Peer Review
Experimental Toxicology Division
NHEERL

# Factors that Influence the Relative Potency of Diesel Exhaust Particles as Adjuvants in Allergic Airway Disease

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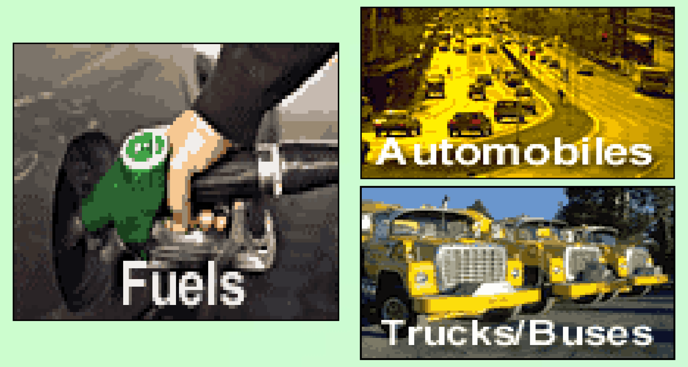
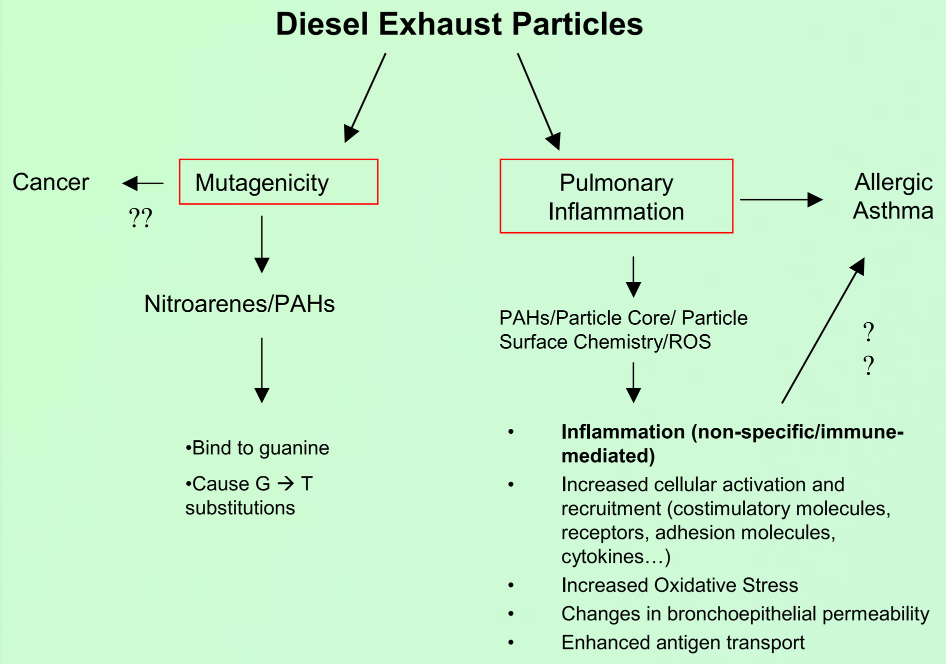
## INTRODUCTION

Diesel exhaust emissions aggravate respiratory and cardiovascular diseases and may increase risks of lung cancer. Studies also suggest that pulmonary exposures to diesel exhaust particles (DEP) promote the development of asthma among susceptible populations. In order to understand how DEP exert their harmful effects in the lung, we wanted to identify the various factors that contribute to their bioactive potencies and relate some of these factors to observed responses to DEP and organic extracts of DEP.




### Mechanisms of Toxicity




## GOAL:

To improve understanding of the relationships between biologically active constituents of DEP and mechanisms of toxicity

## QUESTION:

**Are all DEP samples the same?**

Chemical composition of the particles is influenced by:

- the age of engine
- type of engine and running conditions
- fuel composition
- load characteristics
- lube oil components
- presence and efficiency of control devices
- sampling procedures

## CONSIDERATIONS:

•The biological activities of samples generated and collected under different conditions are likely to be different.

•Knowing chemical features and mutagenic potencies prior to use in experimental and clinical toxicity testing would permit comparisons among different samples and would provide a reference data base.

## METHODS

**Two samples of DEP predominate in health effects research.** Automobile-derived DEP (A-DEP) have been tested extensively for effects in pulmonary inflammation and worsening of allergic asthma-like responses, while standard reference material 2975 DEP (SRM-DEP), derived from an off-road forklift, have been tested widely for mutagenicity. The relative potency of these particles regarding these health effects have never been compared. We designed a comparative study to investigate the bioactivities of these two DEP samples and relate these effects to their physical and chemical characteristics.

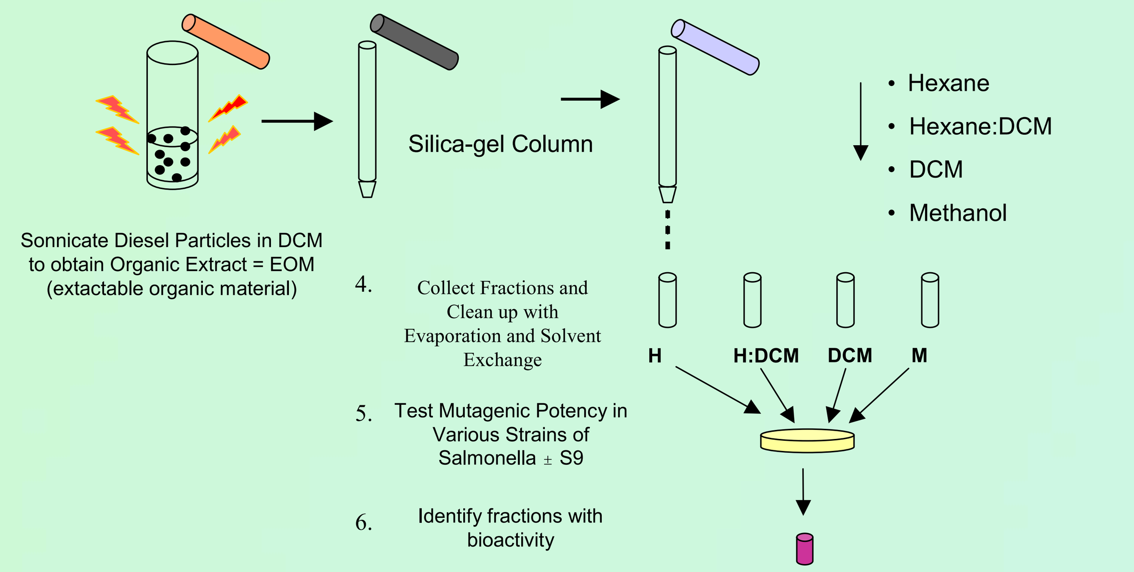
### I. Physicochemical Analyses of Particles

**A-DEP** were obtained from Takahiro Kobayashi, National Institute for Environmental Studies (Tsukuba, Japan). The generation and collection conditions of these particles have been described previously (Kobayashi and Ito, 1995). DEP were collected at a sampling temperature of 50°C onto glass-fiber filters (GD-100R, 203 mm x 254 mm) in a constant-volume sample system fitted at the end of a dilution tunnel. The particles were generated using a light-duty (2740 cc), 4-cylinder, 4JB1-type Isuzu diesel engine. The engine had a torque load of 6 kg/m as generated by an EDYC dynamometer (Meiden-Sya, Tokyo, Japan) and was run at 2000 rpm.

**SRM-DEP** were purchased from the National Institute of Standards Technology, Gaithersburg, MD. The material was obtained using a filtering system designed for diesel forklifts. In clear contrast to the A-DEP material, the SRM-DEP was generated by a heavy-duty diesel engine and collected under hot conditions without the use of a dilution tunnel. The certified analyses of these particles are available through NIST and can be viewed online (http://patapsco.nist.gov/srmcatalog/certificates/2975.pdf).

**Physical and chemical analyses of particles included: -scanning electron micrographs, -elemental carbon to organic carbon ratio, -measurement of pH of suspended particles in saline solution, -sequential organic extraction of particles and calculation of mass distribution among organic fractions, -qualitative gas chromatographic analyses of organic extracts.**

### II. Bioassay-Directed Fractionation




### III. *In Vitro* Salmonella Bioassays of Mutagenic Potency

The EOM and fractions from each DEP sample were evaluated for mutagenicity in the standard plate-incorporation *Salmonella* (Ames) mutagenicity assay. The strains used were the base-substitution strain TA100 (*hisG46, rfa, )uvrB*, pKM101) and the frameshift strain TA98 (*hisD3052, rfa, )uvB*, pKM101). Aroclor-induced Sprague-Dawley rat liver S9 was obtained from Moltox (Boone, NC) and used at 1 mg of S9 protein/plate. Plates were incubated for 3 days, the colonies counted, and linear regressions were calculated over the linear portion of the dose-response curves to determine the mutagenic potencies (revertants/:g). A positive result was defined as a reproducible, dose-related response that at least approached a 2-fold increase in revertants (rev) relative to the control.

### IV. *In Vivo* Acute Pulmonary Toxicity Model: CD-1 Mice

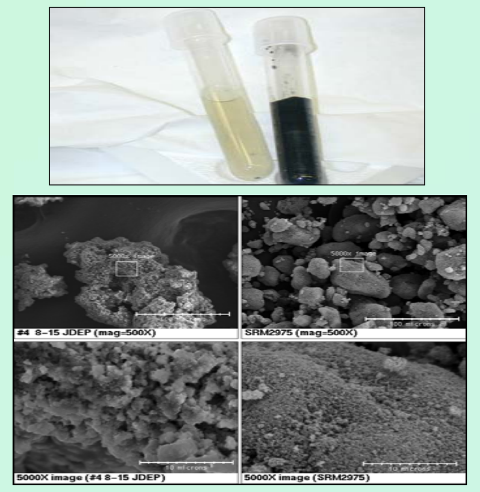
IT Instillation of DEP or Saline Vehicle

Analyses of Bronchoalveolar Lavage Fluid:

- Biochemical indicators of lung injury
- Cellular inflammation
- Chemokines/cytokines

## RESULTS

### Physicochemical Comparison of A-DEP and NIST SRM DEP

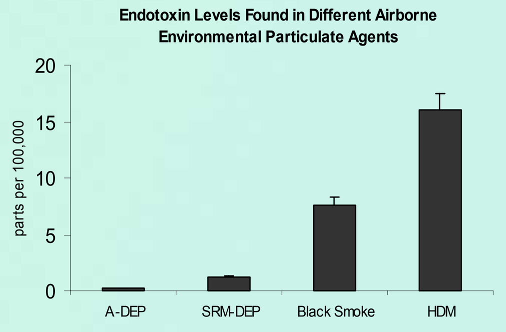


**Differences In:**

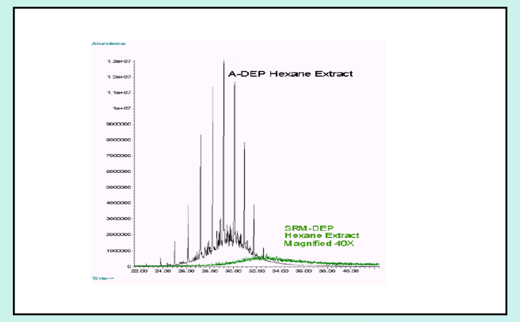
- Solubility
- Texture
- Color
- SEM
- % EOM
- EC:OC
- GC-M
- Endotoxin levels
- Bioassay-directed fractionation

Elemental Carbon:Organic Carbon Analyses

| % of total Mass | NIST Particles | A-DEP Particles |
|---|---|---|
| Elemental Carbon | 60 | 9 |
| Organic Carbon | 5 | 50 |
| Carbonate | <1 | <1 |
| Ash (by difference) | 35 | 41 |
| Extractable Organic Material | 2.7 | 26 |

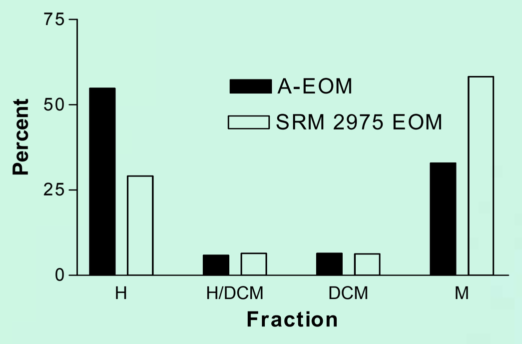



- SRM-DEP has 4.6X more endotoxin than A-DEP
- Both DEP samples have relatively low amounts



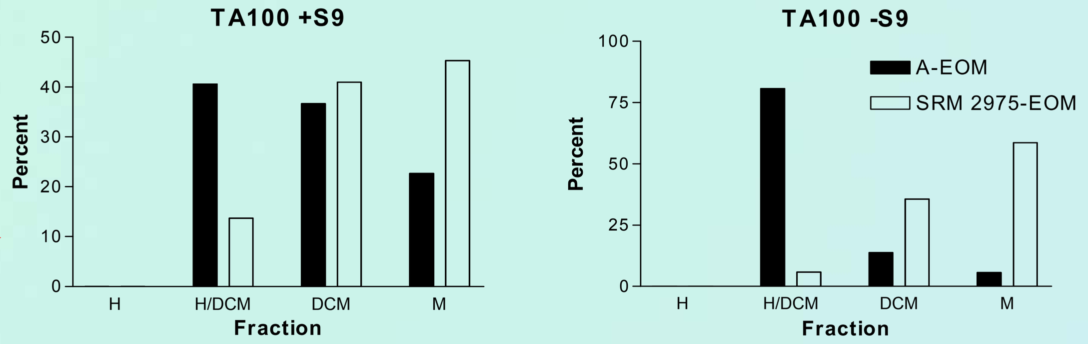

- Almost entire signal of A-DEP = aliphatic hydrocarbons

### Bioassay-Directed Fractionation and Mutagenicity of A-DEP and SRM-DEP



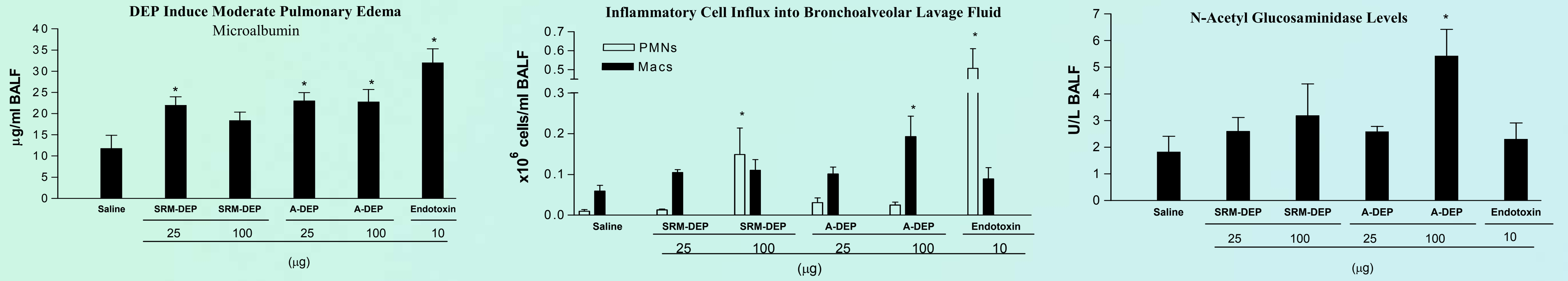

Opposite Mass Distributions of EOM

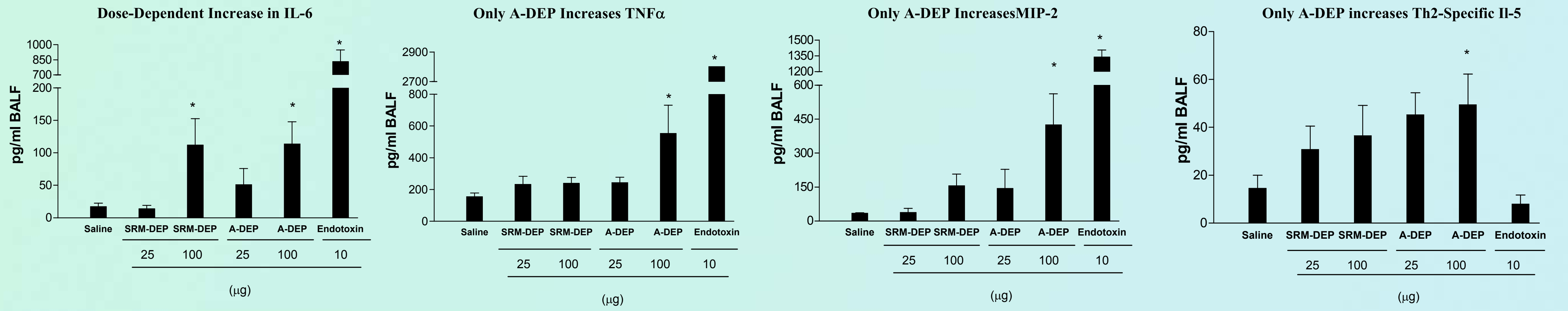
Distribution
Mass
Mutagenicity




Opposite Distributions of Mutagenic Potencies Across Organic Fractions

### Acute Pulmonary Toxicity 4 hr After Involuntary Aspiration of A-DEP and SRM-DEP in CD-1 Mice




RESULTS SUMMARY

- DEP samples differed in %EC/OC, PAH content and % EOM (extractable organic material)
- Peak pulmonary responses occurred at 4 h post aspiration exposure, and generally subsided by 18 h (data not shown)
- Protein (microalbumin) leakage occurred in the airways with exposure to both DEP samples
- SRM-DEP produced neutrophilic inflammation with a relative depletion of antioxidants, while A-DEP produced macrophage influx with increased macrophage activation
- Both DEP induced proinflammatory cytokines IL-6 and MIP-2 however, only A-DEP significantly increased concentrations of TNFα and IL-5
- Taken together, these *in vivo* data suggest that SRM-DEP and A-DEP stimulate different proinflammatory activation pathways the lung, which may in turn influence their relative potencies as allergic adjuvants

## CONCLUSIONS AND IMPACT

CONCLUSIONS: These studies demonstrate that differences in proinflammatory responses induced by each DEP sample are associated with different physicochemical features of the samples. This information provides a rationale for mechanistic studies of the underlying cellular and molecular responses to different DEP leading to the aggravation and possibly the induction of allergic airway disease.

**IMPACT:** These analytical, *in vivo*, and *in vitro* models will allow us to identify the physical and chemical properties of DEP that convey the potential to enhance the risk of developing asthma, or to exacerbate pre-existing disease. By understanding the influence of a variety of factors on the potency of DEP as an allergic adjuvant, it will be possible to make recommendations on how to minimize risk due to exposures to DEP.

## FUTURE DIRECTIONS

Future studies in collaboration with NRMRL engineers will determine the allergic adjuvant effects of acute and chronic inhalation exposures in rodents to diesel exhaust emissions from a variety of diesel fuels and under varying operating conditions. *In vitro* systems with rodent and human lung cells will be used to extrapolate dose response relationships in both species and to elucidate cellular and molecular activation mechanisms.

ACKNOWLEDGEMENTS

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