National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

What differentiates respiratory sensitizers from skin sensitizers?

Implications for predictive toxicity testing

Janine Ezendam
Janine.Ezendam@rivm.nl

Background

- GHS classification and labeling of chemicals: skin and respiratory sensitizers are classified in two different classes

- Skin and respiratory sensitizers share certain properties,
  - protein reactivity, induction of ‘danger’ signals

- Important to identify the unique mechanisms involved in adverse outcome will facilitate development of predictive testing strategies

- Not always black and white picture!

- Complexity illustrated with few examples and outliers based on experimental animal studies

Models using dermal exposure

Local Lymph Node Assay:

- Skin and respiratory sensitizers induce lymphocyte proliferation
- Discrimination may be possible using Th1/Th2 cytokine profiling
  - IL4/IFNγ ratio: respiratory sensitizers > skin sensitizers

- Mouse IgE test
  - Diisocyanates and anhydrides enhance IgE
  - Dependent on protocol / strain – DNCB positive as well

- Read-outs not validated using broad range of respiratory and skin sensitizers

Sensitization phase: respiratory LLNA

- Same protocol as LLNA but inhalation exposure

- Readout: proliferation and cytokine profiles in mandibular LNs

Cell proliferation

- Respiratory sensitizers tested were all positive
- Skin sensitizers DNCB and OXA were positive
- Skin sensitizer formaldehyde was negative
- Irritant methyl salicylate was negative

Arts et al. (2008) Tox Sci 106, De Jong et al (2009) Toxicology 261, Vantriel et al., (2010) Toxicology 269; Ter Burg et al. (2014) Inhalation Toxicology

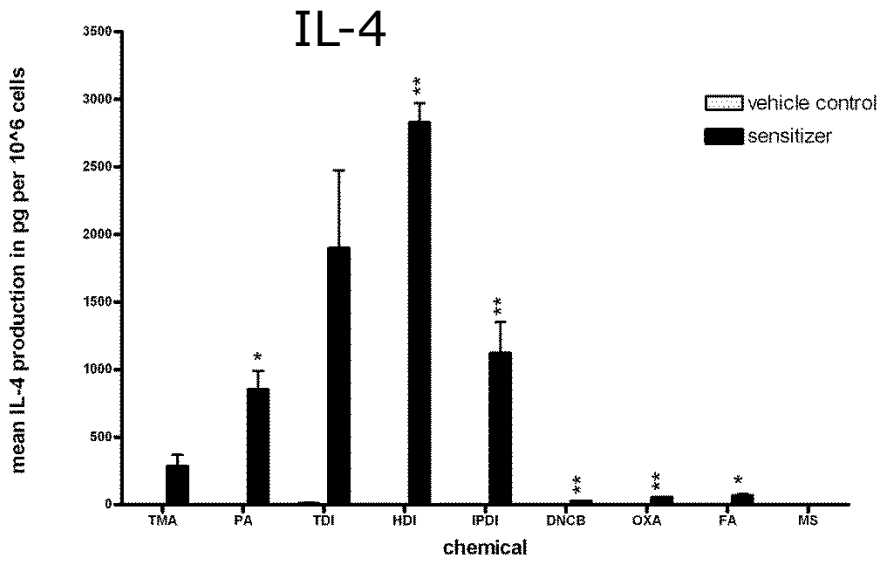
Potency ranking

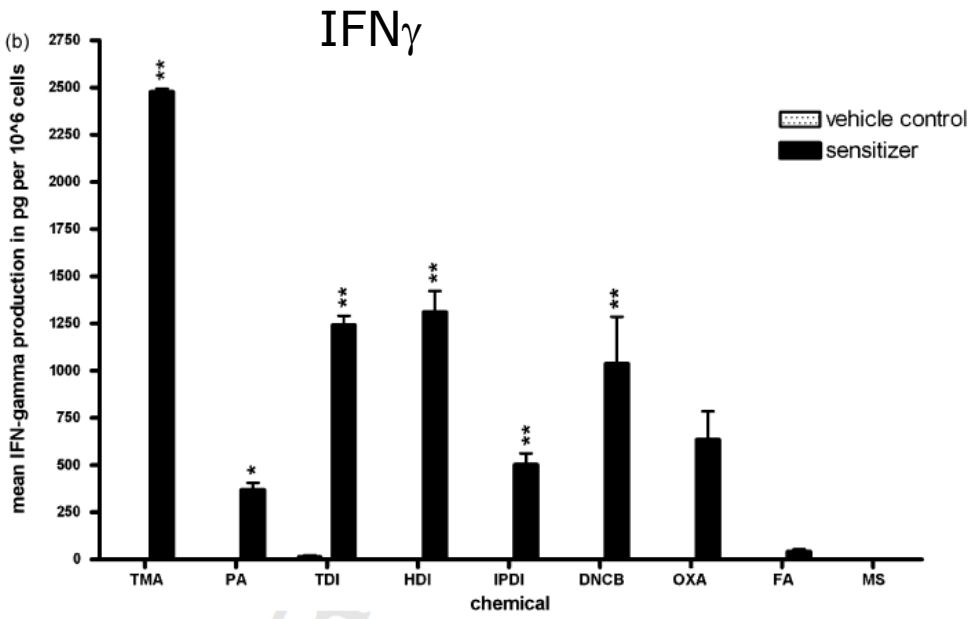
Ranking inhalation	Calculated ED3 (µg)	Ranking dermal	LLNA EC3 (%)
OXA	19	OXA	0.013
TDI	28	DNCB	0.044
PA	63	TDI	0.109
TMA	156	TMA	0.218
DNCB	173	PA	0.357

Route of exposure has some impact on potency ranking

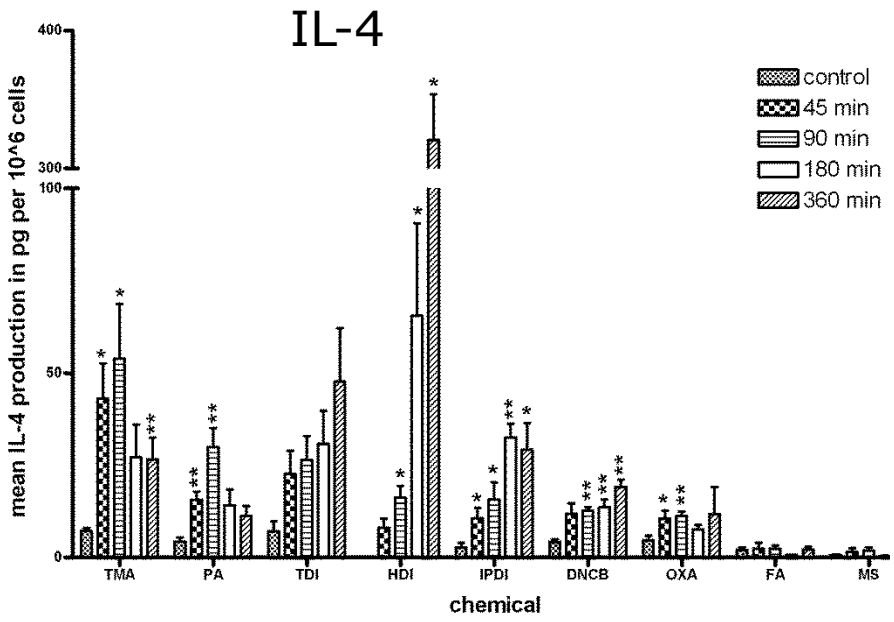
Arts et al. (2008) Tox Sci 106, De Jong et al (2009) Toxicology 261, Vantriel et al., (2010) Toxicology 269; Ter Burg et al. (2014) Inhalation Toxicology

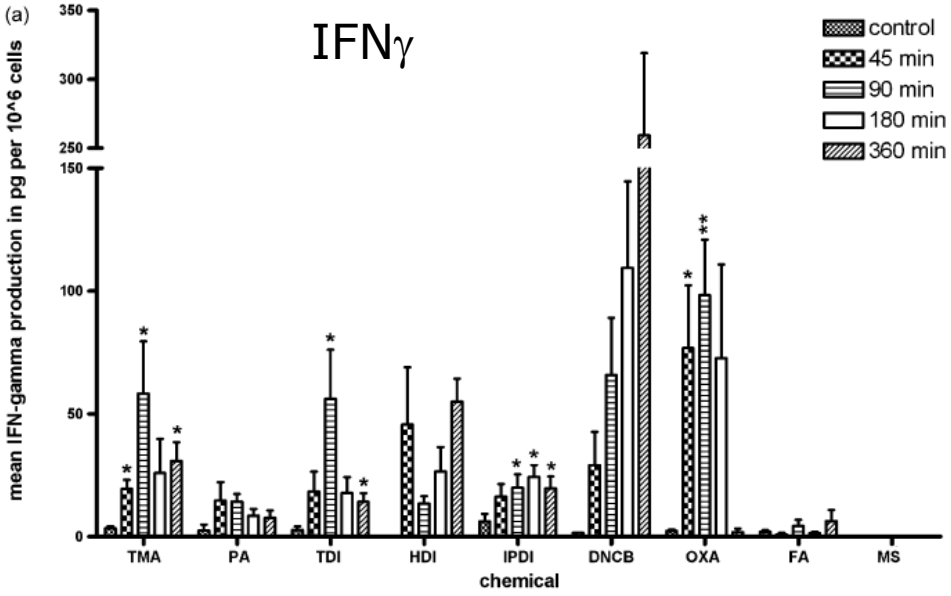
IL-4 and IFNγ dermal LLNA





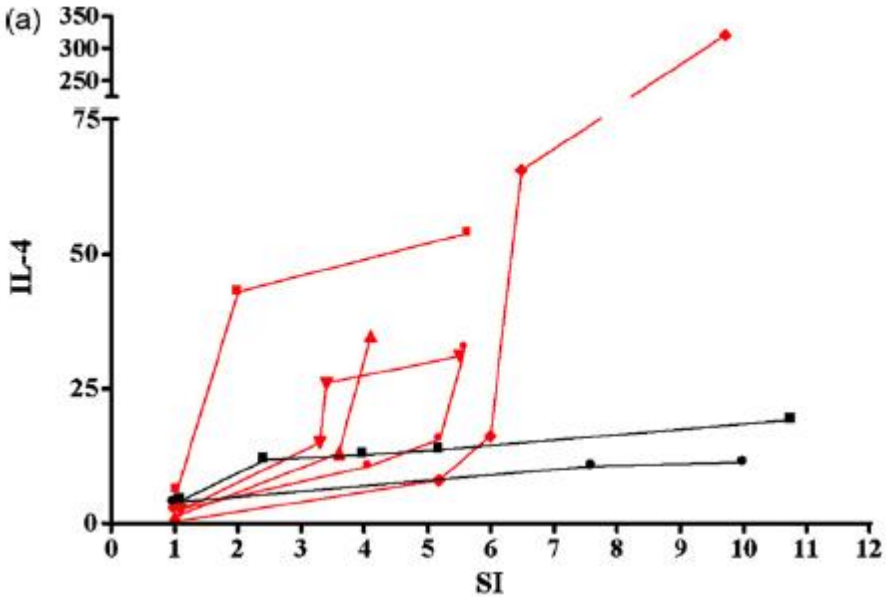
IL-4 and IFNγ respiratory LLNA



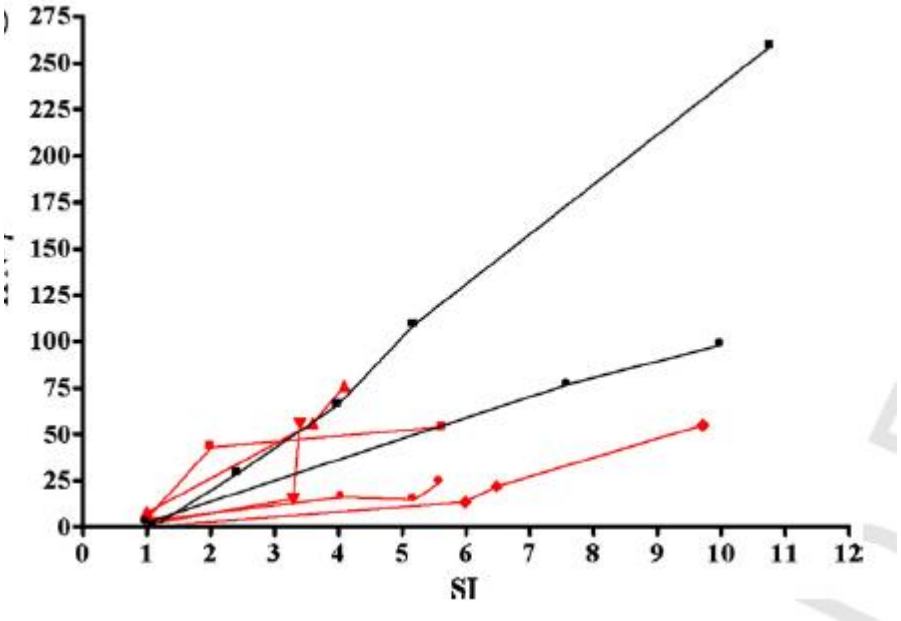


Cytokine production as a function of cell proliferation

IL-4



IFNγ



Glutaraldehyde – an outlier

- GA is a recognized skin and respiratory sensitizer

- Glutaraldehyde was negative upon inhalation in respiratory LLNA
  - Exposure dose too low? → but respiratory irritation

- No respiratory allergy after repeated exposures in guinea pigs

- GA: positive in dermal LLNA + Th2-dominant cytokine response

Hypothesis

- GA is trapped in mucus layer after reacting with peptides → no or low dose reaches immune system

- After dermal sensitization → lower inhalation dose needed for elicitation

Vantriel et al., (2011) Toxicology 279

Elicitation response in Th1 or Th2 prone rats

Days 0 and 7

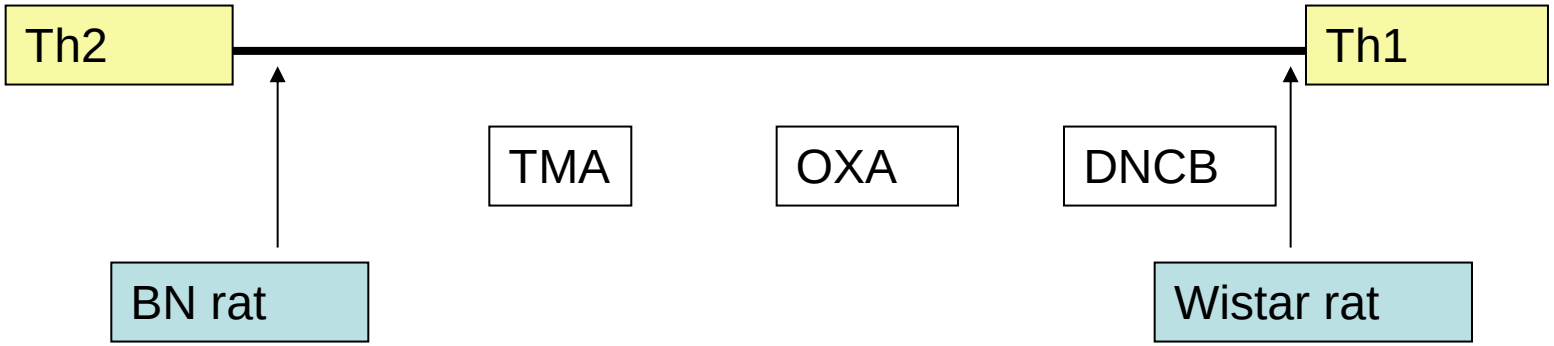
Phase I
Sensitization
via skin
No symptoms

Day 21

Phase II
Challenge
via inhalation
- breathing pattern
- IgE
- BAL

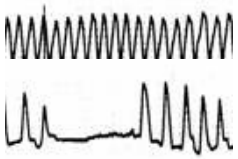
Day 22

Necropsy
- Pathology



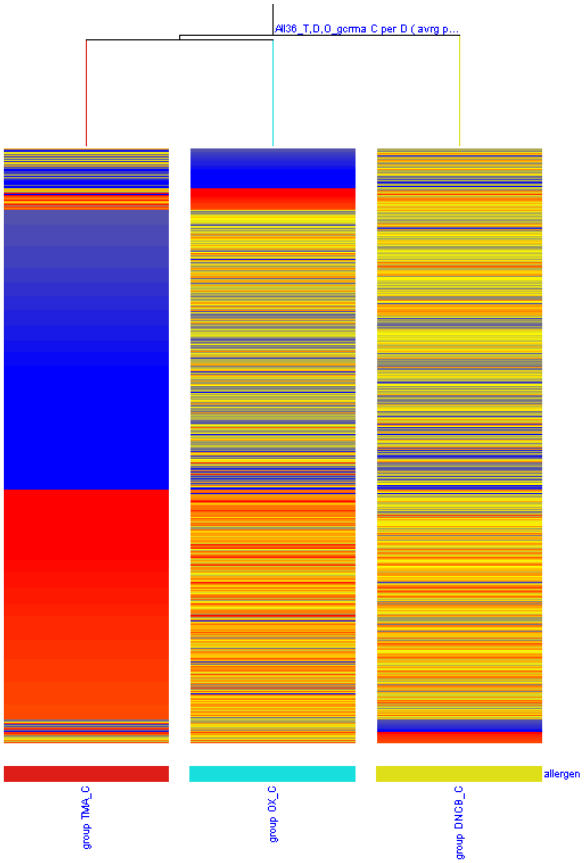
From: Frieke Kuper, TNO

Convential parameters in Th2 rats

	TMA Respiratory sensitizer	**DNCB** Skin sensitizer	**OXA** Skin sensitizer
Breathing functions		-	
BAL cells	Eosinophils, Neutrophils, Macrophages	Neutrophils	Macrophages Neutrophils
Allergic inflammation	Laryngitis	-	Laryngitis
IgE in serum	+	-	+

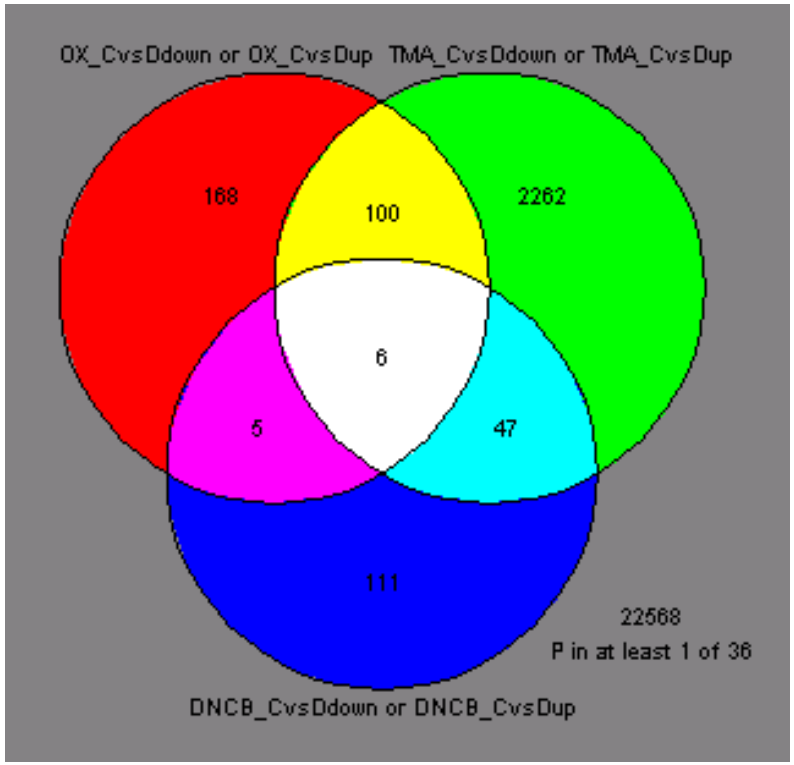
From: Frieke Kuper, TNO

Gene expression lung in allergic BN rats 24h after challenge



TMA OXA DNCB

OXA TMA



DNCB

From: Frieke Kuper, TNO

Significant regulated gene pathways

• Chemokine activity	TMA > OXA >>>> DNCB
• Inflammatory response	TMA > OXA >>>> DNCB
• Immune response	OXA > TMA >>>> DNCB
• T cell receptor signalling	OXA > TMA > DNCB
• Toll-like receptor signalling and Jak-STAT	TMA >>> OXA
• **Extra cellular matrix (airway remodeling)**	TMA

From: Frieke Kuper, TNO

Oxazolone: an outlier

- Conventional parameters (airway hyperresponsiveness, IgE) in BN rats comparable to TMA

- Overlap in gene expression profiles in the lungs but
  - *TMA > OXA Th2 genes*
  - *OXA > TMA Th1 genes*
  - *Lung remodeling restricted to TMA*

- Humans: strong skin sensitizer, no reports on respiratory allergy
  - false-positive in animal model

    OR
  - no or too low inhalation exposure in humans
    - Not a bulk material and presented in large flakes

Kuper et al., (2011) Toxicology 290

Effects of inhalation of DNCB

- DNCB is immunogenic in different respiratory animal models
  - sensitization phase

- Prolonged repeated inhalation exposures in Th1 proneWistar rats
  - Immune priming -- DNCB-specific IgG (no IgE)

  - Allergic inflammation in the upper respiratory tract (larynx)

  - No airway inflammation in lower respiratory tract

  - No respiratory changes

- Cause of concern?

Kuper et al., (2010) Toxicology 269

Wrap-up

- Distinghuishing respiratory from skin sensitizers is difficult in short-term models
- In elicitation models distinct airway and IgE antibody responses and gene expression profiles are observed
- Datapoor -- few compounds tested within models
- Which parameters are indicative for adverse effects?

Food for thought

- Adverse Outcome Pathway group aims at mapping mechanistic knowledge on respiratory sensitizaiton in a structured way
- Understanding mechanisms of toxicity is important for building relevant test methods (animal, non-animal)
- BUT biology is not linear and it is important to distinghuish adaptive versus adverse effects
- Perhaps multiple events have to occur simultaneously in order to lead to adverse outcome

Acknowledgements

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