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, 2014 – July 31, 2015

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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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IV. UW CCAR CLARC Program Collaborations

REPORT OVERVIEW

This Annual Progress Report covers the fifth year of funding to date [8/1/2014 – 7/31/2015] 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.
    - The Year 4 SAC meeting was held on October 6th and 7th 2014.
    - Sanjay Rajagopalan was a late cancellation due to illness and could not attend.

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 5, 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 (now Skype for Business).

Subawards

- The Center's subawards for Lovelace Respiratory Research Institute (LRRI) and the University of New Mexico were renewed for Year 5. The subaward for Washington State University was extended into Year 5 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 controlled exposure studies for Project 4. Instead of the original plan, Project 4 has utilized a typical commute exposure design, where participants are studied while participating in a heavily-trafficked drive with or without an operational filtration system in place. The original health outcomes proposed for Project 4 are being measured. The change in protocol 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, and the commute exposure drives began in December 2014.

- The University of Washington has requested a one-year no cost extension from EPA to finish the scope of work for CCAR.

Changes in Key Personnel

None during this project period.

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 all projects, 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. All projects have submitted Quality Assurance Project Plans (QAPPs) that have been reviewed, revised, and approved by the QAM. 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.

Administrative Core

- Quality Management – The Center will continue to follow up with each individual project, and associated QAO, to review, revise, and archive all relevant SOPs, and their respective QAPPs.

- 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 5 and research activities seeing significant progress across 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.

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, 2015. 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: March 9, 2015 through March 8, 2016

2. IACUC Protocol #2650-08 Annual Approval: February 18, 2014 through February 17, 2015.

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

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

5. 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.

and 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.

Recent work performed under FY15-023, titled "Effects of mixtures of causal components of vehicular emissions and other inhalation toxicants on markers of cardiovascular disease and inflammation in rats and mice," approved January 22, 2015

1. Amendment A was approved on February 11, 2015, to request shortened quarantine for some groups of animals

2. Amendment B was approved on March 30, 2015, to request an additional blood sampling method of retro-orbital bleeding

<u>Project 4</u> – 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), and it was approved by the University of Washington IRB on April 10, 2014 (IRB #46658, annual review March 2015). Three modifications to this application have been approved by University of Washington IRB:

- Modification 1 for amended recruitment scripts and consent was approved 4/10/2014
- Modification 2 for changes to protocol and consent for blood procedures / location change was approved 7/14/2014.
- Modification 3 for amended recruitment materials and consent regarding additional drive and compensation change was approved 12/2/2014.

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 with annual reviews approved December 2013 and December 2014. 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 4 CCAR Annual Report

Current: 7/31/15

Center Publications to Date:

1. **Bergen S, Sheppard L, Kaufman JD, and Szpiro AA. Multi-pollutant measurement error in air pollution epidemiology studies arising from predicting exposures with penalized regression splines. Journal of the Royal Statistical Society, Series C. Submitted.**

2. **Jandarov R, Sheppard L, Sampson PD, and Szpiro AA. A novel principal component analysis for spatially misaligned air pollution data. Journal of the Royal Statistical Society, Series C. Submitted.**

3. **Keller JP, Drton M, Larson T, Kaufman JD, Sandler DP, and Szpiro AA. Covariate-adaptive clustering of exposures for air pollution epidemiology cohorts. Biometrics. Submitted.**

4. **Lee A, Szpiro AA, Kim S-Y, and Sheppard L. Impact of preferential sampling on exposure prediction and health effect inference in the context of air pollution epidemiology. Environmetrics. 2015. Epub ahead of print.**

5. **Wang M, Brunekreef B, Gehring U, Szpiro AA, Hoek G, and Beelen R. A framework for evaluating land use regression models and the effect on health effect estimates. Epidemiology. Submitted.**

6. **Herring CL, McDonald JD, Massoli P, Sueper D, Faiola CL, Erickson MH, Simpson CD, Yost MG, Jobson BT, and VanReken TM. New Methodology for High Resolution Polycyclic Aromatic Hydrocarbon (PAH) Quantification in Diesel – Gasoline Engine Exhaust using HR-ToF-AMS. Environmental Science and Technology. 2015. submitted**

7. **Fintzi JR, Riley EA, Austin A, Schaal L, Gould T, Hartin K, Sasakura M, Sheppard L, Sampson P, Simpson CD, Yost MG, Larson TV. Characterization of On-road Air Quality and Associated Emission Sources Using Principal Component Analysis and Mobile Video Logs. Science of the Total Environment. 2015. Submitted.**

8. **Hudda, N., Gould, T., Hartin, K., Larson, T.V., Fruin, S.A., Emissions from an International Airport Increase Particle Number Concentrations 4-fold at 10 km Downwind. Environ. Sci. Technol. 2014. 48 (12), 6628-6635.**

9. **Galaviz, V.E., Yost, M.G., Simpson, C.D., Camp, J.E., Paulsen, M.H., Elder, J.P., Hoffman, L., Flores, D., Quintana, P.J.E. Traffic pollutant exposures experienced by pedestrians waiting to enter the U.S. at a major U.S.–Mexico border crossing. Atmos. Environ. 2014. 88 (0), 362-369.**

10. **Xu W, Riley E, Austin E, Sasakura M, Schaal L, Gould T, Hartin K, Simpson CD, Sampson PD, Yost M, Larson TV, Xiu G, Vedal S. Use of Mobile and Passive Badge Air Monitoring Data for NO_X and Ozone Air Pollution Spatial Exposure Prediction Models. Atmos Environ. 2015. Submitted.**

11. **Aragon MJ, Chrobak I, Brower J, Roldan L, Fredenburgh LE, McDonald JD, Campen MJ. Inflammatory and Vasoactive Effects of Serum Following Inhalation of Varied Complex Mixtures. Cardiovasc Toxicol. 2015 Apr 22. [Epub ahead of print] PMID: 25900702**

12. **Cosselman KE, Navas-Acien A, Kaufman JD. Environmental Factors in Cardiovascular Disease. Nature Reviews Cardiology. In press.**

13. **Chi, GC, Hajat A, Bird CE, Cullen MR, Griffin BA, Miller KA, Shih RA, Stefanick ML, Vedal S, Whitsel EA, Kaufman JD. Individual and Neighborhood Socioeconomic Status, Long-term Exposure to Air Pollution, and Risk of Cardiovascular Disease. 2015. Environmental Health Perspectives (under review).**

14. **Young, M. T., Sandler, D. P., DeRoo, L. A., Vedal, S., Kaufman, J. D., & London, S. J. (2014). Ambient air pollution exposure and incident adult asthma in a nationwide cohort of US women. American journal of respiratory and critical care medicine, 190(8), 914-921.**

15. **Chan S, Van Hee V, Bergen S, Szpiro AA, DeRoo L, London S, Marshall J, Kaufman JD, and Sandler D. Long term air pollution and exposure and blood pressure in the Sister Study. Environmental Health Perspectives. 2015.**

16. 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. Environmental and Ecological Statistics (2015): 1-31.

17. 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. Environmental health perspectives 123.4 (2015): 301.

18. 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. Environmental Health 14.1 (2015): 49.

19. Kim S-Y, Sheppard L, Larson TV, Kaufman JD, Vedal S. Combining PM2. 5 Component Data from Multiple Sources: Data Consistency and Characteristics Relevant to Epidemiological Analyses of Predicted Long-Term Exposures. Environmental health perspectives (2015).

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

21. 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. The Annals of Applied Statistics 8.4 (2014): 2509-2537.

22. 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. 2014; 180(7):718-28.

23. 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.

24. 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. DOI: 10.1007/s10651-013-0261-4. PMCID: NIHMS520301

25. 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.

26. 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

27. 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.

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

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

30. 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.

31. Gueneron, M., Erickson MH, VanderSchelden G., Jobson BT., PTR-MS Fragmentation Patterns of Gasoline Hydrocarbons, Atmospheric Measurement Techniques, 7, 225-239, 2014.

32. 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 air pollutants adjacent to the I-40 corridor in Albuquerque, NM. Atmos. Environ. 2014. 98 (0), 492-499.

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. 6.3: 615-628.

34. Oppenheim H, Lucero J, Guyot A, Herbert L, McDonald JD, Mabondzo A and Lund AK. 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.

35. Mauderly JL, Kracko D, Brower J, Doyle-Eisele M, Lund AK, McDonald JD and Seilkop SK. 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. Inhalation toxicology 2014. 26.11: 691-696.

36. 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.

37. 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).

38. 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. Toxicol Appl Pharmacol. 2015. Toxicol Sci. 2015 May 11.

39. Schisler J, Campen MJ, Madden M, and Willis MS. Transcriptional Endothelial Biosensor Response to Diesel-Induced Plasma Compositional Changes. Inhalation Toxicology. 2015. 27(5): 272–280.

40. 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.

41. 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.

42. 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.

43. Spalt, E. W., Curl, C.L., Allen, R.W., Cohen, M., Adar, S.D., Hinkley-Stukovsky, K., Avol, E., Castro, C., Nunn, C., Mancera-Cuevas, K., Kaufman, J.D. Time-Location Patterns of a Diverse Population of Older Adults: The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Journal of Exposure Science and Environmental Epidemiology. 2014.

44. 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.

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46. Nicholas T. Contribution of the in-vehicle microenvironment to individual ambient source-derived NO_2 exposure concentration. M.S. Thesis, University of Washington. 2014.

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Center Presentations to Date:

1. **Sampson, PD. Spatio-temporal Modeling for Environmental Monitoring Data. International Symposium on Statistics (ISS). St. John's, Newfoundland, Canada, July 2015.**

2. **Szpiro, A. Dimension Reduction for Spatially Misaligned Multivariate Air Pollution Data. Society for Epidemiologic Research (SER). Denver, CO. June 2015.**

3. **Keller, J. Covariate-adaptive Clustering of Exposures for Air Pollution Epidemiology Cohorts. Western North American Region of The International Biometric Society (WNAR). Boise, ID. June 2015.**

4. **VanderSchelden GS, Fuchs M, Bartoshevich R, Wen M, Jobson BT, Measurements of Diesel Exhaust and its Photoproducts using a PTR-MS and a Photochamber. 2014 Air & Waste Management Association PNWIS Conference, Spokane, WA, November 5-8.**

5. **Fuchs M, VanderSchelden GS, Flyckt CL, Jobson BT. Diesel Exhaust Flow Tube Reactor Characterization. 2014 Air & Waste Management Association PNWIS Conference, Spokane, WA, November 5-8.**

6. **Austin, E. Identification and Classification of Multipollutant Peak Events in Mobile Monitoring Data. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

7. **Riley, E. Multi‐pollutant mixtures identified from a principal component analysis by melding mobile monitoring and integrated passive sampler data. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

8. **Campen, MJ. Endothelial Cell Pattern Recognition Receptors, CD36 and LOX-1, Contribute to Responses to Pollution-Induced Circulating Factors. Society of Toxicology, Phoenix, Arizona, March 2014.**

9. **Hazlehurst, M. Integrating Data from Multiple Time-Location Measurement Methods for Use in Exposure Assessment: the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). International Society for Environmental Epidemiology. Seattle, WA. August 2014.**

10. **Spalt, EW. Patterns in Indoor, Outdoor, In-Vehicle, and Personal Measurements of Volatile Organic Compounds. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

11. **Nicholas, T. Contribution of time in-transit to individual exposure to traffic-related air pollution. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

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

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

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

15. 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.

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

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

18. 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.

19. 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.

20. 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.

21. 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.

22. 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.

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

24. 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

25. 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.

26. 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.

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

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

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

30. 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. Canadian Chemistry Conference and Exhibition, Vancouver, B.C., June 2014

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

32. Austin E. 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, January 2014.

33. 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.

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

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

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

37. McDonald JD, 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.

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

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

Center Posters to Date:

1. **Keller J. Covariate-adaptive Clustering of Exposures for Air Pollution Epidemiology Cohorts. University of Washington DEOHS Student Research Day. Seattle, WA. May 2015.**

2. **Riley E. Black Carbon and Ultrafine Particle Counts Downwind of Two Major Airports. University of Washington DEOHS Student Research Day. Seattle, WA. May 2015.**

3. **Xu W. Use of Mobile and Passive Badge Air Monitoring Data for NO_X and Ozone Air Pollution Spatial Exposure Prediction Models. Annual Conference on**

Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.

4. **Hazlehurst, M. Time‐location measurement methods for use in exposure assessment: the Multi‐Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

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

6. 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.

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

8. 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.

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

10. Riley EA, Sasakura MD, Hartin K, Crampton R, Gould TR, Larson TV, Yost MG, Simpson CD. Principal Component Analysis of Snap-Shot Air Pollutant Measurements In Baltimore, MD. EPA annual Clean Air Research Center Annual Meeting, Seattle, WA July, 2013.

11. Riley EA, Hartin K, Gould T, Larson TV, Yost MG, Simpson CD. Mobile measurements of near-highway air pollutant gradients. Annual Symposium on Environmental, Occupational and Population Health, Semiahmoo, WA, January 2014.

12. 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*, October 2013. ***Winner of a Student Poster Award.*

13. 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.

14. 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.

15. 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.

16. 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.

17. 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.

18. Brower J, Moeller B, Doyle-Eisele M, Stirdivant S, McDonald JD, Campen M. Acute Inhalation Exposure to Mixed Vehicle Emissions Induces Serum Metabolite Changes Related to Oxidative Stress, Lipid Peroxidation, and Energy Metabolism. Society of Toxicology, Phoenix, Arizona, 2014.

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.

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 and through consultation with members of the Project 1 team.

Project 3. Dr. Sheppard's collaboration with Matt Campen on the paper *Ozone Inhalation Enhances Coronary Artery Constriction And Impairs Dilation Via Superoxide-Dependent Mechanisms*, has now been published as an epub ahead of print. 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.

Project 5.
This project has been an important thrust of activities for the Core in Year 5. In the past we constructed preliminary spatial models of the Project 1 multi-pollutant data with the goal of formulating a strategy for predicting multi-pollutant profiles based on Project 1 data at MESA Air subject locations. We will implement this approach:

a. We use k-means clustering to conduct a preliminary identification of clusters in multipollutant datasets comprised of both summaries of mobile monitoring data and badge data, all indexed by fuzzy point locations and season (heating vs. non-heating). (We temporally adjust the mobile monitoring data to prepare it for a purely spatial analysis.)
b. We then use multivariate logistic regression classification algorithm with selected geographic covariates to predict cluster membership at MESA subject locations. We will also explore using support vector machines (SVM) as an alternative classifier, which would enable us to include a larger suite of geographic covariates.
c. Cluster membership will be used as an effect modifier of NO_x, for the role of air pollution on the rate of change of coronary artery calcium (CAC) over time. Details about each step follow.

a. *Cluster Analysis*

We applied traditional k-means cluster analysis methods to the CCAR monitoring data with the goal of identifying spatial differences in the multipollutant sources within each of the cities. We performed the analysis separately within each sampling season for each city, since we have ample reason to believe that source profiles change by season and by city. The four cities studied were Baltimore, Los Angeles, St. Paul and Winston-Salem. All pollutant data in this analysis were normalized by the concentration of NO_X measured at each location.

Data: For each city, passive badge samplers were deployed for a 2-week period at 43 locations. These passive badges measured cumulative VOC concentrations as well as NO_x, NO_2, and Ozone concentrations. In both Baltimore and Los Angeles, a mobile monitoring platform captured real-time afternoon gaseous and particle air concentrations over repeated 6-10 minute samples for these same 43 locations. We accounted for variability due to day-to-day differences in source activity (weekend/weekday) and dispersion factors by subtracting the daily fifth percentile of each pollutant. The pollutants measured by the mobile monitoring platform and included in this analysis were carbon monoxide, black carbon and size-fractioned PM count (25 nm- 400nm, <1

μm, 1-2.5 μm). After the background corrections were applied, we obtained the median pollutant concentrations at each of the 43 monitoring locations and used these in the cluster analysis.

We prepared the pollutant data for clustering by dividing the median concentrations at each monitoring location by its respective badge NO_x concentration. These profiles will eventually be used to investigate modification of the health effects attributed to NO_x between locations with different pollutant profiles. As such, all pollutant concentrations used in this analysis are normalized by the NO_x concentration measured using the 2-week passive sample.
Within each city and season, we normalized pollutant concentrations using z-scores in order to prevent differences in pollutant magnitude from driving the clustering results.

We excluded other pollutant information collected by the mobile monitoring platform from this analysis if: 1) the pollutants were regionally distributed and the contribution of local sources to the variability relative to between day concentration changes was small ($PM_{2.5}$ and ozone) or 2) the coefficient of variation in the concentration of the pollutants was less than 0.1, indicating a lack of spatial gradient (differs by city). We also excluded mobile nitrogen oxide measurements since we are normalizing values by the concentration of NO_x obtained through passive sampling.

Clustering Method: We performed k-means clustering, with Eulerian distance measures, separately by city and season over the 43 different monitoring locations. For Baltimore and Los Angeles, a median concentration of selected mobile monitoring pollutants was included in the clustering in addition to the passive sampling values. In St. Paul and Winston-Salem, we included only passive monitoring in the clustering due to data quality or data completeness issues in the mobile monitoring.

We selected the number of clusters using 3 criteria: 1) Minimizing the number of clusters with fewer than 5 members; 2) Maximizing the separation between the first 2 principal components describing each cluster; and 3) Maximizing the agreement between different validated indices for selecting *k*.

We compared the clustering results between cities and between seasons by plotting mean z-scores for the different solutions as well as by comparing the spatial distribution of the clusters within cities. See the Project 5 progress report for some selected results from our clustering analysis.

In related work (see our description of novel statistical methods developed by the Biostatistics Core below), we are developing *predictive k-means* clustering as an alternative to k-means clustering. The advantage of predictive k-means is that it will identify cluster centers from the monitoring such that we can more accurately predict cluster membership at subject locations. We plan to apply predictive k-means as an enhancement to the exposure modeling and health analysis of Project 5 data described above.

b. *Estimating health effects*:

Predicted cluster memberships at cohort locations partition the subjects by the type of pollution to which they are exposed. As such, cluster membership will be used as an effect modifier of the association between NO_x exposure and measurements of coronary artery calcium (CAC) to

determine whether or not the association varies by multi-pollutant profile (as identified by cluster). The NO_x exposure estimates will be derived from the spatiotemporal exposure prediction models developed for MESA Air.

Following the approach of the MESA Air study, CAC progression will be analyzed using a mixed model that allows for estimation of cross-sectional and longitudinal (progression) associations with NO_x exposure. The mixed-model includes random-intercepts and random-slopes for all time-varying adjustments, each of which varies by participant, and also allows for a fixed effect adjustment for transient confounders. By including cluster membership as an effect modifier in both the cross-sectional and longitudinal terms, we will estimate separate longitudinal associations between CAC and NO_x for each cluster, and thus each multi-pollutant mixture profile. We will restrict the analysis to MESA Air participants in the four sites listed above (Los Angeles, Baltimore, Minneapolis/St. Paul, and Winston-Salem). As the above clustering procedure will result in two sets of cluster identifiers (warm-season and cold-season), we will repeat this analysis using both sets of cluster IDs.

Following the approach of the MESA Air study, we will estimate the association between CAC progression and NO_x by cluster using a staged modeling approach. A staged approach to covariate adjustment considers known relationships between participant characteristics and heart disease, observed relationships between these characteristics and air pollution, and scientific judgment about potential mediators. Our primary model will adjust for age, sex, race/ethnicity, site, scanner type, adiposity, physical activity, smoking and second-hand smoke exposure, employment outside the home, total cholesterol, high density lipoprotein (HDL), triglycerides, statin use (time-varying), neighborhood SES index, education, and income. Simpler models and more fully adjusted models including potential mediators (diabetes, blood pressure and hypertension), and additional potential confounders (C-reactive protein, fibrinogen, creatinine, alcohol use, and family history of premature CVD) will also be evaluated. Measurements from participants after coronary revascularization procedures will be excluded from the analysis.

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

In previous years we have focused on dimension reduction using sparse PCA and predictive sparse PCA, summarized below. Our activities in the past year have expanded to also consider predictive k-means clustering.

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 under revision for the *Journal of the Royal Statistical Society, Series C*..

In a complementary project we are developing methods for predictive k-means clustering. Predictive *k*-means incorporates geographic covariates (e.g. spatial basis functions or land use covariates) to identify clusters in multi-dimensional measurements and predict cluster membership at cohort locations. This procedure can be derived as a mixture of normal distributions and is solved using a version of the expectation-maximization (EM) algorithm. We have compared this approach to k-means clustering followed by spatial prediction. In simulations, we have demonstrated that predictive *k*-means can reduce prediction error by over 40% compared to k-means, with minimal loss in cluster representativeness. The improved prediction accuracy resulted in large gains of 30% or more in power for detecting effect modification by cluster in a simulated health analysis. In a manuscript submitted to *Biometrics*, we applied this approach to the association between systolic blood pressure and long-term fine particulate matter ($PM_{2.5}$) exposure in the NIEHS Sister Study.

For the Project 5 analysis we will compare the *k*-means clusters to clusters found by the novel predictive *k*-means procedure. We plan to predict cluster membership at the cohort locations within each city via multinomial logistic regression using two or three principal component analysis (PCA) scores, derived from GIS variables, as the prediction covariates. We will

explore alternative prediction models such as neural networks and support vector machines, which would allow for more PCA scores or spatial basis functions to be used in the prediction model.

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, Sheppard L, Kaufman JD, and Szpiro AA. Multi-pollutant measurement error in air pollution epidemiology studies arising from predicting exposures with penalized regression splines. Journal of the Royal Statistical Society, Series C. Submitted.**

2. **Jandarov R, Sheppard L, Sampson PD, and Szpiro AA. A novel principal component analysis for spatially misaligned air pollution data. Journal of the Royal Statistical Society, Series C. Submitted.**

3. **Keller JP, Drton M, Larson T, Kaufman JD, Sandler DP, and Szpiro AA. Covariate-adaptive clustering of exposures for air pollution epidemiology cohorts. Biometrics. Submitted.**

4. Lee A, Szpiro AA, Kim S-Y, and Sheppard L. Impact of preferential sampling on exposure prediction and health effect inference in the context of air pollution epidemiology. Environmetrics. 2015. Epub ahead of print.

5. Wang M, Brunekreef B, Gehring U, Szpiro AA, Hoek G, and Beelen R. A framework for evaluating land use regression models and the effect on health effect estimates. Epidemiology. Submitted.

6. 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. Environmental and Ecological Statistics (2015): 1-31.

7. 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. Environmental health perspectives 123.4 (2015): 301.

8. 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. Environmental Health 14.1 (2015): 49.

9. Kim S-Y, Sheppard L, Larson TV, Kaufman JD, Vedal S. Combining PM2. 5 Component Data from Multiple Sources: Data Consistency and Characteristics Relevant to Epidemiological Analyses of Predicted Long-Term Exposures. Environmental health perspectives (2015).

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

11. 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. The Annals of Applied Statistics 8.4 (2014): 2509-2537.

12. 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. 2014; 180(7):718-28.

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. **Sampson, PD. Spatio-temporal Modeling for Environmental Monitoring Data. International Symposium on Statistics (ISS). St. John's, Newfoundland, Canada, July 2015.**

2. **Szpiro, A. Dimension Reduction for Spatially Misaligned Multivariate Air Pollution Data. Society for Epidemiologic Research (SER). Denver, CO. June 2015.**

3. **Keller, J. Covariate-adaptive Clustering of Exposures for Air Pollution Epidemiology Cohorts. Western North American Region of The International Biometric Society (WNAR). Boise, ID. June 2015.**

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

16. 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

17. 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.

18. 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.

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

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

21. 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. **Keller J. Covariate-adaptive Clustering of Exposures for Air Pollution Epidemiology Cohorts. University of Washington DEOHS Student Research Day. Seattle, WA. May 2015.**

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

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, June 2014.

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

5. 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.

6. 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/

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 project. 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. Mobile monitoring and passive sampling measurements was conducted for both heating and non-heating seasons. Due to financial constraints, Winston-Salem only was monitored with passive samplers. During this reporting period we have focused on data analysis of final QC data sets, and providing data for use by investigators in our project and other center investigators. This process has been completed for data collected in Baltimore, Los Angeles, Albuquerque and the 2-week passive sample data for these cities. We also completed a collaborative measurement campaign in Atlanta with the SCAPE center, and have provided preliminary data for this activity (described in the Collaborative Projects section. The final QC data set from this collaborative campaign is under review and being analyzed by both centers.

The instrument platform for mobile monitoring was assembled and tested in Seattle in October of 2011. 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 0.2 µm, particle mean diameter and particle count from 0.03 to 0.2 µm, total particle count >0.1 µm, 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 published in Atmospheric Environment (Riley 2014).

Figure 1. Gradient sampling Data Collected in Albuquerque, NM

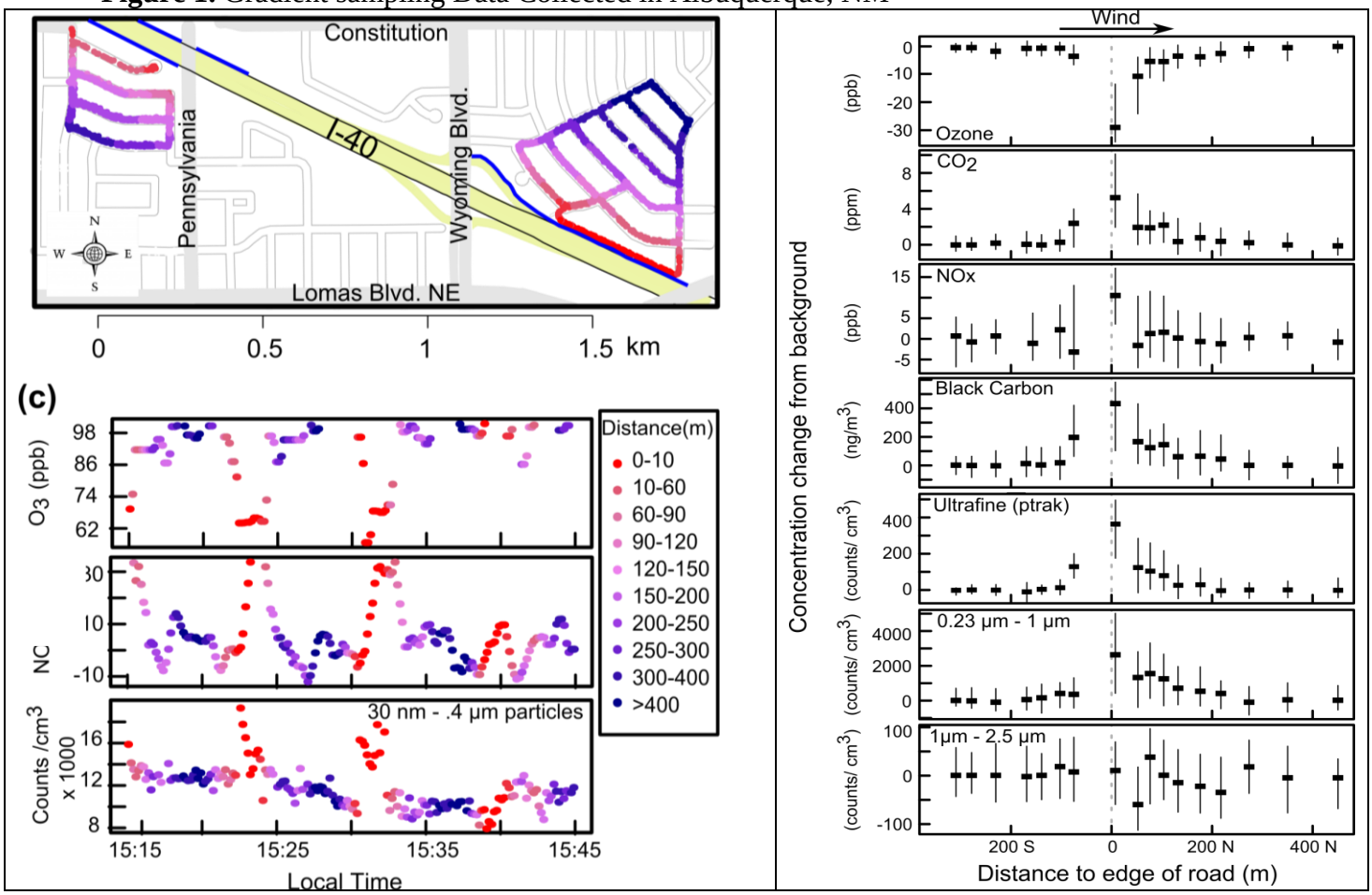


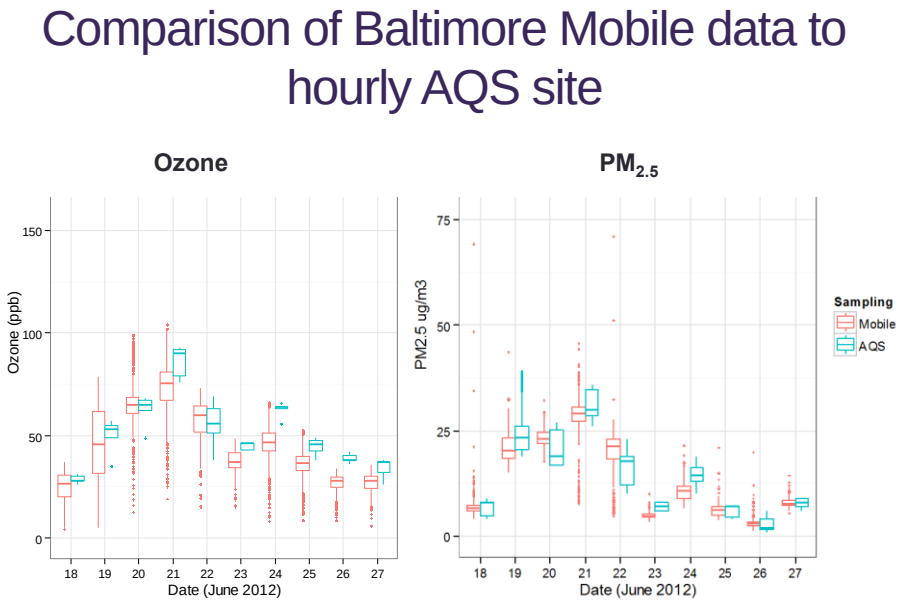
Figure 1 shows results from our gradient sampling tests in Albuquerque, NM over a one week period during May 2012. Mobile monitoring was used to repeatedly sample multi-pollutants near a major interstate roadway (I-40). The 10 sec mobile data collected near the interstate was classified into buffers 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/NO_x scavenging.

Similar patterns in Ozone and NO/NO_x 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 $PM_{2.5}$. The mobile data has excellent agreement with AQS site, and captures the day-to-day variation in these pollutants.

Figure 2



Multivariate analysis of the mobile platform data was done using pilot measurements in Seattle. Traditional PCA with varimax rotation was examined and the resultant factor scores were compared with a photographic record using an onboard camera. A paper on this analysis is under review in Science of the Total Environment (Fintzi et.al. 2015).

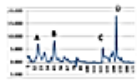
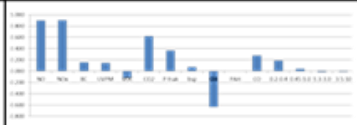
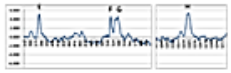
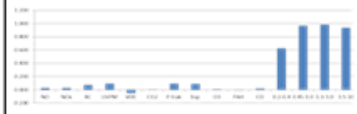
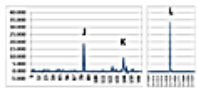
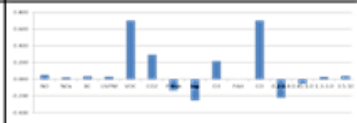
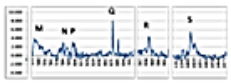
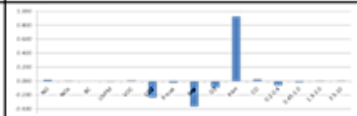
Plausible Source	Feature Loadings*	Selected Feature Scores vs. Time** (time in 10 second intervals)	Onboard Pictures***	Percent of Total Variance
Heavy duty diesel				19
Engines under uphill load				21
Road dust				18
Light duty vehicles				8
Particle bound PAHs			no obvious events	7

Figure 3. Results of PCA analysis with Varimax rotation from Duwamish Pilot study.
Notes: * Factor variable loadings from PCA analysis (see text for variable list)
** Peak events identified from factor scores plotted as a time series
*** Images from onboard video corresponding to peak event for this factor

The results shown in Figure 3 indicate that there are strong latent variables that are logically related to specific roadway sources. We are currently examining whether these features are observed in our MESA cities data.

In the figure, the second column shows factor loadings from a PCA analysis of 10 second mobile monitoring data collected on one afternoon during the pilot study. The measured variables on the horizontal axis from left to right are: NO, NO_x, black carbon via aethalometer, uv channel on aethalometer, VOCs, particle number concentration via P-trak, light scattering coefficient with integrating nephelometer, O_3, particle bound PAHs, CO, and total particle volume concentration within the following optical particle diameter size ranges : >0.2-0.4, >0.4-1.0, >1.0-3.0, >3.0 to 10 micrometers.

The third column shows a time series of the factor score for each factor in the PCA analysis. The labeled peaks correspond to the following events as determined by an onboard camera (events labeled in bold letters correspond to the pictures shown in the fourth column): {A} at red light behind truck; {B} behind truck under freeway; {C} following school bus; {D} at red light behind school bus; {E} truck passing uphill; {F} in uphill traffic; {G & H} roadside next to uphill traffic; {J} sample inlet adjustments (experimental artifact); {K} behind large vehicle; {L} dust plume from off-road truck; {M} next to minivan uphill; {N} idling vehicle; {P} idling

pickup truck; {Q} industrial site (no CO observed); {R} residential street; {S} traffic pulse after stop light.

The fourth column of Figure 3 shows pictures taken with onboard camera at time of the peak event identified by the factor score time series. These video images help to clarity the interpretation of the factor loadings, in terms of possible on-road sources of multi-pollutants. Further work is underway to extend this analysis to longer time scales and to integrate additional information collected during the mobile sampling campaigns.

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

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).

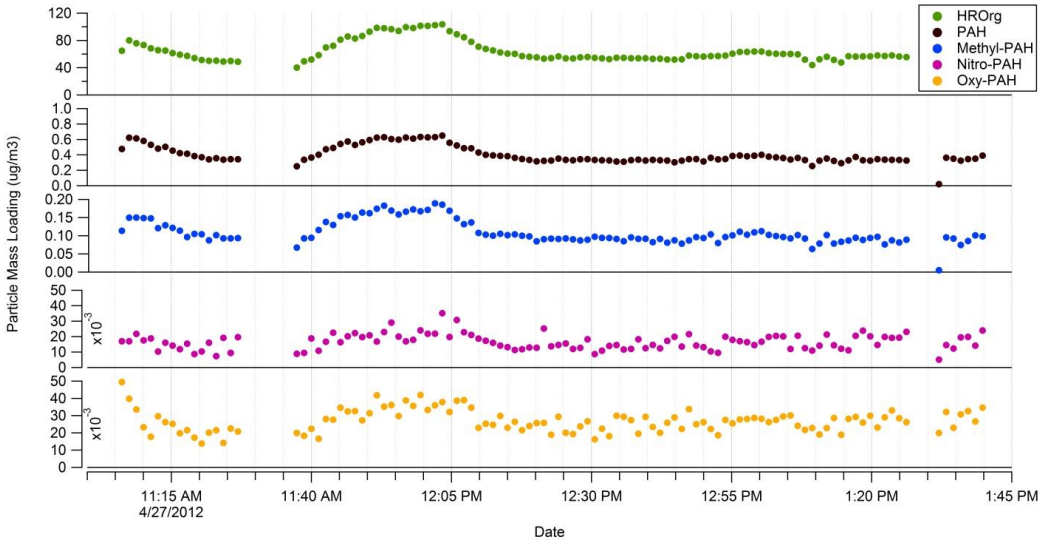


Figure 4. High resolution data for a chamber experiment of 100% diesel exhaust under typical engine load. Trend of mass loadings over the course of a 2 hour sampling, showing the total Organics with respect to the four PAH categories. Where PAH, Methyl-PAH, Nitro-PAH, and Oxy-PAH refer to the particle 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.

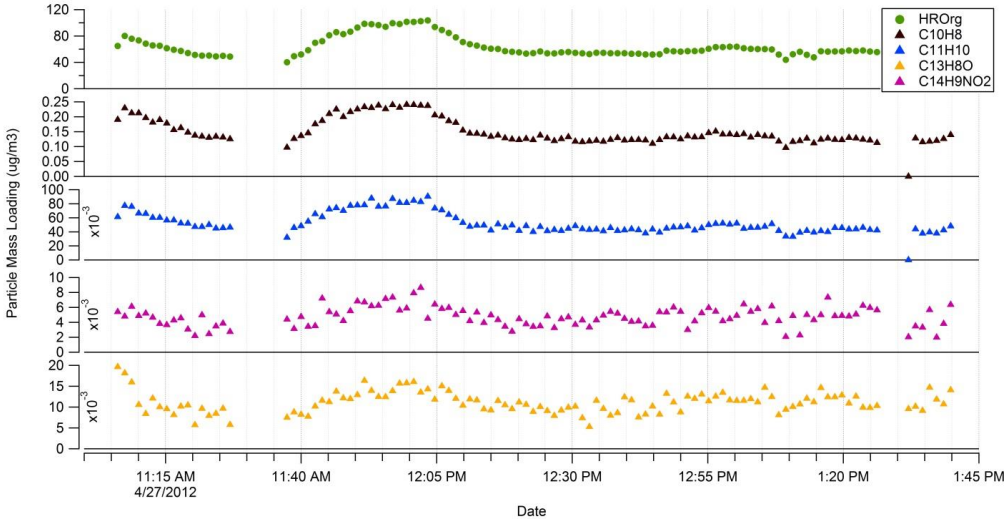


Figure 5. High resolution data for a chamber experiment of 100% diesel exhaust under typical engine load. Trend of mass loadings over the course of a 2 hour sampling, showing the total Organics with respect to the four PAH ions. $C_{10}H_8$ = Naphthalene, $C_{11}H_{10}$ = Methyl-naphthalene, $C_{13}H_8O$ = Fluornone, and $C_{14}H_9NO_2$ = Nitro-phenathrene/ Nitro-anthracene

WSU has continued to focus on the composition of the controlled mixtures of diesel and gasoline exhaust generated at the LRRI exposure facility. The test atmospheres have been found to contain strong signals from polycyclic aromatic hydrocarbons (PAHs), and we have developed a new analytical procedure to examine the contributions of individual PAH compounds to the aerosol under varying test conditions. This procedure, called PAHs by Molecular Ion Proxy (P-

MIP), relies on the fact that PAHs are relatively resistant to fragmentation from electron impact ionization, and thus that significant signal remains at the molecular ion. After quantifying the molecular ion signal and taking into account potential interferences, the contributions of the parent PAH to the aerosol may be inferred. Using the dataset from LRRI, we identified and quantified the molecular ions associated with 53 PAH species, including both unsubstituted and functionalized species. For this data set, the observed interferences were typically less than 1.2% of the observed signal. This application should have broad applicability, particularly once follow-up work establishing standard HR-AMS spectra for PAH compounds is complete. In the LRRI chamber data, we found that the fractional PAH molecular ion signal remained stable despite dramatic temporal variability of the total particulate organic signal, and that the fractional contributions of grouped PAH species and even individual PAH ions were remarkably consistent across experiments. The distribution of PAHs showed no apparent dependence on engine load or exhaust type. Comparison of particle-phase PAH concentrations against gas-phase PAH concentrations for four species suggests a strong enhancement of the particle-phase over what is predicted by absorptive partitioning theory. This work is currently in review for publication in *Aerosol Science & Technology*.

Laboratory work on characterizing the PTR-MS sampling of IVOCs continued. Specifically tests were done to determine the potential for positive interferences from n-aldehydes present in diesel exhaust on the measurement of naphthalene and alkly substituted naphthalenes. This interference has bearing on the interpretation of the gas-particle partitioning analysis using the combined PTR-MS and AMS data sets. Laboratory tests were conducted to establish product ions produced from H_3O^+ reaction with aldehydes as a function of drift field strength. Laboratory test were conducted to evaluate NO+ as a reagent ion to more effectively distinguish between aldehydes, alkanes, and substituted naphthalene compounds. Figure 6 shows the NO+ product ion mass spectrum for selected alkanes, illustrating the low fragmentation and unique M-H product ion

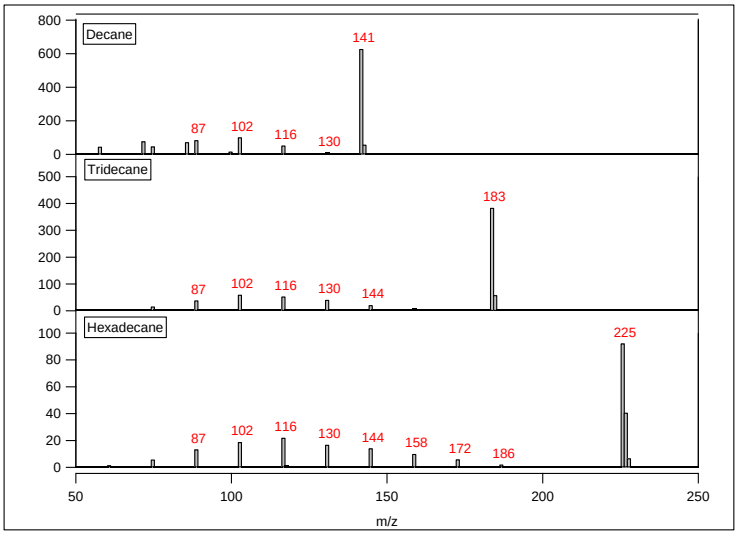


Figure 6. Mass Spectra of three selected n-alkanes using NO^+ as the reagent ion. Units are Hz/MHz NO^+ and experiments were conducted at 80 Td drift conditions.

Diesel exhaust was sampled using NO^+ to contrast information content of the mass spectrum against that obtained with H_3O^+. It was determined that using NO^+ in IVOC mode would produce a more interpretable mass spectrum. These results are currently being written up as a manuscript to be submitted for publication.

Publications / Presentations / Posters

<u>Publications to Date:</u>

1. **Herring CL, McDonald JD, Massoli P, Sueper D, Faiola CL, Erickson MH, Simpson CD, Yost MG, Jobson BT, and VanReken TM. New Methodology for High Resolution Polycyclic Aromatic Hydrocarbon (PAH) Quantification in Diesel – Gasoline Engine Exhaust using HR-ToF-AMS. Environmental Science and Technology. 2015. submitted**

2. **Fintzi JR, Riley EA, Austin A, Schaal L, Gould T, Hartin K, Sasakura M, Sheppard L, Sampson P, Simpson CD, Yost MG, Larson TV. Characterization of On-road Air Quality and Associated Emission Sources Using Principal Component Analysis and Mobile Video Logs. Science of the Total Environment. 2015. Submitted.**

3. **Hudda, N., Gould, T., Hartin, K., Larson, T.V., Fruin, S.A., Emissions from an International Airport Increase Particle Number Concentrations 4-fold at 10 km Downwind. Environ. Sci. Technol. 2014. 48 (12), 6628-6635.**

4. **Galaviz, V.E., Yost, M.G., Simpson, C.D., Camp, J.E., Paulsen, M.H., Elder, J.P., Hoffman, L., Flores, D., Quintana, P.J.E. Traffic pollutant exposures experienced by pedestrians waiting to enter the U.S. at a major U.S.–Mexico border crossing. Atmos. Environ. 2014. 88 (0), 362-369.**

5. **Xu W, Riley E, Austin E, Sasakura M, Schaal L, Gould T, Hartin K, Simpson CD, Sampson PD, Yost M, Larson TV, Xiu G, Vedal S. Use of Mobile and Passive Badge Air Monitoring Data for NO_X and Ozone Air Pollution Spatial Exposure Prediction Models. Atmos Environ. 2015. Submitted.**

6. 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.

7. Gueneron, M., Erickson MH, VanderSchelden G., Jobson BT., PTR-MS Fragmentation Patterns of Gasoline Hydrocarbons, Atmospheric Measurement Techniques, 7, 225-239, 2014.

8. 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 air pollutants adjacent to the I-40 corridor in Albuquerque, NM. Atmos. Environ. 2014. 98 (0), 492-499.

Presentations to Date:

1. **VanderSchelden GS, Fuchs M, Bartoshevich R, Wen M, Jobson BT, Measurements of Diesel Exhaust and its Photoproducts using a PTR-MS and a Photochamber. 2014 Air & Waste Management Association PNWIS Conference, Spokane, WA, November 5-8.**

2. **Fuchs M, VanderSchelden GS, Flyckt CL, Jobson BT. Diesel Exhaust Flow Tube Reactor Characterization. 2014 Air & Waste Management Association PNWIS Conference, Spokane, WA, November 5-8.**

3. **Austin E. Identification and Classification of Multipollutant Peak Events in Mobile Monitoring Data. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

4. **Riley E. Multi‐pollutant mixtures identified from a principal component analysis by melding mobile monitoring and integrated passive sampler data. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

5. 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. Canadian Chemistry Conference and Exhibition, Vancouver, B.C., June 2014

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

7. Austin E. 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, January 2014.

8. 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.

9. 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. **Riley E. Black Carbon and Ultrafine Particle Counts Downwind of Two Major Airports. University of Washington DEOHS Student Research Day. Seattle, WA. May 2015.**

2. **Xu W. Use of Mobile and Passive Badge Air Monitoring Data for NO_X and Ozone Air Pollution Spatial Exposure Prediction Models. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

3. Riley EA, Sasakura MD, Hartin K, Crampton R, Gould TR, Larson TV, Yost MG,

Simpson CD. Principal Component Analysis of Snap-Shot Air Pollutant Measurements in Baltimore, MD. EPA annual Clean Air Research Center Annual Meeting, Seattle, WA July, 2013.

4. Riley EA, Hartin K, Gould T, Larson TV, Yost MG, Simpson CD. Mobile measurements of near-highway air pollutant gradients. Annual Symposium on Environmental, Occupational and Population Health, Semiahmoo, WA, January 2014.

5. 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*, October 2013. ***Winner of a Student Poster Award.*

6. 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.

7. 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.

8. 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). We had planned to include as **Aim 3**, a study of inhalation exposures of human subjects in an effort to compare significant pathophysiological findings from our animal model exposures to responses in humans. Due to human subjects issues related to Project 4, Aim 3 was dropped.

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 rodents.

Research Performed - Progress Summary/Accomplishments

Over the past year we have focused on continuing to evaluate the endothelial cell and myography assays with Project 3, and to extend additional endpoints to confirm the cardiovascular response of putative pollutants. These results are described in Project 3. Project 2 developed novel atmospheres that continue to evaluate the role of gas-particle interactions and the particle size on the toxicity of inhaled mixtures. This was done by developing novel atmospheres that focus on better understanding the gaseous-particle and size components of motor vehicle exhaust (MVE). Atmospheres included:

- MVE minus gases: A denuder that removes gas phase organics was employed.

- MVE minus semi-volatile organics-catalytic stripper with catalyst that removes gaseous and semi-volatile organics from the aerosol was applied to the test atmosphere to evaluate gases/-organic particles versus solid particle residuals.
- MVE benchmark
- Wood smoke

Based on the previous studies of multiple test atmospheres and the response in the endothelial cell assays, it was determined that the two most potent atmospheres were MVe and wood smoke. As a result, we prioritized these test atmospheres for the longer term studies that were performed this year. These include the studies with the test atmospheres developed above. The samples from these studies were generated using the LRRI protocols defined below, and the overall study design in terms of the distribution of samples to UCLA and UNM.

- FY15-023A – February 2015. 132 ApoE-/- mice + HFD - exposed to woodsmoke, MVE100, MVE300, MVE-SVOC, MVE-gases for 50 days
- FY15-023B – July 2015. 24 C57Bl/6 mice – exposed to woodsmoke.

Protocol for 50 day exposures:
Atmospheres based on results from 1d serum bioactivity assay with both ApoE and C57 mice, 5% serum on mCECs. Myography suggested to study the following atmospheres.

ApoE-/- mice, male, 6 weeks at beginning of study. Diet HT88137 for a week prior. 6 h/d x 50 d, 300 µg PM/m^3 equivalents

- Filtered air
- MVE
- MVE-PM (catalytic stripper)
- Woodsmoke

For UCLA: N=10 (40 mice total)
Serum - HDL
Liver – cholesterol endpoints
Lung – inflammatory endpoints
BALF (lipidomics)
Gut – (Lund)
Aorta – en face ORO staining
Heart – rv/lvs, sectioning of aortic outflow for histopath

For UNM: N=12 (48 mice total)
Serum - myography
Liver – cholesterol endpoints
Lung – inflammatory endpoints, histo
BALF (cell counts, diffs, total protein)
Adipose – Maybe for UCLA
Aorta – en face ORO staining
Heart – rv/lvs, sectioning of aortic outflow for histopath, freeze RV and remaining LVS for PCR

The study atmospheres for the MVE and MVE minus SVOC are shown below in Table 1 below (only last week shown). Note that the catalytic stripper oxidized the CO to CO^2, providing the benefit of removing CO along with the gaseous and semivolatile material for these studies. The analysis of the biological response from these studies is under way in collaboration with Project 3.

Date	Chamber 2-MVE 100ug			BOX for GASES(Denuder)-300ug			BOX for SVOC(Stripper)-300ug			Chamber 5-MVE 300ug		
	NOX	CO Target ppm	Particle Mass Target 100 µg/m^3	NOX	CO Target ppm	Particle Mass Target 300 µg/m^3	NOX	CO Target ppm	Particle Mass Target 300 µg/m^3	NOX	CO Target ppm	Particle Mass Target 300 µg/m^3
4/2/2015	3	8	76	9	29	301	18	1	353	14	19	233
4/3/2015	4	14	113	11	30	299	22	1	370	14	32	329
4/4/2015	5	13	116	17	50	335	27	1	325	14	34	310
4/5/2015	2	11	96	9	37	361	14	1	298	14	44	305
4/6/2015	2	8	130	12	42	312	16	2	313	14	189	296
4/7/2015	3	12	97	10	32	319	20	1	319	14	70	287
4/8/2015	2	10	91	6	28	298	9	1	289	14	25	228
Average	9	14	121	15	36	318	25	1	314	18	50	311
Std Deviation	9	12	108	6	12	49	12	3	40	7	34	35
% CV	106	86	89	37	33	15	47	219	13	37	67	11
% Target	9	14	121	15	36	106	25	1	105	18	50	104

Table 1. Study Atmospheres for MVE and MVE minus SVOC.

New Atmosphere Development

Follow-on atmospheres have been under development to better study the impact of particle size and surface area in terms of biological response. The goal is to evaluate the role of gas interactions with PM as it relates to the size/surface area of the particles with the design described below. These studies are starting in August 2015.

MVE surface area assessment (study initiating in August 2015)

Comparing interaction of MVE gases with different size PM:

Study Design:

- *Filtered Air*
- MVE ultrafine (UF) particles: MVe generated with engines and then gases removed
- MVE Fine (F) particles: Resuspended MVe particles in larger size range generated (no gases present)
- MVE UF + gases ** (gases added to mixture after removal)
- MVE F + gases (gases added to mixture)

1 day (6h, immediate sac) – C57, serum collection, lavage (cell count, diffs, total protein), freeze lungs. N=10

50 day (6h/d) – ApoE, HT88137diet
Serum
Aorta
Heart
Gut (Lund)
Liver
Brain (1/2 frozen, half fixed in paraformaldehyde)
Lung
Balf (cell count, diffs, total protein, save for lipidomics)

Aerosol Generation Data for Atmosphere Development
Summary Notes of MVE 1-2µm Generation

- MVE (300 µg/m^3) used earlier is all Ultrafine.
- With MVE there is nothing on APS from H-1000 sample (H-1000 is used to pull sample for smaller lexan chambers)
- Using a Wright Dust Feeder (WDF) with deposited material from the diesel exhaust line, larger particles can be generated (as measured by APS)
- We used a cyclone to get rid of most of the particles above 3 µm.
- Using wright dust feed aerosol generator we still see ultrafines on FMPS with a similar size distribution (Fig 1 below), however, their concentration is lower compared to a similar conc. (300 µg/m^3) MVE (Fig 2 and Fig 3)
- APS data- MVE vs WDF. MVE concentrations are negligible (leakage issues in lexan chamber)
- APS data for WDF. The ratio of number, surface area and mass conc. for <0.5um and >0.5um particles is very small.

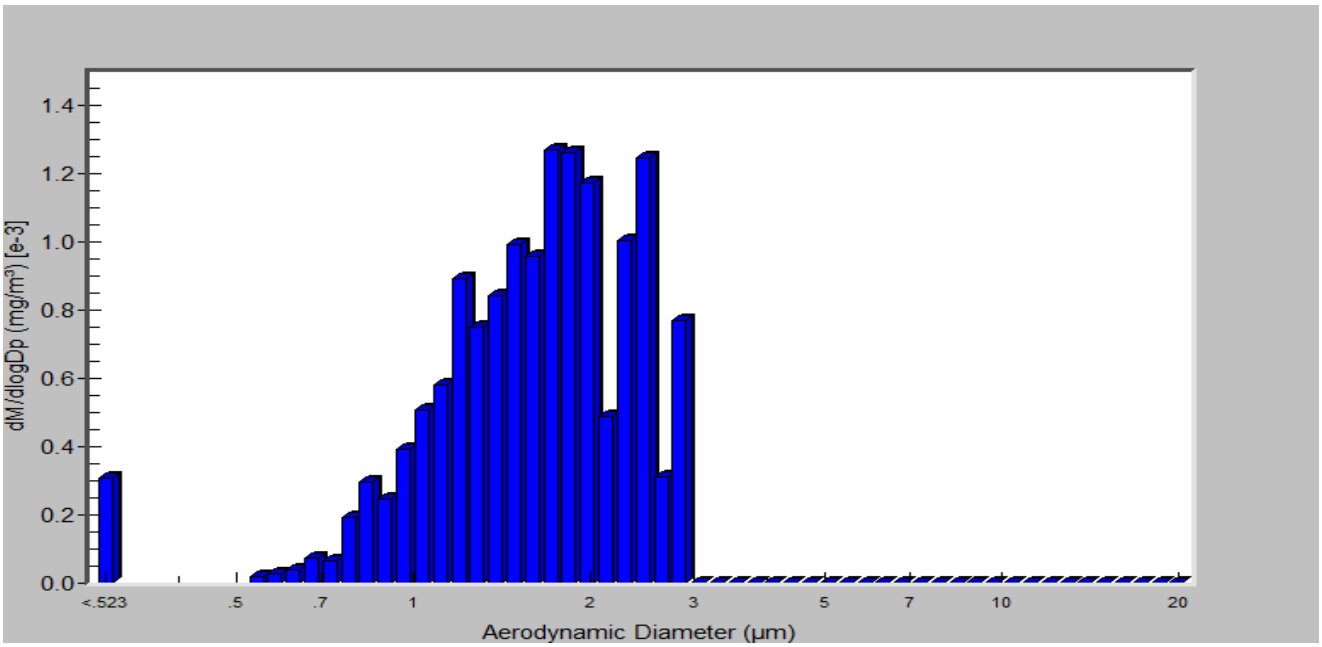


Figure 1. MVE particulate material generated with wright dust feeder to reach 1-2 microns. PM was collected from dilution tunnel, harvested and re-aerosolized to achieve larger particle size.

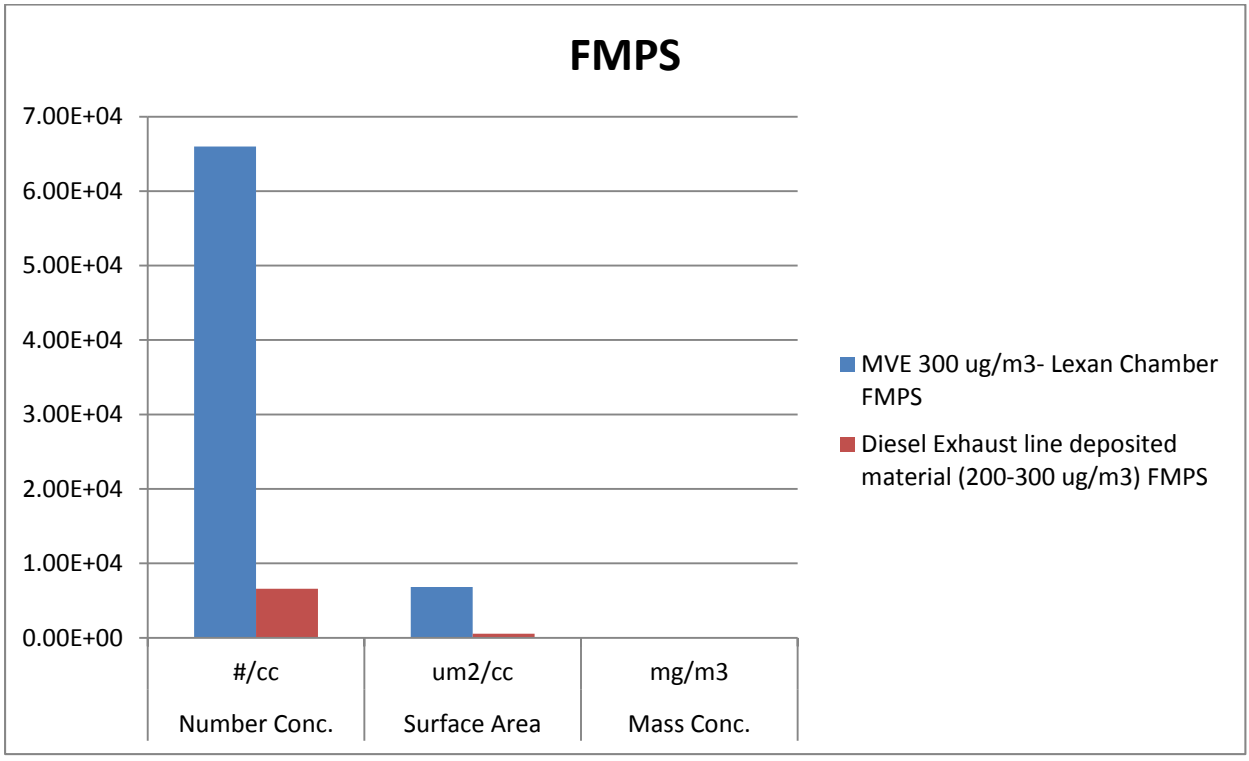


Figure 2. Difference in particle number and surface area concentration in MVE chamber (blue) versus resuspended MVE PM (red). MVE has much higher number, smaller particle size and larger surface area compared to larger material that is resuspended.

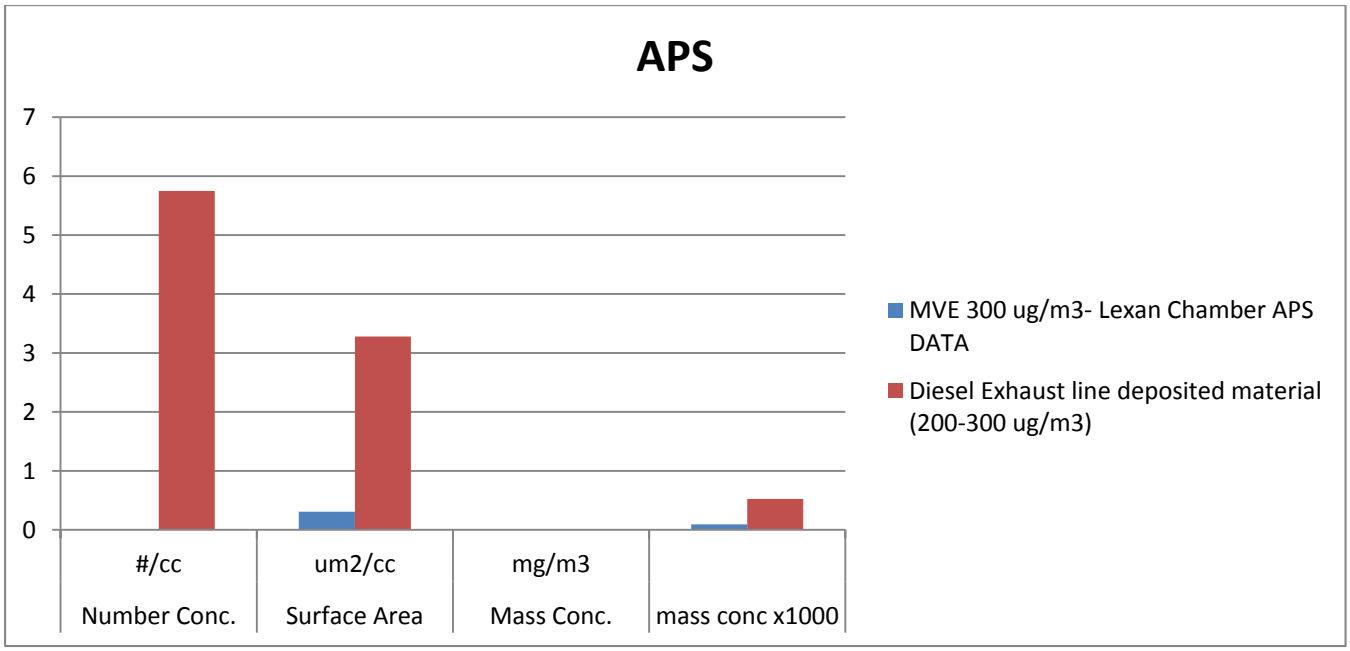


Figure 3. Number concentration/surface area for particles larger than 0.5 microns measured by APS. Resuspended MVE particle number is higher in the higher particle size.

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. 2013. 6.3: 615-628.

2. Oppenheim H, Lucero J, Guyot A, Herbert L, McDonald JD, Mabondzo A and Lund AK. 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.

3. Mauderly JL, Kracko D, Brower J, Doyle-Eisele M, Lund AK, McDonald JD and Seilkop SK. 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. Inhalation toxicology 2014. 26.11: 691-696.

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. 6: 309-324.

Presentations to Date:

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

2. Doyle-Eisele M, Rohr A, Knipping E, Lund A, Brower J, McDonald JD. 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. McDonald JD, 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.

2. Brower J, Moeller B, Doyle-Eisele M, Stirdivant S, McDonald JD, Campen M. Acute Inhalation Exposure to Mixed Vehicle Emissions Induces Serum Metabolite Changes Related to Oxidative Stress, Lipid Peroxidation, and Energy Metabolism. Society of Toxicology, Phoenix, Arizona, 2014.

Future Activities

The next round of studies will continue the follow up on long-term assays, confirming the effect differentials related to surface area and gas-particle interactions.

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.

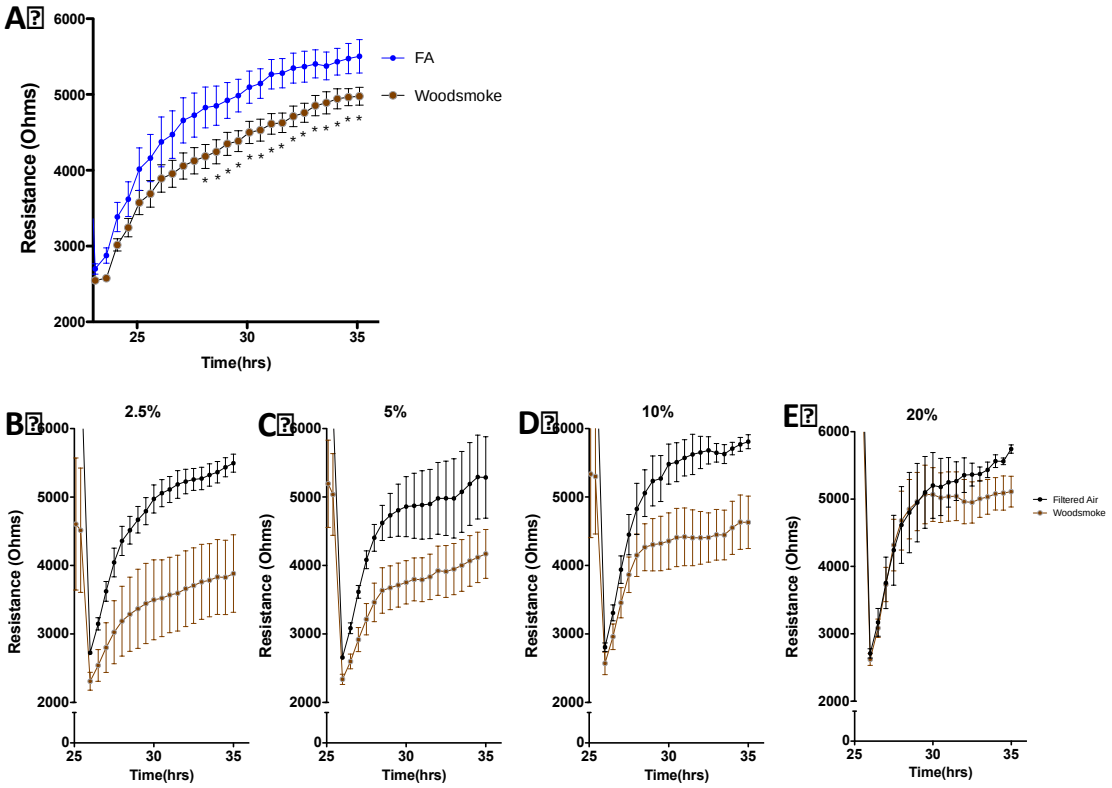
Research Performed - Progress Summary/Accomplishments

Following up on the previous year's observation of the role of blood-borne ligands and bioactivity in terms of driving endothelial cell activation or dysfunction following ozone exposure, we identified a potential for altered serum biochemicals to scavenge nitric oxide, which was associated with lower levels of circulating nitrites/nitrates and also elevated nitrosothiols (Paffett et al, Toxicol Sci, 2015). This finding offers a complementary mechanism to previous observations of ligand-receptor dependent alterations impacting vasodilation (Robertson et al., Toxicol Sci, 2013). Ongoing research into the chemical changes in the blood have offered paradigm-shifting insights. For one, we typically see minimal, if any, changes in cytokine levels following even moderately high levels of pollutants. However, we routinely observe increases in fragmented and adducted proteins, and metabolomics studies suggest small molecule changes may also be numerous and contributory. A recent observation with serum from woodsmoke-exposed mice suggests that it is in fact a *loss* of some factor, rather than induction of higher levels of some mediator or mediators, that leads to indirect vascular pathology.

In the first study, we examined further the bioactivity of O_3 on coronary arteries from rats. Following inhalation of 1 ppm 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.

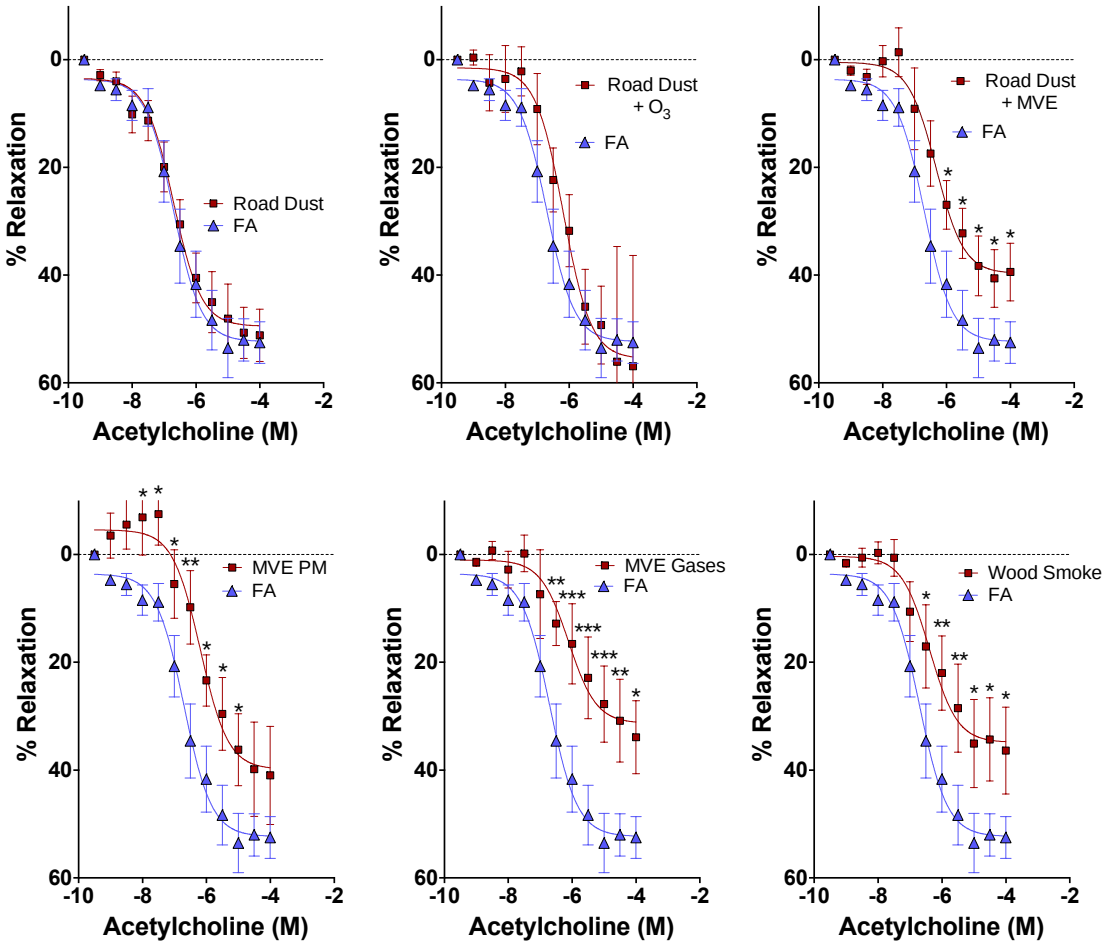
To test the degree of bioactivity conferred to serum following complex mixtures exposures, wildtype (C57BL/6) mice were exposed to woodsmoke, mixed vehicle emissions (MVE), or road dust for a single 6h period (Aragon et al, 2015). Serum obtained from mice 24 h after these exposures was used as a stimulus to assess inflammatory potential in two assays: incubated with primary murine cerebrovascular endothelial cells for 4 h to measure inflammatory gene expression or applied to naïve aortic rings in an *ex vivo* myographic preparation. Road dust and wood smoke exposures were most potent at inducing inflammatory gene expression, while MVE atmospheres and wood smoke were most potent at impairing vasorelaxation to acetylcholine. Responses are consistent with recent reports on MVE toxicity, but reveal novel serum bioactivity related to wood smoke and road dust. Ongoing work with serum from wood smoke exposed mice suggests that bioactivity changes in the serum, at 10% dilution in media, can impair regrowth in a wound healing assay (Figure 1A). Follow up studies with different concentrations of serum show that the regrowth is normalized at higher concentration (30%) and potentially even worse at 2.5% (Figure 1B-E), suggesting that the adverse effects are not caused by an

induced factor, but rather one that is reduced in concentration.



Serum from MVE-exposed mice remained the most potent at inhibiting endothelium-dependent vasodilation (Figure 2). This effect seemed to be largely driven by gaseous components, as serum from mice exposed to the MVE-filtered atmosphere (with PM removed) was the most potent inhibition of dilation, while the road dust exposures had only limited impact. While we had previously demonstrated a receptor-ligand linkage to explain this effect with ozone, the recent data suggest that scavenging nitric oxide by novel serum components may also be a concern (Paffett et al., 2015). Further research on this will be conducted in the coming year.

Ongoing studies to delineate the role of PM surface-adsorbed semivolatile organic compounds (SVOCs) are underway. These employ a catalytic stripper in addition to denuder to more completely remove adsorbed SVOCs. Serum samples have been found to induce endothelial VCAM and cause small impairments in endothelial regrowth, but studies remain in process.



Publications / Presentations / Posters

Publications to Date:

1. **Aragon MJ, Chrobak I, Brower J, Roldan L, Fredenburgh LE, McDonald JD, Campen MJ. Inflammatory and Vasoactive Effects of Serum Following Inhalation of Varied Complex Mixtures. Cardiovasc Toxicol. 2015 Apr 22. [Epub ahead of print] PMID: 25900702**

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. 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. Toxicol Appl Pharmacol. 2015. Toxicol Sci. 2015 May 11.

4. Schisler J, Campen MJ, Madden M, and Willis MS. Transcriptional Endothelial Biosensor Response to Diesel-Induced Plasma Compositional Changes. Inhalation Toxicology. 2015. 27(5): 272–280.

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:

1. **Campen, MJ. Endothelial Cell Pattern Recognition Receptors, CD36 and LOX-1, Contribute to Responses to Pollution-Induced Circulating Factors. Society of Toxicology, Phoenix, Arizona, March 2014.**

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.

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
Tim Larson	University of Washington

Objective of Research

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. 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 was launched in Year 4 of the Center. In this project, we 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 16 subjects, randomized to order. Using an innovative approach in which contrasts of in-vehicle exposure and 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.

Screening

Subjects are screened to determine eligibility. At screening, subjects are required to be in the normal range for BMI, blood sugar, cholesterol and triglyceride levels, lung function, blood pressure, and ECG. Subjects also fill out questionnaires describing past illness, health history, traffic and chemical exposure, smoking history, and occupation. Buccal swab samples are

collected in order to achieve a balance of the TRPV1 (SNP I-585V) gene, which is related to responsiveness to traffic-related air pollution.

Eligible subjects are randomized to three 2-hour commutes that travel I-5, extending from North Seattle to roadways in South Seattle (e.g. Duwamish Valley). During each drive, subjects are accompanied by research staff responsible for collecting subject health measurements and monitoring conditions of the drive. Each drive is separated by at least 3 weeks. During drives, the cabin air and HEPA filters are configured to reflect the randomized exposure conditions (i.e., on-road ambient or filtered air exposure). The cabin ventilation controls are adjusted such that air is entrained and directed to the floor vents, and the temperature inside the vehicle is comfortable for the occupants. Van windows remain closed during the drive and subjects wear N95 masks while transitioning from the lab to the UW van regardless of drive condition.

Enrollment

To date, three subjects have completed all three drives and one subject has dropped out due to scheduling difficulties. Nineteen subjects have been screened and 15 are waitlisted to begin.

Health Measurements

Subjects complete health measurements at baseline, during the drive, immediately after the drive, 3 hours later, 5 hours later and 24 hours later. These health measurements include: questionnaires, blood markers, Holter ECG, ambulatory blood pressure, 24 hour urine, brachial artery reactivity, retinal photography, and Finometer measurements. The frequency of health measurements are shown in Table 1. All subjects provide a urine sample for a cotinine test and, if female, a pregnancy test.

Table 1. Experimental Session Timeline for CCAR, Project Four

AM/PM	**AM**						**PM**						
Time (hour)	10:00pm-7:00 am	7:00	8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00		9:00 am (24hr)
Overnight fast	X												X
Urine collection		X	X	X	X	X	X	X	X	X	X	X	X
Vitals BP, PR, RR		X		XX	XXX		X		X		X		X
ABP							X	X	X	X	X	X	X
Holter Monitor 11 min record		XX	X	X	XX	X	XX	X	X	X	XX	X	XX
Blood Draw			X								X		X
BAR Roosevelt			X			X							
Symptom Questions			X		X		X		X		X		X
Commute Drive													
Finometer			X	X	X	X	X		X		X		X
Retinal Photography			X			X							
Lunch							X						

Air Monitoring

This study involves in-vehicle monitoring for 48 drives involving 16 participants in Seattle. Each day of monitoring will include the following suite of monitors in order to collect real-time measurements of the pollutants: $PM_{2.5}$ (Nephelometer, Radiance Research), black carbon (microAethelometer, Aeth Labs), particle count (P-Trak, TSI Inc), PAHs (PAS 2000CE, EcoChem), NO_2 (CAPS, Aerodyne Research Inc), NO_X (UV absorbance Model 410, 2B Technologies), ozone (chemiluminescence 3.02P, Optec), CO (CO T15n, Langan), CO_2 (CO_2 K-30-FS Sensor, CO_2 Meter.com), temp/RH (Precon HS-2000, Kele Precision Mfg), location (GPS BU-353, US GlobalSat). Filters and air monitors inside the car are powered by gel cell batteries connected to power inverters.

Pilot Testing

During the last year, CCAR Project 4 prepared and extensively pilot tested the UW vehicle in order to provide reduced particle counts and black carbon concentrations to participants. The pilot data from these drives is summarized below.

The on-road concentrations of some air pollutants can be dramatically higher than concentrations of the same pollutants even a short distance from a major roadway. These pollutant gradients are one of the rationales for conducting on-road measurements inside a vehicle where the study subject can have both physiological responses and air pollutant exposures characterized. Important operational concerns include the ability to create a different "control" no or minimal exposure case for baseline comparison with the pollutant exposure situation. The areas with high traffic-related pollutants need to be identified so a route can be formulated which will take the subject through areas where the on-road concentrations of pollutants are significant.

Toward these ends, we conducted pilot studies that began with an assessment of pollutant exposures along major highways in the Seattle area, and then continued with an investigation of methods for distinguishing the clean filtered air control case from the exposure condition inside the vehicle. Multiple test drives were conducted to refine the route with exposure to higher concentrations, and to diesel exhaust from heavy truck traffic, specifically.

Drive Route Location

The air monitoring system inside the vehicle is adapted from a mobile monitoring study conducted in CCAR project 1 in five cities throughout the nation from 2011 to 2013. The initial route characterization pilot study for project 4 was conducted in December 2012. Pollutants were measured outside the vehicle as it was driven on major limited-access highways. Geographic plots coded by quintile of the particle count larger than 50 nm and the fine PM relative concentration by light scattering are shown in Figures 1 and 2, respectively.

The initial three days of drives in 2012 confirmed that I-5 through downtown Seattle experiences high levels of particulate matter concentration and particle counts. This information coupled with logistical concerns about maintaining a consistent 2 hour exposure time for the subject in the vehicle led us to focus on the I-5 corridor past downtown Seattle as a principal part of the commute exposure drive route. The route also goes past the Washington Department of Ecology recently deployed near-road air monitoring station at 10^{th} Ave. S. and S. Weller St. on the east side of I-5.

We used results from mobile monitoring conducted for the Diesel Exhaust Exposure in the Duwamish Study (DEEDS) to identify the Port of Seattle freight truck terminal access routes as an area of interest with higher than normal levels of diesel engine exhaust. A segment of the drive route was added which loops past the truck terminal entrances at the south end of Harbor Island and underneath the West Seattle Bridge. The route shown in Figure 3, which displays particle count by quintile measured inside the vehicle without filtration in place, was selected for its highway pollutant levels along I-5, the higher incidence of diesel engine exhaust by Harbor Island, and ease of access to and from the University of Washington clinic to enable consistent duration exposure sessions among subjects regardless of traffic conditions on a given exposure day.

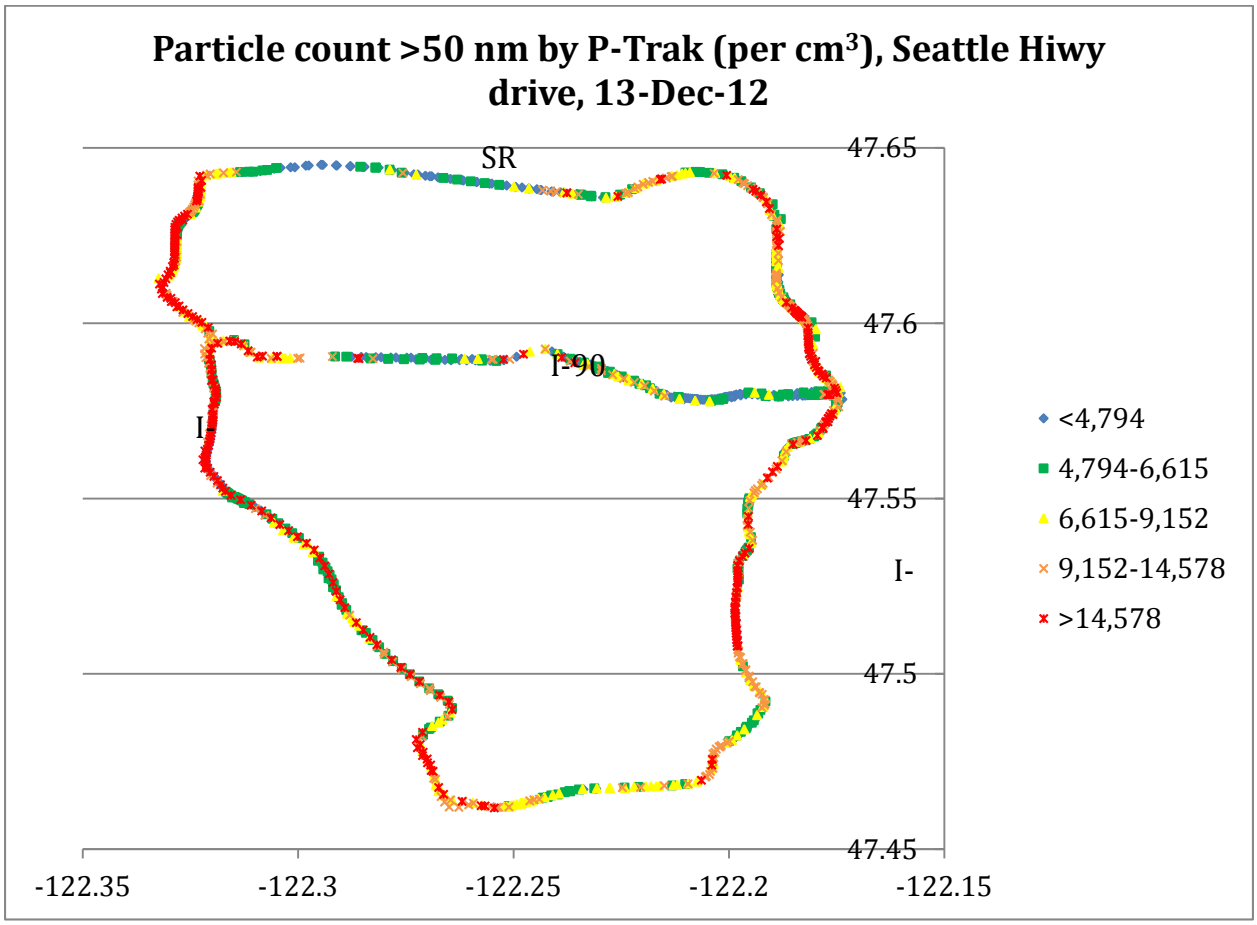


Figure 1. Particle count quintiles during December 2012 mobile monitoring drive in Seattle area. Routes traveled included I-5 past downtown Seattle as far south as Southcenter, I-405 between Southcenter and the northern edge of downtown Bellevue, and both I-90 and SR 520 across Lake Washington between the two north-south routes.

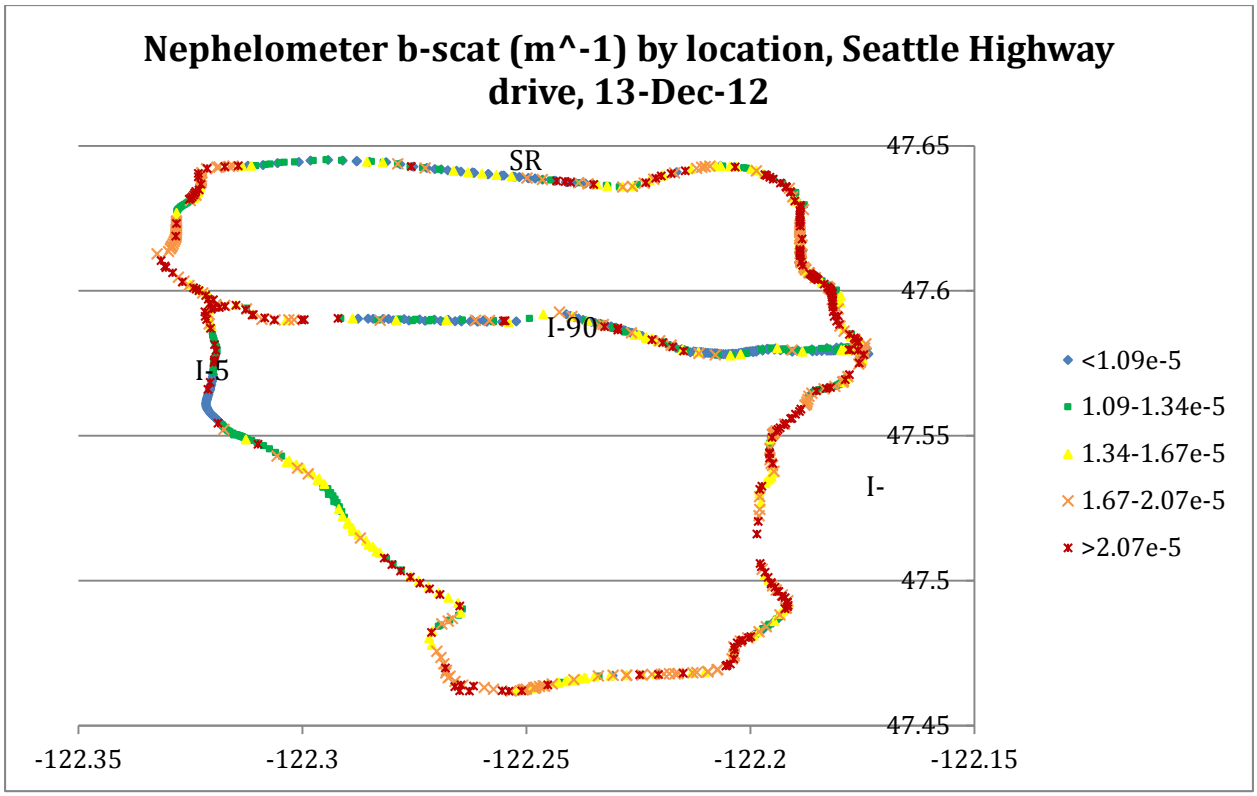


Figure 2. PM2.5 proxy measurements by light scattering nephelometer presented in quintiles during December 2012 mobile monitoring drive in Seattle area. Routes traveled included I-5 past downtown Seattle as far south as Southcenter, I-405 between Southcenter and the northern edge of downtown Bellevue, and both I-90 and SR 520 across Lake Washington between the two north-south routes.

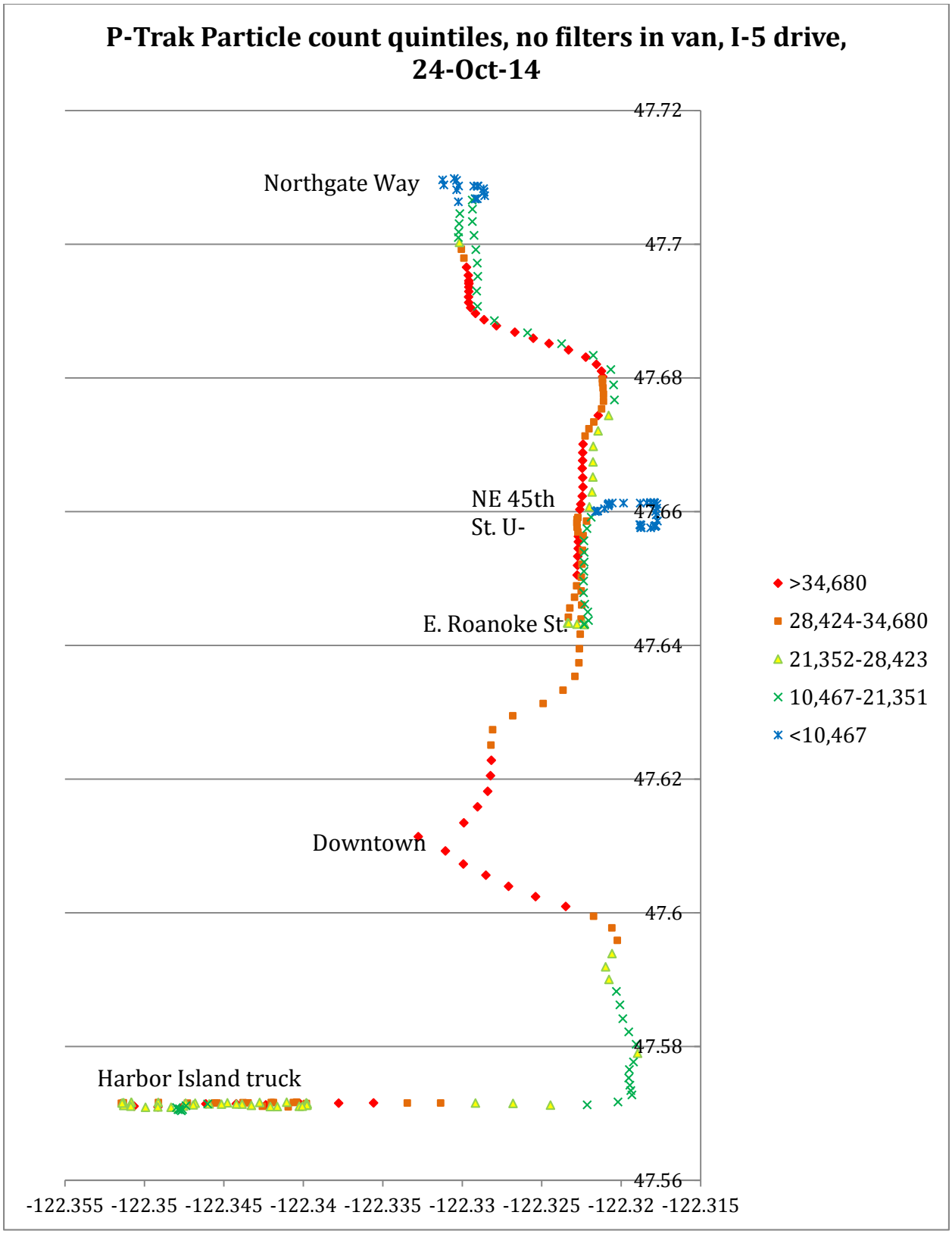


Figure 3. Particle count quintiles during October 2014 in-vehicle monitoring drive along the consolidated Seattle route selected for the subject commute exposure study on-road sessions.

Filtration to Ensure Control Case

With the study designed to compare the physiological effects from exposure and filtered air scenarios, a reliable and consistent way of ensuring a large difference in exposure levels is needed to distinguish the resultant effects. For the filtered air case, we chose to use supplemental filtration in addition to the vehicle's own ventilation system filter. The pilot study evaluated first an existing HEPA filtration unit that had been used for filtering particulate matter in previous exposure and control studies. The Honeywell Envircaire, model 11520 unit is configured to discharge filtered air in a circular pattern radiating away from its round housing. This presented challenges for use in the cabin of the Caravan to direct as much of the filtered air as possible toward the subject in the left-side middle row passenger seat. Baffles were used to narrow the discharge to a smaller cross sectional area where instruments were positioned to evaluate the Envircaire unit performance in reducing particle loadings. A comparison of averaged PM measurements both with and without the filtration media inside the device found the following reductions of particulate matter in the filtered air scenario relative to the no-filtration configuration:

Particle Count, by P-Trak	33.6%
Black Carbon conc., by microAeth	66.2%

Improved particulate matter reduction was sought by using another HEPA filter unit designed to discharge its filtered air stream in one direction at the top of the rectangular cross-section device. This Whirlpool Whispure model AP51030K proved to be more effective at reducing particle loadings at higher fan speeds than the Envircaire unit as indicated by these particle count reductions measured by a P-Trak and black carbon concentration differences by microAeth at four different HEPA unit fan speeds:

HEPA Fan speed:	Low	Medium	High	Turbo
Particle count Reduction	46.1%	51.1%	55.5%	60.4%
Black Carbon Reduction	47.8%	60.6%	68.5%	74.9%

The Whispure performance for light-scattering based fine PM was only 29% to 43% reduction in scattering coefficient (by nephelometer) as the fan speed varied from low to turbo setting. Entrainment of non-filtered air into the discharged stream of filtered air was a significant cause of lowering the particle removal efficiency based on varying the inlet location of the particle instruments in real time. The effect of entrained air mixing with the HEPA unit discharge stream was evident for measurements only 6" from the Whispure outlet air discharge as shown in Figure 4. By comparison, the particle removal efficiency was much better when the measurements were made in the directed air stream exiting the HEPA discharge vent by using a baffle or straight stream line diffuser, as illustrated in Figure 5.

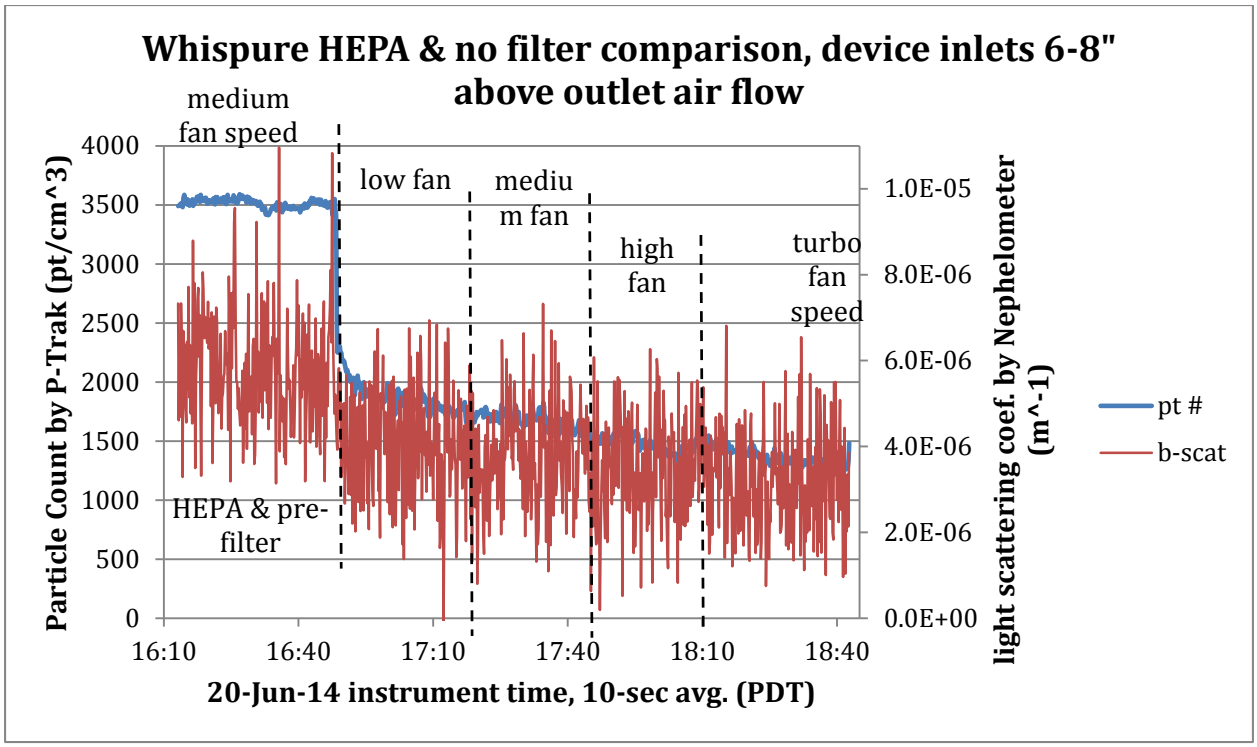


Figure 4. Comparison of non-filter and filter conditions in discharge from Whispure HEPA unit for configuration where entrainment of non-filtered air affects the particulate removal efficiency.

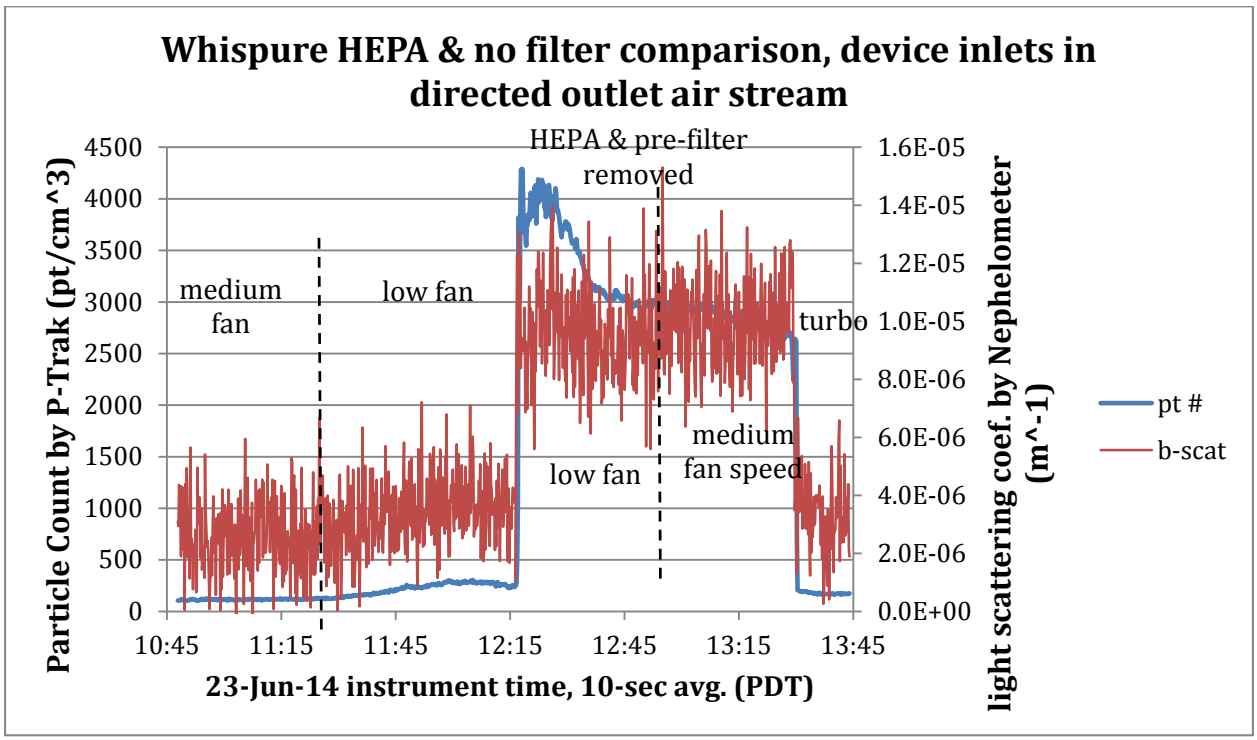


Figure 5. Comparison of non-filter and filter conditions in discharge from Whispure HEPA unit with streamlines maintained to minimize the influence of non-filtered air entrainment.

To ensure the filtered air at the subject's breathing zone is characterized by high particle removal efficiency compared with the exposure scenario, the streamlines of the air exiting the HEPA filter need to be maintained in a laminar, straight-line condition to prevent entrainment of surrounding air that is not HEPA filtered. By measuring the particulate levels in the discharge air stream with and without the filtration elements in the HEPA unit, we found the Whispure could achieve high levels of particulate matter removal. At medium fan speed, we found these reductions to be:

Particle Count, by P-Trak	96%
PM Light scattering, by Nephelometer	73%
Black Carbon conc., by microAeth	71%

The Whispure HEPA filter was therefore used to provide the particulate matter removal inside the study vehicle for creating the "filtered air" scenario that serves as the control case against which the exposure conditions can be compared. It is subsequently equipped with a manifold to gather the discharge flow which then is conveyed via a flex duct to a straight streamline diffuser which is directed toward the subject's breathing zone and the instrument inlets next to the subject's shoulder.

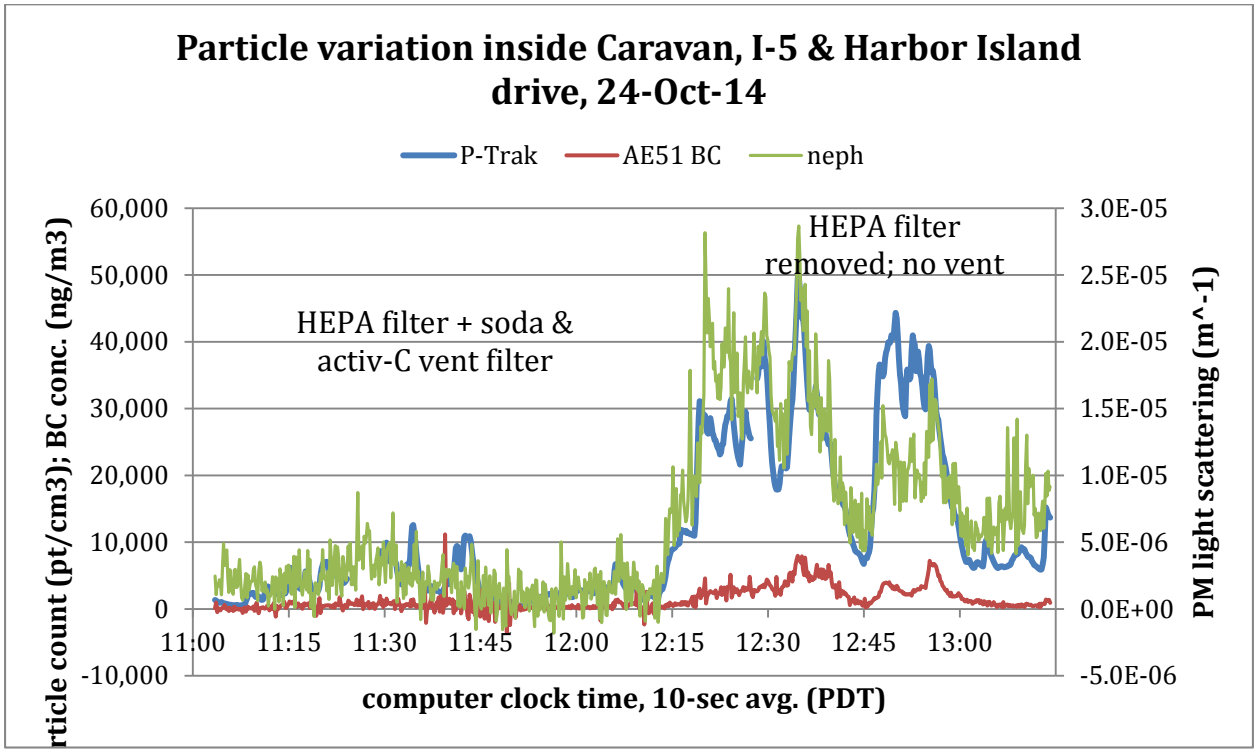


Figure 6. Sequential comparison of particulate matter levels inside study Caravan during on-highway drive.

Reduction of gaseous pollutants is not nearly as effective as particulate matter. Sorption of vapors by use of a specialized baking soda and activated charcoal filter in place of the standard vehicle ventilation system filters is the means of gas reduction for the control filtered air scenario. The contact time is short for the air flow passing through this filter so the reduction efficiencies observed for the particles cannot be realized for gaseous pollutants. Still, we have

been able to show a 24% reduction in NO_2 as measured inside the vehicle by the Aerodyne CAPS analyzer relative to the full exposure configuration in which both the vehicle ventilation filter and HEPA unit filter element are removed.

Publications/Presentations

Publications to Date:

1. **Cosselman KE, Navas-Acien A, Kaufman JD. Environmental Factors in Cardiovascular Disease. Nature Reviews Cardiology. In press.**

Future Activities

We plan to continue the commute exposure study in Year 5 and complete it during the no-cost extension year and will conduct all health analyses in the no-cost extension year.

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

Aim 1: 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. As described in the Biostats Core Section above, we plan to use cluster membership as an effect modifier for the health analyses of the association between NO_2 exposure and measurements of coronary artery calcium (CAC). We have assigned clusters using predictive k-means clustering for each of the four cities sampled as part of Project 1.

Baltimore

Results for the cluster analysis in Baltimore are presented in Figure 1 and Figure 2. As described above in the Biostatistics Core section, the pollutants included in the analysis have been normalized by the NO_x concentration measured at each of the 43 sampling locations. The spatial categorization between the winter and summer seasons differ, although there is some similarity (Rand Index of 0.6 representing moderate agreement). In both the winter and summer sampling periods, we identify a cluster that represents low proportions of all the measured pollutants per ppb of measured NO_x. This likely corresponds to locations with smaller contribution of local traffic related pollutants (Cluster 1 for both seasons). Another common profile between summer and winter months is the identification of clusters representing a higher proportion of low-molecular weight VOCs per ppb of NO_x in combination with a lower proportion of particles counts and higher molecular weight VOCs. This likely corresponds to locations where there is a higher proportion of local car traffic compared to heavier duty diesel vehicles. Cluster 3 in the winter shows higher proportions of particle counts as well as higher molecular weight VOCs likely corresponding to locations with higher local truck traffic. In the summer, cluster 3 seems to capture locations that are more highly impacted by secondary air pollutants as evidenced by the higher proportion of NO_2, O_3, and particles compared to NO_x. Cluster 4, represents locations with a higher proportion of heavier molecular weight VOCs and particles, again likely to be related to higher diesel traffic in these locations. The overall proportions though differ from the winter profile, perhaps due to differences in ambient temperature. The last summer cluster, cluster 5, represents 2 outlier locations characterized by extremely high proportion of VOCs. These two locations are located in close spatial proximity to each other suggesting they are impacted by the same unidentified source.

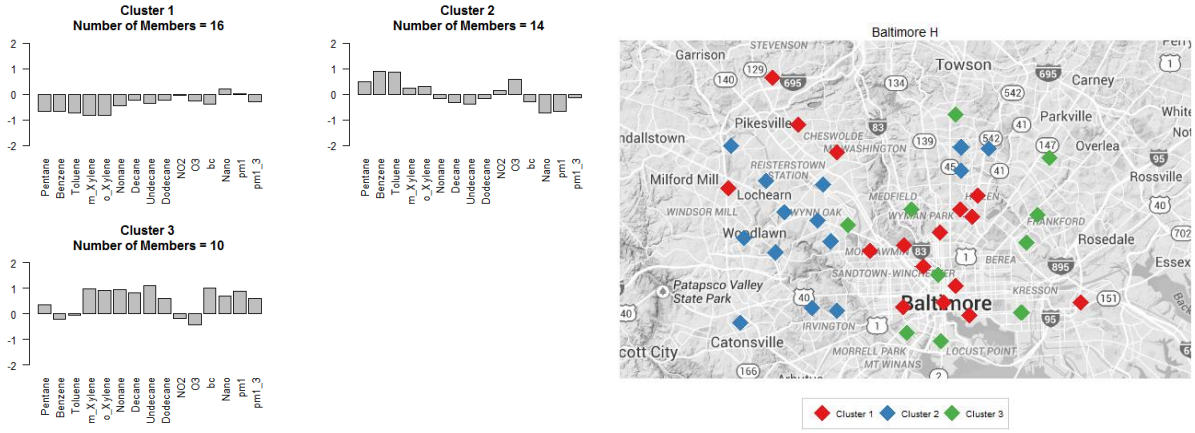


Figure 1 – Baltimore, winter: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

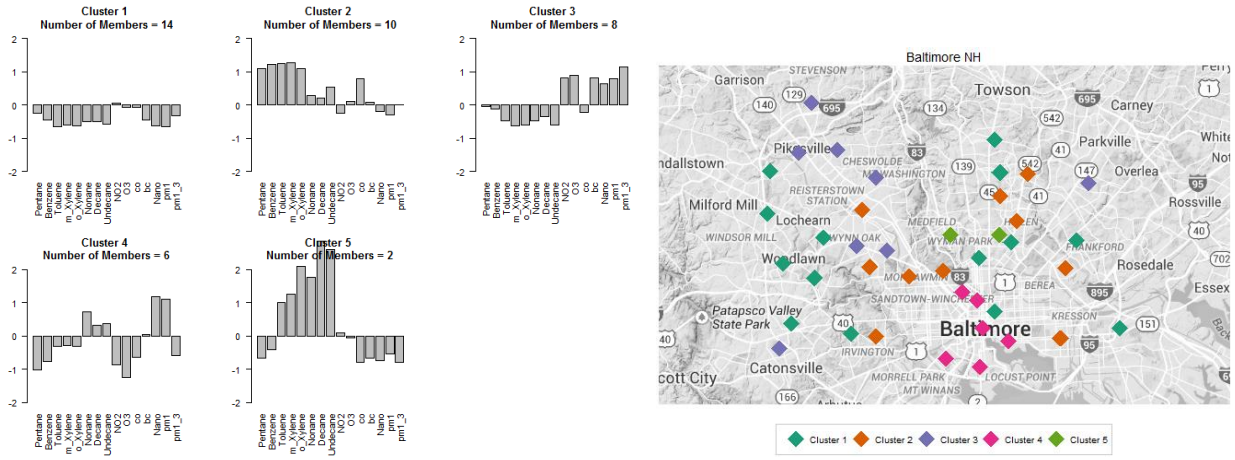


Figure 2 - Baltimore, summer: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

Los Angeles

Results for the cluster analysis in Los Angeles are presented in Figure 3 and Figure 4. Similar to the result in Baltimore, both the composition and spatial distribution of the clusters varies by season. The spatial distribution between the seasons is only moderately comparable (Rand index of 0.6). One location (the intersection of E 47th Pl and Wall St) emerges as an extreme outlier in both seasons, with high proportions of high molecular weight VOCs, although the reason for this is unclear simply based on geography and point source locations. Similar to Baltimore, in both seasons there is a cluster that is characterized by low proportions of most measured pollutants, likely corresponding to lower local traffic locations (Cluster 1). In the summertime, this cluster does show some contribution of particles. The second cluster in both seasons appears to indicate higher proportion of gas vehicle traffic based on the higher proportion of low-molecular weight VOCs and lower proportion of particles. In the winter time, a clear diesel related cluster emerges (cluster 3) that is not apparent during the summer sampling period.

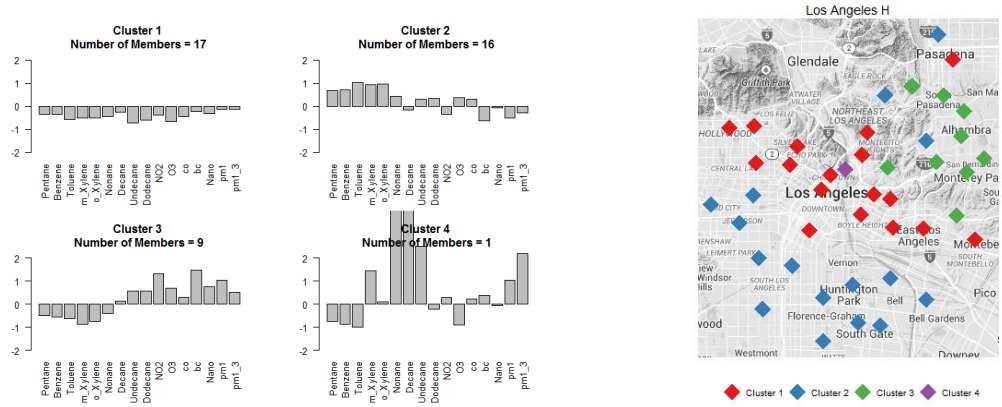


Figure 3 – Los Angeles, winter: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

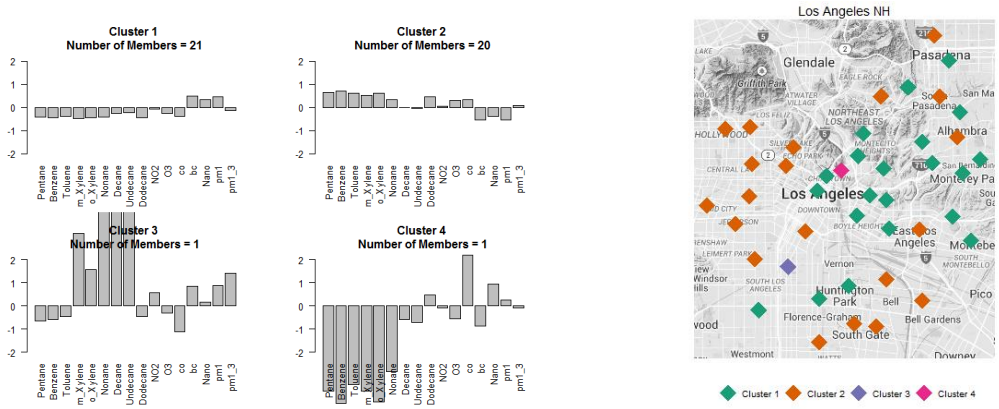


Figure 4 - Los Angeles, summer: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

St. Paul and Winston Salem

For both St. Paul and Winston Salem clustering was applied to only the passive sampling data. Because there was a lack of information on particle composition for these sites, the interpretation of the clustering results is more limited. In St. Paul, for both seasons we observe clusters with a higher proportion of low molecular weight VOCs, perhaps indicating higher proportion of car traffic at these locations (similar to the observations in LA and Baltimore). This type of cluster does not emerge in Winston-Salem. In addition, we observe for both these cities, locations with lower proportions of all measured pollutants that emerge distinctly from other sampling locations.

Figure 5 – St. Paul, winter: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

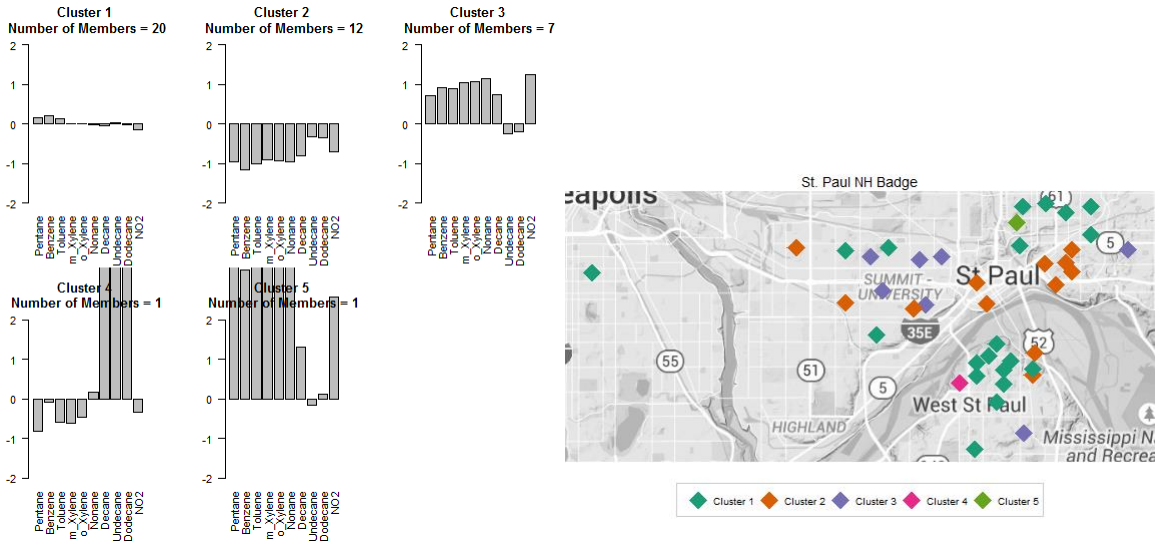


Figure 6 - St. Paul, summer: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

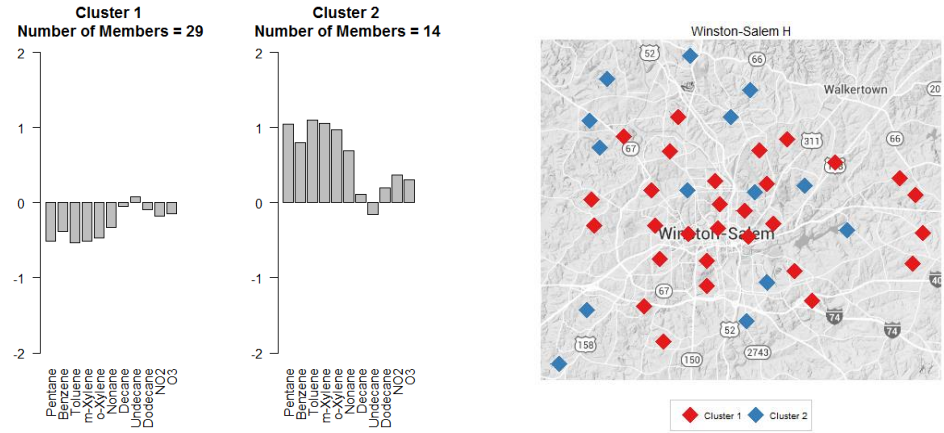


Figure 7 – Winston-Salem, winter: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

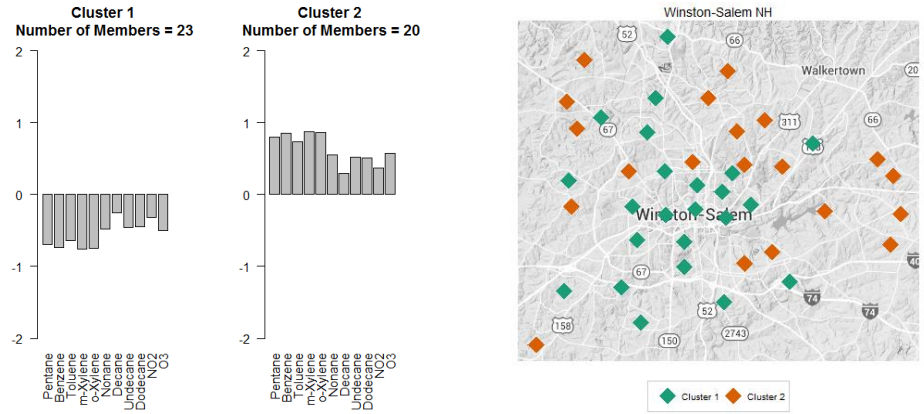


Figure 8 – Winston-Salem, summer: Cluster specific z-score of normalized pollutant concentrations and spatial distribution of clusters

Aim 2: 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 seeking to understand the influence of time spent in transit on personal exposure, which will improve our individual-level exposure estimates and contribute to our epidemiological analysis.

All four exposure campaigns have been completed. These campaigns occurred twice in two seasons each in Winston-Salem, NC and Los Angeles, CA, and involved 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 designed and built in-vehicle passive monitoring devices that capture exposures during driving. We also designed and built proximity monitors, which record time spent in specific microenvironments (inside the residence and inside the vehicle), and we 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 CCAR Project 5 field campaigns and 2) the complete MESA Air cohort at Exam 5. Overall, the participants in this study matched the MESA Air cohort as a whole well with a couple of exceptions. Sampled participants tended to be a little younger and were more likely to be employed. These differences can be attributed to our requirement that sampled participants drive at least 30 min/day on average. We also ended up with fewer Chinese participants, partly because we restricted participation to English speakers and partly because there were no Chinese participants in Winston-Salem by design in the parent MESA study.

Table 1. Demographics for Project 5 Field Campaigns Compared with the MESA Air Cohort as a whole.

	Winston-Salem		**Los Angeles**		**MESA Air***
	Winter (n=46)	Summer (n=47)	Winter (n=47)	Summer (n=46)	(n=4920)
Gender (%)					
Male	46	49	49	59	53
Female	54	51	51	41	47
Race (%)					
White	43	45	30	33	42
Chinese	0	0	6	11	11
Black	57	55	19	15	26
Hispanic	0	0	45	41	21
Age Group* (%)					
45-54	2	2	4	4	2
55-64	20	30	45	34	33
64-74	39	40	34	45	32
75-84	33	26	17	13	26
85+	7	2	0	2	7
Employed* (%)	40	56	60	61	45

*At MESA Exam 5 (2010-2012).

Some characteristics of the first three exposure campaigns (Winston-Salem heating and non-heating and Los Angeles heating) were provided in the last report. The fourth and final field campaign occurred in Los Angeles from July 6 – July 31, 2014. This campaign included 46 participants (96% of goal). Of the 46 participants, 28 returned from the February field campaign and 18 were new participants. We deployed 184 Ogawa and 184 3M samplers (46 each of personal, indoor residential, outdoor residential, and in-vehicle), and measured the same pollutants as during previous campaigns. We also deployed 20 blank samples (11%) and 15 duplicate samples (11% of possible maximum, as no personal duplicates were intended to be deployed, to reduce participant burden). As with the previous campaigns, the highest concentrations for most analytes were found in the vehicle samples (Table 2). The only exception was ozone, where the highest concentration was found outside.

Table 2 presents median concentrations for all measured analytes for all four campaigns. Because SO_2 was only detected in a handful of samples across all field campaigns, at levels just above the detection limit, 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, 0 and 5 samples from Los Angeles heating, and 0 and 8 samples from Los Angeles non-heating were invalidated due to field or laboratory error.

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

	WS Heating				WS Non-heating				LA Heating				LA Non-heating			
	Out	In	Pers	Veh	Out	In	Pers	Veh	Out	In	Pers	Veh	Out	In	Pers	Veh
NO2	9.32	5.86	6.95	87.9	4.33	4.62	5.21	54.8	20.4	19.1	18.3	104	12.7	15.4	15.3	117
NOx	14.4	13.4	16.7	114	5.40	9.89	12.4	61.7	43.7	55.1	57.4	193	16.6	19.6	24.4	83.0
Ozone	32.3	0.46	1.55	29.9	23.3	0.44	1.30	17.59	15.7	0.98	1.55	13.8	25.9	4.37	5.75	39.6
pentanes	14.5	38.9	69.2	304	21.1	85.5	204	786	40.7	48.1	58.9	313	18.2	22.1	28.7	245
isoprene	0.00	0.02	0.11	0.03	0.07	0.07	0.08	1.90	0.07	0.07	0.07	1.77	0.07	0.07	0.07	1.81
nonane	0.01	0.09	0.11	1.07	0.01	0.22	0.29	1.16	0.05	0.08	0.11	0.69	0.02	0.04	0.06	0.61
decane	0.01	0.11	0.14	2.48	0.02	0.22	0.21	4.14	0.03	0.06	0.08	4.73	0.02	0.05	0.07	4.21
undecane	0.01	0.21	0.24	3.97	0.02	0.21	0.23	9.33	0.03	0.06	0.07	14.1	0.01	0.04	0.05	13.5
dodecane	0.18	0.07	0.10	4.38	0.09	0.18	0.17	13.3	0.23	0.05	0.06	12.6	0.17	0.02	0.03	17.8
benzene	0.18	0.26	0.31	1.86	0.09	0.45	0.51	2.52	0.23	0.25	0.25	2.10	0.17	0.16	0.22	2.68
toluene	0.21	1.15	1.55	8.53	0.23	3.52	3.72	20.9	0.62	0.92	1.16	7.80	0.41	0.67	0.94	8.90
m-xylene	0.07	0.33	0.36	5.02	0.07	0.65	0.63	7.27	0.25	0.32	0.41	3.69	0.13	0.19	0.29	4.22
o-xylene	0.04	0.14	0.18	2.52	0.03	0.31	0.28	3.46	0.13	0.16	0.20	2.01	0.07	0.10	0.13	2.23

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. After reviewing the results for all four campaigns, the results for the in-vehicle samples seem higher than expected. We are conducting experiments to ensure that we are capturing the correct sampling time for the in-vehicle 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, an automated rule-based method was developed to process the large quantity of GPS data collected. Each period of time during the monitoring campaign was classified as at 'home,' 'in-vehicle,' or 'other' using this automated process.

The output from this automated processing method for GPS data was compared to the time reported in these locations on the TLD. On average, participants spent the most time at home and the least time in other locations during the winter sampling in Winston-Salem. Time spent in-vehicle ranged from 3.2 to 4.0% when measured by GPS only and from 4.9 to 6.2% when reported via TLD. The Spearman rank correlation between these two methods for each city, season, and location category range from 0.61 to 0.91. Though these two methods produced similar estimates of time in each location category, several errors were identified in the GPS tracking data that could not be addressed with the automated classification procedure. Such errors were flagged and investigated; the time associated with those points was assigned to the appropriate location category using the corresponding TLD.

To produce the single best estimate of time-location patterns during the monitoring periods, the GPS and TLD measurements were integrated in order to capitalize on the strengths of each tool. The GPS measurements of time at home and in other locations was divided into indoors and outdoors based on proportions indoors and outdoors reported in the TLD. The GPS measurement of time spent in-vehicle was used after manual adjustment to fill in gaps in the data. On average, participants spent 4-5 percent of their time in vehicles, 2-6 percent of their time outdoors, and the remainder (89-94 percent) indoors (Figure 1).

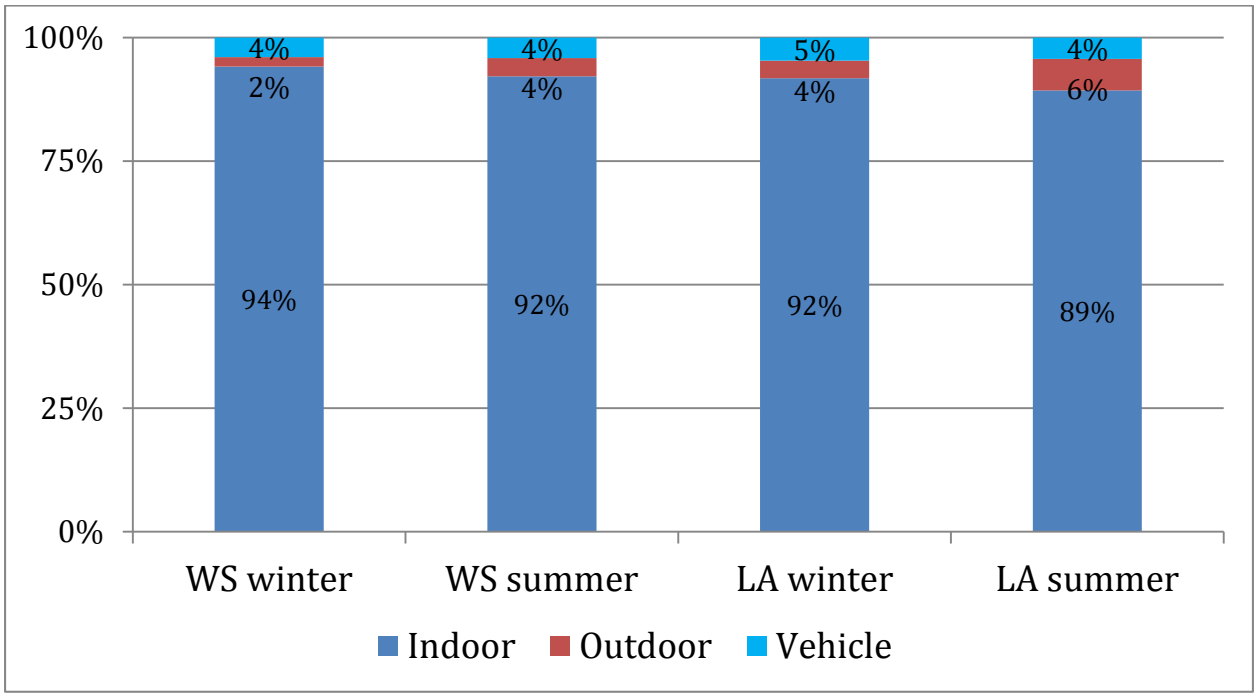


Figure 1. Percent of time spent in vehicle, outdoors, and in-vehicle by sampling campaign.

The percent of time spent indoors, outdoors, and in-vehicle based on this integration of intensive two-week measurement methods was compared to questionnaire data previously collected as part of the MESA Air study. On this questionnaire, participants were asked to describe how much time they spent in a variety of microenvironments, including at home indoors and outdoors, locations away from home indoors and outdoors, and in transit, for each day of the week during typical weeks in the summer and the winter. The best estimate was compared to reported typical time-location patterns derived from the MAQ. The magnitude of the average amount of time spent in each microenvironment, particularly time spent in-vehicle, is similar across measurement methods. A manuscript for this work is currently in progress and is expected to be submitted for publication in the upcoming months. Our group also presented these analyses, along with other results from our Project 5 exposure assessment work, during a Work-in-Progress Webinar on June 8, 2015.

Aim 3: 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 (CAC) as ascertained by CT, intima-medial thickness as measured by ultrasound, and DNA methylation. As described in the Biostatistics section above, cluster membership will be used as an effect modifier of the association between NO_x exposure and measurements of coronary artery calcium (CAC) to determine whether or not the association varies by multi-pollutant profile (as identified by cluster).

Much of the work for Aim 3 thus far has focused on DNA methylation. At the CCAR SAC meeting in October, we presented results from the regression analyses regarding exposure to ambient air pollution and DNA methylation in monocytes using data from 1,207 MESA-Air participants. Since that meeting, additional work has been conducted for the analyses regarding exposure to ambient air pollution and DNA methylation in monocytes using data from 1,207 MESA-Air participants. For the analyses involving 2,713 expression-associated gene methylation sites and air pollution, the committee suggested using a less strict false discovery rate. To this end, we used a less stringent false discovery rate of 0.05 and identified 400 and 513 expression-associated methylation sites associated with $PM_{2.5}$ and NO_X, respectively.

The committee also suggested that we incorporate gene expression data into our analyses. Since all 2,713 methylation sites have previously been associated with expression of nearby genes, we were interested if gene expression of the paired transcripts of the significant air pollution-associated methylation sites were also associated with air pollution. Of these, 190 and 191methylation sites had paired transcripts with expression also associated with $PM_{2.5}$ and NO_X, respectively. The resulting significant genes participate in various cellular functions including cell morphogenesis, migration, differentiation, proliferation, and apoptosis.

To gain biologic insight into genes where both DNA methylation and gene expression are associated with air pollution, we conducted pathway analyses using two publicly available databases – the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). We conducted overrepresentation analyses comparing the set of genes where both DNA methylation and gene expression were associated with air pollution with the total 2,713 genes in

the study. The analyses identified several overrepresented pathways from the KEGG pathway, including leukocyte transendothelial migration and B cell receptor signaling pathway for NO_X and Notch signaling for $PM_{2.5}$. These results provide evidence that air pollution-associated atherosclerosis may be mediated in part by aberrant gene expression driven by alterations in DNA methylation. No significantly overrepresented GO pathways were identified.

Additional analyses are underway to see if air pollution-associated methylation sites are also associated with subclinical atherosclerosis. If so, there are plans to conduct a formal mediation analysis.

Publications / Presentations / Posters

Publications to Date:

1. **Chi, GC, Hajat A, Bird CE, Cullen MR, Griffin BA, Miller KA, Shih RA, Stefanick ML, Vedal S, Whitsel EA, Kaufman JD. Individual and Neighborhood Socioeconomic Status, Long-term Exposure to Air Pollution, and Risk of Cardiovascular Disease. 2015. Environmental Health Perspectives (under review).**

2. **Young, M. T., Sandler, D. P., DeRoo, L. A., Vedal, S., Kaufman, J. D., & London, S. J. (2014). Ambient air pollution exposure and incident adult asthma in a nationwide cohort of US women. American journal of respiratory and critical care medicine, 190(8), 914-921.**

3. **Chan S, Van Hee V, Bergen S, Szpiro AA, DeRoo L, London S, Marshall J, Kaufman JD, and Sandler D. Long term air pollution and exposure and blood pressure in the Sister Study. Environmental Health Perspectives. 2015.**

4. Spalt, E. W., Curl, C.L., Allen, R.W., Cohen, M., Adar, S.D., Hinkley-Stukovsky, K., Avol, E., Castro, C., Nunn, C., Mancera-Cuevas, K., Kaufman, J.D. Time-Location Patterns of a Diverse Population of Older Adults: The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Journal of Exposure Science and Environmental Epidemiology. 2014.

5. 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.

6. Szpiro AA, Sheppard L, Adar SD, and Kaufman JD. Estimating Acute Air Pollution Health Effects from Cohort Study Data. Biometrics. 2014; 40(1):164-174.

7. Nicholas T. Contribution of the in-vehicle microenvironment to individual ambient source-derived NO_2 exposure concentration. M.S. Thesis, University of Washington. 2014.

8. Spalt EW, Curl CL, Allen RW, Cohen M, Williams K, Hirsh JA, Adar, SD, Kaufman JD. Factors influencing time-location patterns and their impact on estimates of exposure: The Multi-Ethnic Study of Atherosclerosis and Air Pollution. Journal of Exposure Science and Environmental Epidemiology. 2014.

Presentations to Date:

1. **Hazlehurst, M. Integrating Data from Multiple Time-Location Measurement Methods for Use in Exposure Assessment: the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). International Society for Environmental Epidemiology. Seattle, WA. August 2014.**

2. **Spalt, EW. Patterns in Indoor, Outdoor, In-Vehicle, and Personal Measurements of Volatile Organic Compounds. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

3. **Nicholas, T. Contribution of time in-transit to individual exposure to traffic-related air pollution. Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

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

5. 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.

Posters to Date

1. **Hazlehurst, M. Time‐location measurement methods for use in exposure assessment: the Multi‐Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Annual Conference on Environmental, Occupational, and Population Health. Semiahmoo, WA. January 2015.**

Future Activities

We are in the process of preparing three manuscripts related to Aim 2: 1) a comparison of the three time-location measurement methods utilized in this study (draft in review by co-authors), 2) an analysis of the relative contributions of each microenvironment to overall exposure to ambient-source nitrogen dioxide, and 3) a manuscript discussing the results of all of traffic-related air pollutant sampling.

For the remainder of year 5 and the no-cost extension year, effort will focus on 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. Included as part of our collaborative budget are funds to support the preparation of a collaborative manuscript on commuting study designs. This manuscript is being developed with researchers from the SCAPE CLARC. Additionally, brief summaries for each project are included detailing the progress to date and future planned activities.

UW CCAR Involvement in the CLARC Collaborative Projects				
PROJECTS	**CCAR Investigators**	**Activities**	**Period**	**Estimated total budget**
#1 Circulating Inflammatory Potential of Inhaled Coarse PM	Matt Campen Jake McDonald	• Ex Vivo Endothelial Cell Assays • High Fructose Rat Model in Designed Exposure Atmospheres	10/12-11/16	$100,000
#2 Mobile and Fixed Site Characterization of Vehicle Emission Impacts in Atlanta	Tim Larson Mike Yost	• Mobile and Fixed Site Monitoring Campaign in Atlanta	10/12-11/16	$64,000
#3 Measurement Error for Air Pollution Cohort Studies: Application and Comparison of Several Statistical Methods to Georgia Birth Cohort Data	Adam Szpiro	• Measurement Error Correction Approach to Georgia Birth Cohort	12/12-11/16	$47,000
#4 Inter-comparison of ambient $PM_{2.5}$ estimation models in NC	Paul Sampson	• Satellite PM Metric Addition to the PM Spatio-Temporal Model in North Carolina	12/12-11/16	$24,000
Collaborative Manuscript: Commuting Study Designs	Joel Kaufman Tim Larson Sverre Vedal Adam Szpiro		7/15-11/16	$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.

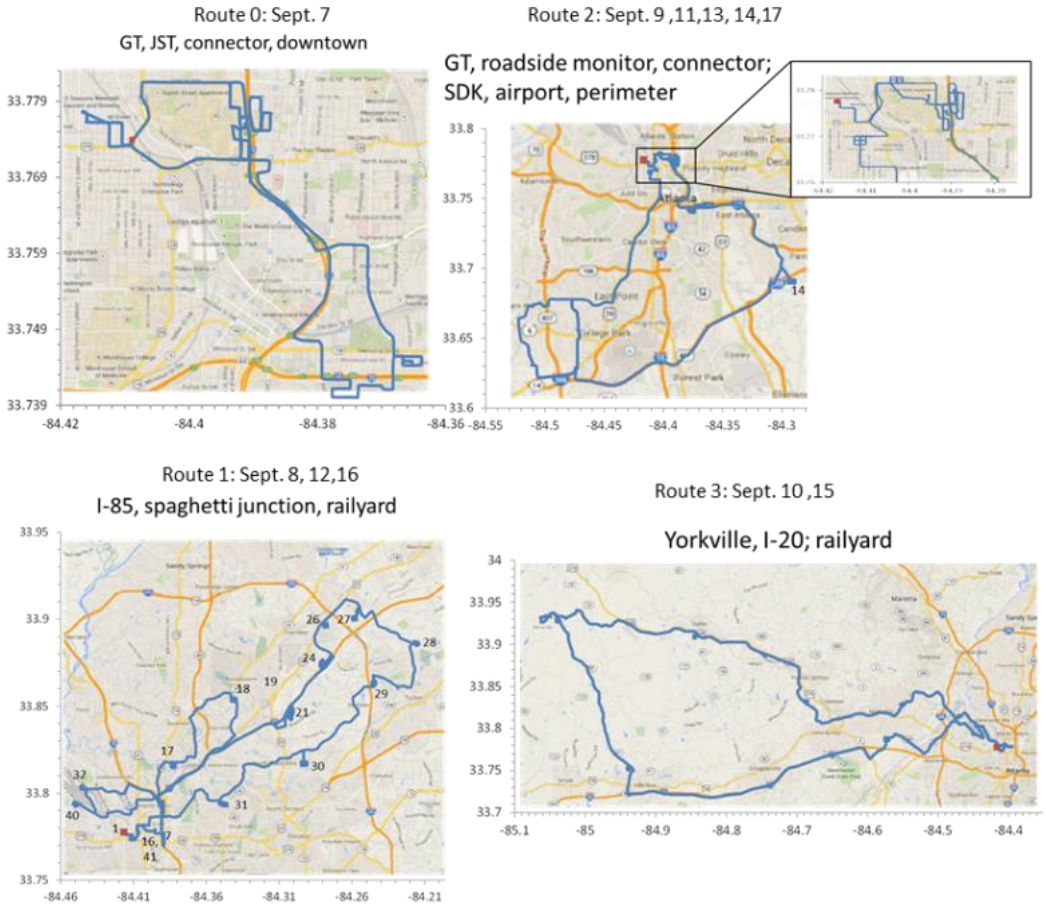


Figure 1: Mobile Platform sampling Routes in Atlanta.

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.

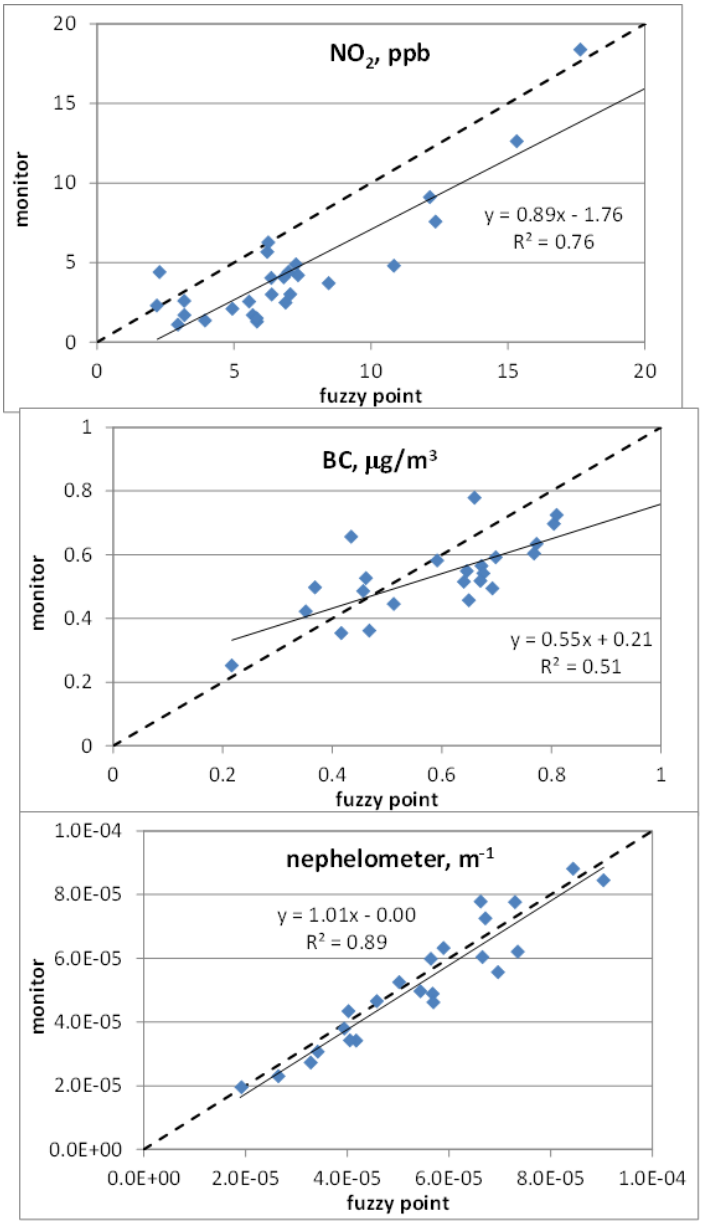


Figure 2. Fuzzy point median values vs. ambient monitor values for selected species (Jefferson St., Yorkville and DeKalb sites)

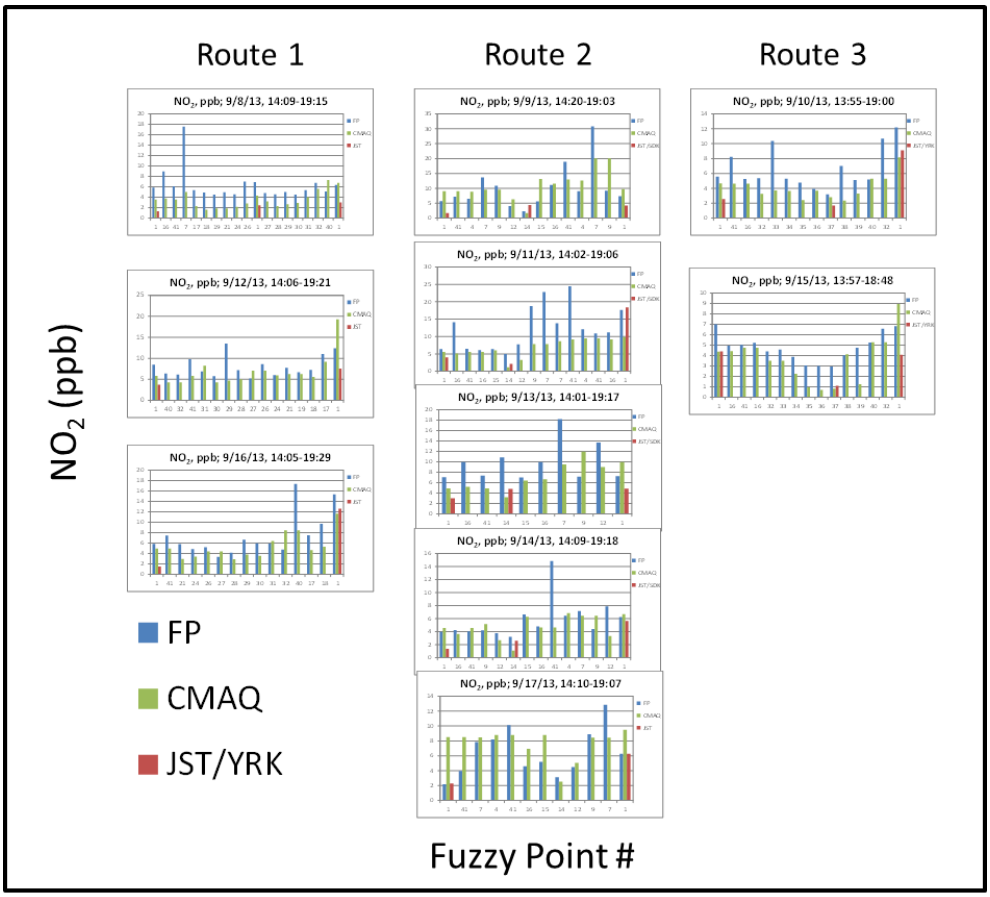


Figure 3. Fuzzy Point (FP) medians vs. CMAQ predictions for NO_2.

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

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

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. UW has begun replicating Emory's analysis and has put in place software and procedures for applying the parameter bootstrap to assess bias and/or inflated standard errors from measurement error.

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 was compiled by the Liu group, including MODIS total AOD values, derived meteorological parameters and, from the UW database, GIS-based spatial covariates. This was distributed to all participating research teams. A common master modeling grid at 10-km resolution was developed by the Liu group and shared by all teams. A set of common procedures and statistics will be jointly developed by all participating teams to evaluate model performance. After preliminary results are generated, each team will document their model development in sufficient detail for other teams to reproduce their results. The estimated deliverable of this project will be a manuscript to report evaluation results.

As of June 2015, the Emory team has worked with the Harvard team to generate the final model development and prediction datasets using MODIS collection 6 AOD data at 10 km resolution over North Carolina for the proposed study period. Quality flags are included to mark potential outliers. The Emory team has completed model development with the updated dataset using Chang's spatial downscaler and Liu group's mixed effects model. The Harvard and UW teams will complete their model runs in August. National scale evaluation of the quality of various MODIS collection 6 AOD parameters are underway. In addition, the Emory team is working to process Georgia Tech's CMAQ output.

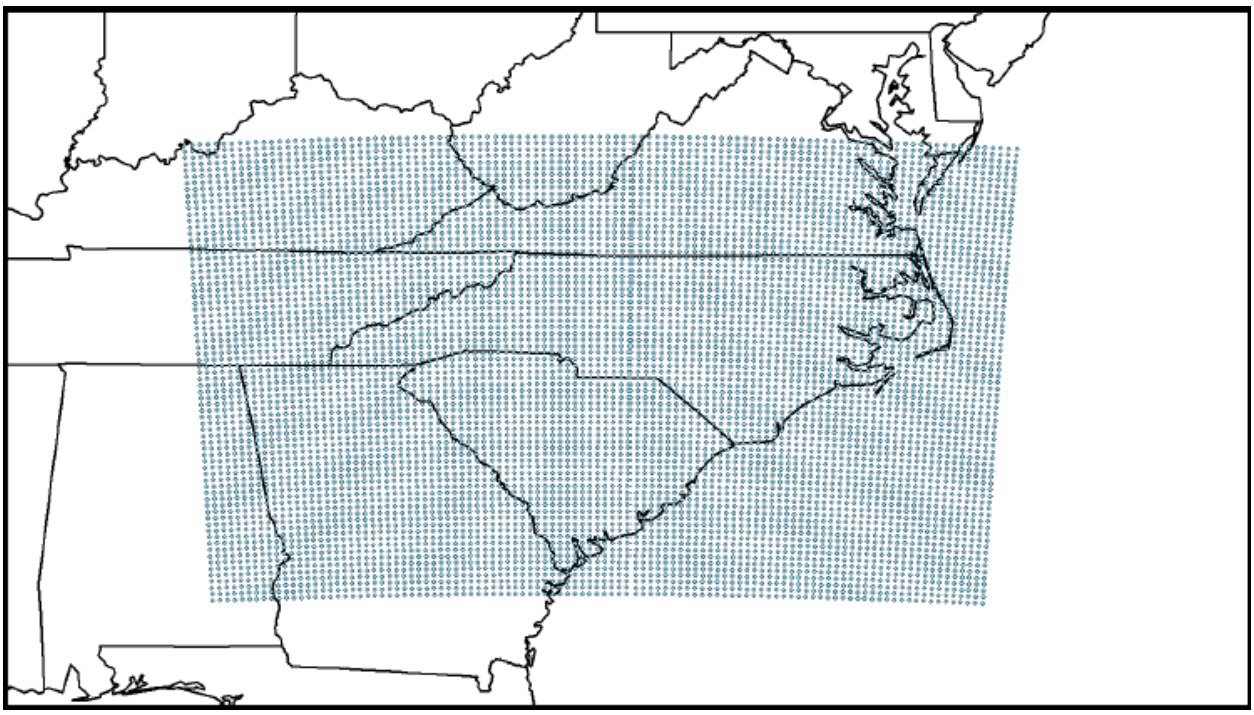


Figure 2