Predicting the toxic effects observed *in vivo* after acute exposure to poorly soluble and inhalable nanomaterials by using more complex *in vitro* models

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NANoREG A common European approach to the regulatory testing of nanomaterials

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Context

- **Inhalation**: important route of exposure for metallic and poorly soluble NMs, including TiO_2 and CeO_2
- Need to evaluate the potential toxicity at the lung level
- **The animal model** is a reliable tool to predict potential adverse effects in human after exposure to NMs
- **However**, not possible to rely only on animal experimentation
  - Considering the diversity of the thousands of existing NMs
  - For ethical and financial reasons

Need for reliable alternatives to assess the pulmonary toxicity of Nanomaterials (NMs)

Context

- ***In vitro* studies using lung cells represent alternatives to assess the pulmonary toxicity after acute exposure to NMs**

- **Nevertheless**, the capacity of the *in vitro* to predict the biological responses *in vivo* in animals remains unclear
  - How to compare accurately the *in vitro* to the *in vivo*? : importance of the dose metrics (Teeguarden et al. 2014, PFT)
  - Does using classical *in vitro* conditions allows mimicking accurately the responses observed *in vivo*?
  - Is it possible to improve the predictivity of the *in vitro?,* by using more realistic exposure conditions and methods?

Objectives

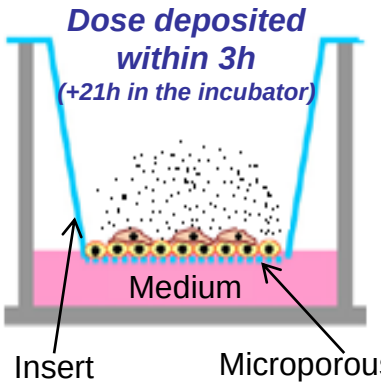
- Our objective was to provide answers about how to predict *in vitro* the biological responses observed *in vivo* in lungs after acute exposure to poorly soluble NMs
  - We assessed the acute pulmonary toxicity of TiO_2 and CeO_2 NMs using more or less realistic *in vitro* exposure conditions (Loret et al. 2016, PFT)
  - We performed *in vivo* experiments with the same NMs and we compared the biological responses *in vitro* to those observed *in vivo* in rodent lungs after acute exposure

Methods

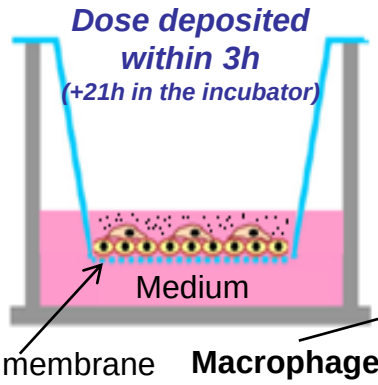
Experimental conditions to compare the *in vitro* to the *in vivo*

In vitro exposure

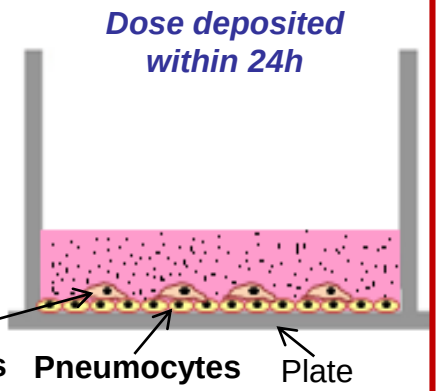
Aerosol exposure at the ALI



Suspension exposure in inserts

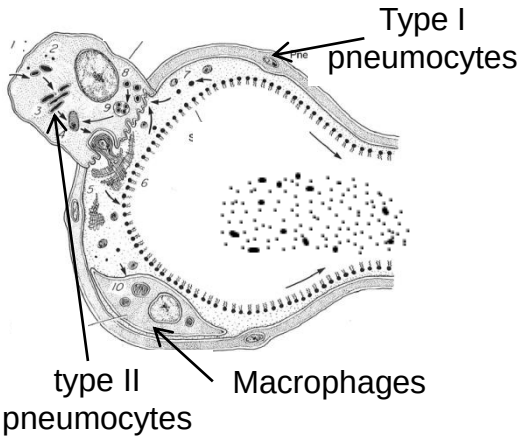


Suspension exposure in plates



In vivo exposure

Intratracheal instillation after hyperventilation



Three TiO_2 (NM105: 21 nm; NM101: 8 nm, coating; NM100: 100 nm) and **one CeO_2** (NM212: 28 nm)

Comparison of biological responses after 24h of exposure to nanomaterials

(Inflammation, oxidative stress, cytotoxicity)

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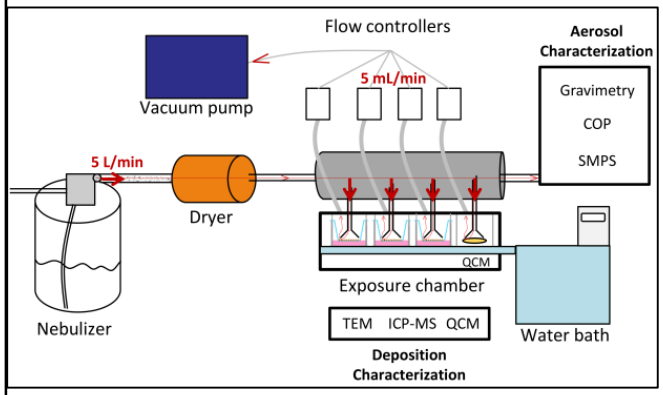
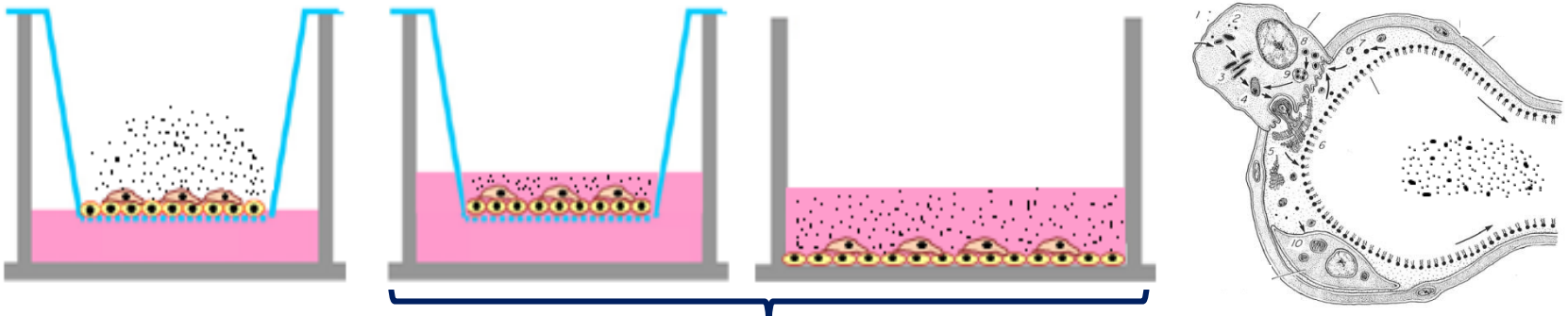
Methods

Assessment of the deposited dose (µg)



Aerosol exposure **Suspension exposure** ***In vivo* exposure**



ICP-MS

Modelisation (ISDD)

(In vitro Sedimentation Diffusion and Dosimetry) (Hinderlitter et al. 2010, PFT)

ICP-MS

After measurement of
the effective density and the Hydrodynamic diameter
(Deloid et al. 2014, Nat Commun)

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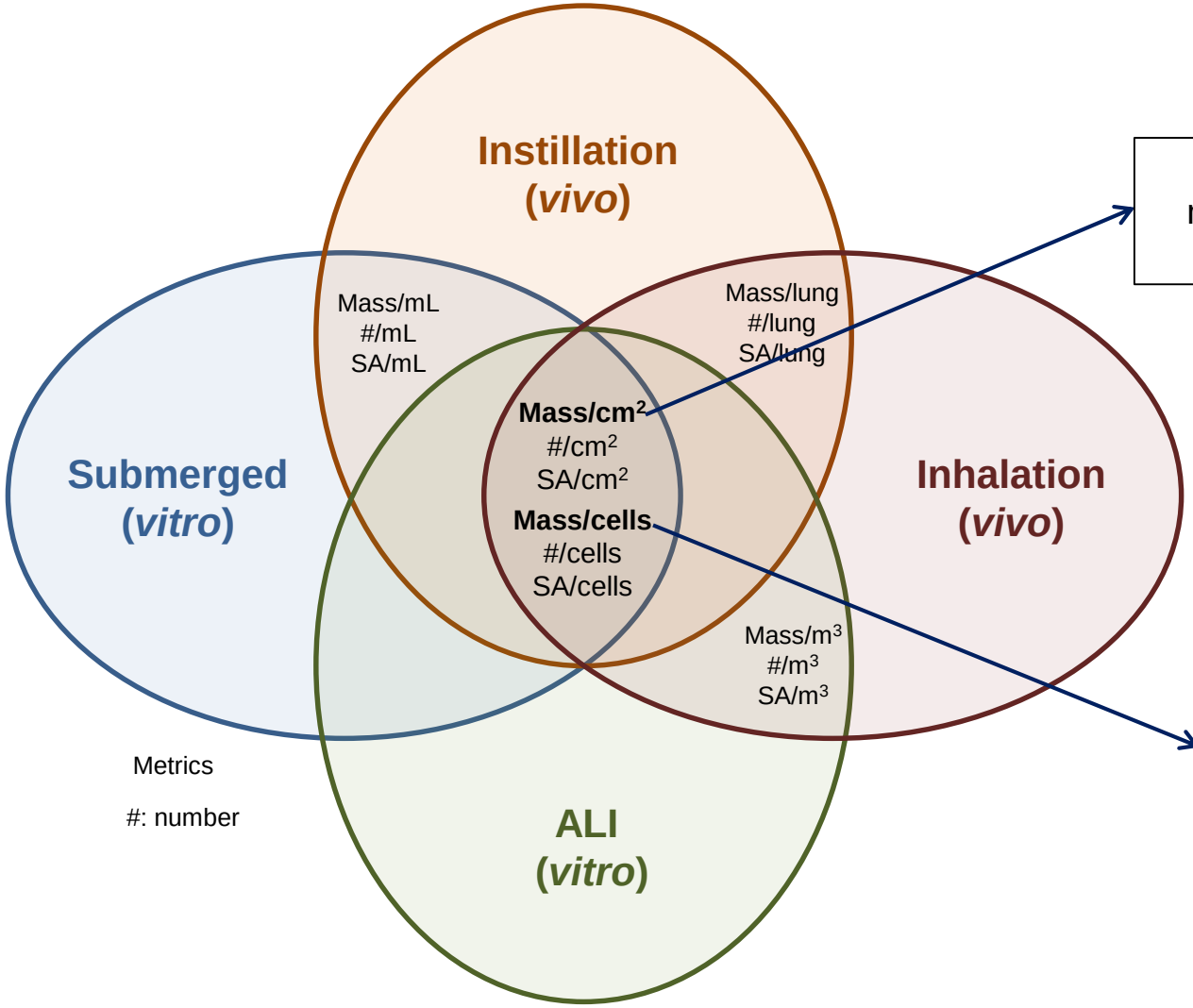
Methods

Selection of relevant dose metrics



Poorly soluble NMs are mainly active due to their surface activity

Macrophages are highly involved in the acute pro-inflammatory response and similar pneumocyte/macrophage ratio were used in vivo and in vitro

Results

Comparisons of methods using the doses normalized by the surface (µg/cm²):

In vitro

Normalization by the surface of the cell layer

(4,7 cm² in inserts or 2 cm² in plates)

In vivo

Normalization by the total alveolar surface

(~4000 cm², Stone et al. 1990)

LOAEL in µg/cm²		**Inflammation**				
		In vitro (cytokines IL-1β, IL-6, IL-8, TNF-α)			*In vivo* (cytokines)	*In vivo* (Neutrophils)
		ALI (3h+21h)	Submerged (3h+21h)	Submerged (24h)	IT	IT
TiO_2	NM105	**1**	**3**	**10 - 20**	**0.1**	**0.1**
	NM101	**1**	**3**	**10**	**0.1**	**0.1**
	NM100	**3**	> 10	> 20	> 0.1	> 0.1
CeO_2	NM212	**1-3**	**10**	> 20	**0.1**	> 0.1

significant effects allowing the determination of a LOAEL (µg/cm²)

no significant effects observed

***In vivo* (IT)** < ALI (3h+21h) < Submerged (3h+21h) < Submerged (24h)

>10x >30 x > 100 x

Results

Comparisons using doses normalized by the number of macrophages (µg/10^6 macrophages):

In vitro

Normalization by the number of macrophages

(60 000/cm² in inserts or 25000 cm² in plates)

In vivo

Normalization by the number of alveolar macrophages in BALF

(10 Millions/lungs)

LOAEL in µg/10^6 macrophages		**Inflammation**				
		In vitro (cytokines IL-1β, IL-6, IL-8, TNF-α)			*In vivo* (cytokines)	*In vivo* (Neutrophils)
		ALI (3h+21h)	Submerged (3h+21h)	Submerged (24h)	IT	IT
TiO_2	NM105	**16.7**	**50**	**400 - 800**	**50**	**50**
	NM101	**16.7**	**50**	**400**	**50**	**50**
	NM100	**50**	> 167	> 800	> 50	> 50
CeO_2	NM212	**16.7-50**	**167**	> 800	**50**	> 50

significant effects allowing the determination of a LOAEL (µg/10^6 macrophages)

no significant effects observed

ALI (3h+21h) < ***In vivo* (IT)** < Submerged (3h+21h) < Submerged (24h)

-3x – 0x

0 x – 3x

8 x – 16x

Results

Ranking of the NMs *in vitro* and *in vivo* using mass as dose metric

LOAEL in µg/10^6 macrophages		Inflammation				
		In vitro (cytokines IL-1β, IL-6, IL-8, TNF-α)			*In vivo* (cytokines)	*In vivo* (Neutrophils)
		ALI (3h+21h)	Submerged (3h+21h)	Submerged (24h)	IT	IT
TiO_2	NM105	**16.7**	**50**	**400 - 800**	**50**	**50**
	NM101	**16.7**	**50**	**400**	**50**	**50**
	NM100	**50**	> 167	> 800	> 50	> 50
CeO_2	NM212	**16.7-50**	**167**	> 800	**50**	> 50

significant effects allowing the determination of a LOAEL (µg/10^6 macrophages)
no significant effects observed

Relative toxicity

NM105 = NM101 > NM212 > NM100

20 nm | 8 nm | 28 nm | 100 nm

0

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Conclusions

How to improve the *in vitro* predictivity after acute exposure to NMs?

- Using compatible and relevant dose metrics is critical
  - We showed better *vivo-vitro* correlations when normalizing the doses by the number of macrophages rather than by the surface
- It seems very important to use similar timing of the dose delivery and exposure duration *in vitro* and *in vivo*
  - We showed much better *vivo-vitro* correlations when delivering the final dose *in vitro* in 3h rather than in 24h
- It seems very important to use realistic cell models (macrophages ++) and exposure methods
  - Nevertheless, further clarifications are still needed to assess whether ALI methods better predicts the biological responses observed *in vivo*
- *In vitro* methods can be used to rank poorly soluble NMs according to their toxicity
  - The NMs were ranked similarly *in vivo* and *in vitro*, whatever the *in vitro* method used

Perspectives

To confirm our observations

- To perform exposure of rats to NMs aerosol for *vivo-vitro* comparisons
- To assess more accurately the doses *in vivo* (which region of the lung?, doses at hot spots?), and *in vitro*
- To find other relevant dose metrics?
- To use more realistic cell models and exposure methods?

Thank you for your attention

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