NANOHEALTH - Research on Safety of Nanoparticles

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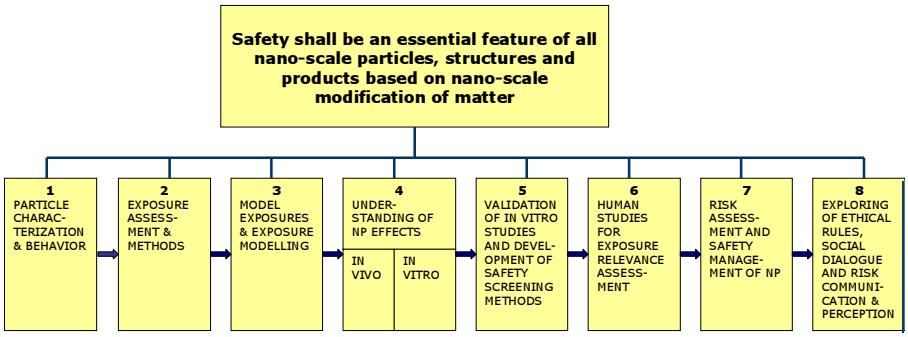
Some concerns regarding the safety of nanomaterials

- The small size of nanoparticles renders them with unique properties utilized in their industrial applications
  - Nanoparticles may also have unique and unpredictable toxic effects and thus pose a health hazard
- Manganese nanoparticles can enter the brain through exposure via the nose through neuronal pathways, i.e. the dendrites of the primary olfactory neurons
- Some nanoparticles can disturb blood clotting and thereby microcirculation with a potential of affecting multiple organ systems
- When inhaled, some nanoparticles induce lung cancer in rats more efficiently than larger particles of the same material
- Carbon nanotubes (CNTs) can induce mesothelioma in mice upon i.p. instillation, in a similar manner as asbestos
  - Are CNTs new asbestos?

Kai Savolainen, Hannu Norppa

Process leading to safety of nanotechnology, nanomaterials and nanoparticles



Kai Savolainen

NANOHEALTH

Engineered Nanoparticles: Synthesis, Characterization, Exposure and Health Hazards

1 January, 2007 – 31 December, 2010

- NANOHEALTH-consortium coordinated by Kai Savolainen Finnish Institute of Occupational Health
- Supported by the Academy of Finland, FinNano Program
- Partners:
  - Finnish Institute of Occupational Health
  - Department of Physics, University of Helsinki
  - Department of Environmental Sciences, University of Kuopio

NANOHEALTH - Aims

- Creation of a reliable and sound foundation for assessing the safety of nanoparticles
- Synthesis of nanoparticles to be studied and characterization of features important for their safety
  - size, morphology, dispersions, agglomeration, etc
- Evaluation of human exposure to nanoparticles at workplaces
  - methods for exposure assessment
  - exposure levels
- Assessment of the health effects of nanoparticles
  - airway irritation
  - inflammatory responses of the lungs
  - genotoxicity (potential carcinogenicity)

NANOHEALTH Consortium

WP1
Synthesis, characterization and behavior of nanoparticles

Prof. Kaarle Hämeri
University of Helsinki
Dept Physics
Finnish Institute of Occupational Health
New Technologies and Risks

Dr Jorma Joutsensaari
University of Kuopio
Dept Environ Sci
Fine Particle and Aerosol Technology Laboratory

WP2
Physiological responses of respiratory tract caused by nanoparticles and exposure assessment

Prof. Pertti Pasanen
University of Kuopio
Dept Environ Sci
Indoor Air Hygiene

WP3
Pulmonary inflammation

Prof. Harri Alenius
Finnish Institute of Occupational Health
Unit of Excellence for Immunotoxicology

WP4
Genotoxicity of nanoparticles

Dr Hannu Norppa
Finnish Institute of Occupational Health
New Technologies and Risks

NANOSH (NMP4-CT-2006-032777)

Inflammatory and Genotoxic Effects of Engineered Nanomaterials (6th FP)

1 November, 2006 – 31 October, 2009

- Coordinated by Kai Savolainen
- Other Partners:
  - Institute for Surgical Research, University of Munich, Germany
  - Central Institute for Labour Protection – National Research Institute, Warsaw, Poland
  - TNO, Zeist, Netherlands
  - Health and Safety Laboratory, Buxton, UK
  - BG Institute for Occupational Safety and Health, Sankt Augustin, Germany
  - Cancer Biomarkers and Prevention Group, University of Leicester, UK

Factors that may affect nanomaterial toxicity and health effects

- size, agglomeration, aggregation
- surface area, surface properties, charge
- shape (particles, fibres, membrane)
- surface modifications, coating, functional groups
- chemical composition, crystallinity
- solubility, dispersibility, bio-persistence
- impurities, catalysts

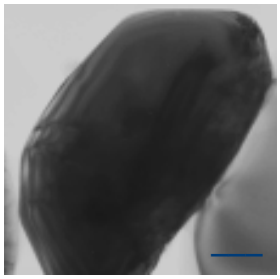
- type of cells studied, their function, capacity to phagocytize, endocytosis, other means of penetration to cells and nucleus

- It is not well understood which characteristics are important for toxicity and health effects of nanomaterials

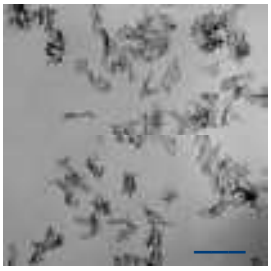
- Existing information on the health effects of and toxicity of particulate matter of larger size is useful but not adequate

- Nanomaterials are very variable

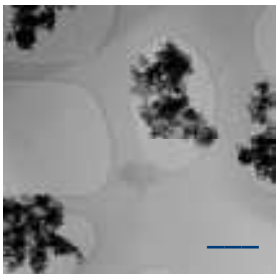
Some TiO_2 particles under study



Fine rutile <5 µm
(Sigma-Aldrich)



SiO_2-coated rutile
10 x 40 nm
(Sigma-Aldrich)

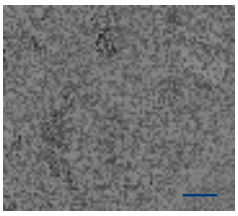


Anatase <25 nm
(Sigma-Aldrich)

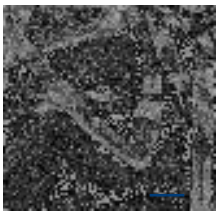
Bar: 150 nm

Photos: Esa Vanhala

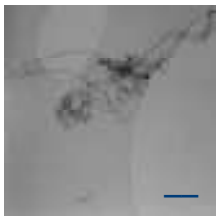
Some carbon nanomaterials under study



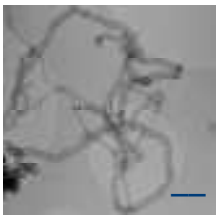
Single-wall CNT >50%, other CNT 40%
1.1 nm x 0.5-100 µm
(Sigma-Aldrich)



Graphite nanofibers 95%; ~4% catalyst metals; outer diam. 100-200 nm, inner diam. 30-50 nm, length 5-20 µm
(Sigma-Aldrich)



Single-wall CNT
<2 nm x 1-5 µm
(SES Research)



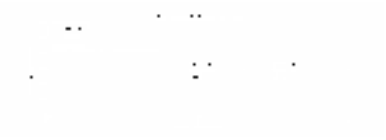
Multi-wall CNT
10-30 nm x 1-2 µm
(SES Research)

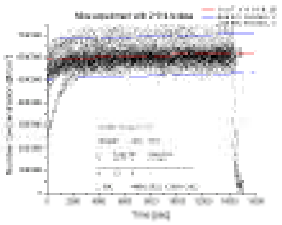
Photos: Esa Vanhala

Bar: 150 nm

Inhalation exposure of mice to TiO_2

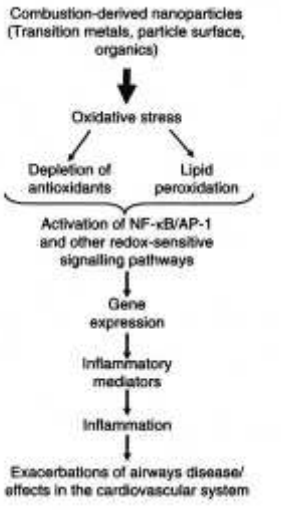
- Exposure chamber with a dust generator (brush)
- TiO_2 generator constructed by Kuopio University (for anatase)
- Constant production of TiO_2 aerosol at 10 mg/m^3
- Exposure of mice:
  - acute (1 d, 4-5 days)
  - longer-term (3 weeks)

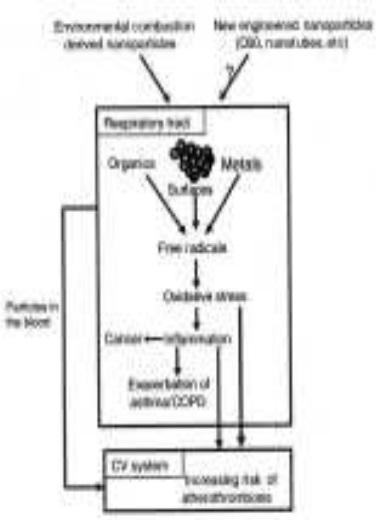




Joonas Koivisto

Nanoparticle-induced events leading to inflammation and disease outcome

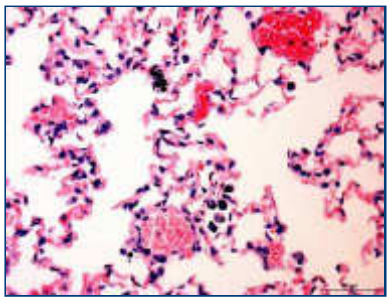




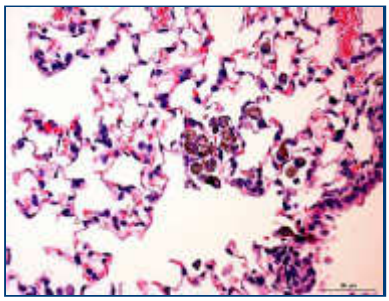
Donaldson et al 2007

Pulmonary histology after TiO_2 rutile inhalation

Fine TiO_2



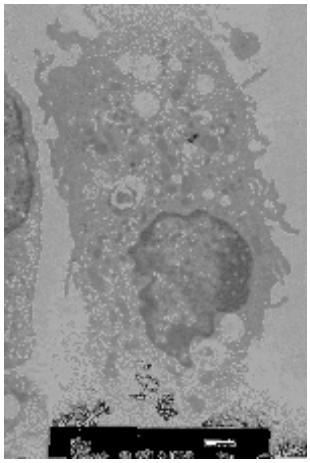
SiO_2-coated Nano-TiO_2



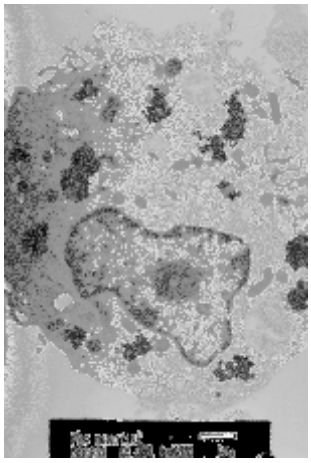
40x lens

Photos: Henrik Wolff

Pulmonary macrophage after inhalation of SiO_2-coated nano-TiO_2 rutile (TEM)



Control

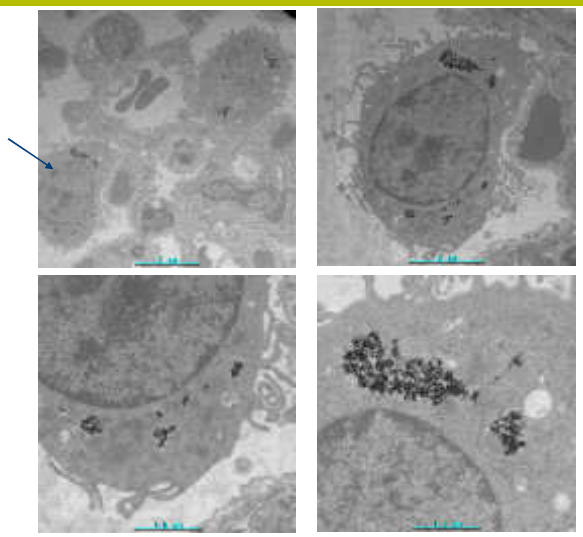


Nano-TiO_2

bar: 1 μm

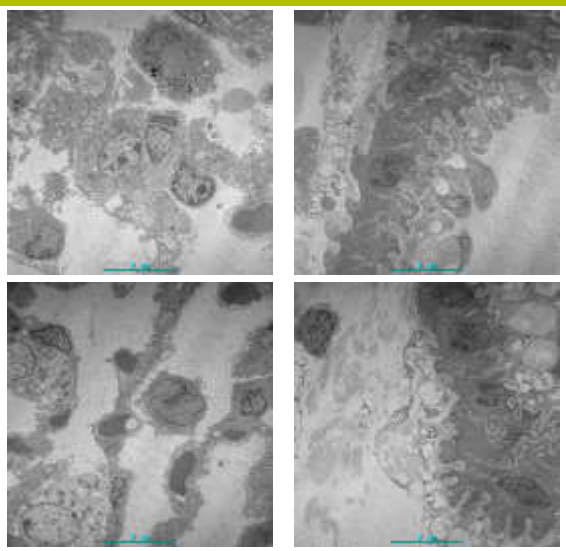
Photos: Esa Vanhala

Nano-TiO_2 in a pulmonary macrophage



TEM-images: Esa Vanhala

SiO_2-coated nano-TiO_2 rutile particles did not accumulate in bronchial epithelial cells



TEM-images: Esa Vanhala

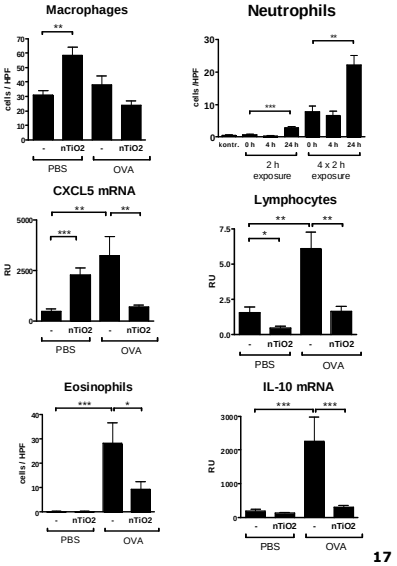
Inhalation of SiO_2-coated TiO_2 rutile Inflammatory effect and asthma model

Healthy mice

- Influx of macrophages and neutrophils in the lungs
- Increased expression of neutrophil-attracting chemokine CXCL5

Asthmatic mice

- Inhibition of pulmonary changes linked to allergic asthma
  - Airway reactivity to Metachol. ↓↓
  - Eosinophils and lymphocytes in lungs ↓↓
  - Mucus secretion ↓↓
  - Cytokine and chemokine expression ↓↓



Inflammation and asthma - Conclusions

Acute inhalation of SiO_2-coated nano-TiO_2 rutile in healthy mice

- Strong initial activation of macrophages or epithelial cells → expression of cytokines and chemokines → neutrophil infiltration
- When exposure continues macrophages or epithelial cells become non-responsive → chemokine/cytokine expression stops
- No inflammatory effect by non-coated nano-rutile, nano-anatase, fine rutile

3-week inhalation of SiO_2-coated nano-TiO_2 rutile in asthmatic mice

- Strong inhibition of pulmonary changes linked to allergic asthma (AHR, eosinophils, lymphocytes, mucus production, cytokine and chemokine expression)
- Exposure to nano-TiO_2 induces macrophage or epithelial cell non-responsiveness → chemokine and cytokine expression stops → no recruitment of inflammatory cells to lungs

Genotoxicity studies in NANOHEALTH

- Genotoxicity is a short-term indication of carcinogenic activity and ability to induce heritable alteration

1. Do nanoparticles increase DNA strand breakage in pulmonary cells *in vitro* and *in vivo* (FIOH)?
2. Do nanoparticles induce chromosomal damage in pulmonary cells *in vitro* and *in vivo* (FIOH)?
3. Does exposure to nanoparticles lead to oxidative DNA damage in pulmonary cells *in vitro* and *in vivo* (University of Leicester - within NANOSH)

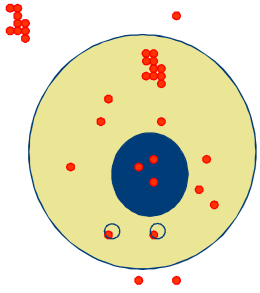
Nanoparticles and genotoxicity

- Due to their small size, nanoparticles may have unpredictable genotoxic properties
- Nanoparticles may pass through cellular membranes and get access to the nucleus
- Elimination of nano-sized particles *in vivo* may be ineffective, leading to accumulation of the particles in cells – this may contribute to genotoxic effects in target tissues
- Until recently, there has been little information on the genotoxicity of nanomaterials

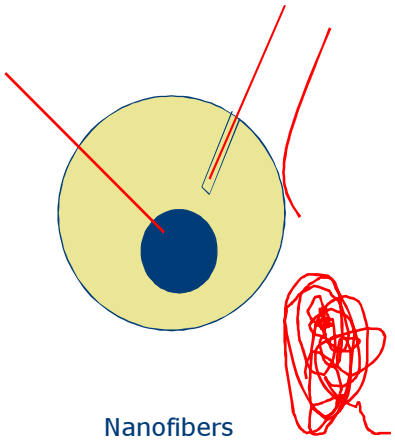
Nanoparticles might be genotoxic

- Directly
  - reactions or interference (in nanoscale) with DNA
  - reactions or interference (in nanoscale) with the mitotic apparatus (microtubules, kinetochores, centrioles, etc)
- Indirectly through oxidative stress within the cell
  - effects on membranes
  - effect on mitochondria: interference with antioxidant defence
- Indirectly through inflammatory effects
- In connection with light - photogenotoxicity

Nanomaterial shape

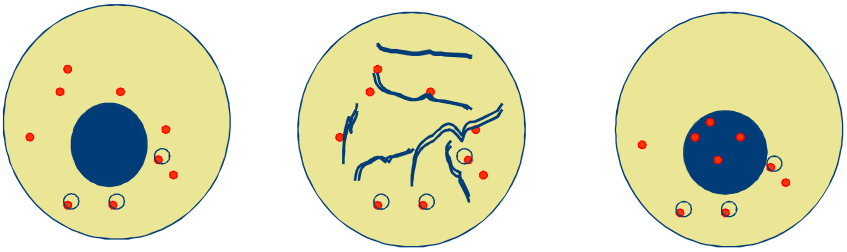


Spherical nanoparticles



Nanofibers

Access to the nucleus in cell division



Nanoparticles that do not go to nucleus in interphase cells

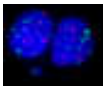
In cell division, nuclear envelope disappears - nanoparticles get access to nucleus

Nanoparticles inside nucleus, potentially able to interfere with interphase DNA

Assay timing should allow cell division and potential contact of nanomaterial with DNA in the interphase following the cell division

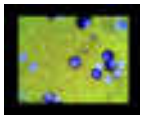
Genotoxicity of nanomaterials - methods

- DNA strand breaks (SBs) *in vitro* and *in vivo* by single cell gel electrophoresis technique (comet assay)
- Micronuclei (MN) *in vitro* (cytokinesis-block technique) and *in vivo*.
- Structural chromosomal aberrations (CAs) *in vitro* in cultures of human lymphocytes



- 8-oxo-2'-deoxyguanosine (8-oxodG) *in vitro* by LC-MS/MS (University of Leicester)
- Malondialdehyde-2'-deoxyguanosine adduct (M1dG) *in vitro* and *in vivo* by immunoslot blot assay (University of Leicester)

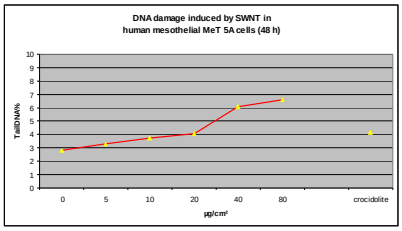
- Bronchial epithelial cells (BEAS 2B), mesothelial cells (MET 5A); human lymphocytes (for CA) *in vitro*
- Mouse type II pneumocytes and Clara cells *in vivo*
- Behaviour of nanoparticles in cell division

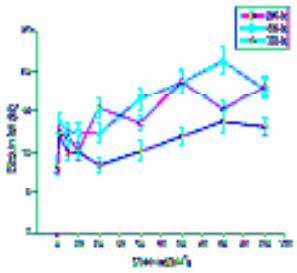


Genotoxicity of nanomaterials - summary

- **Human bronchial epithelial BEAS 2B cells *in vitro***
  - DNA damage (comet assay) induced by TiO_2 (nano-anatase, nano-rutile, fine-rutile), carbon nanotubes (mixed single-wall and others), graphite nanofibers
  - Micronuclei induced by nano-anatase TiO_2, carbon nanotubes (mixed), graphite nanofibers
  - Oxidative DNA damage in progress
  - Under analysis: single-wall and multi-wall carbon nanotubes
- **Human mesothelial Met 5A cells *in vitro***
  - DNA damage induced by single-wall and multi-wall carbon nanotubes
- **Human lymphocytes *in vitro***
  - Chromosomal aberrations induced by single-wall and multi-wall carbon nanotubes
  - Micronuclei induced by nano-anatase
- **Mouse lung cells *in vivo***
  - Nano-anatase TiO_2 inhlation
  - DNA damage *not* detected at about 7 mg/m^3
  - Oxidative DNA damage, micronuclei under analysis

Falck, Lindberg, Hannukainen, Norppa





DNA damage induced by carbon nanotubes (mixed) in human bronchial epithelial BEAS 2B cells

Concluding remarks

- Safety should come first, but regulations are unfinished
- Present regulatory (geno)toxicity assays have been designed for (soluble) chemicals and may not be suitable for nanomaterials or particulate substances in general
- Nanomaterials will differ widely from each other:
  some will be hazardous, same will not
- The wide variety of nanoparticles sets an enourmous task for toxicological research and risk assessment
- Nanomaterial characteristics critical for toxic effects should be identified
- Importance of practical issues of safety:
  - Can exposure occur and in which situations?
  - Is the route of human exposure dangerous?
  - How should exposure be measured (NANODEVICE project)?
  - How can exposure be prevented?