State of Science for Respiratory Sensitization

Dose-Response Models for the OEL-Derivation of Chemicals

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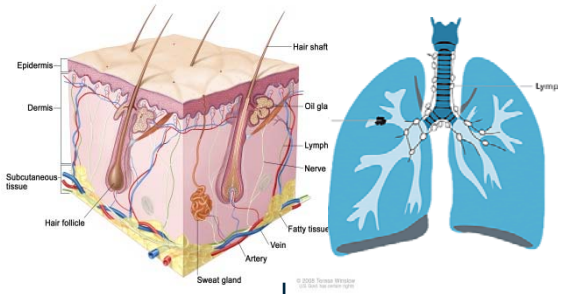
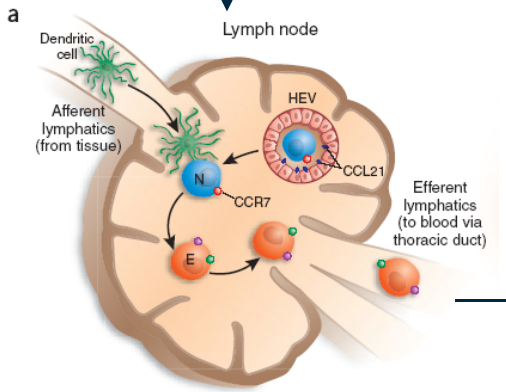
Outline

- Hypotheses, mechanisms & etiopathological relationship of induction and elicitation
- Test approaches & protocols
- Respiratory irritation vs. sensitization vs. allergy
- Metrics & dose
- Derivation of OELs for irritant asthmagens such as TDI-vapor & MDI-aerosol

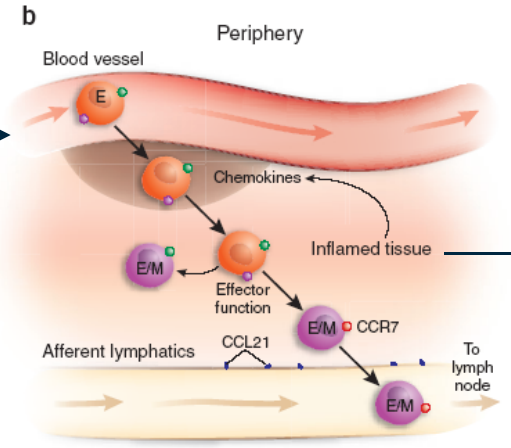
Hypothesis: What do we intend to model and how do we define Sensitization?

Skin and/or
inhalation exposure





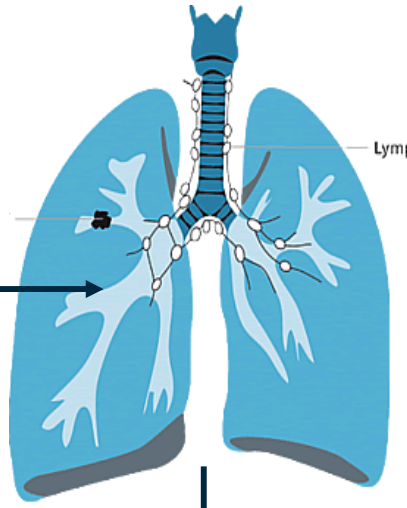
Lymph node priming
& Th-cell polarization



Systemic sensitization and induction of *status asthmaticus* are inter-concatenated but in-dependent processes!

Inhalation
exposure

ΣC x t



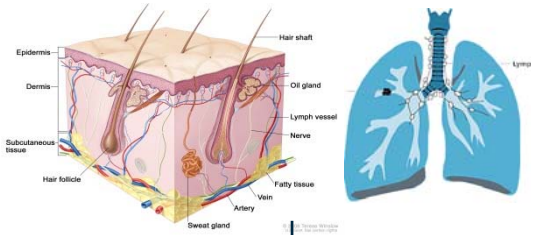
Occupational Asthma

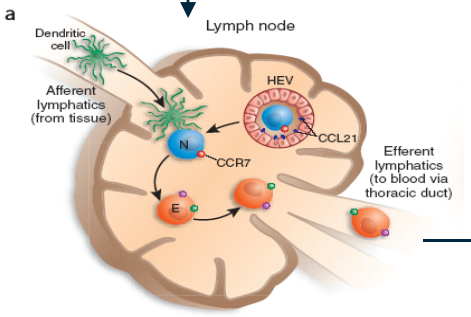
What can be quantified and controlled?

Skin induction quantifiable: **NO**

Lung induction quantifiable-**TWA: YES**
Excursions: NO

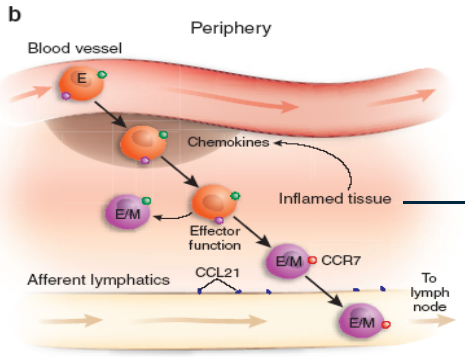
Inhalation exposure quantifiable: **YES**





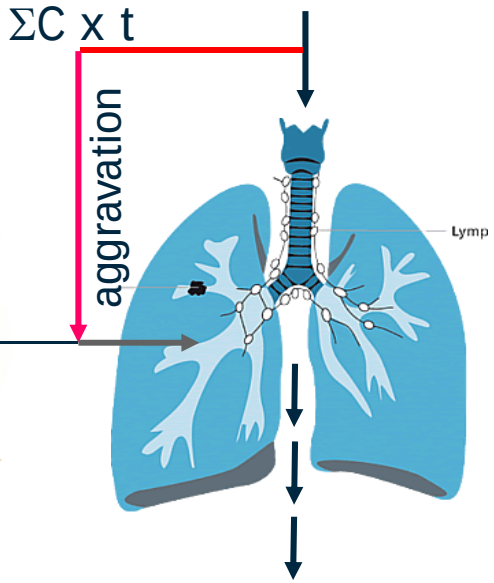
Lymph node priming
& Th-cell polarization

Expression of cytokines
irritation vs. allergy: **NO**



Systemic sensitization

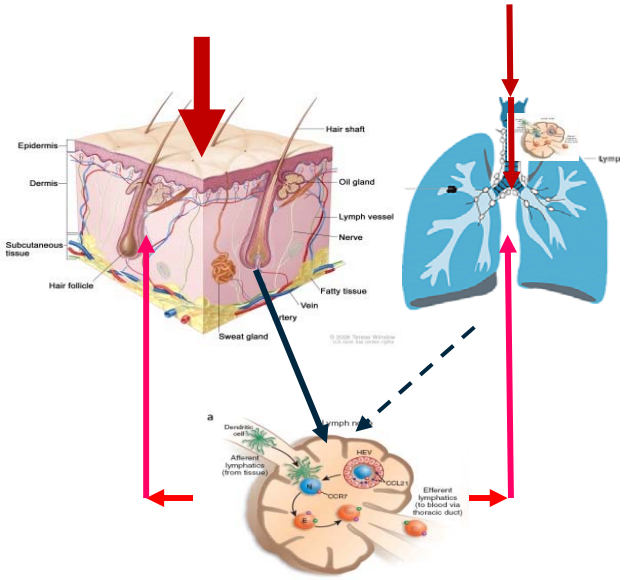
Compartmentalized
expression of immuno-
globulines, e.g. IgE/G
specific or total: **NO**



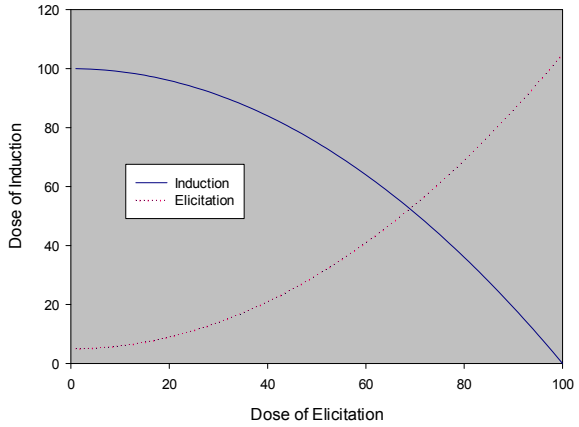
Occupational Asthma

Integrating phenotypes
of OA: **YES**

Skin/Lung Induction: Dose-Metric-Regimen

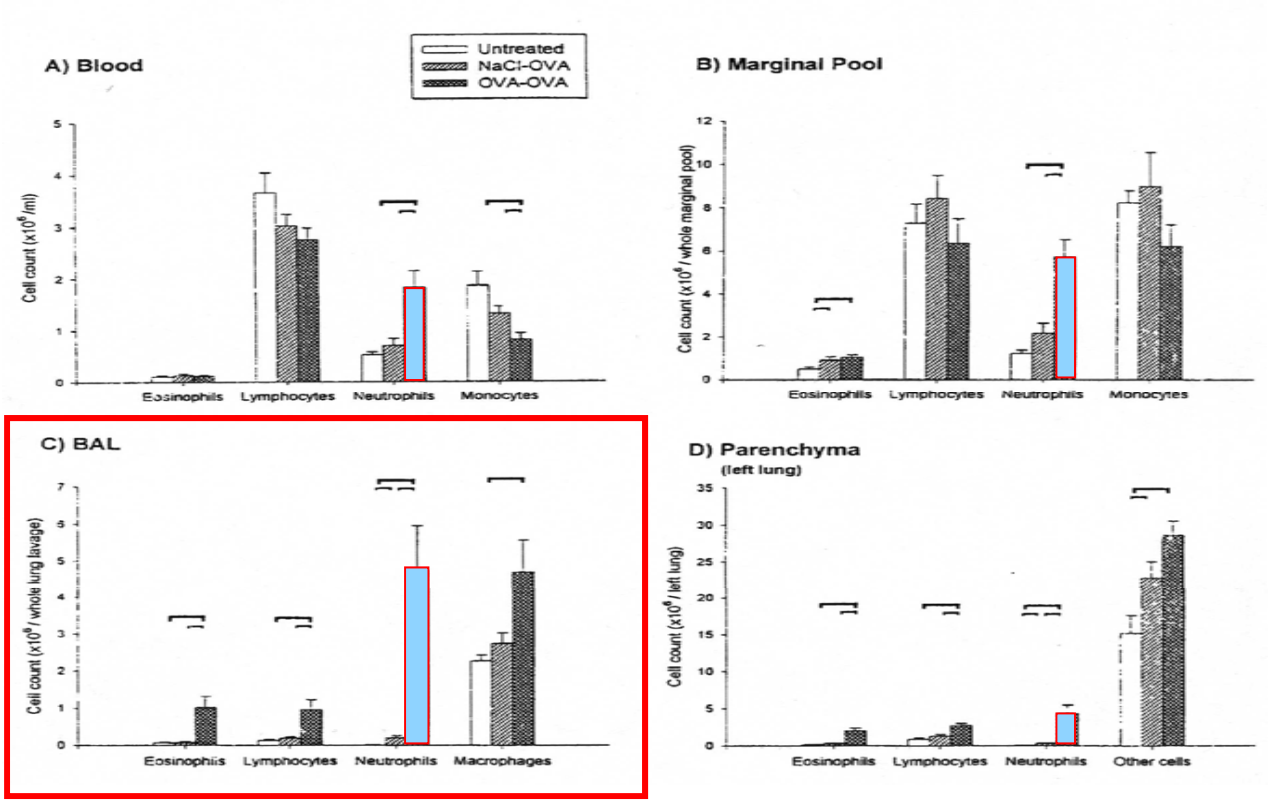


Reciprocal Relationship of Induction and Elicitation



- Biomarker of susceptibility vs. adversity
- Compartmentalization
- Inflammation response to irritant and/or allergic stimulus
- Neurogenic modulation
- Dose metrics and induction site-specific constraints
- Interrelationship of irritation, sensitization, and elicitation of sensitization
- Human relevance

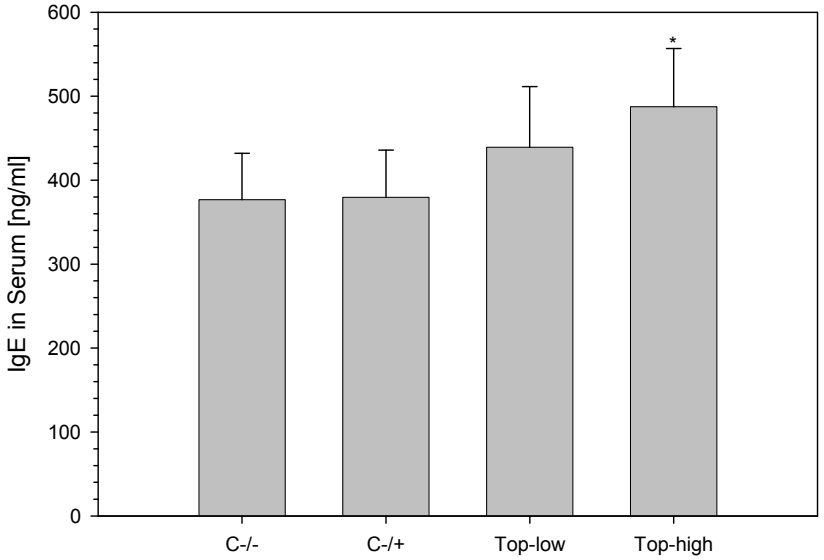
Compartmentalization of PMNs in the BNR-Ova-Model



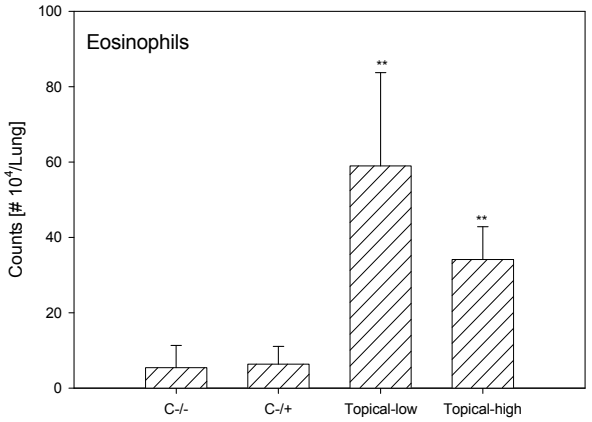
Duplicated from Schuster et al. (2000)

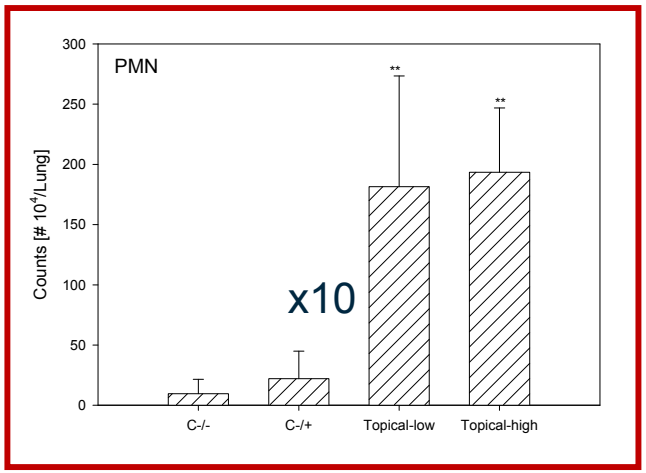
Serum total IgE an Index of Inflammation or Allergy?

Topical induction – 4 x 40 mg MDI / m³ x 30 min



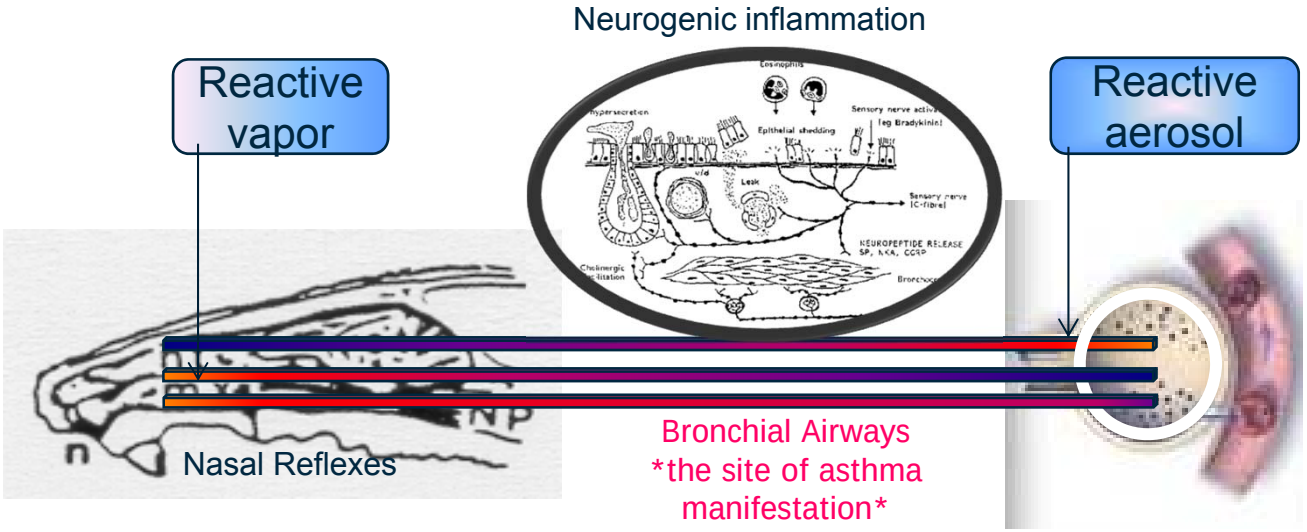
Low/high: 40/150 µl MDI on both days 0 and 7





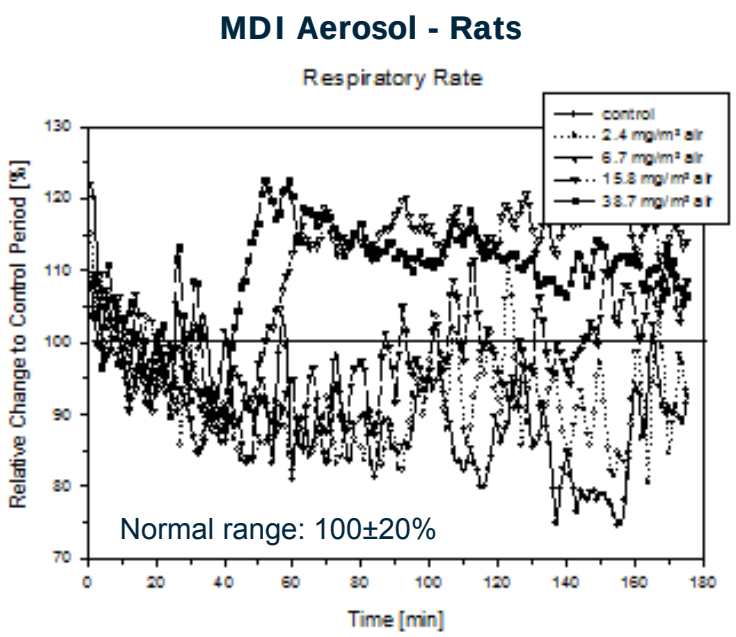
- The most discriminatory endpoint of respiratory allergy is PMN in BAL (in BN rats).

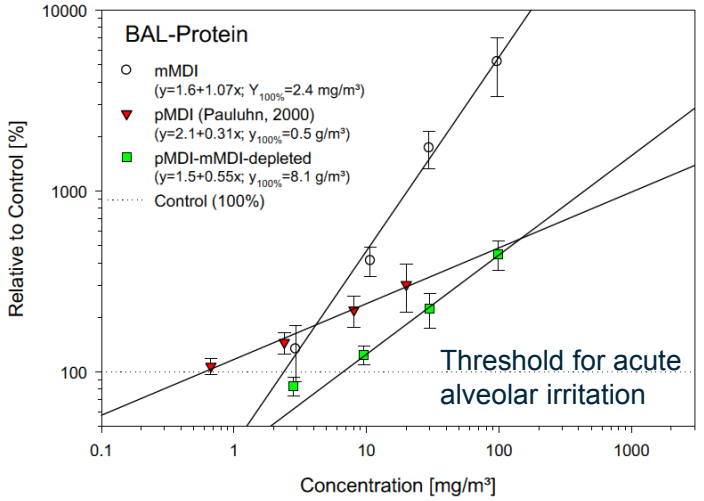
Situation more complex for reactive Vapors: C vs. C x t - Relationships

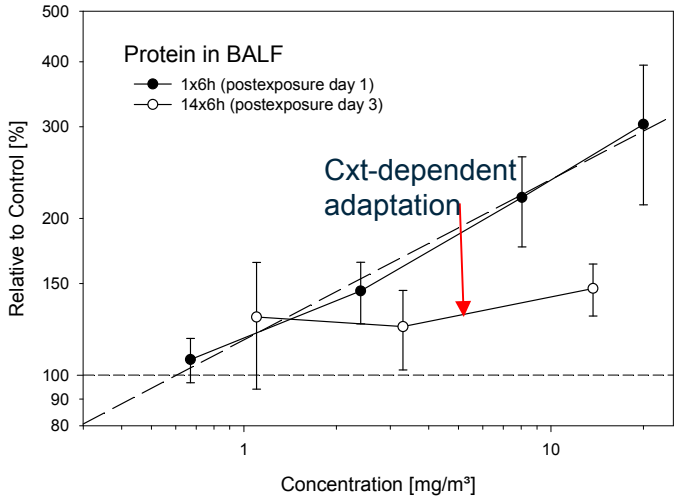


- **Reactive aerosols:** Site of deposition is aerosol size-dependent, the lung dose and POD is Cxt-dependent.
- **Reactive vapors:** The degree of penetration into lower airways is C-dependent. C determines the depth of penetration into the lung & Cxt the site-specific dose.

Respiratory Tract Irritation: Similarities to Skin? Definitely NOT!







Regulatory stumbling blocks:

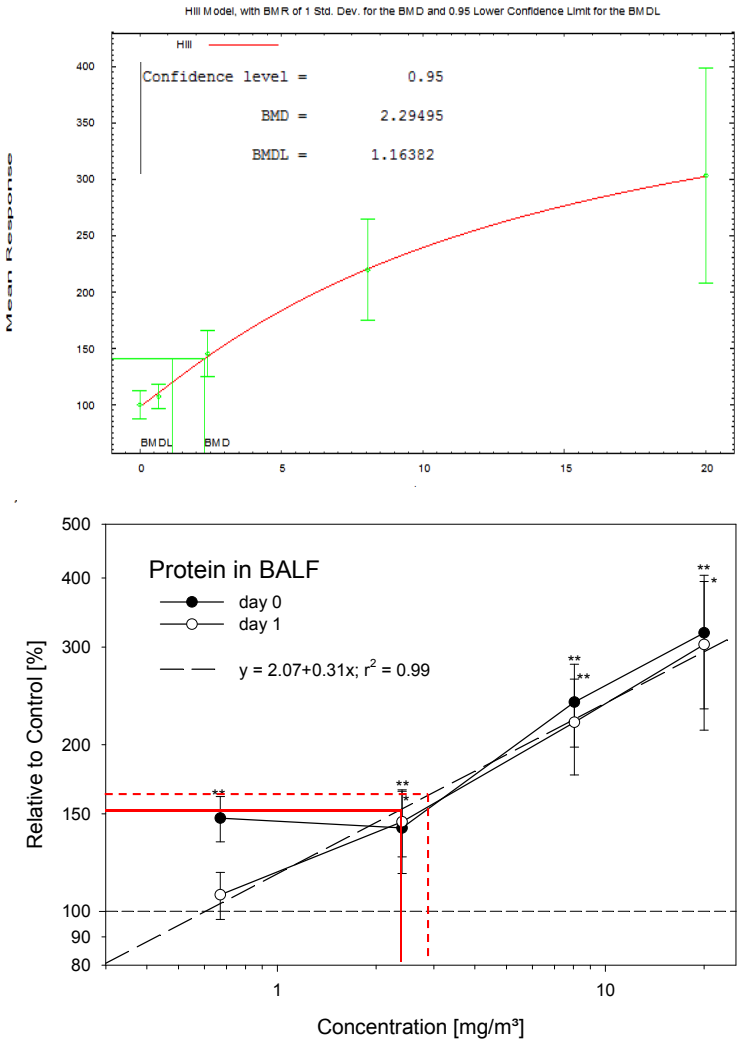
- ❑ RT = ET + T + P [US: separated, EU: combined]
- ❑ Aerosols & vapors dosimetrically different. Also the response to injury is entirely different
- ❑ ET-T-P irritation sites have to be identified

Respiratory Tract Irritation: Selection of Challenge Cxt for Aerosols

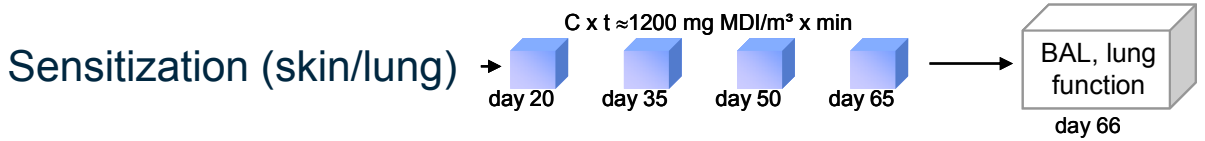
Protocol:

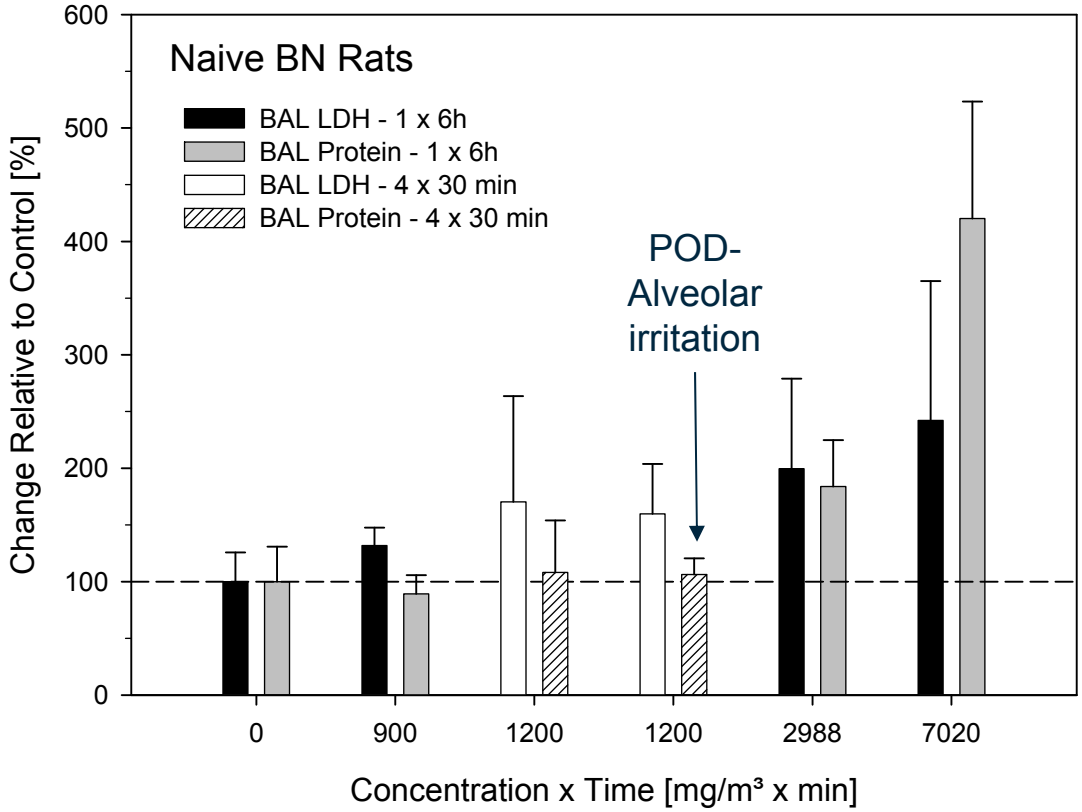
- Single 6h exposure of naïve BN- or Wistar rats to MDI-aerosol
- C-dependence of BAL-protein after and 1-day post-exposure

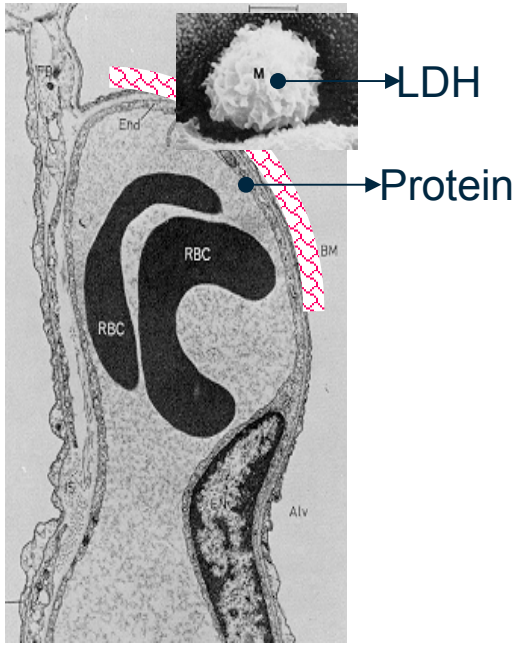
- Acute LRT-threshold: 0.5 mg/m³ x 6 h (180 mg MDI/m³ x min)
- Minimal LRT-threshold 3 mg/m³ x 6 h (1080 mg MDI/m³ x min)
- Converted to challenge duration: 36 mg/m³ x 30 min
- Experimental validation of Cxt needed



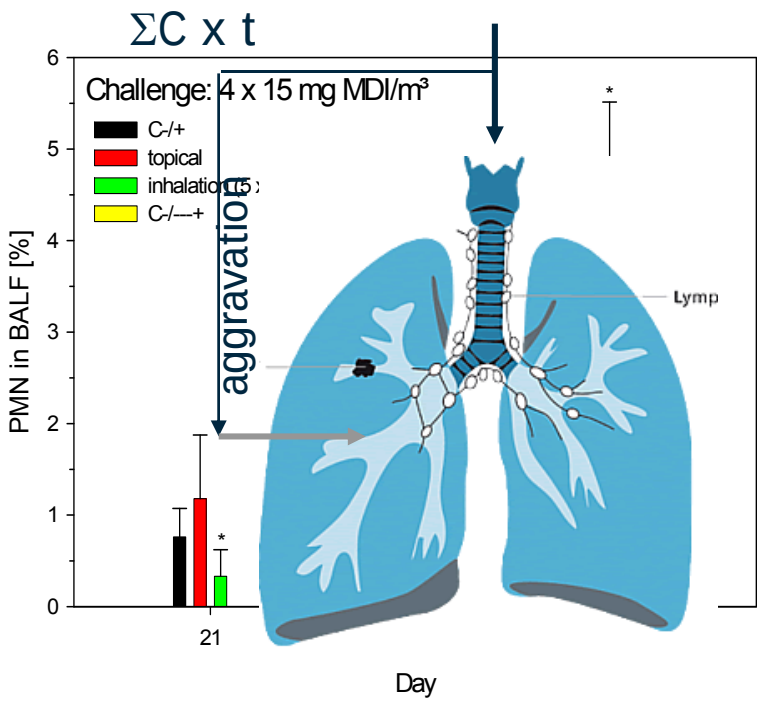
Searching for the POD of Airway Irritation for Aerosols



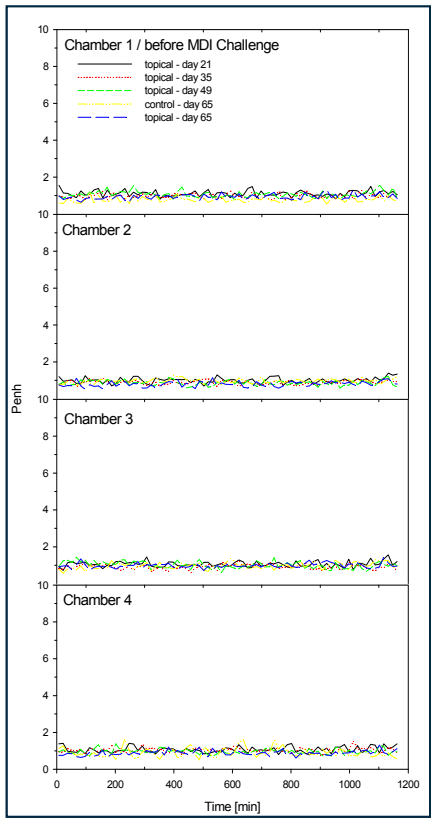




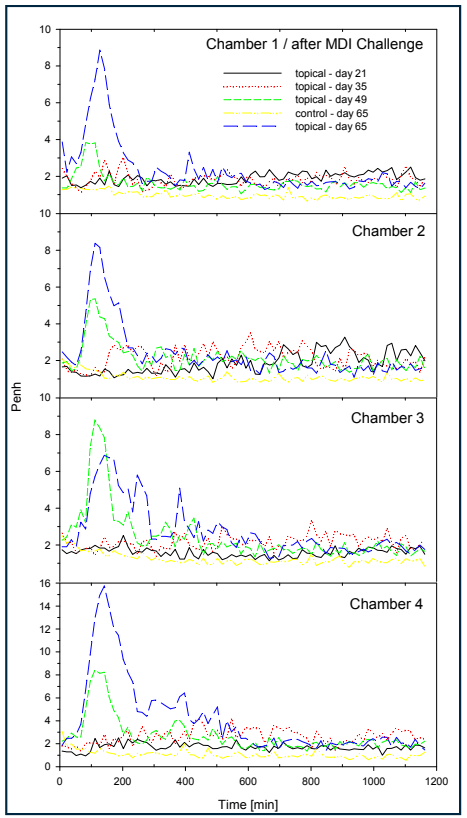
The Induction of Phenotypes of Asthma require a repeated Challenge Protocol



Before Challenge

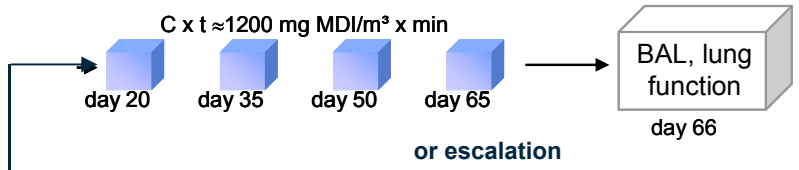


After Challenge

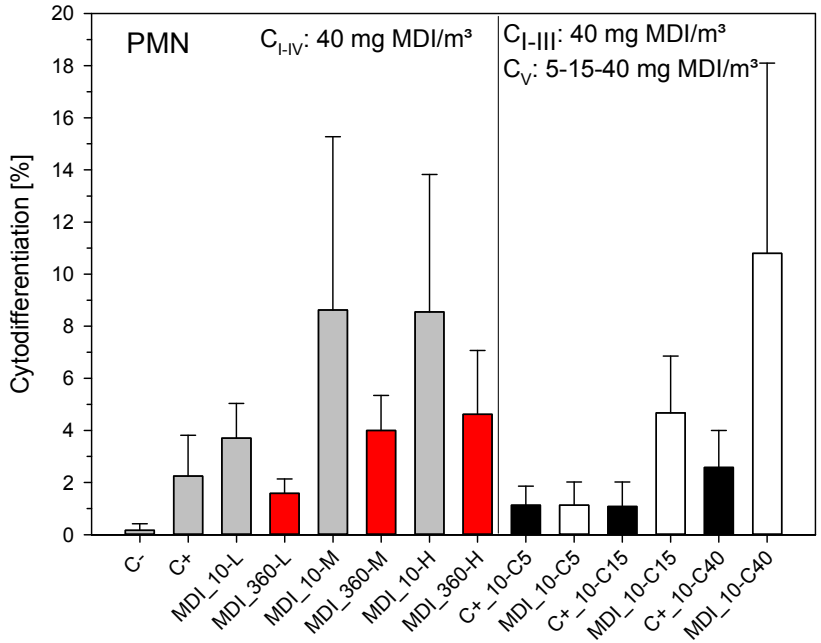


MDI aerosol: optimally 4 inhalation challenges are required to demonstrate the asthma response

Sensitization Efficacy (MDI): Topical vs. Inhalation



Dose	Induction		Priming / Chall.
C x t_{day} (mg/m³ x min)	5x10-min (mg/m³)	5x360-min (mg/m³)	4x30-min
1000 (L)	100	3	40-40-40-40
5000 (M)	500	15	40-40-40-40
10000 (H)	1000	30	40-40-40-40
10000 (H)	1000	--	40-40-40-5-15-40

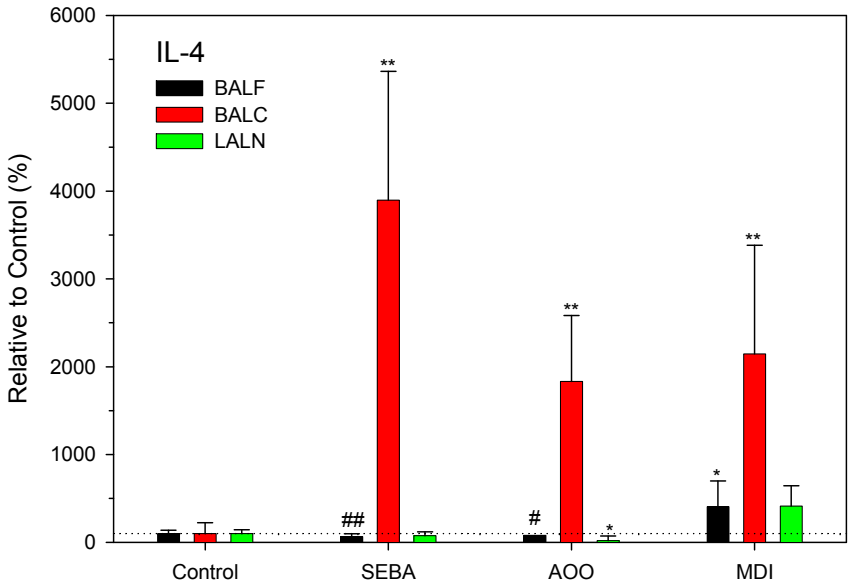


Topical (2x) vs. Inhalation (5x): Topical (SD.±30%) > inhalation (SD.±50%); high-concentration x short-time protocol > low-concentration x long-time protocol; elicitation Cxt-dependent

Compartmentalization and Impact of Vehicle: BNR-MDI-Model

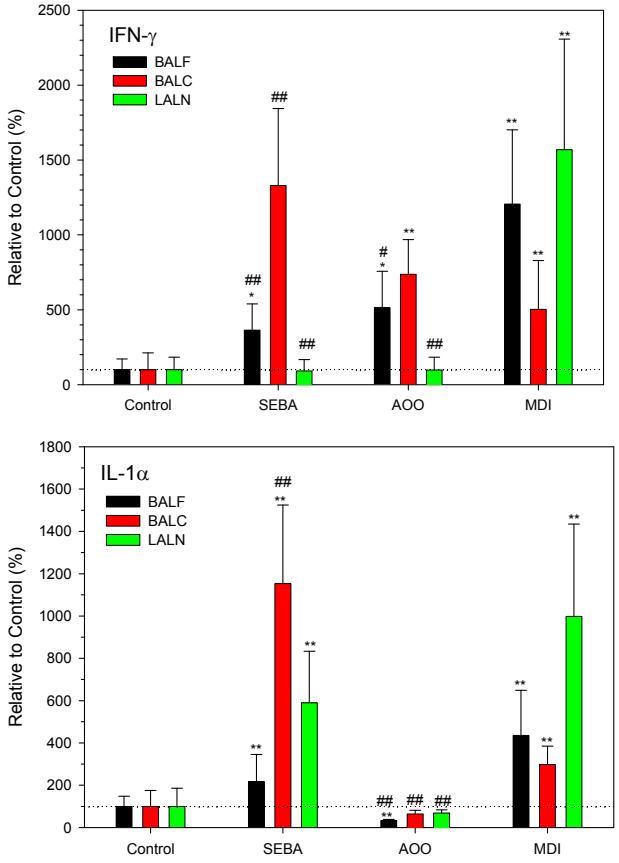
Th_2-Priming

Topical induction – 4 x 40 mg MDI / m³ x 30 min



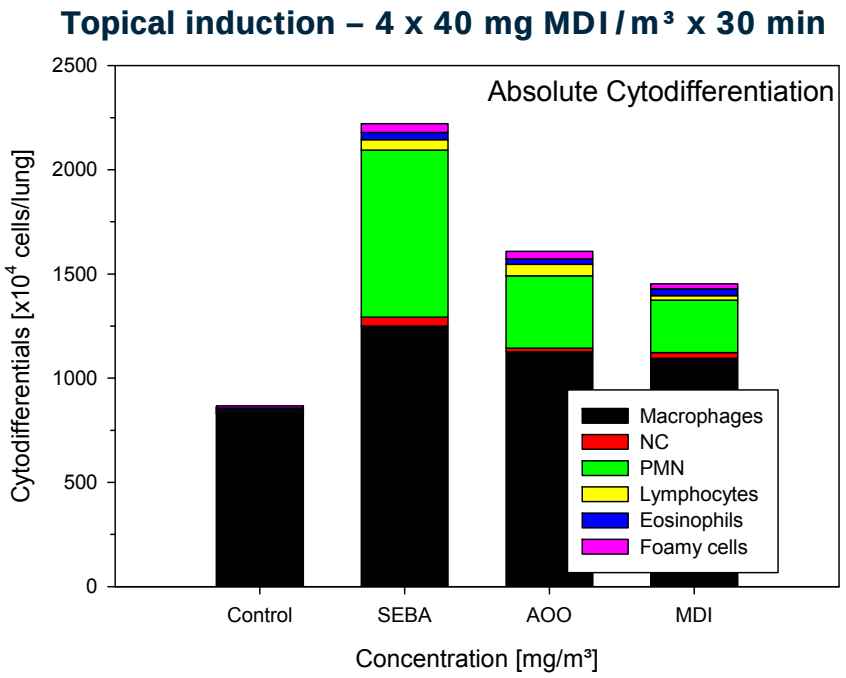
SEBA: di-n-octyl sebacic acid ester (20%)
AOO: acetone:olive oil (20%)
MDI: neat (same dose in all groups)

Th_1-Priming



Pro-inflammatory cytokines show different vehicle-effect relationships from one compartment to another.

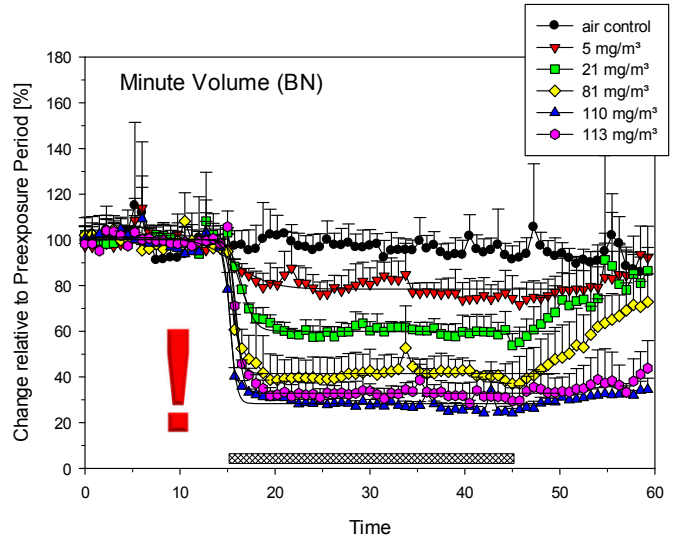
Summary: Rationalization of Protocol

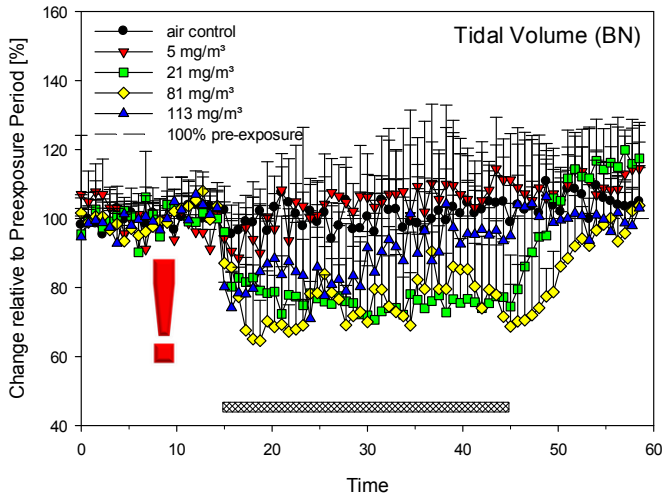


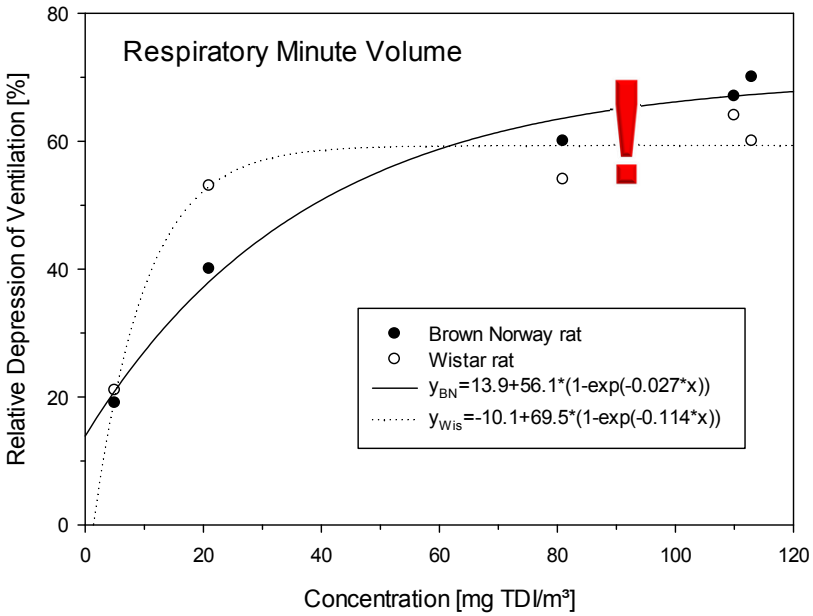
SEBA: di-n-octyl sebacic acid ester (20%)
AOO: acetone:olive oil (20%)
MDI: neat (same dose in all groups)

- Each compartment of the lung gives a different read-out.
- The response in LALNs using different vehicles for sensitization shows variable outcomes.
- BAL-PMN most appropriate effect-based discriminator for both irritation and sensitization.
- Lung priming & elicitation threshold: repeated encounters above irritation threshold Cxt required.

Searching for the POD of Airway Irritation of Vapors: Acute Exposure (TDI)

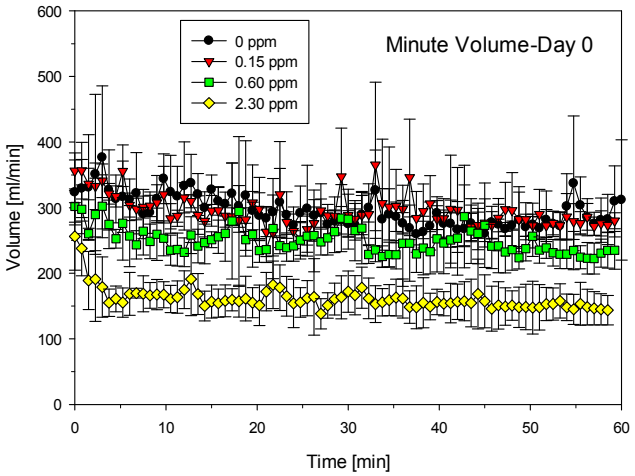


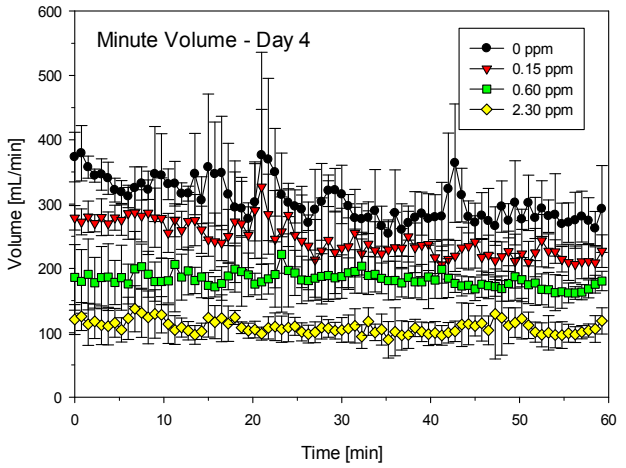


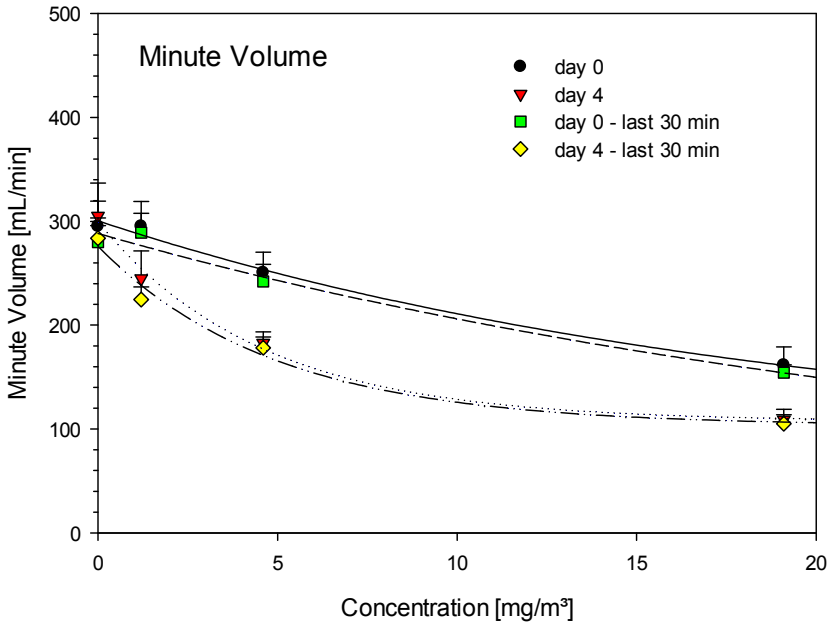


- C MUST be high enough to reach the alveoli
- The depression of ventilation MUST converge against stable breathing

Searching for the POD of Airway Irritation of Vapors: Recurrent Exposure (TDI)

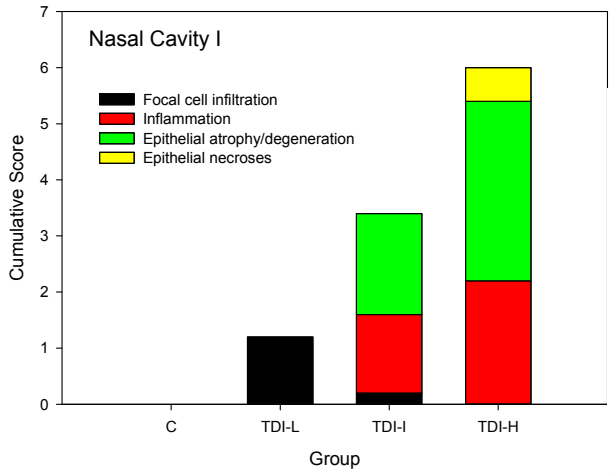


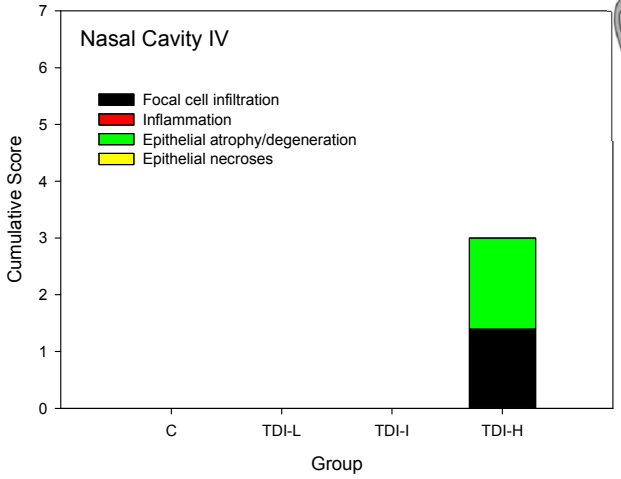


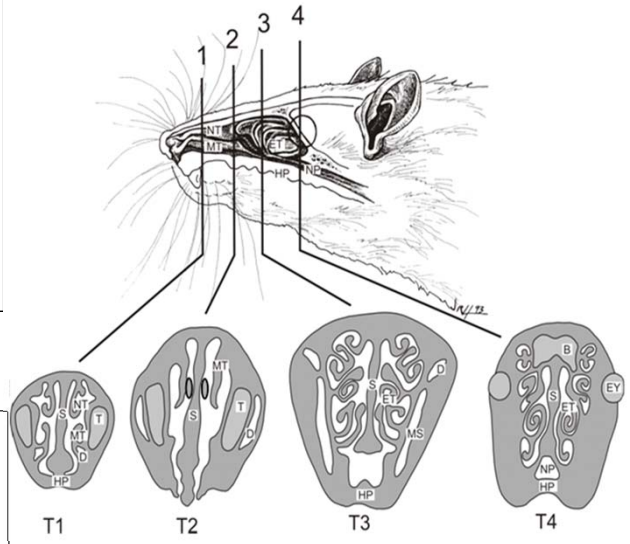


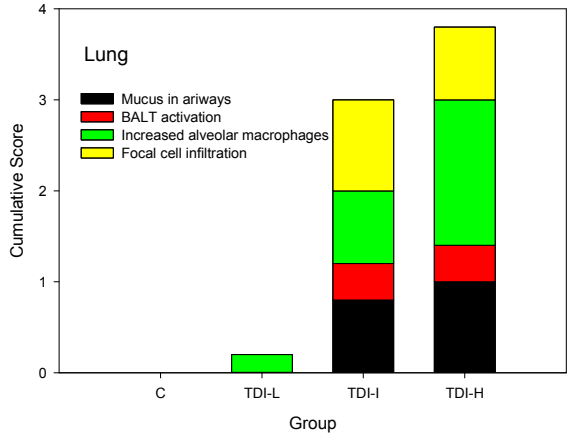
- Single exposure: POD = 0.03 x RD_{50} ~0.1 ppm.
- Repeated exposure: POD based on BMDL(95%) = 0.035 ppm (1st day) and 0.028 ppm (4th day).
- Reason: apparent expression of TRPA receptors on pulmonary C-fibers.

Searching for the POD of Airway Irritation of Vapors: Recurrent Exposure (TDI)









- POD-URTI = 0.028 ppm (4th day).
- Most TDI scrubbed between location 1-4
- What we see in the lung is response to injury but not irritant-inflammation

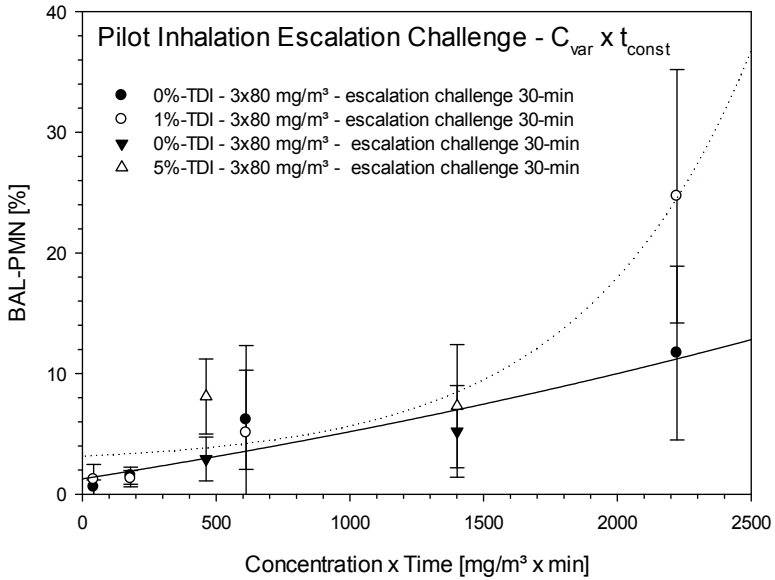
Conceptual Approach for a Respiratory "Sensitization"/ Elicitation Protocol for TDI-Vapor

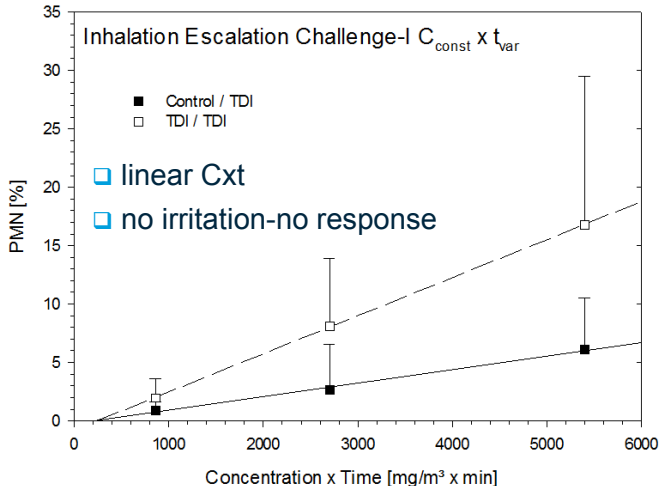
- Prevailing experimental evidence suggests that "*Respiratory Sensitization*" is a multi-step process depending on two independent processes:
  - Induction of a state of increased susceptibility to future encounters. This process is potentially reversible (apart from 'memory effect').
  - The induction of this process requires irritant (inflammatory) encounters at high doses. This can most suitably be achieved by skin exposure(s).
  - This state is evidenced by the determination of pro-inflammatory factors which do not necessarily distinguish the irritant and allergic etiopathologies.

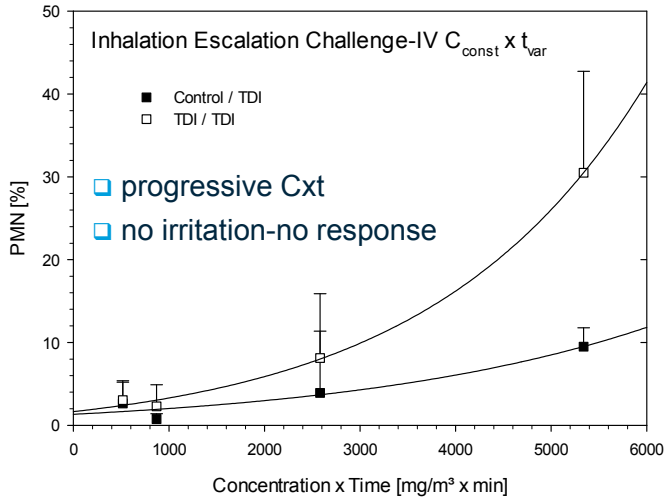
Conceptual Approach for a Respiratory "Sensitization"/ Elicitation Protocol for TDI-Vapor - Continuation

- Hence any "*Respiratory Sensitization*" can only be revealed and quantified by "*Respiratory Elicitation*" in animals "*predisposed to asthma*"
  - Repeated inhalation elicitation encounters above the lung irritant threshold dose (Cxt) are needed for progression & aggravation.
  - The induction of this process requires multiple highly rationalized irritant (mildly inflammatory) encounters at defined Cxt's to produce an 'asthmatic rat'.
  - The elicitation-threshold in asthmatic rats is irritation (Cxt)-dependent.

Comparison of Respiratory Sensitization/Elicitation Protocols for TDI-Vapor





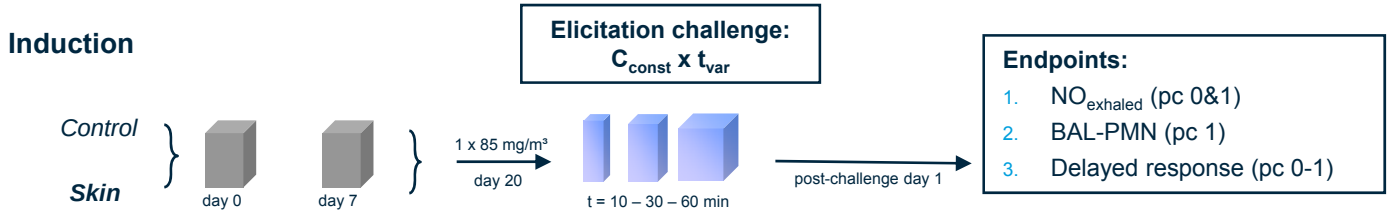


- **Aerosols:** Dosimetrically & physiologically reproducible (MDI)
- **Vapors:** Dosimetrically & physiologically very complex (TDI)
- **Hence, C_{const} x t_{var} protocols are deemed to be most robust for both aerosols & vapors**

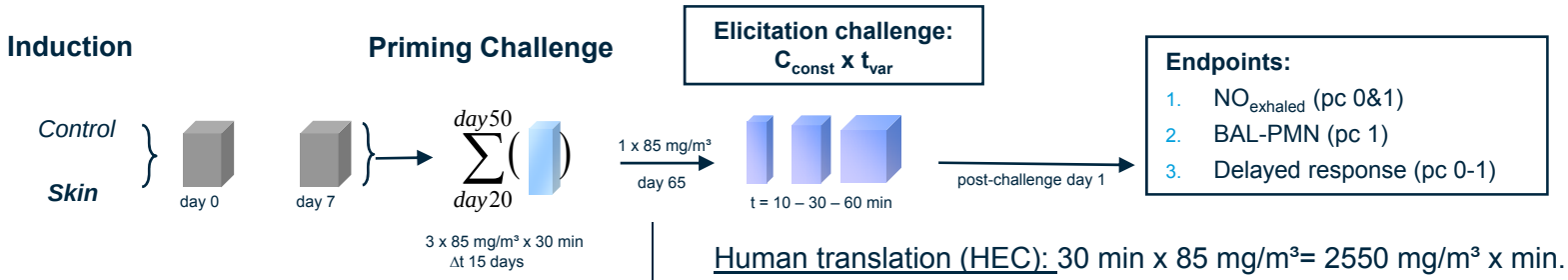
Conceptual Approach for a Respiratory "Sensitization"/ Elicitation Protocol for TDI-Vapor

Pre-step: Irritation inhalation assays as typically used in inhalation toxicology as ancillary studies for the dose-selection of repeated inhalation exposure studies.

Step I: Elicitation threshold after first inhalation challenge

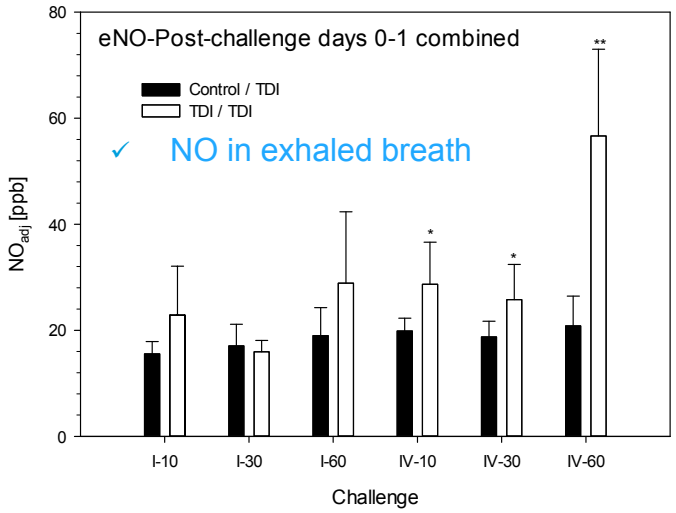


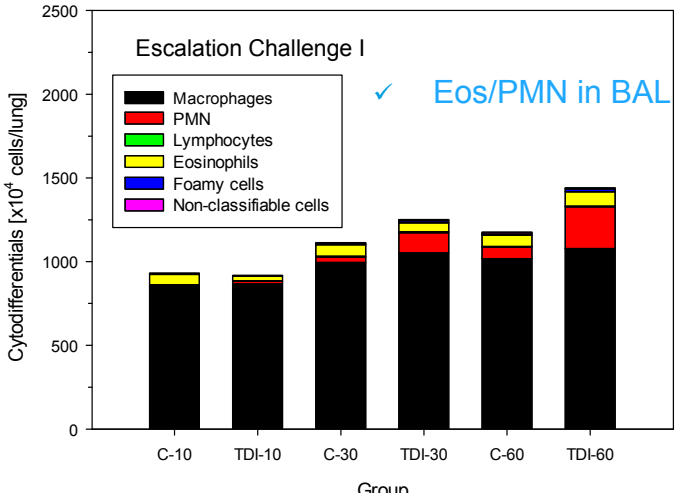
Step II: Elicitation threshold after repeated inhalation challenges

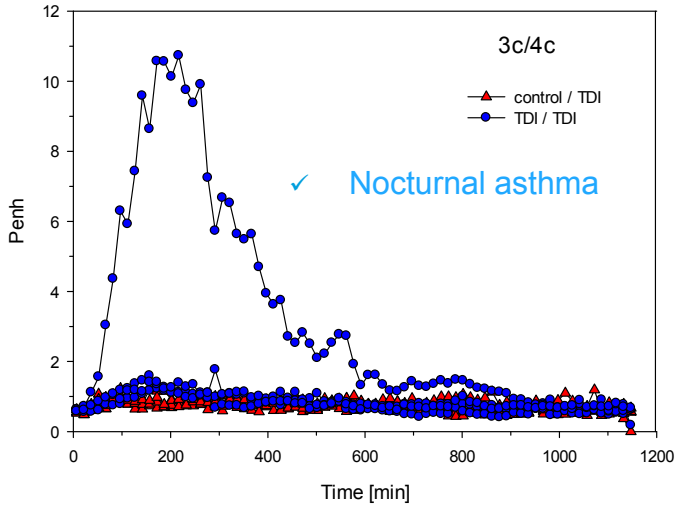


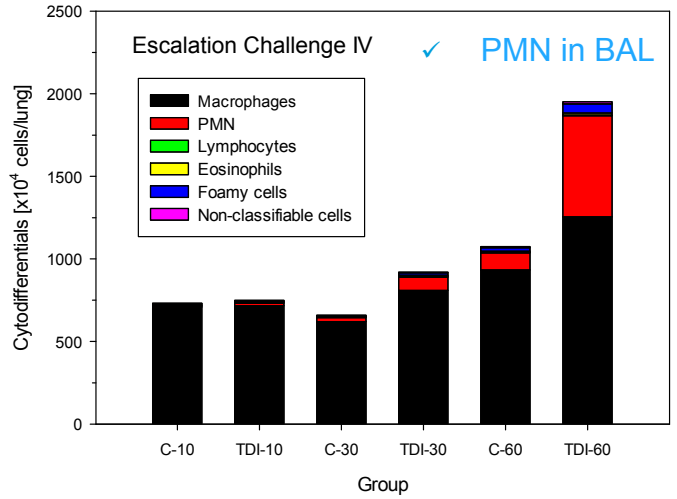
Human translation (HEC): 30 min x 85 mg/m³= 2550 mg/m³ x min.
→ HEC=2550x1/7x1/(10x3x3)=0.04 ppmV x 90 min; 0.04 ppmV x 90 min resulted in 33% responders in workers with TDI-asthma

Translational patho(physio)logical Hallmarks of Human and BNR-Asthma

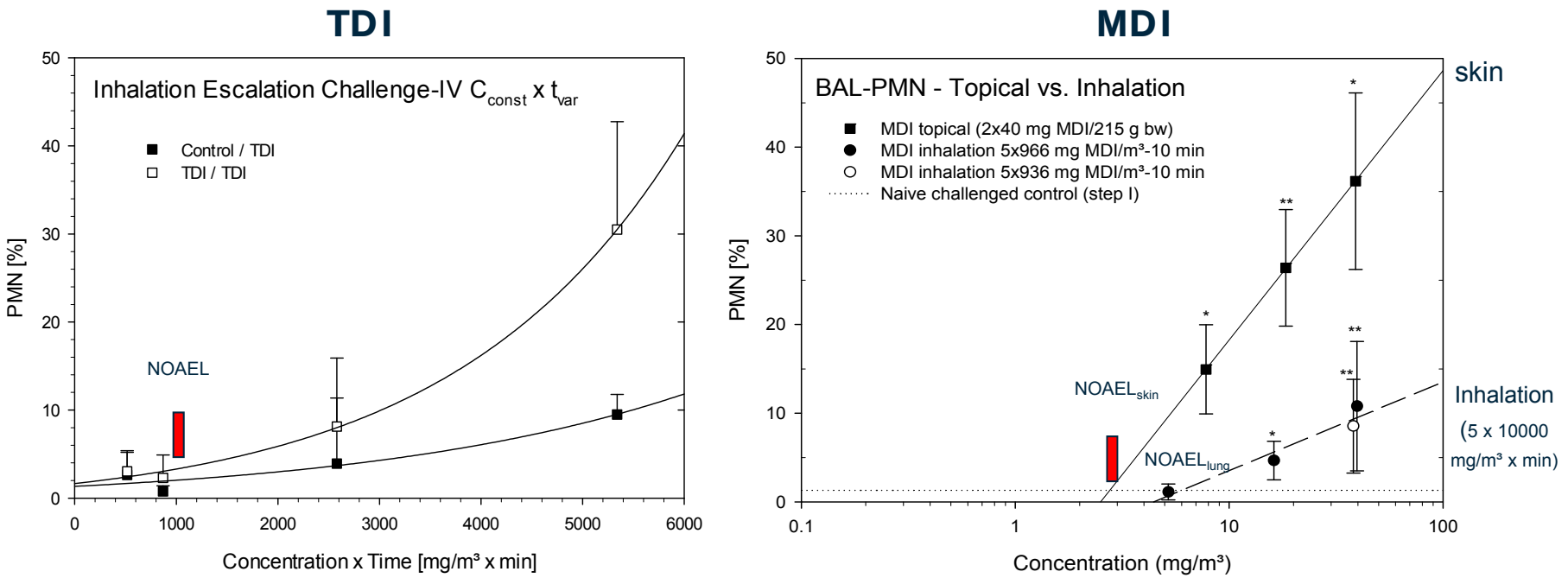






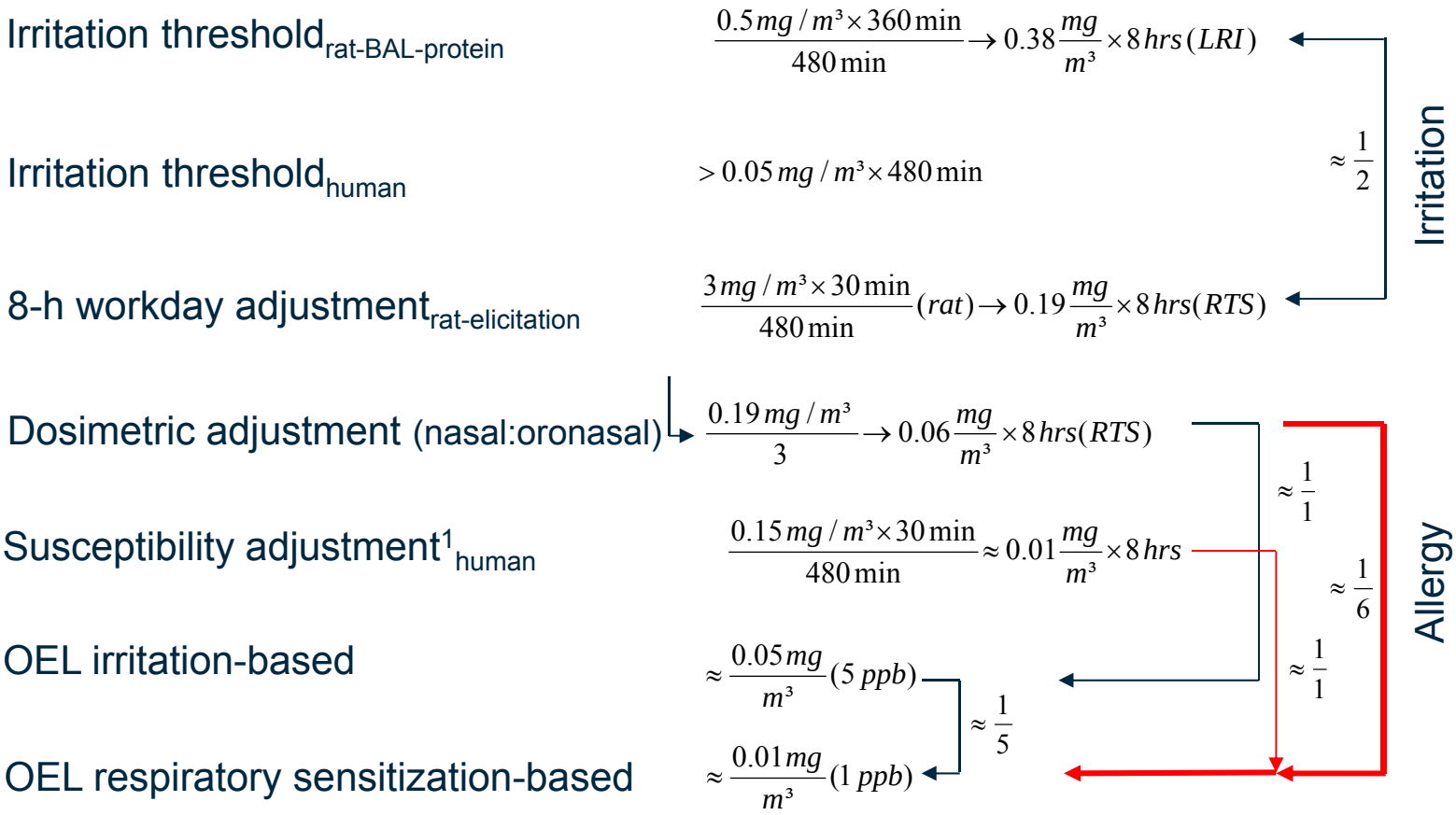


Dose-Response Analysis: TDI-Vapor & MDI-Aerosol



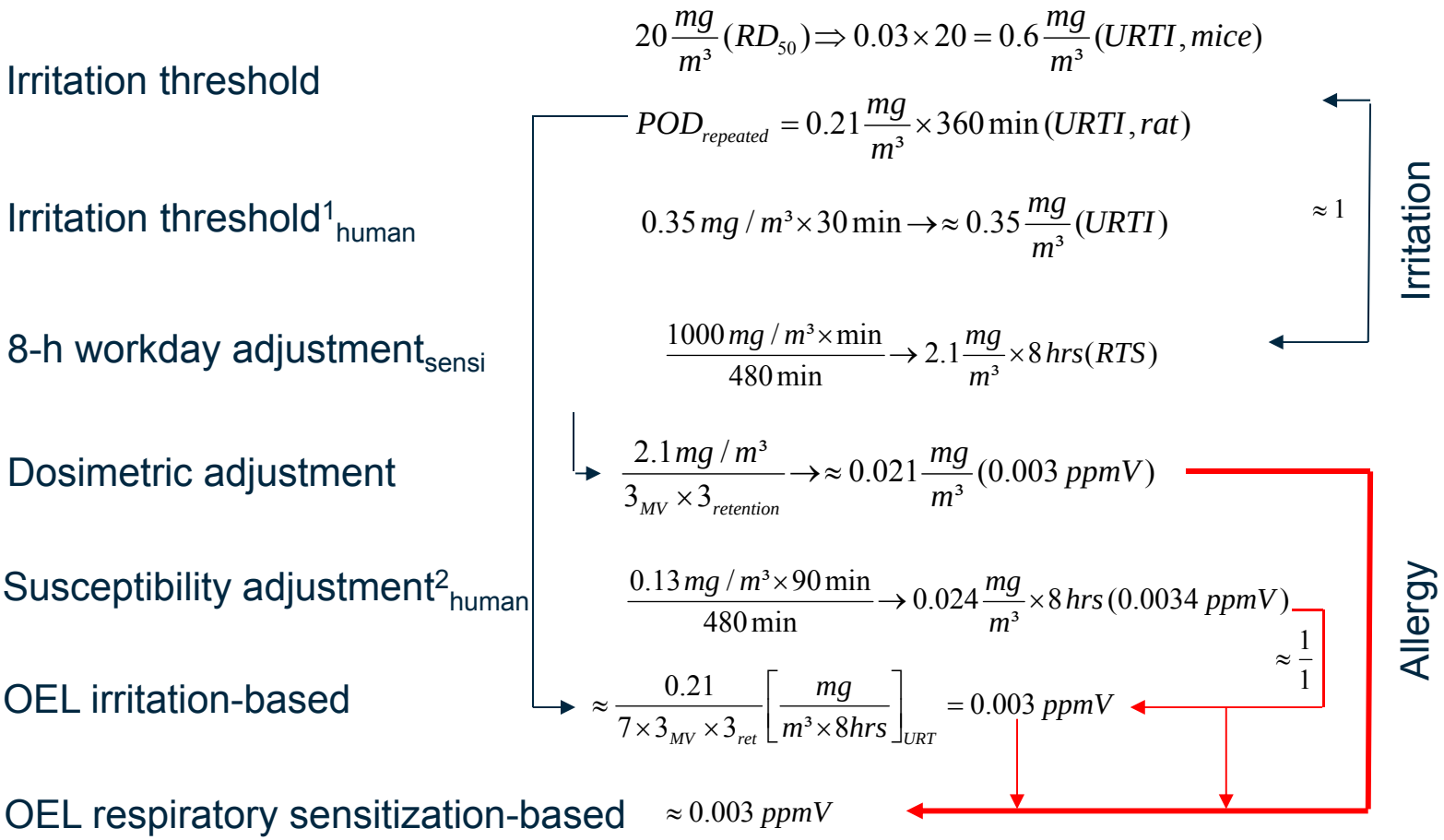
- **TDI:** The acute irritant threshold, which is 0.035 ppm x 6h for TDI, corresponds to ~10-times the elicitation threshold of 1000/(7x360) mg/m³ x min = 0.4 ppm x 6h .
- **MDI:** The acute irritant threshold, which is 0.5 mg/m³ x 6 h for MDI (180 mg/m³ x min), corresponds to ~2-times an elicitation threshold of 90 mg/m³ x min.
- These apparent differences in potency are related to dosimetric differences (vapor vs. aerosol)

MDI: Dosimetric Adjustment & Species Extrapolation



RTS: Respiratory Tract Sensitization; 1) Leroyer et al. (1998) Specific bronchoprovocation test

TDI: Dosimetric Adjustment & Species Extrapolation



RTS: Respiratory Tract Sensitization; 1) Henschler et al., 1962; 2) Vandenplas et al. (1992), Sastre et al. 2003); RD_0: Barrow et al. (1978)

Summary

- The major prejudice of “Respiratory Sensitization” is believed to depend on inhalation sensitization. It is likely more an inhalation priming of already predisposed/sensitized subjects.
- The protocols devised duplicate the key hallmarks of human asthma, including the structure & dosing protocols used in human inhalation bronchial challenges.
- All endpoints measured are quantifiable in terms of dose and integrated effect.
- For MDI-aerosol and TDI-vapor the dosimetrically-adjusted irritant dose was remarkably close to the effective human challenge dose.
- To be effective, elicitation doses must be above the irritant threshold Cxt to prime the respiratory tract for “Respiratory Sensitization”.

Conclusion

- Respiratory sensitization requires recurrent irritant inhalation exposures to induce asthma.
- Both irritation and elicitation of respiratory sensitization are clearly threshold dose- (and NOT concentration-) dependent.
- Protection from irritation (conventional basis of NOAEL) protects also from “sensitizing” the respiratory tract.
- OELs and DNELs can be derived using standard inhalation bioassays.
- Proof-of-principle studies may require a slightly higher degree of sophistication.