HEALTH EFFECTS OF COMBUSTION GENERATED PARTICLES

HOW COMBUSTION GENERATED NANOPARTICLES (UFP) CAN ENTER THE HUMAN ORGANISM – SIZE MATTERS

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LUNG FUNCTION OF ASTHMATICS WHILE WALKING ALONG THE DIESEL BUS ROUTE OXFORD STREET, THROUGH HYDE PARK

McCreanor et al, NEJM 2007

WHO (IARC):
- Diesel exhaust is carcinogenic, June 12, 2012
- Air pollution is carcinogenic, October 17, 2013

Courtesy: Nino Künzli
Swiss Tropical and Public Health Institute
Basel, Switzerland
MAIN PORTAL OF ENTRY: LUNG

Gehr et al., Respir. Physiol. 1978
ALVEOLI, INTERALVEOLAR SEPTA

Gehr et al., Respir. Physiol. 1978
DID YOU KNOW THIS ABOUT THE HUMAN LUNG?

Tennis field
450 Mill. alveoli (M. Ochs, Univ. of Bern) with a surface area of 140 m² (diameter ¼ mm, gas-exchange region 80-90%)

Red wine glass
Volume of capillary blood involved in gas exchange: 210cm³

1/50 of the thickness of a women’s hair
Thickness of tissue barrier: <1µm

(B. Rothen-Rutishauser, Universität Bern)

Gehr et al., Respir. Physiol., 1978
**SURFACTANT, AQUEOUS HYPOPHASE**

- **Surfactant** (Surface active agent)
- Aqueous hypophase
- Type II pneumocytes

Conference on Air Pollution, AQM, Teheran, 12.1.2016, P. Gehr
PARTICLES DEPOSITED IN THE LUNGS → DISPLACEMENT

Gehr et al., J. Aerosol Med., 1990
Schürch et al., Respir. Physiol., 1990
STRUCTURE AND DISPLACEMENT
(FILTER FUNCTION: SURFACTANT)

Gehr et al., J. Aerosol Med., 1990
Schürch et al., Respir. Physiol., 1990
Gehr et al., J. Aerosol Med., 1996
TRANSLOCATION OF NANOARTICLES (UFP) FROM AIR INTO BLOOD

E.R. Weibel, University of Bern

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IN VITRO MODEL: BREATHING MOVEMENTS MAY STIMULATE NANOPARTICLE UPTAKE BY CELLS

Courtesy David Schürch, Adolphe Merkle Institute, University of Fribourg

Captive bubble surfactometer

- Plunger
- Glass chamber
- Agarose
- Epithelial cell layer
- Air bubble
- Surfactant + nanoparticles injected here
- Cell medium

Epithelial cell layer

Membrane

Surfactant + nanoparticles

5x increase of intracellular NPs

Static

Dynamic

Control

Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland

Schürch et al., Langmuir, 2014

Courtesy: Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland
TRANSLOCATION
WITH BLOOD TO OTHER ORGANS

Nanoparticles

Brain → Nose

Lung → Skin → GI-Tract → Blood

Blood Vessels

Bone Marrow → Spleen → Wall

Liver → Heart → Placenta → Fetus

Degenerative Changes
Intratracheal instillation in WKY rats
1-10 µg ¹⁹⁸Au particles in 50 µL saline, negative ionic surface charge
# of particles: 
1 \times 10^{14} (1.4 \text{ nm cluster}) 
2 \times 10^{11} (18 \text{ nm colloid})
G. Schmid, Univ. of Essen, Germany

Mass fractions of gold nanoparticles in different organs after 24 h

Semmler-Behnke et al., Small, 2008
WHAT HAS TO BE CONSIDERED OF NANOPARTICLES FROM COMBUSTION AEROSOLS

- **Size of particles** (nanoparticles)
- **Displacement of nanoparticles** towards epithelial layer (surfactant, surface forces)
- **Distance to capillaries** (translocation)
- **Distance to sensitive cells** (interaction), effect: immune modulation?
- **Interaction with cells** (uptake/penetration, effect: immune modulation, oxidative stress, inflammatory reaction a.o.?)
MAIN ACTORS ARE CELLS
EPITHELIAL CELLS, MACROPHAGES, DENDRITIC CELLS ...
… DO THEY COLLABORATE?
THE CELL MODEL TO TEST THIS
THE TRIPLE CELL CO-CULTURE MODEL

Rothen-Rutishauser et al., *Am. J. Respir Cell Mol. Biol.* 32: 281-899, 2005
STRUCTURAL VICINITY OF DENDRITIC CELLS AND MAKROPHAGES (THROUGH THE EPITHELIAL CELL LAYER)

Deconvolution technique
IMARIS 3D&4D Image Analysis Software
Bitplane AG, Scientific Software

Blank et al., AJRCMB 36: 669-677, 2007
CELL-CELL INTERACTIONS
CELLULAR INTERPLAY ->THE CELLS DO COLLABORATE!

A BURNING QUESTION:
HOW DO NANOPARTICLES ENTER CELLS?

(A: Phagocytosis)
B: Macropinocytosis
C: Clathrin-mediated endocytosis
D: Clathrin and caveolae independent endocytic pathways
E: Caveolae-mediated endocytosis
F: Adhesive interaction (entering): interaction of nanoparticles with cell membrane, effect on fluidity, nanoparticles may slip into cell between phospholipid molecules  
(→ U. Nienhaus, KIT)

Brandenberger et al., Small, 2010
... AND AN ANSWER:
ELECTROCHEMISTRY AND SURFACE-ENHANCED INFRARED ABSORPTION SPECTROSCOPY ON MODEL MEMBRANES (DAP-QDs)

Electrochemistry: voltammograms indicate that lipid layers do not conduct current upon DPA-QD exposure \(\rightarrow\) no holes formed!

SEIRAS: Membrane flexibility is enhanced in the presence of DPA-QDs

Lipid composition of the inner or outer RBC membrane leaflets

1-Dodecanethiol (DT)

IR beam

Courtesy: G.U. Nienhaus, Institute of Applied Physics, KIT

Wang et al., ACS Nano 6 (2012) 1251-1259
NANOPARTICLES IN CELLS

Plain Au nanoparticles

PEG coated Au nanoparticles

Membrane bound (in vesicles/lysosomes)

Free (in cytosol)

→ more nanoparticles in cytosol

Brandenberger et al., Small 2010

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EFFECTS OF DIESEL EXHAUST ON BIOLOGICAL SYSTEMS

- Opel Astra X20DTL, 35 km/h
- Fuel: low sulfur diesel (>10mg/kg, Greenergy SA)
- Lube oil (V10.237, Motorex)
- Exhaust dilution 1:10

⇒ Without particle filter
⇒ With a silicon carbide diesel particle filter

Steiner et al. Tox Letters 2012

Courtesy:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland
DIESEL EXHAUST
PARTICLE SIZE DISTRIBUTION, FILTER EFFECT

counts $dN/d\log D_p$ [1/cm³]

d [nm]

Without Filter
With diesel particle filters

Courtesy: Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland

Steiner et al., Atmos. Environ., 2013
DIESEL EXHAUST
INFLAMMATORY REACTION, FILTER EFFECT

Confocal light micrograph
(blue: nuclei, red: actin)

TNF-α Sekretion, an inflammatory cytokine

Reference
Exhaust
Without filter
With diesel particle filter

Steiner et al., Atmos. Environ., 2013

Courtesy:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland
WHAT SHOULD BE CONSIDERED
SIZE MATTERS! UFP CAN TRANSLOCATE INTO BLOOD IN LUNGS!

- Diesel exhaust, air pollution were declared carcinogenic (many UFP)
- Distance to source of air pollution (e.g. traffic) is crucial
- Filters contribute substantially to reducing adverse health effects from diesel exhaust particles (>99% removed form exhaust)
- UFP enter cells and tissue very easily
- UFP can translocate into blood in the lungs, translocation to secondary organs -> lung is main portal of entry for UFP
- Effects on lungs:
  - Reduced pulmonary function in adults (asthmatics) (1st slide)
  - Reduced development and function of lungs in neonates (not shown)
- Speculations (Translocation through internal tissue barriers) e.g.:
  - Blood-brain-barrier (Alzheimer’s disease?)
  - Blood testis barrier (Development/maturation of sperms?)
  - Blood thymus barrier (Development of T-lymphocytes?)
Traffic related PM from Highway 405 cause atherosclerosis in mice
Araujo et al, Circul Res 2008

Exposure:
40 days
5h / day
3 days / week

Toxicology example

Picture from
Nino Künzli, MD, PhD; MPH
Professor and Deputy Director
Swiss Tropical and Public Health Institute, Basel
Dean, Swiss School of Public Health, Zurich
Switzerland

Conference on Air Pollution, AQM, Teheran, 12.1.2016, P. Gehr
HEALTH EFFECTS UFP?

- **Ischemic Heart Disease mortality in the Californian Teacher’s Study**
  Ostro et al; Env H Perspect 2015

- **Risk of mortality in association to long-term exposure to traffic-related air pollution. European Studies**

- **Elemental carbon or Black Smoke are associated with all-cause mortality (8 cohort studies)**
  Review by Hoek et al, Env Health 2013

- **Life expectancy of reduction in PM2.5 mass**

- **Lung growth was affected by home outdoor levels of PM2.5**
  (Southern Californian Children’s Health Study, Gauderman et al, NEJM 2004)

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**A NEW long-term effect study with UFP available!**
But no 2-pollutant model with UFP, controlling for PM2.5

**Are «effects of NO2» due to ultrafine particles...?**
... are «effects of EC» explained by (unmeasured) UFP?
... are «effects of EC» explained by (unmeasured) UFP?
... but same associations with elemental carbon... and... no UFP data available... – but might look similar... ?!

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Confirmed by Nino Künzli, MD, PhD; MPH
Professor and Deputy Director
Swiss Tropical and Public Health Institute, Basel, Switzerland
CONCLUSIONS
HEALTH EFFECTS CAUSED BY UFP

- Experimental evidence for long-term effects of UFP
- Epidemiological studies: very few with UFP data, thus, no final interpretation possible
- New evidence for possibly high correlation of exposure to UFP with exposure to other «classic pollutants»
- Need to understand to what extent abundant evidence of long-term effects of PM is (in part?) explained by UFP
- Need state-of-the-art exposure assessment for UFP as well – incl. consideration of exposure from outdoor origin while indoors

Courtesy
Nino Künzli, MD, PhD; MPH
Professor and Deputy Director
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KEEP IN MIND
WHEN WORKING WITH NANOPARTICLES (UFP)

• risk = f(hazard, exposure$^{\text{time}}$) for a given size
• effect = f(dose, time$^{\text{after exposure}}$) for a given size

• **Interaction** of nanoparticles with biological systems is primarily a function of **size**: 
  size matters!:
  penetration, translocation, effect/reaction

• Important are furthermore:
  material, corona, agglomeration, time$^{\text{after exposure}}$ etc.
CONCENTRATION OF PARTICLES AND HEALTH - DISTANCE FROM BUSY ROAD

Relative Concentration

- Fine PM, homogenously distributed
- Ultrafine PM - and other primary toxicants - strong dependance on distance from source

Chronic cough or phlegm
Chronic cough
Wheezing w/o colds
Wheezing with breathing problems

Adjusted prevalence [%]

0 m
100 m
200 m
300 m

Relative concentrations of fine particulates and ultrafines in relation to distance from highway 405 - Los Angeles
Zhu et al, J Air Waste Manage Assoc, 2002; 52: 1032

Air-blood tissue barrier
- 1 μm
- 0.1 μm (and smaller)

Concentration of particles

Capillaries
Erythrocytes
Interalveolar septum
Alveolar air
Capillary blood
Erythrocyte
Blood Plasma
Residential distance from highway [m]

Zhu et al, J Air Waste Manage Assoc, 2002; 52: 1032

Pictures from
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