

Annual Progress Report of the
NATIONAL ENVIRONMENTAL RESPIRATORY CENTER

November 1, 2008 – December 9, 2009



Lovelace Respiratory Research Institute

2425 Ridgecrest Dr. SE, Albuquerque, NM 87108



December 2009

ANNUAL REPORT
Of the
NATIONAL ENVIRONMENTAL RESPIRATORY CENTER

November 1, 2008 – December 9, 2009

Joe L. Mauderly, DVM, Director

Lovelace Respiratory Research Institute
2425 Ridgecrest Dr. SE
Albuquerque, NM 87108
505-348-9432
jmauderl@lrri.org

December 2009

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
I. EXECUTIVE SUMMARY	4
II. INTRODUCTION AND BACKGROUND	5
A. Purpose and Nature of the Report	5
B. Origin and Strategy of the NERC Program	5
1. Our Multi-pollutant Air Quality Dilemma.....	5
2. Origin of the NERC Program.....	6
3. Funding and Management Strategy	6
4. Research Strategy.....	7
5. Quality Assurance Strategy.....	11
6. Animal Use Strategy	11
7. Other Strategic Elements of the Program	11
C. Status of the Program	12
1. Status of the Research	12
2. Future of the Program	13
III. ACTIVITIES AND PROGRESS	13
A. Progress Prior to This Reporting Period	13
1. Diesel Engine Exhaust	14
2. Hardwood Smoke.....	16
3. Gasoline Engine Exhaust	18
4. Simulated Downwind Components of Coal Combustion Emissions.....	20
B. Progress during This Reporting Period	22
1. Evaluation of Feasibility of Conducting a Study of Marine HFO Exhaust.....	22
2. Completion of Data from Coal Study	23
3. Integrated Analysis of Data from Four Studies	24
4. Other Accomplishments.....	24
C. Response to Guidance from the External Scientific Advisory Committee	25
1. Future Studies	25
2. Strategic Planning/Marketing Document.....	25
3. Statistical Analysis Workshop	26
D. Sponsorship and Funding	26
1. Sponsorship.....	26
2. Changes in Sponsorship.....	27

IV.	ANTICIPATED ACTIVITIES DURING THE NEXT REPORTING PERIOD.....	28
A.	Completion and Publication of Current Data Sets.....	28
1.	Coal Emissions Study	28
2.	Repeat Studies of Diesel Exhaust and Hardwood Smoke.....	28
3.	Composition of Paved Road Dust	28
4.	Integrated Analysis of Data from Four Studies	28
B.	Workshop on Statistical Strategies	28
V.	REFERENCES	28
VI.	APPENDICES.....	31

LIST OF APPENDICES

<u>Appendix</u>	<u>Page</u>
A. Publications from the National Environmental Respiratory Center.....	A-1
B. Summary of the Workshop on Generation of an Exposure Atmosphere Simulating Ship Main Engine Exhaust	B-1
C. 2009 Guidance Letter from the NERC External Scientific Advisory Committee	C-1

I. EXECUTIVE SUMMARY

This report summarizes the purpose and strategy, past accomplishments, current activities and progress, and the future of the National Environmental Respiratory Center (NERC) multi-pollutant research program. The report focuses on progress during the period between September 1, 2007 and October 31, 2008. The report is summary in nature. Plans, progress, experimental details, interim results, and pre-publication final results are communicated to the advisory committee and sponsors throughout the year. The Center's core product is the publication of findings in the peer-reviewed literature.

NERC is a laboratory research program sponsored jointly by government and industry with the goal of improving the understanding of the contributions of individual air contaminants, their combinations, and their sources to the adverse respiratory and cardiovascular health effects associated with air pollution. The program was created in view of the single-pollutant, single-source orientation of most programs, and the need to understand the air quality-health relationship from a multi-pollutant perspective. The number and type of sponsors vary from year to year. The program is managed by the independent, not-for-profit Lovelace Respiratory Research Institute (LRRI), the research is conducted by a multi-institutional team of investigators and collaborators, and the program is guided by an independent External Scientific Advisory Committee (ESAC) of experts from academia, government, and industry.

The NERC research strategy is to develop a composition-concentration-response database by conducting identically designed, subchronic studies of animals exposed by inhalation to mixtures of common air pollutants having different but overlapping composition, and then to analyze the combined data from these studies to identify pollutants that co-vary most strongly with different respiratory and cardiovascular health effects. The compositions of the exposures are measured in detail, and exposure concentrations extend downward to environmentally-relevant levels. Effects of exposure are characterized using multiple animal models of human respiratory and cardiovascular responses. Common source emissions are used for the exposures, which encompasses many air contaminants of current concern and allows comparisons among sources.

During the past year, the program was funded by 9 sponsors, all non-government corporations or industry associations. A total of \$815,360 was received, although some funds were intended as multi-year support. The principal accomplishments were: 1) evaluation of strategies for conducting a study of a mixture simulating emissions from a ship main engine burning heavy fuel oil; 2) completion of data from the study of simulated downwind coal combustion emissions; and 3) preparation and submission of publications.

Principal activities for the coming year will be: 1) submission of several publications reporting results from the coal study, the pilot study of road dust, the repeat studies of diesel exhaust and hardwood smoke, and the initial results from integrated analysis of the results from the first four studies; 2) a workshop on statistical strategies for analyzing the combined NERC database.

II. INTRODUCTION AND BACKGROUND

A. Purpose and Nature of the Report

This report describes the progress of the National Environmental Respiratory Center (NERC) between November 1, 2008 and December 9, 2009. This report describes the progress of the program in general terms. It is not intended as a vehicle for communicating detailed experimental results or extensive summaries of key findings. Completed NERC results are communicated in final form in scientific publications; less formally in presentations at scientific meetings; and in preliminary form at the annual meeting of the Center's advisors, sponsors, and investigators. Current sponsors of the program receive continual updates throughout the year, including pre-publication results and copies of papers in press. This report summarizes Center objectives, strategy, schedule, progress, difficulties, funding, and other issues as a complement to the more detailed information communicated via other avenues.

B. Origin and Strategy of the NERC Program

1. Our Multi-pollutant Air Quality Dilemma

We live in a multi-pollutant world, but we understand the health risks from air pollution largely from a single-pollutant perspective. The current approach to implementing the U.S. Clean Air Act involves repeated cycles of review of the National Ambient Air Quality Standards (NAAQS) for the six "criteria" pollutants (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides), which in turn stimulates debates and research agendas focused primarily on single pollutants and consuming the majority of air quality health research resources. Fewer resources are directed toward the 33 "urban air toxics" designated as most important among the 188 "hazardous air pollutants" (HAPs); fewer yet are directed toward the remaining 155 HAPs; and very little attention is given to the many other natural and man-made air contaminant species that are not on these lists. In parallel, promulgation of source-specific emission standards (e.g., diesel engines, coal-fired power plants) tends to focus research on only a few of the many sources of air contaminants and seldom clarifies the most toxic components of complex source emissions. Although regulatory structures differ among countries, the situation is generally similar throughout the world; the majority of attention is directed to a minority of pollutants.

Of course, people actually breathe very complex, continuously varying mixtures of hundreds of air contaminants from many man-made and natural outdoor and indoor sources. Even single pollutant classes, such as particulate matter (PM) and volatile organic compounds (VOCs), are almost always encountered as complex mixtures of components from multiple sources. We have few biological markers that can link observed health effects to specific pollutant species, classes, or sources. We know that many pollutants (and other factors) can cause the same biological effects (e.g., oxidative stress, inflammation) or health outcomes (e.g., reduced lung function, increased clinical visits for respiratory and cardiovascular disorders). We also know that nonadditive effects of pollution mixtures are plausible, although largely untested. There are several types of potential interactions among pollutants in mixtures, including both synergy and antagonism (Mauderly and Samet, 2008). However, unless there are data to the contrary, the default regulatory assumption must be that the health risks of exposures to combinations of pollutants are additive. In ignorance of pollutant interactions, it is plausible

that we may either overestimate or underestimate the effects of mixtures, and may ascribe at least some portion of health risks to the wrong pollutants.

The U.S. air quality management landscape is shifting toward a multi-pollutant perspective. Encouraged by multiple advisory groups such as the NRC Committee on Research Priorities for Airborne Particulate Matter (NRC, 2004b) and the NRC Committee on Air Quality Management in the United States (NRC, 2004a), the EPA is exploring potential multi-pollutant regulatory and accountability strategies. Several air quality rules and acts in recent years have dealt with more than one emitted pollutant. Recently, NARSTO conducted an assessment of the potential for multi-pollutant management and accountability strategies (publication in press, see www.narsto.org/mpacc.src). It is clear that the current trend is toward a broader management of air quality to achieve the greatest aggregate health and welfare benefits, although it is not clear how far, and how fast, that trend will progress.

The more we shift toward multi-pollutant air quality management and accountability, the more we will need data on the relative effects of different air contaminants and the effects of pollutant combinations. The NERC Program was created for that purpose, but neither NERC nor any other single program could meet this research need alone. A multi-pollutant perspective needs to pervade the entire spectrum of air quality health research, regardless of whether individual studies address single or multiple air contaminants. NERC was explicitly intended as a spearheading effort that could simultaneously provide valuable information through its own strategy and also catalyze a broader shift in research perspective. The Center has been successful as a stimulus for an evolution of research and regulatory perspectives, and is also providing some of the information that will be important to guide future air quality management decision making.

2. Origin of the NERC Program

The NERC Program (www.nercenter.org) was initiated by Congress in 1998 explicitly to establish a forward-looking, multi-pollutant-oriented laboratory research program aimed at placing the relative health hazards of different air contaminants, their combinations, and their sources into clearer context. This action followed congressional hearings on the 1997 PM and ozone standards in which it became clear that there was no federal research program aimed specifically at disentangling the contributions of all air contaminants to the health effects of air pollution, apart from current regulation-specific concerns. With seed funding from EPA, LRRI recruited an advisory committee, developed the Center's research strategy, began recruiting additional financial support, and initiated research activities.

3. Funding and Management Strategy

From the outset, it was intended that the NERC Program would be funded jointly by EPA, other federal and state agencies, and industry. EPA funding to date has resulted from congressional appropriations. Funding from multiple offices of DOE and DOT has been provided at the initiative of those agencies, or through competitive solicitations, ongoing programs of related research, or specific initiatives. States and non-government entities have contributed voluntarily to the program. Entities contributing \$25,000 or more per year are designated "Affiliate Sponsors" and have open access to planning, interim results, and pre-publication papers in press.

The NERC program is based at the independent, not-for-profit Lovelace Respiratory Research Institute (LRRI) in Albuquerque, New Mexico, USA, which has research

staff, facilities, project management experience, and multi-institutional collaborative arrangements uniquely well-suited to the task. The program is managed by Dr. Joe Mauderly, an LRRRI Senior Scientist having widespread and longstanding recognition in the air pollution health research field. Dr. Jacob McDonald of LRRRI, whose expertise integrates atmospheric and analytical chemistry, characterization of source emissions, inhalation exposure operations, and toxicology, serves as Co-Principal Investigator. The research is conducted by a multidisciplinary team of investigators at LRRRI and several other institutions across the United States.

An External Scientific Advisory Committee (ESAC, see text box) was formed to work with LRRRI to identify an appropriate strategy for meeting this challenging research need, and the Committee has actively guided the program since its inception. To separate the conduct of research and interpretation of results from the sometimes-divergent views among sponsors, the ESAC is independent from sponsorship and is vested with approval authority over major program decisions. In this manner, the ESAC functions more like a board of directors than like a typical research advisory committee. Input on major decisions is sought from all Affiliate sponsors, but final decisions are approved by the ESAC. Contributions to the program do not provide membership on the ESAC, nor is advice from sponsors prioritized on the basis of the amount of their contributions.

NERC External Scientific Advisory Committee

Current Members:

Morton Lippmann, PhD, New York University (Chair)
Michael Bird, MSc, PhD, DABT, C. Chem, FRSC, Exxon
Biomedical Sciences
Bill Bunn, MD, JD, MPH, International Truck & Engine Co.
Judy Chow, PhD, Desert Research Institute
Ian Gilmour, PhD, U.S. Environmental Protection Agency
Gerald VanBelle, PhD, University of Washington
Sverre Vedal, MD, University of Washington
Ron White, MST, Johns Hopkins University
Ron Wyzga, MS, ScD, Electric Power Research Institute

Former Members Guiding the Program during Earlier Stages:

Glen Cass, PhD, Georgia Tech University
Dan Costa, ScD, U.S. Environmental Protection Agency
Jonathan Samet, MD, MS, Johns Hopkins University
John Vandenberg, PhD, U.S. Environmental Protection Agency

4. Research Strategy

The NERC research strategy addresses two interrelated fundamental information gaps: 1) an exposure composition-concentration-response database adequate to support multivariate analysis of the roles of different pollutants in different health effects (when inhaled in mixtures); and 2) contemporary, detailed, head-to-head comparisons of the health effects of different anthropogenic combustion source emissions of significance to air quality management. These needs are met by conducting identically designed inhalation toxicology studies of several source-based mixtures having different, but overlapping compositions (Table 1). The strategy for generating each source emission is based on consensus of scientific and technical experts, achieved by NERC workshops convened for that purpose. Given an adequate database for which both exposures and health outcomes are known in detail, several multivariate analytical approaches, such as principal component analysis (PCA), partial least squares regression (PLS; Malinowski, 2002), parallel factor analysis (PARFAC; Kiers et al., 1999), and Tucker-3 (Tucker, 1966), can be used to determine links between physical chemical species (and their combinations) and health outcomes. The feasibility and utility of this general approach was demonstrated by LRRRI studies using PCA/PLS to identify engine oil components, rather than fuel combustion components, as the primary drivers of lung inflammatory responses to samples of diesel, gasoline, and compressed natural gas engine emissions (McDonald et al., 2004a;

Seagrave et al., 2005a) and identifying traffic and industrial emissions as important contributors to the toxicity of southeastern U.S. ambient PM samples (Seagrave et al., 2006).

Table 1. Matrix of Exposures and Health Outcomes

Exposure Atmospheres	Inflammation, Cytotoxicity, & Tissue Damage	Allergies, Asthma	Defenses Against Infection	Heart & Vessel Effects	DNA Injury
Diesel engine exhaust (completed)	+	+	+	+	+
Hardwood smoke (completed)	+	+	+	+	+
Gasoline engine exhaust (completed)	+	+	+	+	+
Simulated “downwind” coal combustion emissions (completed)	+	+	+	+	+
Respirable paved road dust (recommended)	+	+	+	+	+
Residual oil emissions (considered)	+	+	+	+	+
Secondary organic aerosol (considered)	+	+	+	+	+

The underlying hypothesis is that composition-response relationships can be identified that hold true regardless of the source of the pollutants. If this proves true on a program scale, as it has in our hands on a smaller study scale (McDonald et al., 2004a; Seagrave et al., 2006), the results of the program will be an important step toward a more integrated, multi-pollutant understanding of air quality-health relationships.

Separate subchronic inhalation studies are conducted for each mixture (exposure atmosphere) using a consistent protocol that allows not only direct comparisons of effects among mixtures, but also combining data from all studies into a single database for analysis. Each study contributes a “layer” of composition-concentration-response data to the aggregate database. Animals are exposed in whole-body chambers (Figure 1) 6 hours/day, 7 days/week for up to 6 months to one of three concentrations of the mixture, to the highest concentration with PM removed by filtration, or to clean air as controls. In the first three studies (diesel exhaust, hardwood smoke, and gasoline), the four exposure atmospheres were all dilutions of whole emissions from combustion sources. In the coal study, multiple components, including combustion products of coal, were mixed in prescribed ratios to achieve a target mixture simulating the key coal-derived products of downwind air pollution. All exposure levels are intended to fall within the range of plausible human exposures, although the higher levels represent occupational or “hot spot” conditions rather than widespread environmental exposures. Including multiple exposure levels in each study allows testing the significance of exposure-response trends and provides a glimpse of the nature of the exposure-response curve, including potential identification of no-effects levels.



Figure 1. Whole-body inhalation exposure chambers.

The NERC exposures are characterized at the greatest practical level of detail, encompassing the general physical-chemical classes summarized in Table 2 (McDonald et al., 2004b; 2006a,b; 2008). Most of the classes listed in the table include many species.

Table 2. Classes of Analytes Used to Characterize Exposures

Particles:		Gases:
Mass concentration		CO
Size distribution		CO ₂
Number counts		NO _x
Morphology		SO ₂
Size-specific chemistry		HC
Extractable fraction (and OC/EC)		NH ₃
Mutagenicity of extracts		
Biological material (road dust)		
Particle Extract and Vapor-Phase SVOC:		
Ammonium	n-alkanes, cycloalkanes	organic acids
Sulfate	alkenes	alkaloids
Nitrate	Branched alkanes, alkenes	PAHs (+oxy, nitro)
Elements (metals)	Furans, benzofurans	Hopanes
	Terpenes	Steranes
	Volatile aromatics	Aliphatic alcohols
	Phenols (+methoxy)	Carbohydrates
	Carbonyls	

Health responses are measured using the animal models and endpoints summarized in Table 3 and described in detail in publications (Campen et al., 2003; Day et al., 2008; Harrod et al., 2005; Barrett et al., 2005; Seagrave et al., 2005b; Reed et al., 2006, 2008; Lund et al., 2007). The goal is to employ outcome models and endpoints that span the general categories of health outcomes that have been associated statistically with air pollution by epidemiology.

The research is conducted by a team of LRRI and non-LRRI investigators who produce, analyze, interpret, and publish data (Table 4). The Center does not consist of research cores supporting multiple individual projects as is typical of most “center” programs; rather, it is a completely integrated effort in which all investigators participate in the same studies.

The NERC Program incorporates the best current thinking and broad scientific and stakeholder consensus into designing the research. This is done not only by open discussion with the ESAC and sponsor representatives at the annual meeting, but also by conducting peer expert workshops to review options and develop recommendations for key components of the program. The first workshop optimized the experimental design to facilitate analysis of the resulting database, and was followed by workshops to define the health assessment protocol, the generation of each exposure atmosphere, and strategies for statistical analyses.

Table 3. Assays of Health Responses

General toxicity: F344 rats and A/J mice	Morbidity, mortality, body and organ weight, hematology, serum chemistry, bronchoalveolar lavage, histopathology
Respiratory immune responses: BALBc mice	Development of neo-allergic responses Exacerbation of pre-existing allergic responses
Resistance to bacterial respiratory infection: C57BL/6 mice	Clearance of <i>Pseudomonas aeruginosa</i>
Cardiovascular effects: SHR rats and ApoE ^{-/-} mice	Heart rate and variability Waveform (conductivity) abnormalities Heart and vessel histopathology Pro-atherosclerotic vascular responses Serum clotting factors
Carcinogenic potential: F344 rats and A/J mice	DNA methylation Oxidative DNA damage Micronuclei in circulating reticulocytes

Table 4. Key Participants^a and Roles**Management^a**

Joe Mauderly, DVM (LRR)	Center Director (Principal Investigator)
Jake McDonald, PhD (LRR)	Principal Co-Investigator
Nancy Crowley, PhD (LRR)	Database Manager
Mericka Lehman, BS (LRR)	QA Auditor

Investigators

Ted Barrett, PhD (LRR)	Respiratory immune responses
Edward Bedrick, PhD (University of New Mexico)	Statistical analysis
Matt Campen, PhD (University of New Mexico)	Cardiovascular responses
Steve Belinsky, PhD (LRR)	DNA methylation
Andrew Gigliotti, DVM, PhD, DACVP (LRR)	Necropsy and histopathology
Jake McDonald, PhD (LRR)	Exposure and exposure characterization
Matt Reed, PhD, DABT (LRR)	General toxicity, resistance to infection
JeanClare Seagrave, PhD (LRR)	Bronchoalveolar lavage
Steve Seilkop, MS (SKS Consultants)	Statistical analyses
Jim Swenberg, PhD (University of North Carolina)	DNA oxidative injury
Richard White, BS (LRR)	Exposure supervisor

Chemical Analysis Contractors

Judy Chow, ScD (Desert Research Institute)	Analysis of ions
Eric Grosjean, PhD (DGA, Inc.)	Analysis of carbonyls and organic acids
Jordan Peccia, PhD (Yale University)	Analysis of bioaerosols
Jamie Schauer, PhD (University of Wisconsin)	Analysis of elements
Barbara Zielinska, PhD (Desert Research Institute)	Analysis of organic compounds

5. Quality Assurance Strategy

Considerable effort has been expended to ensure the quality of NERC studies, resulting data, and publications. The work is conducted in an environment and by personnel accustomed to performing research under the strictest of quality assurance (QA) requirements. The program is operated according to a formal Quality Management Plan (QMP), which meets the requirements of 40 CFR 30.54 and EPA QA/G-1STAR “Guidance on Satisfying EPA Quality System Requirements for STAR grants.” The studies follow formal protocols which are approved by the ESAC and distributed to sponsors. The protocols cite Standard Operating Procedures (SOPs) and Study-Specific Procedures (SSPs) that establish standard, step-wise procedures for most repetitive animal care, analytical, and data handling procedures. Data sets are audited by an independent auditor in the LRRRI QA Unit, and are only added to the final NERC database after all audit findings (if any) are corrected. The secure, fully backed-up, electronic database is the sole source for statistical analyses and summaries that are published or presented as “final” data. NERC research is also conducted in compliance with LRRRI requirements, including those stated in the LRRRI QMP and LRRRI Policy No. 601, “Research Quality Assurance”.

6. Animal Use Strategy

The Lovelace Respiratory Research Institute (LRRRI) performs all research in strict compliance with applicable local, state, federal and sponsor regulations and policies pertaining to animal care and use. LRRRI is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, has a current Animal Welfare Assurance filed with the National Institutes of Health (NIH), Office of Laboratory Animal Welfare (Assurance #A3083-01), and is a registered Class R research facility under the *Animal Welfare Act* (U.S. Department of Agriculture [USDA] Registration #85-R-003). All animal procedures are conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and under protocols approved by the LRRRI Institutional Animal Care and Use Committee (IACUC).

7. Other Strategic Elements of the Program

a. Leveraging NERC Resources for Ancillary Studies

In addition to conducting research, it is an explicit goal of the NERC Program to leverage its usefulness by providing information, research resources, and data to the broader research community. These resources provide opportunities for non-NERC investigators to conduct research that would be impossible in their own laboratories. The Center encourages and facilitates the use of exposures and biological samples in order to conduct ancillary studies that extend the scope of the program beyond the core study protocols. Resources that are made available on a case-by-case, merit, noninterference, no-added-cost basis include: 1) exposure of ancillary, special-purpose animals or *in vitro* biological systems on a space-available basis; 2) provision of excess biological samples or samples from the exposure atmospheres; and 3) addition of sample collections or measurements of health endpoints in protocol animals. Several exploratory efforts supported in this manner have resulted in lines of investigation that have benefited both NERC and other programs.

b. Making NERC Data Broadly Available

NERC makes its original data available to other investigators and the public through its web site (www.nercenter.org). Once summary findings from each study are published in the peer-reviewed literature, the complete underlying data sets are made available upon request. Available data sets are listed on the web site. Granting requests requires agreement not to use the data for commercial purposes and to make NERC aware of new findings and publications that result from re-analysis of NERC data. Data sets are extracted from the well-secured NERC electronic database and provided to requestors electronically, along with descriptions of the data fields. No direct external access to the secure database is allowed.

C. Status of the Program

The greatest challenge to the success of the NERC program is conducting the number of studies required to create a database sufficiently robust to support productive multivariate analyses of the importance of different pollutant species and combinations. At the outset, the ESAC proposed 12 different exposure atmospheres, but only four have been studied to date. As designed, the individual studies are lengthy and costly, and financial support has been less than originally envisioned. At this point, it appears unlikely that additional studies will be added to the database. Exploratory integrated analyses of the database containing results from the first three studies began in 2007, and detailed analyses of data from the first four studies will occur during 2010. It is not expected that a database containing only four mixtures will support multivariate analytical techniques having substantive power to disentangle causal components, but comparisons of responses among the first four mixtures have already proven informative. The next two mixtures to be studied (paved road dust and ship main engine emissions) have been prioritized by the ESAC, but more substantive funding than is presently in sight would be required to conduct either.

It is expected that the composition-concentration-response relationships revealed by integrative analyses of the multi-study database will provide a foundation for designing more focused (limited-scale) studies to test hypotheses directly. Such follow-on research has already begun under other programs and could be considered an extension of the NERC Program.

1. Status of the Research

The program has proceeded in accordance with the original strategy, shaped by guidance from the ESAC and advice from sponsors, and evolving as new findings arise and methods are refined. The primary focus to date has been on building the exposure-response database by conducting subchronic inhalation studies. Subchronic studies of diesel engine exhaust, hardwood smoke, gasoline engine exhaust, and simulated downwind coal emissions have been completed, as described below. The results from the diesel, wood smoke, and gasoline studies have been published or are in press. Results from the coal study are being prepared for publication at the time of this report. Results from the repeat studies of diesel exhaust and hardwood smoke are also being prepared for publication.

The integrated analysis of results from multiple NERC studies began in 2007 by exploring statistical strategies. The strategies focus on: 1) identifying the most meaningful biological effects among the many statistically significant findings; 2) comparing effects among the exposures (source emissions); and 3) identifying the physical-chemical classes

of pollutants that drove the different types of biological effects. This effort will be the chief activity during 2010.

The ESAC recommended conducting a study of paved road dust, in lieu of a study of ship main engine emissions. The technology for conducting a road dust study is well in hand at LRRI and has been demonstrated in previous small studies. An agreement among the ESAC and sponsors has been reached on the strategy for collecting the dust. However, efforts to secure funding adequate for the study have not been successful to date. The required funding is substantially greater than supported by the current non-government sponsors of NERC. Despite some interest, commitments for funding have not been obtained from EPA or DOT.

2. Future of the Program

At this time, it appears probable that the NERC program will end with an integrated analysis of existing data and publication of results. Continued sponsorship is needed during 2010 to accomplish those tasks, together with a portion of previous funds that was carried over from FY 2009. Unless substantial additional sponsorship is acquired during 2010, the program will end at that point.

III. ACTIVITIES AND PROGRESS

A. Progress Prior to This Reporting Period

This section summarizes progress to date, but does not present results in detail. Progress, methodology, and both preliminary and final results are reviewed in detail at the annual meeting of the ESAC and sponsors with Center management and investigators, and copies of all papers published or accepted for publication have been provided to the ESAC and sponsors. Only example findings are presented here.

When describing results in this report, the term “significant” means that univariate statistical analyses demonstrated both a significant monotonic trend with exposure concentration and mean values at one or both of the two highest exposure concentrations significantly different from the control mean value for that particular response variable. Of course, many response patterns are possible, and the possibility of meaningful non-monotonic responses cannot be excluded. The criterion for statistical significance was set at $p = 0.05$ for the standard analyses that are applied to all single-study results. Many, but not all, response variables have demonstrated significant effects at the highest exposure levels. Nearly all, but not all, response variables have demonstrated no significant effects at the lowest exposure concentrations. These findings suggest that the exposure concentrations have successfully bracketed the most meaningful range of responses.

The results of the four studies are summarized in following sections. To place those results in context, the composition of the four exposure atmospheres at the highest exposure level of each is summarized in Table 5 and Figure 2.

Table 5. Composition of the Exposure Atmospheres at the Highest Exposure Level

	<u>Diesel</u>	<u>Smoke</u>	<u>Gasoline</u>	<u>Coal</u>
Dilution factor	10	300	10	--
Total mass (mg/m ³)	84	17	116	2.7
Particles				
Mass (µg/m ³)	1005	1041	60	1015
Number (10 ⁶ /cc)	1.0	0.3	0.5	1.5
Size (MMAD, µm)	0.15	0.36	0.15	0.10-1.0
%OC	22	95	18.9	1.7
%EC	63.9	4.4	46.6	0.1
%sulfate	6	0.1	20.5	84.8
%nitrate	4	0.1	0.8	2.1
%ammonium	4	0.2	12.3	10.4
%elements (ash)	0.1	0.2	0.9	0.9
Gases & Vapors				
CO (ppm)	30	22	80	0.1
NO (ppm)	45	0	18	0.6
NO ₂ (ppm)	4	0	1	0.3
SO ₂ (ppm)	0.4	0	0.6	0.2
THC (ppm)	2	3	12	0.4

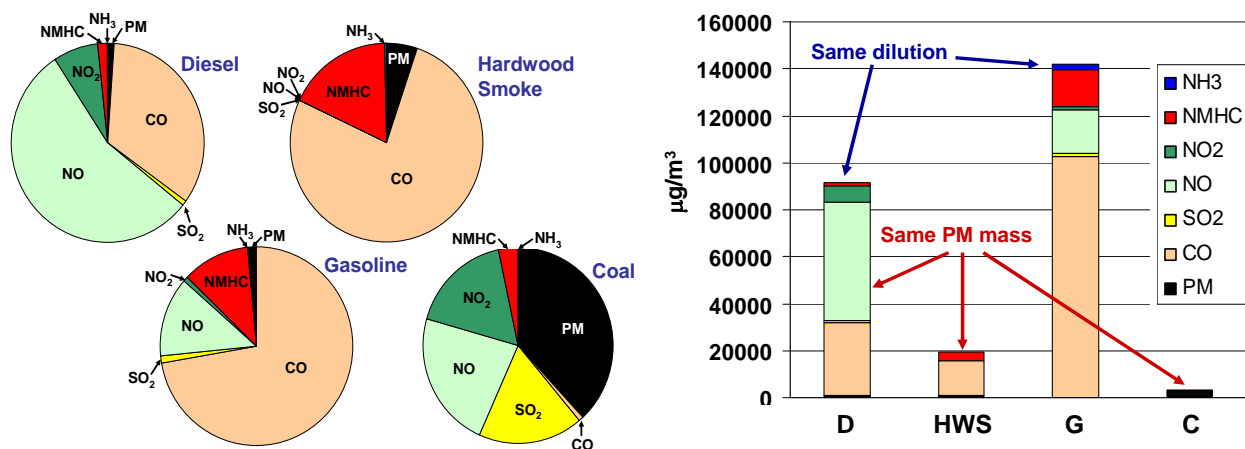


Figure 2. Mass composition of the four exposure atmospheres, less CO₂, CH₄, and H₂O. The pie charts on the left show mass by percentage, and the bars on the right show absolute mass. D = diesel, HWS = hardwood smoke, G = gasoline, and C = coal.

1. Diesel Engine Exhaust

The results of the diesel study are complete and published with the exception of the DNA assays, which are combined with those from the wood smoke study in a

draft paper yet to be submitted, and effects on the ApoE^{-/-} vascular and BALBc *in utero* immune development mouse models, which were evaluated in the repeated exposures during the past year. Those results will be published in 2010.

c. Exposures

Diesel engine exhaust was generated using two Cummins 2000 model 5.9L ISB Series turbocharged engines operated continuously on the U.S. EPA heavy-duty certification cycle (FTP, hot start), burning D-2 diesel control national certification fuel (350 ppm sulfur, 30% aromatics), and using Shell Rotella-T[®] 15W-40 crankcase oil (Figure 3). Animals were exposed 6 hours/day, 7 days/week for times dependent on the health assay up to 6 months in whole-body inhalation chambers. Whole exhaust was diluted to PM mass concentrations of 1000, 300, 100, and 30 µg/m³, or to clean air as controls. Dilution ratios ranged from 10:1 to 300:1. The initial exposures were completed in June 2001. The detailed composition of the exposure atmosphere was reported (McDonald et al., 2004b, 2006a). Exposures of the mouse vascular and immune development models were completed in 2008, using the higher three concentrations and including filtered exhaust at the highest concentration.

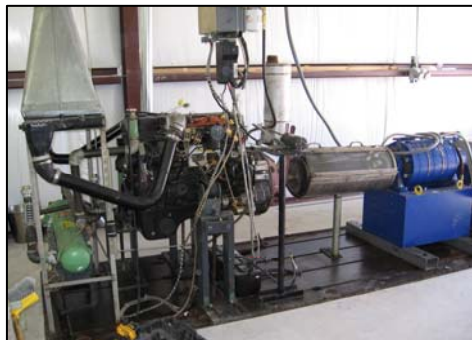


Figure 3. Diesel engine connected to dynamometer

d. Health Effects

The experimental design and many responses in F344 rats and A/J mice were reported by Reed et al. (2004). As with other NERC exposures, effects were slight to modest; there were no overt manifestations of illness, no mortality, and no significant impacts on body weight. Lung histopathology consisted only of increased numbers of alveolar macrophages, with most containing PM. There were no visible tissue structural changes. Kidney and lung weights of both rats and mice were significantly increased at the highest level after 6 months of exposure. Several serum chemistry variables were altered slightly, but significantly. Reductions of serum cholesterol in rats after both 1 week and 6 months of exposure were consistent with findings in most studies of repeated exposure to diesel exhaust in which cholesterol was measured. This effect has yet to be explained. Hematology was affected little. Among the blood clotting variables, Factor VII was reduced in rats at both 1 week and 6 months. Neither the frequency of micronuclei in circulating reticulocytes nor the lung adenoma incidence was altered in A/J mice.

Effects of exposure on indicators measured in bronchoalveolar lavage fluid of F344 rats were reported by Seagrave et al. (2005b). A modest cytotoxic effect was indicated by an exposure-related trend toward increased lactate dehydrogenase (LDH). Alkaline phosphatase (APase) was also increased. Interestingly, two indicators of pro-inflammatory activity, macrophage inflammatory protein 2 (MIP-2) and tumor necrosis factor alpha (TNF α) were decreased, rather than increased. These differences were only significant in females. Exposure did not affect lung glutathione or cell counts.

Effects of exposure on clearance of a non-lethal dose of *Pseudomonas aeruginosa* (*Pa*) instilled into lungs of C57Bl/6 mice after 1 week or 6 months of exposure were

reported by Harrod et al. (2005). Clearance was retarded somewhat at both times, but only significantly at 1 week. At that time, the effect was evident at the lowest exposure level, became significant at the 100 $\mu\text{g PM}/\text{m}^3$ level, and then did not increase further at higher levels. The modest histopathology induced by the infection was correspondingly greater in exposed mice in a dose-related manner.

Effects of a 1-week exposure on the cardiac function of spontaneously hypertensive (SHR) rats were reported by Campen et al. (2003). There were significant exposure-related trends toward increased heart rate and an increased P-Q interval of the electrocardiogram. The increased heart rate persisted during non-exposure hours during the exposure period, but did not persist after the 1-week exposure ended.

Barrett et al. reported effects of exposure on the development (2002a) and exacerbation (2002b) of respiratory allergic responses of young adult BALBc mice induced by sensitization with the neoallergen, ovalbumin (OVA). Exposure concurrent with sensitization did not significantly affect the ability of mice to develop allergic sensitization to OVA. A 3-day exposure before OVA challenge in previously sensitized mice decreased the allergic response to OVA, but exposure after OVA challenge increased the response.

Exposure affected DNA methylation and oxidative injury in both F344 rats and A/J mice, although the effects were not consistently dose-related. Genomic global methylation levels of rats were increased after both 1 week and 6 months of exposure. However, methylation levels were increased at the three lower exposure levels, but not at the highest level. Methylation levels were both increased and decreased in mice, and the small magnitude and variable nature of the responses did not suggest a clear exposure effect. Oxidative DNA damage as reflected by the levels of abasic (apurinic and apyrimidinic) sites (AP). AP sites were increased at 1 week in rats, but not significantly at 6 months. As for methylation, the effect on AP was not observed at the highest exposure level. The level of AP sites was reduced in male mice at 1 week, but not at 6 months. There was no consistent effect on AP sites in female mice.

2. Hardwood Smoke

The results of the wood smoke study are complete and published with the exception of the DNA assays, which are being published together with those from the diesel study, and effects on the ApoE^{-/-} vascular and BALBc *in utero* immune development mouse models, which were evaluated in the repeat exposures during the past year. Those results are being summarized at this time.

e. Exposures

Smoke was generated from a conventional uncertified wood stove (Pineridge Model 27000, 0.06 m³ firebox) placed in a 600 ft² single-room building adjacent to the exposure laboratory (Figure 4). The building was sized and air conditioned to simulate the proper heating scale for the stove. The fuel was a mixture of seasoned black (60%) and white (40%) oak wood harvested in Missouri, split to specifications, and stored in a temperature- and humidity-controlled environment. Moisture content was measured periodically, and maintained at 20-25%. The stove was operated on a three-phase burn cycle that spanned the 6-hour exposure period, including a kindling phase of



Figure 4. Wood stove and flue

20 minutes, a high burn phase of 90 minutes, and a low burn phase for the remainder of the daily exposure. The fire was kindled using unprinted newspaper stock and small pieces of the oak. The stove had a flue height of 4.6 m and terminated in a controlled-environment enclosure. A portion of the smoke was extracted approximately 0.3 m from the top of the flue and diluted to desired concentrations. The dilution ratios ranged from 300:1 to 3,000:1, resulting in PM mass exposure concentrations of 1000, 300, 100, and 30 $\mu\text{g}/\text{m}^3$. Animals were exposed 6 hours/day, 7 days/week to smoke or to clean air as controls. The detailed composition of the exposure atmosphere was reported (McDonald et al., 2006b).

f. Health Effects

The experimental design and many responses in F344 rats and A/J mice were reported by Reed et al. (2006). As with other NERC exposures, effects were slight to modest; there were no overt manifestations of illness, no mortality, and no significant impacts on body weight. Lung histopathology consisted only of slightly increased numbers of alveolar macrophages, with most containing brown pigment assumed to be smoke PM; there were no visible tissue structural changes. The only consistent effect on organ weight was a slight, but significant, reduction in liver weight in rats at both 1 week and 6 months. Several serum chemistry variables in rats were significantly different from control in isolated elements of the gender-time matrix. The only variables that seemed to form consistent patterns of change were a reduction in Apase in both genders at both 1 week and 6 months and a reduction in blood urea nitrogen, also in both genders at both times. Increases, rather than decreases, in those variables are typically interpreted as adverse effects. Hematological effects in both genders of rats were limited to increases in platelets at 1 week and decreases in neutrophils at 6 months. Blood clotting factors were unaffected. As for diesel exhaust, neither the frequency of micronuclei in circulating reticulocytes nor the lung adenoma incidence was altered in A/J mice.

Effects of exposure on clearance of a non-lethal dose of *Pa* instilled into lungs of C57Bl/6 mice after 1 week or 6 months of exposure were included in the report by Reed et al. (2006). Clearance was retarded somewhat at 6 months, but not at 1 week. The effect was not statistically significant by the dual criteria due to high variability.

Effects of a 1-week exposure on the cardiac function of SHR rats were also included in the report by Reed et al. (2006). There were no significant effects on any feature of heart rate or the electrocardiogram.

Effects of 6 months of exposure on indicators measured in bronchoalveolar lavage fluid of F344 rats were reported by Seagrave et al. (2005b). A modest non-monotonic cytotoxic effect was indicated by increases in LDH at the two middle exposure levels in males, but the mean value at the highest level was not significantly different from control. APase and total glutathione were reduced in both genders. As with diesel exhaust, MIP-2 was decreased in both genders. There was no consistent exposure-related trend in alveolar macrophages or other cell counts.

Barrett et al. (2005) reported effects of exposure on the exacerbation of respiratory allergic responses of young adult BALBc mice induced by sensitization with OVA. A 3-day exposure before OVA challenge in previously sensitized mice did not affect the allergic response to OVA, but exposure after OVA challenge increased the response, as reflected by increased numbers of eosinophils in bronchoalveolar lavage fluid. Although not reported in that paper, exposure during sensitization did not alter the development of allergic responses to OVA in young adult mice.

Exposure had no significant effect on DNA methylation and oxidative injury in F344 rats. Exposure significantly decreased DNA methylation levels in female A/J mice at 1 week, but not at 6 months. Exposure increased methylation in male mice at 6 months, but not at 1 week. Both responses were only significant at the middle exposure levels, and not at the highest level. Exposure did not affect AP site levels in mice at either time in a consistent manner.

3. Gasoline Engine Exhaust

The results of the gasoline exhaust study are complete and published in their entirety (Lund et al., 2007, 2008; Day et al., 2008, McDonald et al., 2008, Reed et al., 2008). Because exposure to a filtered atmosphere at the highest concentration and inclusion of the ApoE^{-/-} and *in utero* exposure BALBc mouse models were initiated with this study, there was no need to repeat the exposures to complete the data set as done for diesel and wood smoke.

g. Exposures

Emissions were generated using two 1996 model 4.3L V-6 engines removed from in-use, mid-mileage (50,000-70,000 miles) Chevrolet S-10 compact trucks purchased locally after ascertaining that the engines were in good condition and the vehicles passed local emissions standards (McDonald et al., 2008). The normalcy of emissions was verified in detail by operating the vehicles on a standard duty cycle on a chassis dynamometer at Southwest Research Institute in San Antonio, TX. The engines were removed from the vehicles, configured for test stand operation, and mounted on test stands at LRRRI with new mufflers and their original catalytic converters (Figure 5). The engines were operated on the California Unified Driving Cycle, which simulates urban driving conditions. The fuel was special-blended to the average specifications of U.S. regular unleaded, non-oxygenated, non-reformed gasoline in the summer and winter of 2001-2002. Pennzoil 10W-30 crankcase oil was used. Two engines were used for 3 hours each in order to include two cold starts in each 6-hour exposure day. Animals were exposed 6 hours/day, 7 days/week for up to 6 months to whole emissions diluted at ratios of 10:1, 15:1, and 90:1, or to clean air as controls. These dilutions resulted in PM mass concentrations of 59, 30, and 7 $\mu\text{g}/\text{m}^3$. The detailed composition of the exposure atmosphere was reported (McDonald et al., 2008).



Figure 5. Gasoline engine connected to dynamometer

h. Health Effects

The experimental design and many responses in F344 rats and A/J mice were reported by Reed et al. (2008). As noted in preceding sections, the protocol was modified for this study by eliminating the A/J mouse tumorigenesis assay, and by adding the ApoE^{-/-} mouse assay of vascular damage and the BALBc mouse assay of the development of respiratory allergy beginning with exposure *in utero*. As noted above, the exposure included only three levels of exposure to diluted whole exhaust, but included a filtered (minus exhaust PM) group at the highest exposure level.

As with other NERC exposures, effects were modest; there were no overt manifestations of illness, no mortality, and no significant impacts on body weight. Lung

histopathology in F344 rats and A/J mice consisted only of slightly increased numbers of alveolar macrophages, some of which contained pigment suggesting PM. No tissue structural changes were visible by light microscopy, and even the macrophage response was too slight to merit scoring. There were no significant effects on organ weights of rats or mice at 1 week. The most consistent effect on organ weight at 6 months was an increase in heart weight in both genders of rats, but the effect met the significance criteria only in males. Removal of PM only slightly diminished the effect. Heart weight was not significantly increased in mice. Lung weights and excised lung volumes were not affected.

Exposure for 1 week did not affect serum chemistry variables in F344 rats. At 6 months, three chemistry variables were significantly altered in females, but not males. Because all three variables were reduced rather than increased, the differences were difficult to interpret as adverse effects.

The most striking effect on hematology was an increase in the red blood cell count in both genders at both 1 week and 6 months, which also resulted in increased hemoglobin and hematocrit. Removal of PM partially diminished the effect, except in males at 6 months. These observations were consistent with findings in earlier studies of gasoline emissions, and were ascribed to the exposure to CO. The only alteration of blood clotting factors was an increase in fibrinogen in males at 1 week, but not in females nor in either gender at 6 months.

Bronchoalveolar lavage of F344 rats revealed multiple significant exposure-related effects. Cytotoxicity and pro-inflammatory responses were indicated by increases in LDH and MIP-2, respectively, at 6 months, but not at 1 week. The production of reactive oxygen species by isolated macrophages was reduced at both 1 week and 6 months. These effects were stronger in females than in males.

Exposure affected DNA methylation somewhat in both F344 rats and A/J mice, but the effects were not consistent among species, genders, and times. Methylation levels were decreased in female mice at 1 week, and removal of PM did not influence the effect. Methylation levels were increased in male rats at 6 months, and removal of PM completely eliminated the effect.

There were no significant effects of 1 week of exposure on clearance of *Pa* from lungs of C57Bl/6 mice or of 6 months of exposure on micronuclei in circulating reticulocytes of A/J mice.

Exposure for 1 week did not significantly affect heart rate or electrocardiogram variables in SHR rats. The concentration of lipid peroxides was significantly increased in males, but not females. The concentration of thrombin-antithrombin complex was significantly decreased in females, but not males.

There was some, but little, evidence that exposure for 7 weeks beginning *in utero* affected the development of respiratory allergic responses of young adult BALBc mice. The most convincing effect was an exposure-related increase in serum total IgE, for which the trend was highly significant and the concentration of IgE at the highest exposure level was twice that of the control group. Removal of PM did not influence the response. No other antibody or respiratory function variables reflected significant changes. The effects of exposure on the exacerbation of responses in pre-sensitized mice were variable and modest (Day et al., 2008). Exposure before OVA challenge increased OVA-specific IgG₁, had no other significant effects. Exposure after OVA challenge did not alter the allergic response.

One of the most consistent patterns of biological response to exposure was the alteration of several indicators of vascular damage and pro-atherosclerotic responses in ApoE^{-/-} mice exposed for 7 weeks, as reported by Lund et al. (2007, 2008). These responses were measured in the proximal aorta as either tissue concentrations of indicator compounds or as gene activation as indicated by amplification of mRNA expression. Tissue concentrations of thiobarbituric acid reactive substances (TBARS) and hemoxygenase 1 (HO-1) reflected oxidative stress. Increased mRNA levels for endothelin 1 (ET-1) and several matrix metalloproteinases (MMP-3, -7, and -9) and tissue inhibitors of metalloproteinase 2 reflected transcriptional up-regulation of vascular remodeling processes. Most of these responses were changed little or not at all by removal of PM from the exposure atmosphere.

4. Simulated Downwind Components of Coal Combustion Emissions

The “coal” exposures ended in late 2007. The exposure system and some preliminary composition and response results were presented in the 2007 and 2008 Annual Reports. A paper reporting the generation and composition of the exposure atmosphere has been submitted (McDonald et al., 2009). The health results are being summarized for publication.

i. Exposures

The exposure atmosphere not only included diluted fresh emissions from combustion of coal, but also included additional components that were added to simulate a more distant exposure scenario. The composition of the major components of the atmosphere followed the consensus recommendations of an expert workshop convened for that purpose. The mixture was intended to simulate the major components contributed by coal combustion to the pollution that populations would breathe at tens to a hundred miles downwind from a conventional coal-fired power plant.

The system for generating the exposure atmosphere consisted of multiple sub-systems that contributed the different components necessary to achieve the target mixture (Figures 6 and 7). Coal obtained from the Black Thunder Mine in Campbell County, Wyoming, was pulverized and fed into a pre-heated air stream entering the top of an electric “drop-tube” furnace. The coal was combusted while passing through a quartz tube in the center of the furnace, maintained at 1400°C. The furnace effluent was cooled using a heat exchanger and introduced into the primary ash mixing chamber under controlled dilution. The furnace-derived aerosol was merged with a sulfate aerosol prior to entry into a chamber where all components are mixed. Sulfate was generated by nebulizing a 0.5% sulfuric acid solution, followed by vaporization, condensation, and dilution to achieve the proper concentration and particle size. Gases including SO₂, NO, and NO₂ were metered into the mixing chamber from cylinders of certified compressed gas. The exposure mixture passed through the wall between the generation and exposure rooms and into a manifold that had lines connected to each

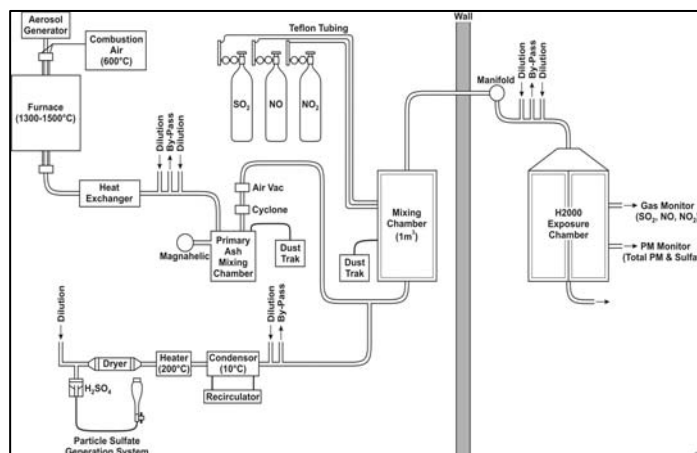


Figure 6. Schematic diagram of the components of the system used to generate the exposure mixture and expose animals.

exposure chamber. The lines to each chamber were equipped for dilution to achieve the desired concentrations. Lines to two chambers carried the lowest dilution (highest concentration), and a HEPA filter in one of those lines removed PM. Animals were exposed 6 hours/day, 7 days/week at dilutions producing PM mass concentrations of 1000, 300, or 100 $\mu\text{g}/\text{m}^3$, to the highest concentration with PM removed, or to clean air as controls.

j. Health Effects

Overall, the coal emissions exposure was perhaps the most innocuous of all NERC exposure atmospheres to date, although that was not true for all response variables. Few significant health responses were observed, and those were generally mild. Because PM was a major component and the PM was predominately sulfate, this suggests that sulfate PM has low toxicity in most of the health assays used in the NERC program. To the extent that some outcomes respond more to non-PM components, the lower concentration of those components in the coal study than in the other studies could have contributed to the lower toxicity. If health effects respond to the total pollution mass concentration, the lower total mass concentration in the coal study might also have resulted in the lower toxicity even though PM concentrations were equivalent. The majority of the few significant responses that were observed appeared to be driven primarily by non-PM components.

There was no evidence of illness, mortality, or impacts on body weight. Lung histopathology consisted only of a slight increase of alveolar macrophages, some of which contained pigment that was probably PM. The only effect on organ weights in rats and mice consistent in both genders was an increased kidney weight in rats at 6 mo. There was no pathology in the kidneys that was apparent by light microscopy.

There were no significant effects of exposure on serum chemistry, hematology, or blood clotting factors of rats at either 1 wk or 6 mo. There were also no significant effects on clearance of bacteria in mice, vascular responses in ApoE^{-/-} mice, or the frequency of micronuclei in circulating reticulocytes of mice.

The exposure caused some significant effects in bronchoalveolar lavage fluid of rats, but some were not in directions typically interpreted as adverse. At 1 week, β -glucuronidase and MIP-2 were increased in both genders. TNF- α was decreased in both genders at 1 wk.. MIP-2 was decreased in females, but not males, at 6 mo. We have no cogent rationale for the decreases in the two pro-inflammatory chemical mediators. The only effect consistent in both genders at both measurement times was a decrease in the production of peroxide radicals by isolated, unstimulated alveolar macrophages. That would suggest a potential reduction of macrophage defenses (although the normal clearance of bacteria in mice was not consistent with impaired macrophage function).

Exposure altered some responses of allergic mice to inhaled allergen. Mice were sensitized to ovalbumin (OVA) by repeated intraperitoneal injection, and then challenged with inhaled OVA to elicit an allergic respiratory response. One-half of the mice

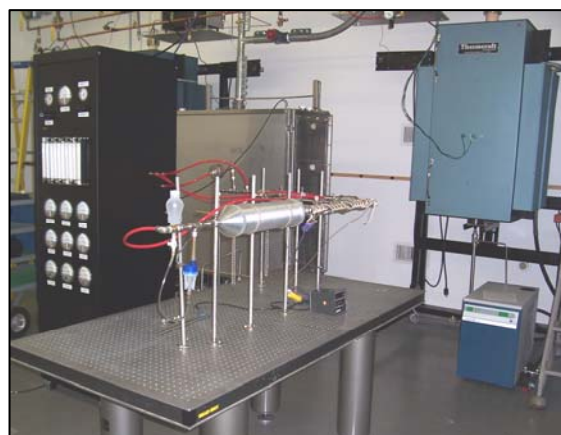


Figure 7. Photograph of the generation system showing the electric furnace on the right, the sulfate generation system in the center, the gas control panel on the left, and the mixing chamber in the background.

were then exposed for three days to the coal atmosphere to see if exposure after receiving the allergen altered inflammatory and antibody responses. The other half of the mice were exposed for three days, and then challenged with OVA to see if exposure before receiving the final allergen challenge altered the responses. Exposure both before and after OVA challenge increased the allergic inflammation as reflected by increased numbers of eosinophils (Figure 8) and macrophages in lung fluid sampled by bronchoalveolar lavage (BAL). Particles seemed to be the principal cause of the effect of exposure before challenge, but did not appear to be responsible for the effect of exposure after challenge. OVA-specific IgE antibody also reflected an exposure order difference (Figure 9). Exposure before OVA challenge caused an increase in IgE at the highest exposure level, and particles appeared to be responsible for a large portion of the effect. Exposure after OVA challenge reduced IgE somewhat (non-significant), and removing particles did not reduce the effect. These results suggest that the importance of particles in modifying allergic responses depends on whether pollutant exposure occurs before or after encountering the allergen.

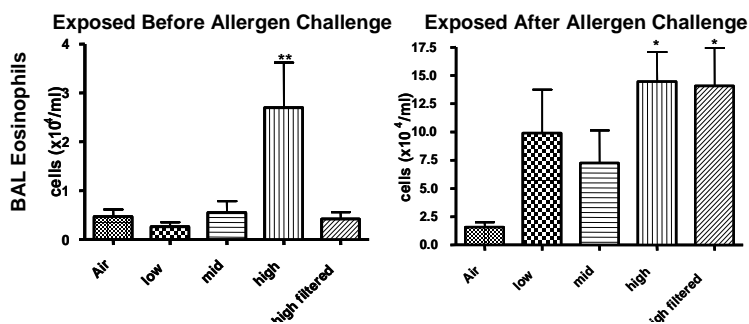


Figure 8. Effect of exposure on eosinophil counts in BAL fluid of allergic mice.

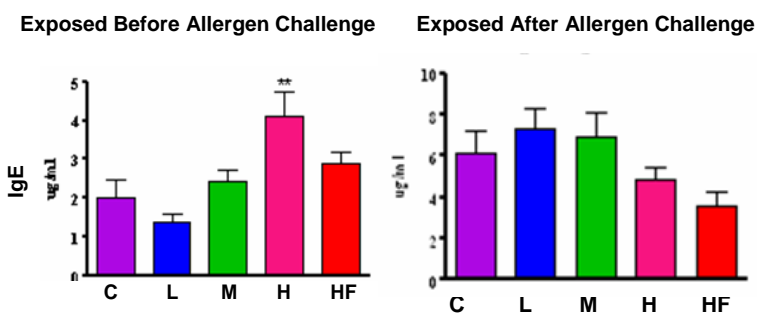


Figure 9. Effect of exposure on OVA-specific IgE antibody.

B. Progress during This Reporting Period

1. Evaluation of Feasibility of Conducting a Study of Marine HFO Exhaust

Considerable effort occurred during 2008-2009 to collect information on emissions from combustion of heavy fuel oil (“HFO”, aka residual oil) in marine main and auxiliary engines, and to determine how such emissions could be created or simulated in the laboratory. A workshop was convened on December 8-9, 2008 to discuss the target composition of such an exposure atmosphere and alternative methods for generating the mixture. The participants are listed in the text box and the workshop report is attached as Appendix A.

A key outcome of the workshop was agreement on a strategy for developing more extensive data on the composition of existing samples, in order to establish a target composition for the particulate material to be used in the study. Dr. Cocker

Workshop Participants
Kjeld Aabo, MAN B&W Diesel, Copenhagen, Denmark
Judy Chow, Desert Research Institute, Reno, NV
David Cocker, University of California, Riverside
Bill Linak, EPA, Research Triangle Park, NC
Joe Mauderly, LRRI, Albuquerque, NM
Jake McDonald, LRRI, Albuquerque, NM
Gary Minsavage, CONCAWE, Brussels, Belgium
Will Ollison, American Petroleum Institute, Washington, DC
Pete Roberts, CONCAWE, Brussels, Belgium
Bob Weeks, Chevron Shipping, San Ramon, CA

agreed to provide an inventory of ship emission samples collected by the UCR group, and to determine the potential availability of portions of the samples for analyses that had not already been performed. He also agreed to explore the availability of information on the fuels and cylinder oils that were used by the engines producing the samples. It was planned to measure thermograms of some of the samples to see if a common pattern emerged that could serve as an index of the suitability of particulate that was generated in the laboratory by LRRI. Strategies for further organic speciation of the samples and analysis of the forms of metal components were to be explored. In short, the UCR group was unresponsive thereafter and none of these actions was accomplished.

Kjeld Aabo of the MAN B&W Marine Engine Division followed up by providing additional information on ship engine operating conditions and cylinder oil use. Dr. Mauderly consulted with MAN B&W technical experts at their headquarters and research facilities in Copenhagen, Denmark in January 2009. Additional technical insights and data were provided, but the result of these discussions was serious doubt that a suitable emissions mixture, and especially a representative particulate fraction, could be produced at atmospheric pressure in the laboratory.

Laboratory trials were conducted at LRRI using an electric furnace to combust HFO. The coal study demonstrated that the gaseous and sulfate components of a complex combustion mixture could be simulated using auxiliary methods, but production of a representative spectrum of particulate matter depended on combustion of HFO. Two trials using different fuels and conditions produced particulate that was not sufficiently representative of ship emissions. In view of opinion from engine technical experts that the electric furnace strategy would not work, and the failure to develop better information on target composition, no further laboratory trials were conducted.

We reached an apparent “dead end” in trying to develop an HFO-based exposure atmosphere that reasonably simulates actual ship engine exhaust. Production engines of the required type are too large for use in the laboratory at a practical cost. Experimental single-cylinder engines exist, but are neither available for loan nor practical to construct within cost constraints. Laboratory-scale combustion at ambient pressure did not produce a spectrum of particles adequately simulating particles in ship exhaust, and we failed to develop adequate information on the target composition of the particulate.

In summary, we concluded that it would be impractical to attempt a study of a mixture simulating exhaust from a ship main engine burning HFO.

2. Completion of Data from Coal Study

Effort continued during the past year to complete and publish data from the study of simulated downwind coal emissions. Most of the data have been developed and undergone QA audit and are in final form for analysis. Certain data sets are not yet complete. The electrocardiogram data from hypertensive rats are still being produced. Because of a software failure, the raw data must be processed by a much slower method that occupies a single computer for extended lengths of time. That analysis is still underway. The DNA data are not yet complete. Dr. Swenberg extracted the samples and is performing analysis of oxidative damage. Those data should be complete by the end of December 2009. Methylation of DNA will need to be analyzed by a method different from that used for previous samples. Although the results should be comparable, the analyses (using DNA extracted by Dr. Swenberg) have not begun. Progress on developing data for the respiratory allergic responses has been much slower

than desired, due to competing demands on staff and equipment. Those data are just now being completed. We should have all of the coal data except electrocardiogram and DNA methylation in hand in January 2010. Statistical analysis will be rapid, and publications should be produced in the spring of 2010.

3. Integrated Analysis of Data from Four Studies

The initial exploration of strategies for reducing the dimensionality of the NERC database was described in the 2008 Annual Report. No significant effort to analyze the database has occurred since. We decided to wait until the coal data were complete before expending substantial effort on the task. In view of reduced funding and uncertainty about continuation of the program, we elected not to spend funds on preliminary efforts that did not involve the four-study data set.

4. Other Accomplishments

k. Publications and Presentations

The current listing of NERC publications is presented in Appendix B. Three papers were submitted that deal directly with NERC results or results from studies using NERC resources. McDonald et al. (2009) submitted a paper reporting the generation strategy and composition of exposure atmospheres for the coal study. Campen et al. (2009a,b) submitted two papers dealing with the components of complex exposures causing vascular effects in the mouse model of atherosclerosis.

Three papers were submitted that dealt with multipollutant issues and were supported in part by NERC funds. A Mauderly and Samet (2009) commentary published in *Environmental Health Perspectives* reviewed evidence for the existence of synergistic interactions among ambient air pollutants. They found that a review of papers reporting studies of the interactions between ozone and other pollutants was sufficient to illustrate that synergies do occur, although many combinations are either additive or antagonistic.

Dr. Mauderly (Mauderly et al., 2009a), along with NERC ESAC member Dr. Wyzga, expended considerable effort during the year to coordinate a review by several Canadian, Mexican, and U.S. authors of the issues faced in understanding the health effects of air pollution in a multipollutant context, and the nature of research necessary to improve our understanding. This effort developed a large chapter in an in-press text by NARSTO on multipollutant air quality management. Drs. Mauderly and Wyzga developed a somewhat shorter version for publication in the peer-reviewed literature, and that paper is currently in co-author review before submission.

During the past year, Dr. Mauderly (Mauderly et al., 2009b), with partial salary support from NERC, coordinated the completion and submission of a paper exploring the synergism between inhaled cigarette smoke and plutonium in producing lung tumors in rats. The data resulted from an earlier DOE-funded study at LRRRI in which rats were exposed once to plutonium-239 dioxide to simulate an accidental exposure, and exposed chronically to mainstream cigarette smoke. Both exposures alone increased the incidence of lung tumors, but the interesting issue was dissecting the combined effects. Extensive statistical analysis by NERC investigator Steve Seilkop demonstrated a clear synergy (greater than additive effect) that was due in part to increased radiation dose from a smoke-induced slowing of plutonium clearance, and in part to an unexplained component. Although the study was not conducted by NERC, the

analysis of the interactions between the two exposures was consistent with NERC's multipollutant emphasis and demonstrated a strategy for disentangling the contributions of multiple factors to the interaction.

Several presentations of NERC results were made at national and international meetings during the past year. As noted in Appendix B, one published abstract resulted from a presentation at the annual American Thoracic Society meeting, and two resulted from presentations at the annual Society of Toxicology meeting. Mauderly gave seven additional formal presentations in Belgium, Denmark, Germany, and the U.S. during the year, and other NERC investigators also presented NERC results at meetings.

1. Laying Groundwork for Continued Multipollutant Research

The NERC program has continued to serve well as a catalyst for non-NERC multipollutant research at LRRRI (and elsewhere). "Spin-off" projects at LRRRI during the past year have included an EPRI-funded study of the toxicity of secondary organic aerosols, and an HEI-funded study of the components of engine emissions and other aerosols causing vascular effects in the ApoE^{-/-} mouse model of atherosclerosis. The latter is conducted jointly with Dr. Vedal and colleagues at the University of Washington, who are undertaking a companion epidemiological component. Other recent and ongoing LRRRI studies of air pollutants also have roots in NERC findings and research resources. LRRRI and the University of Washington recently submitted a joint application for an EPA-funded air pollution research center in response to the EPA solicitation for sequels to its particulate matter centers program. If funded, this center would likely be the most substantial sequel to the NERC program.

C. Response to Guidance from the External Scientific Advisory Committee

Written guidance from the ESAC following the June 2009 annual meeting by teleconference was communicated in a July 6, 2008 letter from the Chairman, Dr. Lippmann (Appendix C). NERC management's response to the key points of guidance is as follows:

1. Future Studies

The ESAC recommended conducting additional studies using the full NERC protocol, if sufficient funding can be identified. It recommended continuing to explore strategies for a study of a mixture simulating emissions from a ship main engine burning HFO, but acknowledged that technology was readily at hand for conducting a study of paved road dust.

As described above, we did not identify a suitable means of generating a mixture adequately simulating ship engine emissions. Our explorations with EPA and DOT of the possibility of funding a study of paved road dust have not been successful. At this time, it appears that the NERC program in its present form will conclude with integrated analysis and reporting of results from four studies.

2. Strategic Planning/Marketing Document

To enhance decision-making concerning the future of the NERC program and to inform potential sponsors, the ESAC recommended that a document be developed that: 1) summarized findings from the first four studies; 2) discussed the significance of findings to

date in furthering the state-of-science in the field; and 3) described the benefits to be gained by studying additional source emissions.

NERC management agrees with the thrust of the suggestion, but the recommended document has not yet been developed at this time. As described above, we are just now completing results from the last of the four studies, so we have not yet been able to develop a synthesis of all results to date. Moreover, the combination of reduced non-federal funding during the past year, lack of any encouragement of likely federal funding, the embarrassingly slow pace of NERC investigators' completion of data from the coal study (with other priorities superseding NERC), and the planned retirement of Dr. Mauderly in late 2010 conspire to offer low probability of continuing the NERC program beyond analysis of data from the first four studies.

3. Statistical Analysis Workshop

To facilitate analysis of the NERC database, the ESAC recommended the convening of a workshop to discuss analytical approaches. Guidance was offered for developing the list of participants, agenda, and report.

NERC management agrees with the guidance and, as has been our intent, plans to convene such a workshop in early 2010.

D. Sponsorship and Funding

1. Sponsorship

Funds received for the NERC Program since the last report (11/1/2008 – 12/9/2009) are listed in Table 6. Because funds are provided by sponsors at different times during the year, the amount of funding listed in the table is not identical to the funding that was available for work during the reporting period. Some funds provided late in the previous year were expended during this reporting period, and some funds provided late during this period are being spent during the following period. In addition, some sponsors provide funding to encompass a multiyear period, but the full amount is listed in the year received. During the past year, this applied to Southern Company, whose contributions during 2009 also encompassed portions of 2008 and 2010, and EUGT (Europäische Forschungsvereinigung für Umwelt und Gesundheit im Transportsektor e.V.) , whose 2009 contribution was intended to support NERC during 2009-2011.

Funding received during this reporting period was increased 22% above the amount received in the preceding year. The receipts of \$815,360 were provided by five individual corporations and four industry associations. No federal or state funding was received during the year.

Table 6. Funds Received During 11/1/2008 – 12/9/2009

Government	None
Non-Government	
ChevronTexaco	60,000
CONCAWE (European petroleum industry association)	150,000
EUGT (European association for research on impacts of transportation on environment and health)	105,360
John Deere	15,000
Electric Power Research Institute	50,000
ExxonMobil	85,000
Navistar (International Truck & Engine Company)	30,000
Japan Automobile Manufacturer's Association	20,000
Southern Company	300,000
Total Receipts	815,360

As noted last year, the full LRRI indirect cost rate will apply to NERC funds spent during FY 2010 (beginning October 1, 2009). This substantially affects the spending power of non-federal contributions to NERC. In past years, LRRI President and CEO Dr. Bob Rubin waived the majority of indirect (overhead) charges on non-government funds received for NERC as charitable contributions to the not-for-profit [501(c)(3)] LRRI. This generous cost-sharing by LRRI substantially leveraged the program's ability to conduct research and because of this commitment, LRRI itself has been by far the largest single non-government sponsor of the NERC Program. In part in view of the lack of federal funding (which partially supported indirect costs) and in part in view of the challenging financial climate, the waiver of indirect costs has ended. This circumstance, albeit fair and understandable, reduces the amount of research activity that can be accomplished per dollar contributed. Of course, sponsors can still receive the tax advantage of donations to the program.

2. Changes in Sponsorship

We are very pleased that EUGT joined the sponsors of NERC during the past year. Four sponsors in the previous year did not provide support for the NERC program during the past year. These included the American Petroleum Institute, Chrysler Foundation, Cummins Engine Company, Ford Motor Company, and FAT (German Auto Manufacturers' Research Association). We regret the loss of sponsorship, but it is not surprising that organizations facing financial extremis would reduce voluntary contributions to external programs.

IV. ANTICIPATED ACTIVITIES DURING THE NEXT REPORTING PERIOD

A. Completion and Publication of Current Data Sets

1. Coal Emissions Study

The final results from the coal emissions study will be published. The status of the data is summarized above. All publications pertaining solely to this study will be submitted during the coming year.

2. Repeat Studies of Diesel Exhaust and Hardwood Smoke

All results from the ApoE^{-/-} mouse vascular model and BALBc mouse model of the development of allergic responses will be submitted for publication during the next reporting period. A portion of the results from the vascular model is included in papers already submitted.

3. Composition of Paved Road Dust

A paper reporting the pilot study of road dust has been drafted and only requires final touches for submission.

4. Integrated Analysis of Data from Four Studies

At least one paper will be submitted as a result of integrated analysis of the complete NERC database. The effort will result in more than one publication, and some results from analyses during 2010 may be submitted in the following year.

B. Workshop on Statistical Strategies

A workshop will be convened during 2010 to discuss strategies for the integrated analysis of the NERC database. Specific approaches and products will be identified, and the workshop participants will comprise a working group to follow and advise the effort as it progresses.

V. REFERENCES

- Barrett, E G., M.D. Reed, T. Espindola and R. Henson (2002a) Diesel Exhaust Exposure in Conjunction with Periodic Allergen Challenge Does Not Lead to Allergic Sensitization. *Am. J. Respir. Crit. Care Med.* 165: A303.
- Barrett, E.G., M.D. Reed, T. Espindola and R. Henson (2002b) Allergic Airway Responses Following Diesel Exhaust Exposure: Exacerbation vs. Attenuation. *Am. J. Respir. Crit. Care Med.* 165: A414.
- Barrett, E.G., R.D. Henson, B. Welsh, S.K. Seilkop, J.D. McDonald, and M.D. Reed (2005) Hardwood Smoke Exposure Exacerbates Allergic Airway Inflammation. *Inhal. Toxicol* 18: 33-43.

- Barrett, E.G., M.D. Reed, J.D. McDonald, S. Shinnik, and M. Anaya: Effects of Gasoline Engine Emissions on Pre-Existing Allergic Airway Responses (2007) *Am. J. Respir. Crit. Care. Med.* 175 (abstract issue):A543.
- Campen, M.J., J.D. McDonald, A.P. Gigliotti, S.K. Seilkop, M.D. Reed, and J.M. Benson (2003) Cardiovascular Effects of Inhaled Diesel Exhaust in Spontaneously Hypertensive Rats. *Cardiovasc. Toxicol.* 3(4): 353-361.
- Campen MJ, Lund AK, Knuckles TL, Conklin DJ, Bishop B, Young D, Seilkop SK, Seagrave JC, Reed MD, and Jacob D. McDonald JD (2009a) Inhaled Diesel Emissions Alter Atherosclerotic Plaque Composition in ApoE-/- Mice. *Toxicol Appl. Pharmacol.* (in press).
- Campen, M.J., A.K. Lund, M.L. Doyle-Eisele, J.D. McDonald, T.L. Knuckles, A.C. Rohr, E.M. Knipping, and J.L. Mauderly (2009b) A Comparison of Vascular Effects From Complex and Individual Air Pollutants Indicates a Role for Monoxide Gases And Volatile Hydrocarbons. *Environ. Health Perspect.* (submitted).
- Day, K. C., M.D. Reed, J.D. McDonald, S.K. Seilkop, and E.G. Barrett: Effects of Gasoline Emissions on Pre-Existing Allergic Airway Responses in Mice (2008) *Inhal. Toxicol.* 20:1145-1155.
- Harrod, K.S., R.J. Jaramillo, J.A. Berger, A.P. Gigliotti, S.K. Seilkop, and M.D. Reed (2005) Inhaled Diesel Engine Emissions Reduce Bacterial Clearance and Exacerbate Lung Disease to *Pseudomonas aeruginosa* Infection. *Toxicol. Sci.* 83: 155-165.
- Kiers, H.A.L., J.M.F. ten Berge, and R. Bro (1999) PARAFAC2 - Part I. A direct fitting algorithm for the PARAFAC2 model. *J. Chemom.* 13: 275-294.
- Lund, A.K., T.L. Knuckles, C.O. Akata, R. Shohet, J.D. McDonald, A. Gigliotti, J.C. Seagrave, and M.J. Campen (2007) Gasoline Exhaust Emissions Induce Vascular Remodeling Pathways Involved in Atherosclerosis. *Toxicol. Sci.* 95: 485-494.
- Lund, A.K., J. Lucero, S. Lucas, M.C. Madden, J.D. McDonald, J. Seagrave, T.L. Knuckles, and M.J. Campen (2008) Vehicular Emissions Induce Vascular MMP-9 Expression and Activity via Endothelin-1 Mediated Pathways. *Arterio. Thrombosis Vasc. Biol.* 29:00 (on-line, DOI:10.1161/ATVBAHA.108.176107).
- Mauderly, J.L., R. Burnett, M. Castillejos, H. Özkaynak, J. Samet, D. Stieb, S. Vedal, and R. Wyzga (2009a) Health Assessment Aspects of Multi-Pollutant, Results-Based Air Quality Management. Chapter in NARSTO review of multi-pollutant air quality management, (in press).
- Mauderly, J.L., S. K. Seilkop, E. B. Barr, A. P. Gigliotti, F. F. Hahn, C. H. Hobbs, and G. L. Finch (2009b) Carcinogenic Interactions Between a Single Inhalation of ²³⁹PuO₂ and Chronic Exposure to Cigarette Smoke. *Radiat. Res.* (in press).
- Mauderly, J. L., and J. M. Samet (2009) Is There Evidence for Synergy Among Air Pollutants in Causing Health Effects? *Environ. Health Perspect.* 117(1): 1-6.
- Malinowski, E.R. (2002) *Factor Analysis in Chemistry*, Third Edition. John Wiley & Sons, New York, 414 pp.

- McDonald, J.D., I. Eide, J.C. Seagrave, B. Zielinska, K. Whitney, D.R. Lawson, and J.L. Mauderly (2004a) Relationship Between Composition and Toxicity of Engine Emission Samples. *Environ. Health Perspect.* 112: 1527-1538.
- McDonald, J.D., E.B. Barr, R.K. White, J.C. Chow, J.J. Schauer, B. Zielinska, and E. Grosjean (2004b) Generation and Characterization of Four Dilutions of Diesel Engine Exhaust for a Subchronic Inhalation Study. *Environ. Sci. Technol.* 38(9): 2513-2522.
- McDonald, J. D. and J. Costanzo (2006a) Particle Size and Organic Phase Distribution of Four Dilutions of Diesel Engine Emissions. *Atmospheric Environ.* (in press).
- McDonald, J.D., R.K. White, E.B. Barr, B. Zielinska, J.C. Chow, and E. Grosjean (2006b) Generation and Characterization of Hardwood Smoke Inhalation Exposure Atmospheres. *Aerosol. Sci. Technol.* 40: 573-584.
- McDonald, J.D., E.B. Barr, R. K. White, D. Kracko, J.C. Chow, B. Zielinska, and E. Grosjean (2008) Generation and Characterization of Gasoline Engine Exhaust Inhalation Exposure Atmospheres. *Inhal. Toxicol.* 20:1157-1168.
- McDonald, J.D., R.K. White, T. Holmes, J.L. Mauderly, J. Laumb, B. Zielinska, J.C. Chow, and E. Grosjean (2009) Simulated Downwind Coal Emissions for Laboratory Inhalation Exposure Atmospheres. *J. Air Waste Man. Assoc.* (submitted).
- National Research Council (NRC) National Academy of Sciences (2004a) *Research Priorities for Airborne Particulate Matter IV. Continuing Research Progress*, National Academy Press, Washington, DC.
- National Research Council (NRC), National Academy of Sciences (2004b) *Air Quality Management in the United States*. National Academy Press, Washington, DC.
- Reed, M.D., A.P. Gigliotti, J.D. McDonald, J.C. Seagrave, S.K. Seilkop, and J.L. Mauderly (2004) Health Effects of Subchronic Exposure to Environmental Levels of Diesel Exhaust. *Inhal. Toxicol.* 16: 177-193.
- Reed, M.D., M.J. Campen, A.P. Gigliotti, K.S. Harrod, J.D. McDonald, J.C. Seagrave, S.K. Seilkop, and J.L. Mauderly (2006) Health Effects of Subchronic Exposure to Environmental Levels of Hardwood Smoke. *Inhal. Toxicol.* 18: 523-539.
- Reed, M.D., E.G. Barrett, M.J. Campen, K.K. Divine, A.P. Gigliotti, J.D. McDonald, J.C. Seagrave, S.K. Seilkop, J.A. Swenberg, and J.L. Mauderly (2008) Health Effects of Subchronic Inhalation Exposure to Gasoline Engine Emissions. *Inhal. Toxicol.* 20: 1125-1143.
- Seagrave, J.C., A. Gigliotti, J.D. McDonald, S.K. Seilkop, K.A. Whitney, B. Zielinska, and J.L. Mauderly (2005a) Composition, Toxicity, and Mutagenicity of Particulate and Semivolatile Emissions from Heavy-Duty Compressed Natural Gas-Powered Vehicles. *Toxicol. Sci.* 87: 232-241.
- Seagrave, J.C., J.D. McDonald, M.D. Reed, S.K. Seilkop, and J.L. Mauderly (2005b) Responses to Subchronic Inhalation of Low Concentrations of Diesel Exhaust and Hardwood Smoke Measured in Rat Bronchoalveolar Lavage Fluid. *Inhal. Toxicol.* 17: 657-670.

Seagrave, J.C., J.D. McDonald, E. Bedrick, E.S. Edgerton, A.P. Gigliotti, J.J. Jansen, L. Ke, L.P. Naeher, S.K. Seilkop, M. Zheng, and J.L. Mauderly (2006) Lung Toxicity of Ambient Particulate Matter from Southeastern U.S. Cities with Different Contributing Sources: Relationships Between Composition and Effects. *Environ. Health Perspect.* 114: 1387-1394.

Tucker, L.R. (1966) Some Mathematical Notes on Three-Mode Factor Analysis. *Psychometrika* 31: 279-311.

VI. APPENDICES

- A. Report of December 2008 Workshop on Marine HFO Emissions
- B. Publications from the National Environmental Respiratory Center
- C. Letter From ESAC Providing Guidance Following the 2009 Annual Meeting

Appendix A

Publications from the National Environmental Respiratory Center

PUBLICATIONS
from the
NATIONAL ENVIRONMENTAL RESPIRATORY CENTER

Lovelace Respiratory Research Institute
2425 Ridgecrest Dr. SE
Albuquerque, NM 87108

Papers and Abstracts of Work Supported Wholly or in Significant Part by NERC

(As of December 9, 2009)

Papers (by date of publication from most recent):

Campen, M.J., A.K. Lund, M.L. Doyle-Eisele, J.D. McDonald, T.L. Knuckles, A.C. Rohr, E.M. Knipping, and J.L. Mauderly: A Comparison of Vascular Effects From Complex and Individual Air Pollutants Indicates a Role for Monoxide Gases And Volatile Hydrocarbons. *Environ. Health Perspect.* (submitted).

McDonald, J.D., R.K. White, T. Holmes, J.L. Mauderly, J. Laumb, B. Zielinska, J.C. Chow, and E. Grosjean: Simulated Downwind Coal Emissions for Laboratory Inhalation Exposure Atmospheres. *J. Air Waste Man. Assoc.* (submitted).

Campen MJ, Lund AK, Knuckles TL, Conklin DJ, Bishop B, Young D, Seilkop SK, Seagrave JC, Reed MD, and Jacob D. McDonald JD. Inhaled Diesel Emissions Alter Atherosclerotic Plaque Composition in ApoE^{-/-} Mice. *Toxicol Appl. Pharmacol.* in press.

Mauderly, J.L., R. Burnett, M. Castillejos, H. Özkaynak, J. Samet, D. Stieb, S. Vedal, and R. Wyzga: Health Assessment Aspects of Multi-Pollutant, Results-Based Air Quality Management. Chapter in NARSTO review of multi-pollutant air quality management, (in press).

Mauderly, J. L., and J. M. Samet: Is There Evidence for Synergy Among Air Pollutants in Causing Health Effects? *Environ. Health Perspect.* 117(1): 1-6, 2009

Mauderly, J.L. and E. Garshick: Diesel Exhaust. Chapter 17 in: *Environmental Toxicants*, Lippmann, Ed., Wiley, New York, pp. 551-631, 2009.

Lund, A.K., J. Lucero, S. Lucas, M.C. Madden, J.D. McDonald, J. Seagrave, T.L. Knuckles, and M.J. Campen: Vehicular Emissions Induce Vascular MMP-9 Expression and Activity via Endothelin-1 Mediated Pathways. *Arterio. Thrombosis Vasc. Biol.* 29:00 (on-line, DOI:10.1161/ATVBAHA.108.176107).

McDonald, J.D., E.B. Barr, R. K. White, D. Kracko, J.C. Chow, B. Zielinska, and E. Grosjean: Generation and Characterization of Gasoline Engine Exhaust Inhalation Exposure Atmospheres. *Inhal. Toxicol.* 20:1157-1168, 2008.

Day, K. C., M.D. Reed, J.D. McDonald, S.K. Seilkop, and E.G. Barrett: Effects of Gasoline Emissions on Pre-Existing Allergic Airway Responses in Mice. *Inhal. Toxicol.* 20:1145-1155, 2008.

Reed, M.D., E.G. Barrett, M.J. Campen, K.K. Divine, A.P. Gigliotti, J.D. McDonald, J.C. Seagrave, S.K. Seilkop, J.A. Swenberg, and J.L. Mauderly: Health Effects of Subchronic Inhalation Exposure to Gasoline Engine Emissions. *Inhal. Toxicol.* 20: 1125-1143, 2008.

Mauderly, J. L., and J. M. Samet: Is There Evidence for Synergy Among Air Pollutants in Causing Health Effects? *Environ. Health Perspect.* 117 (1): 1-6, 2009.

Seagrave, J., M.J. Campen, J.D. McDonald, J.L. Mauderly, and A.C. Rohr: Oxidative Stress, Inflammation and Pulmonary Function Assessment in Rats Exposed to Laboratory-Generated Pollutant Mixtures. *J. Toxicol. Environ. Health* 71: 1352-1.

Mauderly, J.L. and J.C. Chow: Health Effects of Organic Aerosols. *Inhal Toxicol.* 20:257-288, 2008.

Rowan, W.H., M. J. Campen, L. B. Wichers, and W.P. Watkinson: Heart Rate Variability in Rodents: Uses and Caveats in Toxicological Studies. *Cardiovasc. Toxicol.* 7:28-51.

McDonald, J.D., M.D. Reed, M.J. Campen, E.G. Barrett, J.C. Seagrave, and J.L. Mauderly: Health Effects of Inhaled Gasoline Engine Emissions. *Inhal. Toxicol.* 19(Suppl. 1): 107-116, 2007.

Seagrave, J.C., S. Dunaway, P. Hayden, J.D. McDonald, C. Stidley, and J.L. Mauderly: Responses of Differentiated Primary Human Lung Epithelial Cells to Exposure to Diesel Exhaust at an Air-Liquid Interface. *Exper. Lung Res.* 33:27-51, 2007.

Lund, A.K., T.L. Knuckles, C.O. Akata, R. Shohet, J.D. McDonald, A. Gigliotti, J.C. Seagrave, and M.J. Campen: Gasoline Exhaust Emissions Induce Vascular Remodeling Pathways Involved in Atherosclerosis. *Toxicol. Sci.* 95: 485-494, 2007.

Campen, M.J., J. D. McDonald, M.D. Reed, and J.C. Seagrave: Fresh Gasoline Emissions, Not Paved Road Dust, Trigger Alterations in Cardiac Repolarization in ApoE^{-/-} Mice. *Cardiovasc. Toxicol.* 6: 199-210, 2006.

Chow, J.C., J. G. Watson, J.L. Mauderly, D.L. Costa, R.E. Wyzga, S. Vedal, G.M. Hidy, S.L. Altshuler, D. Marrack, J.M. Heuss, G.T. Wolff, C. A. Pope, and D.W. Dockery: Health Effects of Fine Particulate Matter Air Pollution: Lines that Connect. *J. Air Waste Man.* 56:1368-1380, 2006.

McDonald, J.D., R.K. White, E.B. Barr, B. Zielinska, J.C. Chow, and E. Grosjean: Generation and Characterization of Hardwood Smoke Inhalation Exposure Atmospheres. *Aerosol. Sci. Technol.* 40: 573-584, 2006.

Reed, M. D., M. J. Campen, A. P. Gigliotti, K. S. Harrod, J. D. McDonald, J.C. Seagrave, S. K. Seilkop, and J. L. Mauderly: Health Effects of Subchronic Exposure to Environmental Levels of Hardwood Smoke. *Inhal. Toxicol.* 18:523-539, 2006.

Mauderly, J.L.: Health Hazards of Complex Environmental Exposures: A Difficult Challenge to Inhalation Toxicology. *Inhal. Toxicol.* 18: 137-141, 2006.

Barrett, E.G., R.D. Henson, B. Welsh, S.K. Seilkop, J.D. McDonald, and M.D. Reed: Effects of Hardwood Smoke Exposure on Allergic Airway Inflammation in Mice. *Inhal. Toxicol.* 18: 33-43, 2006.

Seagrave, J.C., J.D. McDonald, M.D. Reed, S.K. Seilkop, and J.L. Mauderly: Responses to Subchronic Inhalation of Low Concentrations of Diesel Exhaust and Hardwood Smoke Measured in Rat Bronchoalveolar Lavage Fluid. *Inhal. Toxicol.* 17: 657-670, 2005.

Burchiel, S.W., F.T. Lauer, S.L. Dunaway, J. Zawadzki, J.D. McDonald, and M.D. Reed: Hardwood Smoke Alters Murine Splenic T Cell Responses to Mitogens Following a Six Month Whole Body Inhalation Exposure. *Toxicol. Appl. Pharmacol.* 202 (3): 229-236, 2005.

Seagrave, J.C., J. D. McDonald, and J. L. Mauderly: In Vitro Versus In Vivo Exposure to Combustion Emissions. *Exp. Toxicol. Pathol.* 57: 233-238, 2005.

Tesfaigzi, Y., J. D. McDonald, M. D. Reed, S. P. Singh, G. DeSanctis, P. R. Eynott, F. F. Hahn, M. J. Campen and J. L. Mauderly: Low-Level Subchronic Exposure to Wood Smoke Exacerbates Inflammatory Responses in Allergic Rats. *Toxicol. Sci.* 88(2): 505-513, 2005.

Harrod, K.S., R.J. Jaramillo, J.A. Berger, A.P. Gigliotti, S.K. Seilkop, and M.D. Reed: Inhaled Diesel Engine Emissions Reduce Bacterial Clearance and Exacerbate Lung Disease to *Pseudomonas aeruginosa* Infection. *Toxicol. Sci.* 83: 155-165, 2005.

Mauderly, J. L.: Health Effects of Complex Mixtures: Where Are We and Where Do We Need to Be? In: *Effects of Air Contaminants on the Respiratory Tract – Interpretations from Molecules to Meta Analysis*, INIS Monographs, Fraunhofer IRB Verlag, pp. 43-52, 2004.

Seagrave, J.C., J. D. McDonald, M. D. Reed: Respiratory Toxicity Testing: Alternatives to Inhalation Exposure. In: *Effects of Air Contaminants on the Respiratory Tract – Interpretations from Molecules to Meta Analysis*. INIS Monographs, Fraunhofer IRB Verlag, pp. 205-217, 2004.

McDonald, J.D., E.B. Barr, R.K. White, J.C. Chow, J.J. Schauer, B. Zielinska, and E. Grosjean: Generation and Characterization of Four Dilutions of Diesel Engine Exhaust for a Subchronic Inhalation Study. *Environ. Sci. Technol.* 38(9): 2513-2522, 2004a.

Burchiel, S.W., F.T. Lauer, J.D. McDonald, and M.D. Reed: Systemic Immunotoxicity in AJ Mice following 6-Month Whole Body Inhalation Exposure to Diesel Exhaust. *Toxicol. Appl. Pharmacol.* 196: 337-345, 2004.

Reed, M.D., A.P. Gigliotti, J.D. McDonald, JC Seagrave, S.K. Seilkop, and J.L. Mauderly: Health Effects of Subchronic Exposure to Environmental Levels of Diesel Exhaust. *Inhal. Toxicol.* 16: 177-193, 2004.

Seagrave JC. E. B. Barr, and T.H. March: Effects of Cigarette Smoke Exposure and Cessation on Inflammatory Cells and Matrix Metalloproteinase Activity in Mice. *Experimental Lung Res.* 30: 1-15, 2004.

Campen, M.J., J.D. McDonald, A.P. Gigliotti, S.K. Seilkop, M.D. Reed, and J.M. Benson: Cardiovascular Effects of Inhaled Diesel Exhaust in Spontaneously Hypertensive Rats. *Cardiovascular Toxicology* 03(4): 353-361, 2003.

Mauderly, J.L.: Health Effects of Air Pollution: The Struggle for Context. *Environmental Progress* 22(3): 2-4, 2003.

Muggenburg, B.A., J. M. Benson, E.B. Barr, J. Kubatko and L. P. Tilley: Short-Term Inhalation of Particulate Transition Metals Has Little Effect on the Electrocardiograms of Dogs Having Pre-Existing Cardiac Abnormalities. *Inhal. Toxicol.*15:357-371, 2003.

Harrod, K.S., R.J. Jaramillo, C.L. Rosenberger, S.Z. Wang, J.A. Berger and M.D. Reed: Increased Susceptibility to RSV Infection by Exposure to Inhaled Diesel Engine Emissions. *Am. J. Respir. Cell Mol. Biol.* 28: 451-463, 2003.

Barrett, E. G., K. Rudolph, L. E. Bowen, B. A. Muggenburg and D. E. Bice: Effect of Inhaled Ultrafine Carbon Particles on the Allergic Airway Response in Ragweed Sensitized Dogs. *Inhal. Toxicol.* 15:151-165, 2003.

March, T. H., E. B. Barr, G. L. Finch, K. J. Nikula and JC. Seagrave: Effects of Concurrent Ozone Exposure on the Pathogenesis of Cigarette Smoke-Induced Emphysema in B6C3F1 Mice. *Inhal. Toxicol.* 14:101-127, 2002.

Mauderly, J. L.: Linkages Between Outdoor and Indoor Air Quality Issues: Pollutants and Research Problems Crossing the Threshold. *Indoor Air 2002*, Volume 1, Hal Levin, Ed., International Conference on Indoor Air Quality and Climate, Santa Cruz, CA, ISBN 0-9721832-05, pp. 12-21, 2002.

Tesfaigzi, Y., S. P. Singh, J. E. Foster, J. Kubatko, E. B. Barr, P. M. Fine, J. D. McDonald, F. F. Hahn and J. L. Mauderly: Health Effects of Subchronic Exposure to Low Levels of Wood Smoke In Rats. *Toxicol. Sci.* 65: 115-125, 2002.

Mauderly, J. L.: Diesel Emissions: Is More Health Research Still Needed? *Toxicol. Sci.* 62: 6-9, 2001.

McDonald, J. D., J. Costanzo, E. B. Barr, J. L. Mauderly, J. J. Schauer, B. Zielinska, J. C. Sagebiel, J. C. Chow, E. Grosjean and D. Grosjean: Characterization of Laboratory Exposure Atmospheres

for Health Effects Studies. *Proceedings of Air and Waste Management Annual Conference*, Paper No. 281 on CD-ROM for the National Air and Waste Management Meeting, June 2001.

Redman, T. K., K. Rudolph, E. B. Barr, L. E. Bowen, B. A. Muggenburg and D. E. Bice: Pulmonary Immunity to Ragweed in a Beagle Dog Model of Allergic Asthma. *Exp. Lung Res.* 27(5): 433-451, 2001.

Mauderly, J. L.: The Big Picture. *Environmental Protection magazine* 11(5): 14-18, May 2000.

Mauderly, J. L.: An Evolution of Perspectives: Summary of the Third Colloquium on Particulate Air Pollution and Human Health, Durham, North Carolina, June 6-8, 1999. *Inhal. Toxicol.* 12 (Suppl. 1): 7-12, 2000.

Mauderly, J. L.: Animal Models for the Effect of Age on Susceptibility to Inhaled Particulate Matter. *Inhal. Toxicol.* 12: 863-900, 2000.

Published Abstracts by year:

2010

Mauderly, J.L. and J.D. McDonald: comparison of Composition of PM_{2.5} from Paved Roads and in Ambient Air. *The Toxicologist* (accepted for 2010 meeting of Society of Toxicology).

2009

Day, K.C., Shinnick, S., Lucas, S., McDonald, J.D., Mauderly, J.L., Campen, M.J., and E. G. Barrett: Pulmonary Effects of Mixed Vehicular Exhaust Exposure in Mice. *Am. J. Respir. Crit. Care. Med.* (submitted for May, 2009, ATS meeting).

Campen, M.J., Lund, A. K., McDonald, J. D., Reed, M. D., and J. L. Mauderly: Vascular Lipid Peroxidation and Indices of Plaque Remodeling Are Promoted by Inhalation Exposure to Vehicular Emissions, but Not Hardwood Smoke or a Simulated Coal Combustion Atmosphere. *The Toxicologist* 108: 267, 2009.

Mauderly, J.L., Barrett, E.G., Campen, M. J., Gigliotti, A. P. McDonald, J. D., Reed, M. D., Seagrave, J., and S.K. Seilkop: Comparative Health Effects of Inhaled Diesel and Gasoline Exhaust, Hardwood Smoke, and "Downwind" Coal Emissions. *The Toxicologist* 108: 119, 2009.

2008:

Campen, M.J., A.K. Lund, M.C. Madden, J. Lucero, T.L. Knuckles, M. Doyle-Eisele, S. Allen, J.D. McDonald, M.D. Reed, J. Seagrave, and J.L. Mauderly: Inhaled Complex Combustion Emissions Upregulate Transcription and Activity of Systemic Matrix Metalloproteinase-9 (MMP-9): Evidence in Murine and Human Models. *The Toxicologist* 102 (1): 150 (abstract 738), 2008.

Lund, A.K., J. Lucero, S. Lucas, T.L. Knuckles, and M.J. Campen: Gasoline Engine Emissions Exposure Results in Upregulation of Endothelin-1-Mediated Vascular Matrix Metalloproteinase Activity. *The Toxicologist* 102 (1): 150 (abstract 739), 2008.

Mauderly, J.L., E.G. Barrett, M.J. Campen, A.P. Gigliotti, J.D. McDonald, J. Seagrave, M.D. Reed, and S.K. Seilkop: Comparative Effects of Subchronic Inhalation Exposure of Rodents to Diesel and Gasoline Engine Emissions, Hardwood Smoke, and Simulated Downwind Coal Emissions. *The Toxicologist* 102 (1): 221 (abstract 1071), 2008.

2007:

Mauderly, J.L., J.D. McDonald, J.C. Seagrave, S.K. Seilkop, and E.J. Bedrick: Effect of Chemical and biological composition on the In Vitro Toxicity of Fine and Coarse Respirable Paved Road Dust. *Am. J. Respir. Crit. Care. Med.* 175 (abstract issue):A174, 2007

Barrett, E.G., M.D. Reed, J.D. McDonald, S. Shinnik, and M. Anaya: Effects of Gasoline Engine Emissions on Pre-Existing Allergic Airway Responses. *Am. J. Respir. Crit. Care. Med.* 175 (abstract issue):A543, 2007.

Mauderly, J.L., E.J. Bedrick, J.L. Peccia, J.C. Seagrave, and J.D. McDonald: Influence of Biological Components on In Vitro Oxidative and Inflammatory Potentials of Fine and Coarse PM From Paved Roads. *The Toxicologist* 96(1): 107, 2007.

Seagrave, J.C., M.J. Campen, S. Dunaway, G. Herbert, J.L. Mauderly, J.D. McDonald, and A.C. Rohr: Exposure to Gasoline Engine Exhaust Causes oxidative Stress in Rats. *The Toxicologist* 96(1): 103, 2007.

McDonald, J.D., J.C. Seagrave, L. Mitchell, A. Gigliotti, and J.L. Mauderly. Pulmonary Inflammatory and Systemic Immune Responses to Inhaled Oil Nanocondensates. *The Toxicologist*, 96(1): 230, 2007.

Lund, A.K., T. Knuckles, J.C. Seagrave, C.O. Akata, J.D. McDonald, M.J. Campen: Exposure to Whole Gasoline Engine Emissions Results in Alterations of Molecular Pathways Involved in Progression of Atherosclerosis. *The Toxicologist* 96(1): 138, 2007.

Reed, M.D., A. P. Gigliotti, and J.A. Berger: Gasoline Emissions Affect Clearance of Intratracheally Instilled *Pseudomonas aeruginosa*. *The Toxicologist* 96(1): 103, 2007.

2006:

Mauderly, J.L.: Strategies for Disentangling the Causal Components of PM and Contributions of PM and Co-Pollutants. In: *Proceedings of the 2006 Annual Conference of the Air and Waste Management Association*. New Orleans, LA, June 20-23, 2006, Extended abstract No. CR-E2.

Mauderly, J. L., J. D. McDonald, J.C. Seagrave, and E. J. Bedrick: Relationship Between Composition and In Vitro Toxicity of Fine and Coarse Respirable Fractions of Re-Suspended Paved Road Dust. *Am. J. Respir. Crit. Care Med.* 3(April): 389, 2006.

Seagrave, J.C., and J. L. Mauderly: In vitro Oxidant Capacity of Particulate Matter (PM) Correlates with Cytokine Induction in Human Blood. *Am. J. Respir. Crit. Care Med.* 3(April): 551, 2006.

McDonald, J. D., J.C. Seagrave, J. L. Mauderly, and B. Zielinska: Approaches to Characterizing the Toxicity of Atmospheric Transformations of Diesel and Coal Combustion Emissions. *The Toxicologist* 90: 2212, 2006.

McDonald, J. D., J.C. Seagrave, and J. L. Mauderly: Characterization of Coarse (PM₁₀-PM_{2.5}) and Fine (<PM_{2.5}) Resuspended Dust in the Northeast, Southeast, Southwest, and Western U.S. *The Toxicologist* 90: 241, 2006.

Reed, M. D., J. A. and Berger: Real Time RT-PCR Assessment of Clearance of Respiratory Syncytial Virus Altered by Exposure to Diesel Exhaust and Hardwood Smoke. *The Toxicologist* 90: 232, 2006.

Seagrave, J.C., S. Dunaway, P. Hayden, J. D. McDonald, C. Stidley, and J. L. Mauderly: Responses of Differentiated Primary *Human Lung Epithelial Cells to Exposure to Diesel Exhaust at an Air-Liquid Interface*. *The Toxicologist* 90: 1201, 2006.

2005:

McDonald, J. D., E. Barr and J. L. Mauderly: Collection, Aerosolization, and Characterization of Paved Road Dust for an Inhalation Study. *The Toxicologist* 84 (Suppl. 1) 90, 2005.

Reed, M. D. and K. S. Harrod: Diesel and Hardwood Smoke Emissions Differentially Affect Clearance and Inflammation of Intratracheally Instilled *Pseudomonas Aeruginosa*. *The Toxicologist* 84 (Suppl. 1) 94, 2005.

Seagrave, J.C. and C. Knall: Relative Efficacy of Combustion Emission Particulate Matter to Adsorb the Neutrophil-Attracting Chemokine IL-8. *The Toxicologist* 84 (Suppl. 1): 93, 2005.

2004:

Barrett, E. G., M. D. Reed, R. Henson, and B. Welsh: Hardwood Smoke Exacerbates Preexisting Allergic Airway Responses. *Am. J. Respir. Crit Care Med.* 169: 652, 2004.

Reed, M. D., S. A. Belinsky, M. J. Campen, K. K. Divine, A. P. Gigliotti, J. D. McDonald, J. C. Seagrave, S. K. Seilkop, J. A. Swenberg, and J. L. Mauderly: Health Effects of Subchronic Diesel Exhaust and Hardwood Smoke Exposure. *Am. J. Respir. Crit Care Med.* 169: 651, 2004.

Mauderly, J. L., J. D. McDonald, and J.C. Seagrave: Health Effects of Diesel Emissions: Evolving Questions for an Evolving Issue. *The Toxicologist* 78: 338, 2004.

Seagrave, J.C., S. K. Seilkop and J. L. Mauderly: Responses to Subchronic Inhalation of Diesel Exhaust (DE) and Hardwood Smoke (HWS) Measured in Rat Bronchoalveolar Lavage Fluid. *The Toxicologist* 78: 1380, 2004.

2003:

Campen, M. J., A. Gigliotti, B. Tibbetts, C. Elliott, E. B. Barr, S. K. Seilkop, M. D. Reed, J. L. Mauderly, and J. M. Benson: Cardiovascular Effects of Diesel Exhaust Inhalation in Spontaneously Hypertensive (SH) Rats. *The Toxicologist* 72: 41, 2003.

Harrod, K. S., J. A. Berger, M. D. Reed, and J. D. McDonald: Increased Lung Disease From Respiratory Syncytial Virus By Inhaled Diesel Engine Emissions. *The Toxicologist* 72: 122, 2003.

McDonald, J. D., L. Bowen, J. Mauderly, and M. Lomask: Observations and Recommendations Regarding the Buxco Aerosol Delivery/Unrestrained Plethysmograph Systems. *The Toxicologist* 72: 295, 2003.

2002:

Barrett, E. G., M. D. Reed, T. Espindola and R. Henson: Diesel Exhaust Exposure in Conjunction with Periodic Allergen Challenge Does Not Lead to Allergic Sensitization. *Am. J. Respir. Crit. Care Med.* 165: A303, 2002.

Barrett, E. G., M. D. Reed, T. Espindola and R. Henson: Allergic Airway Responses Following Diesel Exhaust Exposure: Exacerbation vs. Attenuation. *Am. J. Respir. Crit. Care Med.* 165: A414, 2002.

Gigliotti, A., M. Walsh and M. Reed: Tumorigenesis in the A/J Mouse Model After Subchronic Inhalation Exposure to Diesel Exhaust. *The Toxicologist* 66 (1-S): 6, 2002.

Harrod, K. S., R. J. Jaramillo, C. L. Rosenberger and M. D. Reed: RSV Pathogenesis is Exacerbated by Exposure to Inhaled Diesel Engine Emissions. *Am. J. Respir. Crit. Care Med.* 165: A414, 2002.

Mauderly, J. L.: Health Effects of Mixtures of Air Pollutants. Air Quality and Health: State of the Science, *Proceedings of the Clean Air Strategic Alliance Symposium*, Red Deer, Alberta, Canada, June 3-4, 2002.

McDonald, J. D: You Can't Dilute Fresh Combustion Emissions Without Altering Their Composition. *The Toxicologist* 60: 360, 2002.

McDonald, J. D., E. B. Barr, R. K. White, J. Costanzo and A. Myren: Design and Characterization of a Wood Smoke Inhalation Exposure System. *Proceedings of the American Association of Aerosol Research Annual Meeting*, 2002..

Reed, M. D.: Diluted Diesel Exhaust Emissions Do Not Induce Changes in Peripheral Blood Micronuclei Levels in Subchronically Exposed Male and Female A/J Mice. *The Toxicologist* 60: 71, 2002.

Reed, M. D., E. G. Barrett, J. M. Benson, A. P. Gigliotti, K. S. Harrod, J.C. Seagrave and J. L. Mauderly: Health Effects of Fresh Diluted Diesel Emissions. *Proceedings of the American Association of Aerosol Research Annual Meeting*, 2002.

Reed, M. D. and J. C. Seagrave: Alterations in Clotting Parameters Induced by Subchronic Diesel Exhaust Exposure. *Am. J. Respir. Crit. Care Med.* 165: A414, 2002.

Seagrave, J.C., K. J. Nikula, T. H. March and E. Barr: Inflammatory Cells and MMP Activity in Bronchoalveolar Lavage Fluid of Mice During and After Exposure to Cigarette Smoke. *Am. J. Respir. Crit. Care Med.* 165: A601, 2002.

2001:

Benson, J. M., B. A. Muggenburg, L. P. Tilly, M. J. Campen, W. P. Watkinson, Q. W. Powell, E. B. Barr and J. L. Mauderly: Effects of Inhaled Metals on Electrocardiograms of Aged Beagle Dogs and F344/N Rats. *The Toxicologist* 60(1): 191, 2001.

Bowen, L. E., E. G. Barrett, E. B. Barr, J. D. McDonald and J. L. Mauderly: Aerosol Characterization of a Buxco Inhalation Exposure System. *The Toxicologist* 60(1): 70, 2001.

McDonald, J. D., E. B. Barr, J. Costanzo and J. L. Mauderly: Particle Size Distribution at Four Dilution Levels of Diesel Exhaust. *Proceedings of the American Association of Aerosol Research Annual Meeting*, 2001.

McDonald, J. D., E. B. Barr and J. L. Mauderly: Organic Phase Distribution and Composition at Four Dilution Levels of Diesel Exhaust. *Proceedings of the American Association of Aerosol Research Annual Meeting*, 2001.

McDonald, J. D., Q. H. Powell, L. E. Bowen and J. L. Mauderly: Organic Carbon Content of Aerosols Produced from a Palas Carbon Generation System. *The Toxicologist* 60(1): 430, 2001.

Rudolph, K., L. E. Bowen, B. A. Muggenburg, M. Nysus and D. E. Bice: Inhalation of Ultrafine Particles Has No Effect on Immune Response and Airway Reactivity in a Beagle Dog Model of Allergic Asthma. *Am. J. Respir. Crit. Care Med.* 163(5): 172, 2001.

Seagrave, J., K. J. Nikula, T. H. March, and E. Barr: Inflammatory Cells and MMP Activity in Bronchoalveolar Lavage Fluid of Mice During and Following Exposure to Cigarette Smoke. In *Proceedings of the Lovelace Respiratory Research Institute's International Symposium: Remodeling and Repair in Respiratory Diseases*, p. 142, Lovelace Respiratory Research Institute, Albuquerque, NM, 2001.

2000:

Graham, J. A. and J. L. Mauderly: Airborne Particulate Matter: Physico-Chemical Characteristics and Human Exposure Issues Related to Health Effects Research and Assessment: *The Toxicologist* 54(1): 64, 2000.

March, T. H., J. C. Seagrave, J. A. Berger, E. B. Barr, G. L. Finch, M. G. Ménache and K. J. Nikula: Effects of Chronic Ozone Exposure in a Mouse Model of Cigarette Smoke-Induced Emphysema. In Proceedings of the Lovelace Respiratory Research Institute's International Symposium: Susceptibility Factors for Respiratory Diseases, p. 110, Lovelace Respiratory Research Institute, Albuquerque, NM, 2000.

Nikula, K. J., J. C. Seagrave, E. B. Barr, J. A. Berger, G. L. Finch, T. H. March and M. G. Ménache: Combined Effects of Cigarette Smoke and Ozone in a Mouse Model of Smoke-Induced Emphysema. *The Toxicologist* 54(1): 313, 2000.

Nikula, K. J., J. C. Seagrave, J. A. Berger, E. B. Barr, G. L. Finch, T. H. March and M. G. Ménache: Ozone Modulates Lung Toxicity in a Mouse Model of Smoke-Induced Emphysema. *Am. J. Respir. Crit. Care Med.* 161(3): A173, 2000.

Rudolph, K., L. Bowen, B. Muggenburg, M. Nysus and D. E. Bice: The Inhalation of Ultrafine Particles Has No Effect on Immune Response and Airway Reactivity in a Beagle Dog Model of Allergic Asthma. In: *Proceedings of the Lovelace Respiratory Research Institute's International Symposium: Susceptibility Factors for Respiratory Diseases*, p. 114, Lovelace Respiratory Research Institute, Albuquerque, NM, 2000.

1999:

Ménache, M. G., R. C. Graham, M. DeVito and L. S. Birnbaum: An Empirical Approach to Predicting Biological Responses Following Exposure to Mixtures. *The Toxicologist* 48(1-S): 23, 1999.

Appendix B

Summary of the Workshop on Generation of an Exposure Atmosphere Simulating Ship Main Engine Exhaust

NATIONAL ENVIRONMENTAL RESPIRATORY CENTER

Summary of the

**Workshop on Generation of an Exposure Atmosphere Simulating Ship
Main Engine Exhaust**

December 8-9, 2008

Lovelace Respiratory Research Institute
Albuquerque, NM

Contact:

Joe L. Mauderly, DVM, Director
National Environmental Respiratory Center
Lovelace Respiratory Research Institute
2425 Ridgecrest Dr. SE
Albuquerque, NM 87108
Phone: 505-348-9432
E-mail: jmauderl@lrri.org

Executive Summary

A workshop was convened in Albuquerque, New Mexico on December 8-9, 2008 to discuss the generation of a residual oil combustion atmosphere for a NERC subchronic inhalation study. Nine participants from the U.S. and Europe attended. Consensus was reached on key points regarding the exposure scenario to be simulated and the general approach to selecting a target composition for the exposure mixture. The target exposure mixture will simulate fresh exhaust from a ship main engine burning heavy fuel oil (HFO, aka residual oil, residual fuel oil, bunker oil) containing approximately 1% sulfur (S) and representative concentrations of nickel (Ni) and vanadium (V). It was concluded that information gaps precluded prescribing the target composition in detail, but the participants agreed on a strategy for obtaining the necessary information. Specific tasks will be undertaken by participants, and the on-board sampling studies of the University of California, Riverside will serve as the core source of data and samples for additional analysis. It was agreed that using an actual engine or conducting exposures on board a vessel were impractical; and thus, that the most plausible strategy for generating the exposure atmosphere would involve combustion of HFO in an electric drop-tube furnace and modifying the effluent as necessary to achieve a target mixture. The participants agreed to serve as a working group during the activities necessary to determine whether or not an acceptable exposure atmosphere can be generated.

I. Background

A. Rationale for Studying HFO Combustion Emissions

The National Environmental Respiratory Center (NERC) is a laboratory research program sponsored jointly by government and industry with the goal of improving the understanding of the contributions of individual air contaminants, their combinations, and their sources to the adverse respiratory and cardiovascular health effects associated with environmental air pollution. The NERC research strategy is to achieve this by analyzing a new exposure-response database created by conducting identically designed, subchronic studies of animals exposed by inhalation to mixtures of common air pollutants having different but overlapping composition. The compositions of the exposures are measured in detail and effects of exposure are characterized using several animal models of human respiratory and cardiovascular responses. The power of statistical analyses of the combined database from all studies is enhanced in proportion to the number of different mixtures and the compositional contrast among the mixtures.

The mixtures used for the NERC exposures are intended to simulate reasonable cases of common source emissions. These mixtures encompass many (although certainly not all) air contaminants of concern for air quality management; thus, attributing causality among components of these mixtures would be significant progress. Using source emissions also allows comparisons of the nature and magnitude of effects among source types, even though each source is represented by only one of myriad possible variations.

Exposures completed to date include on-road diesel and gasoline engine exhaust, hardwood smoke, and simulated “downwind” components of coal combustion emissions. The first three exposure atmospheres were generated by dilution of fresh

effluent from engines and a wood stove. The coal atmosphere was synthesized by burning coal in an electric “drop-tube” furnace and adding other components to achieve a mixture having a composition recommended by a workshop. In contrast to fresh emissions, that atmosphere was designed to simulate exposures to key pollutants contributed by coal-fired power plants to exposures tens to hundreds of miles downwind.

During the past three years, consensus developed among the Center’s investigators, independent External Scientific Advisory Committee (ESAC), and sponsors that the next two studies should address emissions from combustion of HFO and paved road dust. These choices were based on the contrasts in exposure composition that these atmospheres would add to the NERC database, and the fact that the health hazards of these two source emissions are of current interest. HFO emissions contrast with previous exposures in several ways, but especially in the larger portions of organic material and reactive metals (especially Ni and V) in the particulate phase. Both epidemiological and toxicological studies have pointed toward the potential importance of Ni and V. HFO is used largely in two applications: 1) to fuel the main (and sometimes auxiliary) internal combustion engines of ships; and 2) to fuel boilers for generating steam for electrical power generation, process heat, and space heating. These two applications generate emissions having different characteristics, although both types of emissions contain higher Ni and V concentrations than preceding NERC exposure atmospheres.

B. Recommendations From June 2008 Annual NERC Meeting

Possible approaches to generating a HFO combustion exposure atmosphere were discussed at the 2008 annual NERC meeting. As noted below, a summary of background information and a literature review had been previously distributed, and presentations on marine HFO emissions and our preliminary results from laboratory trials with the electric furnace were presented. Sponsors expressed interest in providing the funding necessary to conduct a study of an atmosphere simulating exhaust from a ship main engine burning HFO, if an approach to generating an acceptable atmosphere could be developed.

In its June 28, 2008 guidance letter, the ESAC recommended that the study be pursued, and that a workshop be convened to consider: 1) the most appropriate target mixture; and 2) approaches to generating that mixture using either an engine or the electric furnace that had been used for the coal study.

II. Preparation for the Workshop

A. Review of Published Information

Preparation for the workshop began well in advance. Literature was reviewed to gather information on the composition of HFO combustion emissions, with a focus on exhaust from ship main engines burning HFO. Only a few published journal articles were found, and many described emissions from medium-speed 4-cycle engines such as those used for auxiliary power on ships or for land-based electrical generators. Exhaust from those engines tends to be more similar to exhaust from large on-road diesel engines than to exhaust from ship main engines. Ship main engines are extremely large, very low-speed, 2-cycle diesel engines having separate cylinder and crankcase lubricating

systems, and their exhaust differs markedly from that of typical on-road diesel engines. Particulate matter (PM) from ship main engines consists largely of sulfate and organic carbon, with minor amounts of black (elemental) carbon, and few agglomerate “soot” particles. The composition of emissions from HFO-fired boilers was also reviewed, and especially the extensive set of studies performed by Dr. Bill Linak and colleagues at the U.S. EPA laboratory in Research Triangle Park. Boiler emissions differ considerably from ship exhaust.

The most relevant data found were those produced the Dr. David Cocker and his colleagues at the University of California, Riverside College of Engineering Center for Environmental Research and Technology (CE-CERT). Those investigators sampled stack emissions from multiple ships during actual voyages. CE-CERT data from two ships were summarized and used to generate a “straw-man” composition of the PM and key gaseous components of ship main engine exhaust. The results of the literature review were summarized in a February 7, 2008 document distributed to the NERC ESAC and sponsors (Mauderly and McDonald, 2008).

We also developed other contacts familiar with ship operating practices, marine emissions, and regulatory strategies. Key among these was Capt. Bob Weeks of Chevron Shipping, who provided useful information and also pointed us toward other useful contacts. Through him, we identified Greg Levin of U.S. Combustion Products, a company active in the marine salvage market. From Mr. Levin, we learned that although used medium-speed auxiliary engines are available as skid-mounted generator sets, it is not practical to consider obtaining and operating even the smallest of the ship main engines or land-based generators of that type.

C. Trial Combustion of HFO in the NERC Electric Furnace

We obtained a sample of HFO drawn from a marine bunkering source in a U.S., port and burned it in the electric furnace. The sample contained 3.2% sulfur. The fuel was fed into the heated intake air stream of the furnace using a simple syringe pump, and emissions were sampled. Cylinder lubricating oil (unused Mobilgard 570) was mixed with the fuel in a volumetric ratio simulating the advertised relative consumption rates of oil and fuel by typical engines. The furnace was operated at approximately 1,400°C. The effluent was cooled, passed through a mixing chamber, and sampled in an animal exposure chamber in the same exposure system used for the coal study. Particle and gas concentrations, particle size, and particle morphology were examined. The results were presented at the NERC annual meeting and also at the workshop. This trial demonstrated that HFO could be burned in the furnace, but also indicated that the particulate matter resulting from that method of introducing the HFO may not closely represent the distribution of particle size and type typical of marine engines. It was found that the furnace effluent would probably need to be modified to approximate the mixture of gases, vapors, and particles indicated by the CE-CERT on-board sampling results.

Subsequent to these initial furnace trials, we received a second HFO sample containing 1.35% S. Future trials will use the more recent, lower-S fuel.

D. Presentations and Discussion at the June 2008 Annual NERC Meeting

Both Bob Weeks and David Cocker were invited to speak at the 2008 annual NERC meeting. Bob presented a review of ship operations, regulatory issues, and trends. David summarized their on-board sampling studies, including not only data from the two ships we cited in developing our preliminary “straw man” mixture, but also results from a greater range of vessels and air quality data from locations inland from the Los Angeles area ports. Information on the possibility of obtaining a suitable used engine was reviewed, as was a quote for a new medium-speed HFO-burning generator set. It was generally agreed that:

- using an actual ship main engine was impractical from cost and logistical viewpoints;
- using a medium-speed four-cycle engine was not desirable because the exhaust would offer less compositional contrast to previous NERC exposures than exhaust from a ship main engine;
- using a one-cylinder research engine (as employed in the European HERCULES research program) might be desirable if one could be obtained within the limits of practical resources;
- the CE-CERT data were likely to be the most appropriate source for developing a target composition for the exposure mixture;
- the interest of sponsors and needs of the program were best served by focusing on ship main engine emissions rather than a mixture simulating near-port, multi-source environmental air pollution or boiler emissions;
- using the electric furnace to generate a synthetic mixture was appropriate if a mixture having acceptable characteristics could be generated by that approach; and
- a workshop should be convened, to include a small group of individuals having expertise with ship engines and emissions, sampling and characterization of emissions, and sponsor interests.

III. Workshop Participants and Agenda

The attendees are listed in Table 1. Three additional participants were sought, but could not attend. Bob Weeks had planned to attend, but was prevented by last-minute business circumstances. The marine engine manufacturer Wärtsilä was contacted, but did not send a representative. Dr. Martti Larimi of the Internal Combustion Engine Research Group at the Helsinki University of Technology provided helpful information on their use of a single-cylinder engine in the HERCULES program, but neither he nor any of his senior colleagues could attend within the range of selected dates.

The workshop followed the one and one-half day format common to previous NERC workshops and annual meetings. Materials distributed to attendees prior to the event included the recent NERC Annual Report and the February 2008 review mentioned above. Scheduled presentations occupied a minority of the time; the majority was devoted to information exchange, discussion of alternatives, and building consensus about strategies. Overall, the workshop was very successful in achieving its primary goals.

Table 1. Workshop Attendees

Kjeld Aabo (ship engine manufacturer representative - emission controls)
Research Centre, MAN B&W Diesel A/S
Copenhagen, Denmark

Judy Chow (ESAC member – sampling and analytical methods)
Atmospheric Sciences Division, Desert Research Institute
Reno, Nevada

David Cocker (sampling on-board emissions)
CE-CERT, University of California, Riverside
Riverside, California

Bill Linak (operating and sampling HFO-fired laboratory boilers)
Air Pollution Technology Branch, U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Joe Mauderly (NERC Director and workshop facilitator)
National Environmental Respiratory Center, LRRI
Albuquerque, New Mexico

Jake McDonald (manager of NERC exposure operations - analytical chemistry)
National Environmental Respiratory Center, LRRI
Albuquerque, New Mexico

Gary Minsavage (technical coordinator for health)
CONCAWE
Brussels, Belgium

Will Ollison (regulatory analysis and scientific affairs)
American Petroleum Institute
Washington, DC

Pete Roberts (technical coordinator for air quality)
CONCAWE
Brussels, Belgium.

IV. Key Considerations and Constraints

A. Target Composition

The primary issue to resolve is the target composition of the exposure mixture. As was true for the other NERC exposure atmospheres, there is a wide range of fuels, operating variables, and source (engine) types. Operation of ships near land would be of the greatest concern for human exposures. This is done at reduced power.

Considering the scant published data for ship main engine exhaust, it was agreed that the CE-CERT database from on-board sampling of the exhaust from the main engines of several vessels was the richest source of information useful for designing a target composition for the NERC exposure. The target composition proposed in the February 2008 review was deemed correct in its general characteristics, but it was evident

that the CE-CERT group had a much larger database from which to draw. Further review and summary of that database was recommended before the most appropriate target composition could be identified. In contrast to the NERC coal workshop therefore, a detailed “recipe” for the mixture was not developed during this workshop. It will be developed through follow-on efforts, and presented to the NERC ESAC and sponsors for review.

B. Generation Method

Selecting the best method for generating the mixture would logically follow specification of the target composition. In this case however, the approach to generation was defined by practical considerations. It was readily agreed that it was impractical from both the logistical and cost viewpoints to consider using an actual low-speed, two-cycle engine of the type used as ship main engines. Conducting the exposures on-board a ship was also readily dismissed in view of the logistical requirements of animal studies and the need for exposures 7 days per week for up to 8 months. It was also agreed that a medium-speed 4-cycle engine would not represent the target source or produce the target exposure mixture. It was agreed that a single-cylinder research engine would probably be useful, but we could not confirm the existence of such an engine except for the one used in Helsinki. Apparently, only one engine was constructed in a local shop for the HERCULES study, and it is still in use. Based on Dr Larmi’s input, the cost and lead-time for construction of a new single-cylinder engine were considered prohibitive for the NERC study. This left only one apparent choice, which was to begin creating the target mixture using the NERC electric furnace to burn HFO, followed by adjusting the effluent as necessary to achieve the desired mixture. Our strategy therefore, will be to determine whether an appropriate mixture can be generated by this approach. If that proves unsuccessful, it is likely that the study will not be conducted.

V. Summary of Conclusions, Recommendations, and Next Steps

A. Use the Electric Furnace to combust HFO and adjust the effluent to achieve the desired mixture.

The electric furnace appears to be the only practical alternative for combusting the HFO for the study. The furnace and associated power supply and cooling system have been moved from the laboratory where the coal exposure was conducted, and the system is again operable. We have resumed generation trials with initial emphasis on more realistic introduction of HFO and cylinder oil, concurrent with the other steps being taken to resolve the target mixture.

Action:

- Jake McDonald will conduct iterative trials aimed at introducing the HFO as a spray and adding cylinder oil in a more realistic manner. The fundamental indicator of success will be a realistic distribution of particle type and size. Until the study fuel is obtained, these trials will be done using the 1.35% S fuel already on hand.

B. Use 1% sulfur fuel containing substantial nickel and vanadium

A regulatory-driven trend toward increased use of distillate fuels in main and auxiliary engines in and near ports is imminent, but use of the main engines burning HFO is still typical of contemporary practice. However, many ports now require switching to a low-sulfur fuel at some distance from the port. At this time, a representative low-sulfur fuel would contain approximately 1% sulfur.

There is evidence that the selection of feedstocks and the refining processes aimed at reducing the sulfur content of HFO also tend to reduce the Ni and V contents. We should use fuel having substantial concentrations of Ni and V in order to serve one of the purposes of including this exposure in the NERC database. Therefore, we need to identify a fuel having an approximately 1% sulfur content, but still having substantial concentrations of Ni and V. The artificial addition of Ni and V to the fuel was rejected by the group as a first choice. Although no specifications for concentrations of the metals were defined, it was agreed to use a fuel actually on the market. Selection of fuel will follow a review of fuel types and compositions available world-wide. If the electric furnace is used, the amount of fuel required for the study will not be large (one or two barrels should suffice); thus, it should be possible to obtain a sufficient quantity regardless of location of the source.

Actions:

- Kjeld Aabo will seek information on the distribution of fuel types and their relative usage worldwide.
- Based on this survey and information from the refining industry, Will Ollison and Gary Minsavage will recommend a source of fuel. This fuel will be obtained in sufficient quantity to conduct the final generation trials and, if approved by the NERC ESAC, the animal exposures.

C. Incorporation of the Contribution from Cylinder Oil

In the first furnace trial, unused cylinder oil was mixed into the HFO in a volumetric ratio typical for oil vs. fuel consumption of contemporary ship engines. This resulted in burning the oil in the same manner as the fuel. That strategy only partially represents the contribution of oil to ship exhaust. Cylinder oil is added through small pores in the lower cylinder wall and is consumed in part by direct contact with the flame front during fuel combustion, in part by “distillation” in the high-temperature environment, and in part by drainage and disposal of drippage from below the level of piston travel.

Discussions about the composition of ship exhaust, and especially the range and proportions of particle types, led to the conclusion that simply adding oil to the fuel may not achieve adequate simulation. It may be necessary to introduce the contribution from cylinder oil at least in part separately from the primary combustion of fuel. A portion of the oil contribution may need to be generated by a vaporization-pyrolysis process and then added to the furnace effluent. LRRI has experience with the vaporization and condensation of lubricating oil aerosols for inhalation exposures, and can explore this approach during upcoming generation trials.

We had no data on the portions of oil contributed to exhaust by combustion, pyrolysis, and distillation. Kjeld Aabo indicated that cylinder wall temperatures likely range from 250°C to 280°C. We will seek information on cylinder wall temperatures and the portions of oil contributed to exhaust by different processes.

We had no information on the composition of cylinder oil, or the relative worldwide usage of different oil brands and types. This information would be useful in specifying the oil to be used in the study.

Actions:

- Kjeld Aabo will seek information within MAN on cylinder wall temperatures and the likely proportions among the different pathways for oil contribution to exhaust.

NOTE: Joe Mauderly visited MAN B&W in Copenhagen on January 21. As a result, we received information from MAN that approximately 75-80% of the cylinder oil is typically emitted in some form via the exhaust, and only 20-25% is discarded as drip oil.

- Kjeld Aabo will seek market information on the use of cylinder oil types and compositions.

NOTE: Joe Mauderly visited with Gary Minsavage (CONCAWE) and Erick Rushton (Exxon) in Brussels on January 23, and they agreed to also seek information on cylinder oil composition and the distribution of usage.

- Kjeld Aabo will ask if there is information on the relative contributions of fuel and oil to the organic components of exhaust particulate matter.

NOTE: During his visit to MAN B&W, Joe Mauderly learned that MAN had no information on the proportional contributions of cylinder oil and fuel to the organic content of exhaust particles. They agreed that much of the organic content most likely comes from cylinder oil, but had not determined the proportions. Moreover, they could offer no information on the detailed speciation of either the vapor-phase or particulate organic material. Such data are not required for certification of engines, and have not been used to date in developing engine technology or optimizing engine performance.

D. Method of Feeding HFO Into the Furnace

In the initial furnace trial, HFO was fed into a heated inlet air stream using a syringe pump. Although the droplet size was not measured, it was unquestionably much larger than the droplet size resulting from spraying a high-pressure stream of heated HFO through an engine fuel injector. A realistic combustion scenario would require a smaller fuel particle size. Small-scale aerosolization strategies will be explored. A satisfactory fuel aerosolization device may generate aerosol at a rate too high in proportion to furnace flow; thus, much of the aerosol may have to be discarded or collected for re-use.

Actions:

- Jake McDonald will explore methods for introducing the HFO in a manner that achieves a more realistic particle size, focusing on approaches for generating a fuel spray.
- Joe Mauderly will inquire of MAN B&W about typical fuel aerosol particle size and the temperature of fuel at the injector.

E. Information Gaps Concerning the Target Mixture

Several gaps were identified in the information we had upon which to base a target mixture. These gaps and potential approaches to obtaining the information were discussed at length. The general composition of the particulate matter and ratios of gases are known. The particulate mass should be comprised of 40-60% sulfate, 3-6% ash, and the remainder organic carbon. However, other important compositional targets remained unknown.

Overall, it was agreed that the mixture could not be specified in detail until more knowledge was gained. It was also agreed that the CE-CERT database and archived samples from sampling exhaust from several ships provided the best starting point for gaining the necessary knowledge. Because additional sampling trials are planned, it is possible to collect new samples to fill gaps, if necessary. However, it would be desirable if we could use existing samples. The analysis of the CE-CERT samples has been limited because the funding for the program did not provide for detailed speciation of particulate organic material or characterizing the form of key metals.

The key information gaps fall into two general categories: 1) the detailed composition of the particulate organic phase, and its variability among vessels; and 2) the form (valence state and solubility) of the metals, with emphasis on Ni and V. The organic content of the CE-CERT particle samples has not been analyzed in detail, and the form of the metals has not been explored. The particle organic composition is considered important for guiding the furnace trials. It will be useful to know more about the form of metals in the animal exposure atmosphere, but this is of secondary importance in guiding the furnace trials. Another area of sparse information is the variability of the vapor-phase organic material. Because organic vapors contribute only a small component of ship exhaust, and we already know from the CE-CERT results that carbonyls predominate, additional information on organic vapors is of tertiary importance for guiding the furnace trials.

Additional analyses of particulate organic matter are clearly needed. Analyzing the several existing CE-CERT samples is preferable to collecting new samples, because of their availability in adequate number. However, permission would need to be obtained from the sponsors of the CE-CERT studies, for at least some types of analyses. David Cocker agreed to review the sample archive, explore the necessary permissions, and provide samples to the extent practical,

Some of the CE-CERT samples have been characterized by thermograms, and analyzing additional samples in this manner may be a useful way to explore the degree of variability in composition among vessels and operating conditions. CE-CERT can

perform the thermograms. If a characteristic thermogram pattern emerges, that could be helpful for guiding the development of a representative mixture at LRRRI.

Bill Linak described the methods used to determine the form of metals in the EPA boiler samples, noting that relatively large quantities of material were required. The group felt that there may be other methods that use less material, but did not have specific information at hand. Judy Chow agreed to inquire about alternate analytical methods, and to perform analyses for which the Desert Research Institute may have established methods. The potential cost of the new analyses was discussed, but that issue could not be resolved until the methods and number of samples were resolved. It was decided that existing NERC funds can cover modest costs, and sponsors will be consulted if costs prove to be large.

Gary Minsavage noted that there may be an opportunity to gain more information about the fuels and cylinder oils used in the different ships during sampling by CE-CERT by examining engine operating records. He explained that each fuel and oil should be traceable back to a Material Safety Data Sheet (MSDS) and/or company that produced the product. The MSDSs may be helpful in characterizing the fuels and lubes used during sample collection, and especially if they contain one or more Chemical Abstract Services (CAS) numbers. He reviewed the HFO CAS numbering system (see CANCAWE HFO Product Dossier and/or HPV Chemical Challenge Program Heavy Fuel Oils Category Test Plan). He noted that a single generic CAS number may be associated with any purchased HFO. As cited in the HPV Test Plan:

“Because of the variability inherent in the processing of petroleum materials, the definitions API (American Petroleum Institute) developed for the CAS numbers are qualitative in nature, written in broad, general terms. The definitions often contain only ranges of values for carbon numbers with little if any quantitative analytical information or concern for possible compositional overlaps. As a result, the CAS descriptions are not useful in determining the exact composition of any specific refinery stream.”

However, obtaining CAS numbers of blend components of the HFO rather than a generic HFO CAS number, and/or discussions with the producers of the fuels and lubes may give a better idea of the composition of the HFO. This could help understanding linkages among fuels, lubes, and emissions.

Overall, the discussion revealed that considerable work remained before the group was willing to specify a target exposure composition in more detail than the general composition summarized in the February 2008 review.

Actions:

- David Cocker will provide a sample inventory, indicating the materials and amounts available from each ship, and analyses done to date.
- David Cocker will determine the availability of existing samples and the range of analyses that might be performed, in view of limitations that might derive from the sponsors of the collections.

- David Cocker will explore the availability of information on the fuels and cylinder oils used in the ships that were sampled (e.g., source, MSDS sheets, and CAS numbers).
- If it is possible to use the filters for this purpose, David Cocker will obtain thermograms of filter material from his 8-ship matrix of complete samples, to assess variability and to determine whether a generalized pattern emerges.
- Judy Chow will inquire about methods that might be used to obtain the information we need on organic speciation and the form of metals, suitable for the amount of sample available.
- If permission can be obtained, analyses of organics and metals will be performed on existing CE-CERT filters, using the most appropriate method. If this occurs, costs will be estimated and the source of funds will be defined.

F. Continuity of the Working Group

It was agreed by all participants to continue operating as a working group. Most workshop participants have active roles in the efforts to resolve the information gaps, and all participants have strong interest in both those activities and their outcomes. Although many issues remain to be resolved, there was a high degree of consensus among the participants regarding the goals of the effort, the key steps that need to be taken, and the allocation of responsibility among individuals. We were fortunate to have assembled this group, which although small, encompassed sufficient expertise to move the project forward.

Actions:

- Joe Mauderly will track and coordinate progress in accomplishing the above actions in a timely manner and ensuring adequate communication within the working group, and among that group, the ESAC, and sponsors.

References

Mauderly, J. and J. McDonald. "Target Composition of Residual Oil Emissions Exposure Atmosphere". Unpublished review circulated to NERC ESAC and Sponsors, February 7, 2008.

Appendix C

2009 Guidance Letter from the NERC External Scientific Advisory Committee



NEW YORK UNIVERSITY SCHOOL OF MEDICINE

Morton Lippmann, Ph.D.
Department of Environmental Medicine

57 Old Forge Road, Tuxedo, NY 10987-5007
Telephone: (845) 731-3558
Facsimile: (845) 351-5472
morton.lippmann@nyumc.org

July 6, 2009

Dr. Joe L. Mauderly
Lovelace Respiratory Research Institute
2425 Ridgecrest Dr.
Albuquerque, NM 87108

Dear Joe:

On behalf of the External Scientific Advisory Committee (ESAC) to the NERC program at LRRI, I want to thank you and your staff for the opportunity to review the progress on the NERC complex mixtures research program over the past year. We recognize that you have had to cope with a substantially reduced level of extramural support in recent years, and commend you for your foresight in preserving sufficient funds for the completion of the remaining assays on the first four complex mixtures and for the integrated analysis of the databank. The June 1, 2009 Status Report that you provided to us was very helpful in preparing us for this year's teleconference meeting. We hope your continuing efforts to obtain funding sufficient to support one or more full-scale complex mixture studies will be successful, enabling the NERC team to expand the databank in terms of including mixtures containing additional chemical components.

We reiterate our previous advice that, should funding become available, future NERC source-related studies be done with resuspended road dust and/or a mixture comparable to the diluted combustion effluent from large shipboard engines. It was disappointing to hear that your preliminary investigation to identify a technically and economically feasible means of creating, from a tube furnace, a suitable test atmosphere for a marine engine burning heavy oil was unsuccessful. However we encourage your continued efforts to look for a feasible method for generating representative a combustion effluent from marine engines. Since substantial preliminary work on obtaining and analyzing resuspended road dust samples has been successfully completed, and no technical and economic restraints on performing a full NERC mixture study on resuspended road dust are anticipated, the ESAC supports the selection of unsuspended road dust for the next NERC mixture study should unrestricted funds become available.

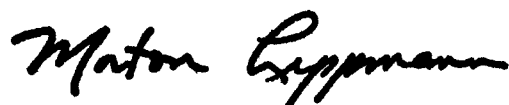
To enhance decision-making by current and potential future sponsors regarding support for the NERC source-related mixtures program, the ESAC recommends that a document be developed that provides a concise summary of the program's objectives and results to date, its significance for understanding the potential health impacts from emission sources with a broad cross-section of constituents, and what information would potentially be gained from support for additional subchronic studies of source-related mixtures. Specifically the document should include: 1) a non-technical summary description of the health outcome findings for each of the first four source emission categories; 2) a brief discussion of the interpretation of the health findings from the program to date, with an emphasis on their significance for the state-of-the-science; and 3) a discussion of what scientific benefit would potentially accrue from inclusion of additional source emissions such as resuspended road dust and/or ship engine emissions in the NERC program's integrated analysis of sources and health outcomes. We leave it to you to decide the best format of the document and the means of its distribution

ESAC discussed the desirability of convening a small working group to assist NERC in development of a statistical analysis plan for the database of results of the four completed exposures. The ESAC noted that the previous workshop on this topic had limited impact for a variety of reasons, and the ESAC recommends that:

1. NERC convene a small working group of statisticians to collaborate with NERC scientists in developing specific plans for the statistical analyses of the compositional and effects assay database matrix for the four completed source-related atmospheres.
2. The working group should include Steve Seilkop, Gerald van Belle, Ron Wyzga, and additional statisticians with expertise in classification and regression trees as well as partial least squares statistical methods.
3. An agenda for the meeting should be developed by the working group and approved by ESAC.
4. As part of the agenda, the working group should indicate specific products that will result from the workshop.
5. A report summarizing the results of the workshop should be prepared within one month of the meeting and sent to the ESAC and current NERC program sponsors.

We remain highly supportive of the NERC research program, and look forward to receiving further reports on the progress of the program. Let us know if we can be of further assistance.

Sincerely,

A handwritten signature in black ink that reads "Morton Lippmann". The signature is written in a cursive, flowing style.

Morton Lippmann, PhD
Chair, NERC ESAC

Contact: Joe L. Mauderly, DVM
Director, National Environmental Respiratory Center
Vice President, Lovelace Respiratory Research Institute
2425 Ridgecrest Dr. SE, Albuquerque, NM 87108 USA
Phone: 505-348-9432; Mobile: 505-250-4065; E-mail: jmauderl@LRRRI.org