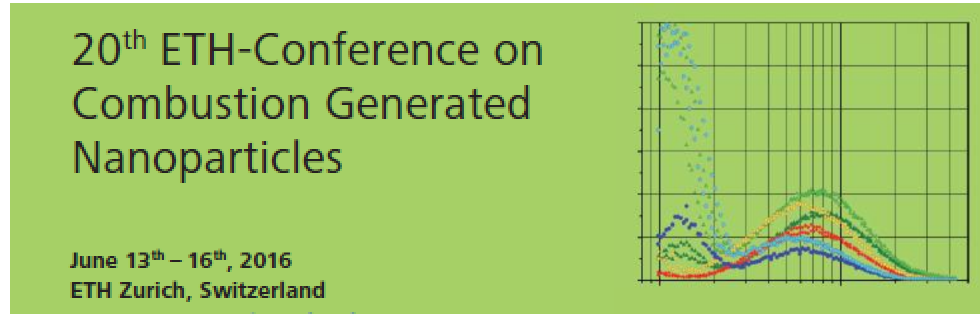

Session 6B: Health effects

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

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OVERVIEW

- Background and motivation
- Materials and methods
  - Sampling
  - Analytical determinations
  - Biological models
- Results
  - Combustion tests
  - Biological effects
- Conclusions

Background & motivation

- 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

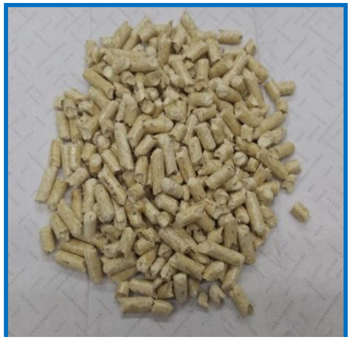
Here presented:

- **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

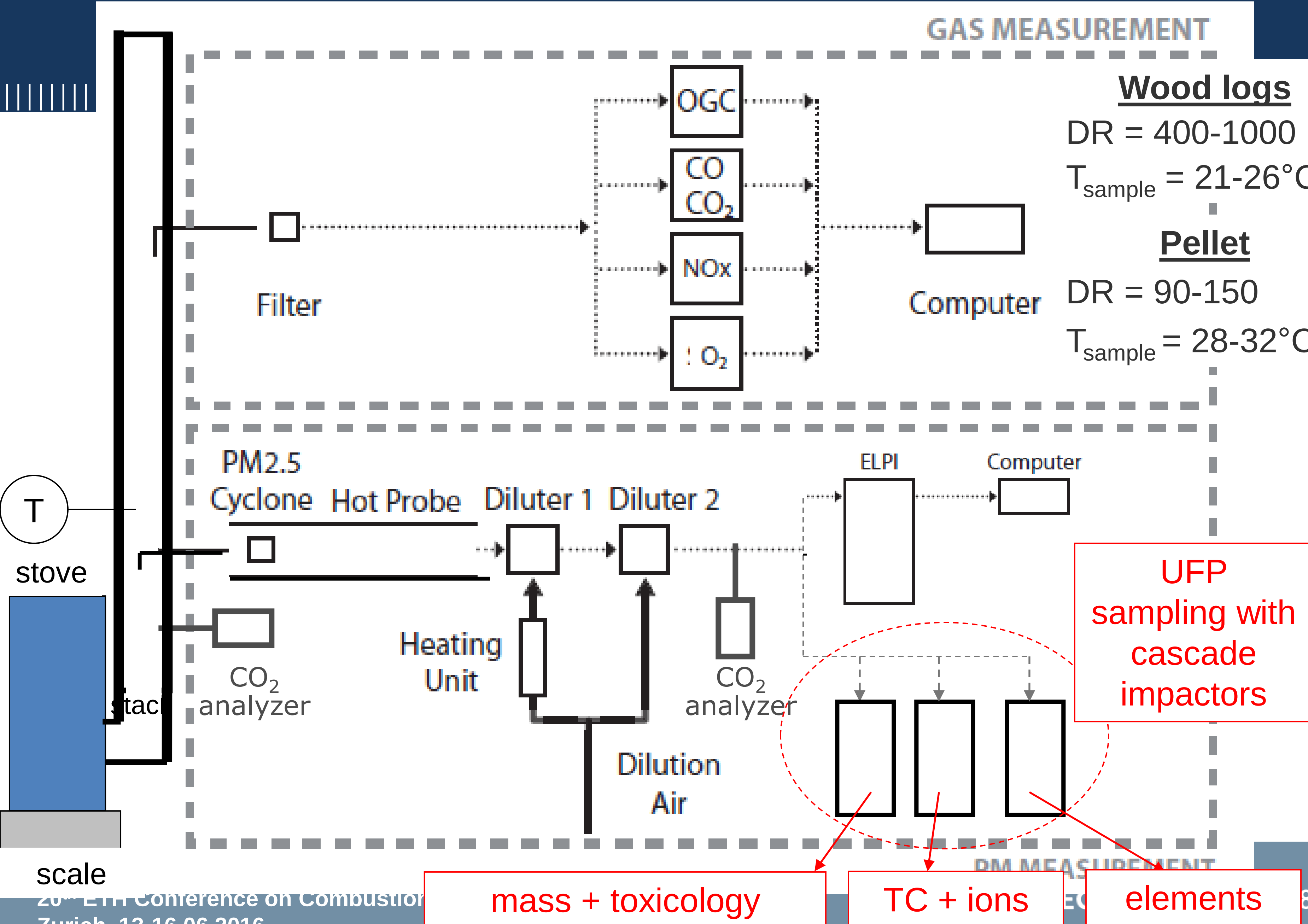
Stove technical data:

- 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

Fir
(softwood)

Beech
(hardwood)

EXPERIMENTAL SET UP FOR UFP STACK SAMPLING



Wood logs

DR = 400-1000

T_{sample} = 21-26°C

Pellet

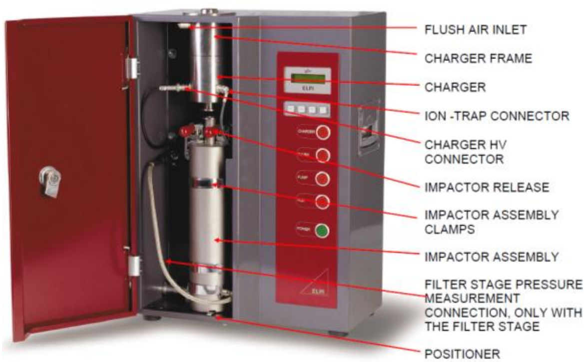
DR = 90-150

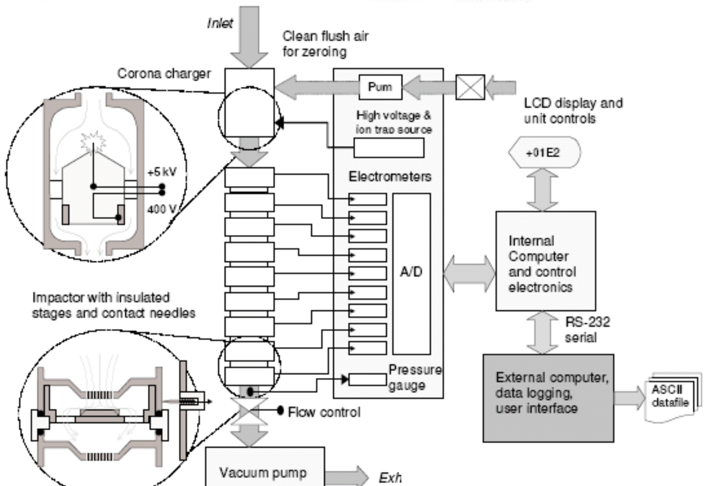
T_{sample} = 28-32°C

EXPERIMENTAL SET UP FOR UFP STACK SAMPLING

Size distribution

Electrostatic Low-Pressure Impactor
Dekati Ltd





Size range: 7 nm – 10 µm

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

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

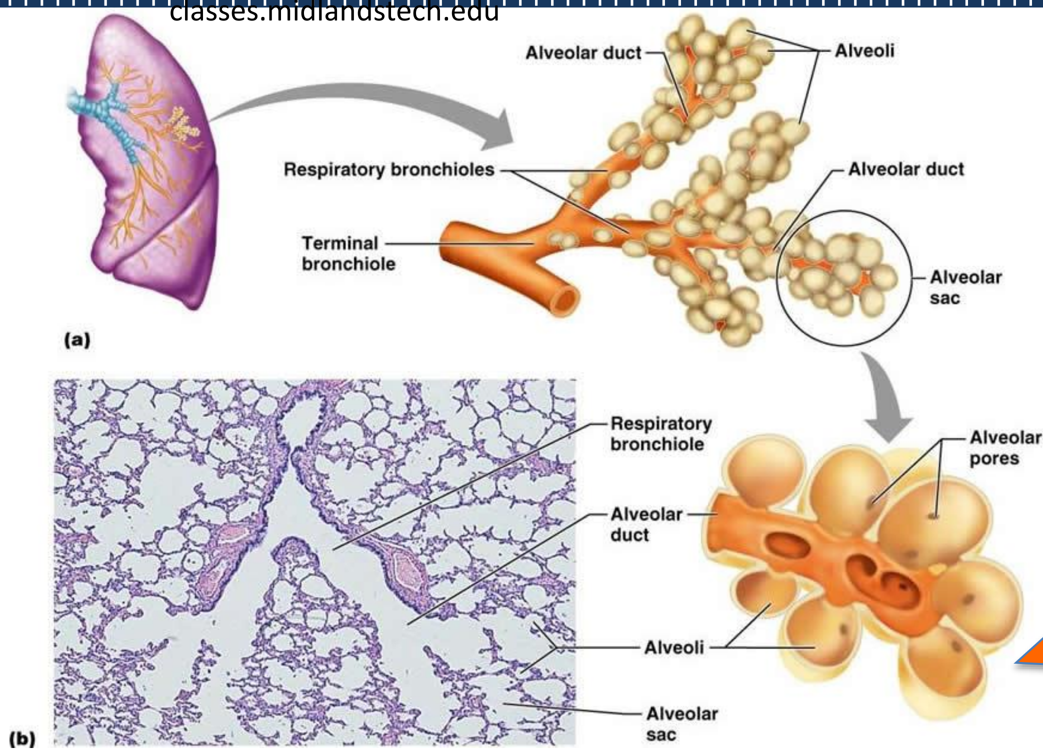
ICP-AES(4) on polycarbonate substrates → elements (Al ,As, Ba, Cd, Co, Cu, Fe, Mn, Mo, Ni, P, Pb, Sr, Ti, V, Zn)

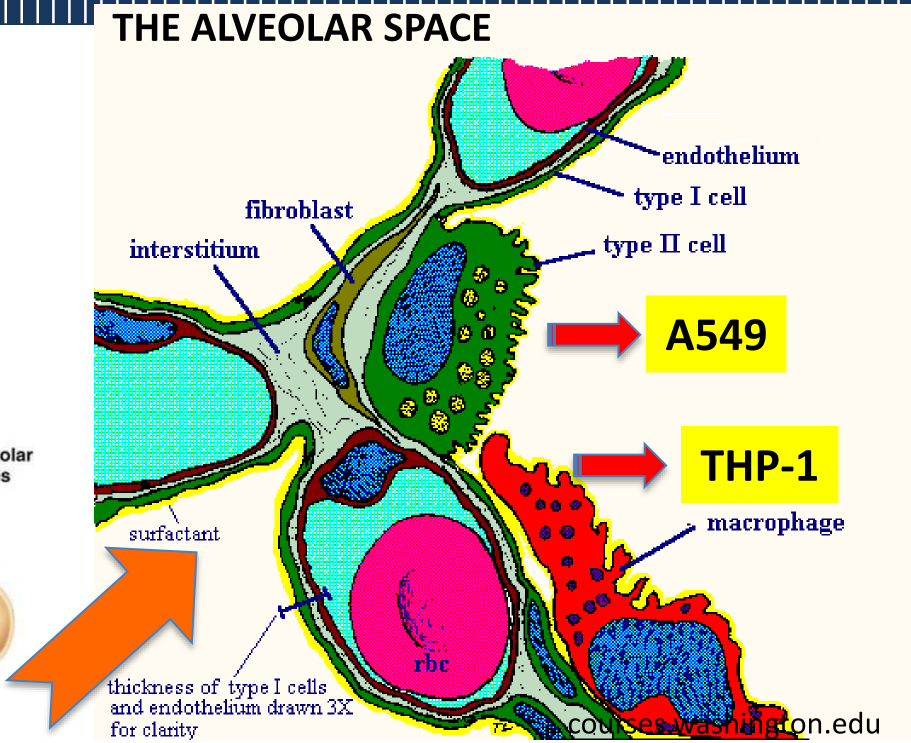
GC-MS(5) on quartz fiber filters → PAHs (Anthracene, Benzanthracene, Benzo[a]pyrene, Benzofluoranthene, Fluoranthene, Chrysene, Phenanthrene, Pyrene)

(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)

Materials and methods
BIOLOGICAL MODELS

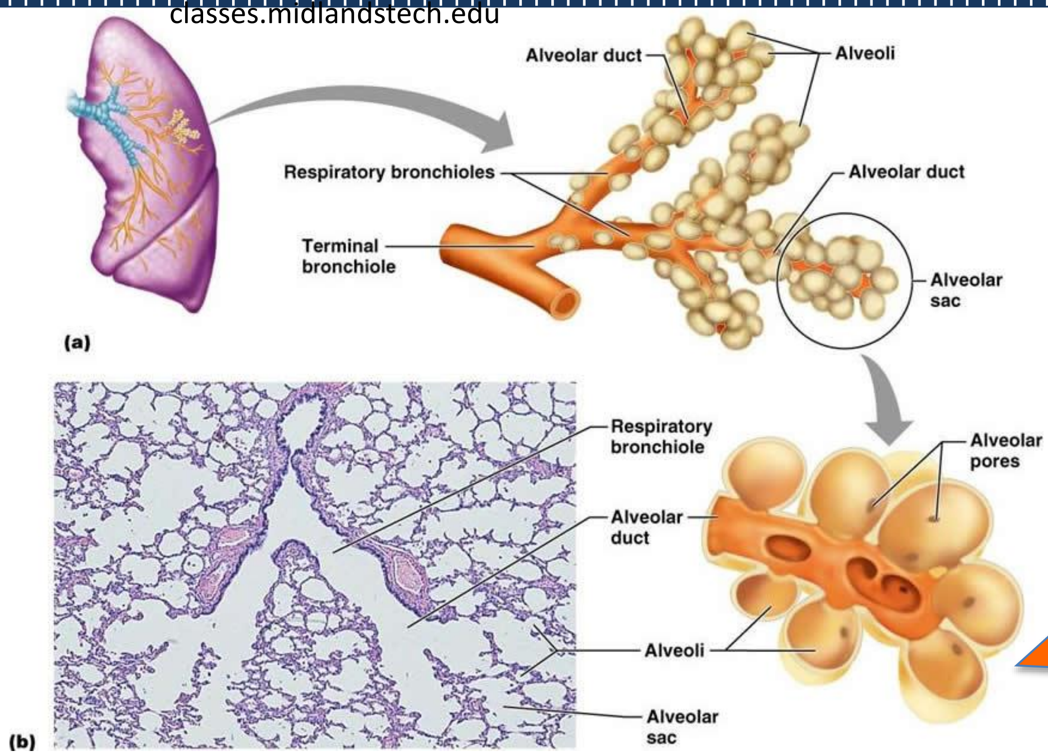


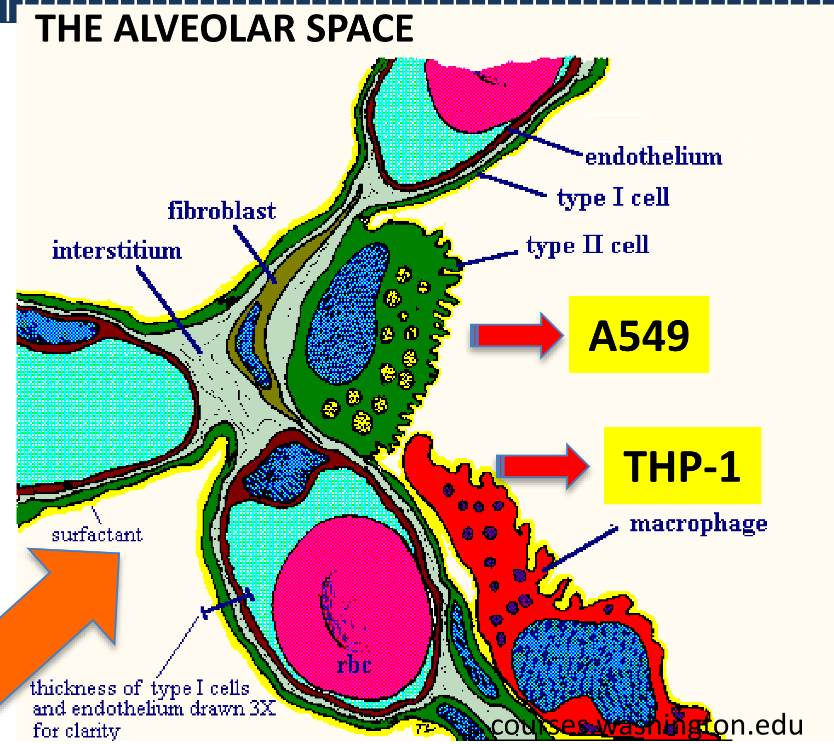


Experimental models:

- 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:

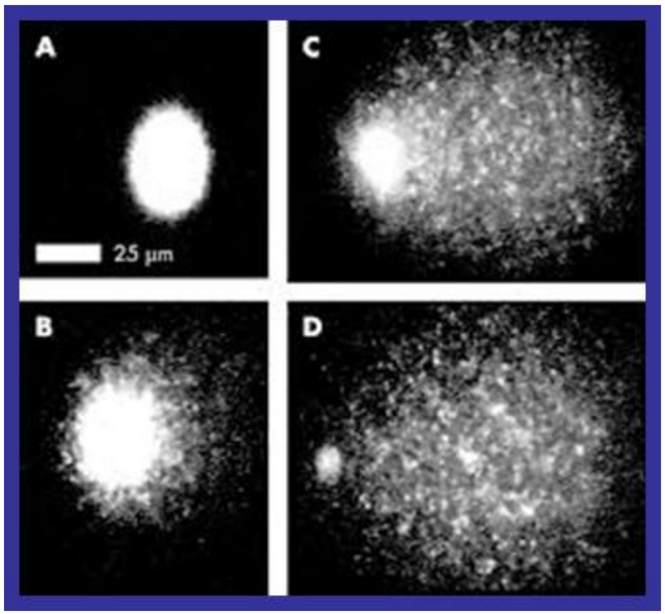
- Cell viability: MTT test and lactate dehydrogenase leakage
- **Inflammatory marker**: **interleukin-8** (IL-8) release by ELISA
- Cellular uptake**:** FACS analysis
- **Genotoxicity**: **Comet assay, ɣH2AX**

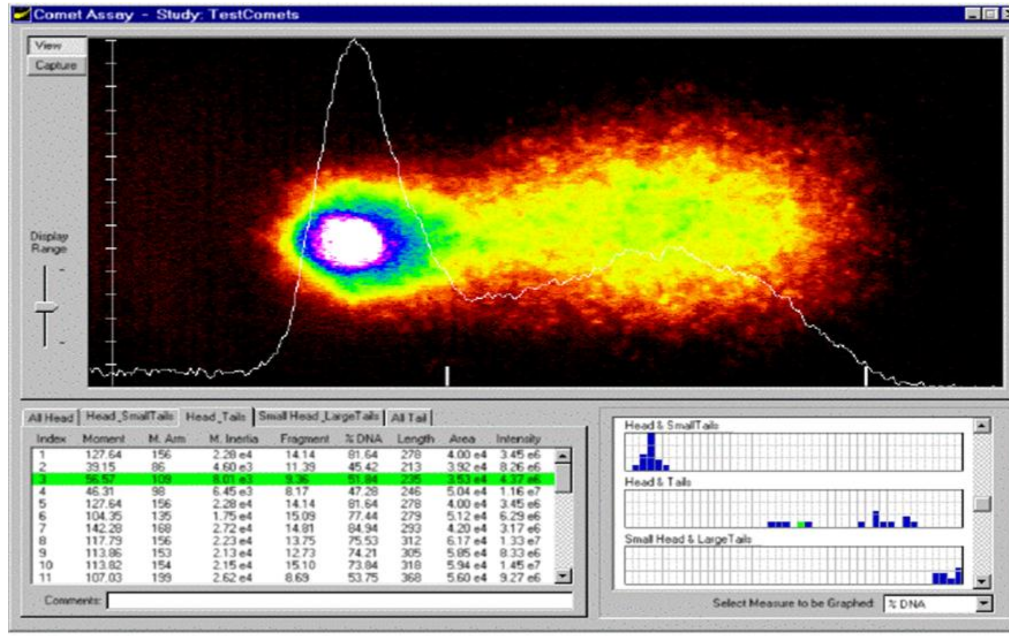
Corsini et al., in preparation

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:

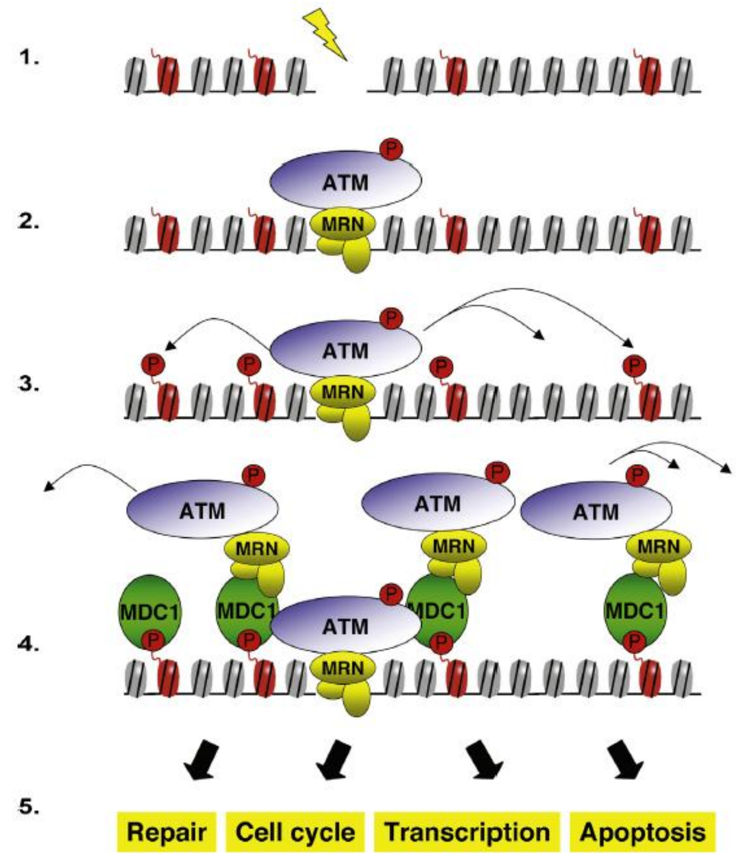
- tail length (µm)
- % DNA
- tail moment (µm)

Materials and methods
BIOLOGICAL MODELS

ɣH2AX 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



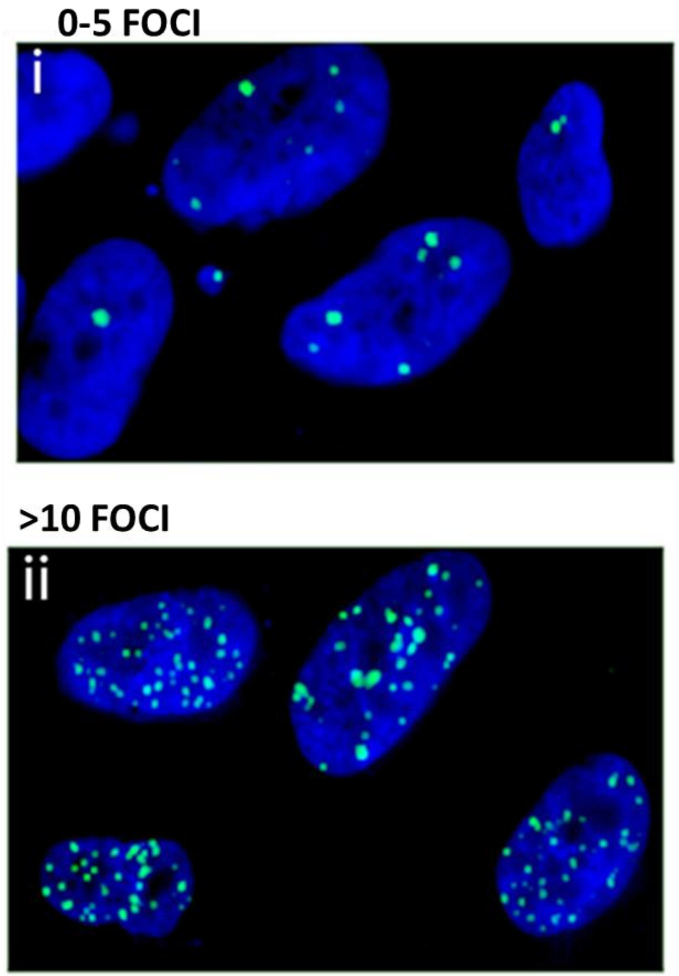
from Valdiglesias et al., Mutation Research (2013)

Materials and methods
BIOLOGICAL MODELS

<u>ɣH2AX assay:</u>

> ➢ **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

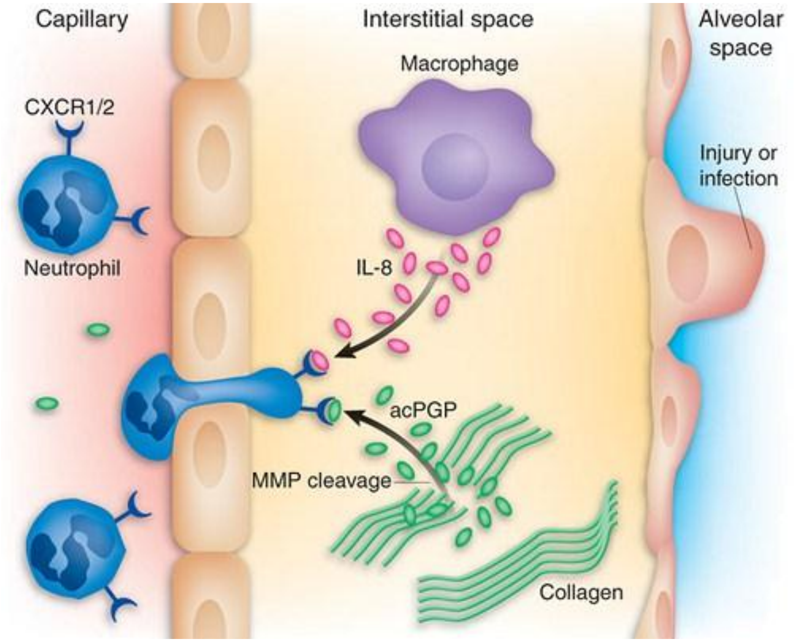
➢ **Localized inflammation**

- 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

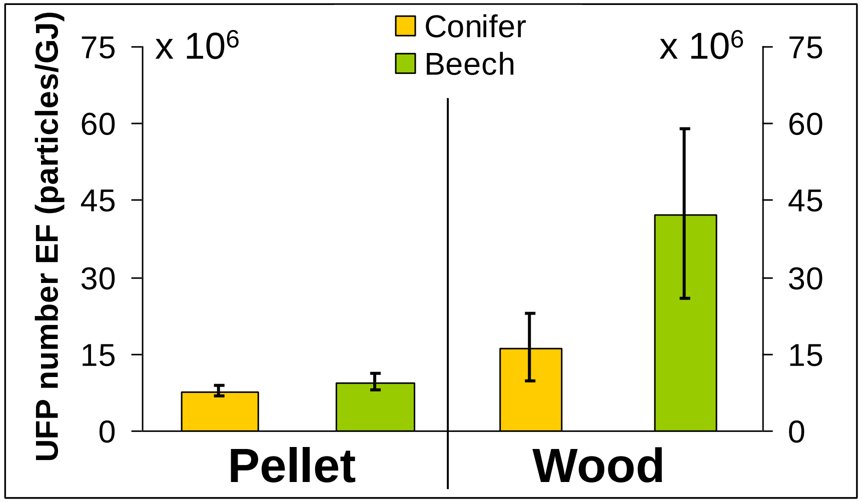
- IL-8 induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection.

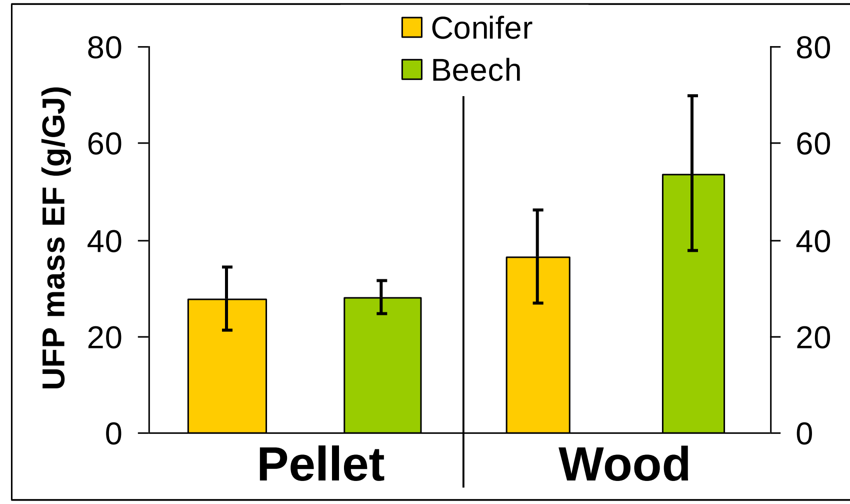
- IL-8 also induces phagocytosis once they have arrived



Results

UFP EMISSION FACTORS

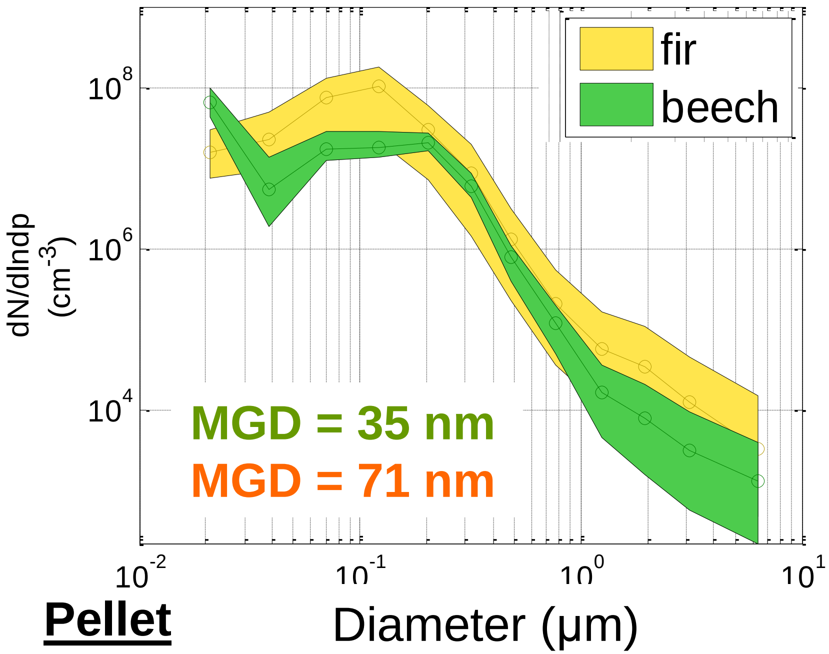




- ➢Lower UFP emissions from pellet (both number and mass)
- ➢Emission insensitive to the pellet kind
- ➢Highest emissions from beech wood

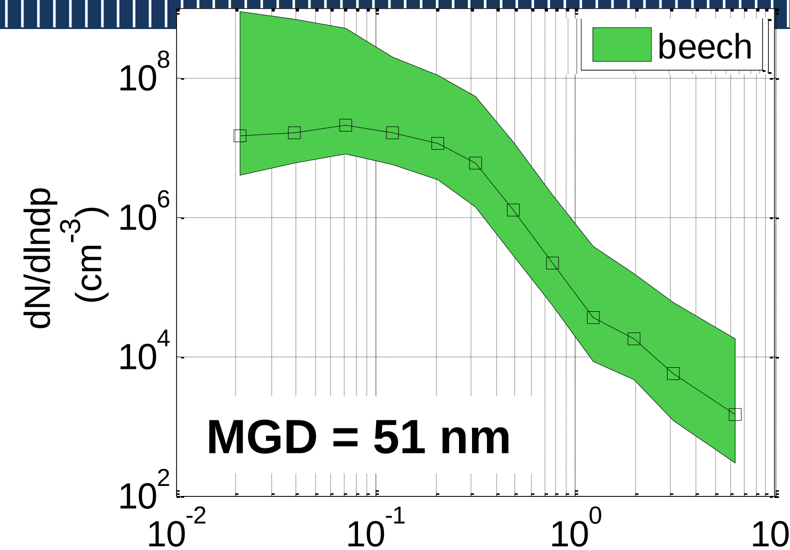
	Pellet	Wood logs
Stack concentrations: (@NTP,13% O_2)	12 - 56 mg/m^3	32 - 107 mg/m^3
	5 - 16 · 10^7 #/cm^3	23 - 60 · 10^7 #/cm^3

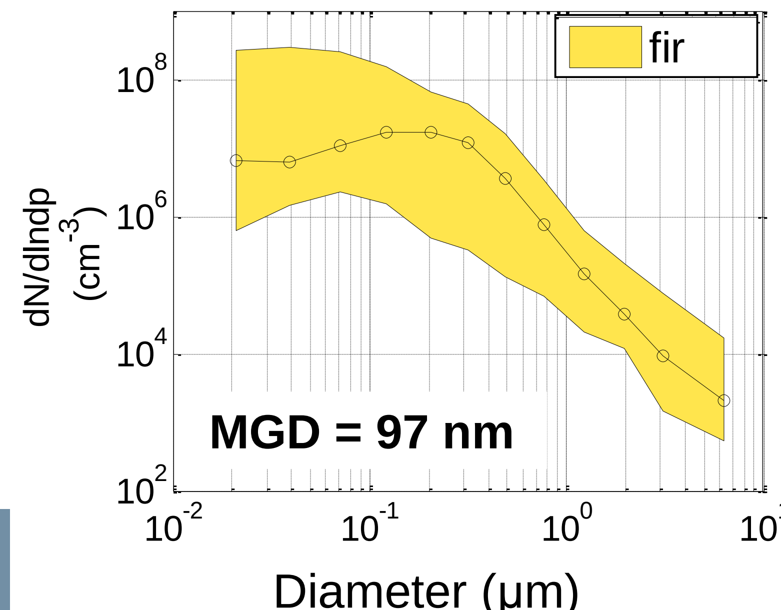
Results
PARTICLE SIZE DISTRIBUTION



Pellet

Wood





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

Results
BIOLOGICAL EFFECTS: GENOTOXICITY

Summary table for genotoxicity tests on A549 cells:

Assay	Pellet		Wood log		DEP
	Conifer	Beech	Conifer	Beech	
Comet (tail length)	* ↑	*** ↑↑	*** ↑↑	** ↑↑	** ↑↑
ɣH2AX (5-10% foci)	↑	** ↑	** ↑	** ↑	*** ↑↑

*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

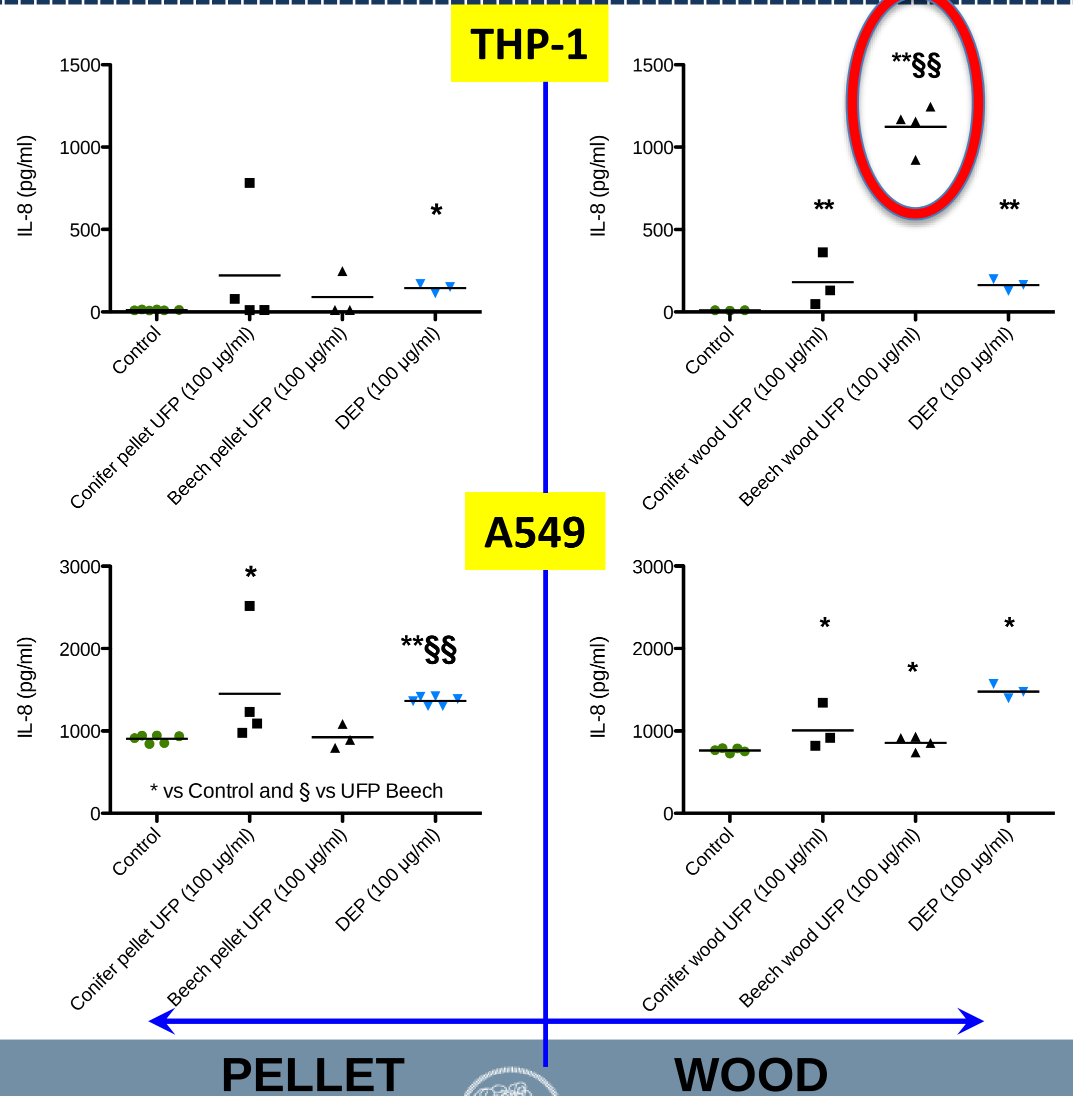
- Comet assays show strong potential for DNA damage (SSB damage)
- UFP from wood log combustion displays stronger effect than pellet
- Similar effects compared to DEP (Diesel Exhaust Particle) control

Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release:

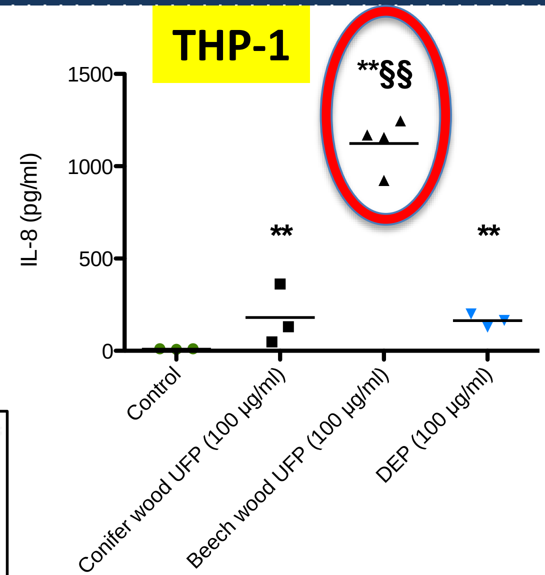
- Pellet UFP less powerful than wood log UFP in both cell lines and for both type of essence

- UFP from beech wood logs more effective on IL-8 release in THP-1 cells

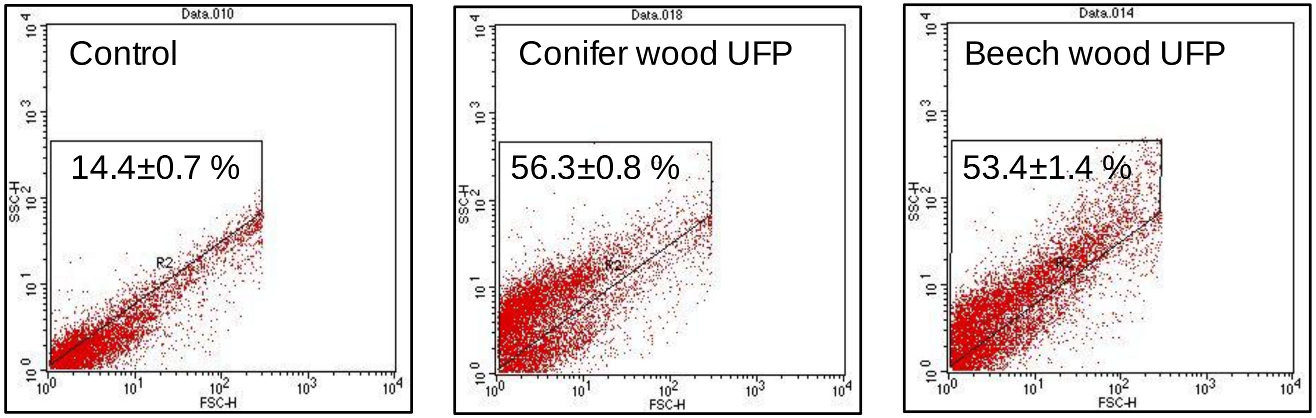


Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood logs combustion



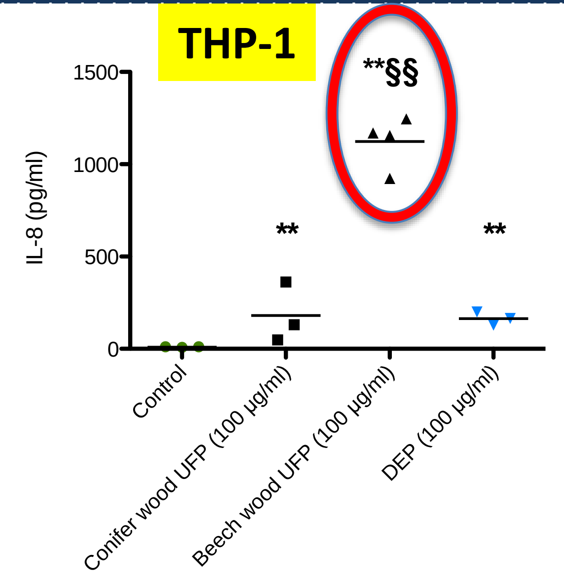
- Different cellullar uptake?



NO, based on FACS analyses

Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood logs combustion



- Different sensitivity to UFP components?

Compounds	Unit	Beech pellets	Conifer pellets	Beech logwood	Conifer logwood
Levoglucosan	%	0.005	0.006	6.548	1.470
Mannosan	%	0.003	0.005	0.451	0.487
Galactosan	%	-	0.001	0.233	0.076
Al	%	0.212	0.255	0.033	0.039
As	%	0.001	0.001	0.001	0.002
Ba	%	0.006	0.011	0.003	0.003
Cd	%	0.001	0.001	0.003	0.003
Co	%	-	0.001	0.001	0.001
Cr	%	-	-	0.041	0.044
Cu	%	0.022	0.043	0.010	0.008
Fe	%	0.216	0.256	0.083	0.088
Ni	%	0.009	0.042	0.012	0.007
Zn	%	0.240	0.493	0.186	0.279
V	%	-	-	0.001	0.001
Ti	%	0.003	0.004	0.003	0.002
Sr	%	0.002	0.002	0.004	0.004
Mo	%	0.037	0.001	0.001	0.001
Pb	%	0.032	0.020	0.006	0.010
Na+	%	0.170	0.231	0.180	0.158
NH4+	%	0.016	0.024	0.3000	0.362
K+	%	25.443	23,180	2.255	0.319
Mg++	%	0.034	0.044	0.002	0.001
NO3-	%	0.626	0.891	0.540	0.317
SO4--	%	9.188	11.920	3.585	4.033
TC	%	3.64	12.74	48.80	87.11

- PAHs? **NO**

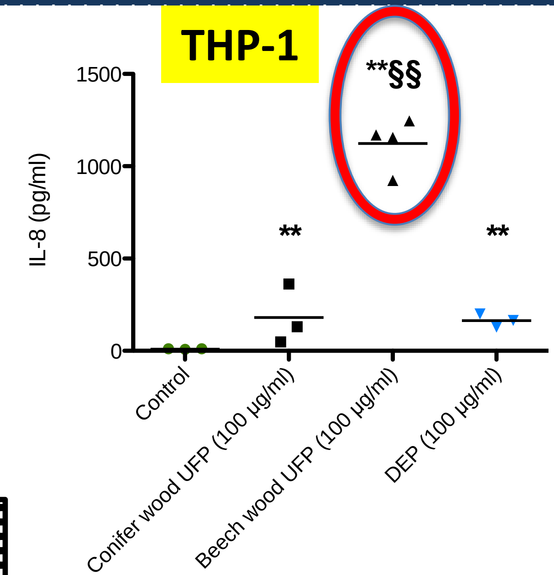
Compounds	Unit	Beech wood log	Conifer wood log
ΣPAHs	%	0.206 ± 0.183	3.591 ± 0.659

- Sugars? **YES**

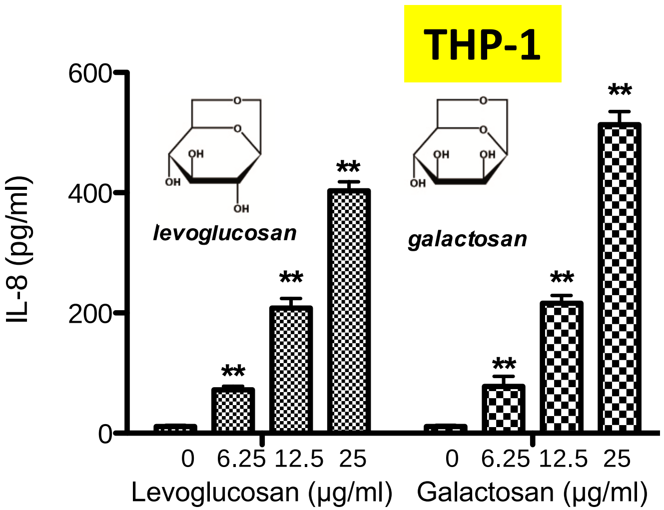
Compounds	Unit	Beech wood log	Conifer wood log
Levoglucosan	%	6.548 ± 0.005	1.470 ± 0.011
Galactosan	%	0.233 ± 0.001	0.076 ± 0.001

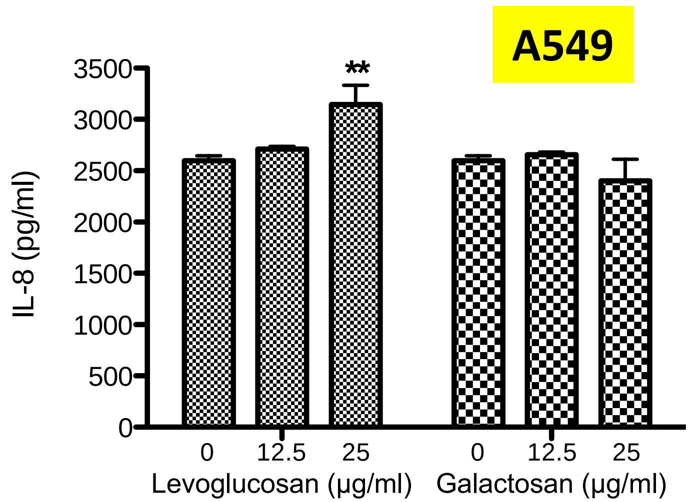
Results
BIOLOGICAL EFFECTS: INFLAMMATION

IL-8 release from beech wood combustion



- Different cell sensitivity to sugar components





- 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

fondazione cariplo for funding the TOBICUP project (Grant 2013-1040)

GRUPPO PIAZZETTA CAMINETTI & STUFE 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

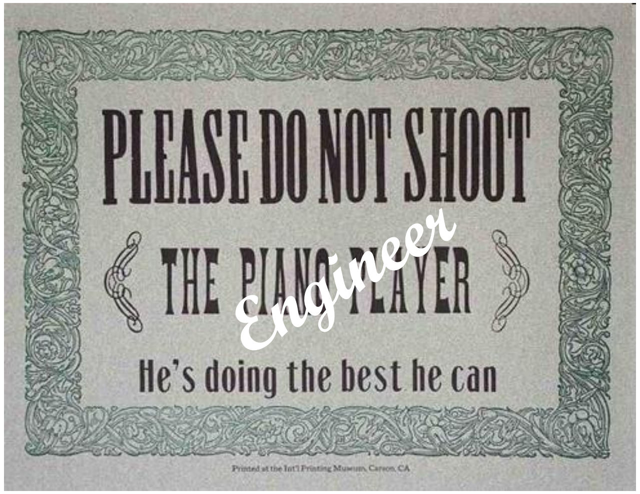
(*Dept. of Physics, Università degli Studi di Milano*),

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

S. Signorini (*Energy and Environment Laboratory, Piacenza*).

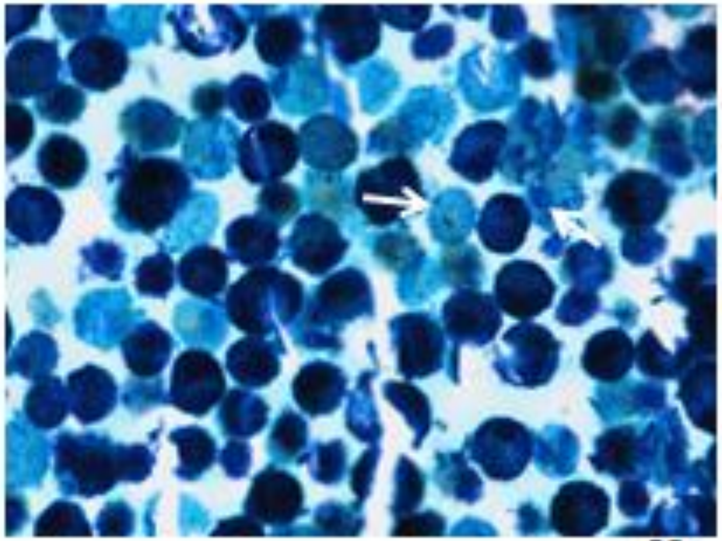
Acknowledgments

Thank you
for your attention

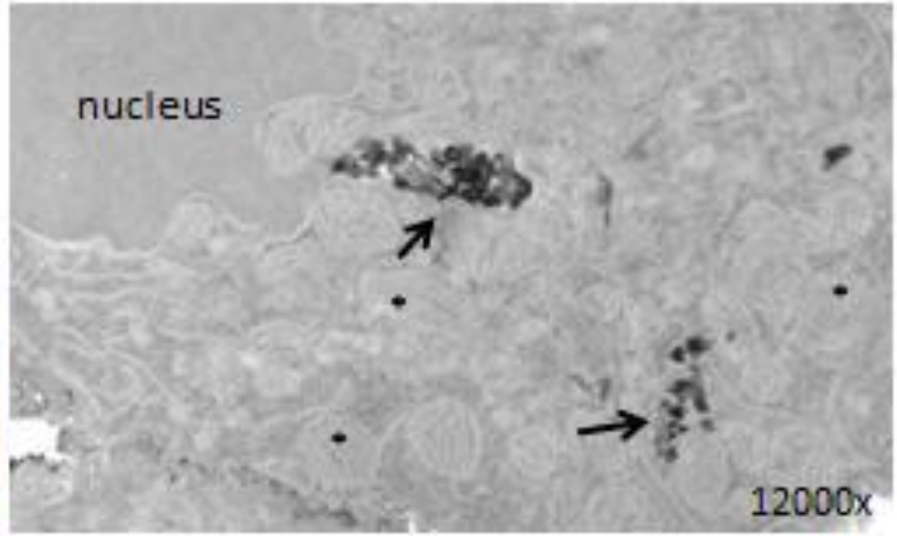


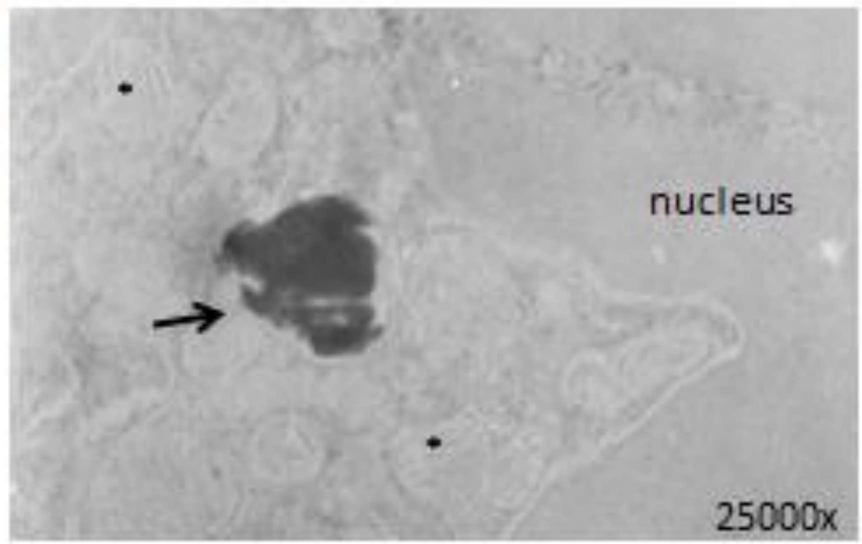
TOBICUP's Toxicology group

Beech Pellet (LT06)



60x

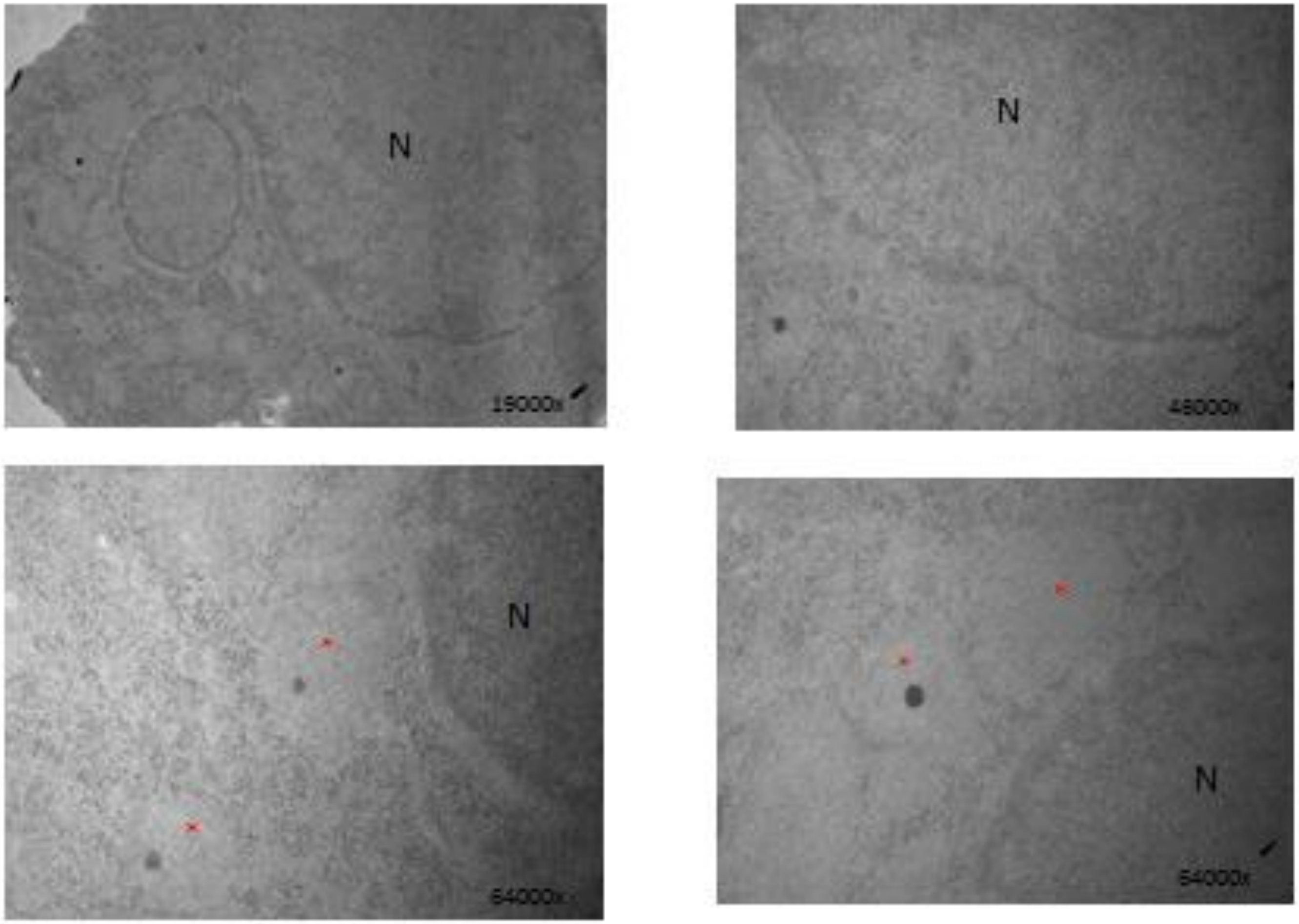




•: mithocondria
Black arrows: nanoparticles
White arrows: nanoparticles inside cells

Fir Pellet

LT01



N: Nucleo *: mitocondri

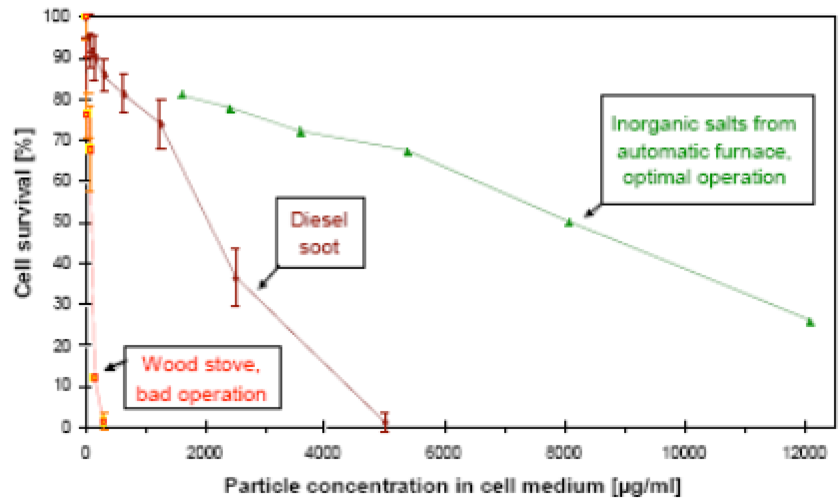
Conclusions

HEALTH RELEVANCE OF PARTICLES FROM WOOD COMBUSTION IN COMPARISON TO DIESEL SOOT

N. Klippel[1] and T. Nussbaumer[1,2]
[1]Verenum, Langmauerstrasse 109, CH-8006 Zurich (Switzerland), www.verenum.ch
[2]Hochschule für Technik + Architektur (HTA) Luzern, CH-6048 Horw, (Switzerland), www.hta.fhz.ch

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

Test biologici su tossicità cellulare e aberrazioni cromosomiche

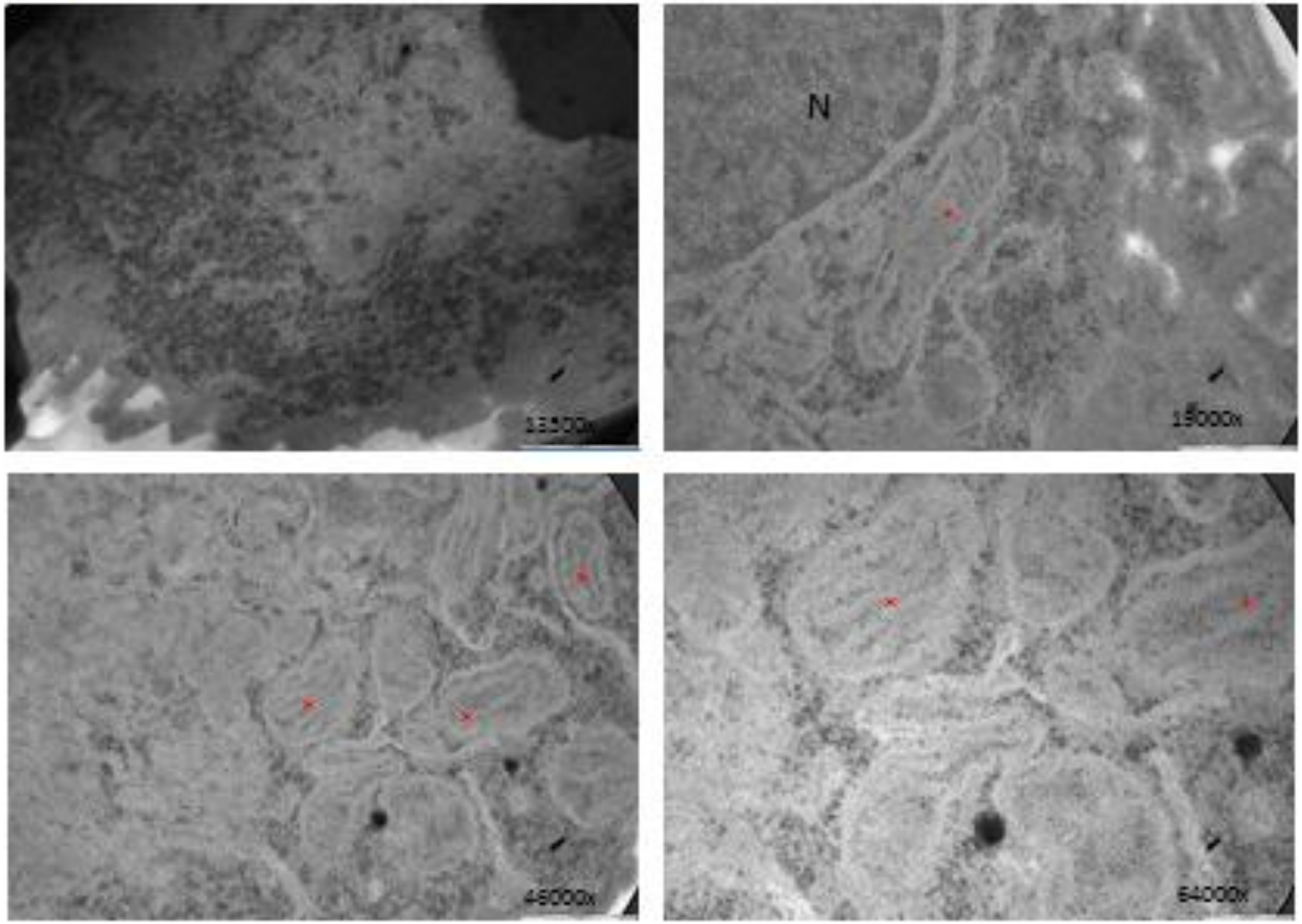


Risultati: il PM da combustione completa ha un effetto tossicologico 5 volte inferiore al PM diesel. Il PM da combustione incompleta ha una tossicità 15 volte superiore al PM da diesel ed un contenuto di IPA 20 volte superiore

POLITECNICO DI MILANO

Beech wood

LT08

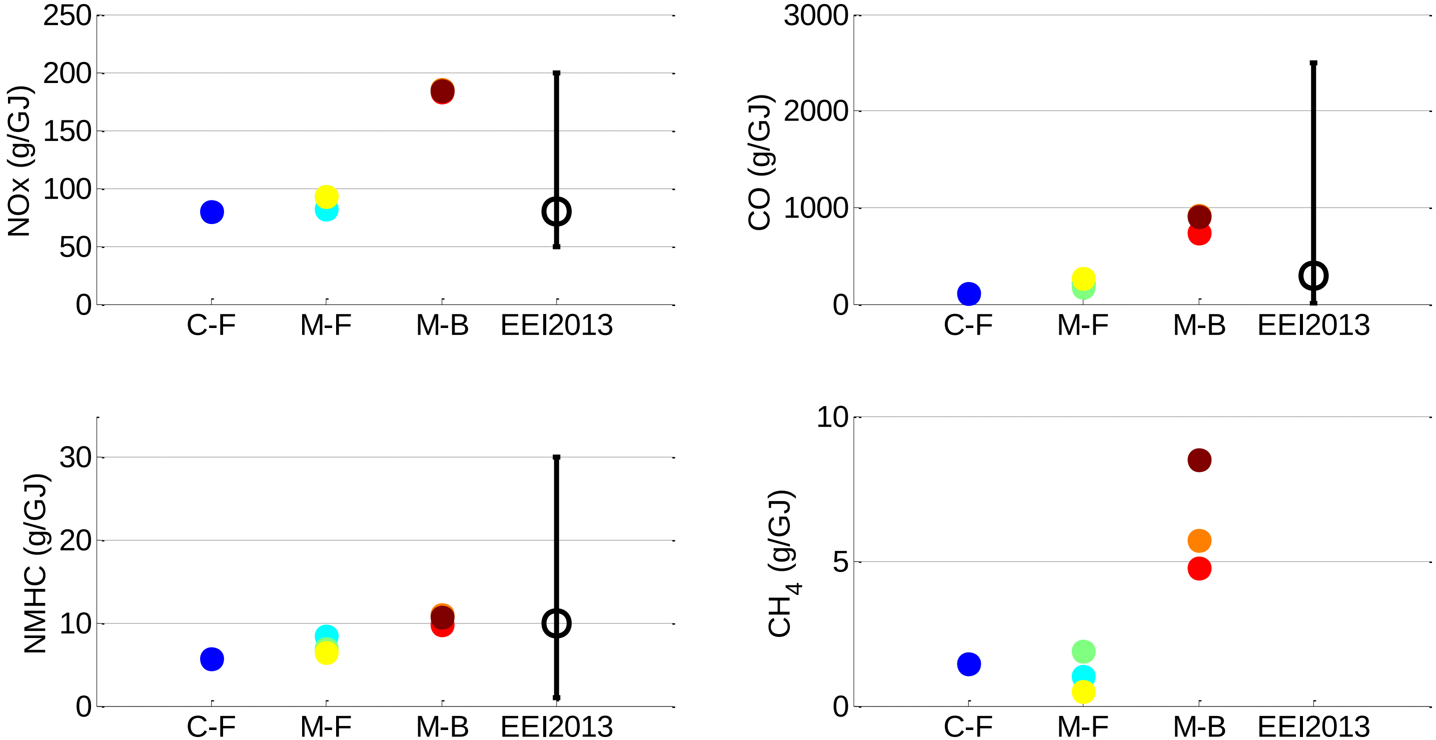


N: Nucleo *: mitocondri

EXPERIMENTAL RESULTS – gaseous pollutants

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)

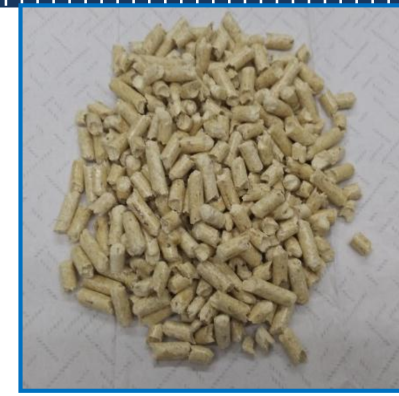
TESTED FUEL CHARACTERISTICS

Pellet

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

- tested pellets are commercially available
- fir pellets comply with EN ISO 17225-2:2014 class A1

EN ISO 17225-2:2014 - Solid biofuels - Fuel specifications and classes Part 2: Graded wood pellets (ISO 17225-2:2014)



Fir pellet (A1) (softwood)

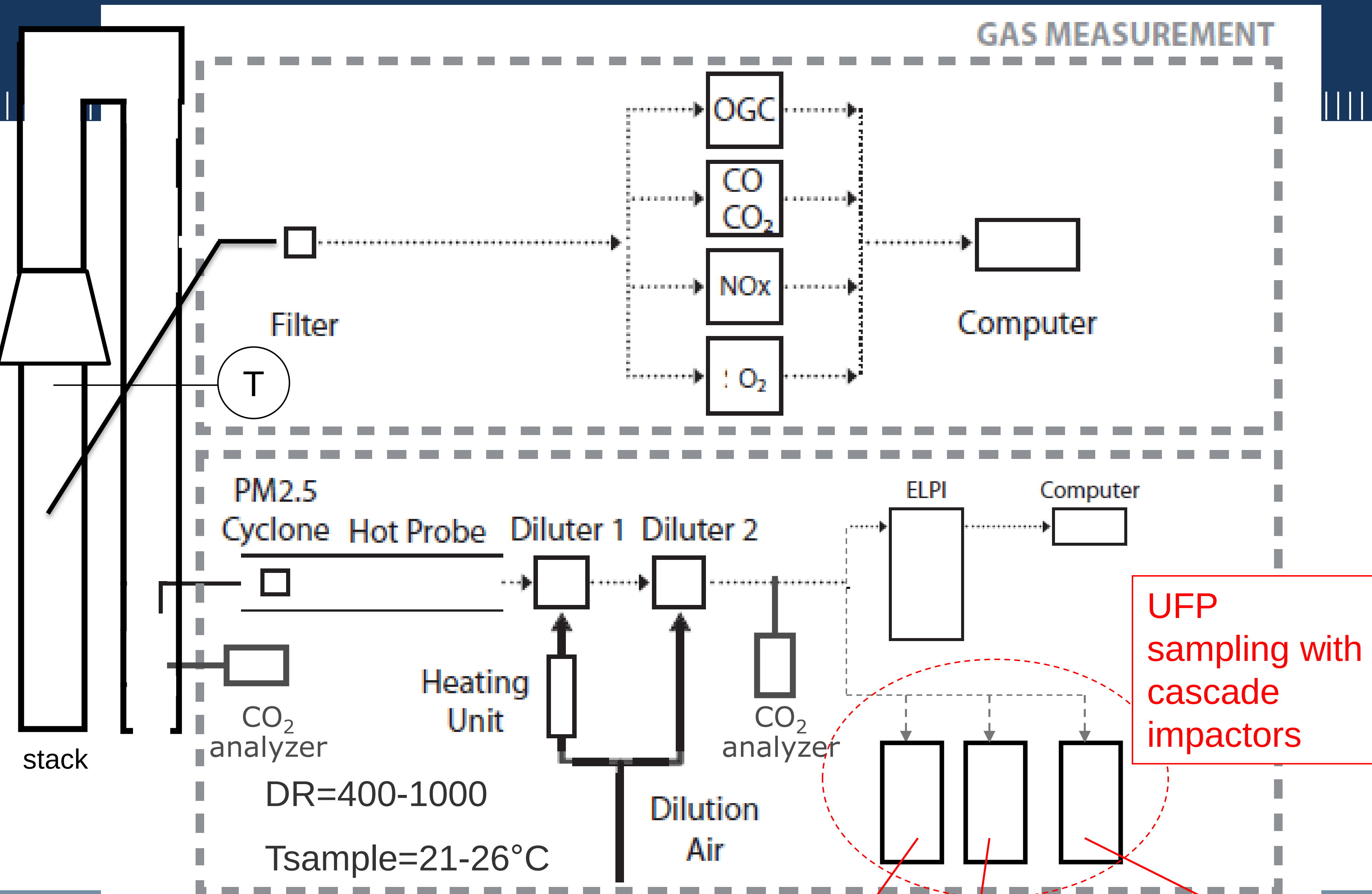
Beech pellet (hardwood)

TESTED FUEL CHARACTERISTICS

Wood log

	Fir pellets (A1)	Beech pellets
Water content (% w/w)	10.7	11.0
Ash (@ 550° C)	0.2	0.6
Carbon (% w/w)	46.4	43.6
Hydrogen (% w/w)	5.7	5.4
Nitrogen (% w/w)	0.5	0.45
Sulfur (mg/kg)	50	90
Chlorine (mg/kg)	<20	<20
HHV (MJ/kg)	18.8	17.4
LHV (MJ/kg)	17.3	16.0

EXPERIMENTAL SET UP and UFP SAMPLING – wood stove



DR: dilution ratio

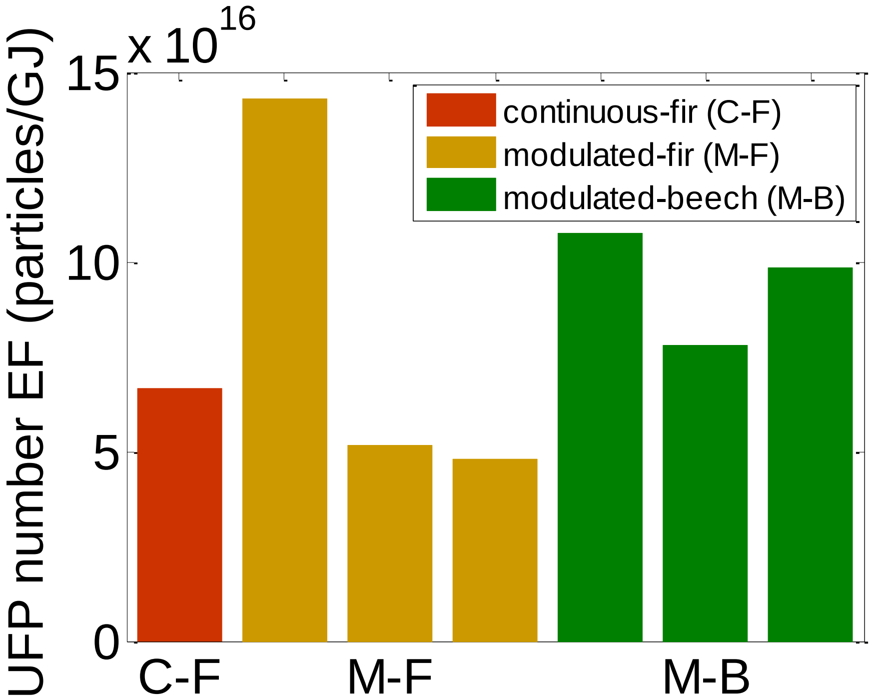
mass + toxic

TC + ions

elements

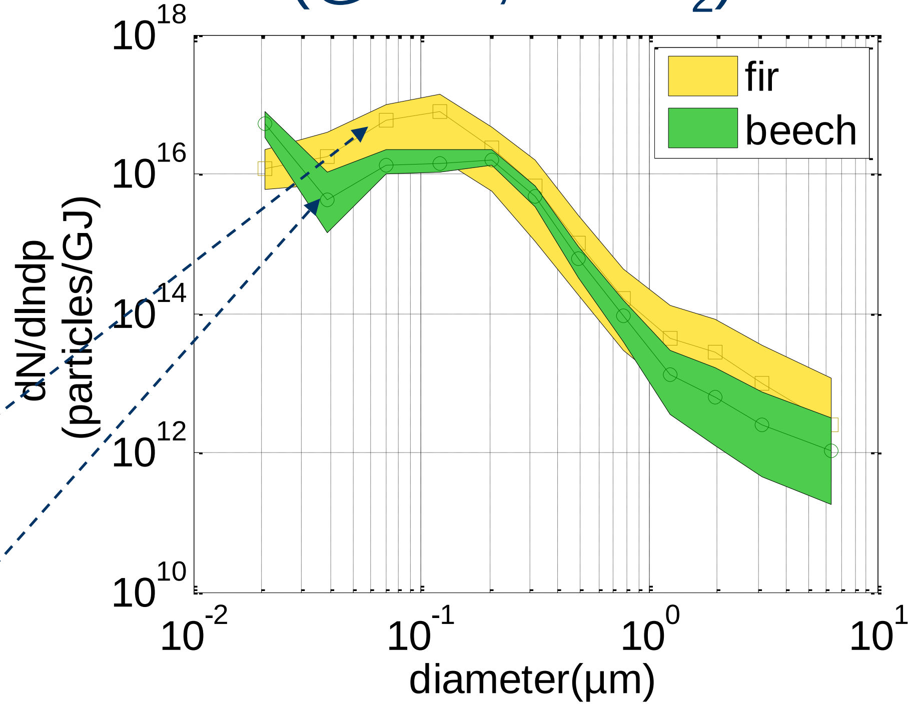
Results

PELLET STOVE – UFP NUMBER



UFP number concentration

$4.7 - 16 \cdot 10^7$ particles/cm^3 (@NTP,13%O_2)

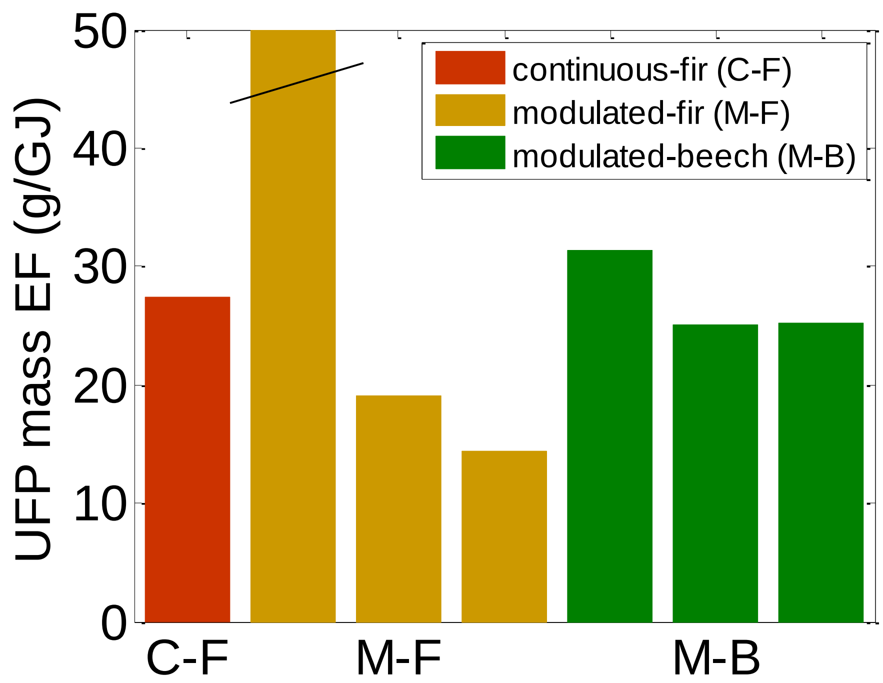


GMD_{fir} = 71 nm

GMD_{beech} = 35 nm

GMD: geometrical mean diameter

Results
PELLET STOVE – UFP MASS



UFP mass concentration

12 - 56 mg/m^3

(@NTP,13%O_2)