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Pulmonary toxicity and genotoxicity of carbon nanotubes in rat, a subacute inhalation study

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A common European approach to the regulatory testing of nanomaterials

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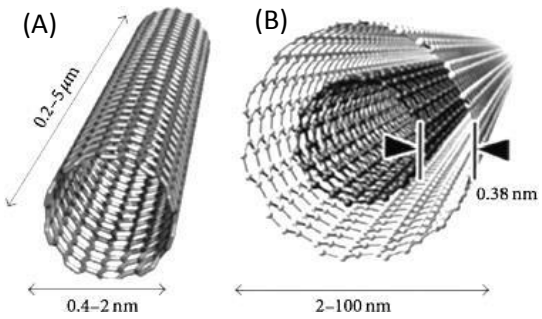
Carbon nanotubes



Unique physical properties

- Mechanical
- Electrical

Industrial et medical applications

- Nanoelectronics
- Composite materials
- Sport goods
- Hydrogen storage
- Drug carriers

Toxicological properties

- Large variety of CNTs→ Different toxicological profiles
  - Number of « walls »
  - Length, diameter
  - Fonctionnalization (COOH, OH…)
  - Impureties
  - …

- Toxicological properties
  - Pulmonary inflammation
  - Granulomas, pulmonary fibrosis
  - DNA damages
  - Mesothelioma (intraperitoneal and endotracheal instillations)

- IARC
  - MWNT-7: category 2B (Possibly carcinogenic for humans)
  - Other CNTs: category 3 (Not classifiable as to their carcinogenicity to humans)

Objective

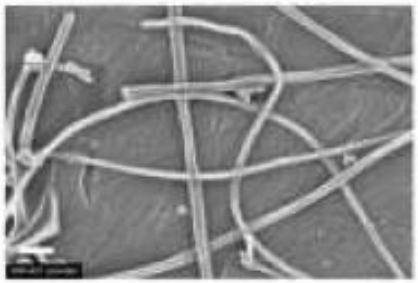
- Comparison of the pulmonary toxicity of two distinct carbon nanotubes (NM-401 and NM-403).

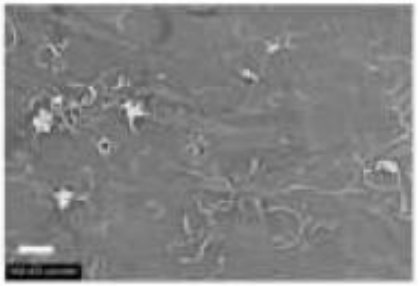
- Sub-acute nose-only inhalation study in rats

- Part of a larger project with NRCWE, DK (U Vogel, H Wallin, KB Knudsen)
  - Comparison of pulmonary toxicity of 30-40 CNTs following intratracheal instillation (Structure activity relationship)
  - Comparison of pulmonary toxicity of 2 CNTs: intratracheal instillation vs. inhalation

Main characteristics of CNTs

	NM-401	NM-403
	Pristine	Pristine
	MWCNT	MWCNT
	« Long and thick »	« Short and thin »
Length (µm)	4.0 ± 0.37	0.4 ± 0.03
Diameter (nm)	67 (24-138)	12 (5-37)
Specific surface area BET (m^2/g)	18	189
Bulk density (g/cm^3)	0.02	0.17
Purety (%)	98	96.9





Experimental protocol

Female
Sprague
Dawley Rats

Nose-only inhalation exposure

(2x3h) / day,
5 days / week,
4 weeks

Control
+ 2 doses
(0.5 and 1.5 mg/m^3)

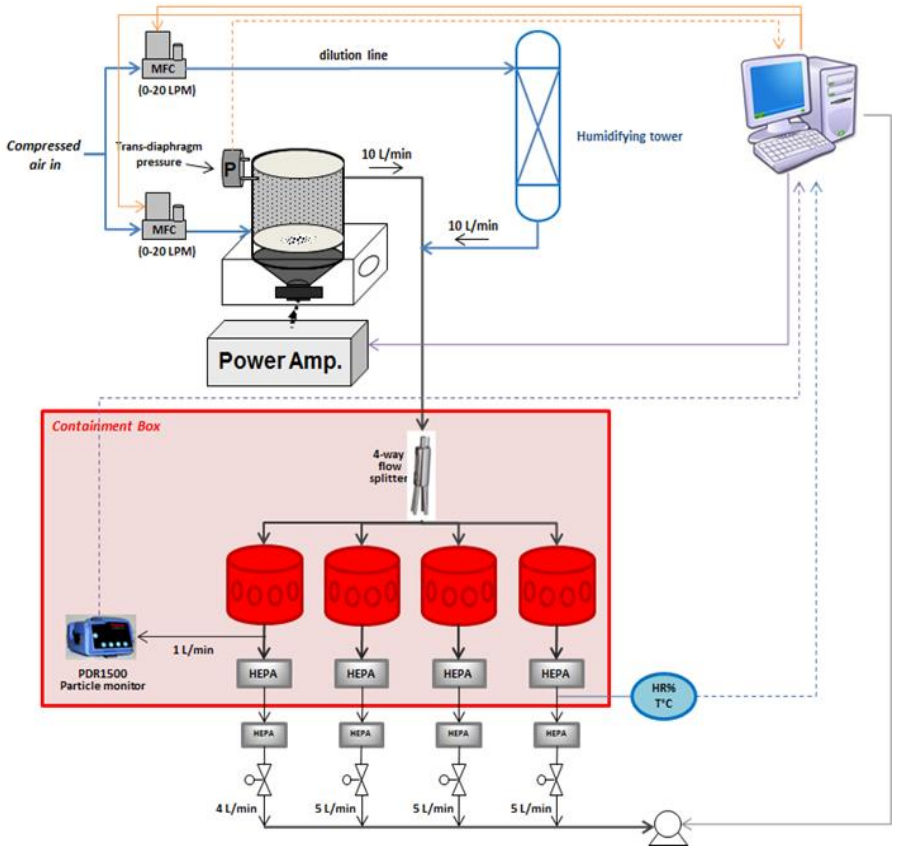
Tissue sampling

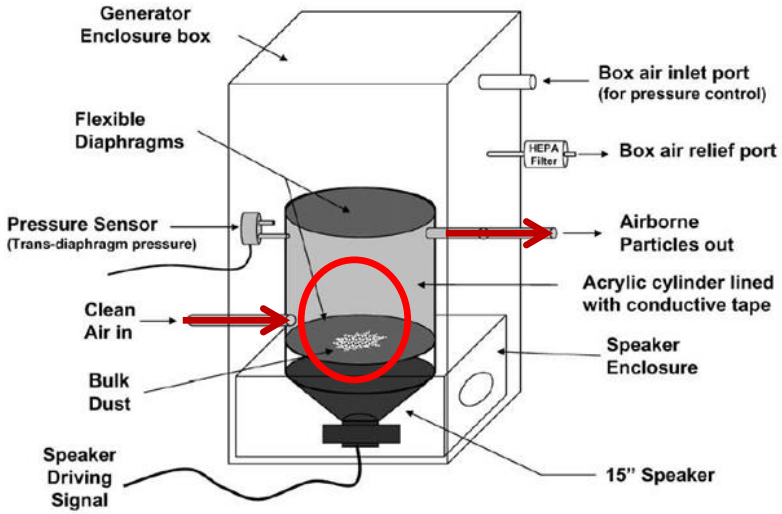
3 30 90 180

Post exposure time (days)

Inflammatory response (BALF)
Biochemical markers
Histopathology (H&E and Trichrome Masson)
Genotoxicity (comet assay)

Experimental set-up

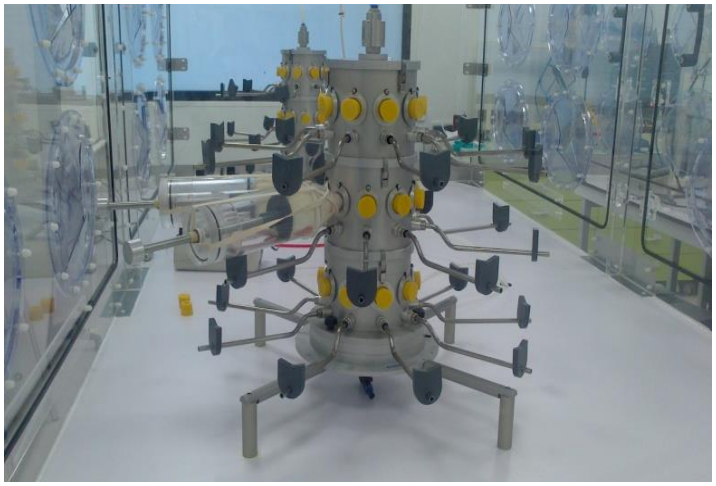


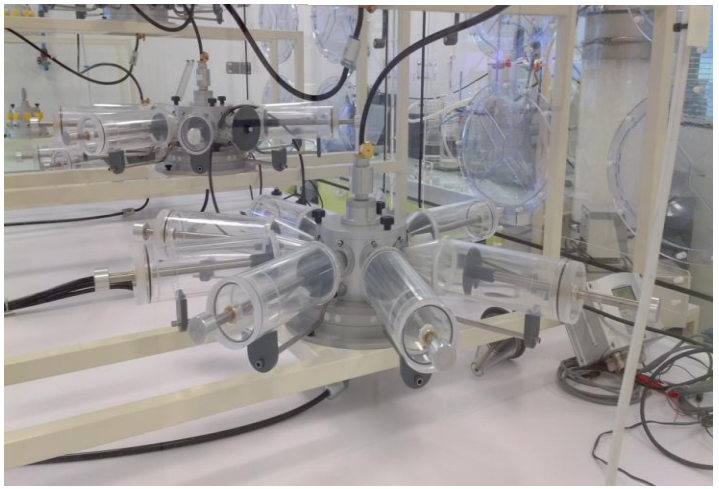


Mc Kinney et al, 2009

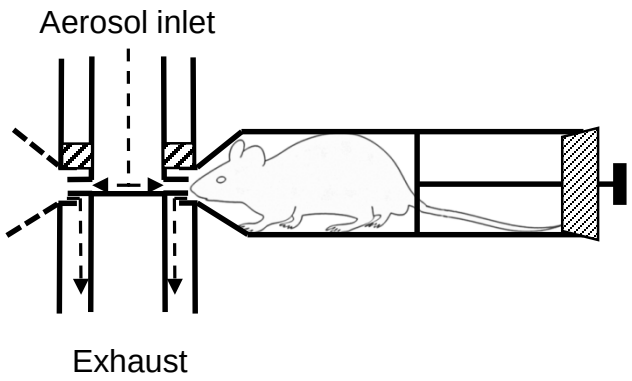
Experimental set-up

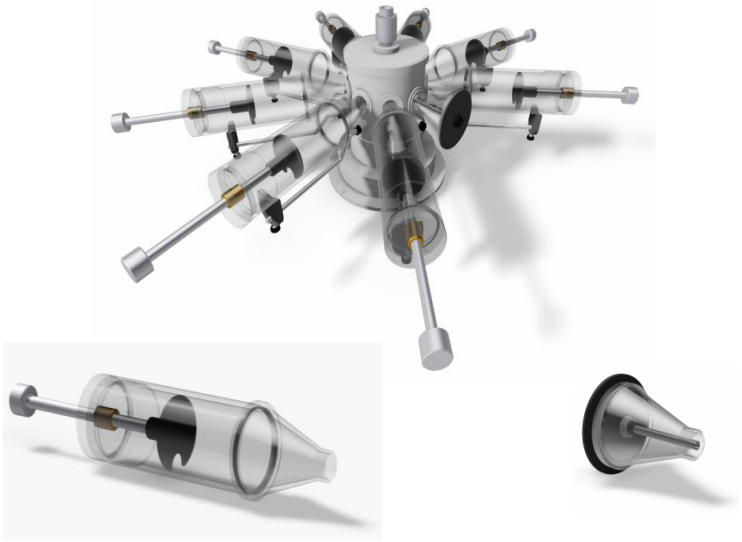
« Exposed »





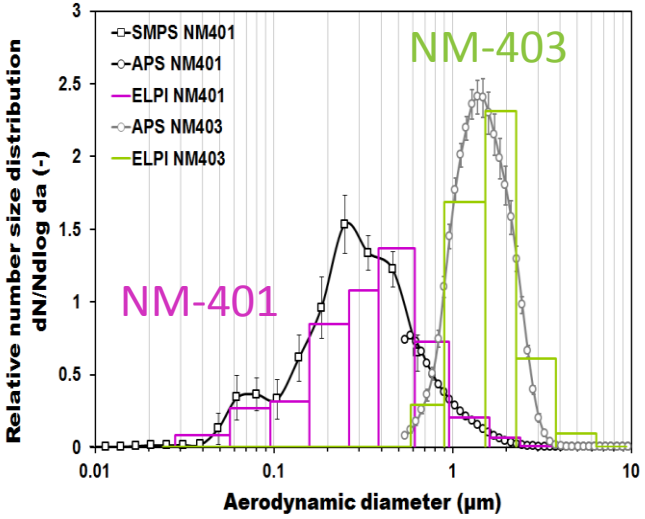
« Control »



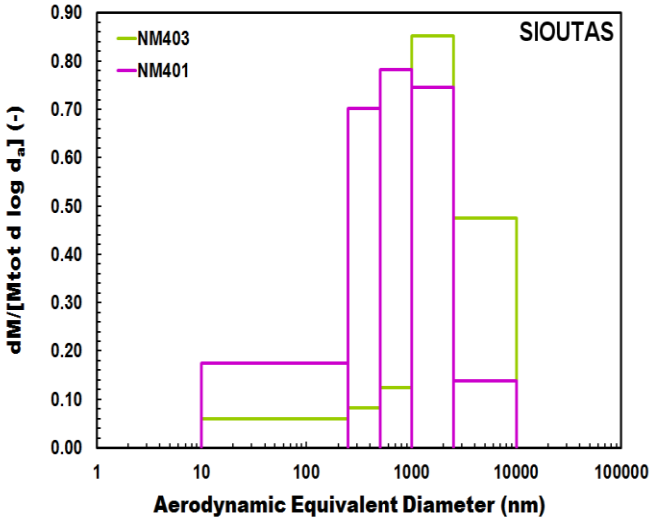


Aerosol characterisation

Number size distribution



Mass size distribution

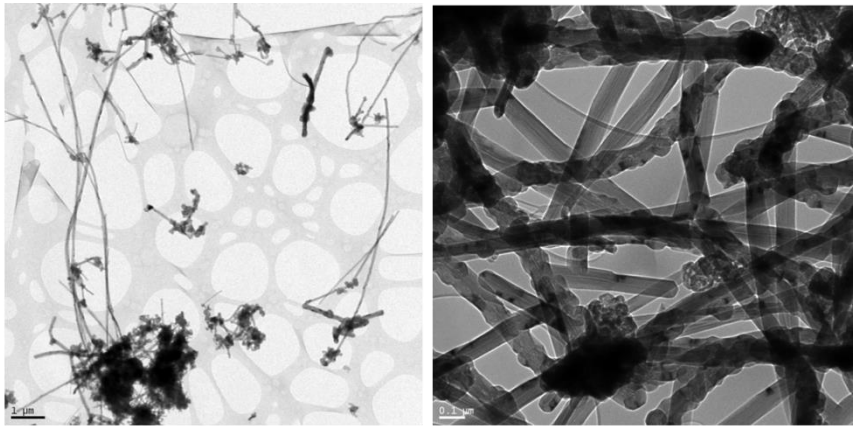


	Target concentration (mg/m³)	Concentration (mg/m³)	Concentration (particles/cm³)	MMAD (nm)	NMAD (nm)	Pulmonary fraction *	Pulmonary deposited dose* (µg)
NM-401	0.5	0.54 ± 0.11	~815	790	280	0.099-0.103	120-125
	1.5	1.59 ± 0.24	~2200				362-377
NM-403	0.5	0.50 ± 0.14	~130	1940	1440	0.035-0.053	42-64
	1.5	1.48 ± 0.63	~540				128-192

* MPPD v3.04 model (www.ara.com)

Aerosol characterisation

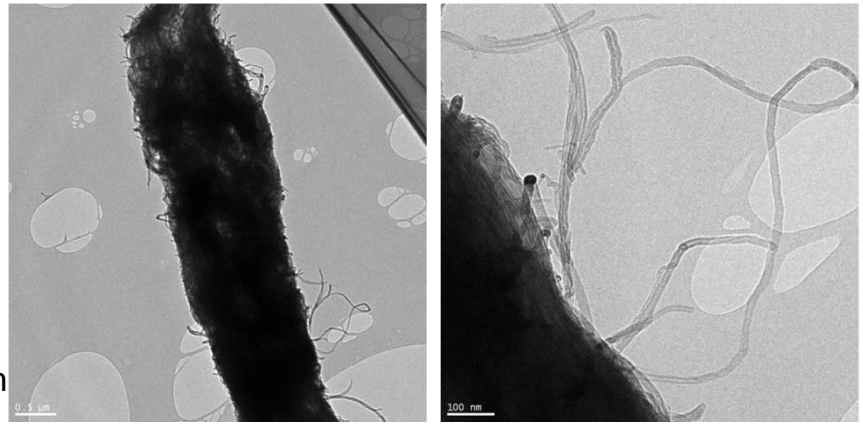
NM-401



1 µm

100 nm

NM-403



0,5 µm

100 nm

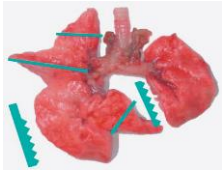
Experimental protocol

Female
Sprague
Dawley Rat

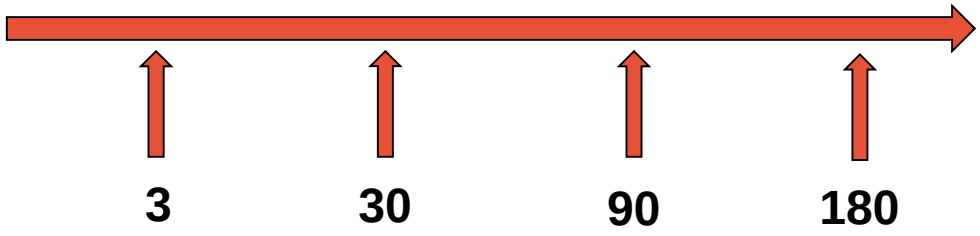
Nose-only inhalation exposure

(2x3h) / day,
5 days / week,
4 weeks

Control
+ 2 doses
(0.5 and 1.5 mg/m^3)



Tissue sampling

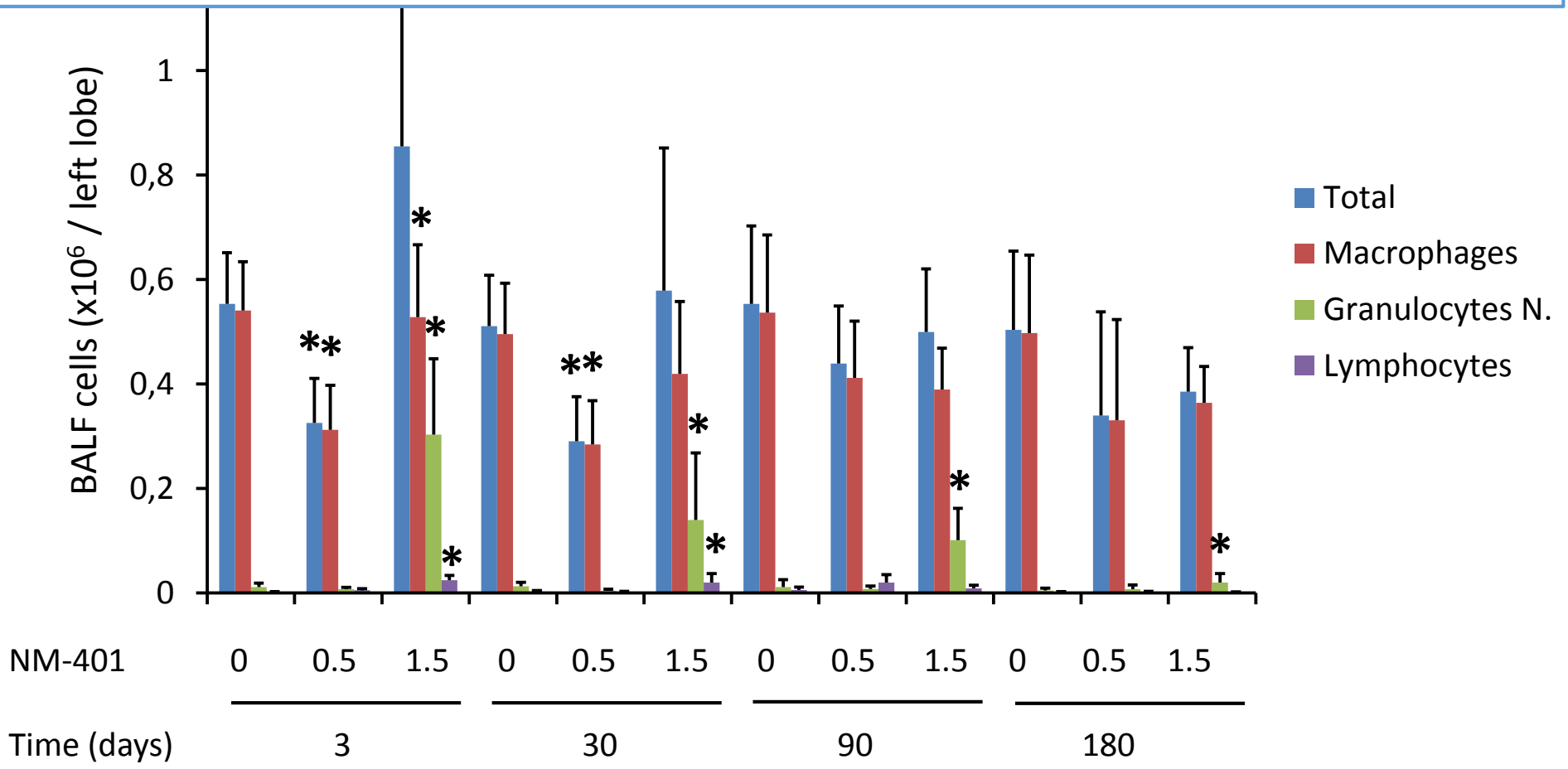


Post exposure time (days)

Inflammatory response (BALF)
Biochemical markers
Histopathology (H&E and Trichrome Masson)
Genotoxicity (comet assay)

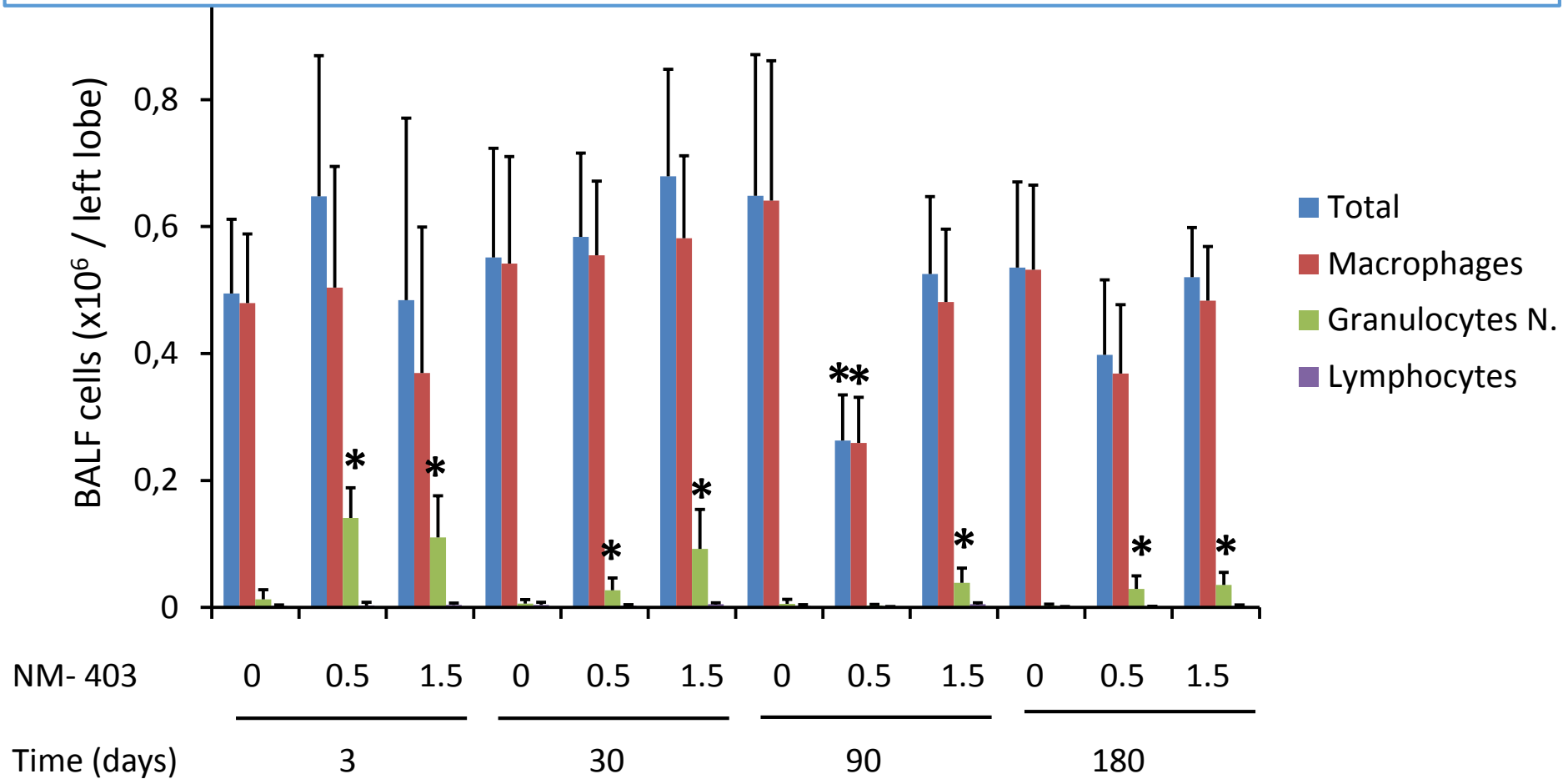
BALF cytology (NM-401)

NM-401, at 1.5 mg/m^3 induced an alveolar influx of granulocytes and lymphocytes that decreased overtime
NM-401, at 0.5 mg/m^3 did not induce such an influx but decreased the number of alveolar macrophages



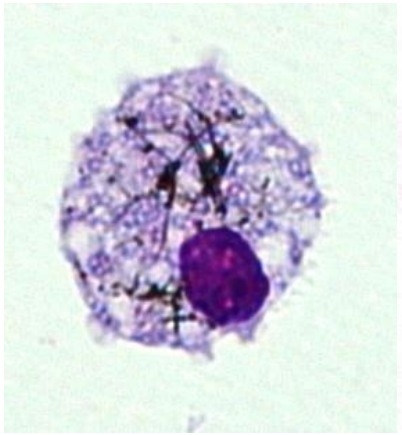
BALF cytology (NM-403)

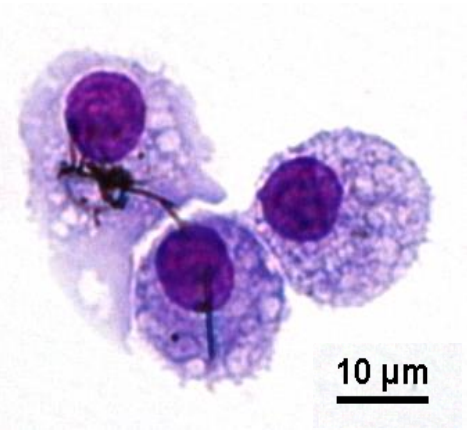
NM-403, at both concentrations induced an alveolar influx of granulocytes that decreased overtime

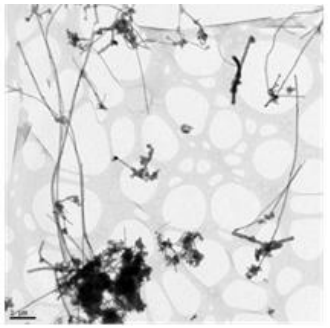


BALF cytology

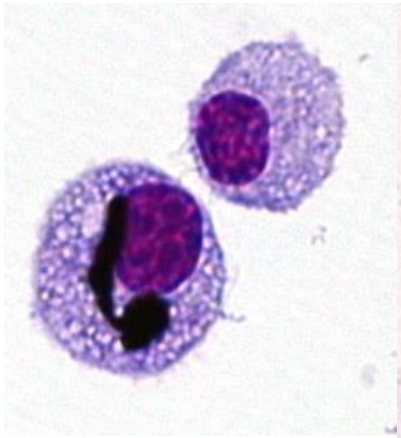
NM-401

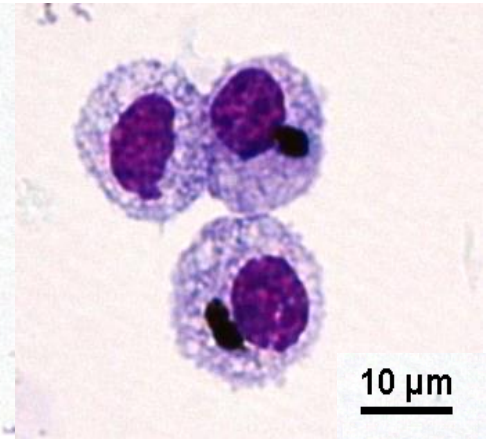


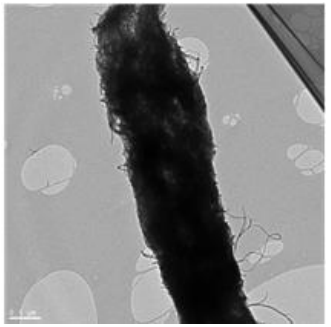




NM-403

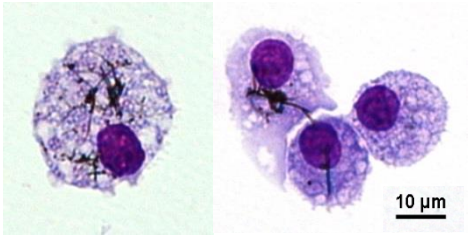




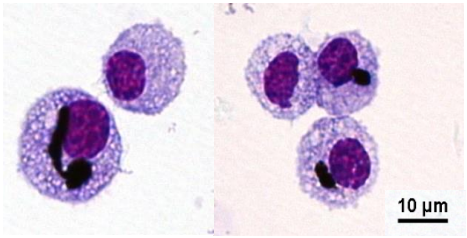


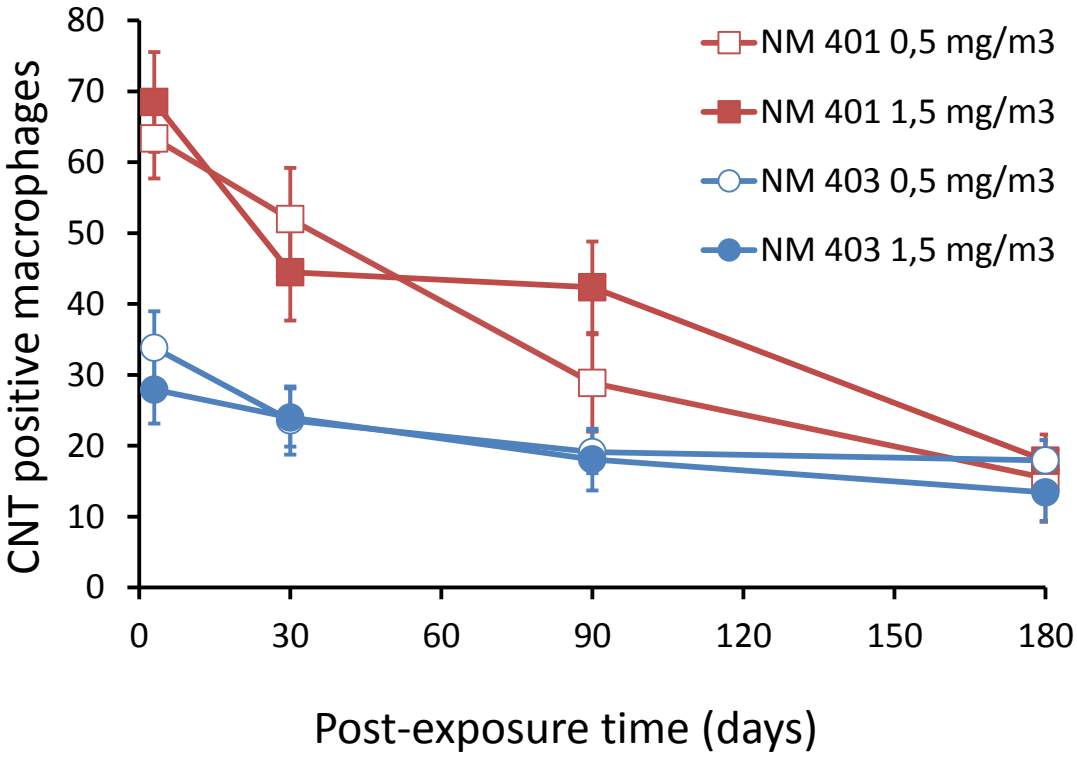
BALF cytology

NM-401



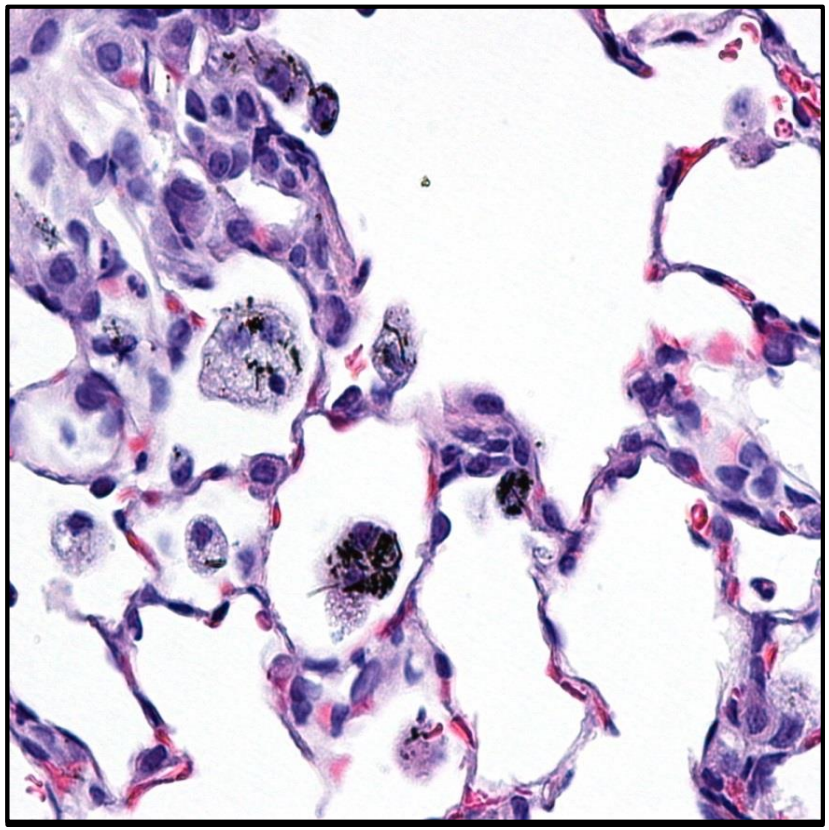
NM-403



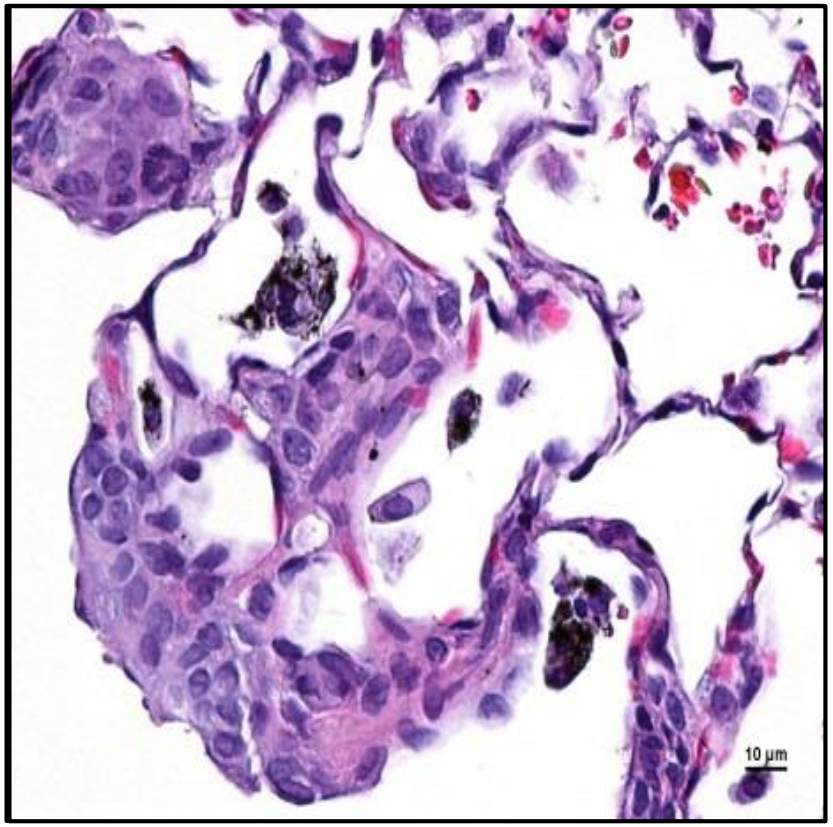


Lung histology (NM-401)

Day 3

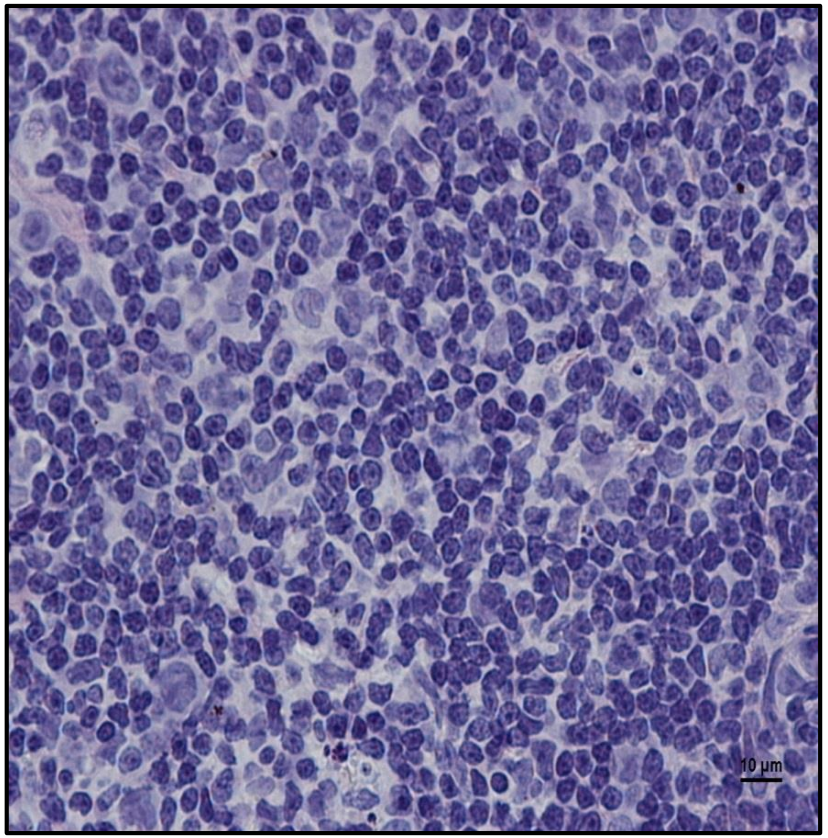


Day 180

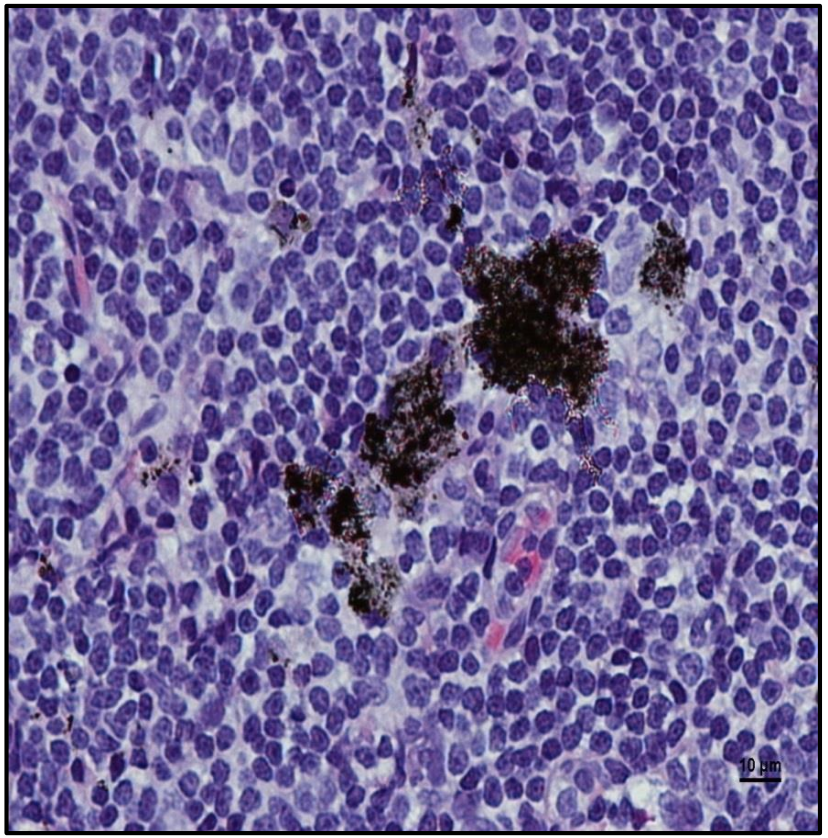


Lung associated lymph node histology (NM-401)

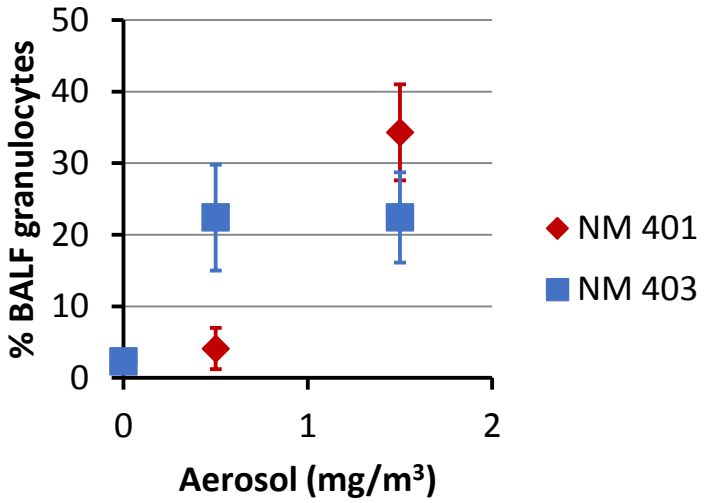
Day 3

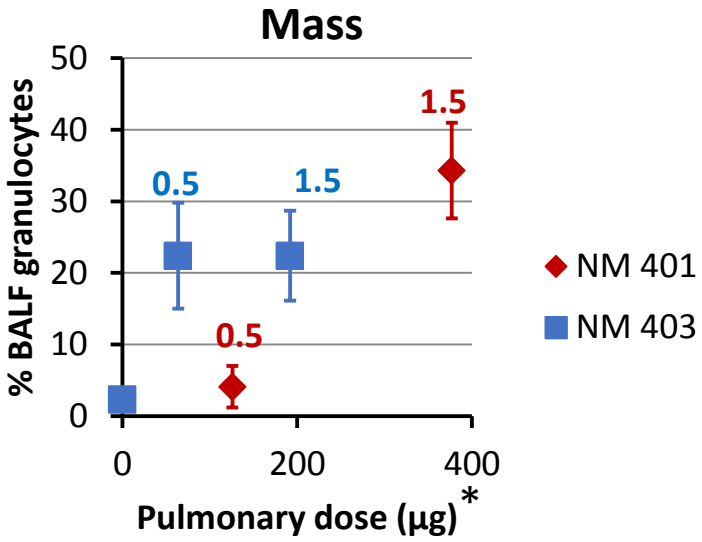


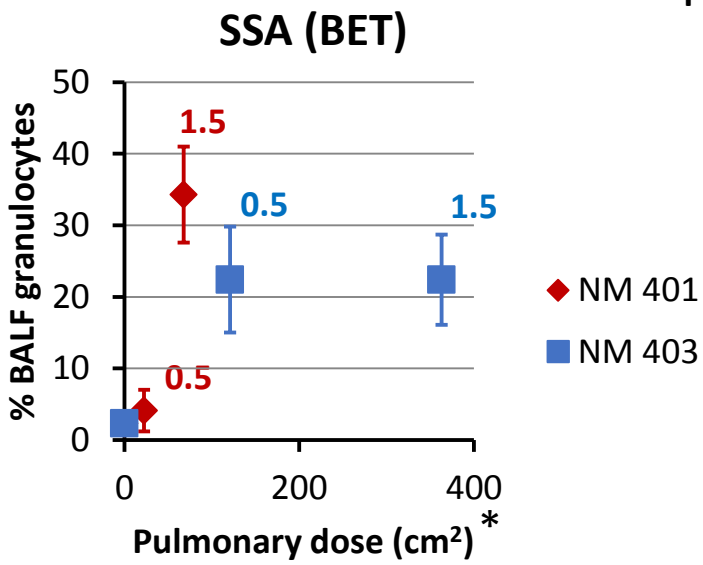
Day 180



Pulmonary inflammation: dose metrics







* MPPD v3.04 model (www.ara.com)

Conclusions et perspectives

- Both CNTs induced an acute pulmonary inflammatory response
  - Influence of the specific surface area ?
  - No significant histopathological changes (fibrosis or granuloma)
  - Induction of DNA damage difficult to interpret (absence of dose effect or time effect)

- Quantification of retained CNTs in lungs (TGA method)
- Localization of CNTs in lung tissue section (dark field and hyperspectral microscopy)

- Assessment of the toxicological properties of CNTs by high throughput and high content screening methods
  - Transcriptomics
  - Proteomics

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Smart Tools for Gauging Nano Hazards

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Thanks for your attention

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