

ACES PHASE 3: Chronic Inhalation Bioassay



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ANIMAL STUDIES

Chronic Carcinogenicity Bioassay of Wistar Rats:

- Expose 288/group 16 hr/day, 5 days/wk for 24+ months
- 3 dilutions of whole emissions + clean air controls
- 166/group committed to carcinogenesis bioassay
- 122/group allocated for evaluation at 1, 3, 12, & 24 months

Pulmonary function
Pulmonary toxicity assays
Hematology & serum chemistry
Histopathology
Ancillary studies

Subacute 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

Pulmonary toxicity assays
Hematology & serum chemistry
Histopathology
Ancillary studies



PROJECT TEAM

Joe Mauderly	LRRRI	Principal Investigator
Ed Barr	"	Engine & Exposure Systems Engineer
Matt Campen	"	Respiratory Physiology
Nancy Crowley	"	Database Manager
Andrew Gigliotti	"	Necropsy, Histology, & Histopathology
David Grlego	"	Facilities Engineer
Jake McDonald	"	Exposure & Characterization Manager
Matt Reed	"	Study Director
JeanClare Seagrave	"	Bronchoalveolar Lavage & Cell Proliferation
Sonya Swing	"	Attending Veterinarian & Animal Care
Stephanie Taulbee	"	Quality Assurance Manager
Richard White	"	Daily Exposure Supervisor
David Bartels	ACS	Engine Facility Engineer
Matt Jorgensen	"	" " "
Greg Rees	"	" " "
Barbara Zielinska	DRI	Analytical Chemistry
Rodney Miller	EPL	Histopathology
Steve Sellkop	SKS	Data Analysis

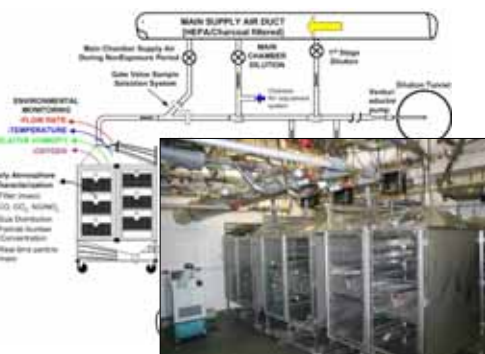


EXPOSURE ATMOSPHERES

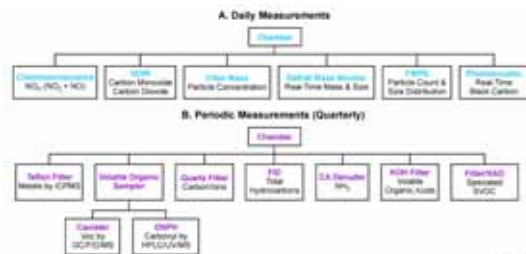
- Whole diluted emissions from engine selected from Phase 1
On test stand connected to AC dynamometer
2007-compliant fuel supplied in bulk
Manufacturer's recommended oil, filters, & maintenance
- Continuously-repeated variable-duty cycle as used in Phase 1
Room temperature cold start included each day
- Constant flow primary dilution tunnel
Charcoal & HEPA-filtered ambient air for combustion, dilution, and control exposure
- Secondary dilution to 3 exposure concentrations
Tentatively 1:10, 1:30, 1:100
Temperature and humidity in animal chambers likely to set lowest dilution



DILUTION-EXPOSURE SYSTEM



CHARACTERIZATION OF EXPOSURE



RAT BIOASSAY

Male and Females, 6 wk old at start of Exposure

- Quarantined and conditioned 2 wk before exposure
- Assigned semi-randomly by weight and identified by microchip
- Biostatus monitored by serology and sentinels
- Housed continuously in inhalation exposure chambers
- NTP-2000 diet and water *ad libitum*

Allocation Within Each Treatment Group

Months	1	3	12	18	24	30	Total
Chronic Bioassay	-	-	-	-	-	166	166
Intermediate Sacrifice	20	20	20	-	28	-	88
Unassigned	-	16	-	-	18	-	34
Total Used at Interval	20	36	20	-	46	166	
Cumulative Total Used	20	56	76	76	122	288	288
Remaining in Chambers	268	232	212	212	166	0	

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MEASUREMENTS AT 1, 3, 12 & 24 MONTHS

Pulmonary function

Quasistatic pressure-volume (vital capacity, compliance)
Forced vital capacity (FEV_{0.1}, peak flow, mean midexpiratory flow)
CO diffusing capacity

Bronchoalveolar lavage

Cells
Lactate dehydrogenase
Protein
Glutathione
Alkaline phosphatase
Cytokines (IL-1 β , TNF α)

Mice at 1 & 3 months

Cell proliferation

Immunohistochemistry using nuclear antigen (Ki67)

Hematology

RBC, WBC, differential, Hb, Hct, etc.

Clinical Chemistry

Proteins, enzymes, cholesterol, BUN, etc.

Necropsy and Histopathology

Gross lesions & organ weights
Light microscopy of standard sections of multiple tissues

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WHY RATS?

1. Rats are the animal species that "brought us to the table" in regard to both cancer and non-cancer effects of diesel emissions
2. Rats are the standard inhalation cancer bioassay species (e.g., NTP)
Guidelines now constrain exposures at and below MTD
3. Rats are the species mandated for testing of emissions from new fuels
(CAA, Section 211(b), CFR 79 Title 40)
4. The "overload" issue that negated previous diesel bioassay results for framing human cancer risk pertained to overwhelming deposition of particles. This is not anticipated to occur with 2007 emissions
Mauderly, Inhal. Toxicol. 8(suppl):1-28, 1996
Nikula et al., Fundam. Appl. Toxicol. 37:37-53, 1997
5. Rats have proven responsive to most inhaled atmospheres that are carcinogenic in humans
Fewer false negatives compared to IARC category 1 than mice or S. Hamsters
Rats more sensitive than mice or S. hamsters to particulate carcinogens
Rats negative and mice positive for some gases & vapors
Mauderly, Environ. Health Perspect. 105(suppl. 5): 1337-1346, 1997
Mauderly et al., Toxicol. Sci. 81:280-292, 2004
6. If you think rats are "over-responsive", then this is an "acid test"

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ACCOMMODATION OF ANCILLARY STUDIES

Contract includes facilitation of ancillary studies

- Accommodation of treatment & measurement protocols
- Collection & shipping of samples
- Provision of on-site laboratory space & coordination of logistics
- Accommodation of special animals

Rats

- Ancillary measurements of 20 interim sacrifice rats/group/time
Excess tissues, blood, lavage fluid
No opportunity to pre-treat or instrument animals
- Special Studies using unassigned rats
Must be removed by designated times

Mice

- Ancillary measurements of 20 interim sacrifice mice/group/time
- Broader opportunity to use additional 20-40 mice/group/time designated for ancillary studies
Could be same or other strains
Some flexibility in instrumentation, exposure length, and measurement times

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