



Progress with Structure–Activity Relationship modelling of occupational chemical respiratory sensitizers

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Purpose of review

This appraises currently available computer-based (*‘in silico’*) models relating the molecular structure of low molecular weight compounds to their respiratory sensitization hazard. The present review places focus on the two main applications of such structure–activity relationship (SAR) models: hypotheses on disease mechanisms and toxicological prediction.

Recent findings

Analyses of the chemical structures of low molecular weight organic compounds known to have caused occupational asthma has led to the development of mechanistic alerts usually based on electrophilic reaction chemistry and protein cross-linking potential. Protein cross-linking potential has also been found to be a consistent feature of chemicals that have caused human cases of hypersensitivity pneumonitis. Stepwise iteration of quantitative SAR (QSAR) modelling has shown appreciable improvements in predictivity for occupational asthma hazard and useful prospects for practical application. A good case has also been made for the potential use of structural alert-based mechanistic SARs in predictive toxicology.

Summary

Further understanding of the molecular interactions between chemical respiratory sensitizers and components of human proteins have been obtained from in-vitro and in-silico techniques. There have been developments in both qualitative (mechanistic) SARs and QSARs, which offer potential for use in a predictive algorithm for the toxicological screening of industrial chemicals for respiratory sensitization potential.

Keywords

chemical respiratory sensitizers, hypersensitivity pneumonitis, occupational asthma, QSAR models, structure–activity relationships

INTRODUCTION

Occupational respiratory sensitizers can cause rhinitis, asthma or pneumonitis when inhaled by a worker who has acquired hypersensitivity after a latent period of exposure. The immunopathologic mechanisms for these disease end-points include immediate (type I) hypersensitivity for asthma and rhinitis, as well as immune complex (type III) and delayed (type IV) hypersensitivity for pneumonitis. The complete range of immunopathologic mechanisms are not yet fully elucidated. For example, many cases of occupational asthma or rhinitis caused by chemical respiratory sensitizers are characterized by an isolated late fall in forced expiratory volume in 1 s (FEV1) on specific inhalation challenge (SIC), rather than an immediate response typical of type I hypersensitivity. Specific IgE may be difficult or impossible to demonstrate [1].

The potential for even more diversity in molecular mechanisms for apparent respiratory hypersensitivity to LMW chemicals is apparent when nonimmunologic pathological processes are also considered. These might be pharmacological, as with asthma and rhinitis resulting from the direct

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KEY POINTS

- Recent studies have further characterized the mechanistic chemistry likely to be involved in the early immunopathological processes of respiratory sensitization.
- Electrophilic reaction chemistry and protein cross-linking are thought to be key mechanisms in this process.
- Developments in mechanistic understanding and quantitative structure–activity relationship (QSAR) models are likely to find increasing application in toxicological screening protocols (curtailing the any need for animal testing) for the respiratory sensitization hazard of low molecular weight industrial chemicals.
- QSAR models also demonstrate excellent productivity for use by clinicians investigating cases of occupational asthma where the causal exposure could include a previously unrecognized asthmagen.

release of inflammatory mediators from mast cells caused by inhaled morphine acting on cell surface receptors [2]. Occupational asthma, clinically indistinguishable from that caused by immunologic sensitization, can also result from repeated low-dose exposure to an irritant chemical [3].

Study of the relationship between a chemical's structure and its activity in terms of respiratory hypersensitivity therefore needs to take account of the potential for pathophysiological diversity. The purposes of such study are twofold: first, to generate mechanistic insights into the molecular processes of chemical respiratory sensitization; and second, to generate models for their prediction. Moreover, when the respiratory sensitizer is a chemical of low molecular weight (LMW; a categorization usually defined by a molecular mass less than 1 kDa), it is feasible to study such relationships. The models described herein will focus on LMW organic compounds.

TYPES OF STRUCTURE–ACTIVITY RELATIONSHIP MODELS

Structure–activity relationship (SAR) models can be either mechanistic (qualitative) or statistical (quantitative), the latter usually being termed QSARs. QSARs are generated from statistical comparisons of the molecular features of chemicals with and without the biological activity in question. In the context of respiratory sensitization active chemicals are those that have been shown to be capable of causing rhinitis, asthma or pneumonitis by hypersensitivity in humans, although most of the focus has been on asthma. Inactive or control chemicals are generally deemed to be those without a

demonstrated potential to cause respiratory hypersensitivity in humans. The QSAR models that have been published for respiratory sensitization differ in their selection criteria for learning dataset chemicals, and these differences inevitably impact on their validity.

Mechanistic SARs are based on prediction of a chemical's potential to cause a certain biological activity by virtue of its ability to partake in an essential molecular reaction required for the toxicological process. In the context of respiratory sensitization the formation of covalent bonds between molecules of the chemical and amino acid residues such as lysine, present within the structure of native human protein molecules, has generally been assumed to be the essential prerequisite [4,5].

MECHANISTIC INSIGHTS FROM STRUCTURE–ACTIVITY RELATIONSHIP MODELLING

Insights into how exposure to LMW chemical respiratory sensitizers might result in antigen presentation to the immune system during the sensitization phase, as well as triggering effector mechanisms during the elicitation phase have been obtained from both QSARs and mechanistic SARs. Early QSARs [6,7] involving simple comparisons of the molecular features of LMW organic compounds in asthmagenic and control chemicals showed that respiratory sensitizers were statistically more likely to contain heteroatom (notably nitrogen or oxygen) containing functional groups than nonsensitizers, and particularly when two or more groups containing such heteroatoms were present in the same molecules. Jarvis *et al.* [8,9] later confirmed this observation statistically using larger learning datasets with similar selection criteria.

For LMW respiratory sensitizers with only one functional group capable of reacting with proteins expertise in mechanistic chemistry [4,5,10] has offered explanations in terms of electrophilic reaction chemistry. Acid anhydrides, which are potent respiratory sensitizers, have a high 'electrophilic index' allowing them to react with the electron dense amine groups in the side chains of amino acids, notably lysine (which has a second 'free' amino group). This chemical reaction forms a covalent bond between the anhydride molecule and a native protein generating a hapten–protein conjugate.

There are other possible ways in which a chemical respiratory sensitizer containing more than one functional group, capable of reacting with a native human protein, could potentially interact with 'native' human macromolecules, thus leading to disease. The work of Enoch *et al.* has suggested that

in the case of, for example, diisocyanates each functional (reactive) isocyanate group is not sufficiently electrophilic to bind to protein. They suggest, however, that electrochemically stable covalent bonds can be achieved when both isocyanate groups in a given molecule react with protein. In-vitro studies have demonstrated that both hexamethylene diisocyanate (HDI) and toluene diisocyanate (TDI) can, in fact, bind to human serum albumin through either one or both functional (in this case isocyanate) groups [11]. The relative degree of haptenation and protein cross-linking has subsequently been shown to depend on the molecular species of diisocyanate and the carrier protein [12²]. Such potential for diversity of chemical interaction with proteins could be a reason for the heterogeneity of pathophysiological responses seen in occupational asthma caused by diisocyanates and other LMW chemical asthmagens.

The simultaneous reaction of two functional groups in each molecule of a chemical respiratory sensitizer with different parts of the same or different proteins could lead to intra- or intermolecular protein cross-linking as previously hypothesized [13]. Formation of cross-links within the same protein molecule could lead to change in the protein's conformation or tertiary structure displaying 'cryptic' autoantigenic epitopes to the immune system (Fig. 1). Thus the epitopes resulting from this mechanism would not contain the bound hapten itself and would essentially be completely autoantigenic. Such a mechanism of autoantigen formation might explain components of the immunopathological mechanisms of chemically induced hypersensitivity pneumonitis as illustrated

in Fig. 2. Some support for this hypothesis was gained from the finding that nine out of the 10 chemicals, which were the attributed cause of reported human cases of hypersensitivity pneumonitis, had protein cross-linking potential [14]. This proportion was significantly greater than that for the larger set of chemicals reported to cause occupational asthma but not hypersensitivity pneumonitis. In the same comparative study it was also found that chemical respiratory sensitizers reported to have caused hypersensitivity pneumonitis tended to be more lipophilic than those known to cause asthma but not hypersensitivity pneumonitis. A possible explanation for this observation may be that the surfactant lining of the alveoli is more lipid based than the epithelial lining of the bronchioles.

If the two reactive groups of a chemical respiratory sensitizer were able to react with different protein molecules then intermolecular cross-linking could result. In the case report of hypersensitivity pneumonitis and asthma caused by pyromellitic dianhydride [15], immunoserological testing showed evidence of antibodies to a dimer of immunoglobulin G (IgG), perhaps suggesting that the two highly electrophilic groups in a molecule of pyromellitic dianhydride had cross-linked two IgG macromolecules to create an autoantigen.

STRUCTURE-ACTIVITY RELATIONSHIP MODELS FOR RESPIRATORY SENSITIZATION HAZARD PREDICTION

A variety of SAR approaches have been applied to the prediction of LMW organic chemical respiratory sensitizers in models for which the development

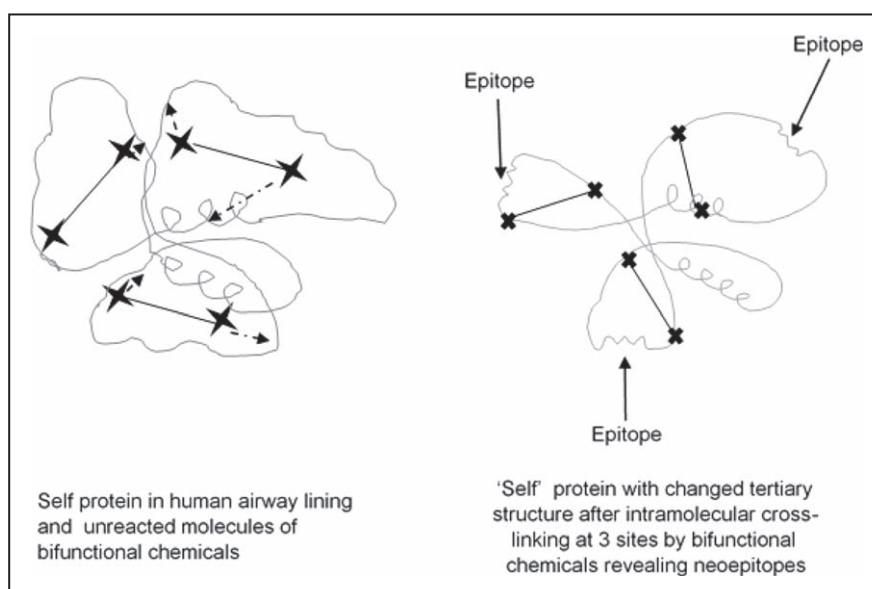


FIGURE 1. A hypothetical mechanism by which bifunctional respiratory sensitizers could render a self protein antigenic.

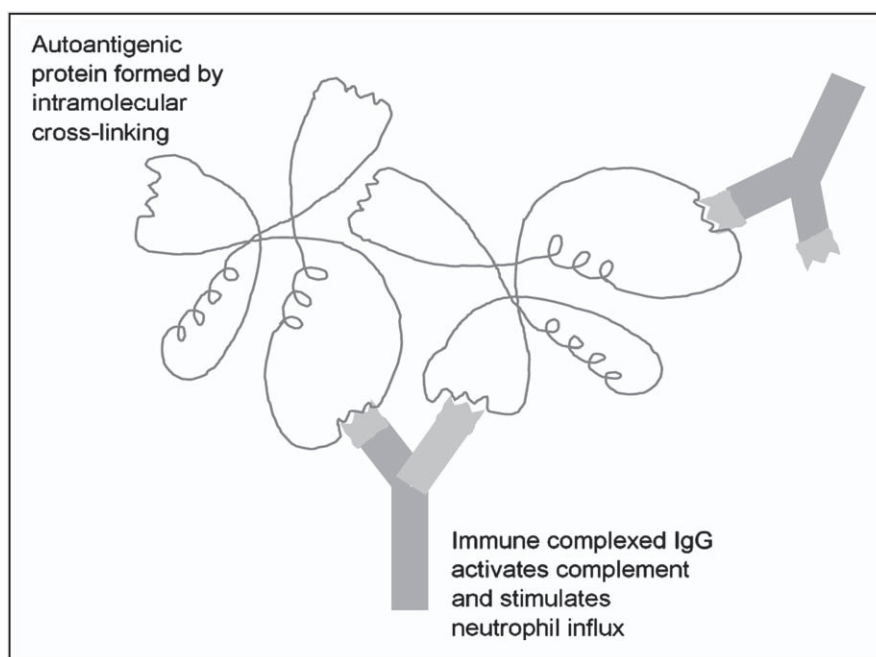


FIGURE 2. Possible means by which type III hypersensitivity results from a chemical respiratory sensitizer that has altered the tertiary structure of a self protein to render it (auto)antigenic.

and validation processes have been detailed for scrutiny in peer-reviewed publications. These have included models utilizing CASE-MultiCASE methodologies [16], cat-SAR [17], and a logistic regression method by Jarvis *et al.* [8]. Since the key differences in learning datasets and predictive performances of these (Q)SAR models were previously described [9] there have been several further developments.

The set of mechanism-based structural alerts developed by Enoch *et al.* offer an excellent model for corroborating the positive prediction or identification of a chemical respiratory sensitizer. However, the absence of an identified structural alert in a molecule does not have negative predictive value [10]. Mechanistic SARs are likely to have a place in predictive algorithms, at least for the purposes of regulatory toxicology, as one of the OECD principles is that QSARs should have ‘a mechanistic interpretation, if possible’. Indeed Mekenyan *et al.* [18] have proposed a ‘pipeline workflow’ algorithm which utilizes predictions of electrophilic reactivity, cross-linking potential and other potentially relevant chemical steps, such as autooxidation in the lung to a compound which has the qualitative predictors of protein binding. They demonstrated a good sensitivity but high false-positive rate when this workflow was tested against 120 human respiratory sensitizers and 82 human respiratory nonsensitizers. Addition of a stage to this workflow of *in-vitro* analysis of lysine binding [19] increased the sensitivity from 84 to 89% but correspondingly marginally decreased the specificity from 54 to 52%.

Warne *et al.* [20] studied the molecular characteristics that differentiated skin and respiratory sensitizers (identified from regulatory labels R42 or R43) from a set of chemicals with neither risk label. However their training set contained only 17 respiratory sensitizers (R42-active compounds) and the external validation set limited to only two R42 labelled chemicals.

Jarvis *et al.* [21] developed their second QSAR model, using similar methods of selection of learning datasets and logistic regression computational techniques to their first model, which determined a probability that a test chemical had asthmagenic potential – this value being termed the chemical’s ‘asthma hazard index’. An updated and expanded database of active compounds comprised those which had been reported in peer-reviewed literature, before the end of 2012, to have caused at least one case of occupational asthma thought to be because of sensitization following a latent period of exposure. As in their first model they used occupational exposure limit tables to identify control compounds to which humans had been extensively exposed in industry but for which there were no known case reports of occupational asthma. After 10% of active and control compounds had been excluded by random selection for subsequent use as an external validation set the learning dataset comprised 99 asthmagenic and 204 control compounds. Nine of the 10 validation astmagens and 23 of the 24 validation controls were correctly predicted, using a cut-point hazard index of 0.5, giving

an estimated sensitivity of 90% and specificity 96%. The area under the receiver operating characteristic (ROC) curve was found to be 0.95 for the revised QSAR [21], compared to 0.86 for the original model [8] showing improved predictive performances over the range of cut-point hazard indices

As already stated one of the key determinants of the predictive accuracy of any QSAR model is the relevance of the learning dataset chemicals to the biological outcome being studied. Whilst most of the QSAR models that have been proposed for predicting respiratory sensitization potential have utilized human respiratory sensitizers for their learning dataset 'active' chemicals there remains disagreement about how to select appropriate 'control' chemicals for comparison of molecular features. The best approach, in the opinion of the current authors, is to use chemicals to which humans have been extensively exposed in industry but for which there have been no cases reported of occupational asthma, rhinitis or hypersensitivity pneumonitis. Examples of QSARs utilizing this source of control chemicals include those developed by Jarvis *et al.* [8,21] and Karol *et al.* [22].

An alternative source of controls, used by other groups developing QSAR models for respiratory sensitization, has consisted of chemicals found to have a negative result by the local lymph node assay (LLNA). The LLNA is an in-vivo test in mice developed for the purpose of screening compounds for skin sensitization potential. Dearman *et al.* [23] make a case for the LLNA being a source of data to identify human respiratory nonsensitizers based on a nonrandomly selected set of 23 respiratory sensitizers which all had a positive result using the LLNA. Whilst the authors acknowledge that a positive LLNA result for a chemical does not imply that it is a respiratory sensitizer their concluding words are, '... this implication is that a chemical which fails to register positive in a well characterized and properly conducted test for skin sensitization will lack the potential to induce either skin sensitization or sensitization of the respiratory tract'. However there are exceptions – notably benzalkonium chloride, a biocide widely used in cleaning agents. Despite reported negative LLNA results [24,25] there have been a number of reported cases of occupational asthma attributed to benzalkonium chloride [26,27].

The ideal method for comparing the predictive performances of the available SARs would be to use the same groups of chemical respiratory sensitizers and control compounds as the test compounds in a common external validation set. The limited numbers of active compounds, however, make this virtually impossible given that very few compounds

will have appeared in the learning datasets of none of the available SAR models. Dik *et al.* [28] undertook a comparative analysis for this purpose creating an aggregate set of 138 respiratory sensitizers and 521 controls which comprised the pooled learning dataset compounds of five models, together with an additional 168 chemicals which had tested negative for the mouse LLNA.

The five models being compared by Dik *et al.* were the mechanistic SAR developed by Enoch *et al.* [10], the QSAR developed by Jarvis *et al.* [8], cat-SAR [17], and two other models whose details have not been published; 'The Derek Nexus knowledge base' (a qualitative SAR based on structural alerts using software from a company whose website is Lhasa Ltd. [29]) and 'MULTICASE' (a model similar to that described by Graham *et al.* but apparently using updated learning datasets and the computer technology described by Klopman [30]). The study concluded that a screening algorithm starting with 'Derek' would pick out 58 of the 659 chemicals in the aggregated dataset as respiratory sensitizers (including four false positives) followed by 'MULTICASE' identifying 175 as nonsensitizers (including five false negatives) leaving 426 compounds for which neither model could give a prediction. The study has various methodological flaws including:

- (1) A disproportionate number of controls were identified from a negative mouse LLNA result, which, as already discussed, is not necessarily consistent with the human experience.
- (2) The expansion of the control set using 168 LLNA negative compounds will inevitably give favourable validation results to those models which used similar control selection in their learning datasets.
- (3) In determining predictive values the authors make the assumption that one in two compounds in 'chemical space' are respiratory sensitizers. No rationale for this sweeping assumption was given.

APPLICATIONS OF PREDICTIVE STRUCTURE-ACTIVITY RELATIONSHIP MODELS

An analogy can be made between a QSAR model used in predictive toxicology to distinguish between chemicals with or without a hazardous property and a diagnostic test based on a quantitative measurement (e.g. a serological assay) used in clinical medicine to distinguish between patients with and without disease. For both types of test an appropriate cut-point value can be obtained from an ROC curve depending on the optimal balance between

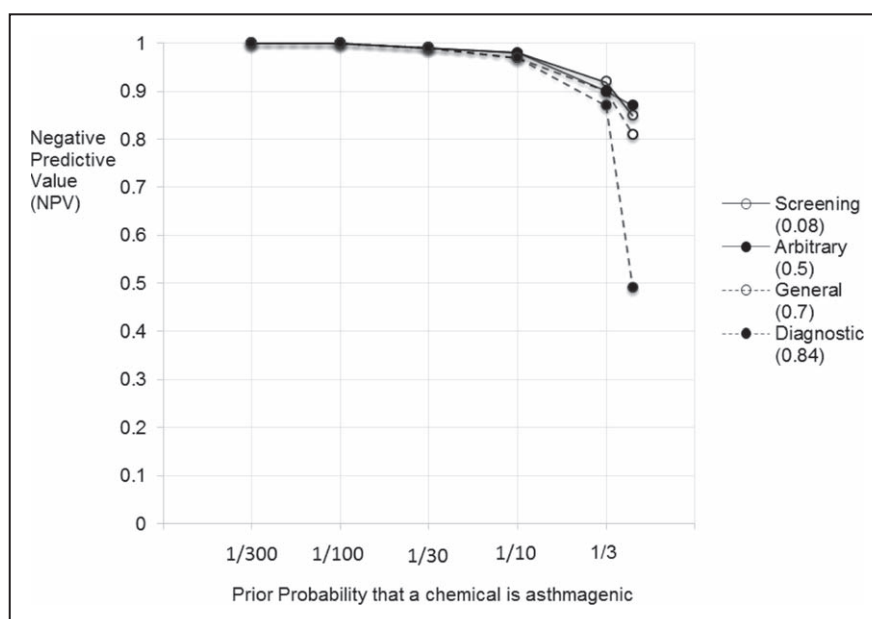


FIGURE 3. Variation in Negative Predictive Value (NPV) with prior probability for different cut-point hazard indices.

sensitivity and specificity for the particular context. Thus for screening purposes the priority in setting a cut-point would be to maximize sensitivity, so as to minimize the frequency of ‘missed’ cases (or respiratory sensitizers) whilst accepting that a patient (or chemical being screened) with a positive result is likely to need further investigation to confirm the presence of disease (or respiratory sensitization potential). This approach contrasts with the diagnostic situation in which a cut-point is more likely to be chosen to maximize diagnostic specificity.

Whatever cut-point value is set for a given situation the predictive values will vary as a function of the sensitivity and specificity and the disease prevalence (or prior probability that a given chemical is a respiratory sensitizer) as previously described [9]. Figures 3 and 4 illustrate how predictive values vary with prior probability for the initial asthma hazard prediction model developed by Jarvis *et al.* [8] using four different cut-point hazard indices. A hazard index is a value between 0 and 1 representing the probability, as determined by the model, that a given test chemical is asthmagenic.

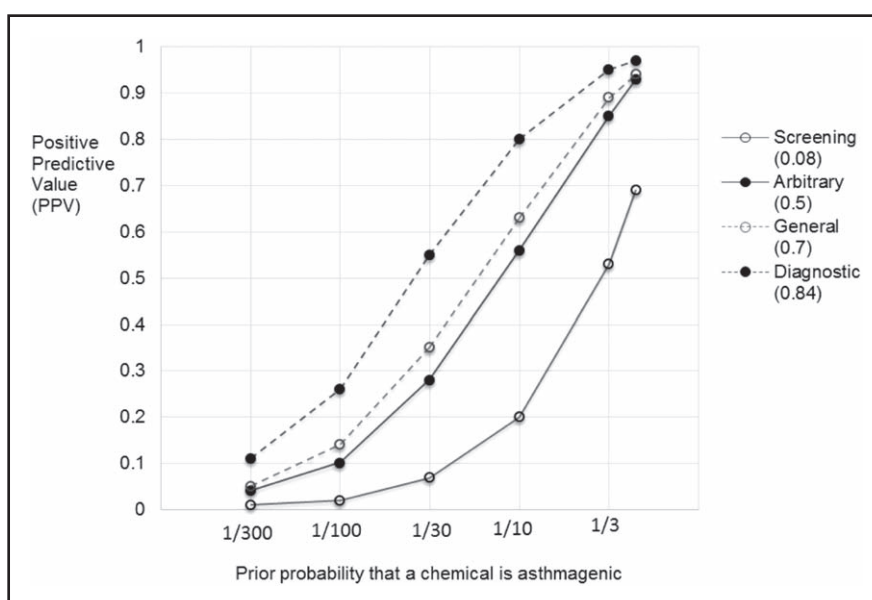


FIGURE 4. Variation in Positive Predictive Value (PPV) with prior probability for different cut-point hazard indices.

When screening undifferentiated LMW organic compounds we suggest that the prior probability of any given chemical being a true respiratory sensitizer is closer to a range between 1 in 300 and 1 in 30 rather than the one in two estimated by Dik *et al.* [28]. In this range of prior probabilities the Jarvis *et al.* model has a positive predictive value (PPV) of greater than 0.95, regardless of the cut-point hazard index, but a negative predictive value (NPV) of only 0.55 at best. This has been the basis for previous arguments [9,10] that a negative result could have potential for eliminating the need for any further testing for the vast majority of chemicals screened whilst a positive result would need further corroboration, perhaps from a mechanistic SAR or in-vitro testing.

As the prior probability approaches one in three there is a large fall in the NPV but a corresponding rise in PPV. The Jarvis *et al.* [8] QSAR model therefore has potential utility in assisting clinicians investigating a possible novel cause of occupational asthma from a small number of potential candidates. Several case reports have been published in which the clinician has used a positive QSAR result as corroboration of their clinical identification of a novel asthmagen [31–33]. Of potentially more value in the clinic is the possibility for using QSAR generated predictions of potential asthmagenicity of chemicals to which a worker with occupational asthma has been exposed. This has significant potential when the causative agent might be a previously unrecognized asthmagen within a range of exposures in the workplace. QSAR data, in combination with a thorough occupational hygiene assessment [34] could help to reveal candidate chemical asthmagens with which to perform a SIC test on the patient.

Other authors have quoted hazard indices generated by the Jarvis *et al.* model in their work studying the chemistry of the indoor atmosphere. Several studies [35–37] have analysed the reaction products of volatile organic compounds, for example with atmospheric ozone, and found a number of them to be positively predicted as respiratory sensitizers by the Jarvis *et al.* model. Of note Forrester and Wells [38] have investigated the oxidation products of limonene, the most widely used fragrance compound, and described several structures containing one or more carbonyl groups and which have hazard indices greater than 0.5.

CONCLUSION

There is significant potential for QSARs, perhaps complimented by qualitative SARs, to play a key part in regulatory toxicological screening of

industrial chemicals for respiratory sensitization hazard although they have not so far demonstrated a practical impact on policy decisions. However further development of the models that have so far shown greatest potential, as summarized in this review, is likely to lead to toxicological predictive tools that can be used for regulatory screening protocols which are long overdue for the prospective identification of respiratory sensitization hazards of industrial chemicals. SAR approaches also offer scope for prediction of, and development of mechanistic insights into a more diverse range of disease end-points than simply asthma caused by sensitization. These include allergic rhinitis and hypersensitivity pneumonitis, as well as asthma resulting from irritant effects of chemicals.

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None.

Conflicts of interest

The authors cite some of their own publications in this review but have tried to be as objective as possible in summarizing relevant published material.

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