Central European Biomass Conference 2011
27.1.2011 Graz, Austria

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

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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

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

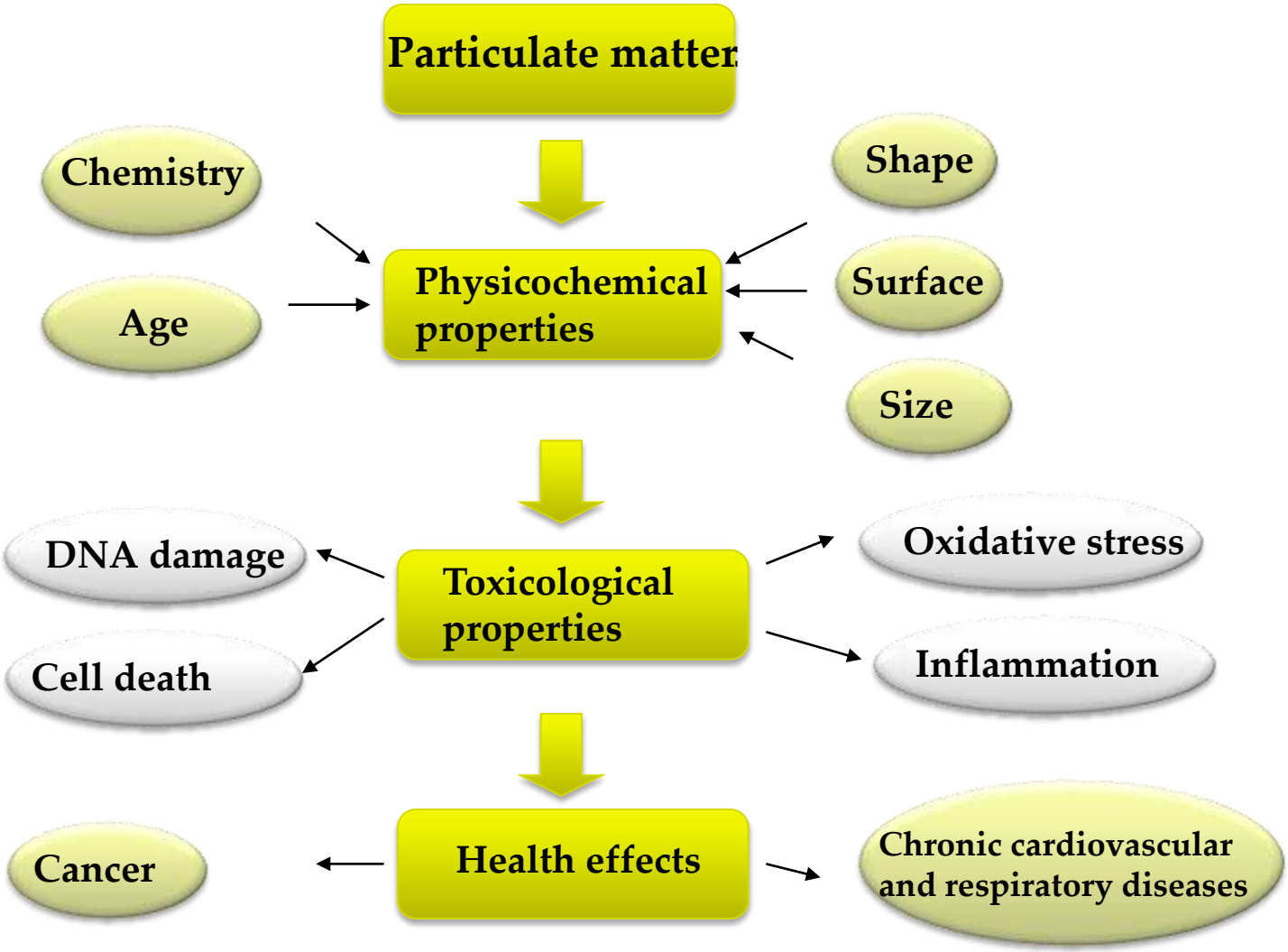
However, PM chemical compositions responsible for the extensive public health impacts are insufficiently known

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



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 impalance 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

Studied appliances

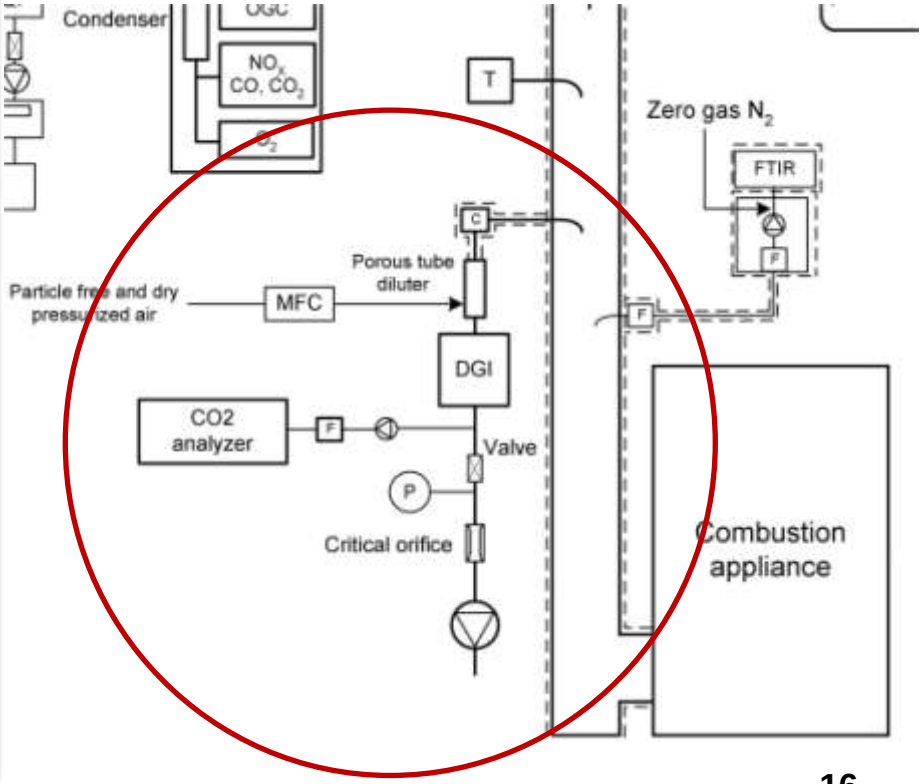
- **Masonry heater**
  - Conventional masonry heater
  - Air inlet through the grate and the door
  - Three batches combustion, 55 min
  - Small soapstone heater

- **Pellet boiler**
  - modern 25 kW small-scale pellet boiler
  - microprocessor controlled continuous combustion

- **Sauna stove**
  - ingition and 2. batch
  - simple combustion technology- bad combustion conditions
  - small and light weight
  - traditional Finnish sauna stove

Particle collection
(University of Eastern Finland, FINE)

- 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

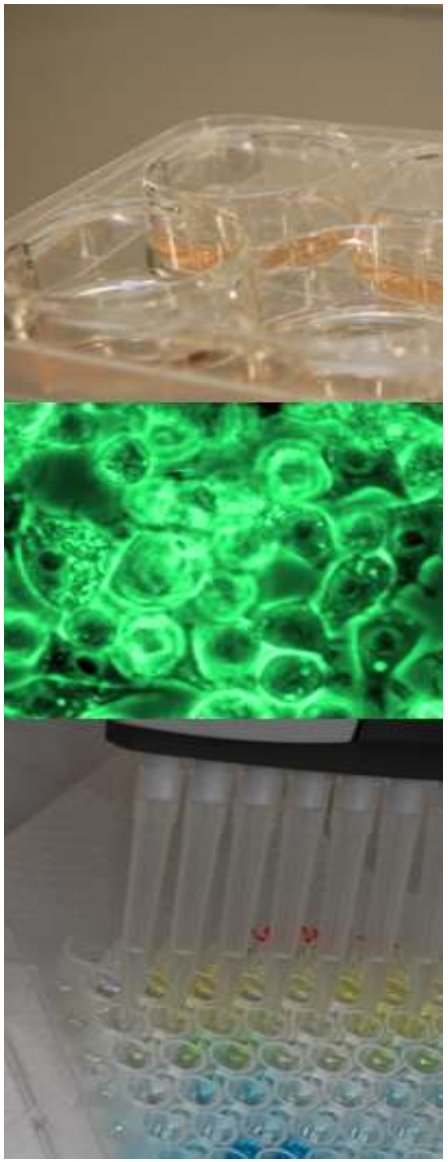
They are target cells in PM induced immunotoxicity

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

Exposure time: 24 hours

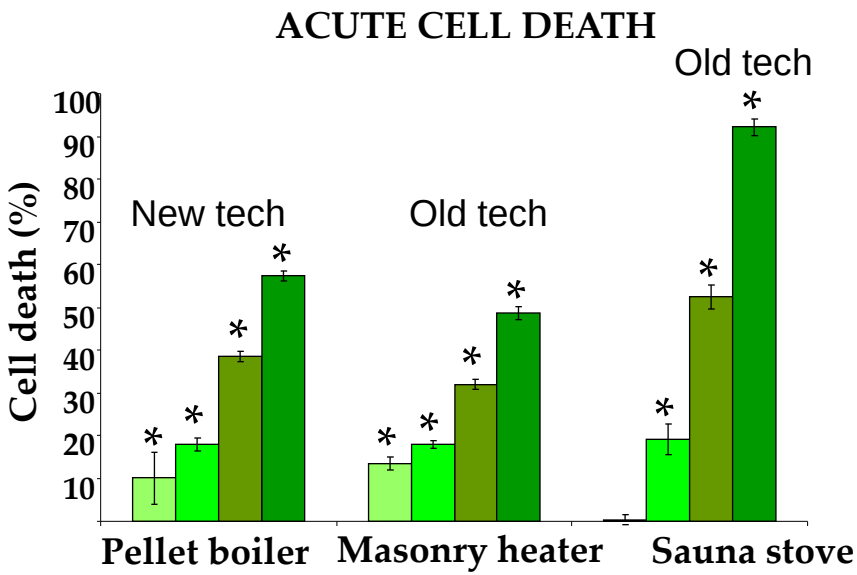
Detected endpoints:

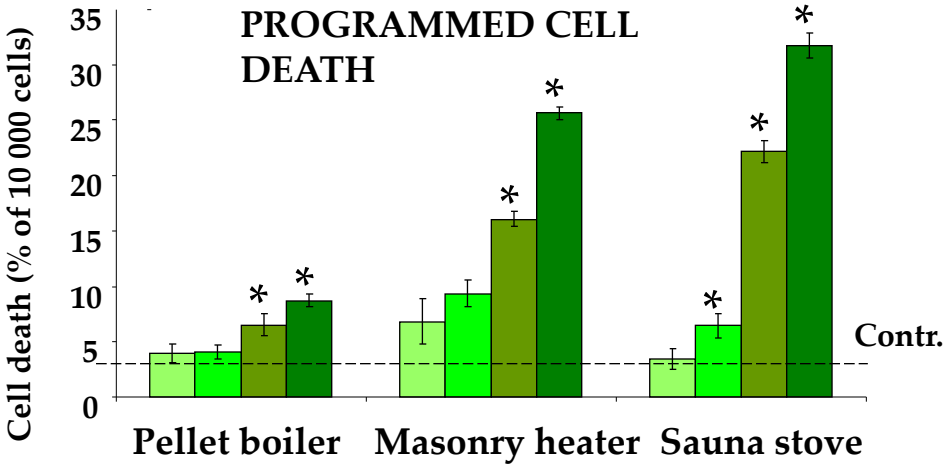
- Cell death (acute and programmed)
- Inflammatory mediators (e.g.MIP-2, TNFα)
- DNA damage



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

Tapanainen et al, submitted

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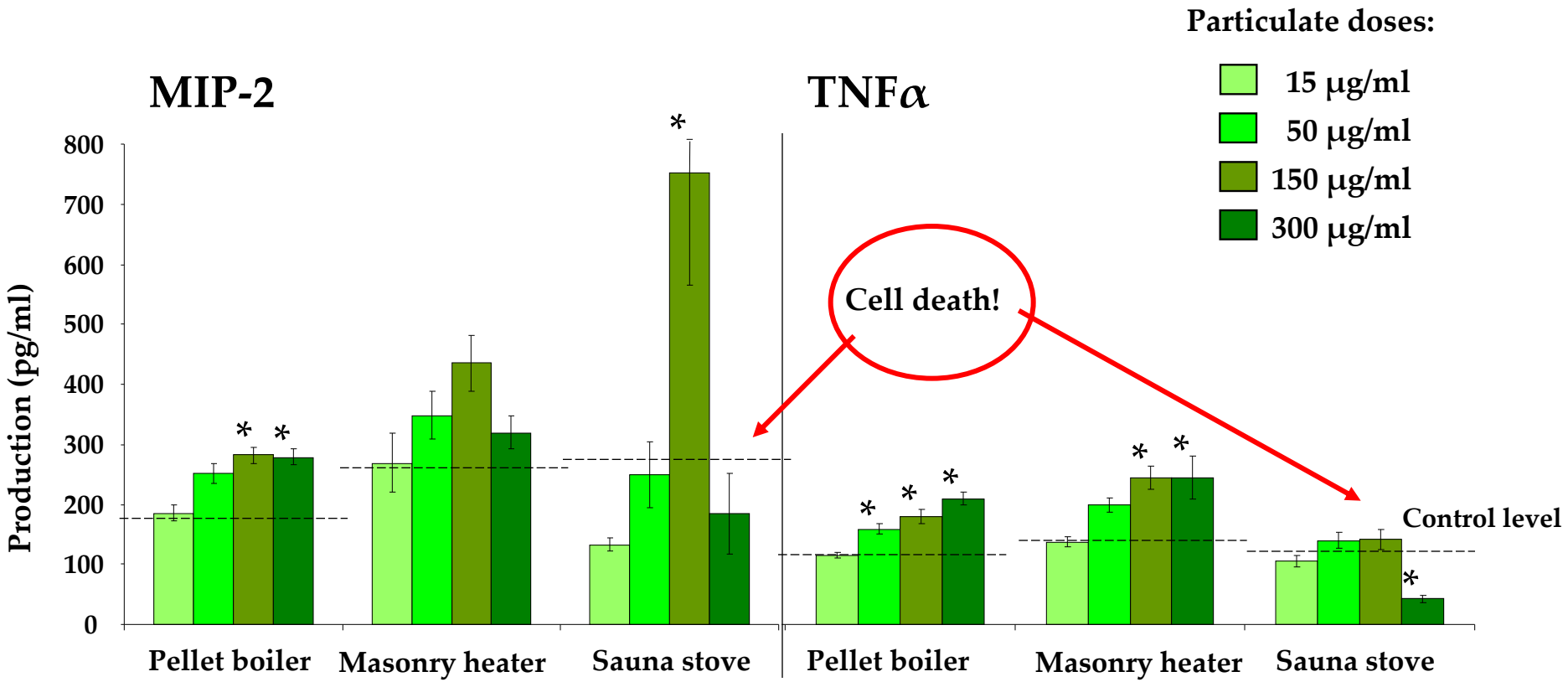
Collaboration: Professor Jokiniemi

Wood combustion particles induce weak inflammatory responses in macrophages



Tapanainen et al, submitted

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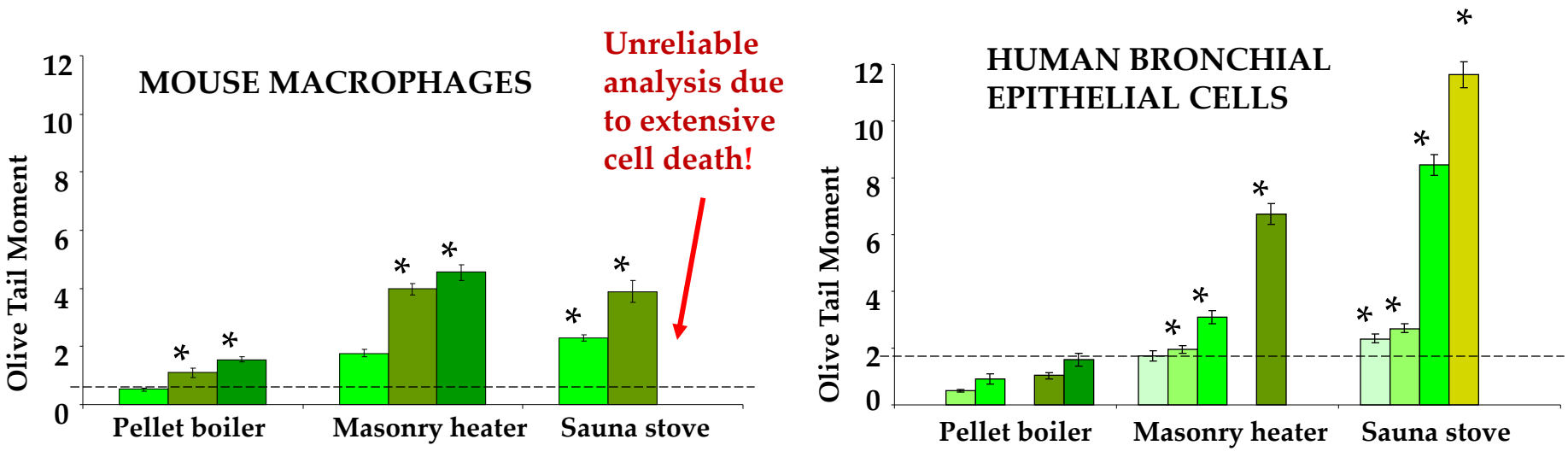
Collaboration: Professor Jokiniemi

Genotoxicity and chemical content of the combustion particles



* Statistical significant difference compared to control

	PAH content (ng/mg) Total	Genotoxic*	PM_1(mg/MJ)	OC (mg/MJ)
Pellet boiler	6	3	16	1
Masonry heater	19.000	10.000	52	11
Sauna Stove	83.000	34.000	260	165

Particulate doses:

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

Tapanainen et al, submitted

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

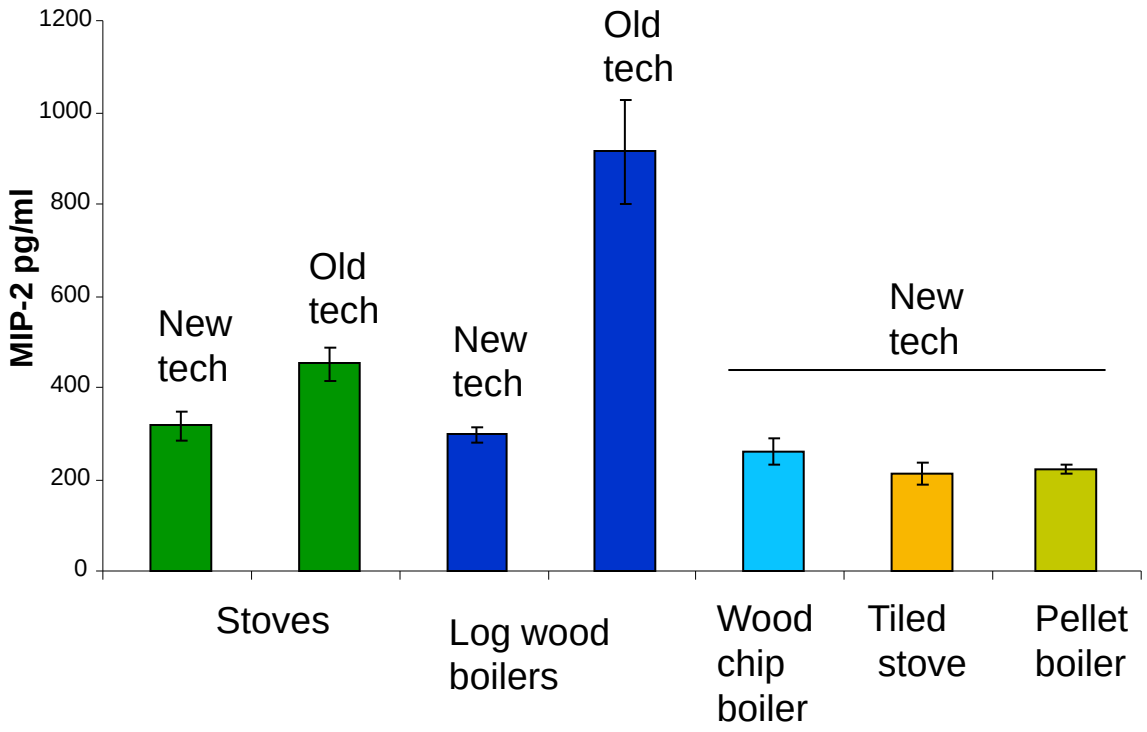
1. stove
2. log wood boiler
3. tiled stove
4. pellet boiler
5. wood chip boiler

Old technology

1. stove
2. log wood boiler

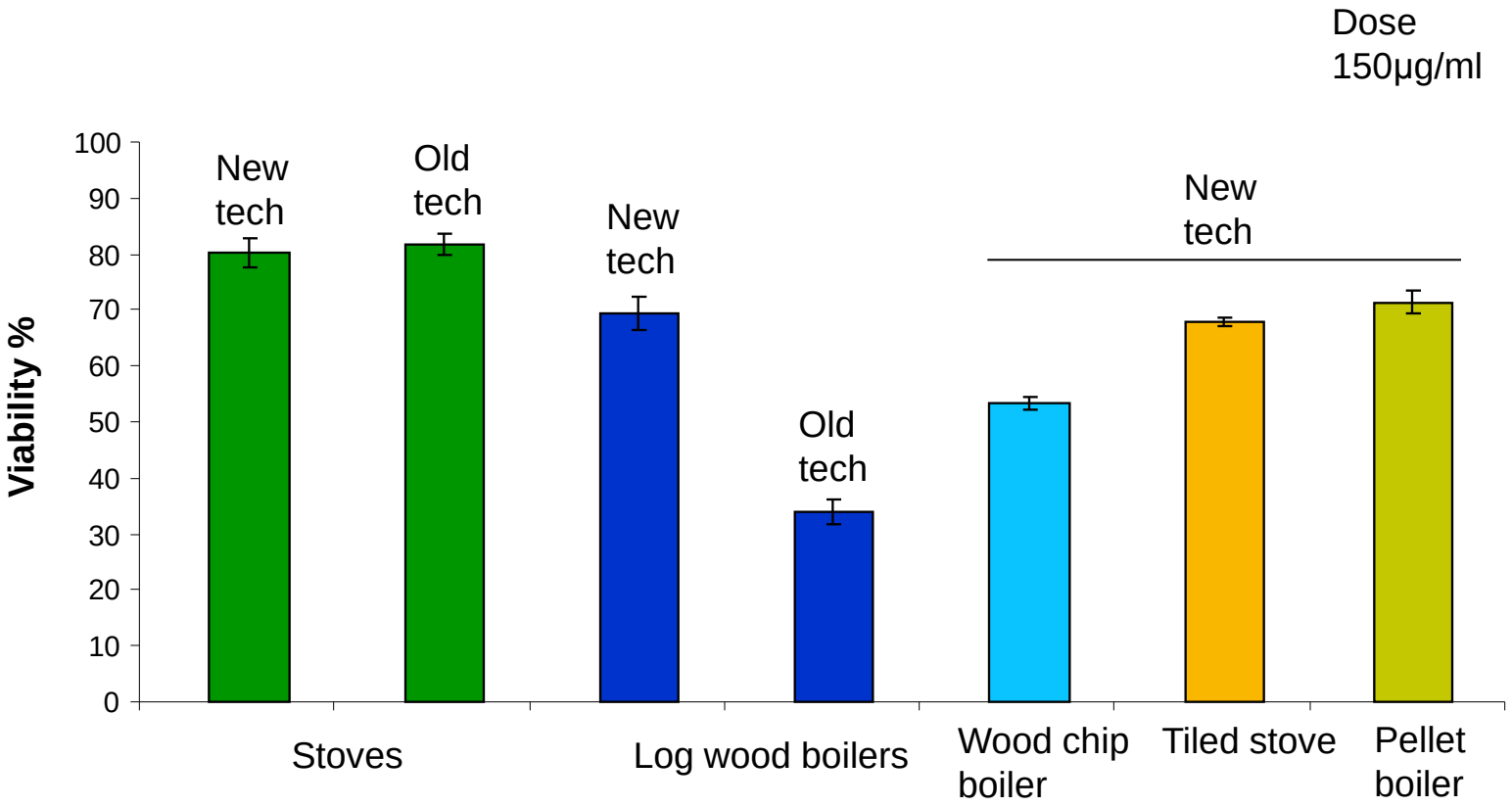
Inflammatory responses

Dose 150µg/ml



Unpublished data

Cytotoxicity (MTT test)



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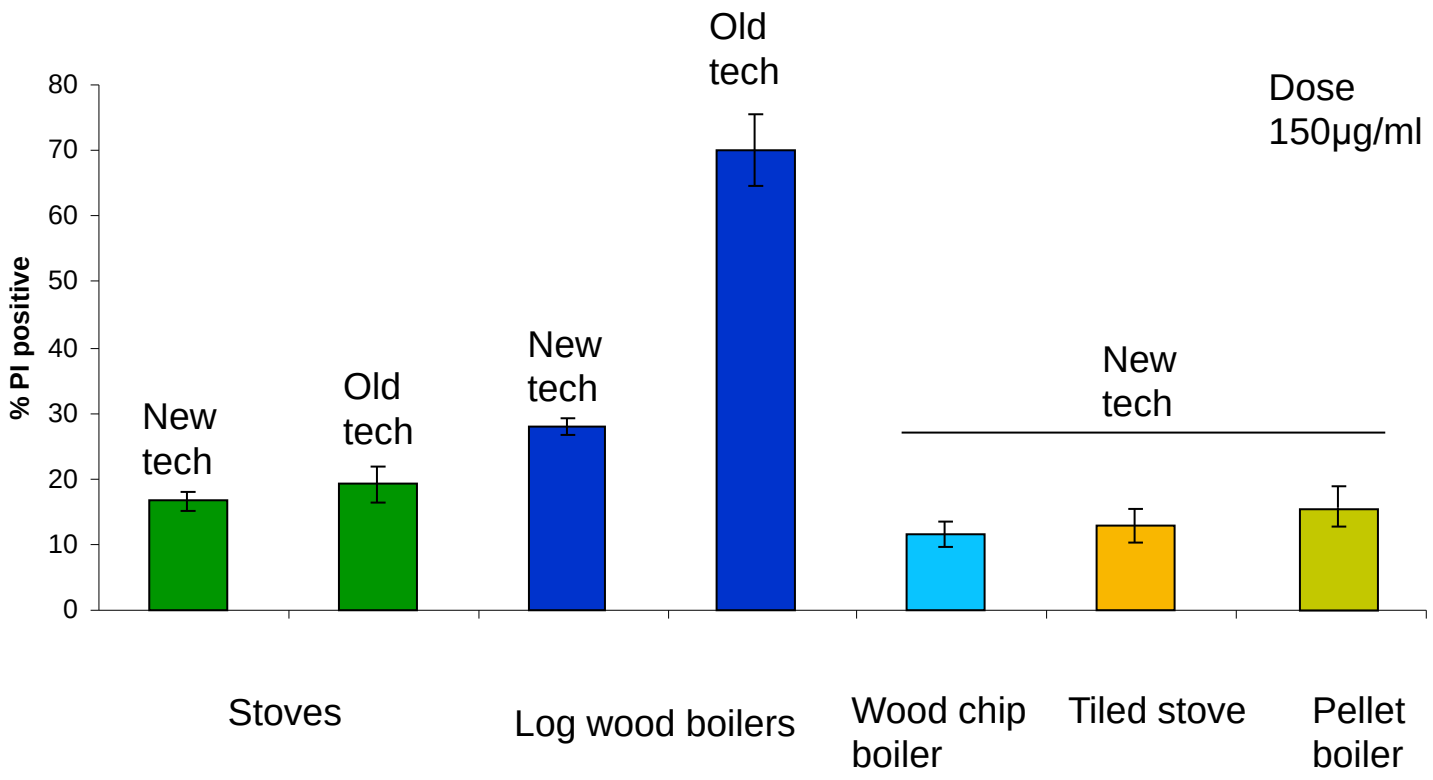
Cytotoxicity /Cell membrane permeability



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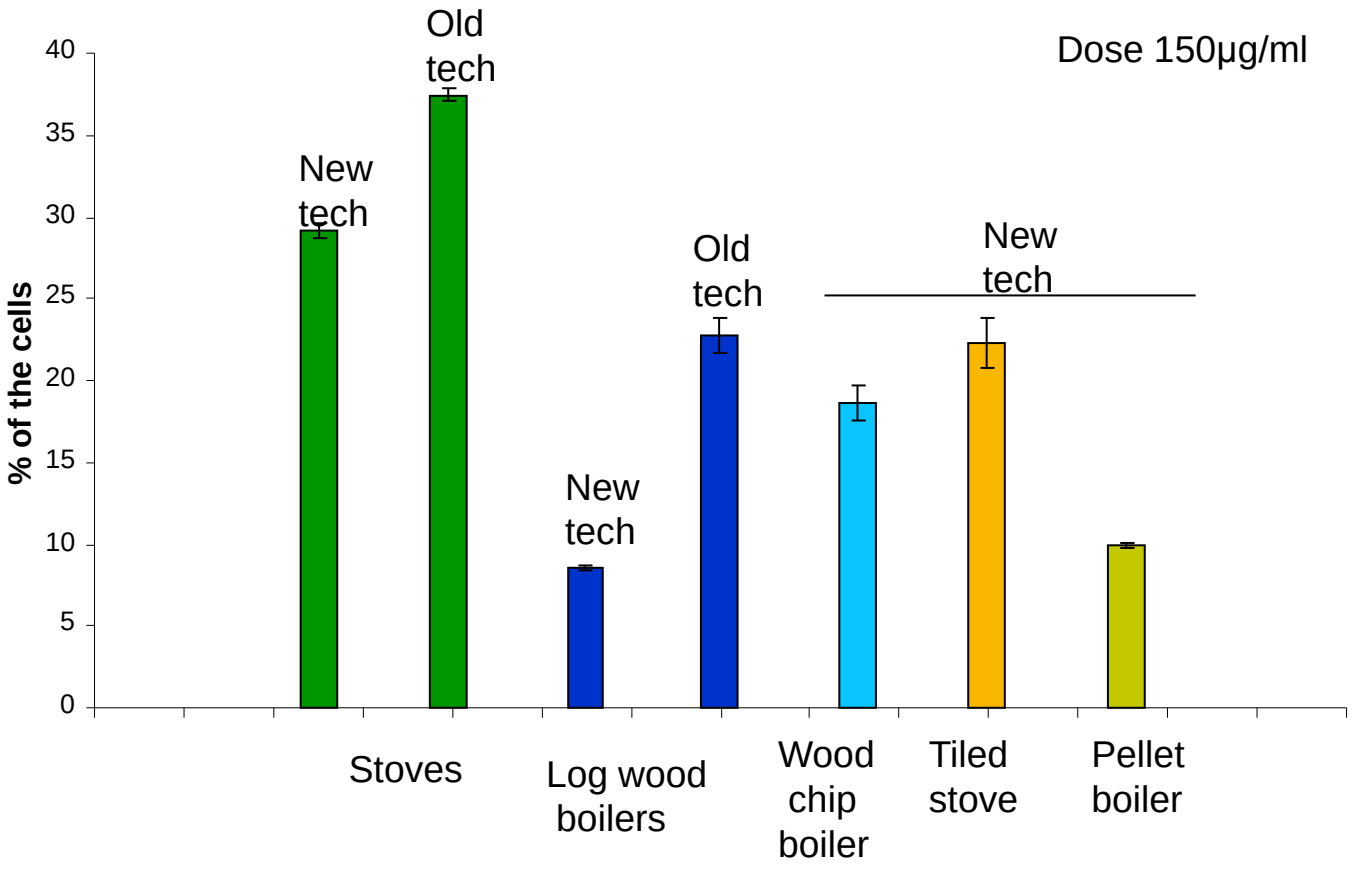
Programmed cell death



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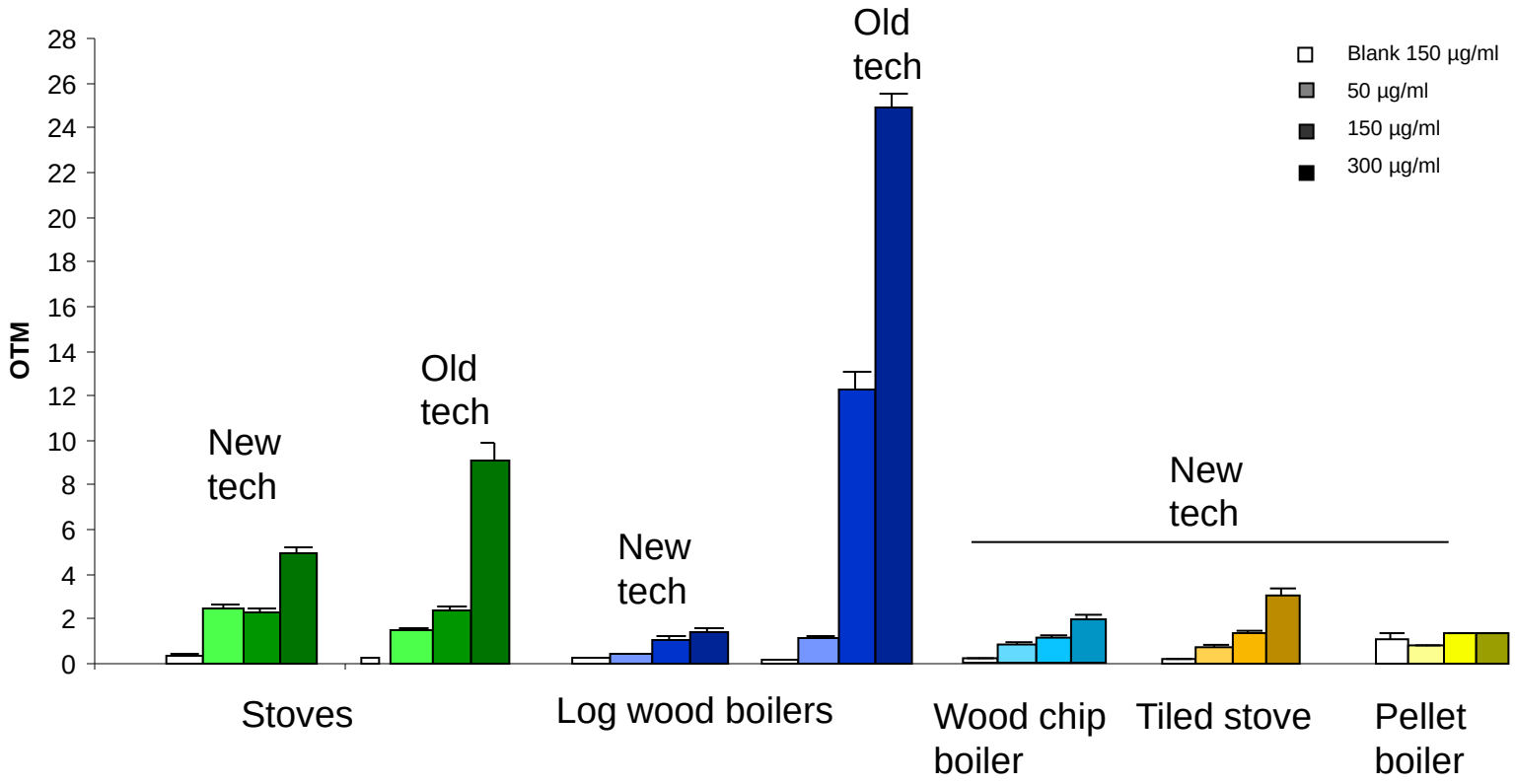
Genotoxicity /Comet assay



Effect of chemical composition on toxicological responses

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

The PAH composition was in a key role in activated toxicological responses

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

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
- slighly increased Inflammatory responses

New technology stove

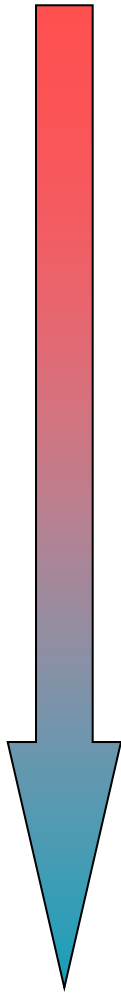
- Same activated cellular mechanisms as by the PM emissions from old tech stove but response level is lower

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!

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Acknowlegements

- Graz University of Technology, Austria
  - **Prof. Obernberger** and research group
- University of Eeastern Finland, Finland
  - **Prof. Jokiniemi**, and research group