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

Loretta L. Müller1, Pierre Comte2, Jan Czerwinski2, Markus Kasper3, Andreas C.R. Mayer4, Jean-Paul Morin5, Peter Gehr1, and Barbara Rother-Rutishauser1

1. University of Bern, Institute of Anatomy, Bern, Switzerland; 2. AFHBI, University of Applied Sciences, Biet-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
groups: 3 cases, worst cases compared to best cases
- results are means of 3 exposures with each 3 cell cultures (n = 3 x 3 = 9)

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

Material and Methods
Cell cultures: The triple cell co-cultures were prepared as described in [2], as epithelial cells (Hbha 14a) were
used. For the experiments 0.5x10⁶ 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 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.

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

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% CO₂ & humidification (80% relative humidity)
- entrance of exhaust and reference air to heated box
- control measurements (p, T, CO₂, 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)

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

Particle size Distribution
- worst cases bigger particles than best cases
- carburettor smaller particles than TSDI

Cytotoxicity (lactate dehydrogenase (LDH))
- TSDI best case, 8h post-exposure time: higher in exposure than control
- carburettor worst case, 8h post-exposure time: higher in exposure than control and reference

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 and reference
- All conditions: tendency (not statistically significant) for higher concentrations in exposures than in controls

Literature

Acknowledgement
We thank Barbara Tschirren for her excellent technical assistance. This work was supported by the Federal Office for the Environment (FOEN).