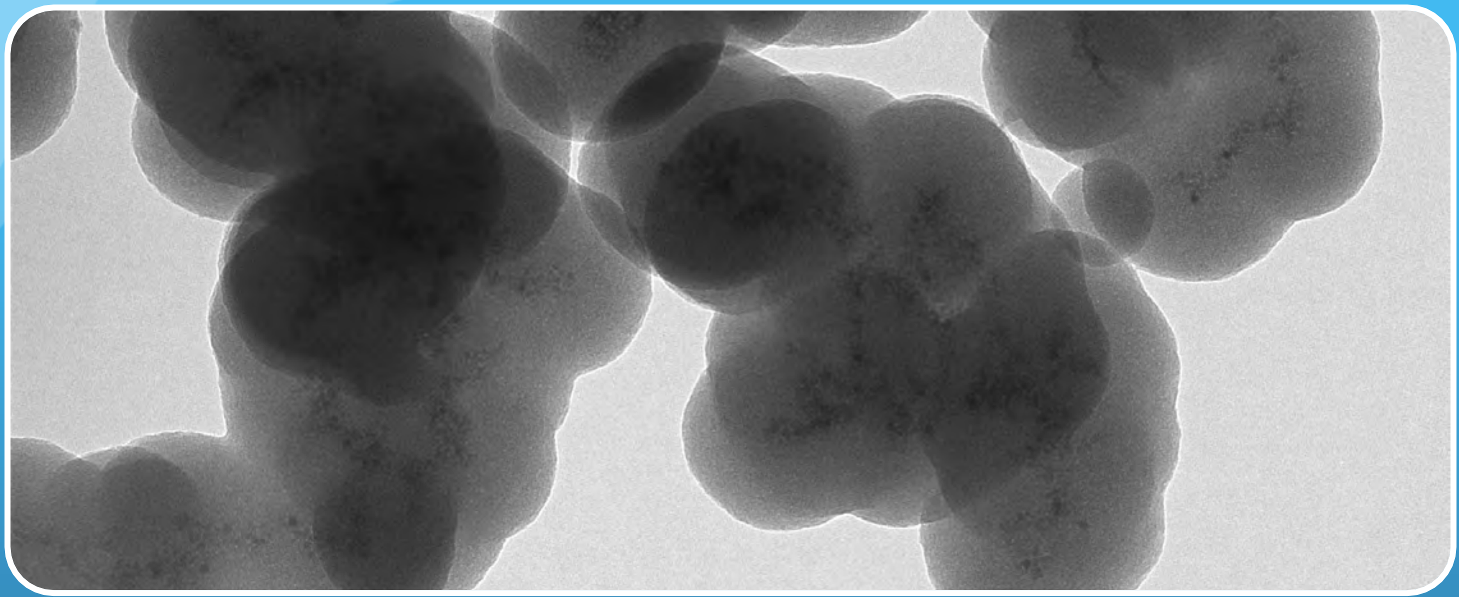


Nanosafety in Research Laboratories

Jesus Santamaria

Instituto de Nanociencia de Aragón (INA) – Zaragoza

Our Institute: INA

- **Nanoscience Institute of Aragon:** Research in Nanoscience and Nanotechnology
- Established in **2004**
- Located in **Zaragoza** (Aragón, Spain)
- 165 employees (71 Doctors, 66 PhD students)
- Hosts the **Advanced Microscopy Laboratory**
- Over **20 MM€ in state of the art equipment**

Summary

- Some basic concepts. Risks and nanotechnology.
- Handling engineeered nanomaterials (ENs): Not enough precautions taken.
- Research on nanosafety at INA.
- Recommendations and perspectives.

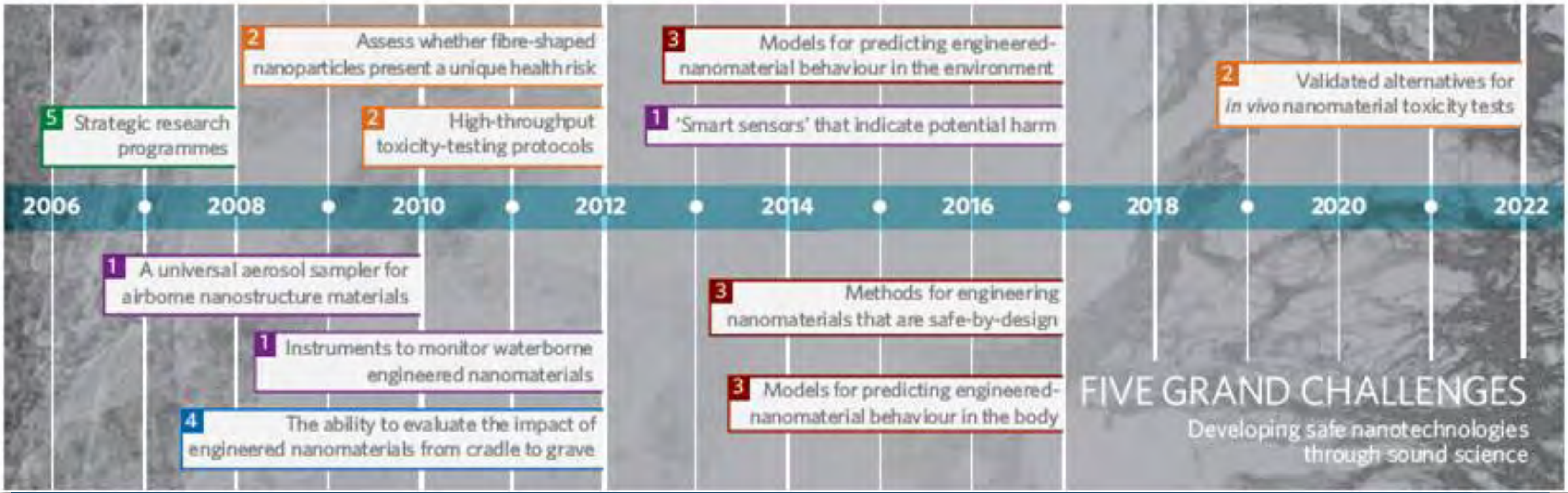
Risk perception – Risk management

- Risk perception is one´s opinion of the likelihood of facing harm associated with a certain activity. Risk management refer to the ways in which we deal with that risk

- Risk is a normal part of everyone daily life; there is no such thing as zero risk. Everyday activities such as crossing a street involves a certain health risk. This can be reduced to accepable levels by safety precautions such as looking for traffic before crossing

- Risk assessment becomes more difficult in the presence of uncertainties regarding the potencial consequences or the likelyhood of a certain event. This is often the case with new technologies: biotechnology, nanotechnology…

Risk of Engineered Nanomaterials could cause a backlash against Nanotechnology

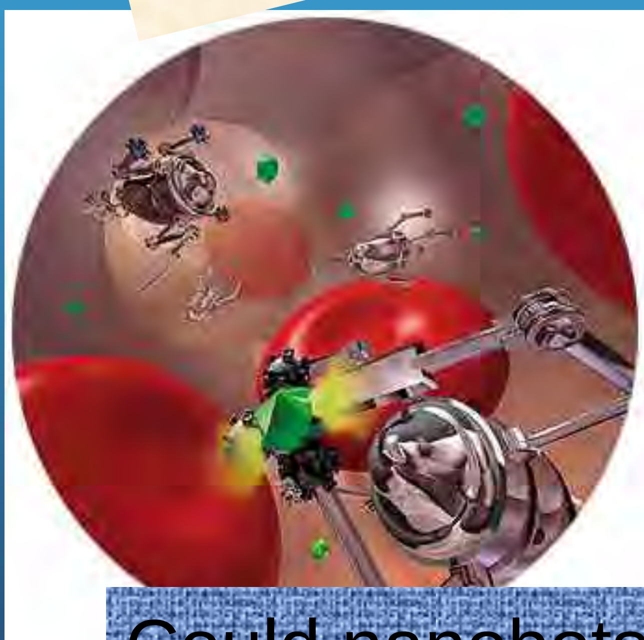


"The spectre of possible harm — whether real or imagined — is threatening to slow the development of nanotechnology unless sound, independent and authoritative information is developed on what the risks are, and how to avoid them"

Maynard, Nature 2006, 444, 267

Already happening?

Nanotechnology Group to Address Safety Concerns The NanoBusiness Alliance, a trade group for businesses at work on nanotechnology, plans to announce a new task force today to address health and environmental concerns



Could nanobots go wild?

Technology's Future: A Look at the Dark Side

AOL NEWS SPECIAL REPORT

THE NANOTECH GAMBLE

BOLD SCIENCE. BIG MONEY. GROWING RISKS.

Amid Nanotech's Dazzling Promise, Health Risks Grow

Getty Images

Nano-Foods Coming to a Store Near You

To the industry, they're the ultimate secret ingredient. And scientists say nanomaterials are already in the U.S. food supply.

Nanoparticles are in the air...

- Natural aerosols: Due to the action of earth volcanoes, desert storms, smoke from (unintentional) wildfires

- "Take a breath in the average office today, and you will inhale about 30 million nanoparticles!" (Human Subjects Risk in New Technologies. In: *DOE Protecting Human Subjects Newsletter, No. 13*. Spring 2006)

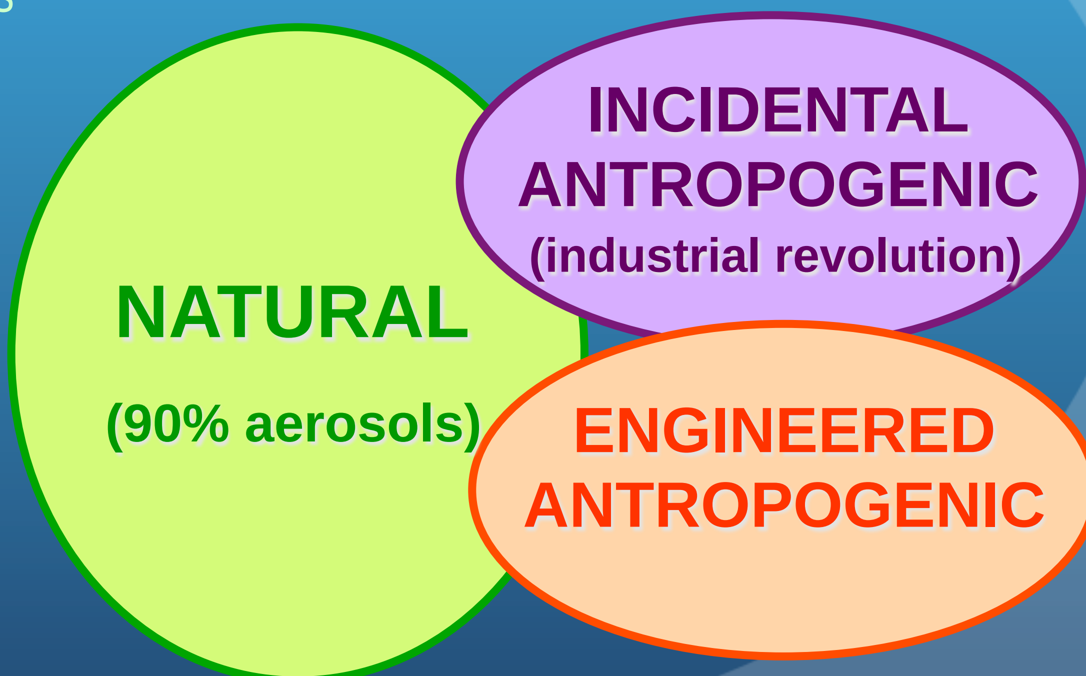
Sandstorm in Sahara

Wildfire

Volcano eruption

Nanoparticles are in the air…

- Artificial aerosols:
  - Anthropogenic aerosols: Produced by the industrial activities, motor soots (diesel), paintings, industrial aerosols
  - Engineered-nanoparticle aerosols: Specific synthesis of nanomaterials during engineering and scientific activities



And have been with us a long time...

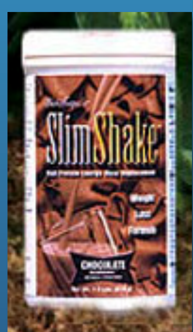
✓ 5300 yr old Ice Man: TEM on pulmonar tissue showed TiO_2 and SiO_2 nanoparticles. (L.E. Murr et al. J. Mat. Sci. 15 (2009) 237-247).

✓ Other:

- Carbon nanotubes in Damascus swords
- Lycurgus cup
- Stained glas windows in Cathedrals...

… We also find them in everyday goods

Over 2000 products

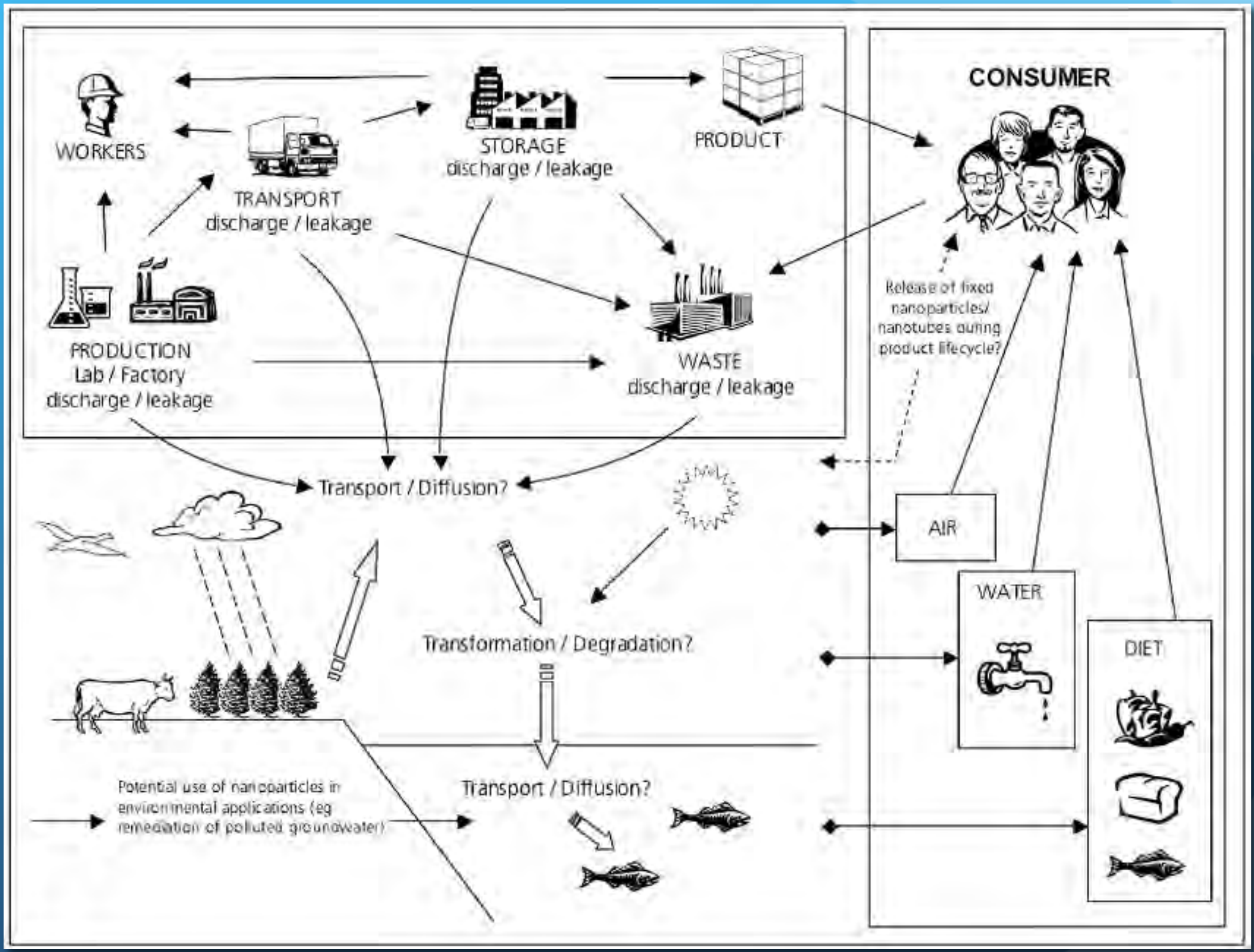


- Cosmetics and personal care (~ 60 %)
- Paints and coatings (~ 10%)
- Catalysts and lubricants
- Safety printing
- Textile and Sports
- Health and medicine
- Dietary supplies (~ 10%)
- Food wrappings
- Agricultural reagents and compounds
- Veterinary
- Water decontaminants
- Building materials
- Electronics (~ 10%)
- Fuel cells and batteries
- Paper manufacture
- Weapons and explosives

Beware: some of their properties are really new

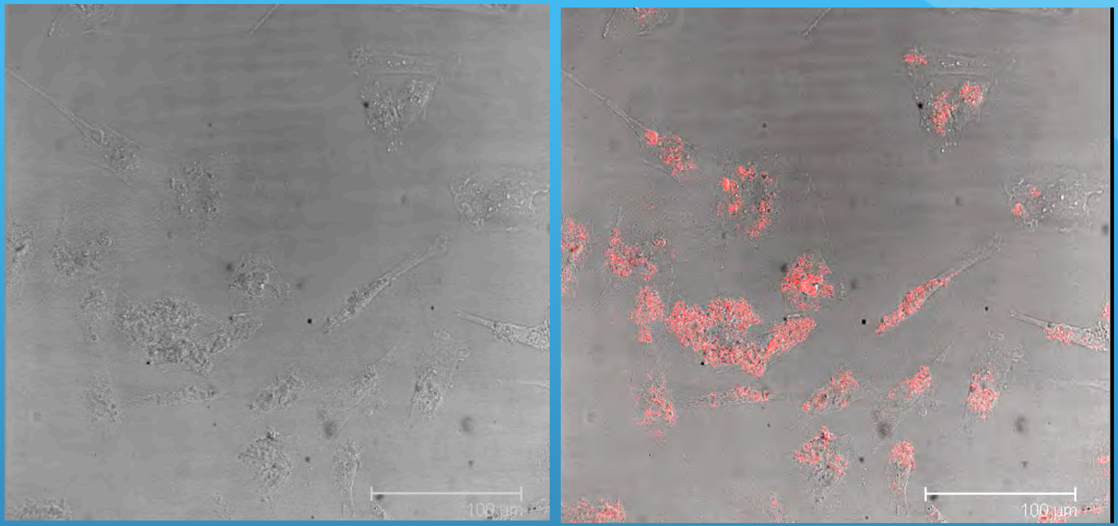
- Ability to penetrate tissues and cells within tissues
- Elicit response of the immune system. Accumulation in certain tissues/organs. May activate cancer pathways
- High reactivity --- high surface area
- Interaction with energy in new ways
- ...

Exposure routes to ENs



National Institute for Resources and Environment, Japan (www.nire.go.jp/eco_tec_e/hyouka_e.htm)

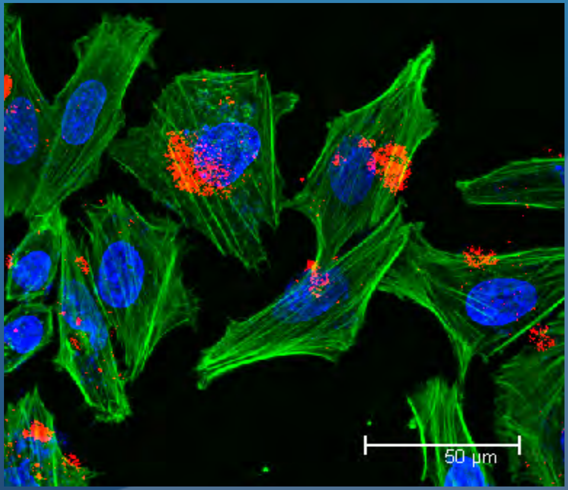
ENs are able to enter cells



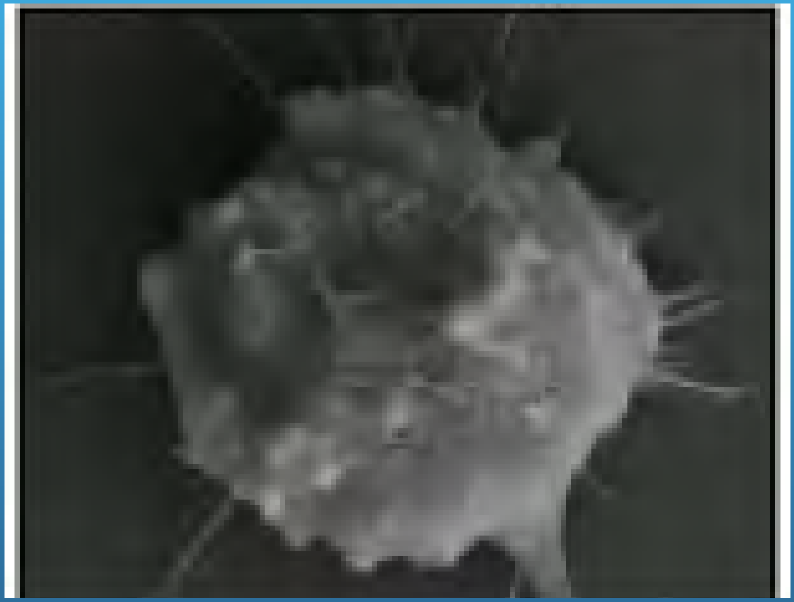
SaOS + 10 ng/cel SiO2-NH2 100 nm

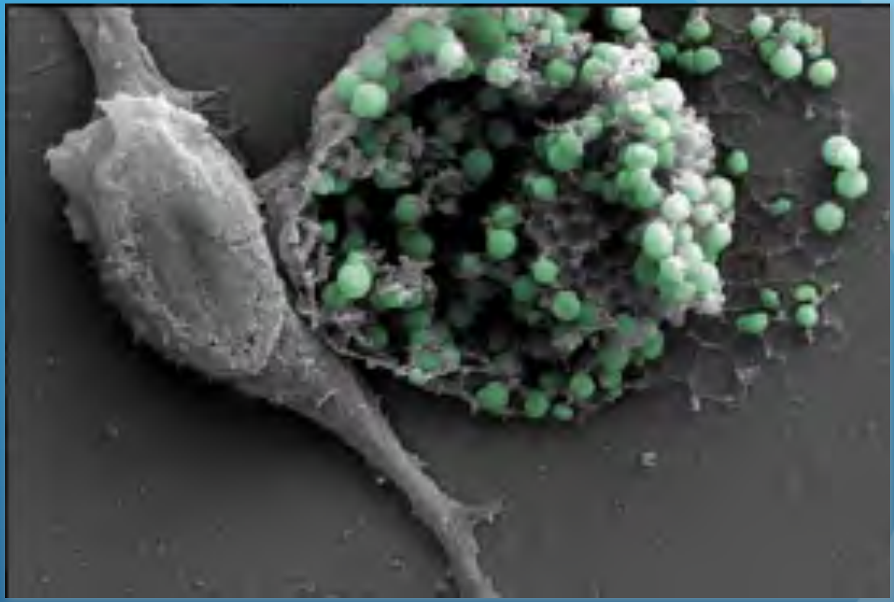
- …and sometimes even cell nuclei → transfection

(Collaboration with Dr Villaboa Hospital La Paz, Madrid)



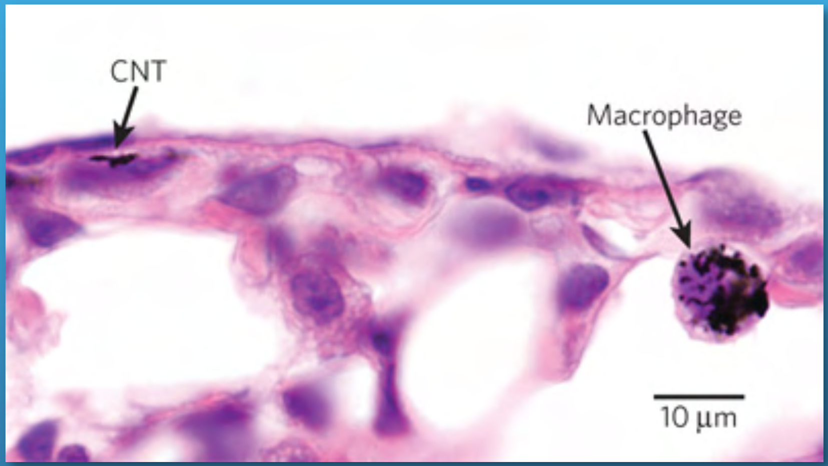
Are often taken up by macrophages





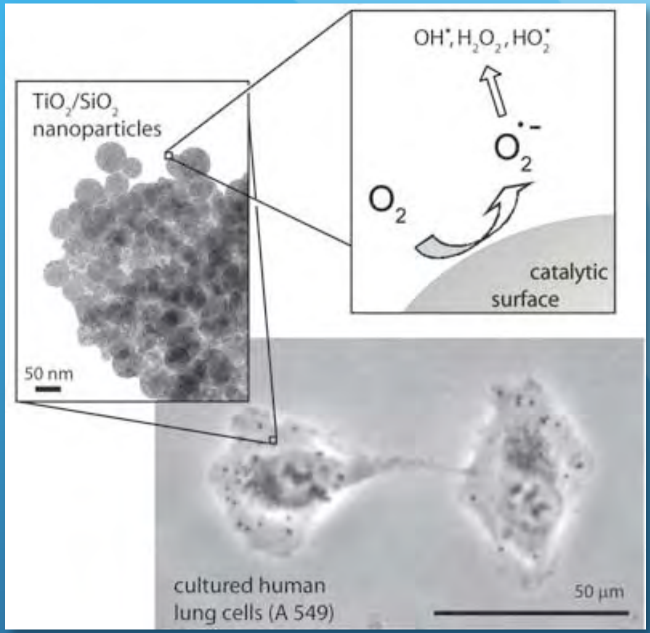
(Collaboration with Africa Gonzalez, University of Vigo)

Harmful effects of ENs have been demonstrated. Respiratory diseases



When inhaled by mice, multiwalled carbon nanotubes (CNTs) can embed themselves in the lining of the lung

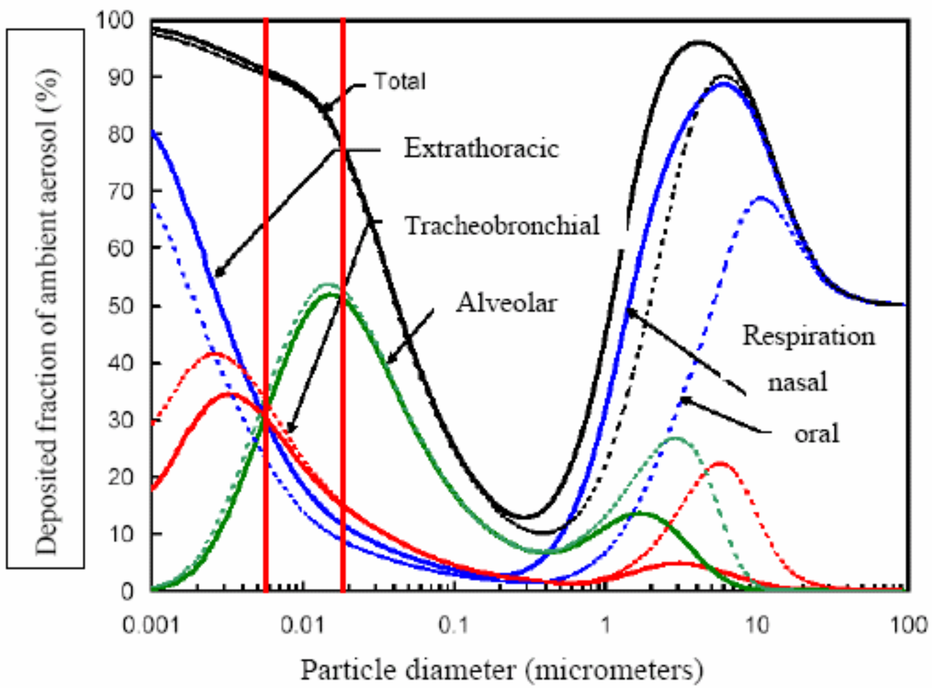
Ryman-Rasmussen. Nat. Nanotech. 2009, 4, 747



When human lung epithelial cells (bottom) take up metal-containing nanoparticles (left), harmful molecules can be generated in reactions at the nanoparticle surface (right)

Limbach, Env. Sci. Technol. 2007, 41, 4158

Can they reach the lung alveoli?



Witschger and Fabriès (2005) (Institut National de Recherche Scientifique (INRS), France

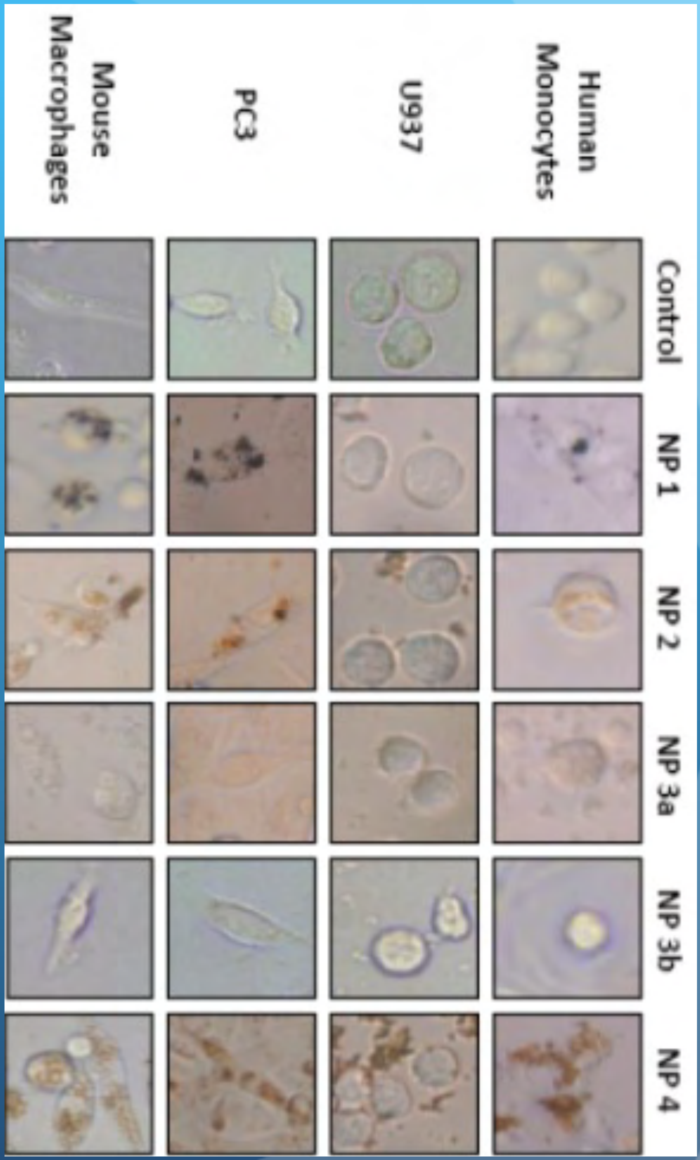
- Shape matters (particles or fibres)
- 1 nm NPs do not reach alveoli. 80% are deposited in nose and pharynx.
- At 5 nm 90% of all inhaled particles are retained and then are deposited in equal proportions in the three regions
- At 20 nm particles 80% are retained, more than 50% deposited in the alveolar region.

NPs in alveoli → Macrophage Phagocytosys → Attempt to degrade → Removed to the mucose → Granuloma

100 días

How to measure cell toxicity (in vitro)?

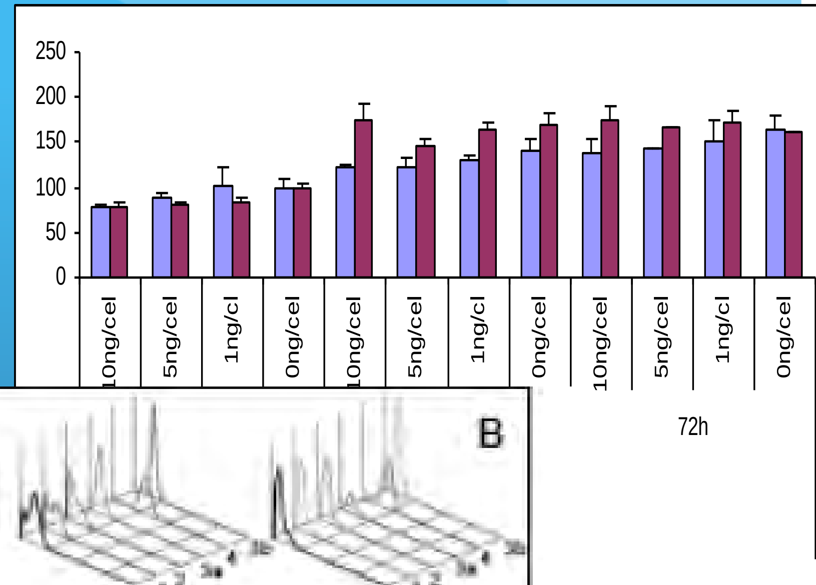
- No standardized protocol
- Key factors for measured toxicity:
  - NP characteristics: size, shape, charge, surface area, composition…
  - Method of measurement
  - Cell type(s)

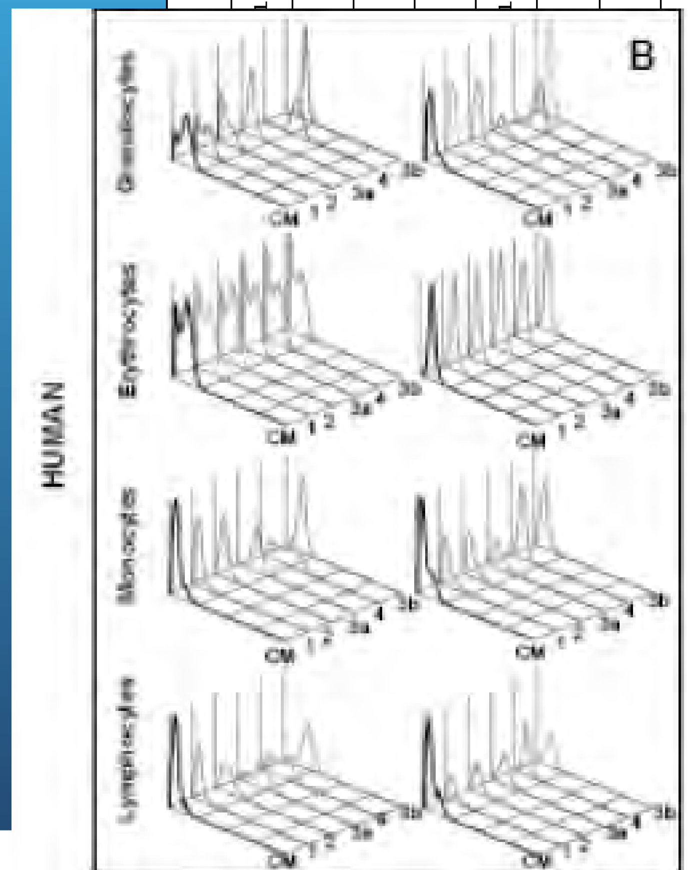


(Collaboration with Africa Gonzalez, University of Vigo) – *Small* (2008)

What to measure?

- Viability
  - metabolism, e.g. MTT assay
  - death=loss of membrane integrity, e.g. Trypan blue exclusion assay
- Cell stress (e.g. ROS production)
- Other: e.g. immune response

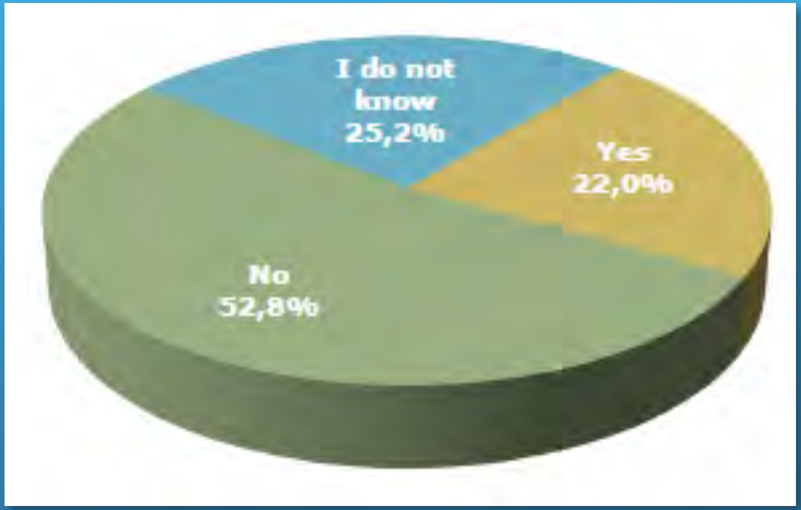




Small, (2008)

→Results sometimes do not correlate!
→A battery of tests and statistical analysis is required

Are we (as scientists) conscious of these risks when we handle ENs in the lab?



Can your nanoparticles become airborne during processing?

- An internet survey performed from April to June 2009 revealed that about a quarter of the respondents did not know whether the nanomaterials they made would become airborne during synthesis, 22% reported knowing that the nanomaterials would become airborne

Balas et al, Nature Nanotech. (2010)

Laboratory protection methods

Table 1 | Relationship between laboratory protection measures and knowledge of nanomaterials becoming airborne during synthesis.

		General laboratory safety during synthesis and handling (%)					
		No special protection	Local extraction on lab bench	Standard fume hood	Fume hood with nanosize filters (for example, HEPA)	Special nanosafe fume hood	Other
May nanomaterials become airborne at any stage of synthesis? (%)	Yes	20.8±5.0	16.7±4.7	50.0±5.9	6.3±3.4	4.2±3.0	2.1±2.0
	No	22.1±3.1	16.8±2.8	49.6±3.6	7.1±2.1	2.7±1.5	1.8±1.2
	Don't know	34.6±5.4	11.5±4.0	44.2±4.0	7.7±3.5	1.9±1.3	
	Overall	24.0±1.6	15.2±1.4	47.5±1.8	7.8±1.1	2.8±0.8	2.8±0.8

The researchers recognized that their nanomaterials could become airborne, but many used little to no laboratory protection. Confidence intervals are given as ±standard error, with $100(1-\alpha) = 68.3\%$. The row labelled overall summarizes the answers from all respondents.

- Nearly 40% of researchers reported using none or only weak means of general laboratory protection, even when they recognized that their materials could become airborne, (20.8% for no protection and 16.7% for local extraction)
- No statistically significant difference regarding the method of general protection chosen between researchers responding yes or no to the potential presence of airborne particles

Balas et al., Nature Nanotech. (2010)

Personal protection methods

Table 2 | Relationship between the use of personal respiratory protection devices and knowledge of nanomaterials becoming airborne during synthesis.

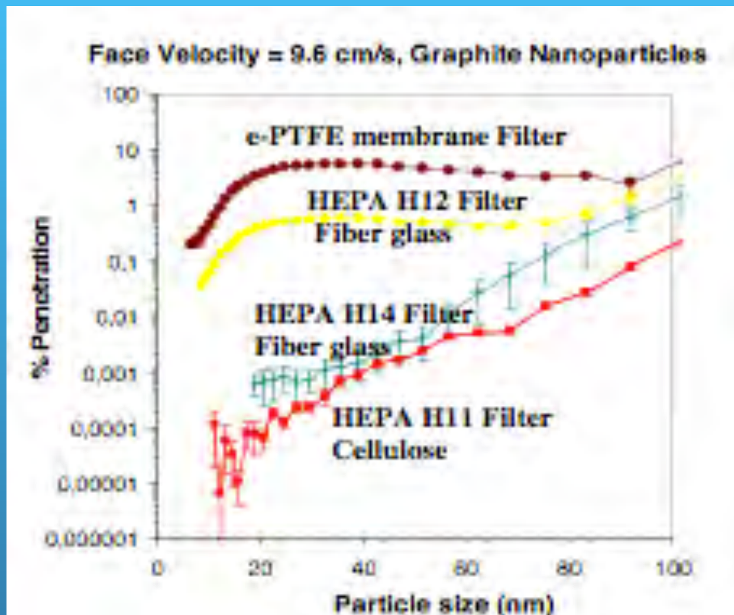
		Personal protection equipment when handling nanomaterials (%)					
		None	Mouth mask without filters	Respiratory mask with standard filters	Full face-shield with standard filters	Mask or shield with specially designed filters	Full-body protective equipment
May nanomaterials become airborne at any stage of synthesis? (%)	Yes	29.8±5.6	36.2±5.8	27.7±5.5		4.3±3.1	2.1±1.9
	No	57.7±3.6	17.1±2.9	16.2±2.9	4.5±1.8	2.7±1.5	1.8±1.3
	Don't know	50.9±5.6	28.3±2.3	15.1±5.1	1.9±1.5		3.8±2.5
	Overall	48.8±1.8	24.4±1.6	18.4±1.5	2.8±0.8	2.8±0.8	2.8±0.8

Many respondents had poor personal protection despite having declared that the nanomaterials they synthesized could become airborne. Confidence intervals are given as ±standard error, with $100(1-\alpha) = 68.3\%$. The row labelled overall summarizes the answers from all respondents.

- 48.8% reported not using any type of respiratory protection and 24.4% used a mouth mask without filters (ineffective protection)
- Respondents with poor personal protection (29.8% using no protection and 36.2% using a mask without filters) were high, despite having declared that the nanomaterials they synthesized could become airborne

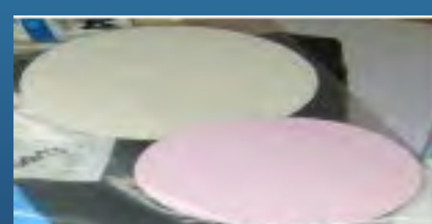
Balas et al., Nature Nanotech. (2010)

Excellent performance of mask filters for NPs



- Tests performed with graphite particles confirm for major conventional HEPA filters that nanoaerosol penetration rates decrease drastically for smaller particles.
- Main hazard coming from inadequate mask fitting

Nanosafe



Excellent performance of gloves for NP aerosols

Several types of gloves have been tested by Helium diffusion test. All the tested gloves are made of porous material which differs according to the material and thickness. On the other hand, after exposure to a high concentration of graphite aerosols, ranging from 20 to 100nm, no particle penetration can be measured.

Nanosafe

Dissemination report
January 2008
DR-325/326-200801-1

Project ID NMP2-CT-2005-515843

No se puede mostrar la imagen. Puede que su equipo no tenga suficiente memoria para abrir la imagen o que ésta esté dañada. Reinicie el equipo y, a continuación, abra el archivo de nuevo. Si sigue apareciendo la x roja, puede que tenga que ... de nuevo.

Research needs in Nanosafety

How harmful?

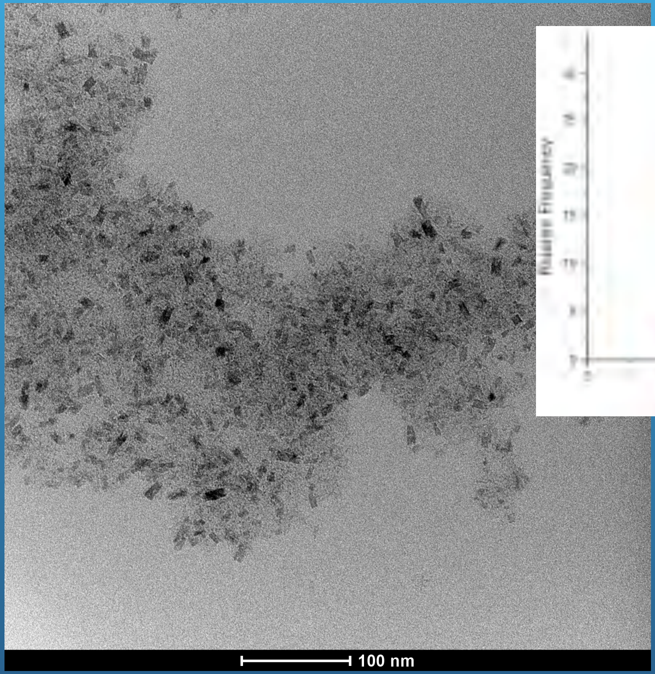
- Nanotoxicology in vitro, in vivo
- Epidemiological studies

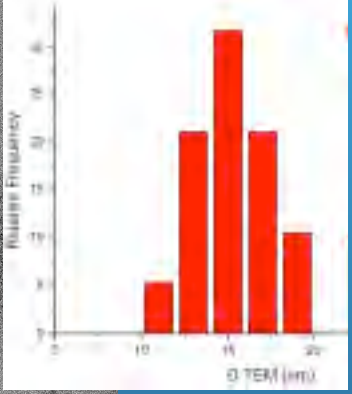
Can ENs be controlled?

- Dispersion and characterization of NP aerosols
- NP monitoring (domestic, industrial, environment)
- Life cycle analysis
- Safe handling, trapping, disposal

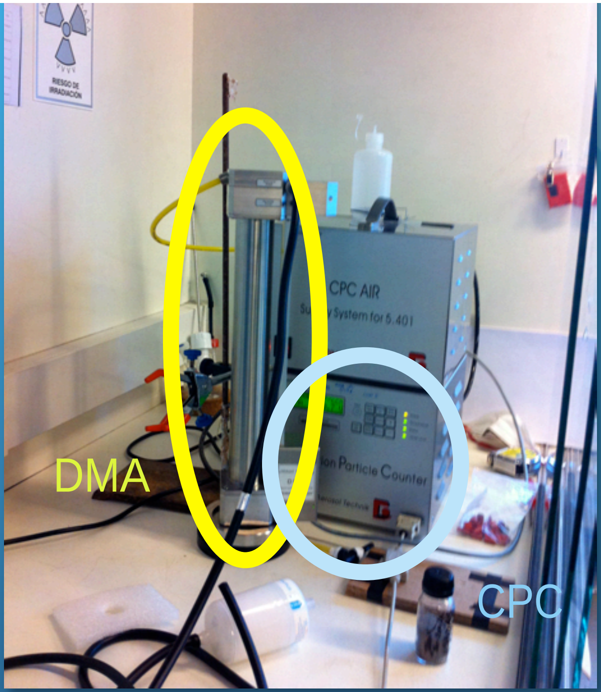
Research in Nanosafety at INA:
Characterization of EN aerosols

"Traditional" electron microscopy: TEM, SEM



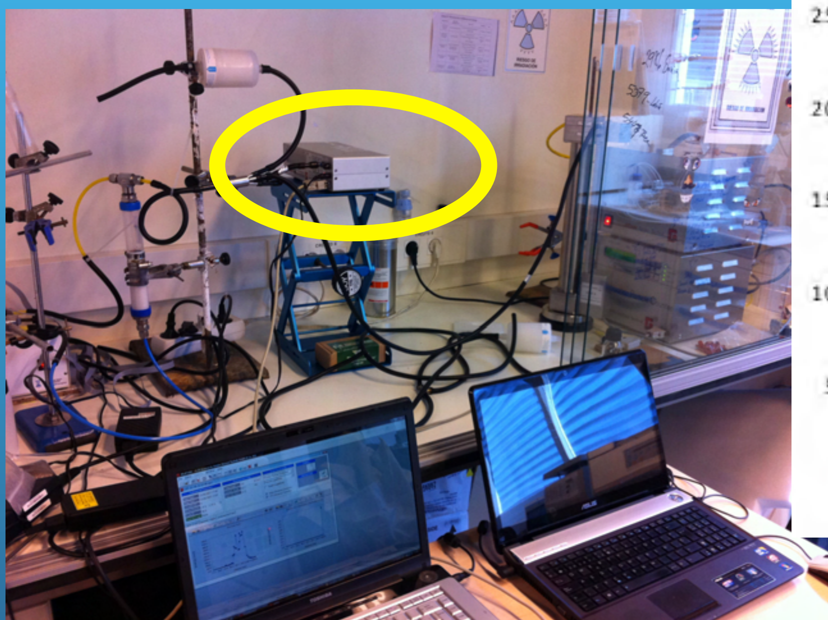


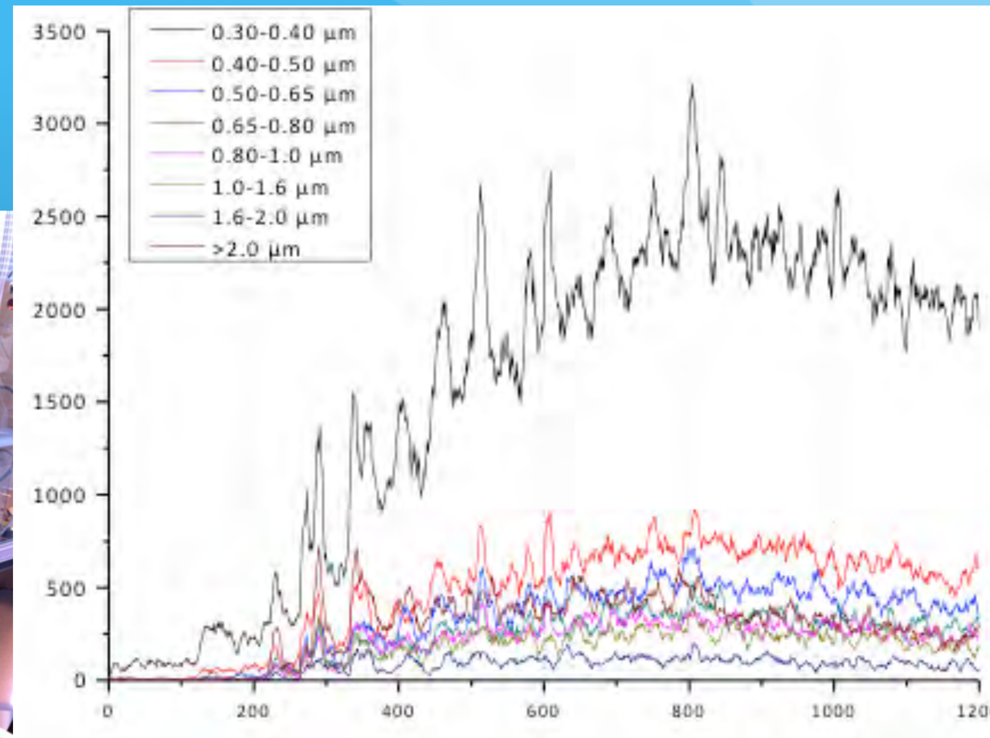
Scanning Mobility Particle Sizing (SMPS) 5-1100 nm



- Electrostatic classification (Differential mobility analyzer, DMA)
- Optical detection (Condensation particle counter, CPC

Characterization of EN aerosols (2)

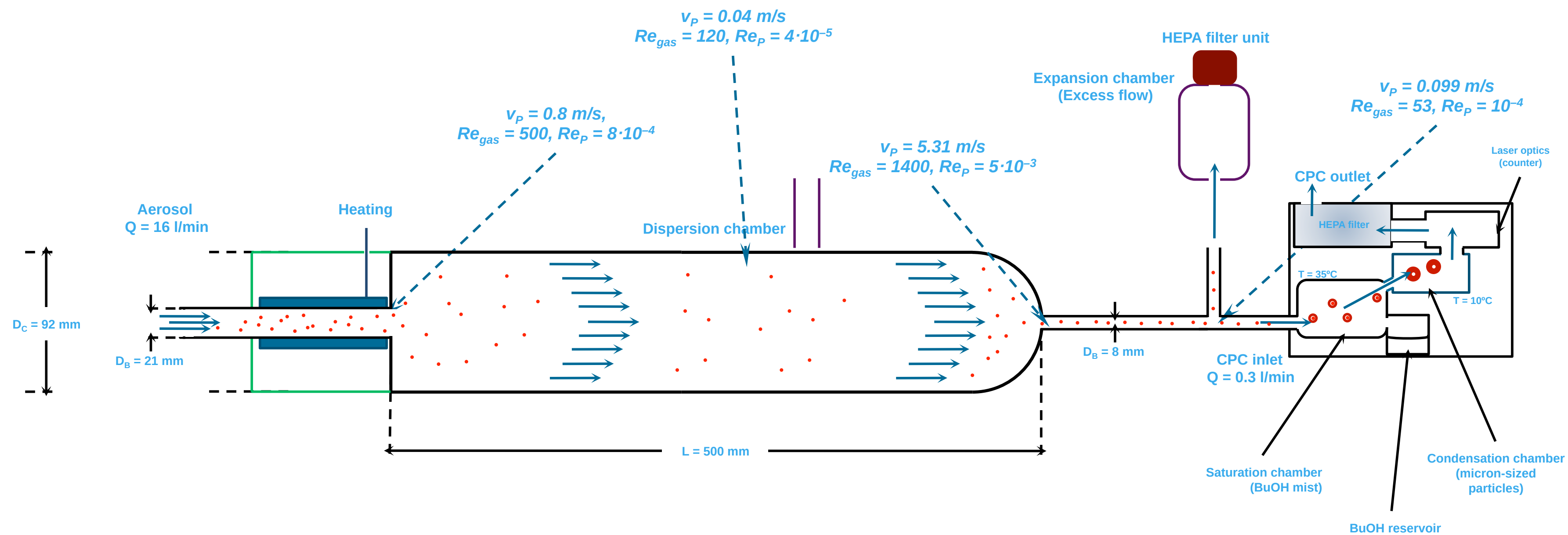




Optical Particle Counter (OPC)

- Optical detection
- D_P > 300 nm Similar to CPC quantification without condensation stage

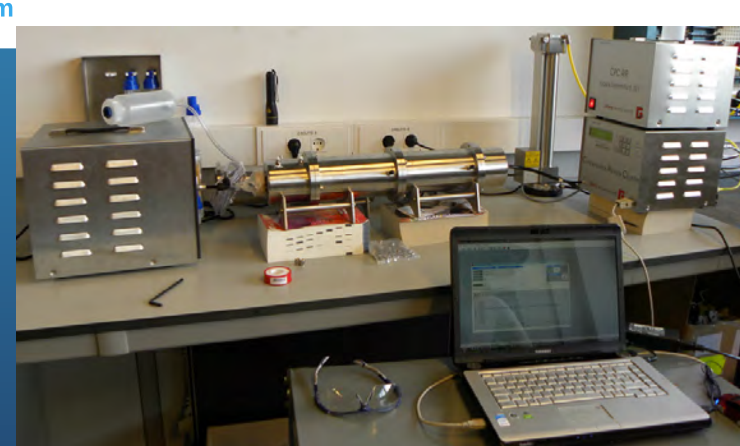
Dispersion/agglomeration behaviour of ENs under controlled conditions



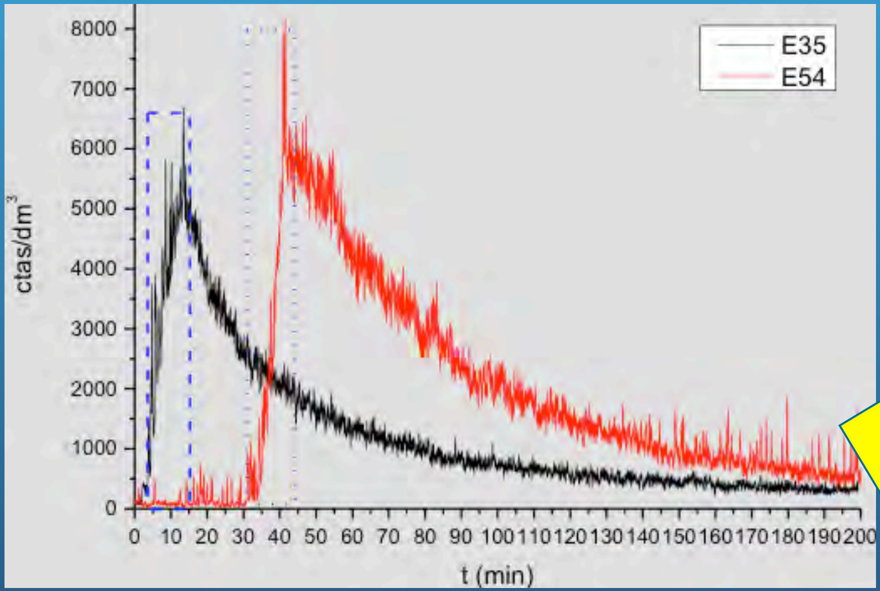
Test conditions: dp 0 15 nm at 298 K and 1 atm

Dispersion tube for nanoparticle aerosol testing

- Agglomeration/aggregation in dry/humid air
- Nanoparticle surface charge
- Deposition in different surfaces and mechanical elements



EN aerosol generation in common handling operations-Dispersion chamber 1



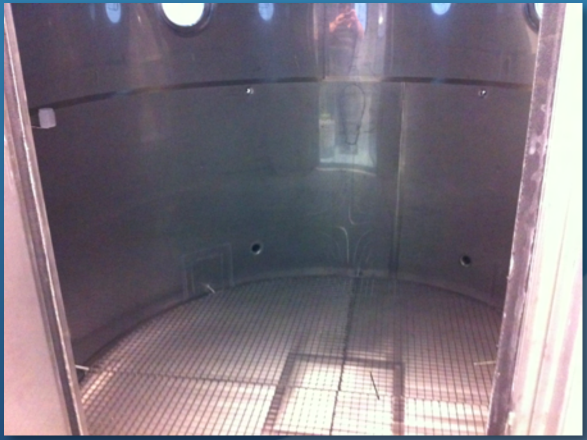
EN aerosol generation in common handling operations-Dispersion chamber 2

Self-cleaning installation for dispersing engineered nanoparticles for occupational health and safety studies

EN aerosol generation in common handling operations-Dispersion chamber 2

Installation for dispersion of nanoparticle aerosols

- Effect of nanoparticle properties (in aggregation, dispersion, deposition…)
- Effect of atmosphere conditions in nanoparticle aerosols (humidity, temperature, pressure…)
- Assessment of laboratory operations in nanotechnology
- Environmental impact of nanoparticles
- Health impact of nanoparticles (Animal tests)



Labelling of ENs

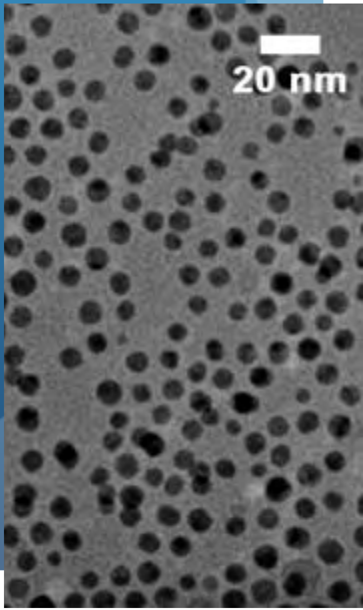
Not the same objective or techniques as biolabelling

Discriminate ENs from NPs existing in the environment

- Labelling must not affect the behaviour aspect to be studied
- Investigate dispersion/agglomeration under realistic conditions
- Efficiency of trapping methods
- Monitor ENs in industrial or laboratory environments
- Environmental impact of nanoparticles

High sensitivity needed

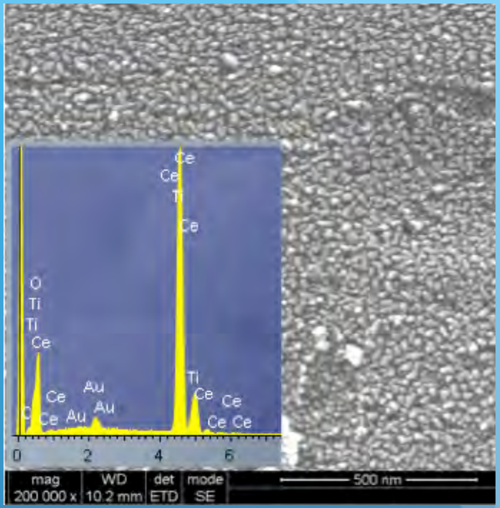
- Monitor ENs at trace concentration levels
- In the presence of a myriad of NPs



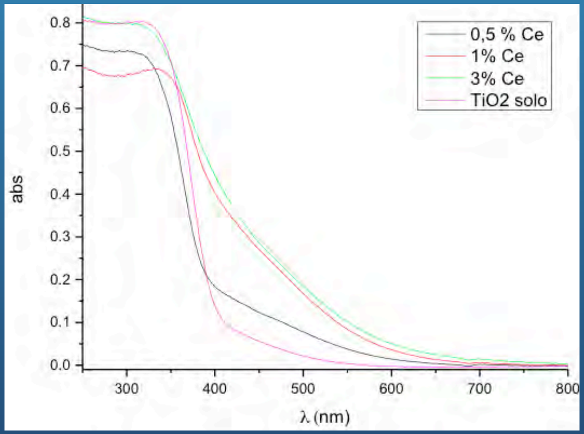
Labelling of ENs

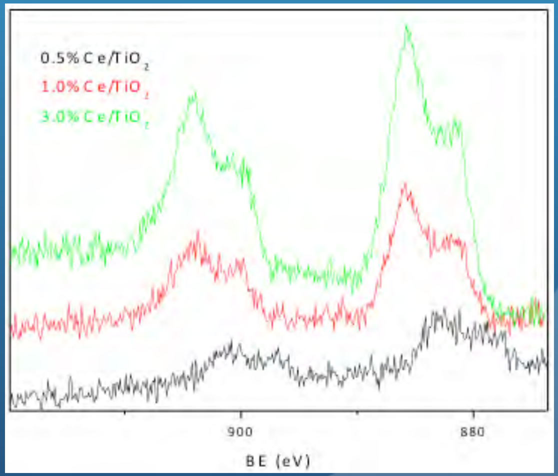
Some techniques being investigated:

- SEM-EDX
- XPS
- FTIR
- X-Ray Fluorescence
- UV-VIS



Example: TiO_2 NPs labelled with Ce





Recommendations and perspectives

- A strong research effort in Nanosafety is urgently needed.
- A multidisciplinary approach: engineering, surface chemistry, biology, toxicology…
- Emerging field. Lots of opportunities
- Scientists should self-regulate because they are the ones who decide how nanomaterials are handled in the laboratory.
- Until safe handling conditions for different ENs can be established, the precautionary principle should be used.

Let us face the problems that we can solve:

- Make sure nanomaterials are handled safely
  - Worker exposure (includes researchers!)
  - Public exposure

- Make sure nanomaterials are disposed of safely
  - Specialised disposal/trapping may become necessary

- Make sure products containing nanomaterials are monitored for possible losses.
  - Not as easy as it looks. May require labelling.

- Make sure the life cycle of products containing nanomaterials is analysed
  - Not easy either, recovery of nanomaterials may be difficult

Data are INDEED needed...

- With Nanotechnology this may require extensive testing of new materials.
  - Expensive
  - Time consuming
  - Sometimes inconclusive
- A similar situation was (is) faced for other chemicals (e.g. suspected carcinogens)
  - Apply precautionary principle: Avoid unnecessary exposure, minimize risks... until the product is proven safe
- Let us make sure that uncertainties do not impede Nanotechnology development
  - Find conditions that allow safe production, use and disposal

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