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Lung cell responses upon diesel and GDI vehicle exposures

VERT Focus event

March 16th 2018

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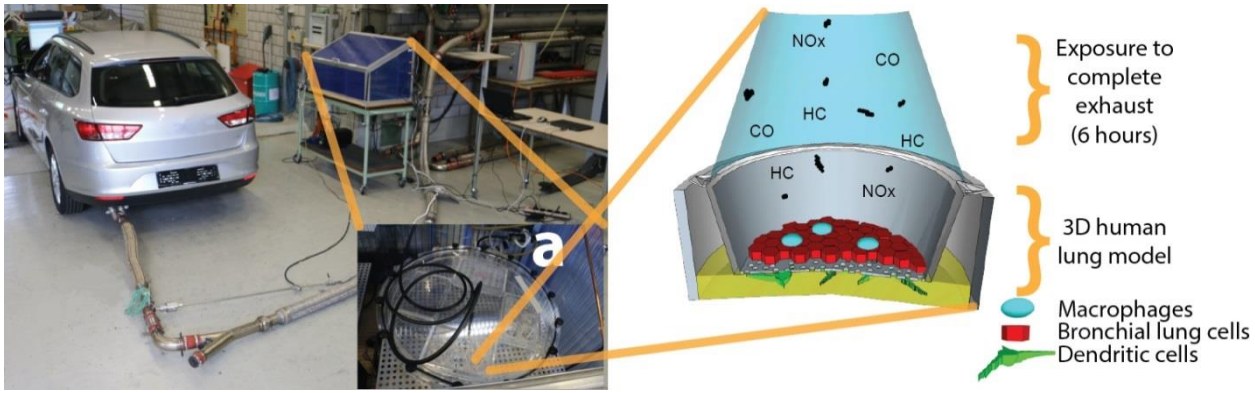

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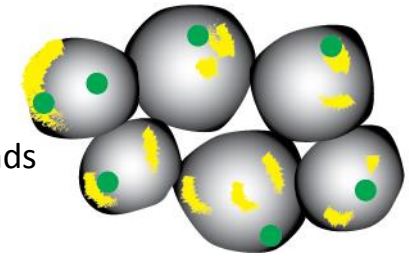
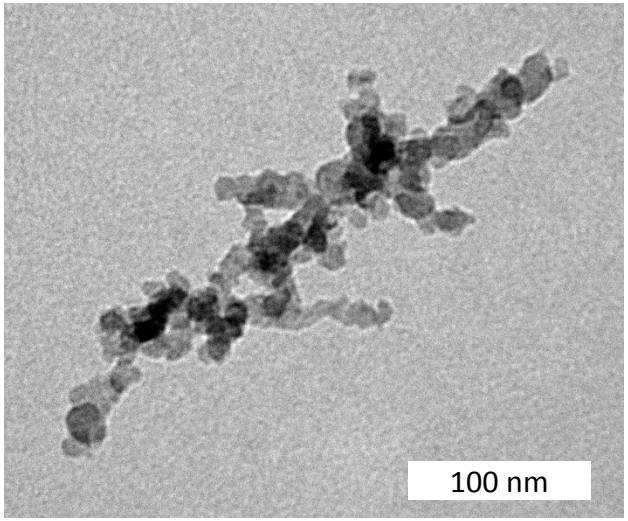
Main sources of air pollution in cities

Diesel Exhaust	Gasoline exhaust
Numerous studies since 1980s	Few studies
Adverse effects known	Not much known
25 % of passenger cars (CH[1])	75 % of passenger cars (CH[1])
Efficient exhaust after-treatment systems	Particle number emissions high (new technology GDI vs new diesel)

Particles
Elemental carbon
Metals
Organic compounds



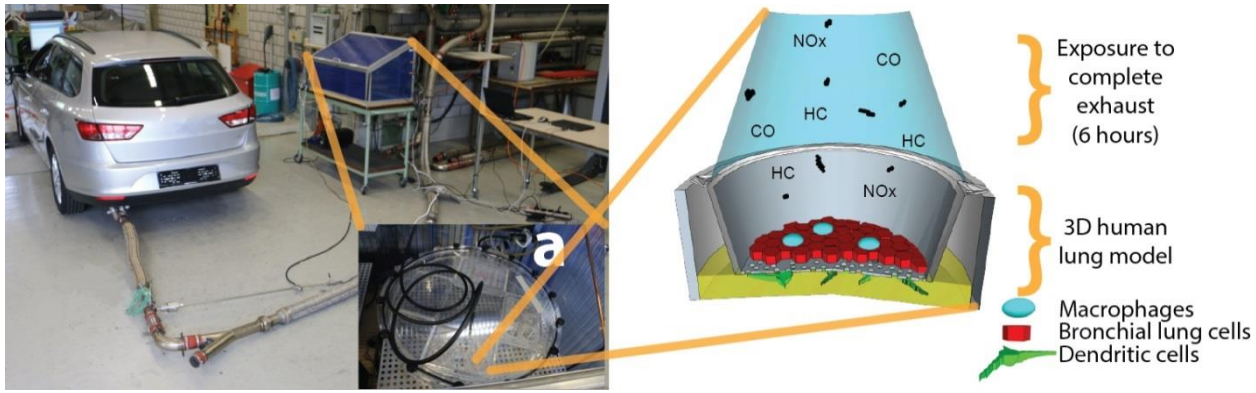
Gases
Carbon monoxide (CO)
Nitrogen oxides (NOx)
Hydrocarbons (HC)



[1] Bundesamt für Statistik. «Mobilität und Verkehr 2013»
GDI: gasoline direct injection

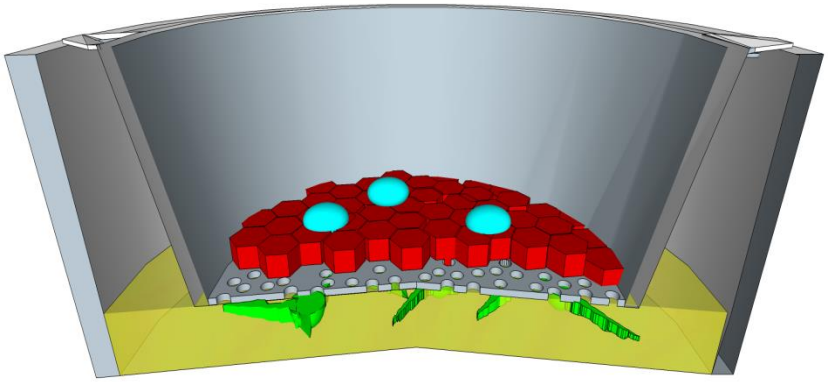
Aim of my PhD thesis

- Aim: study adverse effects from different gasoline engine exhaust types
  - Only one parameter (fuel, filter) changed at a time
  - Comparison to a suitable reference vehicle
  - Exposure to human lung cells *in vitro*
- Hypothesis: also gasoline car exhaust leads to adverse effects in human lung cells.



In vitro cell culture model

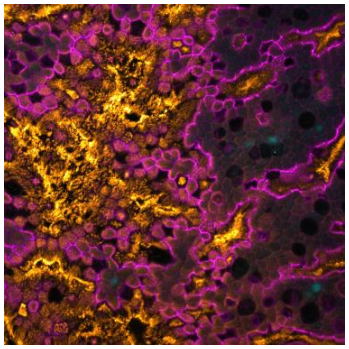
3D **human** cell model of the epithelial **airway** barrier

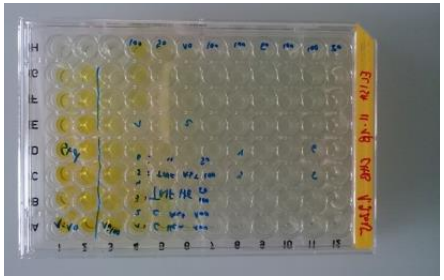


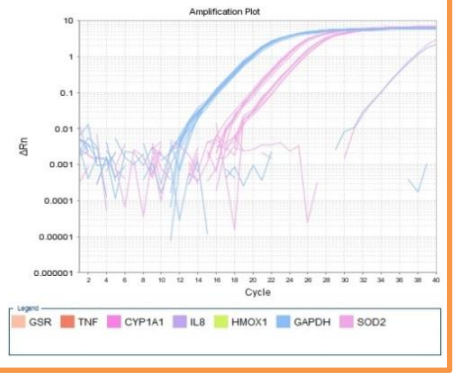
Macrophages (from human blood)
Bronchial epithelial cells (16HBE14o- cell line)
Dendritic cells (from human blood)

References co-culture model:
Rothen-Rutishauser B, *et al.,* 2005. Am. J. Respir. Cell Mol. Biol
Blank F, *et al.,* 2006. J Aerosol Med
Blank F, *et al.,* 2007. Am. J. Respir. Cell Mol. Biol

In vitro testing strategy:







Vehicle exhaust exposure system

Exhaust generation
- 4 cars tested

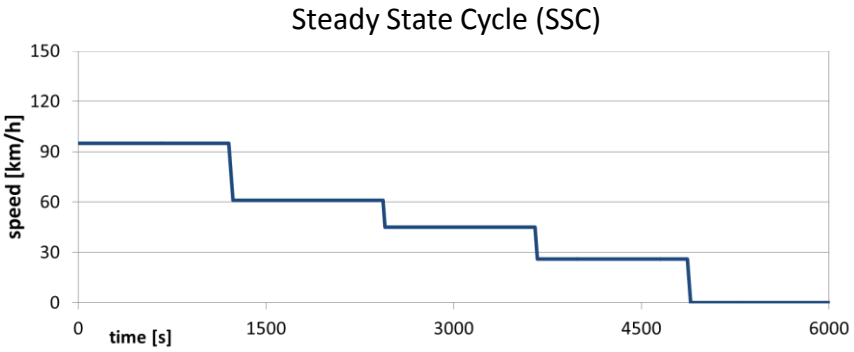
Exhaust collection
- At tailpipe

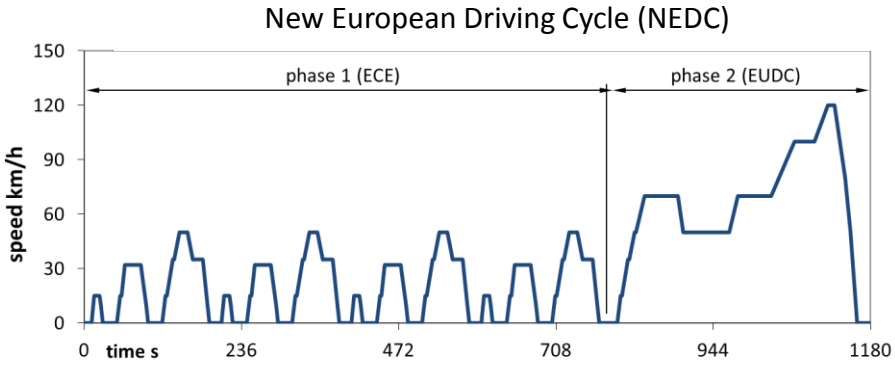
Exhaust characterization
- Online
- Particles (condensation particle counter)
- Gases (NO_x, CO, CO_2, THC)

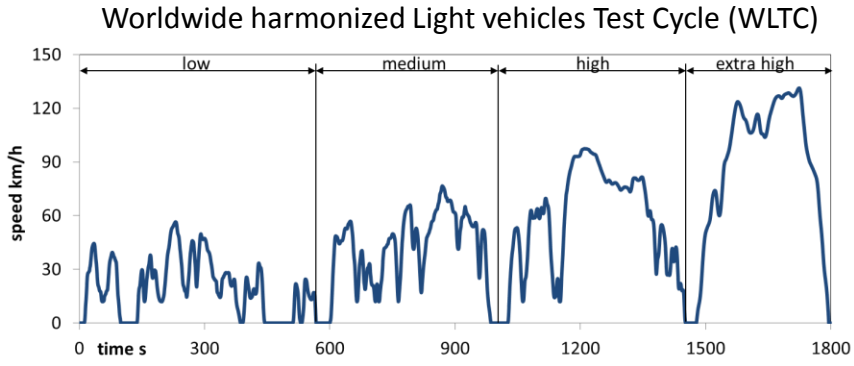
Exhaust exposure to cells
- T = 37 °C
- CO_2 conc = 5 %
- rH = 70-90 %
- Flow = 2 L/min

Exhaust dosing
- 1:10 dilution
- 6 hrs

Different driving cycles







- Different driving cycles → different exhaust emissions

Tested vehicles and parameters

GDI1

- Cycle: NEDC
- Parameters:

Filter (Uncoated GPF)

GDI2

- Cycle: SSC, WLTC
- Parameters:

Filter (Uncoated GPF, coated GPF)

Ethanol (0%, 10%, 85%)

Repeated exposure

Diesel

- Cycle: WLTC
- Parameters:

Unfiltered only

GDI3

- Cycle: WLTC
- Parameters:

Lubrication oil addition (**high ash** and **low ash**)

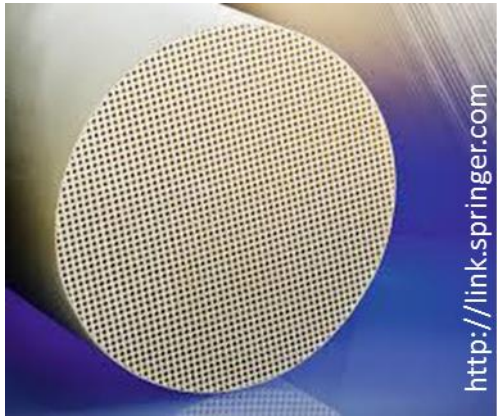
Abbreviations:
GDI: Gasoline Direct Injection
GPF: Gasoline Particle Filter
NEDC: New European Driving Cycle
SSC: Steady State Cycle
WLTC: Worldwide harmonized Light vehicles Test Cycle

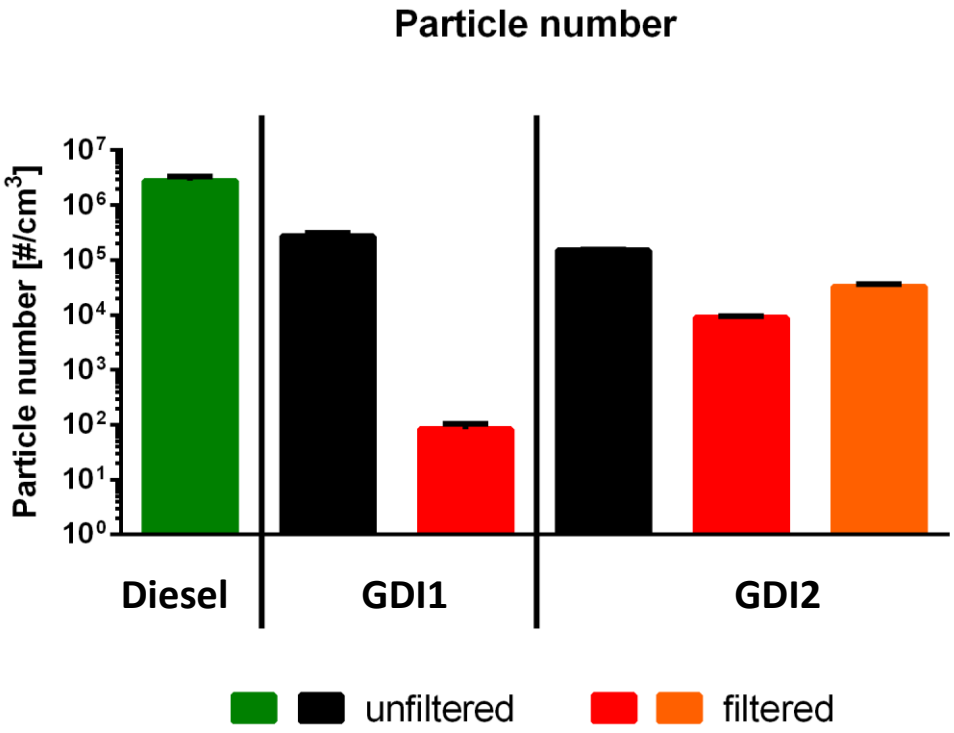
Publications:

Bisig C, *et al.,* **2015**. Emission Ctrl Sci and Tech;
Bisig C, *et al.,* **2016**. Env Research;
Bisig C, *et al.,* **2018**. Env Pollution;

Results – Vehicle exhaust analysis

Wallflow Filter



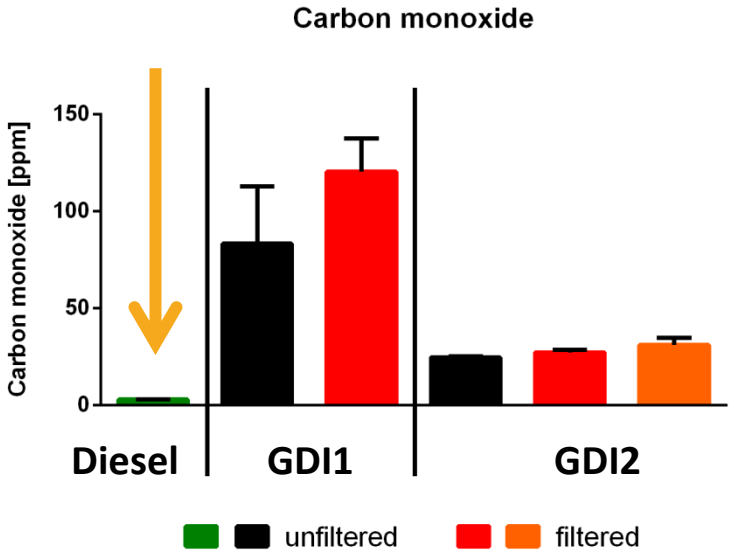


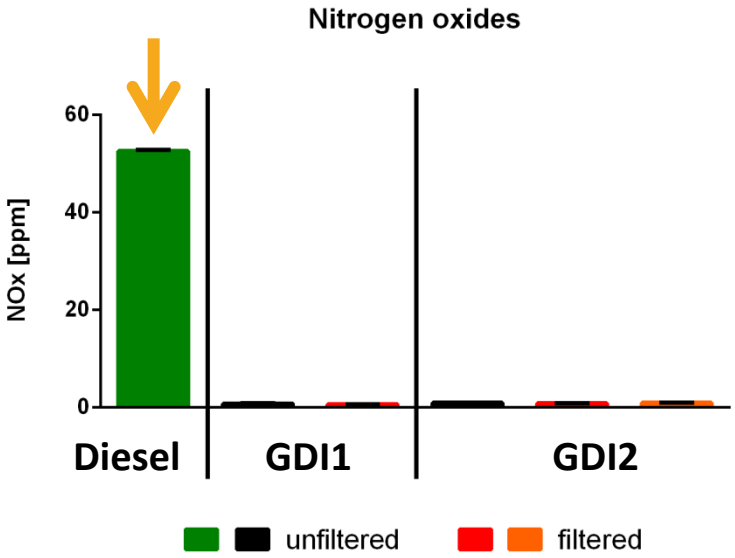
- Higher PN in Diesel exhaust
- Lower PN in filtered exhaust

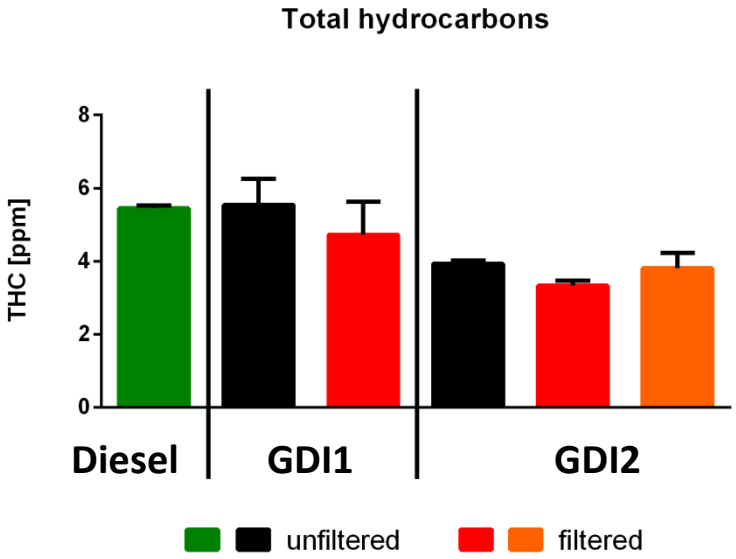
WLTC: Worldwide harmonized Light vehicles Test Cycle
NEDC: New European Driving Cycle
PN: Particle Number
Principle Wallflow Filter: www.auto-umwelt.at

Bisig C, *et al.,* 2015. Emission Ctrl Sci and Tech; **Bisig C**, *et al.,* 2016. Env Research; **Bisig C**, *et al.,* 2018. Env Pollution;

Results – Vehicle exhaust analysis



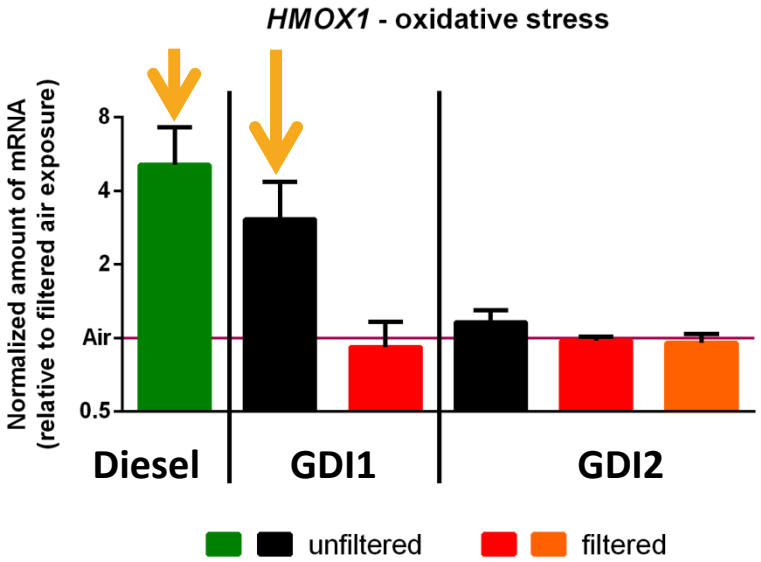


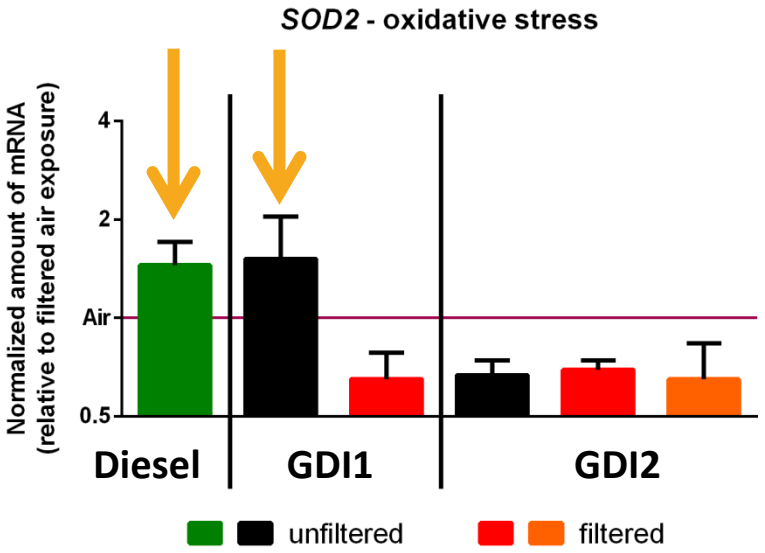


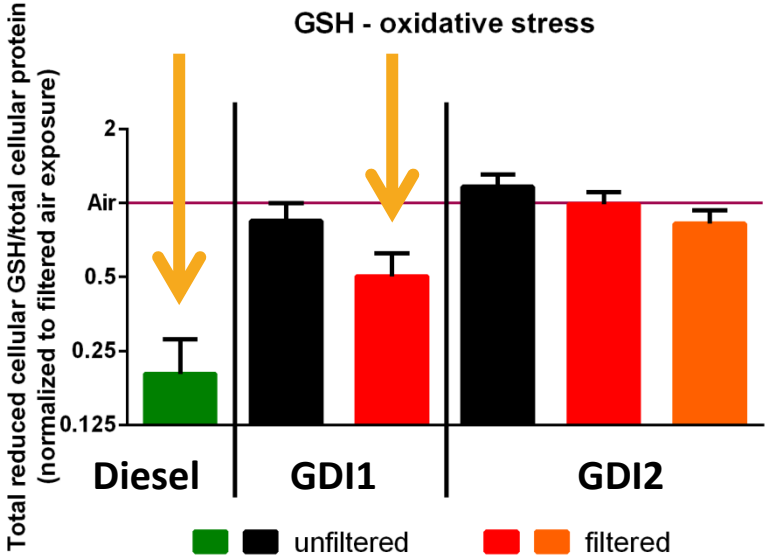
- Diesel: low CO but high NO_X
- GDI1/2: higher CO, low NOx
- Hydrocarbons: at the same level

Bisig C, *et al.,* 2015. Emission Ctrl Sci and Tech;
Bisig C, *et al.,* 2016. Env Research;
Bisig C, *et al.,* 2018. Env Pollution;

Unfiltered *vs.* filtered gasoline exhaust







- Diesel exhaust: high oxidative stress
- GDI1: oxidative stress
- GDI2: no oxidative stress

Bisig C, *et al.,* 2015. Emission Ctrl Sci and Tech;
Bisig C, *et al.,* 2016. Env Research;
Bisig C, *et al.,* 2018. Env Pollution;

Tested vehicles and parameters

- Cycle: NEDC
- Parameters:

Filter (Uncoated GPF)

- Cycle: SSC, WLTC
- Parameters:

Filter (Uncoated GPF, coated GPF)

Ethanol (0%, 10%, 85%)

Repeated exposure

- Cycle: WLTC
- Parameters:

Unfiltered only

- Cycle: WLTC
- Parameters:

Lubrication oil addition (**high ash** and **low ash**)

Abbreviations:
GDI: Gasoline Direct Injection
GPF: Gasoline Particle Filter
NEDC: New European Driving Cycle
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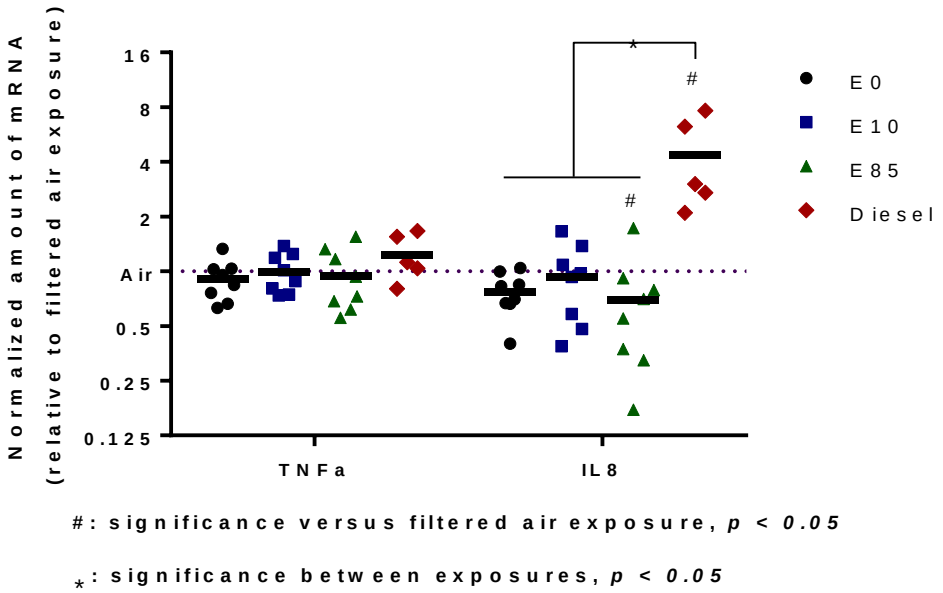
Publications:

Bisig C, *et al.,* **2015**. Emission Ctrl Sci and Tech;
Bisig C, *et al.,* **2016**. Env Research;
Bisig C, *et al.,* **2018**. Env Pollution;

Different fuel-blends

- Ethanol is widely used as a fuel-supplement (e.g. USA, Brazil)



- No adverse cell responses in exhausts of gasoline-ethanol blends

Bisig C, *et al*. 2016, Env Research

Tested vehicles and parameters

- Cycle: NEDC
- Parameters:

-Filter (Uncoated GPF)

- Cycles: SSC, WLTC
- Parameters:

-Filter (Uncoated GPF, coated GPF)

-Ethanol (0%, 10%, 85%)

-Repeated exposure

- Cycle: WLTC
- Parameters:

-Unfiltered only

- Cycle: WLTC
- Parameters:

-Lubrication oil addition (**high ash** and **low ash**)

Abbreviations:
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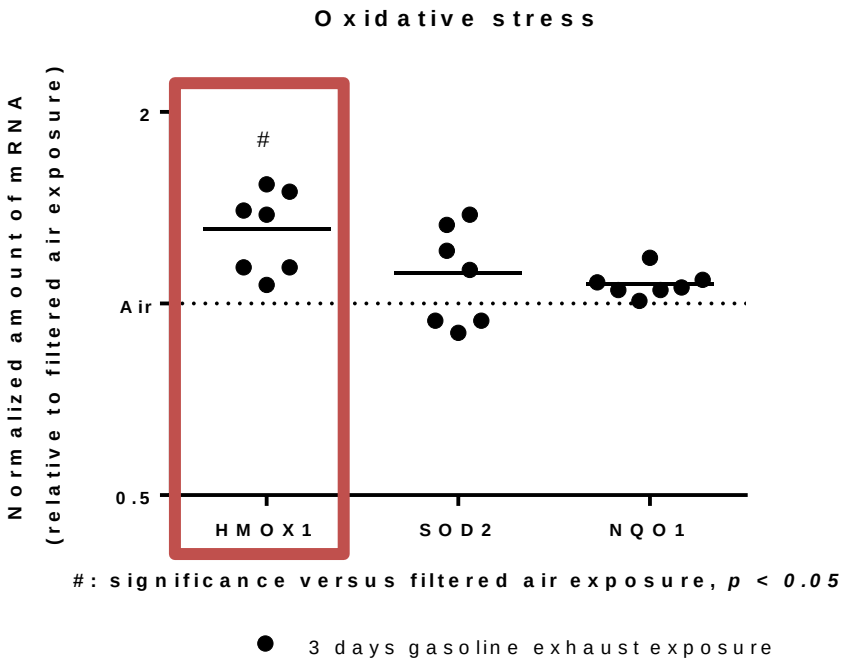
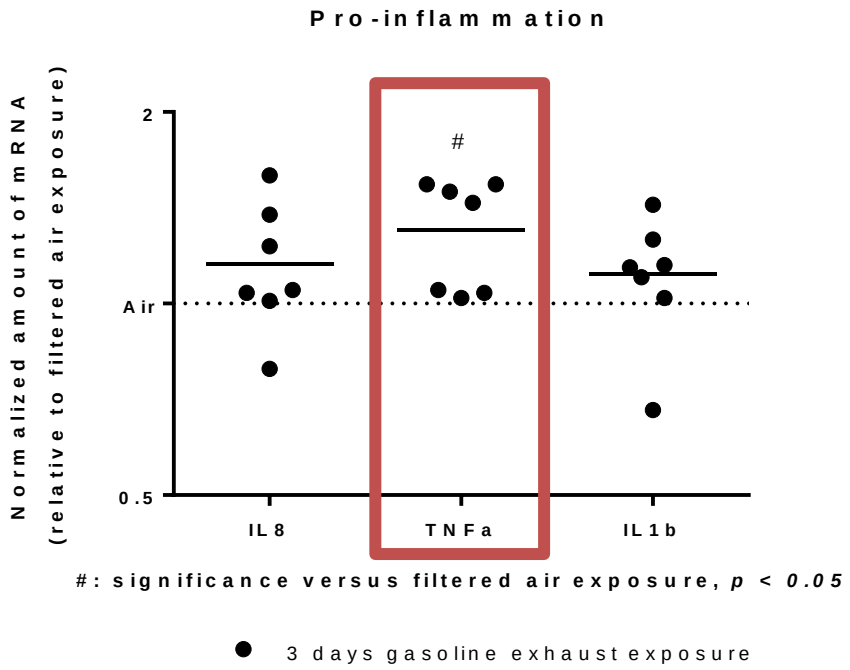
Bisig C, *et al.,* **2018**. Env Pollution;

Repeated gasoline exhaust exposure

- 3 x 6h exhaust exposure (unfiltered exhaust)





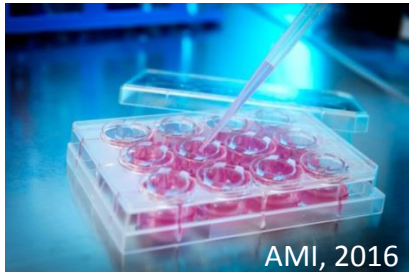
- Indications for **oxidative stress** and **pro-inflammation** compared to filtered air exposure

Bisig C, *et al.,* 2018. Env Pollution;

Summary gasoline exhaust studies

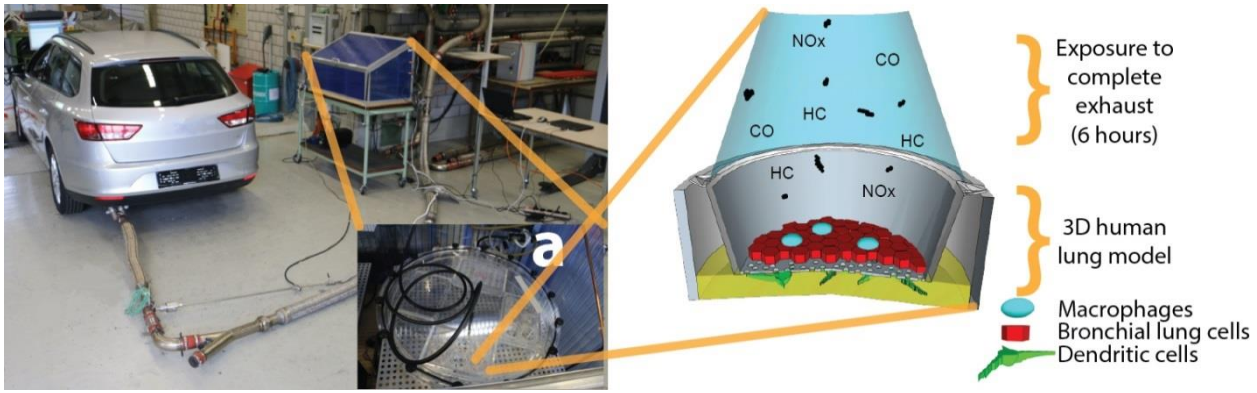
Marker	Diesel unf.	GDI1 unf.	GDI1 uncoat. GPF	GDI2 unf.		GDI3 unf.	GDI3 Lube-oil
Particle number [#/cm^3]	~10^6	~10^5	~10^2		~10^5	~10^5	~10^6
CO [ppm]	~3	~80	~120		~25	~20	~50
NOx [ppm]	~50	~1	~1		~1	~1	~1
Cytotoxicity (LDH)	(↑)	-	-	-	-	-	-
Morphology (Microscopy)	-	-	-	-	-	-	-
Oxidative stress (PCR, GSH)	↑	↑	(↑)	-	(↑)	-	-
Pro-inflammation (PCR)	↑	-	-	-	(↑)	-	-
Mutagenicity (DNA adducts)	-	Not performed		-		Not performed	

- Adverse effects influenced by vehicle
- Filter not sufficient for complete exhaust detoxification
- Particle number not the only important exhaust parameter



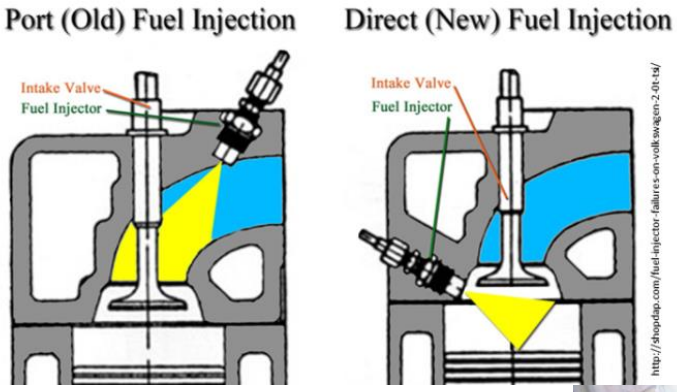
Bisig C, *et al.,* 2015. Emission Ctrl Sci and Tech; **Bisig C**, *et al.,* 2016. Env Research; **Bisig C**, *et al.,* 2018. Env Pollution

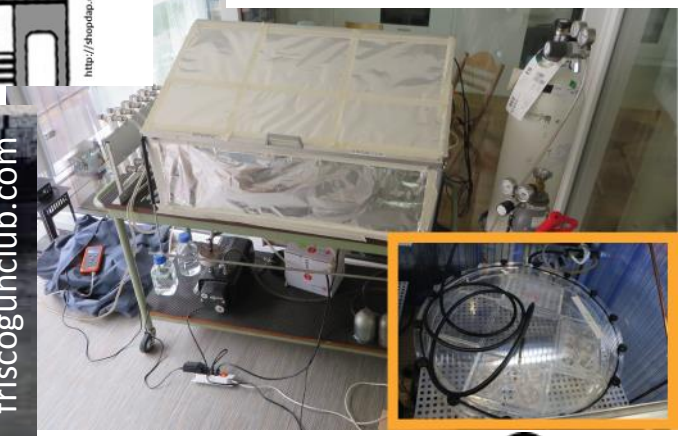
Final conclusions

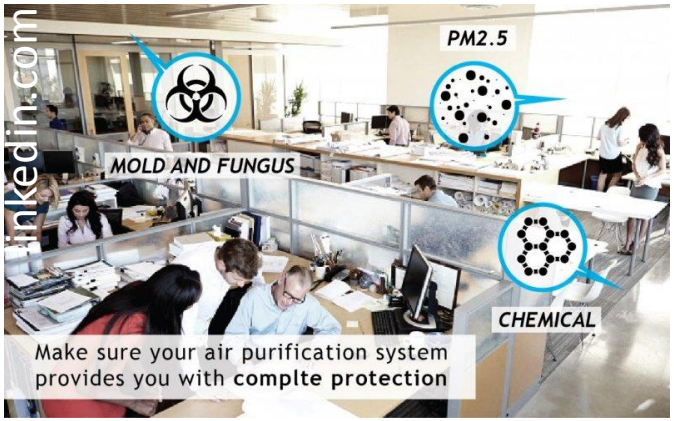


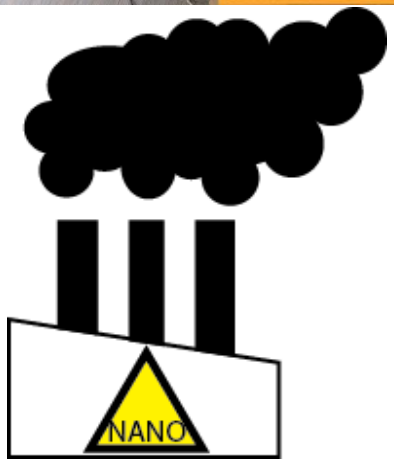
- The presented exposure system allows to assess the *in vitro* effects of the **complete exhausts** with reproducible and reliable results
- Main results
  - Adverse effects are influenced by vehicle, particle filter, and driving cycle
  - **Gasoline** exhaust induced **less effects** *in vitro* compared to diesel exhaust
  - A gasoline particle filter is **not sufficient** for exhaust detoxification
  - **Ethanol**-supplemented fuel did **not induce adverse effects** and might be a good compromise (fossil fuel vs renewable energy)
  - **Repeated exposure** was successful, but longterm (> 3 days) are necessary to assess adverse effects of car exhausts

Outlook





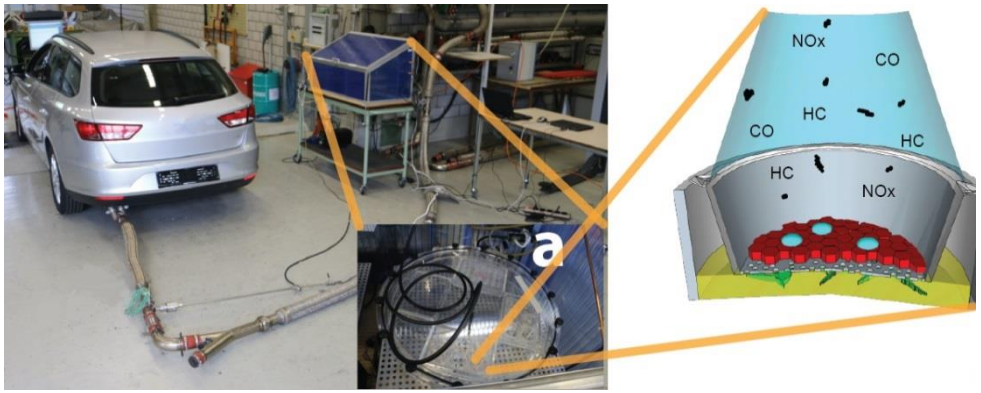




My future:

- PostDoc Mobility in Munich
- CAST Aerosols and their toxicity
- Development of an optimized *in vitro* exposure protocol

Thank you for your attention



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VERT Focus event; March 16th 2018; christoph.bisig@unifr.ch