Evaluating Chemical and Clinical Spaces: The Bifunctional Reactivity of Respiratory Sensitizers in Comparison to Skin Sensitizers and Non-Sensitizing Irritants

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Introduction: Respiratory sensitization is a complex condition characterized by a spectrum of clinical symptoms, ranging from mild rhinitis to severe, debilitating asthma. In contrast to skin sensitizers, the chemical space of low molecular weight respiratory sensitizers is minimal, largely dominated by diisocyanate and anhydride functional groups. Currently, no validated methods exist that are specific to the detection of respiratory sensitizers. Although the proposed adverse outcome pathway (AOP) for respiratory sensitization shares similarities with that of skin sensitization, it has been hypothesized that the mechanisms of chemical reactivity involved in hapten formation, identified as the first key event, differ between respiratory and skin sensitizers. In this study, eighteen chemicals (including non-fragrance skin sensitizers, fragrance skin sensitizers, respiratory sensitizers, and non-sensitizing irritants) were reviewed for existing clinical case summaries, evaluated for chemical reactivity mechanisms, and analyzed using the TIMES (TIssue MEtabolism Simulator) *in silico* model to predict their metabolic transformations. The goal was to identify differences in chemical reactivity mechanisms, compare metabolic processes in the skin and lung environments, assess their impact on sensitization potential, and align these findings with clinical observations to distinguish between skin sensitizers, respiratory sensitizers, and non-sensitizing irritants.

Methods: Existing reviews summarizing clinical data on selected eighteen chemicals were evaluated to document clinical cases of skin sensitization and respiratory sensitization. These chemicals were also examined for predicting chemical reactivity mechanisms and metabolic transformations using the TIMES software. Two models were applied:

1. In vivo, non-kinetic skin sensitization with autooxidation
2. In vitro, lung metabolism

Both models predicted the most probable metabolic pathways and the relative quantity of metabolites.

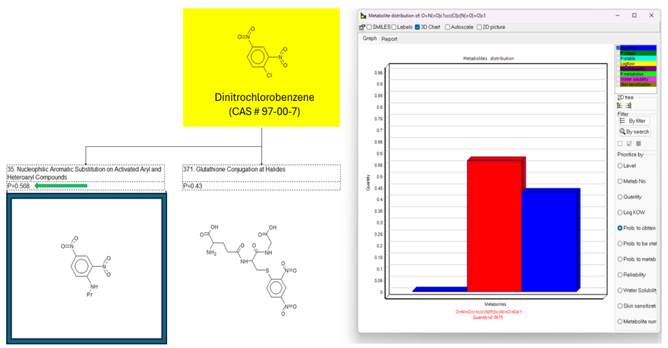

Figure 1: TIMES simulation showing metabolic predictions with probable mechanisms

Limitations: The *in silico* simulations had two key limitations: (1) the skin sensitization model was trained on a substantially larger dataset (1,405 chemicals) compared to the lung model (150 chemicals), and (2) the skin model was developed using historical *in vivo* data, whereas the lung model relied on *in vitro* data. Despite these constraints, the work demonstrates a promising framework for advancing new approach methodologies (NAMs) to support the development of respiratory sensitization assays.

Low–molecular-weight respiratory sensitizers commonly share bifunctional electrophilic groups, which may drive targeted reactivity within lung tissue and emphasize the pivotal role of lung-specific immune cells in the respiratory sensitization AOP.

Table 1: Chemical Space Evaluation from TIMES

CAS #	Most Probable Pathway-Skin	Metabolite Structure	Most Probable Pathway-Lung	Metabolite Structure
Non-Fragrance Skin Sensitizers				
97-00-7	S_NAr nucleophilic addition at halide.		Nitro group reduction.	
2682-20-4	Ring opening S_N2 reaction via protein.		S-N bond hydrolysis.	
35691-65-7	Glutathione conjugation at halides.		Hydroxylation at alpha carbon.	
Fragrance Skin Sensitizers				
104-55-2	Michael addition on α,β-unsaturated carbonyl.		Oxidation to Carboxylic Acid.	
17369-59-4	Ring opening via nucleophilic attack by protein.		Hydroxylation at terminal methyl.	
97-53-0	Dealkylation followed by quinone formation.		Quinone methide formation.	
97-54-1	Quinone methide formation.		Epoxidation of conjugated alkene.	
118-58-1	Ester aminolysis via direct protein interaction.		Ester hydrolysis.	
Respiratory Sensitizers				
127-65-1	S_N2 reaction at halide via protein.		Hydroxylation at terminal methyl.	
Respiratory Sensitizers				
101-68-8	Nucleophilic attack of isocyanate via protein.		Isocyanate hydrolysis.	
26471-62-5	Nucleophilic attack of isocyanate via protein.		Isocyanate hydrolysis.	
552-30-7	Ring-opening S_N2 reaction via protein.		Ester hydrolysis.	
85-42-7	Ring opening S_N2 reaction via protein.		Ester hydrolysis.	
822-06-0	Nucleophilic attack of isocyanate via protein.		Isocyanate hydrolysis.	
110-85-0	Oxidative cleavage to glyoxal. Probability – 65%		Oxidative cleavage to glyoxal. Probability – 85%	
Non-sensitizing Irritants				
69-72-7	Sulphation at alcohol.		Sulphation at alcohol.	
100-06-1	Dealkylation followed by sulphation.		Dealkylation followed by sulphation.	
150-13-0	Acetylation of amines.		Acetylation of amines.	

Results: A review of **clinical case summaries** for the eighteen chemicals revealed that patients with skin sensitization symptoms from skin sensitizers did not exhibit concurrent respiratory issues. Similarly, workers occupationally sensitized to respiratory sensitizers did not exhibit simultaneous skin sensitization. No clinical cases for either skin sensitization or respiratory sensitization were reported from exposure to non-sensitizing irritants. These observations do not take into consideration the patients' medical history of skin or respiratory allergies. **Table 1** highlights the results of **chemical space evaluations** using the TIMES *in silico* model.

Table 2: Conclusions From Chemical Space Evaluation Using TIMES

S_NAr nucleophilic addition at halide	Chemicals defined as strong skin sensitizers displayed direct interaction with skin proteins.
	Pro-haptens were metabolically activated to electrophilic metabolites.
	Non-sensitizing irritants had a high probability for phase II conjugation.

Future direction: Future efforts will focus on expanding the chemical space to include a more diverse representation of chemicals within the three categories, thereby improving insight into their underlying mechanistic drivers. Additionally, strategic testing using advanced non-animal models will help refine biological insights while supporting more ethical and human-relevant approaches. Together, these efforts aim to strengthen predictive frameworks and accelerate progress toward more accurate respiratory sensitization assessment.

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