Toxicity and risks for human health of nanomaterials

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INERIS

General organization of presentation

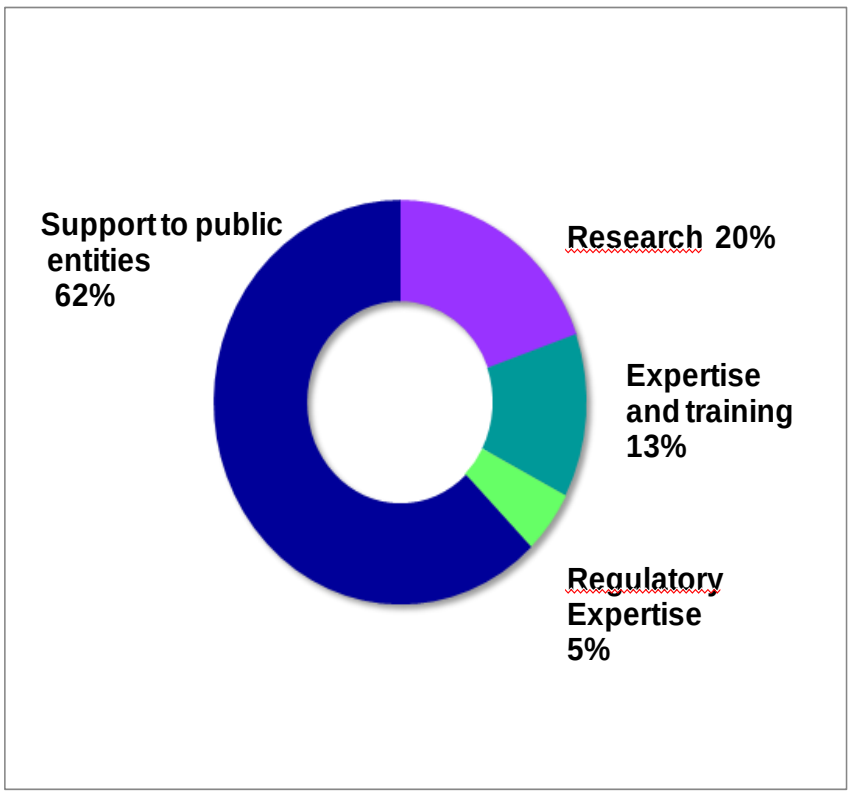
1) What is INERIS?
2) Toxicology and Nanotoxicology: definitions and context
3) Effects of nanoparticles on human health
    - 3.1. Entry routes
    - 3.2. Biological effects
4) Conclusion

1) What is INERIS?

National Institute for industrial Environment and RISks.

INERIS: Public Institute with Industrial and Commercial goals.

- Founded in 1960
- 600 employees, 50 PhD students
- 70 M€ revenues



- 60% funding from the French Ministry of Ecology -governance
- 40% contracts with private or public entities (including Europe)

INERIS

***Controlling risks** for sustainable development*

www.ineris.fr

600 people (+ 50 in PhD) 60 M€

Human being

Health
Safety

Industrial or natural sites

Environnemental impacts
Hazards

Environment

Chronic

Ground/ underground

Accidental

Experimentation

Laboratory

Pilot

Full scale tests

Sites, factories

Feed back

Modélisation

Research

* Public
* Private
* Europe

Service

* Technical support for authorities
* Companies
* Local administrations

Analyses

Studies
Assistance for studies
Guidelines

Regulatory dossiers
Industrial sites
Risk Prevention Plans
Third party expertise

Consulting
Risk management, audits
HSSE

Normalisation

Regulation watch

Certification
ATEX, TDM, Explosives

Training

Research - Expertise - Consulting - Training

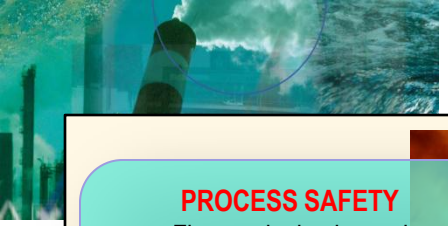
INERIS

Controlling risk for sustainable development

- Prevention of industrial and technological risks.

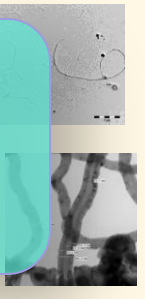
- Prevention of risks associated with chemical substances.

- Participation in the development, improvement , and sharing of best technologies and best practices.



PROCESS SAFETY
- Fire, explosion hazards
- Loss of containment
- Safety parameters (MIE, Kst…)
- Molecular modeling QSPRs

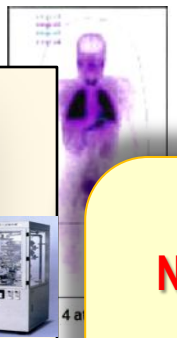
INSTRUMENT DEVELOPMENTS
- On-line monitoring (LIBS, LII, LIF)
- Sampling techniques(microscopy, TEM)
- Instrumental calibration, perf. evaluation
- Nano generation and aerosolisation modules



PRODUCT CHARACTERIZATION
NANOBENCH
- Physico-chemical properties (TEM, SEM…)
- Dedicated equipments :

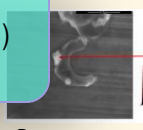
Use and ageing (mechanical stress…)
End of life (recycling)

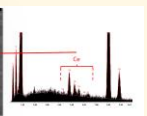
Dedicated Installation for safe manipulation of nanoparticles (Halle nano, NanoBench)



TOXICOLOGY
- Experimentation (stress, inflammation, genotoxicity..)
- In vitro & In-silico (QSARs, TK, PBPK…)
- Dedicated equipments (in-vitro, in-vivo inhalation)

ECOTOXICOLOGY
- Experimental (Ageing, OECD tests…)
- Dedicated equipments (mesocosm)



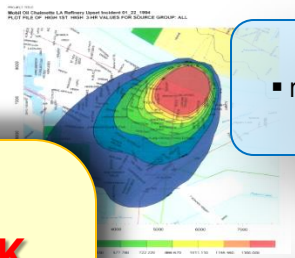


Dedicated GLP platforms

INERIS TASK FORCE ON NANOTECHNOLOGIES RISK ASSESSMENT

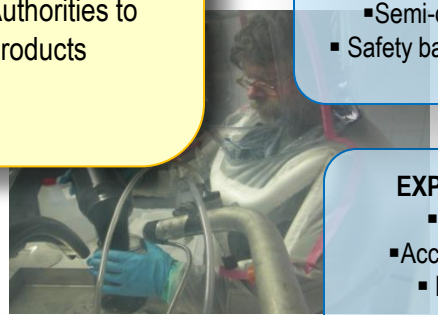
Help Industries, Laboratories and Authorities to develop safer processes and products

(emeric.frejafon@ineris.fr)



MODELISATION
- nucleation, agglomeration
- Reactivity-Transport

RISK ANALYSIS
- "Control banding" tools
- Semi-quantitative risk assessment
- Safety barriers management & definition
- Intrinsic safety



EXPOSITION ASSESSMENT
- Occupational exposure
- Accidental exposure scenarios
- Environmental exposure

EXPERTISE
- Industries, public authorities
- nanoREACH, ANSES
- ISO, CEN & AFNOR
- OECD – WPNM

SOCIO-ECONOMIC ANALYSIS
Integration of LCA and LCC results
- Cost benefit & cost-efficiency analysis (CBA, CEA)
- Multi-criteria analysis (MCA)

TRAINING
- Workers, HS department, regulators
- nanosafety

CERTIFICATION
- NanoCert Workers
- NanoCert MTD

Main goals of the Experimental Toxicology Unit

Assessment and prevention of hazards produced by chemical and physical agents to human health

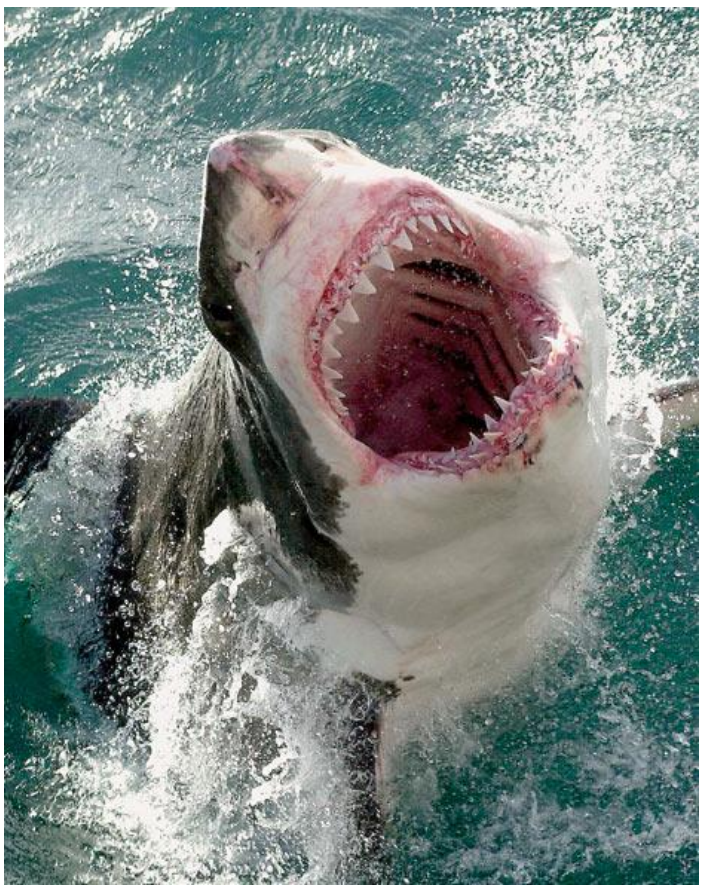
- Assessment of the risks associated with exposure to non-ionising radiation (**electromagnetic fields**) via experimentation
- Assessment of toxicologic effects of **nanomaterials** via experimentation.
- Contribution (national level) to the implementation of the European legislation on chemical substances (**REACh**)

2) Toxicology and Nanotoxicology: Definitions and Context

INERIS

Definitions

- **Toxicology =** the science evaluating inherent **dangers** of substances.



- **Risk = exposure x danger**

Ultrafine or Nanoparticles – General Notions

Ultrafine Particles:

- defined by industry/ air pollution field (approx. 1990) as subfraction of generated particulate matter
- size < 100 nm
- incidental

Ultrafine Particles

Combustion processes (23%)

Industrial

Domestic

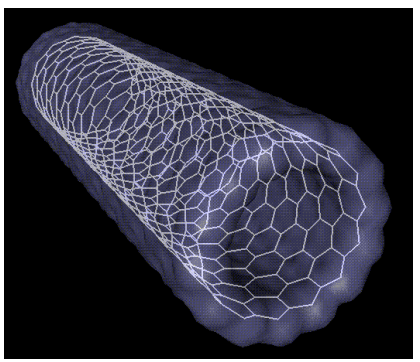
Transport (60%)

Ultrafine or Nanoparticles – General Notions

Manufactured Nanoparticles:

- linked to the development of nanotechnologies
- Engineered particles
- Nanomaterial = OECD definition

« Material composed of one or several components having at least one dimension is in the nanometric order of magnitude »



Nanoparticles

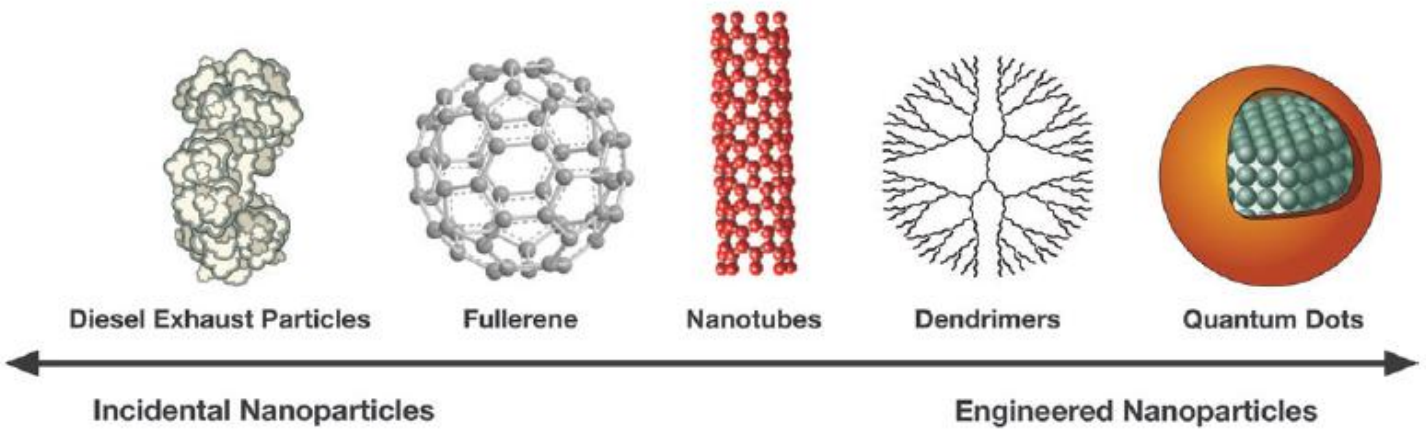


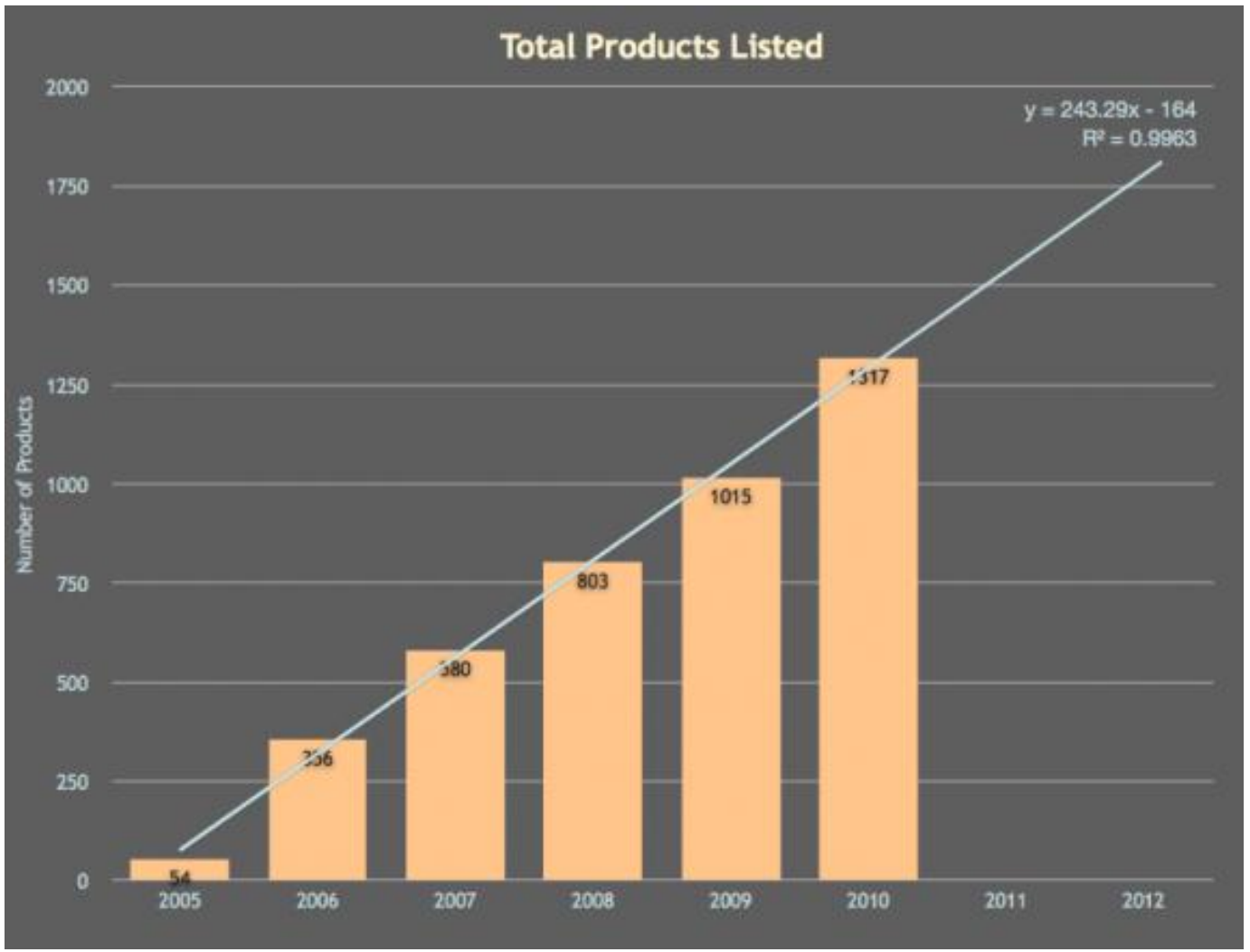
FIG. 1. Examples of incidental and engineered nanoparticles.

Recent wording and definitions, but interactions between life and nanoparticles are not as recent...

- Carbon Nanotubes found in glacier carrots in Greenland (néolithic - 10 000 years)
- Nanotubes and fullerenes , found from combustion processes (natural gaz, propane...)

Stern ST, McNeil SE (2008). Nanotechnology safety concerns revisited. *Toxicological Sciences*, **101(1)**, 4-21.

Nanotechnology consumer products inventory

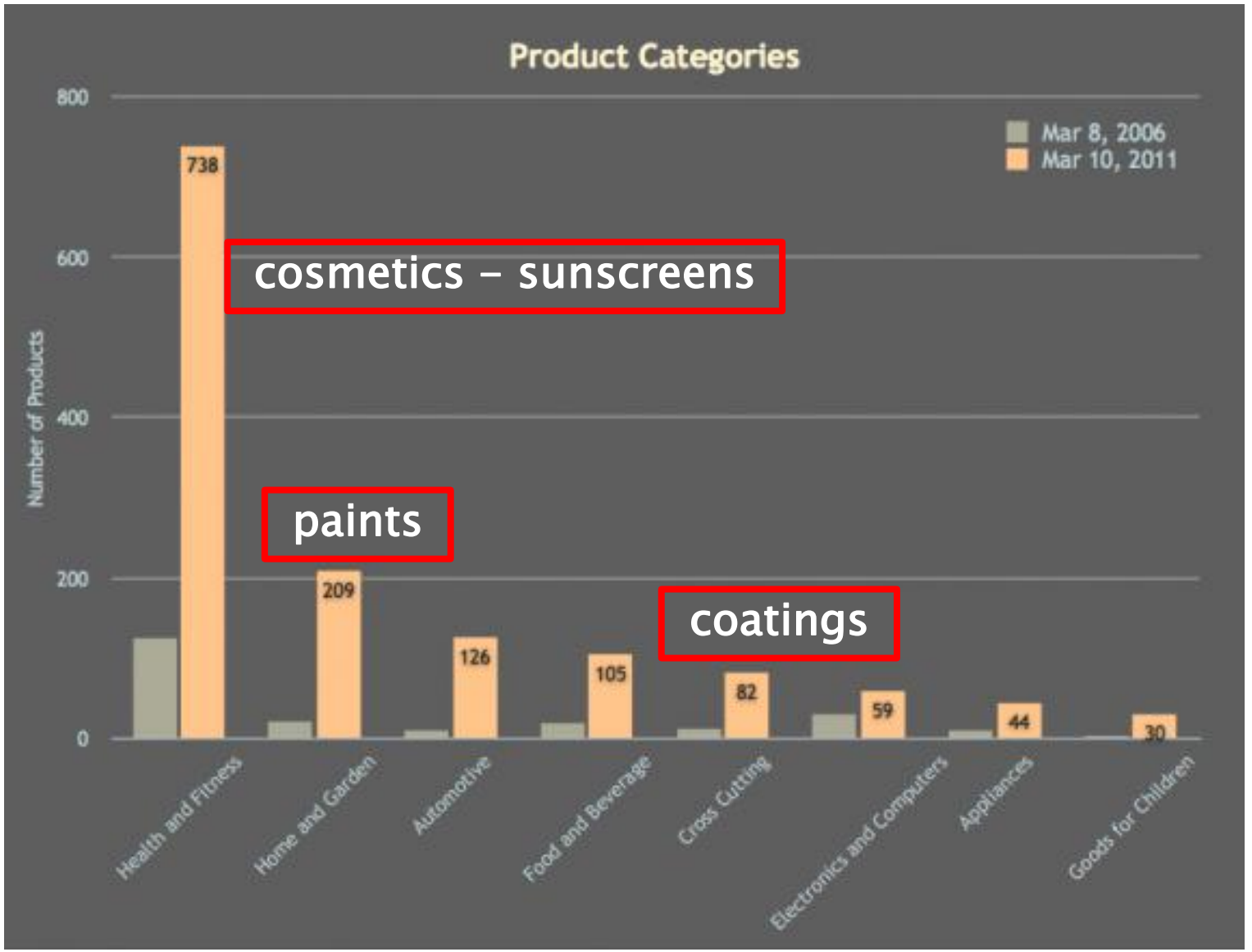


March 10, 2011:
1317 products or product lines.

521% growth
March 2006 =>March 2011

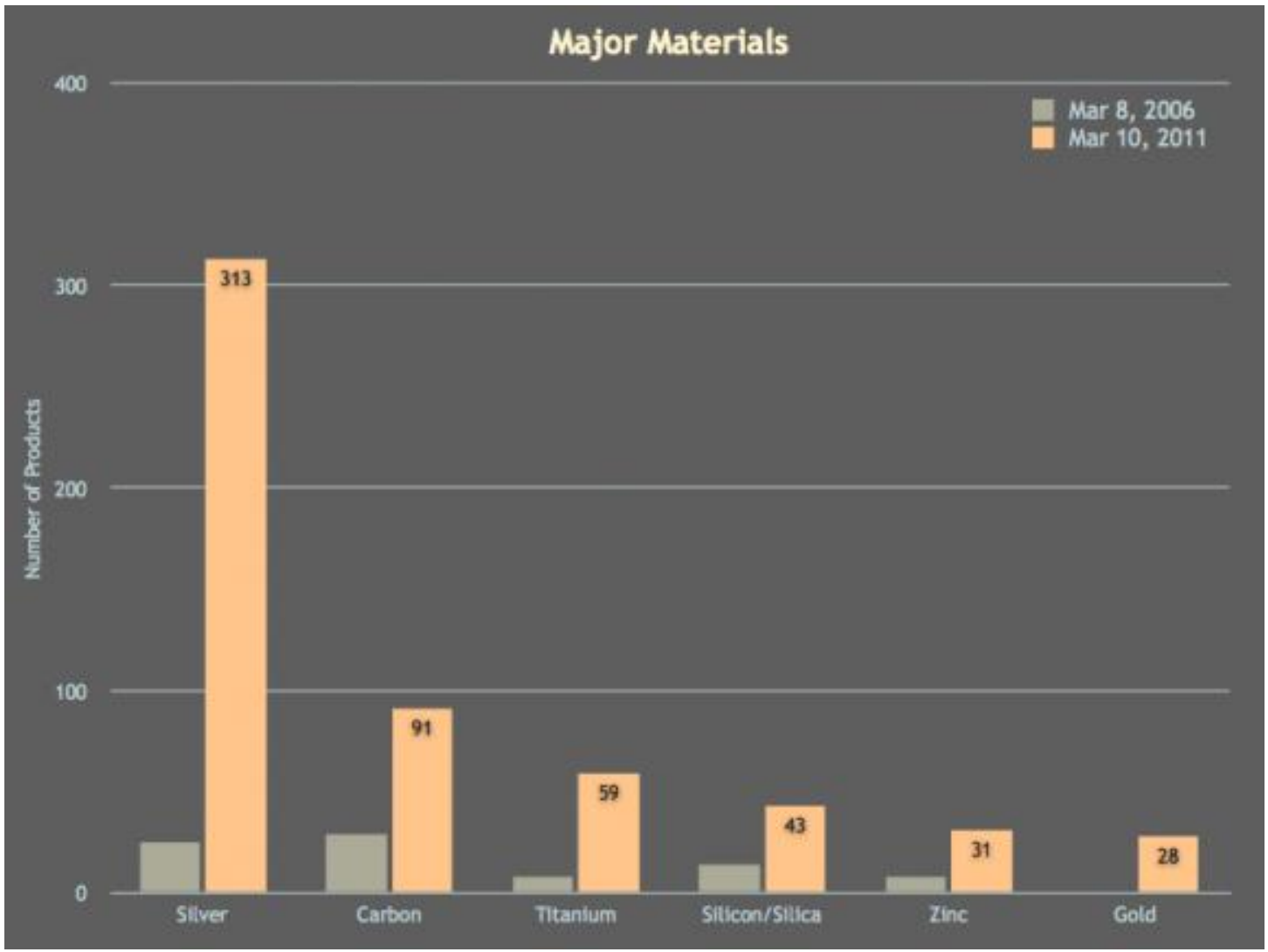
http://www.nanotechproject.org/inventories/consumer/analysis_draft/

Nanoparticles in consumer products



http://www.nanotechproject.org/inventories/consumer/analysis_draft/

Nanoparticles in consumer products



Most common material :
silver

Carbon (including fullerenes)

titanium (including titanium dioxide)

silica

zinc (including zinc oxide)

gold

http://www.nanotechproject.org/inventories/consumer/analysis_draft/

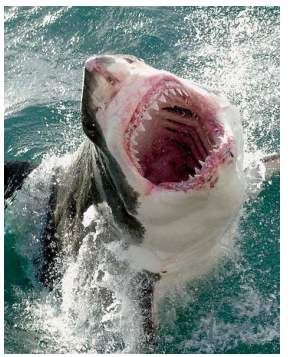
Why is risk evaluation of nanoparticles necessary?

Exponential development of nanotechnologies,

increased new particles/extreme diversity,

increased presence of nanomaterials in usual consumer products,

Risk = exposure x danger



→ Increased exposure of people (professional and general population)

« Is the field moving so fast that it's destined to repeat the mistakes of earlier technological revolutions? »

Service RF (2004). Nanotechnology grows up. *Science*, **304**, 1732-1734.

EDITORIAL Occup Environ Med 2004;61:727–728.

Toxicology

Nanotoxicology

K Donaldson, V Stone, C L Tran, W Kreyling, P J A Borm

A new frontier in particle toxicology relevant to both the workplace and general environment and to consumer safety

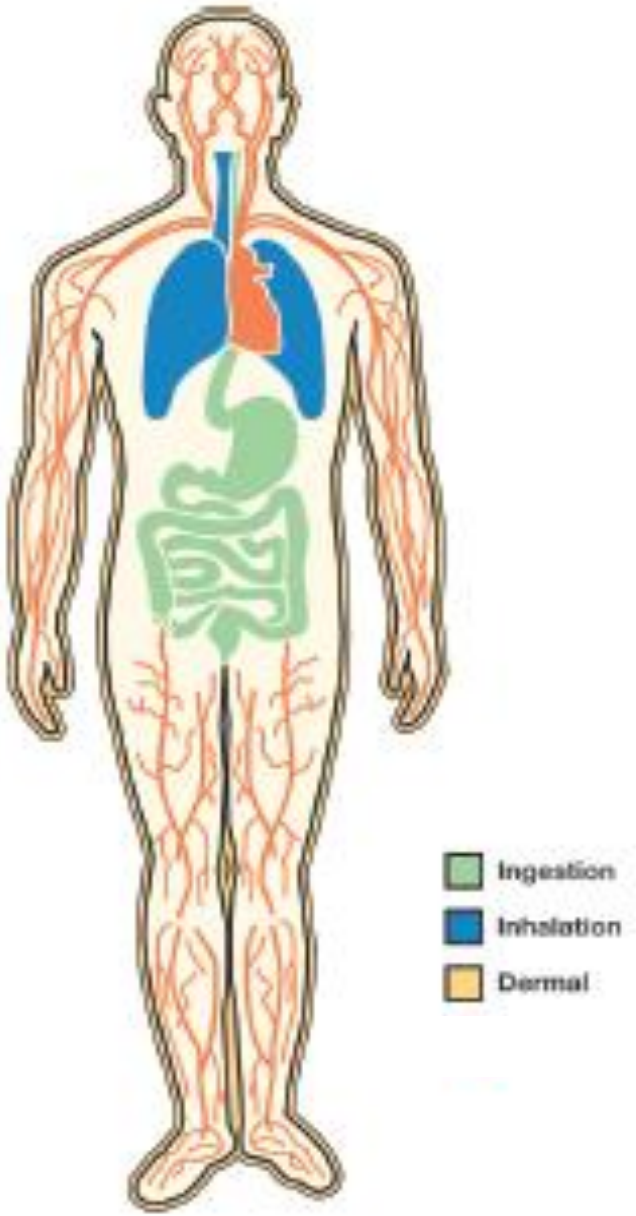
"We therefore propose that a new subcategory of toxicology—namely nanotoxicology—be defined to address gaps in knowledge and to specifically address the special problems likely to be caused by nanoparticles".

3) Effects on human health

3.1. Entry routes

Absorption/Distribution (Target organs determination) /Metabolism/Elimination

Entry routes



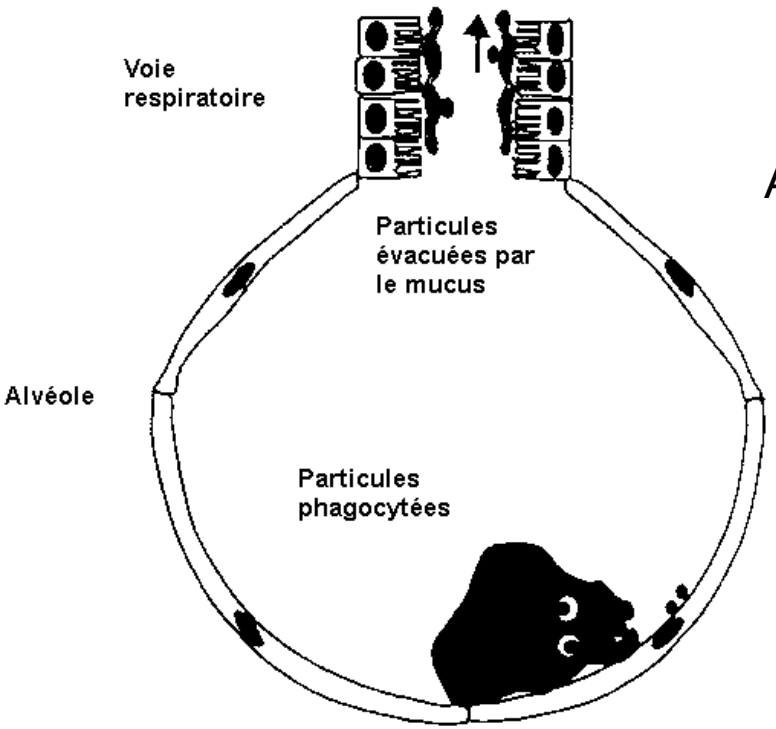
- Inhalation : main route

- Oral route: via feeding (eg food packaging nanoparticles), via ingestion of inhaled particles

- Dermal route : via cosmetics...

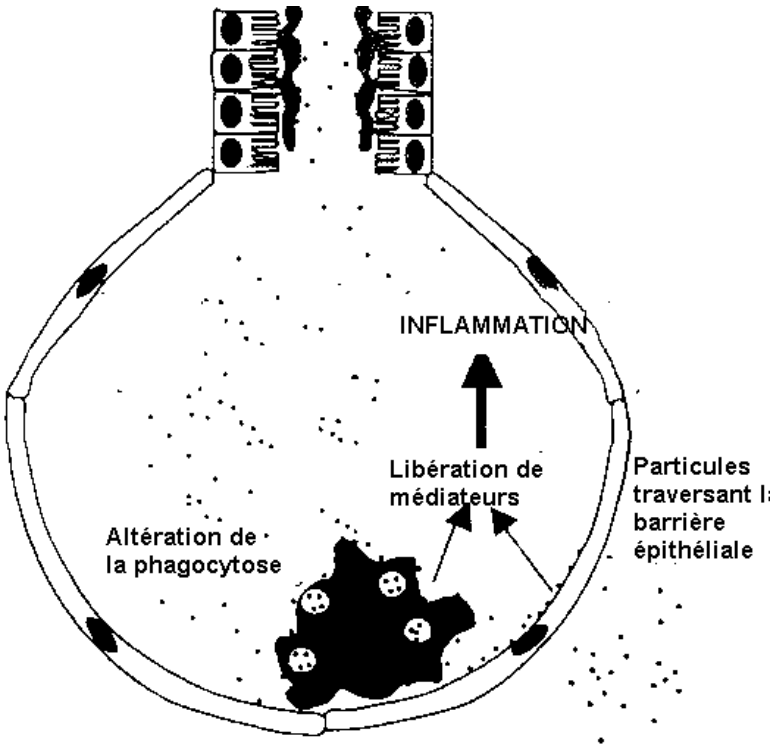
Inhalation: Nanoparticles elimination

Pulmonarian clearance :
mucociliated escalator
phagocytosis by macrophages



Fine particles

Alveolar cells



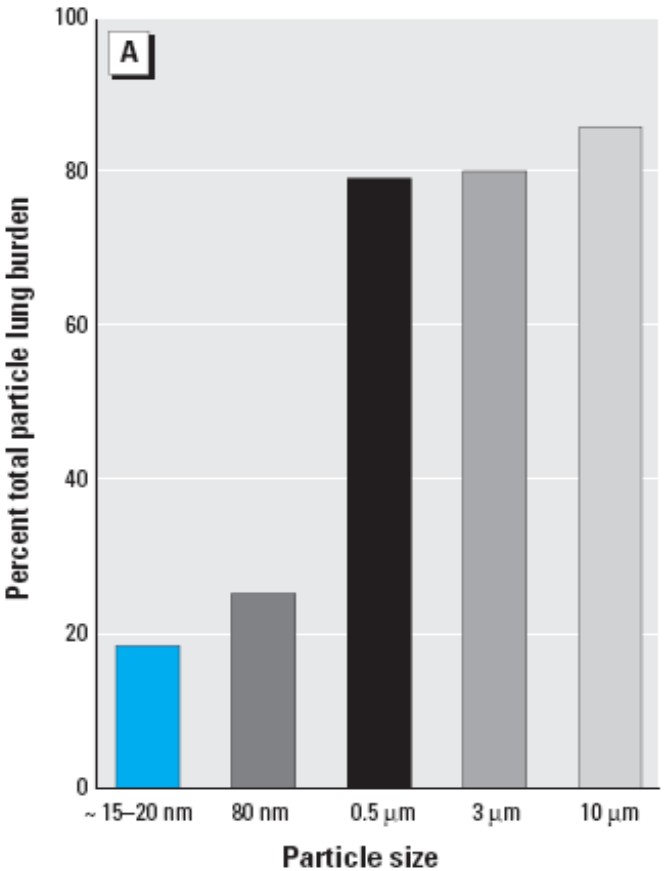
Nanoparticles

For the same mass of particles:
=> More deposition
=> Increased passage
=> Macrophages behavior?

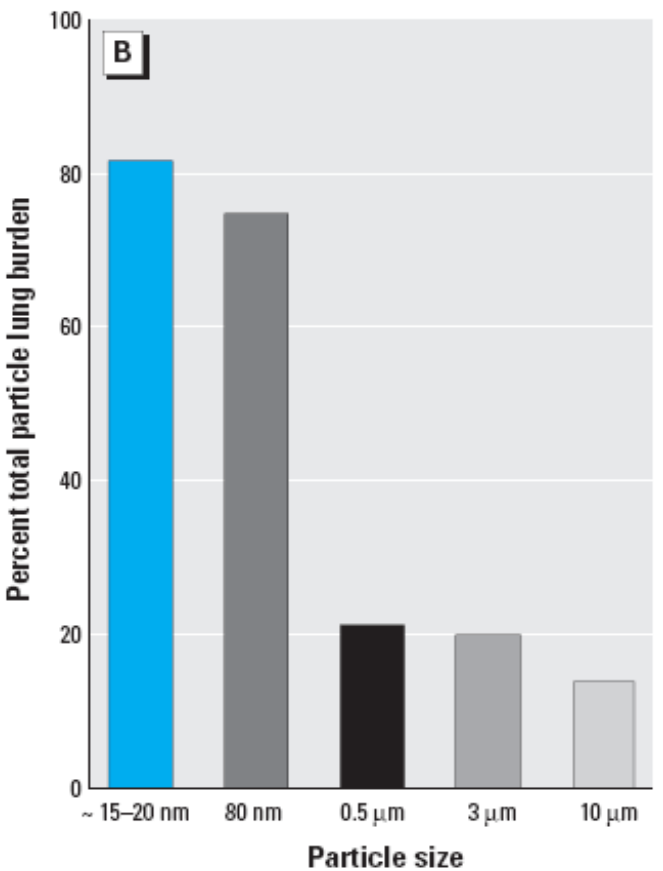
Inhalation:
Nanoparticles elimination vs transfer to the body

Studies on rodents have shown that the nanoparticles deposited in the lungs can enter into the pulmonary interstitium

Macrophages



Pulmonary interstitium



(Oberdörster, 1992, 2000)

Inhalation:
Nanoparticles transfer to the body

Concerning transfer of nanoparticles, the evidence is conflicting…

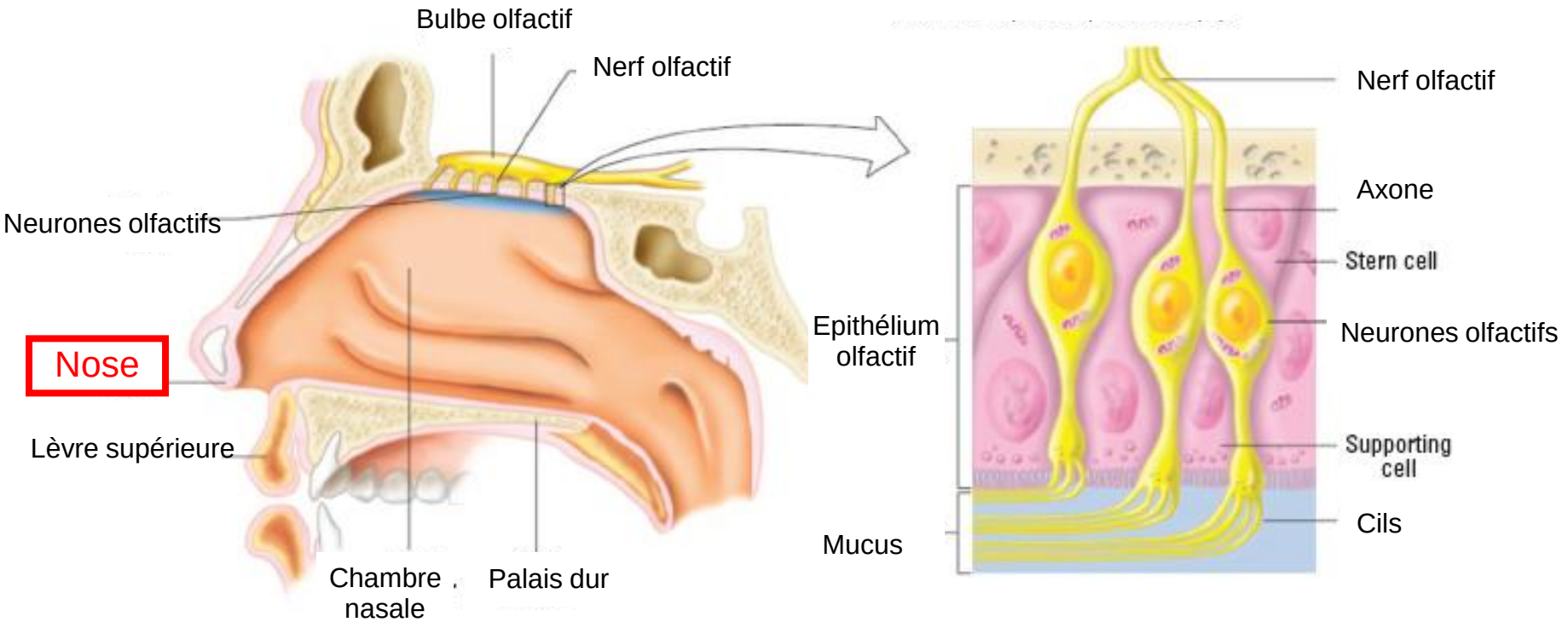
- Oberdorster, 2002 : 50% transfer (liver). NPs ^{13}C (20-29 nm)
- Kreyling, 2002 : < 1% transfer (Ir, 15 et 80 nm, IT)
- Takenaka, 2005 : < 1% transfer (Au, 10 nm)

⇒ Nanoparticles can deposit in the respiratory tract after inhalation.

Nanoparticle translocation into the systemic circulation may occur after inhalation, but conflicting evidence is present on the extent of translocation.

=> additional studies are needed to further elucidate these findings and to characterize the physiological impact.

A particular entry route: neuronal translocation



A few reports indicate uptake of nanoparticles in the brain via the olfactory epithelium.

D'après Oberdörster, 1994

Dermal route

Numerous studies of nanoparticles penetration through the skin:

- TiO_2 – sunscreens

  No clear penetration beyond stratum corneum
- Beryllium (500 nm - 1 µm)

  With skin agitation: penetration to the dermis (pig, in vitro)
- Quantum dots

  Limited penetration to the dermis (pig, *in vitro*), depending on size/shape/charge

⇒ Little evidence that dermal applications of metal oxide nanoparticles used in sunscreens lead to systemic exposure. However, usual testing is done with healthy, intact skin… BUT, what if the skin presents lesions???

Absorption through the gut

- By definition, function of the gut is to absorb exogenous substances
- Charge of particles is crucial (mucus negatively charged)
- Penetration speed correlates with the size (14 nm/2 min, 415 nm/30 min, 1µm/0) (Szentkuti, 1997)
- Passage possible through lymphatic vessels and capillaries

  (eg: polystyrene : 50 nm 34%, 100 nm 26%) (Jani, 1990)
- Systemic absorption is low (Jani, 1990)

⇒ Uptake of nanoparticles in the gastrointestinal tract after oral uptake exists.
Sometimes intentionally made in the design of food and pharmacological components.

3) Effects on human health

3.2. Biological effects
(cellular stress, inflammation, tumours…)

Toxicity and potential mechanisms

- Toxicity evaluation: human (epidemiology); animal models (in vivo); cellular models (in vitro); computer modelization (in silico)

Epidemiology: downstream

In vivo: still most accurate results, but death assessment is the key parameter

In vitro: assessment of cytotoxicity(cell death)… predictivity?

In silico: still requires other approaches in combination

- In general, two processes are involved in the toxicity of particles:

Inflammatory processes

Oxydative stress
(generation of oxygen radicals)

→ Pulmonary diseases, tumours…

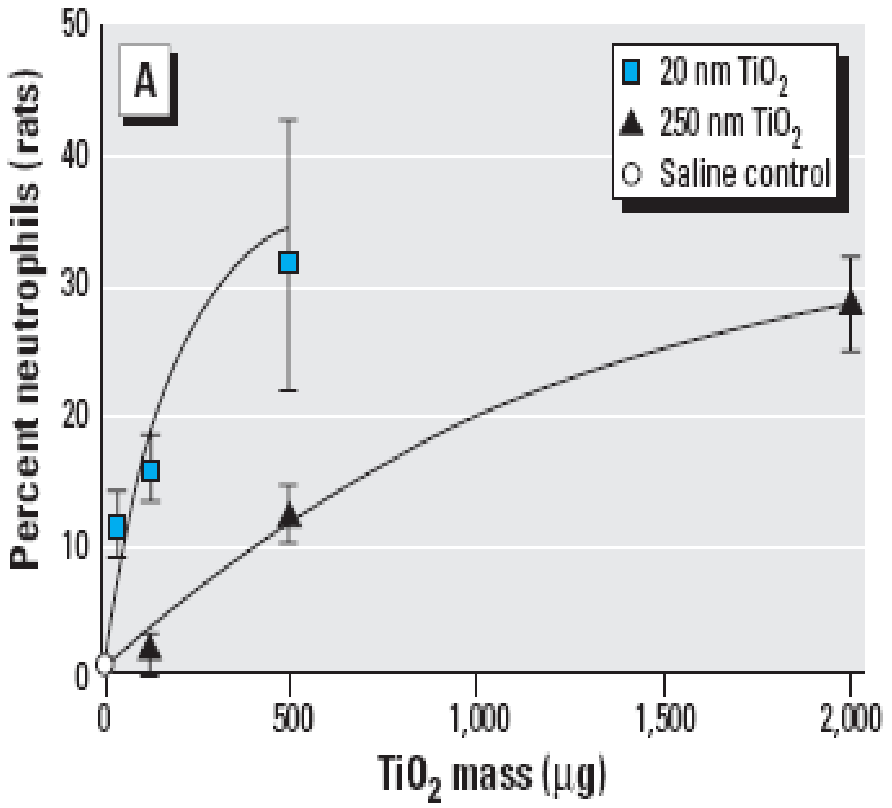
Inhalation toxicity

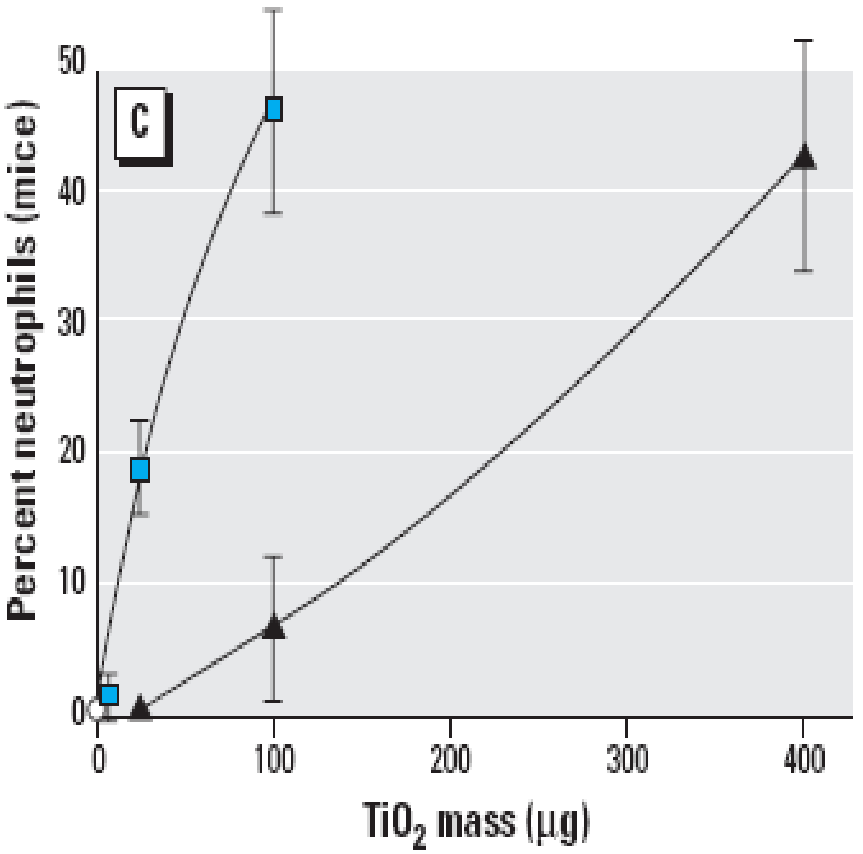
- For most manufactured nanoparticles, no toxicity data is available.
- Available toxicity data is generated with a small set of nanoparticles, known for years, high production level:
  - carbon black (CB),
  - titanium dioxide (TiO2),
  - iron oxides,
  - amorphous silica.
- Observations: upon prolonged exposure (inhalation) in rats, inflammation and lung tumours can occur.

Example: effects of TiO2 nanoparticles on inflammation, after respiratory exposure

For a same mass, and similar chemical composition (TiO_2, carbone black), nanoparticles (14-21 nm) are more toxic than bigger particles (250-320 nm) from the same nature (Ferin, 1992 ; Oberdorster, 1994 ; Li, 1999…)

Marker of inflammation

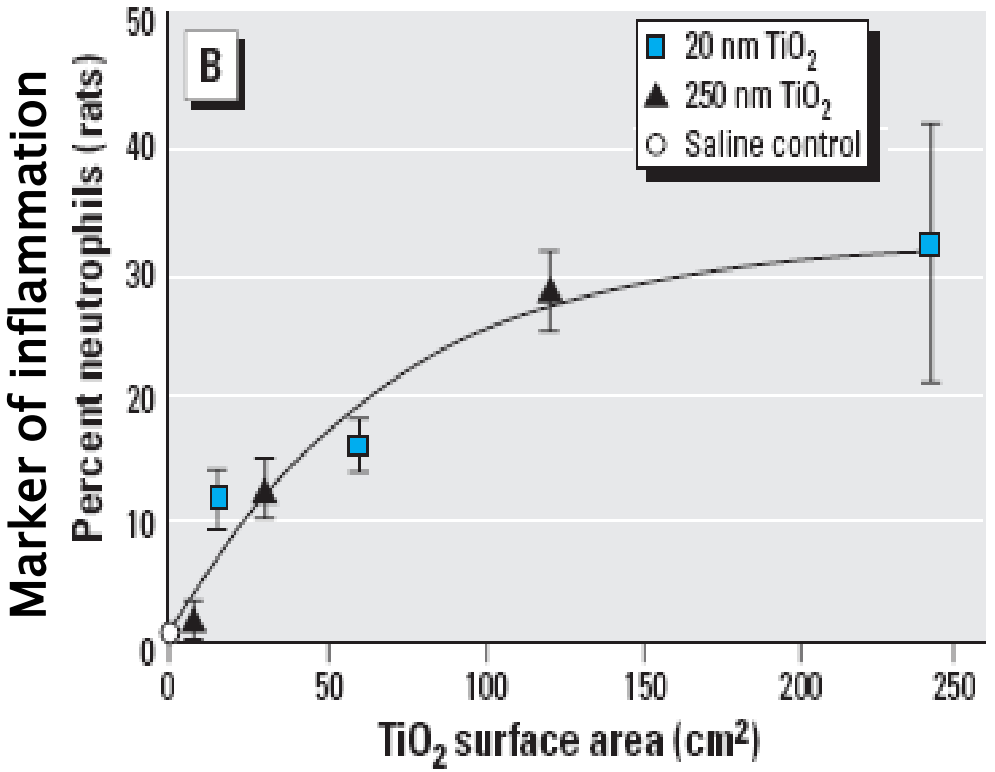


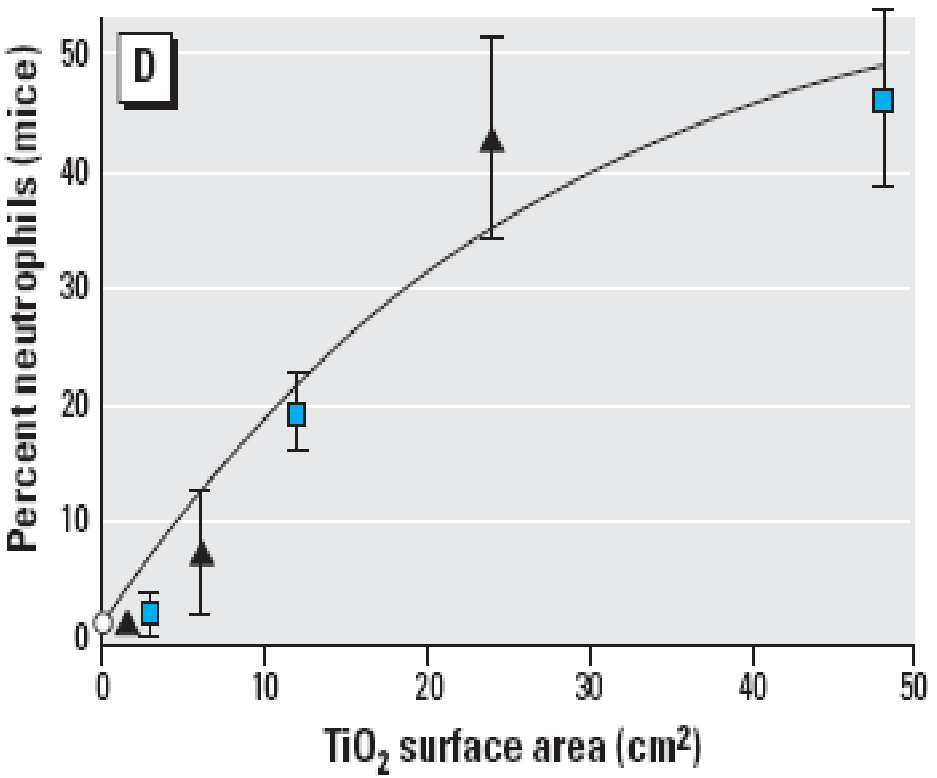


Adapted from Oberdörster, 1994

Example: effects of TiO2 nanoparticles on inflammation, after respiratory exposure

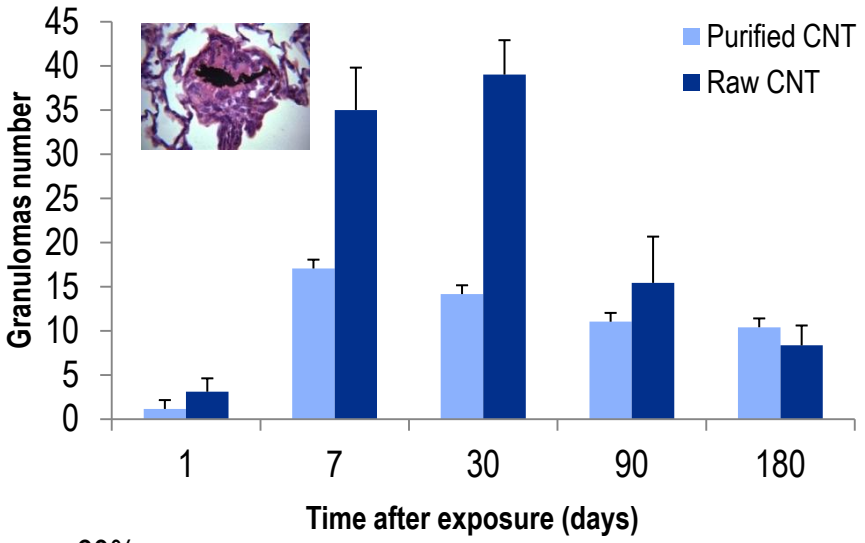
If the same results are expressed in particle surface area, dose-response curves are similar, suggesting surface area is involved in pulmonary inflammation

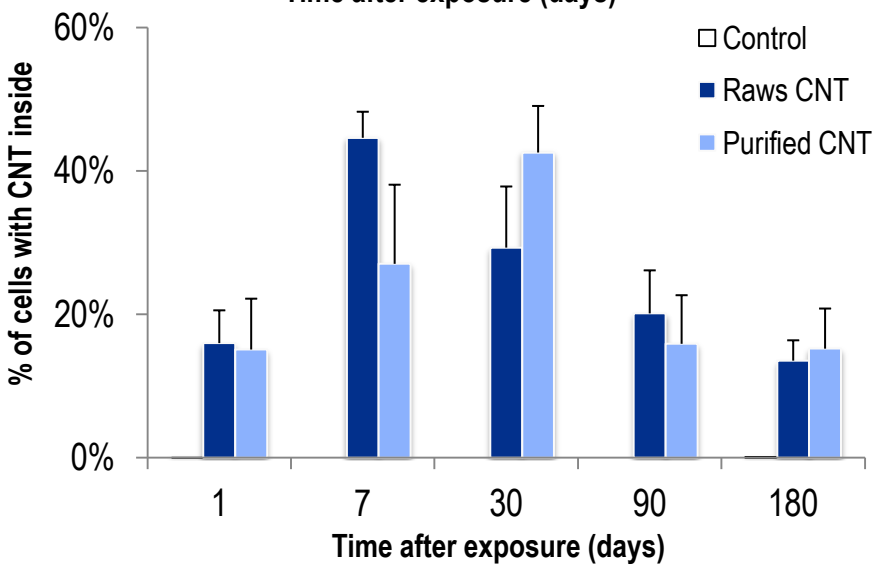




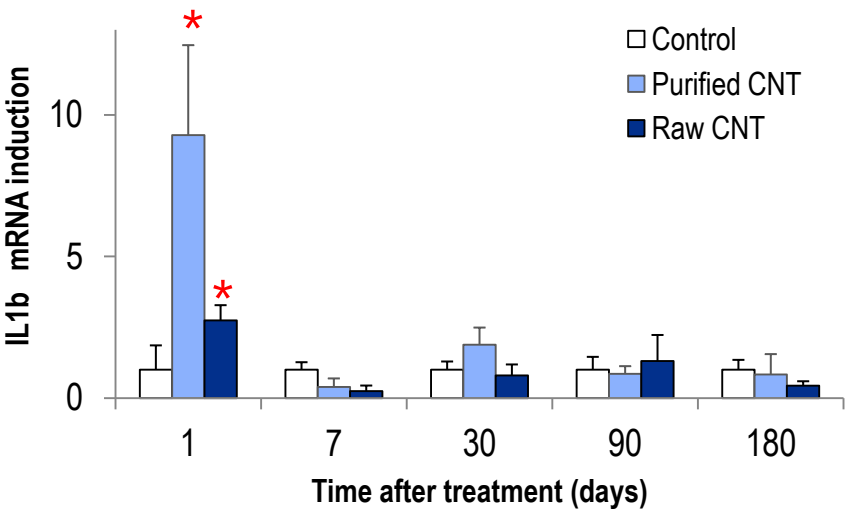
Adapted from Oberdörster, 1994

Effect of carbon nanotubes purity on pulmonary toxicity after respiratory exposure (rats)





Elgrabli D, Trouiller B, Rogerieux F and Lacroix G.
SwCNT raw (20% iron) or purified (2% iron) (CNI)
1 instillation (200 µg)



➔Macrophages phagocyte raw CNTs faster than purified CNTs

➔Raw CNTs induce more granulomas (and bigger) than purified CNTs.

➔Markers of oxydative stress, inflammation and apoptosis are increased after instillation of purified CNTs.

Respiratory effects of nanomaterials : conclusion

However, again, this can not be generalized…

- 2006 (Sayes *et al*.) : the cytotoxic and inflammatory effects of TiO_2 nanoparticles are independent from size and surface area of the particle.

- 2006, 2007 (Warheit *et al*.) : inflammatory effects of quartz (12-500 nm) nanoparticles, in rats, are not correlated with size or surface area, but with surface reactivity. Changes in surface reactivity lead to different hemolytic potential.

=> Surface properties (not only surface area) are crucial

Purity and other specifics also play a role

Systemic effects of nanoparticules

- Limited number of studies: on specific nanomaterials. mostly acute studies done; few long-term chronic studies.
- Target organs not always identified
- Tested materials not sufficiently characterized
- Examples of tested nanomaterials: fullerenes, CNT, dendrimers, Fe_3O_4, Zn, Cu, TiO_2, quantum dots...

- General observations:
  - Toxic dose (DL_{50}) :order of magnitude: mg-g/kg: slightly to moderately toxic
  - Usually no mortality
  - Target organs: liver, spleen and kidney

Nanoparticles and cancer: examples

- Fullerenes

  Dermal route

  - Application on skin of mice (Nelson, 1993)

    No tumours found (dose of 200 µg of fullerene, 2 times/week, 24 weeks)
  - Confirmed by *in vitro* mutagenesis tests (dose --> 5 mg/ml) (Mori, 2006)

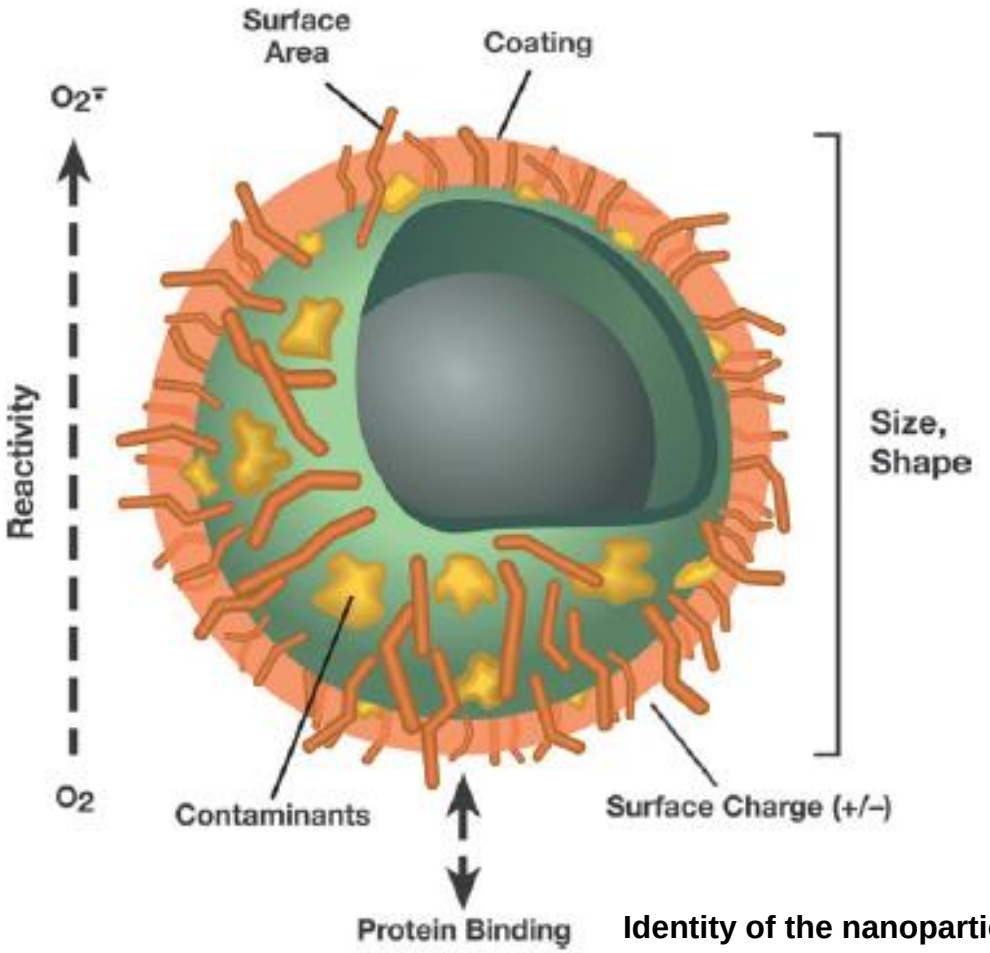
- Multiwall Carbon Nanotubes

  Injection in abdominal mesothelium in mice: similar response than with asbestos (Takaji, 2008 ; Poland, 2008)

  - Tissue lesions similar to mesothelioma (25 weeks after injection) . No effect of fullerenes (Takaji, 2008).
  - Short term effects: Inflammation (24h) and granulomas (7 days) observed after exposure to long, straight, carbon nanotubes and asbestos. No effect seen with short, non fibrous, nanotubes and carbon black (Poland, 2008).

=> Variable results based on the nanoparticles specific characteristics

Conclusion: Nanoparticles physico-chemical properties that may influence toxicity



Identity of the nanoparticle when in a biological medium

FIG. 3. Physicochemical properties of nanoparticles that may influence biocompatibility.

CAUTION: Big difference between physico-chemical identity of a nanoparticle and its biological identity

To reach accurate danger evaluation:

- Importance of thorough and exhaustive characterization of physico-chemical properties of the studied nanoparticles (size, shape, agglomeration, solubility, surface properties…)

- Experimental conditions and how they can influence/modify the nanoparticles properties need to be defined (eg: addition of dispersion agents…)

⇨ Identification of properties that determine the toxicity of the nanomaterials

⇨ Allow/facilitate comparison between studies

What next?

- A lot of work to do!!!
  - Identify toxic effects
  - Clarify the specifics that determine toxicity => try to define nanoparticles families => « safer by design » nanoparticles
  - exposure evaluation: occupational + general population
  - Lifecycle

- Given the notable lack of information, current recommendations are largely based on common sense:
  - minimize exposure and hazards,
  - use the knowledge by analogy to ultrafine material toxicity
  - general health and safety recommendations (fume hoods, protective equipment…)

Conclusion

- Experimental studies with some bulk nanoparticles (carbon black, titanium dioxide, iron oxides) used for decades suggest various adverse effects.
- Engineered nanomaterials with new chemical and physical properties are being produced constantly and the toxicity of these is unknown.
- NO blanket statements about human toxicity can be given at this time.
- AND… impact of Nanoparticles on ecosystems and environnment??