ACES: Evaluation of Tissue Response to Inhaled 2007-Compliant Diesel Exhaust

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And
The ACES Team

Presented by:
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Health Effects Institute

DEER 2012 Conference
October 19, 2012

Sponsors and Partners include:

- U.S. Department of Energy (DOE)
- Engine Manufacturers Association (EMA)
- U.S. Environmental Protection Agency (EPA)
- American Petroleum Institute (API)
- After-treatment Manufacturers
- California Air Resources Board (CARB)

- Health Effects Institute (HEI)
- Coordinating Research Council (CRC)
- Southwest Research Institute (SwRI)
- Lovelace Respiratory Research Institute (LRRI)

LRRI Animal Toxicity Study Team

Jake McDonald	LRRI	Principal Investigator and Exposure Operations
Judy Chow	DRI	Analytical Chemistry
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Rodney Miller	EPL	Histopathology
JeanClare Seagrave	LRRI	Bronchopulmonary Lavage & Cell Proliferation
Steve Seilkop	SKS	Biostatistician
Cheryl DiCarlo	LRRI	Attending Veterinarian & Animal Care
Barbara Zielinska	DRI	Analytical Chemistry

The Advanced Collaborative Emissions Study (ACES)

OVERALL OBJECTIVES

To characterize emissions and possible health effects of new advanced heavy duty engine and Emission control systems in the market 2007 – 2010

- **PHASE I**: Detailed Characterization of four 2007-compliant heavy duty engines. Results published by the Coordinating Research Council (2009) and in a paper in JAWMA (2011)

- **PHASE 2**: Detailed characterization of three 2010-compliant HD diesel engine. Testing in progress. Report in Spring 2013.
  - Chris Tenant presenting later in this session

- **PHASE 3**: Health effects testing in rodents chronically exposed to emissions from a 2007 engine. Rats exposures for 24-30 months; mice for 3 months. Interim results released mid-April 2012
  - This is the focus of my talk

Diesel Emissions and Carcinogenicity

- 1960s – 70s: Early years: carcinogenic compounds in diesel soot – determined by in vitro and some in vivo studies
- 1980s: Life time exposure of rodents to diesel emissions: lung cancer findings, but role of overload was a concern
- 1980s – 90s: Occupational epidemiology studies: Suggestive
- 1989: International Agency for Research on Cancer: Ranks diesel emissions in group 2A category – "probably carcinogenic to humans"
- 1999: HEI review of diesel epidemiology: Exposure information not sufficient for quantitative risk assessment
- 2000s – 2010s: New epidemiology studies – improved exposure assessment
- 2012: IARC revisits and finds diesel exhaust emissions as "carcinogenic to humans" (Category 1)
- 2013-2014: HEI plans to evaluate diesel epidemiology studies

New Diesel Emissions and Health Effects Studies

- 1990s and early 2000s: Improvements in diesel engine technology resulting in lower PM emissions
- Mid - late 2000s: New after-treatment technology introduced to the market, with 100X to 1,000X reductions in PM emissions
- Mid-2000s: HEI plans the ACES program
- Are the *new* emissions carcinogenic? How do we find out:
  - Human Epidemiology – Not now and probably never
  - Animals – feasible to study
    - This is the focus of ACES Phase 3 study

Design of ACES Animal Studies

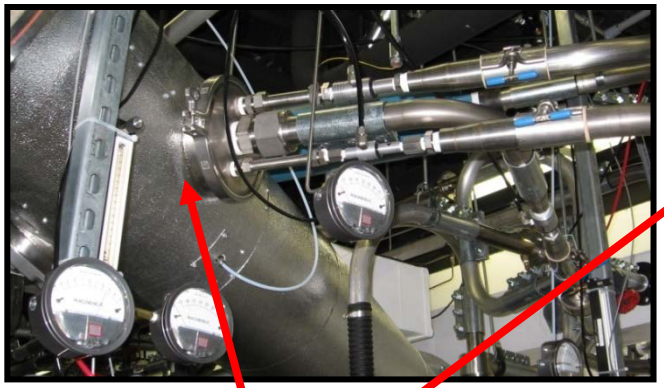
- Expose appropriate strain of rodent(s) for life time to new technology emissions and study health effects
- Design:
  - Use a 2007 engine (part of ACES Phase 1)
  - Use a rat strain (Wistar Han), employing as many animals as practical, both genders
  - Exposure:
    - Use 3 dilutions of emissions, plus clean air, for exposure
    - Expose animals 16/hrs day, 5 days/week, for their life-time (24 to 30 months)
    - Use a very demanding, specially developed 16-hour cycle
    - Characterize emissions throughout the exposure period
  - Sacrifice animals for interim evaluations (1, 3, 12 and 24 months)

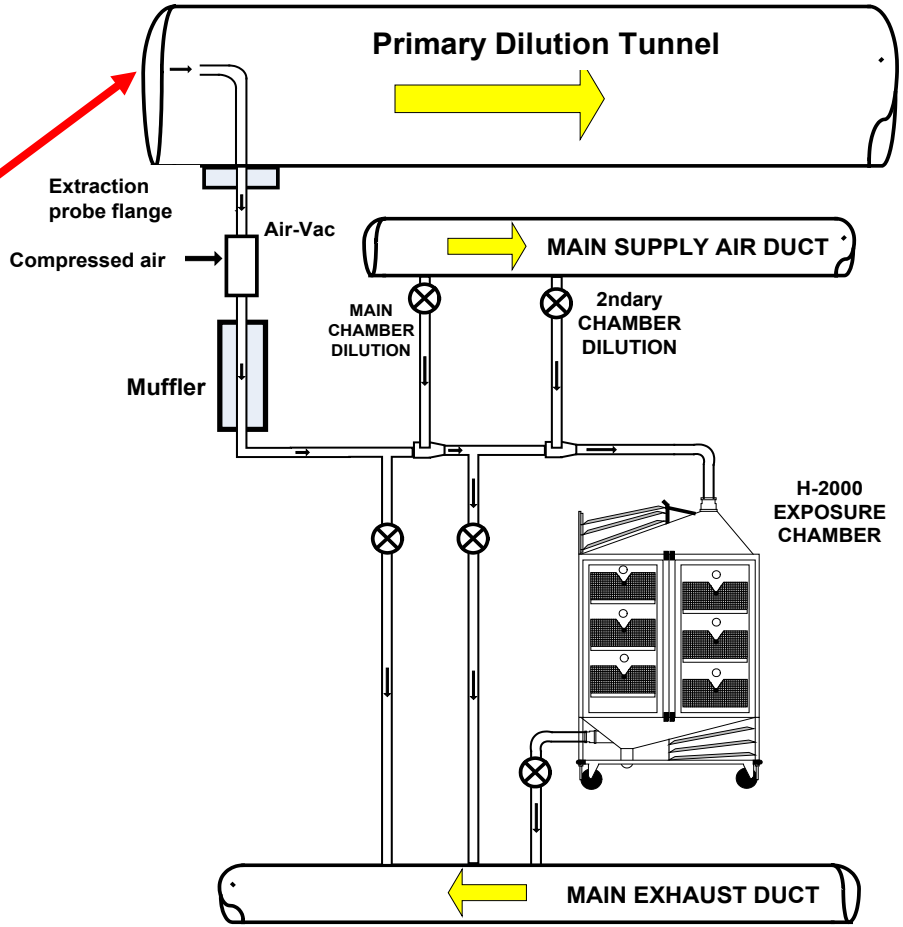
Features of the Study

- Characterize exposure levels throughout
- Study appropriate end point
  - Histopathology (to see if cancerous or pre-cancerous lesions develop)
  - Genotoxic markers (indicators of cancer)
  - Pulmonary function (to see if it is affected)
  - Lung lavage (to ascertain the state of lung tissues and cell proliferation)
  - Hematology and serum chemistry
  - Oxidative and Inflammatory markers (indicators of a host of health effects, including cardiovascular)

Massive Undertaking

Animals Are Exposed in Whole-Body Inhalation Systems



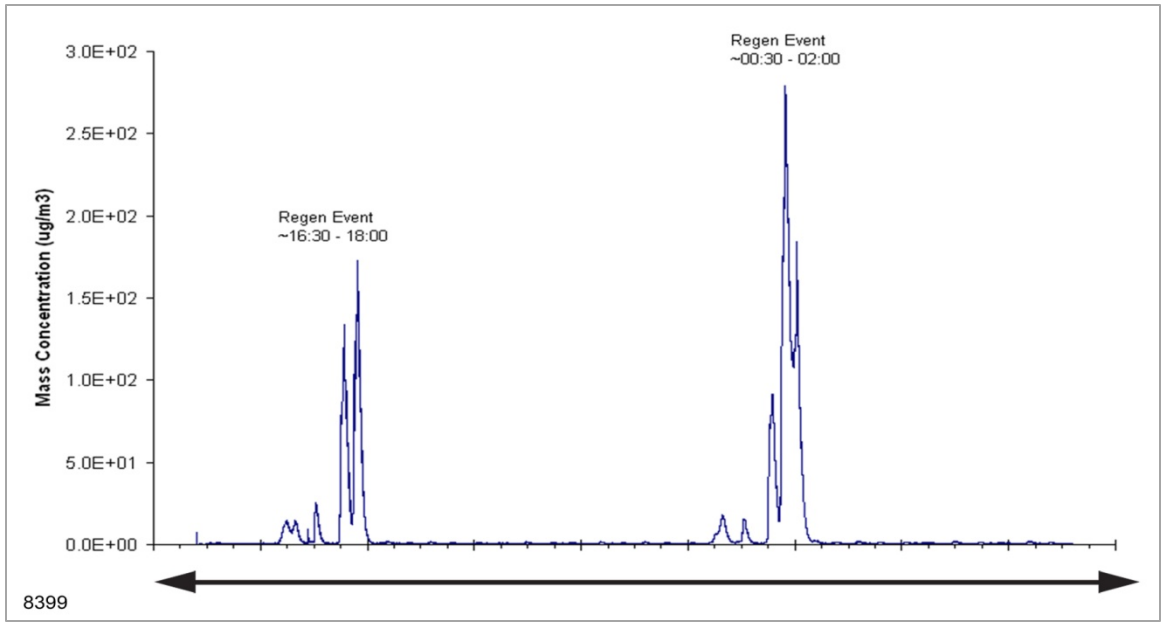


(Note: Drawing is not to scale)

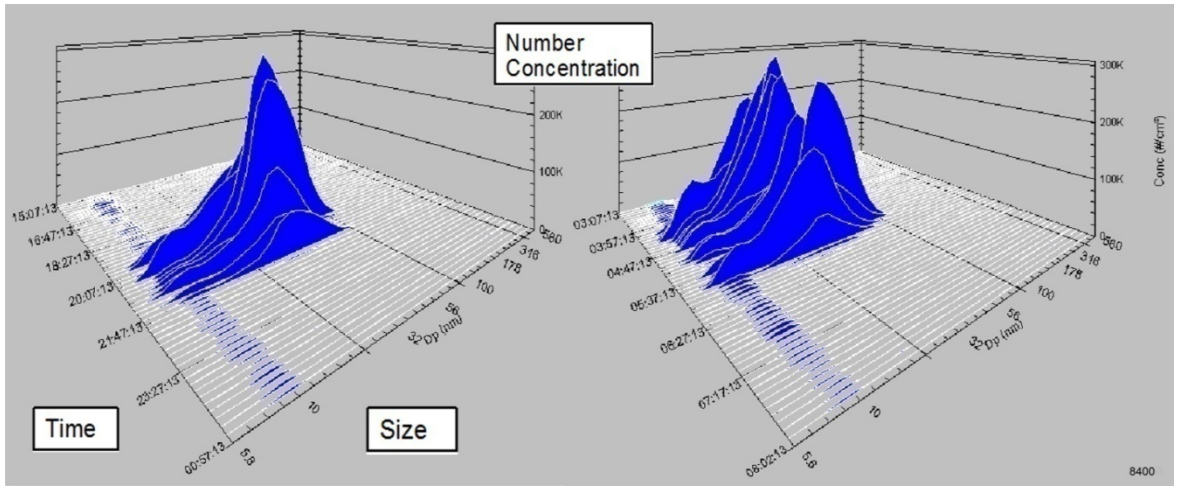
AVERAGE EXPOSURE CONCENTRATIONS: 12 MONTHS

	High		Mid		Low	
Gases:	Mean	Stdev	Mean	Stdev	Mean	Stdev
NO_2 (ppm)	4.2	0.5	0.91	0.11	0.109	0.013
NO (ppm)	5.8	1.1	1.40	0.23	0.293	0.160
NOx (ppm)	9.9	1.4	2.30	0.29	0.402	0.159
CO (ppm)	6.8	2.9	n/a	n/a	n/a	n/a
THC (ppm)	0.5	0.4	n/a	n/a	n/a	n/a
SO_2 (ppb)	23.9	4.4				
PM ($\mu g/m^3$):						
Chamber Inlet (filter)	9	5	3	3	2	1
Chamber (filter)	27	10	31	20	21	12

ATMOSPHERE COMPOSITION



Real-time particle mass



Real-time particle number

DISCLAIMER

- 3 and 12-month results have been reviewed and published
- 24 month results have NOT been reviewed and are preliminary
- In old DE studies, tumors generally seen between 24 and 30 months of exposure; we are currently at 27 months

Any conclusions reached now
are preliminary and may change

Lung Histopathology - Summary

- No treatment-related lung lesions in low or mid dose groups
- Some lung lesions observed in animals exposed to the highest levels, but:
  - Little progression of lesions up to 12 and 24 months
  - Severity of the lungs lesions, determined to be minimal to mild (on a 1 – 4 scale)
- No tumors or pre-neoplastic changes observed (up to 24 months)

Histopathology in Male Rats at 3 and 12 Months

Incidence and Types of Findings

Males 3 Month

Lung	Control	Low	Mid	High
Hyperplasia Epithelium Periacinar	0/10	0/10	0/10	10/10
Accumulation Macrophage	0/10	0/10	0/10	3/10
Fibrosis Interstitial	0/10	0/10	0/10	4/10

Males 12 Months

Lung	Control	Low	Mid	High
Hyperplasia Epithelium Periacinar	0/10	0/10	0/10	10/10
Accumulation Macrophage	0/10	0/10	0/10	4/10
Fibrosis Interstitial	0/10	0/10	0/10	10/10

DEFINITIONS:

- **Hyperplasia:** An increase in the number of cells in a tissue, often an early stage in the development of cancer.
- **Macrophage**: Cells that engulf and digest cellular debris and pathogens
- **Fibrosis**: Formation of excess connective tissue; a sign of a repair or reactive process

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Histopathology in Rats at 24 Months

Incidence and Types of Findings

Males

Lung	Control	Low	Mid	High
Hyperplasia Epithelium Periacinar	0/10	0/10	0/10	10/10
Bronchiolization	0/10	0/10	0/10	1/10
Fibrosis Interstitial	0/10	0/10	0/10	10/10

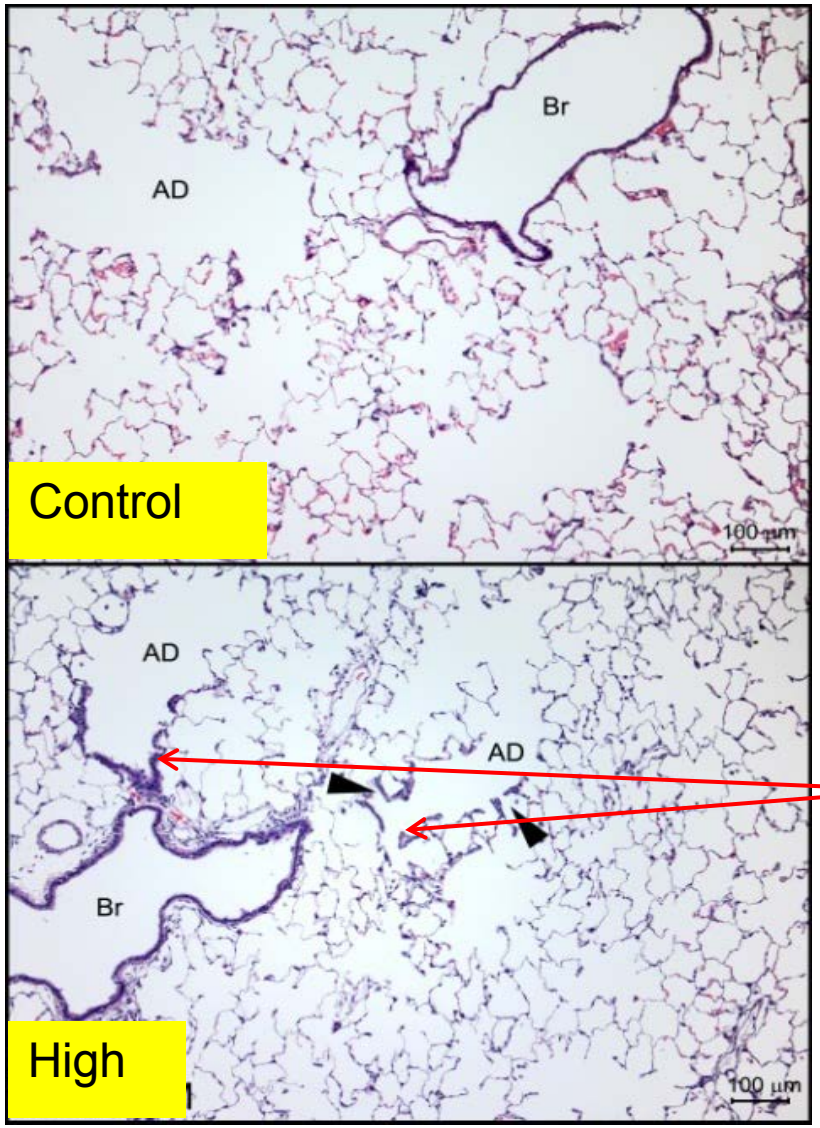
Females

Lung	Control	Low	Mid	High
Hyperplasia Epithelium Periacinar	0/10	0/10	0/10	10/10
Bronchiolizatoin	0/10	0/10	0/10	1/10
Fibrosis Interstitial	0/10	0/10	0/10	10/10

DEFINITIONS:

- **Hyperplasia:** An increase in the number of cells in a tissue, often an early stage in the development of cancer.
- **Bronchiolization:** A change in the normal flat epithelium, rendering it cuboidal and similar to cells lining the terminal bronchioles.
- **Fibrosis**: Formation of excess connective tissue; a sign of a repair or reactive process

Minimal Epithelial Hyperplasia



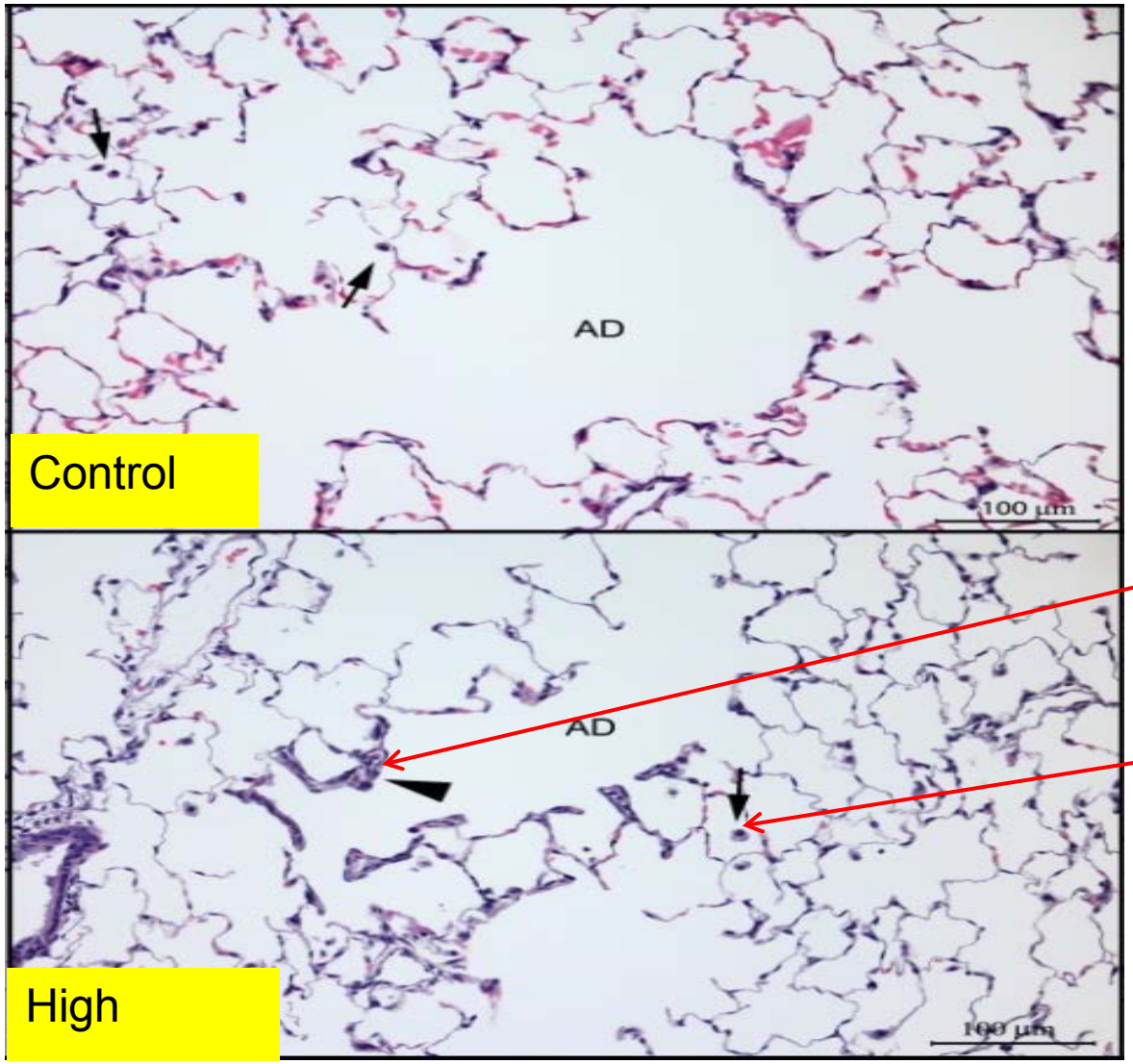
Epithelial hyperplasia observed at high exposure level (associated with alveolar ducts)

Findings generally mild

Thickening of alveolar duct septae

AD = Alveolar Duct; Br = Bronchiole

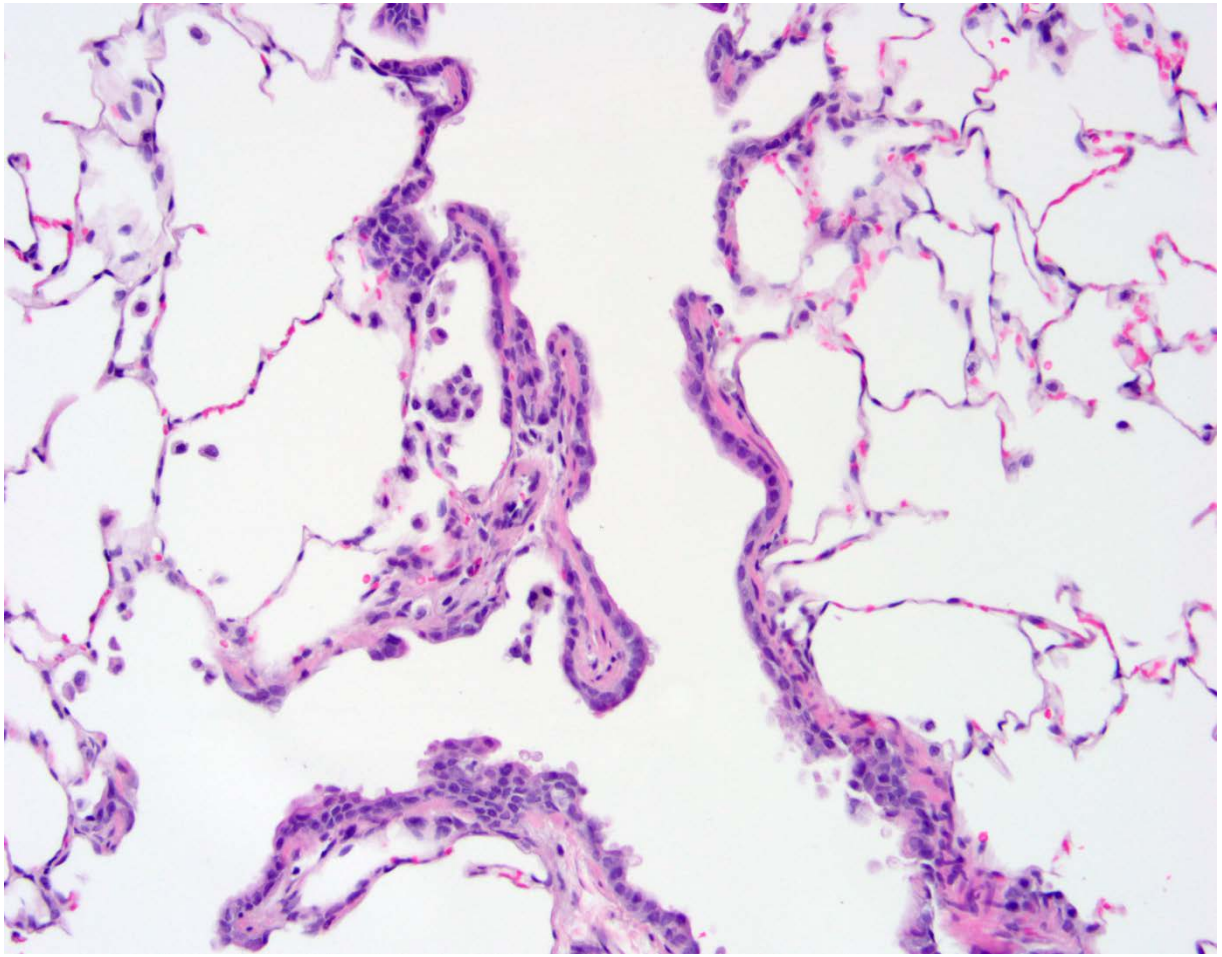
Higher Power View of Previous Slide



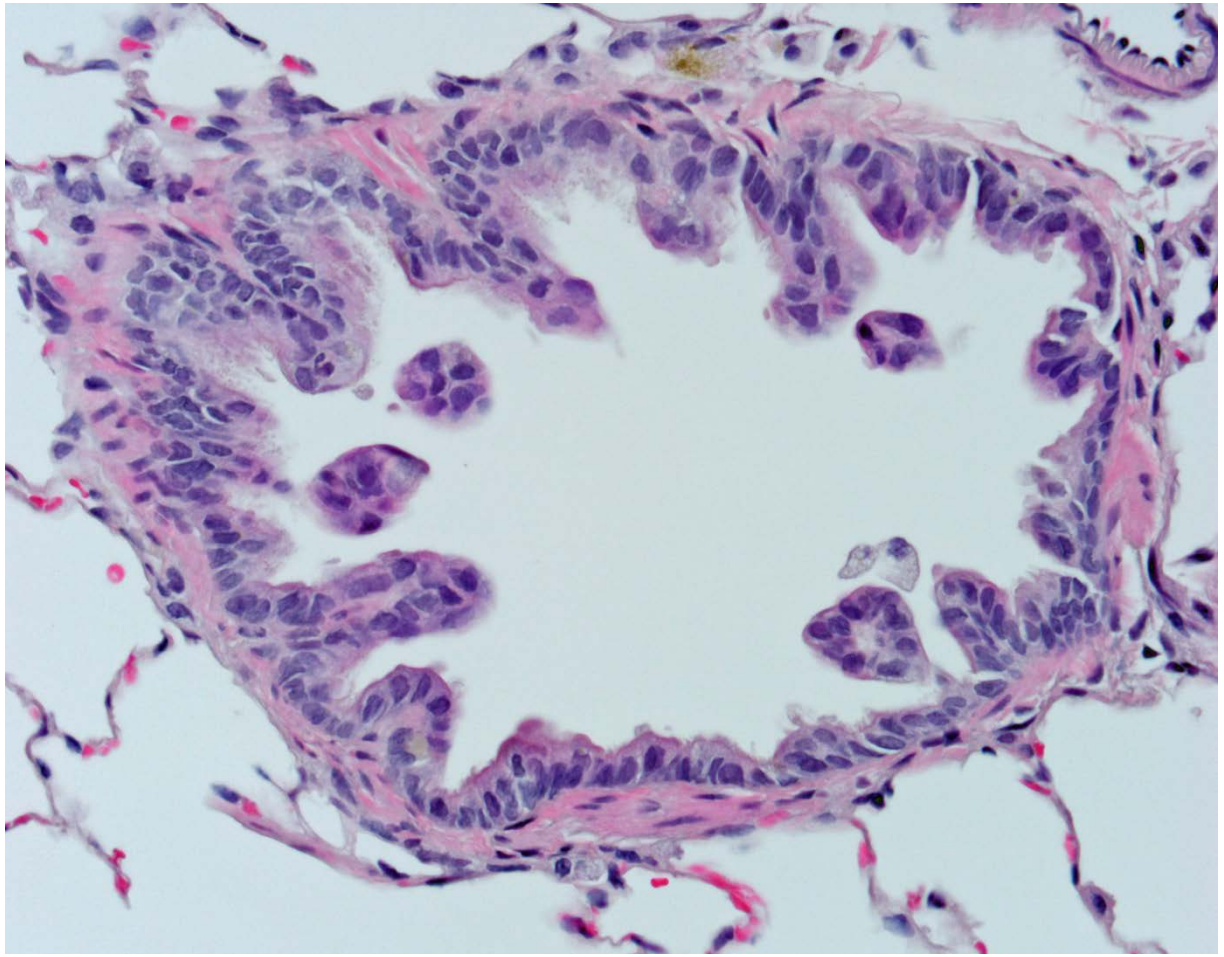
Thickening of alveolar duct septae

Macrophage

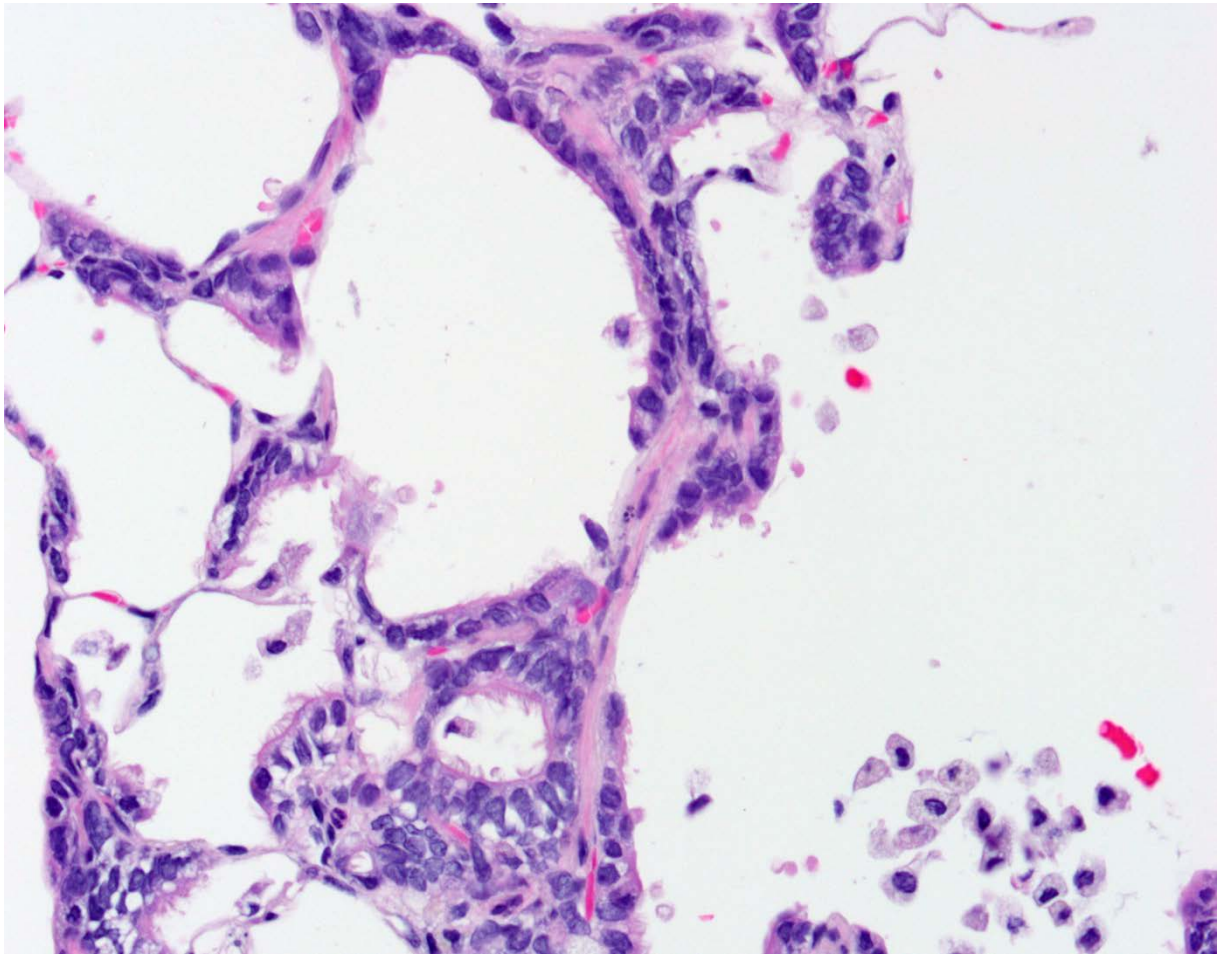
Mild Inflammation and Alveolar Duct Thickening in Terminal Brochioles



Mild Protrusion of Epithelial Cells to Bronchioles



Macrophage Accumulation



Summary: 24 Month Rat Histopathology

- Minimal lesions at 24 months; are similar to minimal lesions at 12 months
- **Some** mild lesions at 24 months now occur a little more proximal in the bronchioles and have piling up of epithelial cells that project slightly into some lumina (compared to 12 months). All mind; none considered to be moderate.
- Minimal amount of inflammatory reaction within the lesions
- No identifiable soot-like particulate, cannot distinguish a difference in macrophages seen in control animals with those seen in high dose rats
- No lesions seen that may represent a typical preneoplastic lesion

Possible Cause of Toxicity at the High Dose

- Significant amounts of NO_2 in 2007 diesel emissions
  - High dose exposure level (4.2 ppm NO_2) was selected to minimize NO_2 toxicity
  - Expectation: Some NO_2 related toxicity may be seen at the high dose
- What do we know about toxicity of NO_2 at exposure levels used in this study?
  - HEI funded Mauderly et al 1989 study
  - F344 Rats, exposed for similar ppm-hours
    [17,290 in Mauderly; 17,472 in ACES at 12 months]
  - Findings: NO_2 caused epithelial hyperplasia, thickening of walls of terminal bronchioles, inflammation, and oxidative stress. There was little effect on respiratory function. Effects at 12 months were not significantly different than at 24 months
- Note parallels to the ACES findings

SUMMARY

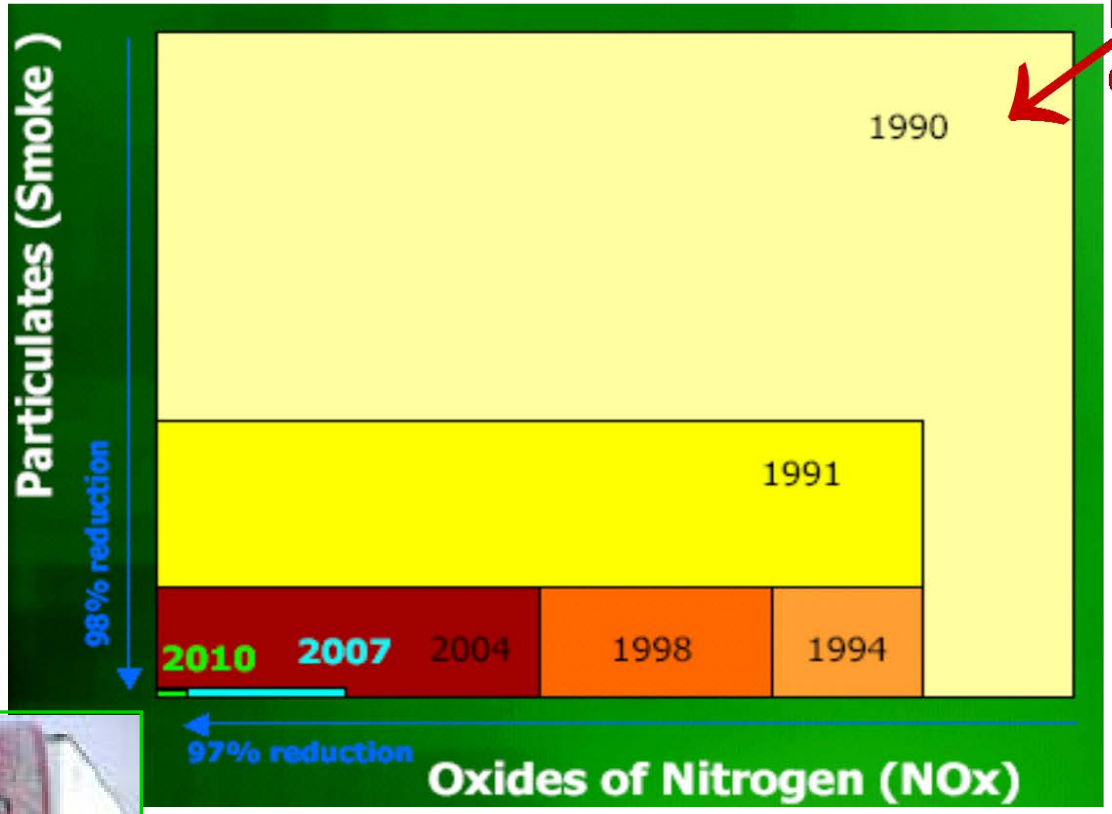
- Exposures produced minimal inflammatory and tissue remodeling in lungs of rats
- Lung injury:
  - Minimal to mild at 3 and 12 months (1 on scale of 1-4). Minimal lesions at 24 months are similar to minimal lesions at 12 months
  - Some mild lesions at 24 months
  - No pre-neoplastic or neoplastic lesions observed
- No 'soot' accumulation in macrophages (this was a hallmark of *traditional diesel exhaust experiments* due to high soot exposure levels)

Remainder of study under way

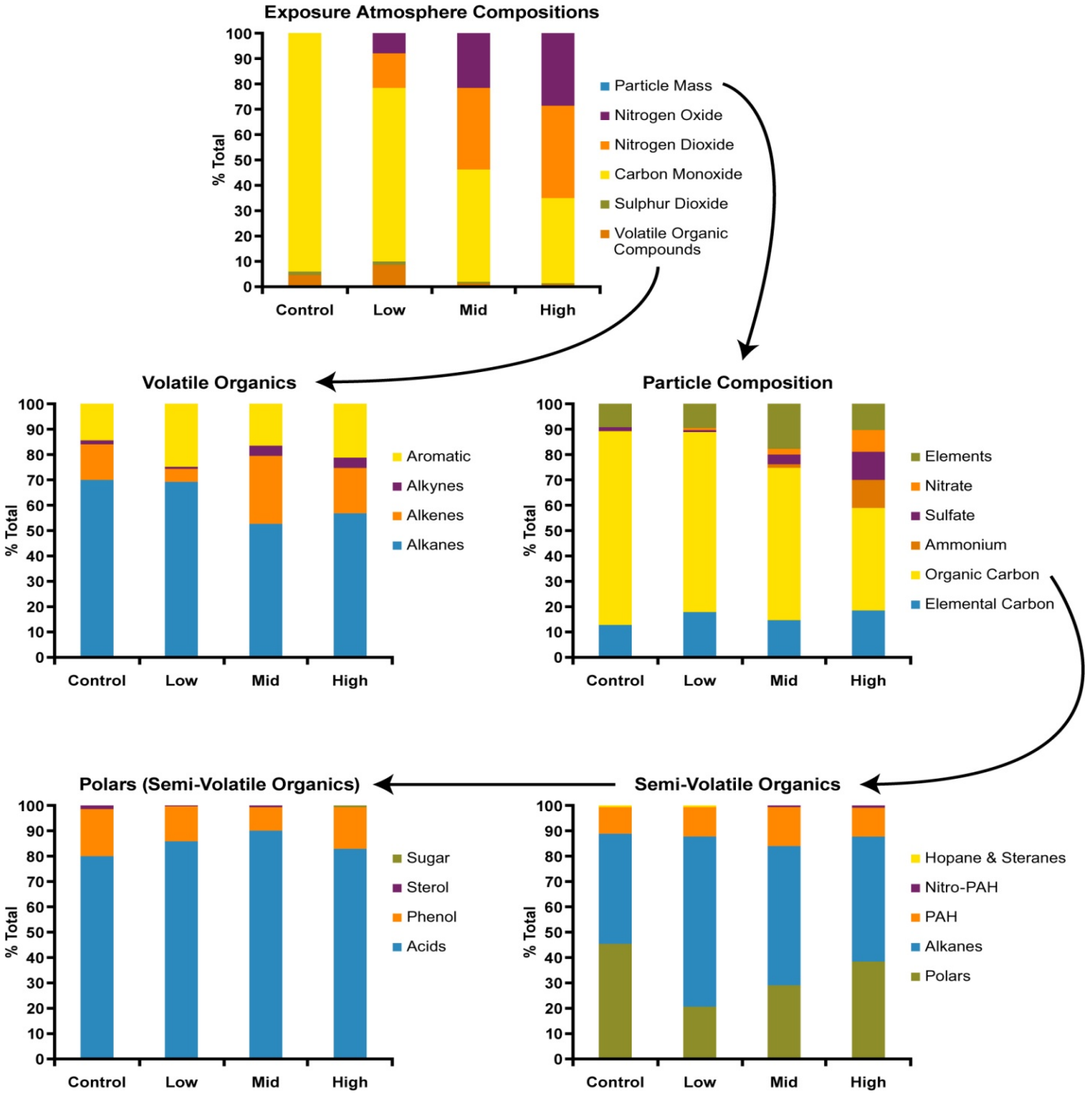
Note: In previous TDE studies, significant lung tumors not observed until after 24 months

Thank you!!!

Traditional Diesel Exhaust≠ New Technology Diesel Exhaust



Big changes!


Exposure Atmosphere Compositions
% Total
Control
Low
Mid
High
Particle Mass
Nitrogen Oxide
Nitrogen Dioxide
Carbon Monoxide
Sulphur Dioxide
Volatile Organic Compounds
Volatile Organics
Aromatic
Alkynes
Alkenes
Alkanes
Particle Composition
Elements
Nitrate
Sulfate
Ammonium
Organic Carbon
Elemental Carbon
Polars (Semi-Volatile Organics)
Sugar
Sterol
Phenol
Acids
Semi-Volatile Organics
Hopane & Steranes
Nitro-PAH
PAH
Alkanes
Polars

Role of NO_2 in Observed Effects?

When HEI designed the study, it was expected that at the high concentration (16 hr/day 4.2 ppm NO_2) some NO_2-related effects may be observed. This was based on results of previous studies, including:

HEI Study (Mauderly et al., 1989) F344 rats exposed (7hr/day, 5 days/week) to 9.5 ppm NO2

Pulmonary function, histopathology, and, immune response assessed after 12, 18, 24 mo (1820, 2730, 3640 hr) of exposure

Findings: **NO_2 caused epithelial hyperplasia, thickening of walls of terminal bronchioles, inflammation, and oxidative stress. There was little effect on respiratory function.**

Effects at 12 mo not significantly different than at 24 months

How do the NO_2 "doses" compare at 12 mo?

Mauderly et al:17,290 ppm-hr.

ACES: 17,472 ppm-hr

Biological Response Indicators

Hematology
Red Blood Cell Count
Hemoglobin
Hematocrit
Mean Corpuscular Volume
Mean Corpuscular Hemoglobin Concentration
Mean Corpuscular Hemoglobin
Platelet Count
Percent Reticulocytes
White Blood Cell Count and Absolute Differential
White Blood Cell Count Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Large Unstained Cells
Coagulation
Partial Thromboplastin Time
Prothrombin Time

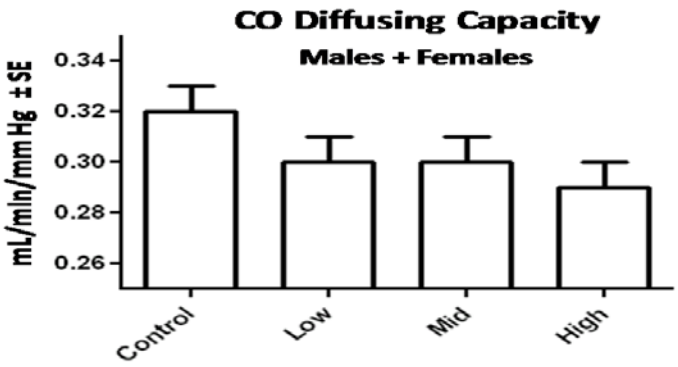
Serum Chemistry
Alanine Aminotransferase (Alanine Transaminase)-Serum
Albumin
Aspartate Aminotransferase (Aspartate Transaminase)-Serum
Bilirubin (Total)
Blood Urea Nitrogen
Calcium
Chloride (Serum)
Cholesterol (Total)
Creatinine (Serum)
Glucose
Gamma Glutamyltransferase
Alkaline Phosphatase
Phosphates
Potassium (Serum)
Protein (Total)
Sodium (Serum)
Triglycerides
Calculated Variables and Ratios
Albumin/Globulin
Blood Urea Nitrogen/Creatinine
Globulin

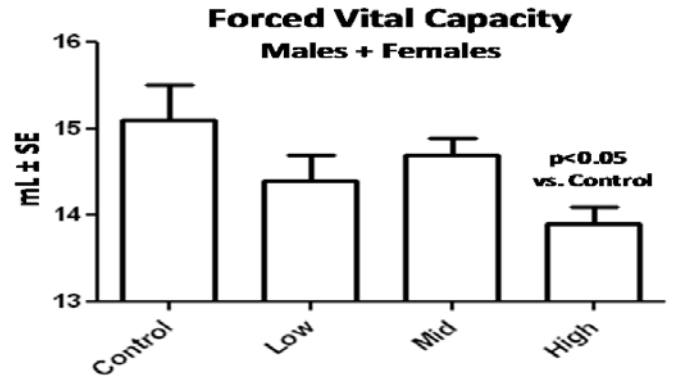
Biological Response Indicators

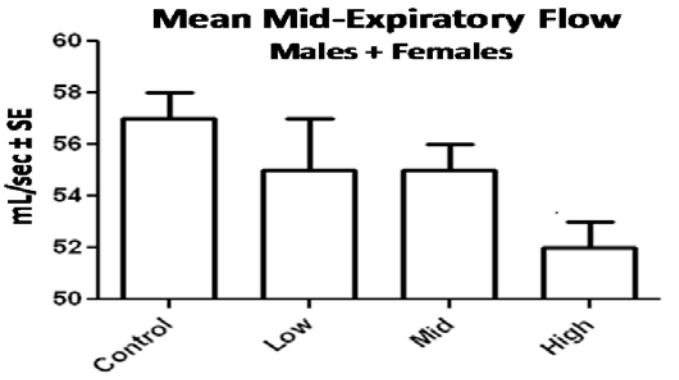
Lung Lavage
Lactate dehydrogenase activity
Protein
Albumin
Hemoglobin
Alkaline Phosphatase
Total cell counts/differentials
Total antioxidant capacity
Sodium (Serum)
Triglycerides
Lung Tissue
IL-1β
TNFα
MIP-2
KC
IL-6
Oxidized/Reduced Glutathione
Heme oxygenase-1
8-Hydroxy-Guanosine
Cell proliferation

Pulmonary Function (Rats only)
Quasistatic Chord Compliance
CO Diffusing Capacity/Alveolar Volume
Forced Expiratory Flow
Mean Mid Expiratory Flow
Quasistatic vital capacity
Forced Vital Capacity
Other
Clinical Observations
Mortality
Body Weight
Organ Weights
Tissue Histopathology

Respiratory Function in Rats at 3 Months







Significant ($p<0.05$) <u>trend</u> observed for each of these endpoints

Findings were generally mild

Example:

8 % decline in forced vital capacity

>20 % of predicted would typically be considered clinically significant

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