Electrophilic Reaction Chemistry of Low Molecular Weight Respiratory Sensitizers

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Received April 20, 2009

Certain types of low molecular weight chemicals have the ability to cause respiratory sensitization via haptenation of carrier proteins. It has been suggested that such chemicals must contain multiple "reactive" functional groups to elicit an immune response. In contrast to the well-developed electrophilic reaction chemistry ideas detailing the initial haptenation event for skin sensitization, no detailed mechanistic chemistry analysis has been performed for respiratory sensitization. The aim of this study, therefore, was to perform an electrophilic reaction chemistry analysis to explain the differing respiratory sensitizing potentials of 16 chemicals containing both single and multiple functional groups. The analysis has been supported by quantum chemical calculations probing the electrophilicities of the reactive chemicals. These calculations suggest that within each mechanistic category differing "reactivity thresholds" exist that must be passed for respiratory sensitization to occur. In addition, this study highlights how such mechanistically driven category formation could be used as an in silico hazard identification tool.

Introduction

A number of low molecular weight (LMW) industrial chemicals have been shown to cause respiratory sensitization in humans (*1, 2*). Despite the risk to human health, no well-validated or widely accepted test method currently exists to enable the identification of the respiratory sensitization potential of such chemicals for regulatory purposes (*3*). It has been suggested that in order for a LMW industrial chemical to be capable of eliciting respiratory sensitization it must be able to bind covalently to proteins in a similar fashion to the formation of protein−haptan adducts that lead to skin sensitization (*3, 4*). This hypothesis is supported by the high number of respiratory sensitizers that test positive in the local lymph node assay (LLNA) for skin. However, not all respiratory sensitizers are positive in the LLNA, and not all chemicals positive in the LLNA are respiratory sensitizers (*5*).

A number of workers have investigated the mechanistic relationship between skin and respiratory sensitization for LMW chemicals (*5*−*7*). These studies have highlighted the need for haptenation of a carrier protein in order for an immune response to be elicited. It has been shown that differing cytokine secretion profiles are responsible for skin and respiratory sensitization. For instance, binding studies between cysteine, lysine, and dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA) indicate that a difference in the ability to bind to either cellular or soluble proteins could be responsible for the differing skin and respiratory-sensitizing abilities of the two chemicals (*7*). It has been shown that DNCB, which causes skin sensitization but not respiratory sensitization, binds preferentially to the model nucleophile N-acetyl cysteine. In contrast TMA, which causes both skin and respiratory sensitization, binds preferentially to the model nucleophile N-acetyl lysine. Despite these differences in protein-binding profiles, the authors (*7*) caution against the suggestion that the capability of a chemical to bind to lysine is a prerequisite for respiratory sensitization. It is likely that further structure−activity investigations are required to confirm the importance of differing protein binding profiles for chemicals able to cause skin and respiratory sensitization.

Previous studies have attempted to produce in silico models to allow the identification of LMW chemicals capable of causing respiratory sensitization (*2, 3, 8, 9*). The focus of these studies has been to attempt to identify the structural features of the known LMW respiratory sensitizers that might be associated with their ability to cause respiratory sensitization. The resulting models from these studies frequently highlight the importance of more than a single "reactive" functional group such as more than a single amine or isocyanate group to elicit respiratory sensitization. The authors of these studies assign the mechanistic rationale as being that such chemicals are capable of reacting with more than a single protein side chain and thus obtain their respiratory sensitization potency from being able to cross-link protein chains. None of the authors of these studies attempt to offer a detailed mechanistic rationale based on the likely electrophilic reaction chemistry (*2, 3, 8, 9*).

Despite the fact that skin and respiratory sensitization are different hypersensitivity phenomena, with differences in the cellular mechanisms, in the cytokine and chemokine profiles, and in the profiles of the types of T-cells involved (*10*), an understanding of the electrophilic reaction chemistry allows for the formation of mechanistic chemical categories. This is due to the covalent binding to a protein being the essential step that is required for both types of sensitization. Given that it has been suggested that detailed mechanistic knowledge is required if chemical categories are going to be useful in regulatory toxicology (*11*) and the recent definition of transparent mechanistic categories for skin sensitization (*12*), then a similar analysis for respiratory sensitization is likely to be beneficial in the development of in silico hazard identification tools.

The aim of this study, therefore, was to investigate the likely electrophilic reaction chemistry for 10 LMW respiratory sensitizers. In addition, the electrophilic reaction chemistry of six chemicals that do not cause respiratory sensitization was also investigated. Quantum chemical calculations were also utilized

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10.1021/tx9001463 CCC: $40.75 © 2009 American Chemical Society
Published on Web 07/17/2009

to probe the differing electrophilicities of the studied chemicals within the categories formed. The respiratory-sensitizing activity of these chemicals had previously been accounted for due to the presence of more than a single "reactive" functional group. The analysis in this study was carried out using the methods previously used to understand the electrophilic reaction chemistry leading to skin sensitization (*13*). It is likely that the ability to offer an analogous electrophilic chemistry-based mechanistic understanding of LMW respiratory sensitization is going to be of clear benefit to both regulators and industry for the risk assessment of chemicals from rational and transparent in silico methods.

Materials and Methods

Data. The chemistry of a total of 16 chemicals was investigated in this study. These chemicals were collected from several sources (*1, 3, 14*−*17*). Ten chemicals were included based on their frequent occurrence in the literature as being examples of chemicals that caused respiratory sensitization. A further six chemicals often cited as being examples of nonrespiratory-sensitizing monofunctional chemicals were also included.

Assignments of Electrophilic Mechanisms. The chemistry of the respiratory-sensitizing chemicals was rationalized on the basis of electrophilic mechanisms. These mechanisms were assigned to the chemicals on the basis of previous knowledge from skin sensitization (*13*). In keeping with the skin sensitization work, the assignment of an electrophilic reaction mechanism also involved suggesting appropriate metabolic pathways that could produce sufficiently electrophilic daughter molecules that could be responsible for respiratory sensitization. Finally, example skin sensitization (*18*) data were utilized to highlight differing electrophilic reactivities between chemicals with one or two functional groups.

Computational Chemical Calculations. All calculations on chemical structure were performed using the Gaussian03 package of programs utilizing the B3LYP/6-31G(d) level of theory (*19*). Structures were drawn using the GausView application within Gaussian03; chemical structures were then optimized using the following criteria: maximum force, <0.000450; rms (root-mean-square) force, <0.000300; maximum displacement, <0.001800; and rms displacement, <0.001200.

The global electrophilicity parameter (ω) was then calculated for each optimized chemical as described previously using eq 1 (*20*).

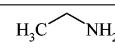
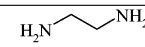
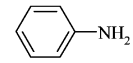
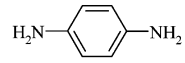
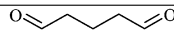
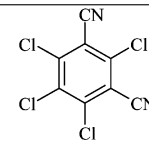
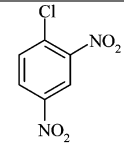
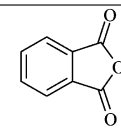
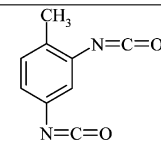
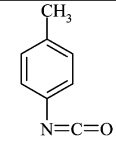
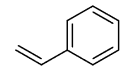
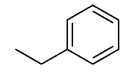
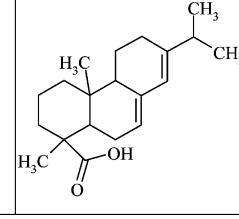
$$\text{electrophilicity index } (\omega) = [(E_{\text{HOMO}} + E_{\text{LUMO}})/2]^2/[2 \times (E_{\text{LUMO}} - E_{\text{HOMO}})] \quad (1)$$

where E_{HOMO} and E_{LUMO} are the one-electron energies of the highest occupied and lowest unoccupied molecular orbitals, respectively.

Results and Discussion

Respiratory sensitization is an important end point hampered not only by a lack of data but also by lack of a standard assay, both of which have limited the development of in silico tools. A small number of in silico studies have suggested that the respiratory sensitization potential of chemicals might be related to the presence of multiple functional groups and the ability of such groups to enable the cross-linking of protein chains (*1, 3, 4*). However, there are also a number of chemicals that are known to cause respiratory sensitization that do not contain an obvious source of multiple functional groups and thus do not fit this hypothesis. These chemicals are phthalic anhydride, vinyl benzene, and abietic acid (Table 1). The aim of this study therefore was to investigate these chemicals in terms of the electrophilic reaction chemistry principles that have been previously used to understand the mechanisms responsible for skin sensitization (*13, 21*). The wider aim of this study was to develop mechanistically driven chemical categories that could

Table 1. Chemicals Investigated in This Study, Their Respiratory Sensitization Potential, and Putative Electrophilic Mechanism of Action

Name	Structure	Respiratory sensitiser	Putative mechanism
Ethylamine		No	-
Ethylenediamine		Yes	Schiff base
Piperidine		No	-
Piperazine		Yes	Schiff base
Aniline		No	-
4-Phenylenediamine		Yes	Michael addition
Formaldehyde		Yes	Schiff base
Glutaraldehyde		Yes	Schiff base
Tetrachloroisophthalonitrile		Yes	S_NAr
Dinitrochlorobenzene		No	S_NAr
Phthalic anhydride		Yes	Acylation
2,4-Diisocyanate toluene		Yes	Acylation
4-Isocyanate toluene		No	Acylation
Vinyl benzene		Yes	S_N2
Ethyl benzene		No	-
Abietic acid		Yes	S_N1 / S_N2

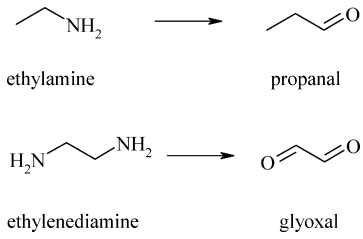


Figure 1. Oxidation deamination mechanism for ethylamine and ethylenediamine.

be used as an in silico hazard identification tool. The known respiratory sensitizers in this study can be assigned to one of the six electrophilic mechanistic domains that have been used previously for skin sensitization (*13*). The following sections illustrate how the chemicals investigated in this study can be assigned to one of these domains and how this information can be used in hazard identification. The suggested chemical categories are supported by quantum chemical calculations.

Schiff Base Formation

Formaldehyde and Glutaraldehyde. An electrophilic reaction chemistry analysis of these two chemicals offers a more detailed mechanistic insight into their ability to cause respiratory sensitization. It is has been previously demonstrated that aliphatic aldehydes undergo fast and reversible Schiff base formation with proteins, and it is likely that this is the initial step in the sensitization process (*22, 23*). Given the facile and reversible nature of this process, it is likely that for all three chemicals the protein cross-linking step is the controlling factor in the resulting level of respiratory sensitization. It is relatively easy to envisage glutaraldehyde undergoing a second facile Schiff base reaction with a further lysine unit resulting in cross-linking. In contrast, formaldehyde cannot undergo a second Schiff base reaction; however, it has been shown that a number of cross-linking reactions can occur, one example being via an electrophilic attack upon aromatic protein side chains such as tyrosine (*23*).

Ethylamine and Ethylenediamine. It is unlikely that the amine moieties in either ethylamine or ethylenediamine undergo direct reaction with protein residues containing nucleophilic centers (primarily sulfur and nitrogen atoms). Instead, it is more likely that both chemicals are metabolized into aldehydes by oxidative deamination (Figure 1). This is the same metabolic process that has been suggested to be responsible for the positive skin sensitization response for ethylenediamine and diethylenetriamine in the LLNA (where the corresponding aldehydes undergo Schiff base formation) (*13*).

If one assumes that both chemicals are metabolized in equal amounts to the corresponding aldehyde, then a simple explanation based on the differing reactivities relating to Schiff base formation is able to explain the differing respiratory sensitization potentials of the parent compounds. In the case of ethylenediamine, one of the likely metabolites is glyoxal, while for ethylamine, it is acetaldehyde. Inspection of LLNA skin sensitization data reveals glyoxal to be on the boundary between being a moderate and being a strong skin sensitizer. While there are no corresponding LLNA data for acetaldehyde, a number of aliphatic aldehydes have been tested, and all of them are weak or very weak skin sensitizers (*18*).

Piperidine and Piperazine. A similar mechanistic rationale can be developed to explain the differing activities of piperidine and piperazine, based on the formation of protein reactive metabolites (Figure 2). Studies in the rat have shown that

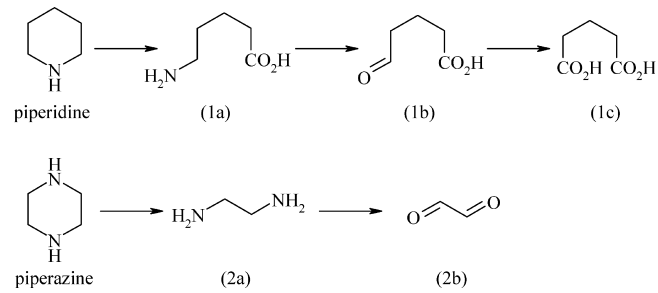


Figure 2. Potential metabolic pathways for piperidine and piperazine.

Table 2. Electrophilic Index Values for Chemicals within the Schiff Base Domain

chemical	electrophilic index (ω) (eV)
glyoxal	3.24
formaldehyde	1.46
acetaldehyde	1.20
diethyl acetaldehyde	1.05

piperazine can be metabolized into ethylenediamine (metabolite 2a in Figure 2), which can then undergo oxidative deamination to produce the protein reactive dialdehyde glyoxal (metabolite 2b) (*24*). Metabolism studies upon piperidine in bacteria have shown that it can also be metabolized via an oxidative deamination process, producing a dicarboxylic acid (*25*).

Given that the proposed protein reactive species from both piperazine and ethylenediamine is glyoxal, one would expect cross-reactivity between the two chemicals. Unfortunately, no respiratory sensitization cross-reactivity studies exist to test this mechanistic hypothesis. However, the hypothesis is supported by skin sensitization guinea pig data in which studies have shown alkylene diamines, including ethylenediamine and piperazine, to cross-react with each other (*26*).

The above analysis of the Schiff base domain suggests that chemicals capable of causing respiratory sensitization need to pass a "reactivity threshold", which can be exceeded via a combination of electrophilicity and adduct stability gained from protein cross-linking. One can calculate the electrophilic portion of this reactivity threshold using the electrophilic index (ω). This has been used previously to model the electrophilicities of skin-sensitizing chemicals in the Michael domain (*27*). A comparison of the electrophilic index values for glyoxal, formaldehyde, acetaldehyde, and diethyl acetaldehyde reveals glyoxal to be the most electrophilic of the four (Table 2). Interestingly, the calculated electrophilic indices for the remaining three chemicals are below the value of 1.50 eV that has been previously suggested to be required for a chemical to be considered as a strong electrophile (*28*). The calculations suggest that glyoxal is sufficiently electrophilic to cause respiratory sensitization without necessarily needing the additional stability gained from protein cross-linking [although it clearly can undergo such reactions (*22*)]. In contrast, the monoaldehydes such as formaldehyde are insufficiently electrophilic and thus require the additional adduct stability gained from protein cross-linking. Given that the initial Schiff base formation is controlled by the electrophilic portion of the "reactivity threshold", it is likely that if the parent aldehyde is insufficiently electrophilic no amount of protein cross-linking will result in a respiratory sensitization response. The calculated decrease in electrophilicity upon lengthening the alkyl chain helps rationalize why the metabolites of ethylamine and piperidine do not cause respiratory sensitization.

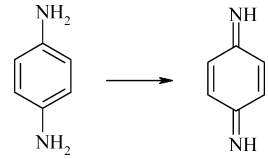


Figure 3. Oxidation of 4-phenylenediamine to the reactive *p*-benzoquinone diimine species.

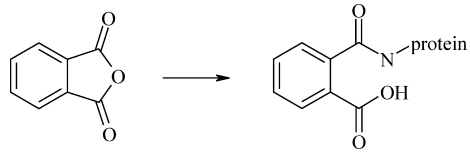


Figure 4. Acylation mechanism for phthalic anhydride.

Michael Addition

Aniline and 4-Phenylenediamine. The difference in respiratory sensitization between aniline and 4-phenylenediamine can be explained by their ability to give rise to compounds that undergo the Michael reaction. The ability of a chemical to undergo the Michael reaction with proteins has been suggested to be responsible for a range of toxicities including skin sensitization and acute aquatic toxicity (*29, 30*). Previous analysis of the same two chemicals in the LLNA revealed that aniline was a very weak skin sensitizer, while 4-phenylenediamine was a strong skin sensitizer (*18*). Subsequent analysis suggested that the aniline result was more than likely a false positive result due to the ability of aniline to act as an irritant (irritants often act as false positives in the LLNA) (*13*). The differing activities for both skin and respiratory sensitization can be explained in terms of the ability of 4-phenylenediamine to be oxidized to 4-benzoquinone diimine reactive species (Figure 3). An analogous oxidation is not possible for aniline.

Acylation

Phthalic Anhydride, 2,4-Diisocyanate Toluene, and 4-Isocyanate Toluene. Phthalic anhydride and related acid anhydrides have been suggested to be capable of causing respiratory sensitization (*17*). On first inspection, phthalic anhydride appears to have the ubiquitous multiple functional groups often quoted as being required for respiratory sensitization. However, inspection of the electrophilic reaction chemistry suggests an irreversible acylation mechanism, in which the anhydride unit acts as a single monofunctional reaction center, to be more likely (Figure 4). This type of mechanism has been suggested previously to explain the ability of related chemicals to cause skin sensitization (*13*). Such chemicals (including phthalic anhydride itself) that are capable of acting via an acylation mechanism are frequently moderate to strong skin sensitizers, indicating their high degree of protein reactivity (*18, 31*).

A similar rationale can be applied to the ability of 2,4-diisocyanate toluene to cause respiratory sensitization. This chemical can also be assigned to the acylation mechanistic domain. In contrast to the reported respiratory sensitization ability of diisocyanate chemicals, the equivalent monoisocyanates are used widely in industry and are not considered to be asthmagens. This difference in respiratory sensitization can be understood in a similar fashion to the Schiff base domain in that a "reactivity threshold" must be passed to elicit an immune response. Inspection of the calculated electrophilicity indices for the three chemicals in the acylation domain shows phthalic anhydride to be significantly more electrophilic than the either of the isocyanates (Table 3). As with multifunctional Schiff base chemicals, it appears that the ability to cross-link proteins enables the diisocyanates to become sufficiently reactive to cause a respiratory sensitization response.

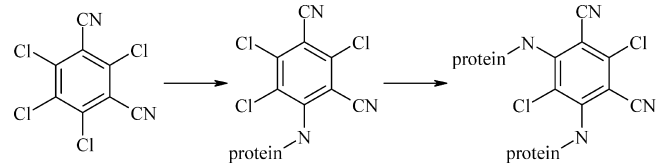


Figure 5. S_NAr mechanism leading to respiratory sensitization for TCPN.

Table 3. Electrophilic Index Values for Chemicals within the Acylation Domain

chemical	electrophilic index (ω) (eV)
phthalic anhydride	2.61
2,4-diisocynate toluene	1.23
4-isocynate toluene	1.00

Table 4. Electrophilic Index and Hammett/Taft Values for Chemicals within the S_NAr Domain

chemical	electrophilic index (ω) (eV)	Hammett/Taft (*13*)
TCPN	3.06	4.95
DNCB	3.32	4.02

S_NAr

Tetrachloroisophthalonitrile (TCPN) and DNCB. The fungicide TCPN is known to cause both skin and respiratory sensitization, while in contrast DNCB only causes skin sensitization (*4, 13*). Both of these chemicals can be assigned to the nucleophilic aromatic substitution mechanistic domain (S_NAr) with, in both cases, the electron-withdrawing cyano and nitro groups activating the aromatic ring, making it potentially protein reactive (Figure 5). The high reactivity of these two chemicals is supported by their calculated electrophilic indices, which show DNCB to be the more electrophilic of the two (Table 4). Experimental skin sensitization potentials (LLNA) show TCPN to be a stronger skin sensitizer than DNCB (*18, 32*). Although the nitro group is more strongly activating than the cyano group, in the case of TCPN, the chloro-substituents also have an activating effect. In the absence of experimental reactivity data, one cannot be certain whether TCPN or DNCB is the more reactive. On the basis of Hammett/Taft substituent constants, TCPN is predicted to be the more reactive (*13*), whereas on the basis of their ω indices, DNCB is predicted to be the more electrophilic of the two (Table 4). In any event, TCPN, unlike DNCB, is able to undergo multiple S_NAr reactions (Figure 5), and this will contribute substantially to its skin sensitization potency. This argument can be extended to understand the differing respiratory sensitization potentials of the two chemicals. Comparison of the electrophilic indices of TCPN and DNCB to those of chemicals within either the Schiff base or the acylation domains suggests that they should both be sufficiently electrophilic as to cause respiratory sensitization (Table 4). However, it appears that chemicals within the S_NAr domain have a higher "reactivity threshold" than those in the other mechanistic domains. A likely explanation for this can be found in hard−soft acid base theory. If one considers that the nucleophilic aromatic system is very soft (high electron density spread over a large area), then, it is clear that such systems will be more reactive toward softer electrophiles such as cysteine rather than the harder lysine. It is therefore likely that the ability of TCPN

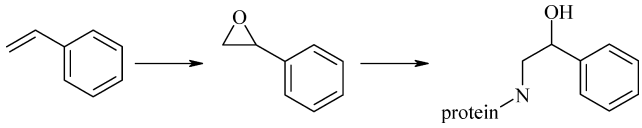


Figure 6. Epoxidation followed by S_N2 ring-opening mechanism for vinyl benzene.

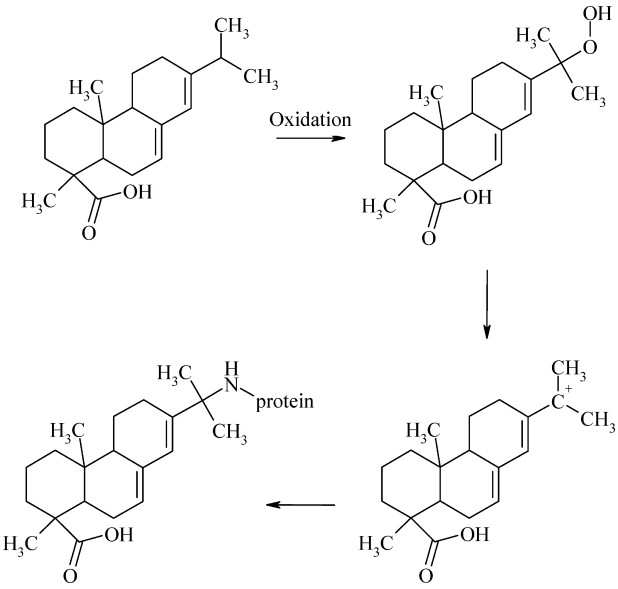


Figure 7. Oxidation mechanism leading to reactive intermediates for abietic acid.

to undergo multiple S_NAr reactions and thus cross-link proteins results in this chemical passing the higher "reactivity threshold" required for respiratory sensitization within this domain.

S_N1/S_N2

Styrene and Abietic Acid. Styrene has also been suggested to be capable of causing respiratory sensitization, while, in contrast, ethyl benzene was demonstrated not to be capable of causing asthma (*15*). Vinyl benzene only has a single reactive group, which is a polarized alkene moiety; such polarized alkenes have been suggested to be capable of undergoing Michael addition leading to skin sensitization and excess aquatic toxicity (*13, 32*). However, the benzene ring alone is not usually considered as sufficiently polarizing to result in significant Michael addition. An alternative and more likely mechanistic explanation is that the alkene bond undergoes epoxidation and then subsequent ring opening via an S_N2 reaction; such a mechanism is clearly not possible for ethyl benzene (Figure 6). In contrast to the Michael addition reaction, the S_N2 reaction is irreversible, thus leading to stable adducts capable of triggering respiratory sensitization.

Abietic acid is a common industrial respiratory sensitizer occurring widely as part of colophony (*14*). In contrast to its ability to cause respiratory sensitization, abietic acid has been tested in the LLNA and found to be only weakly sensitizing (*18*). Previous studies have shown that abietic acid-related chemicals can undergo free radical oxidation to produce several highly reactive intermediates capable of either S_N1 or S_N2 reactions with protein side chains (*33*) (Figure 7).

If one considers the hypothesis that weakly skin-sensitizing chemicals (i.e., those only capable of weak protein binding) do not cause respiratory sensitization unless they can also cause protein cross-linking, then an explanation is required for the differing ability of abietic acid to cause respiratory sensitization despite it only being a weak skin sensitizer. The most likely explanation for the weak skin-sensitizing potential of abietic acid is that insufficient amounts of it are oxidized to the protein reactive intermediates in the skin. In contrast, the lung is a very oxidizing environment; thus, significantly larger amounts of abietic acid are presumably oxidized, producing the very reactive intermediates that result in respiratory sensitization.

Protein Reactivity and Cross-Linking

The analyses presented in this study suggest that two categories of chemicals exist that are capable of causing respiratory sensitization. The first category contains chemicals for which a plausible protein cross-linking mechanism cannot be envisaged; such chemicals include phthalic anhydride, vinyl benzene, and abietic acid. The second category includes chemicals in which multiple mechanisms that can lead to protein cross-linking can be envisaged. These two categories can be understood if one considers that a "reactivity threshold" must be passed in order for a respiratory sensitization response to be provoked. The analysis suggests that this threshold requires a combination of electrophilicity and protein cross-linking ability, with only extremely electrophilic chemicals not requiring the additional reactivity gained from being able to react with multiple protein chains (chemicals in the first category). In addition, the analysis suggests that differing mechanistic domains have differing "reactivity thresholds". This is illustrated by the high electrophilicities of chemicals within the S_NAr domain, which, despite being more electrophilic than chemicals in the other domains, still require the additional reactivity gained from protein cross-linking to cause respiratory sensitization. This also suggests that (at least for the S_NAr domain) the "reactivity threshold" required for respiratory sensitization is higher than that required for skin sensitization.

Mechanism-Based Hazard Identification

The analysis presented in this study represents the beginnings of a methodology for the prediction of respiratory sensitization without the use of animals. This is especially important given the lack of a validated animal model for this important end point. One can envisage a series of steps that could form part of an intelligent testing strategy for respiratory sensitization of an untested chemical, X (Figure 8).

The first step in assessing the respiratory-sensitizing potential of chemical X would be to assign it to one of the protein-binding mechanistic domains (step 1 in Figure 8). Having assigned X to its most likely domain, one would then calculate its electrophilicity (ω) (step 2). Given the mechanistic domain and calculated electrophilicity information, one could make a first assessment of the likelihood of X being a respiratory sensitizer (step 3a in Figure 8). As discussed, the degree of electrophilicity required to pass the "reactivity threshold" varies depending on the mechanistic domain. For example, if X was assigned to the acylation domain and had a calculated electrophilicity value of 2.60 eV (which is approximately equal to that of phthalic anhydride), then the likelihood would be that X would be a respiratory sensitizer (with the answer to step 3a being "yes"). This is due to fact that X is sufficiently electrophilic that the "reactivity threshold" for this domain is passed without the need for protein cross-linking. In contrast, if chemical X had been assigned to the S_NAr domain with a calculated electrophilicity value of 3.00 eV (approximately equal to TCPN), then the answer to step 3a would be "no" and an assessment of the ability of X to protein cross-link would be required (step 3b). If the

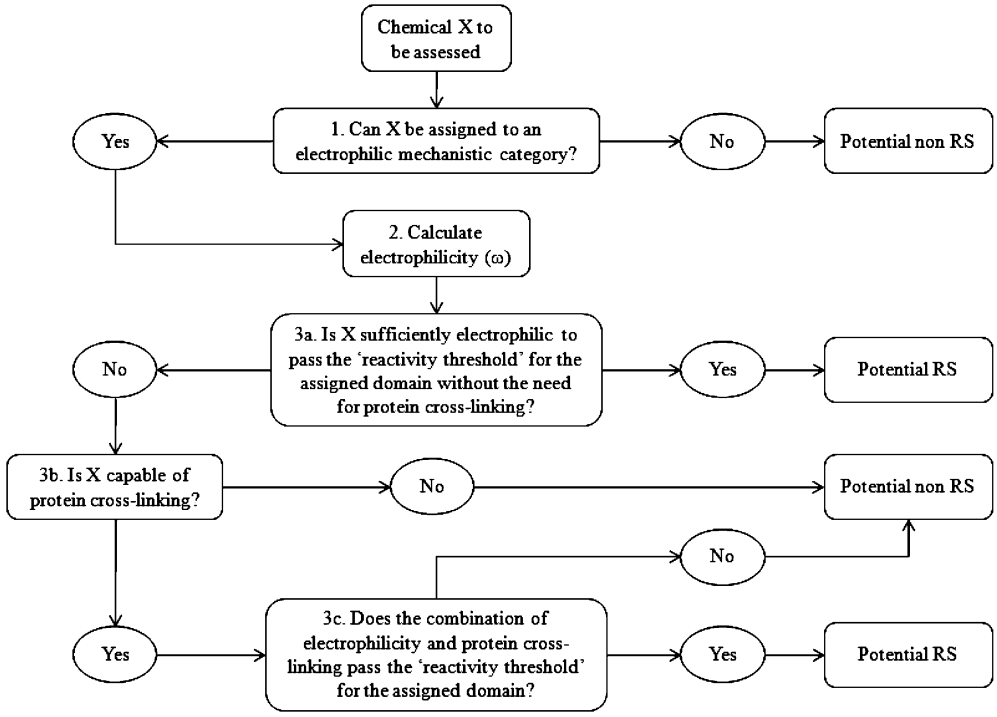


Figure 8. Flowchart for the in silico hazard identification of respiratory sensitization (RS) for a new chemical, X.

answer to step 3b is "yes" and X is able to protein cross-link and the answer to step 3c is also "yes", then the higher "reactivity threshold" for the S_NAr domain would be passed and X would likely be a respiratory sensitizer. However, if X could either not protein cross-link (answer to step 3b being "no") or was insufficiently electrophilic despite being able to protein cross-link (answer to step 3c being "no"), then the "reactivity threshold" would not be passed and X is likely to be a non-respiratory sensitizer.

The above analysis and the use of the flowchart require each of the mechanistic domains to be populated with experimental data. This is needed in order that the calculated electrophilicity values can be utilized to make predictions about whether chemical X passes the "reactivity threshold" required for respiratory sensitization within a given mechanistic domain. This is clearly the limiting factor in the application of this type of approach; however, the methodology is transparent and mechanistically based.

Conclusions

This study has demonstrated that electrophilic reaction chemistry principles can be utilized to offer a mechanistic rationale for the respiratory sensitization potential of LMW chemicals. It is clear from the examples discussed that the processes leading to respiratory sensitization are more complex than the presence of multiple functional groups within a chemical. The analysis suggests that a chemical's ability to cause respiratory sensitization is related to a combination of electrophilicity and protein cross-linking ability. Importantly, the study has shown that within certain mechanistic domains highly electrophilic chemicals can cause respiratory sensitization without the need for the additional reactivity gained from protein cross-linking. In addition, the analysis has also highlighted that within each of the mechanistic domains differing combinations of electrophilicity and protein cross-linking reactivity are required to cause respiratory sensitization. The analysis presented in this study has demonstrated that knowledge of the potential electrophilic reactions of a LMW chemical (and its metabolites) can lead to a mechanism-driven evaluation of respiratory sensitization potential. This study provides a mechanism-based framework supported by mechanistically relevant calculations allowing for the prediction of respiratory sensitization. Such mechanistically transparent methods are of a clear benefit to regulators to enable the reduction in the number of animals used in toxicological assessment. In addition, they increase the knowledge for, and application of, simple and transparent in silico technologies.

Acknowledgment. The comments and suggestions of the anonymous reviewers are gratefully acknowledged for the significant improvements that resulted due to their time and effort during the review process. The funding of the European Union Sixth Framework CAESAR Specific Targeted Project (SSPI-022674-CAESAR) and the European Chemicals Agency (EChA) Service Contract No. ECHA/2008/20/ECA.203 is gratefully acknowledged.

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