1. CENTER OBJECTIVES AND STRATEGY

1.1 Objectives: The research agenda of our Center is designed to address four of the six research priorities set by EPA in the solicitation to establish Clean Air Centers. The primary objective is to investigate health effects of air pollution mixtures, sources, and individual pollutants across life stages (prenatal to senescence). To meet this objective we propose 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.

1.2 Strategy: Figure 1 provides the Center framework. Below we present its components.

![Figure 1: Center Framework](image)

1.2.1 Health outcomes and susceptibility/vulnerability: Our research spans from laboratory animals to human cohorts to large populations focusing on similar important health outcomes (Figure 1). Projects using human subjects (2 through 5) will systematically investigate the modifying effects of individual susceptibility (clinical/biologic measures) and individual vulnerability (social milieu). Our three cohorts (Projects 2 to 4) offer a unique opportunity to study cognitive and vascular health effects in different age groups. Cognitive decline in the
elderly, cognitive deficits, behavior/developmental issues in children, and vascular diseases in the elderly and in children can all impose serious burdens to our society. Air pollution’s role needs to be defined.

1.2.2 Exposure Metrics and Methods for Assessing and Generating Exposures: In addition to focusing on common health outcomes and effect modifiers, all human Projects will address a common set of exposure assessment-driven hypotheses. We will investigate the effects of the same six exposure metrics (short- and long-term exposures to individual pollutants, sources and mixtures). First, we will determine effects associated with individual pollutants using either monitoring and satellite data or model estimates. Second, we will examine the effects of individual pollution sources using source contribution estimates derived from source apportionment models. Third, we propose a new paradigm for assessing the health effects of multiple pollutants that directly estimates the joint effects of the entire pollutant mixture. Finally, exposure assessments for the human studies and exposure generation of the animal toxicology study will be performed by the three technical Cores, which will use an array of existing and new methods, presented in Fig 1.

1.2.3 The New England and National Studies: We will investigate the effects of air pollution on populations living in New England and across the US. New England is impacted by several mixture types depending upon prevailing winds and meteorological conditions resulting in pollution levels and composition that exhibit considerable variability. Therefore, our region is especially well suited to perform health effects research and to test new hypotheses for novel outcomes using three prominent cohorts (Projects 2, 3 and 4). In addition, the National study (Project 5) will include thousands of zip codes across the US, which are impacted by different sources and air pollution mixtures, thus providing the opportunity to investigate the health effects of air pollution mixtures and sources beyond those observed in the New England.

1.3 Research Program: Description of the five Center Projects:

1.3.1 Project 1 (Animal Toxicological Study) will investigate the effects of air pollutant mixtures in Boston. Specifically, we will focus on the identification of mixture characteristics that are mostly responsible for their toxic effects, including: individual components, combinations of components, formation processes or source types. These components include both particles and gases that are emitted directly from sources (primary) or are formed in the atmosphere (secondary). We will generate exposures to realistic pollutant mixtures using a novel integration of our ambient particle concentrator and photochemical chamber technologies. We will expose Sprague-Dawley rats to air pollution components in combination and separately. Our exposure plan features three concurrent types of exposures (Sham, Control Exposure and Exposure) in a design that allows us to control for variability in ambient particle composition and also for inter-subject variability in biological response. Toxicity will be assessed by changes in: in vivo oxidant response, blood pressure and inflammation. We will also use a novel assessment of vascular reactivity using fluorescent microspheres to measure vascular flow and resistance in every organ with emphasis on the heart and brain. Results from these studies will inform the other projects as to potential mechanisms of actions that may explain observed findings in human populations.

1.3.2 Project 2 (NAS Study) will build on our previous success using the Normative Aging Study (NAS) cohort, a prospective cohort living in New England. We have studied this cohort of elderly individuals as part of our previous two Centers, and have gained valuable knowledge on air pollution health effects. For this new Center, we propose to investigate pollution effects on: 1) cognitive and neuropsychological function (chronic effects), as measured by the Mini Mental State
Exam (MMSE) and selected tests from other methods (CERAD, WAIS-R and NES2); 2) cardiovascular and endothelial function (acute and chronic effects), as measured by pulse wave analysis and blood pressure; 3) inflammation, endothelial function, and oxidative stress (acute and chronic effects), as reflected in serum biomarkers; and 4) telomere length as a surrogate for biological aging. We will determine how these effects differ depending on the concentrations of individual pollutants, the composition of multi-pollutant mixtures, and types of air pollution sources. Further, we will investigate the modifying effect of measures of susceptibility (clinical/biologic measures) and vulnerability (social milieu), on the previously established relationships between exposures and outcomes. Finally, we will explore the exposure-response relationships for any significant associations to be discovered.

1.3.3 Project 3 (Framingham Study) will investigate the effects of air pollution on cognitive, vascular and endothelial function among middle-aged and older adults enrolled in the Framingham Offspring and Third Generation cohorts. This is a well-known cohort of individuals, many of whom live in New England. We will investigate the effects of long-term exposures on cognitive impairment, as measured by MMSE and the CERAD Word List Memory test, and cognitive interference, as measured by the Victoria Stroop test. In addition, we will investigate the effects of short- and long-term exposures on vascular and endothelial function as manifest by: 1) Decreased brachial artery diameter and flow mediated dilatation; 2) Decreased digital pulse amplitude response to hyperemia; and 3) Increased systolic, diastolic and pulse pressure. Further, we will determine how these effects differ depending on the concentrations of individual pollutants, the composition of multi-pollutant mixtures, and types of sources. Finally, we will examine the modifying effect of measures of susceptibility (gender, diabetes, obesity, chronic obstructive lung disease) and vulnerability (socioeconomic position (SEP), depression).

1.3.4 Project 4 (Viva Study) will investigate the effects of air pollution on somatic growth, blood pressure, cardiovascular fitness, and cognition among children in the Viva cohort. This is a unique ongoing pre-birth cohort study of over 1,300 children from Eastern Massachusetts, with longitudinal repeated health measurements. Families were recruited during the first trimester of pregnancy between 1999 and 2002. We will investigate the effects of pre- and post-natal exposures on: 1) birth weight and longitudinal growth including, weight-for-length z-score and change in weight-for-length (birth through age two) and body-mass index (BMI) z-score and change in BMI (ages two through ten); 2) blood pressure, measured at birth, six months, three years, and seven years; and cardiovascular fitness, assessed by Step Testing at age seven; and 3) cognition, assessed as visual memory at six months, and at three and seven years; language at three and seven years; intelligence at seven years; and behavior at seven years. We will examine whether acute and chronic effects vary by pollutant, source and mixture, and by the timing of pre- and post-natal exposures. Finally, we will examine whether increased vulnerability or susceptibility to pollution effects is caused by socioeconomic disparities, stress and violence, tobacco smoke, and reduced maternal and child omega-3 fatty acid intake.

1.3.5 Project 5 (National Study) will build on the statistical methods we have developed and databases we have acquired to perform large epidemiological studies in the Region and across the Nation. In the National studies we will estimate mortality and hospitalization risks associated with short- and long-term exposures to individual pollutants, source types and pollutant mixtures. We will use the largest available collection of national datasets including: 1) mortality and hospital admissions for hundreds of counties and thousands of zip codes; 2) measures of vulnerability and susceptibility including individual- and area-level information on socio-economic and behavioral factors; and 3) air pollution and weather data from national networks.
The Regional studies will investigate two cohorts and will estimate risks associated with exposures to individual pollutants, sources, mixtures adjusted by individual- and area-level risk factors: 1) we will estimate risks of adverse birth outcomes using approximately 700,000 live births and; 2) we will assess mortality and morbidity risks using 2.3 million Medicare enrollees. Together, these studies will use administrative databases to: 1) identify individual and area-level factors that explain the geographical heterogeneity of the air pollution health risks across the US; 2) investigate effect modification by susceptibility, vulnerability, and air pollution mixtures; and 3) examine the shape of exposure-response relationships.

1.4 Center Participants: The Center will involve researchers from the following institutions i) Harvard School of Public Health and Medical School; ii) Beth Israel and Deaconess Medical Center; iii) Veterans Administration Boston Hospital; and iv) Yale University.

1.5 Existing and Future Center Expertise: We have been involved in the field of air pollution health effects since the 70s when the original Harvard Six Cities study was launched. Over the last decade we are privileged to host one of the five EPA Centers which has enabled us to continue our contributions to this field. As a result of these efforts we have sustained a multi-disciplinary research team which can carry out multi-faceted research programs. As we indicate in the Administrative Core, our team has been very productive and we have published 191 peer reviewed papers as part of our previous and current Center. For the proposed Center we will comprise a team of over twenty Investigators across a large number of disciplines including: Exposure Assessment and Air Pollution (Kang, Koutrakis, Lawrence and Wolfson), Epidemiology (Bell, Gold, Schwartz and Speizer), Cardiovascular (Gillman, Mittleman and Vokonas), Pulmonary Health (Gold and Speizer), Toxicology (Godleski and Diaz), Psychology (Kubzansky), Neurology (Bellinger and Oken) and Biostatistics (Coull, Dominici and Zanobetti). Many of these investigators have been collaborating very closely for more than a decade as part of our previous and current Centers and other Projects. However, several prominent Faculty members have joined our team: Dominici and Bell will conduct the National Study; Mittleman will lead the Framingham Study; Bellinger, Oken and Kubzansky will support the investigation of cognitive and neurotoxic effects; and Gillman will participate in the investigation of effects on blood pressure in children. Importantly, these new members have established collaborations with existing Center investigators. Our field has been evolving rapidly at a fast pace since the inception of the EPA Centers. New evidence suggests that air pollution can have far-reaching effects beyond the lung and heart. As a result, our Center focus has expanded to address cognitive, vascular, and developmental health issues. To meet these needs, the new faculty members from our School and the Medical School listed above have been added to our Center. At the same time, we have outstanding faculty members from our Department who are not included in the Center but are available as needed (e.g. Dockery, Kobzik, Spengler, Brain).

1.6 Center Infrastructure and Effectiveness: We propose an ambitious research agenda which requires many resources. Thus, its realization poses a great challenge, especially, considering the budgetary constraints. However, we are convinced we can pursue this cutting-edge research with the resources available for the following reasons: 1) we will use similar exposure metrics, outcomes and effects modifiers for the different Projects; 2) we will rely on air pollution or health data that we have already collected or to which we will have access and we will obtain the biomedical data sets from the three cohorts using modest resources; 3) we will employ the same exposure assessments and statistical models for all cohorts participants because they live in the same Region; 4) we will use our existing infrastructure such as the HSPH Boston Supersite, sampling devices, statistical models and inhalation facilities; and 5) as always, we will rely on
the high caliber of Harvard doctoral and post-doctoral students, many of whom are supported by
training grants or University resources.

1.7 Center Administration: The Center Director will be Petros Koutrakis, who is the Director of
the existing EPA Center. He is a Professor of Environmental Sciences and the Director of the
Exposure, Epidemiology and Risk (EER) Program at Harvard. He will be assisted by Deputy
Center Director John Godleski, Associate Professor of Pathology at the Harvard Medical School.

1.8 Research Questions: Our Center will address four of the six questions of the RFA.

RQ5) Effects of pollutants and mixtures: Investigation of the effects of individual pollutants,
sources, and pollutant mixtures is the major thrust of our proposed Center. Our animal studies
will use novel exposure generation systems to investigate the toxicity of components or
combinations, including primary and secondary particulate and gaseous pollutants. All human
studies will investigate the effects of pollutants, sources and mixtures. We will use a new
paradigm to determine the effects of mixtures by incorporating temporal and regional clustering
to investigate days with similar pollutant profiles, for the acute effects, or areas with similar
pollutant profiles for chronic effects. Specifically we will: identify mixture compositions which
are more potent; examine the additive or synergistic effect of individual pollutants present in the
mixture and; finally, we will be able to link mixtures to sources and/or atmospheric processes.

RQ3) Sub-populations that are at increased risk: All four human Projects will investigate the
modifying effect of susceptibility and vulnerability. We will examine associations between six
exposures metrics and numerous outcomes in each of the human studies. For these potential
associations we will investigate the modifying effect of gender, diabetes and obesity as well as
socioeconomic disparities, stress, depression, violence, tobacco smoke and omega-3 fatty acid
intake. The distinctive feature of our studies is that they include many new outcomes for which
these effect modifications have not been evaluated. Importantly, our cohort studies will provide a
unique opportunity to study these risk factors simultaneously in children, adults and elderly who
are exposed to the same pollution mixtures. Of paramount importance will be our efforts to
examine the impact of pre- and post-natal exposures. During this critical stage of development
exposures may result in life-long alterations in health. Specifically, our Viva study will examine
growth, cardiovascular function, and cognition through age 10.

RQ1) Regional and temporal differences in air pollution risk: In the National Study we will
investigate mortality and hospitalization risks in hundreds of counties across the US using the
largest available collection datasets. We will investigate whether regional heterogeneity and
temporal variability can be explained by differences in pollutant mixtures. Briefly, all counties
will be grouped based on their average pollutant profiles using spatial clustering. Effects
estimates will be compared among groups of counties rather than contiguous geographic areas.
Subsequently, for selected counties we will perform temporal clustering to group days with
similar pollution mixtures. Effect estimates will be compared across groups of days with similar
pollution characteristics rather than monthly or seasonally. Finally, we will examine individual-
and area-level factors that may explain geographic heterogeneity.

RQ4) Shape of exposure/concentration-response relationships: Our Center will investigate
potential exposure-response associations stemming from the use of multiple exposure metrics,
outcomes and populations. For each of the Projects we will investigate the shapes of these
relationships. Our specific comparisons of such relationships will differentiate among single
pollutants, sources and mixtures. Most importantly, our Center has developed statistical methods
to define exposure-response relationships and these will be applied directly in our studies.