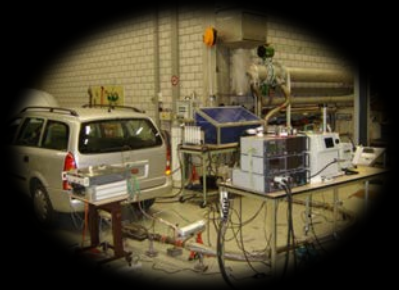
adolphe merkle institute
excellence in pure and applied nanoscience
UNIVERSITY OF FRIBOURG SWITZERLAND

Feinstaub und Lunge
Klinische Aspekte und Aktuelles aus der Forschung

Barbara Rothen-Rutishauser
Chair BioNanomaterials
Adolphe Merkle Institute
University of Fribourg
Marly, Switzerland



The Adolphe Merkle Institute in Fribourg (CH)

Polymer Chemistry & Materials
(Weder)

Soft Matter Physics
(Steiner)

BioNanomaterials
(Fink/Rothen)

Surface Science
(2015)

Assembly (Lattuada)

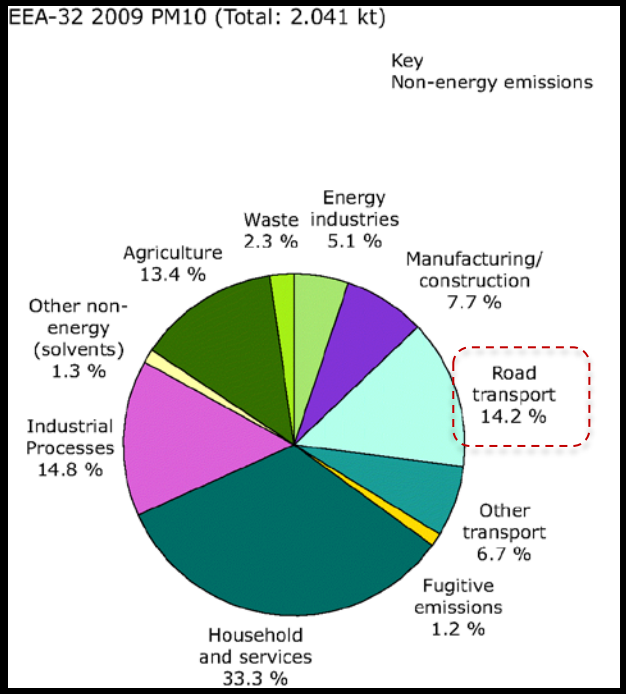
Scattering (Balog)

Macromolecular Chemistry (Nico Bruns)

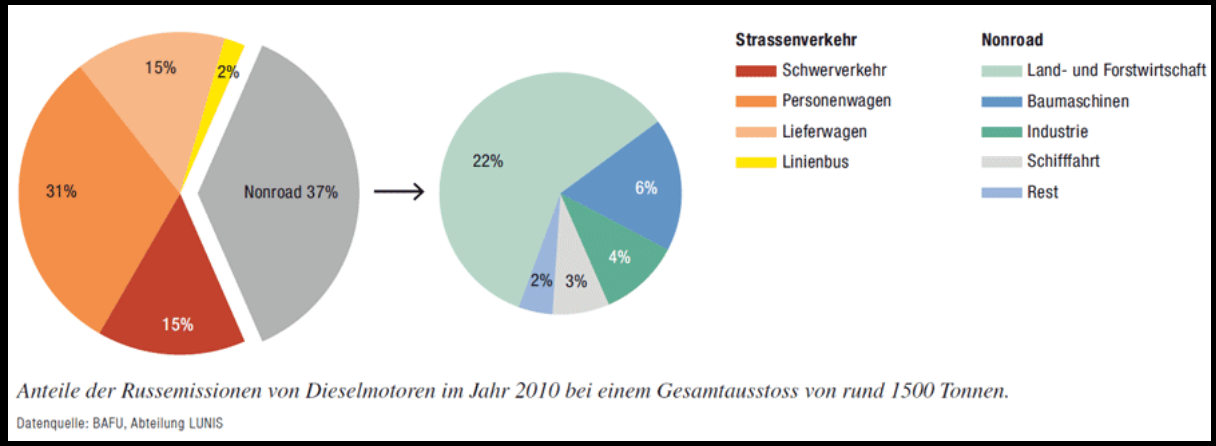
Particulate Matter 10 (PM_{10})

www.swissinfo.ch

What is PM_{10}?



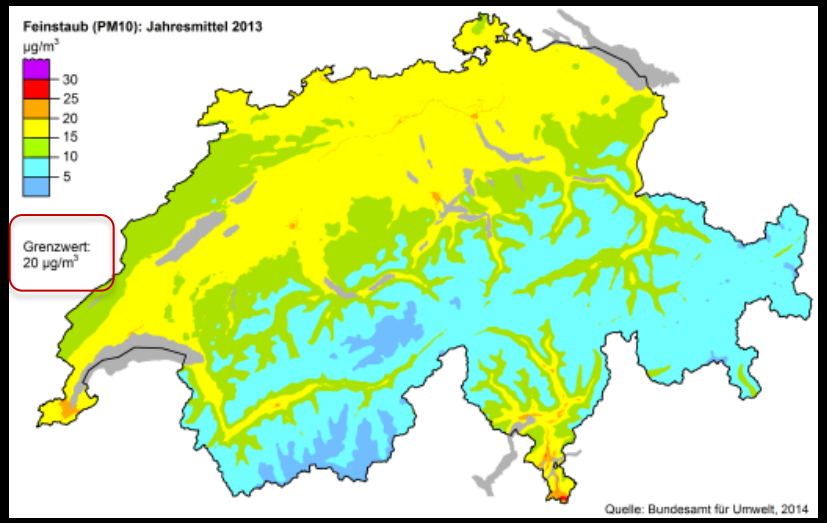
www.eea.europa.eu

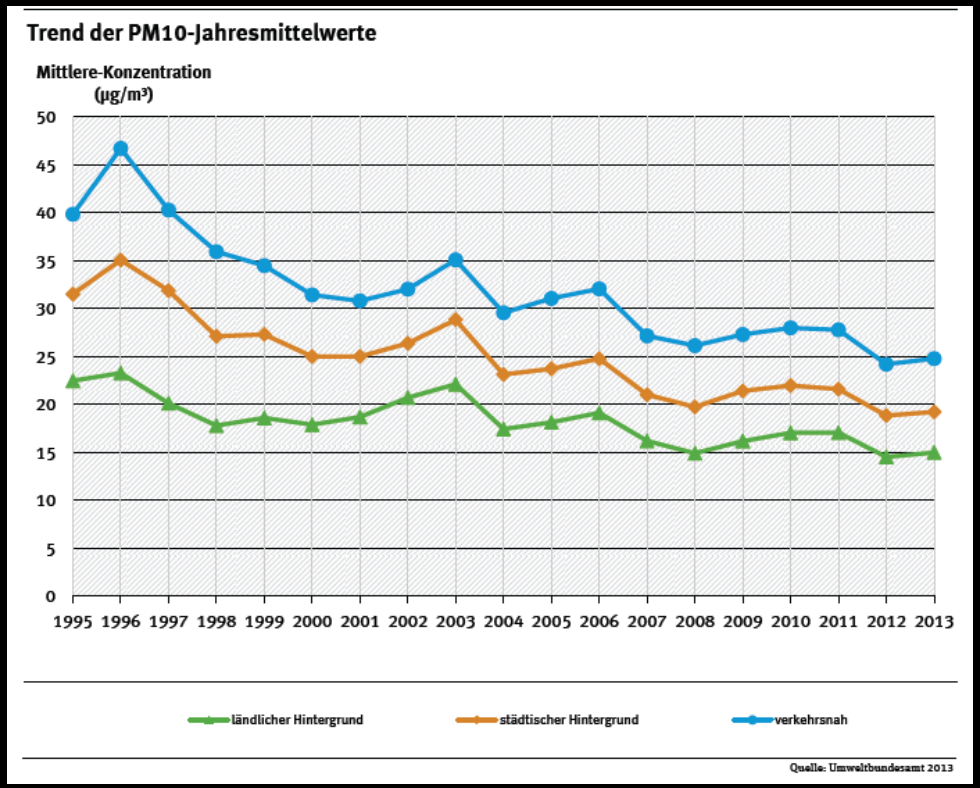


Anteile der Russemissionen von Dieselmotoren im Jahr 2010 bei einem Gesamtausstoss von rund 1500 Tonnen.

Datenquelle: BAFU, Abteilung LUNIS

PM_{10} limit values – CH/D





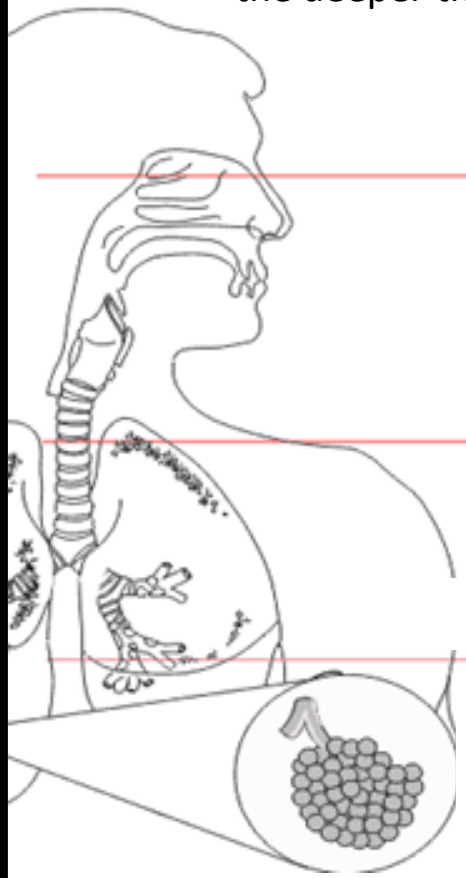
Luftqualitätsstandards für $PM_{2,5}$

Zielwert: 25 µg/m³ als Jahresmittelwert, sollte ab 1.1.2010 erreicht sein
Grenzwert Stufe 1: 25 µg/m³ als Jahresmittelwert, der ab 1.1.2015 einzuhalten ist
Grenzwert Stufe 2: 20 µg/m³ Jahresmittelwert, der ab 1.1.2020 einzuhalten ist

www.umweltbundesamt.de

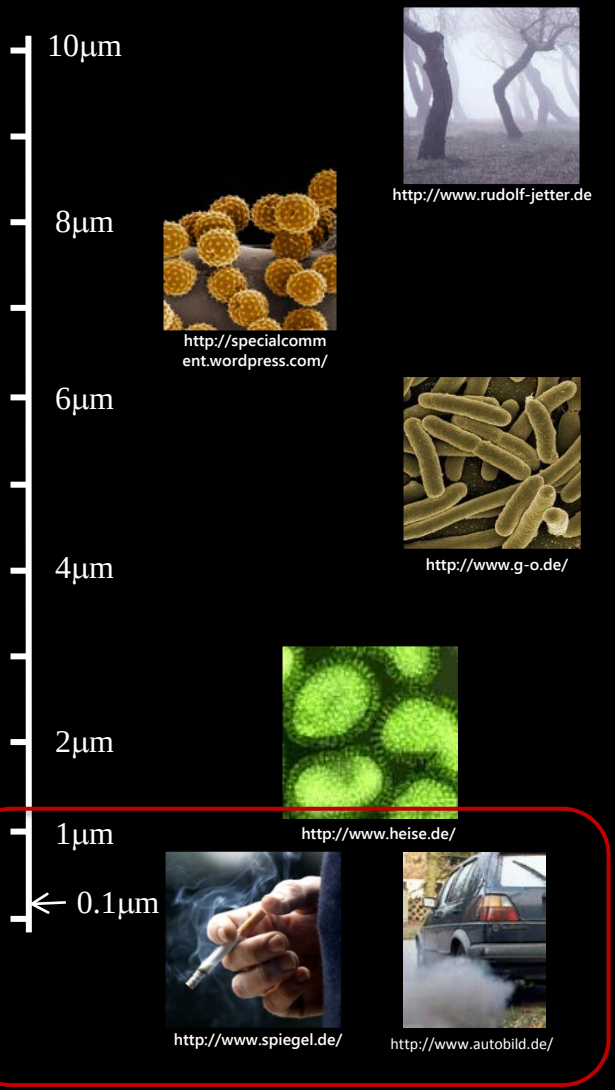
Interaction of PM_{10} with the lung

The smaller the particles and the less water-soluble a gas is the deeper they penetrate into the lung

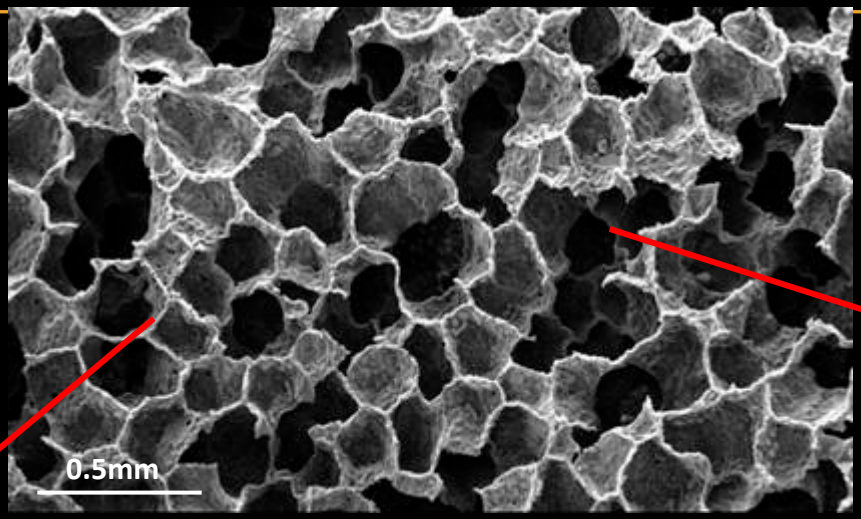


Location	
Naso-pharynx	Particles < 30 µm
Trachea	
Bronchi	Particles < 10 µm
	SO_2, NO_2, Ozon
Bronchioli	
Alveoli	Particles < 2-3 µm
	NO_2, Ozon

Dr. P. Straehl, BAFU, Abt. Luft-reinhaltung und NIS and Dr. Regula Rapp, Swiss TPH



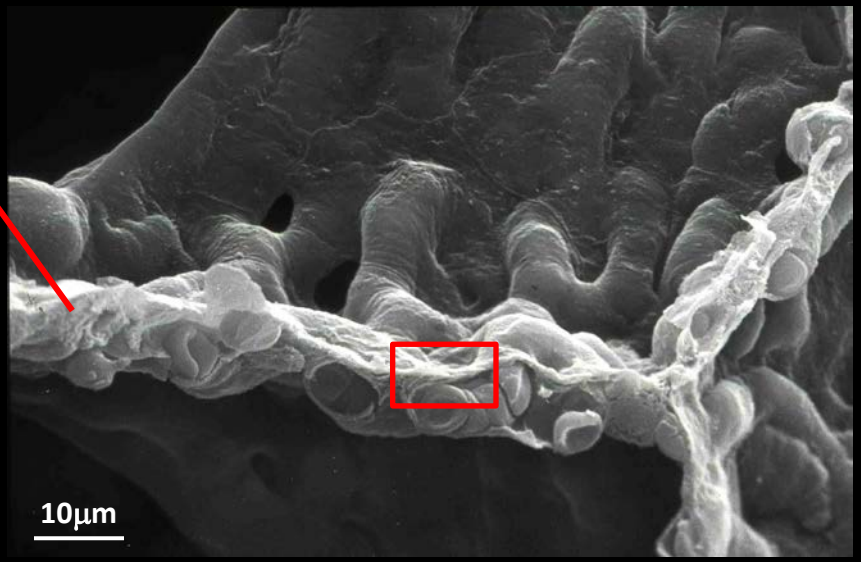
The human lung- Microscopic



P. Gehr, Uni Bern

Alveoli

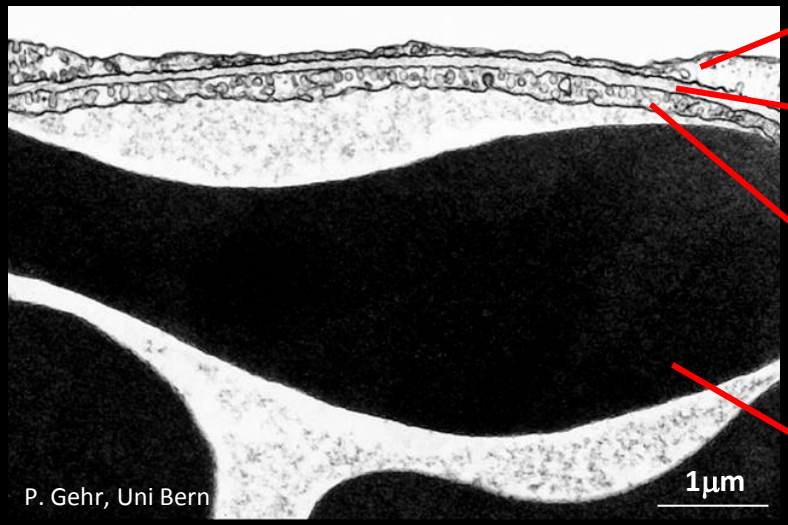
Interalveolar Septa



E. Weibel, Uni Bern

The human lung- Air-blood barrier

Alveolus (Air)



Capillary

Epithelium (Typ I cell)

Basal lamina

Endothelium

Red blood cell

500 millions alveoli
Diameter 1/4mm
Gas exchange reion 80-90%
Surface 140 m^2

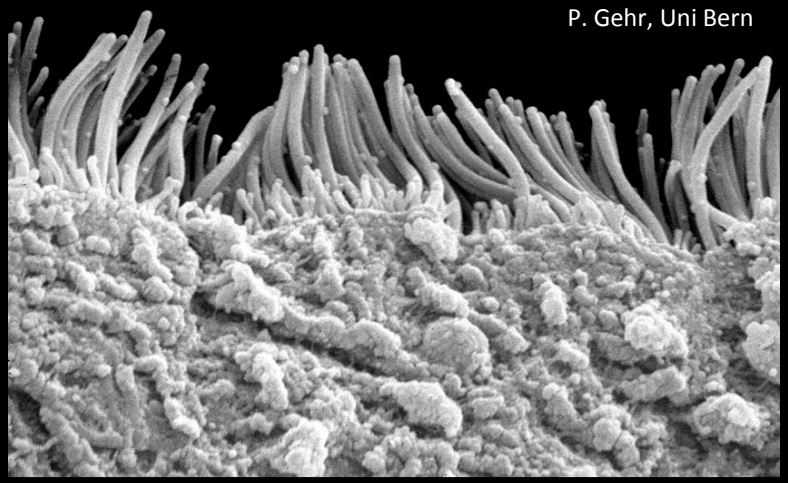
Capillary volume 210cm^3

Air-blood barrier <1µm

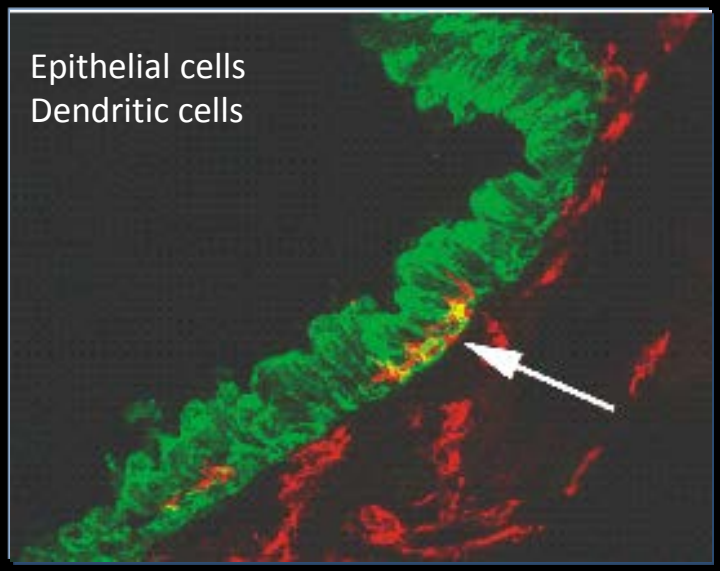
Gehr et al. Resp Physiol (1978)
Ochs and Weibel. Fishman´s Pulmonary Diseases and Disorders, New York (2008)

The human lung- Clearing mechanisms

Conducting airways
Mucociliar transport
(fast, minutes)



Lung parenchyma
The immune system
(Slow, days-weeks)



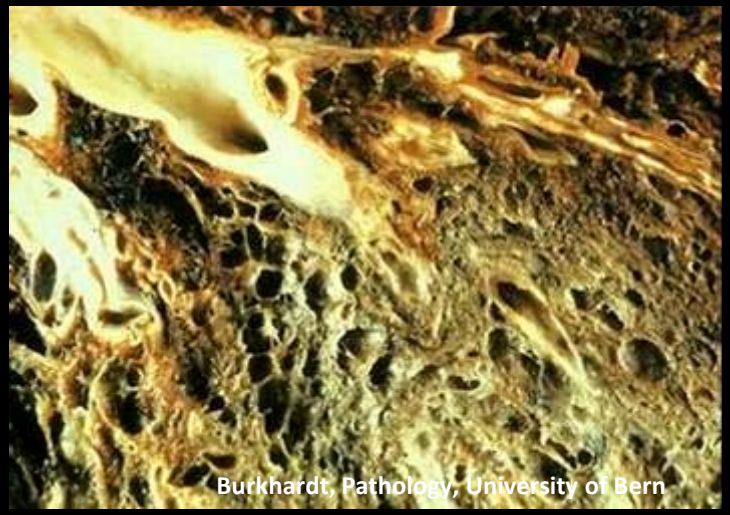
Photos courtesy P. Stumbles / S. Napoli (Blank et al. Exp Rev Resp Med 2008)

Macrophages:
Professional phagocytotic cells

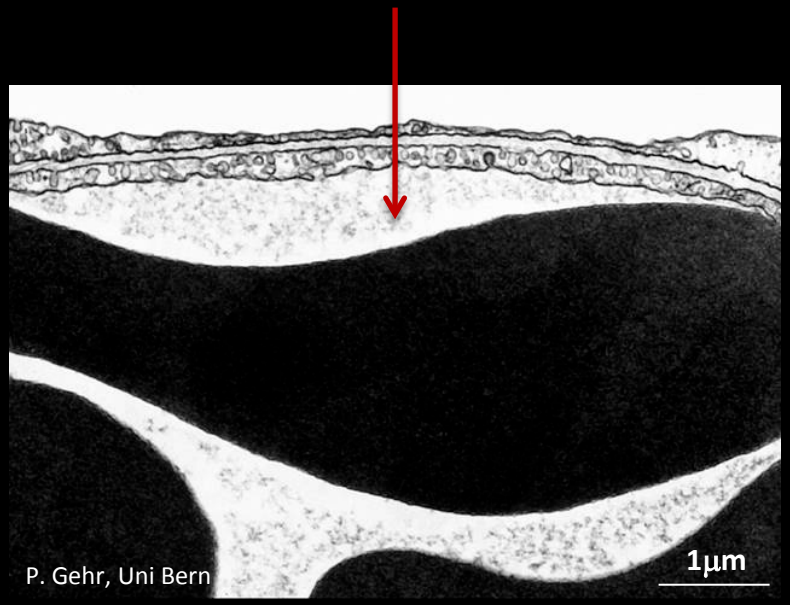
Dendritic cells:
Professional antigen-presenting cells

The human lung- Clearing mechanisms

Healthy and diseased lung



Translocation of nanoparticles

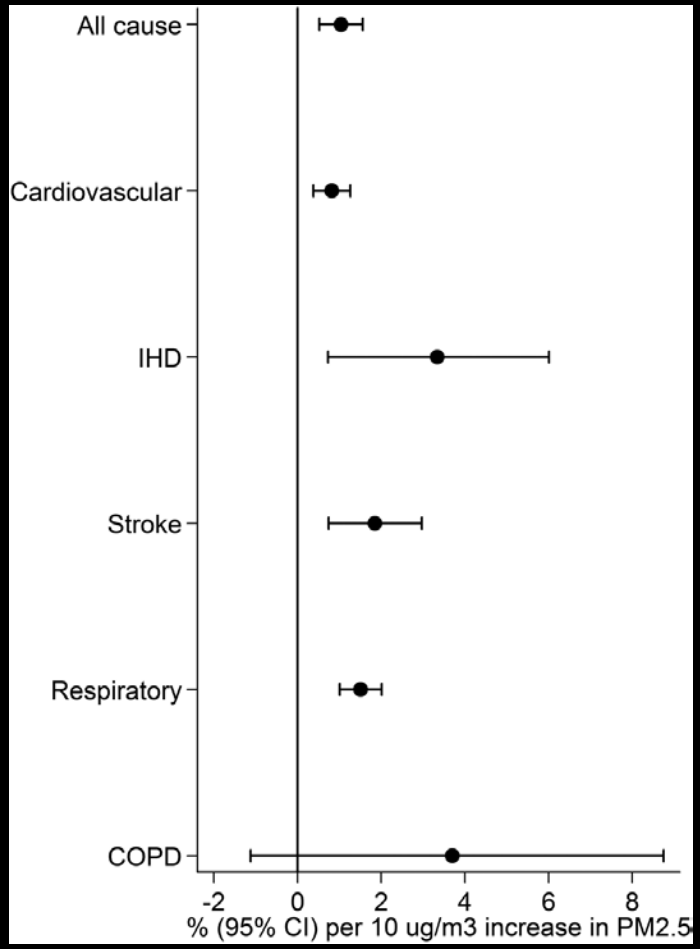


Nanoparticle can translocate across the lung tissue barrier => secondary organs via the blood circulation

⇒ Liver
⇒ Kidney
⇒ Heart
⇒ Brain
⇒ Fetus

Mills et al. 2009 Nat Clin Pract Cardiovasc Med
Muehlfeld et al. 2008 Swiss Med Wkly
Semmler-Behnke et al. 2007 Environ Health Perspect
Peters et al. 2006 Part Fibre Toxicol
Ryman-Rasmussen et al. 2009 Nat Nanotech
Wick et al. 2010 Environ Health Perspect

$PM_{2.5}$- daily mortality and hospital admissions

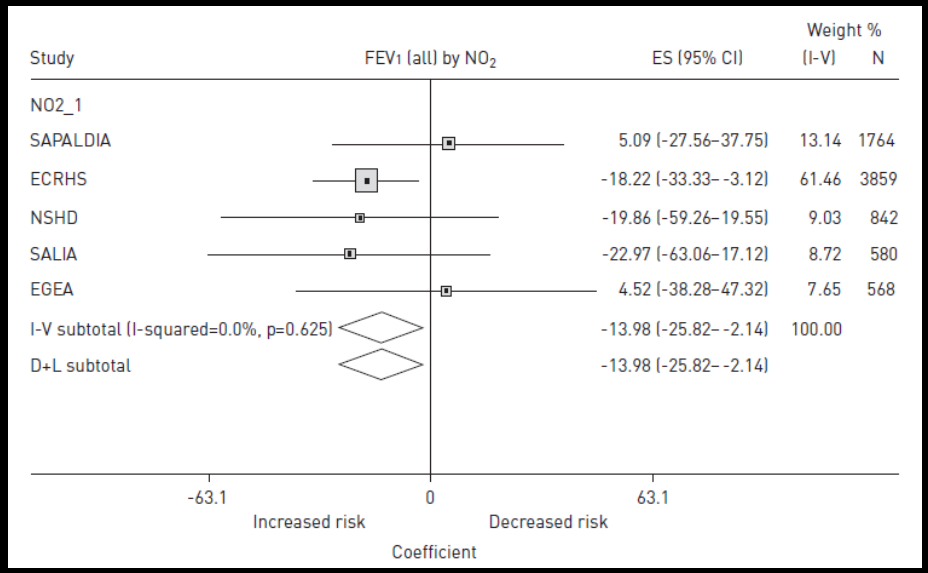


A systematic review and meta-analysis of 110 peer-reviewed time series studies

=> Adverse health effects of short-term exposure to $PM_{2.5}$: 10 $\mu g/m^3$ increment associated with 1.04% (95% CI 0.52% to 1.56%) increase in the risk of death

Atkinson et al. Thorax 2014

European Study of Cohorts for Air Pollution Effects (ESCAPE)



Association of long-term exposure to ambient air pollution with lung function in adult participants from five cohorts

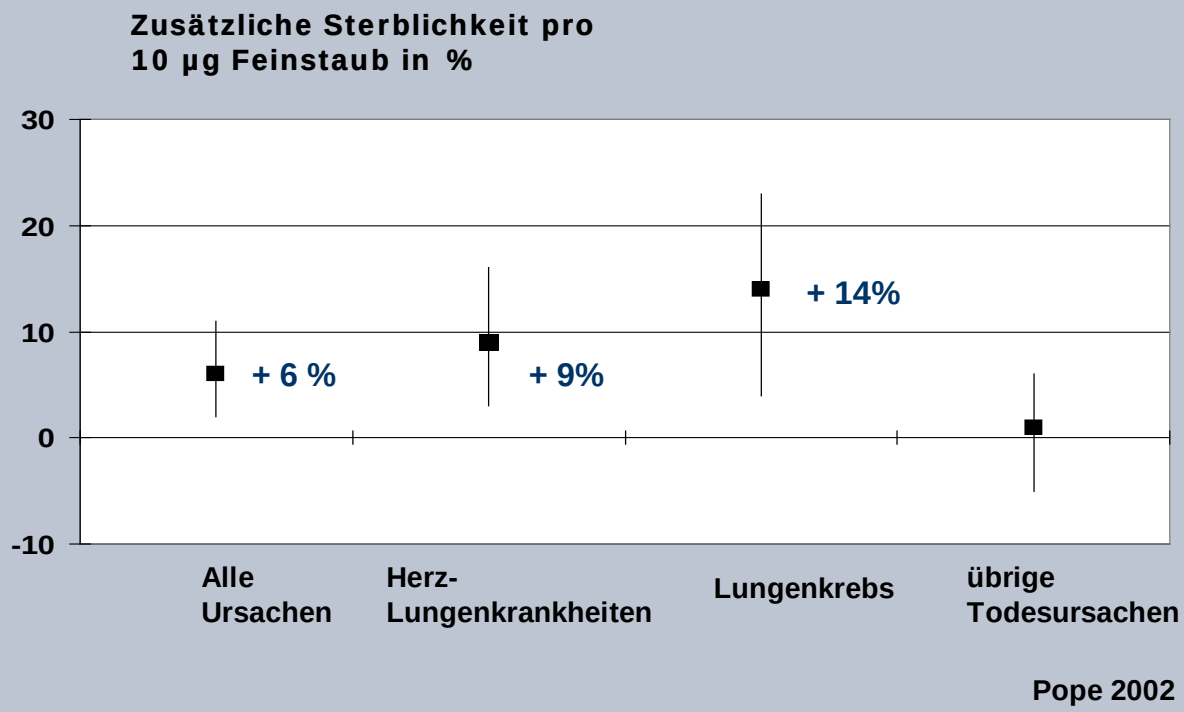
⇒ 10 µg/m^3 increase in NO_2 exposure was associated with lower levels of FEV1

⇒ increase of 10 µg/m^3 in PM_{10} was associated with a lower level of FEV1

Adam et al. EJR 2014 (in press)

Chronic effects of PM_{10} exposure

bei 500‘000 Personen der amerikanischen Krebsstudie, 1982-2002



Pope et al., J. Am. Med. Assoc., 2002

Chronic effects of PM_{10} exposure

International Agency for Research on Cancer

World Health Organization

PRESS RELEASE
N° 213

12 June 2012

IARC: DIESEL ENGINE EXHAUST CARCINOGENIC

Lyon, France, June 12, 2012 -- After a week-long meeting of international experts, the International Agency for Research on Cancer (IARC), which is part of the World Health Organization (WHO), today classified diesel engine exhaust as **carcinogenic to humans (Group 1)**, based on sufficient evidence that exposure is associated with an increased risk for lung cancer.

Models to study risk assessment of PM_{10}

Epidemiology:

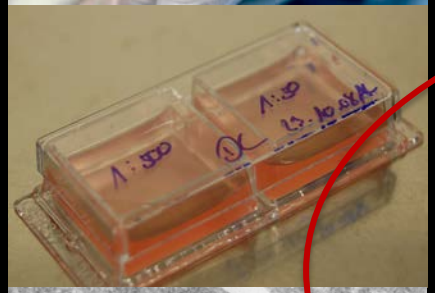
The study of the distribution and determinants of disease frequency in human populations and the application of this study to control health problems

http://publichealth.jbpub.com/ Overview of epidemiologic study design

Animal experiments:

in vivo testing

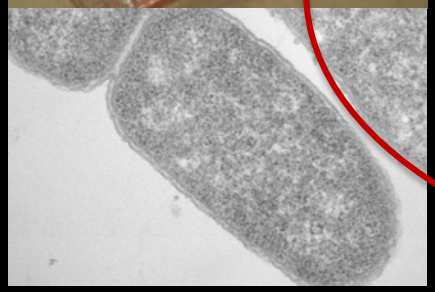
http://www.aboutanimaltesting.co.uk/using-animals-testing-pros-versus-cons.html



Cell culture experiments:

in vitro testing

Rothen-Rutishauser et al. Exp Opin Drug Metabol Toxicol 2008



The Ames test:

in vitro testing with bacteria

Ames et al. Proc Nat Acad Sci USA 1973

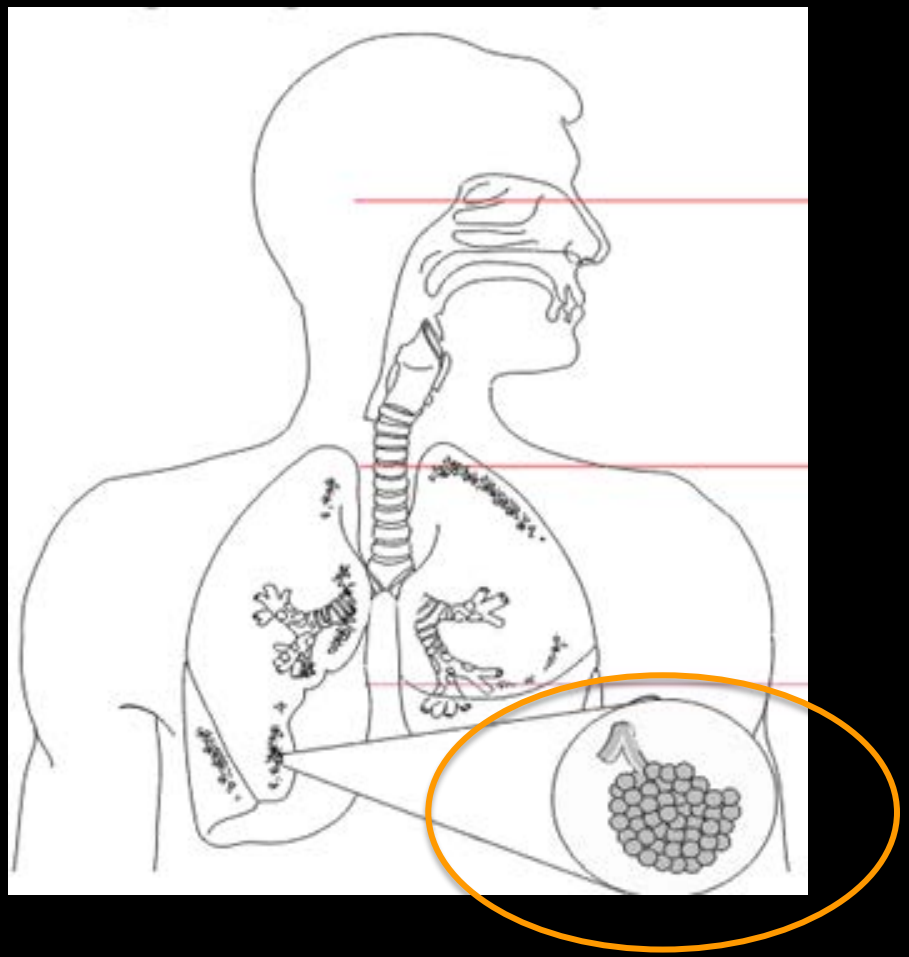
Realistic risk assessment systems

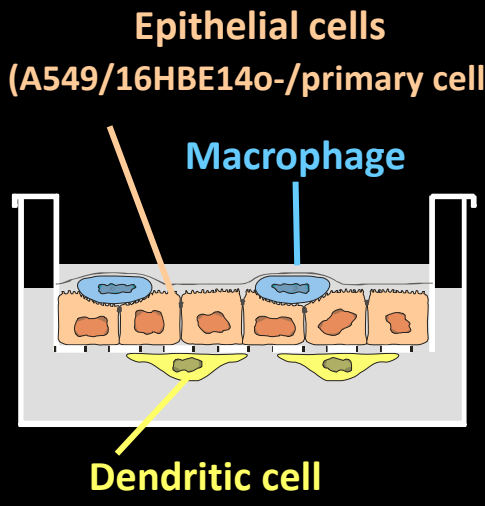
Definition of realistic:
”resembling or simulating real life”
dictionary.com

www.sott.net

Cell responses

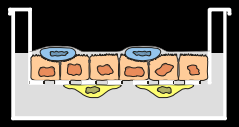
3D human epithelial airway barrier model

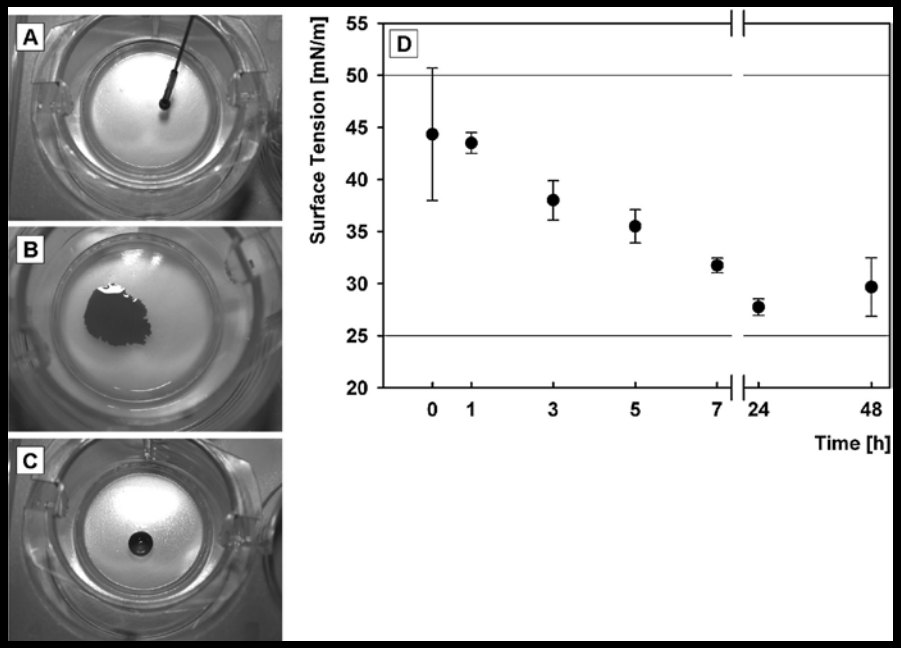


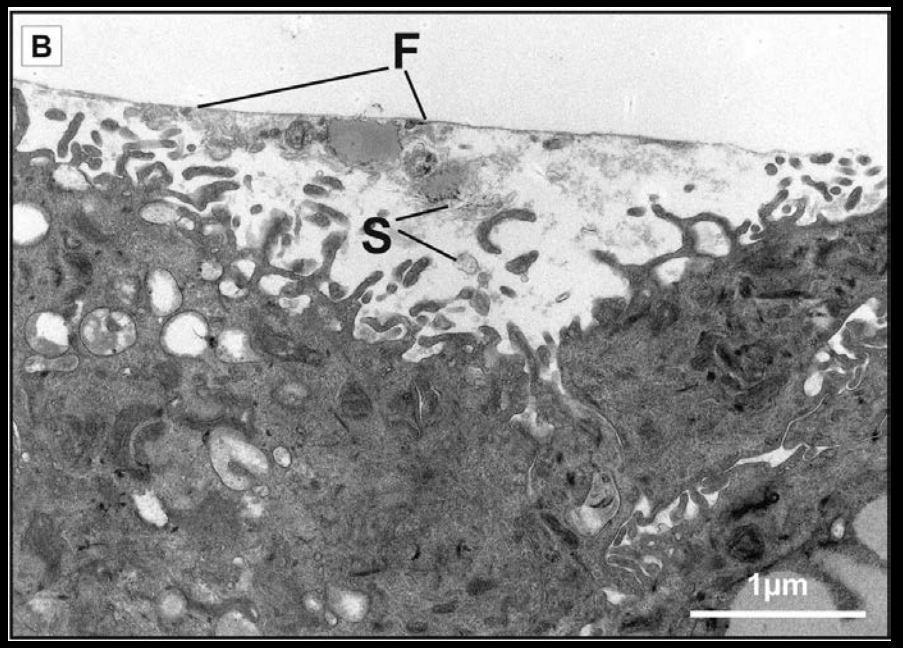


Rothen-Rutishauser et al. Am J Respir Cell Mol Biol 2005;
Blank et al. Am J Respir Cell Mol Biol 2007
Rothen-Rutishauser et al. Review, Exp Opin Drug Metab Toxicol 2008
Lehmann et al. Eur J Pharm Biopharm 2010

Air-liquid cultures

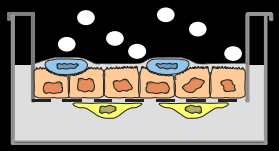


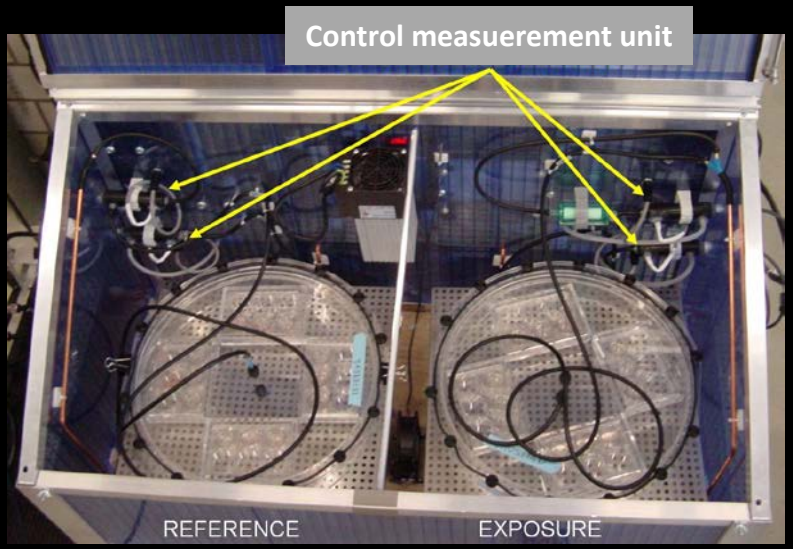




Blank et al. J Aerosol Med 2006; Blank et al. Am J Respir Cell Mol Biol 2007

Exhaust emission exposure system

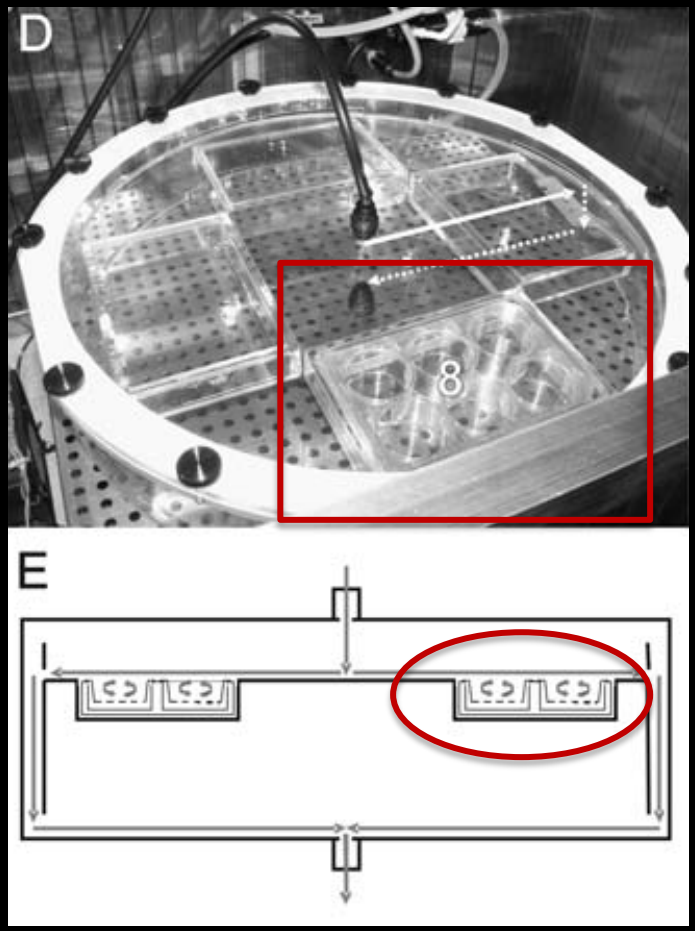




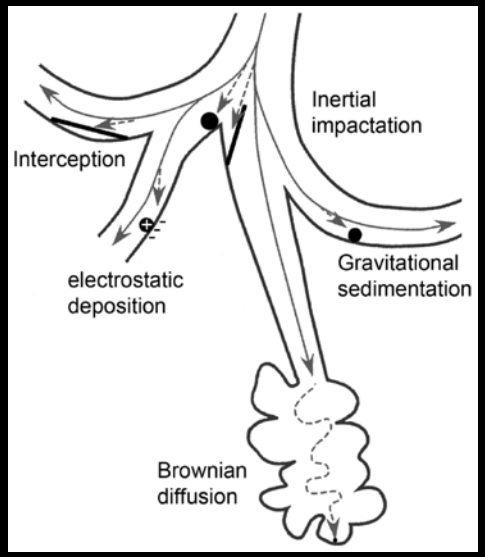
Müller et al. Environ Sci Technol 2009

Müller et al. Tox Env Chem 2012

Flow pattern in the exposure chambers



Müller et al. Environ Sci Technol 2009
Supporting Info



Fröhlichl et al. Int. J Mol Sci 2014

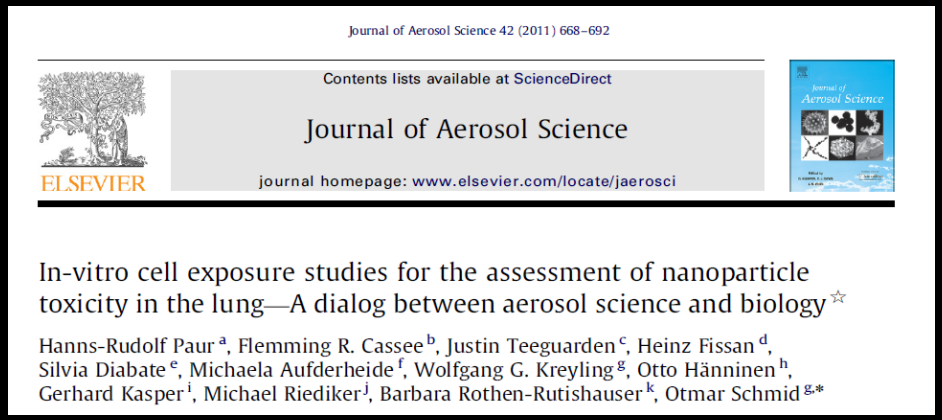
Realistic *in vitro* dose levels

Ambient urban exposure and **occupational exposure**

5 mg/m^3 (maximum level allowed by Occupational Safety and Health Administration (OSHA))

3x10^{-5}-5x10^{-3} µg per h per cm^2 of lung tissue

2-300 particles per h per (epithelial) cells



Journal of Aerosol Science 42 (2011) 668–692

Contents lists available at ScienceDirect

Journal of Aerosol Science

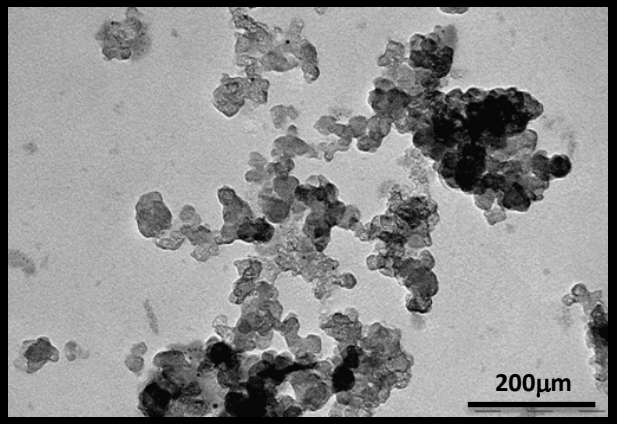
journal homepage: www.elsevier.com/locate/jaerosci

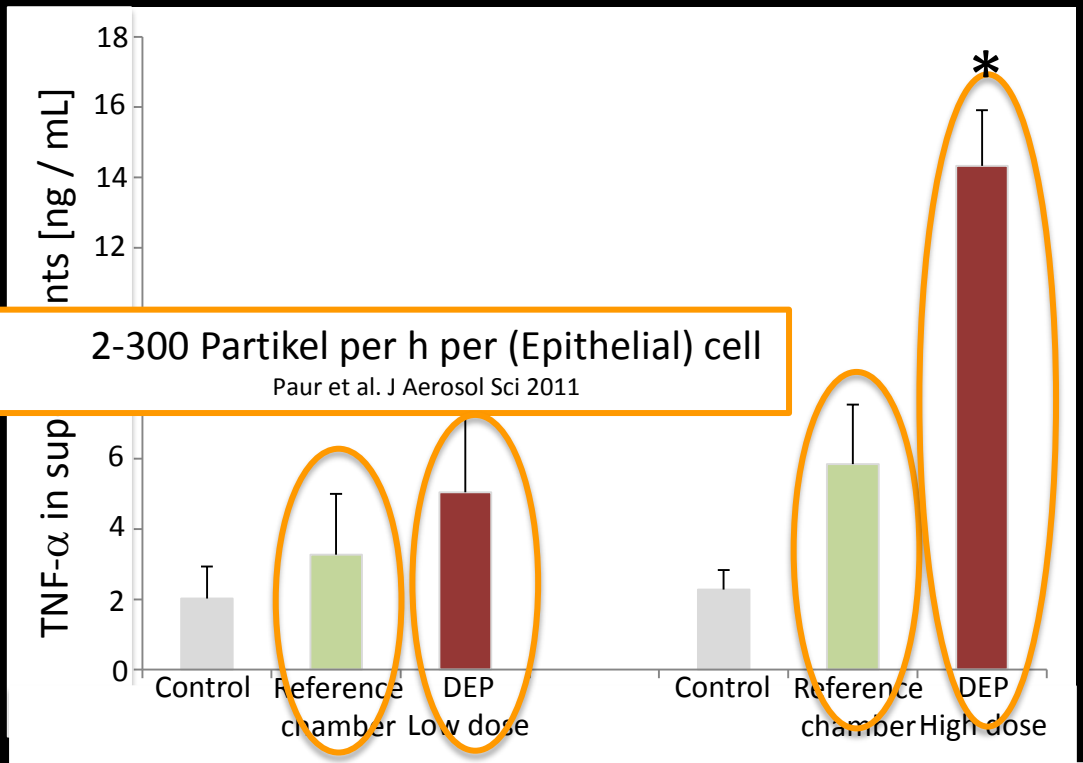
ELSEVIER

In-vitro cell exposure studies for the assessment of nanoparticle toxicity in the lung—A dialog between aerosol science and biology ☆

Hanns-Rudolf Paur [a], Flemming R. Cassee [b], Justin Teeguarden [c], Heinz Fissan [d], Silvia Diabate [e], Michaela Aufderheide [f], Wolfgang G. Kreyling [g], Otto Hänninen [h], Gerhard Kasper [i], Michael Riediker [j], Barbara Rothen-Rutishauser [k], Otmar Schmid [g,*]

Particle deposition





2-300 Partikel per h per (Epithelial) cell

Paur et al. J Aerosol Sci 2011

1.7 x 10^7 Particle / cm^2
=> 134 Particle /cell

7.48 x 10^7 Particle / cm^2
=> 587 Particle /cell

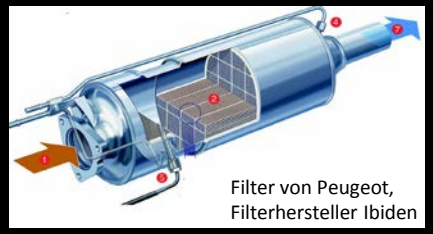
Müller et al. Environ Sci Technol 2009;
Steiner et al. Tox Letters 2012

Effect of non-catalyzed diesel particle filter

Müller et al. Environ Sci Technol 2009
Steiner et al. Tox Letters 2012

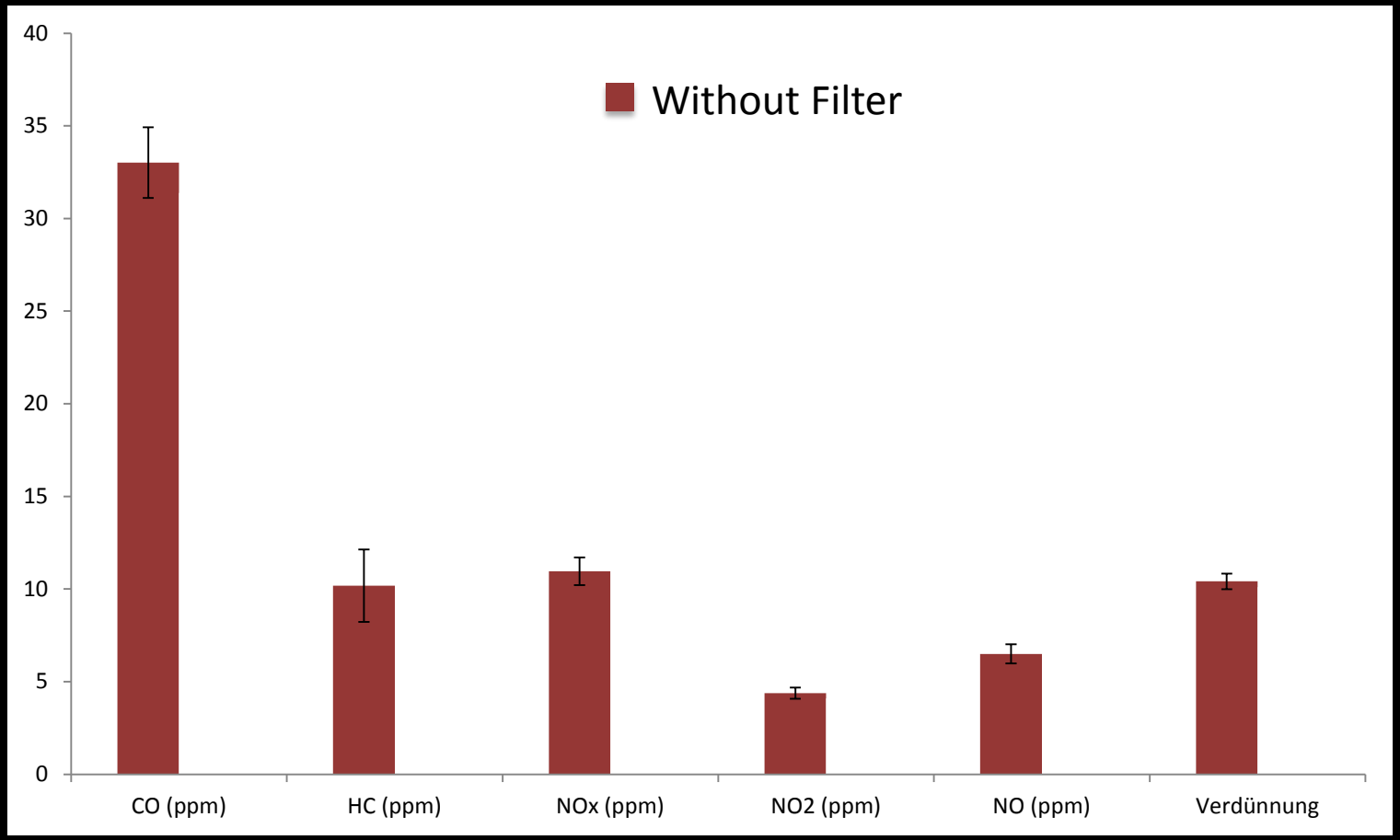
- Opel Astra X20DTL, 35 km/h
- Fuel: low sulfur diesel (>10mg/kg, Greenergy SA)
- Lube oil (V10.237, Motorex)
- Exhaust dilution 1:10

=> Without filter
=> With a silicon carbide Diesel particle filter



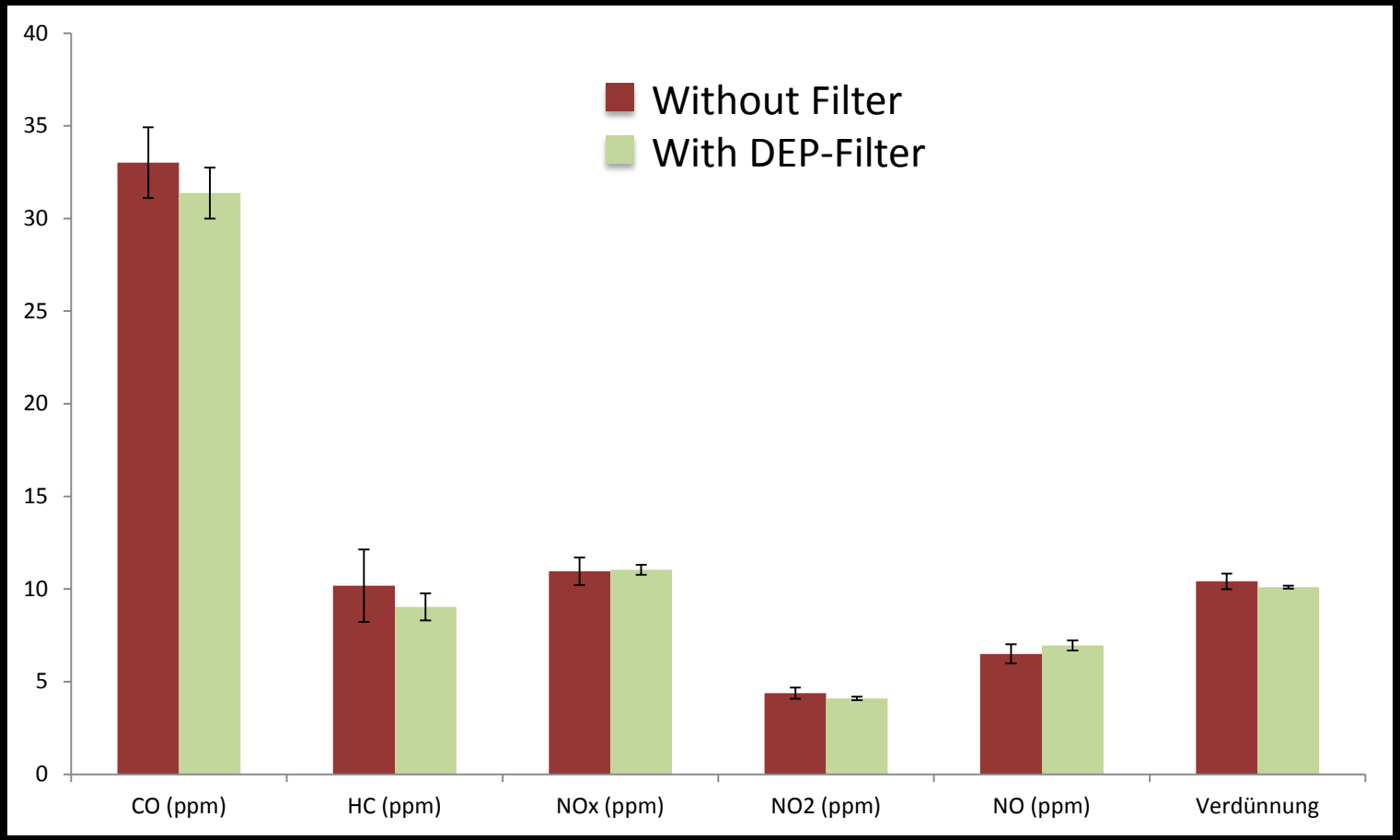
Filter von Peugeot, Filterhersteller Ibiden

Exhaust characterisation



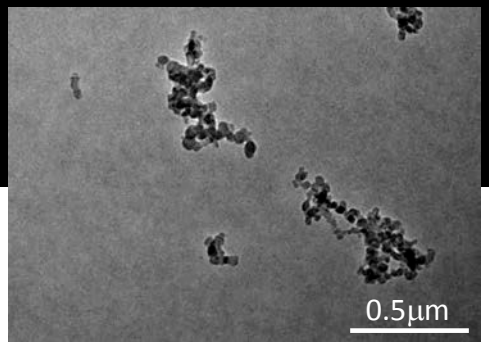
Steiner et al. 2013 Atmos Environ

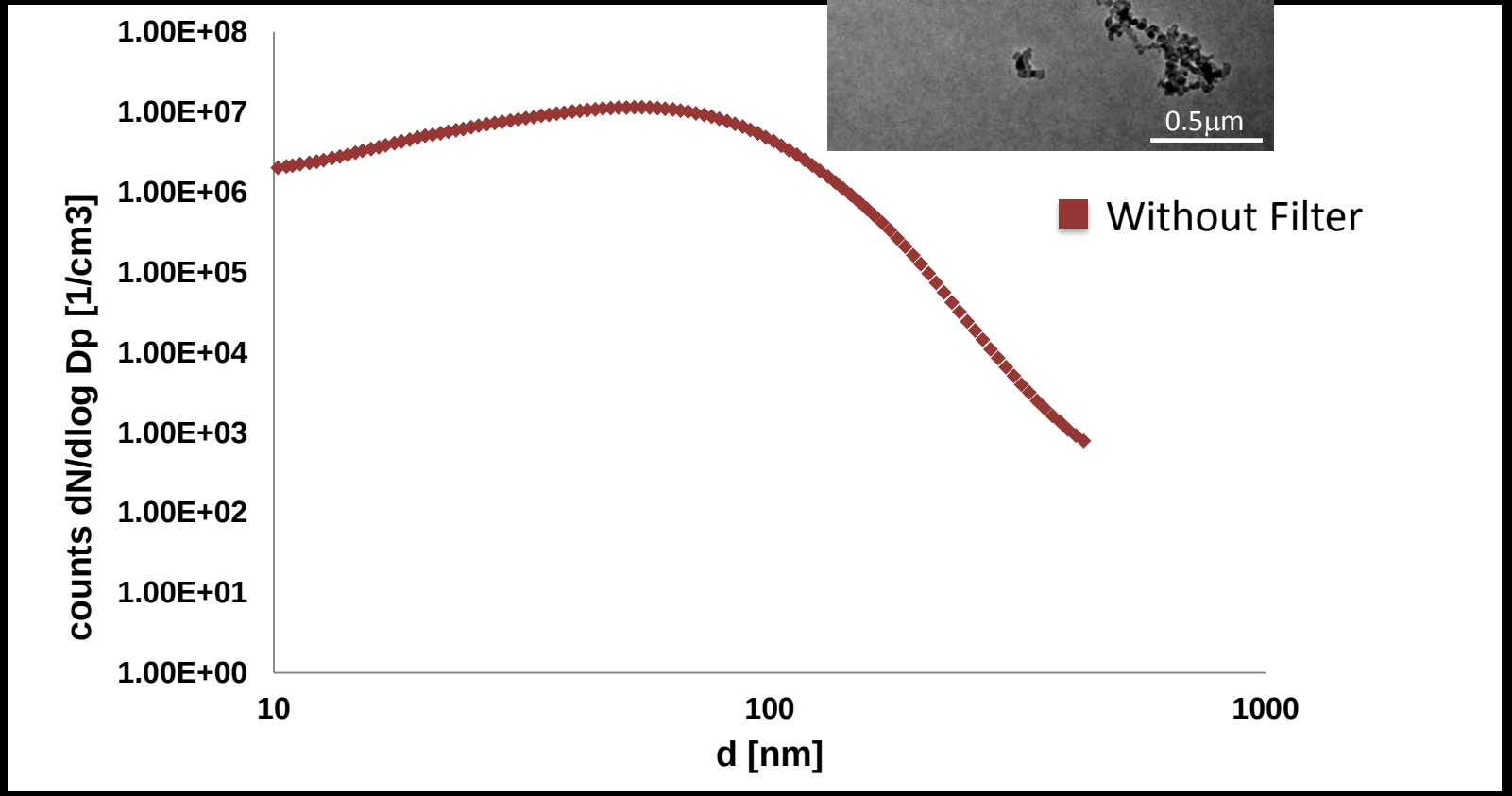
Exhaust characterisation



Steiner et al. 2013 Atmos Environ

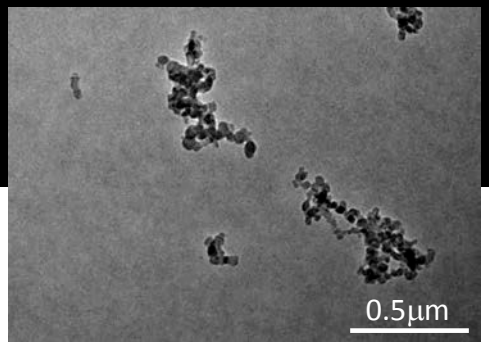
Particle size distribution

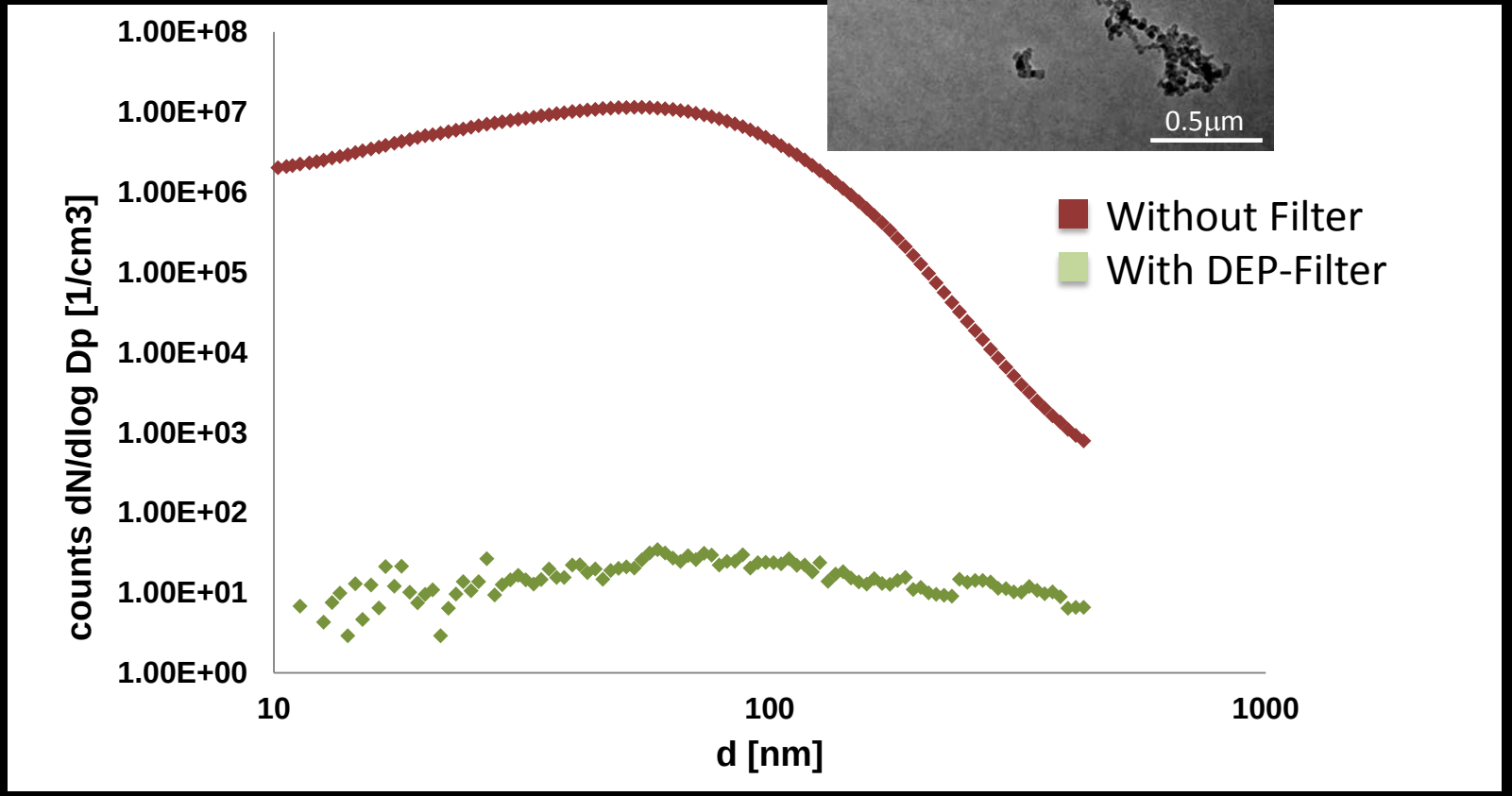




Steiner et al. 2013 Atmos Environ

Particle size distribution



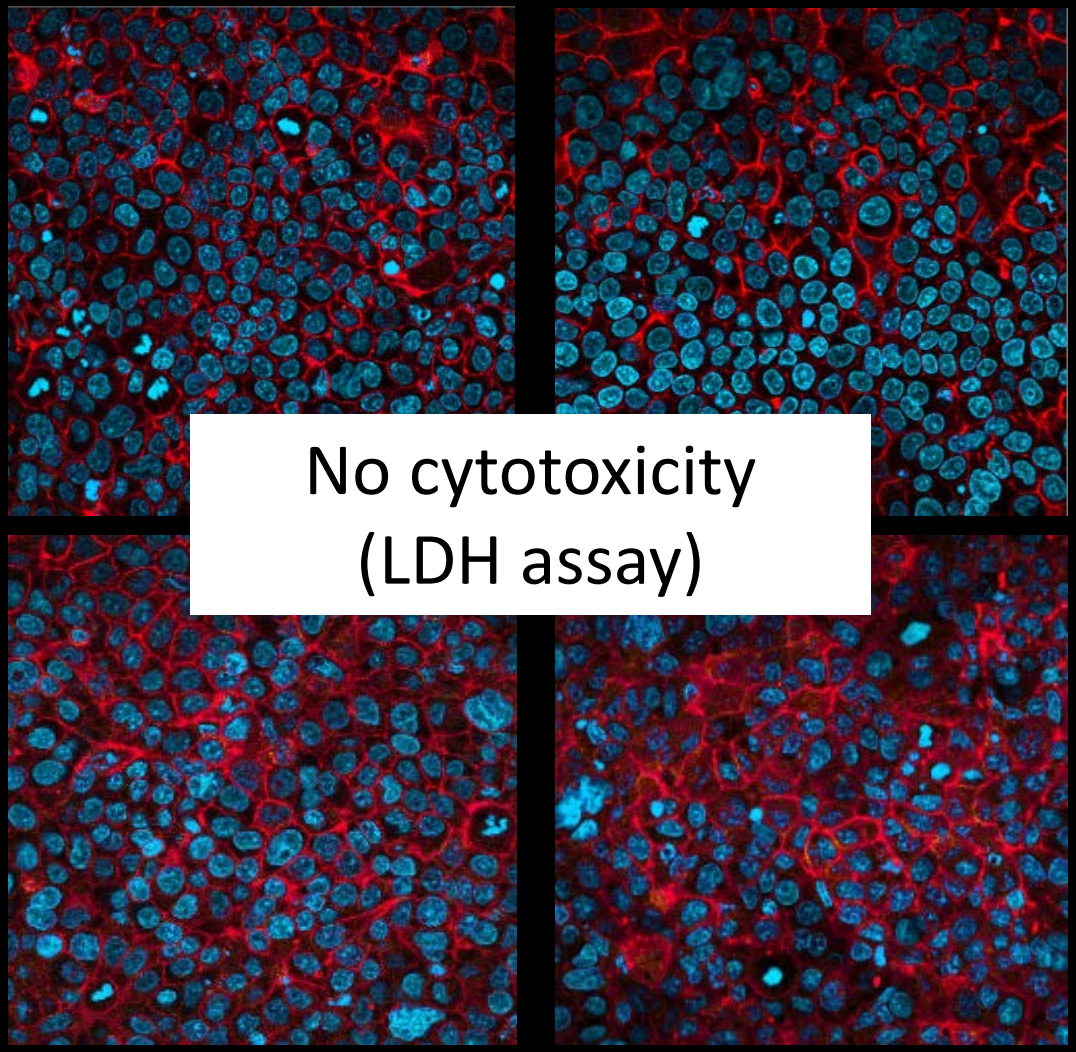


Steiner et al. 2013 Atmos Environ

Cell morphology and cytotoxicity

Control in the incubator

Clean air 6h



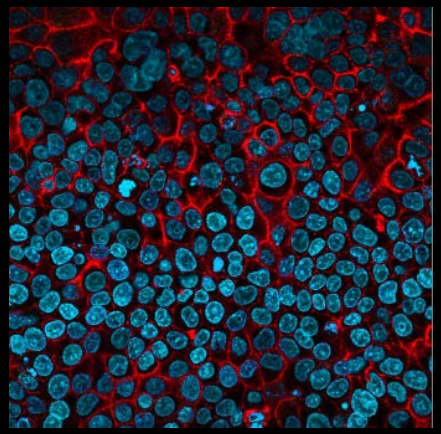
No cytotoxicity (LDH assay)

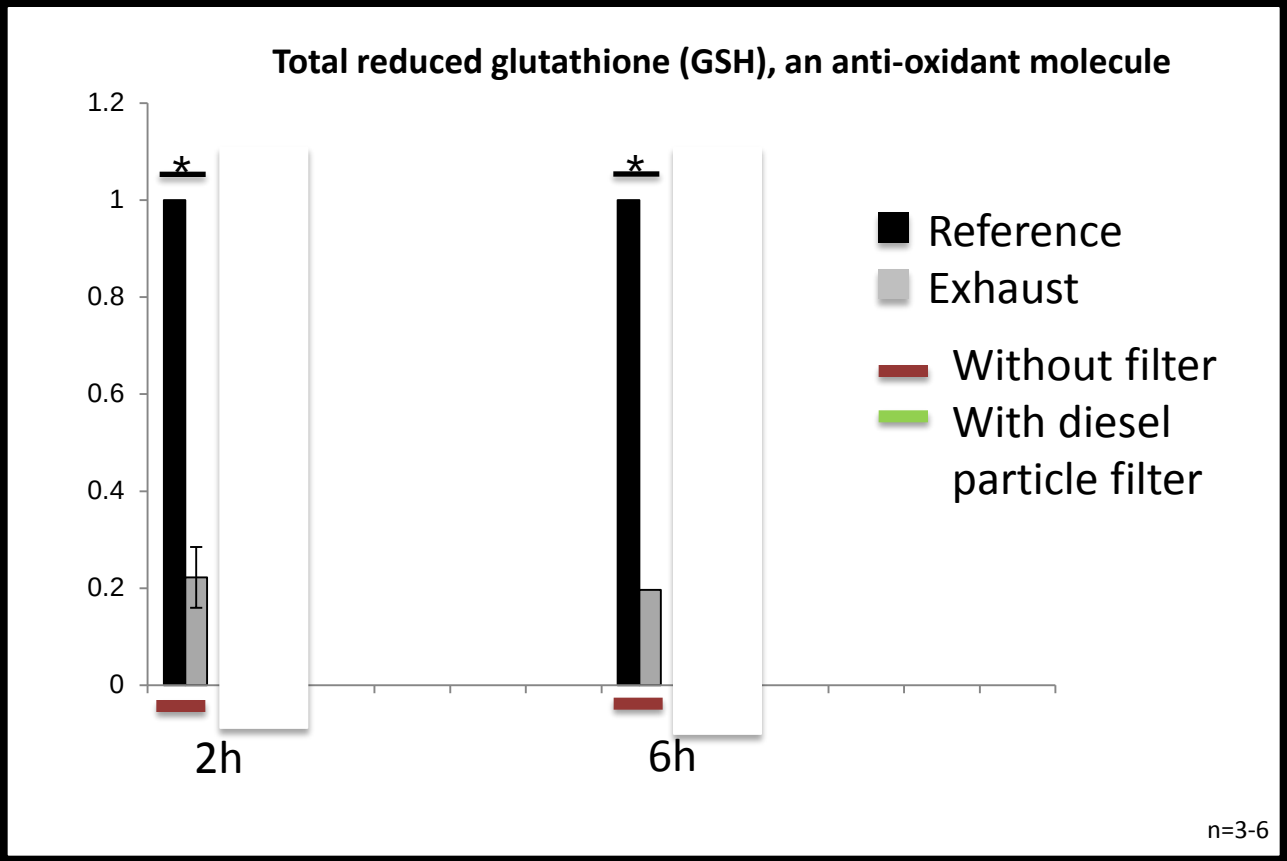
Without filter 6h

With diesel particle filter 6h

Steiner et al. 2013 Atmos Environ

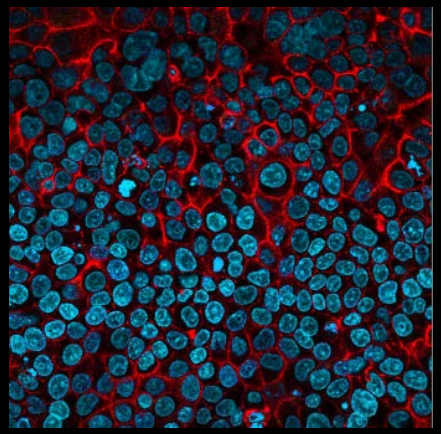
Antioxidative response

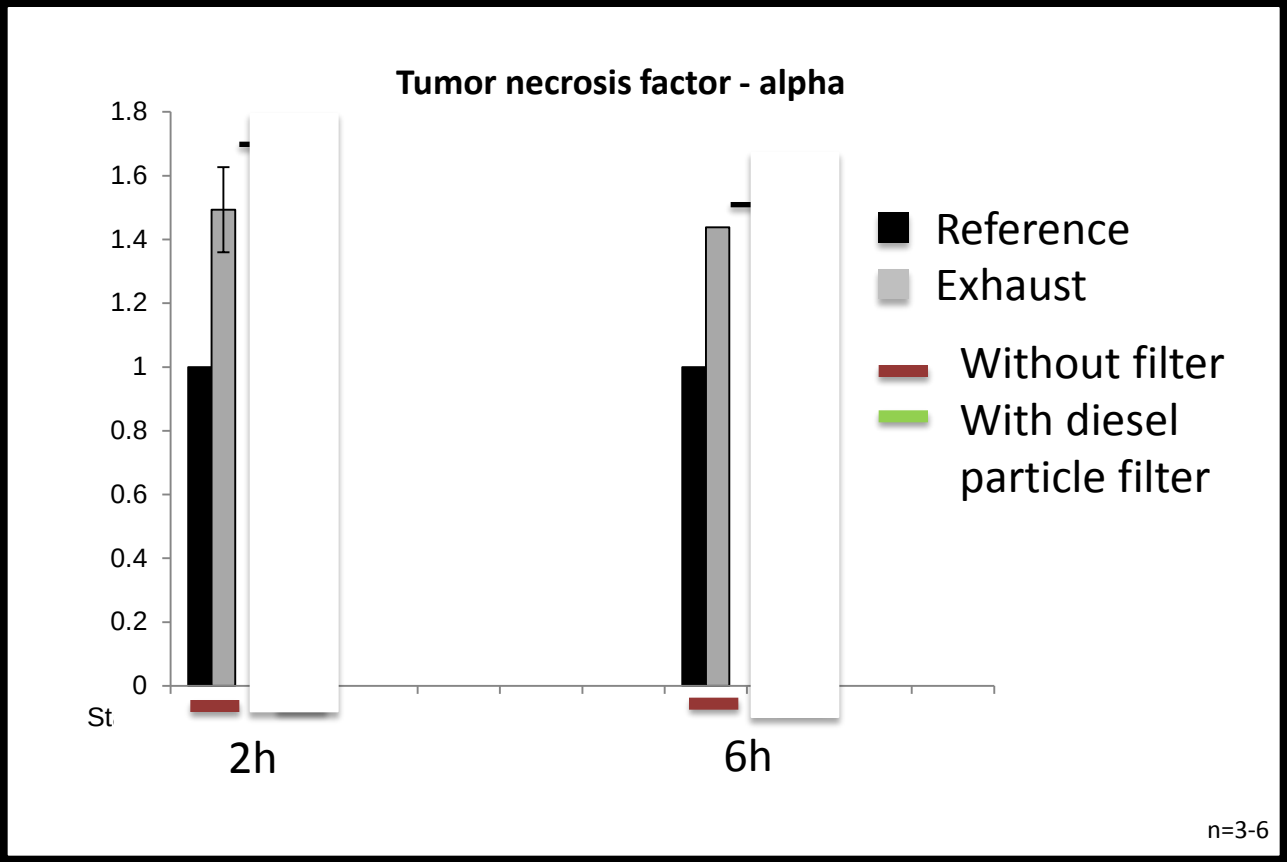




Steiner et al. 2013 Atmos Environ

Pro-inflammatory response





Steiner et al. 2013 Atmos Environ

Models to study risk assessment of PM_{10}

Epidemiology:

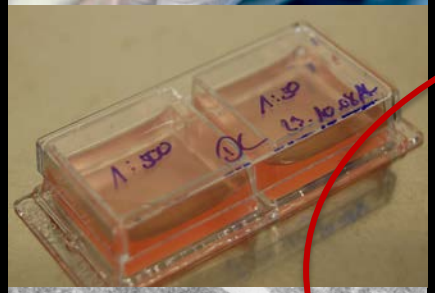
The study of the distribution and determinants of disease frequency in human populations and the application of this study to control health problems

http://publichealth.jbpub.com/ Overview of epidemiologic study design

Animal experiments:

in vivo testing

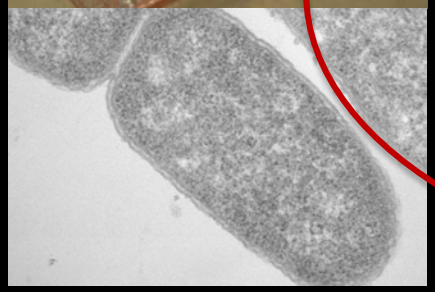
http://www.aboutanimaltesting.co.uk/using-animals-testing-pros-versus-cons.html



Cell culture experiments:

in vitro testing

Rothen-Rutishauser et al. Exp Opin Drug Metabol Toxicol 2008

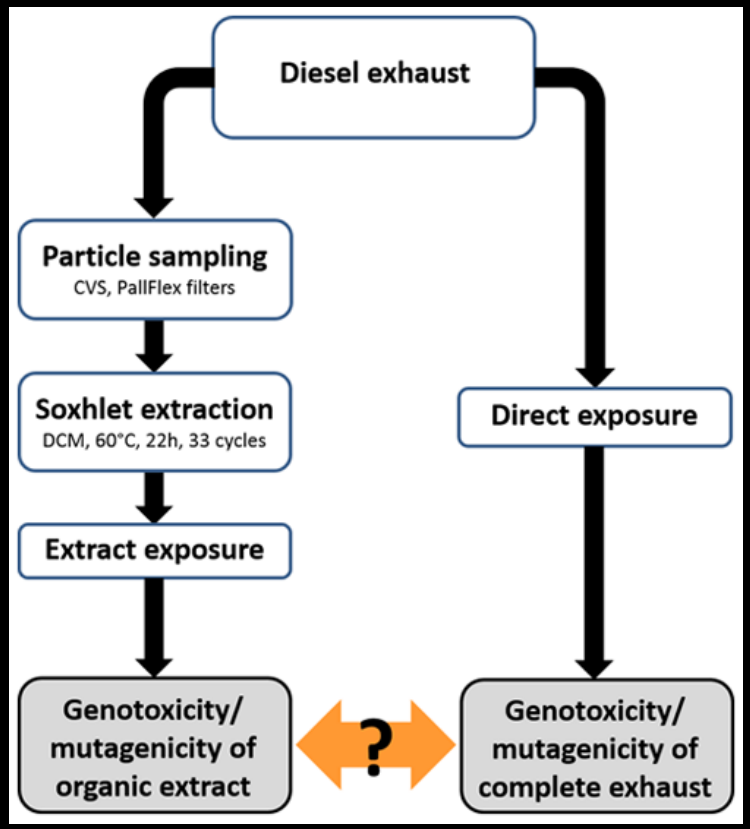


The Ames test:

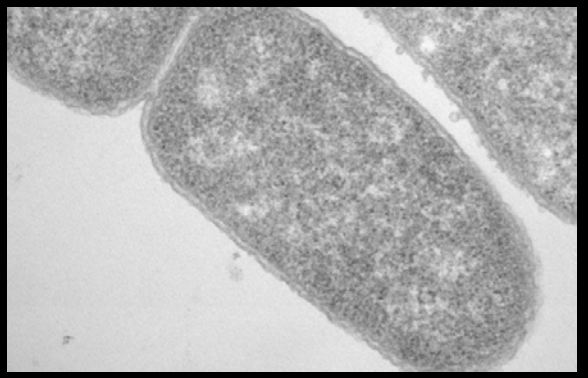
in vitro testing with bacteria

Ames et al. Proc Nat Acad Sci USA 1973

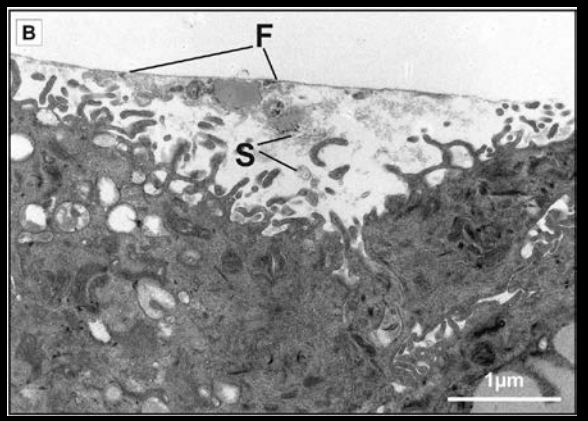
Comparison of tests



Ames test (bacteria)

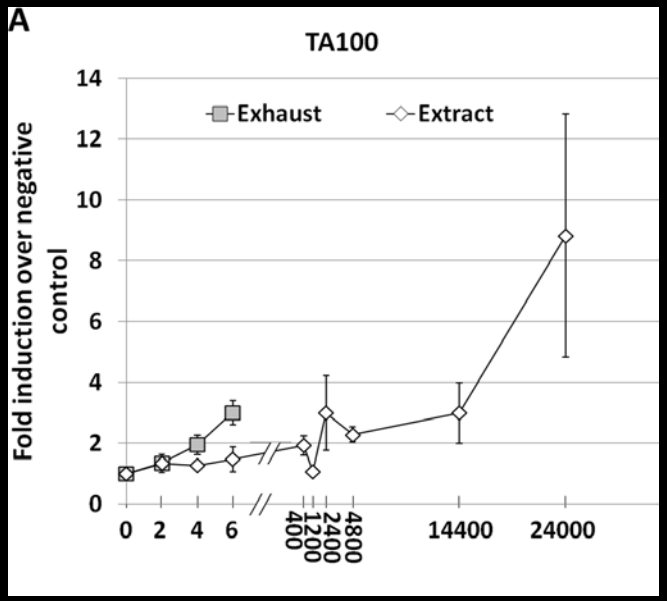


Human lung cell model



Steiner et al. Env Sci Technol 2014

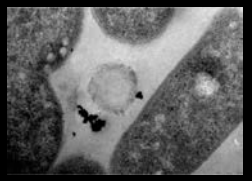
Ames test – human lung cells



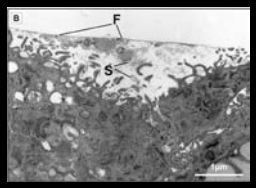
Clift et al. Nanotoxicology 2013; Steiner et al. Env Sci Technol 2014

Conclusions

The **Ames test** in combination with **organic extracts** is **not a reliable method** to assess the risk of exhaust emission



Realistic cell culture models combined with **a controlled deposition of nanomaterials** at **the air-liquid interface** of lung cells



Stable and reproducible system to study the effect of exhaust emissions *in vitro* under **realistic conditions**

=> Differentation between gaseous compounds and particles

Conclusions

	Without filter (Gas and particles)	With filter (Gas)
Cytotoxicity	No	No
Oxidative stress (GSH and *HMOX1*)	↑	↑
Pro-inflammatory reactions (TNFa, IL8)	↑	No

⇒ **Diesel particle filter** significantly **reduces (pro-)inflammation** *in vitro*

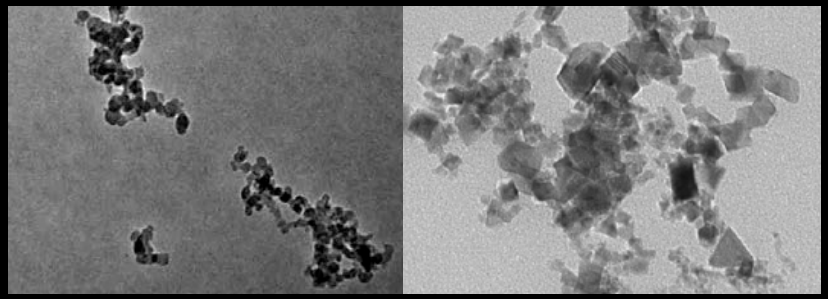
⇒ However, the **gaseous components** also need to be reduced by filter technology

Outlook: Reliable and versatile system

Two-stroke scooter exhaust

Cytotoxicity and pro-inflammatory reactions

Müller et al. 2010 Environ Sci Technol



DEP and CeO2 co-exposures

CeO2 reduces oxidative stress effects observed upon DEP exposure

Steiner et al. 2012 Tox Letters

http://ec.europa.eu/energy/renewables/biofuels/

B0, B20, B100

Reduced cell response only for B20

Steiner et al. 2013 Atmos Environ

Acknowledgments

The BioNano-Group

Sandro Steiner

Loretta Müller

EngToxDi Team
Jan Czerwinski
Pierre Comte
Andreas Mayer
Norbert Heeb

Institute of Anatomy, University Bern
Peter Gehr
Former team members

Technion – Israel Institute of Technology Haifa - Israel
Josue Sznitman
Jana Tenenbaum-Katan

BIO-INSPIRED MATERIALS
NATIONAL CENTER OF COMPETENCE IN RESEARCH

EV|UP ERDÖL-VEREINIGUNG UNION PÉTROLIÈRE

FNSNF
SCHWEIZERISCHER NATIONALFONDS ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

VSS lubes

LUNGENLIGA Leben heisst atmen

NOVARTIS

Adolphe Merkle Foundation

Bundesverwaltung admin.ch
Departement für Umwelt, Verkehr, Energie und Kommunikation
Bundesamt für Umwelt BAFU