Toxic Potential of 2- and 4-stroke Scooter and Diesel Car Exhaust Emissions in Lung Cells *In Vitro*

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Introduction

A growing number of scooters (small two-wheeled vehicles, maximum speed of 45 km/h, engine capacity of 50 cm^3) produce an increasing amount of potentially harmful emissions. Comparisons between cars (Euro 3 standard) and various scooters show that two-stroke scooters can emit 10-23 times more carbon monoxide (CO), 10-171 times more total hydrocarbons (HC) and 3-8 times more nitrogen oxides (NO_x), depending on the scooter type (Rüdy and Weilenmann, 2006). Further high emissions of polycyclic aromatic HCs (PAH) and very high amounts of particulate matter (PM) in the nanoscale range are typical for scooter exhaust emissions (Czerwinski and Schramm, 2006; Rijkeboer et al, 2005). Therefore, scooters are significant contributors to air pollution (McDonald et al, 2005) and have to be considered as so-called super-polluters (McDonald et al, 2005; Siegmann et al, 2008).

PM, HC, PAH, NO_x, CO and also diesel exhaust particles (DEP) are known to have adverse effects on lung cells via the pathway of oxidative stress and (pro-) inflammatory responses by the release of tumor necrosis factor alpha (TNF-α) and interleukin 8 (IL-8) (Brandsma et al, 2008; Brunekreef and Holgate, 2002; Chhikara et al, 2009; Sawyer et al, 2010; Scapellato and Lotti, 2007). In addition, these pollutants are associated with the development of pulmonary and cardiovascular diseases (Becker et al, 2005; Brauner et al, 2007; Donaldson et al, 2005; Pan et al, 2004; Xiao et al, 2003).

Methods

The toxicity of exhaust emissions was evaluated using a newly developed exposure system (Muller et al, 2010): Triple cell co-cultures (Rothen-Rutishauser et al, 2005), simulating the human epithelial airway barrier, were directly exposed at the air-liquid interface to exhaust emissions (2h exposure, dilution of 1:100, constant conditions of 5% CO_2, 37°C and 80% relative humidity). Parallel to the exposure chamber, a reference chamber was also treated equally, but with ultra clean air only. Particle number, total particle surface area, mean particle diameter, CO, HC and NO_x concentrations were measured. Cellular responses were assessed by looking at the cell morphology (staining of the actin cytoskeleton, cell nuclei, tight junction protein occludin) and by measuring the cytotoxicity (release of lactate dehydrogenase (LDH)) and (pro-) inflammatory responses (TNF-α, IL-8) after 8 and 24h post-exposure time.

Statistical analysis for physical characterization was conducted using ANOVA on Ranks test, followed by Dunn's test in case of significance or by using the Wilcoxon test in the case of only two comparison groups. The effects of emission parameters on biological parameters were analyzed using linear regression.

The emissions of two 2-stroke scooters (each with four different set-ups) and a 4-stroke scooter, and two diesel cars were compared (for technical details see Table 1).

Results

The highest particle number was found for the worst cases of 2-stroke direct injection (TSDI) and 2-stroke carburetor scooters. The particle number was statistically significantly reduced following filtration of the removed exhaust. For both 2-stroke scooters, the particle number was reduced in best case and even more in absolute best case conditions. The 4-stroke scooter emitted fewer particles than the 2-stroke scooters, but more than the diesel car with a diesel particle filter (DPF). The size of the particles was smaller for the carburetor scooter than for the TSDI scooter, and only very small particles were emitted in absolute best cases.

Table 1. Technical details of the vehicles and set-ups used

Scooter Peugeot 2-stroke direct injection	**worst case** • unleaded fuel • Swiss army oil • 100% oil ratio • dummy muffler	**worst case – filtered**	**best case** • Aspen fuel • Motorex oil • 50% oil ratio • oxi cat & wire mesh filter catalyst	**absolute best case** • Aspen fuel • Motorex oil • 50% oil ratio • coated particle filter
Scooter Peugeot 2-stroke carburetor				
Aprilia 4-stroke scooter	**normal conditions** (original, without catalyst)			
Diesel car	**with particle filter**		**without particle filter**	

The NO_x concentrations were highest for TSDI emissions, followed by the diesel cars and 4-stroke scooter, and were lowest for the carburetor 2-stroke scooter. With the application of best and absolute best case optimizations, not only the particle number but also the NO_x concentrations could be reduced.

In addition to the particle number concentration, the particle number-size distribution and the NO_x concentrations, the total active surface area of the particles, the mean diameter, and the CO and HC concentrations were measured.

Concerning the biological effects, the following correlation could be shown.

- The concentration of NO_x was negatively correlated with the TNF-α and IL-8 concentration after 8h post-exposure time. This was the opposite of what was expected. A possible explanation is the probable high amount of NO in the exhaust of 2-stroke scooters. As NO is known to activate the immune response (Fishman et al, 2008), this could protect the cell cultures.
- The mean particle diameter and the particle number between 10-100nm were positively correlated with the IL-8 concentration after 8h post-exposure time.
- The HC concentration was positively correlated with the TNF-α concentration after 24h post-exposure time.
- The TNF-α concentration was significantly reduced in TSDI absolute best case compared to 2-stroke direct injection worst case.
- The main influence on toxic potential was found for particle number between 10-100nm: A reduction of particle number of 99.9% resulted in an 8% lower toxic potential (after 2h exposure). The reduction would probably be even higher after a longer exposure duration.

Conclusions

- There is a high potential for emission reduction (particulate & gaseous compounds) through technical optimizations (particle filter, better oil & fuel)
- The highest influence on toxic potential was found for particle number between 10-100nm

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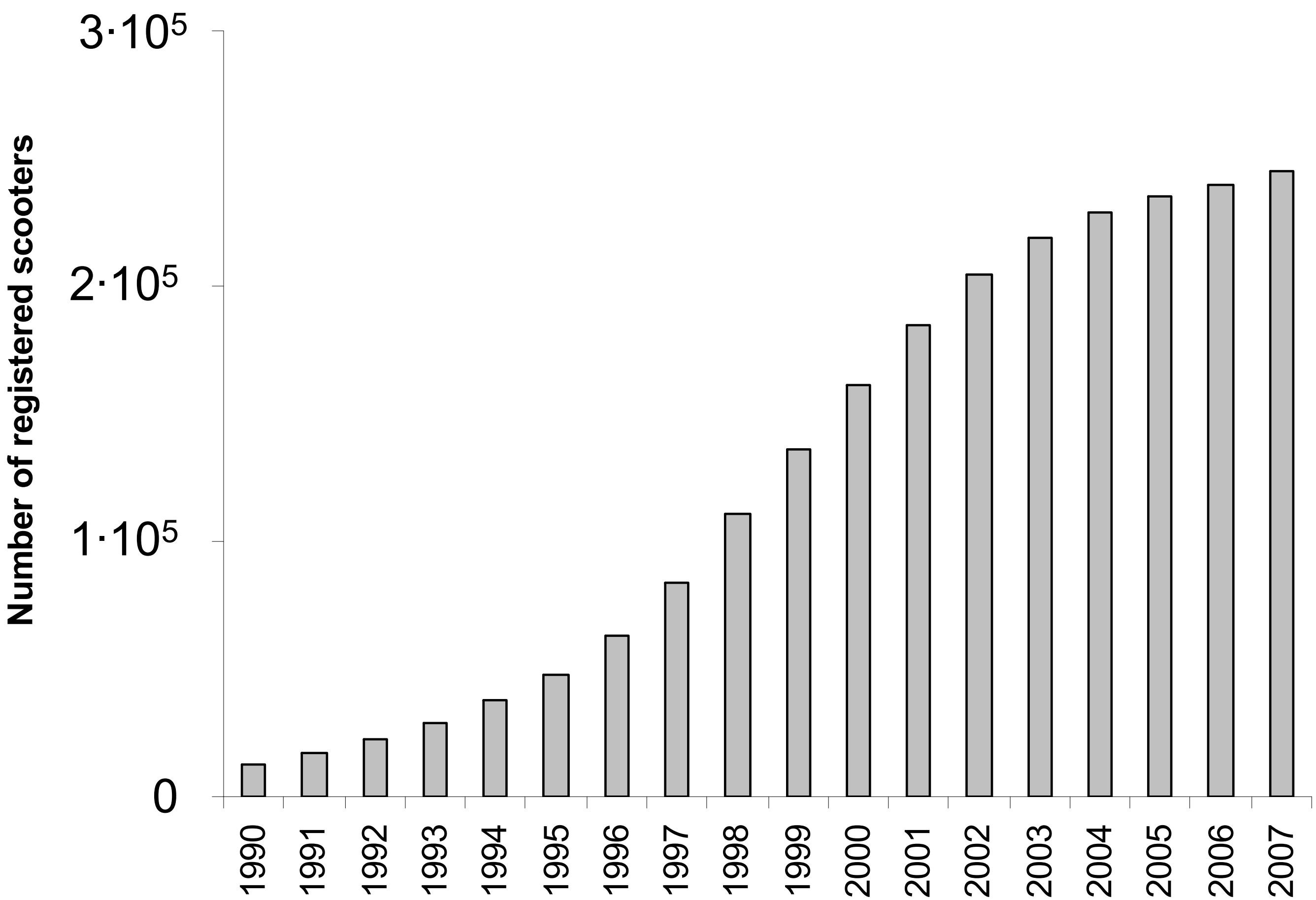
14th ETH Conference on Combustion Generated Nanoparticles

Zurich, 3rd August 2010

Loretta Müller
University of Bern, Institute of Anatomy

Increasing number of scooters

- Increasing number of registered scooters in Switzerland



Swiss Statistics, www.admin.ch

Scooters as “super-polluters”?

- Increasing number of registered scooters in Switzerland

- Predictions
  - Switzerland 2010: all scooters together will emit more **volatile organic compounds** than all cars together

    (BUWAL Schriftenreihe, Umwelt Nr. 355)

  - Europe: by 2020 scooters will emit more **total hydrocarbons** than all other vehicle categories

    (EC report No. 08.RE.0019.V4)

- 2-stroke scooters may be “**super-polluters**”

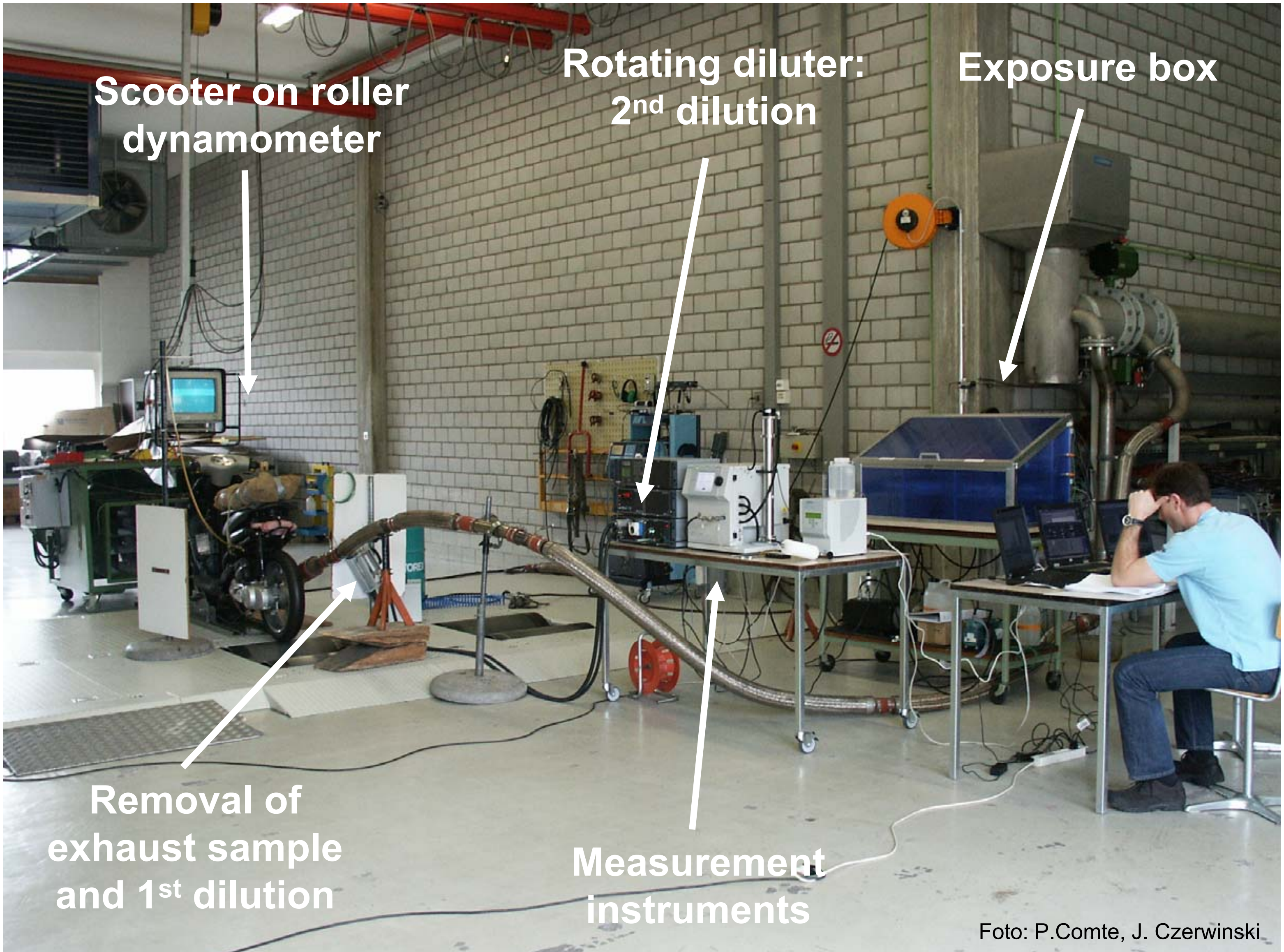
  (Delft Outlook (2006); Siegmann et al (2008) Atmos Environ)

Without a comment…

Scooter jam in Taipeh, Taiwan

(Blick am Abend, 29.10.2009)

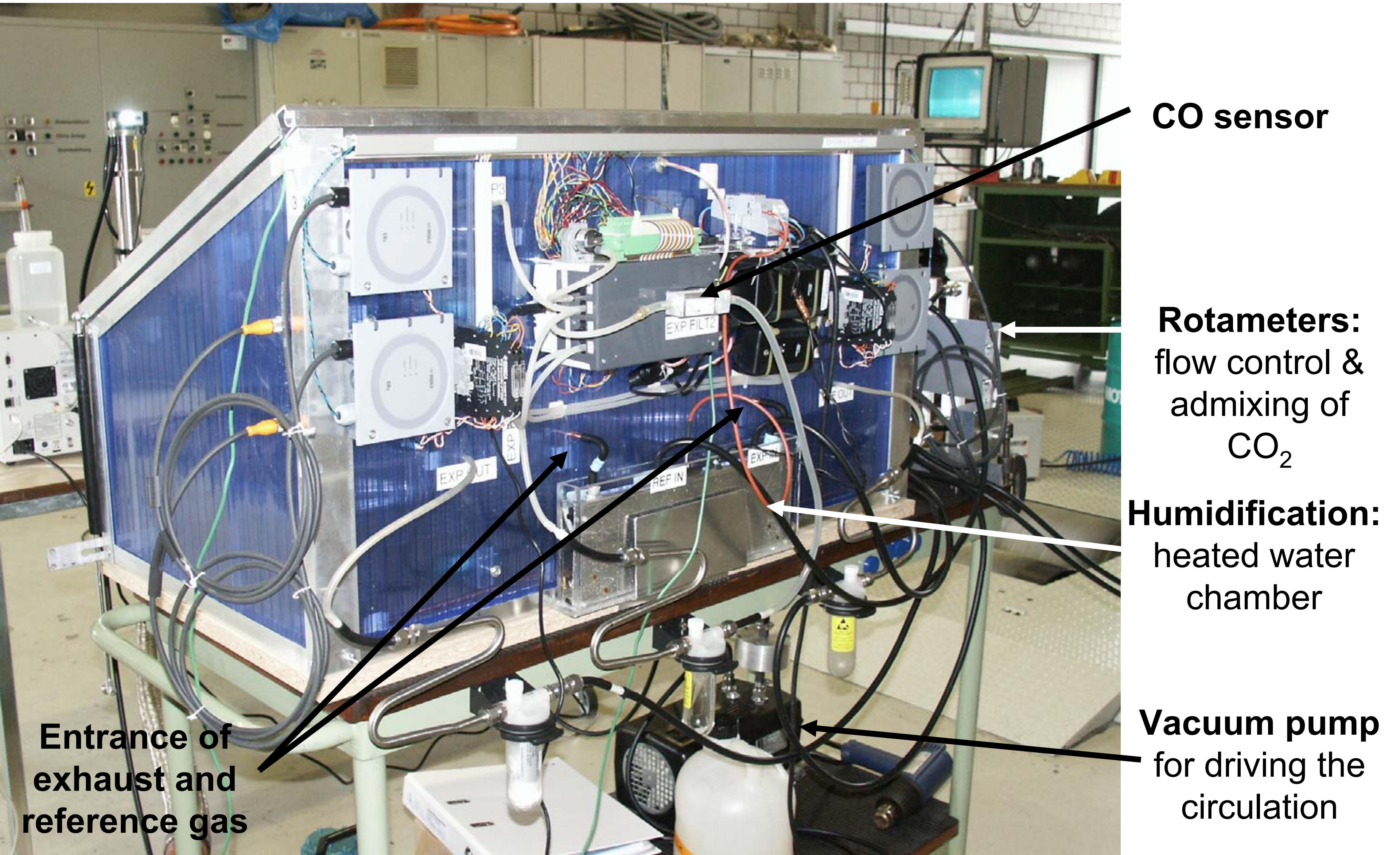
Overview of the exposure system



Constant velocity of 40 km/h

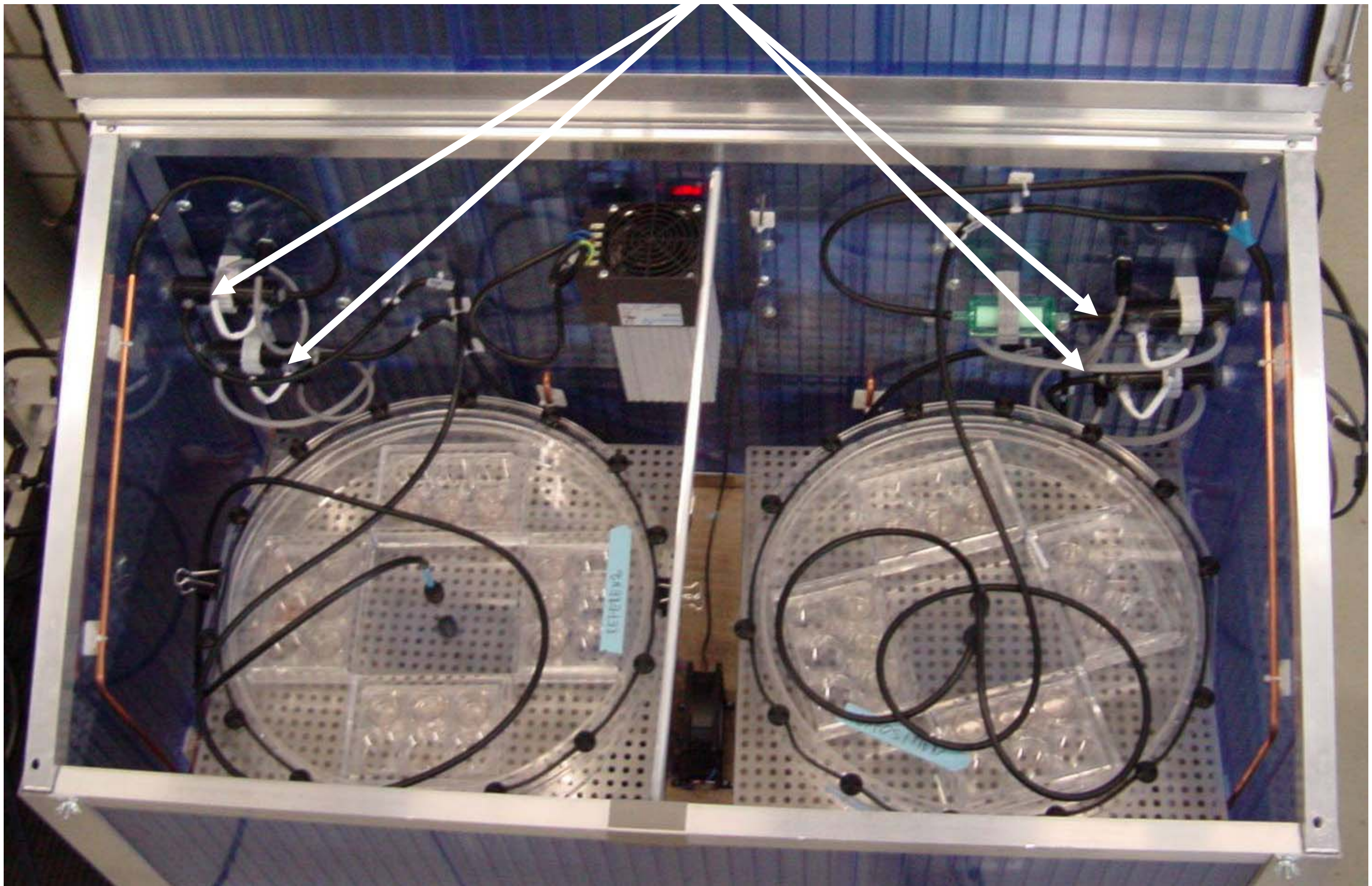
(Muller et al (2010) Environ Sci Technol)

Heated box


CO sensor
Rotameters: flow control & admixing of CO_2
Humidification: heated water chamber
Vacuum pump for driving the circulation
Entrance of exhaust and reference gas
EXP FILT2
EXP OUT
REF IN
P3

Exposure chamber

Control measurement unit
(pressure differences, temperature, CO_2 concentration, relative humidity)



Reference **Exposure**

Control
(in the incubator)

(Muller et al (2010) Environ Sci Technol)

Comparison of different (scooter) emissions

<table>
<tr><td>Scooter Peugeot 2-stroke direct injection</td><td rowspan="2">worst case
• unleaded fuel
• Swiss army oil
• 100% oil ratio
• dummy muffler</td><td rowspan="2">worst case
–
filtered</td><td rowspan="2">best case
• Aspen fuel
• Motorex oil
• 50% oil ratio
• oxi cat & wire mesh filter catalyst</td><td rowspan="2">absolute best case
• Aspen fuel
• Motorex oil
• 50% oil ratio
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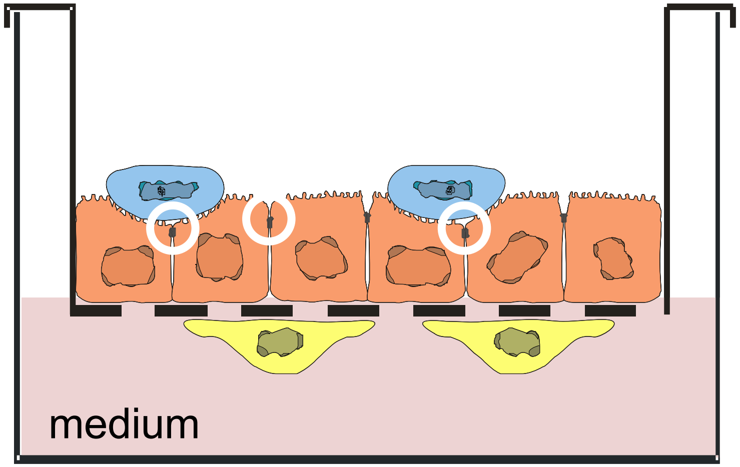
<tr><td>Scooter Peugeot 2-stroke carburetor</td></tr>
<tr><td>Aprilia 4-stroke scooter</td><td colspan="4">normal conditions (original, without catalyst)</td></tr>
<tr><td>Diesel car</td><td colspan="2">with particle filter</td><td colspan="2">without particle filter</td></tr>
</table>

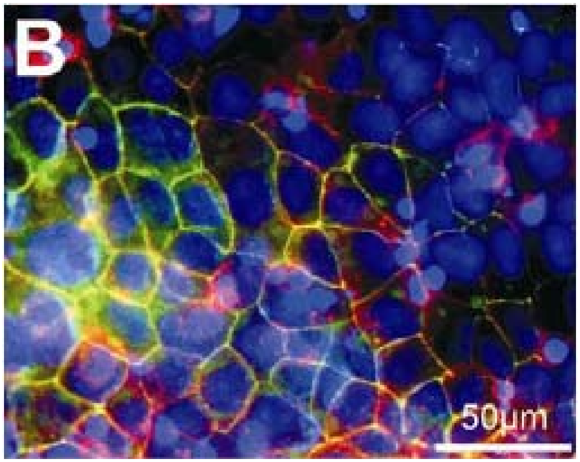
- Each 3 exposures with 3 different cell cultures (n=3)
- 2h exposure time, 1:100 dilution, 8h & 24h post-incubation

Biological analysis



Co-cultures of human

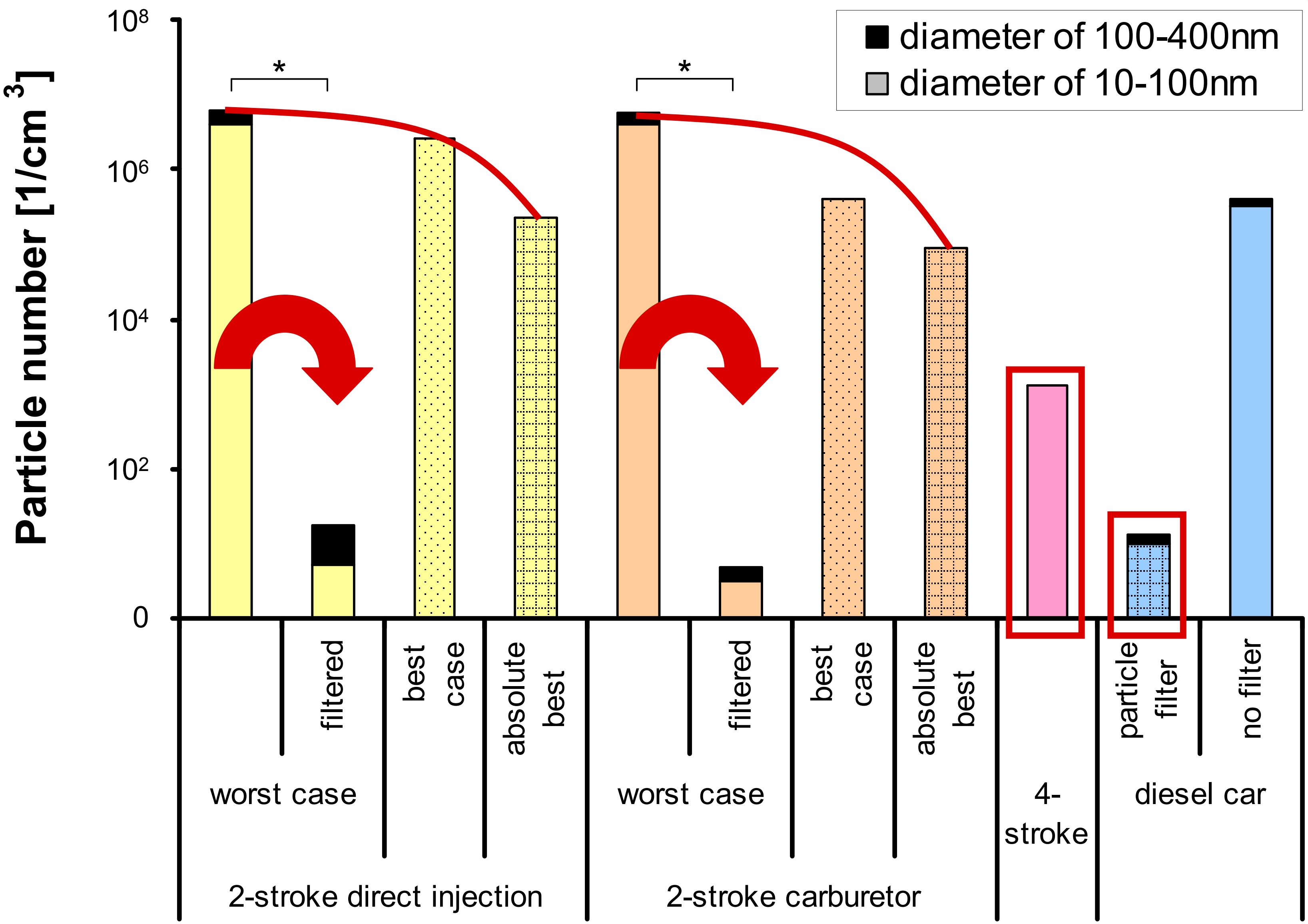
- bronchial **epithelial cells** (16HBE14o⁻ cell line)
- monocyte-derived **macrophages**
- monocyte derived **dendritic cells**



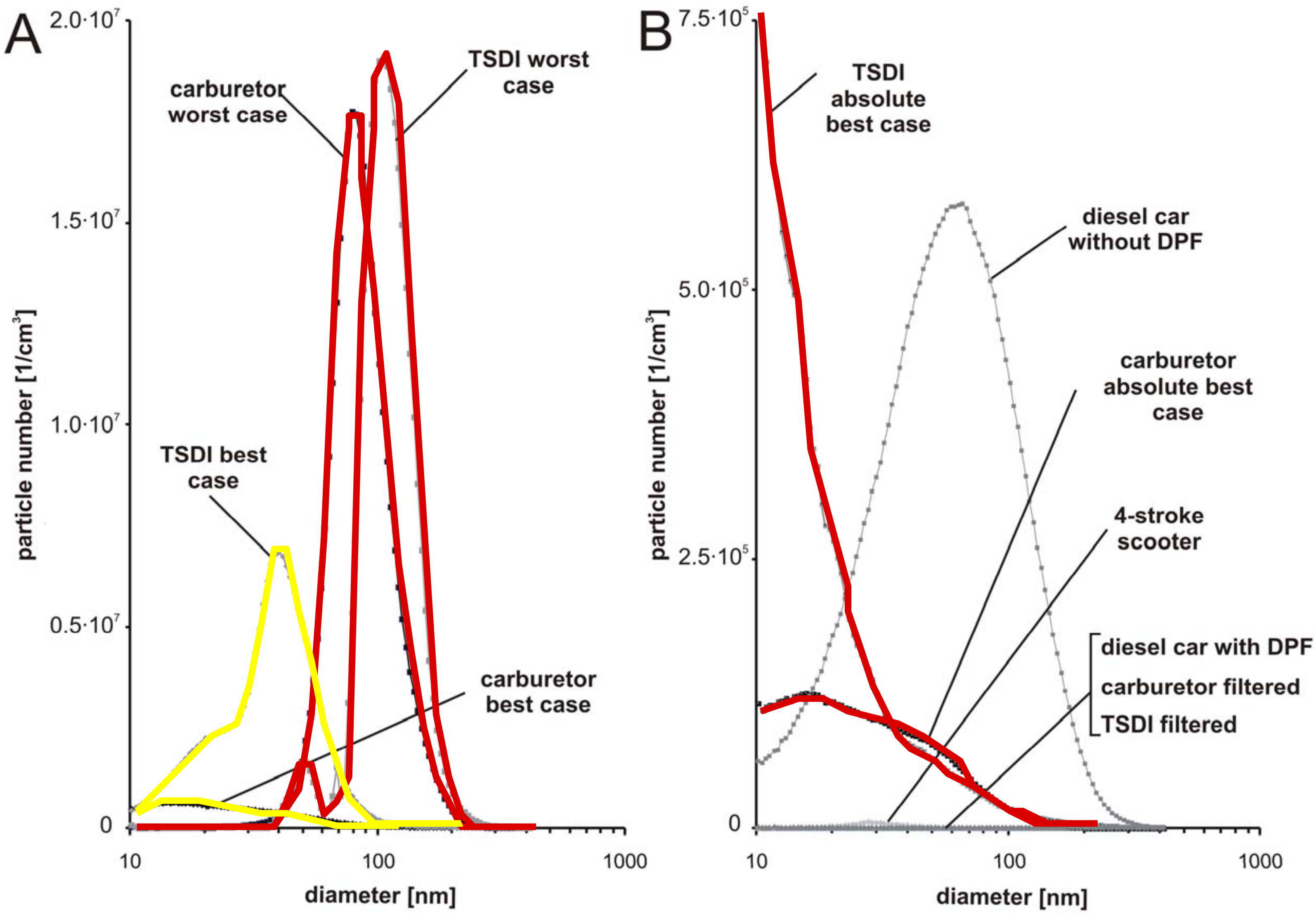
(Muller et al (2010) Environ Sci Technol)

- **Cell morphology**: actin cytoskeleton, cell nuclei and tight junction protein occludin
- **Cytotoxicity** (release of lactate dehydrogenase)
- **(Pro-)inflammatory reaction** (release of tumor necrosis factor α and release of interleukin 8 (IL-8))

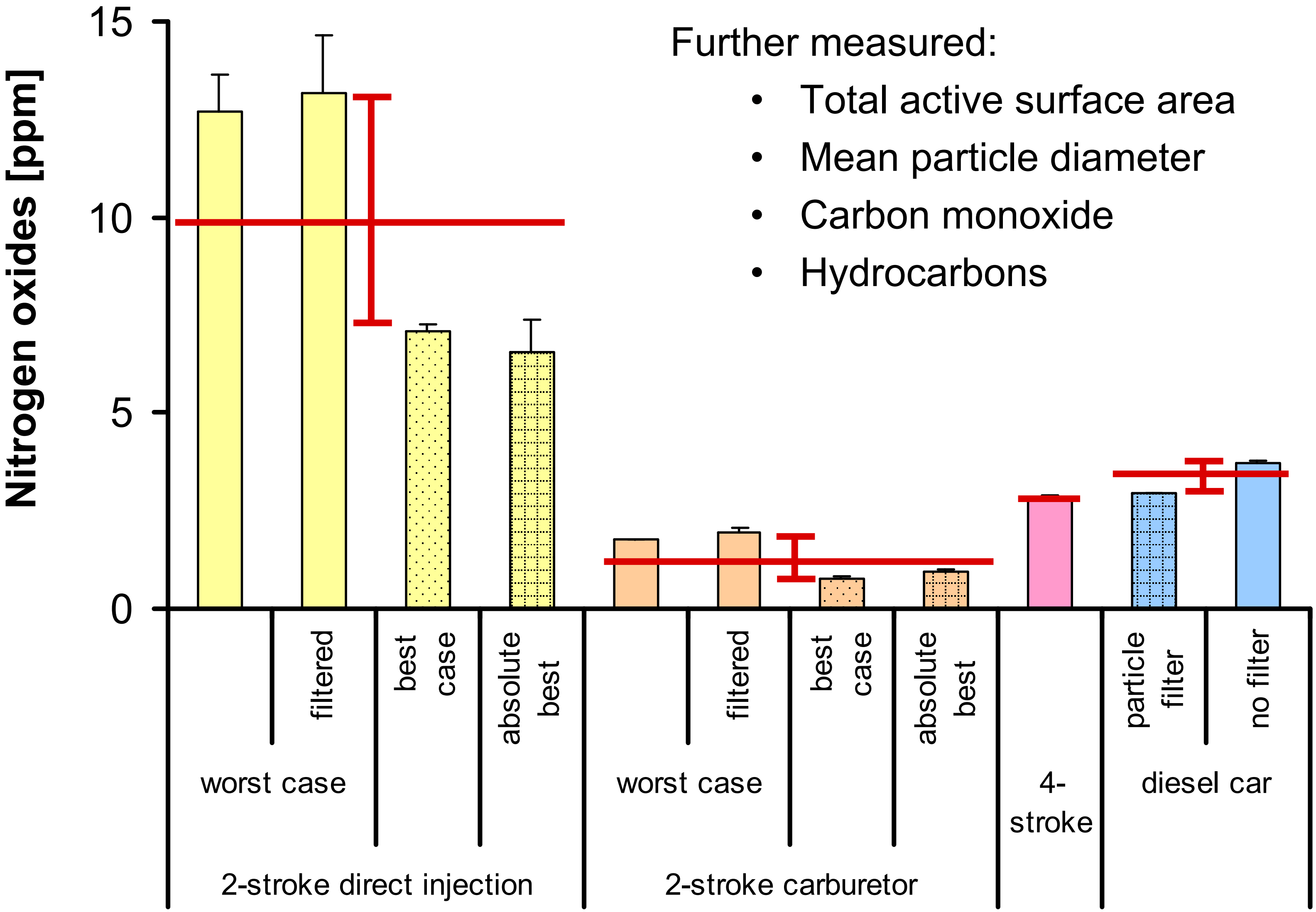
Results: particles



Results: particle size distribution



Results: gases



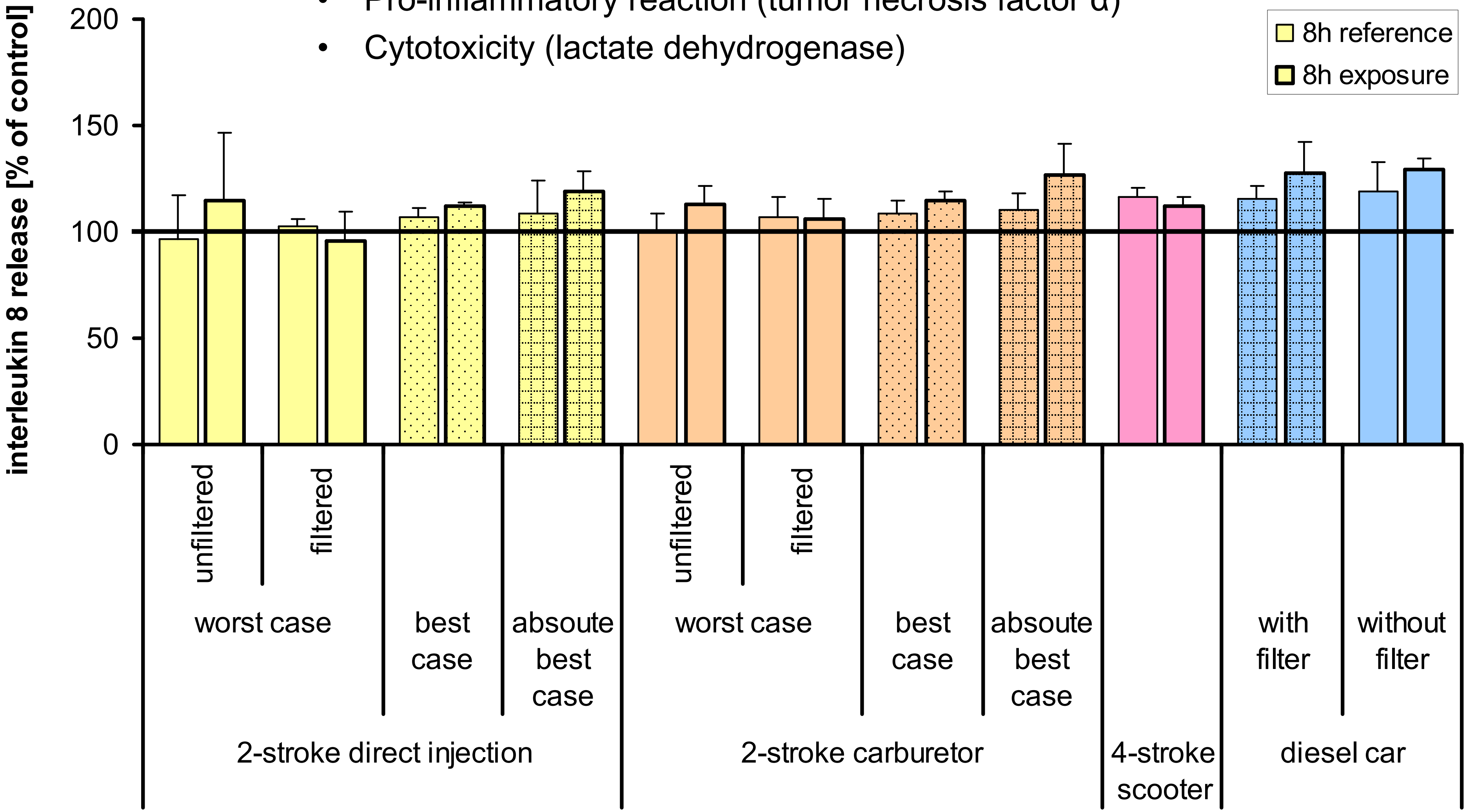
Further measured:

- Total active surface area
- Mean particle diameter
- Carbon monoxide
- Hydrocarbons

Results: toxic potential (inflammatory reaction)

Further measured:

- Pro-inflammatory reaction (tumor necrosis factor α)
- Cytotoxicity (lactate dehydrogenase)



Results: biological effects

- Concentration of **nitrogen oxides** is **negatively correlated** with the **tumor necrosis factor α** and **interleukin 8** concentration after 8h post-exposure time
  - > Maybe mostly NO, which could activate the immune response
- **Mean particle diameter** and **particle number between 10-100nm** are positively **correlated** with the **interleukin 8** concentration after **8h** post-exposure time
- **Hydrocarbon** concentration is positively correlated with the **tumor necrosis factor α** concentration after **24h** post-exposure time
- **Tumor necrosis factor α** concentration was significantly **reduced** in **2-stroke direct injection absolute best case** compared to 2-stroke direct injection worst case
- **Main influence** on toxic potential for **particle number between 10-100nm**: 99.9% **reduction of particle number** results in a 8% **lower toxic potential** (after 2h exposure)

Conclusion & Outlook

- High potential for emission reduction (particulate & gaseous compounds) through **technical optimizations** (particle filter, better oil & fuel)
- Tendency **for higher toxic potential of 2-stroke** scooters than for 4-stroke scooter and diesel cars
- Highest influence on toxic potential found for **particle number between 10-100nm**
- **Experimental optimizations:** More repetitions, longer exposure duration (chronic & acute manner)
- Measuring of **nitrogen dioxide** and **nitrogen monoxide** concentration (not only nitrogen oxides)

Special Thank to…

... to everybody from the group of **P.Gehr & B.Rothen-Rutishauser** (Institute of Anatomy, University of Bern)

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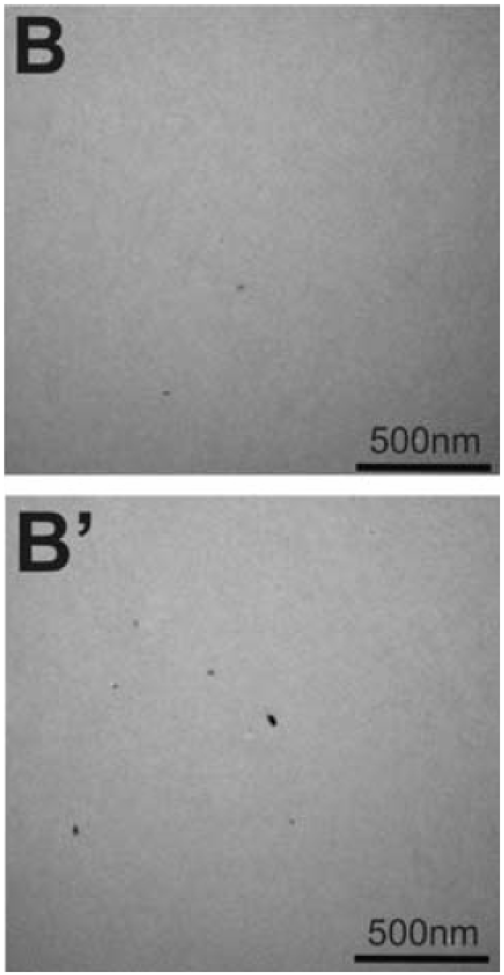
... to everybody I forgot and you!


B
500nm
B'
500nm

Comparison of different vehicles

- 2h exposure time
- Dilution of 1:100, flow of 2 l/min
- Fixation of cells 8h & 24h post-exposure
- **Co-cultures:** human bronchial epithelial cell line 16HBE14o$^-$ with monocyte-derived macrophages and dendritic cells
- 3 x 2h exposure with each 3 different cell cultures
- Different vehicles