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Influence of ozone initiated processing on the toxicity of aerosol particles from small scale wood combustion

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HIGHLIGHTS

- The PAH fraction of biomass combustion aerosol decreases due to ozone aging.
- Nominal combustion PM induced less cell death compared to PM from hot air starved combustion.
- Aging alters the toxicological effects of biomass combustion PM.

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ABSTRACT

Black carbon containing emissions from biomass combustion are being transformed in the atmosphere upon processing induced by tropospheric ozone and UV. The knowledge today is very limited on how atmospheric processing affects the toxicological properties of the emissions.

The aim of this study was to investigate the influence of ozone initiated (dark) atmospheric processing on the physicochemical and toxicological properties of particulate emissions from wood combustion.

Emissions from a conventional wood stove operated at two combustion conditions (nominal and hot air starved) were diluted and transferred to a chamber. Particulate matter (PM) was collected before and after ozone addition to the chamber using an impactor. Detailed chemical and physical characterization was performed on chamber air and collected PM. The collected PM was investigated toxicologically *in vitro* with a mouse macrophage model, endpoints included: cell cycle analysis, viability, inflammation and genotoxicity.

The results suggest that changes in the organic fraction, including polycyclic aromatic hydrocarbons (PAHs) are the main driver for differences in obtained toxicological effects. Fresh hot air starved emissions containing a higher organic and PAH mass-fraction affected cell viability stronger than fresh emissions from nominal combustion. The PAH mass fractions decreased upon aging due to chemical degradation. Dark aging increased genotoxicity, reduced viability and reduced release of inflammatory markers. These differences were statistically significant for single doses and typically less pronounced. We hypothesize that the alterations in toxicity upon simulated dark aging in the atmosphere may be caused by reaction products that form when PAHs and other organic compounds react with ozone and nitrate radicals.

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1. Introduction

Exposure to small scale biomass combustion aerosol is considered a major health issue globally. There is cumulative evidence from both *in vitro* and *in vivo* experiments including controlled human exposure studies, where exposure to fresh biomass combustion particles from incomplete combustion conditions, have been shown to cause adverse health effects. The endpoints include inflammation, cytotoxicity, genotoxicity, oxidative stress and arterial stiffness (Barregard et al., 2006; Jalava et al., 2012; Uski et al., 2012; Happo et al., 2013; Unosson et al., 2013). Polycyclic aromatic hydrocarbons (PAHs) are suspected to be mediators of adverse health effects in exposure to this kind of biomass combustion aerosol (Binkova et al., 2007). Several publications demonstrated that elevated levels of PAHs and soot (black carbon) are emitted from wood stoves operated at hot air starved conditions (Pettersson et al., 2011; Orasche et al., 2013; Eriksson et al., 2014).

The chemical composition of ambient particles is regarded an important factor behind toxicological responses (Rohr and Wyzga, 2012). The composition varies rapidly over time in batch-wise biomass combustion (e.g. wood stoves) and between different combustion systems as the combustion conditions changes (Bolling et al., 2009; Elsasser et al., 2013; Pagels et al., 2013). Recent studies have demonstrated that the combustion conditions and the associated changes in chemical composition of fresh emission particles results in large differences in toxicological responses (Danielsen et al., 2009; Bolling et al., 2012; Jalava et al., 2012).

Upon emission to the atmosphere the particle characteristics changes due to atmospheric processing. Processing initiated by UV radiation, ozone or nitrate radicals leads to formation of additional secondary organic aerosol (SOA), mass and chemical transformation of the primary aerosol can also occur due to surface reactions. There are indications from field studies that atmospheric processing significantly affects the toxicity of ambient particulate matter (Jalava et al., 2006). Since a large proportion of residential biomass combustion is performed at night or in winter time when the UV radiation intensity is low, atmospheric aging initiated by tropospheric ozone becomes highly relevant.

There are only a few studies published on the toxicology of aged biomass combustion aerosol, Kunzi et al. (2013) reported mild effects, typically not statistically significant neither for fresh nor for aged biomass combustion aerosol, on a range of endpoints. The low effects might be due to comparatively low particle doses used to expose cells at the air liquid interface. In real life situations humans are exposed to both fresh and aged aerosols, in different locations and meteorological conditions. Hence, it is important to link combustion condition, aerosol aging processes and concomitant chemistry of PM to the toxicological effects.

The aim of this study was to investigate the influence of dark aging and combustion conditions on the physicochemical and toxicological properties of particulate emissions from small-scale biomass combustion.

2. Materials and methods

A conventional wood stove was used to generate the emissions (Pettersson et al., 2011), the fuel was birch wood. Two combustion modes were defined, “normal” firing procedure at nominal load (NOM) and hot air starved combustion (HAS). Particles were collected for toxicological analysis during two replicate experiments for each combustion mode. In each experiment a fresh and an aged sample was collected. The two replicate samples were pooled to one sample for the toxicological investigation.

2.1. Combustion aerosol generation

The nominal combustion case was defined as a full combustion cycle (from fuel addition on glowing embers until the oxygen concentration reached ~16%), the stove was operated as instructed by the manufacturer. This resulted in comparatively low emissions, with an aerosol dominated by black/elemental carbon. The hot air starved combustion aimed to reflect adverse combustion conditions with a higher OA and PAH fraction. This was achieved by adding small amounts of wood logs, cut in relatively small pieces every 5–6 min to the fire. This procedure generated intermittent periods of 1) high organic aerosol emissions following fuel addition and 2) “overload” hot air starved conditions with very low O_2 concentrations (<5%), resulting in high PAH emissions (Eriksson et al., 2014). A similar combustion mode was also recently used in controlled human exposures in the same laboratory by Unosson et al. (2013). The experiment average concentrations of O_2, CO and NO is shown in Table 1.

2.2. Experimental procedure

The emissions were diluted and sampled to a 15.3 m^3 stainless steel reaction chamber, according to principles further described in the Supporting Information (SI). The emissions were added to the chamber for a period of 30–60 min until a mass concentration of about 3000 μg m^{-3} was achieved. A cascade impactor (DGI, Dekati Ltd, Finland) was used for collection of samples for chemical and toxicological analysis. First fresh aerosol was sampled for about 1 h. After the fresh aerosol sampling period had ended, ozone was added to the chamber at concentrations that corresponded to 1200 ppb in an empty chamber for the hot air starved experiments and 1900 ppb in the nominal combustion experiments due to higher concentration of NO in the latter case. After ozone addition, the aged aerosol was collected on a new filter set until the mass concentration in the reaction chamber was 200–300 μg m^{-3}, or just before the filters in the DGI had become overloaded.

2.3. Aerosol characterization and instrumentation

The mass concentration (PM_1) in the chamber was measured by a tapered element oscillating microbalance (TEOM) at an operating temperature of 30 °C (series 1400, Ruprecht & Patchnik, USA). Filter sampling for organic carbon (OC) and elemental carbon (EC) was carried out and analyzed with a thermal-optical analyzer according to the EUSAAR-2 protocol (Cavalli et al., 2010). OC was converted to organic matter (OM) to include the hydrogen and oxygen bound in the organic compounds. The conversion factor was directly measured with aerosol mass spectrometry. The ozone concentration in the reaction chamber was monitored using a UV spectrophotometer (model 49i, Thermo Scientific, USA). The NO and NO_2 (NO_y–NO) concentration was measured by a NO_x monitor (model CLD-700-AL, Ecophysics, USA).

Separate sampling for particle and gas phase PAHs was performed for fresh emissions in the chamber with an integrated sample unit including polyurethane foam plugs (gas phase) and

Table 1
The experiment average concentration of O_2, CO and NO_x measured in undiluted flue gases.

Experiment	O_2 (%)	CO (mg MJ^{-1})	NO_x (mg MJ^{-1})
HAS#1	11.4	1760	61
HAS#2	9.9	1900	65
NOM#1	10.9	930	64
NOM#2	13.8	1050	73

Teflon filters (PM). An online liquid chromatography-gas chromatography/mass spectrometry (LC-GC/MS) system was used for analyzing the collected material using a method further described in the SI.

A high resolution time of flight aerosol mass spectrometer (SP-AMS, Aerodyne research Inc., USA) was operated in the dual vaporizer mode, that is the conventional tungsten vaporizer and an additional laser vaporizer for quantification of refractory material (Onasch et al., 2012). The SP-AMS was deployed in a replicate experiment to further characterize the fresh and aged aerosol for the hot air starved combustion mode, see the SI for more information.

2.4. Extraction and chemical analysis of collected PM from the DGI

The DGI filter samples were prepared according to a previously validated procedure (Tapanainen et al., 2011). The aerosol was extracted by dissolving the filters in methanol. The extracts from the three lowest stages from the two experiments of each combustion situation were pooled together to form a PM_1 sample (NOM-fresh, NOM-aged, HAS-fresh, HAS-aged). Analysis for elements (inductively coupled plasma mass spectrometer, ICP-MS), water soluble ions (ion chromatography) and PAHs (GC–MS) were performed on parts of the collected DGI-filters, a total of 6 ions and 31 elements (Tables S1 and S2 in the SI) were analyzed. See SI for more information about preparation and chemical analysis of the filters.

2.5. Study design of toxicological analyses

Mouse RAW264.7 macrophages (ATCC, USA) were exposed to four doses (15, 50, 150 and 300 μg ml^{-1}) of particles from each combustion situation. The doses were chosen according to our previous studies on wood combustion particles and time point by a time-dependency study with various different particulate samples (Jalava et al., 2005, 2010a; Tapanainen et al., 2011). The preparation of the cells and particles is further described in the SI, together with a short description of the biological and statistical terms used in this paper.

2.5.1. Apoptosis and cell cycle analysis

The cell cycle phases and the apoptotic cell death were measured with flow cytometric total DNA content analysis. Apoptotic cells are the recognized number of hypodiploid cells in sub G_1 peak after staining DNA with propidium iodide. A total of 10,000 events were analyzed per sample using Summit software version 4.3 (Beckman Coulter Inc., USA). The method is further described in the SI.

2.5.2. Cell viability

Cell viability was measured using the MTT test which detects the amount of functioning mitochondria and endoplasmic reticulum in the cell suspension. The results were analyzed using WorkOut 2.0 software (Dazdaq Ltd., UK). The method is further described in the SI. Results were calculated as a percentage by comparing absorbances from cell suspensions exposed to particulate samples with those from corresponding control cells.

2.5.3. Production of inflammatory mediators

The productions of proinflammatory cytokine TNF-α and chemokine MIP-2 were analyzed from cell culture medium using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA). The analysis was performed according to instructions of the manufacturer. The absorbances were detected at wavelength 450 nm with the multilabel plate reader (Victor^3). The concentrations of the inflammatory mediators were calculated by interpolation from the standard curve using WorkOut 2.0 software. The method is further described in the SI.

2.5.4. Genotoxicity

DNA damage was assessed by the alkaline single cell gel electrophoresis (comet assay) as previously described by (Jalava et al., 2010b). The nuclei were analyzed in a blinded manner (100 nuclei per dose) using an image analysis system (Comet assay IV, Perceptive Instruments Ltd., UK). The Olive tail moment ([(tail mean-head mean) × tail % DNA/100]) was the parameter used in the statistical analysis. The method is further described in the SI.

2.6. Statistical analysis

The cellular responses induced by the particles emitted from different experimental situations were compared to control and corresponding blank samples and with regard to particle dose. More information about the statistical analysis is available in the SI.

3. Results

3.1. Chemical characteristics of the aerosol

The major components of the particles before and after aging for the two combustion cases are summarized in Table 2 (all other elemental analysis is given in SI). The carbonaceous fraction (OM + EC) dominated in all experiments. The fractions of zinc, potassium, sulfates and chlorides were about four times higher for the nominal compared to the hot air starved experiments. In the hot air starved case the burn rate is high and fuel is frequently added which results in increased absolute emissions of products of incomplete combustion and higher fractions of organic carbon and PAHs (Eriksson et al., 2014). This is one contributing factor why the relative content of inorganic constituents was four times lower in the NOM mode compared to the HAS mode. Relatively high Zinc mass fractions were found (0.2–0.8%), while other toxicologically relevant metals such as Pb, Cd, Ni, Co showed abundances at least 20 times lower than Zinc (Table S2 in SI).

For the hot air starved case, the average OM fraction increased from 57 to 61% upon aging and the average EC fraction decreased from 40 to 33%, consistent with condensation of organic components that were oxidized in the gas-phase in the chamber. For the nominal combustion case, the average OM fraction decreased from 19 to 9%, due to aging mechanisms. First, reaction products of heterogeneous fragmentation reactions in the particle phase (e.g. with ozone) may partly yield higher vapor pressure products that evaporates which decreases the OC particle fraction. Secondly, it is possible that semi-volatile organic material in the particle phase evaporates to the gas-phase when the aerosol concentration decreases due to dilution and wall losses of the gas-phase. The average $PM_{1.0}$ concentration was ~2.5 mg m^{-3} for the fresh samples and ~0.8 mg m^{-3} for the aged samples for the hot air starved case,

Table 2
Chemical composition of the fresh and aged aerosol. The total fresh PM1 emissions were 40 mg MJ^{-1} for NOM and 80 mg MJ^{-1} for HAS.

Experiment	OM (%)	EC (%)	NO_3 (%)	Zn (%)	K + Cl + SO_4 (%)	Others (%)	PAHs (%)
HAS-fresh	56.9	39.7	0.7	0.2	2.1	0.4	0.4
HAS-aged	60.9	33.3	3.1	0.2	1.3	1.2	0.2
NOM-fresh	19.2	69.2	0.8	0.7	8.2	1.9	0.1
NOM-aged	8.9	76.0	6.2	0.8	6.8	1.3	–[a]

[a] Data missing.

the corresponding numbers for the nominal case were 3.0 and 2.0 mg m^{-3}. Most likely, the hot air-starved case resulted in a higher concentration of SOA precursors, for example methoxy-phenols (Yee et al., 2013), light aromatics and 2-3 ring PAHs, which upon oxidation in the chamber adds to a net increase of the OM fraction. The relative fraction of nitrate ions increased in the aged samples both for the nominal and hot air starved case. This can be explained by formation of secondary particulate nitrates which originate from NO_x oxidized in the aging process.

Fig. 1a shows the ratio between total organic aerosol and rBC for a hot air starved experiment. After ozone addition, the organics to rBC ratio increases by about 50%, which is slightly higher than for the thermal optical analysis (~30% increase). The time scale for this secondary organic aerosol formation process is less than 30 min and after this the OA to rBC ratio remains constant. The ratio between oxygen and carbon atoms (O:C) in the organic aerosol increased from 0.22 to 0.27 upon aging, indicating a transformation towards a more oxidized, possibly also more polar organic aerosol.

3.2. Polycyclic aromatic hydrocarbons

The PAH mass fraction deduced from the collected impactor substrates in the HAS-fresh sample was 0.4% and in the HAS-aged sample it was 0.2%. The PAH fraction was substantially lower in the NOM-fresh case, 0.1%, unfortunately there is no data available for NOM-aged. Fig. 1b shows the PAHs to rBC ratio measured on-line with the SP-AMS during the course of a hot air starved aging experiment. There is a clear trend that the PAH to rBC ratio decreases over a time-scale of less than 30 min upon ozone addition, after which it stabilizes at a lower ratio. This is consistent with chemical degradation of PAHs rather than vaporization. The decrease in the PAH to rBC ratio of 30% from the SP-AMS is largely consistent with the off-line analysis that showed a 40% decrease in the PAH to EC ratio.

Fig. 2 shows the relative mass concentration of the major quantified PAH compounds, ordered from left to right according to the specific compound ozone reactivity. There is a clear trend that the compounds with the highest ozone reactivity were lost to a higher degree in the aging process. For example, the mass fraction of benzo(a)pyrene which is highly reactive with ozone has been reduced by about 75% upon aging, while benzo(e)pyrene that has almost identical vapor pressure (Haftka et al., 2006) but a much lower reactivity towards ozone has only been reduced by about 30% of its initial concentration. This suggests that chemical degradation

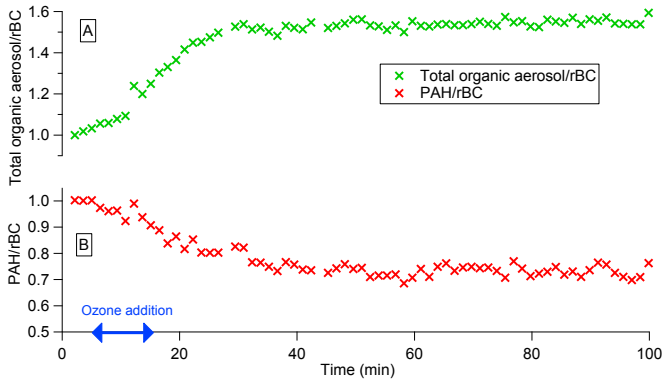

Fig. 1. (A) Ratio (normalized) of total organic aerosol (OA) to rBC and (B) particulate phase PAHs to rBC during ozone aging of hot air starved (HAS) combustion aerosol measured by SP-AMS. Ozone was added to the reaction chamber between minute 5–15.

rather than pure vaporization is the major cause of the decrease of the PAH fraction observed upon aging. LC-GC/MS analysis of particulate PAHs from the filter setup showed that the mass fraction of PAHs was ~4 times higher for the hot air starved experiments than the nominal experiments. This technique was only used for analyzing particulate phase PAHs in fresh aerosol, due to possible reaction of ozone with PM associated PAH on the filter during collection of aged aerosol.

3.3. Toxicological results

3.3.1. Cytotoxicity and cell cycle phases

Aerosol samples from all combustion situations induced a dose-dependent and statistically significant decrease in cell viability in MTT test (Fig. 3). There was only minor difference at lowest dose level (15 μg ml^{-1}) and no difference at all at second lowest dose (50 μg ml^{-1}) between studied PM samples. However, the dose of 150 μg ml^{-1} caused statistically significant difference between NOM and HAS emission samples. There was also statistically significant difference between HAS-fresh and HAS-aged PM samples. The highest dose (300 μg ml^{-1}) induced a clear statically significant difference between NOM and HAS combustion situations. A statistically signified difference between NOM-fresh and NOM-aged was detected, but not between HAS-fresh and HAS-aged for the highest dose.

A statistically significant and dose dependent increase in the amount of apoptotic cells was detected after the exposure to PM from NOM combustion situation (Fig. 4). However, this was not the case at highest dose level (300 μg ml^{-1}) of HAS combustion aerosol where a decreased amount of apoptotic cells was detected compared to lower doses. When combustion situations were compared to each other, a statically significant difference was detected between NOM and HAS combustion cases with doses 15, 150 and 300 μg ml^{-1}. In addition, this difference was also seen in the lower amount of cells in the G_1 phase after the exposure to the hot air starved combustion particles at dose of 150 μg ml^{-1}. Also systematic difference between emission samples (NOM and HAS) was detected when cells S-G_2/M cell cycle phase was analyzed. Proportion of cells in S-G_2/M cell cycle phase decreased with increasing dose of NOM-fresh and NOM-aged-combustion PM, whereas there was an opposite trend after the exposure to HAS combustion samples, this difference was also statistically significant for the highest dose.

3.3.2. Production of inflammatory mediators

Particulate samples derived from all situations triggered a dose-dependent production of the proinflammatory mediator TNF-α in RAW264.7 macrophages (Fig. 5A). Detected responses were the highest in NOM combustion case. All doses of NOM-fresh combustion derived particles produced statistically significant response when compared to control. With NOM-aged combustion particles only the response by largest dose reached the level of statistical significance. TNF-α production in macrophages was substantially lower by HAS combustion samples than those of the NOM case. For both combustion modes, fresh particles induced larger increase in TNF-α production than the aged. In HAS conditions, dose of 150 μg ml^{-1} produced statistically significant response compared to control, instead, when the highest dose (300 μg ml^{-1}) was introduced to cells, statistically significantly lower TNF-α concentrations were received when compared to the control level.

Chemokine MIP-2 response to emission particles was highly similar to the TNF-α response (Fig. 5B). The highest MIP-2 production induced by the emission samples was evoked by the fresh particles at the dose of 150 μg ml^{-1}. This effect differed statistically significantly from the responses induced by the aged samples from

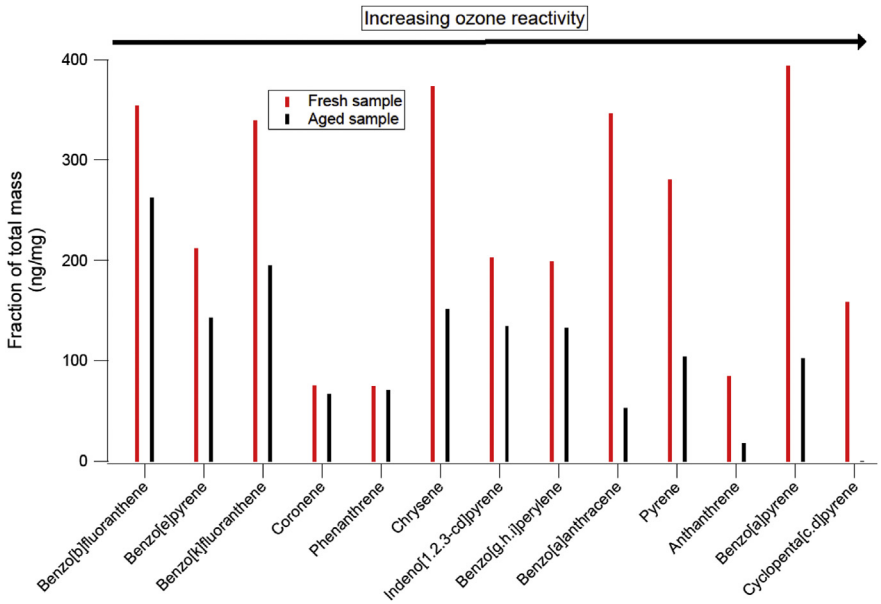

Fig. 2. Mass fractions of the major particle phase PAHs from the DGI filters collected at the fresh and aged hot air starved experiments. The PAHs are ordered from left to right according to increasing ozone reactivity (Tsapakis and Stephanou, 2003).

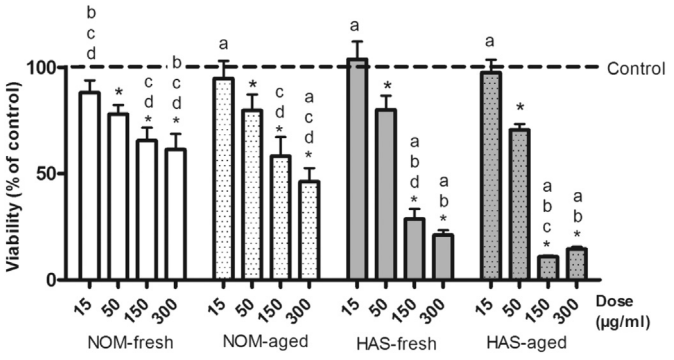

Fig. 3. Cell viability assessed with MTT test after 24 h exposure to PM_1 emission samples from different combustion samples. Bars present four doses (15, 50, 150 and 300 μg ml^{-1}) and whiskers the standard error of mean. Asterisks indicate statistical significance compared to control ($p < 0.05$) ANOVA and Dunnett's test. Letters indicate statistically larger response ($p < 0.05$) ANOVA, Tukey's test compared to aerosol. a) NOM-fresh; b) NOM-aged 2; c) HAS-fresh and d) HAS-aged.

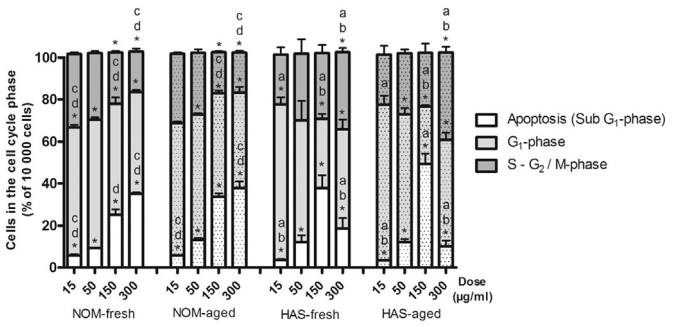

Fig. 4. The percentages of mouse RAW264.7 macrophages in the different phases of the cell cycle (SubG1, G_1 and S-G_2/M) after exposure to different doses (15–300 μg ml^{-1}) of emission particles from differed combustion situations. Asterisks indicate statistical significance compared to control ($p < 0.05$) analyzed by non-parametric Kruskal–Wallis test. Letters indicate statistically larger response ($p < 0.05$) ANOVA, Tukey's test compared to other samples. a) NOM-fresh; b) NOM-aged 2; c) HAS-fresh and d) HAS-aged.

both NOM and HAS combustion. When the highest dose was introduced to cells the MIP-2 production was increased after the

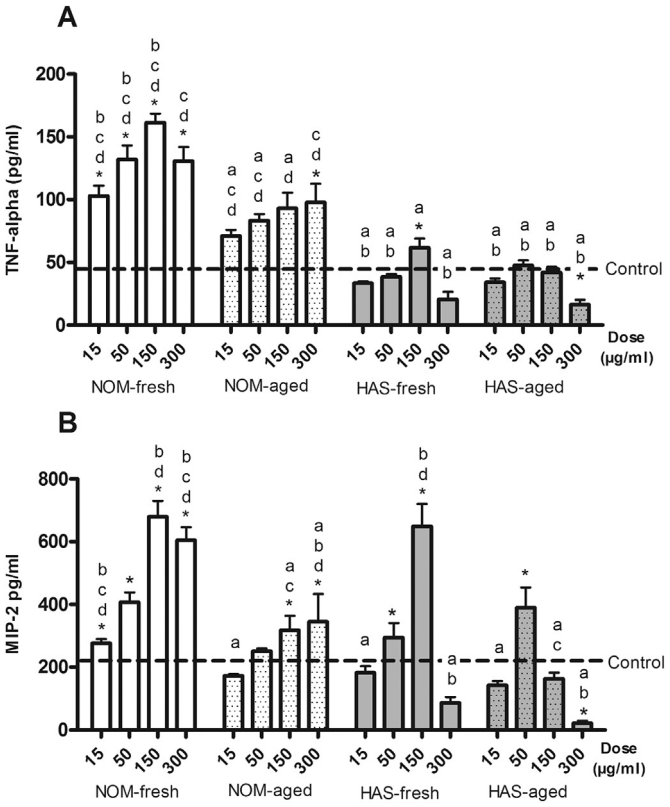

Fig. 5. Tumor necrosis factor alpha (TNF-α) (A) and Macrophage inflammatory protein 2 (MIP-2) (B) responses (pg ml^{-1}) after 24 h exposure of the RAW264.7 macrophages to PM_1 samples. Bars present four doses (15, 50, 150 and 300 μg ml^{-1}) and whiskers the standard error of mean. Asterisks indicate statistical significance compared to control ($p < 0.05$) ANOVA and Dunnett's test. Letters indicate statistically larger response ($p < 0.05$) ANOVA, Tukey's test compared to other samples. a) NOM-fresh; b) NOM-aged; c) HAS-fresh and d) HAS-aged.

exposure to samples from the NOM combustion situation, the opposite trend was observed for the samples from HAS.

3.3.3. Genotoxicity

All the studied particulate samples induced dose-dependent DNA damage in the comet assay when doses where compared to control cells (Fig. 6). The particles from the aged hot air starved combustion was statistically significantly more potent inducers of DNA damage than particles from other three emission samples at 150 μg ml^{-1} dose. Because severe cytotoxicity induced by hot air starved combustion samples, the responses by the highest doses (300 μg ml^{-1}) could not be analyzed.

4. Discussion

4.1. Cytotoxicity

In this study, combustion conditions had a stronger effect on cytotoxicity than ozone aging. Still, statistically significant higher cytotoxicity (MTT-test) was detected when aged samples were compared to fresh aerosol at dose level 150 μg ml^{-1} (HAS) and 300 μg ml^{-1} (NOM). Generally the results agree well with previous *in vitro* studies (Jalava et al., 2010a; Tapanainen et al., 2011; Jalava et al., 2012) where particles from incomplete wood combustion are more potent inducers of cell death and apoptosis than those emitted from the more complete combustion process. The HAS combustion PM was a very potent inducer of cell death at the two highest doses. This may be linked to the higher fraction of organic components including PAHs. Tapanainen et al. (2011) also linked cytotoxic effects with the fraction of water-soluble alkali metals and zinc. However, the differences in organic mass fraction (19 vs. 57%) when comparing NOM and HAS combustion has presumably a more pronounced effect in this study than the differences in the sum of soluble alkali metals and zinc (9 and 3% respectively).

PM induced DNA damage in mammalian cells can cause alteration in the cell cycle. Damaged DNA activates DNA-repair machinery which tries to restore cells in normal stage, if the process fails, the cell cycle can be blocked permanently and ultimately lead to apoptotic or necrotic cell death (Miller and Ramos, 2001). Previous studies (Tapanainen et al., 2011; Jalava et al., 2012; Tapanainen et al., 2012) have detected apoptotic cell death in cell cycle analysis (SubG1 phase) with PAH-rich wood combustion samples as also seen in this study. Moreover, all PM samples caused a reduction of the cell proportion in the normal resting phase (G1) of cells. Aging of aerosol did not cause any difference in cell cycle analysis when results were compared to the fresh sample. However, the portion of cells in S-G2/M (cells dividing) was decreased in cells after exposure to nominal combustion PM. Instead, increase in S-G2/M with hot air starved combustion PM sample was evident. This may be due to cell cycle arrest that occurs when cells are trying to repair damaged DNA. In addition, considerable reduction of apoptotic cells was detected between 150 μg ml^{-1} and 300 μg ml^{-1} doses with HAS combustion PM sample. It can be interpreted as that the cells are too damaged to undergo apoptotic cell death, but are dying by necrosis.

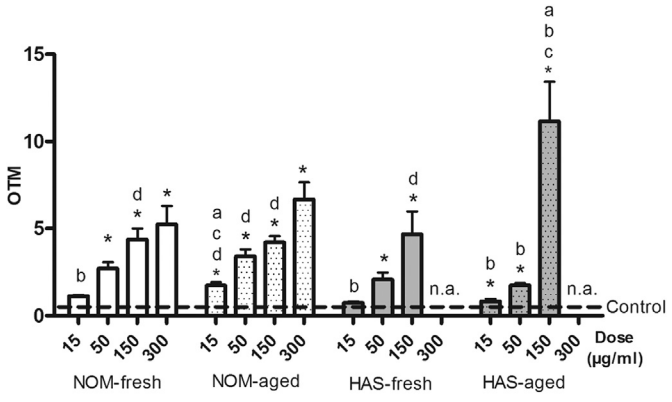

Fig. 6. Genotoxicity assessed with comet assay after 24 h exposure to PM_1 from different combustion samples. Bars represent four doses (15, 50, 150 and 300 μg ml^{-1}) and whiskers the standard error of mean (SEM). Asterisks indicate statistical significance compared to control ($p < 0.05$) analyzed by non-parametric Kruskal–Wallis test. Letters indicate statistically larger response ($p < 0.05$) ANOVA, Tukey's test compared to other samples. a) NOM-fresh; b) NOM-aged 2; c) HAS-fresh and d) HAS-aged.

4.2. Production of inflammatory markers

Exposure to fresh and aged biomass combustion aerosols for 24 h affected dose dependently the production of the inflammatory markers TNF-α and MIP-2. Relatively clear dose response relationships were generally found for fresh and aged aerosol from nominal combustion (except for the highest dose of fresh aerosol). The reduced concentrations of inflammatory markers for high concentrations of hot air starved combustion aerosol can most likely be linked with the high degree of cell death at these high doses.

Moreover, PAHs have previously been linked to immunosuppressive effects (van Grevenynghe et al., 2003). This may also contribute to the generally low responses for TNF-α in HAS-fresh and HAS-aged samples. The clear decrease in the inflammatory response of aged particles for the nominal combustion case is an interesting observation. It suggests that the secondary reaction products in PM (for example degradation products of PAHs) also may have an immune suppressing effect, even in a case where the organic fraction is low and the PAH concentration is moderate.

4.3. Genotoxicity

In this study DNA damage was significantly elevated for doses of 50 μg ml^{-1} and higher for all aerosol types. Fresh biomass combustion aerosol has previously been shown to induce DNA damage (Tapanainen et al., 2011; Jalava et al., 2012; Tapanainen et al., 2012), which has been linked to the PAH content of the wood smoke. The greatest damage on DNA (about double or more than for all other cases) was induced by the 150 μg ml^{-1} dose of HAS-aged PM sample. A possible explanation can be found in the reaction products from PAHs in aerosol that is subjected to atmospheric dark aging. PAHs present on soot surfaces can react with ozone to form secondary quinones (oxy-PAHs) at a timescale of minutes (Shiraiwa et al., 2012). Further nitrate radicals are formed by reactions between NO_2 and ozone in the dark. Nitrate radicals can transform PAHs to nitro-PAH derivatives which have very high mutagenic potential (FinlaysonPitts and Pitts, 1997). Quinones formed from ozone aging have the ability to reduce oxygen to reactive oxygen species (ROS) (Shiraiwa et al., 2012), which is known for inducing DNA damage (Danielsen et al., 2009). In the present study the PAH fraction was reduced by about a factor of two when comparing the fresh and the aged sample for the HAS case. It is therefore likely that a substantial fraction of the reacted PAHs were transformed to nitro or oxy-PAHs.

4.4. Atmospheric implications

Wood combustion aerosol gives a significant contribution to ambient air PM pollution in many environments worldwide. The type of wood stove used here and the two investigated combustion conditions are expected to reasonably well represent emissions from log wood combustion for residential heating purpose. There is

currently a debate whether the toxicity of fresh carbonaceous combustion particles is driven by the elemental/black carbon core (Janssen et al., 2012) or the organic coating. Our study supports the hypothesis that the organic coating including aromatic components such as PAHs is the main driver of the effects.

At night and in areas where the UV exposure in the atmosphere is low, a significant part of the atmospheric transformations will take place due to dark aging initiated by reactions with ozone as studied here. It is important to derive the time-scales for the transformation of the particle properties. We estimate based on our measured ozone concentrations that the aging in this study corresponds to 10–15 h in the atmosphere at a 24 h average winter time ozone concentration of 30 ppb. Thus exposures within the immediate vicinity of the emission spot may best be represented by the fresh emissions studied here, while exposures several hours downwind the emission spot may be better represented by the case with aged samples.

Aged biomass combustion aerosol is of importance in several parts of the world, one example is the Asian brown cloud in the outflow of Southeast Asia which originates to about 2/3 from biomass combustion sources (Sheesley et al., 2012). This study and previous studies have shown clear evidence of transformation in the organic composition, for example increased oxygen to carbon ratio.

5. Summary and conclusions

We studied the aging of biomass combustion aerosol and the toxicological responses of fresh and aged aerosol from: 1) A full combustion cycle at nominal burn rate (NOM), resulting in particles dominated by elemental carbon, with low to moderate PAH content. 2) Hot air starved (HAS) combustion at high burn rates including frequent addition of log wood, which produced an aerosol with a high fraction of organics and a moderate to high fraction of PAHs. A stronger cytotoxic effect was found for HAS compared to NOM which we hypothesize to be linked to the higher organic fraction (including PAHs). The PAH mass fractions decreased upon aging due to chemical degradation. Dark aging increased genotoxicity, reduced viability and reduced the release of inflammatory markers. These differences were statistically significant for single doses, but typically less pronounced. We hypothesize that the alterations in toxicity upon simulated dark aging in the atmosphere may be caused by reaction products that form when PAHs and other organic compounds react with ozone and nitrate radicals.

Clearly, there is a need for more detailed studies that links the composition and transformation of the organic fraction (including PAHs and their reaction products) upon aging (under both dark and UV exposure conditions) with toxicological responses from the cell level up to controlled human exposure studies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2014.11.068.

References

Barregard, L., Sallsten, G., Gustafson, P., Andersson, L., Johansson, L., Basu, S., Stigendal, L., 2006. Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal. Toxicol. 18 (11), 845–853.

Binkova, B., Chvatalova, I., Lnenickova, Z., Milcova, A., Tulupova, E., Farmer, P.B., Sram, R.J., 2007. PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. Mutat. Res-Fund Mol. M. 620 (1–2), 49–61.

Bolling, A.K., Pagels, J., Yttri, K.E., Barregard, L., Sallsten, G., Schwarze, P.E., Boman, C., 2009. Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Part Fibre Toxicol. 6.

Bolling, A.K., Totlandsdal, A.I., Sallsten, G., Braun, A., Westerholm, R., Bergvall, C., Boman, J., Dahlman, H.J., Sehlstedt, M., Cassee, F., Sandstrom, T., Schwarze, P.E., Herseth, J.I., 2012. Wood smoke particles from different combustion phases induce similar pro-inflammatory effects in a co-culture of monocyte and pneumocyte cell lines. Part Fibre Toxicol. 9.

Cavalli, F., Viana, M., Yttri, K.E., Genberg, J., Putaud, J.P., 2010. Toward a standardised thermal-optical protocol for measuring atmospheric organic and elemental carbon: the EUSAAR protocol. Atmos. Meas. Tech. 3 (1), 79–89.

Danielsen, P., Loft, S., Kocbach, A., Schwarze, P.E., Moller, P., 2009. Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP1 cell lines. Mutagenesis 24 (1), 104.

Elsasser, M., Busch, C., Orasche, J., Schon, C., Hartmann, H., Schnelle-Kreis, J., Zimmermann, R., 2013. Dynamic changes of the aerosol composition and concentration during different burning phases of Wood combustion. Energ. Fuel 27 (8), 4959–4968.

Eriksson, A.C., Nordin, E.Z., Nyström, R., Pettersson, E., Swietlicki, E., Bergvall, C., Westerholm, R., Boman, C., Pagels, J.H., 2014. Particulate PAH emissions from biomass combustion – time-resolved analysis with aerosol mass spectrometry. Environ. Sci. Technol. 48 (12), 7143–7150.

FinlaysonPitts, B.J., Pitts, J.N., 1997. Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles. Science 276 (5315), 1045–1052.

van Grevenynghe, J., Rion, S., Le Ferrec, E., Le Vee, M., Amiot, L., Fauchet, R., Fardel, O., 2003. Polycyclic aromatic hydrocarbons inhibit differentiation of human monocytes into macrophages. J. Immunol. 170 (5), 2374–2381.

Haftka, J.J.H., Parsons, J.R., Govers, H.A.J., 2006. Supercooled liquid vapour pressures and related thermodynamic properties of polycyclic aromatic hydrocarbons determined by gas chromatography. J. Chromatogr. A 1135 (1), 91–100.

Happo, M.S., Uski, O., Jalava, P.I., Kelz, J., Brunner, T., Hakulinen, P., Maki-Paakkanen, J., Kosma, V.M., Jokiniemi, J., Obernberger, I., Hirvonen, M.R., 2013. Pulmonary inflammation and tissue damage in the mouse lung after exposure to PM samples from biomass heating appliances of old and modern technologies. Sci. Total Environ. 443, 256–266.

Jalava, P., Salonen, R.O., Halinen, A.I., Sillanpaa, M., Sandell, E., Hirvonen, M.R., 2005. Effects of sample preparation on chemistry, cytotoxicity, and inflammatory responses induced by air particulate matter. Inhal. Toxicol. 17 (2), 107–117.

Jalava, P.I., Salonen, R.O., Halinen, A.I., Penttinen, P., Pennanen, A.S., Sillanpaa, M., Sandell, E., Hillamo, R., Hirvonen, M.R., 2006. In vitro inflammatory and cytotoxic effects of size-segregated particulate samples collected during long-range transport of wildfire smoke to Helsinki. Toxicol. Appl. Pharm. 215 (3), 341–353.

Jalava, P.I., Salonen, R.O., Nuutinen, K., Pennanen, A.S., Happo, M.S., Tissari, J., Frey, A., Hillamo, R., Jokiniemi, J., Hirvonen, M.R., 2010a. Effect of combustion condition on cytotoxic and inflammatory activity of residential wood combustion particles. Atmos. Environ. 44 (13), 1691–1698.

Jalava, P.I., Tapanainen, M., Kuuspalo, K., Markkanen, A., Hakulinen, P., Happo, M.S., Pennanen, A.S., Ihalainen, M., Yli-Pirila, P., Makkonen, U., Teinila, K., Maki-Paakkanen, J., Salonen, R.O., Jokiniemi, J., Hirvonen, M.R., 2010b. Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. Inhal. Toxicol. 22, 48–58.

Jalava, P.I., Happo, M.S., Kelz, J., Brunner, T., Hakulinen, P., Maki-Paakkanen, J., Hukkanen, A., Jokiniemi, J., Obernberger, I., Hirvonen, M.R., 2012. In vitro toxicological characterization of particulate emissions from residential biomass heating systems based on old and new technologies. Atmos. Environ. 50, 24–35.

Janssen, N.A., Gerlofs-Nijland, M.E., Lanki, T., Salonen, R.O., Cassee, F., Hoek, G., Fischer, P., Brunekreef, B., Krzyzanowski, M., 2012. Health Effects of Black Carbon. World Health Organization, Copenhagen, Denmark.

Kunzi, L., Mertes, P., Schneider, S., Jeannet, N., Menzi, C., Dommen, J., Baltensperger, U., Prevot, A.S.H., Salathe, M., Kalberer, M., Geiser, M., 2013. Responses of lung cells to realistic exposure of primary and aged carbonaceous aerosols. Atmos. Environ. 68, 143–150.

Miller, K.P., Ramos, K.S., 2001. Impact of cellular metabolism on the biological effects of benzo[a]pyrene and related hydrocarbons. Drug Metab. Rev. 33 (1), 1–35.

Onasch, T.B., Trimborn, A., Fortner, E.C., Jayne, J.T., Kok, G.L., Williams, L.R., Davidovits, P., Worsnop, D.R., 2012. Soot particle aerosol mass spectrometer: development, validation, and initial application. Aerosol Sci. Tech. 46 (7), 804–817.

Orasche, J., Schnelle-Kreis, J., Schon, C., Hartmann, H., Ruppert, H., Arteaga-Salas, J.M., Zimmermann, R., 2013. Comparison of emissions from wood combustion. Part 2: impact of combustion conditions on emission factors and

characteristics of particle-bound organic species and polycyclic aromatic hydrocarbon (PAH)-related toxicological potential. Energ. Fuel 27 (3), 1482–1491.
Pagels, J., Dutcher, D.D., Stolzenburg, M.R., McMurry, P.H., Galli, M.E., Gross, D.S., 2013. Fine-particle emissions from solid biofuel combustion studied with single-particle mass spectrometry: identification of markers for organics, soot, and ash components. J. Geophys Res. Atmos. 118 (2), 859–870.
Pettersson, E., Boman, C., Westerholm, R., Bostrom, D., Nordin, A., 2011. Stove performance and emission characteristics in residential wood log and pellet combustion, Part 2: wood stove. Energ. Fuel 25, 315–323.
Rohr, A.C., Wyzga, R.E., 2012. Attributing health effects to individual particulate matter constituents. Atmos. Environ. 62, 130–152.
Sheesley, R.J., Kirillova, E., Andersson, A., Krusa, M., Praveen, P.S., Budhavant, K., Safai, P.D., Rao, P.S.P., Gustafsson, O., 2012. Year-round radiocarbon-based source apportionment of carbonaceous aerosols at two background sites in South Asia. J. Geophys Res. Atmos. 117.
Shiraiwa, M., Selzle, K., Poschl, U., 2012. Hazardous components and health effects of atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic compounds and allergenic proteins. Free Radic. Res. 46 (8), 927–939.
Tapanainen, M., Jalava, P.I., Maki-Paakkanen, J., Hakulinen, P., Happo, M.S., Lamberg, H., Ruusunen, J., Tissari, J., Nuutinen, K., Yli-Pirila, P., Hillamo, R., Salonen, R.O., Jokiniemi, J., Hirvonen, M.R., 2011. In vitro immunotoxic and genotoxic activities of particles emitted from two different small-scale wood combustion appliances. Atmos. Environ. 45 (40), 7546–7554.
Tapanainen, M., Jalava, P.I., Maki-Paakkanen, J., Hakulinen, P., Lamberg, H., Ruusunen, J., Tissari, J., Jokiniemi, J., Hirvonen, M.R., 2012. Efficiency of log wood combustion affects the toxicological and chemical properties of emission particles. Inhal. Toxicol. 24 (6), 343–355.
Tsapakis, M., Stephanou, E.G., 2003. Collection of gas and particle semi-volatile organic compounds: use of an oxidant denuder to minimize polycyclic aromatic hydrocarbons degradation during high-volume air sampling. Atmos. Environ. 37 (35), 4935–4944.
Unosson, J., Blomberg, A., Sandstrom, T., Muala, A., Boman, C., Nystrom, R., Westerholm, R., Mills, N.L., Newby, D.E., Langrish, J.P., Bosson, J.A., 2013. Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. Part Fibre Toxicol. 10.
Uski, O.J., Happo, M.S., Jalava, P.I., Brunner, T., Kelz, J., Obernberger, I., Jokiniemi, J., Hirvonen, M.R., 2012. Acute systemic and lung inflammation in C57Bl/6J mice after intratracheal aspiration of particulate matter from small-scale biomass combustion appliances based on old and modern technologies. Inhal. Toxicol. 24 (14), 952–965.
Yee, L.D., Kautzman, K.E., Loza, C.L., Schilling, K.A., Coggon, M.M., Chhabra, P.S., Chan, M.N., Chan, A.W.H., Hersey, S.P., Crounse, J.D., Wennberg, P.O., Flagan, R.C., Seinfeld, J.H., 2013. Secondary organic aerosol formation from biomass burning intermediates: phenol and methoxyphenols. Atmos. Chem. Phys. 13 (16), 8019–8043.