University of Washington, EPA Clean Air Research Center
Year 1 - Annual Progress Report
Reporting Period: December 1, 2010 – July 31, 2011

Date of Report: July 31, 2011
EPA Agreement Number: RD-83479601 / EPA-RC2009-STAR-C1
Center Name: UW CCAR, Center for Clean Air Research
Center Director: Sverre Vedal
Full Project Period: 12/1/2010 – 11/30/2015

Collaborating Institutions	Location
University of Washington	Seattle, WA
Washington State University	Pullman, WA
Lovelace Respiratory Research Institute	Albuquerque, NM
University of New Mexico	Albuquerque, NM

REPORT OVERVIEW

This Annual Progress Report covers the first partial year of funding to date [12/1/2010 – 7/31/2011] for the University of Washington's Clean Air Research Center, termed the "UW CCAR - Center for Clean Air Research."

Each of the four individual EPA Clean Air Research Centers aim to advance understanding of the human health effects of exposures to complex (multipollutant) air pollution mixtures. The UW Center, specifically, is examining how pollution from roadways affects cardiovascular health. The research will integrate exposure, epidemiological, toxicological, clinical, and statistical sciences to study the cardiovascular hazards of recent and aged roadway emissions.

The Center consists of two core function groups providing biostatistical analysis support and administrative activities. There are four separate institutions, contributing to five distinct but integrated research projects, with a sixth project being carried out in the Biostatistics Core. The projects and core groups are listed below and will be described in individual summaries. Center investigators and their respective institutions will be listed with their associated research projects.

The summaries include outlines of objectives, project status, changes or difficulties encountered, and future activities. The Administrative Core summary serves as the overall Center's review. Additionally, there is a separate financial report, which provides a more detailed summary of the Center's financial activities to date.

Research Projects & Core Groups:

- Administrative Core
- Biostatistics Core
- Project 1 - Exposure Mapping – Characterization of Gases and Particles for Exposure Assessment in Health Effects and Laboratory Studies
- Project 2 - Simulated Roadway Exposure Atmospheres for Laboratory Animal and Human Studies
- Project 3 - Cardiovascular Consequences of Immune Modification by Traffic-Related Emissions
- Project 4 - Vascular Response to Traffic-Derived Inhalation in Humans
- Project 5 - Effects of long-term exposure to traffic-derived aerosols and gases on subclinical measures of cardiovascular disease and DNA methylation in a multi-ethnic cohort

INDIVIDUAL PROJECT/CORE SUMMARIES

Administrative Core

Individual Project Title: Administrative Core

Member	**Institution**
Sverre Vedal – Center Director	University of Washington
Jacob McDonald – Center Deputy Director #1	Lovelace Respiratory Research Institute
Timothy Larson - Center Deputy Director #2	University of Washington
Amanda Gassett – Center Quality Assurance Manager	University of Washington
Mark Davey – Center Manager	University of Washington

Objective of Research

The UW CCAR is focused on the cardiovascular health effects of near-roadway pollution, a complex mixture of particle, vapor and gas phase components that vary by vehicle emission source, road surface, extent of physical aging and the type and degree of atmospheric processing and photochemical reactions. This exposure scenario is not only known to be of considerable health importance, but also serves as a prototypical case for developing research approaches to dealing with multi-pollutant exposure-effect relationships. Our aim is to integrate exposure, epidemiological, toxicological, clinical, and statistical sciences to study cardiovascular hazards of fresh and aged roadway emissions and significantly advance our understanding of the components and reaction products that cause these effects.

The Center consists of five highly integrated research projects and two facility cores (including a Biostatistics Core) that together are pursuing the following six aims:

1. To characterize real-world near-roadway pollutant concentrations, particle size distributions and chemical composition

2. To simulate realistic contrasting near-roadway multi-pollutant exposure atmospheres for laboratory animal and human studies

3. To identify cardiovascular and immunologic effects and the pathogenic mechanisms of near-roadway exposures using animal models

4. To identify cardiovascular and immunologic effects of near-roadway exposures in human clinical studies

5. To identify effects of long-term exposure to traffic-derived particles and gases on sub-clinical measures of cardiovascular disease and DNA methylation in a multi-ethnic population

6. To develop a statistical and methodological framework for studying health effects of multi-pollutant mixtures

Research Performed - Progress Summary/Accomplishments

Committees and Meetings

- Investigators Committee – The investigators committee is comprised of key members from all five research projects, as well as representatives from the Biostatistics and Administrative Cores. This group meets approximately every two months for status reports and to discuss the day to day scientific activities of the Center and its individual projects. To date, this committee has met four times, with a fifth meeting scheduled for early August.

- Internal Steering Committee – The Internal Steering Committee (ISC) is comprised of the Center Director, Deputy Directors, project and core PI's, the Center Quality Assurance Manager (QAM), and the Center Manager. This group meets quarterly to discuss finances, budgets, resource allocation, and collaborations. To date, this committee has met once, with a second meeting scheduled for late August. The ISC also serves as the Cross Collaboration Committee, convening to discuss multi Center and institution collaborative opportunities. (See Below)

- Scientific Advisory Committee – The Scientific Advisory Committee (SAC) is composed of ten scientists representing varying specialties and institutions. A list of the committee members and their associated institutions appears in Table 1. The committee is scheduled to convene in Seattle for the first annual "UW CCAR" SAC meeting on September 26th and 27th. All ten members have confirmed their attendance and are expected to participate and offer constructive advice on the Center's progress, direction, focus, and future.

Table 1

Expertise	Member	Institution
Exposure Science	Michael Brauer	University of British Columbia
Exposure Science	Thomas Peters	University of Iowa
Exposure Science	Barbara Turpin	Rutgers University
Epidemiology	Arden Pope	Brigham Young University
Toxicology	Ian Gilmour	US EPA
Toxicology	Jake Lusis	University of California, Los Angeles
Toxicology	Sanjay Rajagopalan	Ohio State University (Michigan State University CLARC Member)
Statistics	Brent Coull	Havard University (Harvard University CLARC Member)
Clinical Studies	John Balmes	University of California, San Francisco (Chair)
Clinical Studies	Nicholas Mills	University of Edinburgh, UK

Quality Control

- The Center Quality Management Plan (QMP) was submitted and approved, within 60 days of the award, by the EPA Quality Assurance Manager in March 2011. That document has been circulated to all Center personnel and resides on the Center server and public web site. There have been minor revisions to that document for two changes in project quality assurance personnel, which will be discussed in the "changes in personnel" section. The revised plan will be forwarded to the EPA Quality Assurance Manager for formal documentation purposes after the Year 1 Annual Progress Report is submitted. As part of the QMP, the Center Manager has been in contact with all projects, confirming that all project personnel have the appropriate basic and specific training and certifications needed to perform the scheduled activities.

- Each individual research project within the Center has appointed its own Quality Assurance Officer (QAO). That person is responsible for creating, submitting, and archiving all relevant Quality Management materials. Each project has been gathering and revising existing Standard Operating Procedures (SOPs) or creating new SOPs for Center specific activities.

- The QAO is also responsible for creating their project specific Quality Assurance Project Plans (QAPPs), which have been started for Projects 1, 2, and 3. These projects will be the first to generate analytical datasets. To remain in compliance, each QAO and project PI has been instructed to complete their respective QAPPs and have them approved by the Center Quality Assurance Manager (QAM) before any analytical data collection can begin.

Subawards

- Three Center subawards have been initiated and completed for the following collaborating institutions: Washington State University, Lovelace Respiratory Research Institute (LRRI), and the University of New Mexico. Financial information relating to the subawards can be found in the separate Center Financial Report.

Information Technology

- The University of Washington's Department of Environmental and Occupational Health Sciences (DEOHS) has established a temporary Center server to create immediate functionality. This server is maintained, secured, and archived by DEOHS IT personnel and has been made password accessible to all Center/project personnel. The server exceeds current demands and is expandable if required.

- A Structured Query Language (SQL) server will be customized for the Center's internal and external demands when capacities and data sharing protocols are finalized. Consideration is being given to expanding the current MESA Air server, for a more cost effective infrastructure and ease of access, or designing an entirely separate system for added flexibility. This process should be initiated in the fall of 2011.

- A web site has been created to inform visitors on the UW CCAR and CLARC activities. The site will go live as of 8/1/2011, initially with abbreviated content. As time allows, the site and content will be progressively and routinely revised to form a comprehensive internal and external informational tool. The web site displays the new UW CCAR logo.

Human Subjects & IACUC

Below is a summary of the Human Subjects and Institutional Animal Care and Use Committee (IACUC) considerations and status for each individual research project. The Center Manager has been in contact with each project and confirmed that all projects and personnel have the appropriate certifications and training required for the scheduled activities. UW CCAR projects utilizing Human Subjects or confidential information are not scheduled to start until at least Year 2 of the Center's activities. Most of the Human Subjects activities in CCAR will exploit existing approvals already in place for MESA Air.

- Administrative Core - All Human Subjects training and certifications are current and documented with the Center Manager. Institutional IACUC approval for the University of Washington and LRRI are on file with the UW CCAR Manager and the CLARC EPA Project Officer.

- Biostatistics Core - There are no immediate Human Subjects or IACUC concerns for the Biostatistics Core.

- Project 1 - Integration sampling with Project 5 and their subject population is being proposed. Human Subjects concerns relating to modifying MESA Air IRB approvals

were recently discussed and should be clarified in August of 2011. In anticipation, all Project 1 personnel have confirmed current Human Subjects training and certifications. There are no immediate IACUC concerns for Project 1.

- Project 2 - There are no immediate Human Subjects or IACUC concerns for Project 2.

- Project 3 - Institutional IACUC approval for the University of Washington and LRRI are on file with the UW CCAR Manager and the CLARC EPA Project Officer. An amendment for adding one laboratory person was submitted and approved in June 2011 for the LRRI IACUC plan and is on file with the Center Manager.

- Project 4 - Because the project's Human Subjects activities are not scheduled to begin until Year 3 of the Center, there are no immediate Human Subjects or IACUC concerns for Project 4.

- Project 5 - During Year 2 of this project, the Center and Project 5 will work with the Collaborative Health Studies Coordinating Center (CHSCC) and the MESA field centers at UCLA and Wake Forest University to acquire appropriate Human Subjects approvals for the personal and residential sampling included in this project. There are no IACUC concerns for Project 5.

Collaboration Opportunities

In keeping with the EPA emphasis on multi-Center collaborations, the UW CCAR has been discussing and exploring several potential collaborative ideas:

1. Emory University/Georgia Tech University – Extend the Project 1 mobile monitoring campaign to include Atlanta, GA in late summer 2013. This opportunity benefits from field sampling ending in Winston-Salem, NC around August 20, 2013 and having the instrumentation in close proximity. This additional sampling would generate data to complement both centers by collecting roadway measurements of analytes that were not originally planned by each center alone.

2. Harvard University – Bring the LRRI group together with the Harvard group involved in the irradiation chamber to initially exchange information with consideration for designing a project of value to both centers.

3. Michigan State University (with Ohio State component) – Initially exchange information on toxicology study protocols and animal models in order to design a study that integrates well with both center aims.

Difficulties Encountered and Revised Goals

Note: Individual project summaries detail any difficulties or delays encountered, requiring revision of originally proposed goals.

Overall, the Center has been on schedule with minimal difficulties. Progress was slow initially due to establishing subaward contracts, financial accounts, and budgets. The Center is now structured with a solid administrative foundation and positioned to concentrate on the proposed research and collaborations. The Administrative Core will now focus on reinforcing internal and external documentation, providing structured financial controls with the subawards, and quickly establishing the required quality control measures and documentation. As increased spending occurs and data is collected, emphasis will be placed on more frequent contact with the project financial and quality management personnel.

Changes in Key Personnel

Below is a summary of personnel changes within the Center. There is some overlap with the project summaries.

- Administrative Core - No significant personnel changes in the Administrative Core.

- Biostatistics Core - Biostatistics Core Co-investigator Paul Sampson will replace Assaf Oron as the Project 5 Quality QAO. Dr. Sampson brings greater experience and knowledge to the position and will be working on protocols for documenting software and analysis programs, as well as peer review procedures of results.

- Project 1 - Dr. Dan Bon has been hired by Washington State University as post-doc. Dr. Bon will start in August 2011 and has a background in atmospheric chemistry measurements. He has extensive experience with the PTR-MS and AMS instruments, which will be deployed for use in the chamber studies.

- Project 2 - Dr. Joe Mauderly, who was initially proposed as the PI for Project 2, retired from Center activities. Dr. Jacob McDonald has assumed the PI role for Project 2. Melissa Porter, from LRRI, has been named as the Quality Assurance Officer for Project 2, replacing Melanie Doyle-Eisele.

- Project 3 - No significant personnel changes in Project 3.

- Project 4 - No significant personnel changes in Project 4.

- Project 5 - Sverre Vedal was originally proposed as the PI for Project 5. Due to his Center Director commitments, Joel Kaufman being the PI of MESA Air, and from EPA peer review comments indicating that it was reasonable that Joel Kaufman take on the PI duties for this project, it was agreed that Joel Kaufman should assume the role of PI for Project 5. This change was approved and documented by the EPA Project Officer.

- The proposed Project 5 Quality Assurance Officer (QAO), Jim Sullivan, is no longer with the University of Washington. Cynthia Curl has been appointed his replacement and also serves as the UW MESA Air Center Manager.

Unexpected Cost Increases

Note: Individual project financial information is detailed in the separate Center Financial Report

Overall the Center has been slow in initiating purchases and allotting salary. This can be attributed to the delayed funding confirmation at the start of the grant but also to the investigators reorganizing their separate commitments, schedules, and staffing. Currently, all projects are staffed and expenditures and progress have increased to an expected level. Discussion of unexpected costs will focus on Project 1, which is the first Center project to begin data collection.

Planned Activities for the Subsequent Reporting Period

Administrative Core

- Committee Meetings - Investigators Committee, Internal Steering Committee, and the Scientific Advisory Committee meetings are all scheduled for the August-September 2011 period, as well as throughout the next reporting period. Internal Steering Committee discussion will also focus on creating, developing, and implementing cross-center collaboration ideas.

- Quality Management – The Center will follow up with each individual project and associated QAO to develop and archive all relevant SOPs. Additionally, all projects will be responsible for submitting individual QAPPs for a consolidated Center plan.

- Information Technology – As the amount of data generated across the Center is better defined, server capacity, configuration, access, and cost will be specified and a system purchased and integrated. Additionally, the Center Web site will be continually revised and updated for calendar events, publications, documents, and other items of interest and information.

- Human Subjects – The appropriate Human Subjects modifications and approvals will be pursued for Project 1 and Project 5's research interests as soon as the sampling design is finalized.

- Finances - Internal and external documentation will be reinforced, providing structured financial controls with the subawards. As increased spending occurs, emphasis will be placed on more frequent contact with the project financial personnel.

Biostatistics Core

- Finalize the hiring of a postdoctoral fellow to support the methodological research program.

- Review the exposure prediction models, relying on the foundation provided by the spatio-temporal model and assessing whether the multipollutant data collected will support this model.

- Support Project 1 investigators as pilot data become available and assess whether the sampling plan can be improved further.
- Continue to support all projects on an as needed basis.

Individual Research Projects

- Project 1 – With the instrumentation suite complete, focus will shift to field operations, coordinating with exposure Projects 1, 2, 3, and 5, and characterizing the various exposure chamber atmospheres.

- Project 2 – Activities will involve continued dose and mixture exposure studies with Project 3, as well as chamber studies defining the physical and chemical transformation processes and atmosphere characterizations.

- Project 3 – Continued animal exposures and assays associated with the specified aims and refining the exposure conditions with feedback from Projects 1 and 2.

- Project 4 – Work closely with Projects 2 and 3 to define the experimental protocols in preparation for the scheduled exposures.

- Project 5 - Work with the CHSCC and the MESA field centers to acquire appropriate Human Subjects approvals for the personal and residential sampling included in this project, as well as finalizing data collection from MESA/MESA Air activities for future Project 5 and Center use.

Publications/Presentations

There have been no Center presentations or publications to date, other than the March 2011 SOT Conference CLARC announcement in Washington DC and the April 2011 EPA CLARC Kickoff meeting in Raleigh, NC.

Supplemental Keywords

Atherosclerosis, Epidemiology, Toxicology, Transportation

Relevant Web Sites

http://depts.washington.edu/uwccar/

Biostatistics Core

Individual Project Title: Biostatistics Core

Investigator	Institution
Elizabeth A. (Lianne) Sheppard	University of Washington
Paul Sampson	University of Washington
Adam Szpiro	University of Washington

Objective of Research

The overall objective is to support the statistical needs of all Center projects. This will be achieved through five specific objectives:

1. Advise Center projects on data management and compilation. This Core will provide advice on database design, forms design, data entry support, data quality review, data storage, back-up and documentation.

2. Ensure quality statistical design and analysis of Center research. The Core members meet regularly (weekly); these meetings ensure regular attention to the vast array of (bio)statistical needs of the Center, including: study design, power calculations, data collection, statistical analysis plan development, statistical analysis, and interpretation of results.

3. Implement novel statistical methods that are required for Center projects. Novel statistical methods are incorporated into Projects 5 and 1 to jointly model and then estimate health effects for mixtures of pollutants.

4. Identify additional statistical methodological research that will advance Center projects and seek resources to perform such research. The active participation of statisticians in the scientific research of the Center enables identification of innovative statistical methods and approaches to statistical analyses to deal with the most pressing scientific needs of the Center. The Center will catalyze (but not fund) solicitation of resources for new methodological research.

5. Communicate and disseminate Center findings. This Core will aid in the interpretation of Center findings and use examples from Center research in statistical papers on methodological development and implementation. The Core will also collaborate on manuscripts, foster understanding of statistical methods, and engage statisticians in air pollution research.

Research Performed - Progress Summary/Accomplishments

The primary activities to date of the Biostatistics Core have fallen in three areas: participation in overall UW CCAR and CLARC start-up activities, supporting the design of the mobile monitoring research being conducted by Project 1, and advancing statistical methodological research.

Overall CCAR and CLARC Start-up Activities

Dr. Sheppard attended the February kick-off meeting and co-led (with Brent Coull of Harvard) the break-out session "Statistical Challenges in Analyzing Multipollutant Studies". This meeting provided useful perspective on the work of all of the new Centers and of the opportunities for future collaborations. The statistical break-out session discussed a number of diverse topics and suggested as recommendations: convening a workshop on statistical challenges in multipollutant exposures, advance planning to ensure comparable statistical approaches across centers, and a project to explore different approaches to dimension reduction in health studies.

Support of Project 1

The monitoring campaigns to be conducted by Project 1 involves two different activities in each of four cities: a mobile monitoring campaign that involves continuous monitoring at a single fixed site coupled with repeated sampling of multiple intersections along 3 pre-determined routes over 9 days within a two-week period, and a passive sampling campaign at a fixed number of stationary sites for a two-week period. Project 1 investigators have met with Biostatistics Core faculty and staff to discuss the objectives and design of their campaigns. The Core recommended significantly increasing the number of locations sampled in the passive campaign; currently Project 1 is planning to collect at least double the initial 20 planned. Follow-up discussions regarding design of the mobile campaign are planned for late summer.

Advancing Methodological Research

We continue to refine our spatio-temporal model for ambient pollution concentrations. This model is comprised of a spatio-temporal mean field and a spatio-temporal variance field. The mean field is made up of a linear combination of temporal basis functions, with spatially varying coefficients estimated from a universal kriging model. Estimation from this model is based on maximum likelihood and can be accomplished using the R package SpatioTemporal that we developed. We have been fitting this model based extensive NOx, NO_2, and $PM_{2.5}$ data collected in 6 MESA Air cities under the auspices of the MESA Air study. We are applying this experience to predicting PM component data from these cities collected under the auspices of the HEI-funded NPACT study. We have found that while it is possible to fit this model in settings where there are limited PM component data, the performance statistics suggest it is difficult to predict pollutant concentrations in such data sparse situations.

Publications/Presentations

There have been no Biostatistics Core presentations or publications to date.

Future Activities

We are actively recruiting for a postdoctoral fellow to support our methodological research program. With this enhanced staffing, we will focus on methods to quantify the health effects of multipollutant mixtures in a cohort study. We will focus on exposure prediction models, relying to the degree possible on the foundation provided by our currently developed spatio-temporal model. We will assess whether the multipollutant data we will be using will be rich enough to support this model or whether alternative approaches will be necessary. For instance, we may consider using a data reduction approach first, e.g. principal components analysis, as initially performed.

We will continue Core activities to support all projects on an as needed basis. In the short term we expect the bulk of our effort will support Project 1 investigators and staff as their pilot data become available. One pilot study activity will to assess whether the sampling plan can be improved further.

Supplemental Keywords

Environmental Policy, Exposure Modeling, Epidemiologic Inference, Health Effects, Air Pollution Exposure

Project 1

Individual Project Title: Exposure Mapping – Characterization of Gases and Particles for Exposure Assessment in Health Effects and Laboratory Studies

Investigator	Institution
Michael Yost (PI)	University of Washington
Timothy Larson	University of Washington
Christopher Simpson	University of Washington
Thomas Jobson	Washington State University
Timothy Vanreken	Washington State University

Objective of Research

Roadway-source air pollutants encompass a diversity of chemicals, including both particulate and gas phase components which are transformed by chemical and physical reactions as they age in the environment. Consequently, human exposures to air pollutants can range from relatively un-aged to highly aged components that vary with respect to particle size and the chemical composition of particle and gas phase components. To obtain a more comprehensive understanding of the seasonal and spatial variability in the concentration and composition of air

pollutant exposures within MESA-Air cities, we employ mobile and fixed site monitoring to assess both gas and particle components of these pollutants as they age from roadway sources to population areas. The main project objectives are:

1. Characterize spatial and temporal gradients of selected air pollutants along roadways and within neighborhoods in MESA cities using a mobile platform;
2. Measure spatial variation in concentrations of selected air pollutants at two-week average stationary sites in coordination with the mobile measurements.
3. Characterize aging of air pollutant components as they are transported from roadway sources to neighborhood receptor locations;
4. Provide detailed characterization of laboratory exposure conditions available for toxicology testing, and identify likely conditions that mimic those found in urban settings.

Research Performed - Progress Summary/Accomplishments

Aims 1 and 2 have been the main focus of activities in this period. During this startup phase a field sampling schedule has been defined across all cities, through 2013. We have been working on assembling the instrument platform for mobile monitoring that will be conducted in the 4 MESA Air cities: Minneapolis/St. Paul, MN, Baltimore, MD, Los Angeles, CA and Winston-Salem, NC. Each city will be measured during the heating and non-heating season, during a 2 week long sampling period. This mobile monitoring instrument platform is designed to measure concentrations of particles and gases while continuously on the move with position information simultaneously logged by a real time GPS. We have ordered and received most of the instruments and are programming a custom Lab View interface to perform the data acquisition to a laptop.

Data collection will include the following components: optical particle size in 31 size bins from 10 to 0.2um, particle mean diameter and particle count from 0.03 to 0.2um, total particle count >0.1um, particle light scattering coefficient, particle light absorption (black carbon), NO/NO_2, O_3, CO, CO_2 and total VOCs.

Planned driving routes are being created for each city, arranged into 3 sectors with ~15 measurement waypoints in each sector for measurement. The routes and waypoints will coincide with the 2-week passive sampling campaigns conducted at the same time as the mobile monitoring. Pilot testing of the particle instrument interface and GPS logging will commence in late August; the interfaces for other devices is planned for late September. The initial measurement campaign is planned to start in late November, 2011 and will continue over the next 2 years. In addition to the mobile platform measurements in the four cities, we are also scheduled to make these same measurements of the controlled exposure atmospheres at LRRI in late spring, 2012 in collaboration with WSU as part of Objective 4.

Washington State University (WSU) activities have included the following: (1) Post Doc Search: The search was successful and we have hired Dr. Dan Bon who brings experience with both PTR-MS and AMS instruments that will be used in the exposure chamber studies. (2) Preparation of Standard Operating Procedures (SOP): SOPs were prepared for both the PTR-MS

and ToF-HR-AMS instruments and sent to CCAR staff. (3) Meetings: WSU has participated via conference calls on the CCAR Investigators Meetings. (4) Scheduling utilization of PTR-MS and TofF instrumentation.

Relatively few problems have been encountered to date that have required modifications in the project aims. After consulting with the Biostatistical Core, we determined that more passive sampling was needed to provide an adequate description of spatial variability in pollutants, and to reflect study subject residence concentrations. The main change has been to expand the number of passive samplers from 20 to ~40 in each city. Samplers will be placed based on a sampling frame designed to capture both near roadway levels and roadway gradients in pollutants and to reflect study subject residential concentrations. A standardized vehicle platform also was needed to improve logistics of the field sampling and to improve data QC. We have elected to use a hybrid vehicle, which will operate on electric power at low speeds to enable a more accurate measurement of roadway pollutants in traffic. The same make/model vehicle will be rented in each city during the measurement sessions. Power for the instrument package will be provided by a 120 VAC inverter system that operates on a deep cycle battery, with partial recharging from the vehicle during operation.

Because of funding limitations associated with MESA Air and Project 5, epigenetic analyses are only available from a subset of the originally proposed MESA Air cities (See Project 5 Summary). As a result, both Project 5 and Project 1 altered the sample design to conduct air monitoring in the current four cities: St. Paul, Baltimore, Winston-Salem, and Los Angeles, while dropping New York and Chicago. This change, although not ideal with respect to epigenetics, does provide opportunities for monitoring increased pollutant concentrations in Los Angeles and more comprehensive participant health measurement.

Publications/Presentations

There have been no Project 1 presentations or publications to date.

Future Activities

Two pilot projects are scheduled for equipment testing. The first, a limited equipment test, will take place in the first week of August 2011 in the local Seattle neighborhoods. The second, a full-scale systems test will take place in Tacoma, WA, retracing areas where the previous Tacoma mobile monitoring study took place. Additionally, we are working with current UW students and staff in 2 of the 4 cities [Baltimore and Los Angeles] scheduled to be sampled, having them drive example mobile monitoring routes for time and driving feasibility. They will also define criteria for selecting and documenting fixed sampling site locations. We anticipate starting field measurements in November and continuing over the next 2 years. The laboratory test environment measurement campaign currently is planned for the fall of 2012.

Supplemental Keywords

Exposure science, Community Exposures, Chemical Transport, Mobile Monitoring

Project 2

Individual Project Title: Simulated Roadway Exposure Atmospheres for Laboratory Animal and Human Studies

Investigator	Institution
Jacob McDonald (PI)	Lovelace Respiratory Research Institute
Amie Lund	Lovelace Respiratory Research Institute

Objective of Research

An integral component of the Center is the development of laboratory-generated atmospheres for experimental exposures of animals and humans. This Project will develop these atmospheres, with the primary objective of simulating environments containing key components of roadway emissions and the products of environmental factors that transform them. The exposures will help determine air contaminants that cause or potentiate the toxicity of roadway emissions or confound interpretations based on exposure defined by roadway proximity alone. The work that is currently under way is related to Aims 1 and 2 defined below.

Aim 1: Develop and characterize laboratory-generated exposure atmospheres simulating the key components of near-roadway exposures, including transformed emissions and co-exposures.

Aim 1 will modify and optimize existing LRRI exposure systems that have been previously utilized. We will modify the existing Motor Vehicle Emissions (MVE) exposure system to permit studies that compare atmospheres that mimic near roadway exposure to atmospheres that are physically aged or chemically transformed to mimic downwind exposures. The MVE atmospheres will be studied both as near as realistically practical to the point of emission, and after atmospheric aging simulating time-related particle nucleation and agglomeration. Next, the atmospheres will be chemically transformed in an irradiation (smog) chamber. The ability of a representative background pollutant mix to potentiate the toxic effects of roadway emissions will then be determined. The background mix will be simulated by combining inorganic ions, metals, secondary organics volatile hydrocarbons and ozone in realistic proportions.

Aim 2: Conduct inhalation exposures of laboratory animals.

Aim 2 will integrate with the animal toxicology project. Building on previous findings that show synergistic increases in mouse vascular response when gasoline and diesel emissions are

combined, we will investigate permutations to assess the effects of the near-roadway scenarios developed in Aim 1 and define the biological potency based on lipid peroxidation in $ApoE^{-/-}$ mice (Ref: Project 3). The first phase of this work is screening potency of motor vehicle emissions under different exposure combinations that may reflect scenarios observed in Project 1.

Research Performed - Progress Summary/Accomplishments

Three separate gasoline:diesel mixtures have been developed, and the initial set of experiments (animal inhalation exposures) are currently under way. The next phase of this research will evaluate the role of physical and chemical transformations on the potency of the motor vehicle atmospheres. The dilution system has been modified to enable physical transformation/aging and agglomeration of the mixed motor vehicle exhaust, and the characterization is under way. Next, the motor vehicle exhaust system has been coupled to the irradiation chamber that will allow study of chemical transformation. Finally, a NOx denuder system has been procured from the Desert Research Institute. This denuder will remove NOx that would prohibit successful atmospheric transformation experiments.

Protocols and SOP's for the conduct of this work have been completed, and a quality assurance plan that describes use of SOPs and data management is underway.

Publications/Presentations

There have been no Project 2 presentations or publications to date.

Future Activities

The dose and mixture response studies will be completed in conjunction with Project 3. In parallel, mixed atmospheres will be generated to determine the physical and chemical transformation. These atmospheres will be characterized in collaboration with Project 1, and the results will be related to field observations from Project 1.

Supplemental Keywords

Air Pollution, Motor Vehicle, Mixtures, Particulate Matter

Project 3

Individual Project Title: Cardiovascular Consequences of Immune Modification by Traffic-Related Emissions

Investigator	Institution
Matthew Campen (Co-PI)	University of New Mexico
Michael Rosenfeld (Co-PI)	University of Washington
Jacob McDonald	Lovelace Respiratory Research Institute
Amie Lund	Lovelace Respiratory Research Institute

Objective of Research

Traffic-related emissions are associated with the incidence and progression of acute and chronic cardiovascular sequelae in human population studies. Such phenomena of near-roadway health effects have yet to be characterized toxicologically. Because of potentially complex confounding issues related to noise, socioeconomic status, ethnicity, etc., there is a need to better understand the biological underpinnings of the hypothesis that fresh mixtures of vehicular emissions have a more potent than expected impact on human health. We hypothesize that the complex mixtures produced by traffic are inherently more toxic due to the combined presence of both particulates and volatile organic emissions. Furthermore, we hypothesize that emissions-induced oxidation of certain endogenous phospholipids, presumably from the pulmonary surfactant, can stimulate the activity of immune cells through such receptors and in turn promote the invasion of existing vascular lesions. This project will use complex roadway mixtures as generated and characterized in the laboratory (Project 2) to pursue the following aims:

Aim 1: Ascertain 1) the potentiating effects of physical and photochemical aging on fresh emissions and 2) interactions of vehicular emissions with pertinent copollutants (ozone, road dust) driving systemic vascular oxidative stress**.**

Aim 2: Examine effects of the emissions-induced oxidative modifications to endogenous phospholipids in activating immune-modulating receptors such as LOX-1, CD-36, TLR-2, and TLR-4. This Aim will utilize transgenic models to examine the roles of these receptors, as well as characterize the lipidomic alterations in various tissues.

Aim 3: Further explore the role of specific immune cell populations as participants in the innate and adaptive responses to emissions-induced phospholipid modifications. Here we utilize mouse models of immunodeficiency, including SCID and B-Cell deficient models. Additionally, we will pursue bone-marrow transplants from mice lacking those receptors described in Aim 2 to mechanistically establish the involvement of the oxidatively-modified phospholipids.

Findings will 1) indicate the most potent combinations of urban roadway and background copollutants in terms of vascular toxicity and 2) detail the role of the immune system in mechanistically driving the systemic effects of inhaled pollutants.

Research Performed - Progress Summary/Accomplishments

At present, we have ordered the first cohort of mice for exposures. We are planning to carry out

the first profile of exposures, detailed in Aim 1, in July-September 2011.

The first pilot studies are slated to begin late July 2011, in which we will start assessing the interactions between vehicular source pollutants (gasoline and diesel engine emissions) in driving vascular oxidative stress. Three ratios of emission composition will be tested among 2 strains (ApoE-null and LDLR-null) of mice and 2 diets (high fat and normal). Results from these early studies will focus efforts for future work in Aims 2 and 3. All exposures will be carried out using standardized exposure protocols established in Project 2.
After initial delay, due in part to overlapping commitments in the engine rooms, we are on target to begin a series of weeklong exposures and to transfer tissues to the respective investigators for expedited biological assays in the Fall 2011-Spring 2012 timeframe.

Publications/Presentations

There have been no Project 3 presentations or publications to date.

Future Activities

To complete accelerated exposures pertaining to Aim 1, and the vascular injury assays from those exposed animals, and complete breeding of double knockout mice. Continue interactions with Projects 1 and 2 in refining the exposure conditions.

Supplemental Keywords

Coronary Artery Disease, Oxidized Phospholipids, Atherosclerosis, Particulate Matter, Volatile Organic Compounds, Carbon Monoxide, Ozone, Toxicology

Project 4

Individual Project Title: Vascular Response to Traffic-Derived Inhalation in Humans

Investigator	Institution
Joel Kaufman (PI)	University of Washington
Jacob McDonald	Lovelace Respiratory Research Institute
Amie Lund	Lovelace Respiratory Research Institute

Objective of Research

Project 4 examines the acute vascular effects of model traffic-derived inhalation exposures in human subjects, in a multi-pollutant context. The project uses controlled clinical exposures to examine specific hypotheses based on the premise that traffic-related air pollutants acutely trigger increased arterial reactivity, vasoconstriction, and increased blood pressure in humans, and that these responses will vary depending on the components and sources of those exposures. We will test the hypothesis that traffic (e.g., diesel and gasoline engine) –derived aerosols exert vascular effects in human subjects, and provide insight into the most toxic components and mechanisms underlying epidemiological observations of cardiovascular disease events and mortality.

Research Performed - Progress Summary/Accomplishments

Project 4 is planned to begin human studies in Year 3 of the Center. The experiments will be customized based on findings in Center Projects 1-3.

Building on data derived from animal studies and exposure characterization studies in Center years 1 and 2, and by customizing exposures to capitalize on those findings, we plan clinical experiments nested within a crossover trial to be primarily conducted in Center years 3 and 4. In healthy subjects, we will test whether a traffic-derived laboratory-generated high-potency pollution atmosphere, as suggested through other Center projects, causes an increased vascular response (brachial artery vasoconstriction and increased blood pressure) compared with both a roadway-derived exposure of hypothesized lower potency, and with filtered air.

Projects funded from other sources are ongoing in the human exposure facility, and we anticipate no new obstacles to completing the proposed Center-funded work. We have recently completed one experimental protocol, are conducting data analysis on those results, and are currently launching a new experimental protocol which we plan to have completed by Center Year 3. The new protocol will permit us to conduct pilot evaluations of the proposed procedures to be used in Center Project 4, as we ramp up Center activities in the laboratory in anticipation of the launch of the Center-funded protocol in Year 3. We are now conducting pilot scale investigations regarding DNA methylation procedures as proposed in the Center application.
The new experimental protocol (supported primarily by NIEHS 5P50ES015915) being launched has received IRB approval without controversy, and we do not anticipate difficulties with approval for the Center-funded activities that will follow this protocol. However, it remains premature to proceed with an IRB application for Center-funded project as we can yet anticipate protocol modifications, nor define the to-be-used exposure situation, which will be an underpinning of that IRB application.

There are currently no changes to the aims or procedures as described in the grant application. In the 18 months subsequent to filing this report, we anticipate that we will need to adjust our planned approach to reflect the results of the animal experiments and interval scientific knowledge.

Publications/Presentations

There have been no Project 4 presentations or publications to date.

Future Activities

During Year 2 of this project, we will continue to collaborate with Center investigators and staff from Projects 2 and 3 to refine our plans for the experimental protocol so that we can launch the Center-sponsored experimental protocol on time and with all procedures adequately pilot-tested.

Supplemental Keywords

Cardiovascular Health, Diesel Exhaust, Gasoline Exhaust, Fine Particles, Volatile Organic Compounds, Blood Pressure

Project 5

Individual Project Title: Effects of long-term exposure to traffic-derived aerosols and gases on subclinical measures of cardiovascular disease and DNA methylation in a multi-ethnic cohort

Investigator	Institution
Joel Kaufman (PI)	University of Washington
Sverre Vedal	University of Washington
Timothy Larson	University of Washington
Michael Yost	University of Washington
Elizabeth A. (Lianne) Sheppard	University of Washington
Paul Sampson	University of Washington
Adam Szpiro	University of Washington

Objective of Research

Project 5 has three primary objectives:

1. Employ the small-scale gradient data acquired as part of the mobile monitoring campaign in Project 1, in conjunction with central fixed site data, regulatory monitoring data, and geographic covariates, to build a multi-pollutant exposure model for traffic-derived air pollutants. This model will incorporate complex spatial information on primary and secondary traffic-derived particles and gases.

2. Develop and validate individual-level exposure estimates for traffic-derived air pollutants, integrating: i) the outdoor residential concentration estimates from the multi-pollutant model; ii) estimates of residential infiltration rates; iii) road class- and traffic condition-specific estimates of on-roadway concentrations; and iv) individual-level questionnaire-derived time-location information. These individual-level exposure

estimates will also utilize personal monitoring data designed to clarify the in-transit component of total exposure.

3. Estimate the effect of individual-level exposure to traffic-derived air pollution on subclinical cardiovascular disease using these exposure models. Health outcomes will include left ventricular myocardial mass as ascertained by MRI, arteriolar diameters as measured by retinal photography, coronary artery calcium as ascertained by CT, intima-medial thickness as measured by ultrasound, and DNA methylation.

Research Performed - Progress Summary/Accomplishments

The main activities of Project 5 relate to the epidemiologic health study that will begin only in subsequent years. Activities during the first year of the project have included project planning, including protocol development, participation in quality assurance planning, scheduling, coordination with the MESA field centers, and harmonization with the Collaborative Health Studies Coordinating Center (CHSCC). Planning work has included development of a sampling schedule in coordination with the MESA field centers; discussions with the CHSCC regarding strategies related to human subjects approvals; and coordination with Project 1 on sampling locations based on mapping characteristics of MESA participants' residential locations.

One difficulty that has arisen has been related to the epigenetic analyses included in Aim 3. Our original proposal stated that genome-wide DNA methylation analysis would be conducted as part of the NIH Roadmap Initiative grant to Yongmei Liu at Wake Forest University (ES107650-01, "Epigenome-Wide Association Study of DNA Methylation and Atherosclerosis"), on specially prepared blood samples collected in all MESA participants at Exam 5. Due to funding limitations, the scope of the Epigenome-Wide Association Study was altered; at present, the DNA methylation analysis is occurring on 3800 participants from four field centers (in Baltimore, New York, St. Paul, and Winston-Salem), rather than the expected 5400 from all six field centers. Chicago and Los Angeles, both of which were originally included in Projects 1 and 5, are the two centers in which epigenetic testing is not occurring.

We are addressing this change in two ways. First, we have altered the cities included in air monitoring activities in this Center. As described in the Project 1 summary, the mobile monitoring campaign originally scheduled for Baltimore, Chicago, Los Angeles, and Winston-Salem will now occur in Baltimore, Los Angeles, St. Paul and Winston-Salem. The personal and residential monitoring included in Project 5 was originally planned for Chicago and LA; because of the change in the epigenetic testing, we will now include Winston-Salem and Los Angeles. While ideally these cities would be measuring all health outcomes of interest, retaining Los Angeles in the monitoring campaigns of both Projects 1 and 5 allows us to capture some of the highest air pollutant concentrations in the country, and substituting St. Paul for Chicago (in Project 1) and Winston-Salem for Chicago (in Project 5) maximizes the number of participants for which we have data on all proposed health outcomes.

Our second approach for dealing with this change is to work together with MESA and MESA Air investigators to process and store blood samples from a subset of approximately 150 Los

Angeles participants to potentially allow for future epigenetic analyses. Specifically, we are proposing a modified protocol by which clinic staff would draw blood into PBMC isolation tubes, the blood would be centrifuged and the PBMC fraction washed and cryopreserved at the site, and then shipped frozen to Wake Forest University for storage.
Overall, epidemiological analyses, including left ventricular myocardial mass as ascertained by MRI, arteriolar diameters as measured by retinal photography, coronary artery calcium as ascertained by CT, and intima-medial thickness as measured by ultrasound will occur for participants in Baltimore, Los Angeles, St Paul, and Winston-Salem. The epidemiological analyses including DNA methylation outcomes will include participants in Baltimore, St. Paul, Winston-Salem, and likely a subset from Los Angeles.

There has also been one important change in the timeline for Project 5. Originally, it was expected that the personal monitoring and field work activities associated with this project would occur in Year 2. However, since it is critical that this project align with the activities in Project 1, and since the Project 1 mobile monitoring campaigns in Winston-Salem and Los Angeles do not begin until January of 2013, the personal and residential monitoring associated with Project 5 will be delayed until Project Year 3.

There have been no other problems, delays, or adverse conditions that could impair the ability of this project to meet the results specified in the application. There have also been no absences or changes of key personnel, nor any costs significantly higher than originally estimated. While no data have been collected to date, Project 5 personnel are working closely with the Quality Assurance Manager to develop appropriate QA plans and policies. Since the residential monitoring and epidemiological analyses will not begin until Year 3, we have not yet begun application for Human Subject approval.

Publications/Presentations

There have been no Project 5 presentations or publications to date.

Future Activities

During Year 2 of this project, we intend to work with the CHSCC and the MESA field centers at UCLA and Wake Forest University to acquire appropriate Human Subjects approvals for the personal and residential sampling included in this project. We will also develop Standard Operating Procedures and Quality Assurance Plans to govern these sampling efforts. We will collaborate with the MESA and MESA Air studies as the fifth and final clinic exam comes to a close, during which the health outcome data included in this project is being collected. We will also work with the MESA and MESA Air studies to attempt to collect blood samples from a subset of participants in Los Angeles to allow for future epigenetic analyses.

Supplemental Keywords

Cardiovascular Health, Epidemiology, Volatile Organic Compounds, Subclinical, Atherosclerosis