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Alternatives for Skin Sensitization testing
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Case study 6:
The artificial neural network model for predicting LLNA EC3

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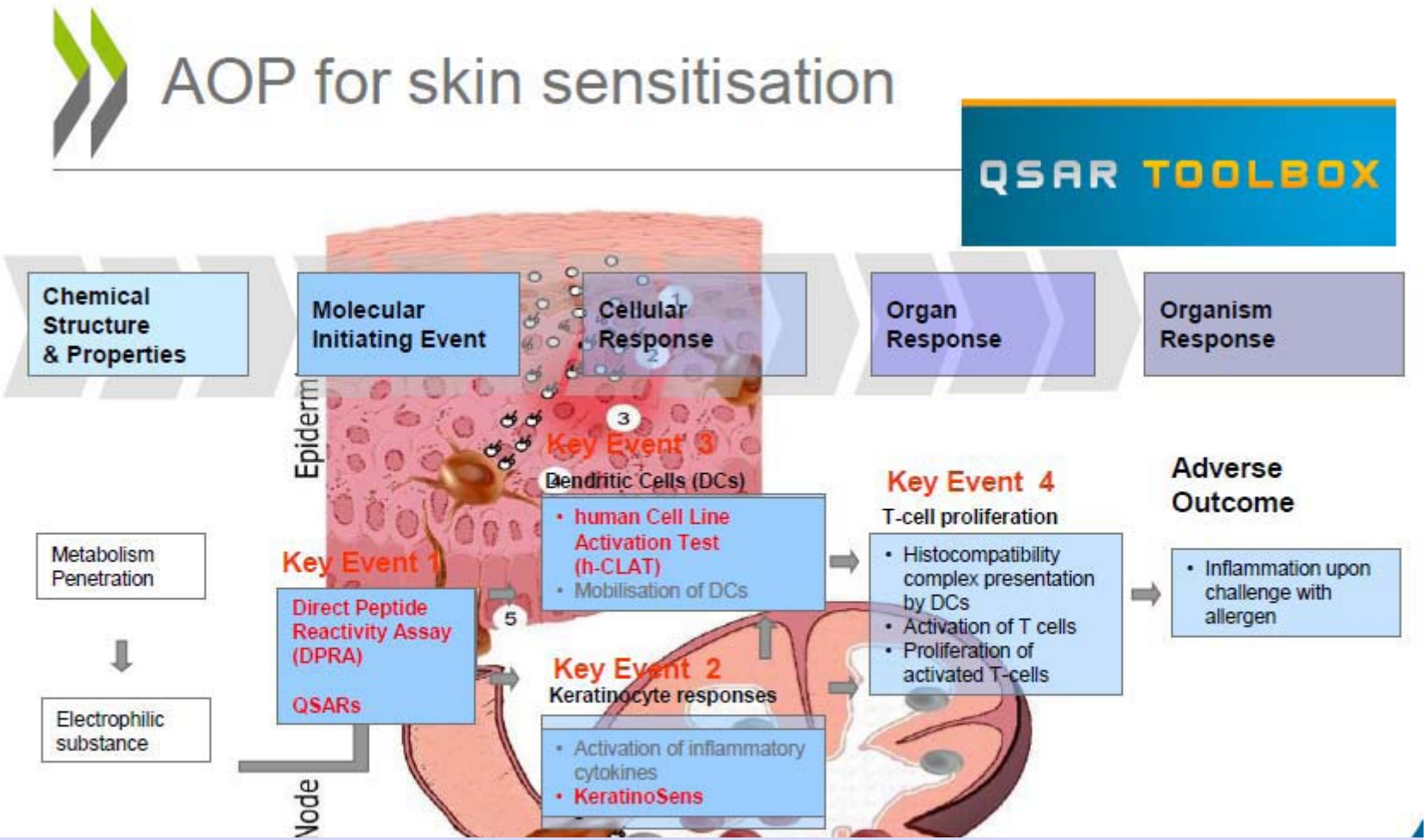
Aim of the study

Due to regulatory constrains and ethical concerns, alternatives to animal testing are needed to predict skin sensitizing potential of chemicals.

To do risk assessment, potency evaluation is essential and the relative potency of targeted chemicals has been mainly valuated by calculating EC3 value, which is obtained from LLNA.

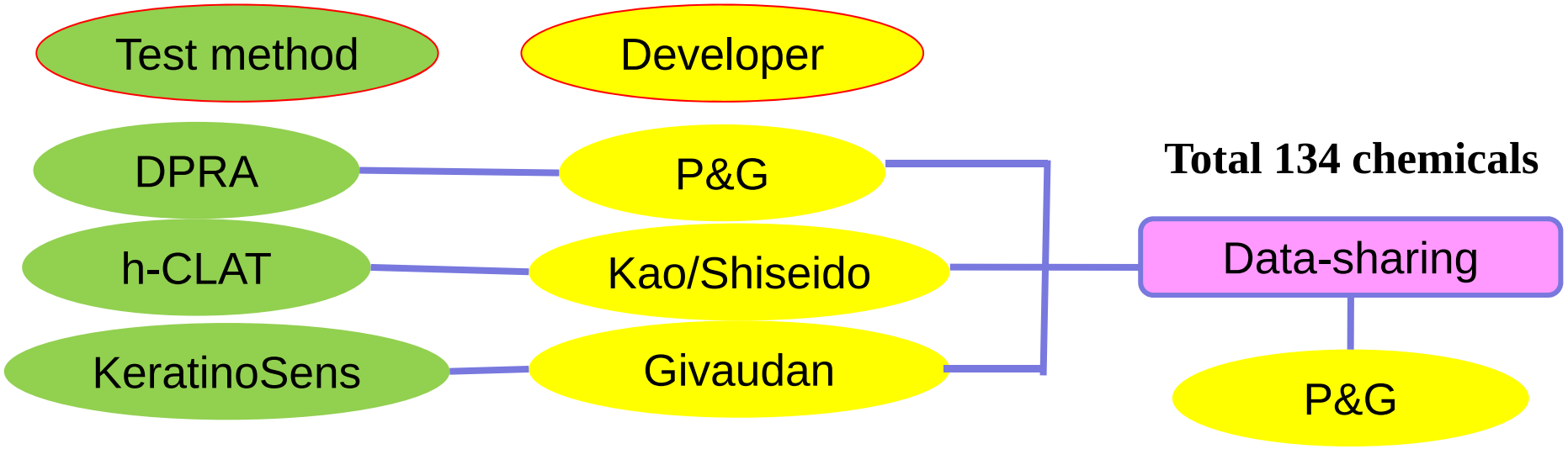
In this study, I propose a skin sensitization potency prediction model using artificial neural network analysis of data from multiple in vitro assays. EC3 value can be predicted by using this model, and the predicted EC3 value can be applied for prediction of a safe level of human exposure using a Quantitative Risk Assessment (QRA) approach.

Key events of the AOP and corresponding methods



Key event 1: Peptide reactivity (DPRA or SH test)
Key event 2: Keratinocyte response (KeratinoSens™ or ARE assay)
Key event 3: Dendritic cell activation (h-CLAT)

Dataset used in this study

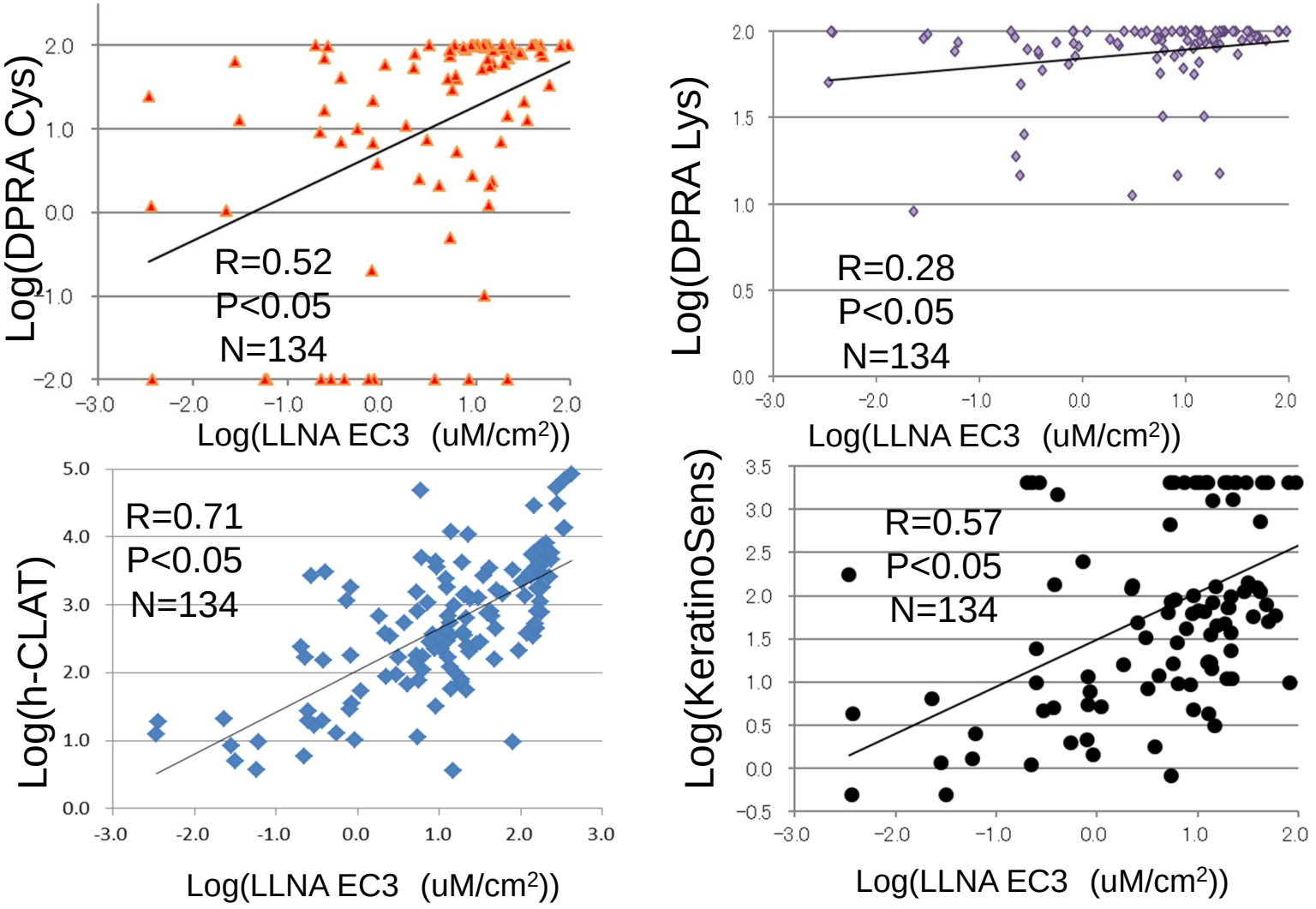


1. **All 134 chemicals were used for model development. Then 10-fold cross (inner) validation was conducted.**

2. **10 new (un-learned) chemicals were evaluated to test the model.**

Many thanks P&G, Givaudan and Kao for permission to use this dataset.

Relationship between in vitro parameters and EC3



Each descriptor derived from DPRA (Cys), DPRA (Lys), h-CLAT and KeratinoSens correlated with LLNA EC3, significantly. However, each single indicator seems to be not enough for risk assessment due to variability. How should we fuse them?

Artificial Neural Network (ANN) analysis

In vitro test data

- h-CLAT
- DPRA-Cys
- DPRA-Lys
- **KeratinoSens**
- Other biomarkers

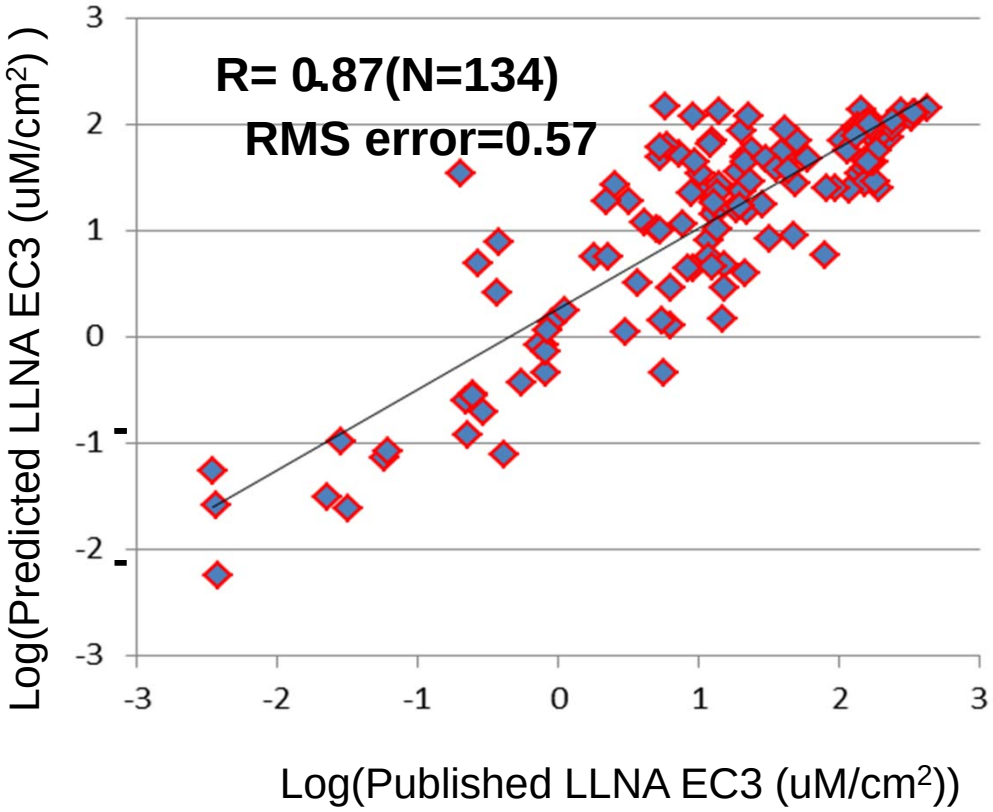
In vivo test data

LLNA EC3

- EC3 values for LLNA positive chemicals
- For LLNA negative chemicals, 101% was used as EC3 value (converted to μM/cm^2 unit).

Input layer | Hidden layer | Output layer

Correlation between LLNA and the ANN prediction



After 10-fold cross-validation

*Root mean square (RMS) error $= \sqrt{\sum((\text{measured value} - \text{predicted value})^2) / \text{number of data}}$

This ANN model showed a good correlation between predicted values and actual values.

Category predictive capacity of the ANN model

Predicted	LLNA				
	5	4	3	2	1
	Extreme	Strong	Moderate	Weak	NS
5	7	1	0	0	0
4	1	10	3	0	0
3	0	4	20	6	0
2	0	0	9	18	9
Negative(2/3)	0	3	7	6	30

EC3≤0.1% ; Extreme, 0.1%<EC3≤1%; Strong, 1%<EC3≤10%; Moderate, EC3>10%; Weak, NS; not-sensitizing in LLNA or Negative in in vitro tests. Positive/negative identification: 2 out of 3 approach.

For five category classification (extreme, strong, moderate, weak and non-sensitizer), the accuracy is 63%.

Test the ANN model by newly evaluated chemicals

This work has been conducted as a joint study between Merck (Dr. Thomas Broschard) and Shiseido.

	Actual LLNA data		Estimated results (In vitro and ANN)	
Substance	EC3 (%)	Category	EC3 (%)	Category
Item1	-	Negative	Not soluble in DPRA	-
Item2	28.6	Weak	33.6	Weak
Item3	-	Negative	0.2	Strong
Item4	-	Negative	Not soluble in DPRA	-
Item5	-	Negative	45.8	Weak
Item6	(GPMT)	Negative	76.6	Negative
Item7	29.4	Weak	56.2	Weak
Item8	47.7	Weak	37.4	Weak
Item9	-	Negative	24.9	Weak
Item10	14.5	Weak	Co-elusion in DPRA	-

3 of 10 chemicals were out of applicability domain of DPRA.

6 of the 7 chemicals were predicted almost correctly (weak or non-sensitizer).

1 chemical (all three in vitro tests positive) was overestimated with our model.

Main limitations and uncertainties of the approach

- ✓ Because these in vitro methods are submerged cell-based assay or HPLC assay, water-insoluble chemicals are difficult to evaluate correctly.

- ✓ Because of limited metabolic capability of the cell lines and experimental conditions, pro-haptens (i.e. chemicals requiring enzymatic activation) and pre-haptens (i.e. chemicals requiring oxidation) are thought to be out of the applicability domain.

- ✓ This approach is currently not designed to predict complex mixtures.

- ✓ Though the artificial neural network is thought to be a useful tool especially for prediction of complex biological response, there is a so-called "black box" in the process of the decision. Furthermore, we need to discuss how we show its validity.

Conclusions and future…

- Each event in the AOP of skin sensitization (protein-binding, dendritic cell activation and keratinocyte response) might affect the potency.
- The correlation between actual EC3 value and predicted one was good. Therefore, our ANN model can contribute to building a new QRA evaluation system which is using no animals.
- The results of the newly tested chemicals showed both the usefulness and limitations of this approach.
- We should discuss more how to prove the validity of the ANN model.