Health related toxicological effects of particulate emissions from small –scale biomass combustion systems

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1University of Eastern Finland
2National Institute for Health and Welfare
Background

• Biomass combustion contributes substantial proportion of the urban particulate concentrations which are associated with a large health burden across the world

• In Europe approximately 350,000 annual premature deaths occur due to the particulate air pollution

• In addition, adverse health effects of particulate air pollution has also large economic impacts due to worsening of symptoms of cardio-respiratory diseases, hospitalizations, loss of working days, etc.

• Even the small particulate concentrations may cause severe health impacts
Biomass combustion emissions contains thousands of chemicals, many of which have well-documented adverse human health effects including

- commonly regulated pollutants such as fine particles, carbon monoxide (CO), nitrogen oxides (NOx)
- ciliatoxic respiratory irritants such as phenols, cresols, acrolein, and acetaldehyde
- carcinogenic organic compounds such as benzene, formaldehyde, and 1,3 butadiene
- carcinogenic cyclic compounds such as PAHs

E.g. Wood smoke is classified as human carcinogens by the International Agency for Research on Cancer (IARC), probable human carcinogens (Group 2A) (Straif et al., 2006).
Evaluation of health risks are based on

- Epidemiological studies
- Human exposure studies (ethical limitations)
- *In vivo* animal studies
- *In vitro* studies on cellular level
Findings in epidemiological studies
(mostly USA and New Zealand)

• **Asthmatic subjects**: the best defined susceptible population group
  – Increased symptoms and decreased lung functions
  – Increased hospital emergency room visits due to asthma attacks
  – The estimated contribution of wood combustion to the outdoor air PM$_{10}$ or PM$_{2.5}$ concentration is 20-90% during the study periods

• **In developed countries**: residential wood combustion is associated with increase of respiratory diseases (asthma and chronic obstructive pulmonary disease (COPD) and recently also shown association with cardiovascular health.

• **In developing countries**: there is strong evidence on acute lower respiratory infections (ALRIs) in children and (COPD) in women.
Cont..

• Experimental human exposure studies
  – increased oxidative stress
  – lung inflammation and damage
  – systemic inflammation in blood and increased tendency to blood coagulation

• Experimental animal and cell studies
  – oxidative stress,
  – cytotoxicity,
  – DNA-damage,
  – inflammation
  -> impaired host defence against bacterial infections

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Because of extensive adverse health effects, there are pressures in many European countries to start regulation of combustion emissions.

However, PM chemical compositions responsible for the extensive public health impacts are insufficiently known.
Why interest on toxic effects of exposure to biomass combustion particles?

The chemical composition of biomass PM is different from those derived from fossil fuel combustion.

These particles may pose different kind and level of health risk than other ambient particles of similar size.
Identification of mechanisms behind the health effects

Particulate matter

- Chemistry
- Age

Physicochemical properties

- Shape
- Surface
- Size

Toxicological properties

- Oxidative stress
- Inflammation

Health effects

- DNA damage
- Cell death
- Cancer
- Chronic cardiovascular and respiratory diseases

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Toxicological methods

(1) Inflammation

In PM experimental setups, inflammation is the main studied endpoint

• A normal protective response to destroy, dilute, or isolate foreign agents and promote the repair of injured tissue.

• Particulate-induced inflammation is suggested as the main mechanism causing exacerbations of air pollution related respiratory and cardiac diseases
  – increased symptoms of obstructive lung diseases (COPD, asthma) that are of inflammatory origin
  – cardiovascular effects, such as atherosclerosis, blood coagulation, decrease in heart rate variability, ST-segment depression
(2) Cytotoxicity

• Cytotoxicity is related to airway remodeling in chronic respiratory diseases and it has also possible effects in the development of cardiac diseases.
  – Inflammation induced epithelial damage is associated with asthma pathogenesis in human lung.
  – Cytotoxic activity of lymphocytes has affected the impairment of COPD.
  – Cytotoxicity in natural killer cells have been in association with coronary artery disease.
  – An important role of apoptosis in fibrotic lung diseases.

• Experimental animal studies have shown particulate exposure induced tissue damage in lungs.
(3) Genotoxicity

Genotoxicity (DNA damage) in mammalian cells is associated with cell cycle arrest, a process which activates the DNA repair machinery. If the process fails, the cell cycle can be blocked permanently, triggering apoptotic cell death.

Genotoxicity is associated to air pollution in various studies.

- in Prague high concentrations of PAH compounds in the air caused chromosomal aberrations in exposed subjects
- oxidative DNA damage was detected in subjects living in Eastern European cities (Prague, Kosiče, Sofia) in areas with high PAH-concentration
- water-soluble metal and organic soluble PAHs have been in association to micronuclei formation in human epithelial cells after exposure to Mexico City particulate samples
(4) Oxidative stress

Increased production of intracellular oxygen radicals can lead to cell death, DNA damage and inflammation.

- Oxidative stress has been implicated in a number of human diseases e.g. atherosclerosis, diabetes, ischemia-reperfusion, cancer, inflammatory diseases, Parkinson's disease and Alzheimer's disease

- It is an imbalance between the formation of free radicals and the ability of antioxidant system to remove these reactive molecules in biological organisms.

- It can result from 1) diminished amount of antioxidants or 2) increased production of reactive oxygen (ROS) or nitrogen species

- The production of ROS and cytokines are closely related with each other.
Multidiciplinary collaboration

**Rereacrh groups**

**Aerosol technology:**
- Professor Jokiniemi, University of Eastern Finland
- Professor Obernberger, Graz University of Technology and BIOENERGY 2020+ GmbH

**Aerosol toxicology:**
- Professor Hirvonen, University of Eastern Finland
Toxicological properties of PM samples from different combustion conditions
Collaboration: Professor Jokiniemi, University of Eastern Finland

<table>
<thead>
<tr>
<th>Studied appliances</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Masonry heater</strong></td>
<td></td>
</tr>
<tr>
<td>• Conventional masonry heater</td>
<td></td>
</tr>
<tr>
<td>• Air inlet through the grate and the door</td>
<td></td>
</tr>
<tr>
<td>• Three batches combustion, 55 min</td>
<td></td>
</tr>
<tr>
<td>• Small soapstone heater</td>
<td></td>
</tr>
<tr>
<td><strong>Pellet boiler</strong></td>
<td></td>
</tr>
<tr>
<td>• modern 25 kW small-scale pellet boiler</td>
<td></td>
</tr>
<tr>
<td>• microprocessor controlled continuous combustion</td>
<td></td>
</tr>
<tr>
<td><strong>Sauna stove</strong></td>
<td></td>
</tr>
<tr>
<td>• ignition and 2. batch</td>
<td></td>
</tr>
<tr>
<td>• simple combustion technology- bad combustion conditions</td>
<td></td>
</tr>
<tr>
<td>• small and light weight</td>
<td></td>
</tr>
<tr>
<td>• traditional Finnish sauna stove</td>
<td></td>
</tr>
</tbody>
</table>
Particle samples were collected to filters with a Dekati Gravimetric Impactor (DGI)
- sample diluted with porous tube diluter
- DR 13-26
Sample preparation

1. Weighing of filters
2. Methanol extraction (sonication)
3. Evaporation of additional methanol
4. Dispensing the particle suspension to glass tubes on mass basis
5. Drying under nitrogen flow
6. Storing at -20 °C

Before exposure of cells:
6. Dissolving particles to DMSO and water
7. Sonication for 30 minutes
Exposure to particulate matter

Cell lines:
- Mouse RAW264.7 macrophages,
- Human BEAS-2B cells

They are target cells in PM induced immunotoxicity

Particulate doses: 15, 50, 150 and 300 µg/ml

Exposure time: 24 hours

Detected endpoints:
- Cell death (acute and programmed)
- Inflammatory mediators (e.g. MIP-2, TNFα)
- DNA damage
Particles produced in poor combustion conditions induce extensive cell death

- All the studied emission particles caused acute and programmed cell death in macrophages
- The particles emitted from sauna stove were the most cytotoxic

**Particulate doses:**
- 15 µg/ml
- 50 µg/ml
- 150 µg/ml
- 300 µg/ml

**ACUTE CELL DEATH**
- Pellet boiler
- Masonry heater
- Sauna stove

**PROGRAMMED CELL DEATH**
- Pellet boiler
- Masonry heater
- Sauna stove

Collaboration: Professor Jokiniemi
Wood combustion particles induce weak inflammatory responses in macrophages

Particulate doses:
- 15 µg/ml
- 50 µg/ml
- 150 µg/ml
- 300 µg/ml

MIP-2

Production (pg/ml)

Pellet boiler  Masonry heater  Sauna stove  Pellet boiler  Masonry heater  Sauna stove

TNFα

Cell death!

Collaboration: Professor Jokiniemi

Tapanainen et al, submitted
Genotoxicity and chemical content of the combustion particles

*Statistical significant difference compared to control

**Unreliable analysis due to extensive cell death!**

### PAH content (ng/mg)

<table>
<thead>
<tr>
<th>Source</th>
<th>Total</th>
<th>Genotoxic*</th>
<th>PM$_{10}$ (mg/MJ)</th>
<th>OC (mg/MJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet boiler</td>
<td>6</td>
<td>3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Masonry heater</td>
<td>19.000</td>
<td>10.000</td>
<td>52</td>
<td>11</td>
</tr>
<tr>
<td>Sauna Stove</td>
<td>83.000</td>
<td>34.000</td>
<td>260</td>
<td>165</td>
</tr>
</tbody>
</table>

### Particulate doses:

- 7,5 µg/ml
- 75 µg/ml
- 15 µg/ml
- 150 µg/ml
- 50 µg/ml
- 300 µg/ml

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Collaboration: Professor Jokiniemi

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28/01/2011
Toxicological properties of PM samples from seven different small-scale biomass heating systems

Collaboration: Professor Obernberger
Graz University of Technology and BIOENERGY 2020+ GmbH

**Studied appliances**

**New technology**
1. stove
2. log wood boiler
3. tiled stove
4. pellet boiler
5. wood chip boiler

**Old technology**
1. stove
2. log wood boiler
Inflammatory responses

Dose 150μg/ml

MIP-2 pg/ml

New tech
Old tech
New tech
Old tech
New tech

Stoves
Log wood boilers
Wood chip boiler
Tiled stove
Pellet boiler

Unpublished data

Collaboration: Professor Obernberger

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Cytotoxicity (MTT test)

Viability %

- **Stoves**
  - New tech: 90%
  - Old tech: 85%

- **Log wood boilers**
  - New tech: 75%
  - Old tech: 30%

- **Wood chip boiler**
  - Old tech: 55%

- **Tiled stove**
  - New tech: 60%

- **Pellet boiler**
  - New tech: 70%

Dose: 150μg/ml

Unpublished data

Collaboration: Professor Obernberger

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Cytotoxicity /Cell membrane permeability

![Graph showing cytotoxicity and cell membrane permeability for different types of stoves and boilers.](image)

- New tech
- Old tech

Dose: 150μg/ml

**Unpublished data**

Collaboration: Professor Obernberger

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Programmed cell death

% of the cells

Stoves  Log wood boilers  Wood chip boiler  Tiled stove  Pellet boiler

New tech  Old tech  New tech  Old tech  New tech

Dose 150µg/ml

Unpublished data

Collaboration: Professor Obernberger
Genotoxicity / Comet assay

Blank 150 µg/ml
50 µg/ml
150 µg/ml
300 µg/ml

OTM

New tech
Old tech

Stoves
Log wood boilers
Wood chip boiler
Tiled stove
Pellet boiler

Collaboration: Professor Obernberger
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Effect of chemical composition on toxicological responses

<table>
<thead>
<tr>
<th></th>
<th>MTT</th>
<th>TNFα</th>
<th>MIP-2</th>
<th>PI</th>
<th>SubG1</th>
<th>G1</th>
<th>S/G2M</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>-0.270</td>
<td>-0.013</td>
<td>0.445</td>
<td>0.451</td>
<td>0.456</td>
<td>-0.454</td>
<td>0.275</td>
</tr>
<tr>
<td>EC</td>
<td>0.440</td>
<td>0.492</td>
<td><strong>0.612</strong></td>
<td>0.382</td>
<td><strong>0.763</strong></td>
<td>-0.527</td>
<td>-0.332</td>
</tr>
<tr>
<td>Ca</td>
<td>-0.244</td>
<td>-0.433</td>
<td><strong>-0.705</strong></td>
<td><strong>-0.749</strong></td>
<td><strong>-0.793</strong></td>
<td><strong>0.833</strong></td>
<td>-0.262</td>
</tr>
<tr>
<td>Mg</td>
<td>-0.165</td>
<td><strong>-0.550</strong></td>
<td><strong>-0.783</strong></td>
<td><strong>-0.759</strong></td>
<td><strong>-0.724</strong></td>
<td><strong>0.772</strong></td>
<td>-0.220</td>
</tr>
<tr>
<td>Mn</td>
<td>-0.285</td>
<td>-0.510</td>
<td><strong>-0.766</strong></td>
<td><strong>-0.659</strong></td>
<td><strong>-0.798</strong></td>
<td><strong>-0.731</strong></td>
<td>0.065</td>
</tr>
<tr>
<td>K</td>
<td>0.051</td>
<td>-0.495</td>
<td><strong>-0.802</strong></td>
<td><strong>-0.670</strong></td>
<td><strong>-0.657</strong></td>
<td><strong>0.666</strong></td>
<td>-0.138</td>
</tr>
<tr>
<td>Na</td>
<td>-0.033</td>
<td>-0.332</td>
<td><strong>-0.653</strong></td>
<td><strong>-0.705</strong></td>
<td><strong>-0.776</strong></td>
<td><strong>0.824</strong></td>
<td>-0.301</td>
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<tr>
<td>Zn</td>
<td>-0.077</td>
<td>-0.515</td>
<td><strong>-0.789</strong></td>
<td><strong>-0.725</strong></td>
<td><strong>-0.641</strong></td>
<td><strong>0.969</strong></td>
<td>-0.194</td>
</tr>
<tr>
<td>S</td>
<td>-0.029</td>
<td>-0.455</td>
<td><strong>-0.758</strong></td>
<td><strong>-0.688</strong></td>
<td><strong>-0.622</strong></td>
<td><strong>0.662</strong></td>
<td>-0.152</td>
</tr>
<tr>
<td>Cl</td>
<td>-0.136</td>
<td>-0.493</td>
<td><strong>-0.711</strong></td>
<td><strong>-0.700</strong></td>
<td><strong>-0.587</strong></td>
<td><strong>0.695</strong></td>
<td>-0.163</td>
</tr>
<tr>
<td>Cd</td>
<td>-0.062</td>
<td>-0.251</td>
<td><strong>-0.556</strong></td>
<td><strong>-0.602</strong></td>
<td>-0.507</td>
<td><strong>0.629</strong></td>
<td>-0.389</td>
</tr>
</tbody>
</table>
The PAH composition was in a key role in activated toxicological responses

<table>
<thead>
<tr>
<th>Six criteria PAH (EC/2004)</th>
<th>MTT</th>
<th>TNF-α</th>
<th>MIP-2</th>
<th>PI</th>
<th>SubG1</th>
<th>G1</th>
<th>S/G2M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]anthracene</td>
<td>0.354</td>
<td>0.587*</td>
<td>0.697**</td>
<td>0.697**</td>
<td>0.648*</td>
<td>-0.662**</td>
<td>0.116</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.323</td>
<td>0.556*</td>
<td>0.705**</td>
<td>0.688**</td>
<td>0.692**</td>
<td>-0.692**</td>
<td>0.130</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.214</td>
<td>0.762*</td>
<td>0.619</td>
<td>0.310</td>
<td>0.405</td>
<td>-0.357</td>
<td>-0.286</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>0.341</td>
<td>0.569*</td>
<td>0.714**</td>
<td>0.679**</td>
<td>0.666**</td>
<td>-0.670**</td>
<td>0.103</td>
</tr>
<tr>
<td>Indeno[1.2.3-cd]pyrene</td>
<td>0.204</td>
<td>0.266</td>
<td>0.495</td>
<td>0.530</td>
<td>0.543*</td>
<td>-0.596*</td>
<td>0.358</td>
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<tr>
<td>Dibenzo[a.h]anthracene</td>
<td>0.049</td>
<td>0.119</td>
<td>0.399</td>
<td>0.448</td>
<td>0.434</td>
<td>-0.594*</td>
<td>0.329</td>
</tr>
</tbody>
</table>
Toxicological potency in cells between the studied appliances

Old technology log wood boiler
- rather high inflammatory response
- high cytotoxicity, especially with PI-method
- Genotoxicity is dramatically increased.
- This appliance type may affect all the proposed disease mechanisms.

Old technology Stove
- Increased  genotoxicity
- slightly increased Inflammatory responses

New technology stove
- Same activated cellular mechanisms as by the PM emissions from old tech stove but response level is lower

Tiled stove, wood chip boiler and new technology log wood boiler
- At least some of the toxicological parameters are increased

Pellet boiler
- Most of the toxicological parameters were only slightly increased, and the genotoxic response was negligible.
Conclusions

• Overall, the combustion derived particles showed small inflammatory potency
• Cytotoxic and genotoxic effects were detected by most of the combustion particles
• The PAH composition was in a key role in activated toxicological responses
• Combustion technology caused significant differences in the toxic potency of the emitted particles

The present data suggests that emissions and health related toxicological effects of fine combustion particles can be reduced by using appropriate appliances in biomass combustion
Thank you for your attention!
Acknowledgements

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• University of Eeastern Finland, Finland
  • **Prof. Jokiniemi**, and research group