

# Diagnosis and monitoring of allergy for LMW agents:

## *di-isocyanate sensitisation in spray painters*

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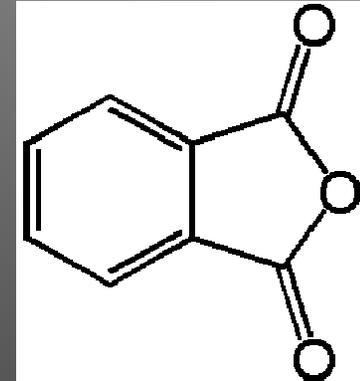
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# LMW occupational allergens -1

Mostly industrial chemicals of < 500-1,000 Da

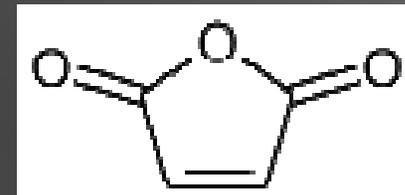
Many used as cross-linkers in polymer production  
highly reactive with eg. OH- or NH<sub>2</sub>-groups  
bi-functional



Volatile or inhaled in solvent-aerosols

*Examples:*

phthalic and maleic anhydride,  
aldehydes, di-isocyanates



Highly reactive with host tissues and (macro-)molecules  
→ acute toxicity in high concentrations



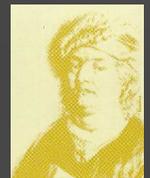
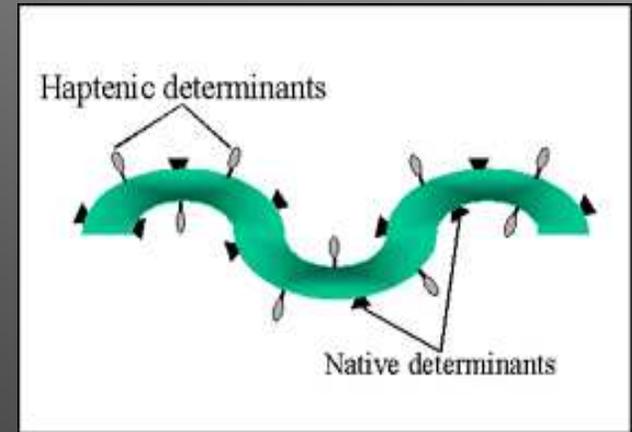
# LMW occupational allergens - 2

LMW agents: as such *non-immunogenic*

Covalent coupling to host macromolecules:

- formation of HMW 'haptene-carrier' complexes
- immune response to these 'non-self' ('altered self') macromolecules
- haptens function as T and B cell epitopes
- multivalent antigens with repeated epitopes

➔ *To which host macromolecules??*



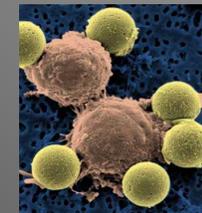
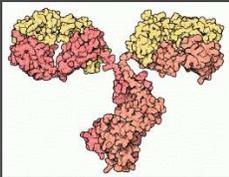
# Occupational asthma due to LMW agents

Typical features of **ALLERGY**:

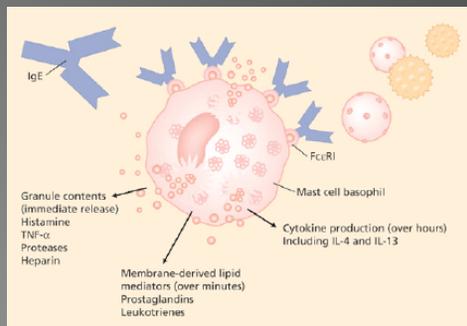
- only **in minority** of exposed workers
  - subpopulation of hypersensitive subjects ?
- **latency** period:
  - sensitisation phase of months to years
- allergen – **specificity**:
  - only reactions to sensitising or closely related agents



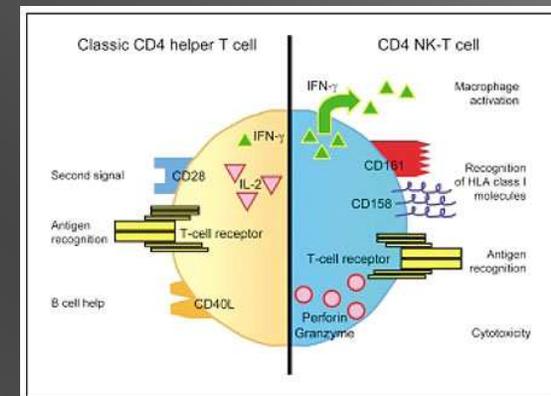
# Occupational asthma due to LMW agents: **ALLERGY?**



## *Allergen-specific immune responses?*



- IgE (Type I) ?
- IgG (type III) ?
- T cells (Type IV) ?



....other mechanisms??



# Di-isocyanates as occupational sensitizers

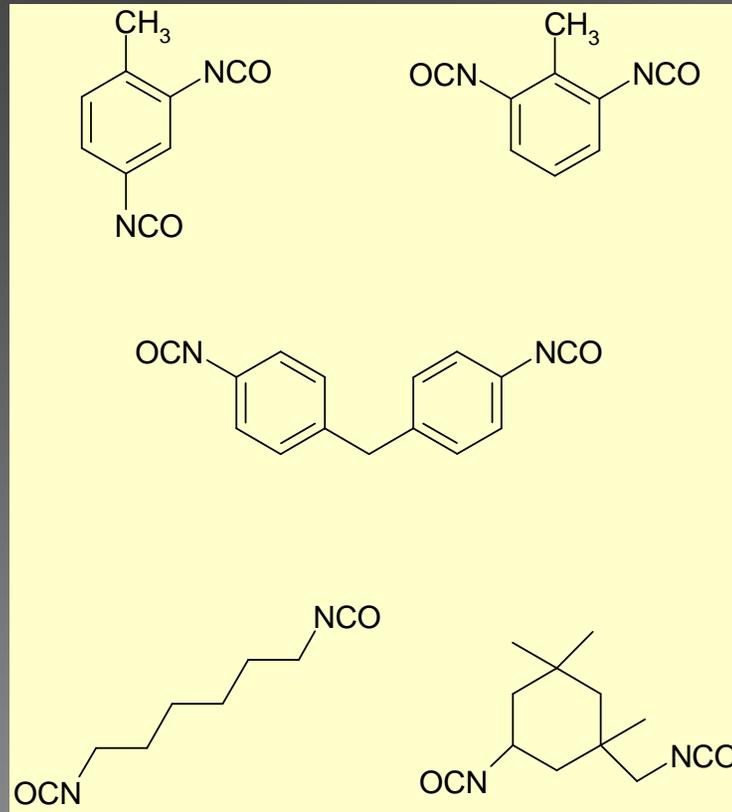
- NCO as functional group: reactive with eg.  $\text{-NH}_2$
- Cross-linkers used in polymerization processes
- applications in production of plastics, foams (polyurethane) and adhesives, coatings, spray painting
- most frequently reported LMW agent causing occupational asthma



# Di-isocyanates : .....*molecular variations on a theme*.....

2,4 and 2,6-TDI: toluene di-isocyanate

4,4 MDI



1,6 HDI

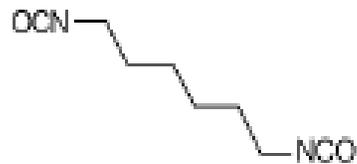
IPDI



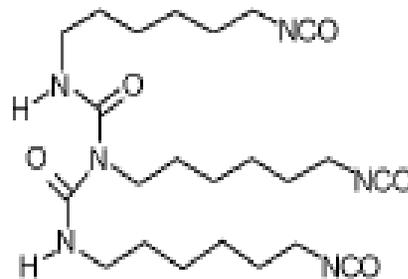
# Oligomers of hexamethylene di-isocyanate (HDI)

Higher MW → less volatile

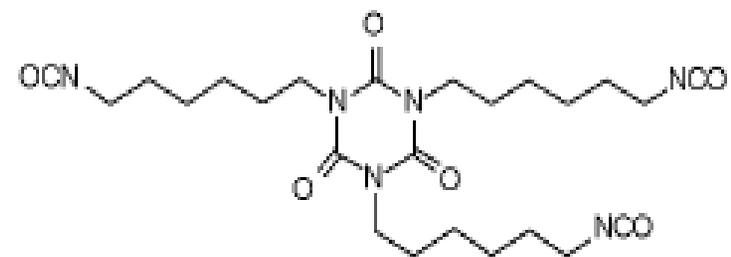
Introduced to reduce inhalatory exposure



Hexamethylene diisocyanate (HDI)



HDI Biuret



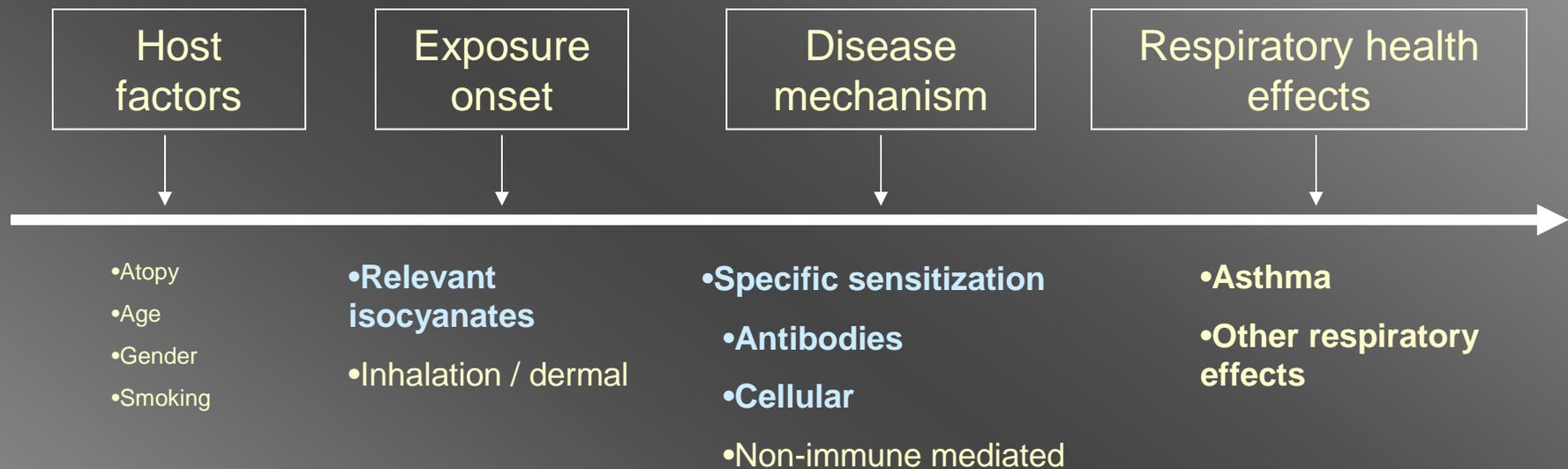
HDI Isocyanurate

*Potency as sensitizers and inducers of asthma?*

*Cross-reactivity?*



# Isocyanate exposure and respiratory health effects in the spray painting industry



PhD study Anjoeka Pronk; UU nov 2007

- Dick Heederik, Gert Doekes, IRAS, UU

- Liesbeth Preller, TNO

- Jan Willem Lammers, Jos Rooijackers, UMCU

- Adam Wisnewski, Yale SPH, USA - Monika Raulf-Heimsoth, BGFA, Bochum, GE



# Isocyanates and respiratory health in 581 workers in the Dutch spray painting industry

Detailed exposure assessment: mainly HDI oligomer exposure

- Health end points
  - Health Questionnaire
  - Anti-HDI antibodies : IgE and IgG
    - ImmunoCAP
    - EIA using HDI<sub>L</sub>-HSA, HDI<sub>V</sub>-HSA, N3300-HSA, N100-HSA
  - Subset (215 workers)
    - BHR: Methacholine challenge (PD20)
    - Baseline spirometry: reference values



# Respiratory health in the spray painting industry

	Office workers	Others	Spray painters
<b>Asthma-like symptoms</b> Wheezing or chest tightness	8.0	20.6	26.1*
<b>COPD-like symptoms</b> Chronic cough/phlegm or shortness of breath	14.0	28.0*	33.6*
<b>Work related rhinitis</b>	14.3	15.0	19.8
<b>Work related chest tightness</b>	2.0	4.0	8.3

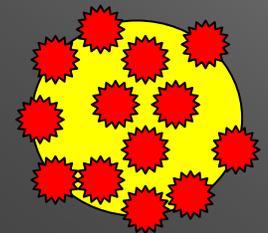
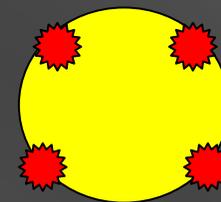
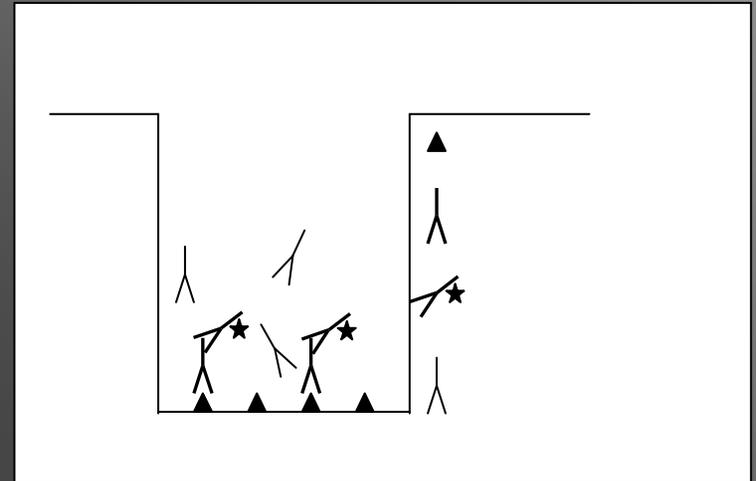


*Relation with immune sensitisation to isocyanates?*



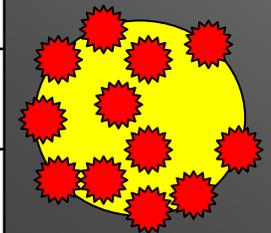
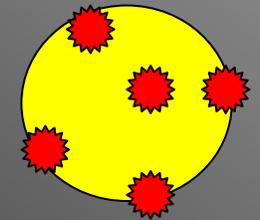
# Immunoassays for IgE and IgG anti-isocyanate sensitization

- CAP® assay or EIA?
- Which (di-isocyanate?)
  - Monomeric HDI
  - Oligomers
- Which carrier protein?
  - human serum albumin(HSA)
  - airway mucosal protein? Which?
- Coupling procedure?
  - In fluid phase
  - At liquid/vapour phase boundary surface
- Molar isocyanate/protein ratio?



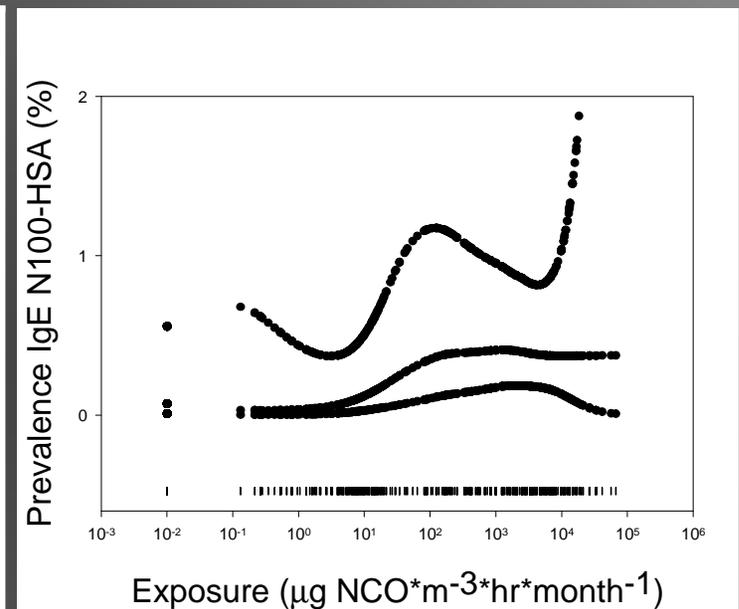
# Immunoassays for anti-isocyanate IgE and IgG

Conjugate	Source*	Carrier	Phase isocyanate	HDI/HSA ratio	test system
<i>HDI-immunoCAP</i>	Phadia	CAP (solid phase)	-	-	Immuno-CAP®
<i>HDI<sub>L</sub>-HSA</i>	IRAS	HSA	Liquid	25 - 30	EIA
<i>HDI<sub>v</sub>-HSA</i>	Yale	HSA	Vapor	9 - 10	EIA
<i>N3300<sub>0.1%</sub>-HSA</i>	Yale	HSA	Liquid	8 - 8.5	EIA
<i>N3300<sub>1.0%</sub>-HSA</i>	Yale	HSA	Liquid	8 - 8.5	EIA
<i>N100<sub>0.1%</sub>-HSA</i>	Yale	HSA	Liquid	5.5 - 6	EIA
<i>N100<sub>1.0%</sub>-HSA</i>	Yale	HSA	Liquid	5.5 - 6	EIA



# Specific IgE antibodies (%)

	Office workers	Others	Spray painters
HDI ImmunoCAP	0	1.0	2.1
HDI <sub>L</sub> -HSA	0	3.5	2.9
HDI <sub>V</sub> -HSA	0	0.7	0.4
N3300-HSA	0	1.0	2.1
N100-HSA	0	2.1	4.2



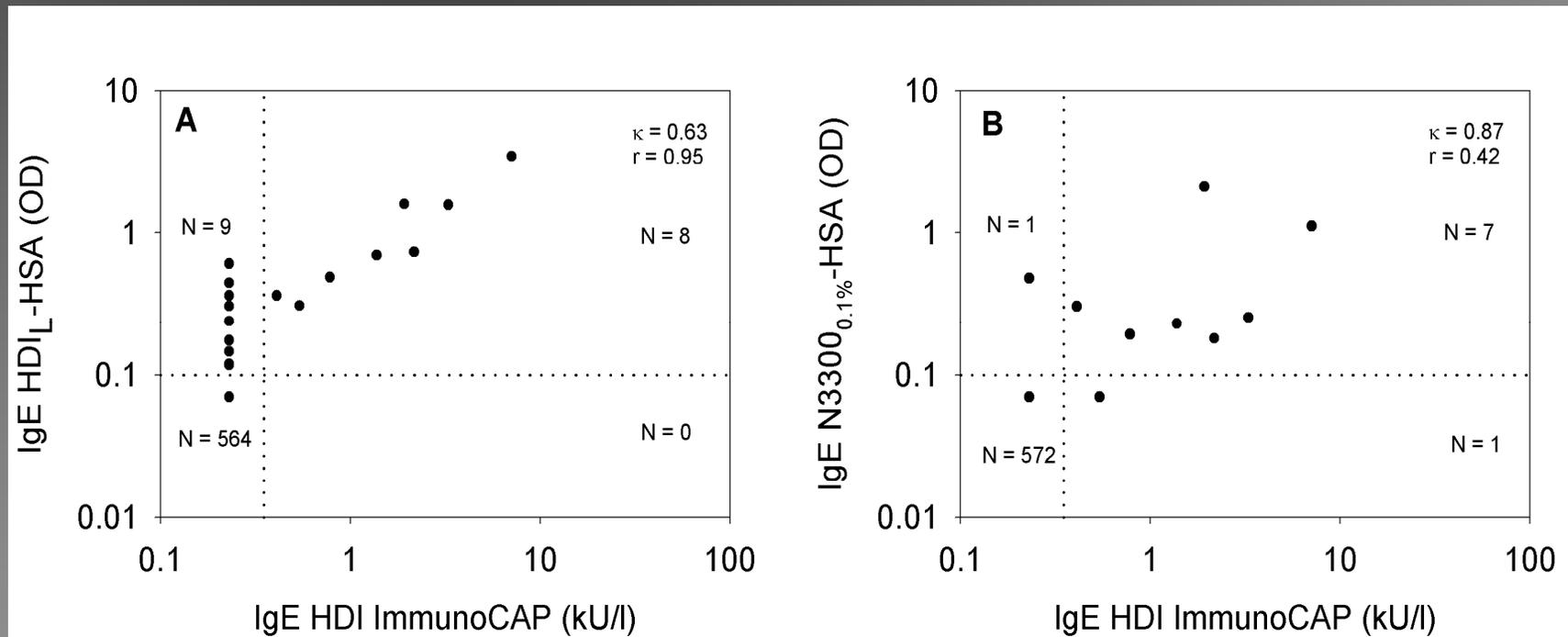
PR IQR: 3.0 (1.1-8.4)



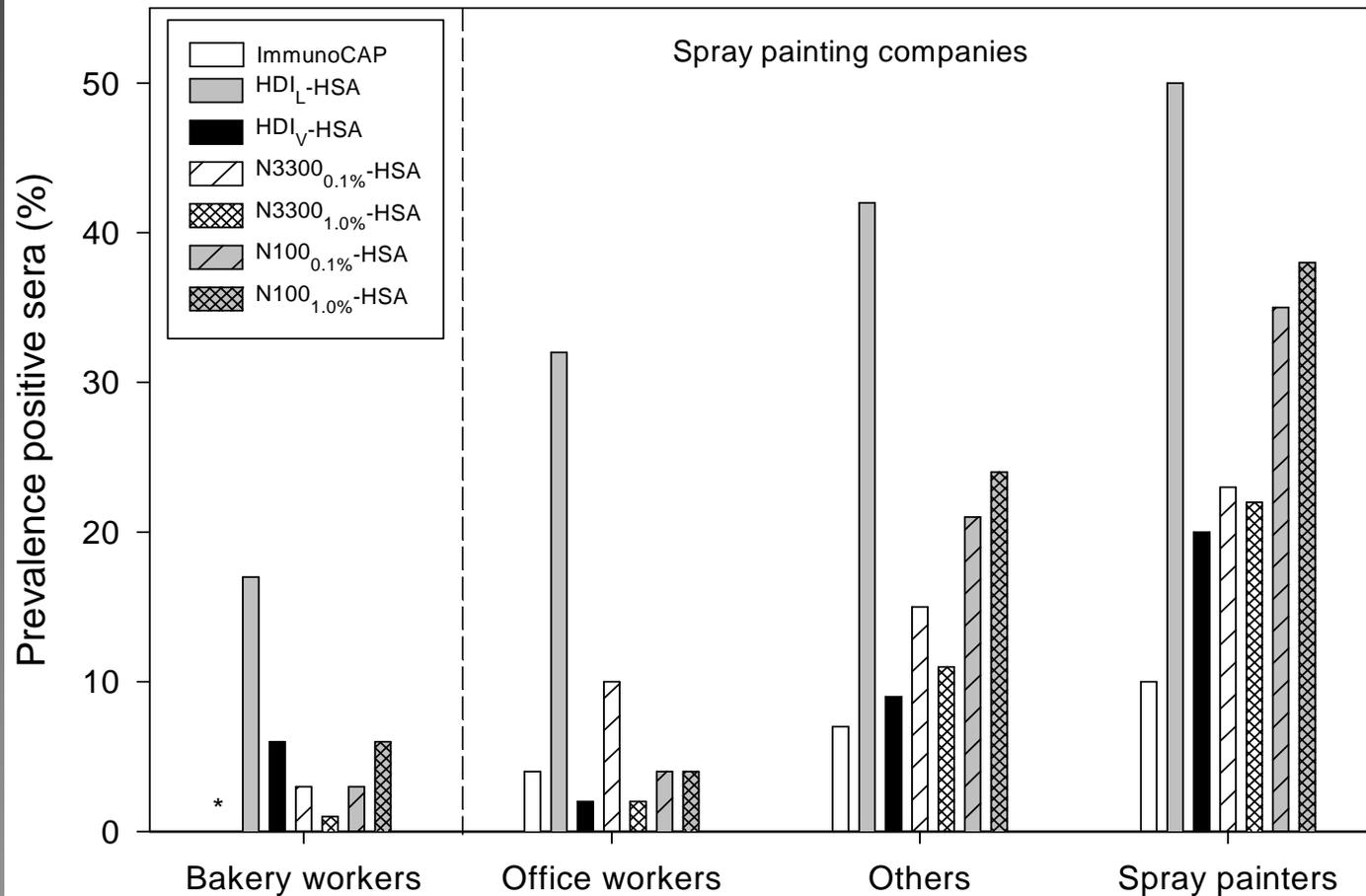
Adjusted for age, gender, current smoking and atopy



# Specific anti-isocyanate IgE antibodies: *correlation between immunoassays*

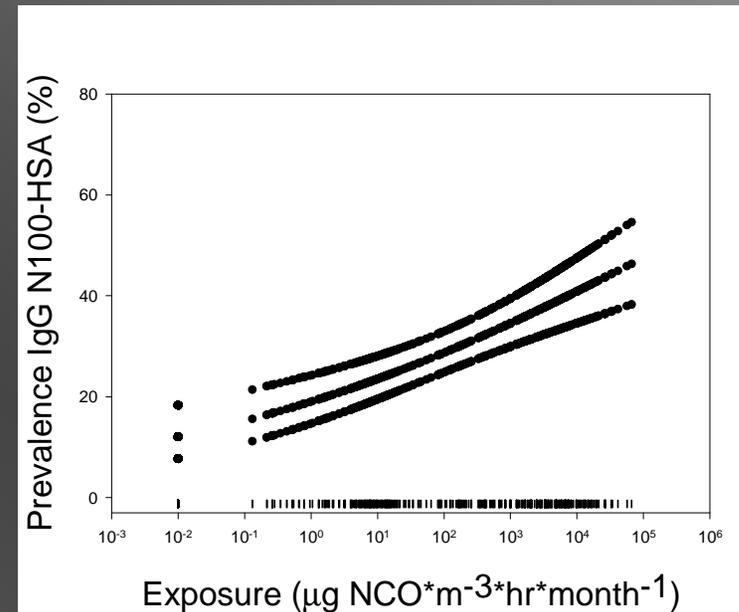


# Anti-isocyanate IgG in spray painters and controls



# Specific IgG antibodies (%)

	Office workers	Others	Spray painters
HDI ImmunoCAP	4.0	7.2	9.5
HDI <sub>L</sub> -HSA	32.0	41.5	50.4*
HDI <sub>V</sub> -HSA	2.0	9.3	20.0*
N3300-HSA	10.0	15.1	23.3
N100-HSA	4.0	21.5*	34.6*



PR IQR: 2.0 (1.5-2.6)



Adjusted for age, gender, current smoking and atopy



# Assays for anti-isocyanate IgG: concordance (*kappa*) and correlation (*r*)

	HDI <sub>L</sub> -HSA	HDI <sub>V</sub> -HSA	N3300 <sub>0.1%</sub> - HSA	N3300 <sub>1.0%</sub> - HSA	N100 <sub>0.1%</sub> - HSA	N100 <sub>1.0%</sub> - HSA	Immuno- CAP
HDI <sub>L</sub> -HSA	**	0.25	0.36	0.30	0.46	0.50	0.09
HDI <sub>V</sub> -HSA	<i>0.50 (67)</i>	**	0.57	0.61	0.44	0.45	0.23
N3300 <sub>0.1%</sub> -HSA	<i>0.61 (94)</i>	<i>0.50 (57)</i>	**	0.81	0.68	0.65	0.19
N3300 <sub>1.0%</sub> -HSA	<i>0.47 (79)</i>	<i>0.64 (54)</i>	<i>0.95 (80)</i>	**	0.61	0.56	0.19
N100 <sub>0.1%</sub> -HSA	<i>0.81 (128)</i>	<i>0.44 (60)</i>	<i>0.65 (93)</i>	<i>0.59 (80)</i>	**	0.84	0.15
N100 <sub>1.0%</sub> -HSA	<i>0.82 (139)</i>	<i>0.48 (65)</i>	<i>0.65 (95)</i>	<i>0.58 (80)</i>	<i>0.98 (135)</i>	**	0.16
Immuno-CAP	<i>0.43 (32)</i>	<i>0.27 (19)</i>	<i>0.12 (21)</i>	<i>0.15 (18)</i>	<i>0.11 (24)</i>	<i>0.28 (27)</i>	**



# Serology – health effects (PR 95%CI)

	IgE		IgG	
	WR chest tightness	WR rhinitis	WR chest tightness	WR rhinitis
HDI-ImmunoCAP	1.6 (0.2-10.30)	<b>2.6 (1.4-4.8)</b>	0.8 (0.2-3.2)	1.5 (0.8-2.0)
HDI <sub>L</sub> -HSA	1.8 (0.5-6.9)	<b>2.0 (1.1-3.6)</b>	1.4 (0.7-3.0)	1.4 (0.9-2.0)
HDI <sub>V</sub> -HSA	4.3 (0.8-23.1)	<b>2.8 (1.1-6.7)</b>	1.2 (0.5-3.0)	1.2 (0.8-2.0)
N3300-HSA	1.5 (0.2-10.2)	<b>2.1 (1.0-3.4)</b>	1.0 (0.4-2.3)	1.3 (0.3-1.0)
N100-HSA	<b>1.8 (1.0-3.4)</b>	<b>1.8 (1.0-3.4)</b>	1.7 (0.8-3.5)	<b>1.5 (1.1-2.2)</b>



Adjusted for age, gender, current smoking and atopy



# Serology in isocyanate-exposed workers - 1

- EIAs with various HSA-IC conjugates produce concordant and correlating results
- Concordance and correlations due to genuine cross-reactivity between various IC-conjugates (not shown)
- Also correlating with results of ImmunoCAP®
- .....results of assays are however far from 100% identical  
Assays are not simply interchangeable.  
Differences in sensitivity and specificity



# Serology in isocyanate-exposed workers - 2

- IgE anti-IC only in small %, related to exposure
- May explain small % of (wr) symptoms; most DIC-associated reported illness must be due to other mechanisms
- IgG anti-IC
  - in much higher % of all workers
  - some assays may also be positive in controls
  - related to exposure
  - not related to symptoms

