

Towards an IATA for Chemical Respiratory Sensitization: Establishment of Reference Chemicals to Evaluate *In Vitro* and *In Silico* Approaches

Kristie Sullivan¹, Nancy Baker², Stella Cochrane³, Steven J. Enoch⁴, Janine Ezendam⁵, Grace Patlewicz⁶, Erwin L. Roggen⁷, Raja Settivari⁸, Katherina Sewald⁹

1. Physicians Committee for Responsible Medicine, 2. Leidos, 3. SEAC, Unilever, 4. Liverpool John Moores University, 5. RIVM, 6. US EPA, 7. Novozymes, 8. The Dow Chemical Company, 9. Fraunhofer ITEM.

ABSTRACT

Public health and regulatory needs require approaches to detect and discriminate respiratory sensitizers from dermal sensitizers; however no single method or strategy is generally accepted. An Adverse Outcome Pathway (AOP) for respiratory tract sensitization by low molecular weight organic chemicals has been published and is proceeding through the Organization for Economic Cooperation and Development review process. This AOP is a useful foundation for the development and assessment of *in vitro* and *in silico* test methods and combinations thereof within one or more Integrated Approaches to Testing and Assessment (IATA). The published AOP identified several promising approaches; however most of these have only been assessed with a few well-known respiratory sensitizers (e.g., toluene diisocyanate or trimellitic anhydride). To further evaluate the utility of these and other approaches, we have set out to build a more comprehensive list of reference chemicals, including known respiratory irritants, non-sensitizers, and dermal sensitizers. The ideal list of respiratory sensitizers should cover a range of chemical classes and include “challenging” chemicals, such as respiratory sensitizers thought to elicit effects through dermal exposure and those for which specific-IgE has not been detected in humans. To build the list, we are conducting a review of established structure-based profilers, recent literature, and human clinical reports, focusing on data verified in humans for translatability to human health outcomes. We are also making use of the Abstract Sifter literature review tool (Baker et al., (2017)) to identify additional potential respiratory sensitizers. Briefly, a set of PubMed MeSH terms describing adverse effects (AEs) for 92 likely sensitizers was used to query a large database of chemicals and AEs, yielding over 7000 chemicals of potential interest. The top 500 ranked chemicals (based on article counts) are currently undergoing manual review. This reference chemical list is an important step towards the assessment of potential test methods and the creation of internationally-harmonized integrated approaches for the detection of chemical respiratory sensitizers.

METHODS

The lack of a widely used or regulatory-accepted *in vivo* method for discriminating respiratory sensitizers from dermal sensitizers leads to challenges in determining known positive chemicals, but it also presents an opportunity to take a weight of evidence approach. We are gathering any information which might indicate a chemical is a respiratory sensitizer, from structural alerts, in chemico and in vitro assays through to human clinical reports to build a comprehensive dataset. Clear respiratory sensitizers and challenging negatives can be pulled from such a dataset. Careful documentation of source material, criteria for listing determination, and inconsistencies are essential considerations. A template was devised to facilitate the systematic collection of information.

Other information fields collected but not shown below include molecular weight, solubility, physical form, and metabolic activation.

Manual literature review: Papers investigating the predictivity of a number of *in silico*, *in vitro* and *in vivo* methods to detect respiratory sensitizers were surveyed to capture chemicals others have considered for their “reference lists”. Several hallmark sensitizers are often used at the method development and optimization stage and data gathered in those studies are collected. A number of respiratory sensitizers have been assessed in *in vitro* tests validated for dermal sensitization and the resulting data is illustrative. Finally, a number of case reports have been collected to build an *in silico* profiler; these data are also considered for this list. The manual list currently stands at > 120 chemicals.

Automated literature review: The Abstract Sifter literature search tool allows the expansion of an initial list of respiratory sensitizers by first determining the adverse effects associated with well-established respiratory sensitizers in the published literature. The tool then identifies chemicals associated with those same asthma-related adverse effects and ranks them based on article counts, highlighting potential previously unidentified sources of data from manual searching.

Database and Regulatory list review: Chemicals appearing on occupational, classification, or other regulatory lists as chemicals of concern for respiratory sensitization are potential respiratory sensitizers, and there may be human data associated with them. For example, the ECHA CHEM database contains 194 unique substances indicating a concern for respiratory sensitization, many with multiple lines of supporting evidence.

CONSIDERATIONS FOR A COMPREHENSIVE LIST

- Positive chemicals**
 - Major mechanistic domains represented
 - Pro- and pre-haptens
 - Volatile, water- or DMSO-soluble and insoluble chemicals
 - Chemicals representative of sector- or agency-specific needs
 - Chemicals for which IgE antibodies have not been detected
 - Chemicals identified as causing respiratory sensitization via dermal exposure
- Negative chemicals**
 - Dermal Sensitizers
 - Respiratory irritants
 - Non-irritant, non-sensitizers

DISCUSSION POINTS

- There is a clear need for a list of verified positive and negative respiratory sensitizers with which to assess the predictive capacity of methods and approaches
- Such a list should be created in consultation with regulatory agencies and validation bodies. Authors have already begun to engage OECD, ICCVAM, NICEATM, EURL ECVAM, and regional regulatory agencies.
- This list is a work in progress and the chemicals appearing in the table below should not necessarily be considered respiratory sensitizers.
- The list and supporting data should ultimately be hosted on a publicly-accessible platform to facilitate further method assessment and development.
- Unpublished data would augment this analysis.
- The data upholding regulatory or classification list designation should be manually checked. For example, the evidence behind a designation of “respiratory sensitizer” in the ECHA CHEM database is diverse, and for a number of chemicals experimental evidence is solely, upon manual inspection, available for the dermal, but not the respiratory, sensitization endpoint.

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DISCLAIMER: Does not necessarily represent U.S. EPA policy.

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COLLABORATORS WANTED

Please contact us if you would like to contribute data or collaborate on this effort.

Chemical	Structural alert	in chemico result	KeratinoSens/LuSens	h-CLAT	Other	In vivo animal result	In vivo human result
1,1,3-Tributylthiourea (TBTU)							
1,2-Benzisothiazolin-3-one							
1,3-Bis(isocyanatomethyl)cyclohexane							
1,3,5-Triazine-2,4-diamine, 6-chloro-N, N'-bis(2,2,6,6-tetramethyl-4-piperidinyl)							
1,3,5-Tris(6-isocyanatohexyl)-1,3,5-triazinane-2,4,6-trione							
1,5-Naphthalene diisocyanate							
2-Diethylethanolamine							
2-Methyl-3,5-dinitrobenzamide							
2,4-Dichloro-5-chlorosulfonyl-benzoic acid							
2,4,5,6-Tetrachlorobenzene-1,3-dicarbonitrile							
3-Amino-5-mercapto-1,2,4-triazole							
3-Carene							
3-Dimethylaminopropylamine							
5-Aminosalicylic acid							
6-Aminopenicillanic acid							
7-Aminocephalosporanic acid							
Abietic acid							
Acetic acid							
Adipic acid							
Aminoethylethanolamine							
Aminophylline							
Ammonium hexachloroplatinate				+CCL5 & IL-8 in human alveolar epithelial/endothelial coculture			
Ammonium persulfate							
Ammonium tetrachloroplatinate		NA		+			+
Amoxicillin							
Ampicillin		- (20m), + (24h)					
Amprolium hydrochloride							
Azodicarbonamide							
Basic Blue 99		+	NA	NA		+(LLNA)	
Biuret of hexamethylene diisocyanate							
Captadol							
Cefadroxil							
Ceftazidime		+					
Cefteram pivoxil							
Cephalexin							
Chloramine T							
Chlorendic anhydride	anhydride	+	+	+	+(predictive gene signature)	+(LLNA)	+
Chlorhexidine							+
Chloroxylonol							
Cimetidine							
Cyanuric chloride		+	NA	NA		+(LLNA)	
Diacetyl morphine							
Diethanolamine		-					
Diglycidyl ether of bisphenol A							
Dimethylethanolamine		-					
Diethylphthalate		+		+			+
Diocetylphthalate							
Diphenylmethane-4,4'- diisocyanate		+	NA	NA		+(LLNA)	
Dobutamine HCl							
Dodecanedioic acid							
Epigallocatechin gallate							
Ethanolamine		-					
Ethoxylated bisphenol A diacrylate							
Ethyl acrylate		+					
Ethyl cyanoacrylate							
Ethylene oxide							
Ethylenediamine		-	+	+		+(LLNA)	+
Eugenol							

TABLES 1 & 2

These tables are an slice of the total data being collected, and only reflect potential positive respiratory sensitizers as identified by cited references. A lack of data does not indicate data does not exist but rather we have not yet recorded it.

Chemical	Structural alert	in chemico result	KeratinoSens/LuSens	h-CLAT	Other	In vivo animal result	In vivo human result
Fenthion							
Fluazinam							
Fluorescein Isothiocyanate							
Formaldehyde		+		+			+
Furfuryl alcohol							
Glutaraldehyde		+	+	+		+(LLNA)	+
Glycyl compound							
Hexachlorophene							
Hexahydrophthalic anhydride (HHPA)	anhydride	+	-	+		+(LLNA)	+
Hexamethylene diisocyanate		+	+	NA		+(LLNA)	
Hexamethylenetetramine							
Himic anhydride	anhydride						+
Hydralazine							
Hydroquinone		+					
Indigotine							
Isoniazid							
Isothorone diisocyanate		+	+	NA	+(predictive gene signature)	+(LLNA)	
Maleic anhydride	anhydride	+	-	+		+(LLNA)	+
Methyl blue							
Methyl DOPA							
Methyl hexahydrophthalic anhydride (MHHPA)	anhydride	NA	NA	NA			+
Methyl methacrylate							
Methyl-2-cyanoacrylate							
Methyltetrahydrophthalic anhydride (MTHPA)	anhydride	NA	NA	NA		+(LLNA)	+
Mitoxanthrone							
MM22383							
Morphine hydrochloride							
N-methylmorpholamine							
Ninhydrin		+					
Orange 3R Reactive Dye		+	NA	NA		NA	
P-phenylenediamine							
Pauli's reagent (4-diazobenzenesulphonic acid)		- (20m), + (24h)					
Penicillamine							
Benzyipenicillin (Penicillin G)		+	-	+		+(LLNA)	
Phenyglycine acid chloride							
Phtalic anhydride (PA)	anhydride	+	-	-	+(predictive gene signature)	+(LLNA)	+
Piperacillin							
Piperazine							
Plicatic acid							
Potassium dichromate							
Reactive Black 5		+	NA	NA		+(LLNA)	
Reactive Orange					+(predictive gene signature)		
Reactive Red 123		NA	NA	NA		+(LLNA)	
Reactive Yellow 39		+	NA	NA		+(LLNA)	
Red-BBN Reactive Dye							
Salbutamol							
Spiramycin							
Styrene							
Sulfathiazole							
Tetrachlorophthalic anhydride (TCPA)	anhydride	+	NA	NA		NA	+
Tetracycline							
Tetramethrin							
Thiamine							
Thiamphenicol							
Toluene diisocyanate		+	+	NA		+(LLNA)	+
Trientine							
Triethylenetetramine		-					
Triglycidyl isocyanurate							
Trimellitic anhydride (TMA)	anhydride	+ / -	-	+	+CCL5 & IL-8 in human alveolar epithelial/endothelial coculture	+(LLNA)	+
Trimethylolpropane triacrylate							
Triphenylmethane trisocyanate							
Tylosin							