

Development of a Reference List of Chemical Respiratory Sensitizers to Facilitate Evaluation of Integrated Approaches to Testing and Assessment

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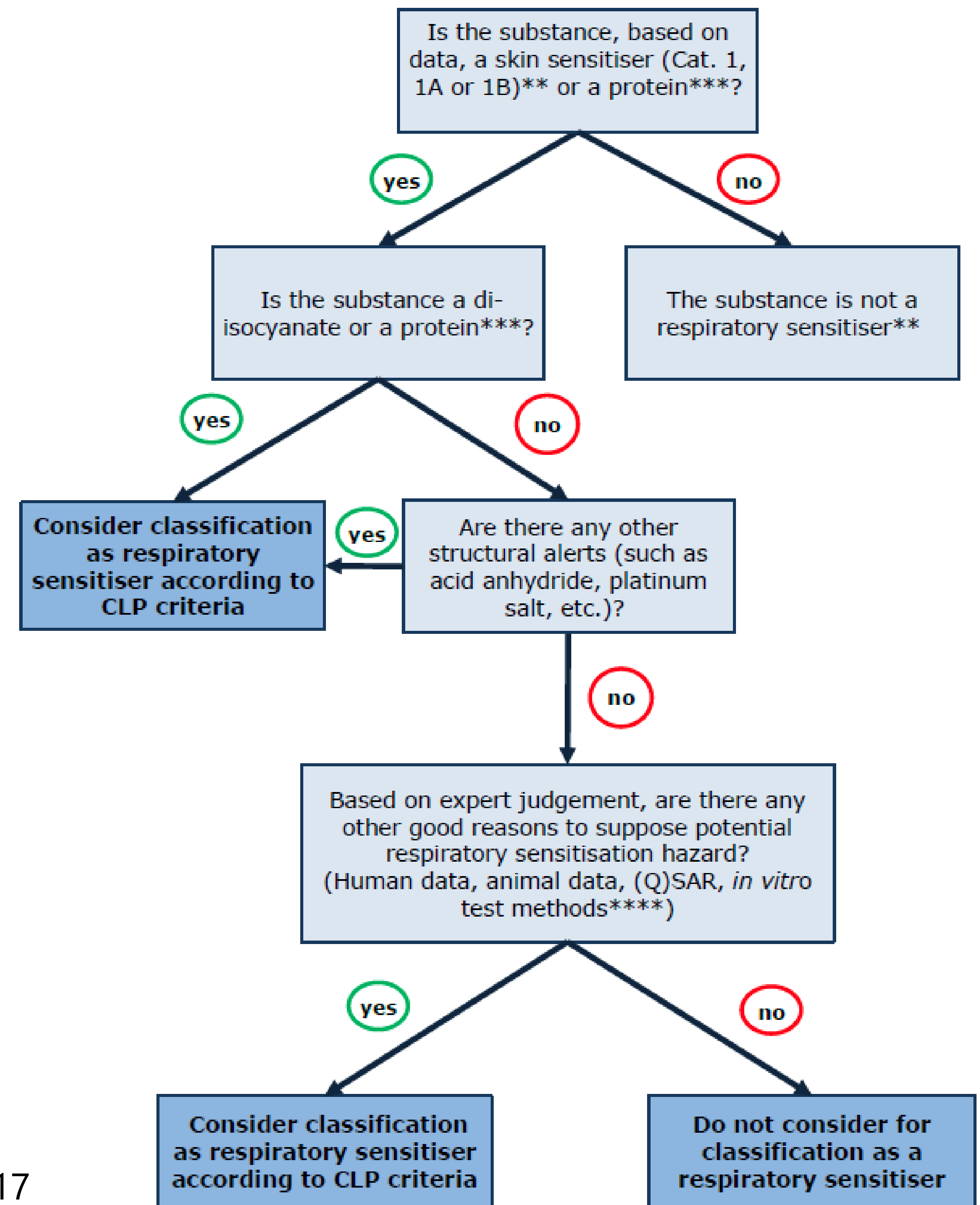
Physicians
Committee
for Responsible Medicine

Outline

1. Adverse Outcome Pathway has been developed
2. Human and other data collected on a preliminary set of potential respiratory sensitizers
3. Pursuing additional strategies to include more chemicals
4. Aiming for some testing in 2021
5. Your feedback is sought and welcomed

Regulatory Context

- No assays currently accepted for regulatory use
- ECHA and US EPA take a weight-of-evidence approach
- Approaches needed to discriminate sensitizers and irritants and dermal from respiratory sensitizers
- More testing needed to understand predictive capacity of potential approaches



Project: Sensitization of the respiratory tract AOP and IATA development

Contributors

Nancy Baker, US EPA

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Erwin L. Roggen, 3Rs Mgmt & Consulting

Raja Settivari, Corteva

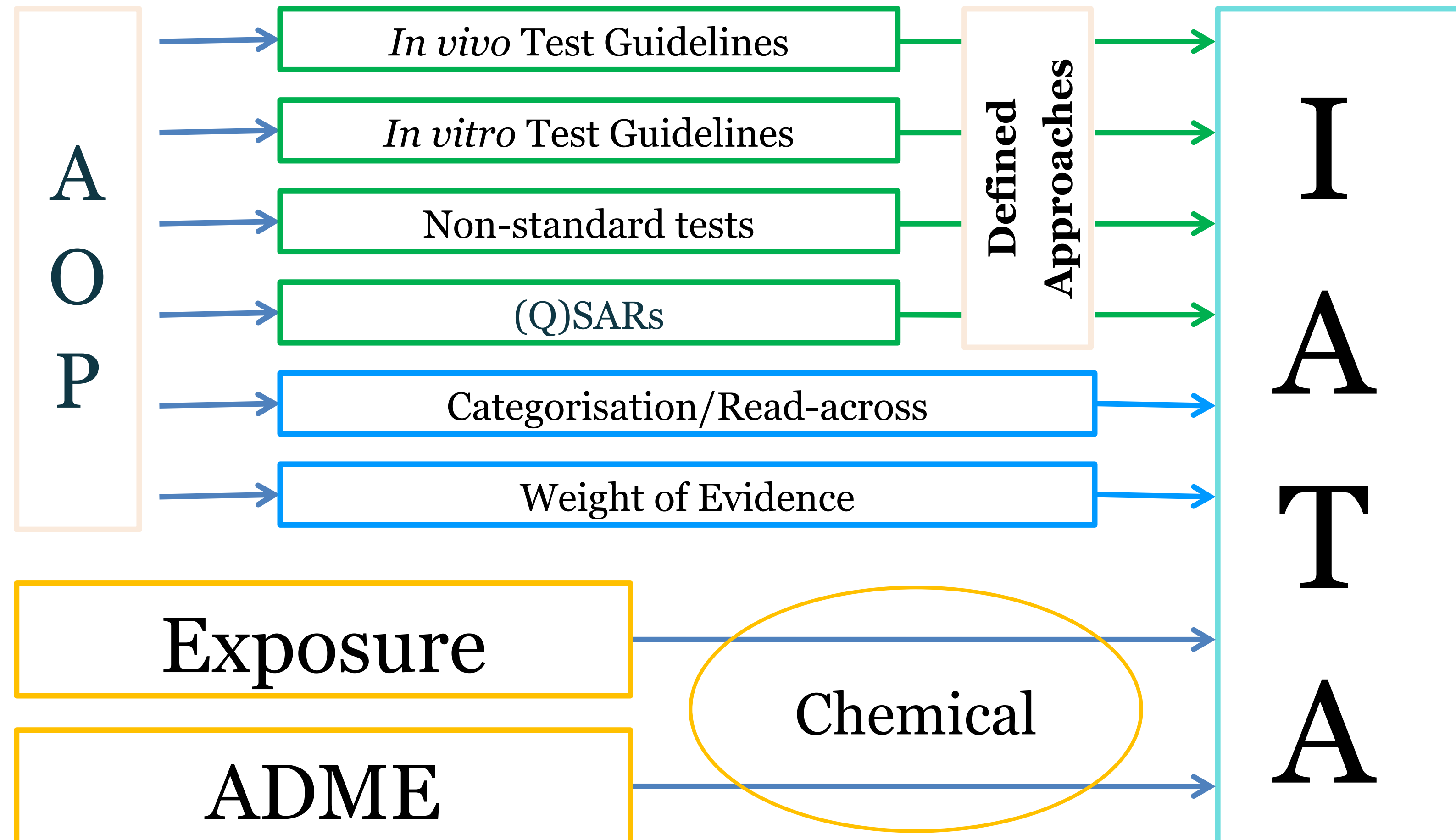
Katherina Sewald, Fraunhofer ITEM

Madhuri Singal, AeroTox Consulting

Project Elements

- Publish AOP ✓
- Seek endorsement of AOP at OECD >>>
- Develop list of reference chemicals >>>
- Collaborate to optimize and test in vitro and in silico methods >>>
- Develop IATA for regulatory use

An AOP to IATA Approach



Domain: LMW Organic Chemicals

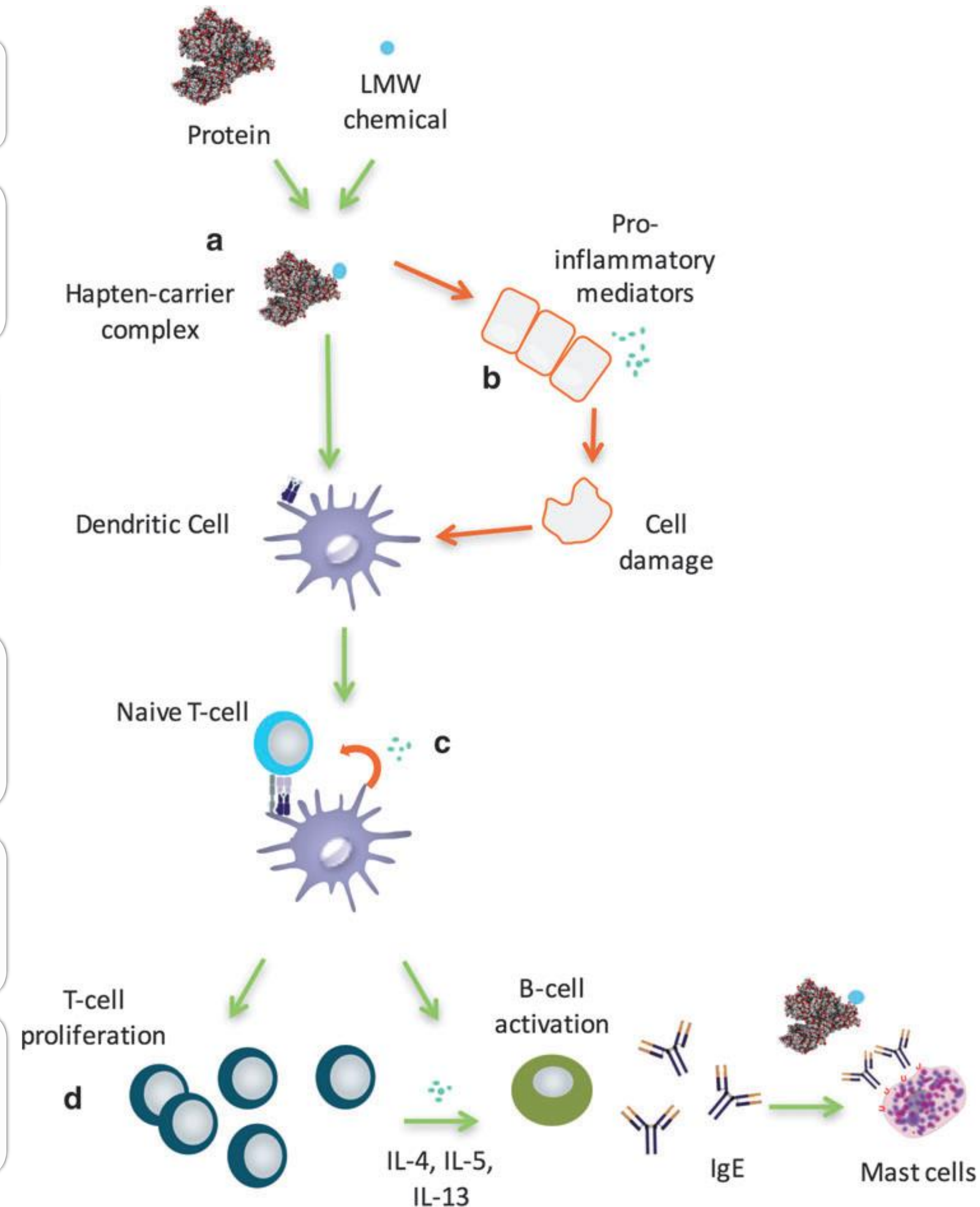
MIE: Covalent Binding Preference to Lysine Residues on Proteins

Cellular Danger Signals: Activation of Inflammatory Cytokines and Chemokines and Cytoprotective Gene Pathways (Th2)

Dendritic Cell Activation (Th2 skewing) and Migration

T-cell Activation-Proliferation-Polarization (Th2)

AO: Sensitization of the Respiratory Tract and Allergic Asthma upon Challenge

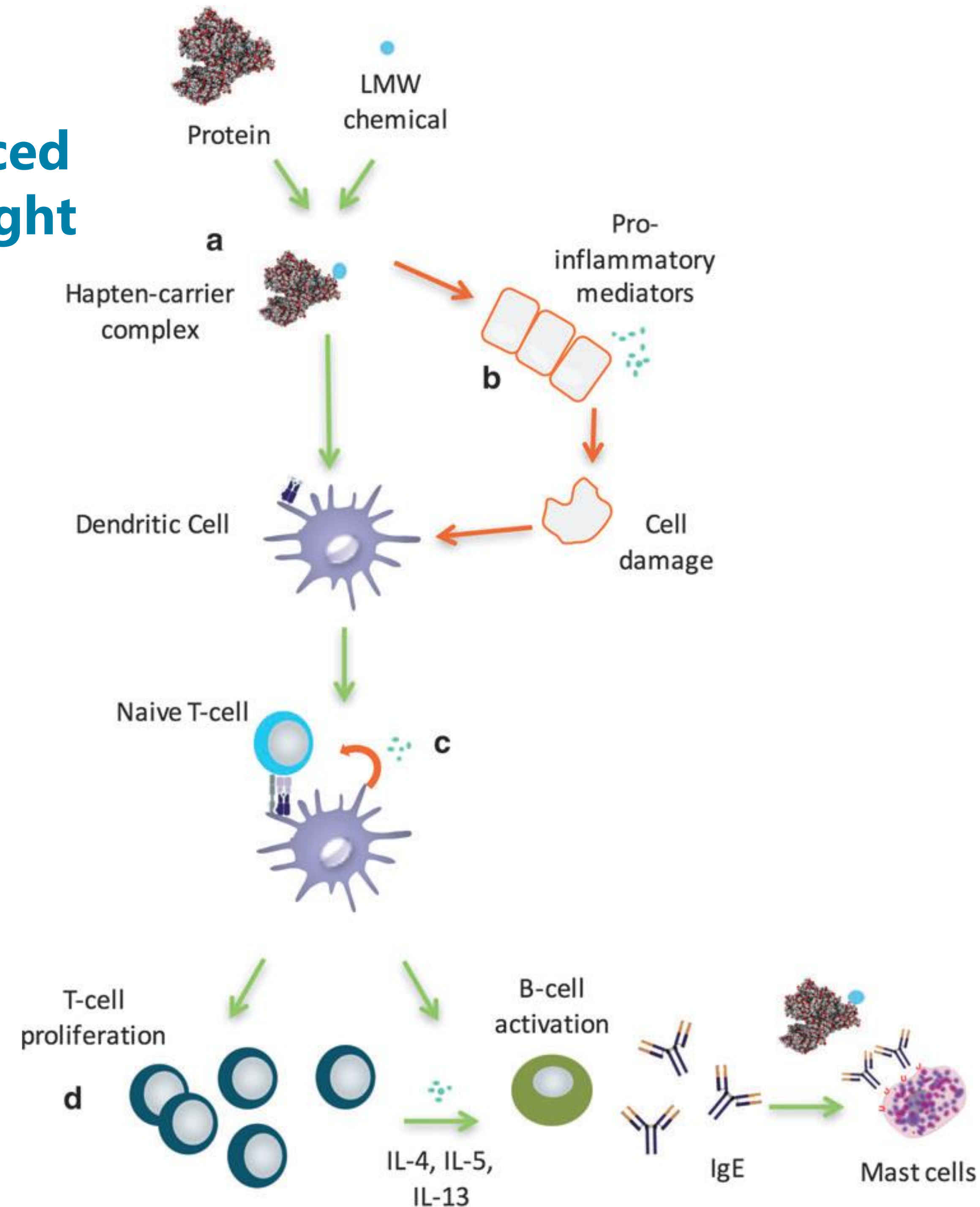


Sensitisation of the Respiratory Tract induced by Covalent Binding of Low Molecular Weight Organic Chemicals to Proteins

Sullivan et al. 2017. Applied in vitro
Toxicology 3(3): 1-14. DOI:
10.1089/aivt.2017.0010

OECD AOP WIKI

AOP 39: Covalent Binding of Low
Molecular Weight Organic Chemicals to
Proteins leads to Sensitisation
(Sensitization) of the Respiratory Tract
<https://aopwiki.org/aops/39>



Reference List Characteristics

Positive set:

- Human Data available
- Major mechanistic domains represented
- Pro- and pre-haptens, volatile, soluble, & insoluble chemicals

Negative set:

- Human Data available
- Dermal sensitizers
- Respiratory Irritants
- Non-sensitizers

Reference List Development

- 120 chemicals identified with human clinical indication of respiratory sensitization, primarily from Enoch et al. *Chem Res Toxicol* 2012, 25:2490-2498.
- Have collected phys/chem, metabolism, structural alert, *in silico*, *in vitro*, and *in vivo* animal and human data
- Animal data was obtained via manual PubMed searches with the chemical name + respiratory + animal (or guinea pig or LLNA) OR chemical name + asthma
- *In vitro* data obtained via manual PubMed searches with the chemical name + *in vitro*. Includes DPRA Lys/Cys ratio, Keratinosens, H-Clat, GardAir, Resp. co-culture
- Human data obtained using EPA-developed Abstract Sifter tool, that automates broad literature searching via PubMed

Baker N et al. *F1000Research* 2017, 6(Chem Inf Sci):2164

ftp://newftp.epa.gov/COMPTOX/Sustainable_Chemistry_Data/Chemistry_Dashboard/Abstract_Sifter/

Criteria for classification of human data:

No information

There is no information to evaluate the compound

Either absent from the literature

Or the available literature is irrelevant to human respiratory symptoms

No

The clinical literature demonstrates that the compound is not a respiratory sensitizer in humans

Significant occupational exposure and investigation of asthmatic symptoms rules out immune-mediated occupational asthma/respiratory allergy caused by the compound

Equivocal

There is clinical evidence of respiratory symptoms after exposure, but available evidence does not conclusively demonstrate sensitization

Either there is no evidence of immune-mediated response to distinguish respiratory sensitization from respiratory irritation

Or there is conflicting evidence of immune-mediated response or significant confounding exposure

Criteria for classification of human data:

Yes ***There is significant clinical evidence that the compound has caused respiratory sensitization in at least one patient, as defined by one of the following scenarios:***

Patient history of exposure with positive specific bronchial challenge, combined with evidence of specific IgE and/or IgG immune-mediated response as determined by exposure to the compound:

- _____
Skin-prick test (SPT)
- _____
Radioallergosorbent test (RAST)
- _____
Enzyme-linked immunosorbent assay (ELISA)

Patient history of exposure with positive nonspecific bronchial challenge, combined with evidence of IgE and/or IgG immune-mediated response paired with negative controls to eliminate confounding exposures

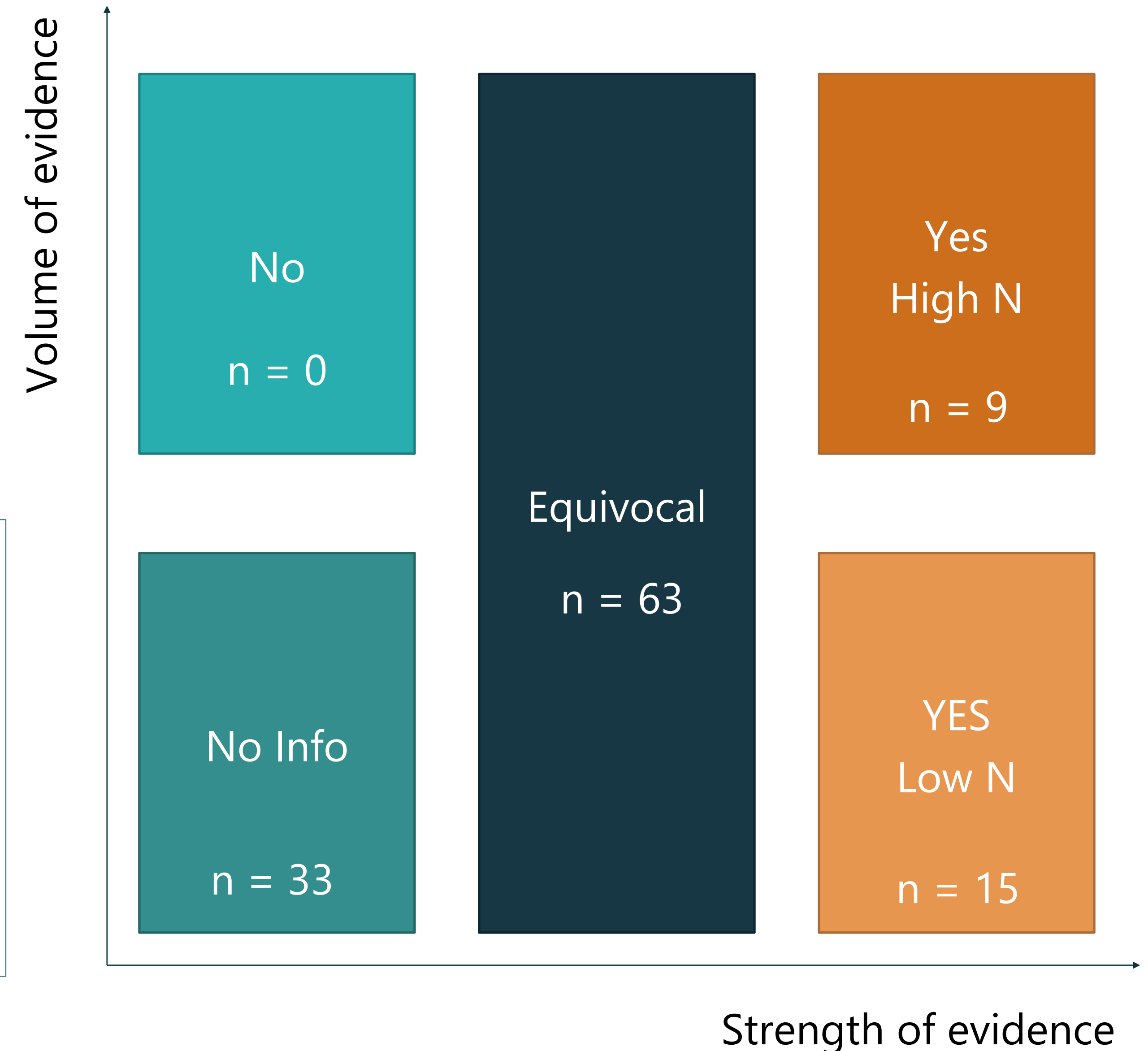
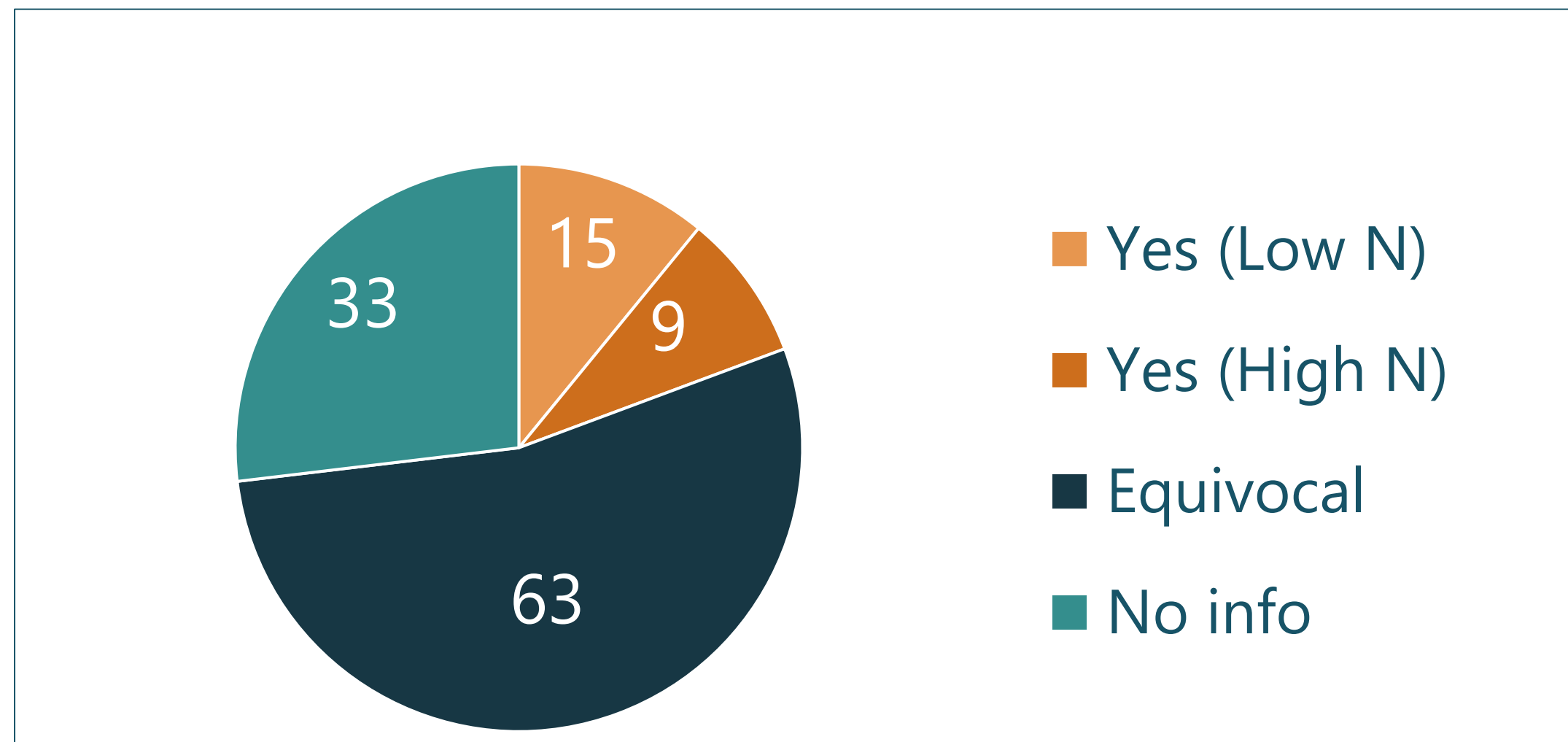
$1 \leq N \leq 10$: **Low N**

Additionally, the quantity of cases identified in the available literature is indicated for all compounds in this category:

$N > 10$: **High N**

Human Data Results

Has this compound been shown to cause respiratory sensitization from human exposures in the literature?

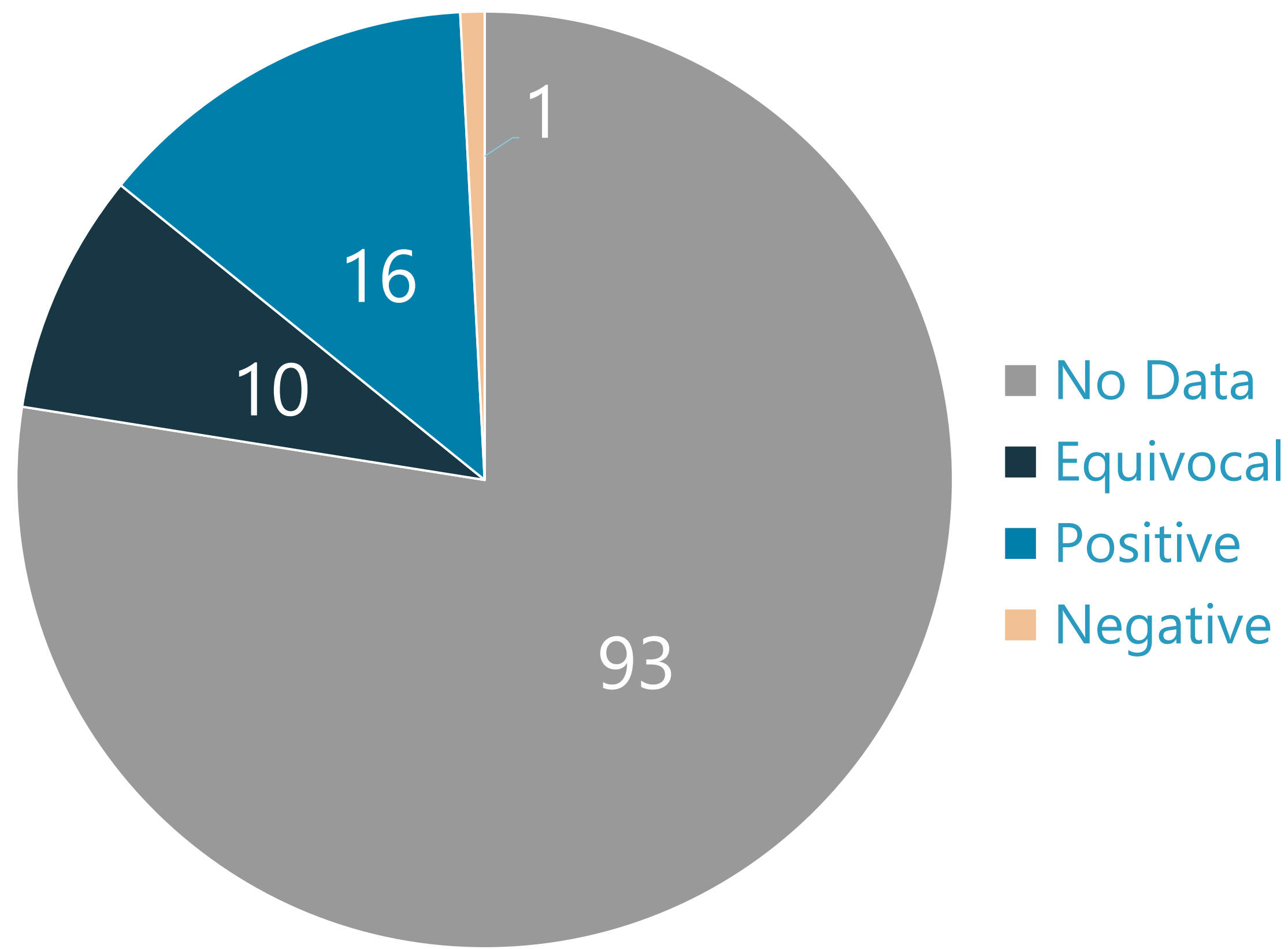


Animal weight of evidence

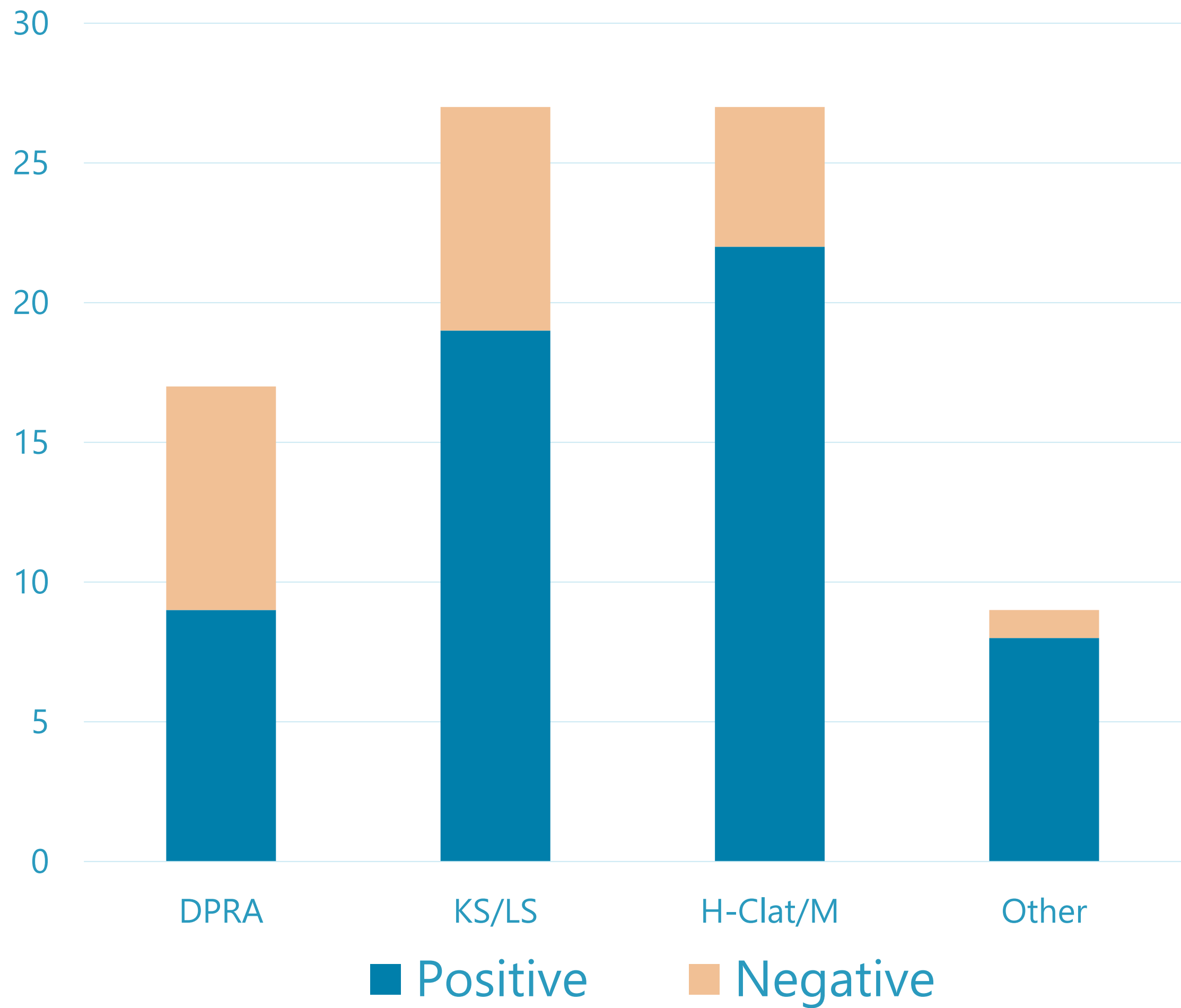
Scoring	Experimental Model	Positive Parameters	Call
++	Model that uses induction (dermal/inhalation) and challenge (inhalation) with the chemical under investigation	Th2 parameters (cytokines) Immunoglobulins (IgG, IgE) as a measure of sensitization Lung function measurements Lung inflammation (pathology, BAL fluid)	Yes
++	Mouse IgE test	IgE in serum	Yes
++	LLNA with cytokine fingerprinting	Positive LLNA with evidence for Th2 skewing based on cytokine profiles	Yes
+	Traditional LLNA	Positive LLNA in the absence of cytokine profiles	Equiv
+	Guinea Pig assays (Buehler, GPMT)	Positive outcome	Equiv
Exclusion	Inhalation tox studies (28/90 days)	Do not provide evidence for sensitization. Lung effects may be caused by irritation.	Lack of data

Animal and *In Vitro* Results

**ANIMAL CALLS:
RESPIRATORY SENSITIZATION**



**IN VITRO RESULTS:
SENSITIZATION GENERALLY**



Full List Decision Basis

Is this chemical a probable
respiratory sensitizer based on
human and other evidence?

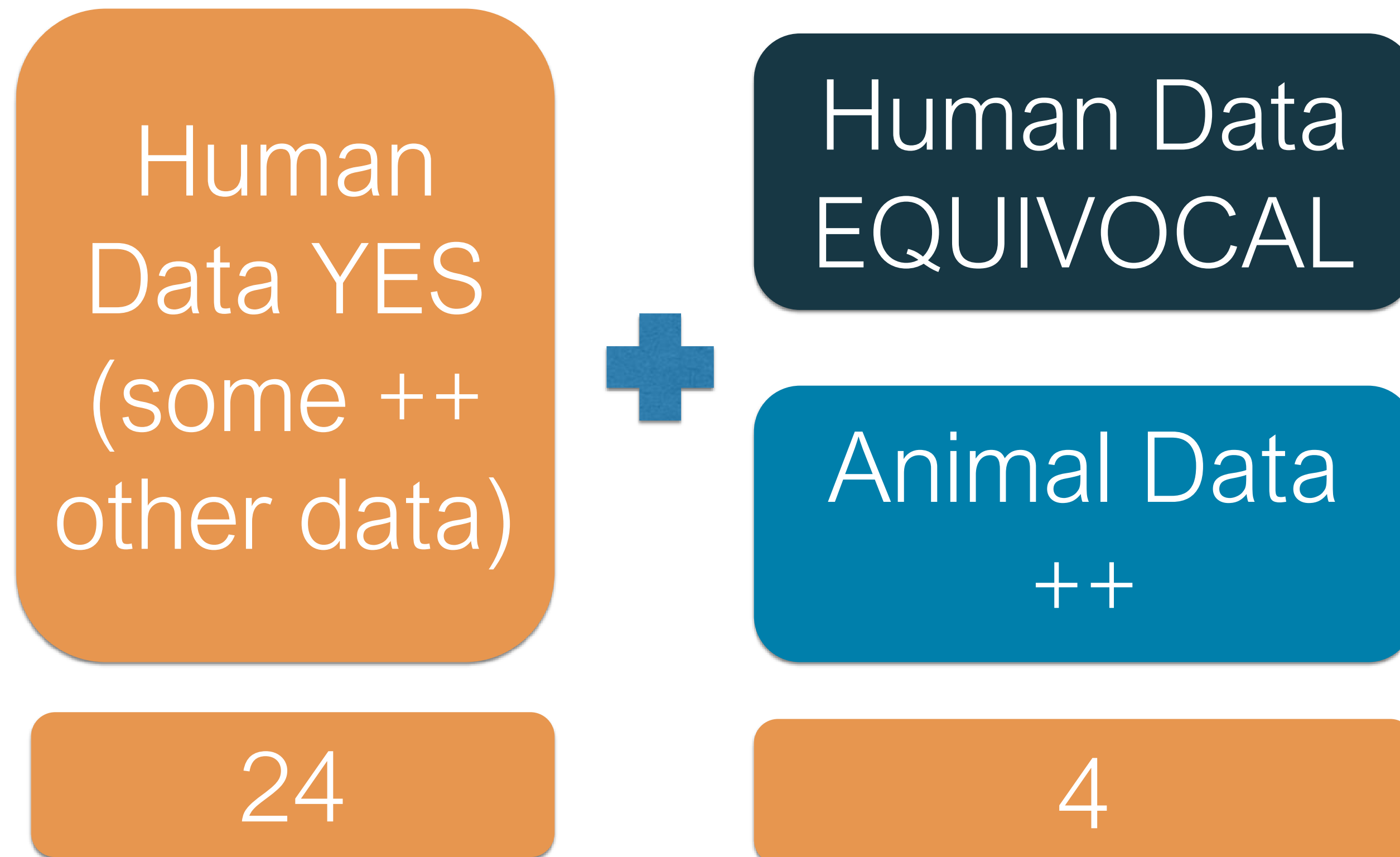
Human
Data YES
(some ++
other data)

24

Chemical	DPRA indicates RS	DPRA RATIO LYS/CYS	KeratinoSens/ LuSens	h-CLAT/ MUSST	Other in vitro	Animal	Human
POSITIVE (DATA + SA EVIDENCE)							
Ammonium hexachloroplatinate			-	-	+	++	YES - HIGH N
Glutaraldehyde	+	2.8	+	+		++	YES - HIGH N
Hexahydrophthalic anhydride (HHPA)	+	10.7	-	+		++	YES - HIGH N
Methyltetrahydrophthalic anhydride (MTHPA)						++	YES - HIGH N
Phtalic anhydride (PA)	+	39.5	-	-	+	++	YES - HIGH N
Piperazine	-	not reactive			-	++	YES - HIGH N
Plicatic acid							YES - HIGH N
Toluene diisocyanate	-	0.4	+			++	YES - HIGH N
Trimellitic anhydride (TMA)	+	39.7	-	+	+/-	++	YES - HIGH N
2,4-Dichloro-5-chlorsulfonyl-benzoic acid							YES - LOW N
7-Aminocephalosporanic acid							YES - LOW N
Ammonium persulfate					+	++	YES - LOW N
Ampicillin							YES - LOW N
Cefadroxil							YES - LOW N
Cefteram pivoxil							YES - LOW N
Chloramine T			+	+	+	++	YES - LOW N
Formaldehyde	-	0.2		+			YES - LOW N
Hexamethylene diisocyanate	-	0.4	+	+	+	++	YES - LOW N
Pauli's reagent (4-diazobenzenesulphonic acid)							YES - LOW N
Phenylglycine acid chloride							YES - LOW N
Piperacillin							YES - LOW N
Potassium dichromate			+	+			YES - LOW N
Thiamphenicol							YES - LOW N
1,1,3-Tributylthiourea (TBTU)							YES - LOW N

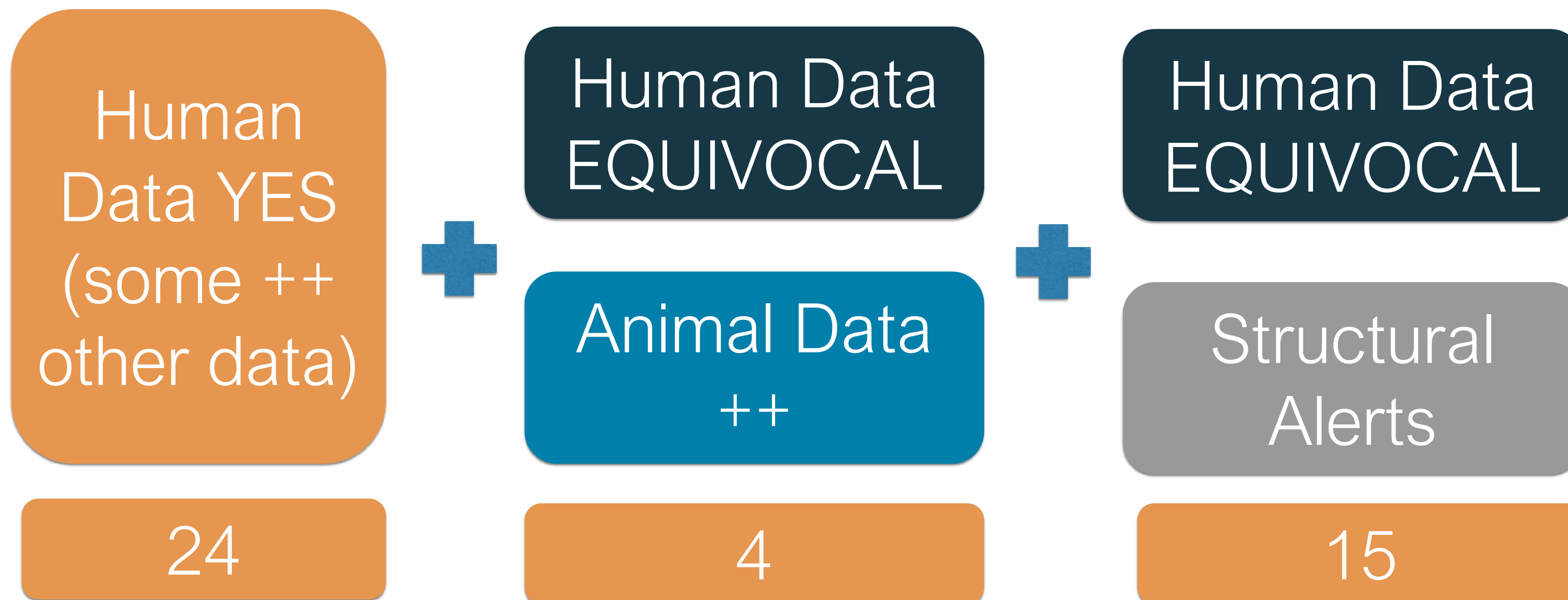
Full List Decision Basis

Is this chemical a probable
respiratory sensitizer based on
human and other evidence?



Full List Decision Basis

Is this chemical a probable
respiratory sensitizer based on
human and other evidence?



Equivocal + WOE

Chemical	OECD QSAR TB Respiratory sensitization	DPRA indicates RS	Other in vitro	Animal	Human
POSITIVE (DATA + SA EVIDENCE)					
3-Amino-5-mercapto-1,2,4-triazole	No alert found			++	EQUIVOCAL
Diphenylmethane-4-4' diisocyanate	Acylation >> Isocyanates and related >> Di-isocyanates			++	EQUIVOCAL
Isophorone diisocyanate	Acylation >> Isocyanates and related >> Di-isocyanates		+	++	EQUIVOCAL
Maleic anhydride	Acylation >> Ring opening acylation at a carbonyl >> Anhydrides	+	-/+	++	EQUIVOCAL
1,5-Naphthalene diisocyanate	Acylation >> Isocyanates and related >> Di-isocyanates				EQUIVOCAL
5-Aminosalicylic acid	Pro-Michael Addition >> Pro-quinone and related >> Aminophenols				EQUIVOCAL
6-Aminopenicillanic acid	Acylation >> Ring opening acylation at a carbonyl >> Lactams				EQUIVOCAL
Biuret of hexamethylene diisocyanate	No alert found;;Acylation >> Isocyanates and related >> Di-isocyanates				EQUIVOCAL
Ceftazidime	Acylation >> Ring opening acylation at a carbonyl >> Lactams				EQUIVOCAL
Cephalexin	Acylation >> Ring opening acylation at a carbonyl >> Lactams				EQUIVOCAL
Chlorendic anhydride	Acylation >> Ring opening acylation at a carbonyl >> Anhydrides				EQUIVOCAL
Ethylenediamine	Pro-Schiff base formation >> Pro-cross linking Schiff base >> Ethylenediamines	-	+		EQUIVOCAL
Eugenol	Pro-Michael Addition >> Pro-quinone-methide >> 4-Allylphenols		+	EQUIVOCAL	EQUIVOCAL
Himic anhydride	Acylation >> Ring opening acylation at a carbonyl >> Anhydrides			EQUIVOCAL	EQUIVOCAL
Hydroquinone	Pro-Michael Addition >> Pro-quinone and related >> Hydroquinones		+	EQUIVOCAL	EQUIVOCAL
P-phenylenediamine	Pro-Michael Addition >> Pro-quinone and related >> Phenylenediamines		+	EQUIVOCAL	EQUIVOCAL
Benzylpenicillin (Penicillin G)	Acylation >> Ring opening acylation at a carbonyl >> Lactams		+	EQUIVOCAL	EQUIVOCAL
Tetrachlorophthalic anhydride (TCPA)	Acylation >> Ring opening acylation at a carbonyl >> Anhydrides	+			EQUIVOCAL
Triphenylmethane triisocyanate	Acylation >> Isocyanates and related >> Di-isocyanates				EQUIVOCAL

Total currently positive chemicals: 43

Equivocal + WOE

Compound	Occupational Asthma	Specific IgE/IgG	Confounders	Human Data	Animal Data	Structural Alerts
Maleic anhydride	(+) history for asthma (+) specific bronchial challenge	untested	phthalic acid, controlled (negative bronchial challenge)	Equivocal	++	Ring opening acylation at a carbonyl >> Anhydrides
1,5-Naphthalene diisocyanate	(+) history for asthma and allergic alveolitis (+) specific inhalative challenge	(-) sIgE, sIgG	none	Equivocal	none	Acylation >> Isocyanates and related >> Di-isocyanates
Eugenol	(+) history for asthma and rhinitis (+) specific inhalation challenge	nonspecific IgE elevated	perfume	Equivocal	+	Pro-quinone, hydroquinone
P-phenylenediamine	(+) history for asthma and rhinitis (+) specific inhalation challenge	unclear	hair dyes	Equivocal	+	Pro-quinone and related >> Phenylenediamines
Benzylpenicillin (Penicillin G)	(+) history for asthma (+) specific bronchial challenge	untested	none	Equivocal	+	Ring opening acylation at a carbonyl

Reference List Characteristics

Positive set:

- Major mechanistic domains represented ✓
- Pro- and pre-haptens, volatile, soluble, & insoluble chemicals ✓
- Human Data available ✓
- Chemicals representative of sector- or agency-specific needs, and representing use scenarios of concern >>>

Negative set:

- Dermal sensitizers
- Respiratory Irritants >>>
- Non-sensitizers

Next steps

- Create criteria for tiered WOE
- Refine additional equivocal chemicals
- Widen the net
- Determine Negative chemicals
- Publish List
- Update OECD Toolbox Profiler
- Test candidate NAMs

Next steps: Refine Equivocals

- WOE: Could chemicals with A) respiratory effects but not clear sensitization effects (perhaps because of no IgE detection or testing) AND 2) clear sensitization potential from other assays be considered positive?
- Pattern recognition: Are any positive and equivocal chemicals tested in ToxCast and can we learn anything from those assays, which might align with the Key Events from the AOP?
- Structure-based patterns: Can read across and other structural similarity techniques be employed to refine equivocal chemicals?

Next steps: Find additional chemicals

- Using *Abstract Sifter* selected the top ten chemicals with High N positive data and identified associated key words within PubMed: asthma, respiratory hypersensitivity, and bronchial hyperreactivity
- Retrieved any chemical tagged with one of these three terms: 583 chemicals
- Sorted by additional relevant key words for top 10 chemicals
- Chemicals will then need to be evaluated for available data

MeSH Chemical Name (hyperlinked)	Asthma	Respiratory	Hyperbaric Hyperventilation	Antibody Formation	Antibody Specificity	Antigen Presentation	Basophils	Erythrocytes	Immunoglobulin E	Immunoglobulin G	Lymphocyte Activation	Lymphocyte Binding Kinetics	Time Factors	Pulmonary Alveoli	Bronchi	Lung	Trachea	
Latex	40	20	2	0	4	0	1	0	23	2	0	0	1	0	4	0	5	0
Chlorine	30	6	10	0	0	0	0	0	2	0	0	1	0	0	10	4	12	0
Ethanol	17	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	0
rosin	17	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Tartrazine	16	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
Polyvinyl Chloride	15	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	0
Ammonium Sulfate	14	1	6	0	0	0	0	0	2	0	1	1	0	0	1	0	1	0
Nicotine	13	0	1	0	0	0	0	0	1	1	0	0	0	0	1	2	7	3
sodium metabisulfite	12	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
phthalic acid	12	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Methacholine Chloride	11	4	7	0	1	0	2	0	15	0	0	0	0	0	37	63	46	8
isocyanic acid	11	3	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0
Psyllium	11	2	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0
Chromium	10	4	1	0	1	0	0	0	1	0	0	2	0	0	1	0	1	0
metabisulfite	10	4	0	0	0	0	0	0	2	0	0	0	0	0	0	1	1	0
Tea	10	0	0	0	0	0	0	0	5	2	0	0	0	0	0	1	3	0
Carmine	9	1	0	0	1	0	0	0	7	1	0	0	0	0	0	0	0	0
bisphenol A	8	2	4	0	0	0	0	0	2	0	0	0	0	0	1	0	6	0
Folic Acid	8	0	0	0	0	0	0	0	4	0	0	1	0	0	0	0	4	0
2,6-diisocyanatotoluene	7	2	1	0	1	0	0	0	3	3	0	0	0	0	2	0	2	0
potassium persulfate	7	1	1	0	0	0	0	0	2	0	0	1	0	0	2	0	0	0

Next steps: Determine Negatives

Negative set:

- Non-sensitizers: From OECD reference lists or literature
- Dermal sensitizers: From OECD reference lists or literature
- Respiratory Irritants: Our Analysis + Literature

Summary

- We have defined 43 probable respiratory sensitizers based primarily on human clinical data
- Additional animal and structural information contributed to these determinations
- Large set of chemicals for which the human data remains equivocal; we are pursuing strategies to further refine
- Testing will proceed based on most certain positive chemicals, considering chemical properties, practical issues, and coverage
- We were not able to confirm chemicals from this list were not respiratory sensitizers based on human data

Feedback Welcome! (acknowledgements)

Human Data Search & Eval.

- Jessica Ponder, PCRM
- Ramya Rajagopal, Unilever
- Madhuri Singal, AeroTox Consulting
- Stella Cochrane, Unilever

In vitro data Search & Eval.

- Raja Settivari, Dow Chemical Company
- Erwin L. Roggen, 3Rs Management & Consulting

ksullivan@pcrm.org

Animal data Search & Eval.

- Janine Ezendam, RIVM
- Katherina Sewald, Fraunhofer ITEM

In silico data, structural alerts, Abstract Sifter, phys/chem, ToxCast, read across

- Steven J. Enoch, Liverpool John Moores U.
- Grace Patlewicz, US EPA
- Nancy Baker, US EPA

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