Inhalation of nanoparticles and health effects

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Urban air particles - a health hazard

- Extensive epidemiological studies have demonstrated an association between air pollution particles and mortality and morbidity of lung- and cardiovascular diseases
  - Acute exposure
  - Chronic exposure

Much focus on the importance of the nano-sized fraction (ultrafine) of urban air particles
Ultrafine particles: particles with aerodynamic diameter lower than 100 nm

Engineered nanoparticles: with at least one dimension lower than 100 nm.

Suh et al. (in press)
Deposition of nanoparticles in the respiratory system

NPs in lung: different deposition according to particles dimension
Other particle characteristics important for adverse health effects

- Biopersistence in the lung
- Surface area/ reactivity
- Shape (fiber)
- Binding of proteins in the lining fluids of the lung
- Agglomeration/ aggregation properties

No single particle characteristic as a hallmark indicator for fate and pulmonary toxicity has been identified
Inflammation
– Crucial for health effects induced by particles

Release of inflammatory mediators (eg IL-6)

Dilatation & leakage from capillaries

Attraction of immune cells

Production of reactive oxygen species (ROS)

Development of tissue damage
Lung inflammation

• Lung inflammation plays a key role in development and aggravation of lung diseases such as asthma, chronic obstructive pulmonary disease, silicosis/fibrosis and during lung infections

• Barrier disruption with increased particle translocation
Mechanisms of disease and death induced by particulate matter

Particles

- Particles and components enter the circulation
- Release of inflammatory mediators to the circulation

Particles → Inflammation responses in lung

Inflammation responses in lung → Lung disease

Lung disease → Cardiovascular diseases

- Stress responses
- Remodulation of the heart
- Changes of heart rate variability
- Blood coagulation
- Atherosclerosis
Lung exposure to nanoparticles

- Human inhalation chambers
  - Mainly diesel exhaust particles
- Animal inhalation studies
  (acute, subacute, subchronic, chronic)
- Intratracheal instillation
  - Similar effects as with inhalation studies
- Use of lung cells culture (*in vitro*)
Human inhalation chamber

• Diesel exhaust
  – High level of nanoparticles
  – Short term changes of lung and systemic inflammation, thrombogenesis, vascular function and brain activity
  – Uncertainty about which diesel exhaust component that is responsible

• Ultrafine carbon particles
  – Subtle effects on vascular endothelial function
  – Effects on heart rate variability

• Zinc oxide nanoparticles
  – No acute systemic effects in healthy subjects
Animal inhalation studies

• **Acute**
  – Nanosilver (18-20 nm): No significant effects (750 μg/m³) (Sung et al 2011)
  – Nickel nanoparticles: Endotelial disruption and impaired vasorelaxation from 100 μg/m³ (Cuevas et al. 2010)

• **Subacute** (OECD 412)
  – Amorphous silica (38 nm): Pulmonary and cardiovascular alterations in old rats (Chen et al. 2008)
  – Nanosilver (~10 nm): Minimal inflammatory response and cytotoxicity (Stebounova et al. 2011)

• **Subchronic** (OECD 413)
  – Ultrafine TiO₂ (21 nm): Prolongation of lung retention and acute inflammatory response (Ferin et al. 1992)
  – Ultrafine TiO₂: Rats developed a more severe inflammatory response than mice and hamsters (Bermudez et al. 2004)
  – Nanosilver (18-19 nm): Lesions in rat lung and liver, NOAEL 100 μg/m³ (Sung et al 2009)
  – Gold nanoparticles (4-5 nm): Small changes in lung histopathology and function in high-dose rats, NOAEL 0.38 μg/m³ (Sung et al 2011)
Higher lung inflammatory response after exposure to TiO$_2$-D (21 nm) than TiO$_2$-F (250 nm)

**TABLE 4**

*Polymorphonuclear leukocytes in lavage fluid during and after 3 mo of inhalation*

<table>
<thead>
<tr>
<th>Time from Start of Exposure (wk)</th>
<th>Control</th>
<th>TiO$_2$-D</th>
<th>TiO$_2$-F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. x 10$^{-3}$</td>
<td>95% CI</td>
<td>No. x 10$^{-3}$</td>
</tr>
<tr>
<td>4</td>
<td>0.92 ± 0.3</td>
<td>(0.65, 1.19)</td>
<td>3.59 ± 1.0</td>
</tr>
<tr>
<td>8</td>
<td>0.61 ± 0.3</td>
<td>(0.39, 0.83)</td>
<td>47.16 ± 8.5</td>
</tr>
<tr>
<td>12</td>
<td>0.68 ± 0.3</td>
<td>(0.40, 0.95)</td>
<td>87.38 ± 19.2</td>
</tr>
<tr>
<td>41</td>
<td>0.82 ± 0.3</td>
<td>(0.61, 1.04)</td>
<td>12.84 ± 5.7</td>
</tr>
<tr>
<td>64</td>
<td>1.01 ± 0.3</td>
<td>(0.51, 1.43)</td>
<td>2.63 ± 1.7</td>
</tr>
</tbody>
</table>

*Definition of abbreviation: CI = confidence interval.
*Values are mean ± SD; n = 5 to 8 per group.*

Ferin et al.1992, Am J Respir Cell Mol Biol
Subchronic inhalation of gold NPs (4-5 nm)

Table 11: Histopathologic observations for female rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Control</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>10</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>No microscopic findings</td>
<td>9/10</td>
<td>90</td>
<td>9/10</td>
<td>90</td>
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<tr>
<td>Abnormality</td>
<td>1/10</td>
<td>10</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2/10</td>
<td>20</td>
<td>3/10</td>
<td>30</td>
</tr>
<tr>
<td>Liver Sign</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal minimum</td>
<td>0/10</td>
<td>0</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>mild</td>
<td>1/10</td>
<td>10</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>Necrosis</td>
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<td>Focal minimum</td>
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<td>0/10</td>
<td>0</td>
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<tr>
<td>Vacuolization</td>
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<tr>
<td>Hepatocellular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>minimum</td>
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<td>0</td>
<td>0/10</td>
<td>0</td>
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<tr>
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<td>0</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>Lungs Sign</td>
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<td>No microscopic findings</td>
<td>10/10</td>
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<td>100</td>
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<tr>
<td>Abnormality**</td>
<td>0/10</td>
<td>0</td>
<td>0/10</td>
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<tr>
<td>Inflammation**</td>
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</tr>
<tr>
<td>Focal minimum</td>
<td>0/10</td>
<td>0</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>mild</td>
<td>0/10</td>
<td>0</td>
<td>0/10</td>
<td>0</td>
</tr>
</tbody>
</table>

**, p < 0.01, compared with control. Abnormality** refers to such changes as inflammation, vacuolization and necrosis upon histopathological examination. One abnormality is counted even if inflammation and necrosis are present simultaneously.
Inhalation of carbon nanotubes (SWCNT) induced both pro-inflammatory and fibrogenic responses

- Do carbon nanotube have hazards similar to asbestos?
- Asbestos causes fibrosis and mesothelioma (cancer in the pleural mesothelia)


Donaldson et al. 2010 PFT
Instillation of particles

- Much used surrogate for inhalation route
- Predict the potential for inhaled particles to produce lung hazard effects
- Similar effects as with inhalation studies

Donaldson et al. 2002, J Aero Med
Instillation of metal oxide nanoparticles

- Equal-surface-area doses
- The different NPs have different types of inflammation
- NPs can not be viewed as a single hazard entity

CHO et al 2010, EHP
Instillation of carbon nanotubes (MWCNT)

Muller et al 2005, Tox Appl Pharm
Silica nanoparticles 30 nm in an epithelial lung culture

-/- without BSA in both stock solution and in media
+/- BSA in stock solution, not in media
++/+ BSA in stock solution and in media (0.1%)

Gualtieri M et al 2011, Nanotox
Cytokine responses without uptake of silica nanoparticles (50 nm labelled with rhodamine)

IL-6

Gene expression (fold increase) vs Time of exposure (min)

Time of exposure: 0, 90, 180, 360, 600, 1200 min

Gene expression (fold increase)

Time of exposure (min)

IL-8

Gene expression (fold increase) vs Time of exposure (min)

Gene expression (fold increase)

Time of exposure (min)

Confocal microscopy

3 hours

Gualtieri M et al 2011, Nanotox
Inflammatory responses by carbon nanoparticles in lung cell culture enhanced effects in cardiac cell culture.
Potentiating effects of NPs on ongoing inflammatory processes?

- Adverse health effects of urban particles (PM) effects are primarily seen in individuals with pre-disposing factors, such as asthma, COPD, atherosclerosis - diseases known to involve inflammatory processes.

- How is potentiating effects of NPs with such pre-disposing factors?
  - Allergy-elicited lung inflammation?
Intratracheal administration of ovalbumin (1 µg every 2 week for 6 weeks), carbon black (50 µg every week for 6 weeks); 

Inoue et al 2005
Conclusions/considerations

- Nanoparticles have without doubt a potential to induce health effects and inflammation seems to be crucial.
- Nanoparticles have to be assessed separately in the hazard identification.
- However, the experimental studies have been performed with high concentrations of NPs.
- The exposure levels are critical for the human health risk assessment.
- Different nanoparticles may augment lung inflammation related to pre-existing lung diseases such as allergy, which may induce inflammatory response at lower concentrations of NPs than in "healthy" individuals - more relevant in relationship to exposure levels?
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