Health & Safety Aspects of Nanoparticles

Lorentz Workshop
2007
Outline

• Particle characteristics
• Lung physiology
• Interaction with membranes
• Chemical and biochemical processes
• Physiological consequences
  – cardiovascular
  – Immune system
  – brain
• Methods to assess toxicology *in vitro*
• Recommendations to Dutch government concerning safety
Historical development of particle toxicology

Lung disease in miners in Bohemia & Austria - Agricola & Paracelsus (1500-1550)

Particle concentrations from mining & industry

- Till mid 20th century: up to 40 mg/m³,
- now: 2 mg/m³

Research levels: 10-50 µg/m³

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Types of particulate matter (PM) and associated health issues

Urban, rural & technogenic Particles

- PM$_{10}$-PM$_{2.5}$, PM$_{2.5}$-PM$_{0.1}$, nanoparticles
- Residual oil fly ash
- Soot
- Smog – mineral particles surrounded by water layer
- Amorphous fumed silicon dioxide
- Diatomaceous earth
- Glass fiber/MMMF
- Metal aerosols (welding)

- Asthma, pneumonia, bronchitis, heart failure
- Asthma, pneumonia, bronchitis
- Asthma, pneumonia, bronchitis, radiological changes, skin cancer
- Pulmonary inflammation, sudden cardiac death
- Inflammation, oedema, fibrosis
- Fibrosis, silicosis
- Inflammation, occupational asthma, pleural plaques
- Inflammation, lung cancer, metal fume fever
# Natural PM and associated health issues

**Silicate minerals**
- Crocidolite: asbestosis, lung cancer, pleural plaques
- Chrysotile: asbestosis, lung cancer, pleural tumors
- Talc: pneumoconiosis (talcosis), pulmonary oedema
- Quartz: fibrosis, silicosis, lung cancer
- Zeolites: lung cancer

**Non-Silicate minerals**
- Apatite: impaired pulmonary function, hemolytic activity
- Bauxite: Shaver’s disease
- Graphite: graphite pneumoconiosis, fibrosis
Characteristics of PM important in health considerations

- mass concentration
- number concentration
- surface area
- chemical reactivity – acidity and solubility
- particle charge & surface chemistry
- particle core chemistry
- metals & oxidation state
- carbon (organic and black)
- shape
- size and size distribution
For 100 ng spherical particles of unit density

<table>
<thead>
<tr>
<th>Particle diameter (nm)</th>
<th>Particle number</th>
<th>Surface area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.4 x 10^{13}</td>
<td>300</td>
</tr>
<tr>
<td>20</td>
<td>2.4 x 10^{10}</td>
<td>30</td>
</tr>
<tr>
<td>1000</td>
<td>190000</td>
<td>0.006</td>
</tr>
</tbody>
</table>

10x greater than the number of cells in a lung weighing 100g
Particle deposition and removal mechanisms in the respiratory tract during in- & exhaling

- Particles > 1.5μm aerodynamic diameter - impaction caused by their inertial mass in branching airways
- Particles 0.5 – 1.5 μm aerodynamic diameter - sedimentation by gravitational forces
- Particles < 0.5μm - diffusion by thermal motion of air molecules
- Clearance: dependent on particle size:
  - Larger particles via mucociliary escalator: velocity towards the trachea (10-20 mm/min.)
  - Ultra fine and nanoparticles - particle entrapment between the cilia, phagocytosis or translocation into the epithelium
Alveoli anatomy

- Bronchioles
- Alveoli
- Alveolar duct
- Alveolar sacs
- Carbon Dioxide is dropped off
- Oxygen is picked up
- Wall of the air sac
- Capillary
- Red Blood Cell

BiMaDe
Bio-Organic Materials and Devices
Membrane barrier system of alveoli

S – surfactant film
ALL – aqueous lining layer
AM – alveolar macrophage
DC – dendritic cell
BM – basal membrane
CT – connective tissue
C – capillary endothelium
TJ – tight junction
AEPT1 – alveolar epithelium type 1
Routes for large and small macromolecules across alveolar Type 1 epithelial cells

(a) Transcytosis

(b) Tight junctions

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Permeability of phagocytic cell membranes to polystyrene spheres

Alveolar macrophages

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Permeability of non-phagocytic cell membranes to polystyrene spheres

Red Blood cells

- Synthetic polymers, uncharged or negatively charged
- 25 nm gold particles
- Ultrafine titanium dioxide
- Metals, metal oxides

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Particle retention after a single one hour inhalation of 15nm iridium particles

<table>
<thead>
<tr>
<th>Organ</th>
<th>Retained Mass Fraction</th>
<th>Retained Particles Number</th>
<th>Particle Surface Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One Week</td>
<td>Six Months</td>
<td>One Week</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.6</td>
<td>0.06</td>
<td>7.00×10^{11}</td>
</tr>
<tr>
<td>Liver</td>
<td>0.006</td>
<td>0.0005</td>
<td>7.00×10^9</td>
</tr>
<tr>
<td>Splccn</td>
<td>0.004</td>
<td>0.0003</td>
<td>4.66×10^9</td>
</tr>
<tr>
<td>Heart</td>
<td>0.004</td>
<td>0.0005</td>
<td>4.66×10^9</td>
</tr>
<tr>
<td>Brain</td>
<td>0.003</td>
<td>0.0005</td>
<td>3.50×10^9</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.006</td>
<td>0.0001</td>
<td>7.00×10^9</td>
</tr>
</tbody>
</table>
Mechanisms of adverse health effects
The primary effect of PM is oxidative stress

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Sources of reactive oxidizing species (ROS)

- **Particle-mediated - Non-cellular**
  - *Silica* - Si\cdot SiO\cdot Si^+ SiO^- – lipid peroxidation, DNA damage
  - *Coal dust* – iron-generated carbon-centered radicals
  - *Asbestos* –
    - corcidolite & amosite – iron as part of crystal lattice
      \[ \text{Na}_2 \text{Fe}_3^{2+} \text{Fe}_2^{3+}\text{Si}_8 \text{O}_{22} (\text{OH})_2 \]
    - chrysotile – iron contaminant on fiber surface
  - *Fossil fuel combustion*
    - iron and other transition metals → \cdot \text{OH}
    - Polycyclic aromatic hydrocarbon

- **Cellular ROS**
  - *Mitochondria* - unpaired electrons from semiquinones of the e-transport chain during normal respiration - donated to molecular oxygen to form superoxide radicals.
  - *NADPH oxidase* - multienzyme complex → superoxide radicals
5 pathways to ROS

- Redox metals generating superoxide, peroxide and hydroxyl radicals
- Quinones on particle surface redox cycle to produce semiquinone radical which then go on to produce superoxide and hydroxyl radical
- Bacterial endotoxins from bacteria on particle surface trigger inflammation responses = infiltration immune cells in tissue (macrophages) → up-regulation cytb245 NADPH oxidase → superoxide → hydroxyl radicals
- Polyaromatic hydrocarbons undergo bio-transformations via cytP450 to yield reactive electrophiles and superoxide → hydroxyl radicals
- Particle surface itself causes oxidative stress (mechanism unknown)
Fenton reaction

\[
\text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{3+} + \text{O}_2^-
\]

\[
2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{HO}^- + \text{OH}^-
\]

Similar processes for other redox metals
• Cu(II)/Cu(I)
• V(V)/V(IV)
• Cr(VI)/Cr(V)
• QH°/Q
5 pathways to ROS

• Redox metals generating superoxide, peroxide and hydroxyl radicals

• Quinones on particle surface redox cycle to produce semiquinone radical which then go on to produce superoxide and hydroxyl radical

• Bacterial endotoxins from bacteria on particle surface trigger → inflammation responses = infiltration immune cells in tissue (macrophages) → up-regulation cytP245 NADPH oxidase → superoxide → hydroxyl radicals

• Polyaromatic hydrocarbons undergo bio-transformations via cytP450 to yield reactive electrophiles and superoxide → hydroxyl radicals

• Particle surface itself causes oxidative stress (mechanism unknown)
Stimulation of ROS production by alveolar macrophages harvested from particle exposed rats

<table>
<thead>
<tr>
<th>Particle</th>
<th>Exposure</th>
<th>Chemiluminescence (Increase From Control)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diesel exhaust</td>
<td>IT (5 mg/kg BW); 3 days post</td>
<td>2.3-Fold zymosan-stimulated</td>
<td>Yang et al. (2001)</td>
</tr>
<tr>
<td>Carbon black</td>
<td>IT (5 mg/kg BW); 3 days post</td>
<td>2.3-Fold zymosan-stimulated</td>
<td>Yang et al. (2001)</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>IT (5 mg/100 g BW); 1 day post</td>
<td>3.0-Fold zymosan-stimulated</td>
<td>Blackford et al. (1997)</td>
</tr>
<tr>
<td>Carbonyl iron</td>
<td>IT (5 mg/100 g BW); 1 days post</td>
<td>1.6-Fold zymosan-stimulated</td>
<td>Blackford et al. (1997)</td>
</tr>
<tr>
<td>Residual oil fly ash (ROFA)</td>
<td>IT (1 mg/100 g BW); 1 days post</td>
<td>9–11-Fold PMA-stimulated</td>
<td>Antonini et al. (2004), Lewis et al. (2003)</td>
</tr>
<tr>
<td>Residual oil fly ash (ROFA)</td>
<td>IT (2 mg/rat); 1 days post</td>
<td>3.0-Fold zymosan-stimulated</td>
<td>Nurkiewicz et al. (2004)</td>
</tr>
<tr>
<td>Welding fume (manual metal arc/stainless sleet electrode)</td>
<td>IT (5 mg/rat BW); 3 days post</td>
<td>2.5-Fold zymosan-stimulated</td>
<td>Antonini et al. (2004)</td>
</tr>
</tbody>
</table>

IT – intratracheal instillation

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
RNS

NO\(^{-}\) via NO synthase (NOS) from L-arginine

- 3 NOS enzymes – 2 constitutive, 1 inducable (iNOS)
- Elevated levels NO\(^{-}\) in alveolar magrophages recovered from silica-exposed subjects (animals and humans)
  - Coal Dust
  - Asbestos
  - Titanium dioxide
  - Diesel exhaust

- Strong correlation between increased radical levels and iNOS m-RNA & protein levels
Maintaining the redox balance
Physiological consequences

Respiratory System
Cardiovascular system
Immune system
Brain
PM leads to small airway remodelling

Vancouver
PM$_{10}$ = 25 μg/m$^3$

Mexico city
PM$_{10}$ = 66 μg/m$^3$

Low magnification
High magnification

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Airway remodelling reflected in increased procollagen expression in tracheal explants

Effects of iron loading on fine titanium dioxide (120 nm) induced expression of procollagen
Effects of PM on cardiovascular system

Particle Toxicology ed. Donaldson & Borm, CRC Press, 2007
Effect of PM on immune system

- Reduced alveolar macrophage phagocytosis and Impaired pulmonary clearance of inhaled bacteria
  - Intracellular overloading of particulate
  - Direct toxicity of internalized particles
  - Co-production of suppressive mediators (prostaglandins, corticosteroids)
  - Cytoskeletal dysfunction (impaired phagosome transport and increased cytoskeletal stiffness)
- Depressed expression levels Toll-like receptor (TLR2)
  - recognize mycobacterial components and other tuberculosis like organisms
- Enhanced allergic and asthmatic responses resulting from ROS effects
Impairment of alveolar macrophage phagocytosis by particles

White arrows – 250 nm CB particles  Black arrows – latex indicator beads

Macrophages which:

a. Could still take up indicator beads after taking up particles (PM)
b. Were unable to take up beads following uptake of particles (PIP)
c. Could not take up beads or particles (NPM)
d. Could take up beads but not particles (PC)

Impairment of alveolar macrophage phagocytosis by particles

% macrophages unable to phagocytose particles or latex beads

PARTICLE TYPES

PARTICLE DOSE (μg/mm²)
- 0.0975
- 0.39
- 0.195
- 0.78

Effect of PM on immune system

• Reduced alveolar macrophage phagocytosis and Impaired pulmonary clearance of inhaled bacteria
  – Intracellular overloading of particulate
  – Direct toxicity of internalized particles
  – Co-production of suppressive mediators (prostaglandins, corticosteroids)
  – Cytoskeletal dysfunction (impaired phagosome transport and increased cytoskeletal stiffness)

• Depressed expression levels Toll-like receptor (TLR2)
  – recognize mycobacterial components and other tuberculosis like organisms

• Enhanced allergic and asthmatic responses resulting from ROS effects
PM and human brain pathology

Mexico city environment:
• 2000 km²
• 20 million residents
• 35,000 industrial facilities
• 3.5 million vehicles
• 2.6 million tons of particulate and gaseous air pollutants (ozone and PM$_{<2.5}$) annually
• Limited natural ventilation
Brain Pathology

• In Mexico city dogs
  – Up-regulation of COX2 in olfactory bulb, frontal cortex, hippocampus
  – Activation of neuronal NFκB and elevated levels of iNOS
  – Breakdown of blood brain barrier
  – Accumulation of Aβ42 in neurons, glial cells and blood vessels

• In Mexico city lifelong residents *versus* residents of low pollution cities
  – Elevated COX2 expression in frontal cortex & hippocampus
  – Elevated DNA damage in frontal cortex
  – Elevated levels of Aβ42 in frontal cortex and hippocampus
  – Presence of Aβ42 plaques in frontal cortex
  – Breakdown of blood brain barrier – extravascular red blood cells
Assessment of PM toxicology *in vitro*

Can be done in any lab equipped to do cell culture and standard biochemical analyses

- Cytotoxicity - MTT assay - tetrazole dye turns purple by activity of mitochondrial reductases
- Cell stimulation / proliferation – incorporation of 5-bromo 2’-deoxyuridine into DNA – assayed by immunostaining
- Cytokine production – ELISA (enzyme-linked immuno assay)
- Oxidative stress – measurement of intracellular GSH/GSSG
- Alteration of cell signalling cascades
  - Changes in levels of intracellular Ca, MAPK phosphorylation
  - transcription factor NFkB, AP-1 activation
- Genotoxicity – assays for mammalian cell transformation, DNA breaks, inhibition of DNA repair, etc
Advice for the Health Council of the Netherlands & Dutch Cabinet

• Health Significance of Nanotechnologies, 2006
  – [www.healthcouncil.nl](http://www.healthcouncil.nl)

• White paper on Risk Governance-towards an integrative approach
  – Geneva International Risk Governance Council, 2005
### Examples from medical applications of nanoparticles

<table>
<thead>
<tr>
<th>Nanotechnological applications in</th>
<th>State of development</th>
<th>Possible benefits</th>
<th>Points of attention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medicine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scientific research</td>
<td>Already fully underway</td>
<td>New research possibilities</td>
<td>Nanoparticles possibly toxic for humans and the environment</td>
</tr>
<tr>
<td>Pharmacological research</td>
<td>Not much yet</td>
<td>More efficient screening candidate substances, fewer laboratory animals required</td>
<td>Nanoparticles possibly toxic for humans and the environment</td>
</tr>
<tr>
<td>Antiseptic surfaces</td>
<td>First (clinical) applications</td>
<td>More effective disinfection, resistance less likely, fewer chemicals required</td>
<td>Nanoparticles possibly toxic for humans and the environment</td>
</tr>
<tr>
<td>Diagnostics <em>in vitro</em></td>
<td>First clinical applications</td>
<td>High sensitivity, earlier detection, cheaper</td>
<td>Nanoparticles possibly toxic for humans and the environment, Increase in gap between detection and therapy, Privacy, Doctor-patient relationship</td>
</tr>
<tr>
<td>Diagnostics <em>in vivo</em> (imaging)</td>
<td>First applications in patients</td>
<td>High sensitivity, earlier detection</td>
<td>Nanoparticles possibly toxic for humans and the environment, Increase in gap between detection and therapy, Privacy</td>
</tr>
<tr>
<td>Drug delivery systems</td>
<td>First (still relatively simple) systems already used on patients</td>
<td>More effective medicines, fewer side effects</td>
<td>Nanoparticles possibly toxic for humans and the environment, Just distribution</td>
</tr>
<tr>
<td>Therapy using nanoparticles</td>
<td>First applications in patients (antibiotics, experimental treatment of brain tumours)</td>
<td>More effective medicines</td>
<td>Nanoparticles possibly toxic for humans and the environment, Just distribution</td>
</tr>
<tr>
<td>Tissue engineering</td>
<td>First simple products on the market (bone formation, artificial skin)</td>
<td>More rapid repair of broken bones and skin wounds, new organs</td>
<td>Nanoparticles possibly toxic for humans and the environment, Just distribution</td>
</tr>
<tr>
<td>Coatings on passive implants</td>
<td>Still in development, partially in clinical test phase</td>
<td>More wear resistant, smoother and more biocompatible</td>
<td>Nanoparticles possibly toxic for humans and the environment</td>
</tr>
<tr>
<td>Active implants</td>
<td>Already used clinically (e.g. pacemaker, cochlear implant)</td>
<td>Better function, more biocompatible</td>
<td>Nanoparticles possibly toxic for humans and the environment, Ethical questions concerning autonomy and enhancement, Just distribution, Arms race</td>
</tr>
</tbody>
</table>
The risk handling chain

Management sphere:
Decision on & implementation of actions

- Risk management
  - Implementation
    - Option realisation
    - Monitoring & control
    - Feedback from risk management practice
  - Decision making
    - Option identification & generation
    - Option assessment
    - Option evaluation & selection

Assessment sphere:
Generation of knowledge

- Pre-assessment
  - Problem framing
  - Early warning
  - Screening
  - Determination of scientific conventions

Risk appraisal
- Risk assessment
  - Hazard identification & estimation
  - Exposure & vulnerability assessment
  - Risk estimation
  - Concern assessment
  - Risk perceptions
  - Social concerns
  - Socio-economic impacts

Communication

Tolerability & Acceptability judgement

- Risk evaluation
  - Judging the tolerability & acceptability
  - Need for risk reduction measures

- Risk characterisation
  - Risk profile
  - Judgement of the seriousness of risk
  - Conclusions & risk reduction options
Categorization of risk problems arising from or reinforced by nanotech applications (IRGC)

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Risk problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>Privacy</td>
</tr>
<tr>
<td></td>
<td>Self-tests</td>
</tr>
<tr>
<td></td>
<td>Toxicity - readily degradable np</td>
</tr>
<tr>
<td>Complex</td>
<td>Sustainability</td>
</tr>
<tr>
<td></td>
<td>Gap between rich and poor</td>
</tr>
<tr>
<td>Uncertain</td>
<td>Toxicity - poorly degradable np</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>Gap between diagnostics and therapy</td>
</tr>
<tr>
<td></td>
<td>Advanced home-care technology</td>
</tr>
<tr>
<td></td>
<td>Enhancement</td>
</tr>
<tr>
<td></td>
<td>Some military applications</td>
</tr>
</tbody>
</table>
Dealing with “uncertain”

- Precaution-based
  - Using hazard characteristics as tools for risk estimates
    - persistence, ubiquity, etc.
  - Tools: containment
    - ALARA – As Low As Reasonably Available
    - BACT – Best Available Control Technology

- Resilience focused
  - Improving ability to cope with surprises
  - Avoid high vulnerability
  - Allow flexible response
  - Preparedness for adaptation
“All things are poison and nothing is without poison, only the dose permits something to be not poisonous”
(Paracelsus 1493-1541)

Thank you
Effects of PM on cardiovascular system

- Impaired smooth muscle relaxation, impaired vasodilatation & decreased blood flow due to loss of NO via $\text{O}_2^- + \text{NO} \rightarrow \text{OONO}^-$
- Impaired degradation of intravascular fibrin due to reduced release of t-PA from endothelium (diesel study)
- Up-regulation of tissue factor expression $\rightarrow$ thrombus formation
- Platelet aggregation $\rightarrow$ arterial & venous thrombus formation
- Direct effect on autonomic nervous system causing reduced control in the variation of the interval between consecutive heart-beats