Chemical Research in Toxicology

Article

pubs.acs.org/crt

Development of Mechanism-Based Structural Alerts for Respiratory Sensitization Hazard Identification

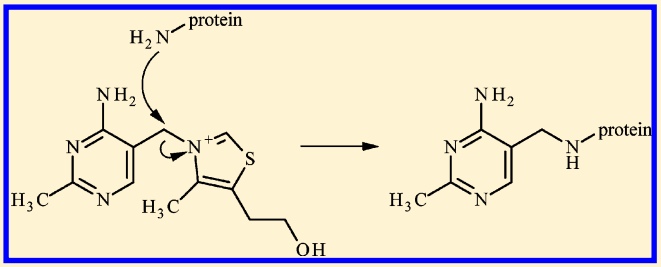
S. J. Enoch,*[,†] M. J. Seed,[‡] D. W. Roberts,[†] M. T. D. Cronin,[†] S. J. Stocks,[‡] and R. M. Agius[‡]

[†]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, England L3 3AF

[‡]Centre for Occupational and Environmental Health, University of Manchester, Manchester, England M13 9PL

Supporting Information

ABSTRACT: This study outlines how mechanistic organic chemistry related to covalent bond formation can be used to rationalize the ability of low molecular weight chemicals to cause respiratory sensitization. The results of an analysis of 104 chemicals which have been reported to cause respiratory sensitization in humans showed that most of the sensitizing chemicals could be distinguished from 82 control chemicals for which no clinical reports of respiratory sensitization exist. This study resulted in the development of a set of mechanism-based structural alerts for chemicals with the potential to cause respiratory sensitization. Their potential for use in a predictive algorithm for this purpose alongside an externally validated quantitative structure−activity relationship model is discussed.



INTRODUCTION

The term respiratory sensitizer has been defined by the Globally Harmonised System for Classification and Labelling of Chemicals as a substance that will induce hypersensitivity of the airways following inhalation.[1] The system places emphasis on human evidence where "hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered." More than 300 substances have been shown to cause occupational asthma, and a large proportion of these are low molecular weight (LMW) organic compounds.[2]

There is currently no widely accepted test method that is able to identify potential LMW respiratory sensitizers for regulatory purposes, despite the risk to human health. A number of previous studies have shown that the formation of a covalent bond between a protein in the lung and a LMW chemical is the key first step (the so-called molecular initiating event) leading to respiratory sensitization in humans.[3−5] This hypothesis is supported by the number of respiratory sensitizers that test positive in the local lymph node assay (LLNA) for skin sensitization. The presence of more than one reactive group in a compound making protein cross-linking possible as an initial step in chemical respiratory sensitization had also been hypothesized previously following statistical structure−activity relationship (SAR) analyses.[6−8] Such a statistical approach has been used to develop a quantitative SAR (QSAR) model for the purpose of asthma hazard prediction. Because of its high negative predictive value, it has been proposed as an efficient initial screening tool for eliminating the need for further consideration of respiratory sensitization hazard for the vast majority of compounds.[9] However, its lower positive predictive value means that further consideration may be required before labeling a chemical as a respiratory sensitizer. A mechanistic SAR approach may offer an important means of substantiating suspicion of respiratory sensitization potential for a chemical.

Previous mechanistic chemistry studies have shown that chemicals able to cause respiratory sensitization can be assigned to one of six electrophilic mechanistic domains.[3,4,10] These electrophilic mechanistic domains cover areas of chemistry similar to those defined for skin sensitization.[11] However, these studies showed there to be some key mechanistic differences (in terms of the chemistry) between skin and respiratory sensitization.[4] For example, in the case of respiratory sensitization, the harder electrophilic mechanisms such as acylation and Schiff base formation were shown to be more prevalent than the softer mechanisms such as Michael addition. The hypothesis for this difference is that the harder nucleophile lysine acts as the predominant biological nucleophile in the lung (the assumption being that cysteine is unavailable due to it being oxidized in the respiratory tract to disulfide). The importance of lysine as the biological nucleophile in respiratory sensitization has been confirmed experimentally in a number of studies.[12−14] In addition, these studies also suggested that a protein modification threshold exists for respiratory sensitization. This threshold can be exceeded due to the compound's intrinsic electrophilicity alone or by a combination of electrophilicity and cross-linking ability. Thus, extremely electrophilic chemicals do not need to be able to protein cross-link in order to cause respiratory sensitization. This is in contrast to skin sensitization where a combination of

Received: July 6, 2012
Published: October 11, 2012

electrophilicity and (for some mechanistic domains) hydrophobicity are the key drivers of sensitization potential.[15−17]

A number of studies have described structural alerts (molecular substructures or fragment) that relate an electrophilic or pro-electrophilic group within a molecule to covalent protein binding for skin sensitization and skin irritation.[15,18−23] However, there have been no published sets of structural alerts developed specifically for the mechanistic chemistry applicable within the more oxidizing environment of the lung. Previous mechanistic chemistry studies into respiratory sensitization have outlined potential mechanisms but have not compiled structural alerts.[3,4] The development of mechanism-based structural alerts is important as they allow mechanistic information to be used for hazard identification (for example, as found in commercial software such as Derek Nexus; www.lhasalimited.org). In addition, such structural alerts have also been incorporated into a number of freely available tools such as Toxtree (freely available from http://toxtree.sourceforge.net/). Clearly, the expansion of these freely available tools to other end points of regulatory interest is of benefit and is an important ongoing effort.

Therefore, the aim of this study was to develop a series of mechanism-based structural alerts for respiratory sensitization (hazard identification). This was undertaken by a mechanistic chemistry analysis of a data set of chemicals identified as being respiratory sensitizers in humans. This analysis allowed a set of structural alerts related to covalent protein binding in the lung to be developed.

MATERIALS AND METHODS

Respiratory Sensitizing Chemicals. 104 organic chemicals with molecular weights less than 1000 g/mol for which structures could be clearly identified were extracted from the literature as being associated with respiratory sensitization in humans (the 104 chemicals include the chemicals previously published[3,4]). These chemicals were defined as low molecular weight (LMW) respiratory sensitizers and were identified from the literature if a physician had, in a peer-reviewed report, clearly diagnosed occupational asthma following a latent period of exposure. Medline literature search techniques were similar to those used to identify a previously published set of respiratory sensitizers,[8] but the time period was extended to include all case reports published before October 2011. Not all of these respiratory sensitizers had been confirmed by bronchial challenge testing, widely considered among respiratory physicians to be the gold standard for the diagnosis of occupational asthma attributed to a specific causative agent.[24] However, the process of peer-review provides a good level of corroboration that a novel chemical respiratory sensitizer has been correctly identified by the physician publishing the case. By including these case reports, the number of chemicals from which structural alerts can be generated for hazard prediction is significantly greater than if only those published case reports with a positive bronchial challenge test are used.

Control Chemicals. A set of 82 organic chemicals with molecular weight ranges less than 1000 g/mol were identified as control chemicals. These chemicals were assumed not to cause respiratory sensitization in humans as no clinical reports of occupational asthma were documented in the literature. This being the case despite the fact that for these chemicals industrial exposure experience is sufficient for workplace exposure limits to be set. These controls were selected at random from the 401 compounds for which a workplace exposure limit was listed in the Health & Safety Executive document EH40 (available from http://www.hse.gov.uk/pubns/books/eh40.htm) and matched as far as possible with the set of respiratory sensitizers by molecular weight banding. It could be argued that any chemical has the potential to cause human respiratory hypersensitivity given the right exposure circumstances and that it is impossible to prove that any chemical is a nonrespiratory sensitizer. By identifying controls from workplace exposure limit tables and which had never been reported to cause occupational asthma, there is at least evidence that humans have had fairly extensive industrial inhalational exposure. Such tables were also the source of controls for the learning data set used in the development of the QSAR model by Jarvis et al.[8] The full data set of respiratory sensitizers and control chemicals is available in the Supporting Information.

Mechanistic Assignments. Analysis of the chemistry related to covalent binding in the lung relevant to respiratory sensitization was carried out by expert analysis. This analysis consisted of two stages, the first being the development of a set of structural alerts based on the previously published mechanistic chemistry for respiratory sensitization.[3,4] These structural alerts were used to assign electrophilic mechanisms to the chemicals in the data set. Chemicals for which no electrophilic mechanism could be identified were subjected to additional expert analysis. This analysis resulted in the definition of additional structural alerts related to covalent protein binding in the lung. All structural alerts developed in this study were encoded as SMARTS patterns and used in conjunction with an in-house KNIME workflow. All SMARTS patterns are available in the Supporting Information.

Structural Alert Development. All structural alerts were developed from an analysis of the organic chemistry related to the molecular initiating event of covalent protein binding. A set of 22 structural alerts were developed from an analysis of the previously published mechanistic chemistry relating to respiratory sensitization.[3,4] These 22 structural alerts had not been published previously. The 22 structural alerts were used to screen the 104 respiratory sensitizing chemicals. An analysis of the respiratory sensitizing chemicals not containing an alert resulted in the identification of a further 30 structural alerts. All 52 structural alerts were related to the organic chemistry of covalent protein binding. The ability of the structural alerts to distinguish respiratory sensitizers from the control chemicals was also investigated.

RESULTS AND DISCUSSION

The aim of this study was to develop a set of structural alerts suitable for the identification of potential respiratory sensitizing chemicals (hazard identification). The set of structural alerts was based on an analysis of a data set of chemicals reported to cause respiratory sensitization in humans. An initial set of structural alerts was developed based on the previously published mechanistic chemistry related to covalent protein binding in the lung.[3,4] The applicability domain covered by these structural alerts was then evaluated by investigating their ability to identify respiratory sensitizers from the data set of 104 chemicals. Finally, the applicability domain of the structural alerts was expanded by analyzing the respiratory sensitizing chemicals not identified in this initial evaluation. All analyses were undertaken from the hypothesis that the molecular initiating event for chemicals with a molecular weight below 1000g/mol was the formation of a covalent protein adduct in the lung.

Structural Alerts Related to Previously Published Mechanistic Chemistry. A set of 22 structural alerts were identified for the profiler for respiratory sensitization from an analysis of mechanistic chemistry published previously.[3,4] The applicability domain covered by the initial profiler was investigated by assessing the number of chemicals identified as having a mechanism related to covalent protein binding in the new (expanded) data set of respiratory sensitizing chemicals considered in this study. Table 1 illustrates the ability of the mechanistically derived structural alerts to distinguish respiratory sensitizers from the control group of chemicals. Table 2 shows a detailed breakdown of the number of respiratory

Table 1. Contingency Table Showing the Classification of Respiratory Sensitiser and Controls Based on the Initial Set of 22 Structural Alerts

	structural alert	no structural alert
respiratory sensitizer (104)	59	45
control (82)	2	80

Table 2. Structural Alerts Present in the Initial Profiler along with the Number of Chemicals Identified by Each Structural Alert from the Data Set Used in the Current Study

structural alert	number of chemicals	cross-linking required?
di-isocyanates	9	Y
quinone-and-related	0	N
hydroquinone-and-related	8	N
anhydrides	8	N
lactams	11	N
cyano-acrylates	2	N
piperazines	1	Y
ethylenediamines	3	Y
ethanolamines	5	Y
azocarbonamides	1	N
tetrachloroisophthalonitrile	1	N
formaldehyde	1	Y
dialdehydes	1	Y
vinyl-benzenes	1	N
vinyl-sulphones	0	N
pro-vinyl-sulphones	0	N
glyoxal	0	Y
chlorhexidine-and-related	1	N
epoxides	3	N
chloro-nitrogen	1	N
acyl chlorides	1	N
phenyl-acetates	2	N

sensitizing chemicals identified by each of the 22 structural alerts (59 of the 104 chemicals; N.B. chemical 114 triggered two structural alerts).

The 45 respiratory sensitizing chemicals that were not identified as having a mechanism related to covalent protein binding by the initial profiler were subject to mechanistic chemistry analysis. The analysis allowed for the expansion of the applicability domain covered by the profiler through the identification of additional structural alerts related to covalent protein binding. The new structural alerts are described in the following sections.

Nucleophilic Aromatic Substitution (S_NAr). Of the respiratory sensitizers which are not categorized by the initial set of structural alerts, a nucleophilic aromatic substitution mechanism can be proposed for fluazinam; 4-diazobenzenesulphonic acid; 1,3,5-triazine-2,4-diamine-6-chloro-*N*,*N*′-bis-(2,2,6,6-tetramethyl-4-piperidinyl); sulfathiazole; and hexachlorophene (chemicals 1−5, respectively, in Figure 1). In addition, hexachlorophene has the potential to cross-link protein chains as it contains two reactive sites. This helps explain the toxicity of this compound because a single chlorination phenolic ring system would not normally be expected to be very reactive toward nucleophiles. The requirement to cross-link is especially evident given that the more reactive chemical dinitro chlorobenzene does not cause respiratory sensitization.[3]

Michael Addition. A Michael addition mechanism can be postulated for 10 chemicals that were not identified by the initial set of structural alerts. Eugenol and salbutamol can be activated to quinone-methides (Figures 2 and 3, respectively).

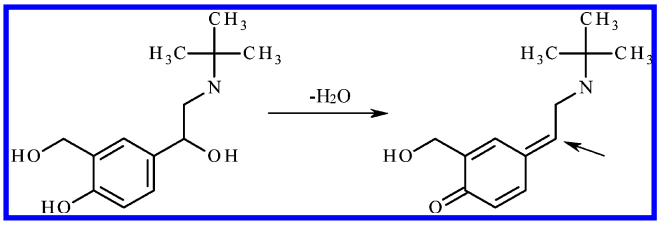


Figure 2. Dehydration of salbutamol to a quinone-methide containing species (reactive site as indicated).

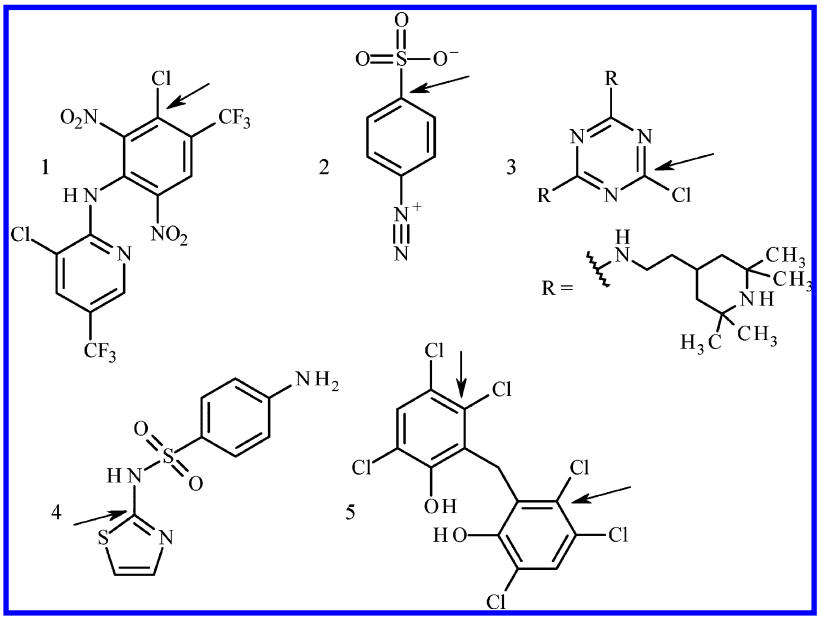


Figure 1. Chemicals suggested as being capable of undergoing a S_NAr mechanism leading to covalent adduct formation (reactive site as indicated).

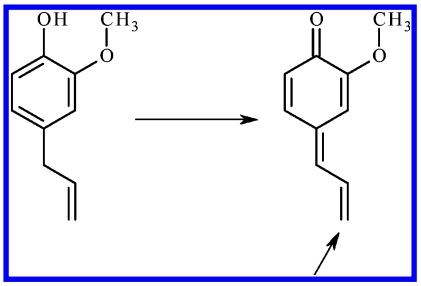


Figure 3. Oxidation of eugenol to a quinone-methide containing species (reactive site as indicated).

In the case of eugenol, this is an oxidation reaction, while for salbutamol the quinone-methide is produced via a dehydration reaction. Morphine hydrochloride can also undergo an oxidation reaction to produce a cyclohexanone ring system capable of acting as an electrophile (Figure 4). BBN reactive

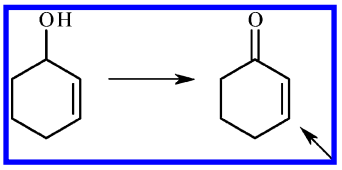


Figure 4. Oxidation of the ring system 2-cyclohexanol (present in morphine hydrochloride) to cyclohexanone (reactive site as indicated).

dye can undergo a tautomerization to produce a quinone-imine (Figure 5). Finally, methyl blue and indigotine are able to act as direct acting electrophiles (Figure 6 and 7, respectively).

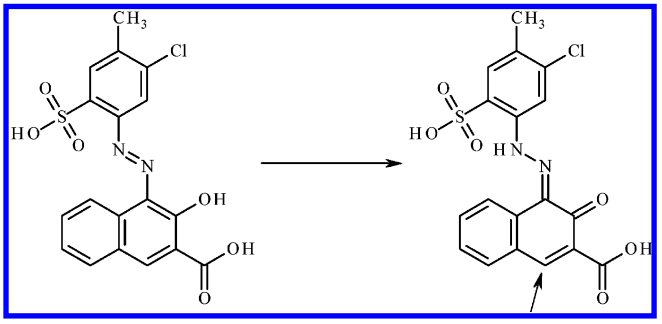


Figure 5. Tautomerization of Red BBN reactive dye into a quinone-imine (reactive site as indicated).

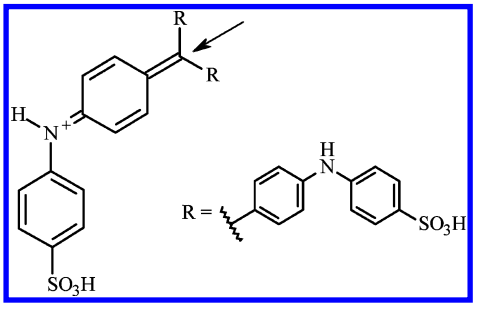


Figure 6. Structure of methyl blue that contains a nitrogen derivative of a quinone-methide (reactive site as indicated).

Methyl methacrylate, ethoxylated bis-phenol-A diacrylate and trimethylolpropane triacrylate can act via Michael addition due to the presence of an acrylate moiety (Figure 8). The ability of these chemicals to cause respiratory sensitization is perhaps

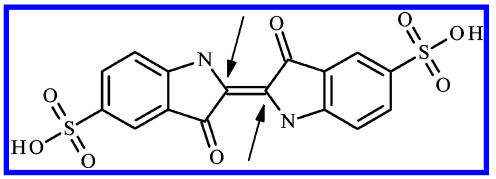


Figure 7. Structure of indigotine indicating the presence of the electrophilic sites for Michael addition.

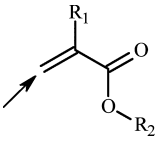


Figure 8. Structure of acrylate (R1 = H, R2 = C) and methacrylate (R1 and R2 = C) containing chemicals (reactive sites as indicated).

surprising given that acrylates and methacrylates have been reported as being relatively weak skin sensitizers.[25] Acrylates and methacrylates are in fact relatively reactive such that they polymerize to poly acrylates and poly methacrylates rapidly in solution. It is this polymerization reaction that reduces their ability to cause skin sensitization.[26] However, in the case of respiratory sensitization these chemicals will be in the vapor phase and thus will not be polymerized to the same degree. (The lining of the lung is an aqueous environment such that some polymerization is likely to occur when an acrylate or methacrylate comes into contact with the membrane. In contrast, in terms of skin sensitization the polymerization reaction is likely to have extensively occurred in the aqueous vehicle used in the assay before the chemical comes into contact with the skin.) Thus, the presence of the reactive monomer units in the lung results in respiratory sensitization. In addition, ethoxylated bis-phenol-A diacrylate and trimethylolpropane triacrylate contain multiple acrylate units allowing for the cross-linking of protein chains.

The final additional chemical that can be assigned to the Michael addition domain is 2-methyl-3,4-dinitrobenzamide. This chemical can undergo tautomerism to produce an aci-tautomer capable of reacting with a nucleophile. The tautomerization and subsequent reaction are as shown in Figure 9.

Bimolecular Nucleophilic Substitution (S_N2). An additional 10 chemicals not identified by the initial set of structural alerts can act via a bimolecular nucleophilic substitution at either a carbon or a sulfur atom. Amprolium hydrochloride and thiamine can undergo an S_N2 reaction at an activated carbon atom in which a positively charged aromatic system acts as the leaving group (Figure 10). 3-Carene and abietic acid can potentially undergo an S_N2 mechanism after epoxidation of the cyclohexene ring (Figure 11). However, for these two chemicals an alternative mechanism involving the formation of a hydroperoxide cannot be ruled out (mechanism not shown). These oxidation reactions producing epoxides (or hydroperoxides) have been previously shown to occur in a variety of oxygenated systems.[27−31] It is therefore likely that such species will be readily produced in the lung. 1,2-Benzisothiazolin-3-one, 3-amino-5-mercapto-1,2,4-triazole, captafol, and penicillamine all contain sulfur atoms that can undergo covalent binding via disulfide exchange (Figure 12). Upon first inspection, the presence of this mechanism is perhaps unusual as it is generally considered that the biological nucleophile in the lung is nitrogen-based.[14] However, covalent

dx.doi.org/10.1021/tx3003092 | Chem. Res. Toxicol. 2012, 25, 2490−2498

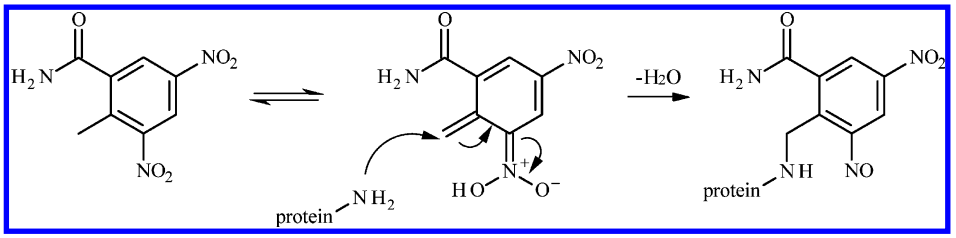


Figure 9. Tautomerism of 2-methyl-3,4-dinitrobenzamide to produce an aci-tautomer capable of undergoing Michael addition.

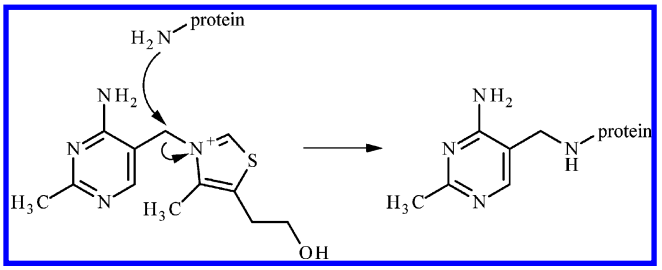


Figure 10. S_N2 mechanism at an activated carbon atom involving a positively charged aromatic system acting as the leaving group (reaction with thiamine is shown).

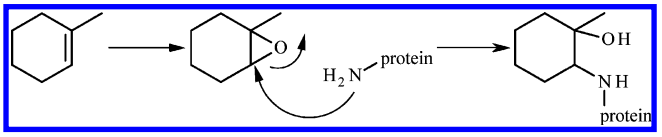


Figure 11. S_N2 mechanism via epoxidation of a cyclohexene ring.

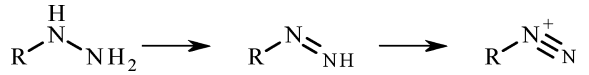


Figure 13. S_N2 mechanism for hydrazine derivatives (R = alkyl, aryl).

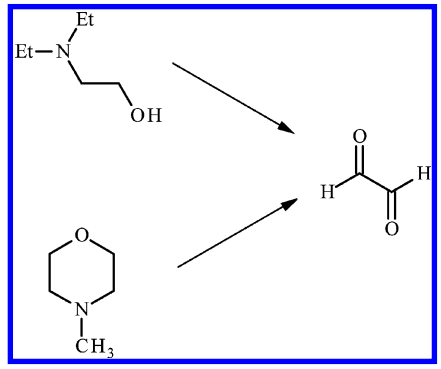


Figure 14. Oxidative deamination of 2-diethylethanolamine and *N*-methylmorpholine into glyoxal.

bond formation via a disulfide exchange has been shown to be important in a number of toxicity end points.[21,32,33] In this mechanism, the chemical is acting as the electron rich nucleophile due to the presence of the lone pair on the thiol. This is in contrast to the usual situation in which exogenous chemicals behave as electron deficient electrophiles. Thus, given the clear association with activity in the current analysis this mechanism is also clearly important for respiratory sensitization.

There are two hydrazine derivatives in the data set both of which are reported as respiratory sensitizers, hydralazine and isoniazid. These chemicals are likely to act as very hard electrophiles capable of binding to proteins via an S_N2 reaction involving N_2 as a leaving group. This mechanism is summarized in Figure 13.

Schiff Base Formation. A Schiff base mechanism can be postulated for nine of the respiratory sensitizing chemicals not assigned a mechanism in Table 2. 2-Diethylethanolamine and *N*-methylmorpholine can be oxidatively deaminated to produce glyoxal (Figure 14). Glyoxal is an extremely reactive Schiff base former that has been shown to be able to cross-link protein chains.[34] In addition, thiamphenicol contains a dichloroacetamide moiety that can undergo hydrolysis to produce a glyoxal-type species (Figure 15). An oxidative deamination mechanism can be applied to 3-dimethylaminopropylamine resulting in the formation of the dialdehyde propanedial (Figure 16). This chemical is capable of cross-linking proteins due to the two carbonyl groups.

Methanamine has been reported to be a formaldehyde releaser[35,36] resulting in indirectly causing respiratory sensitization as formaldehyde is able to covalently cross-link protein chains.[34] The ability of a chemical to release formaldehyde resulting in respiratory sensitization is clearly of concern, and thus, other chemical classes that have been reported as being capable of the same mechanism should also be considered as potential sensitizers. A recent review reported a series of structural alerts for chemicals capable of releasing formaldehyde.[32] These chemical classes are summarized in Figure 17.

Cimetidine and 1,1,3-tributylthiourea can undergo a Schiff base reaction at the imidourea and thiourea moieties, respectively. This reaction is analogous to the reaction that can occur at a carbonyl group and is as shown in Figure 18. In addition, furfuryl alcohol can be oxidized to the monocarbonyl containing furfuryl aldehyde (Figure 19). In contrast to the

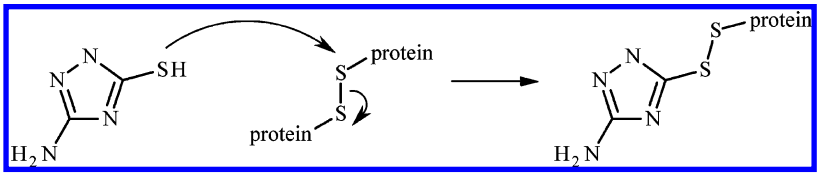


Figure 12. Disulfide exchange type mechanism for chemicals assigned to the S_N2 at a sulfur atom category (3-amino-5-mercapto-1,2,4-triazole is shown).

dx.doi.org/10.1021/tx3003092 | *Chem. Res. Toxicol.* 2012, 25, 2490−2498

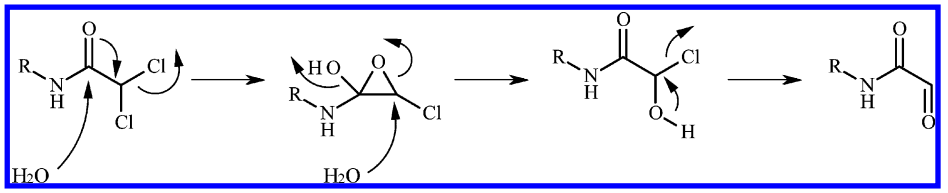


Figure 15. Hydrolysis of dichloroacetamide (present in thiamphenicol) to produce a glyoxal-type species (R = carbon).

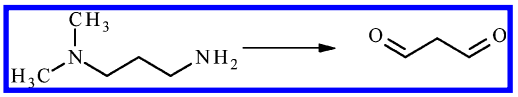


Figure 16. Oxidative deamination of 3-dimethylaminopropylamine into propanedial.

majority of chemicals assigned to the Schiff base domain, these chemicals contain only a single reactive group. It is likely, therefore, that these chemicals are sufficiently electrophilic in order to be able to elicit a sensitization response without the need for the additional reactivity gained by being able to cross-link protein chains.

The final chemical that can be assigned to the Schiff base domain is ninhydrin. This chemical can undergo a dehydration reaction resulting in the production of indane-1,2,3-trione that is capable of multiple Schiff base reactions resulting in sensitization.[37,38] The dehydration of ninhydrin into indane-1,2,3-trione is as shown in Figure 20.

Acylation. Two additional chemicals can be assigned to the acylation domain: fenthion and tetramethrin. Fenthion can undergo an acylation mechanism in which a biological nucleophile attacks the phosphorus−sulfur double bond with a phenolate ion acting as a leaving group (Figures 21). It is likely that this mechanism also applies to the phosphate analogues in which the carbon−sulfur double bond is replaced by a carbon−oxygen double bond. This is due to the fact that, in general, the carbon−oxygen double bond is the more reactive of the two. Tetramethrin is able to undergo an acylation reaction in which a nucleophile attacks the carbonyl group (Figure 22). This mechanism also results in the production of formaldehyde, which as discussed is a potent respiratory sensitizer. Thus, chemicals able to undergo the same mechanism as tetramethrin are of particular concern as potent respiratory sensitizers.

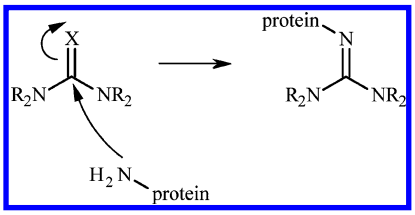


Figure 18. Schiff base reaction for chemicals containing either an imidourea (X = N-CN) or thiourea moiety (X = S). R = hydrogen or carbon.

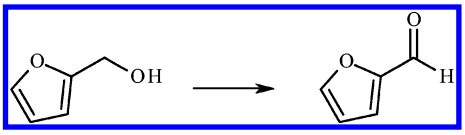


Figure 19. Oxidation of furfuryl alcohol to furfuryl aldehyde.

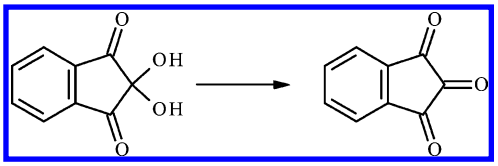


Figure 20. Dehydration of ninhydrin into indane-1,2,3-trione.

Chemicals Causing Respiratory Sensitization via Possible Noncovalent Mechanisms. Tylosin, tetracycline, and spiramycin are macrolide antibiotics that inhibit peptidyl transferase in the ribosomal complex.[39,40] Chemicals of this type do not necessarily cause respiratory symptoms through covalent bonding, in turn leading to sensitization. Given the lack of clearly identifiable structural alerts, it is possible that noncovalent electrostatic interactions might be important as a molecular initiating event. A separate mechanistic profiler might

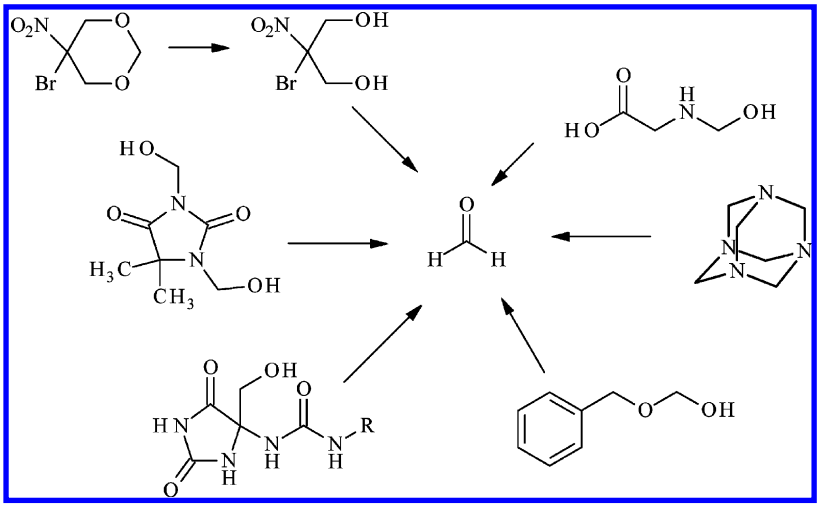


Figure 17. Chemical classes, including methamine, that can release formaldehyde and thus are potential respiratory sensitizers.

dx.doi.org/10.1021/tx3003092 | Chem. Res. Toxicol. 2012, 25, 2490−2498

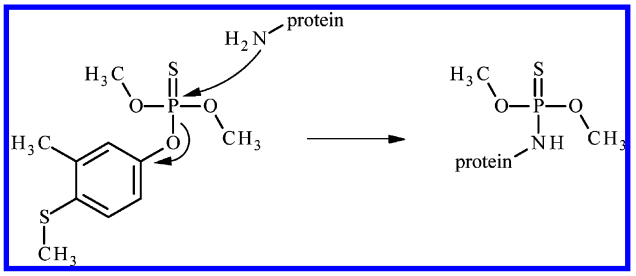


Figure 21. Acylation mechanism for fenthion.

be required to correctly identify chemicals capable of causing respiratory sensitization via such alternative mechanisms.

False Positive Chemicals: Irritants. The data set contains five acids all of which are reported to cause sensitization in the lung: acetic acid, adipic acid, dodecanedioic acid, chloroxylenol, and 2,4-dichloro-5-chlorsulfonyl-benzoic acid. It is possible that these chemicals act as irritants to the respiratory tract rather than true sensitizers, given the recognized difficulty in distinguishing clinically between these mechanisms (in terms of the chemistry, a LMW chemical must be able to form a covalent bond with a protein in order to trigger a sensitization response).[41] This hypothesis is supported by the fact that acids have been reported as false positives in skin sensitization studies due to their ability to act as irritants.[16,17,42]

Summary of the Mechanism-Based Structural Alerts for Respiratory Sensitization. The analysis presented above results in the development of 52 structural alerts related to the electrophilic chemistry associated with covalent protein binding in the lung. These structural alerts are summarized as SMARTS patterns in the Supporting Information and are suitable for hazard identification. In total, the structural alerts developed in this study were able to assign an electrophilic mechanism to 95 of the 104 chemicals in the data set. As discussed, there are five acids in the data set and three chemicals for which respiratory sensitization might be initiated via a noncovalent mechanism. The final chemical not assigned an electrophilic mechanism was dioctylphthalate. Investigations into this chemical suggest that it is not an electrophile and thus should not cause respiratory sensitization. This hypothesis is supported by the 11 phthalates listed in the control group for which no clinical reports of respiratory sensitization have been reported. One possible explanation for the apparent activity of this chemical is the presence of phthalic anhydride as an impurity. This chemical is a known respiratory sensitizer that covalently binds to proteins via an acylation mechanism. Despite these chemicals, the analysis clearly illustrates that the ability to covalently bind to proteins in the lung is the key event that must occur in order for respiratory sensitization to take place for low molecular weight chemicals. The ability of the extended set of structural alerts to distinguish respiratory sensitizers from the control group of chemicals is as shown in Table 3.

Table 3. Contingency Table Showing the Classification of Respiratory Sensitiser and Controls Based on the Final Set of 52 Structural Alerts

	structural alert	no structural alert
respiratory sensitizer (104)	95	9
control chemicals (82)	7	75

Mechanism-Based Structural Alerts and Hazard Identification. This article has focused on the development of structural alerts for respiratory sensitization hazard identification. The structural alerts presented give an indication of the potential of a chemical to form a covalent bond in the lung via one (or more) of the identified mechanisms. However, importantly the absence of all of the alerts from a chemical does not allow for the prediction of the absence of respiratory sensitization potential. This is due to the fact that no matter how extensive a set of structural alerts are for a given molecular initiating event there will be areas of the applicability domain that have not been analyzed. These unexplored areas of the applicability domain may contain new structural alerts. Given this, the mechanism-based structural alerts outlined in this study should be used alongside other *in silico* methods in order to develop a predictive algorithm for regulatory screening for the respiratory sensitization hazard of chemicals. Such an algorithm should comply with the OECD principles for (Q)SAR validation,[43] these being (1) a defined end point; (2) an unambiguous algorithm; (3) a defined domain of applicability; (4) an appropriate measures of goodness-of-fit, robustness, and predictivity; and (5) a mechanistic interpretation, if possible.

The accompanying OECD explanatory notes state that predictivity is determined by external validation. The externally validated QSAR model[8] mentioned in the introduction, which is freely available on the Internet (http://www.coeh.man.ac.uk/asthma/register.php) has been shown to have negative predictive value close to 100%.[44] Therefore, it has potential for the initial regulatory screening of chemicals for respiratory sensitization hazard. The mechanistic chemistry presented in the current study enables point five of the OECD QSAR principles to be covered for chemicals that cause respiratory sensitization via the formation of a covalent bond. Used together, these two SAR approaches could be developed into a screening algorithm with excellent predictive power such that a

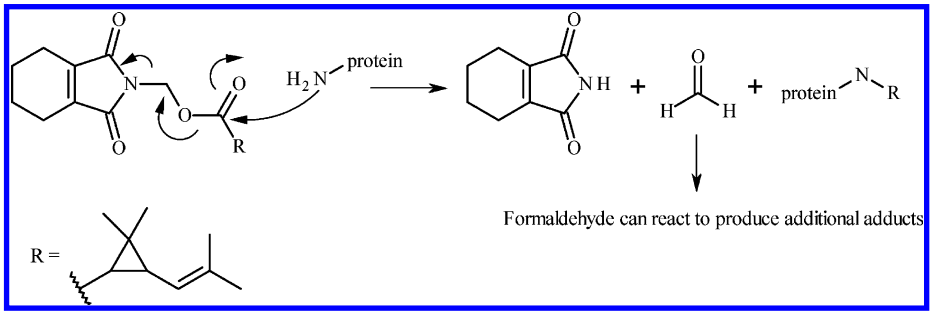


Figure 22. Acylation mechanism for tetramethrin resulting in protein formation and the production of formaldehyde.

mechanistic explanation is provided for a chemical ultimately labeled as a respiratory sensitizer.

CONCLUSIONS

This study has outlined how a data set of 104 chemicals for which clinical reports of respiratory sensitization have been identified from the literature can be rationalized in terms of electrophilic chemistry. The analysis has resulted in the development of a mechanism-based profiler for respiratory sensitization containing 52 structural alerts related to covalent protein binding in the lung. These structural alerts are designed for hazard identification. Thus, the respiratory sensitization profiler and the electrophilic chemistry contained within it are likely to be of use in regulatory risk assessment. Such mechanistic approaches and existing externally validated QSAR models have the potential to complement each other in the development of a SAR algorithm for the prediction of chemical respiratory sensitization hazard.

ASSOCIATED CONTENT

Supporting Information

Full data set and structural alerts encoded as SMARTS patterns. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 44 151 231 2164. Fax: + 44 151 231 2170. E-mail: s.j.enoch@ljmu.ac.uk.

Funding

Funding was received for the initial development of the QSAR model for predicting chemical asthma hazard from the Colt Foundation (http://www.coltfoundation.org.uk/) who are also supporting its ongoing development at the University of Manchester. The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement number 266835 and from Cosmetics Europe.

Notes

This article represents the views of the authors and not necessarily those of the funding bodies.

The authors declare no competing financial interest.

REFERENCES

(1) United Nations (2005) Globally Harmonised System for Classification & Labelling, http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev01/English/00e_intro.pdf.

(2) Mapp, C. E., Boschetto, P., Maestrelli, P., and Fabbri, L. M. (2005) Occupational asthma. *Am. J. Respir. Crit. Care Med. 172*, 280–305.

(3) Enoch, S. J., Roberts, D. W., and Cronin, M. T. D. (2009) Electrophilic reaction chemistry of low molecular weight respiratory sensitisers. *Chem. Res. Toxicol. 22*, 1447–1453.

(4) Enoch, S. J., Roberts, D. W., and Cronin, M. T. D. (2010) Mechanistic category formation for the prediction of respiratory sensitisation. *Chem. Res. Toxicol. 23*, 1547–1555.

(5) Lalko, J. F., Kimber, I., Dearman, R. J., Gerberick, G. F., Sarlo, K., and Api, A. M. (2011) Chemical reactivity measurements: Potential for characterisation of respiratory chemical allergens. *Toxicol. in Vitro 25*, 433–445.

(6) Agius, R. M., Elton, R. A., Sawyer, L., and Taylor, P. (1994) Occupational asthma and the chemical properties of low molecular weight organic substances. *Occup. Med. 44*, 34–36.

(7) Agius, R. M., Nee, J., McGovern, B., and Robertson, A. (1991) Structure activity hypotheses in occupational asthma caused by low molecular weight substances. *Ann. Occup. Hyg. 35*, 129–137.

(8) Jarvis, J., Seed, M. J., Elton, R., Sawyer, L., and Agius, R. (2005) Relationship between chemical structure and the occupational asthma hazard of low molecular weight organic compounds. *Occup. Environ. Med. 62*, 243–250.

(9) Seed, M. J., Cullinan, P., and Agius, R. M. (2008) Methods for the prediction of low-molecular-weight occupational respiratory sensitisers. *Curr. Opin. Allergy Clin. Immunol. 8*, 103–109.

(10) Aptula, A. O., and Roberts, D. W. (2006) Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: General principles and application to reactive toxicity. *Chem. Res. Toxicol. 19*, 1097–1105.

(11) Aptula, A. O., Patlewicz, G., and Roberts, D. W. (2005) Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol. 18*, 1420–1426.

(12) Holsapple, M. P., Jones, D., Kawabata, T. T., Kimber, I., Sarlo, K., Selgrade, M. K., Shah, J., and Woolhiser, M. R. (2006) Assessing the potential to induce respiratory hypersensitivity. *Toxicol. Sci. 91*, 4–13.

(13) Hopkins, J. E., Naisbitt, D. J., Kitteringham, N. R., Dearman, R. J., Kimber, I., and Park, B. K. (2005) Selective haptenation of cellular or extracellular protein by chemical allergens: Association with cytokine polarization. *Chem. Res. Toxicol. 18*, 375–381.

(14) Kimber, I., Agius, R., Basketter, D. A., Corsini, E., Cullinan, P., Dearman, R. J., Gimenez-Arnau, E., Greenwell, L., Hartung, T., Kuper, F., Maestrelli, P., Roggen, E., and Rovida, C. (2007) Chemical respiratory allergy: Opportunities for hazard identification and characterisation. *ATLA 35*, 243–265.

(15) Patlewicz, G., Dimitrov, S. D., Low, L. K., Kern, P. S., Dimitrova, G. D., Comber, M. I. H., Aptula, A. O., Phillips, R. D., Niemela, J., Madsen, C., Wedebye, E. B., Roberts, D. W., Bailey, P. T., and Mekenyan, O. G. (2007) TIMES-SS: A promising tool for the assessment of skin sensitization hazard. A characterization with repect to the OECD validation principles for (Q)SARs and an external evaluation for predictivity. *Regul. Toxicol. Pharmacol. 48*, 225–239.

(16) Roberts, D. W., Aptula, A. O., and Patlewicz, G. (2007) Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol. 20*, 44–60.

(17) Roberts, D. W., Patlewicz, G., Kern, P. S., Gerberick, F., Kimber, I., Dearman, R. J., Ryan, C. A., Basketter, D. A., and Aptula, A. O. (2007) Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem. Res. Toxicol. 20*, 1019–1030.

(18) Barratt, M. D., and Langowski, J. J. (1999) Validation and subsequent development of the DEREK skin sensitization rulebase by analysis of the BgVV list of contact allergens. *J. Chem. Inf. Comput. Sci. 39*, 294–298.

(19) Dimitrov, S. D., Low, L. K., Patlewicz, G. Y., Kern, P. S., Dimitrova, G. D., Comber, M. H. I., Phillips, R. D., Niemela, J., Bailey, P. T., and Mekenyan, O. G. (2005) Skin sensitization: Modeling based on skin metabolism simulation and formation of protein conjugates. *Int. J. Toxicol. 24*, 189–204.

(20) Enoch, S. J., Madden, J. C., and Cronin, M. T. D. (2008) Identification of mechanisms of toxic action for skin sensitisation using a SMARTS pattern based approach SAR QSAR. *Environ. Res. 19*, 555–578.

(21) Gerner, I., Barratt, M. D., Zinke, S., Schlegel, K., and Schlede, E. (2004) Development and prevalidation of a list of structure-activity relationship rules to be used in expert systems for prediction of the skin-sensitising properties of chemicals. *ATLA 32*, 487–509.

(22) Hulzebos, E., Walker, J. D., Gerner, I., and Schlegel, K. (2005) Use of structural alerts to develop rules for identifying chemical substances with skin irritation or skin corrosion potential. *QSAR Comb. Sci. 24*, 332–342.

(23) Schultz, T. W., Rogers, K., and Aptula, A. O. (2009) Read-across to rank skin sensitisation potential: subcategories for the Michael acceptor domain. *Contact Dermatitis 60*, 21−31.
(24) Tarlo, S. M., Balmes, J., Balkissoon, R., Beach, J., Beckett, W., Bernstein, D., Blanc, P. D., Brooks, S. M., Cowl, C. T., Daroowalla, F., Harber, P., Lemiere, C., Liss, G. M., Pacheco, K. A., Redlich, C. A., Rowe, B., and Heitzer, J. (2008) Diagnosis and management of work-related asthma: American colleage of chest physicians consensus statement. *Chest 134*, S1−S41.
(25) 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.
(26) Roberts, D. W., and Natsch, A. (2009) High throughput kinetic profiling approach for covalent binding to peptides: Application to skin sensitisation potency of Michael acceptor electrophiles. *Chem. Res. Toxicol. 22*, 592−603.
(27) Dvorakova, M., Valterova, I., Saman, D., and Vanek, T. (2011) Biotransformation of (1S)-2-carene and (1S)-3-carene by picea abies suspension culture. *Molecules 16*, 10541−10555.
(28) Martins, R. R. L., Neves, M. G. P. M. S., Silvestre, A. J. D., Simoes, M. M. Q., Silva, A. M. S., Tome, A. C., Cavaleiro, J. A. S., Tagliatesta, P., and Crestini, C. (2001) Oxidation of unsaturated monoterpenes with hydrogen perocide catalysed by manganese(III) porphyrin complexes. *J. Mol. Catal. A: Chem. 172*, 33−42.
(29) Miyazawa, M., and Kano, H. (2010) Regio- and stereoselective oxidation of (+)-Δ^3-carene by the larvae of common cutworm (spodoptera litura). *J. Agricul. Fd Chem. 58*, 3855−3858.
(30) Prinz, S., Mllner, U., Heilmann, J., Winkelmann, K., Sticher, O., Haslinger, E., and Hfner, A. (2002) Oxidation products of abietic acid and its methyl ester. *J. Nat. Prod. 65*, 1530−1534.
(31) Gafvert, E., Shao, L. P., Karlberg, A.-T., Nilsson, U., and Nilsson, J. L. G. (1994) Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. *Chem. Res. Toxicol. 7*, 260−266.
(32) Enoch, S. J., Ellison, C. M., Schultz, T. W., and Cronin, M. T. D. (2011) A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol. 41*, 783−802.
(33) Hermens, J. L. M. (1990) Electrophiles and acute toxicity to fish. *Environ. Health Perspect. 87*, 219−225.
(34) Marqui, C. (2001) Chemical reactions in cottonseed protein cross-linking by formaldehyde, glutaraldehyde, and glyoxal for the formation of protein films with enhanced mechanical properties. *J. Agric. Food Chem. 49*, 4676−4681.
(35) de Groot, A. C., Flyvholm, M.-A., Lensen, G., Menne, T., and Coenraads, P.-J. (2009) Formaldehyde-releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. *Contact Dermatitis 61*, 63−85.
(36) de Groot, A. C., White, I. R., Flyvholm, M.-A., Lensen, G., and Coenraads, P.-J. (2010) Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. *Contact Dermatitis 62*, 2−17.
(37) Kaiser, E., Colescott, R. L., Bossinger, C. D., and Cook, P. I. (1970) Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem. 34*, 595−598.
(38) Piirila, P., Estlander, T., Hytonen, M., Keskinen, H., Tupasela, O., and Tuppurainen, M. (1997) Rhinitis caused by ninhydrin develops into occupational asthma. *Eur. Respir. J. 10*, 1919−1921.
(39) Schlunzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. (2001) Structural basis for the identification of antibiotics with the peptidyl transferase centre in eubacteria. *Nature 413*, 814−821.
(40) Goldman, R. C., Fesik, S. W., and Doran, C. C. (1990) Role of protonated and neutral forms of macrolides in binding to ribosomes from gram-positive and gram-negative bacteria. *Antimicrob. Agents Chemother. 34*, 426−431.
(41) Burge, P. S., Moore, V. C., and A.S., R. (2012) Sensitisation and irritant induced occupational asthma with latency are clinically indistinguishable. *Occup. Med. 62*, 129−133.
(42) Basketter, D., Darlenski, R., and Fluhr, J. W. (2008) Skin irritation and sensitisation: Mechanisms and new approaches for risk assessment. *Skin Pharmacol. Physiol. 21*, 191−202.
(43) OECD (2004) *The Report from the Expert Group on (Quantitative) Structure-Activity Relationship ([Q]SARs) on the Principles for the Validation of (Q)SARs*, OECD Series on Testing and Assessment No. 49, ENV/JM/MONO(2004)/24 (available from http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/seriesontestingandassessmentpublicationsbynumber.htm).
(44) Seed, M., and Agius, R. (2010) Further validation of computer-based prediction of chemical asthma hazard. *Occup. Med. 60*, 115−120.