for autoxidation reaction.

Model validation & performance: Confusion matrices were applied to assess iSafeRat® CLASS v1.1 & QSTB v4.5 DASS performances on the internal database and/or the OECD dataset.

Table 1. *Validation data of iSafeRat® CLASS v1.1 implemented in iSafeRat® Desktop v4.2.19.*

Dataset	Size (N)	Accuracy (%)	Balanced accuracy (%)	Sensitivity (%)	Specificity (%)
Training	506	67.8	67.2	72.0	62.5
External validation	141	75.9	74.4	88.5	60.3

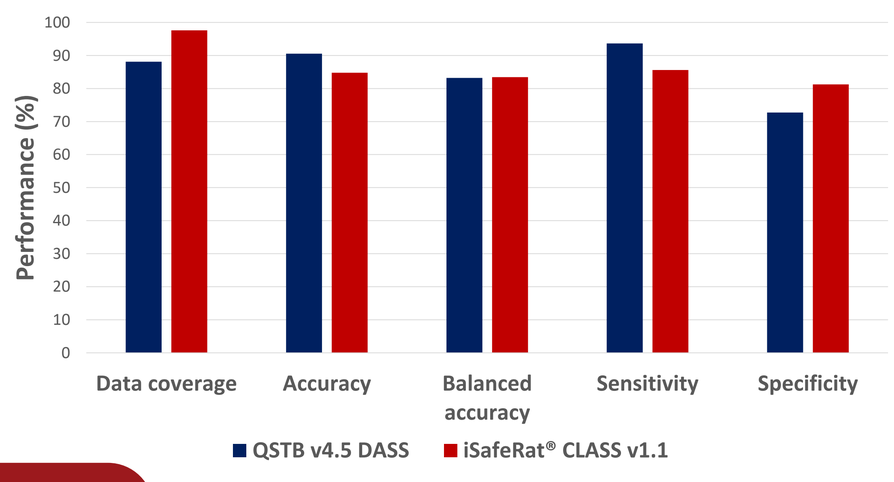
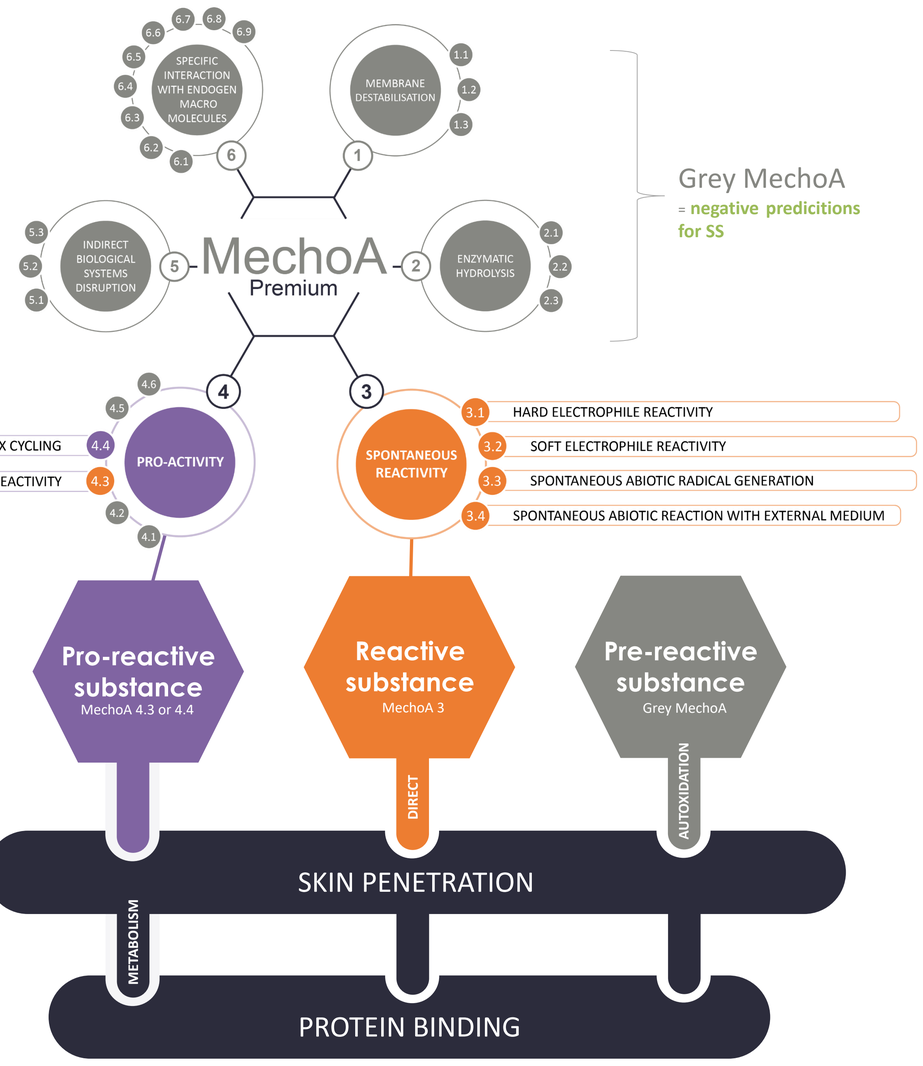


Fig 1. *Comparison of iSafeRat® CLASS v1.1 (iSafeRat® Desktop v4.2.19) and the DASS model (QSTB v4.5) performances toward the OECD LLNA dataset (N = 168).*



***In Silico* CLASSIFICATION and LABELLING ASSESSMENT for SKIN SENSITISATION (iSafeRat® CLASS) COMBINING 3 CONCEPTS:**

1) ISafeRat® MechoA 2) Autoxidation profiler

3) Skin penetration predictions

Results

The performances were conveyed in terms of **data coverage** (*i.e.*, percentage of substances of a dataset that are within the applicability domain of a model), **accuracy** (*i.e.*, percentage of accurate predictions of model), **balanced accuracy** (*i.e.*, [sensitivity + specificity]/2), **sensitivity** (*i.e.*, percentage of sensitisers that are predicted as such by a model) and **specificity** (*i.e.*, percentage of non-sensitisers predicted as such by a model).

The validation data of CLASS performed well when challenged with a large diversity of structures (Table 1). Variations in performances were observed between the training and the external validation set. Overall, the **accuracy**, **balanced accuracy** and the **sensitivity** were higher with the test set, while the **specificity** was conserved regardless of the dataset. These differences partially arise from the enrichment of the training set in mono-substance clusters that are misclassified by the model.

The performance of CLASS and the QSTB on the LLNA dataset established by the OECD 497 guideline group were compared (Fig. 1). Both models demonstrated high performance with LLNA outcomes predictions as illustrated by the **balanced accuracy**. The **data coverage** of CLASS was superior by 10 points, demonstrating a larger applicability domain. The **specificity** of CLASS was 10% higher compared to the QSTB while its **sensitivity** was 8% lower. However, a strict comparison of both models is difficult since the outcomes of QSTB are either a prediction (13.5% of outcomes) or a read-across (86.5% of outcomes).

Conclusion

This poster describes the development and validation of a new conceptual model, **iSafeRat® CLASS**, combining three different SARs to elucidate the ability of a substance to trigger the first key events leading to skin sensitisation in the AOP. In addition, another SAR highlights the cases where LLNA results diverge from the other *in vivo* tests (*e.g.*, Buehler, GPMT).

The model was highly predictive of LLNA outcomes for organic substances including some organo-halogens, with accuracy and sensitivity reaching over 70%. Moreover, performances of our model are highly comparable with recognized models such as the DASS automated workflow of the QSTB. However, iSafeRat® CLASS is an AOP integrative model, while the outcome from QSTB which can be based on a read-across. In this case, the approach must be strongly justified in a RAAF document in a REACH dossier.

Finally, **iSafeRat® CLASS** is in the process of being recognized as an alternative in combination with *in vitro* studies according to strategies described in the next published OECD Defined Approach (DASS) 497.

References

1. Bauer, F.J *et al.* (2018). High-accuracy prediction of Mechanisms of Action using structural alerts. Comput. Toxicol., 7, 36–45.
2. MechoApedia: https://www.kreatis.eu/MechoA/
3. Hoffmann, S. *et al.* (2022). Expansion of the Cosmetics Europe skin sensitisation database with new substances and PPRA data. Regul Toxicol Pharmacol., 131 105-169.
4. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7c: Endpoint specific guidance Version 3.0. European Chemical Agency (2017).

Scan QR code to download the poster