

Further evaluation of quantitative structure–activity relationship models for the prediction of the skin sensitization potency of selected fragrance allergens

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Fragrance substances represent a very diverse group of chemicals; a proportion of them are associated with the ability to cause allergic reactions in the skin. Efforts to find substitute materials are hindered by the need to undertake animal testing for determining both skin sensitization hazard and potency. One strategy to avoid such testing is through an understanding of the relationships between chemical structure and skin sensitization, so-called structure–activity relationships. In recent work, we evaluated 2 groups of fragrance chemicals – saturated aldehydes and α,β -unsaturated aldehydes. Simple quantitative structure–activity relationship (QSAR) models relating the EC3 values [derived from the local lymph node assay (LLNA)] to physicochemical properties were developed for both sets of aldehydes. In the current study, we evaluated an additional group of carbonyl-containing compounds to test the predictive power of the developed QSARs and to extend their scope. The QSAR models were used to predict EC3 values of 10 newly selected compounds. Local lymph node assay data generated for these compounds demonstrated that the original QSARs were fairly accurate, but still required improvement. Development of these QSAR models has provided us with a better understanding of the potential mechanisms of action for aldehydes, and hence how to avoid or limit allergy. Knowledge generated from this work is being incorporated into new/improved rules for sensitization in the expert toxicity prediction system, deductive estimation of risk from existing knowledge (DEREK).

Key words: aldehydes; ketones; local lymph node assay; mechanisms; Michael addition; quantitative structure–activity relationships; relative alkylation index; Schiff base. © Blackwell Munksgaard, 2004.

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Allergic contact dermatitis is a condition that can have a serious impact on the quality of life. The manifestation of allergic contact dermatitis is dependent on skin contact with a specific chemical. However, once an individual has become sensitized then often only minute amounts are required to elicit an allergic reaction. The identification of chemical allergens and evaluation of their potency are important in helping to manage the risks associated with exposure (1, 2).

At present, the only validated approaches to identify sensitization hazard conclusively are *in vivo* models, such as the local lymph node assay (LLNA). There is an urgent need to develop novel approaches or risk assessment strategies to replace

animal testing, especially in view of forthcoming legislation in the cosmetics arena, which poses a ban on animal testing (3). One such approach is that of quantitative structure–activity relationships (QSARs). There are recent reviews outlining progress in this area (4, 5).

Previously (6, 7), we have investigated the feasibility of developing QSARs for the skin sensitization potential of fragrance aldehydes – saturated and α,β -unsaturated. Simple QSAR models relating the EC3 values to LogP and σ^* were developed for both sets of aldehydes [σ^* modelled reactivity and LogP (octanol–water partition coefficient) modelled the lipophilicity]. The work presented here outlines the steps

taken to explore the predictive power of the QSARs for compounds, we knew to be within the domain and to extend the scope of the developed QSARs.

Materials and Methods

Michael addition aldehydes

In our previous study, the following QSAR equation was developed for the 9 Michael addition aldehydes tested:

$$\text{pEC3} = 0.54 + 0.17\text{LogP} + 1.31R'\sigma^*(\alpha) + 0.49R\sigma^*(\beta)$$

(where α and β specify the positions of the alkyl groups relative to the carbonyl group); p(EC3) represents the negative logarithm of the molar EC3; that is, $\text{Log}(1/\text{EC3}') = \text{Log EC3} - \text{Log MW}$.

The next stage of our investigation involved identifying further aldehydes for testing in the LLNA that would either test the predictive power of the QSAR or extend its range. Originally, as diverse a set of chemicals (in terms of their structure) was selected to ensure a broad spread of observable potencies. The observed breadth of sensitization potency was less than anticipated. The strongest allergen tested was cinnamic aldehyde [104-55-2], which in fact is classified as a 'moderate' allergen, and the weakest fragrance allergen was citral [5392-40-5]. There was an absence of both strong and weakly sensitizing Michael addition aldehydes. It is perhaps worth mentioning here where these 'classifications' arise. The EC3 value can provide a simple route through which allergens can be graded and has been described as such in a number of publications (8–10). The predictive identification and discrimination of classes of compounds were proposed by Basketter et al. (11) for the following groups: strong, moderate, weak and extremely weak allergens. The thresholds for these classifications were selected on the basis of comparison with experimental results in humans, and they are as follows: EC3 lower than 0.1% for strong, EC3 between 0.1 and 10% for moderate, EC between 10 and 30% for weak, EC3 between 30 and 50% for extremely weak and EC3 greater than 50% for non-sensitizer.

Compounds that were most likely to extend the training space of the QSAR model were identified for testing in the LLNA. The steps aimed to achieve this are described below:

- (1) explore the chemical space as defined by the training ranges of the parameters.
- (2) explore how the tested chemicals fit this space.

- (3) inspect using the regression equation which parameters had the greatest impact in modifying the sensitization response.
- (4) explore which chemical fragments would lead to those changes.
- (5) search for commercially available chemicals.

Hypothetical molecules were simulated by computing as many combinations of the descriptors ($R'\sigma^*(\alpha)$, $R\sigma^*(\beta)$ and LogP) as possible. This produced a three-dimensional pictorial representation of the chemical space as defined by the training ranges of the QSAR model (data not shown). The QSAR regression equation was inspected to establish which parameter had the greatest influence on the sensitization response. $R'\sigma^*(\alpha)$ value had the greatest impact on predicted EC3 value, in which more potent Michael addition aldehydes had correspondingly lower $R'\sigma^*(\alpha)$ values and vice versa. Perrin's table of σ^* values (12) was used in order to determine those fragments and hence potential aldehydes that were most likely to fit the experimental data gaps. Table 1 lists some examples of fragments and their associated σ^* values. Potential aldehydes were drawn using the Chemdraw package (Version 6.0, CambridgeSoft, MA, USA) (chemicals A–D in Fig. 1), and other input parameters (LogP and molecular weight) were calculated. Chemical catalogues, such as Sigma–Aldrich, were searched to identify commercial chemicals matching chemicals A–D as far as possible. 4 chemicals were selected for LLNA testing, which were considered to broaden the range of sensitization potencies. These 4 chemicals (as shown in Fig. 2) were:

- (1) 5-methyl-2-phenyl-hex-2-enal [21834-92-4].
- (2) 2,4-hexadienal [142-83-6].

Table 1. Fragments contributing to lower sensitizing potency and higher sensitizing potency

	Value
Lower sensitizing potency	
$R'\sigma^*$	
C_2H_5	−0.1
$\text{CH}_2\text{CH}_2\text{CH}_3$	−0.12
$\text{CH}(\text{CH}_3)\text{CH}_3$	−0.19
$(\text{CH}_2)_3\text{CH}_3$	−0.25
$\text{C}(\text{CH}_3)_3$	−0.3
$(\text{CH}_2)_5\text{CH}_3$	−0.25
Higher sensitizing potency	
$R'\sigma$	
$\text{CH}=\text{CH}_2$	0.56
$\text{CH}_2\text{C}=\text{CH}$	0.81
$(\text{C}_6\text{H}_4)_2\text{Br}$	1.07
$\text{C}_6\text{H}_4\text{-pNO}_2$	1.26

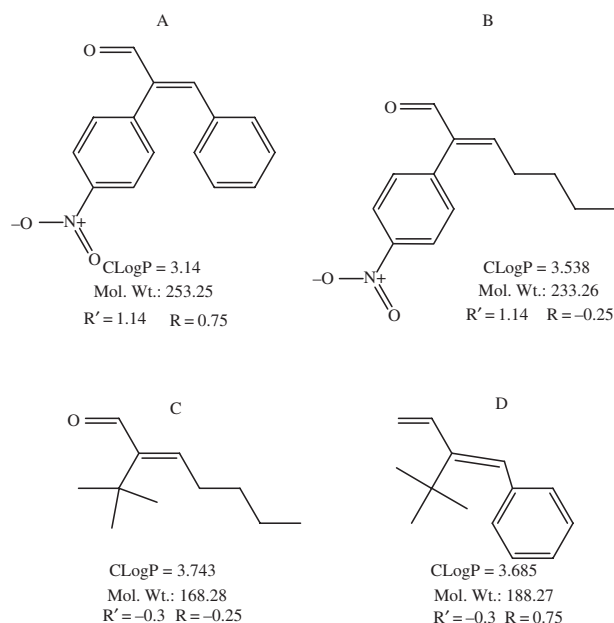


Fig. 1. Hypothetical Michael aldehydes that should extend the breadth of the quantitative structure–activity relationship model.

- (3) 2-ethyl-but-2-enal [19780-25-7] or [63883-69-2].
- (4) β -phenylcinnamaldehyde [13702-35-7].

pEC3 estimates were made using the QSAR model for the 4 chemicals identified. Figure 3 reflects these estimates relative to those predictions for chemicals A–D and the actual values for the originally tested Michael addition aldehydes.

Sourcing [19780-25-7] or [63883-69-2] proved difficult and as a consequence an alternative com-

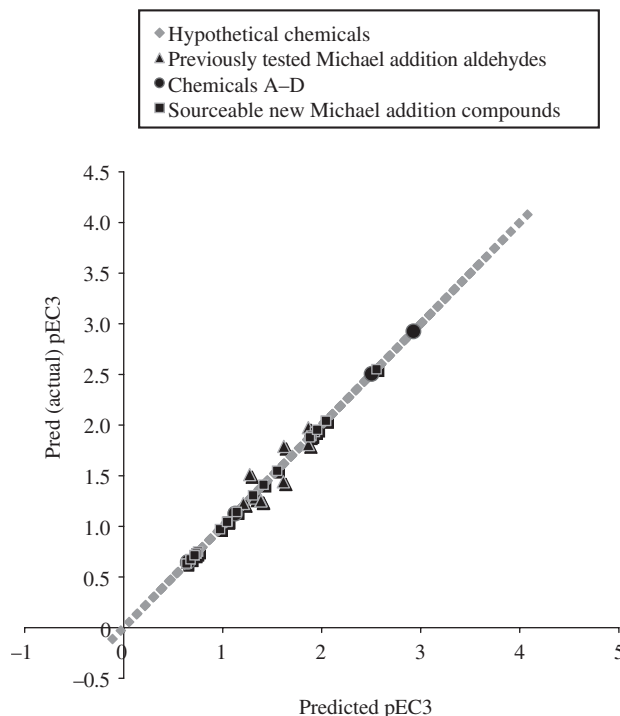


Fig. 3. Prediction for previously tested fragrance Michael aldehydes, chemicals A–D and sourceable new compounds.

pound was selected for testing, *trans*-2-methyl-2-butenal [497-03-0].

These 4 aldehydes were then tested in the LLNA using the same protocol as previously.

Methodology for the Schiff base model

The same procedure was performed in order to help to identify suitable compounds to extend and test the Schiff base QSAR model. The QSAR model derived previously was:

$$\text{pEC3} = 0.25 + 0.28^* \text{LogP} + 0.86^* R\sigma^*$$

An investigation of the current chemical space covered by the model was undertaken, which confirmed that there was a reasonable breadth of sensitization potency in the aldehydes tested. However, there was a large data gap of experimental values between pEC3 values of 0.2 and 0.8 (EC3 values corresponding to 25 and 70). Probing the QSAR model revealed that weaker sensitizers (high EC3 values and low pEC3 values) had low σ^* values (in the range of 0–0.5), whereas stronger sensitizers tended to have higher σ^* values (1.5–3). Perrin's table of σ^* values (12) was used to determine possible fragments, and hence carbonyl-containing compounds that were most likely to fit the experimental data gaps. Commercial catalogues were searched to identify

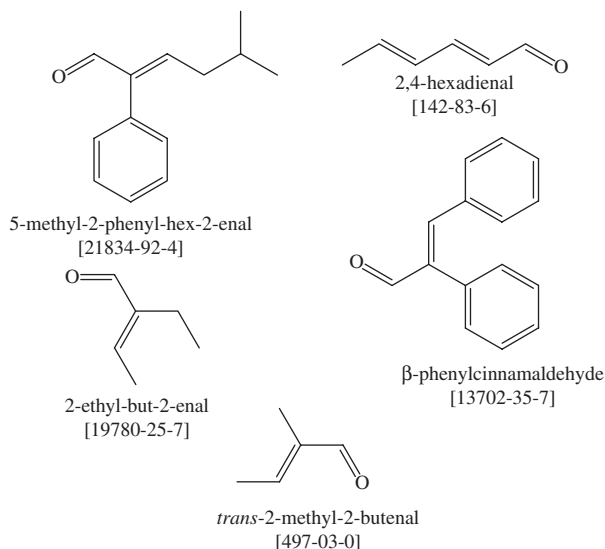


Fig. 2. Selected Michael addition compounds.

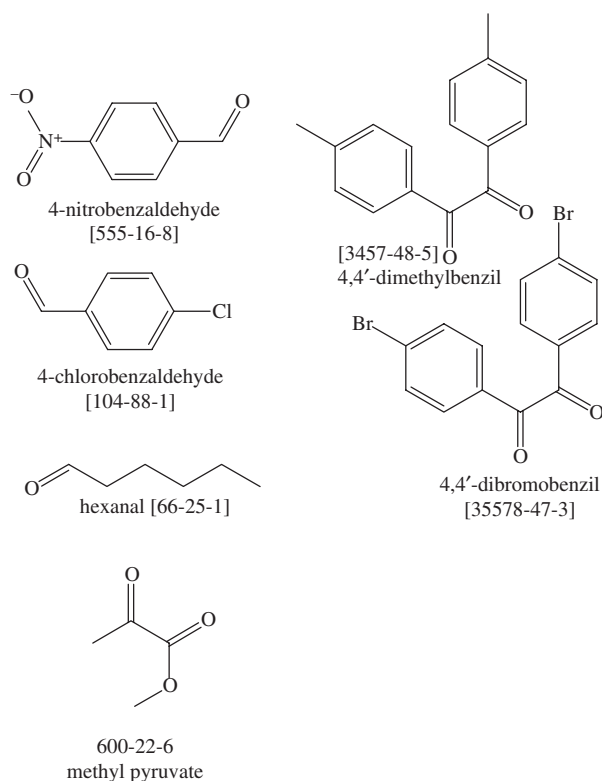


Fig. 4. Selected Schiff base compounds.

suitable candidates for testing. The following compounds (Fig. 4) were selected for further LLNA testing:

- (1) 4-chlorobenzaldehyde [104-88-1].
- (2) 4-nitrobenzaldehyde [555-16-8]
- (3) hexanal [66-25-1]
- (4) 4,4'-dibromobenzil [35578-47-3]
- (5) 4,4'-dimethylbenzil [3457-48-5]
- (6) methyl pyruvate [600-22-6]

Two 1,2-diketones were added to the suggested list of Schiff base aldehydes on account of previous work by Roberts et al. and Patlewicz et al. (13, 14). Here, 1,2-diketones were found to be well predicted by the current Schiff base model when a correction factor for the σ^* for H was included. This suggested that the actual Schiff base model was perhaps not limited to aldehydes alone, but was more indicative of mechanisms of action. As a result, 2 diketones were included in

the current set of new compounds, in addition to 1 ketoester.

Results and Discussion

Michael addition model

Results for the selected Michael addition aldehydes are listed in Table 2 and are shown in Fig. 5a. On the whole, the agreement is fair. The predicted value for *trans*-2-methyl-2-butenal [497-03-0] was poor. The QSAR model predicted this aldehyde to be a moderate sensitizer, whereas it was in fact weak to non-sensitizing ($EC3 > 50\%$). The reason for this discrepancy is thought to be the very low molecular weight and LogP relative to the other aldehydes. Compounds 5-methyl-2-phenyl-2-hexenal [21834-92-4] and β -phenyl cinnamic aldehyde [13702-35-7] were predicted to be stronger than they were in reality. Both of these possessed phenyl groups in the α position of the α,β -unsaturated aldehyde system. Conjugation with the carbon-carbon π -bond lowers the energy of the starting material more than it lowers the energy of the transition state (TS) (in which the carbon-carbon π -bond is almost non-existent), and this effect is not modelled by σ^* . The situation is analogous to, but not the same as, what happens with aromatic aldehydes (for which there is a loss of phenyl-carbonyl conjugation in the TS). The pEC3 values of the Michael addition aldehydes and the newly tested aldehydes were then combined to investigate the feasibility of a new overall QSAR (Fig. 6a).

The regression equation takes the form:

$$pEC3 = 0.55 + 0.14\text{LogP} + 0.51R\sigma^*(\beta) + 1.07R'\sigma^*(\alpha)$$

$$N = 13 \quad R^2 = 0.73 \quad R^2_{\text{adj}} = 0.64 \quad s = 0.2695 \quad F = 8.16$$

Schiff base model

Results for the selected Schiff base compounds are given in Table 3 and are shown in Fig. 5b. Initially, the agreement looks poor. Hexanal [66-25-1] is well predicted by the model. Methyl pyruvate [600-22-6] is also well predicted, which supports the premise that other carbonyl-containing compounds in addition to aldehydes could perhaps be predicted using this QSAR model. The

Table 2. Results for new Michael addition aldehydes

Name	CAS #	Actual EC3	Predicted EC3
β -phenyl cinnamic aldehyde	13702-35-7	2.5	0.6
5-methyl-2-phenyl-2-hexenal	21834-92-4	4.4	1.7
2,4-hexadienal	142-83-6	3.5	2.7
<i>Trans</i> -2-methyl-2-butenal	497-03-0	>50	17.8

two 1,2-diketones tested were poorly estimated by the model in contrast to other 1,2-diketones previously tested (14). However, this is, no doubt, on account of the resonance effects of the benzene rings. This was expected because the reacting carbonyl group is conjugated with an aryl group and the effect of the loss of this conjugation in the TS is not modelled by σ^* . An additional descriptor or alternative descriptor to σ^* would be needed to account for both inductive and resonance effects. 2 aromatic aldehydes with strong withdrawing groups were selected for LLNA testing in order to investigate whether a strong

inductive effect would overcome the stabilizing resonance effect of the aromatic ring resulting in greater sensitization. The aromatic aldehydes studied so far (6) have all had relatively electron-rich π -systems and have been weak to non-sensitizing. The mesomeric electron donation stabilizes the starting material, but not the TS. We reasoned that a strongly electronegative substituent (where NO_2 has a strong withdrawing resonance and inductive effect) in the para position would reduce this electron donation, so that the starting material would be less stabilized relative to the TS. We wanted to test whether this reduction in the resonance effect would be sufficient to make these compounds sensitize in line with the QSAR predictions. These aldehydes were poorly predicted by the QSAR model, and in fact were

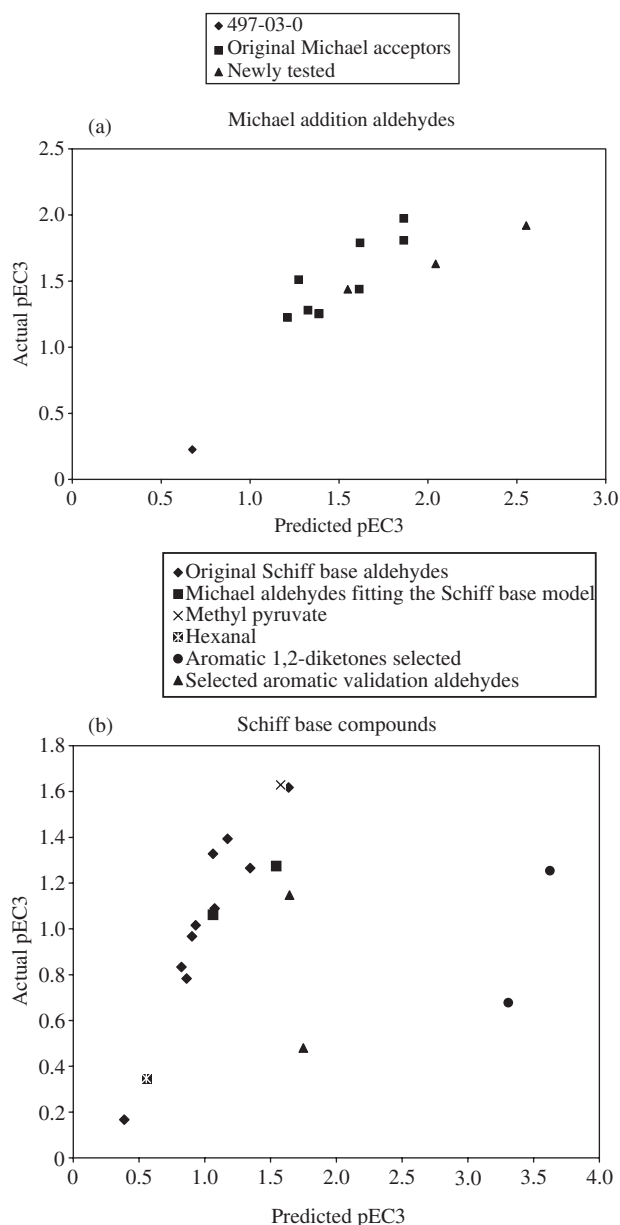


Fig. 5. (a) Predicted and actual Michael addition fragrance allergens (■), new aldehydes (▲) and [497-03-0] (◆). An arbitrary value of 50 for the EC3 was used in the PEC3 plot for [497-03-0]; (b) Predicted and actual Schiff base compounds.

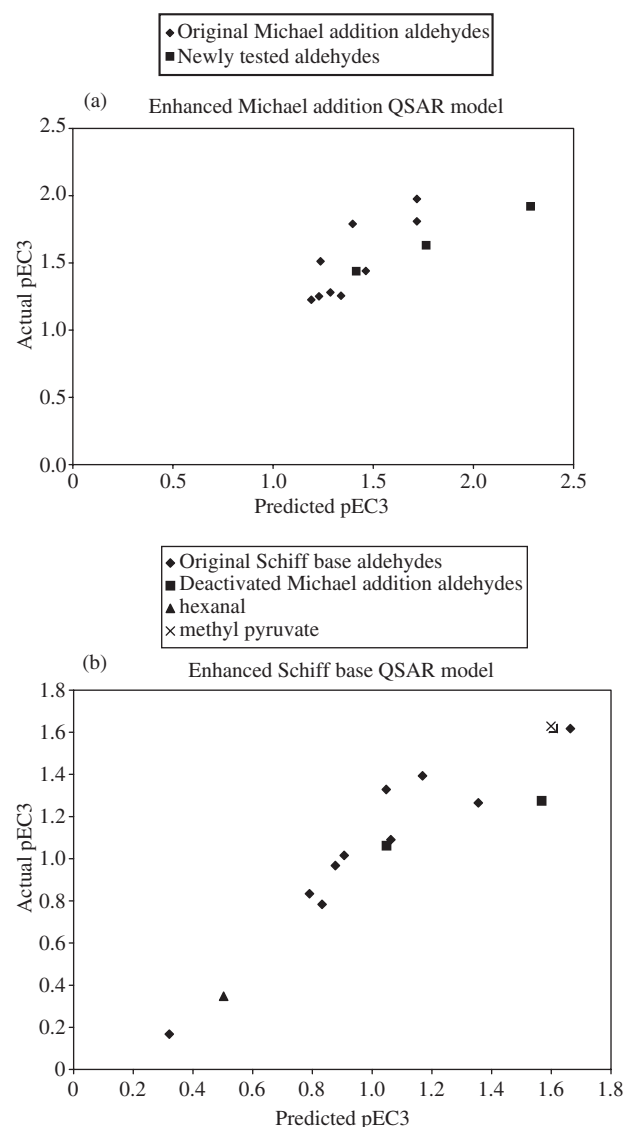


Fig. 6. (a) Enhanced Michael addition QSAR model. (b) Enhanced Schiff base QSAR model.

Table 3. Results for new Schiff base compounds

Name	CAS #	Actual EC3	Predicted EC3
4-nitrobenzaldehyde	555-16-8	>50	2.7
4-chlorobenzaldehyde	104-88-1	>10	3.2
4,4'-dimethylbenzil	3457-48-5	>50	0.1
4,4'-dibromobenzil	35578-47-3	20.5	0.1
Hexanal	66-25-1	45	27.9
Methyl pyruvate	600-22-6	2.4	2.7

found to be weakly or non-sensitizing. This finding shows that not even a p-NO₂ group diminishes the resonance effect sufficiently to lead to significant sensitization potential.

The pEC3 values of the Schiff base aldehydes and carbonyls (hexanal and methyl pyruvate) were then combined into a new QSAR (Fig. 6b).

The regression equation takes the form of

$$\text{pEC3} = 0.17 + 0.93\sigma^* + 0.30\text{LogP};$$

$$N = 14 \quad R^2 = 0.87 \quad R^2_{\text{adj}} = 0.85 \quad s = 0.165 \quad F = 37.7$$

Conclusions

In this study, we have aimed to increase the breadth of each QSAR and to understand its scope by selecting further compounds for testing in the LLNA. 4 further Michael addition aldehydes and 6 Schiff base-formers were selected for testing. Local lymph node assay data generated for these 10 new compounds showed that the original QSARs were reasonable, but still required improvement; (for example, resonance effects of aromatic systems). Aromatic carbonyls are weaker than the Schiff base QSAR predicts even for electronegatively substituted benzaldehydes. Although other diketones have been shown to be well predicted (13, 14), the two 1,2-diketones tested were not well predicted by the Schiff base model. One ketoester was also included in this set to investigate the general applicability of the Schiff base QSAR model and was found to be well predicted. Physico-chemical properties were used in combination with all LLNA EC3 values, in order to develop enhanced QSAR models for the two classes of compounds.

Our understanding of aldehyde allergy is improved in terms of the potential mechanisms of action for aldehydes and for risk assessment. Knowledge generated from this work is currently being incorporated into new/improved rules for sensitization in the expert toxicity prediction system, deductive estimation of risk from existing knowledge (DEREK) (15).

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