Nanomaterials - Nanoparticles

How can we contribute to a safe development?

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• What is a nanomaterial?
• Why bother?
• A worthy challenge
• Hazards
• Risks and control
• Integrative and supportive approaches
Nanoparticles/-materials

- Engineered
  - Silver
  - Carbon-based
    - Nanotubes
    - Nanofibers
    - Platelets
  - Metal oxides etc

- Natural
  - Tubular clay minerals e.g. Imogolite

- Incidental
  - Engine exhaust
  - Welding fumes
  - Candle smoke
  - Carbon black
Nanosize (< 100 nm)

Short and long nanowires

Macrophage - metaloxide NPs

Nanoplatelets

Carbon nanotubes

Nanomaterial

> 1 dimension < 100 nm

Nanoparticle

3 dimensions < 100 nm
Search nanomaterials and health in PubMed

Most publications refer to the therapeutic potential
Why bother?

Key enabling technologies

- Nanotechnology
- Micro- och nanoelectronics
- Advanced materials
- Photonics
- Biotechnology
- Advanced manufacturing

Growth - number of nanoenabled products in commerce

European Commission

An expanding key sector
- A key area for worker health

0.2 billion Euro 2009
2 billion Euro 2015

SchultePA et al Nanotoxicology 2016
A worthy challenge

Nanosize = new properties

• Endless variation
  – Size, shape, coating, surface activity…..

• Engineered - Safety by design!

Key body organs and cell functions

Multiple effects

Osman et al, Nanotoxicology 2020
Hazards?

Yes, from some (multiple effects).
Multiwalled nanotubes (MWNT-7) - lung cancer (inhalation, rat)

<table>
<thead>
<tr>
<th>Air conc. (mg/m³)</th>
<th>Lung cancer – males (50 animals/exposure group)</th>
<th>Lung cancer – females (50 animals/exposure group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>2%</td>
<td>-</td>
</tr>
<tr>
<td>0.02</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>0.2</td>
<td>16% (p \leq 0.05)</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>22% (p \leq 0.01)</td>
<td>16% (p \leq 0.01)</td>
</tr>
<tr>
<td>Trend</td>
<td>(p \leq 0.01)</td>
<td>(p \leq 0.01)</td>
</tr>
</tbody>
</table>

Hyperplasia, granuloma, localized fibrosis in both sexes. No general toxicity.
Cancer promotion

- MWCNT (mice, 5 mg/m³ for 15 days; l=1-6 µm)
- Dramatic effect following i.p initiator
- Dose relevant to human exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice (n)</th>
<th>% with tumors</th>
<th>Tumors (n; total)</th>
<th>Tumors/animal</th>
<th>AC (n) per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered air</td>
<td>56</td>
<td>23</td>
<td>17</td>
<td>0.25 (±0.17)</td>
<td>7</td>
</tr>
<tr>
<td>MCA (i.p.)</td>
<td>54</td>
<td>52*</td>
<td>41</td>
<td>0.81 (±0.17)</td>
<td>14*</td>
</tr>
<tr>
<td>MWCNT (inhal)</td>
<td>49</td>
<td>27</td>
<td>20</td>
<td>0.38 (±0.19)</td>
<td>7</td>
</tr>
<tr>
<td>MCA+MWCNT</td>
<td>42</td>
<td>91</td>
<td>133</td>
<td>2.9 (±0.39)*</td>
<td>56*</td>
</tr>
</tbody>
</table>

Note: *p>0.0001; AC= Adenocarcinoma; MCA=methylcholanthrene; MWCNT=multi-walled carbon nanotubes
Five malignant serosal tumours in the MCA+MWCNT, and one in the MCA, group.
Progressive fibrosis in both the MWCNT only and MCA+MWCNT groups.

Sargent et al 2014
MWCNT-7 – mesothelioma (Wistar rat)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
<th>Combined</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>MWCNT-7</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Crocidolite</td>
<td>19</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant mesothelioma</td>
<td>18**</td>
<td>95**</td>
</tr>
</tbody>
</table>

*Significantly different from the vehicle group at *P* < 0.05, *P* < 0.001.

Intratracheally: 0.13 µg/dose, 1/w, 12 wks. Sacrificed at 104 wks

Numano et al 2019
Human studies (cross sectional)

• Biomarkers for fibrosis increased
  – Sputum (KL-6)
  – Blood
• Oxidative stress
  – 8-OH-dG, SOD
• No effect on lung function or FENO
• Effect on CVD risk markers
  – ICAM-1, VCAM-1, CRP

Reviewed by Schulte et al 2019
Conclusions CNT

- Carcinogenic in experimental animals
  - 2-y inhalation (lung; low dose)
  - 2-y intratracheal (mesothelioma)
- Fibrosis (localized and progressive) in rodents (inhalation)
- Suggestions for inflammatory and fibrotic response in exposed workers
  - Not overt disease
- Biomarkers indicating CVD risk
Shape: The key paradox

Particle too big for macrophages, but aerodynamic diameter still respirable
"Asbestos paradigm"
HARN-hypothesis

Paradigm for determinants of fiber toxicity

Asbestos

Carbon nanotubes

Clearance

Inflammation

Frustrated phagocytosis

HARN= High Aspect Ratio Nano Particles; Aspect Ratio= length/diameter ratio

Donaldson et al. (2010)
Frustrated phagocytosis: A: in pleural space, B: in cell culture

Note: respirable particle diameter <25-30µm! (gives aerodynamic diameter 3 µm)
Cytokine release: Vehicle (VC), carbon black (CB), graphene nanoplatelets (GP)

Note: Carbon black is compact nanoform of graphene
Confirmed by platelet size by Roberts et al 2016 for d >5 µm
Structure - surface activity

- Nanotubes
  - Germanium-imogolite
- High aspect ratio
  - but <5 µm
- Intratracheal instillation, Wistar rat
- Fibrotic response (60 d)
  - persistent inflammation
- HARN not only frustrated phagocytosis?
  - Need to test also HARN<5µm
Shape and size new knowledge

Frustrated phagocytosis not necessarily high aspect ratio

High aspect ratio effect at <5µm
Generic concerns
Dustiness

The pigment TiO$_2$ is 300 times more dusty as nanosized (20 nm) compared to the bulk form (150 nm)


Courtesy of professor Ulla Vogel, NRCWE, DK
Surface area

• Outdoor particulate exposure (exhaust gases)
  – Effects at low mass concentrations (µg/m³)
    • Analogy nanoparticles – ultrafine particles
      » Stone V et al 2017

• 1 mg of spheric coal particles
  – d=10 µm, $10^{12}$ particles, surface area 270 m²
  – d=10 nm, $10^{21}$ particles, surface area 270 000 m²
    » Maynard et al 2011

• Early nanotoxikology
  – Surface area determines small insoluble particle toxicity
    » Oberdörster 1990 and 2000

Dust with low toxicity as fine particles has higher toxicity as nano/ultrafine particles
Same mass different response – but linear response with surface area

Rat. Different particle size of carbon black, latex and TiO2

Donaldson et al., 2002

Courtesy of professor Ulla Vogel, NRCWE, DK
Early concerns for nano versus conventional size

- Enhanced dustiness
  - $300 \times$ för TiO2
- Enhanced lung deposition
  - $1-3 \times$
- Enhanced adverse effect. Inflammation proportional to particle surface area
  - $100 \times$

professor Ulla Vogel, NRCWE, DK
Surface reactivity – Zeta potential

- Phagolysosomes (pH 5.6) in macrophages
- Hypothesis: NPs with a high positive Z-potential (acid) destabilise lysosomes
- Trigger inflammation - cell death
- Better fit with observations than oxidative potential

Zeta potential: electric potential created between the surface of a particle and the suspension medium

Donaldson et al 2012
Solubility - toxic ion release

- High solubility NPs deposit on the lung lining (neutral pH)
- Develop surfactant corona
- Taken up into macrophage phagolysosomes (acidic pH)
- Accelerated dissolution rate – toxic ions (Zn, Cu) accumulate
- Lysosomal membrane destabilization and inflammation.

Donaldson et al 2012
Generic..

Poorly soluble low toxicity particles
(TiO$_2$)
TiO$_2$ ultrafine (nano)

- Lung function decline (within normal)
- Airway/lung inflammation/damage
  - Surfactant protein D increase
  - Increase in exhaled NO (FENO)

- Oxidative stress (e.g. ROS, 8-OHdG)
- Systemic inflammation
- Increase in CVD risk markers
  - Lipid oxidation, VCAM-1, ICAM-1, etc
- Carcinogenic in experimental animals (lung; inhalation)
  - Overload and/or specific interaction?

Schulte et al 2019
Exposure scenarios

From Nanex
Exposure scenarios

Some exposure data*

Few human effects studies

* Compiled in Ev@lutil
"Nanometric particles document database

Sparse exposure data
Bulk of exposed population

Recycling/re-use

from Nanex Project
Environmental exposures

Microplastics

- Polystyrene 1-2 μm spheric
- Cytoxic to human epithelial cells in vitro
- ROS-formation
- Barrier impaired
- Alfa1-antritrypsin decrease

Dong C-H, J Haz Mat 2020

Revel, *Curr Opin Env Science & Health* 2018

Gasperi, *Curr Opin Env Science & Health* 2018
Risk assessment

One exposure – multiple outcomes
Multiple outcomes

Known (CNT)
• Mesothelioma
• Lung cancer
• Fibrosis

Air pollution analogy
• Lung cancer
• Asthma
• COPD
• Myocardial infarction
• Stroke
• Atrial fibrillation
• Intrauterine growth
• Pre-eclampsia
• Dementia?
• Mood change?

Research need
Key characteristics in exposed populations

Other NCDs
- COPD
- CVD
- Dementia
- Reproductive outcomes
- Diabetes
- ....
- .....  

Carcinogens (IARC)
- Electrophilic/metabolically activated
- Genotoxic
- Alters DNA repair/genomic stability
- Epigenetic alterations
- Oxidative stress
- Induces chronic inflammation
- Immunosuppressive
- Modulates receptor-mediated effects
- Causes immortalization
- Alters cell proliferation, cell death, or nutrient supply

Smith MT EHP 2016
Guyton KZ Carcinogenesis 2018

Helpful when latency times insufficient and/or small exposed populations
Control
Safety by design = substitution
Permissible exposure levels

Photos from CL Gercia, NIOSH
Photo from Methner et al 2008
## Suggested guidance values

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>OEL/guidance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNT</td>
<td>6 µg/m³</td>
<td>Lee et al 2019</td>
</tr>
<tr>
<td>MWCNT</td>
<td>0.05 µg/m³</td>
<td>Fukushima 2018</td>
</tr>
<tr>
<td>MWCNT</td>
<td>&lt;1 µg/m³</td>
<td>NIOSH 2013</td>
</tr>
<tr>
<td>MWCNT</td>
<td>50 µg/m³</td>
<td>Bayer, 2010</td>
</tr>
<tr>
<td>MWCNT</td>
<td>7 µg/m³</td>
<td>Schulte et al., 2010</td>
</tr>
<tr>
<td>MWCNT</td>
<td>2.5 µg/m³</td>
<td>Nanocyl, 2009</td>
</tr>
<tr>
<td>CNT/Nanocellulose</td>
<td>0.01 f/ml</td>
<td>Scaffold proj. 2014</td>
</tr>
<tr>
<td>CNT</td>
<td>0.01 fiber/cm³</td>
<td>IFA, 2009</td>
</tr>
<tr>
<td>Graphene</td>
<td>18 µg/m³</td>
<td>Lee et al 2019</td>
</tr>
<tr>
<td>TiO₂-ultrafine</td>
<td>0.3 mg/m³</td>
<td>NIOSH 2011</td>
</tr>
<tr>
<td>TiO₂-ultrafine</td>
<td>0.1 mg/m³</td>
<td>Scaffold proj. 2014</td>
</tr>
</tbody>
</table>
Control?

Non- or extremely low threshold and multiple outcomes:
Challenge to regulatory praxis
Respirable crystalline silica

- One exposure – multiple outcomes
  - Lung cancer, silicosis, pneumonia, COPD, autoimmune disease, kidney disease, myocardial infarction
- Non-threshold carcinogen paradigm (40ys exposure)
  - Acceptable risk 4/100 000 excess death risk (life-long)
  - maximum tolerable risk 4/1000
- Cancer directive BOELV 0.1 mg/m$^3$
  - 45y working-life life-long lung cancer excess death risk 33/1000
- Adding non-malignant respiratory disease (85/1000), and kidney disease (39/1000) the full working life excess death risk is around 160/1000 (16%!)
  - Myocardial infarction not quantifiable (evident at ¼ of BOELV)

Albin M, Gustavsson P. Scand J Work Env Health Jan 2020
The way forward?

INTEGRATIVE AND SUPPORTIVE APPROACHES
One item fallacy

• One item assessment
  – Key for causality
  – Insufficient for regulation/implementation

• One exposure – multiple outcomes
  – E.g. crystalline silica, diesel exhaust, PAHs

• Multiple exposures – same target
  – Multiple solvents
    • hygienic effect for CNS
  – Ototoxic chemicals and noise
    • permissible level 5dBA lower
  – Frequent for (nano-) particles (e.g. cardiovascular)
Need for integrated regulatory concepts

• One exposure - multiple outcomes
  – Integrate all outcomes for given exposure
    • Epidemiologists should state excess risk
  – Start with mortality
  – Apply limits for tolerable/acceptable excess deaths from exposure independent of disease, but with same rigour for evidence

• Same target organ – multiple exposure
  – Apply ”noise-ototoxic chemicals” model to e.g. noise+particles for CVD?
Supportive approaches

- Outdoor – occupational environment
- Nanomaterials – other particles
- Agreement on key characteristics in exposed populations for main outcomes
  - Experimental – field studies (repeated)
    - Less delay first warning to control
      - Shortcut to agree on causality
      - Sufficient power in smaller populations than for "hard endpoints"
Gaps

• Nanotechnology – what actually happens in working life
  – Exposure (non-uniform materials, down-stream use, waste)
  – Who is exposed?
    • Extended information obligations Jan 2020
      https://echa.europa.eu/regulations/nanomaterials

• Findings challenge occupational (and environmental) health, research and regulation
  – Illustrates also other gaps

• Current knowledge insufficiently integrated
  – In new research across domains
  – In regulatory approaches
WE CAN CONTRIBUTE MORE...
How?

• Use potential between research domains
  – particles size, experimental/observational, occupational/environmental
  – Agreement on key characteristics
  – Use ownership of research boundaries!

• Challenge current regulatory failure in integrating evidence
  – Individual worker protection from all mortality
  – Claim/take back risk assessment ownership
  • Develop easily communicated risk measures