Ultrafine Dust and Nanoparticles: Hazard Identification in vitro

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Institute of Toxicology and Genetics
The London Smog Disaster, Dec. 3.-11., 1952

Over 4,000 Deaths were attributable to the Great London Smog

### Comparison of Time-Series Study Estimates of the Total Mortality RR of a 100 µg/m³ PM$_{10}$ Increase

<table>
<thead>
<tr>
<th>Study Area (Reference)</th>
<th>Mean PM$_{10}$ (µg/m³)</th>
<th>Maximum PM$_{10}$ (µg/m³)</th>
<th>100 µg/m³ RR</th>
<th>100 µg/m³ (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utah Valley, UT (Pope et al., 1992)</td>
<td>47</td>
<td>297</td>
<td>1.16*</td>
<td>(1.10-1.22)</td>
</tr>
<tr>
<td>St. Louis, MO (Dockery et al., 1992)</td>
<td>28</td>
<td>97</td>
<td>1.16*</td>
<td>(1.01-1.33)</td>
</tr>
<tr>
<td>Kingston, TN (Dockery et al., 1992)</td>
<td>30</td>
<td>67</td>
<td>1.17*</td>
<td>(0.88-1.57)</td>
</tr>
<tr>
<td>Birmingham, AL (Schwartz, 1993)</td>
<td>48</td>
<td>163</td>
<td>1.11*</td>
<td>(1.02-1.20)</td>
</tr>
<tr>
<td>Athens, Greece (Touloumi et al., 1994)</td>
<td>78</td>
<td>306</td>
<td>1.07*</td>
<td>(1.05-1.09)</td>
</tr>
<tr>
<td>Toronto, Canada (Özkaynak et al., 1994)</td>
<td>40</td>
<td>96</td>
<td>1.07*</td>
<td>(1.05-1.09)</td>
</tr>
<tr>
<td>Los Angeles, CA (Kinney et al., 1995)</td>
<td>58</td>
<td>177</td>
<td>1.05*</td>
<td>(1.00-1.11)</td>
</tr>
<tr>
<td>Chicago, IL (Ito, et al., 1995)</td>
<td>38</td>
<td>128</td>
<td>1.05*</td>
<td>(0.98-1.09)</td>
</tr>
<tr>
<td>Santiago, Chile (Ostro et al., 1995)</td>
<td>115</td>
<td>367</td>
<td>1.08*</td>
<td>(1.06-1.12)</td>
</tr>
</tbody>
</table>

* Single pollutant model (i.e., PM$_{10}$)
** Multiple pollutant model (i.e., PM$_{10}$ and other pollutants simultaneously)
† One-day mean PM$_{10}$-concentration employed
‡ Multiple-day mean PM$_{10}$-concentration employed

PM$_{10}$ = particulate matter < 10 µm
Estimated city-specific mortality rate ratios adjusted for age, sex, smoking, education, and body mass index, plotted against mean PM$_{2.5}$ concentrations in six U.S. Cities.

The U.S. Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: A Midcourse Report of Status, Progress, and Plans


Figure 1. PM health effects pathway

Figure 2. Hypotheses for health effects of PM.
Cellular Responses to UFP

Ultrafine Particles → ROS! → [Ca^{2+}]_i! → Respiratory Burst

Lipid Peroxidation
GSH → MDA → GSSG

Nuclear Signalling Pathways!
- NFκB
- AP-1
- Nrf2

Anti-oxidative Response!

Lipid Mediator Response!

Inflammatory Mediators!

Particle Properties

Introduction

Cellular Responses

In vitro Exposure

Hypothesis

Nel et al. (2006) Science 311: 622-627
The Hierarchical Oxidative Stress Model

- **Introduction**
  - Celluar Responses
  - In vitro Exposure
  - Hypothesis

**Response pathways:**
- Normal
- Anti-oxidant defense
- Inflammation
- Cytotoxicity

**Signaling pathway:**
- Nrf-2
- MAP Kinase NF-κB cascade
- Mitochondrial PT pore

**Genetic response:**
- Anti-oxidant response element
- AP-1 NF-κB
- N/A

**Outcome:**
- Phase II enzymes
- Cytokines Chemokines
- Apoptosis

Nel et al. (2006) Science 311: 622-627
**Situation in the Lung**

**Fate of Particles**

- **LPS** (Pseudomonas aeruginosa)
- Alveolar Makrophage
- NO, TNF-$\alpha$, IL-6, IL-8, Lipid Mediators
- Epithelial Cells
- Basal Membrane
- Endothelial Cells
- Adhesion Molecules
- TNF-$\alpha$, IL-6, IL-8, Lipid Mediators

**Introduction**

**Cellular Responses**

**In vitro Exposure**

**Hypothesis**

**ETH Zürich 2006**

Harald F. Krug
Ultrafine Particles - Nanoparticles

Fly Ash

Hematite

Carbon Black (Soot)

Silicasol

In vitro Exposure

Hypothesis
Fly Ash as Model Particles for Real-Life Emissions

Medium Mass of the Components [% of total Mass]:
• ca. 75 % Sulfates and Chlorides of Na, K and Ca
• ca. 6 % Metals (Cd, Cu, Fe, Pb, Sb, Sn, Ti, Zn)
• ca. 1 % Carbon
• ca. 70% are watersoluble Constituents (pH 7,4)

**Introduction**

**Cellular Responses**

**In vitro Exposure Hypothesis**

**3D Cell Model**

**Exposure System**

- Particle from Source
- AEOLA
- Cyclon
- Sample drawing reactor
- Particle analysis
- Mass-flow controller
- Vacuum pump
- Filter
- Rotameter
- Fan

**In cooperation with:**

ITC, Forschungszentrum Karlsruhe,
(H.R. Paur/S. Müllhopt)
3D-Cell Model
Exposure to Particles at the Air-Liquid-Interface

Introduction

Cellular Responses

In vitro Exposure

Hypothesis
The Cultex/FZK-System

Gas inlets

www.vitrocell.com
Survival time during Air-Exposure

Lab Air without Flow

<table>
<thead>
<tr>
<th>Exposure Time [h]</th>
<th>Submers</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability [% of Submers-Control]</td>
<td>100</td>
<td>95</td>
<td>90</td>
<td>85</td>
<td>80</td>
</tr>
</tbody>
</table>

Labcontrol
Filtered Air
Fly ash

<table>
<thead>
<tr>
<th>Exposure Time [h]</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability [% of Submers-Control]</td>
<td>105</td>
<td>100</td>
<td>95</td>
<td>90</td>
</tr>
</tbody>
</table>

Introduction
Cellular Responses
In vitro Exposure
Hypothesis
Interleukin-Production after Fly Ash Exposure

Exposure to air flow at 100 ml/min -/+ fly ash

Exposure to lab air without flow at 37°C
# Induction of Hemoxygenase-1

## Antioxidative Protection

### BEAS-2B Cells in Medium-Culture

<table>
<thead>
<tr>
<th>K</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>[µg/ml] [µg/cm²]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,1</td>
<td>4,2</td>
<td>6,3</td>
<td>8,4</td>
<td>10,5</td>
<td></td>
</tr>
</tbody>
</table>

**Fly Ash [20 h]**

- **HO-1**
- **PCNA**

### BEAS-2B + THP-1

**Air/Liquid-Interface**

- **Lab Air**
- **filtered Air**
- **Fly Ash Aerosol**
- **filtered Air**
- **Fly Ash Aerosol**
- **Fly Ash submers**

- **Lab Air 4h**
- **filtered Air 2h**
- **Fly Ash Aerosol 2h**
- **filtered Air 4h**
- **Fly Ash Aerosol 4h**
- **Fly Ash submers 30 µg/ml**

**HO-1**

**PCNA**

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Harald F. Krug 18
Fly Ash induces Arachidonic Acid Release in RAW264.7 Macrophages

Introduction

Cellular Responses

In vitro Exposure

Hypothesis
In Vitro Studies by

Ultrafine Particles

ROS!

[Ca^{2+}]_{i}!

Respiratory Burst

Vicki Stone (Edinburgh)
Calcium-Homeostasis

Peter Hoet (Leuven)
Translocation and pro-
Thrombotic Effects

Inflammatory
Mediators!

Krug (Karlsruhe)
3D-Cell Models/ Uptake
and Translocation
Cellular Mechanisms

Inflammation
Mediators!

Resident Macrophages/ Role of
Dendritic Cells

Beck-Speier (Munich)
Lipid Mediators

Diabaté (Karlsruhe)
Genotoxicity

Cellular
Signalling Pathways!

MDA
NADH

GSSG

NFkB
AP-1
Nrf2

Jaspers (Chapel Hill)
Cellular Signaling/Cytokine
Production

Inflammatory
Mediators!

Lipid Peroxidation
GSH

Resident Macrophages/ Role of
Dendritic Cells

Diabaté (Karlsruhe)
Lipid Mediators/(anti-oxidative
Responses

Roel Schins (Düsseldorf)
Genotoxicity

Intoxication
Hypothesis

ETH
Zürich
2006
Hypothesized pathways leading to adverse cardiovascular health effects from exposure to UFPs

Delfino et al. (2005) Environ. Health Perspect. 113: 934-946
Characteristics of Combustion Derived Nanoparticles (CDNP)

Table 1: Characteristics of the CDNP considered in this review

<table>
<thead>
<tr>
<th>CDNP</th>
<th>Origin</th>
<th>Reported health effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diesel exhaust particles</td>
<td>Combustion of diesel oil</td>
<td>Inflammation, fibrosis, cancer,</td>
</tr>
<tr>
<td>Welding fume</td>
<td>Welding processes</td>
<td>Inflammation; translocation of metals to</td>
</tr>
<tr>
<td>Fly-ash</td>
<td>Combustion of coal or oil</td>
<td>the brain</td>
</tr>
<tr>
<td>NP Carbon black</td>
<td>Combustion of heavy fuel oil</td>
<td>Inflammation, lung cancer; translocation of particles to the brain</td>
</tr>
</tbody>
</table>

Co-operation:

Electron Microscopy:
H. Zöltzer - Universität Kassel
H. Thiele - ITU Karlsruhe

Antibodies and transfected Cells:
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M. Peter - DKFZ Heidelberg
S. Korsmeyer - Washington University, St. Louis
V. Dixit - University of Michigan
J. Blenis - Harvard Medical School
D. Green - La Jolla

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Funded by:
DFG-CFN
BWPLUS B.-W.
BfR
EU
BMBF
HGF

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