Original Article

***In silico* risk assessment for skin sensitization using artificial neural network analysis**

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ABSTRACT — The sensitizing potential of chemicals is usually identified and characterized using *in vivo* methods such as the murine local lymph node assay (LLNA). Due to regulatory constraints and ethical concerns, alternatives to animal testing are needed to predict the skin sensitization potential of chemicals. For this purpose, an integrated evaluation system employing multiple *in vitro* and *in silico* parameters that reflect different aspects of the sensitization process seems promising. We previously reported that LLNA thresholds could be well predicted by using an artificial neural network (ANN) model, designated iSENS ver. 2 (integrating *in vitro* sensitization tests version 2), to analyze data obtained from *in vitro* tests focused on different aspects of skin sensitization. Here, we examined whether LLNA thresholds could be predicted by ANN using *in silico*-calculated descriptors of the three-dimensional structures of chemicals. We obtained a good correlation between predicted LLNA thresholds and reported values. Furthermore, combining the results of the *in vitro* (iSENS ver. 2) and *in silico* models reduced the number of chemicals for which the potency category was under-estimated. In conclusion, the ANN model using *in silico* parameters was shown to have useful predictive performance. Further, our results indicate that the combination of this model with a predictive model using *in vitro* data represents a promising approach for integrated risk assessment of skin sensitization potential of chemicals.

Key words: Skin sensitization, LLNA, Risk assessment, Artificial neural network, *In silico*

INTRODUCTION

Tests for skin sensitization potential are required prior to the introduction of new cosmetic ingredients. Historically, animal models such as the guinea pig maximization test (Magnusson and Kligman, 1969) were used to determine whether a chemical has the potential to induce skin sensitization in humans. More recently, the murine local lymph node assay (LLNA), in which cellular proliferation in the draining lymph node is measured after repeated topical application of the test compound onto the ears, has been employed (Basketter *et al.*, 2002). The relative potency of a chemical sensitizer is measured in terms of the EC3 value, which is the concentration of the test chemical necessary to produce threshold stimulation of proliferation in draining lymph nodes as compared with concurrent vehicle controls (Basketter *et al.*, 2002). Based on EC3 values, chemicals can be classified into five potency categories (extreme, strong, moderate,

weak and non-sensitizers), and it is also possible to predict a safe level of human exposure using a quantitative risk assessment approach (Api *et al.*, 2008). However, a recent EU cosmetic directive has prohibited the sale of cosmetic products containing ingredients that have been tested in animals. For this reason, and because of ethical concerns, great efforts have been made to develop alternatives to animal testing to predict the skin sensitization potential of chemicals. Most of these alternative methods use the results of LLNA as *in vivo* data for prediction. This is since LLNA can provide a relative sensitization potency; information that is pivotal to evaluate the human health risks. Furthermore, it was reported that there was a linear relationship between LLNA EC3 values and human repeated insult patch tests thresholds (Basketter *et al.*, 2005).

Skin sensitization is a complex immunological response consisting of two phases, the induction phase and elicitation phase, and has been well studied over several dec-

ades (Kimber *et al.*, 2002; Aeby *et al.*, 2010; Adler *et al.*, 2011). The mechanisms involved in the induction phase, designated as the adverse outcome pathway (AOP), were reviewed recently in an OECD report (OECD, 2012). Among them, hapten-protein complex formation, production of danger signals and inflammatory mediators by keratinocytes, activation of dendritic cells and T-cell proliferation are recognized as key events. Various *in vitro* methods focused on these processes have been developed (Gerberick *et al.*, 2007; Suzuki *et al.*, 2009; Natsch and Emter, 2008; Bauch *et al.*, 2012; Emter *et al.*, 2010; Ashikaga *et al.*, 2006; Sakaguchi *et al.*, 2006; Python *et al.*, 2007; Azam *et al.*, 2006; Takahashi *et al.*, 2011; Johansson *et al.*, 2013; Lambrechts *et al.*, 2010; Vacanson *et al.*, 2014). But, because of the complexity of the biological response, it has been recognized that a single *in vitro* test is insufficient to replace animal testing and that integration of results from different *in vitro* tests, as well as *in silico* methods, is needed for prediction of the skin sensitization potential of chemicals (Jowsey *et al.*, 2006; Natsch *et al.*, 2009; Jaworska *et al.*, 2011).

There have also been many reports on prediction of skin sensitization of chemicals by *in silico* methods (Fedorowicz *et al.*, 2005; Patlewicz *et al.*, 2008; Patlewicz and Worth, 2008). One approach is to use (quantitative) structure-activity relationship ((Q)SAR) models that find mathematical relationships between various physical-chemical or structural properties and biological activity. ADMEWORKS (Fujitsu Kyushu Systems Limited, Fukuoka, Japan), TOPKAT (Accelrys, San Diego, CA, USA), TIMES-SS (University of Bourgas, Bulgaria) and Case Ultra (MULTICASE, Cleveland, OH, USA) are representative QSAR models. Some utilize fragment structures and functional groups considered to be associated with skin sensitization potential as structural alerts in an expert system, as in DEREK for Windows (LHASA Ltd., Leeds, UK). Another approach is read-across, in which chemicals are classified into groups that are considered to exhibit similar or regular patterns of physical-chemical or toxicological properties. Then, the data available for chemicals in the same category may be used for read-across to predict missing toxicological data. The OECD QSAR Toolbox (<http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm>) is a representative read-across tool. Recently, Teubner *et al.* (2013) evaluated 100 chemicals (45 sensitizers and 55 non-sensitizers) using 7 *in silico* models, including those mentioned above. The best performance was achieved by TIMES-SS, but only 16% of the test chemicals fell within its reliability domain. There were some models that could provide predictions for almost all compounds, but these

gave many false positive and false negative results. They concluded that current *in silico* models were not sufficiently accurate for broad application to predict skin sensitization potentials.

There have also been attempts to predict the skin sensitization potency of chemicals by using *in silico* evaluation as part of an integrated testing strategy (Natsch *et al.*, 2009; Jaworska *et al.*, 2011; Nukada *et al.*, 2013). In general, better predictions were obtained when an *in silico* method was used in combination with other tests in a weight-of-evidence approach, as compared with the use of the other tests alone.

We previously reported that LLNA thresholds (EC3 for LLNA-positive chemicals and maximum test concentrations for LLNA-negative chemicals) could be well predicted by using a combination of *in vitro* test data focused on protein binding, danger signaling of keratinocytes and activation of dendritic cells in an artificial neural network (ANN) analysis, designated iSENS ver. 2 (integrating *in vitro* sensitization tests version 2) (Hirota *et al.*, 2013; Tsujita-Inoue *et al.*, 2014). In the present article, we set out to predict the LLNA threshold of chemicals by means of ANN analysis using chemical structure-based determinants obtained by means of molecular orbital (MO) calculations. We also investigated whether the combination of *in vitro* and *in silico* ANN models improved the predictive performance for skin sensitization potential.

MATERIALS AND METHODS

Databases

We selected a total of 206 chemicals (164 sensitizers and 42 non-sensitizers) (Supplemental data). All the chemicals had previously been evaluated and classified with the LLNA (Gerberick *et al.*, 2005; Basketter *et al.*, 2007; Basketter and Kimber, 2011).

Extraction of descriptors for predicting skin sensitization

The structures of chemicals were built with Chem 3D Ultra 10.0 (CambridgeSoft Co., Cambridge, MA, USA) and modeled in their neutral forms. Geometry optimization was performed and then molecular-orbital descriptors were calculated using the PM3 Hamiltonian of a semi-empirical MO package (MOPAC2002). The calculated descriptors were as follows: Sum of charges of carbon, hydrogen, oxygen, and nitrogen (Sum C, Sum H, Sum O, Sum N), Core-Core repulsion, COSMO area, COSMO volume, dipole moment, heat of formation, electronic energy, ionization potential, total energy, polarizability α , β , γ , and molecular weight. These descriptors

were grouped by correlation coefficient, and independent descriptors that were significantly correlated with LLNA thresholds were selected for the ANN input layer.

Artificial neural network (ANN) analysis

In this study, calculated molecular descriptors and LLNA thresholds were correlated using an artificial neural network. A typical ANN consists of an input layer, a hidden layer, and an output layer, as previously reported (Hirota *et al.*, 2013). The input layer consisted of six molecular descriptors (Sum H, Sum N, heat of formation, ionization potential, polarizability β , and polarizability γ) that were correlated with LLNA thresholds, and the output layer consisted of LLNA thresholds. We used two hidden layers in this study and all calculations were performed using QwikNet Ver.2.23. A preliminary study indicated that an ANN model with a configuration of 6-4-2-1 for the input, 1st hidden, 2nd hidden and output layers was optimal in terms of RMS error. An online back-propagation algorithm was used for network training, with values of the learning rate and momentum parameter of 0.02 and 0.7, respectively. The range of weights was set to be -5 to 5 (weight perturbation; 50%).

The predictive ability of the neural network model was evaluated by means of a 10-fold cross-validation procedure (Hirota *et al.*, 2013). This method systematically removed data successively from the training set. A network model was then constructed on the basis of this reduced data set and was subsequently used to predict the removed data. This procedure was repeated for all data to obtain a complete set of predicted values. The goodness-of-fit was evaluated in terms of root-mean-square (RMS) error, defined as follows:

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

Potency estimation in LLNA and in the ANN prediction model

Skin sensitization potency classes of chemicals were taken from previous reports (Gerberick *et al.*, 2005; Basketter *et al.*, 2007; Basketter and Kimber, 2011). Sensitization potency was classified into 4 categories: extreme (EC3 in LLNA or predicted LLNA threshold < 0.1%), strong (EC3 in LLNA or predicted LLNA threshold, $\geq 0.1\%$ to < 1%), moderate (EC3 in LLNA or predicted LLNA threshold, $\geq 1\%$ to < 10%), and weak or nonsensitizer (EC3 in LLNA or predicted LLNA threshold, $\geq 10\%$ to 100%) in the ANN model.

Statistical analysis

The correlation coefficient (*r* value) was determined

by the use of Pearson's correlation statistics. Correlation analysis was performed by Excel Statistics 2004 software (Social Survey Research Information, Tokyo, Japan).

RESULTS

Extraction of molecular orbital (MO)-calculated descriptors

LLNA thresholds (EC3 for LLNA-positive chemicals and maximum test concentrations for LLNA-negative chemicals) and MO-calculated descriptors for all compounds were summarized in Supplemental Data. The compounds included 12 extreme, 20 strong, 68 moderate, 65 weak (including 1 false positive) sensitizers and 41 non-sensitizers based on reported LLNA potency classifications (Gerberick *et al.*, 2005; Basketter *et al.*, 2007; Basketter and Kimber, 2011).

The choice of the descriptors is important for ANN analysis. A larger number of descriptors improves the predictive performance in the training data set, but reduces the predictive performance for test compounds because of the vulnerability of the model. Therefore, we classified descriptors having high similarity into groups and selected descriptors that were statistically significantly correlated with LLNA thresholds from independent groups for the ANN input layer. Table 1 shows the simple correlation coefficients between MO-calculated descriptors and LLNA thresholds. Sum H, sum N, heat of formation, ionization potential, polarizability β and polarizability γ were found to have weak but significant correlations with LLNA thresholds (Table 1, Fig. 1). Then, we classified descriptors with high similarity into groups. Table 2 shows the simple correlation coefficients between MO-calculated descriptors. Correlation coefficients of less than -0.8 or more than 0.8 are highlighted. Sum C, sum H, COSMO area and COSMO volume as well as COSMO area, COSMO volume, molecular weight, polarizability α , electronic energy and total energy were classified into the same groups because of their high similarity. Finally, we identified 6 apparently independent descriptors (Sum H, sum N, heat of formation, ionization potential, polarizability β and polarizability γ) that showed significant correlations with LLNA thresholds, and used these for further ANN analysis.

Establishment of the ANN model for predicting LLNA thresholds

Using the above descriptors as the input layer and *in vivo* LLNA thresholds as the output layer in ANN analysis, we obtained a good correlation between predicted and reported values. The correlation coefficient (*r* value)

Fig. 1. Relationship between LLNA thresholds and MO-calculated descriptors showing a significant correlation in Table 1. Relationship between LLNA thresholds and Sum H (A), Sum N (B), heat of formation (C), ionization potential (D), polarizability β (E) and polarizability γ (F).

and root mean square (RMS) error were 0.791 and 0.571, respectively (Fig. 2A). When we assessed this model by 10-fold cross-validation ($N = 3$), the correlation coefficient (r value) and RMS error were 0.708 ± 0.018 and 0.660 ± 0.017 , respectively (Fig. 2B).

We further classified test samples into 4 potency categories (extreme, strong, moderate and weak sensitizer or non-sensitizer) based on the predicted LLNA thresholds (Table 3A). As shown in Table 3B, accuracy was 55%. The rates of over-prediction and under-prediction of

potency categories were 23% and 22%, respectively.

Integrated evaluation using *in vitro* and *in silico* prediction models

We previously reported that LLNA thresholds could be well predicted with iSENS ver. 2 using a combination of *in vitro* SH test, ARE assay and h-CLAT, which focus on protein binding, danger signals of keratinocytes and activation of dendritic cells, respectively (Tsujita-Inoue *et al.*, 2014). In this study, we selected 62 chemi-

Table 1. Correlation coefficient between LLNA thresholds and MO-calculated descriptors.

Correlation coefficient	Sum C	Sum H	Sum O	Sum N	Core-Core repulsion	COSMO area	COSMO volume	MW	Polarizability α	Electronic energy	Heat of formation	Ionization potential	MW	Polarizability α	Polarizability β	Polarizability γ	Total energy
log (LLNA thresholds (%))	-0.1204	0.1985	-0.0003	-0.1998	0.0908	0.1049	0.1032	0.0827	-0.0566	-0.2704	**	0.2187	0.0254	-0.1015	-0.1533	-0.4226	-0.0312

*: $p < 0.05$, **: $p < 0.01$ **Table 2.** Correlation coefficient between MO-calculated descriptors.

	Sum C	Sum H	COSMO area	COSMO volume	MW	Polarizability α	Electronic energy	Total energy	Core-Core repulsion	Sum O	Ionization potential	Heat of formation	Polarizability β	Polarizability γ	Dipole moment	Sum N
Sum C	1.0000	-0.8832	-0.8373	-0.8177	-0.7111	-0.6719	0.5466	0.5463	-0.4861	-0.1262	-0.1262	0.1241	-0.0704	-0.0566	-0.0433	-0.0058
Sum H	-0.8832	1.0000	0.9569	0.9512	0.8532	0.7849	-0.7922	-0.7925	0.7034	-0.0925	0.1956	-0.4402	0.0872	0.0719	0.0781	-0.1447
COSMO area	-0.8373	0.9569	1.0000	0.9923	0.9400	0.8579	-0.8426	-0.8805	0.6882	-0.1793	0.1973	-0.4769	0.1337	0.1918	0.1547	-0.1042
COSMO volume	-0.8177	0.9512	0.9923	1.0000	0.9572	0.8865	-0.8920	-0.9092	0.7653	-0.2123	0.1538	-0.4924	0.1332	0.2096	0.1773	-0.1083
MW	-0.7111	0.8532	0.9400	0.9572	1.0000	0.8990	-0.8972	-0.9264	0.7506	-0.2594	0.0971	-0.4619	0.1740	0.2969	0.1985	-0.0669
Polarizability α	-0.6719	0.7849	0.8579	0.8865	0.8990	1.0000	-0.8760	-0.8804	0.7715	-0.1748	-0.1856	-0.2610	0.1932	0.5197	0.2145	-0.0555
Electronic energy	0.5466	-0.7922	-0.8426	-0.8920	-0.8972	-0.8760	1.0000	0.9735	-0.9309	0.4429	-0.0060	0.5940	-0.1398	-0.2871	-0.2446	0.1073
Total energy	0.5463	-0.7925	-0.8805	-0.9092	-0.9264	-0.8804	0.9735	1.0000	-0.8229	0.4765	-0.0609	0.6268	-0.1636	-0.3196	-0.2659	0.0750
Core-Core repulsion	-0.4861	0.7034	0.6882	0.7653	0.7506	0.7715	-0.9309	-0.8229	1.0000	-0.3398	-0.0824	-0.4754	0.0863	0.2033	0.1833	-0.1469
Sum O	-0.1262	-0.0925	-0.1793	-0.2123	-0.2594	-0.1748	0.4429	0.4765	-0.3398	1.0000	-0.1376	0.7428	-0.0726	-0.0382	-0.4353	-0.0944
Ionization potential	-0.1262	0.1956	0.1973	0.1538	0.0971	-0.1856	-0.0060	-0.0609	1.0000	-0.1376	1.0000	-0.3922	-0.0017	-0.4196	-0.0866	-0.0991
Heat of formation	0.1241	-0.4402	-0.4769	-0.4924	-0.4619	-0.2610	0.5940	0.6268	-0.4754	0.7428	-0.3922	1.0000	0.0005	0.1230	-0.2607	0.1946
Polarizability β	-0.0704	0.0872	0.1337	0.1332	0.1740	0.1932	-0.1398	-0.1636	0.0863	-0.0726	-0.0017	0.0005	1.0000	0.2832	0.1414	0.2356
Polarizability γ	-0.0566	0.0719	0.1918	0.2096	0.2969	0.5197	-0.2871	-0.3196	0.2033	-0.0382	-0.4196	0.1230	0.2832	1.0000	0.1258	0.0429
Dipole moment	-0.0433	0.0781	0.1547	0.1773	0.1985	0.2145	-0.2446	-0.2659	0.1833	-0.4353	-0.0866	-0.2607	0.1414	0.1258	1.0000	0.1492
Sum N	-0.0058	-0.1447	-0.1042	-0.1083	-0.0669	-0.0555	0.1073	0.0750	-0.1469	-0.0944	-0.0991	0.1946	0.2356	0.0429	0.1492	1.0000

Correlation coefficients of less than -0.8 or more than 0.8 are shown as highlighted.

Fig. 2. Correlation between LLNA and ANN prediction results based on MO descriptors of the dataset of 206 chemicals. Six descriptors shown in Fig. 1 were used as the input layer and reported LLNA threshold was used as the output layer. The r values and RMS errors of the ANN prediction model were 0.791 and 0.571 (before cross-validation (A)) and 0.708 ± 0.018 and 0.660 ± 0.017 (after cross-validation (B) ($N=3$)).

cals from the iSENS ver. 2 dataset (Table 4A). It was not possible to obtain MO-calculated descriptors of metal-containing chemicals (potassium dichromate, cobalt chloride and nickel sulfate) with MOPAC 2002. Fig. 3 compares the predicted and reported LLNA thresholds. For the 53 chemicals in the training dataset we used the predicted LLNA thresholds obtained after 10-fold cross-validation, and for the other 6 (diphenylcyclopropenone, phthalic anhydride, maleic anhydride, benzoyl peroxide, propyl gallate, and 4-chloroaniline) we used the predicted thresholds. The *in silico* model gave a good correlation between predicted and reported LLNA thresholds for these 59 chemicals (Fig. 3B). As shown in Fig. 3A and B, about half the chemicals showed predicted LLNA thresholds higher than the reported values, which indicates that skin sensitization potency is underestimated. From the viewpoint of safety assurance, it is important to reduce the number of under-estimated chemicals. Therefore, we adopted the lower of the predicted LLNA thresholds in the two models and compared the resulting values with reported data (Fig. 3C). In the combined model, all the predicted LLNA thresholds were the same as or less than in iSENS ver. 2. The predictivity of iSENS ver. 2, the *in silico* model and the combined model is summarized in Table 4B. It should be noted that under-prediction

of potency category was reduced to 8% with the combination of the *in vitro* (iSENS ver. 2) and *in silico* models.

DISCUSSION

We previously reported that LLNA threshold as a continuous value was well predicted using descriptors derived from *in vitro* test results by ANN analysis with our systems iSENS ver. 1 and iSENS ver. 2 (Hirota *et al.*, 2013; Tsujita-Inoue *et al.*, 2014). In the AOP of skin sensitization (OECD, 2012), initial molecular events such as protein-binding and cellular responses of keratinocytes and dendritic cells play important roles. Thus, the descriptors in these ANN models were selected from tests considered to represent the AOP key events. On the other hand, it is well known from practical experience that certain kinds of chemical structure are associated with skin sensitization potential. For example, Schiff base or Michael addition functionality tends to be associated with skin sensitization. Indeed, the knowledge-based DEREK system employs structural alerts. However, it would be difficult to quantitatively predict skin sensitization potency on the basis of structural alerts. Therefore, we aimed to construct an ANN model for predicting LLNA thresholds by using structure-derived molecular orbital (MO)-calcu-

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Table 3 (A) . Predicted LLNA thresholds derived from *in silico* model.

Test chemicals	CAS No.	LLNA			MO-calculated descriptors				In silico model		
		Potency category	EC3 values (%)	LLNA threshold (%)	SumH	Sum N	Heat of formation	Ionization potential	Polarizability β	Polarizability γ	Predicted LLNA thresholds (%)
2-Amino-6-chloro-4-nitrophenol 2-(4-Amino-2-nitro-phenylamino)-ethanol (HC Red No.3) 2-Aminophenol 3-Aminophenol a-Amyl cinnamic aldehyde 2-(4-tert-Amylcyclohexyl) acetaldehyde (QRM2113) trans-Anethol Aniline Benzaldehyde 1,2,4-Benzenetricarboxylic anhydride (trimellitic anhydride) 1,2-Benzisothiazolin-3-one (Proxel active) Benzo[alpyrene Benzocaine p-Benzquinone Benzyl benzoate Benzyl bromide Benzylidene acetone (4-phenyl-3-buten-2-one) Bis-1,3-(2',5'-dimethylphenyl)-propane-1,3-dione Bisphenol A-diglycidyl ether 1-Bromobutane 1-Bromododecane 1-Bromododecanoic acid 12-Bromo-1-dodecanol 1-Bromoeicosane 1-Bromohexadecane 1-Bromohexadecane 1-Bromohexane 1-Bromononane 1-Bromooctadecane 1-Bromopentadecane 1-Bromotetradecane 7-Bromotetradecane 2-Bromotetradecanoic acid 1-Bromotridecane 1-Bromoundecane 2,3-Butanediol 4,31-03-8 1-Butanol a-Butyl cinnamic aldehyde p-tert-Butyl-a-ethyl hydrocinnamal (Lilial) Butyl glycidyl ether	514-10-3 874-23-7 140-67-0 6358-09-4 2871-01-41 95-55-6 591-27-5 122-40-7 620159-84-4 104-46-1 62-53-3 100-52-7 552-30-7 2634-33-5 50-32-8 94-09-7 106-51-4 120-51-4 100-39-0 122-57-6 1675-54-3 109-65-9 6938-66-5 143-15-7 73367-80-3 3344-77-2 4276-49-7 3508-00-7 112-82-3 111-25-1 693-58-3 112-89-0 629-72-1 112-71-0 74036-97-8 10520-81-7 765-09-3 693-67-4 431-03-8 71-36-3 7492-44-6 80-54-6 2426-08-6	Weak Nonsensitizer Weak Moderate Moderate Strong Moderate Weak Nonsensitizer Moderate Moderate Weak Extreme Weak Extreme Weak Strong Moderate Nonsensitizer Moderate Nonsensitizer Moderate Moderate Weak Weak Moderate Moderate Moderate Nonsensitizer Weak Moderate Moderate Weak Weak Weak Nonsensitizer Weak Weak	15 >40 18 2.2 2.2 0.4 3.2 11 37 2.3 89 >25 9.2 2.3 0.0009 22 0.0099 17 0.2 3.7 2.3 0.0009 22 8.3 18 18 6.9 6.1 4.8 2.3 10 >25 15 5.1 9.2 21 3.4 10 20 11 >20 11 19 31	15 40 18 2.2 2.2 0.4 3.2 11 37 2.3 89 25 9.2 2.3 0.0009 22 0.0099 17 0.2 3.7 2.3 0.0009 22 8.3 18 18 6.9 6.1 4.8 2.3 10 >25 15 5.1 9.2 21 3.4 10 20 11 >20 11 19 31	4.17794 1.827811 2.063591 0.986395 1.836101 1.324233 1.358643 2.769804 3.004586 1.987744 1.25566 1.232655 1.001345 1.142443 2.607168 1.728098 0.935705 2.451026 1.441993 1.952773 3.14279 4.159228 1.206415 5.52666 3.141607 3.266588 3.304551 5.07784 4.352451 4.110641 1.689737 2.415914 4.59445 3.868609 3.626195 3.65391 3.628925 3.384153 2.899815 0.937814 1.267243 2.528821 2.851994 1.848101	0 0 0 1.407555 1.335052 -0.040596 -0.04788 0 0 0 -0.050346 0 -0.227867 0 -0.030963 0					

Table 3 (A). (Continued).

Test chemicals	CAS No.	Potency category	LLNA		MO-calculated descriptors				In silico model			
			EC3 values (%)	LLNA threshold (%)	SumH	Sum N	Heat of formation	Ionization potential	Polarizability β	Polarizability γ	Predicted LLNA thresholds (%)	Predictive potency category
C4 Azlactone	176664-99-6	Moderate	1.8	1.8	1.999504	-0.892202	-100.83469	251.9916	-74.10530667	7431.179883	11 $\pm$ 1.40	Weak or NS
C6 Azlactone	176665-02-4	Moderate	1.3	1.3	2.488113	-0.289608	-112.11038	254.7044	70.19607	10657.24474	11 $\pm$ 1.32	Weak or NS
C9 Azlactone	176665-04-6	Moderate	2.8	2.8	3.216013	-0.310006	-127.5531	250.2942	76.82141333	11957.5769	8.7 $\pm$ 0.83	Moderate
C11 Azlactone	176665-06-8	Weak	16	16	3.697638	-0.290177	-139.38974	254.7666	46.71165667	16003.27159	8.6 $\pm$ 0.85	Moderate
C15 Azlactone	176665-09-1	Weak	18	18	4.667401	-0.31006	-160.31068	250.3244	68.45478	17143.16691	7.3 $\pm$ 0.24	Moderate
C17 Azlactone	17665-11-5	Weak	19	19	5.150168	-0.290088	-172.17861	254.7715	66.53472667	20515.501	7.5 $\pm$ 0.35	Moderate
C19 Azlactone		Weak	26	26	5.635872	-0.290072	-183.10743	254.7491	70.52120333	22207.39539	7.7 $\pm$ 1.31	Moderate
Chlorobenzene	108-90-7	Nonsensitizer	>25	25	1.09745	0	15.28319	219.9502	-138.7317667	9479.978303	1.9 $\pm$ 0.20	Moderate
1-Chloro-2,4-dinitrobenzene	97-00-7	Extreme	0.05	0.05	0.764738	2.875511	-14.53071	231.9709	371.3608067	38492.40408	0.009 $\pm$ 0.00	Extreme
1-Chlorohexadecane	4860-03-1	Moderate	9.1	9.1	4.059957	0	-99.62869	247.1204	9.877733333	12559.1067	8.6 $\pm$ 0.37	Moderate
5-Chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	Extreme	0.009	0.009	0.713699	-0.198336	-25.65773	214.8394	211.86575	25220.73827	1.5 $\pm$ 0.13	Moderate
1-Chloromethylpyrene	1086-00-6	Extreme	0.005	0.005	2.30622	0	48.66469	195.2555	-38.89539333	63416.42626	0.029 $\pm$ 0.01	Extreme
1-Chlorononane	2473-01-1	Nonsensitizer	>50	50	2.365666	0	-61.40649	247.1043	9.152146667	6340.80485	13 $\pm$ 1.86	Weak or NS
1-Chlorooctadecane	3386-33-2	Weak	16	16	4.544406	0	-110.55059	247.1137	10.00070667	14348.22616	8.2 $\pm$ 0.45	Moderate
1-Chlorotetradecane	2425-54-9	Weak	20	20	3.575516	0	-88.70629	247.1107	9.70088	10778.08771	8.8 $\pm$ 0.74	Moderate
Cinnamic alcohol	104-54-1	Weak	21	21	1.951945	0	-12.97708	210.0413	103.7156733	36473.47913	3.2 $\pm$ 0.23	Moderate
Cinnamic aldehyde	104-55-2	Moderate	3	3	1.653182	0	-2.02001	217.6435	-320.2166767	55310.65134	4.0 $\pm$ 3.37	Moderate
Citral	5392-40-5	Weak	13	13	2.251399	0	-46.5184	219.655	-94.48053667	13170.01041	21 $\pm$ 1.98	Weak or NS
Clotrimazole	23593-75-1	Moderate	4.8	4.8	3.902171	-0.033158	116.46839	219.8485	85.98244	20212.57281	5.8 $\pm$ 0.32	Moderate
Coumarin	991-64-5	Nonsensitizer	>25	25	1.376642	0	-45.17033	219.3772	35.83949333	14217.21835	5.8 $\pm$ 0.59	Moderate
Cyclamen aldehyde	103-95-7	Weak	22	22	2.630527	0	-50.24161	218.9409	4.11776	12613.70959	32 $\pm$ 2.69	Weak or NS
trans-2-Decenal	3913-71-1	Moderate	2.5	2.5	2.405866	0	-65.89384	238.0198	114.23269	13446.84025	17 $\pm$ 2.20	Weak or NS
2,2-Dibromo-2,4-dicyanobutane	35691-65-7	Strong	0.9	0.9	1.171427	-0.334127	48.90681	242.5259	3.612196667	242.5259	7.0 $\pm$ 1.91	Moderate
Diethyl acetaldehyde	97-96-1	Weak	76	76	1.506365	0	-67.94238	244.9216	9.21003	2819.355973	5.4 $\pm$ 1.21	Moderate
Diethylenetriamine	111-40-0	Moderate	5.8	5.8	1.439864	-0.483568	-11.97672	219.5024	-2.492056667	3601.04941	2.7 $\pm$ 0.61	Moderate
Diethyl maleate	141-05-9	Moderate	5.8	5.8	1.76751	0	-165.16579	262.3617	-3.970003333	9679.846413	15 $\pm$ 0.17	Weak or NS
1-(2',5'-Diethylphenyl)butane-1,3-dione	167998-76-7	Moderate	9.6	9.6	2.748827	0	-90.19339	220.368	-24.98133333	12789.36861	31 $\pm$ 1.99	Weak or NS
Diethylphthalate	84-66-2	Nonsensitizer	Negative *	100	2.21011	0	-156.62826	239.97	11.77116333	8049.76699	21 $\pm$ 1.38	Weak or NS
Diethyl sulfate	64-67-5	Moderate	3.3	3.3	1.19325	0	-191.94961	263.3738	-2.871293333	8398.30885	23 $\pm$ 1.05	Weak or NS
3,4-Dihydrocoumarin	119-84-6	Moderate	5.6	5.6	1.567551	0	-68.61903	220.3263	42.39005	9948.044993	12 $\pm$ 0.97	Weak or NS
Dihydroeugenol	2785-87-7	Moderate	6.8	6.8	2.127498	0	-80.3827	205.4796	39.43347	9247.90517	12 $\pm$ 1.56	Weak or NS
1,4-Dihydroquinone	123-31-9	Strong	0.11	0.11	1.430979	0	-72.54572	204.8083	0.002893333	6388.34026	4.8 $\pm$ 0.28	Moderate
3-Dimethylaminopropylamine	109-55-7	Moderate	2.2	2.2	1.607835	-0.30069	-19.37885	215.2955	-9.39635	4070.05955	5.5 $\pm$ 0.69	Moderate
7,12-Dimethylbenz[<i>a</i>]anthracene	57-97-6	Extreme	0.006	0.006	2.945881	0	62.45231	192.3112	32.63961	100935.9531	0.010 $\pm$ 0.01	Extreme
5,5-Dimethyl-3-methylene-dihydro-2(3H)-furanone	29043-97-8	Moderate	1.8	1.8	1.492409	0	-88.31679	244.8455	-75.34246667	5292.042313	9.0 $\pm$ 1.21	Moderate
1-(2',5'-Dimethylphenyl)butane-1,3-dione	56290-55-2	Weak	13	13	2.278022	0	-81.78061	219.875	-19.2802	12964.93277	32 $\pm$ 1.24	Weak or NS
Dimethyl sulfate	77-78-1	Strong	0.19	0.19	0.637108	0	-186.13958	260.0375	33.83118333	3253.36486	1.3 $\pm$ 0.25	Moderate
Dimethylsulfoxide	67-68-5	Weak	72	72	1.077198	0	-51.37911	221.1968	30.4511	827.4006367	4.4 $\pm$ 0.63	Moderate
Dodecyl methane sulfonate	51323-71-8	Moderate	8.8	8.8	3.650617	0	-199.47656	258.5676	-11.98662667	13112.82478	9.1 $\pm$ 0.14	Moderate
4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone)	15646-46-5	Extreme	0.003	0.003	1.986577	-0.181498	-65.61422	212.7117	-23.91056333	93257.80437	0.02 $\pm$ 0.01	Extreme
3-Ethoxy-1-(2',3',4',5'-tetramethylphenyl)propane-1,3-dione	170928-69-5	Weak	33	33	2.813717	0	-143.50175	217.7583	-42.97723333	21852.72278	28 $\pm$ 5.85	Weak or NS
Ethyl acrylate	140-8805	Weak	28	28	1.239177	0	-76.86707	253.9794	-30.87552667	4817.925653	6.4 $\pm$ 1.82	Moderate
Ethyl benzoacetate	94-02-0	Nonsensitizer	>40	40	2.126923	0	-107.47575	231.688	-100.3926233	16552.33093	22 $\pm$ 1.31	Weak or NS
Ethylenediamine free base	107-15-3	Moderate	2.2	2.2	0.819924	-0.327308	-8.95505	221.75	0.011853333	1281.614867	2.6 $\pm$ 0.13	Moderate
Ethylene glycol dimethacrylate	97-90-5	Weak	28	28	2.059218	0	-147.92082	242.6834	0.010783333	15715.47572	22 $\pm$ 1.18	Weak or NS
N-Ethyl-N-nitrosourea	759-73-9	Moderate	1.1	1.1	0.972597	0.133303	-31.85502	231.1057	-61.13751333	6743.23198	0.74 $\pm$ 0.13	Strong
Ethyl vanillin	121-32-4	Nonsensitizer	>50	50	1.726067	0	-101.30397	220.1758	-204.9202067	15906.99276	35 $\pm$ 0.91	Weak or NS

In silico risk assessment for skin sensitization

Table 3 (A). (Continued).

Test chemicals	CAS No.	Potency category	LLNA		MO-calculated descriptors				In silico model			
			EC3 values (%)	LLNA threshold (%)	SumH	Sum N	Heat of formation	Ionization potential	Polarizability β	Polarizability γ	Predicted LLNA thresholds (%)	Predictive potency category
Eugenol	97-53-0	Weak	13	13	2.131193	0	-50.6765	205.4212	-101.1315033	9579.28436	26 $\pm$ 3.07	Weak or NS
Farnesal	502-67-0	Weak	12	12	3.291597	0	-49.5672	215.7205	-0.51317	11332.535	17 $\pm$ 1.31	Weak or NS
Fluorescein-5-isothiocyanate	3326-32-7	Strong	0.14	0.14	2.683235	0.049599	-81.09924	210.2419	424.0407633	125434.5165	1.6 $\pm$ 0.06	Moderate
Formaldehyde	50-00-0	Strong	0.61	0.61	0.200601	0	-38.44633	245.1112	-14.67187333	61.32354333	0.34 $\pm$ 0.05	Strong
Furil	492-94-4	Nonsensitizer	>25	25	1.574614	0	-67.78443	224.7716	-43.06415333	54774.48238	4.1 $\pm$ 1.41	Moderate
Geraniol	106-24-1	Weak	26	26	2.482258	0	-52.74959	215.7489	50.21719667	11101.88479	35 $\pm$ 0.24	Weak or NS
Glutaraldehyde	111-30-8	Strong	0.1	0.1	1.236322	0	-94.14422	247.2994	21.72401	3162.134027	5.2 $\pm$ 1.33	Moderate
Glycerol	56-81-5	Nonsensitizer	Negative *	100	1.383949	0	-150.37417	256.6982	1.903786667	1596.206813	19 $\pm$ 4.37	Weak or NS
Glyoxal	107-22-2	Moderate	1.4	1.4	0.344527	0	-70.73389	240.6613	-0.003203333	1243.072577	1.1 $\pm$ 0.23	Moderate
Heptadienal	5910-85-0	Moderate	4	4	1.595985	0	-24.71534	217.5919	-392.5511533	46285.26218	7.8 $\pm$ 0.43	Moderate
Hexane	110-54-3	Nonsensitizer	Negative *	100	1.587637	0	-40.13214	260.7551	0.040106667	2639.947053	16 $\pm$ 2.27	Weak or NS
trans-2-Hexanal	6728-26-3	Moderate	5.5	5.5	1.438857	0	-44.08345	237.9096	108.8685867	9729.585533	21 $\pm$ 3.59	Weak or NS
Hexyl cinnamic aldehyde	101-86-0	Weak	11	11	3.006363	0	-33.21079	216.8798	15.92324	28222.73938	14 $\pm$ 2.38	Weak or NS
Hydroxybenzoic acid	99-96-7	Nonsensitizer	>25	25	1.478877	0	-120.88055	220.4817	-298.1866067	21733.46864	19 $\pm$ 2.19	Weak or NS
Hydroxycitronellal	107-75-5	Weak	33	33	2.700814	0	-135.13361	249.2269	-7.50034	6496.744403	11 $\pm$ 0.92	Weak or NS
2-Hydroxyethyl acrylate	818-61-1	Moderate	1.4	1.4	1.350673	0	-117.53386	253.4552	15.85570333	5720.69897	12 $\pm$ 1.01	Weak or NS
3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	31906-04-4	Weak	17	17	3.115401	0	-120.12787	219.4383	-26.50763	9709.092907	20 $\pm$ 0.49	Weak or NS
2-Hydroxypropyl methacrylate	923-26-2	Nonsensitizer	>50	50	1.805031	0	-132.76807	243.5369	-1.038236667	7434.111437	22 $\pm$ 2.06	Weak or NS
Imidazolidinylurea	3923-64-9	Weak	24	24	3.05346	-1.044258	-340.73222	226.4314	174.09673	45777.93501	4.7 $\pm$ 0.88	Moderate
1-Iodododecane	4292-19-7	Weak	13	13	3.147588	0	-53.84983	227.7062	43.86541	9312.727347	12 $\pm$ 0.73	Weak or NS
1-Iodobexadecane	544-77-4	Weak	19	19	4.115446	0	-75.69116	227.7106	46.03383333	13037.98318	7.8 $\pm$ 0.12	Moderate
1-Iodohexane	638-45-9	Nonsensitizer	>50	50	1.695197	0	-21.09536	227.6924	33.96197667	3192.14431	12 $\pm$ 3.10	Weak or NS
1-Iodononane	4282-42-2	Weak	24	24	2.421356	0	-37.47111	227.7062	41.24249	6310.983253	27 $\pm$ 1.38	Weak or NS
1-Iodooctadecane	629-93-6	Nonsensitizer	>25	25	4.599626	0	-86.61144	227.709	46.67263667	14872.33352	7.4 $\pm$ 1.09	Moderate
1-Iodotetradecane	19218-94-1	Weak	14	14	3.631358	0	-64.77048	227.7067	45.0989	11184.20194	9.1 $\pm$ 0.65	Moderate
Isoeugenol	97-54-1	Moderate	1.2	1.2	2.052157	0	-57.45296	200.3421	-72.94051667	44165.19317	2.5 $\pm$ 0.81	Moderate
Isononanoyl chloride	57077-36-8	Moderate	2.7	2.7	2.184463	0	-92.74493	257.3581	8.08581	4728.371053	14 $\pm$ 0.97	Weak or NS
Isopropanol	67-63-0	Nonsensitizer	>50	50	1.072212	0	-67.26317	257.1312	-3.259173333	1129.40763	2.2 $\pm$ 0.43	Moderate
Isopropyl eugenol	51474-90-9	Nonsensitizer	>59	59	2.824734	0	-63.37651	204.0162	145.8869933	13016.19815	23 $\pm$ 4.20	Weak or NS
Isopropyl isoeugenol	2953-00-7	Strong	0.6	0.6	2.666544	0	-59.91728	201.083	-24.39454333	66308.46383	0.57 $\pm$ 0.24	Strong
Isopropyl myristate	110-27-0	Weak	44	44	4.260562	0	-171.70004	253.3524	-2.41918	11837.38993	8.3 $\pm$ 0.71	Moderate
Kanamycin	59-01-8	Nonsensitizer	>25	25	5.720149	-0.618394	-483.63464	222.6687	-27.0228	20954.6403	5.9 $\pm$ 0.61	Moderate
Lactic acid	50-21-5	Nonsensitizer	>25	25	1.138513	0	-154.41191	262.5116	20.07222333	2070.59274	16 $\pm$ 2.32	Weak or NS
Lauryl gallate (dodecyl gallate)	1166-52-5	Strong	0.3	0.3	4.34909	0	-260.29631	218.1375	59.16441	22029.87528	9.0 $\pm$ 0.62	Moderate
R(R+)-Limonene	5989-27-5	Weak	69	69	2.168352	0	-2.44621	216.5765	7.776946667	4453.504027	18 $\pm$ 3.07	Weak or NS
2-Mercaptobenzothiazole	78-70-6	Weak	30	30	2.628162	0	-51.93813	218.7193	10.72098333	7919.571343	33 $\pm$ 1.45	Weak or NS
4-Methoxyacetophenone	149-30-4	Moderate	1.7	1.7	1.118477	0.304396	54.13268	207.5621	293.1411967	17373.05416	6.0 $\pm$ 1.84	Moderate
2-Methoxy-4-methyl-phenol	100-06-1	Nonsensitizer	>50	>50	1.71312	0	-62.56091	218.9067	325.6788167	29263.35766	18 $\pm$ 2.36	Weak or NS
1-(p-Methoxyphenyl)-1-penten-3-one	93-51-6	Moderate	5.8	5.8	1.648086	0	-71.4609	205.0783	-78.84979333	6194.56237	12 $\pm$ 1.67	Weak or NS
4-(methylamino)phenol sulfate (Metol)	104-27-8	Moderate	9.3	9.3	2.346038	0	-54.20198	208.9019	667.9539133	103223.9982	0.18 $\pm$ 0.03	Strong
2-Methyl-4H,3,1-benzoxazin-4-one (Product 2040)	55-55-0	Strong	0.8	0.8	2.507018	-0.081865	-189.04561	210.7117	323.5772967	49149.86928	7.0 $\pm$ 2.15	Moderate
a-Methyl cinnamic aldehyde	525-76-8	Strong	0.7	0.7	1.368801	-0.240402	-52.26284	221.0156	-36.46772	19293.76097	5.8 $\pm$ 0.73	Moderate
6-Methylcoumarin	101-39-3	Moderate	4.5	4.5	1.804044	0	-7.81479	216.91	-184.26864	43598.1036	6.9 $\pm$ 2.51	Moderate
Methyl dodecane sulfonate	92-48-8	Nonsensitizer	>25	25	1.531971	0	-54.53611	215.9435	-72.02888	18876.73134	8.6 $\pm$ 1.68	Moderate
2,5-Diamino-toluene	2374-65-4	Strong	0.39	0.39	3.536169	0	-195.85729	256.0735	43.52160333	13767.65409	9.1 $\pm$ 1.06	Moderate
3-Methylleugenol	95-70-5	Strong	0.2	0.2	1.397842	-0.103454	5.62357	190.0423	7.730586667	21157.82465	0.23 $\pm$ 0.11	Strong
5-Methylleugenol	186743-26-0	Weak	32	32	2.242629	0	-57.76082	210.8009	-34.00974333	13521.20565	24 $\pm$ 1.24	Weak or NS
6-Methylleugenol	186743-25-9	Weak	13	13	2.262332	0	-59.33771	209.0181	-32.77056333	11146.74789	27 $\pm$ 0.93	Weak or NS
5-Methylleugenol	186743-24-8	Weak	17	17	2.263355	0	-56.25588	204.7645	4.93628	15171.98014	15 $\pm$ 2.78	Weak or NS

Table 3 (A). (Continued).

Test chemicals	CAS No.	Potency category	LLNA		MO-calculated descriptors					In silico model		
			EC3 values (%)	LLNA threshold (%)	SumH	Sum N	Heat of formation	Ionization potential	Polarizability β	Polarizability γ	Predicted LLNA thresholds (%)	Predictive potency category
Methyl hexadecene sulfonate Methyl hexadecyl sulfonate 5-Methyl-2,3-hexanedione p-Methylthiodicinnamic aldehyde Methyl 4-hydroxybenzoate (methylparaben)	26452-48-2	Strong	0.8	0.8	4.322169	0	-194.25445	249.9372	-26.60576333	19104.33004	9.3 $\pm$ 0.54	Moderate
	4230-15-3	Nonsensitizer	>25	>25	4.504523	0	-217.71221	256.0453	40.27917333	17586.29809	8.4 $\pm$ 0.36	Moderate
	13706-86-0	Weak	26	26	1.663449	0	-105.38205	245.2217	19.52573333	4490.027493	18 $\pm$ 0.64	Weak or NS
	5406-12-2	Weak	14	14	1.98241	0	-35.15144	216.0941	-13.3282	12131.4083	16 $\pm$ 1.13	Weak or NS
	99-76-3	Nonsensitizer	>50	>50	1.561174	0	-109.8478	221.1332	-244.20667	21182.97941	17 $\pm$ 0.65	Weak or NS
	55302-96-0	Strong	0.4	0.4	2.152925	-0.044447	-85.0916	199.1339	134.03021	24463.19819	2.0 $\pm$ 0.34	Moderate
	186743-29-3	Moderate	3.6	3.6	2.189071	0	-64.59977	201.8981	7.010066667	45294.00749	1.5 $\pm$ 0.28	Moderate
	13041-12-8	Moderate	1.6	1.6	2.203865	0	-64.92806	200.26	-66.64309333	47322.89207	2.6 $\pm$ 0.06	Moderate
	2682-20-4	Moderate	1.9	1.9	0.974619	-0.211909	-23.63002	211.7616	-80.04202667	5099.78106	2.7 $\pm$ 0.72	Moderate
	66-27-3	Moderate	8.1	8.1	0.950592	0	-139.66201	265.6233	47.40539667	3088.545303	10 $\pm$ 1.55	Weak or NS
1-Methyl-3-nitro-1-nitrosoguanidine N-Methyl-N-nitrosourea Methyl 2-nonynoate a-Methylphenylacetaldehyde 3-Methyl-4-phenyl-1,2,5-thiadiazole-1,1-dioxide (MPT) Methyl salicylate Methyl 2-sulphophenyl octadecanoate	70-25-7	Extreme	0.03	0.03	0.857746	1.02999	41.84375	239.0448	-15.93956333	5504.43352	0.02 $\pm$ 0.00	Extreme
	684-93-5	Extreme	0.05	0.05	0.707701	0.141407	-26.51137	231.12	-65.21352333	5227.316363	0.93 $\pm$ 0.17	Strong
	111-80-8	Moderate	2.5	2.5	1.993524	0	-72.23821	257.4255	-86.50727667	14284.04275	16 $\pm$ 1.24	Weak or NS
	93-53-8	Moderate	6.3	6.3	1.530243	0	-105.74757	218.7419	146.2639867	10916.64396	15 $\pm$ 0.94	Weak or NS
	3775-21-1	Moderate	1.4	1.4	1.57061	-0.600616	-25.94584	230.8297	327.1042467	37289.4656	2.1 $\pm$ 0.67	Moderate
	119-36-8	Nonsensitizer	>20	>20	1.53032	0	-105.74287	218.7377	-3.713643333	10906.81909	12 $\pm$ 0.82	Weak or NS
		Moderate	2	2	5.79527	0	-280.04657	229.7853	-15.06709	24032.94715	8.4 $\pm$ 0.91	Moderate
	110-41-8	Weak	10	10	2.956541	0	-102.07774	247.0646	21.24494667	9312.35488	10 $\pm$ 0.73	Weak or NS
	90-15-3	Moderate	1.3	1.3	1.784407	0	-7.39508	200.9144	-38.47436	15450.92097	3.5 $\pm$ 0.16	Moderate
	100-11-8	Extreme	0.05	0.05	1.336509	1.428041	3.38463	237.4348	224.1944467	19267.5273	0.07 $\pm$ 0.03	Extreme
2-Nitro-p-phenylenediamine Nonanoyl chloride cis-6-Nonenal Octanoic acid Oleyl methane sulfonate Oxalic acid Palmitoyl chloride Patonic 138C (sodium lauroyl lactylate) Penicillin G Pentachlorophenol Perillaldehyde Phenylacetaldehyde Phenyl benzoate 1,4-Phenylenediamine 3-Phenylenediamine 1-Phenyl-2-methylbutane-1,3-dione 1-Phenyl-octane-1,3-dione 1-Phenyl-1,2-propanedione 3-Phenyl propenal b-Propiolactone Propylene glycol 3-Propylenephthalide Propylparaben Pyridine Resorcinol Saccharin Salicylic acid	5307-14-2	Strong	0.5	0.5	1.158064	1.344466	-1.72273	193.4958	99.65285	16278.26208	3.8 $\pm$ 0.81	Moderate
	764-85-2	Moderate	1.8	1.8	2.18823	0	-93.50969	258.8379	34.94529	4957.206747	12 $\pm$ 0.83	Weak or NS
	2277-19-2	Weak	23	23	2.22596	0	-59.8638	224.9983	-26.26749	4749.126267	27 $\pm$ 2.83	Weak or NS
	124-07-2	Nonsensitizer	>50	>50	2.19387	0	-139.68316	261.2463	15.87326667	5442.910667	12 $\pm$ 0.83	Weak or NS
	35709-09-2	Weak	25	25	5.068902	0	-205.79189	225.0024	16.87668333	15224.12119	8.1 $\pm$ 0.26	Moderate
	144-62-7	Weak	15	15	0.604114	0	-185.49629	269.9849	0.00521	1695.296547	4.5 $\pm$ 0.24	Moderate
	112-67-4	Moderate	8.8	8.8	3.882822	0	-131.71952	258.8612	-1.919713333	12288.09318	8.7 $\pm$ 0.60	Moderate
	13557-75-0	Weak	15	15	4.085016	0	-498.72016	244.127	-18.41675	15609.40457	9.0 $\pm$ 0.54	Moderate
	61-33-6	Weak	30	30	3.143341	-0.235792	-297.58613	224.4854	-68.51511667	18714.65925	14 $\pm$ 1.61	Weak or NS
	87-86-5	Weak	20	20	0.275373	0	-51.31147	212.7677	44.06555667	38414.60332	0.4 $\pm$ 0.10	Strong
2111-75-3 122-78-1 93-99-2 106-50-3 108-45-2 6668-24-2 55846-68-1 579-07-7 14371-10-9 57-57-8 57-55-6 17369-59-4 94-13-3 110-86-1 108-46-3 81-07-2 69-72-7		Moderate	8.1	8.1	2.036172	0	-31.68469	228.9381	-77.47423	13457.62348	13 $\pm$ 2.82	Weak or NS
		Moderate	3	3	1.566812	0	-20.66244	223.0522	58.30382667	5211.37032	11 $\pm$ 1.48	Weak or NS
		Weak	20	20	2.233816	0	-23.76561	220.2788	196.0031533	35807.42025	14 $\pm$ 2.79	Weak or NS
		Strong	0.16	0.16	1.247475	-0.10536	13.73639	190.6107	-0.09856667	24117.45948	0.15 $\pm$ 0.06	Strong
		Strong	0.49	0.49	1.267023	-0.102491	13.19818	198.307	-11.74843333	10460.20971	1.0 $\pm$ 0.08	Moderate
		Weak	29	29	2.15034	0	-66.492	231.3096	-34.40097667	7695.825163	21 $\pm$ 1.81	Weak or NS
		Weak	11	11	2.888548	0	-82.76448	231.923	-49.38861667	13302.03733	14 $\pm$ 1.13	Weak or NS
		Moderate	1.3	1.3	1.569584	0	-55.67243	232.8523	-50.43803667	11339.58782	6.7 $\pm$ 1.20	Moderate
		Moderate	1.4	1.4	1.652233	0	-2.01137	217.6375	297.26514	55372.40139	2.1 $\pm$ 0.14	Moderate
		Strong	0.15	0.15	0.693813	0	-68.91276	265.9099	-15.45211	713.2180367	3.8 $\pm$ 1.15	Moderate
3-Propylenephthalide Propylparaben Pyridine Resorcinol Saccharin Salicylic acid		Nonsensitizer	Negative *	100	1.252402	0	-110.46684	252.8852	15.70012667	1311.959877	6.5 $\pm$ 0.31	Moderate
		Moderate	3.7	3.7	1.732975	0	-48.16055	214.4103	202.2236833	34118.13735	10 $\pm$ 2.41	Weak or NS
		Nonsensitizer	>25	>25	2.053811	0	-119.96564	221.3248	-172.3307	14831.76053	23 $\pm$ 1.11	Weak or NS
		Weak	72	72	1.12371	-0.189072	26.64976	234.08	1.73079	911.6438467	2.0 $\pm$ 0.38	Moderate
81-07-2 69-72-7		Moderate	5.5	5.5	1.446446	0	-73.60873	212.401	-0.933856667	3097.916783	7.5 $\pm$ 1.38	Moderate
		Nonsensitizer	>75	>75	1.164417	-0.606912	-91.57541	236.9443	23.82632333	5769.320407	10 $\pm$ 2.32	Weak or NS
		Nonsensitizer	>25	>25	1.44616	0	-117.26273	218.6432	-64.16003333	10544.15797	15 $\pm$ 0.19	Weak or NS
		Nonsensitizer	>25	>25	1.44616	0	-117.26273	218.6432	-64.16003333	10544.15797	15 $\pm$ 0.19	Weak or NS

Table 3 (A). (Continued).

Test chemicals	CAS No.	LLNA			MO-calculated descriptors				In silico model			
		Potency category	EC3 values (%)	LLNA threshold (%)	SumH	Sum N	Heat of formation	Ionization potential	Polarizability β	Polarizability γ	Predicted LLNA thresholds (%)	Predictive potency category
Sodium lauryl sulfate	151-21-3	Weak (false positive)	14	14	2.936797	0	-428.07001	257.2149	-6.198956667	11752.41575	10 ± 0.57	Weak or NS
Sodium 3,5,5-trimethylhexanoyloxy benzenesulfonate	94612-91-6	Moderate	6.4	6.4	3.417979	0	-347.12317	226.7584	76.72803	16088.96181	11 ± 0.95	Weak or NS
Sulfanilamide	63-74-1	Nonsensitizer	>50	>50	1.470133	-0.607726	-63.32628	206.319	-376.19483	26580.6413	19 ± 9.94	Weak or NS
Sulfanilic acid	121-57-3	Nonsensitizer	>25	>25	1.440988	-0.019933	-118.66523	206.6591	-417.7220933	27330.80709	44 ± 1.78	Weak or NS
Tartaric acid	87-69-4	Moderate	8.7	8.7	1.544146	0	-282.49365	264.768	49.11718667	3373.077827	20 ± 1.64	Weak or NS
Tetrachlorosalicylanilide	1154-59-2	Extreme	0.04	0.04	1.693753	0.055004	-63.25578	205.3661	265.84375	18963.14306	0.05 ± 0.08	Extreme
2,2,6,6-Tetramethyl-heptane-3,5-dione	1118-71-4	Weak	27	27	2.58702	0	-125.00519	247.6833	-14.79837667	7935.478857	12 ± 0.63	Weak or NS
1-(2',3',4',5'-Tetramethylphenyl)-3-(4'-tertbutylphenyl)propane-1,3-dione	55846-68-9	Nonsensitizer	>40	>40	4.086069	0	-87.58364	217.7155	-9.707433333	31155.83496	6.9 ± 0.69	Moderate
1-(2',3',4',5'-Tetramethylphenyl)butane-1,3-dione	167998-73-4	Moderate	8.3	8.3	2.620928	0	-98.67389	217.5028	-66.2433	19828.24104	34 ± 1.71	Weak or NS
Tetramethylthiuram disulfide	137-26-8	Moderate	5.2	5.2	2.1112	0.709771	51.59425	198.4435	2.344566667	12839.79623	4.4 ± 0.59	Moderate
2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride)	108-77-0	Extreme	0.09	0.09	0	-0.693694	18.62007	242.9763	-409.4944233	52173.50979	1.5 ± 1.04	Moderate
4,4,4-Trifluoro-1-phenylbutane-1,3-dione	326-06-7	Weak	20	20	1.533524	0	-208.38006	232.3159	-36.70211	8421.94909	27 ± 3.61	Weak or NS
1-(3',4',5'-Trimethoxyphenyl)-4-dimethylpentane-1,3-dione	135099-98-8	Nonsensitizer	>40	>40	3.041322	0	-187.80939	218.2484	-120.69614	27957.5201	20 ± 2.40	Weak or NS
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safrol)	116-26-7	Moderate	7.5	7.5	1.942235	0	-33.77956	212.5595	-110.9422067	13846.44995	17 ± 0.54	Weak or NS
3,5,5-Trimethylhexanoyl chloride	36727-29-4	Moderate	2.7	2.7	1.629498	0	-43.05584	205.9802	-8.617413333	8288.998047	5.1 ± 0.85	Moderate
Undec-10-enal	112-45-8	Moderate	6.8	6.8	2.648082	0	-71.3549	238.0315	-130.9253933	14119.77224	19 ± 1.67	Weak or NS
Vanillin	121-33-5	Nonsensitizer	>50	>50	1.468173	0	-99.94615	211.1293	-194.2741867	15780.93912	25 ± 2.29	Weak or NS
Vinylidene dichloride	75-35-4	Nonsensitizer	>50	>50	0.415012	0	2.33688	225.9689	-71.95304667	4859.922757	1.8 ± 0.26	Moderate
Vinyl pyridine	1337-81-1	Moderate	1.6	1.6	1.483567	-0.189392	42.11282	224.7571	-84.82495	16829.24265	2.9 ± 0.16	Moderate

Table 3 (B). Comparison between reported *in vivo* LLNA classification and predicted LLNA classification using *in silico* model.

		<i>in silico</i> model classification			
		Extreme	Strong	Moderate	Weak or NS
LLNA classification (<i>in vivo</i>)	Extreme	8	2	2	0
	Strong	0	4	16	0
	Moderate	0	4	40	25
	Weak or NS	0	3	40	62

Accuracy: 55%
Over-prediction of potency category: 23%
Under-prediction of potency category: 22%

NS; non-sensitizer.

Table 4 (A). Prediction of LLNA thresholds using iSENS ver. 2 and *in silico* model.

Test samples	CAS	LLNA			iSENS ver. 2		<i>in silico</i> model		Combined model	
		Potency category	EC3 values (%)	LLNA thresholds (%)	Predicted LLNA thresholds (%)	Predictive potency category	Predicted LLNA thresholds (%)	Predictive potency category	Minimum of predicted LLNA thresholds (%)	Predictive potency category
Oxazolone	15646-46-5	Extreme	0.003	0.003	0.08 ± 0.02	Extreme	0.02 ± 0.01	Extreme	0.02	Extreme
Diphenylcyclopropenone	886-38-4	Extreme	0.003	0.003	0.013 ± 0.002	Extreme	0.004	Extreme	0.004	Extreme
p-Benzoquinone	106-51-4	Extreme	0.0099	0.0099	0.03 ± 0.004	Extreme	0.64 ± 0.08	Strong	0.03	Extreme
Tetrachlorosalicylanilide	1154-59-2	Extreme	0.04	0.04	0.025 ± 0.006	Extreme	0.05 ± 0.08	Extreme	0.025	Extreme
2,4-Dinitrochlorobenzene	97-00-7	Extreme	0.05	0.05	0.32 ± 0.09	Extreme	0.009 ± 0.002	Extreme	0.009	Extreme
Potassium dichromate	7778-50-9	Extreme	0.08	0.08	0.09 ± 0.05	Extreme	N.D.	Extreme	0.09	Extreme
Glutaraldehyde (act. 50%)	111-30-8	Strong	0.1	0.1	0.10 ± 0.02	Extreme	5.2 ± 1.33	Moderate	0.10	Extreme
1,4-Dihydroquinone	123-31-9	Strong	0.11	0.11	0.02 ± 0.00	Extreme	4.8 ± 0.28	Moderate	0.02	Extreme
1,4-Phenylenediamine	106-50-3	Strong	0.16	0.16	0.19 ± 0.05	Strong	0.15 ± 0.06	Strong	0.15	Strong
Phthalic anhydride	85-44-9	Strong	0.16	0.16	21 ± 2.06	Weak	0.77	Strong	0.77	Strong
Maleic anhydride	108-31-6	Strong	0.16	0.16	8.7 ± 1.65	Moderate	0.38	Strong	0.38	Strong
Benzoyl peroxide	94-36-0	Strong	0.30	0.3	6.8 ± 1.57	Moderate	15	Weak or NS	6.8	Moderate
Propyl gallate	121-79-9	Strong	0.32	0.32	4.8 ± 0.44	Moderate	45	Weak or NS	4.8	Moderate
Cobalt chloride	1332-82-7	Strong	0.4	0.4	3.2 ± 0.10	Moderate	N.D.	Moderate	3.2	Moderate
2-Aminophenol	95-55-6	Strong	0.4	0.4	0.42 ± 0.13	Strong	6.8 ± 0.20	Moderate	0.42	Strong
2-Nitro-1,4-phenylenediamine	5307-14-2	Strong	0.5	0.5	4.3 ± 0.83	Moderate	3.8 ± 0.81	Moderate	3.8	Moderate
Formaldehyde (act. 37%)	50-00-0	Strong	0.61	0.61	0.79 ± 0.19	Strong	0.34 ± 0.05	Strong	0.34	Strong
Methylidibromoglutaronitrile	35691-65-7	Strong	0.9	0.9	0.27 ± 0.05	Strong	7.0 ± 1.91	Moderate	0.27	Strong
Isoeugenol	97-54-1	Moderate	1.2	1.2	9.0 ± 1.35	Moderate	2.5 ± 0.81	Moderate	2.5	Moderate
1-Naphthol	90-15-3	Moderate	1.3	1.3	3.6 ± 0.5	Moderate	3.5 ± 0.16	Moderate	3.5	Moderate
2-Mercaptobenzothiazole	149-30-4	Moderate	1.7	1.7	3.6 ± 1.00	Moderate	6.0 ± 1.84	Moderate	3.6	Moderate
Ethylene diamine	107-15-3	Moderate	2.2	2.2	7.1 ± 0.72	Moderate	2.6 ± 0.13	Moderate	2.6	Moderate
Cinnamic aldehyde	104-55-2	Moderate	3.0	3	1.6 ± 0.29	Moderate	4.0 ± 3.37	Moderate	1.6	Moderate
Phenylacetaldehyde	122-78-1	Moderate	3.0	3	2.7 ± 0.97	Moderate	11 ± 1.48	Weak or NS	2.7	Moderate
3-Aminophenol	591-27-5	Moderate	3.2	3.2	5.2 ± 0.47	Moderate	4.0 ± 0.45	Moderate	4.0	Moderate
Nickel sulfate	10101-97-0	Moderate	4.8	4.8	2.6 ± 0.27	Moderate		Moderate	2.6	Moderate
Tetramethylthiuram disulfide	137-26-8	Moderate	5.2	5.2	0.38 ± 0.12	Strong	4.4 ± 0.59	Moderate	0.38	Strong
Resorcinol	108-46-3	Moderate	5.5	5.5	6.7 ± 0.66	Moderate	7.5 ± 1.38	Moderate	6.7	Moderate
3,4-Dihydrocoumarin	119-84-6	Moderate	5.6	5.6	11 ± 0.44	Weak	12 ± 0.97	Weak or NS	11	Weak or NS
2-Methoxy-4-methyl-phenol	93-51-6	Moderate	5.8	5.8	6.1 ± 0.53	Moderate	12 ± 1.67	Weak or NS	6.1	Moderate
4-Chloroaniline	106-47-8	Moderate	6.5	6.5	4.7 ± 0.54	Moderate	0.13	Strong	0.13	Strong
Trimellitic anhydride	552-30-7	Moderate	9.2	9.2	9.7 ± 1.63	Moderate	11 ± 3.71	Weak or NS	9.7	Moderate
1-Bromohexane	111-25-1	Weak	10	10	17 ± 0.89	Weak	16 ± 0.52	Weak or NS	16	Weak or NS
Hexyl cinnamic aldehyde	101-86-0	Weak	11	11	5.6 ± 0.75	Moderate	14 ± 2.38	Weak or NS	5.6	Moderate
Citral	5392-40-5	Weak	13	13	3.0 ± 0.55	Moderate	21 ± 1.98	Weak or NS	3.0	Moderate
Eugenol	97-53-0	Weak	13	13	8.2 ± 0.56	Moderate	26 ± 3.07	Weak or NS	8.2	Moderate
Abietic acid(AB) (act. 70%)	514-10-3	Weak	15	15	22 ± 3.81	Weak	8.4 ± 0.30	Moderate	8.4	Moderate
Oxalic acid	144-62-7	Weak	15	15	7.2 ± 0.53	Moderate	4.5 ± 0.24	Moderate	4.5	Moderate
Cinnamic alcohol	104-54-1	Weak	21	21	4.5 ± 0.45	Moderate	3.2 ± 0.23	Moderate	3.2	Moderate
Benzocaine	94-09-7	Weak	22	22	5.0 ± 0.53	Moderate	0.81 ± 0.41	Strong	0.81	Strong
Geraniol	106-24-1	Weak	26	26	9.0 ± 0.68	Moderate	35 ± 0.24	Weak or NS	9.0	Moderate
Ethyleneglycol dimethacrylate	97-90-5	Weak	28	28	4.8 ± 1.80	Moderate	22 ± 1.18	Weak or NS	4.8	Moderate
Linalool	78-70-6	Weak	30	30	25 ± 31.51	Weak	33 ± 1.45	Weak or NS	25	Weak or NS
Penicillin G	61-33-6	Weak	30	30	43 ± 31.17	Weak	14 ± 1.61	Weak or NS	14	Weak or NS
Butyl glycidyl ether	2426-08-6	Weak	31	31	4.3 ± 0.61	Moderate	18 ± 2.49	Weak or NS	4.3	Moderate
Hydroxycitronellal	107-75-5	Weak	33	33	2.5 ± 0.30	Moderate	11 ± 0.92	Weak or NS	2.5	Moderate
Pyridine	110-86-1	Weak	72	72	29 ± 4.19	Weak	2.0 ± 0.38	Moderate	2.0	Moderate
Dimethylsulfoxide	67-68-5	Weak	72	72	36 ± 2.39	Negative	4.4 ± 0.63	Moderate	4.4	Moderate

Table 4 (A). (Continued).

Test samples	LLNA			iSENS ver. 2			<i>in silico</i> model		Combined model
	CAS	Potency category	EC3 values (%)	LLNA thresholds (%)	Predicted LLNA thresholds (%)	Predictive potency category	Predicted LLNA thresholds (%)	Predictive potency category	
1-Butanol	71-36-3	NS	>20	20	31 ± 0.94	Negative	4.9 ± 1.27	Moderate	Minimum of predicted LLNA thresholds (%)
Chlorobenzene	108-90-7	NS	>25	25	19 ± 2.41	Weak	1.9 ± 0.20	Moderate	4.9
Diethylphthalate	84-66-2	NS	>100	100	19 ± 3.59	Weak	21 ± 1.38	Weak or NS	1.9
Glycerol	56-81-5	NS	>100	100	36 ± 2.58	Negative	19 ± 4.37	Weak or NS	19
4-Hydroxybenzoic acid	99-96-7	NS	>25	25	47 ± 7.31	Negative	19 ± 2.19	Weak or NS	19
2-Hydroxypropyl methacrylate	923-26-2	NS	>50	50	15 ± 1.91	Weak	22 ± 2.06	Weak or NS	15
Isopropanol	67-63-0	NS	>50	50	52 ± 4.38	Negative	2.2 ± 0.43	Moderate	2.2
Lactic acid	50-21-5	NS	>25	25	49 ± 2.00	Negative	16 ± 2.32	Weak or NS	16
Methyl salicylate	119-36-8	NS	>20	20	30 ± 3.09	Negative	12 ± 0.82	Weak or NS	12
Octanoic acid	124-07-2	NS	>50	50	6.8 ± 0.78	Moderate	12 ± 0.83	Weak or NS	6.8
Propylene glycol	57-55-6	NS	>100	100	31 ± 1.17	Negative	6.5 ± 0.31	Moderate	6.5
Saccharin	81-07-2	NS	>75	75	42 ± 2.06	Negative	10 ± 2.32	Weak or NS	10
Vanillin	121-33-5	NS	>50	50	16 ± 0.79	Weak	25 ± 2.29	Weak or NS	16
Sodium dodecyl sulfate	151-21-3	False Positive	14	14	13 ± 1.80	Negative	10 ± 0.57	Weak or NS	10

Table 4 (B). Comparison between reported *in vivo* LLNA classification and predicted LLNA classification using iSENS ver. 2, *in silico* model and combined model.

Reported LLNA classification (<i>in vivo</i>)	iSENS ver. 2 classification						<i>in silico</i> model classification						Combined model classification					
	Extreme	Strong	Moderate	Weak or NS	Extreme	Strong	Extreme	Strong	Moderate	Weak or NS	Extreme	Strong	Extreme	Strong	Moderate	Weak or NS	Extreme	Strong
	6	0	0	0	4	1	4	1	0	0	0	0	6	0	0	0	6	0
Strong	2	4	5	1	0	4	0	4	5	2	2	6	2	6	4	0	2	6
Moderate	0	1	12	1	0	1	0	1	8	4	0	2	0	2	11	1	0	2
Weak or NS	0	0	11	19	0	1	0	1	9	20	0	1	0	1	17	12	0	1
Accuracy: 66%																		
Over-prediction of potency category: 23%																		
Under-prediction of potency category: 11%																		
Accuracy: 61%																		
Over-prediction of potency category: 19%																		
Under-prediction of potency category: 20%																		
Accuracy: 56%																		
Over-prediction of potency category: 35%																		
Under-prediction of potency category: 8%																		

NS; non-sensitizer. N.D.; No data.

Fig. 3. Correlation between LLNA and ANN prediction results of iSENS ver. 2 and *in silico* model. (A) ANN prediction results of iSENS ver. 2 for 62 chemicals reported previously. (B) ANN prediction results of *in silico* model for 59 chemicals. (C) Correlation between reported LLNA threshold and minimum of (predicted LLNA threshold of iSENS ver. 2 and *in silico* model).

lated descriptors in place of structural alerts. We selected Sum H, Sum N, heat of formation, ionization potential, polarizability β and polarizability γ as independent, statistically significant descriptors and showed that ANN using these descriptors enables us to predict skin sensitization potency of chemicals. The reason why this approach works may be that the descriptors are related to physico-chemical properties known to be important for skin sensitization.

For example, liposolubility is related to polarizability, molecular volume and hydrogen bonding property, and

so it is reasonable to consider that the relevant descriptors (polarizability β , polarizability γ , Sum H, Sum N and ionization potential) are correlated with skin absorption and binding affinity to protein. Sum H, Sum N and ionization potential are also related to electronegativity, and therefore to nucleophilic and electrophilic reactivity. On the other hand, ionization potential, heat of formation, and total energy are expected to influence the stability of chemicals, and so might be related to metabolic degradability. Therefore, the descriptors selected in this study are likely to reflect biological processes that are important in

skin sensitization.

The ITS concept integrates various types of *in vitro* and *in silico* information, including biological, biochemical and chemical factors that impact on the allergenic properties of chemicals and the acquisition of skin sensitization potency, for prediction of skin sensitization potential (Jowsey *et al.*, 2006). Several studies have reported hazard identification or potency categorization of skin sensitization by means of majority vote or score-based systems or a Bayesian network (Bauch *et al.*, 2012; Natsch *et al.*, 2013; Nukada *et al.*, 2013; Jaworska *et al.*, 2011, 2013). In the present study, we performed ANN analysis with *in silico* descriptors, and integrated the predictions of iSENS ver. 2 and this *in silico* model. Since it is important to reduce the number of under-estimations from the viewpoint of safety assurance, we adopted the lower of the values predicted by these two models as the LLNA threshold of the combined model. As shown in Table 4B, 7 chemicals were under-predicted in potency category classification by iSENS ver. 2. Among them, it was not possible to calculate MO descriptors of cobalt chloride, as previously mentioned. For benzoyl peroxide, propyl gallate and 3,4-dihydrocoumarin, the predicted LLNA thresholds were higher in the *in silico* model than in iSENS ver. 2, so that they were still under-predicted by the combined model. For phthalic anhydride, maleic anhydride and 2-nitro-1,4-phenylenediamine, the predicted LLNA thresholds were lower in the *in silico* model than in iSENS ver. 2. As a result, the predicted potency categories of these chemicals became consistent with the reported category in LLNA, except in the case of 2-nitro-1,4-phenylenediamine, which was still under-predicted in the combined model. Phthalic anhydride, which was reported to be strong sensitizer in LLNA, was positive in the SH test, but negative in h-CLAT and ARE assay. In iSENS ver. 2, it was predicted as a weak sensitizer. Thus, the combination of *in vitro* and *in silico* models is expected to be very useful for chemicals that are false negative in some *in vitro* studies. As described above, the number of under-estimated chemicals was decreased with the combined model (Table 4B). When evaluating chemicals without animal testing, comprehensive risk assessment before application to humans is extremely important, especially for sensitizing materials. Therefore, there is a strong requirement for the combined model to minimize the number of under-estimated chemicals, and the issue of over-estimated chemicals may be less critical. Among 10 chemicals predicted as non-sensitizers in iSENS ver. 2, only dimethylsulfoxide and sodium dodecyl sulfate were false negatives. Dimethylsulfoxide was rated a weak sensitizer in LLNA with a high EC3 of 77%, while sodi-

Fig. 4. Proposed evaluation scheme for skin sensitization risk using our ANN model.

um dodecyl sulfate was rated as false positive in LLNA. Since iSENS ver. 2 generates very few false negatives, it may be a realistic approach to apply the combined model including the ANN system only to chemicals predicted as positive by iSENS ver. 2, as shown in Fig. 4.

In conclusion, our ANN model using descriptors calculated from chemical structures was effective to predict LLNA thresholds. This is a new approach for *in silico* prediction, because most previous *in silico* models aimed at predicting skin sensitization were constructed only for hazard identification. The combination of our previous ANN model using *in vitro* data with the present *in silico* model appears to be an effective approach for predicting sensitizing potency. Furthermore, our ANN models can be used to calculate an acceptable human exposure level, using a quantitative risk assessment approach (Api *et al.*, 2008). Our results indicate that further development of combined *in vitro* and *in silico* tools for non-animal risk assessment of skin sensitization would be worthwhile.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

- Adler, S., Basketter, D., Creton, S., Pelkonen, O., van Benthem, J., Zuang, V., Andersen, K.E., Angers-Loustau, A., Aptula, A., Bal-Price, A., Benfenati, E., Bernauer, U., Bessems, J., Bois, F.Y., 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, S.B., 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. and Zaldivar, J.M. (2011): Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. *Arch. Toxicol.*, **85**, 367-485.
- Aeby, P., Ashikaga, T., Bessou-Touya, S., Schepky, A., Gerberick, F., Kern, P., Marrec-Fairley, M., Maxwell, G., Ovigne, J.M., Sakaguchi, H., Reisinger, K., Tailhardat, M., Martinozzi-Teissier, S. and Winkler, P. (2010): Identifying and characterizing chemical skin sensitizers without animal testing: Colipa's research and method development program. *Toxicol. In Vitro*, **24**, 1465-1473.
- Api, A.M., Basketter, D.A., Cadby, P.A., Cano, M.F., Ellis, G., Gerberick, G.F., Griem, P., McNamee, P.M., Ryan, C.A. and Safford, R. (2008): Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. *Regul. Toxicol. Pharm.*, **52**, 3-23.
- Ashikaga, T., Yoshida, Y., Hirota, M., Yoneyama, K., Itagaki, H., Sakaguchi, H., Miyazawa, M., Ito, Y., Suzuki, H. and Toyoda, H. (2006): Development of an *in vitro* skin sensitization test using human cell lines: the human cell line activation test (h-CLAT). I. Optimization of the h-CLAT protocol. *Toxicol. In Vitro*, **20**, 767-773.
- Azam, P., Peiffer, J.L., Chamousset, D., Tissier, M.H., Bonnet, P.A., Vian, L., Fabre, I. and Ourlin, J.C. (2006): The cytokine-dependent MUTZ-3 cell line as an *in vitro* model for the screening of contact sensitizers. *Toxicol. Appl. Pharmacol.*, **212**, 14-23.
- Basketter, D.A., Clapp, C., Jefferies, D., Safford, B., Ryan, C.A., Gerberick, F., Dearman, R.J. and Kimber, I. (2005): Predictive identification of human skin sensitization thresholds. *Contact Dermatitis*, **53**, 260-267.
- Basketter, D.A., Evans, P., Fielder, R.J., Gerberick, G.F., Dearman, R.J. and Kimber, I. (2002): Local lymph node assay - validation, conduct and use in practice. *Food Chem. Toxicol.*, **40**, 593-598.
- Basketter, D.A. and Kimber, I. (2011): Predictive tests for irritants and allergens and their use in quantitative risk assessment. In "Contact Dermatitis", 5th edition, chapter 13. Springer, Berlin, Eds Johansen, J.D., Frosch, P.F. and Lepoittevin, J-P., pp.229-240.
- Basketter, D.A., Sanders, D. and Jowsey, I.R. (2007): The skin sensitization potential of resorcinol: Experience with the local lymph node assay. *Contact Dermatitis*, **56**, 196-200.
- Bauch, C., Kolle, S.N., Ramirez, T., Eltze, T., Fabian, E., Mehling, A., Teubner, W., van Ravenzwaay, B. and Landsiedel, R. (2012): Putting the parts together: combining *in vitro* methods to test for skin sensitizing potentials. *Regul. Toxicol. Pharm.*, **63**, 489-504.
- Emter, R., Ellis, G. and Natsch, A. (2010): Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers *in vitro*. *Toxicol. Appl. Pharmacol.*, **245**, 281-290.
- Fedorowicz, A., Singh, H., Soderholm, S. and Demchuk, E. (2005): Structure-activity models for contact sensitization. *Chem. Res. Toxicol.*, **18**, 954-969.
- Gerberick, G.F., Ryan, C.A., Kern, P.S., Schlatter, H., Dearman, R.J., Kimber, I., Patlewicz, G.Y. and Basketter, D.A. (2005): Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis*, **16**, 157-202.
- Gerberick, G.F., Vassallo, J.D., Foertsch, L.M., Price, B.B., Chaney, J.G. and Lepoittevin, J.P. (2007): Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. *Toxicol. Sci.*, **97**, 417-427.
- Hirota, M., Kouzuki, H., Ashikaga, T., Sono, S., Tsujita, K., Sasa, H. and Aiba, S. (2013): Artificial neural network analysis of data from multiple *in vitro* assays for prediction of skin sensitization potency of chemicals. *Toxicol. In Vitro*, **27**, 1233-1246.
- Jaworska, J., Harol, A., Kern, P.S. and Gerberick, G.F. (2011): Integrating non-animal test information into an adaptive testing strategy - skin sensitization proof of concept case. *ALTEX*, **28**, 211-225.
- Jaworska, J., Dancik, Y., Kern, P., Gerberick, F. and Natsch, A. (2013): Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice. *J. Appl. Toxicol.*, **33**, 1353-1364.
- Johansson, H., Albrekt, A.S., Borrebaeck, C.A. and Lindstedt, M. (2013): The GARD assay for assessment of chemical skin sensitizers. *Toxicol. In Vitro*, **27**, 1163-1169.
- Jowsey, I.R., Basketter, D.A., Westmoreland, C. and Kimber, I. (2006): A future approach to measuring relative skin sensitising potency: a proposal. *J. Appl. Toxicol.*, **26**, 341-350.
- Kimber, I., Basketter, D.A., Gerberick, F. and Dearman, R.J. (2002): Allergic contact dermatitis. *Int. Immunopharmacol.*, **2**, 201-211.
- Lambrechts, N., Vanheel, H., Nelissen, I., Witters, H., Van Den Heuvel, R., Van Tendeloo, V., Schoeters, G. and Hooyberghs, J. (2010): Assessment of chemical skin-sensitizing potency by an *in vitro* assay based on human dendritic cells. *Toxicol. Sci.*, **116**, 112-129.
- Magnusson, B. and Kligman, A.M. (1969): The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Dermatol.*, **52**, 268-276.
- Natsch, A. and Emter, R. (2008): Skin sensitizers induce antioxidant response element dependent genes: Application to the *in vitro* testing of the sensitization potential of chemicals. *Toxicol. Sci.*, **102**, 110-119.
- Natsch, A., Emter, R. and Ellis, G. (2009): Filling the concept with data: integrating data from different *in vitro* and *in silico* assays on skin sensitizers to explore the battery approach for animal-free skin sensitization testing. *Toxicol. Sci.*, **107**, 106-121.
- Natsch, A., Ryan, C.A., Foertsch, L., Emter, R., Jaworska, J., Gerberick, F. and Kern, P. (2013): A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. *J. Appl. Toxicol.*, **33**, 1337-1352.
- Nukada, Y., Miyazawa, M., Saitou, K., Sakaguchi, H. and Nishiyama, N. (2013): Data integration of non-animal tests for the development of a test battery to predict the skin sensitizing potential and potency of chemicals. *Toxicol. In Vitro*, **27**, 609-618.
- OECD (2012): The adverse outcome pathway for skin sensitization initiated by covalent binding to proteins. Part 1: Scientific evidence. OECD environment, health and safety publications series on testing and assessment. **168**, 1-46.
- Patlewicz, G., Aptula, A.O., Roberts, D.W. and Uriarte, E. (2008): A minireview of available skin sensitisation (Q)SARs/Expert sys-

In silico risk assessment for skin sensitization

- tems. QSAR Comb. Sci., **27**, 60-76.
- Patlewicz, G. and Worth, A. (2008): Review of data sources, QSARs and integrated testing strategies for skin sensitization. JRC Scientific and technical reports. EUR 23225 EN – 2008. <http://publications.jrc.ec.europa.eu/repository/handle/111111111/998>
- Python, F., Goebel, C. and Aeby, P. (2007): Assessment of the U937 cell line for the detection of contact allergens. Toxicol. Appl. Pharmacol., **220**, 113-124.
- Sakaguchi, H., Ashikaga, T., Miyazawa, M., Yoshida, Y., Ito, Y., Yoneyama, K., Hirota, M., Itagaki, H., Toyoda, H. and Suzuki, H. (2006): Development of an *in vitro* skin sensitization test using human cell lines; human cell line activation test (h-CLAT). II. An inter-laboratory study of the h-CLAT. Toxicol. In Vitro, **20**, 774-784.
- Suzuki, M., Hirota, M., Hagino, S., Itagaki, H. and Aiba, S. (2009): Evaluation of changes of cell-surface thiols as a new biomarker for *in vitro* sensitization test. Toxicol. In Vitro, **23**, 687-696.
- Takahashi, T., Kimura, Y., Saito, R., Nakajima, Y., Ohmiya, Y., Yamasaki, K. and Aiba, S. (2011): An *in vitro* test to screen skin sensitizers using a stable THP-1-derived IL-8 reporter cell line, THP-G8. Toxicol. Sci., **124**, 359-369.
- Teubner, W., Mehling, A., Schuster, P.X., Guth, K., Worth, A., Burton, J., van Ravenzwaay, B. and Landsiedel, R. (2013): Computer models versus reality: How well do *in silico* models currently predict the sensitization potential of a substance. Regul. Toxicol. Pharm., **67**, 468-485.
- Tsujita-Inoue, K., Hirota, M., Ashikaga, T., Atobe, T., Kouzuki, H. and Aiba, S. (2014): Skin sensitization risk assessment model using artificial neural network analysis of data from multiple *in vitro* assays. Toxicol. In Vitro, **28**, 626-639.
- Vacanson, M., Achachi, A., Mutez, V., Cluzel-Tailhardat, M., Varlet, B.L., Rozières, A., Fournier, P. and Nicolas, J.F. (2014): Human T cell priming assay: depletion of peripheral blood lymphocytes in CD25(+) cells improves the *in vitro* detection of weak allergen-specific T cells. Experientia Suppl., **104**, 89-100.