WORKSHOP:

Design of an *in vitro* system to assess the inhalation toxicity of nanomaterials

State-of-the-science aerosol generation and characterization

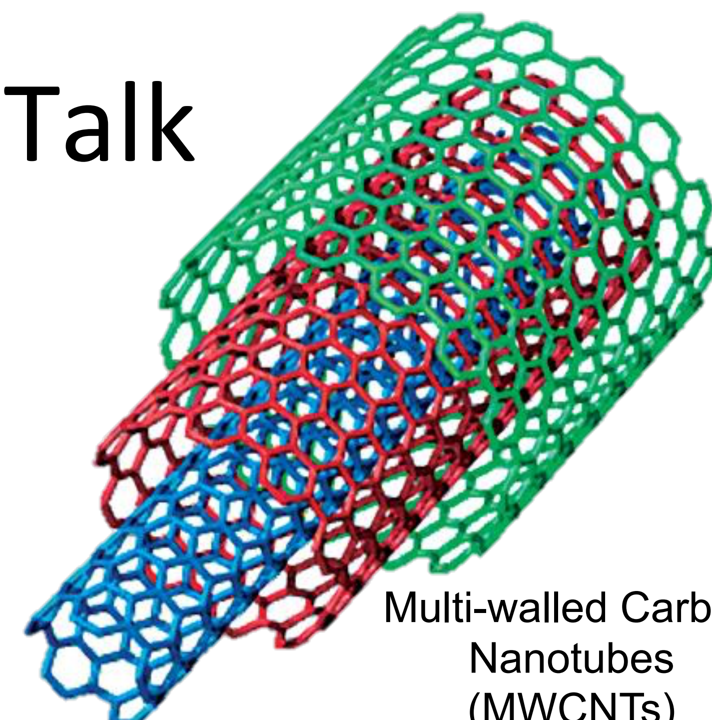
February 24-25, 2015

Goal of Workshop

To review the state-of-the-science and determine the technical needs to develop a testing strategy that will reduce or replace the use of animals in studies to assess the inhalation toxicity of nanomaterials.

Outline of Talk

- Aerosol Generation
- Aerosol Characterization
- Test Materials of Interest

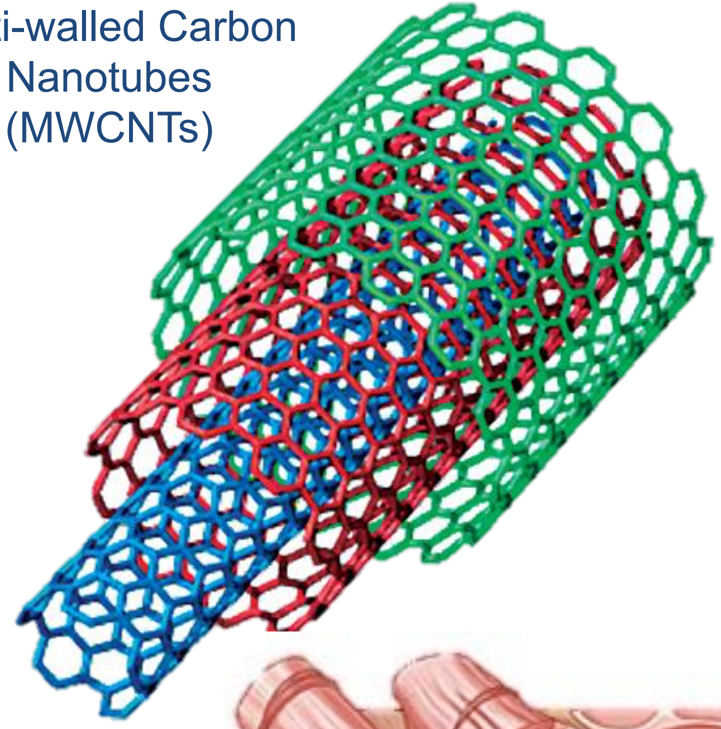


Multi-walled Carbon Nanotubes (MWCNTs)

Overall Experimental Design

1. **Identify the synthesis methods used to produce each MWCNT**
2. **Characterize the MWCNT physical and chemical properties, including property changes throughout the toxicological evaluation**
3. **Classify the MWCNT aggregation state in a variety of matrices ranging from chemically-relevant to physiologically-relevant**

4. Establish a hazard profile of MWCNT that characterizes the particle's potential toxicity
5. Identify what MWCNT properties induce toxic responses
6. Validate cytotoxicity results with historical *in vivo* histopathological assessment



Multi-walled Carbon Nanotubes (MWCNTs)

Lung Fibrosis

AEROSOL GENERATION

What are Aerosols?

- Aerosols are ensembles of
  - Solid particles suspended in a gas
  - Liquid particles suspended in a gas
  - Multi-phase solid/liquid particles suspended in a gas

- In contrast to colloidal particles, aerosol particles cannot be made to be stable as a function of time
  - Colloidal particles can be stored
  - Aerosol particles cannot be stored

- Time frame of aerosol stability varies from particle to particle
  Aerosol particles change as a function of time

- Aerosol particles are characterized by their size distribution

Special Issues with Aerosol Nanoparticles

- Low charging efficiency
- Extremely high diffusivity

$$D = \frac{k_B T C_c}{3\pi\mu D_p}$$

Stokes-Einstein Relation

- For smaller particles, particle mass if often negligible
  - Very long sampling times
  - Mass based detection often inaccurate due to negligible particle mass

Particle Diameter (nm)	Distance Fallen Due to Gravity (μm)	Distance Moved Due to Diffusion (μm)
10	0.2	200
100	4	20
1000	200	4

Compounding Effects…

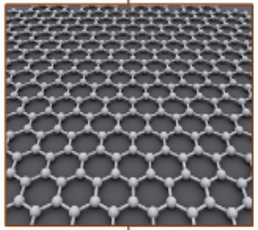
- Thermophoresis
  - Thermal gradients that cause net migration of particles from higher temperature to lower temperature regions of gas
- Photophoresis
  - Non-uniform heating of particles exposed to light
- Diffusiophoresis
  - Presence of gradient of vapor molecules that are either heavier or lighter than surrounding gas molecules
- Electrophoresis
  - Charged particles follow electric field lines; charged particles collect onto surfaces

AEROSOL CHARACTERIZATION

Characterization of the engineered materials used in the study should be conducted in three (3) distinct phases in the experimental approach

Before Exposure

- Pristine Nanomaterial Characterization
- Aerosolized Material
- Exposure Chamber Design and Operation
- Aerosol Characterization

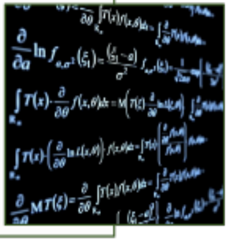


During Exposure

- Dose Metrics
- Cell Culture
- Exposure to Cells

After Exposure

- Early Endpoint Analyses
- Late Endpoint Analyses
- Statistical analysis



Pristine Particle vs. Aerosolized Particle

PARTICLE SYNTHESIS

- Nano = < 100 nm
- Ultrafine = < 100 nm
- Fine = 100 nm - 3 μm

AEROSOL GENERATION

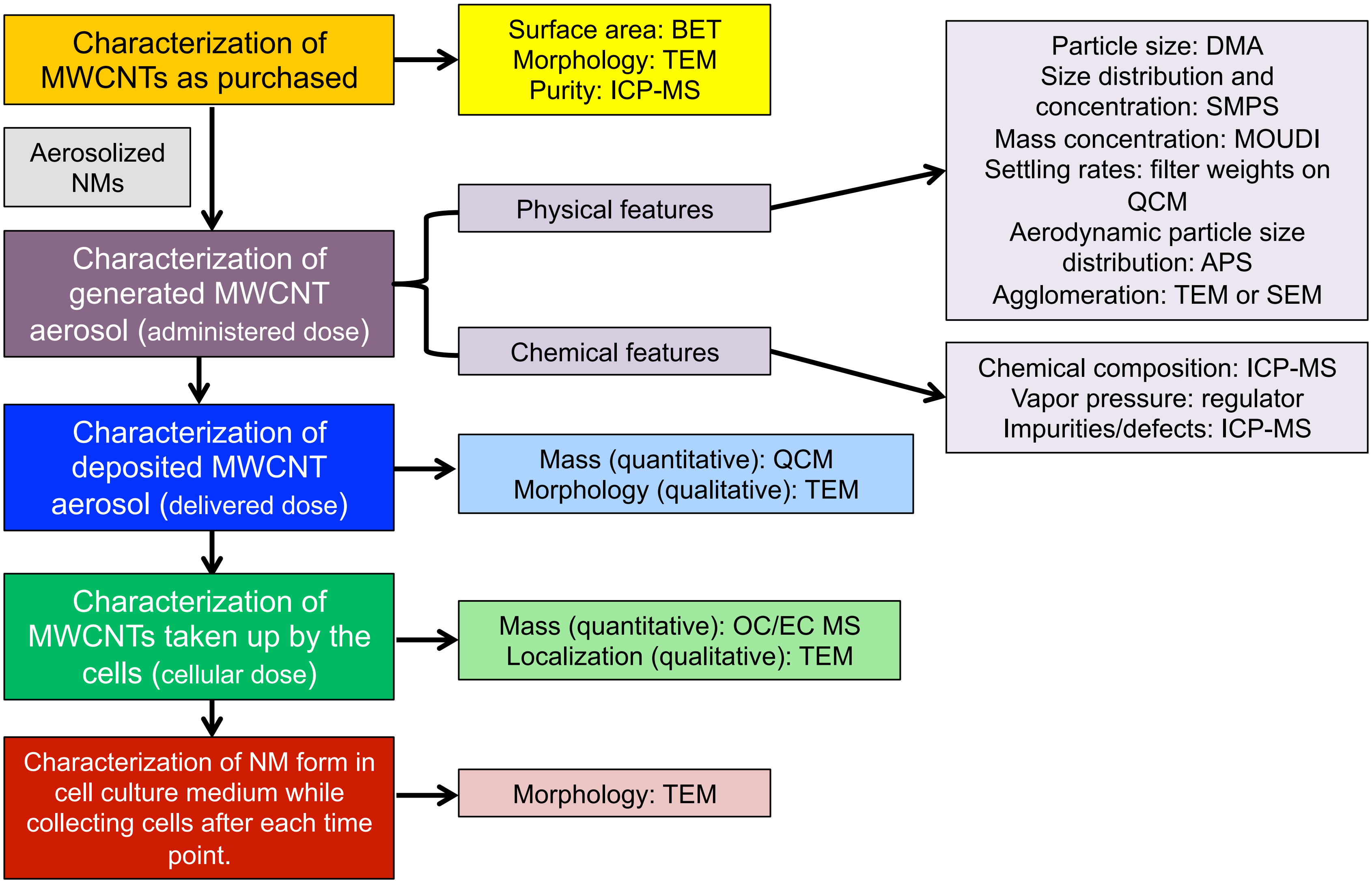
- Respirable (rat) = $\leq$3 μm (max = 5 μm)
- Respirable (human) = < 5 μm (max = 10 μm)

- Common Dogma -

- Nanoparticles are more toxic (inflammogenic, tumorigenic, fibrogenic) than fine-sized particles of identical composition.
- Concept generally based on systematic comparisons of only 2 particle-types:
  - titanium dioxide particles
  - carbon black particles
  - Now…carbon nanotubes

Carbon Nanotube Characterization

Techniques



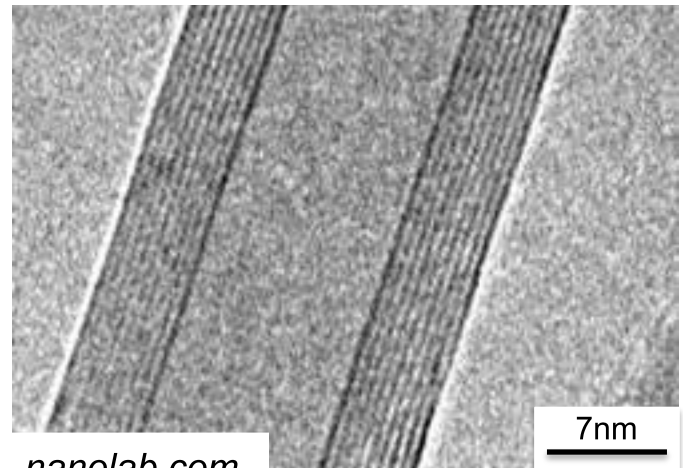
Carbon Nanotube Characterization

Techniques

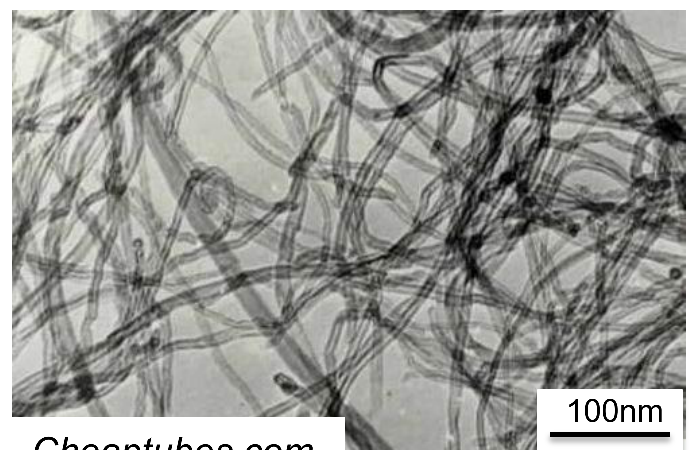
Characterization of MWCNTs as purchased

↓

Surface area: BET
Morphology: TEM
Purity: ICP-MS



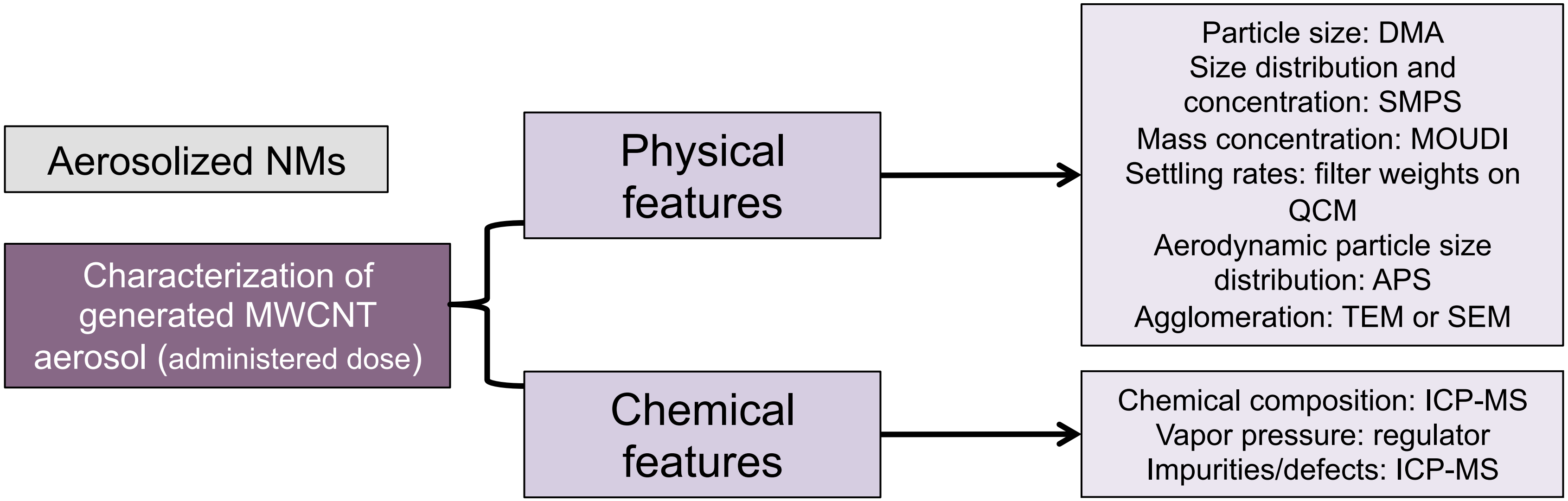
nanolab.com



Cheaptubes.com

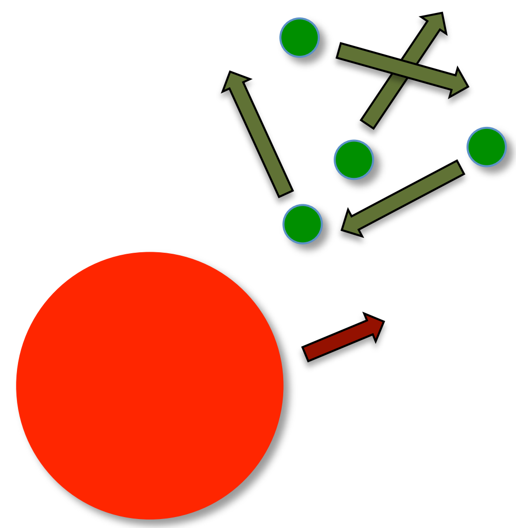
Carbon Nanotube Characterization

Techniques



<u>Aerosol Particle Coagulation - Nanoparticles</u>

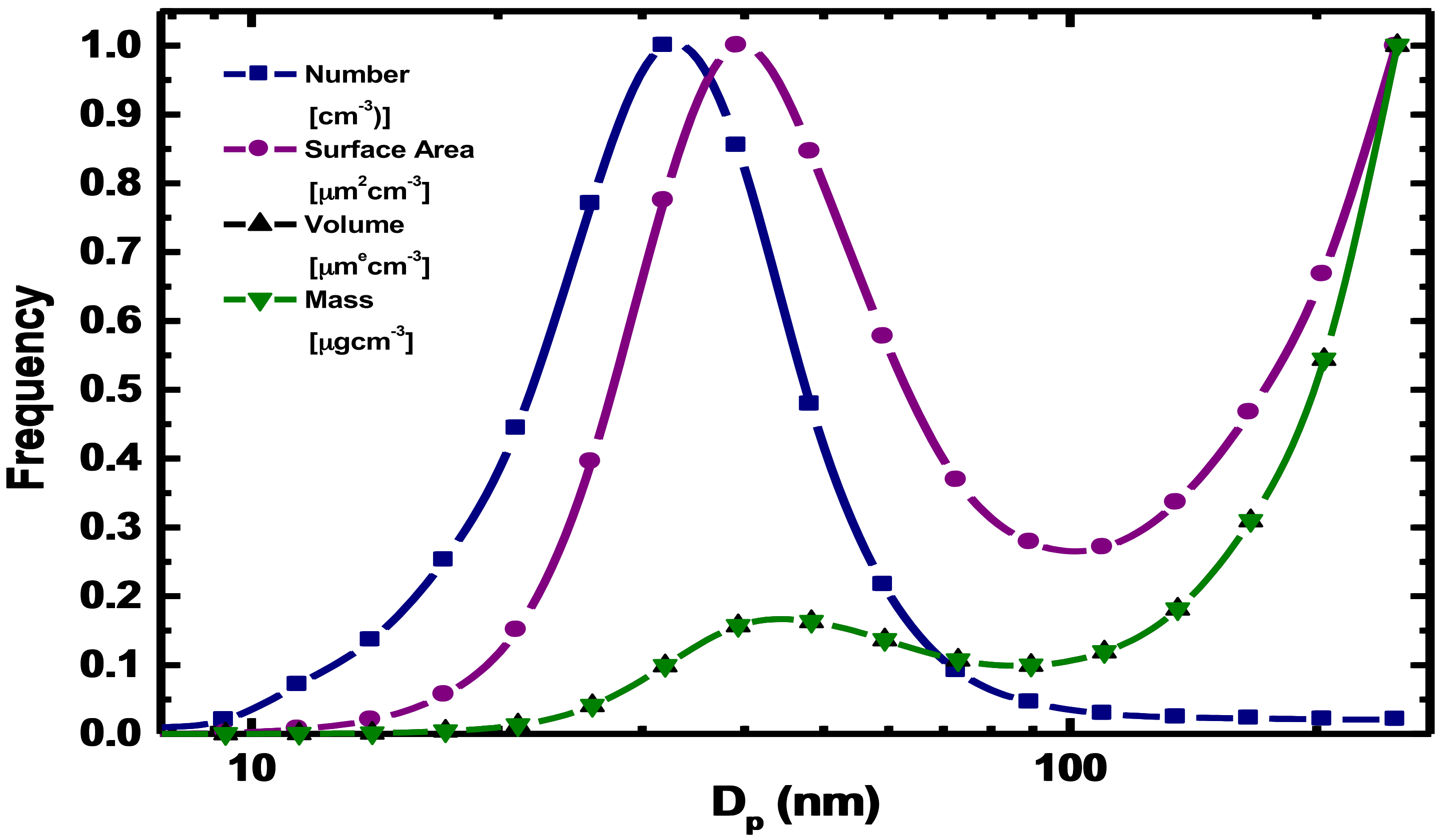
- Brownian Coagulation Coefficient K (D_{p1}, D_{p2})
  - **Nanoparticles**
    - High diffusivity - move quickly and often
    - Insignificant collision cross-section
  - **Larger particles**
    - Low diffusivity - move slowly
    - Large collision cross-section



Carbon Nanotube Characterization

Techniques

Number, Surface Area, and Mass



Number:
97.4% < 100 nm
2.6% > 100 nm

Surface Area:
62.0% < 100 nm
38.0% > 100 nm

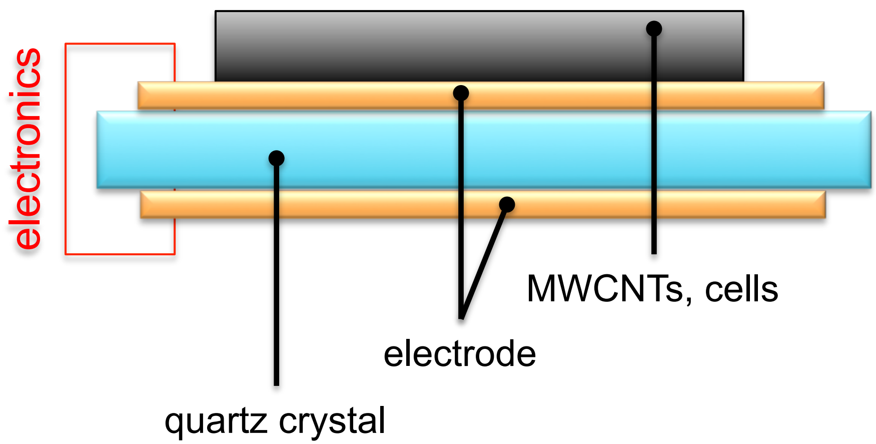
Mass:
27.6% < 100 nm
72.4% > 100 nm

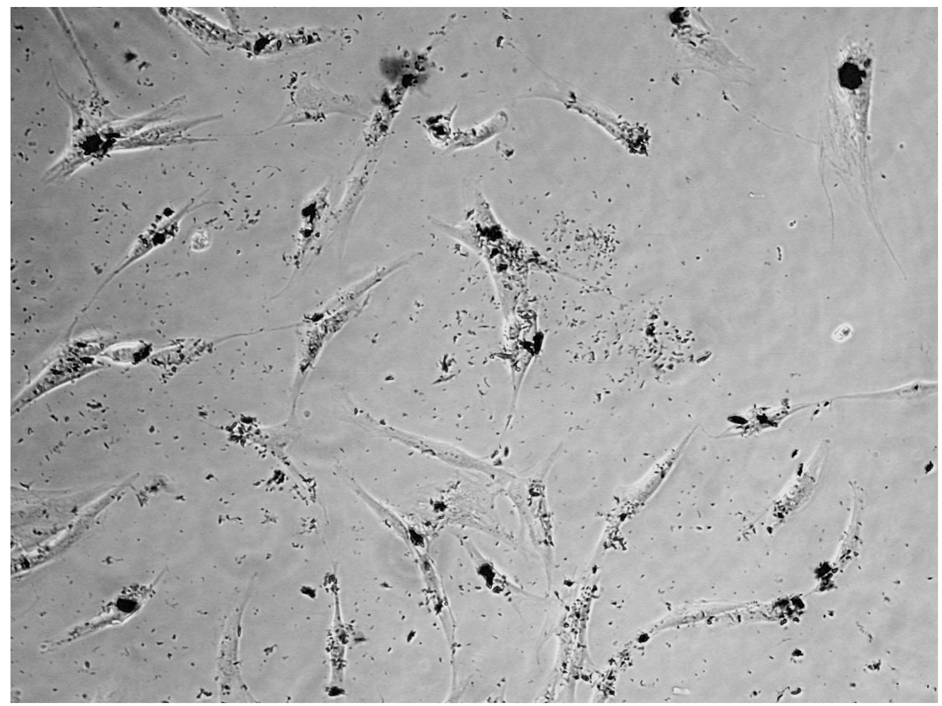
Nanoparticles have almost no mass

Carbon Nanotube Characterization
Techniques

Characterization of deposited MWCNT aerosol (delivered dose) → Mass (quantitative): QCM
Morphology (qualitative): TEM

Quartz Crystal Microbalance



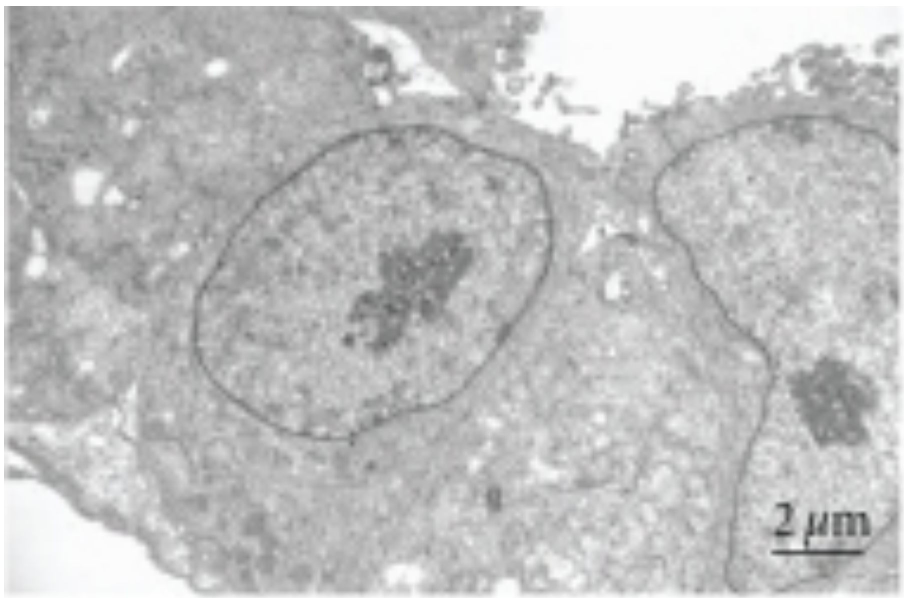


Multi-walled carbon nanotubes associate with cellular membranes
(unpublished data)

Carbon Nanotube Characterization

Techniques

Characterization of MWCNTs taken up by the cells (cellular dose) → Mass (quantitative): OC/EC MS
Localization (qualitative): TEM



(a)

(b)

(a) HeLa cells in culture before CNT inoculation
(b) HeLa cells in culture after CNT inoculation

Elhissi AM, Ahmed W, Hassan IU, Dhanak VR, D'Emanuele. "A Carbon Nanotubes in Cancer Therapy and Drug Delivery". *J Drug Delivery* (2011).

TEST MATERIALS

Characterization information for Mitsui 7

REFERENCES:
Evaluation of the health effects of carbon nanotubes) Taquahashi, Y., Y. Ogawa, et al. (2013)

Improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals." J Toxicol Sci **38**(4): 619-628. Sargent, L. M., D. W. Porter, et al. (2014)

Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes." Part Fibre Toxicol 11: 3.

Variable	Short MWCNTs	Long tangled MWCNTs	Long needle-like MWCNTs	Carbon black	Asbestos
Trade name	Baytubes C150 HP	MWCNT 8-15 nm	Mitsui MWCNT-7	Printex 90®	Crocidolite asbestos
Manufacturer	Bayer Material Science	Cheaptubes, Inc.	Mitsui & Co. Ltd	Evonik Industries	Pneumoco-niosis Research Centre
Characteristics of primary fibres or particles (provided by manufacturer)	OD 2-20 nm Length 1->10 µm	OD 8-15 nm Length 10-50 µm SSA 233 m^2/g	OD >50 nm Length ~13 µm	Average size 14 nm SSA 300 m^2/g	ø 180 nm Length 4.6 µm
Composition measured by TEM + EDS, average of 5 measurements	Carbon content >99 % (w/w) Residual catalyst metals: Co <0.2 % (w/w)	Carbon content >99 % (w/w) Residual catalyst metals: Co, Fe, Ni <0.5 % (w/w)	Carbon content >99 % (w/w) Residual catalyst metals: < 0.1 % (w/w; detection limit)	Carbon content ~ 100 % (w/w)	Not applicable

Compositional analysis shown is the average of five separate analyses by transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS). MWCNTs, multi-walled carbon nanotubes; OD, outer diameter; SSA, specific surface area.

Characterization information for Nanocyl 7000 and Arkema available through JRC

	NM400 (Nanocyl)	NM402 (Arkema)
d (nm)	5 – 35	6 - 20
L (µm)	0.7 - 3	0.7 - 4
Impurities % Al, % Co	5.38 .24, 0.58	3.16 3.00×10^{-4}, 2.39
Extent of defects	1.20	1.12

REFERENCES

Detailed characterization information for JRC materials is available online Vietti, G., S. Ibouraadaten, et al. (2013)

Towards predicting the lung fibrogenic activity of nanomaterials: experimental validation of an in vitro fibroblast proliferation assay." Part Fibre Toxicol **10**: 52.

Test Nanomaterial Charge Questions

It was suggested by the steering committee (SC) to focus on multi-walled carbon nanotubes (MWCNTs) for the proposed method development. The decision was based on the fact that the majority of PMNs received by EPA include MWCNTs.

1. Are the identified MWCNTs available in sufficient quantities for the test? What is the test amount needed?

2. Do the test agents require special modification (e.g. radioisotope labeling) for any stage of testing? If so, is it available and feasible within the experimental design?

Test Nanomaterial Charge Questions

3. Are the identified CNTs relevant in the regulatory space? If not, is a comparable substitute, relevant in this space, available?

4. Is there an optimal test range of physicochemical properties that would provide data necessary and / or sufficient to build a QSAR model?

(De)agglomeration potential	**Chemical generation method**	**Water solubility**
Length	**Impurity**	**Rigidity**
Density	**Aspect ratio**	**Other**