Measurements of oxidative capacity of combustion generated nanoparticles using profluorescent nitroxide probes

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Oxidative stress caused by generation of free radicals and related reactive oxygen species (ROS) at the sites of deposition has been proposed as a mechanism for many of the adverse health outcomes associated with exposure to particulate matter (PM). In addition to particle-induced generation of ROS in lung tissue cells, several recent studies have shown that particles may also contain ROS. As such, they present a direct cause of oxidative stress and related adverse health effects. Recently, a new profluorescent nitroxide molecular probe (bis(phenylethynyl)anthracene-nitroxide; BPEAnit) (Fairfull-Smith and Bottle 2008), developed at QUT was applied in an entirely novel, rapid and non-cell based assay for assessing the oxidative potential of particles (i.e. potential of particles to induce oxidative stress). The technique was applied on particles produced by several combustion sources, namely cigarette smoke, diesel exhaust and wood smoke (Miljevic, Fairfull-Smith et al. 2010; Miljevic, Heringa et al. 2010; Surawski, Miljevic et al. 2010)). Profluorescent nitroxides have a very low fluorescence emission, but upon radical trapping or redox activity, a strong fluorescence is observed. One of the main findings from the initial studies undertaken at QUT was that the oxidative potential per PM mass significantly varies for different combustion sources as well as the type of fuel used and combustion conditions.

However, possibly the most important finding from our studies was that there was a strong correlation between the organic fraction of particles and the oxidative potential measured by the PFN assay, which clearly highlights the importance of organic species in particle-induced toxicity (Miljevic, Heringa et al. 2010; Surawski, Miljevic et al. 2010).

To further explore this correlation we have focused our research on investigating the role of various fuels and diesel injection technologies on the oxidative capacity (ROS concentration) of diesel particles. In the first study (Surawski, Miljevic et al. 2011) we have investigated 3 different fuels (biodiesel, synthetic diesel and petro-diesel) with 2 different injection technologies (common rail and direct injection). In the second study (Surawski, Miljevic et al. 2011) we have investigated the role of 3 different biodiesel fuel feedstocks (soy, tallow and canola) at 4 different blend percentages (20%, 40%, 60% and 80%). The first study showed a stronger influence of the engine technology than fuel type on particle oxidative capacity. While a significant decrease of PM was observed for the newer common rail technology it was interesting to observe an increase in the particle oxidative capacity. Given that rather large increases in ROS emissions occurred with the common rail injection configuration (in some cases a more than 10-fold increase was observed), this has significant implications for the overall toxicological properties of the emitted particles. The somewhat disturbing overall conclusion from the first study was that the injection technology which produces fewer particles (i.e., common rail injection) has significantly more oxidative and genotoxic material available on the surface of the particle, potentially causing health effects that are not captured by considering only particle number, or particle mass based emissions.

In the second study we observed that ROS concentrations increased monotonically with biodiesel blend percentage, but did not exhibit strong feedstock variability. Furthermore, the ROS concentrations, and therefore the oxidative capacity, correlated quite well with the organic volume percentage of particles – a quantity that increased with increasing blend percentage. The increase in the blend percentage from 20 to 80% has on one side resulted in a significant decrease in particle mass and particle surface but on the other side has resulted in an increase in the particle oxidative capacity. Our results have implications for the regulation of DPM using only physical properties such as mass, surface or even number based metric. Regulating purely the particle surface area or mass would not be able to detect results such as these, as the surface chemistry of particles is not explicitly considered. Therefore, not only the raw surface area of particles but also the surface chemistry of particles is important for assessing the health impacts of DPM. These results suggest that the development of instrumentation (and standards) that enable the internal mixing status of particles to be determined (within a surface area framework) are potentially required.

References

Fairfull-Smith, K. E. and S. E. Bottle (2008). "The Synthesis and Physical Properties of Novel Polyaromatic Profluorescent Isoindoline Nitroxide Probes." Eur J Org Chem **32**: 5391-5400.

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Surawski, N. C., B. Miljevic, et al. (2010). "Particle emissions, volatility and toxicity from an ethanol fumigated compression ignition engine." Environmental Science & Technology **44**(1): 229-235.

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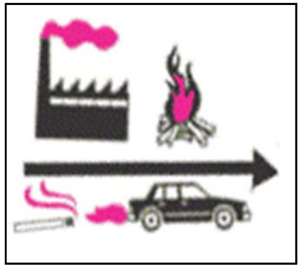
PM & health effects

- Epidemiological studies - strong associations between levels of ambient particulate matter (PM) and increased respiratory and cardiovascular disease morbidity and mortality
- mechanism(s) by which particles induce adverse health effects are still not entirely understood

Proposed mechanism: Oxidative stress hypothesis

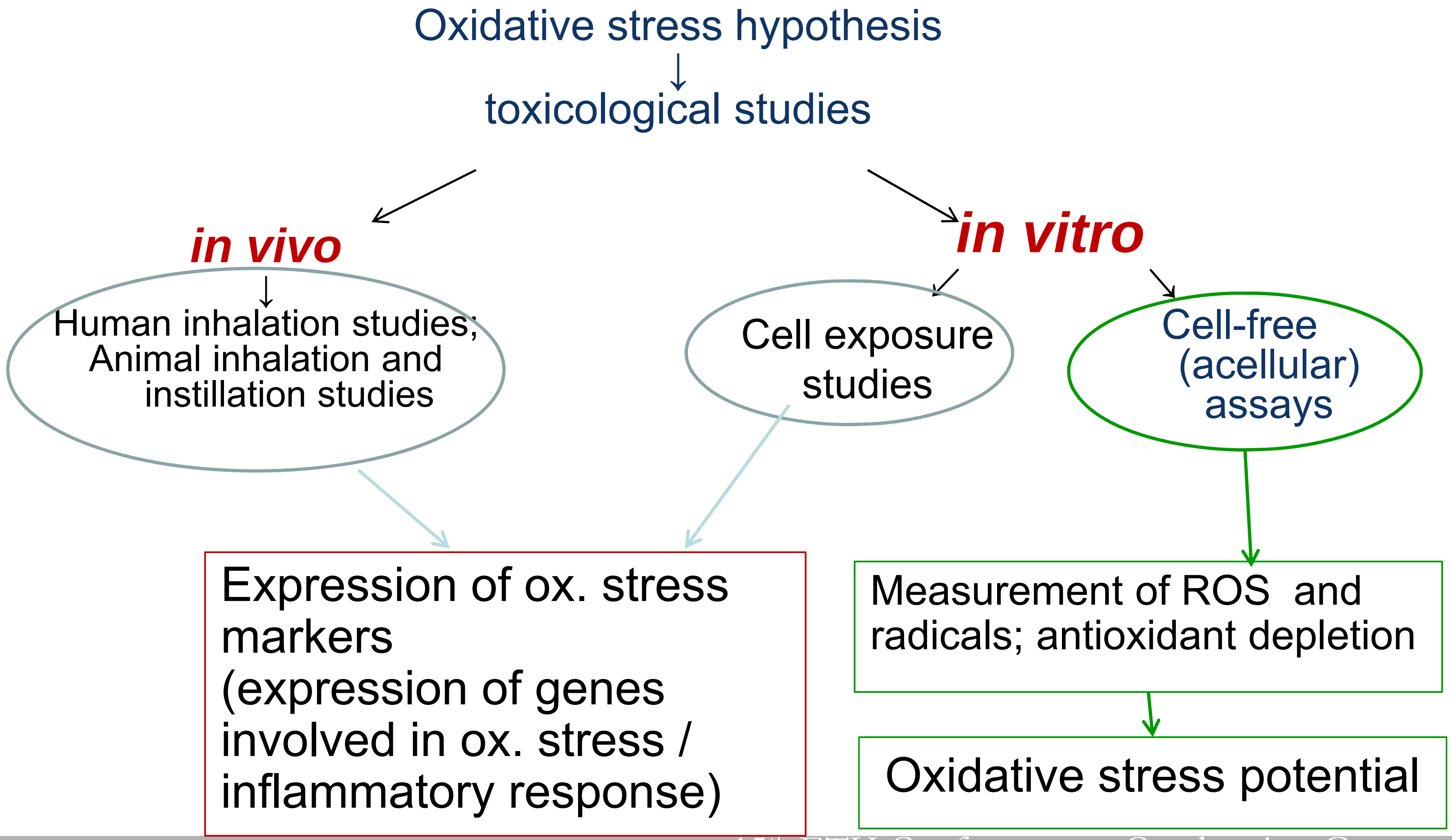


PM → free radicals; ROS → oxidative stress → inflammation

↓

cell injury / death

PM & health effects



Oxidative stress hypothesis

↓

toxicological studies

in vivo

↓

Human inhalation studies; Animal inhalation and instillation studies

in vitro

Cell exposure studies

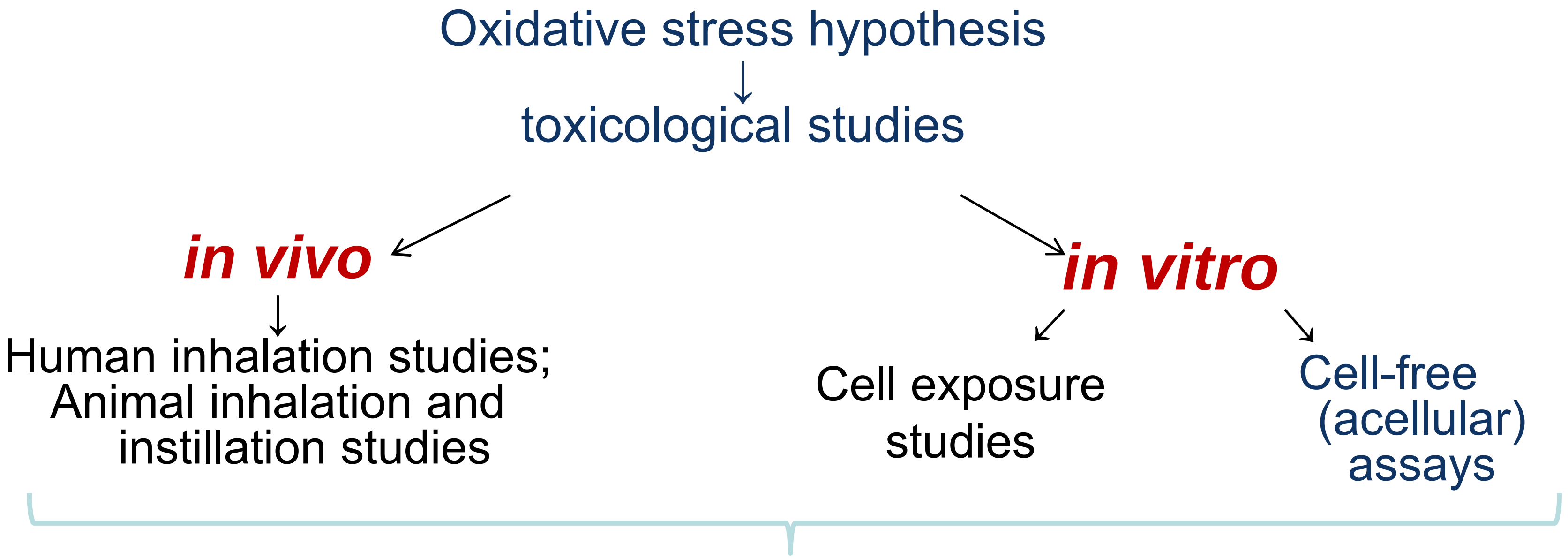
Cell-free (acellular) assays

Expression of ox. stress markers (expression of genes involved in ox. stress / inflammatory response)

Measurement of ROS and radicals; antioxidant depletion

Oxidative stress potential

PM & health effects



Oxidative stress hypothesis

↓

toxicological studies

in vivo

↓

Human inhalation studies;
Animal inhalation and
instillation studies

in vitro

Cell exposure
studies

Cell-free
(acellular)
assays

Properties of particles relevant for the observed health effects?

-size

-composition:

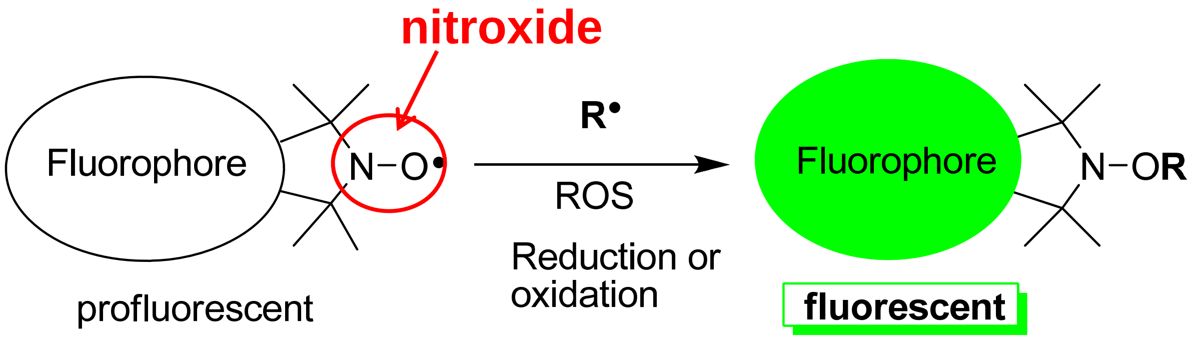
1. transition metals
   $(M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + \cdot OH + OH^-)$
2. organics (PAHs; quinones)
3. ROS ***inherent, exogeneous* – direct cause of ox. stress**

Generation of ROS in cells – *endogenous ROS*

Motivation of the study

- Develop a cell-free assay for rapid and routine screenings of the oxidative potential of PM – ROS concentration.
- Establish a relationship between particle size/surface area and ROS concentration.
- Establish the relationship between the volatile organic fraction of PM and ROS concentration for various airborne particle (pollution) sources.

Profluorescent nitroxides (PFNs)



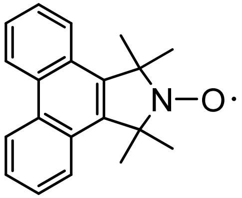
- powerful optical sensors applicable as detectors of radicals and redox active agents
- Nitroxides: -trap C- , S- and P- centred radicals – stable adducts

  - scavenge O-centred radicals through redox mechanisms

  - applied in biological studies as antioxidants, detection of hydroxyl and peroxyl radicals, monitoring thermo-oxidative polymer degradation and photogeneration of radicals in polymer films

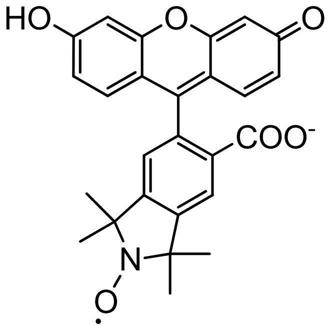
Profluorescent nitroxides synthesised at QUT

BPEAnit



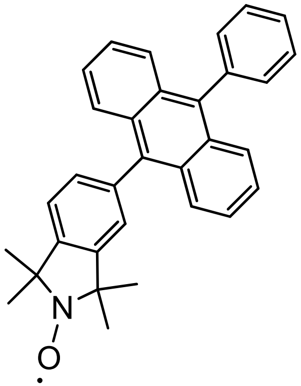
Phenanthrene-nitroxide

λ_{ex}= 294 nm
λ_{em}=355 nm
372 nm



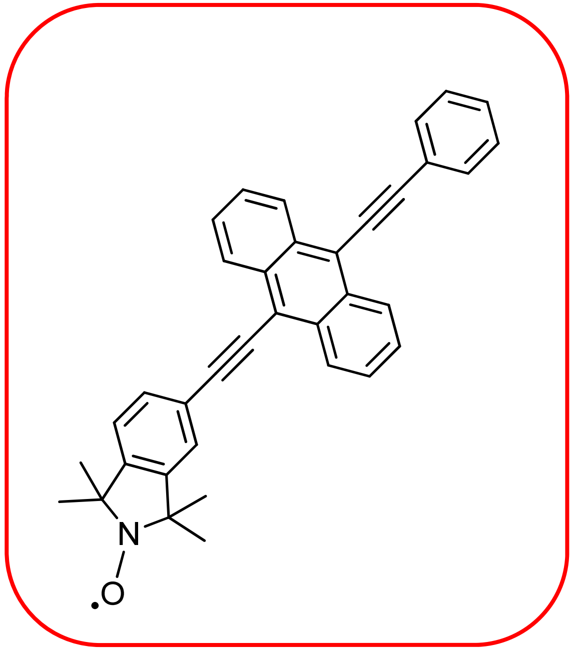
Flourescein-nitroxide

λ_{ex}= 495 nm
λ_{em}=515 nm



9,10–diphenylanthracene-nitroxide

λ_{ex}= 395 nm
λ_{em}=410 nm
430 nm



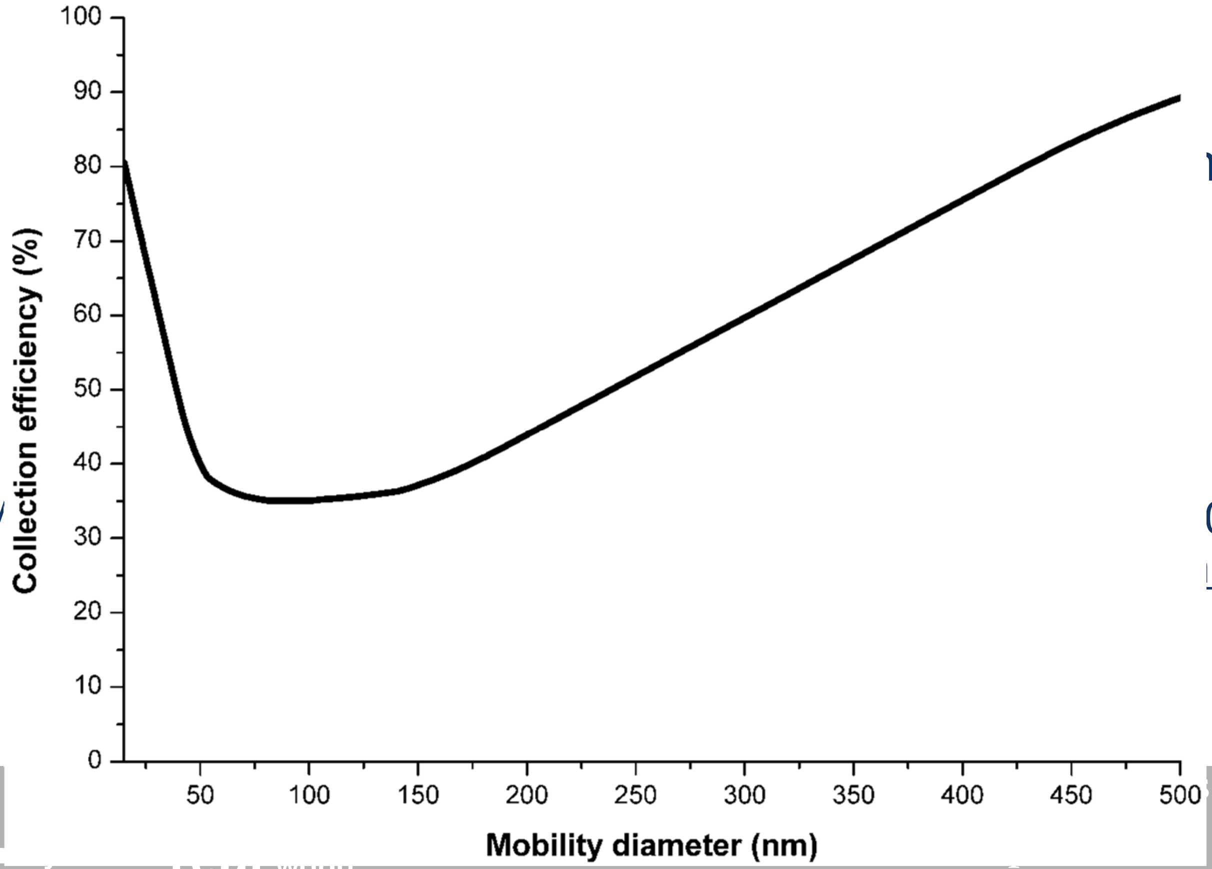
9,10-bis(phenylethynyl)anthracene-Nitroxide (BPEAnit)

λ_{ex}= 430 nm
λ_{em}=485 nm
510 nm

Fairfull-Smith and Bottle. Eur J Org Chem (2008) (32) pp. 5391-5400

Sampling methodology

- Bubbling aerosol through an impinger with fritted nozzle tip containing PFN solution, followed by fluorescence measurement



- sampling chemical pling and

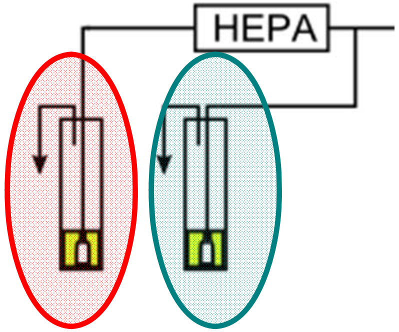
- collection

M 09. On the of ultrafine

-

BPEAnit assay – sampling:

- bubbling aerosol through an impinger with fritted nozzle tip containing BPEAnit solution fluorescence measurement
- solvent – dimethylsulfoxide (DMSO)
- test & HEPA-filtered control sample taken
- I_{485nm}(test) - I_{485nm}(ctrl) ⟶ $I_{485nm}(ROS_{particle})$



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calibration curve

↓

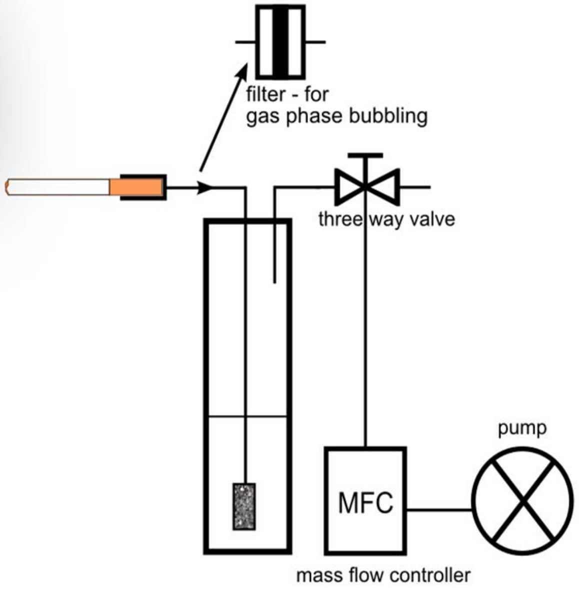
n $(ROS_{particle})$

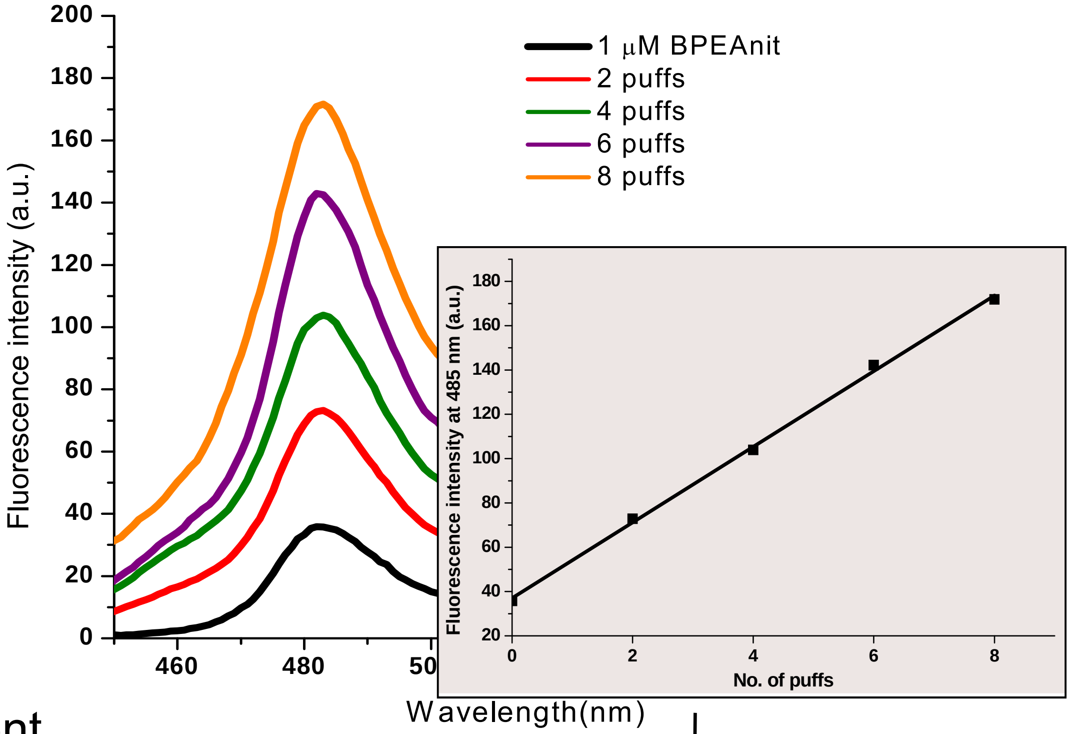
↓

Normalized to the measured particle mass

Cigarette smoke - mainstream

- Linearity, reproducibility?
- Puffs – equal volumes of aerosol of the same physicochemical properties





Miljevic et al., Atmospheric Environment (2010) vol. 44 (18) pp. 2224-2230

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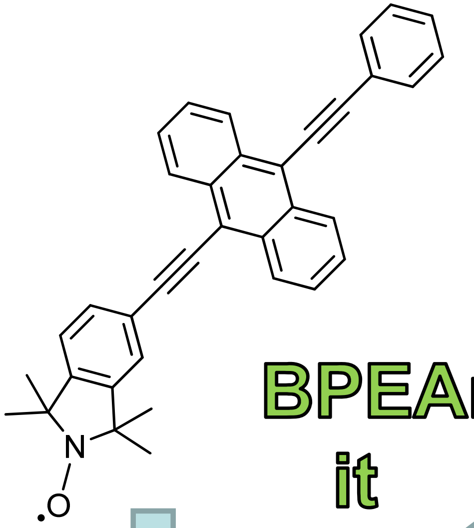
Linear, reproducible



BPEAn it

Cigarette smoke

Diesel exhaust

Wood smoke

Wood smoke

Automatic pellet boiler:

- Automatically fed
- More controlled combustion conditions (air supply and amount of fuel) – more efficient
- Low particle emissions – dominated by alkali metal salts (KCl, K2SO4)

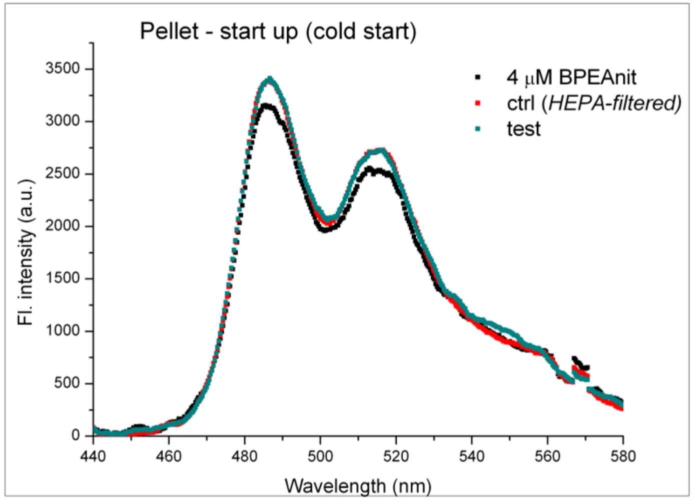
Log wood stove:

- Manually fed (batch-wise combustion)
- less controlled combustion
- highly variable emissions – higher percentage of organics and soot

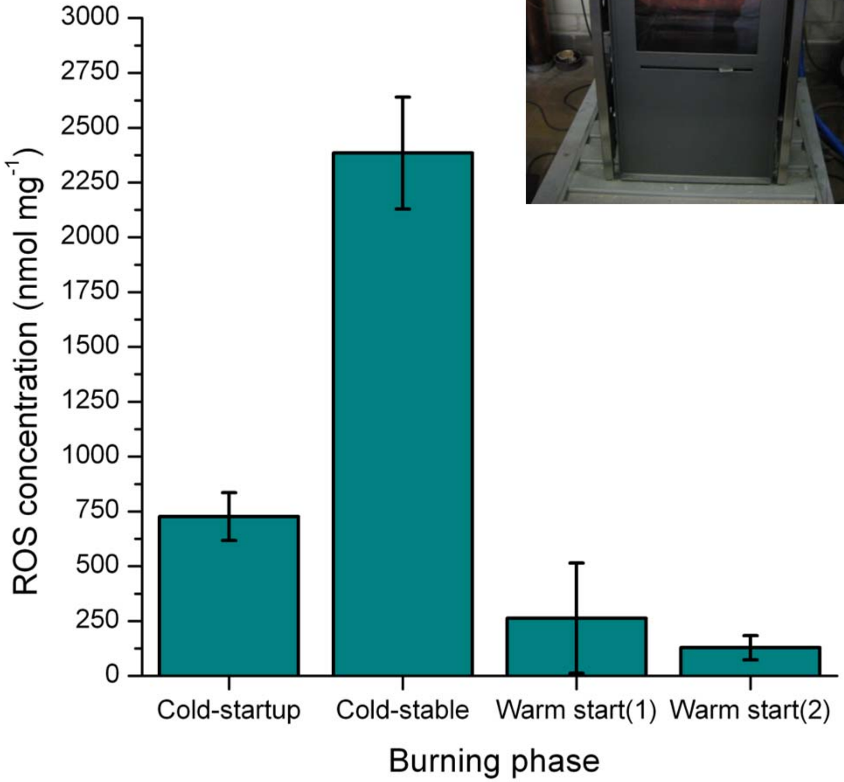
Oxidative potential?

Wood smoke

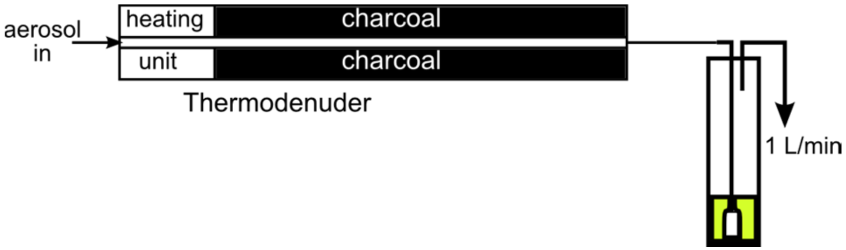
Automatic pellet boiler:

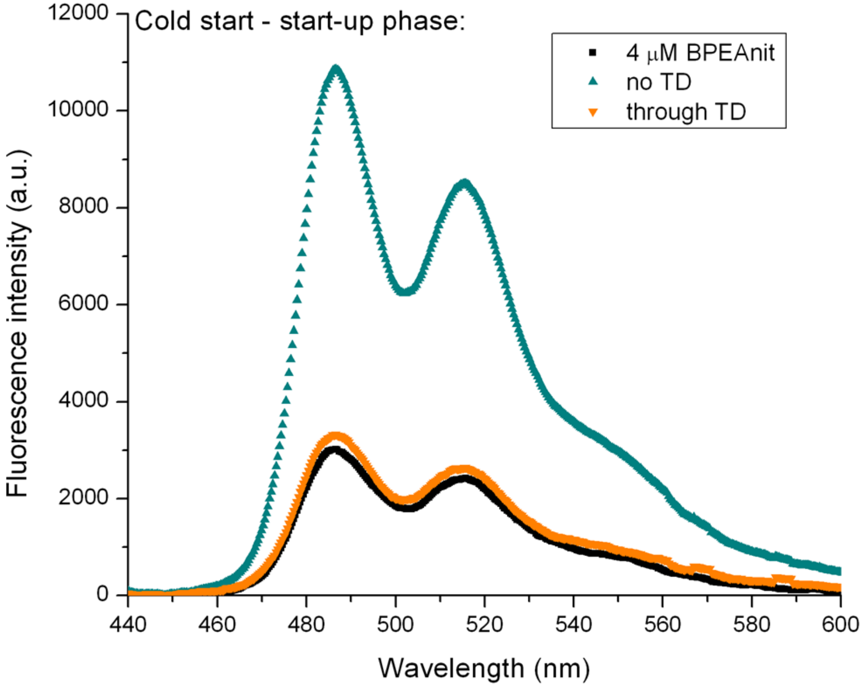


Log wood stove:



Results: Sampling through thermodenuder (TD)

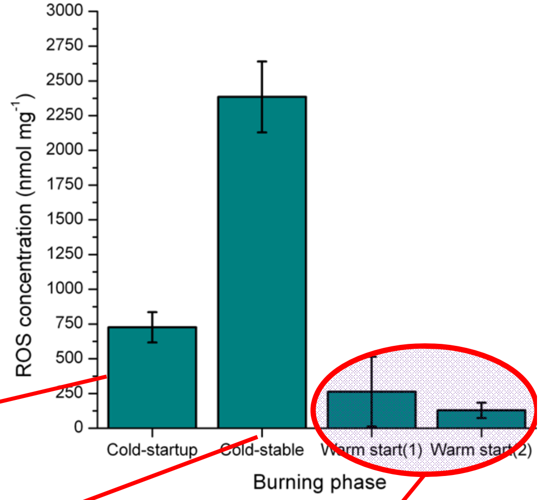


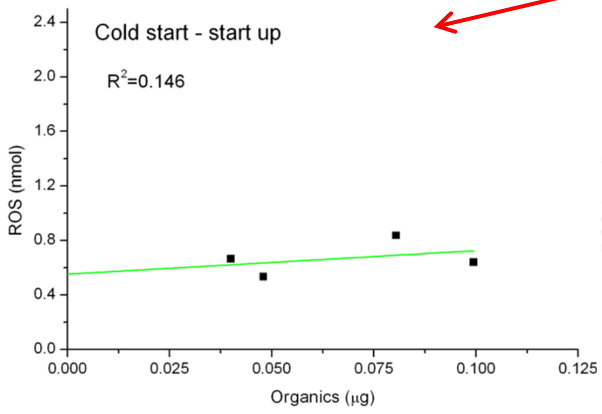


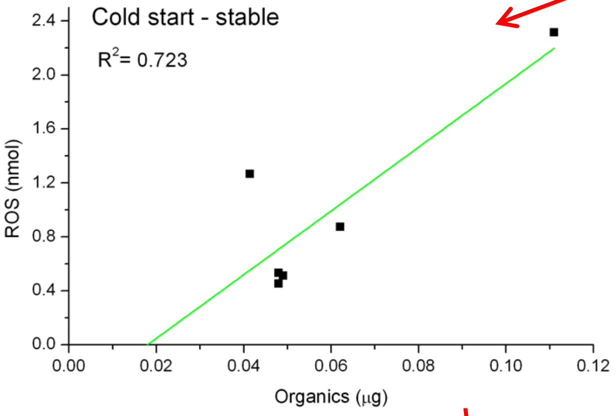
Importance of organics in oxidative potential of particles !

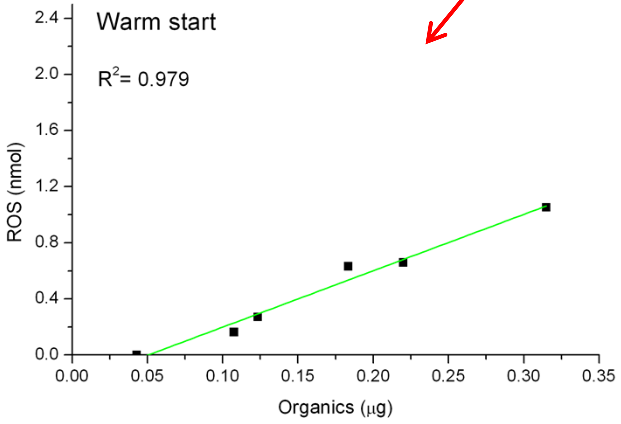
Results: ROS vs. organics

- Organics – from the AMS





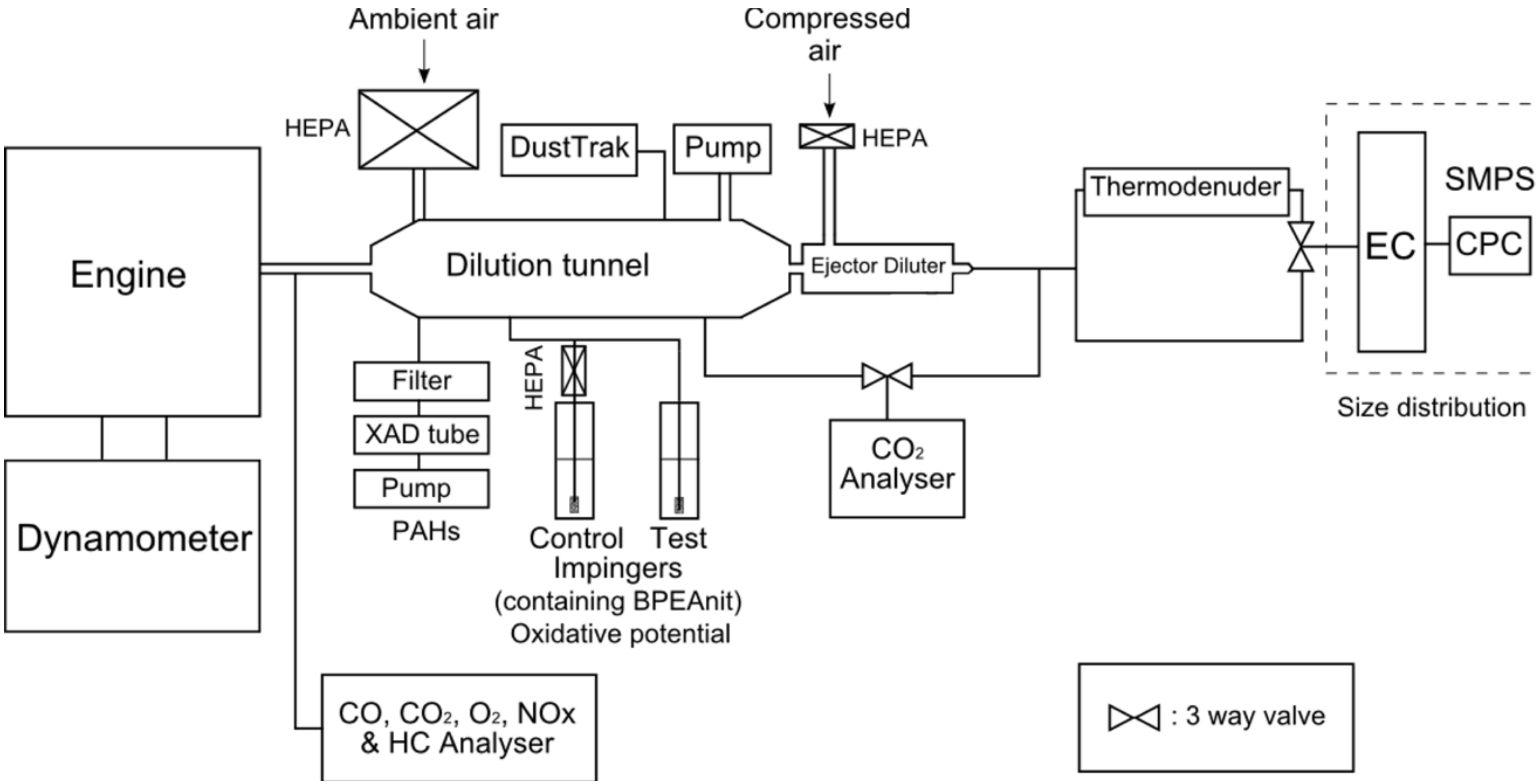




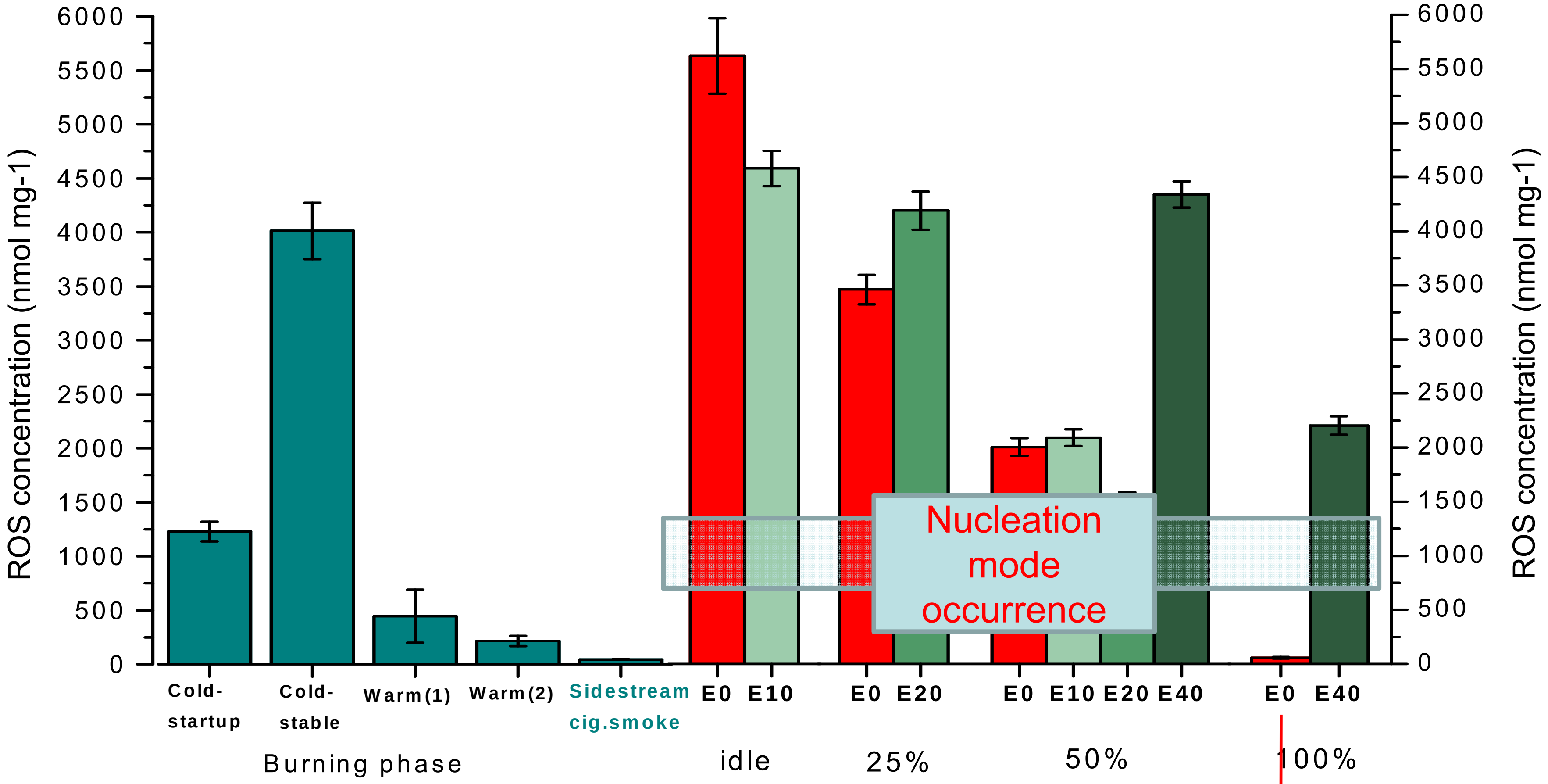
Miljevic et al. Environ Sci Technol (2010)
vol. 44 (17) pp. 6601-6607

More “reactive”

Experimental Setup for Engine Emissions Measurements



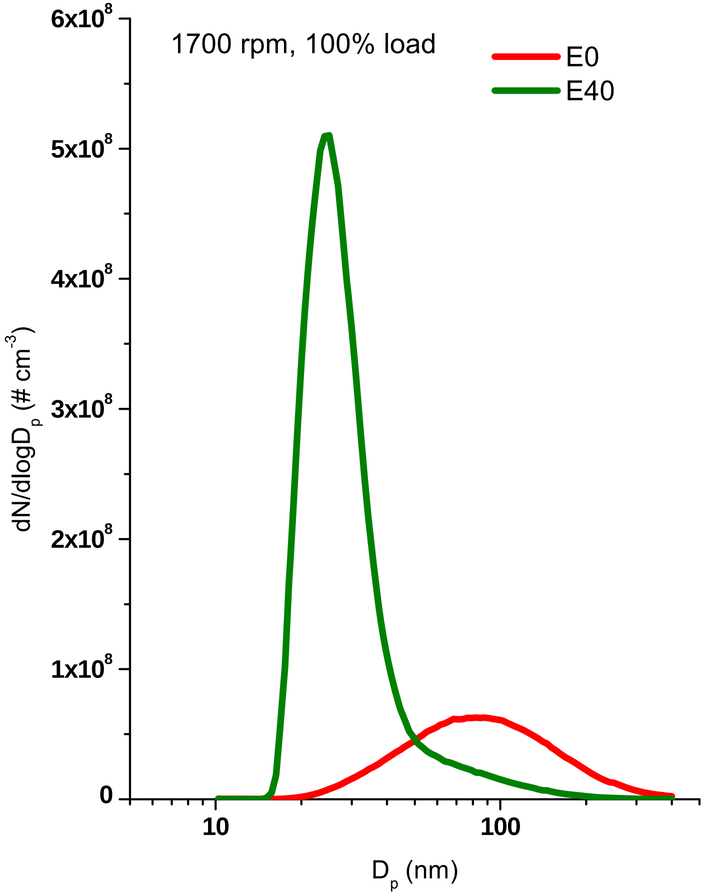
Summary:

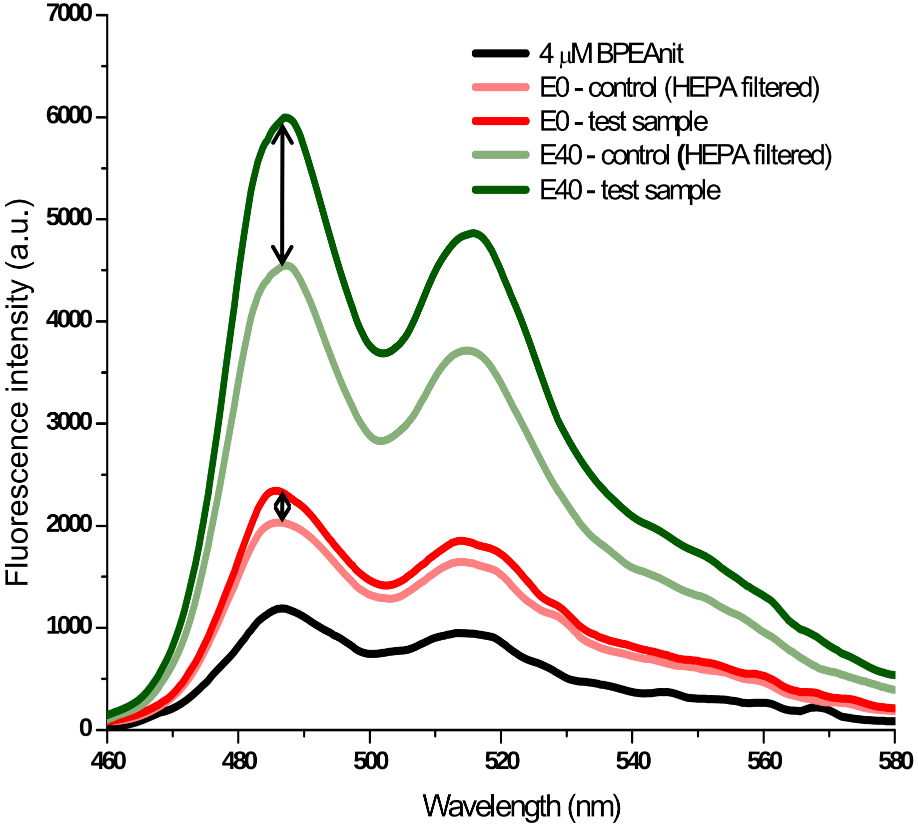


Nucleation mode occurrence

Accumulation mode

Ethanol fumigation

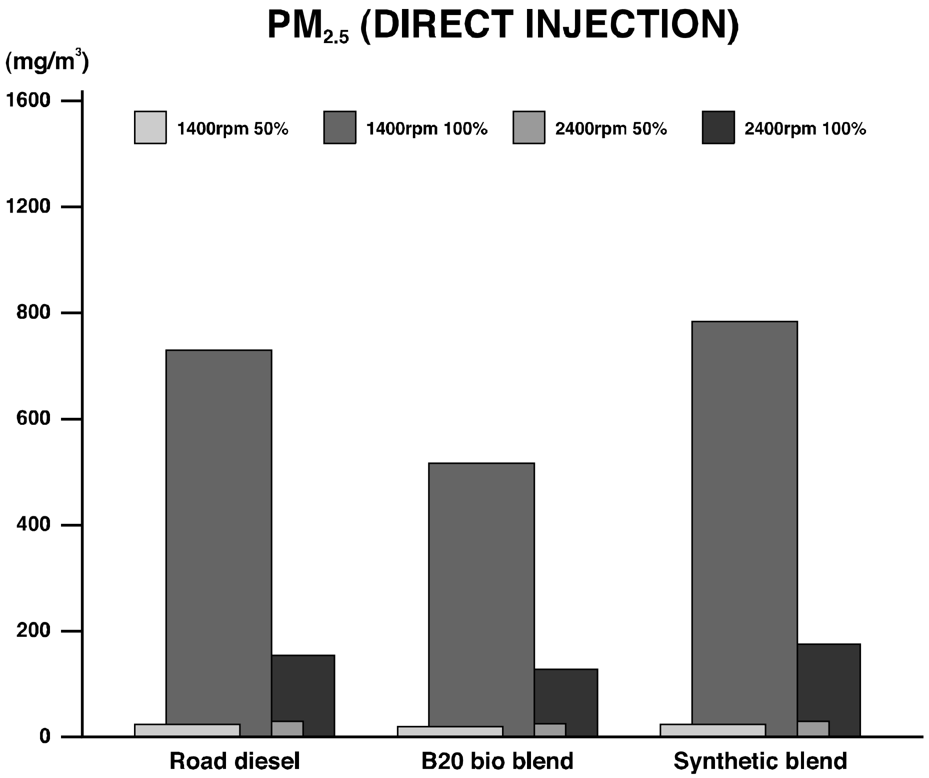


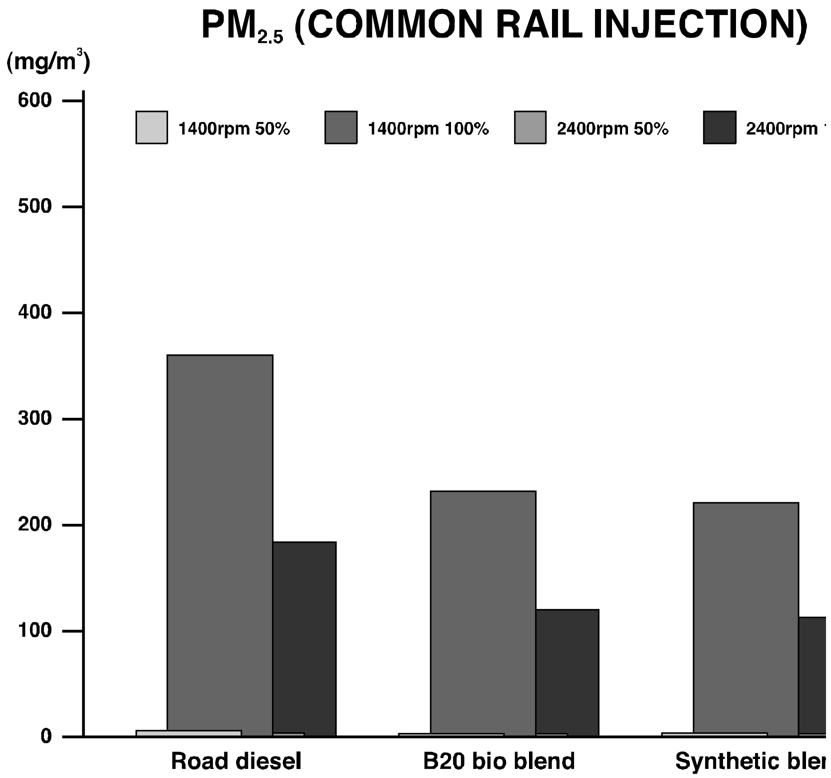


Surawski et al. Environ Sci Technol (2010) vol. 44 (1) pp. 229-235

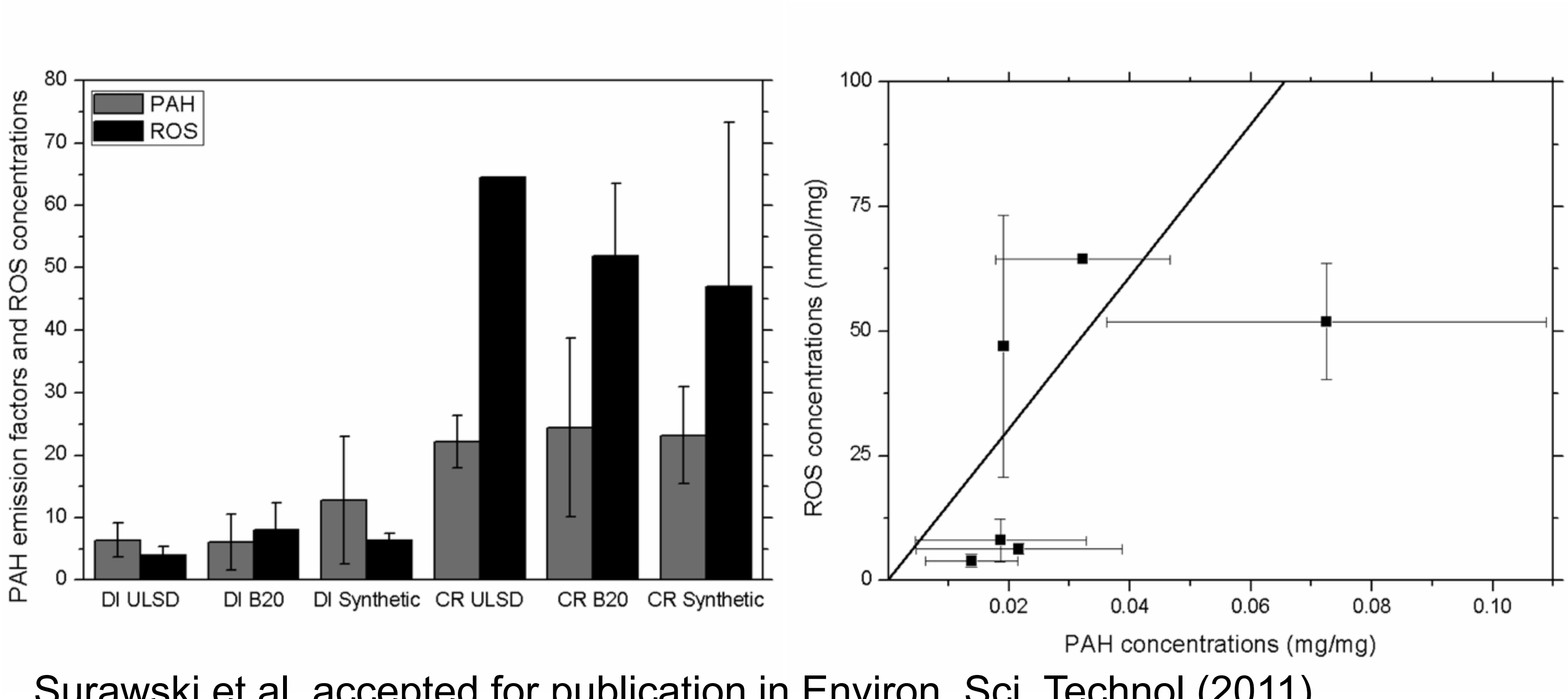
$I_{485nm}(ROS_{particle})$ = 4.5 x
$I_{485nm}(ROS_{particle})$

Two injection technologies and three fuels (Diesel, B20, FT blend)



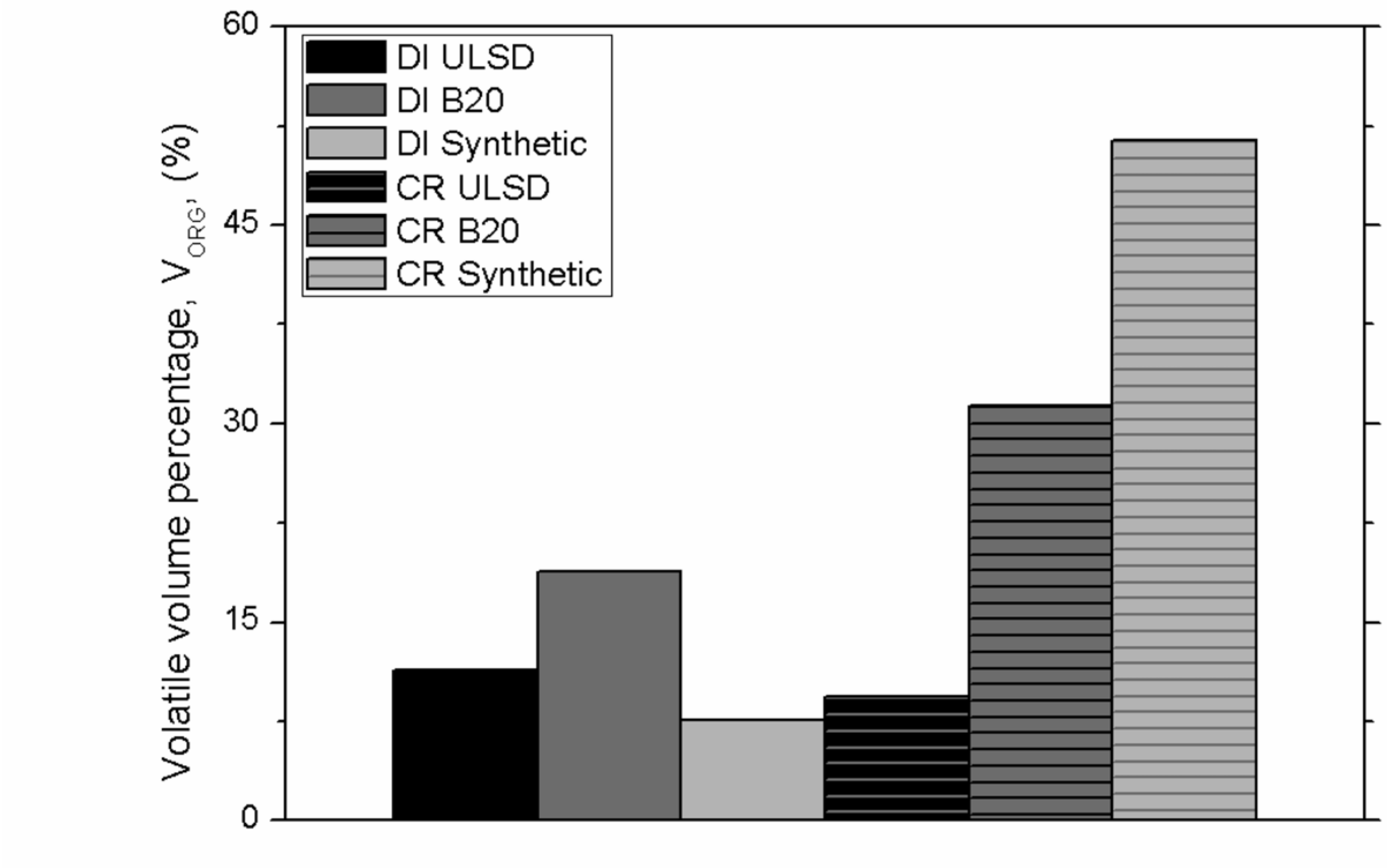


Two injection technologies and three fuels (Diesel, B20, FT blend)



Surawski et al. accepted for publication in Environ. Sci. Technol (2011)
DOI: 10.1021/es200388f

Two injection technologies and three fuels (Diesel, B20, FT blend)



Influence of biodiesel feedstock

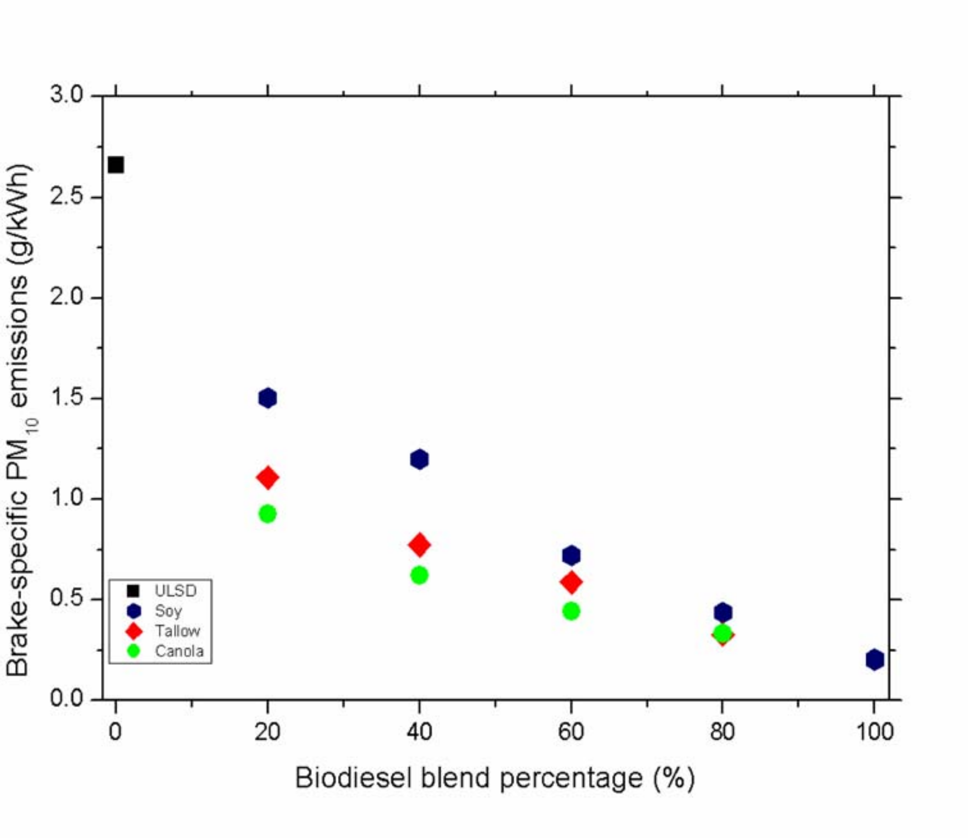
3 different feedstock's:

- Soy
- Tallow
- Canola
- 20, 40, 60, 80 and 100% (only soy)

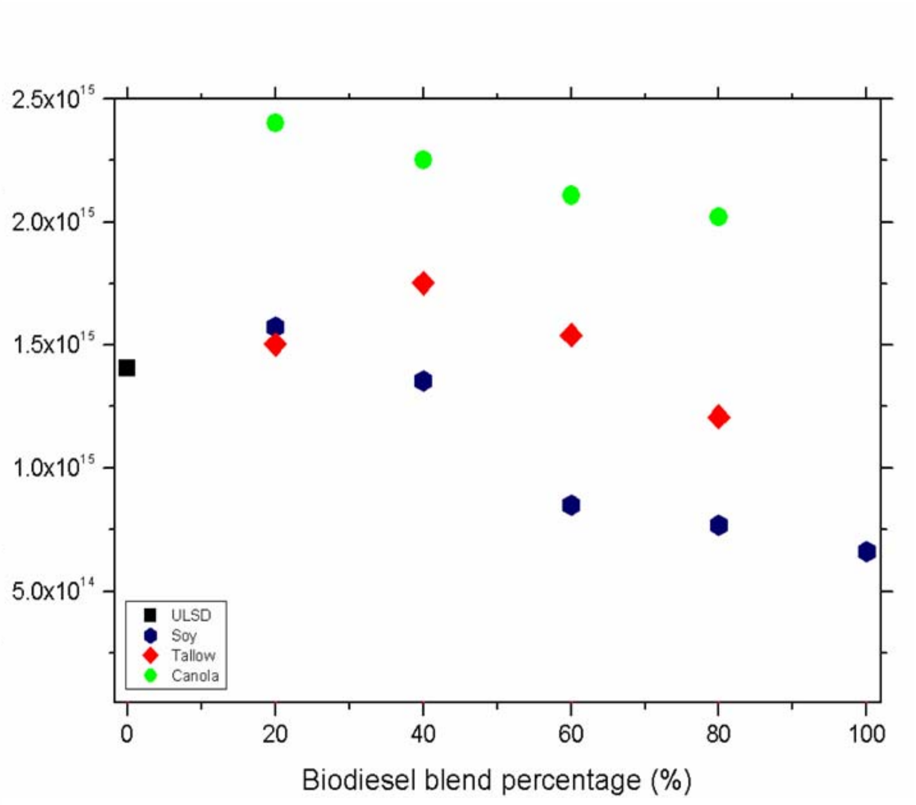
Measured:

- Particle mass, number and surface (NSAM)
- PAH, ROS (only for 20% and 80% blends)
- Organic (volatile) volume percentage

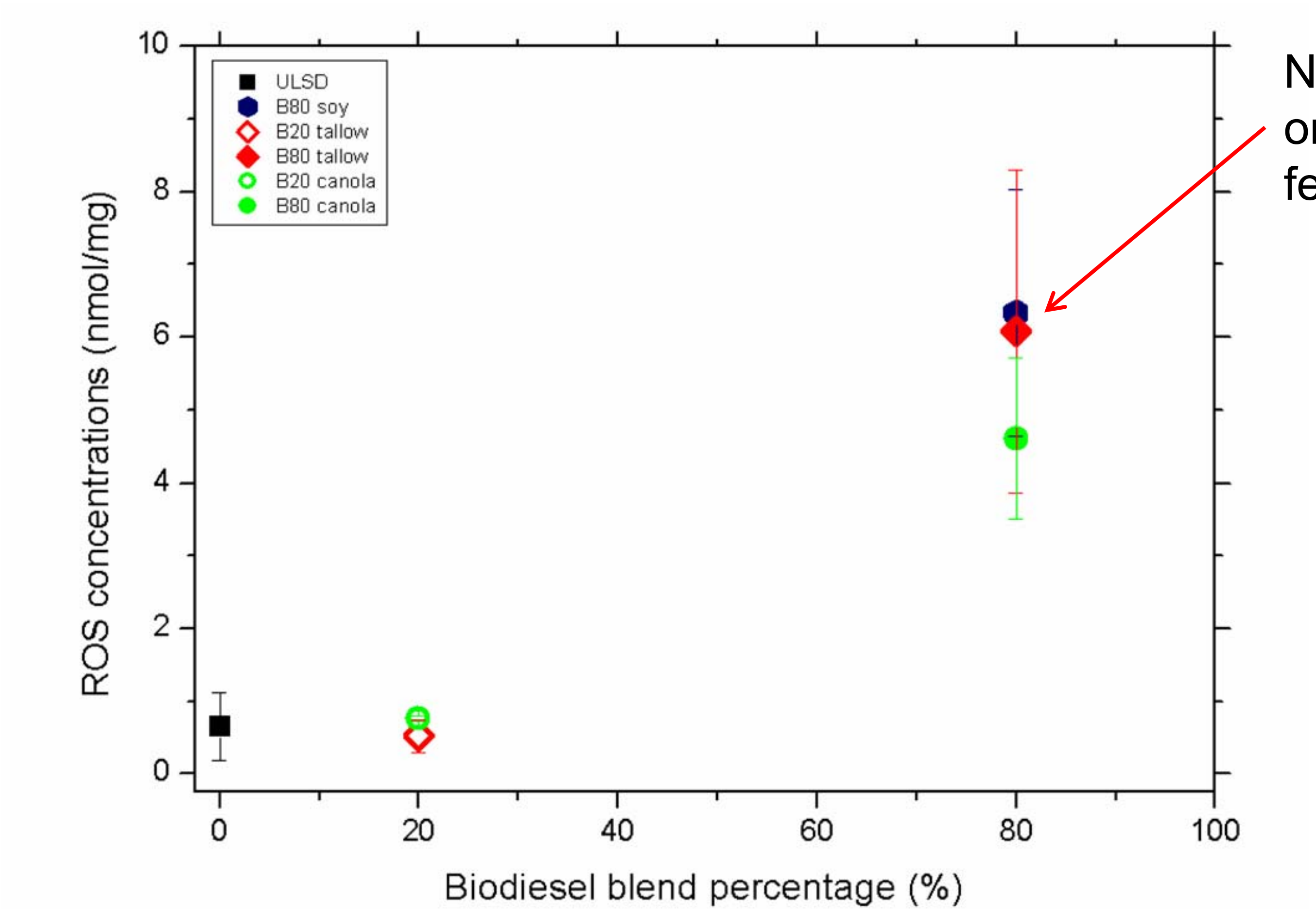
Particle mass



Particle number

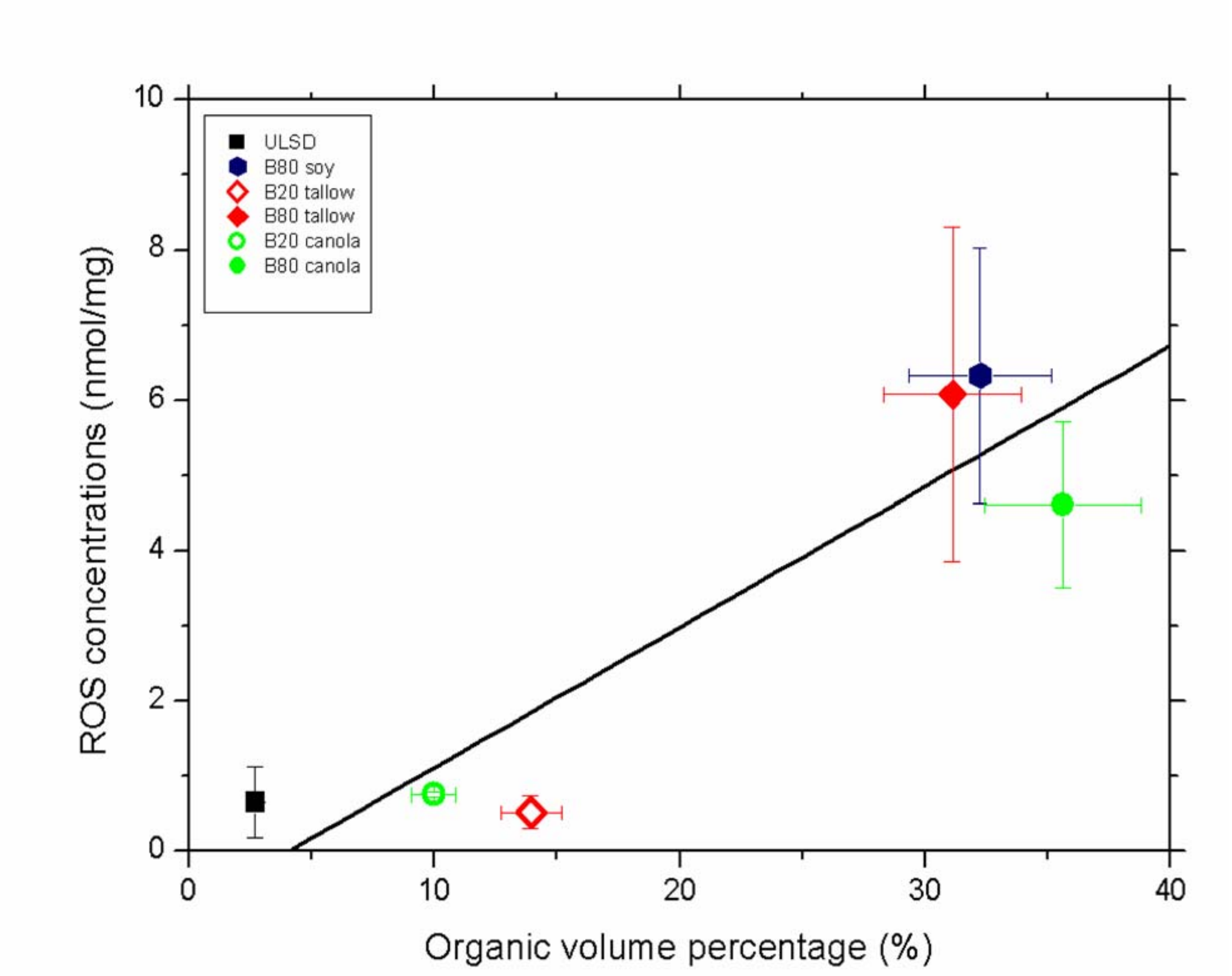


ROS concentrations



No dependence on the feedstock

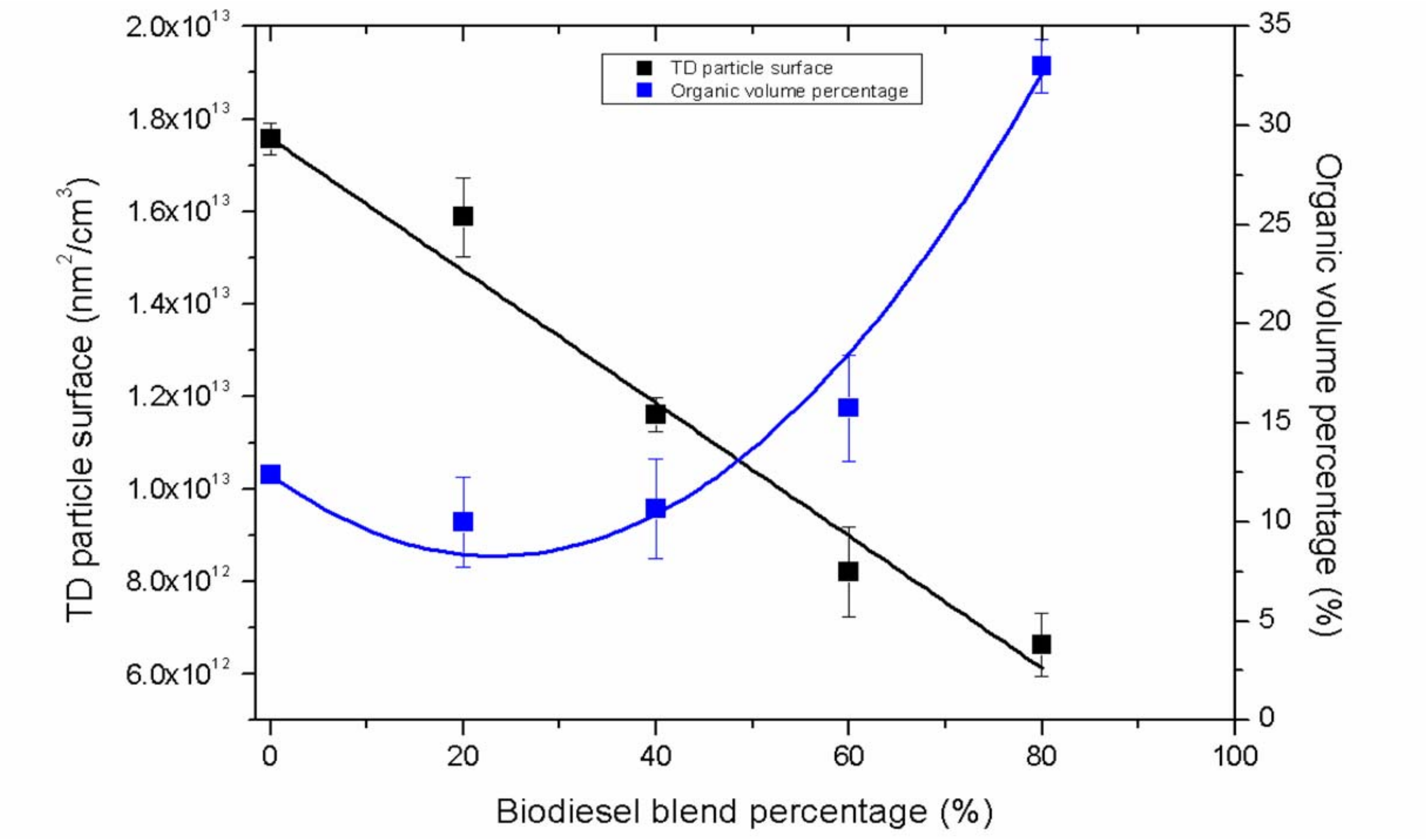
ROS v.s. organic volume percentage



Consequences

- In underground mines or tunnels the ventilation rate is limited.
- Very often the air quality limits the number of vehicles that can be used and therefore the productivity.
- If the biodiesels were used due to their much smaller mass emissions they would have enabled a larger number of vehicles to be used until the ambient PM mass level would reach the maximum allowed.
- Although we would have the same ambient PM the particles would have a much larger oxidative capacity – more toxic.

Particle Surface area as a metric?



Surawski et al. submitted to Environ. Sci. Technol (2011)

- Similar results on the role of organic/volatile fraction on particle oxidative capacity also observed by others (see for example Biswas et al, EST, 2009) but with a different probe DTT.
- A decrease in particle mass/surface emission is very often followed by an increase in the volatile particle component – increase in the oxidative capacity of particles.
- Regulating only physical metrics mass/number/surface area would not be able to detect results such as those presented here.
- Not only the raw surface area of particles but also the surface chemistry of particles is important for assessing the health impacts of DPM.

Acknowledgement

- SkillPro Services Pty Ltd
- Australian Coal Association Research Program for funding project C18014
- Australian Research Council Center for Free Radical Chemistry and Biotechnology.