Effects of direct exposure of engine exhaust nanoparticles on human lung cell immune response

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Objectives

- **To experiment with cellular exposure/response through airborne particle deposition**
- **To correlate response of human lung cells to particle diameter and/or chemistry**
- **To observe difference in the response of cells, normal or primed, to particles**

Materials and Methods

- **Cells**
  - Human lung carcinoma cell line, A549, epithelial from ATCC
  - Density - 80,000 cells per each transwell membrane
  - Incubation period before exposure: 48 hours
  - Primed cells: add 25 ng/cc Human Tumor Necrosis Factor (TNF)-α agent for 24 hours before replacing medium for exposure experiments
  - Placed on top of a COSTAR transwell filter membrane with medium underneath in a well throughout exposure experiment
  - Number of cells counted right before an exposure experiment

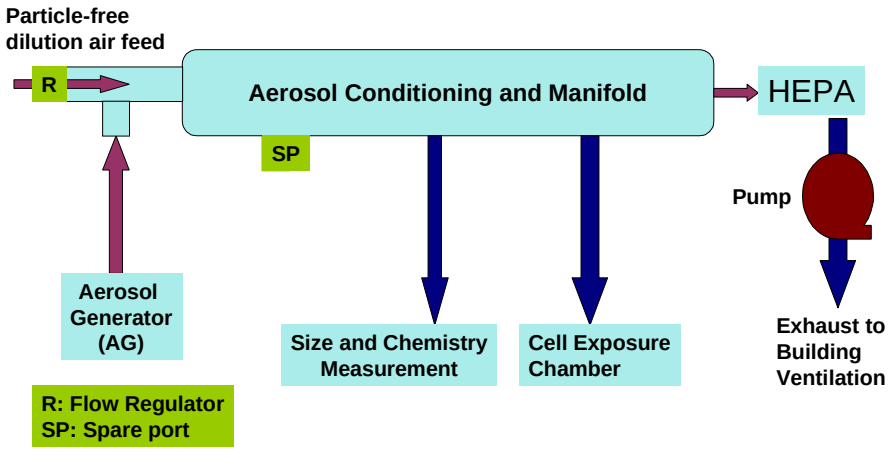
Materials and Methods (cont.)

- **Synthetic Particles**
  - Monodisperse nanoparticles generated by electrospray generator
  - Monodisperse supermicron particles generated by vibrating orifice generator
  - Polydisperse particles generated by supersonic jet nebulizer
  - Solutions prepared with Nanopure double DI-water, and filtered with a 0.4 µm filter before use in particle generator
- **Engine Generated Particles**
  - gasoline SIDI, CARB-II reformulated fuel
  - 1998-spec heavy-duty diesel engine, DECSE fuel
- **Measured by scanning mobility particle sizer (SMPS) for nanometer and aerodynamic particle sizer for supermicron particles**

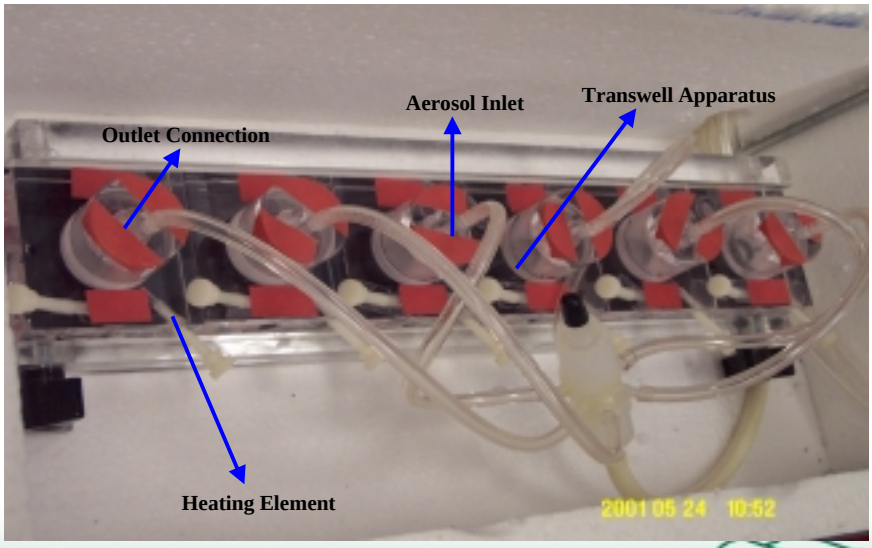
Materials and Methods (cont.)

- **ELISA - IL 8**
  - Material from Pharmingen, OptEIA set, Human IL-8 analysis kit
  - Establish calibration curve for each exposure experiment
  - 2 curves each experiment
  - Accepts ± 10%
- **Cell death**
  - Microscopy, Trypsin stained

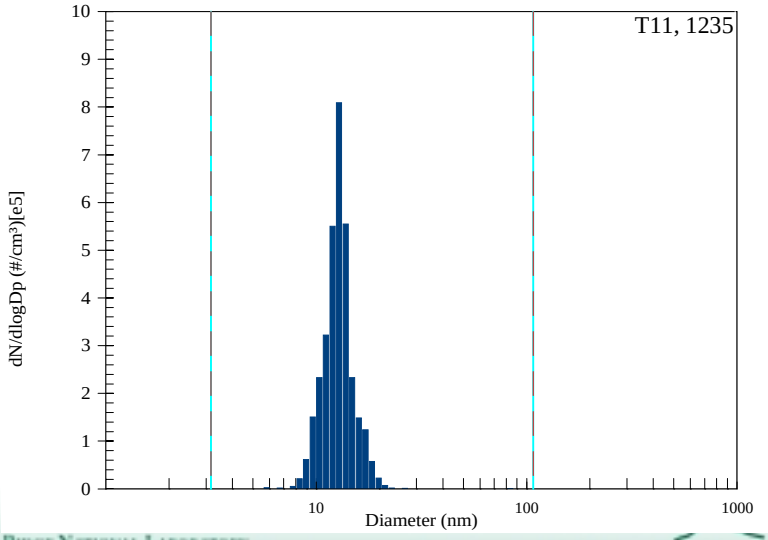
Block Diagram of Cell Exposure



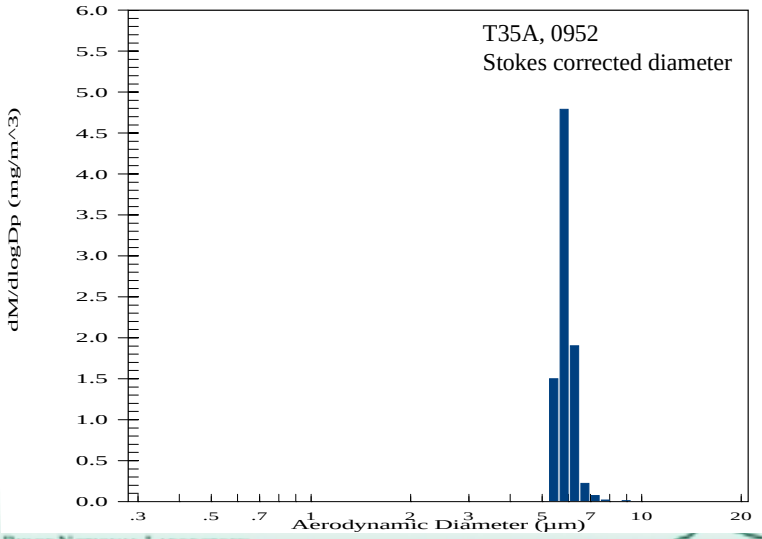
Cell Exposure Chamber



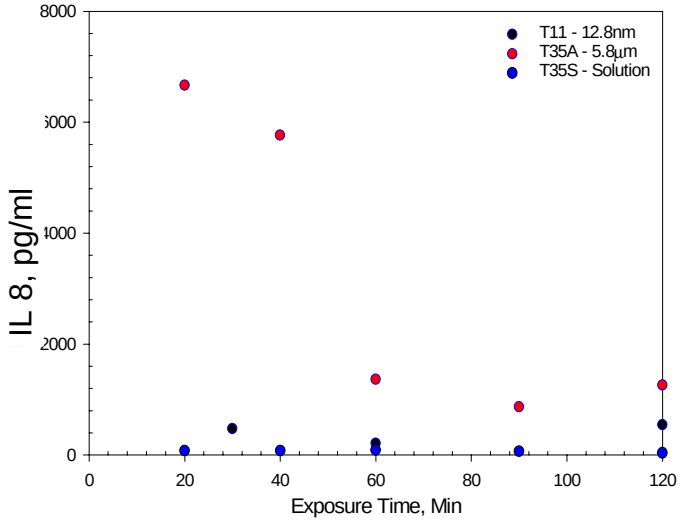
Size Distribution of Monodisperse nanometer particles from 0.125% (v/v) $NiCl_2$ solution



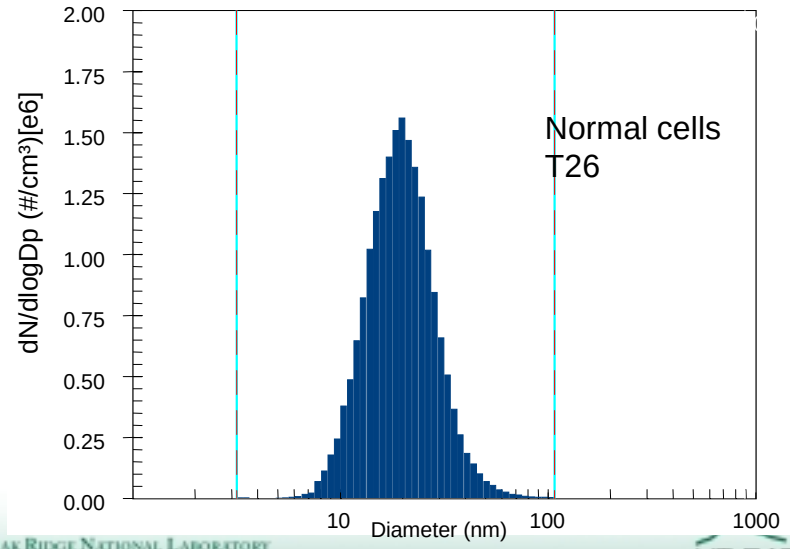
Size Distribution of Monodisperse supermicrometer particles from 0.125% (v/v) $NiCl_2$ solution



Exposure to monodisperse $NiCl_2$ particles and solution



Size Distribution of Diesel Emission Particles (DEP)
Low Sulfur Fuel, Engine Idle



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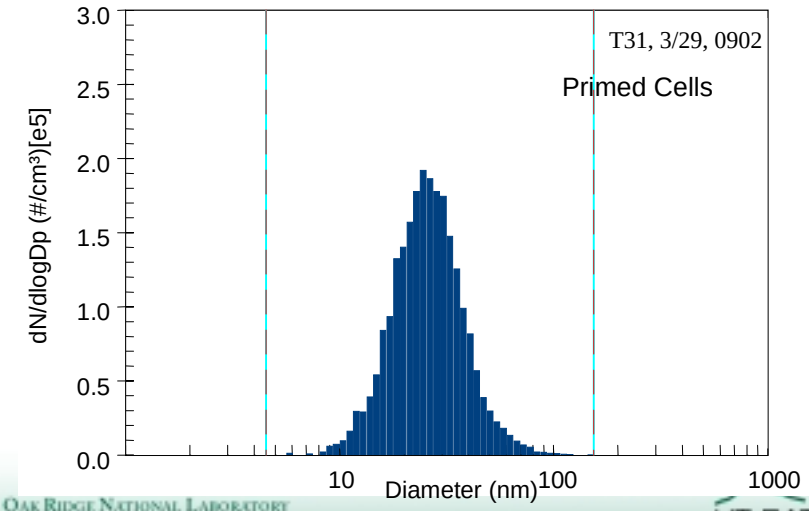
Size Distribution of Diesel Emission Particles (DEP)
Low Sulfur Fuel, Engine Idle,with CDPF



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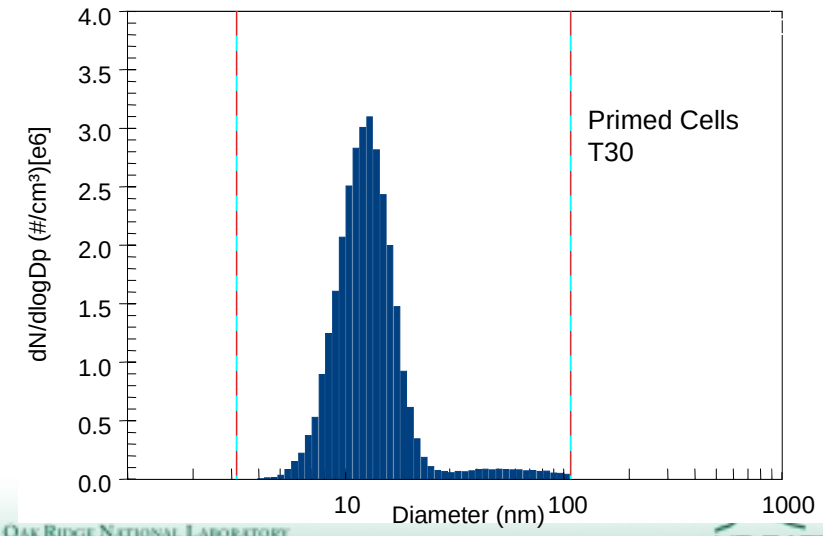
Size Distribution of Diesel Emission Particles (DEP)
40 ppm Sulfur Fuel, low load



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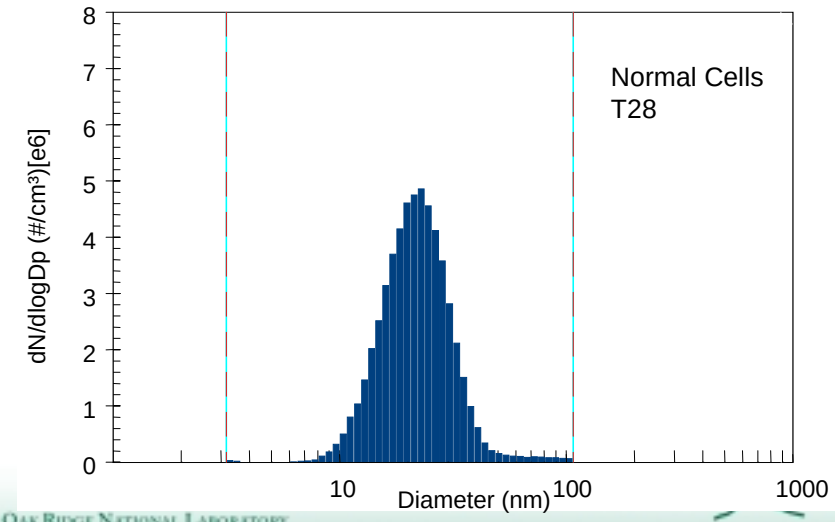
Size Distribution of Diesel Emission Particles (DEP)
40 ppm Sulfur Fuel, Idle



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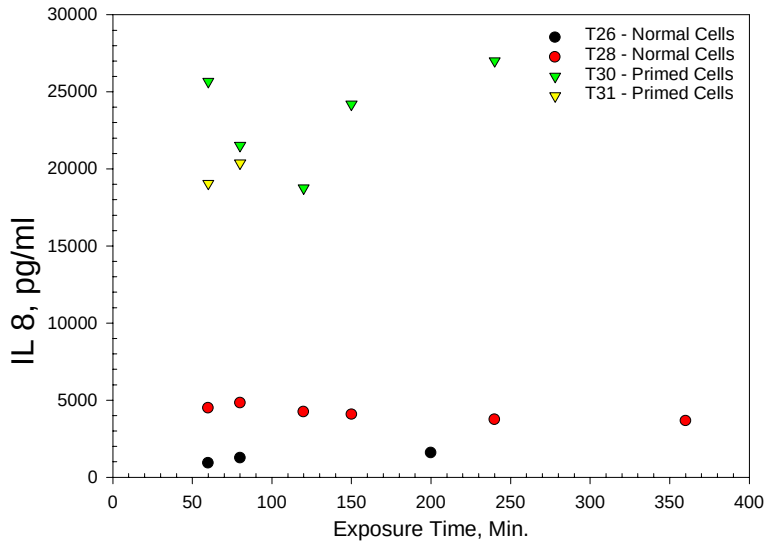
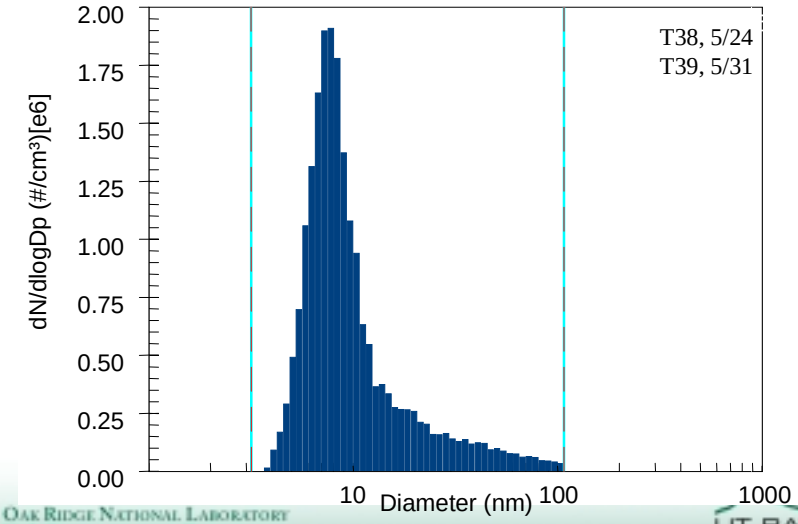
Exposure to Diesel Emission Particles (DEP)

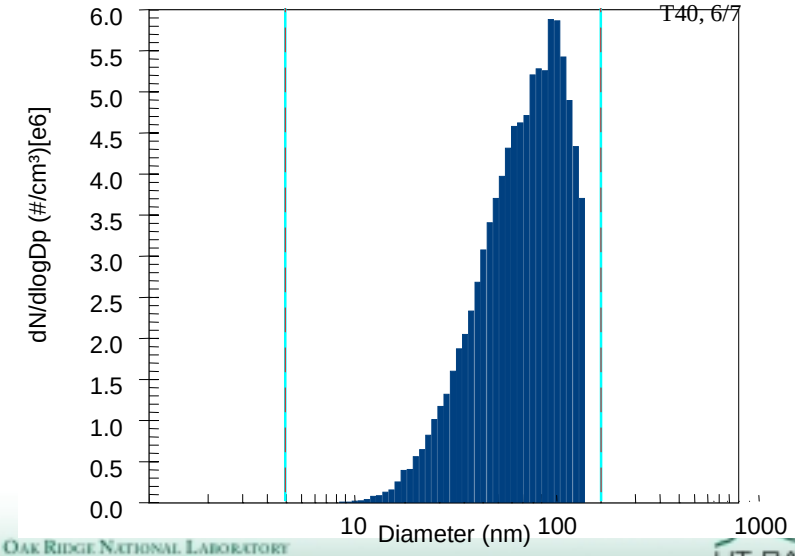


Size Distribution of Gasoline Engine Emission Particles

SIDI Cell exposure, 950 rpm, 16.7 ft.lbs, DR=9



Size Distribution of Gasoline Engine Emission Particles
2400 RPM, 70% load



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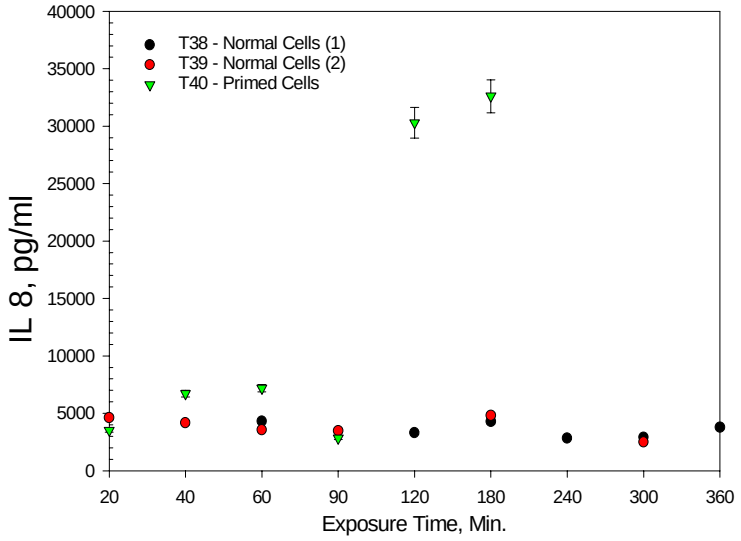
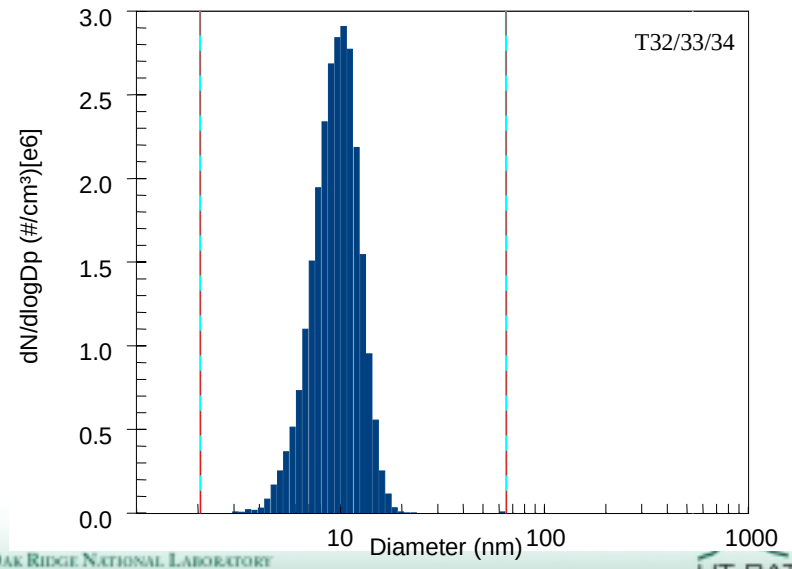
Exposure to Gasoline Engine Emission Particles



Size Distribution of Monodisperse Nanometer H_2SO_4 Particles



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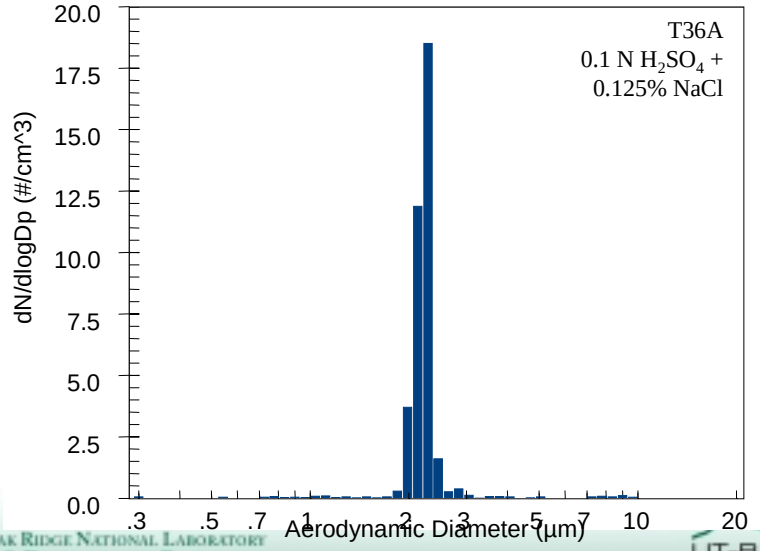
Size Distribution of Monodisperse Supermicrometer H_2SO_4-Based Particles



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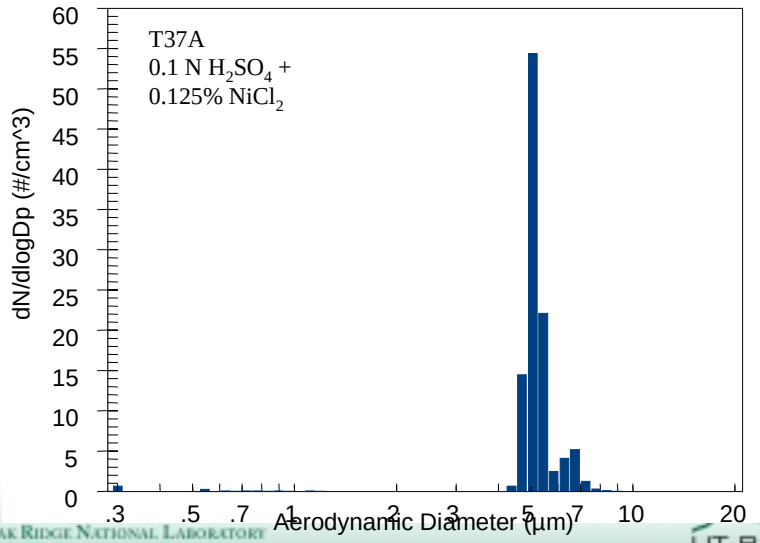
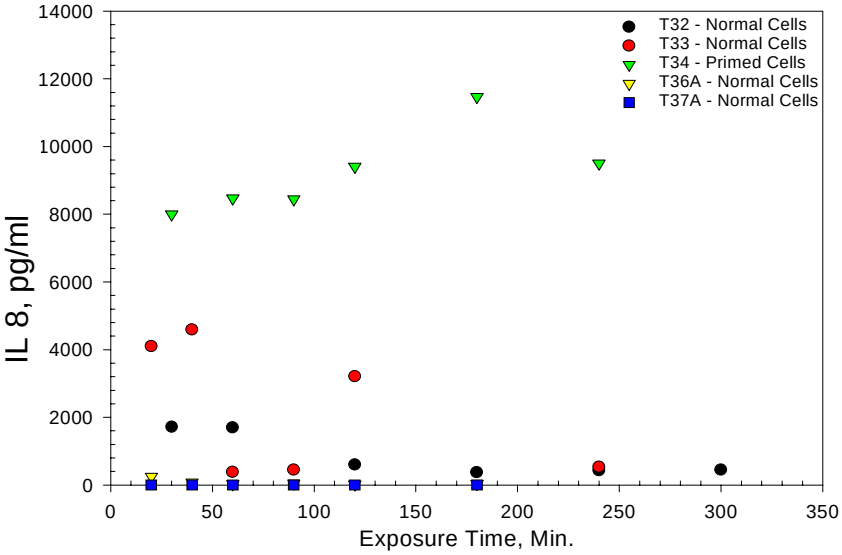
Size Distribution of Monodisperse Supermicrometer H_2SO_4-Based Particles



Exposure to Monodisperse H_2SO_4-Based Particles



Summary

- A field portable apparatus has been developed and successfully employed to investigate responses of human lung cells (specifically A549 epithelial cell line) to direct deposition of airborne particles
- Primed and normal A549 cells appear to be a good model for investigating the toxicity of airborne particles
- IL-8 expression as a result of direct deposition of $NiCl_2$ particles was higher for supermicron than for nanometer, consistent with literature data of exposure to residual oil particles
- IL-8 expressions for H_2SO_4 and its mixture particles however was in reverse of $NiCl_2$ particles indicating nanometer particles induced higher IL-8 than supermicron

Summary - Continued

- For normal lung cells, the IL-8 expressions appear to be in the same magnitude for exposure to diesel and gasoline particles
- For primed lung cells, however, the IL-8 expression was approx. 50% higher in the exposure to gasoline than to diesel particles.
  - However, the response time of primed cells was shorter (in an hour) for diesel than for gasoline particles (in 2 hours)
- Future studies will look at shorter exposure times, lower number concentration

Acknowledgements

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