National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

Influence of NO_2 on pulmonary toxicity in mice sub-chronically exposed to diluted diesel engine exhaust

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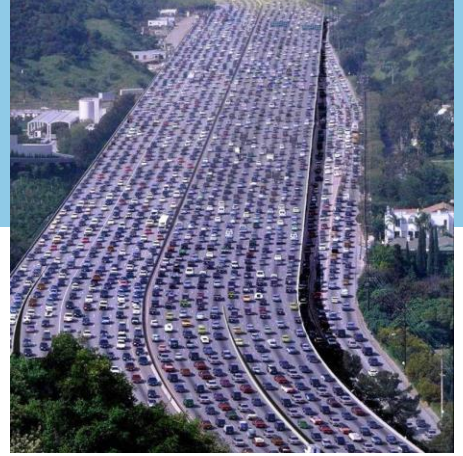
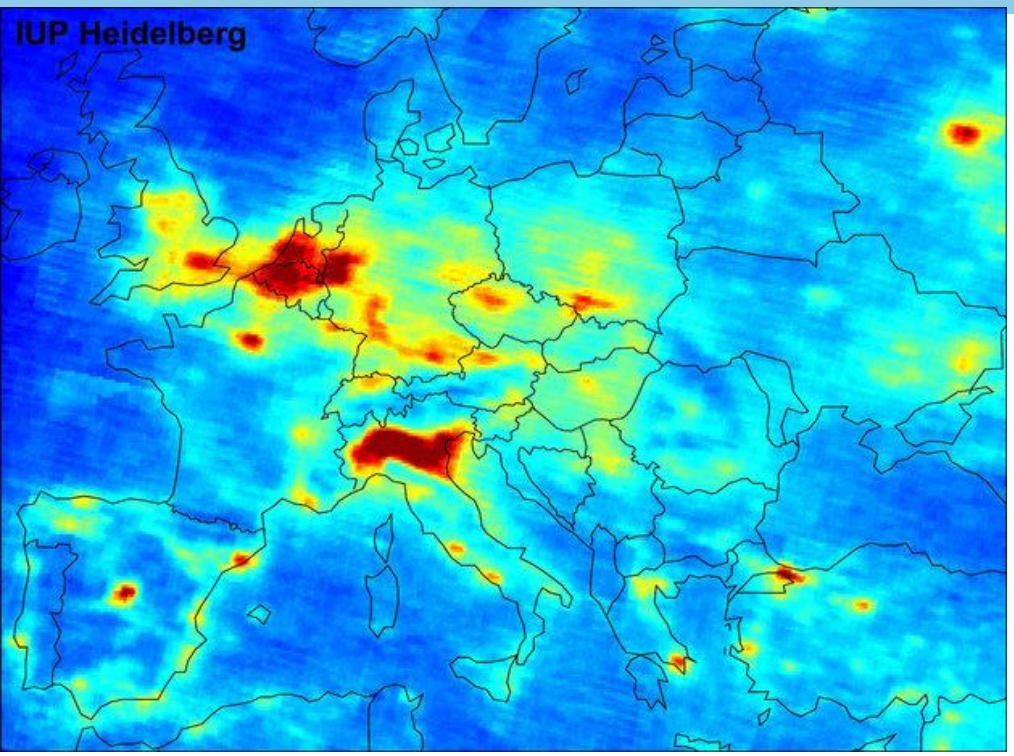


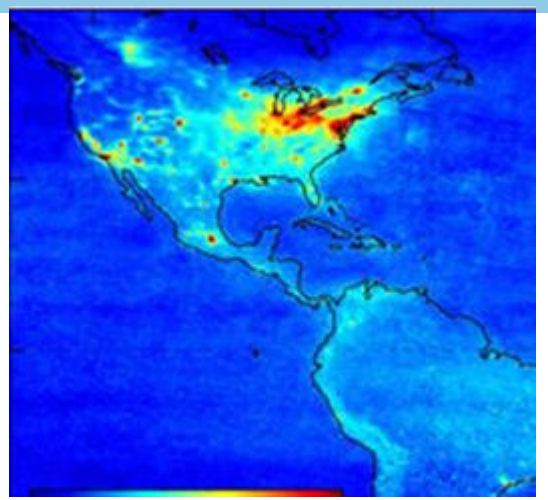
NO_2 and health effects

- Road traffic major source of NO_2

- Associations between day-to-day variations as well as long-term exposure to NO_2 and variations in mortality, hospital admissions, and respiratory symptoms

- Clinical and toxicological evidence provides some mechanistic support for a causal interpretation of the respiratory effects





Exceedances current standards

EU limit values

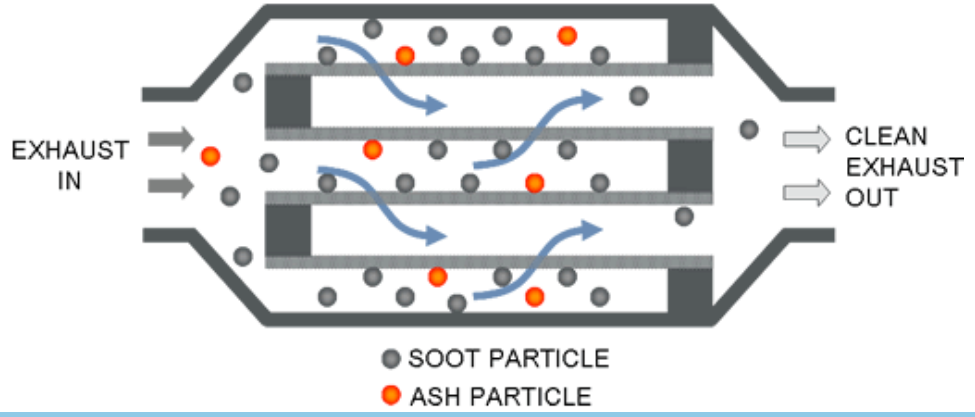
NO_2 hourly mean value may not exceed 200 µg/m^3 more than 18 times in a year

NO_2 annual mean value may not exceed 40 µg/m^3

The problem with NO_2 - uncertainties

- **WHO:** NO_2 might be more than an indicator for traffic-related air pollution – mechanistic evidence (respiratory) and the weight of evidence on short-term associations is **<u>suggestive</u>** of a causal relationship

- Increase direct NO_2 in ambient air due to PM emission control technologies



NO_2 and health effects

- So, how does NO_2 affect effects of complex mixtures?

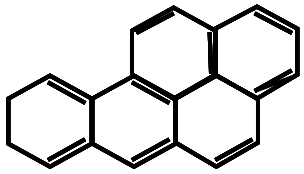
Hypothesis

NO_2 will accelerate effects of other components in the air pollution mixture

Inhalation study in mice

Sub-chronically exposure study to disentangle the possible adverse effects of NO_2 from other components in the air pollution mixture

Oxidative stress and inflammation caused by combustion-derived nanoparticles

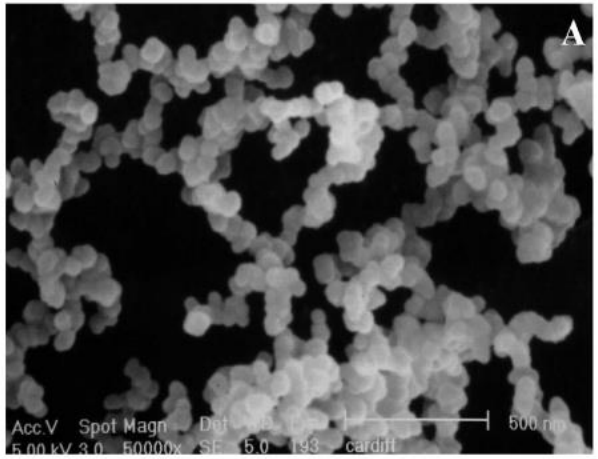


Organics

Fuel combustion

Surfaces

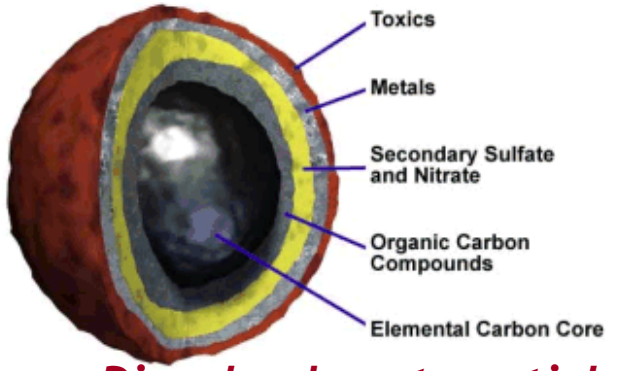
Nitrogen dioxide



Free radicals

Oxidative stress

Inflammation

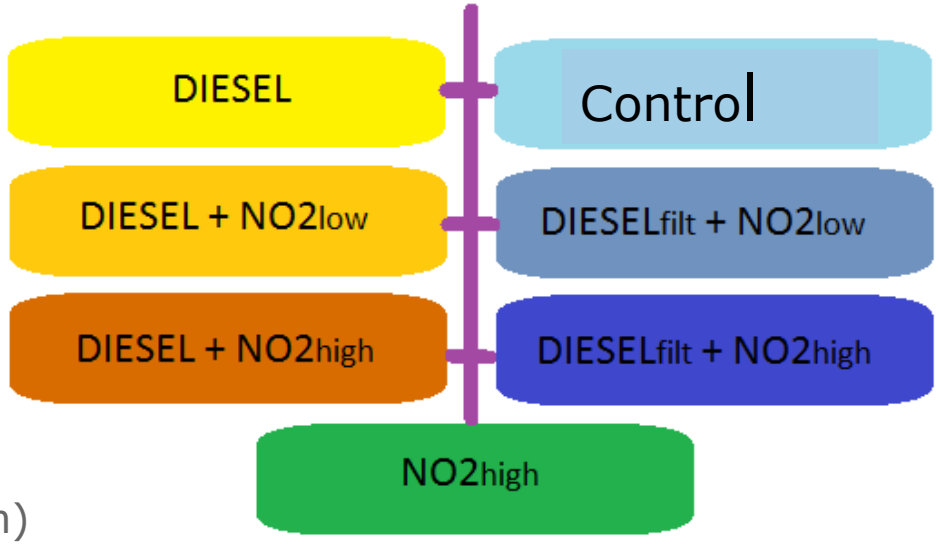


Diesel exhaust particle

Donaldson et al 2005, *Particle and Fibre Toxicology*

Design of complex mixture inhalation study

- Diesel engine exhaust
- Doped with NO_2



•Exposure groups

Group 1 - Clean air (control)
Group 2 - diesel (1 mg/m^3), NO_2 (2 ppm)
Group 3 - diesel (1 mg/m^3) + low NO_2 (4 ppm)
Group 4 - diesel (1mg/m^3) + high NO_2 (15 ppm)
Group 5 - filtered diesel (1 mg/m^3) + low NO_2
Group 6 - filtered diesel (1 mg/m^3) + high NO_2
Group 7 - high NO_2

1 ppm = 1.8 mg/m^3

Exposure

- 13 week inhalation study, 6 hours/day, 5 days/week
- 70 male C57/Bl6 mice (SPF, aged 7-8 wk)

Exposure characteristics

- *diesel generation: 110 KVA Genset 1500 rpm, 35 kw generator load, EN590 diesel*
- *Filtering of diesel to remove soot → reaction of particles with gases before filtration*

Diesel - average mass of 1077 µg/m^3, 1.1x10^6 particles/cm^3

NO_2 - 2, 4 and 15 ppm

Effect assessment

Necropsy was performed one day after the last exposure

- collect blood,
- lung (pathology, mRNA expression),
- brain (mRNA expression in specific regions including cortex),
- heart, spleen, liver, and kidney

Effect assessment - results

- No effects on body weight or weight of the organs heart, liver and spleen

- However, the lung weights were significantly increased in animals exposed to test atmospheres with high NO_2,
  - irrespective the presence of DE in the test atmosphere

Effect assessment – results histopathology

Increased septal cellularity in the lungs of

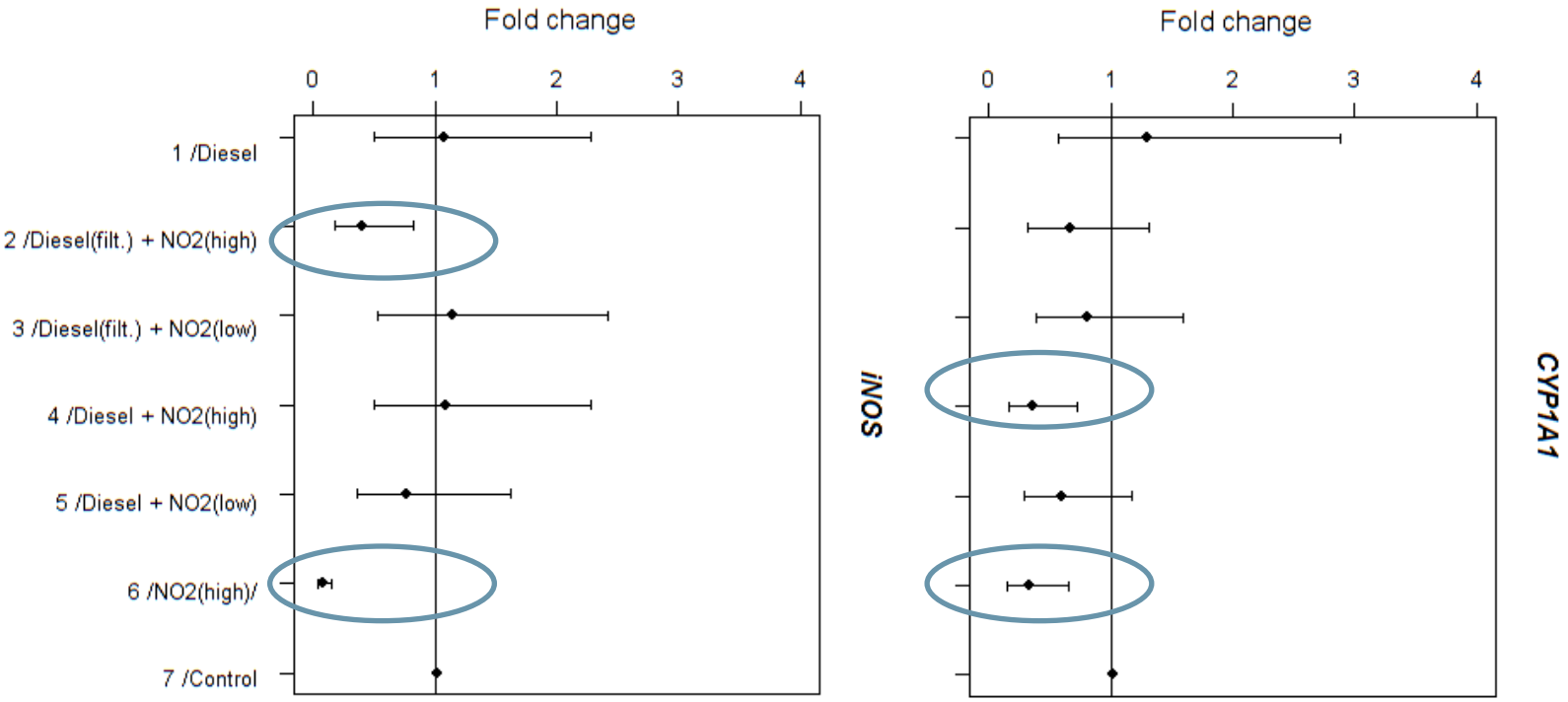
- 2/5 animals exposed to DEE + high NO_2 or filtered DEE + high NO_2
- 4/5 animals exposed to high NO_2 alone

- lungs of animals exposed to diesel engine exhaust showed a diffuse accumulation of black pigmented macrophage
- Bottum line: very mild effects mostly related to NO_2

Selected genes

Gene	Function
CYP1A1 *{Cytochrome p450}*	xenobiotic and drug metabolism Activity ↓by antibiotics (fluoroquinolones, macrolides) ↑ by aromatic hydrocarbons (e.g., diesel exhaust).
iNOS *{Inducible nitric oxide synthase}*	immune defence catalyzes the production of NO (vascular tone, insulin secretion, airway tone etc.)
TNFα *{Tumor necrosis factor alpha}*	cytokine (involved in cell communication), takes part in the acute phase reaction (inflammation) and in the systemic inflammation,
COX-2 *{Cyclooxygenase-2}*	elevated during inflammation
HO-1 *{Heme oxygenase (decycling)1}*	Sensitive biomarker of oxidative stress
ICAM-1 *{Intercellular Adhesion Molecule 1)}*	activated leukocytes bind to endothelial cells via ICAM-1/LFA-1 and then transmigrate into tissues.

Results – lung mRNA expression



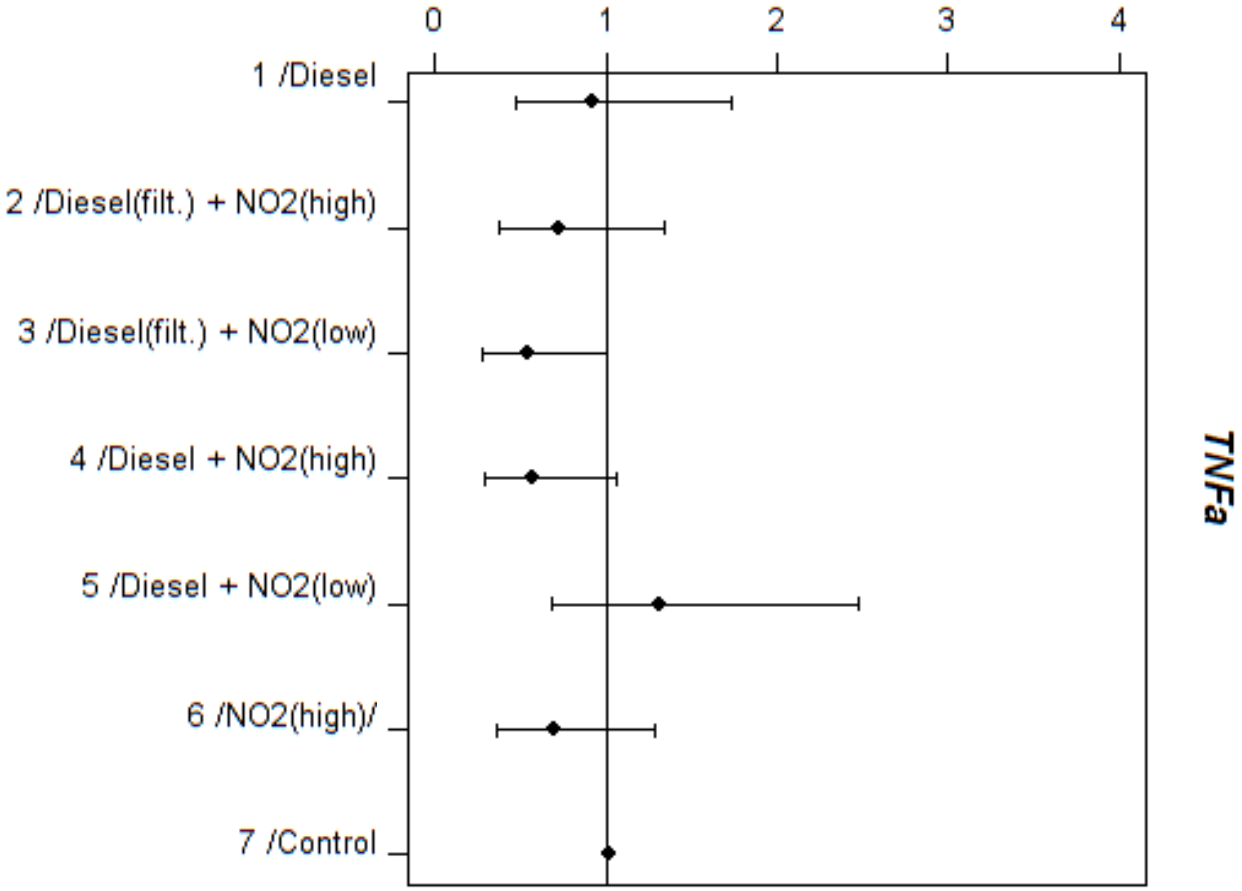
iNOS

inducible nitric oxide synthase

CYP1A1

cytochrome P450

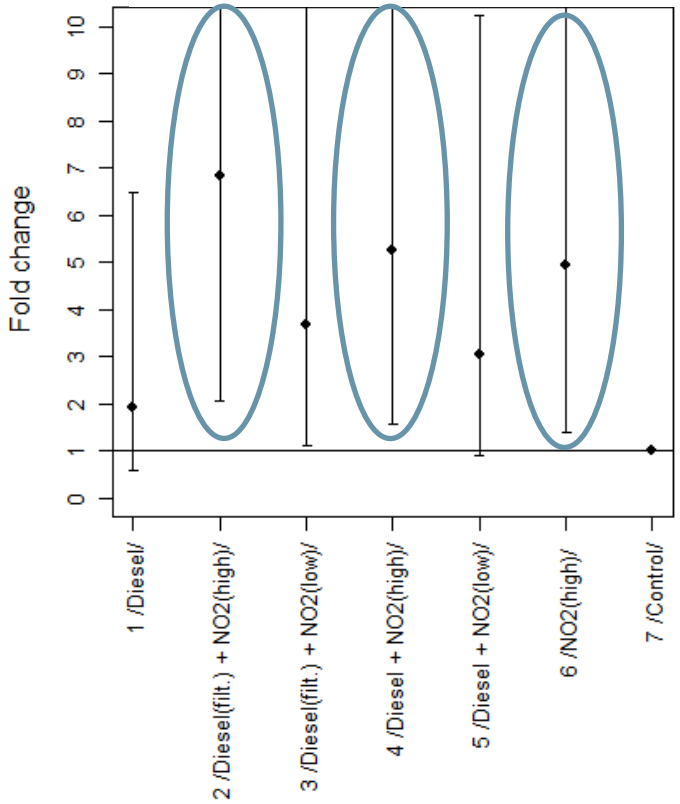
Results – lung mRNA expression



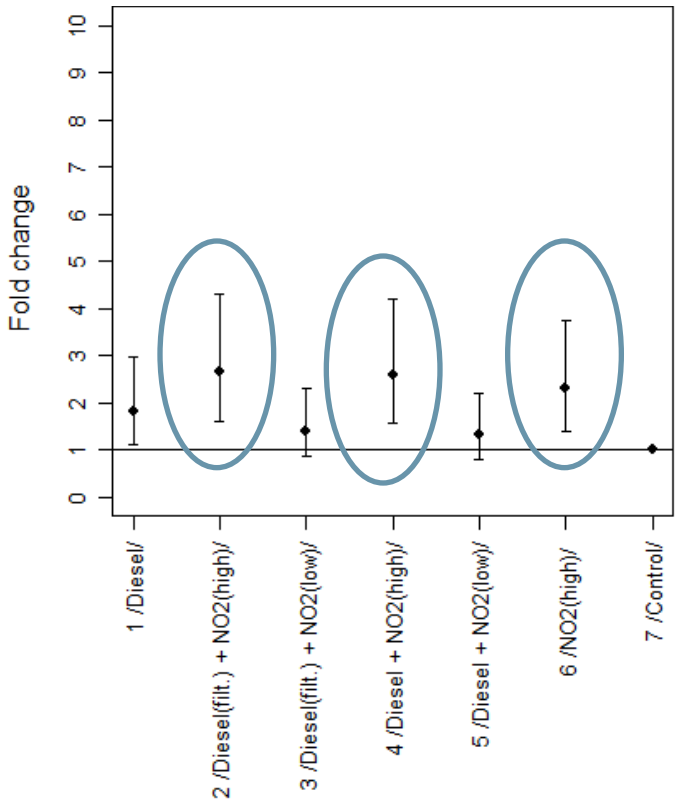
TNFa - tumor necrosis factor alpha

Results – lung mRNA expression

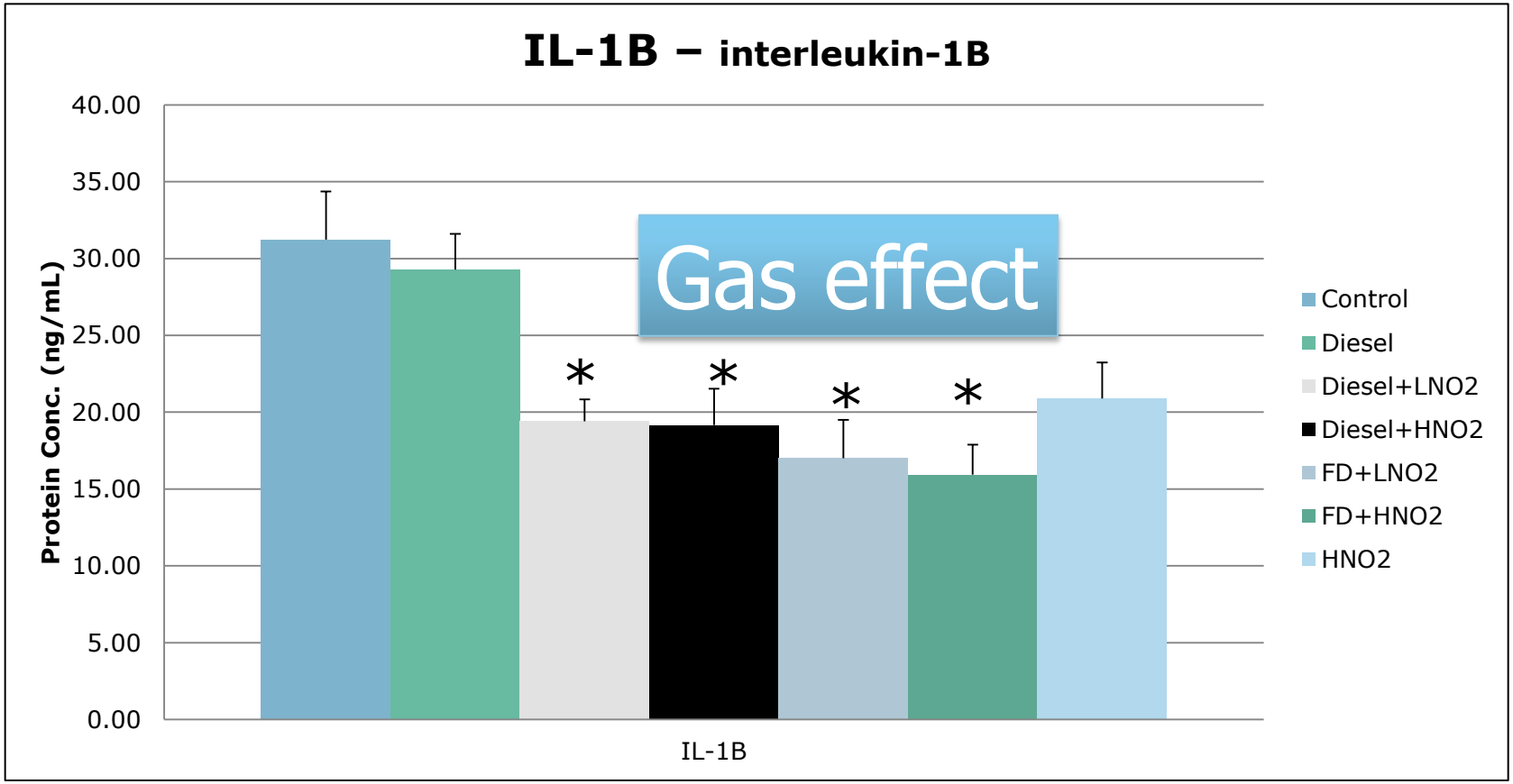
ICAM - intercellular adhesion molecule



NQO1 - NAD(P)H dehydrogenase (quinone 1)



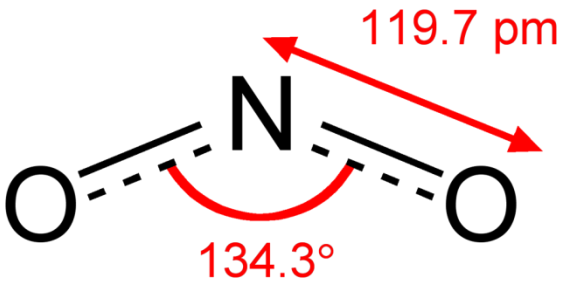
Gene expressions in brain

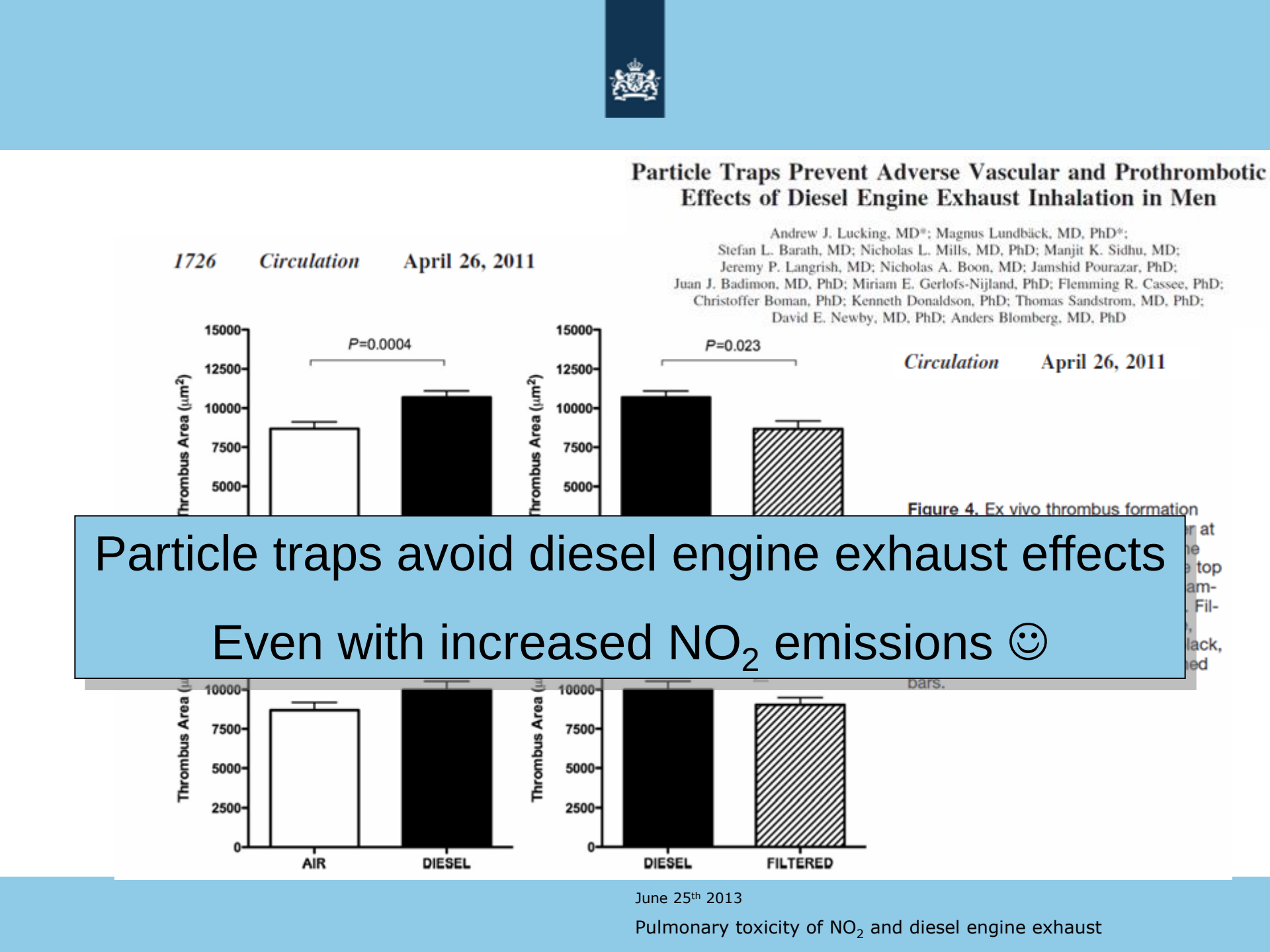


* = Significantly different from Control

Conclusions

- No marked pathology: new diesel technology clean?
- Higher concentrations of NO_2 tend to affect expression of the genes studied
- Possible dose-response effect of NO_2




Particle Traps Prevent Adverse Vascular and Prothrombotic Effects of Diesel Engine Exhaust Inhalation in Men
Andrew J. Lucking, MD*; Magnus Lundbäck, MD, PhD*; Stefan L. Barath, MD; Nicholas L. Mills, MD, PhD; Manjit K. Sidhu, MD; Jeremy P. Langrish, MD; Nicholas A. Boon, MD; Jamshid Pourazar, PhD; Juan J. Badimon, MD, PhD; Miriam E. Gerlofs-Nijland, PhD; Flemming R. Cassee, PhD; Christoffer Boman, PhD; Kenneth Donaldson, PhD; Thomas Sandstrom, MD, PhD; David E. Newby, MD, PhD; Anders Blomberg, MD, PhD
1726 Circulation April 26, 2011
Circulation April 26, 2011
P=0.0004
P=0.023
Thrombus Area (μm²)
15000
12500
10000
7500
5000
2500
0
AIR
DIESEL
FILTERED
Figure 4. Ex vivo thrombus formation
Particle traps avoid diesel engine exhaust effects
Even with increased NO2 emissions ☺

Acknowledgement

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