Strengthening skin sensitisation predictions combining enhanced MechoA and *in silico* skin penetration predictions

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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 detects pro-reactive substances, which are both responsible for protein adducts. MechoA 4.4 detects chemicals which can undergo cyclic metabolic oxidation and reductions generating reactive radicals such as thiyl radicals capable of forming disulfide bonds with proteins. Such categorisations constitute a necessary, but insufficient MIE of the adverse outcome pathway (AOP) for skin sensitisation (SS).

MechoA was previously tested for its performance to identify Skin Sensitisation potential of the substances listed in the Cosmetics Europe Skin Sensitisation Database (CESSD)[3]. In this study, iSafeRat® MechoA has been combined with other *in silico* tools, such as an in-house autoxidation profiler and skin penetration predictions, to improve the level of accuracy previously observed to identify the CESSD skin sensitisers.

METHODS

In the 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 is considered **negative** if only MechoAs different than these MechoA classes were triggered (grey MechoAs in Figure 1).

In this study, we have developed an autoxidation profiler and skin penetration prediction tool and combined them with the MechoA model. First, we determine if the substance can be autoxidated to form reactive products. Then, we predict the MechoA of the parent substance. If positive MechoAs are triggered or autoxidation products generated, the rate of skin penetration (i.e. low, moderate or high) is estimated. If a positive MechoA is triggered together with a moderate or high skin penetration, then the substance is predicted to be a skin sensitiser. This method was applied to CESSD substances to evaluate its performance.

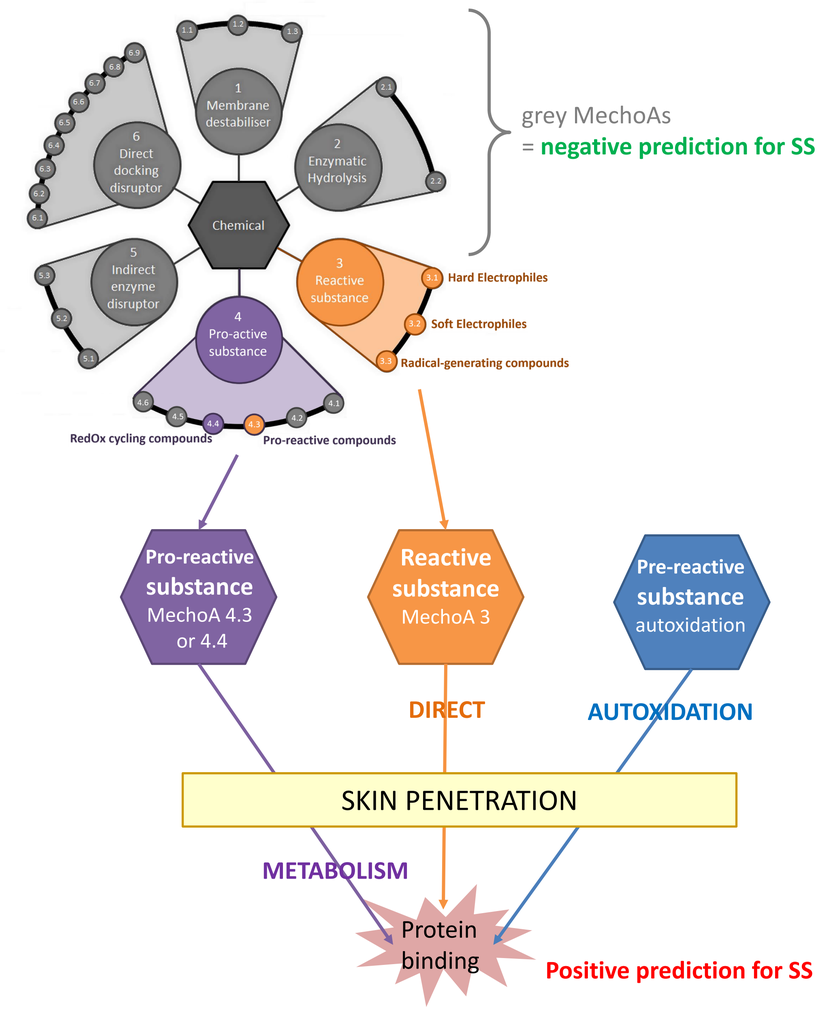

Figure 1: Positive or negative predictions for SS depending on MechoA of parent substance or autoxidation product(s).

HIGH-ACCURACY PREDICTION OF SKIN SENSITISATION, COMBINING 3 CONCEPTS :

1) MechoA model 2) Autoxidation profiler 3) Skin penetration predictions

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	120	61	55	77
in vivo	Human potency	120	66	59	80
Main study					
in vivo	LLNA	120	77	70	**92**
in vivo	Human potency	120	77	71	**89**

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	116	53	37	76
Main study					
in chemico	DPRA	116	63	59	68

RESULTS

In a preliminary study, a raw comparison of predictions for SS potential based on MechoA classes was performed. 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 55 and 59% respectively. MechoA 3 only correlated with 37% DPRA positives which are restricted to the detection of electrophilic parent substances in the absence of metabolism in the test.

In the main study, the gains in specificity and sensitivity are presented in Tables 1 & 2. Considering the autoxidation potential of substances (*e.g.* terpenes) considerably increased the sensitivity of iSafeRat® model for SS relative to experimental studies (LLNA, Human patch test, Table 1) and DPRA (Table 2). However, the autoxidation of terpenes also decreased the specificity of the predictions relative to DPRA due to inconsistent experimental results. These may greatly depend on the autoxidation kinetics of test substances incubated during 24h in lab-dependent lighting conditions. Taking into account the skin penetration of substances enhanced the specificity of the model relative *to in vivo* studies. For instance, hexane, initially predicted MechoA 4.3 (*i.e.* positive for SS following metabolism) showed negative results in the *in vivo* experiments. Considering its high volatility (based on vapour pressure), the amount of substance reaching viable epidermis following skin penetration, as predicted by the model is insufficient to induce SS. As a result, model predictions have substantially gained from combining MechoA with skin penetration prediction and the autoxidation profiler, with sensitivity and specificity reaching respectively circa 70% and 90% relative to LLNA and human clinical data.

CONCLUSIONS

This poster describes a new integrated MechoA model, iSafeRat® SS model, specifically refined to predict with high accuracy the skin sensitisation potential of:

- Substances which are not reactive, but could undergo autoxidation leading to the formation of electrophiles capable of SS following skin penetration
- Substances which are direct electrophiles or as pro-actives but without inducing SS based on insufficient rate of skin penetration

iSafeRat® SS model can now predict a substance as non skin sensitising with high accuracy (89-92%) or as skin sensitiser with good reliability (around 70%) and we will continue to improve performance with the inclusion of further parameters than those discussed here. Currently, the use of this model can provide useful help in prioritizing and decision making in Research & Development contexts.

REFERENCES

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