

One-Atmosphere Research Program for Urban Gaseous/Particulate Matter and Human Health Effects Studies



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Support from

American Chemistry Council

US Environmental Protection Agency

**Does atmospheric photochemistry contribute
significantly to human lung cell damage?**

Can we describe (model) any contribution?

Reaction and Exposure Systems at UNC



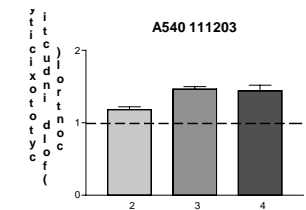
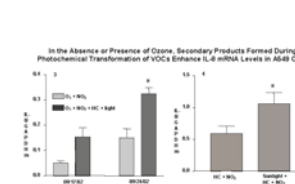
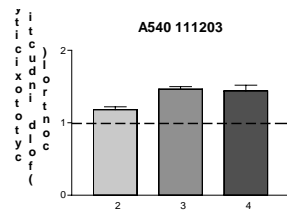
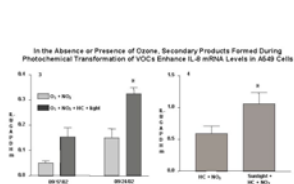
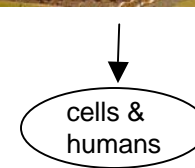
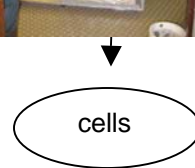
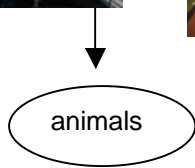
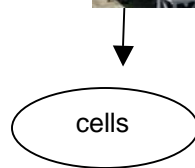
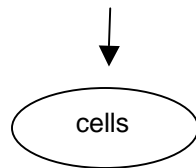
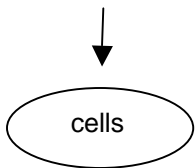
Mixtures or Components of Sources, and Photochemistry

Gas Only

PM & Gas

PM & Gas

PM & Gas



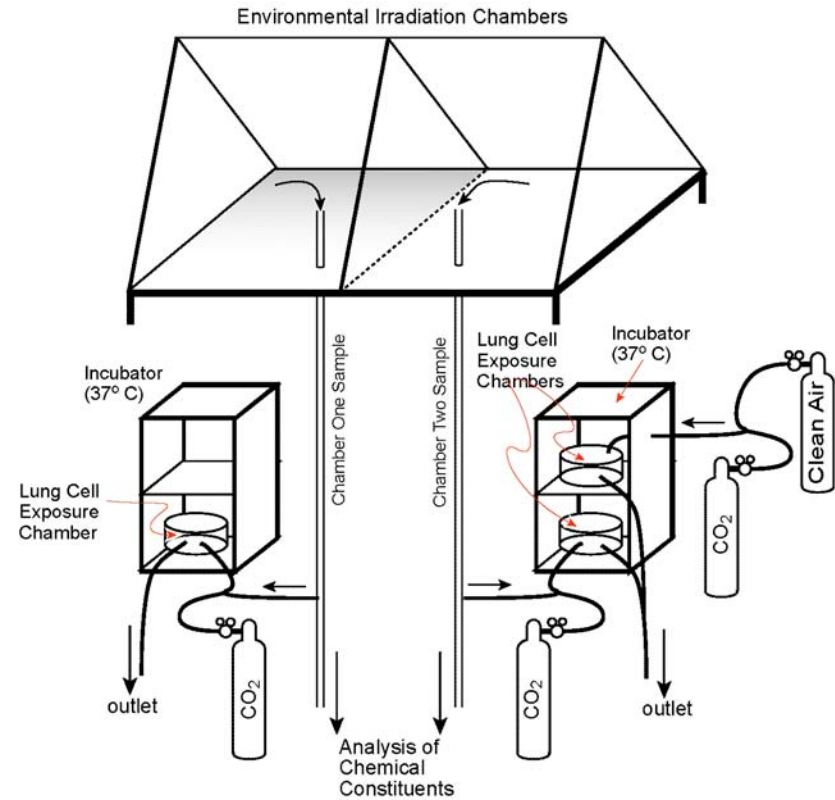
1000's of gas and particle experiments

new systems

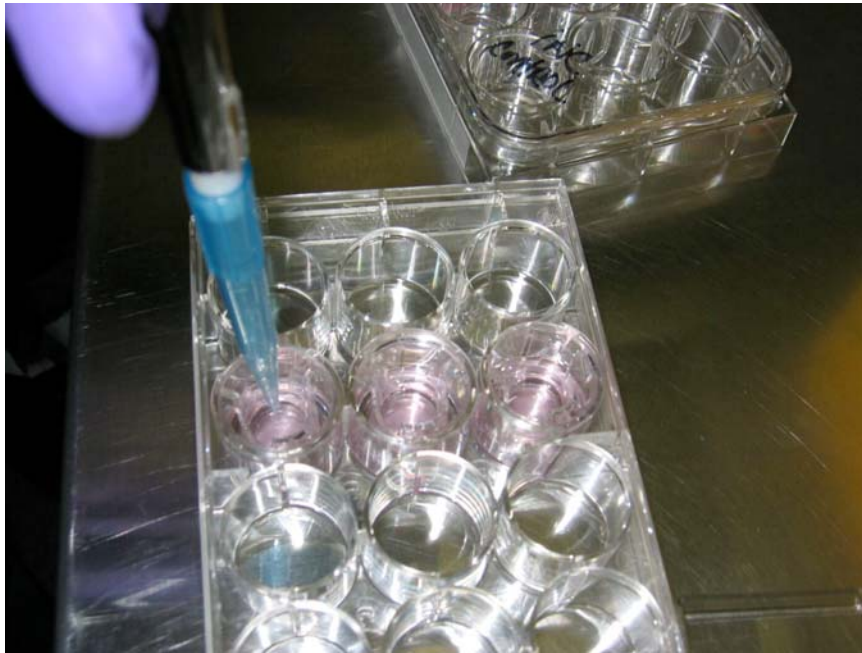
Large Body of Results and Experience



UNC Outdoor Teflon Film Smog Chambers



Smog Chamber – In Vitro Exposure System



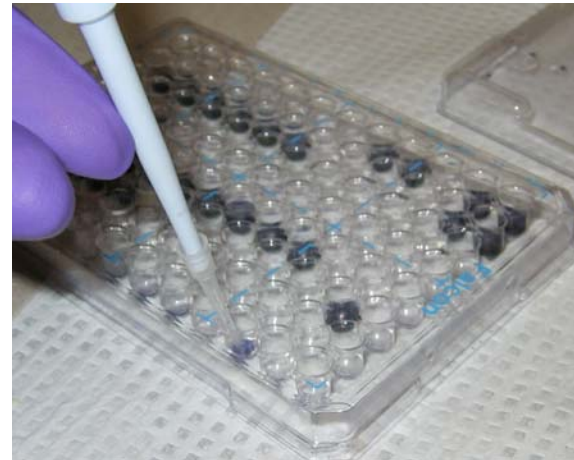
In vitro exposure system: materials

Plates holding membrane wells,
Living Human Respiratory Epithelial Cells
(aka lung cells) on membranes,
Saucers to hold plates with correct
gas environment

Incubator (body temp) Exposure Chamber.



Toxicological Detection Methods

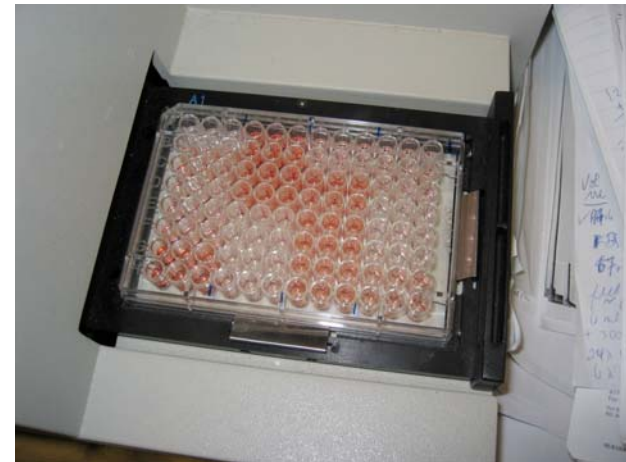


Cytotoxicity or
Cell Viability, LDH

cytokine mRNA via RT-PCR analysis IL-8



ELISA: cytokine protein levels, IL-8, IL-6, others

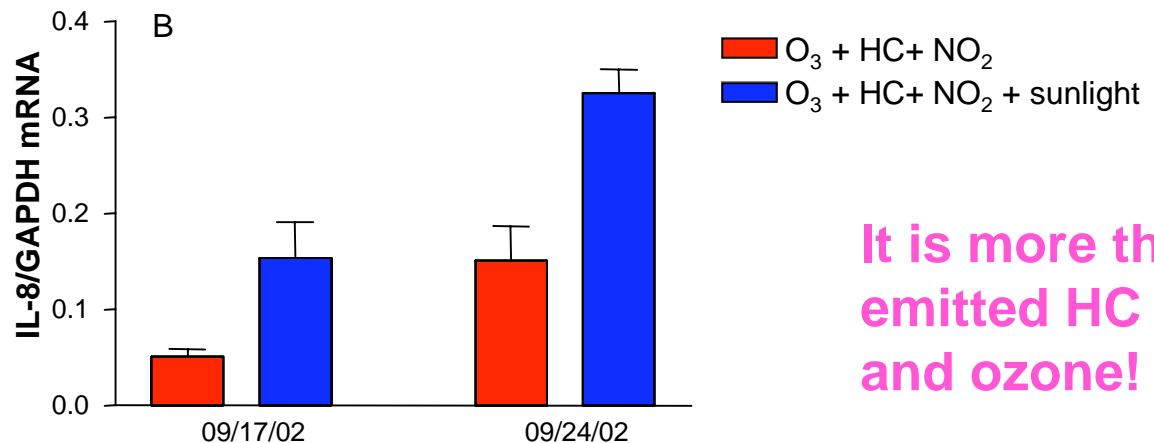
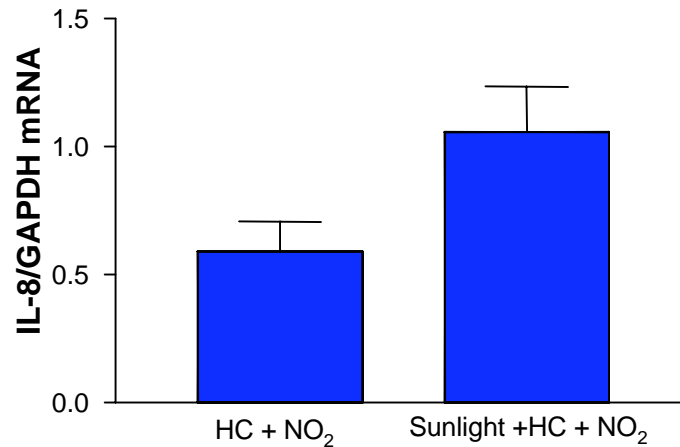


Is photochemistry important to effects?

Synthetic Urban Mix: Lung Cell Inflammatory Response

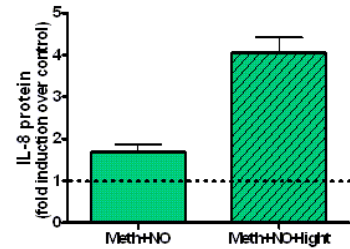
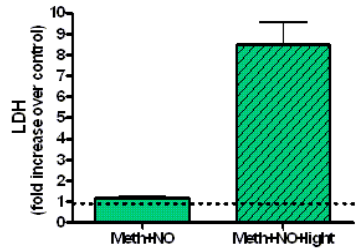
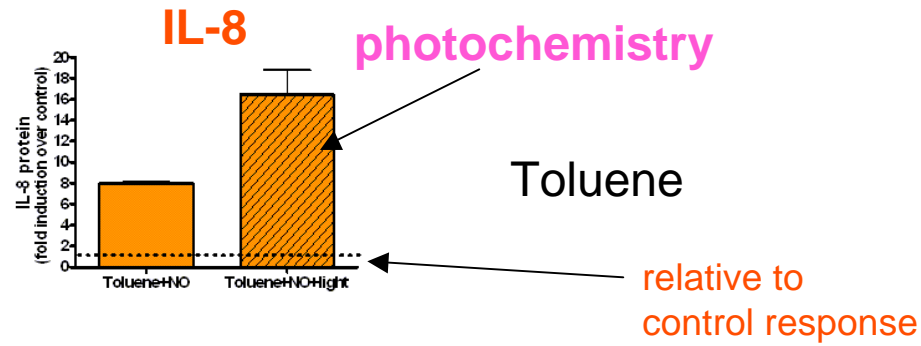
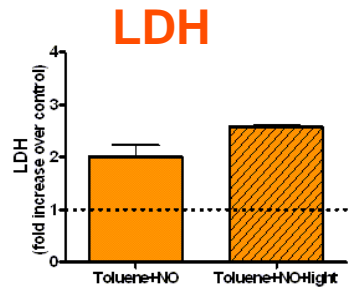
In the Absence or Presence of Ozone, Secondary Products Formed During Photochemical Transformation of VOCs Enhance IL-8 mRNA Levels in A549 Cells

absolute response

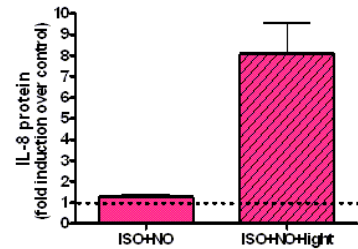
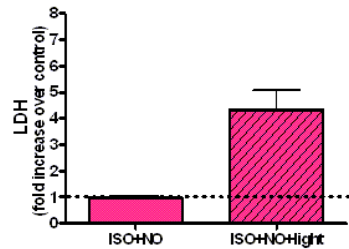


It is more than emitted HC and ozone!

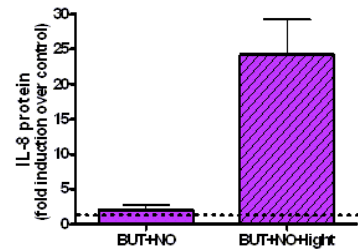
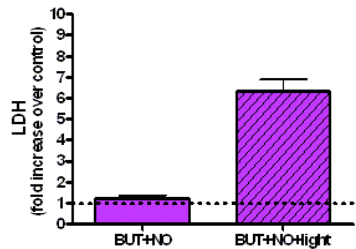
Is photochemistry important?



Methanol



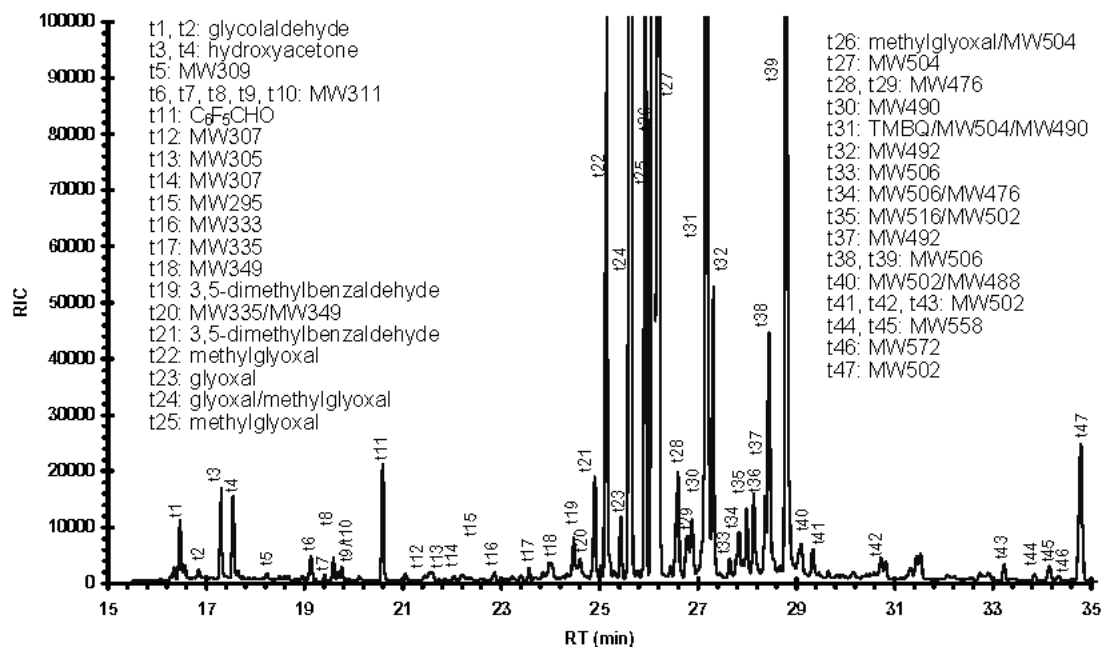
Isoprene



1,3-Butadiene

LDH = lactate dehydrogenase
Related to cell viability

Advanced Analysis of Exposure

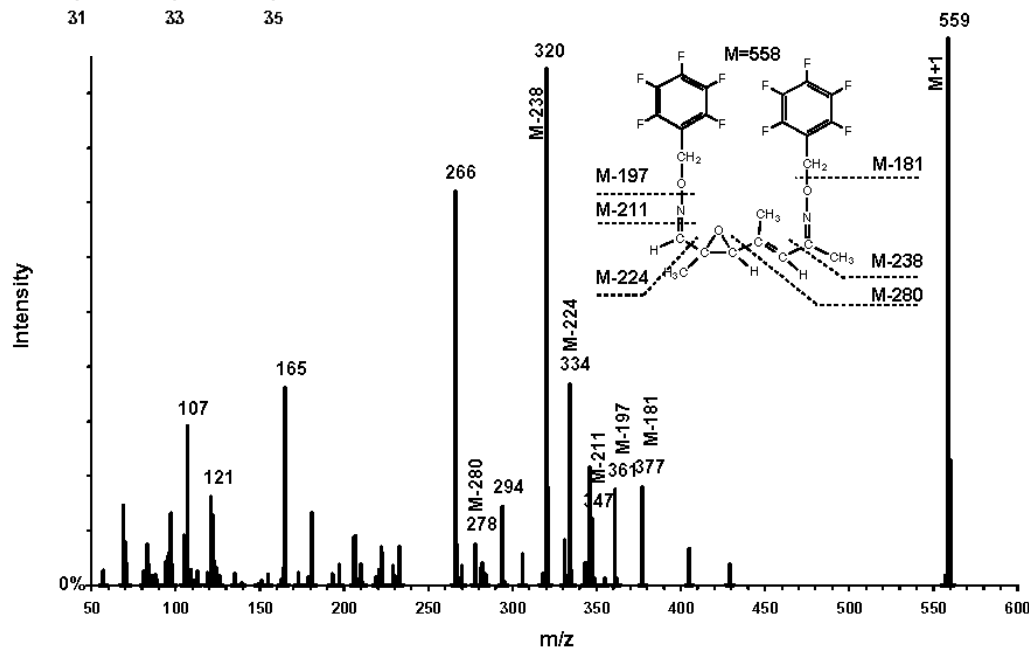


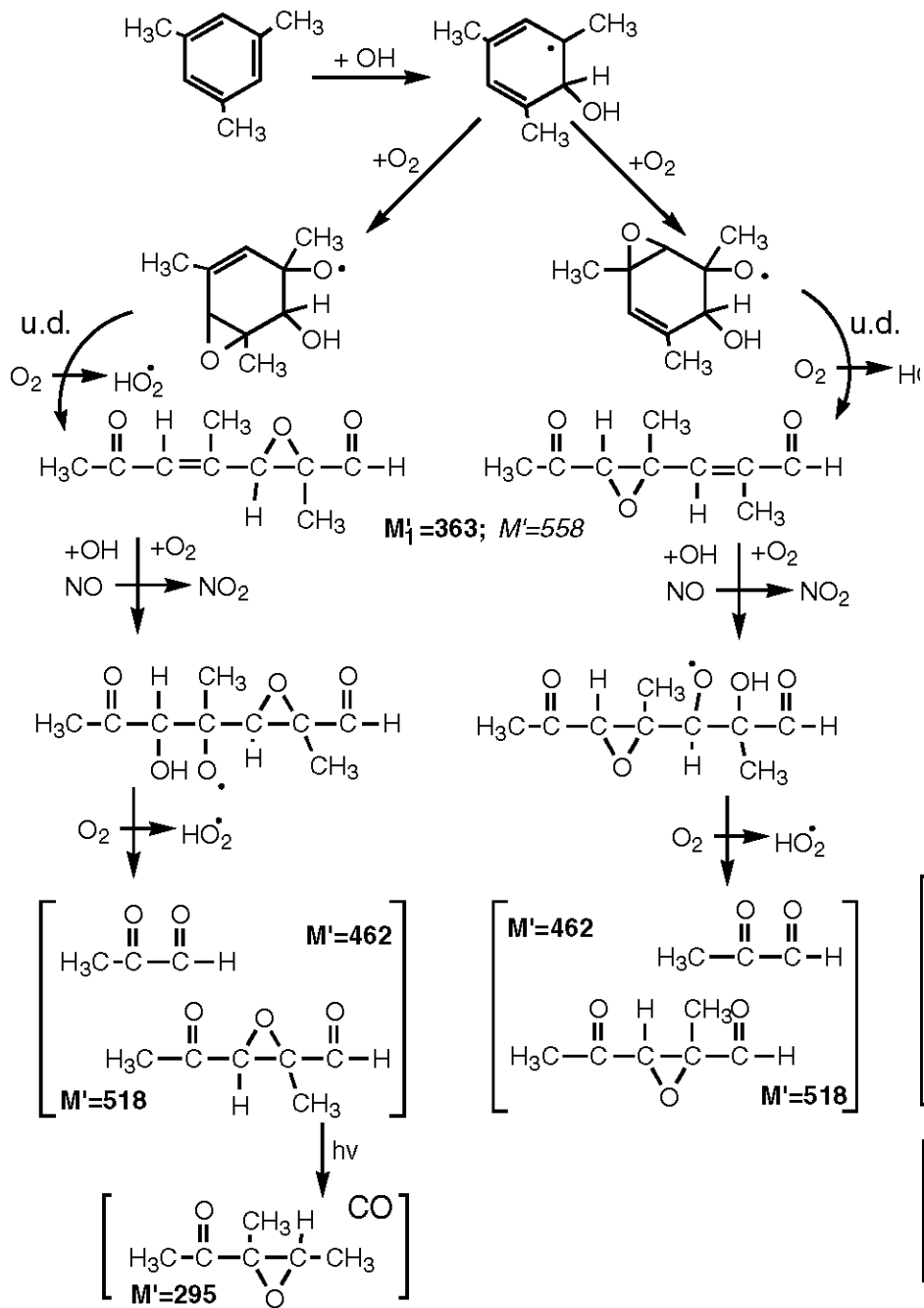
Identification of unknown species that have respiratory health effects.

Minor products can have major toxicity.

Gas chromatogram of 1,3,5 trimethylbenzene/NOx photochemical reaction mixture

Mass spectrum of t44/t45 peak

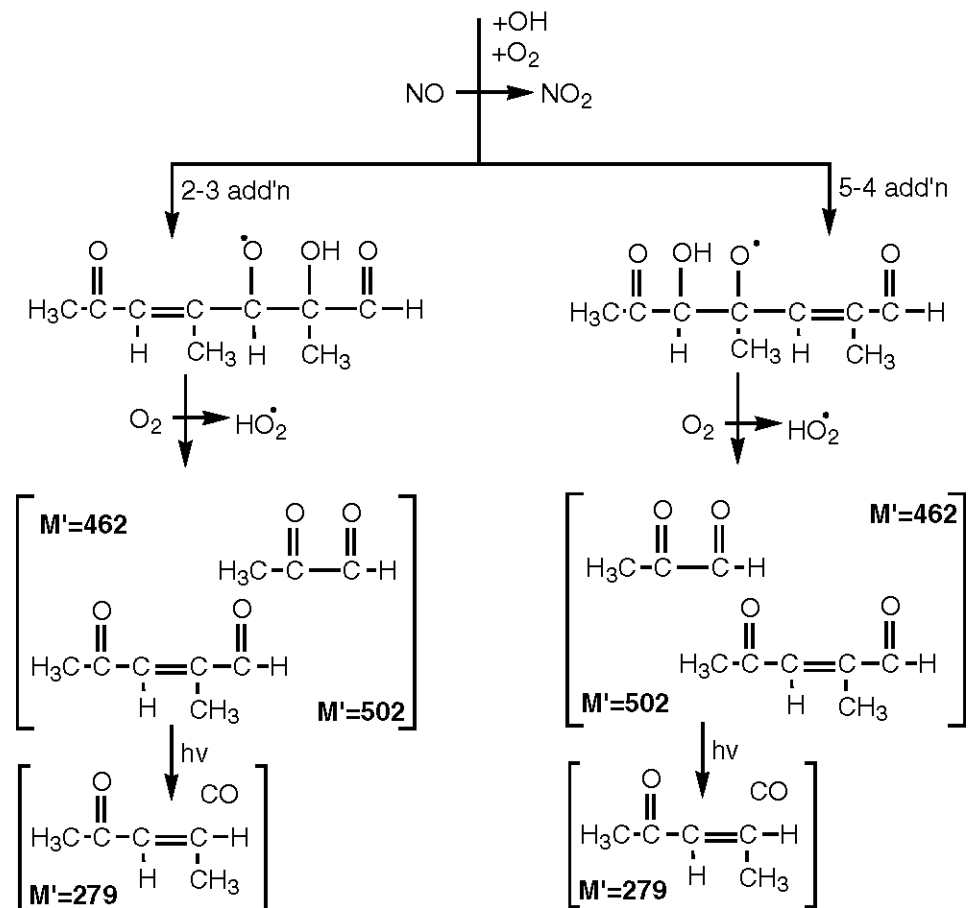
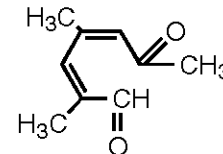




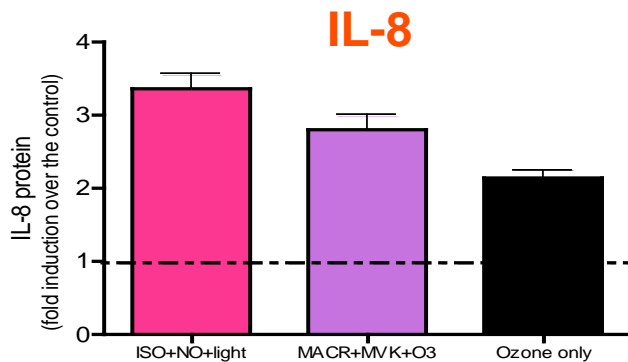
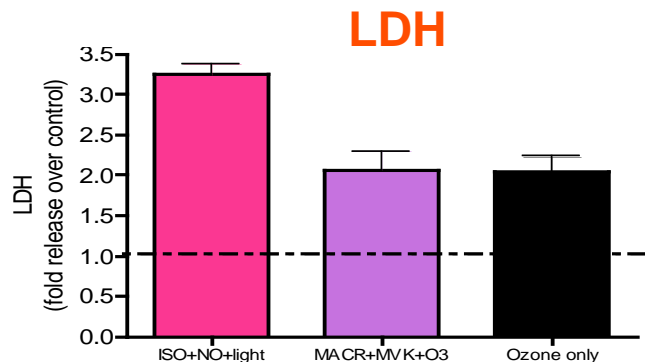
Identification of unknown species that may have respiratory health effects.

2,4-dimethyl-6-oxo-2,4-heptadienal

$M_1 = 347$; $M' = 542$

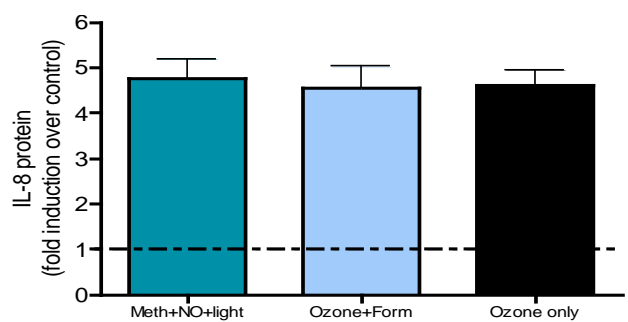
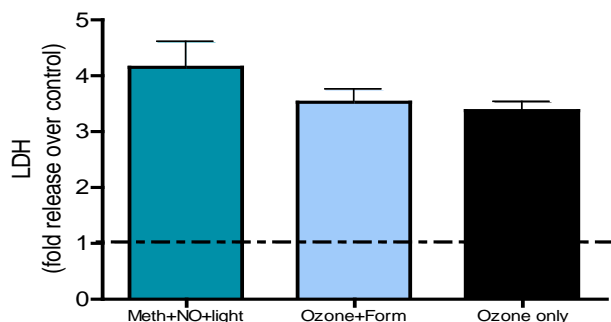


Examining the Inflammatory Responses of HAPS: The Role of Ozone and other Photochemical Transformation Products

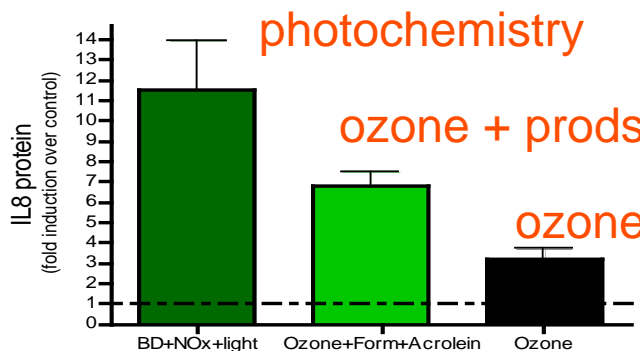
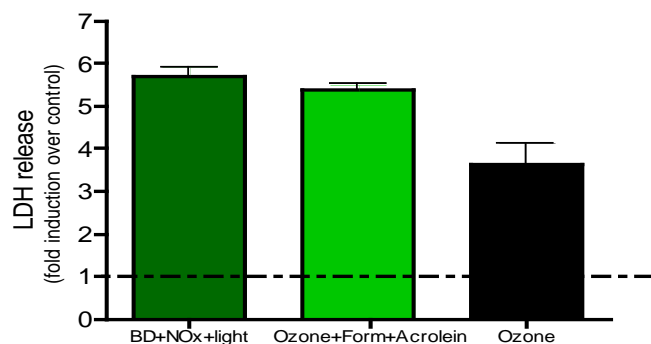


Isoprene

relative to control response



Methanol



photochemistry

ozone + prods

ozone

1,3-Butadiene

Examining the Inflammatory Responses of HAPS: The Role of Ozone and Other Organic Photochemical Transformation Products

- Exposure to the photochemically generated products of BD or ISO *significantly increased cytotoxicity and cytokine gene expression* compared to their injected primary photochemical transformation products, such as acrolein, formaldehyde and ozone for BD and methacrolein, methyl vinyl ketone, and ozone for ISO.
- Exposure to the equivalent levels of **ozone** generated during the photochemical transformation of BD or ISO *did not* induce the same level of inflammatory cytokine release, suggesting that ozone alone is not the sole inducer of inflammatory responses in this system.
- In the photochemical transformation of **methanol**, generating primarily ozone and formaldehyde, *ozone was the main inducer* for both inflammation and cytotoxicity.
- In more complex atmospheric mixtures, **ozone alone does not significantly account** for the effects seen, and therefore **full photochemical transformations and interactions** must be carefully evaluated when investigating adverse health effects induced by exposure to HAPS.

BD == 1,3-butadiene ISO == isoprene

Particles and *in vitro* Lung Cells

- Conventional methodologies are **unable to expose** lung cells *in vitro* simultaneously **to both particulate and gaseous pollutants** that are being formed in the ambient air.
- To expose cells to particles, **current methods collect the particles in solvents** or on a filter (and subsequently washed with solvent) and the solvent mixture is applied to cells. This likely **modifies the particle's chemistry** and its effect on cells.
- A new method for exposure of cells to such mixtures is to use **electrostatic precipitation**. We modified a TSI model 3100 Electrostatic Aerosol Sampler (EAS) to **deposit particles directly onto respiratory epithelial cells** grown on membranes and placed inside the EAS.
- We tested the EAS with **diesel exhaust** (DE) from a 1980 Mercedes-Benz model 300SD diluted with room air to a moderate particle concentration.
- The EAS achieved an overall **average collection efficiency of 97%** for all particles in the measured size range, When EAS was off the collection efficiency was under 2%.
- Cells **exposed to the EAS** system alone or DE with the EAS off showed **minimal cytotoxicity and release of IL-8** that was indistinguishable from the incubator controls.
- Cells **exposed to DE with the EAS turned on** produced a **threefold** increase in LDH and IL-8 release as compared to the control.

Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles

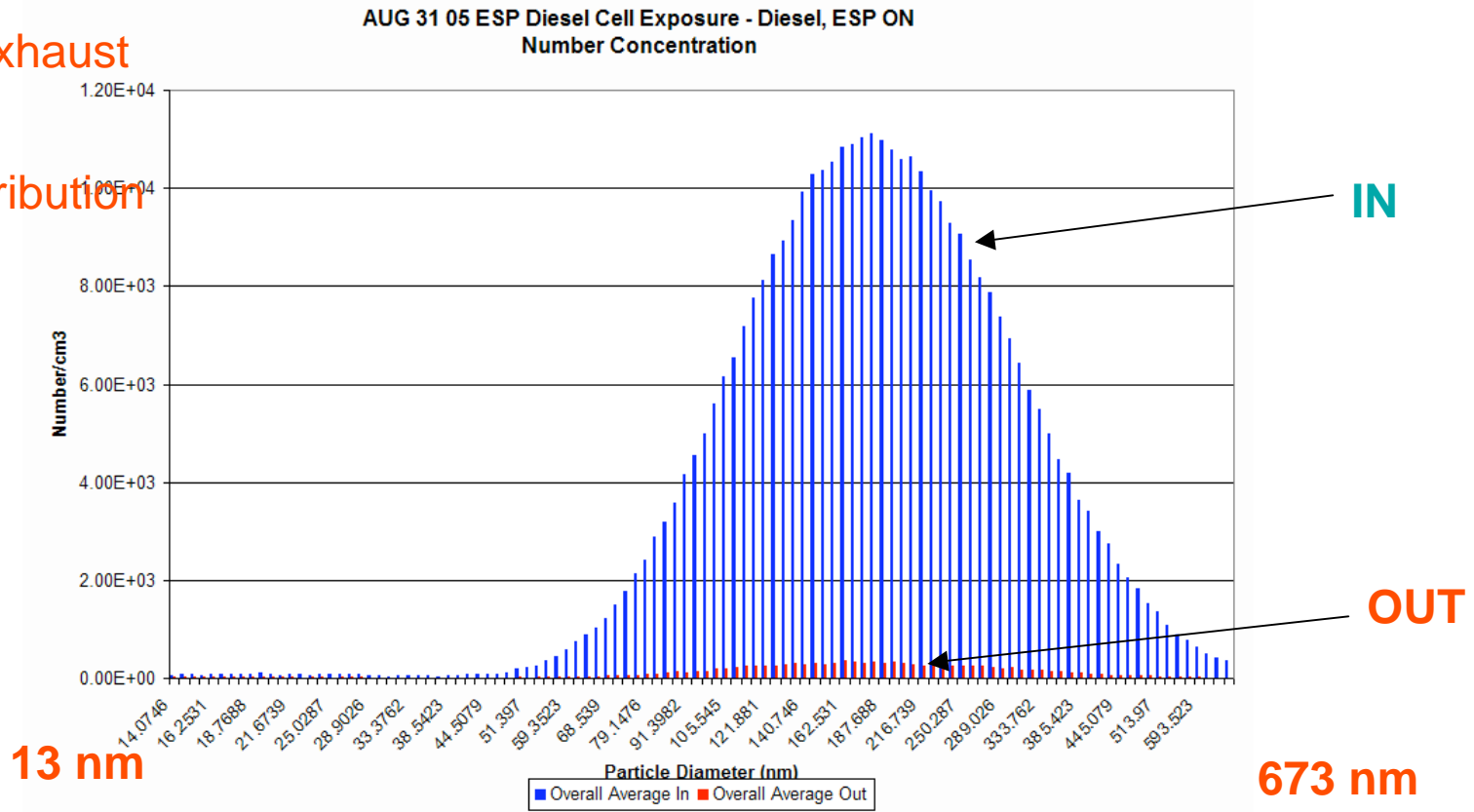


TSI 3100 Electrostatic Aerosol Sampler (EAS) was modified to improve the deposition of particles onto respiratory epithelial cells grown on membranes

Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles

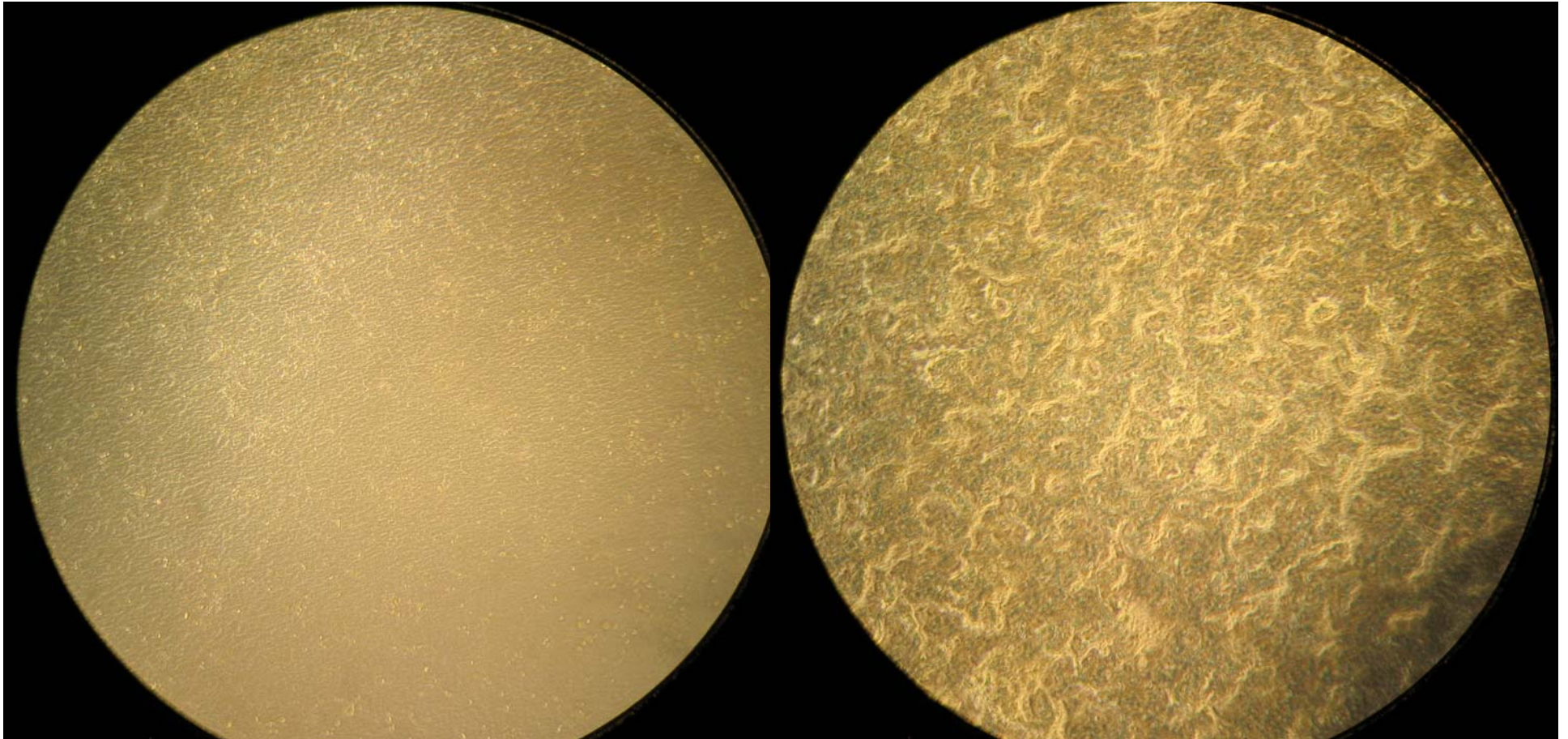
$3 \times 10^{12} \text{ nm}^3/\text{cm}^3$

Diesel exhaust particles size distribution



ESP Collection Efficiency 97%

Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles

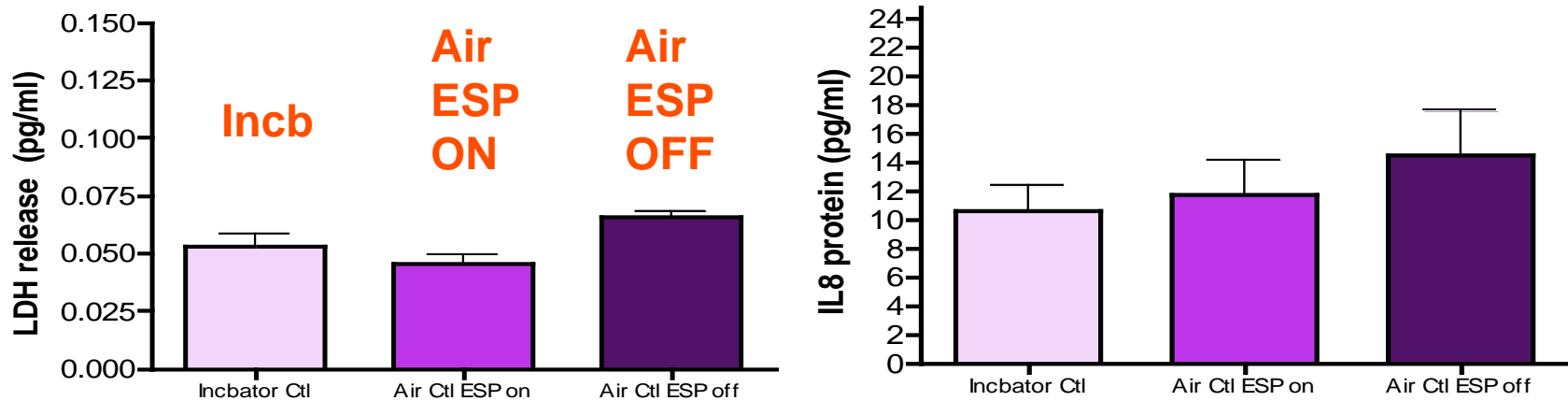


incubator control

Diesel PM EAS 1 hr exposure

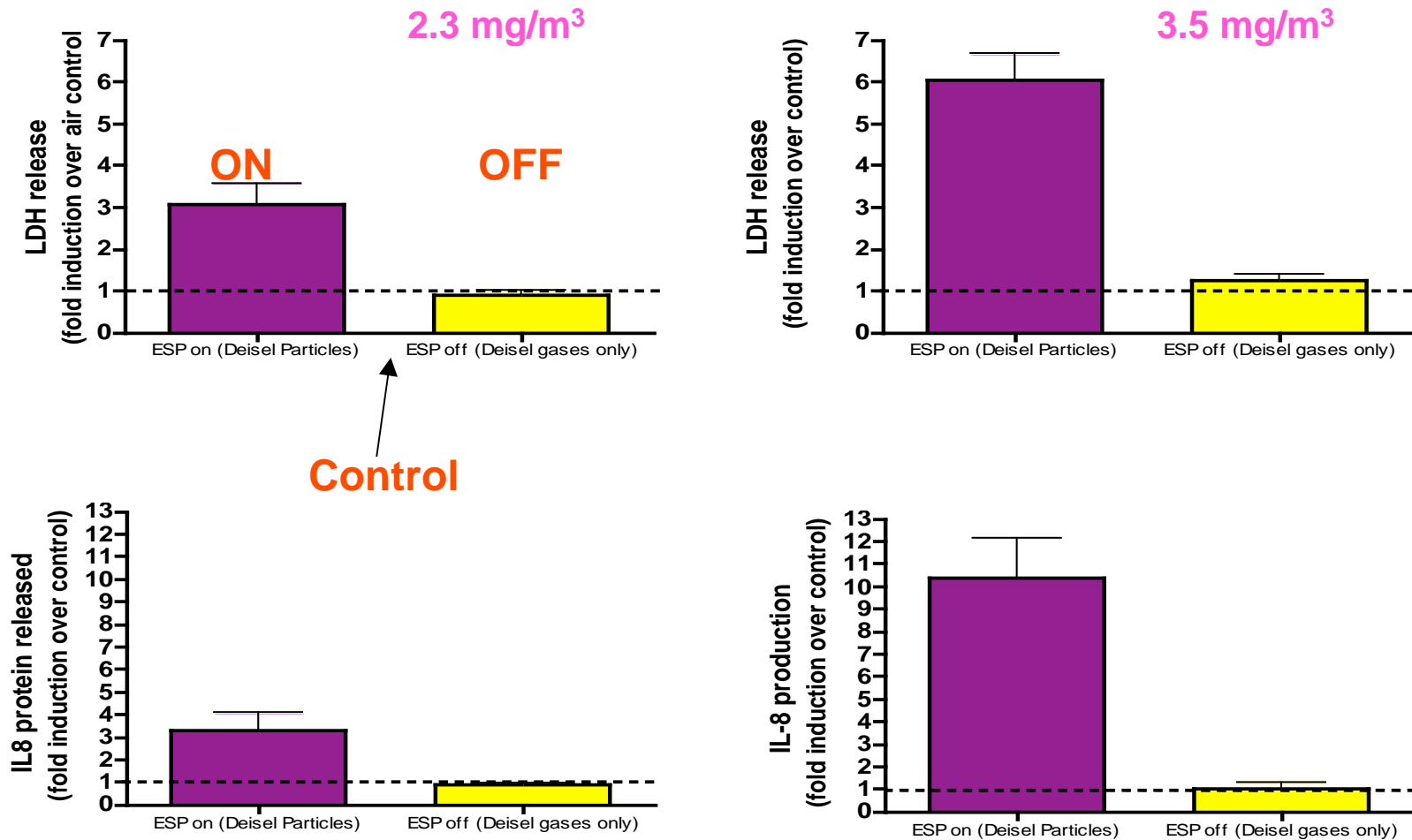
BEAS lung cells

Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles: **clean air controls**



No statistical difference in cytotoxicity and inflammatory cytokine release found between the incubator controls, the air controls with the ESP turned on, or the air controls with the ESP turned off

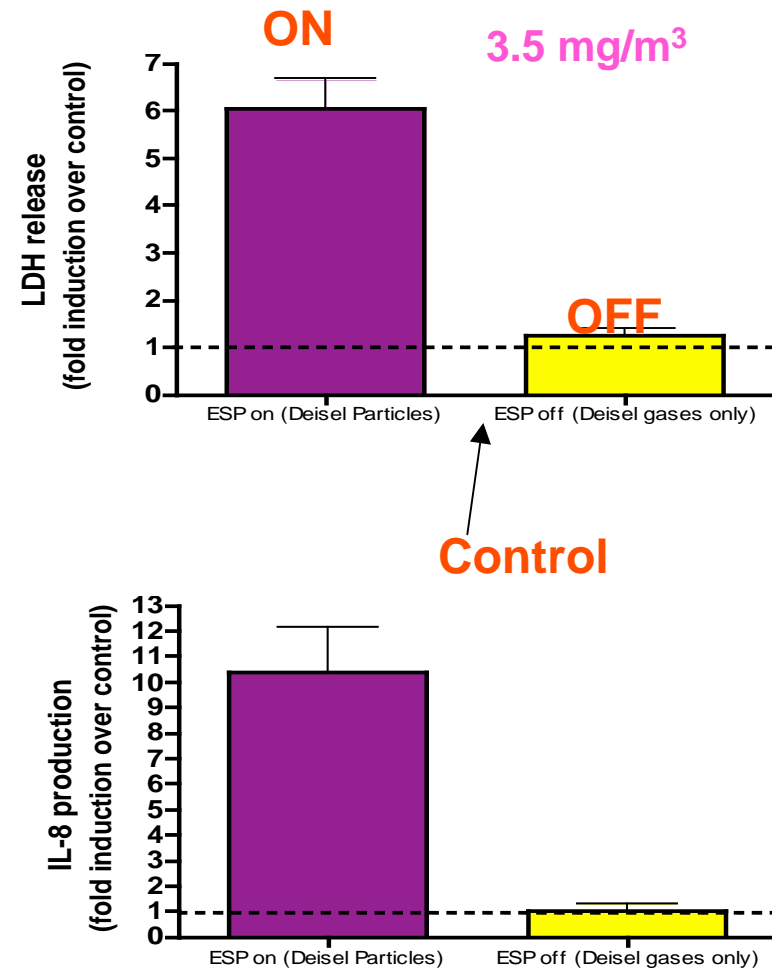
Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles



Diesel Exhaust from a 1980 Mercedes-Benz model 300SD

Electrostatic Precipitation as an Alternative Method for *In Vitro* Exposures to Mixtures of Gases and Particles

- We are able to directly expose respiratory epithelial cells to DE particles **without prior collection** in a separate medium that will significantly alter the particles' characteristics.
- These results suggest that the EAS system can be used to determine the full toxic potential of both gaseous and particulate components of air pollution mixtures, while also distinguishing the adverse effects of each component separately.



Diesel Exhaust from a 1980 Mercedes-Benz model 300SD