J. P. Morin
University Rouen
Rouen
France

8

In vitro lung toxicity of diesel exhausts using continuous flow sampling and exposure devices

In vitro lung toxicity of diesel exhausts using continuous flow sampling and exposure devices

Jean-Paul MORIN - INSERM E9920 Fac Medecine Pharmacie de ROUEN 22 bd Gambetta 76181 ROUEN Cedex - FRANCE
Frederic DIONNET – CERTAM 1 rue Joseph FOURIER 76800 SAINT ETIENNE du ROUVRAY – France

As recently published by our group, we have developed a new model of precision-cut rat lung slices in organotypic culture, placed in a biphasic air/liquid system, which allows to perform pathological and histochemical studies as well as cell and molecular biology investigations after exposures to complex atmospheres (Morin et al 199 a, b), LePrieur etal 2000 a, b).

Authors have paid particular attention to the development of engine exhaust sampling and dilution in order to compromise between physical (temperature, pressure, dilution ratio, pollutant condensation, particle size distribution) and biological (O2 and CO2 tensions, temperature, hygrometry, flow rates medium pH) requisites we have developed a specific sampling-dilution-exposure system which allows to deliver in a stable and reproducible way a constant flow of diluted engine exhausts to biological samples in vitro maintaining both gaseous and particulate matter physicochemical properties. (plate 1)

Briefly, continuous isokinetic sampling was performed from the exhaust line, followed by a two step dilution system. The primary dilution tunnel had three concomitant functions namely i) cooling exhausts gases down to 45°C without pollutant condensation by dilution with dry clean air; ii) oxygen tension correction and regulation to 19.5% to be compatible with biological viability; iii) carbon dioxide tension correction and regulation to 5% in order to maintain culture medium pH based on the equilibrium of CO2/bicarbonate buffer. (plate 2)

Five parallel secondary dilution systems consisted in the addition of a constantly monitored and regulated flow of clean air/CO2 (95:5) gas (plate 3) prior to delivery of diluted exhausts into constant flow through rotating exposure chambers in which rat lung slices are exposed to the test atmospheres.

The whole flow is regulated individually for each exposure chamber by a light depression generated by pumps coupled to massic flow rate regulators. This allows to avoid any physical hindrance to both gaseous and particulate matter during the process of exhaust sampling and dilution which was one of the most important pre-requisite of our development.

The whole system has been validated by the monitoring of carbon monoxide concentrations used as a marker of gaseous compounds (plate 5) and by the monitoring of particulate matter number and size distribution using the SMPS technology (plate 6). These results show a very good linear proportionality between expected dilution ratio and actual carbon monoxide concentration thus pointing out the perfect control of the gas dilution processes. The characterization of the particule number, fractional volume, and size distribution showed that almost no alteration of particulate matter was evidenced prior to the entry of the exposure chambers due to the careful control of high turbulence (Reynolds number >4000) and the lack of water and pollutant condensation during the dilution process which could be the two major phenomenon's leading to particle size distribution alterations. Furthermore, a very good linear proportionality between the expected dilution ratio and the particle number or fractional volume was seen, pointing out that the dilution process was as effective for gaseous compounds as for particulate matter.

This device of continuous sampling and dilution of engine exhausts allows us to state that the biological material used in this study is exposed to complex atmospheres containing both well characterized gaseous and particulate matter. This makes this newly designed system most promising for air quality and environmental toxicology assessment.

Plate 9 shows the occurence of DNA breaks labelled by the TUNEL method on lunf slice cuts after diesel exhaust exposure.

Plate 10 shows a short overview of the biological endpoints assessed to date in this system. The difference in toxicity pattern recorded after either whole exhausts or filtered exhaust exposures point out the major contribution of particulate matter compared to the gaseous fraction in the induction of the toxic response in lung tissue exposed in vitro to a a continuous flow of diluted raw exhausts.

For more detailed informations of the biological impacts of diesel engine exhaust on lung tissue in vitro, see the above listed references.

Le PRIEUR E., MORIN J.P, BION A., GOURIOU F., DIONNET F. « Toxicological Impact of Diesel Fuel supplementation with rapseed Methylester (RME) on the lung toxic potential of Diesel exhausts » SAE Technical Paper 2000-01- 2060 Paris **2000**.

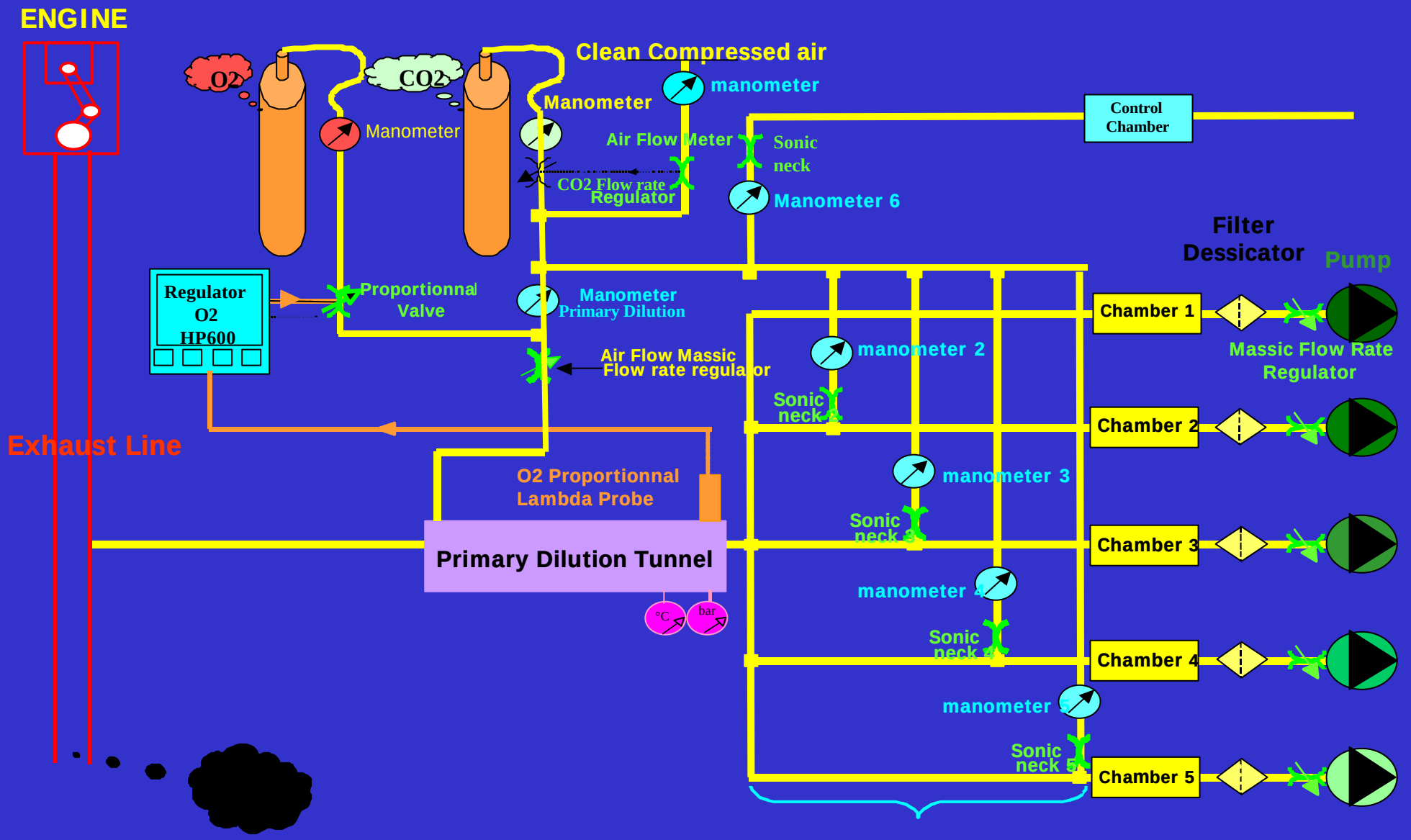
Le PRIEUR E., MORIN J.P., VAZ E., BION A., DIONNET F. « Toxicity of diesel engineexhaust: induction of pro-inflammatory response and apoptosis in an in vitro

model of lung slices in bi-phasic organotypic culture » SAE Technical Paper 2000-01-1928 Paris **2000**.

MORIN JP, Le PRIEUR E, DIONNET F, ROBIN L. " The influence of a particle trap on the in vitro lung toxicity response to continuous exposure to diesel exhaust emission." SAE Technical Paper 1999-01-2710 Vancouver **1999.**

MORIN JP, FOUQUET F, MONTEIL C, LePRIEUR E, VAZ E, DIONNET F. Development of a new in vitro system for continuous in vitro exposure of lung tissue to complex atmospheres : Application to diesel exhaust toxicology. Cell Biol. Toxicology 15 : 143-152 **1999.**

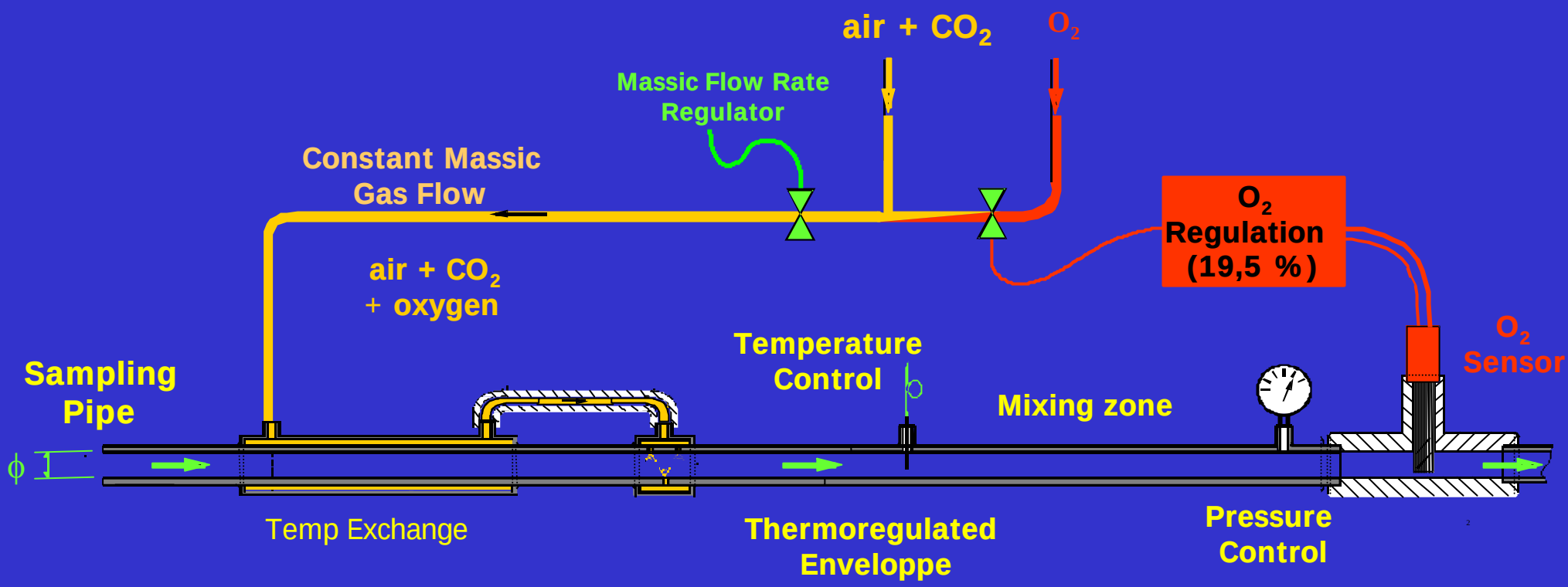
Sampling and Exposure Scheme



CERTAM

Inserm

Primary Dilution Tunnel Scheme



$$\textit{Reynolds Number} \quad : Re = \frac{v\ f}{n} > 4000 \qquad L^{3}\ 20\ f$$

Turbulent Reynolds was obtained by adjusting tunnel diameter according to the desired flow rate

CERTAM

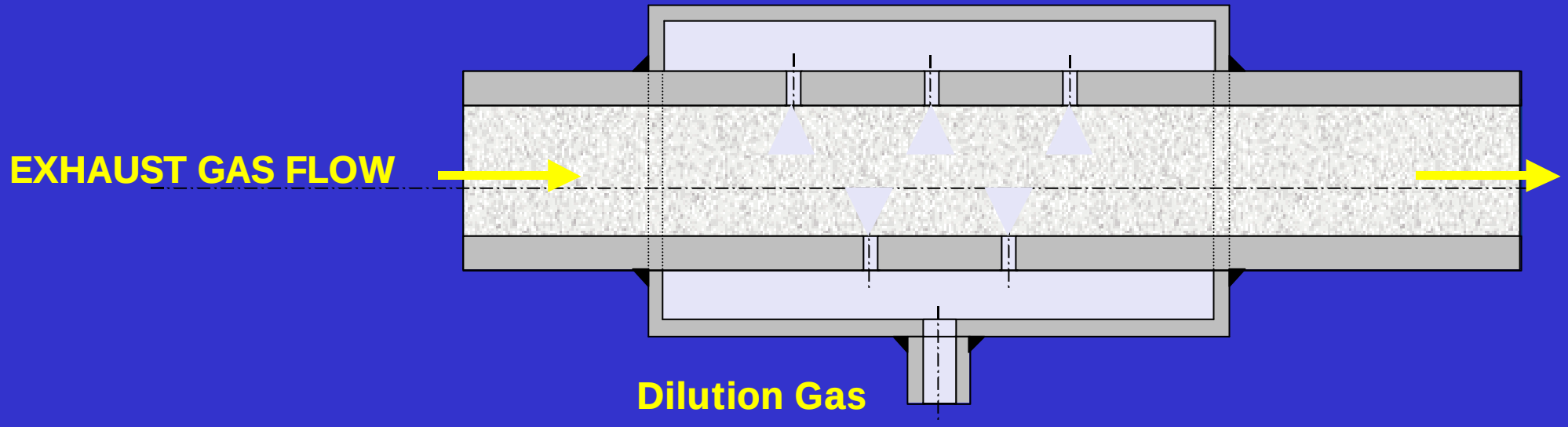
Inserm

Secondary Dilution Scheme

- DESIGNED WITH ORTHOGONAL DILUTION GAS INJECTION INLETS ALLOWING TURBULENCE GENERATION

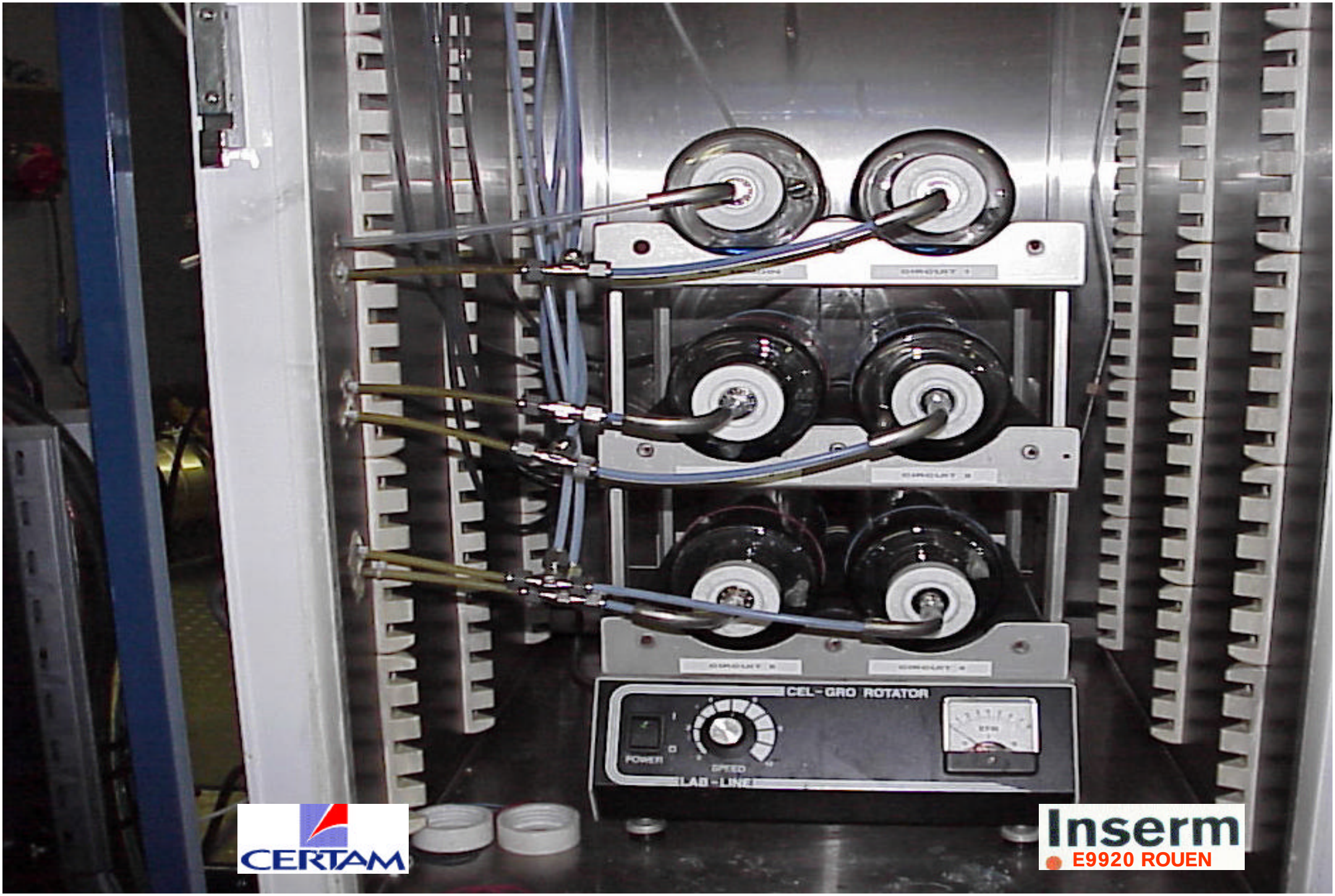
- CALIBRATED INLET DIAMETERS

- NUMBER OF INLETS ADJUSTED FOR GIVEN FLOW RATE

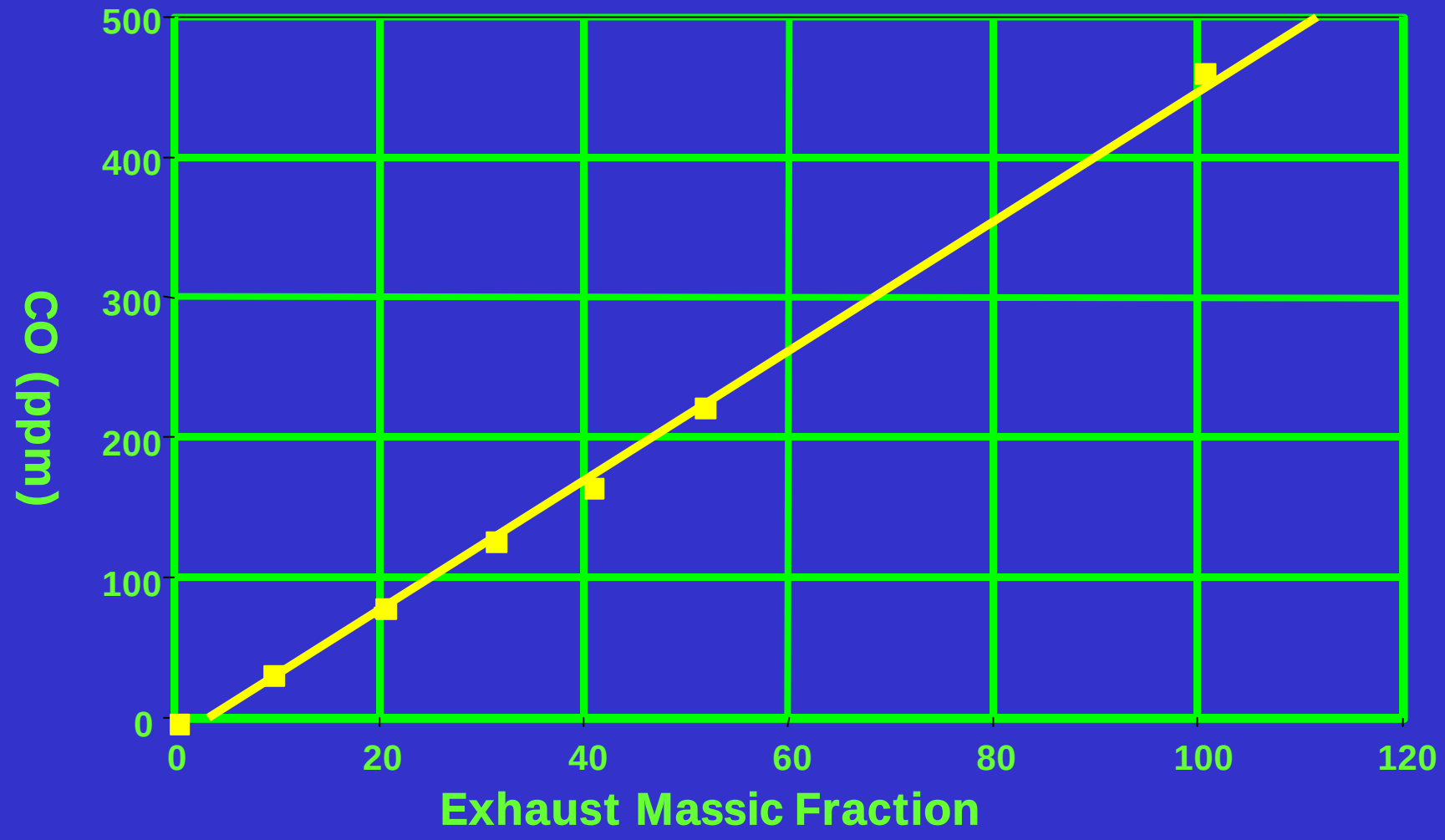


CERTAM

Inserm


CEL-GRO ROTATOR
POWER
SPEED
LAB-LINE
CERTAM
Inserm
E9920 ROUEN

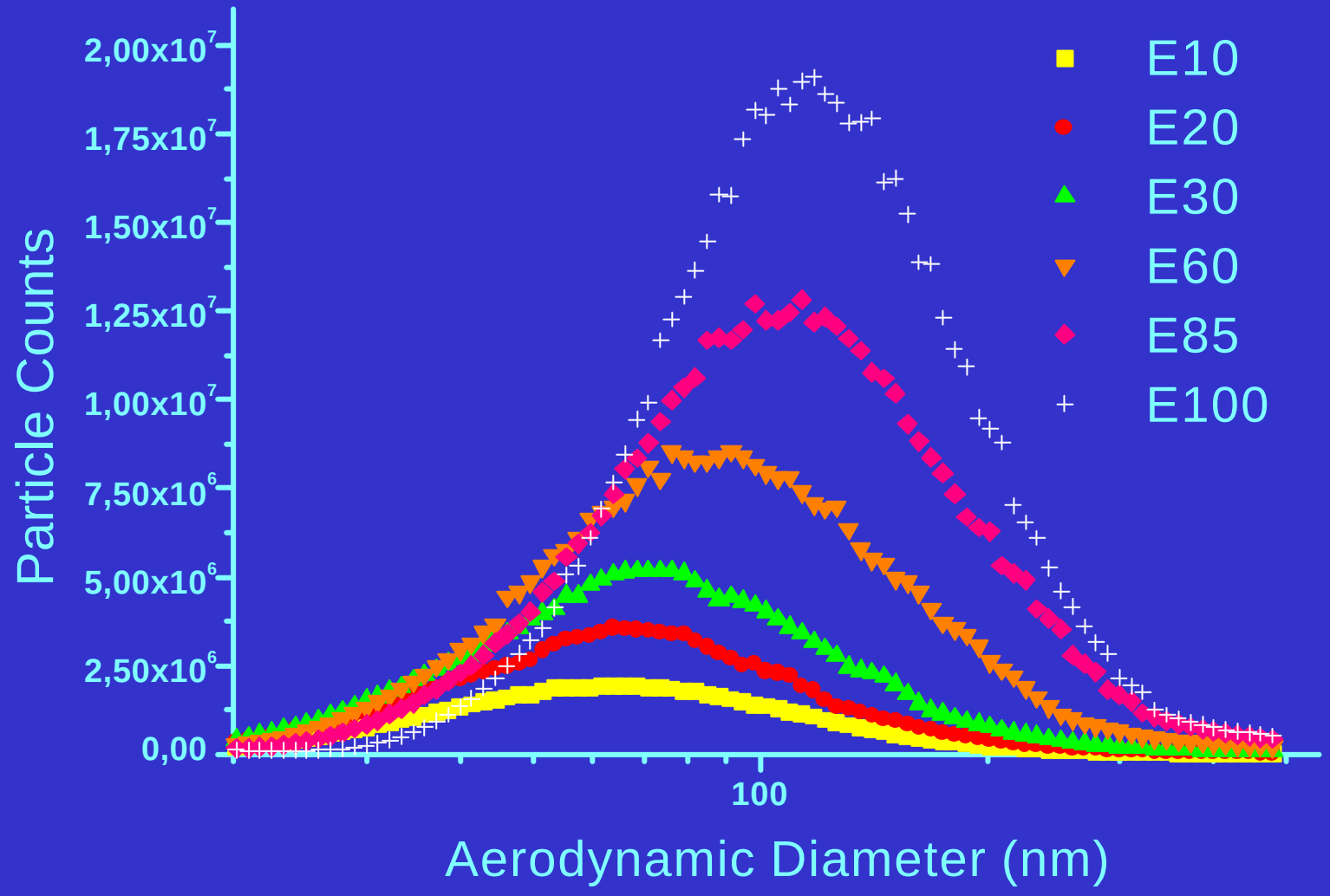
Validation of Dilution Efficiency (CO Concentrations)



CERTAM

Inserm

Particle Numbers and Size Distributions As a Function of Diesel Exhaust Dilution Ratio



ADVANTAGES OF THE EXPOSURE DESIGN

Continuous exposure to whole raw exhausts (both gazeous and particulate)

Minimal impaction of particulate matter between engine and biological samples

No requirement for solubilization procedure of particulate matter = No alteration of pollutant biodisponibility

No alteration of gazeous nor particulate matter physicochemical properties.

CERTAM

Inserm

ADVANTAGES OF ORGANOTYPIC CULTURES

Presence of all lung cell types as potential targets

Preservation of anatomical intercellular interactions

Preservation of tissue architecture

High degree of tissue specific differenciation over the experimental period

Well adapted for Cellular and Molecular Biology investigations

Suitable for pathological investigations

CERTAM

Inserm

Diesel Exhaust Impact on Rat Lung Slices

	Whole	Filtered
SOD	+	+
Catalase	=	=
GPX	=	+
GSH	---	-
Surfactant	-	-
Polyamines	=	=
TNF a	++	=
Nucleosomes	+	=

CERTAM

Inserm