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How to assess respiratory sensitisation?

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Definitions used in this presentation

Allergy is a hypersensitivity reaction initiated by immunologic mechanisms

Contact/respiratory allergy are hypersensitivity reactions in the skin/airways following dermal/inhalation exposure to an allergen, which is initiated by immunologic mechanisms induced by that specific allergen

Phase I: initiation = **sensitisation phase**, systemic, independent of route of exposure
Phase II: hypersensitivity reaction = manifestation of allergy upon **elicitation/challenge**, adverse, local

Asthmagen is a substance causing adverse respiratory effects by non-immunological mechanisms (Kimber et al., 2001)

Respiratory sensitisers
CLP- Regulation 1272/2008

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H317: May cause an allergic skin reaction

H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled

➨**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.**

In regulatory terms:

- There is a clear distinction between skin sensitisation resulting in allergic contact dermatitis (H317) versus irritant contact dermatitis that is not associated with, or dependent upon, allergic sensitisation (H315)

- A similar clear distinction between chemical respiratory allergens and asthmagens that induce effects through non-immunological mechanisms is long overdue

Important issues:

- Respiratory allergy can be severe/life-threatening

- No widely accepted or validated animal tests on respiratory sensitisation

- So far, classification only based on respiratory effects/complaints in humans

How to assess respiratory sensitisation?

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(a) In silico/in vitro?
(b) Experimental studies in animals
(c) Clinical/workplace observations and investigations

Methods for respiratory sensitisation and elicitation
(Arts et al., Crit Rev Tox, 2006)

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Species	Sensitisation method (airways)	Elicitation method (airways)	Parameters evaluated
Guinea pig	Multiple inhalation	Inhalation (hapten, protein, hapten-protein)	Airway responses
Guinea pig	1x inhalation	Inhalation (protein)	Airway responses
Rat	Multiple inhalation	Inhalation (hapten)	Airway responses
Guinea pig	1x Inhalation	-	Antibody
Guinea pig/rat	Multiple inhalation	-	Antibody
Guinea pig	Multiple intratracheal	-	Antibody

Note: also animal studies with respiratory sensitisation and dermal elicitation – evaluation of skin effects

How to assess respiratory sensitisation?

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Note: so far all known respiratory allergens have tested positive in a skin sensitisaton test (LLNA, GPMT, and/or Buehler test)

Question 1: Can the dermal sensitisation route be used to test for respiratory allergy?

Question 2: What about typical skin sensitisers in a respiratory test?

Methods for dermal sensitisation and inhalation elicitation

(Arts et al., Crit Rev Tox, 2006)

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Species	Sensitisation method (skin)	Elicitation method (airways)	Parameters evaluated
Guinea pig	1x ID	Inhalation (hapten-protein)	Airway responses
Guinea pig	1x ID	Inhalation (hapten)	Airway responses
Guinea pig	Multiple ID	Inhalation (hapten)	Airway responses/pathology
Rat	Multiple topical	Inhalation (hapten)	Airway responses/pathology
Guinea pig	1x ID	Intratracheal instillation	Airway responses, dye extravasation
Mouse	Multiple topical	Intratracheal instillation	Airway histopathology

Dermal exposure, and therefore sensitisation including of the respiratory tract, despite respiratory protection???

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LLNA – dermal/respiratory

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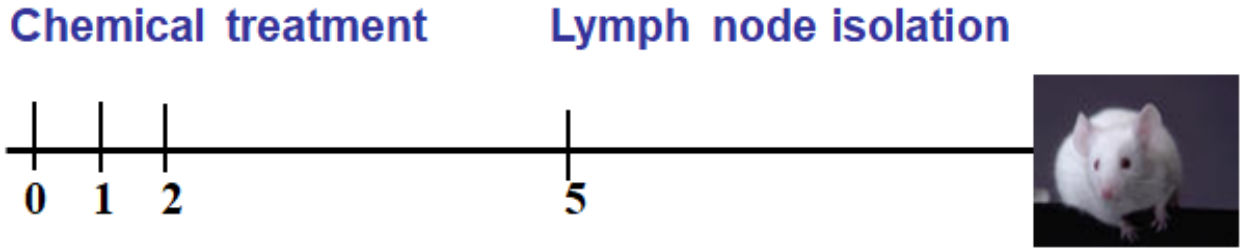


Days 0-1-2 treatment of male BALB/c mice by inhalation or dermal exposure

Day 5 isolation of draining lymph nodes and lymph node cells

In vitro labeling of lymph node cells with ^{3}H-thymidine (proliferation)

Determination of cytokine responses

Comparison reponses after skin (single dose) and inhalation exposures

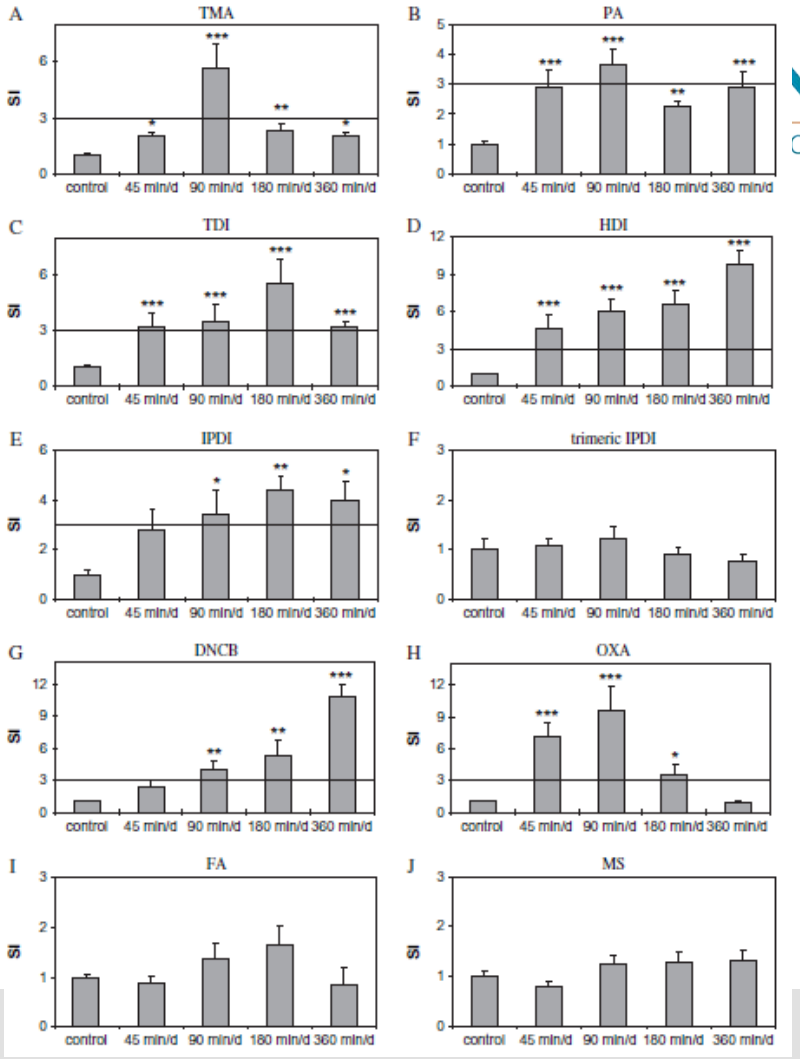
Respiratory LLNA

(Arts et al., Tox Sci, 2008)

	Target concentration (mg/m^3)[a]
TMA	30
PA	15
TDI	7.5 (ca. 1.0 ppm)
HDI	7.5 (ca. 0.9 ppm)
IPDI	7.5 (ca. 1.2 ppm)
Trimeric IPDI	15
DNCB	30
OXA	15
FA	3.6 (ca. 3.0 ppm)
MS	30

GA: 6/18 ppm (25/75 mg/m3); 90 min/day also negative in resp LLNA

(van Triel et al., 2011)



Dermal exposure, and therefore sensitisation including of the respiratory tract, despite respiratory protection!!!

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Animal testing strategy

- All known chemical respiratory allergens tested → positive responses in the LLNA, GPMT and/or Buehler (Dearman et al., 2013)

- They induce T cell activation and proliferation in regional lymph nodes following skin exposure → positive LLNA response

- Differential qualities of T cell response are not registered in the LLNA which simply measures the proliferation of all lymph node cells

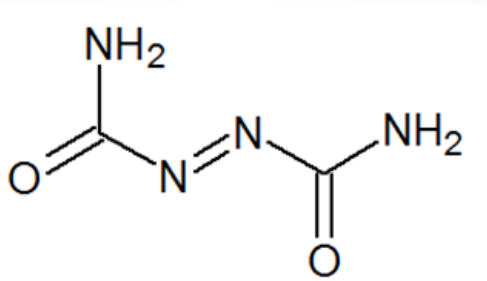
- It is assumed they also elicit positive responses in guinea pigs by provoking T cell responses that drive skin reactions following challenge of sensitised animals

Thus: chemicals that are negative in the dermal LLNA or GPMT/Buehler can be regarded as lacking not only skin sensitising activity, but also the potential to induce sensitisation of the respiratory tract

Human data – the ADCA case

Azodicarbonamide - C,C'-azodi(formamide)
EC 204-650-8 / CAS 123-77-3

Classified as respiratory sensitiser (H334)
but **not** as skin sensitiser (H317)



Animal tests:
Buehler - negative
LLNA - negative
GPMT - negative

Conclusion on animal data: no skin and respiratory sensitiser!

Documented cases – respiratory symptoms (mainly 1960 – 1985)

Data from factory plants: 4 publications

- 11 workers with symptoms in a grinding company (Ferris et al., 1977)
- 151 workers in ADCA production; 28 with symptoms (Slovak, 1981)
- 30 injection molding operators, 18 with symptoms; compared with indirect/no exposed workers (Ahrenholz et al., 1985)
- 136 ADCA exposed workers in manufacturing plastic parts - compared to 34 non exposed - reported complaints more often (Ahrenholz & Anderson, 1985)

- Case reports: 8 (Malo et al., 1985; Valentino & Comai, 1985; Normand et al., 1989; Kim et al., 2004)
  (only 3 well documented)

- National registers: one or more cases reported in UK, SF, NL and Korea (mainly before 2000)

Criteria to confirm a definite diagnosis of allergic occupational asthma (Klees et al., 1990):

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(1) Diagnosis of asthma by a physician
(2) An association between the symptoms of asthma and work
(3) Workplace exposure to an agent or process previously associated with work-related asthma

and at least two of the following criteria:

(4) Significant work-related changes in spirometry
(5) Significant work-related changes in non-specific airway hyperresponsiveness, and
(6) A positive response to an inhalation provocation with the specific agent to which the individual is exposed at work

plus: - preferably with additional immunological measurements
- info on personal or familial atopic history

Observations on ADCA

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- Associations between workplace exposure to ADCA and symptoms of respiratory allergy mainly before 2000
- In only 3 cases were the symptoms and clinical investigations well documented (Malo et al., 1985; Kim et al., 2004)
- Apart from Malo et al. (1985), the respiratory reactions were not typical, viz. “progressive” reactions reaching a maximum after a few hours (also observed following exposure to irritating substances such as chlorine (Burge et al., 2012))
- No or limited info on atopy or predisposing factors
- Source and purity of the chemical used in the provocation tests not reported
- Possibility of co-exposure to respiratory irritants and/or to respiratory allergens cannot be excluded

Conclusions on ADCA

Collectively, the data available from clinical and experimental animal studies indicate that ADCA is not a true respiratory allergen

ADCA may be an asthmagen:

- Work-exacerbated asthma (pre-existing asthma unrelated to work that is made worse by a workplace exposure)
- Irritant-induced asthma (non-allergic, including Reactive Airways Dysfunction Syndrome [RADS])

Final remarks

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Negative animal tests on skin sensitisation should be regarded as also negative for respiratory sensitisation

Respiratory allergy investigations in workers should take the (previously mentioned) criteria into account to confirm a definite diagnosis of allergic occupational asthma

It would be better to have the same classification criteria for skin and respiratory sensitisation (thus involvement of an immunological mechanism)

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Thank you for your attention