Title:
Effects of subchronic inhalation exposure to diesel engine exhaust in the 5xFAD mouse model of Alzheimer’s Disease.

Abstract: (min. 300 – max. 500 words)
The abstracts for papers and posters must contain unpublished information on your research subject: background, investigation methods, results and conclusions. Graphs and references are very welcome. Acronyms should be avoided. Abstracts with < 300 words can not be considered. General information on products which are already commercially available can not be accepted as presentations for the conference but are very welcome at the exhibition of particle filter systems and nanoparticle measurement instruments.

In recent years, it has been suggested that nanoparticles generated from combustion processes, may contribute to the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (AD). We could previously demonstrate that a single, short-term inhalation exposure to diesel engine exhaust (DEE) triggers rapid region-specific gene expression changes in rat brain to an extent comparable to those observed in the lung. The aim of our present study was to investigate the effects of a subchronic exposure to DEE in 5xFAD transgenic mice. This mouse model is characterized by the development of histological, neurological as well as functional impairments which are considered to mimic the development and progression of AD in humans.

Ten weeks old female 5xFAD mice and their non-transgenic littermates were exposed by whole body inhalation to diluted DEE (≈ 1 mg particles/m³) or clean air for 3 or 13 weeks, during 5 days/week and 6 hour/day. At the end of the exposures, all mice were subjected to a series of behavioural tests (e.g. Y-maze, cross-maze, string suspension). At ten days post-exposure, histopathological, biochemical and molecular-biological changes were evaluated in the lungs and brain tissues of the animals.

In line with the expectations, the 5xFAD mice displayed typically age-dependent behavioural deficits and amyloid plaque formation in cortex and hippocampus. A significant DEE exposure-related effect was observed for the string suspension test, representing a measure of motor coordination/grip strength. DEE exposure was also associated with mRNA expression changes of markers of inflammation and oxidative stress in specific brain regions, including the olfactory bulb. Whole brain tissue homogenate levels of Abeta42 protein were found to markedly increased in the 3 weeks DEE
exposed 5xFAD mice in comparison to the sham exposed littermates. After 13 weeks, further increases in whole brain Abeta42 levels were observed in the transgenic animals, but differences between the DEE and clean air exposed animal were absent at this time point. Quantitative histopathology of plaque-load in cortex and hippocampus of all animals is ongoing.

Our current findings support that the central nervous system may be a sensitive target for combustion-derived nanoparticles. Further research is needed to evaluate the impact of long-term low-level exposure on the development and progression of neurodegenerative diseases in humans.

This work is supported by funds from the Research Committee of the Medical Faculty of the University of Düsseldorf (9772-365), the DFG Graduate School GRK1033 and the RIVM - Centre for Environmental Health, Bilthoven, Netherlands.

Short CV: Dr. Roel Schins is a research group leader at the IUF Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany. As a trained toxicologist, his longstanding research interest is to unravel mechanisms whereby particles may cause diseases in humans. His current research focuses on the investigation of nanoparticle effects in the lung, the gastrointestinal tract and the brain. Dr. Schins studied Health Sciences at Maastricht University in The Netherlands and worked as a Postdoctoral Research Fellow in the Biomedicine Research Group at Edinburgh Napier University, UK. He is deputy editor of Particle and Fibre Toxicology and has served as expert on the toxicology of (nano)particles to various organisations and bodies including IARC/WHO, OECD, ECETOC, DFG, and DG-SANCO.

Return by e-mail latest 14th of April 2012 to ttm.a.mayer@bluewin.ch
Effects of Subchronic Inhalation Exposure to Diesel Engine Exhaust in the 5xFAD Mouse Model of Alzheimer’s Disease

Roel Schins¹, Maja Hullmann¹, Catrin Albrecht¹, Damien Van Berlo¹, Miriam Gerlofs-Nijland², Antje Hillmann³, John Boere², Paul Fokkens², Sascha Weggen⁴, Thomas Bayer², Jean Krutmann¹, Flemming Cassee²

¹ IUF - Leibniz Research Institute for Environmental Medicine, Dusseldorf, Germany,
² National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands,
³ Department of Psychiatry, University of Medicine, Goettingen, Germany,
⁴ Department of Neuropathology, Heinrich Heine University Dusseldorf, Germany
Oxidative stress and inflammation caused by combustion-derived nanoparticles

Donaldson et al 2005, Particle and Fibre Toxicology
Effects of air pollution particles on the brain

Observations in human studies
(histopathology, cognition)

Observations in brains of rodents after controlled exposures to e.g. PM, CAPs, diesel exhaust particles, etc.
Carbon nanoparticles translocate to rat brain
Oberdörster et al, 2004 *Inhal Toxicol*
Inhalation of Nanoparticles (<100nm)

- **Nasal deposition**
- **Alveolar deposition**

**TRANSLOCATION**

- **olfactory route**
- **air-blood barrier** ➔ **Blood** ➔ **blood-brain barrier** ➔ **Pulmonary / Systemic inflammation**

**BRAIN**

- **Inflammation**
- **Oxidative stress**
- **Neurodegeneration**

*Van Berlo et al. EXS 2012*
Comparative evaluation of the effects of short-term inhalation exposure to diesel engine exhaust on rat lung and brain

Damien van Berlo1, Catrin Albrecht1, Ad M. Knaapen2,*, Flemming R. Cassee3, Miriam E. Gerlofs-Nijland3, Ingeborg M. Kooter3,*, Nicola Palomero-Gallagher4, Hans-Jürgen Bidmon5, Frederik-Jan van Schooten2, Jean Krutmann1, Roel P.F. Schins1.

Arch Toxicol 2010; 84: 553-562.

Nose-only inhalation 2h 1.9 mg/m³ DEE or filtered air

Investigation of lung and brain tissues

Relative mRNA expression

HO-1

CYP1A1

* indicates significant difference (p < 0.05) compared to control.
BRAIN INFLAMMATION AND ALZHEIMER’S-LIKE PATHOLOGY IN INDIVIDUALS EXPOSED TO SEVERE AIR POLLUTION


Amyloid β deposition
(formation of plaques)
&
Neuronal degradation
Long-term exposure to traffic-related particulate matter impairs cognitive function in the elderly

Ulrich Ranft *, Tamara Schikowski, Dorothee Sugiri, Jean Krutmann, Ursula Krämer

Institut für Umweltmedizinische Forschung (IUF) an der Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Auf’n Hennekamp 50, 40225 Düsseldorf, Germany
Alzheimer’s Disease

• Progressive and fatal brain disorder named after the German neuropathologist *Alois Alzheimer*

• Most common form of dementia

• 27 million people are affected worldwide

  → predicted to nearly double every 20 years
  
  (2009 Word Alzheimer Report)

  → Familial Alzheimer’s Disease (FAD), approx 0.1%

Formation of plaques and neurofibrillary tangles (Amyloid β and Tau proteins)

Role of oxidative stress and inflammation
Amyloid-Precursor-Protein (APP) metabolism

Patterson C et al. CMAJ 2008;178:548-556
Alzheimer Model (5xFAD mice)

→ mutant human Aβ precursor protein (APP) cDNA sequence:
  \[ \text{APP K670N/M671L (Swedish) + I716V (Florida) + V717I (London)} \]

→ mutant human presenilin 1 (PS1) cDNA sequence:
  \[ \text{PS1 M146L + L286V} \]

*Expression of both transgenes is regulated by neural-specific elements of the mouse Thy1 promoter to drive over-expression in the brain.*

Age: 2 months  4 months  6 months  9 months

Study design

Genotyping

5xFAD ♀ n=32
Wildtype ♀ n=20

3 weeks (6h/d, 5d/wk) 1wk
Diesel or clean air

Behavioural tests

Dissections (brains, lungs)

Whole body exposure

Mass dose: ~1 mg/m³
Number dose: 2·10⁶ particles/cm³
Mean size: 81 nm (GSD 1.75)

Genotyping

5xFAD ♀ n=32
Wildtype ♀ n=20

13 weeks, 6h/d, 5d/wk

Behavourial tests

Dissections (brains, lungs)
Y-maze test: Spatial working memory
(Spontaneous alternation behaviour)

3 weeks

13 weeks

![Graph showing data for Y-maze test]

- FAD air
- WT air
- FAD Diesel
- WT diesel

P<0.01

![Graph showing data for Y-maze test]

- FAD air
- WT air
- FAD Diesel
- WT diesel

P<0.01
String suspension task
(Motor coordination, grip strength)

3 weeks

String Suspension

P<0.05

13 weeks

String Suspension

P<0.05

P<0.01

P<0.01
Aβ42 ELISA (whole brain)

3 weeks

<table>
<thead>
<tr>
<th>Condition</th>
<th>5xFAD air</th>
<th>5xFAD Diesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mg Protein</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

P<0.05

13 weeks

<table>
<thead>
<tr>
<th>Condition</th>
<th>5xFAD air</th>
<th>5xFAD Diesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mg Protein</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>
Summary

Development of “Alzheimer-like Pathology” in 5xFAD mice:

- Behaviour tests (Y-maze, X-maze, string suspension,..)
- Age dependent increase in whole brain Aβ42 levels
- Formation of Aβ plaques (cortex, hippocampus)

Diesel engine exhaust exposure (3 weeks, 13 weeks):

- No significant “cognitive impairment” effects in 5xFAD mice or in wildtype mice (Y-maze, X-maze)
- “Motor function impairments” in 5xFAD mice
- Increased whole brain Aβ42 levels after 3 weeks exposure
- Quantitative evaluation of Aβ plaque formation (ongoing)

→ DEE exposure accelerates Alzheimer phenotype in transgenic mice
Hans-Jürgen Bidmon
Nicola Palomero-Gallagher
INM, Research Center Jülich & C & O
Vogt Institute for Brain Research,
HHU Düsseldorf

Sascha Weggen
Guido Reiffenberger
Institut für Neuropathologie,
Heinrich-Heine-University Düsseldorf

Thomas Bayer
Division of Molecular Psychiatry,
Georg-August-University Göttingen

Rijksinstituut voor Volksgezondheid
en Milieu
Ministerie van Volksgezondheid,
Welzijn en Sport

Flemming Cassee,
Miriam Gerlofs-Nijland,
Ilse Gosens, John Boere,
Daan Leesman, Paul Fokkens

Catrin Albrecht
Maja Hullmann
Kirsten Gerloff
Bryan Hellack
Julia Kolling
Agnes Scherbart
Waluree Thongkam
Damien van Berlo
Anton Wessels
Irmgard Förster
Jean Krutmann