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Chemical determinants of occupational hypersensitivity pneumonitis

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Abstract

Background: Workplace inhalational exposures to low molecular weight (LMW) chemicals cause hypersensitivity pneumonitis (HP) as well as the more common manifestation of respiratory hypersensitivity, occupational asthma (OA).

Aims: To explore whether chemical causation of HP is associated with different structural and physico-chemical determinants from OA.

Methods: Chemical causes of human cases of HP and OA were identified from searches of peer-reviewed literature up to the end of 2011. Each chemical was categorised according to whether or not it had been the attributed cause of at least one case of HP. The predicted asthma hazard was determined for each chemical using a previously developed quantitative structure-activity relationship (QSAR) model. The chemicals in both sets were independently and 'blindly' analysed by an expert in mechanistic chemistry for a qualitative prediction of protein cross-linking potential and determination of lipophilicity (log K_{ow}).

Results: Ten HP causing chemicals were identified and had a higher median QSAR predicted asthma hazard than the control group of 101 OA causing chemicals ($p < 0.005$). Nine of ten HP causing chemicals were predicted to be protein cross-linkers compared to 24/92 controls ($p < 0.0001$). The

distributions of log Kow indicated higher values for the HP list (median 3.47) compared to controls (median 0.81) ($p < 0.05$).

Conclusion: These findings suggest that chemicals capable of causing HP tend to have higher predicted asthma hazard, are more lipophilic and are more likely to be protein cross-linkers than those causing OA.

Key words: hypersensitivity pneumonitis, occupational chemicals, occupational respiratory disease, toxic inhalation

Key points:

1. Only a relatively small number of reported cases of occupational hypersensitivity pneumonitis (HP) have been attributed to low molecular weight (LMW) chemicals - as opposed to high molecular weight agents causing HP, or LMW chemicals causing occupational asthma (OA).
2. Quantitative and qualitative approaches to structure-activity relationships offer a valuable means of exploring mechanistic differences between HP and OA when caused by LMW chemicals.
3. Preliminary results suggest that the ability of a chemical respiratory sensitizer to cause HP is associated with lipophilic and protein cross-linking properties.

Introduction

Reports of hypersensitivity pneumonitis (HP) with causal attribution to a chemical of low molecular weight (LMW) show potential for significant respiratory distress [1]. However, as has been described in the context of occupational asthma (OA) due to hypersensitivity [2,3,4], detailed study of the chemical features characteristic of LMW causes of HP might reveal important insights into disease mechanisms and perhaps lead to a model for predicting chemicals with the potential to cause HP. This paper is the first to report as a pilot the possible application of Structure Activity Relationship models in predicting or explaining occupational HP hazard.

A validated quantitative structure-activity relationship (QSAR) model had been developed by a statistical comparison of the molecular descriptors present in chemicals reported to cause OA in humans with those in control chemicals (for which no case reports of asthma had been reported despite extensive human industrial exposure) [5,6]. One of the important structural features found to be statistically associated with chemical asthmagenicity was the presence of two or more reactive or functional groups in a molecule [7,8,9]. A possible reason why such polyfunctionality might be necessary for a chemical to be asthmagenic could be that it confers the potential to cross-link native human proteins, perhaps leading to conformational change and the exposure of epitopes capable of provoking an immune reaction that leads to asthma. Alternatively cross-linking might operate at a cell surface level. Cross-linking of protein molecules by a polyfunctional chemical has also been suggested by experts in mechanistic chemistry to confer electrochemical stability for protein binding that might not be achieved by reaction of a single chemical group alone [10,11]. A preliminary literature search for chemical causes of HP had identified two compounds, toluene *tri*-isocyanate [12] and pyromellitic *di*-anhydride [13], that exhibit a greater degree of polyfunctionality than the more commonly reported asthmagenic compounds in the same groups (a range of di-isocyanates and mono-anhydrides).

The pathology of OA and HP differs in the anatomical site with OA resulting from inflammation of the distal bronchi and bronchioles whilst HP, as indicated by its alternative name “(extrinsic) allergic alveolitis”, predominantly affects the alveoli. It is therefore also plausible that HP causing chemicals differ from OA causing chemicals in toxicokinetic properties such as relative solubility in water and lipid, given that lipophilic compounds would be more likely to partition at the level of the alveoli than at a bronchial level and that its molecules would need to cross a lipid rich surfactant layer in order to cause HP [14]. The objectives of this study were therefore to investigate the following hypotheses:

1. To investigate the application of a QSAR designed for predicting chemicals capable of causing occupational asthma to the prediction of HP causing chemicals.
2. To compare chemical structural and physico-chemical properties of occupational asthmagens and occupational causes of HP with particular reference to protein cross-linking potential and lipophilicity.

Methods

A literature search was undertaken to generate as comprehensive lists as possible of LMW chemicals reported to cause at least one human case of HP (the ‘HP list’) and those reported to cause at least one human case of occupational asthma due to sensitisation but no cases of HP (the ‘OA list’). For a chemical reported as the aetiological agent in either a case of OA or HP to be included in either list it had to be both organic (defined as carbon containing) and of molecular weight less than 1000 Da.

The process of literature searching began with the iteration and expansion of an already existing database of OA causing chemicals [5]. The Medline database was searched for journal articles published up to the end of 2011, which potentially contained case reports or series of OA caused by a LMW chemical. Search terms used either individually or in combination for Keyword, Topic and

Textword based searches included 'asthma', 'occupational asthma', 'respiratory sensitis(z)ation', 'respiratory sensitis(z)er', 'chemical', 'low molecular weight'. Articles that described either a case or case series of OA caused by a LMW organic compound were selected by scrutinising the title, abstract and where indicated the full article as described previously [5]. The criteria for OA diagnosis and causal attribution to a specific chemical were also the same as those previously used [5] in that the case report or series had to describe at least one clear diagnosis of OA attributed to a specific LMW organic compound following a latent period of exposure.

The same database, time frame and search strategy was employed to identify cases of HP with causal attribution to a LMW organic chemical. A different set of search terms was used which included the following either individually or in combination: 'pneumonitis', 'alveolitis', 'hypersensitivity pneumonitis', 'allergic alveolitis', 'respiratory sensitis(z)er', 'chemical', 'low molecular weight'. A second stage involved combining search results using each chemical name from the provisional OA list and 'hypersensitivity pneumonitis' or synonyms. From these search results the title, abstract and where indicated the full article were scrutinised in order to select all possible case reports or series describing HP with causal attribution to a LMW chemical. The search terms 'pneumonitis' and 'alveolitis' were employed without the respective pathophysiological descriptors 'hypersensitivity' and 'allergic' in order to try to screen for as many case reports of HP as possible. Care was then taken in the final selection to ensure that the case report or series definitely described pneumonitis resulting from hypersensitivity rather than a non-immunological type of chemical pneumonitis such as lipoid pneumonitis. Chemicals were therefore only included in the HP list if either the case report(s) clearly specified and discussed hypersensitivity mechanisms and there had been a latent period of exposure to the attributed cause.

When a given chemical had been reported to cause both HP and OA either within the same case or as individual conditions in separate cases the chemical was included in the HP list rather than the OA

list. The reasoning for this was that the number of chemicals reported to cause HP was predicted to be small by comparison with the number that cause OA and the study was aiming to examine the chemical features that differentiated this small subset of HP causing chemical respiratory sensitisers that overlapped with the larger subset of OA causing chemical respiratory sensitisers. A compound was only included in either list if the name used in the case report(s) or series gave it unambiguous identity.

For each compound in either the HP or OA list further information pertaining to CAS Registry Number and chemical structure were obtained from either The Merck Index [15] or one of several web based databases freely available online:

1. The ChemExper Chemical Directory . Available from: <http://www.Chemexper.com>
2. NCBI. PubChem. Available from: <http://pubchem.ncbi.nlm.nih.gov/>
3. Chemical Book . Available from: <http://www.chemicalbook.com>
4. Royal Society of Chemistry. ChemSpider. Available from: <http://www.chemspider.com/>

Using one of these online databases it was usually possible to download the structure of each compound in the form of a 'molfile', which is an alphanumeric textual representation of a chemical structure. When a molfile was not available online for a compound the structure was drawn using ChemDraw software which allows conversion into a molfile format. The molfile for each compound was then opened in text form using Notepad and cut and pasted into the box indicated on the Occupational asthma hazard prediction programme's webpage:

<http://www.coeh.man.ac.uk/asthma/login.php>

On submission of a chemical's molfile this programme generates a value, known as the 'asthma hazard index' (HI), between zero and one representing the determined probability that the given compound has potential to cause asthma by sensitisation.

The qualitative prediction of whether or not a given compound in the HP or OA lists has the potential to cross-link proteins was performed by an expert chemist [SJE] working independently. In order to ensure that for this categorisation the independent investigator was blind to whether a given compound was on the OA list or HP list these lists were pooled and then listed in alphabetical order alongside the identified CAS Registry Number before being sent to the expert chemist.

The expert chemist independently sought the chemical structure for each compound on the pooled HP/OA list using the Royal Society of Chemistry database, ChemSpider. Each compound for which a structure was so identified was categorised as either a potential protein cross-linker or not based on whether or not, in the expert chemist's opinion, it had at least two structural groups with the potential to react with amino acid side chains on endogenous proteins.

The logarithm of the coefficient of partition between octanol and water ($\log K_{ow}$) was used to ascribe a value for lipophilicity to each compound for which a structure was identified by the expert chemist. (The interpretation is that the larger the value of $\log K_{ow}$ for a chemical, the more lipophilic i.e. fat soluble and the less hydrophilic i.e. water soluble it will be) The chosen method for determining this parameter for each compound utilised publicly available computer software known as KOWWIN, developed by the US Environmental Protection Agency [16]. It allows the determination of $\log K_{ow}$ values for any chemical structure utilising an atom/fragment contribution method. Briefly, the approach breaks a molecule into a series of predefined chemical atoms/fragments, each atom/fragment has a defined $\log K_{ow}$ value determined from an analysis of experimental data. This allows simple QSAR models to be used to predict the $\log K_{ow}$ value of the entire chemical by counting the number of atoms/fragments and summing their contributions.

Statistical comparisons between the chemicals in the HP list and those in the OA list were performed for each of the three study parameters; QSAR generated asthma hazard index, cross-linking potential and log Kow. The Mann-Whitney U test was used where the variable was continuous but not thought to have a normal distribution as was the case for QSAR generated asthma hazard index and log Kow. Pearson Chi-square test was used to compare the chemicals on the HP and OA lists where the variable being compared was categorical as was the case for protein cross-linking potential. All statistical analysis was done using SPSS version 20.

Ethical approval was not required for this study as the only data used was existing human and toxicological data that were already in the public domain.

Results

The literature search identified 101 compounds which met the criteria for inclusion in the OA list (supplementary table 1) and 10 compounds which met the criteria for inclusion in the HP list (table 1). Nine of the 10 chemicals on the HP list had also been reported to cause OA due to sensitisation either coexisting with HP or as the sole manifestation of hypersensitivity.

It was possible to generate a HI using the QSAR for all 101 compounds on the OA list (supplementary table 1) and all compounds on the HP list (table 1). The median HI value for the 101 control chemicals ('the OA list') was 0.92 (interquartile range 0.48 to 1). The upper and lower quartile HI values of the 10 HP causing chemicals had values of 1 and the variation of HI across this set of chemicals differed significantly from that of the control set; $p < 0.005$ using the Independent Samples Mann-Whitney U test (figure 1).

The expert chemist working independently, and therefore using independent search strategies to identify chemical structures, was able to identify unambiguous structures for all ten of the compounds on the HP list but only 92 of the 101 controls on the OA list.

Nine of the ten chemicals identified as having caused HP were predicted to be cross-linkers by the expert in mechanistic chemistry [SJE]. The proportion of predicted cross-linkers differed significantly from that in the control set which was 24 out of 92 (25%); $p < 0.0001$ using Pearson Chi-square test (table 2).

The median log Kow value for the 92 control chemicals was 0.81 (interquartile range -0.34 to 2.88). The median log Kow value for the 10 HP causing chemicals was 3.47 (interquartile range 0.86 to 4.80) and the variation across this group differed significantly from the variation in log Kow across the set of 92 controls; $p < 0.05$ using the Independent Samples Mann-Whitney U test (figure 2).

Discussion

This study set out to explore whether low molecular weight organic compounds that have been reported to cause hypersensitivity pneumonitis (HP) have structural features or physico-chemical properties that distinguish them from occupational asthma (OA) causing chemical respiratory sensitisers that do not cause HP. Despite the relatively low number of chemicals reported to cause HP the study results indicate, with statistical significance, that HP causing chemicals tend to have a stronger QSAR predicted asthma hazard, are more lipophilic and have greater potential for protein cross-linking than those causing OA.

The small size of the set of LMW chemicals identified as causative of HP from peer reviewed case reports was expected. HP is a much rarer condition than OA and LMW chemicals are much less frequently reported as causative agents than are high molecular weight substances containing

antigenic proteins e.g. 'Farmer's lung' caused by mouldy hay. Chemical causes tend to be briefly alluded to at the bottom of lists of aetiological agents in review articles of HP in categories such as 'Chemical worker's lung' attributed to limited and generalised chemical groups such as diisocyanates and acid anhydrides [24]. We have identified ten specific LMW organic compounds reported to have caused HP in humans. The report of a diagnosis of hypersensitivity pneumonitis (or allergic alveolitis) and causal attribution to a LMW chemical by a physician in a peer reviewed journal was considered sufficient evidence that the diagnostic criteria had been met for HP. A latent period of exposure was, however, deemed a necessary criterion to exclude causes of lipoid pneumonia since the study was limited to HP and not all causes of chemical pneumonitis (especially since lipophilicity was a property being tested in relation to possible association with HP).

The final list of HP causing chemicals that is shown in table 1 includes several specific diisocyanates, all of which are also well recognised causes of OA. Amongst the acid anhydrides, however, our literature search found sufficient evidence of causation of HP for only pyromellitic dianhydride. The evidence for HP causation by any of the acid anhydrides which are more usually associated with asthma is not conclusive. There are two published case reports of HP associated with polyester powder paint [25,26]. Piirila et al [25] made an assumption that the HP causing agents in their case were acid anhydrides present in the paint fumes in which they detected small concentrations of these chemicals by gas chromatography. Cartier et al [26], however, left the possibility open that the alveolitic reaction in their case was due to linear saturated polyesters or released LMW compounds such as aldehydes. They excluded trimellitic anhydride as the cause by performing a specific inhalation challenge test which was negative. Neither of these case reports were therefore considered as sufficient evidence in this study that either phthalic anhydride or trimellitic anhydride are causes of HP.

Pyromellitic dianhydride was the only chemical identified as a reported cause of HP that has apparently never been reported to cause OA. All the remaining compounds listed in table 1 have either been reported to cause OA as the sole pathology in other cases or, as with triphenylmethane triisocyanate, coexisting in a single case of HP. Interestingly our control group of 101 asthma causing chemical respiratory sensitizers had a lower mean QSAR generated asthma hazard index than the 10 HP causing chemicals. This could be a manifestation of the high representation of diisocyanates which account for 50% of the HP causing chemicals identified. Another possible explanation of this finding is that the molecular descriptors associated with asthmagenicity are even more frequent in molecules of HP causing chemicals, perhaps indicative of the requirements for protein reactivity that lead to subsequent immunopathological events. Asthma, when caused by LMW chemicals, sometimes results from IgE-mediated type I hypersensitivity but also by as yet poorly characterised immunological or other mechanisms. By contrast HP, at least when caused by HMW agents, is thought to result from type III and type IV hypersensitivity mechanisms and IgG antibodies interacting with complement components to trigger the cellular accumulation of macrophages and T-lymphocytes.

The hypothesis that cross-linking of native human proteins is an initial step in chemical asthmagenesis was developed from observations that two or more reactive groups were frequently present in chemical respiratory sensitizers that caused asthma [3]. A chemical respiratory sensitizer is not a complete antigen and is thought to be antigenic by reaction with native human proteins. A single bond with a native protein molecule can produce a hapten-protein conjugate that can function as an epitope that might be responsible for initiating asthma or rhinitis for some chemical respiratory sensitizers. This process is, however, unlikely to be a sufficient trigger for the immunological processes involved in HP which involve immune complex formation. An immune complex could, however, be formed if a chemical forms intramolecular and/or intermolecular cross-links in protein molecules. The formation of intramolecular protein cross-links could expose multiple

self epitopes leading to polyclonal (auto)antibody (IgG) production. Bivalent IgG could then cross-link one protein molecule with another commencing the formation of an immune complex lattice. This would be essentially the same process as that triggered by a HMW antigen, e.g. fungal antigens in mouldy hay, except that the postulated antigen is a self protein made autoantigenic by conformational change from crosslinking by the chemical sensitiser.

The exception to this hypothesis is Pauli's reagent (4-diazobenzenesulphonate) for which there was strong clinical evidence of correct causal attribution, including positive specific inhalation challenge and histopathological features of pneumonitis found following lung biopsy. The case was unusual, however, in that specific IgE was demonstrated by skin prick testing as well as histological findings of immunoglobulin deposition in alveoli and low serum C3. Interestingly specific inhalation challenge resulted in both immediate and late bronchial responses and this could be a case in which a chemical respiratory sensitiser was acting via IgE-mediated mechanisms to cause asthma and other mechanisms to cause pneumonitis which may or may not be immunologically mediated.

The case report of HP caused by pyromellitic acid dianhydride included detailed immunoserological analysis which suggested that serum IgG in the patient exposed to pyromellitic acid dianhydride at work had been cross-linked to form dimers. Further examples of such *in vivo* evidence together with *in vitro* studies would be required to prove that cross-linking of human proteins by a chemical respiratory sensitiser is necessary to generate the antigenic trigger for the immunopathology of HP. The initial chemical reactions are easy to illustrate mechanistically (figure 3).

There is a biologically plausible explanation for lipophilicity being an important requirement for chemicals to cause HP. Type II alveolar cells produce a lipoprotein surfactant layer in order to reduce surface tension of expanding alveoli. It comprises phospholipids such as dipalmitoylphosphatidylcholine which has two hydrophobic tails projecting into the air facing side of

this surfactant layer which is strongly hydrophobic [27]. In order to partition in this layer and enter the alveolar interstitium a chemical would need to be lipophilic [14]. This concept is clearly evident in lipoid pneumonia, a form of chemical pneumonitis in which the pathological mechanism does not involve immunologic hypersensitivity but results from the acute inhalation of certain oil based substances [28]. Lipophilicity, in conjunction with any structural features necessary to confer the required toxicodynamic properties might also be a requirement in the development of chemical induced HP.

The development of a QSAR prediction model for HP analogous to that for asthma [5] is a worthy long term aim. However, as this paper shows the dataset available so far is too limited. There are also only infrequent case reports with which to explore the mechanistic hypotheses. Some agents such as clozapine [29] have been reported as causing pneumonitis but not through hypersensitivity mechanisms. Indeed clozapine is a lipophilic compound (log Kow 3.35) and it scores highly on the asthma QSAR (HI 0.93). For some LMW agents that cause pneumonitis hypersensitivity mechanisms are clearly involved but the specific causative chemical entity has not been identified. An example is pyrethrum [30] which comprises two pyrethrin compounds both of which are lipophilic (figure 4). Application of the hypotheses presented here might lead to speculation that pyrethrin II is the specific HP causing compound because it has two electrophilic centres giving it potential for protein cross-linking, whereas pyrethrin I only appears to have one such centre that could bond with a protein side chain.

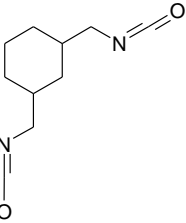
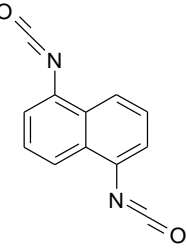
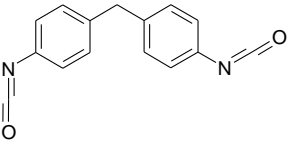

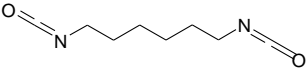
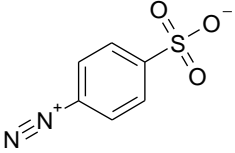
The pilot study reported here has generated plausible hypotheses that lipophilicity and protein cross-linking properties are important requirements for a chemical to be capable of causing hypersensitivity pneumonitis. Corroboration of these suggested disease mechanisms would probably require both laboratory investigation and clinico-epidemiologic studies.

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Table 1. LMW chemicals reported to cause HP in peer-reviewed literature with their structures and study parameters

Chemical Name [example case reference]	CAS Registry Number	Chemical structure	Ever reported to cause OA? Y/N	QSAR generated Hazard Index (0-1)	Cross-linking potential Y/N	Log Kow
1,3-Bis(isocyanatomethyl)cyclohexane [17]	38661-72-2		Y	1	Y	3.92
1,5-Naphthalene diisocyanate [1]	3173-72-6		Y	1	Y	4.37
Diphenylmethane diisocyanate [18]	101-68-8			1	Y	5.22
Formaldehyde [19]	50-00-0			1	Y	0.35
Hexamethylene diisocyanate [20]	822-06-0		Y	1	Y	3.2
4-diazobenzenesulphonate (Pauli's reagent) [21]	305-80-6		Y (Only in conjunction with HP)	0.31	N	-0.8

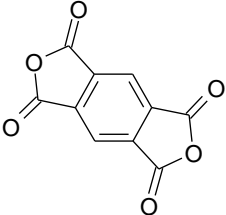
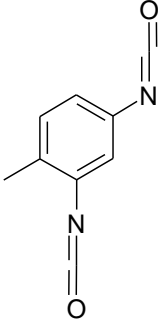
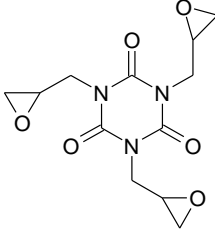
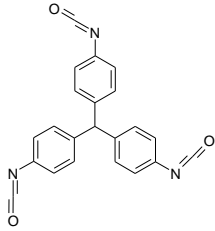
Pyromellitic dianhydride [11]	89-32-7		N	1	Y	2.14
Toluene diisocyanate [22]	584-84-9		Y	1	Y	3.74
Triglycidyl isocyanurate [23]	2451-62-9		Y	1	Y	1.21
Triphenylmethane triisocyanate [10]	2422-91-5		Y (Only in conjunction with HP)	1	Y	7.17

Table 2. Proportions of respiratory sensitisers (RS) predicted to be protein cross-linkers

	Numbers of chemical RS reported to cause HP	Numbers of chemical RS reported to cause OA but not HP	Total
Predicted to have cross-linking potential	9	24	33
Predicted not to have cross-linking potential	1	68	69
Total	10	92	102

p < 0.0001 (Pearson chi-square test)

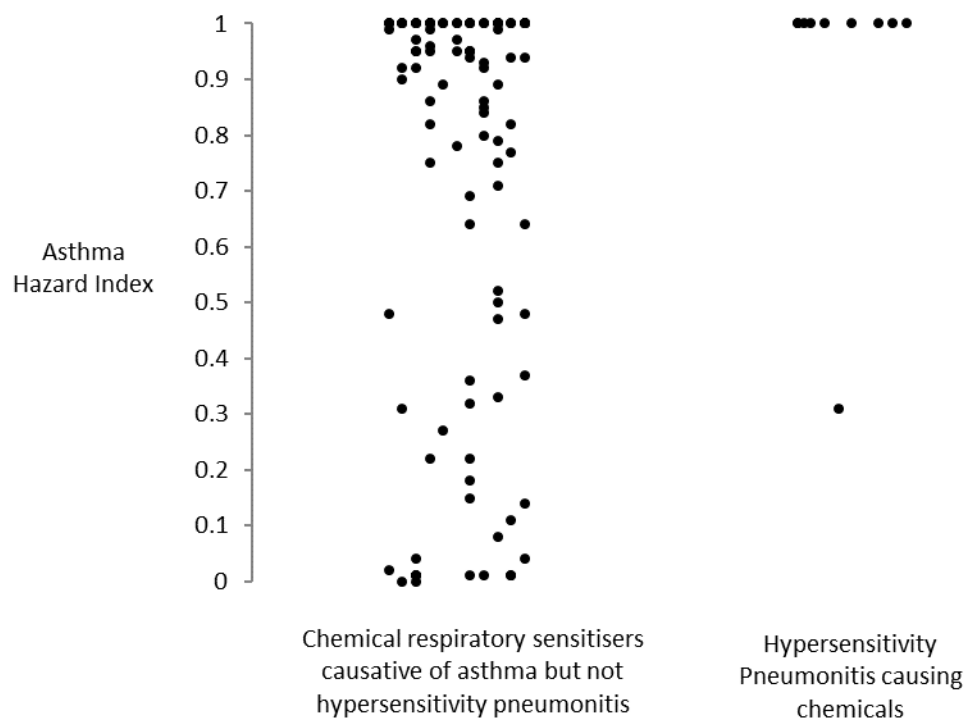


Figure 1. Variation in asthma hazard index for HP causing and control respiratory sensitisers ($p < 0.005$)

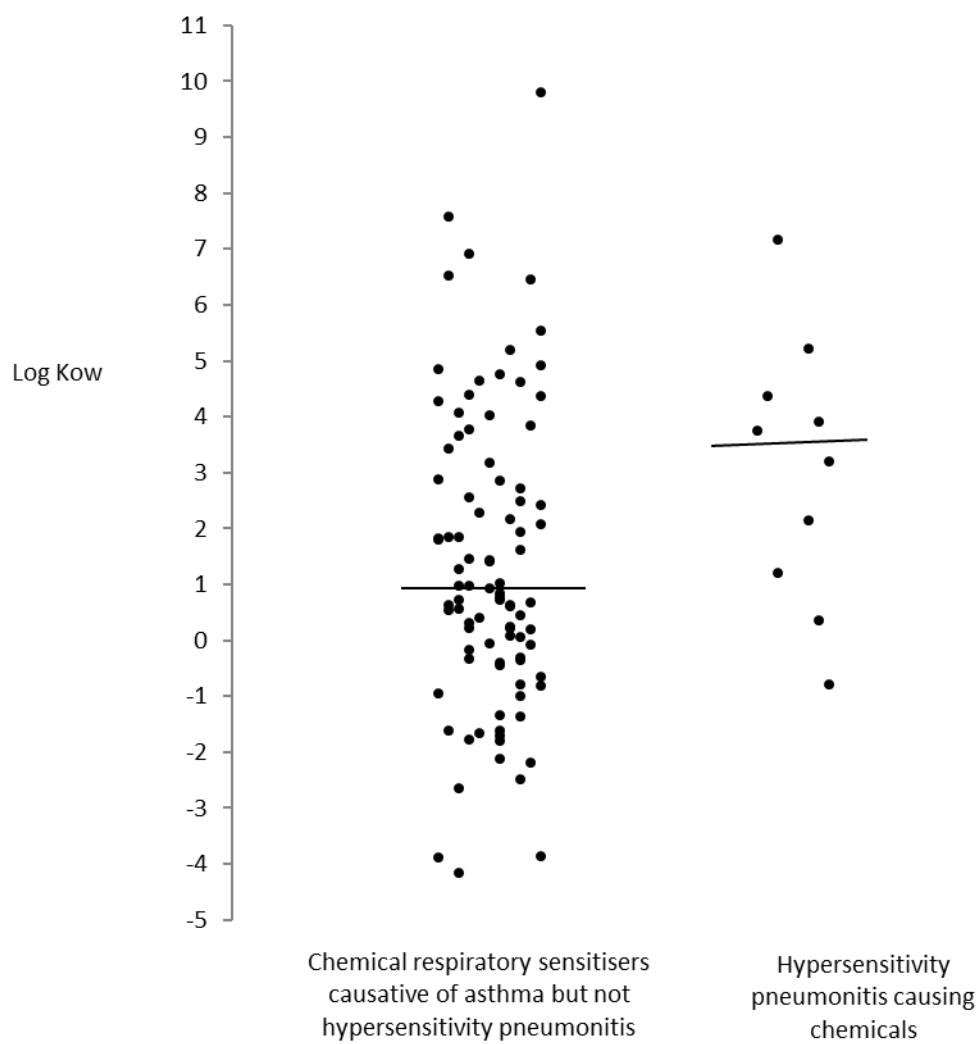


Figure 2. Variation in hydrophobicity (as indicated by Log Kow) for HP causing and control respiratory sensitisers (median values indicated by horizontal line) ($p < 0.05$)

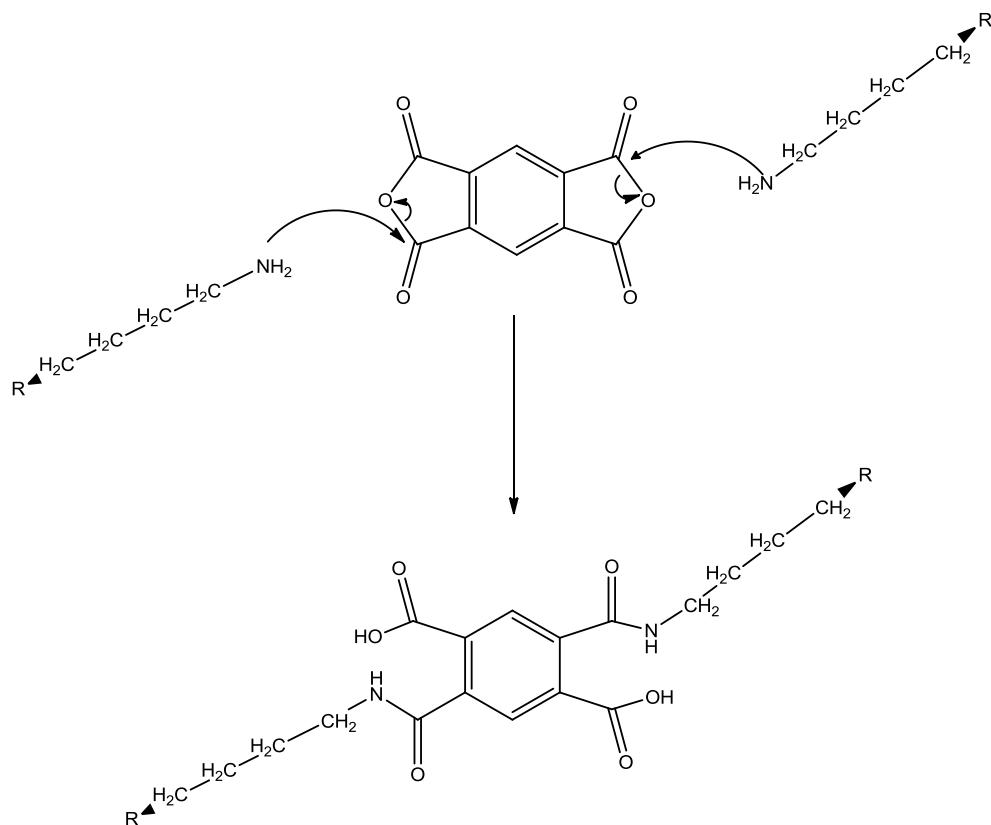
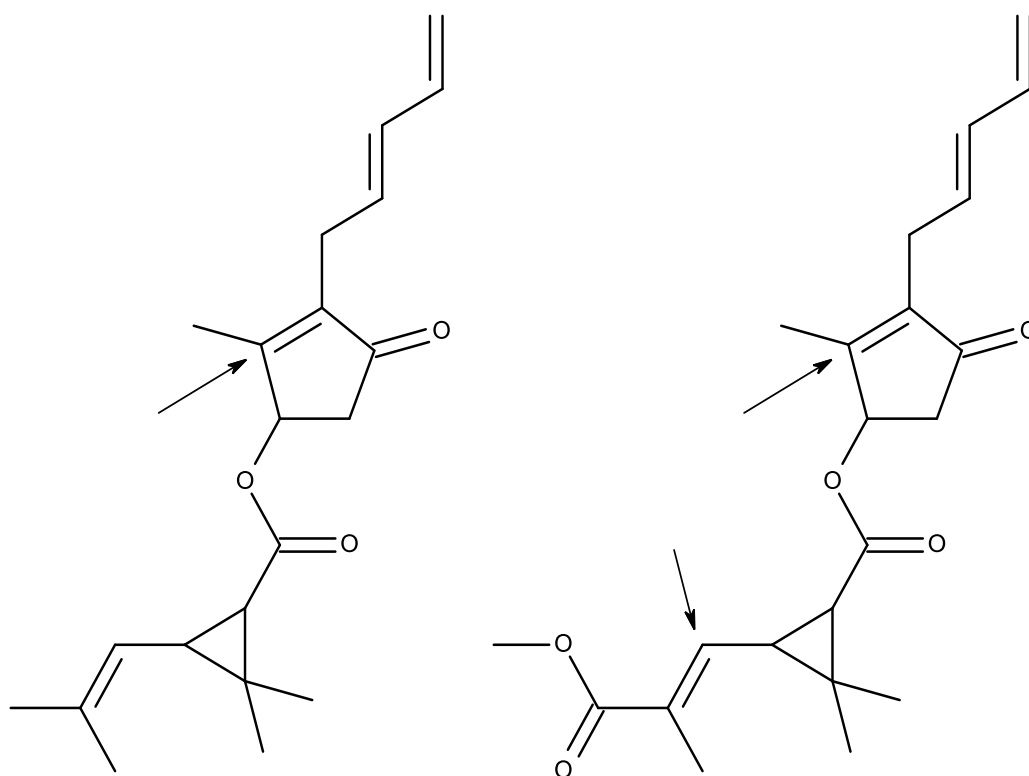


Figure 3. Reaction of a pyromellitic dianhydride molecule with two lysine side chains of either the same protein (causing intramolecular cross-linking) or different proteins (causing intermolecular cross-linking)



Pyrethrin I

Pyrethrin II

Pyrethrin I	(CAS RN 121-21-1)	log Kow = 6.28	HI = 0.89
Pyrethrin II	(CAS RN 121-29-9)	log Kow = 5.33	HI = 0.99

Figure 4. Core structure of pyrethrins (arrows indicate potential sites of nucleophilic attack by nitrogen containing lysine residues)