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 phenomenon 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 m³ of urban air PM10 per square cm² of 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 exhaust 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 peculiarly suitable for testing the biological safety of exhaust after treatment strategies 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

After-Treatment Device

After

Dilution Micro-Tunnels

E-9920 ROUEN
Comparison of standardised distributions without after treatment

- Gasoline (1.3l mp injection)
- Diesel 2l common rail

Aerodynamic Diameter nm

Frequency %

- 83.6 nm
- 28.4 nm
Comparative Particle Numbers and Size Distributions In Engine Exhausts

Gasoline

Diesel

Aerodynamic Diameter nm

$d(N)/d(\log(dP))$
Impact of 3 way Catalysis on PM Number and Size Distribution

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

Before Trap
After Trap

Aerodynamic Diameter nm
Toxicology Experimentation
Engine Test Cell
Continuous flow through exposure chambers

Flow rate regulation
For exhaust dilution control
Cell Viability Assessment

Gasoline

Diesel

Cell Viability Assessment

Gasoline

Diesel
Oxidative Stress Assessment

Gasoline

Diesel

Exhaust Volumic Fraction %

GPX % Control

- NC
- Cata
- % Filter

Exhaust Volumic Fraction %

GPX % Control

Filter
Raw2l
Inflammatory Reaction Assessment

Gasoline

Diesel

Exhaust Volumic Fraction %

TNFα % Control

NC
Cata
% Filter

- E-9920 ROUEN
DNA Alteration Assessment

Gasoline

- Positive Nuclei/field vs Exhaust Volumic Fraction
  - Raw
  - Filter
  - Cata

Diesel

- TUNEL % Control vs Exhaust Volumic Fraction %
  - Raw0.25l
  - Filter
Nitrogen Oxide Dissolution in Culture Medium

Gasoline

- NC4000
- C4000
- NC1500
- C1500

Diesel

- Raw2L
- Filter2L
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 Gaseous 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 NO₂/NO ratio
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
- Decreased by cytotoxicity of uncatalysed gasoline gaseous phase

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 Engine
Exhausts for Biological Safety Assessment

Global exhaust safety assessment