Health effects of inhaled nanomaterials

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Nanosafety at the National Research Centre for the Working Environment, Copenhagen, Denmark

- Government research institute under the Ministry of Employment
- Nanosafety as strategic research area since 2005
- At present 35-40 persons in nanosafety research
- Advisors for the Danish Working Environment Authorities, EPA, EU, OECD, WHO
- Past and present partners in 25 EU projects on (nano)particle safety
Health effects of inhaled nanomaterials – the short version:

Are nanoparticles more hazardous by inhalation compared to larger particles with the same chemical composition?

Yes! (by mass)

Is there occupational and/or environmental exposure to nanomaterials?

Yes

Are exposure levels high enough to pose a potential health risk?

Yes

✓ Need for regulation of (occupational) exposure
Concern about nanomaterials: Association between particulate air pollution and mortality

Direct correlation between mortality and particle concentration ($PM_{2.5}$):

7 deaths/100 000 persons/year/µg/m$^3$ PM 2.5
RISK = Exposure x Hazard
The vision for safe use of nanomaterials

Research

Mechanism-based understanding of toxicological effect

Evidence-based risk assessment. Prediction of the toxicological effects on the basis of physico-chemical properties

Grouping and ranking for regulation
Safe-by-design for innovation

Safe use of engineered and process-generated nanomaterials
Safe-by-design:

- Asbestos
- Paint based on organic solvents
- Mineralwool
- Painters syndrome
- Organic solvents
- Water-based paint
- MAL kodes
- Lung cancer
- Fibre-paradigm
The challenge: Many different nanomaterials with varying physico-chemical properties

- Particle chemistry
- Atom structure
- Shape / morphology
- Size (surface area)
- Functionalisation

"nano"
Inhalation is the most relevant exposure route for nanomaterials for consumers and workers.
Key questions

• What is going on? (the biological mechanisms of action)
• Which physico-chemical properties drive the toxic responses?

Focus on:
• Cardiovascular disease
• Cancer
• Risk assessment
Aerodynamic size in air is the important predictor of pulmonary deposition during inhalation exposure.

Nanomaterials aggregate in air.

Biological relevant size fractions (CEN, 1992):

- Total deposition
- Alveolar
- Head Deposition (larynx)
- Thoracic deposition
Low clearance of nanoparticles from the lung

Airways

- Fine particles
  - Particle deposition and removal by mucociliary clearance
  - Particle phagocytosis by macrophage

- Nanoparticles
  - INFLAMMATION
    - Release of mediators
    - ROS

- Ultrafine particles
  - Inflammatory cells and inflammatory mediators

Marianne Dybdahl
Even lower clearance of High Aspect Ratio nanomaterials

Airways

Fine particles

Particle deposition and removal by mucociliar
Particle clearance
Phagocytosis by macrophage

Nanoparticles
Ultrafine particles

INFLAMMATION
Release of mediators
ROS

Carbon nanotubes

INFLAMMATION
Release of inflammatory mediators
ROS

Frustrated phagocytosis
Very low clearance

Marianne Dybdahl
Inhaled TiO$_2$ nanoparticles in the lung are removed very slowly

Mice inhaled 40 mg/m$^3$ nanosized TiO$_2$ 1 hour daily for 11 days.

TiO$_2$ content in lung tissue was measured by ICP-MS.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Days after exposure</th>
<th>N</th>
<th>TiO$_2$ in lung (mg/kg) (mean ± sd)</th>
<th>Procent of deposited dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO$_2$</td>
<td>5</td>
<td>3</td>
<td>63 ± 10</td>
<td>24%</td>
</tr>
<tr>
<td>Air</td>
<td>5</td>
<td>3</td>
<td>&lt; 8</td>
<td></td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>25</td>
<td>3</td>
<td>55 ± 30</td>
<td>21%</td>
</tr>
<tr>
<td>Air</td>
<td>25</td>
<td>3</td>
<td>&lt; 1</td>
<td></td>
</tr>
</tbody>
</table>

Hougaard et al, PF&T, 2010
Inhalation of nano-TiO$_2$ results in long lasting inflammation

After 5 days

Types and numbers of cells in lung fluid

Hougaard et al, 2010
Global gene expression in lung tissue after inhalation of TiO$_2$ NPs (day 5): acute phase response!

The most differentially regulated genes

<table>
<thead>
<tr>
<th>Acute phase reactants</th>
<th>$P$ value</th>
<th>Fold change$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum amyloid A1</td>
<td>0.00</td>
<td>2.24</td>
</tr>
<tr>
<td>Serum amyloid A3</td>
<td>0.00</td>
<td>4.71</td>
</tr>
<tr>
<td>Complement protein C3</td>
<td>0.00</td>
<td>1.37</td>
</tr>
<tr>
<td>Complement component 1, s (C1s)</td>
<td>0.00</td>
<td>1.28</td>
</tr>
<tr>
<td>Complement component 3a receptor 1 (C3ar1)</td>
<td>0.00</td>
<td>1.15</td>
</tr>
<tr>
<td>Complement component 1, q beta polypeptide (C1qb)</td>
<td>0.00</td>
<td>1.30</td>
</tr>
<tr>
<td>Complement component 1, r subcomponent (C1r)</td>
<td>0.00</td>
<td>1.31</td>
</tr>
<tr>
<td>Complement component C1RB (C1rb)</td>
<td>0.00</td>
<td>1.21</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.01</td>
<td>2.05</td>
</tr>
<tr>
<td>Coagulation factor II (F2)</td>
<td>0.01</td>
<td>1.72</td>
</tr>
<tr>
<td>Mannose binding protein</td>
<td>0.02</td>
<td>1.70</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.01</td>
<td>1.79</td>
</tr>
<tr>
<td>apoA1</td>
<td>0.01</td>
<td>1.51</td>
</tr>
<tr>
<td>apoAII</td>
<td>0.03</td>
<td>1.61</td>
</tr>
<tr>
<td>alpha2-HS glycoprotein</td>
<td>0.00</td>
<td>1.85</td>
</tr>
<tr>
<td>S100A8 (calgranulin A)</td>
<td>0.01</td>
<td>$-1.85$</td>
</tr>
<tr>
<td>Serpina3n</td>
<td>0.00</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Gene names in bold indicate FDR adjusted $P$ value $> 0.05$.

$^a$Average fold change compared with matched controls.
Acute phase response is causally linked to cardiovascular disease

- The acute phase response is the systemic response to acute and chronic inflammatory states caused by fx bacterial infection, trauma and infarction
- Conditions that entail acute phase response are associated with risk of cardiovascular disease including HIV infection, asthma, air pollution exposure
- Acute phase protein Serum Amyloid A is directly implicated in atherosclerosis (Thompson et al, 2018)

![Graph showing the change in plasma concentration of various proteins over time after an inflammatory stimulus.]

**Figure 1.** Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus. Modified from Gitlin and Colten with the permission of the publisher.

*Gabay and Kushner, 1999*
SAA: an acute phase protein that directly promotes foam cells formation and atherosclerosis

- Mice have 3 inducible SAA isogenes (\textit{Saa}1, \textit{Saa}2, \textit{Saa}3)
- SAA induces foam cell formation in macrophages (Lee, 2013).
- Over-expression of SAA3 or SAA1 increases plaque progression (Thompson 2018).
- Inactivation (KO) of all SAA isogenes results in reduced plaque progression (Thompson 2018)

[1] Lee et al, 2013, BBRC
Acute phase proteins CRP & SAA are associated with risk of cardiovascular disease in prospective epidemiological studies

Nurses’ Health Study: 120,000 participants

**TABLE 3. RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>QUARTILE OF PLASMA LEVEL</th>
<th>P VALUE FOR TREND</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median — mg/dl</td>
<td>0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.0</td>
<td>2.1 (1.0–4.5)</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median — mg/dl</td>
<td>0.25</td>
<td>0.43</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.0</td>
<td>1.8 (0.9–3.6)</td>
</tr>
</tbody>
</table>

Ridker et al, NEJM, 2000
Proposed mechanism-of-action for (nano)particle-induced atherosclerosis

Inhaled particles promote atherosclerosis via acute phase response

New knowledge

Established fact

Inhalation of particles below occupational exposure limits → Systemic circulation of acute phase proteins → Atherosclerosis → Cardiovascular diseases

Inflammatory & acute phase response

National Research Centre for the Working Environment
Dose dependent induction of pulmonary acute phase response by different nanomaterials including CNTs

All assessed nanomaterials induce dose-dependent acute phase response in mice
Humans and mice induce acute phase response
Rats have a fundamentally different acute phase response
Human relevance: Inhalation of ZnO induces dose-dependent acute phase response in human volunteers

Study set up:
- 16 volunteers
- Exposed to 0, 0.5, 1 or 2 mg/m³ ZnO particles for 4 h
- OEL: 5 mg/m³ for 8 h
- Acute phase response proteins CRP and SAA

Acute phase response induced at ZnO concentrations well below current OEL

Adapted from Monsé et al Part Fibre Toxicol. 2018 15(1):8
Lung response following pulmonary exposure to two very different CNTs: acute phase response

Strongest transcriptional signal: acute phase response

Property-response comparison. Change in the expression of genes in five highly regulated selected GO biological processes relative to control mice. The genes are organized by the size of changes in expression after exposure to CNTLarge at the 162 μg dose.

SS Poulsen et al TAAP 2015
Close correlation between acute phase response and inflammation across doses, time points, material types, and exposure type.

Acute phase response (Saa3 mRNA levels) vs. Inflammation (#neutrophils in BAL)

- TiO2 and CB instillations
- TiO2 and CB inhalation
- Biofuel dust instillation
- Controls instillation
- Instillation Mitsui
- Instillation Thomas Swan
- Instillation Sigma long

Saber AT et al, Plos One, 2013
Surface area is a predictor of pulmonary inflammation (& acute phase response)

Deposited surface area of particles predicts pulmonary inflammation

Donaldson et al., 2002

Deposited surface area of CNTs predicts inflammation

SS Poulsen et al, 2016 Nanotoxicology
A number of nanomaterials are classified as carcinogenic or possibly carcinogenic by IARC

- Diesel engine exhaust (1)
- Gasoline engine exhaust (2B)
- Welding fumes (1)
- Carbon black (2B)
- Titanium dioxide nanoparticles (2B)
- Mitsui-7 carbon nanotubes (2B)
Proposed key characteristics of carcinogens

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Examples of relevant evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is electrophilic or can be metabolically activated</td>
<td>Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts</td>
</tr>
<tr>
<td>2. Is genotoxic</td>
<td>DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)</td>
</tr>
<tr>
<td>3. Afters DNA repair or causes genomic instability</td>
<td>Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)</td>
</tr>
<tr>
<td>4. Induces epigenetic alterations</td>
<td>DNA methylation, histone modification, microRNA expression</td>
</tr>
<tr>
<td>5. Induces oxidative stress</td>
<td>Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)</td>
</tr>
<tr>
<td>6. Induces chronic inflammation</td>
<td>Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production</td>
</tr>
<tr>
<td>7. Is immunosuppressive</td>
<td>Decreased immunosurveillance, immune system dysfunction</td>
</tr>
<tr>
<td>8. Modulates receptor-mediated effects</td>
<td>Receptor in/activation (e.g., ER, PPAR, AR) or modulation of endogenous ligands (including hormones)</td>
</tr>
<tr>
<td>9. Causes immortalization</td>
<td>Inhibition of senescence, cell transformation</td>
</tr>
<tr>
<td>10. Alters cell proliferation, cell death or nutrient supply</td>
<td>Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis</td>
</tr>
</tbody>
</table>

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Smith MT EHP, 2016, PMID: 26600562
Mutagenicity of carbon nanoparticles: Surface mediated generation of Reactive Oxygen Species

Carbon black (black pigment)

- Carbon black is classified as possibly carcinogenic (2B) by IARC
- Carbon black is mutagenic in vivo and in vitro
- Carbon Black generates reactive oxygen species in vivo and in vitro
- The mutation spectrum is consistent with being caused by ROS
SOME CNTS MAY FIT ‘THE FIBRE PARADIGM’ – the reason for asbestos-induced lung cancer and mesotheliomas

Rule 1 – the fibre is thin enough to be inhaled (D<5 μm)
Rule 2 – the fibre should be so long that the macrophages cannot engulf the fibre (L>5-15 μm)
Rule 3 – the fibre should be insoluble in the lung
Sub-chronic inhalation study of carbon nanotubes

- 4 published sub-chronic (13 weeks) inhalation studies in the open literature

- Pauluhn, Tox Sci 2010:
  - Rats were exposed on 6 h/day, 5 days per week for 13 consecutive weeks to 0, 0.1, 0.4, 1.5, and 6 mg/m³.
  - Biomarkers assessed 4, 13 and 26 weeks after exposure

- Baytubes, MWCNT

- A number of biomarkers of lung inflammation and toxicity

Pauluhn 2010
Conclusions:
No effects observed at the lowest dose, 0.1 mg/m³
Half-life of CNT in lung ca. 1 year
Suggested OEL based on this work: 0.001 mg/m³
Dose-dependent CNT-induced cancer
A short and thin MWCNT did not cause cancer

- Dose-dependent induction of mesothelioma after IP injection (Takagi et al, 2012)
- One specific thick and long MWCNT (MWNT-7) is classified as possibly carcinogenic (2B) by IARC (WHO’s cancer research institute)
- Other thick and long MWCNTs also induce adenomas
- A short and thin MWCNT did not induce cancer after IP injection (20 mg/animal) in a two year cancer study

MWNT-7
Dose dependent MWCNT-induced lung cancer following inhalation exposure in rats

2 year inhalation study in rats
MWNT-7 MWCNT
• Male and female F344/DuCrIcrIj (N=50/group)
• 0, 0.02, 0.2, 2 mg/m$^3$
• 6h/day, 5 days/week for 104 weeks
• Aerosolised as single fibers
• Very low levels of contaminations

Results:
• 2 year inhalation study:
• 6 h/day, 5 days/week
• Lung cancer at 2 (M, F) and 0.2 mg/m$^3$ (M)
• Biopersistence: Accumulation of MWCNTs in lung throughout the exposure period
• Scaling to a risk acceptance level of 1:1000 (instead of 22%), and 40 years exposure (instead of 2), the acceptable air concentration would be 0.000 045 mg/m$^3$ = 45 ng/m$^3$

Kasai et al, PF&T, 2016, PMID: 27737701
Animal models of particle-induced lung cancer

- Only inhalation studies are (normally) used for risk assessment
- 2 year chronic inhalation studies in rats (no cancer in mice and hamsters)
- We want to estimate human lung cancer risk (0.1% - 0.001%) during 40 years of exposure based on groups of 50-100 rats exposed for 2 years (the detection limit is ca. 5% cancer), so the air concentrations should at least be 50 (5%/0.1%) x 20 (40 years/2 years) = 1000 fold higher in the animal studies
- Concern has been raised that impaired clearance (overload) will lead to over-estimation of cancer risk
Inhalation and instillation of two different CNTs gives the same dose response relationship for inflammation in rats

L. Gaté, et al.

Fig. 7. Correlation between the percentage of BALF neutrophils and the pulmonary MWCNT-deposited surface area. (a) Percent neutrophils in BAL fluid on day 1 (IT) or 3 (inhalation) as function of pulmonary deposited surface area (estimated in the lung at the end of exposure) of rats exposed to NM-401 and NM-403 by inhalation (full square) or IT (open square). (b) Percentage of neutrophils in BAL fluid on day 28 (IT) or 30 (inhalation) as a function of pulmonary deposited surface area (estimated in the lung at the end of exposure) of rats exposed to NM-401 and NM-403 by inhalation (full square) or IT (open square). The logarithmic curves “models” were established from the set of individual data.
### Nanosize matters: increased carcinogenic potency in chronic inhalation studies of TiO₂

<table>
<thead>
<tr>
<th></th>
<th>Air concentration</th>
<th>Total number of lung tumors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine, rutile TiO₂</td>
<td>250 mg/m³</td>
<td>39/151 rats = 26%</td>
<td>Lee et al (1985)</td>
</tr>
<tr>
<td>Nano TiO₂ (P25)</td>
<td>10 mg/m³</td>
<td>32/100 rats = 32%</td>
<td>Heinrich et al (1995)</td>
</tr>
</tbody>
</table>

Fine, rutile TiO₂ did not cause cancer in 2-year inhalation studies at 5, 10 or 50 mg/m³ (Muhle et al 1991, Lee et al, 1985)
Concern: the overload hypothesis

- Rats develop lung cancer after particle inhalation (2 years)
- Mice and hamsters do not
- Dose-dependent differences in particle retention
- Overload is seen for CB Printex90 at 50 mg/m³ (13 weeks)
- It is argued that rats therefore overestimate human cancer risk

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Particle Retention Half Times for Rats, Mice, and Hamsters following 13 Weeks of Exposure to Carbon Black</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rats</td>
</tr>
<tr>
<td>HSCb, 1 mg/m³</td>
<td>64⁴</td>
</tr>
<tr>
<td>HSCb, 7 mg/m³</td>
<td>115</td>
</tr>
<tr>
<td>HSCb, 50 mg/m³</td>
<td>No significant clearance</td>
</tr>
</tbody>
</table>

⁴Times are reported in days and were calculated from the retention curves using a two-parameter monoexponential decay function. For the mice, the half times were estimated from the retention curves in Figure 4b.

Elder et al, 2005, Tox Sci, 88(2) 614
Two year inhalation study in female (and male) rats exposed to diesel exhaust (DE), nano-TiO$_2$ (P25) and nano-CB (Printex90) (Heinrich et al., 1995).
Clearence half-times for CB (Printex90), TiO$_2$ (P25) and diesel exhaust particles in the two year inhalation study

**TABLE 9. Half-Times of Pulmonary Tracer Clearance ($^{59}$Fe)**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>3 mo</th>
<th>12 mo</th>
<th>18 mo</th>
<th>18 mo Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61</td>
<td>72</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>Diesel soot, 0.8 mg/m$^3$</td>
<td>94$^a$</td>
<td>121$^a$</td>
<td>221$^a$</td>
<td>n.d.$^b$</td>
</tr>
<tr>
<td>Diesel soot, 2.5 mg/m$^3$</td>
<td>119$^a$</td>
<td>254$^a$</td>
<td>272$^a$</td>
<td>n.d.$^b$</td>
</tr>
<tr>
<td>Diesel soot, 7.0 mg/m$^3$</td>
<td>330$^a$</td>
<td>541$^a$</td>
<td>687$^a$</td>
<td>1068$^a$</td>
</tr>
<tr>
<td>Carbon black</td>
<td>244$^a$</td>
<td>368$^a$</td>
<td>363$^a$</td>
<td>591$^a$</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>208$^a$</td>
<td>403$^a$</td>
<td>357$^a$</td>
<td>368$^a$</td>
</tr>
</tbody>
</table>

$^a$Significant at $p < .01$ (Dunnett’s test).

$^b$n.d., Not determined.

Heinrich et al, 1995
Comparison between rat inhalation and human epidemiology: Cumulative dose-response relationship between diesel exhaust exposure and lung cancer risk in three epidemiological studies

Figure 1. Predicted exposure–response curve based on a log-linear regression model using RR estimates from three cohort studies of DEE and lung cancer mortality. Individual RR estimates (based on HRs reported by Garshick et al. (2012) or ORs reported by Silverman et al. (2012) and Steenland et al. (1998)) are plotted with their 95% CI bounds indicated by the whiskers. The shaded area indicates the 95% CI estimated based on the log-linear model. The insert presents the estimates of the intercept and beta slope factor, the SE of these estimates, and the associated p-values.

Vermeulen et al, 2014, EHP
Risk estimate for DEP based on epidemiological evidence

The EU OEL for DEP is 50 ug/m³
The DECOS estimates that the exposure concentrations of respirable elemental carbon (REC) in the air, which serve as parameter for exposure to diesel engine exhaust powered by petroleum-diesel fuels, and which corresponds to:

• 4 extra death cases of lung cancer per 100,000 (target risk level), for 40 years of occupational exposure, equals to 0.011 µg REC/m$^3$,
• 4 extra death cases of lung cancer per 1,000 (prohibition risk level), for 40 years of occupational exposure, equals to 1.03 µg REC/m$^3$.
• The exposure levels are 8-hour time-weighted average concentrations.

Diesel exhaust as a case:

Diesel exhaust (DE) cause cancer, filtered DE does not.

In chronic (2 year) inhalation studies in rats,

- 2.5 mg/m³ DE induced cancer in 5.5% of the exposed rats
- 40 years instead of 2
- 20:10 000 instead of 5.5%:
- 4.5 ug/m³ will induce 20 excess lung cancer cases
- Epidemiological meta-analysis: 1 ug/m³ induce 17 lung cancers:10 000 exposed
- the chronic inhalation in rats does not over-estimate lung cancer risk for DE
It works: Reduction of particle exposure reduces mortality

EXAMPLE:

- Heating with coal in private households was banned in Dublin, Ireland in 1991:
- Black smoke levels in ambient air were reduced by 0.036 mg/m³
- Mortality rates were reduced by 75 per 100 000 person-years
- 77% cardiovascular (!)
- Effects were adjusted for death rates in the rest of Ireland
- Assuming 45 year work life and that we work 20% of the time, reducing occupational exposure with 1 ug/m³ should save 19 per 100.000 = 2/10 000

(Adapted from Clancy et al, Lancet, 2002)
Take home: Physico-chemical predictors of pulmonary toxicity for nanomaterials

- **Aerosolised size** will determine pulmonary deposition rate and distribution
- **Specific surface area** of retained dose predicts inflammation and acute phase response for insoluble particles
- **Shape** and **size** are strong determinants of clearance rates (and hence deposited dose)
- **High aspect ratio** may predict cancer risk
- **Surface-mediated generation of radicals/ROS** may result in genotoxicity
- **Dissolution/solubility** of low toxicity chemicals may lower toxicity
- Dissolution/release of toxic metals/chemicals may enhance toxicity

- Comparison of risk assessment of diesel exhaust suggests that chronic inhalation studies in rats do not overestimate human risk and can be used for risk assessment of nanomaterials
Acknowledgements and funding

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• The Danish NanoSafety Center 1 and 2, grant# 20110092173-3.
• The European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement # 247989 (Nanosustain).
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• European Union’s Horizon 2020 research and innovation programme under grant agreement No. 686098 (SmartNanoTox).
• European Union’s Horizon 2020 research and innovation programme under grant agreement No 686239 (caLIBRAt e)
Example of insoluble particles: Association between air pollution levels and CRP

Association between PM2.5 and CRP levels in 30 000 Taiwanese:
0.014 mg/L CRP pr 5 ug/m³ increment in PM 2.5

Table 2. Associations of CRP with long-term exposure to PM2.5 in baseline analysis among Taiwanese adults:

<table>
<thead>
<tr>
<th></th>
<th>Crude model</th>
<th></th>
<th>Model 1a</th>
<th></th>
<th>Model 2a</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>% difference</td>
<td>P</td>
<td>Mean</td>
<td>% difference</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>(SE)²</td>
<td>(95% CI)</td>
<td></td>
<td>(SE)²</td>
<td>(95% CI)</td>
<td></td>
</tr>
<tr>
<td>Men (N = 17761)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>0.96 (1.01)</td>
<td>Ref</td>
<td>Ref</td>
<td>0.92 (1.03)</td>
<td>Ref</td>
<td>0.98 (1.05)</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>0.96 (1.01)</td>
<td>0.34 (–1.36, 2.05)</td>
<td>0.69</td>
<td>0.95 (1.03)</td>
<td>1.18 (–0.01, 2.89)</td>
<td>0.18</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>0.98 (1.01)</td>
<td>0.93 (–0.77, 2.62)</td>
<td>0.28</td>
<td>0.97 (1.03)</td>
<td>1.94 (0.23, 3.65)</td>
<td>0.03</td>
</tr>
<tr>
<td>4th quartile</td>
<td>1.07 (1.01)</td>
<td>4.78 (3.09, 6.48)</td>
<td>&lt; 0.001</td>
<td>1.03 (1.03)</td>
<td>4.71 (3.01, 6.41)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>trend test³</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.001</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>every 5 μg/m³ increment⁴</td>
<td>-</td>
<td>1.42 (0.96, 1.87)</td>
<td>&lt; 0.001</td>
<td>-</td>
<td>1.35 (0.89, 1.81)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Women (N = 12273)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>0.76 (1.02)</td>
<td>Ref</td>
<td>Ref</td>
<td>0.70 (1.06)</td>
<td>Ref</td>
<td>0.85 (1.07)</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>0.68 (1.02)</td>
<td>–4.79 (–0.01, 2.42)</td>
<td>&lt; 0.001</td>
<td>0.67 (1.06)</td>
<td>–1.52 (–0.01, 0.78)</td>
<td>0.19</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>0.70 (1.02)</td>
<td>–3.54 (–5.91, -1.16)</td>
<td>0.004</td>
<td>0.70 (1.06)</td>
<td>–1.95, 2.70</td>
<td>0.75</td>
</tr>
<tr>
<td>4th quartile</td>
<td>0.82 (1.02)</td>
<td>3.30 (0.92, 5.68)</td>
<td>0.006</td>
<td>0.79 (1.06)</td>
<td>5.19 (2.88, 7.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>trend test³</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.001</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Every 5 μg/m³ increment⁴</td>
<td>-</td>
<td>1.27 (0.66, 1.89)</td>
<td>&lt; 0.001</td>
<td>-</td>
<td>1.64 (1.04, 2.24)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Zhang et al, Int J Epidemiol. 2017
Biodistribution: Inhaled nanomaterials including CNTs translocate and reach distal organs

CNT translocation was studied with $^{14}$C-skeleton labelled MWCNTs:

- Mice were exposed to 20 ug radioactively labelled MWCNT and followed up to 1 year
- 1% of total dose was found in liver and spleen 1 year post-exposure
- No uptake detected after oral exposure

$^{14}$C labelled Few-Layered Graphene (G):

- Biopersistence: Pulmonary dosed G, GO and rGO is found in lung 1-3 mo post-exposure (PMID: 26864058, PMID: 28570647)
- Translocation of G to liver (1%) and spleen (0.2 %) after 1 mo (PMID: 26864058)
- No uptake from oral dosing (PMID: 26864058)

Czarny, ACS nano 2014
Phys-chem-related difference in MWCNT distribution in lung 1 year after IT of 54µg

Thin, short MWCNT (negative in cancer study) (D~11-26nm, L~1µm)
- Black aggregates in macrophages and granuloma
- Chronic low-level inflammation for 7/11 MWCNT

Mitsui-7 and NM-401 (lung cancer by inhalation) (D~70nm, L~5µm)
- Single fibers in interstitium
- Tissue reaction ~ vehicle controls

Knudsen et al, BCPT, 2018
Epidemiological data are not always available and is only feasible for relatively potent carcinogens

Relative risk of lung cancer for carcinogens that cause 1%, 0.1% or 0.01% excess lung cancer risk in a population with the current Danish lung cancer incidence

<table>
<thead>
<tr>
<th>Life time lung cancer risk (0–74 years) 2011-15 in Denmark</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.9%</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Excess lung cancer risk level</th>
<th>RR</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>RR= (4.9+1)/4.9 = 1.20</td>
<td>RR= (4.5+1)/4.5 = 1.22</td>
</tr>
<tr>
<td>2:1000</td>
<td>RR= (49+2)/49 = 1.041</td>
<td>RR= (45+2)/45 = 1.044</td>
</tr>
<tr>
<td>1:1 000</td>
<td>RR= (49+1)/49 = 1.02</td>
<td>RR= (45+1)/45 = 1.02</td>
</tr>
<tr>
<td>1:10 000</td>
<td>RR= (490+1)/490 = 1.002</td>
<td>RR= (450+1)/450 = 1.002</td>
</tr>
<tr>
<td>1:100 000</td>
<td>RR= (4900+1)/4900 = 1.000 2</td>
<td>RR= (4500+1)/4500 = 1.000 2</td>
</tr>
</tbody>
</table>

Power calculation:

Detection of 1% excess cancer incidence with 5% lung cancer incidence: 8 000 participants (with 80% chance of detecting the effect at 5% significance level).

Detection of 0.1% excess lung cancers (1 per 1 000, which is the acceptance level in the US), corresponds to a RR of 1.02, which requires group sizes of 750 000 persons if the background cancer incidence is 5%.
The preferred evidence for regulation

- Identification of critical effects (cancer, cardiovascular disease, reprotoxicity, asthma, COPD etc)
- Human evidence from epidemiological studies with dose-response relationship
- Chronic inhalation studies in rodents (rats)
- Sub-chronic inhalation studies
- Mechanism-of-action:
  - Threshold/non-threshold?