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Cytotoxicity of Air Pollutant Emissions from Wood Combustion

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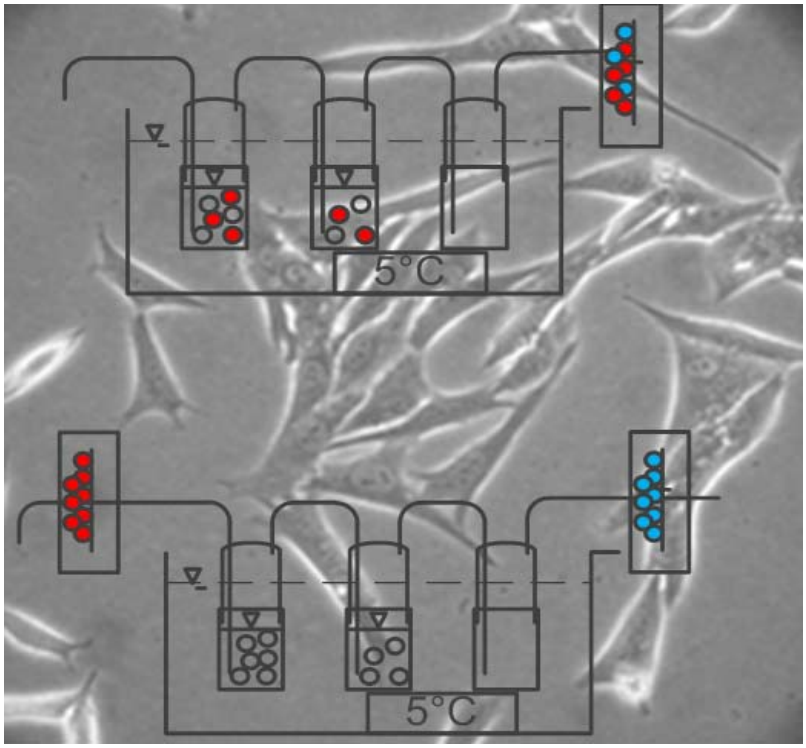

Final report on behalf of the
Swiss Federal Office for the Environment

Horw and Hergiswil, 01 December 2016

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1 Abbreviations and Symbols

A549	Human epithelial lung cell line A549
BC	Black carbon
C2C12	Skeletal mouse muscles cell line C2C12
CC ABST	Competence Centre Aerospace Biomedical Science & Technology
CH_4	Methane
CO	Carbon Monoxide
CO_2	Carbon Dioxide
COC	Condensable organic compounds
CV	Cell viability
CV_{COC}	CV from samples containing only COC
CV_{SP}	CV of solid particles (SP) only ($CV_{SP} = CV_{tot} - CV_{COC}$)
CV_{tot}	CV from samples containing both, COC and PM
DMEM	Dulbecco's modified Eagle's medium high Glucose
ESP	Electrostatic precipitator
EC	Elemental carbon
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FID	Flame ionisation detector
FSC-A	Intensity of diffraction signal
H187	Human epithelial lung cell line H187
$H_{i,atro}$	Heating value of dry wood ($H_{i,atro}$ = 18.3 MJ/kg)
LD_{50}	Lethal dose where 50% of all cells die
Lucerne UASA	Lucerne University of Applied Sciences and Arts
ND-IR	non-dispersive infrared analysis
NMVOC	Non-methane volatile organic compounds (VOC minus CH_4)
NO_x	Nitrogen Oxides
O_2	Oxygen, O_2 content in atmosphere is 21%
$O_{2,ref}$	Reference oxygen content, $O_{2,ref}$= 13% in this study
OAS	Optical aerosol spectrometer
OAPC	Swiss Ordinance on Air Pollution Control
OM	Organic matter
PAH	Polycyclic aromatic hydrocarbons (PAH)
PI	Propidium Iodide
PIA	Primary inorganic aerosol
PM	Particulate matter
$PM_{2.5}$	Particulate matter smaller than 2.5 micrometres
PM_{10}	Particulate matter smaller than 10 micrometres
POA	Primary organic aerosol
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute medium 1640
SIA	Secondary inorganic aerosol

SMPS	Scanning mobility particle sizer
SOA	Secondary organic aerosol
SP	Solid particles
SSC-A	Intensity of scattered light
TOC	Total organic carbon
u	Water content of the wood on dry basis
US-EPA	Environmental Protection Agency of the United States of America
$V_{flue\ gas,min}$	Stoichiometric flue gas volume ($V_{flue\ gas,min}$ = 4.55 m_n^3/kg)
$V_{flue\ gas}$	Flue gas volume used for sampling at 0°C and 1013 Pa
VOC	Volatile organic compounds
ΔCV_{COC}	Difference of CV_{COC} for the same sample between different storage durations
Δh_v	Enthalpy of evaporation of water (Δh_v = 2.5 MJ/kg)
λ	Excess air ratio, $\lambda = 21/(21 - O_{2,flue\ gas})$
λ_{ref}	λ calculated with reference oxygen content of 13%, $\lambda = 21/(21 - O_{2,ref})$
λ_{opt}	Best/optimum combustion conditions in pellet boiler
λ_{++}	Combustion condition with high excess of combustion air
λ_{--}	Combustion condition with lack of combustion air
@ 13% O_2	Results normalised to 13% O_2 content by multiplying with the ratio λ/λ_{ref}

2 Abstract

In this study an economic and less complex method for the collection and subsequent in *vitro cell* analysis of flue gases from biomass combustion, especially for condensable organic compounds (COC) and possibly for solid particles (SP) also, is developed. For this purpose, standard sampling methods US-EPA-5H [1] and VDI-2066 [2] are adapted. Hot flue gases are passed through impingers which are cooled to 5 °C. The rapid cooling induces the condensation of mainly COC, into the impinger fillings, i.e. sterile water and cell growth medium. Subsequently, cells are exposed to these liquids and the cell viability is analysed. Two parallel sampling trains are applied: one with a filter upstream of the impingers to determine the cytotoxicity of COC only and one without a filter upstream of the impingers to determine the cytotoxicity of both, COC and SP.

After characterisation of the sampling method and procedures (investigation of the influence of different parameters, i.e., blanks, type and amount of sampling liquid, sample storage duration, sampling flow rate and different cell types), four combustion devices (log wood and pellet boiler, log wood stove and grate boiler operated with wood chips) with 13 different combustion conditions are compared. In addition to the cell analysis, a detailed characterisation of the flue gas (SP mass, O_2, CO, CH_4, total hydrocarbons) is carried out.

Comparing the cell viability from the investigated combustion devices and conditions no or only a minor cytotoxicity of COC for stationary conditions in a grate boiler and a log wood boiler and a pellet boiler is found. All combustion conditions in the log wood stove and unfavourable conditions in the other devices (lack and high excess of combustion air, cold start and going to standby) induce a significant decrease in cell viability. When the cell viability is based on the amount of organic compounds collected in the sampling liquids there is a difference in LD_{50} of 6–19 mg/l of total organic compounds. This indicates that the same amount of organic compounds emitted from the different combustion devices and conditions exhibit a similar cytotoxicity. If the cell viability is based on the flue gas volume used for sampling (this is directly proportional to the cytotoxicity per MJ of energy supplied) differences up to two orders of magnitude are evident. This is a consequence of the significantly different emission factors of organic compounds between the investigated combustion devices and conditions. The lowest amount of flue gas volume to reach LD_{50}, and consequently exhibiting the highest cytotoxicity, is found during combustion with lack of O_2 in the log wood boiler. However, for all other unfavourable conditions in the log wood stove (cold start, re-fill and operation with wet wood), the log wood boiler (cold start) and the grate boiler (going to standby from part load) the values are similar. Flue gas from stationary conditions in the grate, log wood and pellet boiler shows no or only a minor effect on the cell viability even for large amounts of sampled flue gas.

The comparison of cell viability of samples with COC (sampling method 1) and samples where COC and SP (sampling method 2) were collected shows no significant differences. Thus, no additional cytotoxic effect of SP compared to COC alone is detected. This shows the high health relevance of COCs. On the other hand, with the developed sampling method not the entire SPs are deposited. Water in-soluble particles, i.e. soot which is very health relevant, are not collected. Consequently, the additional SPs collected with sampling method 2 lead on average to only ~10% additional amount of organic compounds within the sampling liquids compared to samples containing COC only (sampling method 1). To analyse the cytotoxic effects of SPs, which is scientifically proven, SPs should be analysed separately and with higher concentrations.

3 Executive summary

Since there is a potential to increase the energy supply from wood combustion by more than 50 % in Switzerland to contribute to the Swiss energy strategy 2050, wood energy consumption is expected to significantly increase within the next years. However, the application of biomass combustion is faced with the drawback of a relevant contribution to the air pollution, especially with respect to inhalable particulate matter smaller than 10 micrometres (PM_{10}), which induces adverse health effects. Consequently, there is a trade-off between air pollution control and the propagation of wood as a renewable energy source.

With respect to air pollutant emissions, different types of combustion devices and combustion modes are related to distinct emission patterns and therefore need to be distinguished. In particular, primary particles from biomass combustion are classified by three different types, namely inorganic particles (mostly salts, dominant at near-complete combustion), soot (formed at high temperatures and lack of oxygen), and condensable organic compounds (COC, formed at low temperatures). COC occurs either in the gas or liquid phase depending on temperature and concentration. In the hot flue gas, COC are in the gas phase and therefore part of the volatile organic compounds (VOC). After condensation in the atmosphere, COC contribute to primary organic aerosols (POA), while VOC can form secondary organic aerosols (SOA) in photochemical reactions in the atmosphere.

For the three basic particle types referred to as salts, soot, and COC, previous investigations by Klippel and Nussbaumer (2007) revealed the highest cytotoxicity and carcinogenicity for COC followed by soot, while salt particles showed the lowest impact [3]. Since the current air pollution control legislation considers emission limits on particulate matter in the hot flue gas, COC and potential secondary organic aerosols (SOA) are usually not directly measured and rarely investigated. Consequently, there is an interest in further information to close the knowledge gap between pollutant emissions at the stack and their relevance to ambient air and finally to human health. Furthermore, experimental methods to investigate the influences on emissions in the stack on health effects applied so far are complex and costly. Hence information available on health issues from flue gases is not only scarce, but typically limited to a small number of specific applications. Therefore, the aim of the present investigation is to develop a less complex method for the collection and subsequent *in vitro* cell analysis of flue gases from biomass combustion which may contain all relevant particle classes, i.e., salts, soot, and COC.

For this purpose, the sampling methods US-EPA 5H and VDI 2066 (the latter is also applied by the Swiss Ordinance on Air Pollution Control) are adapted in order to allow an economic sampling. In this method, hot flue gases are passed through impingers which are cooled to 5 °C. The rapid cooling induces the condensation of mainly COC, into the impinger fillings. Subsequently, cells are exposed to these liquids. Two parallel sampling trains are applied: one with a filter upstream of the impingers to determine the cytotoxicity of COC only (sampling method 1) and one without a filter upstream of the impingers (sampling method 2) to determine the cytotoxicity of COC and solid particles (SP). The cytotoxicity of COC and SP from wood combustion in this study is assessed by measuring the cell viability, denoting the fraction of living cells within a cell population, after a certain time of cell exposure. For the method characterisation, several tests are performed including evaluation of the method for cell viability determination, investigation of the influence of sterility and blanks, type and

amount of sampling liquid, sampling flow rate, sample storage duration, reproducibility and different cell types. The results show the following trends:

- Fluorescence-activated cell sorting (FACS) can be successfully applied to samples generated with the sampling method developed in this study for measuring cell viability. Furthermore, no interferences of suspended particles within the sampling liquids and cell cultures are evident, which is also in line with results from literature.
- No differences in cell viability between sterile and non-sterile sampling equipment as well as blanks (identically prepared sampling liquid without exposure to wood combustion flue gas) and negative control (cell cultures prepared with fresh medium or sterile water and incubated at the same time as exposed samples and blanks) are found.
- Comparing cell growth medium and sterile water as impinger filling used to collect COC, no difference in cell viability between both liquids is evident. Consequently, sterile water is used as sampling liquid for subsequent experiments since it is more feasible for further chemical analysis (e.g. analysis of the total organic content (TOC) within the impingers).
- An investigation of the influence of the time period between sampling and cell exposure with samples stored at 4°C reveals a storage effect with increased cell viability, and consequently decreased cytotoxicity, for samples analysed after two weeks compared to the samples analysed after one night. No change in cell viability is observed up to 31 h of sample storage.
- Investigating the influence of the sampling flow rate and impinger filling amount on the trapping efficiency of COC shows that a sufficient total liquid level (≥ 150 ml) and a high filling in the first impinger, i.e. 80 ml in a 100 ml bottle, as well as flow rates of 5 to 15 l/min should be maintained.
- The reproducibility of the cell viability investigated by the present method is influenced by the variability of the combustion and sampling conditions, and the variability of the cell analysis. Repeated measurements of samples from a pellet boiler reveal a total variability of less than 30% for a cell exposure of 24 h. Since shorter cell exposure results in higher variability, all subsequent cell analyses are performed after 24 h of exposure. However, the total variability varies for different COC concentrations, combustion devices and conditions. Therefore, two or more samples are analysed for each combustion device and condition.
- To test and evaluate the sampling method, the first experiments are performed with C2C12 cells (skeletal muscles cells from mice). To obtain results which are potentially more relevant for humans, human epithelial lung cells H187 are used in subsequent tests. A comparison between the two cell types shows no significant differences in cell viability. Also a limited number of tests (n = 4) is carried out using the A549 cell line (epithelial cells with characteristics of alveolar type II cells). The comparison between the cell viability as function of the TOC of A549 and H187 cells shows that the dose response curves exhibit the same shape, while for A549 cells a much higher TOC amount is needed to induce the same cytotoxicity as for H187 cells.

After establishing the sampling method and confirming its applicability by selected experiments, different combustion devices and conditions are investigated. Therefore, a log wood stove, pellet boiler, moving grate boiler and log wood boiler are used and their cytotoxicity of COC and SP as well as emissions are compared showing the following results:

- The highest emissions (CO, non-methane volatile organic compounds (NMVOC), COC and SP) are found in the log wood stove during cold start with ignition from the bottom followed by the reload conditions. For stationary operation in all investigated devices the emissions are much lower, the organic compounds even by more than an order of magnitude. Other unfavourable conditions (cold start in the log wood boiler, going to standby from part load in the grate boiler, high excess or lack of air in the pellet boiler and lack of air in the log wood boiler) result in high organic, but especially high CO emissions. A high correlation ($R^2 = 0.74$) between total organic carbon (TOC) content determined in the sampling liquid and NMVOC measured in the hot flue gas is evident. Furthermore, COC can exceed the ones of SP measured in the hot flue gas.
- When investigating the cell viability of COC (CV_{COC}) as a function of TOC, no or only a minor cytotoxic effect is observed during stationary conditions (part and full load) in the grate boiler, the log wood boiler during stationary flaming conditions and during optimum combustion in the pellet boiler. For the unfavourable combustion conditions in the pellet boiler (high excess and lack of O_2), the log wood boiler (cold start and lack of O_2) and the moving grate boiler (going to standby from part load) as well as for all combustion conditions in the log wood stove CV_{COC} is significantly decreased with increasing TOC within the sampling solutions. The TOC concentrations inducing a lethal dose of 50 % (LD_{50}), however, only span a range of 6-19 mg/l indicating that the same amount of organic compounds from the different combustion devices and conditions exhibit a similar cytotoxicity.
- CV_{COC} is also analysed as a function of the amount of flue gas ($V_{flue\ gas}$) sampled through the impingers. $V_{flue\ gas}$ is directly proportional to the amount of burned wood and the heat input in MJ or kWh. The comparison of CV_{COC} based on $V_{flue\ gas}$ between the investigated combustion devices and conditions reveal differences up to almost two orders of magnitude. These differences are much more pronounced than for the comparison of CV_{COC} based on TOC, and are due to the large differences in emission factors of organic compounds. The minimum amount of $V_{flue\ gas}$ to reach LD_{50}, and consequently exhibiting the highest cytotoxicity, is found during combustion with lack of O_2 in the log wood boiler. However, LD_{50} from for most of the unfavourable conditions in all devices exhibits similar values. Flue gas from stationary conditions in the grate, log wood and pellet boiler shows no or only a minor effect on CV_{COC} even for large amounts of $V_{flue\ gas}$.
- The comparison of CV_{COC} (sampling method 1) with the one of COC plus SP (CV_{tot}, sampling method 2) reveals no significant differences between CV_{COC} and CV_{tot} for any of the investigated combustion devices and conditions. This indicates that for the investigated concentrations in the sampling liquids no additional cytotoxic effect of SP compared to COC alone is detected. The reason for this is that not the entire SP is collected within the impingers. Water insoluble particles like soot or PAHs, which are strongly health relevant, are not or only to a minor extent precipitated into the sampling solutions. Consequently, samples containing COC and SP only contain on average 10 % more TOC than samples with COC alone. Since the negative controls already contain ~10 % of dead cells the method applied here is most probably not sensitive enough to detect a cytotoxic effect of the small additional TOC amount due to SP. Also no decrease in cell viability is found analysing SP alone obtained by water extraction of filters (before the impingers in sampling method 1) even for TOC concentrations where CV_{COC} shows already a significant decrease. The latter result indicates the high health relevance of COC. However, on the other hand it also shows that with the developed method it is not possible to detect separate health effects of solid SP from wood combustion.

4 Introduction

4.1 Background

Wood combustion currently contributes about 4 % to the total energy consumption of Switzerland [4]. New surveys by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) even project an increase of the wood energy contribution up 7.3 % by 2020 [5]. Furthermore, life cycle analyses show that heat and power from wood are highly effective to replace fossil fuels in the building sector and in industry [6]. Consequently, to reach the goals of the Swiss energy strategy 2050 [7], wood energy utilisation is expected to increase by more than 50 % within the next years.

However, biomass combustion has a high contribution to air pollution. Especially in the cold season, biomass combustion emissions can be the dominating source of particulate matter (PM) smaller than 10 micrometres (PM_{10}) in ambient air as shown by source apportionment studies from the Laboratory of Atmospheric Chemistry at PSI [8-10]. In addition, PM also has a negative impact on human health by possibly causing respiratory and cardiovascular diseases and leading to increased mortality. This was shown by many studies, e.g. by the often cited epidemiological six cities study from Docker et al. (1993) [11] and by more recent summarizing reports from WHO [12, 13]. Particle emission limit values are indicated as mass concentrations and hence do not distinguish between toxic and less harmful substances. However, combustion particles are generally regarded as potentially harmful since they can be carrier of toxic and carcinogenic substances such as polycyclic aromatic hydrocarbons (PAH). Moreover, combustion particles are often found in the size range < 1 µm and often even < 0.1 µm. In many publications (e.g. reviews [14, 15]) such nanoparticles are considered as most health relevant due to their large specific surfaces and the potentially increased mobility.

Consequently, there is a target conflict between air pollution control and the propagation of wood as a renewable energy source. Hence, to mitigate this target conflict, more stringent emission limit values were introduced in 2007 in the Swiss Ordinance on Air Pollution Control (OAPC) [16]. This accentuation mostly affects automatic boilers (> 70 kW), as the PM limit values for such devices were lowered to an extent that electrostatic precipitators or fabric filters become necessary. This was only the case for large combustion units before. Consequently, these new OAPC limit values ensure a decrease of the contribution of automatic wood combustion devices to PM even with increasing numbers of deployed units. On the other hand, the development is uncertain for smaller biomass combustion units (< 70 kW). However, as shown in an experimental study [3] and a more recent literature survey [17] by our group, these devices have a significant impact on air pollution as their emissions of organic compounds, which are considered to have the highest noxious effects, is disproportionately high compared to larger units. Therefore, and since different biomass combustion appliances can emit distinct types of particles the environmental and health relevance of different categories of biomass combustion units is not only determined by the total PM emissions but also by the composition of the total emissions.

4.2 Characterisation of biomass combustion emissions

Wood combustion emissions contain gas and particle phase compounds. Gaseous products from complete combustions consist of CO_2, H_2O and NO_x. During incomplete combustion, gas phase compounds CO and VOC are emitted. VOC undergo photochemical reactions in the atmosphere and can form secondary organic aerosol (SOA) also contributing to ambient PM.

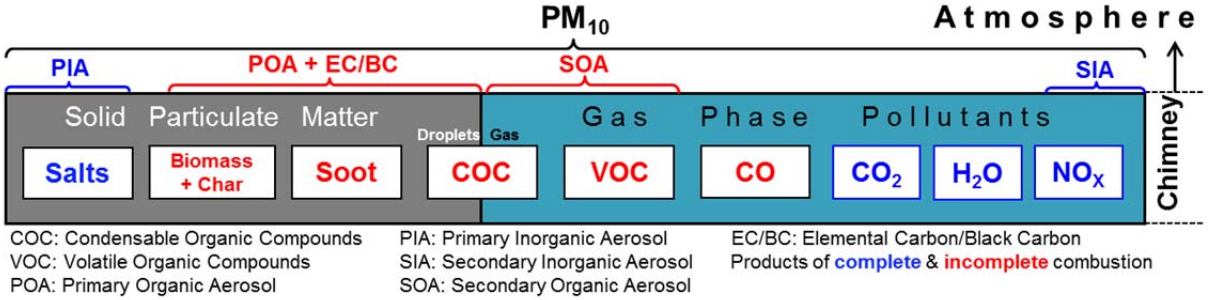

Figure 4.1 General overview of the composition of wood combustion emissions.

Biomass combustion PM is often classified into three different types of primary particles (neglecting metals mainly relevant when using waste wood), which are summarized as “salts”, “soot”, and “condensable organic compounds” (COC), and exhibit completely different physical and chemical properties:

- COC are formed during incomplete combustion and are, in the hot flue gas, mainly in the gas phase and are then part of the VOCs. However, COC rapidly condensate in the ambient air after leaving the stack thereby contributing to POA. COC mainly originate from wood pyrolysis and emerge from subsequent reactions in the following pathways:
    - At low temperatures volatile or condensed organic compounds are formed from pyrolysis with characteristic compounds and properties depending on residence time, heating rate, temperature, and other operation parameters.
    - At moderate temperatures and local lack of oxygen, organic compounds can be converted to secondary and tertiary tars including polycyclic aromatic hydrocarbons (PAHs), which can appear as condensables.
- Soot, mostly black carbon (BC) and to a minor extent POA, is also formed during incomplete combustion from organic precursors in zones of high temperatures and lack of oxygen where volatiles and primary tars react to secondary and tertiary tars and form PAHs, which consequently can form soot particles by further synthesis reaction and agglomeration with release of hydrogen.
- Inorganic particles, mainly consisting of salts containing chloride, potassium, calcium, sulphur, and oxygen (e.g., KCl, K_2SO_4, $CaCO_3$, CaO…), are formed from elements in the fuel. As shown by measurements at two automatic boilers [18] and a literature survey [19] by our group, these particles are dominant at near-complete combustion and thus can only be partially avoided by fuel conversion in the primary zone at low temperature and low oxygen content (low-particle combustion).

Combustion conditions and the resulting PM strongly depend on fuel type, combustion technology and type of operation (Figure 4.2), which was shown in own investigations from 1999 to 2009 [20-23]. In

automatic biomass combustion, nearly complete combustion can be achieved and hence salts are the dominant particle fraction. However, during start-up and in phases of inappropriate operation, COC or soot can also be emitted from automatic appliances. Incomplete combustion is often found in small manual wood combustion devices, where soot or COC can be the dominant PM fraction released to the atmosphere. Due to the different temperature regimes and the different influence of the residence time for soot and COC formation, usually either one of the two particle types dominates the total PM emissions at incomplete combustion.

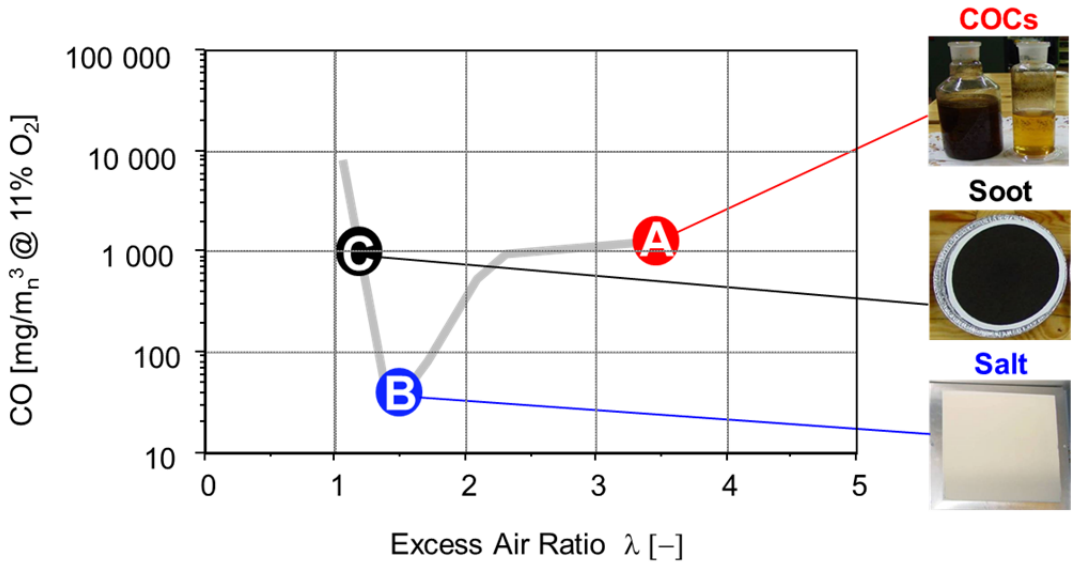

Figure 4.2 CO as function of the excess air ratio (λ) [21]. In typical wood combustion, three different combustion regimes can be distinguished. A (COC): Low temperature as always found during cold start-up and hence cannot be completely avoided. B (salt): Appropriate operation with optimum conditions. C (soot): Lack of oxygen which can occur due to inappropriate operation as for example due to operation with a throttled air inlet.

4.3 Health effects

Many studies (e.g. a review [24]) show strong evidence that airborne particles are related to adverse health effects (Figure 4.3) including increased mortality. Especially, submicron particles (PM_1) are insufficiently filtered by nose and bronchia and consequently can penetrate into the lung inducing several negative effects (Figure 4.3).

Since biomass combustion exhibits a high contribution to air pollution (in the cold season it can be the dominating source of PM_{10} as shown by source apportionment studies from PSI [8-10]) and combustion particles are generally regarded as potentially harmful, several studies investigated potential health effects of biomass combustion emissions using the following methods:

1. Epidemiologic studies (humans only)
2. *In vivo* exposure studies (on animals and on humans)
3. *In vitro* exposure studies (on animal and human lung cells)

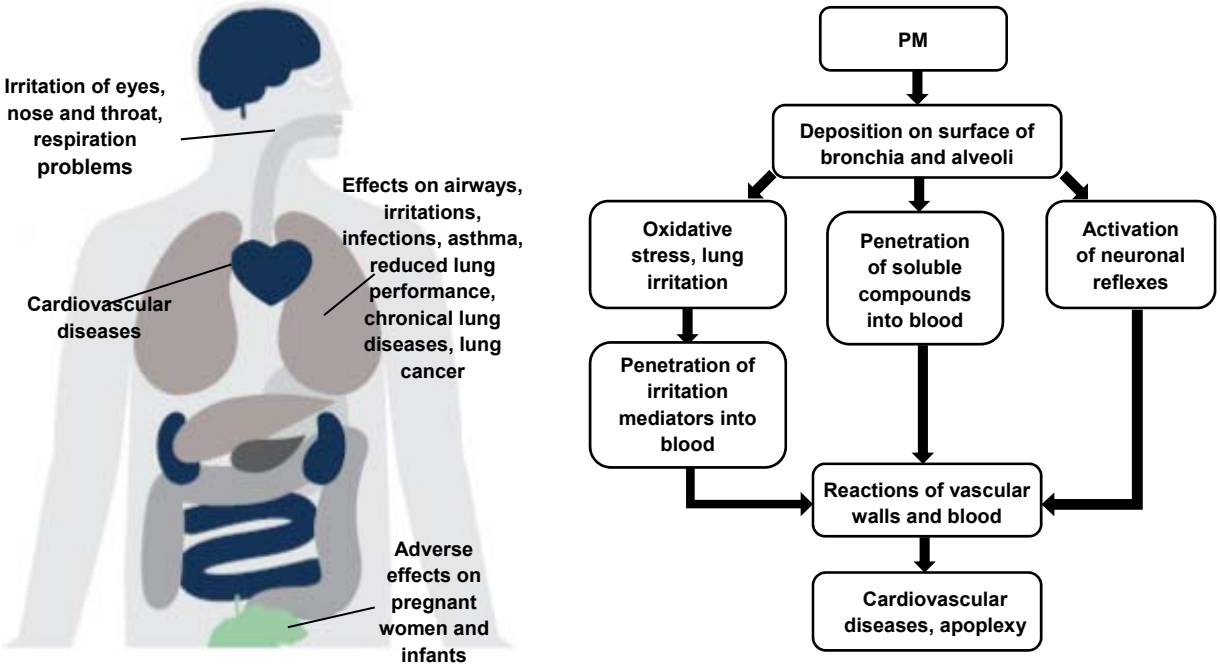

Figure 4.3 Adverse health effects of PM (left panel) and mechanism of noxious effects of PM inside the human body (adapted from [25]).

The different methods have different limitations and advantages (Table 4.1). Nevertheless, it is shown, in both *in vivo* and *in vitro* tests with animals as well as with humans, that biomass combustion particles have adverse health effects (summarised in reviews [15, 26, 27]). Epidemiological studies also found evidence for adverse health effects from biomass combustion PM, however, specific health effects remain difficult to identify using this method [27]. As biomass combustion emissions depend on combustion type and condition (see section 4.2 above) and since the inter-comparability between different health studies is often limited (due to the specific experimental conditions, the complexity of the involved processes), a large number of tests is required to compare important factors influencing specific health effects. This is difficult with *in vivo* experiments and consequently, in this study, *in vitro* experiments are carried out allowing a larger number of tests with highly reproducible and well-controlled conditions.

From *in vivo* and *in vitro* studies (as summarised by reviews [15, 26]) it is evident that biomass combustion emissions are causing cytotoxicity, oxidative stress, inflammation, physiological effects, genotoxicity, and carcinogenicity. In addition, a dose-response effect for particle exposure exists except in case of overloaded biological systems [15, 26]. In this investigation cytotoxicity is used as biological end-point since its determination is relatively fast and economic.

Table 4.1 Advantages and limitations of experimental models used in studies on health impacts from wood combustion emissions (adapted from [28]).

Method	Advantages	Limitations
In vitro	- Exposure can be of large ranges - Well-controlled environment	- Exposure may not be realistic - Not representative of entire body - When animal cells were used, results may not be applicable to humans - Sampling and collecting PM may alter its particle characteristics - Investigation of SOA is difficult
In vivo	- Strong link between effects and exposure - Realistic air to lung exposure is possible	- Exposure duration and concentration are limited - Test subjects are highly variable - Investigation of SOA is difficult - If animals are tested, results may not be applicable to humans
Epide-miology	- Realistic exposure and effects - Long term exposure - Effect of SOA is captured	- The causality is limited and effects cannot be confidently related to one single event - Exposure is not highly specific and hard to quantify - Effects not necessarily caused by exposure

It was shown that combustion type and condition is an important factor influencing specific health effects. For example, incomplete combustion or low oxygen combustion in a conventional soapstone and a masonry heater exhibits a stronger impact on cell toxicity and cell functions than particles from near-complete combustion [29]. Moreover, biomass combustion flue gases contain reactive oxygen species (ROS), which are responsible for oxidative stress and therefore are used as markers for potential health effects. A study carried out from our group in collaboration with the Laboratory of Atmospheric Chemistry from PSI shows, that the ROS content strongly depends on combustion temperature and consequently varies for different combustion devices and conditions [30]. Moreover, old or manual combustion devices can induce higher cytotoxicity and carcinogenicity compared to new combustion technologie and/or automatic devices as shown in two experimental studies where cells where exposed to particle filter extracts [31, 32]. An earlier investigation by our own group, where filter extracts and COC samples were exposed to cells, reveals that PM from different biomass combustion devices and conditions shows decreasing cytotoxicity and carcinogenicity in the following order [3]: COC, soot, and salt particles

However, most of the *in vitro* studies report health effects from biomass combustion particles collected on filters, in impactors or in electrostatic precipitators [3, 29, 31-33] with subsequent cell analysis. COC or secondary organic aerosols (SOA) are usually not investigated due to complex experimental setups. Furthermore, the legislation on biomass combustion relevant for Switzerland and neighbouring countries considers only emission limits of filterable PM in the hot flue gas. Consequently, determining health effects from biomass combustion emissions on PM collected on filters exhibits the following limitations:

- In combustion emissions studies particle collection often is carried out in the hot flue gas and consequently, health effects of COC, mainly leading to POA and partly to SOA in the ambient air, are considered to be highly toxic, cannot be evaluated.

- An own investigation shows that depending on the extraction method of PM from filters (with solvent or grinding the whole filter) not all particles can be extracted or adverse effects due to the filter material can occur [3].

Consequently, with the current knowledge, the total effect of biomass flue gases in real-life conditions cannot be accurately assessed due to scarce information of the potential adverse effecs of COC and SOA. Hence, there is a need of further studies to close the knowledge gap between pollutant emissions at the stack and exposure in the ambient air. Furthermore, new and less complex sampling and experimental methods have to be developed enabling the investigation of COC, SOA and solid particles (SP) from biomass combustion flue gases. Therefore, in this study a sampling line, which allows an economic collection of COC and SP for subsequent *in vitro* cell analysis, is developed and characterised.

4.4 Target

Due to the above mentioned limitations of most of the current experimental procedures for assessing health effects of biomass combustion emissions, a new sampling line shall be developed in this project enabling the assessment of *in vitro* effects of both, SP and COC. Thereby, if possible, standard sampling procedures should be applied. In addition, the experimental method should avoid complex and/or time consuming sample preparation and after treatment procedures prior the cell analysis. With such a method, conducting a larger number of test investigating different biomass combustion devices and conditions shall be possible and carried out in the second part of the project.

5 Methods

5.1 COC and SP sampling method for *in vitro* cell analysis

In order to develop a simple sampling method for the *in vitro* analysis of COC and SP emitted during wood combustion 2 standard sampling methods were combined:

1. VDI 2066 and OPAC for gravimetric SP determination in the hot flue gas.
2. US-EPA method 5H ("Determination of Particulate Matter Emissions from Wood Heaters from a Stack Location" [1]) for sampling SP and condensable compounds (COC, salts, alkali metals).

During sampling, hot flue gas from different wood combustion devices is guided through a heated sampling line which consists of a heated filter, a series of three impingers (borosilicate glass), the first two filled with water and cooled to 5 °C, and a second non-heated filter. The rapid cooling induces the condensation of mainly organic compounds (condensable organic compounds, COC), inorganic salts, water and to a minor extent heavy metals into the impinger fillings [3]. Droplets leaving the impingers due to the sampling air stream and water insoluble particles formed due to the rapid cooling are trapped on the second filter after the impingers. Subsequently, cells are exposed to these liquids and cell viability, used as cytotoxicity indicator in this study, after a certain exposure time is analysed. For the sampling method developed in this project the following two adaptations compared to the standard sampling method US-EPA 5H are applied:

- Instead of water being used in the first two impingers, the use of different cell growth media used for the different cell cultures is investigated as an alternative sampling procedure.
- A second modification compared to the US-EPA 5 method is that the experiments are performed not only with but also without a filter upstream of the impingers. Consequently, in addition to condensable compounds also water soluble SP are trapped. This enables investigating the influence of solid, mainly water soluble, particles precipitated in the impingers.

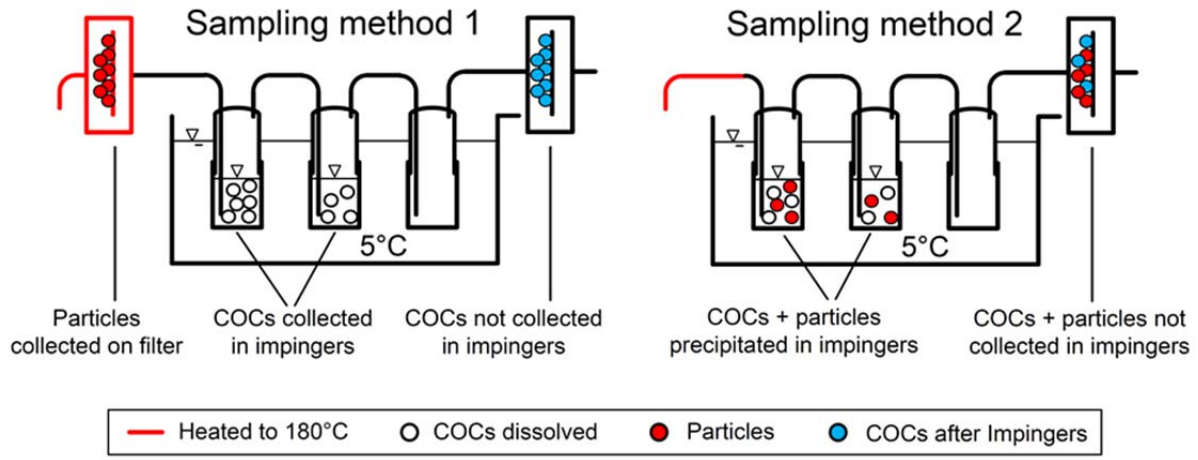

Figure 5.1 Schematic of both sampling lines for cytotoxicity analyses. Sampling method 1 corresponds to the US-EPA 5H method and is used to collect COCs. The heated filter upstream of the impingers is also according to the VDI 2066. Sampling method 2 is a modification of the US-EPA 5H method where the filter upstream of the impingers is omitted and consequently in addition to COCs also particles are precipitated into the impingers.

In order to investigate the cellular effects of both, COC and SP, in flue gases from wood combustion two different sampling lines are set up in parallel which will be referred to as sampling method 1 and sampling method 2, respectively. Sampling method 1 corresponds to the US-EPA 5H method and VDI 2066 (Figure 5.1 and Figure 5.2), whereas in sampling method 2 the filter upstream of the impingers is omitted (Figure 5.1 and Figure 5.2) and in addition to COCs, also SP are precipitated into the impingers. By subtracting the cell viability from sampling method 1 (cell viability of COCs only, referred to as CV_{COC}) from the one of sampling method 2 (cell viability of COC plus SP, referred to as CV_{tot}), the cell viability of SP only (CV_{SP}) can potentially be determined.

$$CV_{SP} = CV_{tot} - CV_{COC} \quad (5.1)$$

For an ideal sampling, two conditions are achieved:

1. Solid particles are quantitatively collected on the filter prior to the impingers (method 1).
2. Solid particles and COCs are quantitatively collected in the impinger fillings (method 2).

Condition 1 can be assumed as valid when applying sampling method 1. However, condition 2 is not achieved for sampling method 2 due to the following reasons:

- Mainly water soluble compounds are trapped within the impinger fillings. BC and PAHs particles which are water insoluble are only precipitated to a minor extent when aerodynamically not able to follow the air stream through the impingers.
- A part of COCs and solid particles, especially water insoluble ones, can penetrate through the impingers and thus are collected on filter 2.

Consequently, the cell viability of SP analysed mainly reflects the cytotoxicity of water soluble SP.

Figure 5.2 Picture of the parallel setup of sampling method 1 and 2. 1) sampling line for gas phase analysis; 2) sampling line method 2; 3) sampling line method 1; 4) heated filter for gravimetric SP determination according to VDI 2066 standard; 5) back up filter to collect COC not trapped in sampling liquid; 6) filter after impingers in sampling method 2 and 7) water bath cooled to 5°C.

5.2 Experimental setup

The experiments consist of two parts. The samples are generated in the combustion laboratory of the Bioenergy Research Group at the campus of the Lucerne University of Applied Sciences and Arts (Lucerne UASA) in Horw (Figure 10.1 in the Appendix). Subsequently, the *in vitro* cell analysis is performed at the laboratory of the Competence Centre Aerospace Biomedical Science & Technology (CC ABSaT) in Hergiswil (Figure 10.2 in the Appendix). The experimental setup in the combustion laboratory is displayed in Figure 5.3.

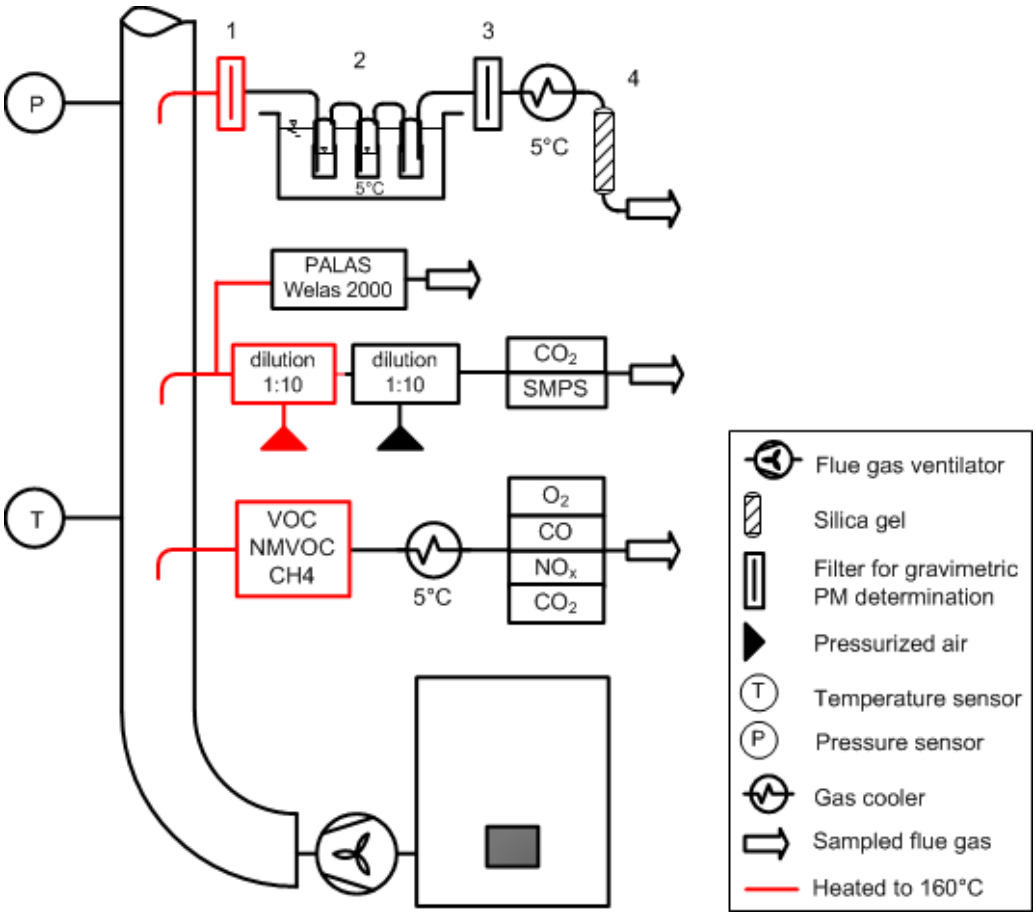

Figure 5.3 Schematic of the experimental setup at the combustion laboratory of Lucerne UASA for the analysis of COCs, gas and particle emissions from different wood combustion devices. The sampling line for COCs consists of the following components: 1) and 3) filter for gravimetric SP determination, 2) impingers cooled in a water bath to 5 °C, 4) pump with gas cooler, water precipitator and silica gel cartridge. The COC sampling line is set up two times in parallel. One time, with a filter upstream (only filter 1) and a second time, with a filter downstream (only filter 2) of the impingers.

In addition to the sampling of COC, a detailed characterisation of the flue gas is conducted covering the following species:

- Gas analysis:
  - O_2, CO, NO_x, CO_2, CH_4, volatile organic compounds (VOC), and non-methane volatile organic compounds (NMVOC). Measuring principles: paramagnetic (O_2), non-dispersive

infrared analysis (ND-IR, CO, CO_2), chemiluminescence (NO_X), and flame ionisation detector (FID, CH_4, VOC and NMVOC).

- Particle analysis:
  - SP mass is determined gravimetrically with isokinetic sampling according to VDI standard and to the Swiss Ordinance on Air Pollution Control (OAPC, [16]) on filter 1 (quartz or glass fibre filters, see Figure 5.3) at 160°C.
  - For some experiments, the particle number concentration and the particle size distribution are measured after two ~1:10 dilution steps using a scanning mobility particle sizer (SMPS) and an optical aerosol spectrometer (OAS, model Welas 2000 from Palas).

Furthermore, flue gas temperature and velocity (determined with a Pitot tube) are measured as well. Since the toxic effect of inorganic salts is much lower than for COCs [3] and heavy metal emissions are of minor importance for natural wood fuels as used here. Cytotoxicity in this study is mainly related to the total organic content (TOC) within the impinger fillings. TOC is measured by thermal oxidation followed by infrared detection according to the reference method DIN EN 1484-H3 performed by Bachema AG, a laboratory for chemical and microbiological analyses of environmental samples.

5.3 Experimental procedure

5.3.1 Combustion laboratory

Before every experiment, the gas analysers are calibrated using standard reference gases. Filters for the gravimetric SP determination (50 mm diameter, quartz and glass fibre filters Whatman QM H and Schleicher&Schuell GF10, respectively) are conditioned for minimum 6 h in a desiccator filled with silica gel and weighted prior to and after sampling. The glass impingers (total volume of bottle 1 is 100 ml and for bottle 2 and 3 250 ml) and connecting sampling tubes are sterilised. All openings are closed with aluminium foil and sampling equipment is then sterilised dry (at 160 °C for 2 h) or using an autoclave system. For most of the experiments 170 ml (80 ml in bottle 1 and 90 ml in bottle 2) are used. US-EPA method 5H suggest 100 ml in each bottle, however, to save cell growth media, less volume is used. In order to provide the longest possible path of the flue gas through the impinger fillings to achieve as high as possible collection efficiencies of COCs, only a 100 ml bottle is used for bottle 1. Impingers are then cooled in a water bath to 5 °C for minimum 15 minutes. The hot flue gas is then guided through the filters and impingers with 5–15 l/min using a pump with gas cooler, water precipitator and silica gel cartridge to determine the sampling volume under dry standard conditions. After sampling the amount of the impinger fillings is measured again and stored in sterile 50 ml conical centrifuge tubes (Falcon™) in a fridge at 5 °C until the cell analysis which is then performed in laboratory of the CC ABSaT in Hergiswil.

5.3.2 Cell analyses

5.3.2.1 Cell cultures

Three different cell lines, human epithelial lung cells H187 (CRL-5804), human epithelial lung cells A549 (CRL-185, alveolar type II cells) and skeletal muscles cells from mice C2C12 cells (CRL-1772), have been tested. All cell lines were obtained from American Type Culture Collection (ATCC; LGC Standards, Molsheim Cedex, France). C2C12 cells are cultured in Dulbecco's modified Eagle's

medium high Glucose (DMEM, Thermo Fisher Scientific, 41966) containing 10 % fetal bovine serum (Brunschwig, CVFSVF00-01).

A549 cells are cultured in Kaighn's modification of ham's F-12 medium (F-12K, Thermo Fisher Scientific, 21127) containing 10 % fetal bovine serum (Brunschwig, CVFSVF00-01). H187 cells are cultured in Roswell Park Memorial Institute medium 1640 (RPMI, Thermo Fisher Scientific, A10491) containing 10 % fetal bovine serum (Brunschwig, CVFSVF00-01). All cultures are maintained in a fully humidified atmosphere, at 37 °C and 5 % CO_2. Cells are passaged every 2 days and are used for experimentation only before the 15^{th} passage. C2C12 and A549 cells are seeded at 5000 cells/cm^2 in 6 well-dishes (Semadeni, 6229), H187 are seeded at a density of 15 000 cells/ml.

The preparation of medium with different concentrations of exposed medium was performed by first adding fetal serum (10 %) separately in medium exposed and medium not exposed. Later, the different media were mixed at the required concentrations. The preparation of medium with different concentrations of exposed sterile water was performed adding to exposed water fetal serum and 10 fold concentrated medium (reconstituted from powder) and water at the required concentrations.

5.3.2.2 Determination of cell viability

The cytotoxicity of wood combustion flue gases in this study is assessed by measuring the cell viability, denoting the fraction of living cells within a cell population, after a certain time of cell exposure. Cells were exposures for 1 h, 6 h, and 24 h to different concentrations of the sampling liquids generated in the combustion laboratory. Since the best reproducibility was found for samples with 24 h cell exposure (see section 6.1.6) and many other *in vitro* studies investigate acute effects of emissions on cells also after 24 h exposure, after the initial tests this 24 h exposure was used in all subsequent experiments. The cell analysis of different sample concentrations (sampling liquid exposed to wood combustion flue gas is mixed with fresh cell growth medium in different concentrations) results in dose-response curves which are compared for the different combustion devices and conditions. Furthermore, from these dose-response curves the LD_{50} (lethal dose for which half of a tested population dies) is determined.

The cell viability is measured by flow cytometry. Analyses are performed using an Accuri C6 system (FACS, ©BD Sciences). This system is equipped with a blue and a red laser for excitation. For detection, there are two scatter detectors and four fluorescence detectors. The system is able to rate 10 000 events per second. The blue laser (488 nm) and the FL2 detector were used for Propidium Iodide (PI) quantification. Dead cells are stained with PI (see Figure 10.3 in the Appendix). PI cannot pass through intact cell membranes, but may freely enter cells with damaged cell membranes. Upon entering dead cells, PI will intercalate into double-stranded DNA or double-stranded RNA. Therefore, PI can be used to stain dead cells. PI is excited at 488 nm and, with a relatively large Stokes shift that can easily be observed using a FACS. Nota Bene: Because this intercalation is mediated by non-covalent forces, PI must remain present in the buffer used to re-suspend cells for data acquisition so that dead cells remain labelled. The detailed staining protocol for the different cell lines can be found in section 10.2 of the Appendix.

To assess the dose response of the cytotoxicity and consequently the LD_{50}, concentrations inducing cell death are used. However, this is not representative for real life conditions where humans are usually not exposed to such high concentrations. Therefore, ideally also other end-points relevant for health effects from biomass combustion flue gas, such as oxidative stress and pro-inflammatory mediators, i.e. Interleukin-8, could be investigated. However, the assessment of these end-points is time consuming, costly and additionally not all necessary infrastructures and experience is available. Therefore, in this project the focus was maintained on studying toxicity of combustion emissions and a method was established, while other end-points were not studied.

5.4 Experimental program

5.4.1 Characterisation of sampling method

To characterise the handling and sampling procedure and examine the influence of different parameters, the following tests (described in detail in the sections below) were performed:

- Sterility and blanks
- Sampling liquid and amount as well as sampling flow rate
- Time between sample generation and start of cell analysis (storage effect)
- Reproducibility.

5.4.2 Combustion devices

For the first tests and the characterisation of the sampling method, a commercial pellet boiler (15 kW, model LPK15 from Liebi LNC AG, Figure 5.4) was used. In addition to an appropriate operation with boiler settings according to the manufacturer ($\lambda_{opt,\ pellet\ boiler}$), modifications of the settings are applied to create combustion conditions with high excess of combustion air ($\lambda_{++,\ pellet\ boiler,}$ resulting in high CO and NMVOC emissions) or with lack of air ($\lambda_{--,\ pellet\ boiler,}$ resulting in high CO and soot emissions). For the former, additional air is blown into the combustion chamber via the ignition tube, whereas for the latter an air inlet is closed. A previous study from our group [34] showed that the generation of these different combustion conditions enables reproducible conditions. This represents, however, an inappropriate operation of the boiler.

Figure 5.4 Images of combustion devices investigated in this study. From left to right: Pellet boiler, log wood stove, moving grate boiler operated with wood chips, log wood boiler.

After the method characterisation with the pellet boiler three additional combustion devices with different combustion conditions (10 in total) were investigated (see Table 2 and Figure 5.4). In the log wood stove for the “Reload” condition always 2 logs with an average weight of 1.5 ± 0.7 kg were used for refilling. The fire in the log wood stove was always started using four small pieces softwood and two pieces of wax-covered wood wool fire starters, which is referred to as ignition module (described in an investigation by our group in [35]), which are put on top of a batch of logs. For the cold start condition with ignition from the bottom a batch of logs were put on top of the ignition module which represents not the ideal case and results in higher cold start emission than ignition from the top [35]. The lack of combustion air condition in the log wood boiler ($\lambda_{--,\ \text{log wood boiler}}$) was achieved by manually closing the inlet for the secondary combustion air and keeping the primary combustion air inlet open. All measurements with the moving grate boiler were conducted with the electrostatic precipitator (ESP) switched off. The combustion conditions with high excess and lack of combustion air in the pellet and log wood boiler were investigated for scientific purposes since these conditions exhibit distinct emissions (COC and soot). In real life operation, usually these conditions for new automatic devices are very scarce and only occur with sever mal-operation.

Table 2 Combustion devices and conditions investigated in this study.

Combustion device	**Nominal heat output**	**Model**	**Supplier**	**Combustion conditions**
Pellet boiler	15 kW	LPK15	Liebi LNC AG	Optimum/best ($\lambda_{opt,\ \text{pellet boiler}}$) Lack of combustion air ($\lambda_{--,\ \text{pellet boiler}}$) High excess of air ($\lambda_{++,\ \text{pellet boiler}}$)
Log wood stove	8 kW	Carena	Tiba AG	Cold start (ignition from bottom) Stationary Reload Dry and wet wood*
Moving grate boiler	150 kW	-	Schmid energy solutions AG	Full load (100%) Part load (30%) Going to standby from 30%
Log wood boiler**	30 kW	Navora	HDG	Cold start Stationary ($\lambda_{opt,\ \text{log wood boiler}}$) Lack of combustion air ($\lambda_{--,\ \text{log wood boiler}}$)

*Beech wood with 16 % and 23.5 % moisture content on dry basis for dry and wet wood, respectively.

**beech wood with a moisture content on dry basis of 13 %.

6 Results

6.1 Method characterisation

6.1.1 FACS analysis

Before determining the cytotoxicity of pollutant emissions from wood combustion flue gases, the applicability of the fluorescence-activated cell sorting (FACS) for measuring cell viability (see section 5.3.2.2), for the samples generated with the sampling method presented above (section 5.1), is investigated.

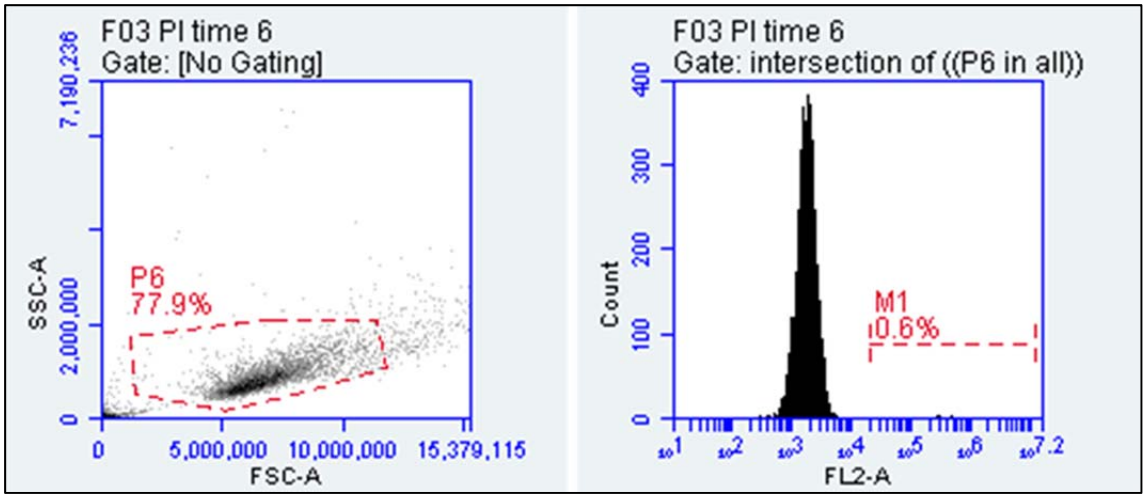

Figure 6.1 Example of the analysis of C2C12 cells by FACS after 6 h incubation. Cells were cultured with DMEM not exposed to wood combustion flue gases (negative control). Cells represent 77.9 % of all events (left panel) and 0.6 % of them are dead (right panel).

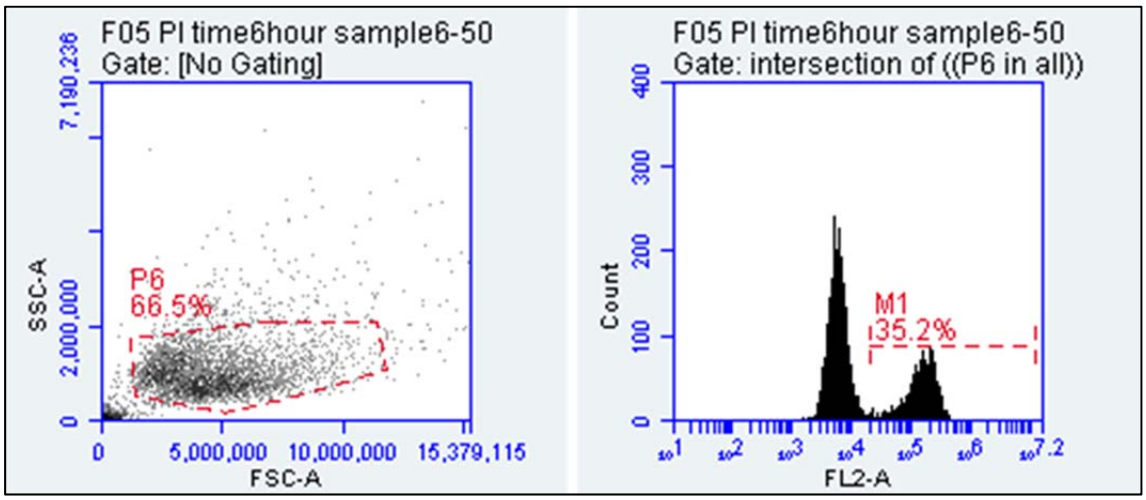

Figure 6.2 Example of the analysis of C2C12 cells by FACS. Cells were cultured with DMEM exposed to higher concentrations of COC within the wood combustion flue gases. Cells represent 66.5 % of all events (left panel) and 35.2 % of them are dead (right panel).

Figure 6.1 shows an example of C2C12 cells, cultured with DMEM not exposed to wood combustion flue gases, after 6 h incubation. A clear area with the signal of the cells can be identified (Figure 6.1 left panel) which is clearly distinct from the debris (lower left corner in Figure 6.1). Dead cells have

different properties compared to living cells. As seen in Figure 6.2, the signal in the SSC-A (intensity of scattered light) and FSC-A (intensity of diffraction signal) space shifts towards the left with a higher fraction of dead cells compared to less dead cells (Figure 6.1). Plotting the number of counts as a function of the intensity (right panels of Figure 6.1 and Figure 6.2) a bimodal signal is evident, from which the fraction of dead cells can be quantified.

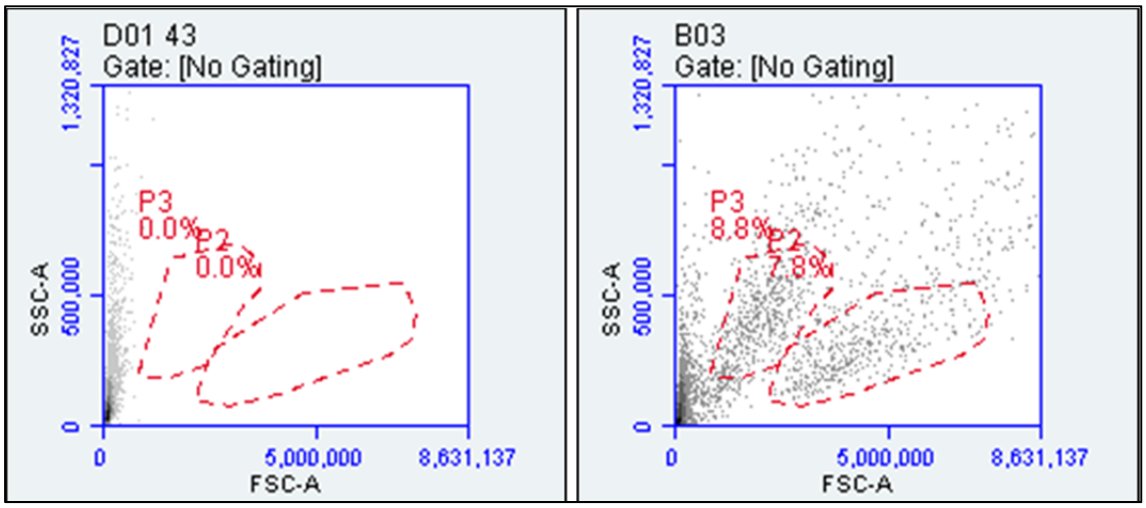

Figure 6.3 Example of the FACS analysis of H187 cells cultured with RPMI exposed to SP and COC from wood combustion (sampling method 2). On the left, medium without added cells is analysed. Events correspond to SP. On the right, cells exposed to SP and COC are shown. SP events do not overlap with cell events. Red dashed circles denote areas for living (right circle) and dead (left circle) cells.

Figure 6.3 shows a comparison of the FACS analysis of the RPMI after exposure to COC and SP generated with sampling method 2 without subsequent cell incubation (left panel) and with H187 cells exposure (right panel). It is evident that the particles within RPMI are not within the area where the cells are found and therefore do not influence the determination of the cell viability. To further investigate, if suspended and not dissolved SP within the sampling liquids influences the FACS analysis, phase images are investigated. First, from Figure 6.4 panels A and E a clear difference in number of suspended SP is evident. Only very few particles can be seen in solution in A (sample TOX 130 generated with sampling method 1 with a filter upstream of the impingers) whereas a large number of suspended particles is found in sample TOX 131 (panel E) which was generated with sampling method 2 without a filter upstream of the impingers. Suspended SP in sample TOX 130 are most probably not due to the sampling of wood combustion flue gas, and rather occur due to minor impurities of the sterile water and/or due to the sample handling (see also Figure 10.6). Investigating the phase images of TOX 131 (panels G and H in Figure 6.4) hardly any particles are visible. They are most probably removed during the PI staining process after incubation (see section 10.2 in the Appendix), especially due to washing and centrifuging the cells several times. Furthermore, also no particles incorporated in the cell aggregates possibly influencing the FACS signal are evident. The FACS analysis and phase images of two more samples (see Appendix section 10.3) confirm the results presented in this section. Consequently, it can be concluded that suspended SP do not influence the quantification of the number of dead cells using the FACS analysis. This is also in line with [29] who also did not find interferences of suspended SP in FACS analysis for cell viability determination.

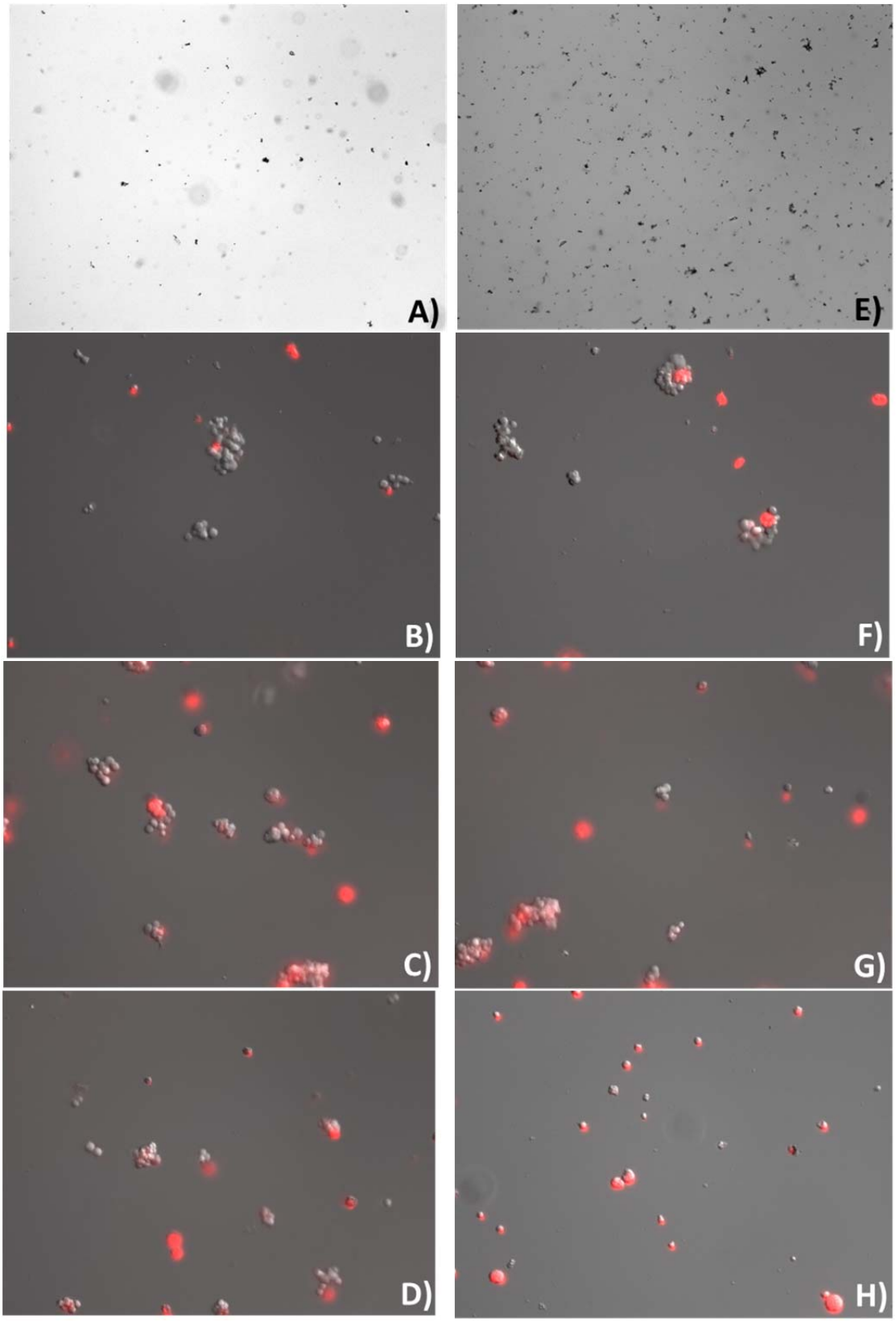

Figure 6.4 Microscope (images A and E) and phase images (B, C, D, F, G and H) of samples TOX 130 (left panels, COC only, sampling method 1) and TOX 131 (right panels, COC plus particles, sampling method 2) after 24 h incubation. Phase images B) and F) represent negative controls with 11.6 % and 12.2 % dead cells, C) and G) are phase images from the original samples diluted to 0.5 % with 37.1 % and 45.6 % dead cells, D) and H) are phase images diluted to 20 % with 83.3 % and 88.9 % of dead cells.

6.1.2 Blanks and sterility

To assess possible interferences with the cytotoxicity tests, e.g. due to contamination with bacteria during preparation of the experiment and/or sampling, two experiments were performed. First, the influence of the sterility of the sampling equipment was investigated. Therefore, two samples (TOX 1 and TOX 3) were generated with sampling method 1. For sample TOX 1, the entire silicon tubes of the sampling line as well as the impingers were cleaned and sterilised using an autoclave system. For sample TOX 3, impingers and tubes were used which were not cleaned (equipment was not used before but standing in the open laboratory for several weeks) or sterilised before sampling. From Figure 6.5 it is evident that there is no influence of the sterility of the sampling equipment as CV_{COC} of samples TOX 1 and TOX 3 is similar for all exposure times and concentrations. Nevertheless, in all subsequent experiments only sterile sampling equipment is used.

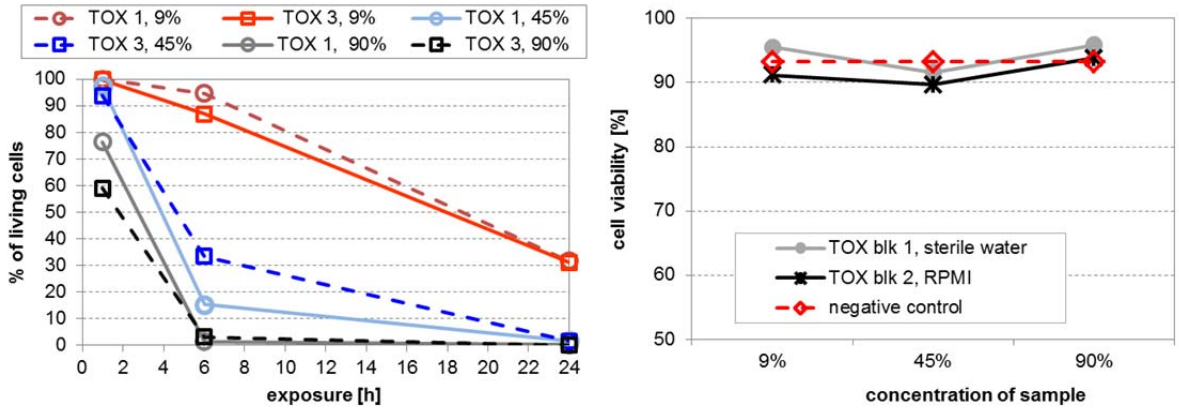

Figure 6.5 Comparison CV_{COC} of C2C12 cells between using sterile and non-sterile/not cleaned sampling equipment (left panel) and cell viability of H187 cells of two procedure blanks (right panel). Three different concentrations (sample diluted to 9 %, 45 % and 90 % of original sample), and negative controls were tested. Sample TOX 1 was generated with sterile and TOX 3 with non-sterile/not cleaned equipment. For both samples $\lambda_{++,\text{pellet boiler}}$ combustion conditions were used.

Table 6.1 Different analysis steps of the exposed sample, procedure blank and negative control.

	in combustion laboratory		in biology laboratory		
step of analysis/ samples	sample preparation*	exposure of sampling liquid to flue gas	preparation of cell culture**	incubation	cell viability measurement
exposed sample	yes	yes	yes	yes	yes
procedure blank	yes	-	yes	yes	yes
negative control	-	-	yes	yes	yes

*sample preparation includes sterilizing of sampling equipment and filling sampling liquid into the impingers which are then cooled in a water bath to 5°C for minimum 15 minutes.

**cell cultures are either prepared with liquid used for sampling in the combustion laboratory or with fresh DMEM, RPMI or sterile water. Preparation of cell cultures for negative controls is performed at the same time as for the other samples.

Second, two procedure blanks (samples TOX 32 and TOX 33) which were identically treated as all other samples, except that they were not exposed to the flue gas, were analysed as well. Cell viability of both samples is similar to the negative control (see Figure 6.5) indicating that there is no effect of the different handling steps (preparation and handling at the combustion laboratory as well as pre-

paring and growing of cell cultures, PI staining and FACS analysis at the biology laboratory) on the cell viability. Table 6.1 shows a more detailed description of the differences between exposed samples, procedures blanks and negative control.

6.1.3 Sampling liquid

In this section possible differences of CV_{COC} of H187 cells between using directly RPMI or sterile water as impinger fillings are investigated due to the following reasons:

1. To test if the hot flue gas (100–180 °C) passing through the impingers alters the RPMI subsequently influencing the cell cultures and cell viability.
2. An in depth chemical analysis of substances precipitated in the impingers can be easier performed on ultra-pure water as the pure RPMI already consists of several organic and inorganic components.

Therefore, three experiments were performed where samples with RPMI and sterile water were generated. From Figure 6.6 it is evident that there is no significant difference in CV_{COC} for samples generated with sterile water and RPMI. Since cell growth media already contain several organic and inorganic compounds, the TOC content of RPMI was analysed for one test sample. It is found that the pure RPMI contains already 3300 ± 200 mg/l TOC. Since the TOC concentrations for all investigated samples is on average ~100 mg/l it is evident that these concentrations fall within the uncertainties of the TOC analysis of 6–12 %, as reported by the laboratory which performed the TOC analyses [36], of the pure RPMI. Consequently, for all subsequent experiments, sterile water is used as sampling liquid in order to enable a quantification of TOC. For samples generated with RPMI and DMEM, TOC is estimated using the average ratio NMVOC/TOC during comparable sampling conditions determined from samples where TOC is measured.

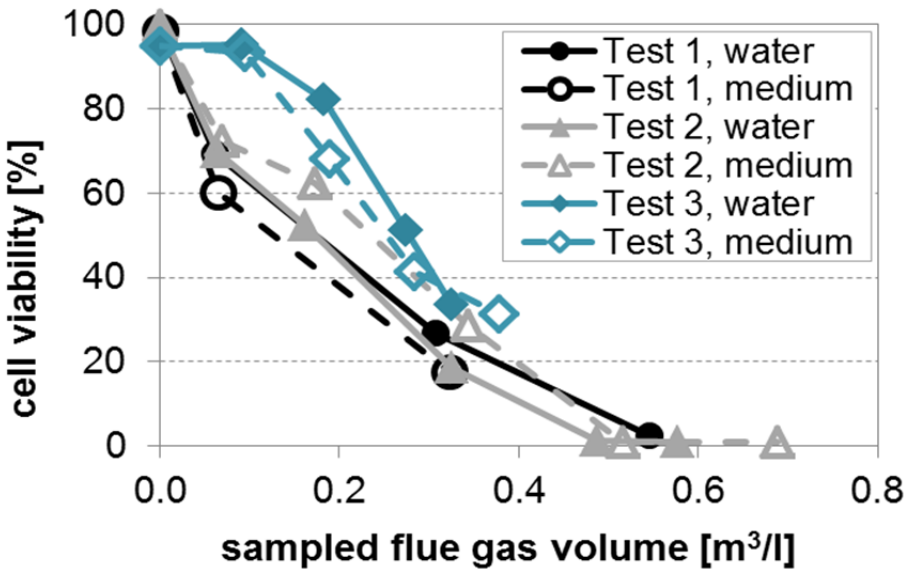

Figure 6.6 Comparison of CV_{COC} of H187 cells after 24 h cell exposure using sterilised water (solid lines) or RPMI (dashed lines) as sampling liquid. Samples were generated with the $\lambda_{++,\ \text{pellet boiler}}$ combustion conditions. Lines with the same colour denote samples which were generated on the same day. The x-axis denotes the volume of flue gas ($V_{flue\ gas}$ at standard conditions and normalised to 13 % O_2) sampled through the impinger fillings.

6.1.4 Storage effect

In a next step, the influence of the time period between sample generation in the combustion laboratory and cell exposure in the laboratory of CC ABSaT was investigated which is referred to as storage effect. All tested samples were generated with sampling method 1 and CV_{COC} of the same samples was analysed after different storage durations. After sample generation samples are transported from the combustion laboratory in Horw to the laboratory of CC ABSaT in Hergiswil (distance of 3.5 km) and stored in a fridge at 4 °C.

From Figure 6.7 it is evident that the cell viability is comparable for different storage durations up to 31 h. However, after sample storage of 14 days, CV_{COC} is significantly increased with low TOC concentrations within the sample. This indicates that the cytotoxicity decreases for longer storage durations. Consequently, in all subsequent experiments the cell analysis in the laboratory of CC ABSaT is always started on the next morning after sample generation in the combustion laboratory which is equivalent to a storage duration of 15–19 h at 4 °C in a fridge.

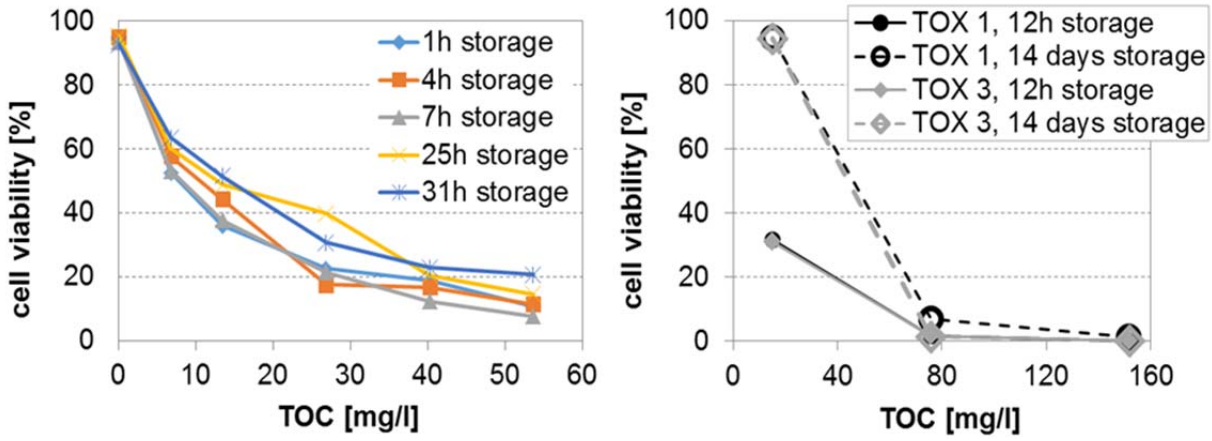

Figure 6.7 CV_{COC} after cell exposure of 24 h of samples TOX 71 (H187 cells, left panel) as well as TOX 1 and TOX 3 (C2C12 cells, right panel) after different storage durations. Samples were generated with $\lambda_{++,\ pellet\ boiler}$ combustion conditions.

6.1.5 Sampling flow rate and filling amount of impingers

Since sterile water, RPMI and DMEM were used as sampling liquids possible differences in the collection efficiency of COCs between the different liquids were investigated. Furthermore, the influence of sampling flow rates and impinger filling volumes on the collection efficiency was tested. Since most of the NMVOCs are condensable two FIDs, one measuring upstream (FID 1) and a second one downstream of the impingers (FID 2) were used. In contrast to FID 1, FID 2 is only capable of measuring total VOCs. However, since CH_4 is basically water-insoluble [37] the collection efficiency is based on total VOCs (see equation 6.1).

$$\Delta VOC = (VOC_{FID\ 1} - VOC_{FID\ 2}) / VOC_{FID\ 1} \quad (6.1)$$

Results (Table 6.2) indicate that there is no significant difference in ΔVOC (1.3 ± 7.0%) using sterile water or RPMI as impinger filling. Furthermore, ΔVOC for the different sampling flow rates (5, 10 and 15 l/min covering the range as specified in the US-EPA 5H method) shows also only small differences (3–5 %). However, comparing ΔVOC for different filling amounts (50, 80, 170 and 280 ml) it is evident

that lower fillings (50 and 80 ml) lead to 8–10 % lower ΔVOC indicating that amounts > 150 ml should be used (US-EPA 5H method suggests to fill bottles 1 and 2 each with 100 ml).

Table 6.2. Mean difference ± standard deviation of ΔVOC between different tests. Numbers in brackets represent the number of performed repetitions. All tests were performed with $\lambda_{++,\text{ pellet boiler}}$ combustion conditions.

test	parameter	$\Delta VOC_{paremter\ 1} - \Delta VOC_{paremter\ 2}$ [%]
Impinger filling	water vs. medium	1.3 ± 7.0 (n = 8)
sampling flow rate	5 l/min vs. 10 l/min	4.0 ± 5.2 (n = 14)
	5 l/min vs. 15 l/min	3.3 ± 3.9 (n = 7)
	10 l/min vs. 15 l/min	4.8 ± 3.9 (n = 7)
Liquid volume in impinger	80 ml vs. 50 ml	4.5 ± 5.8 (n = 7)
	170 ml vs. 80 ml	7.6 ± 5.4 (n = 9)
	280 ml vs. 170 ml	-3.1 ± 4.3 (n = 5)
	170 ml vs. 50 ml	10.2 ± 4.5 (n = 3)
	280 ml vs. 50 ml	8.1 ± 1.5 (n = 3)

6.1.6 Reproducibility

The reproducibility of the cell viability from samples generated at comparable conditions is influenced by the variability of the combustion and sampling conditions as well as the variability of the cell analysis. As described in section 5.3.2.1 cells are passaged every 2 days to a new culture with fresh nutrients. Furthermore, cells were used for experimentation only before the 15th passage to avoid aging effects of the cells. Due to this experimental approach it is not possible to perform all experiments with different combustion devices and conditions with cells from the same passage. Therefore, the variability of the cell viability of cells from different passages (referred to as “biological variability”) also contributes to the total sample-to-sample variability.

Table 6.3 Average ± standard deviation of CV_{COC} for samples TOX 1–TOX 16 at three different concentrations (original sample diluted to 9%, 45% and 90%), two different exposure times (6 h and 24 h) and two different amounts of sampled flue gas volume ($V_{flue\ gas}$) Sampling was carried out with sampling method 1 and combustion conditions $\lambda_{++,\text{ pellet boiler}}$.

$V_{flue\ gas}$	CV_{COC} [%] after 6 h exposure			CV_{COC} [%] after 24 h exposure		
[m_n^3]	diluted to 9%	diluted to 45%	diluted to 90%	diluted to 9%	diluted to 45%	diluted to 90%
40.9 ± 5.6	98.7 ± 0.8	87.0 ± 13.7	56.9 ± 39.5	95.1 ± 3.3	56.3 ± 21.8	16.7 ± 22.7
147.6 ± 22.3	92.9 ± 5.3	47.2 ± 25.1	4.9 ± 4.0	73.9 ± 29.4	11.3 ± 11.0	0.8 ±0.9

To investigate the total variability CV_{COC} of 15 samples exposed to two different flue gas volumes is analysed after cell exposure of 6 h and 24 h for three different concentrations. Results show (see Table 6.3) that the variability (standard deviation of the mean CV_{COC} for the same $V_{flue\ gas}$, concentration range and exposure time) ranges between 0.8-9.5 % and 0.9-29.4 % for 6 h and 24 h cell exposure, respectively. Since the highest variability is < 30 % for the 24 h cell exposure and many other *in vitro* studies (e.g. [29, 31-33, 38]) investigate acute effects of emissions on cells also after 24 h exposure, in subsequent experiments cell viability is only assessed after this time of cell exposure.

Figure 6.8 summarises CV_{COC} after 24 h of cell exposure of all samples (n = 21) where C2C12 cells were use as function of TOC. For every sample CV_{COC} was analysed for 3 to 5 concentrations. Also from this analysis it the variability was found to be max. 26 %. However, since the total variability varies for different ranges of CV_{COC} and dose response curves for different combustion devices and conditions are expected to vary as well, always two or more samples for each combustion device and condition are analysed.

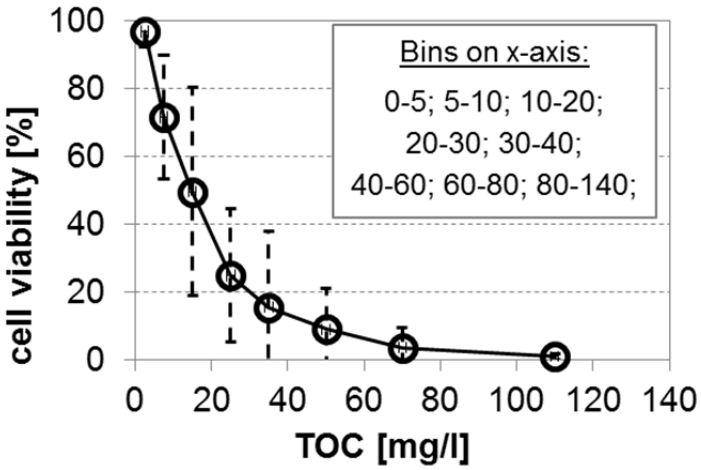

Figure 6.8 CV_{COC} after 24 h of cell exposure of all samples where C2C12 cells were tested. CV_{COC} values were binned into TOC concentration bins and the error bars denote the standard deviation of the mean in each bin. Bin margins were assigned to include at least five CV_{COC} measurements. Samples were generated with $\lambda_{++,\text{ pellet boiler}}$ combustion conditions.

6.1.7 Cell type

To test and evaluate the sampling method the first cytotoxicity tests were performed with C2C12 cells. These cells were used at the beginning for convenience since they were already routinely used at CC ABSaT and no additional procedures or handling steps had to be introduced. Later, to obtain results which are more relevant, the human epithelial lung cells H187 were used. Since different cell types from different parts of the human lung and respiratory tract exhibit distinct responses to exposure of SP (as summarised by review [34]) also a limited number of tests (n = 4) was carried out using the A549 cell line.

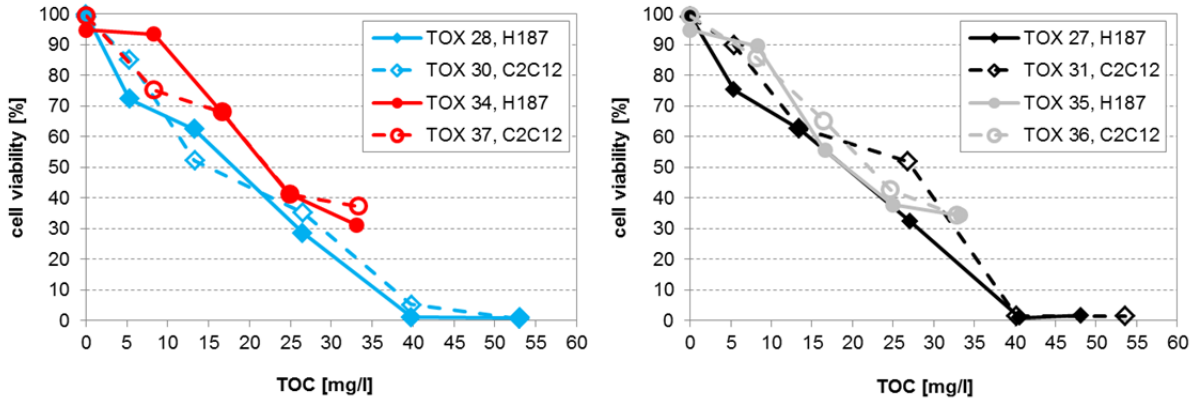

Figure 6.9. Comparison of cytotoxicity of HR187 (solid lines) and C2C12 (dashed lines) during $\lambda_{++,\text{ pellet boiler}}$ combustion conditions. Left panel: CV_{COC} for samples from sampling method 1. Right panel: CV_{tot} for samples from sampling method 2. Lines with same colour denote samples generated on same day.

As presented in Figure 6.9 no significant differences in cell viability between C2C12 and H187 was found. The day-to-day differences originate from the variability in combustion condition, sampling, handling and cell analyses. On the contrary, differences are evident between H187 and A549 cells (Figure 6.10). The dose response curves of the cell viability as function of the TOC content seem to exhibit the same shape, however, for the same cytotoxicity a much higher TOC amount is needed for the A549 cells. For example less than 10 mg/l of TOC is sufficient to induce more than 50 % of dead cells, whereas more than 30 mg/l of TOC are needed for the A549 to induce the same cytotoxicity.

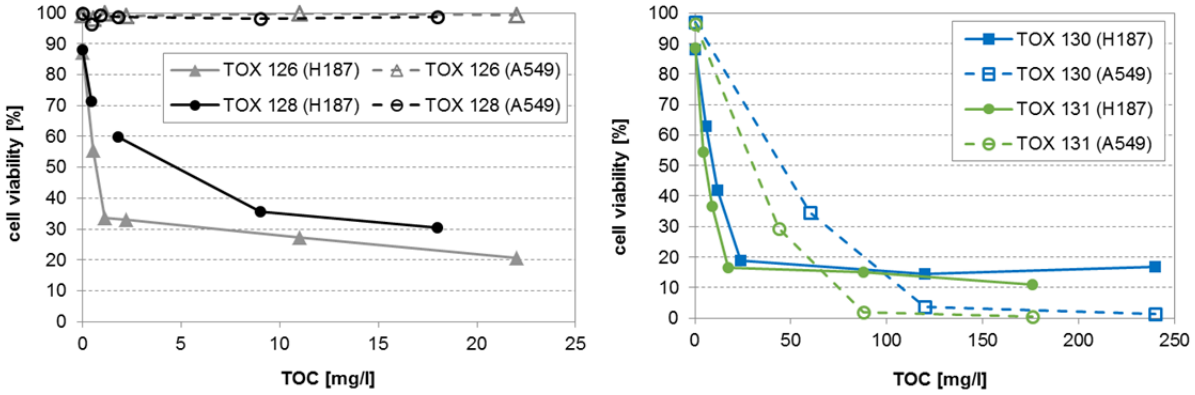

Figure 6.10 Comparison of cytotoxicity results of HR187 (solid lines) and A549 (dashed lines). All samples were generated using the log wood boiler (TOX 116 with cold start and sampling method 1, TOX 126 and TOX 128 with $\lambda_{--,\ log\ wood\ boiler}$ and sampling method 1, TOX 130 and TOX 131 with cold start and $\lambda_{--,\ log\ wood\ boiler}$ with sampling method 1 and 2, respectively).

6.2 Comparison of different combustion devices and conditions

6.2.1 Emissions

Figure 6.11 shows a comparison of the emissions of organic compounds (COC and NMVOC), solid particles (SP) and carbon monoxide (CO) of the investigated combustion devices and conditions. The combustion conditions with high excess and lack of air in the pellet ($\lambda_{++,\ pellet\ boiler}$ and $\lambda_{--,\ pellet\ boiler}$) and log wood boiler ($\lambda_{--,\ lot\ wood\ boiler}$) do not represent a regular operation. These conditions were investigated in order to produce clearly distinct types of SP and COC, i.e. high concentrations of organic compounds and soot for high excess and lack of air, respectively. Experiments in the grate boiler were always performed with the electrostatic precipitator switched off and, consequently, the SP concentrations as presented in Figure 6.11 are much lower in real life conditions. The comparison of the emissions from the different combustion devices and conditions shows the following trends.

- The highest emissions occur in the log wood stove during cold start with ignition from the bottom followed by the reload conditions. For stationary operation, the emissions are much lower, i.e. for organic compounds even by more than one order of magnitude.
- In addition, the other unfavourable conditions (cold start in the log wood boiler, going to standby from 30% in the grate boiler, high excess or lack of air in the pellet boiler and lack of air in the log wood boiler) result in high NMVOC, but especially high CO emissions.
- A strong correlation ($R^2 = 0.74$) between TOC determined in the sampling liquid and NMVOC emissions measured in the hot flue gas is evident (Figure 6.12). Furthermore COC

concentrations (determined from sampling method 1 denoting the sum of TOC measured in the sampling liquid and the mass of COC collected on the filter after the impingers.) can exceed the ones of SP measured in the hot flue gas.

- A strong correlation (R^2 = 0.78) between CO and NMVOC emissions is evident (Figure 6.12). A similar correlation was also observed in a previous study by our own group where the influence of the cold start and part load operation of log wood and pellet boilers on the emissions was investigated [39].

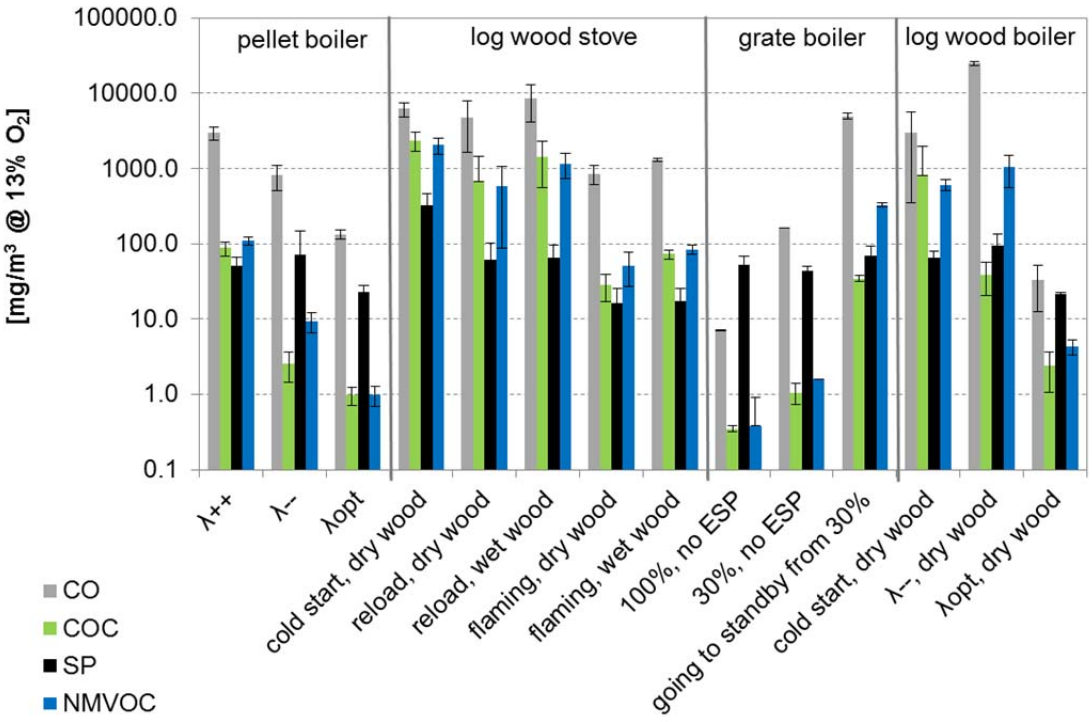

Figure 6.11 Comparison of emissions (average ± standard deviation) from the different combustion devices and conditions. COC are determined from sampling method 1 and are the sum of TOC measured in the sampling liquid and the mass of COC collected on the filter after the impingers.

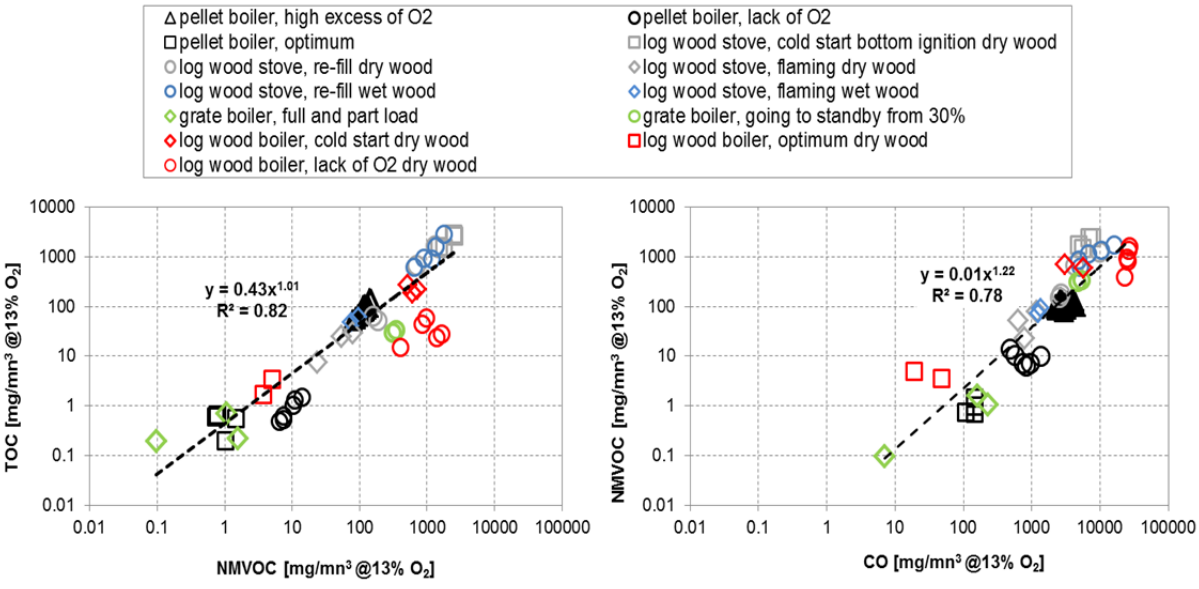

Figure 6.12 Correlations of NMVOC vs. TOC and NMVOC vs. CO from the investigated combustion conditions.

6.2.2 Cytotoxicity of COC

To determine the cytotoxicity of COCs without solid particles samples are generated according to sampling method 1 where a filter upstream of the impingers is installed. To compare the cytotoxicity of organic compounds deposited within the sampling liquids from different combustion devices and conditions, cell viability of COC (CV_{COC}) is related to the amount of TOC detected in the sampling liquids. From Figure 6.13 the following results are evident:

- No toxic effect is observed during part (30%) and full (100%) in the moving grate boiler and the log wood boiler during stationary flaming conditions, even for long sampling durations of 1–3 h. Also for optimum combustion in the pellet boiler only a small decrease of CV_{COC} is evident.
- For the unfavourable combustion conditions in the pellet boiler (λ_{--} and λ_{++}), the log wood boiler (cold start and λ_{--}) and the moving grate boiler (going to standby from 30%) as well as for all combustion conditions in the log wood stove CV_{COC} is significantly decreased with increasing TOC amount within the sampling solutions.
- The TOC concentrations inducing LD_{50}, however, only span a small range of 6 – 19 mg/l. These differences between the different combustion devices and conditions are relevant, however, represent not orders of magnitude.

The LD_{50} was determined by linearly fitting the two data points being closest above and below 50% CV_{COC}. Thereby, values on the x-axis are in the $\log_{10}$ and y-axis values on the linear scale. Before the LD_{50} determination it was accounted for the negative controls and cell viability was scaled up to 100%. It was shown [40] that LD_{50} determination using other methods (e.g. probit) is more accurate, however, due to the following reasons we consider linear fitting appropriate:

- Dose-response functions include only a limited number of different concentrations (n = 4–6) thereby increasing the uncertainty of any fitting method.
- All investigated samples, inducing cell viability < 50%, exhibit a linear area between data points above and below the LD_{50} even without conversion to $\log_{10}$.
- LD_{50} is only determined from the average dose-response functions from the different combustion devices and conditions. The largest standard deviations of the individual data points of the average dose-response curves are 14–35%. These values are expected to be larger than any differences in the LD_{50} determination due to applying different fitting methods.

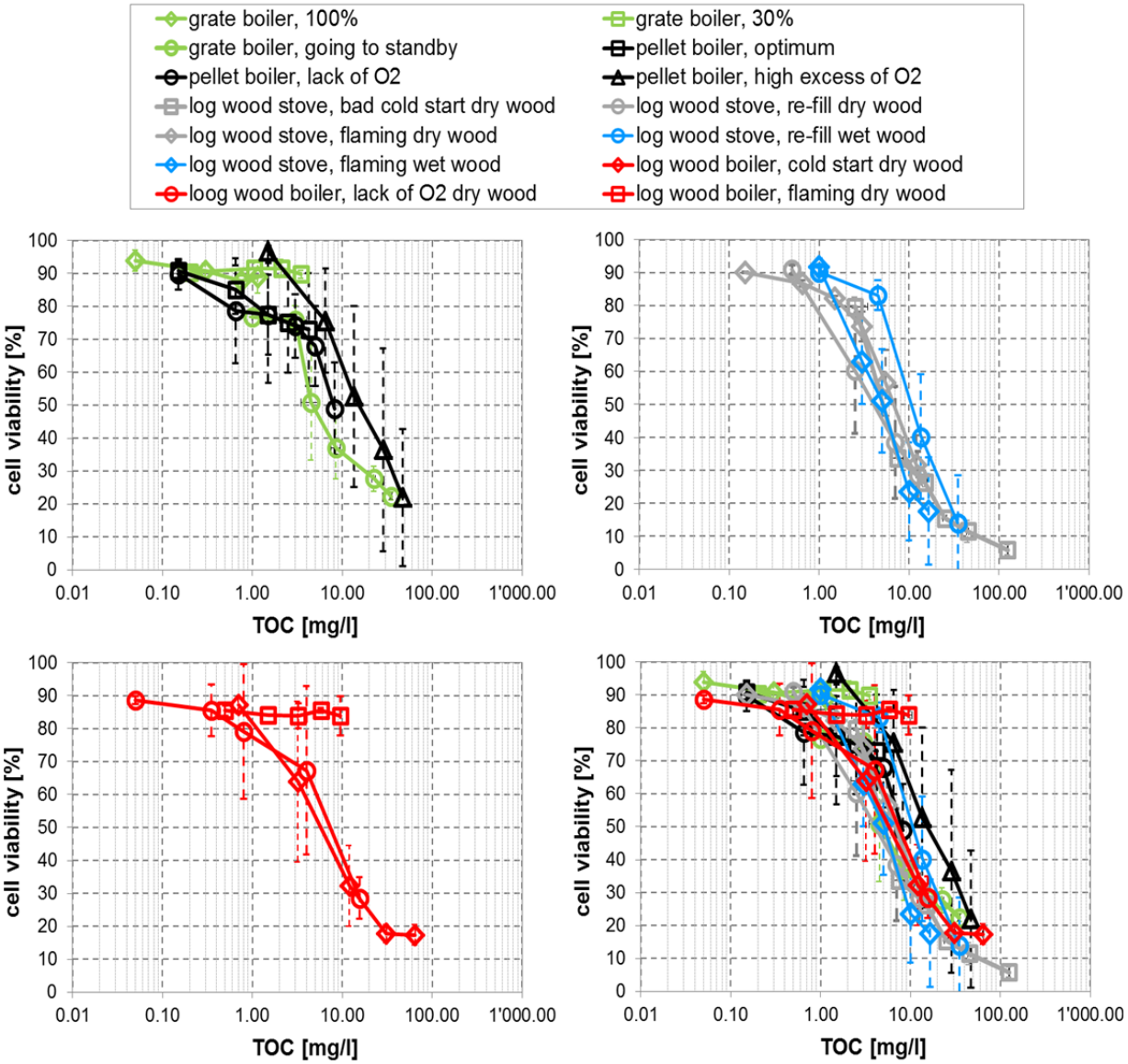

Figure 6.13 Dose response curves of CV_{COC} (H187 cells with 24 h cell exposure) based on TOC within the sampling liquid. CV_{COC} values were binned into TOC concentration bins and the error bars denote the standard deviation of the mean in each bin. Bin margins were assigned for each combustion condition individually. Between two and four samples for each combustion condition were analysed. Upper left panel: Samples from pellet and moving grate boiler. Upper right panel: Samples from log wood stove. Lower left panel: Samples from log wood boiler. Lower right panel: All samples from the different combustion devices and conditions combined.

Since the various combustion devices and conditions have different emissions factors of organic compounds, cell viability is also compared based on the amount of flue gas sampled through the impingers (Figure 6.14). With a known excess air ratio and water content the sampled flue gas volume ($V_{flue\ gas}$) is proportional to the amount of burned wood and consequently proportional to the heat input in MJ or kWh. The comparison of CV_{COC} based on $V_{flue\ gas}$ shows the following results:

- The comparison between the investigated combustion devices and conditions reveals differences of up to almost two orders of magnitude for LD_{50} (Figure 6.14). These differences are much more

pronounced than for the comparison of CV_{COC} based on TOC, and are due to the large differences in emission factors of organic compounds (see section 6.2.1).

- LD_{50} from all unfavourable conditions in the log wood stove (cold start, re-fill and operation with wet wood), the log wood boiler (cold start and combustion with lack of O_2) and the grate boiler (going to standby from part load) exhibit similar values. The minimum amount of $V_{flue\ gas}$ to reach LD_{50}, and consequently exhibiting the highest cytotoxicity, is found during combustion with lack of O_2 in the log wood boiler, followed by going to standby from 30% in the grate boiler and the cold start with ignition from the bottom in the log wood stove (Figure 6.15).
- When using wet wood in the log wood stove ~9 times less amount of flue gas is required inducing LD_{50} compared to dry wood during flaming conditions, which is equal to a 9-fold higher toxicity.
- Flue gas from stationary conditions in the grate boiler (full and part load conditions) and log wood boiler shows no effect on CV_{COC} even for large amounts of $V_{flue\ gas}$ (sampling for 1–3 h with a flow rate of 10 l/min). Also the combustion in the pellet boiler at optimum conditions only shows a minor decrease of CV_{COC}. This decrease, however, occurs only with very large amounts of $V_{flue\ gas}$.

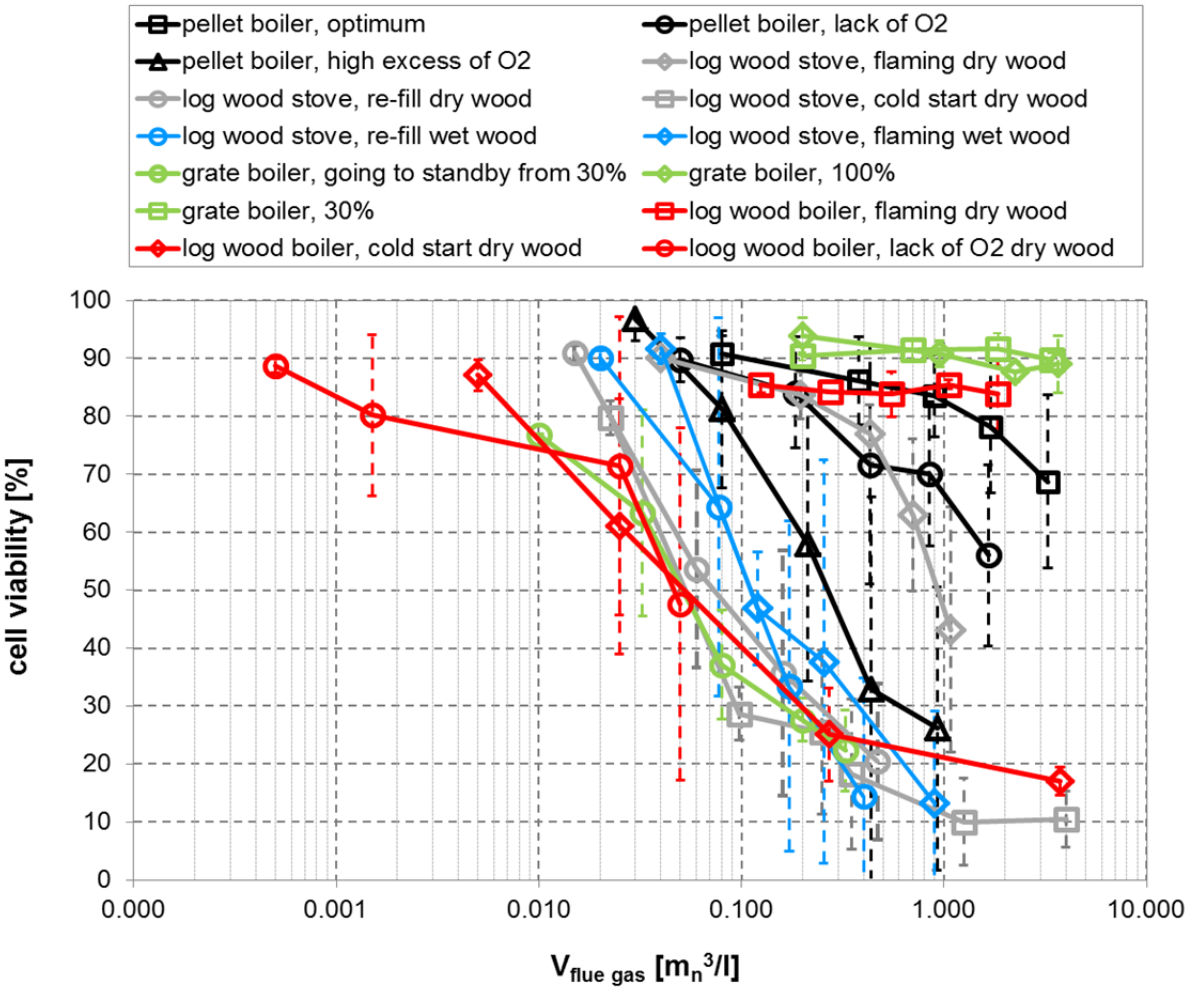

Figure 6.14 Dose response curves of CV_{COC} (H187 cells with 24 h cell exposure) based on the flue gas volume ($V_{flue\ gas,}$ normalised to 13% O_2) sampled through the impinger fillings. CV_{COC} values were binned into $V_{flue\ gas}$ bins and the error bars denote the standard deviation of the mean in each bin. Bin margins were assigned for each combustion condition individually.

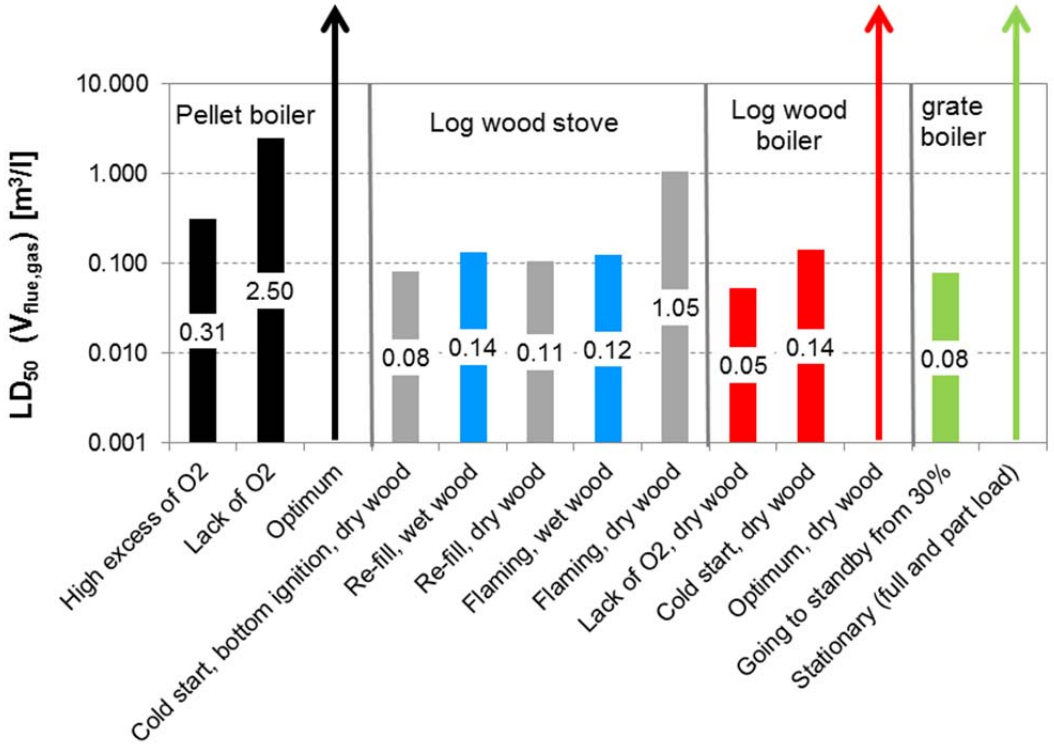

Figure 6.15 Comparison of LD_{50} of CV_{COC} (H187 cells with 24 h cell exposure) based on the flue gas volume ($V_{flue\ gas}$, normalised to 13% O_2) sampled through the impinger fillings. LD_{50} was determined by linearly fitting the two data points being closest above and below 50% CV_{COC}. For the optimum combustion conditions in the pellet, log wood and moving grate boiler no LD_{50} is found, even for large amounts of $V_{flue\ gas}$.

6.2.2.1 Correlations of LD_{50} with emission parameters

Ideally, a relationship between a cytotoxicity indicator and a regularly and simple monitored emission parameter would exist which can be described with a statistical model. Therefore, in this section LD_{50} as determined in Figure 6.14 and Figure 6.15 is compared with different parameters which were measured in the hot flue gas from wood combustion emissions.

From Figure 6.16 it is evident that there is a good correlation between LD_{50} of $V_{flue\ gas}$ and NMVOC ($R^2 = 0.76$) as well as CO ($R^2 = 0.63$). No correlation ($R^2 = 0.16$) is found for LD_{50} vs. SP (Figure 10.7 in the Appendix). This indicates that the cytotoxicity of wood combustion flue gases can be approximated with a power function. However, before such a function should be used to estimate LD_{50} from wood combustion flue gases by only measuring NMVOC and/or CO further combustion devices and conditions, additional cell lines as well as other cytotoxicity indicators should be investigated.

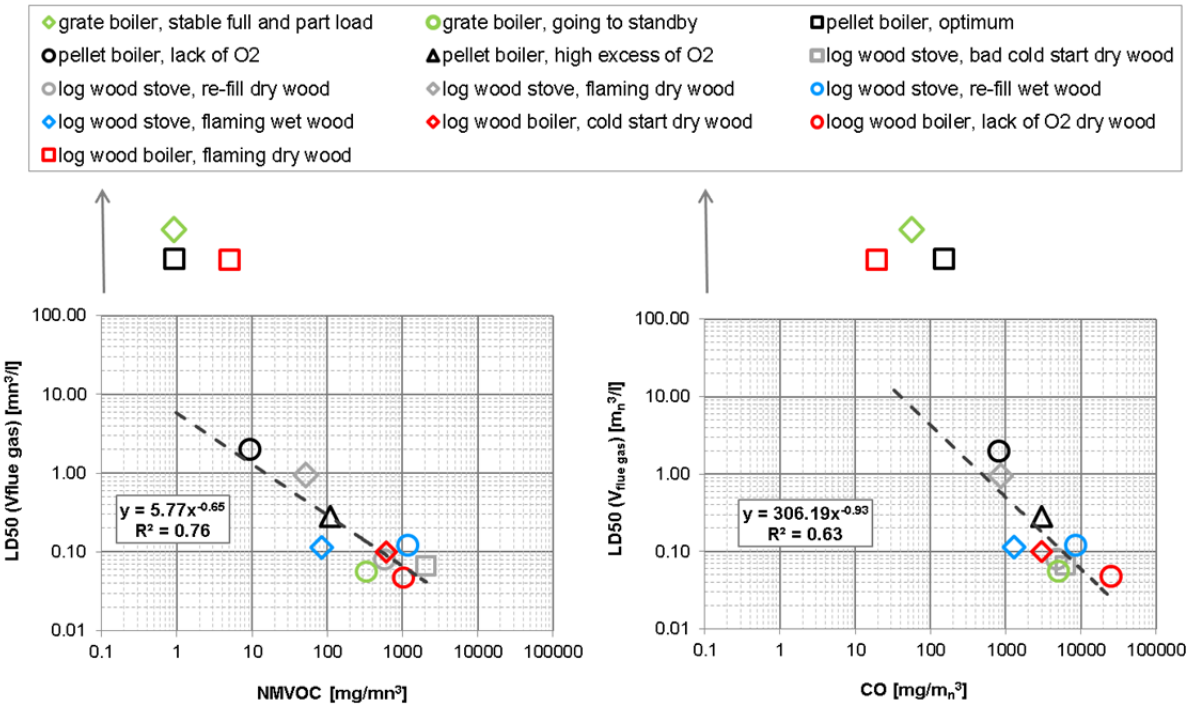

Figure 6.16 Correlations of NMVOC and CO emissions vs. LD_{50} of $V_{flue\ gas}$. NMVOC and CO values represent the averages of the corresponding combustion condition as LD_{50} values shown in Figure 6.15. All values are normalised to 13% O_2. For the optimum combustion conditions in the pellet, log wood and moving grate boiler no LD_{50} is found, even for large amounts of $V_{flue\ gas}$.

6.2.3 Cytotoxicity of particles

To determine the cytotoxicity of SP parallel sampling is performed with method 1 (only COCs are collected since there is a filter upstream of the impingers) and method 2 (no filter upstream of the impingers) where in addition to COCs also SP is precipitated. A comparison of the two sampling methods should reveal whether the additional exposure to SP affects the cytotoxicity.

Figure 6.17 shows a comparison of cell viability of COC only (CV_{COC}, sampling method 1) with the one of COC plus SP (CV_{tot}, sampling method 2) of several experiments. The results reveal no significant differences between CV_{COC} and CV_{tot} for any of the investigated combustion devices and conditions. This indicates that for the investigated concentrations in the sampling liquids no additional cytotoxic effect of SP compared to COC alone is detected.

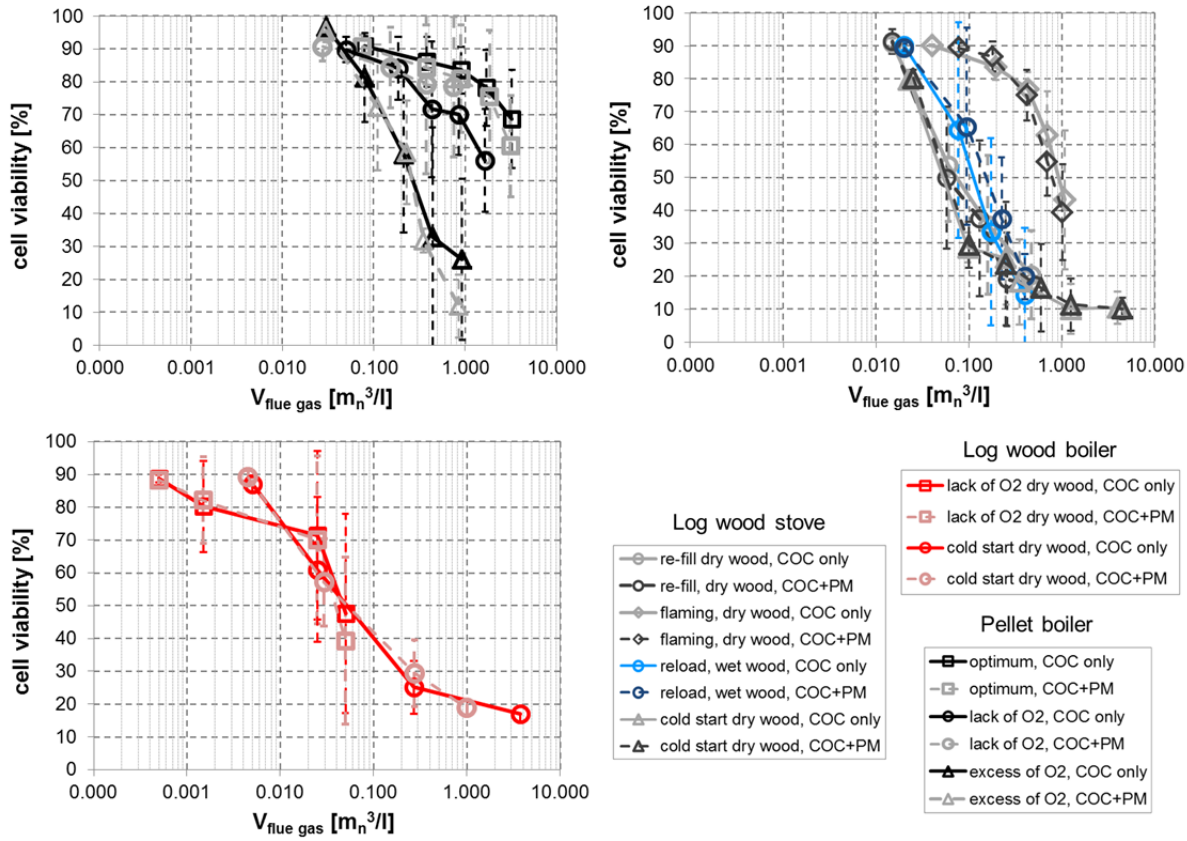

Figure 6.17 Comparison of cell viability (H187 cells with 24 h cell exposure) of COC only (sampling method 1, solid lines) with the one of COC plus SP (sampling method 2, dashed lines) as a function of the amount of flue gas sampled through the sampling liquid ($V_{flue\ gas}$, normalised to 13% O_2). CV_{COC} values were binned into $V_{flue\ gas}$ bins and the error bars denote the standard deviation of the mean in each bin. Bin margins were assigned for each combustion condition individually.

To further investigate the cytotoxic effect of SP for some experiments (n = 7) the SP collected on the filter before the impingers in sampling method 1 was water extracted and cells were exposed to these extracts in the same way as the samples generated with sampling method 1 and 2. Water extraction was carried out according to [41]. In brief, the entire or half the filters were put in 50 ml conical centrifuge tubes (FalconTM), 40 ml of sterile water was added, and the tubes were sonicated in a water bath at 30 °C for 30 min. Also in this analysis (Figure 6.18) no cytotoxicity of SP is detected as cell viability of different TOC concentrations (0–33 mg/l) is not significantly different from the negative controls. On the contrary, LD_{50} for COCs only for the same combustion devices and conditions (except for $\lambda_{opt,\ pellet\ boiler}$) already reached for 6–19 mg/l TOC concentrations. In addition, several studies (e.g. [24] and references therein) have shown that SP induces cytotoxicity. Therefore, in the following some explanations and hypothesis are listed why in this study no cytotoxic effect of SP is detected.

- First, not the entire SP are collected within the impingers. Water insoluble particles like soot or PAHs, which are strongly health relevant, are not or only to a minor extent precipitated into the sampling solutions. In addition, some combustion devices and conditions emit much more BC than organic compounds. This is confirmed by BC (using an Aethalometer) and POA (using an Aerosol Mass Spectrometer) measurements from the same log wood stove in experiments for the NRP70 project at Lucerne UASA [42] where the ratio of POA to BC is found to be ~0.33 [43].

- Second, samples containing COC and SP only contain on average 10 % more TOC than samples with COC alone. Since the negative controls already contain ~10 % of dead cells the method applied here is most probably not sensitive enough to detect a cytotoxic effect of the small additional TOC amount due to SP.
- Third, several studies which investigated cytotoxicity of SP on filters after extraction, found no effects below total SP mass of 20 mg/l [38] and 50 mg/l [29, 31-33]. For these amounts, most COC samples investigated in this study (for all tested cell lines) show already strongly decreased cell viability.

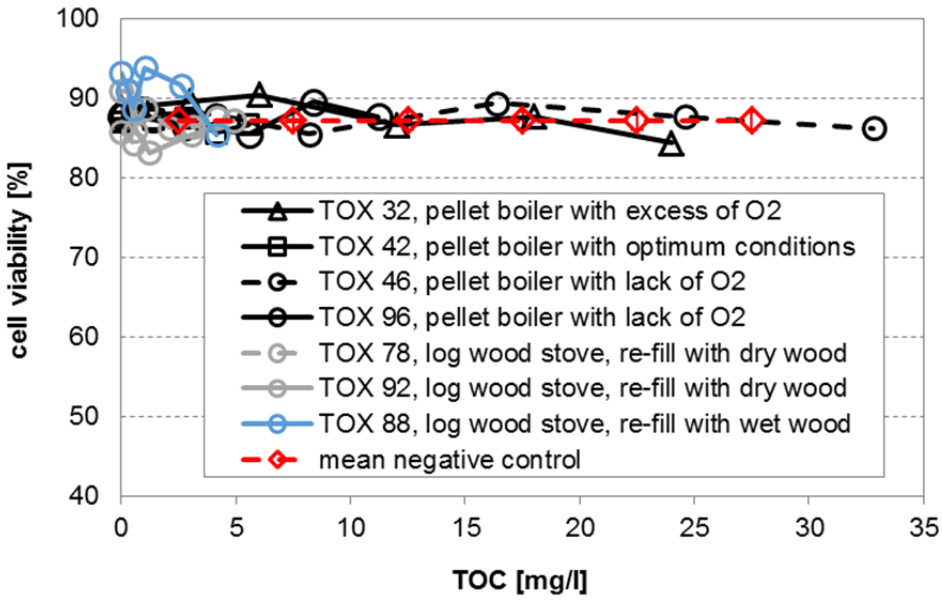

Figure 6.18 Cell viability (H187 cells with 24 h cell exposure) of SP generated by water extraction of filters from sampling method 1 from pellet boiler and log wood stove at various conditions.

The fact that no cytotoxicity was detected for SP, therefore, shows the high health relevance of COC. However, since not all health relevant SP types are collected sufficiently and the cytotoxicity indicator cell viability is not sensitive enough to detect effects below 10%, it is not possible to evaluate separate health effects of solid SP from wood combustion with the developed method. Consequently, additional more sensitive (but also more time-consuming and costly) biological end-points, such as oxidative stress and pro-inflammatory mediators, i.e. Interleukin-8, could be investigated to detect negative cell effects of SP from wood combustion. Furthermore, filter extraction could be performed using organic solvents in order to extract also PAHs and soot. In addition, several filters could be pooled together to reach higher concentrations. This could enable the detection of negative cell effects of SP also by using the cell viability as biological endpoint.

7 Conclusions

In the first part of the project “Cytotoxicity of emissions from wood combustion” a new sampling method was developed enabling the assessment of adverse health effects of sold particles (SP) and condensable organic compounds (COC) from wood combustion flue gases. For this purpose, standard sampling methods (US-EPA 5H method for COC collection as well as VDI-2066 and OAPC standard for SP determination) were adapted in order to allow an economic collection of COC and SP for subsequent *in vitro* cell analysis. After characterising the sampling method a larger number of tests investigating different wood combustion devices and conditions were carried out in the second part of the project. In total 131 samples (122 of them successful) were generated during 39 experiments in the combustion laboratory and subsequently their cytotoxic effect on cells was investigated.

7.1 Method characterisation

To characterise the handling and sampling procedure and examine the influence of different parameters the following tests were performed:

- Applicability of FACS analysis
- Sterility and blanks
- Type of sampling liquid
- Storage time between sample generation and start of cell analysis (storage effect)
- Sampling conditions (amount of liquid, flue gas flow rate through the sampling probe)
- Reproducibility
- Different cell types.

Applicability of FACS analysis

Fluorescence-activated cell sorting (FACS) is applied after staining dead cells with Propidium Iodide for measuring cell viability which is used as measure for the cytotoxicity of COC and SP from different wood combustion devices and conditions. The applicability of the FACS analysis to samples generated with the method developed in this study is investigated in several experiments. Results indicate that the FACS analysis is appropriate and no interferences of suspended particles within the sampling liquids and cell cultures are evident. This is also in line with results from literature [29].

Sterility and blanks

Sterility tests were performed to assess possible influences of handling and procedure steps on the cells, e.g. due to contamination with bacteria, during preparation of the experiment and/or sampling. No effects on the cell viability between sterilised and non-sterile sampling equipment are found. However, sterilising of the sampling equipment is nevertheless performed for all experiments. In addition, no differences in cell viability between a negative control and two procedure blanks are evident.

Type of sampling liquid

Possible differences of the cell viability of H187 cells between using directly RPMI or sterile water subsequently used to produce RPMI as impinger fillings were investigated, since water is advantageous for chemical analysis. No differences in cell viability between both methods are found, and

consequently, for all subsequent experiments, sterile water is used as sampling liquid in order to enable a quantification of the total organic carbon (TOC) content.

Storage effect

The investigation of the influence of the time period between sample generation in the combustion laboratory and the start of the cell analysis in the biological laboratory reveals a relevant storage effect after 14 days only. Cell viability significantly decreased for the same samples analysed after 14 days of storage compared to 15 to 19 hours. No decrease of the cell viability was found within the first 31 h of storage. Therefore, in all subsequent experiments the cell analysis was always started on the next morning after sample generation (after 15 to 19 hours of storage at 4°C).

Amount of sampling liquid, and sampling flow rate

Investigating the influence of the sampling flow rate and impinger filling amount on the collection efficiency of COC reveals that a sufficient total liquid level (≥ 150 ml) and a high filling in the first impinger, as well as flow rates between 5 l/min and 15 l/min should be maintained.

Reproducibility

The reproducibility of the cell viability from samples generated at comparable conditions is influenced by the variability of the combustion and sampling conditions as well as the variability of the cell analysis. Results show that the highest variability is < 30 % for the 24 hours cell exposure. Since the variability is higher for shorter exposure times and many other *in vitro* studies (e.g. [29, 31-33, 38]) investigate acute effects of emissions on cells also after 24 hours exposure, in subsequent experiments cell viability was only assessed after 24 hours exposure. Since, the total variability varies for different CV_{COC} ranges and dose response curves are diverse for different combustion devices and conditions, always two or more samples for each combustion device and condition are analysed.

Different cell types

To test and evaluate the sampling method the first experiments were performed with C2C12 cells (skeletal muscles cells from mice). To obtain results which are more relevant for humans, human epithelial lung cells H187 were used. Since different cell types from different parts of the human lung and respiratory tract exhibit distinct responses to exposure of S also a limited number of tests are carried out using the A549 cell line (epithelial cells with characteristics of alveolar type II cells). The comparison between the C2C12 and H187 cell lines shows no significant differences in cell viability. On the contrary, differences are evident between H187 and A549 cells. The dose response curves of the cell viability as function of the TOC content exhibit the same shape, however, for the same cytotoxicity a much higher TOC amount is needed for the A549 cells. Further investigations to confirm and explain this difference of sensitivity should be performed.

7.2 Emissions and cell viability of COC

After establishing and characterising the sampling method, four combustion devices (log wood stove, pellet boiler, moving grate boiler and log wood boiler) operated at different conditions are investigated. The comparison of their emissions and cytotoxicity show the following results:

- Unfavourable combustion conditions, i.e. cold start, reload, operation with high excess or lack of combustion air, going to standby, result in high emissions, especially CO and non-methane volatile

organic compounds (NMVOC). From the four investigated combustion devices the highest emissions are found in the log wood stove during cold start with ignition from the bottom followed by the reload conditions. For stationary operation in all investigated devices the emissions are much lower, NMVOC even by more than an order of magnitude. Furthermore, COC can exceed SP measured in the hot flue gas.

- Comparing the cell viability of COC based on TOC, stationary conditions (part and full load in the grate boiler, stationary flaming conditions in the log wood and pellet boiler) no or only a minor cytotoxicity is found. Unfavourable combustion conditions in all devices as well as all conditions in the log wood stove, on the other hand, show a significantly decreased cell viability with increasing TOC amount within the sampling solutions. TOC concentrations inducing a lethal dose of 50 % (LD_{50}), however, only span a range of 6–19 mg/l indicating that the same amount of TOC from the different combustion devices and conditions exhibits a similar cytotoxicity.
- CV_{COC} is also analysed as a function of the amount of flue gas ($V_{flue\ gas}$) sampled through the impingers which is directly proportional to the amount of burned wood, the heat input in MJ or kWh. The comparison reveals differences up to almost two orders of magnitude between the investigated combustion devices and conditions. These differences are much more pronounced than for the comparison of CV_{COC} based on TOC, and are due to the large differences in emission factors of NMVOC. The minimum amount of $V_{flue\ gas}$ to reach LD_{50}, and consequently exhibiting the highest cytotoxicity, is found during combustion with lack of oxygen in the log wood boiler. However, LD_{50} from most of the unfavourable conditions in all devices exhibits similar values. When using wet wood in the log wood stove ~9 times less amount of flue gas is required inducing LD_{50} compared to dry wood during flaming conditions, which is equal to a 9-fold higher toxicity. On the other hand, flue gas from stationary conditions in the grate boiler, log wood boiler, and pellet boiler shows no or only a minor effect on CV_{COC} even for large amounts of $V_{flue\ gas}$.
- The results listed above clearly show that an optimum operation of combustion devices as well as the use of appropriate fuel is crucial to minimise negative health and environmental impacts.

7.3 Cell viability of solid particles

The comparison of CV_{COC} (sampling method 1) with the one of COC plus SP (CV_{tot}, sampling method 2) reveals no significant differences between CV_{COC} and CV_{tot} for any of the investigated combustion devices and conditions. This indicates that for the investigated TOC concentrations in the sampling liquids no additional cytotoxic effect of SP compared to COC alone is detected. Possible reasons for that are:

- Water insoluble particles like soot or PAHs, which are strongly health relevant, are not or only to a minor extent precipitated into the sampling solutions. Consequently, samples containing COC and SP only contain on average 10 % more TOC than samples with COC alone.
- In addition, negative controls already contain ~10 % of dead cells and therefore, the method applied here is most probably not sensitive enough to detect a cytotoxic effect of the small additional TOC amount due to SP.

- A further evaluation of the cytotoxicity of SP by analysing SP alone obtained by water extraction of filters (upstream of the impingers in sampling method 1) also shows no cytotoxicity of SPs even for TOC concentrations where CV_{COC} shows already a significant decrease in cell viability. In addition, several other studies found no effects for SP concentrations comparable to the ones tested here.

The fact that no additional cytotoxicity was detected for SP indicates a high health relevance of COC. However, since not all health relevant SP types are collected sufficiently and the cytotoxicity indicator cell viability is not sensitive enough to detect effects below 10% cell death rate, the developed method does not allow an evaluation of separate health effects of solid SP from wood combustion. Consequently, to identify a separate effect of SP, other investigations are needed either by sampling SP only (including all types of SP derived from filter extraction using organic solvents) and/or by application of other biological end-points such as oxidative stress and pro-inflammatory mediators (i.e. Interleukin-8).

8 Outlook

In this project a sampling method was established allowing the *in vitro* assessment of adverse health effects of condensable organic compounds (COC) from wood combustion flue gases. The simple experimental setup allows an economic analysis of a large number of combustion devices and conditions. In addition, this setup could also be easily applied in other laboratories. Therefore, in future studies further combustion devices and conditions could be investigated. Since the results of this study indicate that the same amount of organic compounds from different combustion devices and conditions do not exhibit large differences in cytotoxicity, in the future the quantification of the total organic carbon content within the sampling liquids is not necessary. Cytotoxicity could only be related to the amount of flue gas sampled through the impingers. Consequently, the sampling method becomes even more simple and economic. However, in future studies not only cytotoxicity from additional combustion devices and conditions should be investigated, but also correlations between cytotoxicity and other parameters. For example confirming the relationship between cytotoxicity and non-methane volatile organic compound as well as carbon monoxide emissions would be valuable, since these parameters are measured on a regular basis. Consequently, such findings could result in establishing and/or adjusting emission limit values as well as measurement recommendations for a simple assessment of health effects from wood combustion flue gases.

Further development of the method should be carried out to also enable the assessment of cytotoxicity of SP. For example the implementation of filter extraction procedures as used in literature, which also allow the extraction of water insoluble particles (i.e. polycyclic aromatic hydrocarbons and black carbon) by using organic solvents, could be applied. Furthermore, to reach high enough SP concentrations in the filter extracts, several filter extracts from the same combustion devices and conditions could be combined.

Future experiments could also include the analysis of other cell lines to confirm, explain and better understand the difference of sensitivity of the two human lung cell lines investigated in this study. In addition, cytotoxicity is a biological end-point which is relatively fast, economic and simple to investigate. To assess the dose response of the cytotoxicity and consequently the LD_{50}, concentrations of toxic substances are used which cause cell death. However, in real life conditions cells are not exposed to such high concentrations in the investigated time period. Therefore, ideally also other end-points relevant for biomass combustion flue gas effects at non-toxic concentrations, such as oxidative stress and pro-inflammatory mediators such as Interleukin-8 could be investigated e.g. within a collaboration with the Adolphe Merkle Institute at University of Fribourg.

In this study, cytotoxicity of COC and SP dissolved or suspended in a liquid was investigated. However, in the human respiratory system cells are also directly exposed to gas and particle pollutants in the inhaled air. Therefore, *in vitro* experiments with exposure of cells to COC and SP directly out of an air stream could be performed and compared to results from this study. Such experiments could be performed also in collaboration with the Adolphe Merkle Institute. First, samples as generated with the presented method could be analysed with the air-liquid interface cell exposure system (ALICE). In this system a nebuliser produces a dense cloud of aerosol droplets which is injected into a cell exposure chamber where the particles settle gently onto cells cultivated at the air-liquid interface [44]. Second,

the Adolphe Merkle Institute developed an exposure chamber for *in vitro* cell analysis where emissions are guided through and cells are directly exposed to this air stream [45]. This exposure chamber could be incorporated in the experimental setup in the combustion laboratory at Lucerne UASA in a future project.

9 Literature

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10 Appendix

10.1 Infrastructure and equipment

Figure 10.1 View of the combustion laboratory of the Bioenergy Research Group at the Lucerne UASA campus in Horw. Different measurement devices and workspaces are visible in the front, three chimneys with different combustion devices are in the back.

Figure 10.2 View of the biological laboratory of the CC ABSaT in Hergiswil. On the top right panel the FACS system is displayed whereas the other pictures show work benches used for sample handling.

10.2 PI Staining protocol for the cell lines used in this project

PI staining detailed protocol for C2C12 and A549 cell cultures:

1. Remove medium
2. Wash cells shortly with 200 µl Trypsin-EDTA (37°C)
3. Remove Trypsin-EDTA
4. Resuspend in 200 µl Trypsin-EDTA (37°C)
5. Incubate for 2 minutes at 37°C
6. Add 800 µl of growth medium (37°C)
7. Collect sample in a 1.5 ml Eppendorf tube
8. Centrifugation at 300 x g for 2 minutes
9. Remove supernatant
10. Wash with 1ml PBS1X (37°C), vortex
11. Centrifugation at 300 x g for 2 minutes
12. Remove supernatant
13. Wash with 1ml PBS1X (37°C), vortex
14. Centrifugation at 300 x g for 2 minutes
15. Remove supernatant
16. Add PI solution (10 µg/ml in PBS1X, 37°C), vortex
17. Incubate for 30 minutes in the dark at room temperature
18. Ready for FACS analysis

PI staining detailed protocol for H187 cell cultures:

1. Collect sample in a 1.5 ml Eppendorf tube
2. Centrifugation at 300 x g for 2 minutes
3. Remove supernatant
4. Wash with 1ml PBS1X (37°C), vortex
5. Centrifugation at 300 x g for 2 minutes
6. Remove supernatant
7. Wash with 1ml PBS1X (37°C), vortex
8. Centrifugation at 300 x g for 2 minutes
9. Remove supernatant
10. Add PI solution (10 µg/ml in PBS1X, 37°C), vortex
11. Incubate for 30 minutes in the dark at room temperature
12. Ready for FACS analysis

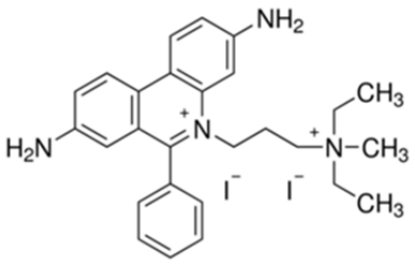

Figure 10.3 3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide

10.3 FACS analysis

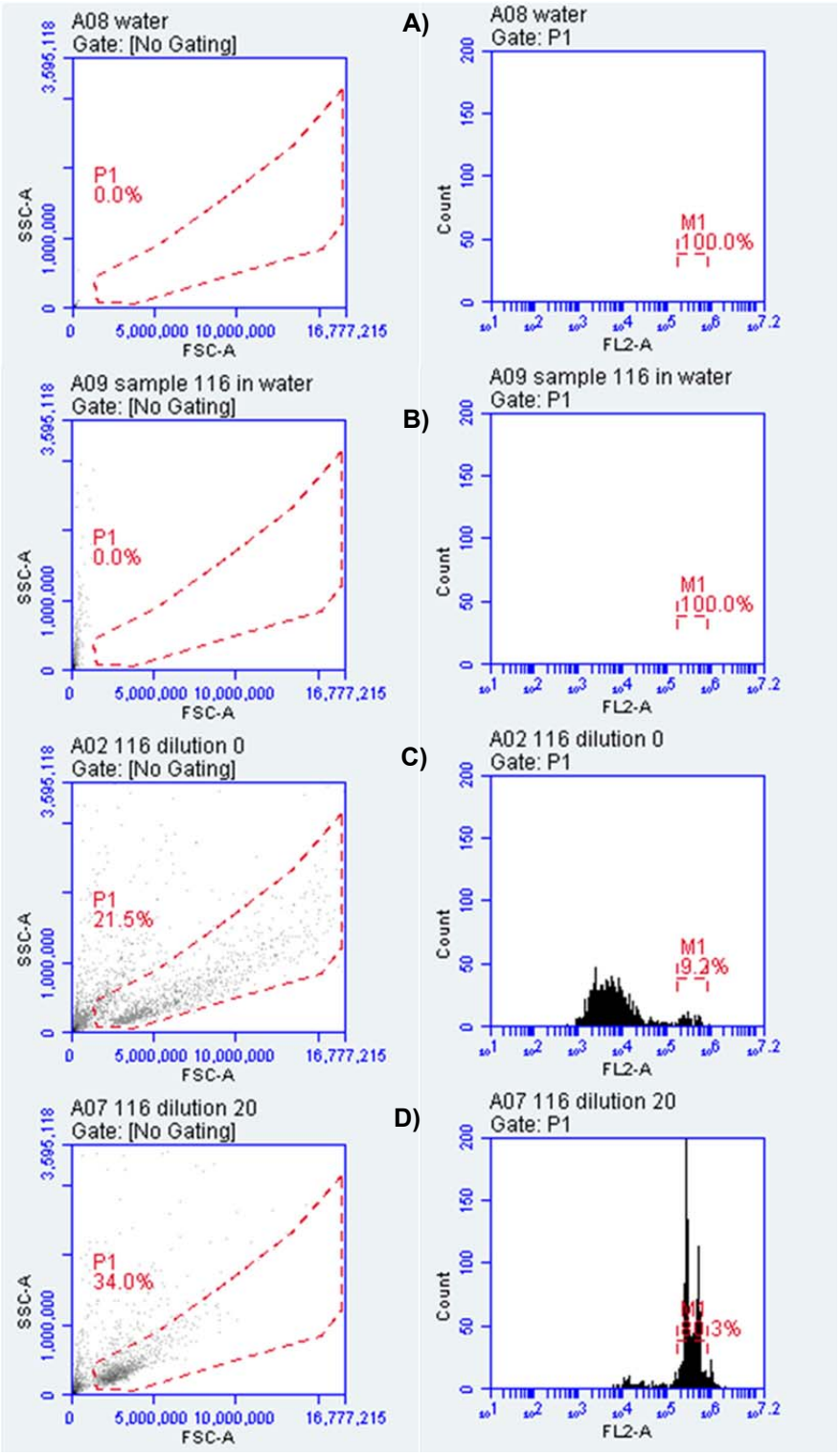

Figure 10.4 FACS analysis of different steps in the cell analysis. Left panels: signal of the cells. Right panels: number of counts as a function of the intensity from which the fraction of dead cells is quantified. A: Control with pure sterile water. Very few particles (black dots close to 0-0 point). Those particles are all out of the cell area. B: Sample TOX 116 (sterile water with COC and PM after exposure to wood combustion flue gas) before mixing with medium and FBS. All particles are out of the cell area. C: H187 negative control stained with PI. 9.3% cells are dead. D: H187 cells exposed for 24 h with medium containing 20% of TOX 116. 80.3% of the cells are dead.

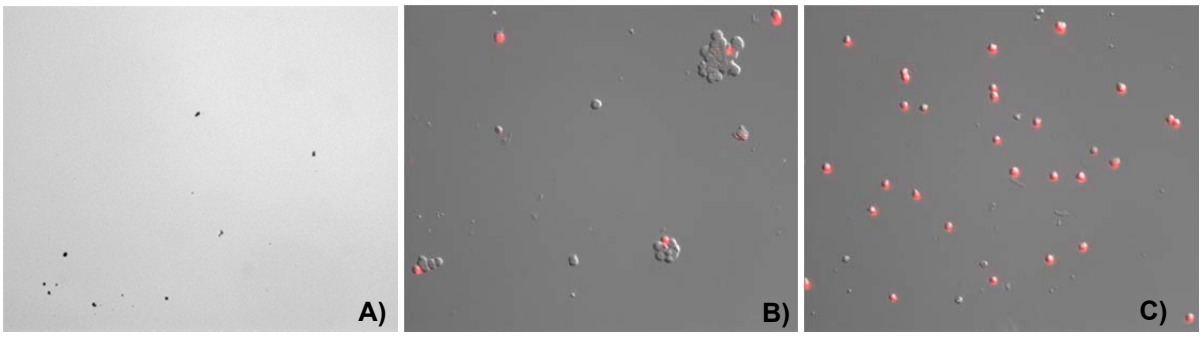

Figure 10.5 Microscope (A) and phase images of negative control (B) and sample diluted to 20% (C) of sample TOX 116. In B) and C) 9.2% and 80.3% of cells are dead, respectively. Only very few particles can be seen in solution in A) which are most probably not due to the sample generation in the combustion laboratory (sampling with sampling method 1 where there is a filter upstream of the impingers) and most probably are rather due to minor impurities of the sterile water and/or due to the sample handling. However, compared to particles in the sample TOX 117 (from parallel sampling method 2, see Figure 10.6) particles in A are much lower.

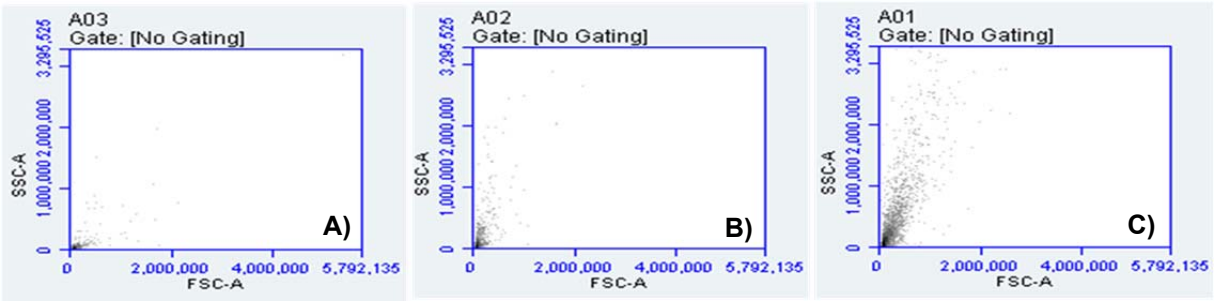

Figure 10.6 FACS analysis of pure sterile water (panel A, 10 µl, 370 counts), sample TOX 116 (panel B, generated with sampling method 1, 10 µl, 719 counts) and sample TOX 117 (panel C, generated with sampling method 2, 10 µl, 2758 counts).

10.4 Emissions

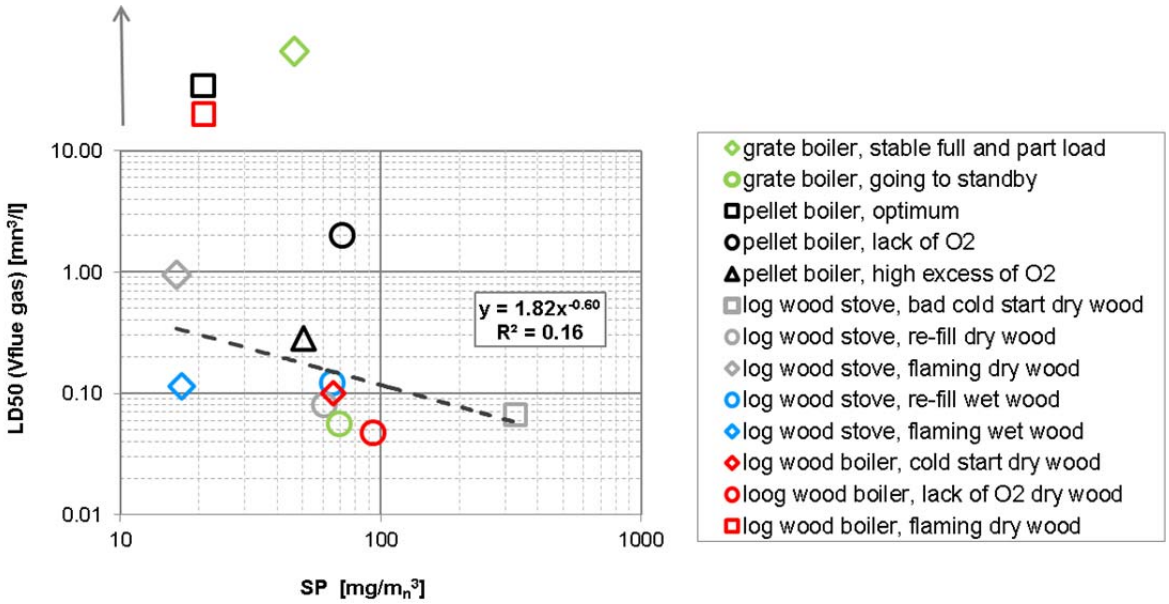

Figure 10.7 Correlations of SP emissions vs. LD_{50} of $V_{flue\ gas}$. SP values represent the averages of the corresponding combustion condition as LD_{50} values shown in Figure 6.15. All values are normalised to 13% O_2. For the optimum combustion conditions in the pellet, log wood and moving grate boiler no LD_{50} is found, even for large amounts of Vflue gas.

10.5 Overview of all samples generated for this study

Table 10.1 Sampling information and results of important emission parameters from all samples generated in this study.

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 1	13.04.2015	pellet boiler	λ++	1	DMEM	C2C12	160	00:13	3.4	116.1	4217.0	36.5	140†	85.0
TOX 3	13.04.2015	pellet boiler	λ++	1	DMEM	C2C12	160	00:16	3.3	102.4	4454.5	36.8	140†	72.0
TOX 6	11.05.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.4	135.5	3891.4	49.0	145†	99.5
TOX 7	11.05.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:09	3.3	119.1	3672.1	53.8	38†	85.0
TOX 8	12.05.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.4	125.2	3702.0	25.9	118†	91.7
TOX 9	12.05.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.3	112.5	3471.4	42.8	365†	79.7
TOX 10	13.05.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.5	131.0	4236.8	50.5	41†	97.5
TOX 11	13.05.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.3	116.2	3895.9	42.1	124†	83.6
TOX 12	15.06.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.1	88.7	3207.9	54.7	28†	59.3
TOX 13	15.06.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.1	79.0	2901.7	49.5	84†	52.4
TOX 14	17.06.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.5	108.8	3019.0	51.5	116†	81.2
TOX 15	17.06.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.4	99.8	2975.1	46.8	31†	73.3
TOX 16	13.07.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.3	102.6	2326.0	81.0	32†	72.8
TOX 17	13.07.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.2	105.4	2887.2	61.2	113†	73.1
TOX 18	15.07.2015	pellet boiler	λ++	1	DMEM	C2C12	100	00:10	3.3	107.1	2333.1	83.6	34†	77.2
TOX 19	15.07.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.4	115.8	2416.3	73.9	124†	84.3
TOX 20	27.07.2015	pellet boiler	λ++	1	sterile water	H187	350	00:35	3.5	117.3	2839.1	48.7	130	90.6
TOX 21	27.07.2015	pellet boiler	λ++	1	sterile water	H187	100	00:10	3.4	112.1	2673.7	52.0	44	103.3
TOX 22	27.07.2015	pellet boiler	λ++	1	DMEM	H187 & C2C12	100	00:10	3.4	116.7	2699.8	67.1	37†	84.3
TOX 23	29.07.2015	pellet boiler	λ++	1	sterile water	H187	100	00:10	3.5	149.7	2620.1	74.1	57	137.9
TOX 24	29.07.2015	pellet boiler	λ++	1	sterile water	H187	350	00:35	3.5	138.1	2722.9	57.1	200	142.5
TOX 25	29.07.2015	pellet boiler	λ++	1	RPMI	H187 & C2C12	350	00:35	3.4	105.6	2211.9	53.7	112†	77.1

Table 10.2 continued

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 26	03.08.2015	pellet boiler	λ++	1	sterile water	H187	350	00:35	3.2	100.3	2337.6	61.8	110	71.1
TOX 27	03.08.2015	pellet boiler	λ++	2	sterile water	H187	287	00:35	3.2	100.3	2337.6	37.6	96	74.6
TOX 28	03.08.2015	pellet boiler	λ++	1	RPMI	H187	350	00:35	3.2	93.9	2213.9	60.3	100†	64.5
TOX 29	03.08.2015	pellet boiler	λ++	2	RPMI	H187	323	00:35	3.2	93.9	2213.9	33.4	93†	64.5
TOX 30	03.08.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.1	87.2	2322.2	56.1	93†	58.5
TOX 31	03.08.2015	pellet boiler	λ++	2	DMEM	C2C12	312	00:35	3.1	87.2	2322.2	32.9	84†	58.5
TOX 32	17.08.2015	pellet boiler	λ++	1	sterile water	H187	350	00:35	3.5	119.0	3215.8	59.5	150	104.6
TOX 33	17.08.2015	pellet boiler	λ++	2	sterile water	H187	341	00:35	3.5	119.0	3215.8	22.8	160	114.4
TOX 34	17.08.2015	pellet boiler	λ++	1	RPMI	H187	350	00:35	3.4	114.7	3141.9	43.6	123†	84.8
TOX 35	17.08.2015	pellet boiler	λ++	2	RPMI	H187	339	00:35	3.4	114.7	3141.9	35.1	119†	84.8
TOX 36	17.08.2015	pellet boiler	λ++	1	DMEM	C2C12	350	00:35	3.4	109.8	3103.7	40.0	117†	79.7
TOX 37	17.08.2015	pellet boiler	λ++	2	DMEM	C2C12	340	00:35	3.4	109.8	3103.7	22.7	115†	79.7
TOX 38	18.08.2015	pellet boiler	optimal	1	sterile water	H187	1501	02:30	1.6	0.8	105.4	23.3	6	0.7
TOX 39	18.08.2015	pellet boiler	optimal	2	sterile water	H187	1555	02:30	1.6	0.8	105.4	16.7	9	1.1
TOX 40	18.08.2015	pellet boiler	λ--	1	sterile water	H187	512	00:51	1.3	10.2	1345.8	57.2	5	1.1
TOX 41	18.08.2015	pellet boiler	λ--	2	sterile water	H187	513	00:51	1.3	10.2	1345.8	48.7	20	4.1
TOX 42	14.09.2016	pellet boiler	optimal	1	sterile water	H187	1970	03:17	1.5	0.7	143.8	25.4	7	0.6
TOX 43	14.09.2016	pellet boiler	optimal	2	sterile water	H187	1880	03:17	1.5	0.7	143.8	22.4	9	0.9
TOX 44	14.09.2016	pellet boiler	λ--	1	sterile water	H187	702	01:10	1.3	6.4	818.5	44.1	3	0.5
TOX 45	14.09.2016	pellet boiler	λ--	2	sterile water	H187	668	01:10	1.3	6.4	818.5	33.4	15	2.5
TOX 46	26.10.2015	pellet boiler	λ--	1	sterile water	H187	1077	01:47	1.3	7.3	731.6	36.3	7	0.6
TOX 47	26.10.2015	pellet boiler	λ--	2	sterile water	H187	1018	01:47	1.3	7.3	731.6	37.7	17	1.9
TOX 48	28.10.2015	pellet boiler	optimal	1	sterile water	H187	2501	04:10	1.6	1.0	143.9	28.7	3	0.2
TOX 49	28.10.2015	pellet boiler	optimal	2	sterile water	H187	2439	04:10	1.6	1.0	143.9	22.6	8	0.6

Table 10.3 continued

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 50	01.12.2015	log wood stove	reload, dry wood	1	sterile water	H187	50	00:05	2.9	185.9	2624.9	22.2	12	51.9
TOX 51	01.12.2015	log wood stove	reload, dry wood	2	sterile water	H187	51	00:05	2.9	185.9	2624.9	34.9	15	63.7
TOX 52	01.12.2015	log wood stove	flaming, dry wood	1	sterile water	H187	150	00:15	4.4	23.3	778.3	26.8	4	7.7
TOX 53	01.12.2015	log wood stove	flaming, dry wood	2	sterile water	H187	162	00:15	4.4	23.3	778.3	13.4	6	11.2
TOX 54	01.12.2015	log wood stove	flaming, dry wood	1	sterile water	H187	100	00:10	3.9	53.2	621.8	14.9	9	25.1
TOX 55	01.12.2015	log wood stove	flaming, dry wood	2	sterile water	H187	102	00:10	3.9	53.2	621.8	23.4	9	26.8
TOX 56	02.12.2015	log wood stove	reload, wet wood	1	sterile water	H187	76	00:07	10.0	872.7	4767.7	44.7	94	944.8
TOX 57	02.12.2015	log wood stove	reload, wet wood	2	sterile water	H187	79	00:07	10.0	872.7	4767.7	142.6	110	1057.0
TOX 58	02.12.2015	log wood stove	reload, wet wood	1	sterile water	H187	76	00:07	6.3	1157.3	6719.8	60.0	150	923.1
TOX 59	02.12.2015	log wood stove	reload, wet wood	2	sterile water	H187	78	00:07	6.3	1157.3	6719.8	227.8	170	1017.3
TOX 60	02.12.2015	log wood stove	flaming, wet wood	1	sterile water	H187	100	00:10	4.1	94.7	1346.7		20	62.1
TOX 61	02.12.2015	log wood stove	flaming, wet wood	2	sterile water	H187	105	00:10	4.1	94.7	1346.7	9.0	23	68.5
TOX 62	09.12.2015	grate boiler	30% load	1	sterile water	H187	1000	02:13	2.1	1.6	162.3	36.6	2	0.2
TOX 63	25.01.2016	pellet boiler	λ++	1	sterile water	H187	151	00:15	3.5	127.1	3379.5	66.1	62	104.4
TOX 64	09.02.2016	pellet boiler	λ++	2	sterile water	H187	252	00:25	3.3	103.2	3438.8	29.1	75	74.5
TOX 65	09.02.2016	pellet boiler	λ++	2	RPMI	H187	251	00:25	3.3	103.2	3438.8	22.7	75	73.7
TOX 66	09.02.2016	pellet boiler	λ++	2	DMEM	C2C12	201	00:20	3.2	80.1	2928.7	12.1	49	55.0
TOX 67	09.02.2016	pellet boiler	optimal	1	sterile water	H187	2500	03:47	1.6	1.4	144.1	29.2	8	0.6
TOX 68	09.02.2016	pellet boiler	optimal	2	sterile water	H187	2798	03:47	1.6	1.4	144.1		11	0.7
TOX 69	23.02.2016	pellet boiler	λ--	1	sterile water	H187	1201	02:02	1.3	7.4	941.4	45.6	6	0.6
TOX 70	23.02.2016	pellet boiler	λ--	2	sterile water	H187	1173	02:02	1.3	7.4	941.4	39.4	20	1.9
TOX 71	24.02.2016	pellet boiler	λ++	1	sterile water	H187	251	00:25	3.3	94.3	2863.1	46.6	67	66.5

Table 10.4 continued

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 72#	08.03.2016	grate boiler	30% load	1	sterile water	H187	1506	03:07	2.1	1.1	225.9	46.2	6	0.7
TOX 73#	08.03.2016	grate boiler	30% load	2	sterile water	H187	1312	03:07	2.1	1.1	225.9	35.1	4	0.6
TOX 74	09.03.2016	grate boiler	100% load	1	sterile water	H187	1501	01:47	2.1	0.1	7.0		2	0.2
TOX 75	09.03.2016	grate boiler	100% load	2	sterile water	H187	1253	01:47	2.1	0.1	7.0	60.2	1	0.2
TOX 76	09.03.2016	grate boiler	100% load	1	sterile water	H187	1302	01:33	2.1	0.0	7.1	77.0	1	0.2
TOX 77	09.03.2016	grate boiler	100% load	2	sterile water	H187	1115	01:33	2.1	0.0	7.1	66.6	1	0.2
TOX 78	14.03.2016	log wood stove	reload, dry wood	1	sterile water	H187	71	00:07	5.9	688.8	4209.5	126.4	120	675.2
TOX 79	14.03.2016	log wood stove	reload, dry wood	2	sterile water	H187	65	00:07	5.9	688.8	4209.5	220.6	130	761.7
TOX 80	14.03.2016	log wood stove	flaming, dry wood	1	sterile water	H187	166	00:15	4.4	78.6	1175.7	25.4	17	29.6
TOX 81	14.03.2016	log wood stove	flaming, dry wood	2	sterile water	H187	142	00:15	4.4	78.6	1175.7	44.1	14	28.3
TOX 82	14.03.2016	log wood stove	reload, dry wood	1	sterile water	H187	71	00:07	3.8	156.1	2463.2	59.7	19	66.3
TOX 83	14.03.2016	log wood stove	reload, dry wood	2	sterile water	H187	69	00:07	3.8	156.1	2463.2	65.8	19	68.3
TOX 84	15.03.2016	log wood stove	reload, wet wood	1	sterile water	H187	71	00:07	5.4	639.4	5051.3	67.4	120	626.4
TOX 85	15.03.2016	log wood stove	reload, wet wood	2	sterile water	H187	69	00:07	5.4	639.4	5051.3	158.6	120	610.3
TOX 86	15.03.2016	log wood stove	flaming, wet wood	1	sterile water	H187	165	00:15	4.7	73.7	1236.6	25.0	26	48.3
TOX 87	15.03.2016	log wood stove	flaming, wet wood	2	sterile water	H187	160	00:15	4.7	73.7	1236.6	28.1	24	46.1
TOX 88	29.03.2016	log wood stove	reload, wet wood	1	sterile water	H187	71	00:07	13.9	1353.4	10291.0	*	130	1599.3
TOX 89	29.03.2016	log wood stove	reload, wet wood	2	sterile water	H187	66	00:07	13.9	1353.4	10291.0	*	130	1659.2
TOX 90	29.03.2016	log wood stove	reload, wet wood	1	sterile water	H187	96	00:09	19.7	1776.5	15734.1	*	230	2851.2
TOX 91	29.03.2016	log wood stove	reload, wet wood	2	sterile water	H187	104	00:09	19.7	1776.5	15734.1	*	280	3243.5
TOX 92	29.03.2016	log wood stove	reload, dry wood	1	sterile water	H187	71	00:07	9.0	1281.8	9604.0	*	220	1713.0

Table 10.5 continued

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 91	29.03.2016	log wood stove	reload, wet wood	2	sterile water	H187	104	00:09	19.7	1776.5	15734.1	*	280	3243.5
TOX 92	29.03.2016	log wood stove	reload, dry wood	1	sterile water	H187	71	00:07	9.0	1281.8	9604.0	*	220	1713.0
TOX 93	29.03.2016	log wood stove	reload, dry wood	2	sterile water	H187	82	00:07	9.0	1281.8	9604.0	*	280	1887.2
TOX 94	06.04.2016	pellet boiler	λ--	1	sterile water	H187	813	01:21	1.3	14.0	471.2	45.1	13	1.5
TOX 95	06.04.2016	pellet boiler	λ--	2	sterile water	H187	930	01:27	1.3	14.0	471.2	35.8	21	2.3
TOX 96	06.04.2016	pellet boiler	λ--	1	sterile water	H187	501	00:50	1.3	10.8	553.6	47.4	9	1.4
TOX 97	06.04.2016	pellet boiler	λ--	2	sterile water	H187	492	00:50	1.3	10.8	553.6	36.6	10	1.6
TOX 98	20.06.2016	log wood stove	cold start von unten	1	sterile water	H187	170	00:21	13.5	2479.3	6830.3	502.5	550	2848.6
TOX 99	20.06.2016	log wood stove	cold start von unten	2	sterile water	H187	184	00:21	13.5	2479.3	6830.3	1256.8	610	2913.4
TOX 100	21.06.2016	log wood stove	cold start von unten	1	sterile water	H187	212	00:30	23.4	2485.2	7698.6	370.2	360	2623.7
TOX 101	21.06.2016	log wood stove	cold start von unten	2	sterile water	H187	243	00:30	23.4	2485.2	7698.6	925.7	400	2498.5
TOX 102	27.06.2016	log wood stove	cold start von unten	1	sterile water	H187	110	00:13	10.1	1739.9	4819.7	204.5	250	1522.4
TOX 103	27.06.2016	log wood stove	cold start von unten	2	sterile water	H187	115	00:13	10.1	1739.9	4819.7	405.3	250	1453.9
TOX 104	27.06.2016	log wood stove	cold start von unten	1	sterile water	H187	230	00:32	23.4	1501.6	5480.2	238.1	240	1605.1
TOX 105	27.06.2016	log wood stove	cold start von unten	2	sterile water	H187	265	00:32	23.4	1501.6	5480.2	484.3	260	1500.5
TOX 106	28.06.2016	grate boiler	going to standby	1	sterile water	H187	125	00:14	1.4	342.8	5308.7	53.4	42	34.0
TOX 107	28.06.2016	grate boiler	going to standby	1	sterile water	H187	151	00:15	1.4	312.1	4628.3	85.3	44	29.3
TOX 108	02.08.2016	log wood boiler	cold start	1	sterile water	H187	155	00:15	5.6	711.9	3049.5	53.5	94	224.2
TOX 109	02.08.2016	log wood boiler	cold start	2	sterile water	H187	146	00:15	5.6	711.9	3049.5	90.4	89	227.1
TOX 110	02.08.2016	log wood boiler	optimum	1	sterile water	H187	1189	01:39	1.5	3.7	46.8	22.1	15	1.7
TOX 111	02.08.2016	log wood boiler	optimum	2	sterile water	H187	1111	01:39	1.5	3.7	46.8	20.0	12	1.5
TOX 114	03.08.2016	log wood boiler	optimum	1	sterile water	H187	1101	01:31	1.5	5.1	18.5	20.4	25	3.4

Table 10.6 continued

sample label*	date	combustion device	combustion condition	sampling method	sampling liquid	cell type	sampling volume	sampling time	λ	NMVOC	CO	PM	TOC	TOC
							[l_n]	[hh:min]	[-]	[mg/m_n^3]	[mg/m_n^3]	[mg/m_n^3]	[mg/l]	[mg/m_n^3]
TOX 115	03.08.2016	log wood boiler	optimum	2	sterile water	H187	1001	01:31	1.5	5.1	18.5	17.3	18	2.8
TOX 116	08.08.2016	log wood boiler	cold start	1	sterile water	H187	317	00:31	3.1	602.9	5623.4	61.6	270	190.3
TOX 117	08.08.2016	log wood boiler	cold start	2	sterile water	H187	310	00:31	3.1	602.9	5623.4	88.8	270	198.8
TOX 118	08.08.2016	log wood boiler	λ--	1	sterile water	H187	101	00:10	1.1	1368.2	25821.7	146.5	34	24.4
TOX 119	08.08.2016	log wood boiler	λ--	2	sterile water	H187	44	00:10	1.1	1368.2	25821.7	162.3	16	25.5
TOX 120	09.08.2016	log wood boiler	cold start	1	sterile water	H187	401	00:30	3.5	507.7	330.8	82.0	430	276.5
TOX 121	09.08.2016	log wood boiler	cold start	2	sterile water	H187	388	00:30	3.5	507.7	330.8	107.4	440	286.2
TOX 122	09.08.2016	log wood boiler	λ--	1	sterile water	H187	130	00:09	1.0	394.4	22400.9	84.8	28	15.5
TOX 123	09.08.2016	log wood boiler	λ--	2	sterile water	H187	121	00:09	1.0	394.4	22400.9	83.0	29	17.3
TOX 124	10.08.2016	log wood boiler	λ--	1	sterile water	H187	116	00:08	1.0	1615.6	26410.3	122.7	47	28.9
TOX 125	10.08.2016	log wood boiler	λ--	2	sterile water	H187	78	00:08	1.0	1615.6	26410.3	139.6	35	31.2
TOX 126	16.08.2016	log wood boiler	λ--	1	sterile water	H187 & A549	150	00:11	1.1	955.3	24544.7	50.7	110	59.3
TOX 127	16.08.2016	log wood boiler	λ--	2	sterile water	H187	141	00:11	1.1	955.3	24544.7	67.7	120	68.8
TOX 128	16.08.2016	log wood boiler	λ--	1	sterile water	H187 & A549	151	00:11	1.1	842.6	25226.0	63.5	90	45.3
TOX 129	16.08.2016	log wood boiler	λ--	2	sterile water	H187	153	00:11	1.1	842.6	25226.0	67.3	97	47.9
TOX 130	26.09.2016	log wood boiler	cold start&λ--	1	sterile water	H187 & A549	509	00:39	1.75	642	4366.3	110.8	1200	373.3
TOX 131	26.09.2016	log wood boiler	cold start&λ--	2	sterile water	H187 & A549	462	00:39	1.75	642	4366.3	47.5	880	288.6

†Estimated using the average ratio NMVOC/TOC = 0.56 determined from all samples from the pellet boiler from sampling method 1 where TOC was measured

*filters used for water extraction

#For the average cell viability of COC only of part load conditions in the grate boiler samples TOX 72 (sampling method 1) and TOX 73 (sampling method 2) were used since no difference between sampling method 1 and sampling method 2 is found.

For TOX 2, 5 61, 62, 69, 70, 90, 91, 98–101, 113 no cell viability data is available due to problems during sampling or cell analysis