Exposure to sub-10nm ‘‘particles’’ emitted from combustion sources: in-vitro toxicity and inflammatory potential

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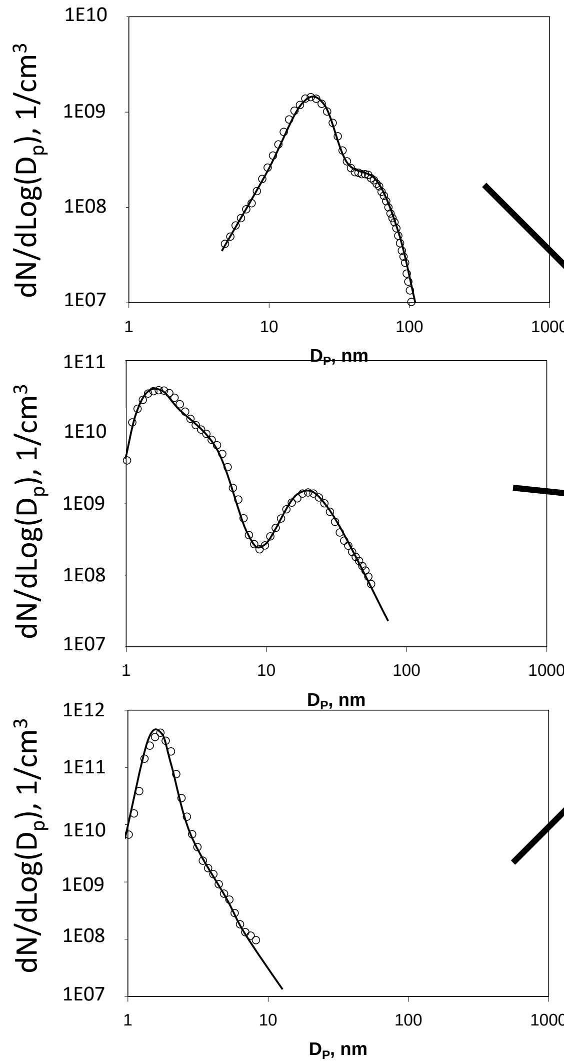
20th ETH- Conference on Combustion Generated Nanoparticles
13-16 June 2016

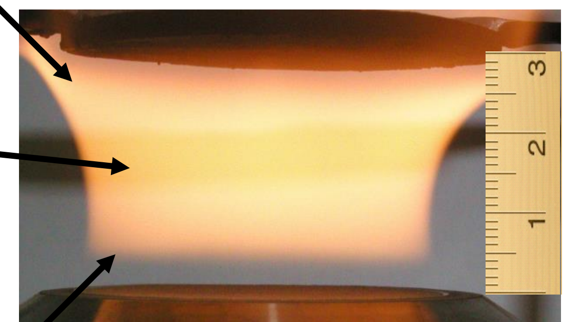
Combustion Generated Nanoparticles

premixed flames (ethylene/air - 1 bar)

DMA measurements

residence time





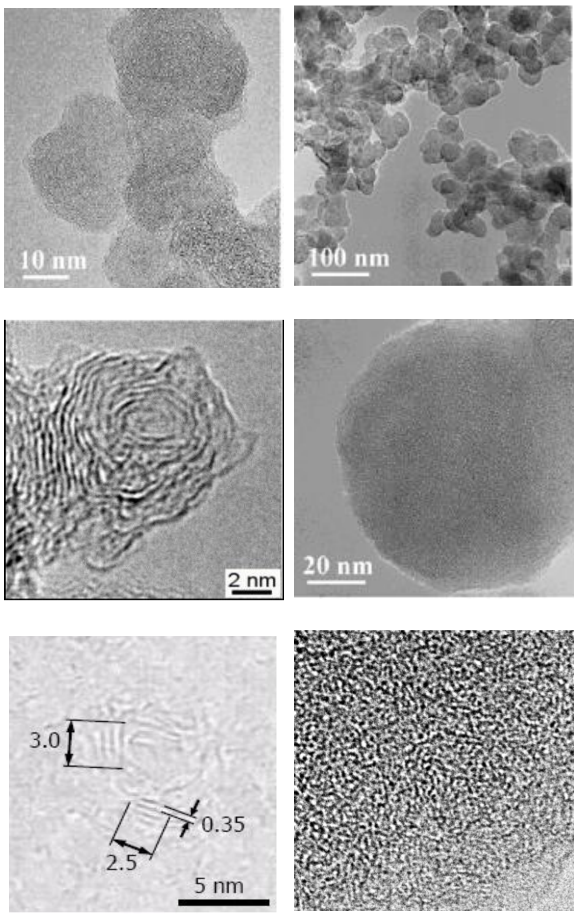
residence time

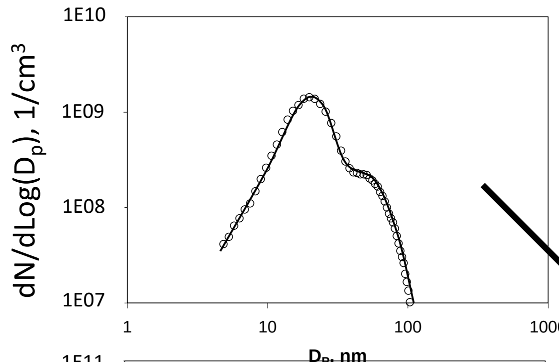
D'Anna, A. "Combustion-formed Nanoparticles", Proc. Combust. Inst. 32:593-613 (2009).

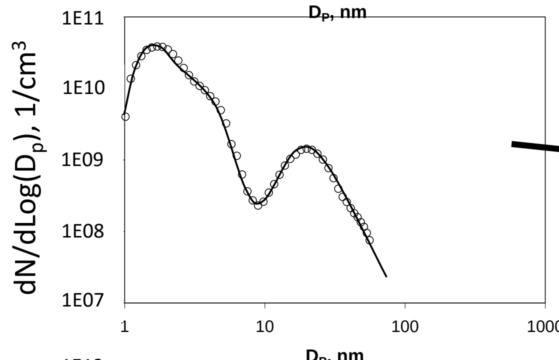
Combustion Generated Nanoparticles

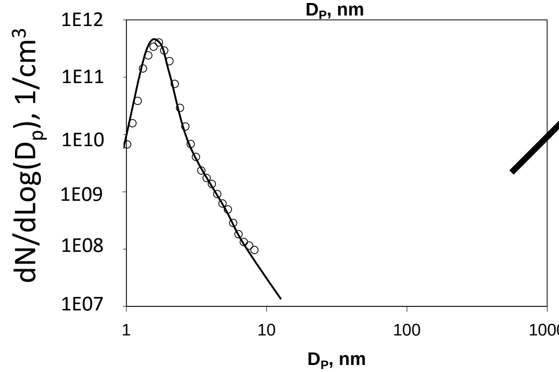
premixed flames (ethylene/air - 1 bar)

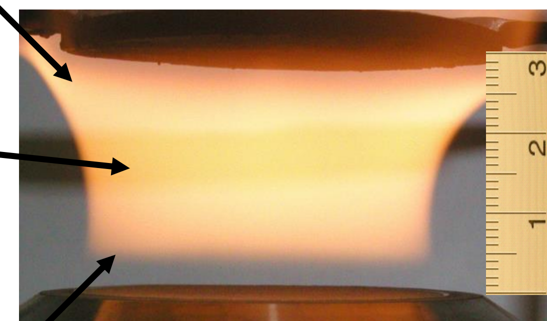
Nano-DMA and HR-TEM











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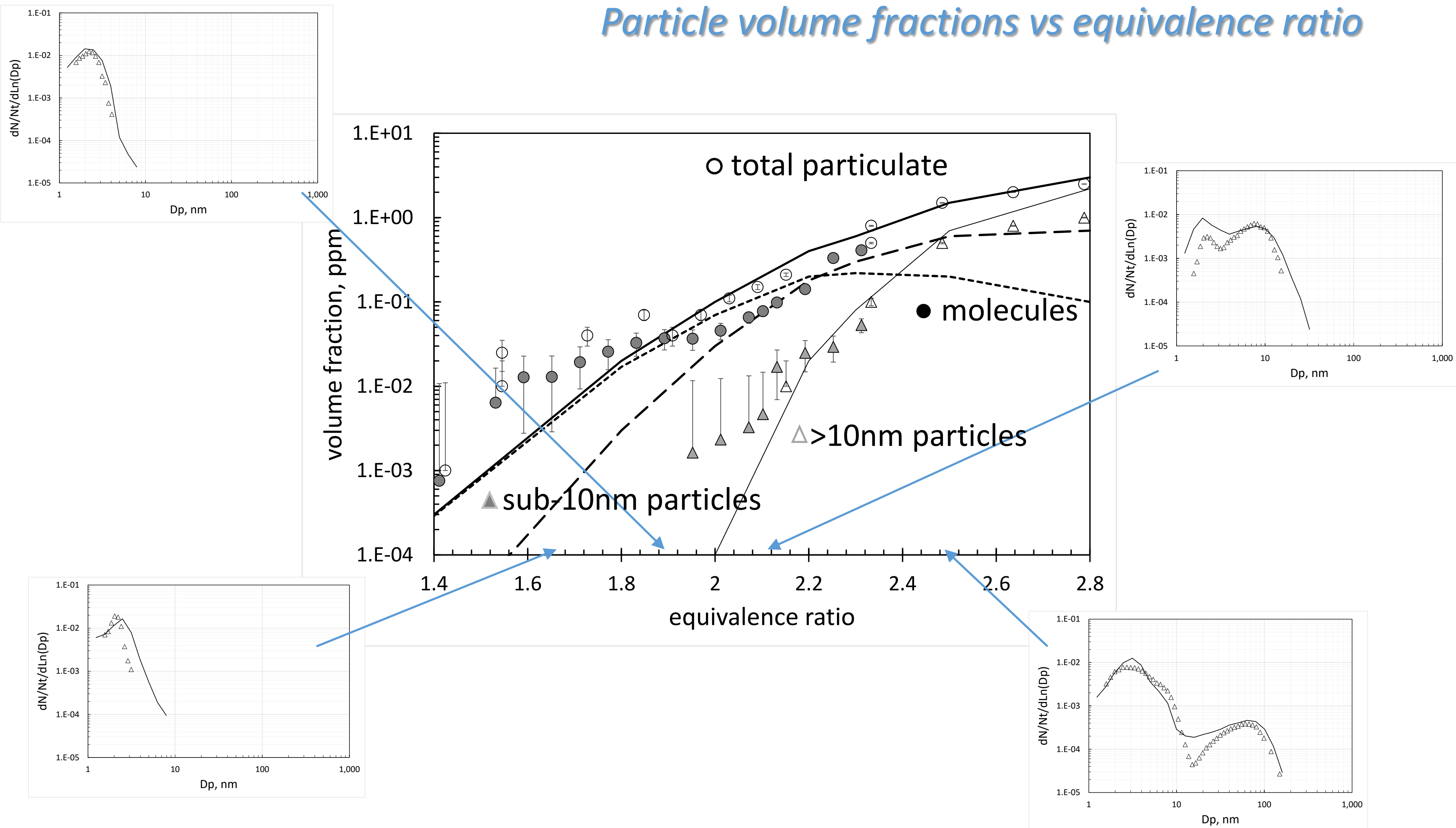
Combustion Generated Nanoparticles

premixed flames (ethylene/air - 1 bar)

Particle volume fractions vs equivalence ratio

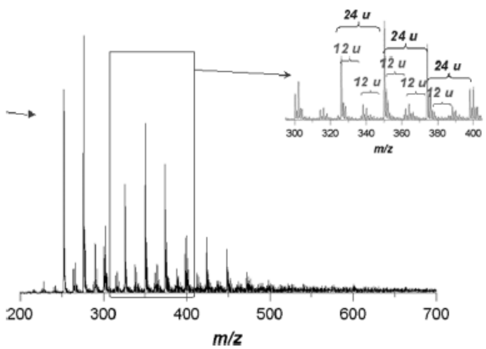


D'Anna A., "Kinetics of Soot Formation". 2015

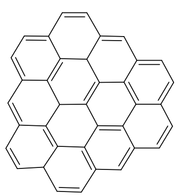
in: Reedijk, J. (Ed.) Elsevier Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Waltham, MA: Elsevier.

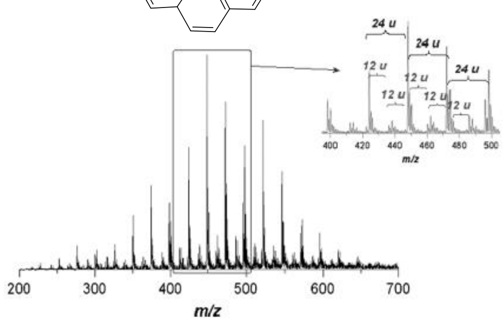
Sub-10 nm "particles"

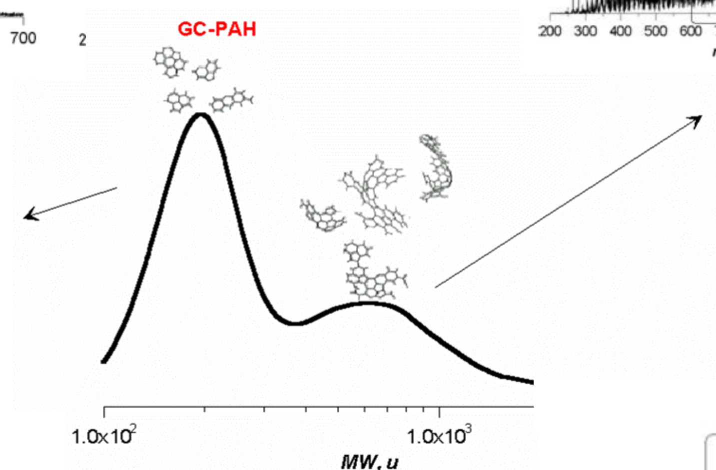
MS – chemical characteristics

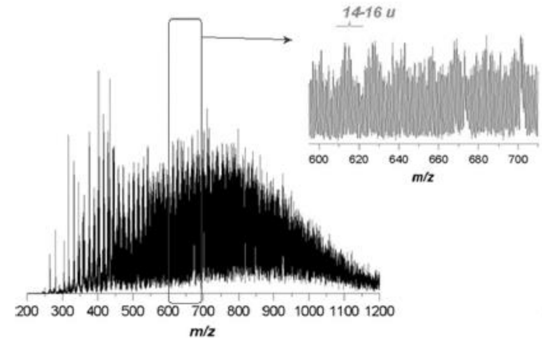


Pericondensed aromatic hydrocarbons (PCAH)

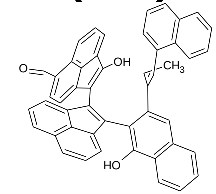


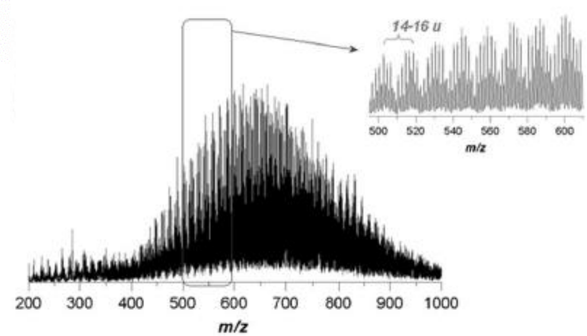






Oligomers of aromatic hydrocarbons (OAH)

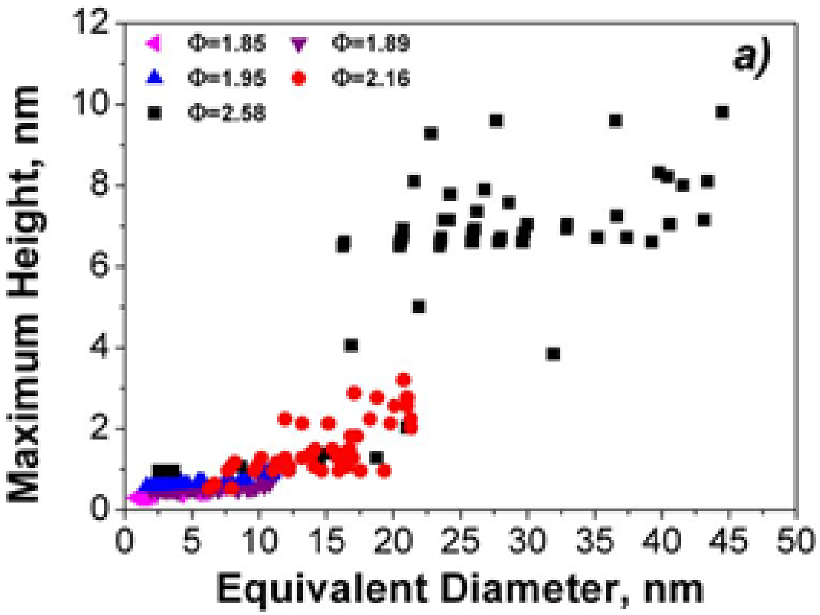


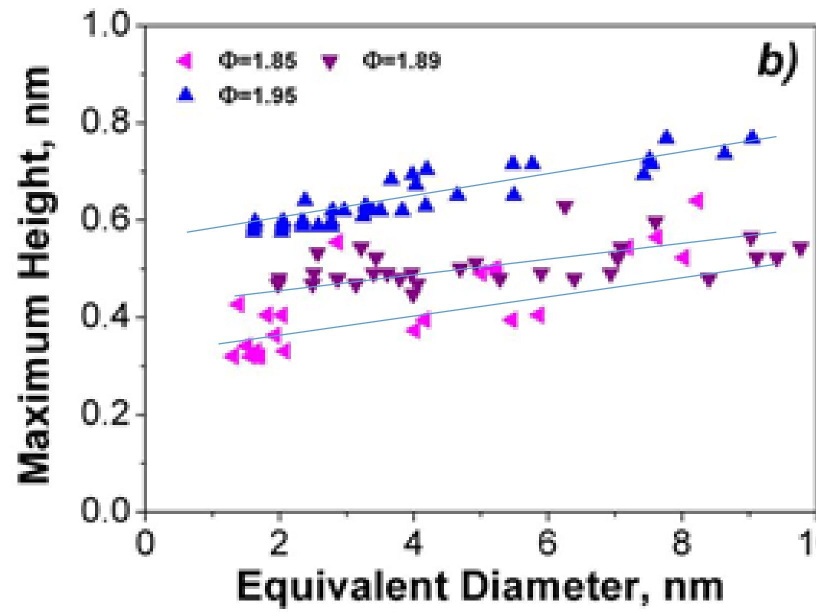


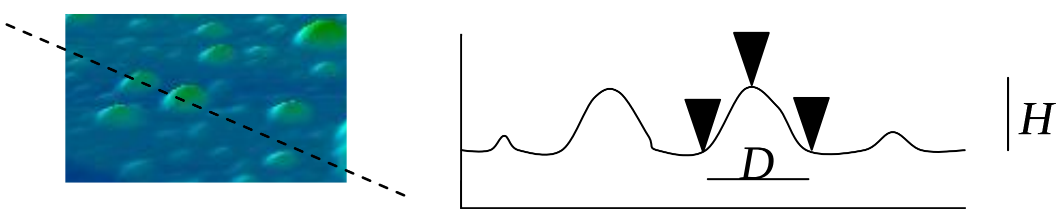
Ciajolo A., "Condensed phases in soot formation process". (2009) Chapt. 21, 333-344
in Combustion Generated Fine Carbonaceous Particles, H. Bockhorn, A. D'Anna, A.F. Sarofim, H. Wang (Eds.), KIT Scientific Publishing,

Sub-10 nm “particles” morphology

Atomic Force Microscopy



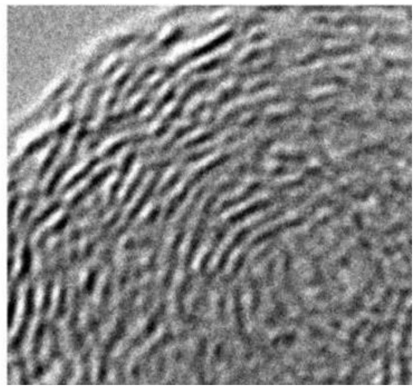


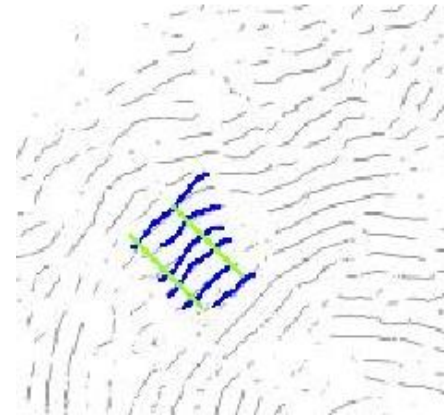


G. De Falco, M. Commodo, P. Minutolo, A. D’Anna “Flame-Formed Carbon Nanoparticles: Morphology, Interaction Forces and Hamaker Constant from AFM”, Aerosol Science and Technology, 49:5, 281-289, 2015.

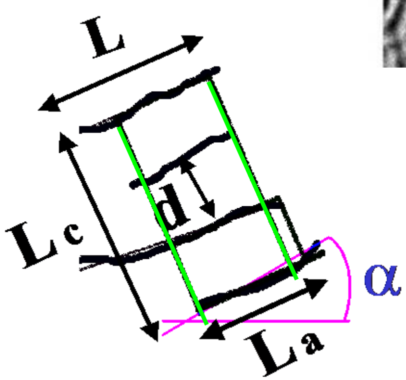
>10 nm particles

HRTEM – morphology (fringe analysis)





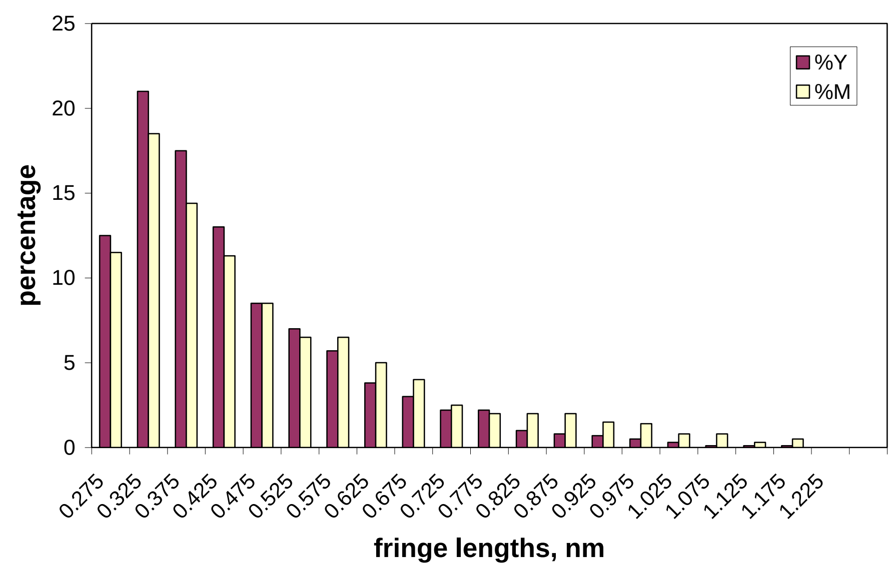
skeletonization



L mean fringe length

La diameter of the coherent domains (BSU)

Lc height of the BSU formed by **N** stacked layers with an interlayer spacing of **d**



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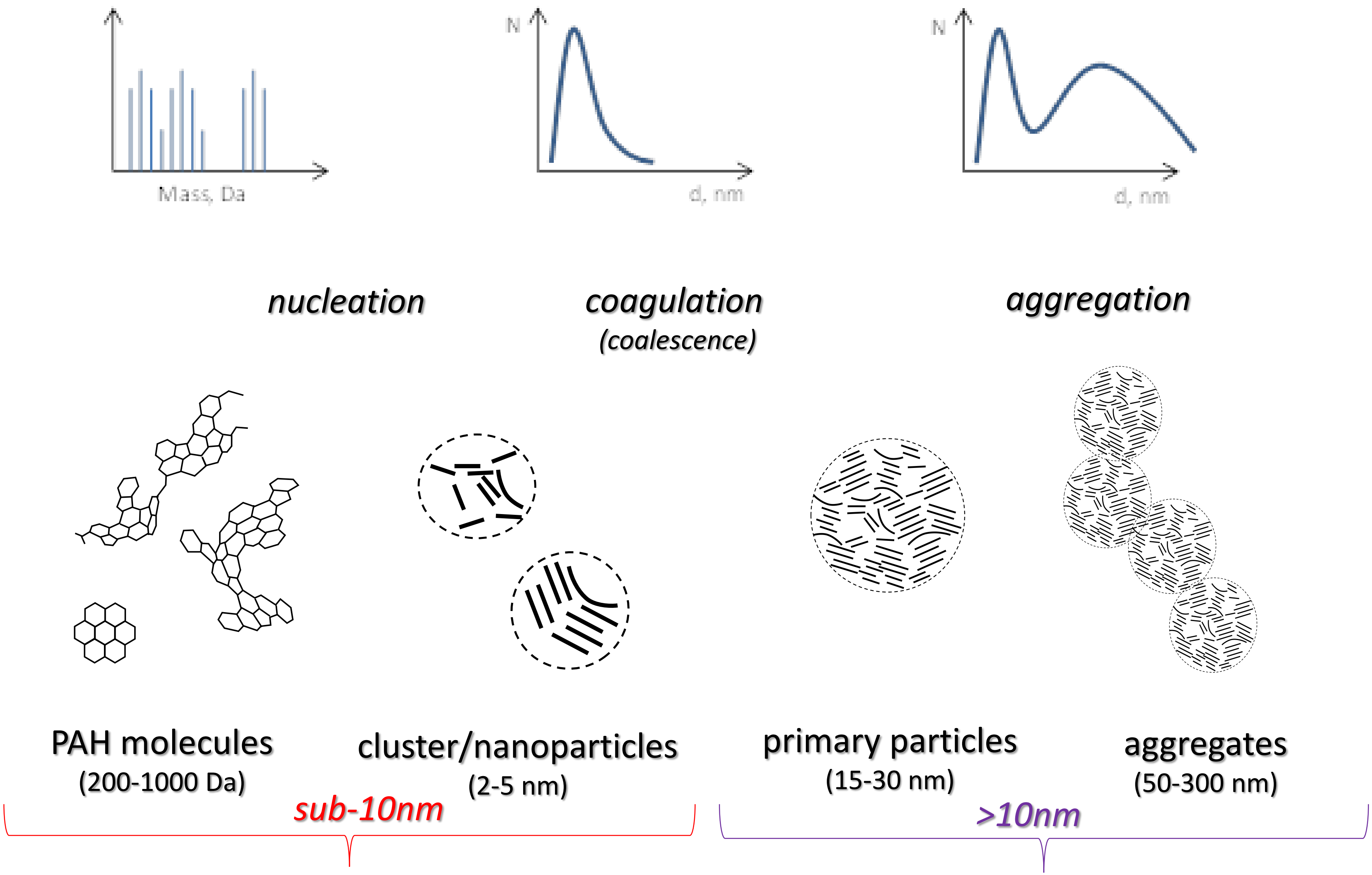
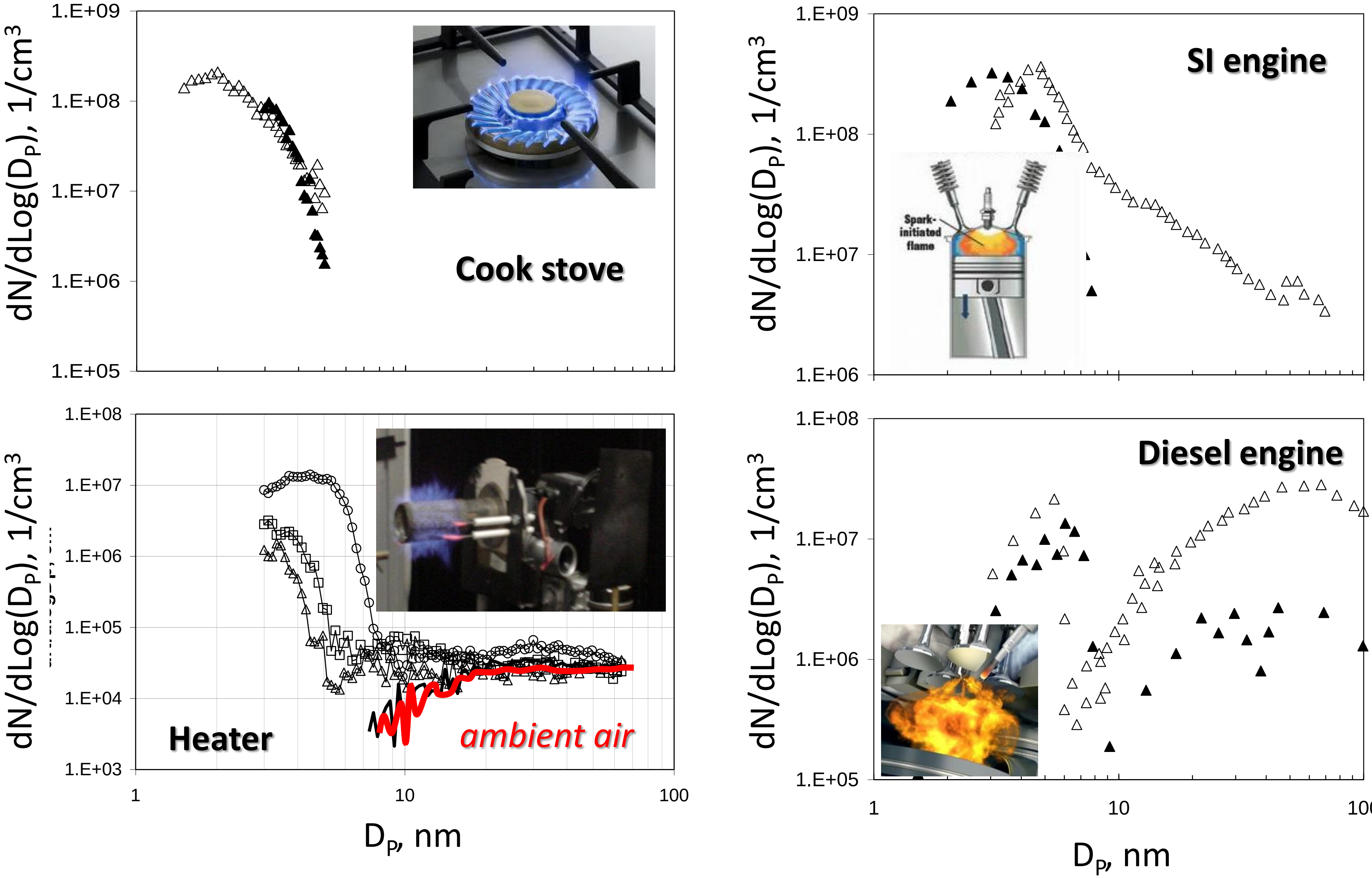
Conceptual model for particle formation



Particle size distributions in practical systems



Sampling and characterization procedures

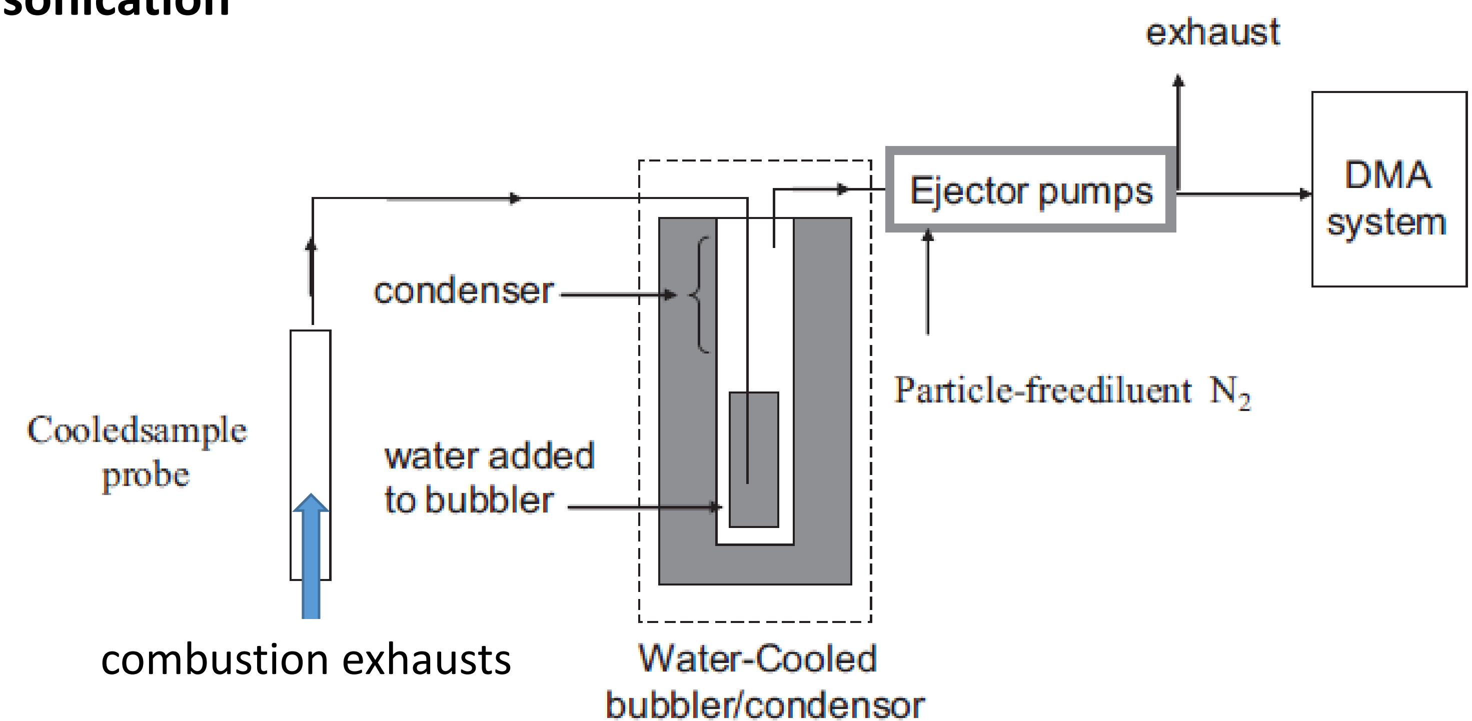
sub-10nm ”particles”

good water affinity

suspended in water with an efficiency of about 50% and isolated from the larger particles

separation of gaseous products by mild evaporation

ultrasonication



L.A. Sgro, A. D’Anna, P. Minutolo, “On the characterization of nanoparticles emitted from combustion sources related to understanding their effects on health and climate”, J. Haz. Mat., 211-212, 420-426, 2012.

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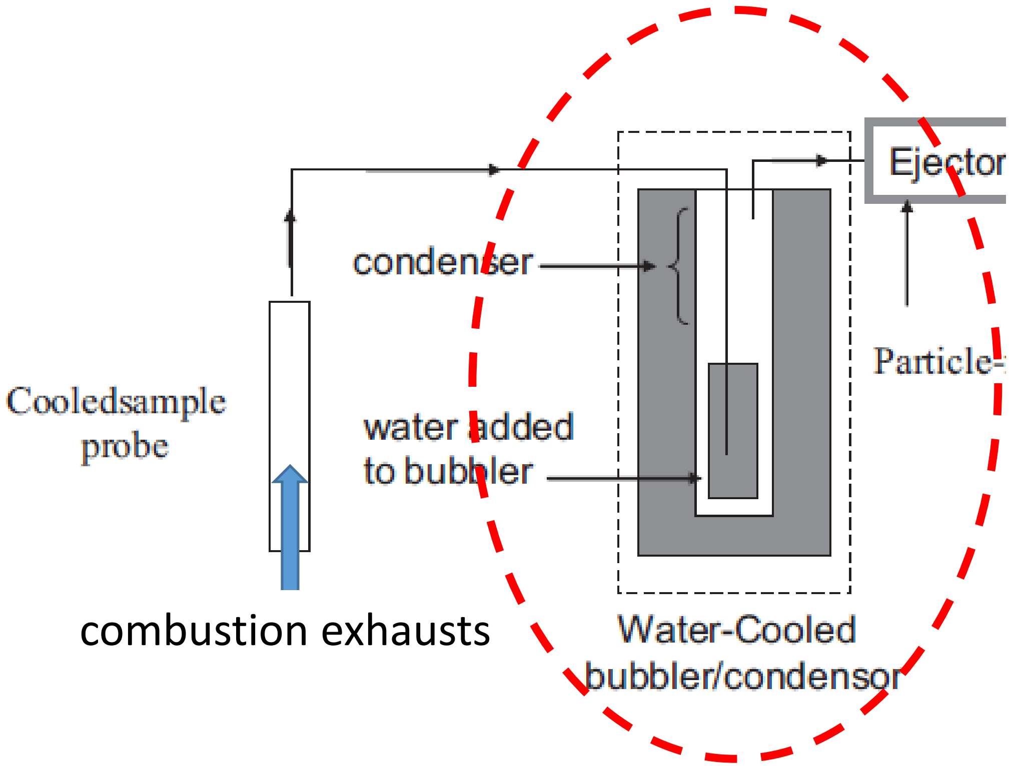


in-vitro tests

size determination by
- **E-DMA**
- **AFM**

chemical characteristics by
- **UV-vis spectroscopy**
- **Fluorescence**
- **TOC**
- **ICP-MS**

Sampling and characterization procedures

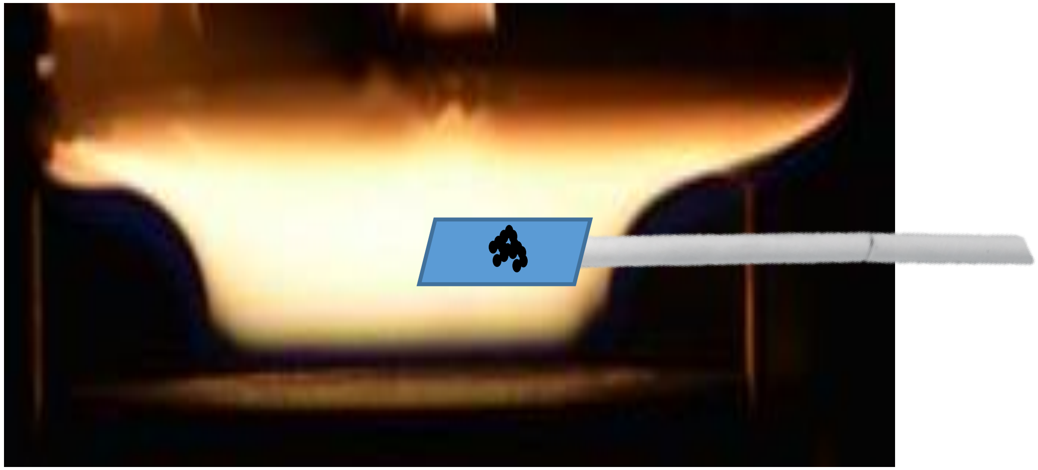
>10nm particles

thermophoretic deposition on quartz plate

mechanical ablation of the solid material

stable suspension in water with 10% of DMSO

dispersion in solvent/solid matrix for structural characterization



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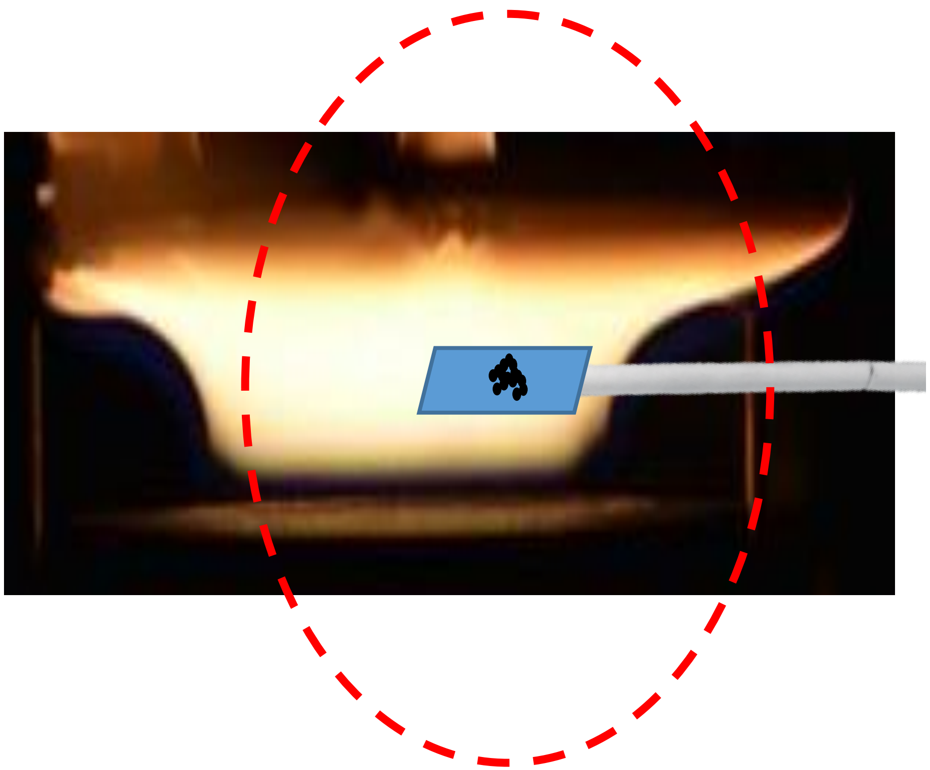


in-vitro tests

size determination by DLS

chemical characteristics by

- UV-vis spectroscopy
- FTIR
- SEC
- MS

Cell culture

human alveolar epithelial-like cells (A549)

immortalized non-tumorigenic human dermal keratinocyte cells (HaCaT)

Cell viability assay

cells were seeded in 96 well plate at a density of $6x10^3$ cells/well

culture media replaced with media containing sub-10nm particles at concentrations from 0.1 to 10 ppm

cells exposed to particles for 24 and 48 h

cell viability evaluated by Cristal Violet Assay

Bioplex cytokine/chemokine detection

-cells were seeded in 6 well plate at a density of $2x10^5$ cells/well in DMEM 10%FCS

-culture media replaced with media containing sub-10nm particles at concentrations of 0.1, 1 ppm (blank test with DMEM)

-cells exposed to particles for 24 h

pro- and anti-inflammatory cytokines released into the culture medium were detected (Bio-Plex Pro Human Cytokine 27-Plex Panel)

Apoptosi detection

-cells were treated with concentration of particles ranging from 0.1 to 10 ppm for 24 and 48 h

Flow Cytometry - Annexin V-FITC apoptosis assay

Western-blot analysis of PARP1

Samples of sub-10 nm "particles"

	Cell viability	cytokine/ chemokine	flow citometry & Annexin V-FITC/PI assay
Bluish, moderately-rich premixed flame	x		x
Cook-top burner	x	x	
Diesel engine exhaust (no DPF)	x	x	

Samples of >10 nm particles

	Cell viability	Western-blot analysis of PARP1
Yellow, rich premixed flame	x	x

Results

Cook-top burner sub-10nm particles show no relevant reduction in cell number on HaCat cells.

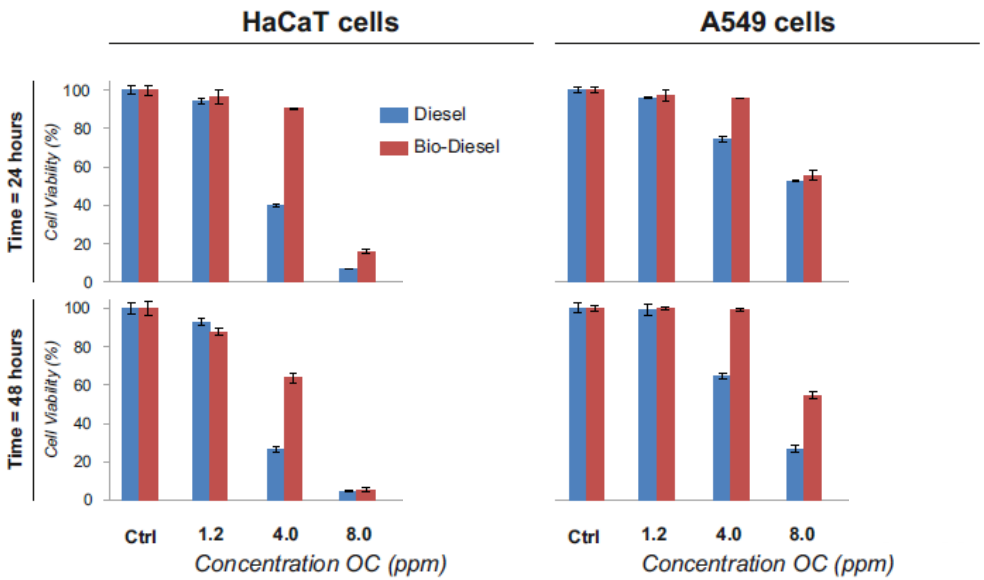
Results of secretome analysis suggest that the pro-inflammatory pathway is not activated for these nanoparticles.

Flame generated sub-10nm particles appear to be significantly more dangerous. They also show to be more dangerous than engineered nanoparticles of the same sizes

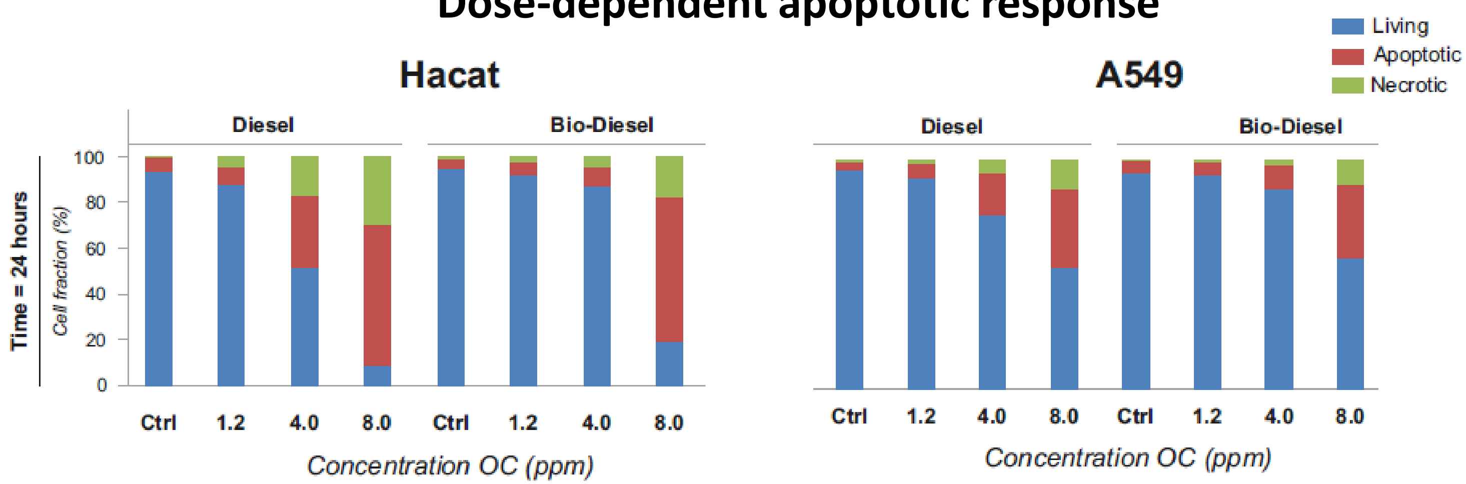
Pedata P, Boccellino M, La Porta R, Napolitano M, Minutolo P, Sgro LA, Zei F, Sannolo N, Quagliuolo L., “Interaction between combustion-generated organic nanoparticles and biological systems: in vitro study of cell toxicity and apoptosis in human keratinocytes”. Nanotoxicology. 2012; 6(4):338-352.

Results

Diesel and biodiesel generated sub-10nm particles induce cytotoxicity at lower concentrations with respect to flame formed particles.



Dose-dependent apoptotic response



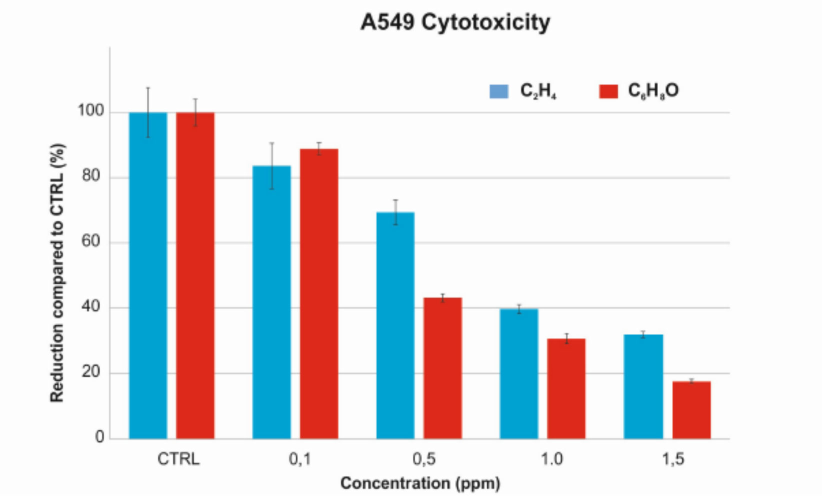
Diesel and Bio-Diesel fueled engine – sub-10 nm particles

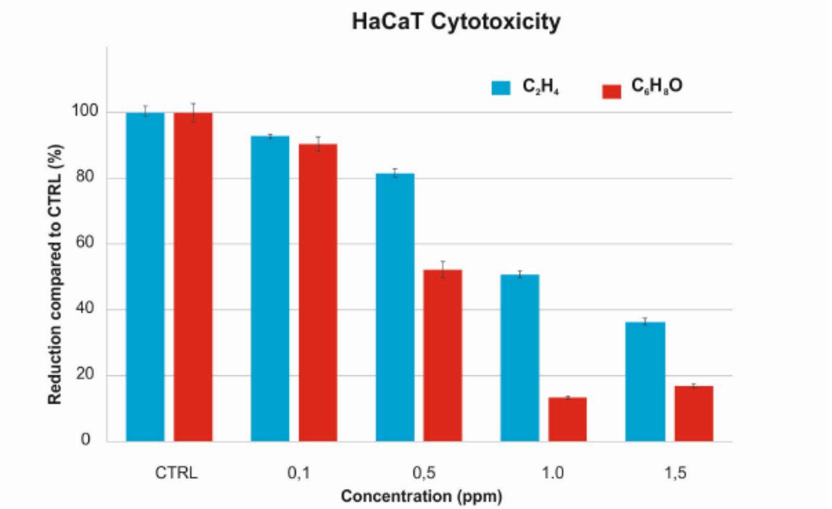
Results

Significant increase of cell mortality following treatment with >10nm particles for 24 hours in both cells lines

Dose-dependent effect

Biofuel >10nm particles seem induce more cytotoxicity compared to those emitted by the ethylene flame





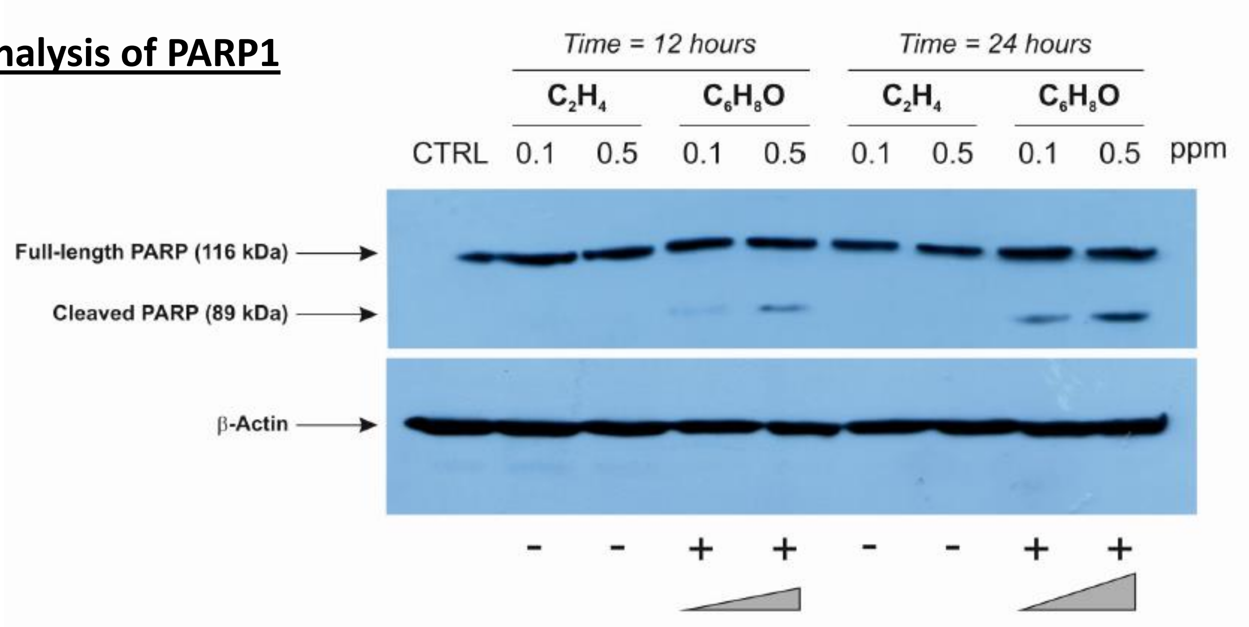
Flame generated particles in ethylene & ethylene/20% 2,5 dimethyfuran flames – >10 nm particles

Results

Evidence of the presence of "p89" fragments of PARP1 in the cells exposed to >10nm particles generated by a ethylene/2,5 dimethylfuran flame

The >10nm particles derived from biofuel combustion have different chemical structure from the ethylene-generated particles: higher amount of oxygen atoms that substitute hydrogen atoms at the edges of the aromatic carbon network

Western-blot analysis of PARP1



Flame generated particles in ethylene & ethylene/20% 2,5 dimethyfuran flames – >10 nm particles

Conclusions

Combustion systems form and eventually emit huge number concentrations of sub-10nm particles which are clusters of aromatic compounds

Toxicity of these ''particles'' has been analyzed *in-vitro* with HaCaT and A549 cells

Toxicity of sub-10nm particles seems to be related to both particle size and particle chemical composition
(in the smaller particles the number of molecules at the surface of the cluster is comparable to the number of molecules constituting the cluster)

Ethylene/2,5dimethyl furan generated particles (>10nm particles) seem to induce stronger cytotoxicity than similar-size, ethylene generated particles
(these particles have higher amount of oxygen atoms that substitute hydrogen atoms at the edges of the aromatic carbon network)