Cytotoxicity and inflammatory potential of two stroke scooter exhaust in human lung cells *in vitro*

Loretta L. Müller[1], Pierre Comte [2], Jan Czerwinski[2], Markus Kasper[3], Andreas C.R. Mayer[4], Jean-Paul Morin[5], Peter Gehr[1], and Barbara Rothen-Rutishauser[1]

1. University of Bern, Institute of Anatomy, Bern, Switzerland; 2. AFHB, University of Applied Sciences, Biel-Bienne, Switzerland; 3. Matter Engineering AG, Nanoparticle Measurement, Wohlen, Switzerland; 4. Technik Thermische Maschinen (TTM), Zurich, Switzerland, 5. INSERM, Université de Rouen, France
E-mail: loretta.mueller@ana.unibe.ch

Introduction

The number of registered scooters in Switzerland and in other countries is increasing every year. In some countries scooters are already the main mean of transport in cities. Because of their simple technology they produce exhaust with a lot of (ultra)fine particles which have the potential to cause adverse health effects [1]. To investigate the toxicity of freshly produced scooter exhaust and various upgrades, e.g catalyst, particle filter, quality of fuel and oil and quantity of oil, we developed a exposure system and tested different scooters.

Exposure Mode

- Peugeot two-stroke direct injection (**TSDI**) & Peugeot **carburettor**
- **worst case:** dummy muffler; worst oil, 100% oil-ratio, unleaded fuel
  best case: oxi cat, wire mesh filter catalyst; best lube oil, 50% oil-ratio; Aspen fuel
- 2h exposure, 8h & 24h after-incubation of cells
- results are means of 3 exposures with each 3 cell cultures (n = 3x3 = 9)

Conclusion

- highest particle number, surface area & elemental carbon in TSDI worst case conditions
- highest biological reactions (cytotoxicity & inflammation) in carburettor worst case

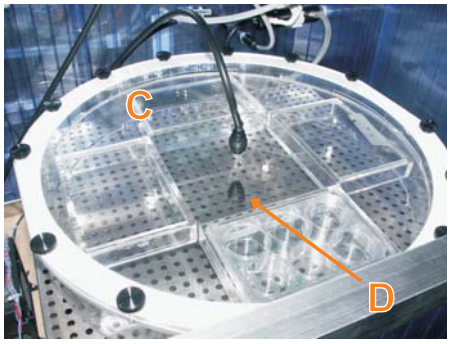
Exposure System

Cell culture system

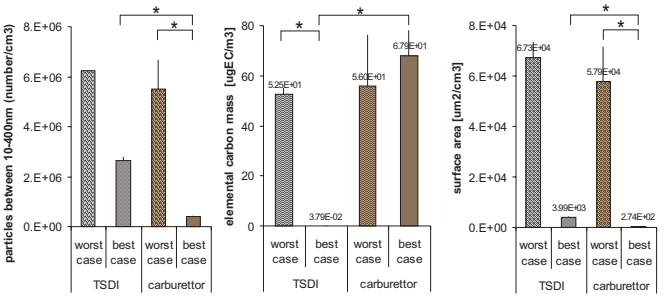
- cell cultivation at air for 18h, warming up of exposure system (90min) & scooter (30min full throttle, 20min at 40km/h)

Processing of exhaust/control air

- 2 dilution steps after removal of exhaust (A, dilution of 1:100)
- measurement of particle properties (B)
- admixing of 5% CO_2 & humidification (80% relative humidity)
- entrance of exhaust and reference air to heated box
- control measurements (p, T, rH, CO_2, CO) before & after exposure chamber
- passing exposure chamber (C; flow of 2 l/min; developed by A. Konstandopoulos): contact of exhaust with cell cultures, suction of the air at the bottom of the chamber (D)



Results: comparison of physical and biological effects between Peugeot TSDI and carburettor



Particle number

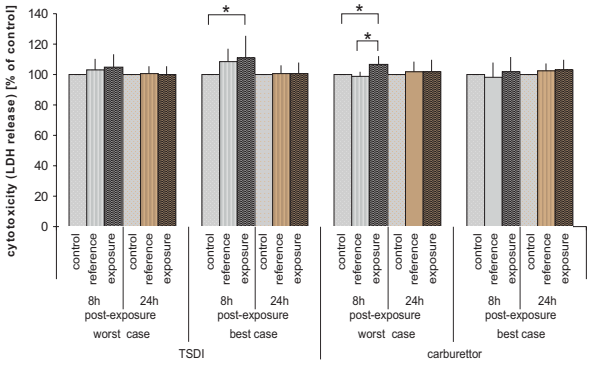
- higher in worst cases than in best cases
- more in TSDI than in carburettor

Elemental carbon mass

- TSDI: worst case higher, best case very low
- carburettor: equal amounts, higher than TSDI

Surface area

- worst cases higher than best cases
- TSDI higher compared to carburettor

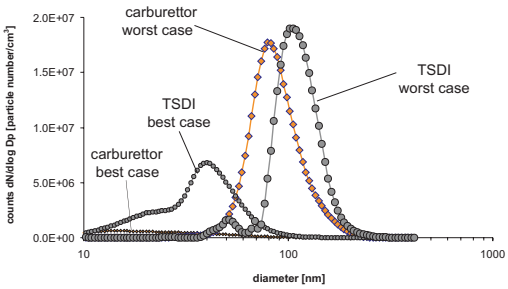


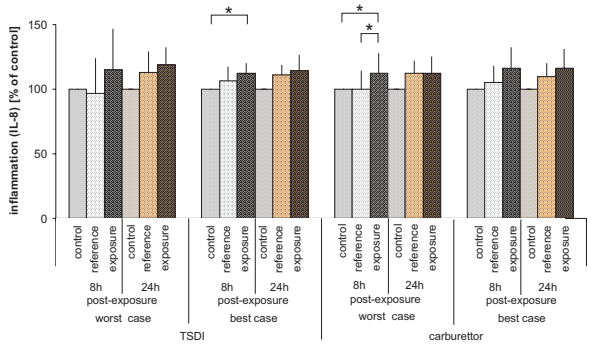
Cytotoxicity (lactate dehydrogenase (LDH))

- TSDI best case,8h post-expsoure time: higher in exposure than control
- carburettor worst case, 8h post-exposure time: higher in exposure than control and reference

Particle size Distribution

- worst cases bigger particles than best cases
- carburettor smaller particles than TSDI





Inflammation marker IL-8

- TSDI best case, 8h post-exposure time: exposure higher than control
- carburettor worst case, 8h post-exposure time: exposure higher than control & reference
- All conditions: tendency (not statistically significant) for higher concentrations in exposures than in controls

Material and Methods

Cell cultures: The **triple cell co-cultures** were prepared as described in [2], as epithelial cells **16hbe 14o** were used. For the experiments 0.5x10^6 cells/ml were added to cell culture inserts in 6well plates and grown submersed in medium for 7d to confluence. For triple cell co-culture on the top **human blood monocyte-derived macrophages** and on the bottom **human blood monocyte-derived dendritic cells** were added [2].
Cytotoxicity: The concentration of lactate dehydrogenase (LDH) in the supernatant of cell cultures was estimated using LDH assay kit (Roche Applie Science) following the user protocol.
Inflammation: As markers for inflammation TNFα and IL-8 concentrations were determined by ELISA (R&D

Literature

[1] Peters, A., Wichmann, H. E., Tuch, T., Heinrich, J., and Heyder, J. (1997). Respiratory effects are associated with the number of ultrafine particles. Am.J.Respir.Crit Care Med., 155, 4, 1376-1383.
[2] Rothen-Rutishauser, B. M., Kiama, S. G., and Gehr, P. (2005). A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. Am J Respir Cell Mol Biol, 32, 4, 281-9.

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