GIORNATE DI STUDIO
4A EDIZIONE

RICERCA E APPLICAZIONE DI METODOLOGIE ECOTOSSICOLOGICHE IN AMBIENTI ACQUATICI

Livorno, 20-22 ottobre 2010

CENTRO INTERUNIVERSITARIO BIOLOGIA MARINA ED ECOLOGIA APPLICATA

Eco-nanotossicologia acquatica

Enrico Sabbioni

ECSIN – European Center for the Sustainable Impact of Nanotechnology, Veneto Nanotech -Rovigo

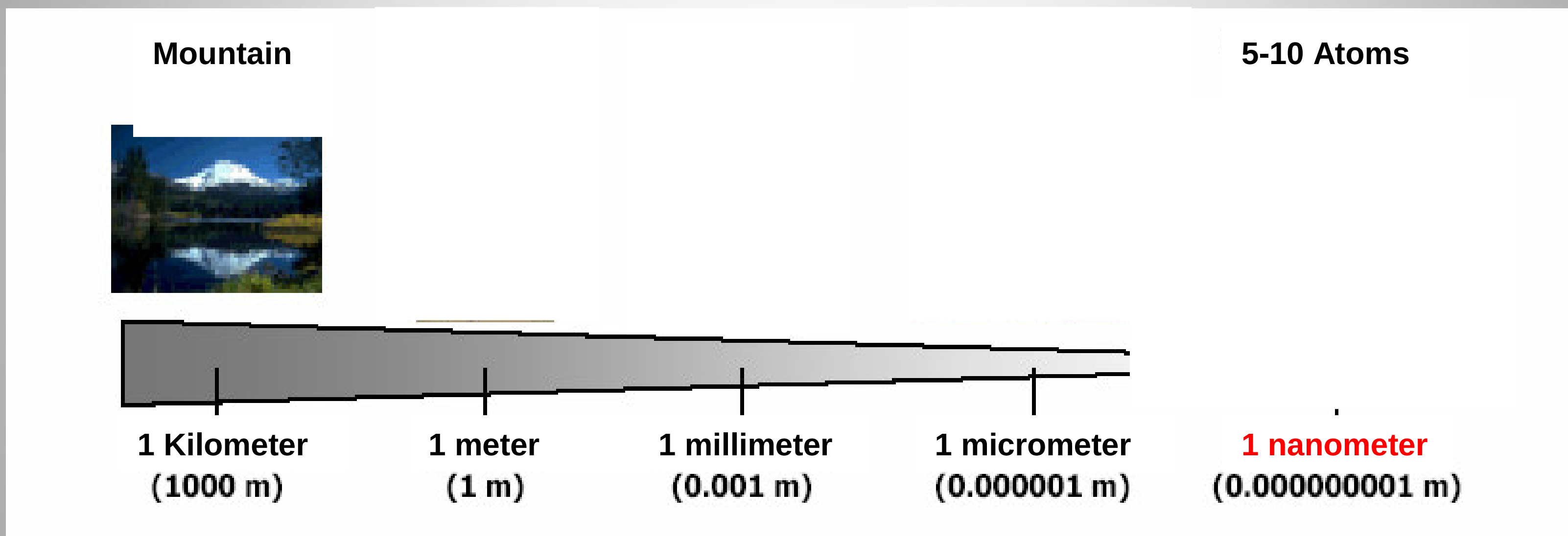
Nanotechnology: why nano is different?

Nanotechnology has an extraordinary potential

- Medicine and Health
- Information technology
- Energy production /Storage
- Materials Science
- Food, Water Environ.
- Remediations methods
- Chemical industry
- Hydrogen fuel cells
- Cosmetics
- Sport

Nanotechnology: the big science of the very small

Nanotechnologies refer to “technology of the tiny”, with dimensions in the range of **1-100 nanometers** (**one billionth, 0.000000001** of a meter).



H_2O molecule:	**0.1nm**
DNA helix:	**about 2 nm diameter**
erythrocytes:	**about 7000 nm wide**
Human hair:	**about 80000nm diameter**

K.M.Kulinowski, Nanotechnology “The Big Science of the Very Small”, CBEN

1 EURO cent... vs Pianeta Terra!

1mm... vs Milano-Taranto!

Defining nanomaterials in an ecotoxicological context

Coarse >2.5µm

Fine 0.1 –2.5µm

Ultrafine <0.1µm

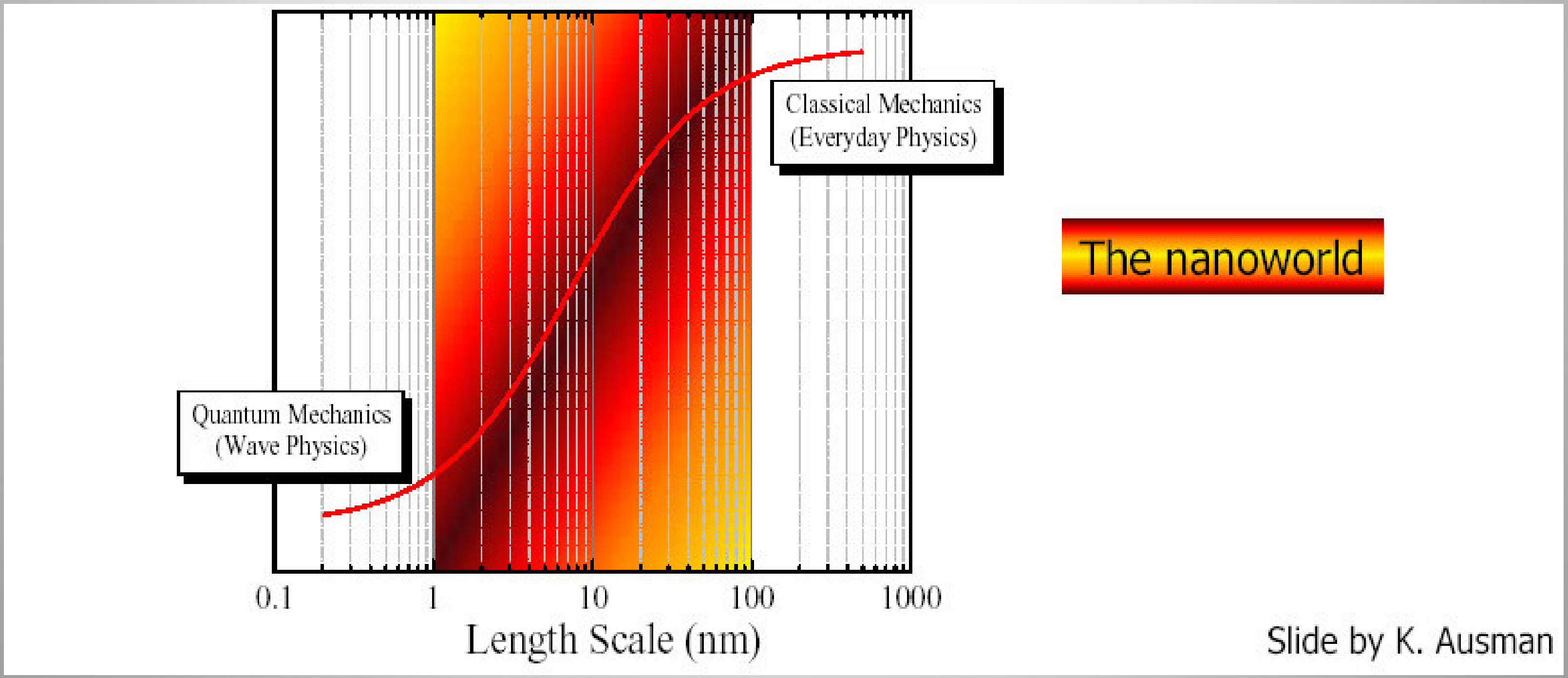
Manufactured NANOPARTCLES <0.1µm

Why is Nano Different?

Micron (1,000 nm) and larger scale: classical physics determines properties.

Angstrom (0.1 nm) scale: quantum mechanics determines properties.

Nanometer scale, fundamental properties depend on exactly how big the particle is.



From K.M.Kulinowski, Nanotechnology "The Big Science of the Very Small", CBEN

Nanophotonics: size matter

Adattato da K.M.Kulinowski, Nanotechnology “The Big Science of the Very Small”, CBEN

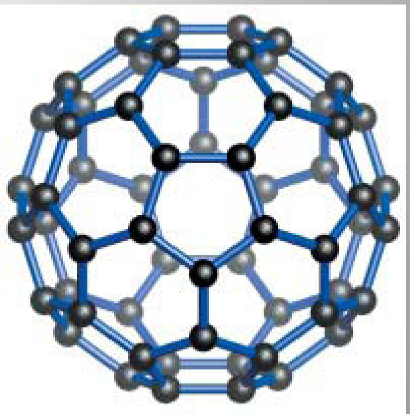
Classification of nanomaterials

- **Carbon nanomaterials** (fullerenes, CNTs, nanocones etc.)
- **Metal oxides** (titanium, zinc, cerium, silica, iron)
- **Quantum dots** (cadmium-selenide,CdSe)
- **Zero-valent metal powder** (Fe,Co,Ni,Ag, Au, Pt)
- **Organic polymers** (dendrites,polystyrene, liposomes)

Fullerenes (C60)

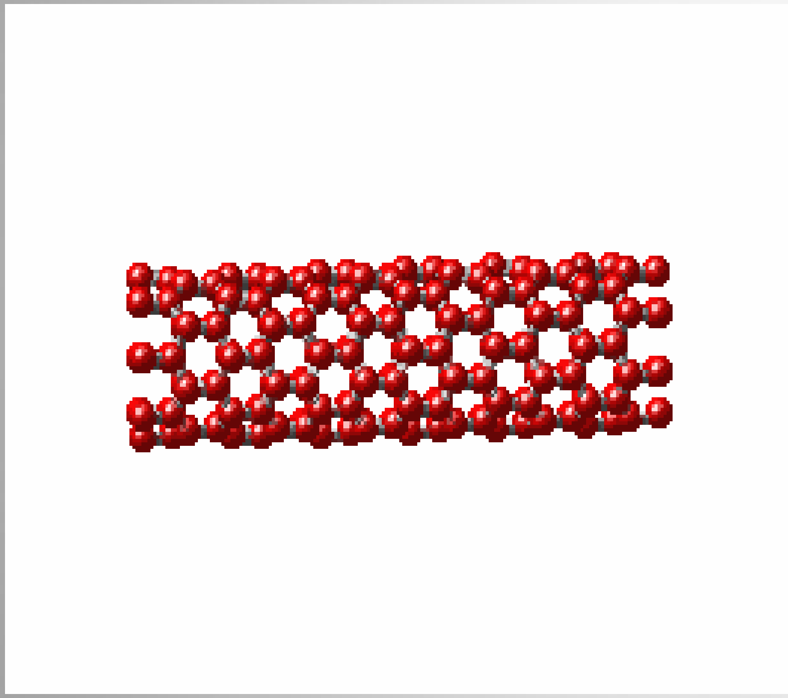
Spheric molecules, 1 nm of diameter with 60 atoms of carbon on 20 hexagons and 12 pentagons (bucky ball)

Drug delivery, electronics, lubricants of surfaces



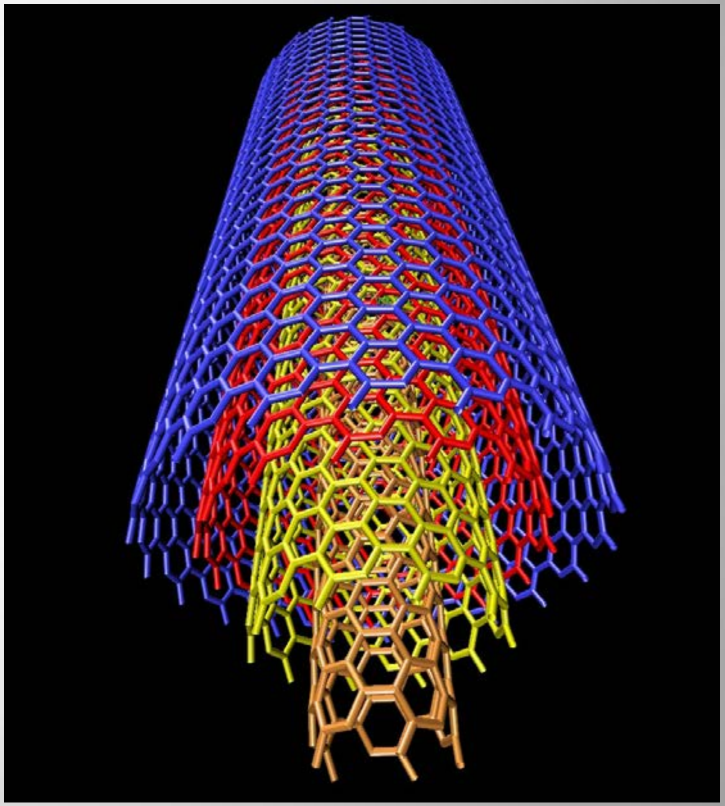
Carbon nanotubes

SWNT



Vicent H. Crespi, University of Pennsilvania

MWNT



Alain Rochefort, CERCA

Cobalto nanoparticles aggregate

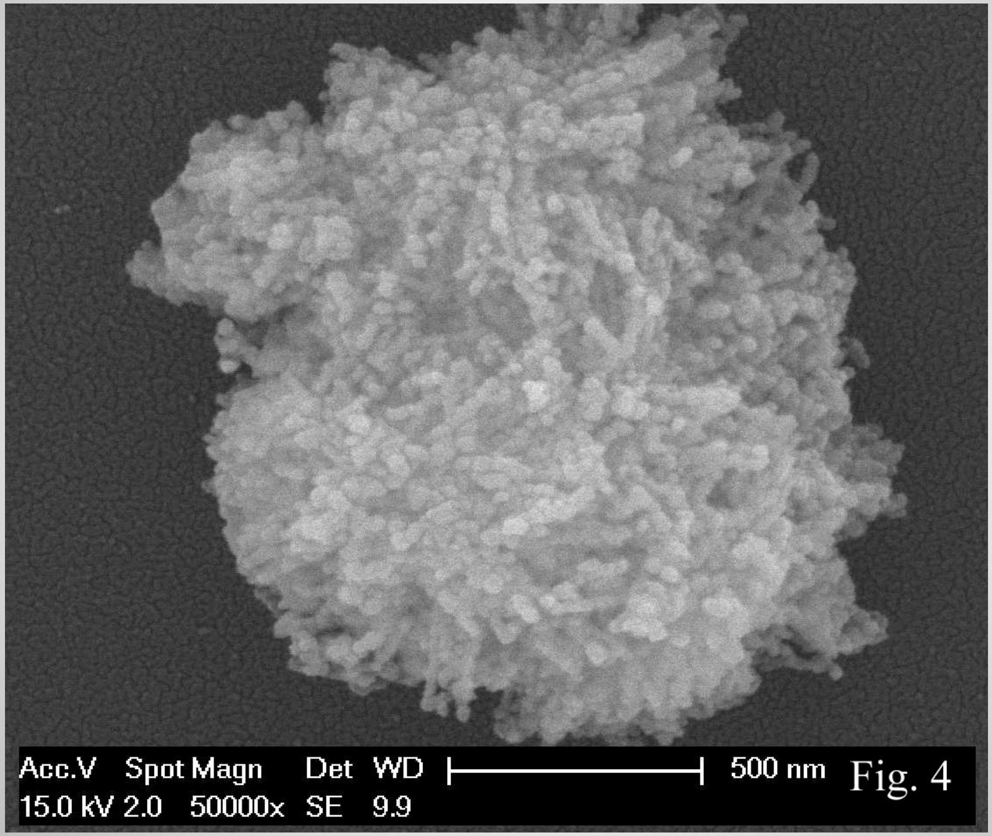


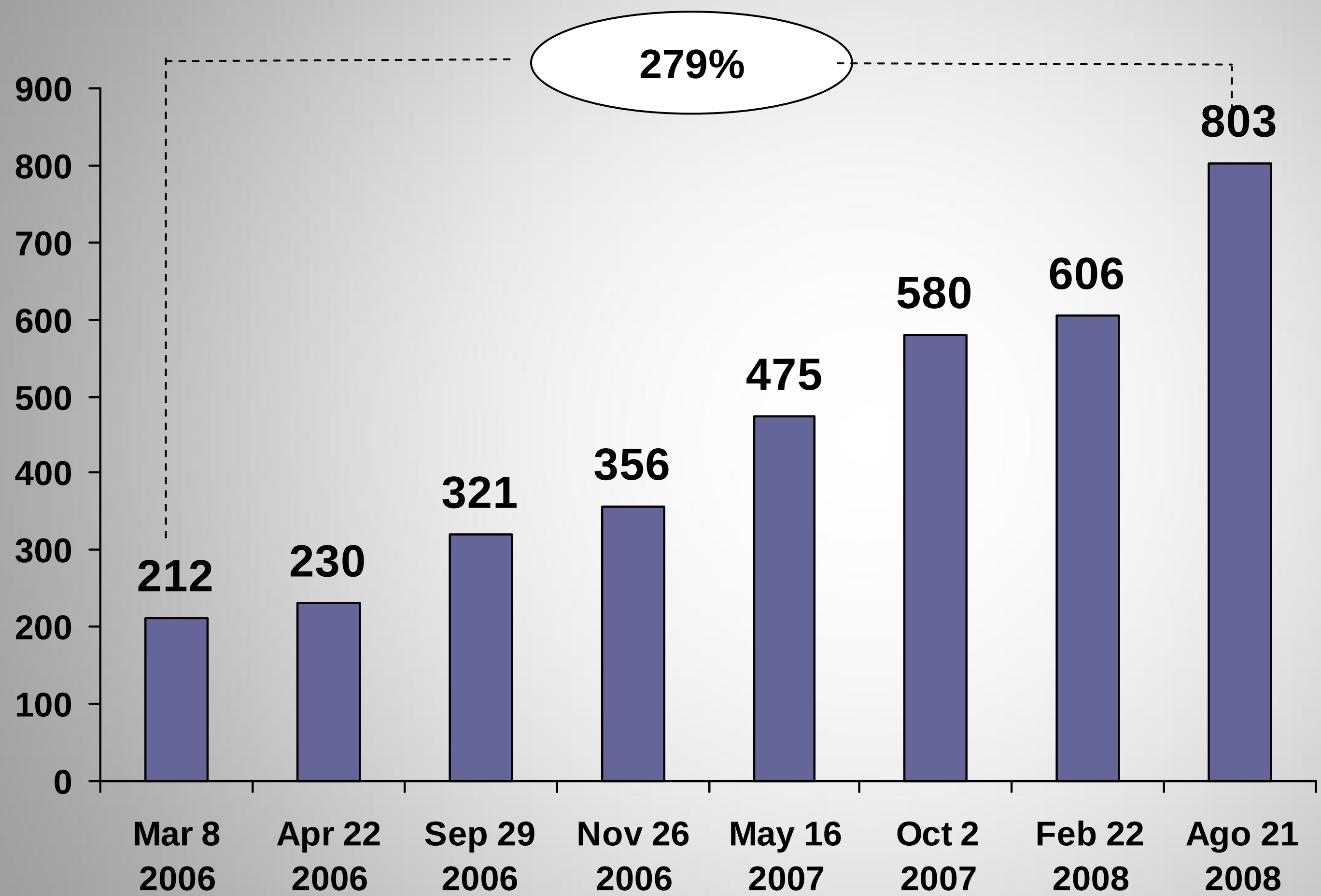
Fig. 4

Why should manufactured nanomaterials be of special concern to ecotoxicologists?

Nanoscale products: sources to the environment

Hundreds of consumer products that incorporate nanoscale materials are already on the market internationally

Number of consumer products on market incorporating NPs (2006 – 2008)



Woodrow Wilson International Center for Scholars

THE PEW CHARITABLE TRUSTS

Project on Emerging Nanotechnologies was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts.
http;//www.nanotechproject.org/inventories/consumer

Consumer products on market incorporating NPs: last inventory

25 Aug 2009: the inventory put the number of consumer products containing nanomaterials at **over 1,000** products, and has grown by nearly **379%** since March 2006 (**259 nanosilver** products, a quarter of all products)

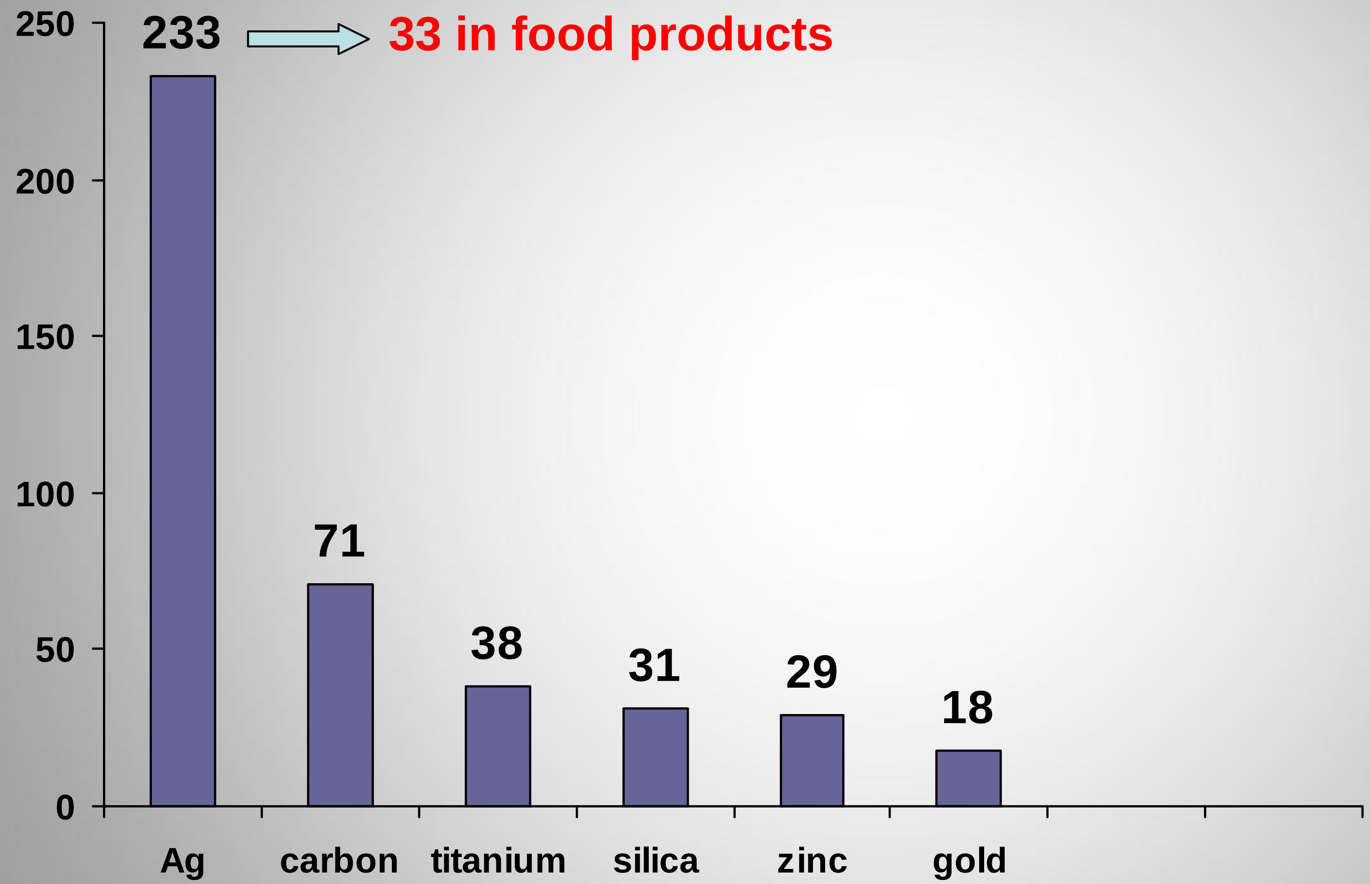
That is acknowledged to be a **very conservative** tally, and the commercial use of nanomaterials is thought **to be much higher**

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

Woodrow Wilson International Center for Scholars

THE PEW CHARITABLE TRUSTS

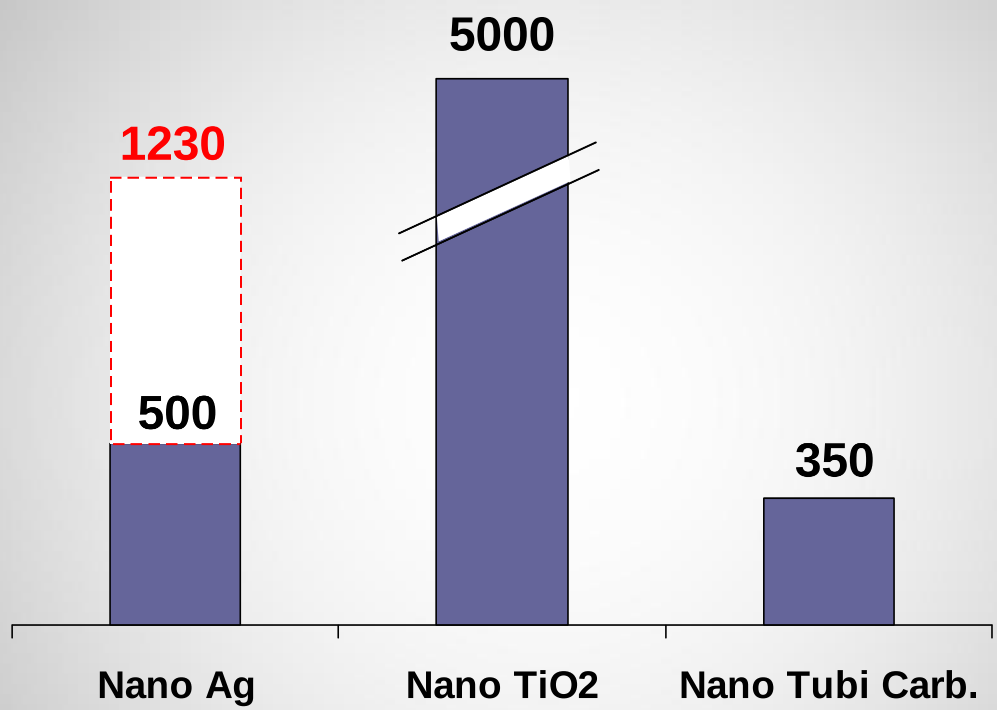
Nanomaterials reported in consumer products on market (2008)



Project on Emerging Nanotechnologies was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts.

http;//www.nanotechproject.org/inventories/consumer

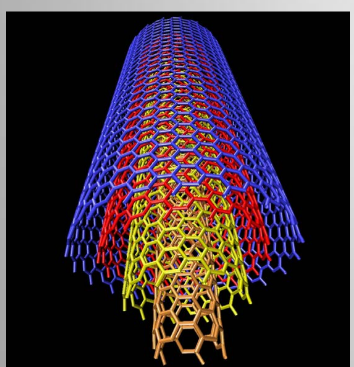
Estimates of world production of some nanoproducts 2007/2008 (t/a)



Müller N., Nanoparticles in the Environment Risk Assessment based on Exposure-Modelling What concentrations of nano titanium dioxide, carbon nanotubes and nano silver are we exposed to?, ETH Zurich, Department of Environmental Sciences, Empa - Material Science & Technology, St Gallen (2007)

A new Bayer MWCNT plant

Since 2007: a pilot facility (annual capacity of **60metric tons**) in Laufenburg (Baden-Württemberg.



The Bayer has invested €22m in the largest facility in the world with an annual capacity of **200 metric tons** which the company is marketing undert the trade name **Baytubes**

CHEMPARK, Leverkused, Germany.

http://www.chempark.com/index.php?page_id=3612

Polimerica

MWCNT:Alain Rochefort, CERCA

Other new CNT plants

Feb 2010

The announcement from Bayer comes the same week that Belgian competitor Nanocyl said it was installing a new reactor for nanotube production that would boost its production capacity to **400 ton/year.**

CNano, headquartered in California but with manufacturing in China, brought online a **500 ton/yr** facility for CNT production.

Source: NextBigFuture (Lifeboat Foundation Technology Research News Website)

Silver nanoparticles: the fastest growing product in the nanotechnology industry,

Due to its antimicrobial activity nanosilver is becoming the 21st century's penicillin

Biological properties:

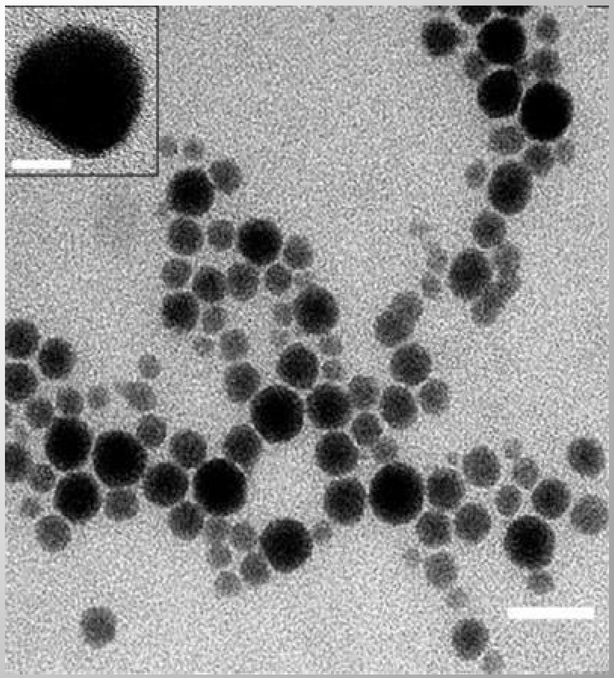
•Consumer products and food technology

•Textiles and fabrication

• Medical applications

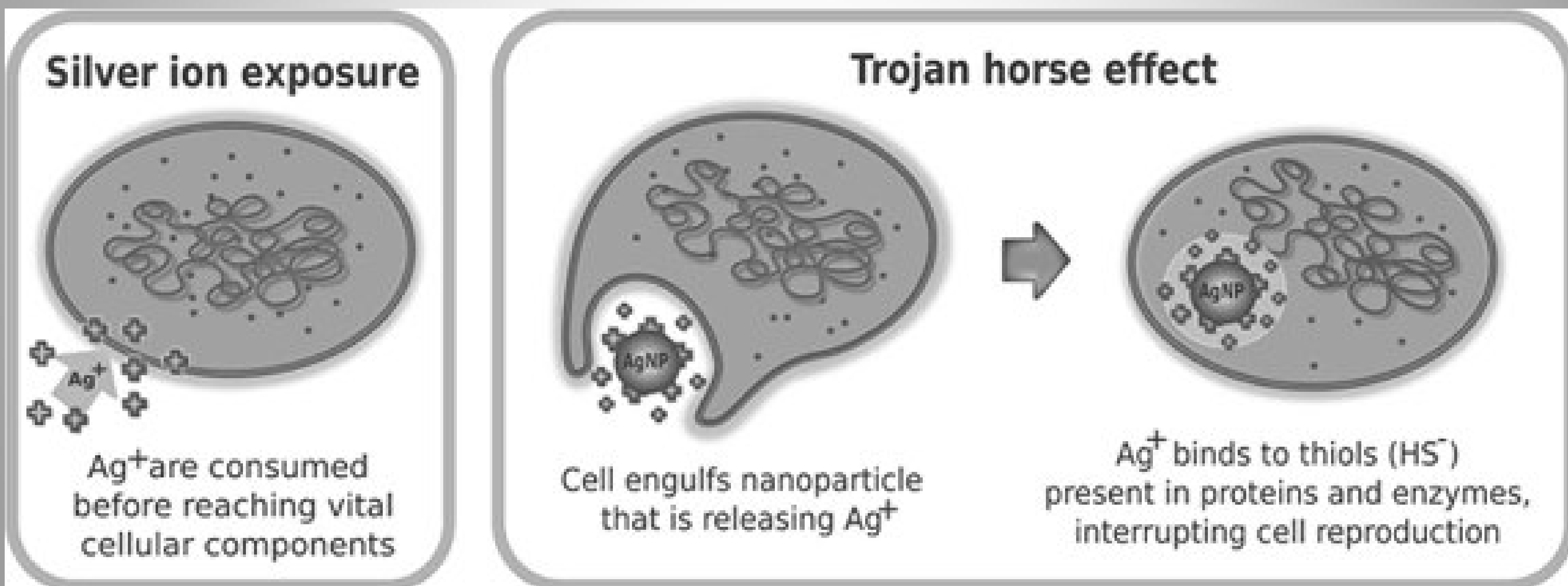
Optical physical properties (not present in microsilver):

• great potential in medical applications (diagnostic,drug delivery, imaging)



The Trojan Horse effect

The nanoformulation of silver may cross biological barriers into the cell. These intracellular nanosilver particles constitute a deposit that continually releases silver ions.



M.E. Quadros and L.C. Marr, Silver ion exposure vs. Trojan-horse effect. Environmental and Human Health Risks of Aerosolized Silver Nanoparticles J. Air & Waste Manage. Assoc. 60:770–781

Release of AgNP from SilverCare washing machines

Samsung SilverCare washing machines.

Release of nanoscale silver particles appears to be an **intentional and routine feature** of these products.

According to the company, the silver particles are generated by “electrolysation” of silver plates inside the washing machine, such that “400 billion nano-sized silver ions are emitted, directly penetrating into fabrics during the wash and final rinse cycles”

2.75 mg of nanosilver are introduced into each **55 L** wash.

Hund-Rinke K, Marscheider-Weidemann F and M Kemper (2008).
“Beurteilung der Gesamtumweltexposition von Silberionen aus Biozid-Produkten”. Forschungsbericht

40http://www.samsung.com/au/silvernano/site.html.

Release of AgNp in laundry water

Nine different textiles, including different brands of commercially available anti-odor socks:

- most of the released particles were **relatively large** and came out of the fabrics during the first wash.

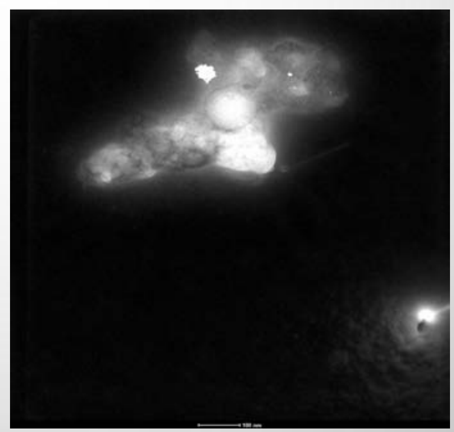
- the total released varied from **1.3 to 35 %** of the total nanosilver in the fabric.

L.Geranio et al The Behavior of Silver Nanotextiles during Washing *Environ. Sci. Technol.* 2009, *43,* 8113–8118

Ag-NP released from facades

A strong leaching of the Ag-NP was observed during the initial runoff events with a maximum concentration of **145 µg/l Ag**.

After a period of one year, **more than 30%** of the Ag-NP were released to the environment



Ralf Kägi, The European Network on the Health and Environmental Impact of Nanomaterials, NanoImpactNet, Lausanne, 10-11 March 2010

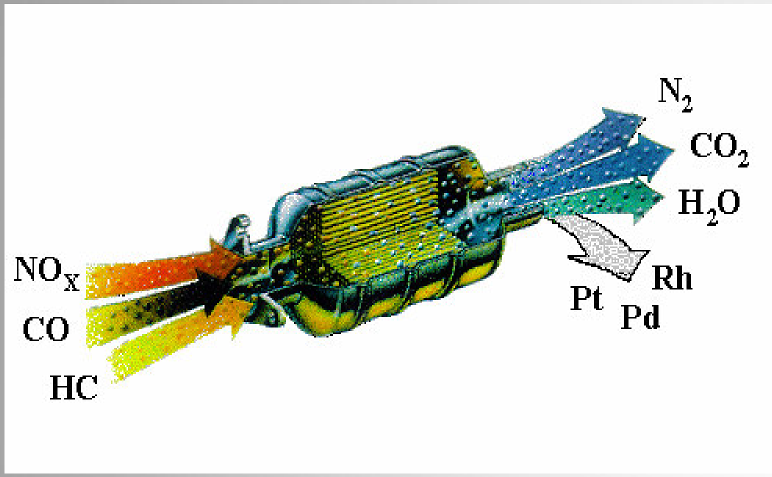
Cultivation of rice using *nano silver* technology

Nanocosmetics

- Titanium dioxide (TiO_2)
- Zinco oxide (ZnO)

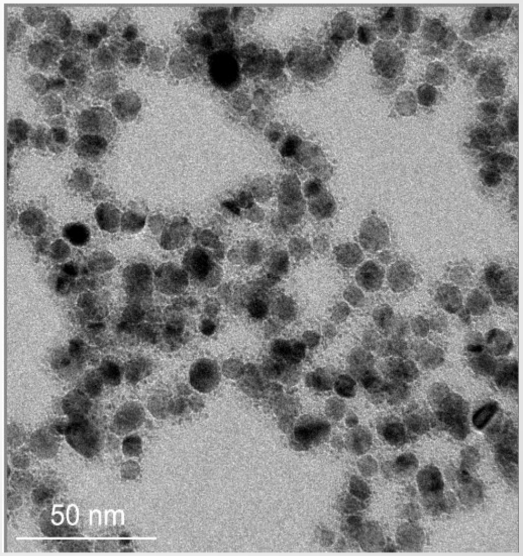
Using 30-60 nm nanoparticles, **Nanophase Technology** has created a product which blocks the UV being completely transparent to visible light

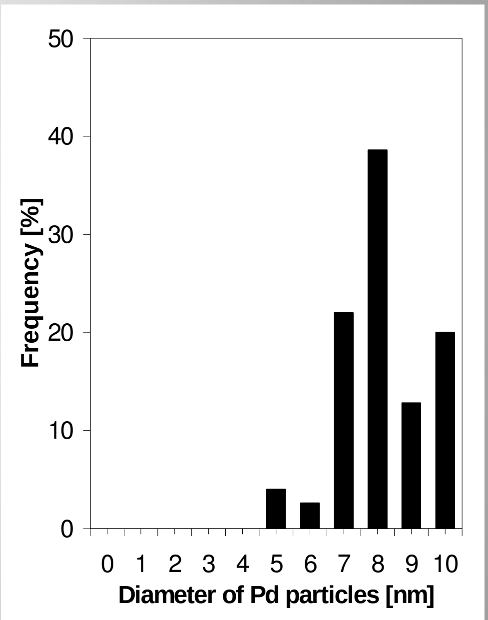
NPs emssion from automotive catalytic converters



Effects of palladium model nanoparticles emitted from catalytic converters on the cytokine release from peripheral blood mononuclear cells of non atopic women, ***P. Boscolo et al. 2009***

Monodispersed Pd NPs 10-20nm





Dr Kerstin Leopold
Technische Universität München
Garching

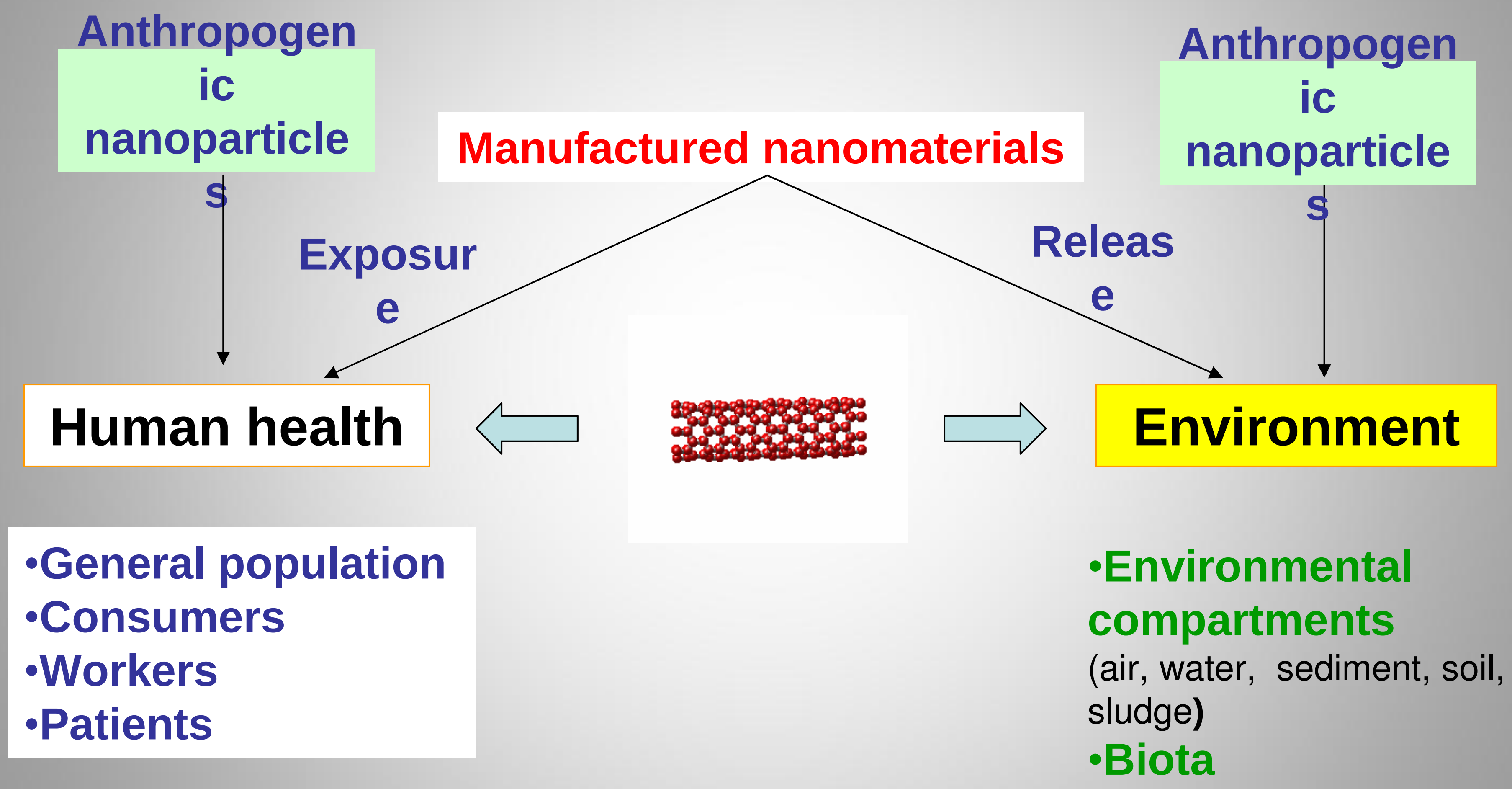
16 marzo 2005

Nanoparticelle di ossido di cerio per marmitte catalitiche più efficienti

Cerium oxide nanoparticles added to fuel as catalyst (m.p. 2600℃):

- migliorano il rendimento del motore
- favoriscono un risparmio sui consumi
- danno luogo a una combustione più pulita del gasolio e delle scorie
- permettono l'ossidazione graduale dei depositi di carbonio accumulati nei motori.

Potential environmental and health impact of nanomaterials



SWCNT: Vicent H. Crespi, University of Pennsilvania

Nanotoxicology:an urgent need

This remarkable and unrestrainable spread of nanomaterials is a great potential for human exposure and their release into the environment with consequent potential adverse effects for both environmental compartments and health

There is the need for accurate, reliable and impartial scientific knowledge on risks and benefits of this new technology

The new discipline, nanotoxicology, including econanotoxicology, meets this need

Definitions

NANOTOXICOLOGY: an emerging discipline evolving from studies of ultrafine particles, aimed at understanding the toxicity of manufactured nanoparticles

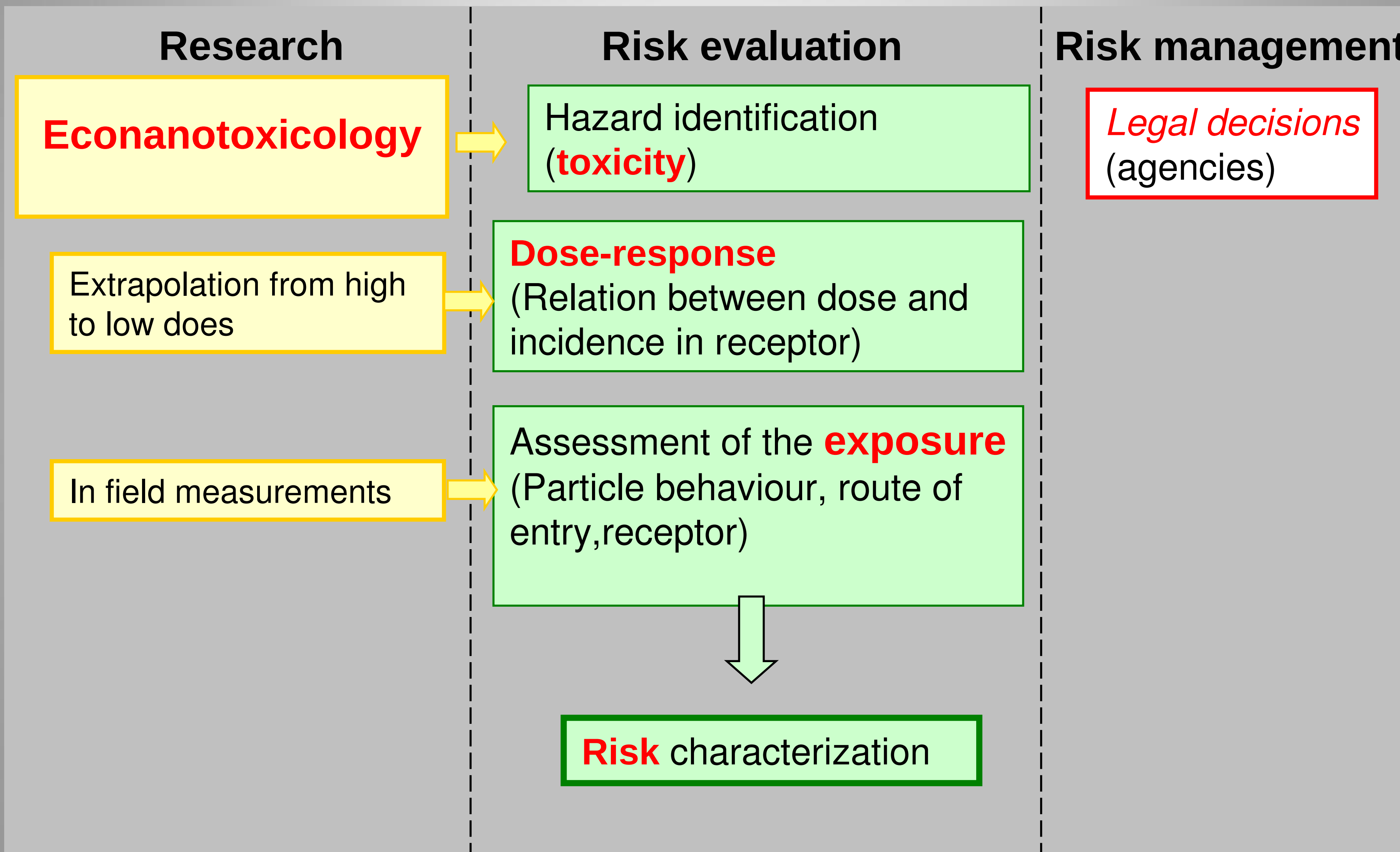
Oberdorster, G et al Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. (2005) 823-839

ECOTOXICOLOGY: the science of contaminants in the biosphere and their effects on costituents of the bioshere

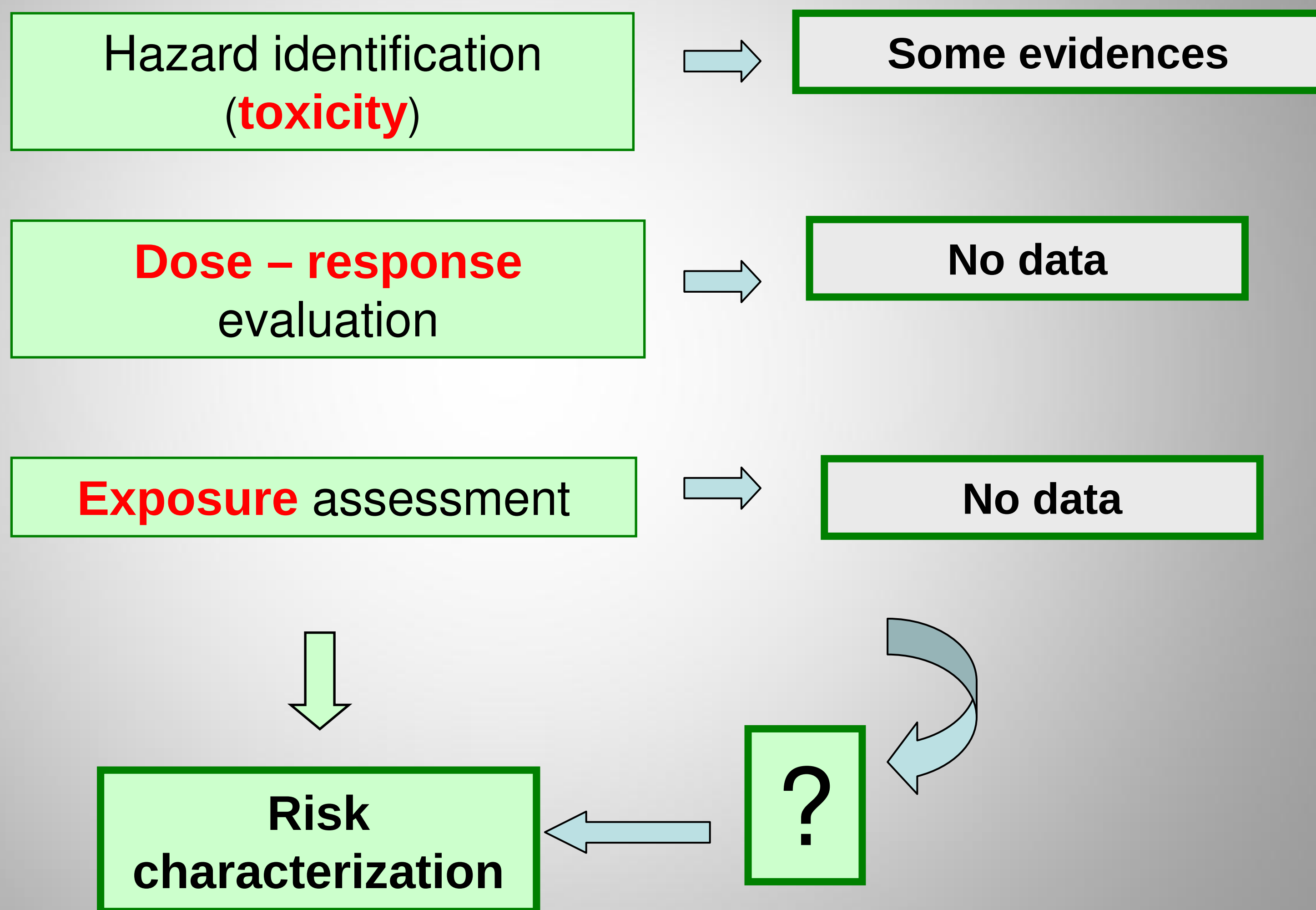
Newmann MC , Ecotoxicology A Sub-discipline of Ecology entry for Encyclopedia of Ecology, Elsevier, Inc.

ECO"nano"TOXICOLOGY the science of manufactured nanomaterials in the biosphere and their effects on costituents of the bioshere

Elements of the ecological risk assessment of nanoparticles



Risk assessment of nanomaterials: where are we?



Dosimetric parameters determining the toxic response of macro- , micro- and nanomaterials

Classical paradigm

Hazard: the dose determines the toxic effect ⇨ **Dosimetric parameters: MASS VOLUME**

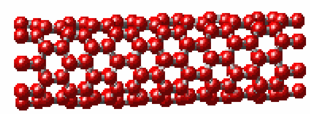
Nanomaterial paradigm

⇩

Hazard: the dose X determines the toxic effect

Paracelsus, Father of toxicology

Chemico-physical parameters determining the toxicological response of nanomaterials



Number, size

Dispersion in

- gas (aerosols)
- liquid (e.g. gels, ferrofluids)
- solid (e.g.matrix material)

Aggregation state

- Single particles
- Aggregates
- Agglomerates

Shape, structure

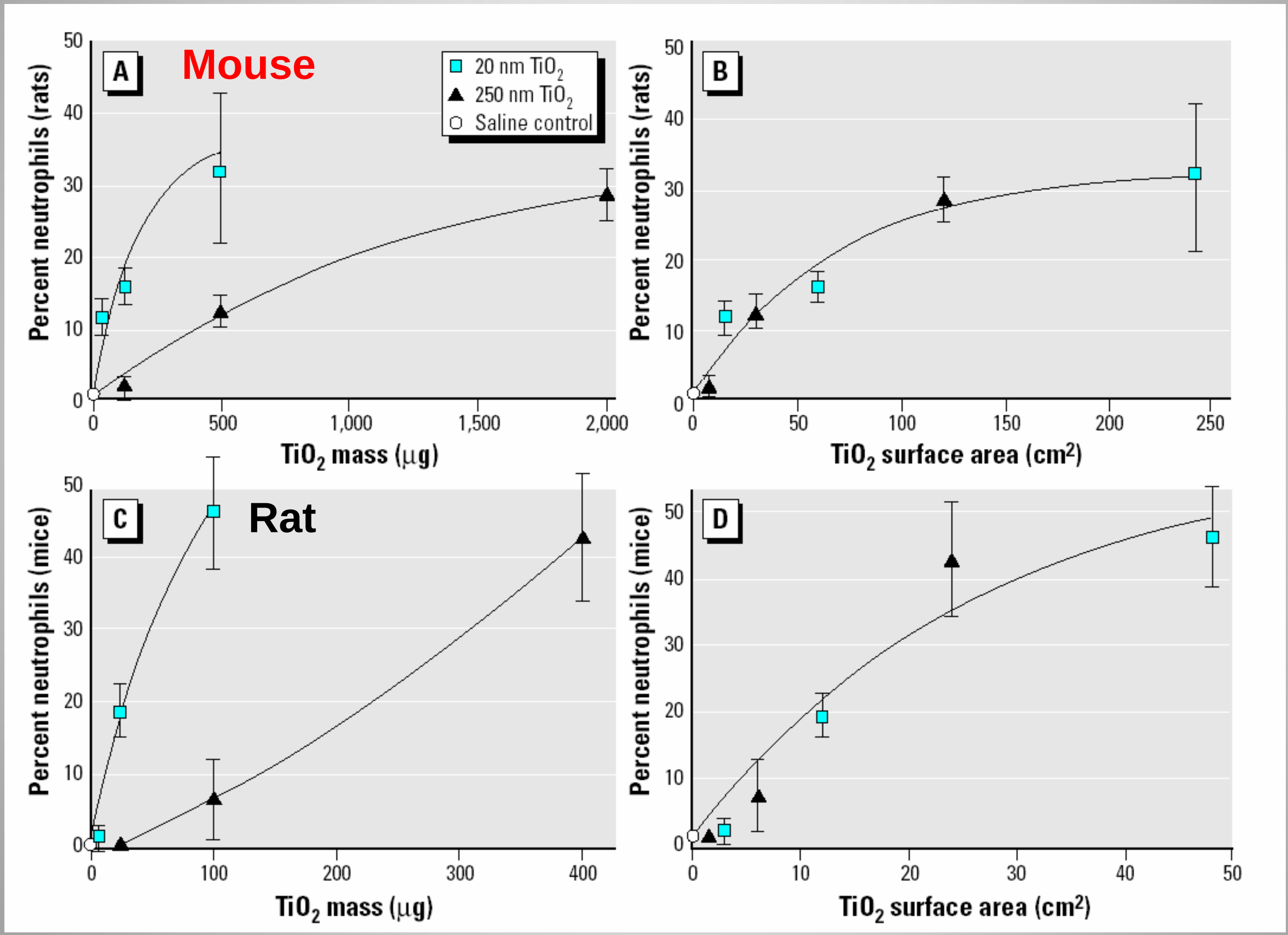
- sphere,needle,tube,disc
- cristallinity
- porosity

Surface area and charge

Surface modification

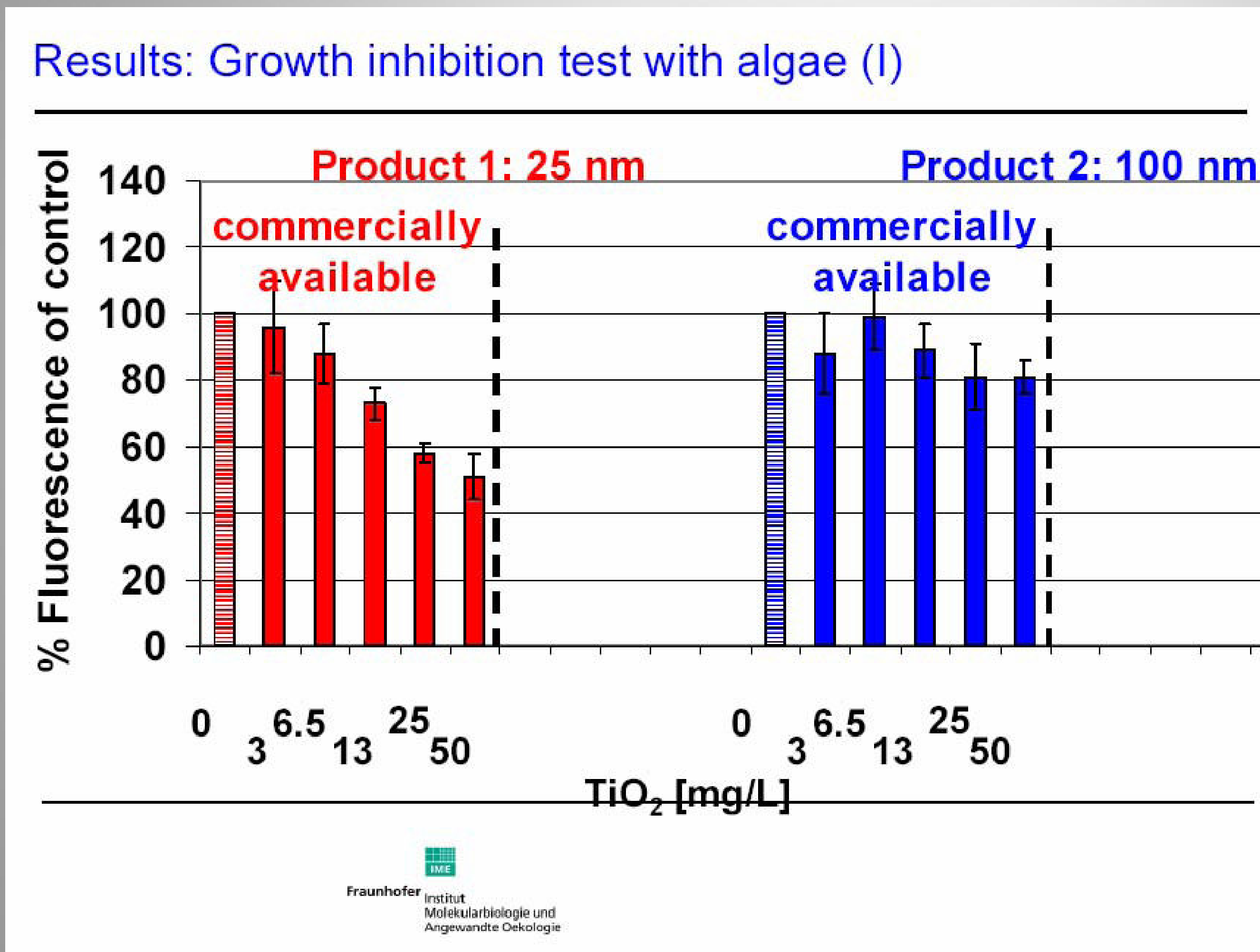
- Untreated (as from the process of production)
- Coated (e.g. coniugated, polymeric films)
- “Core” or shells (e.g. spheres, capsules)

Mass vs. Surface area effect of TiO_2particle size on toxicity in mouse and rats



Oberdörster et al. (2005)

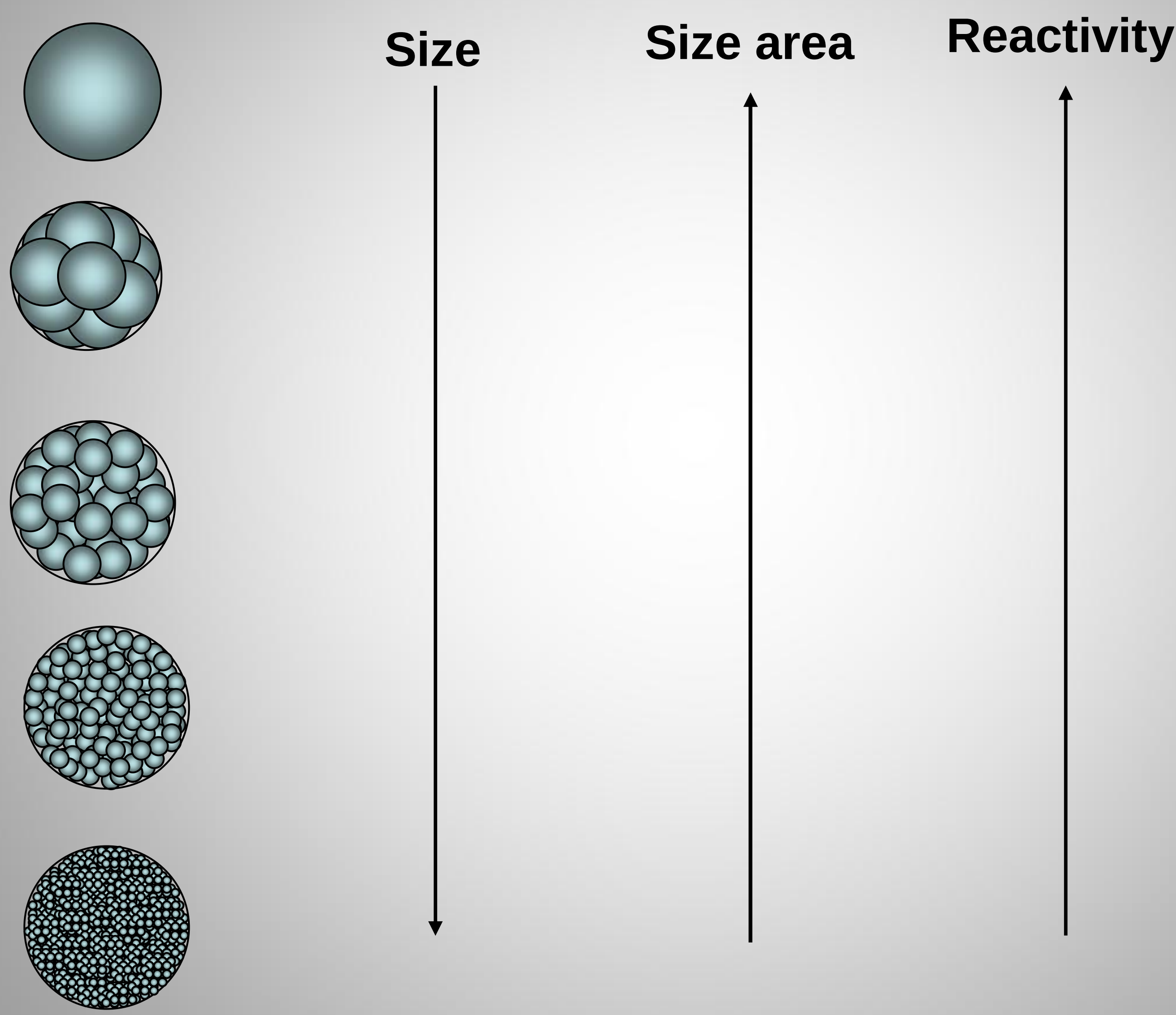
Titanium Dioxide (25 nm vs100 nm) inhibits algal growth (OECD 201)



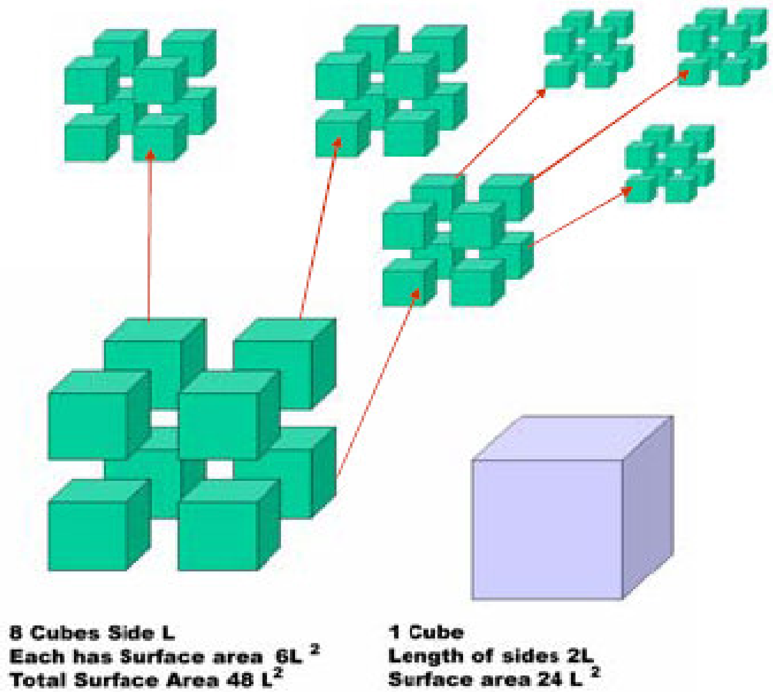
Size-dependent inhibition of algal growth by TiO2

K. Hunde-Rinkeand M. SimonSETAC-EU 2005•

Size-surface area-reactivity paradigm for NPs



Repeat 24 times



From 5 cm^3 - about 1,7 cm per side – of a material divided 24 times smaller cubes are produced. Distributed as monolayer they can cover a football ground

Regulation: workers protection

Framework directive 89/391/EEC

Other directives: 89/656/EEC, 98/24/EC, 1999/38/EC

Present regulatory standards are based on the mass of inhaled particles

However, for NP mass does not seem the most important factor with regard to their biological effects

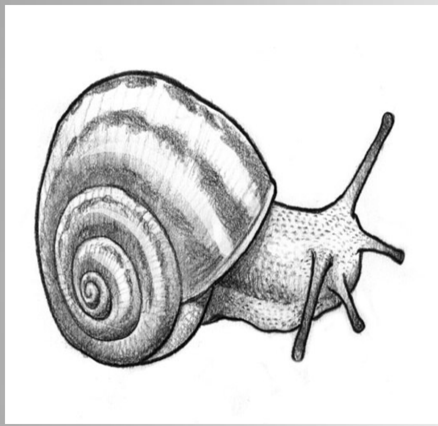
More important are the number of particles, the size, the active surface and the mode of exposure

Since methods for risk assessment concerning workers's protection need to be developed and the risks long-term implications of NP cannot currently be determined **intensive research** is need in order to assess the risks

Ref. The Royal Society and Royal Academy of Engineering; European Commission SCENIHR

Sustainable development of nanotechnology: different velocities

The development of products containing nanomaterials **has been much faster** than any corresponding collection of environmental health data.



A severe gap…

Environmental and health effects of nanomaterials

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Nanotoxicology

Development and production of nanomaterials

Interactions of NP in the environment

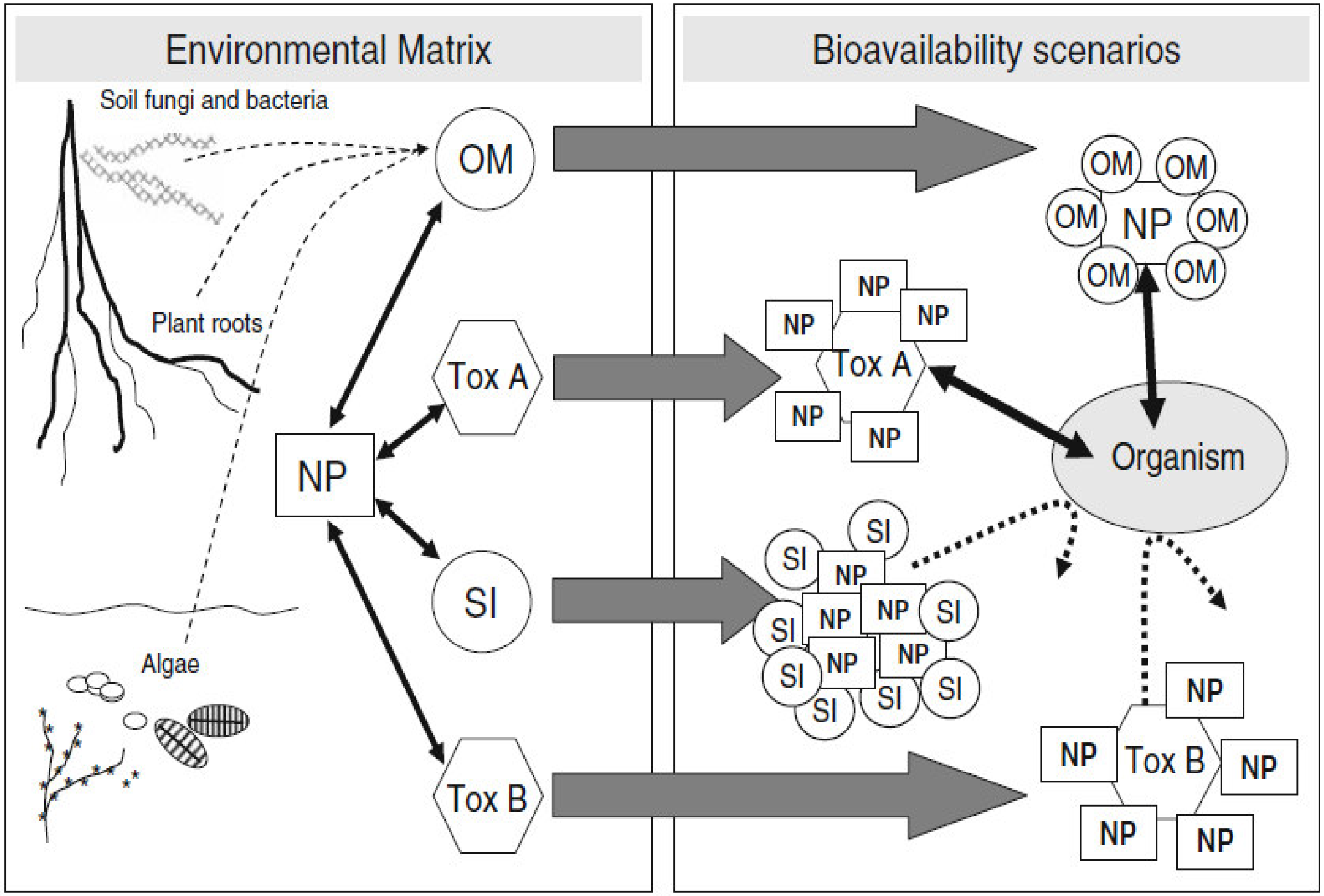


Fig. 3 Scenario of nanoparticles' (NP) interactions with toxicants (Tox A and B), salt ions (SI), and organic matter (OM) such as humic acids or compounds released by plants, fungi, bacteria, and algae. Some compounds present in environmental matrices might increase the NPs' stability (OM) and thus bioavailability (represented as solid arrows entering organisms), whereas others (salt ions) might foster the aggregation of NPs, thus reducing their bioavailability (represented as dotted arrows not entering organisms), or physically restraining NP-organism interactions. In other cases, NPs' bioavailability might be either increased or decreased

Navarro, 2008

Interactions of NP in the environment

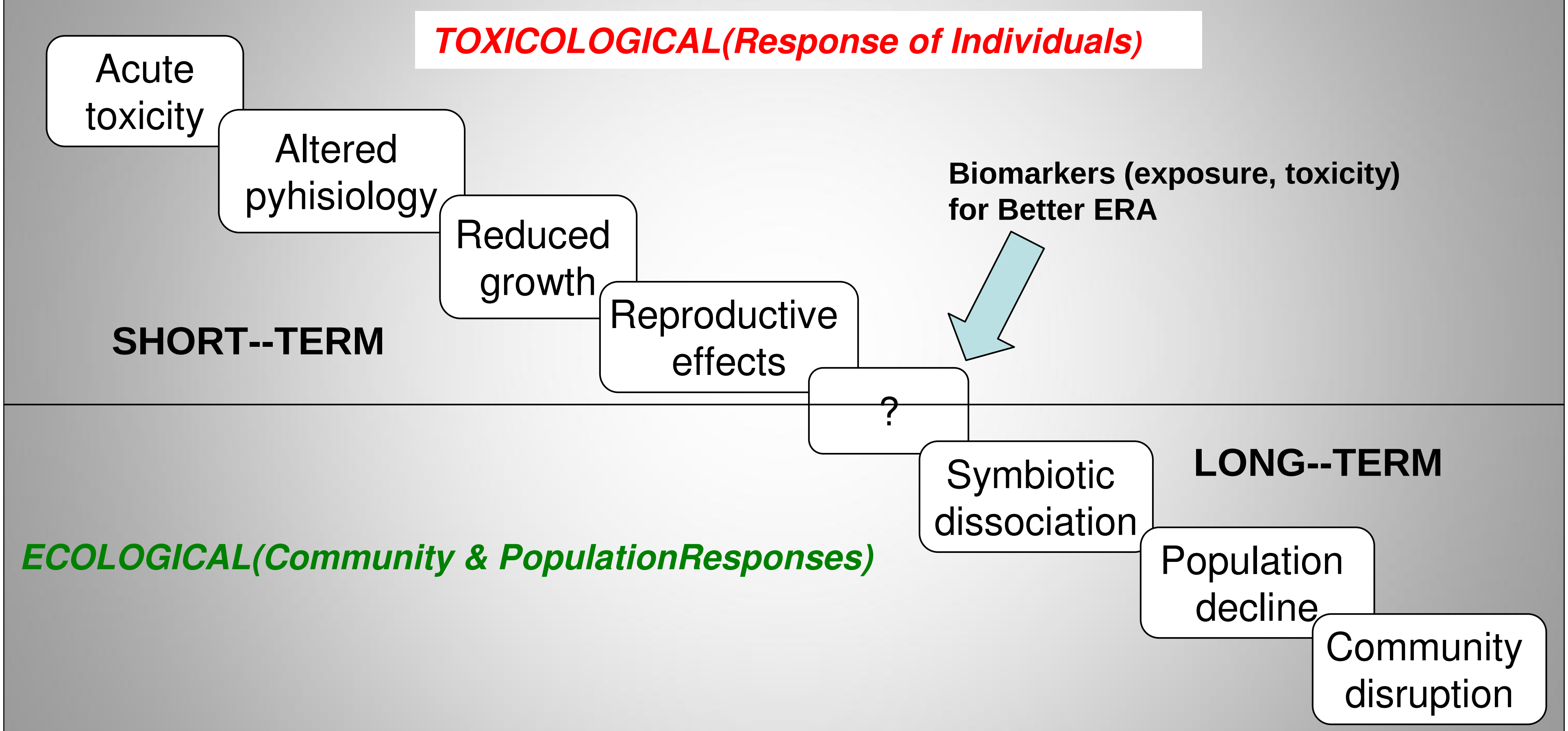
Three aspects seem important when assessing the impact of ENPs as pollutants ending up in the environment:

Mobility (transport and transfer); their ability to move from one place to another or from one recipient to another

Ecotoxicity; the possible harm that ENPs can cause to organisms living in water, sediments and soils that they enter.

Modification; how and to which extent ENPs are modified by contact with the environment (and the consequences of such modifications on ecotoxicity and mobility).

Establishing a chain of ecotoxicological evidence for potential toxicity and bioaccumulation of nanoparticles



Adapted from Gunderson et al. 1997

NPs as carrier of pollutants

.

The large surface area, crystalline structure, and reactivity of some nanoparticles can facilitate **transport of toxic materials** in the environment (Zhang & Masciangioli, 2003).

NPs as carrier of pollutants

2007 Sun et al, *Enhanced Accumulation of Arsenate in Carp in the Presence of Titanium Dioxide Nanoparticles* Water Air Soil Pollut 178:245–254

- TiO_2 nanoparticles had a significant adsorption capacity for As(V).
- When exposed to As(V)-contaminated water in the presence of TiO_2 nanoparticles, carp accumulated considerably more As, and As concentration in carp increased by 132% after 25 days exposure.
- Considerable As and TiO_2 accumulated in intestine, stomach and gills of the fish,and the lowest level of accumulation was found in muscle.

2007 Xuezhi Zhang et al, *Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles* Chemosphere 67, 160–166

- TiO_2 nanoparticles had a significantly stronger adsorption capacity for Cd than SP.
- The presence of SP did not have significant influence on the accumulation of Cd in carp during the 25 d of exposure.
- The presence of TiO_2 nanoparticles greatly enhanced the accumulation of Cd in carp. After 25 d of exposure Cd concentration in carp increased by 146%.
- Considerable Cd and TiO_2 accumulated in viscera and gills of the fish.

Silver Nps are toxic to aquatic organisms

2008 Griffitt et al, Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms, *Environ. Toxicol. Chem.*27:1972—1978

20–30 nm silver nanopowder induced a 48 h LC50 of 7.07–7.20 µg/ml in zebrafish (*Danio rerio*) depending on whether the exposure was to an adult or juvenile fish

2009 Griffitt R.J et al, Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles, *Toxicol. Sci.*,107: 404--415

20–30 nm silver nanopowder induced changes in gene expression, but did not affect gill filament length

2008 Asharani et al., Toxicity of silver nanoparticles in zebrafish models, *Nanotechnology*, 19: 1--8

5–20 nm silver nanoparticles capped with starch or bovine serum albumin induced embryotoxicity

NPs are genotoxic to aquatic organisms

2008 Reeves et al, Hydroxyl radicals (OH) are associated with titanium dioxide (TiO2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells
Mutat. Res., 640: 113--122

2009 Singh et al, NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials, *Biomaterials*, 30: 3891—3914

Titanium nanoparticle-induced genotoxicity in rainbow trout cells

2009 Kühnel D, Agglomeration of tungsten carbide nanoparticles in exposure medium does not prevent uptake and toxicity toward a rainbow trout gill cell line, *Aquat. Toxicol.*, 93:91—99

Nano-sized tungsten carbide particles induce cytotoxicity in a rainbow trout gill cell line

2008 Vevers W.F., Jha A.N., Genotoxic and cytotoxic potential of titanium dioxide (TiO2) nanoparticles on fish cells in vitro,Ecotoxicology,17: 410—420

No chromosomal damage after titanium nanoparticle exposure in the rainbow trout cells measured by a micronucleus assay, N

2010 JP Wise Sr et al,Silver nanospheres are cytotoxic and genotoxic to fish cells, Aquatic Toxicology 97:34-41

30 nm silver nanospheres are <cytotoxic and clastogenic to fish medaka (*Oryzias latipes*) cell line in a concentration-dependent manner. Chromosomal damage using a chromosomal aberration study

Medaka fish have been used extensively as a model for human health Thus,these data have implications for the potential effects of silver nanoparticles on aquatic species, and they also imply that there could be a concern for humans as well

T.M.Scown (2010)

Effects of Aqueous Exposure to Silver Nanoparticles of Different Sizes in Rainbow Trout, Toxicol. Sci. (2010) 115 (2): 521-534.

rainbow trout (*Oncorhynchus mykiss*) were exposed via the water to commercial silver particles of three nominal sizes: 10 nm (N10), 35 nm (N35), and 600–1600 nm (NBulk), and to silver nitrate for 10 days.

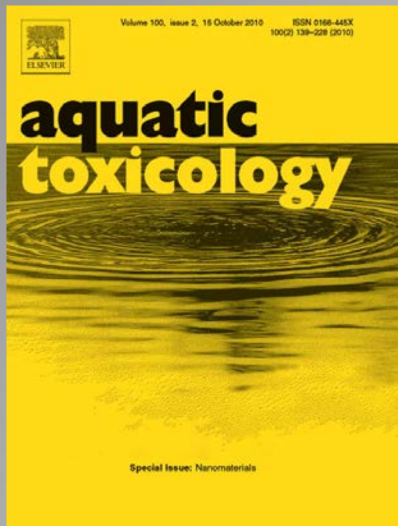
Are NP treated as food by bivalves?

February 22, 2010

In a paper published in *Marine Environmental Research*, Ward et al.used natural seawater to produce **marine snow that contained fluorescently labeled polystyrene nanoparticles.** They then exposed oysters and mussels collected from the Long Island Sound to this snow-filled seawater

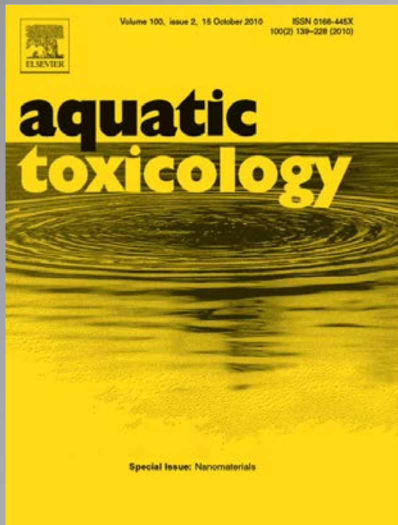
Nanoparticles were **taken up in much higher amounts** when the bivalves were exposed to marine snow. But they also found that when filtered from marine snow, nanoparticles remained in the bivalves' bodies for a much longer time than would be expected for non-nutritive materials: up to three days.

Ward suspects that the particles are being treated as food by the animals, and are being taken up into their digestive cells. **This could be particularly dangerous, he says, since small nanoparticles can circumvent living cells' natural defenses.**



L. Canesi et al. Biomarkers in **Mytilus galloprovincialis** exposed to suspensions of selected nanoparticles (Nano carbon black, C60 fullerene, Nano-TiO2, Nano-SiO2) Aquatic Toxicology 100 (2010) 168–177

Mussels responses to exposure to NP suspensions involve changes in lysosomal and oxidative stress biomarkers in the digestive gland, suggesting **uptake of NP aggregates/agglomerates** mainly through the digestive system.

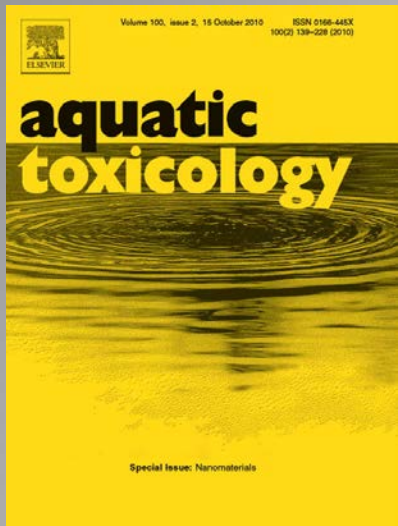


L.Canesi et al In vitro effects of suspensions of selected nanoparticles (C_{60} fullerene, TiO_2, SiO_2) on **Mytilus hemocytes**
Aquatic Toxicology 96 (2010) 151–158

All NP suspensions induced a concentration-dependent **lysozyme release**, **extracellular oxyradical and nitric oxide (NO) production**, to a different extent and with different time courses depending on the concentration and the NP type.

The **inflammatory effects** of NPs were mediated by rapid activation of the stress-activated p38 MAPK.

The results further support the hypothesis that in bivalves **the immune system represents a significant target for NPs.**



L.Canesi et al ,Immunotoxicity of carbon black nanoparticles to blue **mussel hemocytes**
Environment International 34 (2008) 1114–1119

In mussel hemocytes like in mammalian cells NCB exposure can induce **inflammatory processes**, and indicate that bivalve immunocytes can represent a suitable model for investigating the effects and modes of action of nanoparticles in the cells of aquatic invertebrates.

Guidelines for nanotoxicology

Nanotoxicology research and ethical problems:** in vitro* ***and/or *in vivo* ***studies?***

Nanotoxicology will lead to an increase of animal use in research on health effects on nanoparticles oriented to risk assessment.

Ethical problems due to the Directive 86/609/EEC (Reduction, Refinement, Replaceme

Guidelines for nanotoxicology

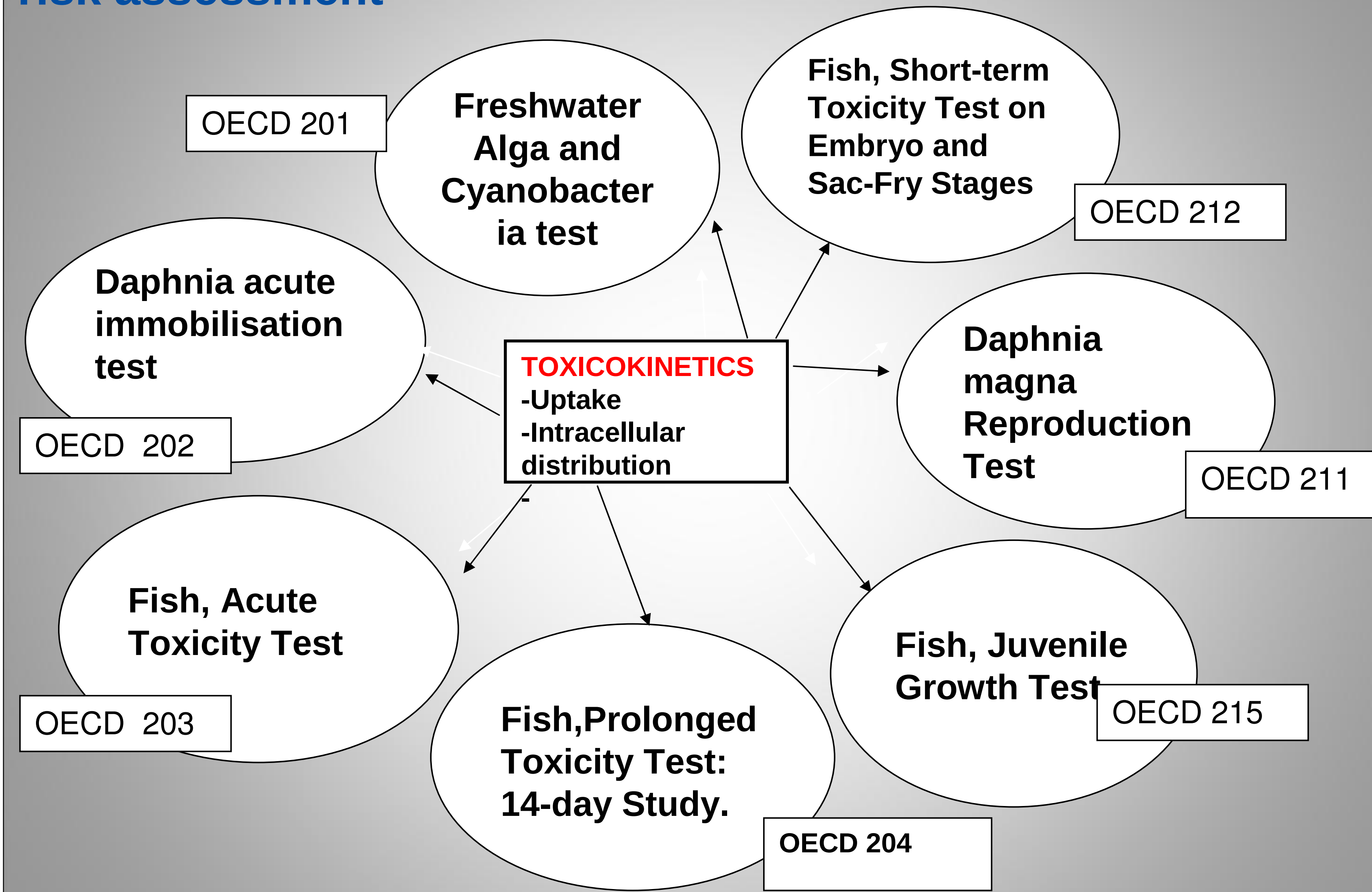
When *in vivo* nanotoxicology research?

…for specific well-oriented purposes such as **biokinetics and identification of target tissues.**

When *in vitro* nanotoxicology research?

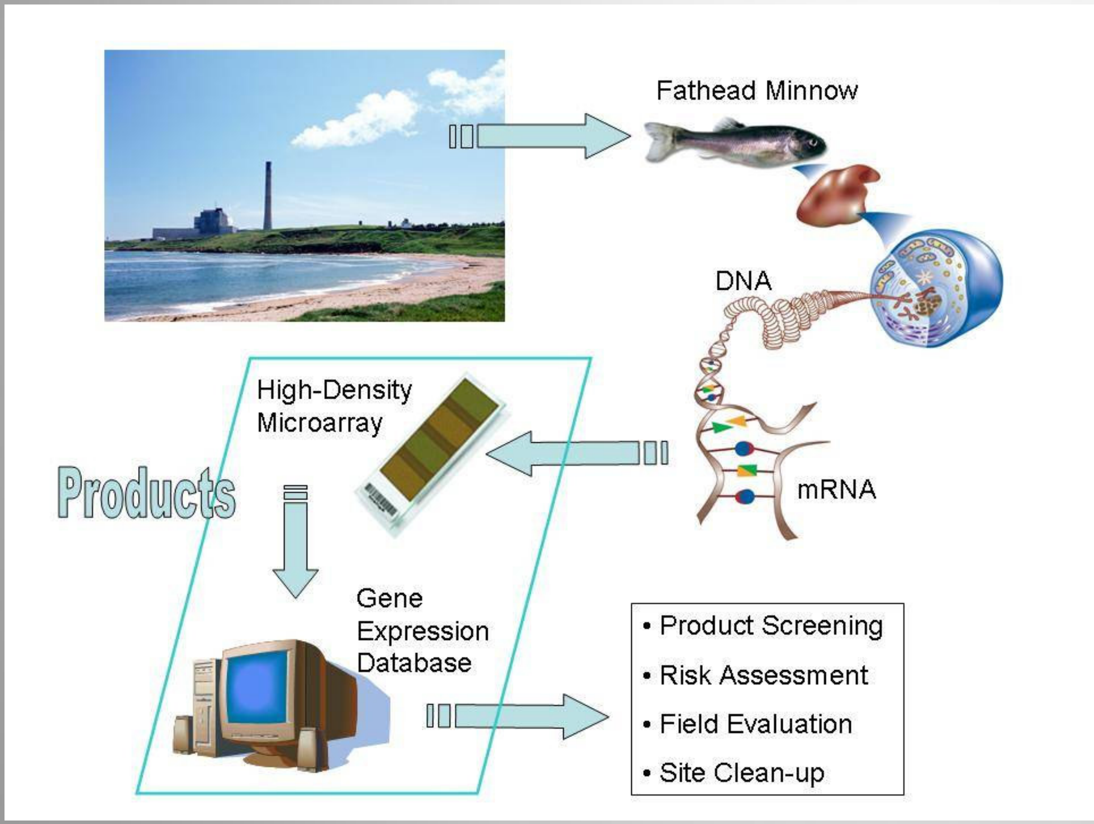
…for **screening** toxicity testing of NP and **mechanistic studies.**

Ecotoxicity test methods and environmental risk assessment



Rapid gene expression screening using a fathead minnow (Pimephales promelas) microarray.

The reactive Nano Iron Particles (RNIP) were iron solids with anaverage particle size of 70 nm,



Very few genes were robustly changed in the RNIP exposed animals.

These included genes that encode proteins involved in **tissue repair, inflammation** (the first line of defense against any foreign chemical or organism), and **anti-oxidant defenses**

These products had been approved for use by the US EPA and presented a baseline of ‘eco-nano-technologies’

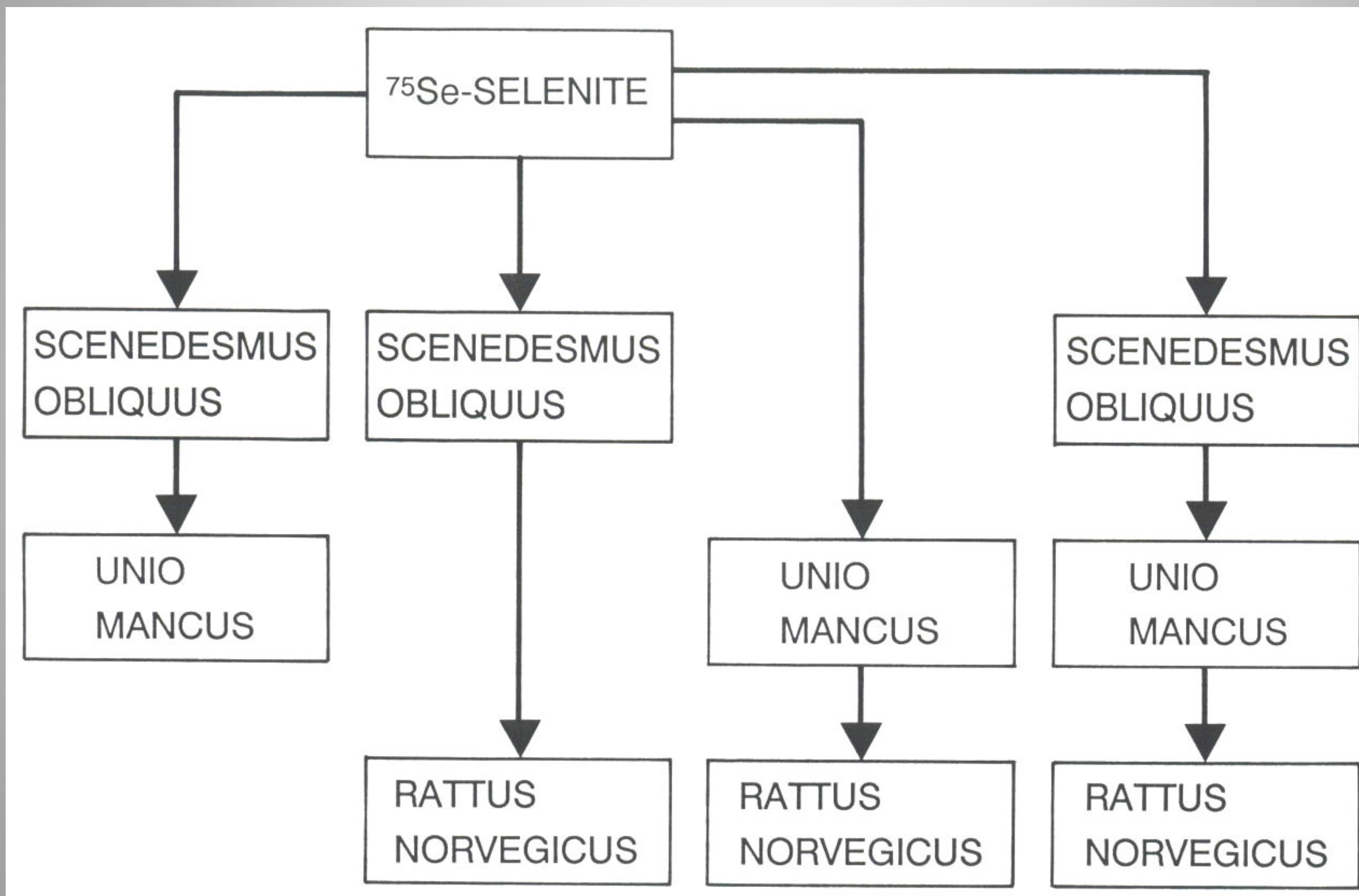
Oberdoster E.2006

The Use of Fish Cells in Ecotoxicology

The Report and Recommendations of ECVAM Workshop 47, ATLA **31**, 317–351, 2003

End point	Cell line	Primary cells
Cellular transformation	BG/F	
Alkaline unwinding assay	BB	
Nick translation		Rainbow trout hepatocytes
Alkaline precipitation asay		Rainbow trout hepatocytes
DNA adduct formation	BF-2, RTG-2, BB	Rainbow trout hepatocytes
Anaphase aberration	RTG-2	
Comet assay	R1, RTG-2, EPC RTL-W1	Trout-Zebrafish hepatocytes, Zebrafish gill cells
Micronucleus induction	BG/F, Ul-h, RTG-2	
Sister-chromatid exchange	ULF-23	
Unscheduled DNA repairsynthesis (UDS)	RTG-2, Ul-h, BB, CAF-MM1	Fish hepatocytes

A food chain experimental model for NPs:a next step



Interaction of NP with the environment

Ecotoxicity studies of ENPs are **scarce and difficult to compare**, and few have taken into account the **modifying effects** of soil, sediment and water constituents.

Data from experiments under simplified conditions indicate that **some ENPs are toxic** to a number of organisms.

This concerns: **fullerenes, silver nanoparticles** and **q-dots**, and to a lesser degree **carbon nanotubes**, and **nanoparticles of Cu, ZnO, TiO_2** and **SiO_2.**

To determine if ENPs represent a risk to organisms and the environment, crucial information is lacking regarding mobility, transfer and uptake as affected by environmental matrices.

Interaction of NP with aquatic organisms

Environmental fate, transport, bioavailabilty.

•Aggregate/agglomerate

•Essential to define the form to which aquatic organisms are exposed in environmentally relevant scenario

Route of exposure (uptake, accumulation)

•At present evidence of acumulation of NPs in fish is very limited (inadequate methods? Uptake to low?)

•If no uptake then can there be toxicity? (tissues surface, gills, gut…) Essential to define the form to which aquatic organisms are exposed in environmentally relevant scenario

… è urgente una nuova figura…

l’econanotossicologo!

Nanotox Day

Pavia, Centro Congressi IRCCS Fondazione Salvatore Maugeri

8 ottobre 2010

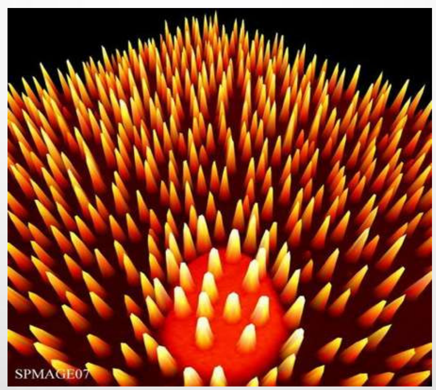
La Società Italiana di Nanotossicologia-SIN

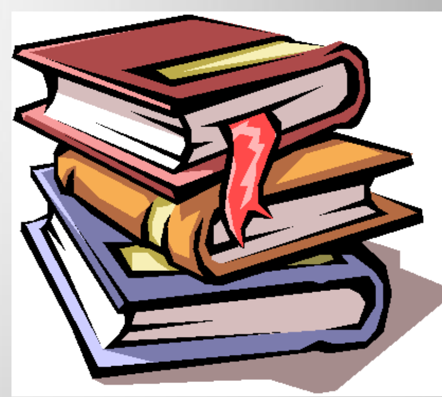
www.sona-it.org/

SIN: mission

Societa' Italiana di Nanotossicologia-SIN, as reference point for the evaluation of the impact of nanotechnologies on environment and health:

Scientific research | **Industrial innovation** | **Legislation**





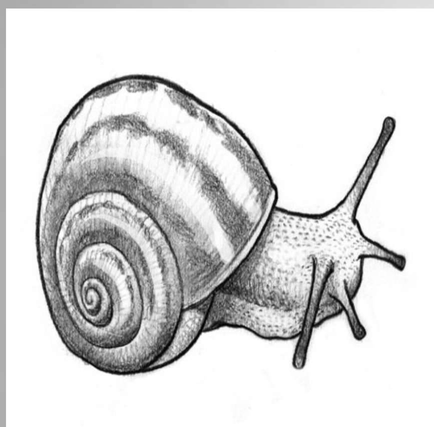
ECSIN-SIN: the vision towards nanomaterials

ECSIN-SIN aim to create conditions that **favour innovation** rather thaa **break on innovation**

Nanomaterial are not neither
"**nano-angels**" nor "**nano-demons**".

Nanotoxicology

econanotoxicology



…a severe gap

Development and production of nanomaterials

SIN

ECSIN

Nanotechnology to serve citizens and satisfy their needs

Safe and sustainable development of nanotechnology

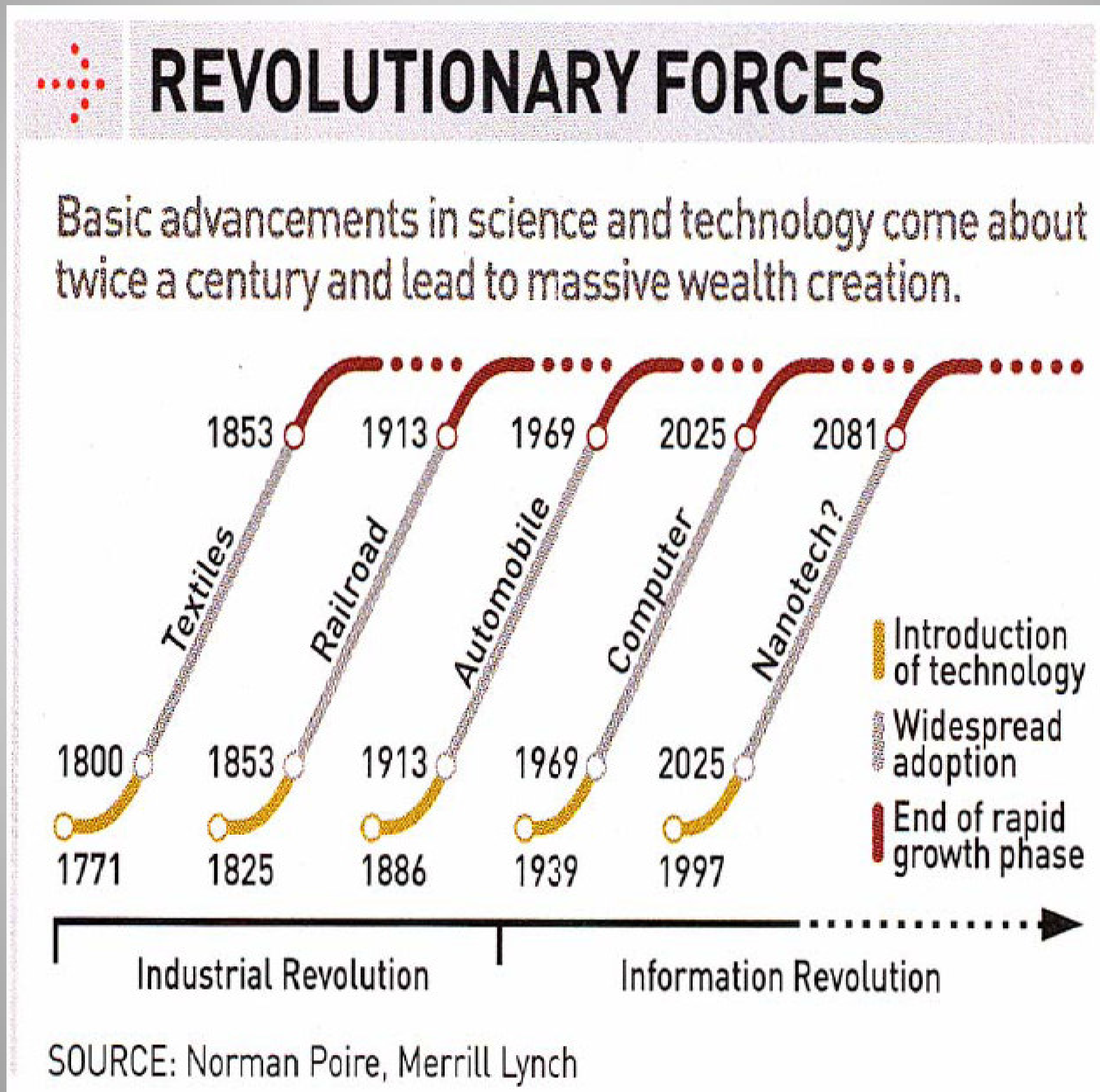
=

Development and production of nanomaterials

+ nanotoxicology +econanotoxicology+ society

Nanotoxicology and econanotoxicology must not considered a "break on innovation", but rather an essential part for a sustainable development of nanotechnology

Nanotoxicology:where are we in the Revolutionary nanotechnology wave?




Thank you for your attention