Carcinogenic effects of airborne particles

Roel Schins

Particle Research Group

Institut für umweltmedizinische Forschung (IUF)
Heinrich-Heine University Düsseldorf,
Germany.

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Health implications of particles

OCCUPATIONAL EXPOSURE

‘Classic’ occupational diseases, e.g. pneumoconiosis, emphysema, chronic bronchitis, pneumonitis, lung cancer (quartz).

AMBIENT EXPOSURE (PM$_{10}$/ PM$_{2.5}$)

Acute: asthma attacks, exacerbations of chronic obstructive pulmonary disease (COPD) and cardiovascular disease

Chronic: immunological effects, cancer

Typical exposures: mg/m$^3$

Typical exposures: µg/m$^3$
Quartz and lung cancer

- Epidemiological studies (reviewed by IARC 1997)

- Toxicological studies in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Tumour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Room air</td>
<td>3</td>
</tr>
<tr>
<td>Quartz</td>
<td>1mg/m³ DQ12 6h/day 5days/week 24months</td>
<td>19</td>
</tr>
</tbody>
</table>

Muhle et al. 1989 Am J Ind Hyg

- Alveolar type II epithelial cells are target for quartz carcinogenesis (Johnson et al., 1987)
# Carcinogenicity studies in rat

<table>
<thead>
<tr>
<th>Particle</th>
<th>Use/Exposure</th>
<th>Durability</th>
<th>Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asbestos</td>
<td>Insulation, mining, shipyard workers</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>Crystalline silica</td>
<td>Quarrying, construction</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>Carbon black</td>
<td>Pigments, toner, tires</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>Titanium dioxide (TiO$_2$)</td>
<td>Pigments, cosmetics, Sunscreen agents</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>NiO, Ni-subsulfide</td>
<td>Exhaust</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>Graphite</td>
<td>Aluminium production</td>
<td>Insoluble</td>
<td>+/-</td>
</tr>
<tr>
<td>Iron oxides (Fe$_x$O$_y$)</td>
<td>Pigments, paramagnetic diagnostics</td>
<td>Insoluble</td>
<td>+/-</td>
</tr>
<tr>
<td>Diesel exhaust</td>
<td>Engines, cars</td>
<td>Partly soluble</td>
<td>+</td>
</tr>
<tr>
<td>Coal mine dust</td>
<td>Mining</td>
<td>Partly soluble</td>
<td>+</td>
</tr>
<tr>
<td>Talc</td>
<td>Cosmetics, mining</td>
<td>Partly soluble</td>
<td>+</td>
</tr>
<tr>
<td>Amorphous silica</td>
<td>Cleaning, paints, adsorbents, drugs</td>
<td>Readily soluble</td>
<td>-</td>
</tr>
<tr>
<td>Cement (CaCO$_3$)</td>
<td>Construction, Building</td>
<td>Soluble</td>
<td>-</td>
</tr>
</tbody>
</table>

Tumour formation in rat inhalation studies is associated with durability of the material (biopersistence) → Poorly soluble particles (PSP)
Pathogenesis

Exposure → inflammation → fibrosis → cancer?

Blood capillary

Lung epithelium

Macrophages

Neutrophils
Particles and inflammation

→ Accumulation of inflammatory cells
    *Neutrophils, macrophages (chronic inflammation)*

→ Increased permeability of epithelium, cell damage

→ Epithelial hyperthrophy and hyperplasia, fibrosis

→ Expression of cytokines, chemokines and adhesion molecules
    e.g. *Tumor Necrosis Factor-alpha (TNF), interleukin-1 (IL-1)*
    *Macrophage Inflammatory Protein-2 (MIP-2), IL-8,*
    *Intercellular Adhesion Molecule-1 (ICAM-1)*
Inflammatory genes:
- $\text{I} \kappa \text{B} \alpha$
- $\text{TNF}$
- $\text{IL-1}$, $\text{IL-2}$
- $\text{IL-6}$, $\text{IL-8}$
- $\text{iNOS}$, $\text{COX-II}$
- $\text{GM-CSF}$, $\text{G-CSF}$
- $\text{ICAM-1}$
- $\text{MIP-1}$
- $\text{RANTES}$, $\text{MCP-1}$

Particles

"Oxidative stress"

Nuclear factor kappa B (NF-$\kappa$B)

A crucial transcription factor in particle-induced inflammation?

Schins and Donaldson (2000).
Neutrophil influx by quartz is preceded by Nuclear Factor kappaB (NFκB) activation in bronchoalveolar lavage macrophages

Quartz treatment causes NFκB activation in macrophages as well as in lung epithelial cells (t=72h)

Alveolar space

Particles

AM

Cytokines

Epithelium

Chemokines

Endothelium

Adhesion molecules

PMN

Pulmonary capillary

Courtesy: Ad Knaapen
Proposed mechanisms for carcinogenicity of poorly soluble particles (PSP)

1. PSP exposure
2. Chronic inflammation and release of ROS/RNS
   - Genotoxicity
   - Cell proliferation
   - Tumour formation

**In vivo genotoxicity of particles (quartz)**

Single intratracheal instillation (2 mg quartz/rat, 5 animals per group)

- Determination of inflammation and DNA damage at 3 days after instillation

**Lavage, isolation**

**Trypsin Digestion**

**Percoll gradient**

**BAL**: Inflammatory cell counts, markers of inflammation and toxicity

**EPI**: DNA damage (comet assay)
**Recruitment and activation of neutrophils**

<table>
<thead>
<tr>
<th>BAL fluid</th>
<th>Control</th>
<th>DQ12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (%)</td>
<td>3.3 (4.9)</td>
<td>47.6 (8.7) **</td>
</tr>
<tr>
<td>Myeloperoxidase (mU/ml)</td>
<td>0.027 (0.02)</td>
<td>99.3 (100.6) *</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/ml)</td>
<td>9.4 (2.5)</td>
<td>12.3 (2.7)</td>
</tr>
</tbody>
</table>

**DNA damage in lung epithelium**
(comet assay following isolation)

- **p<0.001, *p>0.01 vs. PBS**

In vitro co-incubation studies: activated neutrophils cause damage to the DNA of alveolar epithelial cells


Inhibition of DNA damage by antioxidants (catalase, SOD)
Proposed mechanisms for carcinogenicity of PSP

PSP exposure

Chronic inflammation and release of ROS/RNS

Genotoxicity

Cell proliferation

Tumour formation

Quartz particles elicit oxidative DNA damage in RLE rat lung epithelial cells \textit{in vitro}

<table>
<thead>
<tr>
<th></th>
<th>Tail length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>24.2 ± 2.8</td>
</tr>
<tr>
<td>Quartz + Mannitol</td>
<td>19.8 ± 1.7 *</td>
</tr>
<tr>
<td>Quartz + DMSO</td>
<td>20.2 ± 2.4 *</td>
</tr>
<tr>
<td>Control</td>
<td>19.0 ± 0.9</td>
</tr>
<tr>
<td>Mannitol</td>
<td>19.1 ± 1.3</td>
</tr>
<tr>
<td>DMSO</td>
<td>19.5 ± 2.1</td>
</tr>
</tbody>
</table>

8-OHdG

A Control

B Quartz

Ambient particulate matter (PM)

EPIDEMIOLOGICAL OBSERVATIONS

- Exacerbations of airways disease in COPD and asthma (Pope and Dockery, 1999)
- Cardiovascular deaths/hospital admissions (Schwartz and Morris, 1995)
  - Cancer (Beeson et al., 1998: Pope et al., 2002)

8 % increase with a 10 µg/m³ change in PM$_{2.5}$

Susceptible groups ➔ Elderly, diseased individuals,...
CHILDREN (asthma, allergies, cancer ?)
Carcinogenesis of PM?
PM causes inflammation

In vitro studies
- Activation of NFκB pathway, enhanced expression of proinflammatory cytokines (e.g. TNF, MIP-2, IL-6, IL-8)
  (e.g. Becker et al. Toxicol Appl Pharmacol 1996; Frampton et al., Am J Physiol 1999)

Inhalation / intratracheal instillation studies in rats
- Enhanced cytokine/chemokine expression, neutrophil influx
  (e.g. Shukla et al., Am J Respir Cell Mol Biol 2000; Schins et al., Toxicol Appl Pharmacol 2004)

Human volunteer studies (CAPS inhalation, intratracheal instillation of PM)
- Role for transition metals, ultrafine particles and/or endotoxin
  (e.g. Ghio and Devlin, AJRCCM 2002; Schaumann et al. AJRCCM 2004)

- Role for transition metals, ultrafine particles and/or endotoxin
Ambient exposure to PM and cancer?

Exposure to ambient PM

Toxicological evidence (animal experiments & volunteer studies)

? Inflammation

Epidemiology observations

Experimental observations with PSP

Carcinogenicity

Important research questions:

→ Investigation of chronic effects of PM (inflammation, tumorigenesis?)

→ Experiments with model PM to determine the role of constituents and/or characteristics of PM that drive inflammation and genotoxicity
Primary particles (predominantly traffic/combustion)
→ ultrafine particles i.e. <100nm (poorly soluble)
→ organics e.g. polycyclic aromatic hydrocarbons (PAH)
→ transition metals (Fe, Ni, Cu, V, Zn, Cr, ..)

Secondary particles (atmospheric chemistry: SO$_2$, NO$_x$, ammonia, sunlight)
→ Ammonium sulphates, nitrates, chlorides

Crustal minerals (e.g. wind blown)
→ mineral rich: aluminium silicate clays, soil particles

Biologically-derived material
→ Endotoxin
→ Fungal spores, plant parts, bacteria, etc.

Primary genotoxicity?
PAH, nitro-PAH,
metals, ketones,
carboxylic acids,
aldehydes,
peroxides....
PM and DNA damage
Oxidative DNA damage by PM

Damage to ‘naked’ DNA (plasmid unwinding)

<table>
<thead>
<tr>
<th>Control</th>
<th>PM</th>
<th>PMsup</th>
<th>PM sup + Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Damage to ‘naked’ DNA (8-HOdG immunodotblot)

<p>| PM | H$_2$O$_2$ |</p>
<table>
<thead>
<tr>
<th>----</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Reactive oxygen species (ROS) generation by PM

Fenton reaction:

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \cdot\text{OH}$$


$\text{H}_2\text{O}_2(125\text{mM})$
$\text{H}_2\text{O}_2(25\text{mM})$
$\text{H}_2\text{O}_2(5\text{mM})$
without $\text{H}_2\text{O}_2$

15 G
Oxidative DNA damage by PM

Uptake of PM by epithelial cells

DNA strand breakage in epithelial cells

(A549, 3h, 100µg/cm²)

control PM PM DMPO PM DMPO DMSO

Tail moment

0 1 2 3
**8-OHdG immunocytochemistry**

- Untreated cells
- Cells treated with PM2.5

**Hydroxyl-radical generation by PM (EPR)**

- DMPO-OH signal (% control)
  - PM
  - PM + Def

**8-OHdG**

- % of control
  - DFO
  - PM
  - PM + DFO

Role of PAH in genotoxicity of PM?

Mutagenicity of airborne PAH has been clearly established

- Effects of PM filter extracts (organic solvents) in Ames test, SCE test, etc
- Extracts cause bulky DNA adducts in vitro and in vivo

Particles are carriers of PAH into the lungs

- Availability of PAH from PM, in vitro, in vivo?
Bioavailability of PAH from carbon black particles
- Adduct formation in A549 human lung epithelial cells -

- Control
- Blank (DMSO)
- CB - original
- CB - extracted
- Extract of CB
- EPA mixture
- CB (extracted) - coated with EPA-mix

adducts / 10^8 n.t.
PM sampling using high volume cascade impactor (HVCI) in Amsterdam, Athens, Barcelona, Duisburg, Helsinki & Prague

Coarse PM, fine PM, ultrafine particles.

Chemical characterisation e.g. metals, PAH, endotoxin

Inflammation & genotoxicity: in vitro and in vivo
- *in vitro* (macrophages, lung epithelial cells)
- *in vivo* (SHR rats, whole lung, isolated epithelial cells)
PAH-DNA adduct formation by PM in lung epithelial cells in vitro

(\(^{32}\)P-postlabelling)

**Total DNA adduct level**

<table>
<thead>
<tr>
<th>City</th>
<th>Fine PM</th>
<th>Coarse PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prague</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Duisburg</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Helsinki</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Amsterdam</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Barcelona</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Athens</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

*Duffin et al. in preparation*
DNA damage in lung epithelial cells isolated from rats exposed to PM

Particles also induced inflammation: Secondary genotoxicity?

Duffin et al. in preparation
PAH-DNA adduct formation in lung epithelial cells isolated from rats exposed to PM

Duffin et al. in preparation
Proposed mechanisms of genotoxicity of PM (studies with model PM)
PM and childhood cancers

Carcinogenesis beyond the respiratory tract?
Induction of systemic oxidative stress and genotoxicity

1. Biomarker studies:
   - Enhanced oxidative DNA damage (8-OHdG) in peripheral blood leukocytes, and blood antioxidant status of individuals with (chronic) occupational dust exposure, e.g. coal miners (e.g. Schins et al., Int Arch Occup Env Hyg 1995; Schins et al., Biomarkers 1999)
   - Correlation between personal PM2.5 exposure and oxidative DNA damage (e.g. comet) in peripheral blood leukocytes (e.g. Sorensen et al., 2003)

2. Toxicological studies (animal models):
   - Particles (inhalation, instillation) elicit genotoxic/mutagenic effects outside the lungs: Endpoints include DNA strand breaks and oxidative damage, bulky DNA adducts, chromosomal aberrations, sister chromatid exchanges).
   - Various tissues/organs involved such as peripheral blood/bone marrow cells, liver, colons
Systemic effects of ultrafine PM: - Translocation of particles -

Inhaled/instilled particles translocate from the lungs into the blood → implicated in the observed association between PM exposure and cardiovascular effects:

(e.g. Nemmar et al. 2001; 2003; Kreyling et al., 2002)

![Graph showing the translocation of ultrafine albumine particles (80 nm) labelled with $^{99m}$Tc.](image)

Carbon particles (C14, ultrafine) are found in the brain of rats following inhalatory exposure → translocation of particles along the olfactory nerves has been proposed (Oberdorster et al., Inhal Toxicol 2004)
Germline mutagenesis

Reduction of Particulate Air Pollution Lowers the Risk of Heritable Mutations in Mice

Christopher M. Somers,¹ Brian E. McCurry,² Farideh Malek,³ James S. Quinn¹*

Science 2004, 304:1008-1010 (14 May)

→ Germline mutation rates at expanded-simple-tandem repeat (ESTR) DNA loci in mouse pedigrees in association with urban/industrial air pollution (TSP = 40 – 115µg/m³; PAH = 2–30µg/m³)

→ Reduction following particulate-air filtration (> 0.1µm)

Particulate air pollution can cause genetic damage in germ cells & transgenerational effects
How may particles impact on carcinogenesis in various organs other than lungs?

PM (ultrafine component) can translocate from the airways into the blood and thus reach various target cells/tissues. Possible implications:

→ Direct genotoxic effects of particles (surface reactivity, ROS production)

→ Induction of inflammation, apoptotic and/or (compensatory) proliferation

→ Carrier effect: Systemic delivery of mutagens/carcinogens (e.g. PAH)

Particles activate signalling pathways (inflammation/proliferation) within the lung

→ Systemic release of mediators (e.g. cytokines, growth factors, ROS) may impact on processes involved in carcinogenesis (e.g. apoptosis, proliferation)