TNA User Report

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Project title	Investigation of the toxicity of aerosols from wood and engine combustion and mixtures thereof: Direct cell exposure and analysis of combustion aerosol toxicological properties from primary and secondary organic aerosol
Name of the accessed chamber	ILMARI
Number of users in the project	**5**
Project objectives (max 100 words)	It is not completely understood which aerosol fraction or property is responsible for adverse health effects while the link to elevated aerosol concentrations is well established. Atmospheric aging could differentiate the toxicological potential of combustion aerosols. It is likely that the aging process induces changes in the semi-volatile organic compounds, such as PAH and oxy-PAH, which can impact the toxicity. Increase of complexity is required in order to close the gap between well controlled but simplified laboratory experiments and observations in the real atmosphere. The proposed transnational access (TNA) activity will aim to increase realism in chamber experiments by using complex pollutant mixtures. We will investigate the toxicological response of aged and fresh log wood combustion emissions and compare the changes in responses when the logwood emissions are mixed with diesel engine emissions. The effect of atmospheric aging on health effects combining toxicological, chemical and physical properties are investigated simultaneously during aging in the atmospheric simulation chamber. Several air-liquid interface (ALI) cell exposure devices will be applied: 2 HICE ALIs (one from Helmholtz Center Munich and one from Rostock University) and thermo-ALI (UEF), which are connected directly to the ILMARI simulation chamber. In thermo-ALI, a new improved collection system based on thermophoretic deposition is applied to avoid problems of dose classification by size and possible harming effects on the viability of the cells (electrical force field, charging of particles). Comprehensive chemical and physical characterization of the aerosols supports the ALI analyses. Specific attention will be paid to ensure a high level of participation in training and education sessions by early career scientists. Communities in neighbouring fields of research such as environmental and ecosystem science, biodiversity and health and energy research are potential users of the results.

	Objectives and innovative for specified groups: TNA project's objectives are : 1) To find out what are the health impacts of complex pollutant mixtures when atmospherically aged 2) Investigate biological responses of cell cultures for aerosols via "omics" analyses 3) To study changes in the profile of chemical species and linking them to the variation of toxicological responses and combustion aerosol properties during aging 4) To investigate, if aging processes influence significantly the key properties of combustion emissions with respect to their health impact 5) To compare ALI cell exposure devices using different principles for enrichment of particle deposition
Description of work (max 100 words):	Description of the experiments Toxicological responses of aged emissions from different sources (wood, diesel engine and mixture of these emissions) and transformation processes, which are taking place during aging, will be simulated in the aerosol physics, chemistry and toxicology research unit (ILMARI) of the University of Eastern Finland. It includes a 29 m3, air-conditioned FEP Teflon chamber with UV lights, humidification systems and ozone generators. A logwood fired stove and a diesel generator will be used as combustion sources. The experiments will begin with the filling of the chamber with a pre-diluted sample of the first batch of log wood combustion and/or engine exhaust to reach realistic concentration levels. After stabilization and mixing in the chamber, oxidants (O3) are injected into the chamber. The UV lights are switched on and the aerosol is aged with UV for about 4 h representing approx. 1 day of atmospheric aging. The toxicity of the aged aerosols will be analyzed using the air-liquid interface (ALI) techniques as a method of exposing lung cells. Several systems for the enhancement of particle deposition on the lung cell surface will be compared. Two of them use the principle of thermophoresis, one is applying a electrostatic field to increase deposition. After aerosol exposure cell samples will be used to extract metabolites, proteins and RNA for subsequent analyses of the metabolome, proteome and transcriptome, respectively. Additionally, cytotoxicity and genotoxicity tests will be performed. Chemical and physical properties of the aerosols during the aging process will be measured using two aerosol mass spectrometers (SP-HR-ToF-AMS and single particle aerosol mass spectrometer, A-TOF), several gas phase analyzers (PTR-MS, REMPI/SPI-MS and TOF-CIMS) as well as off-line molecular speciation with several mass spectrometry based techniques. The campaign will also include measurements of aerosol concentration and size

	distribution (SMPS), black carbon (Aethalometer AE-4), NOx, CO, O3, chamber RH, and T measurements. The measurement campaign (including preparation) is planned for six weeks in the beginning of 2018 and will consist of chamber experiments with wood combustion and engine emission and mixture of these. Explain why the selected chamber is relevant for the scientific topic addressed. This is a multidisciplinary scientific research project needing multiple approach methods (e.g. combustion unit, dilution of emissions, aging, comprehensive physical and chemical analyses, cell exposure, toxicology) to take full advantage of the research results. These resources are available at ILMARI Research Unit. Combustion sources are installed next to the chamber, which minimizes sample losses in the injection lines. Also, at Ilmari, the ALI techniques can be exploited in the vicinity of the chamber. Indicate the type of support that your project will need in terms of staff, training, additional data, logistics, etc. The project needs expertise in preparing and running the chamber experiments, ALI cell exposures and subsequent cell analyses, and specific instruments (mass spectrometers), as well as the combustion sources. The HICE ALIs and the special cell culture used for HICE ALI exposures as well as mass spectrometrical on-line analyses require personnel from the Helmholtz Center Munich and the University of Rostock.

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[1]Physics; Chemistry; Earth Sciences & Environment; Engineering & Technology; Mathematics; Information & Communication Technologies; Material Sciences; Energy; Social sciences; Humanities.
[2] UNI= University and Other Higher Education Organisation;
RES= Public Research Organisation (including international research organisations and private research organisations controlled by public authority);
SME= Small and Medium Enterprise;
PRV= Other Industrial and/or Profit Private Organisation;
OTH= Other type of organization.

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[3]UND= Undergraduate; PGR= Post graduate; PDOC= Post-doctoral researcher; RES= Researcher ENG= Engineer; ACA= Academic; TEC= Technician.
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Trans-National Access (TNA) Scientific Report

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Instructions

Please limit the report to max 5 pages, you can include tables and figures. Please make sure to address any comments made by the reviewers at the moment of the project evaluation (if applicable, in this case you were informed beforehand). Please do not alter the layout of the document and keep it in Word version. The report will be made available on the eurochamp.org website. Should any information be confidential or not be made public, please inform us accordingly (in this case it will only be accessible by the European Commission, the EUROCHAMP-2020 project partners, and the reviewers). Please include:

- Introduction and motivation
- Scientific objectives
- Reason for choosing the simulation chamber/ calibration facility
- Method and experimental set-up
- Data description
- Preliminary results and conclusions
- Outcome and future studies
- References

Name of the PI: **Prof. Dr. Ralf Zimmermann**
Chamber name and location: **ILMARI, University of Eastern Finland, Kuopio**
Campaign name and period: **Investigation of the toxicity of aerosols from wood and engine combustion and mixtures thereof: direct cell exposure and analysis of combustion aerosol toxicological properties from primary and secondary organic aerosol.**
15.01.2018 – 28.02.2018

Introduction and motivation

Air pollution is the largest environmental health risk in Europe, causing a substantial disease burden. The EU programme "Clean Air for Europe (CAFE)" [1] concluded that an average reduction in life expectancy between six and more than twelve months in central Europe can be attributed to air pollution. Worldwide it contributes to 6.7 % of mortality cases. WHO studies [2] state that570.000 (3 Mio.) premature deaths in Europe (globally) are caused by ambient air pollution, with fine particulate matter ($PM_{2.5}$) being a major concern. The severity of health problems related to air pollution was brought back into public awareness by the increasing number of smog alarms in European cities (e.g. Paris, Milan) and by the recent diesel car emission affair.

It is not completely understood which aerosol fraction or property is responsible for adverse health effects, while the link to elevated aerosol concentrations is well established. Atmospheric aging could differentiate the toxicological potential of combustion aerosols. It is likely that the aging process induces changes in the semi-volatile organic compounds, such as PAH and oxy-PAH, which can impact the toxicity. Increase of complexity is required in order to close the gap between well controlled but simplified laboratory experiments and observations in the real atmosphere.

The activities at ILMARI facility aimed to increase realism in chamber experiments by using complex pollutant mixtures. We will investigate the toxicological response of aged and fresh log wood combustion emissions and compare the changes in responses when the logwood emissions are mixed with engine emissions. The effect of atmospheric aging on health effects combining toxicological, chemical and physical properties are investigated simultaneously during aging in the atmospheric simulation chamber. Several air-liquid interface (ALI) cell exposure devices will be applied: 2 HICE ALIs (one from Helmholtz Center Munich and one from Rostock University) and thermo-ALI (UEF), which are connected directly to the ILMARI simulation chamber (https://www.eurochamp.org/Facilities/SimulationChambers/ILMARI.aspx). In the thermo-ALI, a new improved collection system based on thermophoretic deposition is applied to avoid problems of selective particle enrichment due to chemical composition and size and possible harming effects on the viability of the cells (electrical force field, charging of particles). Comprehensive chemical and physical characterization of the aerosols supports the ALI analyses. Specific attention will be paid to ensure a high level of participation in training and education sessions by early career scientists. Communities in neighbouring fields of research such as environmental and ecosystem science, biodiversity and health and energy research are potential users of the results.

• Scientific objectives

During the campaign in the ILMARI facility at the University of EasternFinland (UEF), Kuopio, during January and February 2018 two types of emissions from a masonry heater fired with spruce wood logs – fresh emissions and emissions aged with the atmospheric simulation chamber - should be assessed regarding their physico-chemical characteristics and their induced toxicological effects *in vitro.* Our primary focus was on determining how well the aging in simulation chamber corresponds to the actual chemical reactions after remissions are released into the atmosphere and how it is suited for toxicological testing and policy making regarding emission control for residential wood heating stoves.

• Reason for choosing the simulation chamber/ calibration facility

The ILMARI facility at the UEF has a unique infrastructure which enables us to assess all parameters of the wood stove emissions at the same time:

- o the complete physicochemical analysis of the masonry heater emissions both online and offline
- o toxicological assessment *in vitro* with two different air-liquid interface exposure systems, a Vitrocell® Exposure system and the novel UEF thermophoresis ALI system

• Method and experimental set-up

For this study, we used a batch combustion of spruce wood logs in a masonry heater. Four batches of 2.5 kg uniform wood logs were combusted for four hours total combustion time. The primary emissions were diluted with compressed filtered air using porous tube and ejector diluters to obtain suitable particle concentrations for the online chemical analysis of PM composition as well as composition of gaseous compounds. Offline filter sampling of the particles was conducted at the same time with the online analyses. The *in* vitro exposure of cells in three different ALI exposure systems (ALI1: RAW cells, ALI2: A549 cells, thermo-ALI: co-culture of A549 and THP-1 cells) was conducted in parallel to the chemical analyses of the emissions. We analyzed the following toxicological parameters : cytotoxicity, genotoxicty, immunotoxicity and performed multi-omics analyses (Transcriptome, Proteome, Metabolome) to gain more detailed information about the sub-acute effects of the masonry heater emissions. Cells exposed to the emission in the thermo-ALI system were allowed to recover for 24 h, then only A549 cells were used in toxicological analyses.Aged aerosols from the ILMARI chamber was enriched upon reaching its maximum mass concentration by a factor of 10–12 with a Versatile Aerosol Concentration Enrichment System (Wang et al., 2012; VACES) and fed to thermo-ALI to expose cell cultures continuously for one hour (Fig. 2).

The aging experiments focused on investigating the effects of UV-light-induced aging of residential-scale wood combustion emissions including mixture with diesel exhaust and were carried out in a 29m^3smogchamber, ILMARI (Leskinen et al., 2015).

• Data description

Chemical data

Chemical composition of single particles was determined by an aerosol time-of-flight mass spectrometer. Particles are sampled and sized by passing through two laser beams on their way to the ion source of the mass spectrometer and subsequent detection of their scattering signal. This allows also calculating their velocity and the time of arrival inside the ion source. Once they have reached the ion source they are hit by anCO_2 laser beam that desorbs organic material from the particle. Subsequently an KrF excimer laser beam ionizes the gaseous aromatic molecules. Afterwards, a third laser beam (ArF excimer laser) hits the core and positive as well as negative ions are produced by a desorption/ionization process.

A soot particle aerosol mass spectrometer (SP-HR-ToF-AMS ; Aerodyne Research Inc.) measured changes in mass concentrations, chemical compositions and size of submicron species during the aging process in the simulation chamber. Two vaporizer configurations, i.e., dual laser and tungsten vaporizers and tungsten vaporizer only modes, were alternated every 120 s; particle time-of-flight (PTOF) modes were operated for 20 s/min.

Toxicological data

Cytotoxicity was assessed with different assays: theAlamar Blue® and the MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-Assays, in which a non-toxic dye is taken up by the cells and the excitation/emission maxima of this dye are changed due to the reducing environment inside the cells. The fluorescence/absorbance signal is directly proportional to the viability of the cells. In the LDH (lactate dehydrogenase) assay the quantity of LDH in the extracellular medium is measured. LDH is ubiquitous in the cytosol, but is only released into the culture medium if the cell membrane is damaged. Cell Viability can also be determined by adding the fluorescent DNA stain4′,6-diamidino-2-phenylindole (DAPI) to the cells. This dye cannot permeate into cells with intact membranes, thus no fluorescent signal will be seen in such cells. To assess the genotoxic potential of the emissions, aliquots of the cells in all three in vitro exposure systems were frozen at -80 °C and will be analysed by the Comet assay. The inflammatory changes will be measured using the enzyme-linked immunosorbent assay (ELISA). Multi-omics (transcriptomics, proteomics and metabolomics) will be carried out after the relevant macromolecules are extracted from the cells.

• Preliminary results and conclusions

Chemical data

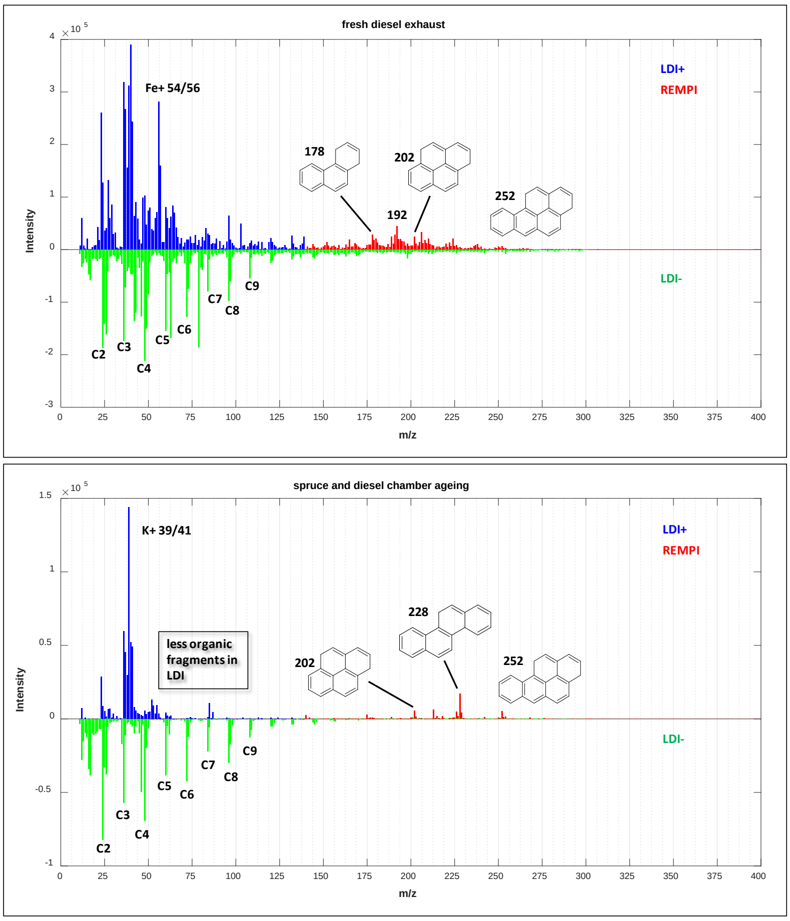

Figure 1 : Single particle aerosol time-of-flight mass spectra from fresh diesel emissions (top) and chamber aged mixture of spruce combustion and diesel emissions (bottom)

Particles emitted directly from a diesel engine (Figure 1 top) show many signals from carbon clusters, indicating a considerable share of soot particles in the emitted particles. Furthermore, three-to five-ring PAH were identified to be present on the particles. Signals from iron ions were also detected along unspecified peaks deriving from organic fragments. With the emissions from chamber aging (Figure 1 bottom) the overall number of signal decreased. There were less organic fragments observed, and the signals from PAHs and carbon clusters were lowered in intensity. Due to spruce combustion, a large signal of potassium was visible.

SP-AMS measurements point out that soot composition from logwood combustion was dominated by refractory black (rBC) carbon and organic compounds (OA, Figure 2). OA/rBC ratio ranged from 0.2-0.3 in the beginning of experiments to 0.4-0.6 at the end of UV aging. Increase of the OA/rBC ratio was due to substantial SOA formation during aging, which was leading typically to twice to initial OA mass, similar to Tiitta et al. 2016.

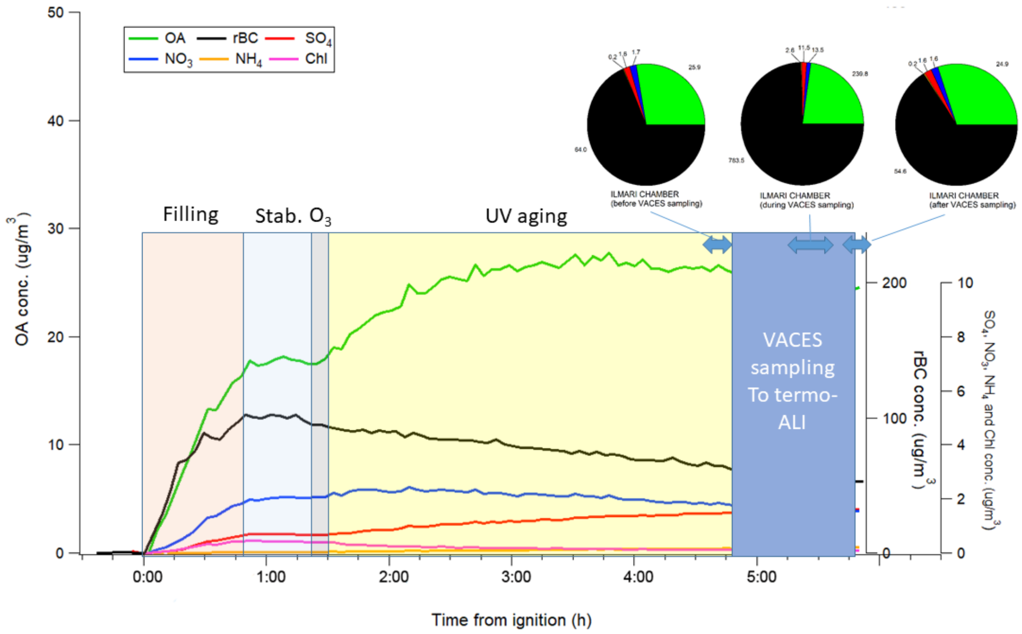

Figure 2 :Example of the evolution of aerosol composition from logwood combustion (1.5 batch of wood) + formation of SOA during UV aging in a ILMARI chamber (14.2.2018) measured using SP-AMS. Stabilization (Stab, 30 min) and ozone injection (O_3, ~20 ppb) was followed by filling of the chamber (50 min). Photochemical age of 0.7 days based on butanol-d9 decay was reached after 4.5 h UV aging. No wall loss corrections were applied.

Toxicological data

We observed clear differences in the cytotoxicty caused by the exposure of the epithelial cells to the emissions from the masonry heater depending on which assay was used. Generally, cytotoxicity was higher in the AlamarBlue® assay, except for the primary emissions, where even an increase in viability of the cells was observed (Figure 3). This was, however, likely due to interactions of the particles with the fluorescent dye. Especially, since this effect was even more pronounced with the high voltage deposition. There a higher deposition of particles on the cell layer and thus a higher interference with the dye could be expected. The viability results from the exposure of a co-culture of A549 and THP-1 cells suggest that primary emissions from spruce log combustion and emissions from spruce logs and spruce logs plus diesel aged in the simulation chamber had no effect on the viability of the cells (Figure 3). However, the combustion and aging of moist birch logs decreased the viability of A549 cells by 15 % compared to the clean air control. Thus from a toxicological perspective, the aging of masonry heater emissions in the atmospheric simulation chamber is suited well for controlled exposure studies *in vitro*.

• Outcome and future studies

Chemistry

For the first time it was possible to perform an analysis of single particles from chamber aging with respect to their chemical composition. Clear differences could be detected between fresh and aged emissions. This offers the possibility to distinguish between different particle types and compare their potential hazardous behaviour. Data from these measurements will be compared with more detailed chemical analyses from filter sampling.

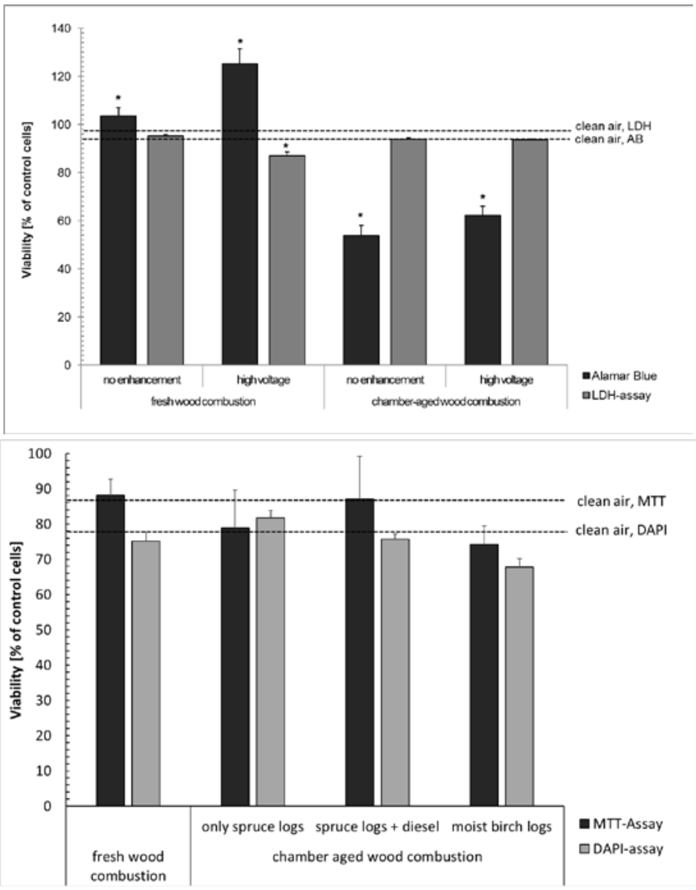

Figure 3: top: Viability of A549 cells assessed with the AlamarBlue®- and LDH-assay after exposure of the cells to emissions from a masonry heater in a Vitrocell® ALI Exposure system; Bottom:Cell viability of A549 cells assessed with the MTT- and DAPI-assay after exposure of the cells in co-culture with THP-1 cells to emissions from a masonry heater in a thermo ALI Exposure system. Cells were exposed to fresh spruce logwood emissions, aged spruce logwood emissions, mixture of aged spruce logwoodand diesel emissions, as well as emissions from moist birch logwood aged in an atmospheric simulation chamber. Bars represent the average of four independent experiments with three technical replicates plus standard error of the mean. Asterisks indicate significant difference to control cells exposed with clean air.

Toxicology

The first results are very promising in regard to toxicity assessment *in vitro*. Even though the particle concentration used in the chamber is rather low, the concentrations seem high enough,with help of VACES enricher, to induce adverse effects in a human alveolar cell line. Thus, we will be able to study in more detail, how the toxicity pathways induced by fresh wood combustion emissions and emissions aged in the atmospheric simulation chamber differ and draw conclusions which constituents of the emissions cause the most severe adverse effects. In further studies, we would like to investigate the effect of aged emissions from a combustion engine (e.g. gasoline car, diesel engine) in combination with aged wood combustion emissions, and also other emerging types of biofuels, such as wood briquettes and/or different types of pellets.

References

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