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

University of Washington CCAR Year 4 Annual Progress Report

Reporting Period: August 1, 2013 – July 31, 2014

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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 fourth year of funding to date [12/1/2013 – 7/31/2014] 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
Elizabeth Spalt – 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 to six 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.

- 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.
    - As reported in the Year 3 Annual Report, 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.
    - The Year 4 SAC Meeting will be held October 6-7, 2014.

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 7/26/13)*
Toxicology	Sanjay Rajagopalan	University of Maryland (Michigan State University CLARC Member)
Statistics	Brent Coull	Harvard University (Harvard University CLARC Member)
Clinical Studies	John Balmes	University of California, San Francisco (Committee Chair)
Clinical Studies	Nicholas Mills	University of Edinburgh, UK

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 4, 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.

- For file and data exchange, we have utilized an online drop box for non-sensitive external file sharing.

- For regular Investigators Meetings, we have utilized webinar programs including Microsoft Lync.

Subawards

- The Center's subawards for Lovelace Respiratory Research Institute (LRRI) and the University of New Mexico were renewed for Year 4. The subaward for Washington State University was extended into Year 4 in order to complete exposure 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.

Changes in Original Study Goals

None for this reporting period.

Challenges and Delays

- The EPA IRB indicated a preference to explore an alternative to the planned exposure chamber studies for Project 4. Instead of the original plan, Project 4 will utilize a typical commute exposure design, where participants will ride in a vehicle on the interstate with or without an operational filtration system in place. The original health outcomes proposed for Project 4 will be measured. The change in protocol has resulted in a delay in the start of this project. IRB approval was obtained from University of Washington in April 2014 and from USEPA in May 2014.

- 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), were originally delayed from April of 2013 to November of 2013 due to scheduling conflicts between the University of Washington, Washington State University, and LRRI. However, it has since been determined that the best use of Washington State University expertise is to conduct monitoring in Seattle using their field unit. These measurements will be conducted at a location along Project 4's typical commute route to help characterize near-road pollution.

Changes in Key Personnel

Admin Core – Mark Davey left University of Washington in August 2013. He was replaced as Center Manager by Elizabeth Spalt.

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 as the data intensive second half of the Center's award period continues.

- With the significant progress of Projects 1, 2, 3, and 5, and the start of Project 4, the CCAR QMP is continuing to undergo 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 fully document their activities. This documentation 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.

<u>Administrative Core</u>

- 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 4 and research activities seeing significant progress across almost all projects, a comprehensive quality review of all Center projects and activities is underway 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, 2014. 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 18, 2014 through February 17, 2015.

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

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

4. 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:

Past work performed under **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.

Recent work performed under **IACUC protocol #FY12-016, November 30, 2011**

1. Amendment E to protocol FY12-016 was approved 20 Sep 2013 to extend possible euthanasia time points post exposure.

2. Amendment F to protocol FY12-016 was approved 25 Nov 2013 to include additional exposure atmospheres.

3. Amendment G to protocol FY12-016 was approved 13 Jan 2014 to include additional mice for further experiments.

4. Amendment I to protocol FY12-016 was approved 22 Apr 2014 to change the strain of approved mice from C57Bl/6 to ApoE -/- mice.

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

Project 4 submitted their Human Subjects application to the University of Washington IRB on January 13, 2014 (Committee D). Approved by the University of Washington Human Subjects Division / Internal Review Board on April 10, 2014 (IRB #46658).

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, IRBs at both Wake Forest University and UCLA have fully approved all activities.

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. As requested by University of Washington Human Subjects also submitted as a separate IRB Application (IRB #44310, Committee EJ). Approved by the University of Washington Human Subjects Division / Internal Review Board on December 28, 2012. Also approved by the Wake Forest University Internal Review Board on December 20, 2012, as Amendment #11 for IRB study # BG05-006. Also approved by UCLA Institutional Review Board on September 16, 2013 as Amendment #13 for IRB #11-001546.

- Includes the CCAR Project 5 sampling campaigns for 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. Also approved by UCLA Institutional Review Board on September 16, 2013 as Amendment #13 for IRB #11-001546 (submitted with initial package to UCLA).

- 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.

Human Subjects / IRB Modification #4

Initially 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. Resubmitted at University of Washington IRB's request as Modification #4 to IRB #44310 and approved on October 17, 2013. Also approved by UCLA Institutional Review Board on September 16, 2013 as Amendment #13 for IRB #11-001546 (submitted with initial package to UCLA).

- 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 3 CCAR Annual Report

Current: 7/31/14

Center Publications to Date:

1. **Campen, M., Robertson, S., Lund, A., Lucero, J. & McDonald, J. Engine exhaust particulate and gas phase contributions to vascular toxicity. Inhal Toxicol 26, 353-360 (2014).**

2. **Paffett ML, Sheppard L, Robertson S, Weaver J, Lucas SN, Campen MJ. Ozone inhalation enhances coronary artery constriction and impairs dilation via superoxide-dependent mechanisms. Submitted to Toxicol Appl Pharmacol, 2014**

3. **Schisler J, Campen MJ, Madden M, and Willis MS. Transcriptional Endothelial Biosensor Response to Diesel-Induced Plasma Compositional Changes. In preparation.**

4. **Lindstrom J, AA Szpiro, PD Sampson, S Bergen, and L Sheppard. SpatioTemporal: An R package for spatio-temporal modeling of air-pollution. (Submitted to *Journal of Statistical Software).* 2014.**

5. **Kim SY, Dutton SJ, Sheppard L, Hannigan MP, Miller SL, Milford JB, Peel J, Vedal S. Components of fine particulate matter and daily mortality in the Denver Aerosol Sources and Health (DASH) study. (Submitted)**

6. **Kim S-Y, Sheppard L, Larson TV, Kaufman JD, Vedal S. Combining Multiple Data Sources for Epidemiological Analysis of Predicted Long-Term Exposures to PM2.5 Species in NPACT. Submitted**

7. **Kim S-Y, Sheppard L, Kaufman JD, Bergen S, Szpiro AA, Larson TV, Adar SD, Diez Roux AV, Polak JF, Vedal S. Individual-level concentrations of fine particulate matter chemical components and subclinical atherosclerosis: A cross-sectional analysis based on two advanced exposure prediction models in the Multi-Ethnic Study of Atherosclerosis. American Journal of Epidemiology, in press.**

8. **Kioumourtzoglou M, Spiegelman D, Szpiro AA, Sheppard L, Kaufman JD, Yanosky JD, Williams R, Laden F, Hong B, Suh H. Measurement error in PM2.5 health effects studies: A pooled analysis of eight personal exposure validation studies. Environmental Health, 2014 Jan 13;13(1):2. PMCID: PMC3922798**

9. **Vedal S, Adar S, Bergen S, Campen MJ, Curl C, Fox JR, Kaufman JD, Kim SY, Larson TV, Lund AK, Mauderly JM, McDonald JD, Miller KA, Sampson PD, Sheppard EA, Simpson CD, Szpiro AA. National Particle Components Toxicity (NPACT) Initiative: integrated epidemiological and toxicological cardiovascular studies to identify toxic components and sources of fine particulate matter. Research Report 178, Health Effects Institute, October 2013.**

10. **Gueneron, M., Erickson MH, VanderSchelden G., Jobson BT., PTR-MS Fragmentation Patterns of Gasoline Hydrocarbons, submitted, International Journal of Mass Spectrometry, June 2014.**

11. **Riley, EA, L Banks, J Fintzi, TR Gould, K Hartin, L Schaal, M Davey, L Sheppard, T Larson, MG Yost, and CD Simpson. Multi-pollutant mobile platform measurements of air pollutants adjacent to the I-40 corridor in Albuquerque, NM. (submitted to Atmospheric Environment). 2014.**

12. **Oppenheim, HA, J Lucero, A-C Guyot, LM Herbert, JD McDonald, A Mabondzo, and AK Lund. Exposure to vehicle emissions results in altered blood brain barrier permeability and expression of matrix metalloproteinases and tight junction proteins in mice. Particle and Fibre Toxicology. 2013. 10:62.**

13. **Spalt, EW, CL Curl, RW Allen, M Cohen, SD Adar, K Hinckley Stukovsky, Ed Avol, Cecilia Castro-Diehl, C Nunn, K Mancera-Cuevas, and JD Kaufman. Time-Location Patterns of a Diverse Population of Older Adults: The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). (submitted to Journal of Exposure Science and Environmental Epidemiology). 2014**

14. **Olives C, Sheppard L, Lindstrom J, Sampson PD, Kaufman JD, and Szpiro AA. Reduced rank spatio-temporal modeling of air pollution concentrations in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Annals of Applied Statistics. 2013. (submitted).**

15. **Bergen S and Szpiro AA. Minimizing the impact of measurement error when using penalized regression to model exposure in two-stage air pollution epidemiology studies. Biostatistics. 2014. (submitted).**

16. **Keller JP, Olives C, Kim S-Y, Sheppard L, Sampson PD, Szpiro AA, Oron AP, Lindström J, Vedal S, and Kaufman JD. A unified spatiotemporal modeling approach for prediction of multiple air pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Environ Health Perspect. 2014. (submitted).**

17. **Hudda N, Gould T, Hartin K, Larson T, Fruin S. Emissions from an international airport increase particle number concentrations fourfold at 10 kilometers downwind. Environmental Science and Technology. 2014. epub ahead of print.**

18. **Galaviz VE, Yost MG, Simpson CD, Camp JE, Paulsen MH, Elder JP, Hoffman L, Flores D, Quintana PJE. Traffic Pollutant Exposures Experienced by Pedestrians Waiting to Enter the U.S. at a Major U.S.-Mexico Border Crossing. Atmospheric Environment. 2014. (accepted for publication).**

19. **McDonald JD, Doyle-Eisele M, Kracko D, Lund A, Surratt JD, Hersey SP, Seinfeld JH, Rohr AC, Knipping EM. Cardiopulmonary Response to Inhalation of Secondary Organic Aerosol Derived from Gas-Phase Oxidation of Toluene. Inhal Toxicol. 2012. 24(11):689-97. doi: 10.3109/08958378.2012.712164**

20. **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.**

21. Nicholas T. Contribution of the in-vehicle microenvironment to individual ambient source-derived NO2 exposure concentration. M.S. Thesis, University of Washington. 2014.

22. Spalt EW, Curl CL, Allen RW, Cohen M, Williams K, Hirsh JA, Kaufman JD. Factors influencing time-location patterns and their impact on estimates of exposure: The Multi-Ethnic Study of Atherosclerosis and Air Pollution. In prep.

23. 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.

24. J. L. Mauderly, D. Kracko, J. Brower, M. Doyle-Eisele, A.K. Lund, J.D. McDonald and S.K. Seilkop. The National Environmental Respiratory Center (NERC) Experiment in Multi-Pollutant Air Quality Health Research: IV. Vascular Effects of Repeated Inhalation Exposure to a Mixture of Five Inorganic Gases. Submitted to Inhalation Tox April 2014

25. Szpiro AA, Sheppard L, Adar SD, and Kaufman JD. Estimating Acute Air Pollution Health Effects from Cohort Study Data. Biometrics, 2014, 70 (1): 164-174. 5 [Epub 10 DEC 2013] PMCID: NIHMS540694

26. **Erickson MH, Gueneron M, Jobson BT. Measuring Long Chain Alkanes in Diesel Engine Exhaust by Thermal Desorption PTR-MS. Atmospheric Measurement Techniques. 2014. 7: 225-239.**

27. 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. 121(9): 1017-1025.

28. 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. August 2013.

29. 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.

30. Szpiro AA and Paciorek CJ. Measurement Error in Two-Stage Analyses, with Application to Air Pollution Epidemiology. Environmetrics. 2013. 24: 501-517.

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

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

33. 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. 6: 615-628.

34. 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. 6: 309-324.

35. 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. 2013. 143(2): 304-311.

36. 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.

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

38. 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.

39. 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. **M. Campen. Endothelial Cell Pattern Recognition Receptors, CD36 and LOX-1, Contribute to Responses to Pollution-Induced Circulating Factors. As part of the Role of Circulating Factors in Mediating Systemic Toxicity of Inhaled Substances Symposium, co-chaired by M. Campen. SOT Annual Meeting, Phoenix, AZ. March 2014.**

2. **Elena Austin. (2014) Identifying multi-pollutant spatial patterns in mobile monitoring data from Baltimore, MD using cluster analysis. Annual Symposium on Environmental, Occupational and Population Health, Semiahmoo, WA**

3. **Austin E., Larson T., Sheppard L., Yost M.. (2014). Pollutant variability and correlations in mobile monitoring data as compared to central site monitoring. EPA Air Sensors Workshop, Research Triangle Park, NC**

4. **Szpiro, A. Does more accurate exposure prediction necessarily improve health effect estimates? International Society for Environmental Epidemiology. Seattle WA, August 2014.**

5. **Jandarov, R. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

6. **Olives, C. Correcting for Spatial Measurement Error in Air Pollution Cohort Studies. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

7. **Keller, J. A Unified Spatiotemporal Modeling Approach for Prediction of Multiple Air Pollutants in MESA Air. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

8. **Sheppard L. Effects of Classical-Like and Berkson-Like Measurement Error on Inference. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

9. **Szpiro, A. Dimension reduction for spatially misaligned multivariate air pollution data. Joint Statistical Meetings. Boston MA, August 2014.**

10. **Bergen, S. Multi-pollutant measurement error in air pollution epidemiology studies arising from predicting exposures with penalized regression splines. Joint Statistical Meetings. Boston MA, August 2014.**

11. **Olives, C. Reduced-rank spatio-temporal modeling of air pollution concentrations in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Joint Statistical Meetings. Boston MA, August 2014.**

12. **Szpiro A. Measurement error in air pollution cohort studies. Planning workshop for RFPA on concentration-response function for adverse health effects of long-term air pollution exposure (invited participant). Health Effects Institute. Boston, MA, June 2014.**

13. **Sampson P. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data (work of Roman Jandarov), presented at the Multivariate Spatial Models workshop of the Pan-American Advanced Study Institute on Spatio-Temporal Statistics, Buzios, RJ, Brazil, June 2014.**

14. **Sampson P. The deformation approach to nonstationary spatial covariance**

modeling incorporating a partial warp parameterization of thin-plate splines, presented at the Pan-American Advanced Study Institute on Spatio-Temporal Statistics, Buzios, RJ, Brazil, June 2014.

15. **Riley EA, Banks L, Fintzi J, Gould TR, Hartin K, Schaal L, Davey M, Sheppard L, Larson T, Yost MG, Simpson CD. "Multi-pollutant Mobile Platform Measurements of Traffic-associated Air Pollutants adjacent to the I-40 Corridor in Albuquerque, NM " 97th Canadian Chemistry Conference and Exhibition in Vancouver, British Columbia, June 1 - 5, 2014.**

16. **Szpiro A. Multipollutant research: challenges and progress (invited panel discussant). Health Effects Institute Annual Meeting. Alexandria VA, May 2014.**

17. **Jadarov R. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data. Work-In-Progress webinar for the Clean Air Research Centers, University of Washington, Seattle, WA, USA, February 2014**

18. **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.**

19. **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.**

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

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

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

23. **T. Holmes, J. McDonald, P. Kuehl, D. Kracko. Characterization of the Blu E-Cigarette to Define the Composition of Inhaled Material. Presented (1202/302) at Society of Toxicology, Phoenix, Arizona, 2014.**

24. **M. Doyle-Eisele, A. Rohr, E. Knipping, A. Lund, J. Brower, J. McDonald. Secondary Organic Aerosols Generated from α-Pinene-Amine Mixtures: Effects on the Cardiovascular System. Presented (1222/322) at Society of Toxicology, Phoenix, Arizona, 2014.**
25. **J. McDonald, Influence of Collection Region and Site Type on the Composition of Paved Road Dust: It's Not Just Dirt!!! Presented (2312) at Society of Toxicology, Phoenix, Arizona, 2014.**

26. 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.

27. 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.

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

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

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

Center Posters to Date:

1. **Erin A. Riley, Miyoko D. Sasakura, Kris Hartin, Robert Crampton, Timothy Gould, Timothy V. Larson, Michael G. Yost, Christopher D. Simpson. "Principal Component Analysis of Snap-Shot Air Pollutant Measurements In Baltimore, MD" EPA annual Clean Air Research Center Annual Meeting July 25th & 26th, 2013 Seattle, WA**

2. **Erin A. Riley, Kris Hartin, Timothy Gould, Timothy V. Larson, Michael G. Yost, Christopher D. Simpson "Mobile measurements of near-highway air pollutant gradients" University of Washington/ UBC/SFU/ UVic Annual Symposium on Environmental, Occupational, and Population Health "Changing Environments and Population Health" January 9 – 10, 2014**

3. **Erin Riley, Elena Austin, Jonathan Fintzi, Timothy Larson, Michael Yost, Lianne Sheppard, Paul Sampson, Christopher Simpson. "Decoupling Regional and Local Sources in Mobile Monitoring of Air Pollutants" Student Research Day, Department of Environmental and Occupational Health, University of Washington, Seattle, May 29, 2014.**

4. **Bergen S, Chan SH, Kaufman JD, Sandler D, Sheppard L, Szpiro AA. Multipollutant measurement error in air pollution epidemiology. ISEE Seattle WA, August 2014.**

5. **Austin E, Larson T, Sheppard L, Yost M. (2014). Pollutant variability and correlations in mobile monitoring data as compared to central site monitoring. EPA**

Air Sensors Workshop, Research Triangle Park, NC, June 2014.

6. **Fintzi, J. Identification and Description of On-Road Emission Sources: Results from Seattle. University of Washington DEOHS Student Research Day. Seattle, WA. May 2014.**

7. **Riley E, Austin E, Fintzi J, Larson TV, Yost MG, Sheppard L, Sampson P, Simpson CD. Decoupling Regional and Local Sources in Mobile Monitoring of Air Pollutants. Student Research Day, Department of Environmental and Occupational Health, University of Washington, Seattle, May 29, 2014.**

8. **S-Y Kim, J.R. Fox, T.V. Larson, L. Sheppard, J.D. Kaufman, S. Vedal. Spatio-temporal modeling of PM2.5 source apportioned factors in one region of the Multi-Ethnic Study of Atherosclerosis. Joint Annual Meeting of International Society for Environmental Epidemiology, International Society of Exposure Science, and International Society of Indoor Air Quality and Climate. Basel, Switzerland (August 2013).**

9. **E. Riley, L. Banks, M. Yost, T. Larson, C.D. Simpson. Mobile monitoring of traffic associated air pollution. UW/UBC/SFU Joint conference on environmental, occupational and public health, Semiahmoo, WA (January 2014).**

10. **Fox JR, Cox DP, Drury BE, Gould TR, Kavanagh TJ, Paulsen MH, Sheppard L, Simpson CD, Stewart JA, Larson TV, Kaufman JD. Chemical characterization and in vitro toxicity of diesel exhaust particulate matter generated under varying conditions. Submitted**

11. **Adar SD, D'Souza J, Mendelsohn-Victor K, Jacobs DR, Cushman M, Thorne PS, Sheppard L, Burke GL, Daviglus M, Szpiro A, Diez-Roux AV, Kaufman JD, Larson TV. Long-Term Exposure to Coarse Particulate Matter, Inflammation, and Coagulation: A Cross-Sectional Analysis from the Multi-Ethnic Study of Atherosclerosis. Submitted**

12. **A. d'Aquino. M. Sasakura, C.D. Simpson. Investigating Stability of Ozone Samples: Ogawa Holding Time Experiment in Seattle, WA. 2012 SACNAS National Conference, Seattle, WA (October 2012).**

13. **Hartin, K. Comparison of Various Components of Diesel Combustion in Environmental Exposure Chambers. UW/UBC/SFU Joint conference on environmental, occupational and public health, Semiahmoo, WA (January 2014).**

14. **J.B. Brower, B.C. Moeller, M. Doyle-Eisele, J.D. McDonald, S. Stirdivant, M.J. Campen. Acute Inhalation Exposure to Mixed Vehicle Emissions Induces Serum Metabolite Changes Related to Oxidative Stress, Lipid Peroxidation, and Energy Metabolism. SOT Annual Meeting, Phoenix, AZ. (March 2014).**

15. **M. Campen, M. Aragon, and A. Erdely. Induction of Serum Inflammatory Potential by Pulmonary Exposure to Multi-Walled Carbon Nanotubes. American Thoracic Society. San Diego, CA (May 2014).**

16. **M. Campen, M. Madden, J. Schisler, and M. Willis. Transcriptional Endothelial Biosensor Response to Diesel-Induced Plasma Compositional Changes. American Thoracic Society. San Diego, CA (May 2014).**

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

18. 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.

19. 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.

20. 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.

21. 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.

22. 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 are:

1. Advise Center projects on data management and compilation
2. Ensure quality statistical design and analysis of Center research
3. Implement novel statistical methods that are required for Center projects: Develop an analytical framework for quantifying the health effects of different mixtures of air pollution components in a cohort study (Project 1 and Project 5)
4. Identify additional statistical methodological research that will advance Center projects
5. Communicate and disseminate Center findings

Research Performed - Progress Summary/Accomplishments

We summarize the activities to date of the Biostatistics Core by specific aim:

1. Advise Center projects on data management and compilation

This Core has continued to support Project 1's data management and compilation needs by providing two graduate student research assistants from Biostatistics (Jon Fintzi and LaNae Schaal) who have done extensive work compiling datasets from multiple monitoring instruments in each campaign, ensuring they are properly aligned temporally, running quality control checks, and maintaining documentation throughout all stages of the process. Each mobile monitoring dataset contains the pollutant data aligned by time of collection and two methods of identifying the spatial location of data collection (GPS, fuzzy point numbering). The LRRI chamber dataset is identified by experiment characteristics rather than spatial location. The fixed monitoring dataset is identified by time only.

All stages of the merging and data cleaning process have been completed for five campaigns: Baltimore mobile monitoring (Summer 2012), Baltimore mobile monitoring (Winter 2012), Seattle mobile (Fall 2012), Albuquerque gradient monitoring (Spring 2012), and Atlanta mobile monitoring (Fall 2013). In addition, the data from the individual instruments have been merged

by time and are being reviewed for quality in the remaining campaigns: Baltimore fixed monitoring (Winter 2012), LRRI chamber data (Spring 2012), Los Angeles mobile (Summer 2013), Los Angeles mobile (Spring 2013), Saint Paul mobile (Summer 2012), and Saint Paul mobile (Winter 2012). While the data cleaning process is being conducted, preliminary data sets and median summaries of the time aligned data have been released for preliminary analyses and proof of concept investigations.

2. Ensure quality statistical design and analysis of Center research

Project 1. The Core has provided extensive input to Project 1 analyses, both through staff support (Jon Fintzi) and through consultation of additional ongoing analyses by other members of the Project 1 team. Mr. Fintzi's early work focused on modeling the mobile monitoring data using a Gaussian process latent dictionary factor analysis framework. This effort, applied to a mobile monitoring dataset from Los Angeles, included characterization of the empirical spatial and temporal patterns in the data and an assessment of the extent to which they are separable. While a promising research avenue, the complexity of the dataset poses unique challenges that have led us to put this project on hold in favor of other research directions that are more tractable in scope. More recently Mr. Fintzi has conducted an analysis of a mobile monitoring data set collected in Seattle synthesizing data collected via the mobile platform's video log with the results of two statistical tools: principal component analysis (PCA) and hierarchical cluster analysis (HCA). The purpose of this analysis is to identify multi-pollutant signatures associated with visible emission sources observed within a subset of the data having high pollutant measurements. After sub setting the data, we used PCA to estimate combinations of pollutants as they co-occur in the subsisted data, and grouped similar measurements together using HCA to help identify broad classes of emission sources. Interpretation is aided by appealing to the platform's video record. Comparisons are also made to summaries of the PCA scores, stratified on various quantitative measurements based on the video such as whether trucks were visible and road type. A paper summarizing this work is being reviewed by coauthors.

Project 3. Dr. Sheppard has collaborated with Matt Campen on the paper *Ozone Inhalation Enhances Coronary Artery Constriction And Impairs Dilation Via Superoxide-Dependent Mechanisms*, being finalized for submission. This work quantifies the constrictive and dilatory responses in the coronary vascular bed to known agonists following O_3 exposure in order to better elucidate underlying pathophysiological mechanisms driving extrapulmonary toxicity. Dr. Sheppard collaborated with co-authors on the statistical and presentation aspects of the paper, specifically the approach to conducting the repeated measures ANOVA and refinements to the data display.

Project 4. The Biostatistics Core has supported the redesign of Project 4 which is poised to begin collecting data.

Project 5. This project will be the main thrust of activities for the Core in Year 5. In the past year we constructed preliminary spatial models of the Project 1 multi-pollutant badge data for the summer of 2013 collected in Baltimore, MD, with the goal of formulating a strategy for predicting multi-pollutant profiles based on Project 1 data at MESA Air subject locations. In this analysis, we had data for 14 pollutants measured at 43 monitoring locations: O_3, NO_2, NOx,

SO_2, pentane, isoprene, nonane, decane, undecane, dodecane, benzene, toluene, meta-xylene, and ortho-xylene. Our goal was to understand spatial predictability of each pollutant using GIS covariates at each location via cross-validation. To address the problem we considered two different approaches (see the discussion below under Aim 3 for more details). First we used a combination of partial least squares (PLS) and universal kriging (UK) in pollutant-specific prediction models. In this approach, PLS scores calculated for each pollutant were a lower dimensional representation of the GIS covariates that we then used to model the mean structure in UK. Second, as an alternative to PLS with UK, we did a two-step variable selection of GIS covariates, again treating each pollutant separately. We used elastic-net penalized regression in the first step of this selection algorithm. Given the selected variables from the first step (i.e. all variables with non-zero coefficients from the penalized regression), in the second step, we further selected the best subset of variables with the highest adjusted R-squared out of the set of variables identified in the first step. The best subset of GIS covariates was then fed into the UK algorithm as the mean structure. Based on results from the Baltimore dataset, the second approach (two-step variable selection followed by UK) resulted in high cross-validated R-squared (indicating good spatial predictability) and performed better than PLS followed by UK. As a next step in this work, we plan to apply our predictive (sparse) PCA approaches to multi-pollutant data from Project 1. Based on the results of our preliminary comparison, we plan to predict the principal scores at the MESA Air subject locations using the second approach. Ultimately, our goal is to use the predicted scores in a health analysis to understand the health effects of multi-pollutant mixtures.

Collorative project on Satellite Data.
The first CLARC annual meeting spawned the collaborative project to compare predictions of ambient PM2.5 using satellite-driven statistical models from the Harvard, SCAPE, and CCAR groups. Most of the models will incorporate MODIS satellite based Aerosol Optical Depth (AOD) measurements in statistical models. The aim is to summarize strengths and weaknesses of the current models and also incorporate comparisons with CMAQ PM2.5 simulations. A specific responsibility of the CCAR group was to provide our GIS-based suite of geographic covariates for use by all the research groups (for those models that involved geographic covariates). The spatial domain for this exercise is a region of approximately 600K km^2 centered on North Carolina and including monitoring data from 126 EPA monitoring sites for the period 2006-2008. Following a series of discussions, the CCAR/MESA Air data team computed and delivered a database of geographic covariates for the 10 km^2 satellite grid. CCAR will first apply the UW spatio-temporal model, without incorporating satellite data, to the task of PM2.5 prediction. This will be a baseline against which to assess the added value of the satellite data. We will then determine the feasibility of incorporating the AOD data as a spatio-temporal covariate, after determining the best procedure to cope with the highly incomplete spatio-temporal coverage of these data. CCAR funding was used to support the delivery of the geographic database and it will soon be called on to support the application and extension of the spatio-temporal model.

Collaborative project on Measurement Error. UW investigators have taken part in ongoing email discussions on the analytic plan for Emory's birthweight analysis, including logistical and methodological aspects of UW's spatio-temporal exposure model. Emory investigators have successfully applied UW's spatio-temporal exposure model in the Atlanta area and have used

predicted exposures to quantify the association between birthweight and PM2.5 exposure. Pending final sensitivity studies, this analysis is complete, and the next step is for UW, Harvard, and Emory investigators to apply their respective measurement error correction methods.

UW has not yet spent money on this project and expects to begin doing so in the coming year.

3. Implement novel statistical methods that are required for Center projects: Develop an analytical framework for quantifying the health effects of different mixtures of air pollution components in a cohort study (Project 1 and Project 5)

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:

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

Dimension reduction and spatial misalignment (3.1 and 3.2): Dimension reduction is often essential for estimating and interpreting associations between a health endpoint and a multivariate exposure, such as a mixture of air pollutants. Principal component analysis (PCA) and sparse PCA are commonly used dimension reduction methods that explain as much variability in the data as possible with linear combinations of a relatively small number of loading vectors (sparseness is desirable to make components more interpretable). The coefficients in this linear representation are known as principal component scores, and they can be used as low-dimensional representations in a health model.

Spatial misalignment occurs when we do not observe exposure data at locations of interest for a health analysis.

To deal with spatial misalignment, we initially tried a sequential approach where we reduce the dimension of the exposure data by sparse PCA (sparse PCA) at monitoring locations and use 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. To resolve this issue, we then developed new methods, termed predictive (sparse) PCA for spatially misaligned data. These methods identify (sparse) principal component loading vectors that explain as much variability in the observed data as possible, while also ensuring the corresponding principal component scores can be predicted accurately by means of spatial statistics at locations where observations are not available. This will make it possible to identify important mixtures of air pollutants and to quantify their health effects in cohort studies, where currently available methods cannot be used.

We demonstrated the utility of predictive (sparse) PCA in simulated data and in an application to annual averages of particulate matter speciation data from national Environmental Protection Agency (EPA) regulatory monitors (CSN and IMPROVE monitors). A manuscript based on this

work is nearly complete and will be submitted to the *Journal of the American Statistical Association: Applications and Case Studies*. The current version of predictive (sparse) PCA 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. Future extensions of this work will include penalized and linked multivariate prediction models for different scores. In a complementary project we are developing methods for predictive k-means clustering.

Using our recently developed framework, we are currently working on understanding the health effects of multi-pollutant mixtures derived from our predictive (sparse) PCA approaches on systolic blood pressure (SBP) in the NIEHS Sisters Study. This builds on previous work in which we found an association between SBP and $PM_{2.5}$ in this cohort. Based on our preliminary results, we are finding that using IMPROVE monitors in addition to CSN for spatial prediction could introduce substantial bias in health estimates. We believe this is because the compatibility condition between monitoring and subject locations, a key condition for valid measurement error corrections when the exposure model is misspecified, is violated by the IMPROVE monitoring network design.

After completing our method refinement and analyses in the Sisters Study and CNS/IMPROVE setting where long-term exposure measurements are available (albeit spatially misaligned), we will take on the 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 (CCAR Project 5), with intra-urban exposure contrasts derived from Project 1 monitoring data.

Measurement error correction (3.3): We have developed a method for measurement error correction with spatially misaligned data, for the general setting where spatial prediction is done by a low-rank penalized regression model. We have extended this work to 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. We have applied our multipollutant measurement error correction to an analysis of PM2.5 and SBP in the Sisters Study and have a paper nearing completion.

4. Identify additional statistical methodological research that will advance Center projects

The process of identifying additional methodological research is most effective when it happens organically. Many fresh ideas are generated during discussions at our weekly Environmental (Bio)statistics Working Group meetings, often while we are critiquing progress and results from ongoing projects. In this report we have included our progress related to this aim in the summaries provided above.

5. Communicate and disseminate Center findings

The Core has been supporting and leading efforts to publish and otherwise disseminate UW CCAR research. Titles of specific publications and presentations are given below.

Publications / Presentations / Posters

Publications to Date:

1. **Bergen S and Szpiro AA. Minimizing the impact of measurement error when using penalized regression to model exposure in two-stage air pollution epidemiology studies. (Submitted)**

2. **Keller JP, Olives C, Kim SY, Sheppard L, Sampson PD, Szpiro AA, Oron A, Vedal S, Kaufman JD. A unified spatiotemporal modeling approach for prediction of multiple air pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Submitted**

3. **Kim SY, Dutton SJ, Sheppard L, Hannigan MP, Miller SL, Milford JB, Peel J, Vedal S. Components of fine particulate matter and daily mortality in the Denver Aerosol Sources and Health (DASH) study. (Submitted)**

4. **Kim S-Y, Sheppard L, Larson TV, Kaufman JD, Vedal S. Combining Multiple Data Sources for Epidemiological Analysis of Predicted Long-Term Exposures to PM2.5 Species in NPACT. Submitted**

5. **Lindström J, Szpiro AA, Sampson PD, Bergen S, Sheppard L. SpatioTemporal: An R Package for Spatio-Temporal Modeling of Air-Pollution. Submitted**

6. **Olives C, Sheppard L, Lindstrom J, Sampson PD, Kaufman JD, Szpiro AA. Reduced-rank spatio-temporal modeling of air pollution concentrations in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Submitted.**

7. **Fox JR, Cox DP, Drury BE, Gould TR, Kavanagh TJ, Paulsen MH, Sheppard L, Simpson CD, Stewart JA, Larson TV, Kaufman JD. Chemical characterization and in vitro toxicity of diesel exhaust particulate matter generated under varying conditions. Submitted**

8. **Adar SD, D'Souza J, Mendelsohn-Victor K, Jacobs DR, Cushman M, Thorne PS, Sheppard L, Burke GL, Daviglus M, Szpiro A, Diez-Roux AV, Kaufman JD, Larson TV. Long-Term Exposure to Coarse Particulate Matter, Inflammation, and Coagulation: A Cross-Sectional Analysis from the Multi-Ethnic Study of Atherosclerosis. Submitted**

9. Riley E, Banks L, Fintzi J, Gould T, Hartin K, Schaal L, Davey M, Sheppard L, Larson TV, Yost M, Simpson CD. Multi-pollutant mobile platform measurements of air pollutants adjacent to the I-40 corridor in Albuquerque, NM. Submitted

10. Kim S-Y, Sheppard L, Kaufman JD, Bergen S, Szpiro AA, Larson TV, Adar SD, Diez Roux AV, Polak JF, Vedal S. Individual-level concentrations of fine particulate matter chemical components and subclinical atherosclerosis: A cross-sectional analysis based on two advanced exposure prediction models in the Multi-Ethnic Study of Atherosclerosis. *American Journal of Epidemiology*, in press.

11. Szpiro AA, Sheppard L, Adar S, Kaufman JD. Estimating acute air pollution health effects from cohort study data. *Biometrics*, 2014, 70 (1): 164–174. 5 [Epub 10 DEC 2013] PMCID: NIHMS540694

12. Kioumourtzoglou M, Spiegelman D, Szpiro AA, Sheppard L, Kaufman JD, Yanosky JD, Williams R, Laden F, Hong B, Suh H. Measurement error in PM2.5 health effects studies: A pooled analysis of eight personal exposure validation studies. *Environmental Health*, 2014 Jan 13;13(1):2. PMCID: PMC3922798

13. Vedal S, Adar S, Bergen S, Campen MJ, Curl C, Fox JR, Kaufman JD, Kim SY, Larson TV, Lund AK, Mauderly JM, McDonald JD, Miller KA, Sampson PD, Sheppard EA, Simpson CD, Szpiro AA. National Particle Components Toxicity (NPACT) Initiative: integrated epidemiological and toxicological cardiovascular studies to identify toxic components and sources of fine particulate matter. Research Report 178, Health Effects Institute, October 2013.

14. 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 1-23, 2013. DOI: 10.1007/s10651-013-0261-4. PMCID: NIHMS520301

15. 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.

16. Szpiro AA and Paciorek CJ. Measurement error in two-stage analyses, with application to air pollution epidemiology (with invited discussion). Environmetrics, Vol 24, 501-517, 2013. PMCID: PMC3994141

17. 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.

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

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

Presentations to Date:

1. **Szpiro, A. Does more accurate exposure prediction necessarily improve health effect estimates? International Society for Environmental Epidemiology. Seattle WA, August 2014.**

2. **Jandarov, R. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

3. **Olives, C. Correcting for Spatial Measurement Error in Air Pollution Cohort Studies. International Society for Environmental Epidemiology. Seattle WA, August 2014.**

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6. **Szpiro, A. Dimension reduction for spatially misaligned multivariate air pollution data. Joint Statistical Meetings. Boston MA, August 2014.**

7. **Bergen, S. Multi-pollutant measurement error in air pollution epidemiology studies arising from predicting exposures with penalized regression splines. Joint Statistical Meetings. Boston MA, August 2014.**

8. **Olives, C. Reduced-rank spatio-temporal modeling of air pollution concentrations in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. Joint Statistical Meetings. Boston MA, August 2014.**

9. **Szpiro A. Measurement error in air pollution cohort studies. Planning workshop for RFPA on concentration-response function for adverse health effects of long-term air pollution exposure (invited participant). Health Effects Institute. Boston, MA, June 2014.**

10. **Sampson P. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data (work of Roman Jandarov), presented at the Multivariate Spatial Models workshop of the Pan-American Advanced Study**

Institute on Spatio-Temporal Statistics, Buzios, RJ, Brazil, June 2014.

11. **Sampson P. The deformation approach to nonstationary spatial covariance modeling incorporating a partial warp parameterization of thin-plate splines, presented at the Pan-American Advanced Study Institute on Spatio-Temporal Statistics, Buzios, RJ, Brazil, June 2014.**

12. **Riley EA, Banks L, Fintzi J, Gould TR, Hartin K, Schaal L, Davey M, Sheppard L, Larson T, Yost MG, Simpson CD. "Multi-pollutant Mobile Platform Measurements of Traffic-associated Air Pollutants adjacent to the I-40 Corridor in Albuquerque, NM " 97th Canadian Chemistry Conference and Exhibition in Vancouver, British Columbia, June 1 - 5, 2014.**

13. **Szpiro A. Multipollutant research: challenges and progress (invited panel discussant). Health Effects Institute Annual Meeting. Alexandria VA, May 2014.**

14. **Jadarov R. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data. Work-In-Progress webinar for the Clean Air Research Centers, University of Washington, Seattle, WA, USA, February 2014**

15. 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.

16. 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.

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

18. Jandarov, R. A novel dimension reduction approach for spatially-misaligned multivariate air pollution data. Joint Statistical Meetings. Montreal Canada, August 2013.

19. 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. **Bergen S, Chan SH, Kaufman JD, Sandler D, Sheppard L, Szpiro AA. Multipollutant measurement error in air pollution epidemiology. ISEE Seattle WA, August 2014.**

2. **Austin E, Larson T, Sheppard L, Yost M. (2014). Pollutant variability and correlations in mobile monitoring data as compared to central site monitoring. EPA**

Air Sensors Workshop, Research Triangle Park, NC, June 2014.

3. **Fintzi, J. Identification and Description of On-Road Emission Sources: Results from Seattle. University of Washington DEOHS Student Research Day. Seattle, WA. May 2014.**

4. **Riley E, Austin E, Fintzi J, Larson TV, Yost MG, Sheppard L, Sampson P, Simpson CD. *Decoupling Regional and Local Sources in Mobile Monitoring of Air Pollutants*. Student Research Day, Department of Environmental and Occupational Health, University of Washington, Seattle, May 29, 2014.**

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

Future Activities

Our plans for the remaining funding period include publishing work currently in progress, wrapping up our collaborative projects, and focusing on developing spatial models of the mobile monitoring data for application to Project 5 analyses. We will continue Core activities to support all projects on an as needed basis.

Supplemental Keywords

Environmental Policy, Exposure Modeling, Epidemiologic Inference, Health Effects, Air Pollution Exposure, Multipollutant, Measurement Error

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

Data collection related to aims 1 and 2 has been completed, and provided the main focus of activities up to the beginning of this reporting period; during this reporting period we have

focused on producing final QC's data sets for use by investigators in our project and other center investigators. This process has been completed for Baltimore and the 2-week passive sample data for the cities. We also completed a collaborative measurement campaign in Atlanta with the SCAPE center, and have provided preliminary data for this activity. The final QC data set from this campaign will be available by early fall of 2014. Field sampling in 4 cities in the MESA-Air cohort was completed: Minneapolis/St. Paul, MN, Baltimore, MD, Los Angeles, CA and Winston-Salem, NC. Due to financial constraints Winston-Salem only was 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 was conducted for both heating and non-heating seasons. 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. A paper describing this work titled "Multi-pollutant mobile platform measurements of air pollutants adjacent to the I-40 corridor in Albuquerque, NM" was submitted to Atmospheric Environment and is under review.

Figure 1 – Gradient sampling Data Collected in Albuquerque, NM

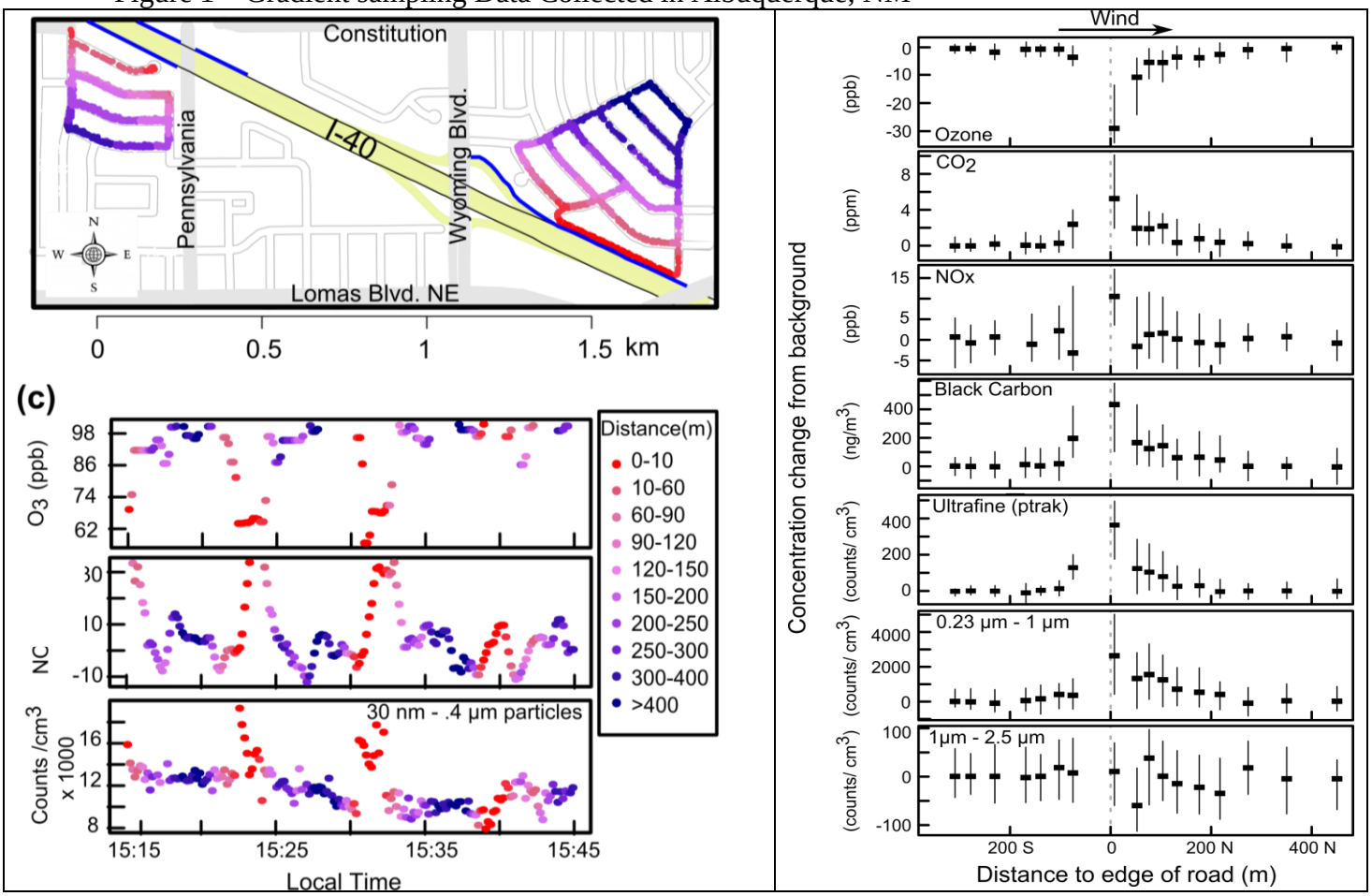


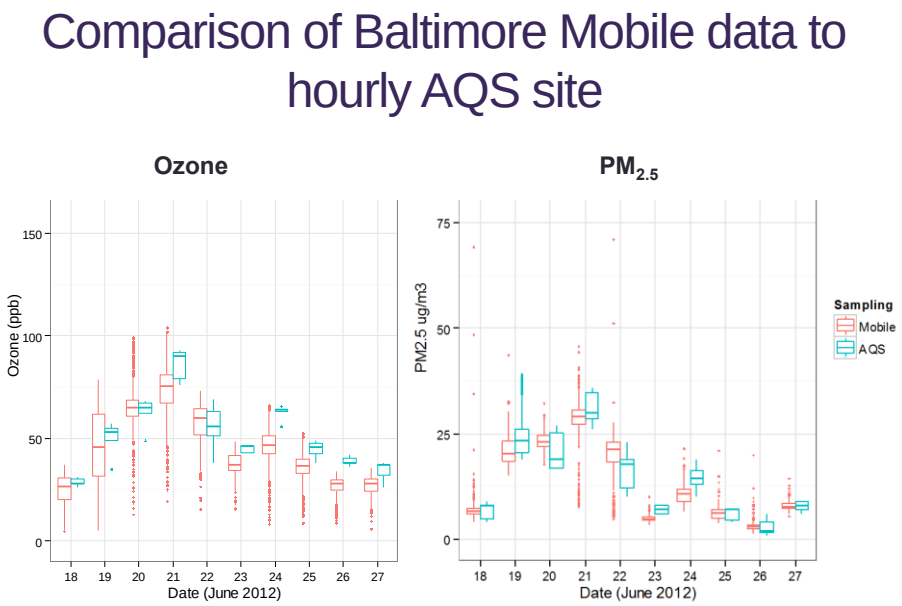
Figure one shows results from our gradient sampling tests in Albuquerque, NM over a one week period during May 2012. Mobile monitoring was used to repeatedly sample multi-pollutants near a major interstate roadway (I-40). The 10 sec mobile data collected near the interstate was classified into buffers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

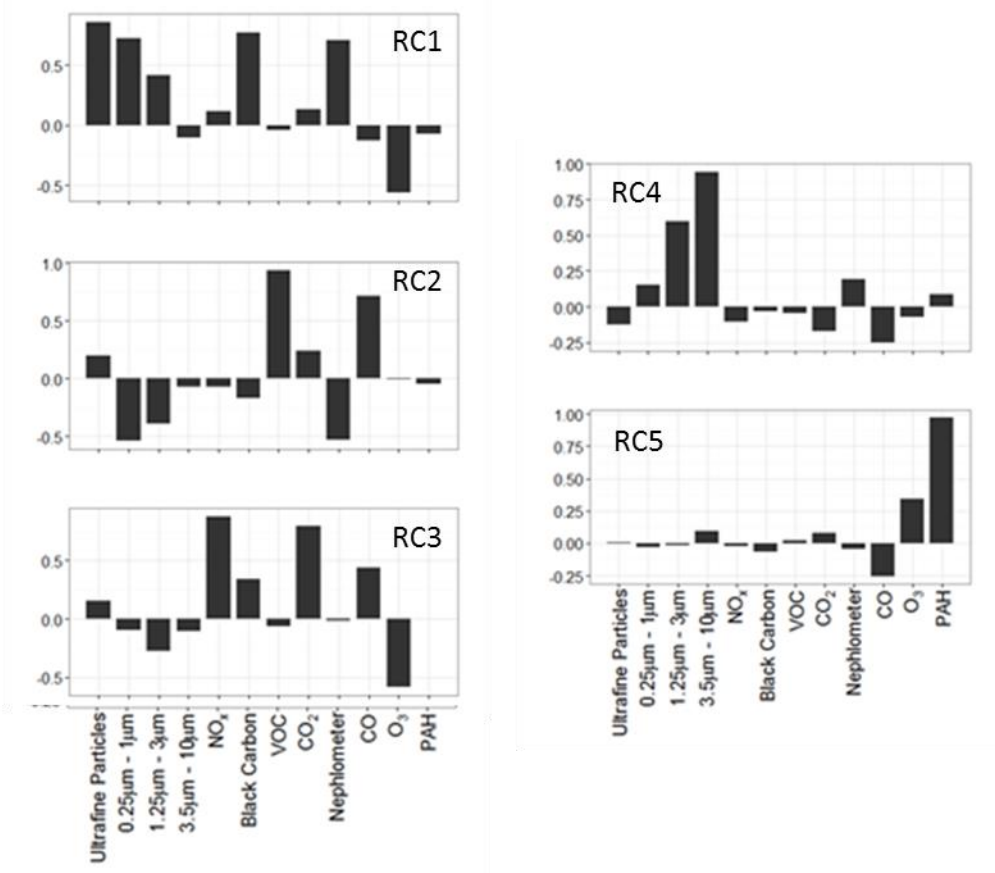
our passive samplers and mobile platform in the other cities. Note that the mobile data only is collected during the evening commute, while the passive badges collect continuously over the 2-week period. Since the mobile platform is often collected during peak traffic and Ozone periods, it may more clearly capture these near roadway effects showing an interaction of the multi-pollutants. Additional comparisons of the data after final QC were made with reference monitors in Baltimore. Figure 2 shows the results of comparing the 30-second averaged data from the mobile platform to hourly data on the same day recorded at the AQS site for Ozone and PM2.5. The mobile data has excellent agreement with AQS site, and captures the day-to-day variation in these pollutants.

Figure 2



Preliminary multivariate analysis of the mobile platform data and its relationship with the video record was done using pilot measurements in Seattle. In contrast with the median values discussed above, this activity has focused more on interpreting the "peaks" in the mobile monitoring record that are observed in all cities, using the Seattle pilot data as a test case. An observed peak is defined as being more than two standard deviations from the overall mean for that sampling day. This produced a subset of measurements comprising ~12% of the data that were then analyzed by PCA with varimax rotation. The five rotated components are shown below (RC1 through RC5 account for 24, 19, 18, 12 and 10 percent of the total variance, respectively). (Figure 3)

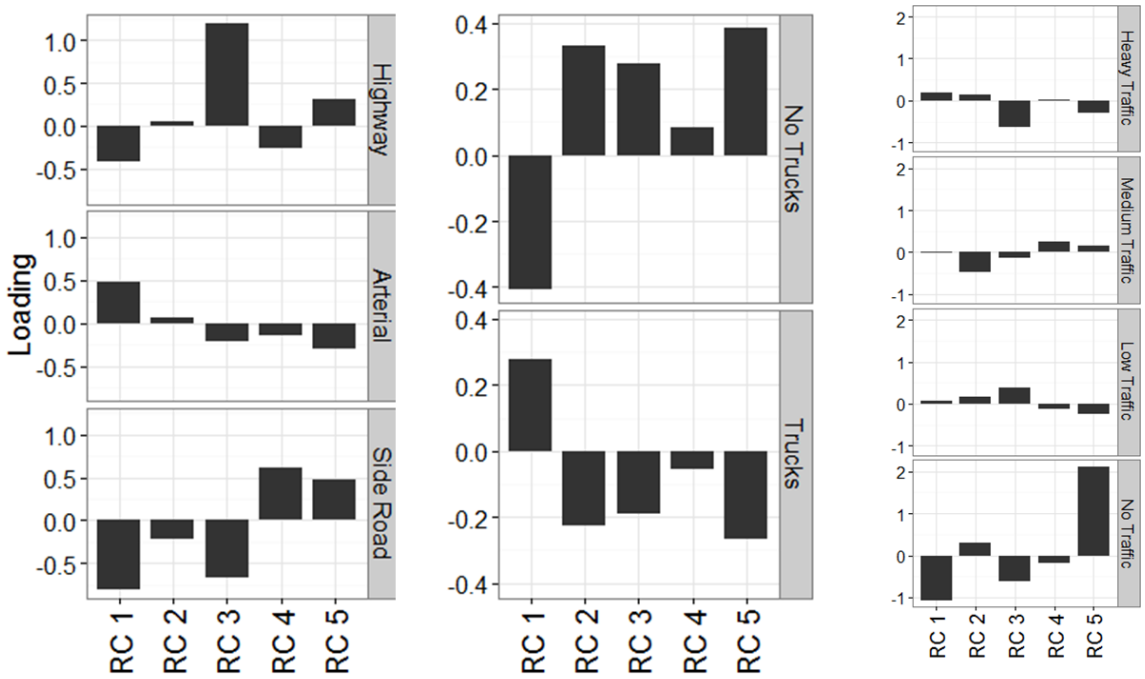
Figure 3



We then summarized the average rotated PCA scores according to different stratifications of the data based on measurements from the video log. The results are shown below.

The left column (Figure 4) indicates that peak measurements observed on highways are characterized by relatively higher scores for the third rotated principal component, which featured high levels of NOx and CO2 and depletion of O3, and relatively lower than average scores for the other five components. The profile for measurements made on arterials indicate high scores for the first principal component, which featured high levels of ultrafine and fine particles as well as black carbon. Side roads tended to have higher than average scores for the fourth and fifth principal components, which featured a tendency towards higher counts in the larger particle size ranges as well as high levels of PAHs and ozone. Side roads also had negative average scores for the first three components, indicating lower levels of NOx, CO, VOCs, Black Carbon, and small particles.

Figure 4



The center column indicates that the profile for peak measurements for which trucks were visible in the video is dominated by high average scores for the first principal component, which is characterized by small sooty particles, and negative average scores for the remaining components. This is highly suggestive of RC 1 as the basis for a multivariate feature that is unique to trucks. Finally, the average rotated PCA scores for all components according to the density of light vehicle traffic is close to zero, except for when no vehicle traffic is observed. All cases where no vehicles were observed occurred on side roads, which themselves had very light traffic. Therefore, the similarity to the average score profile observed on side roads is unsurprising.

Finally, the average rotated PCA scores for all components according to the density of vehicle traffic is close to zero, except for when no vehicle traffic is observed. All cases where no vehicles were observed occurred on side roads, which themselves had very light traffic. Therefore, the similarity to the average score profile observed on side roads is unsurprising.

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.

Our 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 accepted and another submitted for review on the thermal desorption PTR-MS sampling of engine exhausts and fuels.

Detailed analysis of the aerosol mass spectrometer data has continued. Two types of data from the HR-ToF-AMS have been processed. Unit Mass Resolution (UMR) data and the more highly resolved High Resolution (HR) data. The UMR data collapses mass spectral signals into their respective unit masses and uses a fragmentation algorithm to allocate signals at a given UMR between Organic, Nitrate, Sulfate, Ammonium, and Chloride species contributions. The fragmentation algorithm is standard practice within the AMS community and has been corrected using filter data to account for non-ambient conditions observed in the engine exhaust experiments. HR analysis provides a more robust identification of individual ions and thus a direct quantification of contributions between the five main classifications (Organic, Nitrate, Sulfate, Ammonium, and Chloride).

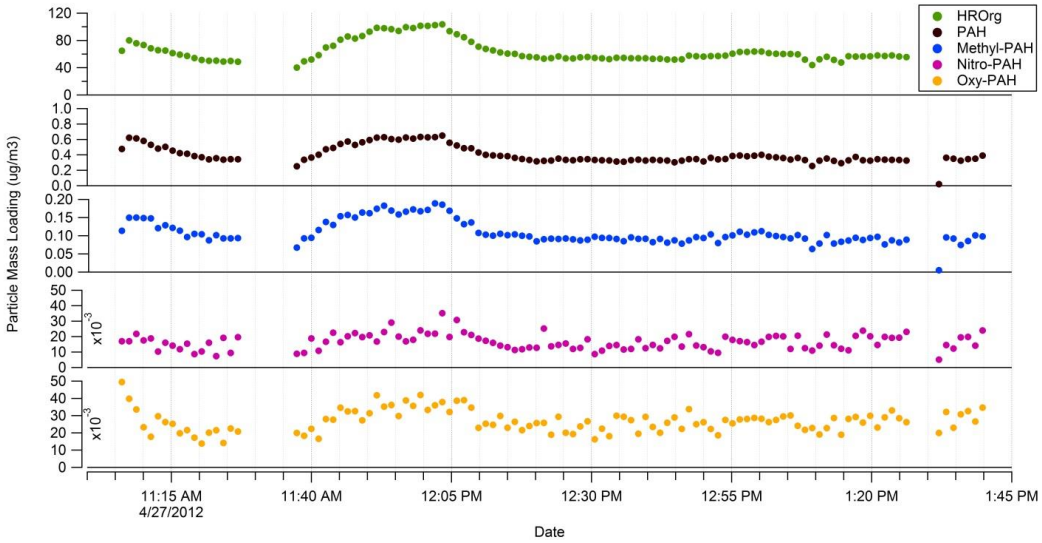


Figure 5. High resolution data for a chamber experiment of 100% diesel exhaust under typical engine load. Trend of mass loadings over the course of a 2 hour sampling, showing the total Organics with respect to the four PAH categories. Where PAH, Methyl-PAH, Nitro-PAH, and Oxy-PAH refer to the particle loading from the summation of ions listed below:
PAH=C10H8+C12H8+C12H10+C13H10+C14H10+C16H10+C18H12+C20H12+C22H12+C22H14;
Methyl-PAH=C11H10+C12H12+C13H12+C14H12+C15H14;
Nitro-PAH=C10H7NO2+C12H9NO2+C13H9NO2+C14H9NO2+C16H9NO2+C18H11NO2;
Oxy-PAH=C13H8O+C13H10O+C15H8O+C14H8O2.

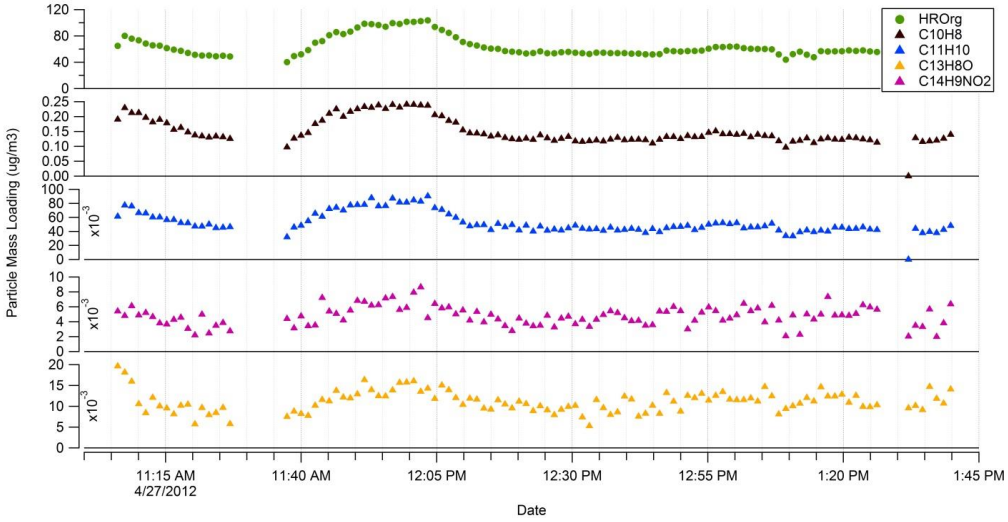


Figure 6. High resolution data for a chamber experiment of 100% diesel exhaust under typical engine load. Trend of mass loadings over the course of a 2 hour sampling, showing the total Organics with respect to the four PAH ions. $C_{10}H_8$ = Naphthalene, $C_{11}H_{10}$ = Methyl-naphthalene, $C_{13}H_8O$ = Fluornone, and $C_{14}H_9NO_2$ = Nitro-phenathrene/ Nitro-anthracene

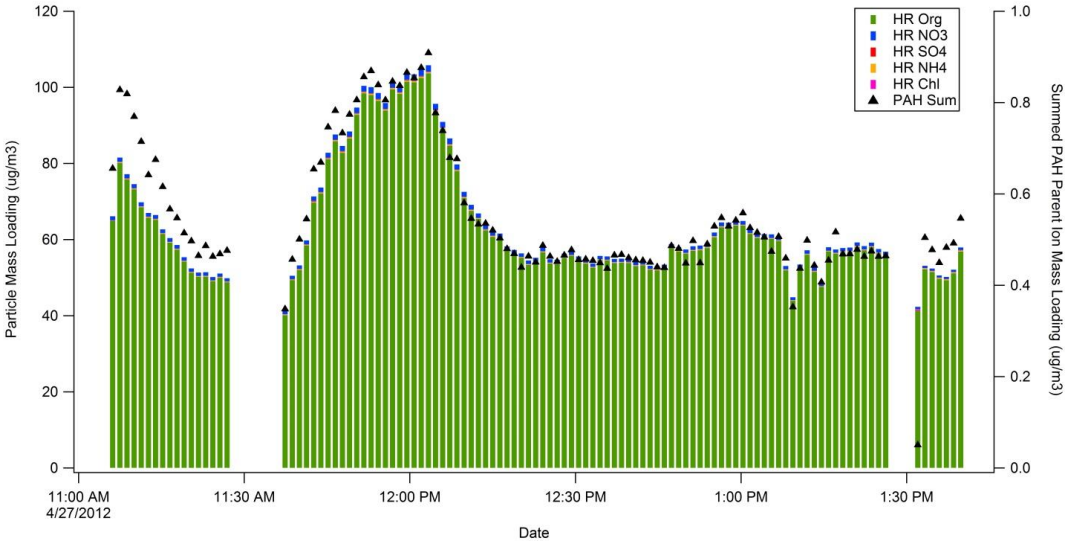


Figure 7. High resolution data for a chamber experiment of 100% diesel exhaust under typical engine load. Trend of mass loadings over the course of a 2 hour sampling , showing the stacked total PM loading of Organic, Nitrate, Sulfate, Ammonium, and Chloride with respect to the Summed PM loading from the 25 PAH target ions

Efforts have been made to validate the total particle mass (PM) loadings measured by the AMS with those measured by other particle-phase instrumentation. Differences seen between the Pallflex filter PM and AMS PM can be attributed to soot which is not measured by the AMS. This difference was verified by comparing the total volume measurements from a scanning mobility particle sizer (SMPS), then assuming particle density based on the known Organic, Nitrate, Sulfate, Ammonium, Chloride, and Black Carbon contributions to the aerosol; to the summation of black carbon (BC) measurements collected by UW with the total AMS PM loading. The addition of the BC measurement to the AMS PM approximated the PM loading

calculated by the SMPS.

A target list of PAH compounds, that were identified using the HR-ToF-AMS data, was populated with PAH compounds that have been most commonly referred in the literature in engine exhaust or engine fuel or have been highlighted for their high carcinogenic risk and negative health impact by the IARC, US Environmental Protection Agency, and the World Health Organization. This list includes 19-PAHs along with 5-methylated PAHs, 4-oxygenated PAHs, and 9-nitro-PAHs. This list of 37 PAHs has been identified within the HR data as 25 individual *parent* ions of these compounds (Figure 5, 6, 7). Though signal from a single *m/z* can be the result of multiple PAH isomers, the AMS is unable to distinguish differences among these isomers. A paper is currently being drafted to demonstrate the capability of the HR-ToF-AMS to measure substantial concentrations from the *parent* ions associated with these compounds.

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 Techniques*, 7, 225-239, 2014.**
2. **Gueneron, M., Erickson MH, VanderSchelden G., Jobson BT., PTR-MS Fragmentation Patterns of Gasoline Hydrocarbons, *submitted, International Journal of Mass Spectrometry*, June 2014.**

3. Erin A. Riley, Lyndsey Banks, Jonathan Fintzi, Timothy R. Gould, Kris Hartin, LaNae Schaal, Mark Davey, Lianne Sheppard, Timothy Larson, Michael G. Yost, Christopher D. Simpson. Multi-pollutant mobile platform measurements of air pollutants adjacent to the I-40 corridor in Albuquerque, NM. Atmospheric Environment (In review)

Presentations to Date:

1. **Erin A. Riley, Lyndsey Banks, Jonathan Fintzi, Timothy R. Gould, Kris Hartin, LaNae Schaal, Mark Davey, Lianne Sheppard, Timothy Larson, Michael G. Yost, Christopher D. Simpson. "Multi-pollutant Mobile Platform Measurements of Traffic-associated Air Pollutants adjacent to the I-40 Corridor in Albuquerque, NM " 97th Canadian Chemistry Conference and Exhibition in Vancouver, British Columbia, June 1 - 5, 2014**

2. Jobson, BT, MH Erickson, Gueneron, M., VanderSchelden, G., Measuring Small Photoproducts and Big Organics by PTR-MS, Canadian Chemistry Conference, June 1-5, 2014, Vancouver, B.C. (invited)

3. **Elena Austin. (2014) Identifying multi-pollutant spatial patterns in mobile monitoring data from Baltimore, MD using cluster analysis. Annual Symposium on Environmental, Occupational and Population Health, Semiahmoo, WA**

4. **Austin E., Larson T., Sheppard L., Yost M.. (2014). Pollutant variability and correlations in mobile monitoring data as compared to central site monitoring. EPA Air Sensors Workshop, Research Triangle Park, NC**

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

6. 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.

7. 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. **Erin A. Riley, Miyoko D. Sasakura, Kris Hartin, Robert Crampton, Timothy Gould, Timothy V. Larson, Michael G. Yost, Christopher D. Simpson. "Principal Component Analysis of Snap-Shot Air Pollutant Measurements In Baltimore, MD" EPA annual Clean Air Research Center Annual Meeting July 25th & 26th, 2013 Seattle, WA**
2. **Erin A. Riley, Kris Hartin, Timothy Gould, Timothy V. Larson, Michael G. Yost, Christopher D. Simpson "Mobile measurements of near-highway air pollutant gradients" University of Washington/ UBC/SFU/ UVic Annual Symposium on Environmental, Occupational, and Population Health "Changing Environments and Population Health" January 9 – 10, 2014**

3. **Erin Riley, Elena Austin, Jonathan Fintzi, Timothy Larson, Michael Yost, Lianne Sheppard, Paul Sampson, Christopher Simpson. "Decoupling Regional and Local Sources in Mobile Monitoring of Air Pollutants" Student Research Day, Department of Environmental and Occupational Health, University of Washington, Seattle, May 29, 2014.**

4. Herring, C., Erickson, M., Gueneron, M., Faiola, C., McDonald, J., Jobson, T., VanReken, T., Hartin, K., Yost, M., and Larson, T., "Characterization of Mixed Diesel and Gasoline Exhaust by HR-ToF-AMS under Varied Engine Load and Dilution Conditions", *AAAR 32nd Annual Conference*, Oct 2013 (poster). ***Winner of a Student Poster Award.*

5. 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.

6. 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.

7. 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 final QC data from the field sampling campaigns, completing the chamber characterization studies. We have completed most of the field work on target and will be assisting project 4 in scripted commute studies for CCAR using instruments from the mobile platform. Data QC and review are underway for the cities that already have been sampled working with the Biostatistics core. Work on publications and dissemination of results is underway.

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

In the previous year we conducted subchronic inhalation studies with several mixed atmospheres in collaboration with Project 3. The results of that work are ongoing as described in the report for Project 3. One of the outcomes of the subchronic work that was presented at the EPA annual meeting for CCAR was the need to conduct studies at more acute exposure durations for the purpose of evaluating multiple mixtures. In collaboration with Project 3, we developed an acute exposure/biosample analysis protocol that used short term predictive bioassays of cardiovascular and systemic response. These included the use of peripheral blood from exposed animals. Peripheral blood was collected and analyzed for vessel constriction in a myography assay, endothelial response with serum applied to endothelial cells, and of metabolites identified from a metabolomics screen. A number of exposures were conducted during the year and the assays continue to be under way. We have a paper near finalization on the results of the metabolomic

screens which are summarized below. In this progress report we emphasize the results of the metabolomics screens from the motor vehicle exposures and provide some preliminary data from the endothelial responses. Additional data are included in Project 3. We also report on published papers and abstracts from this year.

Completion of Exposures and Analysis to Tease Out Components

We conducted acute single day exposures to C57bl6 and Apo E -/- mice , on a high fat diet, to the following atmospheres: (1) MVE (derived from 30 μg PM/m^3 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; road dust, wood smoke, ozone, secondary organic aerosol (SOA) and (5) filtered air (controls). Some data from these exposures are included here, including a detailed analysis of the results of the metabolomics screening. The additional analyses remain under way.

Summary of Metabolomic Profiling after Exposure to Motor Vehicle Exhaust (Development of biomarkers)

Air pollution resulting from motor vehicle emissions has been documented to impact respiratory function and may be linked to other disease pathologies as well. Respiratory inhalation of pollutants may cause direct damage to lung tissue, and with the high level of vascularization in the lungs and exchange of gases, the effects of pollutant inhalation may also be evident in blood. Evidence for pollutant-caused biochemical changes in the systemic blood supply may reflect lung damage and/or alterations to systemic vascular homeostasis, which may contribute to other disease states. This study measured changes in serum metabolites resulting from exposure to mixed vehicle exhaust (MVE). Mice were exposed to two concentrations of MVE for 6 hours, which was followed by an 18 hour "washout" period. Serum was collected after the 6 hour exposure and again after the 18 hour washout period. Metabolite levels in MVE serums were compared to those in serums of mice exposed only to filtered air. Metabolites which are indicators of oxidative stress were increased in 6 hour MVE-exposed mice and the changes were generally diminished after the 18 hr washout period. Other metabolite changes included biochemicals which may be related to cardiovascular function. An increase in branched chain amino acid catabolites may signal a change in whole animal energy metabolism with MVE exposure. The results indicate that MVE exposure causes significant metabolic alterations reflected in metabolite changes in the serum.

Markers of oxidative stress were increased in MVE-exposed serums.

Two markers of oxidative stress, oxidized glutathione and cysteine-glutathione disulfide, were observed to be increased as a result of MVE exposure (Data shown below along with metabolic scheme). Levels of oxidized glutathione and cysteine-glutathione disulfide, in MVE serums, returned to control levels at the 18 hour time point. Taurine, an anti-oxidant, was increased to a statistically significant degree in the MVE-300 group relative to FA controls at 0 hours and may reflect a response to increased oxidative stress. Cysteine, which is an intermediate in taurine and glutathione synthesis was also elevated in the MVE-100 and MVE-300 groups relative to control, but statistical significance was only achieved for the MVE-300 group at a $p<0.1$ level. The cluster of increases in the cysteine/glutathione synthesis pathway may represent increased

systemic oxidative stress along with a compensatory response to generate more anti-oxidants. By 18 hours the differences between MVE exposed serums and FA controls was negligible.

Table1. : Markers of oxidative stress in serum

Sub Pathway	Biochemical Name	Fold Change Two-Way ANOVA Contrasts (no outliers)			
		MVE100-0 FA-0	MVE300-0 FA-0	MVE100-18 FA-18	MVE300-18 FA-18
Methionine, Cysteine, SAM and Taurine Metabolism	S-adenosylhomocysteine (SAH)	1.29	1.47	1.11	0.93
	cysteine	1.24	1.48	0.97	0.96
	taurine	1.13	1.92	0.96	0.72
Glutathione Metabolism	glutathione, oxidized (GSSG)	2.78	1.9	0.81	1.04
	cysteine-glutathione disulfide	1.45	1.3	1.01	1.06

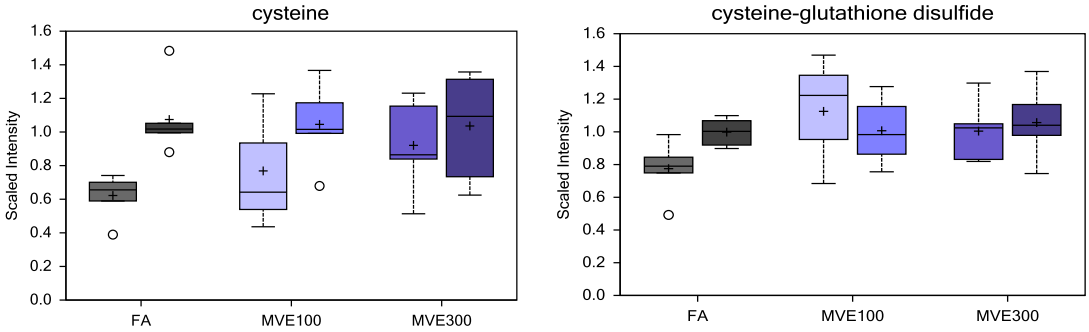


Figure 1. Markers of oxidative stress in serum.

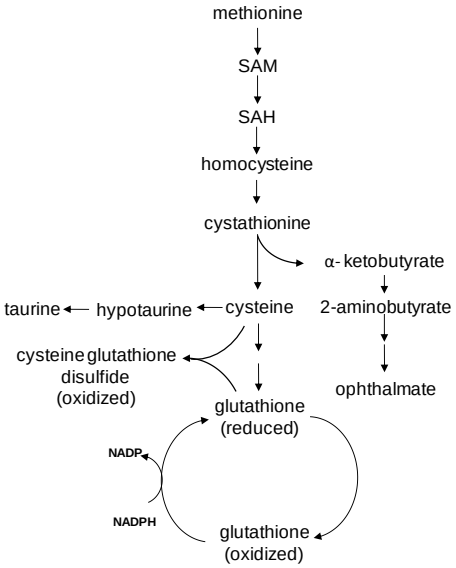


Figure 2: Metabolic scheme

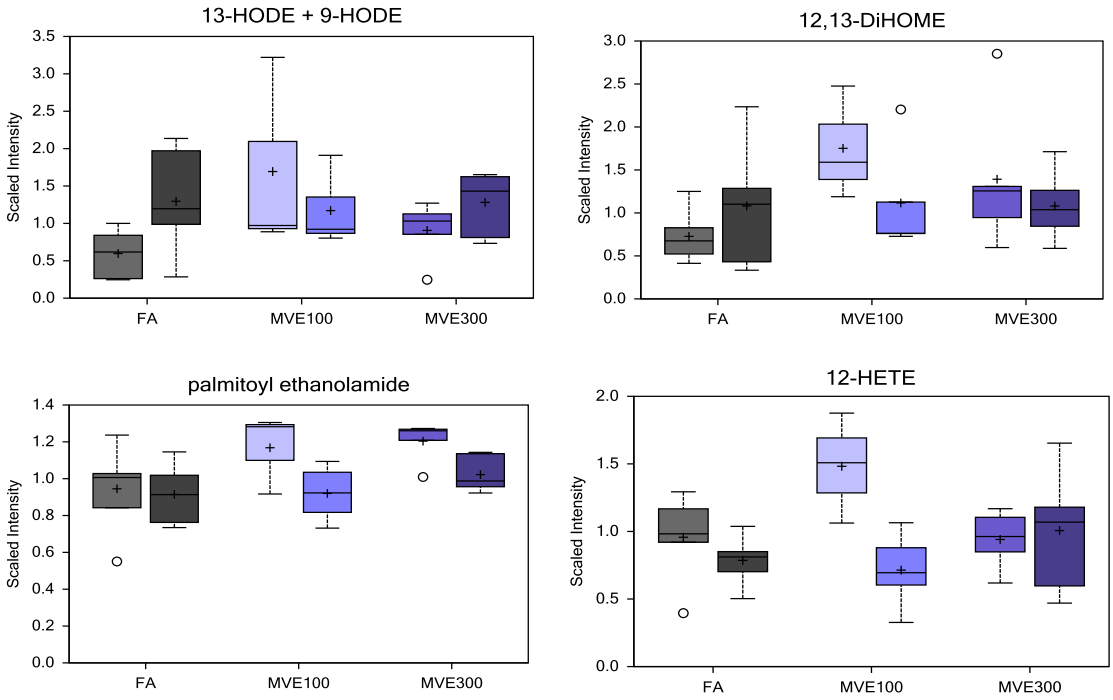
Metabolites associated with lipid peroxidation and inflammation were elevated with MVE exposure.

A number of lipid metabolites associated with lipid peroxidation or inflammation were increased in the MVE-exposed groups; although increases were generally greater in the MVE-100 group (Data shown below). 13-HODE/9-HODE are measures of lipid peroxidation and they were elevated to a statistically significant level in the MVE-100 group relative to FA controls. In

addition, oxalate which has been reported to suppress lipid peroxidation was elevated to a statistically significant level in the MVE-100 0-hour group. 12, 13-DiHOME and palmitoyl ethanolamide are two lipid derivatives reported to be modulators of inflammatory activity. 12, 13-DiHOME has been reported to be a neutrophil attractant and may indicate increased inflammation (HMDB04705). Palmitoyl ethanolamide has anti-inflammatory activity and may accumulate in blood following tissue injury (HMDB02100). In addition, 12-HETE is derived from lipoxygenase activity on arachidonic acid and has been reported to act as a vasoconstrictor (HMDB06111). Overall, the data may suggest that MVE exposure may increase lipid peroxidation and cause modulation of lipid regulated inflammatory mechanisms.

Table 2. Metabolites associated with lipid peroxidation and inflammation.

Sub Pathway	Biochemical Name		Fold Change			
			Two-Way ANOVA Contrasts (no outliers)			
			MVE100-0	MVE300-0	MVE100-18	MVE300-18
			FA-0	FA-0	FA-18	FA-18
Fatty Acid, Monohydroxy	13-HODE + 9-HODE		2.84	1.52	0.9	0.99
Fatty Acid, Dihydroxy	12,13-DiHOME		2.41	1.91	1.03	1
Eicosanoid	12-HETE		1.55	0.98	0.91	1.28
Endocannabinoid	palmitoyl ethanolamide		1.24	1.27	1.01	1.12
Ascorbate and Aldarate Metabolism	oxalate (ethanedioate)		2.19	1.55	0.98	0.92



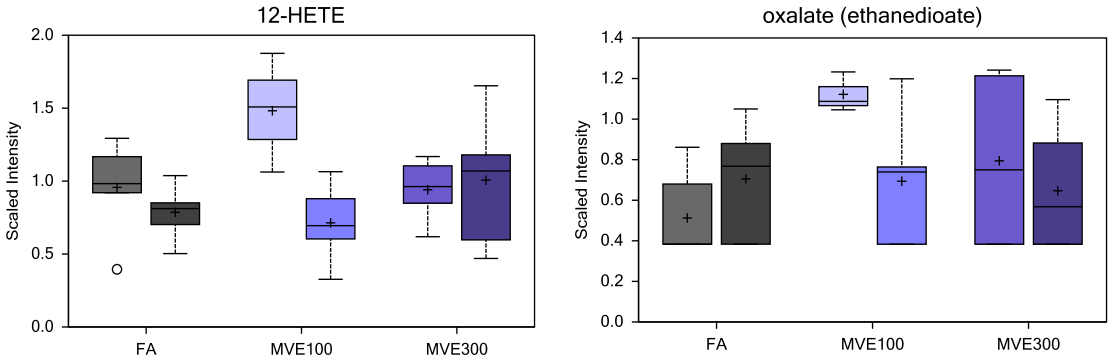
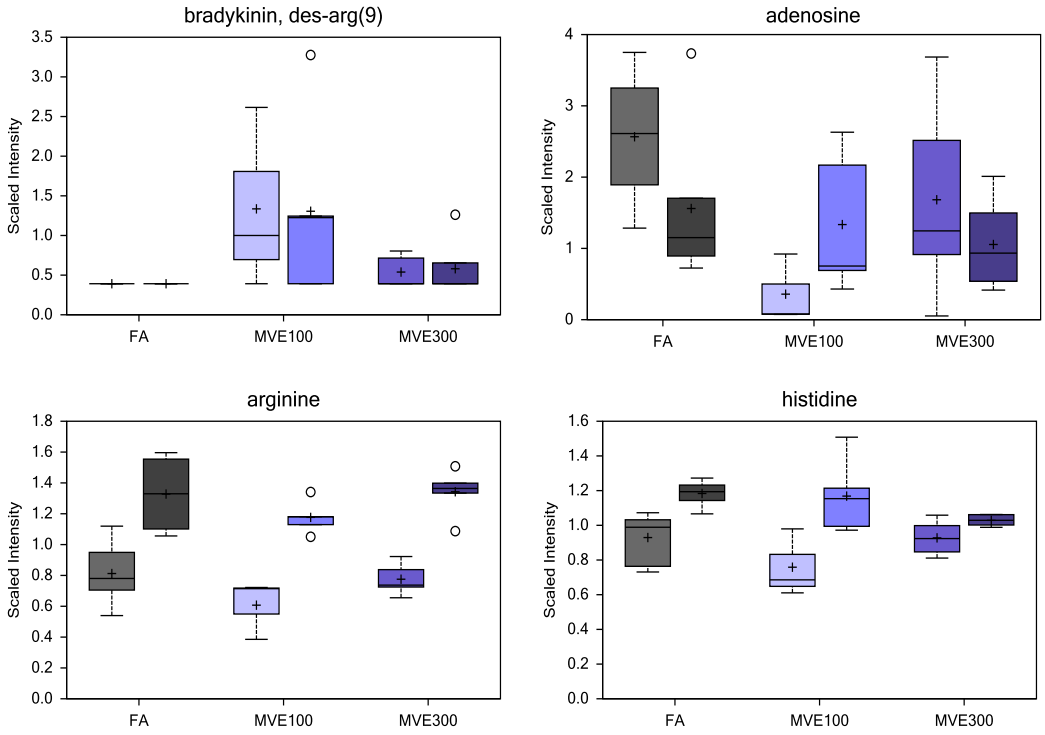


Figure 3. Metabolites associated with lipid peroxidation and inflammation.

Metabolites with potential cardiovascular significance were altered in MVE serums.
The levels of a set of metabolites with cardiovascular activity or that are related to cardiovascular active biochemicals were found to change with MVE exposure (Data shown below). The metabolites bradykinin (HMDB04246) and adenosine (HMDB00050) both have vasodilator activity. In addition, adenosine has also been reported to have vasoconstrictor activity in some tissues. Bradykinin levels were up in both the MVE-100 and MVE-300 0-hour groups, relative to FA-0 hour controls (though only statistically significant for MVE-100). Bradykinin remained elevated at 18 hours in the MVE-100 18-hour serums. Adenosine was dramatically down in the MVE-100 0-hour serums (0.14X; p=0.0006) and also lower in MVE-300 0-hour serums (0.66X; p=0.094). Also of interest was a decrease in histidine with a commensurate rise in 1-methylimidazoleacetate in MVE-100 0-hour serums. The vasodilator histamine is produced from histidine, and 1-methylimidazoleacetate is a degradation product of histamine. It is possible that more histidine is being used to produce histamine; with an increase in 1-methylimidazoleacetate resulting from an increased serum histamine level (histamine was below the level of detection in these serums). Arginine, from which nitric oxide (NO) is derived, was decreased in MVE-100 0-hour serums relative to FA 0-hour controls and might possibly be an indication of increased NO production. Finally, two fibrinogen peptide fragments were elevated in all MVE exposed serums relative to FA controls, but statistical significance was only achieved in the comparison of MVE-300 18-hour serums to FA 18-hour serums. Increases in fibrinogen fragments have been linked to inflammation and injured vessel walls. Increases in fibrinogen fragments might also reflect ECM remodeling or an impact on the vascular clotting system. A note of caution on this set of metabolites: changes were greater in the MVE-100 than in the MVE-300 serums and one might expect an MVE dose response to generate the opposite finding.

Table 3. Metabolites with potential cardiovascular significance.

Sub Pathway	Biochemical Name	Fold Change Two-Way ANOVA Contrasts (no outliers)			
		MVE100-0	MVE300-0	MVE100-18	MVE300-18
		FA-0	FA-0	FA-18	FA-18
Polypeptide	bradykinin, des-arg(9)	3.41	1.38	3.33	1.48
Fibrinogen Cleavage Peptide	TDTEDKGEFLSEGGGV*	1.29	1.14	1.16	1.27
	TDTEDKGEFLSEGGGVR*	3.02	1.22	2.59	1.49
Purine Metabolism, Adenine containing	adenosine	0.14	0.66	0.86	0.68
Urea cycle; Arginine and Proline Metabolism	arginine	0.75	0.95	0.89	1.01
Histidine Metabolism	histidine	0.82	1	0.99	0.87
	1-methylimidazoleacetate	1.74	1.17	1.08	1.11



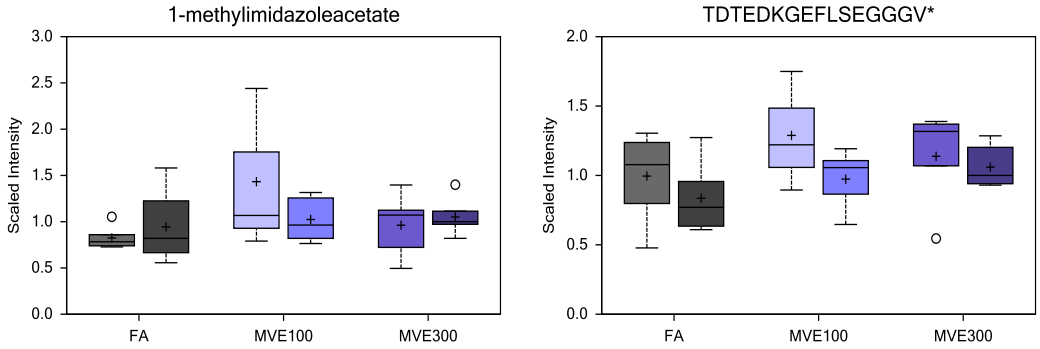


Figure 4. Metabolites with potential cardiovascular significance were altered in MVE serums.

BCAA catabolites were elevated with MVE exposure.

Catabolites of the branched chain amino acids (BCAA) were elevated in MVE 0-hour serums, with a greater number of increases achieving statistical significance with the MVE-300 exposure (Data shown below along with metabolic scheme). BCAA catabolites can feed into the TCA cycle to support energy metabolism when glycolysis and/or fatty acid β-oxidation are insufficient to supply necessary acetyl-CoA. Under some circumstances, increased BCAA catabolites can reflect mitochondrial dysfunction and a backup of the BCAA degradation pathways that feed the TCA cycle. Propionylcarnitine can represent a surrogate for propionyl-CoA generated from valine and isoleucine degradation (as well as odd chain fatty acids) and it was elevated in both 0-hour MVE groups. The increase in BCAA catabolites in the MVE-100 and MVE-300 0-hour serums suggests an alteration in global energy metabolism in the MVE exposed mice. BCAA levels returned to FA levels at the 18 hour time point.

Table 4. BCAA catabolites affected by MVe exposure.

Sub Pathway	Biochemical Name	Fold Change Two-Way ANOVA Contrasts (no outliers)			
		MVE100-0 FA-0	MVE300-0 FA-0	MVE100-18 FA-18	MVE300-18 FA-18
Leucine, Isoleucine and Valine Metabolism	isovalerylglycine	1.42	1.21	0.93	0.91
	3-methylcrotonylglycine	1.58	1.3	1.12	0.93
	alpha-hydroxyisovalerate	1.36	1.25	1.01	0.87
	2-methylbutyrylcarnitine (C5)	1.33	1.13	0.92	0.9
	2-methylbutyrylglycine	1.95	2.42	1.5	1.26
	tigloylglycine	1.22	1.23	1.25	1.12
	2-hydroxy-3-methylvalerate	1.42	1.82	0.8	1.08
	3-methyl-2-oxobutyrate	1.29	1.46	1.11	1.14
	isobutyrylcarnitine	1.28	1.28	1.09	0.91
	isobutyrylglycine	1.27	1.13	0.95	0.74
	alpha-hydroxyisocaproate	0.8	1.63	0.87	0.96
BCAA & FA Metabolism	propionylcarnitine	1.3	1.3	1.02	0.92

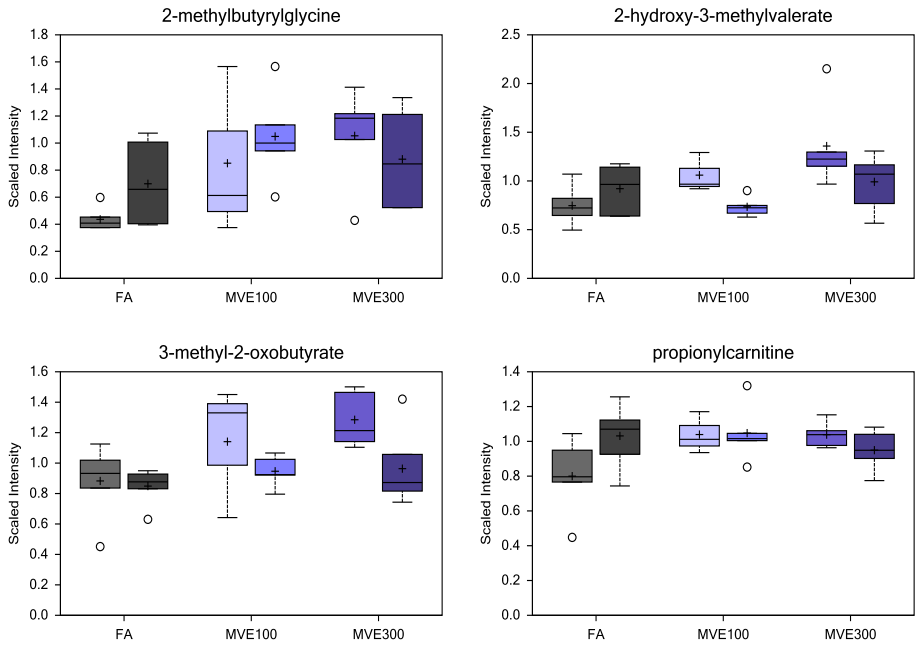


Figure 5. BCAA catabolites affected by MVe exposure.

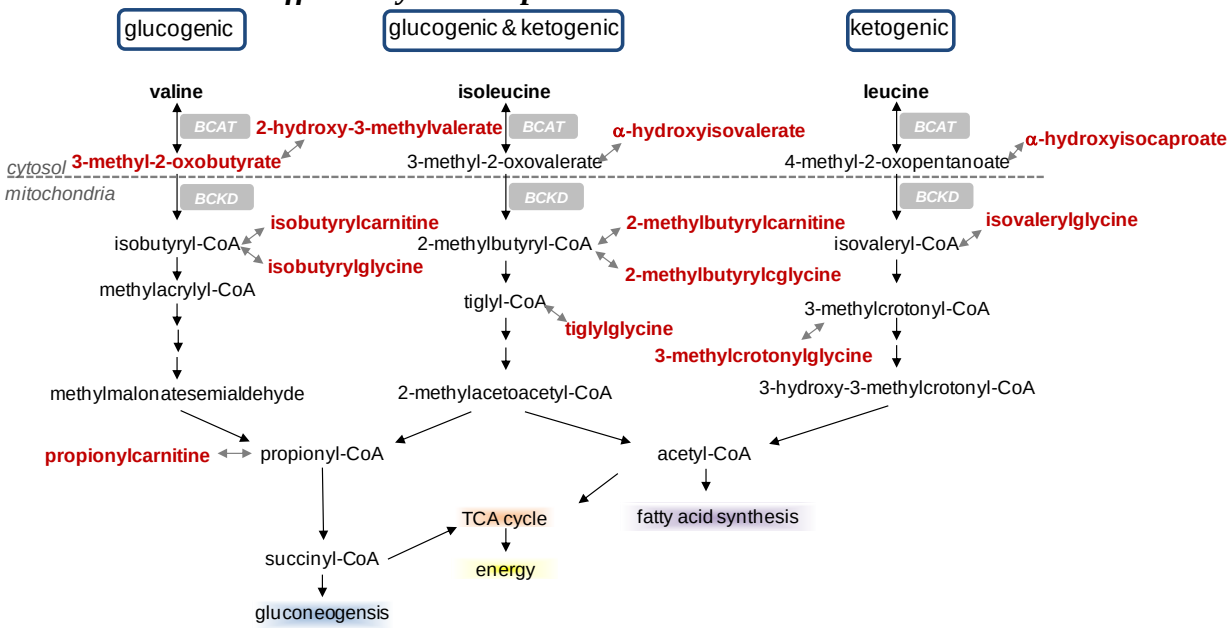


Figure 6. Metabolic scheme.

Free fatty acids increase in MVE-100 serums and trend up in MVE-300 serums.
A general increase in free fatty acids (FFA) was observed in MVE 0-hour serums, although the increases only achieved statistical significance for the MVE-100 0-hour serums. Increases in serum FFAs can reflect increased lipolysis or decreased tissue uptake. The elevations in MVE-exposed serums may indicate a change in energy metabolism in those animals. Increases in FFAs may be consistent with the increases in BCAA catabolites and reflect a shift in carbon sources used for energy metabolism.

Table. 5. Free fatty acids in serum of MVe exposed mice.

Sub Pathway	Biochemical Name	Fold Change Two-Way ANOVA Contrasts (no outliers)			
		MVE100-0 FA-0	MVE300-0 FA-0	MVE100-18 FA-18	MVE300-18 FA-18
Medium Chain Fatty Acid	caproate (6:0)	1.64	1.18	1.47	1.23
	caprylate (8:0)	1.73	1.23	1.02	1.25
	pelargonate (9:0)	1.14	1.03	1.05	0.99
	caprate (10:0)	1.25	0.94	0.98	0.87
Long Chain Fatty Acid	myristate (14:0)	2.32	1.36	0.95	1.25
	myristoleate (14:1n5)	2.75	1.69	1.14	1.4
	palmitate (16:0)	1.52	1.12	0.72	1.03
	palmitoleate (16:1n7)	2.23	1.33	0.75	1.22
	margarate (17:0)	1.31	1.16	0.79	1.03
	10-heptadecenoate (17:1n7)	1.97	1.34	0.74	1.15
	stearate (18:0)	1.21	0.96	0.72	0.99
	oleate (18:1n9)	1.82	1.37	0.87	1.12
	cis-vaccenate (18:1n7)	1.11	1.25	0.93	1.01
	nonadecanoate (19:0)	1.17	0.9	0.66	0.91
	10-nonadecenoate (19:1n9)	2.05	1.45	0.78	1.3
	arachidate (20:0)	1.26	1.18	0.68	0.99
	eicosenoate (20:1n9 or 11)	1.82	1.31	0.65	1.03
	erucate (22:1n9)	1.24	1.36	0.64	1.11
Polyunsaturated Fatty Acid (n3 and n6)	stearidonate (18:4n3)	1.91	1.5	0.64	1.04
	eicosapentaenoate (EPA; 20:5n3)	0.98	0.92	0.61	1.12
	docosapentaenoate (n3 DPA; 22:5n3)	1.9	1.38	0.68	1.16
	docosahexaenoate (DHA; 22:6n3)	1.45	1.2	0.76	1.15
	linoleate (18:2n6)	2.14	1.3	0.78	0.96
	linolenate [alpha or gamma; (18:3n3 or 6)]	2.13	1.45	0.7	1.02
	dihomo-linolenate (20:3n3 or n6)	1.58	1.14	0.59	0.99
	arachidonate (20:4n6)	0.98	1.07	0.86	1.02
	adrenate (22:4n6)	1.62	0.89	0.69	1.18
	docosapentaenoate (n6 DPA; 22:5n6)	1.47	1.37	0.76	1.01
	docosadienoate (22:2n6)	1.58	1.14	0.56	0.9
	dihomo-linoleate (20:2n6)	1.61	1.36	0.77	1.15
	mead acid (20:3n9)	1.19	1.3	0.85	1.12

Fumarate and malate declines in MVE-100 serums may indicate altered TCA cycle function. TCA cycle intermediates fumarate and malate were significantly decreased in MVE-100 0-hour serums relative to FA 0-hour serums (Data shown below along with metabolic scheme). The declines in fumarate and malate were not reproduced in the MVE-300 0-hour serums. Succinate was increased in MVE-100 and MVE-300 0-hour serums, but the increases did not achieve statistical significance relative to FA 0-hour. Increased succinate with coincident decreases in malate and fumarate could be an indication of reduced succinate dehydrogenase activity. Lactate levels increased in the MVE-300 0-hour serums relative to FA 0-hour controls, and may be consistent with a decrease in pyruvate/acetyl-CoA entering the TCA cycle and subsequent shunting of pyruvate into lactate. While the lack of a significant change in malate and fumarate in the MVE-300 serums is reason for caution, the decrease of malate and fumarate in the MVE-100 serums coupled with the increase in MVE-100 fatty acids and BCAAs as well as the lactate increase in MVE-300 serums, may be an indication of mitochondrial dysfunction resulting from MVE exposure.

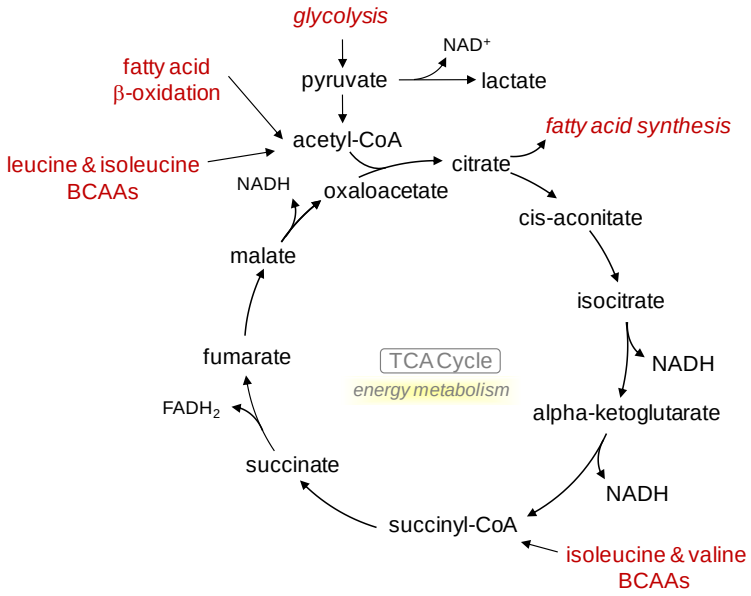


Figure 6. Metabolic scheme

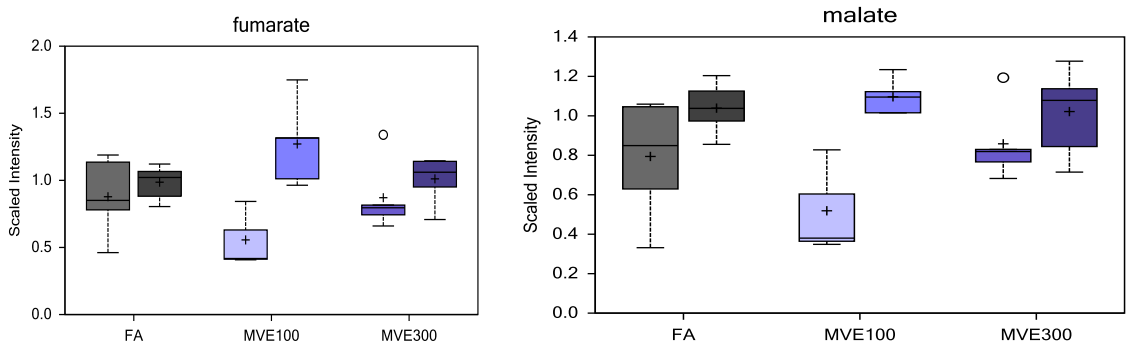


Figure 7. MVe exposure effects on fumarate and malate.

Table 6. MVe impact on measured TCA cycle and glycolysis

Sub Pathway	Biochemical Name		Fold Change Two-Way ANOVA Contrasts (no outliers)			
			MVE100-0	MVE300-0	MVE100-18	MVE300-18
			FA-0	FA-0	FA-18	FA-18
TCA Cycle	citrate		0.97	0.97	0.99	1
	alpha-ketoglutarate		0.83	1.15	1.38	1.29
	succinylcarnitine		1.01	1.26	1.02	1.05
	succinate		1.59	1.44	1.01	1.23
	fumarate		0.63	0.99	1.29	1.03
	malate		0.65	1.08	1.06	0.98
Glycolysis, Gluconeogenesis,	lactate		1.09	1.31	0.91	0.82

Conclusions and Path Forward Regarding Metabolomic Profiling

Inhalation of motor vehicle exhaust by mice produced significant changes in serum global metabolite profiles, although the results may be somewhat confounded by a time-dependent

effect on metabolites that is not MVE-dependent. Global profiling of serum for mice treated with MVE suggests that exposure results in increased oxidative stress and possible inflammatory responses. A set of metabolites with reported cardiovascular activity were altered and may indicate that changes in vascular tone occur with MVE exposure. MVE-dependent changes in energy metabolites were also observed, but a lack of a clear dose response or reproducibility between MVE-100 and MVE-300 groups argues for a cautious appraisal of general significance. Based on the results of these analyses the follow on studies were conducted at 100 ug/m3 due to the clearer signal at that dose. 16 additional test atmospheres were conducted and the analysis is under way.

Endothelial Cell Toxicity/Response

To confirm the phenomenon of serum inflammatory potential related to complex combustion atmosphere exposures, we characterized a number of responses induced by serum from mice exposed to mixed vehicle emissions, wood smoke, road dust, and other mixtures, with serum collected 4h and 24h after exposures. Primary mouse brain microvascular endothelial cells (BMVEC; Cell Biologics) and pulmonary artery endothelial cells (PAEC; Cell Biologics) treated with serum from exposed C57BL mice showed modest elevation of inflammatory chemokines, both in terms of relative mRNA and cell surface expression. Examples of some of the data from the test atmospheres are provided below. Additional analyses, including with APoE mice, are under way.

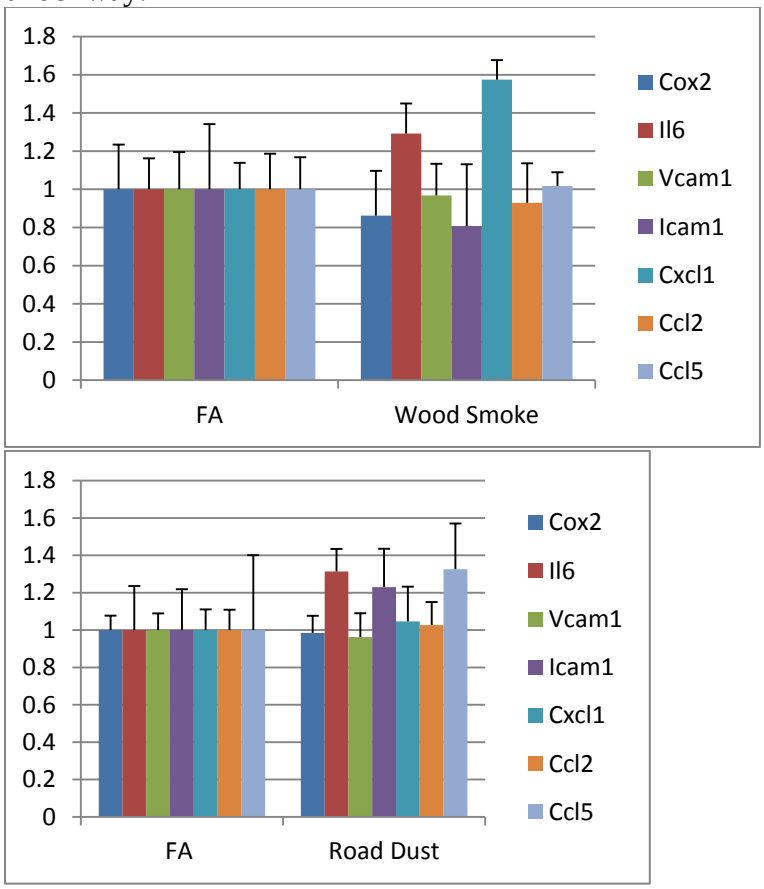
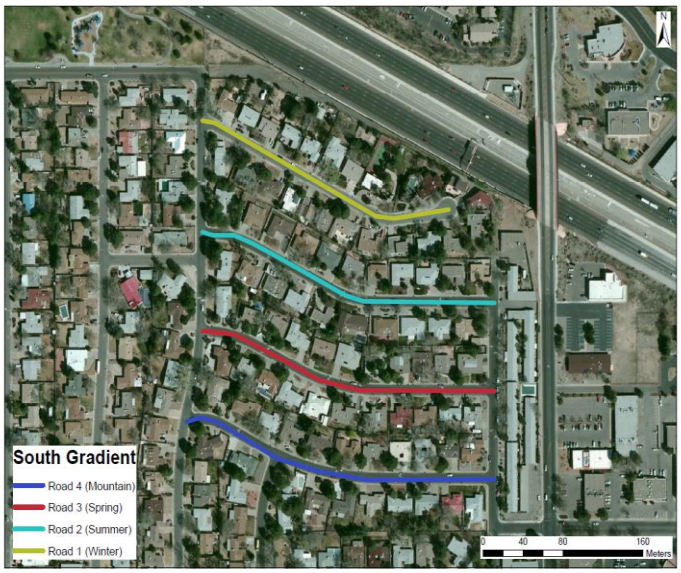


Figure 8. Cytokine responses in endothelial cells challenged with wood smoke and road dust.

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. The figure 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.



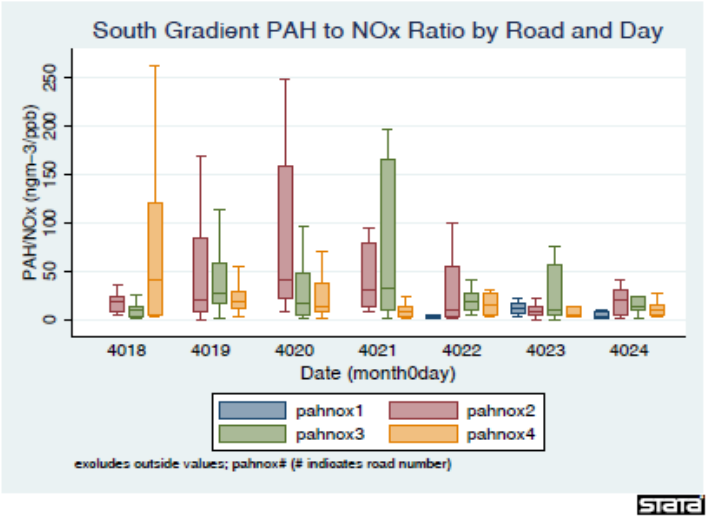


Figure 9. Transect of roadway composition.

Consideration of methods for complex mixture analysis.
One of the activities under Project 2 this year was the publication of final integrative analysis results of the National Environmental Respiratory Center.

Publications / Presentations / Posters

Publications to Date:

There are several publications that are currently in progress to be submitted this quarter, and one that is in press: This was research conducted as a component of the EPA funded NERC program at LRRI. The EPA Center helped to support the final analyses of these data, which are being used to define the test conditions for the road dust test atmospheres.

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. 2014**

2. **Campen, M., Robertson, S., Lund, A., Lucero, J. & McDonald, J. Engine exhaust particulate and gas phase contributions to vascular toxicity. Inhal Toxicol 26, 353-360 (2014).**

3. **H. Oppenheim, J. Lucero, A. Guyot, L. Herbert, J. McDonald, A. Mabondzo and A. Lund. Exposure to vehicle emissions results in altered blood brain barrier permeability and expression of matrix metalloproteinases and tight junction proteins in mice. Particle and Fibre Toxicology 2013 10:62.**

4. **J. L. Mauderly, D. Kracko, J. Brower, M. Doyle-Eisele, A.K. Lund, J.D. McDonald and S.K. Seilkop. The National Environmental Respiratory Center (NERC) Experiment in Multi-Pollutant Air Quality Health Research: IV. Vascular Effects of Repeated Inhalation Exposure to a Mixture of Five Inorganic Gases. Submitted to Inhalation Tox April 2014**

5. **J. Mauderly, E.G. Barrett, K.C. Day, A.P. Gigliotti, J.D. McDonald, K.S. Harrod, A.K. Lund, M.D. Reed, J. Seagrave, M.J. Campen and S.K. Seilkop: National Environmental Respiratory Center (NERC) Experiment in Multipollutant Air Quality Health Research: II. Comparison of Responses to Diesel and Gasoline Engine Exhausts, Hardwood Smoke, and Simulated Downwind Coal Emissions. Inhal. Toxicol. (in press).**

Presentations to Date:

1. **T. Holmes, J. McDonald, P. Kuehl, D. Kracko. Characterization of the Blu E-Cigarette to Define the Composition of Inhaled Material. Presented (1202/302) at Society of Toxicology, Phoenix, Arizona, 2014.**

2. **M. Doyle-Eisele, A. Rohr, E. Knipping, A. Lund, J. Brower, J. McDonald. Secondary Organic Aerosols Generated from α-Pinene-Amine Mixtures: Effects on the Cardiovascular System. Presented (1222/322) at Society of Toxicology, Phoenix, Arizona, 2014.**

3. **J. Brower1, B. Moeller, M. Doyle-Eisele, S. Stirdivant, J. McDonald, M. Campen. Acute Inhalation Exposure to Mixed Vehicle Emissions Induces Serum Metabolite Changes Related to Oxidative Stress, Lipid Peroxidation, and Energy Metabolism. Presented (1242e/354) at Society of Toxicology, Phoenix, Arizona, 2014.**

4. **J. McDonald, Influence of Collection Region and Site Type on the Composition of Paved Road Dust: It's Not Just Dirt!!! Presented (2312) at Society of Toxicology, Phoenix, Arizona, 2014.**

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 continue 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/envhlth/research_center/center.php

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 uses 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 blood-borne ligands and bioactivity in terms of driving endothelial cell activation or dysfunction. In addition to complex combustion mixtures, 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. Furthermore, we have used engineered nanomaterials as another comparison model, as a particle that is not associated with gases nor formed via combustion.

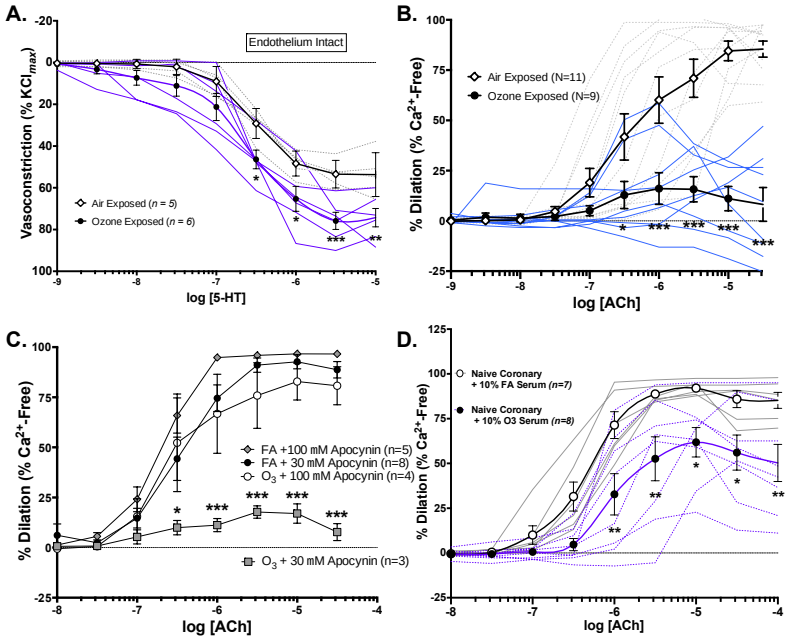


Figure 1. A. Coronary artery constriction to serotonin (5-HT) is enhanced in rats exposed to 1 ppm O_3 for 4h in endothelium-intact vessels (ANOVA interaction p=0.0023). Individual subject responses are shown with dashed (FA) or purple (O_3) lines, along with the symbols representing mean and standard error for each group. **B.** Coronary artery dilation to acetylcholine (ACh) is diminished in rats exposed to 1 ppm O_3 for 4h in endothelium-intact vessels (ANOVA interaction p<0.0001). Individual subject responses are shown with dashed (FA, N=11) or blue (O_3, N=9) lines, along with the symbols representing mean and standard error for each group. **C.** O_3-induced impairment of vasodilation was restored by treatment with 100 μM apocynin, suggesting that endothelial dysfunction may be in part due to NADPH oxidase-derived reactive oxygen species. Asterisks (*) indicate significant difference from FA control rats by a repeated measures two-way ANOVA with Bonferroni's multiple comparison post-hoc test (*P<0.05, **P<0.01, ***P<0.001). **D.** Infusion of 10% serum (in PSS) from rats exposed to O3 into the lumen of isolated naïve (unexposed) coronary arteries induced a significant impairment in vasodilation to acetylcholine (ACh) compared to vessels infused with 10% serum from FA-exposed rats (ANOVA interaction p=0.005).

In the first study, we examined further the bioactivity of O_3 on coronary arteries from rats. Following inhalation of 1ppm O_3, we found that coronaries harvested from exposed rats had a greater propensity to constrict to serotonin and a dramatically reduced ability to dilate to acetylcholine (Figure 1A, B). The impairment in dilation related to intracellular oxidative stress, as full dilation could be recovered with co-treatment with apocynin (Figure 1C) or superoxide dismutase and catalase (not shown). Most importantly, coronary vessels from unexposed (naïve) rats lost dilatory capacity when perfused intraluminally with serum from O_3-exposed rats, as compared to the serum from air-exposed rats (Figure 1D). Thus, the serum components alone could approximate the impairments seen in vivo. These effects were not likely due to cytokines in the serum, as concentrations of 9 measured cytokines did not differ between exposure groups (Figure 2). However, working with a proteomics group at Virginia Commonwealth, we have identified classes of fragmented peptides induced in the serum following O_3 exposure that may drive a systemic inflammatory response consistent with our observations.

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). In mice exposed to mixed vehicular emissions (gasoline and diesel engine), aortas expressed elevated markers of inflammation (MMP9) and oxidative stress (nitrotyrosine); when either the PM or the gas portion of the atmosphere were removed, the effects of the whole emissions disappeared (Fig 3). This and other evidence suggests that the PM-associated VOCs enhance the PM toxicity, possibly by 1) enhancing surface reactivity of the particles, 2) prolonging the residence time of the VOCs, and/or 3) increasing local concentrations of VOCs at points of PM deposition.

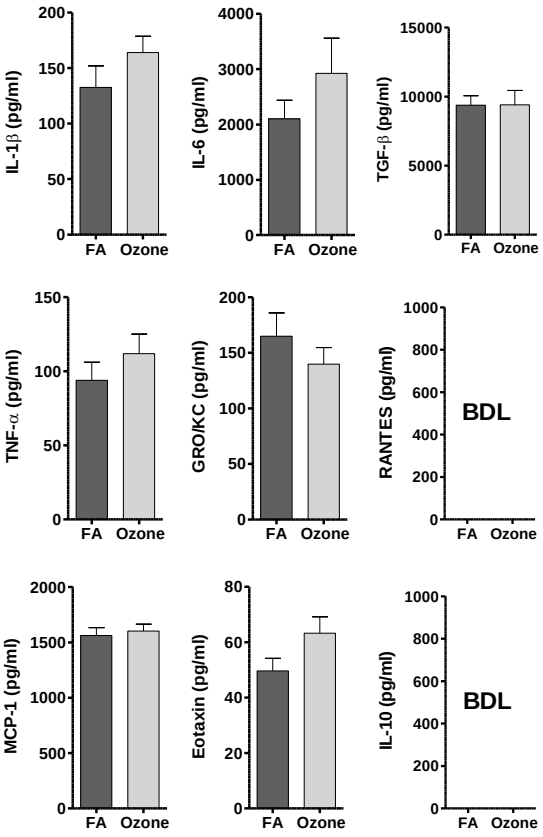


Figure 2. Serum cytokines are unaltered by 1ppm O_3 x 4h exposures, despite potent vasoactivity shown in Figure 2.

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, 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.

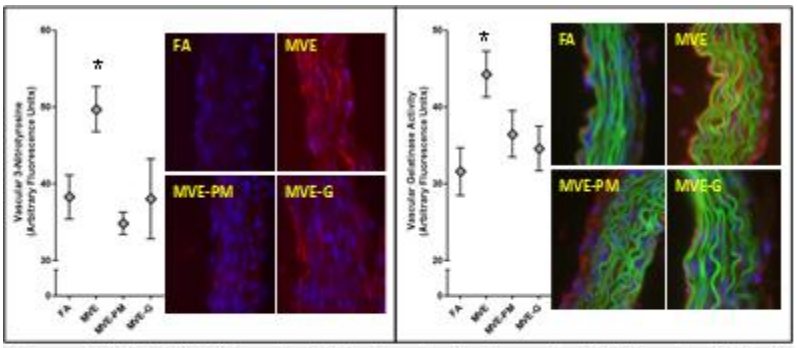


Figure 3. MMP9 (left; red staining) and nitrotyrosine (right; red staining) staining in aortas from ApoE-/- mice exposed to fresh mixed vehicle emissions (MVE) whole **or with PM (-PM) or gases (-G) removed** by filtration or denudation, respectively. MVE induced a significant effect (*P<0.05; N=5/group) that was reduced by –PM & –G permutations.[4,5]

Publications / Presentations / Posters

Publications to Date:

1. **Campen, M., Robertson, S., Lund, A., Lucero, J. & McDonald, J. Engine exhaust particulate and gas phase contributions to vascular toxicity. Inhal Toxicol 26, 353-360 (2014).**

2. **Paffett ML, Sheppard L, Robertson S, Weaver J, Lucas SN, Campen MJ. Ozone inhalation enhances coronary artery constriction and impairs dilation via superoxide-dependent mechanisms. Submitted to Toxicol Appl Pharmacol, 2014**

3. **Schisler J, Campen MJ, Madden M, and Willis MS. Transcriptional Endothelial Biosensor Response to Diesel-Induced Plasma Compositional Changes. In preparation.**

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. 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.

6. 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.

7. 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. This will be the focus of the remainder of the study, interacting closely with Dr. McDonald and Project 2. We will examine the relative systemic inflammatory potential following exposures to complex emissions.

Collaborative studies: 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.

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: Effect of Commute Traffic on Vascular Function

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

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, though the study design is substantially changed.

Project 4 examines the acute vascular effects of commute traffic exhaust exposures in human subjects, in a multi-pollutant context. This double-blind, randomized, controlled crossover trial will test whether traffic-derived mixed pollution atmospheres of diesel exhaust and gasoline engine exhaust, experienced through travel on roadways in a passenger car, causes an increased vascular response (brachial artery vasoconstriction, increased blood pressure, reduced retinal arteriolar diameter) compared with filtered air (FA) in healthy subjects. These nested aims include: whether specific exhaust-related monocytic gene expression effects are mediated by lipid peroxidation; whether traffic-related pollutants' vasoconstrictive effects are increased in subjects with a common SNP variant in the gene coding for TRPV1; and whether monocyte DNA methylation in specific genes is modified with exposure to typical, roadway-derived exposures. This approach differs from what was proposed in our original grant application—which specified a controlled exposure laboratory experiment—based on request of funding agency and following discussion with our center's external scientific advisory committee.

Research Performed - Progress Summary/Accomplishments

Project 4 is planned to begin human studies in Year 4 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 year 4. In this project, we will use a "typical commute" study design and pertinent experience in human exposure studies to advance the Center's research agenda with a double-blind, controlled exposure crossover clinical trial in 24 subjects, randomized to order. Using an innovative approach in which contrasts of in-vehicle exposure and of potential participant susceptibility by genotype are nested in the experiment, we can address several hypotheses in this study. Building on our prior work, we will use a typical commute model to confirm or determine whether traffic (e.g., mixed on-road environment with diesel and gasoline engine exhaust components) -derived aerosols exert demonstrable and important acute vascular effects in human subjects, and whether traffic-derived aerosols acutely

induce increased lipid peroxidation, response to oxidized phospholipids, and result in measurable impacts on gene expression and DNA methylation, in pathways that are related not only to the triggering of acute cardiovascular events, but also to the development and progression of atherosclerosis. Of course, all of the outcomes we measure are completely transient and reversible, and exposures are designed to be those of a typical urban commute path.

During the last year, the CCAR Project 4 was revised, received UW IRB approval without controversy, and was approved by the EPA's Human Subjects Research Review Official on May 23, 2013, so we are permitted to begin expending project funds for this activity. The study vehicle has been prepared and pilot-tested, and we are confident we can provide reduced particle counts and black carbon concentrations to participants. Recruitment of subjects has now begun.

Publications/Presentations

Pending.

Future Activities

We will launch the Center-sponsored study protocol and complete procedures as proposed.

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

Developing spatial exposure model. For Aim 1 of Project 5, we are working closely with Project 1 and Biostatistics Core personnel to develop approaches to their high-dimensional data which can be applied to epidemiological analyses. A preliminary analysis of the Project 1 badge data for the summer of 2013 collected in Baltimore, MD included data for pollutants O_3, NO_2, NO_x, SO_2, Pentane, Isoprene, Nonane, Decane, Undecane, Dodecane, Benzene, Toluene, m-Xylene, and o-Xylene measured at 43 monitoring locations. Our goal was to understand spatial predictability of each pollutant using GIS covariates at each location via cross-validation. For

each pollutant, we used a combination of partial least squares (PLS) and universal kriging (UK) as our prediction model.

Understanding in-vehicle contribution to individual level multi-pollutant exposures. A major effort has been in the field work portion of this project, which will address significant 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 are understanding the influence of time spent in transit on personal exposure, which will improve our individual-level exposure estimates and contribute to our epidemiological analysis.

Three of the four exposure campaigns have been completed, and the fourth is now underway. These campaigns occur twice in two seasons each in Winston-Salem and Los Angeles, and involve 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.

Table 1 shows the demographic characteristics of 1) the subgroups who participated in each of the first three CCAR Project 5 field campaigns and 2) the complete MESA Air cohort at Exam 5 in both Winston-Salem and Los Angeles. As intended, the subgroup we recruited in Winston-Salem is fairly well representative of the MESA Air cohort in that city as a whole. In Los Angeles, participants in Project 5 tended to be younger, and there were fewer Chinese participants than the cohort as a whole. The differences in racial/ethnic composition are due to the decision to include only English speakers in this project.

Table 1. Demographics for Project 5 Field Campaigns Compared with the Winston-Salem and Los Angeles MESA Air Cohorts as a whole.

	CCAR Project 5		MESA Air	CCAR Project 5	MESA Air
	Winston-Salem Heating	**Winston-Salem Non-Heating**	**Winston-Salem Cohort at Exam 5**	**Los Angeles Heating**	**Los Angeles Cohort at Exam 5**
	n (%)	n (%)	n (%)	n (%)	n (%)
Gender					
Male	21 (46%)	23 (49%)	348 (46%)	23 (49%)	445 (49%)
Female	25 (54%)	24 (51%)	415 (54%)	24 (51%)	462 (51%)
Race					
White	20 (43%)	21 (45%)	413 (54%)	14 (30%)	165 (18%)
Black	26 (57%)	26 (55%)	348 (46%)	9 (19%)	111 (12%)
Chinese	0 (0%)	0 (0%)	0 (0%)	3 (6%)	294 (32%)
Hispanic	0 (0%)	0 (0%)	2 (0%)	21 (45%)	337 (37%)
Age Group*					
45-54	1 (2%)	1 (2%)	8 (1%)	2 (4%)	12 (1%)
55-64	9 (20%)	14 (30%)	236 (31%)	21 (45%)	310 (34%)
64-74	18 (39%)	19 (40%)	256 (34%)	16 (34%)	284 (31%)
75-84	15 (33%)	12 (26%)	215 (28%)	8 (17%)	227 (25%)
85+	3 (7%)	1 (2%)	48 (6%)	0 (0%)	74 (8%)
Median Age*	72	69	70	65	69
Age range*	54 - 89	54 - 93	54 - 93	54 - 83	54 - 93

*At MESA Exam 5 (2010-2012).

Some characteristics of the first exposure campaign (Winston-Salem heating season) were provided in the last report. Table 2 presents median concentrations for all measured analytes from that campaign, as well as median concentrations from the non-heating campaign in Winston-Salem and heating season campaign in Los Angeles. Because SO_2 was only detected in one sample during the Winston-Salem heating campaign and two samples in the Los Angeles heating campaign at levels just above the detection limit, and not detected in any samples during the Winston-Salem non-heating campaign, results are not presented here. For each pollutant, between 0 and 5 samples from Winston-Salem heating, 0 and 10 samples from Winston-Salem non-heating, and 0 and 5 samples from Los Angeles heating, were invalidated due to field or laboratory error.

Table 2. Median Concentrations by Sampling Location: Residential Indoors, Residential Outdoors, Personal, and In-vehicle.

	Winston-Salem heating				Winston-Salem non-heating				Los Angeles heating			
Parameter	**Out**	**In**	**Pers.**	**Veh.**	**Out**	**In**	**Pers.**	**Veh.**	**Out**	**In**	**Pers.**	**Veh.**
NO_2	9.32	5.86	6.95	74.1	4.33	4.62	5.21	46.1	20.4	19.1	18.0	71.1
NO_x	14.4	13.4	16.7	114	5.40	9.89	12.4	61.7	43.7	55.1	57.6	196
O_3	32.3	0.46	1.55	29.9	23.3	0.44	1.30	17.59	15.7	0.98	1.55	14.1
Pentanes	14.6	38.9	69.3	280	21.1	85.5	204	587	40.7	48.1	58.9	234
Isoprene	0.00	0.02	0.11	0.02	0.07	0.07	0.08	1.59	0.07	0.07	0.07	1.32
Nonane	0.01	0.09	0.11	0.86	0.01	0.22	0.29	0.91	0.05	0.08	0.11	0.53
n-Decane	0.01	0.11	0.14	1.98	0.02	0.22	0.21	3.29	0.03	0.06	0.08	3.64
n-Undecane	0.01	0.21	0.24	3.01	0.02	0.21	0.23	6.97	0.03	0.06	0.07	11.7
n-Dodecane	0.03	0.06	0.09	3.82	0.03	0.18	0.17	10.24	0.02	0.05	0.06	9.77
Benzene	0.18	0.26	0.31	1.57	0.09	0.45	0.51	2.09	0.23	0.25	0.25	1.41
Toluene	0.21	1.15	1.55	7.21	0.23	3.52	3.72	15.03	0.62	0.92	1.16	5.75
m-Xylene	0.07	0.33	0.36	4.25	0.07	0.65	0.63	6.23	0.25	0.32	0.41	2.64
o-Xylene	0.04	0.14	0.18	2.21	0.03	0.31	0.28	2.99	0.13	0.16	0.20	1.46

For all analytes except for ozone and isoprene, the highest concentrations were found in the vehicle samples during the Winston-Salem heating campaign. For ozone and isoprene, the highest concentrations were found in the outdoor and personal samples, respectively.

The second field campaign occurred in Winston-Salem from July 29 – August 24, 2013. This campaign included 47 participants (98% of goal). Of the 47 participants, 29 returned from the February field campaign and 18 were new participants. We deployed 188 Ogawa and 188 3M samplers (47 each of personal, indoor residential, outdoor residential, and in-vehicle), and measured the same pollutants as during the heating season campaign. We also deployed 19 blank samples (10%) and 15 duplicate samples (11% of possible maximum, as no personal duplicates were intended to be deployed, to reduce participant burden). As with the Winston-Salem heating season campaign, the highest concentrations for most analytes were found in the vehicle samples for the Winston-Salem non-heating season campaign (Table 1). The only exception was ozone, where the highest concentration was found outside.
The third field campaign occurred in Los Angeles from January 27 – February 20, 2014. This campaign included 47 participants (98% of goal). We deployed 188 Ogawa and 188 3M samplers (47 each of personal, indoor residential, outdoor residential, and in-vehicle), as well as 20 blank samples (11%) and 14 duplicate samples (10% of possible maximum,). For all analytes except for ozone, the highest concentrations were found in the vehicle samples. For ozone, the highest concentrations were found in the outdoor samples.

In addition to the air monitoring described above, each of these three field campaigns also included an intensive location-tracking component focused on time spent in five different microenvironments: at home indoors, at home outdoors, away from home indoors, away from home outdoors, and in a motor vehicle. Time-location data were collected using three different methods simultaneously: time-location diaries, Global Positioning System (GPS) tracking, and

proximity monitors. In order to analyze the GPS tracking data, a rule-based model using speed and location information is being developed to categorize each GPS point recorded into the microenvironments of interest.

Epidemiological Analyses. Several analyses relating to Aim 3 of Project 5 are in progress. These include analyses using the following outcomes: 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. Analyses thus far rely on exposure estimates from prior work, but new analyses will incorporate the information gained in this project's Aims 1 and 2.

Publications / Presentations / Posters

Publications to Date:

1. **Spalt EW, CL Curl, RW Allen, M Cohen, SD Adar, K Hinckley Stukovsky, Ed Avol, Cecilia Castro-Diehl, C Nunn, K Mancera-Cuevas, and JD Kaufman. Time-Location Patterns of a Diverse Population of Older Adults: The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). (submitted to Journal of Exposure Science and Environmental Epidemiology). 2014.**

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. **Nicholas T. Contribution of the in-vehicle microenvironment to individual ambient source-derived NO2 exposure concentration. M.S. Thesis, University of Washington. 2014.**

5. **Spalt EW, Curl CL, Allen RW, Cohen M, Williams K, Hirsh JA, Kaufman JD. Factors influencing time-location patterns and their impact on estimates of exposure: The Multi-Ethnic Study of Atherosclerosis and Air Pollution. In prep.**

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.

Future Activities

The final field campaign in the "non-heating" season in Los Angeles is currently underway and will be complete by August 1, 2014. We have complete IRB approval at the University of Washington, Wake Forest University, and UCLA for all of these activities.

In addition to focusing a significant amount of effort on these field campaigns, our immediate next goals are to continue analyzing the data collected in the first three 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. We are also in the process of preparing three manuscripts on the study design and methods, a comparison of the three time-location measurement methods utilized in this study, and an analysis of the relative contributions of each microenvironment to overall exposure to ambient-source nitrogen dioxide.

The next project year will see effort transition primarily to Aim 3: epidemiological analyses incorporating the exposure information gained through Aims 1 and 2.

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	10/12-12/15	$90,000
#2 Mobile and Fixed Site Characterization of Vehicle Emission Impacts in Atlanta	Tim Larson Mike Yost	• Mobile and Fixed Site Monitoring Campaign in Atlanta	10/12-12/15	$55,000
#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	12/12-12/15	$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	12/12-12/15	$15,000

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 & Progress

The goal of this collaborative project is to compare a limited set of spatially intensive mobile and fixed site measurements of selected pollutant with downscaled CMAQ predictions in Atlanta, Georgia.

To this end, we have performed mobile platform and fixed-site monitoring in greater Atlanta, GA over a two-week period in September, 2013. The final QC data set from this campaign will be available in early autumn of 2014. For this project, mobile monitoring and passive sampling measurements were conducted during an approximate two-week period in September 2013. The mobile monitoring platform measured 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 (see Figure 1). Data collection included 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 >0.05 um, particle light scattering coefficient, particle light absorption (black carbon), NO_2, O_3, CO, CO_2 and total VOCs The mobile platform was continuously moving during the measuring periods, which were done from about 2-7 pm each evening. The mobile measurements include ozone, nitrogen dioxide, black carbon, carbon dioxide, particle light scattering, and ultrafine particle count. The mobile measurements were referenced to a fixed site which simultaneously collected data with a second set of instruments. Sampling loops were designed to capture both regional and small scale variability for comparison with CMAQ down-scaled predictions.

We have also made predictions at these fuzzy points for selected species using a downscaled version of CMAQ. The measurements are being compared with this enhanced CMAQ model's 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. Here we report preliminary results for selected mobile platform measurements.

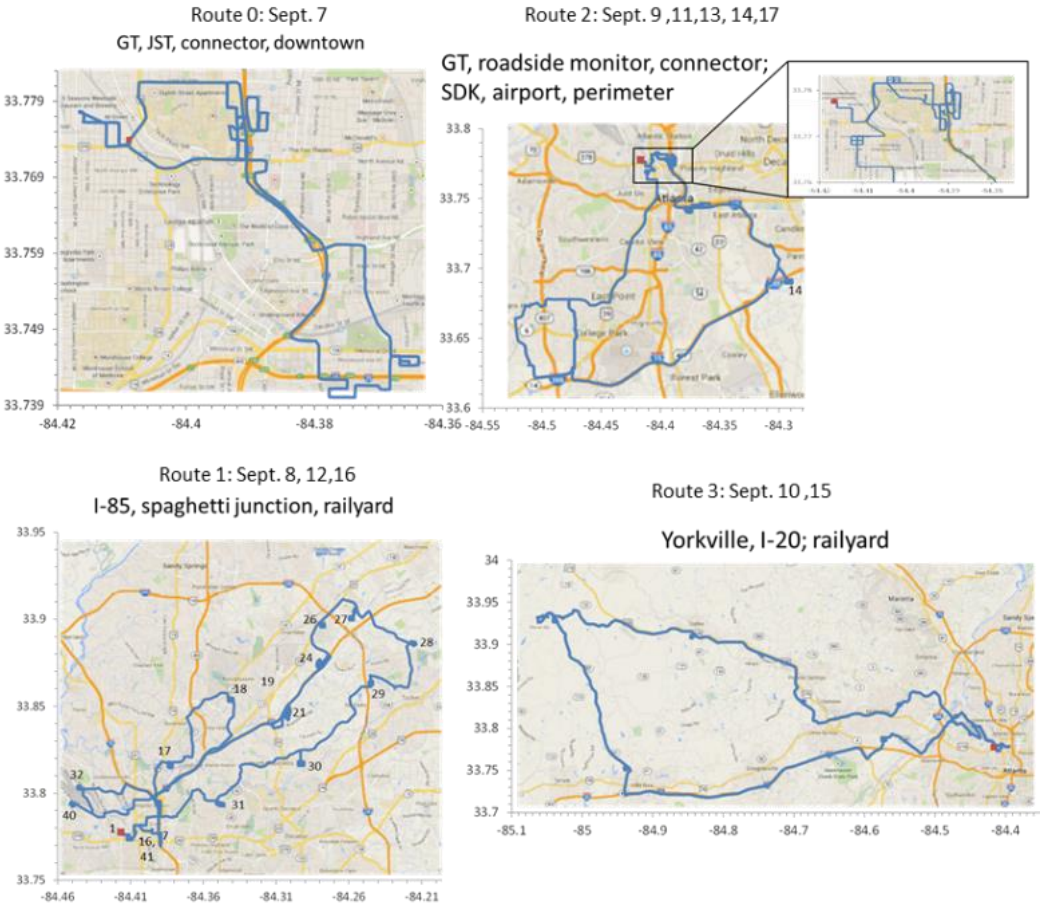


Figure 1: Mobile Platform sampling Routes in Atlanta.

Twenty eight mobile monitoring fuzzy point medians for NO_2, black carbon and particle light scattering were compared with simultaneous ambient monitoring data at the Jefferson St. (JST), Yorkville (YRK) and South DeKalb St ambient monitors as shown in Figure 2. The mobile platform ozone data are still undergoing QA analysis due to systematic denuder effects from the nafion drier inlet that tended to lower the observed readings. In addition, the NOx values from the 2B Tech instrument have known interferences with hydrocarbons, unlike the CAPS NO_2 instrument. Final QC results will be available in early Autumn, 2014.

Preliminary downscaled CMAQ predictions for NO_2 versus fuzzy point medians are shown in Figure 3. There is reasonable agreement between the two values across both time and space. Additional analyses are ongoing.

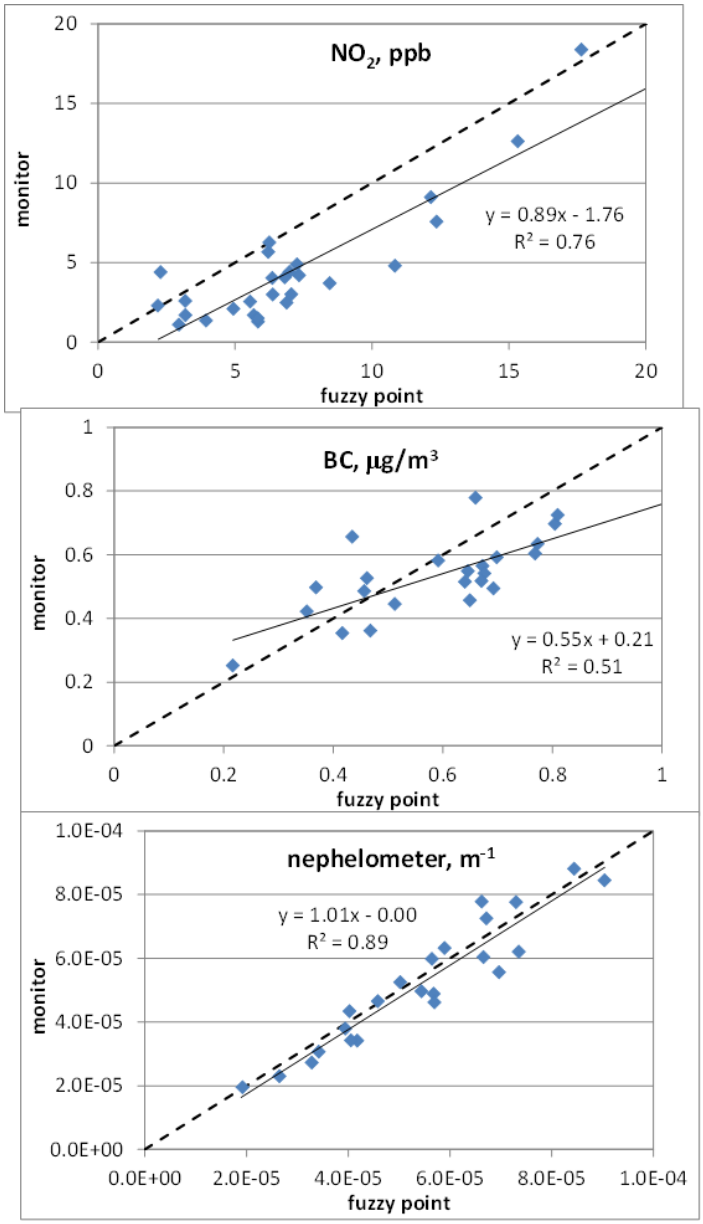


Figure 2. Fuzzy point median values vs. ambient monitor values for selected species (Jefferson St., Yorkville and DeKalb sites)

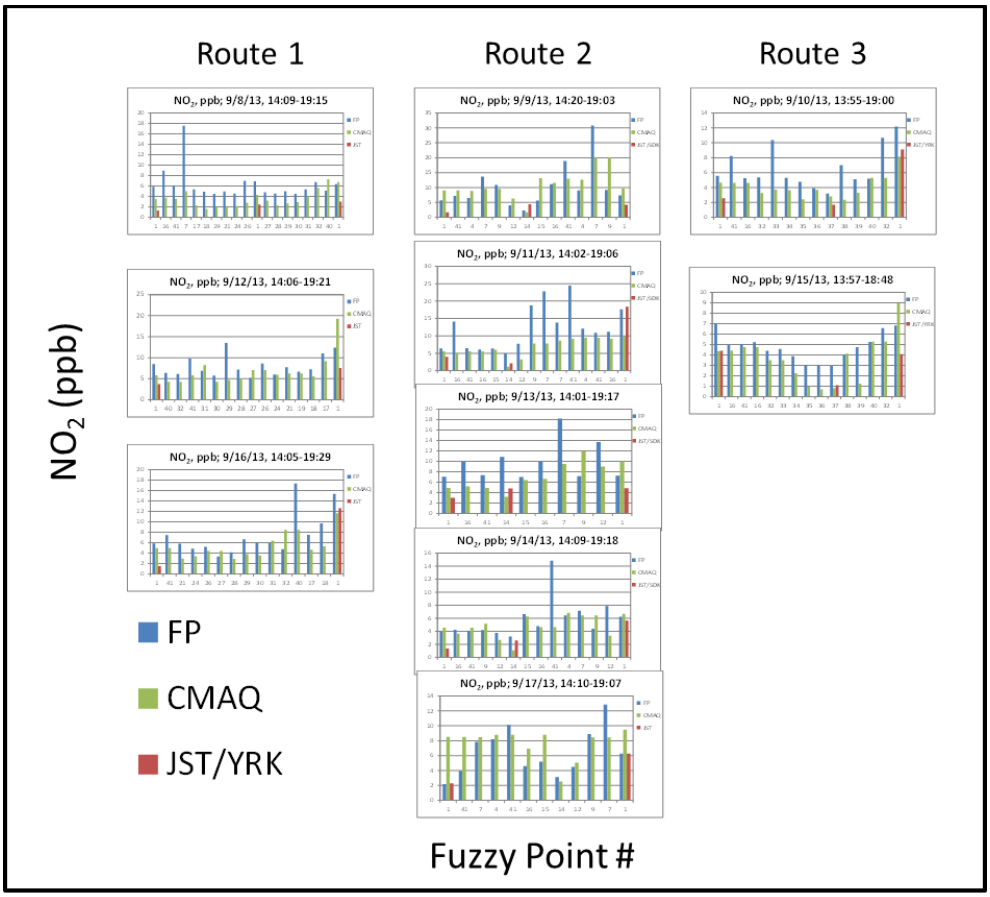


Figure 3. Fuzzy Point (FP) medians vs. CMAQ predictions for NO_2.

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

Objective of Research

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 UW, Harvard, and Emory are participating in this project.

Planned Activities

We will utilize Emory's birthweight cohort data as a testbed for applying measurement error correction techniques developed at the three participating centers. The birthweight data will be derived from administrative records for all singleton live births in Georgia from 2001-2006. 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.

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 and survival

Research Performed / Progress Summary

UW investigators have taken part in ongoing email discussions on the analytic plan for Emory's birthweight analysis, including logistical and methodological aspects of UW's spatio-temporal exposure model. Emory investigators have successfully applied UW's spatio-temporal exposure model in the Atlanta area and have used predicted exposures to quantify the association between birthweight and PM2.5 exposure. Pending final sensitivity studies, this analysis is complete, and the next step is for UW, Harvard, and Emory investigators to apply their respective measurement error correction methods.

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.

The spatial domain for this exercise is a region of approximately 600K km^2 centered on North Carolina and including monitoring data from 126 EPA monitoring sites for the period 2006-2008, as shown in the following figure (Figure 1).

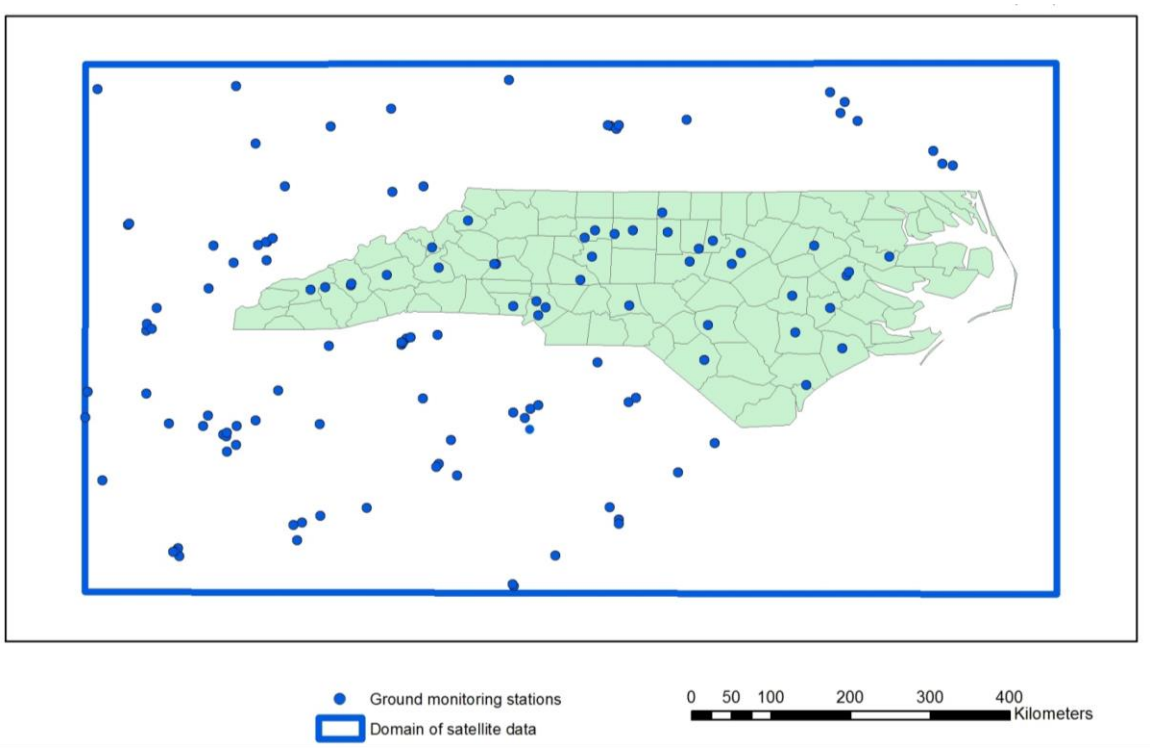


Figure 1

The Emory team has compiled MODIS collection 6 AOD data on a 10 km resolution grid over the modeling domain for 2006-2008 as shown in the figure below (Figure 2). These data were prepared through collaboration with NASA's Goddard Space Flight Center. Computer codes were developed to extract and format various AOD parameters and their QA flags.

To facilitate model cross-comparison, a common input dataset including geographic covariates and meteorological data, as well as satellite AOD measurements, is being used by all the modeling groups. Following a series of discussions, the U.W. CCAR/MESA Air data team computed and delivered a database of geographic covariates for the 10 km^2 satellite grid. The Liu group has combined these with meteorological data and the satellite observations for distribution to all participating research teams. A set of common procedures and statistics has been proposed to evaluate model performance statistics via out-of-sample cross-validation. After preliminary results are generated, each team will document their model development in sufficient detail for other teams to reproduce their results. The expected deliverable of this project will be a manuscript to report evaluation results.

Model fitting is now in progress by all the research groups, with the U.W. CCAR group beginning with a spatio-temporal model not including AOD as a baseline for assessing the value added by the satellite data.

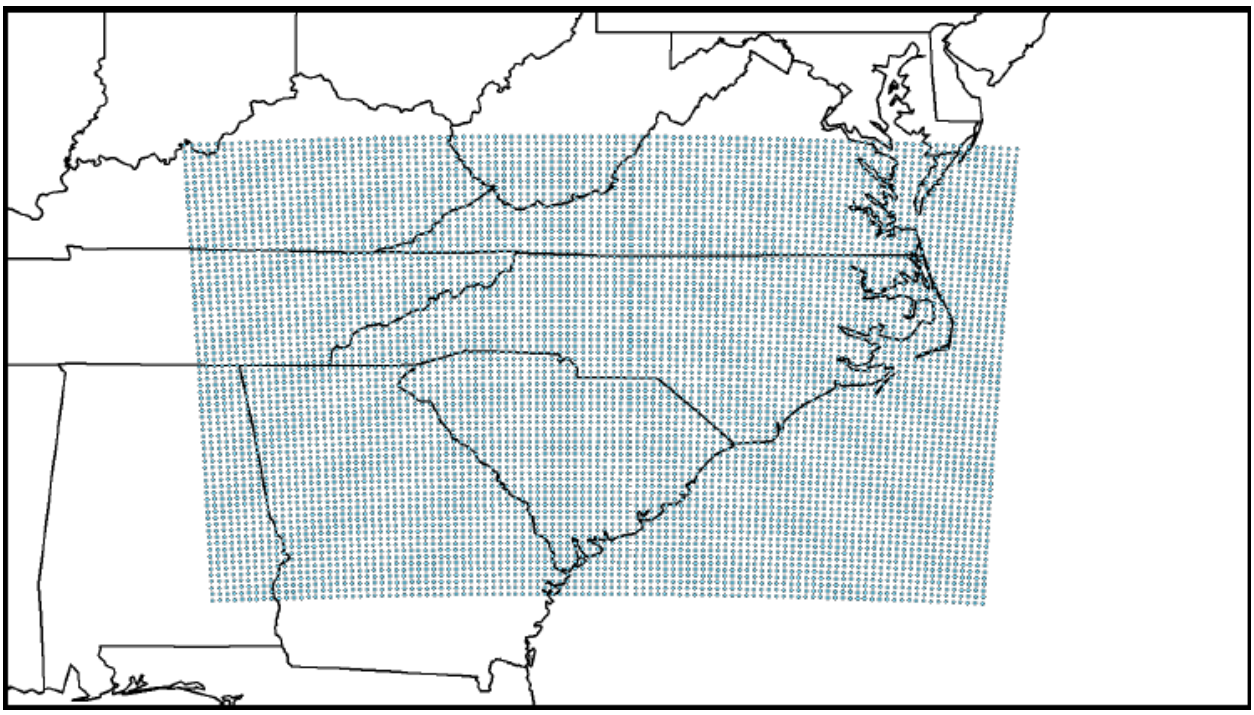


Figure 2