A New Exposure System to Evaluate the Toxicity of Scooter Emission in Lung Cells *In Vitro*

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It is known that diesel exhaust particles (DEP) have the potential to induce adverse health effects associated with pulmonary and cardiovascular diseases (Brunekreef et al, 2002) by inducing oxidative stress (Xiao et al, 2003), inflammatory reactions (Becker et al, 2005), and there is a link between exposure to diesel soot and lung cancer (Donaldson et al, 2005). The toxicity of DEP was studied by using an epithelial airway model (Rothen-Rutishauser et al, 2005). We have shown that DEP in suspension resulted in an increase of both of reactive oxygen species and of the pro-inflammatory chemokine, the tumor-necrosis factor alpha (TNFα, figure 1).

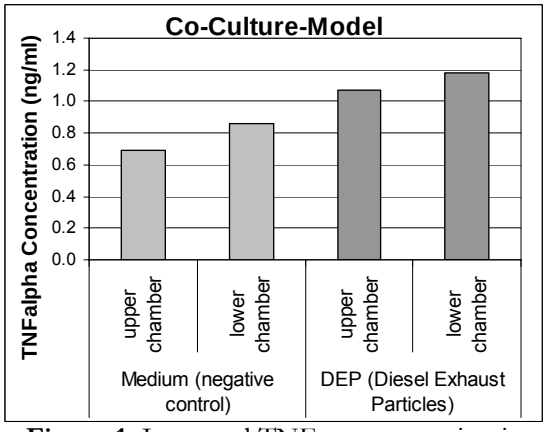

Figure 1. Increased TNFα concentration in co-cultures after DEP exposition in suspension.

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 an exhaust with a lot of fine and ultrafine particles which have a potential to cause adverse health effects (Peters et al, 1997).

For a realistic exposure of cell cultures, a box was developed in which these cultures can be exposed at air-liquid interface directly to exhaust emissions of scooters. The exhaust is discharged and directed to a mass regulator, where it is diluted 1:100 with absolute clean air. Before passing the cell cultures in a round exposure chamber which was developed especially for exhaust exposure (Morin et al, 1999; Papaioannou et al, 2006), the diluted exhaust emission is heated to 37°C, enriched with CO_2 to an end concentration of 5% CO_2 and humidified to a relative humidity of 80%. Directly before the entering to the exposure chamber control measurements (CO and CO_2 concentration, temperature, pressure, relative humidity) are conducted. On the top of the round exposure chamber, which is located in the isolated and heated box (37°C), the scooter exhaust enters with a flow of 2 l/min and is spread evenly over the four exposed 6-well plates. The air is sucked at the bottom of the exposure chamber and again CO_2 concentration, temperature, pressure and relative humidity are measured. Parallel to the exhaust exposure experiments control experiments are conducted in a reference exposure chamber, where cell cultures are exposed to absolute clean air enriched with CO_2, humidified and heated. The conditions in the exposure chamber during the exposure are stable with 80-85% of humidity, 36.5-37.5°C and about 5% CO_2.

Parallel to the exposure of the cells, the exhaust is analyzed by measuring the elementar carbon mass with a photoelectric aerosol sensor (PAS), the total particles

surface with a diffusion charging particle sensor (DC), the number of particles and the distribution of particles between 10-400nm with an electrostatic classifier with differential mobility analyser (DMA) in combination with a condensation particle counter. First measures and calculation showed that about 20% of the total particle number passing the exposure chamber stay in the chamber.

We exposed monocultures of 16HBE human bronchial epithelial cells and triple cell co-cultures (monolayer of 16HBE epithelial cells with dendritic cells and macrophages (Rothen-Rutishauser et al, 2005)) to a Peugeot two stroke direct injection scooter. We investigated two different cases: best case (oxi cat and wire-mesh filter catalyst, best lube oil with a ratio of 50% and Aspen fuel) and worst case (dummy muffler, worst oil with a ratio of 100%, unleaded fuel). After a warm-up phase of 90 minutes for the exposure system and 45 minutes for the scooter the cells were exposed during 2 hours with a dilution of 1:100 and a flow of 2 litres/min. After the exposure we incubated the cell cultures for 4h in a CO_2 incubator before we removed the medium and fixed the cells for toxicity analysis.

Two independent preliminary experiments give the following results:

- The LDH cytotoxicity test show higher cytotoxicity in triple cell co-cultures than in monocultures. The tendency of higher cytotoxicity in best case conditions than in worst case condition is too small to make a conclusion.
- We see a tendency for more oxidative damage in the DNA of mono-cultures than of triple cell co-cultures and a tendency for more oxidative damage in worst case conditions than in best case conditions (results of only one experiment).
- The evaluation of inflammation markers shows higher TNFα concentrations in co-cultures than in mono-cultures and more TNFα in worst case than in best case conditions. The IL-8 concentrations are higher in co-cultures than in mono-cultures and the IL-8 levels of co-cultures are higher for best case than for worst case conditions.

We can summary that mostly the co-cultures show higher reactions than mono-cultures. The only exception is oxidative damage. The results concerning the two cases are contrary: some parameters are higher in best case conditions (IL-8) and some in worst case conditions (oxidative damage, TNFα). The reasons therefore have still to be found. One possible reason could be the amount of ultrafine particles (diameter < 100nm) in the exhaust, as we have more ultrafine particles in best case conditions than in worst case conditions (Figure 2). This factor we have to study further.

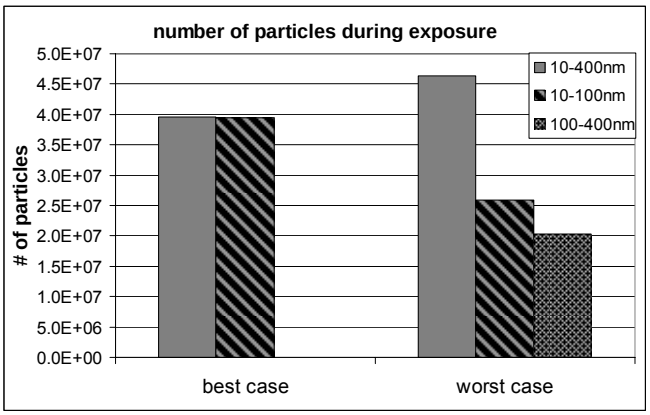

Figure 2. Fractions of particle numbers during exposure.

To complete this study and to finish the evaluation of the exposure system we need to do a third repetition of the experiments, we need to check the particle number in the reference chamber and we have to test other scooter types and other improvements.

References

Becker, S. et al (2005). Toxicol Appl Pharmacol.
Brunekreef, B. et al (2002). Lancet.
Donaldson, K. et al (2005). Part Fibre Toxicol.
Morin, J. P. et al (1999). Cell Biol.Toxicol.
Papaioannou, E. et al (2006). SAE International.
Peters, A. et al (1997). Am.J.Respir.Crit Care Med.
Rothen-Rutishauser, B. M. et al (2005). Am J Respir Cell Mol Biol.
Xiao, G. G. et al (2003). J Biol Chem.

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A System to Evaluate the Toxicity of Scooter Emission in Lung Cells In vitro

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Introduction: why scooter emissions?

Foto: J. Czerwinski

Scooter Avalanche in Taipeh

Overview of the Exposure System

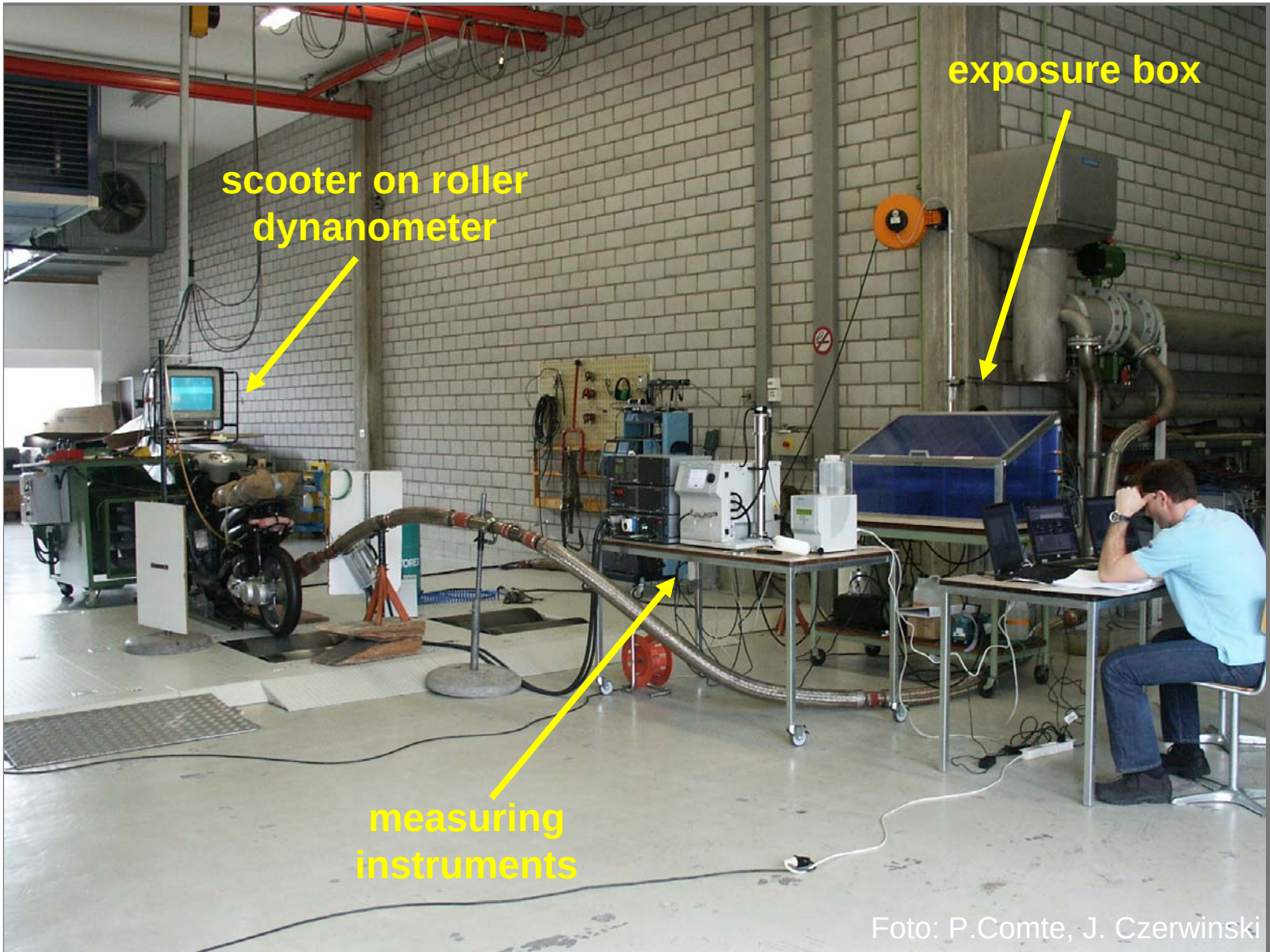
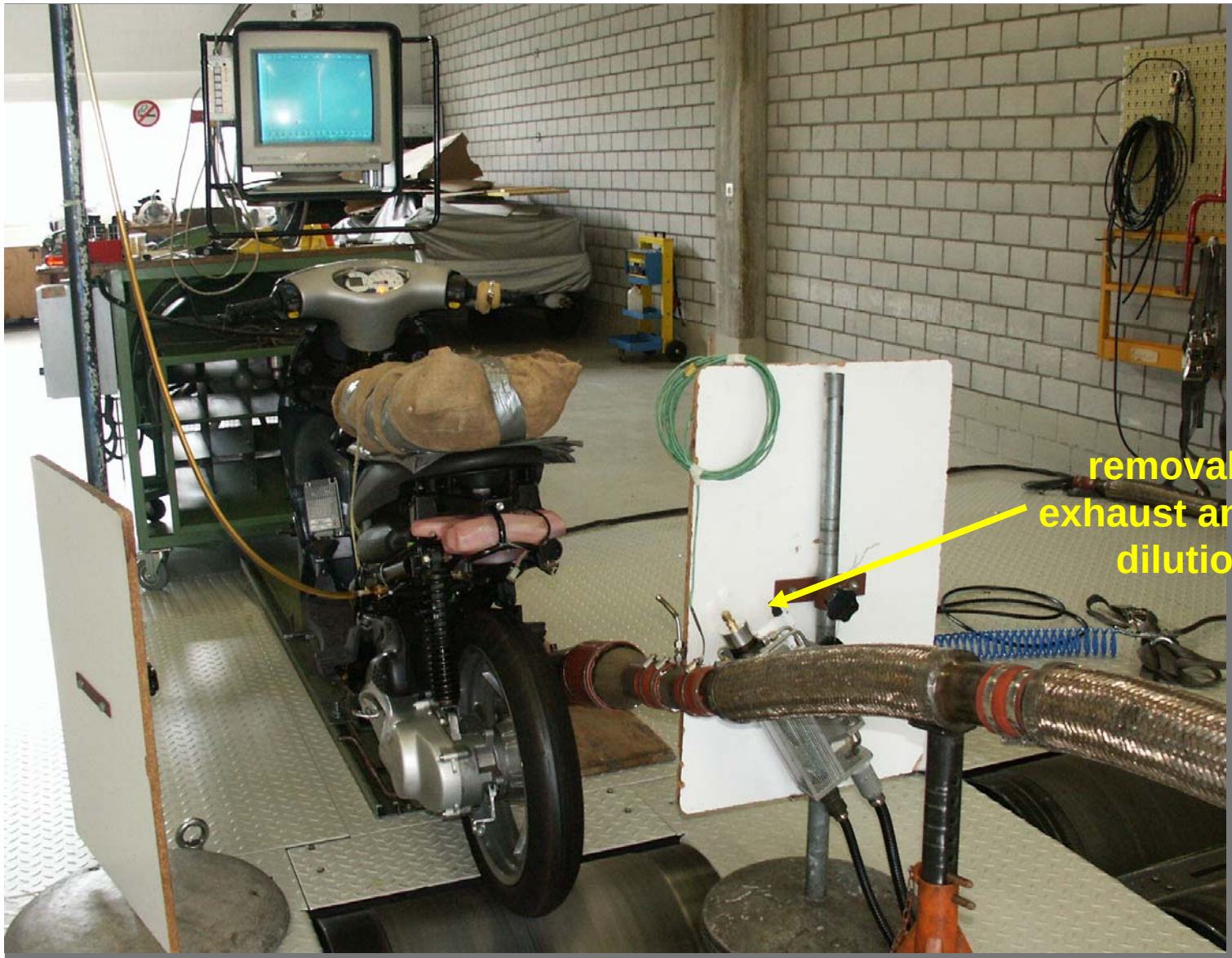


Foto: P.Comte, J. Czerwinski

Scooter and first dilution step

constant velocity of 40km/h



Measuring instruments for particles

> elementar carbon mass
> total particle surface

photoelectric aerosol sensor (PAS) and diffusion charging (DC) particle sensor

rotation diluter: 2nd dilution

electrostatic classifier with differential mobility analyser (DMA)
> "sorting" of particles

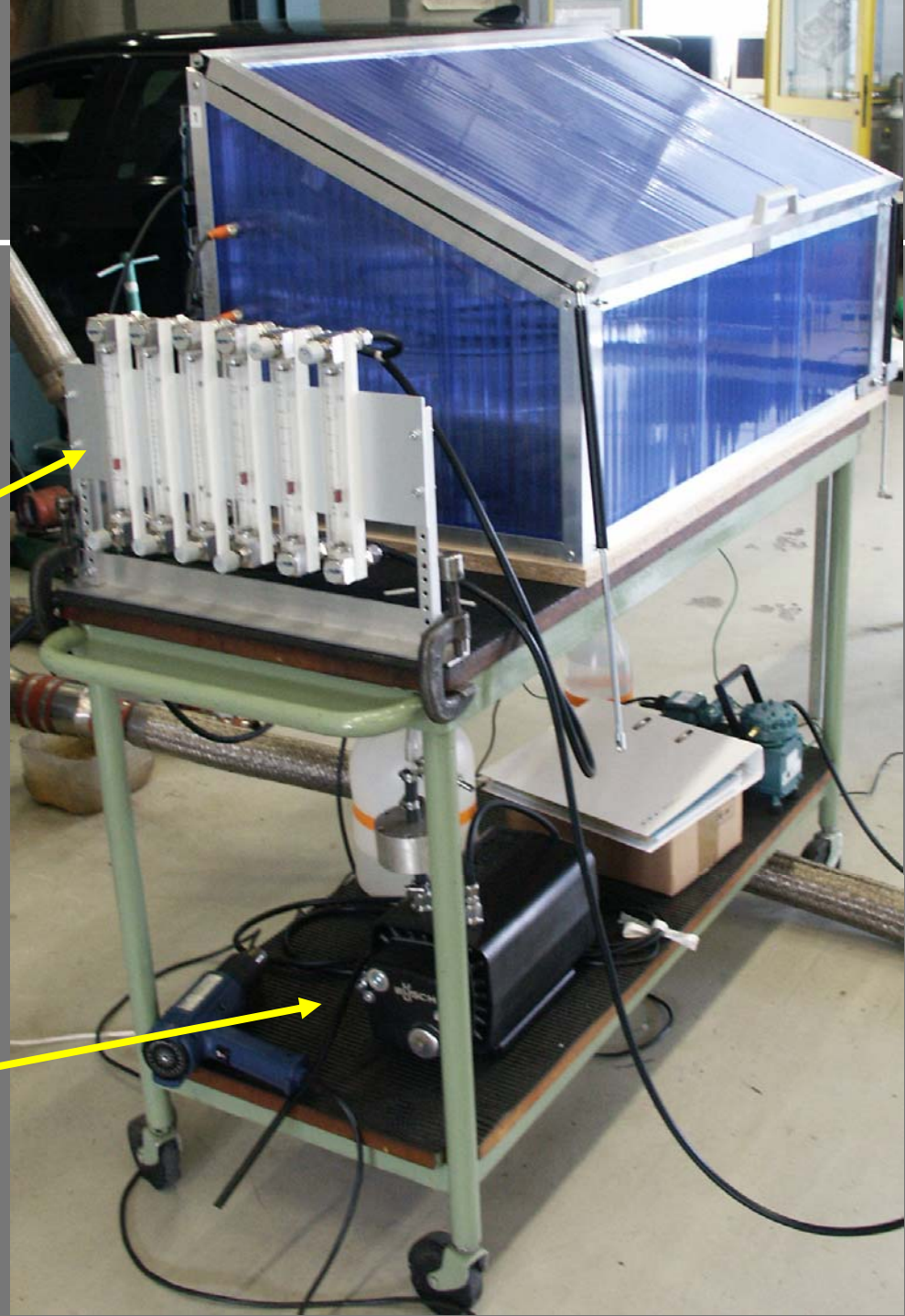
condensation particle counter
> particle number

distribution of particles between 10-400nm

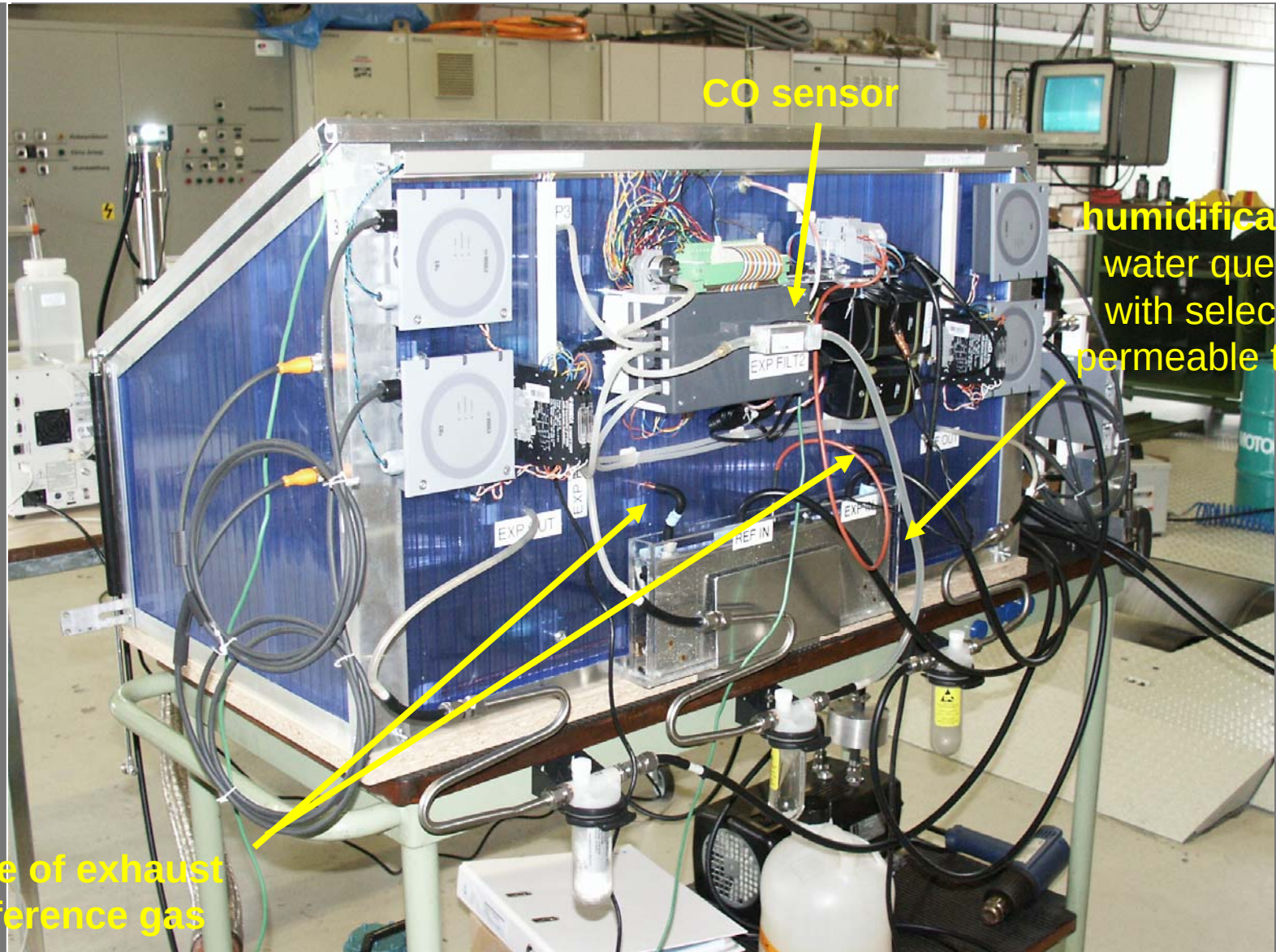
Flow control

rotameters for flow control and admixing of CO_2

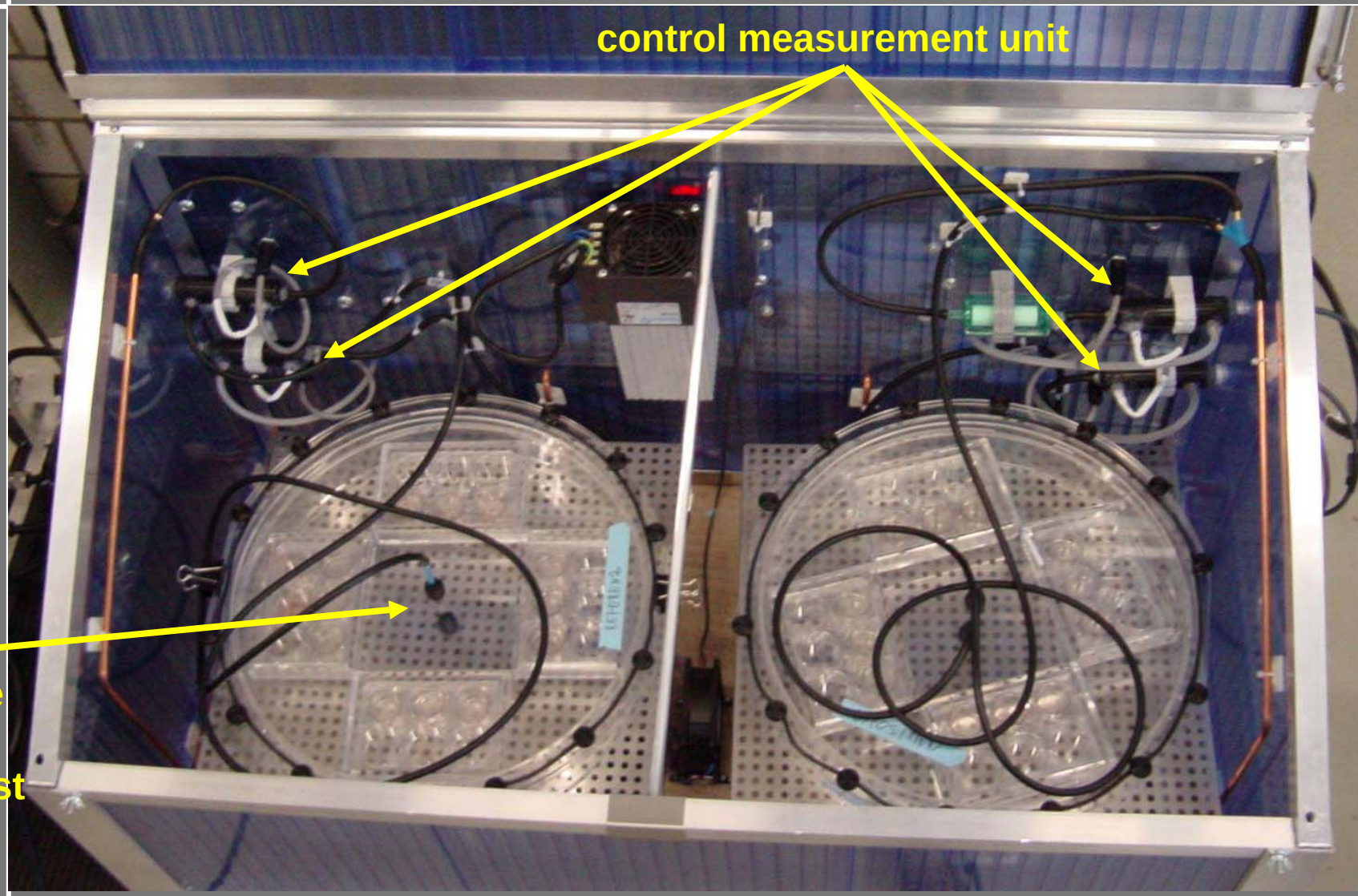
vacuum pump for driving the circulation



Heated box with air processing and control measurements

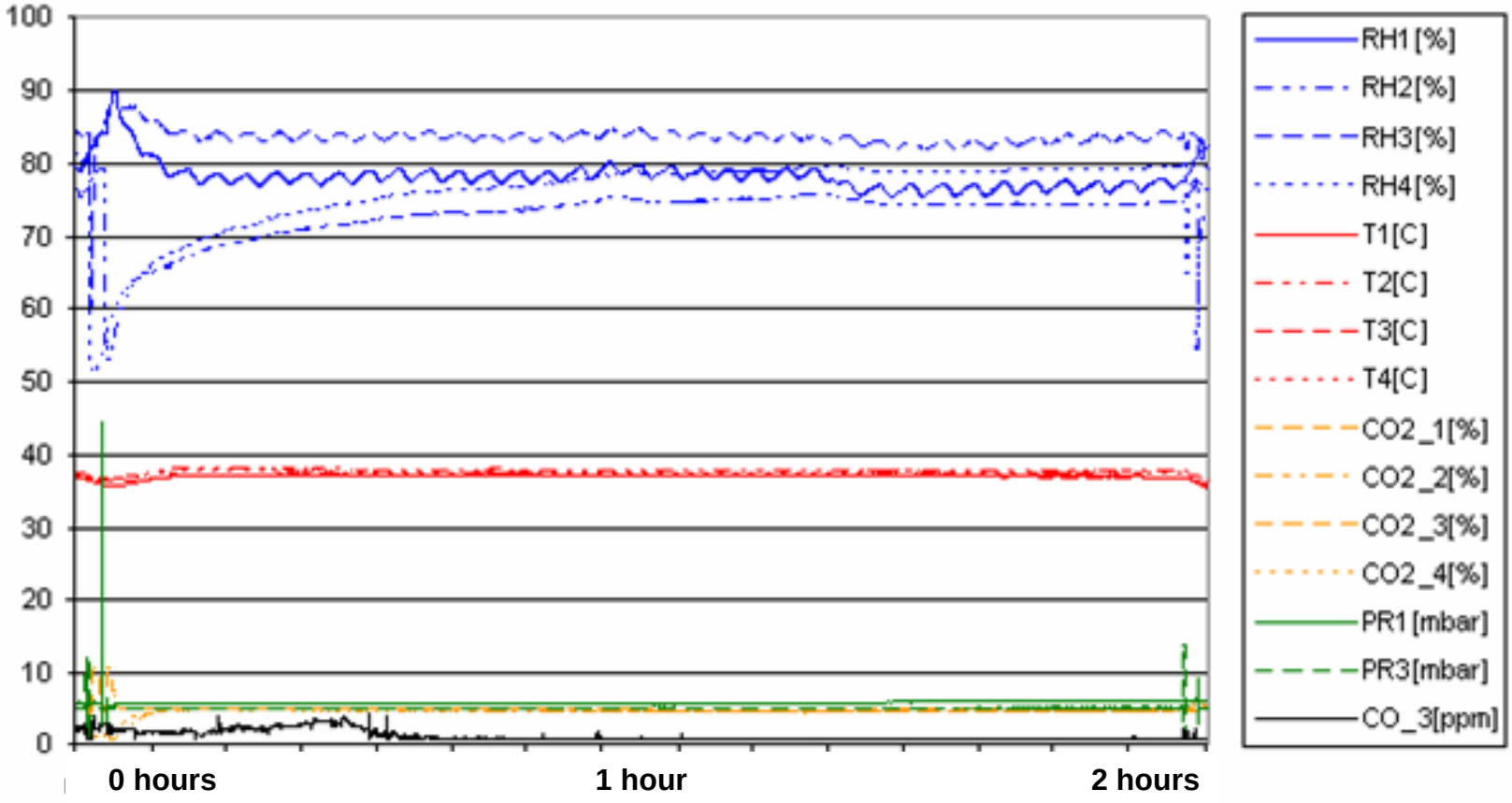


Exposure chamber (from A.Konstandopoulos, J.P. Morin)



Control measurements

Recording of conditions (T, p, rH, CO_2, CO) during the whole exposure

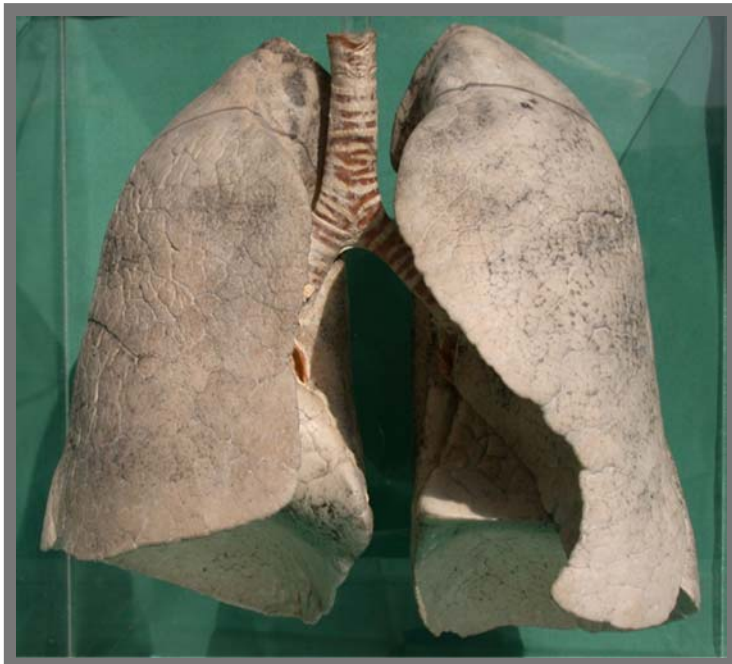


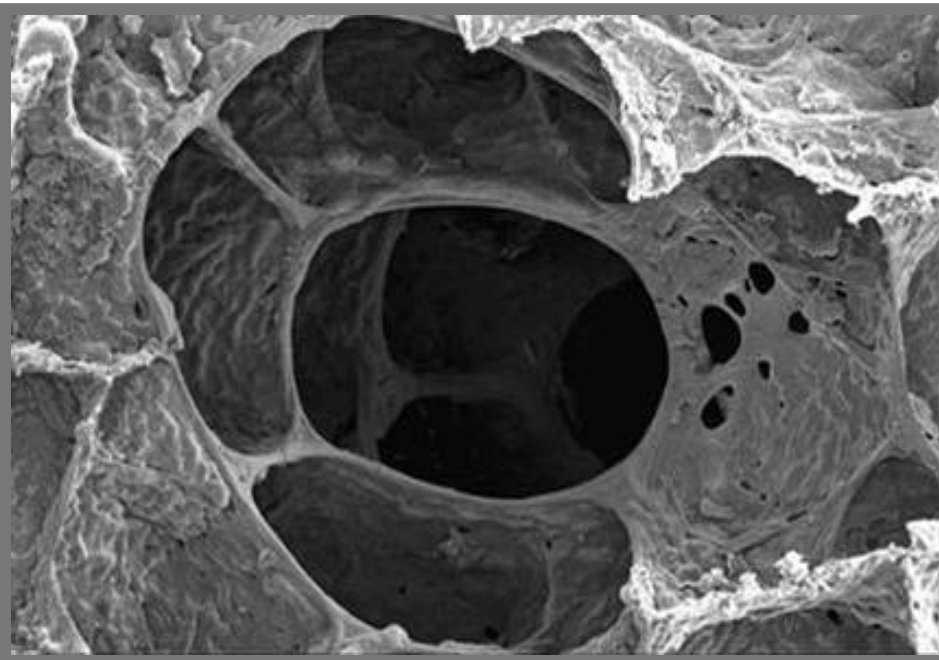
Here: Peugeot TSDI, best case, exposition of 2h , 08.05.2008

Conclusion I: exposure system

- Well working exposure system
- Stable conditions:
  - 80-85% relative humidity
  - 36.5-37.5°C
  - ~ 5% CO_2
- About 20% of the particles stay in the exposure chamber

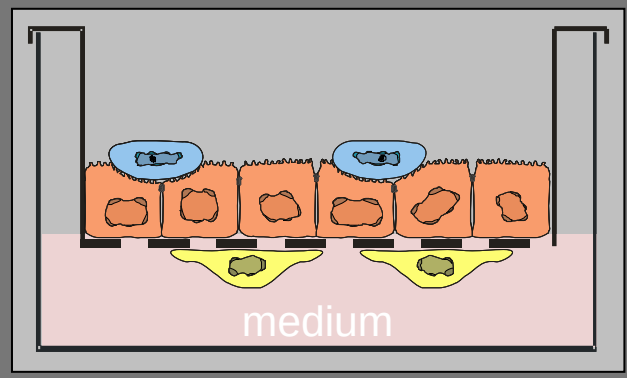
Introduction: structure of the lung





Cell types:

- **Monocultures** of 16HBE human bronchial **epithelial cells**
- **Co-cultures** of **epithelial cells**, **macrophages** and **dendritic cells**



Rothen-Rutishauser *et al.*
Am J Respir Cell Mol Biol. 2005

First Results: exposure conditions

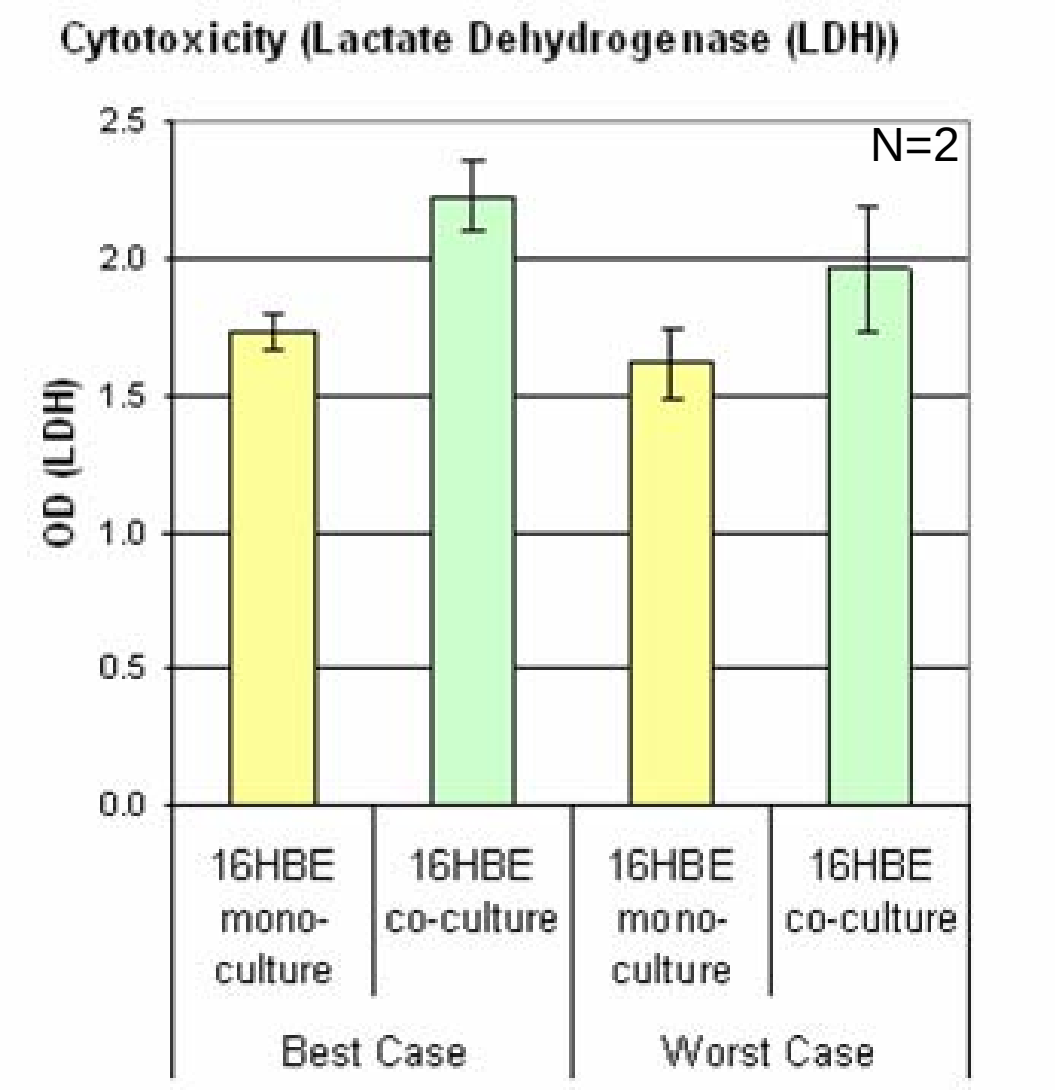
Exposure conditions:

- Warming up: **exposure system** (90min.) and **scooter (**45min.)
- **Exposure for 2h** with **1:100 dilution** and a flow of **2 litres/min.**
- Incubation of 4h after exposure

Scooter:

- Peugeot TSDI (two stroke direct injection)
- **Best case:** oxi cat + WFC; best lube oil, 50% ratio, Aspen fuel
- **Worst case:** dummy muffler; worst oil, 100% ratio, unleaded fuel

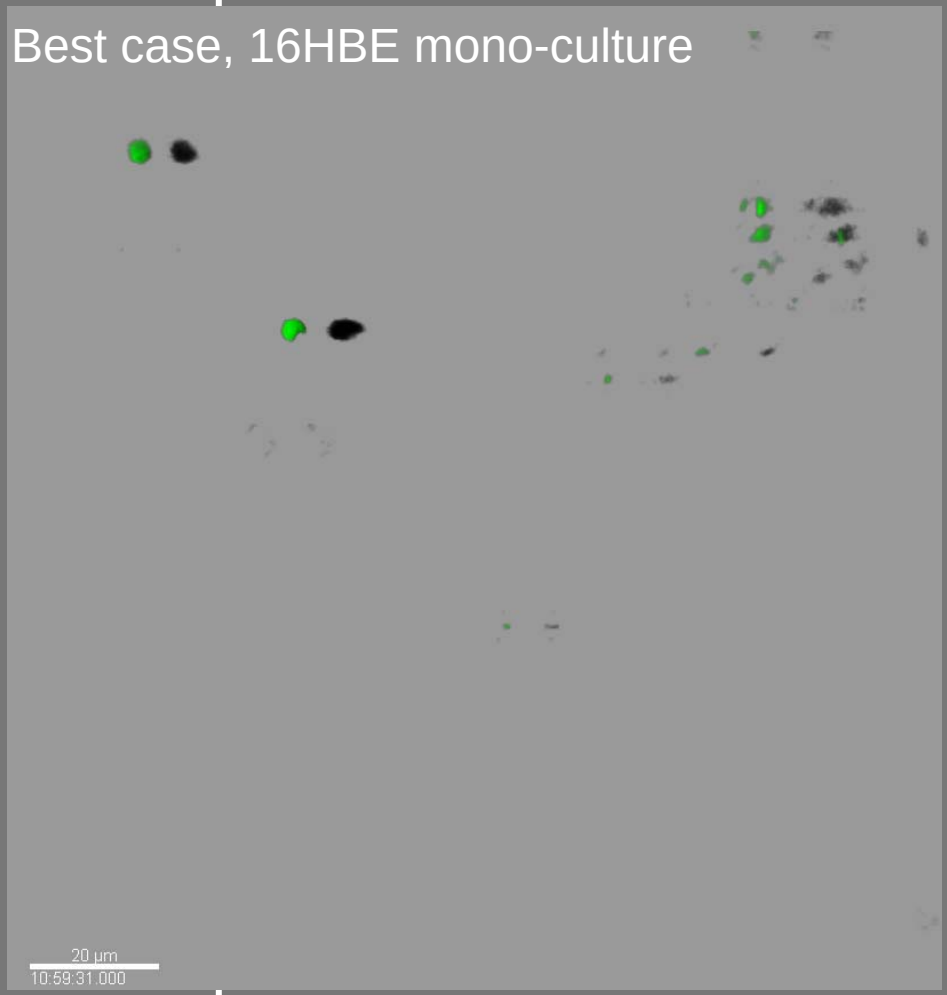
First Results: cytotoxicity



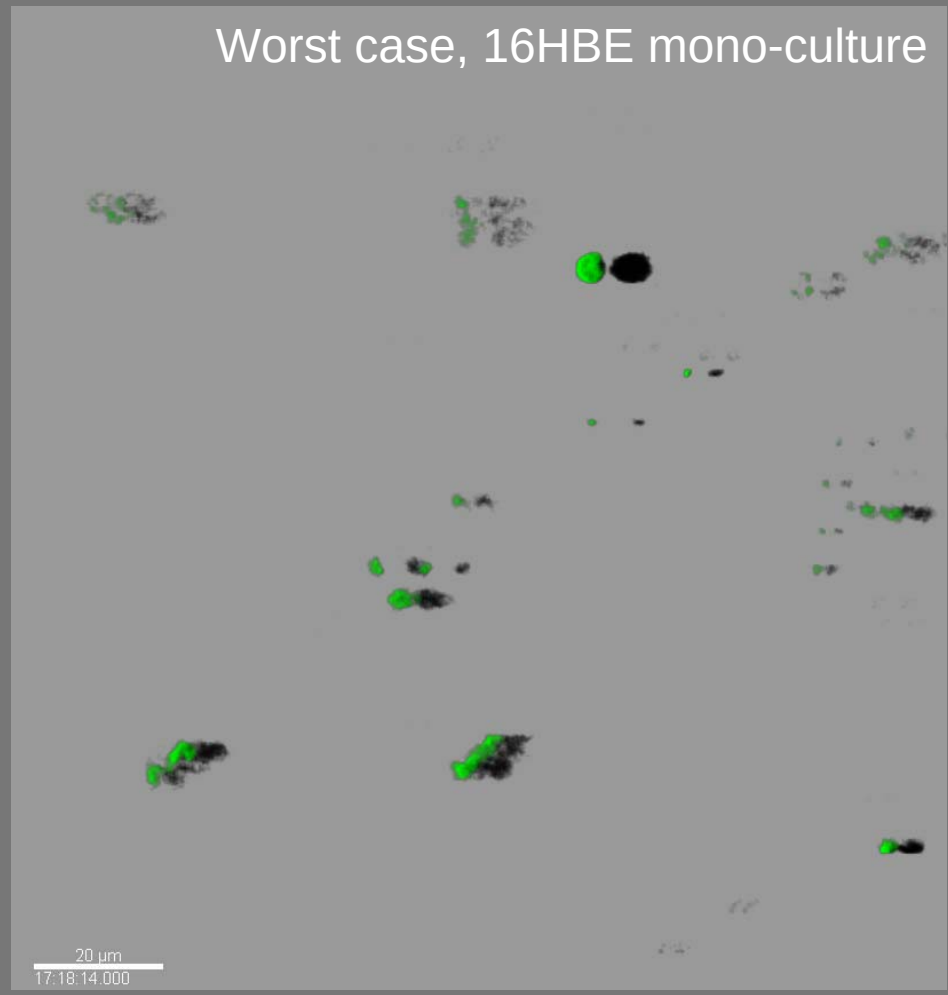
- More cytotoxicity in co-cultures than in mono-cultures

First Results: oxidative damage

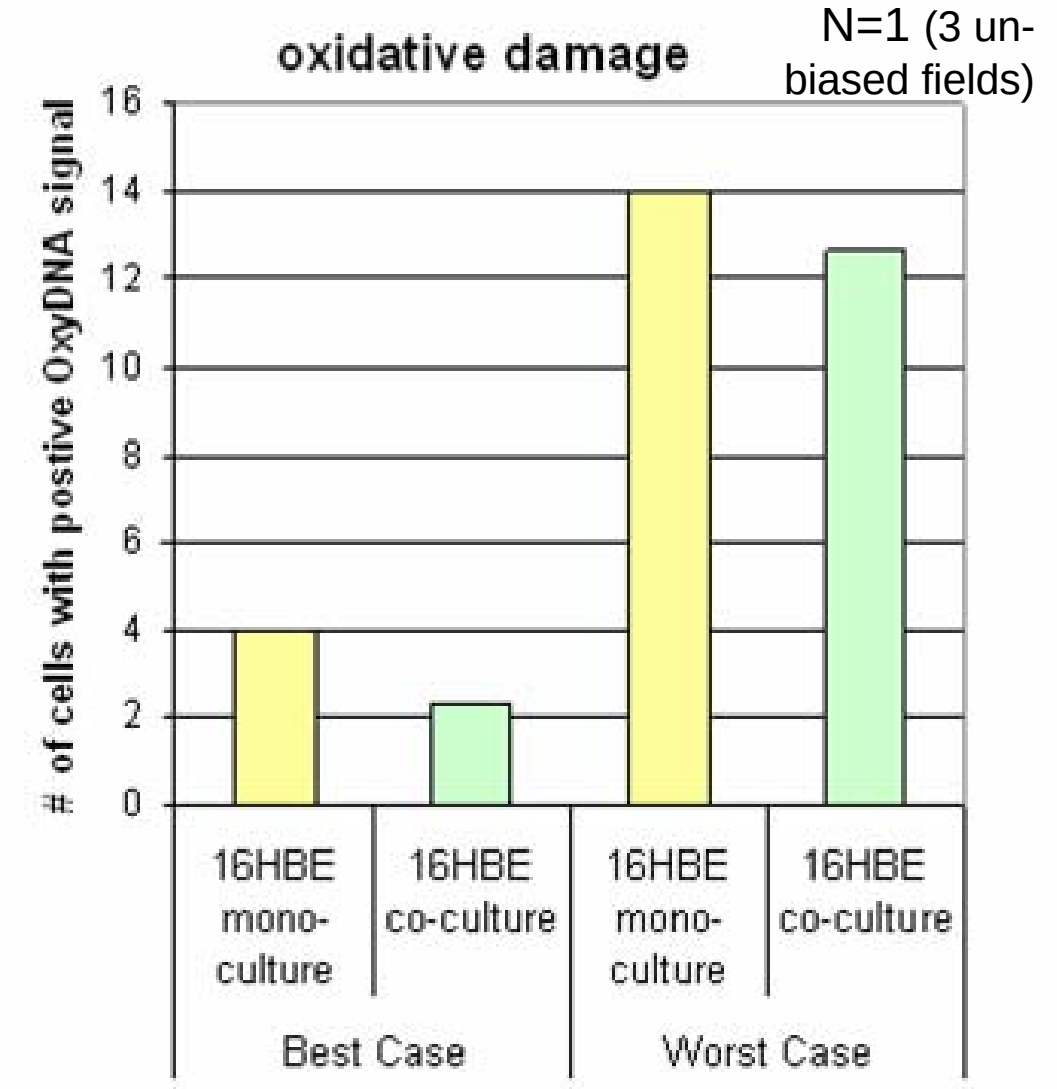
Best case, 16HBE mono-culture



Worst case, 16HBE mono-culture

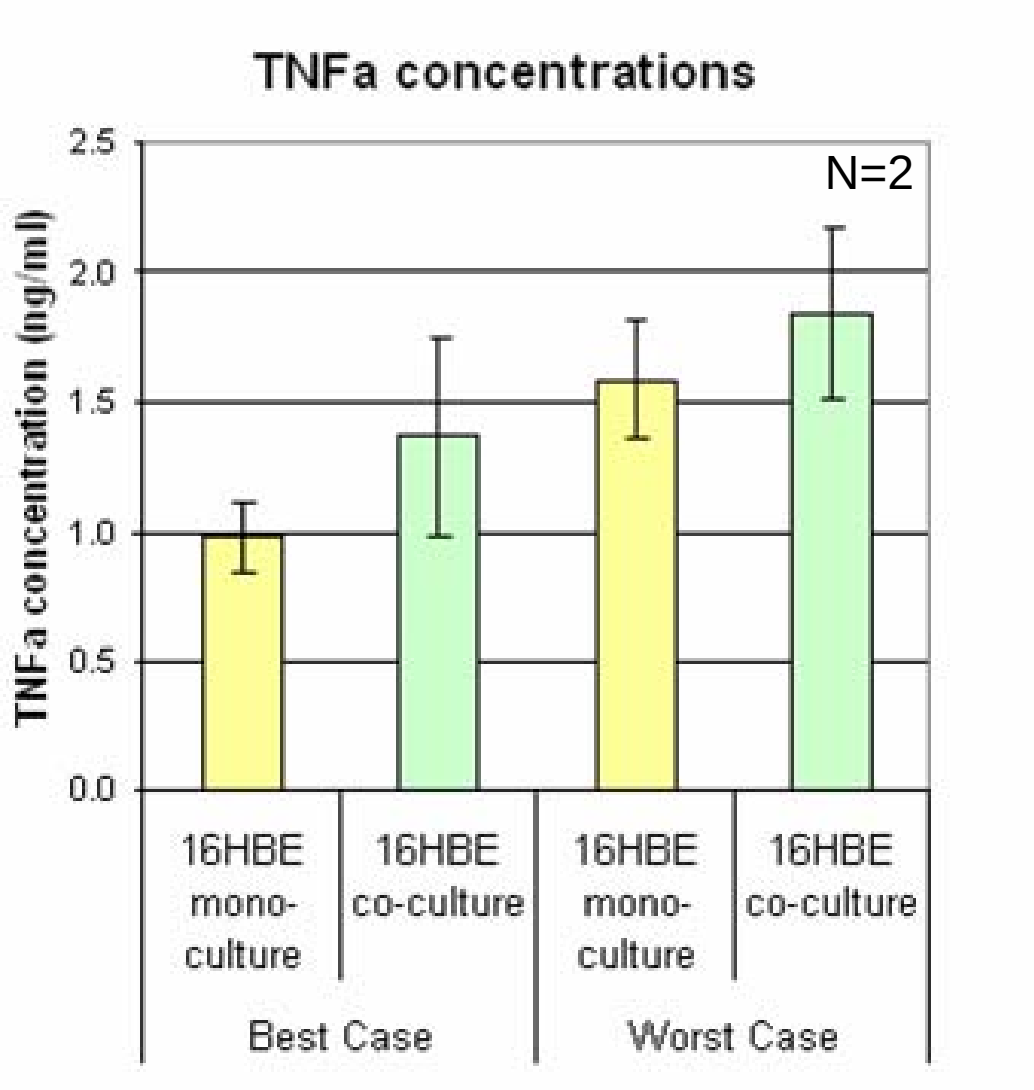


First Results: oxidative damage



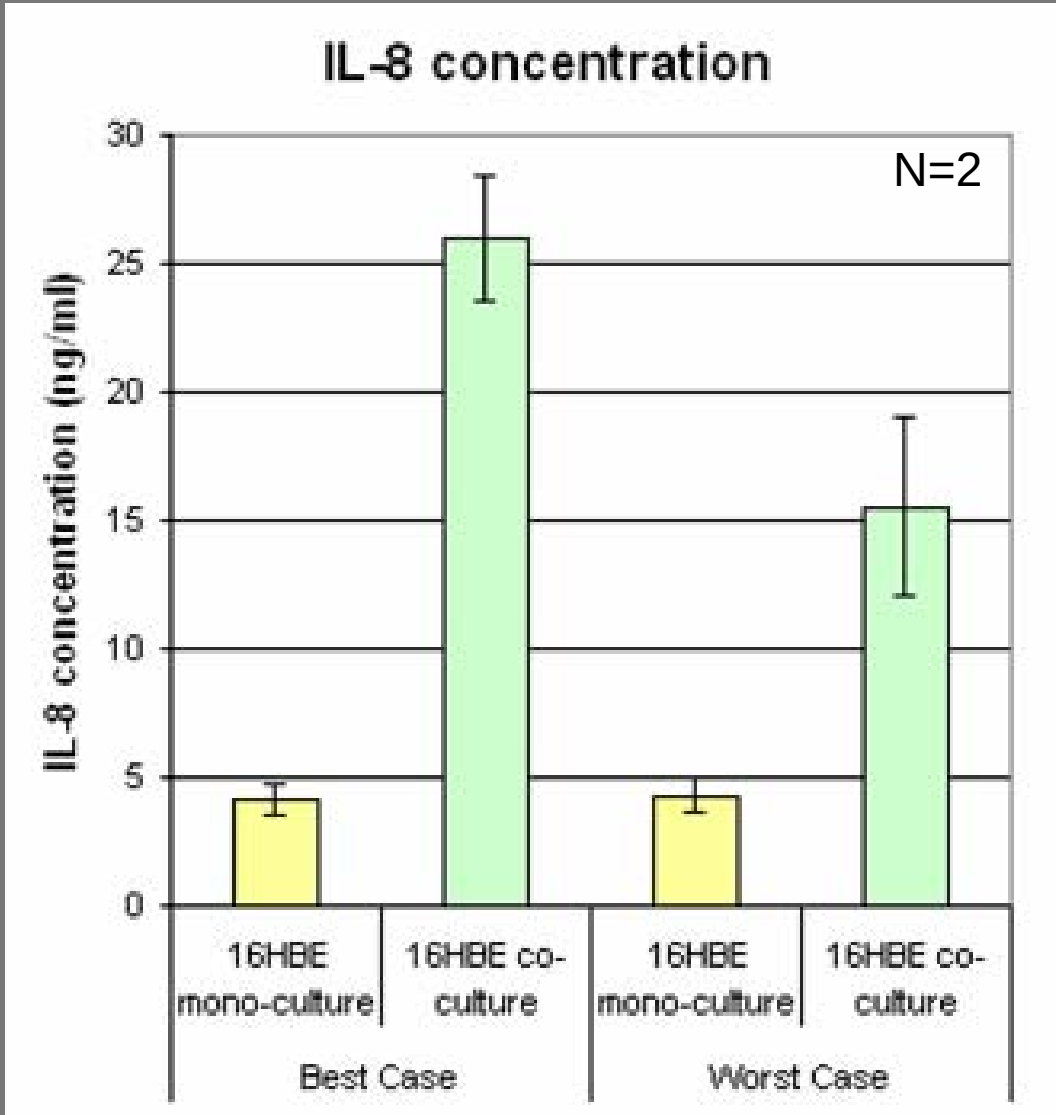
- Tendency for more oxidative damage in mono-cultures than in co-cultures
- Tendency for more oxidative damage in worst case than in best case

First Results: inflammation



- Higher TNFa concentrations in co-cultures than in mono-cultures
- Higher TNFa concentrations in worst case than in best case

First Results: inflammation



- Higher IL-8 concentrations in co-cultures than in mono-cultures
- In co-cultures: higher concentrations for best case than worst case

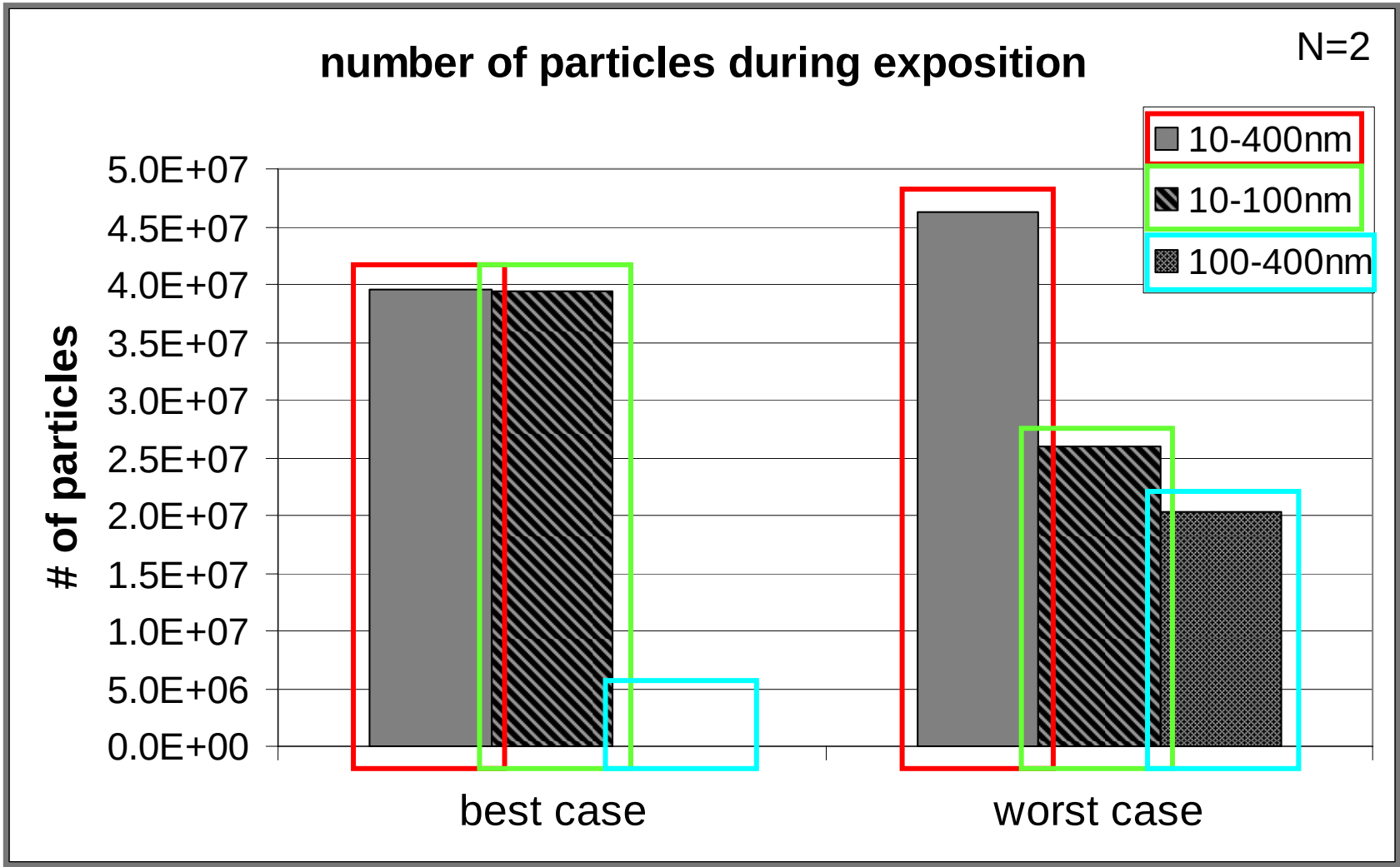
Conclusion II: toxicology

- Higher reactions in co-cultures than in mono-cultures (not in oxidative damage experiments)

  Contrary results concerning the two cases:

	Best case	Worst case
Cytotoxicity	**+**	**+**
Oxidative damage		**+**
TNFa		**+**
IL-8	**+**	

First Results: particle number



> More ultrafine particles (10-100nm) in best case than in worst case!

Outlook

- 3rd repetition of experiments
- Measurement of particle number in reference chamber
- Checking the effects of number of ultrafine particles in best and worst case
- Test other scooter types and other improvements

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