Traffic-related non-exhaust emission UFPs toxicological potentials

10 June 2024

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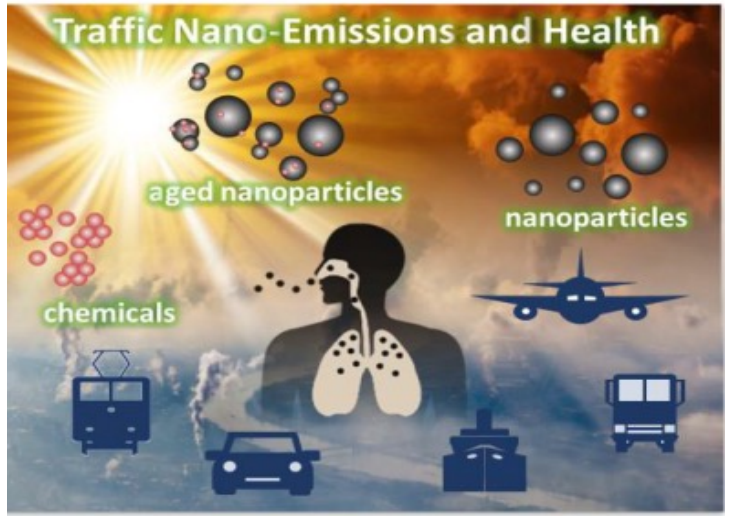
ULtrafine Particles from TRansportation – Health Assessment of Sources

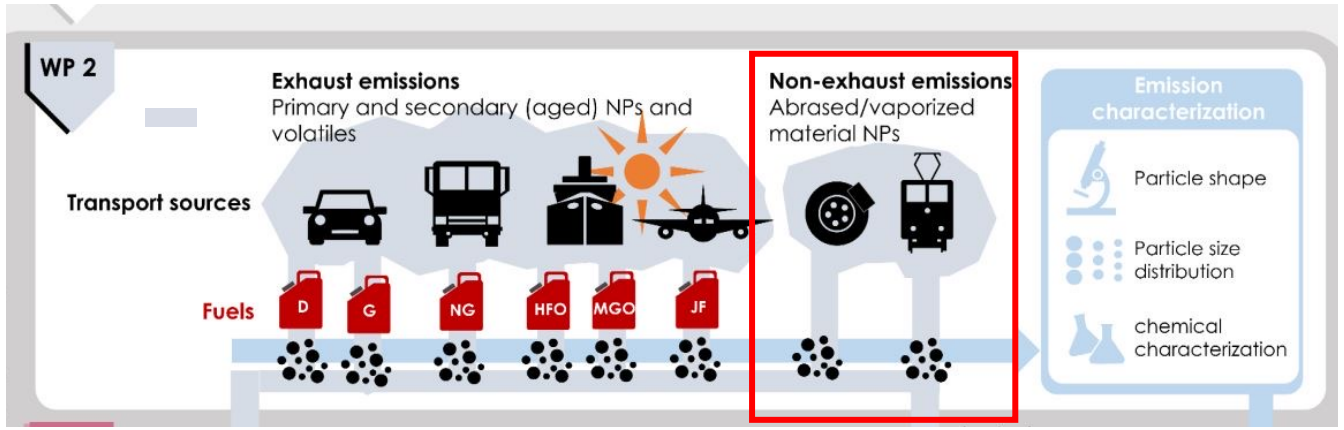
Hazard of transport mode emissions

Ultrafine particles Ø < 100 nm

Non-combustion derived emission significantly contribute to transport emissions

Brake-pads and rail wail catenary sparking





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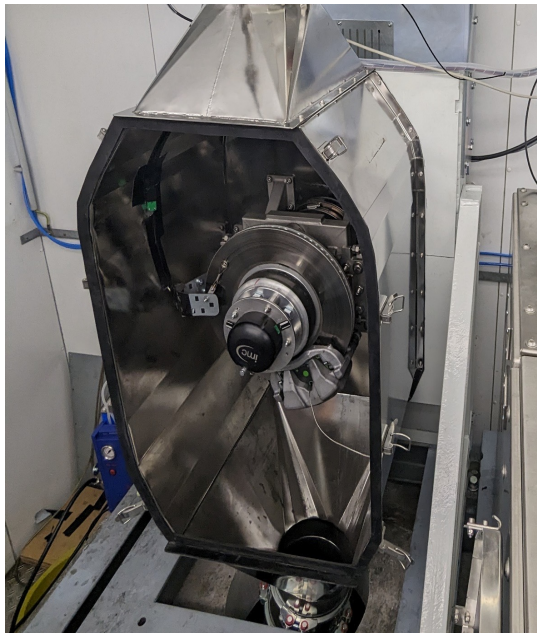
Aerosol generation

Brake dyno



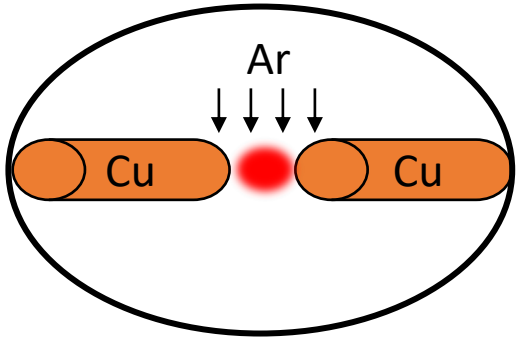
- Orientated towards GTR / EURO 7 standards
- **Non-asbestos organic** (**NAO**) brake pad
- **Low-metallic** (**LM**) brake pad
- WLTP cycle, ≈4.3h + (24h post incubation)

Session 8 – today 15:30

«*Phys. and chem. characterization of emissions from a EURO 7 brake dyno*»

C. Neukirchen

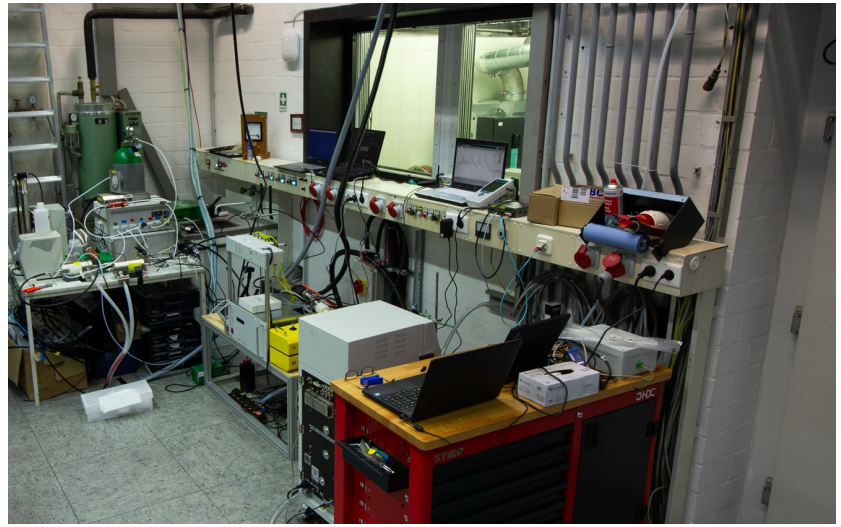
Spark Generator



4h Exposure

Copper electrodes

Spark ablation



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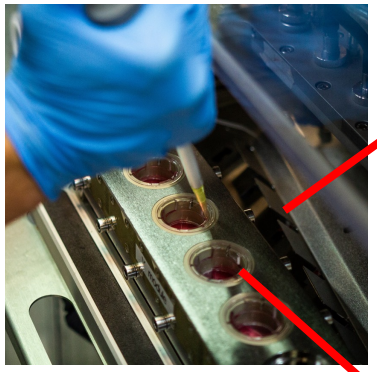
Cell Exposure

- Automated exposure system
- Air-liquid interphase
- Co-Culture cell model (CC)
- Alveolar epithelial cells (A549)
- Clean Air (CA) control
- Aerosol flow rate : 100/60/20 mL/min
  - → Quantification by ICP-MS

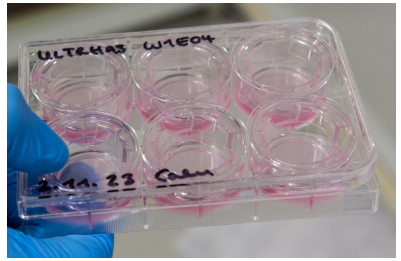
PM 2.5

Exposure system

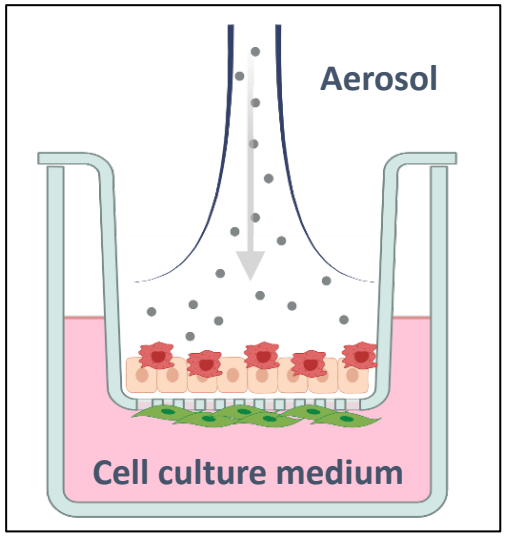
VITROCELL SYSTEMS GmbH, Waldkirchen, GERMANY



Cell model



Air-liquid-interphase



IC
Incubator Ctr.

CA
Clean Air

NAO
Non-asbestos organic

LM
Low-metallic

Cu
Spark Generator

100/60/20 mL*min^{-1}

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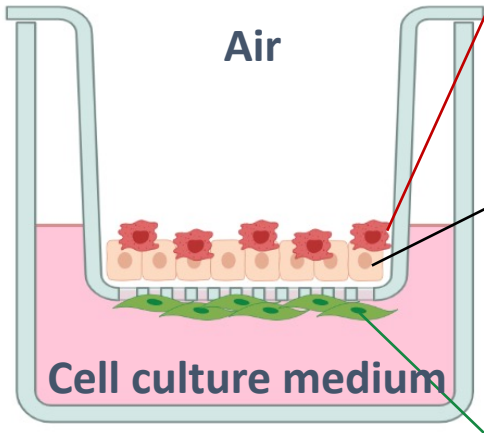
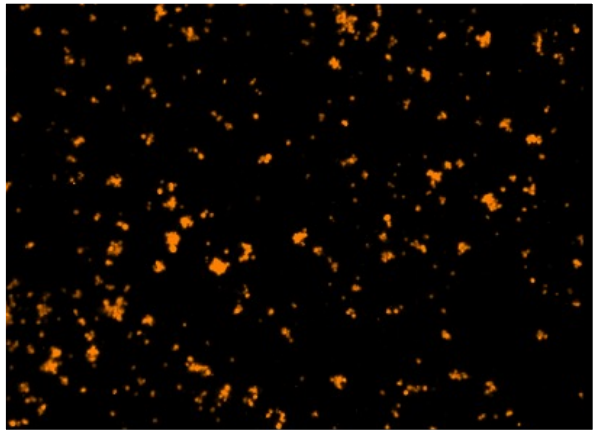
Co Culture (CC) Cell-Model



Replica of Air-Blood barrier

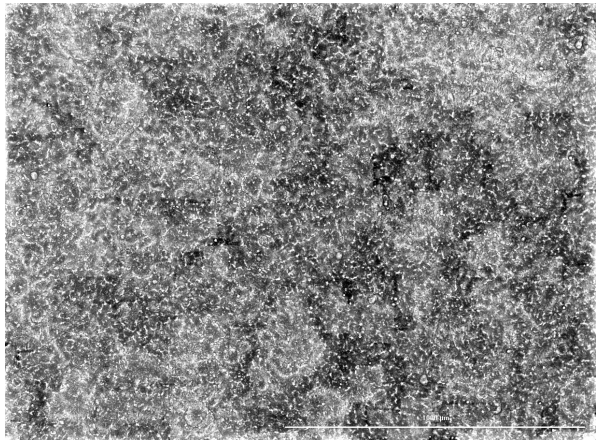
Macrophages (THP-1 derived)

- Immune competence
- Secretion of inflammatory mediators
- Particle phagocytosis



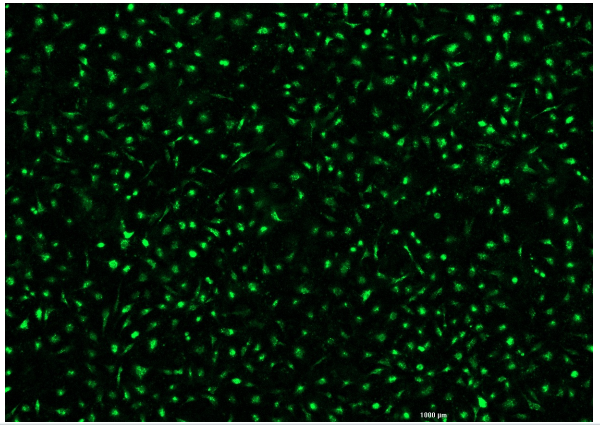
Bronchial epithelial cells (Calu-3)

- Barrier integrity
- Particle deposition
- Lung cancer cell line
- Established preclinical model



Vascular endothelial cells (EA.hy926)

- Blood vesicles
- Endothelial/circulatory effects
- Secondary effects



ULTRHAS

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Aerosol Characteristics

	NAO	LM
GMD (nm)	126 ± 2	133 ± 2
PN (#/cm3)	≈2.6E03	≈1.2E03
PN (#/km)	2.11E+05	9.76E+04

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«*Phys. and chem. characterization of emissions from a EURO 7 brake dyno*»

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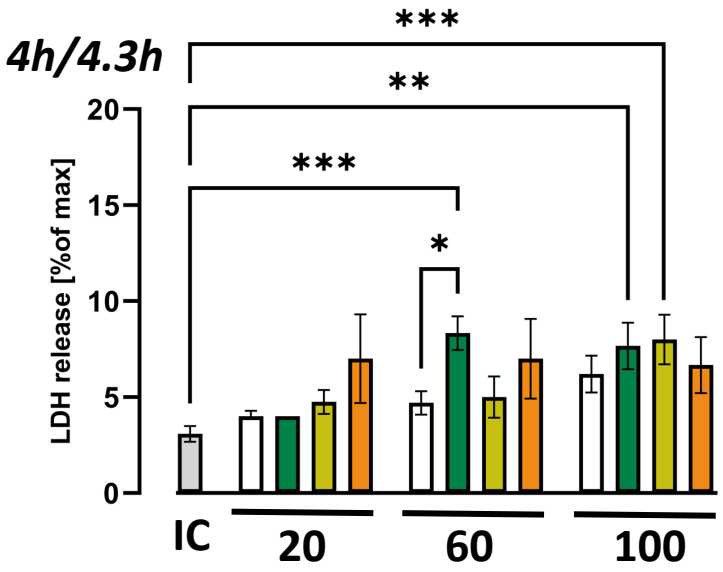
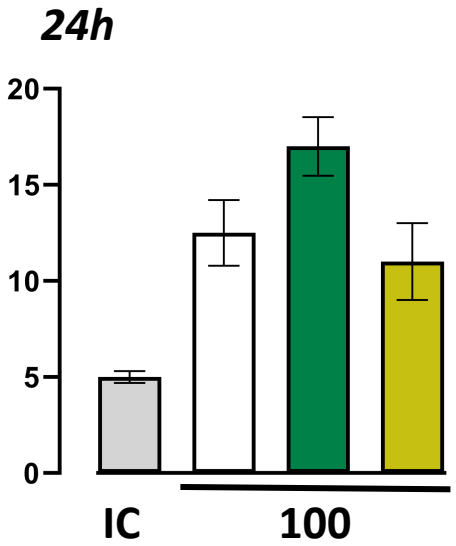
Cytotoxicity – LDH assay

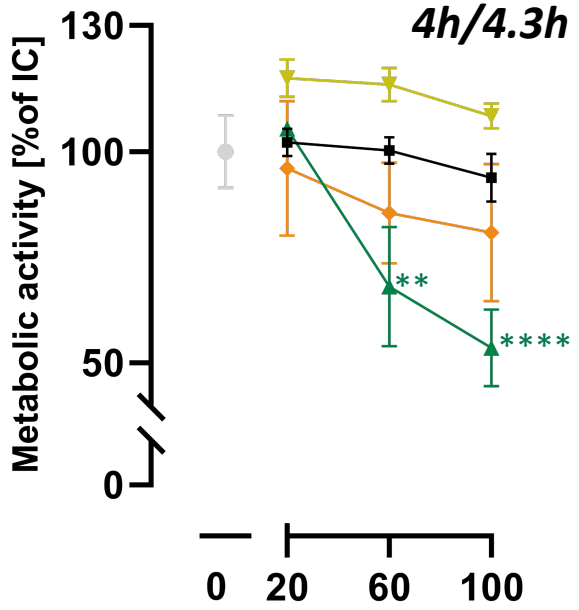
Metabolic activity

ULTRHAS

Co-Culture







IC
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CA
Clean Air

NAO
Non-asbestos organic

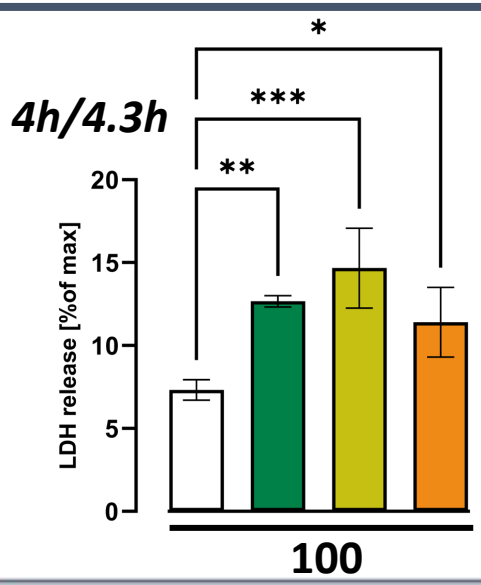
LM
Low-metallic

Cu
Spark Generator

100/60/20
mL*min^{-1}

Mean ± SEM [n= 9 (IC,CA), n=3]
**** p< 0.0001
*** p< 0.0005
** p< 0.01
* p< 0.05

A549



Cytotoxic effect of NAO and LM at 60 resp. 100 mL/min

NAO and Cu decrease metabolic activity

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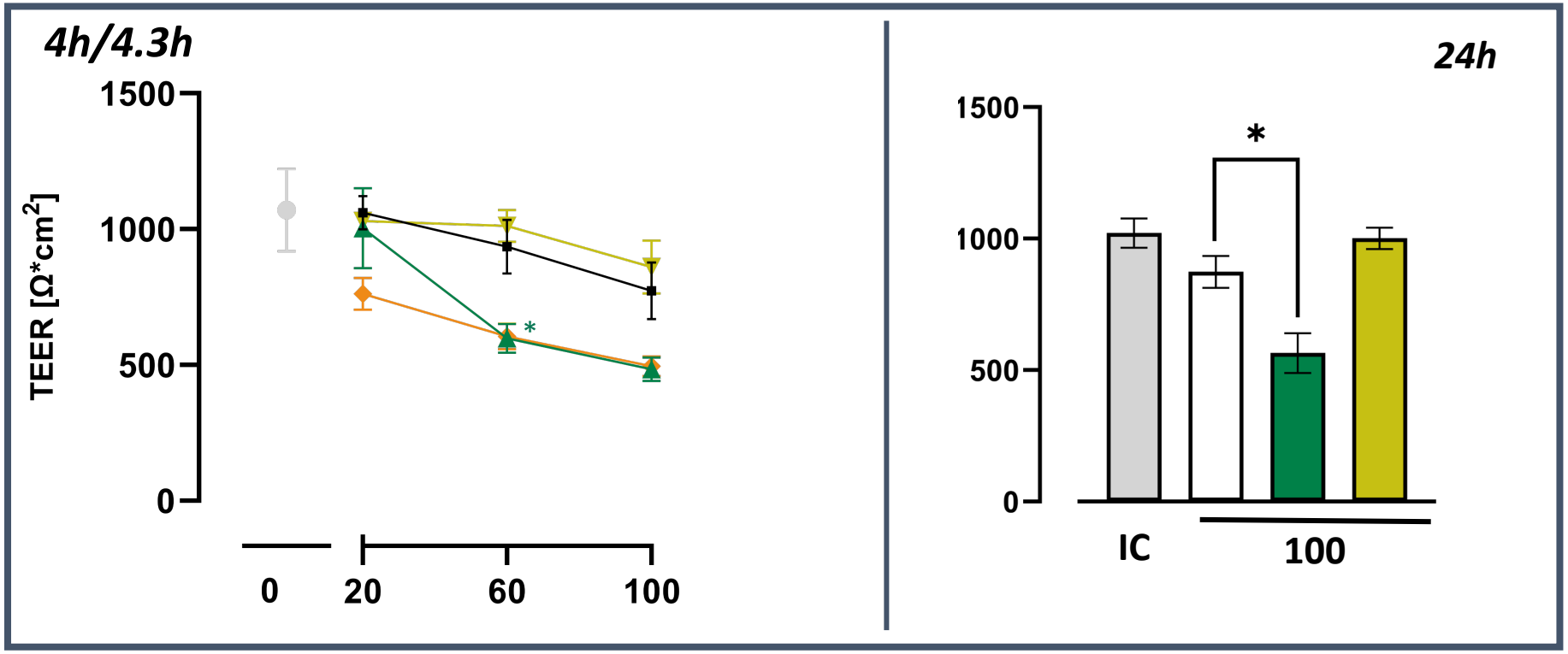
Barrier Integrity - Trans Epithelial Electrical Resistance (TEER)



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Mean ± SEM [n= 9 (IC,CA), n=3]
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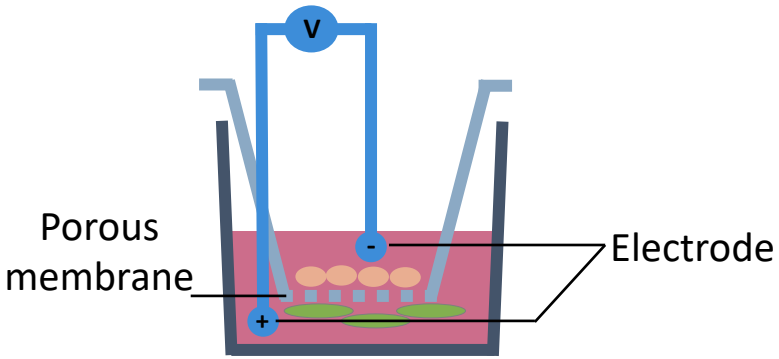

➡ NAO and Cu impair barrier integrity

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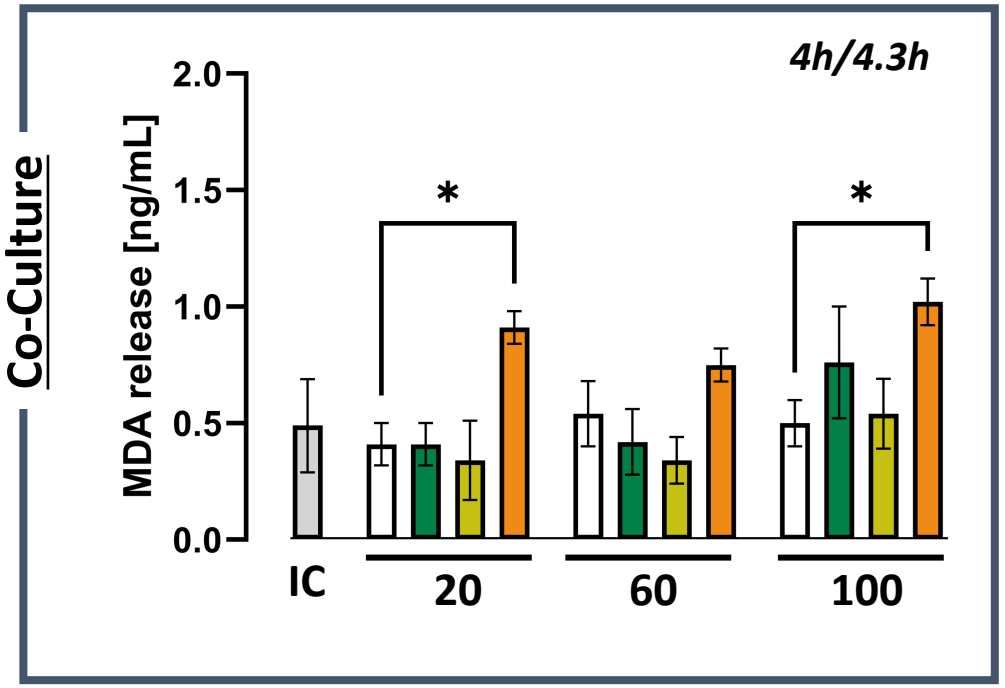
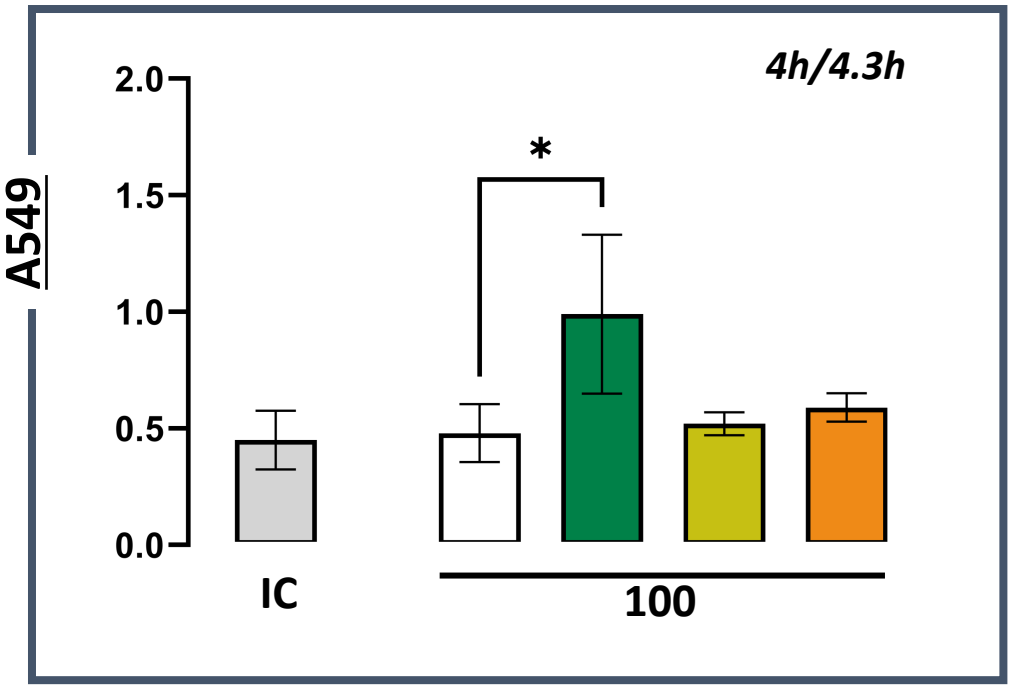
Oxidative Stress – Malondialdehyde (MDA) release





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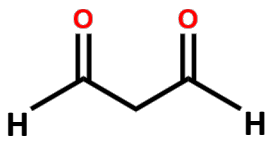
Mean ± SEM [n= 9 (IC,CA), n=3]
* p< 0.05

Lipid peroxidation end-product



MALONDIALDEHYDE

Cu particles induce ox. Stress in Co-culture

NAO indues effect in A549

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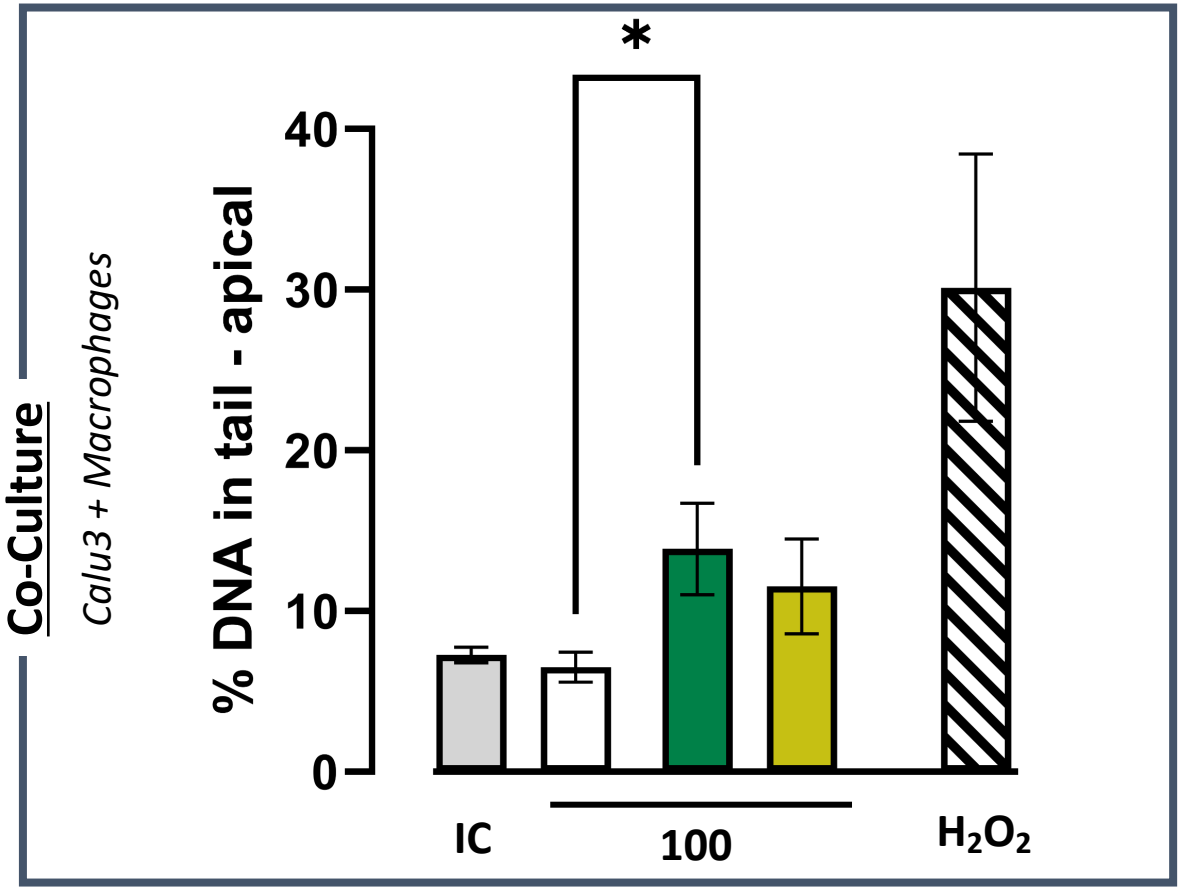
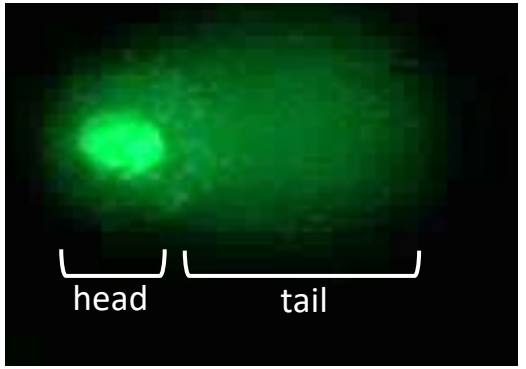
DNA damage – Comet assay alkaline version



Comet assay



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Cu
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100/60/20
mL*min^{-1}

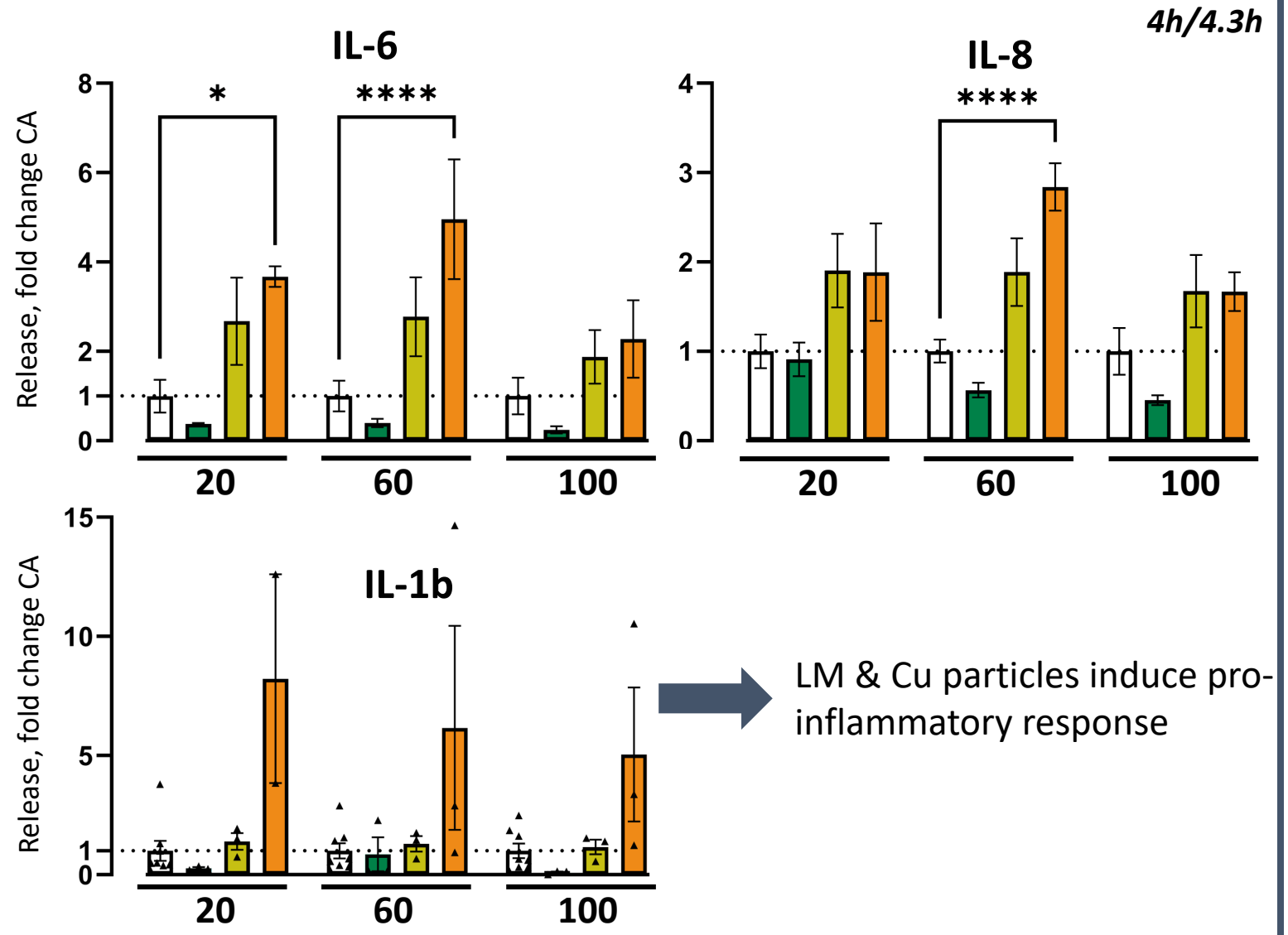
Mean ± SEM [n= 4 (IC,CA), n=3]
* p< 0.05

→ NAO particles induce DNA double strand breaks

This project has received funding from the European Union's Horizon 2020 research and innovation

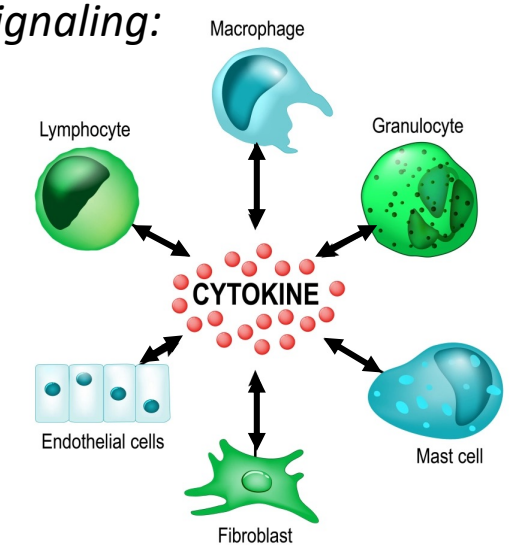
Inflammatory response – Cytokine Release

Co-Culture



LM & Cu particles induce pro-inflammatory response

Signaling:



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100/60/20 mL*min^{-1}

Mean ± SEM [n= 9 (IC,CA), n=3]
**** p< 0.0001
*** p< 0.0005
** p< 0.01
* p< 0.05

Pro-inflammatory signaling in response to stress stimuli

https://blog.healthmatters.io/2018/08/23/what-is-interleukin-6/

This project has received funding from the European Union's Horizon 2020 research and innovation

Conclusion

	NAO	LM	Cu
Cytotoxic	+	+	+
Barrier Integrity	+		+
Metabolic Activity	++		+
Ox. Stress	+		+
DNA damage	+		
Inflammation	-	+	++

Similar particle characteristics in NAO and LM

→ TPN: NAO ≈ 1/2 TPN: LM

Metal deposition

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Outlook

- Secondary genotoxicity
- ICP-MS dose determination of metals
- Alteration of gene expression in exposed cells (RNA seq.)
- Aerosol characterization of Spark Generator
- Comparison of emissions in the ULTRHAS framework

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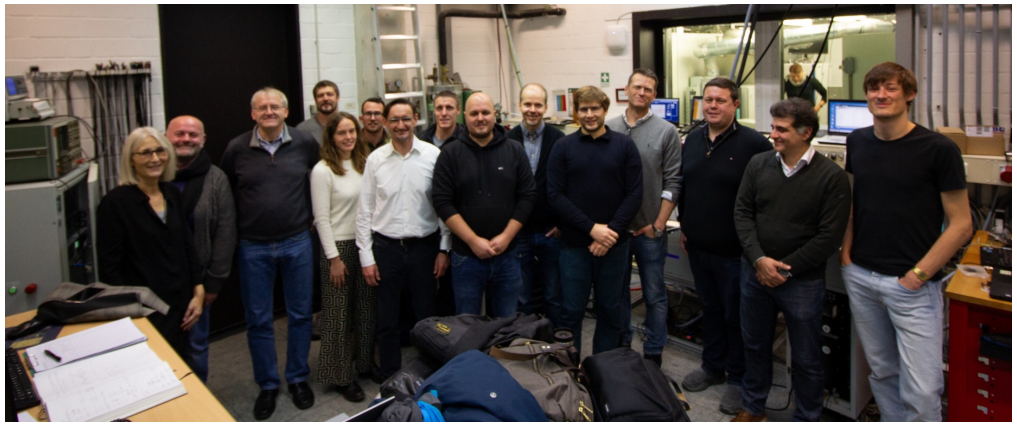
Thank you for your attention !

Thanks to all collaborators and the ULTRHAS consortium!



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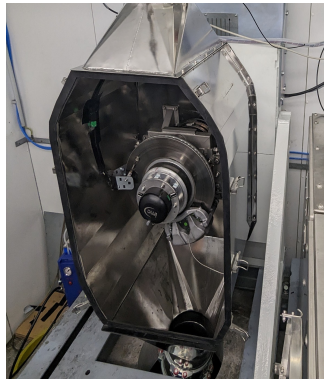
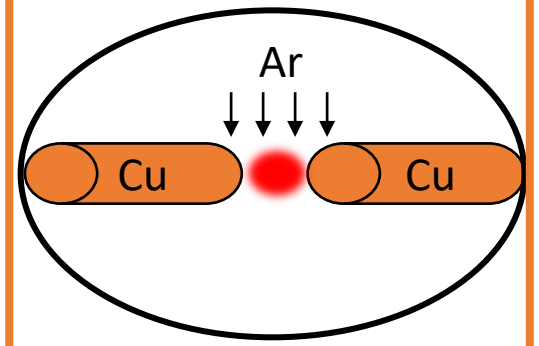
Aerosol generation

Brake dyno



- Non-asbestos organic (NAO)
- Low-metallic (LM)

Spark Generator



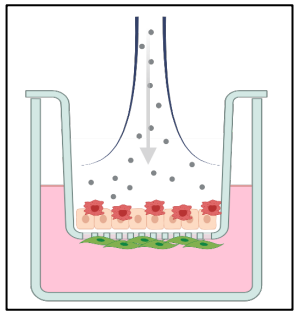
Cell exposure

Co-Culture cell model (CC)

Alveolar epithelial cells(A549)

Air-Flow : 100/60/20 mL/min

Air-liquid-interphase



PM 2.5

VITROCELL SYSTEMS GmbH, Waldkirchen, GERMANY

Sampling

Brake Dyno

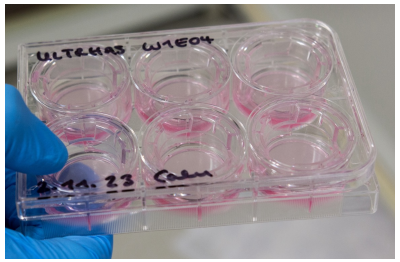
WLTP cycle, (4.3 h)

[+24h post incubation (PE)]

Spark Generator

4h exposure

Cu



- Cytotoxicity
- Metabolic activity
- Inflammatory activation
- DNA damage
- Oxidative stress

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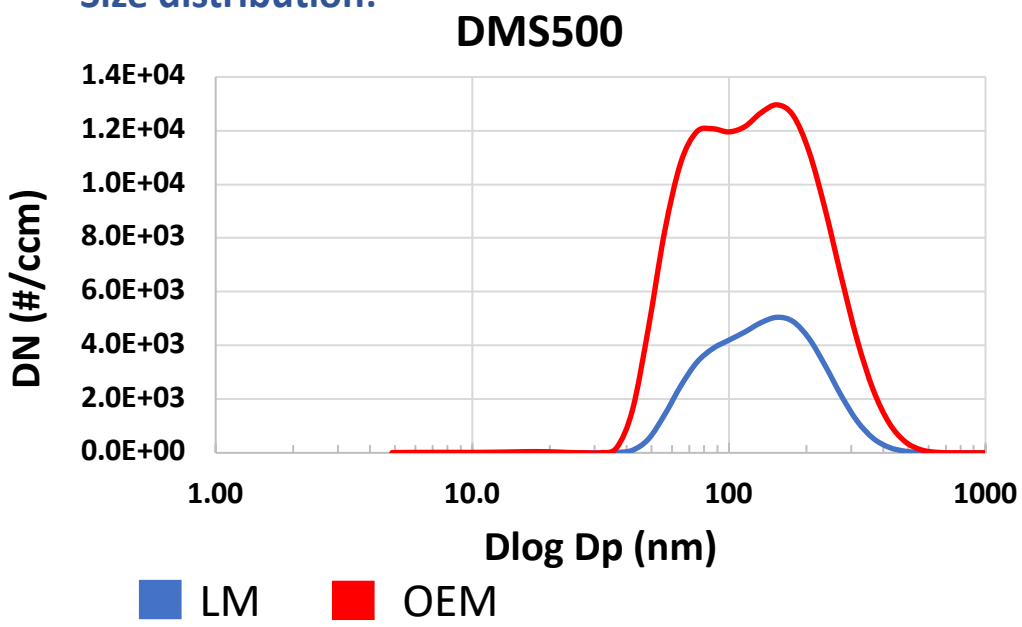
Carsten Neukirchen, JMSC seminar 24th April

Aerosol Characteristics:

Brake pad	NAO	LM
geometric mean (nm)	126	133
TPN (#/km/brake)	7.66×10^{10}	3.55×10^{10}
PM10 (mg/km/brake)	4.39	3.93
PM2.5 (mg/km/brake)	1.80	1.95
PM10/PM2.5	2.43	2.01

Size distribution:



- Similar PN size distribution for both pads
- Considerable fraction of particles in the < 100 nm range

C. Neukirchen, et al. (Universität d. Bundeswehr), JMSC Seminar 2024.

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