

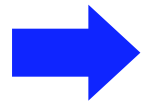
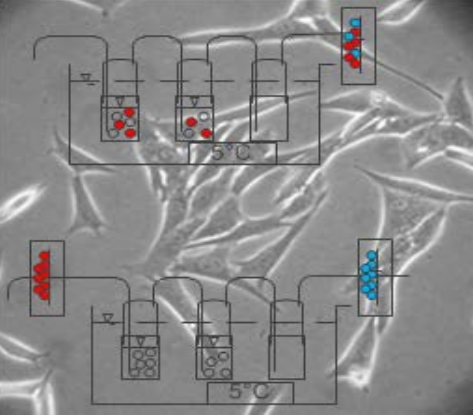
A simple sampling method to analyze cell toxicity of PM & COC from biomass combustion

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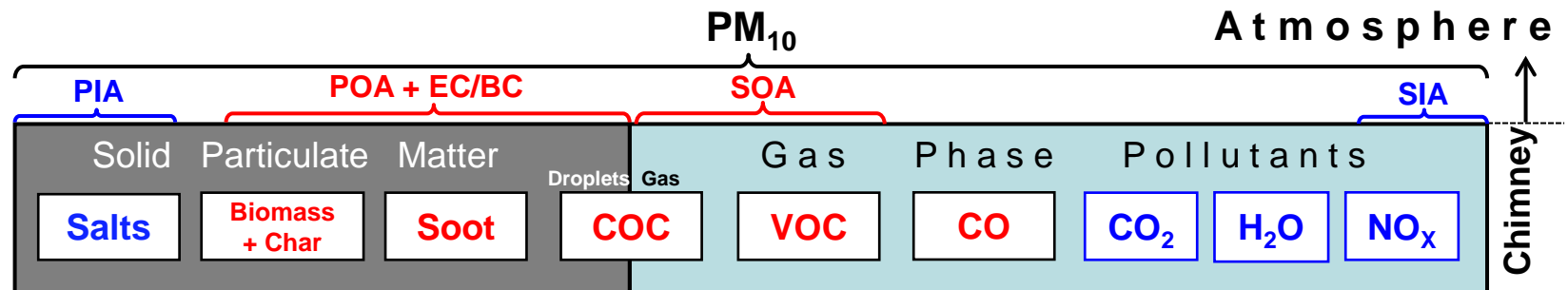
20th ETH-Conference on Combustion Generated Nanoparticles
Zürich, June 15 2016



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Introduction - Background

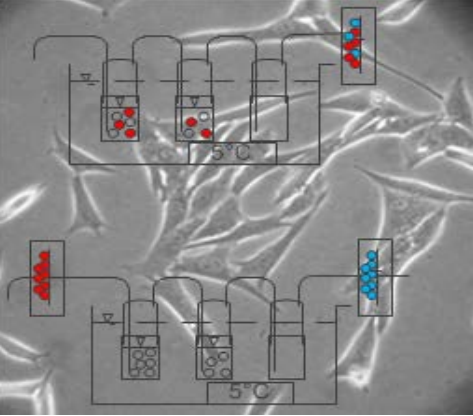
- Wood combustion is a renewable energy source, however it contributes to air pollution and negatively impacts human health
 - Wood combustion contributes > 30% to carbonaceous matter in Europe and is the dominant source in Swiss Plateau and Alpine regions (Sandradewi et al., 2008; Lanz et al., 2010)
 - Fine particulate matter (PM) is linked to cardiovascular diseases, allergic & inflammatory conditions of the lung and increased mortality
- Wood combustion emissions:



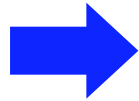
- In current emission legislation only solid PM in the hot flue gas is considered
- SOA and COC can exceed primary emissions

Introduction - Target

- Develop a method to characterize the cytotoxicity of wood combustion emissions, in particular with respect to COC
- The method should be simple to enable economically analysing a large number of samples for a comparison of many different combustion devices and conditions
- In addition, the effects of COC and solid PM shall be distinguished



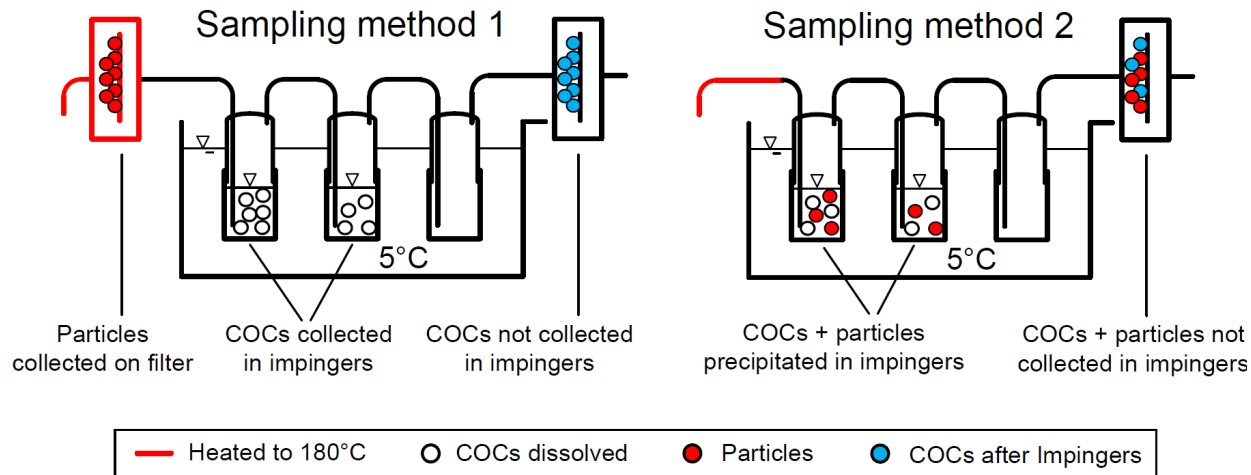
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Methods – Development Approach

- Combine standard sampling procedures
 - *US EPA 5H for PM and COC with quenching of hot flue gas into water*
 - *VDI 2066 for solid PM on a heated filter (e.g. for Swiss OAPC)*
- Sampling in the Bioenergy laboratory in Horw
- Subsequent *in vitro* cell analysis in Biomedical laboratory in Hergiswil

Methods – Sampling



Picture of Impingers



- Impinger fillings:

- Cell growth medium
- Sterile water

- 2 parallel sampling lines:

- Filter upstream of impingers
→ COC only
- No filter upstream of impingers
→ COC plus solid PM

Picture of Sampling Setup



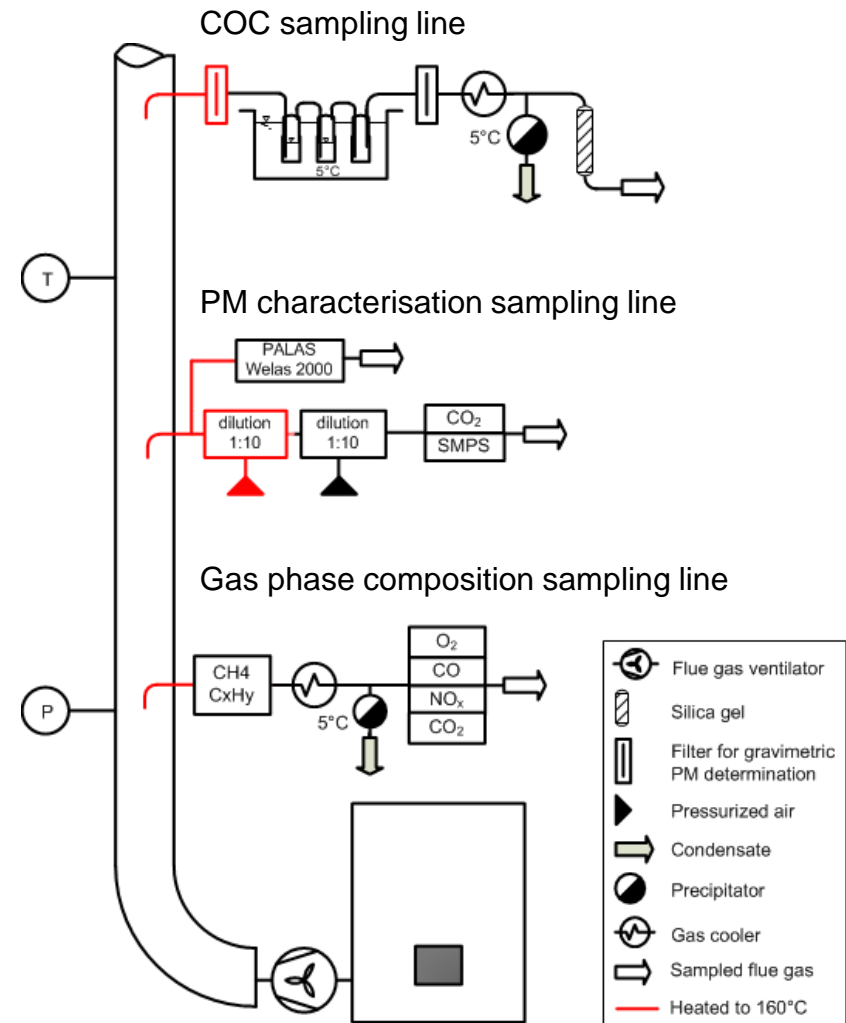
Methods – Experimental Setup

- Gas phase emissions:

- *Combustion regime and efficiency*
 - O_2 , CO_2 , CO
- *Organic compounds with FID:*
 - CH_4 , VOC, NMVOC
- *Nitric oxide emissions: NO*

- Particles:

- Solid PM mass according to VDI
- Particle number concentration
- Particle size distribution

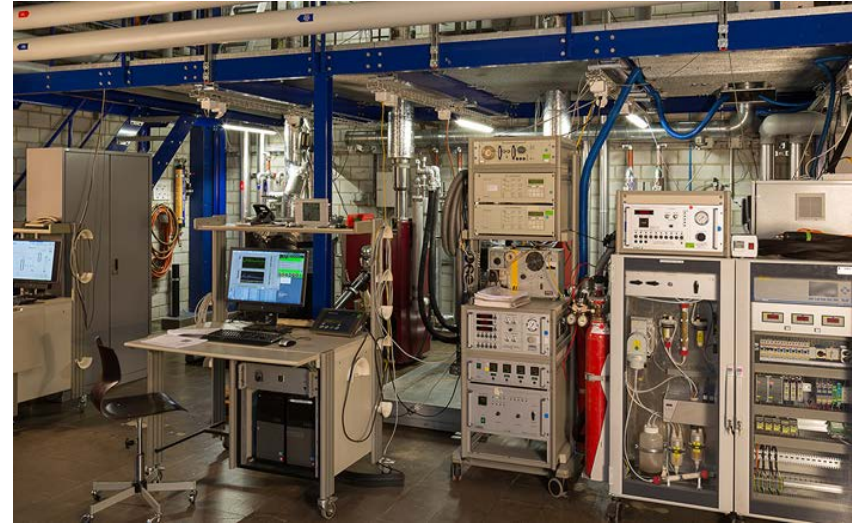


Methods – Combustion Devices

Combustion devices

- *Log wood stove (8 kW)*
 - Reload and flaming
- *Pellet boiler (15 kW)*
 - Combustion air: optimum (λ_{opt}), lack (λ_{-}) and high excess (λ_{++})
- *Semi-industrial moving grate boiler (150 kW)*
 - Operated with wood chips
 - 100% and 30% heat output

Picture of Combustion Laboratory



Log wood stove



Pellet boiler



Semi-industrial grate boiler



**HOCHSCHULE
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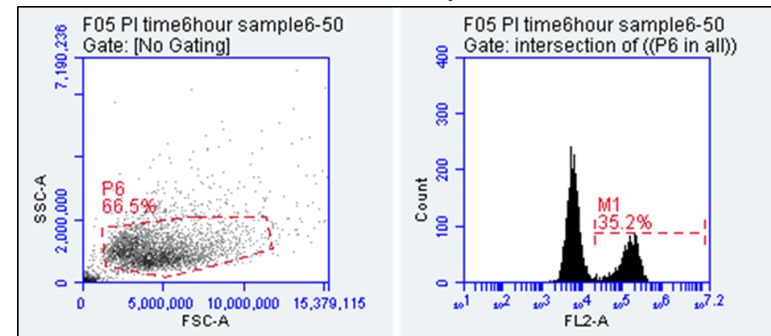
Methods – In Vitro Cell Analysis

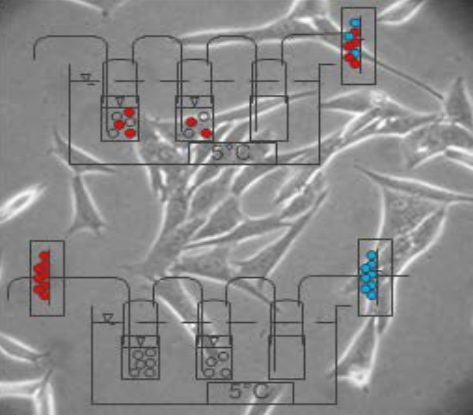
- C2C12 cell line
 - Skeletal muscles cells from mice (5000 cells/cm²)
 - Used for initial tests
- H187 cell line
 - Human epithelial lung cells (15 000 cells/ml)
 - Used for comparison of different devices
- Cell analysis and biological endpoint:
 - Mixing of exposed liquids with “fresh” medium at different concentrations
 - 24h cell exposure
 - Staining of dead cells Propidium Iodide (PI)
 - Number of dead cells is measured with FACS

Biomedical Laboratory



FACS Analysis





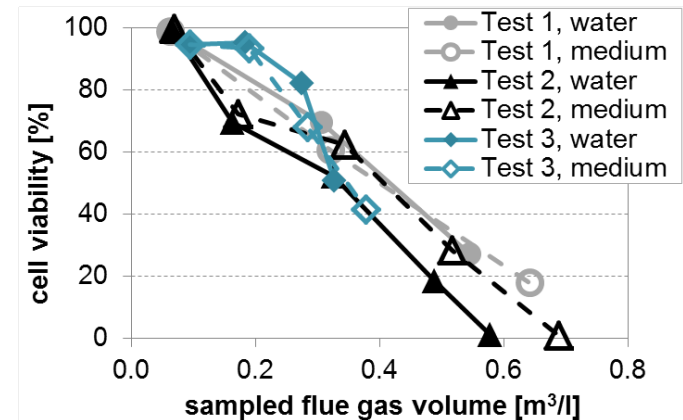
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Results – Method Characterisation

- Characterisation performed with the pellet boiler due to its well reproducible conditions
- No difference in cell viability between C2C12 and H187 cell lines found

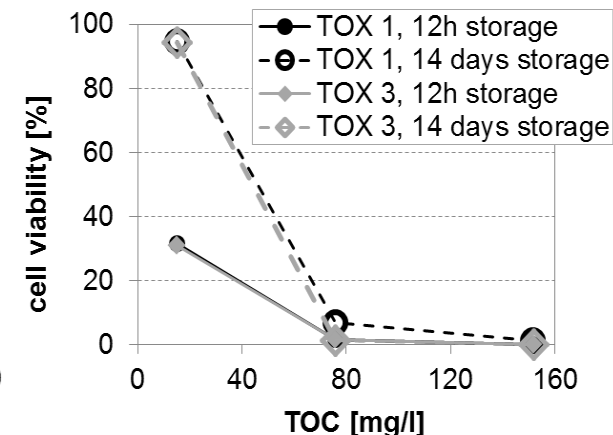
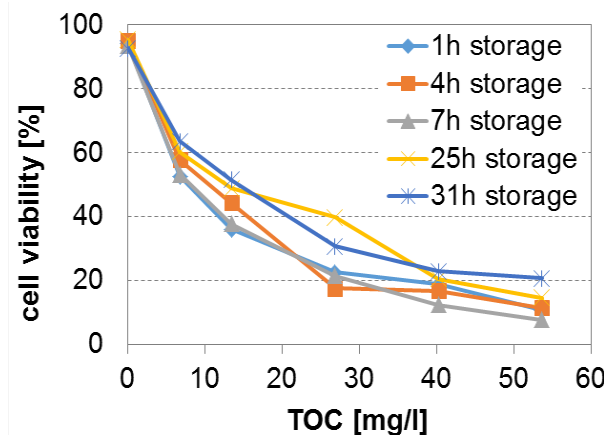
- Sampling liquid (sterile water vs. RPMI)

- No influence on cell viability
- Sterile water used in subsequent experiments to enable TOC analysis

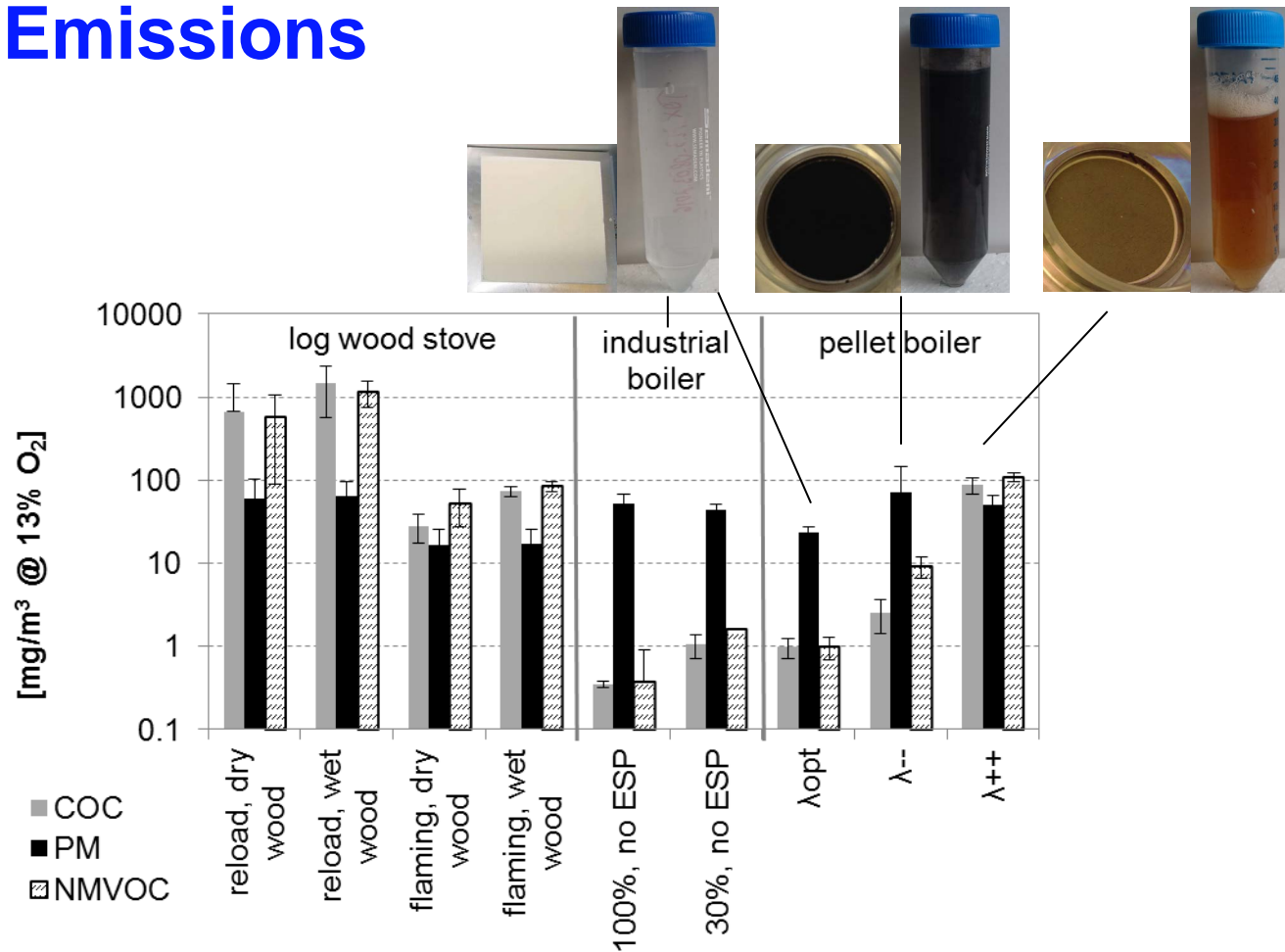


- Storage effect

- Duration between sampling and cell exposure
 - No difference within first 31h
 - Decreased cell viability after 2 weeks
- Start of cell exposure ~12h after sampling



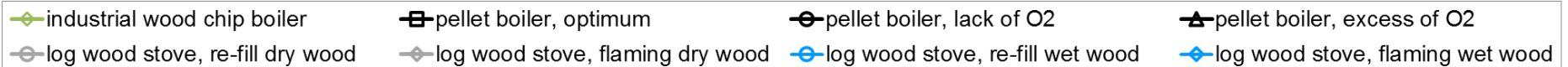
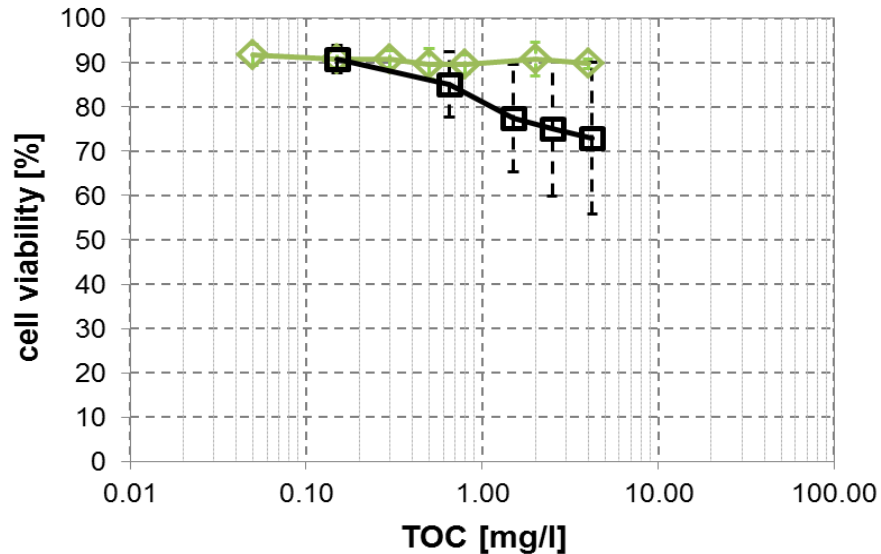
Results – Emissions



- Highest emissions in log wood stove and non-ideal operation in pellet boiler
- High COC with high NMVOC emissions
- $\text{COC} > \text{solid PM}$ in hot flue gas for several conditions

Results – Cell Viability of COC

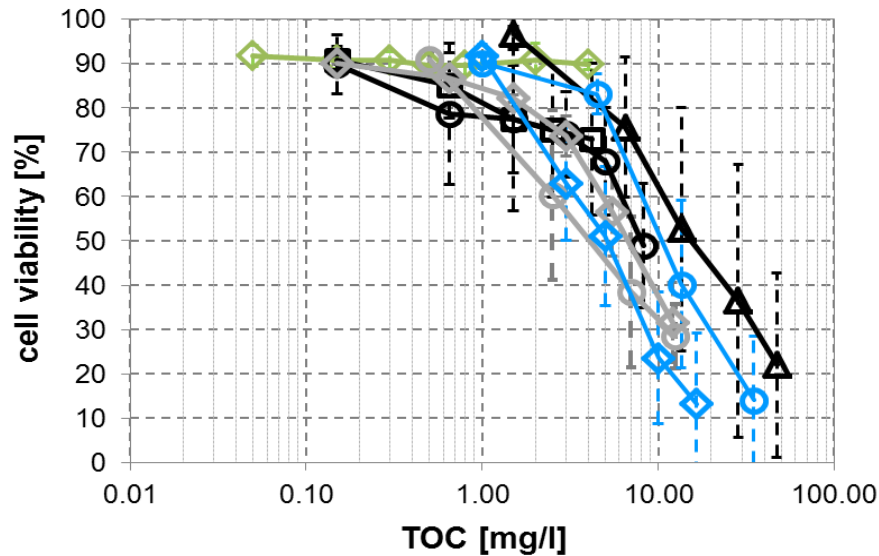
Cytotoxicity based on TOC amount:



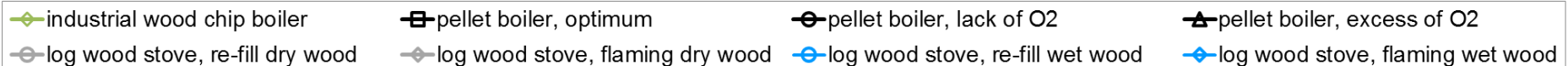
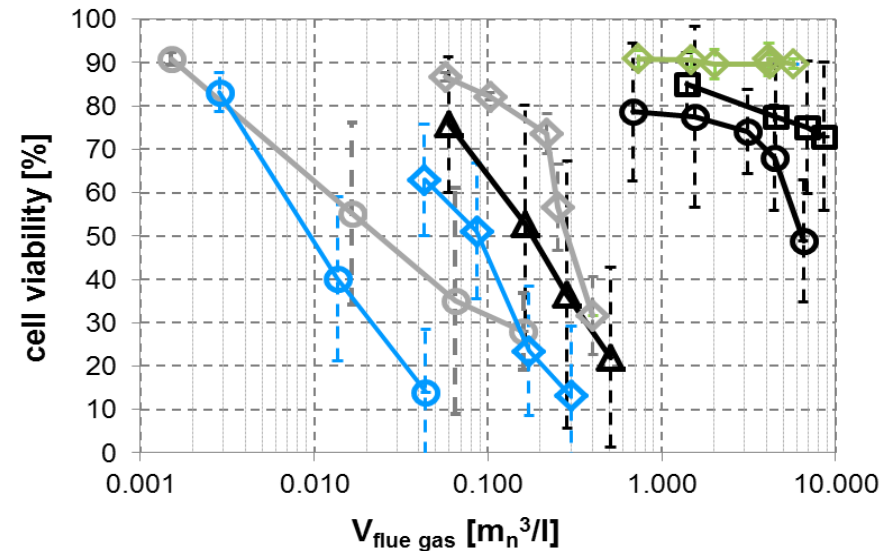
- No and low effect for industrial and pellet boiler with λ_{opt}

Results – Cell Viability of COC

Cytotoxicity based on TOC amount:



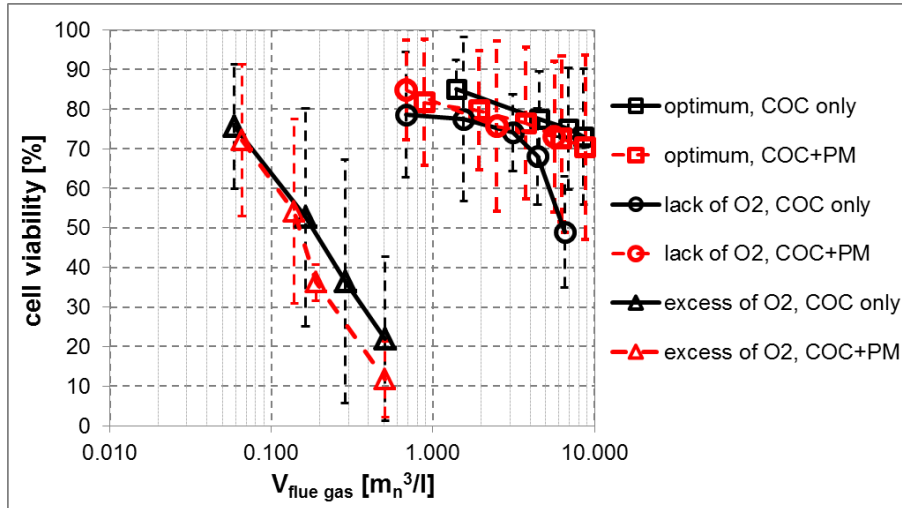
Cytotoxicity based on flue gas volume:



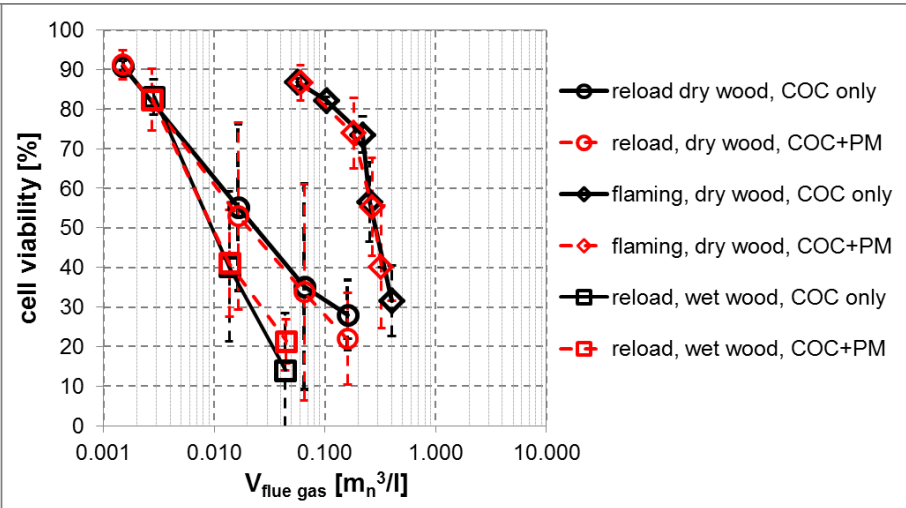
- No and low effect for industrial and pellet boiler with λ_{opt}
- Significant cytotoxicity for stove and λ_{++} and λ_{--} conditions in pellet boiler
 - Differences between these conditions are low ($\text{LD50} \sim 4 - 15 [\text{mg/l}]$)
- Clear differences between combustion devices and conditions
- Cytotoxicity ordered accordingly from highest to lowest NMVOC concentrations in flue gas

Results – Cell Viability of solid PM

Pellet boiler



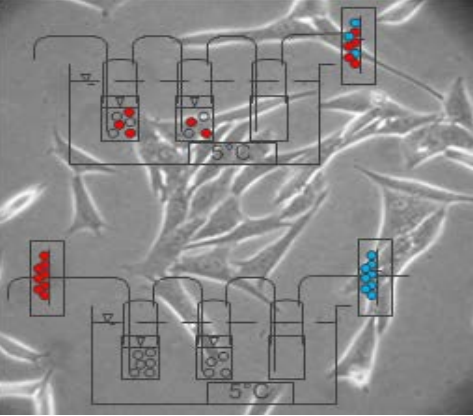
Log wood stove



- Samples containing PM+COC reveal similar cytotoxicity as samples with COC only

→ Cytotoxicity of solid PM compared to COC seems negligible, but applied in-vitro method might not be sensitive enough to detect effect from solid PM

- Fraction of TOC from solid PM to total TOC in sampling solution is < 20%
- Negative controls contain on average already ~ 10% dead cells



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Conclusions

- Method for assessing cytotoxicity of wood combustion emissions by in vitro cell analysis of COC and PM was successfully established and characterised
- Simple setup and procedures allow an economically analysis of a large number of samples enabling a comparison of different combustion types
- Three different combustion devices with 9 different conditions were investigated
- Cytotoxicity based on TOC amount:
 - *No and low effect for industrial moving grate and pellet boiler during optimum operation*
 - *Significant for log wood stove and non-ideal conditions in the pellet boiler but differences between these conditions are low*
- Cytotoxicity based on sampled flue gas volume:
 - *Clear difference between combustion devices and conditions (2-3 orders of magnitude)*
 - *Higher cytotoxicity for conditions with higher NMVOC emissions*
- Samples with PM+COC reveal similar cytotoxicity as for COC only indicating the importance of COC



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Combustion device manufactures:

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energy solutions

Tiba 

Liebi 

Attika Feuer AG (future tests) 

Sigmatic AG (future tests) 
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THANK YOU FOR
YOUR ATTENTION