Community Assessment of Freeway Exposure and Health (CAFEH) Study  
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PROTOCOL  

I. Aims and Hypotheses  

Specific aim 1) to assess the association between exposure to air pollutants emanating from highway traffic and cardiac health in communities that about highways. We will compare measurements of ultrafine particulates (UFP) and other pollutants with measures of health including C-reactive protein (CRP) a measure of systemic inflammation in adults. We will measure changes in air pollution levels and health impacts as a function of distance from highways in six communities in Boston, a city with 2 major highways.  

Specific Aim 2) to investigate the community and cultural understanding of the health effects of air pollution among people living in the neighborhoods in our study that are adjacent to major highways. From this we will develop and field test culturally appropriate educational modules that raise awareness of risks and countermeasures.  

H1: That CRP levels in blood of adults age 40+ (N=390; mean of 4 blood draws/person) will be statistically higher with higher exposure to near highway pollutants, particularly UFP.  

H2: That cultural proclivities previously shown to be predictive of risk perception from environmental hazards will vary among ethnic groups in the study population; and that this variance will be associated with varying perceptions of risk from near highway pollution.  

II. Background  

Substantial evidence suggests that ambient fine particulate pollution (PM2.5) is associated with adverse cardiovascular health outcomes, including mortality. These studies usually assess exposure for geographic areas that range from a municipality to several subsections of a municipality, sometimes interpolating exposure between ambient air monitoring stations. Additional evidence suggests that measures of “near source” exposure to air pollution, most notably nitrogen dioxide and proximity to highways or high traffic density, increase risk of adverse health outcomes.  

From another perspective, environmental exposure studies have demonstrated that there are steep gradients of pollution (often exponential decay with distance) next to heavily trafficked highways. The pollutants that have the sharpest gradients include ultrafine particulates (particle number count; UFP), carbon monoxide (CO), and nitric oxide (NO). PM2.5 level is influenced only slightly in the vicinity of highways while nitrogen dioxide has an intermediate profile, with a gradient that is less pronounced than UFP, CO or NO.  

Toxicology studies support the notion that UFP are particularly toxic for a given mass compared to PM2.5 and that UFP effectively penetrate deep into the lungs. The increased toxicity appears to be because of the “freshly generated” nature of UFP and their large surface area which carries
high levels of available polyaromatic hydrocarbons (PAH) into the lungs. Additionally, there is some evidence that UFP and PAHs (but not intact PM2.5) are able to cross into the systemic circulation and travel to other parts of the body.

Currently, three mechanistic pathways have been proposed for PM to affect cardiovascular health. One entails generating inflammatory and pro-oxidative signals within the lung that then travel to the heart and circulatory system. A second involves stimulation of the autonomic nervous system from within the lung that, in turn, would affect heart rate variability and other nervous system driven responses. A third involves UFP and/or PAHs traveling from the lungs to the heart and circulatory system in the blood and having a direct effect.

Our study seeks to measure near highway pollution gradients and markers of cardiovascular disease in people living in neighborhoods near highways in Boston and Somerville, Massachusetts and test associations between exposure (UFP and other pollutants) and health measures (specifically, C-reactive protein, other markers of inflammation (possibly fibrinogen, a final decision to be made at a later date), lipid profile, blood pressure, ankle brachial artery test [uses a blood pressure cuff] and diagnosis of cardiovascular diseases).

III. Research Plan

CAFEH is a community-based participatory research project that has full participation of the community partners in all aspects of the science, including developing the proposal, leading the study, collecting, analyzing and interpreting the data. Our plan is to work in three neighborhoods in succession, each for one year. The target neighborhoods, in order, are Somerville, Boston Chinatown and South Boston, although the South Boston neighborhood is not yet certain. We have community partners based in or with strong ties to each of these communities.

Here we submit for approval to work in Somerville only. We will submit amendments to the application prior to the start of work in each subsequent neighborhood.

Scientifically, the study is an observational cross sectional approach with in depth exposure monitoring for the study area and two levels of personal data collection:

1) In year one of data collection we will survey a random sample of addresses in each of three boundaries (within 100 meters, 100-400 meters and >1000 meters) set relative to I-93 in Somerville (we may employ different boundaries in subsequent neighborhoods). The questionnaire (attached) will ask about:
   a. Demographics (age, sex, etc.)
   b. Time activity
   c. Aspects of their house (windows open, smoking, etc.)
   d. Aspects of their job (especially exposure to combustion products/dusts)
   e. Noise exposure
   f. Smoking (including direct smoking and second hand exposure)
   g. Diet (brief)
   h. Physical activity
   i. Psychosocial stress
j. Cardiovascular health conditions with which they have been diagnosed

2) Survey respondents will be invited to participate in collection of biological samples/monitoring. We will collect:
   a) Supplemental questions (see attached) on:
      a) Illnesses in the past week
      b) Use of medication in the past week
      c) Major life events in the past month
      d) Experience with discrimination
      e) Exposure to smoke and dust in the past week
      f) Diet
      g) Alcohol consumption
   b) Height and weight
   c) Blood pressure
   d) Ankle brachial artery measurement (using a blood pressure cuff)
   e) A finger prick (for real-time lipid profile)
   f) A blood sample for subsequent laboratory analysis

Random Sample

We have enumerated all of the addresses in the Somerville study area (and will do the same for each subsequent neighborhood). We are in the process of geocoding all addresses. Using the geographic coordinates, we will place addresses into three groups: 1) within 100 m of I-93; 2) between 100 and 400 m from I-93; and 3) in a “background” site > 1000 m from any highway. One third of our random sample for Somerville will be drawn from each of these geographic areas.

We assume that 50% of households have a person over 40 years of age (33% of people in the Somerville neighborhoods are over 40). This almost certainly means that a higher percentage of households have someone over 40 (see map of over 40 by census block below with hatched area being a 400 m buffer on I-93). With some failure to reach a person at home, ineligible household and refusals to complete the survey, we estimate that we need a random sample of 600 addresses to obtain about 250 completed surveys with 150 of these persons also giving blood.

Samples of comparable size will be generated for each subsequent neighborhood in a similar fashion.

Our maximum sample size participants completing surveys is: 1,000

Our maximum sample size for blood draws, blood pressure and other biological data is: 600

Study area:

The map on the next page shows the study area next to I-93 in Somerville. The green area is the 400 meter buffer around the highway. The area within the Somerville City Limit that is within
400 meters to the highway will be the study area for “exposed” participants. We will define geographic boundaries for Chinatown and the third neighborhood (tentatively identified as South Boston) prior to starting work in those neighborhoods in subsequent years and we will include this information in an amendment to the IRB an advance of recruitment in these neighborhoods. The boundaries will be determined partly based on pilot air pollution monitoring that we will conduct.

We will draw our background sample from the Somerville population who are both more than 400 meters from major highways, and more than 100 meters from other significant Somerville transportation emissions sources – the three diesel rail corridors, local collector streets and the boundaries of local shopping districts. Local collector streets are a roadway class defined by the regional transportation planners. Local shopping districts are areas with visible contiguous retail and retail parking areas, whether organized as linear main streets or more compact shopping areas.
IRB training and partner agreements

All will complete the mandatory IRB training prior to administering surveys for the study. **NOTE** that because we will hire community-based individuals, some of whom may find the CITI site and the required reading difficult (partly because of English language capacity), we will have two routes for completing the training:

1) The normal method of taking the training individually without assistance
2) A modified training, which will entail the field staff person taking the training with one of our staff, for example, the project manager, who will help them understand content of the readings.

Our community partners, the Chinese Progressive Association, the Chinatown Resident Association, the Somerville Transportation Equity Partnership and the Committee for Boston Public Housing have all signed agreements with the Tufts IRB covering this research project.

Recruitment

We will hire and train a team of surveyors from the community, including members of our community partners. These field staff will receive a 20 hour training course that we have developed. They will be followed closely in the field by the project manager at the start of their work and then supervised closely thereafter. Before recruiting from the study sample, each surveyor will recruit from an adjacent neighborhood for a half day in order to get experience.

In advance of approaching addresses on our sample we will launch attempts to prepare potential participants for being recruited. We will issue a press release to local community media outlets (attached) and run a public service announcement (attached) on the local public access TV station. We will also drop a flier (attached) at the door of each address in our sample in advance of actually knocking on the doors.

Addresses from the random sample will be approached at multiple times of the day and days of the week in attempts to reach someone at home. We will eliminate any addresses that do not have a resident person age 40 or older from further consideration.

Inclusion criteria for taking the survey are:

1) Age 40 or older because we expect to see indicators of cardiovascular disease more readily in older individuals.
2) Living at a residence in the study area that is on our random sample
3) Signing the ICF

Field staff will be taught to make a determination as to whether the person that they are dealing with is capable of giving informed consent. They will be instructed to be alert for individuals who seem to not understand the basic concept of the survey and consent process and to avoid starting the consent process or to terminate the interview once they figure out that they are dealing with someone of, for example, very low intelligence.
Participants in the blood draw must first complete the survey. In addition, we will enforce the following exclusions because they may interfere with seeing markers of inflammation associated with air pollution exposure:

1) Collagen vascular disease (including rheumatoid arthritis, lupus, scleroderma), irritable bowel disease, Crohn’s disease or on high doses of anti-inflammatory medications including steroids
2) Patients with medical history of heart attack or stroke (with history of admission to hospital for these illnesses)
3) Severely ill patients with kidney disease or liver disease
4) Patients on chemotherapy for cancer
5) Hemophiliacs and those with burns/wounds/scars etc on arms and those who had chemotherapy in last 4 weeks.

Transportation:

We will contract with SCM, a transportation company based in Somerville (http://www.scmtransportation.org/), to provide rides to subjects who either do not have a car or are unable to drive.

Compensation

Gift cards to specific stores (grocery or department) will be provided for compensation ($20 at visit 1 and $25 at both visit 2 and 3).

Withdrawal/Termination Criteria

This is basically a cross sectional study, however, the individual participants in the study who give blood and other biological measures will be seen three times. These individuals will take the core survey at home and then come twice to give blood and have other non-invasive measures taken. They may be excluded from blood draws based on the exclusion criteria above.

Participation in other research studies

Participants in our study are free to participate in any other research studies they choose and we will not seek information on this point.

Languages/English proficiency

Ultimately, we plan to conduct surveys in English, Spanish, Portuguese, Cantonese, and Haitian Creole. First, we are submitting this application in English to the IRB. Once the documents are approved in English, we will do the Spanish and Portuguese translation and back-translation (the most common non-English languages spoken in Somerville) in time to get approval before we go into the field.
Next, after we safely have those three languages under control and we are ready to get into the field – and depending on how things go, we accept that we may already be in the field – we will work on Haitian and Chinese versions and submit these as a further amendment to the IRB. Should we encounter Haitian or Chinese speaking respondents before we have those versions ready, we will keep them in the sample, but defer them.

For other preferred languages, we will use a quick screen for English ability which will entail asking about activities they did yesterday (an informal version of the time activity part of the survey that is relatively complex) and see whether the person can converse effectively about this. If not, we will try to arrange to return with an interpreter (Note that for languages for which we have capability, English ability is normally beside the point. We will ask people what language they prefer to be interviewed in, and that’s what we’ll do.)

For people who cannot speak any of the five languages well enough to do an interview, we will try to contract with a professional interpreter – possibly including interpreters from the service at Cambridge Health Alliance. For analysis and reporting, we will note that the reliability of the interviews in these languages is not guaranteed. We will conduct analyses with and without them to see whether their inclusion makes any difference.

Biological samples/monitoring

Persons completing the survey will be invited to participate in the biological sampling/monitoring part of the study. Those who accept will be scheduled to attend sessions at a central location in the community and will be given an instruction sheet to help them prepare and arrive at the correct location and time (appended). They will complete a second consent form for the biological part of the study and, upon arrival at the location, fill out the set of supplemental questions (see above and attached).

We will collect biological data at the Mystic Activity Center at 30 Memorial Road in Somerville, a location within our 400 m study area that has parking and ample space for 3-5 stations, privacy screens, bathrooms, chairs and tables and the ability to store some materials on site (we will remove and restore hazardous items (needles) and valuable items (equipment).

- **Blood Draw**: We will take blood from a vein in the arm during each study visits. About 5 cc (or 1 teaspoon) will be drawn. We will use this blood to check the level of CRP or C-reactive protein and other markers of inflammation as well as levels of cholesterol and fats. Should the participant agree, we will draw an additional 5 cc (about 1 teaspoon) to store and be able to add tests for other things, including genetic information that might affect their response to air pollution. If they sign the optional consent a total of 10 ccc (about 2 teaspoons) will be drawn at each blood draw visit.

- **Blood pressure**: we will perform blood pressure measurements during the visit. Before taking the BP we will make sure that participants sit quietly for 5 minutes, and if the reading is elevated, give them another 5 minutes and take it again. This measurement will be taken using appropriate sized cuffs that will be wrapped around the left arm. In addition, another blood pressure measurement will be performed on the ankle. We will do this to compare the
difference in blood pressure between the arm and ankle. Blood pressure readings will be provided to the participant right away.

- **Finger prick** blood will be collected to provide a real time measure of blood lipids.

### Reporting findings to participants:

Only the blood lipid values from the finger prick and blood pressure will be reported to the study participants. This is only a screening value that will be given to them with standard educational materials (appended).

There is no emergency level of cholesterol, so we can simply hand participants the fact sheet and if their total cholesterol is above 200 we will advise them to talk with their physician if they have not done so already.

For blood pressure, values up to 140/90 are considered normal (although it’s desirable to have it a bit lower, i.e. 120/85). For people with values from 140/90 to 160/95, we will advise people to talk to their doctor about their blood pressure (if they have not done so already). From 160/95 to 200/100, we will advise people to see a doctor soon, i.e. within a week if possible. If it’s above that, the RN (TBH) will refer the patient to and call an ER.

For the ankle/brachial artery index (ABI), if it’s above 1, there is no concern. For ABI below .9 we will advise people to see their doctor. An ABI of .4-.9 suggests a degree of arterial obstruction often associated with claudication. We will advise to see a doctor soon. An ABI < .4 represents advanced ischemia. Somebody in this condition is likely to be symptomatic and already involved in medical care; but if we have undocumented people or people who for whatever other reason really have no medical care the RN will refer them to an ER and call ahead for them.

### Fate of blood samples

Blood will be drawn into EDTA tubes and labeled with a bar code. Dr. Brugge will retain the key to the coding of samples. Coded samples, without names or identifiers, will be delivered to the laboratory of Dr. Paul Ridker at Brigham and Women’s Hospital. Upon receipt at the laboratory, samples will be logged into the computer by scanning a bar code imprinted on each package and kept chilled until processed. After centrifugation for 20 minutes (2500 rpm, 4 ºC), each plasma sample will be pipetted into ten 2 ml Nunc vials. Buffy coat will be stored in 2 ml tubes and frozen; the red blood cells from the EDTA tubes will also be stored in a 2 ml tube and frozen. The computer printed labels for the vials will be assigned multiple locations across 2 or more separate nitrogen freezers (maintained at -170 ºC) for security. The entire process will be completed within several hours of receipt of the specimens to ensure that samples are frozen within 30-36 hours after venipuncture.

### Laboratory analysis

**Lipoprotein Profile:** The determination of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations will be
assessed by the Ridker laboratory which is certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program.

**C-Reactive Protein (CRP):** The concentration of CRP will be determined using an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from Denka Seiken (Niigata, Japan). In this assay, an antigen-antibody reaction occurs between CRP in the sample and an anti-CRP antibody that has been sensitized to latex particles, and agglutination results. This antigen-antibody complex causes an increase in light scattering, which is detected spectrophotometrically, with the magnitude of the change being proportional to the concentration of CRP in the sample. This assay has a sensitivity of 0.03 mg/L. The day-to-day variabilities of the assay at concentrations of 0.91, 3.07 and 13.38 mg/L are 2.81, 1.61 and 1.1%, respectively.

**Fibrinogen:** The concentration of fibrinogen will be determined using an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from Kamiya Biomedical Co. (Seattle, WA). In this assay, an antigen-antibody reaction occurs between fibrinogen in the sample and an anti-fibrinogen antibody, and agglutination results, forming an insoluble immune complex. The antigen-antibody complex increases light scattering, which is detected spectrophotometrically, with the magnitude of the change being proportional to the concentration of fibrinogen. The day-to-day variabilities of the assay at concentrations of 167.4, 323.6 and 554.1 mg/dL are 0.94, 1.06, and 1.50%, respectively.

**Data control/confidentiality**

Each address in our random sample will be assigned a data control sheet (see attached) and case ID number with which we will track the attempts to recruit at the address and record visible information such as type of housing and proximity and orientation to the highway. Completed questionnaires, ICFs and data control forms will be retained in a locked office in the Department of Public Health and Family Medicine at Tufts University School of Medicine. Data from the forms will be double entered into an electronic database and checked for errors. Addresses and case ID numbers, but not names, will be included in the database which will be on a password coded computer in the same office with the hard copies of documents.

**Risk/Benefit assessment**

There are no direct benefits to participants in the survey and the main risk is breach of confidentiality since some of the information we will ask about is personal information that they might not want shared beyond the research team.

For those participating in the biological samples, there is the benefit of the free cholesterol and blood pressure screening. Again, the main risk is breach of confidentiality, but also there may be some discomfort, bruising, and potential infection associated with the blood collection.

See ICFs for detailed language on risk and benefit.

**Exposure assessment**
Pollutants: The air pollutants we will monitor include UFP (number and size), PM$_{2.5}$ and BC (mass), NO/NO$_2$/NOx, CO$_2$ and CO. These pollutants were selected because they are associated with cardiac and/or pulmonary health effects and are present in highway vehicle exhaust. In addition to these pollutants, we will also make real-time measurements of total particle-bound polycyclic aromatic hydrocarbons (PAH). PAH are of interest because of their toxicity and because they are also commonly found in vehicular exhaust.

Spatial and temporal variation: To capture spatial trends in highway-generated air pollutants, measurements will be made in detail in each neighborhood in our study. It has been observed that pollutant concentrations were highest within 10-50 m of highways and decreased exponentially to background levels by about 300 m. We will make measurements both continuously and in stationary locations in the study areas. Measurements will be made as often as 2-3 times per week, including weekends, throughout the calendar year so as to capture major temporal and seasonal variations in highway pollutant levels. In addition, we will measure wind speed and direction because these will greatly impact highway pollutant levels at the monitoring sites, and we will collect video recordings of highway traffic so that vehicle counts (both number and type) can be performed and related to the air pollutant measurements. We will coordinate monitoring in neighborhoods with blood drawing so as to have an acute exposure measure, after a 48-hour lag time (as indicated by time series studies), to associate with CRP levels.

Outdoor versus indoor air pollution: UFP, BC and NOx, have been shown to penetrate buildings, with interior UFP often around 50% of outside values, however, we will make confirmatory indoor measurements inside a subset of residences. We will measure UFP and NOx indoors. Indoor measurements will be made in residences at roughly the same distances from highways as the outdoor measurements. At least 5 sets of indoor measurements will be collected per neighborhood. Outdoor measurements will be made as near as possible to the time that the indoor measurements are made to enable comparison between indoor and outdoor air pollutant levels. We will collect indoor measurements in rooms and during times of the day when contributions from indoor sources (e.g., cooking activities, furnaces, car garages, water heaters) will not affect measurements.

History of exposure: In the last 20 years, there have been changes in the number and composition of vehicles traveling on Boston highways. There have also been changes in mandated vehicle air pollution control technology as well as the chemistry of vehicle fuels. We will have access to extensive public data from the state and the Massachusetts Planning Organization on vehicle volume and mix on I-93 our primary highway. We will use this to create a timeline indicating significant changes in traffic volumes, fleet composition, air pollution control technology, and fuel chemistry. We will use the timeline to place residents into broad categories of historical exposure based on how long they lived near a highway and distance of their residence from the highway. We will create time periods of exposure (e.g., 0-3 years, 4-8 years and >9 years) and divide each period based on distance of residences from highways (e.g., 0-100 m, 100-200 m, and >200 m). Exposure weighting factors will be assigned to each period depending on whether exposure was likely to be high or low compared to the present. The creation of such exposure categories will exceed the level of detail in most, if not all, previous studies that measured near-highway pollutants, and will allow correlation of our biological measurements with past pollutant levels.

Research vehicle: All of the air monitoring equipment will be housed in a motor vehicle (a converted RV) to facilitate rapid characterization of pollutant gradients in the neighborhoods.

Analysis Plan

Baseline descriptive analyses will be conducted and reported accordingly. In addition, bivariate analyses, multivariate regression models and structured equation modeling (SEM) will be conducted.
Bivariate analyses: Consideration will be made for the four measures of CRP. Non-independence will be adjusted for to obtain robust standard errors for all bivariate analyses. All potential confounders will be systematically compared to CRP, the main outcome and UFP, the main predictor in this analysis, although we will also test other measured pollutants to assure that UFP is the most predictive.

Multivariate analyses: The relationship between UFP the main predictor and CRP will be adjusted for confounders. Effect modifiers of the relationships between UFP and the outcome or between those of any of the significant confounders and the outcome will be sought. For each model, potential confounders and interactions will be added sequentially into the multivariate regression model in a manual stepwise selection process. Special consideration will be made for repeated measures using mixed modeling linear regression.

Structural equation modeling: SEM is a multi-tier regression modeling technique for characterizing explanatory or causal pathways between dependent and independent latent variables that takes into account interactions, non-linearities, correlated independents, measurement error, correlated error terms, and multiple latent independents each measured by multiple indicators. We will employ SEM to assess environmental, behavioral and biological factors associated with heart disease risks in adults. Our preliminary model for heart disease risk will serve as our starting point for the heart disease model and will subsequently build alternative models based on changes suggested by SEM modification indexes.

**Theoretical framework for the latent variables in the heart disease SEM**

<table>
<thead>
<tr>
<th>Role in the SEM</th>
<th>Factor represented by latent variable</th>
<th>Potential indicator variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent latent variable</td>
<td>Heart disease risk</td>
<td>CRP, cholesterol level, saturated fats, unsaturated fats, BMI, smoking history, co-morbidities</td>
</tr>
<tr>
<td>Independent latent variable</td>
<td>Pollution exposure</td>
<td>UFP, NOx, CO, BC, PPAH from highway as modified by time spent, in home, commuting, occupational exposure, other pollution sources</td>
</tr>
<tr>
<td>Independent latent variable</td>
<td>Lifestyle</td>
<td>BMI, exercise levels</td>
</tr>
</tbody>
</table>

The SEM will be conducted for adults (heart disease risk relationship with the environment and exposure to pollutants; note that at a later date we may analyze stored DNA samples and add genetics). Latent variables will be identified and regressed to determine and characterize any relationships between the dependent latent variable and the independent latent variables. Measurements data from the study questionnaire, laboratory results and pollutant measurements will be used as potential indicator variables. The correlation coefficient cut-off for latent variable contribution by indicator variables criteria will be set at r=0.35. Latent variables will be defined as those with Eigen values >1.0 and constituting three or more indicator variables. We anticipate that the model will have one endogenous latent variable (with other latent variables determinants) and two exogenous latent variables (those without latent variable determinants) and their related indicators identified for the heart disease risk SEM will correspond to those shown in the table and figure above.

**Dissemination**

Publications, reports and presentations will present only composite data. That is, there will be no reporting of individual responses or biological values. We will also not report the names or
addresses of any participants. We may publish maps that show the general distribution of homes relative to, for example, the nearest highways. Should we publish maps of this sort, we will do so without local streets and at low enough resolution that individual addresses cannot be discerned.