

Responses of Cultured Lung Epithelial Cells to Airborne Diesel Emissions

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PURPOSE

- **Rapid assessment of biological effects of airborne emissions under more nearly physiological conditions**
 - **Real time exposure to realistic concentrations**
 - **Whole exhaust**
 - **Includes gas phase, semi-volatiles, and particles of various size classes in natural proportions**
 - **Potential for fractionation**
 - **Air-liquid interface culture**
 - **No loss, dilution, dissolution, or reaction of constituents with medium**

Topics

- **Diesel exhaust characterization**
- **Exposure system**
- **Biological responses**

Diesel Exhaust Generation

➤ Engine

- 2000 model Cummins ISB 5.9 L turbo

➤ Fuel

- U.S. certification fuel

➤ Crankcase Oil

- Shell Rotella[®] T 15W 40

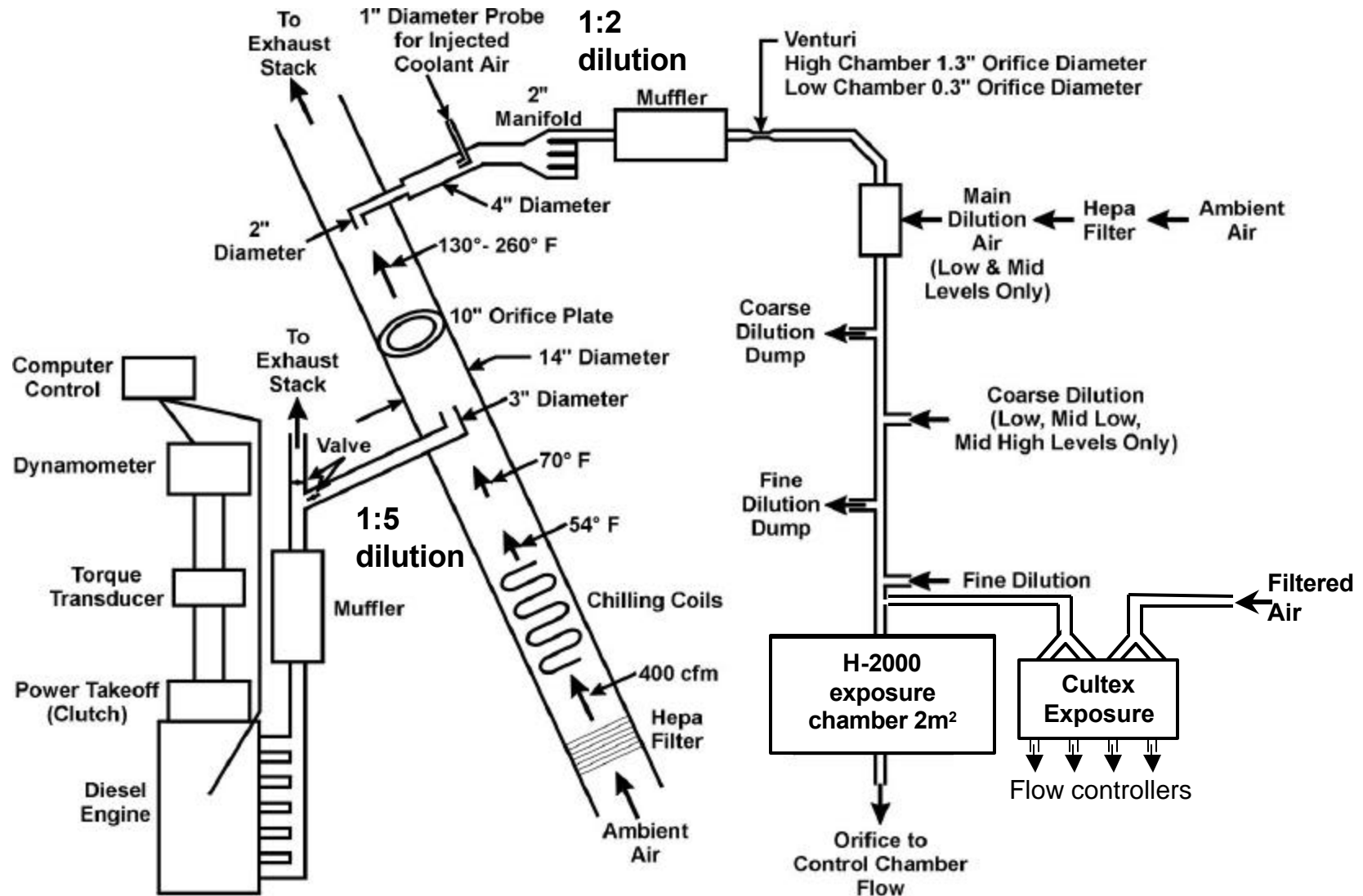
➤ Exhaust system

- Simulated Dodge Ram (no aftertreatment)

➤ Operating cycle

- Repeating heavy-duty certification duty cycle

Diesel Exposure System



Exposure Atmosphere

Mean Concentrations

➤ Particles (filter mass) 1000 $\mu\text{g}/\text{m}^3$

➤ Gases

CO 30 ppm

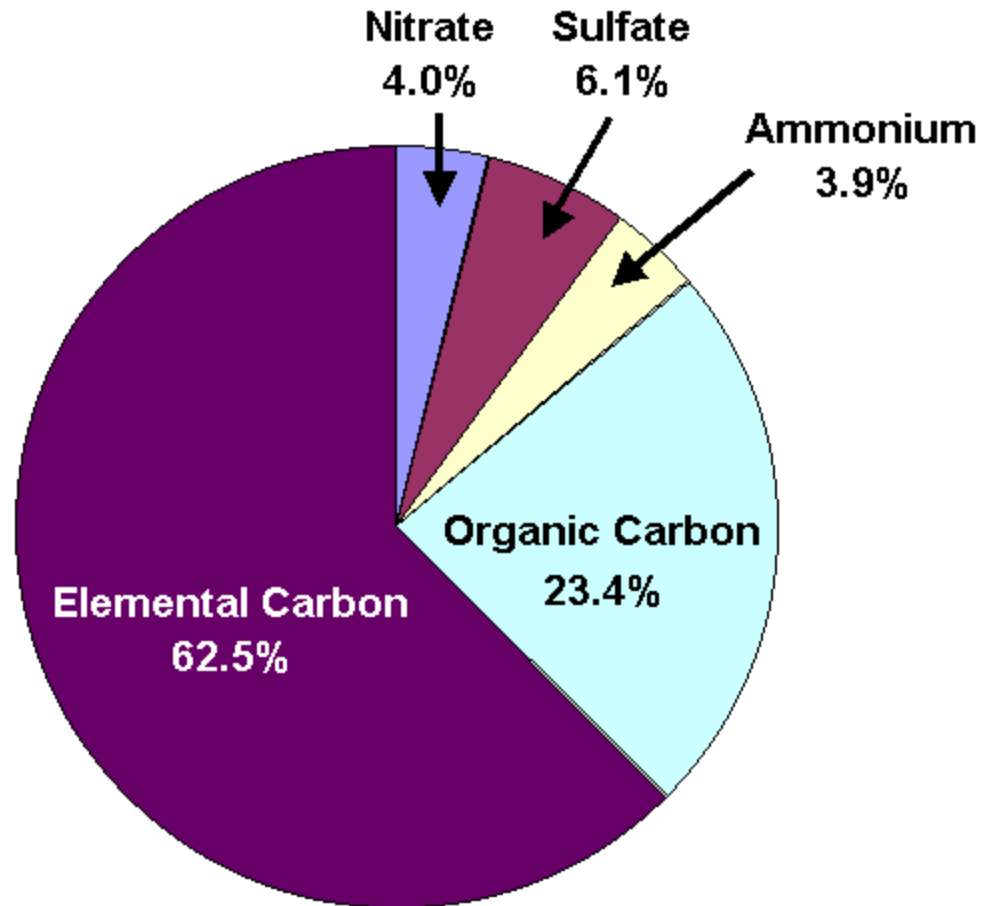
NO 42 ppm

NO₂ 5 ppm

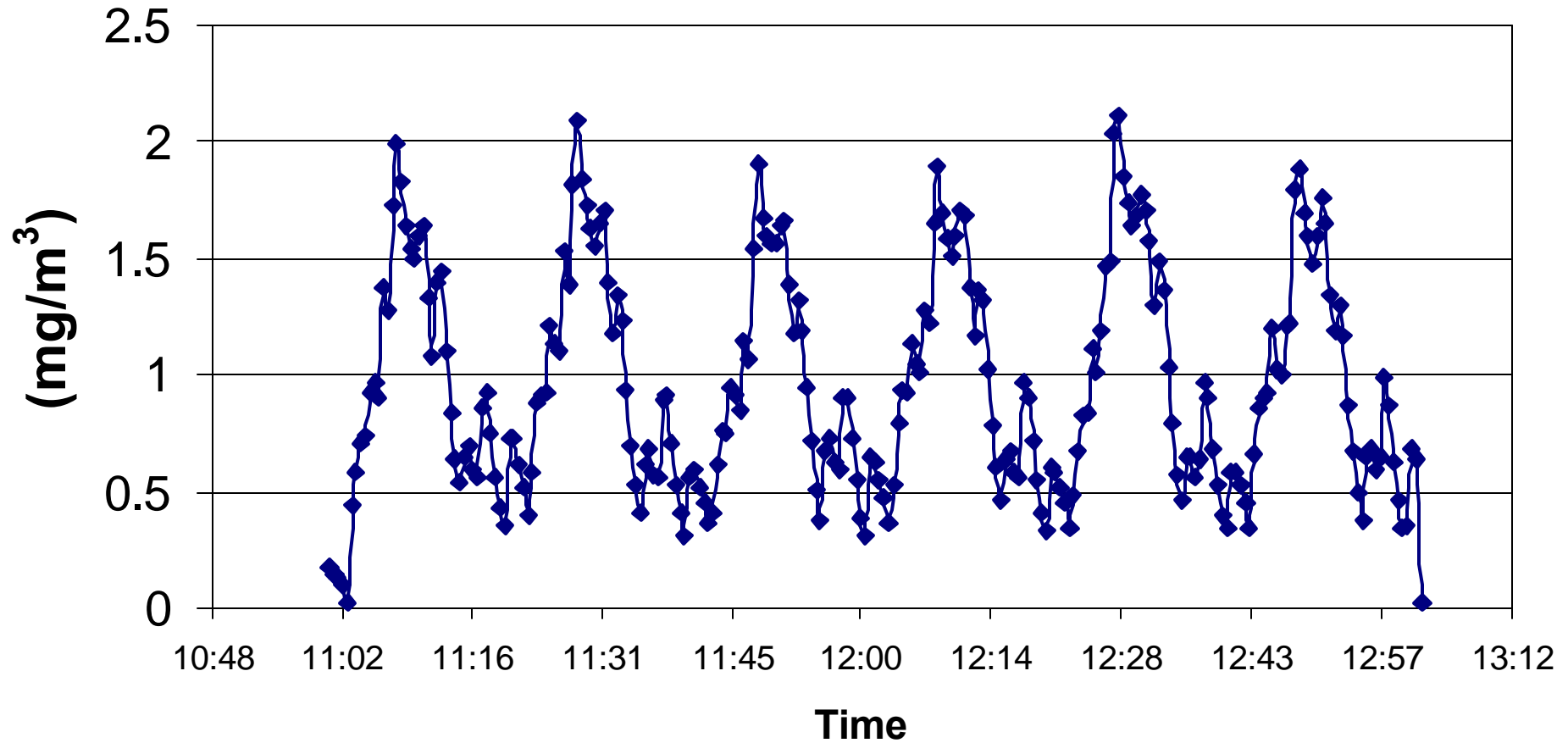
Analyses in progress

➤ Particulate and volatile organics

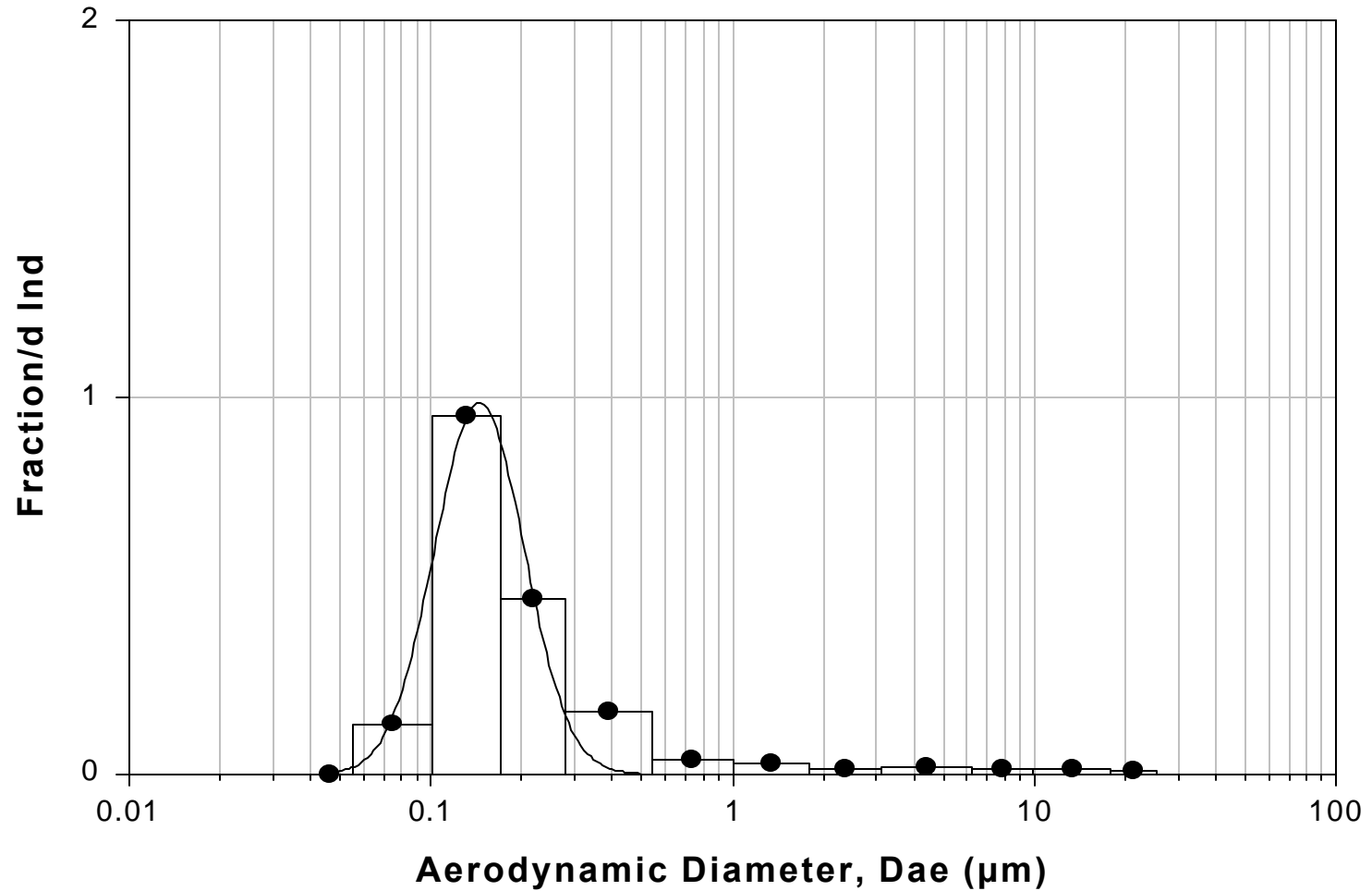
Bulk Composition of Particles



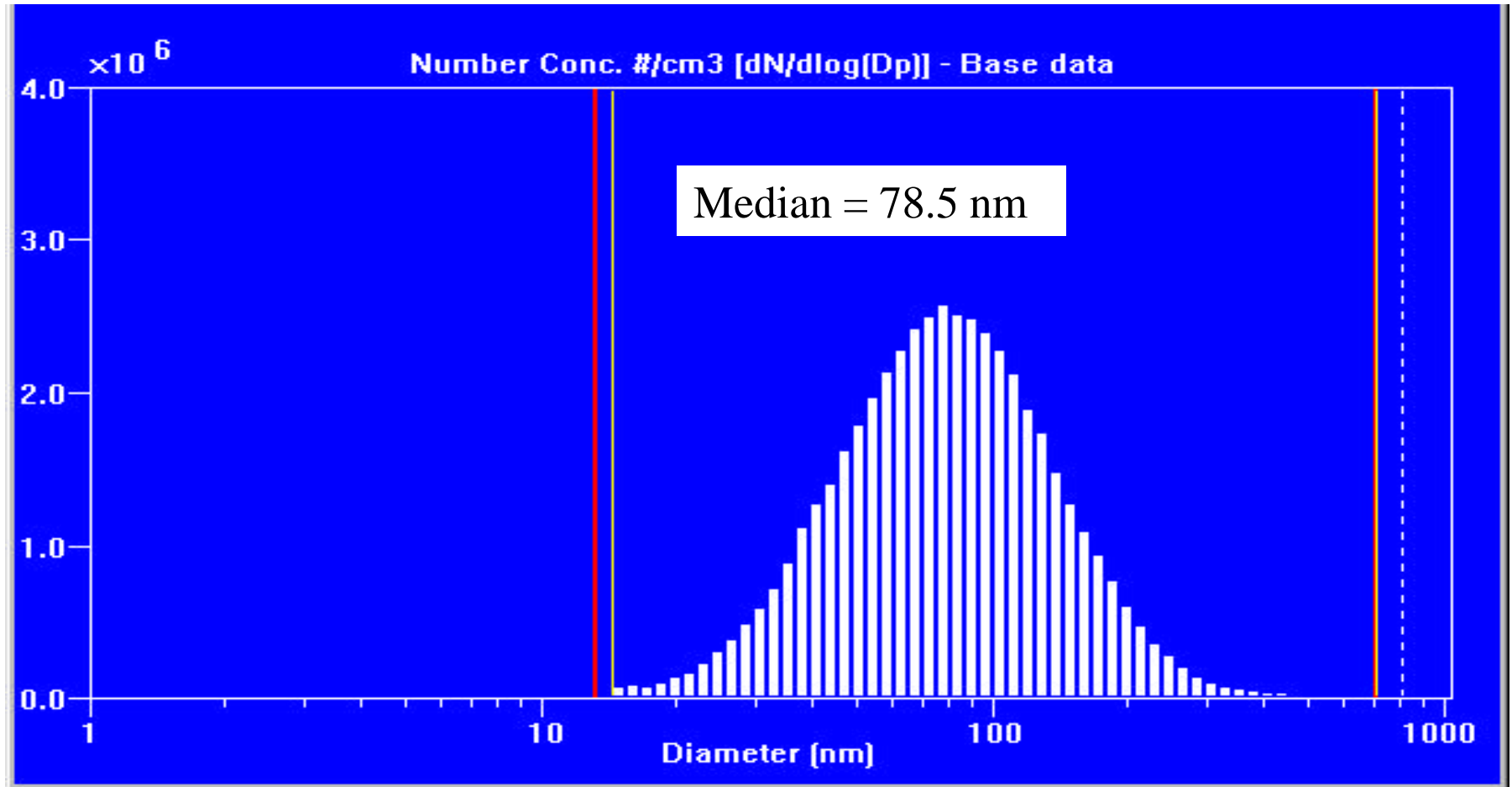
Real-Time Mass Concentration



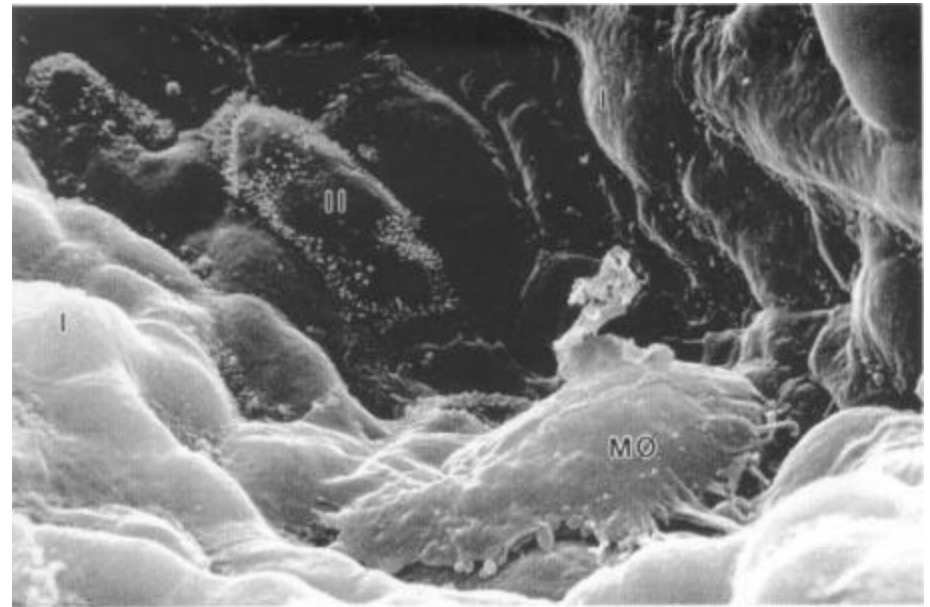
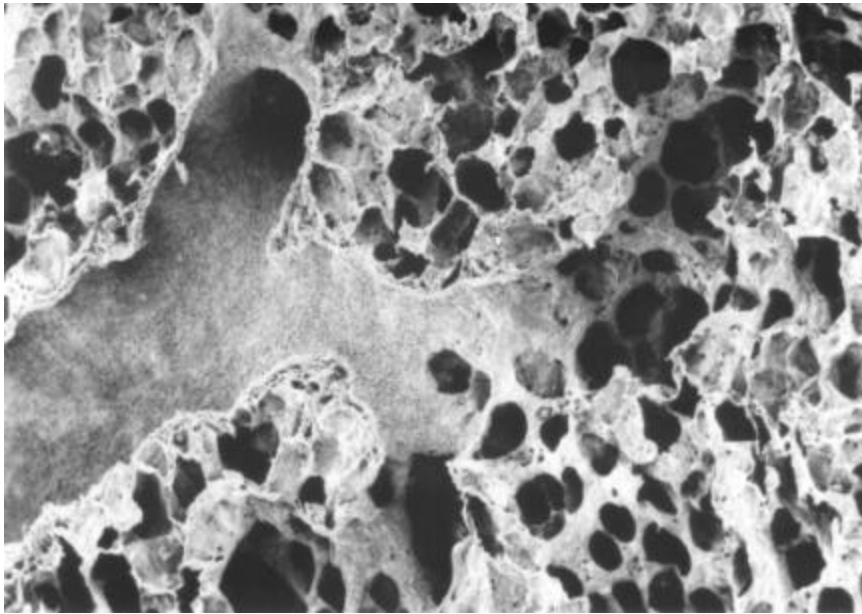
Distribution by Mass



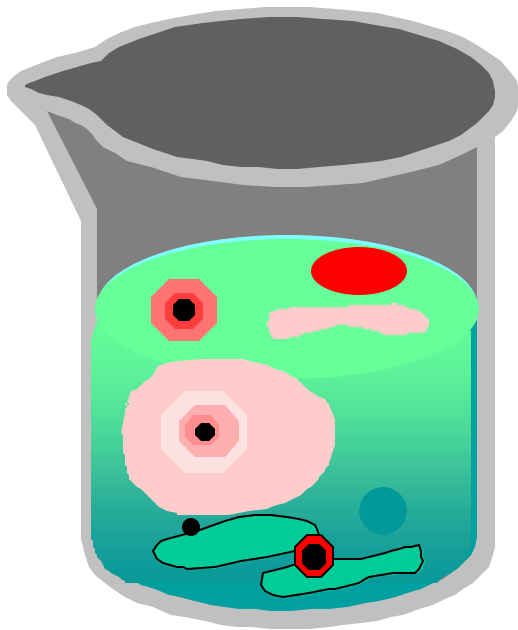
Distribution by Number



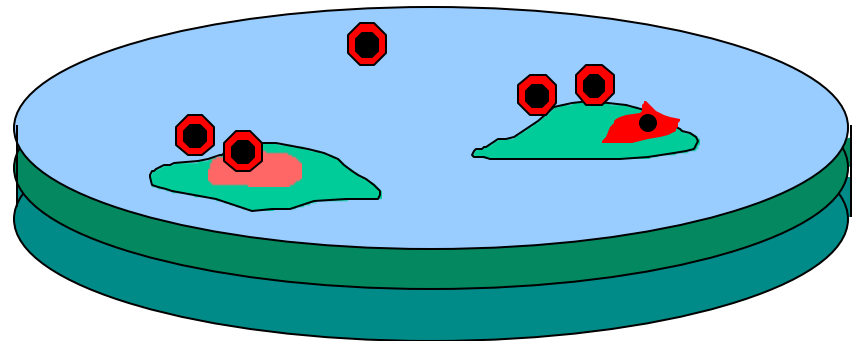
Anatomy of an Alveolus



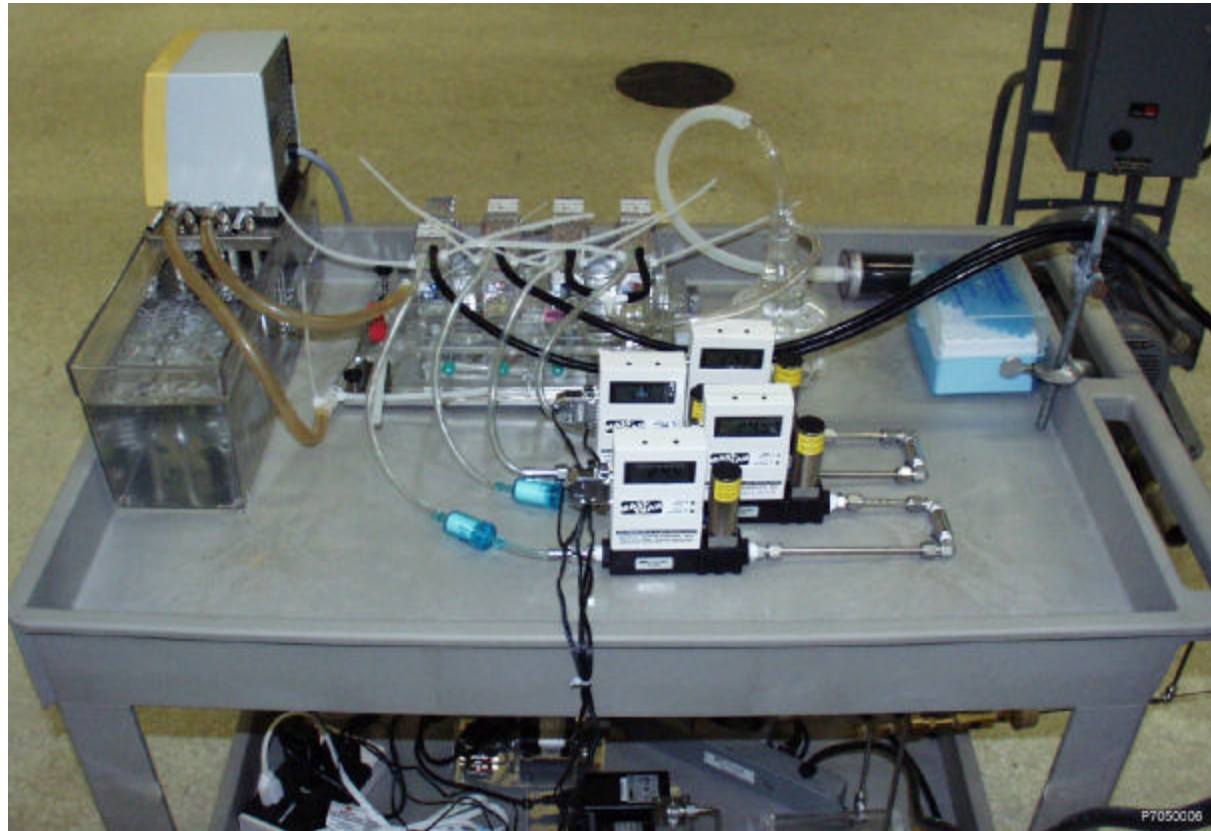
Conventional Submersion Culture



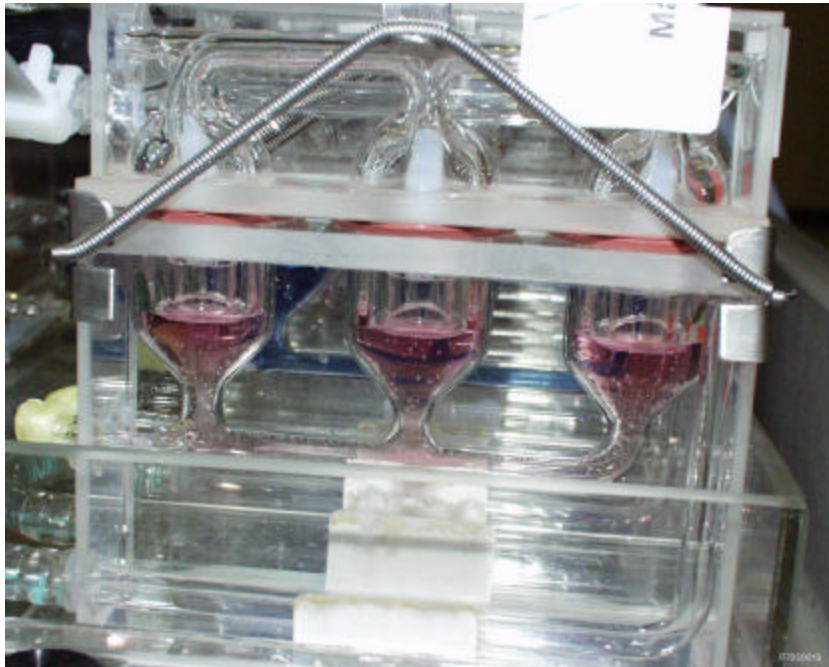
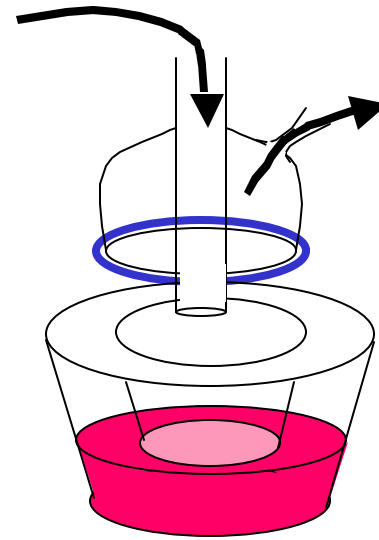
Air/Liquid Interface Culture

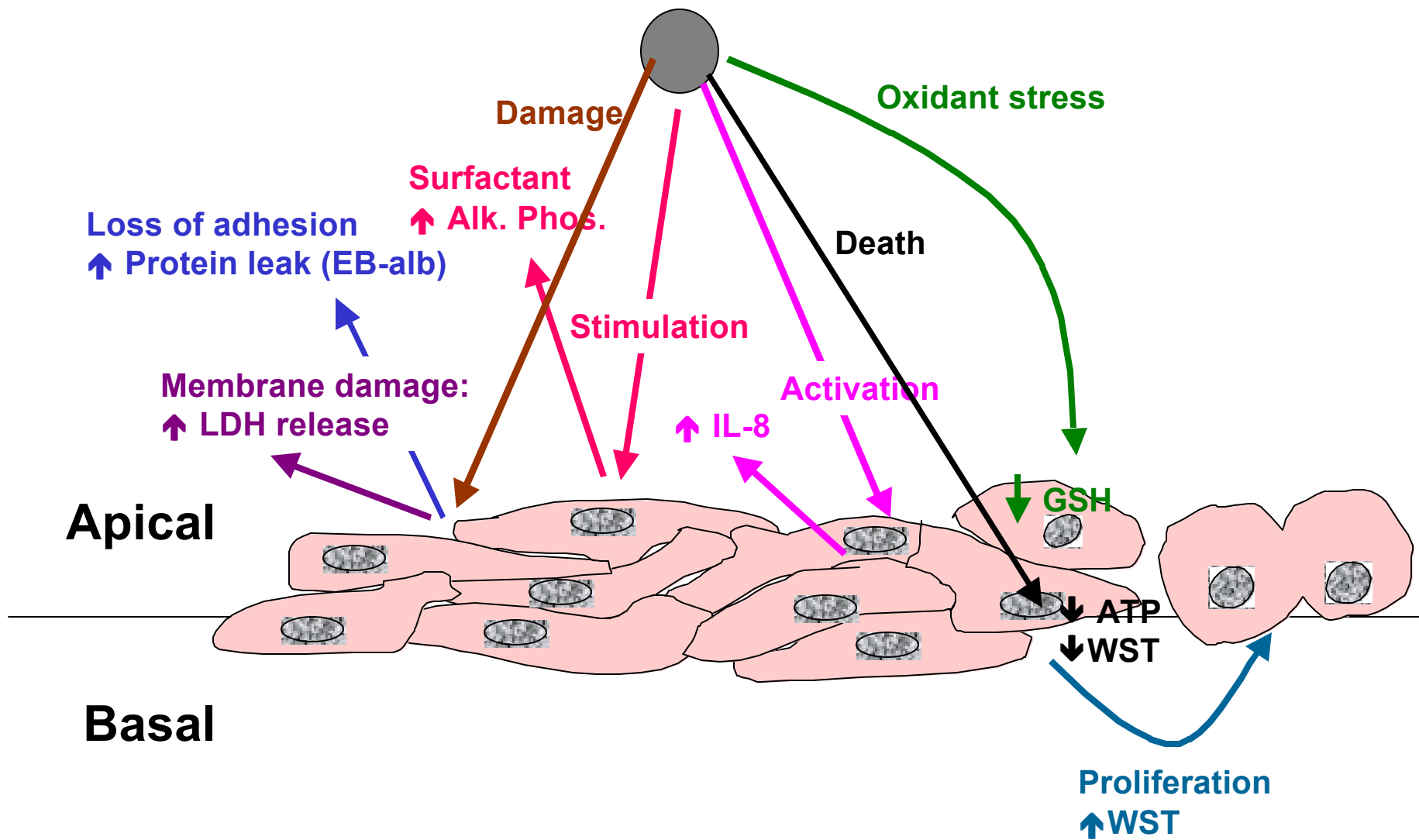


Air-Liquid Interface Exposure

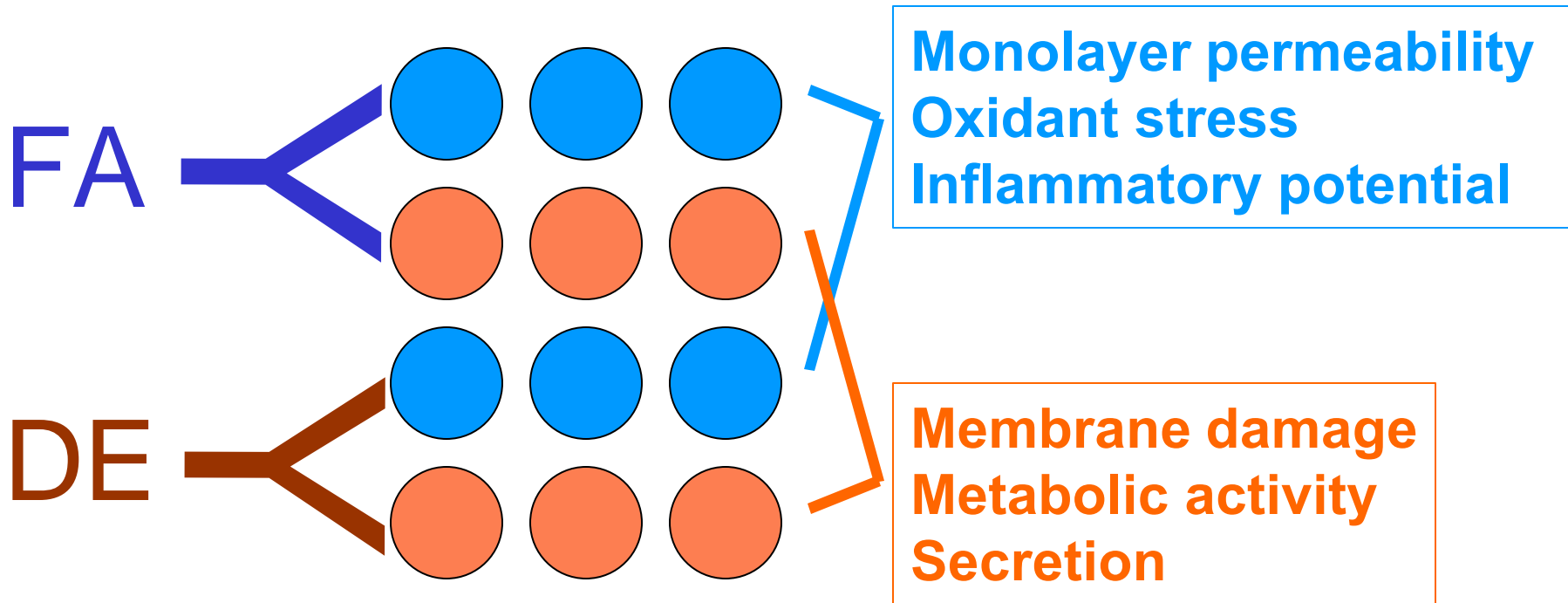


Temperature controlled by circulating waterbath
Flow controlled by parallel flow regulators
2 modules exposed to diesel exhaust, 2 modules
exposed to filtered/scrubbed air, 3 transwells/module





Responses Measured



Exposed for 1 hr or 3 hr to whole exhaust,
or 3 hr to filtered exhaust

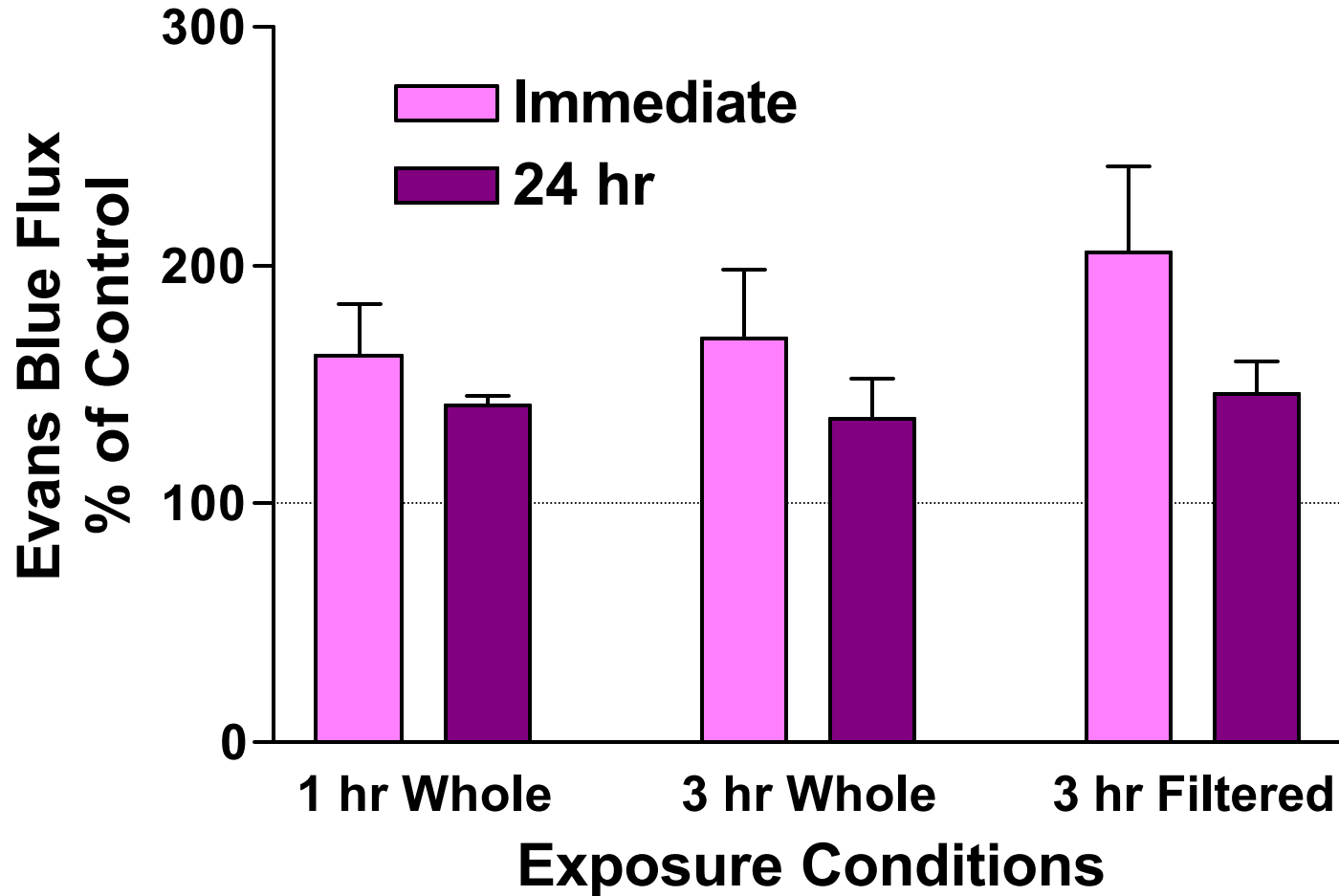
Responses measured 1 hr or 24 hr after exposure

Acute Toxicity and Metabolic Responses

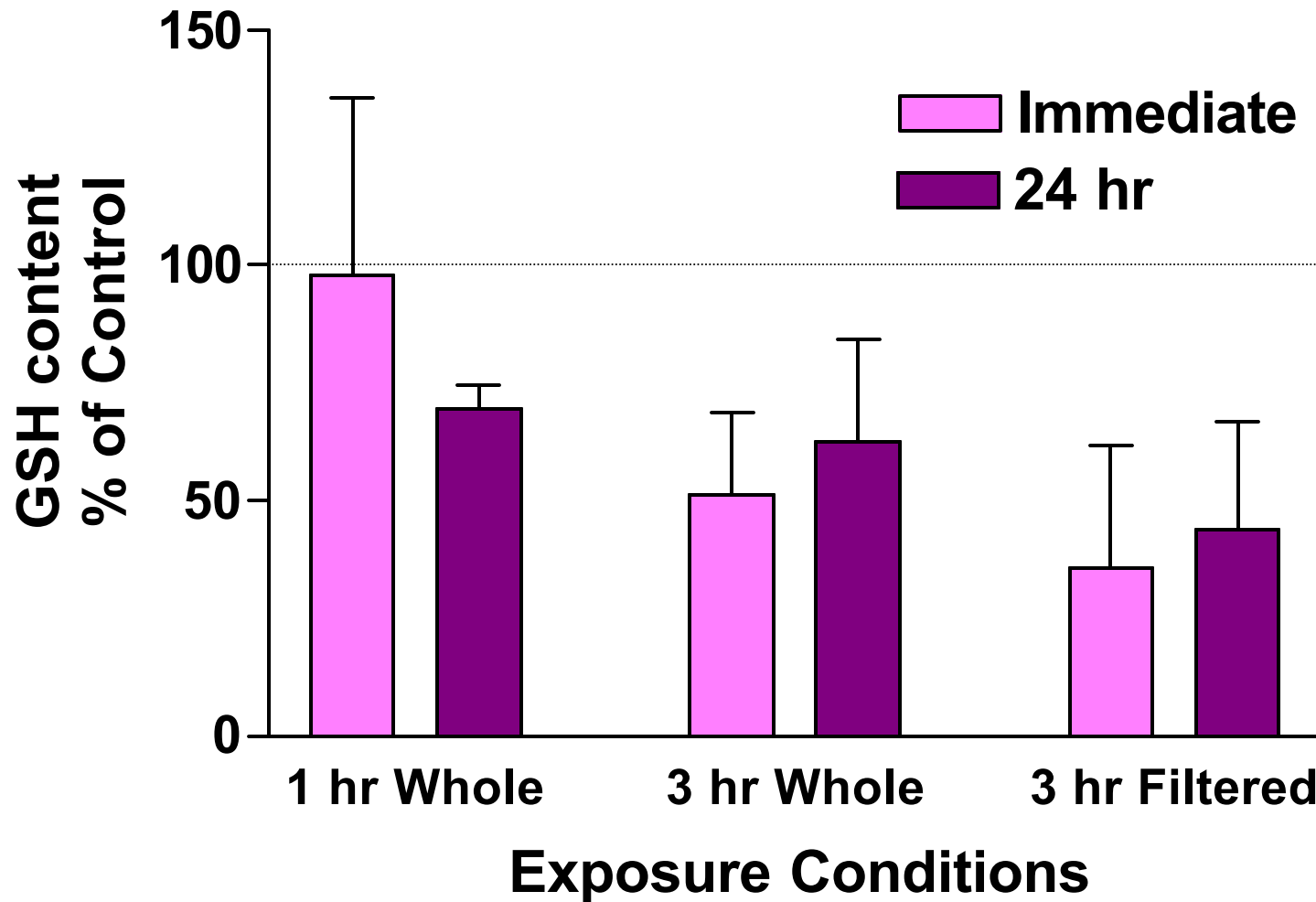
No significant effects of 1 hr or 3 hours of whole exhaust, or 3 hr of filtered exhaust, on:

- **ATP content**
- **Metabolic activity/ cell # by WST**
- **LDH release**
- **Alkaline Phosphatase**

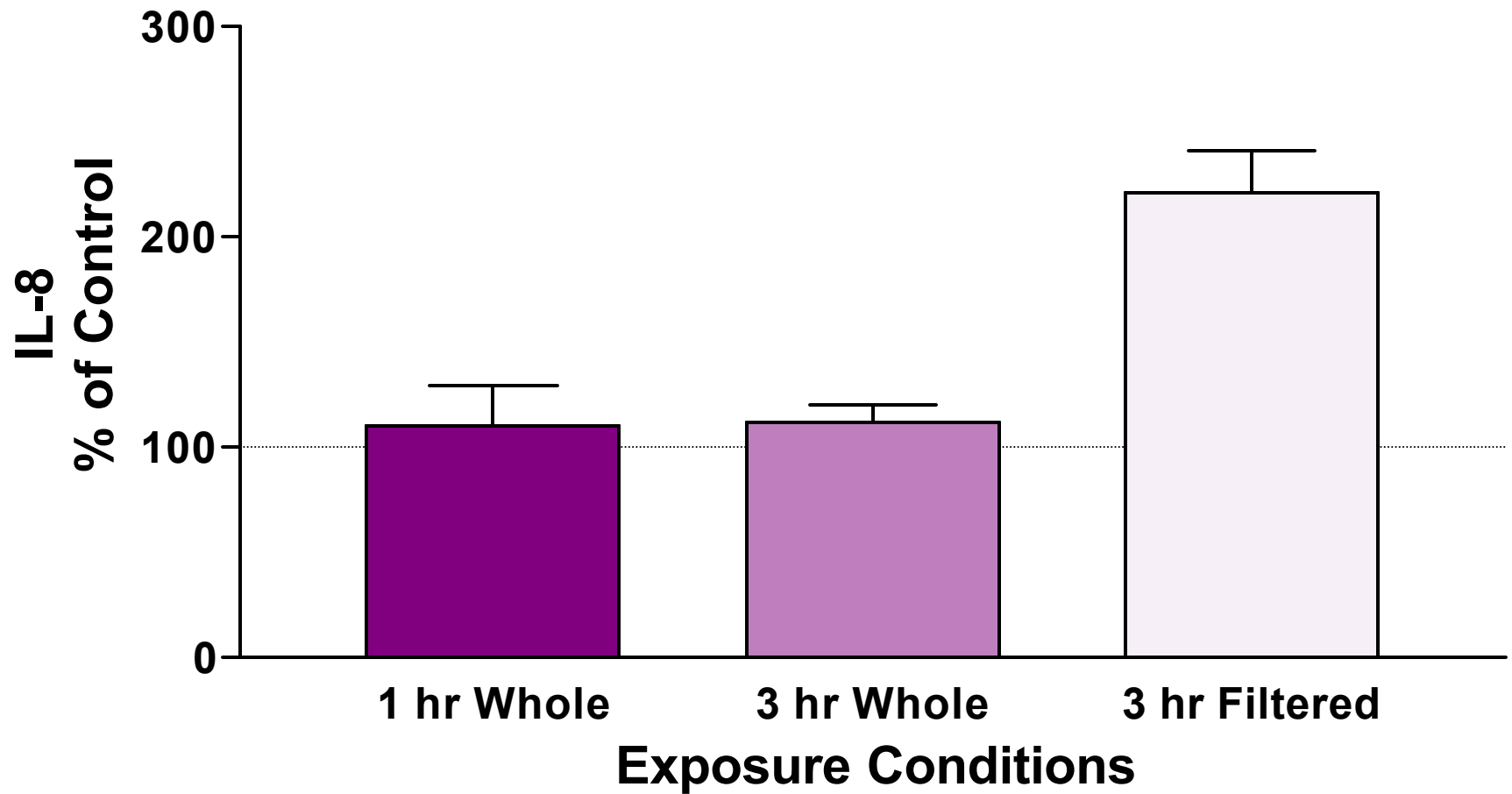
Epithelial Permeability



Cellular Glutathione



IL-8 Release



SUMMARY

- This method can be used to detect biological effects of engine emissions
 - In range of occupational exposures
 - Short times
- Non-cytotoxic levels of emissions:
 - Increase epithelial permeability measured by basolateral to apical passage of labeled protein
 - Induce oxidant stress as measured by decreases in glutathione content
 - Increase synthesis and secretion of an inflammatory mediator (IL-8)

NEXT STEPS

- **Characterize actual doses at the interface**
- **Extend observations to lower doses**
- **Further fractionation/model constituents:**
 - **Particles (coarse, fine, UF) vs. vapor/gas**
 - **Nanoparticles**
- **Effects of fuels, aftertreatments, other engine modifications**
 - **Potential for on-site evaluations**