A high accuracy predictor of LLNA skin sensitisation outcome

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INTRODUCTION

iSafeRat® Mechanism of toxic Action (MechoA) profiler[1], included in the OECD Toolbox 4.5, is a robust and efficient Structure Activity Relationship (SAR) model which predicts Molecular Initiating Events (MIE) and further key events in many cases[2]. MechoA 3 class detects reactive electrophilic parent compounds, while MechoA 4.3 sub-class detects pro-reactive substances, which are both responsible for protein adducts. MechoA 4.4 sub-class detects chemicals which can undergo cyclic metabolic oxidation and reductions generating reactive radicals capable of averse reactions with proteins. Other classes are predicted non-sensitising. The scheme constitutes a useful, but insufficient predictor of MIE of the adverse outcome pathway (AOP) for skin sensitisation (SS). This was demonstrated when tested on a previous version of the Cosmetics Europe SS Database (CESSD)[3].

In this study, an enhanced version of iSafeRat® MechoA has been combined with other in-house *in silico* tools, including an autoxidation profiler and skin penetration predictions, and was compared with skin sensitisation data in the updated version of the CESSD[4] with 169 substances.

METHODS

In an earlier preliminary study, MechoA predictions for the CESSD substances were considered as **positive** for SS if MechoA 3 and/or 4.3 and/or 4.4 was triggered when comparing with **LLNA** and **Human data** where metabolism can occur. No metabolism system is included in the **DPRA** method. Therefore, only MechoA 3 predictions triggered an alert when comparing with this method. A prediction was considered **negative** if other MechoA classes were triggered (grey MechoAs in Figure 1).

In this study, we developed an autoxidation profiler and skin penetration prediction tool and combined them with the MechoA model. First, we predicted the MechoA of the parent substance. Then, for substances from MechoA classes expected to be non-sensitisers we determined if the substance contains structures likely to be autoxidised, forming reactive products and leading to positive experimental results. For MechoAs predicted as **positive** and when autoxidation products were generated, the rate of skin penetration (*i.e.* low, moderate or high) was estimated. If a MechoA was predicted **positive** with moderate or high skin penetration, then the substance was predicted to be a skin sensitiser. Otherwise the prediction was considered **negative**.

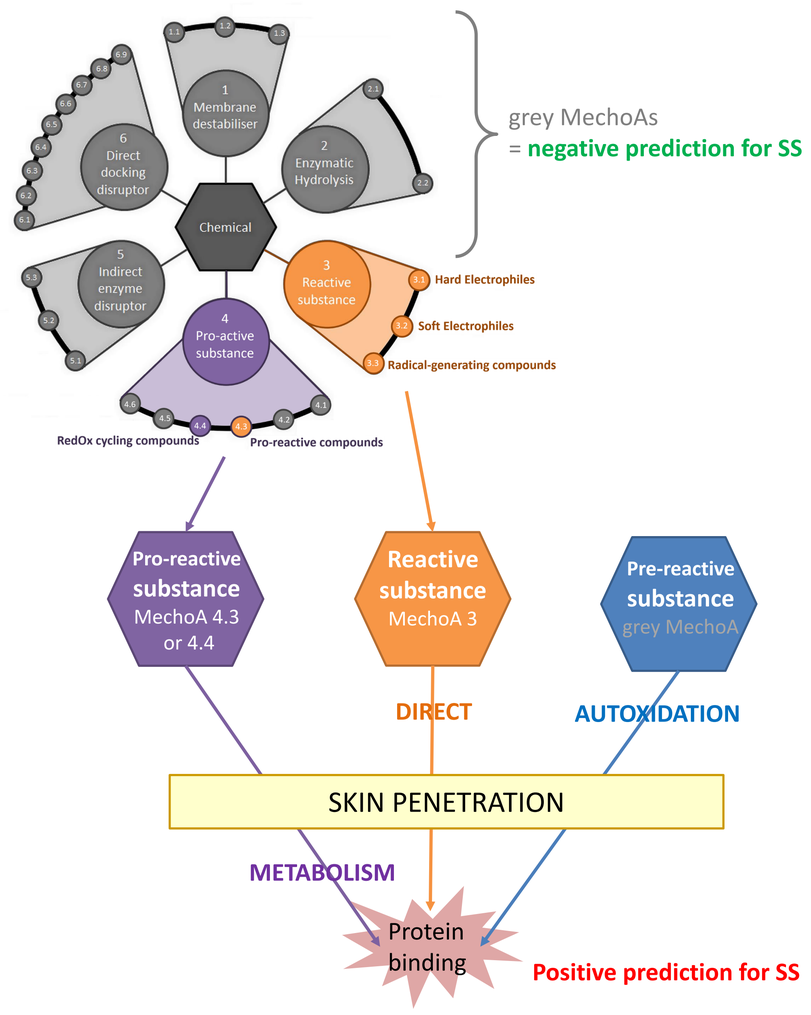

Figure 1: Positive or negative predictions for SS depending on MechoA of parent substance, autoxidation product(s) or metabolites.

In Silico CLASSIFICATION and LABELLING ASSESSMENT for SKIN SENSITISATION (iSafeRat® CLASS)

COMBINING 3 CONCEPTS:

1) iSafeRat® MechoA 2) Autoxidation profiler 3) Skin penetration predictions

Reaches an accuracy, sensitivity and specificity ca. 95% for the LLNA study

Table 1: Sensitivity and specificity of Enhanced iSafeRat® MechoA scheme model relative to *in vivo* studies using CESSD database.

Method type	MechoA 3 &/OR 4.3 &/OR 4.4 vs.	Inside AD*	Accuracy (%)	Sensitivity (%)	Specificity (%)
Preliminary study					
in vivo	LLNA	160	61	57	70
in vivo	Human potency	139	63	58	69
Main study					
in vivo	**LLNA**	**162**	**96**	**96**	**94**
in vivo	Human potency	141	77	**87**	61

Table 2: Sensitivity and specificity of Enhanced iSafeRat® MechoA scheme model relative to DPRA using CESSD database.

Method type	MechoA 3	Inside AD*	Accuracy (%)	Sensitivity (%)	Specificity (%)
Preliminary study					
in chemico	DPRA	147	47	30	75
Main study					
in chemico	DPRA	149	62	57	72

RESULTS

In the preliminary study, a correlation was observed between chemicals triggering MechoA 3 and/or 4.3 and/or 4.4 and positive results observed for SS in LLNA and with Human data with a sensitivity of **57** and **58%** respectively (see Preliminary study Table 1). MechoA 3 only correlated with **30%** DPRA positives which are restricted to the detection of electrophilic parent substances in the absence of metabolism in the test (see Preliminary study Table 2).

In this study, the gains in specificity and sensitivity are presented (see Main Study Tables 1 & 2). Structural MIE updates made to the MechoA profiler together with assessment of autoxidation potential of substances (*e.g.,* terpenes) considerably increased the sensitivity of iSafeRat® model for Skin Sensitisation relative to *in vivo* studies (LLNA, Human tests, Table 1) and DPRA (Table 2). However, the implementation of the latter also decreased the specificity of the model relative to Human Data and DPRA. This decrease is likely due to the heterogenicity of human data which are generated using three different methods (HMT, HRIPT and DPT) and to reduced concentrations sometimes employed in human studies, while LLNA is always performed according to OECD 429 up to 100% in the absence of skin irritation. Regarding DPRA, the improvement may stem from autoxidation kinetics in lab-dependent lighting conditions or the formation of non-specific substance-peptide weak bonds during the experiment. Considering the skin penetration of substances enhanced the specificity of the model relative to LLNA studies. For instance, hexane, initially predicted skin sensitiser showed negative results in the *in vivo* experiments. Because of its high volatility, the amount of substance reaching viable epidermis as predicted by the model, is insufficient to induce SS. As a result of these developments, the performances of the new enhanced model, named « **iSafeRat CLASS** » substantially increased, especially relative to LLNA with **sensitivity and specificity** reaching circa **95%**.

CONCLUSIONS

This poster describes a new model, **iSafeRat® CLASS**, using the MechoA profiler refined to predict with high accuracy the SS potential of parent substances or substances that undergo autoxidation or skin metabolism leading to the formation of electrophile products which can penetrate the skin at sufficient rate to induce SS.

The model was highly predictive of LLNA outcomes for organic substances including some organo-halogens, with accuracy, sensitivity and specificity all circa 95%. Our efforts are currently focused on the recognition of our model as a validated alternative to *in vivo* experiments for regulatory requirements especially in combination with *in vitro* studies as recommended in the OECD Defined Approach 497. Future enhancements will aim to extend the applicability domain of our model and the classification of substances by predicting EC_3 values.

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