Advanced Collaborative Emissions Study (ACES)

Cooperative multi-party effort to characterize emissions and possible health effects of new advanced heavy duty engine and control systems and fuels in the market 2007 – 2010.

Jake McDonald

ACES Phase 3B: Summary of 1 and 3 Month Exposures

RFP 06-1 primary (null) hypothesis:

Emissions … will have very low pollutant levels and will not cause an increase in tumor formation or substantial toxic effects in rats and mice at the highest concentration of exhaust that can be used … compared to animals exposed to clean air, although some biological effects may occur.

CORE BIOSCREENING STUDY DESIGN

3-Month Exposure of C57BL/6 Mice:

- Expose 120/group 16 hr/day, 5 days/wk for 3 months (13 wk)
- 60/group allocated for evaluation at 1 & 3 months
  - Lung lavage, Lung tissue & cell proliferation
  - Hematology & serum chemistry (3 mo)
  - Histopathology

Chronic Carcinogenicity Bioassay of Wistar Han Rats:

- Expose 288/group 16 hr/day, 5 days/wk for 24-30 months
- 3 dilutions of whole emissions + clean air controls
- 166/group committed to carcinogenesis bioassay
- 122/group allocated for interim evaluations at 1, 3, 12, & 24 months
  - Pulmonary function (3, 12, & 24 mo)
  - Lung lavage, lung tissue & cell proliferation
  - Hematology & serum chemistry (3, 12, & 24 mo)
  - Histopathology

Accommodate ancillary biological studies of rats and mice

- Markers of potential Cancer, vascular inflammation effects

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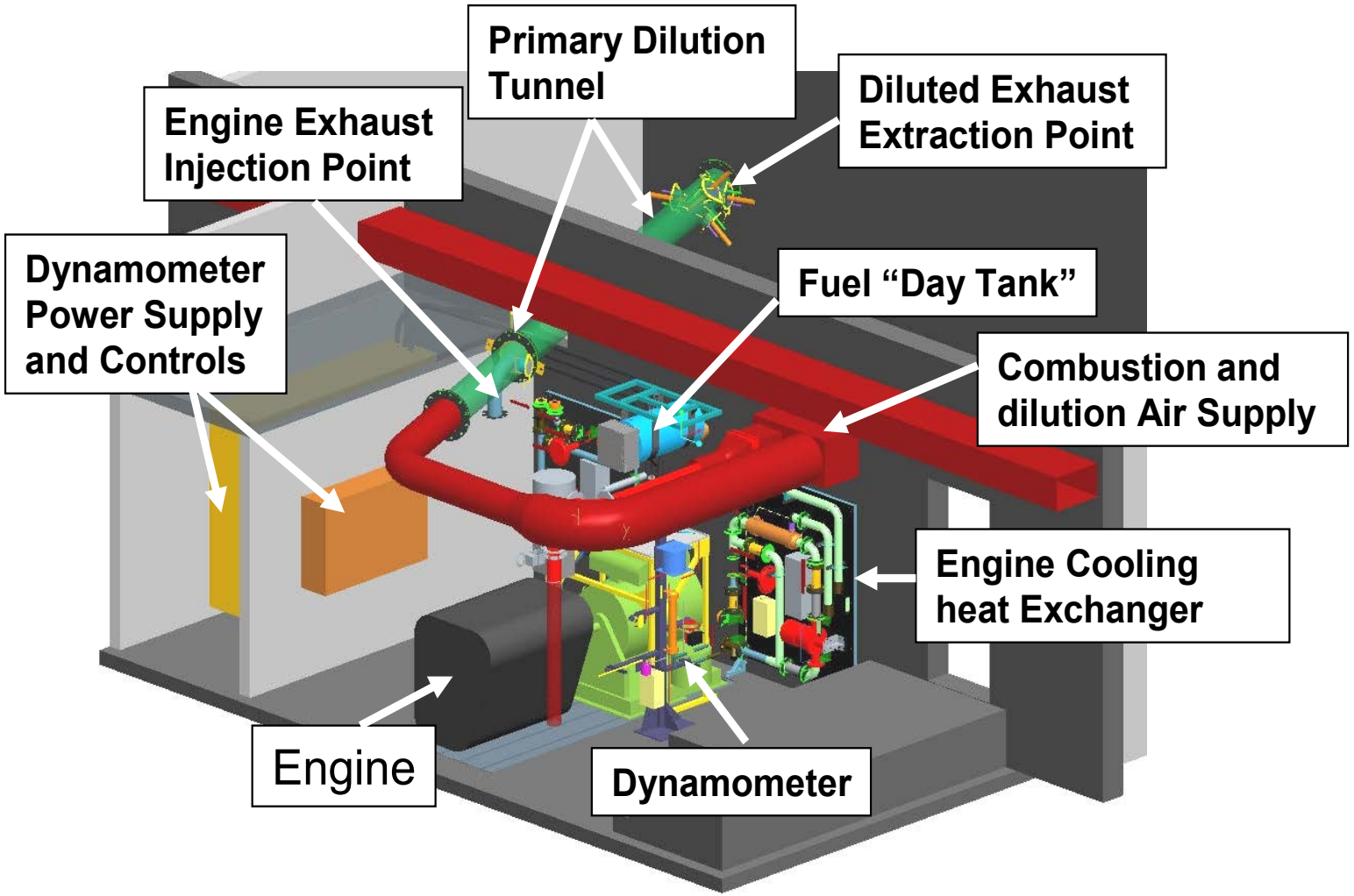
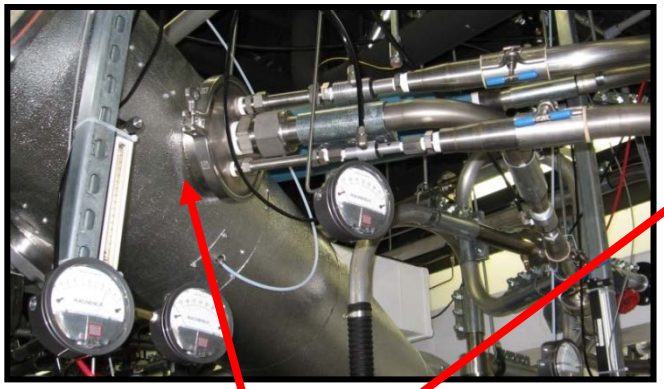
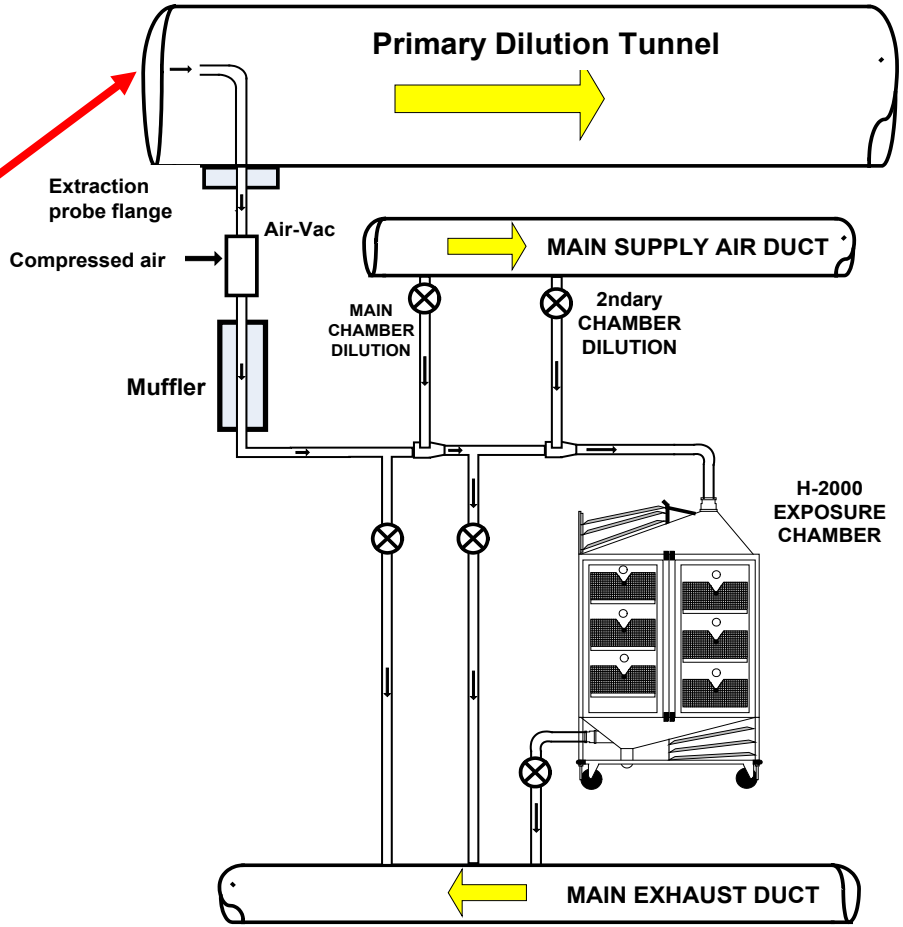
Engine and Primary Dilution System



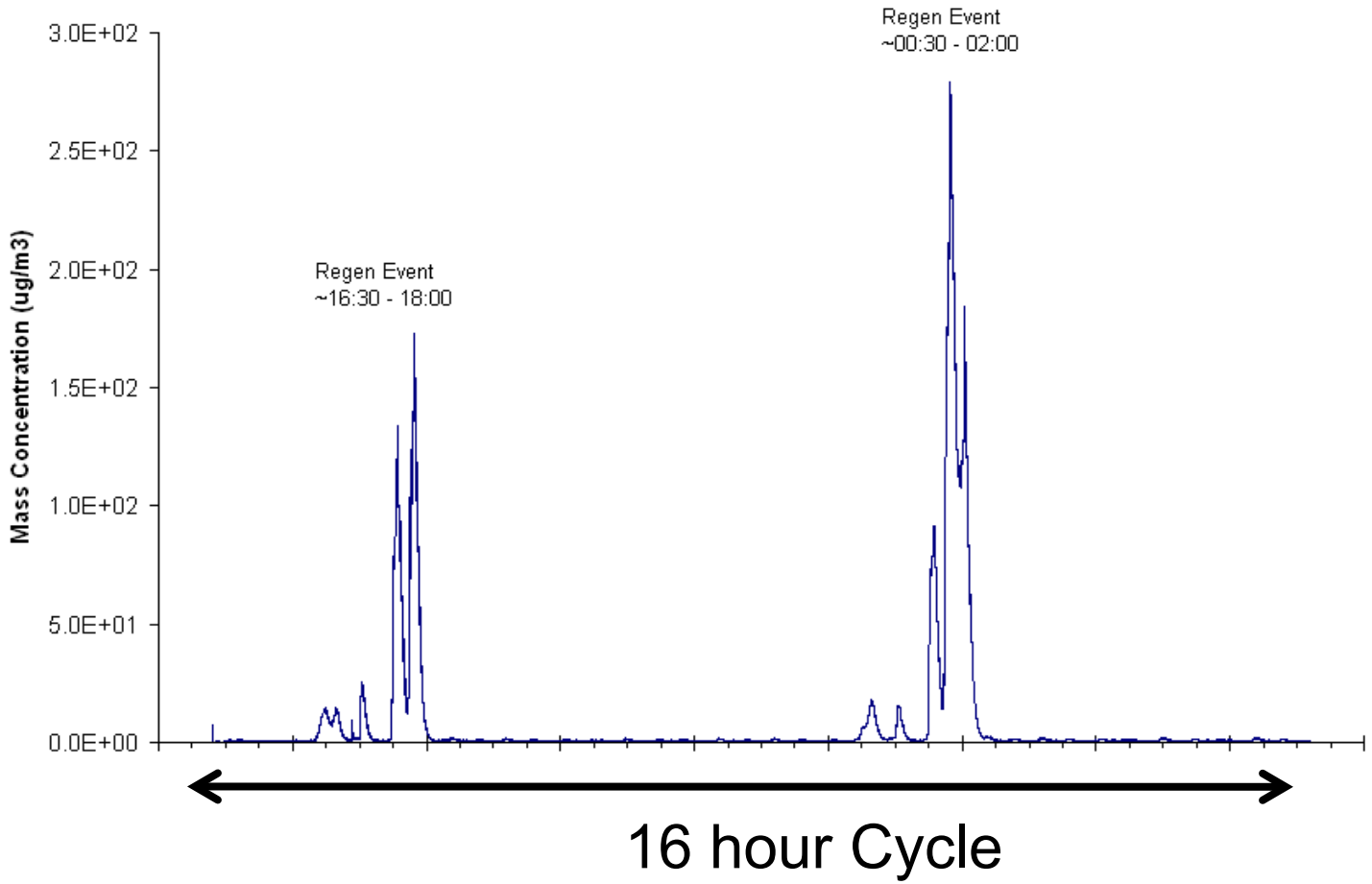
Exhaust Extraction and Secondary Dilution Systems



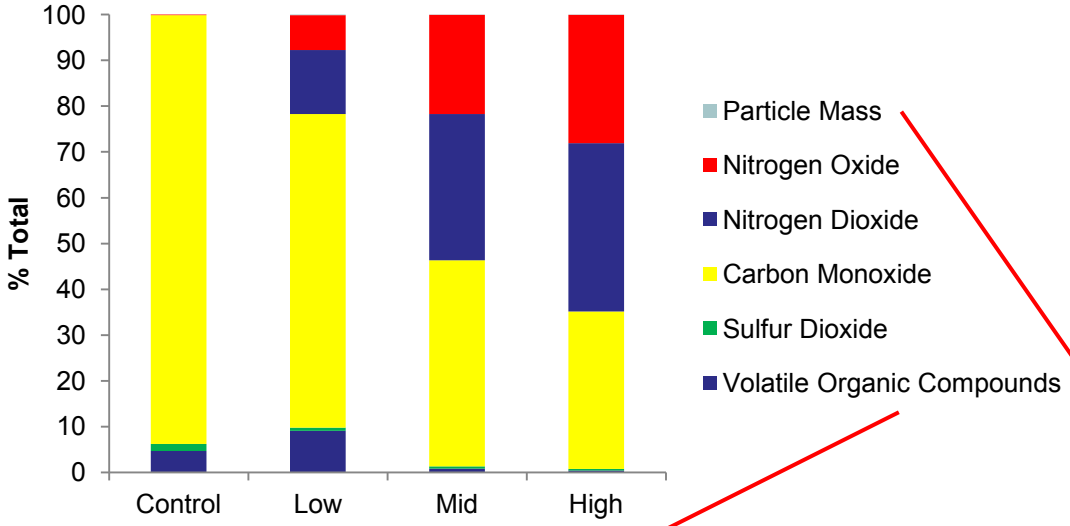


(Note: Drawing is not to scale)

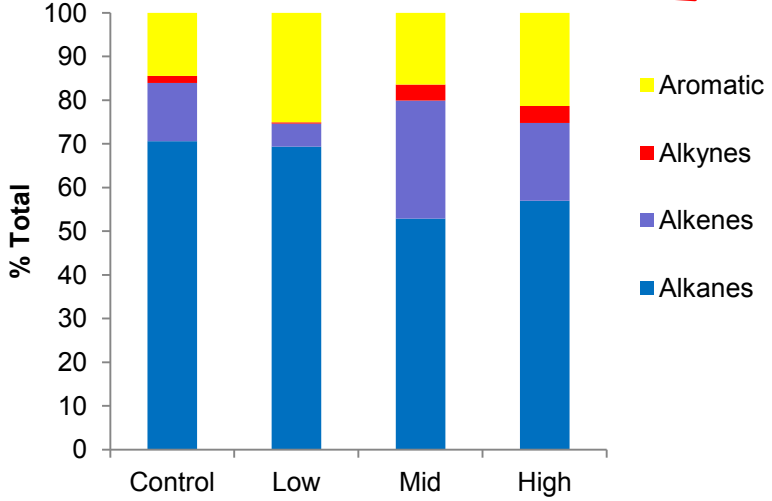
ACES 16-Hr Cycle
Dekati, DMM Mass Concentration



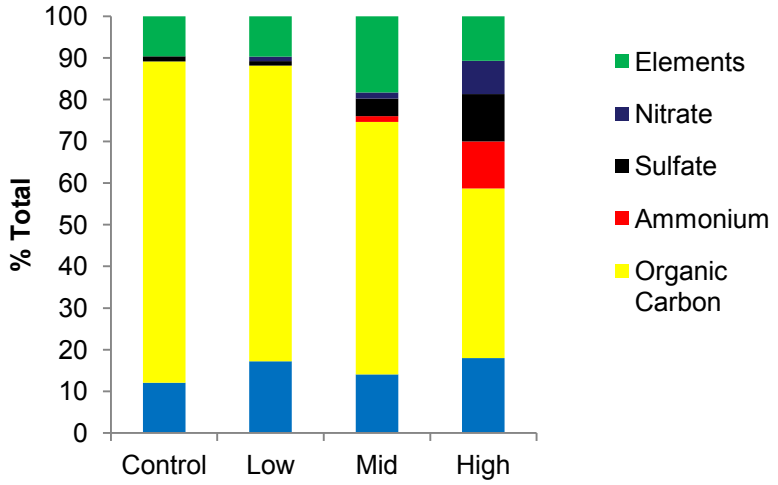
Exposure Atmosphere Compositions



Volatile Organics



Particle Composition



Statistical Approach

ANOVA

- **Experimental group, gender, group:gender interactions**
  - **If no significant gender difference, genders pooled**
  - **Dunnet's multiple comparison procedure used**
  - **Log transformations done on heteroscedastic data**
  - **Significance evaluated at p=0.01 and p=0.05**

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Findings:
Rodent 1 and 3 Month Sacrifices

The majority of the analyses showed no difference between diesel exhaust exposure and clean air control.

Histopathology analysis revealed mild/minimal exposure-related hyperplasia in the rats after 3 months of exposure, but not in mice.

A few statistically significant findings were noted for pathology indicators of pulmonary stress and inflammation in rats and mice (fewer findings in mice).

Pulmonary function assessments in rats showed slight differences in exposed rats compared with control after 3 months of exposure.

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Biological Response in Rats

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

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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

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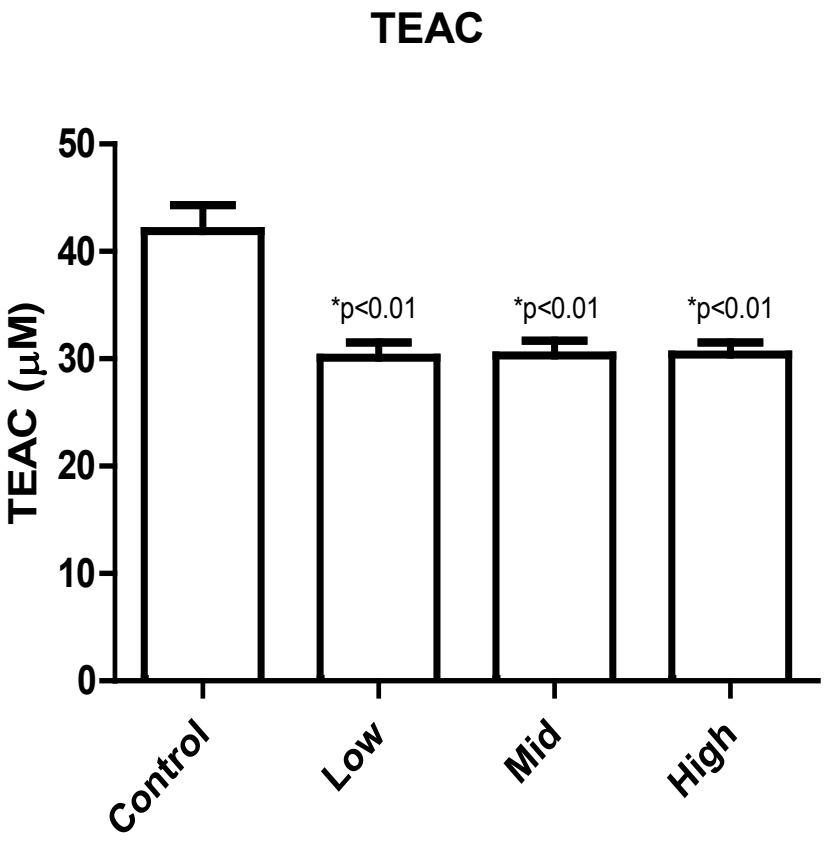
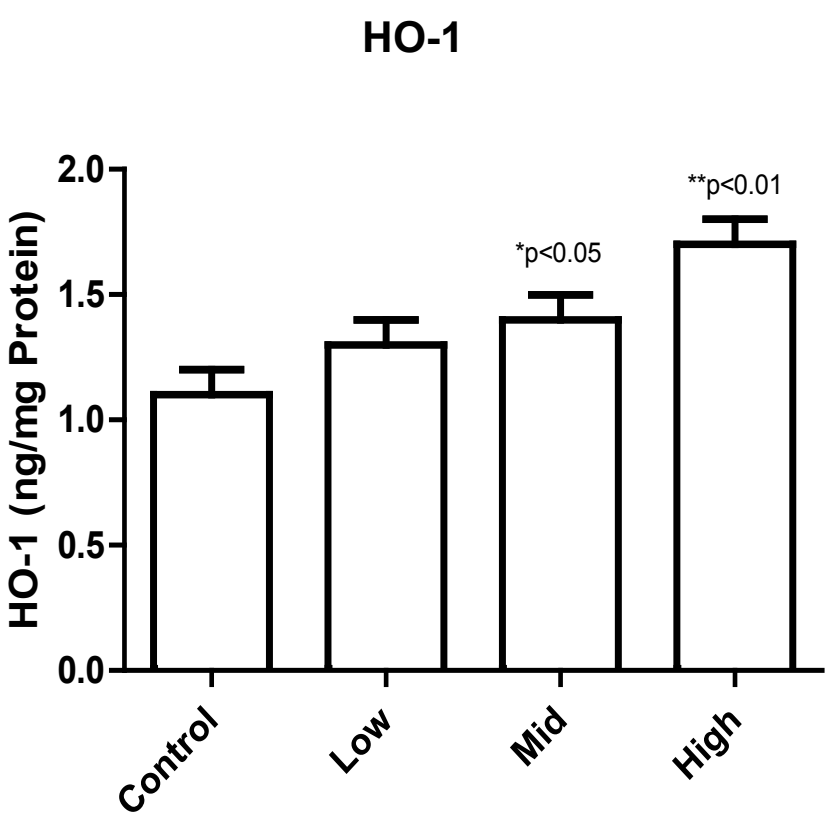
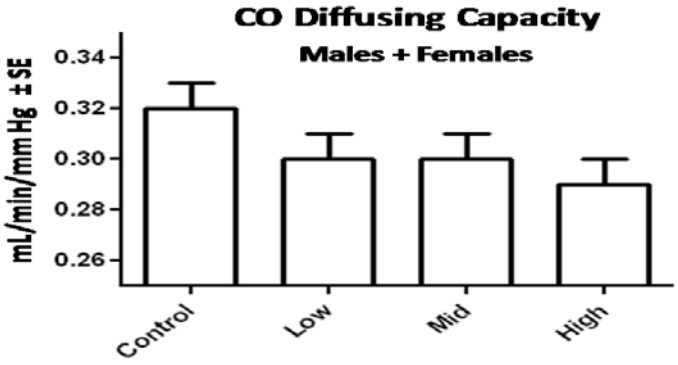
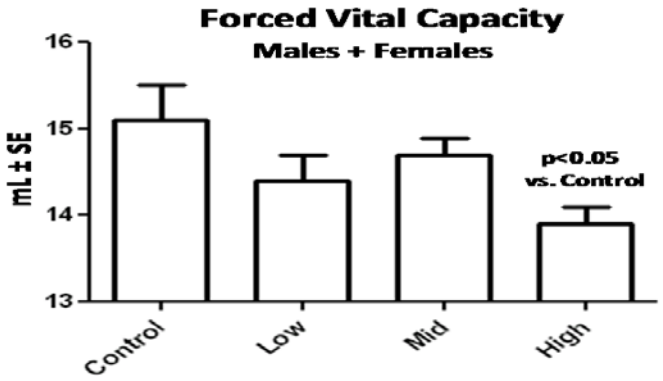
Pulmonary Inflammation/Stress in Rats at 3 Months

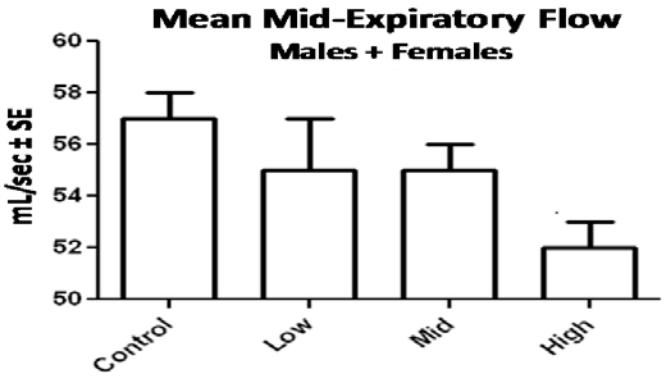




Respiratory Function in Rats at 3 Months







Significant ($p<0.05$) trend 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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Histopathology in Rats at 3 Months

Incidence and Types of Findings

Males

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

Females

Lung	Control	Low	Mid	High
Hyperplasia Epithelium Periacinar	0/10	0/10	0/10	9/10
Accumulation Macrophage	0/10	0/10	0/10	3/10
Fibrosis Interstitial	0/10	0/10	0/10	2/10

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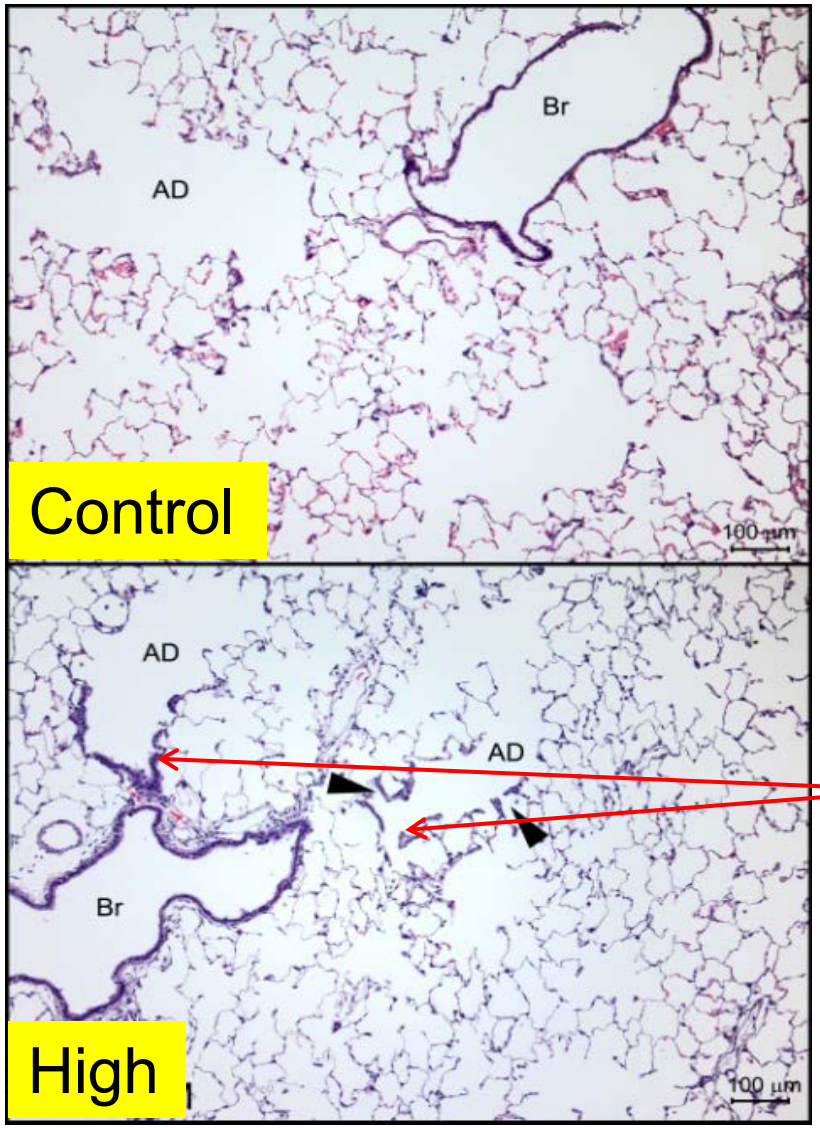
Histopathology in Rats at 3 Months



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

Findings generally mild

Thickening of alveolar duct septae

AD = Alveolar Duct; Br = Bronchiole

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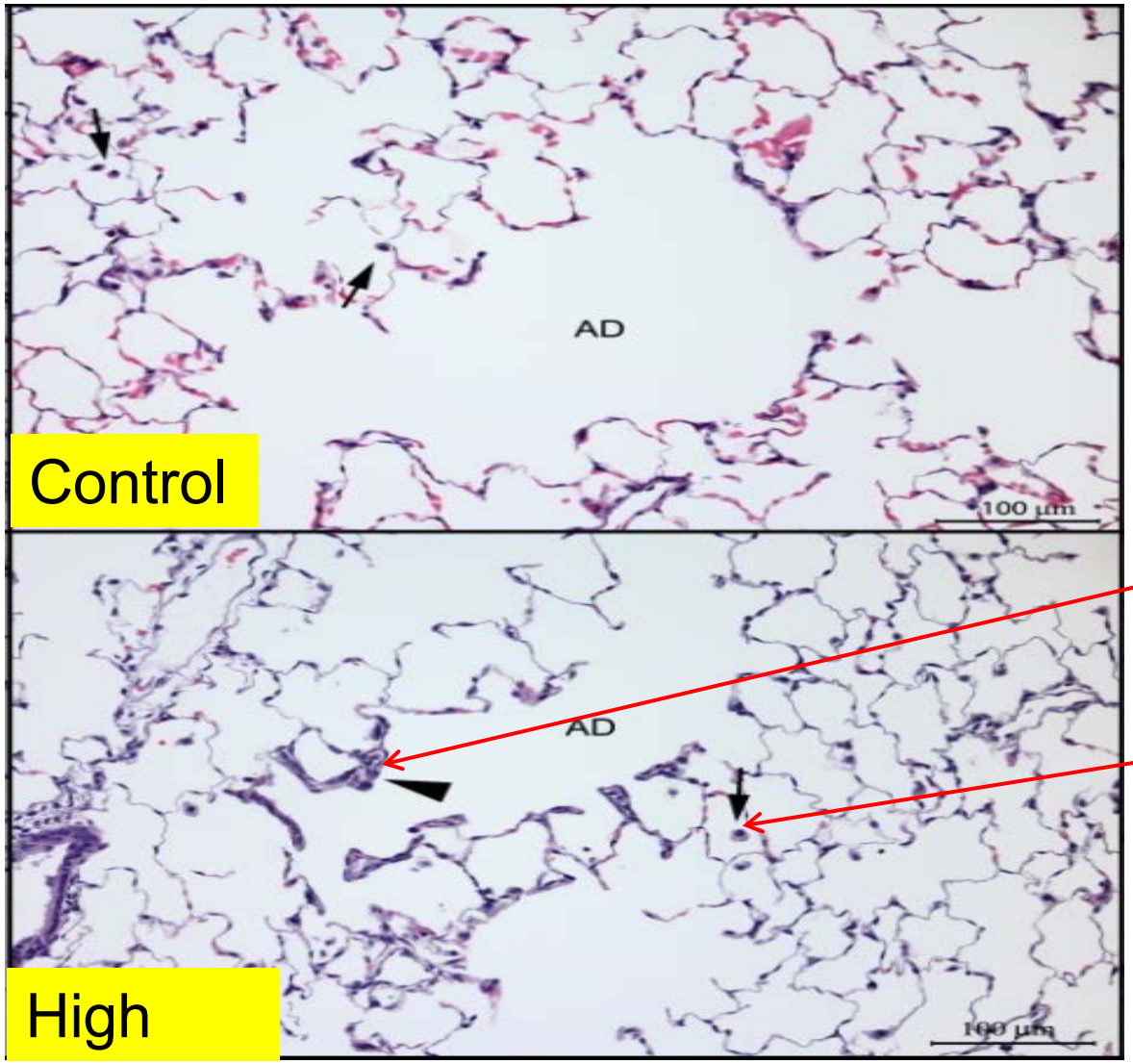
Histopathology in Rats at 3 Months:

Higher Power View of Previous Slide



Thickening of alveolar duct septae

Macrophage

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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 NO_2**
- **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

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SUMMARY

- **The majority of the response variables measured showed no effect**
- **Exposure caused detectable but minimal effects in airway remodeling, respiratory function, and select markers of stress and inflammation in rats.**
- **Mice (data not shown) showed limited to no airway remodeling, and few increases in inflammatory indicators**
- **Results in rats *may* be consistent with effects of NO_2-only exposures seen in other studies**

Additional:

- **Study now at approximately 16 months.**
- **12 month analysis nearly complete**

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