

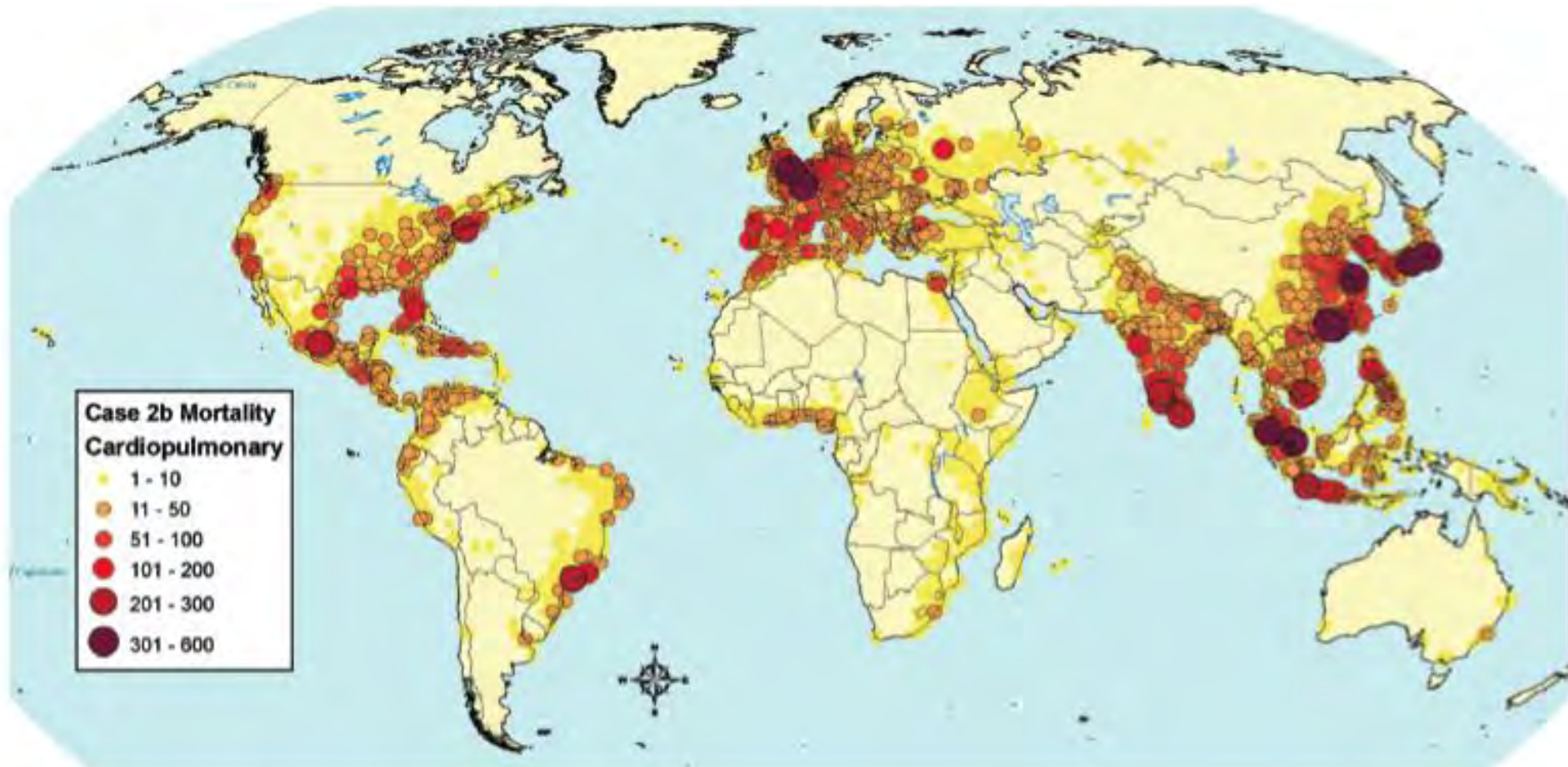
# HEALTH EFFECTS OF AIR POLLUTION IN PORT CITIES

Prof. Dr. Jeroen Buters

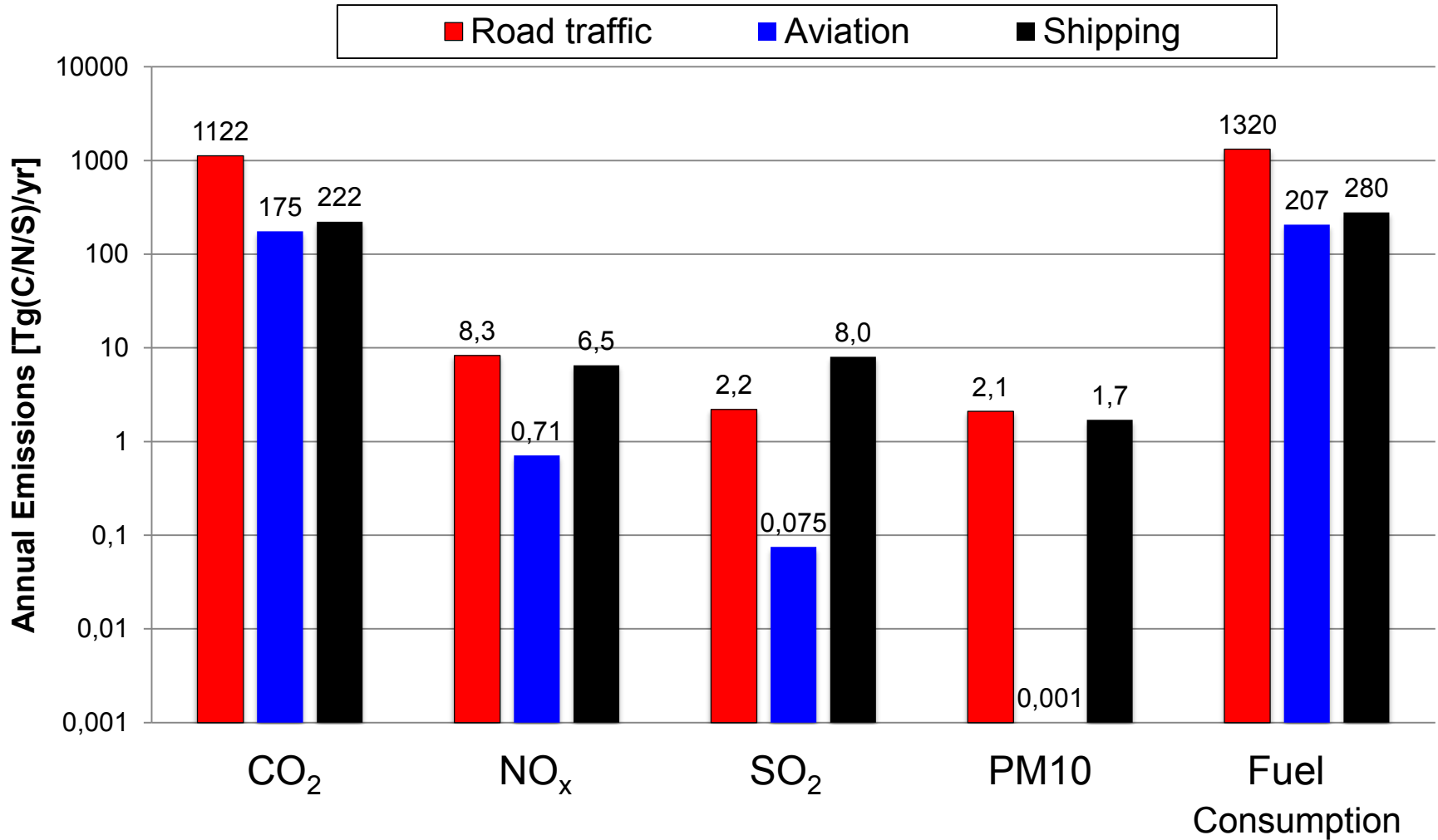
ZAUM- Center for Allergy and Environment  
Helmholtz Zentrum München/  
Technische Universität München

# Ship emissions and human health: a global problem

- Modelled cardiopulmonary mortality due to ship emissions -



# Sources of pollution



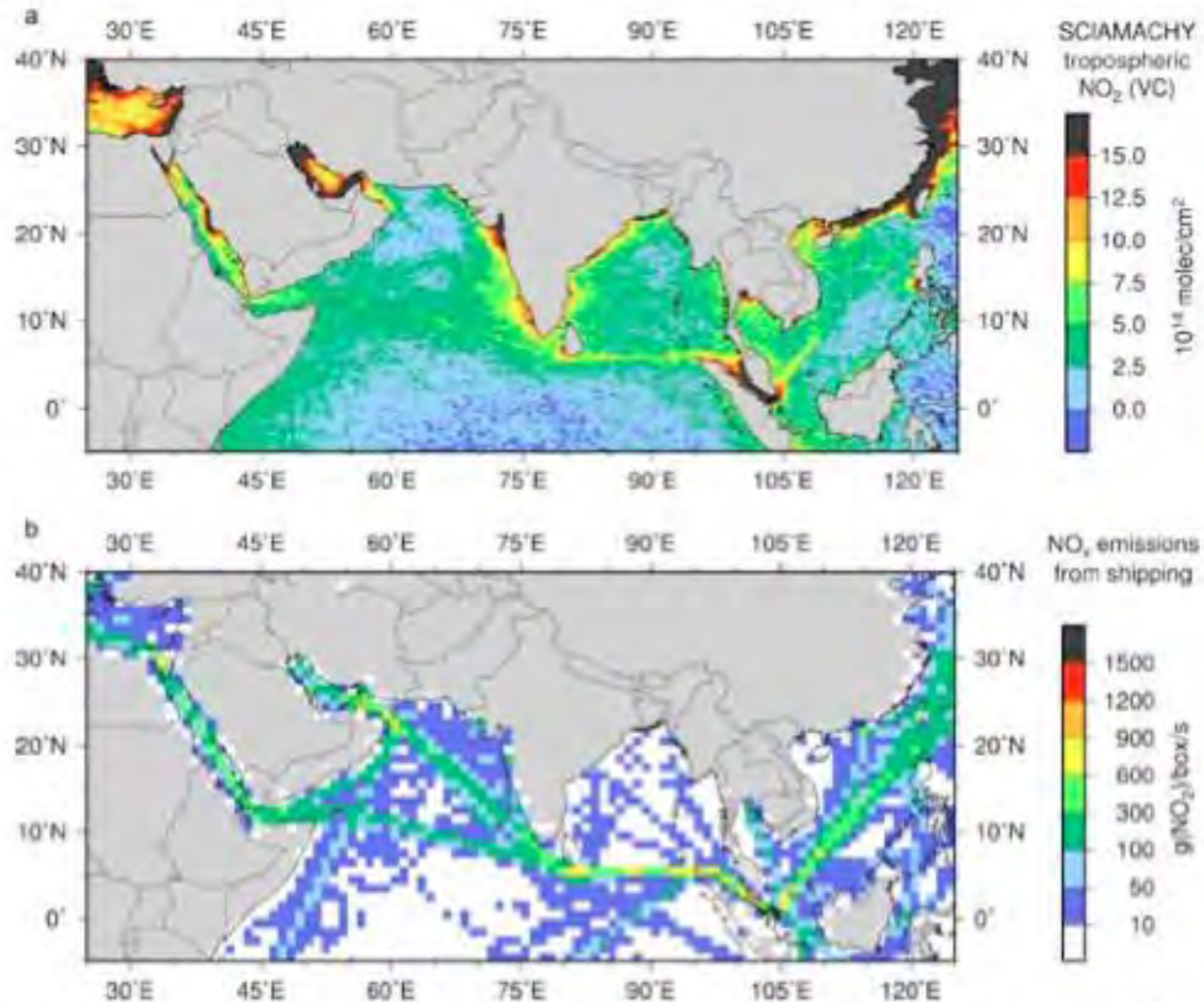
# Sulphur in ship emissions generates clouds



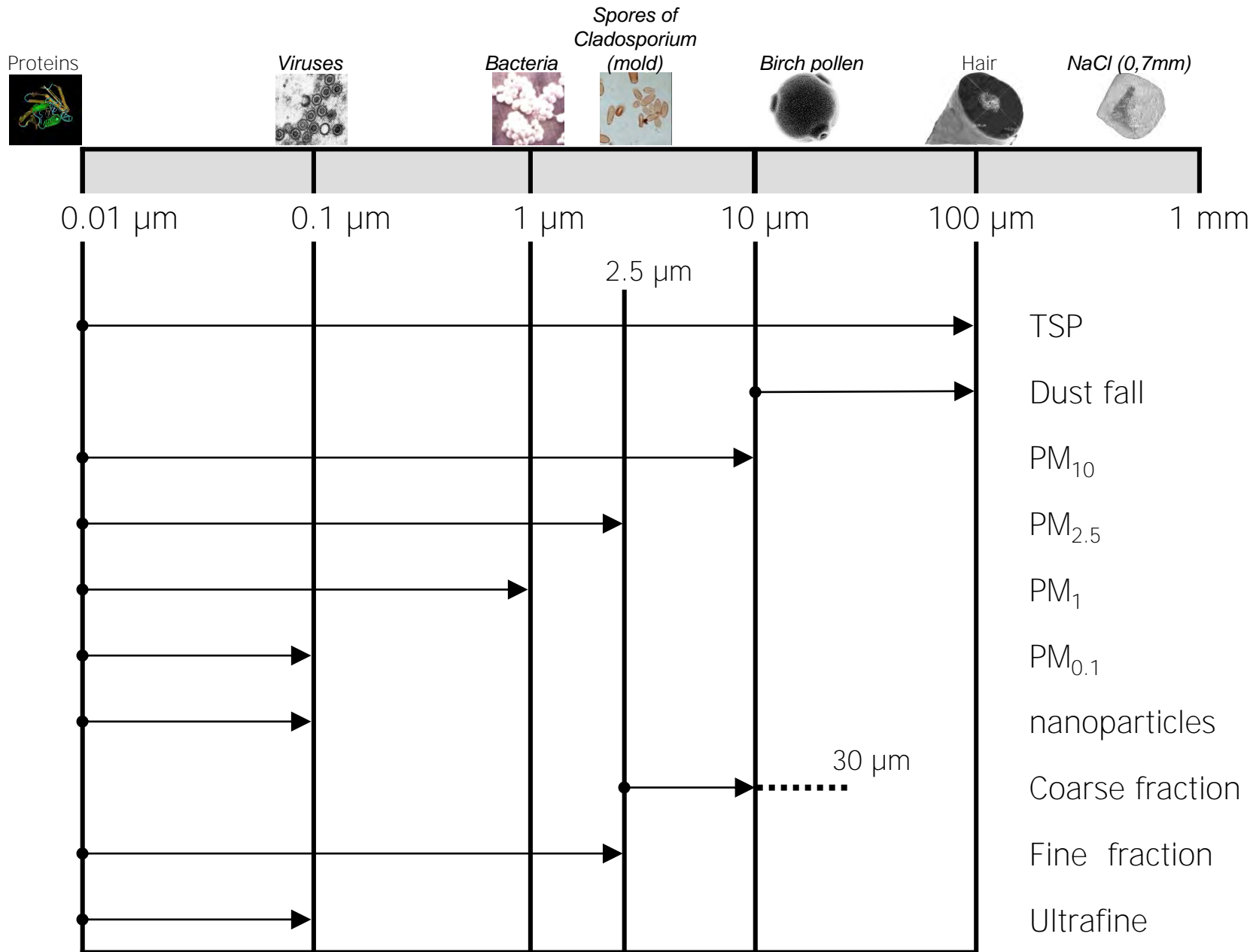
*A satellite image from 4 March 2009 showing ship tracks — the bright streaks of clouds that form around the particles in ship exhaust — over the northeast Pacific Ocean. The ship tracks are brighter than the natural marine clouds around them because they contain lots of small cloud droplets, which you can see in this zoomed-in image. NASA image by the LANCE/EOSDIS MODIS Rapid Response Team.*



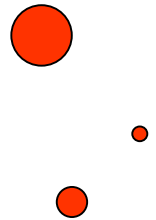
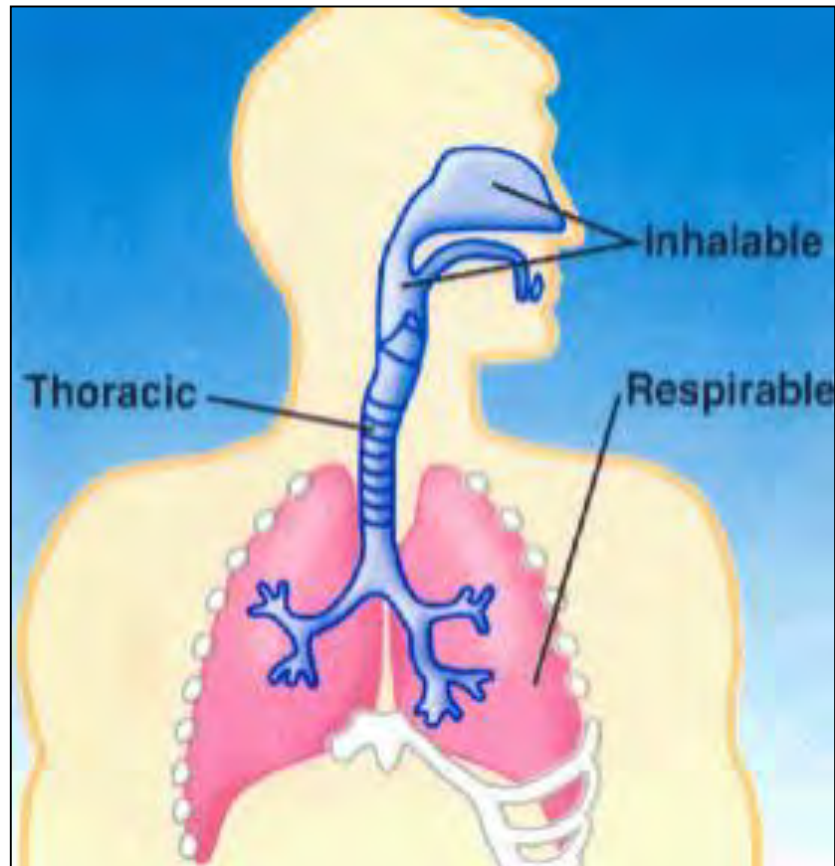




**Figure 3.** NO<sub>x</sub> signature of shipping in the Indian Ocean, as detected by SCIAMACHY (a), and estimated from emission models (b). From *Richter et al.*, (2004).



# Particle deposition in the lung



# London smog 1952

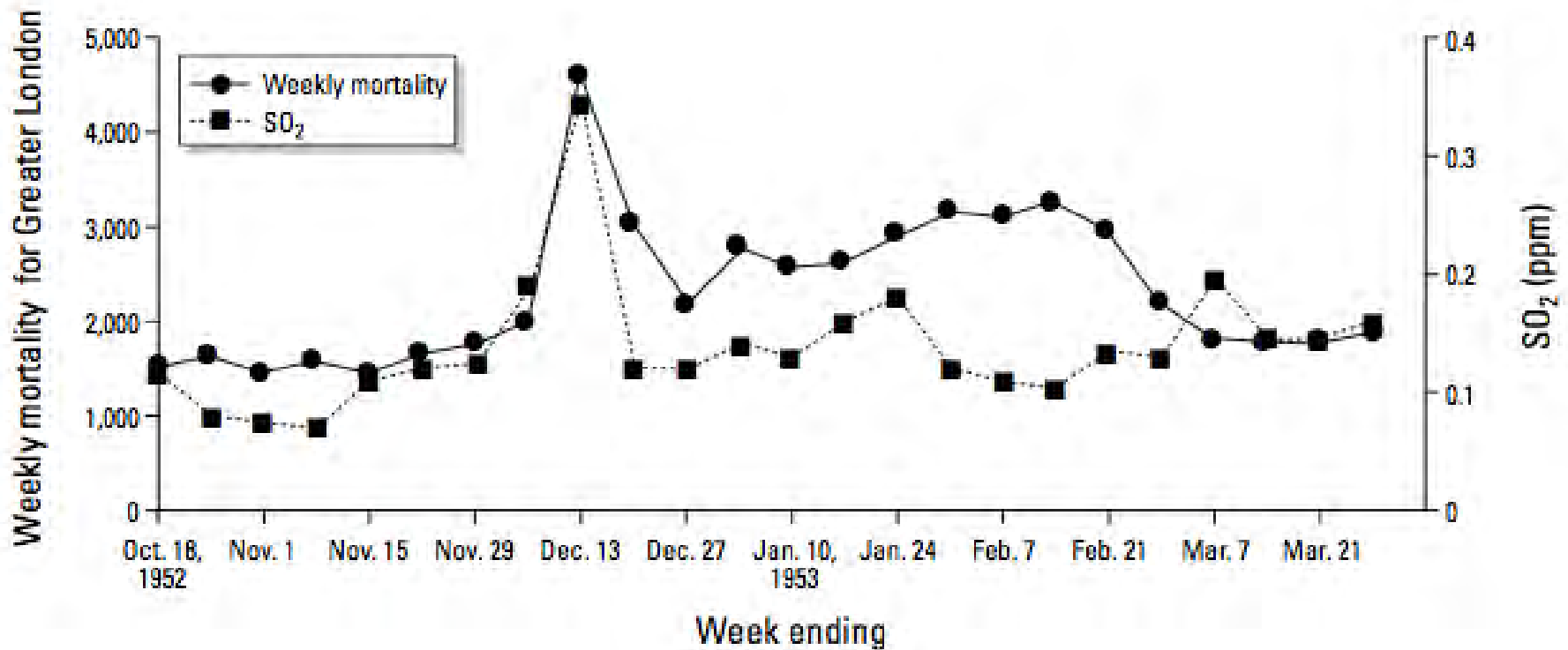


Ship diesel: 1600 mg sulphur/kg

<10mg Schwefel/kg ab Jan 2013 DIN EN 228: 2013-01, standard in Germany since 2003



# London smog: lethality



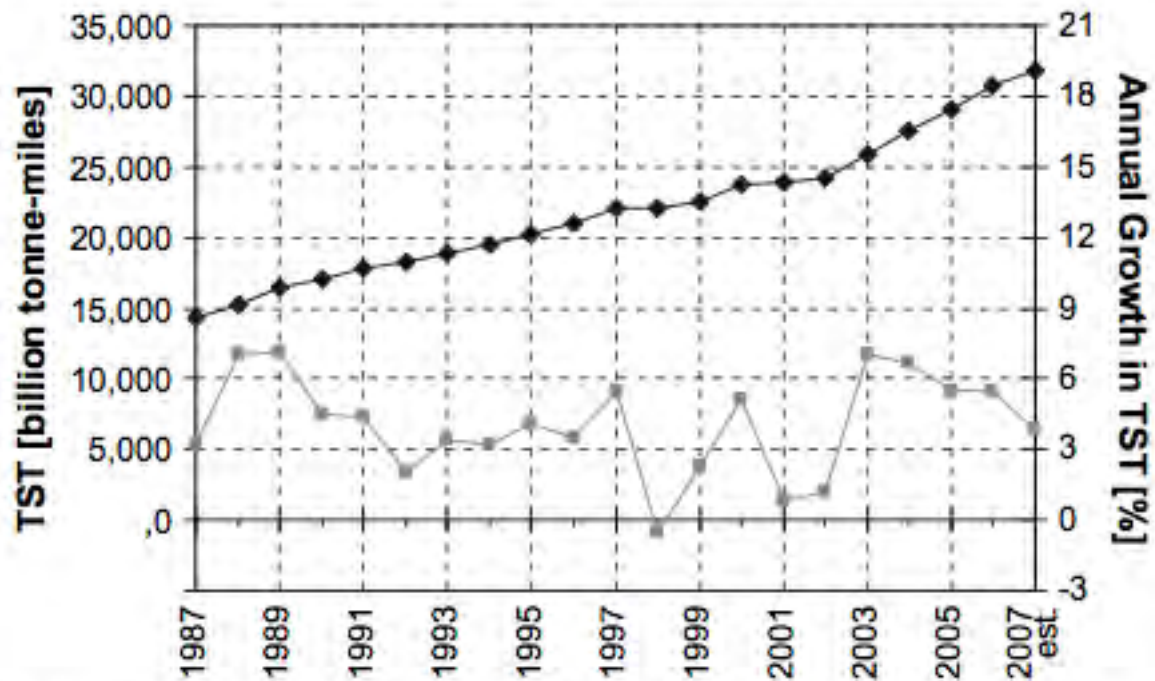
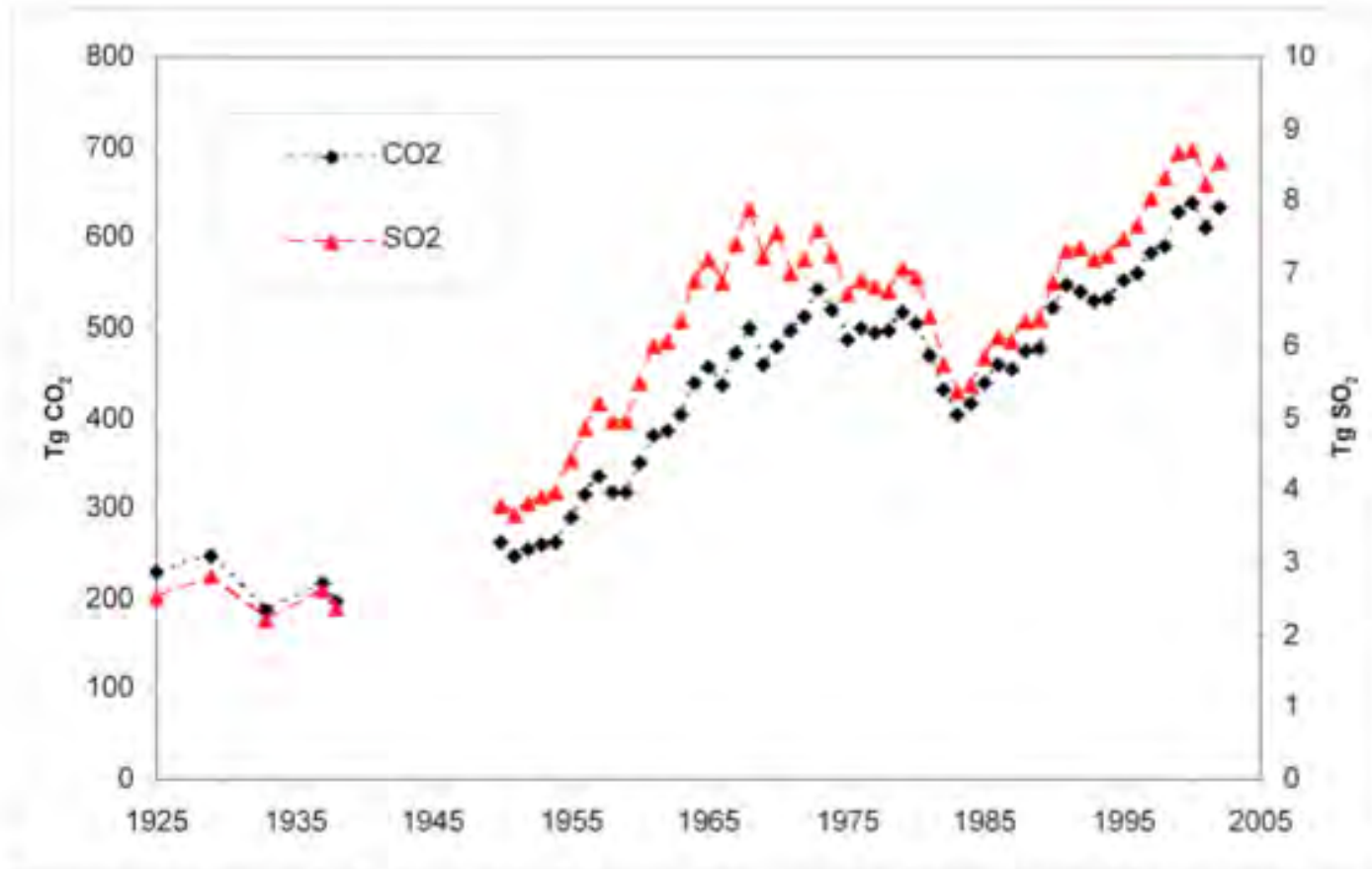


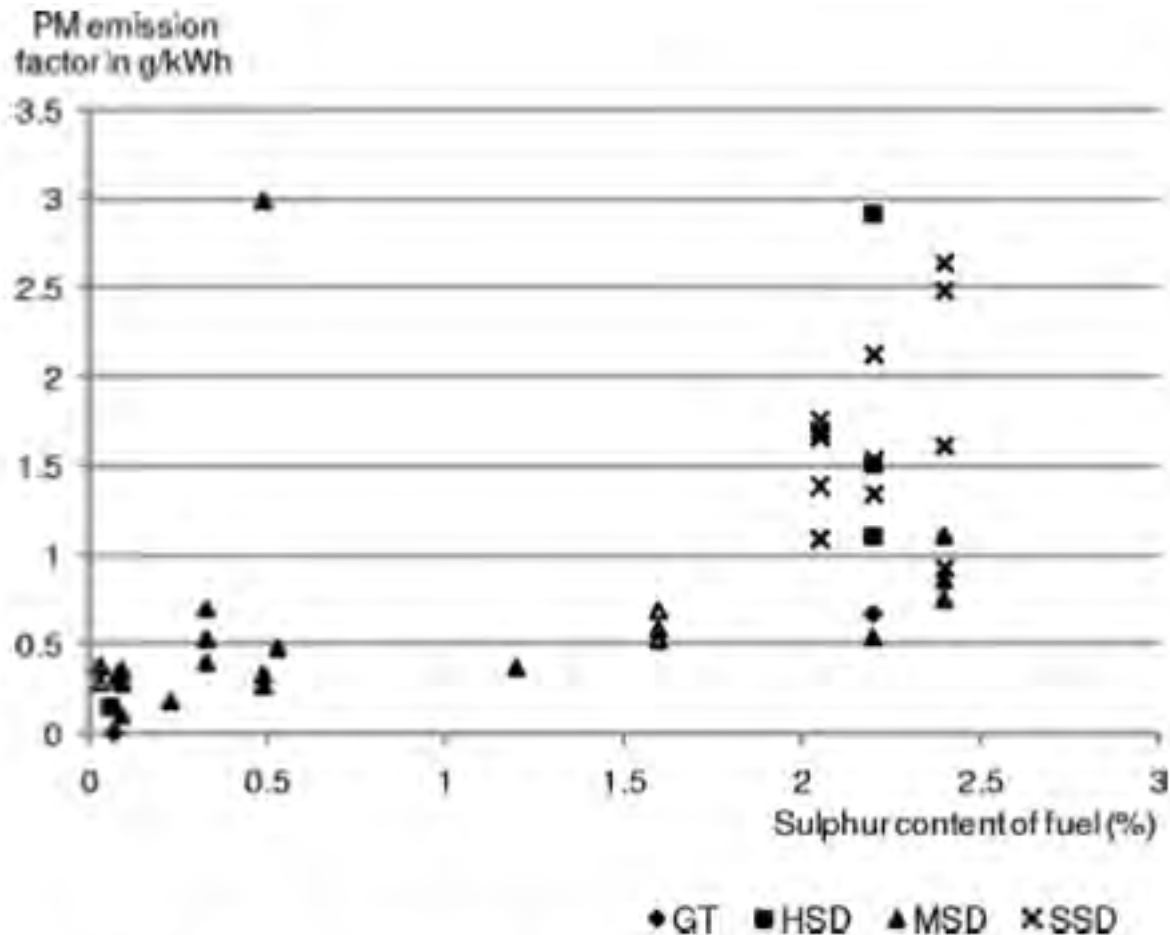
Fig. 4. World seaborne trade (TST) in billion ton-miles and corresponding annual growth rate from 1987 to 2007. Source: Fearnleys (2007).

# Ship emissions will increase



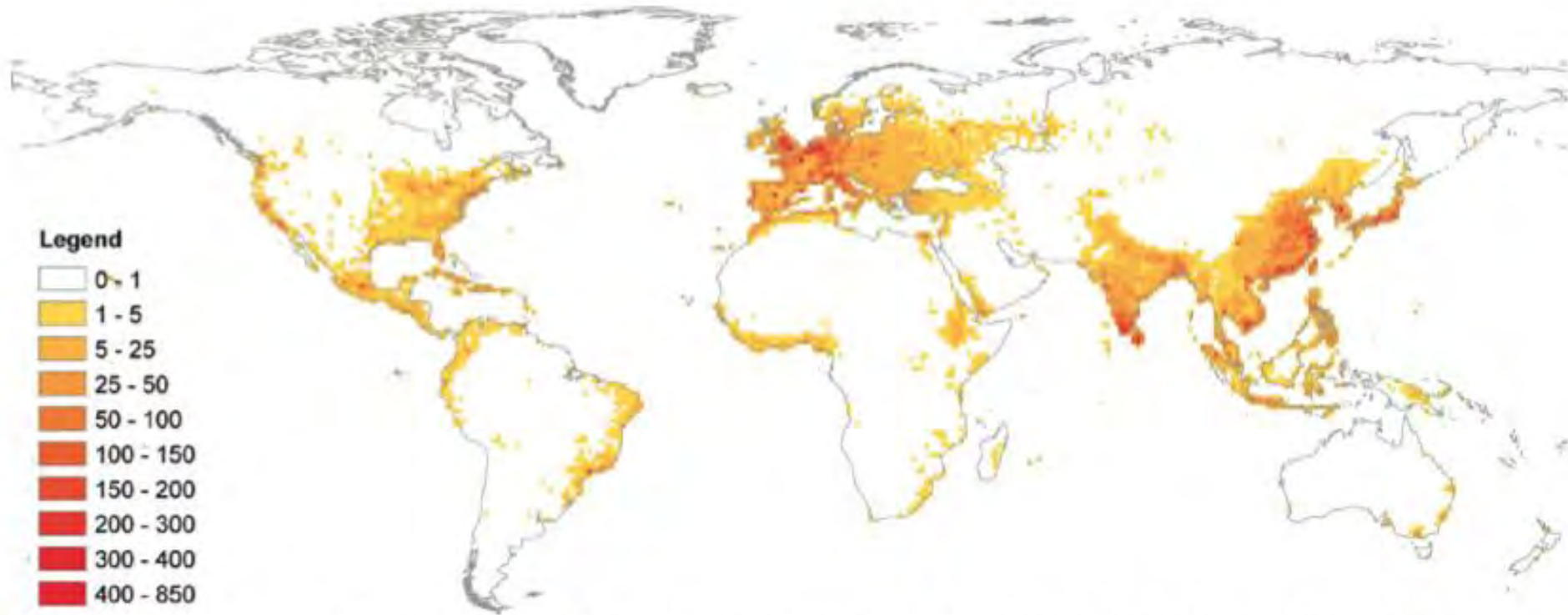
**Figure 1.** Development of CO<sub>2</sub> and SO<sub>2</sub> ships, emissions, based on estimated sales of marine fuel, 1925-2002. Note that no data are available for World War II (from *Endresen et al., 2007*).

# Effect of sulphur removal from ship fuel



**Figure 4.** The variation of particle emissions with sulfur content of the fuel oil. GT = gas turbines, HSD = high-speed diesel, MSD = medium-speed diesel, SSD = slow-speed diesel.<sup>7-9,23</sup> The values from the presented measurements are indicated with open markers.

# Effect of Sulfur in Ship Fuels on Mortality



**FIGURE 2.** Annual premature mortality for the *No Control* scenario compared to a “no shipping” case using ICOADS data.





HFO



DF

# HICE

Testing toxicity of anthropogenic combustion:

ship diesel exhaust

# Studying the causes of health effects of combustion-derived aerosols in the framework of the Virtual Helmholtz Institute HICE: Concept



**HICE** › Aerosols and Health  
 Helmholtz Virtual Institute of Complex  
 Molecular Systems in Environmental Health

R.Zimmermann<sup>1,11</sup>,  
 S.Mülhopt<sup>4</sup>, M.Dilger  
 M.-R.Hirvonen<sup>6,11</sup>, J.  
 A.J.Wlodarczyk<sup>8,11</sup>,  
 T.Streibel<sup>1</sup>, E.Karg<sup>1</sup>,  
 M. Arteaga Salas<sup>1,11</sup>

H.Paur<sup>4,11</sup>, C.Schlager<sup>4</sup>,  
 R. Rabe<sup>5</sup>,  
 A.BeruBe<sup>8,11</sup>,  
 gesbäumker<sup>5,11</sup>,  
 M. Elsasser<sup>1</sup>,

A.Rheda<sup>1</sup>, B.Werner<sup>1,11</sup>, J.Pässig<sup>1,11</sup>, T. Gröger<sup>1</sup>, G.Abbaszade<sup>1</sup>, C.Radischat<sup>1,11</sup>

<sup>1</sup>Joint Mass Spectrometry Centre, Rostock University.&Helmholtz Zentrum München), D

<sup>2</sup>MDC, Berlin, D;

<sup>3</sup>ZAUM, Technical University Munich, D;

<sup>4</sup>KIT (ITC/ITG), Karlsruhe, D;

<sup>5</sup>University of Rostock (Physics), D

<sup>6</sup>University Eastern Finland (FI)

<sup>7</sup>Uni Luxemburg; LUX

<sup>8</sup>Cardiff University, UK

<sup>9</sup>HMGU, D;

<sup>10</sup>Vitrocell GmbH, D;

<sup>11</sup>HICE – Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health-Aerosols and Health

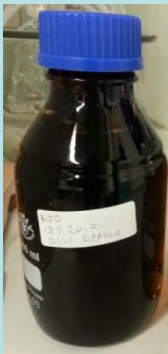


On-line analysis:

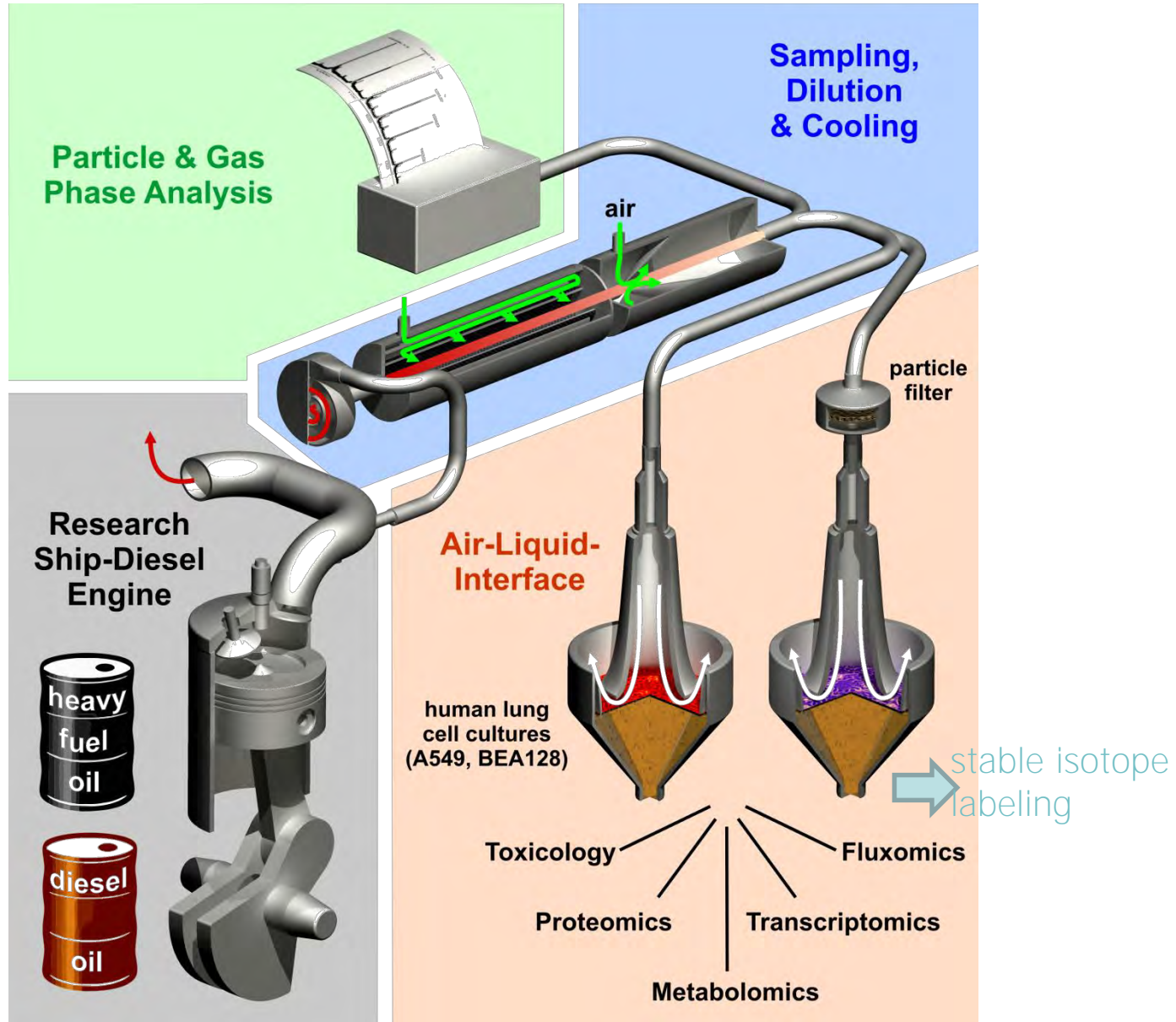
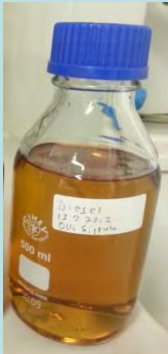
Off-line analysis:

- DAMS-GC-TOFMS
- GC-IC-TOFMS
- GC-HERTOF
- HPLC-MS/MS
- FRET-PMMS
- ESPO-MSIMS
- EPT-MS
- IC-MS
- Sensors (FID..)

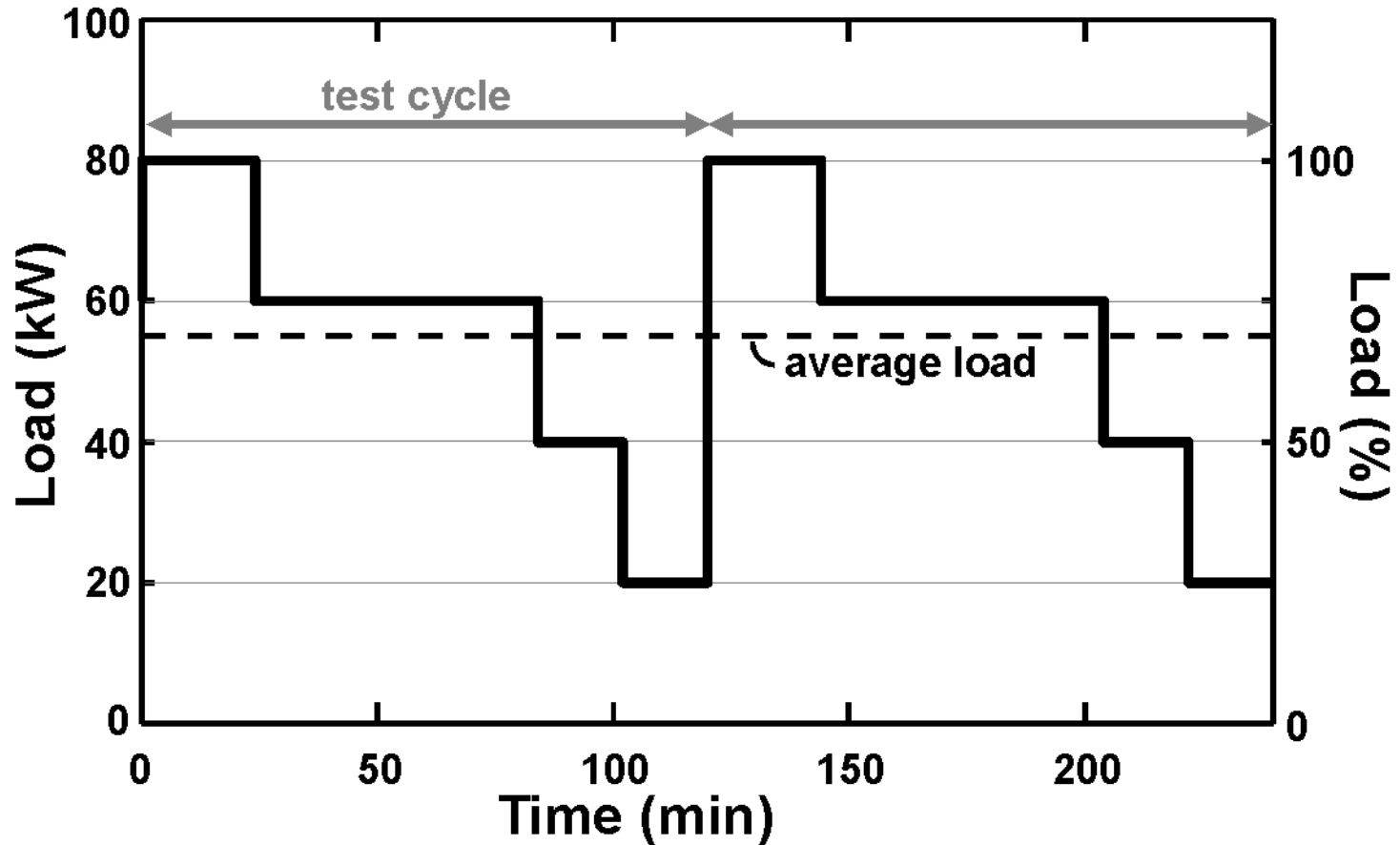
**HFO**



**DF**

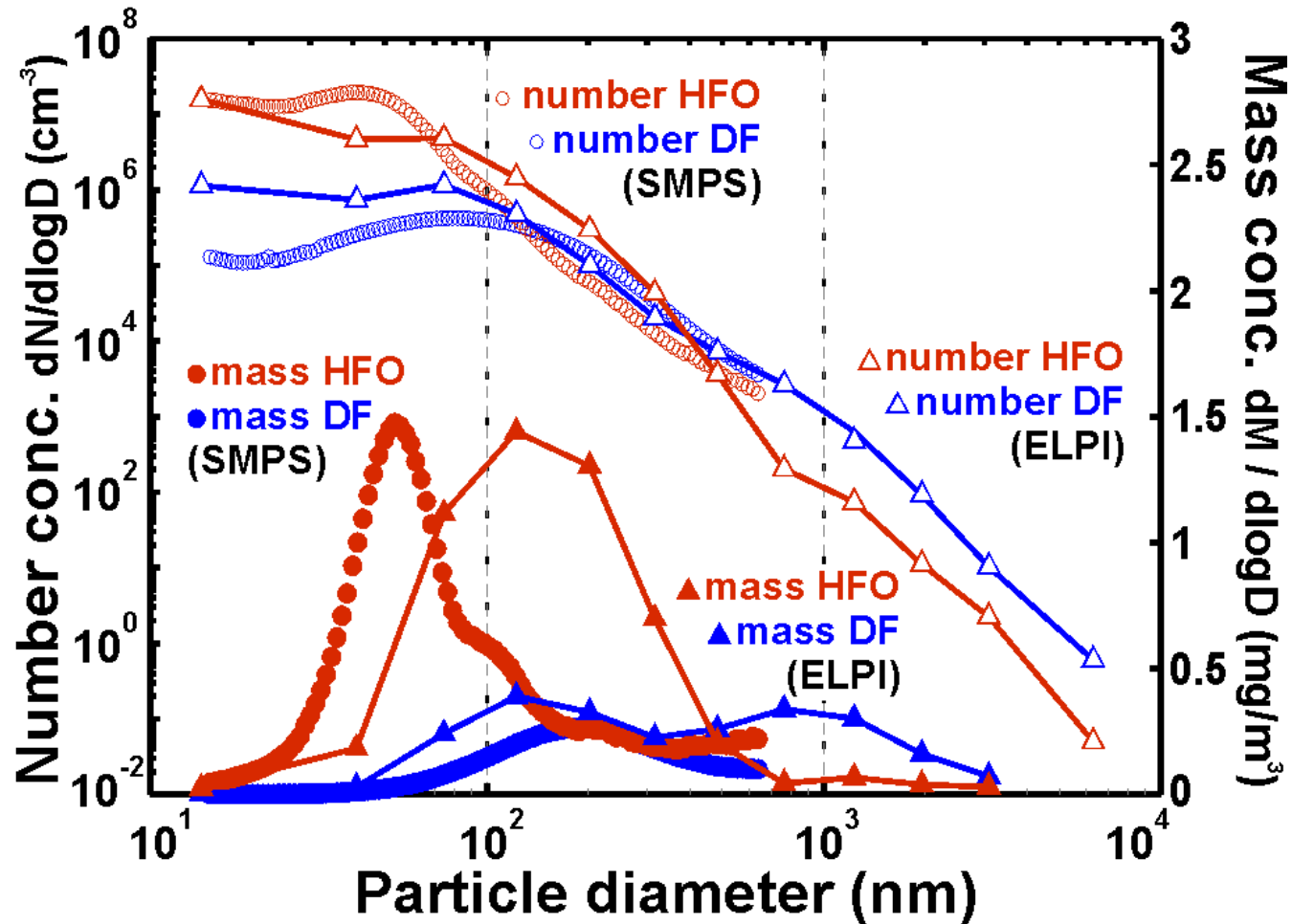


# Harbour manouvering test cycle

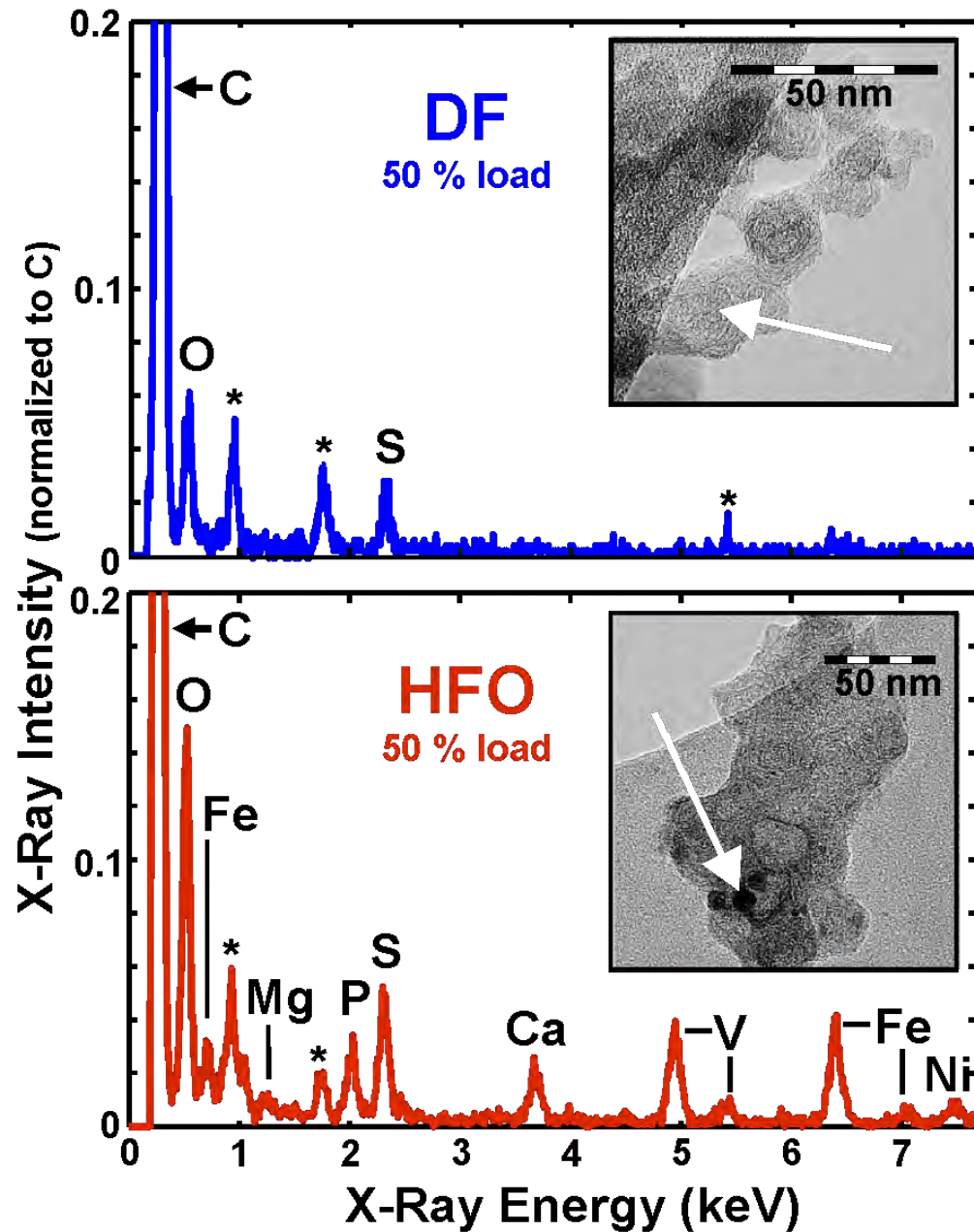


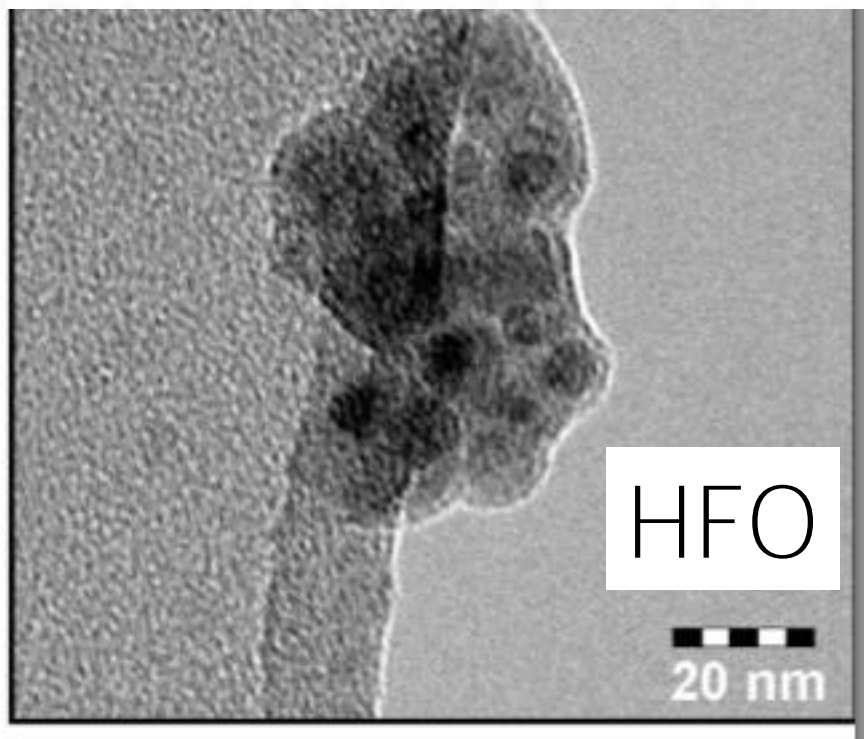
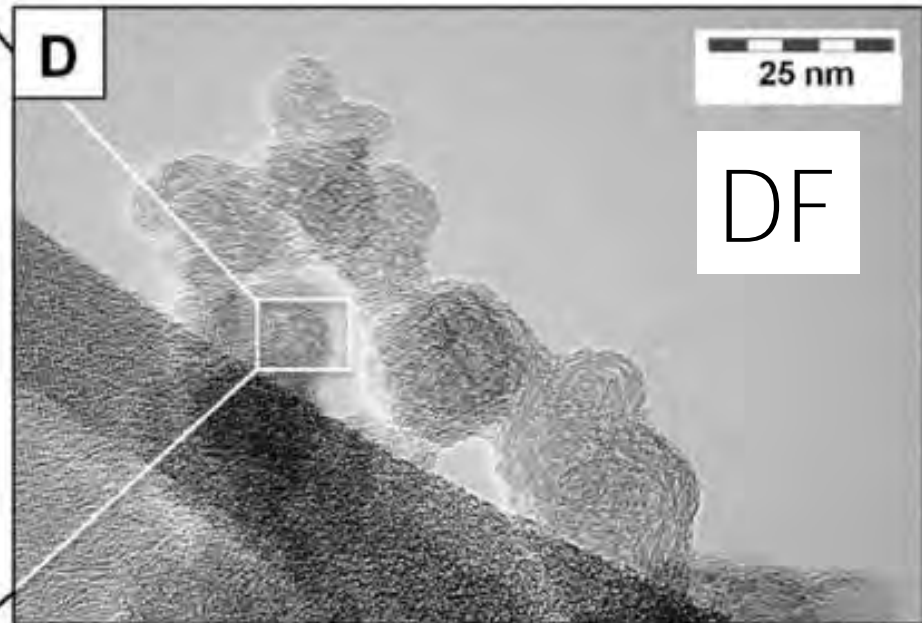


# Particle size distribution

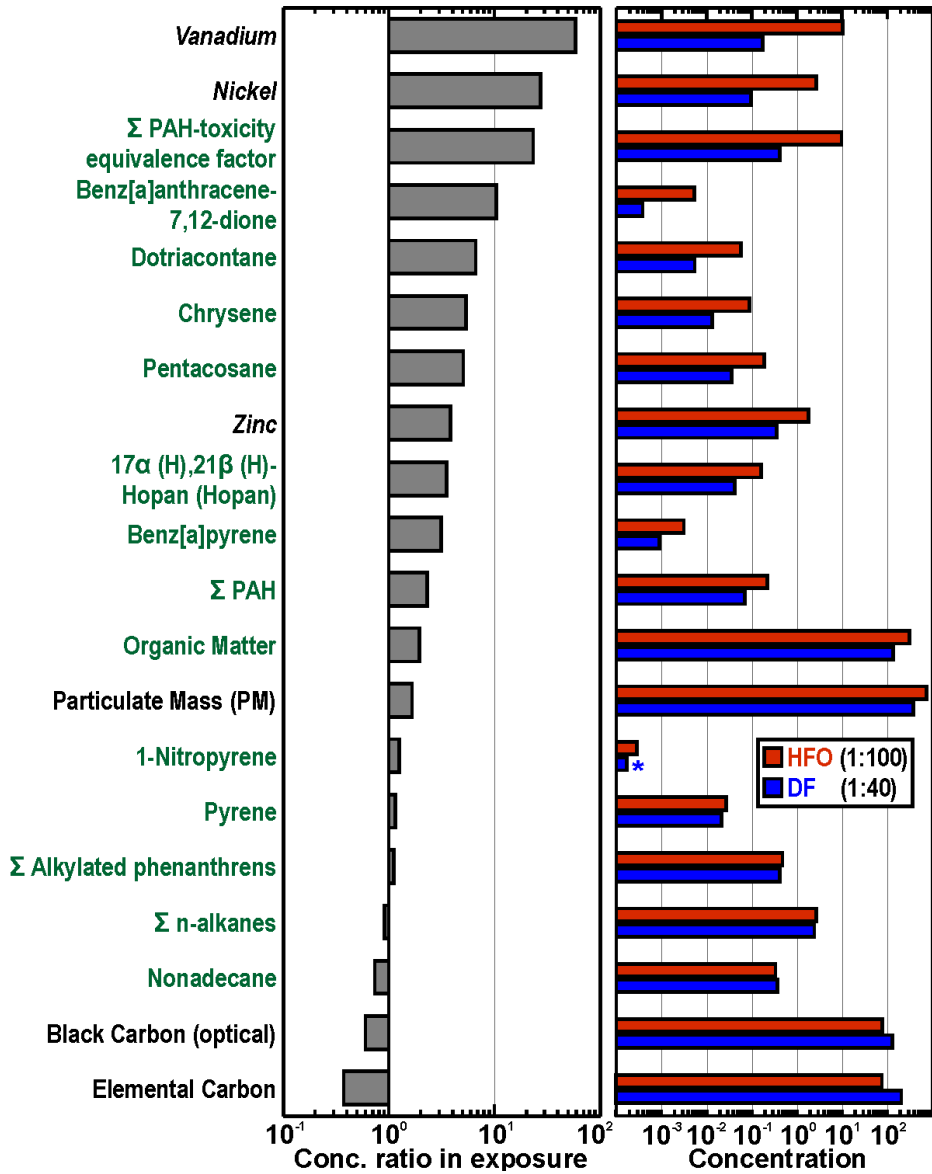


# Anorganic composition of the exhaust particles

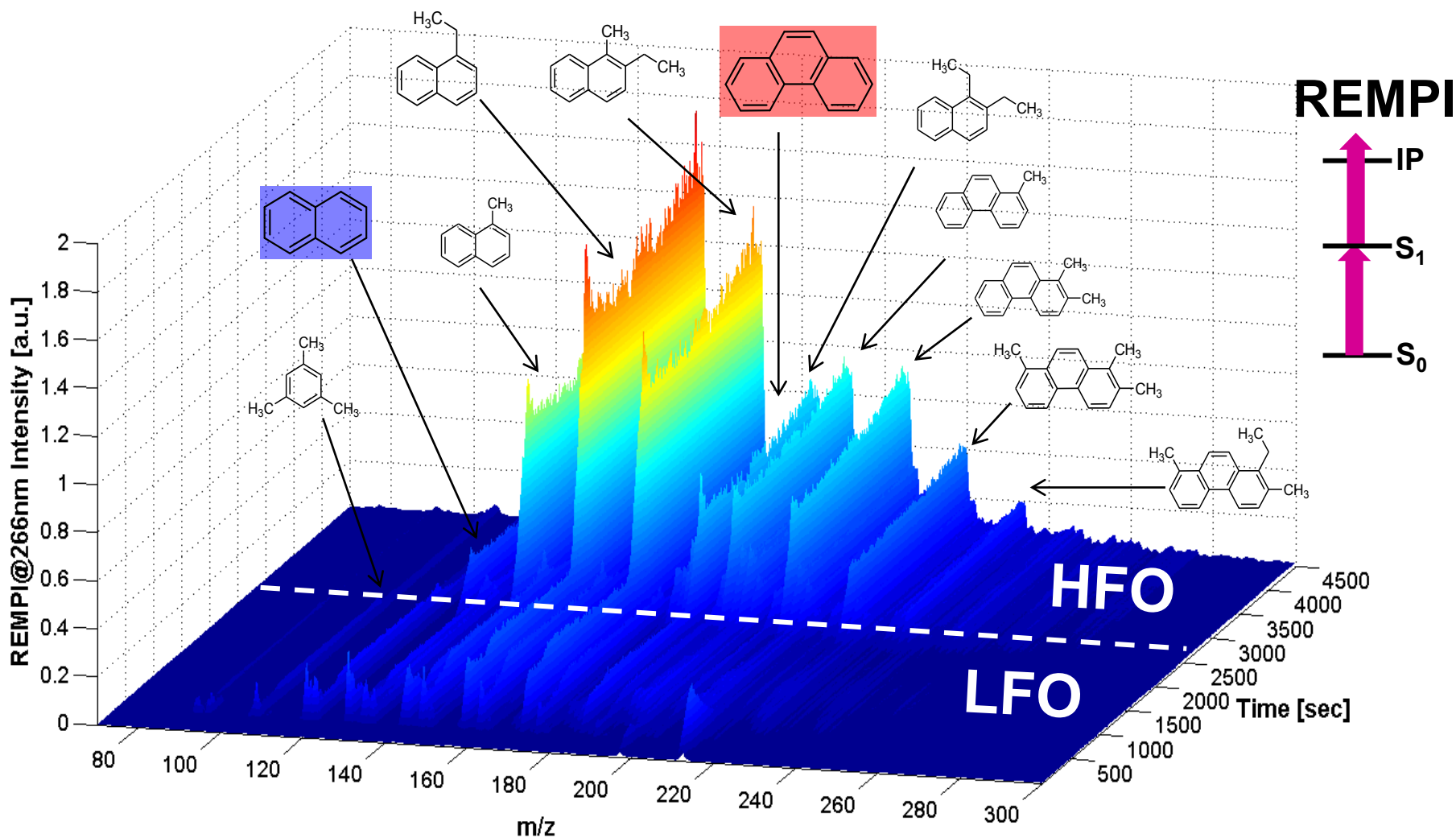




# Organic composition of the exhaust particles

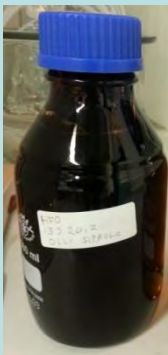


Switching from light fuel oil (LFO) to heavy fuel oil (HFO) →  
Increase of Polycyclic Aromatic Hydrocarbons (PAH) in exhaust gas

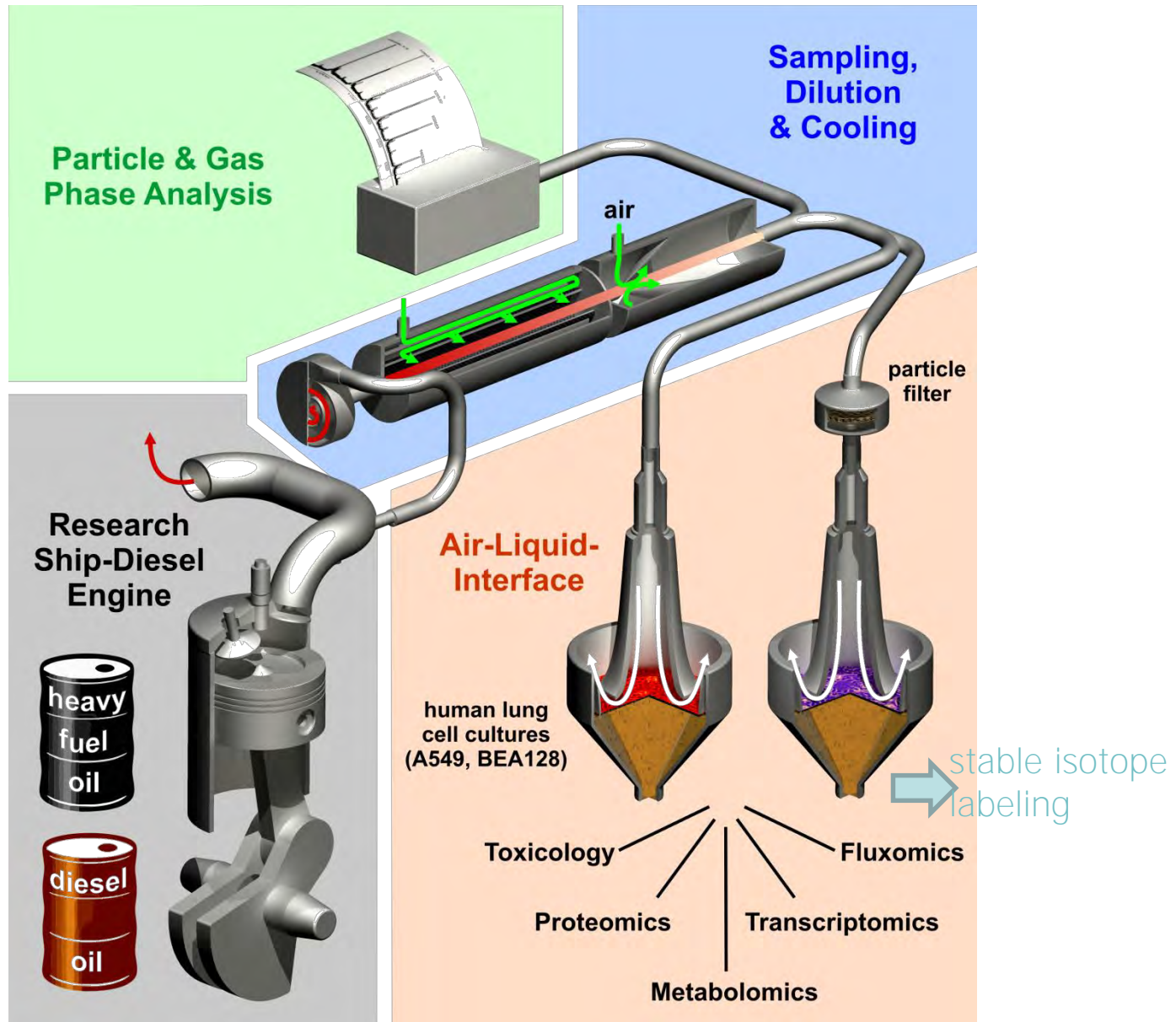
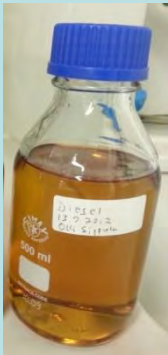




**HFO**

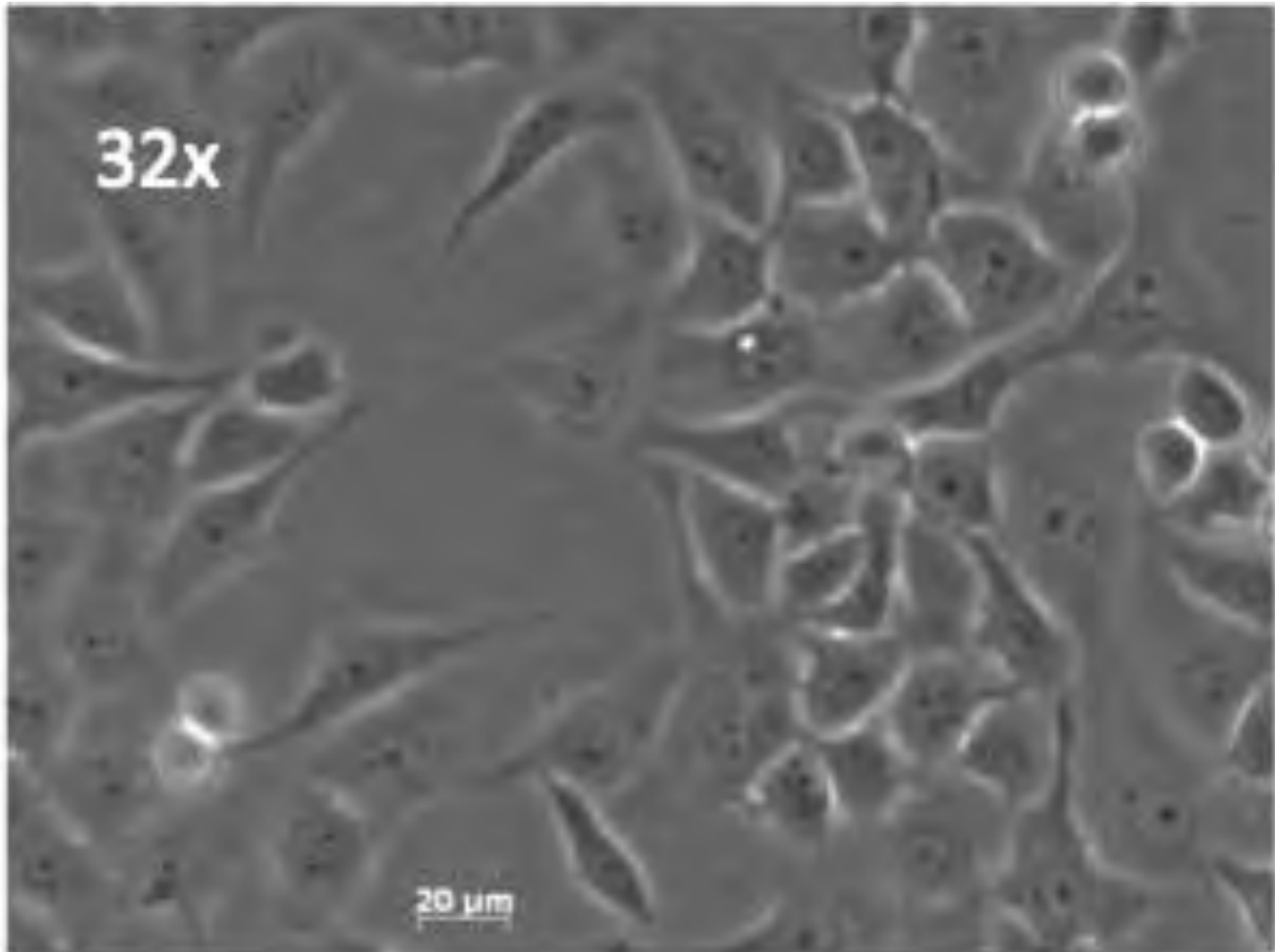


**LFO**

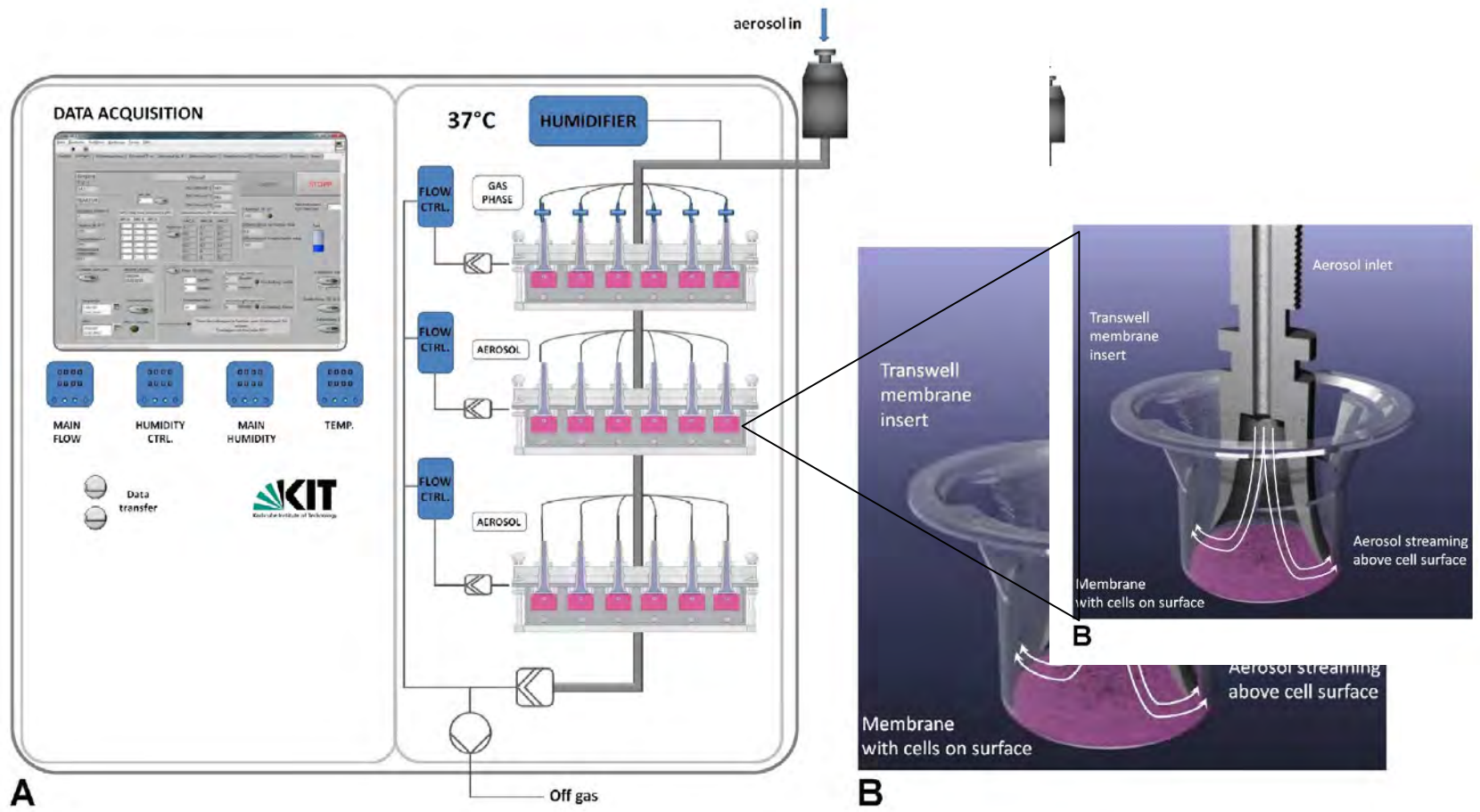


# Human bronchial epithelial cells

- BEAS-2B immortalized -



# Exposing human cells at the air-liquid interface





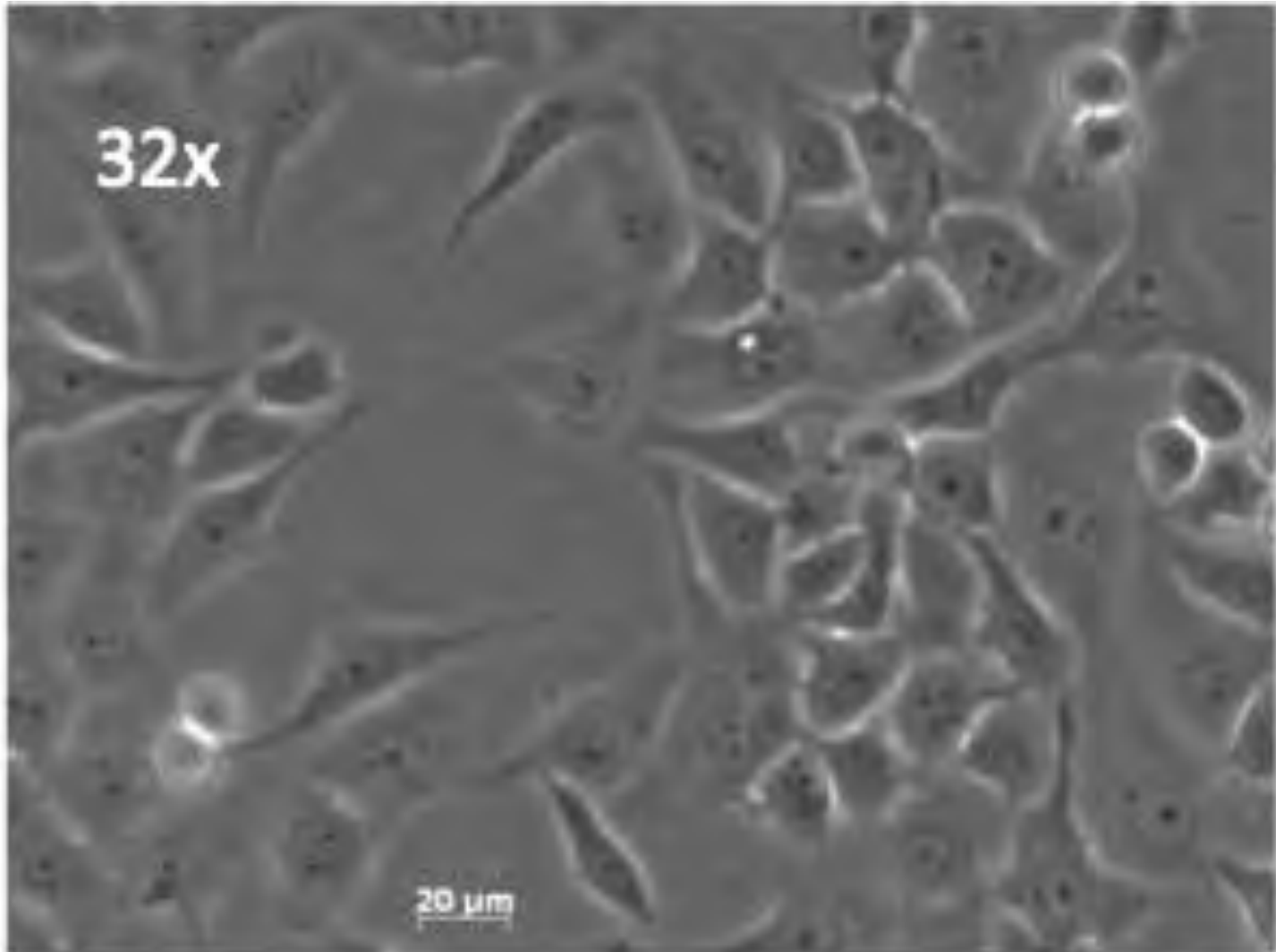


**HICE Aerosols and Health**  
Helmholtz Virtual Institute of Complex  
Molecular Systems in Environmental Health

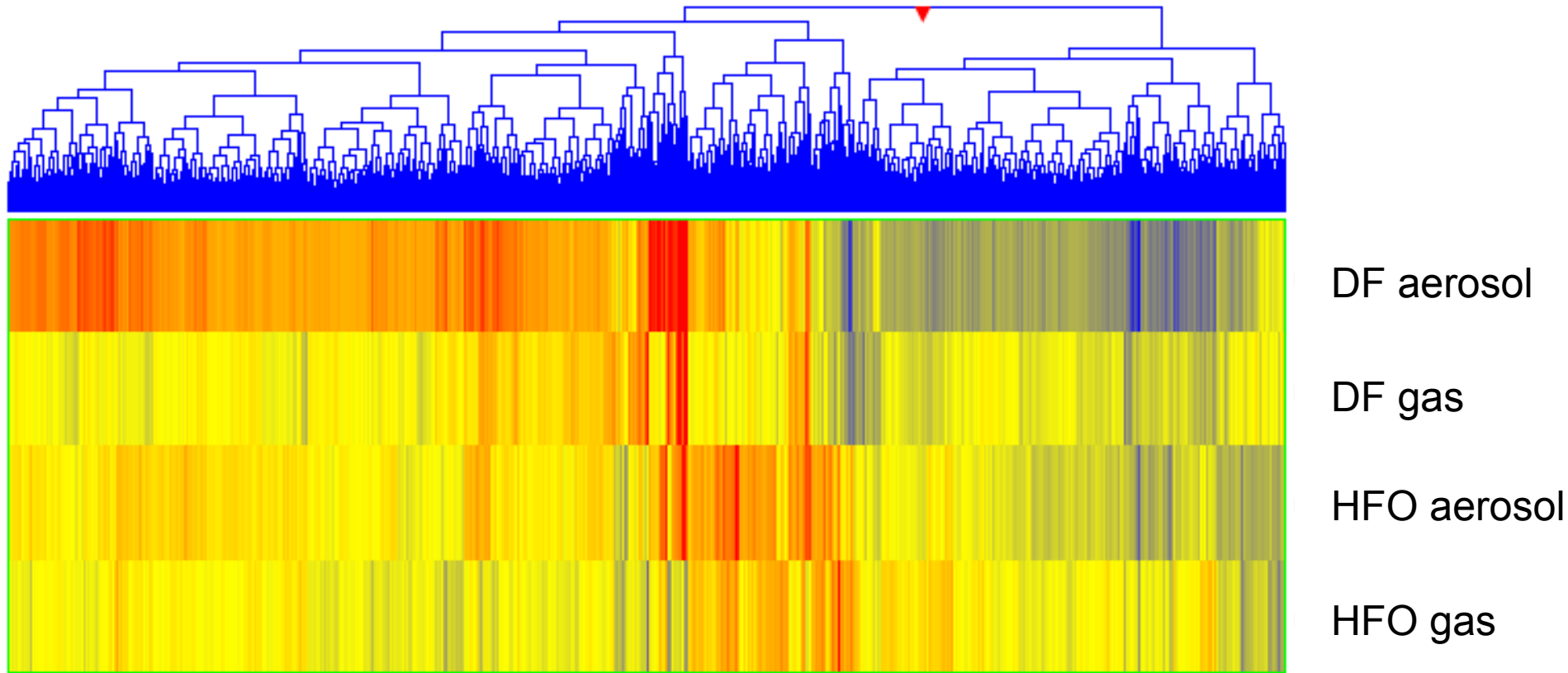
Universität Würzburg  
Helmholtz-Zentrum für Umweltforschung  
SKIT  
EMPH

# Human bronchial epithelial cells

- BEAS-2B immortalized -



# Genome wide RNA target analysis \*



- >1.5-fold Up-regulated genes
- >1.5-fold Down-regulated genes

- about 42,000 genes, against clean air

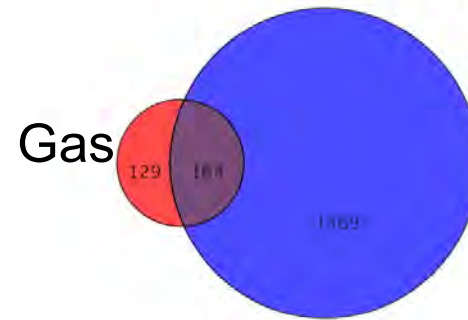
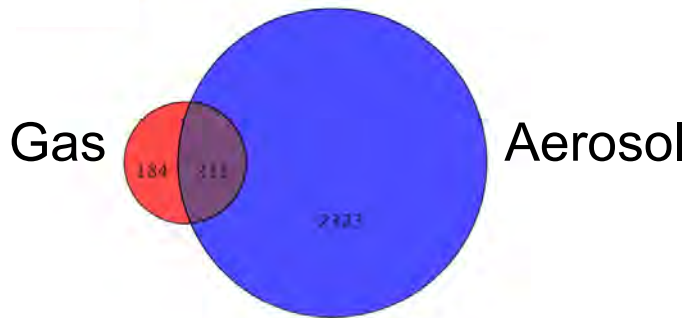


# Number of regulated genes

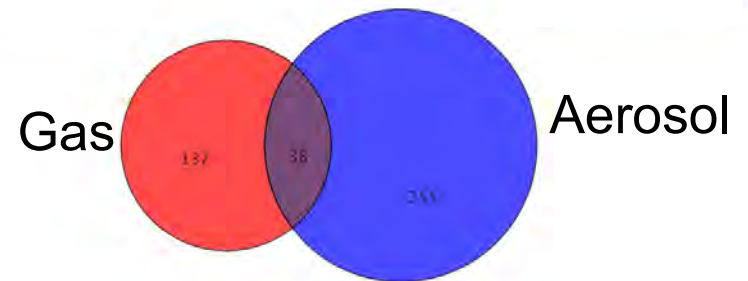
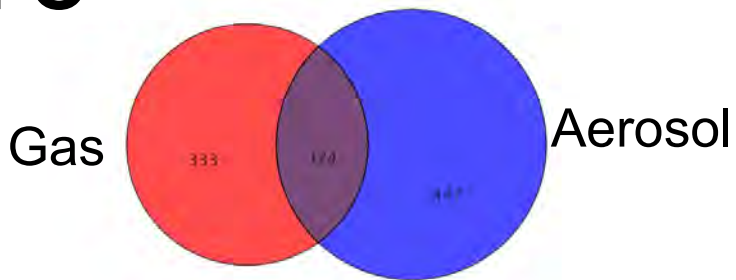
up

down

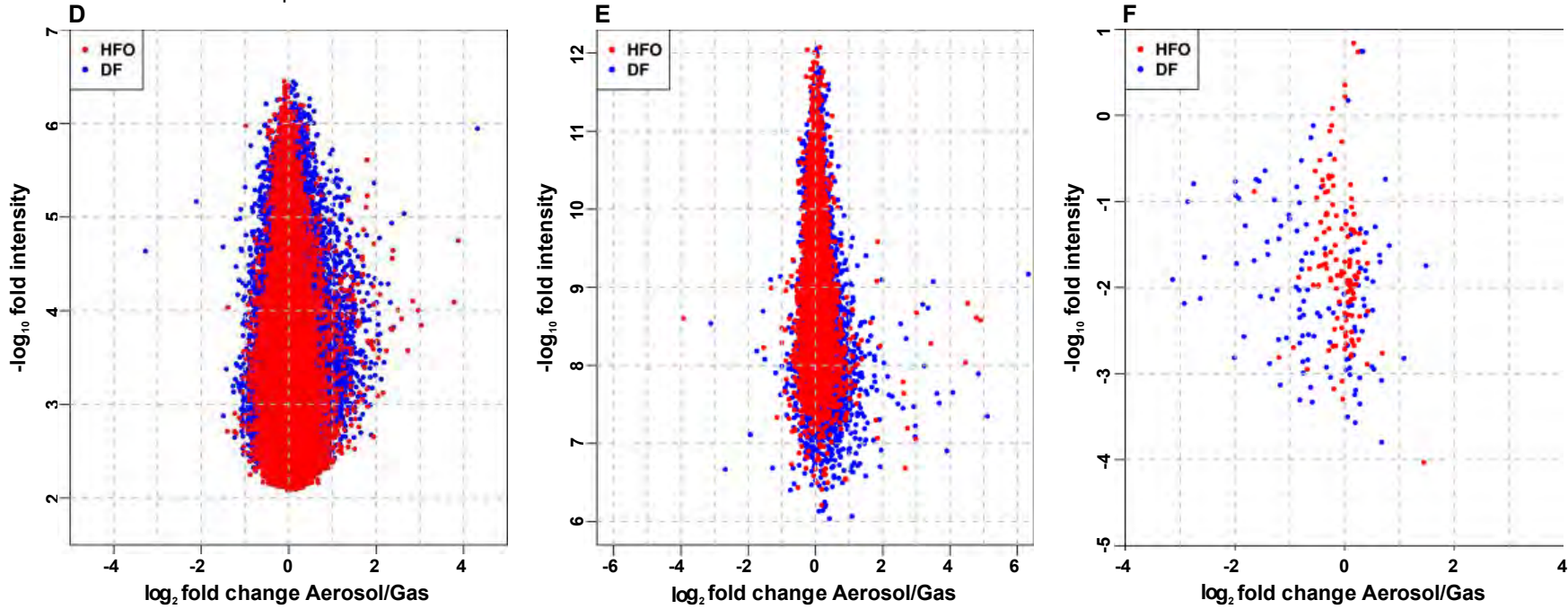
**DF**



**HFO**

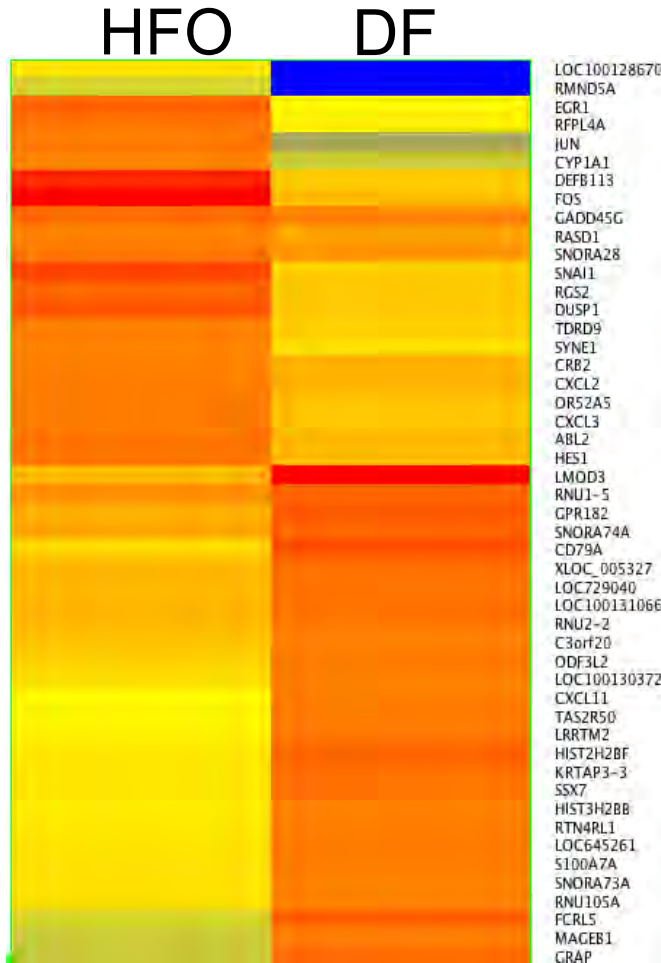


2 Toxicologic evaluation of particles: More responses with DF



■ HFO  
■ DF

# HFO ≠ DF - ≥4-fold regulated genes-



Gene	GO process/component
RMND5A	Unknown
EGR1	Response to mechanical stimulus, drug, isoquinoline alkaloid, hypoxia
RFPL4A	Unknown
JUN	Negative regulation of cell proliferation and transcription, innate immune response
CYP1A1	Xenobiotic metabolism
DEFB113	Immune defense
FOS	Response to oxidative stress, regulator of proliferation
GADD45G	DNA damage response
RASD1	Activator of G-protein signaling, negative regulator of transcription
SNORA28	(small nuclear RNA)
SNAI1	negative regulator of DNA damage response and transcription
RGS2	Negative regulation of G-Protein signaling
DUSP1	Oxidative/ heat stress, apoptosis
TDRD9	DNA methylation, gene silencing, cell differentiation
SYNE1	Cell death, cytoskeletal anchoring at nuclear membrane
CRB2	Calcium ion binding
CXCL2	Inflammation
OR52A5	Sensory perception of smell
CXCL3	Inflammation
ABL2	Regulator of cytoskeleton reorganization, autophagy, and endocytosis
HES1	Cell maturation
LMOD3	cytoskeleton
RNU1-5	unknown
GPR182	G-protein coupled receptor, vasodilation
SNORA74A	(small nuclear RNA)
CD79A	Transmembrane signaling
ODF3L2	unknown
CXCL11	Inflammation
TAS2R50	Sensory perception of taste (bitterness)
LRRTM2	Synapse organization
HIST2H2BF	Nucleosome assembly (histone)
KRTAP3-3	Keratin filament
SSX7	Regulation of transcription
HIST3H2BB	Nucleosome assembly (histone)
RTN4RL1	Cell surface
S100A7A	Inflammation (psoriasis), cell maturation, tumorigenesis
SNORA73A	(small nuclear RNA)
RNU105A	(small nuclear RNA)
FCRL5	Integral to membrane (Fc-receptor-like)
MAGEB1	Expressed in tumors
GRAP	Ras protein signal transduction

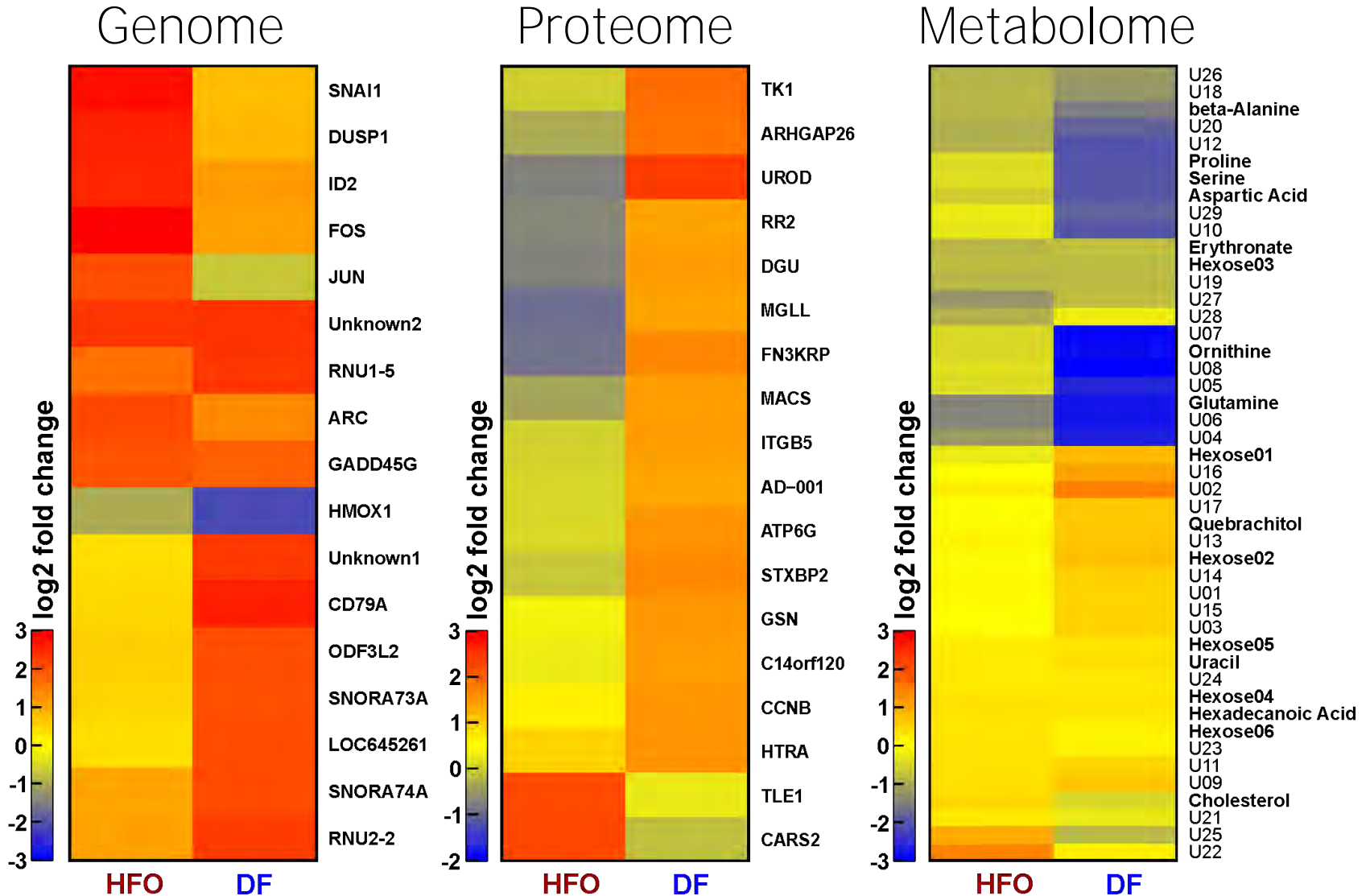
Stronger induced by HFO  
Stronger induced by LFO

Legend - Hierarchical Entity Type on treatment

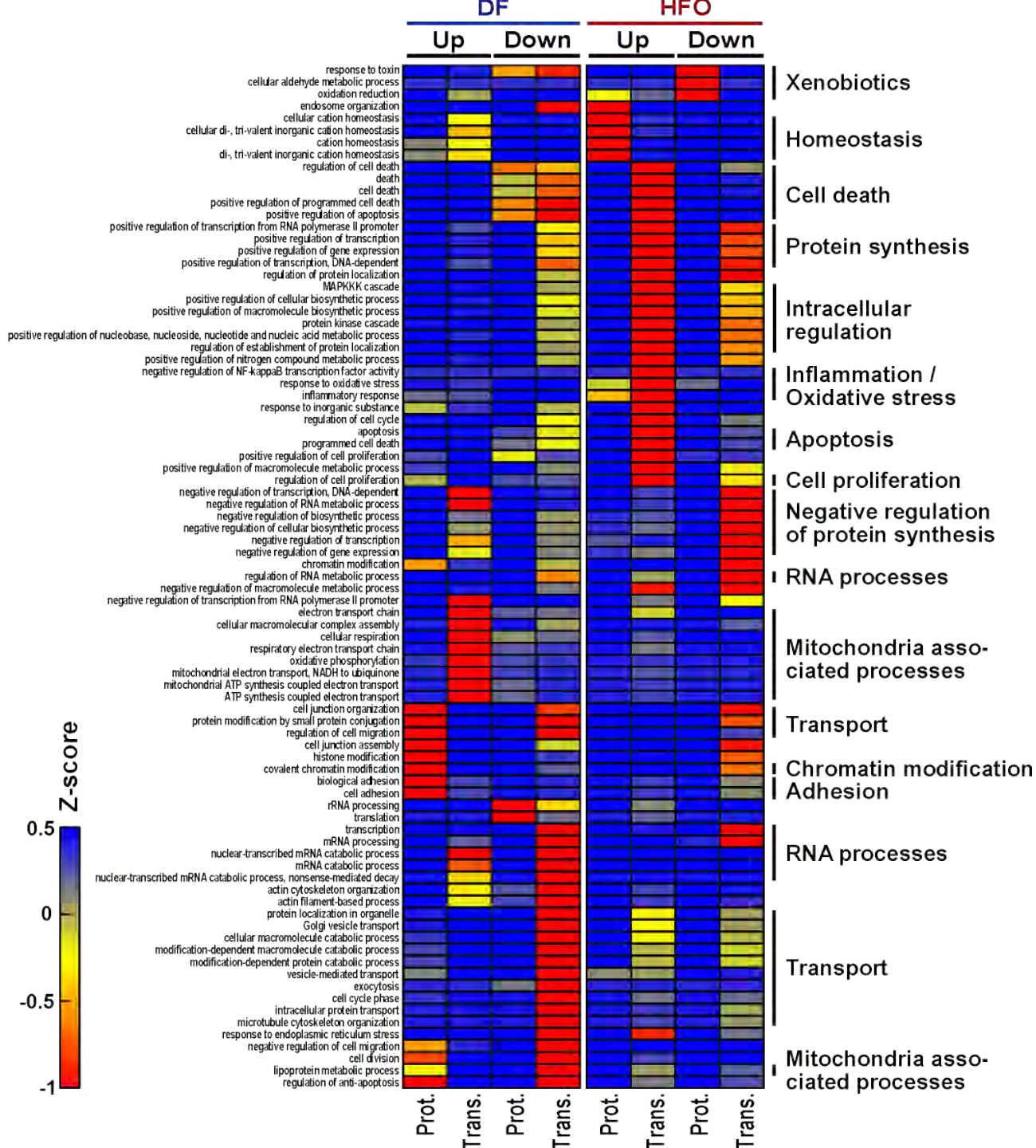
Color range: -2.1 0 4.1

Description:  
Created from Advanced Analysis operation: Clustering.  
Entity List: Fold change >= 4.0  
Interpretation: treatment  
Experiment: LFO HFO complete vs Filter  
Clustering Algorithm: Hierarchical  
Clustered By: Associated values  
Clustered On: Entities  
Similarity Measure: Euclidean  
Linkage Rule: Ward  
Associated Value Columns: [Log FC (HFO complete aerosol) vs (HFO filtered aerosol)], [Log FC (LFO complete aerosol) vs (LFO filtered aerosol)]  
Cluster Within Conditions: 0

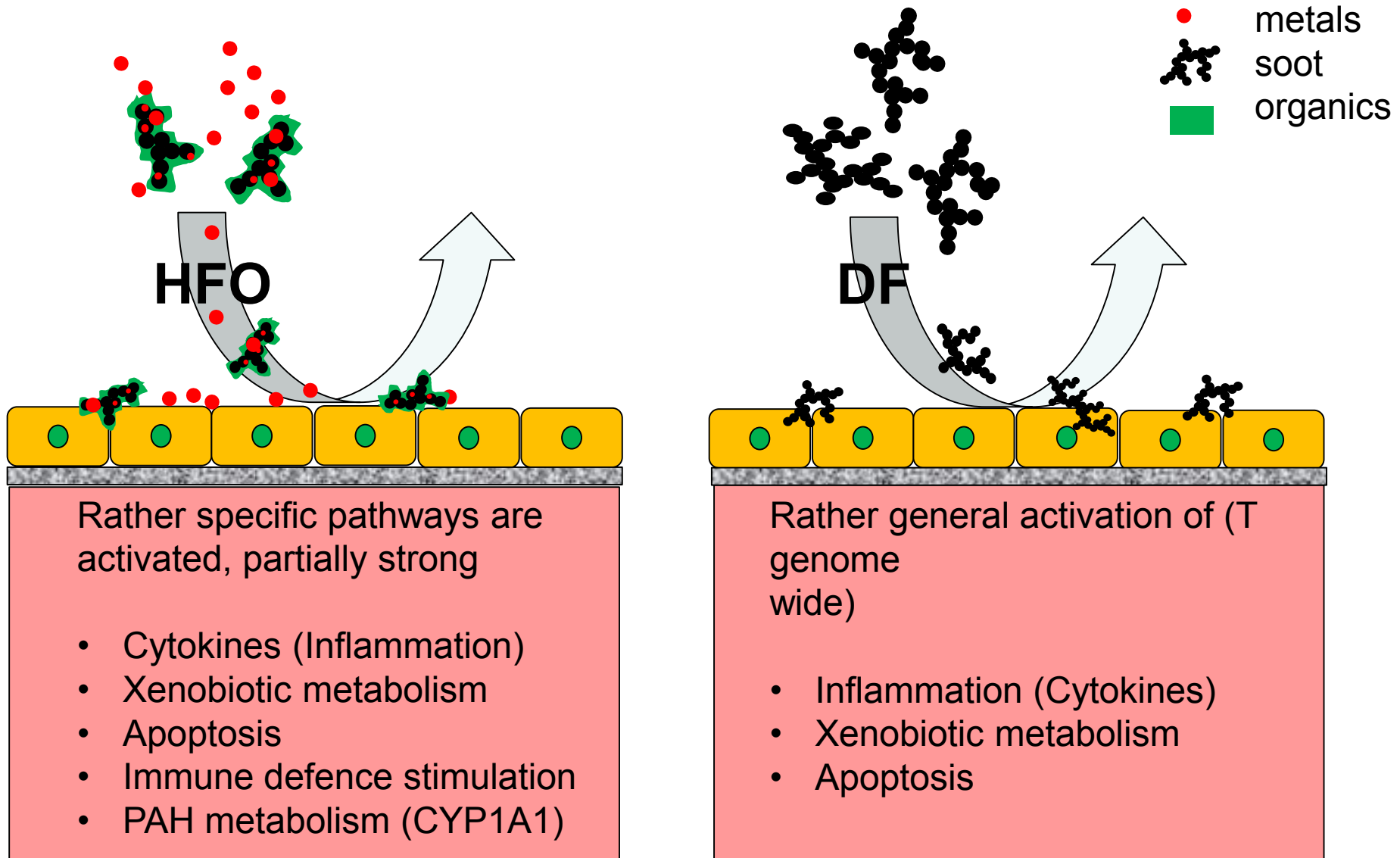
# „top 10“ most regulated







# Summary of Effects





# Conclusions

- To get approx. equal effects HFO was diluted 1:100; LFO 1:40
- Particles were more toxic than the gaseous phase
- **HFO  $\neq$  DF: HFO had more organics and less but stronger toxic effects, DF had more different effects**
- Removing sulphur is not enough to eliminate the adverse health effects
- We expect particles to be the main culprit for the DF health effects. (Oeder *et al.*, PLOSone 2015 in press)

Zimmermann group @  
Joint Mass Spectrometry Centre

- |                       |              |
|-----------------------|--------------|
| Dr. J. Maguhn         | S. Erdmann   |
| Dr. T. Streibel       | B. Weggler   |
| Dr. J. Schnelle-Kreis | B. Werner    |
| Dr. M. Sklorz         | J. Kleeblatt |
| Dr. J. Lintelmann     | S. Otto      |
| Dr. G. Matuschek      | M. Elsasser  |
| Dr. E. Feicht         | H. Grabow    |
| Dr. Kirchner          | K. Kiersch   |
| Dr. D. Diekmann       | C. Krüger    |

JOINT MASS SPECTROMETRY CENTRE




**HICE › Aerosols and Health**  
Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health

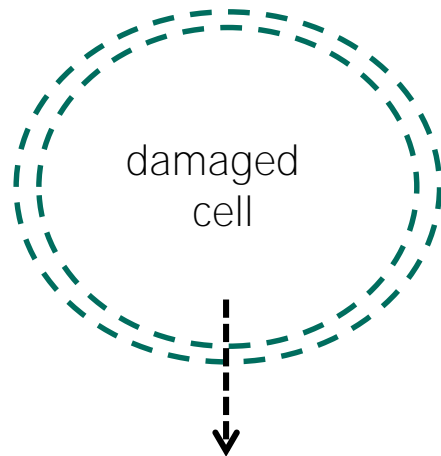
- |               |               |
|---------------|---------------|
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| C. Rüger      | H. Czech      |
| T. Schwemer   | K. Lau        |

- Funding
- Uni Rostock
  - Deutsche Forschungsgemeinschaft
  - Bundesministerium für Bildung und Wissenschaft
  - Bayerische Staatsregierung
  - Deutsche Forschungsgemeinschaft
  - Bundesministerium für Bildung und Wissenschaft
  - Compagnie Airsense
  - Helmholtz HICE)



# Rapid at-site assays for cytotoxicity: Determination of dilution-factor

## LDH-Assay

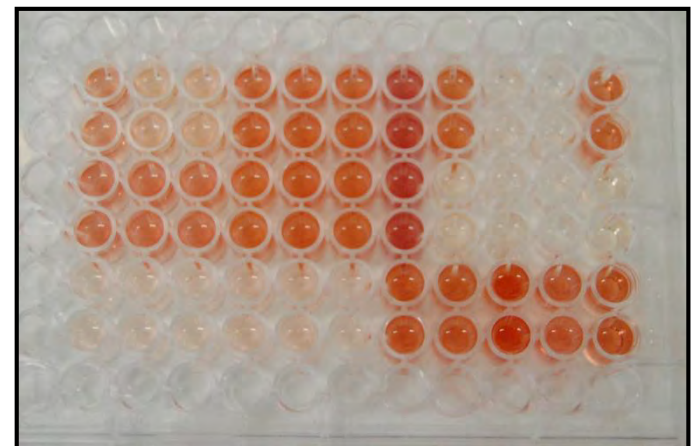
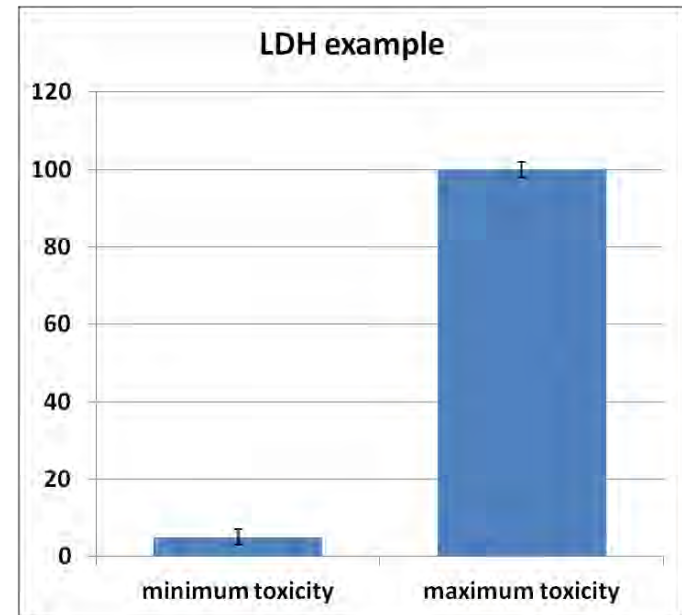


Lactate dehydrogenase (LDH)



quantification with enzymatic assay

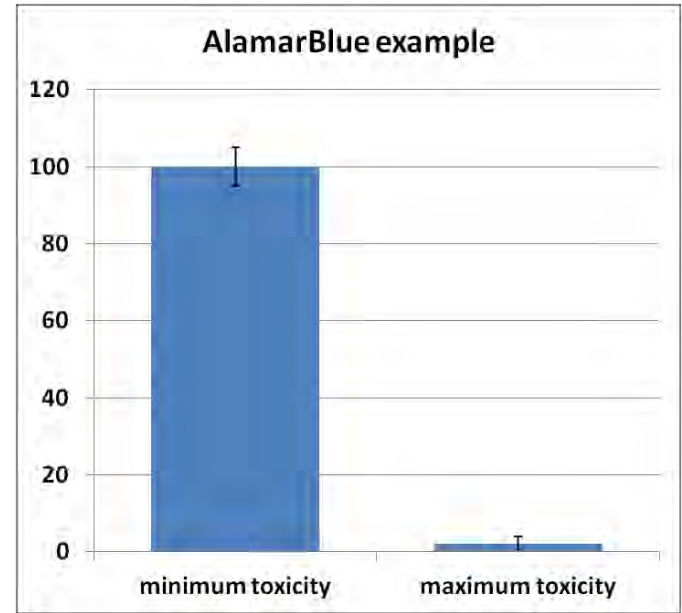
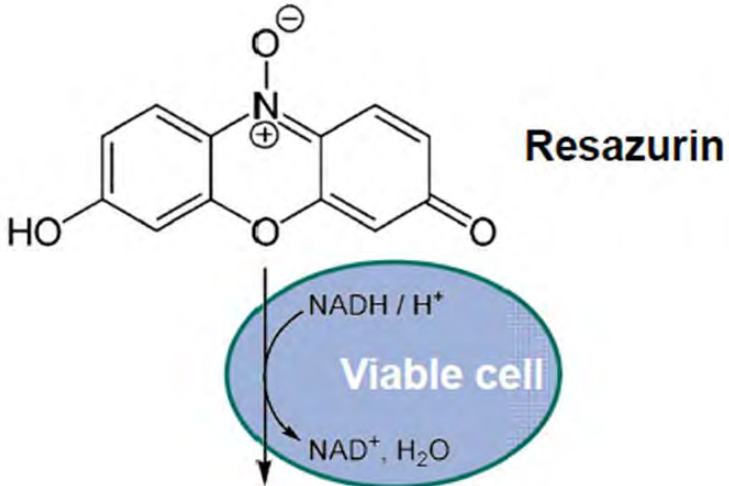
**→ reflects amount of cells with damaged membrane (=dead)**



**high red intensity = high LDH release**

# Rapid at-site assays for cytotoxicity: Determination of dilution-factor

## AlamarBlue Assay



AlamarBlue  
LDH

→ reflects metabolic activity

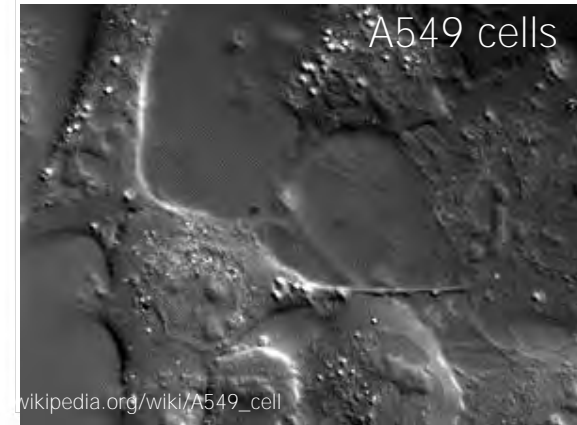
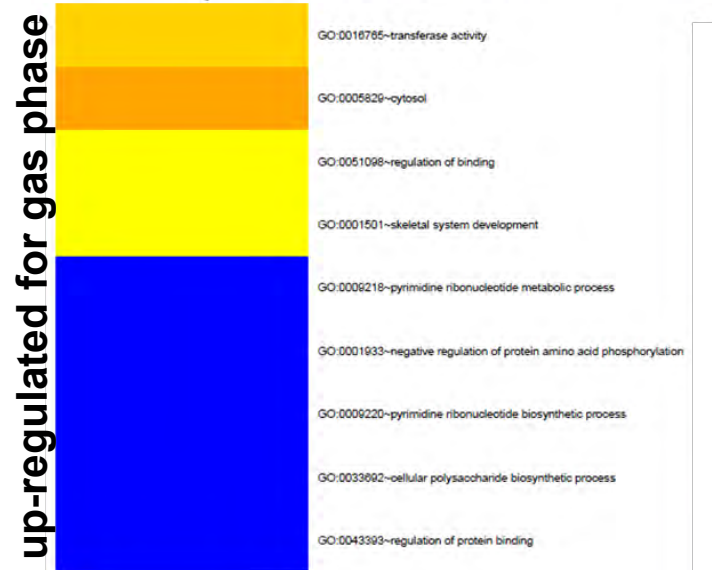
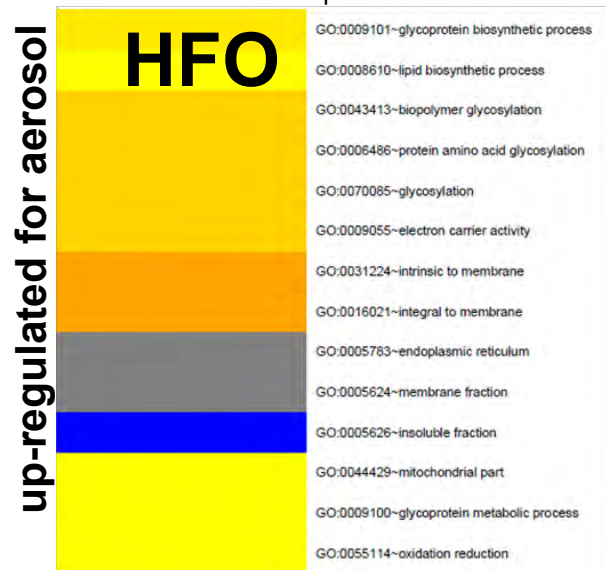
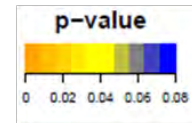
LFO → 1:40  
HFO → 1:100

Low viability = high toxicity

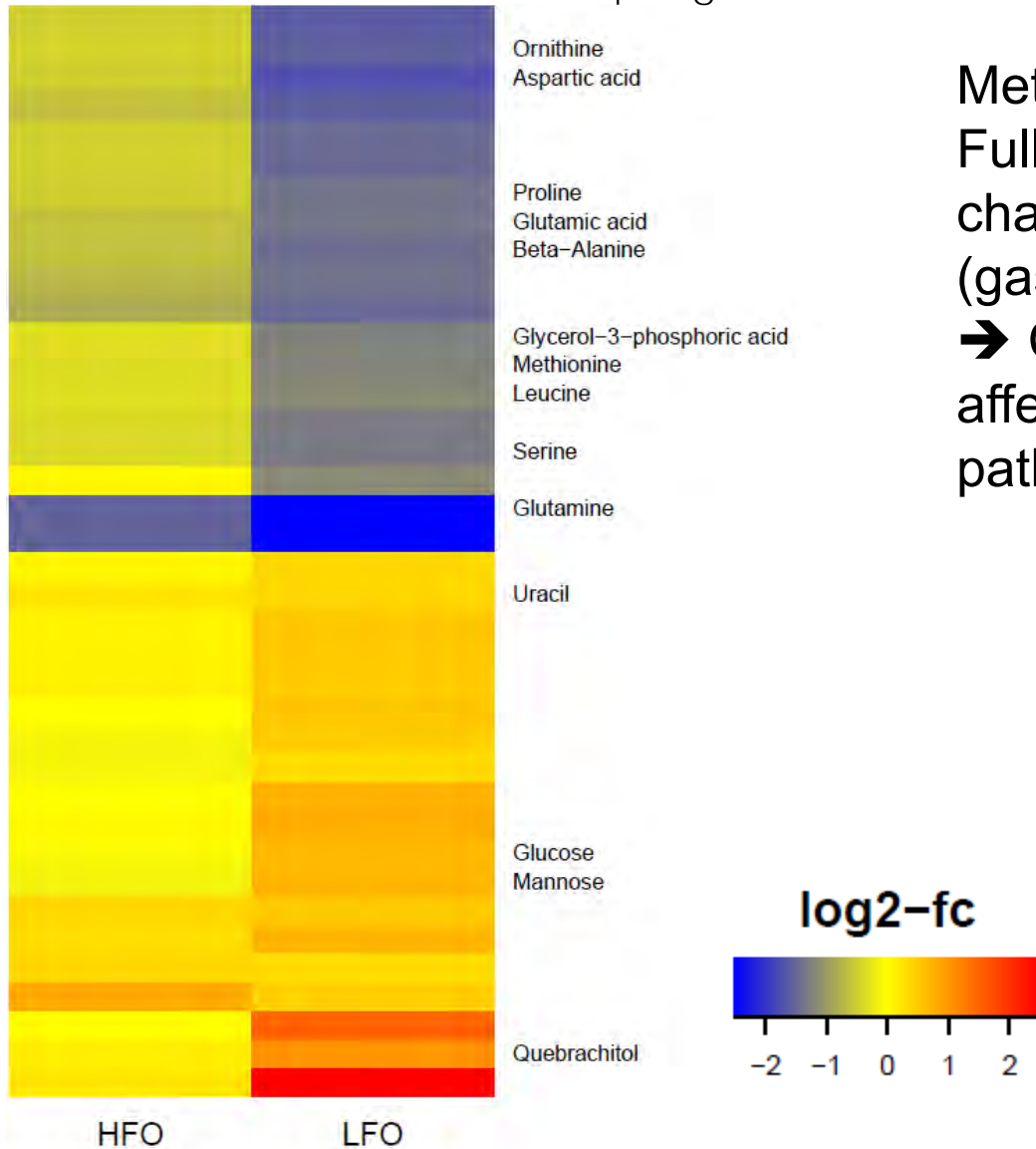
pink = high viability; blue = low viability



Cell Line: A549 - adenocarcinomic human alveolar basal epithelial cells



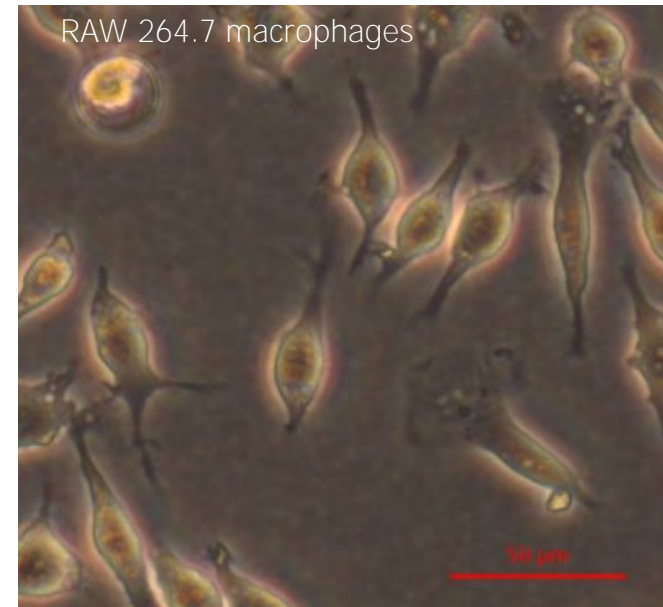
# S 2B - RAW 264.7 murine macrophages



## Metabolomics:

Full aerosol induces stronger changes than the filtered aerosol (gas phase).

→ Central C-metabolism is not affected (labeling!) but other pathways are significantly regulated.

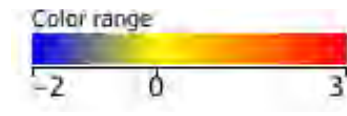




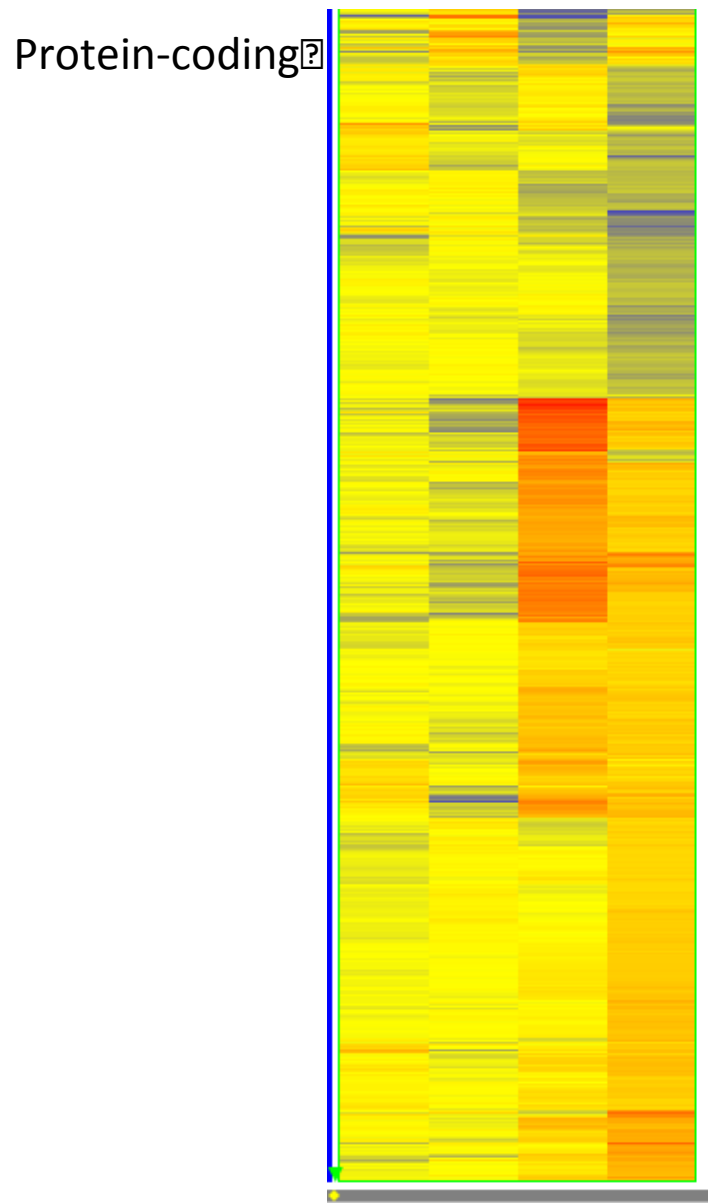
Gene-Name	Gene-Function	associated with
<b>IL1</b>	Regulation of immune responses, inflammatory reactions, and hematopoiesis	<b>Inflammation</b>
<b>CD4</b>	Co-receptor assisting the T cell receptor (TCR) in communicating with an antigen-presenting cell, Inflammation	
<b>GLYAT</b>	Detoxification of a wide range of xenobiotic and endogenous metabolites	<b>Xenobiotic metabolism</b>
<b>CYP1A1</b>	Xenobiotic and drug metabolism, Metabolic activation of aromatic hydrocarbons (polycyclic aromatic hydrocarbons, PAH)	
<b>CYP3A4</b>	Catalyze many reactions involved in drug metabolism	
<b>CYP26C1</b>	Catabolism of all-trans- and 9-cis-retinoic acid, and thus contributes to the regulation of retinoic acid levels in cells and tissues	
<b>CYP26A1</b>	Regulates the cellular level of retinoic acid which is involved in regulation of gene expression in both embryonic and adult tissues	
<b>CYP11B1</b>	Involved in the conversion of 11-deoxycortisol to cortisol in the adrenal cortex	
<b>SULT1E1</b>	Catalyze the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds	
<b>IGF1</b>	Important role in childhood growth and continues to have anabolic effects in adults	<b>Cancer induction</b>
<b>IGF2</b>	Major fetal growth factor	
<b>Jun</b>	Cell cycle progression and anti-apoptotic activity	
<b>Casp10</b>	Execution-phase of cell apoptosis	<b>Apoptosis</b>
<b>CRADD</b>	Induction of cell apoptosis	
<b>HRK</b>	Activator of apoptosis	
<b>CASP7</b>	Apoptosis	
<b>MAPK10</b>	Regulatory roles in the signaling pathways during neuronal apoptosis	
<b>NFKB</b>	Immune response, Cell proliferation, Cell death	
<b>CYCS</b>	component of the electron transport chain in mitochondria, initiation of apoptosis	
<b>CASP3</b>	execution-phase of cell apoptosis	



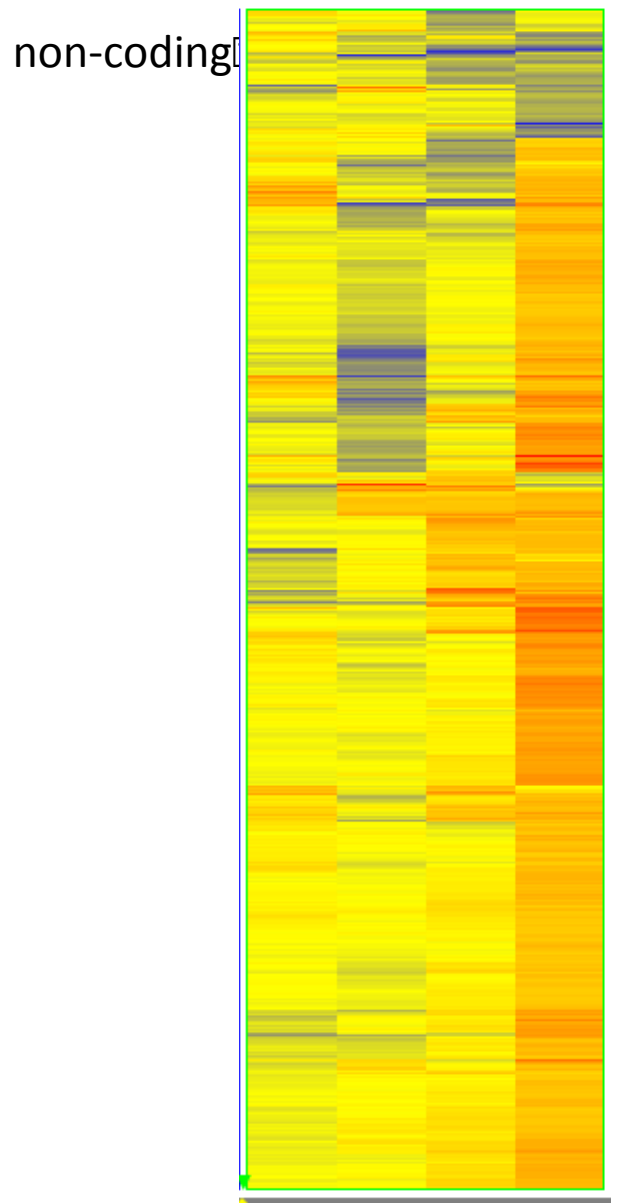
# Global analysis of regulated genes ( $\geq 1.5$ -fold)



Gas HFO Particle HFO HFO HFO  
HFO HFO HFO HFO



Gas HFO Particle HFO HFO HFO  
HFO HFO HFO HFO

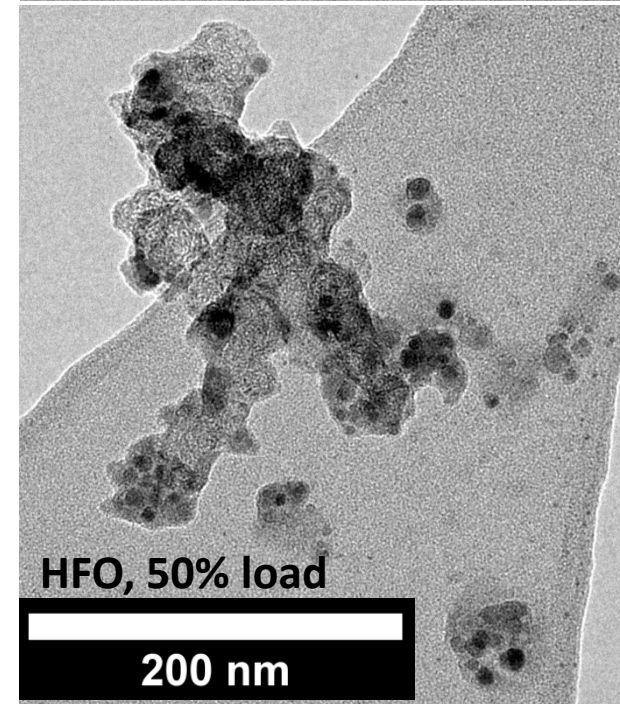
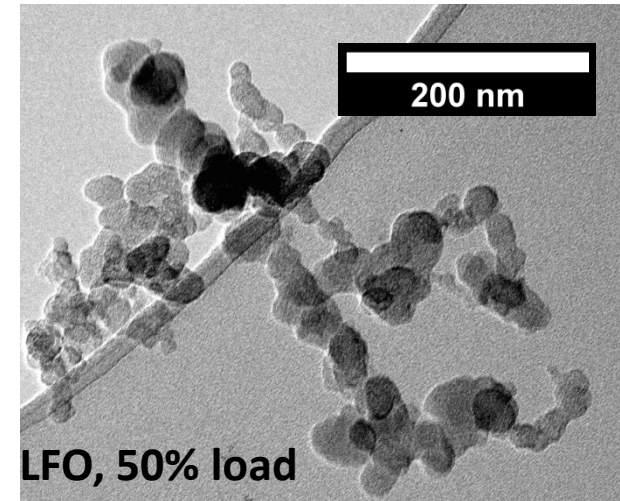
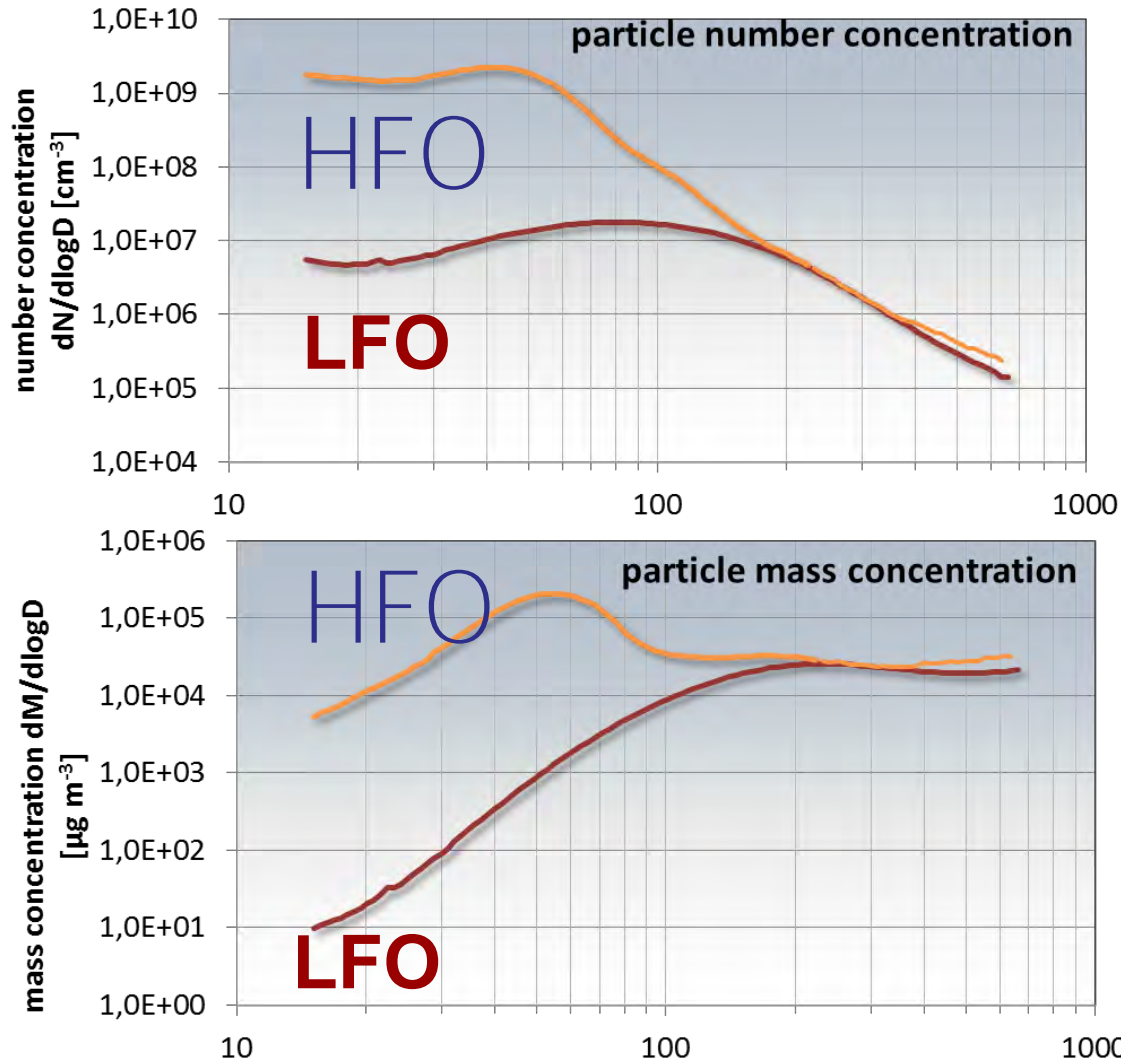


Gas referred to clean air;  
Complete aerosol referred to Gas (=particle)

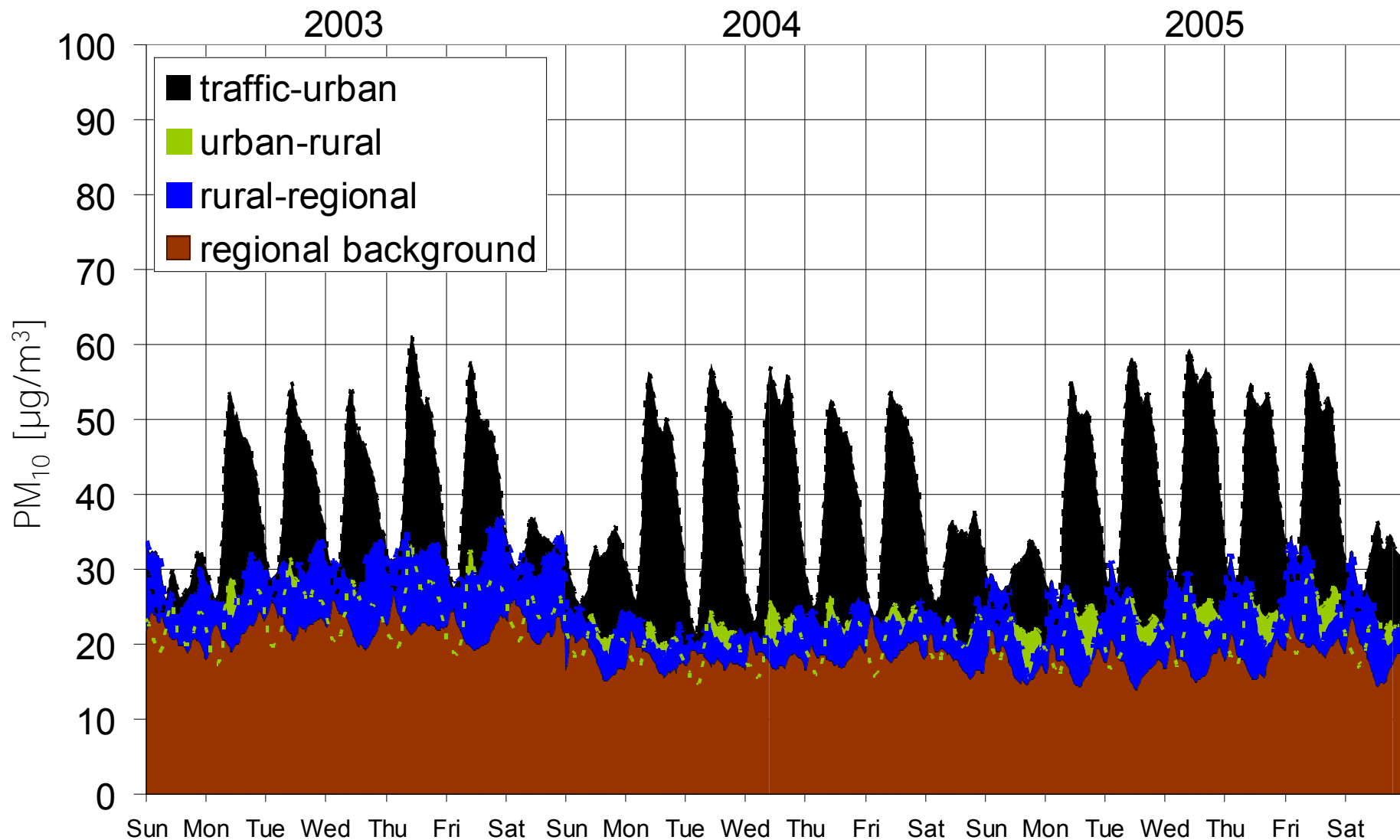
# Particles: Measured Emissions

- at ALI-exposure system -

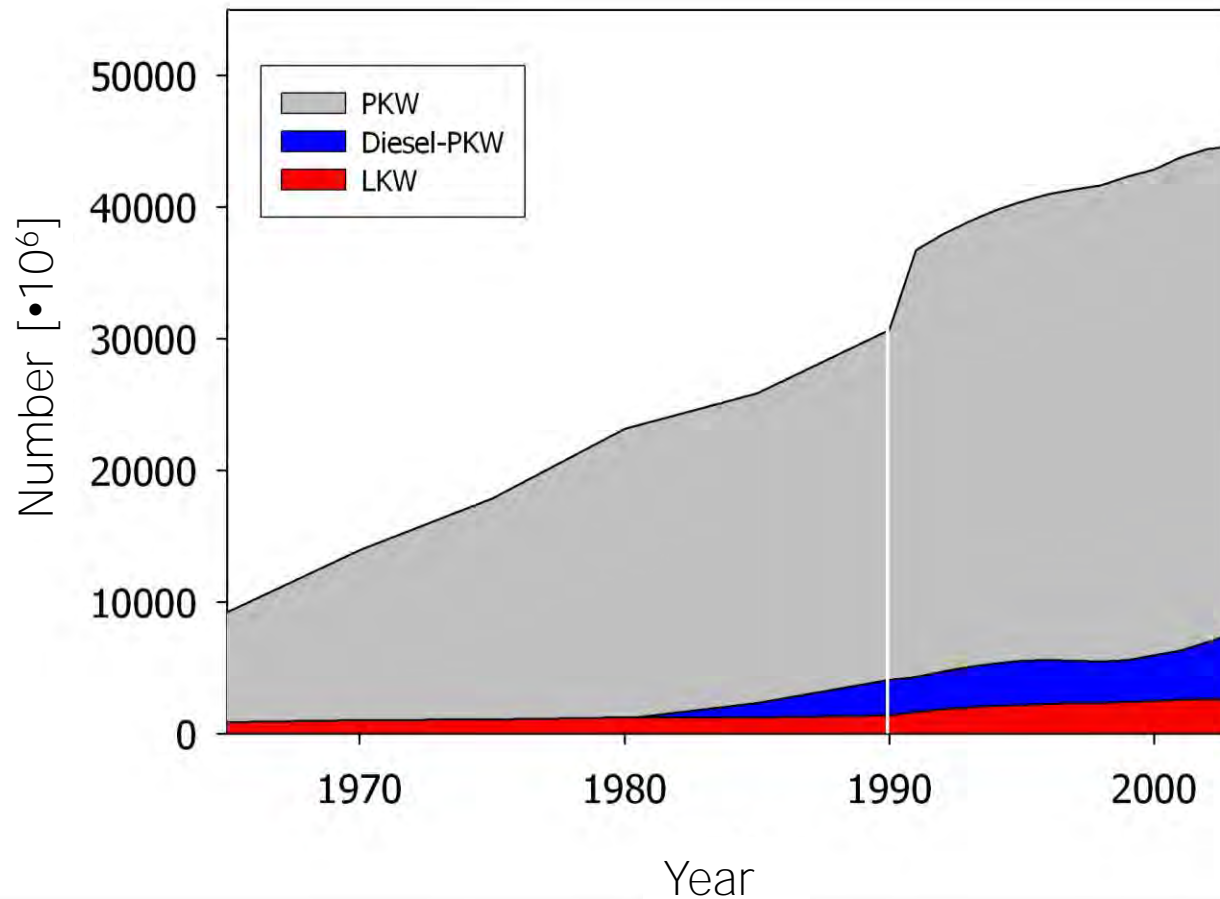
## General parameters: Size distribution



# Diurnal Variation: Bremen (traffic site)



# Vehicle development in Germany







PALFINGER  
700 870000





**HICE Aerosols and Health**  
Helmholtz Virtual Institute of Complex  
Molecular Systems in Environmental Health

Universität  
Rostock  Health in Env  
JOINT MASS E

KIT



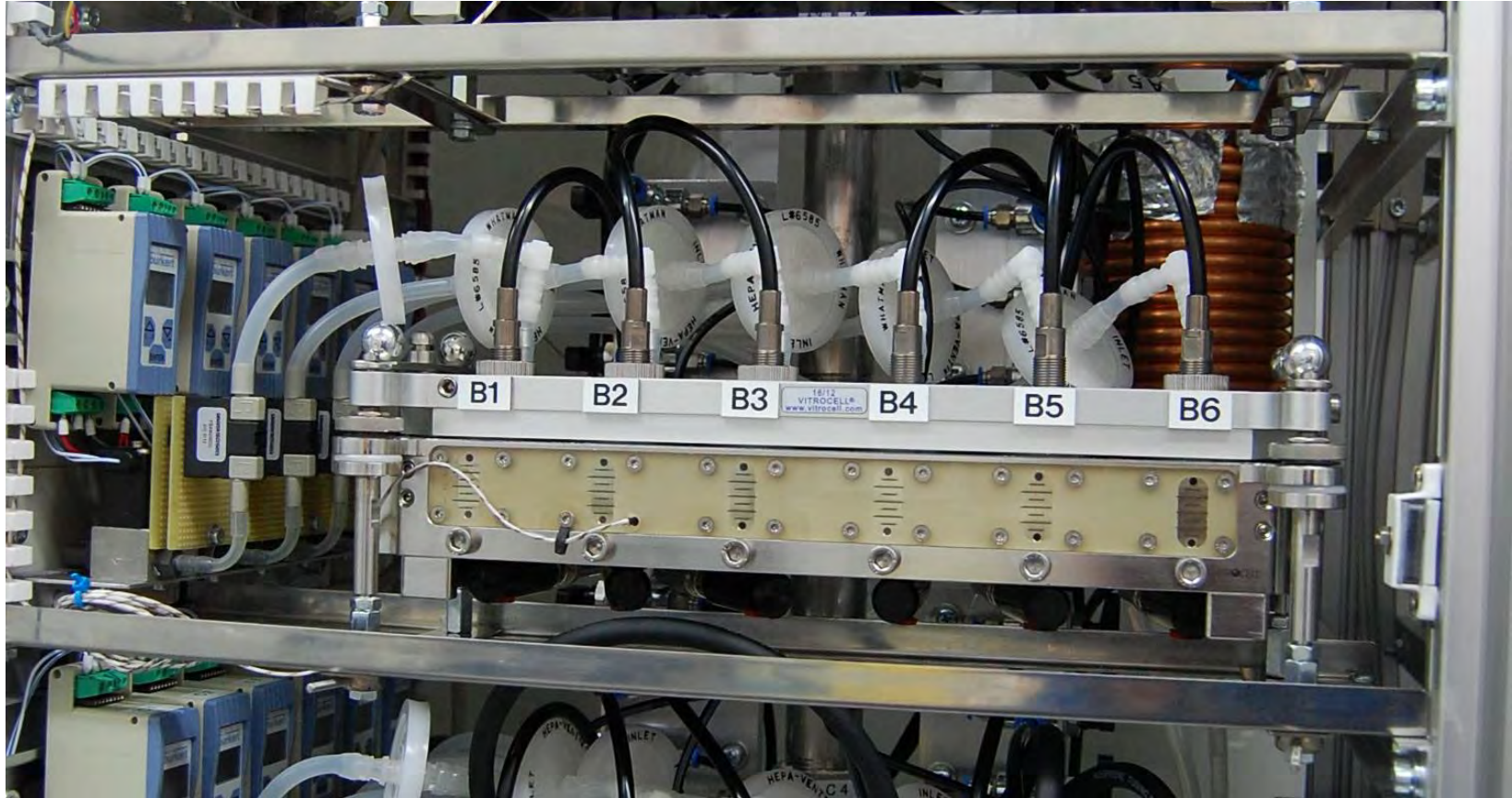


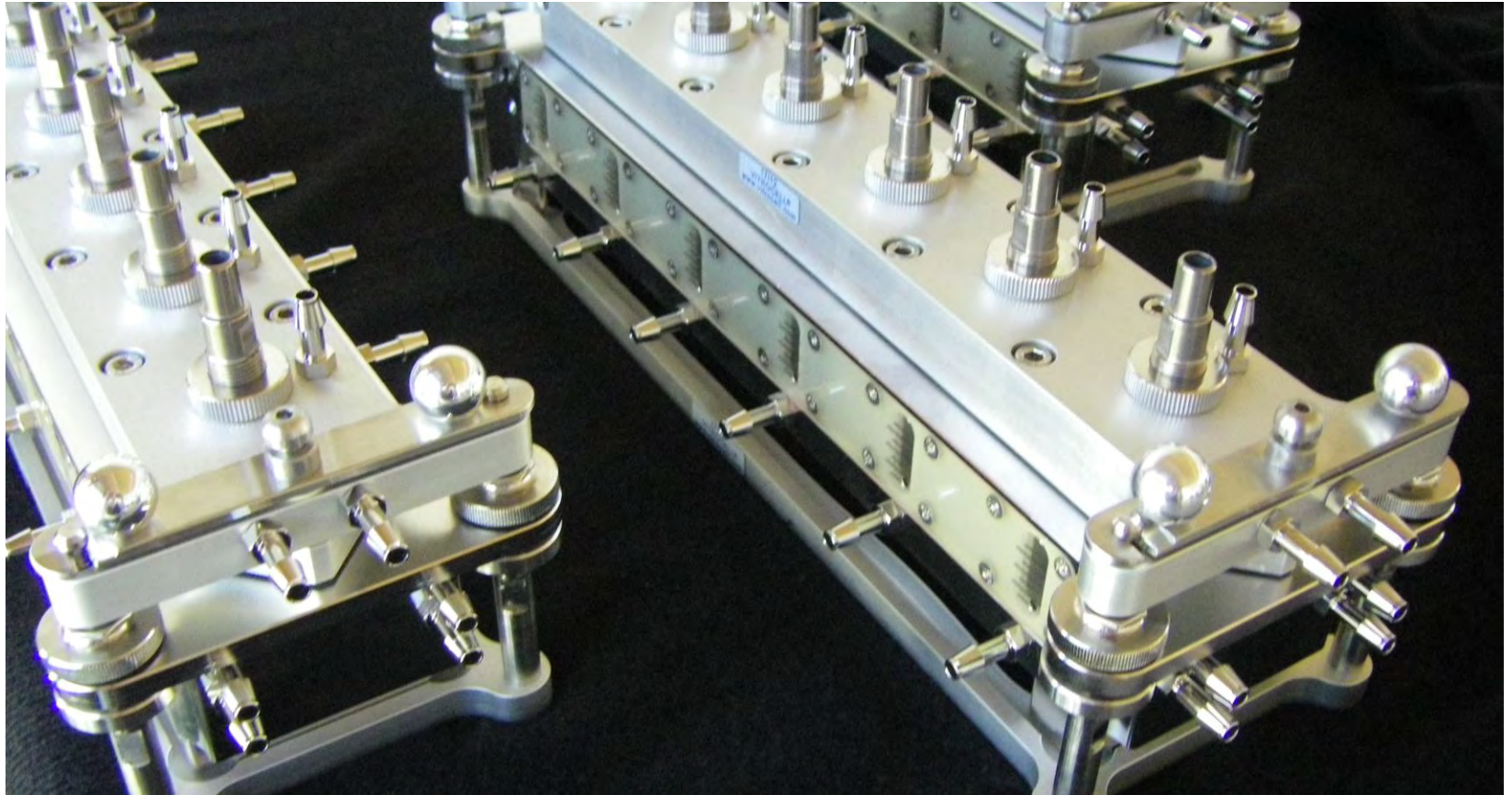






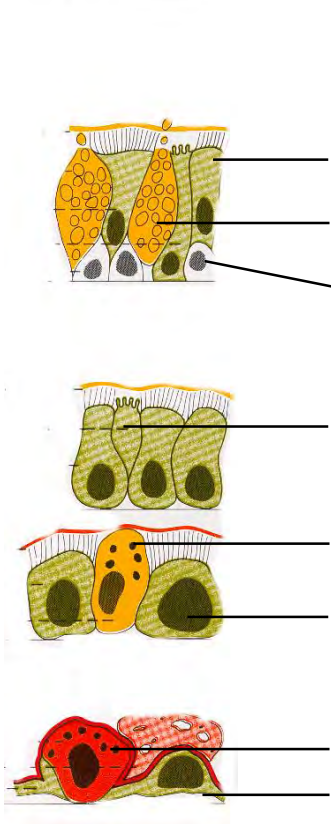
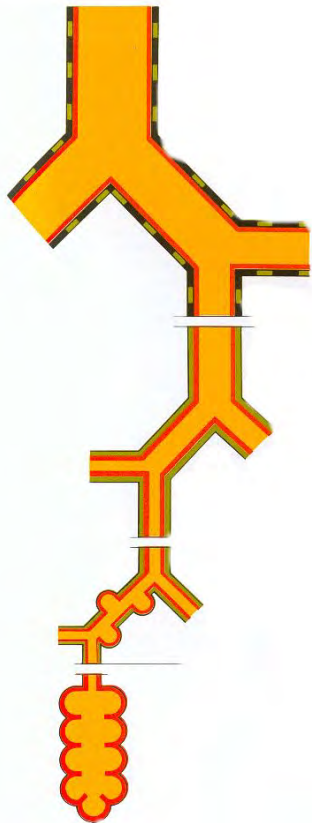






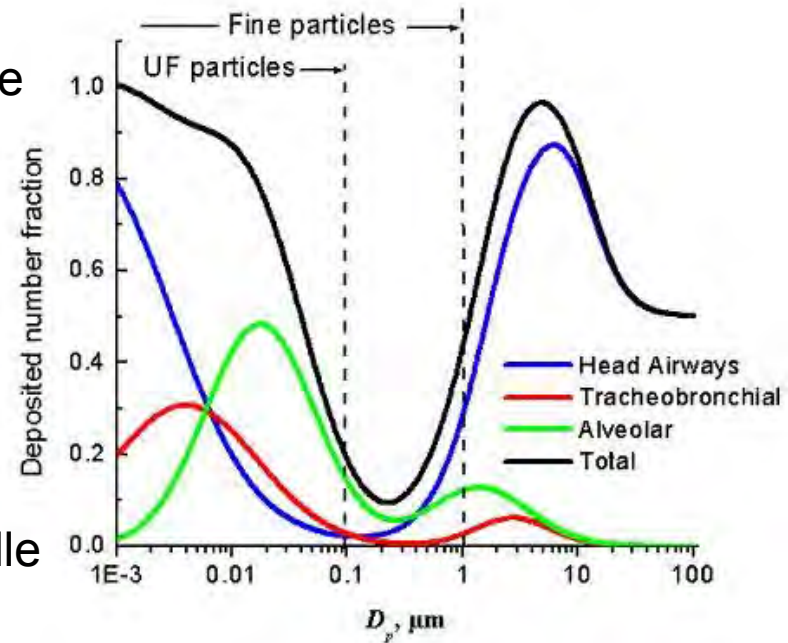


# Respiratorisches Epithelium



- zyl. Flimmerzelle
- Becherzelle
- Basalzelle
- Bürstenzelle
- Clara-Zelle
- kub. Flimmerzelle
- Typ II - Zelle
- Typ I - Zelle

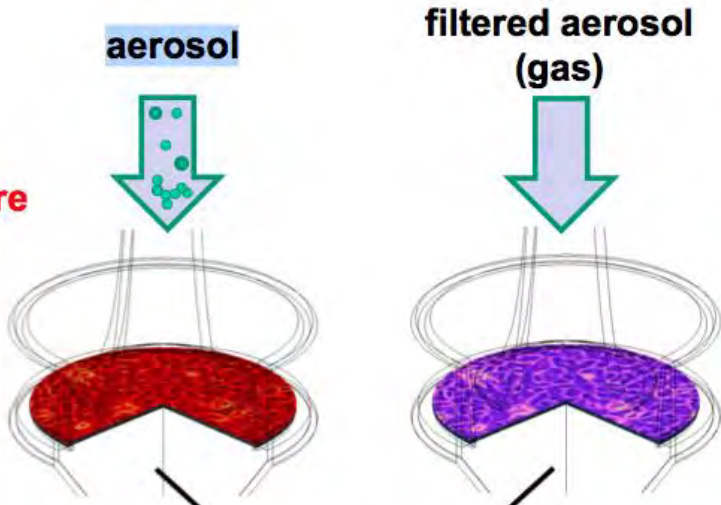
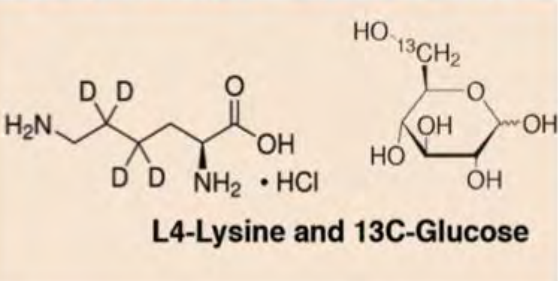
# Deposition von Partikeln



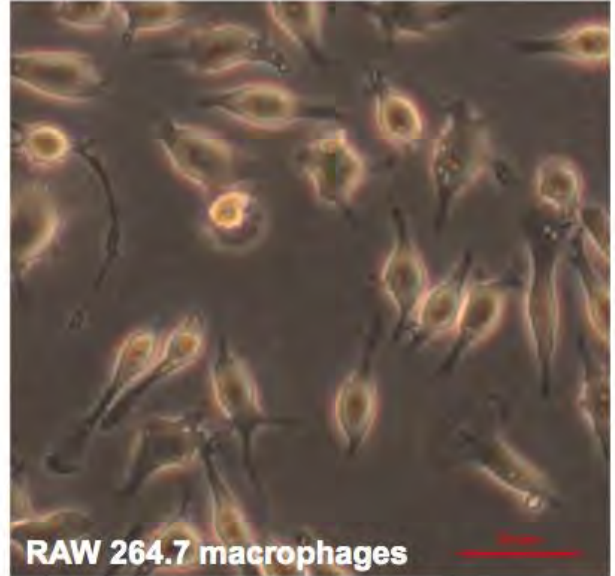
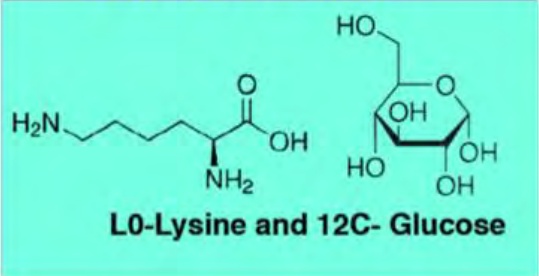
D=Diameter, UF=Ultrafeinstaub

Quelle Figur: Prof. N. Krug, pers. Mitteilung

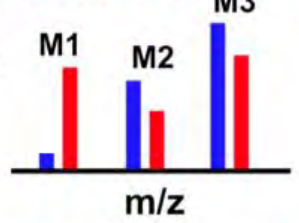
**Stable isotope labeled cell culture**



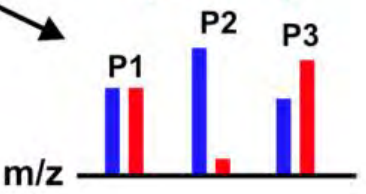
**Native cell culture**



**Metabolomics**



**Proteomics (SILAC)**



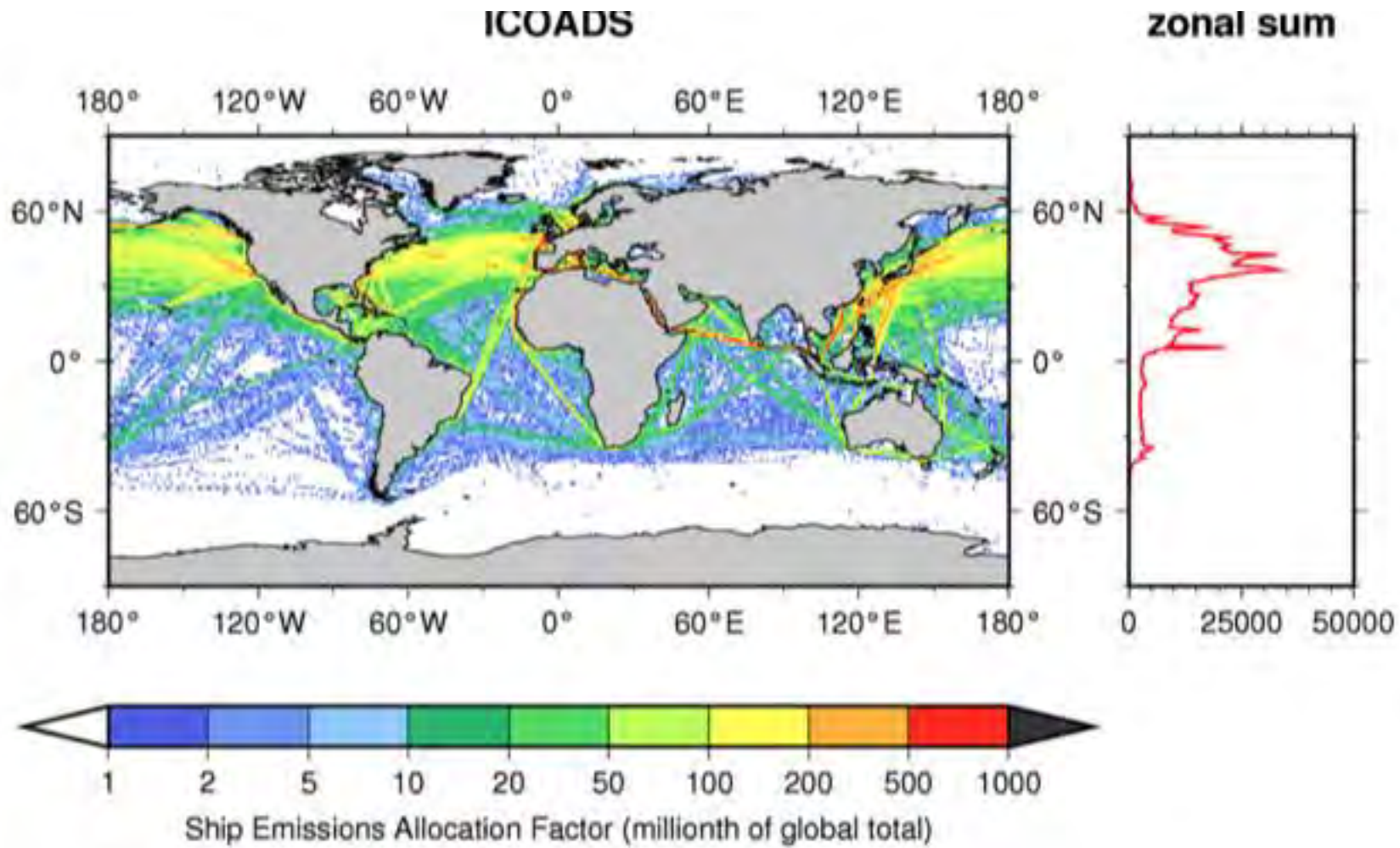
# Ship diesel exhaust components

Component	Heavy Fuel *	Diesel**
SO <sub>2</sub>	400-550ppm	1-10 ppm
NO <sub>2</sub>	1-10 ppm	61-50ppm
CO	50-300 ppm	70-550 ppm
NO <sub>x</sub>	600-750 ppm	600-700 ppm
PM (TSP)	665.0 µg/m <sup>3</sup>	14.4 µg/m <sup>3</sup>
THC	150-270 ppm	200-350 ppm

\* 1.6% Sulphur (world average 2.7%, max. 4.5%)

\*\* Diesel according DIN EN 590: <10 ppm Sulphur

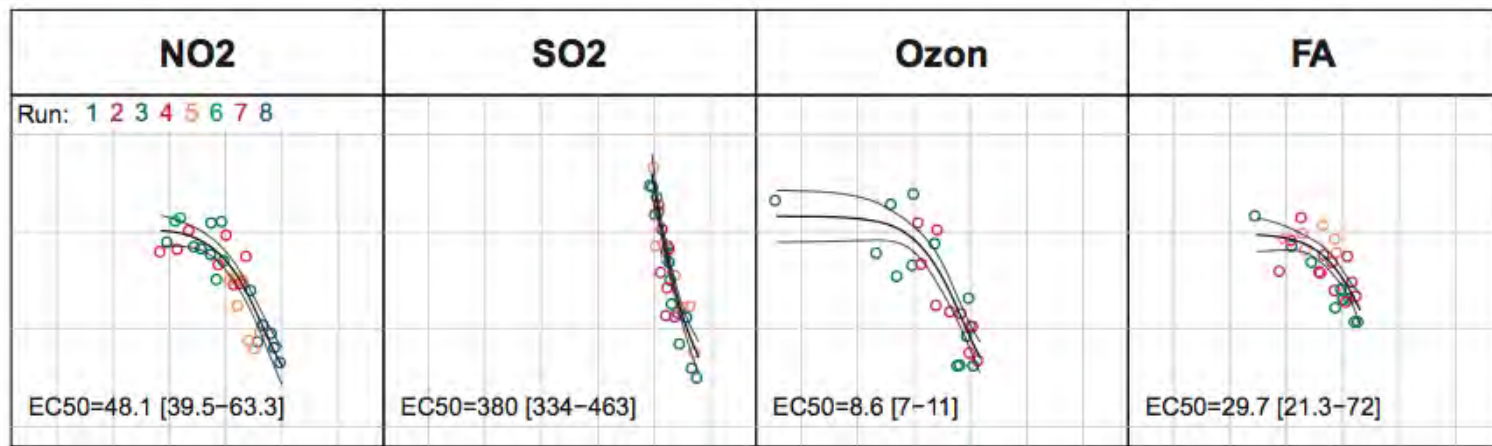




# Toxicity in at the Air Liquid Interface

- 1hr exposure, BfRA -

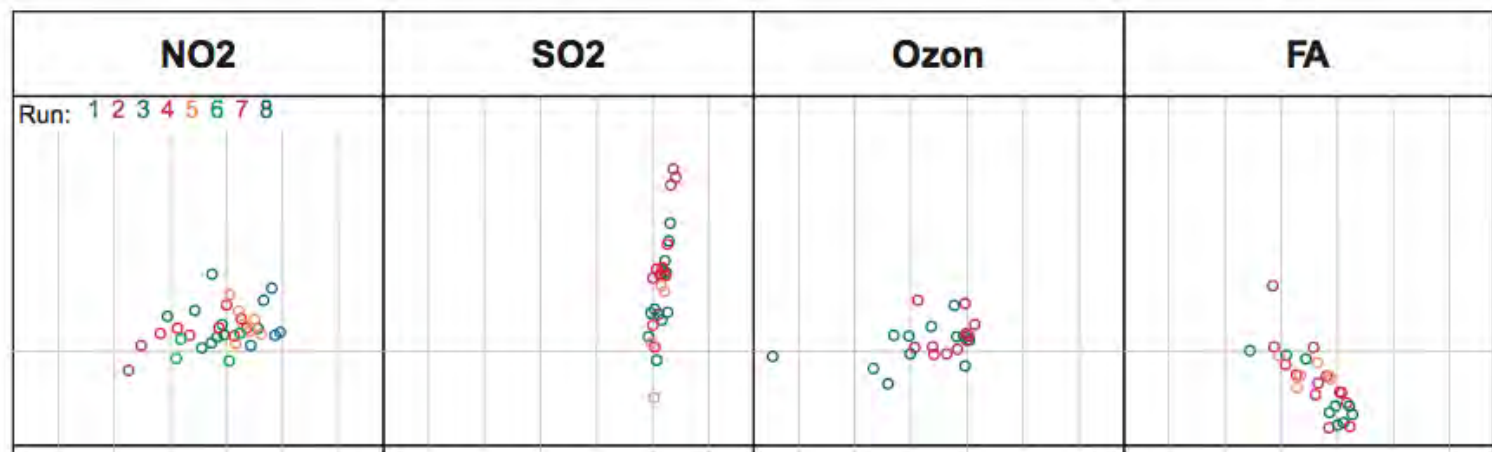
Current Exclusion Method



0.01 1 100ppm

FA= formaldehyde

Comet Assay

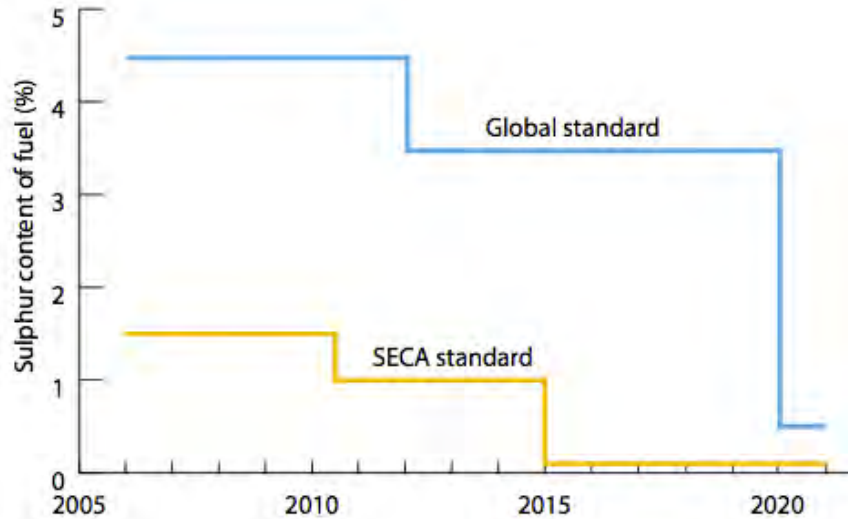


0.01 1 100ppm

Smirnova et al, BfRA 2012

# Regulations of fuels

## Ship-fuel



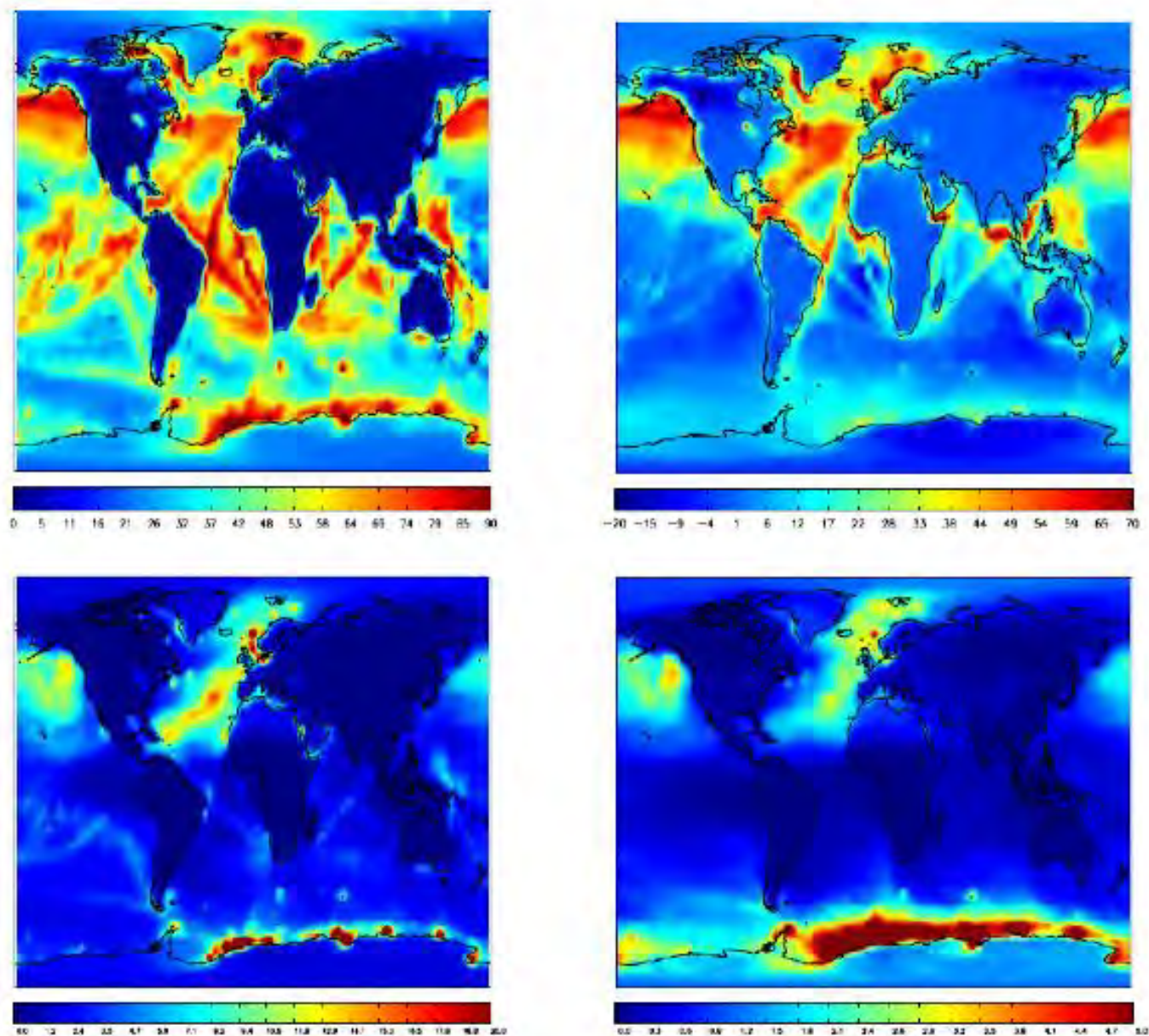
## Automotive-fuel DIN EN 590 \*



Abgasnorm	spätestens	Schwefelgehalt	Cetanzahl
Euro 1	1. Januar 1993	max. 0,200 %	min. 49
Euro 2	1. Januar 1996	max. 0,050 %	min. 49
Euro 3	1. Januar 2001	max. 0,035 %	min. 51
Euro 4	1. Januar 2006	max. 0,005 %	min. 51
Euro 5	1. Januar 2009	max. 0,001 %	min. 51
Euro 6	1. Januar 2014		

\* < 10ppm Sulphur

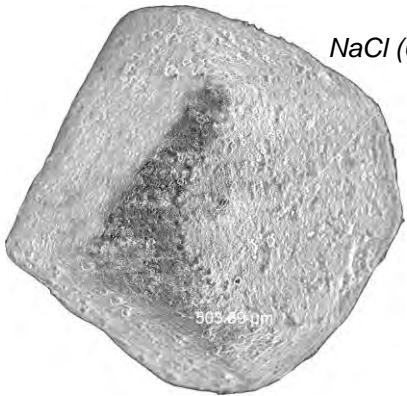




**Fig. 7.** Yearly average contribution (%) from all ships+ports to NO<sub>2</sub> (upper left) SO<sub>2</sub> (upper right), hydrophobic organic carbon (lower left) and hydrophilic organic carbon (lower right).

# Sources of particles

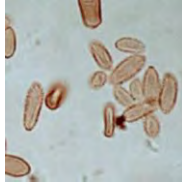
NaCl (0,7mm)



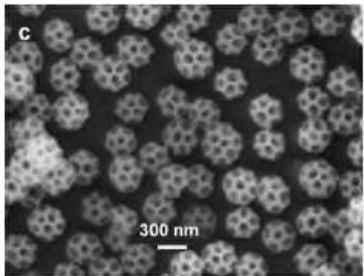
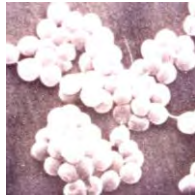
## Biogenic

- Spores
- Bacteria
- Sea salt
- Bronchosomes
- Crustal materiall (Sand etc.)
- Combustion of vegetable Material
- Pollen

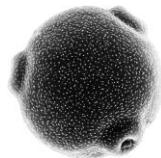
Mold spores



Bacteria

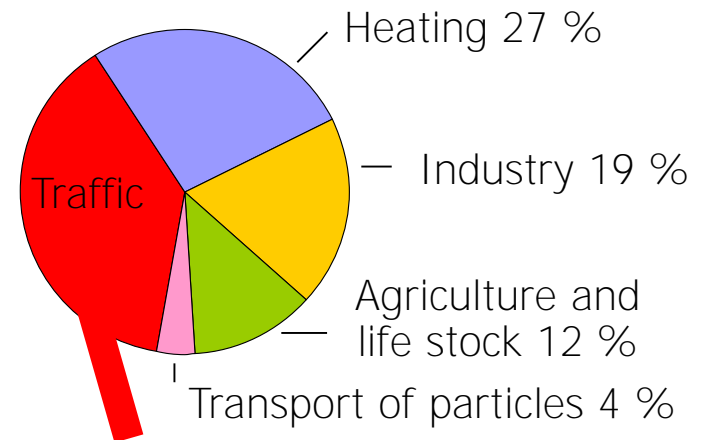


Bronchosomes



Birch pollen

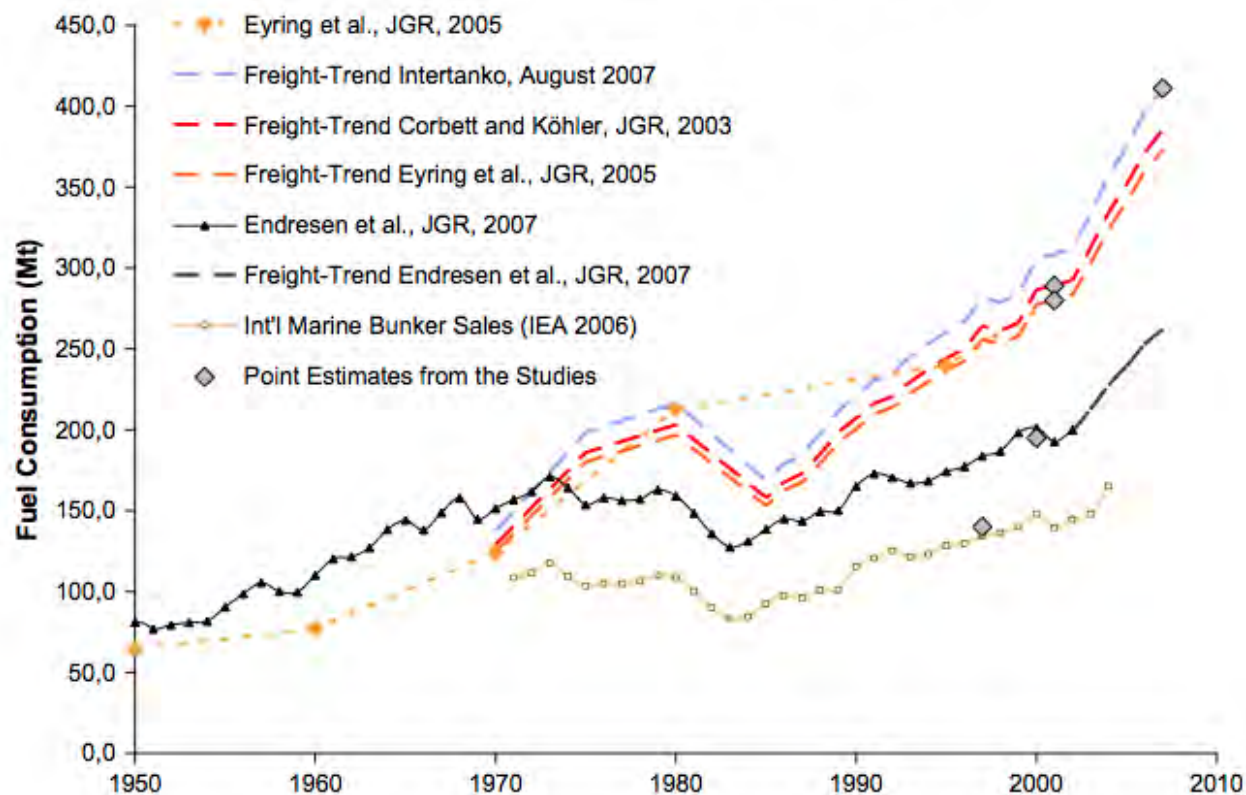
## Anthropogenic



Source: Emissionskataster Bayern, 2004

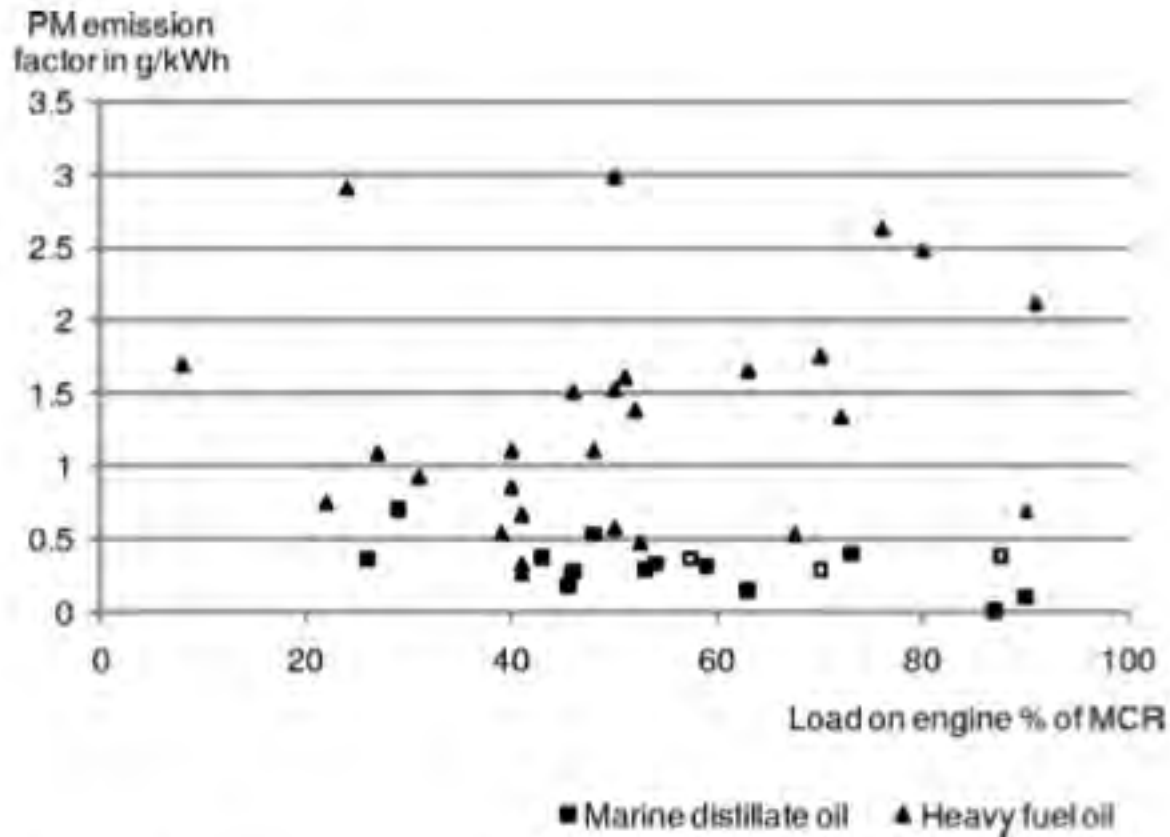
- Traffic:
  - 26% Off-road Diesel
  - 63% On-road vehicles (of those ca. 70 % Dieselfahrzeuge)





**Fig. 5.** World fleet fuel consumption (civilian, military, and auxiliary) and international marine bunker fuel statistics in Mt from different estimates. The symbols indicate the original estimates for individual years and the solid lines show the original trend estimates from these studies. The dashed lines show the back- and forecast calculated from the time evolution of freight ton-miles with the point estimates from 2001 (Corbett and Köhler, 2003; Eyring et al., 2005a), 2000 (Endresen et al., 2007), 2007 (Gunner, 2007), and 1997 (IEA, 2006) taken as the reference year.

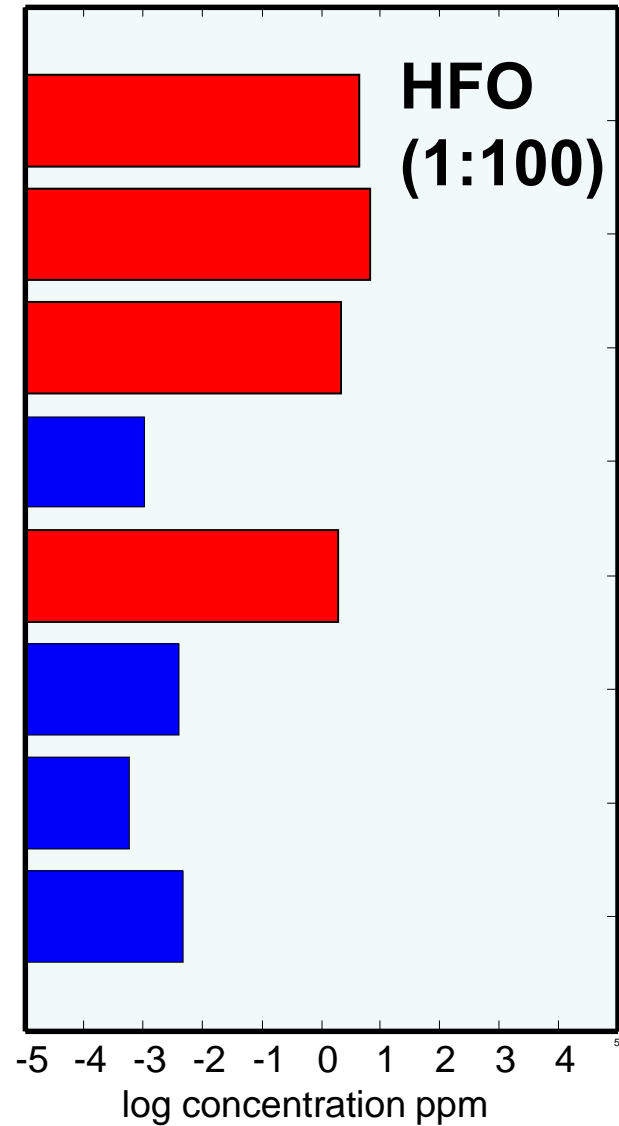
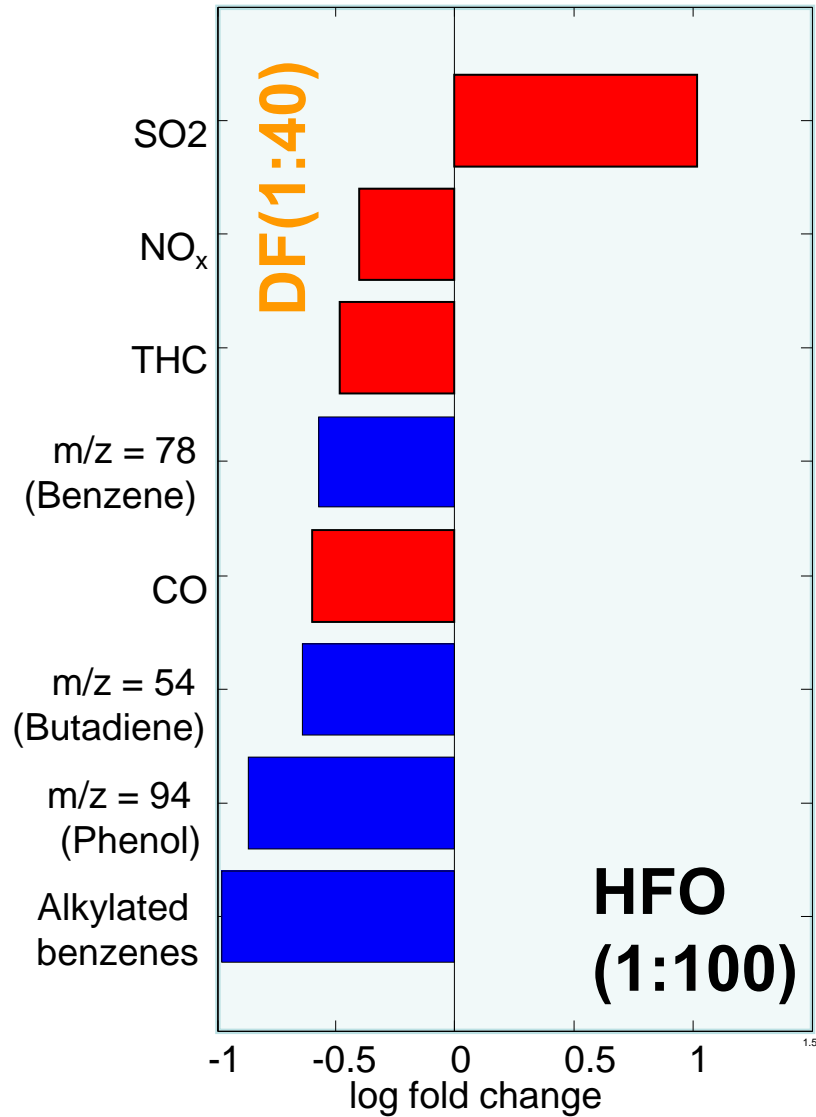
## Winnes and Fridell



**Figure 3.** PM emission factor as a function of engine load. Results from four prior studies and the presented measurements. The values from the presented measurements are indicated with open markers.

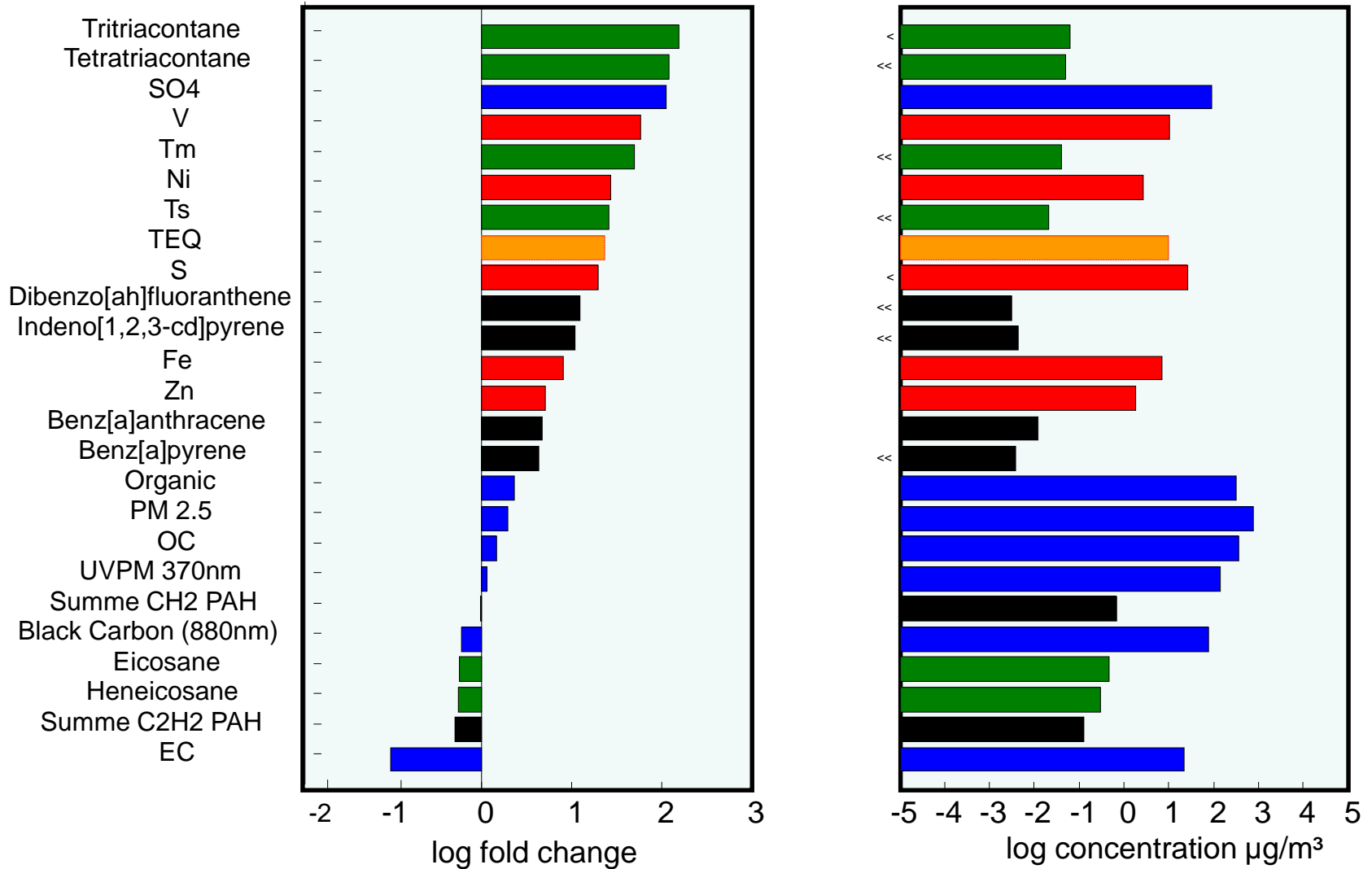
# Measured Emissions

- at ALI-exposure system -



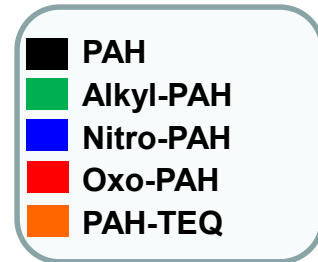
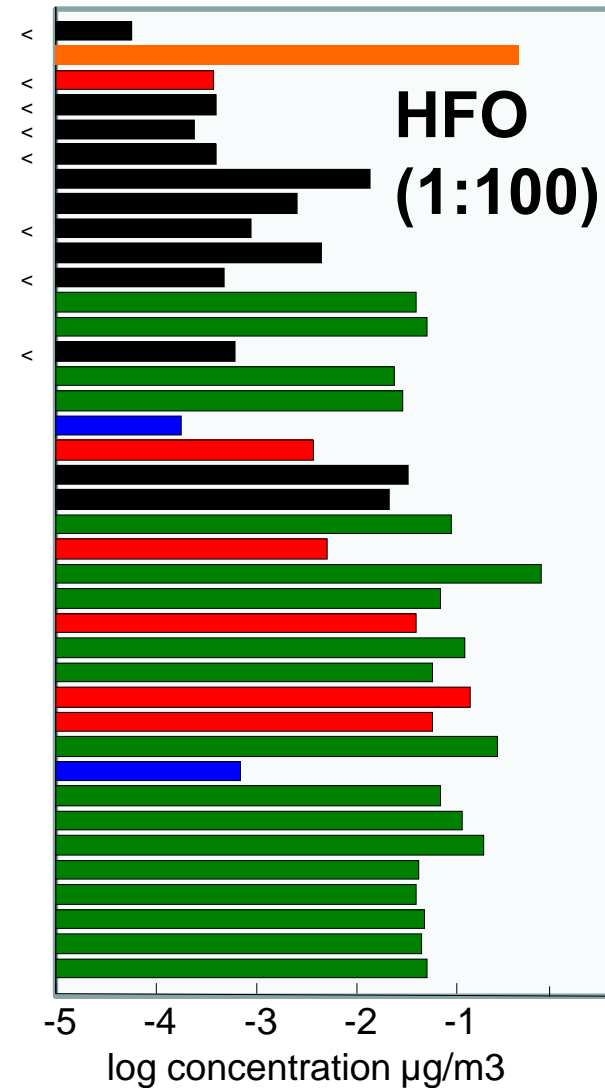
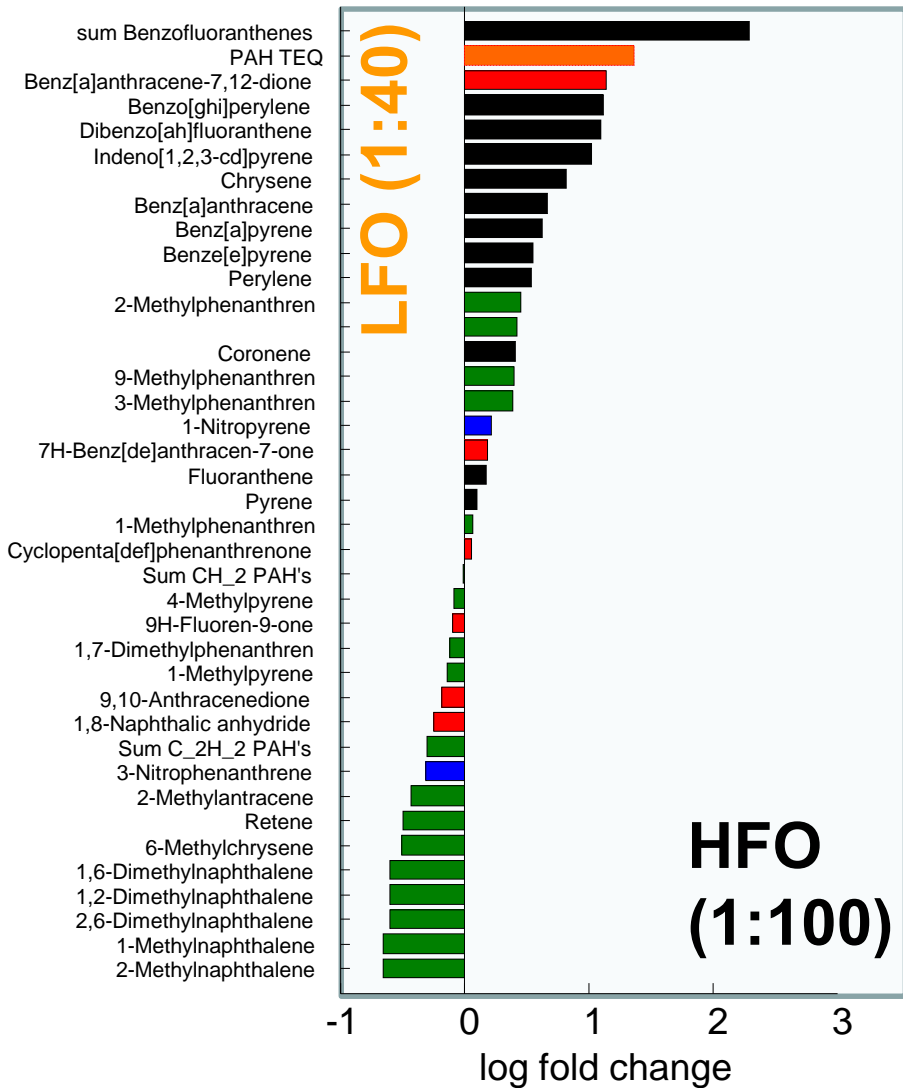
# Measured Emissions

- at ALI-exposure system -



# Measured Emissions

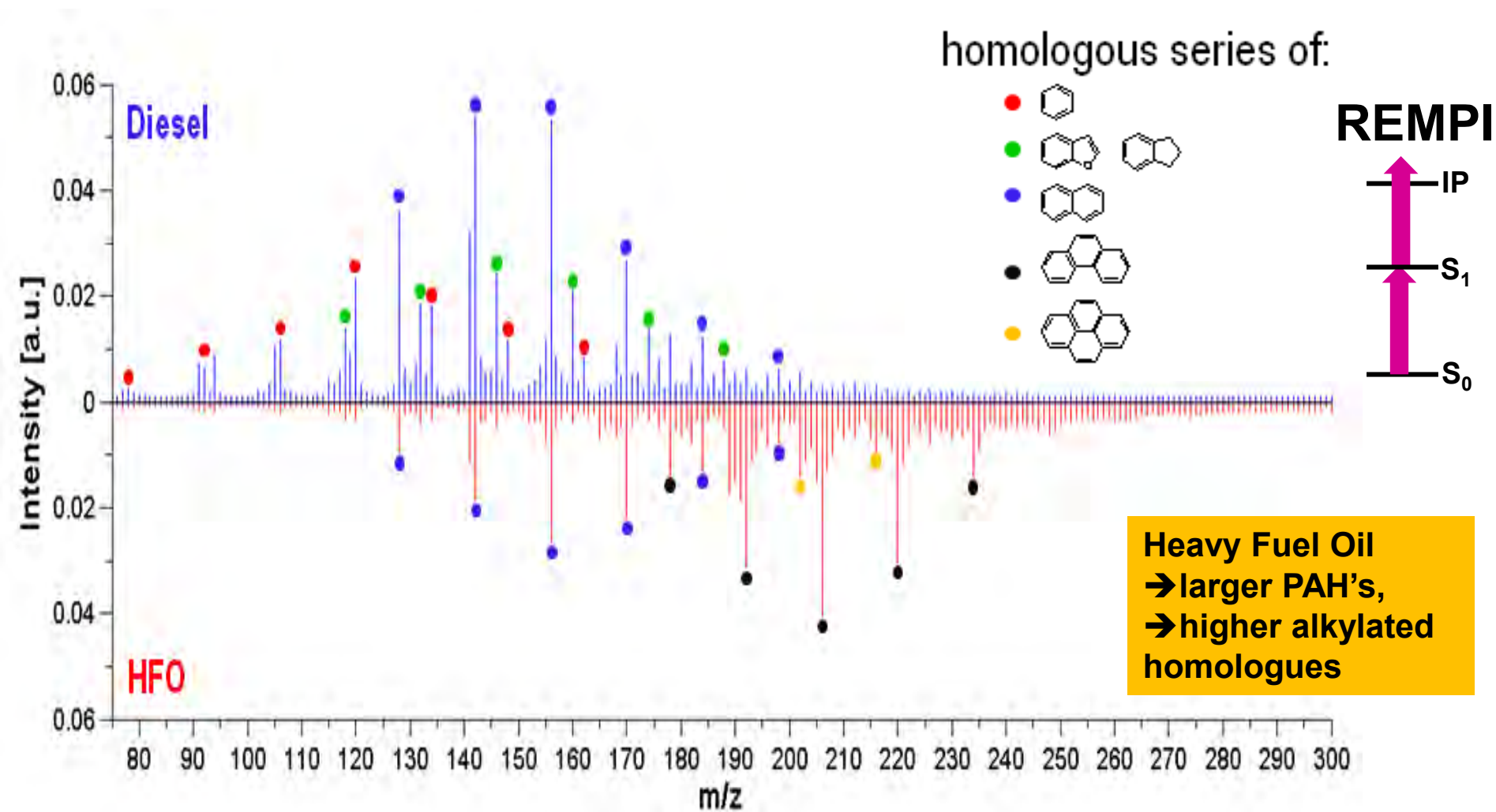
- at ALI-exposure system -



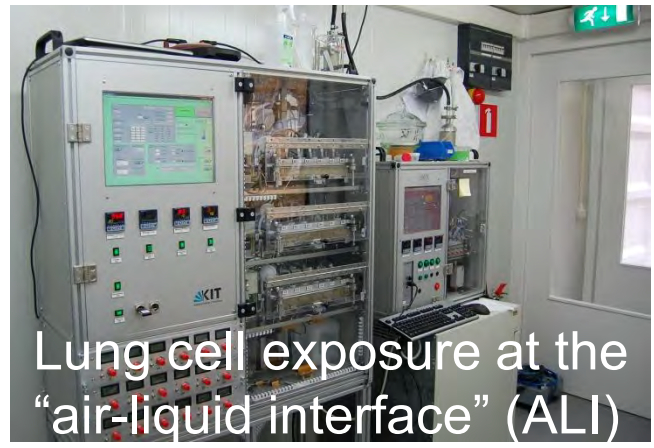


# Measured Emissions

- at ALI-exposure system -

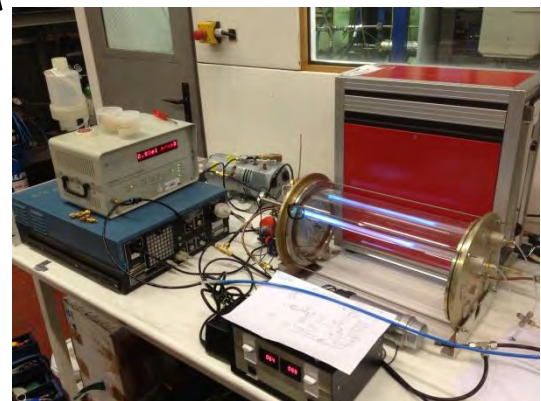
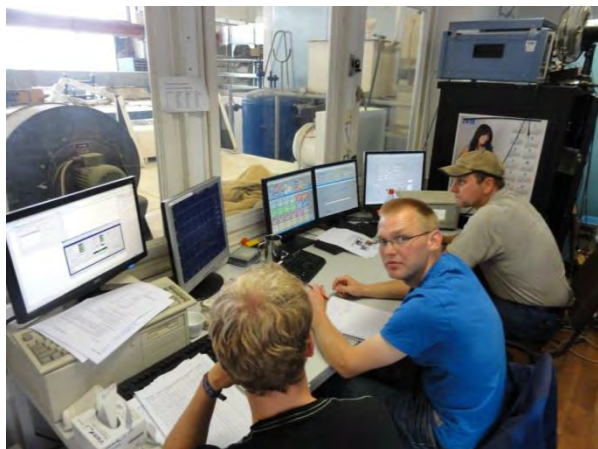
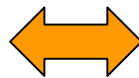
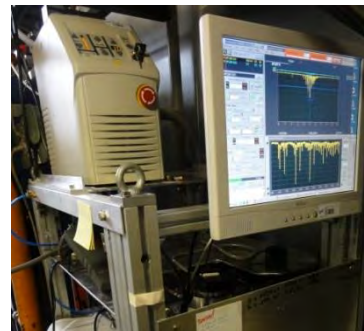
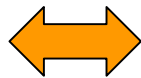


# HICE ship diesel engine campaign: Exposure of human lung cell assays





# HICE ship diesel engine campaign: Aerosol characterization



# Particle deposition in the lung

