PREDICTING IN SILICO THE DIRECT-PEPTIDE-REACTIVITY-ASSAY (DPRA) WITHIN THE ALLERGIC CONTACT DERMATITIS FRAMEWORK

A. Detroyer[b], J. Eilstein[b] C. Piroird[b], S. Imbert[b], A. Del Bufalo[b], H. Ivanova[a], I. Popova[a], C. Kuseva[a], Y. Karakolev[a], S. Dimitrov[a], O. Mekenyan[a]

[a]Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", "Yakimov" St. 1, bourgas 8010, Bulgaria
[b]l'Oréal Research and Innovation, Aulnay-sous-Bois, France.

INTRODUCTION

Allergic Contact Dermatitis (ACD) depends, amongst other parameters, on the ability of chemicals to covalently bind with skin proteins. Thus, *in chemico* methods like Direct-Peptide-Reactivity-Assay (DPRA) were developed as one of the elements of the battery of assays to replace alternatives to the animal ACD tests, i.e Local-Lymph-Node-Assay (LLNA).

In this context, mechanistically based *in silico* DPRA models were built, to potentially be used for the screening/design of new chemicals and to help evaluating experimental DPRA results at the tests' limits (low solubility chemicals, ...).

The modeling concept presented aims at providing a mechanistic justification and a transparency of the predictions, taking into account the experimental DPRA data variability.

CONCLUSION

We set forth to build mechanistically justified DPRA, i.e. Cysteine and Lysine peptide alerts, at the 13% and 42% reactivity level with a 95% confidence. The 4 mechanistic *in silico* DPRA models presented here provide good predictive performance, a high transparency (justifications, applicability domain) but also take into account the experimental DPRA data variability (grey zones of prediction). A simulator of abiotic oxidation is combined with the reactivity model. Hence, the oxidative activation products are also evaluated along with the target chemicals. As such, they comply to the *in silico* model requirements (e.g. from OECD) for their use in the alternative method's framework and are one of the first to reply to the latest trend in regulatory toxicology to include explicitly experimental variability.

Today, the models were built to potentially be used for the screening (classification info) and/or design of new chemicals (mechanistic info) but also to help evaluate experimental DPRA results at the tests' limits (ex. low solubility chemicals,...). To make a clear-cut conclusion on the skin sensitization potency of any chemical, the DPRA result should be combined with complementary information's derived from other assays[c].

References

a- Journal of Applied Toxicology: 'Accounting for data variability, a key factor in in *vivo/in vitro* relationships: application to the skin sensitization potency (*in vivo* LLNA versus in vitro DPRA) example'; S. Dimitrov, A. Detroyer, C. Piroird, C. Gomes, J. Eilstein, T. Pauloin, C. Kuseva, H. Ivanova, I. Popova, Y. Karakolev, S. Ringeissen and O. Mekenyan; DOI:0.1002/jat.3318
b - Gerberick GF, Ryan CA, Dearman RJ, Kimber I. 2007a. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. Methods 41: 54–60.
c - Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E, Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S, Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R, Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhler S, Phrakonkham P, Piersma
A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tähti H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar J-M. 2010. Alternative (non-animal) methods for cosmetics testing: current status and future prospects – 2010. Arch. Toxicol. 85: 367–485.

RESULTS

1 Determining DPRA thresholds taking into account experimental data variability *(grey zones)*

In our published DPRA variability study[a], the variation of the 95% confidence interval of the mean observed % depletion for Cysteine (Ac-RFAACAA-COOH) and Lysine (Ac-RFAAKAA-COOH) peptides, for 29 and 27 mainly overlapping chemicals with five and/or more depletion values was studied (Fig 1)

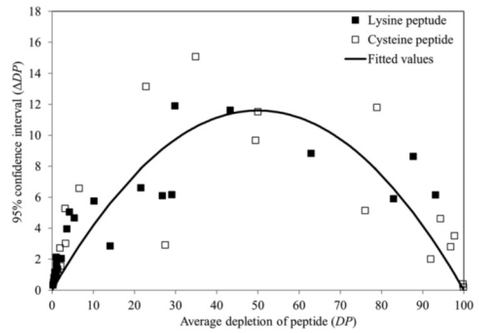

Figure 1

The parabolic trend of the confidence interval was similar for the two synthetic peptides and could be overall fitted with a polynomial function of second order. The following model was derived for a 95% confidence interval:

$$\Delta DP = -0.46\ DP + 0.0046DP^2 \quad \text{with } N = 52\ ;\ R^2 = 0.83\ ;\ s = 2.5$$

where DP is the depletion of the peptide in percent and ΔDP is the half of the 95% confidence interval.
Next, a methodology was developed, introducing data variability into the threshold optimization which could be applied to any set of data. The optimized DPRA (maximum % between Cysteine and Lysine peptide) thresholds best correlating with LLNA predefined thresholds for 80 chemicals (with both DPRA and LLNA data) (respectively 13,3% and 42,3%, see Fig. 2) are in agreement with the already suggested cut-off values for classifying reactivity [a, b].
Moreover, data uncertainty creates 'grey zones' around the thresholds where the true values could be larger or smaller compared to the classification threshold. To determine the grey zones around the DPRA thresholds, we then proposed:

$$DPMin\ Thr + \Delta DP \geq DPThr$$
$$DPMax\ Thr - \Delta DP \leq DPThr$$

where DPThr is the depletion of the peptide threshold in percent and ΔDP is the half of the 95% confidence interval.The obtained optimal DPRA thresholds and their corresponding grey zones with 95% confidence are shown in Fig. 2.

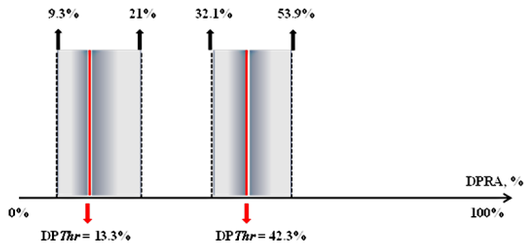

Figure 2: DPRA optimized thresholds DP*Thr* and their corresponding grey zones at 95% confidence

2 Constructing mechanistic binary in silico DPRA models

Based on the obtained optimal thresholds, 4 mechanistically justified DPRA reactivity *in silico* models have been developed for:

- Cysteine (DPRA 13%) (check overview in Fig. 3)
- Cysteine (DPRA 42%)
- Lysine (DPRA 13%)
- Lysine (DPRA 42%)

The training sets of Cysteine and Lysine peptide models comprising more than 150 chemicals with experimental data, cover different chemical classes (e.g. carbonyl and dicarbonyl compounds, carboxylic acid and esters, alcohols, etc.).
Each model comprises chemical subclasses falling in the range of variation of its own experimentally measured peptide depletion data. The sub-classes associated with each binding potency range are characterized by structural boundaries (DPRA structural alerts) justified by theoretical mechanisms on interactions of chemicals with the synthetic peptides. For each model, the DPRA structural alerts have been prioritized into three potency categories based on the depletion data for ex. DPRA threshold of 13% for Cysteine (Fig. 3):

A/DPRA less than 9% (DPRA 13%) - accounting for left boundary of grey zone with 95% confidence
B/Grey zone 9-21% (DPRA 13%) - falling in grey zone with 95% confidence
C/DPRA above 21% (DPRA 13%) - accounting for right boundary of grey zone with 95% confidence

These DPRA alerts (prioritized by their binding potency) are applied on target chemicals and their oxidative derivatives generated by abiotic activation simulation. Subsequently, predictions are provided by applying DPRA model associated with the identified potency alert. The worst case scenario is applied when more than one alert is identified. Similarly, the worst scenario is applied with respect to the set of parent and generated autoxidation products, i.e., the prediction corresponds to most potent structure.

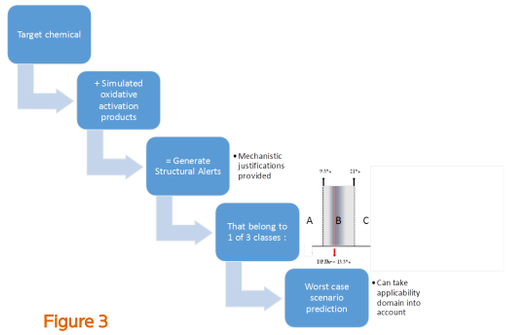

Figure 3

These four models were integrated in the OASIS TIMES platform and predictions were made transparent by the presence of mechanistic justifications and indications for the chemicals' belonging to the applicability domain of the models.

These models present good predictive performance of the different experimental binding results within the training set chemicals (Table 1)

Model	Sensitivity, %	Specificity, %	Accuracy, %
Cys (DPRA 42%)	95	81	87
Cys (DPRA 13%)	93	85	90
Lys (DPRA 42%)	93	96	95
Lys (DPRA 13%)	98	89	93

Table 1

L'ORÉAL Research & Innovation