Pathophysiology of Respiratory Sensitization: Use of Clinical Hallmarks Combined with Adverse Outcome Pathway to Inform the Development of New Approach Methodologies (NAMs) to Identify Chemical Respiratory Allergens

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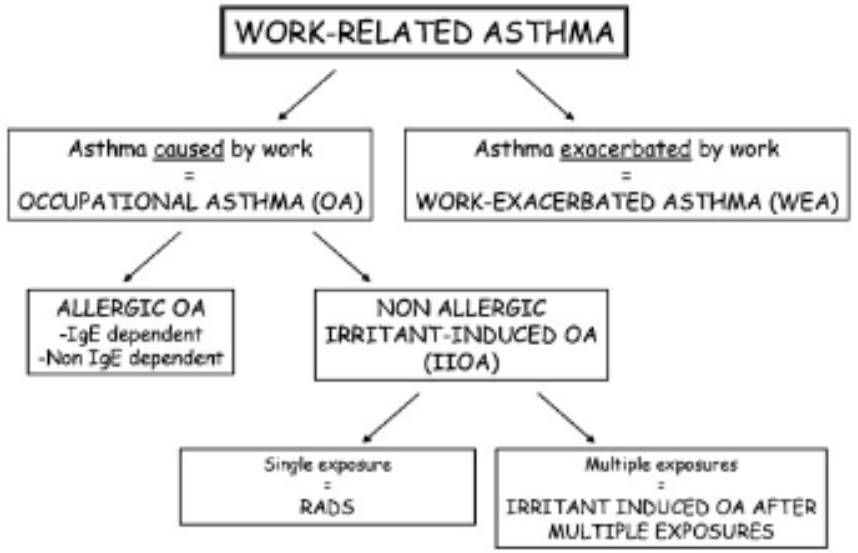
Chemical Respiratory Allergy (CRA)

- The disease develops in 2 phases

- Work exposure to low molecular weight (LMW) chemicals and occupational asthma
- Potential for environmental exposure and risk to consumer health

- Chemicals can cause asthma by both immunological and non-immunological mechanisms
- Classification of work-related asthma



WAO- Diagnosis of Occupational Asthma

- Diagnosis of underlying mechanism is not possible purely based on clinical symptoms
  - Lack of accurate diagnosis results in several challenges; e.g. clinical, regulatory

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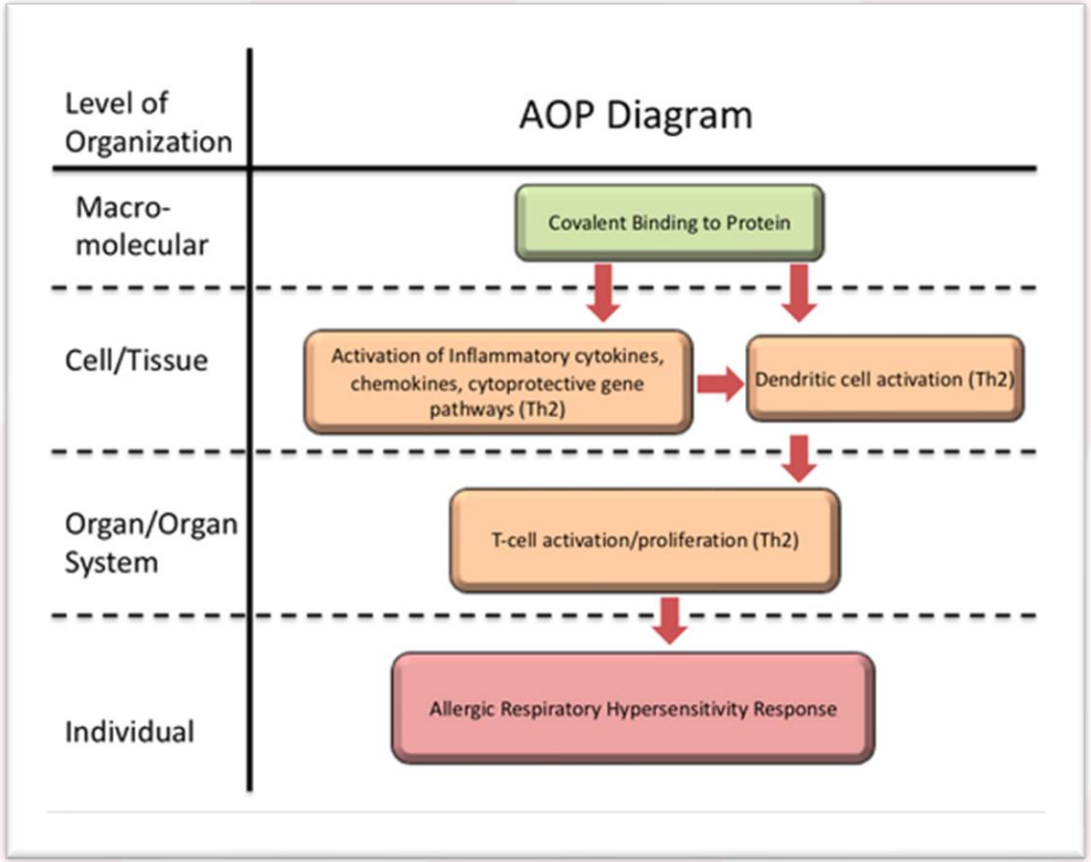
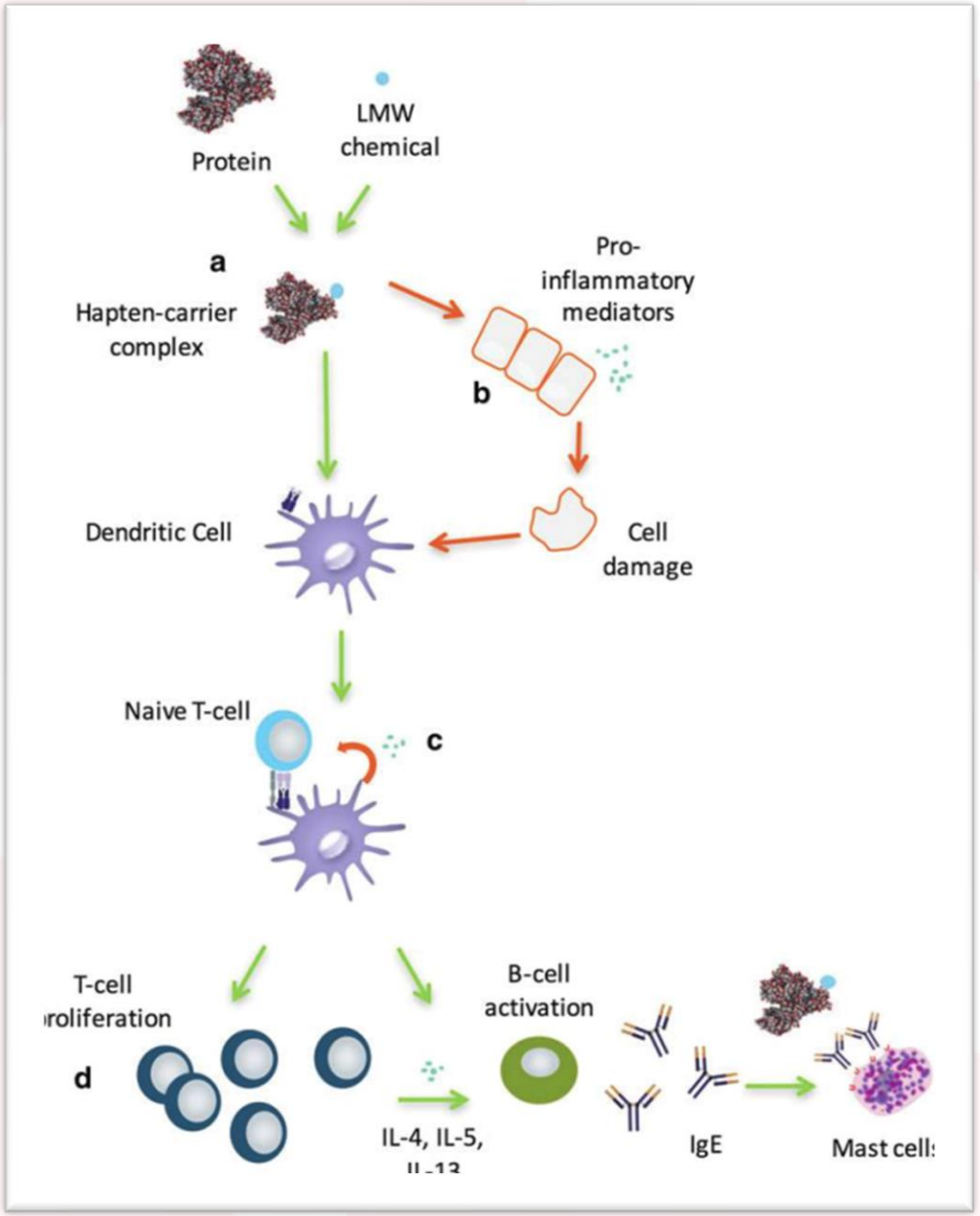
Adverse Outcome Pathway (AOP) for Sensitisation of the Respiratory Tract





[AOP-Wiki; AOP 39](#)
Sullivan et al. APPLIED IN VITRO TOXICOLOGY. 2017, Volume 3, Number 3

Clinical Challenges

- Subjects sensitised by immunological mechanism may differ from subjects with asthma occurring through non-immunological mechanisms

  Immunological mechanism
  - Some individuals may be highly sensitive to allergenic substance
  - Thresholds for elicitation of the allergic reaction may vary with time and between sensitised individuals
  - Lower levels of exposure
  - Occurrence of latency

  Non-immunological mechanism
  - There would not be the same concern about increasing levels of sensitivity associated with allergic responses
  - Much less variation in threshold levels would be expected
  - Higher exposure levels
  - Usually no latency

- There is a potential for cross-sensitization between structurally similar allergens (e.g. for some diisocyanates)

- While one of the approaches to manage occupational asthma (OA) is removing the subject from the work-related exposure, this may not always be helpful or possible especially if there is additional environmental exposure or exposure to cross-reactive allergens. Therefore, a robust diagnosis of the underlying condition is critical for management and prevention of the disease

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Regulatory Challenges

- Respiratory sensitisers are identified as substances of very high concern (SVHC) as perArticle 57(f) of REACH Regulation and hence, are subject to authorisation

- The EU Classification, Labelling and Packaging (CLP) Regulation 7 criterion for a substance to be identified as a respiratory sensitiser is ***a substance that will lead to hypersensitivity of the airways following inhalation of the substance***

Hazard category and sub-categories for respiratory sensitisers	
Category	*Criteria*
Category 1	*Substances shall be classified as respiratory sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:* *(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and /or* *(b) if there are positive results from an appropriate animal test.*
Sub-category 1A:	*Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered.*
Sub-category 1B:	*Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered.*
(1) At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.	

- However, whether it is human evidence or animal data, the immunological mechanism does not have to be demonstrated

Annex I: *3.4.2.1.2.1.* *Evidence that a substance can lead to specific hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. ~~The condition will have the clinical character of an allergic reaction.~~ However, immunological mechanisms do not have to be demonstrated.*

Annex I: *3.4.2.1.3.1.* *Data from appropriate animal studies (*) which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans (**) may include:*

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice;

(b) specific pulmonary responses in guinea pigs.

(*) At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.

(**) ~~The mechanisms by which substances induce symptoms of asthma are not yet fully known.~~ For preventative measures, these substances are considered respiratory sensitisers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyper reactivity, they should not be considered respiratory sensitisers.

- Over-regulation of chemicals, implications of EU Green Deal on respiratory sensitisers

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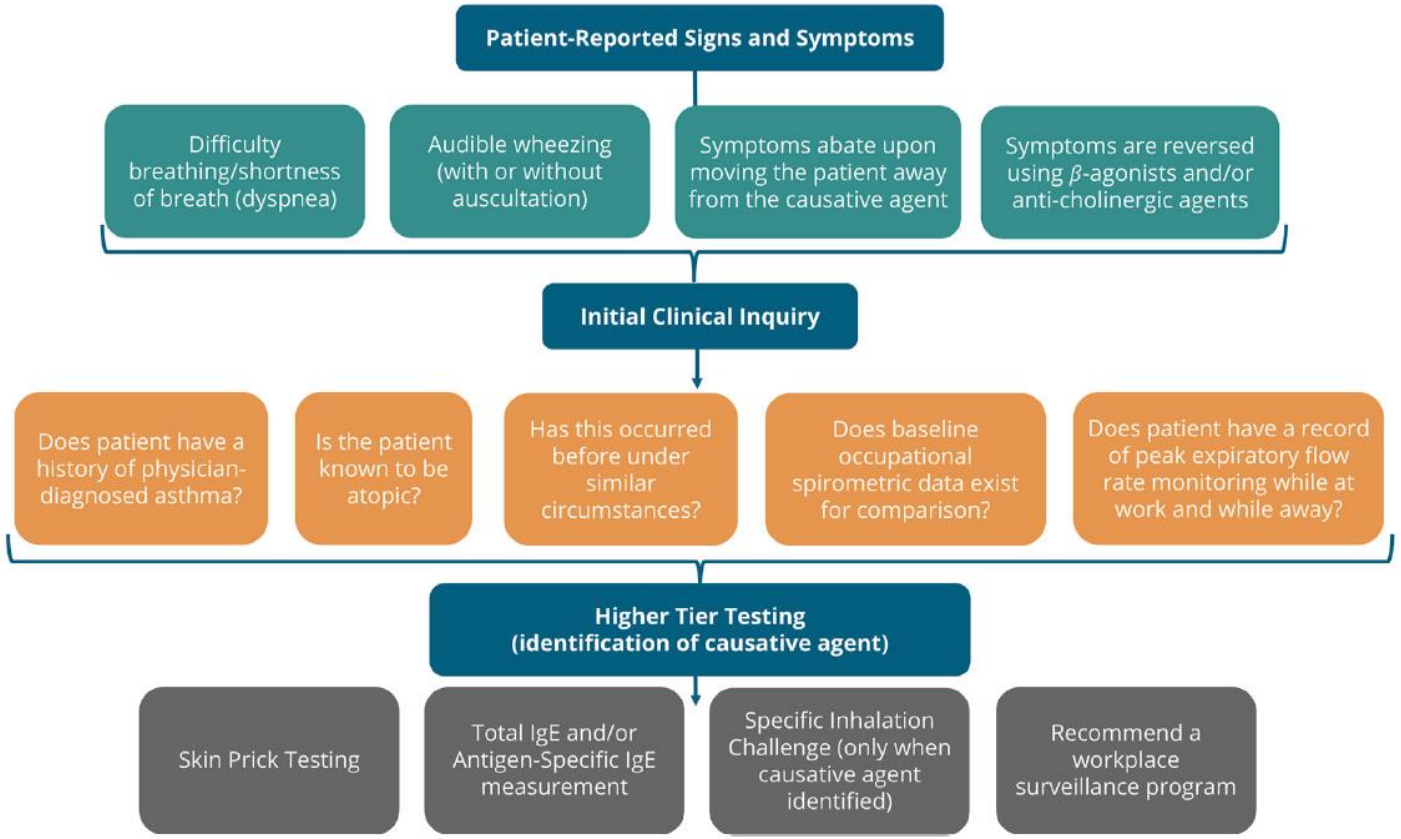
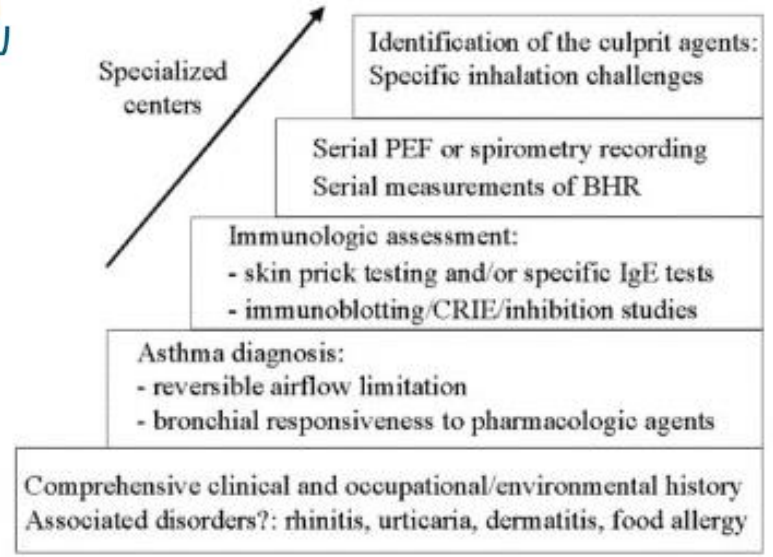
Clinical Investigation of Occupational Asthma



Ponder et al. Frontiers in Toxicology. July 2022, Volume 4, Article 916370



WAO- Diagnosis of Occupational Asthma

Clinical Data & Tests

- Work history and questionnaires – Clinical history focussing on circumstances of the onset of asthma symptoms, severity and persistence of asthma, the temporal relationships between exposures at work and disease exacerbation, the clinical course of asthma, as well as known triggers; can lack specificity

- Immunological testing –
  - Skin prick test (SPT) is the primary mode of skin testing for IgE mediated allergy; it is safe and can be sensitive if standardised hapten conjugates are available
  - Serological tests include measuring specific IgE through radio-allergosorbent assay (RAST) or enzyme-linked immunosorbent assay (ELISA)

  Often, lack of standardised preparations make immunological testing challenging for LMW compounds

- Physiological tests-
  - Non-specific inhalation challenge- In the absence of airflow limitation, bronchial hyperresponsiveness can be confirmed by methacholine or histamine challenge, measuring decrease in forced expiratory volume in 1 second (FEV_1)
  - Serial peak expiratory flow (PEF) monitoring – Prolonged recording of PEF or spirometry for periods at work and off work is a valuable diagnostic tool in OA; although the test results cannot be used alone; challenging to get the subject to cooperate
  - Specific inhalation challenge in laboratory – Regarded as 'gold standard' in diagnosis of OA; specific compound in question is administered in the inhalation challenge and FEV_1 is monitored (≥ 15% decrease considered as positive)
  - Workplace challenge with serial FEV1 measurements – If negative, rules out OA; a positive test may not rule out irritation

- Inflammatory parameters
  - Induced sputum parameters – Eosinophils in sputum, non-invasive, direct evidence of inflammation
  - Fraction exhaled nitric oxide (FeNO) – Measurement of NO as an inflammatory marker

Only specific IgE can be a standalone indicator of sensitisation. Other tests need to be used in combination.

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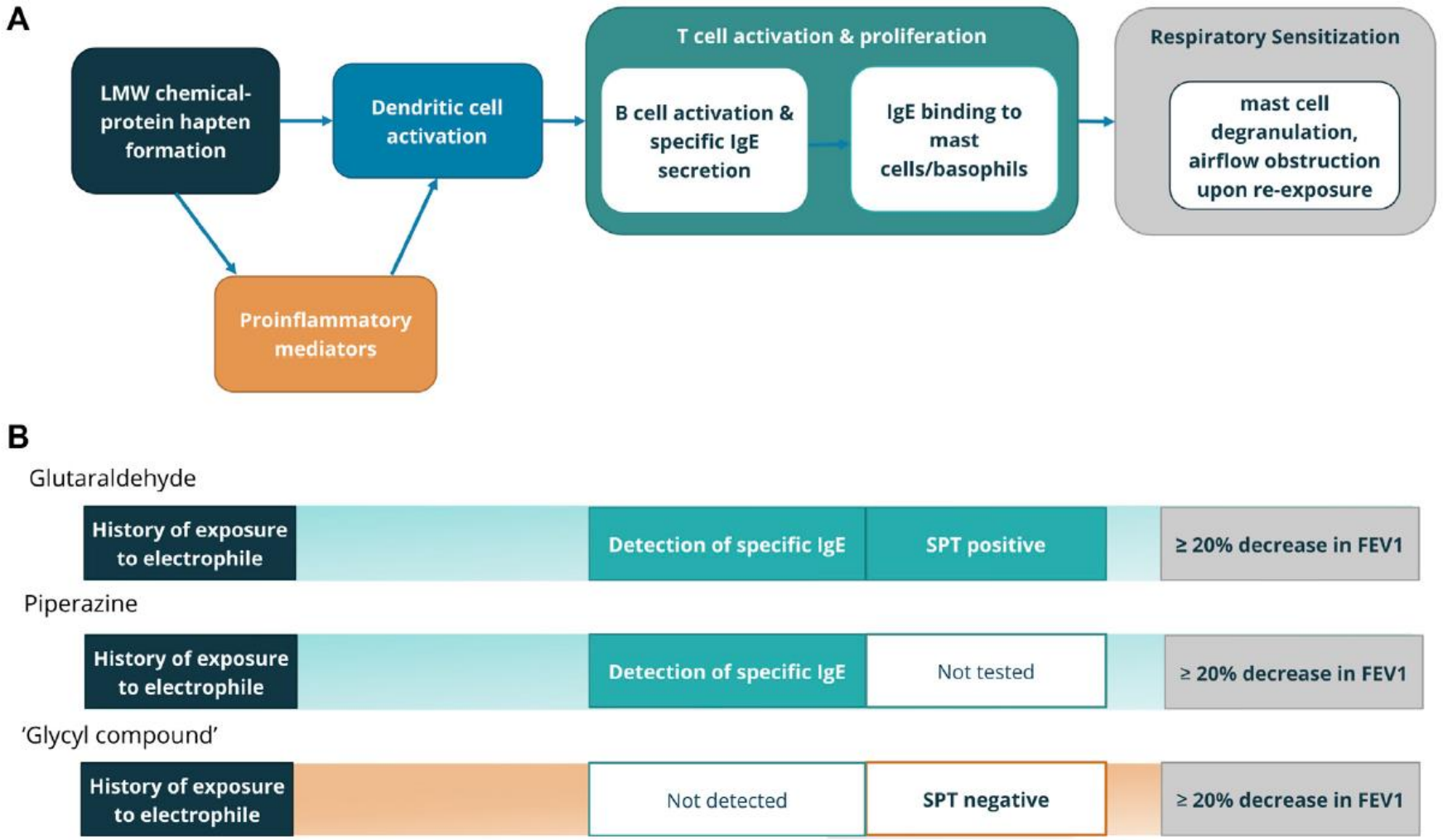
Proposed Criteria for Determining Respiratory Sensitisers using Clinical Data



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Proposed Criteria for Determining Respiratory Sensitisers using Clinical Data

Table 1. Summary of criteria for data evaluation and categorization of reported respiratory sensitizers.

Evidence/data category	Compelling evidence	Reasonable evidence	Inadequate evidence	Questionable evidence
Chemical exposure	Exposure is CONFIRMED by: 1. Documentation of exposure AND 1. Measurement of exposure in environment	Exposure is SUPPORTED by either: 1. Documentation of exposure OR 1. Measurement of exposure in environment But there is a possibility of interference due to other chemical exposures at the same facility	Insufficient data on exposure to responsible chemical OR exposure cannot be confirmed	No documented evidence of exposure AND Missing any alternative assessment to confirm exposure
Exposure route	Confirmation of inhalation exposure by expert analysis of patient documentation.		Suspected inhalation exposure OR Inhalation exposure is not confirmed by other means	No documented evidence of inhalation exposure although inhalation exposure possible
Symptom evaluation	Documented confirmation of a reaction by a medical professional including using pulmonary function testing, skin observations, serum IgE, OR other forms of medically supervised observations during initial presentation.		No medical confirmation of symptoms OR reported by patient	No medical evaluation OR documented symptoms based only on a questionnaire OR Symptoms only patient reported
Confirmatory tests	Documented by a medical professional including using pulmonary function testing, patch tests, skin prick tests, serum IgE, OR other forms of medically supervised observations during initial presentation or re-challenge		No confirmation or evaluation by a medical professional	No existing confirmatory tests
Medical history	No prior incidents of atopy or respiratory issues in majority of subjects	Presence of atopy or respiratory issues in majority of subjects that may complicate exposure responses	Prior incidents of atopy or respiratory issues	
Subjects evaluated in reports	Multiple subjects at multiple facilities OR Multiple subjects in a single facility		Single individual OR reports of single individuals in unrelated scenarios	
Subject age	Adult			

Sadekar et al. Critical Reviews in Toxicology. 2022.

Proposed Criteria for Determining Respiratory Sensitisers using Clinical Data

Hypothesis tested: "Methyl methacrylate (MMA) can cause the development of OA in subjects that were not previously asthmatic."

Table 6. Confidence score in adapted Bradford Hill criteria for case studies.

Adapted BH criteria	Confidence score	Justification
Strength	+	There were no K1 or K2 studies identified. All studies lacked essential details on workplace exposure to assert with confidence that MMA and not other chemicals used was the causative agent.
Consistency	+	The prevalence in orthopaedic and dental sectors is extremely low and inconsistent with other recognised respiratory sensitisers.
Specificity	++	These associations are more likely to be due to the known irritant property of MMA vapour triggering asthma-like symptoms in an asthmatic.
Temporality	++	It is claimed that the OA developed after exposure to MMA but this is not substantiated with evidence. Insufficient data upon which to score.
Biological gradient	+	Insufficient data upon which to score.
Plausibility	+	The causal link between MMA and development of OA is not established neither is an immunological MOA. Observed effects could equally be due to irritation.
Coherence	+	Irritation is known to trigger asthma in asthmatics and could be equally responsible for the pattern of disease.
Experiment		N/A
Analogy	++	There is some evidence that HEMA may be linked with OA in dentists. However, the numbers are small and exposures are mixed so it is uncertain whether this is causally related or coincidental elicitation due to irritation.

MMA: methyl methacrylate; N/A: not applicable; OA: occupational asthma; HEMA: hydroxyethyl methacrylate.

Data reviewed, Bradford Hill (BH) criteria & confidence score applied:

- Worker health studies
- Case study records
- Quantitative Structure-Activity Relationship (QSAR) analyses
- Exposure data
- European National Surveillance data

Table 11. Overall level of confidence in the six lines of evidence.

Adapted BH criteria	Worker health studies	Case studies	QSARs	Exposure	National surveillance data	SIC test data
Strength	++++	+	++	+++	+	+
Consistency	++++	+	+	+++	+	++
Specificity	++++	++	++	++++	+	+
Temporality	++++	++	N/A	N/A	+	++
Biological gradient	+++	+	N/A	N/A	+	+
Plausibility	+++	+	++	N/A	+	++
Coherence	+++	+	+	+++	+	+
Experiment	N/A	N/A	N/A	N/A	N/A	N/A
Analogy	N/A	++	N/A	N/A	N/A	++
Overall level of confidence	++++	+	+	+++	+	+

QSAR: quantitative structure–activity relationship; SIC: Specific Inhalation Challenge.

Pemberton & Kimber. 2022. Critical Reviews in Toxicology, 52:2, 139-166

Learning from Exposure Data to Distinguish Between the Pathological Mechanisms

Latency:

- Single short-term accidental massive exposure or several short-term high-level exposures to a respiratory irritant can cause asthma without a latency period, defined as reactive airways dysfunction syndrome or RADS
- Low-dose irritant asthma (or "low-dose RADS") where respiratory effects including asthma occur from mainly chronic or repeated exposure to a single irritant or mixture without evidence of IgE mediated mechanism; susceptibility group (e.g. with pre-existing non-specific bronchial hyperreactivity/ NSBHR)
- Latency period is hallmark of sensitiser induced asthma
- A systematic review of literature and analyses using the Scottish Intercollegiate Guideline Network (SIGN) grading system and the Royal College of General Practitioners (RCGP) three-star system, identified agents or worksites that cause allergic as well as irritant occupational asthma (Bauer et al. Journal of Occupational Medicine and Toxicology 2013, 8:15)

Levels:

- Data pointing to high historical exposure to MMA; mean as high as 420 mg/m3 (8-h TWA) and peaks well in excess of 1000 mg/m3; in the acrylic sheet industry (Pemberton & Kimber. 2022. Critical Reviews in Toxicology, 52:2, 139-166)
- Measurements from workplaces using isocyanates indicate levels in ppb (Middendorf et al. JOEM Volume 59, Number 12S, December 2017; Plehiers et al. Toxicology and Industrial Health 2020, Vol. 36(11) 876–884; Plehiers et al. Toxicology and Industrial Health 2020, Vol. 36(11) 885–891)

A systematic comparison of occurrence of latency and/or airborne levels leading to allergic symptoms for well known LMW sensitisers and irritants would be valuable

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Importance of Dermal Route of Exposure

- Lipophilic LMW chemicals, including various vapours, gases, liquids, and aerosols, can be absorbed through the skin
- Loss of skin barrier function and allergen sensitisation via the skin, e.g. filaggrin loss-of-function, over-expression of TSLP
- Sensitisation of the respiratory tract to chemical respiratory allergens can occur through skin exposure, e.g. isocyanate; substantial evidence from animal studies and limited human epidemiological data
- A role of cutaneous sensitisation to isocyanates and considering appropriate dermal protective measures is critical, despite the wide use of respiratory protection measures at workplace and individual level, the risk of occupational asthma by isocyanate has plateaued and not further declined (Coureau et al. Int. J. Environ. Res. Public Health 2021, 18, 13181)
- Cutaneous measurements for exposure monitoring, such as using tape-strip sampling, can be challenging (Kent et al. Scand J Work Environ Health 2006;32(3):225–231)

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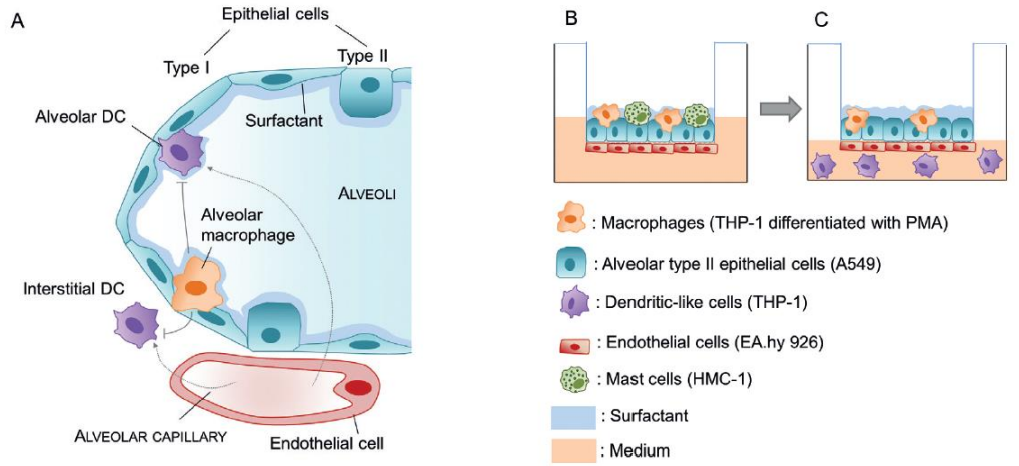
New Approach Methodologies (NAMs)

- Exposure and clinical data can be limiting for newer compounds & hence, the need for NAMs
- However, lack of fit-for-purpose, widely accepted NAMs to identify respiratory sensitisers; not all NAMs developed to identify skin sensitisers can determine respiratory sensitisers

- Direct peptide reactivity assay (DPRA) (OECD TG442C)– As both chemical skin and respiratory sensitisers need to bind a protein, the DPRA could be used for the identification of LMW respiratory sensitisers as well (some exceptions, e.g. piperazine, ethylene diamine)

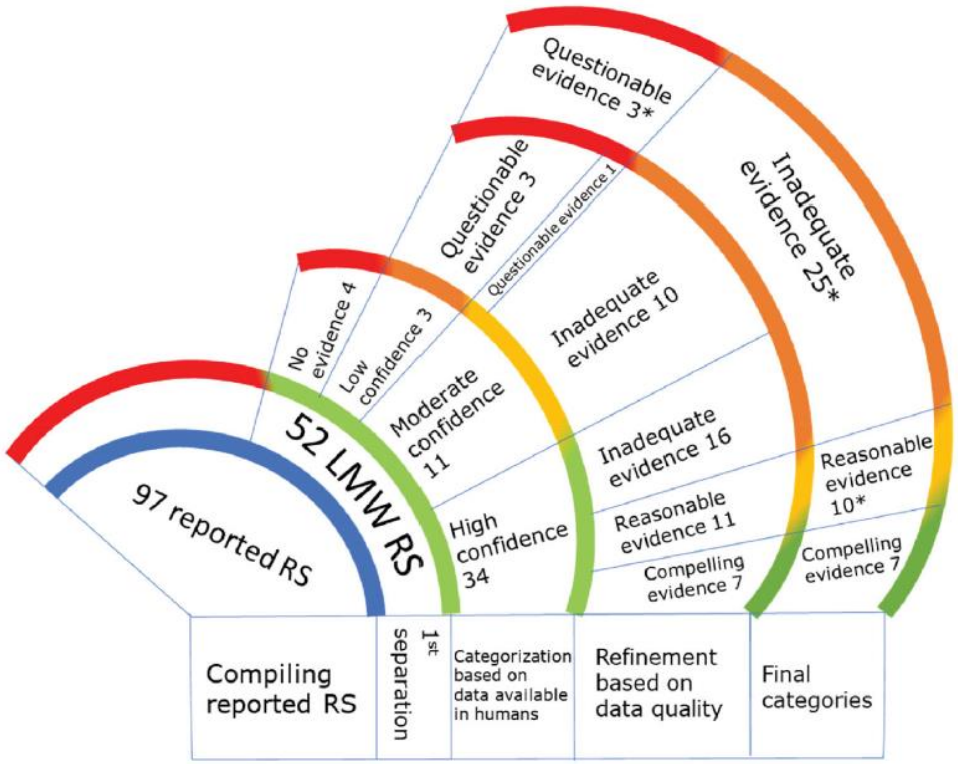
- Human cell line activation test (h-CLAT) (OECD TG442E) – Only few respiratory sensitisers tested; false positives or negative results (e.g. phthalic anhydride); may need inputs from epithelial cells

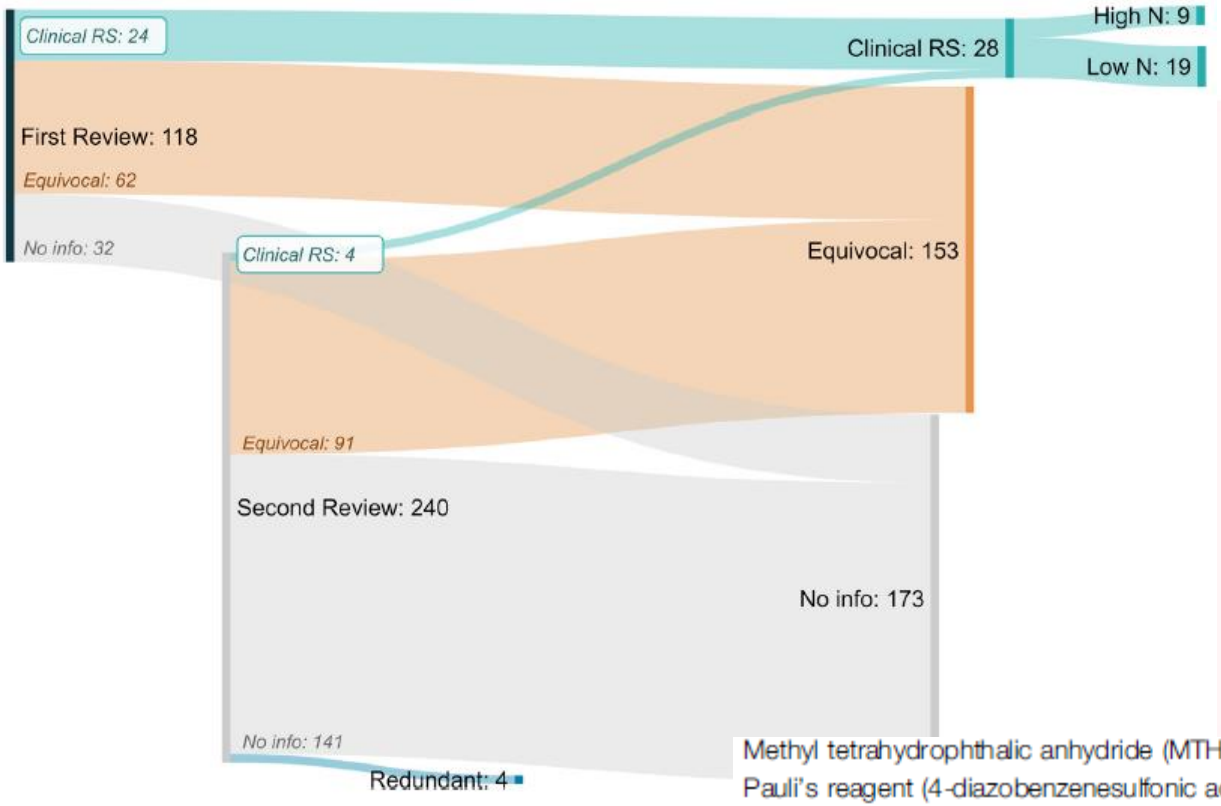
- 3D *in vitro* ALI model – Alveolar type II epithelial cells (A549), endothelial cells (EA.hy926), macrophage-like cells (PMA-differentiated THP-1), and dendritic-like cells (non-differentiated THP-1); measuring cell surface markers, cytokines and gene expression to identify respiratory sensitisers (Chary et al. 2019. ALTEX 36(3), 403-418)



- Genomic Allergen Rapid Detection (GARD)® assay (GARD®air) – Using the MUTZ-3 cells, by testing 10 respiratory sensitisers and 20 non-respiratory sensitisers, identified a biomarker signature containing 389 differentially regulated genes; accuracy, sensitivity and specificity of 84%, 67%, and 89%, respectively (Forerryd et al. March 2015, PLOS ONE)

Reference Compounds to Validate NAMs





Category 1 – Compelling evidence
Chloramine T
Diphenylmethane diisocyanate
Hexahydro phthalic anhydride
Hexamethylene diisocyanate
Piperazine
Toluene diisocyanate
Trimellitic anhydride

Chemical name
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
2,4-dichloro-5-chlorsulfonyl-benzoic acid
7-aminocephalosporanic acid
Ammonium hexachloroplatinate
Ammonium persulfate
Ampicillin
Carmine
Cefadroxil
Cefteram Pivoxil
Chloramine-T (Sodium p-toluenesulfonylchloramide)
Formaldehyde†
Glutaraldehyde†
Hexahydrophthalic anhydride (HHPA)
Hexamethylene diisocyanate (HDI)
Menthol
Methylene diphenyl diisocyanate (MDI)
Methyl tetrahydrophthalic anhydride (MTHPA)
Pauli's reagent (4-diazobenzenesulfonic acid)
Phenylglycine acid chloride
Phthalic anhydride (PA)
Piperacillin
Piperazine
Plicatic acid
Potassium dichromate
Thiamphenicol
Toluene diisocyanate (TDI)
Trimellitic anhydride (TMA)

Sadekar et al. Critical Reviews in Toxicology. 2022.

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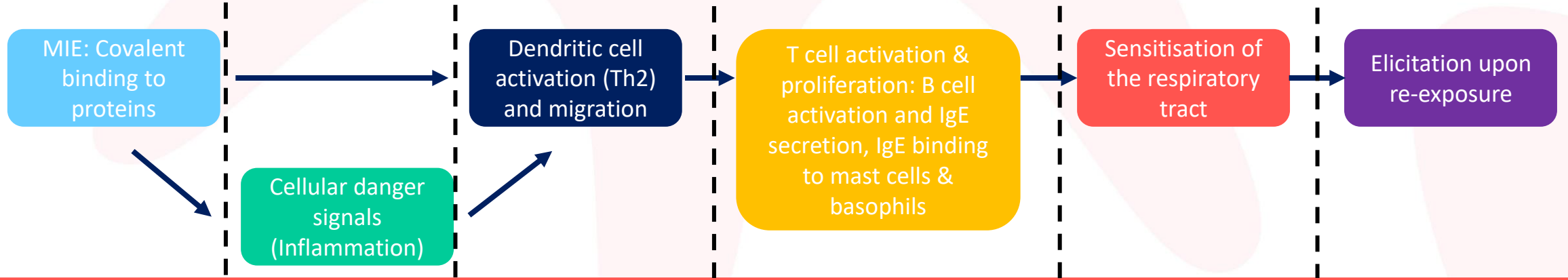
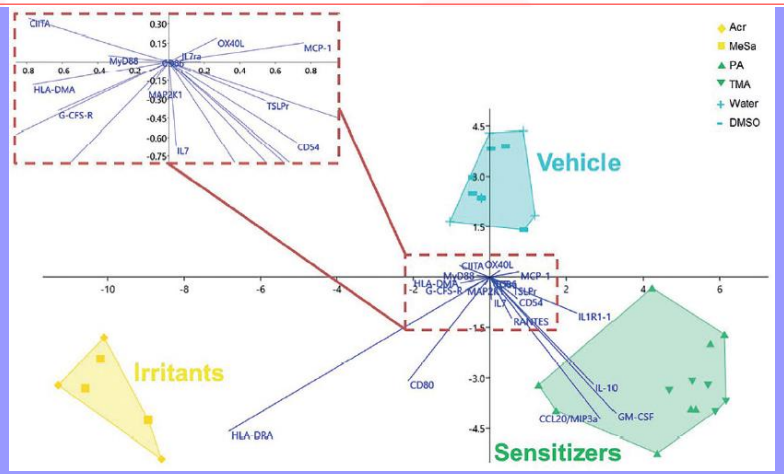
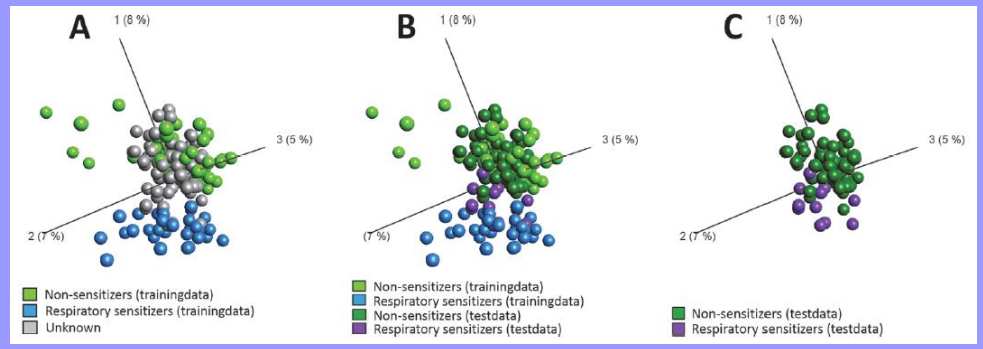
Using AOP to Combine Exposure, Clinical Data and NAMs in an Integrated Approach to Testing and Assessment (IATA)



Clinical Signs	None	None	None	None	None	Asthma, rhinitis, conjunctivitis
Data & Assays	History of exposure, Patterns & Levels, Causal relationship, QSAR, DPRA	Th2 cytokines, 3D in vitro ALI model, GARD®air	hCLAT, 3D in vitro ALI model, GARD®air	Specific IgE, SPT		History of exposure, Causal relationship, SIC, Specific IgE, SPT, Inflammatory markers (sputum eosinophils/ neutrophils, FeNO)



Dosimetry/ Dose Response



Applying the CRA IATA to Novel Compounds Lacking Exposure & Clinical Data

Reference list of compounds for testing NAMs

- Respiratory sensitisers
- Respiratory irritants
- Compounds with both properties, curated using clinical data

Using CRA IATA and specific criteria for differentiating sensitisers from non-sensitisers

- Distinction based on difference in biomarker read-outs
- Differences in dose-response
- Case studies to validate the criteria

Novel compounds lacking clinical data

- Apply biomarker and dose criteria to determine potential for CRA

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Next Steps, Opportunities, and Outlook

- Robust diagnosis of immune-mediated CRA; harmonized framework/ criteria for integrating clinical data into the diagnosis
- Opportunity for toxicologists and clinicians to collaborate, maximize learnings from clinical experience, make available certain types of data, build in standardisation in exposure & clinical data collection, and consider further unexplored or underutilized clinical evidence
- Further understand and characterise the dermal route of exposure contributing to sensitisation
- A systematic comparison of occurrence of latency and/or airborne levels leading to allergic symptoms for well known LMW sensitisers and irritants
- Define and test an IATA to identify compounds with potential to cause CRA by combining exposure and clinical data, NAMs data and insights from dosimetry; benchmark novel compounds as per the specific criteria defined by the IATA

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