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Nanotechnology

What about safety
How do we determine risk?

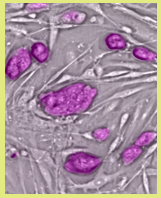
Wim H. de Jong, DVM, PhD

Expected increase in use of nanomaterials

- Possible applications
  - Material science
    - Strenght of materials (especailly CNT)
  - Consumer products
    - Cosmetics (sunscreens)
    - Fabrics
    - .....
  - Food/feed and food technology
    - Packaging
    - Vitamins, supplements
    - ....
  - Medical applications
    - Pharmaceutical (drug delivery, enhanced activity)
    - Medical technology

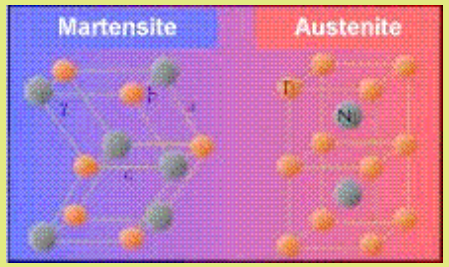
Nano-technologies

Tissue Engineered Products
Gene Therapy
Drug/device combinations
Smart materials
Minimally invasive surgery
Computer-assisted surgery systems
Active medical devices
Artificial organs
Telemedicine
Medical Imaging
Diagnostics (lab on a chip)



Biological sciences

Cognitive sciences



Materials science

Information technology

Converging technologies

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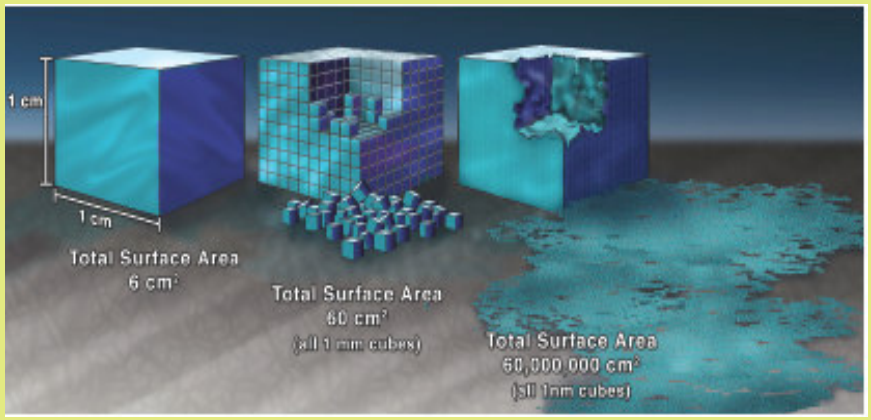
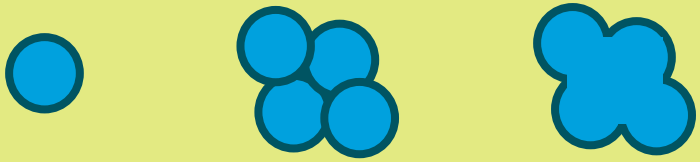
Why do we use nanomaterials?
Decrease in size results in increase in surface area

All: 1 x 1 cm



size	number	Total Surface area
1 cm	1	6 cm^2
1 mm	1000	60 cm^2
1 µm	1 x 10^{12}	6.000cm^2
1 nm	1 x 10^{21}	60.000.000 cm^2 (600km^2)



Increase in surface area >> increase in surface activity,
but also increase in possible contact with cells and tissues

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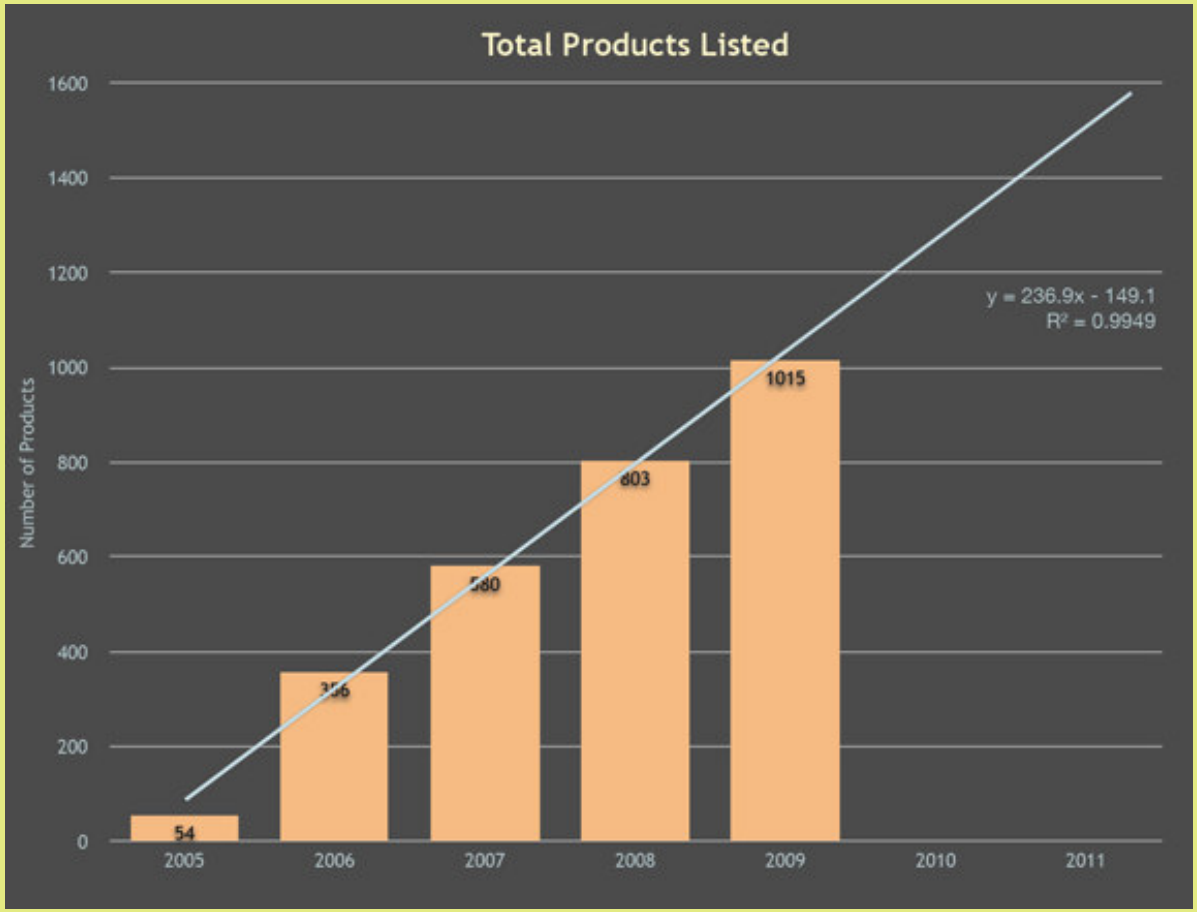
Increase in consumer products with nanoclaim



Number of total products listed, by date of inventory update, with regression analysis. **August 2009**

Nanotechnology Consumer Products Inventory, Woodrow Wilson International Center for Scholars, Washington, USA

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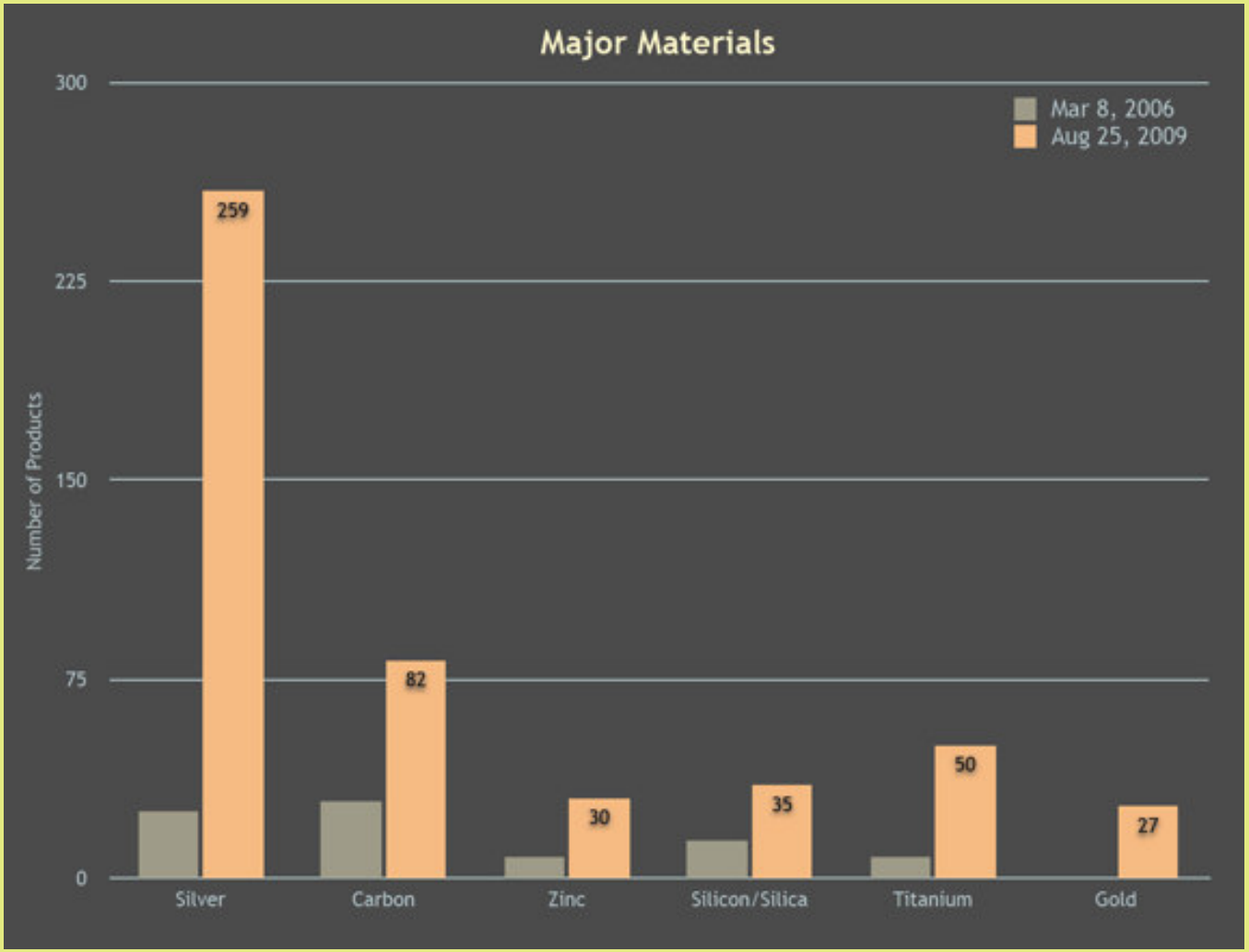
Most commonly used nanomaterials in consumer products



Nanotechnology Consumer Products Inventory, August 2009,

Woodrow Wilson International Center for Scholars, Washington, USA

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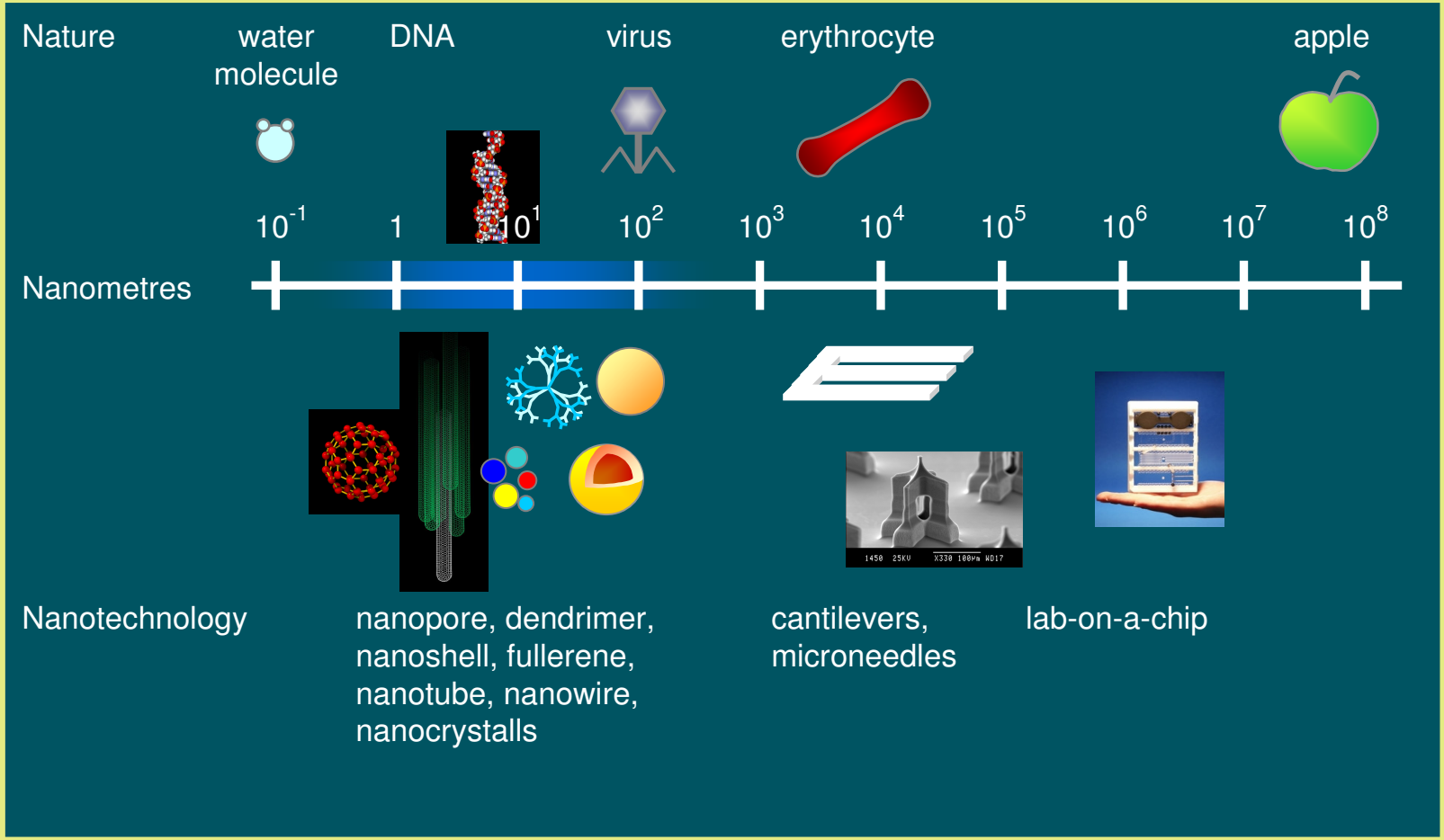
Why are we concerned?



Nanomaterials (nanoparticles) can have sizes similar to structures at subcellular level and (theoretically) can reach and interact with such structures.

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Safety evaluation

- Safety evaluation
  - Identification of substance
  - Hazard characterization
    - Hazard identification
    - Dose response effect (no effect level)
  - Exposure assessment/ treatment dose
- What is risk?
  - Risk, combination of likelyhood of occurrence of harm to health and the severity of that harm
  - Margin of safety (no effect level / effective treatment dose)
  - (No exposure >>>>> No risk)
- Residual risk
  - Risk benefit analysis
- Risk is a possibility, not an absolute value !

How do you determine risk?

- Hazard, a potential source for harm to health

- *In vitro* studies
  - Indicate possibility for cell damage
  - Mainly used for to screening and mechanistic studies
  - Relevance for risk assessment is limited

- *In vivo* studies
  - Overall “black box”
  - Indications for possible organ specific toxic effects and no effect levels
  - Extrapolation problems (inter- and intraspecies variation)
    - Uncertainty factors
  - More relevant for risk assessment than *in vitro*

Why is safety evaluation and risk assessment of nanomaterials so difficult?

- Diversity of nanomaterials (inorganic, organic, coated,…)
- Solubility, agglomeration/aggregation (stability, size distribution)
- Matrix (interactions, effects on size, digestion)
- Quality of available nanomaterials (polydispersity, purity, concentration)
- Test protocols (dispersion, reproducibility, comparability)
- Choice & preparation of test medium (concentration, solvents)

Key issue in testing and quality control

Detection and characterization of the nanomaterials

For safety evaluation identification is essential

What do we want / need to know for nanoformulations / carriers?

- Chemical composition
- Size
- Size distribution
- Agglomeration / aggregation
- Crystallinity
- Coatings
- Surface charge
- Specific physicochemical characteristics
  - why is this specific nanomaterial used?
    - mainly important for consumer products
- …….

- How is the nanoparticle defined?

How is a nanoparticle/nanomatrial defined? What do we mean by size?

Particle type	Nominal	TEM*	AFM*	FCS^	NTA*	DLS'	FlFFF^
TiO_2 & FA 1mg/L	5	16.1 +/-6.4	3.7 +/-2.1	6.5 +/-2.8	164 +/-32 (30mg/L)	1230 +/-430 (30mg/L)	3.7 (100mg/L)
ZnO & FA 1mg/L	20	12.2 +/-4.5	25.7 +/-8.5	28.5 +/-10.5	130 +/-50 (100mg/L)	870 +/-680 (30mg/L)	228.3 (100mg/L)
QDs 1.92 ug/L	6-10	6.5 +/-1.9	5.9 +/-3.4	81.8 +/-4.9	213 +/-17	234 +/-35	41.5

*number average ^weight average `z average

Domingos *et al.* 2009

Understanding and correlation of size measurement techniques is essential

TEM, transmission electron microscopy; AFM, atomic force microscopy; DLS, dynamic light scattering; FCS, fluorescence correlation spectroscopy; NTA, nanoparticle tracking analysis; FIFFF, flow field flow fractionation

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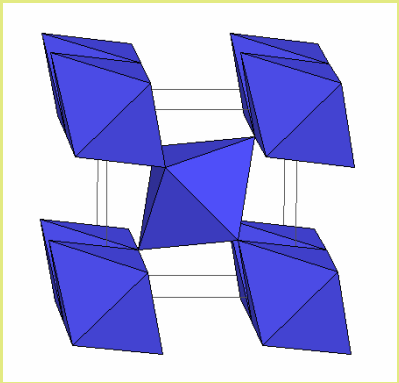
Courtesy of Karin Tiede, FERA, York, UK

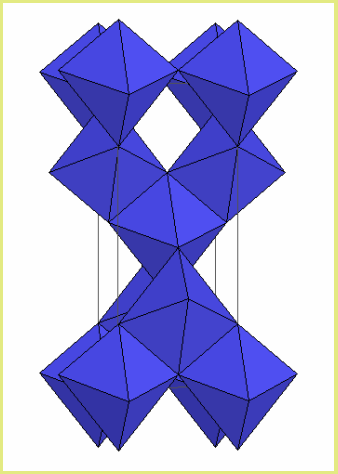
Existing problems in safety evaluation of nanomaterials/nanoparticles

- Identification of nanomaterial is essential
  - Various crystal forms of same material may exist
    - Titanium dioxide; rutile, anatase, brookite crystals
  - Presence of coating on nanomaterials
    - Each different coating can be considered a new formulation / material

Rutile TiO_2

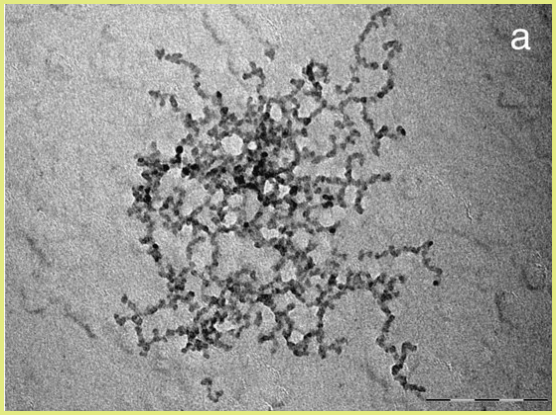


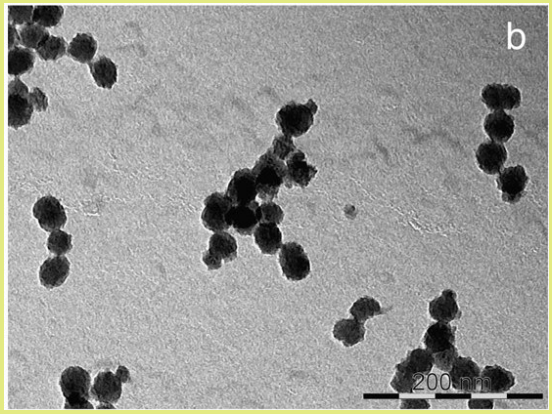
Anatase TiO_2



Particle size and agglomeration
Example of nominal and actual size of silica nanoparticles

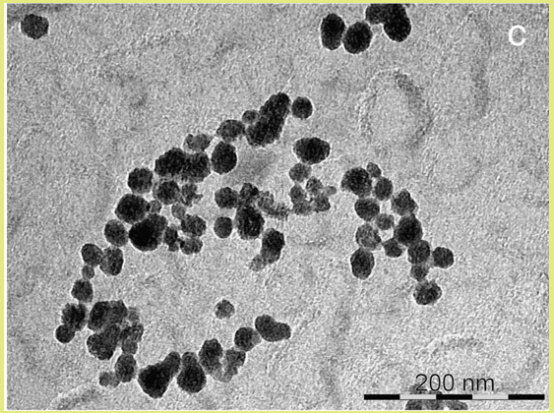
10 (11)

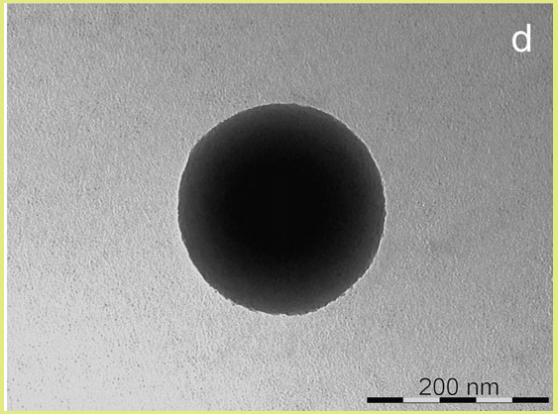




30 (34)

80 (34)





400 (248)

Transmission electron microscopy images of silica nanoparticles deposited from deionized water.

Park et al., Toxicol Appl Pharmacol, 2009

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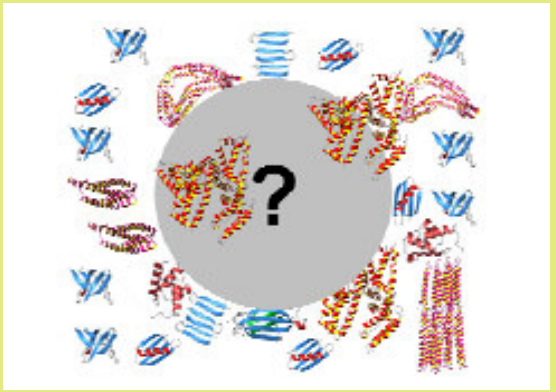
Safety evaluation

- Problems with testing

  - Problems with identification/characterization
  - Problems with dispersion for testing in vitro and/or in vivo
  - Protein adherence, effect of protein corona
  - We now it exists, but we do not know its biological effects



Nanoparticles do not exist as single particle entity, they adsorbe things, e.g. proteins

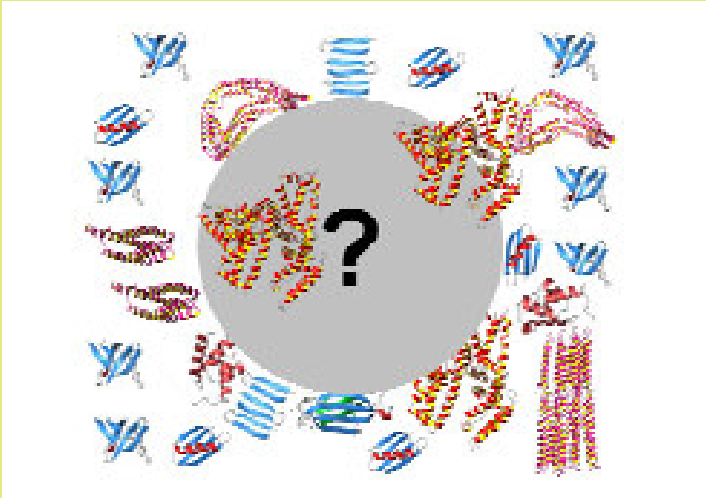
What do we know

- Protein corona is important for biological interactions and cellular recognition
- Corona is not static, proteins get on and off

What do we not know

- Dependence on nanomaterial?
- Dependence on size?
- Dependence on …?

Implications for interpretation of testing



EU FP6 project NanoInteract,

courtesy of Prof Kenneth Dawson, UCD, Dublin, Ireland

What is the dose metric for particle toxicity?

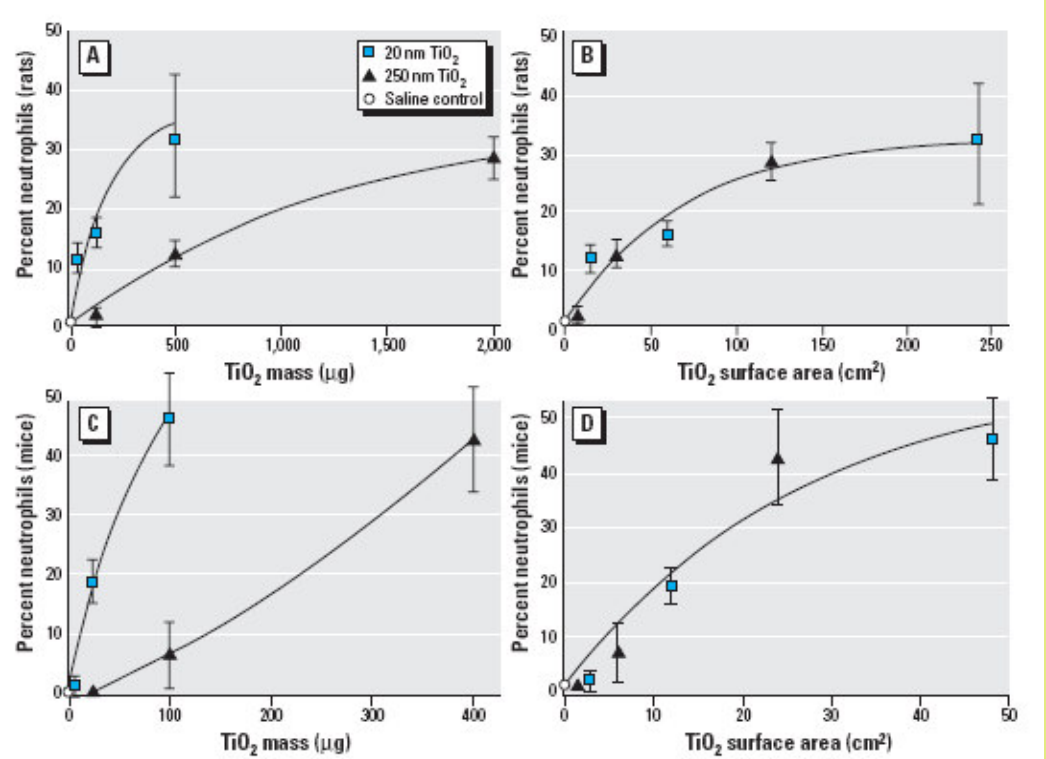


Figure 4. Percentage of neutrophils in lung lavage of rats (*A,B*) and mice (*C,D*) as indicators of inflammation 24 hr after intratracheal instillation of different mass doses of 20-nm and 250-nm TiO_2 particles in rats and mice. (*A,C*) The steeper dose response of nanosized TiO_2 is obvious when the dose is expressed as mass. (*B,D*) The same dose response relationship as in (*A,C*) but with dose expressed as particle surface area; this indicates that particle surface area seems to be a more appropriate dosemetric for comparing effects of different-sized particles, provided they are of the same chemical structure (anatase TiO_2 in this case). Data show mean ± SD.

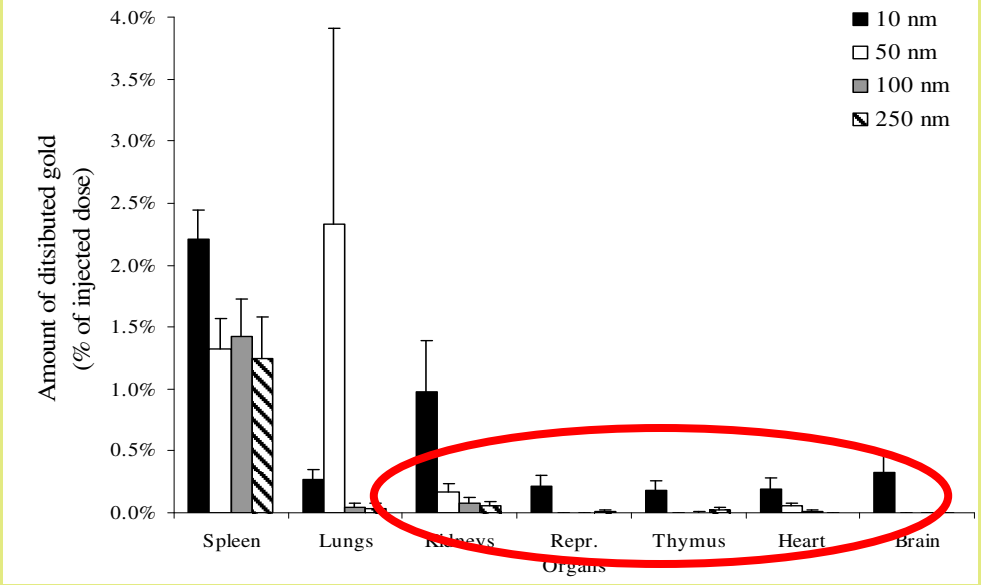
Surface area was demonstrated to be a better descriptor for local effects in the lung after inhalation exposure. What about other routes of exposure (oral, dermal, intravenous)?

Oberdörster et al., Environ Health Perspect 113, 823, 2005

Is dose metric of mass applicable?

- Dose metrics per kg body weight
  - Mass (milligram, gram)
  - Number of particles, as effects may be determined by the particle characteristics
  - surface area, as demonstrated for inhalation toxicity of TiO_2
  - .....something else?

Pharmacological availability
Effect of nanoparticle size on tissue distribution



Gold distribution at 24 h after iv injection in rats as percentage of injected dose (100 µg per animal)

Particle size	10 nm	50 nm	100 nm	250 nm
Number concentration	5.7×10^{12}	4.5×10^{10}	5.6×10^{9}	3.6×10^{8}
Surface area	1.6×10^{15}	3.2×10^{14}	1.7×10^{14}	6.9×10^{13}
Mass injected	85 µg	106 µg	98 µg	120 µg

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De Jong et al., Biomaterials, 2008

Pharmacological availability
Effcets of size on toxicokinetics

Table 6
The average number of gold particles distributed to the various organs (estimated)

Tissue	10 nm, Number of particles (number/g organ)	50 nm, Number of particles (number/g organ)	100 nm, Number of particles (number/g organ)	250 nm, Number of particles (number/g organ)
Blood	1.9E + 12	1.2E + 10	2.2E + 09	4.6E + 07
Liver	2.4E + 12	8.2E + 09	2.3E + 09	9.5E + 07
Spleen	1.1E + 11	5.2E + 08	7.3E + 07	3.8E + 06
Lungs	1.4E + 10	9.0E + 08	2.2E + 06	1.2E + 05
Kidneys	4.9E + 10	6.5E + 07	3.8E + 06	1.6E + 05
Testis	1.1E + 10	–	–	4.2E + 04
Thymus	9.1E + 09	–	–	6.4E + 04
Heart	9.9E + 09	2.2E + 07	4.3E + 05	–
Brain	1.6E + 10	–	–	–
	$N = 7$	$N = 2$	$N = 4$	$N = 5$

Although only a few % of the administered dose
a considerable amount may be present in organs in terms of particle numbers.

What about local accumulation and chronic effects?

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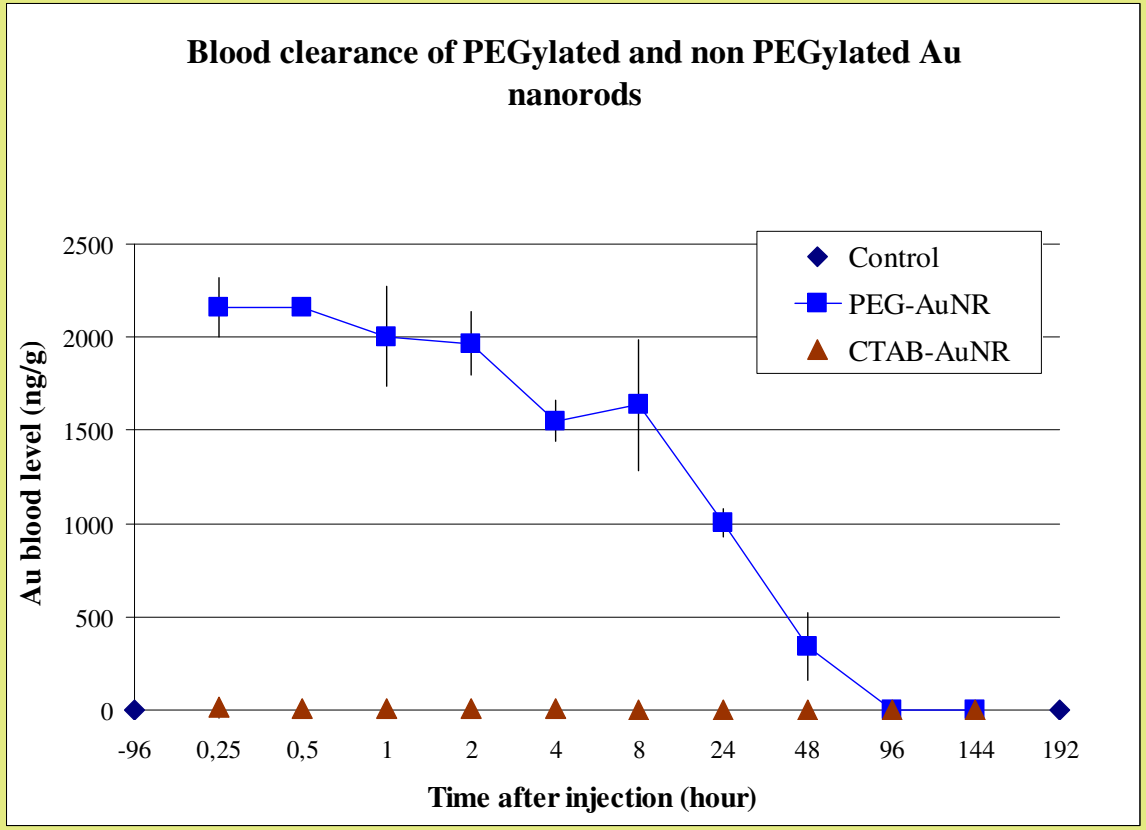
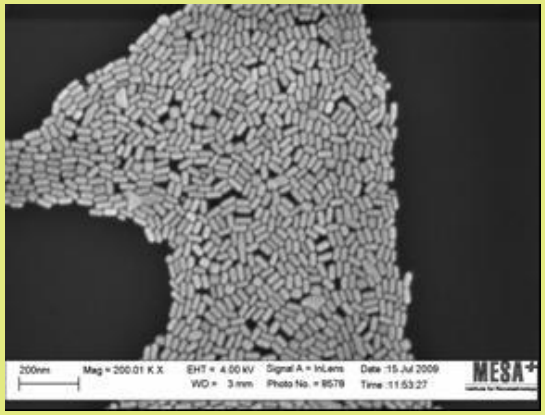
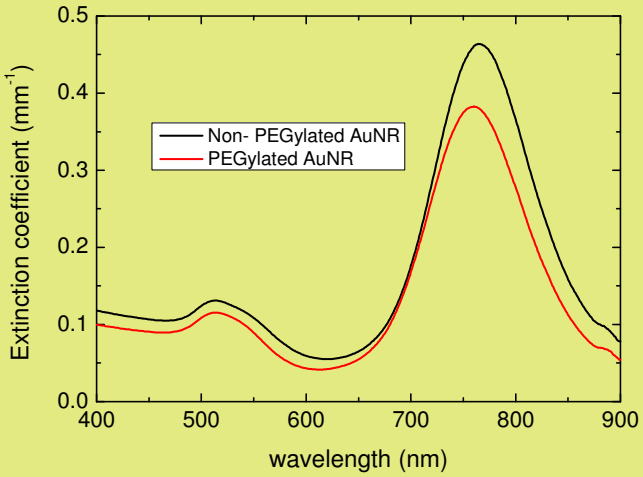
De Jong et al., Biomaterials, 2008

Pharmacological availability
Effects of PEG coating of gold nanorods on toxicokinetics







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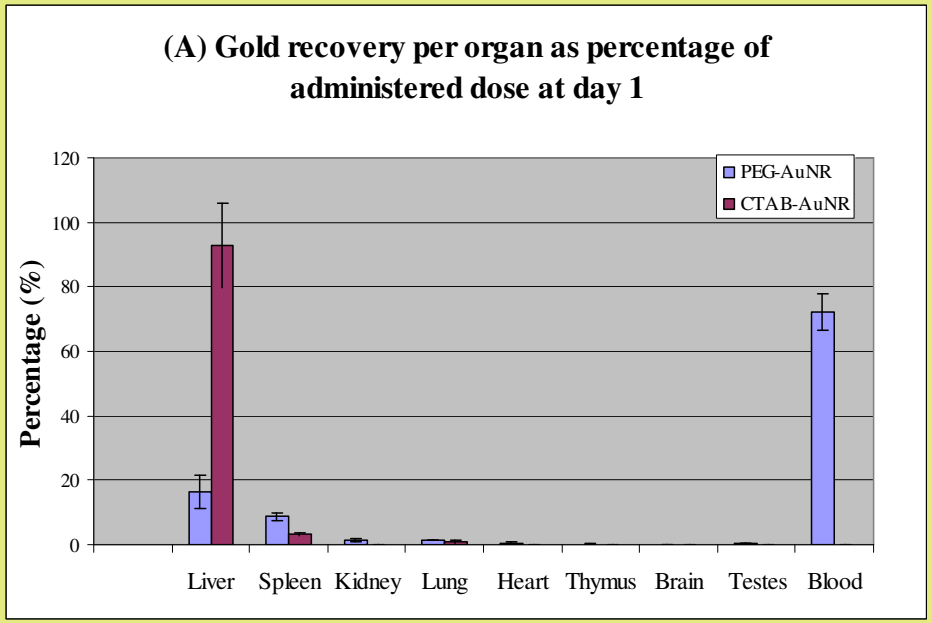
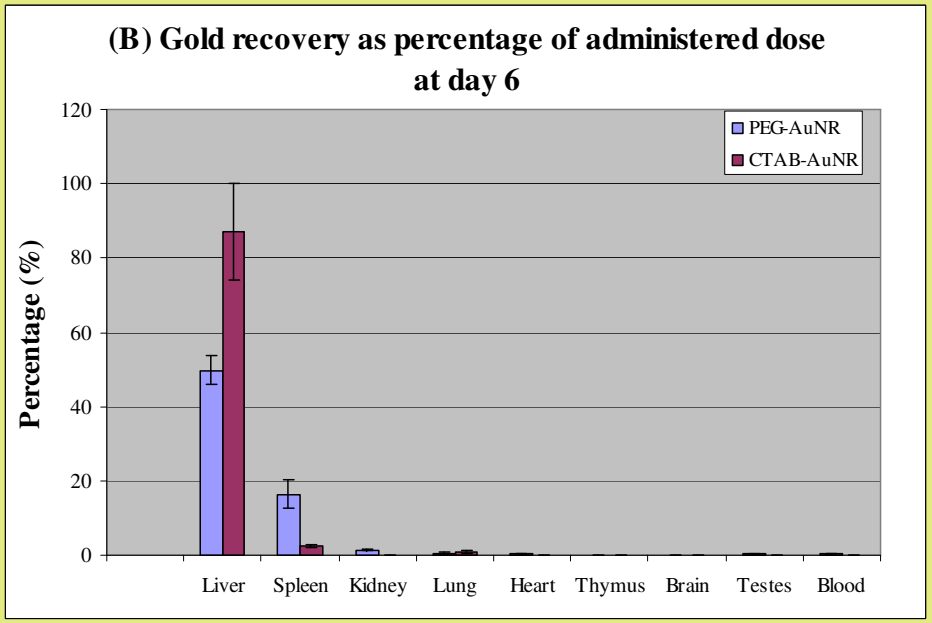
Lankveld et al., Submitted, 2010

Effects of coating of gold nanorods on toxicokinetics





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Lankveld et al., Submitted, 2010

Effects of coating of gold nanorods on toxicokinetics

	DAY 1		DAY 6	
	PEG-AuNR$_{770}$	CTAB-AuNR$_{770}$	PEG-AuNR$_{770}$	CTAB-AuNR$_{770}$
Liver	320 ± 105	2339 ± 390	978 ± 145	2059 ± 299
Spleen	3477 ± 153	1643 ± 236	6644 ± 1973	1132 ± 204
Kidney	183 ± 32	13 ± 1	176 ± 29	5 ± 3
Lung	264 ± 22	239 ± 102	106 ± 17	172 ± 99
Heart	192 ± 5	3 ± 1	104 ± 13	4 ± 3
Thymus	66 ± 19	2 ± 0	66 ± 26	2 ± 0
Brain	27 ± 3	5 ± 6	2 ± 0	2 ± 1
Testes	33 ± 10	2 ± 0	23 ± 6	2 ± 0
Blood	1007 ± 76	3 ± 0	3 ± 1	3 ± 0

Data are presented as gold concentration in ng per gram tissue. Gold nanorods were administered intravenously at day 0. Number of animals (samples) n=3 for day 1 and n=6 for day 6. Tissue samples were prepared by organ digestion before ICP-MS measurement.

For toxicity local organ dose is of importance.

For PEGylated gold nanorods now SPLEEN is target organ with highest exposure dose.

What about local accumulation and chronic effects?

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Lankveld et al., Submitted, 2010

Effect of shape on biological responses

Issue of nanofibres/nanotubes

CNT versus asbestos

nature nanotechnology | VOL 3 | JULY 2008 | 423 LETTERS

Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study

CRAIG A. POLAND[1], RODGER DUFFIN[1], IAN KINLOCH[2], ANDREW MAYNARD[3], WILLIAM A. H. WALLACE[1], ANTHONY SEATON[4], VICKI STONE[5], SIMON BROWN[1], WILLIAM MacNEE[1] AND KEN DONALDSON[1]*

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.33, No.1, 105-116, 2008

Original Article

Induction of mesothelioma in p53+/– mouse by intraperitoneal application of multi-wall carbon nanotube

Atsuya Takagi[1], Akihiko Hirose[2], Tetsuji Nishimura[3], Nobutaka Fukumori[4], Akio Ogata[4], Norio Ohashi[4], Satoshi Kitajima[1] and Jun Kanno[1]

Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, Hirose A, Nishimura T, Ohashi N, Ogata A.

Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fisher 344 rats.

The Journal of Toxicological Sciences, 34, 65-76, 2009

TOXICOLOGICAL SCIENCES 110(2), 442–448 (2009)
doi:10.1093/toxsci/kfp100
Advance Access publication May 8, 2009

Absence of Carcinogenic Response to Multiwall Carbon Nanotubes in a 2-Year Bioassay in the Peritoneal Cavity of the Rat

Julie Muller,* Monique Delos,† Nadtha Panin,* Virginie Rabolli,* François Huaux,* and Dominique Lison*,[1]

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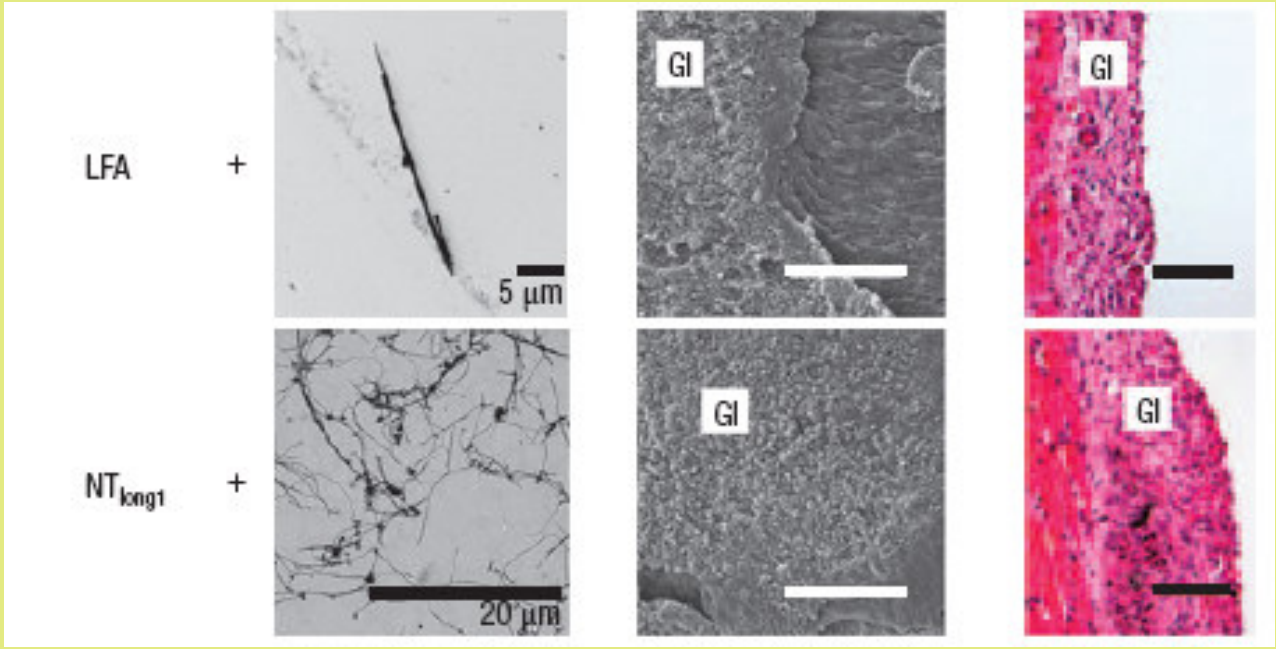
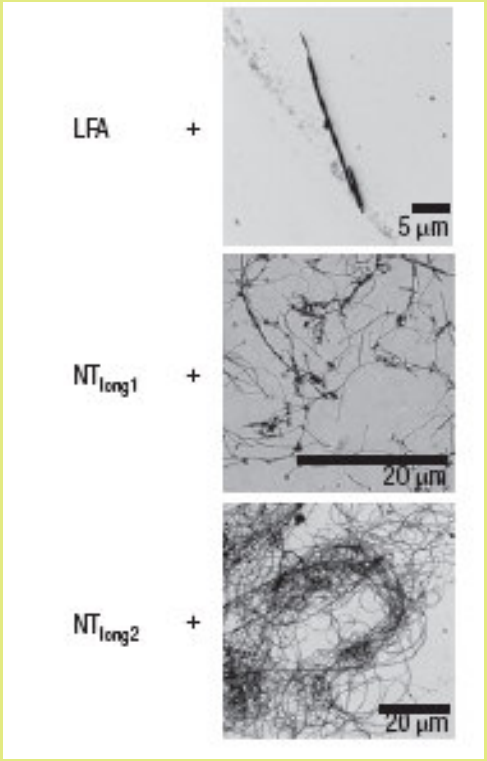
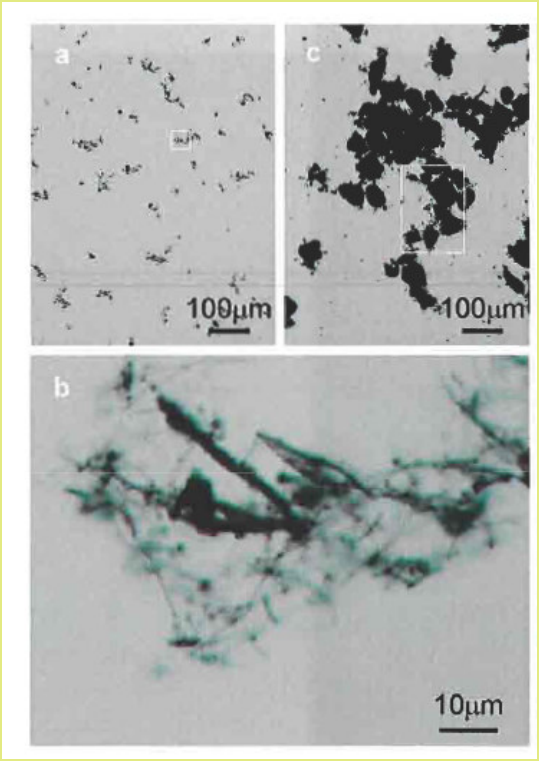
Effect of shape on biological responses

- MWCNT induce a granulomatous inflammation in vivo similar to asbestos fibres



Poland et al., 2008



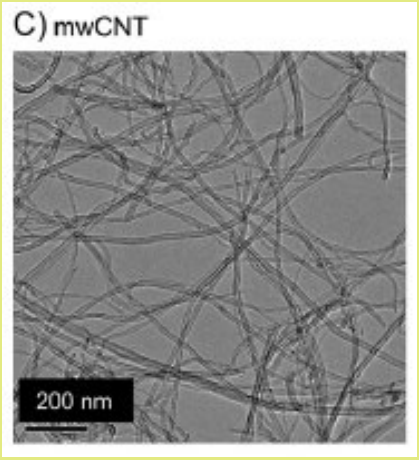


Takagi et al 2008, Sakamoto et 2009

MWCNT induce tumors in P53 mice and F344 rats

Nygaard et al 2009

CNT act as adjuvant



Poland et al 2008

MWCNT induce chronic inflammation

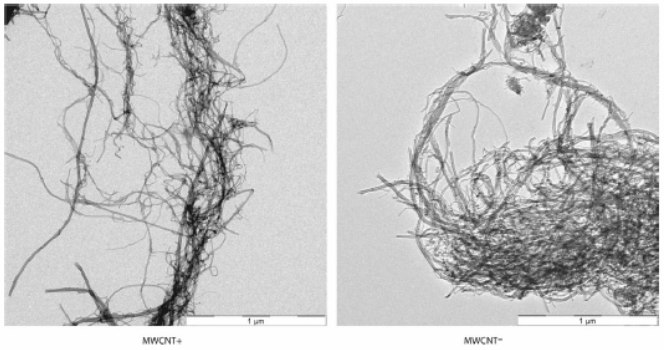


FIG. 1. Transmission electron microscopy images of the carbon nanotubes. MWCNT +, unheated multiwall carbon nanotubes and MWCNT–, multiwalled carbon nanotubes heated at 2400°C under argon. The images were obtained on a Leo 922 (Zeiss), 200 kV.

Muller et al 2009

MWCNT do NOT induce tumors in 2 year study

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Macrophage response to fibres

Effect of shape
CNT versus asbestos

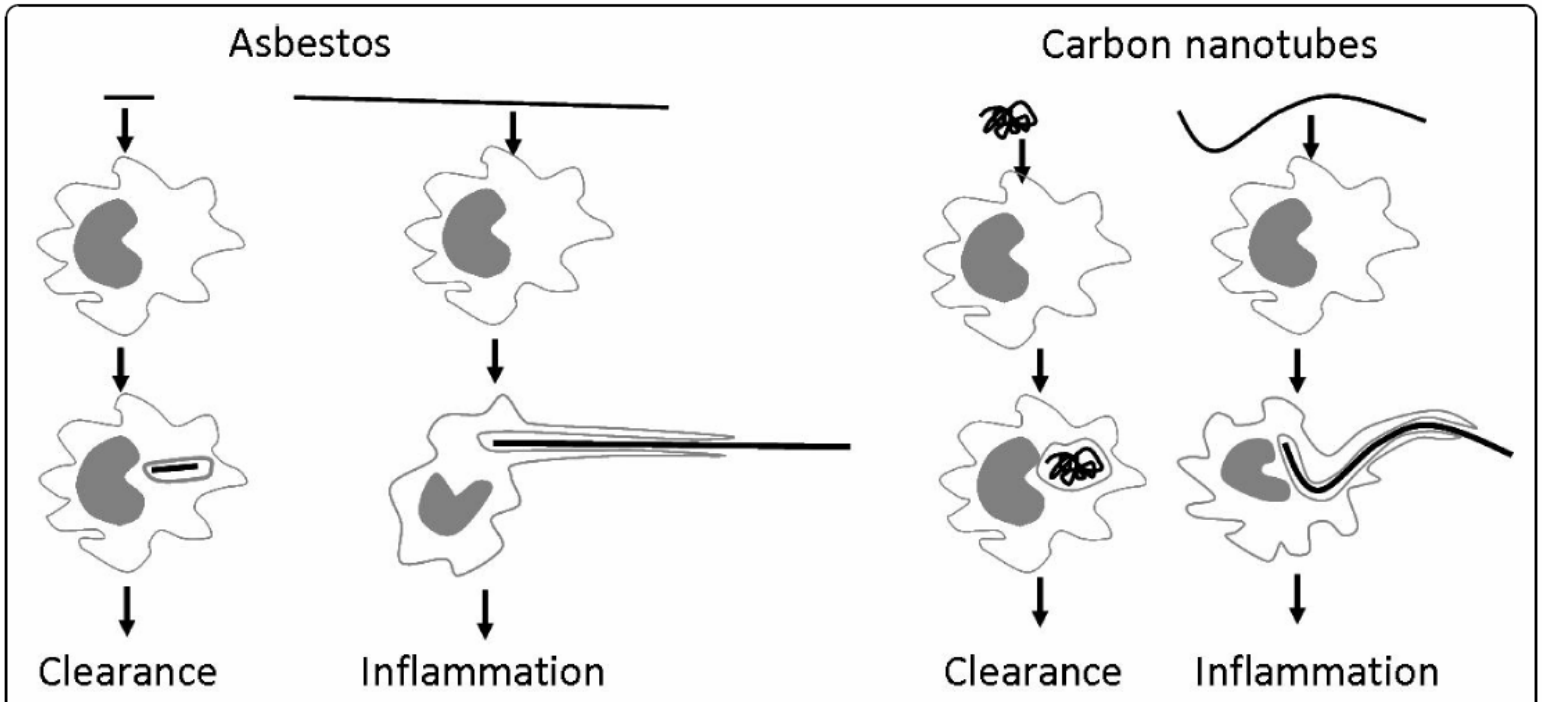

Figure 2 The frustrated phagocytosis paradigm as it relates to long and short fibres of asbestos (left) and various forms of carbon nanotubes (right). When confronted by short asbestos fibres or tangled, compact carbon nanotube 'particles' the macrophage can enclose them and clear them. However the macrophage cannot extend itself sufficiently to enclose long asbestos or long nanotubes, resulting in incomplete or frustrated phagocytosis, which leads to inflammation.

Donaldson et al., Particle and Fibre Toxicology, 2010

Nanofibres, the MWCNT issue

There are different types of MWCNT
"when a fibre has characteristics of brown/blue asbestos
(rigid, non degradable, length >20 µm)
it behaves like brown/blue asbestos" (Poland et al., 2008, Donaldson et al., 2010)

Lesson is NOT
MWCNT behave like asbestos but.....
.....when producing and using MWCNT
or any fibre-like nanomaterial
Check for these specific characteristics
(rigidity, degradability, fibre length)

Perform proper safety evaluation to exclude this specific hazard associated with a certain types of fibres.
Including extensive characterization.

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Where do we stand with nanotechnology?

- High expectations especially in nanomedicine
- Consumer products
  - Multitude of consumer products already available on the market
  - Some labeled, others not
- Various hazards (toxic effects) identified
  - Inhalation exposure most severe hazard and highest risk
- Exposure estimation remains a problem
- Little or no information on possible chronic effects

- Case by case approach for risk assessment advocated

Summary
What do we know about toxicological risk assessment of nanomaterials?

- The particulate nature of nanomaterials influences the toxicokinetics
  - ADME – absorption, distribution, metabolism, excretion
  - Dependent on size, shape, material, etc…
- Physico-chemical and toxicological properties of nanomaterials (and surfaces) different from bulk material – parameters?
  - What value is border/turning point for toxic behaviour?
- Not all nanomaterial formulations are toxic
  - Increase in surface activity does not automatically imply toxicity
- Many factors with varying effects

Continuing issues 2010

- Importance of characterization
  - Size determination and method
  - Example of various crystal forms of same material
    - Titanium dioxide; rutile, anatase, brookite crystals
- Problems with dispersion
- Toxicity of solvents and/or process residues
- Protein adherence, effect of protein corona
- Genotox issue, contradicting results reported
  - Can existing genotox assays be used?
- Dose metrics (mass, number of particles, surface area, …)
  - Also for in vitro: is the dose the concentration (i.e. all particles present) in the liquid, or only the number of particles in contact with the cells?

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