The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
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IAEA
International Atomic Energy Agency

2268-12

Conference on Nanotechnology for Biological and Biomedical Applications (Nano-Bio-Med)

10 - 14 October 2011

Nanotoxicology - Quo Vadis?

Harald F. KRUG
SFLMST, EMPA, Lerchenfeldstr. 5
CH-9014 St Gallen
SWITZERLAND

EMPA

Materials Science & Technology

NANOTOXICOLOGY – QUO VADIS?

Harald F. Krug
Empa, Department Materials Meet Life,
St. Gallen, Switzerland

Health & Performance

Body Performance
Functionalized Fibers
Biotechnology/Biomaterials
MedTech
Materials - EHS

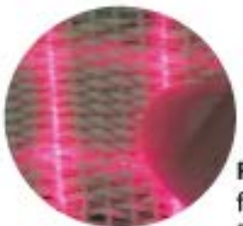
Coating technology
for Nanocomposites
and Fibers

Material safety

Biotechnology
Developing tailor-made
Enzymes for industrial
Applications

Biopolymere
Materials & Coatings

Flame retardant
Materials & Additives



Functionalised clothing
for the Monitoring of
Body Functions

Thomas Hartung, Johns Hopkins University

CAAT Johns Hopkins University Center for Alternatives to Animal Testing

CAAT
Johns Hopkins University
Center for Alternatives to Animal Testing

Promoting for 29 years alternatives to animal testing where they are not fit for purpose

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Thomas Hartung, Johns Hopkins University

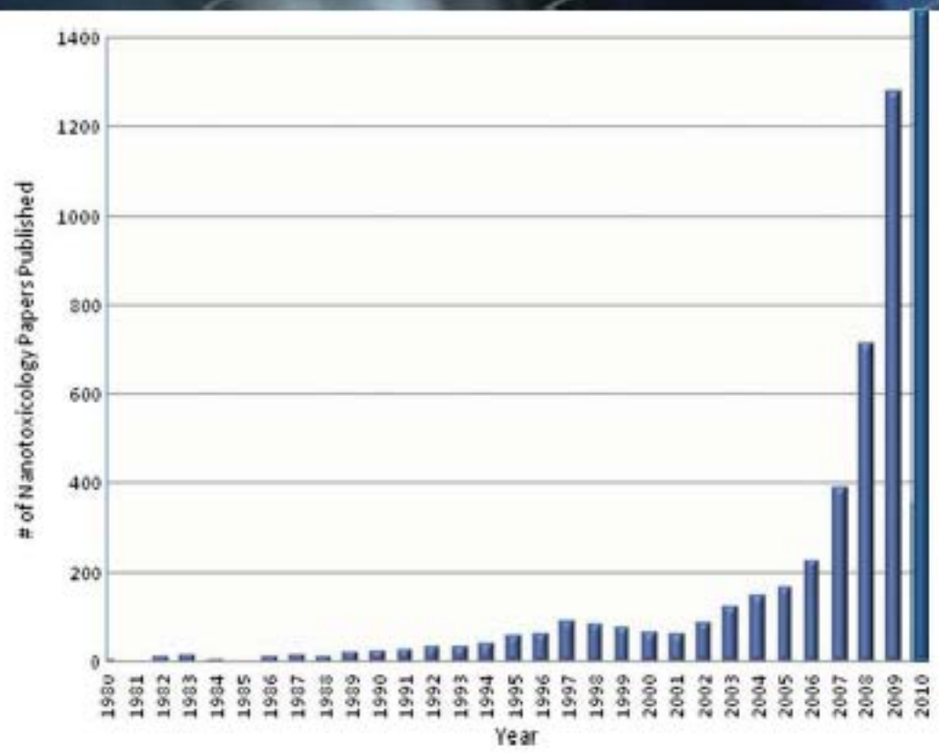
CAAT Johns Hopkins University Center for Alternatives to Animal Testing

R22 harmful if swallowed
(LD_{50} = 150-200mg/kg in rats)
R 36 irritant to eyes
R 37 respiratory irritant
R 38 irritant to skin
Not carcinogenic, but co-carcinogen (promotor)
Unclear mutagenicity
Embryonic malformations in cat, dog, rat, mice, rabbit, monkey

Unlikely to be brought to the market today

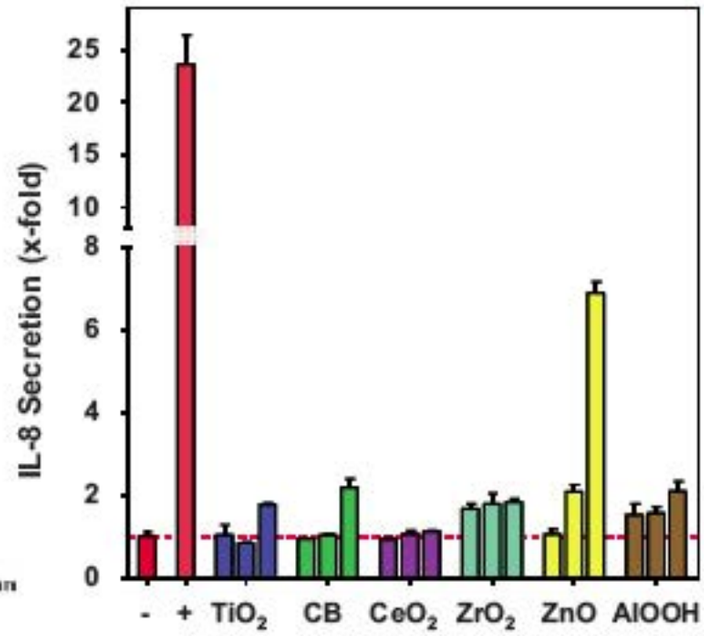
JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

C.L. Haynes (2010): The emerging field of nanotoxicology
Anal. Bioanal. Chem 398, 587-588.



Same Size – Different Materials

nanoCare



Ø:
TiO_2 10-20 nm
Carbon Black (CB) 15 nm
CeO_2 20 nm
ZrO_2 10-25 nm
ZnO 40 nm
AlOOH 40 nm

0.5, 5 and 25 $\mu g/cm^2$

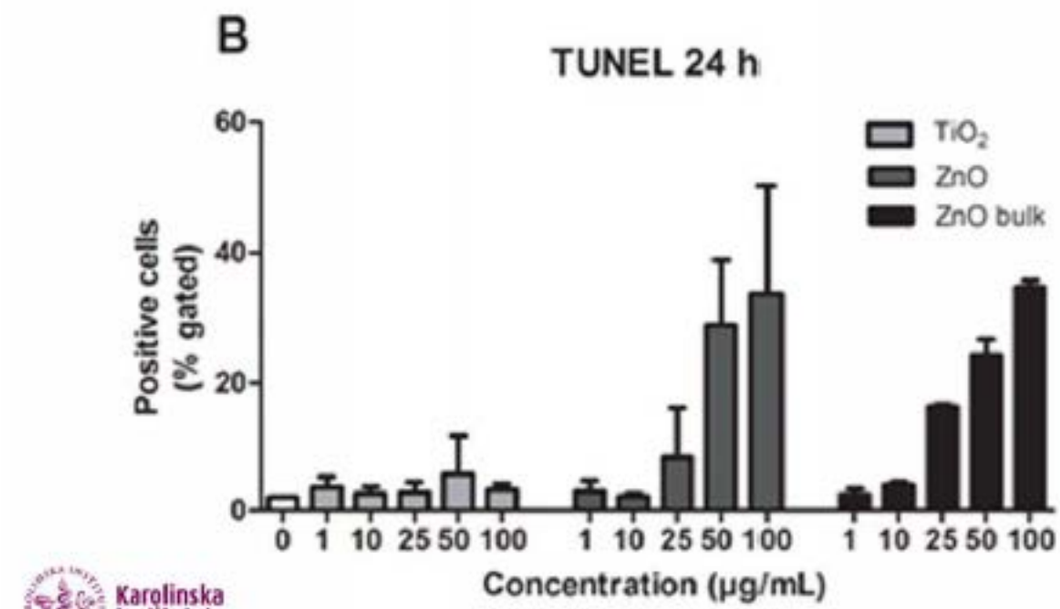
Bundesministerium für Bildung und Forschung

Zinc-Story – essential or toxic on the Nano-Level?

NANOMMUNE
Immune cells respond to zinc nanoparticles and/or zinc ions

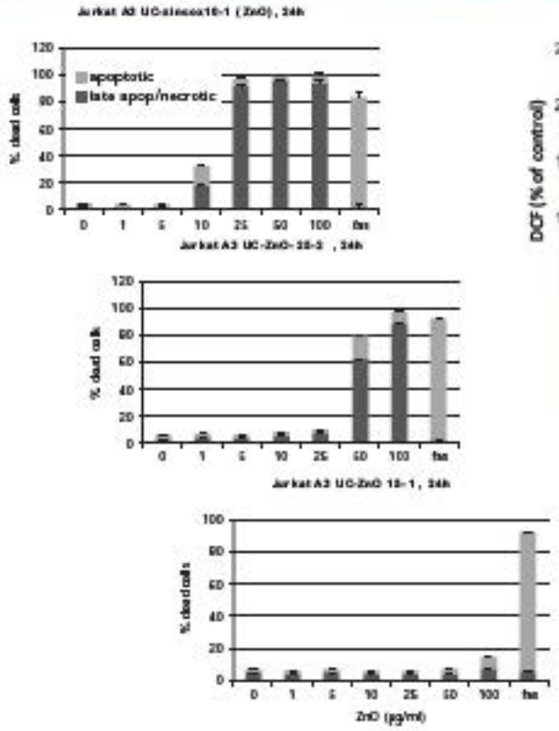
EMPA University of Pittsburgh Karolinska Institutet

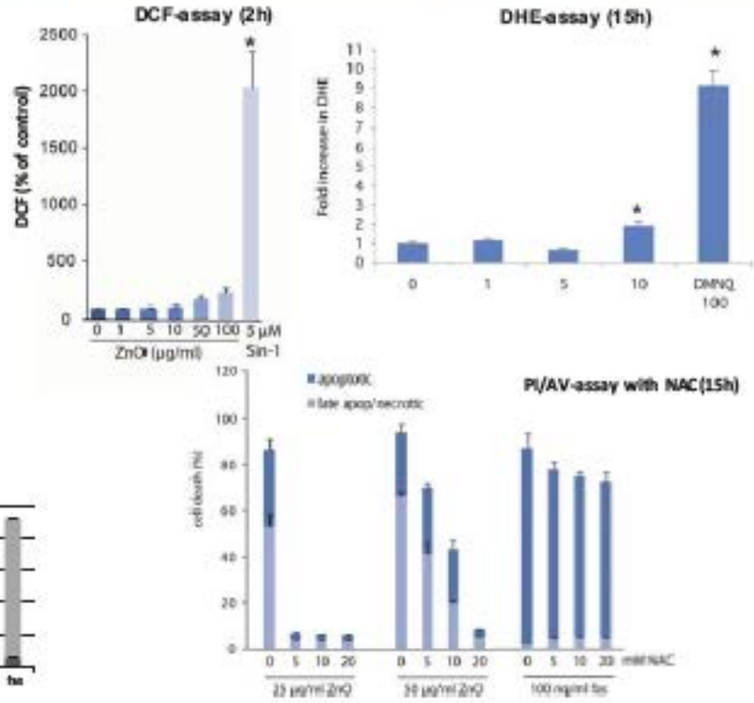
No difference between nano or bulk ZnO particles



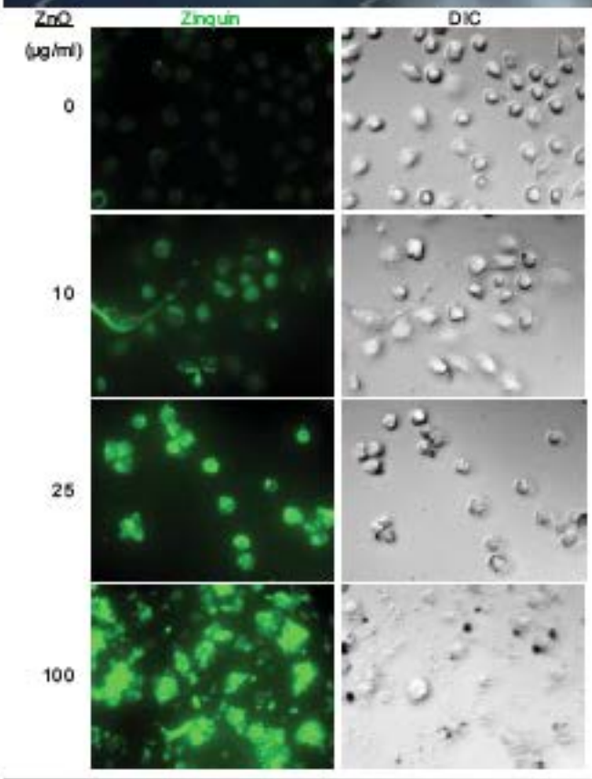
Karolinska Institutet

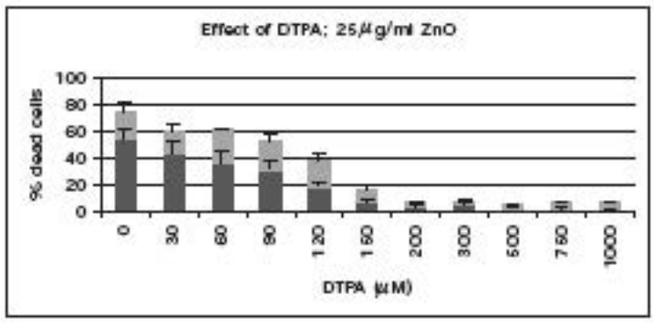
ZnO Toxicity is depending on the ZnO-material

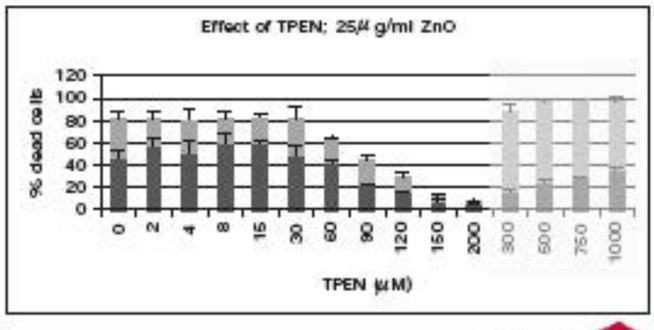




Extracellular and intracellular ZnO/Zn^{2+}

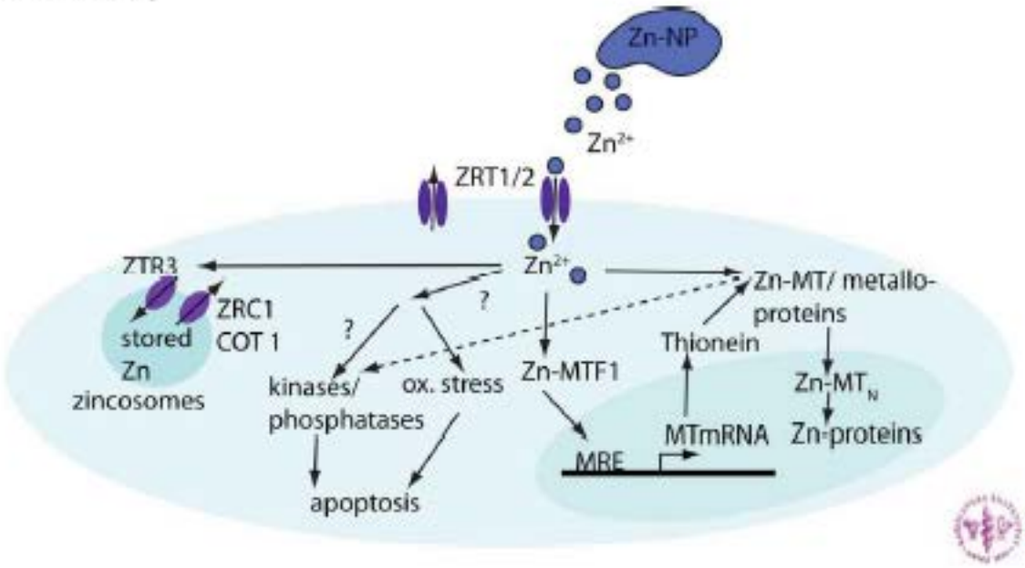




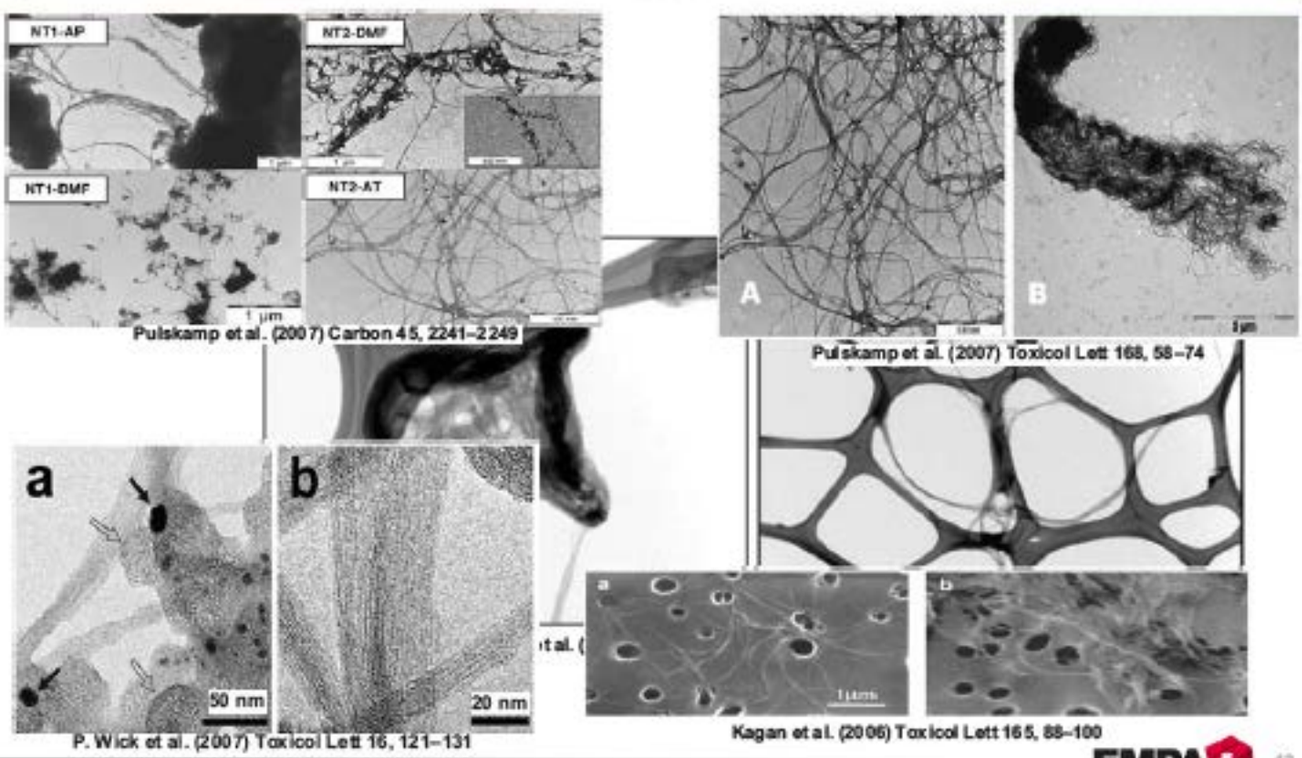


Hypothetical Model of ZnO-NP Toxicity in Jurkat Cells

ZnO-NPs dissolve mainly extracellularly and Zn^{2+} rapidly enters the cells. To cope with the excess free Zn^{2+}, Zn^{2+} is stored in zincosomes and the expression of MT is upregulated. If the concentration of free Zn^{2+} is still too high, apoptotic cell death is initiated by a yet unknown mechanism (not via classical apoptotic pathways but involving the production of ROS and the induction of endonucleases which fragment the DNA).



CNT-Story – Medical Tool or highly Toxic



Pulskamp et al. (2007) Carbon 45, 2241–2249

Pulskamp et al. (2007) Toxicol Lett 168, 58–74

P. Wick et al. (2007) Toxicol Lett 16, 121–131

Kagan et al. (2006) Toxicol Lett 165, 88–100

NANO LETTERS
2006
Vol. 6, No. 6
1261−1268

Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays

J. M. Worle-Knirsch, K. Pulskamp, and H. F. Krug*

Forschungszentrum Karlsruhe, Institute of Toxicology and Genetics, Department of Molecular and Environmental Toxicology, P.O. Box 3640, D-76021 Karlsruhe, Germany

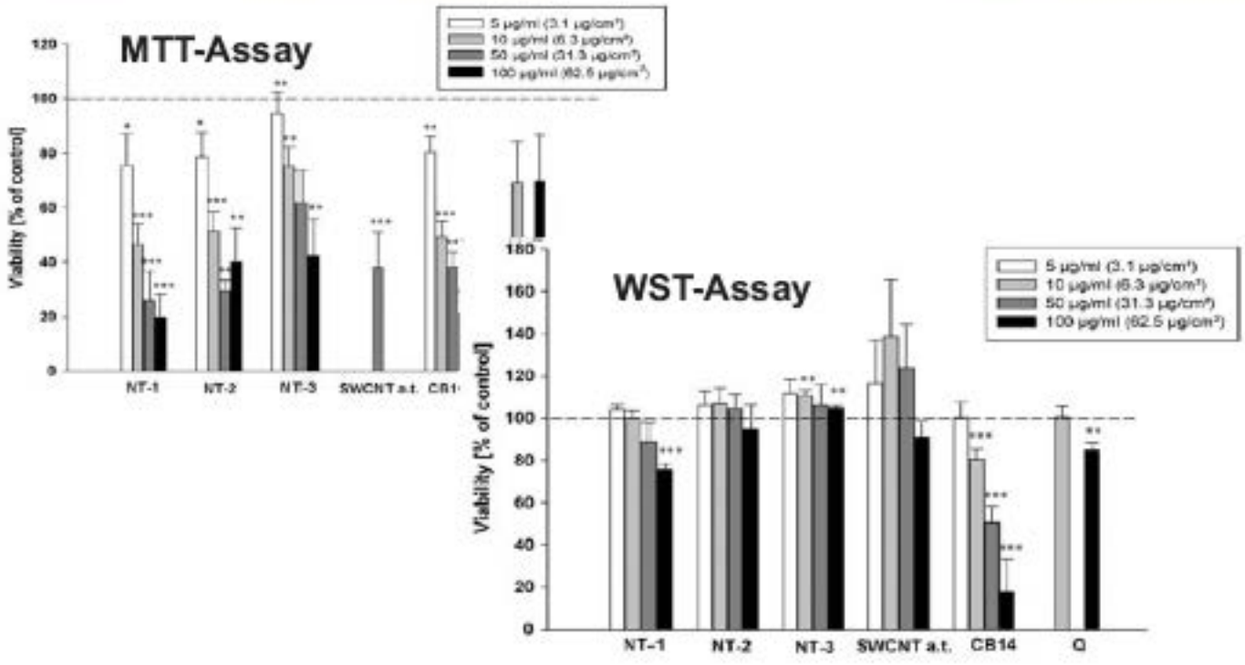
Received January 26, 2006; Revised Manuscript Received March 29, 2006

ABSTRACT

New materials of emerging technological importance are single-walled carbon nanotubes (SWCNTs). Because SWCNTs will be used in commercial products in huge amounts, their effects on human health and the environment have been addressed in several studies. Inhalation studies in vivo and submerse applications in vitro have been described with diverging results. Why some indicate a strong cytotoxicity and some do not is what we report on here. Data from A549 cells incubated with carbon nanotubes take a strong cytotoxic effect within the MTT assay after 24 h that reaches roughly 50%, whereas the same treatment with SWCNTs, but detection with WST-1, reveals no cytotoxicity. LDH, FACS-

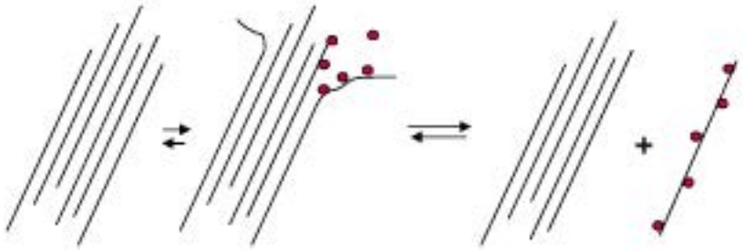
Our findings strongly suggest verifying cytotoxicity data with at least two or more independent test systems for this new class of materials (nanomaterials). Moreover, we intensely recommend standardizing nanotoxicological assays with regard to the material used: there is a clear need for reference materials.

The Mystery of Viability Assays



Pulskamp, K., Diabaté, S., Krug, H.F. (2007) Toxicol. Lett. 168: 58-74

CNT Suspension / Dispersion



adapted from Strano MS et al., 2003 J Nanosci Nanotechnol

Organic Solvents / Surfactants

sodium dodecylbenzene sulfonate NaDDBS
Islam et al., 2003

SDS, TritonX, chloroform, DMF, Methylpyrrolidone NMP, etc.

Biomolecules

designed peptide Nano-1
Dieckmann GR et al., 2003

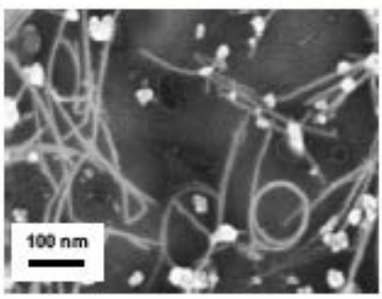
ssDNA of anysequence
Zheng M et al., 2003

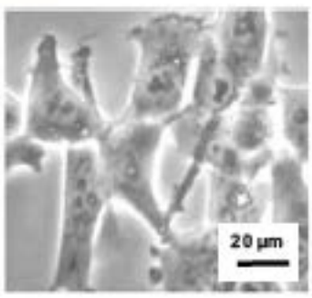
Biocompatible Surfactants

polysorbate 80
Wick P et al., 2007

pluronicF108

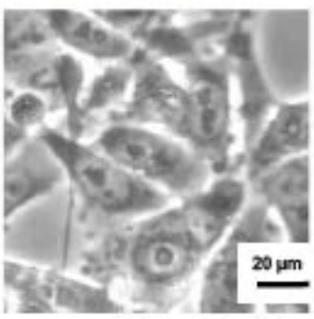
Morphological Changes of Cells



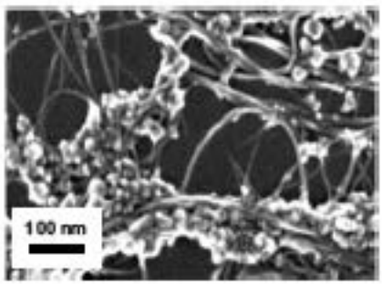


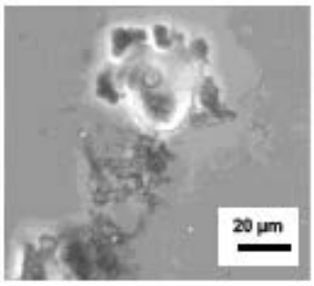
Ni 5.5 wt%
Y 0.7 wt%

SWCNT 50%



control cell culture





Ni 2.4 w%
Y 0.4 w%

SWCNT 48%

incubation time 3 days; 15 µg / ml CNT

Wick et al. (2007) Toxicol. Lett. 168, 121-131

Small Tubes – Big Bundles

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]*

[1]MRC/University of Edinburgh, Centre for Inflammation Research, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK
[2]School of Materials, University of Manchester, Grosvenor Street, Manchester M1 7HS, UK
[3]Woodrow Wilson International Center for Scholars, 1300 Pennsylvania...
[4]Institute of Occupational Medicine, Research Avenue North, Riccarton...
[5]School of Life Sciences, Napier University, Colinton Road, Edinburgh EH...
*e-mail: ken.donaldson@ed.ac.uk

BBC NEWS

'Asbestos warning' on nanotubes

By Jonathan Fildes
Science and technology reporter, BBC News

Carbon nanotubes, the poster child of the burgeoning nanotechnology industry, could trigger diseases similar to those caused by asbestos, a study suggests.

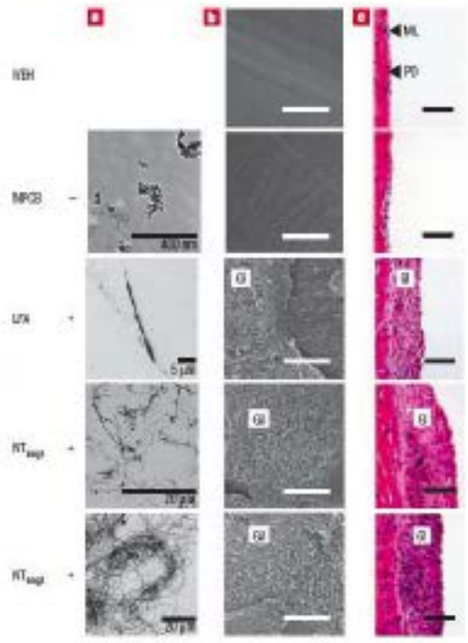
Specific lengths of the tiny fibres were found to cause "asbestos-like" inflammation in the lining of the chest cavity in mice.

Use of asbestos triggered a "pandemic of lung disease" in the 20th Century.

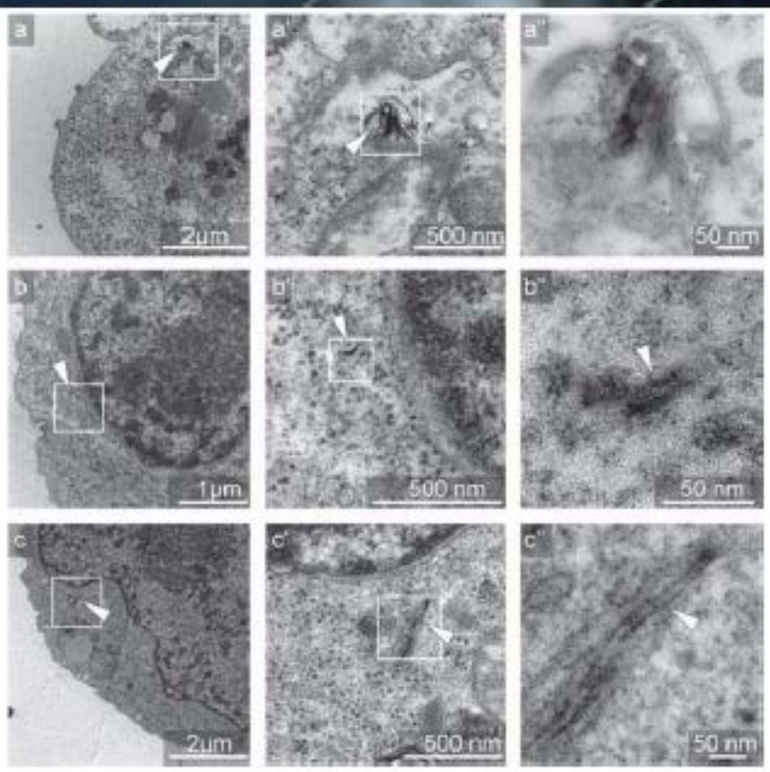
Story from BBC NEWS:
http://news.bbc.co.uk/go/pr/fr/-/2/hi/science/nature/7488705.stm

Published: 2008/05/20 17:00:23 GMT

© BBC MMVIII



Uptake of CNT by Jurkat Cells

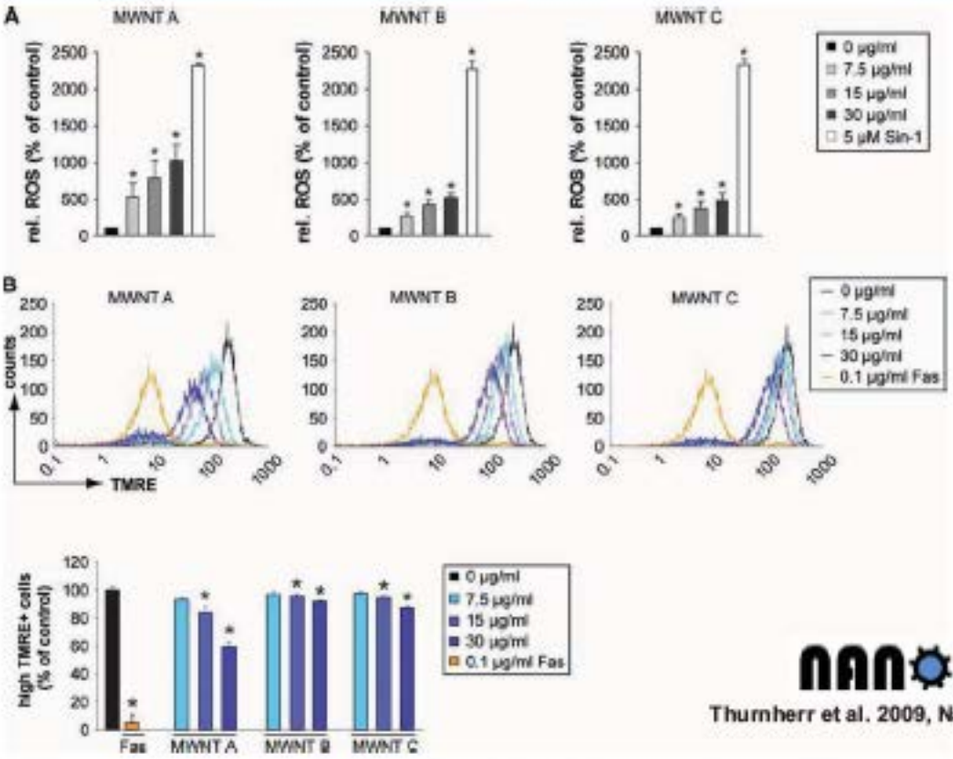


TEM micrographs of Jurkat cells after three days of incubation with 30 mg/ml
MWCNT A (a-a"), Bay-Tubes
MWCNT B (b-b''), Nanocyl SA
MWCNT C (c-c''), Cheap Tubes Inc.
Tubes (arrowheads) are present in the cytoplasm of exposed cells.

NANOMMUNE

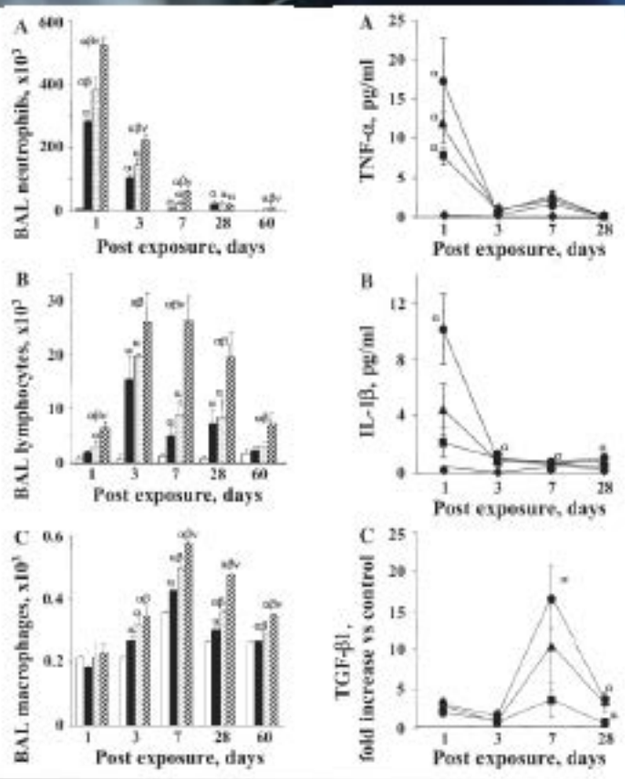
Thurnherr et al. 2009, Nanotoxicology, 3, 319-338

ROS and Mitochondria



Thurnherr et al. 2009, Nanotoxicology, 3, 319-338

Inflammatory Effect of SWCNT



Shvedova et al. 2005
Am. J. Physiol. Lung Cell Mol. Physiol. 289, L698-L708

Doses applied:
PBS
10µg
20µg
40µg

Contaminants in CNTs

DONALDSON ET AL. TOXICOLOGICAL SCIENCES 92(1), 5–22 (2006)

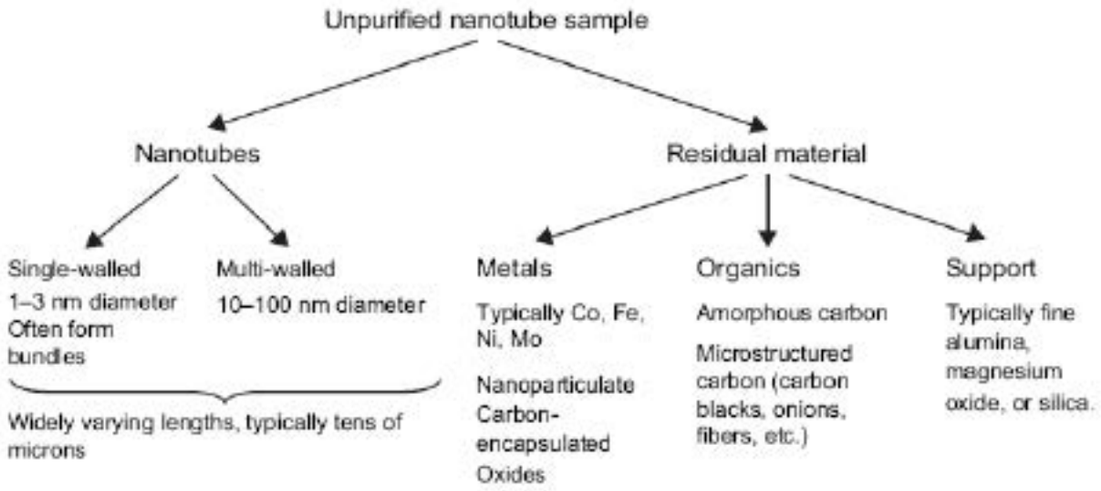
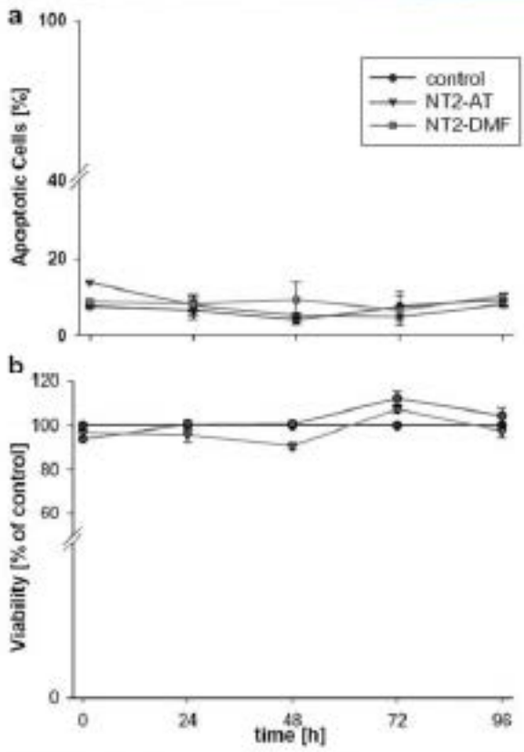


FIG. 4. Possible components in a sample of unpurified CNT.

Contaminants and Acute Toxicity

Pulskamp et al.
Carbon 45, 2241–224 (2007)

No acute cytotoxicity in vitro in human lung cells



Fullerene Paradox: Radical Scavenger

Neurobiology of Disease 5, 129–135 (1998)
Article No. 0013

Buckminsterfullerenol Free Radical Scavengers Reduce Excitotoxic and Apoptotic Death of Cultured Cortical Neurons

Laura L. Dugan,* Joseph K. Gabrielsen,* Shan P. Yu,*
Tien-Sung Lin,† and Dennis W. Choi*,‡,1

*Center for the Study of Nervous System Injury and D
Washington University School of Medicine, St. Louis,
Study of Nervous System Injury and Department of C
St. Louis, Missouri 63130

Journal of Neurochemistry
Lippincott Williams & Wilkins, Inc., Philadelphia

Carboxyfullerene Prevents Iron-Induced Oxidative Stress in Rat Brain

*†Anya M. Y. Lin, *B. Y. Chyi, ‡S. D. Wang, §H.-H. Yu, §P. P. Kanakamma, §T.-Y. Luh, *C. K. Chou, and *L. T. Ho

*Department of Medical Research and Education, Veterans General Hospital–Taipei, †Department of Physiology, National Yang-Ming University, ‡Department of Anatomy, National Defense Medical Center, and §Department of Chemistry, National Taiwan University, Taipei, Taiwan

Fullerene Paradox: Oxidative Stress

Research | Article VOLUME 112 | NUMBER 10 | July 2004 • Environmental Health Perspectives

Manufactured Nanomaterials (Fullerenes, C_{60}) Induce Oxidative Stress in the Brain of Juvenile Largemouth Bass

Eva Oberdörster

Duke University Marine Laboratory, Beaufort, North Carolina, USA; Department of Biology, Southern Methodist University, Dallas, Texas, USA

Materials and Methods

Fullerenes. Uncoated 99.5% pure fullerenes (SES, Houston, TX) were water solubilized using standard methods (Deguchi et al. 2001) by the Center for Biological and Environmental Nanotechnology, Rice University (Houston, TX) and were a generous gift for this study. Briefly, fullerenes (100 mg/L) were dissolved in tetrahydrofuran (THF), sparged with nitrogen, stirred overnight in the dark, and filtered through a 0.22-μm nylon Osmonics filter

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Marine Environmental Research 62 (2006) S5–S9
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvres

Short communication

Toxicity of an engineered nanoparticle (fullerene, C_{60}) in two aquatic species, *Daphnia* and fathead minnow ☆

Shiqian Zhu [a], Eva Oberdörster [b,c], Mary L. Haasch [a,*]

[a] Environmental Toxicology Research Program, National Center for Natural Products Research, Department of Pharmacology, School of Pharmacy, The University of Mississippi, 347 Faser, University, MS 38677-1848, USA
[b] Department of Biology, Southern Methodist University, TX, USA
[c] Duke University Marine Laboratory, Beaufort, NC, USA

Small Balls (C_{60}) – Big Pitfalls

in THF

C_{60} Fluka

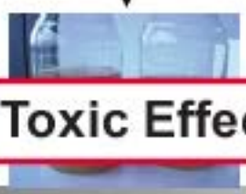
in water

C_{60} in THF susp.

start

transfer THF/water

after weeks



Suspension

Toxic Effect

Spohn P et al. (2009) Environ. Pollut. 157:1134-1139

No Effect

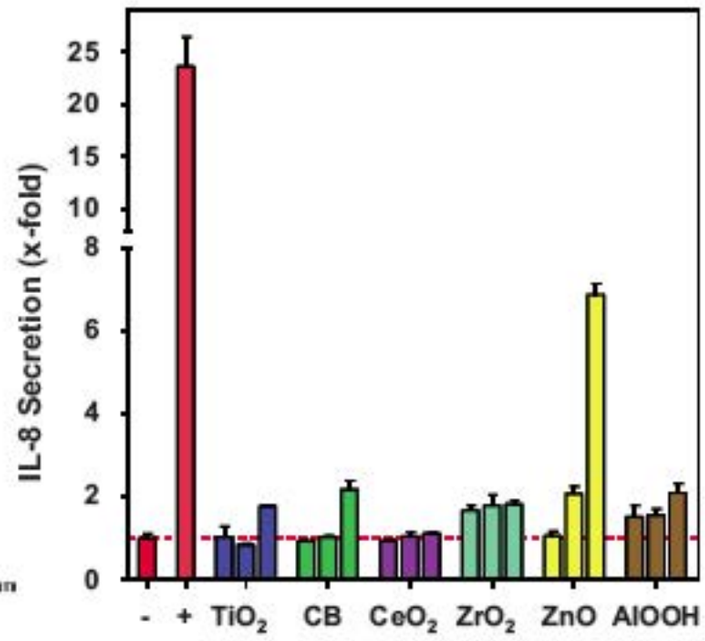
Conclusion for Toxicity of Nanomaterials (1)

- Zinc as an essential element exhibits toxicity in high doses
- ZnO-NP induce toxicity exclusively via the dissolution of Zn^{2+} ions
- Carbon Nanotubes exhibit toxicity in dependence on their contaminant content and length
- If purified (no amorphous carbon, no or less catalytic metals) biological activity is strongly reduced
- Long term studies (several months) within cells in culture reveal no biological alterations or responses but the uptake and accumulation within vesicular structures of the cells
- MWCNTs start to have a biological effect in vitro when highly agglomerated
- In vivo the situation is clearly changed and frustrated macrophages induce severe reactions when exposed to long and rigid CNTs
- Fullerenes seem not to induce any oxidative stress response

More Pitfalls and Flaws

High Dosage/ Concentrations

Same Size – Different Materials

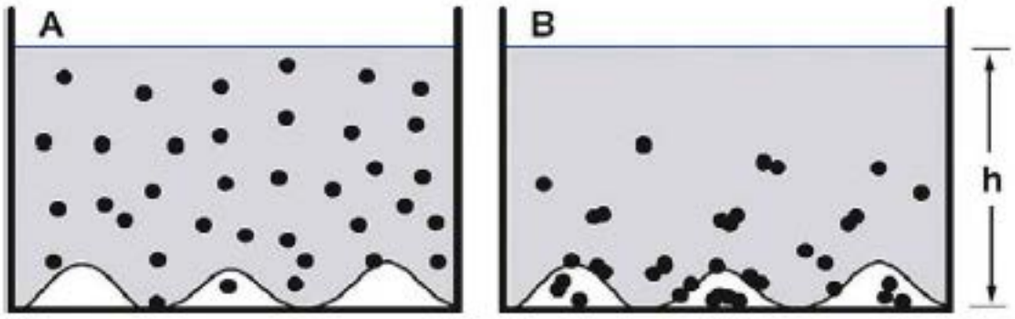


Ø:
TiO_2 10-20 nm
Carbon Black (CB) 15 nm
CeO_2 20 nm
ZrO_2 10-25 nm
ZnO 40 nm
AlOOH 40 nm

0.5, 5 and 25 $\mu g/cm^2$

Bundesministerium für Bildung und Forschung

Excessive Delivery of Nanostructured Matter to Submersed Cells .....



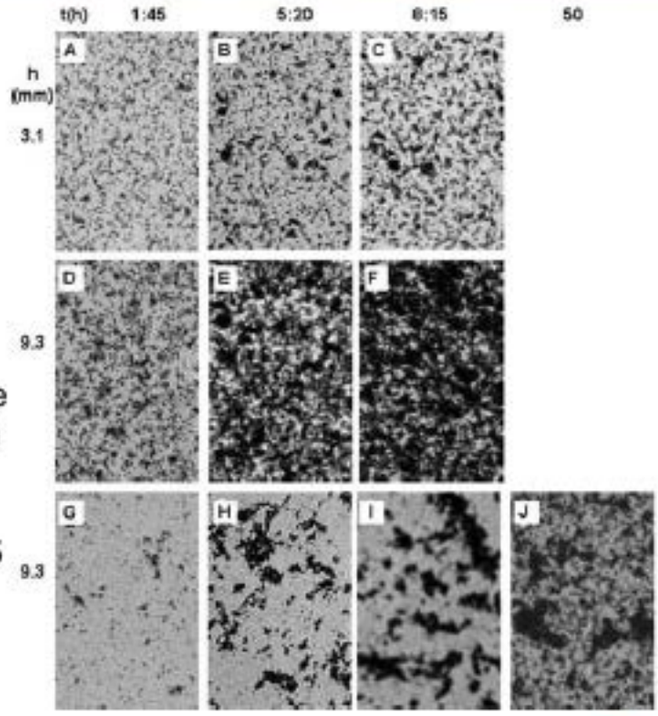
Schematic illustrations of the conceivable interaction of insoluble particles with submersed cells grown at the bottom of a well, filled with an appropriate medium of height h. (A) Previously employed picture, (B) more appropriate concept discussed in this study. The number of particles in (A) and (B) is the same.

Wittmaack, K. (2011) ACS Nano, 5: 3766-3778

Excessive Delivery of Nanostructured Matter to Submersed Cells .....

TiO_2(80) particles (27 µg/mL)

At the quoted heights (h) of the media, the mass concentration converts to maximum achievable areal densities of (A-C) 8.4 µg/cm² and (D-J) 25.2 µg/cm² (corresponding thicknesses 21.5 and 64.5 nm)



Wittmaack, K. (2011) ACS Nano, 5: 3766-3778

J Toxicol Pathol 2009; 22: 71–78

Original

Lung Carcinogenic Bioassay of CuO and TiO_2 Nanoparticles with Intratracheal Instillation Using F344 Male Rats

Masanao Yokohira[1], Nozomi Hashimoto[1], Keiko Yamakawa[1], Satoshi Suzuki[1], Kousuke Saoo[1], Toshiya Kuno[1], and Katsumi Imaida[1]

0.5 mg/rat lung: no evidence for carcinogenicity

Inhalation Toxicology, 2009; 21(S1): 144–157

RESEARCH ARTICLE

Carcinogenicity of inhaled nanoparticles

Markus Roller

Advisory Office for Risk Assessment, Dortmund, Germany

A minimum of 5 X 3 mg/rat lung, maximum 20 x 6 mg/rat lung by instillation ⇒ carcinogenicity!

Calculated Overload

Rat lung weight: 0.9 g

Macrophage count: 1.200.000

0,5mg/lung ⇒ 416 pg / cell: no effects (Yokohira et al., 2009)

15mg/lung ⇒ 12.500 pg / cell
120mg/lung ⇒ 100.000 pg / cell } **tumour induction (Roller, 2009)**

Irreversible Pulmonary Changes Induced in Rat Lung by Dust Overload

Environmental Health Perspectives
Vol. 97, pp. 189-191, 1992

by Bernd Bellmann,[1] Hartwig Muhle,[1] Otto Creutzenberg,[1] and Robert Mermelstein[2]

3mg/lung ⇒ 2.500 pg / cell reflects overload conditions

Experiments and their Consequences

PNAS

Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β

Amir S. Yazdi[a], Greta Guarda[a], Nicolas Riteau[b], Stefan K. Drexler[a], Aubry Tardivel[a], Isabelle Couillin[b], and Jürg Tschopp[a,1]

[a]Department of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland; and [b]Laboratory of Molecular Immunology and Embryology, University of Orleans and Centre National de la Recherche Scientifique, F-45071 Orleans, France

Our data suggest that nano-TiO_2 should be used with greater caution than is currently used. The manufacture of TiO_2 nanoparticles is a huge industry; about 2 million tons per year are produced worldwide. In addition to paint, cosmetics, sunscreen, and vitamins, nanoparticles can be found in toothpaste, food colorants, nutritional supplements, and hundreds of other personal care products. When this widespread usage is considered in light of the data presented above, the potential risk of inflammation-driven cancer is a particular concern, especially for people occupationally exposed to high concentrations of TiO_2 nanoparticles. Better precautions must be taken to limit the ingestion of these particles, both in manufacturing and in everyday contact with nanoparticle-containing substances.

Similar words of caution were expressed in the last century following the first evidence for association between lung inflammation and asbestos inhalation. Yet it took almost 100 y, and countless fatalities, until asbestos was banned from use. Given that asbestos and TiO_2 exert similar proinflammatory activities via IL-1R signaling through the activation of either IL-1α and IL-1β, we hope that future decisions will be made to prevent possible morbidity and perhaps mortality.

Proc. Natl. Acad. Sci U. S. A 107, 19449-19454 (2010)

Concentrations and Doses

In Vitro:
Titaniumdioxide and siliciumdioxide nanoparticle induce the Nlrp3 inflammasom in mouse- and human macrophages and human keratinocytes.
THP1 cells treated with MSU (300 µg/mL), TiO_2 20 nm (200 µg/mL), TiO_2 80 nm (200 µg/mL), SiO_2 15 nm (200 µg/mL), and ZnO 15 nm (200 µg/mL) for 6 h.

In Vivo:
Intranasal application and broncho-alveolar lavage (BAL). TiO_2 (7.5–30 mg/kg) in sodiumchloride solution or only medium were administered via nasal instillation (40 µl with anesthesia).
Peritonitis was induced in 6- to 10-weeks old mice after injection of 1–1.5 mg of the TiO_2 particles.

For comparison: Ken Donaldson and coworkers have injected 50µg CNT or asbestos per mouse, for the measurement of mesothelioma-like alterations under „realistic" conditions (Poland et al., 2008)

Paracelsus neglected

Wrong Conclusions

news@nature.com
The best in science journalism

Published online: 16 June 2006; | doi:10.1038/news060612-14

Nanoparticles in sun creams can stress brain cells

Tiny grains send cells into potentially dangerous overdrive.

Philip Ball

Tiny particles like those found in some sunscreens are linked to stress in mouse cells

Research

Titanium Dioxide (P25) Produces Reactive Oxygen Species in Immortalized Brain Microglia (BV2): Implications for Nanoparticle Neurotoxicity†

THOMAS C. LONG,‡ NAVID SALEH,§ ROBERT D. TILTON,|| GREGORY V. LOWRY,§ AND BELLINA VERONESI*,‡

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599-7431, Department of Civil and Environmental Engineering, Department of Chemical

taz 11.7.2006 Sonnencreme könnte Hirn aufweichen

Seite 1 von 2

die tageszeitung

Sun creams may disintegrate you brain

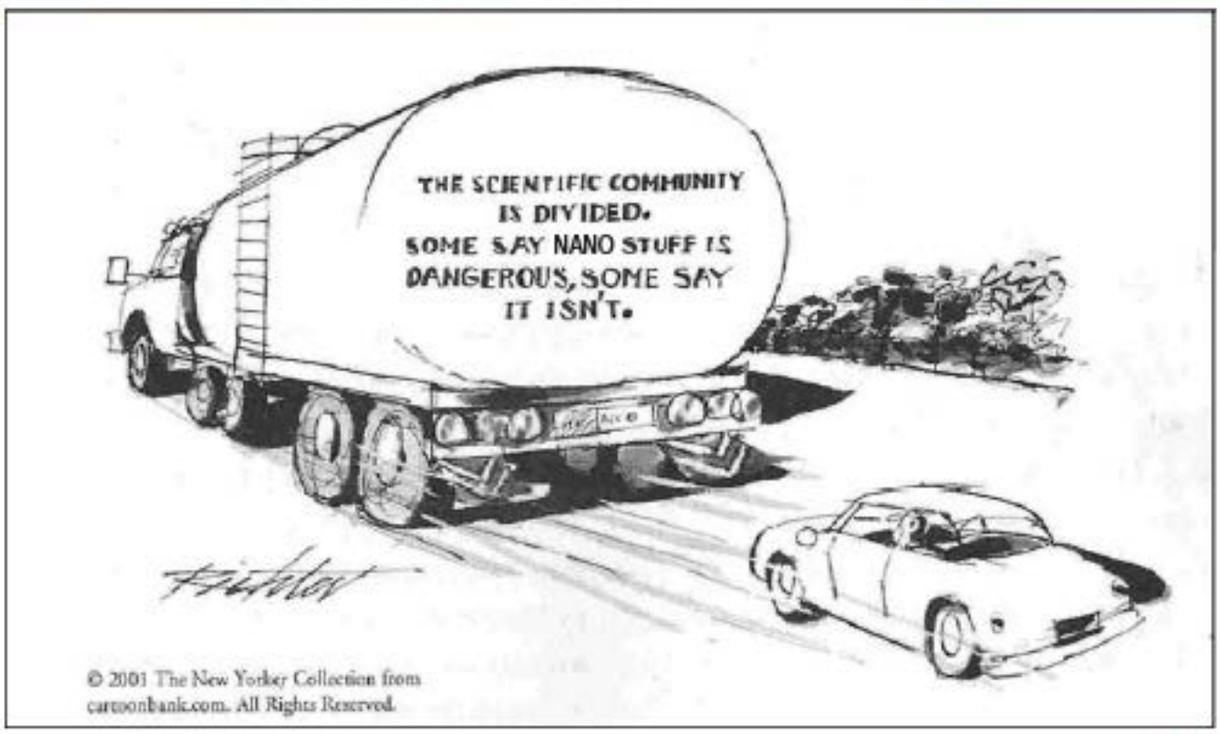
Von wegen bedenkenlos bräunen: Titandioxid, wie es in Hightech-Sonnenschutzmitteln verwendet wird, steht im Verdacht, Nervenzellen zu schädigen. Dazu müssen die Nanopartikel allerdings erst einmal einen Weg durch die Haut finden

VON WOLFGANG LÖHR

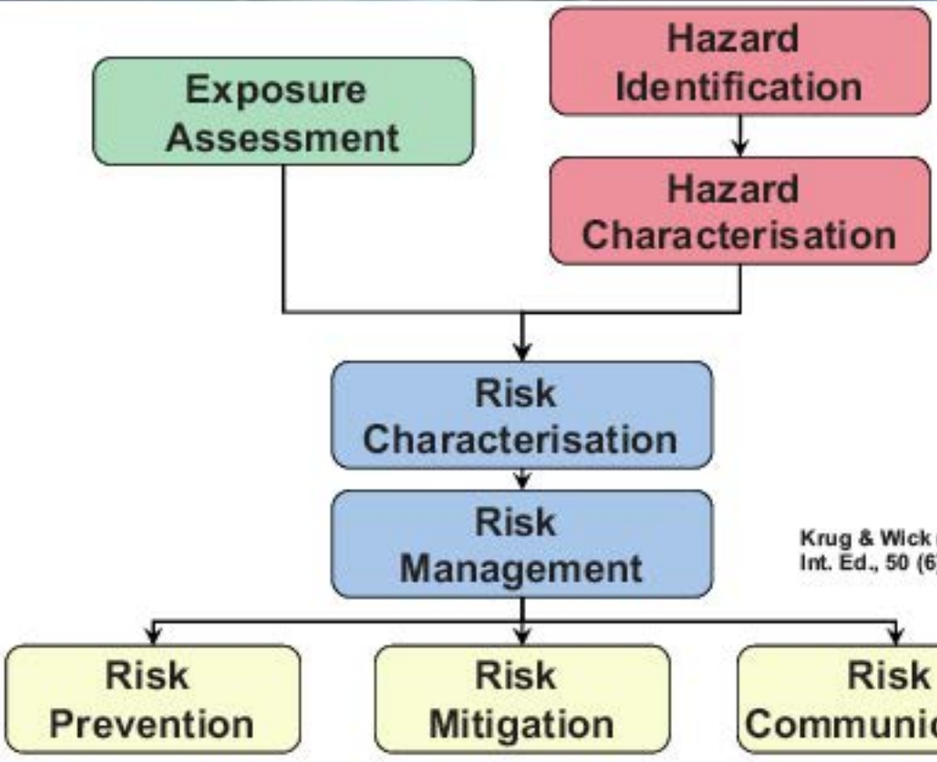
Mirror.co.uk
NEWS

Suncream could cause Alzheimer's

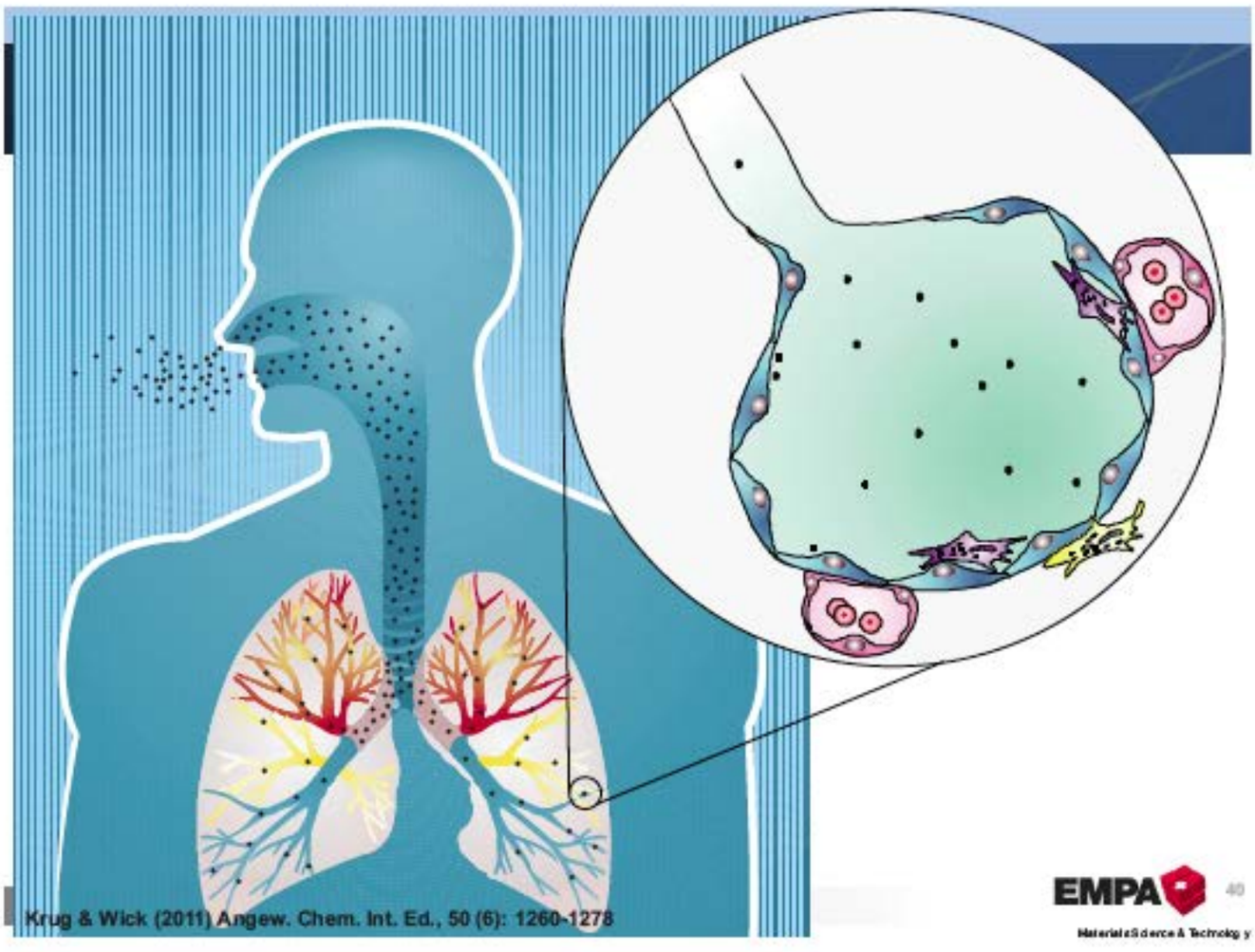
Nanomaterials – the great Uncertainty?



Risk Assessment is Important



Krug & Wick (2011) Angew. Chem. Int. Ed., 50 (6): 1260-1278



Krug & Wick (2011) Angew. Chem. Int. Ed., 50 (6): 1260-1278

Three Nanotox-Principles

- **The Transport-Principle**

  Limbach LK, Wick P, *et al.* (2007). Environ. Sci. Technol. 41:4158-4163

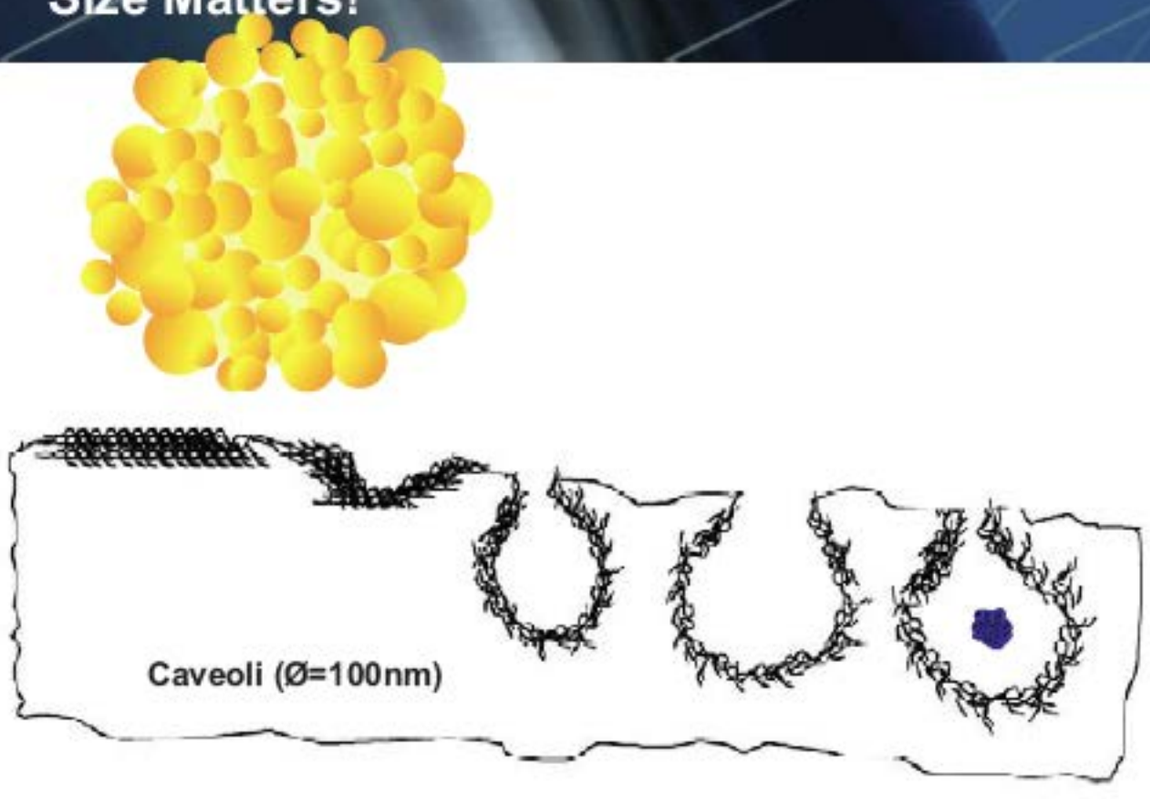
- **The Surface-Principle**

  Nel et al. (2006) Science 311: 622-627
  Oberdörster et al., (2000) HEI-Report 96

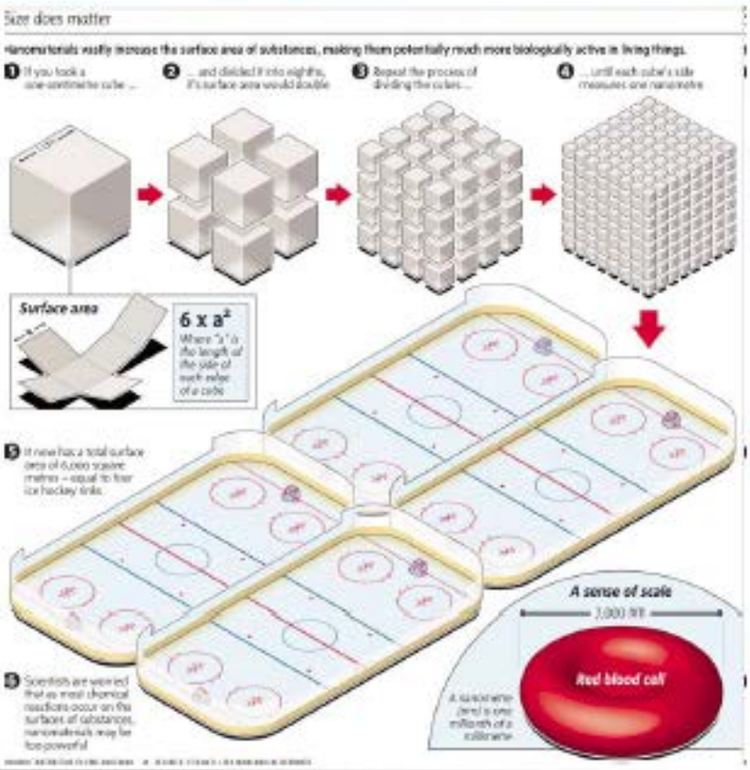
- **The Material-Principle**

Krug & Wick (2011) Angew. Chem. Int. Ed., 50 (6): 1260-1278

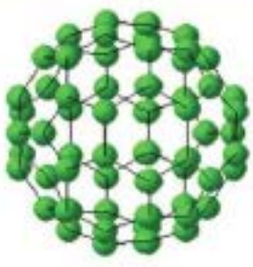
Size Matters!

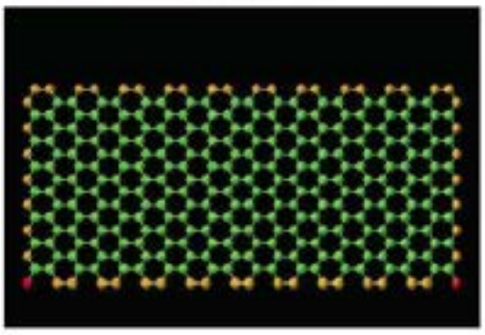


Small Size but very large specific Surface

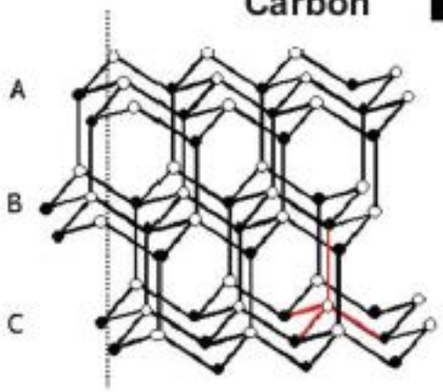


One Material – different Modifications and more…

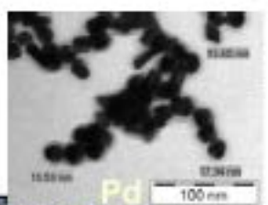


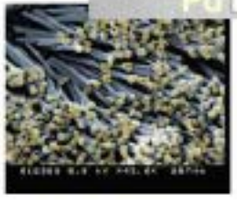


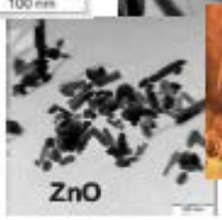
Carbon



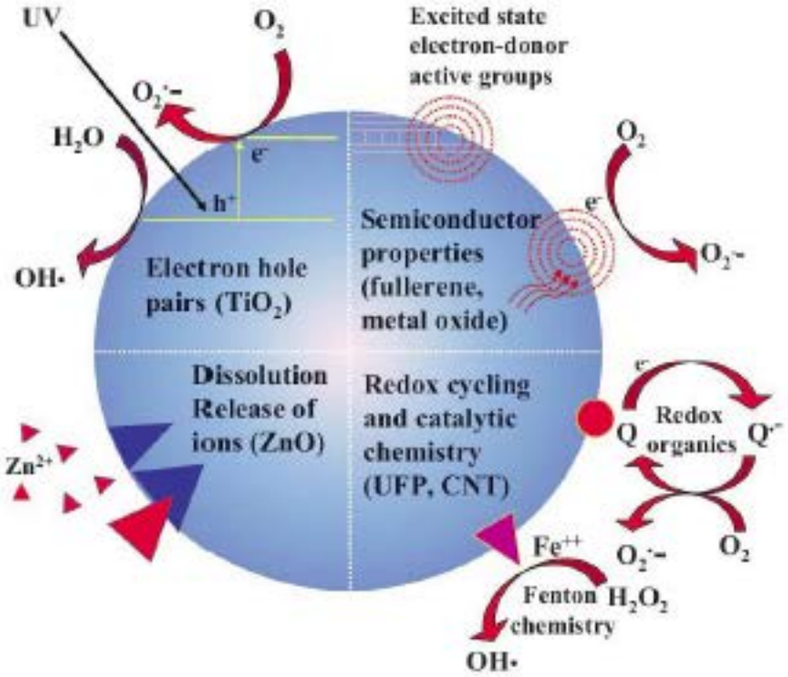
and many more .....







(Material)Surface Properties!

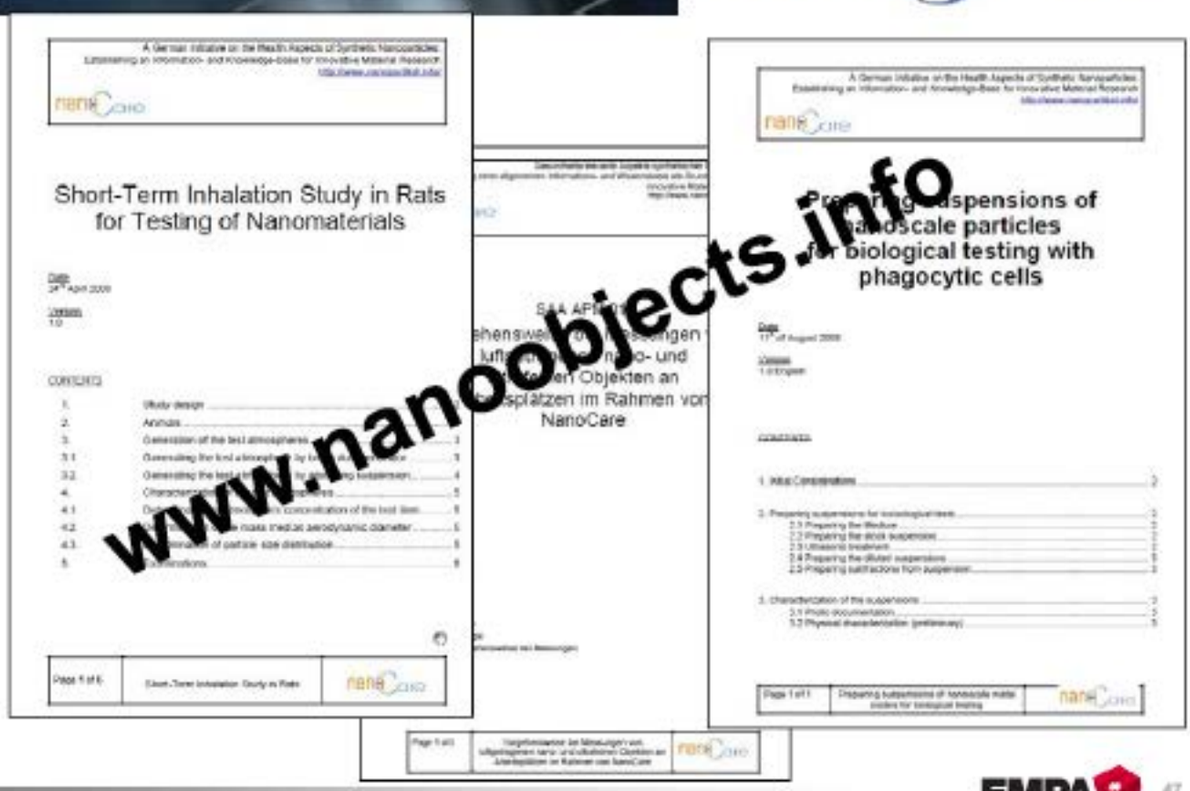


Xia et al. (2009) Annu. Rev. Public Health 30: 137-150

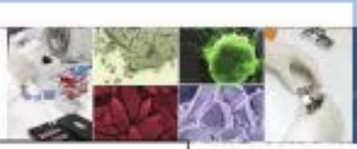
We need .....

new models and harmonizing activities

SOPs



NANOMMUNE



NANOMMUNE

Quality Handbook
10/12/2011

SEVENTH FRAMEWORK PROGRAMME
THEME 4 - NMP – NANOSCIENCES, NANOTECHNOLOGIES, MATERIALS, AND NEW PRODUCTION TECHNOLOGIES (214281)

NANOMMUNE

COMPREHENSIVE ASSESSMENT OF HAZARDOUS EFFECTS OF ENGINEERED NANOMATERIALS ON THE IMMUNE SYSTEM

QUALITY HANDBOOK

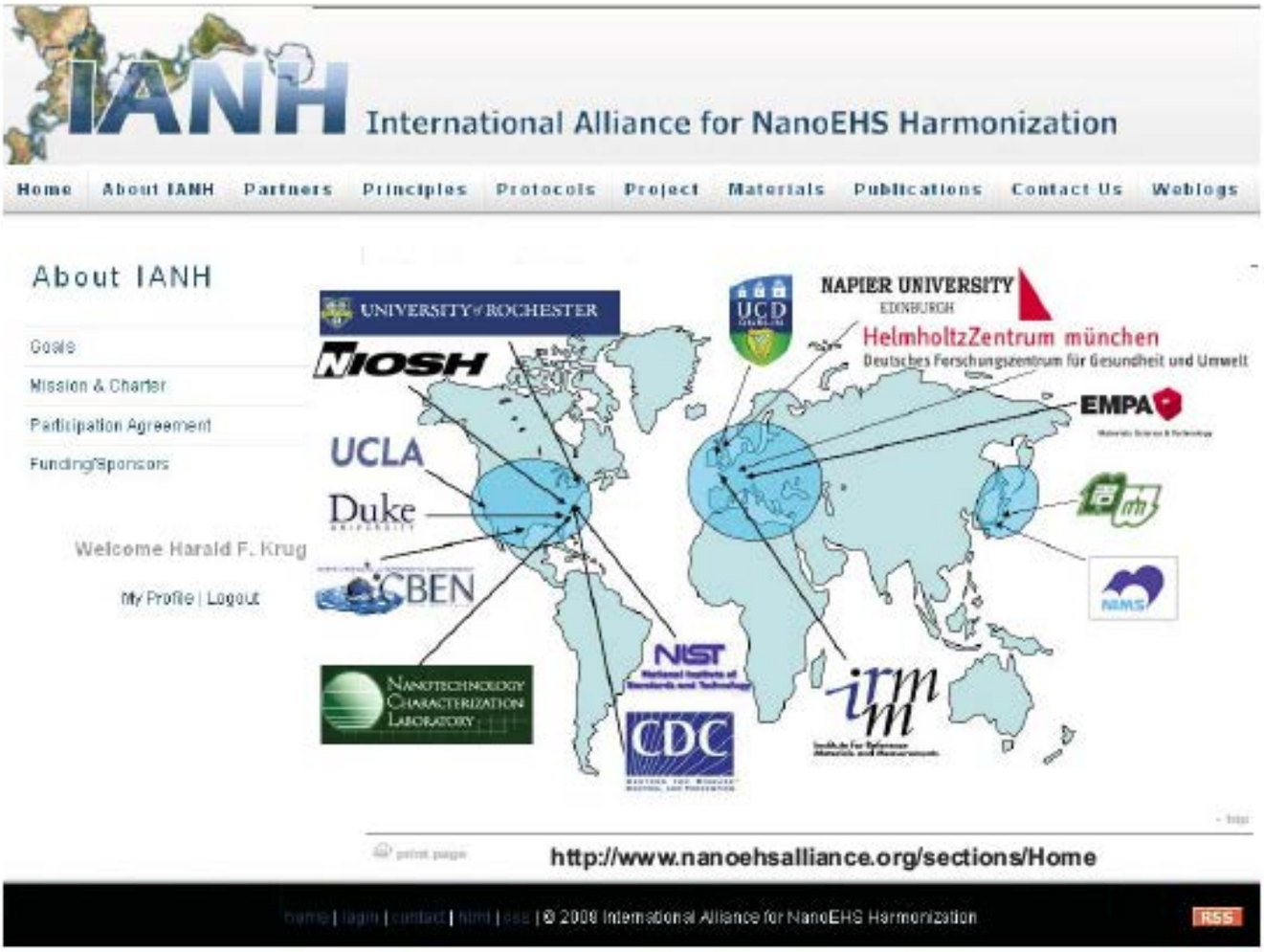
STANDARD PROCEDURES FOR NANOPARTICLE TESTING

[WORK PACKAGES NO. 2,3,4,5,6]

Edited by: Harald F. Krug, Empa

EMPA

Karolinska Institutet



Harmonised(Standardised) Methods

- **V** *Viability*
- **I** *Inflammation*
- **G** *Genotoxicity*
- **O** *Oxidative stress*

EMPA

IG DHS
Interessengemeinschaft
Detailhandel Schweiz

WESSLING

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

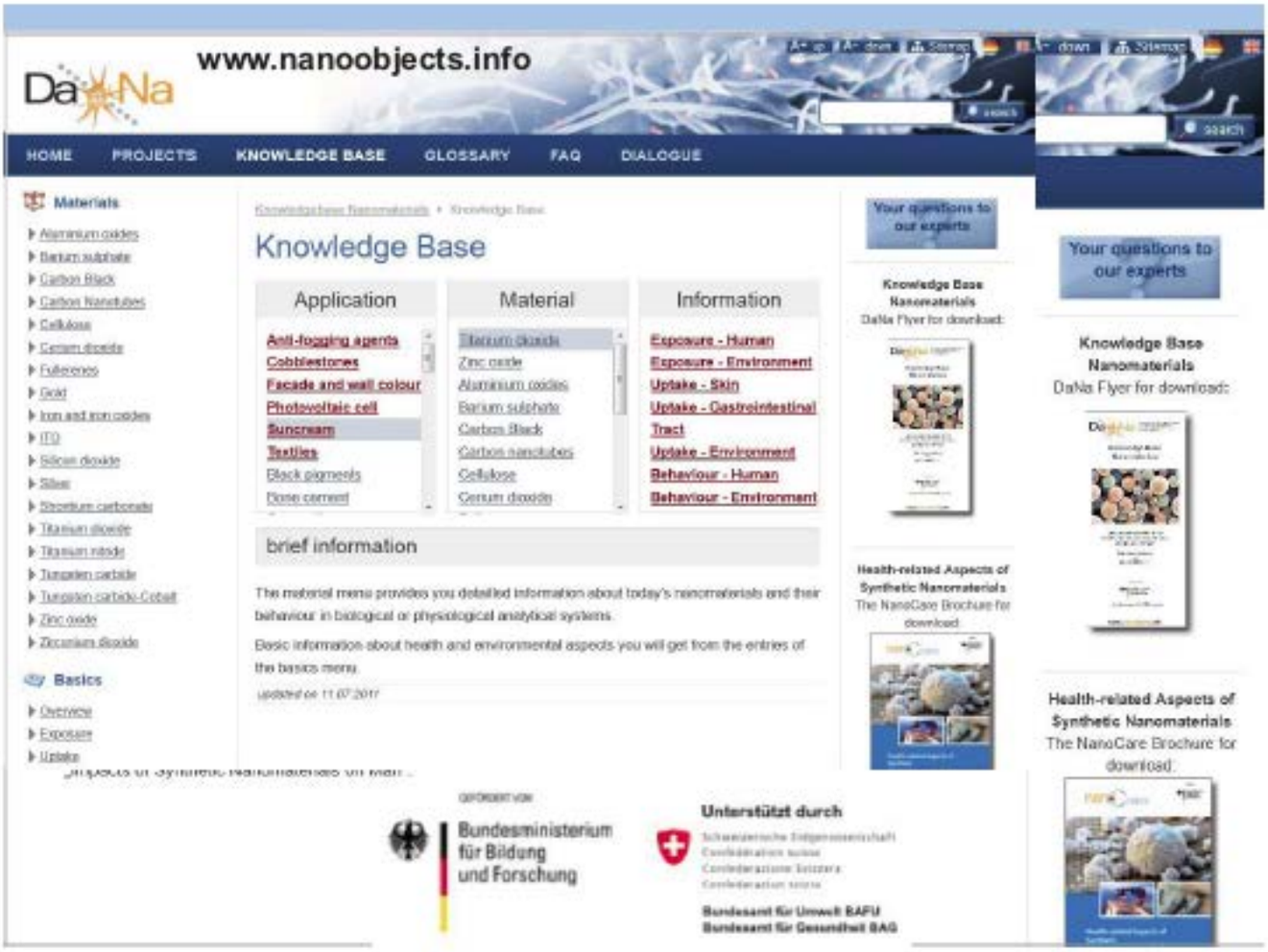
Bundesamt für Umwelt BAFU

Bundesamt für Gesundheit BAG

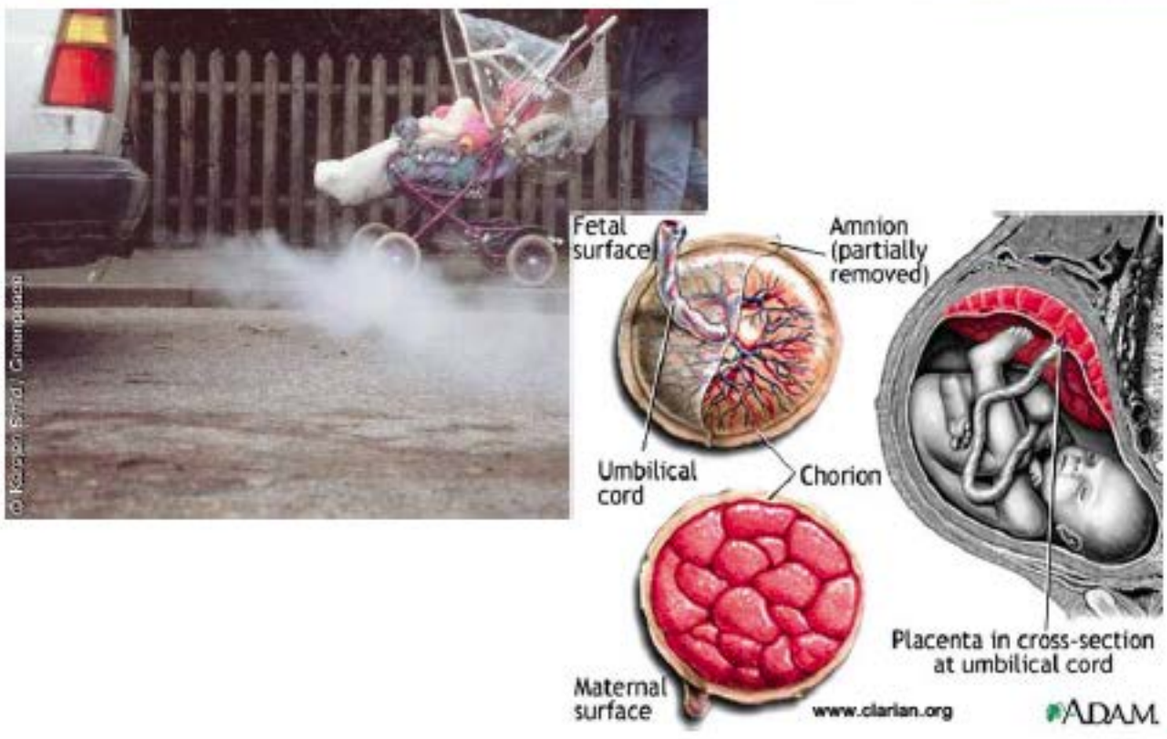
EPFL
ECOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

NOVARTIS

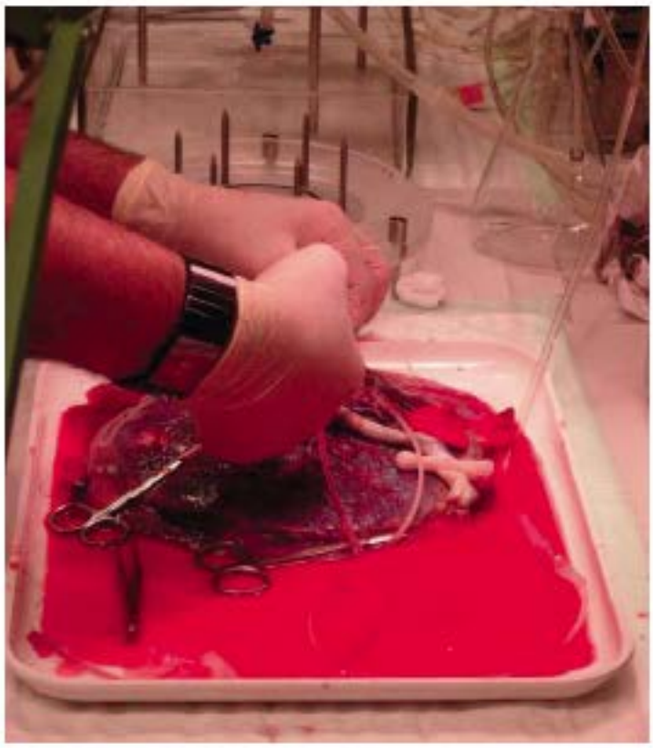
CCMX
Competence Centre for
Materials Science and Technology



Placenta: an efficient barrier for nanoparticles?

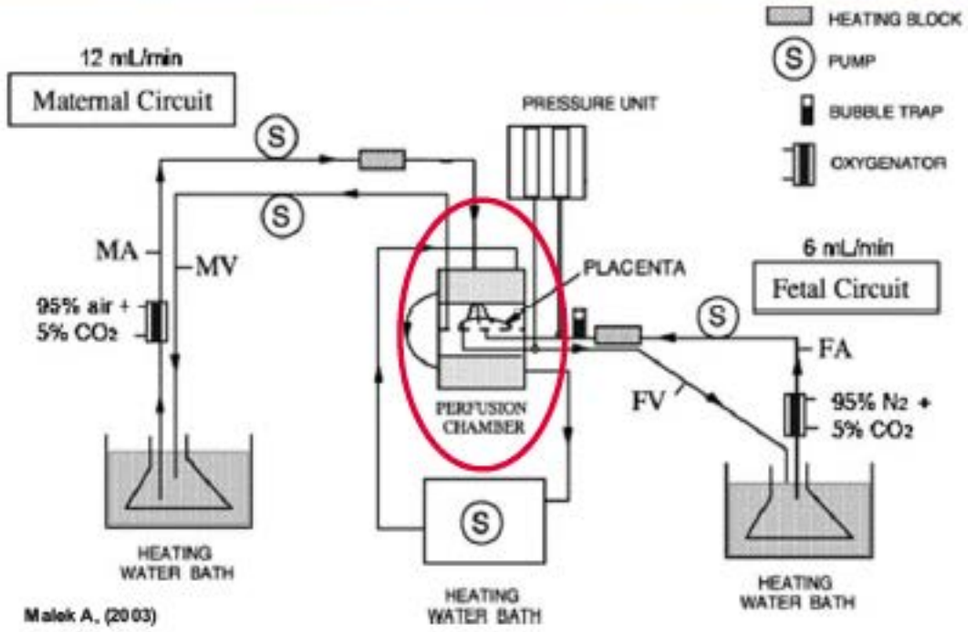


Human Placenta shortly after Delivery



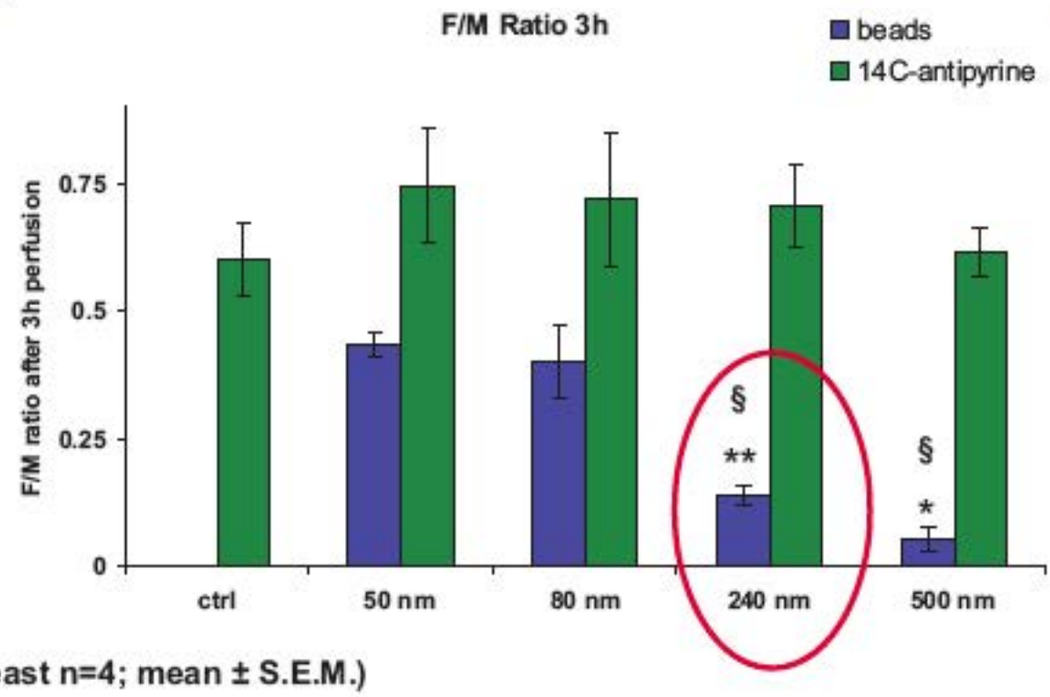
Experimental Set-Up

Re-circulating Placenta Perfusion Model



developed by Panigel M, et 1967 and improved by Schneider et al 1972

Placenta Barrier Capacity is Size Dependent



(at least n=4; mean ± S.E.M.)

Wick et al., 2010, Environ. Health Perspect. 118: 432-436

Future Challenges in Nanotoxicology

Outcome of the 2nd Minisymp. Nanotoxicol. Stockholm 2010

- Exposure & Environment
- Foreign Entities vs. Endogenous Entities
- Don't Believe a Single Method
- Method Reliability
- In Vitro ⇒ in Vivo Correlation
- Nano-Specific Signatures
- REACH
- Challenges for Regulatory Activities
- Fibre Paradigm Revisited
- Crowns/Coronas out of Proteins, Lipids and …?
- How to define "Biopersistence"?

Future Needs of Nanotoxicology

- The huge amount of new chemicals and expected new nanomaterials on the market raises the question for alternative methods for a first screening
- In vitro methods should be reliable, robust, sensitive and predictive
- Without a responsible process of marketing "nanotechnologies" will fail within the next decade
- Support of (nano)toxicological activities/projects is continuously needed; risk characterisation and assessment is crucial
- Comparability of Tox-studies is mandatory and should be achieved by use of standardised methods, reference materials and the appropriate controls in each experimental set-up

Conclusions

There is still research need on "nano-risk" but

- **We should use improved and comparable methods**
- **We should use the correct controls and, if available, a reference material**
- **Concentrations should be kept in the right range**
- **No genotox studies with cytotoxic concentrations or overload conditions**

Important questions for Nanotoxicologists

- Do living organisms "see" the nanomaterial?
- How does coverage of nanoparticles by biological material alter toxicity of NP?
- Is agglomeration/aggregation reducing all nanoeffects?
- How can we overcome interference of nanomaterials with the assay systems?
- Why don't we have an agreement on controls and reference/benchmark materials?
- Is the application of huge concentrations in vitro justified by the limitations in treatment-time?
- What about long-term effects?
- Do we need additional millions of animals to test this?

Changing Paradigms

Vol 444|16 November 2006 nature

COMMENTARY

Safe handling of nanotechnology

The pursuit of responsible nanotechnologies can be tackled through a series of grand challenges, argue **Andrew D. Maynard** and his co-authors.

Particle and Fibre Toxicology

BioMed Central

Particle and Fibre Toxicology 2005, 2:8

Review

Open Access

Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy

Günter Oberdörster[1], Andrew Maynard[2], Ken Donaldson[3], Vincent Castranova[4], Julie Fitzpatrick*[5], Kevin Ausman[6], Janet Carter[7], Barbara Kam[8,9], Wolfgang Kreyling[10], David Lai[11], Stephen Olin[5], Nancy Monteiro-Riviere[12], David Warheit[13], Hong Yang[14] and A report from

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