CENTER FOR CLEAN AIR RESEARCH
UNIVERSITY of WASHINGTON
Department of Environmental and Occupational Health Sciences

University of Washington CCAR Year 3 Annual Progress Report

Reporting Period: December 1, 2012 – July 31, 2013

Submission Date of Report: July 31, 2013 (Revised October 23, 2013)
EPA Agreement Number: RD-83479601 / EPA-RC2009-STAR-C1
Center Name: UW CCAR, Center for Clean Air Research
Center Director: Sverre Vedal

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

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REPORT OVERVIEW

This Annual Progress Report covers the second year of funding to date [12/1/2012 – 7/31/2013] 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 integrates 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 expanded on in individual summaries. Center investigators and their respective institutions will be listed with their associated research projects.

The individual project summaries review objectives and report on changes or difficulties encountered during the reporting period. Progress and preliminary results, as well as discussion about future activities, are included. The Administrative Core summary serves as the overall Center review. Additionally, there is a separate financial report, which provides a more detailed summary of the Center's financial picture 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 & 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

ADMINISTRATIVE CORE – CENTER REVIEW

Member	Institution
Sverre Vedal – Center Director	University of Washington
Jacob McDonald – Center Deputy Director	Lovelace Respiratory Research Institute
Timothy Larson - Center Deputy Director	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 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

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 continues to meet every four weeks for status reports and to discuss the day to day scientific activities of the Center and its individual projects. As the research and data become more developed and integrated across projects, the presentations from rotating investigators have become more valuable for examining preliminary results and shaping progress and direction.

- 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 has met quarterly to discuss finances, budgets, resource allocation, and collaborations. The ISC also serves as the Cross Collaboration Committee and convened recently to discuss the progress and direction of the inter-Center collaboration projects. Additionally, during this meeting, the ISC discussed potential chamber facility modifications and the associated funding sources in support of the planned Projects 1 and 2 November 2013 chamber characterizations and for the start of Project 4, scheduled to begin in the late fall of 2013 or early winter of 2014.

- Scientific Advisory Committee – The Scientific Advisory Committee (SAC) is comprised of ten scientists representing varying specialties and institutions, including the US EPA and members from the other CLARC Centers. A list of the committee members with their associated institutions appears in Table 1.

  The Year 2 SAC meeting was held on September 27th and 28th 2012. All SAC committee members, as well our EPA program and project officers, were present and offered constructive advice on the Center's progress, direction, focus, and future.

  The Year 3 SAC meeting was held on July 23rd and 24th 2013. This small change in timeframe from previous CCAR SAC meetings was the result of the University of Washington's Center also hosting the EPA CLARC Annual Program Meeting on July 25th and 26th. By having these meetings back to back, travel expenses for SAC and CLARC program participants were minimized.

  - The Year 3 SAC meeting was chaired by Sanjay Rajagopalan, instead of John Balmes, who had a scheduling conflict.

  - Both Barbara Turpin and C. Arden Pope were late cancellations due to family concerns and could not attend.

- Jake Lusis asked to be removed from the CCAR Advisory Committee citing a lack of specific background to the research now being conducting within the CCAR Center. Dr. Jesus Araujo from the University of California Los Angeles was suggested as his replacement by the Center's Director and subsequently approved by the CCAR EPA Project Officer Michael Hiscock.

Dr. Michael Hiscock was introduced as the new CCAR EPA Project Officer, effective July 23, 2013. Dr. Hiscock replaces Mel Peffers who is moving to a new position within the EPA. We would like to thank Mel Peffers for her guidance and leadership during her tenure as the CCAR EPA Project Officer.

We thank the committee for evaluating our Center's progress and for sharing their ideas. The insight and suggestions provided during these meetings, and in their follow up SAC comments, are always greatly appreciated and are strongly considered by our investigators.

Table 1 – CCAR Scientific Advisory Committee Members

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	Jesus Araujo	University of California Los Angeles *(Added as of 7/26/13)*
Toxicology	*Jake Lusis*	*University of California, Los Angeles (Replaced as of 7/26/12)*
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 (Committee Chair)
Clinical Studies	Nicholas Mills	University of Edinburgh, UK

- CLARC Program Annual Meeting – As previously mentioned, the University of Washington hosted the Annual CLARC Program Meeting on July 25th and 26th 2013. The four Centers, plus the US EPA, were represented by approximately 90 investigators and researchers.

  Harvard – Harvard School of Public Health
  SCAPE – Georgia Institute of Technology & Emory University
  GLACIER – Michigan State University
  CCAR - University of Washington
  US EPA

This year's Annual Meeting focused less on general overviews from each Center, as the research has progressed significantly into year 3 of the 5 year Center program, and more on targeted highlights from individual projects and research activities. An additional focus was also directed towards further developing the 5 cross-center collaboration projects through individual break-out sessions. Results, progress, and future direction from those sessions were reported back for general discussion.

A lively and productive group discussion was moderated by Dr. Sverre Vedal on the topic of "Effects of Different Air Pollutant Mixtures." Questions such as air pollution mixture toxicity and particle mass component and source toxicity were debated and considered for future research directions.

Dr. Brent Coull, from the Harvard Center, gave a presentation on "Statistical Approaches for Multipollutant Data," to conclude the Annual Meeting.

Information Technology

- The Center continues to utilize the University of Washington's Department of Environmental and Occupational Health Sciences (DEOHS) server space at no cost to the UW Center. Current hardware provides adequate storage and computing space for the foreseeable future of the Center. This resource continues to be appropriately maintained, secured, and archived by DEOHS IT personnel, with password access for approved Center/project personnel.

- As research has progressed into Year 3, Projects 2 and 3 have utilized their own institution or research group IT resources in collecting, processing, analyzing, and storing their respective raw and "intermediate" data. This is appropriate given the physical separation [Albuquerque, NM] of these two projects from the Seattle-based Center. The remaining CCAR Projects are all making use of the DEOHS server system and its security advantages. The Biostatistics Core will perform much of the analysis and modeling on their own IT resources but will also eventually rely heavily on the DEOHS server structure for data sharing and review, and for Center integration activities.

- The Center's web site continues to provide information to the investigators and CLARC Program members, as well as to the general public. Content relating to the Center's calendar, researchers, projects, collaborators, and products remains current. The Web Site is also serving as an information platform for coordinating the EPA CLARC Program Annual Meeting.

- The Center partnered with the DEOHS Continuing Education Program to temporarily use their existing Web Site infrastructure to register attendees for the July 2013 EPA CLARC Program Annual Meeting. Use of this site streamlined the process and provided an internal method for transfer of funds. We thank the CE program for making this available and working closely with us.

- In anticipating an increased need for file and data exchange, and for use with the July 2013 EPA CLARC Program Annual Meeting, we established two methods for external file sharing:

    1. A secure "drop box" platform for larger-scale file sharing. UW DEOHS has provided an on-campus system that runs on a server termed "Kayak." This is a temporary storage service to facilitate file transfer between DEOHS researchers and collaborators outside of DEOHS. Kayak is running the Alfresco content management system on Linux. Access control is provided by CCAR/DEOHS sponsored and controlled UW Web login. All traffic is encrypted and by default, a project's folder space will only be accessible by appointed project administrators.

    2. A commonly used "freeware" online drop box account for non-sensitive materials.

- As travel costs have increased, our EPA project and program officers suggested that providing Webinar access to the Annual Program Meeting would make the content available to a much larger audience. Working with EPA Administrative staff and the University of Washington IT group, specific presentations and discussion periods were made available using an Adobe Connect Webinar environment.

Subawards

- The Center's subawards for Lovelace Respiratory Research Institute (LRRI) and the University of New Mexico were renewed for Year 3. The subaward for Washington State University was extended into Year 3 in order to complete chamber characterization experiments and data analysis. We appreciate their participation and contributions to the Center as the research progresses. Financial information relating to the subawards can be found in the separate Center Annual Financial Report.

Difficulties Encountered and Revised Goals

Note: Individual project summaries detail any additional difficulties encountered, or revisions of originally proposed goals or activities.

- Due to the significant and unexpected expenses associated with Project 1, which is an instrument and travel intensive project, alterations in the sampling design were required. Project 1 was originally scheduled to sample in 4 cities [St. Paul, Baltimore, Winston-Salem, and Los Angeles] each for two seasons [Heating and Non Heating]. After much discussion from the Center's investigators and Internal Steering Committee, it was decided that the mobile and fixed site monitoring components would not be conducted for either season in Winston-Salem.

The Project 1 passive badge deployment at 43 sample sites was still conducted by the Project 5 field team, which was in Winston-Salem during the two seasonal exposure periods. These integrated badges, although not as data-rich as the mobile monitoring continuous instruments, still provide reasonable spatial mapping of targeted pollutants, strengthened by complementary Project 5 sampling.

This change in design, while unfortunate, has allowed for a more comprehensive and creative campaign in Los Angeles where mobile monitoring, fixed site monitoring, passive badge deployment, highway measurements, and near-road gradient transects were conducted. There was also an opportunity to partner with researchers from the University of California at Los Angeles to "characterize" the roadways and neighborhoods surrounding the Los Angeles International Airport using two mobile monitoring platforms simultaneously.

Problems, Delays, Adverse Conditions

- The Center's Director Dr. Sverre Vedal began a 6-month sabbatical in Beijing China on April 1st 2013. Regular Investigators and Internal Steering Committee Meetings have been held seamlessly through online communication. Dr. Vedal has also participated in the monthly Center Directors calls and when not available, the Center's Deputy Directors, Dr. Timothy Larson and Dr. Jacob McDonald have taken his place.

  Dr. Vedal traveled back to Seattle for a one month period in July to plan, host, and participate in the Center's Scientific Advisory Committee Meeting, as well as the EPA CLARC Program's Annual Meeting. He will return to China in August for the remaining 2 months of his sabbatical, completing it in September 2013.

- The University of Washington chamber characterization experiments, similar to what was conducted in April and May of 2012 at the Lovelace Respiratory Research Institute (LRRI), have been delayed from April of 2013 to November of 2013. This shift was due to scheduling conflicts between the University of Washington, Washington State University, and LRRI. This delay has actually afforded more time to better plan and design the proposed experiments and refine what was observed and learned from the LRRI work.

- Project 5 conducted scheduled sampling in Winston-Salem for both the Heating [January and February 2013] and Non Heating [July and August 2013] periods. With a limited link to Project 1's sampling schedule and the desire to process and analyze the Winston-Salem data, the investigators for Project 5 requested that their Los Angeles sampling campaign be moved from 2013 to 2014; more specifically Heating period sampling [February 2014] and Non Heating period [June 2014]. It was collectively agreed that the information from data analysis obtained from Winston-Salem would provide useful knowledge in Los Angeles with little to no impact on the project's or Center's overall timeline.

- Project 4 is proceeding as originally planned, with the understanding that decisions, or lack of decisions, by the EPA IRB could force a complete change in the design of this project.

Changes in Key Personnel

Project 2 – Amie Lund left Lovelace Respiratory Research Institute [LRRI] in Sept. 2012. We would like to thank Dr. Lund for her contributions to the UW Center. Her role has been filled by other LRRI personnel, as well as an increased level of participation from Project 3 and Matt Campen at the University of New Mexico in Albuquerque, NM.

Biostatistics Core – Paul Sampson, who has been the Quality Assurance Officer [QAO] for the Biostatistics Core, will be replaced by Casey Olives. Dr. Olives is a Senior Staff Statistician with a strong Biostatistics background.

Unexpected Cost Increases

Unexpected cost increases and budget reallocations, as well as other relevant financial information, are detailed in the individual project summaries contained in the separate Center Annual Financial Report.

Quality Control / Assurance

- As the research progresses and significant data is collected, there will be a heightened effort to promote the expertise of the Biostatistics Core to all of the individual projects. The Biostatistics Core has their respective aims, but was also created and structured to be a Center resource for consultation and review of questions, materials, methods, and processes. It is anticipated, and expected, that all of the projects and investigators will rely heavily on the Biostatistics Core during the data intensive second half of the Center's award period.

- With the significant progress of Projects 1, 2, 3, and 5, and the anticipated start of Project 4 sometime in the Fall of 2013, the CCAR QMP is currently undergoing a comprehensive review by the Center Manager and Project PI's to confirm the currency of overall goals and objectives, training, procedures and systems, documentation, and data storage and security. This document [QMP Revision 2.0] will be reviewed and approved by the CCAR QAM Amanda Gassett, the CCAR Director Sverre Vedal, and the EPA CLARC Quality Assurance Officer Lisa Doucet. A copy of this file will be sent to the EPA CLARC Project Officer, as well as a copy that will reside on the CCAR internal server and the CCAR public accessible Web Site.

- Each individual research project's Quality Assurance Officer (QAO) is continually creating and revising Standard Operating Procedures (SOPs), as required, as part of an ongoing process to document all Center and project specific activities.

- The Center's Quality Assurance Manager (QAM) has worked closely with the four projects actively collecting research data. Projects 1, 2, 3, and 5 have submitted Quality Assurance Project Plans (QAPPs) that have been reviewed, revised, and approved by the QAM. Project 4 has received materials to create their QAPP and will have its plan submitted and approved by the QAO before any analytical data is collected. When appropriate, the Biostatistics Core will be required to provide a QAPP, or similar plan, to fully document their activities. This plan will also be reviewed and approved by the Center's QAM.

Planned Activities for the Subsequent Reporting Period

The individual project and Biostatistics Core summaries will address planned and future activities.

Administrative Core

- Quality Management – The Center will continue to follow up with each individual project, and associated QAO, to review, revise, and archive all relevant SOPs, and their respective QAPPs. Additionally, Project 4 will be responsible for submitting for approval their individual QAPP before any data is collected.

- Quality Management – To supplement the formal QAPP for each Project, the Center's Quality Assurance Manager has requested that each project collecting research data create a separate QC Report. This report will need to be "customized" to each individual project and data collection method but should contain such items such as, but not limited to:

    1. Summary of sampler type and use/deployment characteristics
    2. Summary of collection media planning, deployment, capture, and validity results
    3. Criteria for usable data or for flagging or voiding suspect data
    4. Information on comparison to existing or available AQS data
    5. Summary statistics in relation to Data Quality Objectives [DQO's]
    6. Laboratory Analysis QC
    7. Method limit of detection results
    8. Method QC results i.e. duplicate and blank samples, standard curves, etc.

- Quality Management - With the Center well into Year 3 and research activities seeing significant progress across almost all projects, a comprehensive quality review of all Center projects and activities is being planned by the QAM. Because of the substantial distances between institutions, the significant differences in types of data collected, the sheer volume of information involved, and the time and effort this undertaking could

require, the design and execution of this review will be a continuing topic of discussion in the investigators meetings, as well as between the QAM, the Center's Director, and the Center's Manager.

- Data Use Requests – As the projects collect, process, and analyze data, discussion has been raised about creating a more formal method for handling data use requests. This idea concerns internal to the Center requests, but also in the longer term, external requests from a wide variety of interested collaborators. This item will be progressively addressed in upcoming Investigators Meetings as well as among the Internal Steering Committee members.

- Manuscript Review – As data does become available to Center end-users, a formal manuscript review process will be needed to evaluate topics and publication content. This item will also be addressed in upcoming Investigators Meetings and a process will be defined and implemented.

Human Subjects & IACUC

Below is a current and historical summary of the Human Subjects and Institutional Animal Care and Use Committee (IACUC) status for each individual research project. The Center Manager has confirmed that all projects and personnel have the appropriate certifications and training required for Year 3, and beyond.

Administrative Core - All Human Subjects training and certifications are current and documented with the UW CCAR Manager, as of July 31, 2013. Institutional IACUC approval for the University of Washington and LRRI are on file with the Center Manager and the CLARC EPA Project Officer.

Biostatistics Core - There are no ongoing or planned Human Subjects or IACUC activities for the Biostatistics Core.

Project 1 - There are no ongoing or planned Human Subjects or IACUC activities for Project 1.

Project 2 - There are no ongoing or planned Human Subjects or IACUC activities for Project 2.

Project 3 – There are no ongoing or planned Human Subjects activities for Project 3.

University of Washington: **IACUC Protocol #2650-08, February 24, 2011**

1. IACUC Protocol #2650-08 Annual Approval: February 14, 2013 through February 23, 2014.

2. IACUC Protocol #2650-08 Annual Approval: February 22, 2012 through February 23, 2013.

3. Significant change approval to Protocol #2650-08 for Biological Use Authorization (BUA) to add "Endotracheal Installation of C. Pneumonia to Mice" was submitted February 23, 2012, approved March 12, 2012, and is on file with the Center Manager.

Lovelace Respiratory Research Institute: **IACUC Protocol #FY11-083, March 18, 2011**

1. Amendment A to Protocol #FY11-083 for adding one laboratory person was submitted for documentation purposes June 20, 2011 and is on file with the Center Manager.

2. Amendment B to Protocol #FY11-083 for adding one new strain, C57B16, and 104 mice was submitted May 11, 2012, approved June 7, 2012, and is on file with the Center Manager.

Project 4 – There are no ongoing or planned IACUC activities for Project 4.

Project 4 is in the process of submitting an application to the UW Human Subjects Division (UWHSD) to acquire the appropriate Human Subjects approvals for human controlled exposures to mixtures of diesel and gasoline exhaust, scheduled to begin late in 2013 in Seattle, WA. As the HSD agreements are already in place from previous similar projects, we do not anticipate any obstacles to this process. All documents will be on file with the Center Manager, when approved and available, and forwarded to NCER for consideration and ethical review.

Project 5 – There are no ongoing or planned IACUC activities for Project 5.

The existing IRB approvals at the University of Washington cover activities at both Wake Forest and at UCLA. As described below, Wake Forest University's IRB has fully approved all activities.

The first field campaign working with UCLA is anticipated for February of 2014. UCLA staff members have just recently submitted an application to the UCLA IRB, but this application is still pending review.

Human Subjects / IRB Modification #39

Submitted November 13, 2012; Modification of the MESA Air Human Subjects Application (IRB #26962, Committee E/G). Approved by the University of Washington Human Subjects Division / Internal Review Board on December 31, 2012. Also approved by the Wake Forest University Internal Review Board on December 20, 2012, as Amendment #11 for IRB study # BG05-006.

- Include the CCAR Project 5 sampling campaigns in 96 MESA Air participants to the main MESA Air IRB application.
- Personal, indoor residential, outdoor residential and in-vehicle air monitoring among a subset of 48 MESA Air participants each in Winston-Salem and Los Angeles (each) in two distinct seasons in each city.
- Location tracking, via time-location diaries, proximity monitors, and GPS units.

- Recruitment brochure, participation tracking logs, contact scripts, eligibility screeners, consent forms, time-location diaries, diary instructions and technician observation forms.

Human Subjects / IRB Modification #40

Submitted April 2, 2013; Modification of the MESA Air Human Subjects Application (IRB #26962, Committee E/G). Approved by the University of Washington Human Subjects Division / Internal Review Board on April 26, 2013. Also approved by the Wake Forest University Internal Review Board on March 28, 2013, as Amendment #13 for Study #BG05-006.

- Addition of a results letter, to provide participants information on the air pollution levels we measured in their homes and the travel routes we observed. This addition was

Human Subjects / IRB Modification #41

Submitted June 4, 2013; Modification of the MESA Air Human Subjects Application (IRB #26962, Committee E/G). Approved by the University of Washington Human Subjects Division / Internal Review Board on June 21, 2013. Also approved by the Wake Forest University Internal Review Board on June 4, 2013, as Amendment #14 for Study #BG05-006.

- Additional materials to be provided to participants during future field campaigns. Photo inserts for the recruitment brochures, a list of "do's and don'ts" regarding the sampling equipment, and a simplified time-location diary and instructions.
- Expand the total number of participants included in this sampling to allow 48 participants to be recruited at each field campaign in each city, rather than 48 total per city.

Publications / Presentations / Posters - Cumulative

The below publications, presentations, and posters are also included in their associated individual project summaries.

* **Bold** denotes new entries, post-Year 2 CCAR Annual Report

Current: 7/31/13

Center Publications to Date:

1. **Bergen S, Sheppard L, Sampson PD, Kim S-Y, Richards M, Vedal S, Kaufman JD, Szpiro AA. A National Prediction Model for Components of $PM_{2.5}$ and Measurement Error Corrected Health Effect Inference. Environ Health Perspect 2013; Jun 11.**

2. **Erickson MH, Gueneron M, Jobson BT. Measuring Long Chain Alkanes in Diesel Engine Exhaust by Thermal Desorption PTR-MS. Atmospheric Measurement Technology Discussions 2013 (submitted).**

3. **Lindstrom J, Szpiro AA, Sampson PD, Oron A, Richards M, Larson TV, Sheppard L. A Flexible Spatio-Temporal Model for Air Pollution with Spatial and Spatio-Temporal Covariates. Environmental and Ecological Statistics 2013 (in press).**

4. **Lund AK, Doyle-Eisele M, Lin Y-H, Arashiro M, Surratt JD, Holmes T, Schilling KA, Seinfeld JH, Rohr AC, Knipping EM, McDonald, JD. The Effects of α-Pinene- vs. Toluene-Derived Secondary Organic Aerosol Exposure on the Expression of Markers Associated with Vascular Disease. Inhalation Toxicology 2013 (in press).**

5. **McDonald JD, Chow JC, Peccia J, Liu Y, Chand R, Hidy GM, Mauderly JL. Influence of Collection Region and Site Type on the Composition of Paved Road Dust. *Air Qual Atmos Health.* 2013 (in press).**

6. **Robertson S, Colombo ES, Lucas SN, Hall PR, Febbraio M, Paffett ML, Campen MJ. CD36 Mediates Endothelial Dysfunction Downstream of Circulating Factors Induced by O3 Exposure. Toxicol Sci. 143(2):304-311, 2013.**

7. **Sampson PD, Richards R, Szpiro AA, Bergen S, Sheppard L, Larson TV, Kaufman JD. A Regionalized National Universal Kriging Model Using Partial Least Squares Regression for Estimating Annual PM2.5 Concentrations in Epidemiology. Atmospheric Environment, 2013, 75:383-392.**

8. **Sun M, Kaufman JD, Kim S-Y, Larson T, Gould T, Polak JF, Budoff MJ, Diez Roux AV, Vedal S. Particulate Matter Components and Subclinical Atherosclerosis: Common Approaches to Estimating Exposure in a Multi-Ethnic Study of Atherosclerosis Cross-Sectional Study. Environ Health 2013; 12: 39.**

9. **Szpiro AA and Paciorek CJ. Measurement Error in Two-Stage Analyses, with Application to Air Pollution Epidemiology. Environmetrics 2013 (submitted).**

10. **Szpiro AA, Sheppard L, Adar SD, and Kaufman JD. Estimating Acute Air Pollution Health Effects from Cohort Study Data. Biometrics 2013 (submitted).**

11. **Yin F, Lawal A, Ricks J, Fox JR, Larson T, Navab M, Fogelman AM, Rosenfeld ME, Araujo JA. Diesel Exhaust Induces Systemic Lipid Peroxidation and Development of Dysfunctional Pro-Oxidant and Pro-Inflammatory High-Density Lipoprotein. Arterioscler Thromb Vasc Biol. 2013 Jun;33(6):1153-61.**

12. Campen MJ, Lund A, Rosenfeld M. Mechanisms Linking Traffic-Related Air Pollution and Atherosclerosis. Curr Opin Pulm Med. 2012 Mar;18(2):155-60. PMID: 22189455.

13. Sheppard L, Burnett RT, Szpiro AA, Kim S-Y, Jerrett M, Pope CA III, Brunekreef B. Confounding and Exposure Measurement Error in Air Pollution Epidemiology, Air Quality, Atmosphere & Health, 2011, Jun;5(2):203-216.

14. Szpiro AA, Paciorek C, Sheppard L. Does More Accurate Exposure Prediction Necessarily Improve Health Effect Estimates? Epidemiology, 2011b, 22:680-685.

15. Szpiro AA, Sheppard L, Lumley T. Efficient Measurement Error Correction with Spatially Misaligned Data. Biostatistics, 2011a, 12:610-23.

16. Vedal S, Kaufman JD. What Does Multi-Pollutant Air Pollution Mean? Am J Resp Crit Care Med 2011; 183: 4-6.

Center Presentations to Date:

1. **Bergen, S. Optimal Penalty Parameter Selection to Minimize the Impact of Exposure Measurement Error in 2-Stage Air Pollution Epidemiology Analyses. ISEE/ISES/ISIAQ. Basel Switzerland, August 2013.**

2. **Bergen, S. Optimal Penalty Parameter Selection to Minimize the Impact of Exposure Measurement Error in 2-Stage Air Pollution Epidemiology Analyses. Joint Statistical Meetings. Montreal Canada, August 2013.**

3. **Jandarov, R. A Novel Principal Component Analysis for Spatially-Misaligned Multivariate Air Pollution Data. Joint Statistical Meetings. Montreal Canada, August 2013.**

4. **Lee, Adel. Impact of Monitoring Network Design on Exposure Prediction and Measurement. Joint Statistical Meetings. Montreal Canada, August 2013.**

5. **Vedal, S. Estimating Exposure and Health Effects of PM2.5 Components. Fudan School of Public Health. Shanghai, China. June 2013.**

6. **Sullivan, MD. Ambient Transition Metals, Lung Density And Lung Function In The Multi-Ethnic Study Of Atherosclerosis (MESA). American Thoracic Society International Conference. Philadelphia, PA, May 2013.**

7. **Vedal S. Multipollutant Data and a Multivariate Modeling Approach for Comparing Cardiovascular Health Effects of Contrasting Air Pollution Mixtures. Symposium (Multipollutant Exposure Metrics and Their Application to Air Pollution Epidemiological Studies). ISES Annual Meeting. Seattle, WA, October 2012.**

8. **Szpiro AA, Paciorek CJ. Model Choice for Spatial Prediction of Multiple Air Pollution Exposures. Joint Statistical Meeting. San Diego, CA, July 2012.**

9. **Vedal S, Szpiro AA. Methods for Estimating Health Effects of Multipollutant Mixtures in Cohort Studies. ISEE Annual Meeting. Barcelona, Spain, September 2011.**

10. CLARC Program Announcement - Society of Toxicology Conference (SOT) – March 2011 (Washington DC).

Center Posters to Date:

1. **Jandarov, RA. Novel Principal Component Analysis for Spatially-Misaligned Multivariate Air Pollution Data. ISEE/ISES/ISIAQ. Basel Switzerland, August 2013.**

2. **Banks LE, Simpson CD, Larson TV, Yost MG. Characterization of Traffic-Related Air Pollutants Near a Major Roadway in Albuquerque Using a Mobile Monitoring Approach. American Industrial Hygiene Conference and Exposition. Montreal, QC, Canada. May 2013.**

3. **Fintzi, J, Sheppard L. Detecting On-Road Emission Sources in Mobile Monitoring Data: A Novel Approach to Thinking About Air Pollution. University of Washington DEOHS Student Research Day. Seattle, WA. May 2013.**

4. Campen MJ, McDonald JM, Rosenfeld ME, Lund AK. Cardiovascular Consequences of Immune Modification by Traffic-Related Emissions. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

5. Keller JP, Sheppard L, Szpiro AA, Sampson PD. Spatial Analysis of a Marker of Roadway Emission Aging. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

6. VanReken T, Jobson T. Chemical Characterization of the LRRI Exhaust Exposure Chambers by PTR-MS and HR-ToF-AMS: Early Results. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

Relevant Web Sites

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

INDIVIDUAL PROJECT/CORE SUMMARIES

Biostatistics Core

Individual Project Title: Biostatistics Core

Investigator	Institution
Elizabeth A. (Lianne) Sheppard	University of Washington
Paul D. Sampson	University of Washington
Adam A. 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. These have been omitted this year because they have not changed from the original proposal and previous progress reports.

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 activities; supporting the design and data management of the mobile monitoring research being conducted by Project 1; and developing new statistical methodology for estimating health effects of multi-pollutant mixtures with spatially misaligned monitoring data.

1. Overall UW CCAR and CLARC Activities

This Core has continued its collaboration with other Centers on two collaborative proposals: one on multipollutant measurement error, the other on modeling pollutant fields using satellite data. Both have made some limited progress in the previous year. We have not spent our funding for these projects to date and anticipate this will happen in the next funding year.

2. Support of Project 1

The monitoring campaigns conducted by Project 1 involve two different activities in each of four cities: 1) a mobile monitoring campaign that includes both continuous monitoring at a single fixed site along with repeated sampling of multiple intersections along 3 pre-determined routes over 9 days within a two-week period during afternoon rush hours, and 2) a concurrent passive sampling campaign at a fixed number of stationary sites for a two-week period. Biostatistics Core faculty and staff continue to support the monitoring activities, mostly through support of a wide range of data-related activities including data cleaning, management, analysis, and presentation. We currently fund two student RAs who work on the Project's data, database management system, and data analyses.

3. Statistical Methods Development

Our goal is to develop a statistical framework for assessing health effects of long-term exposure to multi-pollutant mixtures when health data and monitoring data are spatially misaligned. This entails three primary steps:

1. Dimension reduction of the multi-pollutant exposure surface
2. Spatial prediction of the (reduced dimension) multi-pollutant exposure surface
3. Health effect inference that accounts for uncertainty from prediction (and possibly dimension reduction) in the first two steps

Our work to-date has been grounded in a study of the health effects of multi-pollutant exposure on systolic blood pressure (SBP) in the NIEHS Sisters Study. This builds on previous work in which we found an association between SBP and PM2.5 in this cohort. We are using annual averages from the national EPA STN and IMPROVE networks to estimate exposure.

Once our methods are refined and validated in the Sisters Study and STN/IMPROVE setting where long-term exposure measurements are available (albeit spatially misaligned), we will take on the incrementally more challenging task of incorporating spatio-temporal mobile monitoring data from Project 1. This effort will culminate in analyses of associations between long-term exposure to PM2.5 components and gases and cardiovascular disease outcomes in the MESA cohort, with intra-urban exposure contrasts derived from Project 1 monitoring data.
Our specific progress on methods development it is as follows.

Predictive sparse PCA (Steps 1 and 2): We initially tried reducing the dimension of the exposure data by sparse PCA (SPCA) (sparseness is desirable to make components more interpretable) and then using spatial modeling to predict component scores at subject locations. This did not work well because some of the PC loadings included pollutants that are not well-predicted by GIS covariates and/or spatial smoothing. Therefore, we have developed a modified SPCA algorithm, termed predictive SPCA, which attempts to find PC loadings that lead to spatially predictable scores. We have applied the predictive SPCA method to STN/IMPROVE data and have validated it in simulation studies. The current version of predictive SPCA is based on spatial prediction by unpenalized regression splines (i.e., low-rank splines combined with GIS covariates), with separate unlinked prediction models for each PC score. Planned extensions include penalized and linked multivariate prediction models.

Multi-pollutant spatial prediction (Step 2): We have made substantial progress on a new model for multivariate spatial prediction that, in particular, is well-suited for incorporating in extensions of predictive SPCA and in our multi-pollutant measurement error correction methods. We have derived a low-rank version of the common component co-kriging model of Diggle and Ribeiro. The advantages of this approach compared to competing methods (including the full-rank common component model) are (i) it facilitates sharing of information between pollutants for spatial smoothing and regression on GIS covariates (co-kriging only addresses the spatial smoothing aspect) and (ii) re-expressing it as a low-rank penalized regression, rather than a random effect model, makes sharing of information between pollutants more transparent and eases incorporation in our predictive SPCA and measurement error methods.

Measurement error correction (Step 3): We have developed a measurement for measurement error correction with spatially misaligned data, for the general setting where spatial prediction is done by a low-rank penalized regression model. This includes, for example, unlinked models for multiple pollutants or the low-rank common component model described above. For a linear health model, we have derived analytic estimates of bias from smoothing and estimation error (more penalization results in more smoothing and less estimation error). We have evaluated using these bias estimates to optimally select smoothing parameters. Our results to date suggest that even if this is done, an additional bias correction step is still required. While this works, it appears to be equally efficient to select penalty parameters by REML and then apply the bias correction. We have applied our measurement error correction to an analysis of PM2.5 and SBP in the Sisters Study and have validated it in single pollutant simulation studies. We are refining the details for multi-pollutant models and expect to complete this work in the coming years.

Publications / Presentations / Posters

Publications to Date:

1. **Lindstrom J, Szpiro AA, Sampson PD, Oron A, Richards M, Larson TV, Sheppard L. A Flexible Spatio-Temporal Model for Air Pollution with Spatial and Spatio-Temporal Covariates. Environmental and Ecological Statistics 2013 (in press).**

2. **Sampson PD, Richards R, Szpiro AA, Bergen S, Sheppard L, Larson TV, Kaufman JD. A Regionalized National Universal Kriging Model Using Partial Least Squares Regression for Estimating Annual PM2.5 Concentrations in Epidemiology. Atmospheric Environment, 2013, 75:383-392.**

3. **Szpiro AA and Paciorek CJ. Measurement Error in Two-Stage Analyses, with Application to Air Pollution Epidemiology. Environmetrics 2013 (submitted).**

4. Sheppard L, Burnett RT, Szpiro AA, Kim S-Y, Jerrett M, Pope CA III, Brunekreef B. Confounding and Exposure Measurement Error in Air Pollution Epidemiology, Air Quality, Atmosphere & Health, 2011, Jun;5(2):203-216.

5. Szpiro AA, Paciorek C, Sheppard L. Does More Accurate Exposure Prediction Necessarily Improve Health Effect Estimates? Epidemiology, 2011b, 22:680-685.

6. Szpiro AA, Sheppard L, Lumley T. Efficient Measurement Error Correction with Spatially Misaligned Data. Biostatistics, 2011a, 12:610-23.

Presentations to Date:

1. **Bergen, S. Optimal Penalty Parameter Selection to Minimize the Impact of Exposure Measurement Error in 2-Stage Air Pollution Epidemiology Analyses. ISEE/ISES/ISIAQ. Basel Switzerland, August 2013.**

2. **Bergen, S. Optimal Penalty Parameter Selection to Minimize the Impact of Exposure Measurement Error in 2-Stage Air Pollution Epidemiology Analyses. Joint Statistical Meetings. Montreal Canada, August 2013.**

3. **Lee, Adel. Impact of Monitoring Network Design on Exposure Prediction and Measurement. Joint Statistical Meetings. Montreal Canada, August 2013.**

4. **Szpiro AA, Paciorek CJ. Model Choice for Spatial Prediction of Multiple Air Pollution Exposures. Joint Statistical Meeting. San Diego, CA, July 2012.**

Posters to Date:

1. **Jandarov, RA. Novel Principal Component Analysis for Spatially-Misaligned Multivariate Air Pollution Data. ISEE/ISES/ISIAQ. Basel Switzerland, August 2013.**

Future Activities

Our postdoctoral fellow, Roman Jandarov, has productively advanced our methodological research program. We expect to sustain our progress in the next year on methods to quantify the health effects of multipollutant mixtures in a cohort study. As Dr. Jandarov may not remain with us for longer than two years, we are beginning to look for a new postdoctoral fellow to follow up with his research.

We will continue Core activities to support all projects on an as needed basis. In the next year we expect the bulk of our effort will continue to support Project 1 investigators and staff with their data management and analysis.

Supplemental Keywords

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

Relevant Web Sites

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

References

Bergen S, Sheppard L, Sampson PD, Kim S-Y, Richards M, Vedal S, Kaufman JD, Szpiro AA. A National Prediction Model for Components of $PM_{2.5}$ and Measurement Error Corrected Health Effect Inference. Environ Health Perspect 2013 (in press).

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 continue as the main focus of activities in this year 3 time period. This phase of the study is conducting field sampling through 2013 across 4 cities in the MESA-Air cohort: Minneapolis/St. Paul, MN, Baltimore, MD, Los Angeles, CA and Winston-Salem, NC. Due to

financial constraints a decision was made by the center leadership that Winston-Salem will only be monitored with passive samplers. The instrument platform for mobile monitoring was assembled and tested in Seattle in October of 2011. Mobile monitoring and passive sampling measurements for both heating and non-heating seasons have been completed in Minneapolis/St. Paul, Baltimore and Los Angeles. During each 2-week sampling period the mobile monitoring platform measures concentrations of particles and gases while continuously on the move along a fixed sampling route with position information simultaneously logged by a real time GPS. Data collection includes 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.

Pre-planned driving routes were created for each city, arranged into 3 sectors with 14 measurement intersection waypoints in each sector for measurement, plus a common central reference site. These 43 waypoints were selected in advance, based on a set of route criteria developed in consultation with the Biostatistics Core of the center. The routes were evaluated by the Biostatistics Core for use in the spatial mapping of exposures later in the study. Based on advice from our advisory committee, we also developed a more intensive "roadway gradient" sampling scheme, which modified one of the waypoints. This gradient sampling scheme was pilot tested during our field visit to Albuquerque, NM and the results are shown in Figure 1 below. Similar gradient samples were collected in all cities where mobile monitoring was conducted.

Figure 1 – Gradient sampling Data Collected in Albuquerque, NM

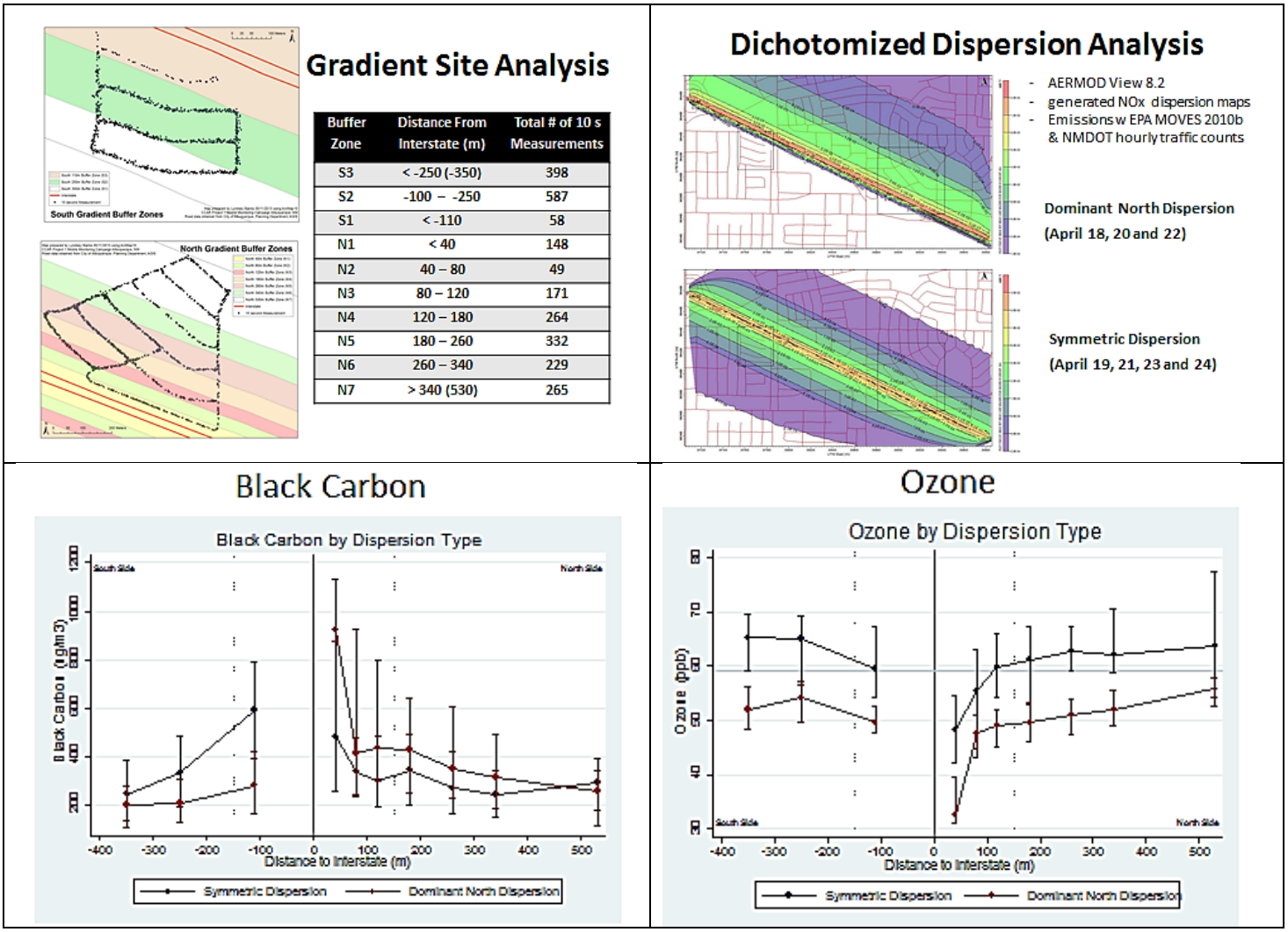


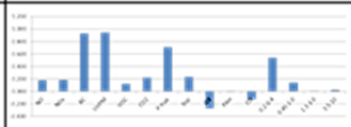
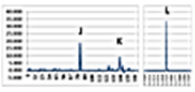
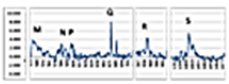
Figure one shows results from our gradient sampling tests in Albuquerque, NM over a one week period during May 2012. Mobile monitoring was used to repeatedly sample multi-pollutants near a major interstate roadway (I-40). The 10 sec mobile data collected near the interstate was classified into buffers corresponding to different distances from the centerline of the interstate.

Next, AERMOD was used to evaluate the dispersion condition near the roadway during the specific days and time that mobile sampling was done. Two major dispersion conditions were identified: a dominant north-side dispersion pattern with winds coming from the south; and a symmetric dispersion condition during more stagnant conditions. Three sampling days corresponded to the dominant north-side pattern (shown in red), and four days corresponded to the symmetric pattern (shown in blue). The data shown represent the median and interquartile range (error bars) for buffer distance over the 7-day period, classified by the dispersion conditions. The data for black carbon (absorbance) and Ozone quite clearly show that the instruments capture the near roadway gradient, and also illustrate the effect of dispersion

conditions on the shape of the gradient. The mobile sampling also clearly captures the near road deficit in Ozone which is likely due to NO/NOx scavenging.
Similar patterns in Ozone and NO/NOx have been observed in larger scale sampling with both our passive samplers and mobile platform in the other cities. Note that the mobile data only is collected during the evening commute, while the passive badges collect continuously over the 2-week period. Since the mobile platform is often collected during peak traffic and Ozone periods, it may more clearly capture these near roadway effects showing an interaction of the multi-pollutants.

Preliminary multivariate analysis of the mobile platform data was done using pilot measurements in Seattle. Traditional PCA with varimax rotation was examined and the resultant factor scores were compared with a photographic record using an onboard camera.

Figure 2. Results of PCA analysis with Varimax rotation from Duwamish Pilot study.

Plausible Source	Feature Loadings*	Selected Feature Scores vs. Time** (time in 10 second intervals)	Onboard Pictures***	Percent of Total Variance
Heavy duty diesel				19
Engines under uphill load				21
Road dust				18
Light duty vehicles				8
Particle bound PAHs			no obvious events	7

* Factor variable loadings from PCA analysis (see text for variable list)
** Peak events identified from factor scores plotted as a time series
*** Images from onboard video corresponding to peak event for this factor

The results shown in Figure 2 below indicate that there are strong latent variables that are logically related to specific roadway sources. We are currently examining whether these features are observed in our MESA cities data.

In the figure, the second column shows factor loadings from a PCA analysis of 10 second mobile monitoring data collected on one afternoon during the pilot study. The measured variables on the horizontal axis from left to right are: NO, NOx, black carbon via aethalometer, uv channel on aethalometer, VOCs, particle number concentration via P-trak, light scattering coefficient with integrating nephelometer, O3, particle bound PAHs, CO, and total particle volume concentration within the following optical particle diameter size ranges : >0.2-0.4, >0.4-1.0, >1.0-3.0, >3.0 to 10 micrometers.

The third column shows a time series of the factor score for each factor in the PCA analysis. The labeled peaks correspond to the following events as determined by an onboard camera (events labeled in bold letters correspond to the pictures shown in the fourth column): {A} at red light behind truck; {B} behind truck under freeway; {C} following school bus; {D} at red light behind school bus; {E} truck passing uphill; {F} in uphill traffic; {G & H} roadside next to uphill traffic; {J} sample inlet adjustments (experimental artifact); {K} behind large vehicle; {L} dust plume from off-road truck; {M} next to minivan uphill; {N} idling vehicle; {P} idling pickup truck; {Q} industrial site (no CO observed); {R} residential street; {S} traffic pulse after stop light.

The fourth column shows pictures taken with onboard camera at time of the peak event identified by the factor score time series. These video images help to clarity the interpretation of the factor loadings, in terms of possible on-road sources of multi-pollutants. Further work is underway to extend this analysis to longer time scales and to integrate additional information collected during the mobile sampling campaigns.

In pursuance of Objective 4, detailed chemical characterization measurements were made of controlled exposure atmospheres at LRRI in May 2012. Over the course of three weeks, nearly 50 distinct exposure atmospheres were sampled. The majority of these test atmospheres were composed of unaged gasoline and diesel exhaust at various loadings and degrees of mixing; a few atmospheres were also sampled where the emissions were photochemically aged prior to sampling. All test atmospheres were sampled by the same instrument platform used for the mobile sampling.

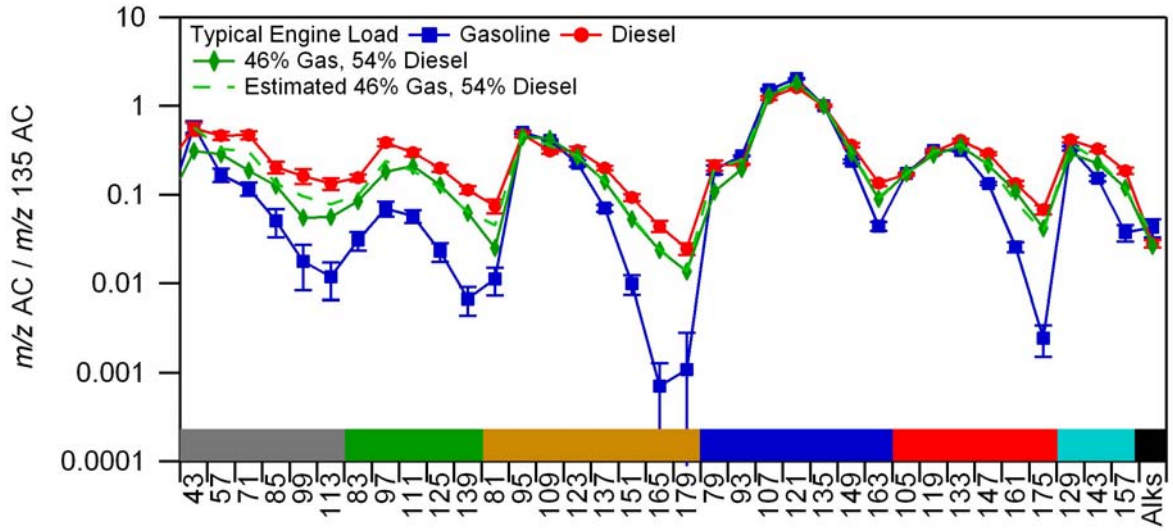
Additionally, the WSU collaborators sampled the test atmospheres with a high resolution time-of-flight aerosol mass spectrometer (HR-ToF-AMS) and a proton transfer reaction mass spectrometer (PTR-MS). The PTR-MS was coupled with a thermal desorption system for analyzing organic compounds with intermediate volatility (IVOCs). The HR-AMS and PTR-MS provided a much more detailed characterization of the particle- and gas-phase organic composition of the test atmospheres, which will yield improved understanding of the chemical characteristics and phase partitioning behavior of exhaust mixtures. Preliminary results from the experiments at LRRI were presented at the CLARC annual meeting and a manuscript has been recently submitted for review on the thermal desorption PTR-MS sampling methodology describing some results from LRRI exposure chamber sampling.

One result from the test chamber sampling is that it seems possible to quantify the relative amounts of diesel and gasoline exhaust in the mixture from analysis of IVOC compound distribution. Significant differences exist between gasoline and diesel engine exhaust IVOC

compound abundance, allowing for a chemical mass balance approach to determine relative contributions as shown in Figure 3. This analysis is useful for exposure chamber experiments and may be usefully applied to ambient air monitoring to better quantify the role of diesel engine emissions in organic compound concentrations in urban air.

Additional work was performed this year in determining PTR-MS fragmentation patterns for organic compounds found in vehicle exhausts and a manuscript is currently being written for submission to a journal this fall. Detailed analysis is ongoing combining results from the PTR-MS and HR-ToF-AMS to examine if gas to particle partitioning of organic constituents can be observed in the data. Multiple publications and presentations are expected in the coming year.

Figure 3. PTR-MS data showing relative abundance of ion signals to *m/z* 135 (C_4-alkylbenzene compounds) for pure diesel (red circle) and gasoline (blue square) exhaust and a mixture of the two (green diamonds). The relative abundance of the mixture can be well estimated (dashed line) from the fractional contributions of each exhaust. Colored bars indicate different compound groups: alkenes (grey), cyclolaknes (green), bicycloalkanes (orange), alkylbenzenes (blue), naphthenic monoaromatics (red), naphthalenes (turquoise), C_{10}-C_{17} alkanes (black).



Relatively few problems have been encountered to date that have required any modifications in the project aims. After consulting with the Biostatistical Core, we determined that more passive samplers were 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 43 in each city. A standardized vehicle platform also was needed to improve logistics of the field sampling and to improve data QC. We attempted to use a hybrid vehicle to enable a more accurate measurement of roadway pollutants in traffic. However, this was not possible because most rental companies either don't offer a hybrid or have very few available. The same make/model vehicle (Ford Escape) is rented in each city during the measurement sessions.

Publications / Presentations / Posters

Publications to Date:

1. **Erickson MH, Gueneron M, Jobson BT. Measuring Long Chain Alkanes in Diesel Engine Exhaust by Thermal Desorption PTR-MS. Atmospheric Measurement Technology Discussions 2013 (submitted).**

Presentations to Date:

1. **Jandarov, R. A Novel Principal Component Analysis for Spatially-Misaligned Multivariate Air Pollution Data. Joint Statistical Meetings. Montreal Canada, August 2013.**

2. **Vedal S. Multipollutant Data and a Multivariate Modeling Approach for Comparing Cardiovascular Health Effects of Contrasting Air Pollution Mixtures. Symposium (Multipollutant Exposure Metrics and Their Application to Air Pollution Epidemiological Studies). ISES Annual Meeting. Seattle, WA, October 2012.**

3. **Vedal S, Szpiro AA. Methods for Estimating Health Effects of Multipollutant Mixtures in Cohort Studies. ISEE Annual Meeting. Barcelona, Spain, September 2011.**

Posters to Date:

1. **Banks LE, Simpson CD, Larson TV, Yost MG. Characterization of Traffic-Related Air Pollutants Near a Major Roadway in Albuquerque Using a Mobile Monitoring Approach. American Industrial Hygiene Conference and Exposition. Montreal, QC, Canada. May 2013.**

2. **Fintzi, J, Sheppard L. Detecting On-Road Emission Sources in Mobile Monitoring Data: A Novel Approach to Thinking About Air Pollution. University of Washington DEOHS Student Research Day. Seattle, WA. May 2013.**

3. Keller JP, Sheppard L, Szpiro AA, Sampson PD. Spatial Analysis of a Marker of Roadway Emission Aging. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

Future Activities

Activities in the next year will focus on analysis of data from the field sampling campaigns, completing the chamber characterization studies, and assembling the data set for further analysis.

We have completed most of the field work on target and will be conducting a cross-center collaboration study with the SCAPE center in Atlanta in September of 2013, using the mobile platform. We also have scheduled the chamber characterization studies in Seattle/UW for October –November of this year. Data cleaning and QC review are underway for the cities that already have been sampled, and working with the Biostatistics core we have partially automated the data QC and review process. Work on publications and dissemination of results for the first year of measurements is underway and nearly ready.

Supplemental Keywords

Exposure science, Community Exposures, Chemical Transport, Mobile Monitoring

Relevant Web Sites

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

Project 2

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

Investigator	Institution
Jacob McDonald (PI)	Lovelace Respiratory Research Institute

Objective of Research

Objectives/Hypothesis: Traffic-related emissions are associated with the incidence and progression of acute and chronic cardiovascular sequelae in human population studies; however, the causal components, subsequent chemical transformation of these components, and their associated toxicity on the cardiovascular system have not yet been determined. Project #2 is in progress to develop atmospheres with the primary objective of simulating environments containing key components of roadway emissions and the products of environmental factors that transform them. Previous, current, and future exposures are designed to determine air contaminants (or components) that cause or potentiate the toxicity of roadway emissions or confound interpretations based on roadway proximity alone.

Approach: This project will generate and characterize multiple complex roadway mixtures for subsequent animal and human exposure-related toxicology studies. In **Aim 1**, we will develop and characterize laboratory-generated exposure atmospheres simulating the key components of near-roadway exposures, including transformed emissions and coexposures. **In Aim 2**, we will

conduct inhalation exposures of laboratory animals (as described in Project 3). Lastly, **in Aim 3**, we will conduct inhalation exposures of human subjects in an effort to compare significant pathophysiological findings from our animal model exposures to responses in humans.

Expected Results: Results from these studies will identify key components, as well as the most potent combinations, of urban roadway and background copollutants that result in toxicological responses in the cardiovascular system of both rodents and humans.

Research Performed - Progress Summary/Accomplishments

This year we completed subchronic inhalation exposures to verify the impact of component/gas mixtures. Further, in collaboration with Project 3 we spent a considerable effort focused on assay development for the bioassays. Finally, in consideration of the next round of experiments that are under consideration currently we overcame some technical hurdles for the conduct of the irradiation chamber experiments and also considered the potential use of Project 1 data for design of additional test atmospheres.

Completion of Exposures and Analysis to Tease Out Components

We conducted 50-day exposure of Apo E -/- mice , on a high fat diet, to the following chemistries: (1) MVE, 300 μg PM/m^3: 30 μg PM/m^3 derived from a gasoline engine combined with 270 μg PM/m^3 derived from a diesel engine; (2) MVE at the 300 μg PM/m^3 concentration with PM filtered; (3) MVE at the 300 μg PM/m^3 concentration with gases filtered, using a denuder; (4) MVE at the 300 μg PM/m^3 concentration with NOx scrubbed out; and (5) filtered air (controls). Studies were conducted to evaluate atmospheres that would allow us to 'tease' out the role of gases versus particles in novel ways, and that further evaluate the role of physical aging of motor vehicle exhaust. Studies were completed with test atmospheres to evaluate:

- Mixed motor vehicle exhaust
- Mixed motor vehicle exhaust minus particles
- Mixed motor vehicle exhaust minus gases (includes particles)
- Mixed motor vehicle exhaust minus NOx and ultrafines (simulates downwind)
- These atmospheres were developed to address key CCAR questions related to transformation and multipolltant components that are most important for toxicity. To develop atmospheres minus particles, HEPA filters are used. The atmospheres remove 99 % of the particles and permit the gases to pass through. The atmosphere with the gases removed was developed with the use of the HARVARD parallel plate denuder. The denuder was loaned to CCAR from the Harvard CLARC. This denuder allows the removal of 95% of all gases with only small (<5%) particle loss, mostly in the ultrafine range. A fourth condition uses the DRI cobalt oxide denuder (see below) to remove NOx and ultrafine particles. The NOx denuder removes 95 % of the NOx and allows other gases to pass through. It also removes the smallest fraction of particles that may agglomerate and be removed in close proximity to roadways. Figures 4 and 5 illustrate the change in particle size resulting from the denuder.

The overall composition of major components from those test atmospheres are described in Figure 1 below. Atmosphere development and characterization activities included the development of test atmospheres that further characterized the gas:particle partitioning and atmospheric processing. The motivation for this work was driven by guidance from the oversight committee, which wanted us to further investigate previous findings of enhanced vascular response after exposure to the mixture of gasoline and diesel exhaust. The hypothesis was that the combination of particle enriched and highly sorptive diesel exhaust with the vapor hydrocarbons and inorganics enhanced the toxicity, perhaps through increase in the delivered dose of materials to the deep lung. Several atmospheres and atmospheric characterization experiments were conducted to better elucidate these findings. These were 50 day experiments and a number of molecular endpoints were evaluated. These are described in the Project 3 report.

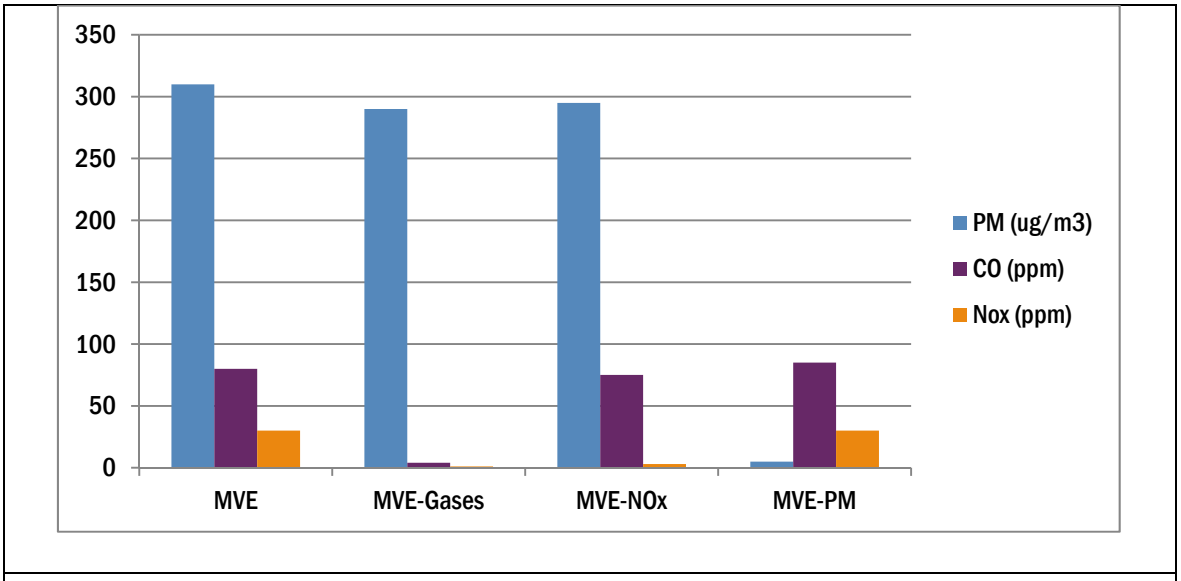


Figure 1. Composition of Test Atmospheres

Overcoming Technical Challenges to Evaluate Irradiated Atmospheres:

One of the atmospheres that we have targeted is in consideration of the atmospheric aging of motor vehicle exhaust. This atmosphere helps to evaluate the potential for aging to alter the biological potency of motor vehicle emissions, and also correlates to the work that is conducted at Harvard and EPA to consider the impact of aging on biological response. The LRRI irradiation chamber has been characterized and used extensively for the evaluation of secondary organic aerosol produced from single component mixtures.

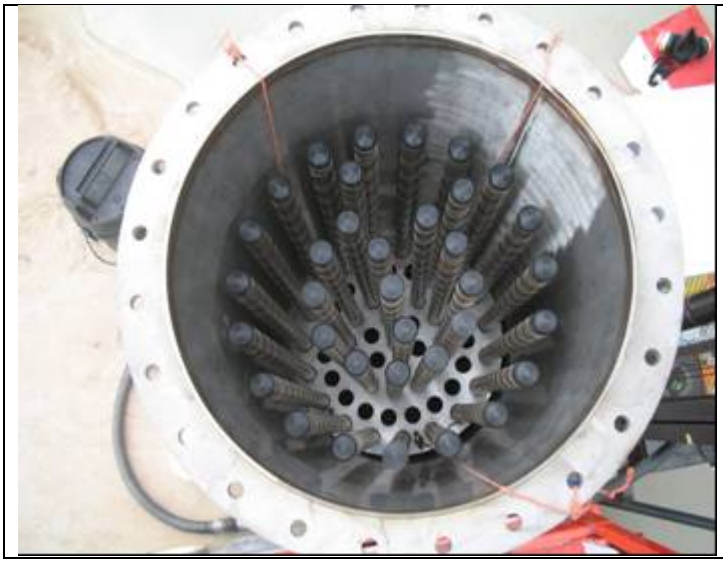
The challenge for motor vehicle exhaust is the large amounts of inorganic gases, especially NOx, that can titrate the chemistry of the test atmosphere is present in large amounts. An ideal ratio for the test gases in an irradiation chamber are approximately 10:1 hydrocarbon to NOx. Further, it is ideal that the concentration of hydrocarbon in these test atmospheres are no more than approximately 1-2 ppm as a starting concentration. Out of the tailpipe, there is an approximate 3:1 ratio of NOx:hydrocarbons. In order to overcome this problem, and to optimize

the ratio of NOx to hydrocarbons, LRRI implemented a cobalt-oxide coated denuder (See image below). This device, developed at the Desert Research Institute, removes NOx while allowing the hydrocarbons and particles to pass through.

A second challenge was in modulating the flow/air balance requirements to move material from the exhaust, to a dilution tunnel, through the denuder and then to the irradiation chamber. There are significant differences in the flow requirements for each of these stages. The flow in the denuder requires approximately 150 liters per minute. Further, the flow through the irradiation chamber must be at 30 liters per minute in total, including the addition of flow for system humidification. We met this challenge by meeting the flow requirements of the denuder, and then subsequently by creating a bypass the removed excess denuded aerosol. The denuded aerosol that would transit to the irradiation chamber was transited to the exposure chamber with a positive displacement roots blower. This allowed us to create the desired test atmosphere under all of these limitations.

Another challenge that occurred with the irradiation chamber is the potential time that it would take to conduct the experiments as originally designed versus the complications of a) running an engine for 2 months consecutively and b) maintaining the 'charge' of the denuder during that time. The irradiation chamber requires that the hydrocarbons and NOx are supplied 24 hr per day. This can be accomplished for a short amount of time, but would be expensive and not feasible for a long duration of time. In addition, the NOx denuder would get saturated with that much use, and without a break we would not be able to recharge it.

Because of these challenges, we considered the irradiation chamber experiments, as designed, would need to be conducted over a short duration (maximum 7 days). To meet this, we worked in collaboration with Project 3 to develop meaningful acute bioassays. These are in the process of being implemented for this atmosphere now.



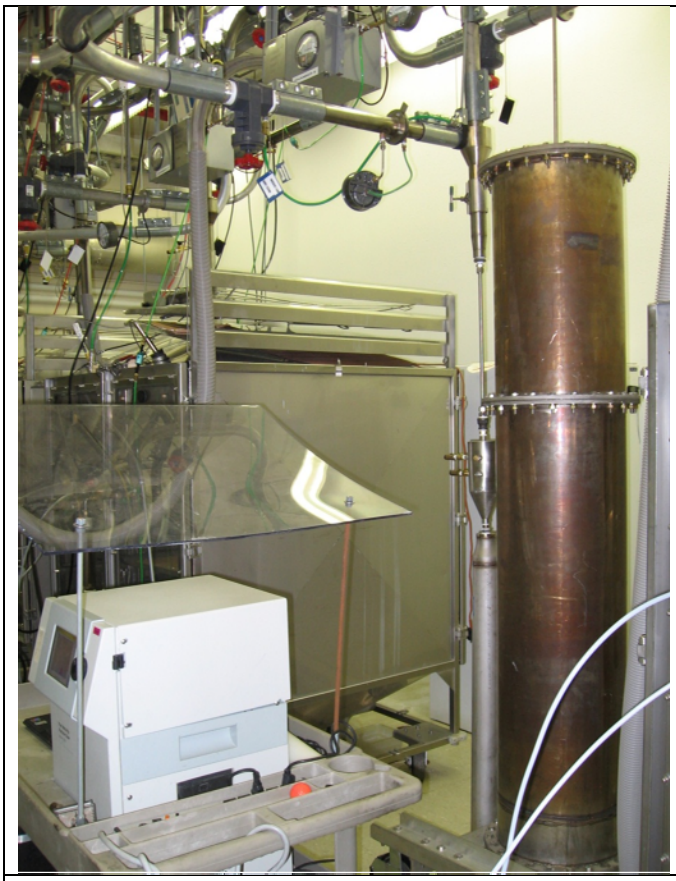
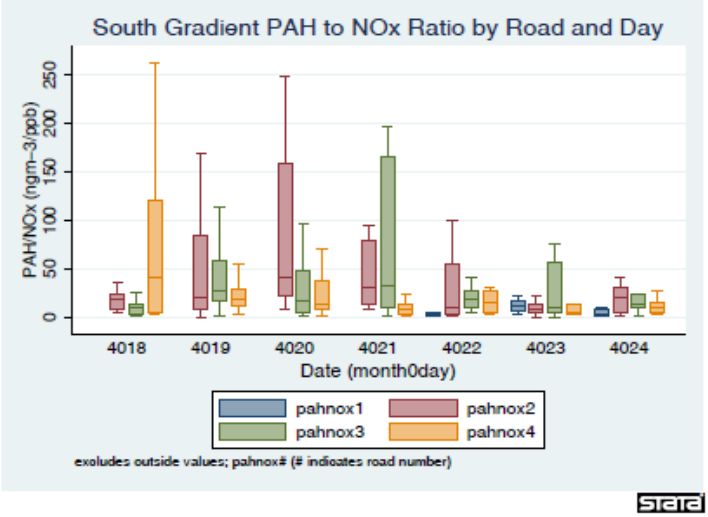


Figure 2. NOx denuder used to remove NOx from motor vehicle exhaust.

Consideration of Project 1 Data for Test Atmosphere Design

Part of the design of the Center was to integrate projects to help design experiments. The initial design of test atmospheres considered an approach where a priori we would evaluate the potentially useful components of roadway emissions and their transport on the change in composition and toxicity that we could model in the laboratory. We have developed on combinations on several of the important urban gas mixtures to meet this a priori goal, and are considering those for studies currently.

We also considered some of the ambient data from Project 1 in the design of test atmospheres. Figure 3 below shows a transect through Albuquerque from a roadway, and a representative set of data (PAH/NOx). As indicated, there were some interesting differences as one transected away from the road. However, it is unclear if these differences would provide enough of a contrast to truly elucidate biological differences in the magnitude of response. Because of this, we have considered the use of the ambient data for the design of toxicology experiments more as a tool in placing the results/atmospheres in context of what they model as opposed to determining how we approach the test atmospheres. This may be a topic of discussion for the annual meeting.



Publications / Presentations / Posters

Publications to Date:

1. **McDonald JD, Chow JC, Peccia J, Liu Y, Chand R, Hidy GM, Mauderly JL. Influence of Collection Region and Site Type on the Composition of Paved Road Dust. Air Qual Atmos Health. 2013 (in press).**

Presentations to Date:

N/A

Posters to Date:

1. VanReken T, Jobson T. Chemical Characterization of the LRRI Exhaust Exposure Chambers by PTR-MS and HR-ToF-AMS: Early Results. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

Future Activities

The next round of studies will utilize the new short term bioassays to include the atmospheric reaction chamber and urban background studies.

Supplemental Keywords

Inhalation Toxicology, Diesel, Gasoline Engine

Relevant Web Sites

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

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

Objective of Research

Objectives/Hypothesis: 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 overlapping issues related to noise, socioeconomic status, ethnicity, etc., there is a need to better understand the biological plausibility 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.

Approach: This project will use complex roadway mixtures as generated and characterized in the laboratory. In **Aim 1**, we will 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), both in terms of driving systemic vascular oxidative stress**. In Aim 2**, we will examine effects of the emissions-induced oxidative modifications to endogenous phospholipids, in terms of 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. Lastly, **in Aim 3**, we will further explore the role of specific immune cell populations as participants in the innate and adaptive responses to emissions-induced phospholipid modifications. In this Aim, we will 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.

Owing to suggestions from the advisory committee, we have focused on the nature and bioactivity of circulating factors induced by pollutant exposures, as these appear to be ligands that interact with the scavenger receptors of interest in Aims 2 and 3. This has been an area of significant progress for the past year.

Expected Results: 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

The primary accomplishment of this past year was an investigation into the role of bloodborne ligands and their interaction with the CD36 scavenger receptor in driving systemic vascular effects resulting from ozone (O_3) exposure. O_3 was used as a model pollutant that has no direct access to the circulation, due to its high reactivity, and also because O_3 is an important contributor to the photochemical smog mixtures being developed in Project 2.

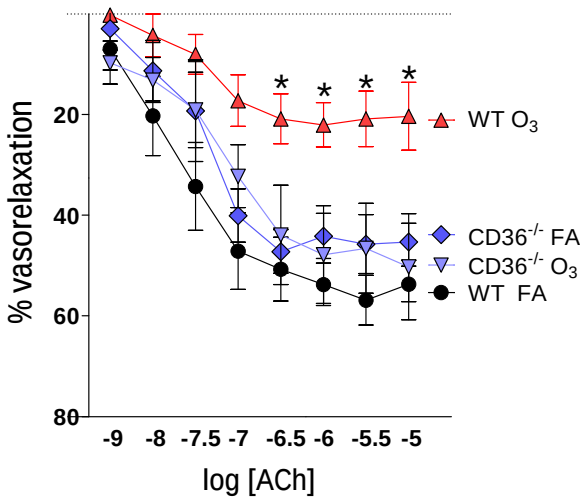


Figure 1. CD36$^{-/-}$ mice (blue) are protected from O_3-induced endothelial dysfunction compared to WT. * denotes difference from other groups ($P<0.05$).

In brief, wildtype (WT) or CD36-null mice were exposed for 4h to 1ppm O_3 and studies were conducted 24h post-exposure. Aortas were harvested and tested for relaxation responses to acetylcholine (ACh), and endothelium-dependent vasodilator. While O_3-exposed WT mice exhibited a reduction in the vasorelaxation response to ACh, CD36-null mice were protected (Figure 1). Follow-up studies revealed that CD36-null mice were also protected from pulmonary inflammation, which required the development of a novel method to test the contribution of circulating ligands on the vascular effects.

To accomplish this, we used a dilute serum mixture in the aortic ring baths, taking serum from exposed mice and treating aortas from unexposed mice. When we used unexposed WT aortas, we found that serum obtained from O_3-exposed mice – both WT and CD36-null – could impair aortic responses to ACh (Figure 2). However, when vessels were obtained from unexposed CD36-null mice, the circulating components failed to elicit a response. Combined, we concluded that 1) CD36 is involved in the pulmonary inflammatory response to O3; 2) neither inflammation nor CD36 are required for the generation of circulating vasoactive factors; and 3) those circulating factors inhibit vasorelaxation via a CD36-dependent pathway.

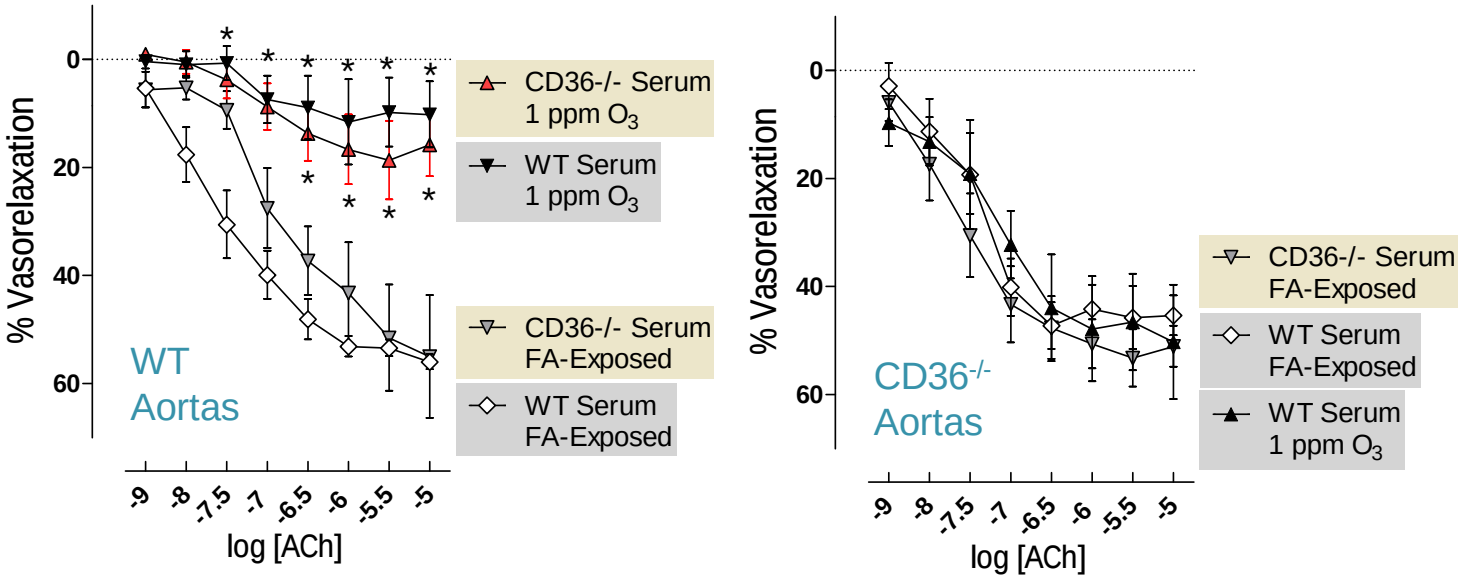


Figure 2. Left: Whole body inhalation of O_3 causes substantial loss of endothelial-dependent vasorelaxation compared to FA control. Pre-incubation of naïve arteries with serum from O_3-exposed mice (2.5% in PSS) impaired relaxation compared to arteries incubated with serum from FA controls. Right: Using CD36-null aortas led to a lack of effect from the serum from O_3-exposed mice.

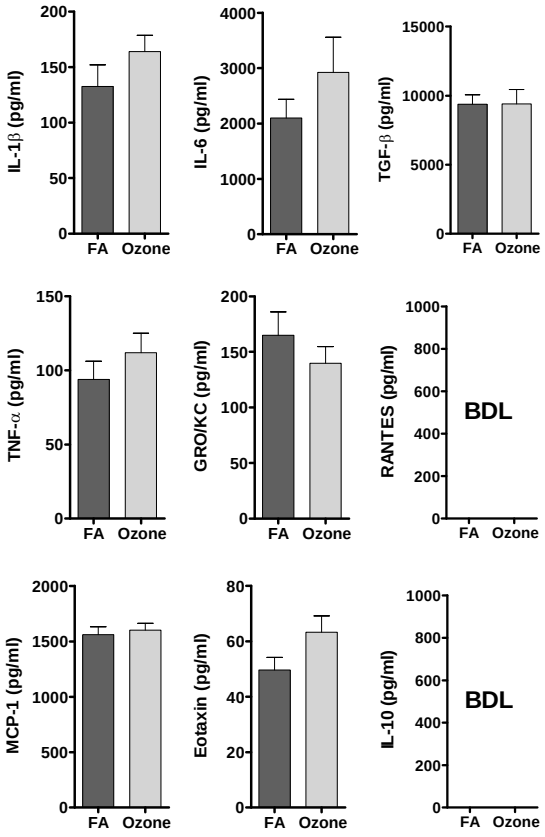


Figure 3. Serum cytokines are unaltered by 1ppm O_3 x 4h exposures, despite potent vasoactivity shown in Figure 2.

In attempting to ascertain the nature of chemical changes in the circulation following pollutant exposure, we have examined cytokine levels via multiplex assay (Millipore). O3 induced no changes in serum levels of all cytokines examined (Figure 3). Additionally, we examined 4-hydroxynonenal adducts on proteins and found those to be significantly elevated on a number of as-yet-unidentified proteins (bands on immunoblots). Proteomics work with Andrew Ottens of Virginia Commonwealth University has reveals a great number of altered small peptides (<5kD) in the serum, and we are investing efforts to link such changes with bioactivity.

Working closely with Project 2, we also implemented 2 long-term (50 day) studies to mixed vehicular emissions (combined gasoline and diesel exhausts). In one study, male ApoE-/- mice were exposed to whole MVE, MVE with particulate matter (PM) removed by filtration (MVE–PM), and MVE with gases removed by denudation (MVE–G). Analysis of those tissues is ongoing, but the results indicate a likely interaction between gases and particulate fractions of the whole emissions. For instance, the effects of MVE on vascular 3-nitrotyrosine and MMP9 protein levels was largely ameliorated by either denudation of gases or filtration of particles. MVE-mediated inflammation appeared reduced by denudation, but not PM filtration, though the statistical conclusions are hampered by a low subject number.

The second study examined the impact of MVE on older (18 months) versus younger (2 months) mice. These assays are still being conducted, but several important conclusions regarding vulnerability are arising. Among the more interesting findings was that serum-induced vasorelaxation impairments due to MVE exposure were only observed with serum from young mice (Figure 4), but mostly due to the fact that serum from old mice exposed to filtered air conditions also induced vasorelaxation impairments. That is, aging leads to the generation of vasoactive factors independent from environmental exposures. Other biological assays are pending, including cardiac function by SPECT/CT, cardiac gene changes by qPCR, and lavage endpoints.

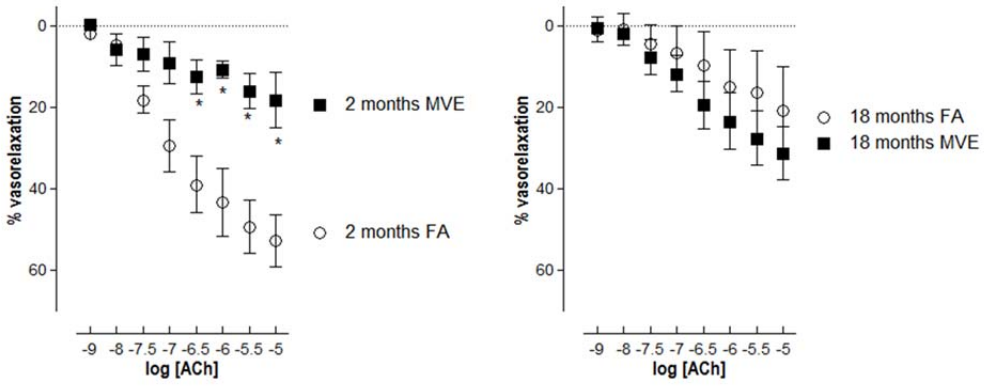


Figure 4. Vasorelaxation responses to serum from young (left) or old (right) mice exposed to filtered air or MVE. In both graphs, aortas were obtained from naïve (unexposed) young mice (2 months old). Serum from MVE-exposed young mice induces a significant impairment of vasorelaxation. However, serum from old mice, regardless of exposure, also induces impairments in vasorelaxation.

Publications / Presentations / Posters

Publications to Date:

1. **Lund AK, Doyle-Eisele M, Lin Y-H, Arashiro M, Surratt JD, Holmes T, Schilling KA, Seinfeld JH, Rohr AC, Knipping EM, McDonald, JD. The Effects of α-Pinene- vs. Toluene-Derived Secondary Organic Aerosol Exposure on the Expression of Markers Associated with Vascular Disease. Inhalation Toxicology 2013 (in press).**

2. **Robertson S, Colombo ES, Lucas SN, Hall PR, Febbraio M, Paffett ML, Campen MJ. CD36 Mediates Endothelial Dysfunction Downstream of Circulating Factors Induced by O3 Exposure. Toxicol Sci. 143(2):304-311, 2013.**

3. **Yin F, Lawal A, Ricks J, Fox JR, Larson T, Navab M, Fogelman AM, Rosenfeld ME, Araujo JA. Diesel Exhaust Induces Systemic Lipid Peroxidation and Development of Dysfunctional Pro-Oxidant and Pro-Inflammatory High-Density Lipoprotein. Arterioscler Thromb Vasc Biol. 2013 Jun;33(6):1153-61.**

4. Campen MJ, Lund A, Rosenfeld M. Mechanisms Linking Traffic-Related Air Pollution and Atherosclerosis. Curr Opin Pulm Med. 2012 Mar;18(2):155-60. PMID: 22189455.

Presentations to Date:

N/A

Posters to Date:

1. Campen MJ, McDonald JM, Rosenfeld ME, Lund AK. Cardiovascular Consequences of Immune Modification by Traffic-Related Emissions. Clean Air Research Centers Annual Meeting. Boston, MA, June 2012.

Future Activities

- Aim 1: Compare potency of mixed emissions and photochemically-transformed emissions in terms of serum inflammatory potential.

- Aim 2: Explore roles of TLR4 and LOX-1 with O3 responses, as per CD36. Consider all KO models with MVE exposures.

- Aim 3: Develop assays to measure monocyte response to serum factors, as a parallel to the endothelial assays.

Supplemental Keywords

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

Relevant Web Sites

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

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

** Italicized text is taken from the CCAR Year 2 Annual Progress Report, used for reference and perspective.*

Objective of Research

There has been significant discussion on the design and direction of Project 4 at both the Center and EPA levels. This has led to a schedule shift with respect to the start of the research but Project 4's active phase will be launched in this upcoming year. Currently, the basic aims have not changed from those described in the previous annual summary, however, significant or radical modification may be required dependent on further discussion.

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. Our External Scientific Advisory Committee suggested that we simplify our Project 4 protocol, and we are taking that suggestion seriously.

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 are currently conducting a different experimental protocol, with exposure to diesel exhaust, which we plan to have completed by Center Year 3. This 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.

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. We modified our consent process slightly to reflect the International Agency for Research on Cancer determination that diesel exhaust emissions represented a human carcinogen. We briefly suspended our exposures while awaiting approval of the new language. Our IRB approved the modification, and participants are being recruited and exposed again using the revised materials. We anticipate moving forward with IRB approval for the CCAR Project 4 protocol shortly, so that it is ready to proceed in Center year 3.

Publications/Presentations

N/A

Future Activities

During Year 3 of this project, we will launch the Center-sponsored experimental protocol on time and with all procedures adequately pilot-tested.

Supplemental Keywords

Relevant Web Sites

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

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 (Lianne) Sheppard	University of Washington
Paul Sampson	University of Washington
Adam Szpiro	University of Washington

Objectives of Research

Project 5 has three primary objectives, which are unchanged from those described previously:

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

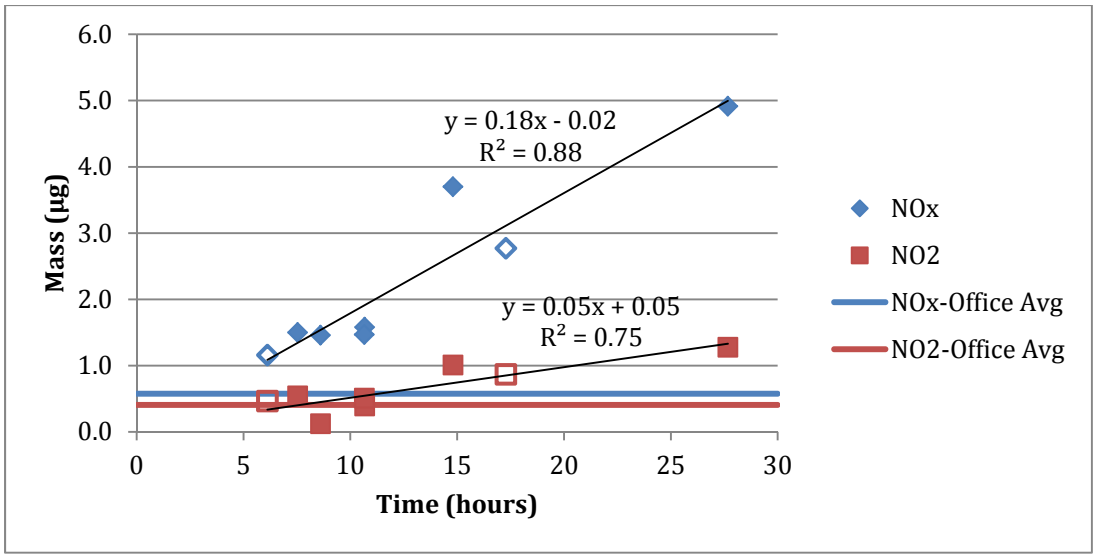
Over the past year, we have focused our efforts on the field work portion of this project, which will address signification portions of the second aim of this project. Specifically, through a combination of personal, residential and in-vehicle sampling, paired with intensive location tracking, we intend to understand the influence of time spent in transit on personal exposure,

which will improve our individual-level exposure estimates and contribute to our epidemiological analysis.

The field work component of this project is planned to occur twice in two seasons each in Winston-Salem and Los Angeles, and involves individual-level air monitoring in multiple microenvironments, GPS tracking over a relatively long duration, and proximity monitoring, each of which required unique methods for novel equipment development. Specifically, we have designed and built in-vehicle passive monitoring devices that capture exposures during driving. We have also designed and built proximity monitors, which record time spent in specific microenvironments (inside the residence and inside the vehicle), and we have customized off-the-shelf GPS units to allow continuous location tracking for periods up to and exceeding two weeks.

All of this equipment was tested during a series of pilot studies. In the first pilot study, we evaluated the ability of an external battery, connect to GPS units through a customized circuit-board, to provide sufficient data acquisition, data quality, and sampling duration. This configuration was successful and we determined that we could track participant locations continuously for up to a month. During a second pilot study, we evaluated the custom-built in-vehicle samplers, which consist of a stainless steel container fitted with a Teflon core and Ogawa and 3M VOC passive sampling badges. Our aims for this pilot study were to determine how much driving time was required to meet sample detection limits, to evaluate sample reproducibility, and to ensure that the equipment did not leak (i.e. that blank samples were, in fact, blank). This pilot study occurred in December of 2012, and included 20 samples (10 sets of duplicates). We observed generally high reproducibility among duplicates, low concentrations in blanks, and determined that detection limits were reliably exceeded in samples of participants with driving times of 30 minutes/day or greater over a two week period. We also observed that our measured concentrations were consistent with those observed in previous studies. A second pilot study was conducted in March of 2013 to ensure that the Teflon cores we were using were not acting as “sinks” for the pollutants, and these results taken together have provided confidence in our equipment.

Figure 1, below, shows the relationship between driving time (during which the samplers were open) and absolute mass (ug) of NO_X and NO_2 measured on the Ogawa filters during the two pilot studies. We observed fairly linear relationships between the time driving and pollutant mass. In this figure, filled symbols indicate samples collected during the first pilot study, and empty symbols indicate samples collected during the second pilot study. The solid colored lines show average concentrations observed in blank samplers stored in closed containers.



After confirming the suitability of our sampling equipment and the reliability of our methods, we conducted the first field campaign in Winston-Salem from January 27 – February 21, 2013. This campaign included 46 participants (96% of goal). We deployed 184 Ogawa and 184 3M samplers (46 each of personal, indoor residential, outdoor residential, and in-vehicle), and measured the following pollutants: oxides of nitrogen (NO_X), nitrogen dioxide (NO_2), ozone (O_3), sulfur dioxide (SO_2), pentanes, isoprene, n-nonane, n-decane, n-undecane, n-dodecane, benzene, toluene, m-xylene, and o-xylene.

We also deployed 17 blank samples (9%) and 13 duplicate samples (9% of possible maximum, as no personal duplicates were intended to be deployed, to reduce participant burden). The samplers were submitted to University of Washington laboratories in late February and we have received the laboratory results in the past week, but the data have not yet been analyzed. Table 1 (below) shows the demographic characteristics of 1) the subgroup who participated in the first CCAR Project 5 field campaign and 2)) the complete MESA Air cohort in Winston-Salem. As intended, the subgroup we recruited is fairly well representative of the Winston-Salem cohort as a whole.

Category	Project 5		W-S Cohort at Exam 5	
	Number	Percent	Number	Percent
Gender				
Male	20	43%	348	46%
Female	26	57%	415	54%
Race				
White, Caucasian	21	46%	413	54%
Black, African-American	25	54%	348	46%
Age Group *				
45-54	1	2%	8	1%
55-64	9	20%	236	31%
64-74	18	39%	256	34%
75-84	15	33%	215	28%
85+	3	7%	48	6%
Median Age	72		70	
Age range	54 - 89		54 - 93	

*At MESA Exam 5 (2010-2012).

Publications / Presentations / Posters

Publications to Date:

1. **Bergen S, Sheppard L, Sampson PD, Kim S-Y, Richards M, Vedal S, Kaufman JD, Szpiro AA. A National Prediction Model for Components of $PM_{2.5}$ and Measurement Error Corrected Health Effect Inference. Environ Health Perspect 2013 (in press).**

2. **Sun M, Kaufman JD, Kim S-Y, Larson T, Gould T, Polak JF, Budoff MJ, Diez Roux AV, Vedal S. Particulate Matter Components and Subclinical Atherosclerosis: Common Approaches to Estimating Exposure in a Multi-Ethnic Study of Atherosclerosis Cross-Sectional Study. Environ Health 2013; 12: 39.**

3. **Szpiro AA, Sheppard L, Adar SD, and Kaufman JD. Estimating Acute Air Pollution Health Effects from Cohort Study Data. Biometrics 2013 (submitted).**

4. Vedal S, Kaufman JD. What Does Multi-Pollutant Air Pollution Mean? Am J Resp Crit Care Med 2011; 183: 4-6.

Presentations to Date:

1. **Vedal, S. Estimating Exposure and Health Effects of PM2.5 Components. Fudan School of Public Health. Shanghai, China. June 2013.**

2. **Sullivan, MD. Ambient Transition Metals, Lung Density And Lung Function In The Multi-Ethnic Study Of Atherosclerosis (MESA). American Thoracic Society International Conference. Philadelphia, PA, May 2013.**

Center Posters to Date:

N/A

Future Activities

The next field campaign will be during the "non-heating" season in Winston-Salem in August 2013. This will be followed by two campaigns in Los Angeles, scheduled for February and June of 2014. We have complete IRB approval at the University of Washington and Wake Forest University for all of these activities and have recently applied for IRB approval at UCLA.

In addition to focusing a significant amount of effort on these field campaigns, our immediate next goals are to analyze the data collected in the first Wake Forest sampling campaign to understand the relative importance of time spent in transit to total personal exposure. This will help us determine how to generate individual exposure estimates for all MESA Air participants, for use in epidemiological analyses.

Supplemental Keywords

Cardiovascular Disease, Subclinical

Relevant Web Sites

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

CCAR CLARC Program Collaborations

CCAR is committed to participating in four of the five CLARC collaborative projects that have been proposed. The individual projects, the investigators from the CCAR team and approximate time periods and total budgets are included in the table below. Additionally, brief summaries are included detailing the progress to date and future planned activities.

UW CCAR Involvement in the CLARC Collaborative Projects				
PROJECTS	**CCAR Investigators**	**Activities**	**Period**	**Estimated total budget**
#1 Circulating Inflammatory Potential of Inhaled Coarse PM	Matt Campen Jake McDonald	• Ex Vivo Endothelial Cell Assays • High Fructose Rat Model in Designed Exposure Atmospheres	10/12-11/13	$75,000
#2 Mobile and Fixed Site Characterization of Vehicle Emission Impacts in Atlanta	Tim Larson Mike Yost	• Mobile and Fixed Site Monitoring Campaign in Atlanta	10/12-11/13	$45,000
#3 Measurement Error for Air Pollution Cohort Studies: Application and Comparison of Several Statistical Methods to Georgia Birth Cohort Data	Adam Szpiro	• Measurement Error Correction Approach to Georgia Birth Cohort	12/12-11/14	$40,000
#4 Inter-comparison of ambient $PM_{2.5}$ estimation models in NC	Paul Sampson	• Satellite PM Metric Addition to the PM Spatio-Temporal Model in North Carolina	12/12-11/13	$10,000

Collaborative Project #1 Summary – Circulating Inflammatory Potential of Inhaled Coarse PM

(Collaborators: GLACIER, Harvard, and CCAR)

Interactions with the GLACIER program are two-fold. First, we have obtained serum samples from Rob Brook at U. Mich. from his human exposures to coarse PM. With these samples we are currently testing a battery of inflammatory potential assays. Data from these studies should be forthcoming in the 4th year of funding. Second, we will be sending tissues to Jesus Araujo to analyze HDL dysfunction and oxidized lipids in serum and bronchoalveolar lavage fluid. Plans for this are still being prepared, in terms of which pollutant atmospheres and which specific animal models to use, but studies will again be undertaken in year 4. These will provide important clues into the nature and origin of the circulating vasoactive species.
We have identified a biological chemist at Virginia Commonwealth University, Andrew Ottens, PhD, who has been examining the proteomic changes in serum following exposures. In the coming year, we will publish his findings related to peptide fragmentation in parallel with

bioactivity related to vascular dysfunction, loss of endothelial barrier integrity, and neuroinflamamtion.

Collaborative Project #2 Summary – Mobile and Fixed Site Characterization of Vehicle Emission Impacts in Atlanta

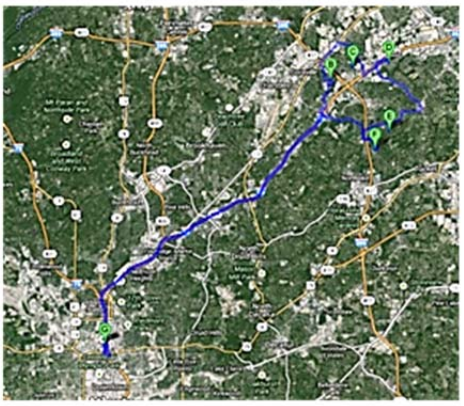
(Collaborators: SCAPE and CCAR)

Investigators: Timothy Larson,[1,2] Chris Simpson,[1] Timothy Gould[2], Kris Hartin[1], Miyoko Sasakura[1], Michael Yost[1] Departments of (1)Environmental & Occupational Health Sciences, and (2) Civil & Environmental Engineering , University of Washington;

Rodney Weber[3], Vishal Verma[3], Laura King[3], Ted Russell[4], Jim Mulholland[4], Heather Holmes[4], Eric Edgerton[5] Schools of (3) Earth & Atmospheric Sciences and (4) Civil & Environmental Engineering, Georgia Institute of Technology; (5) Atmospheric Research and Analysis Inc.

Project Goals

This collaborative project will perform mobile platform and fixed-site monitoring in greater Atlanta, GA over a two-week period in September, 2013. These measurements will obtain continuous fixed site measurements at a central location and 2-week average measurements of selected species at up to 20 fuzzy point locations in Atlanta during the same period. The mobile routes and measurements will be compared with enhanced CMAQ hourly model predictions on a 4km scale, and within grid cells, to 250m downscaled predictions performed with a land use regression model developed for the Atlanta region.



<= Figure 1: Sampling Loops in Atlanta.

Several conference calls have been made to establish the fuzzy point sampling locations, and the mobile routes. The Atlanta project team decided to focus on ~20 locations spread over 3 routes. Two routes will include gradient sampling sites, designed to capture pollutant gradients around a major roadway. These gradient sites will have both passive badges for gas phase components. The mobile route choices were influenced by points of interest within modeling domain

including stationary satellite monitoring sites.

The mobile platform will be continuously moving during the measuring periods, which are done from about 2-7 pm each evening. The mobile measurements are referenced to a fixed site which simultaneously collects data with a second set of instruments. Each sampling loop will be traversed on 3 to 4 different days (with perhaps within-day repeats of Loop 1) on a given night. Loops 1 and 2 are designed to capture both regional and small scale variability for comparison with CMAQ down-scaled predictions. Loop 3 is designed to capture regional scale variability within a CMAQ domain.

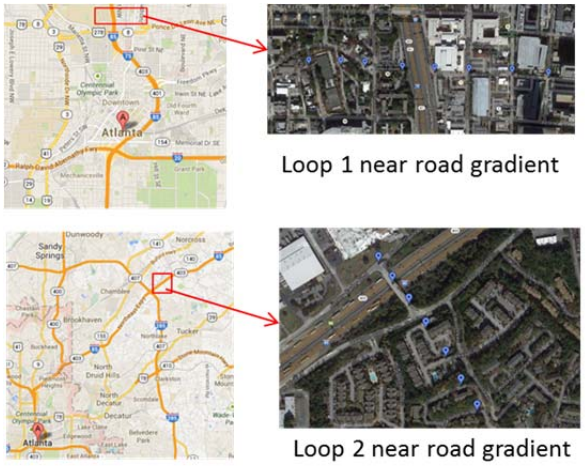
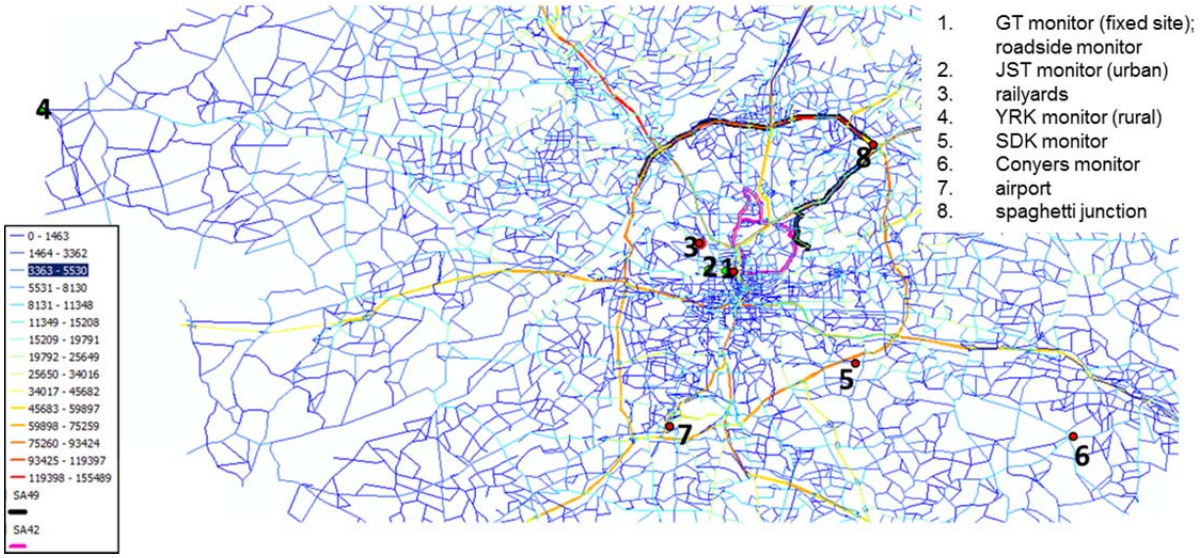


Figure 2: Sampling loop 1 and 3 will have near roadway gradient sites; these are designed to capture spatial gradients with both passive and mobile sample data. Mobile data provide hourly and daily information; passive samples will capture the average over the 2-week sampling period.

Figure 3: Overall sampling and modeling domain in Atlanta GA.



Collaborative Project #3 Summary - Measurement Error for Air Pollution Cohort Studies: Application and Comparison of Several Statistical Methods to Georgia Birth Cohort Data

(Collaborators: SCAPE, Harvard, and CCAR)

The objective of this project is to compare and contrast methods for measurement error with spatially misaligned exposure data in air pollution cohort studies. Investigators from the CLARCs at UWand Emory are currently participating in this project, and investigators from Harvard will participate in future activities.

We will utilize Emory's birth weight cohort data as a testbed for applying measurement error correction techniques developed at the three participating centers. The birth weight data will be derived from administrative records for all singleton live births in Georgia from 2002-2005. Exposures to PM2.5 will be predicted from spatio-temporal models based on regulatory monitoring in Georgia and nearby counties in surrounding states. An initial estimate of the association between PM2.5 exposure (by trimester) will be calculated without accounting for measurement error. Three versions of measurement error correction will be applied to this analysis: parameter bootstrap (UW), SIMEX (Harvard), and Bayesian (Emory). We will also conduct simulation studies to elucidate any differences in findings between the three correction methods.

Note that the scope of this project includes only single pollutant measurement error. Multi-pollutant methods are currently under development at the three centers, and future collaborations will build on the present project to compare and contrast these methods.

Progress has primarily been in the form of conference calls to clarify aims and scope of collaboration and a draft analytic plan (under revision based on ongoing email discussions) with the following features.

- Emory will consider using UW's MESA Air spatio-temporal to predict two-week average concentrations of $PM_{2.5}$
- Evaluating feasibility of fitting a single health model for all data vs. fitting separate models for smaller geographic units and combining in meta-analysis
- Evaluating different approaches to including meteorology in spatio-temporal model (e.g., average over two-week period, stagnation index, dispersion model output)

We understand that Emory has made significant progress on applying UW's spatio-temporal model to their data.

Project Deliverables

- Measurement error corrected findings to be incorporated in a substantive paper on the

risks of air pollution and low birth weight
- A statistical paper comparing and contrasting correction methods

Groundwork for Future Collaboration
- Review paper for applied readers that summarizes what is known about characterizing and correcting for measurement error in air pollution cohort studies, focusing on use of spatio-temporal model predictions (analogous to the Zeger et al. (EHP, 2000) paper for time series studies)
- Extension to multi-pollutant analyses and nonlinear models such as logistic regression & survival

Collaborative Project #4 Summary - Inter-comparison of ambient PM2.5 estimation models in NC

(Collaborators: SCAPE, Harvard, and CCAR)

The goal of this effort is to summarize the strengths and limitations of current satellite-driven PM2.5 exposure models and CMAQ PM2.5 simulations, and to identify directions for future model development and applications in various population-based health effects studies. There are six candidate models to be evaluated: (1) Koutrakis group's mixed effects model, (2) Schwartz group's multi-level model, (3) Chang's spatial downscaler,(4) Liu group's mixed effects model, (5) UW/CCAR group's spatiotemporal model, and (6) Russell group's CMAQ PM2.5 simulation. We propose to design a modeling domain centered in North Carolina for the 2006 – 2008 time period.

To facilitate model cross-comparison, a common input dataset will be compiled by Liu group and distributed to all participating research teams. A common master modeling grid at 3-km resolution will also be developed by Liu group and shared by all teams. A set of common procedures and statistics will be jointly developed by all participating teams to evaluate model performance. After preliminary results are generated, each team will document their model development in sufficient detail for other teams to reproduce their results. The estimated deliverable of this project will be a manuscript to report evaluation results.

As of July 2013, The Emory team has downloaded the MODIS collection 6 data at 3 km and 10 km resolution over North Carolina for the proposed study period through a collaboration with NASA's Goddard Space Flight Center. Computer codes are being developed to extract and format various AOD parameters and their QA flags. Preliminary data analysis is being done to evaluate the consistency and quality of various AOD parameters in this new product.