"Laboratory-generated soot with well-defined organic coating for *in-vitro* cytotoxicity assessment"

Interdisciplinary project funded by the Federal Office for Environment (FOEN, N° 17.0094.PJ)

Zaira Leni
Konstantina Vasilatou, Michaela Ess, Alejandro Keller, Marianne Geiser

ETH Conference
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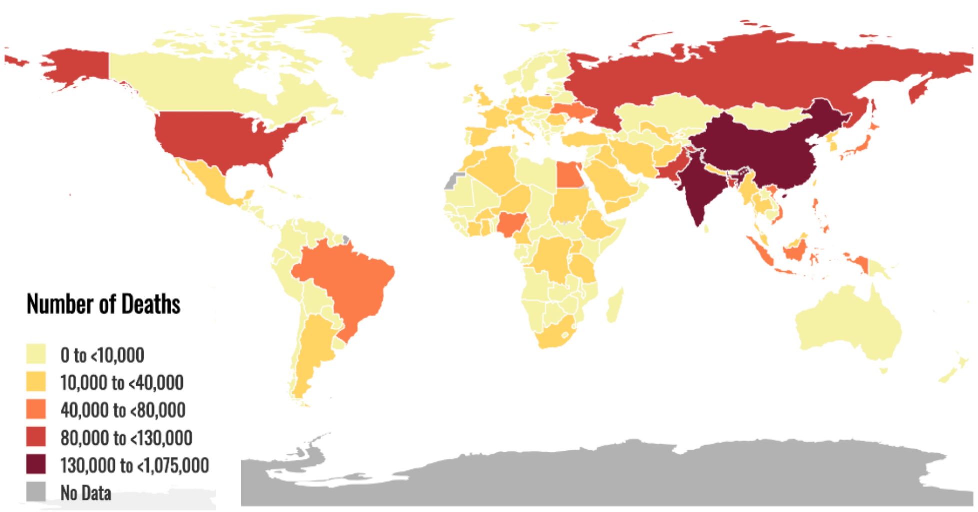
llution is the 6th highest-ranking risk factor for death globally

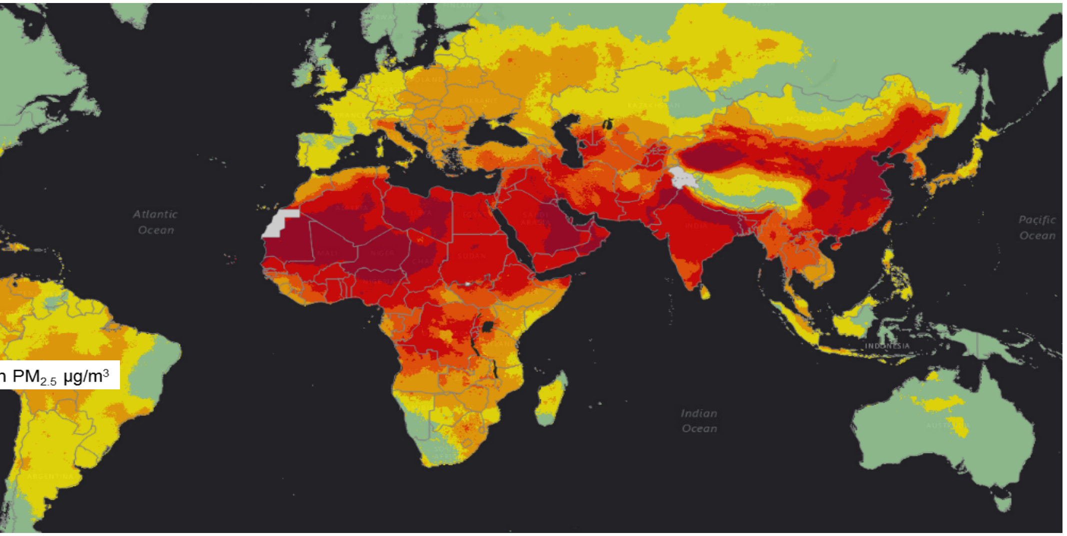
92%
More than 9 out of 10 of the world's population lives in areas **exceeding WHO** guidelines for safe limits.

<1%
In China and India, **less than 1%** of the population lives in areas meeting the WHO guidelines

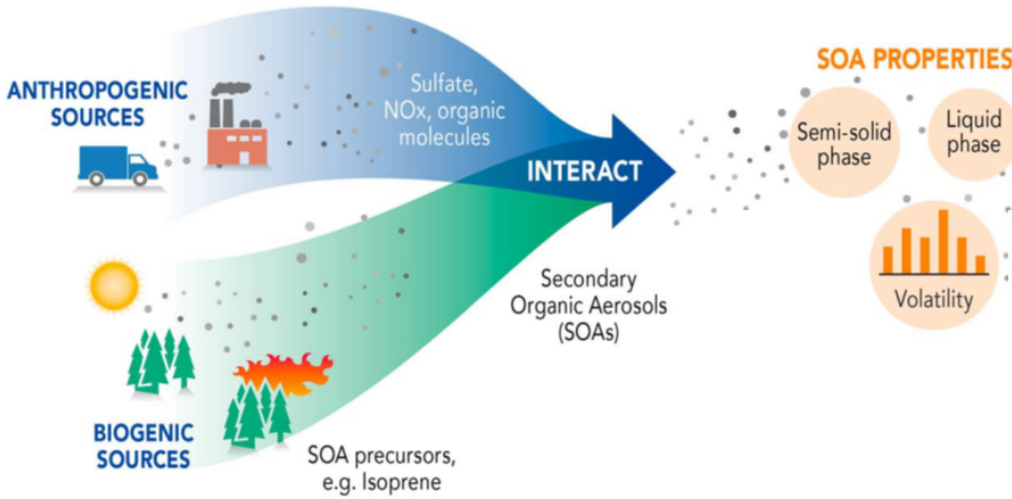
Source: WHO 2016



Health Effects Institute. 2018. State of Global Air 2018. Boston MA



Global map of modelling annual median concentration of $PM_{2.5}$ in $\mu g/m^3$ http://maps.who.int/airpollution/

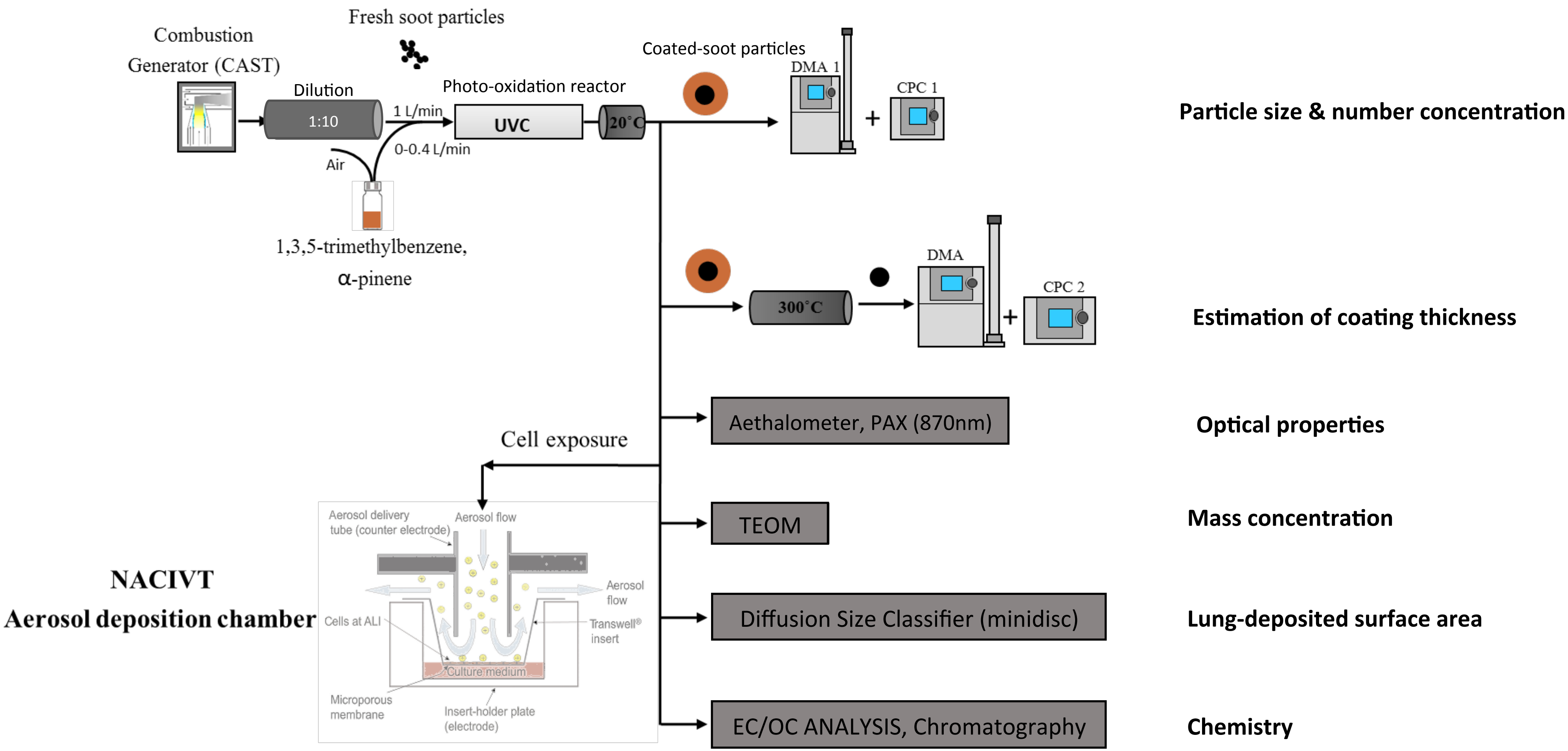


Adapted from "Recent advances in understanding SOA: implications for global climat
Shrivastava

Project goals and experimental setup (I)

eration of well characterized aged soot particles coated with oxidized organic matter

- Anthropogenic origin (1,3,5-trimethylbenzene, urban site)
- Biogenic origin (α-pinene, suburban)

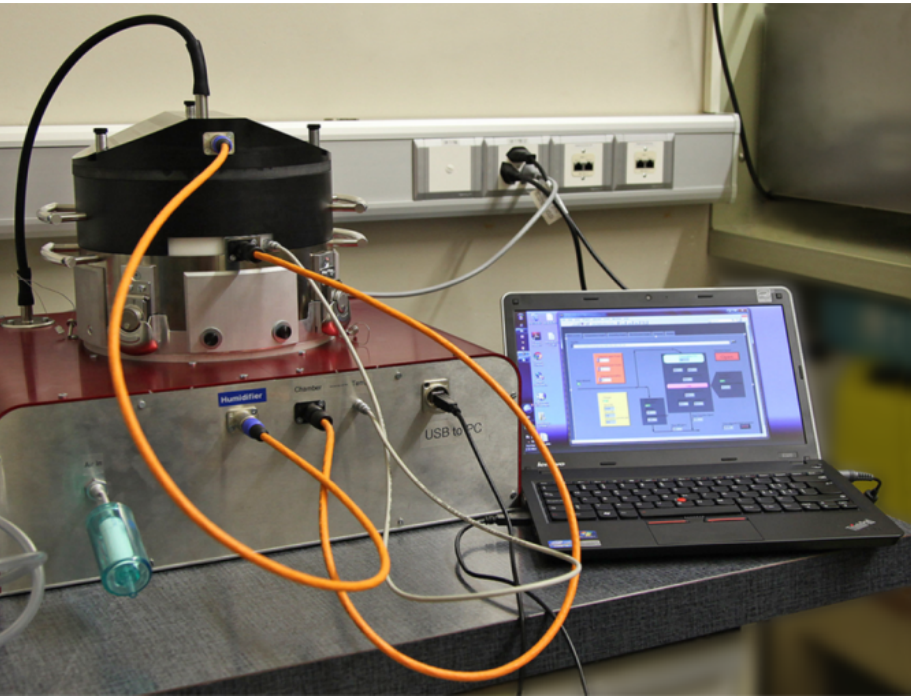


01 Type BC
. Vasilatou, *Aerosol Science and Technology* (2019)

Project goals and experimental setup (II)

(Nano-)Aerosol Chamber for In-Vitro Toxicology, NACIVT (www.nacivt.ch)

54 × 49 × 48 cm (WDH), 30.5 kg



Aerosol (external production/ source)

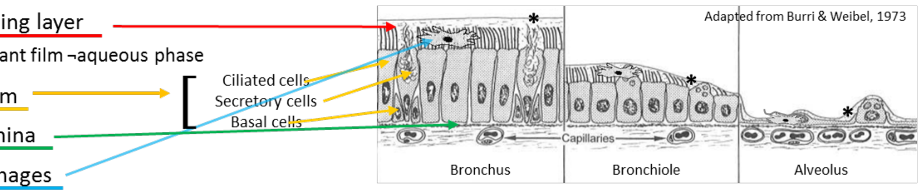
Aerosol conditioning (T; RH)

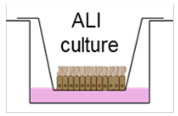
Aerosol distribution to delivery tubes

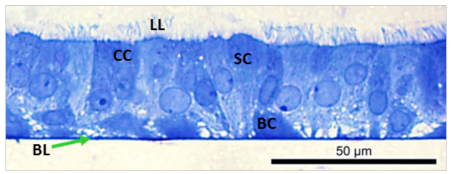
Particle deposition on 24 cell cultures

Custom electronics (aerosol charger, integrated pump, flow control)

- "All-in-one", mobile system for direct at any particle source
- Mimics particle deposition in lungs (T gas composition, air flow, N_P, N_{dep})
- Simultaneous exposure of 24 cell cult
- Controlled & stable conditions allow l term exposures







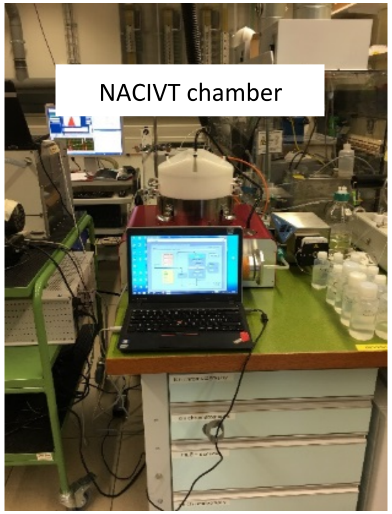
Junctional complexes

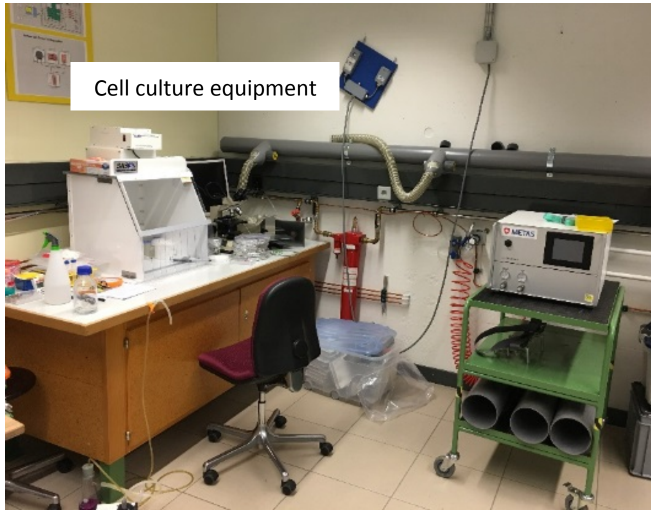
Jeannet N. et al., *Nanotoxicology* , 2015; Geiser M. et al., *Nanom*

Objective and experimental setup (III)

three experimental campaigns took place at Federal Institute of Metrology (METAS)

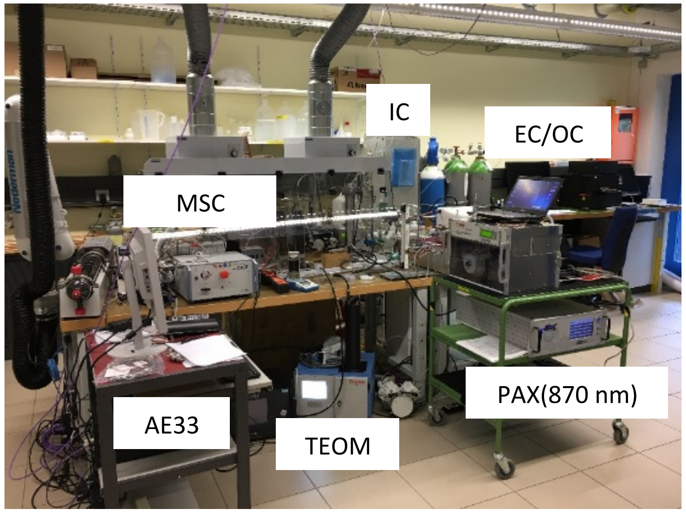
- 1st: 13-16/08/2018 (TMB)
- 2nd: 03-17/12/2018 (TMB)
- 3rd: 13-24/05/2019 (α-pinene)

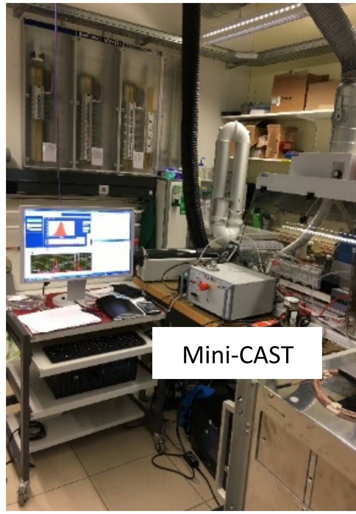




equipment of University of Bern transferred to TAS:

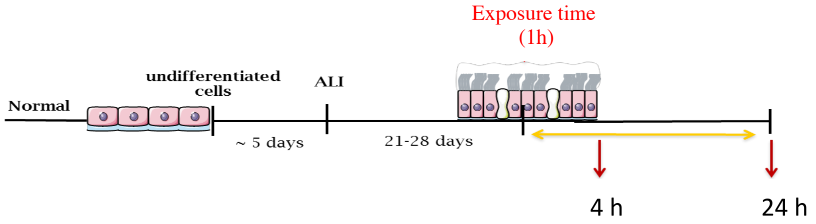
- NACIVT chamber
- Cell culture lab (e.g. sterile bench, cell incubator)
- Light microscope

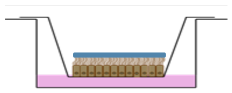


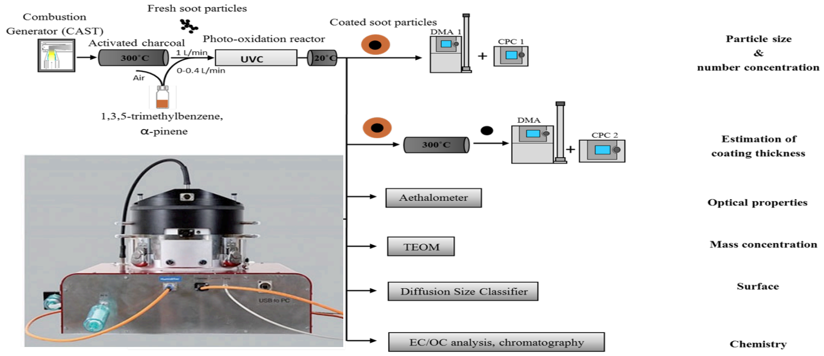


Exposure of realistic cell models to well-controlled, chemically defined synthetic reference aerosols, to identify t aerosol components/properties responsible for adverse respiratory effects.

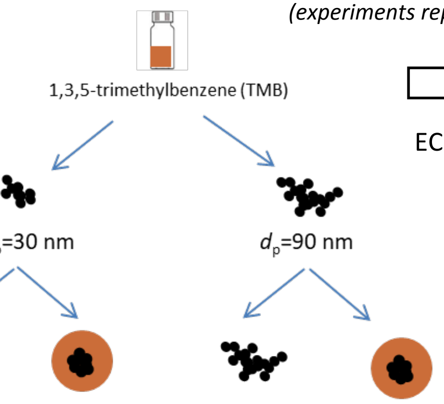
Particle delivery strategy







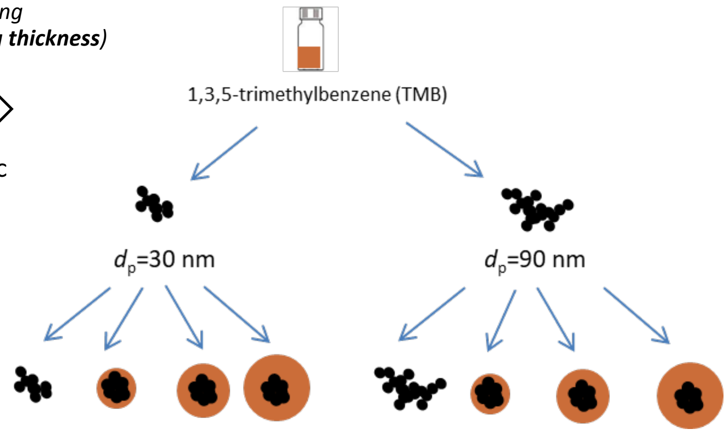
1st campaign



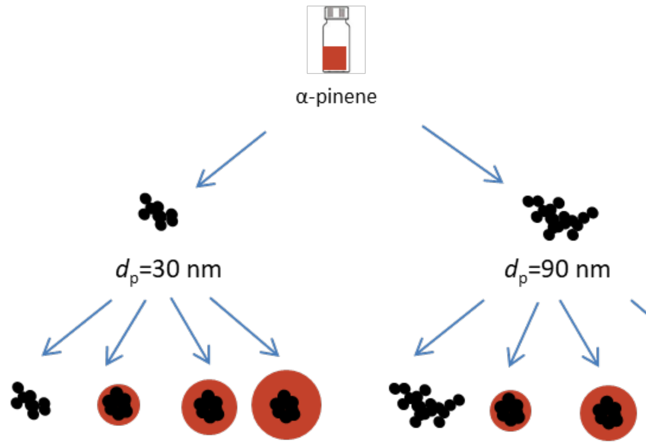
Cytotoxicity depends on organic coating
*(experiments repeated with **additional coating thickness**)*

EC (soot core)→not cytotoxic

2nd campaign



3rd campaign



to evaluate **coating** effects on cytotoxicity

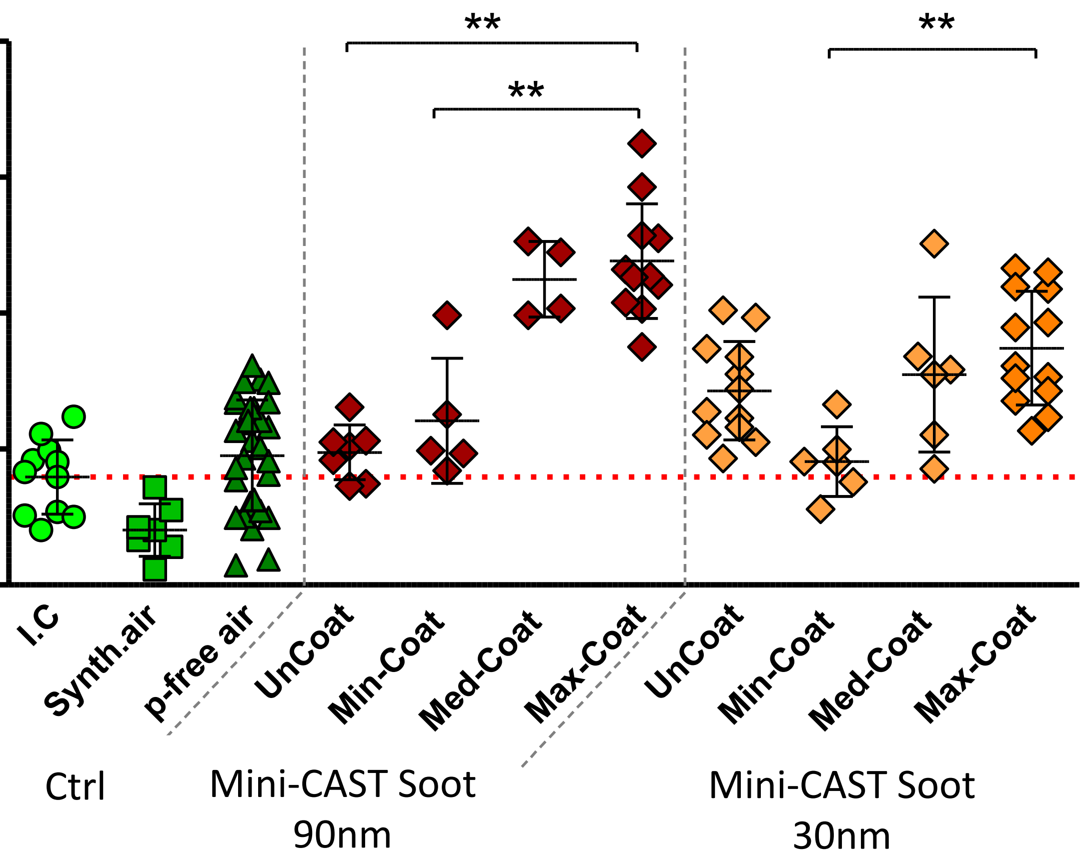
EC/TC(%) 99 54 31
Size (nm) 91 88 103

Particle number concentration remains the same between 30 nm and 90 nm in all three campaigns:

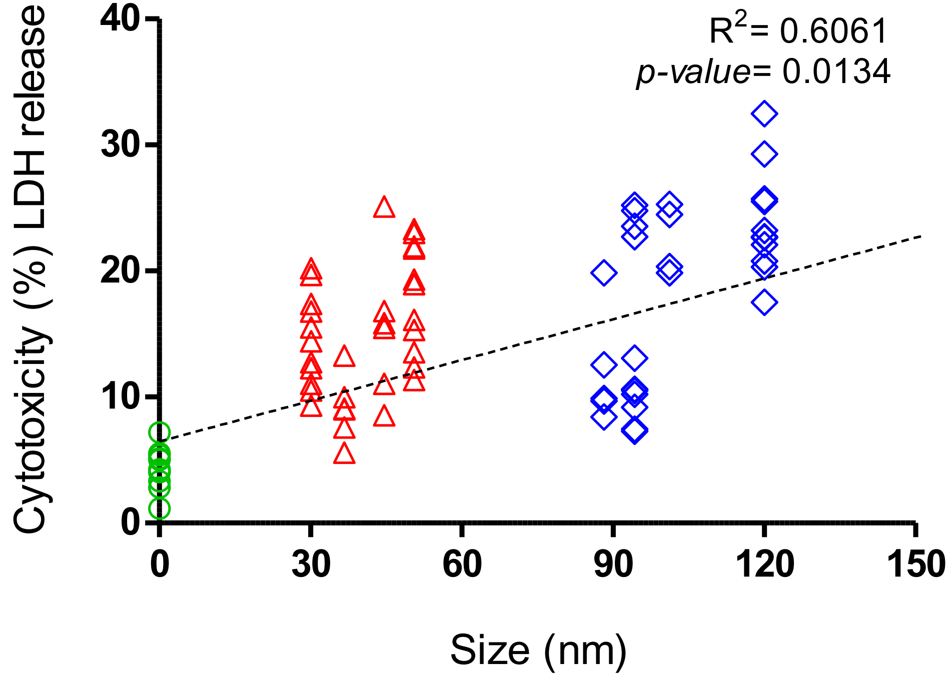
30 nm ~ 4 x 10^5 NP/cm^3

90 nm ~ 1.3 x 10^5 NP/cm^3

1st and 2nd campaign: TMB as SOA-precursor (I)



Correlation Size/Cytotoxicity

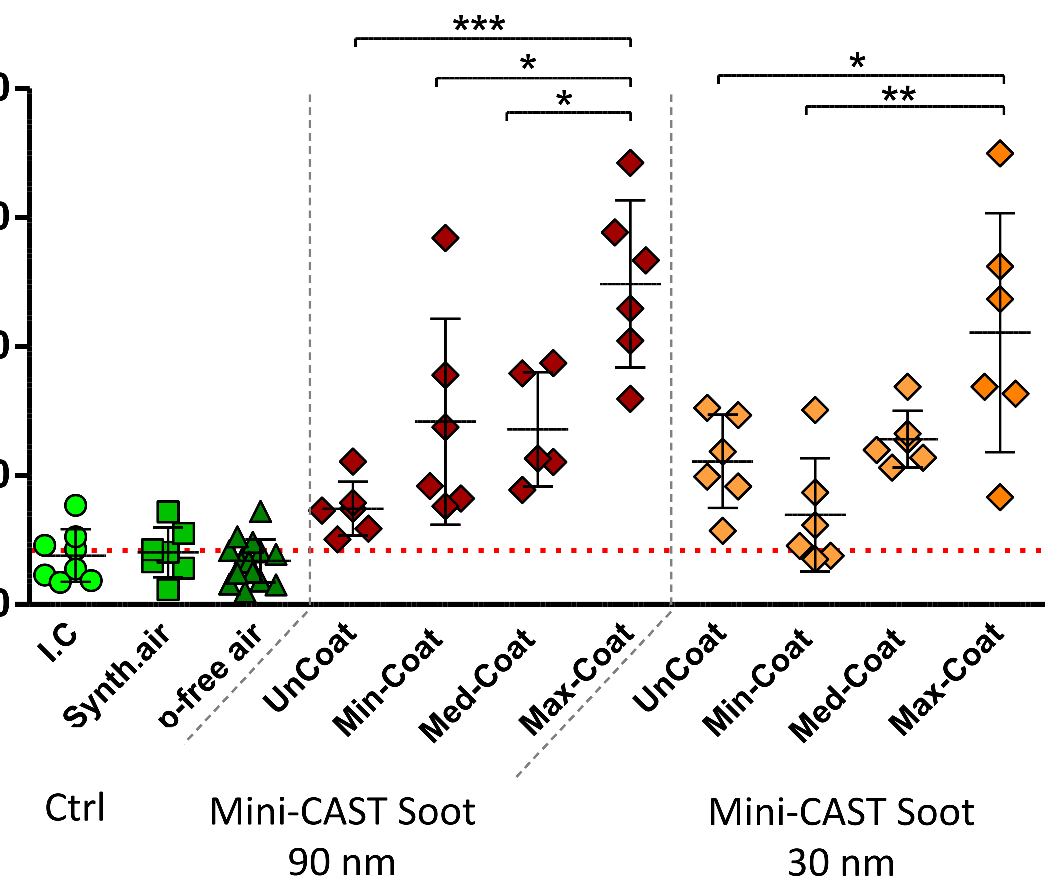


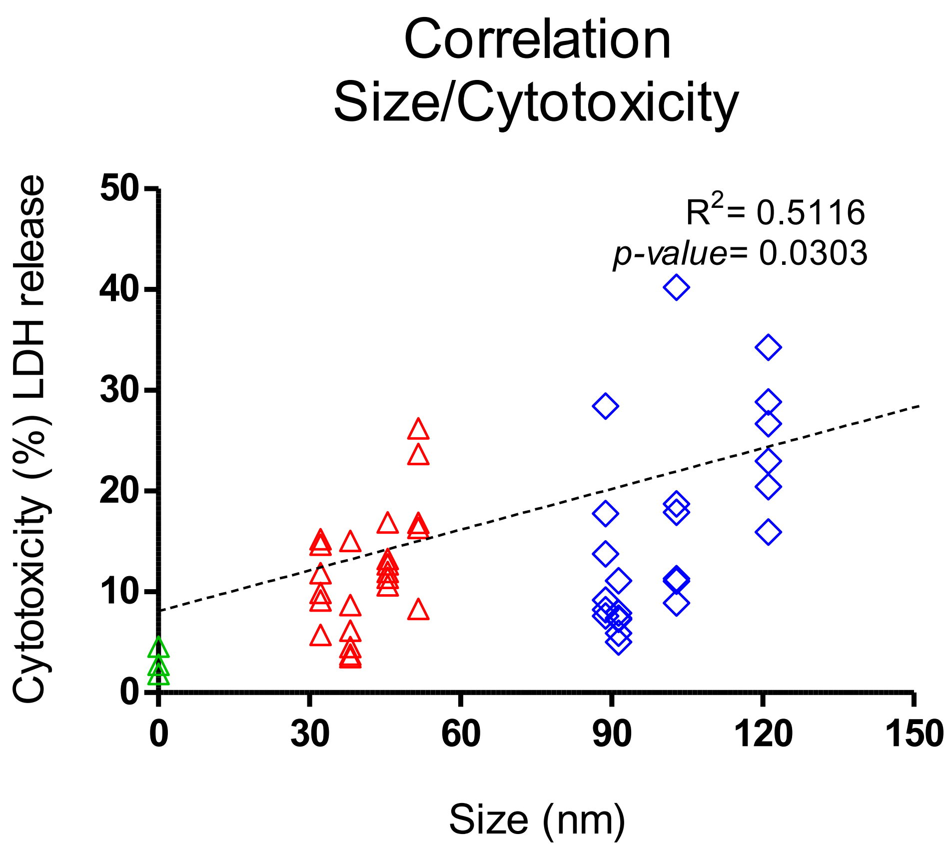
No significant difference in terms of cytotoxicity between incubator control (I.C), p-free air and synthetic air exposed cells

Soot particles consisting only of EC (90 nm UnCoated soot) cause no cytotoxic effects

Cytotoxicity increases with increasing coating thickness. Max-Coat 90 nm induces the highest cytotoxicity, i.e. release of LDH (mean

3rd campaign: α-pinene as SOA-precursor

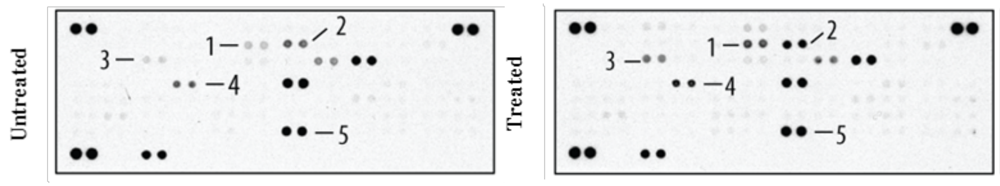




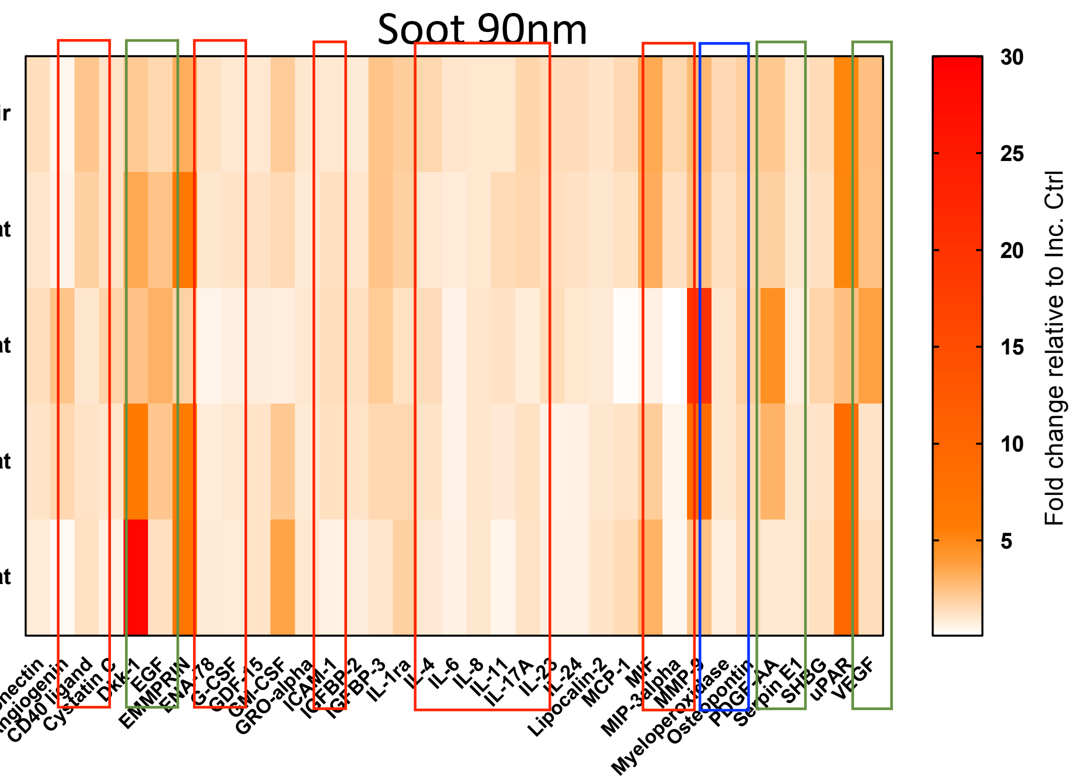
- *Cytotoxicity increases with coating thickness as in the case with TMB as SOA-precursor*
- *General, slightly lower cytotoxic effect with α-pinene as precursor compared to TMB*

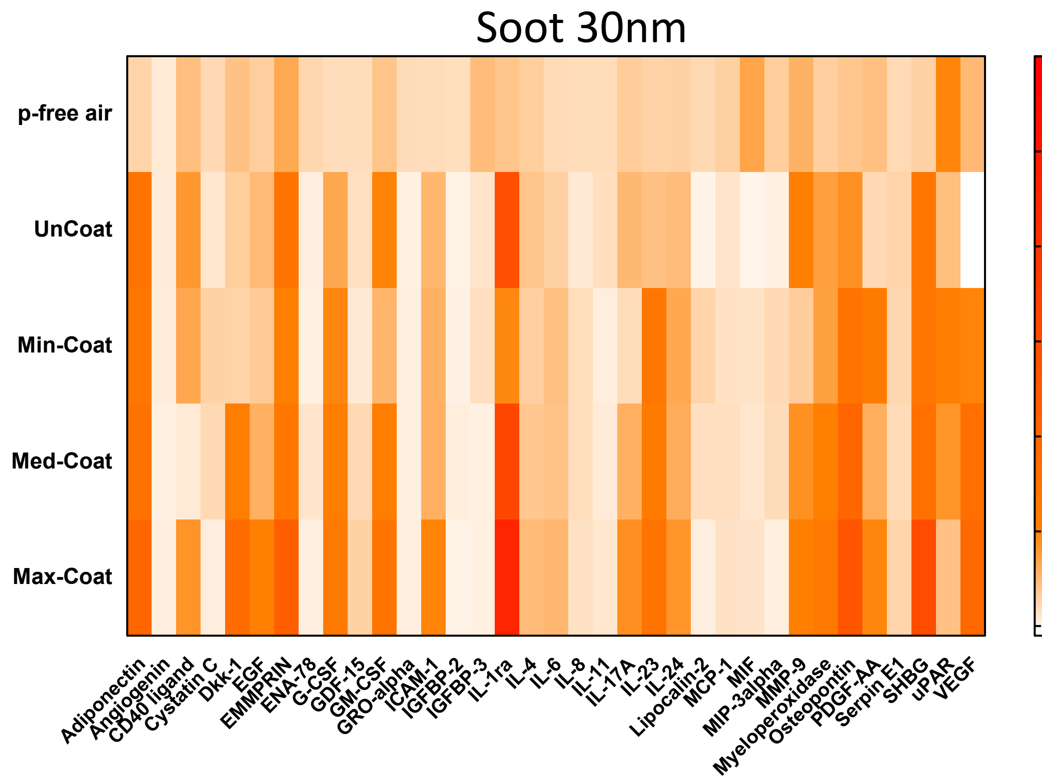
Ongoing analysis: Secretome

102 cytokines analyzed



1[st] and 2[nd] campaign: TMB as SOA-precursor





Out of 102 cytokines and chemokines analyzed, **35** are down- or up-regulated in comparison to incubator control

Conclusion and outlook

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lot study funded by FOEN

t particles consisting **only of EC** (90 nm UnCoated soot) cause no cytotoxic effects.

otoxicity increases with **coating thickness** with TMB and α-pinene as SOA-precursors.
-Coat 90 nm induces the highest cytotoxicity.

inene as SOA-precursor cause slightly lower cytotoxic effect compared to TMB.

ngoing and future activities:

nsmission electron microscopy (TEM) of cell culture sections and of particles sampled on TEM grids.

retome analysis for the 3rd campaign

e pilot study has led to the EU project: EMPIR AeroTox (http://empir.npl.co.uk/aerotox

focal microscopy of samples from 2nd and 3rd campaign at the National Physical Laboratory (NPL, UK).

lysis of the degree of oxidation of the organic matter by Aerosol Mass Spectrometry (NPL, UK).

EMPIR
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METAS

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ETAS

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AeroTox Partners

METAS

FINNISH METEOROLOGICAL INSTITUTE

LUND UNIVERSITY

UNIVERSITÄT BERN

KING'S College LONDON

NPL National Physical Laboratory

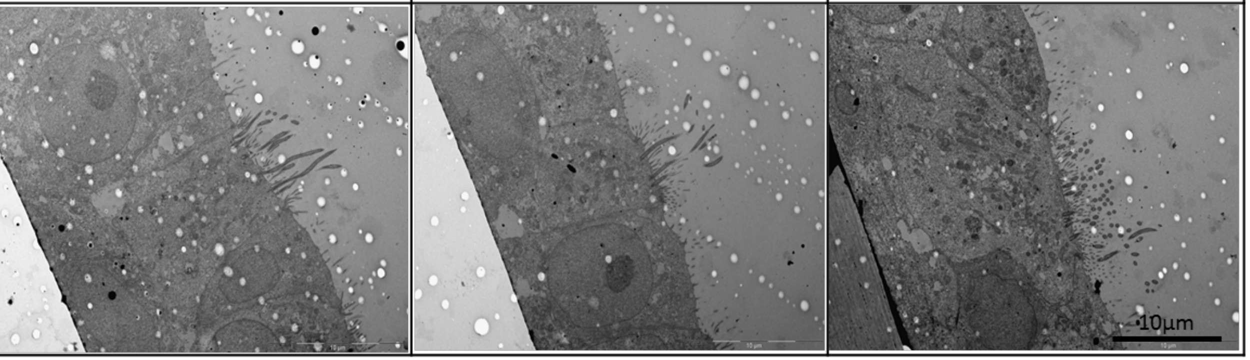
Fachhochschule Nordwestschweiz

BAM

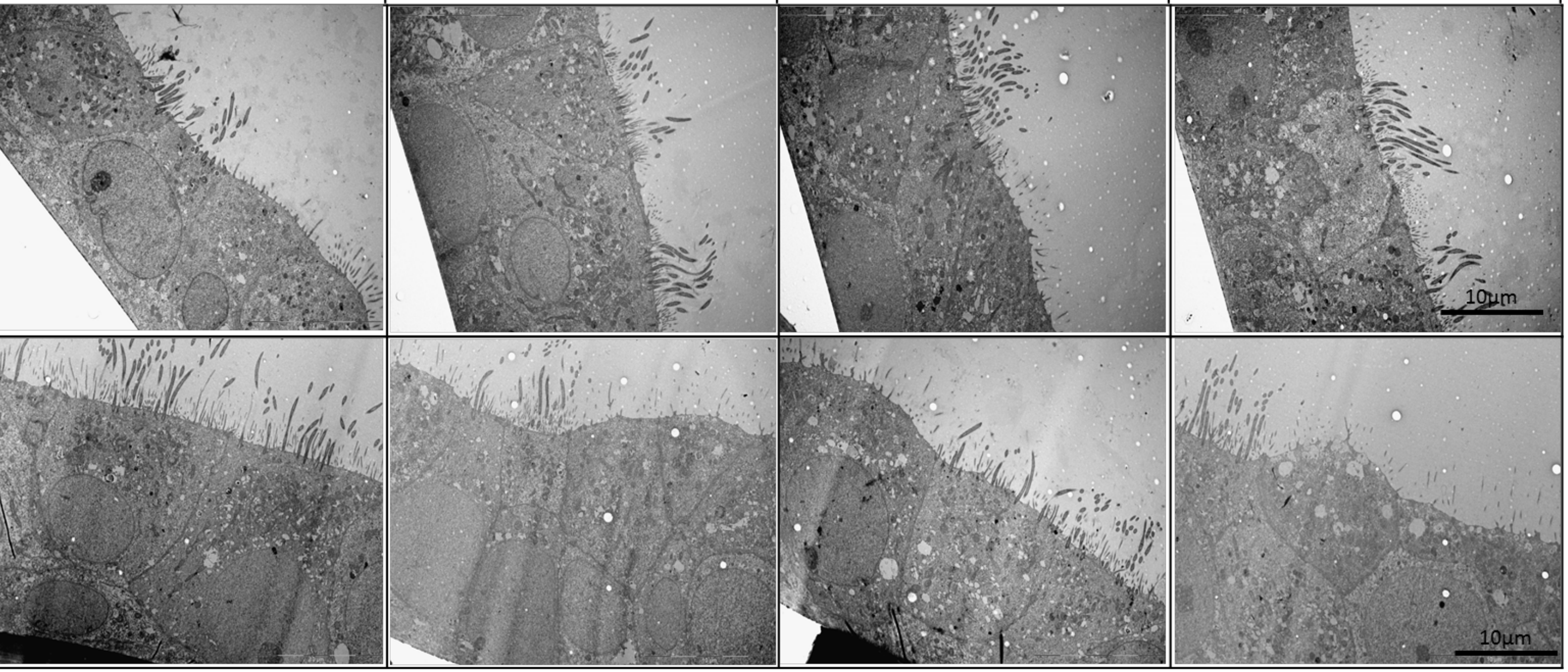
Ongoing analysis: TEM inserts

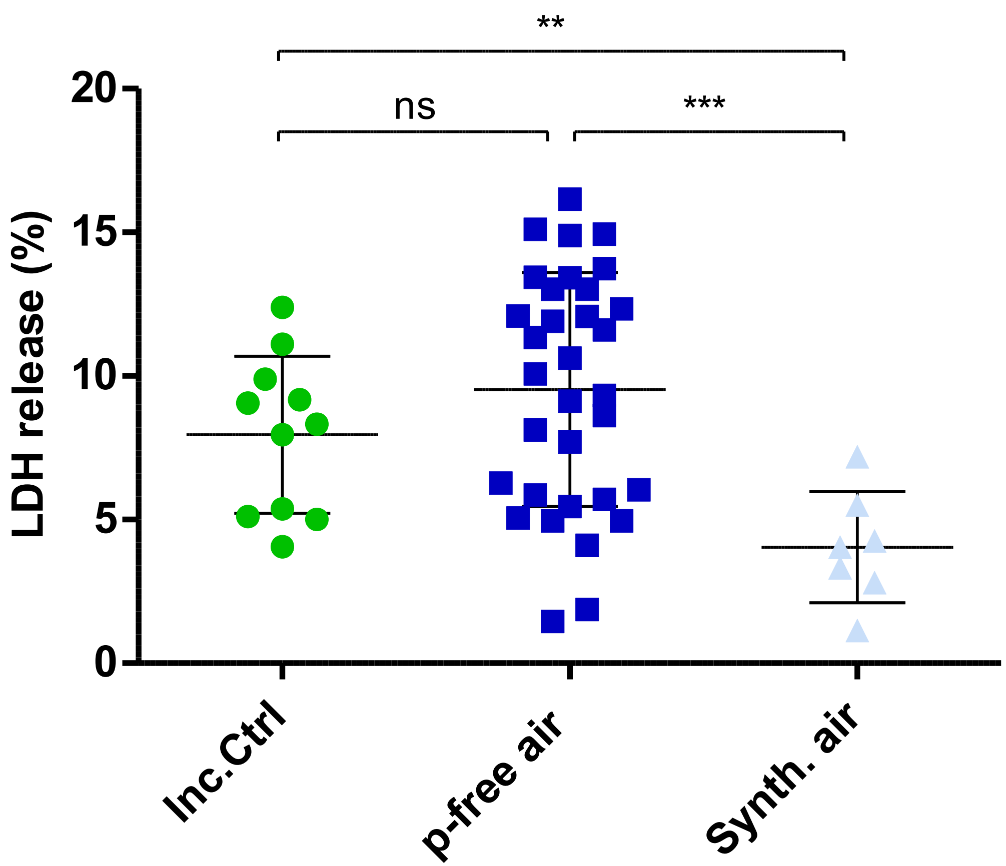
2^{nd} EXPERIMENTAL CAMPAIGN: *TMB as SOA-precursor*

I.C	p-free air	Synth. air



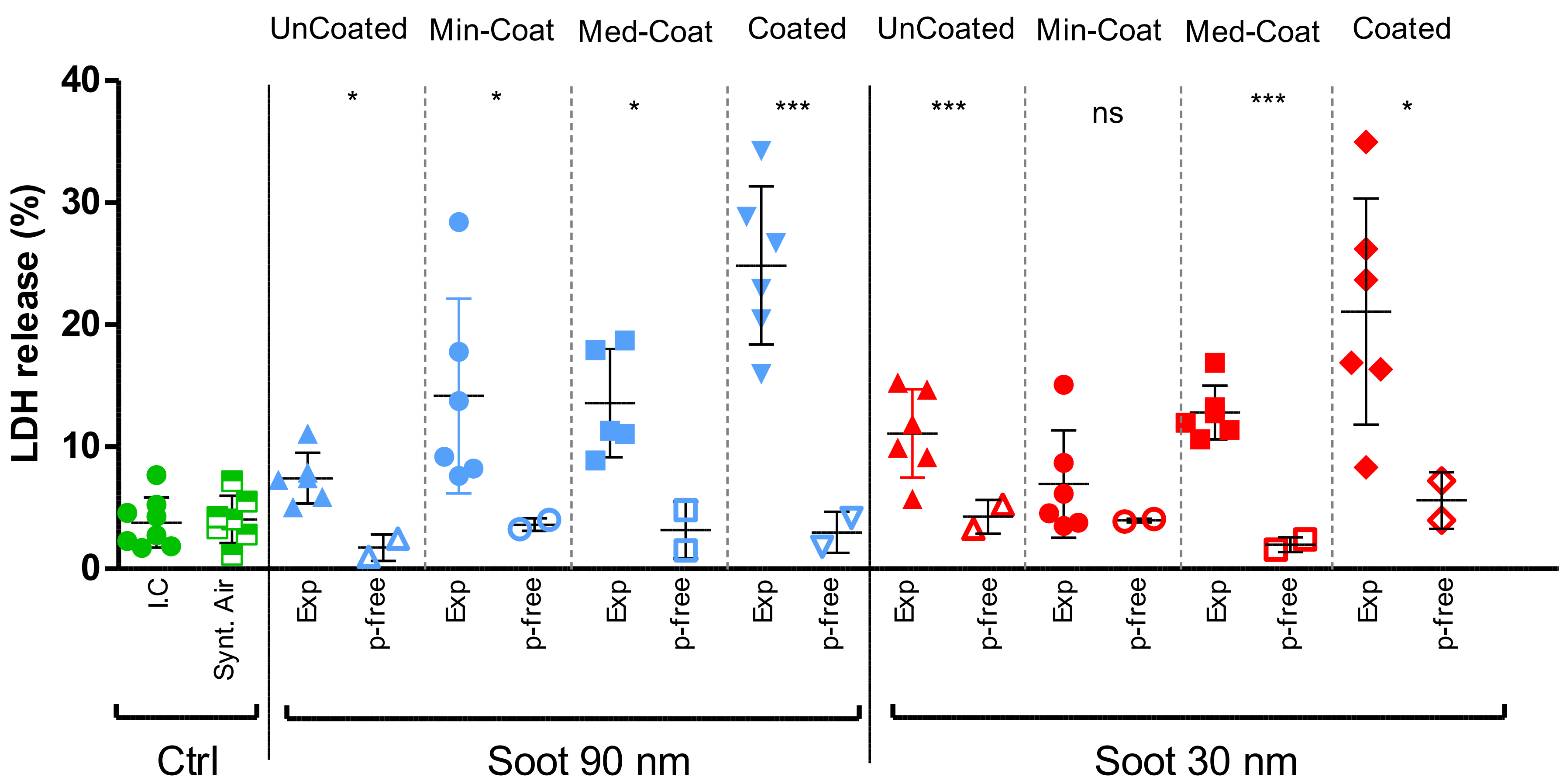
UnC.	Min-Coat	Med-Coat	Max- Coat





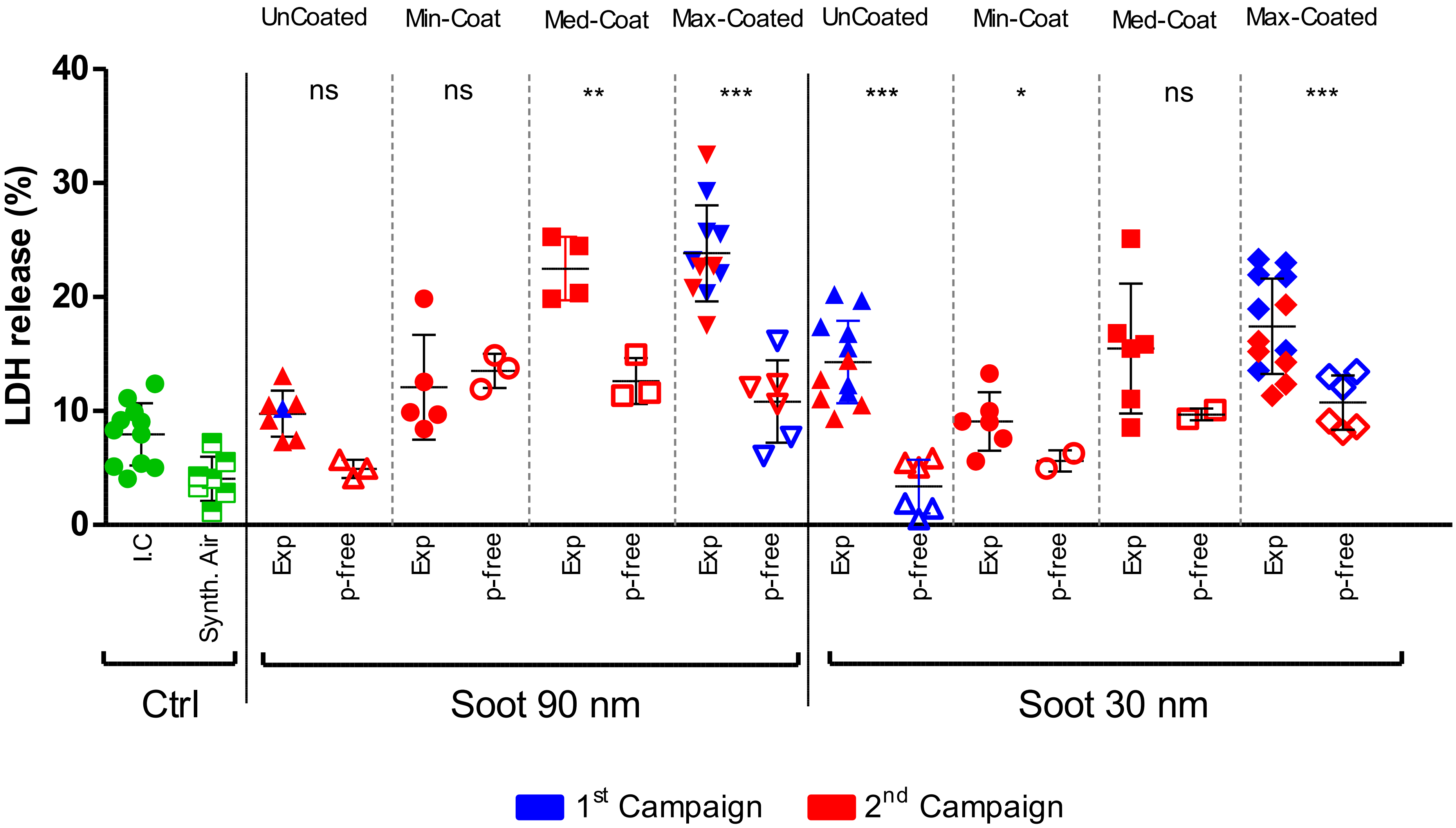
KS normality test			
Passed normality test (alpha=0.05)?	Yes	Yes	Yes
P value summary	ns	ns	ns
D'Agostino & Pearson normality test			
Passed normality test (alpha=0.05)?	Yes	Yes	Yes
P value summary	ns	ns	ns
Shapiro-Wilk normality test			
Passed normality test (alpha=0.05)?	Yes	Yes	Yes
P value summary	ns	ns	ns

3rd campaign: α-pinene as SOA-precursor



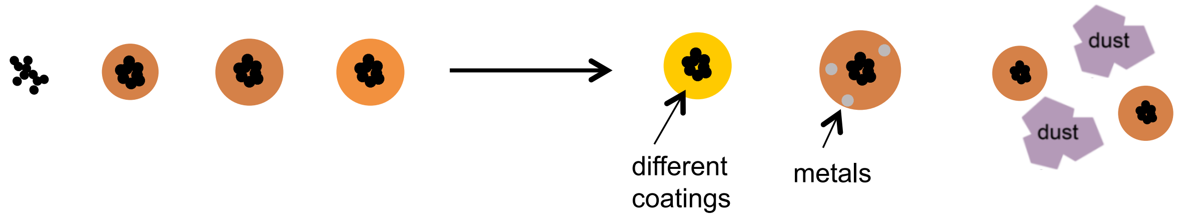
otoxicity upon exposure to coated soot much higher than that of incubator control and particle-free air tests ->
increase in cytotoxicity is indeed caused by coated soot particles

1st and 2nd campaign: TMB as SOA-precursor (II)



Good reproducibility between the 1st and 2nd campaign

Current project | **EMPIR project**



Model aerosols:
internal mixture of soot core particles with coating from secondary organic matter

Properties tested:
uncoated vs. coated particles, role of soot core size, coating thickness and coating chemistry

Spring 2018 - Spring 2019

Further tests to clarify the role of:
- primary vs. secondary organic coating
- secondary inorganic coating
- internal metal admixtures
- externally mixed dust particles (e.g. from brake abrasion)

Biomedical analyses will be complemented with toxicogenomics and particle uptake studies

June 2019 - May 2022

Generation of aged soot aerosol

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SSA=*light scattering/light extinction*