Session 6B: Health effects

In vitro assessment of proinflammatory and genotoxicological effects of wood combustion-generated ultrafine particles

G. Lonati\(^1\), P. Fermo\(^2\), C.L. Galli\(^3\), R. Vecchi\(^4\)

\(^1\)Dept. of Civil and Environmental Engineering, Politecnico di Milano
\(^2\)Dept. of Chemistry, Università degli Studi di Milano
\(^3\)Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano
\(^4\)Dept. of Physics, Università degli Studi di Milano and INFN - Milano
OVERVIEW

- Background and motivation

- Materials and methods
  - Sampling
  - Analytical determinations
  - Biological models

- Results
  - Combustion tests
  - Biological effects

- Conclusions
Lombardy is a European hot-spot for PM pollution
- restrictions to vehicular traffic
- restriction to wood combustion for domestic heating

Previous works demonstrated that wood combustion can generate biologically active PM2.5 particles

Ultrafine particles (UFP, \( d_p < 100 \) nm) are thought to be the best single indicator of the health impacts of most combustion sources

Wood smoke particles are usually within the UFP size range but their chemical composition can depend on several factors (e.g.: the kind of wood used, the combustion process and conditions) and differ from those derived from fossil fuel combustion
TOBICUP project
TOxicity of BIomass Combustion generated Ultrafine Particles

Project aims:
• deeper insight on physiochemical features of UFPs emitted by residential biomass combustion (RBC)
• assessment of toxicological responses of UFP both from source samples and ambient samples dominated by RBC

Project activities:
• tests on small scale domestic woody biomass automatic and manually fed appliances (i.e.: pellet stove and wood stove)
• ambient air sampling in cold and warm season
• quantitative characterization of gaseous pollutants and UFP
• chemical and toxicological characterization of UFP

Here presented:
• Features and effects of UFP from stack samples from domestic pellet and wood log stoves
Materials and methods
PELLET STOVE FEATURES

Stove technical data:
- commercially available wood pellet stove
- **nominal heat output** 11.1 kW
  (nominal fuel consumption 2.4 kg/h, efficiency=89.2%)
- minimum heat output 3.4 kW
  (minimum fuel consumption 0.8 kg/h, efficiency=84.5%)
- internal pellet storage, automatic pellet supply via auger screw, fan assisted flue discharge

Conifer pellet (EN A1) (softwood)
Beech pellet (hardwood)
Materials and methods
WOOD STOVE FEATURES

Stove technical data:
- commercially available wood log stove
- **nominal heat output 8.2 kW**
  (nominal fuel consumption 2.0 kg/h, efficiency=80.8%)
- natural draft
- manual feed

**WOOD STOVE FEATURES**

Fir (softwood)  Beech (hardwood)
EXPERIMENTAL SET UP FOR UFP STACK SAMPLING

Wood logs
- DR = 400-1000
- \( T_{\text{sample}} = 21-26^\circ\text{C} \)

Pellet
- DR = 90-150
- \( T_{\text{sample}} = 28-32^\circ\text{C} \)

UFP sampling with cascade impactors

- mass + toxicology
- TC + ions
- elements

EXPERIMENTAL SET UP FOR UFP STACK SAMPLING
EXPERIMENTAL SET UP FOR UFP STACK SAMPLING

Size distribution

Electrostatic Low-Pressure Impactor
Dekati Ltd

Particle sampling

Micro-Orifice Uniform Deposit Impactor
MPS Corporation

Particles collected on the two last stages of the impactor ($d_{50} = 100$ nm and $d_{50} = 56$ nm) considered for analyses

Ozgen et al., EUBC 2016

Size range: 7 nm – 10 µm
Materials and methods

ANALYTICAL DETERMINATIONS

- **IC**(1) on quartz fiber filters
  - water soluble anions (Cl\(^{-}\), NO\(_2\)^{-}, NO\(_3\)^{-}, SO\(_4\)^{2-}, formate, acetate, propionate, oxalate, methanesulfonic acid) and cations (K\(^{+}\), Ca\(^{2+}\), Na\(^{+}\), NH\(_4\)^{+}, Mg\(^{2+}\))

- **HPAEC–PAD**(2) on quartz fiber filters
  - anhydrosugars (levoglucosan, mannosan and galactosan)

- **TOT**(3) on quartz fiber filters
  - total carbon

- **ICP-AES**(4) on polycarbonate substrates
  - elements (Al, As, Ba, Cd, Co, Cu, Fe, Mn, Mo, Ni, P, Pb, Sr, Ti, V, Zn)

- **GC-MS**(5) on quartz fiber filters
  - PAHs
    - (Anthracene, Benzantracene, Benzo[a]pyrene, Benzofluoranthene, Fluoranthene, Chrysene, Phenanthrene, Pyrene)

**Materials and methods**

**ANALYTICAL DETERMINATIONS**

(1) Ion chromatography
(2) High performance anion-exchange chromatography coupled with pulsed amperometric detection
(3) Thermal optical transmittance
(4) Inductively coupled plasma atomic emission spectroscopy

(Piazzalunga et al., 2013. Anal Bioanal Chem 405:1123–1132)

---

20th ETH Conference on Combustion generated nanoparticles
Zurich, 13-16.06.2016

POLITECNICO MILANO 1863
**Materials and methods**

**BIOLOGICAL MODELS**

**Experimental models:**
- human lung adenocarcinoma epithelial cell line **A549** as a surrogate of type II cells
- promyelocytic cell line **THP-1** as a surrogate of alveolar macrophages
Materials and methods
BIOLOGICAL MODELS

Parameters:
- Cell viability: MTT test and lactate dehydrogenase leakage
- Inflammatory marker: interleukin-8 (IL-8) release by ELISA
- Cellular uptake: FACS analysis
- Genotoxicity: Comet assay, γH2AX

Corsini et al., in preparation
Materials and methods
BIOLOGICAL MODELS

Alkaline Comet assay:
- single and double DNA strand break

Arbitrary classification of nucleotides:
- **Type A**: no damage
- **Type B, C, D**: increasing of DNA damage

Damage quantification based on:
- tail length (µm)
- % DNA
- tail moment (µm)
Materials and methods
BIOLOGICAL MODELS

γH2AX assay:

- double DNA strand break

- DNA double strand breaks (DSB) are the gravest form of DNA damage

- Phosphorylated histone H2AX (γH2AX) is used as a biomarker of cellular response to DSB

- Phosphorylation of histone H2AX leads to the formation of a cluster of proteins (foci) that mediate cellular events including:
  - activation of the DNA damage checkpoint
  - repair of the DNA lesion
  - transcriptional responses

from Valdiglesias et al., Mutation Research (2013)
Materials and methods
BIOLOGICAL MODELS

γH2AX assay:

- double DNA strand break

- DNA double strand breaks (DSB) are the gravest form of DNA damage in eukaryotic cells

- Phosphorylated histone H2AX (γH2AX) is used as a biomarker of cellular response to DSB

- Results are expressed as percentage of cells with 0-5% foci (control) and more than 10% foci (DNA damage)
Materials and methods
BIOLOGICAL MODELS

Interleukin-8 release

➤ Localized inflammation

• IL-8 is a protein produced by macrophages and other cell types such as epithelial cells

• IL-8 secretion is increased by oxidant stress, which thereby cause the recruitment of inflammatory cells and induces a further increase in oxidant stress mediators

• IL-8 induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection.

• IL-8 also induces phagocytosis once they have arrived
Results

UFP EMISSION FACTORS

- Lower UFP emissions from pellet (both number and mass)
- Emission insensitive to the pellet kind
- Highest emissions from beech wood

Stack concentrations:  

**Pellet**  
12 - 56 mg/m$^3$
5 - 16 · 10$^7$ #/cm$^3$

**Wood logs**  
32 - 107 mg/m$^3$
23 - 60 · 10$^7$ #/cm$^3$
**Results**

**PARTICLE SIZE DISTRIBUTION**

- **Wood**
  - MGD = 35 nm
  - MGD = 71 nm

- **Pellet**
  - Smaller particles from pellet
  - Larger variability for wood
  - Beech produces smaller particles

**EXPERIMENTAL RESULTS – ultrafine particles (UFP)**

**Transmission factors**
### Summary table for genotoxicity tests on A549 cells:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Pellet</th>
<th>Wood log</th>
<th>DEP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conifer</td>
<td>Beech</td>
<td>Conifer</td>
</tr>
<tr>
<td>Comet (tail length)</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>γH2AX (5-10% foci)</td>
<td>▶</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

*p<0.05; ** p<0.01; ***p<0.001 vs. control

A549 cells treated for 24 h with 100 µg/ml medium of test particulate

- Comet assays show strong potential for DNA damage (SSB damage)
- UFP from wood log combustion displays stronger effect than pellet
- Similar effects compared to DEP (Diesel Exhaust Particle) control
Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release:

- Pellet UFP less powerful than wood log UFP in both cell lines and for both type of essence
- UFP from beech wood logs more effective on IL-8 release in THP-1 cells
Results

BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood logs combustion

Different cellular uptake?

![Graph showing IL-8 release from control, conifer wood UFP, and beech wood UFP](image.png)

NO, based on FACS analyses
Results

BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood logs combustion

- Different sensitivity to UFP components?

- PAHs? NO

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Unit</th>
<th>Beech pellets</th>
<th>Conifer pellets</th>
<th>Beech logwood</th>
<th>Conifer logwood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levoglucosan</td>
<td>%</td>
<td>0.005</td>
<td>0.006</td>
<td>6.548</td>
<td>1.470</td>
</tr>
<tr>
<td>Mannosan</td>
<td>%</td>
<td>0.003</td>
<td>0.005</td>
<td>0.451</td>
<td>0.487</td>
</tr>
<tr>
<td>Galactosan</td>
<td>%</td>
<td>-</td>
<td>0.001</td>
<td>0.233</td>
<td>0.076</td>
</tr>
<tr>
<td>Al</td>
<td>%</td>
<td>0.212</td>
<td>0.255</td>
<td>0.033</td>
<td>0.039</td>
</tr>
<tr>
<td>As</td>
<td>%</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Ba</td>
<td>%</td>
<td>0.001</td>
<td>0.011</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Cd</td>
<td>%</td>
<td>0.001</td>
<td>0.001</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Co</td>
<td>%</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Cr</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>0.041</td>
<td>0.044</td>
</tr>
<tr>
<td>Cu</td>
<td>%</td>
<td>0.022</td>
<td>0.043</td>
<td>0.010</td>
<td>0.008</td>
</tr>
<tr>
<td>Fe</td>
<td>%</td>
<td>0.216</td>
<td>0.256</td>
<td>0.083</td>
<td>0.088</td>
</tr>
<tr>
<td>Ni</td>
<td>%</td>
<td>0.009</td>
<td>0.042</td>
<td>0.012</td>
<td>0.007</td>
</tr>
<tr>
<td>Zn</td>
<td>%</td>
<td>0.240</td>
<td>0.493</td>
<td>0.186</td>
<td>0.279</td>
</tr>
<tr>
<td>V</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Ti</td>
<td>%</td>
<td>0.003</td>
<td>0.004</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Sr</td>
<td>%</td>
<td>0.002</td>
<td>0.002</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Mo</td>
<td>%</td>
<td>0.037</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Pb</td>
<td>%</td>
<td>0.032</td>
<td>0.020</td>
<td>0.006</td>
<td>0.016</td>
</tr>
<tr>
<td>Na+</td>
<td>%</td>
<td>0.170</td>
<td>0.231</td>
<td>0.180</td>
<td>0.151</td>
</tr>
<tr>
<td>NH4+</td>
<td>%</td>
<td>0.016</td>
<td>0.024</td>
<td>0.3000</td>
<td>0.362</td>
</tr>
<tr>
<td>K+</td>
<td>%</td>
<td>25.443</td>
<td>23.160</td>
<td>2.255</td>
<td>3.150</td>
</tr>
<tr>
<td>Mg++</td>
<td>%</td>
<td>0.034</td>
<td>0.044</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>NO3-</td>
<td>%</td>
<td>0.626</td>
<td>0.891</td>
<td>0.540</td>
<td>0.311</td>
</tr>
<tr>
<td>SO4--</td>
<td>%</td>
<td>9.188</td>
<td>11.920</td>
<td>3.585</td>
<td>4.034</td>
</tr>
<tr>
<td>TC</td>
<td>%</td>
<td>3.64</td>
<td>12.74</td>
<td>48.80</td>
<td>87.1</td>
</tr>
</tbody>
</table>

- Sugars? YES

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Unit</th>
<th>Beech wood log</th>
<th>Conifer wood log</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levoglucosan</td>
<td>%</td>
<td>6.548 ± 0.005</td>
<td>1.470 ± 0.011</td>
</tr>
<tr>
<td>Galactosan</td>
<td>%</td>
<td>0.233 ± 0.001</td>
<td>0.076 ± 0.001</td>
</tr>
</tbody>
</table>
Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood combustion

- Different cell sensitivity to sugar components
  - THP-1
  - A549

- Dose-response relation for sugars in THP-1 cell line
- No effect on A549 lines
Conclusions

Combustion tests:
- Lower emission factors (both UFP mass and number) from pellet
- Higher emissions from beech wood

Genotoxicity:
- UFP from wood combustion displays stronger effect than pellet
- Effects of UFP from wood combustion similar to DEP

Inflammation:
- UFP from wood combustion displays stronger effect than pellet
- Beech wood induces higher IL-8 release in THP-1
- Effect related to sugar components of UFP
Acknowledgments

for funding the TOBICUP project (Grant 2013-1040)

for the pellet and wood stoves used in the study

All the participants to the TOBICUP project:

S. Ozgen, R. Tardivo, E. Tosi (Politecnico di Milano),

L. Corbella and R. Gonzalez (Dept. of Chemistry, Università degli Studi di Milano),

E. Corsini, L. Marabini, M. Marinovich, S. Turacchi
(Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano),

V. Bernardoni, M. Dell’Acqua, G. Valli
(Dept. of Physics, Università degli Studi di Milano),

S. Becagli (Dept. of Chemistry, Università degli Studi di Firenze),

S. Signorini (Energy and Environment Laboratory, Piacenza).
Thank you for your attention

TOBICUP’s Toxicology group
Beech Pellet (LT06)

- mithocondria
- Black arrows: nanoparticles
- White arrows: nanoparticles inside cells

nucleus

12000x

25000x
Fir Pellet

LT01

N: Nucleo  •: mitocondri

19000x  48000x  64000x  64000x
Conclusions

A confronto PM da diesel, da combustione completa ed incompleta della legna

Test biologici su tossicità cellulare e aberrazioni cromosomiche

Risultati il PM da combustione completa ha un effetto tossicologico 5 volte inferiore al PM diesel. Il PM da combustione incompleta ha una tossicità 15 volte superiore al PM da diesel ed un contenuto di IPA 20 volte superiore.
Beech wood
Results

Pellet stove

- Stove gaseous emissions in line with EEA inventory proposed values
- Generally higher emissions for beech

C-F: continuous-fir; M-F: modulated-fir; M-B: modulated-beech;
EEI2013: EMEP/EEA emission inventory guidebook 2013 – small combustion (Table 3-25)
### Tested Fuel Characteristics

**Pellet**

<table>
<thead>
<tr>
<th></th>
<th>Fir pellets (A1)</th>
<th>Beech pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (% w/w)</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Ash (@ 550°C)</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Carbon (% w/w)</td>
<td>47.3</td>
<td>46.3</td>
</tr>
<tr>
<td>Hydrogen (% w/w)</td>
<td>5.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Nitrogen (% w/w)</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Sulfur (mg/kg)</td>
<td>55</td>
<td>130</td>
</tr>
<tr>
<td>Chlorine (mg/kg)</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>HHV (MJ/kg)</td>
<td>18.8</td>
<td>18.34</td>
</tr>
<tr>
<td>LHV (MJ/kg)</td>
<td>17.4</td>
<td>17.005</td>
</tr>
</tbody>
</table>

- Tested pellets are commercially available
- Fir pellets comply with EN ISO 17225-2:2014 class A1

## TESTED FUEL CHARACTERISTICS

### Wood log

<table>
<thead>
<tr>
<th></th>
<th>Fir pellets (A1)</th>
<th>Beech pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (% w/w)</td>
<td>10.7</td>
<td>11.0</td>
</tr>
<tr>
<td>Ash (@ 550° C)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbon (% w/w)</td>
<td>46.4</td>
<td>43.6</td>
</tr>
<tr>
<td>Hydrogen (% w/w)</td>
<td>5.7</td>
<td>5.4</td>
</tr>
<tr>
<td>Nitrogen (% w/w)</td>
<td>0.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Sulfur (mg/kg)</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>Chlorine (mg/kg)</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>HHV (MJ/kg)</td>
<td>18.8</td>
<td>17.4</td>
</tr>
<tr>
<td>LHV (MJ/kg)</td>
<td>17.3</td>
<td>16.0</td>
</tr>
</tbody>
</table>
EXPERIMENTAL SET UP and UFP SAMPLING – wood stove

DR: dilution ratio

DR = 400-1000

Tsamp = 21-26°C

CO₂ analyzer

UFP sampling with cascade impactors

mass + toxic

TC + ions

elements

GAS MEASUREMENT

Filter

OFC

CO

CO₂

NOx

O₂

Diluter 1

Diluter 2

Cyclone

Hot Probe

Dilution Air

PM2.5

Heating Unit

ELPI

Computer

stack
**EXPERIMENTAL RESULTS – ultrafine particles (UFP)**

**PELLET STOVE – UFP NUMBER**

UFP number concentration

\[ 4.7 \cdot 10^7 \text{ particles/cm}^3 \]

(@NTP,13%O₂)

**Results**

**PELLET STOVE – UFP NUMBER**

Average UFP number emission factors

- **GMD**: geometrical mean diameter
  - \( \text{GMD}_{\text{fir}} = 71 \text{ nm} \)
  - \( \text{GMD}_{\text{beech}} = 35 \text{ nm} \)

**GMD**: geometrical mean diameter
Results

PELLET STOVE – UFP MASS

UFP mass concentration

12 - 56 mg/m³
(@NTP, 13%O₂)

Average UFP mass emission factors

UFP mass concentration

C-F M-F M-B

0 10 20 30 40 50
UFP mass EF (g/GJ)

continuous-fir (C-F)
modulated-fir (M-F)
modulated-beech (M-B)

R² = 0.86

UFP number @13%O₂ (cm⁻³)

ufp mass @13%O₂ (mg/m³)

x 10⁷