

# Risk Assessment of the Inflammogenic and Mutagenic Effects of Diesel Exhaust Particulates: A Systems Biology Approach

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## Results/Conclusions

1. Combustion of diesel fuel under specific conditions produces DEP of widely varying chemical composition and physical characteristics. The oxidative capacity of DEP correlates with organic content and potency in inducing antioxidant responses in cells.
2. DEP exposure potentiates allergic lung disease in a rodent model of sensitization. The immunotoxicity of DEP appears to be associated with its PAH content rather than its total organic content.
3. DEP exposure activates critical signal transduction pathways leading to an increase in inflammatory mediator expression. The activation of signaling by DEP is initiated by a loss of the activity of PTPases that are responsible for maintaining signaling quiescence in resting cells.

## Impact and Outcomes

The findings of this study provide biological plausibility in furtherance of our understanding of the health effects of PM, aid in the identification of hazardous PM components and, thereby, facilitate the design of regulatory efforts aimed at mitigating the adverse effects of PM inhalation.

## Future Directions

- Develop imaging approaches amenable to computational modeling of the activation of pivotal signaling events initiated by PM exposure.
- Further link signaling pathway activation to specific adverse outcomes such as inflammatory mediator expression and identify responsible physicochemical properties in PM.
- Expand the study of signaling dysregulation to include nanomaterials.

## References

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2. Cao, D., Tal, T., Graves, L., Gilmour, I., Linak, W., Reed, W., Bromberg, P., and Samet, J. Diesel Exhaust Particulate (DEP)-Induced Activation of Stat3 Requires Activities of EGFR and Src in Airway Epithelial Cells. *American Journal of Physiology: Lung, Cell, & Molecular Physiology*, 292, L422-L429 (2007).
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5. Singh, P., DeMarini, D.M., Dick, C.A.J., Taber, D., Ryan, J., Linak, W.P., Kobayashi, T., and Gilmour, M.L. Bioassay-Directed Fractionation, Physicochemical Characterization, and Pulmonary Toxicity of Automobile and Forklift Diesel Exhaust Particles in Mice. *Environmental Health Perspectives*, 112(8) 820-825 (2004).
6. Stevens, T., Krantz, Q. T., Linak, W.P., Hester, S., and Gilmour, M.L. Increased Transcription of Immune and Metabolic Pathways in Naive and Allergic Mice Exposed to Diesel Exhaust. *Toxicological Sciences*, 102(2), 359-370 (2008).
7. Stevens, T., Linak, W.P., Gilmour, M.L. Differential potentiation of allergic lung disease in mice exposed to chemically distinct diesel samples. *Toxicol. Sci.* 107(2), 522-534.
8. Tal, T., Bromberg, P.A., Kim, Y., and Samet, J.M. (2008). Tyrosine phosphatase inhibition induces epidermal growth factor receptor activation in human airway epithelial cells exposed to diesel exhaust. *Toxicol. Appl. Pharmacol.* 233:382-388.

## Science Question

- Diesel exhaust particles vary considerably in their physicochemical characteristics, such as the ratio of organic to inorganic carbon associated with the particle.
- Exposure to diesel exhaust particles results in immunotoxic and genotoxic effects.
- Immune reactions such as inflammation are known to be regulated by mediator proteins whose expression is under the control of signal transduction pathways.

• These signaling cascades are regulated at a molecular level by the opposing activities of kinases and phosphatases which modulate the levels of phosphoproteins.

• The research in this series of projects was designed to address the following questions:

• Does the toxicity of DEP correlate with specific physicochemical properties?

• What are the key signaling events activated by DEP of varying composition?

• How do DEP of varying composition disregulate intracellular signaling?

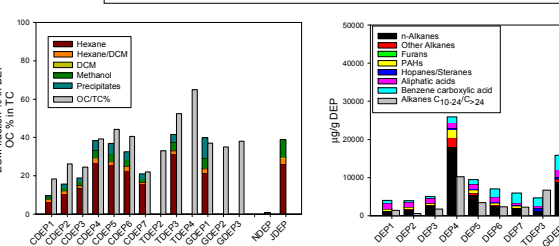
## Research Goals

This project employs an integrated approach towards the development of a computational model of the mechanisms of adverse action of diesel exhaust particle inhalation

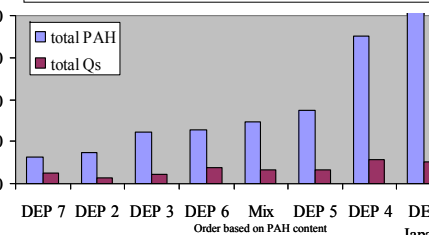
### DIESEL EXHAUST PARTICLE GENERATION

| Engine + compressor            | Manufacturer: DEUTZ<br>4 Cyl.<br>Power: 31 kW    | CDEP1-7 | 2004 - 2006                                  |
|--------------------------------|--|---------|--|
| Light-duty truck + dynamometer | Manufacturer: Detroit Diesel<br>8 Cyl.<br>134 kW | TDEP1-4 | 2006 - present                               |
| Small engine                   | Manufacturer: Yanmar<br>1 Cyl.<br>4.8 kW         | GDEP1-4 | 2006 - present<br>(Biodiesel, Fuel additive) |

### EXTRACTABLE ORGANIC CONTENT OF DEP



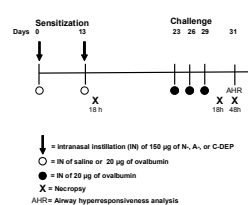
### REACTIVE ORGANIC CONTENT OF DEP



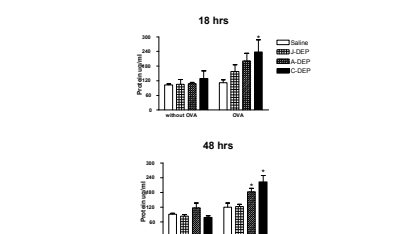
### OXIDATIVE EFFECT OF DEP

| Sample           | Total PAHs (µg/g) | DTT (x10 <sup>-4</sup> activity/ µg) | HO-1 (density/10 <sup>6</sup> µg) |
|------------------|-------------------|--------------------------------------|-----------------------------------|
| CDEP7            | 156               | 36                                   | 0.77                              |
| CDEP2            | 181               | 33                                   | 0.46                              |
| CDEP3            | 303               | 47                                   | 0.86                              |
| CDEP6            | 352               | 66                                   | 1.53                              |
| CDEP-Mix (3+5+7) | 365               | 61                                   | 1.06                              |
| CDEP5            | 439               | 86                                   | 1.61                              |
| CDEP4            | 875               | 92                                   | 6.71                              |
| JDEP             | 3193              | 380                                  |                                   |

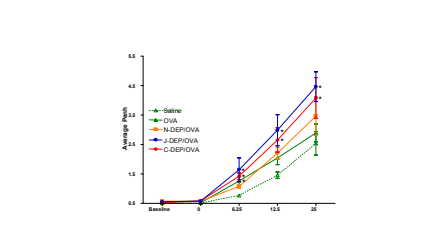
## 2. ASSESSMENT OF DIESEL EXHAUST PARTICLE IMMUNOTOXICITY



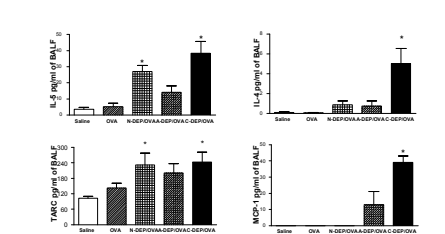
Schematic of Intranasal Exposure Regimen for BALB/c mice



DEP Exposure Increases Protein Levels in the Bronchoalveolar Lavage Fluid. \* p<0.05.

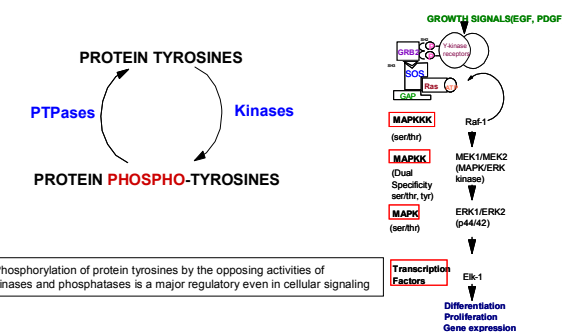


DEP Exposure Increases Airway Hyperresponsiveness to Methacholine Challenge. \* p<0.05.

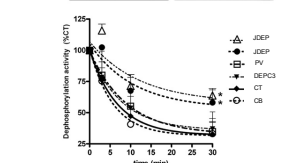
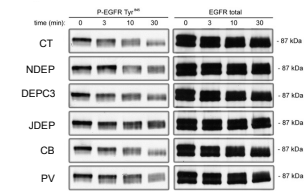


DEP Exposure Potentiates the Release of Cytokines and Chemokines in the Lung. \* p<0.05.

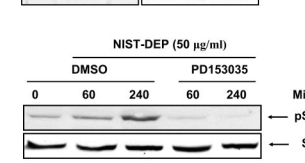
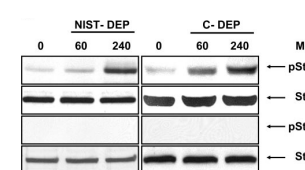
## 3. MOLECULAR MECHANISMS OF DIESEL EXHAUST PARTICLE TOXICITY



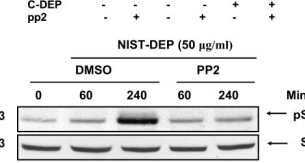
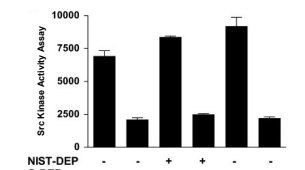
Phosphorylation of protein tyrosines by the opposing activities of kinases and phosphatases is a major regulatory even in cellular signaling



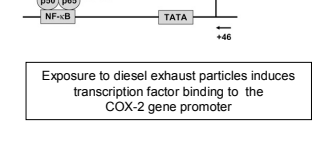
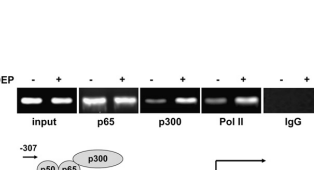
Exposure to diesel exhaust particles of varying organic content results in impaired dephosphorylation of pivotal signaling intermediates in human lung cells



Exposure to diesel exhaust particles of varying organic content activates the transcription factor STAT3 through a mechanism that requires EGFR and Src kinases



Exposure to diesel exhaust particles induces transcription factor binding to the COX-2 gene promoter



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