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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, and 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; Hanno 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 have 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; Orašce 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 change (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 changes due to atmospheric processing. Processing initiated by 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. 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. The procedure generated intermittent periods of 1) high organic aerosol emissions following fuel addition and 2) “overload” hot air starved conditions with very low O2 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 O2, CO, and NO is shown in Table 1.

2.2. Experimental procedure

The emissions were diluted and sampled to a 15.3 m³ 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 mg m⁻³ was achieved. A cascade impactor (DIG, 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 (PM₁₀) in the chamber was measured by a tapered element oscillating microbalance (TEOM) at an operating temperature of 30 °C (series 1400, Ruprecht & Patnikar, 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 NO₂ (NOX-NO) concentration was measured by a NO₂ 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>
<thead>
<tr>
<th>Table 1</th>
<th>The experiment average concentration of O₂, CO and NOₓ measured in undiluted flue gases.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>O₂ (%)</td>
</tr>
<tr>
<td>HAS#1</td>
<td>11.4</td>
</tr>
<tr>
<td>HAS#2</td>
<td>9.9</td>
</tr>
<tr>
<td>NOM#1</td>
<td>10.9</td>
</tr>
<tr>
<td>NOM#2</td>
<td>13.8</td>
</tr>
</tbody>
</table>
Teflon filters (PM). An online liquid chromatography-gas chromato-
graphy/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
drawn by dissolving the filters in methanol. The extracts from
the three lowest stages from the two experiments of each combus-
tion situation were pooled together to form a PM1 sample
(NOM-fresh, NOM-aged, HAS-fresh, HAS-aged). Analysis for ele-
ments (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

the sub G1 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 reticu-
lum 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 particu-
late samples with those from corresponding control cells.

2.5.3. Production of inflammatory mediators

The productions of proinflammatory cytokine TNF-α and che-
mokine 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 in-
structions of the manufacturer. The absorbances were detected at
wavelength 450 nm with the multilabel plate reader (Victor³). 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 elec-
rophoresis (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
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 compo-
ents 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 de-
creases due to dilution and wall losses of the gas-phase. The
average PM10 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](https://example.com/Table2.png)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>OM (%)</th>
<th>EC (%)</th>
<th>NO3 (%)</th>
<th>Zn (%)</th>
<th>K + Cl + SO4 (%)</th>
<th>Others (%)</th>
<th>PAHs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAS-fresh</td>
<td>56.9</td>
<td>39.7</td>
<td>0.7</td>
<td>0.2</td>
<td>2.1</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>HAS-aged</td>
<td>60.9</td>
<td>33.3</td>
<td>3.1</td>
<td>0.2</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>NOM-fresh</td>
<td>19.2</td>
<td>69.2</td>
<td>0.8</td>
<td>0.7</td>
<td>8.2</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>NOM-aged</td>
<td>8.9</td>
<td>76.0</td>
<td>6.2</td>
<td>0.8</td>
<td>6.8</td>
<td>1.3</td>
<td>-</td>
</tr>
</tbody>
</table>

* 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\(_2\) 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 online 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 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 statistically significant difference between NOM and HAS combustion situations. A statistically significant 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 particle 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...
both NOM and HAS combustion. When the highest dose was introduced to cells the MIP-2 production was increased after the

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 $300$ $\mu g$ 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 (SubG$_1$, G$_1$ and S-G$_2$/M) after exposure to different doses ($15–300$ $\mu g$ ml$^{-1}$) of emission particles from differing 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.

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 $300$ $\mu g$ 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.

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 immuno-suppressive 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 NO₂ 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 aerosols give 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 be represented by the fresh emissions studied here, while exposures several hours downwind the emission spot may be better represented by the 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


