A Hypothesis-driven Framework for Screening of Potential Respiratory Sensitizers

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Abstract

Respiratory sensitization following inhalation exposure to low–molecular-weight (LMW) chemicals can cause symptoms ranging from rhinitis to severe asthma. The known chemical space for LMW respiratory sensitizers is relatively narrow, dominated by diisocyanates and anhydrides, and no validated methods exist for their specific identification. Although the proposed adverse outcome pathway (AOP) for respiratory sensitization shares similarities with the skin sensitization AOP, key differences in hapten formation chemistry and early molecular events underscore the need for endpoint-specific approaches. We hypothesized that respiratory sensitizers are enriched for bifunctional electrophilic structures capable of protein crosslinking in airway tissues, leading to neo-antigen formation and immune activation. To test this hypothesis, we reviewed publicly available clinical evidence for 152 chemicals: 108 skin sensitizers, 14 respiratory sensitizers, 18 irritant non-sensitizers, 7 non-irritant non-sensitizers, and 5 unclassified. Clinical case summaries were integrated with in silico skin and lung metabolism predictions generated using the Tissue Metabolism Simulator (TIMES) version 2.34. These combined data informed the development of a hypothesis-driven framework to screen for chemicals with the potential to induce respiratory sensitization and to distinguish them from skin sensitizers and non-sensitizing irritants. Clinical evidence did not indicate overlapping instances of skin and respiratory sensitization within this dataset. Although inhaled irritants can cause symptoms similar to respiratory sensitization, they were not linked to immune-mediated sensitization based on clinical evidence. Bifunctional electrophiles appeared across the inventory, but only a subset showed the reactivity profile of known respiratory sensitizers in TIMES simulations. TIMES predictions indicated that strong skin sensitizers predominantly reacted directly with skin proteins. Whereas, certain respiratory sensitizers (e.g., piperazine) were predicted to require metabolic activation, with a greater likelihood of forming reactive metabolites in the lung than in skin. Nonsensitizing irritants (e.g., alcohols, carboxylic acids) showed a strong tendency toward Phase II metabolic conjugation and elimination. Respiratory sensitizers were frequently characterized by bifunctional electrophilic structures, with their reactivity and protein crosslinking potential emerging as key determinants. Chemical space analysis and clinical review support the importance of bifunctional reactivity within lung tissue and the role of lung-resident immune cells in shaping the respiratory sensitization AOP. Although lung metabolism models are informed by smaller in vitro datasets compared with the more extensive in vivo skin datasets, this integrated approach supports the development of a mechanistically informed screening framework and progression of NAMs tailored to respiratory sensitization.

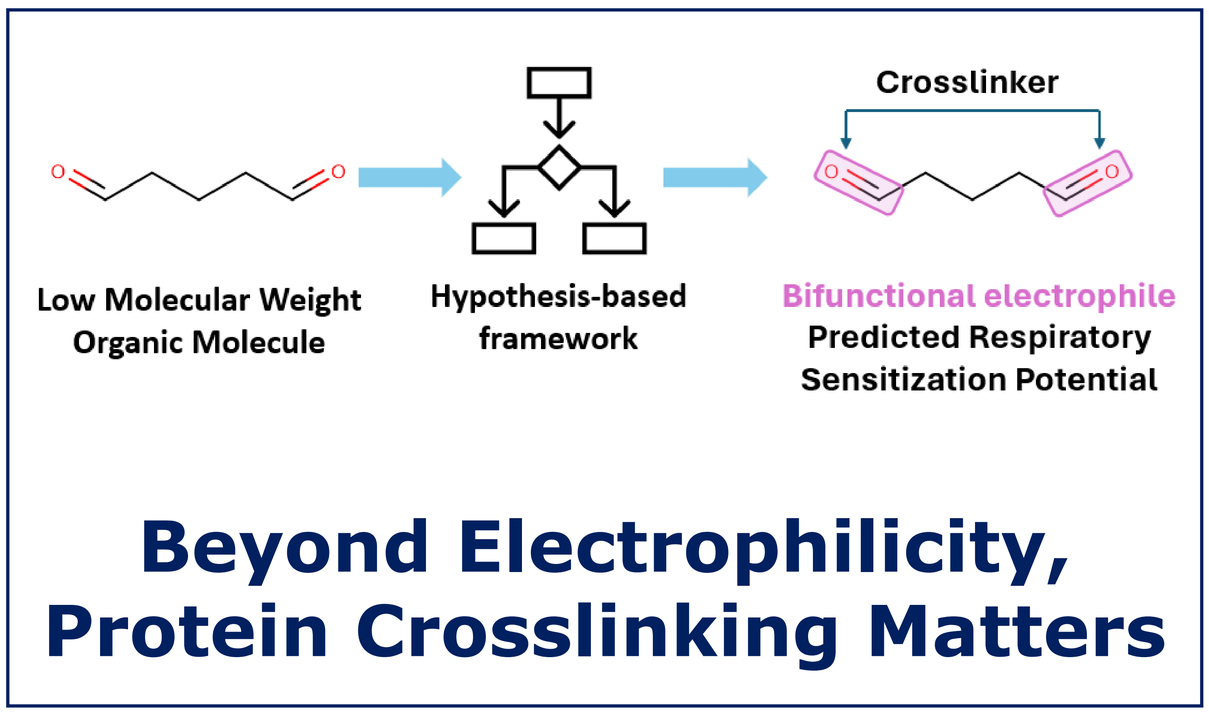
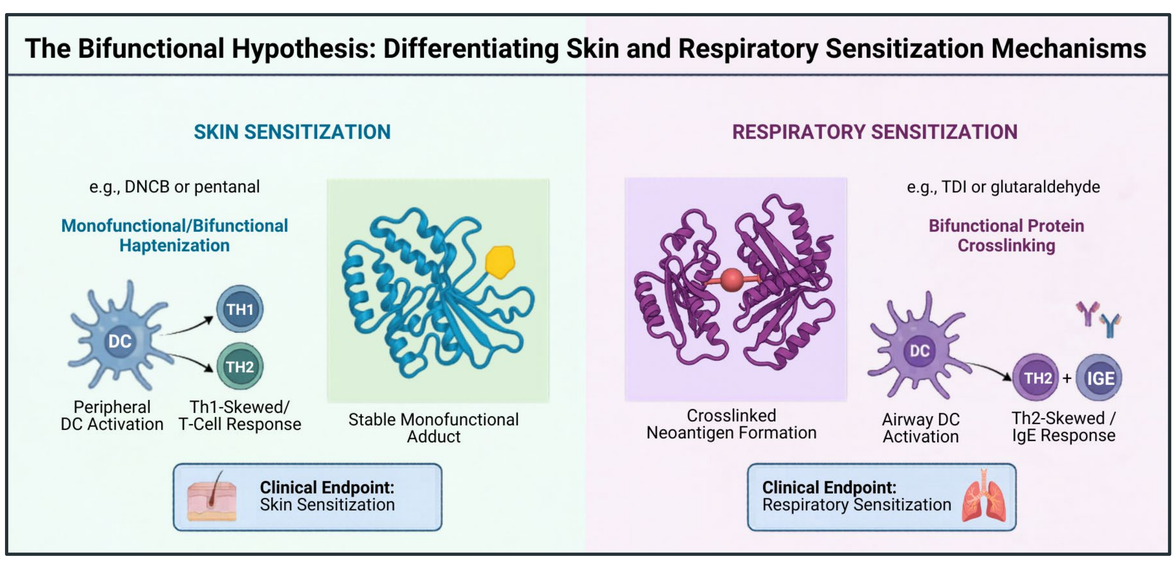


FIGURE 3



Conclusion

- Respiratory sensitizers were consistently associated with protein-crosslinking potential in the respiratory tract.
- Bifunctional electrophiles emerged as a common mechanistic feature, either as parent compounds or following metabolic activation.
- Electrophilicity alone was insufficient to distinguish respiratory sensitizers from skin sensitizers and irritants.
- Monofunctional electrophiles and non-crosslinking bifunctional chemicals were not associated with respiratory sensitization.
- These findings support an AOP-informed framework for respiratory sensitization prediction and development of respiratory sensitization-specific NAMs.

Results

1) Summary of the Review of Clinical Cases (Figure 1)

The dataset comprised 152 chemicals, including 99 skin sensitizers, 14 respiratory sensitizers, 21 non-sensitizing irritants, 16 non-irritant/non-sensitizing substances, and 2 unclassified chemicals (Figure 2). Hazard classifications were obtained from ECHA and PubChem. Review of published clinical evidence indicated that sensitization outcomes were consistently endpoint-specific, with individuals exhibiting either skin or respiratory sensitization, but not both. Respiratory sensitizers with the strongest clinical support were primarily diisocyanates (e.g., TDI, MDI, HDI) and acid anhydrides (e.g., TMA, HHPA), which were associated with occupational asthma, rhinitis, and elevated IgE following inhalation exposure. Similar endpoint exclusivity was observed for other respiratory sensitizers, including glutaraldehyde and ethylenediamine, as well as across published clinical reports for skin sensitizers.

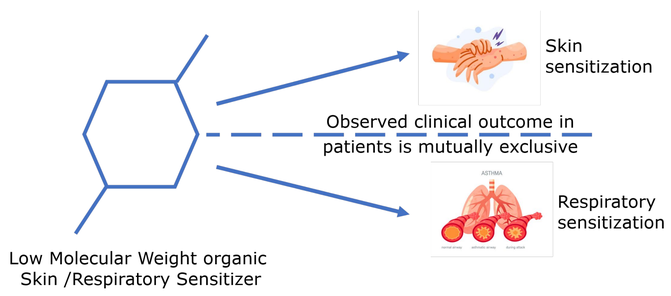
2) Chemical Space Evaluation (Table 1)

Non-Fragrance Skin Sensitizers: Strong skin sensitizers were consistently predicted to act as direct haptens, forming stable monofunctional protein adducts through established electrophilic reaction mechanisms (Figure 3). Examples included DNCB (SNAr reactivity with lysine), methylisothiazolinone (cysteine-directed ring opening), and MDBGN (cysteine thiol substitution). Despite differing chemistries, all were predicted to modify proteins without evidence of bifunctional protein crosslinking, consistent with their skin sensitization profiles and lack of respiratory sensitization potential. **Fragrance Skin Sensitizers:** Fragrance skin sensitizers were predicted to react with skin proteins through monofunctional haptenization mechanisms, such as lysine-directed Schiff base formation (2-methyldecanal) or protein-mediated ring opening (3-propylidene phthalide). Despite their established skin sensitization potential, no clinical evidence of respiratory sensitization was identified for these chemicals, supporting the hypothesis that monofunctional protein binding is sufficient for skin sensitization but may be insufficient to drive the protein crosslinking events proposed for respiratory sensitization. **Respiratory Sensitizers:** All respiratory sensitizers were associated with bifunctional or multifunctional protein-reactive chemistry, either as parent compounds (e.g., TDI, TMA, glutaraldehyde, cyanuric chloride) or following metabolic activation (e.g., piperazine). TIMES predictions indicated the capacity to form crosslinked protein adducts through multiple reactive sites, with piperazine showing preferential formation of the bifunctional metabolite glyoxal in the lung relative to skin. These findings support the hypothesis that protein crosslinking, rather than simple haptenization, is a key mechanistic feature distinguishing respiratory sensitizers from skin sensitizers and irritants. **Non-Sensitizing Irritants:** Non-sensitizing irritants showed a distinct metabolic profile characterized by rapid detoxification and elimination, with little or no predicted formation of reactive protein-binding metabolites. Even when structurally bifunctional, compounds such as methylglyoxal and ethylene brassylate lacked effective protein-crosslinking potential due to reduced reactivity or competing metabolic pathways (e.g., hydrolysis). These findings suggest that structural bifunctionality alone is insufficient for respiratory sensitization and that efficient protein crosslinking may be a critical distinguishing feature of respiratory sensitizers.

3) Proposed Screening Framework (Figure 4)

A hypothesis-driven screening framework was developed by integrating chemical space analysis, clinical evidence, and TIMES metabolism predictions for prospective identification of LMW respiratory sensitizers. **Node 1**: Electrophilic reactivity. Determine whether the chemical is directly electrophilic or can be metabolically activated (prohapten) to a reactive electrophile capable of protein binding. **Node 2:** Functional reactivity. Monofunctional electrophiles are directed toward skin sensitizer/irritant outcomes, while chemicals with two or more reactive centers proceed for further evaluation. **Node 3:** Protein crosslinking potential. Bifunctional or multifunctional chemicals capable of efficient protein crosslinking are predicted as respiratory sensitizers; those lacking effective crosslinking potential are predicted as skin sensitizers or irritants. Protein crosslinking emerges as the key discriminator explaining why only a subset of skin sensitizers exhibit respiratory sensitization.

FIGURE 1: Summary of the clinical case reviews



Framework Process

FIGURE 2: Hazard ID-based Chemical Categories

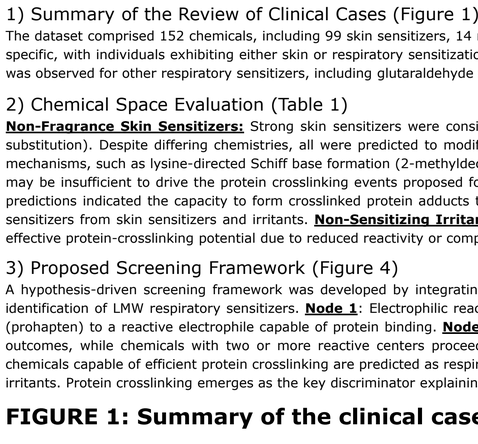


FIGURE 4: Proposed Screening Framework

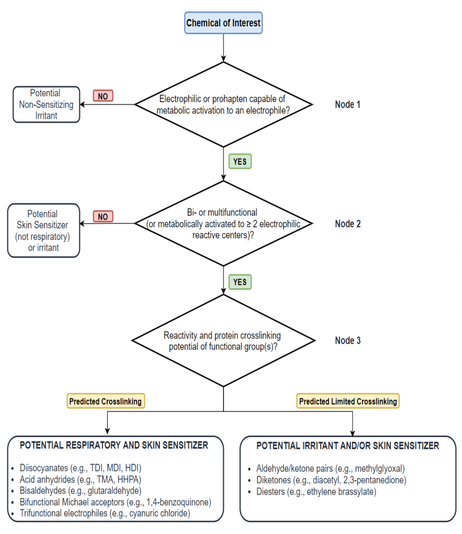
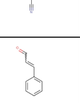
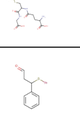
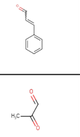
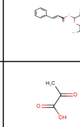
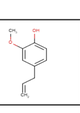
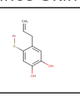
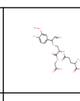
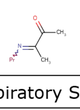
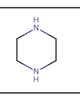
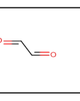


TABLE 1: Hypothesis-driven Predictions

CAS	Structure	Chemical	Most Probable Pathway-Skin	Metabolite Structure	Most Probable Pathway-Lung	Metabolite Structure	Electro-phile?	Bi-functional?	Framework prediction
Non-Fragrance Skin Sensitizers									
97-00-7		Dinitro chlorobenzene	S_NAr nucleophilic addition at halide by protein		Nitro group reduction followed by N-hydroxylation		Y	N	SS/I
2682-20-4		Methyl isothiazolinone	Ring opening by protein addition		S-N bond hydrolysis		Y	N	SS/I
35691-65-7		Methyl dibromo glutaronitrile	Glutathione conjugation at halide		Aliphatic C-hydroxylation		Y	Y	SS/I
14371-10-9		Cinnamic aldehyde	Michael addition via protein		Oxidation followed by glucuronidation of carboxylic acid		Y	Y	SS/I
78-98-8		Methyl glyoxal	Aldehyde oxidation followed by glycination of carboxylic acid		Oxidation of aldehyde		Y	Y	SS/I
Fragrance Skin Sensitizers									
97-53-0		Eugenol	Dealkylation, quinone formation followed by Michael addition by protein		Quinone formation followed by glutathione conjugation		Prohapten to electrophile.	Prohapten to bifunctional	SS/I
105-95-3		Ethylene brassylate	Ester hydrolysis followed by sulphation of alcohol		Ester hydrolysis		Y	Y	SS/I
431-03-8		Diacetyl	Direct acting Schiff base formation with protein		Carbonyl reduction		Y	Y	SS/I
Respiratory Sensitizers									
26471-62-5		Toluene diisocyanate	Nucleophilic attack of isocyanate by protein		Isocyanate hydrolysis and decarboxylation		Y	Y	SS/RS
552-30-7		Trimellitic anhydride	Ring opening by protein followed by glycine conjugation of carboxylic acid		Ester Hydrolysis followed by glucuronidation of carboxylic acid		Y	Y	SS/RS
111-30-8		Glutaraldehyde	Aldehyde oxidation followed by glycine conjugation of carboxylic acid		Aldehyde oxidation		Y	Y	SS/RS
110-85-0		Piperazine	Oxidative deamination to glyoxal		Oxidative deamination to glyoxal		Prohapten to electrophile	Prohapten to bifunctional	SS/RS
108-77-0		2,4,6-trichloro-1,3,5-triazine	S_NAr nucleophilic addition at halide by protein		Glutathione conjugation at halide		Y	Trifunctional	SS/RS

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