Comparative toxicological evaluation of SI and CI engine exhausts in an in vitro model of rat lung slices.

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Context of the study

Major discrepancies in terms of PM induced toxicity profiles are recorded in in vivo experiments between inhalation and instillation modes of exposure to particulate matter.

The most probable explanation are :

- Alteration of PM size distribution during PM sampling and resuspension due to PM aggregate formation
- Adsorption of pollutant condensates from gas phase during PM sampling.
- Alteration of pollutant biodisponibility by liquid suspension procedures involving the use of tensio active agents to stabilize the suspension

These above phenomenom occur in studies dealing with PM instillation while they are lessened or absent during continuous flow inhalation studies as discussed in Bion et al. Cell Biol Tox 2002; 18: 301-314.

The relevance to the in vivo situation of in vitro toxicity studies with toxic atmospheres, suffers from the same discrepancies between experiments where PM are solubilized in culture media using tensio active agents (mimicking instillation) and studies where biological systems are exposed in a bi-phasic air/liquid systems to continuous flows of complex atmospheres (mimicking more closely inhalation).

A second point to be considered for in vitro studies is the relevance of PM concentrations used to be in contact with the biological targets. We made calculations showing that maximal alveolar deposition rate would be in the range of 5-10 pg/cm²/minute (ca. 2 µg in 150 m² of lung alveolar epithelium) and that this deposition will occur essentially by diffusion mechanisms for PM with aerodynamic diameters of 50-100 nm. Usually, in vitro studies dealing with medium suspended PM are performed with 10-100 µg/cm² of epithelial cells which are unrealistic concentrations corresponding to the total PM deposition of ca. 1-5 m^3 of urban air PM10 per square cm²of alveolar epithelium!.

Methods

These previous points lead us to develop a model of bi-phasic organotypic cultures of rat lung tissue to be exposed to a continuous flow of diluted engine exhausts drawn continuopusly from the exhaust pipe of engines driven on test benches. (Presented at the 4th ETH conference on nanoparticle measurement, Zürich 2000).

This model offers the advantage of preserving the physicochemical properties of ehaust components and to very closely mimick the exhaust/lung tissue interactions occurring mainly by diffusion for ultrafine particles. LePrieur et al. Archives Toxicology 2000 74 :460-466.

This model is pecularly suitable for testing the biological safety of exhaust after treatment stategies since exhaust may be simultaneously studied in parallel drawn from two different locations of engine exhaust pipe.

Results

Nanoparticle measurements

In this study, we report the toxicity profiles induced by either gasoline or diesel engines, the impact of exhaust filtration for both engine types and the impact of 3 way catalysis on gasoline exhaust line.

Comparison of gasoline and diesel engine exhausts show that PM size distribution differ significantly : mean aerodynamic diameter being smaller for gasoline compared to diesel.

Gasoline exhaust PM size distribution curve may be decomposed as the sum of two PM populations with respective aerodynamic diameters of 30 nm (condensation aerosol) and 80 nm (soot). Filtration of exhausts with cordierite filter is very efficient on both PM population with 4 and 2 order of magnitude efficacy on 30nm and 80nm populations respectively.3 way catalysis of gasoline exhaust removed almost totally the 30nm PM population while it did not modify the number and size of the 80 nm PM population. Filtration efficacy of diesel exhaust was by two order of magnitude.

Toxicity studies

Gasoline engine exhausts:

uncatalysed gasoline exhaust exert their toxic action essentially by the gaseous phase, filtration did not modify at all the exhaust toxicity.

3way catalysis almost totally suppressed the toxic potential of the exhaust. Exhaust PM after 3 way catalysis is low in concentration and did not exert any significant toxicity under our experimental condition

Diesel engine exhausts :

Exhaust filtration allowed to suppress the inflammatory reaction and DNA alterations observed after exposure to total (PM+ gaseous phase) exhaust, thus showing the major contribution of PM to these adverse effects.

Diesel exhaust gaseous phase may exert some action on oxidative stress since exhaust filtration only partially withdraw the action of total exhausts on oxidative stress.

Conclusions

Although we could not demonstrate toxic effects of SI engine exhausts after 3 way catalysis, these engine may contribute significantly to the PM concentrations in urban aerosols, especially when 3way catalysis is not present or efficient (cold start). It is important to notice that under loaded conditions these engine emit PM aerosol of similar size distributions as diesel soot.

For diesel engines, we confirm that two of the major adverse health effects namely inflammatory reaction and DNA alteration are due to PM fraction of the exhausts. These observations support the development of exhaust treatment strategies reducing the number of PM in the exhausts. In this respect, exhaust filtration or retrofitting should be widely encouraged as well for on road and off road engines.

Comparative toxicological evaluation of gasoline and diesel engine exhausts in an in vitro model of rat lung slices.

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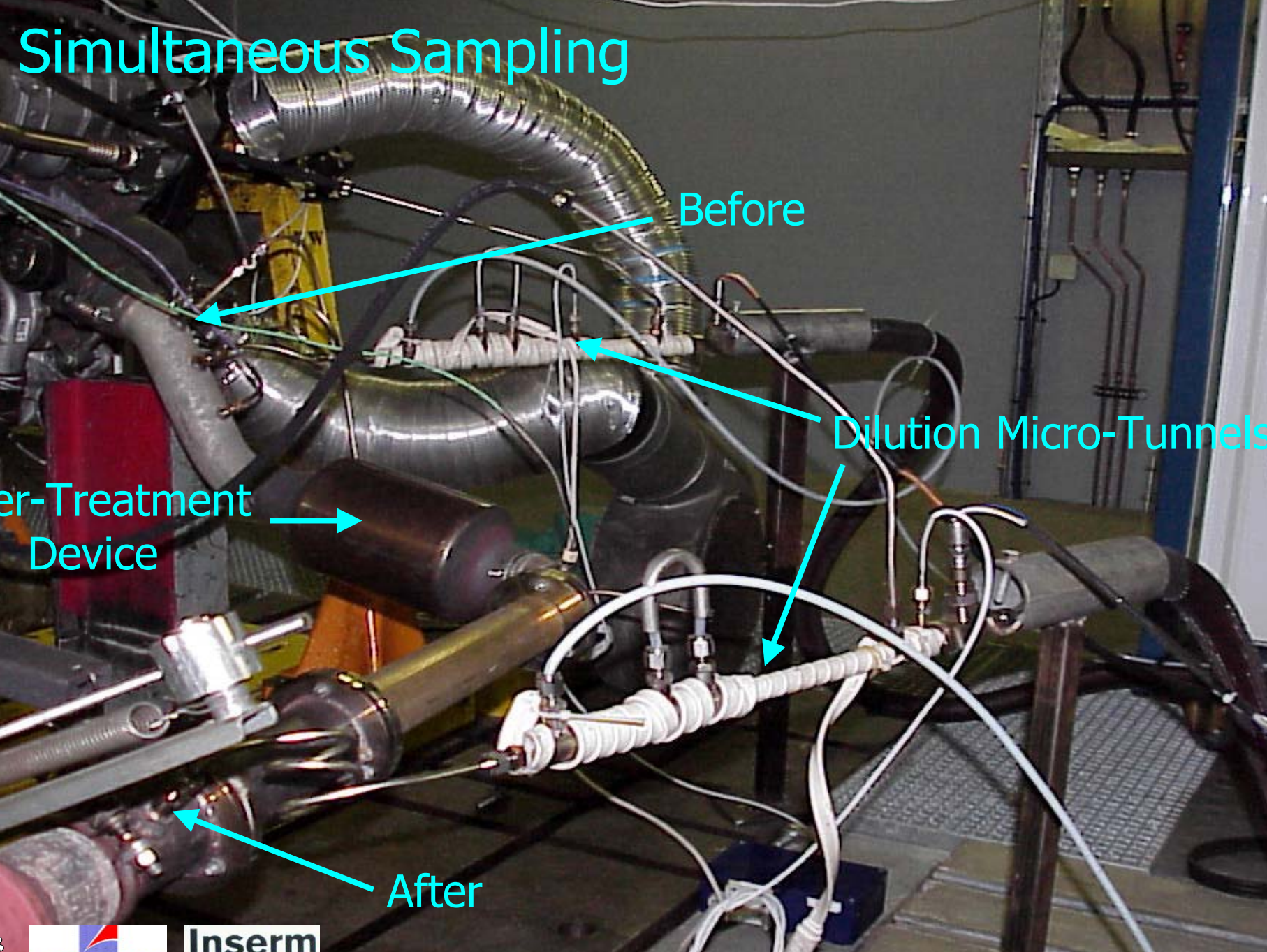
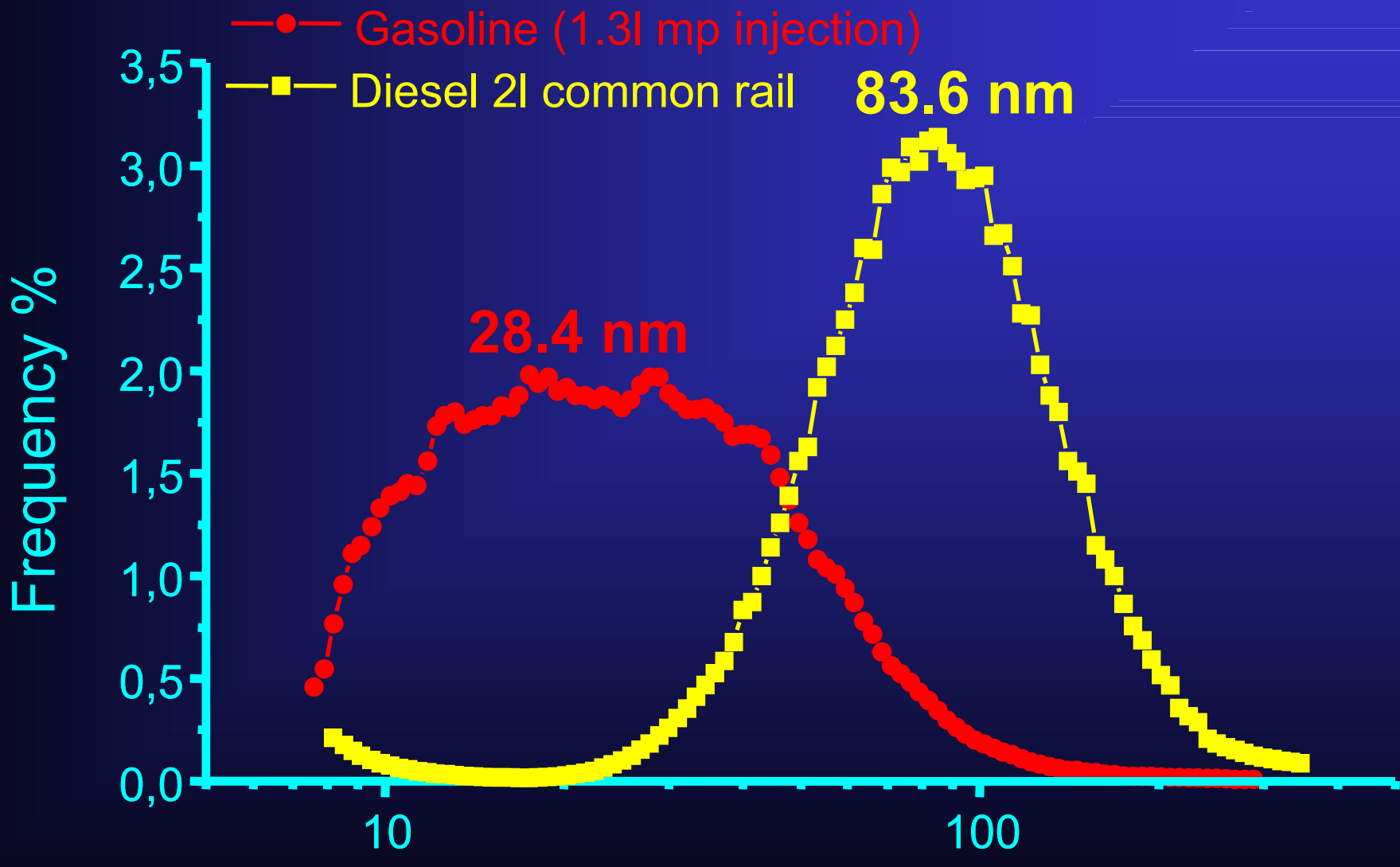
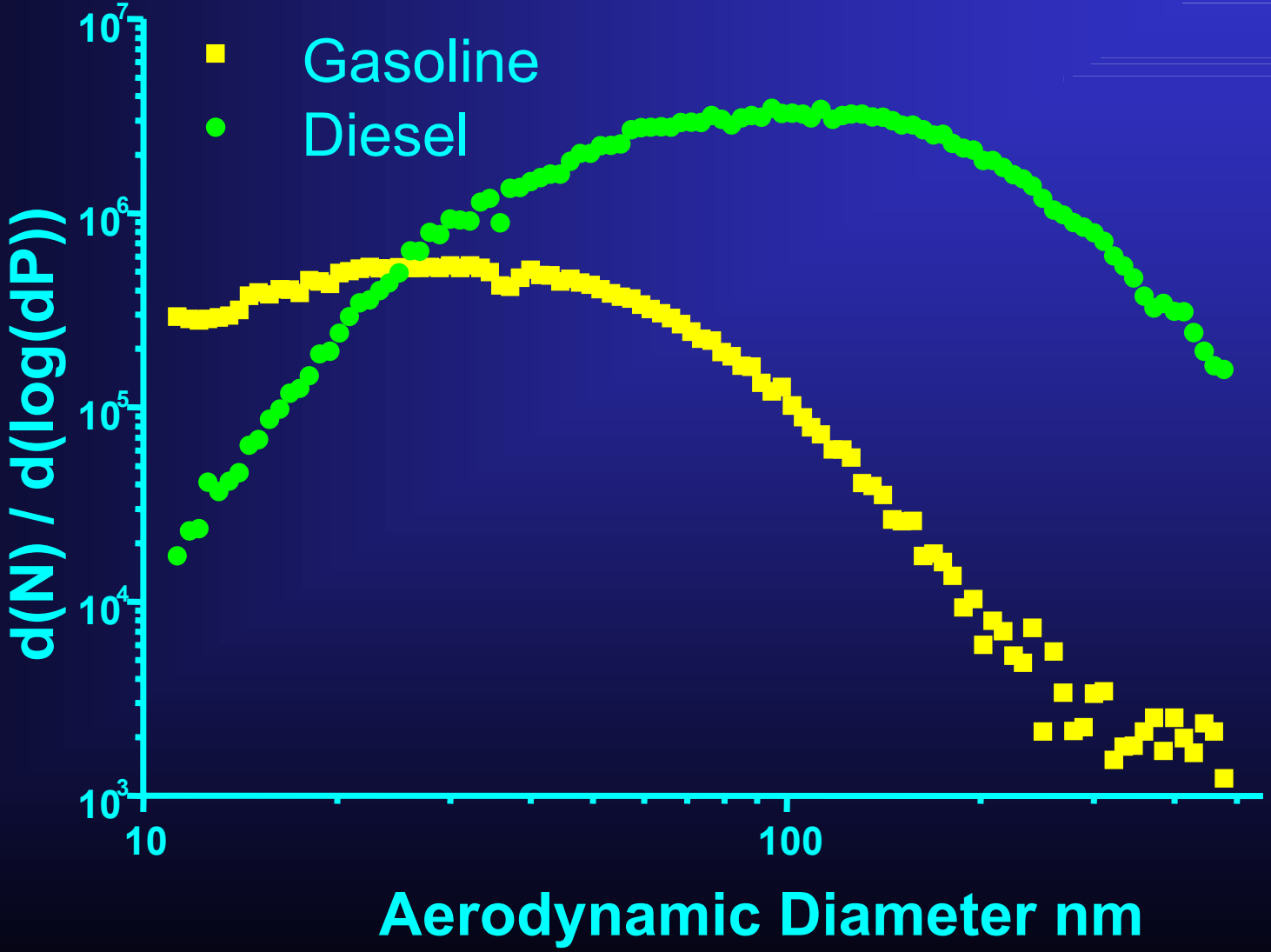

Simultaneous Sampling
Before
Dilution Micro-Tunnels
er-Treatment
Device
After

Comparison of standardised distributions without after treatment

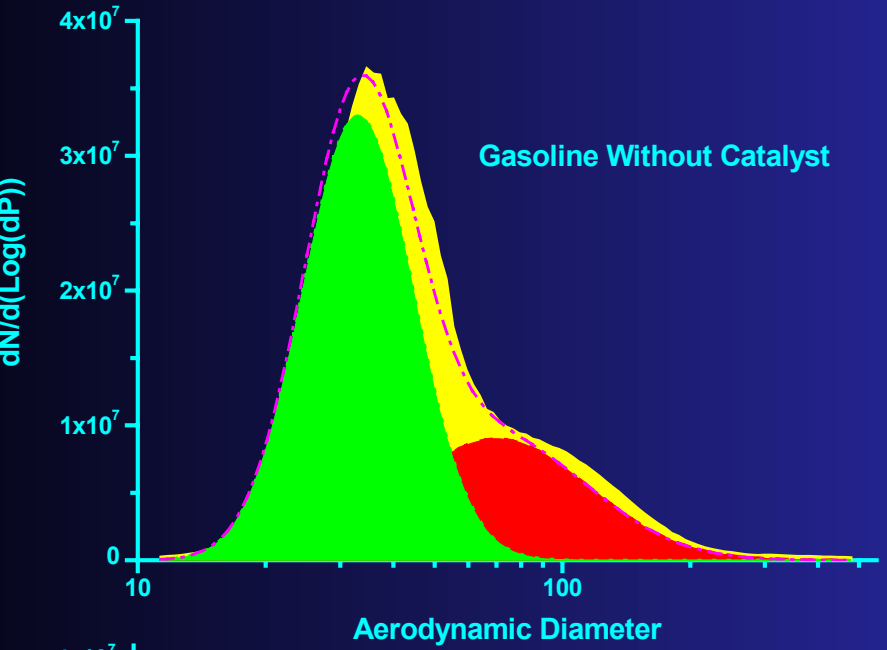


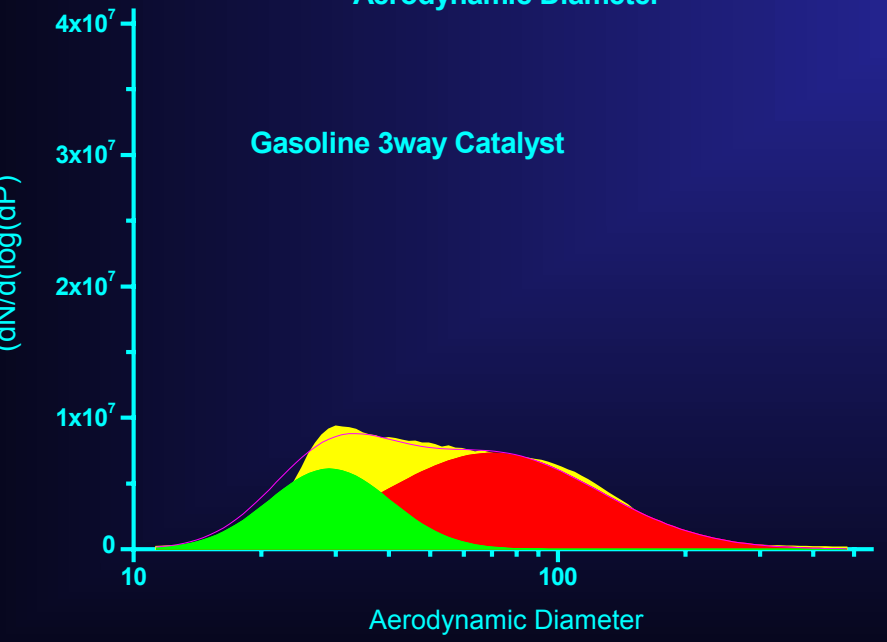
Comparative Particle Numbers and Size Distributions In Engine Exhausts

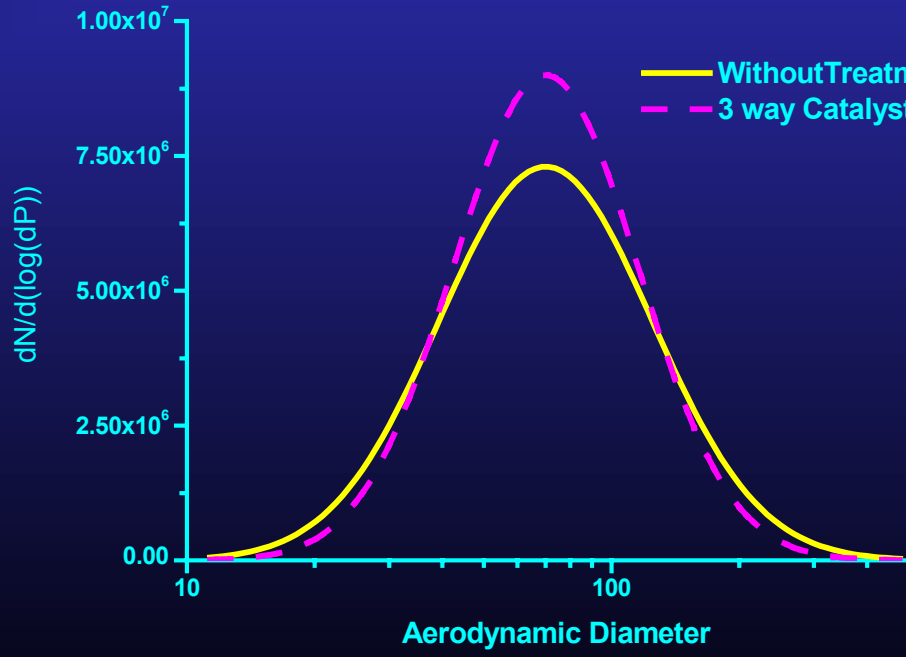


Impact of 3 way Cataly on PM Number and Size Distribution

2l gasoline engine MP injectio







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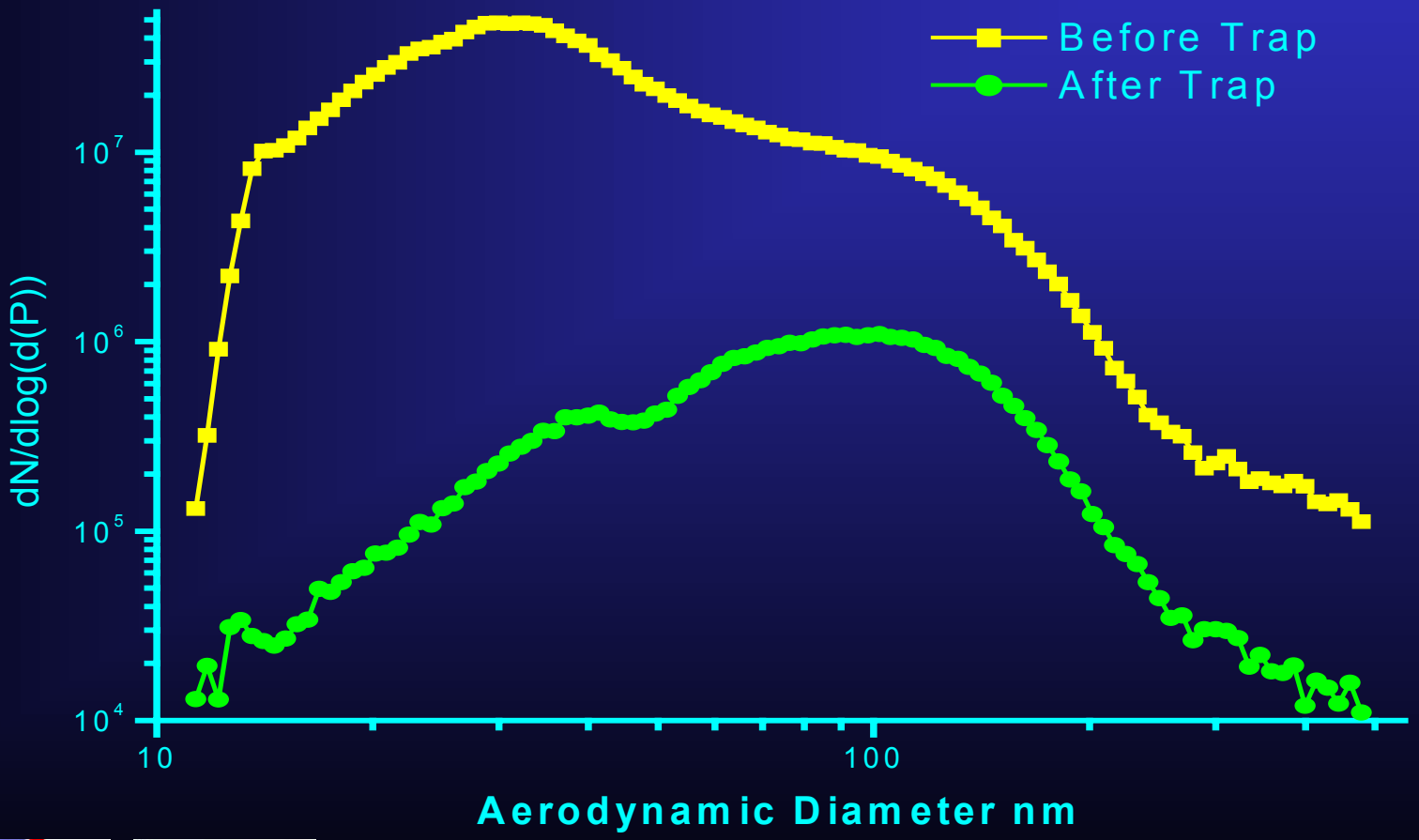
Impact of Cordierite Filter on PM Number and Size Distribution 2l Gasoline engine



Toxicology Experimentation
Engine Test Cell

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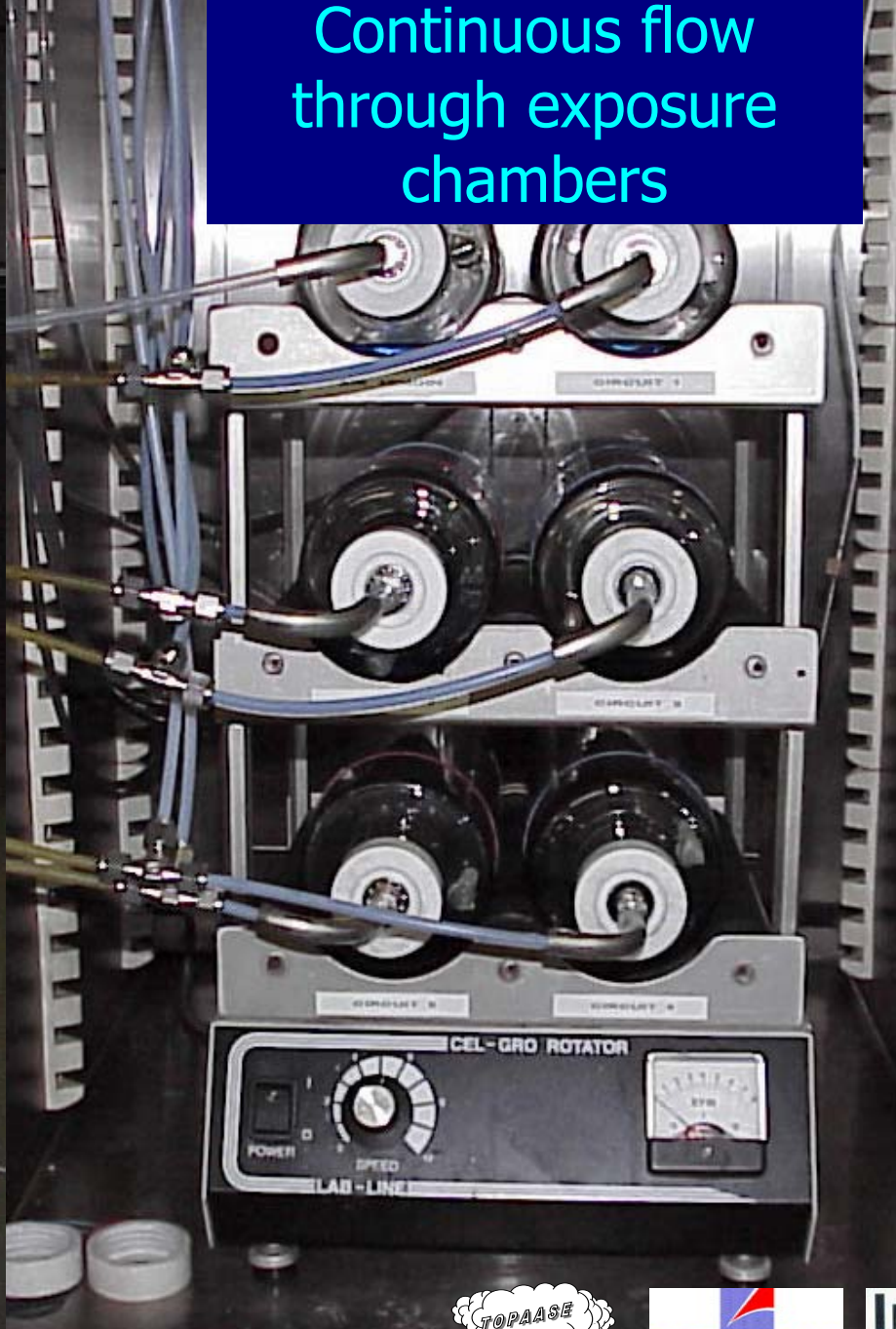
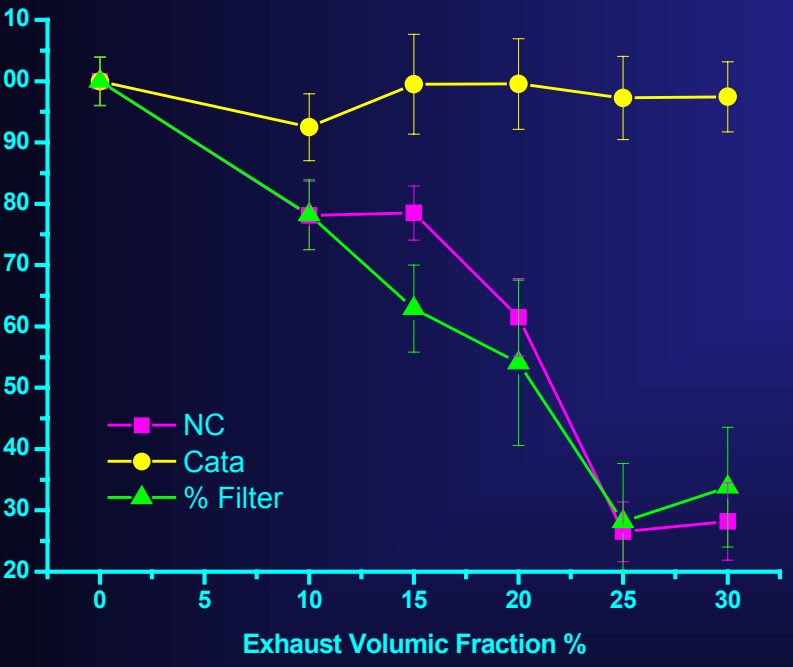
Flow rate regulation
For exhaust dilution control



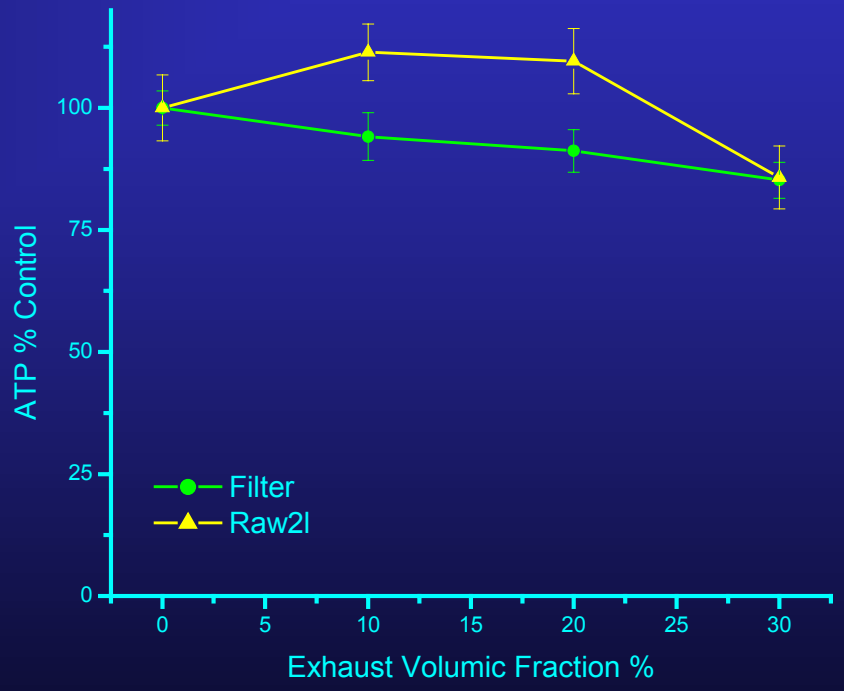
Continuous flow through exposure chambers

Cell Viability Assessment

Gasoline



Diesel



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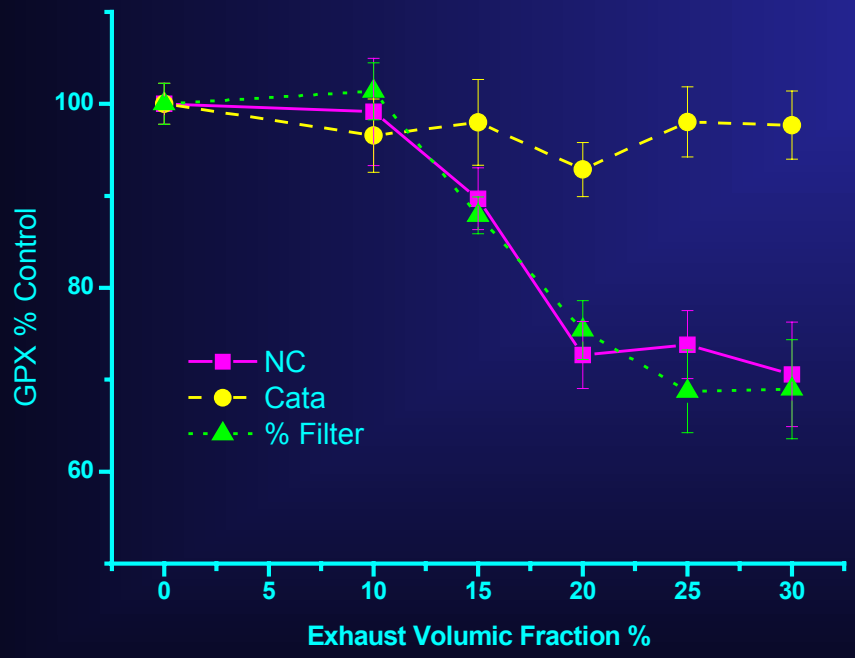
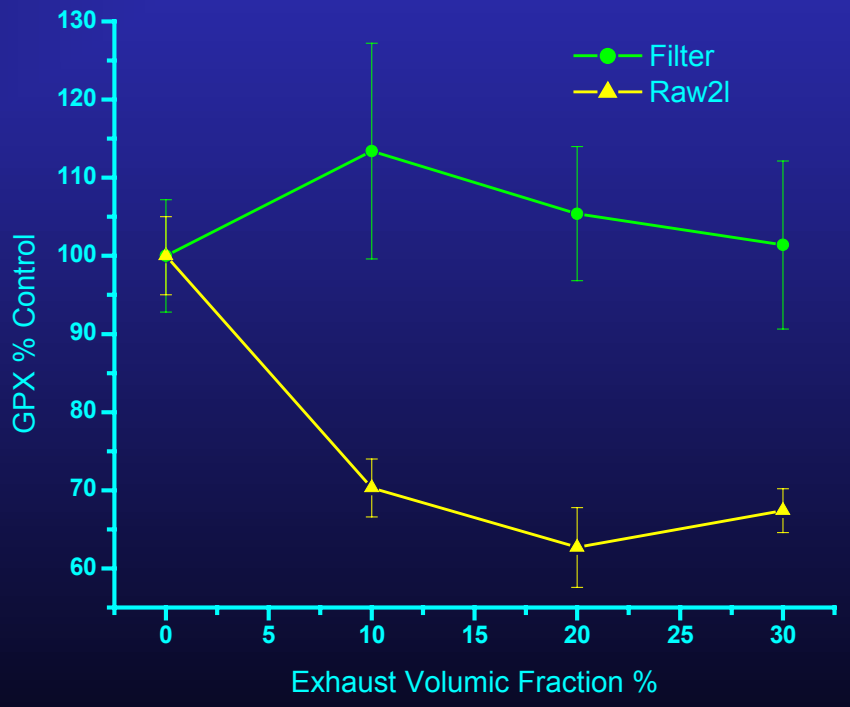
Oxidative Stress Assessment

Gasoline



Diesel



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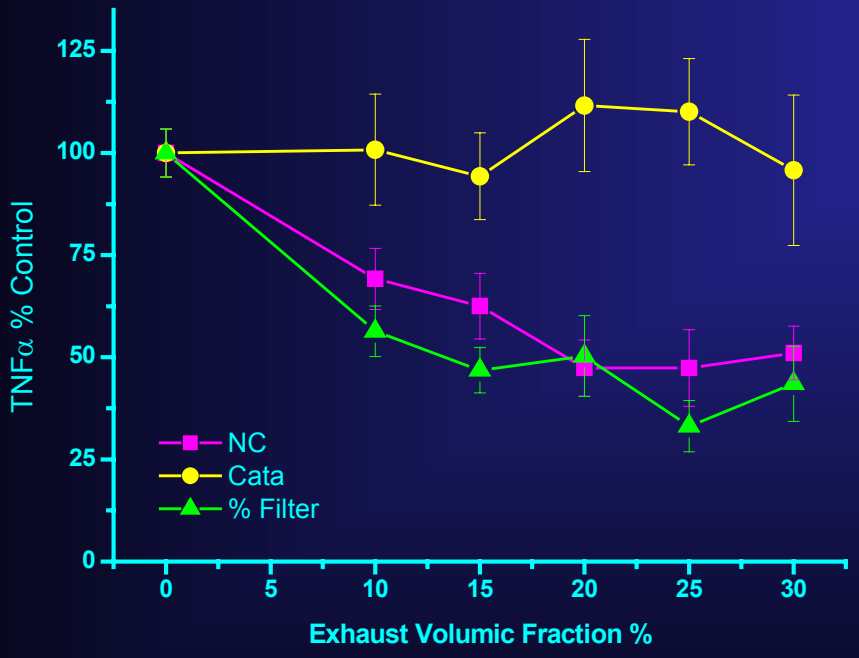
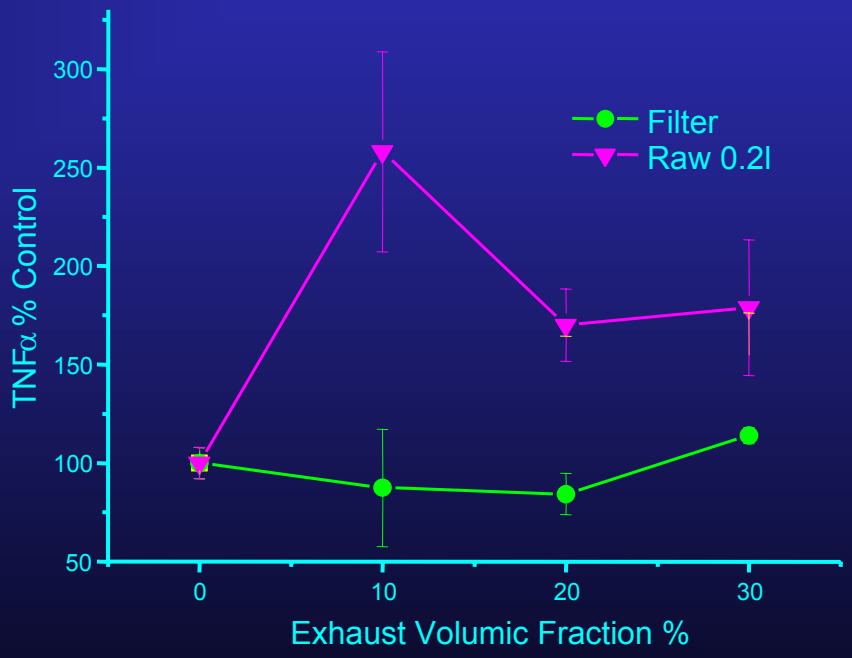
Inflammatory Reaction Assessment

Gasoline



Diesel



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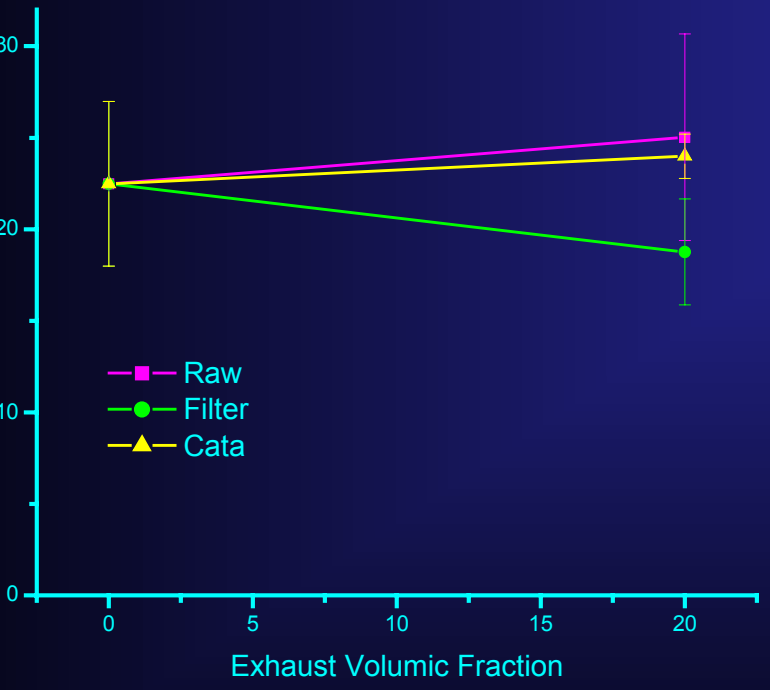
DNA Alteration Assessment

Gasoline



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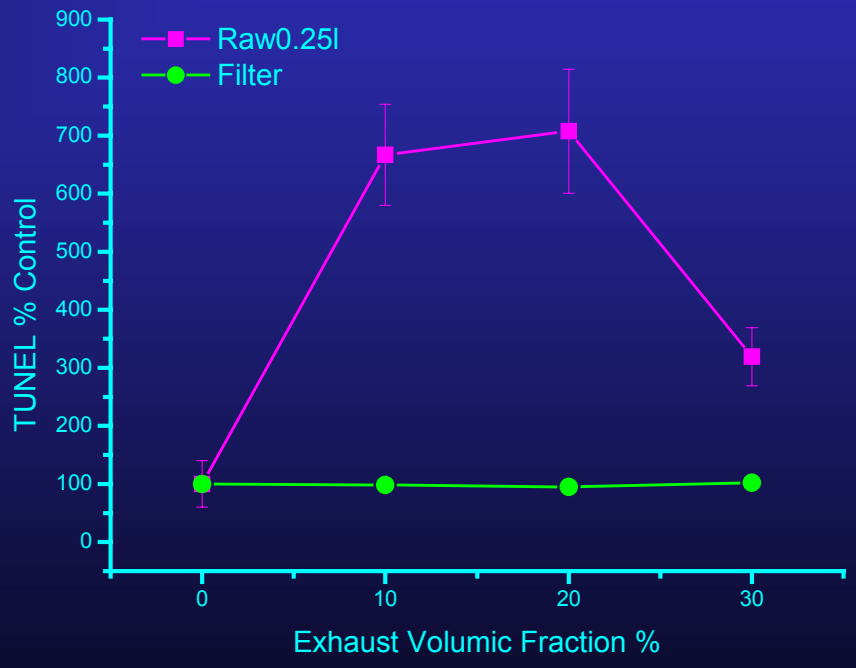

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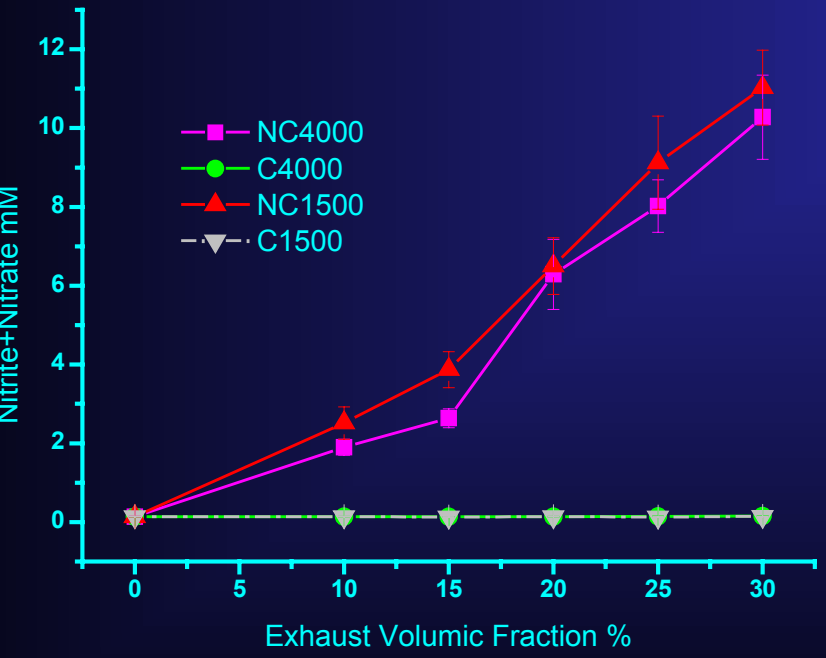
Nitrogen Oxide Dissolution in Culture Medium

Gasoline



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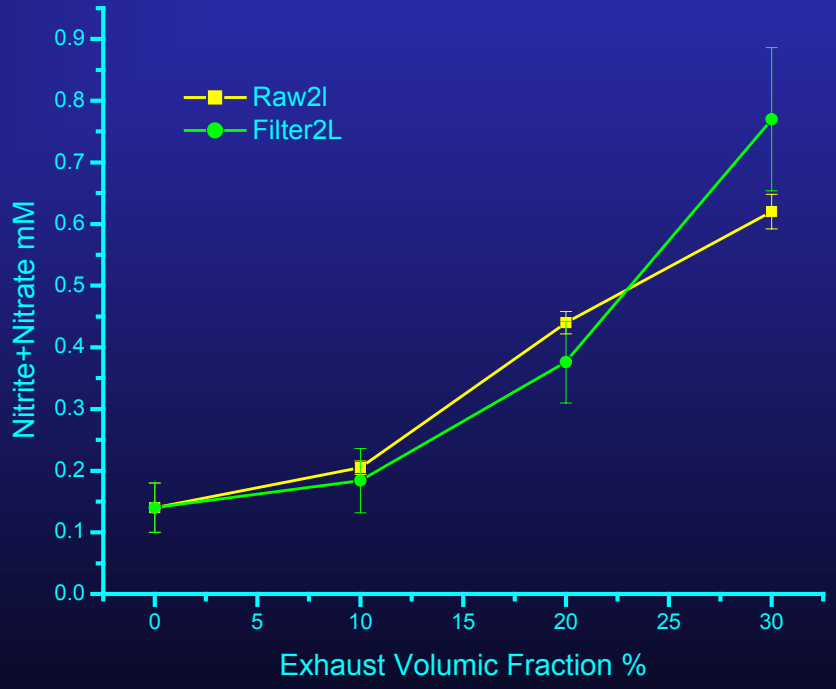

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Discussion Particulate Matter

Gasoline :

major liquid aerosol 30 nm + minor Soot 80 nm
3 way catalysis suppresses the 30 nm aerosol
Particulate trap 3-4 order magnitude on 30nm aerosol
2 order of magnitude on soot

Diesel :

majority of soot 80-100 nm according to dilution ratio
Oxidation catalysis little effect on soot
Particulate trap 2 order of magnitude on soot

Discussion Gazeous Phase

Gasoline :

Without Catalysis :

40 000 ppm CO, 2000 ppm Nox

3 Way Catalysis

Almost complete suppression

Diesel :

Without Catalysis

200 ppm CO, 550 ppm Nox

Oxidation Catalysis

Almost Complete CO suppression

No action on total Nox

Increase of NO_2/NO ratio

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Discussion Toxicity

Cell Viability

Not impaired by diesel

Impaired by uncatalysed gasoline gaseous phase

Oxidative Stress

Essentially impaired by diesel PM

Impaired by uncatalysed gasoline gaseous phase

Inflammation

Increased by diesel PM

reased by cytotoxicity of uncatalysed gasoline gaseous ph

DNA Alteration

Increased by diesel PM

No effect of Gasoline exhausts

Conclusion

We failed to demonstrate Gasoline PM Toxicity
Low amount of soot ? Inoccuity of 30nm Aerosol ?

Necessity to improve analytical knowledge
on PM Quality
Liquid versus Solid Aerosols ?

Due to Technology Improvement for PM Emissions
Necessity to consider the Gaseous phase of Engin
Exhausts for Biological Safety Assessment

Global exhaust safety assessment