Weight-of-Evidence Analysis for the Development of a Reference List of Chemical Respiratory Sensitizers

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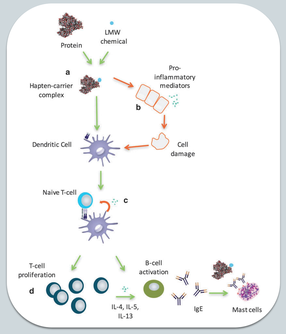
BACKGROUND

Unmet Regulatory Need

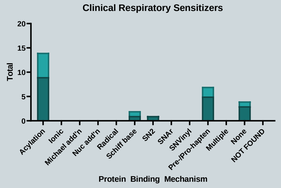
Depending on the regulatory context, approaches are needed for determining the potential of a low molecular weight (LMW) compound to sensitize the respiratory tract and hence the need for hazard labeling, potency assessment, and the definition of sensitization and elicitation thresholds. Approaches are also needed to distinguish respiratory from dermal sensitizers. This unmet need presents a unique opportunity to apply New Approach Methodologies (NAMs) and human biological understanding *ab initio* to develop regulatory guidelines and approaches needed to protect consumer and worker health.

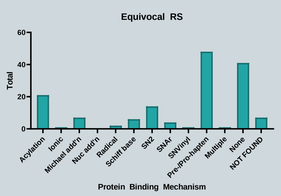
AOP-Driven Criteria

The Adverse Outcome Pathway for respiratory sensitization follows a similar path to dermal sensitization, from protein binding to immune activation [1]. However, these pathways diverge at early key events, resulting in IgE-mediated bronchial hypersensitivity for respiratory sensitizers rather than T cell-mediated contact dermatitis. The biological necessity of Key Events in the AOP was used to identify clinical diagnostic criteria for classifying chemical respiratory sensitizers from clinical literature [2].



"In Litero" Screening to Identify Clinical Respiratory Sensitizers





This approach successfully identified 28 chemicals that can be considered as human respiratory sensitizers and used to evaluate the performance of NAMs as part of a weight of evidence approach to identify novel respiratory sensitizers. A comparison of the protein binding mechanisms of our identified "in litero" clinical respiratory sensitizers shows that acylation is a prevalent protein binding mechanism, in contrast to Michael addition and Schiff base formation common to skin sensitizers [2]. The 153 chemicals with equivocal evidence were prioritized for further evaluation herein based on additional (*in vitro* and *in vivo*) evidence.

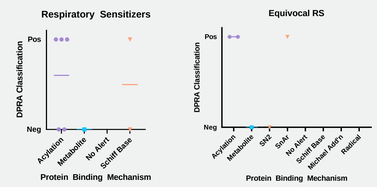
SUMMARY

Using our 28 clinical respiratory sensitizers, we have gathered and integrated available *in vivo* and *in vitro* sensitization data to develop a list of 49 respiratory sensitizers and 16 non-sensitizers that could be used to evaluate NAMs for RS.

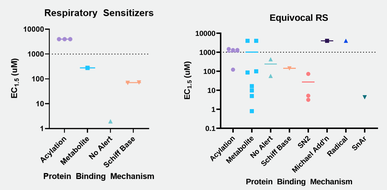
POSITIVE CHEMICALS

In Vitro Dermal Methods are Not Sensitive for Respiratory Sensitizers

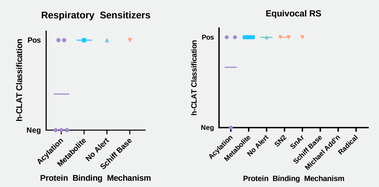
DPRA [3-5]



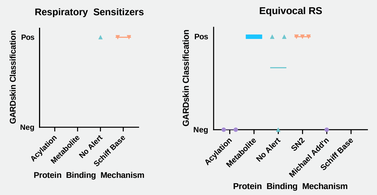
KeratinoSens [6]



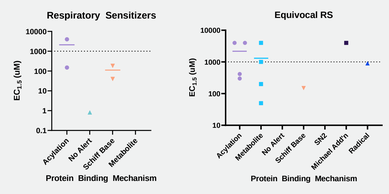
h-CLAT / MUSST [3-5]



GARDskin [6]



LuSens [6]



Low Sensitivity for Softer Electrophiles:

- The 28 clinical respiratory sensitizers [2] were used to determine whether any *in vitro* dermal sensitization method could readily identify respiratory sensitizers. Although these methods are highly sensitive to skin sensitizers, none of these methods can be used alone as an indicator of respiratory sensitization.
- Sensitivity of these methods was strong for hard electrophiles (Schiff bases, Michael acceptors) but false negatives for soft electrophiles and pre-/pro-haptens are common, underscoring the need for RS-specific *in vitro* methods.

Weight-of-Evidence: Identifying Additional Respiratory Sensitizers

Clinical Respiratory Sensitizers

Chemical	Use	In Silico Alert	DPRA	h-CLAT / MUSST	in vivo RS	EC1.5 (uM) KeratinoSens	EC1.5 (uM) LuSens	GARD	EC3 LLNA (%)	EC3 LLNA (%)	EC3 LLNA (%)	S.M.W.N (GPMT)	S.W.A.N (GP 406)
2,4-Dichloro-5-chlorsulfonyl-benzoic acid	Medical	Acylation alert											
7-Aminocephalosporanic acid	Pharmaceutical	Acylation alert											
Ampicillin	Pharmaceutical	Acylation alert											
Cefadroxil	Pharmaceutical	Acylation alert											
Cefteram pivoxil	Pharmaceutical	Acylation alert											
Hexahydrophthalic anhydride	Industrial	Acylation alert	Pos	Neg	High	4000							
Hexamethylene diisocyanate	Industrial	Acylation alert	Neg	Pos	High		100		0.03				
Methyl tetrahydrophthalic anhydride (MTHPA)	Pharmaceutical	Acylation alert			High								
Phenylglycine acid chloride	Pharmaceutical	Acylation alert											
Phthalic anhydride	Industrial	Acylation alert	Pos	Neg	High	4000	>2.4E+03		0.16			Strong sensitizer	Moderately sensitising
Piperacillin	Pharmaceutical	Acylation alert											
Toluene diisocyanate	Industrial	Acylation alert	Neg	Pos	High								
Trimellitic anhydride (TMA)	Industrial	Acylation alert	Pos	Neg	High	4000			9.2	9.2	0.19	Moderate sensitizer	
methylene diisocyanate TBTU (N,N,N',N'-Tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate)	Industrial	Acylation alert											
Chloramine T	Biocide	Metabolite alert		Pos	High	248			0.4	0.4			
Piperazine	Pharmaceutical	Metabolite alert	Neg		High								
Plicatic acid	Industrial	Metabolite alert											
2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate	Industrial	Metabolite alert											
Carmine	Food	Metabolite alert											
Menthol	Pharmaceutical	Metabolite alert											
Thiamphenicol	Pharmaceutical	SN2 alert											
Ammonium hexachloroplatinate	Industrial	No alert			High								
Ammonium persulfate	Industrial	No alert			High								
Paull's reagent (4-diazobenzenesulfonic acid)	Industrial	No alert											
Potassium dichromate	Industrial	No alert		Pos		1.26	0.815	Pos					
Formaldehyde	Industrial	Schiff base alert	Neg			63.2	184	Pos	14.5	12.3	8.2	Strong sensitizer	Moderately sensitising
Glutaraldehyde	Industrial	Schiff base alert	Pos	Pos	Pos	24.3	<45	Pos	2.06	0.2	0.16		

Likely Respiratory Sensitizers

Use	EQUIVOCAL RS	In Silico Alert	DPRA	h-CLAT / MUSST	in vivo RS	EC1.5 (uM) KeratinoSens	EC1.5 (uM) LuSens	GARD	EC3 LLNA (%)	EC3 LLNA (%)	EC3 LLNA (%)	S.M.W.N (GPMT)	S.W.A.N (GP 406)	Rating:
Biocide	unknown	SN2 alert			High				11.6	5.15				**
Industrial	confounders	Acylation alert			High	122			0.08					****
Industrial	no test	Acylation alert		Pos	High		350		0.1			Moderately sensitising		****
Industrial	confounders	Acylation alert	Pos	Neg	High	1480			0.16	0.16		Moderately sensitising		****
Industrial	no test	Metabolite alert		Pos	Med	9.8		Pos	1.67	1.34	0.37	Strong sensitizer		***
Industrial	confounders	Metabolite alert		Pos	Med	5.01	<53	Pos	2.17	0.399	0.206	Strong sensitizer		***
Industrial	no test	SNAr alert		Pos	Med	4.32			0.004					**
Industrial	no test	Metabolite alert		Pos	Med	85.8		Pos	2.32	2.2	2.2			***
Industrial	confounders	SNAr alert	Pos		Med				0.09			Moderately sensitising		**
Industrial	no test	SN2 alert		Pos	Med	5.2		Pos	1.5					**
Industrial	negative	Metabolite alert	Neg	Pos	Med	99.5	>750	Pos	2.7	2.2	2.2			***
Industrial	confounders	Metabolite alert			Med	0.8			<1.25	<0.5	0.0009			**
Food/cosm	no test	Acylation alert			Med	>2E+03	415	Neg	0.22	0.164	0.07			***
Biocide	no test	SN2 alert			Med				6.91					*
Industrial	no test	Metabolite alert			Med				1.02					**
Cosmetic	no test	SN2 alert	Neg	Pos	Med	3.16		Pos	32.4	9	7.79			**
Industrial	confounders	Metabolite alert		Pos	Med	16.6			15	14.7		Strong sensitize		**
Industrial	unknown	Metabolite alert		Pos	Med	>2E+03	<241	Pos	40.9	20.4	19.1	Moderate sensitizer		**
Pharmaceutical	no test	Acylation alert		Pos	Med	1310	>2.4E+03	Neg	46.4	45.2	41.2			**
Industrial	confounders	Metabolite alert			Med									*
Industrial	confounders	Schiff base alert			Med									*

An additional 21 chemicals with equivocal epidemiological evidence and *in silico* protein binding alerts were found to have *in vivo* and/or *in vitro* evidence supporting their classification as likely respiratory sensitizers, including two additional human skin sensitizers.

NEGATIVES

RS-Negative Reference Chemicals

The AOPs for irritation and sensitization overlap at early key events, therefore inclusion of RS-negatives which do and do not cause dermal sensitization and/or respiratory irritation is needed to identify which methods can discriminate between these AOs.

Potential RS negatives	Protein Reactivity	Irritant	Dermal Sensitizer
citric acid		✓	
n-hexane		✓	
benzoic acid		✓	
4-aminobenzoic acid		✓	
4-hydroxybenzoic acid		✓	
isopropanol		✓	
lactic acid		✓	
salicylic acid		✓	
methyl salicylate		✓	
glycerol	✓	✓	
butoxyethanol	✓	✓	
alpha-terpineol	✓	✓	
(+) alpha pinene	✓	✓	
capsaicin	✓	✓	
eugenol	✓	✓	✓
D-limonene	✓	✓	✓

REFERENCE CHEMICAL LIST

- Our list of respiratory sensitizers includes low-molecular weight chemicals known to, or suggested to, cause RS in humans based on epidemiological reports, protein binding alerts for the chemical or its metabolites, and experimental evidence demonstrating induction and/or elicitation of sensitization.
- Additional considerations for RS reference chemicals will be incorporated to finalize an ideal reference chemical list for the development of RS-specific NAMs:
  - Well-defined chemical structures
  - Commercial availability and cost
  - Vehicle solubility and compatibility
  - Representation of material forms: *e.g.* solid, liquid
  - Representation of protein binding reactivity: unreactive, soft electrophiles, hard electrophiles
  - Lack of acute toxicity: minimize hazards of handling and disposal

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