Common Mechanism-based Study Design for Inhalation Toxicity Testing of Poorly Soluble (Nano-/Ultrafine-) Particles: *Are new Testing Strategies and Interpretation Guidelines Needed?*

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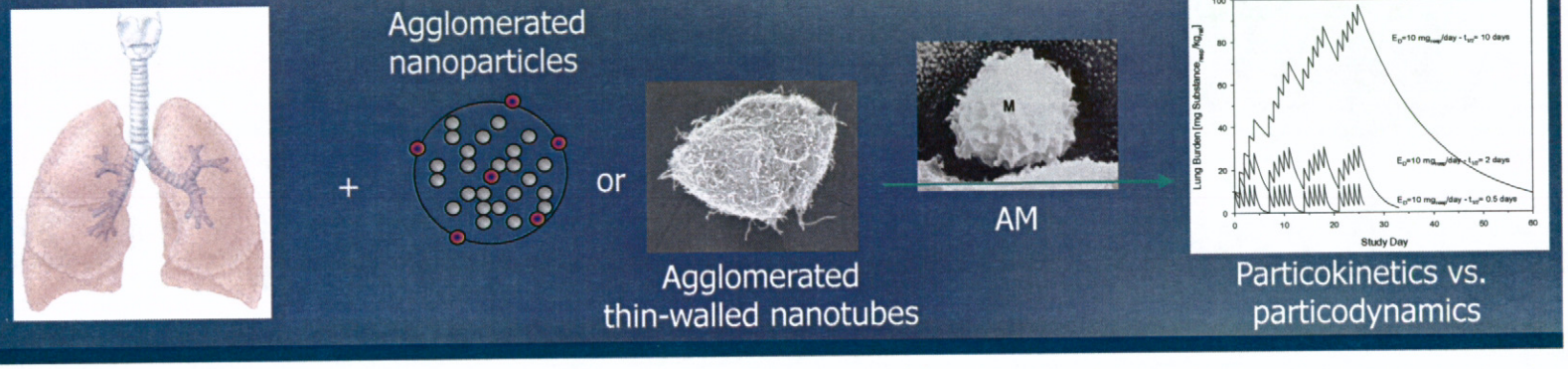
Cincinnati, February 3, 2011

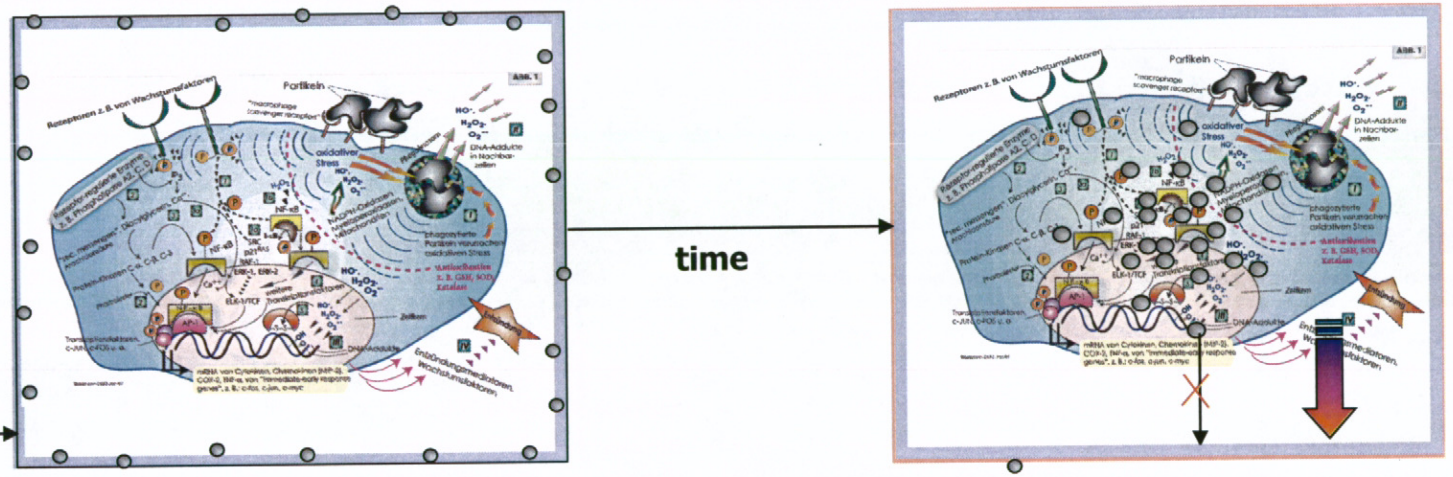


Bayer Healthcare
Institute of Toxicology / Inhalation Toxicology
Wuppertal, Germany

Poorly Soluble Particles: Basic generic Mechanism

Direct mode of action (acute)

Retention-related cell-mediated mode of action (accumulative, chronic)



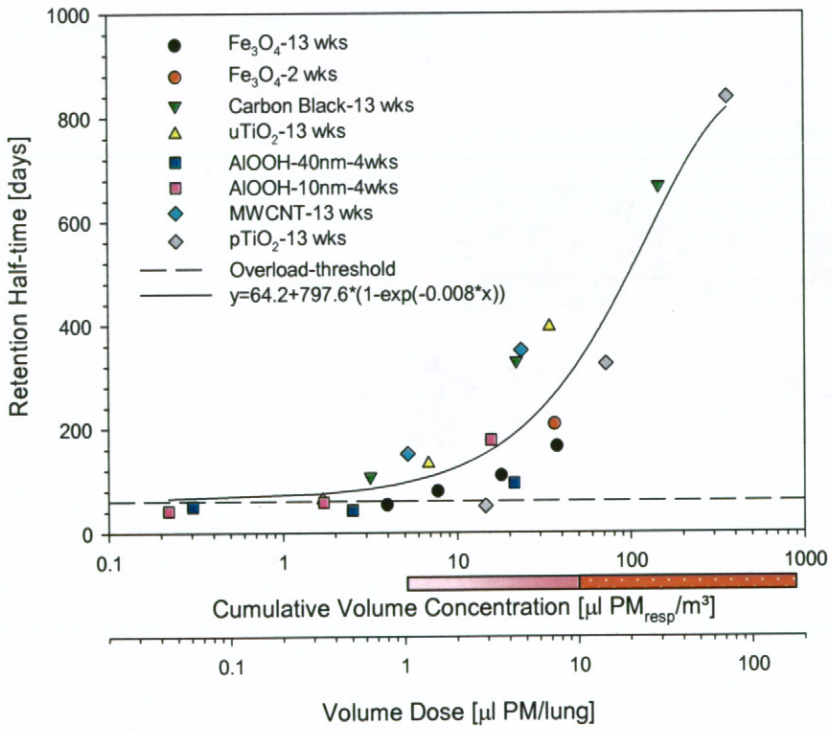
Type II Cell activation

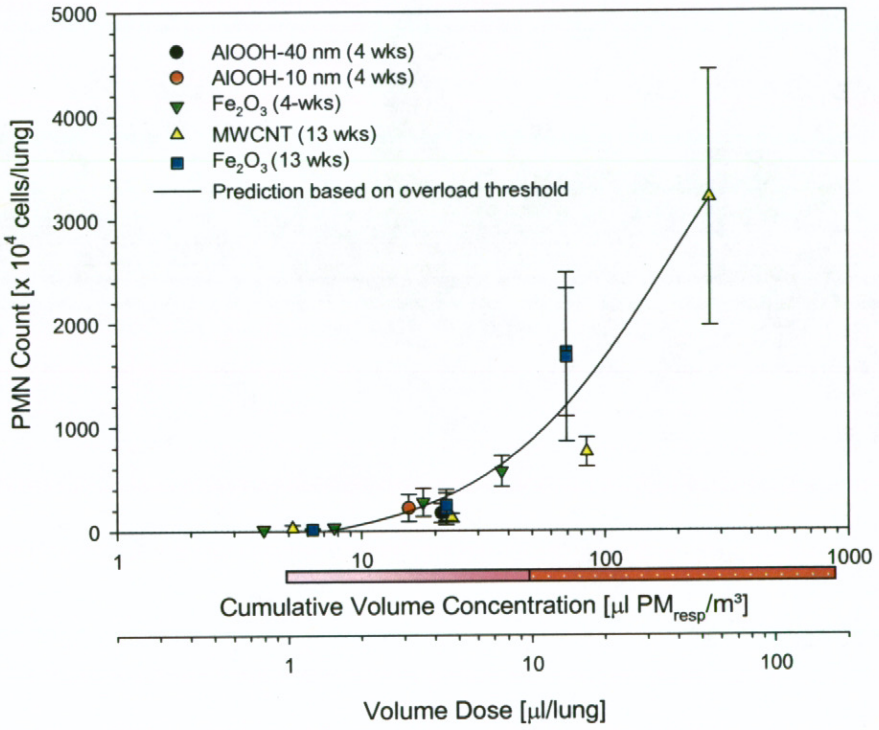
time

- Occupational risk characterization should be based on the chronic, cumulative outcome observing the most critical mode of action.
- Adversity is triggered by the quantity of inflammatory cells and their degree of activation orchestrating the chronic inflammation.
- The magnitude and time course of effects follows the kinetics of particle-laden phagocytes and not the particles themselves.

Correlation of cumulative Lung **Dose** and BAL-PMN **Effect**

$$Cumulative\,volume\,concentration = C_i \times F_a \times E_d / \rho_e \left[\frac{\mu l}{m^3}\right]$$



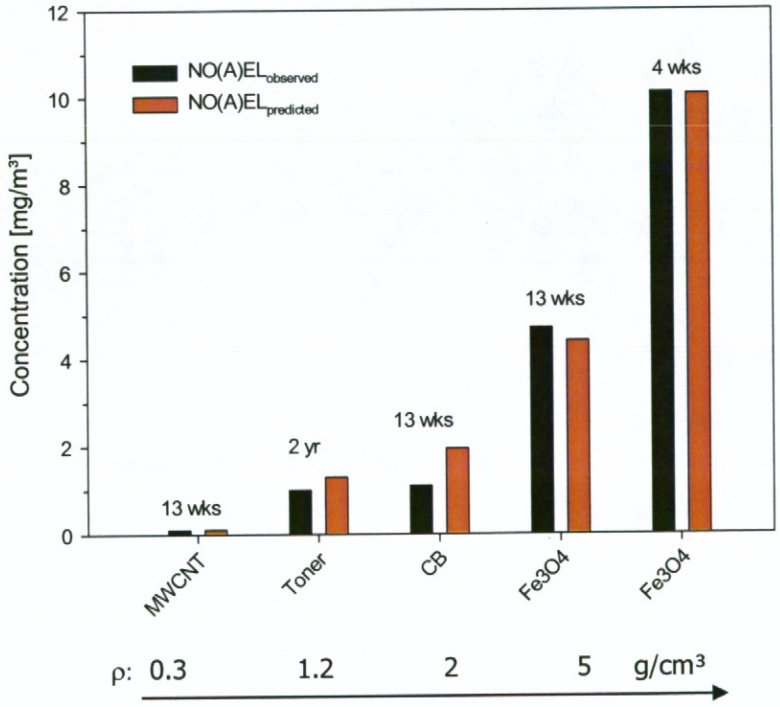


Kinetic threshold (homeostasis) < threshold of inflammation

Conclusion I

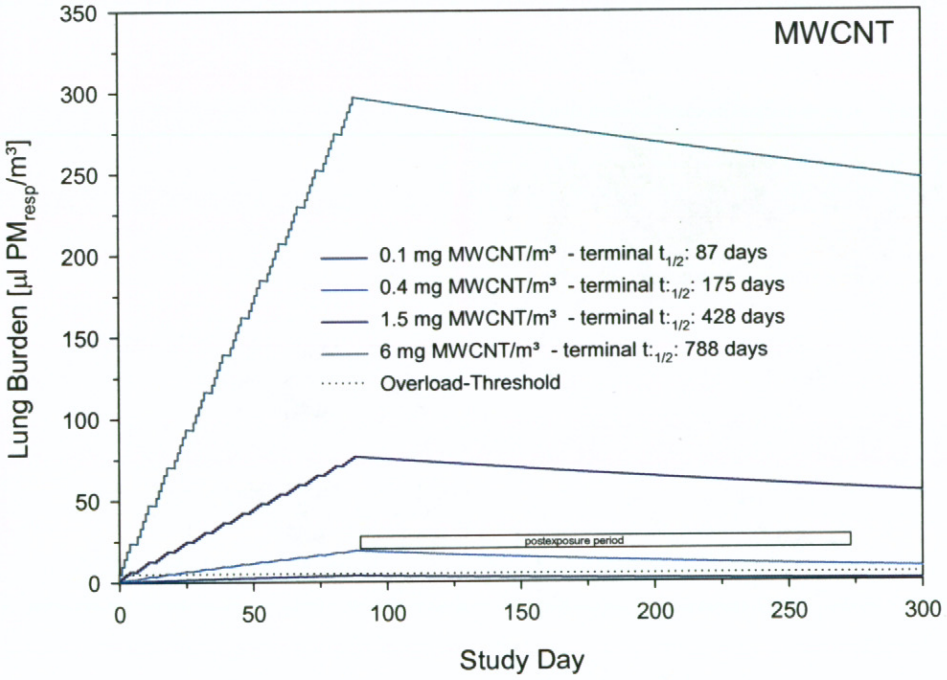
- The unifying metric 'agglomerate volume' appears to be the most favorable metric to predict the $NOAEL_{kinetics}$ of any study.
- NOAELs can be predicted for PSPs covering a wide range of concentrations and specific particle densities.
- The NOAEL appears to be proportional to the specific density of agglomerate (or the more volume at a fixed mass is retained, the higher the toxicity appears to be).
- Long-term pulmonary effects follow the retained cumulative dose.
- This analysis supports a conclusion that the $NOAEL_{PSP}$ solely depends on kinetic factors.

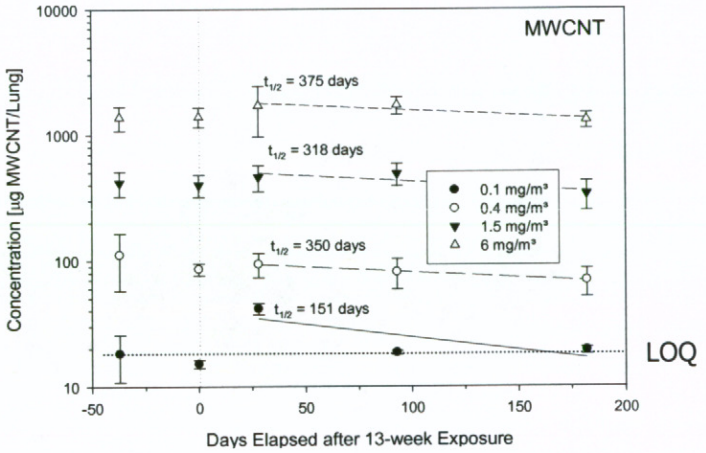
$$NO(A)EL_{predicted_i} = \frac{1\,\mu l}{0.29\,m^3} \times \frac{\rho}{f_{vi}} \times \frac{100}{PM_{resp}} \left[\frac{mg}{m^3}\right]$$

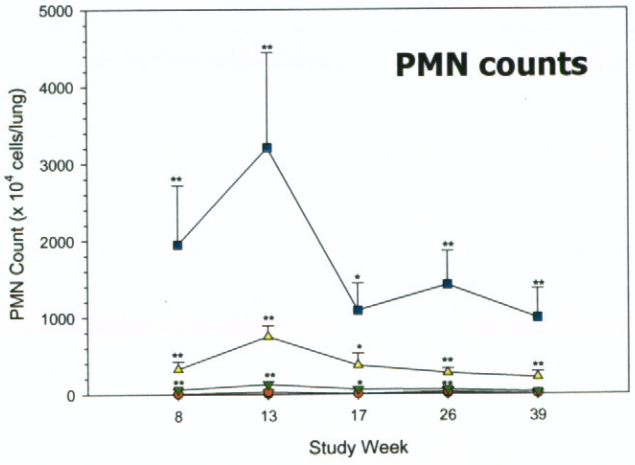


Predicted study-specific NOAEL

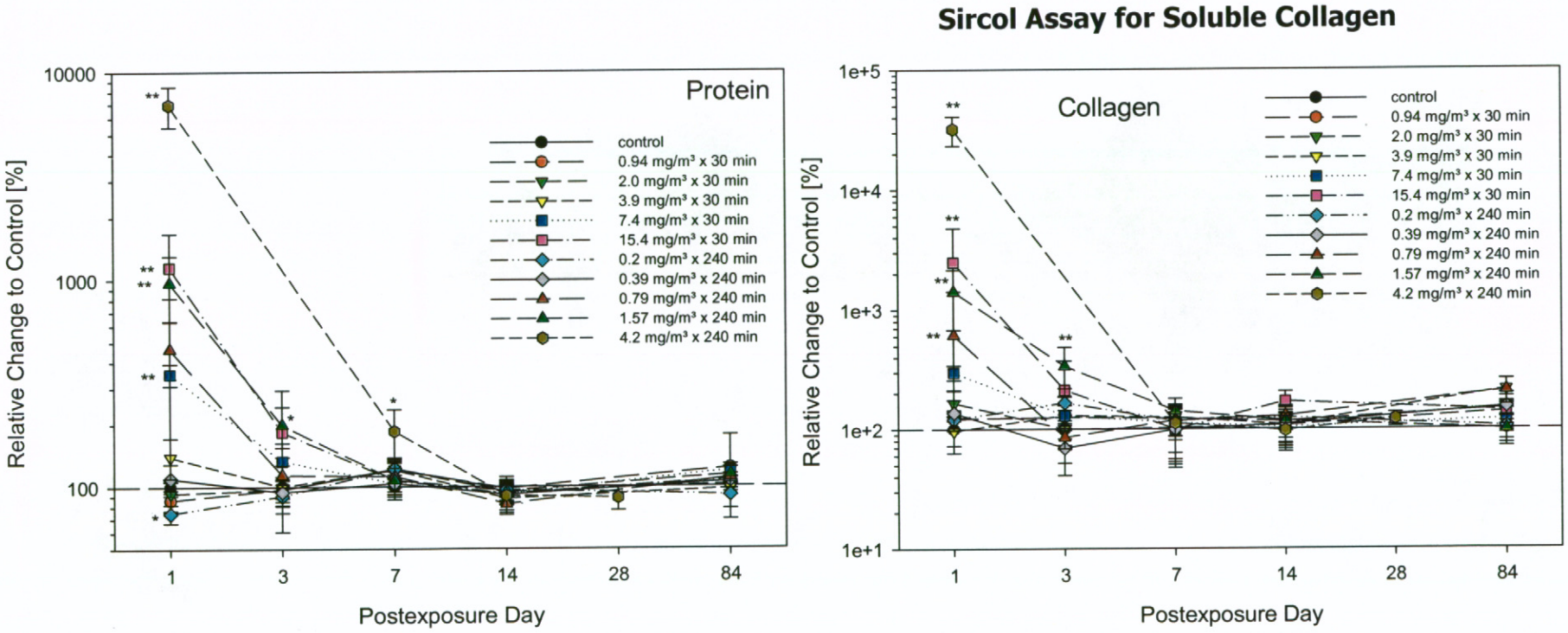
The Kinetic Cornerstones of PSP-related Lung Toxicity





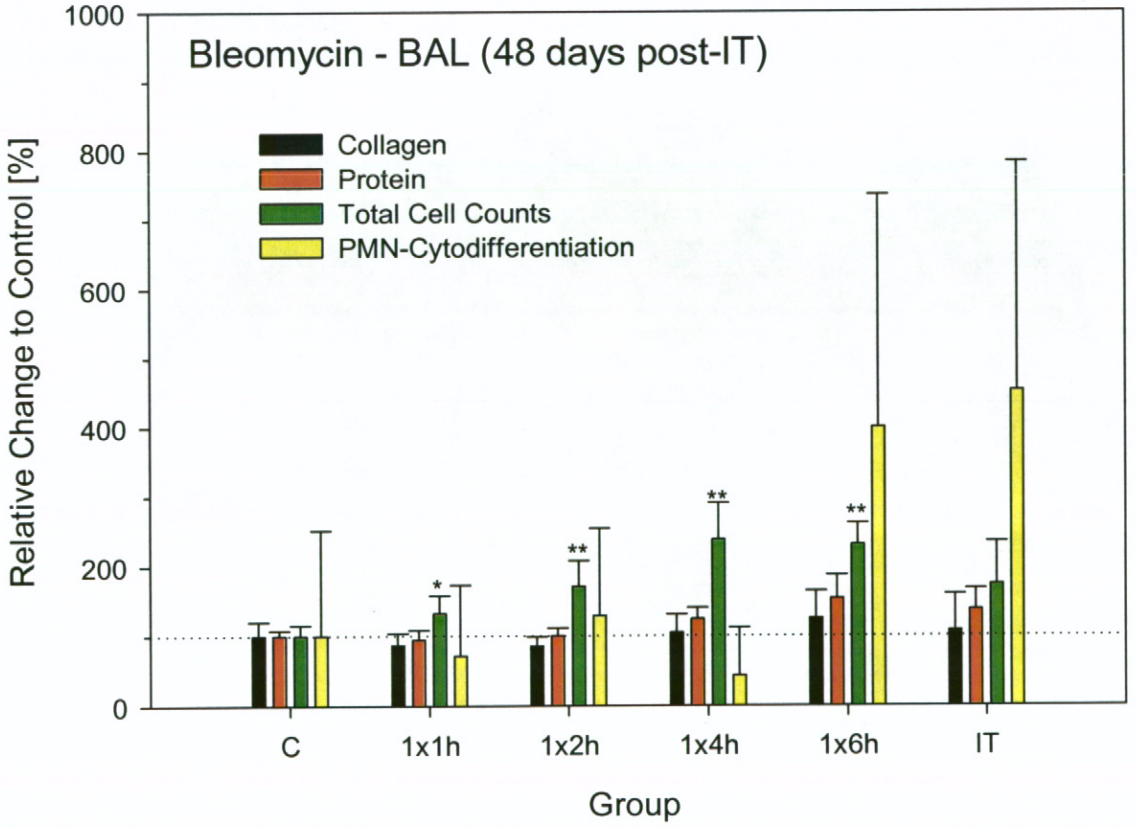


Phosgene: Time-Course Studies do not show Evidence of Chronicity

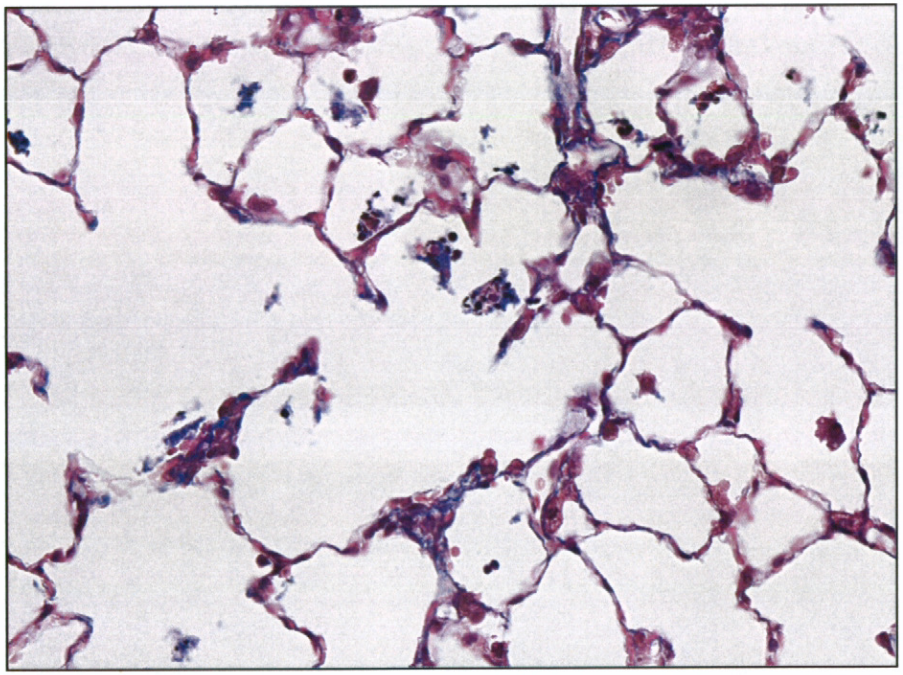


BAL-collagen is evidence of alveolitis and parallels the influx of protein as a result of acute inflammation (alveolitis). At maximum alveolitis BAL-collagen was 300-times above control (MWCNT 16-imes) without any long-term sequelae.

Sircol-Assay (BAL): Bleomycin



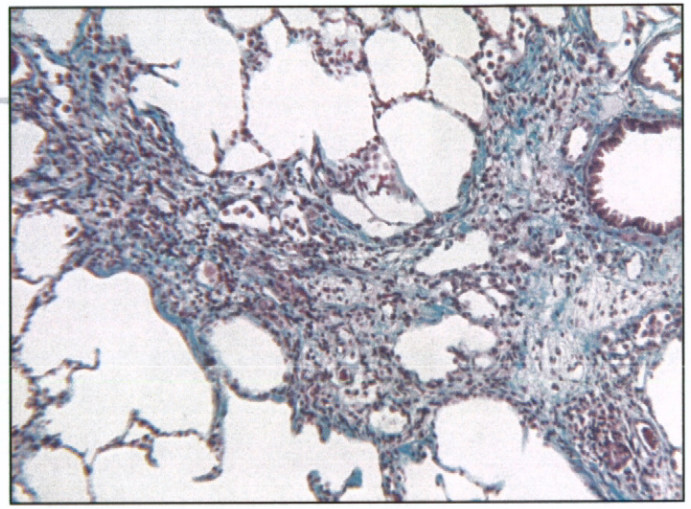
Inflammatory Collagen vs. Fibrosis



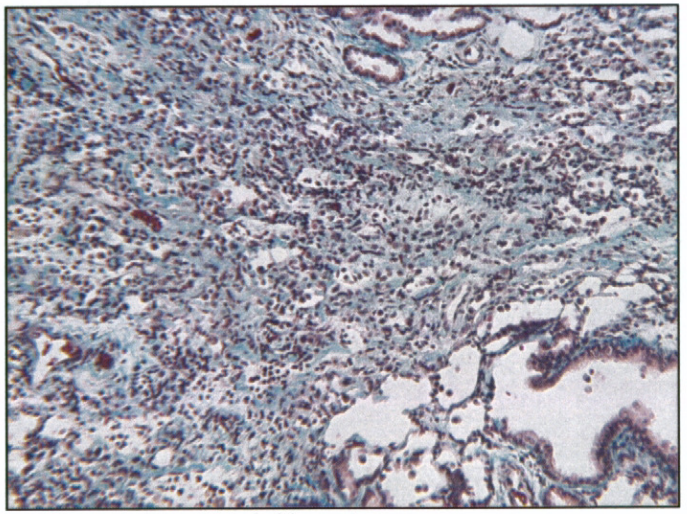
MWCNT, 6 mg/m³ - subchronic exposure

Masson Goldner Stain

Bleomycin
(1xIT, after 6 wks)

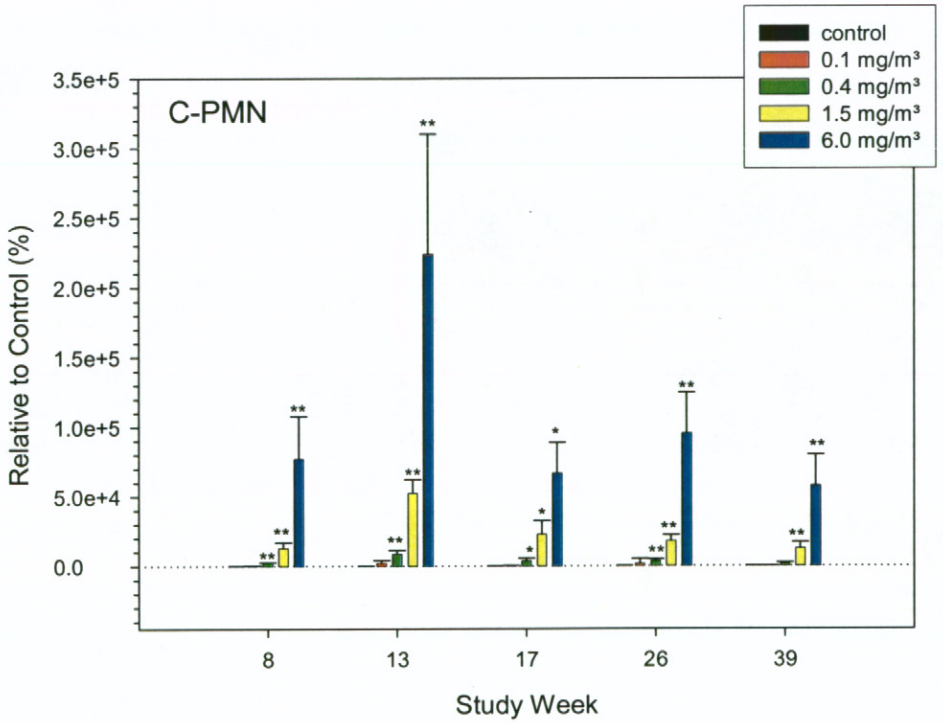


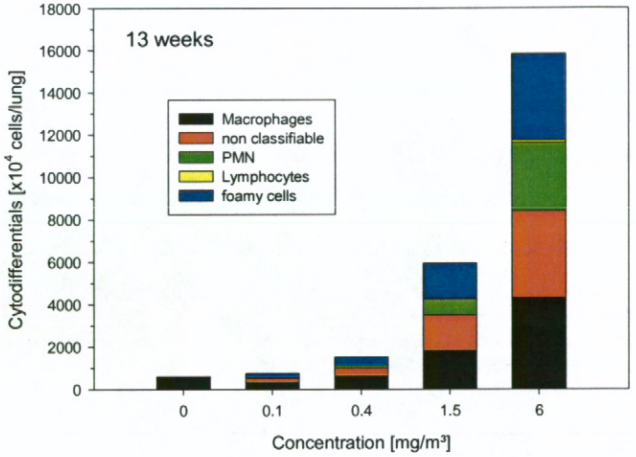
Septal thickening with focal interstitial fibrosis

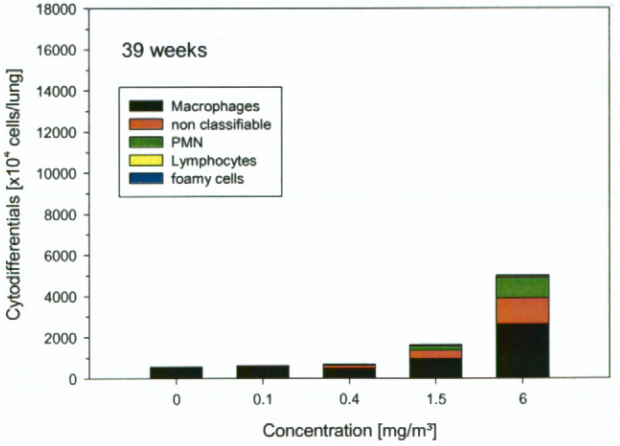


Diffuse interstitial fibrosis

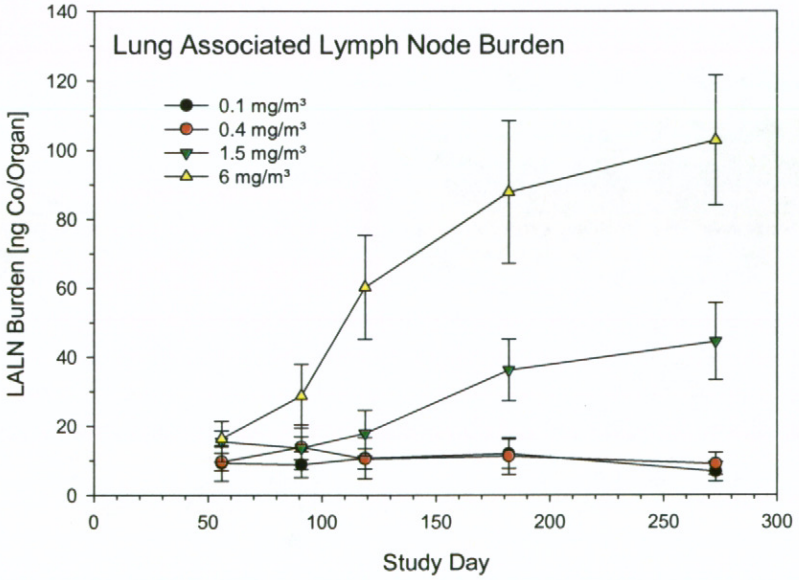
MWCNT 13 wk Study (Baytubes)

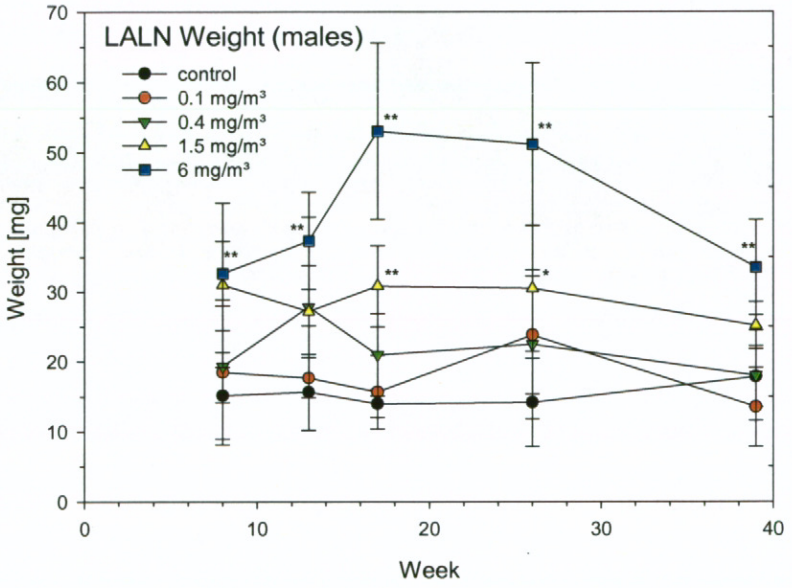






MWCNT 13 wk Study (Baytubes)

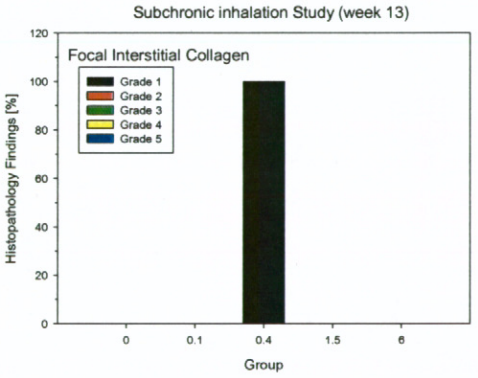


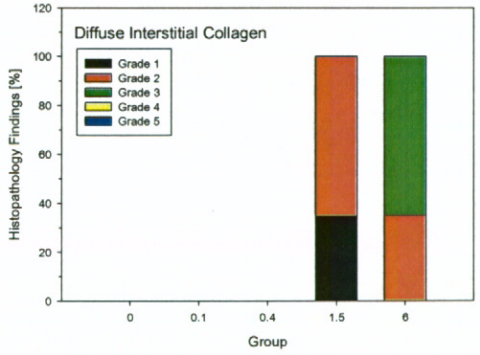


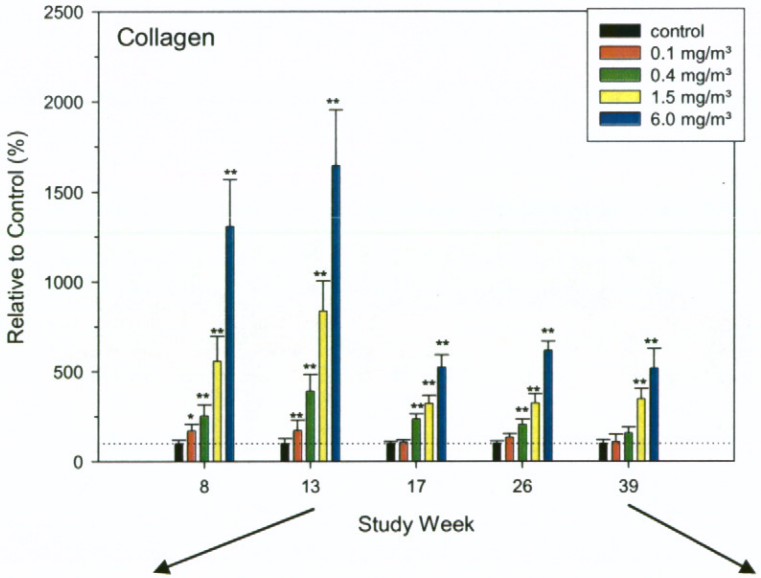
Kinetic data shows increased lymphatic clearance at overload-induced inflammatory lung burdens

MWCNT 13 wk Study (Baytubes)

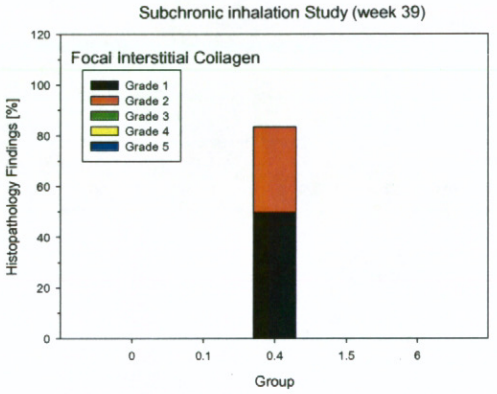
1: minimal; 2: slight/mild; 3: moderate

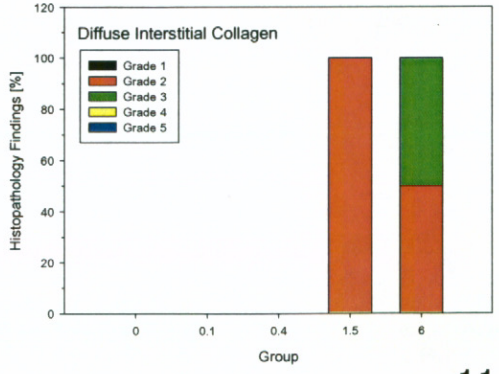






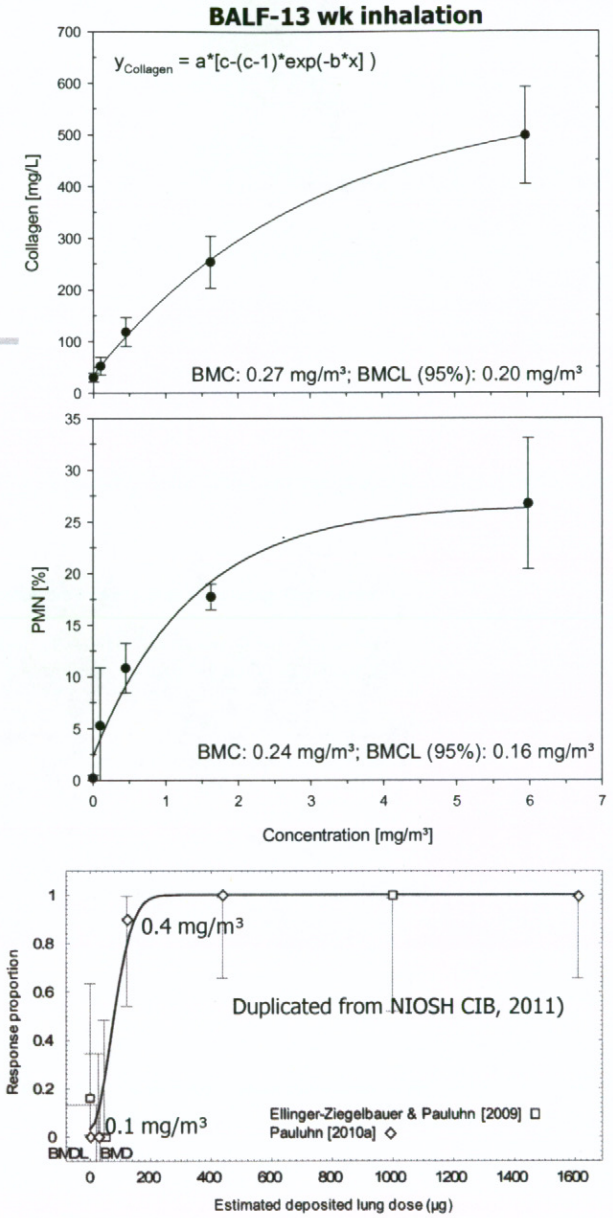
- **Evidence of acute-on-chronic effect with fast recovery due to 'inertization'**
- **Kinetics of recovery matches the kinetics of overload**
- **Resolution of dose-response in BAL-data more amenable for quantitative risk assessment than ordinal data from conventional histopathology.**





PODs of 13-wk Inhalation Study

- $NOAEL_{alveolitis}$: 0.1 mg/m³
  - $NOAEL_{B\text{-}BAL\text{-}PMN\%}$: 0.16 mg/m³
  - $NOAEL_{B\text{-}BAL\text{-}collagen}$: 0.20 mg/m³
- $NOAEL_{histo}$: 0.1 mg/m³
- $NOAEL_{kinetics\&BAL\text{-}TCC}$: 0.1 mg/m³
- $NOAEL_{PBPK}$: 0.12 mg/m³ (δ=0.1 mg/cm³)
- Fibrosis was not found at any exposure level (2 internal + 2 external pathologists)
- <u>Comments:</u>
  - In the absence of definition of ordinal scores histo findings cannot be used for quantitative risk analyses (*sub-site, intensity, abundance*)
  - Incorrect 're-interpretation' of study: no distinction between locally synthesized insoluble cross-linked collagen and extravasated soluble collagen
  - Analysis does not mirror of the dose-response relationship of the primary injury pattern which is alveolitis



"Early-stage pulmonary fibrosis (proportion of rats with minimal or greater focal interstitial thickening)"

Estimation of Human Equivalent Lung Dose

Ventilation x Deposition:

$$AF_{lungburden-A/H} = \frac{V_{E-A} \times F_{a-A}}{V_{E-H} \times F_{a-H}} \times \frac{BW_H}{BW_A} = \frac{0.29 \times 0.075 \times 70}{10 \times 0.164 \times 1} = 0.93$$

: **Ventilation of rat adjusted on 1 kg body weight**

Clearance:

$$AF_{clearance-A/H} = \frac{V_{d-A} \times k_{e-A}}{V_{d-H} \times k_{e-H}} = \frac{V_{d-A} \times t_{1/2-H} \times \ln 2}{V_{d-H} \times t_{1/2-A} \times \ln 2} = \frac{7 \times 10^{10}{}_A \times 400_H}{50 \times 10^{10}{}_H \times 60_R} = 0.93$$

: **400/60=6.7 vs. 700/100 = 7.0**

Parameterization of V_d:

	Average AM Volume (μm^3)	Average AM Counts in Lung	AM Volume per Lung (μm^3/kg bw)
Rat (kg-based)	1166	6*x 10^7	7 x 10^{10}
Human (70 kg)	4990	7.0 x 10^9	5 x 10^{11}

*: empirically confirmed in the current 13-week rat inhalation study

Derivation of OEL

Empirical data-based:

$$OEL = NO(A)EL_A \times \frac{AF_{lung\ burden-A/H}}{AF_{clearance-A/H}} \times \frac{1}{AF_{StudyDuration}} = \frac{0.93}{0.93 \times 1.5} \times NO(A)EL_A$$

$$= \frac{0.16}{1.5} = 0.1 \frac{mg\ MWCNT_{Baytubes}}{m^3} (benchmark)$$

Generic data-based:

$$OEL_{generic} = \frac{0.5\ \mu l\ PM_{respirable}}{m^3} \times \frac{0.93}{0.93} \times \delta_{agglomerate} = 0.5 \times 0.3 \frac{mg}{m^3} = 0.15 \frac{mg}{m^3}$$

Which means the theoretical mechanism-based approach verifies the more conservative empirical approach. NOAEL depends on kinetic factors (dosimetry) not MOA-specific additional uncertainties.

Conclusion

$$OEL_{generic}\ [mg/m^3] = 0.5\ \mu l\ PM_{resp}/m^3 \times \rho$$

- Lung toxicity appears to be dependent on agglomerate or 'assemblage' kinetics rather than on primary particle properties (no fibers found in air or AMs).
- Lung dosimetry must be accompanied by both respiratory tract histopathology and BAL to deliver meaningful data for human risk characterization (site of dose-effect relationships).
- The metric of dose must be defined by the mechanism of target organ injury.
- Inhalation studies should be designed to meet the kinetic cornerstones of Poorly Soluble Particles. This would improve the comparability of studies across different laboratories and reduces animal use at limited resources.
- Hypothesis-based inhalation studies deliver maximum information on the conclusiveness of findings.