# *Diesel Emission Induced Lung Toxicity Responses*

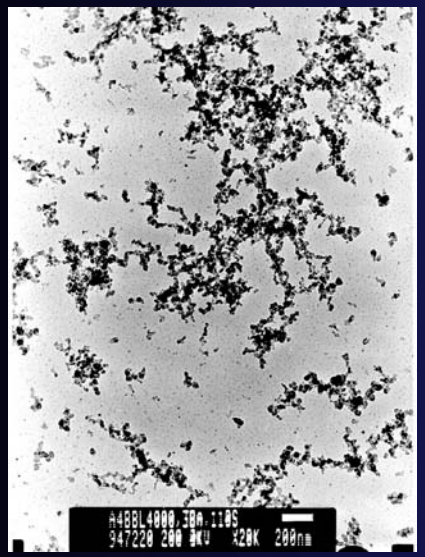
## *$NO_2$/NOx ratio versus Particulate Matter*

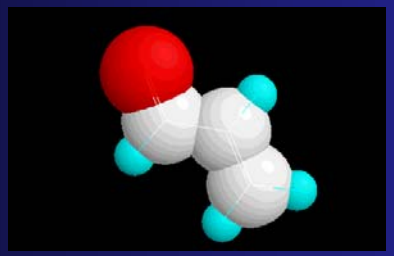
*Jean-Paul MORIN*

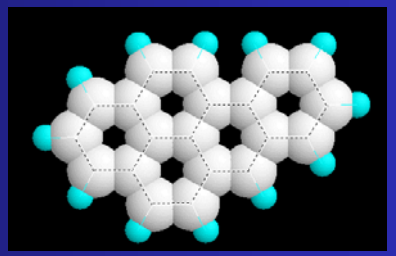
*Inserm U644*
*Université de Rouen - France*

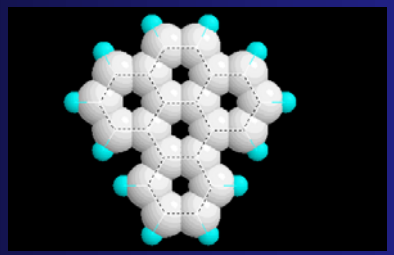
Inserm

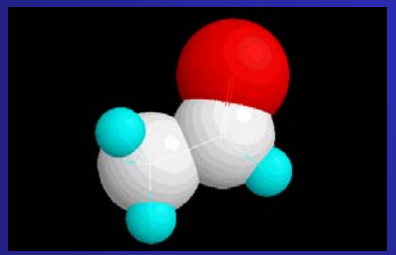
# Impact of Complex Aerosols on Lung Tissue

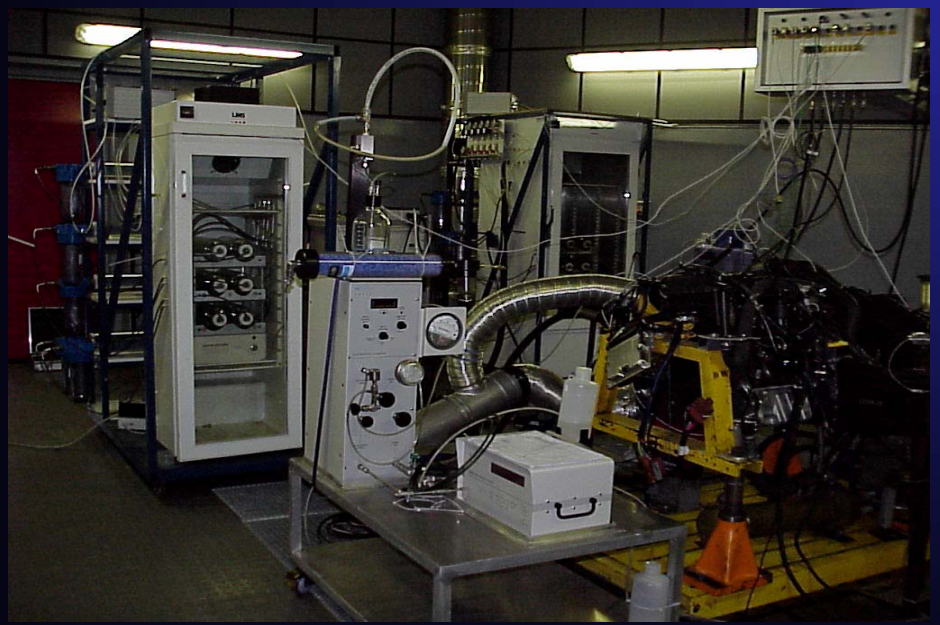


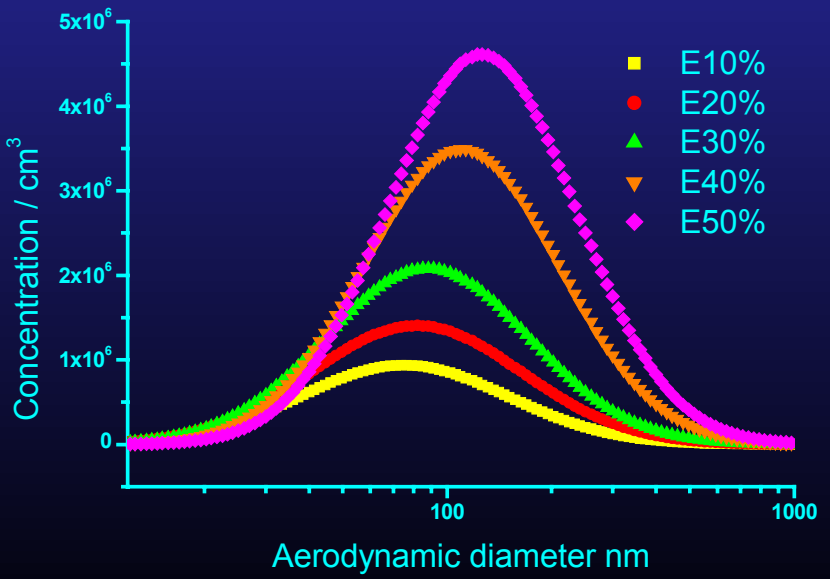








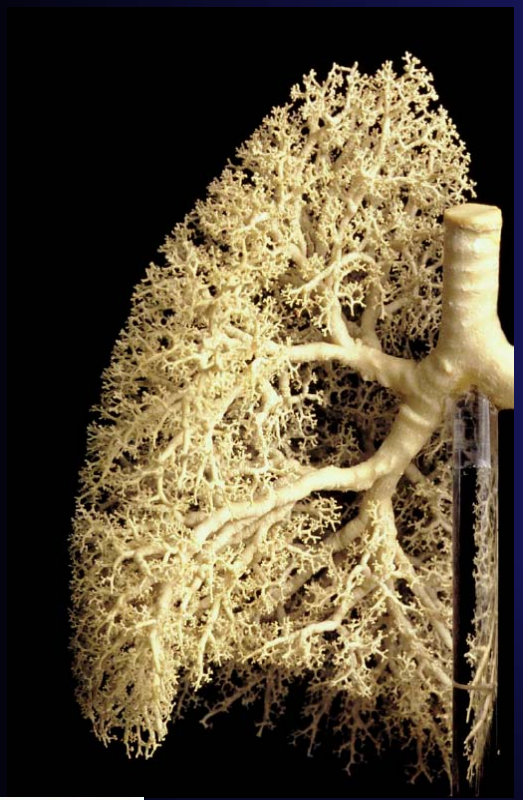





CERTAM

Inserm
U644 ROUEN

# Respiratory tract Gas Velocity and PM deposition



Changement de direction
**Impaction**

**Extra-thoracic**

**Diffusion**

>2 µm
<20 nm

**Tracheo-bronchial**

**Impaction**

↗ Section area
↘ Gas Velocity
**Sedimentation**

**Alveoli**

Velocity Close to Null
**Diffusion**

<100 nm
>20 nm

**Hydrophilic**

**Hydrophilic (Mucus)**

**Hydrophobic (Surfactant)**

**Gas Velocity**

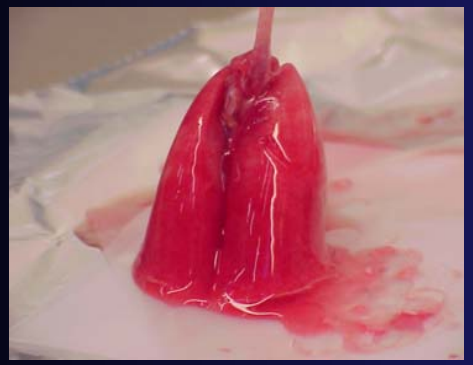
**Section Area**

Inserm

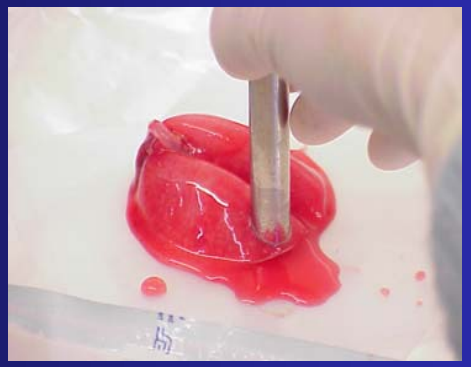
CERTAM

# Rat Lung Slices Preparation And Dynamic Air/Liquid Culture

Agarose filled lungs



Peripheral lung coring



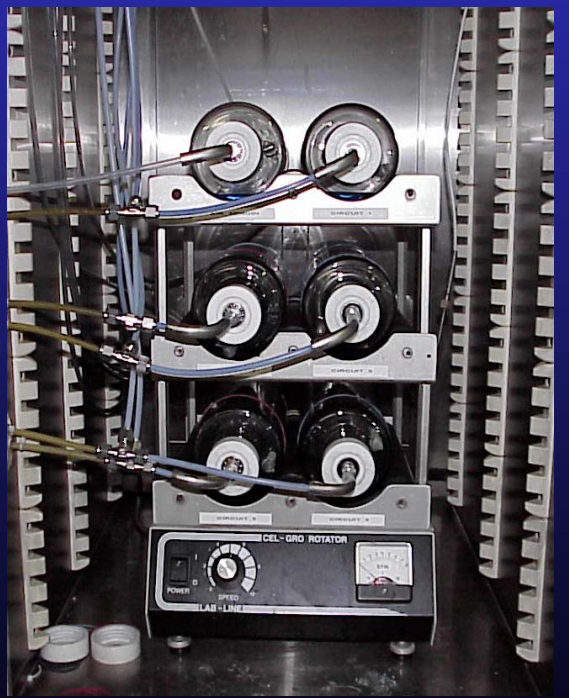
Brendel/Vitron Slicer

Diam 8mm - Thick 1500µm

Opened cap vials

Slices on Cylindrical Inserts

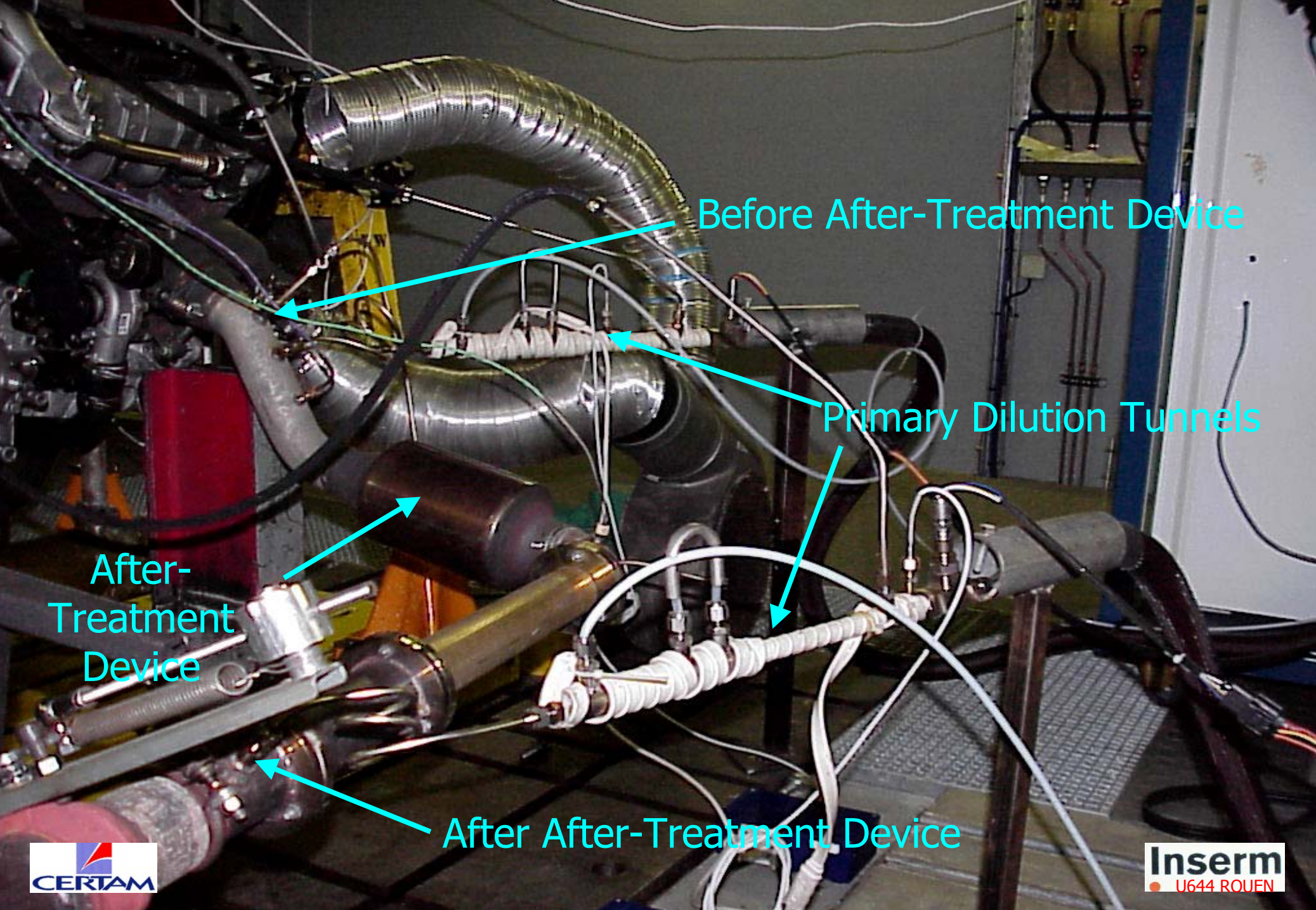
Flow-through Rotating Exposure Chambers



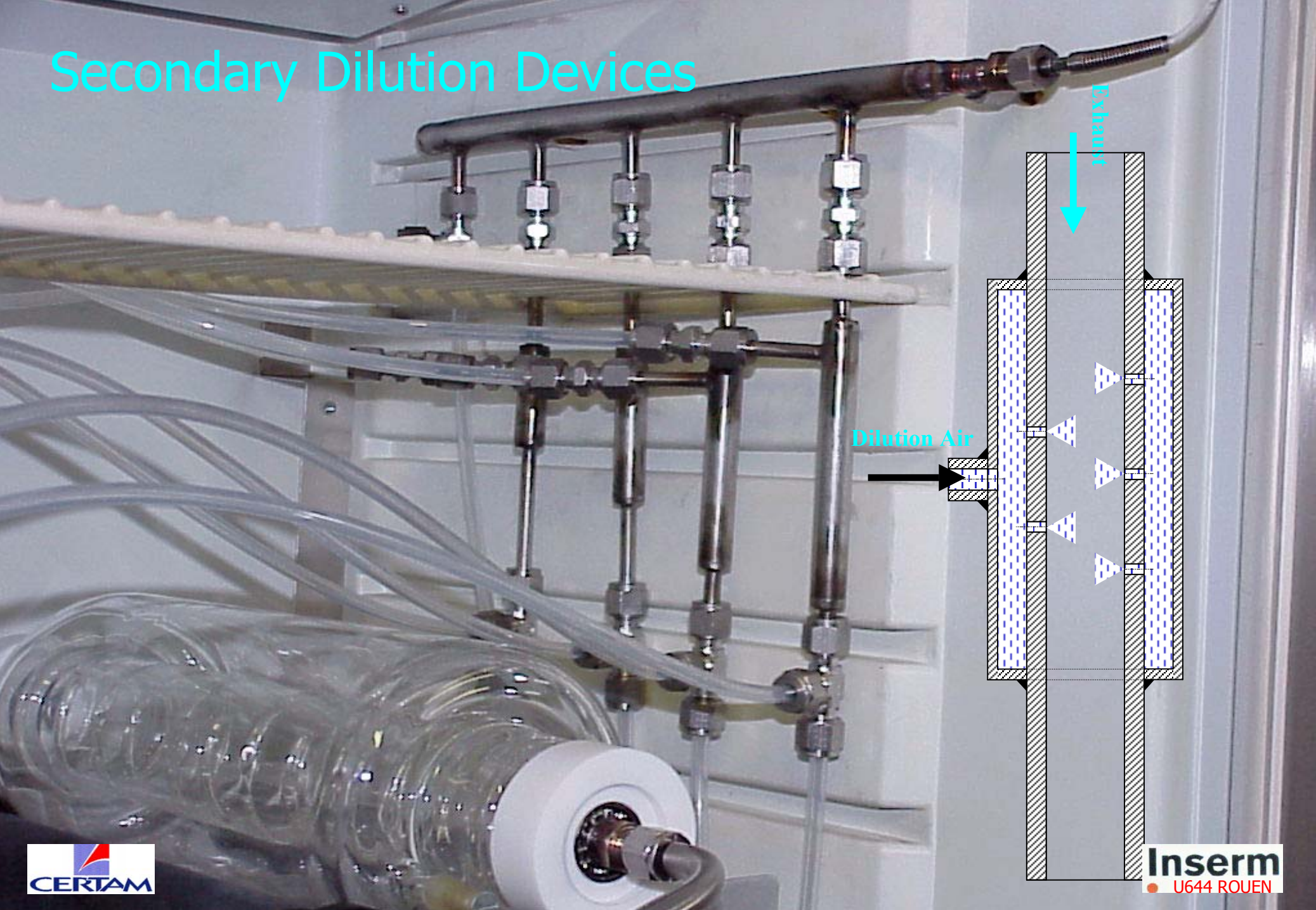

CERTAM

Inserm U644 ROUEN

*OPTIMISED*
*Combustion Aerosol Sampling*
*and*
*Dilution Systems*


Before After-Treatment Device
Primary Dilution Tunnels
After-
Treatment
Device
After After-Treatment Device

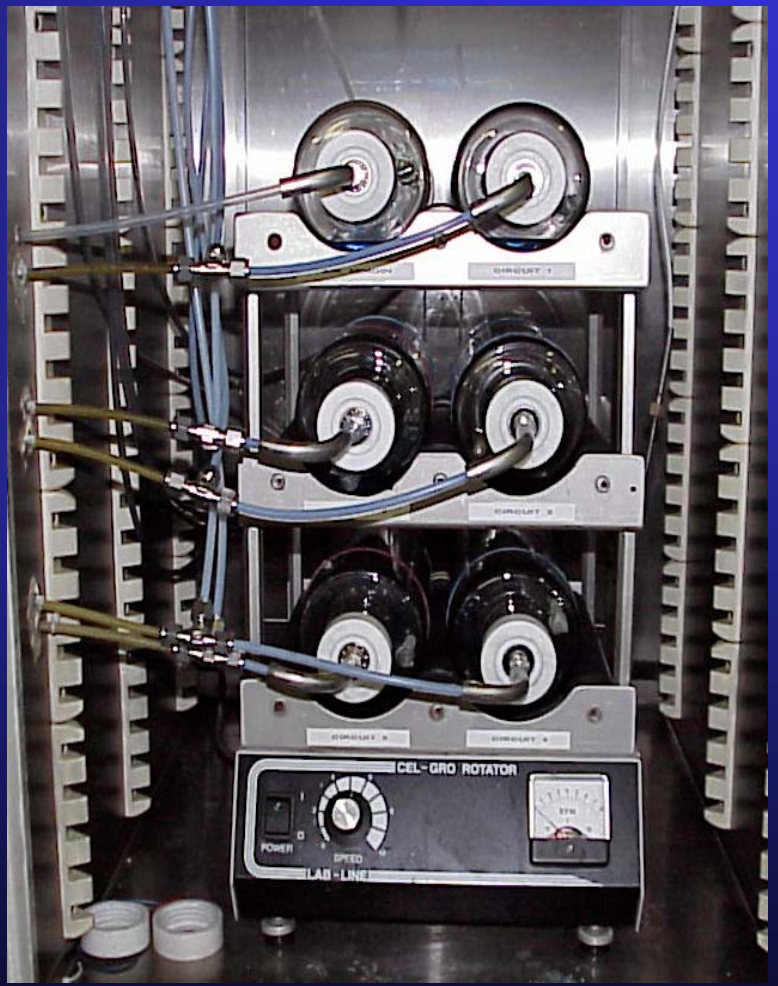
# Secondary Dilution Devices




CERTAM

Inserm
U644 ROUEN

# Standardized Dilution and Exposure Systems




Organotypic Cultures

# Advantages of Sampling and Exposure Systems

*** No alteration of both gazeous phase and PM physicochemical properties**

* Interactions Aerosol/Biological sample mimicking the in vivo situation (sedimentation and diffusion)

* No Alteration of pollutant Bioavailibility

* Global Approach of Exhaust impact

Inserm
U644 ROUEN

CERTAM

# *Toxicity Endpoints*

-**Cell viability** :

Intracellular ATP content

- **Oxidative stress and Detoxication** :

Intracellular glutathione content (GSH)

Enzyme activity of SOD, Catalase, GPx, GST

8-hydroxy-2'-deoxyguanosin (histological staining)

- **Inflammatory response** :

TNF$\alpha$ (release in culture medium)

ICAM-1 (histological staining)

-**Apoptosis :**

-Nucleosome Assay

-TUNEL (histological staining)

-DNA Ladders

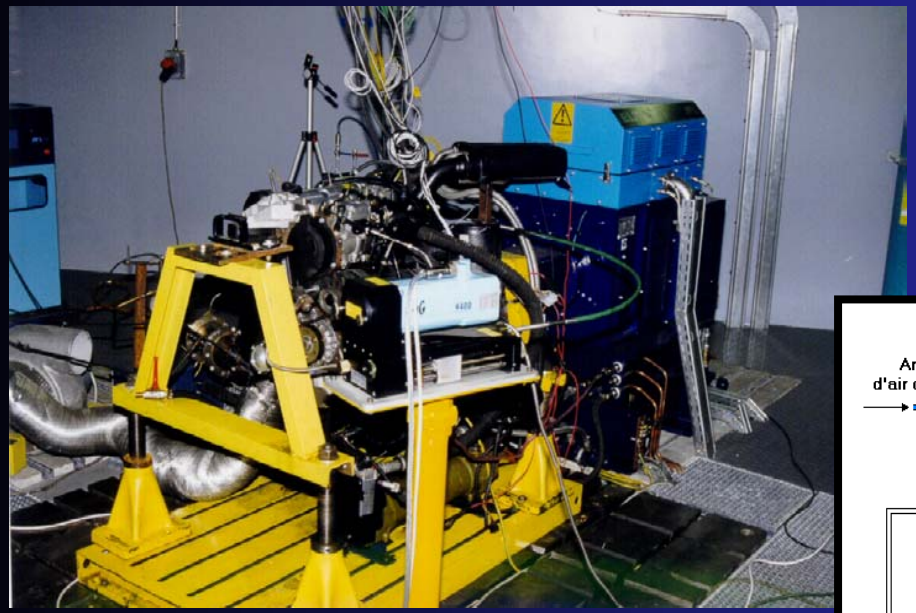
# Comparative Impacts on Lung Slices

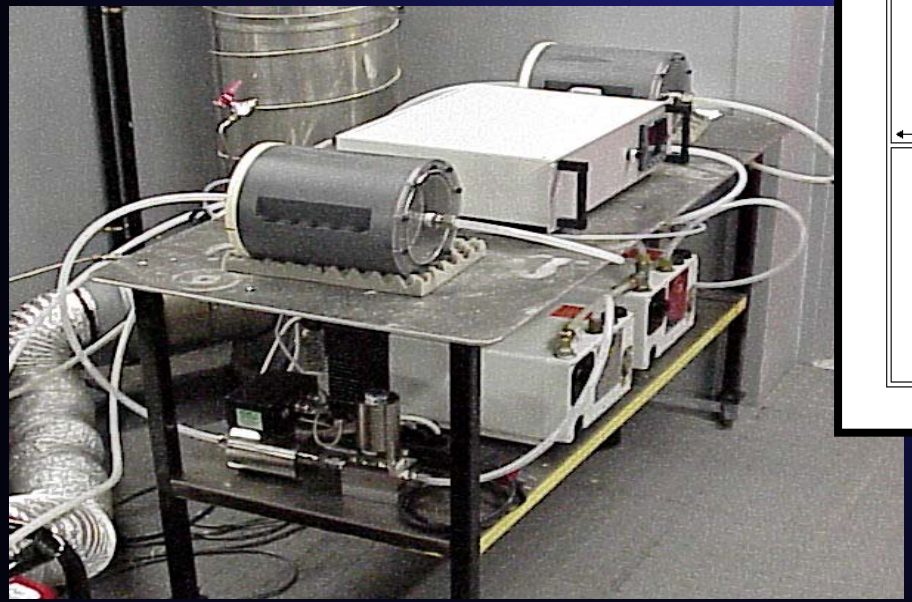
| | Diesel Exhaust (Low $NO_2$/NO ratio) | | Suspended DEP / Medium | Suspended DEP / Tween | |
|---|---|---|---|---|---|
| | Total | Filtered | Total | Total | |
| **Filtrate** | | | | | |
| ATP | = | = | = | = | = |
| GPx | + | + | = | = | = |
| GST | + | + | = | ++ | ++ |
| Catalase | + | + | = | - | -/= |
| GSH | --- | -- | = | = | = |
| SOD | - | - | = | ++ | ++ |
| TNFα | ++ | = | = | =+ | ++ |
| Nucleosomes | ++ | = | = | = | = |
| TUNEL | +++ | = | = | = | = |
| DNA Ladders | ++ | = | = | = | = |
| 8-OxoG | +++ | ++ | = | = | = |
| | | ↑ | ↑ | | ↑ |
| | | Gas Phase | No Effect | | Desorbed |

Inserm CERTAM

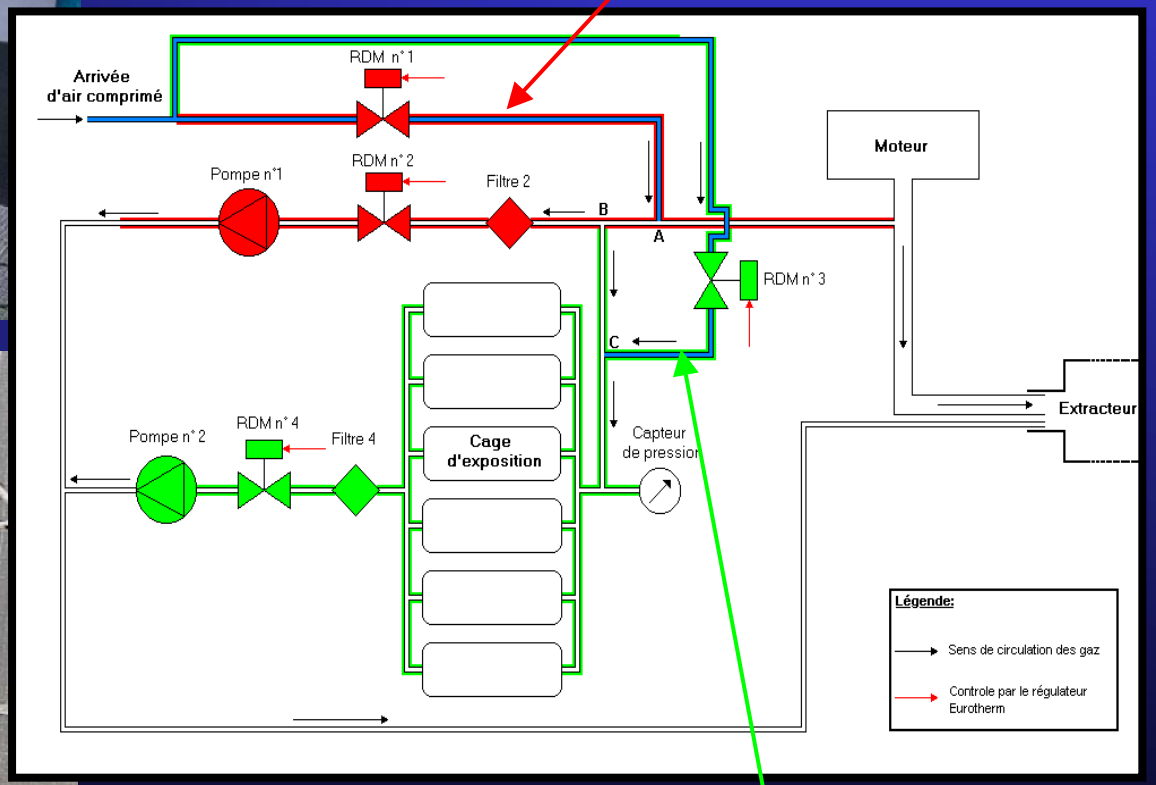
*in vivo*

# Sampling and dilution of Engine Exhausts





*1rst dilution loop*



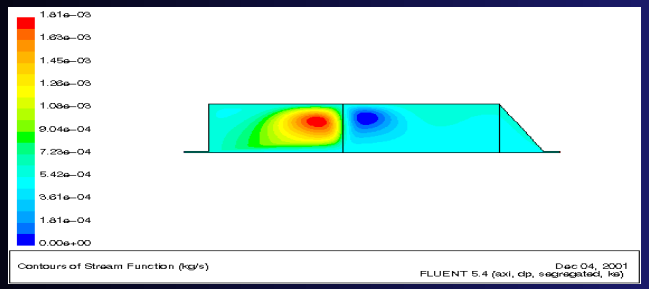

*2nd dilution loop*

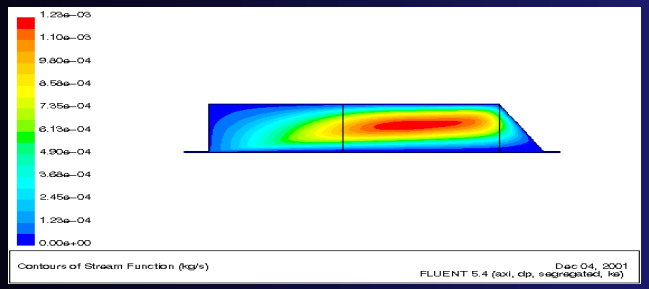
Inserm U644 ROUEN

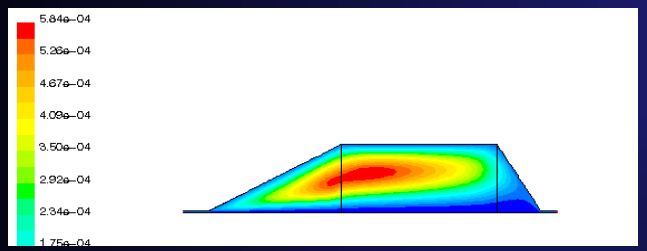
CERTAM

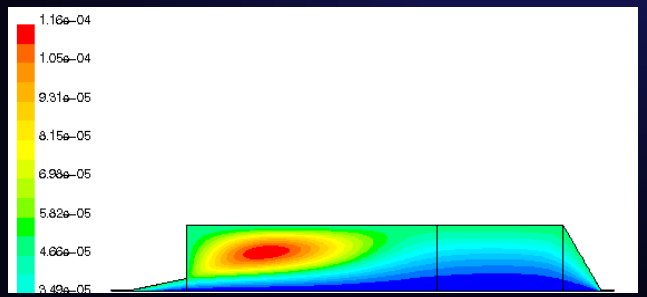
# Design of Inhalation Cages for Vigile Unconstraint Rodents

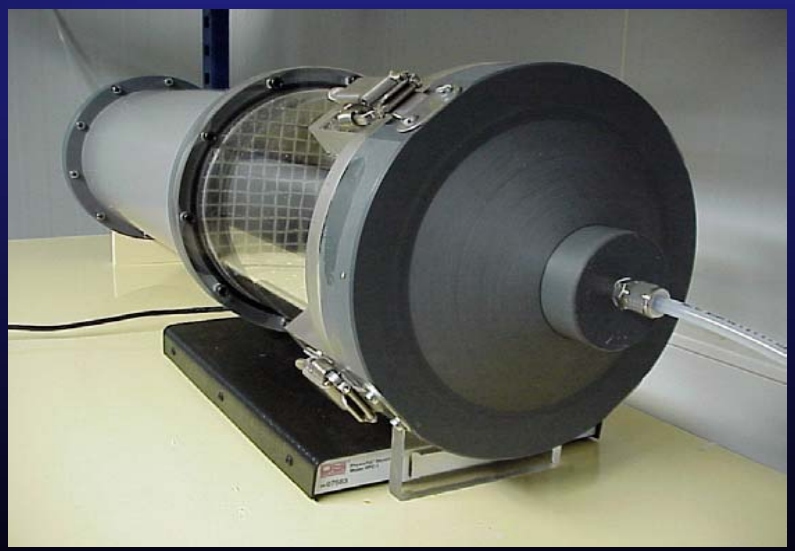
Modelization with FLUENT 2D





Inserm U644 ROUEN

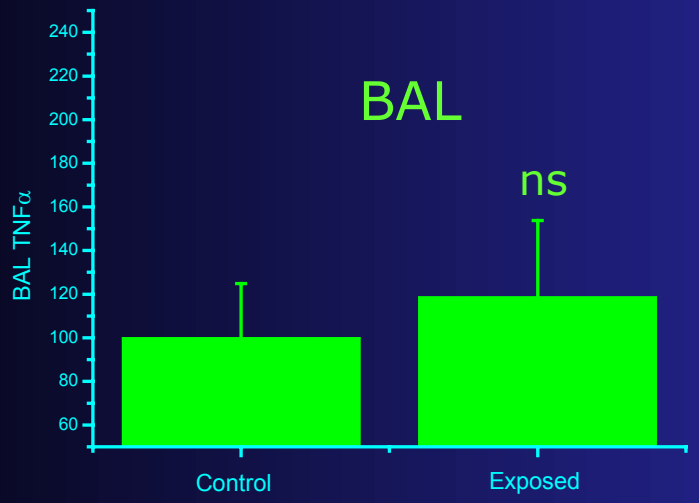
CERTAM

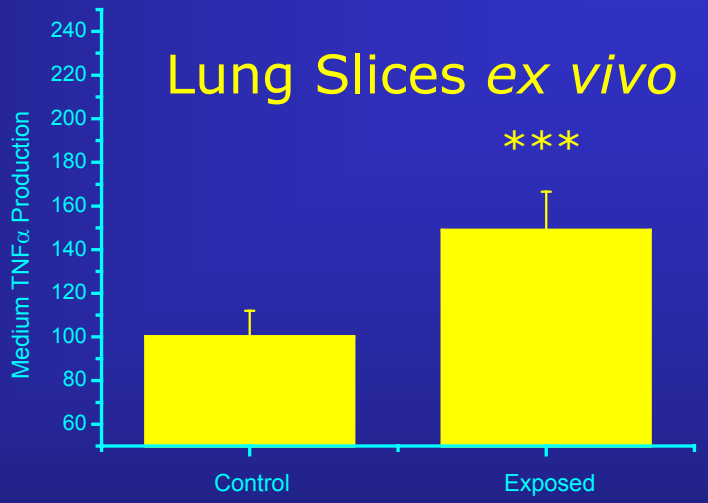
*Comparison*
*of*
*in vivo and in vitro*
*lung responses*

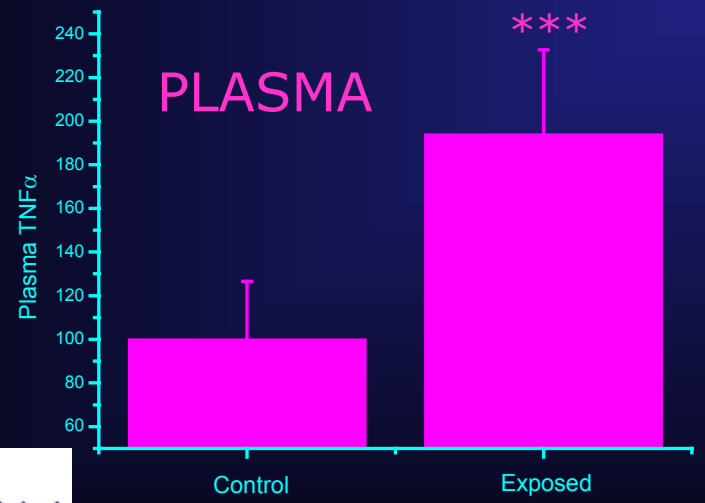
*Could Rat lung slices in bi-phasic air/liquid culture be used as a surrogate for in vivo aerosol inhalation toxicology*

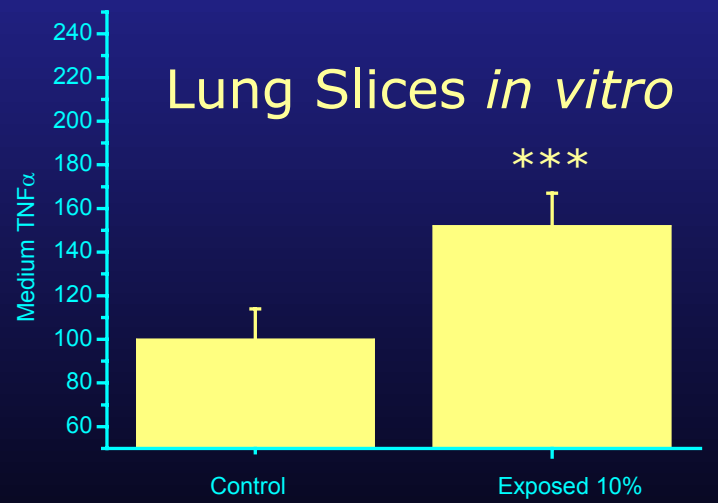
?

# Inflammation : TNFα Production



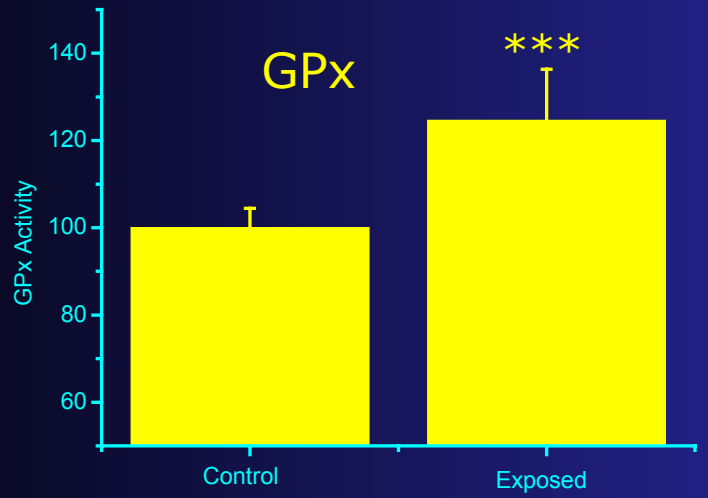



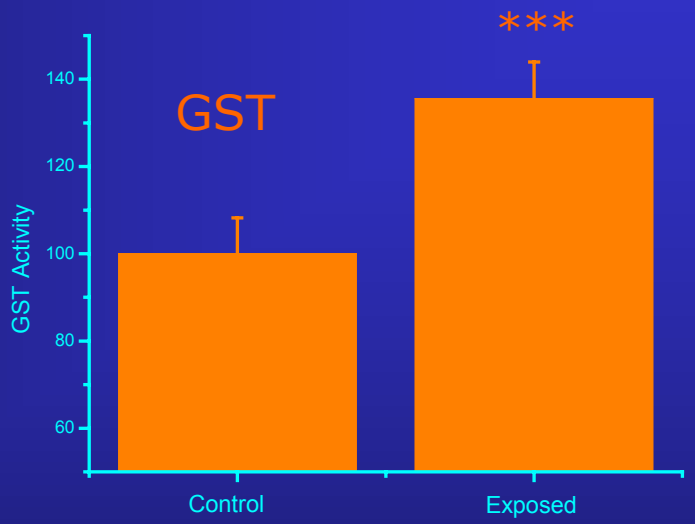



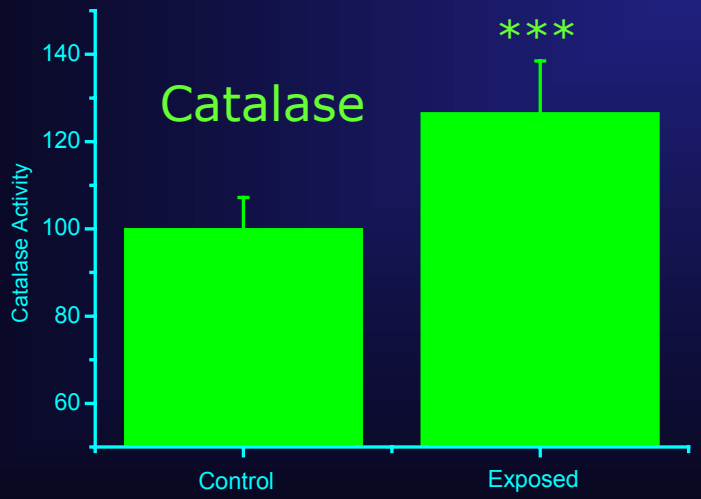

CERTAM

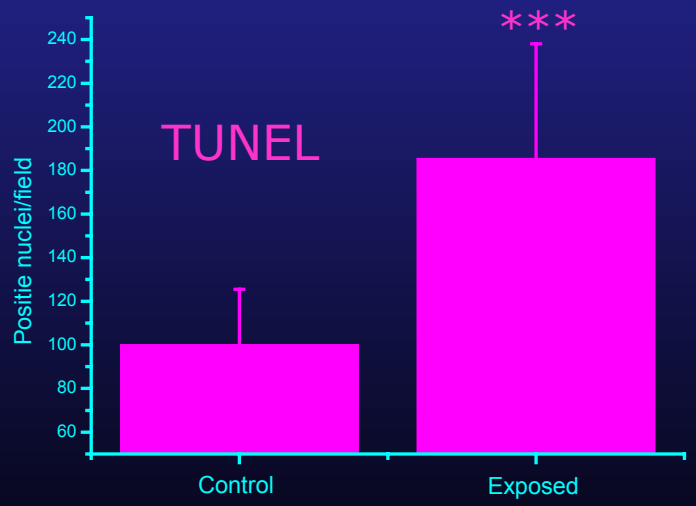
Inserm
U644 ROUEN

# Lung Tissue Investigations




Inserm U644 ROUEN

CERTAM

## Lung Tissue Investigations

## Excellent correlations between *in vitro* and *in vivo* Lung Toxicity patterns with continuous exposure to diluted exhausts

## DNA alteration, Inflammation and Oxidant stress

*Use of Rat Lung Slices in bi-phasic air/liquid culture as a surrogate for in vivo aerosol inhalation toxicology* Yes

Inserm U644 ROUEN

CERTAM

# Modulation of Engine Emissions Quality

## Fuel, Engine load, Filter, « Treatment »

## Modulation of CO, NOx, HC, PM

# Diesel Engine Emissions

## ⇨ Exhaust Characteristics

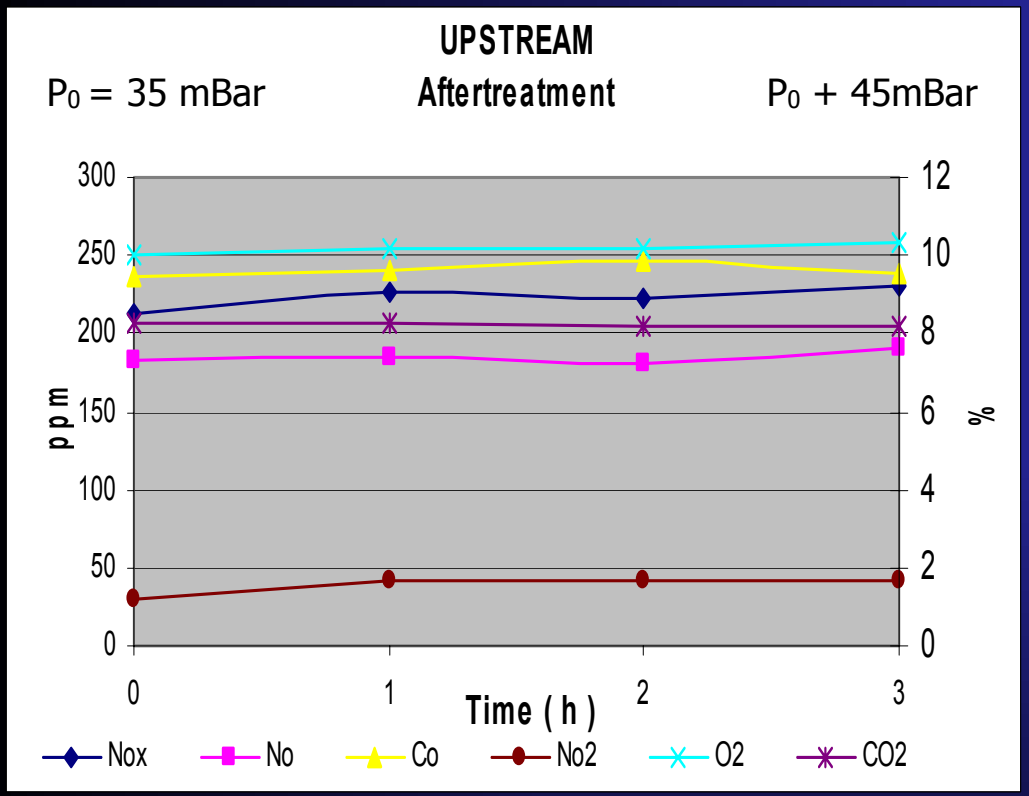
| Gas Phase | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| HC (ppm) | 29 | 29 | 19 | 19 | 10 | 10 | 0 | 0 |
| CO (ppm) | 137 | 137 | 0 | 0 | 0 | 0 | 0 | 0 |
| NOx (ppm) | 423 | 423 | 406 | 406 | 467 | 467 | 484 | 484 |
| $NO_2$ (ppm) | **24** | **24** | **106** | **106** | **191** | **191** | **260** | **260** |
| NO (ppm) | 399 | 399 | 300 | 300 | 277 | 277 | 224 | 224 |
| **$NO_2$/NO** | **0,06** | **0,06** | **0,35** | **0,35** | **0,69** | **0,69** | **1,16** | **1,16** |

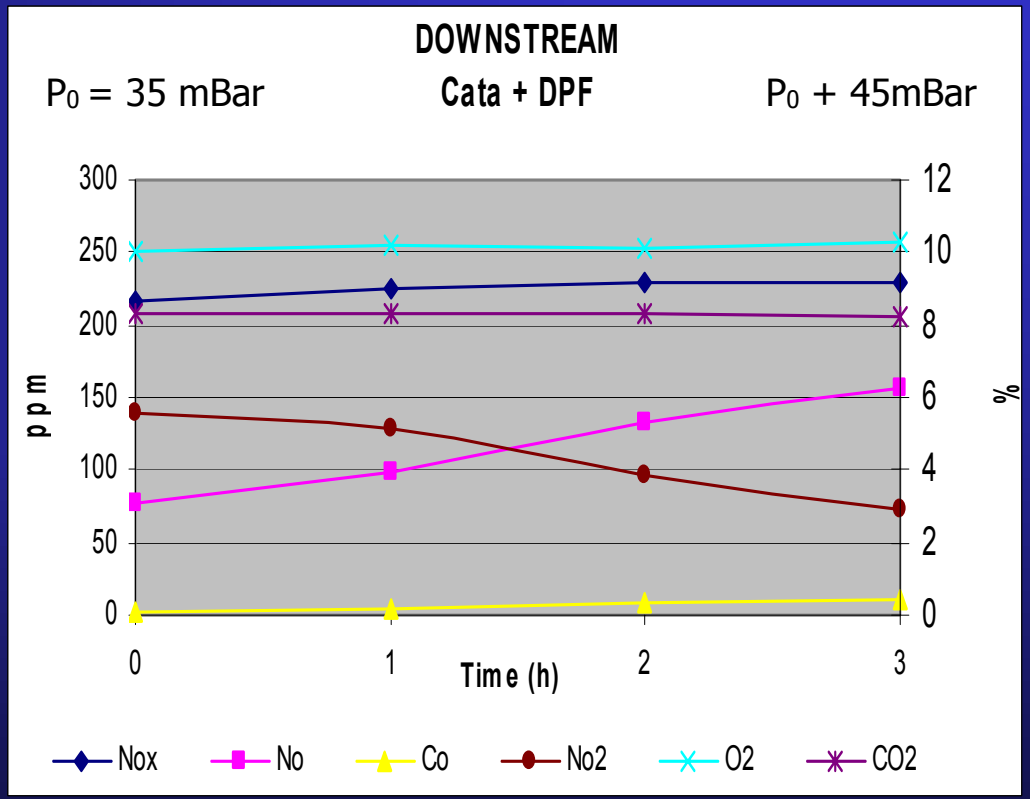
**Oxidant Potential** →

| Smoke Index | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| FSN | 1.8 | ND | 1.8 | ND | 0.7 | ND | 0.7 | ND |
| $mg/m^3$ | 44 | ND | 44 | ND | 12 | ND | 12 | ND |

Inserm E9920 ROUEN CERTAM

# Pollutant Emission Time Evolution



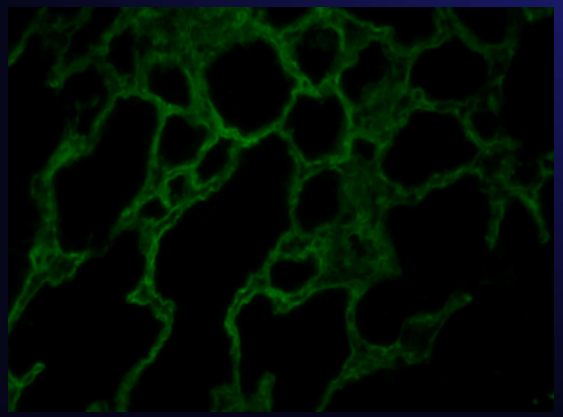




No Change in Total NOx But Changes in $NO_2$ and NO proportions

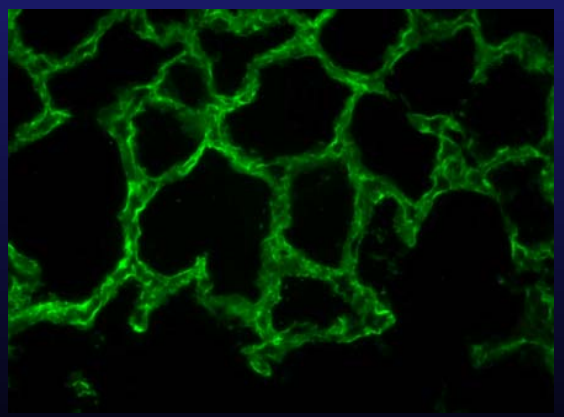
# INFLAMMATORY RESPONSE

| Diesel Emission | TNFalpha Production | ICAM-1 Expression | | |
|---|---|---|---|---|
| A | ↗ | ↗ | ⇨ Almost Exclusively PM Phase | Low $NO_2/NO$ |
| B | = | ↗ | ⇨ Almost Exclusively PM Phase | Low $NO_2/NO$ |
| C,D | ↗ | ↗ | ⇨ Gas Phase | |
| E,F | ↘ | ↗ | ⇨ Gas phase | High $NO_2/NO$ |
| G,H | ↘ | ↗ | ⇨ Gas phase | High $NO_2/NO$ |

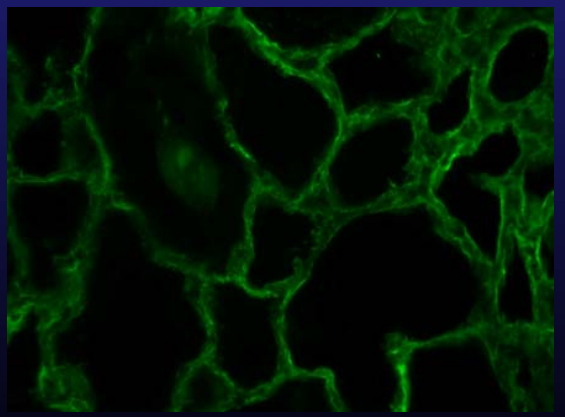
ICAM-1



Control



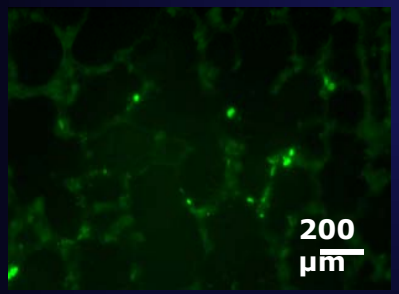
Emissions A, C, D, E, F, G, H



Emission B

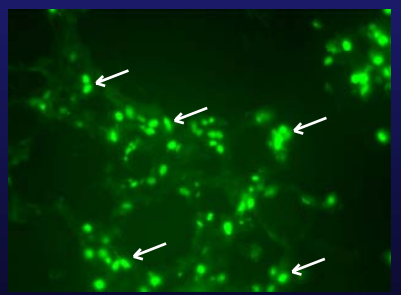
# DIESEL Emissions : APOPTOSIS

| Effluents Diesel | Nucleosomes | DNA Scales | TUNEL | |
|---|---|---|---|---|
| A | ↗ | + | ↗ | ⇨ **PM effect** Low $NO_2/NO$ |
| B | = | - | = | |
| C,D | ↗ | + | ↗ | ⇨ **Gas Phase Effect** |
| E,F | = | - | = | ⇨**No apparent Effect** |
| G,H | = | - | = | ⇨**Necrosis ?** High $NO_2/NO$ |

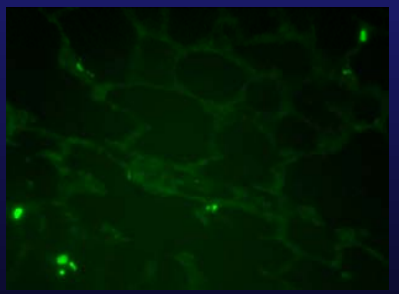



Control

**Tunel Labeling**



A, C and D

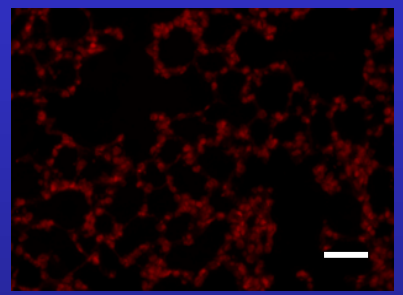


B, E, F, G and H

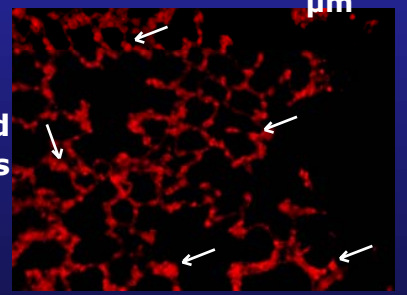
# DIESEL Emissions and DETOXICATION Systems

| | | A,B | C,D | E,F | G,H |
|---|---|---|---|---|---|
| Detoxication Systems | GSH | ↘ | ↘ | ↘ | ↘ |
| | GST | ↗ | ↗ | = | = |
| Oxidant Stress | GPx | ↗ | ↗ | ↗ | ↗ |
| | Catalase | = | = | ↗ | ↗ |
| | MnSOD | ↗ | ↗ | = | = |
| | 8-oxoguanine | + | + | + | + |

Low $NO_2/NO$ | High $NO_2/NO$



Control




Diesel

⇨ **Two Distinct Profiles According to $NO_2/NO$ Ratio**

⇨ **Predominant impact of gaseous phase**

# CONCLUSION AND SUGGESTIONS

**Untreated Raw Diesel Engine Emissions**
**exhibit low oxidant capacity**
**and exert their Toxicity Mainly through PM matter**

## The Apparent Toxic Impact of PM is « Modulated »
## By the Oxidant Potential of the Gas phase

**$NO_2$/NO ratio has been chosen as a marker**
**of exhaust global oxidant potential**

**Inflammation and Oxidant Stress, but no Cytotoxicity,**
**Induced by PM at low $NO_2$/NO ratio (<0.1)**

**Oxidant Stress, Cytotoxicity and**
**No Inflammation**
**Due to the Gaseous Phase**
**No more apparent impact of PM**
**For $NO_2$/NO ratio > 0.2**

Inserm U644 ROUEN
CERTAM

**Several Strategies for After Treating Diesel Engine Emissions rely on increasing the Oxidant potential of the Gas phase. Health Impact Concerns may Arise from these Strategies**

**Beside Total Nox measurements,**
**$NO_2$ and NO proportions should be Carefully monitored as a potential pertinent marker of Diesel Engine Emission « Health Safety »**

**This may help to no regret « health based » industrial strategies and air quality regulations**

# Acknowledgements to Coworkers

*Jean-Paul Morin*
*Anne Bion*
*Stéphane Loriot*
*Mamadou Fall*

*Frédéric Dionnet*
*Frantz Gouriou*
*Denis Farin*
*Veronika Keravec*
*Pascal Ponty*

Inserm U644 ROUEN

CERTAM

## With the Financial Supports of

**MAAPHRI**
**FP5 - EU Contract**
**N° QLK4-CT-2002-02357**

**PRIMEQUAL**
**PREDIT**

**CPER**