Draft Proposal

Synergistic Cardiovascular Health Effects
between Ozone and Source-Oriented Submicron Fine and Ultrafine Particulate Matter

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Table of Contents

Abstract

Epidemiological studies demonstrate associations between air pollutants, such as PM2.5 and ozone, and cardiovascular morbidity and mortality (e.g., Brook et al., 2002). Yet, few studies have examined the mechanistic pathways that link such pollutant exposures to cardiovascular endpoints. Studies of ozone and PM synergies are also limited. Although pulmonary and cardiovascular risk of ambient PM2.5 appears to be higher in the eastern US than the western US (e.g., Dominici et al., 2006), the risk is sufficiently high across the country to warrant concern. Agencies such as CARB and EPA comply with the National Ambient Air Quality Standards (NAAQS) by controlling emissions. These emissions controls are intended to reduce ambient ozone and PM concentrations to meet NAAQS standards without regard for the relative toxicity of the emissions, because (a) the NAAQS are mass-based, not source- or composition-based and (b) the relative cardiovascular toxicity of sources is not yet available. Recently we developed and implemented a new system that samples source-oriented PM from the atmosphere suitable for toxicological studies. In the current project, we will use 18 source-oriented samples collected in Fresno for a prior CARB-funded project plus 4 source-oriented samples collected in the south and middle bores of the Caldicott tunnel during the evening rush. Normal and immune-sensitized mice will be exposed to these samples with and without co-exposure to ozone. Thrombotic, endothelial, arrhythmic and cardiac dysfunction endpoints will be examined. To further explore the mechanisms underpinning these health effects, cardiac myocytes and endothelia will be isolated from the in vivo models. Single-cell phenotyping using flow cytometric analyses will be performed to directly test the signaling pathways activated by in vivo exposure. To relate these mouse-model findings to human health effects, human induced pluripotent stem cell (hiPSCs)-derived endothelial cells and cardiomyocytes will be exposed in vitro. The results of this study will include insights into the mechanisms linking PM and ozone to cardiovascular health effects, including synergies between PM and ozone and synergies of immune sensitization.

Introduction

Numerous epidemiological studies have associated elevations in ozone and particulate matter concentrations (PM) with increased morbidity and mortality. Early thinking was that these health effects are primarily pulmonary in nature because air pollutants first deposit in the lungs. Starting near the turn of the century, evidence has accumulated that the PM-mortality link may be more closely related to cardiovascular endpoints than pulmonary ones (e.g., Samet et al., 2000; Pope et al., 2004). In a seminal crossover study, Brook and colleagues (2002) found acute vasoconstriction in response to ozone, in addition to PM. Since then, the evidence associating ozone with cardiovascular risk has grown, to now include, for instance, chronic exposure (e.g. Perepu et al., 2012) and blood pressure effects of ozone opposite to that for PM (Hoffmann et al., 2012). Ozone is now implicated in cardiovascular health effects via a range of pathways such as oxidative stress, cytokine-mediated systemic inflammation, modified endothelial function, and heart rate variability (Srebot et al., 2009). It is clear that via multiple pathways, acute and chronic exposure to ozone and PM result in cardiovascular dysfunction.

The National Ambient Air Quality Standards (NAAQS) regulate air pollutants to protect public health. The pollutants regulated in the NAAQS include both ozone and PM due to evidence associating them with increased morbidity and mortality and concurring mechanistic studies in animal models. Ozone is not emitted directly into the atmosphere in any substantial quantities. It is formed in the atmosphere due to photochemical reactions between organic compounds and nitrogen oxides released from both natural and anthropogenic sources. As a result, it is not evident from the measurement of ozone, which sources or combinations of sources are responsible for its concentration and spatial and temporal patterns. PM, in contrast, is emitted directly into the atmosphere and formed from atmospheric photochemical reactions. As a result, individual particles in the atmosphere contain chemical signatures of both their emission sources and photochemical transformations that they undergo in the atmosphere. The resulting complex chemical composition of atmospheric particles like results in different particles eliciting different health effects, but the chemical compositions are also signatures for the sources and transformation of the particles. The current NAAQS regulate PM based on its mass concentration, temporal pattern and size range, but not on its chemical composition or source. Yet it is clear that sources lead to different health effects – some are benign and the toxic ones are toxic in different ways (Wexler and Pinkerton, 2012). We have designed, built and implemented in Fresno the first system for sampling PM from the atmosphere associated with different sources and source combinations (Bein et al., 2009). In this proposal, we will use source-oriented and size-resolved samples already collected in Fresno and new ones collected from the Caldicott tunnel to explore the cardiovascular and vascular health effects of different source of PM common in California, and the mechanisms underlying these adverse health effects.

As is evidenced by the increasing prevalence in California of asthma and other diseases related to the immune system, an increasing fraction of the population appears to be immune-sensitized. Although most urban residents in California and many more in rural areas such as the San Joaquin Valley are exposed to concentrations of ozone and PM that exceed the NAAQS, fortunately only a small percentage die or are hospitalized in response to any given event. Yet some genetic predisposition or pre-existing condition likely causes certain individuals to be susceptible to these inhaled insults. We posit that immune sensitization is one such possible cause of susceptibility, so in addition to a normal mouse model, an ova sensitized mouse model will also be employed in the studies proposed here.

In summary, we propose a suite of studies to explore (1) the vascular and cardiovascular effects of (2) ozone and PM alone and in synergy to (3) a normal and an immune-sensitized mouse model, (4) elucidating the underlying mechanisms linking the air pollution insults to the resulting cardiovascular health effects using (5) source-oriented particles collected in California so that the health effects and associated mechanisms can be related to the sources that emitted the pollutants. Because the study is thorough, the budget is commensurately high, likely too high for CARB to afford in the current budget climate. We are offering this suite of experiments as a starting point for discussion with CARB staff. The goal of these discussions is to determine which experiments are most important for the study considering the budgetary constraints and regulatory goals. The Electric Power Research Institute co-funded

with CARB the original source-oriented toxicology study that focused primarily on pulmonary endpoints. We will submit this proposal to EPRI simultaneous with our submission to CARB, and request co-funding between the two agencies.

The proposing team is led by Anthony Wexler, director of the Air Quality Research Center (AQRC) at UC Davis, who developed the concept of sampling source-oriented PM directly from the atmosphere. Keith Bein, a research scientist in the AQRC, built the source-oriented sampling system, fielded it in Fresno, and delivered PM to toxicologists for their studies. Dr. Bein will deliver extracted PM to Drs. Pinkerton and Chiamvimonvat for their studies. Kent Pinkerton directs the Center for Health and the Environment at UC Davis and was the co-PI on the source-oriented toxicology project. His team will perform the aspirations on the animals, examine endothelial endpoints and work with Dr. Chiamvimonvat on the cell culture ozone and PM exposures. Dr. Chiamvimonvat is a cardiologist at the UC Davis Medical Center. She and her team will examine the cardiovascular endpoints for this study.

This proposal is organized as follows. The next page summarizes the specific aims and approaches for the proposed work. The technical plan is organized around the 5 questions posed in the RFP. Following the technical plan are the references, project schedule, team description and vitae, and the draft budget.

Objectives

Specific Aims

There are numerous epidemiological studies establishing the link between ambient particulate matter (PM) and cardiovascular-related morbidity and mortality (CVMM). There is also support for a link between ozone and CVMM but so far the evidence is not as strong as the PM-CVMM link. Furthermore, the mechanistic links between air pollutant exposure and cardiovascular endpoints are not well established. A number of potential mechanisms have been proposed that include platelet aggregation, endothelial dysfunction, accelerated atherosclerotic lesions, increased circulating inflammatory mediators, oxidative stress, and changes in autonomic function. However, there remain significant gaps in our knowledge. Specifically, it is not known how these multiple factors may ultimately result in endothelial and myocardial dysfunction. The signaling pathways activated remain unknown.

Ambient ozone concentrations vary with time, while PM concentrations are also dependent on particle size and composition. **From a regulatory perspective, the most important characteristics of PM are its sources because the emissions from these sources can be controlled through regulation.** From a mechanistic viewpoint, the sources of PM are also important because source governs composition and composition is likely to play a role in the pathways that lead to CVMM. In addition, PM is not inhaled alone because many locations in California with PM levels that violate the NAAQS, such as Fresno and the SoCAB, also have levels of ozone in violation and sometimes these elevated levels occur at the same time.

For this proposal, we will focus our effort on the endothelial cells and cardiomyocytes, both *in vivo* and *in vitro*. We will expose mice and cell preparations to ozone and/or 22 different source-oriented and size-segregated PM samples collected from the atmosphere in Fresno and the Caldicott tunnel. **We hypothesize that particulate and ozone exposures result in an increase in inflammatory mediators in a source-dependent manner, accelerating endothelial and myocardial dysfunction, and ultimately leading to pathogenesis of myocardial infarction (MI), cardiac dysfunction, and arrhythmias.** To test this hypothesis, the following comprehensive Specific Aims are proposed.

Aim 1. Test the hypothesis that exposure to source-oriented particulate matter, ozone, or the combination will result in source-dependent changes in cardiac function in vivo. **Approach**: Mice will be exposed to source-oriented samples of particulate matter and/or ozone for 3 days. Controls will be exposed to filtered air. Cardiac function will be assessed in vivo using echocardiogram, hemodynamic monitoring and measures of endothelial dysfunction. Increases in arrhythmia susceptibility will be tested using in vivo electrophysiology studies. Serum cytokine profiles will be analyzed. Comprehensive assays including homocysteine, C-reactive protein (CRP), platelet activation markers (fibrinogen, P-selectin), platelet function markers (thrombin and cAMP), fibrinolytic markers (t-PA and PAI-1), heme-oxygenase (HO), endothelin-1 (ET-1), and neutrophil adhesion molecules that are associated with MI risks will be performed in different groups of mice compared to controls.

Aim 2. Test the hypothesis that exposure to source-oriented particulate matter, ozone, or the combination will result in cardiomyocyte and endothelial dysfunction. **Approach**: Single-cell phenotyping using flow cytometric analysis will be performed to assess endothelial cells and cardiomyocytes isolated from mice in Aim 1. Endothelial cell and cardiomyocyte-specific markers will be used. The key signaling pathways (NOS, NF-κB, PI3K, p-ERK1/2, and ERK1/2) in endothelial cells and cardiomyocytes will be tested. Endothelial cell markers of the lungs will similarly be examined.

Aim 3. Test the hypothesis that immune sensitization alters the cardiac response to source-oriented particulate matter, ozone, or the combination. **Approach**: Mice will be sensitized to an allergen (ovalbumen) to create an allergic, hyperinflammatory condition in the lungs. Changes in vivo and in vitro will be tested as described in Aims 1 and 2.

Aim 4. Translate the mouse-model findings from prior Aims to human endpoints. **Approach**: We will use human induced pluripotent stem cells (hiPSCs)-derived endothelial cells and cardiomyocytes to test the effects of exposure *in vitro*. The markers and key signaling pathways identified in Aim 2 will be directly tested in hiPSC-derived endothelial cells and cardiomyocytes *in vitro*.

Technical Plan

The "Cardiovascular Effects of Multipollutant Exposure: Mechanisms and Interactions" RFP requested that each proposal respond to 5 questions. Each of these questions is listed below numbered, underlined and in italics followed by our response to each one.

1. An hypothesized mechanistic pathway for PM2.5- and ozone-induced cardiovascular dysfunction. Submitters must include a detailed description or diagram of the hypothesized pathway, and a literature review supporting the hypothesis. They must also clearly indicate the parts of the mechanistic pathway their proposed study will address.

The central hypothesis to be tested in the proposal is that particulate matter and ozone exposures result in inflammatory mediator alterations in a source-dependent manner, accelerating endothelial and myocardial dysfunction, and ultimately leading to pathogenesis of myocardial infarction (MI), cardiac dysfunction, and arrhythmias (Figure 1). Moreover, we hypothesize that immune sensitization will alter the cardiac response to source-oriented particulate matter, ozone, or the combination. For this proposal, we will focus our effort on the endothelial cells and cardiomyocytes, both *in vivo* and *in vitro*.

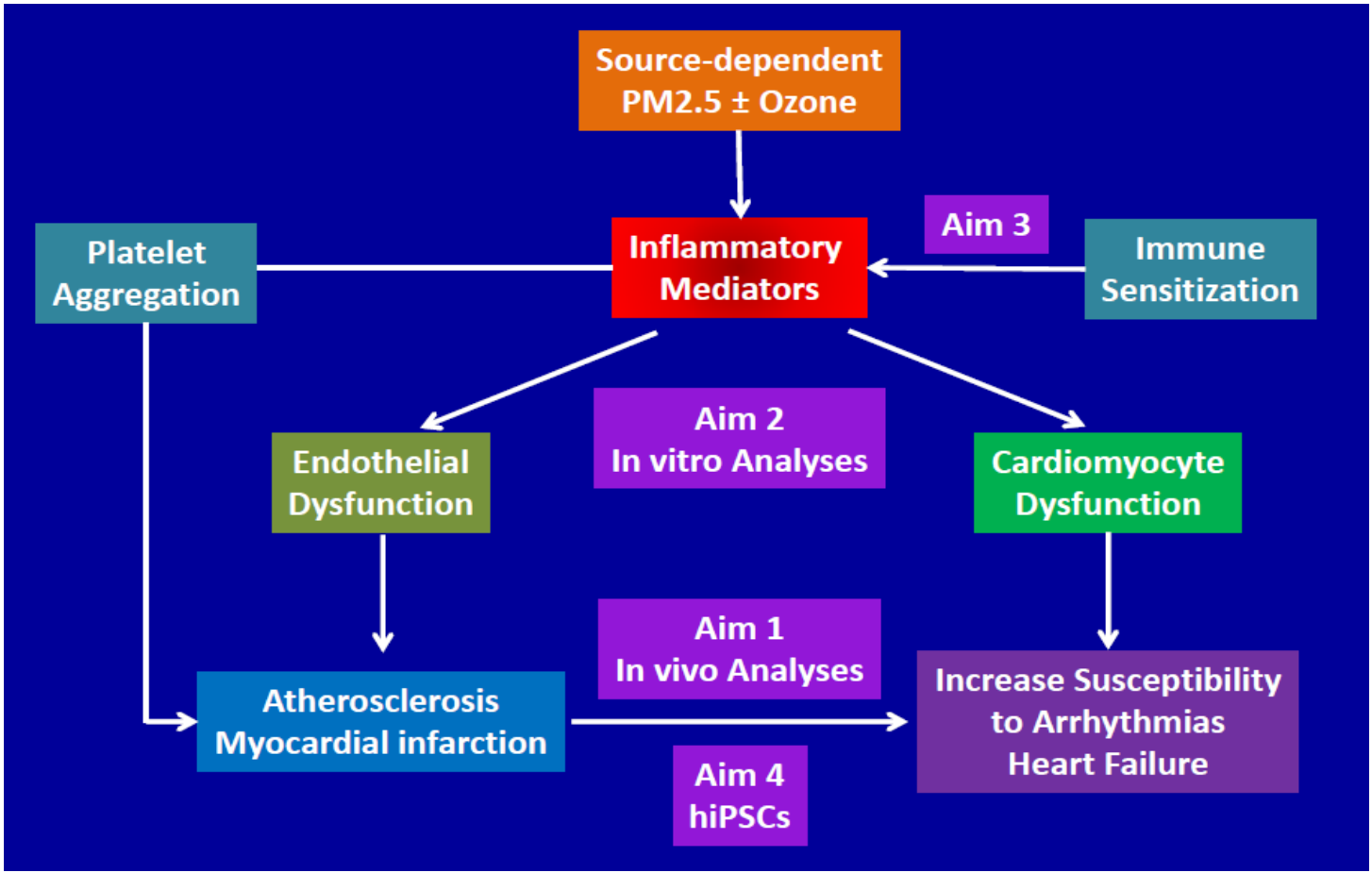

Figure 1. Central Hypothesis and Specific Aims.

The proposed study will utilize unique source-oriented PM samples from the atmosphere suitable for toxicological studies. Moreover, we will use a comprehensive suite of in vivo and vitro analyses, including single-cell phenotyping, to directly quantify the effects of PM and ozone exposure on the endothelial and cardiac myocytes.

2. Description of, and justification for, the proposed animal model(s). This section should also provide the number of groups of animals proposed, and the number of animals in each group.

Background

Growing evidence from epidemiologic (Peters et al., 2004; Pope et al., 2006; Wellenius et al., 2006; Hoffmann et al., 2007, rev. in Brook et al., 2010) and laboratory (Sun et al., 2005; Araujo et al., 2008; Lund et al., 2009; Hazari et al., 2011) studies indicates that exposure to ambient air pollution results in increased risk of myocardial infarction and arrhythmias, progression of cardiovascular disease, and increased morbidity and mortality. These conditions may give rise via exposure from a highly mixed and complex set of criteria air pollutants including gases, vapor and particles.

Factors associated with vascular toxicity, such as endothelin-1 (ET-1), matrix metallopeptidase-9 (MMP-9), and heme oxygenase-1 (HO-1), are involved in the progression of atherosclerosis, which accounts for nearly half of all deaths from cardiovascular disease (Peterson et al., 2005).

Another consequence of cardiovascular change is atherosclerosis, an inflammatory-mediated disease of the vasculature characterized by the presence of atherosclerotic plaques, endothelial dysfunction, and inappropriate vascular remodeling that is mediated by extracellular matrix degradation. The MMP family of endopeptidases is the primary mediator of vascular extracellular matrix degradation, and MMP-9 has specifically been associated with atherosclerotic plaque growth and rupture (Luttun et al., 2004) and acute myocardial infarction (Kai et al., 1998). ET-1 expression is found elevated in atherosclerotic, compared with normal vessels (Ihling et al., 2001). In addition to its potent vasoconstrictive and mitogenic effects, ET-1 likely promotes atherosclerotic plaque growth by its stimulatory activities on neutrophil adhesion and platelet activation (Luscher and Barton, 2000), as well as its ability to stimulate extracellular matrix-degrading MMPs (Ergul et al., 2003). HO-1 is generally expressed at low levels in most tissues under normal basal conditions; however, it is highly upregulated in response to various pathophysiological stresses. HO-1 has potent anti-inflammatory and antioxidative functions, and numerous studies suggest that HO-1 induction is an adaptive defense mechanism to protect cells and tissues against injury in atherosclerosis and other cardiovascular disease states (rev. in Wu et al., 2011).

Study Design (Aims 1 and 3)

Eight- to 10-week old male BALB/c mice, obtained from Harlan Laboratories (Hayward, CA), will be used for the in vivo studies. Animal housing and experiments will be approved by the Institutional Animal Care and Use Committee at University of California, Davis. The entire study will employ 552 mice, as follows:

As described in detail below, 22 Source-Oriented PM samples will be tested on normal and ova-sensitized mice. 6 will aspirate PM and 6 more will be exposed to ozone and aspirate PM for a total of 22 source-oriented samples x 2 (normal+ ova-sensitized) x 12 (6 PM + 6 PM+ozone) = 528 mice. In addition, 6 normal and 6 ova-sensitized mice will breathe fresh air (control) or ozone-only for a total of 12 (6 ova-sensitized + 6 normal) x 2 (fresh air and ozone-only) = 24, giving a grand total of 528+24=552.

We need sufficient PM in each source-oriented sample to conduct this study. Each aspiration uses 50 µg, which will be administered to 6 normal and 6 ova-sensitized mice who will each be exposed to fresh air or ozone during the study, so the total mass needed for each source-oriented sample is 50 µg/dose x 2 (normal+ ova-sensitized) x 12 (6 PM + 6 PM+ozone) = 1.2 mg for the in vivo studies. The in vitro studies need much less: 50 µg per dish for human induced pluripotent stem cells (hiPSCs)-induced endothelial cells or cardiomyocytes for a total of 100 µg for a grand total of 1.3 mg of PM needed from each source-oriented sample. Table 3 below lists the source-oriented samples that have more than this amount of mass remaining.

3. Description of the proposed exposure protocol using PM2.5, ozone and a mixture of both. The description should include method, duration, dose/concentration, and number of days of exposure.

In Vivo Exposure Protocol (Aims 1 and 3; Aim 2 will perform in vitro analysis of cells isolated from the animals from Aims 1 and 3)

Ozone could enhance the cardiovascular effects of PM in at least two ways:

1. Ozone prior to PM exposure could increase permeability and transport of PM into circulation
2. Ozone after PM exposure could increase oxidative response

As a result, we will expose animals to ozone for three days with the PM aspiration on day 2 so that (a) there is ozone exposure both before and after the PM aspiration and (b) there is a 2-day lag between PM aspiration and necropsy which corresponds to the lag found in many human epidemiological studies of CVMM. Table 1 summarizes the exposures.

	Table 1. Normal Mouse Exposure Schedule			
	Ozone and PM Exposure	**PM Exposure**	**Ozone Exposure**	**Fresh Air Control**
Day	**Activity**			
1	Ozone exposure	Fresh air exposure	Ozone exposure	Fresh air exposure
2	Ozone exposure and oropharyngeal aspiration of source-oriented PM	Fresh air exposure and oropharyngeal aspiration of source-oriented PM	Ozone exposure and oropharyngeal aspiration of saline	Fresh air exposure and oropharyngeal aspiration of saline
3	Ozone exposure	Fresh air exposure	Ozone exposure	Fresh air exposure
4	Necropsy	Necropsy	Necropsy	Necropsy

All exposures will take place in animal chambers in the Center for Health and the Environment at UC Davis. Controls will be breath fresh air in the same chambers and conditions as exposed animals. Ozone-only exposed animals will be exposed for 6 hours per day to ozone concentrations of 200 ppb. Animals exposed to ozone and PM will breathe ozone the same as the ozone-only schedule and on day 2, they will aspirate 50 µg of source-oriented PM dissolved in 50 µl of saline; dose response studies in prior work support this 50 µg of source-oriented PM dissolved in 50 µl of saline dose (Wexler and Pinkerton, 2012).

Animals

Male BalbC mice (8-10 weeks old) will be purchased from Charles River Laboratories, Inc. (Raleigh, NC) and shipped to the University of California, Davis. BalbC mice are proposed for this study because they have a propensity for a TH2-mediated immune response that gives rise to a local and systemic inflammatory response (Watanabe et al., 2004). Animals are housed in AAALAC approved facilities in plastic cages with TEK-Chip pelleted paper bedding (Harlan Teklad, Madison, WI). Mice will be acclimated for two weeks and have access to food and water ad libitum on a 12-hr light/12-hr dark cycle throughout the study. Animals are handled in accordance with standards established by the U.S. Animal Welfare Acts as set forth in the National Institutes of Health Guidelines (Institute of Laboratory Animal Resources 1996).

Experimental Design

Groups of six mice will be assigned to either vehicle control or source-oriented PM exposure groups. Size-fractioned source-oriented samples (SOS) will be used for testing cardiovascular effects. Control animals receive vehicle only. Mice are anesthetized via inhalation of isoflurane with oxygen (3:1 ratio) and SOS delivered to the lungs of anesthetized mice via oropharyngeal aspiration using a pipette (Li et al 2009; 2010; Wagner et al 2012). To perform the oropharyngeal aspiration, mice are suspended vertically from the central incisors and tongue restrained to facilitate placement of the SOS suspension at

the back of the throat to ensure aspiration of PM suspension through the trachea. Control mice are exposed to 50 µl of PBS alone. SOS-exposed mice aspirate 50 µg of PM in 50 µl of sterile PBS (Nygaard et al. 2009; Samuelsen et al. 2009). Each SOS is suspended in PBS and sonicated for a minimum of one hour then vortexed for one minute prior to dosing. Following dosing, mice will be closely monitored until they regain normal activity.

Allergic Mouse Model

The murine model of allergic airway inflammation was developed based on previously published research and experiments in our laboratory (Li et al. 2009; Li et al. 2010; Wagner et al 2012; Morishita et al. 2004; Carosino et al. 2012). Mice were randomly divided into exposure groups of eight and exposed to purified ovalbumin (OVA) with or without SOS PM suspended in a sterile buffered saline solution (Wexler and Pinkerton, 2012). Using this exposure protocol, we found a significant elevation in pulmonary inflammation due to OVA enhanced PM exposure (see Figure 2). The exposure protocol here differs from that of the non-sensitized mice because the hypothesis is different. Here we are exploring the role of immune system response in CVMM.

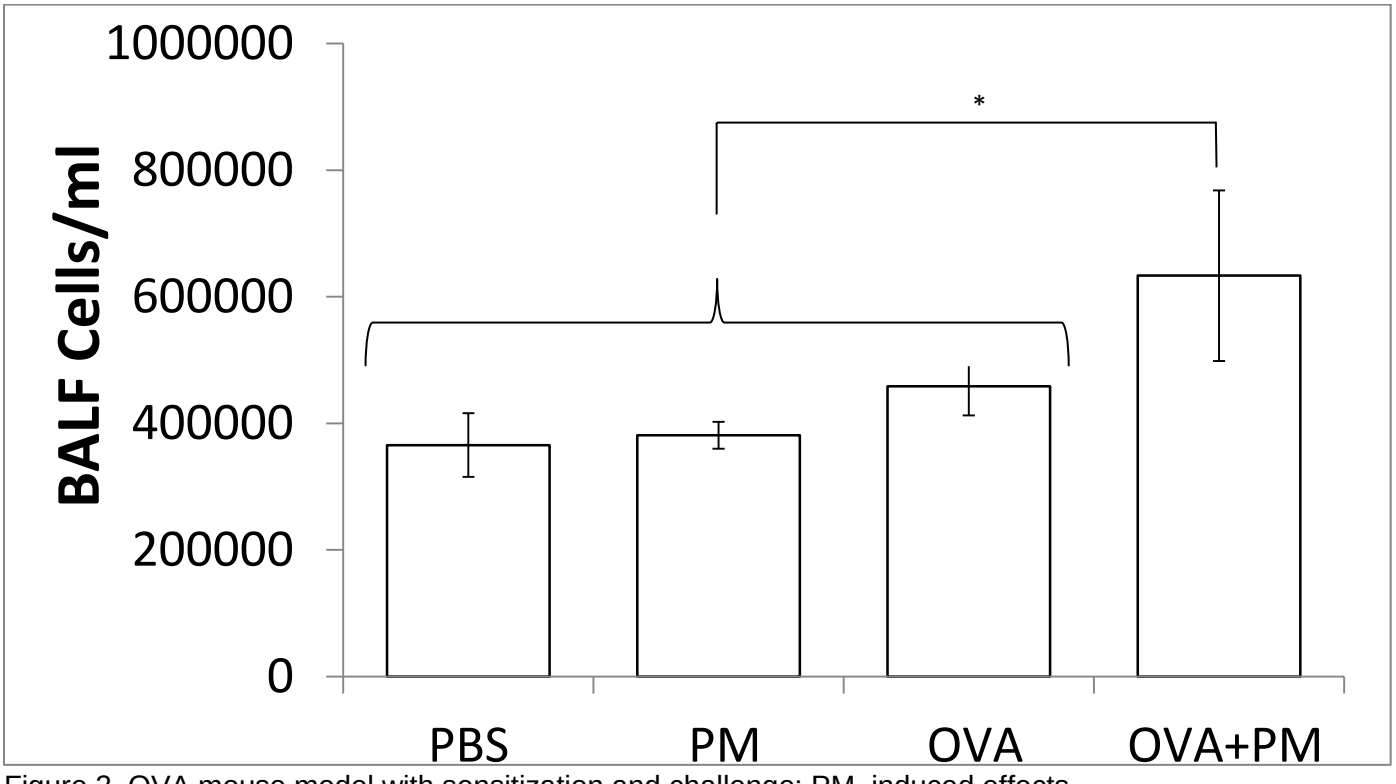

Figure 2. OVA mouse model with sensitization and challenge: PM–induced effects

Eleven days prior to the PM and ozone exposures, animals are ova sensitized, as shown in Table 2. On days 1, 2, and 3, animals are challenged by ova alone or in combination with source-oriented PM, ozone or both. Each exposure is done by placing the mice under a deep plane of anaesthesia with Isoflurane (2% Isoflurane, 1 LPM oxygen). Sensitization to vehicle, PM + vehicle, ova, or ova + PM is performed through intranasal aspiration by suspending the solution above the nares and allowing the mouse to spontaneously inhale the solution by intranasal aspiration. Animals will be necropsied on day 4, twenty-four hours post-challenge.

The target concentration of ozone for all animal studies will be 200 ppb for 6 hours/day for 3 days. Ozone is generated with electric discharge ozonizers (Model 100, Erwin Sander Elektropapparatebau GmbH, Uetze-Eltze, Germany) from medical grade oxygen. Ozone concentration will be monitored with an ultraviolet ozone analyzer (Model 1003AH, Dasibi Environmental Corp., Glendale, CA). Calibra-

tion of the analyzer will be performed according to the national reference method (United States Code, 1988).

	Table 2. Ova Sensitized/Challenged Mouse Exposure Schedule			
	Ozone and PM Exposure	**PM Exposure**	**Ozone Exposure**	**Fresh Air Control**
Day	**Activity**			
-10	Ova Sensitization	Ova Sensitization	Ova Sensitization	Ova sensitization + Fresh air exposure
-8	Ova Sensitization	Ova Sensitization	Ova Sensitization	Ova sensitization + Fresh air exposure
-6	Ova Sensitization	Ova Sensitization	Ova Sensitization	Ova sensitization + Fresh air exposure
. . .	Fresh air exposure	Fresh air exposure	Fresh air exposure	Fresh air exposure
1	Ova challenge + Ozone exposure	Ova challenge + Fresh air exposure	Ova challenge + Ozone exposure	Ova challenge + Fresh air exposure
2	Ova challenge + Ozone exposure + Oropharyngeal aspiration of source-oriented PM	Ova challenge + Fresh air exposure + Oropharyngeal aspiration of source-oriented PM	Ova challenge + Ozone exposure	Ova challenge + Fresh air exposure
3	Ova challenge + Ozone exposure	Ova challenge + Fresh air exposure	Ova challenge + Ozone exposure	Ova challenge + Fresh air exposure
4	Necropsy	Necropsy	Necropsy	Necropsy

In Vitro Exposure Protocol (Aim 2 and 4)

In vitro PM exposures. All PM suspensions will be prepared in cell culture media from PM stock solutions. Briefly, the stock solutions will be vortexed and then sonicated using a water bath sonicator immediately before diluting into complete cell culture medium. Culture media are described in the next section under "Culture and Directed Differentiation of hiPSC into hiPSC-CMs."

In vitro ozone exposures. Monolayers of cells will be exposed to ozone or filtered air in specially designed cylinfrical glass vessels measuring 3.66 liters in volume. Atmospheres in the culture system contain 95% air and 5% CO_2 by volume saturated with water vapor at 37.5^{o}C. This mixture flows through each vessel at a total rate of 15 liters/min. In the lid of the vessel, a diffuser plate with 19 sysmmetrically located holes, each 1.6 mm in diameter, is incorporated to distribute the flow evenly. Exhaust is removed from a central point below a perforated desiccator plate on which the 24-well culture plate is placed. Three experimental vessels for ozone exposure and one control vessel (without provisions for ozone) will be used. Ozone is generated with electric discharge ozonizers (Model 100, Erwin Sander Elektropapparatebau GmbH, Uetze-Eltze, Germany) from medical grade oxygen. Separate ozonizers will be used for each of the three experimental vessels so the ozone levels are independently adjustable.

Ozone concentration in each vessel will be repeatedly monitored for 2 min (during each total cycle of 8 min) with an ultraviolet ozone analyzer (Model 1003AH, Dasibi Environmental Corp., Glendale, CA). The analyzer is connected to computer-controlled valves which cycle from vessel to vessel. Materials in contact with ozone are composed of either Teflon or glass. Concentration data from the ozone analyzer is collected with a computer-based data acquisition system (PC-AT, IBM Corp., Boca Raton, FL and System 4000, ADAC Corp, Woburn, MA) is used to provide statistical reports on the exposure conditions. Calibration of the analyzer will be performed according to the national reference method (United

States Code, 1988) and will be traceable to a National Institutes of Standards and Technology absolute ozone photometer. Since atmospheres sampled by the analyzer will be saturated with moisture at 37.5^{o}C, it will be necessary to raise the internal temperature of the analyzer to 42 ^{o}C to prevent condensation on the optics of the instrument. In addition, as the response of the analyzer to a given ozone concentration is inversely proportional to the absolute temperature of the sample (DeMore et al, 1976), calibrations will be performed at the elevated temperature to eliminate temperature-related differences.

Culture and Directed Differentiation of hiPSC into hiPSC-CMs. Feeder-free hiPSCs (iPS-D19-9-T7, WiCell, Madison, WI) will be cultured with mTeSR media (Stemcell Technologies Inc., Vancouver, Canada) on hESC-qualified matrigel. HiPSCs will be differentiated into hiPSC-derived cardiomyocytes (CMs) using a directed differentiation method (Yang et al., 2008). Briefly, hiPSCs on day 0 will be allowed to form embryoid bodies (EBs) overnight in mTeSR media. Using directed differentiation (DD)-medium specific for each differentiation stage (Yang et al., 2008), we typically observe 50-90% beating clusters of different cardiomyocyte subtypes with ~13% of cardiomyocytes as verified by fluorescence-activated cell sorting (FACS) using cardiomyocyte-specific tropomyosin staining (Figure 3A). The cardiomyocyte population consists of heterogeneous cardiomyocyte subtypes as shown by myosin light chain (MLC)2a staining for all immature cardiomyocytes and MLC2v for ventricular-specific cardiomyocytes (Figure 3BC). Day 60 (maturing cardiomyocytes) will be used for experiments described below.

In vitro exposure of hiPSC-derived cardiomyocytes and endothelial cells. The in vitro studies will require 50 µg per dish of human induced pluripotent stem cells (hiPSCs)-induced endothelial cells or cardiomyocytes. Size-fractioned source-oriented samples (SOS) will be used. Each SOS is suspended in PBS and sonicated for a minimum of one hour then vortexed for one minute prior to dosing. PBS will be used as control. Cells will be treated for one day before harvesting. Single cell phenotyping using flow cytometric analyses will be performed as described in Aim 2.

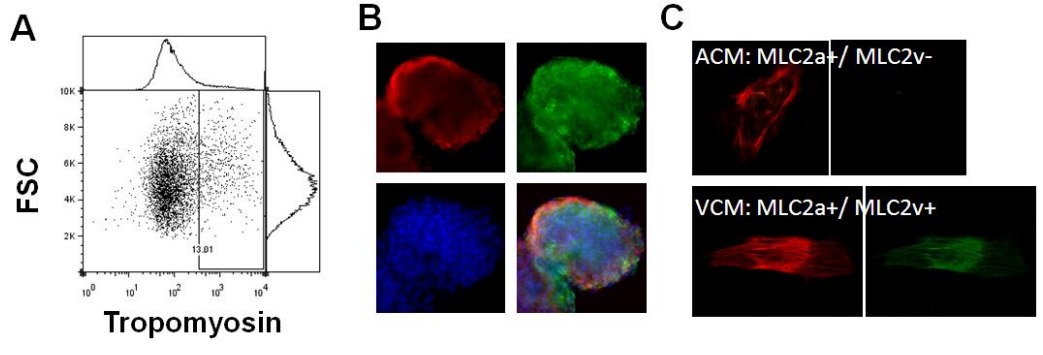

Figure 3. A) Directed cardiac differentiation method yields ~13% hiPSC-CMs 15 days post-differentiation as verified by FACS of CM-specific tropomyosin staining. B) A beating cluster of hiPSC-CMs immunostained with myosin light chain (MLC)2a (red), MLC2v (green), Hoechst (blue) and merged. C) An hiPSC-atrial (A)CM is for positive MLC2a and negative MLC2v and a hiPSC-ventricular (V)CM is positive for MLC2a and MLC2v. MLC2a identifies all immature CMs, while MLC2v identifies ventricular CM subtype.

Source-Oriented Particulate Matter Samples (all Aims)

During a previous research project funded by the California Air Resources Board (Wexler and Pinkerton, 2012), source oriented samples were collected in Fresno California using a novel sampling technique that conditionally samples particles from the atmosphere depending on the sources or source combinations that predominate at the sampling site at a given time. In brief, a single particle mass spectrometer (RSMS-II), operating in the 70-150 nm particle diameter range, continuously provides the chemical composition of individual particles. The mass spectra indicate which sources are currently affecting the site. Ten ChemVol samplers are each assigned one source or source combination and RSMS-II controls which one operates depending on the sources or source combinations observed. Each ChemVol collects both an ultrafine (UF ≡ D_p < 170 nm) and submicron fine (SMF ≡ 170 < D_p < 1000 nm) sample. By running this system for weeks at a time during two separate seasons (Summer 2008 and Winter 2009), sufficient sample was collected by the ChemVols for comparative toxicological studies. Further details on instrument and algorithmic design, implementation and first results from these studies can be found in the work of Bein et al. (2009).

During the prior study, some of the Source Oriented Samples were used up and for a few we have insufficient mass remaining for the studies proposed here. Table 3 gives a brief description of these sample sources, including the season they were collected, the size range of the collected PM, the amount available for the proposed studies and the associated sources or source combinations. Table 3 only lists the 18 samples that have sufficient mass remaining for the studies proposed here.

Table 3. Archived Fresno Source-Oriented PM Samples.
A list of the archived Fresno source-oriented PM samples with sufficient remaining archived mass for the proposed studies, including collection season, associated source(s), size range and mass of PM available. SMF indicates submicron fine (0.17 to 1 um); UF indicates 0 to 0.17 um) particle size.

Dominant Source Combinations	Ultrafine [mg]	Submicron Fine [mg]
Summer 2008		
Local dinnertime cooking emissions	22	19
Highly processed regional background PM	21	33
Daytime mixed layer	75	39
Nighttime nocturnal inversion background	30	30
Winter 2009		
Local residential heating emissions	3.2	
Local vehicular emissions		1.6
Regional source mixture	1.7	
Evening commuter traffic and cooking	1.6	
Morning commuter traffic	7.9	10.4
Daytime mixed layer	12.3	27
Nighttime nocturnal inversion background	10.5	24

Included in this list are several different samples that are dominated by local vehicular emissions. Despite this, there is a general paucity of diesel and freeway related emissions in these samples. A large majority of vehicular emissions impacting the sampling site during the Fresno studies were light-duty gasoline powered vehicles operated at low speeds in stop-and-go driving conditions on commercial and residential roadways. Furthermore, due to the complexity of isolating specific sources from ambient mixtures, the source-oriented samples are not purely primary vehicular emissions and include background PM and components from other sources. For these reasons, and since the EPA recently listed diesel particulate matter as a possible carcinogen, we propose to conduct an additional sampling campaign, as a part of the study being proposed here, to collect diesel and freeway related emissions for inclusion in the cardiovascular studies.

To ensure collection of purely primary vehicular emissions from freeway related driving conditions and samples that are enhanced in heavy-duty diesel emissions relative to light-duty gasoline emissions, the authors propose conducting an additional field study in the Caldecott Tunnel in Oakland, CA, which sits on SR 24 and cuts through the Berkeley Hills between Oakland to the west and Walnut Creek to the east. It is a three-bore, two-lane/bore tunnel where the southern bore services eastbound traffic, the northern bore services westbound traffic and the center bore switches between westbound traffic during the morning commute and eastbound traffic during the evening commute. For eastbound traffic during the evening commute, vehicles traverse a positive vertical gradient, heavy-duty (HD) diesel vehicles are not permitted through the center bore and light-duty (LD) vehicles use both bores. As a result, there is an increased engine load due to the uphill traverse, and the emission mix in the southern bore is enhanced in HD diesel emissions relative to the center bore, which has been observed during several field studies conducted in these tunnels (Geller et al., 2005; Kean et al., 2000; Kirchstetter et al., 1996, 1999a,b; Ning et al., 2008; Phuleria et al., 2006).

For the proposed studies, two ChemVol stacks — each including an afterfilter support and 0.17 μm, 1 μm and 2.5 μm stages (i.e., ultrafine, submicron fine, supermicron fine and coarse) — and blower assemblies will be placed in the tunnel ventilation system. One ChemVol stack will sample directly from the southern bore and the other from the center bore. Although we will collect 4 size fractions, we will only employ the size fractions that match our prior studies (0-0.17 μm and 0.17-1 μm). The coarser stages will be collected to prevent this material depositing on the finer stages. Even though it will not be used for this study, these PM fractions will be archived for possible use in other CARB-sponsored studies.

Sample collection will be conducted during evening rush hour traffic over a period of several days to collect tens of milligrams of vehicular emissions. Other time periods when the ratio of HD to LD vehicles in the southern or northern bores may be larger will also be investigated; e.g., nighttime traffic is often dominated by HD vehicles. Sampling independently, but simultaneously, from both bores provides an excellent opportunity to separate these two source categories for subsequent relative toxicity studies. Furthermore, since all the necessary sampling equipment is already owned by the Air Quality Research Center at U.C. Davis and outfitted in a mobile trailer, this will be a relatively inexpensive sampling campaign to conduct.

4. Description of the proposed endpoints and the methods for measuring them. Proposed endpoints may be biochemical and/or physiological, but must address the specific hypothesis proposed, and be oriented toward demonstrating the influence of PM2.5 and ozone exposure on cardiac function.

In vivo Studies (Aims 1 and 3)

Cardiac function will be monitored before and after exposure by echocardiography and in vivo hemodynamic monitoring. Increases in arrhythmia susceptibility will be tested using in vivo electrophysiologic studies.

Serial Echocardiography

Cardiac echocardiography will be performed before and after exposure to determine the fractional shortening, left ventricular wall thickness, dimension and volume. Diastolic function will be assessed using mitral inflow and tissue Doppler imaging (TDI, Figure 4) (Daneshvar, Wei et al. 2010). Altered patterns of valve annulus motion can be early indicators of diastolic dysfunction.

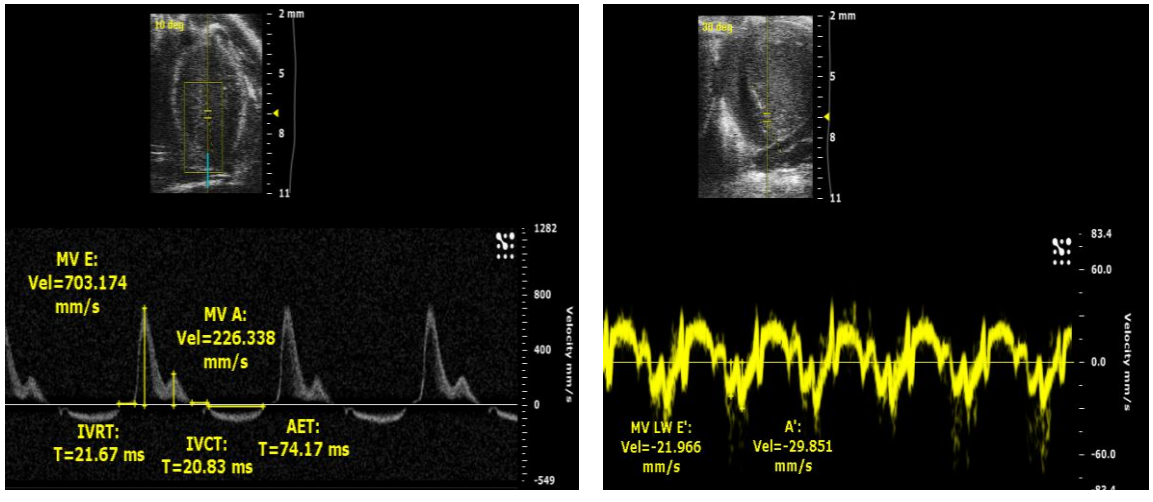

Figure 4. Examples of pulsed-wave mitral inflow analyses (Left Panel) and pulsed-wave tissue Doppler imaging (TDI, Right Panel) in the mouse models. Pulsed-wave TDI was performed at the lateral mitral annulus using apical 4 chamber view.

Pressure-volume (PV) analyses

Intact heart hemodynamic analysis will be performed using a four-electrode PV catheter (Millar Instruments) to record chamber volume by impedance and pressure by micromanometry (Takimoto, Champion et al. 2005). Different parameters will be assessed including LV end diastolic pressure (LVEDP). PV loops will be constructed before and during transient reduction of preload to generate specific systolic and diastolic function indexes, LV afterload (indexed by arterial elastance), ejection fraction, contractile function as assessed through load-independent parameters (maximal power index and preload recruitable stroke work), and diastolic function (LV stiffness constant, tau and peak rate of pressure decline (dP/dt_{min}), see Figure 5) (Esposito, Rapacciuolo et al. 2002; Takimoto, Champion et al. 2005).

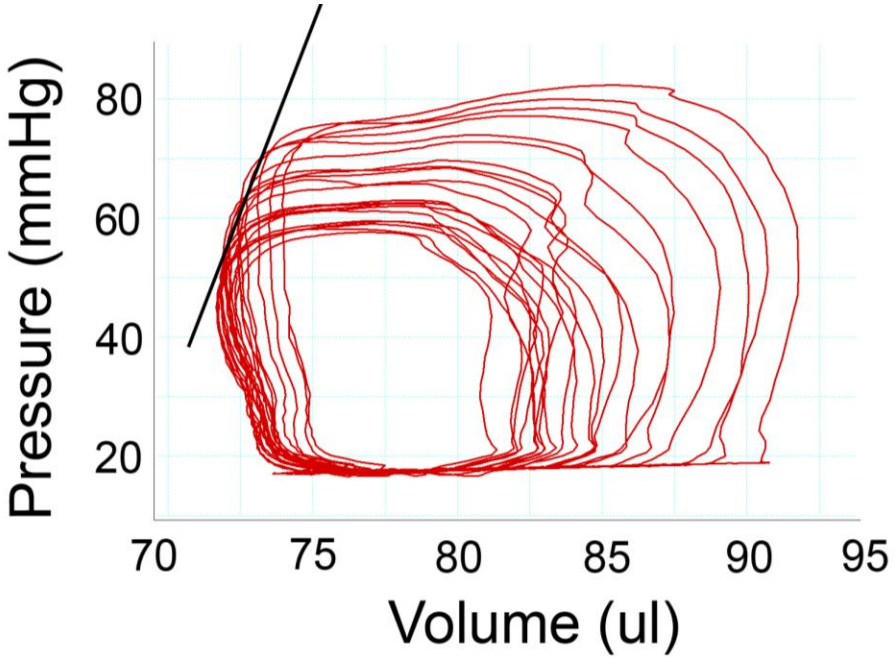

Figure 5. Examples of PV loops in a sham animal.

Electrophysiology studies

In-vivo electrophysiology studies will be performed as previously described.(Berul, Aronovitz et al. 1996) Standard pacing protocols will be used to determine the electrophysiologic parameters, including sinus node recovery time, atrial, AV nodal, and ventricular refractory periods and AV nodal conduction properties. Each animal will undergo an identical pacing and programmed stimulation protocol. The Q-T interval will be determined manually by placing cursors on the beginning of the QRS and the end of the T wave. The rate-corrected QT interval (QT_c) will be calculated using modified Bazett's formula as reported by Mitchell et al, whereby the RR interval will be first expressed as a unitless ratio (RR in ms/100 ms). QT_c interval will be defined as QT interval (in ms)/$(RR/100)^{1/2}$ (Mitchell, Jeron et al. 1998).

To induce atrial and ventricular tachycardia and fibrillation, programmed extra-stimulation techniques and burst pacing will be utilized. Programmed right atrial and right ventricular double and triple extra-stimulation techniques will be performed at 100-ms drive cycle length, down to a minimum coupling interval of 10 ms. Right atrial and right ventricular burst pacing were performed as eight 50-ms and four 30-ms cycle length trains episodes repeated several times, up to a maximum 1-min time limit of total stimulation. For comparison of the inducibility in each mouse, programmed extra-stimulation techniques and stimulation duration of atrial and ventricular burst pacing will be the same in all mice. Reproducibility will be defined as greater than one episode of induced atrial or ventricular tachycardia.

In Vitro Cardiovascular Studies

Serum cytokines Analyses (Aims 1 and 3)

Mice will be sacrificed post exposure after in vivo analyses as described above. Blood will be collected for further analyses. The hearts will be fixed for the further histological examination. Plasma will be used for the analyses of C-reactive protein (CRP), fibrinogen, P-selectin, thrombin, cAMP, *t*-PA, and PAI-1. Each serum sample will be subjected to cytokine assay including eotaxin, fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-monocyte colony-stimulating factor (GM-CSF), interferon-gamma (INF-γ), interleukin (IL)-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 protein 40 (IL-12p40), IL-12 protein 70 (IL-12p70), IL-13, IL-15, IL-17, IL-18, Kuppfer cell

(KC),leukemia inhibitory factor (LIF), monocyte chemotaxtic protein-1 (MCP-1), monocyte colony-stimulating factor (M-CSF), monokine induced by gramma interferon (MIG), macrophage inflammatory protein (MIP)-1α, vascular endothelial growth factor (VEGF), MIP-1β, MIP-2, platelet derived growth factor (PDGF), regulated upon activation, normally T-expressed, and presumably secreted (RANTES), and tumor necrosis factor alpha (TNFα).

Single-cell phenotyping using flow cytometric analyses (Aims 2, 3, 4)

Since the heart contains a mixture of heterogenous populations of cells,(Zak 1973; Eghbali, Czaja et al. 1988) to directly test the hypothesis, single-cell based assays using flow cytometric analyses will be performed as we have previously described.(Sirish, Lopez et al. 2012) Single cells will be isolated from the mouse hearts using enzymatic digestion as we have previously described. The nucleated cells from the fresh myocardial preparation were enumerated based on the incorporation of 7-aminoactinomycin D (7-AAD) (Figure 6). A cardiac-specific TnT antibody will be used to label the myocytes (MC) and allowed us to gate and eliminate the MC population that had higher auto fluorescence from the non-muscle cells (NMC) in the analysis. Biotinylated anti-CD31 antibody will be used to identify endothelial cells. (Figure 6).(Hudon-David, Bouzeghrane et al. 2007) Biotinylated lineage antibodies (CD3e, CD11b, Cd45R, Ly-6C, Ly-6G and TER-119, BD Bioscience) will be used. Cells stained with isotype-matched IgG antibodies will be used as controls. Next, Ki67, a nuclear antigen that is expressed in actively cycling cells,(Epting CL, Lopez JE et al. 2008) will be used to directly test the hypothesis that there is a significant increase in the proliferative capacity. Ki67 is absent in the resting G0 phase, increases during the G1/S phase, and reaches a maximum during the G2/M phase.(Landberg, Tan EM et al. 1990)

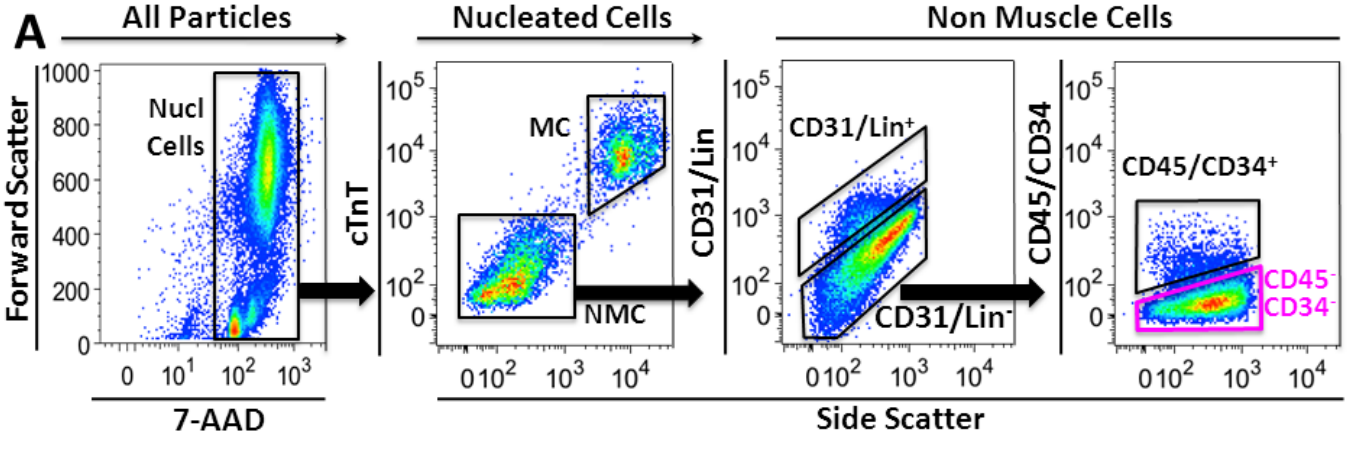

Figure 6. Flow cytometric analysis. The selection of nucleated cells (Nucl cells) based on the incorporation of 7AAD and the separation of myocytes (MCs) from non-muscle cells (NMC) using cTnT antibody. The Thy1.2pos linneg CD31/CD45/ CD34neg cells are identifie d from the NMC population using Thy1.2 and lineage (lin)-specific antibodies. (B) Flow cytometric analysis comparing the percentages of the Thy1.2^{pos} lin^{neg} cells from the four groups of animals. Cells stained with isotype-matched IgG antibodies were used as controls and presented in the left panels. (C, D) Percentages of Thy1.2^{pos} cells and Ki67 positivity in Thy1.2^{pos} cells from four groups of animals. *P < 0.05.

Endothelial cells (ECs) and endothelial progenitor cells (EPCs) will be analyzed by single-cell phenotyping with a flow cytometry. Other endothelial dysfunction markers (ICAM-1, VCAM-1, E-selectin, MCP-1, and PAI-1) in endothelial cells and blood will be analyzed by flow cytometry analyses. The key signaling pathways (NOS, NF-κB, PI_3K, p-ERK1/2, and ERK1/2) in heart tissue will be analyzed using specific antibodies and phosphor-specific antibodies.

Patch-clamp recordings of ventricular myocytes

Ventricular myocytes will be isolated from the animals post exposure. APs will be recorded using perforated patch-clamp techniques to maintain intracellular milieu as we have previously described.(Ahmmed, Dong et al. 2000; Xu, Tuteja et al. 2003; Xu, Zhang et al. 2005; Li, Timofeyev et al. 2009) We have previously documented the existence of several important Ca^{2+}-activated ionic currents in mouse myocytes.(Xu, Dong et al. 2002; Xu, Tuteja et al. 2003; Tuteja, Xu et al. 2005; Lu, Timofeyev et al. 2009; Tuteja, Rafizadeh et al. 2010) Therefore, it will be important to assess the AP profiles without applying intracellular chelators. Next, if indicated by the above findings, we will perform systematic assessment of the inward and outward current density as well as time- and voltage-dependent kinetics. Multiple components of the underlying outward K^+ currents have been reported in mouse atrial myocytes corresponding to different molecular correlates (Table 4) and will be assessed as outlined in our previous studies.(Xu, Dong et al. 2002; Xu, Tuteja et al. 2003; Tuteja, Xu et al. 2005; Xu, Zhang et al. 2005; Lu, Timofeyev et al. 2009; Tuteja, Rafizadeh et al. 2010)

Table 4. Distinct components of outward K^+ currents in mouse myocytes

	Molecular correlates	**Pharmacology**
Rapidly inactivating transient outward K^+ current ($I_{to,f}$)	$K_v4.2/K_v4.3$	4-AP sensitive (> 100 μM) Heteropoda toxin-3 (nM range)
Slowly inactivating transient outward K^+ current ($I_{to,s}$)	$K_v1.4$	4-AP sensitive (> 100 μ M)
Fast activation with slow inactivation outward K^+ current ($I_{K,slow}$), (at least 2 different components)	$K_v1.5$ & $K_v2.1$	4-AP sensitive (10-50 μM) TEA-sensitive (> 25 mM)
Rapidly activating outward K^+ current (I_{Kr})	MERG	E4031-sensitive
Non inactivating steady state current (I_{ss})	?	High concentration of 4-AP, TEA
Ca^{2+}-activated K^+ current	SK1,2,3	Apamin

(Lees-Miller, Kondo et al. 1997; Barry, Xu et al. 1998; London, Jeron et al. 1998; Guo, Xu et al. 1999; Xu, Guo et al. 1999; Xu, Li et al. 1999; Nerbonne 2000; London, Guo et al. 2001; Xu, Tuteja et al. 2003; Tuteja, Xu et al. 2005)

In Vivo Pulmonary/Aortic Studies (Aims 1 and 3)

Along with measures of cardiac function, studies will be performed of the pulmonary/aortic endothelium to understand their role in cardiac dysfunction due to ozone and PM exposure and immune sensitization. Specifically, we will conduct assays to determine oxidative stress as well as other measures of vascular change to include endothelin-1 (ET-1), matrix metallopeptidase-9 (MMP-9), and heme oxygenase-1 (HO-1), all involved in the genesis and progression of atherosclerosis, which accounts for nearly half of all deaths from cardiovascular disease (Peterson et al., 2005).

TBARS assay

Aortic thiobarbituric acid reactive substances (TBARs) levels will be assessed on pulmonary vessels and one-half of each aorta (midsagittal cut, containing ascending, arch, and descending aorta to the bifurcation), n=8, using a TBARS assay kit (OXItek, ZeptoMetrix Corp., Buffalo, NY). TBARS levels will be measured in whole, uncentrifuged aorta homogenates per manufacturer's instructions, as described previously by Lund et al. (2007). Briefly, aortas (one-half, midsagittal cut) are resuspended by diluting 1:10 weight/volume in normal saline. Tissues are homogenized and sonicated for 15 s at 40 V. Duplicate samples are read on a spectrophotometer (Perkin Elmer Lambda 35, Boston, MA) and referenced using a malondialdehyde standard curve, with findings expressed as malondialdehyde equivalents.

Real-time PCR

Real-time PCR will be performed as described by Lund et al. (2011). Total RNA will be isolated from pulmonary vessels and one half of the aorta (midsagittal cut, containing ascending, arch, and descending aorta to the bifurcation), n=6 per group, using All Prep DNA/RNA/protein Mini Kit (Qiagen, Valencia, CA). cDNA will be synthesized from total RNA in a 20-µl final reaction volume per manufacturer's instructions (Biorad, iScript Select cDNA synthesis kit). The mixture is heated at 42°C for 1 h and then cooled to 4°C. Real-time PCR is performed with gene-specific primers in the ABI 7900 (Applied Biosystems, Valencia, CA) sequences which have been reported by Lund et al. (2007). Expression markers of oxidative stress will be measured, including ET-1, MMP-9 and HO-1.

Expected findings:

We predict that there will be a significant increase in serum inflammatory cytokines and chemokines from PM and ozone exposure in a source-dependent manner. We further predict that the increase in serum cytokines and chemokines may result in a source-dependent reduction in the fractional shortening (FS), a surrogate of systolic function, by echocardiography. In vivo hemodynamic monitoring may detect a significant changes in cardiac function as a result of the PM and ozone exposure. The observed changes in signaling lipids, cytokines, CRP, fibrinogen, P-selectin, thrombin and cAMP, *t*-PA, and PAI-1 could result in the increased propensity of thrombosis, and increased inflammation, which supports the hypothesis that PM and ozone exposure causes the increased risks in myocardial infarction. In vivo electrophysiologic studies will be used to test our hypothesis that exposure to PM and ozone will result in an increase in arrhythmia susceptibility and changes in cellular excitability will be further tested using patch-clamp recordings in single isolated ventricular myocytes.

Using single-cell phenotyping by cytometric analysis, we will directly test the effects of PM and ozone exposure on the isolated endothelial cells and cardiomyocytes. Signaling pathways which may be activated by the exposure will be tested to determine the mechanisms for the observed endothelial and myocyte dysfunction. Additionally, using single-cell phenotyping, we will assess the effects of PM and ozone exposure on the number and proliferative capacity of endothelial cells.

5. Description of the statistical methodologies to be used to analyze the results. This section should include the specific statistical tests to be employed, and estimates of the statistical power of the analyses. Submitters should also describe the methods to be used to determine whether or not the data are normally distributed, and if not, what alternative statistical methods will be use

All data will be tested for normality by the Shapiro-Wilk test and homogeneity of variances will be assessed using the Levene's test. Repeated measures of analysis of variance (RM-ANOVA) will be performed with treatment as a between-group variable and time as a within-group variable. Dr. Neil Willits is a biostatistician on campus who works with us on air pollution toxicology studies. We have included him in the budget for this proposal. He will be consulted on study design and data interpretation.

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Takimoto, E., H. C. Champion, et al. (2005). "Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy." Nat Med 11(2): 214-222.

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Tuteja, D., D. Xu, et al. (2005). "Differential isoform expression of small conductance Ca2+-activated K+ channels, SK1, SK2 and SK3 channels in mouse atrial and ventricular myocytes." Am J Physiol Heart Circ Physiol.

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Wagner JG, Morishita M, Keeler GJ, Harkema JR. 2012. Divergent effects of urban particulate air pollution on allergic airway responses in experimental asthma: a comparison of field exposure studies. Environ Health. 6;11:45. doi: 10.1186/1476-069X-11-45. PMID:22768850

Watanabe H, Numata K, Ito T, Takagi K, Matsukawa A. 2004. Innate immune response in Th-1 and Th-2 dominant mouse strains. Shock 22:460–466

Watkins, Alex, et al. "Air Pollution and Arrhythmic Risk: The Smog Is Yet to Clear." Canadian Journal of Cardiology (2012).

Wexler, A. S. and K. E. Pinkerton (2012). Toxicity of Source-Oriented Ambient Submicron Particulate Matter. Final Report to the State of California Air Resources Board, Research Division. Sacramento, California Air Resources Board: 145 for Contract Number 06-331.

Wu ML, Ho YC, Lin CY, Yet SF. 2011. Heme oxygenase-1 in inflammation and cardiovascular disease. Am J Cardiovasc Dis 1:150-158.

Xu, H., W. Guo, et al. (1999). "Four kinetically distinct depolarization-activated K+ currents in adult mouse ventricular myocytes." J Gen Physiol 113(5): 661-678.

Xu, H., H. Li, et al. (1999). "Elimination of the transient outward current and action potential prolongation in mouse atrial myocytes expressing a dominant negative Kv4 alpha subunit." J Physiol 519 Pt 1: 11-21.

Xu, Y., P. H. Dong, et al. (2002). "Presence of a calcium-activated chloride current in mouse ventricular myocytes." Amer J Physiol 283: H302-H314.

Xu, Y., D. Tuteja, et al. (2003). "Molecular identification and functional roles of a Ca2+-activated K+ channel in human and mouse hearts." J Biol Chem 278(49): 49085-49094.

Xu, Y., Z. Zhang, et al. (2005). "The effects of intracellular Ca2+ on cardiac K+ channel expression and activity: novel insights from genetically altered mice." J Physiol 562(Pt 3): 745-758.

Yang, L., M. H. Soonpaa, et al. (2008). "Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population." Nature 453(7194): 524-528.

Zak, R. (1973). "Cell proliferation during cardiac growth." Am J Cardiol 31(2): 211-219.

Project Schedule

Task 1: Collect heavy- and light-duty vehicle Source-Oriented Samples (SOS) from Caldicott tunnel; extract PM from these and existing SOS; prepare for in vivo and in vitro exposures; characterize new SOS.

Task 2: Perform in vivo exposures and assay in vivo endpoints (Aims 1 and 3)

Task 3: In vitro assay for Aims 1, 2, 3 and 4; Set up in vitro exposure apparatus and perform in vitro exposures, assay endpoints

Task 4: Draft final report; submit publications

Task 5: Amend final report; submit publications

	Quarter											
Task	13-3	13-4	14-1	14-2	14-3	14-4	15-1	15-2	15-3	15-4	16-1	16-2
1	■	■	■	■								
2			■	■	■	■	■					
3				■	■	■	■	■				
4									■	■		
5											■	■

Vitae and the Team

Dr. Wexler is the PI of this proposal. He is a professor of Mechanical and Aerospace Engineering, Civil and Environmental Engineering, and Land, Air and Water Resources and director of the Air Quality Research Center and Crocker Nuclear Laboratory. He was the originator of the source-oriented sampling idea and PI on the proposal that implemented it.

Professor of Pediatrics in the School of Medicine and Professor of Anatomy, Physiology and Cell Biology in the School of Veterinary Medicine. He is Director of the Center for Health and the Environment (CHE). The primary research focus of his laboratory is the toxicology of inhaled gases, vapors and particles. He oversees the CHE inhalation facility where all exposures to PM and ozone in animals will be done as proposed in this study. His interest in susceptible animal models, inflammation and allergy to enhance the interaction of the cardiopulmonary system to give rise to cardiovascular abnormality/dysfunction are current areas of research investigation in the Pinkerton laboratory relevant to the topic of ozone/PM effects in vascular disease.

Dr. Chiamvimonvat is a cardiologist and the Roger Tatarian Endowed Professor of Cardiovascular Medicine in the School of Medicine at UC Davis. For over a decade, her laboratory has focused on the molecular mechanisms of cardiac arrhythmias in hypertrophy and failure. Her laboratory will be able to bring a wealth of experience to the current project, including extensive familiarity with *in vivo* studies of cardiac function and arrhythmia inducibility, models of cardiac hypertrophy and failure, single-cell phenotyping using flow cytometric analyses, patch-clamp recordings, confocal microscopy imaging, molecular biological and biochemical techniques. and induced human pluripotent stem cell models *in vitro*.

Dr. Bein is an Assistant Research Scientist in the Air Quality Research Center and a member of the Research Faculty at the Center for Health and the Environment at UC Davis. He developed the source-oriented sampling apparatus, used it in Fresno to sample source-oriented PM from the atmosphere there in the summer of 2008 and winter of 2009. He also developed efficient protocols for extracting PM from the source-oriented sampling substrates that preserve the composition close to what was sampled, as well as the protocols necessary to prepare the extracted PM for subsequent chemical analysis, including ICP-MS, IC, EC/OC and GC-MS. Furthermore, he has extensive experience conducting field studies using a suite of different instrumentation under varying conditions, including the ChemVol sampling train to be used in the Caldecott Tunnel sampling being proposed here.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Wexler, Anthony S.	Professor
eRA COMMONS USER NAME ASWEXLER	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Berkeley	B.S.	1976	Engineering Physics
Massachusetts Institute of Technology	M.S.	1978	Mechanical Engineering
California Institute of Technology	Ph.D.	1990	Mechanical Engineering

A. Personal Statement

My research employs theoretical, mathematical modeling and measurement techniques to investigate gas and particle deposition in the airways of humans and animal models, and the health effects elicited by these pollutants. Although my formal education is in engineering, I have performed biomedical research since the mid-1980's using my quantitative mathematical, physics and chemistry background to understand biological problems. Starting about 8 years ago, I focused my biomedical research on juvenile airway development, using airway geometric parameters and novel approaches developed and published by my lab to identify disruption of airway development due to pollutant exposure. I work with a wide range of faculty and students on campus, bringing together complementary teams of investigators to tackle problems related to aerosol science, including dosimetry and airway development. As director of the Air Quality Research Center and Crocker Nuclear Laboratory at UC Davis and formerly of EPA's San Joaquin Valley Aerosol Health Effects Research Center (SAHERC), I have experience managing substantial funding and personnel. Dr. Van Winkle and I have a recent past history of productive collaboration.

B. Positions and Honors

Positions and Employment

Director, Crocker Nuclear Laboratory, University of California, Davis	2009-present
Director, Air Quality Research Center, University of California, Davis	2005-present
Professor, Departments of Mechanical and Aeronautical Engineering, Civil and Environmental Engineering, and Land, Air and Water Resources, University of California, Davis	2000-present
Professor, Department of Mechanical Engineering, University of Delaware	1999-2000
Assoc. Professor, Department of Mechanical Engineering, University of Delaware	1994-1999
Assistant Professor, Department of Mechanical Engineering, University of Delaware	1991-1994
Research Associate, Department of Physiology and Biophysics, University of Southern California	1984-1990
Engineer, BMDP Statistical Software (Developers of statistical software), Los Angeles, CA	1983-1984
Engineer, Berkeley Solar Group (HVAC energy conservation consultants), Berkeley, CA	1978-1983

Honors and Awards

Elected to Fellow status, American Association for Aerosol Research	2011
Appointed to Editorial Advisory Board, Aerosol Science and Technology	2008
Outstanding Mid-career Research Faculty Award, College of Engineering, UC Davis	2005
President of the American Association for Aerosol Research	2005-2006

C. Selected Peer-reviewed Publications (from a total of 188)

Most relevant to the proposed research (5)

178. Lee, D.Y., C.D. Wallis, L.S. Van Winkle, and A.S. Wexler, Disruption of tracheobronchial airway growth due to postnatal ozone exposure. *Inhal. Toxicol.* 23:520-531, 2011.
176. Lee, D.Y. and A.S. Wexler, Particle deposition in juvenile rat lungs: a model study, J. Aerosol Sci. 42:567-579, 2011.
175. Lee, D.Y., P.K. Srirama, C. Wallis, and A.S. Wexler, Postnatal growth of tracheobronchial airways of Sprague-Dawley rats. J. Anat. 218:717–725, 2011.
172. Lee, D.Y., N. Willits, A.S. Wexler, Detecting alterations in pulmonary airway development with airway-by-airway comparison. Ann. Biomed. Engin. 39: 1805-1814, 2011.
166. Lee, D.Y., C. Wallis, A.S. Wexler, E.S. Schelegle, L.S. Van Winkle, C.G. Plopper, M.V. Fanucchi, B. Kumfer, I.M. Kennedy, J.K.W Chan, Small particles disrupt postnatal airway development. J. Applied Physiol. 109:1115-1124, 2010.

Other publications of importance to the field (10)

182. Srirama, P.K., C.D. Wallis, D.Y. Lee, and A.S. Wexler, Imaging Airways and Particles Deposited in Them: Extra-thoracic Airways of Laboratory Animals, J. Aerosol Sci.45:40-49, 2012.
181. Chan, J.K.W., M.V. Fanucchi, D.S. Anderson, A.D. Abid, C.D. Wallis, D.A. Dickinson, B.M. Kumfer, I.M. Kennedy, A.S. Wexler, and L.S. Van Winkle, Susceptibility to inhaled flame-generated ultrafine soot in neonatal and adult rat lungs. Toxicol. Sci. 124:472-486, 2011, doi: 10.1093/toxsci/kfr233.
180. Lee, D.Y. and A.S. Wexler, Simulated annealing implementation with shorter Markov chain length to reduce computational burden and its application to the analysis of pulmonary airway architecture. Computers in Biology and Medicine 41:707-715, 2011.
168. Van Winkle, L.S., J.K.W. Chan, D.S. Anderson, B.M. Kumfer, I.M. Kennedy, M.J. Kleeman, A.S. Wexler, C. Wallis, A.D. Abid, K.M. Sutherland, and M.V. Fanucchi, Age specific responses to acute inhalation of diffusion flame soot particles: Cellular injury and the airway antioxidant response. Inhal. Toxic. 22(S2):70-83, 2010.
152. Fresconi, F., A.S. Wexler, and A. Prasad, Transport Profiles in the Conducting Airways of the Human Lung. Int´l J. Heat Mass Transfer 51:5552-5561, 2008.
151. Lee, D.Y., M.V. Fanucchi, C.G. Plopper, J. Fung, and A.S. Wexler. Pulmonary architecture in the conducting regions of six rats. Anat. Record 291:916-926, 2008.
150. Lee, D.Y., A.S. Wexler, M.V. Fanucchi, and C.G. Plopper, Expiration Rate Drives Human Airway Design. J. Theor. Biol. 253:381-387, 2008.
149. Lee, D.Y., S.S. Park, G.A. Ban-Weiss, M.V. Fanucchi, C.G. Plopper, and A.S. Wexler. A bifurcation model for characterization of pulmonary architecture. Anat. Record 291:379-389, 2008.
147. Nolte, C.G., P.V. Bhave, R.L. Dennis, J.R. Arnold, K.M. Zhang, A.S. Wexler, Modeling Urban and Regional Aerosols - Application of the CMAQ-UCD aerosol model to Tampa, a coastal urban site. *Atmos. Environ.* 42:3179-3191, 2008.
144. Park, S.S. and A.S. Wexler, Size dependent deposition of particles in the human lung at steady-state breathing J. Aerosol Sci. 39:266-276, 2008.

C. RESEARCH SUPPORT

ACTIVE RESEARCH SUPPORT

Source: Tobacco-Related Disease Research Program Dates: 8/1/12 to 7/31/14
Title: Imaging ETS Deposition in the Airways of Sprague-Dawley Rats
PI: Anthony Wexler
Objective: Map the deposition pattern of Environmental Tobacco Smoke in Rat Airways

Source: Electric Power Research Institute Dates: 11/1/11 to 10/31/12
Title: Photochemistry of Atmospheric Amines
PI: Anthony Wexler
Objective: Develop model of atmospheric amines reactions and products related to animal feeding operations and carbon sequestration

Source: National Science Foundation Dates: 09/1/12 to 08/31/13
Title: NSF-AGS post-doctoral fellowship for Dr. Cari Dutcher

PI: Cari S. Dutcher (at UCD)
Objective: Develop statistical thermodynamic models of solutions relevant to atmospheric aerosols

Source: San Joaquin Valley Air Pollution Control District Dates: 6/1/11 to 12/31/12
Title: A Study of Long Range Transport of Air Pollutants to the San Joaquin Valley
PI: Anthony Wexler
Objective: Measure long range transport of pollutants from Asia at MIRA in Big Sur

Source: California Air Resources Board Dates: 6/1/10 to 5/31/14
Title: Health Effects of Central Valley Particulate Matter
PI: Anthony Wexler
Objective: Study toxicity of air pollution in Sacramento in susceptible rat models

Source: National Science Foundation Dates: 11/01/10 to 10/31/13
Title: Hygroscopic Properties of Aerosol Organics
PI: Simon Clegg (at UCD)
Objective: Develop model of organic aerosol particle growth as a function of RH

Source: Electric Power Research Institute Dates: 6/1/09 to 5/31/13
Title: Thermodynamic Properties and Gas/Aerosol Partitioning of Atmospheric Amines
PI: Simon Clegg (at UCD)
Objective: Develop model of atmospheric amines related to animal feeding operations and carbon sequestration

Source: DOE Dates: 5/08 – 4/13
Title: Process Models of the Equilibrium Size and State of Organic/Inorganic Aerosols, for the Development of Large Scale Atmospheric Models and the Analysis of Field Data
PI: Anthony Wexler
Objective: Improve the AIM thermodynamics model in the range of temperatures for the inorganic compounds, particles size dependence, and others.

<u>RESEARCH SUPPORT COMPLETED WITHIN THE LAST THREE YEARS</u>

Source: California Air Resources Board and Electric Power Research Institute Dates: 6/07 to 6/12
Title: Toxicity of Source-Oriented Ambient Aerosol
PI: Anthony Wexler
Objective: Collect source oriented fine and ultrafine particle samples in Fresno and test their relative toxicity.

Source: TSR&TP Dates: 9/05 – 8/11
Title: Atmospheric Aerosols and Health
PI: Anthony Wexler
Subject: Recruiting and training graduate students in air quality
Originating PI: Anthony Wexler; Managing PI: Cort Anastasio

Source: EPA Dates: 9/05-9/11
Title: San Joaquin Valley Aerosol Health Effects Research Center (SAHERC)
PI: Anthony Wexler
Subject: Health effects of atmospheric particles

Source: EPA Dates: 3/06 – 3/11
Title: Ion Mobility Analysis of Particulate Matter and Gas Phase Precursors
PI: Anthony Wexler
Objective: Develop an ion mobility spectrometer for analyzing ambient gases and particulate composition

Source: NOAA Dates: 7/07 – 6/11

Title: Improving Aerosol-Chemical Effects of Radiative Forcing in Climate Models
PI: Anthony Wexler
Objective: Improve the AIM thermodynamic model to include organic compounds, improve the user interface and include climate change calculations.

Source: National Institutes of Health Dates: 4/1/09 to 3/31/11
Title: A nonhuman primate model for the link between childhood asthma and obesity
PI: Eliot Spindel, Oregon Health Sciences University
Objective: Investigate changes in lung architecture in children born to obese mothers

Source: NSF/SBIR Phase 2 Dates: 1/05 to 12/09
Title: Development of a Broad Spectrum Differential Mobility Aerosol Analyzer for Ambient Aerosol Size Distribution Measurements
PI: Anthony Wexler
Subject: Development of a new aerosol spectrometer based on one of Dr. Wexler's patents
Principle Investigator: Fred Brechtel, Brechtel Manufacturing Inc.

Source: California Air Resources Board Dates: 7/05-6/10
Title: Characterization of Versatile Aerosol Concentration Enrichment System
PI: Anthony Wexler
Subject: Test for artifacts in the VACES

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Chiamvimonvat, Nipavan	Professor of Medicine
eRA COMMONS USER NAME NCHIAMVIMONVAT	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto, Canada		1980	Biology
University of Toronto, Canada	M.D.	1984	Medicine

A. Personal Statement

For over a decade, our laboratory has focused on the molecular mechanisms of cardiac arrhythmias in hypertrophy and failure. More recently, we have demonstrated the beneficial effects of a novel class of compounds, namely inhibitors of soluble epoxide hydrolase (sEH) in the prevention of cardiac hypertrophy, remodeling, and arrhythmias. In collaboration with Dr. Chen and Pinkerton, we have previously demonstrated that short-term secondhand smoke exposure decreases heart rate variability and increases arrhythmia susceptibility in mice. The study was published in American Journal of Physiology in 2009.

My laboratory will be able to bring a wealth of experience to the current project, including extensive familiarity with *in vivo* models of cardiac hypertrophy and failure, echocardiography, *in vivo* hemodynamic monitoring, *in vivo* electrophysiologic studies, single-cell phenotyping using flow cytometric analyses, patch-clamp recordings, immunofluorescence confocal microscopy, molecular biological and biochemical techniques. We have recently been successful in obtaining an NIH Core Equipment Grant to purchase a new ultrasound system (VisualSonics Vevo 2100) which will be extremely timely for the proposed project.

Additionally, the two well qualified postdoctoral researchers (**Drs. Ning Li and Padmini Sirish**) in the project are highly motivated and have extensive experiences in the proposed techniques including animal surgeries, *in vivo* imaging, flow cytometric analyses, and the proposed molecular and biochemical techniques. **Dr. Valeriy Timofeyev**, Staff Research Associate, has extensive track records in patch-clamp recordings. **Dr. Deborah Lieu**, Assistant Professor and my long term collaborator, has extensive experience with pluripotent stem cells. Finally, I have an established track record of productivity and grants management that will ensure the timely and successful completion of the proposed research. I am strongly committed to the proposed study.

B. Positions and Honors.

Positions and Employment

1984-85 Intern in Internal Medicine, University of Toronto, Ontario, Canada
1985-87 Resident in Internal Medicine, University of Toronto, Ontario, Canada
1987-89 Fellow in Cardiology, University of Western Ontario, Ontario, Canada
1989-91 Fellow in Clinical Cardiac Electrophysiology, University of Calgary, Alberta, Canada
1991-93 Heart and Stroke Foundation of Canada Research Fellow, University of Calgary, Alberta, Canada
1993-97 Medical Research Council of Canada Research Fellow, Johns Hopkins University, Baltimore, Maryland
1997-2000 Assistant Professor of Medicine, University of Cincinnati, Cincinnati, Ohio
1997-2000 Staff Cardiologist and CCU Attending, Veteran Administration Medical Center, Cincinnati, Ohio
2000-2005 Associate Professor of Medicine, University of California, Davis, CA
2000-present Staff Cardiologist and CCU Attending, Veteran Administrations Medical Center, Mather, CA
2002-present Associate Chief for Research, Division of Cardiovascular Medicine, UC Davis
2005-present Professor of Medicine, University of California, Davis, CA
2006-present Roger Tatarian Endowed Professor of Cardiovascular Medicine, University of California, Davis

2008-present Director, NIH T32 Training Program in Basic and Translational Cardiovascular Science

Honors

1979 The L.V. Redman Prize in Chemistry, Faculty of Arts and Science, University of Toronto
1980 Ann Shepard Memorial Scholarship, Faculty of Arts and Science, University of Toronto, Canada
1981-83 Dr. C.S. Wainwright Memorial Scholarship, Faculty of Medicine, University of Toronto, Canada
1991-1993 Heart and Stroke Foundation of Canada Research Fellowship
1993-1998 Medical Research Council of Canada Research Fellowship
1996 Runner up for the 1996 Young Investigator Award from North American Society for Pacing and Electrophysiology (NASPE)
1997-98 Beginning Grant-in-Aids, American Heart Association-Ohio Affiliate
1997-98 Faculty of Medicine Development Award, University of Cincinnati
1998-2002 National Scientist Development Grant, American Heart Association

Other Experience and Professional Memberships

1997-present Reviewer for *Cardiovascular Research, Circulation Research, Journal of Molecular and Cellular Cardiology, Circulation, American Journal of Physiology, Journal of American College of Cardiology*
2000-present Member, Society of General Physiologists
2000 Peer Review Committee, Ohio Valley Affiliate, American Heart Association
2001-present Peer Review Committee, CV Pathophysiology Study Group 1, American Heart Association
2002-11 Editorial Board, *Circulation Research*
2002 Special Emphasis Panel, ECS Study Section, NIH
2002-2003 Ad hoc member, Peer Review Committee, CVA Study Section, NIH
2004-2008 Member, Peer Review Committee, ESTA Study Section, NIH
2006-present Editorial Board, *American Journal of Physiology* (Heart and Circulatory)
2007-2009 Vice Chair, Peer Review Committee 2B, American Heart Association, Western Affiliates
2007,09-12 Member, Special Emphasis Panel, ZRG1-CVS-F03 Study Section, NIH
2008 Chair, American Heart Association Western Affiliate, Young Investigator Forum
2009 Vice Chair, AHA Jon Holden DeHaan Cardiac Myogenesis Research Centers Study Group
2009 Member, NIH Special Emphasis Panel
2008-10 Vice Chair, Research Committee, American Heart Association, Western Affiliates
2010-11 Chair, Peer Review Committee 2B, American Heart Association, Western Affiliates
2010-12 Chair, Research Committee, American Heart Association, Western Affiliates
2012-present Member, NHLBI Institutional Training Mechanism Review Committee (NITM)

C. Selected peer-reviewed publications

Most relevant to the current application:

1. Xu D, Li N, He Y, Timofeyev V, Lu L, Tsai HJ, Kim IH, Tuteja D, Mateo RK, Singapuri A, Davis BB, Low R, Hammock BD, **Chiamvimonvat N**. Prevention and reversal of cardiac hypertrophy by soluble epoxide hydrolase inhibitors. *Proc Natl Acad Sci U S A.* 2006;103:18733-18738. PMCID: 1693731

2. Chen CY, Chow D, **Chiamvimonvat N**, Glatter KA, Li N, He Y, Pinkerton KE, Bonham AC. Short-term secondhand smoke exposure decreases heart rate variability and increases arrhythmia susceptibility in mice. *Am J Physiol Heart Circ Physiol.* 2008;295:H632-639.

3. Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, Lu L, Yang J, Mochida H, Low R, Hammock BD, **Chiamvimonvat N**. Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol.* 2009;47:835-845. PMID: 19716829

4. Li N, Liu JY, Qiu H, Harris TR, Sirish P, Hammock BD, Chiamvimonvat N. Use of metabolomic profiling in the study of arachidonic acid metabolism in cardiovascular disease. *Congest Heart Fail*. 2011;17(1):42-46. PMCID: PMC2947894

5. Liu JY, Li N, Yang J, Qiu H, Ai D, **Chiamvimonvat N**, Zhu Y, Hammock BD. Metabolic profiling of murine plasma reveals an unexpected biomarker in rofecoxib-mediated cardiovascular events. *Proc Natl Acad Sci U S A.* 2010;107:17017-17022. PMCID: PMC2947894

Additional recent publications of importance to the field (in chronological order):

1. Ahmmed GU, Dong PH, Song G, Ball NA, Xu Y, Walsh RA, **Chiamvimonvat N**. Changes in Ca^{2+} cycling proteins underlie cardiac action potential prolongation in a pressure-overloaded guinea pig model with cardiac hypertrophy and failure. *Circ Res.* 2000;86:558-570. PMID: 10720418

2. Zhang Z, Xu Y, Song H, Rodriguez J, Tuteja D, Namkung Y, Shin HS, **Chiamvimonvat N**. Functional Roles of $Ca_v1.3$ (α_{1D}) calcium channel in sinoatrial nodes: insight gained using gene-targeted null mutant mice. *Circ Res.* 2002;90:981-987.PMID:18096820

3. Xu Y, Tuteja D, Zhang Z, Xu D, Zhang Y, Rodriguez J, Nie L, Tuxson HR, Young JN, Glatter KA, Vazquez AE, Yamoah EN, **Chiamvimonvat N**. Molecular identification and functional roles of a Ca^{2+}-activated K^+ channel in human and mouse hearts. *J Biol Chem.* 2003;278:49085-49094.PMID:13679367

4. Zhang Z, He Y, Tuteja D, Xu D, Timofeyev V, Zhang Q, Glatter KA, Xu Y, Shin HS, Low R, **Chiamvimonvat N.** Functional roles of $Ca_v1.3(\alpha_{1D})$ calcium channels in atria: insights gained from gene-targeted null mutant mice. *Circulation.* 2005;112:1936-1944. PMID: 16172271

5. Timofeyev V, He Y, Tuteja D, Zhang Q, Roth DM, Hammond HK, **Chiamvimonvat N**. Cardiac-directed expression of adenylyl cyclase reverses electrical remodeling in cardiomyopathy. *J Mol Cell Cardiol*. 2006;41(1):170-81. PMID: 16750219

6. Lu L, Zhang Q, Timofeyev V, Zhang Z, Young JN, Shin HS, Knowlton AA, **Chiamvimonvat N**. Molecular coupling of a Ca^{2+}-activated K^+ channel to L-type Ca^{2+} channels via alpha-actinin2. *Circ Res.* 2007;100:112-120. PMID: 17110593

7. Zhang Q, Timofeyev V, Lu L, Li N, Singapuri A, Long MK, Bond CT, Adelman JP, **Chiamvimonvat N.** Functional roles of a Ca^{2+}-activated K^+ channel in atrioventricular nodes. *Circ Res.* 2008;102:465-471. PMID: 18096820

8. Lu L, Timofeyev V, Li N, Rafizadeh S, Singapuri A, Harris TR, **Chiamvimonvat N.** Alpha-actinin2 cytoskeletal protein is required for the functional membrane localization of a Ca^{2+}-activated K^+ channel (SK2 channel). *Proc Natl Acad Sci U S A.* 2009;106:18402-18407. PMCID: 2775294

9. Zhang Q, Timofeyev V, Qiu H, Lu L, Li N, Singapuri A, Torado CL, Shin HS, **Chiamvimonvat N**. Expression and roles of $Ca_v1.3$ (α_{1D}) L-Type Ca^{2+} Channel in atrioventricular node automaticity. *J Mol Cell Cardiol.* 2011;50:194-202.

10. Sirish P, Lopez JE, Wong A, Timofeyev V, Young JN, Majdi M, Chen H-SV, **Chiamvimonvat N**. MicroRNA Profiling of Cardiac Progenitor Cells Predicts a Variance in The Proliferative Potential of Cells Derived From Neonatal and Adult Hearts. Journal of Molecular and Cellular Cardiology, 2012, 52(1):264-272. PMID: 22062954

Patent Application:

U.S. National Phase Patent Application based on PCT/US2006/022054: Use of Cis-Epoxyeicosatrienoic acids and inhibitors of soluble epoxide hydrolase to reduce cardiomyopathy.

D. Research Support

Ongoing Research Support

1. Veteran Administration Merit Review Grant Chiamvimonvat (PI) 10/01/09-09/30/13

Department of Veteran Affairs; Functional roles of atrial specific ion channels.

The major goals of this project are to study the functional roles of interacting proteins on atrial specific ion channels trafficking using live-cell imaging.

Role: PI

2. NIH R01 HL85844 Chiamvimonvat (PI) 04/15/07-06/30/16

Functional interaction of cardiac ion channels

The major goals of the project are to examine the functional interactions of cardiac calcium channels and calcium-activated potassium channels using yeast two-hybrid screen, co-immunoprecipitation, pull-down assay, siRNA and patch-clamp techniques.

Role: PI

3. NIH R01 HL85727 Chiamvimonvat (PI) 02/01/08-01/31/13

Mechanisms and treatment of cardiac arrhythmias.

The major goals of the project are to examine the beneficial effects of soluble epoxide hydrolase inhibitors in cardiac hypertrophy and failure using murine models.

Role: PI

4. NIH R01 HL85727 (Knowlton, PI) 07/01/08-06/30/13
Human cardiomyopathy and HSP 60
The major goals of the project are to examine the roles of heat shock proteins in cardiomyopathy
Role: Collaborator

5. NIH T32 86350 Chiamvimonvat (PI) 03/01/08-02/28/13
Training program in basic and translational cardiovascular science
The major goals of this project are to train a new cadre of basic and translational cardiovascular sciences at the graduate and post-graduate levels as well as medical students and residents in a multidisciplinary environment.
Role: PI

6. NIH R01 096819 Gomes (PI) 05/01/10-04/30/15
The role of the proteasome in troponin related cardiomyopathies
Role: Collaborator

7. Howard Hughes Medical Institute Med into Grad Training Program 07/01/06-60/30/14
The major goals of this Institutional Training Grant is to train PhD students in biomedical sciences in translational research
PI: Frederich J. Meyers
Role: Co-Director

8. California Institute of Regenerative Medicine 01/01/10-12/01/13
Building cardiac tissue from stem cells and natural matrices
PI: K. McCloskey
Subcontract PI: N. Chiamvimonvat

9. California Institute of Regenerative Medicine 01/01/13-12/31/15
Induction of stem cell-derived pacemaking cells
The goal of the study is to use small molecules to increase the differentiation of human pluripotent stem cells into pacemaking cells.
PI: D. Lieu
Role: Co-investigator

10. NIH S10 RR033106 Chiamvimonvat (PI) 04/01/13-03/31/14
In vivo ultrasound imaging system (VisualSonics Vevo 2100)
This is a core equipment grant for the new in vivo ultrasound imaging system for small animal models.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Pinkerton, Kent E	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) KEPINKERTON	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Brigham Young Univ., Provo, UT	B.S.	1974	Microbiology,Chemistry
Duke University, Durham, NC	M.S.	1978	Pathology
Duke University, Durham, NC	PhD	1982	Pathology

A. Personal Statement - Dr. Pinkerton has authored more than 200 articles and book chapters on the health effects of gases, particles, fibers and tobacco smoke in both indoor and outdoor environments. He is a leading expert in air pollution health and tobacco smoke research, with an emphasis in lung development and the fetal basis of adult-onset respiratory diseases (asthma and chronic obstructive pulmonary disease). He oversees a state-of-the-art inhalation exposure facility to expose laboratory animals to gases, particulates and fibers, along with secondhand smoke. Dr. Pinkerton leads an active research program in environmental pulmonary toxicology with funding from federal, state and private agencies. He has mentored more than 40 graduate students and is a graduate faculty member in comparative pathology, immunology, pharmacology and toxicology at UC Davis.

B. Positions and Honors

Positions and Employment

1982-84 Research Associate, Division of Allergy, Critical Care and Respiratory Medicine, Department of Medicine, Duke University Medical Center
1984-86 Assist. Medical Research Professor, Department of Pathology, Duke University Medical Center
1986-92 Assist. Adjunct Professor, Dept. of Anatomy and Cell Biology, University of California at Davis
1992-98 Assoc. Professor-in-Residence, Dept. of Anatomy, Physiology and Cell Biology, University of California at Davis
1998-present Professor-in-Residence, Dept. of Anatomy, Physiology and Cell Biology, University of California at Davis
2001-present Director, Center for Health and the Environment, University of California at Davis
2005-present Professor, Dept. of Pediatrics School of Medicine, University of California at Davis

Other Experience and Professional Memberships

American Association for the Advancement of Science, American Association of Veterinary Anatomists, American Thoracic Society,Society of Toxicology

Honors

1998/2000 Favorite Teacher Award, School of Veterinary Medicine, Classes of 2001/2003
2006 Faculty Teaching Award, School of Veterinary Medicine
2008 University Citation for Distinguished Teaching, University of California, Davis

C. Selected Relevant Publications (from more than 200):

2012 Amy K. Madl, Stephen V. Teague, Yongquan Qu, Daniel Masiel, James E. Evans, Ting Guo, Kent E. Pinkerton. Aerosolization System for Experimental Inhalation Studies of Carbon-

Based Nanomaterials, Aerosol Science and Technology, 46:1, 94-107.

2012 Plummer LE, Ham WH, Kleeman MJ, Wexler AE, Pinkerton KE. 2012 Influence of Season and Location on Pulmonary Response to California's San Joaquin Valley Airborne Particulate Matter, Journal of Environmental Toxicology and Health, 75:253–271.

2011 Filosto S, Castillo S, Danielson A, Franzi L, Khan E, Kenyon N, Last J, Pinkerton K, Tuder R, Goldkorn T. Neutral sphingomyelinase 2: a novel target in cigarette smoke-induced apoptosis and lung injury. Am J Respir Cell Mol Biol. 44(3):350-60.

2010 Westbrook DG, Anderson PG, Pinkerton KE, Ballinger SW. Perinatal tobacco smoke exposure increases vascular oxidative stress and mitochondrial damage in non-human primates. Cardiovasc Toxicol. 10(3):216-26, 2010.

2009 Bolton, S.J., Pinnion, K., Oreffo, V., Foster, M., and Pinkerton, K.E. Characterization of the proximal airway squamous metaplasia induced by chronic tobacco smoke exposure in spontaneously hypertensive rats. Respiratory Research 10:118.

2009 Madl, A.K., and Pinkerton, K.E. Health effects of inhaled engineered and incidental nanoparticles. Critical Reviews in Toxicology 39:629-658.

2009 Woodruff, P.G., Ellwanger, A., Solon, M., Cambier, C.J., Pinkerton, K.E., and Koth, L.L. Alveolar macrophage recruitment and activation by chronic second hand smoke exposure in mice. COPD: Journal of Chronic Obstructive Pulmonary Disease 6:86-94.

2008 Yu, M., X. Zheng, J. Peake, J.P. Joad, and K.E. Pinkerton. Perinatal environmental tobacco smoke exposure alters the immune response and airway innervation in infant primates. JACI 122:640-647.

2008 Yu, B., U.P. Kodavanti, M. Takeuchi, H. Witschi and K. E. Pinkerton. Acute tobacco smoke-induced airways inflammation in spontaneously hypertensive rats. Inhal Toxicol 20:623-633.

2008 Donaldson, K., P.J.A. Borm, G. Oberdorster, K.E. Pinkerton, V. Stone, and C.L. Tran. Concordance between *in vitro* and *in vivo* dosimetry in the proinflammatory effects of low-toxicity, low-solubility particles: the key role of the proximal alveolar region. Inhal Toxicol 20:53-62.

2007 Cakir, Y., Z. Yang, C.A. Knight, M. Pompilius, D. Westbrook, S.M. Bailey, K.E. Pinkerton, and S.W. Ballinger. Effect of alcohol and tobacco smoke on mtDNA damage and atherogenesis. Free Rad Bio Med 43:1279-1288.

2007 Wang, L.W., J.P. Joad, K. Abel, A. Spinner, S. Smiley-Jewell, H. Liu, and K.E. Pinkerton. Effects of environmental tobacco smoke on the developing immune system of infant monkeys. J Allergy Clin Immunol 120(2):445-451.

2006 Kodavanti, U.P, M.C. Schladweiler, A.D. Ledbetter, R.V. Villalobos-Ortuno, M.C. Suffia, P. Evansky, J.H. Richards, R.H. Jaskot, R. Thomas, E. Karoly, Y-C T. Huang, D.L. Costa, P.S. Gilmour, and K.E. Pinkerton. The spontaneously hypertensive rat: an experimental model of sulfur dioxide-induced airways disease. Toxicol Sci 94(1):193-205.

2005 Zhong, C.Y., *Y.M. Zhou, G.C. Douglas, H. Witschi, and K.E. Pinkerton. MAPK/AP-1 signal pathway in tobacco smoke-induced cell proliferation and squamous metaplasia in the lungs of rats. Carcinogenesis 26(12):2187-2195.

D. RESEARCH SUPPORT

ACTIVE

U01 ES02027 (Pinkerton/Van Winkle) 10/1/10-09/30/15 1.8 cal mo 15%
NIEHS $1,709,079
Engineered Nanomaterials: Linking Physical and Chemical Properties to Biology
The goal of this program is to systematically explore the influence of physicochemical properties of engineered nanomaterials (ENMs). We will define the effect of these physical/chemical properties on how ENMs interact with the intact organism, specific target organs and specific cell types within the target organs. ENMs are tested in a series of systematic examinations of absorption and distribution following inhalation/ingestion exposures.

NIH R01 (Goldkorn/Pinkerton, Co-I) 07/01/09-06/30/14 0.6 cal mo 5%
$9,078

Molecular Characterization of a Novel Lung Sphingomyelinase
Role: Co-I (PI Goldkorn)
The goal of this study is to examine the role of sphingomyelinase in epithelial injury and repair following tobacco smoke exposure. The role of sphingomyelinase parenchymal tissue injury, destruction and remodeling will be examined. Enlargement of the airspaces are highly suggestive of a destructive process that is likely to involve deregulation of apoptotic –controlled signaling pathways.

U50 OH07550 (Schenker, M) 09/30/01-09/29/13 0.60 cal mo 5%
$75,000
Research C: Health Effects of Ambient Airborne Particles from the Sacramento and San Joaquin Valleys
Role: Co-investigator & Co-Director
The major goals are to examine the relationship between mineral dust exposure in the farming industry and histopathological changes occurring in the lungs of the California farm worker, and to determine the respiratory health effects of short-term exposure to concentrated ambient particles of California agriculture in mice and rats.

California Air Resources Board 06/1/10-05/31/13 0.24 cal mo 2%
(Pinkerton and Wexler) $160,000
Toxicity of source-oriented ambient aerosol
Role: Co-PI
The focus of this study establish the relative toxicity ranking of ambient, source-oriented particles using a novel collection system and *in vivo* biological testing.

R01 CA-151601 (Spindel) Oregon Health & Science University: **GPRC00884B** (subaward to UC Davis)
UC Davis (Pinkerton) 5/1/12-2/28/16 $8,950.00 0.36 cal mo 3%
Nicotine, nicotinic receptors and lung cancer
Role: PI of UC Davis subaward
The goal of this subaward is perform exposure of mice to tobacco smoke to examine tumor development.

<u>COMPLETED</u>

Astra Zeneca 09-001448 06/01/09-05/31/12 0.12 cal mo 1%
Epithelial repair in COPD $162,377/year
Role:PI
The goals of this study are to examine the development of epithelial injury and adaptation to progressive exposure to tobacco smoke in the spontaneously hypertensive rat.

UC Tobacco-Related 07/01/09-06/30/12 0.6 cal mo 5%
Disease Research Program 09-00025 $125,000/year
Role: PI
Environmental Tobacco Smoke and Influenza Viral Infection
The goal of this study is to examine the influence of perinatal exposure to environmental tobacco smoke on susceptibility to influenza infection and enhanced danger of secondary bacterial infection.

NIH RC1 ES018232-01 10/01/09-06/30/12 1.2 cal mo 10%
$331,558/year
Novel Approaches to Evaluate Carbon Nanotube Health Impacts
Role: PI
The goal of this study is to examine unique approaches to better understand the mechanisms of toxicity of carbon nanotubes in laboratory rats by inhalation.

P51 RR-00169 (Klein, B) 05/01/05-04/30/12 1.8 cal mo 15%
California National Primate Research Center

Role: Staff Scientist
To serve as a Staff Scientist in the Inhalation Core and provide advice to the Morphology Core of the California National Primate Research Center. No overlap.

RD-831918 (Bonham, A.C.) 08/01/04-7/31/09
EPA
The Role of Air Pollutants in Cardiovascular Disease
Role: Co-Investigator
Examine the health effects of repeated short-term exposure to ambient environmental particles on the autonomic control of the cardiovascular system. Studies are designed to examine pulmonary and cardiovascular changes induced by particles of the California Central Valley in both urban and rural locations.

R01 ES012957 (Chen, Chao-Yin) 5/1/04-3/31/09
NIH
Particulate Matter Exposure: Cardiovascular Mechanisms
Role: Co-Investigator
Determine if short-term (3-day) exposure to $PM_{2.5}$ results in a reduced HRV due to decreases in the intrinsic membrane properties and/or synaptic excitability of anatomically and functionally identified CNS cardiac vagal neurons in the nucleus ambiguous that regulate HRV.

KEITH J. BEIN
Assistant Research Scientist
Air Quality Research Center
One Shields Avenue
University of California
Davis, CA 95616
Phone: (530) 754-4963
Fax: (530) 754-4962
E-mail: kjbein@ucdavis.edu

EDUCATION

Ph.D., Atmospheric Sciences — June, 2007
University of California, Davis

B.S., Physics — May, 2001
California State University, Chico

B.S., Chemistry — May, 2001
California State University, Chico
ACS Certified

EMPLOYMENT EXPERIENCE

Center for Health and the Environment — 2012-present
University of California, Davis
Title: Research Faculty

Air Quality Research Center — 2009-present
University of California, Davis
Title: Assistant Research Scientist

Air Quality Research Center — 2007-2009
University of California, Davis
Title: Postdoctoral Scholar

Department of Land, Air and Water Resources, — 2001-2007
University of California, Davis
Title: Research Assistant

HONORS AND AWARDS

The National Dean's List, 2007
ACCESS Colloquium Participant, 2007
Gordon Research Conference Participant, 2007
Travel Award, American Association for Aerosol Research, 2005
Travel Award, U.C. Davis, 2004

Honorable Mention, National Science Foundation, 2003
Jastro Shields Scholarship, U.C. Davis, 2002, 2003
Honors in general education, C.S.U. Chico, 2001
Honors in chemistry, C.S.U. Chico, 2001
Honors in physics, C.S.U. Chico, 2001
Floyd L. English Scholarship, C.S.U. Chico, 1998, 1999
Outstanding Student Award, C.S.U. Chico, 1997, 1998, 1999
Dean's List, C.S.U. Chico, 1995, 1996, 1997, 1998, 1999, 2000

SELECTED REFEREED PUBLICATIONS

1. Bein, K.J., Zhao, Y., Wexler, A.S., and M.V. Johnston (2005), Speciation of size-resolved individual ultrafine particles in Pittsburgh, Pennsylvania, *JGR – Atmospheres*, 110, D07S05.

2. Zhao, Y., Bein, K.J., Wexler, A.S., Misra, C., Fine, P.M. and C. Sioutas (2005), Field evaluation of the versatile aerosol concentration enrichment system (VACES) particle concentrator coupled to the rapid single particle mass spectrometer (RSMS-3), *JGR - Atmospheres*, 110, D07S02.

3. Pekney, N.J., Bein, K.J., Davidson, C.I., Wexler, A.S., and M.V. Johnston (2006), Identification of sources of atmospheric PM at the Pittsburgh Supersite. Part I: Single particle analysis and filter-based Positive Matrix Factorization, *Atmospheric Environment*, 40(S2), S411-S423.

4. Bein, K.J., Zhao, Y., Pekney, N.J., Davidson, C.I., Johnston, M.V., and A.S. Wexler (2006), Identification of sources of atmospheric PM at the Pittsburgh Supersite. Part II: Quantitative comparisons of single particle, particle number, and particle mass measurements, *Atmospheric Environment*, 40(S2), S424-S444.

5. Bein, K.J., Zhao, Y., Johnston, M.V., and A.S. Wexler (2007), Identification of sources of atmospheric PM at the Pittsburgh Supersite. Part III: Source characterization, *Atmospheric Environment*, 41(19), 3974-3992.

6. Bein, K.J., and A.S. Wexler (2007), Activity interpretation in H_2O-H_2SO_4 binary nucleation, *Journal of Chemical Physics*, 127, 124316.

7. Bein, K.J., Zhao, Y., Johnston, M.V., and A.S. Wexler (2008), Interactions between boreal wildfire and urban emissions, *JGR – Atmospheres*, 113(D7), D07304.

8. Bein, K.J., Zhao, Y., Johnston, M.V., Evans, G.J., and A.S. Wexler (2008), Extratropical waves transport boreal wildfire emissions and drive regional air quality dynamics, *JGR – Atmospheres, 113*, D23213.

9. Bein, K.J., Zhao, Y. and A.S. Wexler, Conditional sampling for source-oriented toxicological studies using a single particle mass spectrometer (2009), *Environmental Science & Technology*, 43(24), 9445-9452.

Preliminary Cost Proposal

Task	Labor	Fringe	Travel	Supplies	Analyses	Overhead	Total
1	48,115	14,435	1,000	10,000	10,000	8,255	90,804
2	241,686	71,902	1,000	50,000		36,359	399,947
3	179,731	57,443	1,000	250,000		48,717	535,892
4	117,418	35,956			10,000	16,337	179,711
5	117,418	35,956				15,337	168,711
Total	704,368	215,692	3,000	310,000	20,000	125,006	1,378,066

Budget note: We propose here (1) a comprehensive set of endpoint measurements to explore the range of mechanisms whereby ozone and PM could lead to cardiac dysfunction and (2) the use of source-oriented PM collected in Fresno and the Caldicott Tunnel so that the health effects and mechanisms can be related to responsible sources relevant to California. The result is a budget that may not be affordable for CARB. In parallel with submitting this proposal to CARB, we are also submitting it to EPRI to request cost-sharing. EPRI co-funded the original source-oriented toxicology study with CARB so they may be inclined to continue this partnership.