SECOND YEAR REPORT

Harvard University Clean Air Research Center
Air Pollution Mixtures: Health Effects across Life Stages
Annual Center Progress Report
Period: August 1, 2011– July 31, 2012

This report presents an overview and summary of the progress and achievements of the first year of funding for the Harvard University Clean Air Research Center. The primary objective of the Center is to estimate health effects of air pollution mixtures, sources, and individual pollutants across life stages (prenatal to senescence). The Harvard CLARC addresses four of the six research priorities of the EPA solicitation to establish Clean Air Centers. The Center: 1) investigates the effects of pollutants and mixtures through animal and human studies; 2) identifies sub-populations that are at increased risk through the investigation of the modifying effects of gender, diabetes, obesity, socioeconomic disparities, stress, depression, violence, smoking, and omega-3 fatty acid intake in children, adults, and elderly; 3) explains regional and temporal differences in air pollution risks; and 4) examines the shapes of exposure-response relationships for associations resulting from the use of multiple exposure metrics, outcomes and populations.

The Harvard CLARC has assembled a multidisciplinary team with expertise in a wide range of fields, including epidemiology, biostatistics, air pollution, exposure assessment, cell biology, pathology, and toxicology. To meet our main objective, we have developed five research Projects that will be supported by three technical Cores. We have developed a common set of hypotheses that will be tested in the different Projects using a synergistic and integrative approach. Importantly, as part of the proposed Center, we will examine the consistency of findings across different health outcomes and across populations at different stages of life by: 1) relying on a common set of exposure metrics; 2) exploring the same set of susceptibility and vulnerability factors; and 3) integrating results from laboratory animal experiments with cohort and population based studies. Specifically, we plan to: 1) investigate the acute and chronic effects of short- and long-term exposures to individual pollutants, pollution sources and multi-pollutant mixtures on: cognitive/neuropsychological function, cardiovascular/endothelial function, inflammation, birth weight/growth, and CVD-related hospitalization/mortality across life stages; and 2) identify susceptibility and vulnerability factors that modify these effects.

In the sections that follow, each project is discussed separately. For each project, we summarize the objectives, progress and achievements, publications and presentations, and future activities. This report concludes with sections discussing our quality assurance and human subject requirements, changes to key personnel, and center collaborations. A financial report is submitted separately.
Project 1: Relative Toxicity of Air Pollution Mixtures. PI: John J. Godleski.

1.1 Objective of Research: Project 1 is an inhalation toxicological animal exposure study that investigates the relative toxicity of different component concentration combinations of air pollution mixtures. These components include both particles and gases that are emitted directly from sources (primary species) or are formed in the atmosphere through a series of reactions that are predominantly photochemical (secondary species). The project uses source-specific emissions as well as ambient air or concentrated ambient particles and our photochemical chamber technologies to generate realistic mixtures. We are testing the biological responses of exposure to fresh, aged primary, and secondary pollutants (both gas and particle phase) formed from the photochemical oxidation of traffic emissions or Boston ambient air. We will investigate whether the toxicological effects of exposure to aged primary or secondary particles. Toxicity is assessed in Sprague-Dawley rats by changes in 1) \textit{in vivo} oxidant response, 2) blood pressure, 3) measures of inflammation, and 4) vascular blood flow/resistance.

Progress Summary/Accomplishments: During the reporting period we have completed a group of 3 papers which are \textit{in press} in the journal Air Quality, Atmosphere, and Health. These papers describe the reaction chamber and exposure system used for study of emissions derived from traffic using a northeastern traffic tunnel as the source of mixed vehicular emissions, and compared the effects of the emissions with or without simulation of atmospheric aging by photochemistry and formation of secondary particles on respiratory, cardiovascular, and systemic outcomes.

We have investigations examined the relative toxicity of these air pollution mixtures, focusing on traffic. All exposures were done at a target dose of 50 micrograms per cubic meter. Sprague Dawley rats were repeatedly exposed to primary only (P), secondary organic aerosol only (SOA), a combination of the two (P+SOA) or filtered air for 5-hours/day for 3 weeks. BP was measured continuously using implanted telemetry. Mixed effects models were used to compare responses of systolic (SBP), diastolic blood pressure (DBP) and heart rate (HR).

Exposure to P increased and maintained an effect on SBP and DBP across weeks showing a significant dose response. SOA exposure on the first day resulted in a significant increase in both SBP and DBP (p=0.001, p=.0003) becoming strongly negative by week 3. With P+SOA, significant increases in SBP and DBP of 13 mmHg were observed on the first day of exposure (p=0.04, p=0.0015) which was maintained through week 1 for SBP and through week 2 for DBP. Sham exposures in the SOA and P+SOA groups after 3 weeks showed compensatory decreases in both SBP and DBP. No exposure had a significant effect on HR. Both primary and secondary traffic derived aerosols can substantially increase SBP and DBP but these increases are eventually lost.

Respiratory outcome included breathing pattern, in-vivo chemiluminescence (IVCL), broncho-alveolar lavage, and complete blood count. Although all exposures produced decreases in tidal/minute volumes and inspiratory/expiratory flows, there were differences in inflammatory changes in BAL, with increased neutrophils for SOA and for P+SOA and increased lymphocytes for P and P+SOA, without changes in total protein, ß-NAG or IVCL.

Publications/Presentations:


Papapostolou V, Lawrence J, Ferguson S, Wolfson JM, Diaz EA, Godleski JJ, Koutrakis P. Development of an exposure generation system to investigate the health effects of fresh and aged vehicular emissions. Air Quality, Atmosphere and Health. 2012 In press. DOI:


Godleski, JJ.Invited Speaker. Pulmonary dysfunction and air pollution. Symposium on Air Pollution. Experimental Biology Meeting San Diego, CA April 2012


Future Activities: We will conduct exposures to fresh and photochemically-aged source traffic emissions with and without ozone and other secondary gases. Toxicity of exposures will be assessed in rats using a variety of outcomes including changes in vivo chemi-luminescence, blood pressure, inflammation, and vascular flow/resistance.

Changes/problems/delays in proposed work: There are no substantive changes, problems, or delays in proposed work to report at this time.
Project 2: Cognitive Decline, Cardiovascular Changes, and Biological Aging in Response to Air Pollution. PI: Joel Schwartz, HSPH.

Objective of Research: In this Project we investigate the acute and chronic effects of air pollution on cognitive and neurological impairments, systemic inflammation, and vascular dysfunction. We will determine how these effects differ depending on the composition of multi-pollutant mixtures and the source contributions to PM composition. We will then ascertain the level of increased effects in susceptible and vulnerable subpopulations by examining modifying factors of obesity, diabetes, diet, socioeconomic position, and psychosocial stress.

Progress Summary/Accomplishments: We have published a number of papers. A key part of this project and of all the cohort studies is development of a spatio-temporal model predicting daily PM$_{2.5}$ concentrations in New England using land use regression terms and satellite remote sensing. We have successfully developed a model that predicts daily PM$_{2.5}$ at the spatial resolution of a 10x10 km grid across New-England. The model was used to investigate both the long- and short-term effects of PM$_{2.5}$ exposures on hospital admissions across New-England. We found that chronic exposure to particles is associated with substantially larger increases in hospital admissions than acute exposure and both can be detected simultaneously using our exposure model.

We have evaluated the relationship between premature birth and birth weight with exposure to PM$_{2.5}$ levels during pregnancy in Massachusetts for a 9-year period (2000-2008), and we found that exposure to PM$_{2.5}$ during the last month of pregnancy contributes to risks for lower birth weight and preterm birth in infants.

We have studied the cross-sectional association between DNA methylation in nine inflammatory genes and lung function in the Normative Aging Study cohort of 756 elderly men living in the metropolitan area of Boston. In the published a paper we show that DNA methylation may be part of the biological processes underlying the lung function decline and that IFNγ and IL6 may have ambivalent roles through activation of negative feedback. Furthermore, in the Normative Aging Study we investigated the short- and intermediate term air pollution effects on repeated measurements of: fibrinogen, C-reactive protein, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), and epigene-environment interactions by DNA methylation of $Alu$, $LINE-1$, tissue factor ($F3$), Toll-like receptor 2 ($TLR-2$), and $ICAM-1$. We observed associations of traffic-related pollutants on fibrinogen, and both traffic and secondary particles on C-reactive protein, ICAM-1, and VCAM-1. There was effect modification by DNA methylation status, indicating that epigenetic states can convey susceptibility to air pollution.

We published the results of a further follow-up of the Harvard Six City Study where we replicated our previous analysis on the association between PM$_{2.5}$ and mortality; we further examined different time lags, the shape of the concentration-response relationship, changes in the slope of the relation over time, and the impact of time-varying effects for smoking, sex, and education on the results.

Publications/Presentations:

Kloog I, Koutrakis P, Coull BA, Lee HJ Schwartz J. Assessing temporally and spatially resolved PM$_{2.5}$ exposures for epidemiological studies using satellite aerosol optical depth measurements. Atmos. Env. 2011; 45:6267-6275.

Kloog I, Melly SJ, Ridgway WL, Coull BA, Schwartz J. Using New Satellite Based Exposure Methods to Study the Association between Pregnancy PM_{2.5} Exposure, Premature Birth And Birth Weight In Massachusetts Using new satellite based exposure methods to study the association between pregnancy pm2.5 exposure, premature birth and birth weight in Massachusetts. Environ Health. 2012 Jun 18;11(1):40.


**Future Activities:** We will continue to work on the activities proposed in our grant application. In addition, we received a grant from NIEHS to investigate epigenomic effects of environmental exposures. This will allow us to continue examining exposure and epigenetic effects in the Normative Aging Study.

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.
Project 3: Identifying the cognitive and vascular effects of air pollution sources and mixtures in the Framingham Offspring and Third Generation Cohorts. PI: Murray Mittleman, HSPH.

Objective of Research: Long- and short-term exposures to ambient air pollution are associated with adverse acute and chronic cardiovascular and perhaps cognitive function, but these effects are poorly understood. Using data from the Framingham Offspring and Third Generation Cohorts, well-characterized populations that have not been previously investigated in association with ambient environmental exposures, we will: 1) determine whether long-term exposures to ambient pollutants and mixtures are associated with cognitive impairment and cognitive interference; 2) test whether short-term and long-term exposures to pollutants, mixtures and sources are associated with acute and chronic vascular and endothelial function; and 3) consider whether markers of biological susceptibility and vulnerability differentially influence these associations, allowing us to identify subpopulations at increased risk for harmful effects of air pollution.

Progress Summary/Accomplishments: We have conducted preliminary analyses of the effects of air pollution exposures on vascular outcomes including flow-mediated dilation, flow velocity and blood pressure in the Framingham Offspring and Third Generation Cohorts. We also conducted preliminary analyses evaluating the impact of residential distance from an A1 or A2 road on cognitive function as assessed by the Mini-Mental State Exam (MMSE) in the Framingham Offspring Study (n=3,678). Preliminary evidence suggests a non-linear association between distance to major road and lower cognitive function, but associations did not meet nominal statistical significance.

While working on exposure assignment for the Framingham cohorts, we have completed related work which builds on the aims of our project. We evaluated the association between short-term meteorological exposures on biomarkers of inflammation, endothelial function, and heart failure control in a cohort of 100 patients with Class II and III heart failure. In this study we found that higher two-day moving average of apparent temperature was associated with elevated levels of BNP. Finally, CRP followed a similar pattern after three day moving average.

In another study, we followed a cohort of 3,886 individuals hospitalized for AMI in 64 Centers across the United States from 1989 to 1996. Addresses were geocoded, and distance to the nearest major roadway was assigned. Cox regression was used to calculate hazard ratios, with adjustment for personal, clinical and neighborhood-level characteristics. There were 1,071 deaths after 10 years of follow-up. In the fully adjusted model, compared with living >1,000 m, hazard ratios (95% confidence interval) for living ≤100 m were 1.27 (1.01-1.60), for 100 to ≤200 m were 1.19 (0.93-1.60), and for 200 to ≤1,000 m were 1.13 (0.99-1.30) (P(trend)=0.016).

We reviewed the medical records of 1,705 Boston area patients hospitalized with neurologist-confirmed ischemic stroke and abstracted data on the time of symptom onset and clinical characteristics. The estimated odds ratio (OR) of ischemic stroke onset was 1.34 (95% CI, 1.13-1.58) (P < 0.001) following a 24-hour period classified as moderate (PM$_{2.5}$ 15-40 μg/m$^3$) by the EPA Air Quality Index compared with a 24-hour period classified as good (≤15 μg/m$^3$). Considering PM$_{2.5}$ levels as a continuous variable, we found the estimated odds ratio of ischemic stroke onset to be 1.11 (95% CI, 1.03-1.20) (P = 0.006) per interquartile range increase in PM$_{2.5}$ levels (6.4 μg/m$^3$). The increase in risk was greatest within 12 to 14 hours of exposure to PM$_{2.5}$ and was most strongly associated with markers of traffic-related pollution.

We also evaluated the impact of long-term near-roadway exposure on renal dysfunction in a susceptible population. In this study we found that living closer to a major roadway was associated with a significantly lower estimated glomerular filtration rate compared with living farther away. This work is currently under review.
Publications/Presentations:


Future Activities: Future work will include completion of all modeled air pollution data in the Framingham master dataset for Offspring Cycles 6, 7, 8 and Generation 3 Cycles 1 and 2. We will complete analyses and publish manuscripts on vascular outcomes including flow mediated dilation, blood pressure, flow rates, peripheral arterial tonometry and biomarkers of inflammation, oxidative stress and hemostasis.

Changes/problems/delays in proposed work: There are no substantive changes, problems, or delays in proposed work to report at this time.
Objective of Research: The main aim of this project is to determine the health effects of prenatal and postnatal exposures to individual pollutants, sources, and pollutant mixtures on somatic growth, cardiovascular risk (blood pressure, exercise tolerance) and cognition in children. The strength of chronic and acute effects of individual pollutants will vary by source and mixture, as well as the timing of prenatal and postnatal exposures. Increased vulnerability or susceptibility to pollution effects on these adverse health outcomes will also result from socioeconomic disparities, stress and violence, environmental tobacco smoke, and reduced maternal and child omega-3 fatty acid intake measured in the prenatal as well as postnatal periods.

Progress Summary/Accomplishments: We have geocoded the entire longitudinal address history and have used this to link to longitudinal GIS and census data. With these data we have created a longitudinal data set with: 1) estimated spatially and temporally resolved BC and PM$_{2.5}$; and 2) neighborhood-level SES variables. While addresses before and after birth are known and geocoded, for the purpose of improving estimation of birth outcome health data, the address at birth is currently being verified and geocoded. We have performed initial analyses of the associations of measured and estimated individual pollution and traffic exposures (temporally or spatially and temporally resolved) with the following outcomes: maternal (glucose tolerance, hypertension in pregnancy); birth weight and longitudinal development of adiposity; blood pressure (birth, 6 months, 3 years, 7 years); cognition; respiratory illness in early life; sleep duration; and markers of inflammation. We have evaluated whether socioeconomic exposures confound or modify the relation of pollution to these exposures. We have expanded our research team to include many senior and junior investigators from Harvard Pilgrim; the Department of Population Medicine’s Obesity Prevention Program; Boston Children’s Hospital; Beth Israel Deaconness Medical Center; Harvard Medical School; Massachusetts General Hospital; the Brigham and Women’s Hospital; and the University of Utrecht in the Netherlands. In the coming year, in addition to completing analyses and papers on the relation of pre-birth and postnatal distance to roadway, BC, and PM on our many health outcomes, we will begin to: 1) evaluate effects of pollution sources and mixtures; 2) extend our evaluation of effect modification by family and neighborhood SES, and evaluate modification by nutrition and genes and; 3) conduct pathway analyses incorporating multiple outcomes and exposures.

Our most outstanding findings were highlighted in nine posters presented at our external advisory committee meeting. Elevated traffic exposures in pregnancy predict abnormal glucose tolerance during pregnancy. Closer distance to roadway was associated with increased sleep deprivation. Increased black carbon and urbanicity predicted lower fetal growth; increased traffic density predicted development of adiposity by 6 months of age. Increased residence-level black carbon levels or closer distance to roadway in pregnancy predicted reduced cognition by age 7, but associations were confounded by SES. Increased black carbon in the last month of pregnancy predicted higher neonatal blood pressure, but increased ozone in that period predicted lower neonatal blood pressure.

Publications/Presentations:


**Future Activities:** In the coming year, in addition to completing analyses and papers on the relation of pre-birth and postnatal distance to roadway, BC, and PM on our many health outcomes, we will: 1) begin to evaluate effects of pollution sources and mixtures; 2) extend our evaluation of effect modification by family and neighborhood SES, and begin evaluation of modification by nutrition and genes; 3) begin pathway analyses incorporating multiple outcomes and exposures.

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.
**Project 5: A National Study to Assess Susceptibility, Vulnerability, and Effect Modification of Air Pollution Health Risks. PI: Francesca Dominici, HSPH.**

**Objective of Research:** This National study is aimed at identifying factors that explain the heterogeneity of health risks associated with air pollution exposure. We hypothesize that such factors include medical and social conditions, conditions that modify exposure, and differences in pollution composition that modify exposure toxicity. Moreover, we hypothesize that the relevant factors vary among different health outcomes. Our research will be fully interactive with the other Center projects. Our previous results (e.g., diabetic susceptibility) have guided their analyses, and their results have generated specific hypotheses that we will test. We have 3 objectives. In Aims 1 and 2, we will conduct national studies of short- and long-term exposures to individual pollutants, sources, and mixtures. A main focus of our Center is to study established cohorts (NAS, Framingham, and Viva) in Massachusetts and surrounding states using novel, validated approaches to assess exposure. In Aim 3, we will complement those cohort studies, by establishing a cohort of 2.3 million Medicare enrollees residing in the same region and following its members prospectively for cause-specific hospital admissions and mortality for the period 2000-2014, and also by studying all live births in Eastern MA, geo-coded to exact address and followed for adverse birth outcomes.

**Progress Summary/Accomplishments:**

We have estimated temporal and geographical trends in the incidence and treatment of cardiovascular diseases in the Medicare population for the period 1998 to 2008. This is the period where Medicare data Part A is available to us for 100% of the Medicare population. Now the Medicare data has been extended to the year 2009 and 2010. We sought to examine: 1) whether there have been significant regional disparities in MI incidence, treatment and outcomes during this time period; and 2) whether these regional disparities have changed over time. In our study population, which includes nearly 300 million Medicare fee-for-service beneficiary-years, we found: 1) statistically significant declines in the incidence of MI in all U.S. Census Divisions; 2) statistically significant differences in the incidence of MI between geographic regions at the start of the study period which increased over time between 2000 and 2008; 3) strong evidence of a wide and persistent variation in rates of cardiac catheterization and revascularization after MI across regions. To our knowledge, this is the largest study to date that examines geographic differences of trends in acute MI in the U.S. elderly, and the first that examines geographic differences in MI incidence, procedures and 30-day mortality. As always it will require some serious considerations on how to incorporate the temporal changes in cardiovascular disease management into our models of air pollution exposure (short- and long-term) and mortality and morbidity outcomes. We will continue to investigate this issue in year 3.

We have applied Bayesian stochastic search variable selection (SSVS) to ambient PM$_{2.5}$ components in multi-pollutant models of emergency hospital admissions for cardiovascular disease (CVD) in a population of Medicare enrollees older than 65 in each of 118 U.S. counties, 2000-2008. Our SSVS method allows us to evaluate a large number of possible statistical models in each county (we consider 6 of the most common PM$_{2.5}$ components: sulfate, nitrate, silicon, elemental carbon, organic carbon matter, and sodium ion) to identify, within each county, the combination of PM$_{2.5}$ components most associated with CVD hospital admissions. Complete results and extensive tools for reproducibility are made freely available online at [www.ddiez.com/epissvs](http://www.ddiez.com/epissvs) (login: map, password: Review65).

We have performed a literature review and conducted a multi-site time-series analysis of hospital admissions and exposure to PM$_{2.5}$ constituents (elemental carbon, organic carbon matter, sulfate, and nitrate) in a population of 12 million U.S. Medicare enrollees for the period 2000 to 2008. The literature review illustrated a general lack of multi-constituent models or insight about probabilities of differential impacts per unit concentration change. Consistent with previous results, the multi-site time-series analysis
found statistically significant associations between short-term changes in elemental carbon and cardiovascular hospital admissions. Posterior probabilities from multi-constituent models provided evidence that some individual constituents were more toxic than others. Ratios of constituent toxicities, commonly used in risk assessment to describe differential toxicity, were extremely uncertain for all comparisons. These analyses emphasize the subtlety of the statistical techniques and epidemiological studies necessary to inform risk assessments of particle constituents.

In an analysis of 545 US counties for the period 2000 to 2007, we investigate as whether more recent and slower declines in PM$_{2.5}$ levels continue to improve life expectancy. We found that reductions in PM$_{2.5}$ were significantly associated with improvements in life expectancy for the period 2000 to 2007. These results demonstrated that air pollution control in the last decade continues to have a positive impact to public health.

Publications/Presentations:


Bobb JF, Dominici F, Peng R Reduced Bayesian Hierarchical Models: Estimating Health Effects of Simultaneous Exposure to Multiple Pollutants, Journal of the Royal Statistical Society, Series C (tentatively accepted)

Correia A, Pope CA, Dockery D, Ezzati M, Dominici F The Effect of Air Pollution Control on Life Expectancy in the United States: An Analysis of 545 US counties for the period 2000 to 2007, Epidemiology (tentatively accepted)

**Future Activities:** We will continue to develop methods for multi-pollutant models and we will extend them to the multi-pollutant analyses of metals. We will continue the development and analysis of a cohort of Medicare enrollees in Massachusetts and surrounding states for cause-specific hospital admissions and mortality; studying all live births in Eastern MA, geo-coded to exact address and followed for adverse birth outcomes.

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.

**Center Collaborations:**

**Collaboration 1:** Assessment of common outcomes from exposures of a reproducible animal model of cardio-metabolic syndrome to traffic-related multi-pollutant atmospheres used at four CLARC facilities

**Lead Harvard Investigator:** John Godleski

**Collaborative Centers:** GLACIER, University of Washington CLARCs, and EPA

In collaboration with our Project 1, GLACIER, University of Washington CLARCs, and EPA in-house scientist, we have planned to study animals with diet-induced cardio-metabolic syndrome (CMS) using the traffic-related multi-pollutant exposure system in use at each Center. We will use our facility to generate exposures to vehicular emissions from an urban tunnel. This system can differentiate primary and secondary particles as a model of traffic-related ambient urban pollution (TRAUP). Protocols for these studies were developed through a series of emails following the 2011 Center Director’s meeting and finalized at the 2012 meeting. Project 1 scientists played a leadership role in organizing this study and coordinating among the various CLARCs participating in this collaboration.

Using an established high fructose-diet-fed rat model of CMS established at GLACIER, the proposed collaborative study is designed to: 1) determine if this dysfunctional cardiometabolic condition predisposes to the toxic effects of traffic-related air pollution and; 2) identify underlying toxicological modes of action by which this may occur. We will be able to discern if CMS renders the laboratory animal more susceptible to the cardiovascular, autonomic, and airway toxicity of a multi-pollutant mixture of primary particles and secondary organic aerosols derived from traffic emissions in the Boston Tunnel. CMS is defined as three or more of the following abnormalities: abdominal obesity; increased blood pressure; elevated blood glucose; and hyperlipidemia. Common coexisting pathologically linked abnormalities include impaired vascular endothelial function, autonomic nervous system imbalance, systemic inflammation, and oxidative stress. Though it represents an emergent phenotype due to interactions among multiple physiological systems, pre-existing genetic propensity, and environmental factors (e.g., obesity, diet, lifestyle), defects in insulin sensitivity among organs/tissues is a fundamental underlying cause. The animal model is established in Sprague-Dawley rats, the species and strain that have been used at our CLARC so that we have considerable data for comparison between this model of susceptibility and normal animals. At the same time, the GLACIER CLARC has found that exposure to concentrated ambient particles plus ozone produces a reduction in heart rate and blood pressure in this model, the opposite of what we have found with our traffic exposures in normal animals. How this model will respond to exposures used at the University of Washington CLARC and EPA laboratories will produce an extensive data set for direct comparison of responses. The GLACIER CLARC will provide the diet and protocol to each collaborating laboratory, as well as provide data from their laboratory for
In summary, this collaborative project will assess the various traffic-related exposures in use in different CLARCs on one susceptible model for which extensive data exists on the normal counterpart of this model. This collaborative project will use similar outcomes in all CLARCs and will use serum samples from a subset of animals for an in vitro study using endothelial cells. This study will add considerable value to individual studies within the CLARCs. For the Harvard CLARC, it provides an opportunity to use not only an important model of susceptibility, but precisely the same model used in another CLARC with which very different responses from our findings in normal animals have been obtained.

**Collaboration 2: Characterization of primary and secondary traffic related particles**

**Lead Harvard Investigator:** Petros Koutrakis

**Collaborating Center:** SCAPE

The objective of this collaborative project is to obtain near real-time chemical characterization of the tunnel primary, secondary, and aged primary plus secondary aerosols generated for exposures in our toxicology study (Project 1). This collaboration will also provide information about the atmosphere inside the photochemical chamber and how the secondary products relate to those found in the atmosphere. To do this study, we are working with Dr. Sally Ng from Georgia Institute of Technology (GIT). She is using an Aerodyne Aerosol Chemical Speciation Monitor (ACSM). For some of the exposure atmospheres, the ACSM will be operated simultaneously with animal exposures. It will provide complementary chemical data such as the near real-time determination of the contribution of aerosol sources (e.g., primary vs. secondary) and extent of oxidation over the course of each experiment. Furthermore, as numerous chamber studies have been conducted in laboratory settings to study SOA formation previously, this collaboration also provides a unique opportunity for linking the chemical properties of laboratory-generated chamber aerosols to ambient aerosols and their consequent health effects. Such information would be invaluable for designing future laboratory chamber studies to mimic the ambient atmosphere with relevant health outcomes.

Researchers from GIT, Dr. Sally Ng and doctoral student, Matthew Kollman, will operate the ACSM at the Harvard tunnel study from July 23rd – August 10th, 2012. Aerodyne Research Inc. will provide the ACSM for this study. The ACSM provides quantitative measurement of non-refractory submicron aerosol composition with a time resolution of 15 to 30 min. The species that are measured by ACSM include organics, nitrate, sulfate, ammonium, and chloride. The ACSM will be operated in continuous sampling mode, providing mass concentration and mass spectra of each of the species. The ACSM will sample three different chamber/exposure atmospheres simultaneously with animal exposures: primary particles (P), primary particles plus secondary organic aerosols (P+SOA), and SOA only. The extent of oxidation of chamber aerosols can be determined from the mass spectra. Specifically, the evolution of O/C over the course of each experiment can be calculated from the fraction of m/z 44 (CO$_2$). Positive Matrix Factorization (PMF) will also be applied to the organics data to de-convolute the organics mass spectra into different factors to provide further insights into the chemical evolution of organic aerosols.
In addition to the ACSM, we will also be collecting information about the gas phase organics during these collaborative studies. While the ACSM is operating, we will collect SUMMA canister samples of each atmosphere (primary, SOA, and P+SOA). Canisters will be analyzed for TO-15 organics by a local commercial laboratory.

**Collaboration 3:** Obesity and diet as sources of susceptibility to pollution: Collaborative insights derived from birth cohort, animal model and controlled human exposure studies

**Lead Harvard Investigator:** Diane Gold

**Collaborating Centers:** GLACIER, EPA

We plan a collaborative project amongst our Project 4, GLACIER, and EPA in-house scientists: to evaluate obesity and diet (high fructose, high caloric, or high fat) as sources of susceptibility to systemic and specific airway inflammatory/oxidative stress responses to PM$_{2.5}$, O$_3$, and multi-pollutant mixtures. Airway responses relevant to asthma or COPD risk that will be measured in Project 4 children will include FeNO, EBC 8-isoprostanes, leukotrienes and counterbalancing lipoxins. Obesity and diet will also be considered as sources of susceptibility to autonomic dysregulation relevant to cardiovascular and pulmonary outcomes. Through initial collaboration with GLACIER, it has already become apparent that their rodent model findings will inform and guide interpretation of birth cohort findings and planning of future work. While our main focus will be on prenatal and early life obesity/diet as a risk factor for adverse responses in children/rodent offspring, we intend to compare obesity/diet as a source of susceptibility across the life stages, in controlled human exposure studies as well as in epidemiologic studies.

**Collaboration 4:** Ambient PM$_{2.5}$ predictions in North Carolina using several spatio-temporal models

**Lead Harvard Investigator:** Petros Koutrakis

**Collaborating Centers:** SCAPE, CCAR

Satellites offer a new resource for air quality management, providing regular observations of air pollutants with wide spatial coverage. Among various satellite retrieved parameters, aerosol optical depth (AOD) has been used extensively in estimating ground level PM2.5 concentrations. This collaboration will evaluate the performance of various satellite-driven PM2.5 exposure models as well as CMAQ PM$_{2.5}$ in North Carolina. Details about this study will be discussed in future meetings among the collaborating investigators.

**Collaboration 5:** Cluster analysis: a multi-pollutant approach in environmental epidemiology

**Lead Harvard Investigator:** Antonella Zanobetti

**Collaborating Centers:** TBD

We are proposing to collaborate with the other CLARC centers on the application of cluster analysis in environmental epidemiology. Developing a multi-pollutant approach is extremely challenging due to the
highly complex interactions between source emissions, atmospheric processes and effects on human health and ecosystems.

Cluster analysis is a novel method that allows to group days with similar chemical profiles in order to identify days with common physico-chemical properties and meteorological conditions that can then be separately described and investigated. The collaboration would include first applying different statistical techniques to define the clusters; then the clusters will be applied to epidemiological data.

The identified clusters, which define different pollutant profiles, can be used in health effects studies of PM$_{2.5}$ mass; the acute health effects observed on different groupings of days will identify the mixtures posing higher health risks. This approach will permit identification of toxic multi-pollutant mixtures, thus enhancing our understanding of pollutant combinations that pose higher risks to public health. In the near future we will start contacting the investigators from other CLARC centers to propose an outline for this collaboration.
2012 ANNUAL REPORT

Date of Report: July 31, 2012
EPA Agreement Number: EPA-RC2009-STAR-C1
Center Name & Internal Number: Harvard University Clean Air Research Center: Air Pollution Mixtures: Health Effects Across Life Stages. EPA grant number 83479801.
Project Title: Relative Toxicity of Air Pollution Mixtures
Investigator(s): (PI) John Godleski
(Co-PI) Petros Koutrakis
Joy Lawrence
Mike Wolfson
Edgar Diaz
Institution(s) of PI(s):
Harvard School of Public Health, Boston, MA

Research Category: Air Quality and Air Toxics
Project Period: August 1, 2011 – July 31, 2012

Objective of Research: Project 1 is an inhalation toxicological animal exposure study that investigates the relative toxicity of different component concentration combinations of air pollution mixtures. These components include both particles and gases that are emitted directly from sources (primary species) or are formed in the atmosphere through a series of reactions that are predominantly photochemical (secondary species). The project uses source specific emissions as well as ambient air or concentrated ambient particulate and our photochemical chamber technologies to generate realistic mixtures. We are testing the following specific hypotheses: (i) secondary gaseous pollutants formed from the photochemical oxidation of traffic emissions or Boston ambient gases can induce biological responses; (ii) aging Boston concentrated ambient particles (CAPs) or source specific primary particles in the photochemical chamber enhances their toxicity; (iii) toxicological effects of photochemically-aged source emissions, ambient air, or CAPs are exacerbated by co-exposure to ozone and other secondary gases; and (iv) mixture composition and toxicity exhibit inter- and intra-seasonal variability due to changes in source emissions and weather conditions. Toxicity will be assessed in Sprague-Dawley rats by changes in 1) in vivo oxidant response; 2) blood pressure; 3) measures of inflammation; and 4) vascular blood flow/resistance.

Progress Summary/Accomplishments: During the reporting period we have completed three papers which are in press in the Journal Air Quality, Atmosphere, and Health. These papers describe the reaction chamber and exposure system used for study the effects of primary and secondary particles derived from a traffic tunnel on respiratory, cardiovascular and systemic outcomes.

The Toxicological Evaluation of Realistic Emissions of Source Aerosols approach was adapted to investigate the health effects of fresh and aged vehicular particulate (PM) emissions. A ventilation stack of a large urban highway tunnel in the northeastern US was used as the source of primary vehicular emissions. The system used to prepare aerosols for inhalation toxicology studies consists of: 1) a sampling system to extract the effluent from the plenum of the ventilation stack; 2) a photochemical chamber to simulate atmospheric aging; and 3) a countercurrent parallel-plate membrane diffusion denuder that efficiently and non-selectively removes gaseous pollutants. To form SOA, direct plenum primary vehicular emissions were diluted with clean air inside the chamber. O₃ was added to titrate the baseline NO in the chamber to enhance photochemical reactions. Our methods made possible to generate reproducible exposures.

These investigations examine the relative toxicity of these air pollution mixtures, focusing on source-specific emissions, secondary particles derived from these, and combinations of primary and secondary
particles from traffic derived particles. All exposures were done at a target dose of 50 micrograms per cubic meter. Sprague Dawley rats were repeatedly exposed to primary only (P), secondary organic aerosol only (SOA), a combination of the two (P+SOA) or filtered air for 5-hours/day for 3 weeks. BP was measured continuously using implanted telemetry. Mixed effects models were used to compare responses of systolic (SBP), diastolic blood pressure (DBP) and heart rate (HR).

Exposure to P increased and maintained an effect on SBP and DBP across weeks showing a significant dose response. SOA exposure on the first day resulted in a significant increase in both SBP and DBP (p=0.001, p=0.0003) becoming strongly negative by week 3. With P+SOA, significant increases in SBP and DBP of 13 mmHg were observed on the first day of exposure (p=0.04, p=0.0015) which was maintained through week 1 for SBP and through week 2 for DBP. Sham exposures in the SOA and P+SOA groups after 3 weeks showed compensatory decreases in both SBP and DBP. No exposure had a significant effect on HR. Both primary and secondary traffic derived aerosols can substantially increase SBP and DBP but these increases are eventually lost.

Effects resulting from these low levels of exposure are remarkable, since humans may be acutely exposed to such levels near highways and are likely to be exposed to higher concentrations for longer durations over a lifetime. Significant changes in blood pressure are demonstrated here as a result of exposure to relatively small yet stable concentrations of pollutants. Sustained increases in blood pressure were observed and were able to be maintained not only across entire days of exposure, but over weeks of exposure. In addition, an unexpected but highly significant compensatory response causing a dramatic decrease in both systolic and diastolic blood pressures was also seen after previously exposed animals were exposed to filtered air. This suggests a biological protective effect with repeated exposures that cannot be explained by simple autonomic nervous system activation. These results confirm the adverse health effects associated with inhalation of fine particles and give insight into a potential biological adaptation to repeated and anticipated pollutant exposure to maintain blood pressure.

We also examined how exposure to primary and secondary traffic-related particles affect a number of blood pressure control mechanism including DNA methylation of the promoter of endothelial nitric oxide synthase (eNOS), a gene implicated in vasoconstriction. Overall trends indicate a decrease in DNA methylation with exposure. Although this is a cross-sectional sample, the decrease in DNA methylation in the eNOS promoter suggests activation of the gene. This may result in a more rapid vasodilatory effect that supports the development of a compensatory response. Manuscripts from this study are currently in preparation.

The studies of respiratory outcomes, including breathing pattern, in-vivo chemi-luminescence (IVCL), broncho-alveolar lavage, and complete blood count, were equally different among the three exposures. Although all produced decreases in tidal/minute volumes and inspiratory/expiratory flows, there were differences in inflammatory changes in BAL, with increased neutrophils for SOA and for P+SOA and increased lymphocytes for P and P+SOA, without changes in total protein, β-NAG or IVCL.

Both Primary and secondary particles derived from the tunnel resulted in adverse respiratory, inflammatory and cardiovascular responses. A pattern of adaptation to the exposure was found with sub-chronic exposures. Irradiation of the primary particles and gases enhanced the toxicity of the traffic emissions producing inflammatory changes in BAL, decreasing flows and volumes in breathing pattern and increasing diastolic blood pressure. Respiratory changes were augmented with repeated exposure to the SOA and P+SOA scenarios, suggesting a cumulative effect and a central response to the pollutants manifested by a decrease in tidal volume. P+SOA had a greater overall response than SOA only; suggesting that the particles and the more complex agglomerates formed in this multi-pollutant scenario greatly enhanced the toxicity of primary traffic derived particles.
We are adding a rat model of vascular flow and resistance to our exposures, with progress in the development of the preparation of rats with implanted hardware for these microsphere studies. Specifically, this includes catheterizing the left ventricle for perfusion and the thoracic aorta for sampling, and connecting these catheters to access ports implanted subcutaneously in the posterior intra-scapular area. In this year, we have changed from a thoracic approach to implant the cardiac hardware to an abdominal approach and for the aortic sampling we have changed to an approach using the external carotid. These changes should have less surgical morbidity. In addition, studies were started to investigate the pathophysiological mechanisms by which these exposures resulted in the blood pressure changes observed.

Publications/Presentations:


Future Activities: We will continue with our investigations as described above. Exposures to fresh and photochemically aged source emissions/CAPs, with and without ozone and other secondary gases, will be conducted. Toxicity of exposures will be assessed in rats using a variety of outcomes, as described above including changes in vivo chemiluminescence, blood pressure, inflammation, and vascular flow/resistance. This will make possible to determine the contribution of different components of the exposure mixture to observed biological effects.

Changes/problems/delays in proposed work: There are no substantive changes, problems, or delays in proposed work to report at this time.

Supplemental Keywords: pollution, particles, mixtures, oxidative stress, inflammation, vascular flow, blood pressure, epigenetics, pulmonary inflammation, vehicular emissions, secondary aerosols
Relevant Web Sites: [http://www.hsph.harvard.edu/clarc/]
2012 ANNUAL REPORT

Date of Report: July 31, 2012
Center Name & Internal Number: Harvard University Clean Air Research Center: Air Pollution Mixtures: Health Effects Across Life Stages. EPA grant number 83479801.
Project Title: Cognitive Decline, Cardiovascular Changes, and Biological Aging in Response to Air Pollution

Investigator(s): Joel Schwartz, Murray Mittleman
Institution(s) of PI(s): Harvard School of Public Health, Boston, MA

Research Category: Air Quality and Air Toxics
Project Period: August 1, 2011 – July 31, 2012

Objective of Research: In this Project we investigate the acute and chronic effects of air pollution on cognitive and neurological impairments, systemic inflammation, and vascular dysfunction. We will determine how these effects differ depending on the composition of multi-pollutant mixtures and the source contributions to PM composition. We will then ascertain the level of increased effects in susceptible and vulnerable subpopulations by examining modifying factors of obesity, diabetes, diet, socioeconomic position, and psychosocial stress.

Progress Summary/Accomplishments: We have a number of accomplishments to report.

A key part of this project and of all the cohort studies is development of a spatio-temporal model predicting daily PM$_{2.5}$ concentrations in New England using land use regression terms and satellite remote sensing. We have successfully developed a model and we published a paper describing it (1). Using our novel prediction models for predicting daily PM$_{2.5}$ at the spatial resolution of a 10x10 km grid across New-England we published two papers.

In one (2) we investigated both the long and short term effects of PM$_{2.5}$ exposures on hospital admissions across New-England and we found that chronic exposure to particles is associated with substantially larger increases in hospital admissions than acute exposure and both can be detected simultaneously using our exposure models.

In the other paper (3) we evaluated the relationship between premature birth and birth weight with exposure to PM$_{2.5}$ levels during pregnancy in Massachusetts for a 9-year period (2000-2008), and we found that exposure to PM$_{2.5}$ during the last month of pregnancy contributes to risks for lower birth weight and preterm birth in infants.

We studied (4) the cross-sectional association between DNA methylation in nine inflammatory genes and lung function in the Normative Aging Study cohort of 756 elderly men living in the metropolitan area of Boston. In the published a paper we show that DNA methylation may be part of the biological processes underlying the lung function decline and that IFN$\gamma$ and IL6 may have ambivalent roles through activation of negative feedback.

We published (5) the results of a further follow-up of the Harvard Six City Study where we replicated our previous analysis on the association between PM$_{2.5}$ and mortality; we further examined different time lags, the shape of the concentration-response relationship, changes in the slope of the relation over time, and the impact of time-varying effects for smoking, sex, and education on the results.
We published another paper (6) using data from the Normative Aging Study where we investigated short- and intermediate term air pollution effects on repeated measurements of fibrinogen, C-reactive protein, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), and epigen-env-ironment interactions by DNA methylation of Alu, LINE-1, tissue factor (F3), Toll-like receptor 2 (TLR-2), and ICAM-1. We observed associations of traffic-related pollutants on fibrinogen, and both traffic and secondary particles on C-reactive protein, ICAM-1, and VCAM-1. There was effect modification by DNA methylation status, indicating that epigenetic states can convey susceptibility to air pollution.

We published a paper (7) using our novel approach to within city survival analysis to examine the association of temperature variability and ozone in survival in four cohorts of potentially susceptible individuals, showing that long-term increases in temperature variability may elevate the risk of mortality in different subgroups of susceptible older populations.

We have also published a paper in another elderly cohort in Boston (MOBILIZE) demonstrating an effect of distance to road on cognitive function (8). Finally, we have used our model for long-term exposure to Black Carbon to show that long-term exposure to traffic particles is associated with higher blood pressure (9).

Publications/Presentations:

**Future Activities:** We will continue to work on the activities proposed in our grant application. In addition, we received a grant from NIEHS to investigate epigenomic effects of environmental exposures. This will allow us to continue examining exposure and epigenetic effects in the Normative Aging Study.

**Supplemental Keywords:** Air pollution, ambient particles, multi-pollutants, cognitive effects, vascular function, inflammation, neurological impairment, susceptibility, susceptibility and vulnerability

**Relevant Web Sites:** [http://www.hsph.harvard.edu/clarc/](http://www.hsph.harvard.edu/clarc/)

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.
2012 ANNUAL REPORT

Date of Report: July 31, 2012
EPA Agreement Number: EPA-RC2009-STAR-C1
Center Name & Internal Number: Harvard University Clean Air Research Center: Air Pollution Mixtures: Health Effects Across Life Stages. EPA grant number 83479801.
Project Title: Identifying the cognitive and vascular effects of air pollution sources and mixtures in the Framingham Offspring and Third Generation Cohorts.

Investigator(s): (PI) Murray Mittleman
            (Co-) Joel Schwartz
            Diane Gold

Institution(s) of PI(s):
Harvard School of Public Health, Boston MA

Research Category: Air Quality and Air Toxics

Project Period: August 1, 2011 – July 31, 2012

Objective of Research: Long- and short-term exposures to ambient air pollution are associated with adverse acute and chronic cardiovascular and perhaps cognitive function, but these effects are poorly understood. Using data from the Framingham Offspring and Third Generation Cohorts, well-characterized populations that have not been previously investigated in association with ambient environmental exposures, we will: 1) determine whether long-term exposures to ambient pollutants and mixtures are associated with cognitive impairment and cognitive interference; 2) test whether short- and long-term exposures to pollutants, mixtures and sources are associated with acute and chronic vascular and endothelial function; and 3) consider whether markers of biological susceptibility and vulnerability differentially influence these associations, allowing us to identify subpopulations at increased risk for harmful effects of air pollution.

Progress Summary/Accomplishments: Since funding began, we have worked closely with the Framingham data coordinators to geocode all participants from the Framingham Offspring Cohort Cycles 6-8 (complete) and Third Generation Cycles 1-2. We are working with the exposure assessment team to complete assignment of spatio-temporally resolved residential address-level modeled exposure to particulate matter and black carbon (expected completion September 2012).

In the past year, we have conducted preliminary analyses of the effects of air pollution exposures on vascular outcomes including flow-mediated dilation, flow velocity and blood pressure in the Offspring and Third Generation Cohorts. We also conducted preliminary analyses evaluating the impact of residential distance from an A1 or A2 road on cognitive function as assessed by the Mini-Mental State Exam (MMSE) in the Framingham Offspring Study (n=3,678). Preliminary evidence suggests a non-linear association between distance to major road and lower cognitive function, but associations did not meet nominal statistical significance. Future analyses will incorporate tests of cognitive function from a more comprehensive neuropsychological battery, including measures such as the Trail Making Test, the Weschler Memory Scale (WMS), Weschler Adult Intelligence Scale (WAIS), and the Wide Range Achievement Test (WRAT).

We have also expanded the aims of the Framingham project and received approval to evaluate markers of inflammation, oxidative stress and hemostasis including: CD40 Ligand, CRP, fibrinogen, IL-6, IL-18, isoprostanes (8-epiPGF2a), LpPla2 (mass and activity), MCP-1, MPO, OPG, P-selectin, TNF-receptor II.

While working on exposure assignment for the Framingham cohorts, we have completed related work which builds on the aims of our project. We evaluated the association between short-term meteorological
exposures on biomarkers of inflammation, endothelial function, and heart failure control in a cohort of 100 patients with Class II and III heart failure. In this study we found that higher two-day moving average of apparent temperature was associated with elevated levels of BNP. Finally, CRP followed a similar pattern after three day moving average.

In another study, followed a cohort of 3,886 individuals hospitalized for AMI in 64 centers across the United States from 1989 to 1996. Institutionalized patients, those providing only post office boxes, and those whose addresses could not be geocoded were excluded, leaving 3,547 patients eligible for analysis. Addresses were geocoded, and distance to the nearest major roadway was assigned. Cox regression was used to calculate hazard ratios, with adjustment for personal characteristics (age, sex, race, education, marital status, distance to nearest acute care hospital), clinical characteristics (smoking, body mass index, comorbidities, medications), and neighborhood-level characteristics derived from U.S. Census block group data (household income, education, urbanicity). There were 1,071 deaths after 10 years of follow-up. In the fully adjusted model, compared with living >1,000 m, hazard ratios (95% confidence interval) for living ≤100 m were 1.27 (1.01-1.60), for 100 to ≤200 m were 1.19 (0.93-1.60), and for 200 to ≤1,000 m were 1.13 (0.99-1.30) (P(trend)=0.016).

We completed analysis of our ongoing study of acute effects of PM$_{2.5}$ and black carbon, measured at our Boston supersite, in addition to temporal-spatially resolved residential modeled exposure to black carbon on acute ischemic stroke onset. We reviewed the medical records of 1,705 Boston area patients hospitalized with neurologist-confirmed ischemic stroke and abstracted data on the time of symptom onset and clinical characteristics. The estimated odds ratio (OR) of ischemic stroke onset was 1.34 (95% CI, 1.13-1.58) (P < 0.001) following a 24-hour period classified as moderate (PM$_{2.5}$ 15-40 μg/m$^3$) by the U.S. Environmental Protection Agency's (EPA) Air Quality Index compared with a 24-hour period classified as good (≤15 μg/m$^3$). Considering PM$_{2.5}$ levels as a continuous variable, we found the estimated odds ratio of ischemic stroke onset to be 1.11 (95% CI, 1.03-1.20) (P = 0.006) per interquartile range increase in PM$_{2.5}$ levels (6.4 μg/m$^3$). The increase in risk was greatest within 12 to 14 hours of exposure to PM$_{2.5}$ and was most strongly associated with markers of traffic-related pollution.

We also evaluated the impact of long-term near-roadway exposure on renal dysfunction in a susceptible population. In this study we found that living closer to a major roadway was associated with a significantly lower estimated glomerular filtration rate compared with living farther away. This work is currently under review.

Publications/Presentations:


Future Activities: Future work will include completion of all modeled air pollution data in the Framingham master dataset for Offspring Cycles 6, 7, 8 and Generation 3 Cycles 1 and 2. We will complete analyses and publish manuscripts on vascular outcomes including flow mediated dilation, blood
pressure, flow rates, peripheral arterial tonometry and biomarkers of inflammation, oxidative stress and hemostasis.

We will extend the aims of our project to evaluate chronic effects of long-term exposure on atherosclerosis in the aorta based on MRI data collected on the Framingham Offspring Cohort from 2002–2006. We will also examine sub-clinical and CT based pulmonary outcomes and a more extensive battery of neurocognitive assessments originally proposed.

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.

**Supplemental Keywords:** Air pollution, ambient particles, multi-pollutant mixtures, cognitive function, vascular function, inflammation, susceptibility, vulnerability

**Relevant Web Sites:** [http://www.hsph.harvard.edu/clarc/index.html](http://www.hsph.harvard.edu/clarc/index.html)
Objective of Research: The main aim of this project is to determine the health effects of prenatal and postnatal exposures to individual pollutants, sources, and pollutant mixtures on somatic growth, cardiovascular risk (blood pressure, exercise tolerance) and cognition in children. The strength of chronic and acute effects of individual pollutants will vary by source and mixture, as well as the timing of prenatal and postnatal exposures. Increased vulnerability or susceptibility to pollution effects on these adverse health outcomes will also result from socioeconomic disparities, stress and violence, environmental tobacco smoke, and reduced maternal and child omega-3 fatty acid intake measured in the prenatal as well as postnatal periods.

Progress Summary/Accomplishments: We have geocoded the entire longitudinal address history and have used this to link to longitudinal GIS and census data. With these data we have created a longitudinal data set with: 1) estimated spatially and temporally resolved BC and PM$_{2.5}$; and 2) neighborhood-level SES variables. While addresses before and after birth are known and geocoded, for the purpose of improving estimation of birth outcome health data, the address at birth is currently being verified and geocoded. We have performed initial analyses of the associations of measured and estimated individual pollution and traffic exposures (temporally or spatially and temporally resolved) with the following outcomes: maternal (glucose tolerance, hypertension in pregnancy); birth weight and longitudinal development of adiposity; blood pressure (birth, 6 months, 3 years, 7 years); cognition; respiratory illness in early life; sleep duration; and markers of inflammation. We have evaluated whether socioeconomic exposures confound or modify the relation of pollution to these exposures. We have expanded our research team to include many senior and junior investigators from Harvard Pilgrim; the Department of Population Medicine’s Obesity Prevention Program; Boston Children’s Hospital; Beth Israel Deaconess Medical Center; Harvard Medical School; Massachusetts General Hospital; the Brigham and Women’s Hospital; and the University of Utrecht in the Netherlands. In the coming year, in addition to completing analyses and papers on the relation of pre-birth and postnatal distance to roadway, BC, and PM on our many health outcomes, we will begin to: 1) evaluate effects of pollution sources and mixtures; 2) extend our evaluation of effect modification by family and neighborhood SES, and evaluate modification by nutrition and genes and; 3) conduct pathway analyses incorporating multiple outcomes and exposures.

Our most outstanding findings were highlighted in nine posters presented at our external advisory committee meeting. Elevated traffic exposures in pregnancy predict abnormal glucose tolerance during pregnancy. Closer distance to roadway was associated with increased sleep deprivation. Increased black carbon and urbanicity predicted lower fetal growth; increased traffic density predicted development of adiposity by 6 months of age. Increased residence-level black carbon levels or closer distance to roadway
in pregnancy predicted reduced cognition by age 7, but associations were confounded by SES. Increased black carbon in the last month of pregnancy predicted higher neonatal blood pressure, but increased ozone in that period predicted lower neonatal blood pressure.

Publications/Presentations:


Future Activities: In the coming year, in addition to completing analyses and papers on the relation of prebirth and postnatal distance to roadway, BC, and PM on our many health outcomes, we will: 1) begin to evaluate effects of pollution sources and mixtures; 2) extend our evaluation of effect modification by family and neighborhood SES, and begin evaluation of modification by nutrition and genes; 3) begin pathway analyses incorporating multiple outcomes and exposures.

Supplemental Keywords: Air pollution, child health, pregnancy, growth, blood pressure, cognition, inflammation, environmental justice, vulnerability, susceptibility
Changes/problems/delays in proposed work: There are no substantive changes, problems, or delays in proposed work to report at this time.

Relevant websites:  http://www.hsph.harvard.edu/clarc/
2012 ANNUAL REPORT

Date of Report: July 31, 2012  
EPA Agreement Number: EPA-RC2009-STAR-C1  
Center Name & Internal Number: Harvard University Clean Air Research Center: Air Pollution Mixtures: Health Effects Across Life Stages. EPA grant number 83479801.  
Project Title: A National Study to Assess Susceptibility, Vulnerability, and Effect Modification of Air Pollution Health Risks  
Investigator(s):  
(PI) Francesca Dominici  Harvard School of Public Health, Boston, MA  
(Co-PI) Joel Schwartz  Harvard School of Public Health, Boston, MA  
(Co-PI) Michelle Bell  Yale University, School of Forestry, New Haven, CT  
Antonella Zanobetti  Harvard School of Public Health, Boston, MA  
Research Category: Air Quality and Air Toxics  
Project Period: August 1, 2011 – July 31, 2012

Objective of Research: This National study is aimed at identifying factors that explain the heterogeneity of health risks associated with air pollution exposure. We hypothesize that such factors include medical and social conditions, conditions that modify exposure, and differences in pollution composition that modify exposure toxicity. Moreover, we hypothesize that the relevant factors vary among different health outcomes. Our research will be fully interactive with the other Center projects. Our previous results (e.g., diabetic susceptibility) have guided their analyses, and their results have generated specific hypotheses that we will test. We have 3 objectives. In Aims 1 and 2, we will conduct national studies of short- and long-term exposures to individual pollutants, sources, and mixtures. A main focus of our Center is to study established cohorts (NAS, Framingham, and Viva) in Massachusetts and surrounding states using novel, validated approaches to assess exposure. In Aim 3, we will complement those cohort studies, by establishing a cohort of 2.3 million Medicare enrollees residing in the same region and following its members prospectively for cause-specific hospital admissions and mortality for the period 2000-2014, and also by studying all live births in Eastern MA, geo-coded to exact address and followed for adverse birth outcomes.

Progress Summary/Accomplishments: Project 5 has three aims; progress is reported for each aim.

Aim 1. Develop statistical methods and conduct national studies to estimate mortality and hospitalization risks associated with short-term exposures to individual pollutants, source types and air pollution mixtures.

Temporal changes in cardiovascular disease management in the US as a potential effect modifier in our air pollution studies. In a recent paper (Geographical Disparities in Hospitalized Myocardial Infarction Incidence and Outcomes: Does a Rising Tide Lift All Boats? By Yeh, Normand, Wang, Barr, and Dominici, published in Circ. Cardiovascular Quality and Outcomes) we have estimated temporal and geographical trends in the incidence and treatment of cardiovascular diseases in the Medicare population for the period 1998 to 2008. This is the period where Medicare data Part A is available to us for 100% of the Medicare population. Now the Medicare data has been extended to the year 2009 and 2010. We sought to examine: 1) whether there have been significant regional disparities in MI incidence, treatment and outcomes during this time period; and 2) whether these regional disparities have changed over time. In our study population, which includes nearly 300 million Medicare fee-for-service beneficiary-years, we found: 1) statistically significant declines in the incidence of MI in all U.S. Census Divisions; 2)
statistically significant differences in the incidence of MI between geographic regions at the start of the study period which increased over time between 2000 and 2008; 3) strong evidence of a wide and persistent variation in rates of cardiac catheterization and revascularization after MI across regions. To our knowledge, this is the largest study to date that examines geographic differences of trends in acute MI in the U.S. elderly, and the first that examines geographic differences in MI incidence, procedures and 30-day mortality. As always it will require some serious considerations on how to incorporate the temporal changes in cardiovascular disease management into our models of air pollution exposure (short- and long-term) and mortality and morbidity outcomes. We will continue to investigate this issue in year 3.

**Variable selection for multi-pollutant risk estimation:** We have submitted a manuscript entitled “Stochastic search variable selection in multi-pollutant models: a study of fine particulate matter components in 118 U.S. counties, 2000-2008” Barr CD, Diez, DM, Dou Y, Wang Y, Bell M, Dominici F, Peng RD. In this paper we apply Bayesian stochastic search variable selection (SSVS) to ambient PM$_{2.5}$ components in multi-pollutant models of emergency hospital admissions for cardiovascular disease (CVD) in a population of Medicare enrollees older than 65 in each of 118 U.S. counties, 2000-2008. Our SSVS method allows us to evaluate a large number of possible statistical models in each county (we consider 6 of the most common PM$_{2.5}$ components: sulfate, nitrate, silicon, elemental carbon, organic carbon matter, and sodium ion) to identify, within each county, the combination of PM$_{2.5}$ components most associated with CVD hospital admissions. Complete results and extensive tools for reproducibility are made freely available online at [www.ddiez.com/epissvs](http://www.ddiez.com/epissvs) (login: map, password: Review65). We have also published a paper by Levy et al 2012 (Levy J, Diez D, Dou Y, Barr C, Dominici F. (2012) A Meta-Analysis and Multisite Time-Series Analysis of the Differential Toxicity of Major Fine Particulate Matter Constituents, American Journal of Epidemiology 175 (11): 1091-1099) where we performed a literature review and conducted a multi-site time-series analysis of hospital admissions and exposure to PM$_{2.5}$ constituents (elemental carbon, organic carbon matter, sulfate, and nitrate) in a population of 12 million U.S. Medicare enrollees for the period 2000 to 2008. The literature review illustrated a general lack of multi-constituent models or insight about probabilities of differential impacts per unit concentration change. Consistent with previous results, the multi-site time-series analysis found statistically significant associations between short-term changes in elemental carbon and cardiovascular hospital admissions. Posterior probabilities from multi-constituent models provided evidence that some individual constituents were more toxic than others. Ratios of constituent toxicities, commonly used in risk assessment to describe differential toxicity, were extremely uncertain for all comparisons. These analyses emphasize the subtlety of the statistical techniques and epidemiological studies necessary to inform risk assessments of particle constituents.

**Adjustment for Confounding in time series analyses:** In Wang et. al., 2012 (Wang C, Parmigiani G, Dominici F. (2012) Bayesian effect estimation accounting for adjustment uncertainty Biometrics), we have developed an innovative Bayesian approach to account for the uncertainty in how to adjust for confounders in time series studies.

**Aim 2. Develop statistical methods and conduct national studies to estimate mortality and hospitalization risks associated with long-term exposures to individual pollutants, source types and air pollution mixtures.**

**Long term exposure to PM$_{2.5}$ and Life Expectancy:** In Correia et al (Correia A, Pope CA, Dockery D, Ezzati M, Dominici F The Effect of Air Pollution Control on Life Expectancy in the United States: An Analysis of 545 US counties for the period 2000 to 2007, Epidemiology (under revision) we investigate as whether more recent and slower declines in PM$_{2.5}$ levels continue to improve life expectancy. We assembled a dataset for 545 U.S. counties consisting of yearly county-specific average PM$_{2.5}$, yearly
county-specific life expectancy, and several potentially confounding variables related to both socioeconomic status and demographic characteristics for the years 2000 and 2007. We used regression models to estimate the adjusted effect of reductions in PM$_{2.5}$ on changes in life expectancy for the period 2000 to 2007. We found that reductions in PM$_{2.5}$ were significantly associated with improvements in life expectancy for the period 2000 to 2007. These results demonstrated that air pollution control in the last decade continues to have a positive impact to public health.

Causal inference methods for assessing the public health impact of air quality regulations. In Zigler et al (Zigler C, Dominici F, Wang Y (2012) Estimating Causal Effects of Air Quality Regulations Using Principal Stratification for Spatially-Correlated Multivariate Intermediate Outcomes. Biostatistics, 13: 289-302) we have developed a new approach to evaluate the effect of air quality intervention on ambient levels of air pollution and on health outcomes. We apply our method to examine whether the 1990 Clean Air Act Amendments causally affected Medicare mortality through affecting ambient concentrations of particulate matter and ozone. We also published a commentary in JAMA (Dominici F and Mittleman M China’s Dilemma: Reconciling Economic Growth With Environmental Protection (2012), Journal of American Medical Association; 307(19):2100-2102. doi:10.1001/jama.2012.4601) to discuss the paper by Zhang et al 2012, a study of the relationship between air pollutants and biomarkers of inflammation and thrombosis in 125 medical students, before, during, and after the Olympics. Zhang, et al report substantial reductions in the mean concentration of SO$_2$ (-60%), CO (-48%), NO$_2$ (-43%), EC (-36%), PM$_{2.5}$ (-27%), OC (-22%), and sulfate (-13%). These changes in air quality were accompanied by statistically significant improvements in biomarkers related to platelet adhesion and activation including a 34.0% decrease in sCD62P and a 13.1% decrease in von Willebrand's Factor during the period of the Olympics that returned toward baseline after the air pollution controls were removed.

Aim 3. Conduct two cohort studies in Massachusetts and surrounding states to estimate health risks associated with long-term exposures to individual pollutants, sources, and air pollution mixtures. We are currently developing the data set and conducting exploratory analyses to estimate the long term effects of PM$_{2.5}$ on cardiovascular and lung cancer hospital admissions. In close collaboration with the Exposure Core we have obtained estimates of ambient exposure to PM$_{2.5}$ from satellite data and we are not developing statistical modeling for estimating health effects.

Publications/Presentations:


**Future Activities:**  *Aims 1 and 2:* continue analysis of national short- and long-term exposures to individual pollutants, sources, and mixtures. We are continuing to develop methods for multi-pollutant models (Bobb JF, Dominici F, Peng R “Reduced Bayesian Hierarchical Models: Estimating Health Effects of Simultaneous Exposure to Multiple Pollutants” *Journal of the Royal Statistical Society, Series C* (under revision) and we are extending multi-pollutant analyses of metals.  *Aim 3:* continue development and analysis of a cohort of Medicare enrollees in Massachusetts and surrounding states for cause-specific hospital admissions and mortality; studying all live births in Eastern MA, geo-coded to exact address and followed for adverse birth outcomes.

**Supplemental Keywords:** National studies, air pollution, heterogeneity, vulnerability, susceptibility

**Changes/problems/delays in proposed work:** There are no substantive changes, problems, or delays in proposed work to report at this time.

**Relevant web sites:** [http://www.ddiez.com/epissvs/](http://www.ddiez.com/epissvs/)  
Login: Map, password: Review 65
Quality Assurance and Human Subjects

There are no quality assurance issues that will affect data quality to report. The Quality Management Program is in place. Audits are conducted on an annual basis. The next scheduled audits are for the fall of 2012. Emphasis on these audits will be on documentation and archiving of statistical analysis. On human subjects Project 1 is an animal project, Projects 2 and 3 were determined to be exempt. Project 4 has an Institutional Authorization Agreement with Partners Health Care. Partners IRB reviewed the project on 02/08/2012 and it was approved until 02/07/2013. Project 5 is exempt except for a small study pertaining to births in Massachusetts. This project was reviewed by the HSPH IRB of Feb 14, 2012 and is valid until Feb 13, 2012. Copies of these two approvals for Projects 4 and 5 are attached to the end of this report.
Notification of IRB Review: Continuing Review

February 2, 2012

Joel Schwartz, PhD
Harvard School of Public Health
Epidemiology
Landmark Center, Room 415
Boston, MA 02115

Protocol Title: Traffic related air pollution and adverse birth outcomes and adult mortality in eastern Massachusetts
Protocol Number: 20222-102
Sponsor: EPA
IRB Review Date: February 2, 2012
Effective Date: February 14, 2012
Expiration Date: February 13, 2013
IRB Review Type: Expedited
IRB Review Action: Approved

Dear Dr. Schwartz:

On February 2, 2012, the IRB approved your Continuing Review Application for the above-referenced protocol. Please note that the approval for this protocol will lapse on February 13, 2013.

This approval includes the following:
- Protocol Summary, version 1/14/12
- Study Personnel Form, 12/22/10

The IRB made the following determinations:
- Research Information Security Level, The research is classified, using Harvard’s Data Security Policy, as Level 2 Data.

If you have any questions, please contact me at alanders@hsph.harvard.edu.

Sincerely,

Andrea Landers, CIP
IRB Review Specialist

cc: Jose Vallarino
Continuing Review: Notification of IRB Approval/Activation

Protocol #: 2005-P-001418/9; BWH

Date: 02/08/2011

To: Augusto Litonjua, MD, MPH
   Medicine
   181 Longwood MCP-4

From: Deena G Segal
   PHS Research Management
   116 Huntington Ave Suite 1002

Title of Protocol: Effects of Prenatal Diet on Mother and Child (Project VIVA) Ancillary Genetics Study

Version/Number: 1 R01 HL/HD075504 PI Gillman, M.W. (Harvard Pilgrim Health Care)

Version Date: 07/12/2005

Sponsor/Funding Support: Harvard Pilgrim Health Care

IRB Continuing Review #: 6

IRB Review Type: Expedited

Minimal Risk: 45 CFR46.110 and 21 CFR56.110

Expedited Category/ies: (5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).

IRB Approval Date: 02/07/2011

Approval Effective Date: 02/08/2011

IRB Expiration Date: 02/07/2012

This Project has been reviewed and approved by the BWH IRB. During the review of this Project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for securing and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

NOTES: The following document has been reviewed and approved by the IRB:
- Continuing Review for Secondary Use of Samples/Data
- Use of Samples/Data ongoing

As Principal Investigator you are responsible for the following:

1. Submission in writing of any and all changes to this project (e.g., protocol, recruitment materials, consent form, study completion, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB.

2. Submission in writing of any and all adverse event(s) that occur during the course of this project in accordance with the IRB’s policy on adverse event reporting.
3. Submission in writing of any and all unanticipated problems involving risks to subjects or others.
4. Use of only IRB approved copies of the consent form(s), questionnaire(s), letter(s), advertisement(s), etc. in your research. Do not use expired consent forms.
5. Informing all physicians listed on the project of changes, adverse events, and unanticipated problems.

The IRB can and will terminate projects that are not in compliance with these requirements. Direct questions, correspondence and forms (e.g., continuing reviews, amendments, adverse events, safety reports) to Deena G Segal, (617) 424-4114.

cc:
Leslie A Moser, MPH, Harvard School of Public Health 1552 Tremont Street
Dean H. Katica, Medicine, 181 Longwood Ave.