TECHNICAL PROPOSAL

CARDIOVASCULAR EFFECTS OF MULTIPOLLUTANT EXPOSURE: MECHANISMS AND INTERACTIONS OF OZONE AND PM

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This proposal uses animal subjects X

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Abstract

Many studies have reported a significant association between fine PM2.5 and adverse cardiovascular effects. Our recent studies, which were funded in part by the California Air Resources Board, have demonstrated that exposure to quasi-ultrafine (UFP; PM0.15) and fine (FP; PM2.5) accelerate atherosclerosis and impair autonomic control of heart rate and heart rate variability (HRV) in mice that are genetically prone to developing cardiovascular disease, through modes of action that include inflammatory and oxidative stress-related pathways. These studies have also shown that the PM-induced cardiovascular effects can be blocked if the organic constituents are removed from the particles using a thermal denuder before the particles are inhaled by the mice suggesting that these organic constituents may play an important role. The mode of action may involve inflammation, formation of free radicals and oxidative stress.

California residents are often exposed to particles in mixtures with ozone (O_3). Some recent studies have suggested that O_3 exposure, which is well-known to induce respiratory effects, may also adversely affect the cardiovascular system. Ozone is a strong oxidant and has been shown to induce oxidative changes in the lung and to promote respiratory system inflammation, hence there may be some mechanistic overlap between the effects of PM and those of O_3. This overlap suggests that there could be potential interactions between inhaled PM and O_3, specifically with respect to effects on heart pathophysiology and function that could modulate the individual effects of either pollutant. Therefore, the overall objectives of this study are to: 1) to elucidate the mechanistic pathways through which concurrent PM2.5 and ozone exposures induce cardiovascular effects, and 2) to determine whether there are additive or synergistic interactions between these two air pollutants.

Sioutas and colleagues previously developed the capacity to couple a thermal denuder to a Versatile Aerosol Concentration Enrichment System (VACES). The thermal denuder heats the aerosol to a specified temperature to evaporate and remove semi-volatile components, and then returns the aerosol to the original temperature. The VACES can be used to increase the concentration of the processed aerosol by factors of 10 to 20 to provide adequate concentrations for performing acute in vivo toxicology exposure studies. We will use our in-vivo rodent exposure system in combination with the VACES-thermal denuder technology to separately study the cardiopulmonary effects of PM ± O_3, before and after the removal of the semi-volatile components to test three hypotheses. (1) Concurrent exposure to a mixture of concentrated ambient FP (CAPs) and O_3 will, through modes of action involving systemic inflammation, oxidative stress and lipid peroxidation produce greater acceleration of atherosclerosis and reduction in HRV than will exposure to either CAPs alone or O_3 alone; (2) CAPs generated during periods of high ambient photochemical activity (i.e. summer) will be more potent than CAPs generated during periods of low ambient photochemical activity (i.e. winter) with respect to CAPs-induced acceleration of atherosclerosis and impairment of HRV through mechanisms of oxidation of serum lipids and LDL leading to foam cell formation and incorporation into arterial plaques and oxidative stress leading to arterial wall thickening and tissue damage; and (3) the removal of organic constituents of PM2.5 using a thermal denuder will block the effects of PM but not those of O3 with respect to acceleration of atherosclerosis and impairment of HRV through mechanisms of oxidative stress and lipid peroxidation. To test these hypotheses we will measure markers of inflammation, measure arterial plaques and lipid incorporation, measure serum lipids and low density lipoprotein associated cholesterol (LDL), oxidized LDL and monitor changes in heart function using implanted electrocardiograpic transponders in mice genetically modified to be susceptible to developing atherosclerosis. These experiments will be conducted over a period of 3 years.

Introduction

Residents of California have been exposed historically to high ambient concentrations of both PM and O_3, albeit not always at the same time. Air quality in California has been greatly improved over the past two decades but National Ambient Air Quality Standards (NAAQS) and California standards for both pollutants continue to be exceeded, at times. Epidemiologic studies, which are the health-related basis for the NAAQS, have shown that PM-related health effects on the cardiovascular system are large and clinically significant, but there are substantial gaps and uncertainties in our understanding of how inhaled particles that are deposited in the lung can have large effects on more distal organs such as the brain and the heart. To date, mechanistic studies investigating how inhaled PM induces adverse health effects have focused on generic, non-specific modes of action (e.g., oxidative stress and inflammation) that are not unique to air pollution.

In contrast, the ozone NAAQS is primarily based on human exposure studies that have investigated the relationship between well-defined ozone exposures and changes in clinical endpoints, primarily of the respiratory system. While mechanistic pathways through which ozone exposure affects respiratory health effects have been studied, recent research suggests that ozone exposure may also have cardiovascular effects. However, little is known about potential biological mechanisms for ozone-induced cardiovascular effects. In addition, although humans are often exposed to both PM and O_3 as parts of a complex mixture of ambient air pollutants, little is known as to whether or not there are interactions or synergisms among these two important ambient pollutants. Understanding these potential interactions will provide critical data that can assist in the development of air quality policies that can efficiently and effectively protect public health.

Epidemiological and in vivo exposure studies demonstrate that particles (fine and ultrafine) in close proximity to mobile source emissions are more toxic than particles in the air more distant from the source and that they accelerate the development of atherosclerotic plaque which is a major contributor to cardiovascular disease and deaths associated with heart disease. Heart disease is arguably the most important cause of nan-accidental deaths; approximately 50% of deaths can be attributed to heart disease. Associations of O_3 with mortality, and specifically with heart-related mortality, have been reported (Brook et al., 2004; Henrotin et al., 2010; Ito, 2011) but are less strongly established than those for PM (Schwartz and Morris, 1995). This may be due in part to the co-variation of PM and O_3 and to the seasonal variations of O_3 ambient concentrations which might obscure relationships to some disease outcomes. However, there is evidence from animal studies that inhaled O_3 can induce vascular dysfunction, mitochondrial damage and initiate development of atherosclerosis (Chuang et al., 2009). O_3 exposure can also increase myocardial work and impair pulmonary gas exchange to a degree that might be clinically important in persons with significant preexisting cardiovascular impairment (Gong et al., 1998). Mechanistically, both O_3 and PM cause inflammation and can induce oxidative stress when inhaled which suggests that in combination they might act in an additive or perhaps synergistic manner.

Therefore, the objectives of this study are to: 1) to elucidate the mechanistic pathways through which concurrent PM2.5 and ozone exposures induce cardiovascular effects, and 2) to determine whether there are additive or synergistic interactions between these two air pollutants.

Preliminary Results

O_3, or ozone reaction products on PM, can affect heart function: Rats were implanted with DSI telemetry devices and exposed to CAPs in Riverside for 8 weeks during a period of high photochemical activity. It is important to note that the particle concentrator, in addition to increasing the particle concentration, efficiently (~80 to 100%) scrubs O_3 from the CAPs exposure atmosphere. During these exposures, Dr. Paulson from UCLA measured the hydrogen peroxide (H2O2) concentration in the particles and also acquired pollutant and meteorological data from a nearby air monitoring station (Rubidoux, CA). Table1 shows correlations particularly between UFP concentrations and H_2O_2 and Rubidoux relative humidity (RH) and O_3, and the product of the two. The RH×O_3 product is significantly correlated with H_2O_2 generation by UF particles confirming that ambient O_3 contributes to the formation of particle phase oxidant species.

Table 1 Correlation between ultrafine H_2O_2, O_3, RH and the product of O_3 and RH.
Table 4-1-2.

		RH (%)	O_3(ppm)	RH×O_3
H_2O_2 (ng/m^3)	R	0.174	0.343	0.427
	P	0.415	0.101	0.038
	N	24	24	24

This leads to the possibility that even though gas phase O_3 is removed from CAPs exposure atmospheres, atmospheric reactions between O_3 and PM could enrich PM in oxidant species that penetrate the concentrator and can be inhaled. If so, one might see a correlation between ambient O_3 and changes in heart functions as well as a relationship between particle-bound H_2O_2 and changes in heart functions. Table 2 shows correlations between ultrafine H_2O_2 and particle number measured at the Riverside site, and O_3, NO_2, and CO from the Rubidoux site, with cardiovascular parameters (blood pressure, rate of pressure development [dP/dt] and the interbeat interval [IBI; inverse of the heart rate]) in animals exposed to concentrated ultrafine particles. Data shown represent during exposure (same day) and the evening period after the exposure (half day lag). H_2O_2 was correlated with increased diastolic blood pressure during exposures (p=0.046) and decreased developed pressure (p=0.066). Ambient O_3, however, was associated with decreased blood pressure and increased heart rate (reduced IBI). In contrast, CPC correlations increase for blood pressure measurements but decrease for dPdt and heart rate.

Table 2 Correlations between ultrafine H2O2 and particle number measured at the CR-CAES site, and O3, NO2, and CO from the Rubidoux site, with cardiovascular parameters in rats exposed to concentrated ultrafine particles and purified air [1,2]

		Diastolic Pressure (mmHg)		Mean Pressure (mmHg)		Systolic Pressure (mmHg)		dPdt (mmHg / sec)		IBI (seconds / beat)	
		Same Day	Half Day Lag	Same Day	Half Day Lag	Same Day	Half Day Lag	Same Day	Half Day Lag	Same Day	Half Day Lag
H_2O_2	R	**0.410**	0.327	0.373	0.307	0.331	0.294	-0.381	-0.266	0.046	0.014
	P	0.046	0.119	0.073	0.144	0.114	0.163	0.066	0.210	0.831	0.949
	N	24	24	24	24	24	24	24	24	24	24
CPC	R	0.132	**0.434**	0.109	**0.411**	0.115	**0.385**	-0.230	-0.277	0.420	-0.100
	P	0.386	0.003	0.476	0.005	0.454	0.009	0.129	0.065	0.004	0.515
	N	45	45	45	45	45	45	45	45	45	45
O_3	R	**-0.403**	**-0.511**	**-0.403**	**-0.514**	**-0.413**	**-0.509**	0.177	0.282	**-0.342**	-0.123
	P	0.006	0.000	0.006	0.000	0.005	0.000	0.246	0.060	0.022	0.421
	N	45	45	45	45	45	45	45	45	45	45
NO_2	R	**-0.461**	-0.135	**-0.451**	-0.101	**-0.420**	-0.074	-0.026	0.127	-0.008	-0.252
	P	0.001	0.375	0.002	0.511	0.004	0.631	0.866	0.404	0.959	0.095
	N	45	45	45	45	45	45	45	45	45	45
CO	R	-0.275	0.048	**-0.302**	0.073	**-0.304**	0.091	**-0.312**	-0.104	0.079	-0.125
	P	0.068	0.754	0.044	0.634	0.042	0.553	0.037	0.495	0.606	0.413
	N	45	45	45	45	45	45	45	45	45	45
H_2O_2*CPC	R	**0.499**	**0.410**	**0.449**	0.382	0.394	0.359	**-0.507**	-0.325	0.024	-0.001
	P	0.013	0.046	0.028	0.065	0.057	0.085	0.011	0.121	0.913	0.996
	N	24	24	24	24	24	24	24	24	24	24

[1]r is the Pearson Correlation Coefficient. P is the significance (2-tailed). This value, if multiplied by 100, gives the probability that the correlation occurred randomly. Values below 0.05 are considered significant, and values below 0.01 very significant. N is the number of measurements.

[2]Values in bold indicate significant or very significant correlations.

Figure 1 shows the correlation of day to day changes in diastolic pressure over an 8 week period with the daily H_2O_2*Particle Number (CPC) product. While these results are preliminary, given that the exposure study was not designed to elucidate the role of ambient O_3 and particle-bound oxidants, they are suggestive of potential O_3 and particle interactions that could affect the heart.

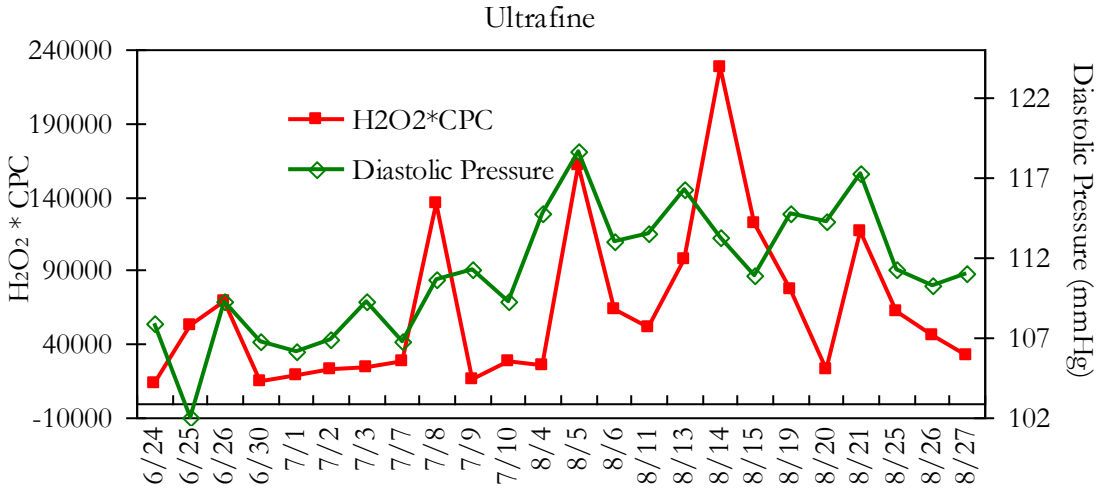

Figure 1 Time series of positively correlated H_2O_2×particle number and diastolic pressure in exposed animals.

EC and OC are important components of ambient PM with respect to respiratory effects: Our previous studies of mice exposed to fine and ultrafine concentrated ambient particles (CAPs) ~50 m downwind of a freeway showed that these exposures had significant biological activity in which appearance of airway allergy biomarkers was associated with elemental and organic carbon (EC and OC, respectively) fractions of the aerosol. When exposures were performed 150 m downwind of the freeway the biological activity was greatly diminished and there were no exposure-related effects (Kleinman *et al.*, 2005). These associations can be seen in Figure 2.

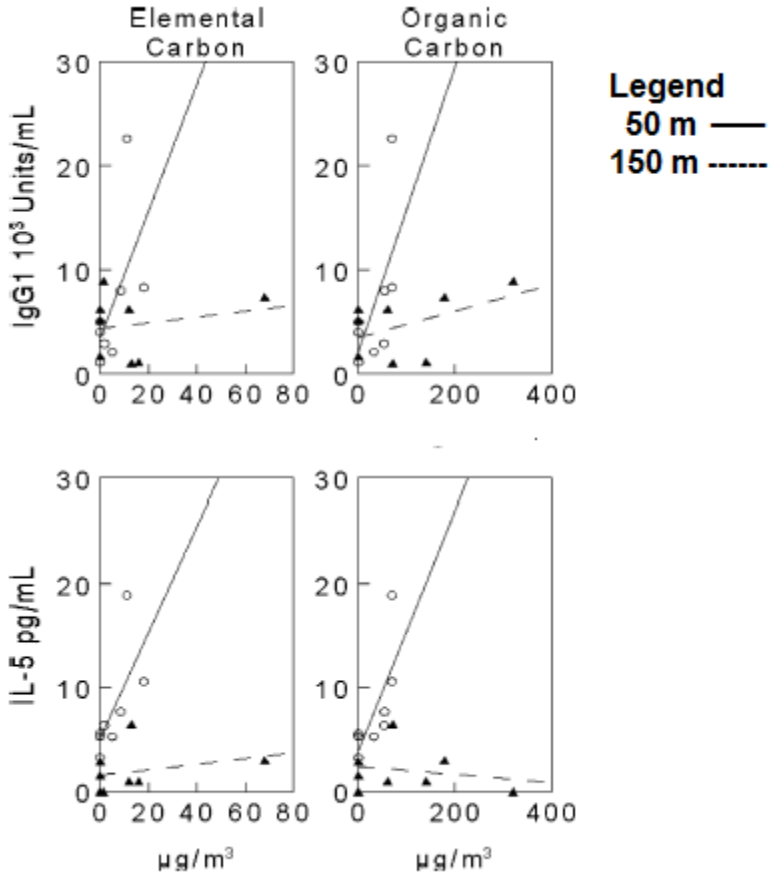

Figure 2 Exposure-response relationships between PM components and biomarkers of allergic response significant associations with EC and OC when mice are exposed 50 downwind of a freeway but not when the mice were exposed 150 m downwind. No significant associations were found between particle number, particle mass or total metal concentrations at either downwind distance (data not shown) (Kleinman *et al.*, 2007).

Exposure to CAPs Accelerates Atherosclerosis and Effects are Blocked by Removal of Organic Constituents: Exposures of mice with genetic impairment of lipid metabolism and increased susceptibility to development of atherosclerotic plaques showed that plaque development was accelerated in mice exposed to CAPs at a site influenced by PM constituents associated with motor vehicle emissions, elemental carbon and organic carbon. Using a thermal denuder coupled to a particle concentrator (VACES), our recently completed, ARB-funded research project, found that removal of the semivolatile constituents of CAPs from the aerosol blocked the acceleration of atherosclerotic plaque development. The thermal denuder heats the aerosol to a specified temperature to evaporate and remove semi-volatile components, and then returns the aerosol to the original temperature.

As seen in Figure 3, passage of CAPs through the thermal denuder greatly reduced concentrations of polycyclic aromatic hydrocarbons (components of PM that are implicated in both heart disease and cancer) and also reduced the ability of the particles to generate free radicals, as measured by the dithiothreitol (DTT) assay.

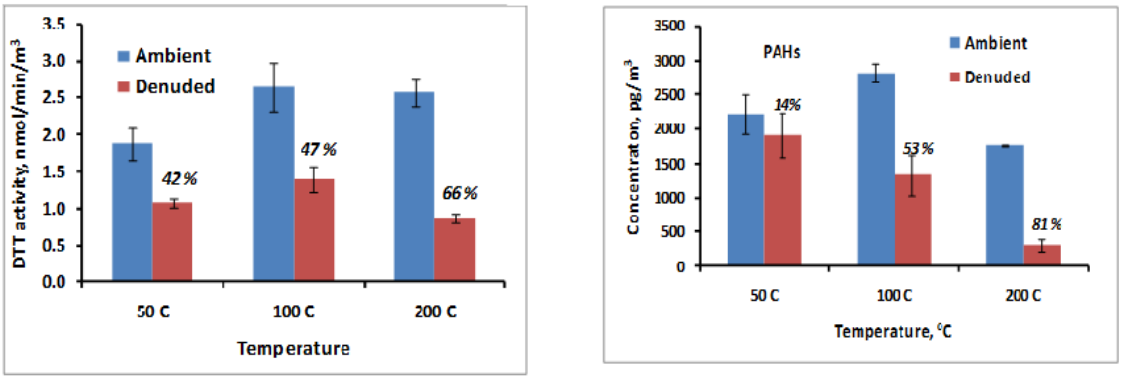

Figure 3 Thermally denuded particles have reduced ability to generate free radicals in an in vitro DTT assay and contain smaller concentrations of PAHs.

Figure 4 shows that in mice exposed to undenuded CAPs the formation of atherosclerotic plaque was accelerated compared to that in mice exposed to either purified air or to denuded CAPs. Both the plaque area and the lipid content of the arterial walls were greater in the arteries of mice exposed to the undenuded CAPs. In addition, while both denuded and undenuded CAPs increased levels of LDL, compared to purified air, only the undenuded CAPs induced lipid peroxidation.

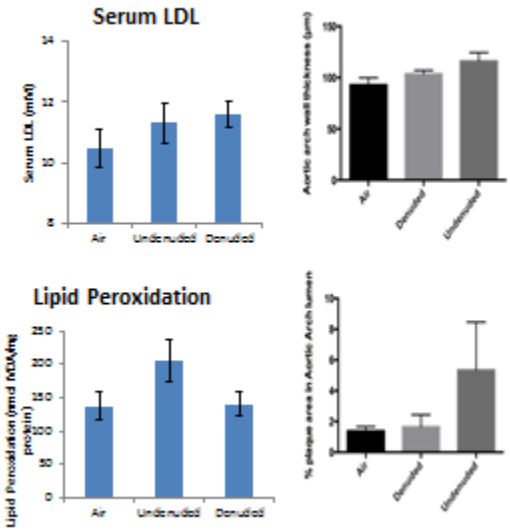

Figure 4 Exposure of apoE-/- mice to CAPs accelerates the formation of atherosclerotic plaque which can be blocked by removal of organic constituents using a thermal denuder.

Plaque area measured in histological sections

Lipid content of artery measured in homogenates

The data in Figure 4 demonstrated effects of UFP. We have also examined the effects of FP exposures in the apoE-/- mice. Mice exposed to concentrated $PM_{2.5}$ (CAPs) showed more rapid development of atherosclerotic plaques after 8 weeks of exposure than did mice exposed to purified air. As shown in Figure 5, we measured the thickness of the arterial wall and the arterial lumen using a non-invasive ultrasound microscopic imaging technique. The wall thickness was increased and the lumen area was decreased in APOE -/- mice exposed to CAPs compared to those in mice exposed to purified air.

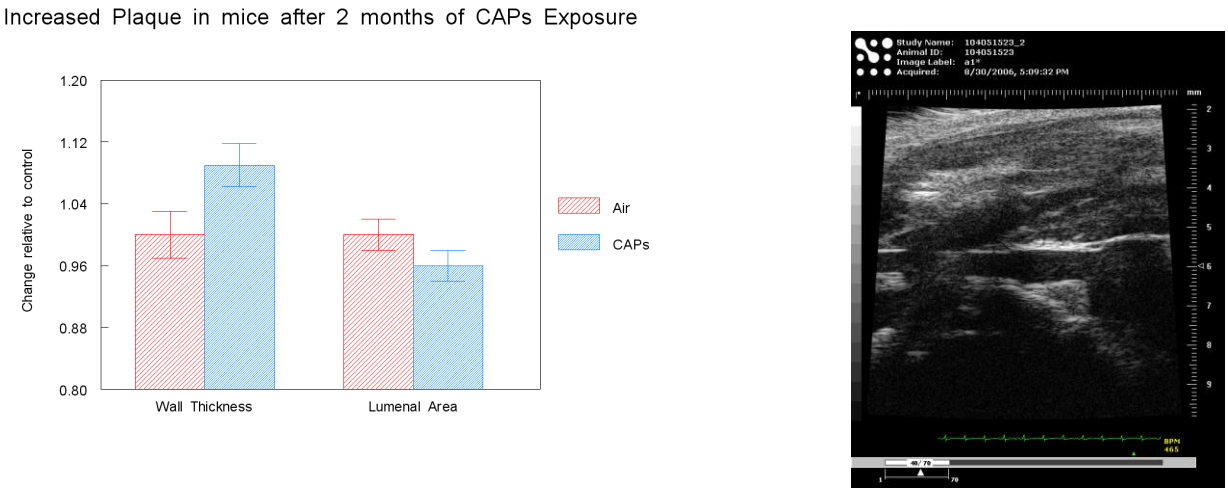

Figure 5. Ultrasound imaging of arteries in CAPs-exposed mice reveals increased wall thickness and decreased arterial lumens. A longitudinal section through an artery in a typical image is shown in the right-hand panel of the figure.

Objectives

The objectives of this study are to: 1) to elucidate the mechanistic pathways through which concurrent PM2.5 and ozone exposures induce cardiovascular effects, and 2) to determine whether there are additive or synergistic interactions between these two air pollutants. It will be important to determine how the toxicity of mixtures containing O_3 and UFP depends on the ambient concentration of O_3, whether seasonal variations in ambient O3 will modify the toxicity of UFP and whether the removal of semi-volatile components from the PM will block atherosclerosis acceleration in the presence and absence of O_3. We will use our in-vivo rodent exposure system in combination with the VACES-thermal denuder technology to separately study the cardiopulmonary effects of PM, before and after the removal of the semi-volatile components, with and without added O_3. Particle exposures and sample collections will take place at the University of California, Irvine (UCI), which is impacted by regional mixed pollutants and is situated between two heavily trafficked freeways, hence is influenced by motor vehicle emissions as well. Ambient O_3 levels are reasonably low, and the VACES removes most of the ozone and oxidant gasses from the aerosol during the concentration process, which will improve the control of O_3 levels in exposures where O_3 will be added. Studies performed at the UCI site have demonstrated significant accelerations of the formation of atherosclerotic plaques in mice exposed to UFP.

. Acute and chronic cardiopulmonary inflammation, vascular injury and myocardial function will be examined using genetically susceptible mice implanted with ECG telemetry devices to test the hypothesis that exposure to ultrafine particles composed of semi-volatile compounds causes inflammation and oxidative stress resulting in pulmonary and cardiovascular injuries that contribute to the development of coronary heart disease. The study will address the question of whether adverse effects associated with exposures in close proximity to sources of mobile source emissions are due to specific classes of reactive organic compounds such as aldehydes or other oxygenated hydrocarbons. Endpoints will include markers of inflammation, histological examinations for evidence of vascular and myocardial pathology, ventricular hypertrophy and biomarkers of lipid, protein and DNA oxidation. The in vivo biological

responses will be correlated with physical and chemical composition of the particles and the in vitro potential of these particles to produce free radicals and induce cytotoxicity. These experiments will be conducted over a period of 3 years.

Technical Plan

Hypotheses

We will test three hypotheses:

1. Concurrent exposure to a mixture of concentrated ambient FP (CAPs) and O3 will, through modes of action involving systemic inflammation, oxidative stress and lipid peroxidation produce greater acceleration of atherosclerosis and reduction in HRV than will exposure to either CAPs alone or O3 alone;
2. CAPs generated during periods of high ambient photochemical activity (i.e. summer) will be more potent than CAPs generated during periods of low ambient photochemical activity (i.e. winter) with respect to CAPs-induced acceleration of atherosclerosis and impairment of HRV through mechanisms of oxidation of serum lipids and LDL leading to foam cell formation and incorporation into arterial plaques and oxidative stress leading to arterial wall thickening and tissue damage; and
3. The removal of organic constituents of PM2.5 using a thermal denuder will block the effects of PM but not those of O3 with respect to acceleration of atherosclerosis and impairment of HRV through mechanisms of oxidative stress and lipid peroxidation

Specific Aims

1. To address hypothesis (1) we will expose apoE-/- mice to purified air, concentrated ambient FP (CAPs), CAPs + 0.2 ppm O_3, and 0.2 ppm O_3 without CAPs to determine the degree to which combined O_3 and CAPs exposure modifies the CAPs-induced acceleration of atherosclerosis and impairment of HRV compared to CAPs alone and O_3 alone. These exposures will be 5 hr per day, 4 days per week for 8 weeks and will be performed during a period of high ambient photochemical activity (O_3 0.07-0.12 ppm).
2. To address hypothesis (2) we will expose apoE-/- mice to purified air, concentrated ambient FP (CAPs), CAPs + 0.2 ppm O_3, and 0.2 ppm O_3 without CAPs to determine the degree to which combined O_3 and CAPs exposure modifies the CAPs-induced acceleration of atherosclerosis and impairment of HRV compared to CAPs alone and O_3 alone. These exposures will be 5 hr per day, 4 days per week for 8 weeks and will be performed during a period of low ambient photochemical activity (O_3 0.03 – 0.06 ppm).
3. To address hypothesis (3) we will expose mice apoE-/- mice to purified air, 0.2 ppm O_3, denuded CAPs, and 0.2 ppm O_3 + denuded CAPs to determine the degree to which removal of the organic constituents modifies the CAPs-induced acceleration of atherosclerosis and impairment of HRV compared to denuded CAPs alone and O_3 alone. These exposures will be 5 hr per day, 4 days per week for 8 weeks and will be performed during a period of high ambient photochemical activity (O_3 0.07-0.12 ppm).

Description of Experimental Techniques

We will use our in-vivo rodent exposure system in combination with the VACES-thermal denuder technology to separately study the cardiopulmonary effects of PM, before and after the removal of the semi-volatile components, with and without added

O_3. Particle exposures and sample collections will take place at the University of California, Irvine (UCI), which is impacted by regional mixed pollutants and is situated between two heavily trafficked freeways, hence is influenced by motor vehicle emissions as well. Ambient O_3 levels are reasonably low, and the VACES removes most of the ozone and oxidant gasses from the aerosol during the concentration process, which will improve the control of O_3 levels in exposures where O_3 will be added. Studies performed at the UCI site have demonstrated significant accelerations of the formation of atherosclerotic plaques in mice exposed to UFP.

. Acute and chronic cardiopulmonary inflammation, vascular injury and myocardial function will be examined using genetically susceptible mice implanted with ECG telemetry devices to test the hypothesis that exposure to ultrafine particles composed of ith semi-volatile compounds causes inflammation and oxidative stress resulting in pulmonary and cardiovascular injuries that contribute to the development of coronary heart disease. The study will address the question of whether adverse effects associated with exposures in close proximity to sources of mobile source emissions are due to specific classes of reactive organic compounds such as aldehydes or other oxygenated hydrocarbons. Endpoints will include markers of inflammation, histological examinations for evidence of vascular and myocardial pathology, ventricular hypertrophy and biomarkers of lipid, protein and DNA oxidation. The in vivo biological responses will be correlated with physical and chemical composition of the particles and the in vitro potential of these particles to produce free radicals and induce cytotoxicity.

The study will use apoE-/- mice that are genetically susceptible to development of atherosclerosis. We have more than 10 years of experience in the exposure and husbandry of these animals. All animal protocols, including surgery to implant transponder ECG units, have been approved by the UC Irvine Animal Care and Use Committee (Protocol # 2001-2242). Based on previous studies we have performed power calculations, backed up by experimental results, that we can see a 50% change in plaque area and/or lipid content in arterial homogenates at the $\alpha = 0.05$ level with 80% power, comparing 12 exposed mice to 12 mice exposed to purified air. To cover contingencies we will use 16 mice per group which will provide adequate numbers in case of sample losses during the study.

Proposed Tasks:

1. Concentrated ambient particle (CAP) exposures of mice that are genetically susceptible to adverse effects of exposure to total and nonvolatile fine PM in mixtures with O_3.
2. Chemical characterization of ambient and exposure atmospheres.
3. Examination of acute and chronic cardiovascular inflammation and injury to test the hypothesis that exposure to fine particles composed of semi-volatile compounds causes inflammation and oxidative stress resulting in pulmonary and cardiovascular injury. This will be done by exposing groups of mice to FP ± Volatile Organics ± O_3.
4. Conduct data analysis, prepare quarterly progress reports for CARB and prepare manuscripts to be submitted for publication in the peer-reviewed literature.
5. Prepare a final report at the end of the project

Details of Conduct of Major Tasks

Task 1: Conduct Concentrated ambient particle (CAP) exposures of mice that are genetically susceptible to adverse effects of exposure to total and non volatile ultrafine PM in the presence and absence of ozone (O_3).

Exposure Procedure

Animals will be exposed to concentrated fine ambient particles (CAPs) using the UCI mobile exposure chambers connected to the VACES particle concentration system. Mice will be conditioned to the exposure system in purified air at least one week before beginning CAPs exposures. We will monitor heart rate in the implanted animals. Exposures will start after stable baseline levels are achieved. For exposures, the mice are placed into sealed, compartmentalized exposure chambers that will be connected to the outlet of the VACES system. The mice (n=16) will be exposed to ultrafine concentrated ambient particles (CAPs) or vapor-denuded CAPs (n=16) for 4 hours per day, 4 days per week for 8 weeks. Control animals (n=16) will receive purified air under conditions identical to those of the animals exposed to CAPs. Temperature will be monitored continuously during the exposures and held to 75 ± 5°C. Animals will be observed throughout the exposure period for signs of distress. Between exposures, mice will be housed in the UCI vivarium and will receive water and food, ad libitum. We will use the APOE -/- mouse which have already established will be sensitive to the atherogenic effects of CAPs and which has high levels of LDL lipoproteins. We have shown that in the APOE-/- mouse PM exposure leads to a loss of protective capability of the HDL even though the ratios of LDL to HDL are reasonable.

Generation of Concentrated PM Samples for Biological and Toxicological Evaluation

Concentrated ambient ultrafine particles will be generated using a portable Versatile Aerosol Concentration Enrichment System (VACES) which will enrich the concentration of ambient particles in the size range of 0.02- 10 μm by a factor 10 (Kim *et al.*, 2001a; Kim *et al.*, 2001b). We have previously demonstrated that 8 week exposures of apoE-/- mice to CAPs at the UCI site accelerated the development of atherosclerotic plaques and also modifies autonomic influences on cardiac physiology resulting in decreased heart rate variability (HRV).

In the VACES, the air stream is saturated with water vapor in a humidifier, which is a 10 L aluminum vessel half-filled with water and maintained at 38ºC. The air stream is directed at, and passes above, the water surface. The residence time in the humidifier is about 3 s. Doubly de-mineralized water (18.1MΩ/cm) is used in the humidifier. By passing through the humidifier the air is saturated with water vapor and warmed up to about 30ºC (Kim *et al.*, 2001b). After leaving the humidifier the air enters a condenser, a stainless steel pipe that is surrounded by a mixture of water and isopropanol, which is continuously circulated by means of a chiller. The temperature of the cooling mixture in the condenser is −3 ± 0.5ºC and checked using a digital thermometer. The actual temperature of the air stream in the condenser is 20–21ºC (Kim *et al.*, 2001b). Due to the sharp drop in temperature (about 10ºC) the air in the condenser becomes strongly supersaturated. The supersaturation causes water vapor to condense onto particles as small as 20 nm in size, which rapidly grow to 2.5–3 μm water droplets. These droplets are subsequently concentrated by a virtual impactor, exiting via its minor air flow. After leaving the virtual impactor, the droplets are dried with

a silica-gel diffusion dryer, brings the concentrated aerosol particles to nearly their original size.

Generation of Concentrated Ambient Fine Particles by Means of the VACES

The general protocol to produce concentrated CAPs material for in vivo and the concurrent in vitro studies will be similar for each sampling experiment. The system is shown in Figure 8. For the in vivo CAPS exposures, the set up is shown in Figure 8. In each sampling line of the concentrator (shown schematically in Figure 8) PM will be concentrated from an intake flow of 120 liters per minute (lpm) to a minor flow of 6 lpm, enriched in concentration by a factor of 20. Particles will be first drawn through a PM2.5 pre-selector to remove coarse particles. From the 6 lpm concentrated flow containing semi volatile and non-volatile ultrafine PM, 5 lpm will be drawn through a diffusion dryer and then supplied to the animal exposure chamber. An O3 generator will generate O3 which will be metered into the CAPs aerosol after drying to achieve the necessary concentrations of O3 in the exposure chambers. O3 will be monitored throughout the exposures. Ambient O3 data will be obtained from the closest air monitoring station (Anaheim) and will be monitored onsite.

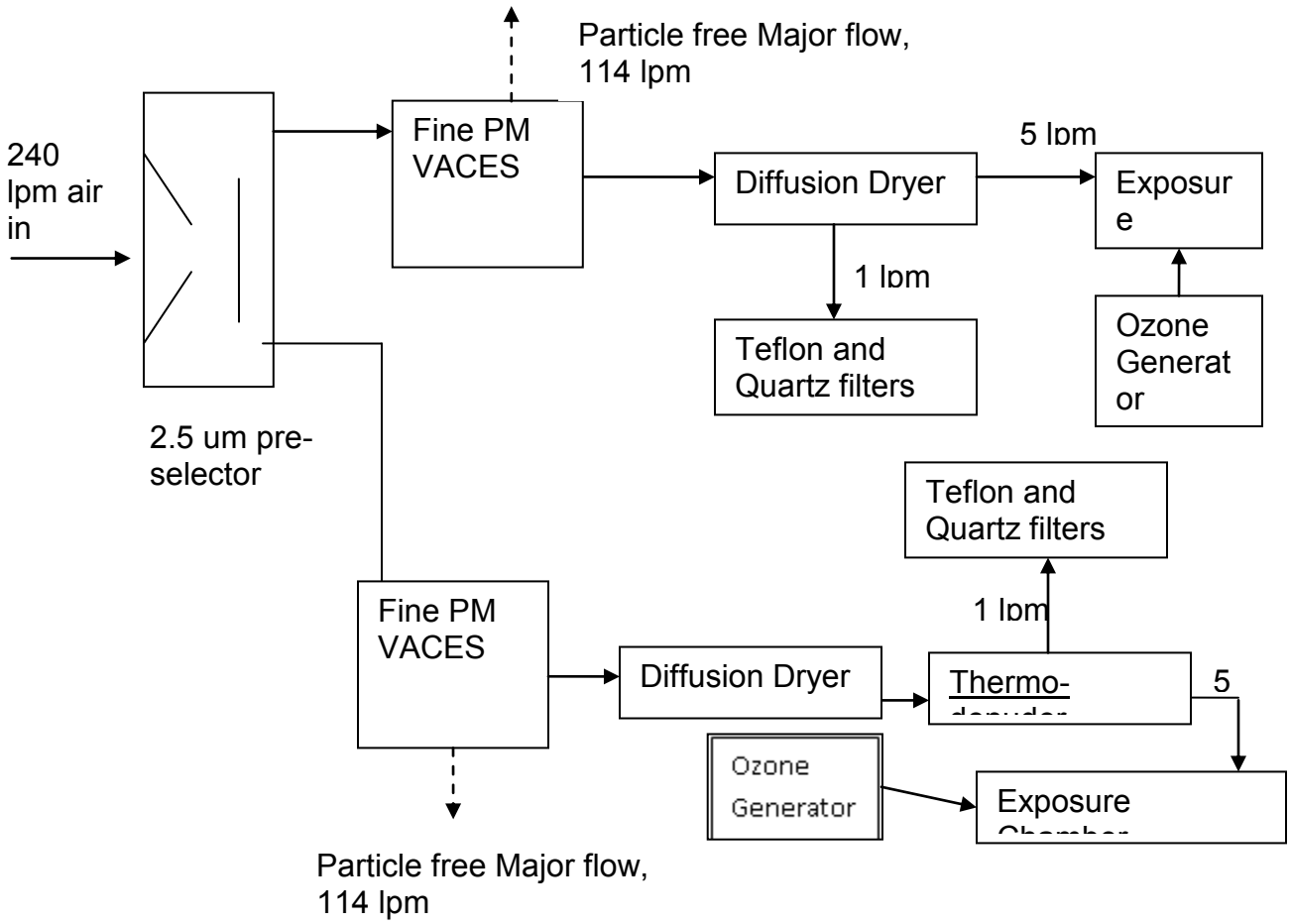

Figure 6. Schematic of the VACES Concentrator System for Generating the CAPs + O_3 atmospheres for the In Vivo Exposures.

The remaining 1 lpm of concentrated particles will be then split into two equal halves of 0.5 lpm, each diverted into a filter sampler consisting of either a 47 mm Teflon filter (2 μm pore, PTFE Teflon, Gelman Science, Ann Arbor, MI) or a 47 mm prebaked quartz filter (Pallflex Corp., Putnam, CT). The Teflon filters will be used to determine particle mass and the trace element and metal content, whereas the quartz filters will be used to determine the PM content of elemental and organic carbon (EC and OC), PAH and inorganic ions (sulfate and nitrate). For the latter measurements, a central portion of the quart filter of about 1 cm2 will be removed for EC-OC measurements. The remainder of the filter will be split in two unequal parts; ¾ of it will be extracted using dichloromethane, DCM, for PAH analysis, whereas ¼ will be extracted with a mixture of ultrapure (milliQ) water and ethanol (5 ml: 0.15 ml, respectively), for analysis or inorganic anions and cations by means of ion chromatography. These analyses are described in greater detail in the following section.

The concentrated aerosol line containing non-volatile only ultrafine PM will pass first through a thermodenuder (a device that is described in the following section) before it enters the exposure chamber. The thermodenuder removes all but the non volatile particles from the air sample. The size distribution of the denuded particles may be slightly different from that of the un-denuded particles. For example if the mass of particles in the range of 50 to 100 nm is about 30 due to volatile organic constituents, removal of the organics will shrink the particle diameter by about 10%. On the other hand, some fraction of the smaller nanoparticles (10 – 50 nm) will be almost entirely volatile. Removal of these particles will shift the overall number distribution to a higher median diameter, but will have little effect on the mass median diameter. For the toxicological studies, we are primarily concerned with the mass distribution and the thermodenuder will do little to alter this. The toxicological effects will be normalized per unit mass, so the bulk loss of material due to the thermodenuder is a more appropriate metric than single particle changes, which are beyond the scope of the proposed work. We will carefully monitor changes in the number and mass size distributions for un-denuded and denuded aerosols and will take any such changes into account as we interpret the data.

This proposed study will use transgenic mice lacking apolipoprotein E (apoE-/-). The mice will be obtained from The Jackson Laboratory (Bar Harbor, ME). The apoE-/- mice developed atherosclerotic lesions in coronary arteries and in the aorta (after 2 months exposure to FP in Irvine, CA). Groups of 16 mice each will be exposed to concentrated FP or to purified air. Changes in cardiac physiology in these mice will be monitored over the course of the study in a subset of 5 FP and 5 purified air-exposed mice with implanted ECG transmitters. Histological assessment of plaque size and markers of vascular and myocardial injury will be measured at the end of exposure. Animals will be housed 4 to a cage in ALAAAC accredited animal housing facility at the UCI vivarium. The mice that are implanted with telemetry devices will be housed singly so that ECG parameters can be monitored before and after exposures. Animals will be provided with food and water ad libitum. Animals will be placed in exposure chambers on each exposure day and exposed for 5 hr per day, 4 days per week for a total of 8 weeks.

Separation of Non Volatile from Volatile PM Using the Thermodenuder

As noted earlier, one of the major thrusts of this proposal is to determine how the toxicity and the characteristics of the semi-volatile fraction of ultrafine PM differs from the nonvolatile fraction. To accomplish this aim, we will use a thermodenuder, in conjunction with the particle concentrator and the parallel filter samplers, to investigate the relative toxicity of PM of different volatilities.

The Dekati thermodenuder is designed to remove volatile/semivolatile compounds from engine exhaust samples. These compounds are known to cause variations in particle measurements through nucleation and condensation. The thermodenuder heats the sample aerosol up to a maximum temperature of 300°C and volatilizes the unwanted compounds. The volatilized compounds are subsequently collected in an activated charcoal adsorber section. Since the particles in the sample have much slower diffusional deposition rates (for 10 nm particles < 1/100) than the vaporized compounds, the vaporized volatiles are collected efficiently, while the sample aerosol particles follow the gas streamlines unaffected. Water driven through the cooling channels cools the sample aerosol in the adsorber.

Task 2: Physical and chemical characterization of PM of Ambient and Exposure Atmospheres.

Mass Concentrations - The PTFE filter samples from the thermo-denuded and undenuded VACES will be weighed three times before and after the weekly sampling using a microbalance (Model MT-5, Mettler Toledo, Inc., Highstown, NJ) after allowing at least 24 hours of equilibration in a controlled environment with temperature of 22-24°C and relative humidity of 40-45%. The mass concentrations of aerosol particles will be determined by the net filter weight gained after sampling

Elemental analysis will be performed by means of Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) using a microwave-assisted digestion that uses a mixed acid of nitric acid, hydrochloric acid, and hydrofluoric acid (Lough *et al.*, 2005; Dillner *et al.*, 2007).

Elemental Carbon (EC), Organic Carbon (OC) and Inorganic Ions- The quartz filter samples of ultrafine particles from the thermo-denuded and undenuded VACES will be cut into 3 unequal fractions for chemical composition analysis. ¼ of the filters will be used for analysis of inorganic ions by means of ion chromatography to determine the PM concentrations of inorganic ions, including sulfate, nitrate, ammonium, potassium, sodium, and chlorine. Another fraction of 1 cm^2 punches from the remaining ¾ of each filter will be analyzed for EC and OC using a Thermal Evolution/Optical Transmittance (TOT) analytic method (Birch and Cary, 1996a; Birch and Cary, 1996b).

Organic speciation analysis- The remaining portion of the ¾ of the quartz filters will be analyzed for organic speciation. The analysis method for the quantification of individual organic compounds in the collected aerosol samples is based on earlier established solvent extraction methods (Fine *et al.*, 2004). Procedures for sample extraction and molecular quantification for the organic tracers have been described in detail (Phuleria *et al.*, 2006), and only a brief summary is presented here. Filter sample aliquots from the high-volume sampler are spiked with known amounts of isotope labeled internal standard compounds and extracted in dichloromethane and methanol. Extracts will be combined and reduced in volume to 100-250 µL by rotary evaporation

followed by pure nitrogen evaporation. The final target volume will be determined based on the amount of organic carbon mass in each sample. The extracts will be derivatized, using diazomethane to convert organic acids to their methyl esters, and analyzed by gas chromatography using a mass spectrometer detection system (GCMS). A separate aliquot of the sample extract will be silylated (Nolte *et al.*, 2002) and run on the GCMS to measure levoglucosan.

Size-resolved Particle Number Concentrations – A TSI Scanning Mobility Particle Sizer (SMPS) will be used to measure ultrafine particle concentrations in that site. The instrument's scanning time will be adjusted to 15 minute cycles. Particles in the range of 5 -250 nm will be measured. The 15 min cycle data will be averaged to time periods that will match the sampling collection periods of the proposed study In addition to the SMPS, a condensation particle counter (TSI 3022 CPC) will be used in parallel to measure total particle number concentrations.

Size-resolved Aerosol Organic and Inorganic Constituent Composition – The Aerodyne Aerosol Mass Spectrometer (AMS) will be used to provide size and chemical mass loading information in real-time for non-refractory sub-micron aerosol particles. The AMS couples size-resolved particle sampling and mass spectrometric techniques into a single real-time measurement system and will be used to analyze exposure and ambient aerosol characteristics during the exposure studies to determine differences in characteristics before and after denuding and before and after addition of O_3 to the exposure atmospheres.

Ozone will be monitored using a UV Absorption Monitor (Dasibi Model 1003-AH) which will be checked daily against a calibrated transfer standard.

Task 3: Biological Assays to Determine Adverse Health Effects.

Bioassay and Data Analysis Methods

1. Blood: serum samples will be collected from each animal from the descending aorta for cytokine, lipoprotein, acute phase protein and oxidized lipoprotein levels.
2. Coronary Arteries and Aorta: Samples of these blood vessels will be collected for histological or immunohistochemical assays and for biochemical assays.
3. Vascular and Cardiac Histology

All morphological assessments will be done without knowledge of the treatment group. At sacrifice, 9 mice from each group will be euthanized with an overdose of pentobarbital and the heart and aorta perfused with 4% paraformaldehyde. The heart and thoracic and abdominal aorta will be removed en bloc for histological and immunohistochemical analyses. The remaining proximal and distal aorta segments will be cut longitudinally. One section will be frozen in OTC and reserved for future laser capture microdissection and genomic and proteomic analysis (not proposed for funding in this proposed study). The remaining aorta sections will be fixed in buffered formaldehyde and examined for total atherosclerotic lesion areas, lipid contents, and cellularity (Wadsworth *et al.*, 2002). Sections of the heart, coronary arteries, aorta (proximal, central and distal areas), liver, spleen, lungs and brain will be harvested from the remaining 9 mice. These will be snap frozen and stored in liquid nitrogen. The frozen samples will be homogenized and used for assays of gene expression, and for analyses of biomarkers of vascular inflammation and oxidative stress.

Atherosclerotic Lesion Characterization

Characterization of mouse atherosclerotic lesions will be performed as described by Sukhova (Sukhova *et al.*, 2003). Lesion area will be measured as lipid deposition using an en face preparation of abdominal aortae (oil red O staining). Longitudinal sections of aortic arches embedded in OCT will be stained for lipids (oil red O), elastin (Verhoeff-van Gieson), collagen (Sirius-Red), macrophages (Mac-3), T cells (CD4), and proliferating cells (Ki67 nuclear antigen) and analyzed as described by Sukhova et al. (2003). Total cholesterol, HDL, triglyceride, and LDL will be determined using the methods described by Sukhova et al. (2003). An example of the lesion observed in APOE -/- mice exposed to UFP at USC is shown in Figure 13.

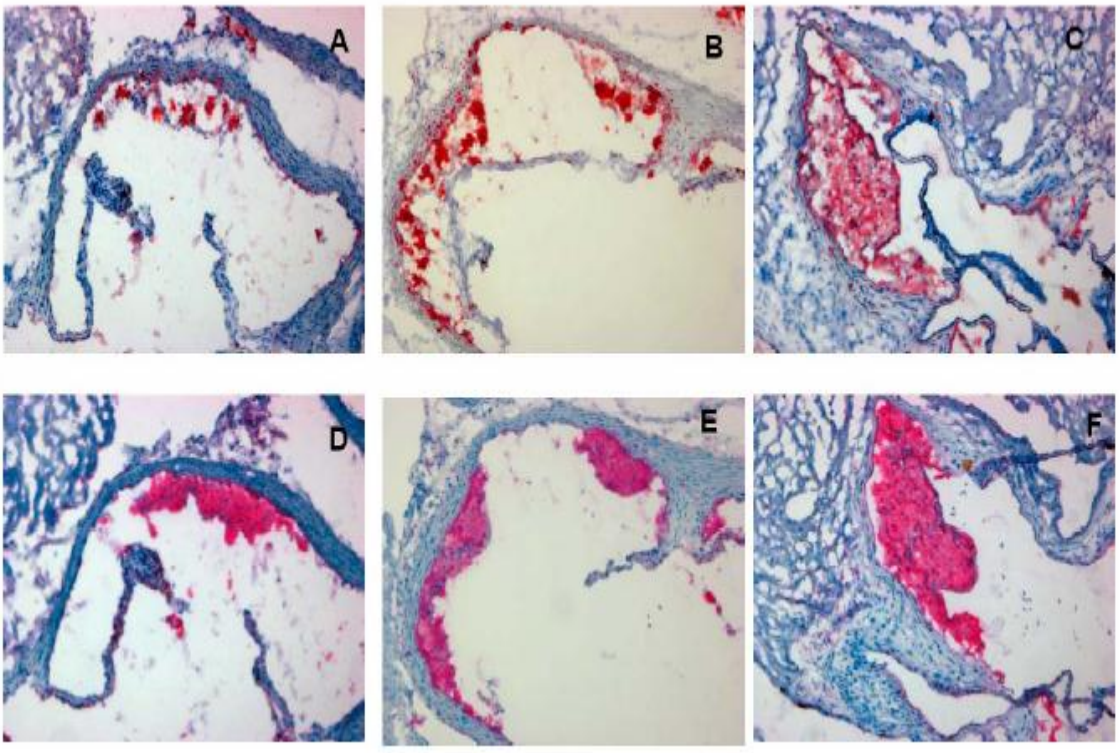

Figure 7. Aortic root sections from CAPs-exposed APOE -/- mice stained to show atherosclerotic lesions. Aortae from UFP-exposed mice (C,F) exhibit more intense and more occlusive lesions than seen in sections from FA (A,D) and FP (B,E) mice (Araujo *et al.*, 2007).

Inflammatory and anti-inflammatory cytokines and markers of vascular inflammation
Homogenates of heart and aorta samples will be analyzed for inflammatory cytokines (IL-6, TNFα) and the acute phase protein CRP, using multiplexed bead assays (Luminex System 100, Lincoplex Assay Kits). The Luminex assay system uses fluorescently tagged beads to which antibodies to specific proteins are attached. After incubation with the sample, the beads are incubated with detection antibodies and then analyzed using a fluorescent flow sorting system (similar in working principle to flow cytometry) and the data are recorded and quantified using an online computer.

ECG Measurement and Analysis:

The telemetry system (PhysioTel Telemetry system, Data Sciences International, St. Paul, MN) is designed to measure biopotential (ECG tracings), temperature, and physical activity in mice. The devices will be implanted using an asceptic technique. Isofluorene will be administered via inhalation to anesthetize the mice. A ~1 cm midline abdominal incision will be made. The contents of the abdomen will be exposed using a retractor. The body of the telemetry device will be placed on top of the intestines and a skin incision will be made at the site of negative lead placement. Using a blunt pointed instrument, called a trocar, a tunnel will be made subcutaneously from the abdominal incision to approximately 1 cm beyond the lead incision through which plastic sleeve will be guided. The trocar will be removed leaving the sleeve in place. A 14-gauge needle will be passed through the abdominal wall lateral to the cranial aspect of the incision going from the outside into the abdominal cavity. One of the leads will be passed through the needle and out of the abdomen. The needle will be withdrawn leaving the lead externalized. The lead will be passed through the waiting plastic sleeve to the desired site, and the sleeve will be removed. The lead will be stripped, leaving at least 1 cm of the wire exposed, and will be secured by suturing the muscle tissue up over the lead using 4-0 non-absorbable suture. These steps will be repeated for the positive lead. The device body will be secured in place by closing the abdominal incision and incorporating the suture rib on the device into the closure using non-absorbable sutures. The skin incision will be closed using skin staples. After surgery, the animal will be placed into a warm environment and the breathing air will be supplemented with additional oxygen. The animal's recovery will be monitored until it is fully awake. Analgesia, buprenorphine (.01 - .05 mg/kg subcutaneously every 12 hours for three days) is provided to all animals post surgery. Enrofloxacin (Baytril) 3 mg/kg BW will be administered by subcutaneous injection twice per day for 7 days.

Tasks 4-5: Conduct data analysis, prepare quality progress reports, final report as well as manuscripts to be submitted for publication to the peer-reviewed literature

Data Management Plan

The DataQuest A.R.T. system will be used to detect, collect and analyze biopotential, body core temperature and activity telemetry signals from each implanted animal. ECG waveforms will be stored on a dedicated computer in data files for subsequent analysis. Analysis of the ECG waveform will be used to extract heart rate, incidence of abnormal heart beats (arrhythmias) and measures of heart rate variability (HRV) which is the magnitude of variance explained (power) in the heart's rhythm across different frequency bands (spectra) of periodic oscillations in heart rate. Portions of these spectra reflect different autonomic influences on heart rate. The high frequency (HF) band (0.15-0.40 Hz) of the heart period power spectrum has been used to estimate cardiac vagal control (Liao *et al.*, 1996). Decreased cardiac vagal activity in humans is associated with an increased risk of coronary atherosclerosis (Hayano *et al.*, 1991). Heart period oscillations at lower frequencies (LF, 0.04-0.15 Hz) are less well understood. They may represent mixed sympathetic-parasympathetic and thermoregulatory influences (Lossius *et al.*, 1994; Fleisher *et al.*, 1996). We will use the criteria defined by Hayano et al. (1991) to examine the incidences of respiratory sinus

arrhythmias which have been associated with the extent of coronary atheromatosis (Hayano *et al.*, 1991). The numbers of premature atrial and ventricular beats will also be determined by analysis of the ECG waveform. Premature ventricular beats have been seen in rats with myocardial infarctions exposed to residual oil fly ash (Wellenius *et al.*, 2002) and will therefore be monitored in this study.

Arterial plaque measurements will be made on stained histological sections. Sections will be viewed under light microscopy and digitized. Image J software, made available through the NIH, will be used to measure the arterial lumen and the area of lumen occluded by plaque. In addition, the number of smooth muscle cells in a specific area of the arterial wall will be made using the Image J point counting program. Histology slides will be read blind. The cellularity/unit area and the plaque area as a percent of arterial lumen will be calculated. Differences between the exposure group means will be determined by ANOVA.

Serum samples will be analyzed for the inflammatory cytokine tumor necrosis factor α (TNF-α), the systemic inflammation indicator C Reactive Protein (CRP), total lipids, LDL and oxidized LDL. Differences between the exposure group means will be determined by ANOVA.

Statistical analysis of the Tasks 2 data will describe the mean values of each chemical aspect or species characterized in the un-denuded and denuded aerosol samples. Chemical composition will be expressed both in terms of airborne concentration (in PM mass per unit volume of air) as well as a percentage of PM mass. The latter is particularly useful for in the in vitro experiments, for which toxicity is expressed per mass of PM.

Much of the emphasis in Task 2 is upon determining the impact of the volatile organic components of ambient aerosols on biological significance of particle exposure as measured by biological activity. As emphasized throughout the project, information developed from these studies concerning the biological impact of particles will help in the understanding of the roles of primary and secondary organic species as well as potentially identifying specific organic constituents that might be associated with effects on health. Task 2 will characterize particle chemistries (OC, EC, PAH, metals) to add new dimensions (beyond size and mass distributions) to the comparison of ambient aerosols. The information about the biological significance of particles with and without the volatile constituents, as assayed in this project, will improve the prioritization of further scientific work towards understanding exactly which chemical characteristics most directly impact heath and how multiple pollutants such as PM and O3 might interact, either physically by generating reaction products on particles or biologically by inducing biological responses and modifying factors such as biological availability of air contaminants.

Data analyses in Task 4 will include statistical evaluations including analyses of variance, ANOVA, and regression analyses to isolate potential trends over the eight week exposure periods. For these analyses significance will be attributed at the $p \leq 0.05$ level.

Finally, through Tasks 5 and 6, we will submit quarterly progress reports, in accordance to CARB's policies, and a final report at the end of this project. We also anticipate that several manuscripts suitable for publication in the refereed literature will be generated through this project and will be part of our activities under Tasks 1-4.

Quality Assurance

We will implement our Quality Assurance and Quality Control (QA/QC) procedures for this program, in the context of the existing procedures utilized at the Air Pollution Health Effect Laboratory. Data for each sampling cycle-period will be analyzed and submitted approximately three months after the end of this period to ensure complete acquisition and analysis of all chemical speciation data as well as biological data. When issues are found during audits or when data problems are identified, the PI will be notified rapidly so that corrective action can be taken. At the end of the measurement period, reports will be submitted by the Co-Investigators to the PI. These reports will summarize the activities associated with the sampling operations and the exposure studies. In addition to the activities reports, data quality summary reports (DQSR) will be submitted for each major measurement type. These reports will summarize the completeness, accuracy, precision, and the minimum detection limit (MDL) for each measurement over the entire study period.

Teflon filters will be equilibrated inside the weighing room with controlled temperature and humidity before use. After being neutralized by Polonium-210 sources, the filters are weighed by the microbalance up to 1 □g resolution. The Microbalance should be checked in advance by using a 100.000-mg stainless steel standard and 47mm blank filter. The Microbalance reading after a 30-second stabilization time is recorded in the logbook together with temperature and relative humidity value at that time. Finally, weighed filters are transferred to labeled petri dishes using sterile tweezers.

Filter handling of quartz and glass fiber filters is similar to Teflon. The filters will be taken from the vacuum desiccators and refrigerator, respectively, where they are stored and placed into the filter holders. Quartz filters (only) will be prebaked in an oven and subsequently stored in a refrigerator in Teflon petri dish containers lined with prefired aluminum foil to avoid carbon contamination prior to field use. Solvent rinsed stainless steel tweezers should be used for all the work with respect to filters.

Mass and chemical concentrations of PM will be determined using the analytical methods discussed in the main text of the proposal that describes the core activities. Each analysis will include daily calibration, 10% replicates, standards, and blanks, and re-analyses when performance tolerances or data validation criteria are not met. Remaining sample sections will be archived under refrigeration for the duration of the project for potential re-analysis or analysis for other species.

. Calibration procedures and performance evaluation is incorporated into all SOPs. Traceability to recognized standards is necessary for calibrations and performance evaluations. Calibrations of the commercially available continuous particle monitors (e.g., SMPS, CPC) will be conducted using NIST traceable monodisperse aerosols in the size range of 0.03 to 10 μm (Bangs laboratories, Carmel, IN). The concentrations measured by each instrument will be compared to those measured by a parallel sampling filter used as the reference sampler.

All instrumentation will be calibrated according to manufacturer's specifications. The laboratory balances will be maintained and calibrated on a continual basis. Audits will verify that the balances are functioning within the manufacturer's specifications.

Particle number concentrations for both ambient and concentrated PM are measured by means of the 3022 Condensation Particle Counter (TSI Inc., St. Paul, MN). These instruments measure concentrations every one minute. For the purposes of the concentrator tests, mass concentration readings are grouped in 15-minute time periods and the average is recorded. At the end of every 15-minute cycle, the

concentration is measured downstream of the concentrator for 5 minutes and subsequently the concentration is measured immediately downstream of the second stage. The expected concentration enrichment is 10 (±3). Any deviation in the enrichment factor values indicates a malfunction in the operation of the system. The following steps will have to be taken in the event that the enrichment values do not fall in the aforementioned ranges:

(a) Check for leaks in minor or major flows.
(b) Check for increased pressure drop across the minor flows of each stage.
(c) Check for high relative humidity (i.e., above 80%) and temperature.
(d) Check for very low ambient levels (e.g., less than 10 ug/m^3 in terms of mass), or very high number concentrations of particles (> 10^6 particles/cm^3 after dilution), which would make the concentrated aerosol exceed the upper limit of the 3022 CPC monitor.

References to Publications Describing Relevant Work and Collaborations by the Investigators

Related Research
As examples of related research, the following current and recently completed studies are summarized below.

Current Studies
California Air Resources Board (Kleinman, PI) (4/1/08-5/31/13)
"Contribution of vapor phase reactive organic compounds in ambient air to cardiopulmonary health effects."

Vapor phase components of ambient pollution can increase the toxicity of inhaled particles. Atmospheric reactions of emitted polycyclic aromatic hydrocarbons (PAH), primarily with OH radical and NOx, result in the formation of nitroarenes that affect human health more than the PAH emissions themselves. We examined the toxicity ambient pollutants in the presence and absence of vapor phase components to examine differences in development of atherosclerosis and changes in cardiac physiology.

California Air Resources Board (Kleinman, PI) (2/1/10-3/31/13)
"Neurotoxic Effects of Inhaled Fine Particles in APOE-/- mice."

Central nervous system impacts were assessed following chronic exposure to concentrated PM2.5 particles in mice exposed in 5 different cities with major differences in pollutant source characteristics and emissions.

Recently Completed Projects
USEPA (Froines, PI [UCLA]; UCI Subcontract: Kleinman, PI) 01/01/02-12/31/12

Southern California Particle Center: We examined the effects of ambient particle exposures on development of cardiovascular and allergic airway diseases. Animal models of asthma and atherosclerosis were exposed at various field locations in the LA

Basin and mice were examined for evidences of immunological and cardiovascular impacts.

Health Effects Institute (Kleinman, PI –subcontract) (4/1/10 – 3/31/11)
"Effects of Inhaled Fine Particles on Cardiovascular Function"
We examined the effects of fine particle exposures on development of atherosclerosis and modification of autonomic function in genetically susceptible mice.

Relevant Publications

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Facilities and Resources
Laboratory:
The Air Pollution Health Effects Laboratory (APHEL), is a self-contained research unit on the UCI North Campus, that is equipped and staffed to provide controlled inhalation exposures for cardiopulmonary toxicology and physiology studies as well as to support basic research. Onsite support includes air barrier animal housing, state of the art atmosphere generation and characterization facilities, radioisotope containment and detection capabilities, and facilities necessary for measurements of cardiopulmonary physiology, histopathology, lung morphometry, assessment of immunological effects, and data processing.

Other on-site support facilities include a mechanical shop, electronic shop, physics and chemistry areas. Shared departmental facilities include a tissue culture room with 2 laminar flow hoods and two CO_2 incubators, glassware washing room, autoclaves, centrifuges, scintillation counter and darkroom. Electron microscopes, EDAX trace element analysis systems and fluorescent live cell imaging systems are available on a recharge basis if needed for the project.

Animal:
Animals will be housed in APHEL vivarium facilities with HEPA filtered clean air isolation units prior to implantation surgery. The animals' health, care and maintenance is overseen by a staff Animal Health Technician under the direction of a staff scientist and a University veterinarian. The animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). A surgical suite and anesthesia facilities are available for the implantation of transponders for the proposed study. After recovery, the animals will be transferred to the USC Vivarium which is also AAALAC accredited. All procedures involving mice will be approved in advance by the Institutional Animal Care and Use Committees (IACUCs) at UCI. The APOE -/- genetically modified mice are available in adequate numbers as standard products from Jackson Laboratories and will not require us to breed animals for this study. Prior to shipment, all animals will be certified by the supplier to be free of selected pathogens.

Computer:
UCI project staff are connected to an institution-wide network for electronic mail and file transfer, with access to standard software packages for word processing, database management, spreadsheet, desktop publishing and graphics.

Major Equipment:

Dr. Kleinman has access to following in the ***Air Pollution Health Effects Laboratory of UCI****:* Laminar flow isolation hoods, -80°C freezers for sample storage (Revco Scientific, Asheville, NC), temperature controlled incubators, a gyrorotory waterbath shaker (New Brunswick Scientific, Edison, NJ), an inverted stage light and fluorescence

microscopes (Nikon, Garden City, NY), a cyto-centrifuge and microtiter plate washers and readers for ELISA, colorimetric and fluorescence based assays. A confocal microscope and an inverted microscope with epifluorescence optical capabilities will be used for histology and immunohistochemistry. A 96-well, microtiter plate reader (SLT Labinstruments, Salzburg, Austria) is used for ELISA and spectrophotometric assays, 1-d and 2-d gel electrophoresis is used for DNA, RNA, and protein speciation (BRL Life Technologies, Gaithersberg, MD), and an atomic absorption spectrophotometer (Instrumentation Laboratory, Lexington, MA), equipped for both flame and thermal atomic atomization analyses for metals. A Luminex 100 Protein Array analyzer is used for cytokine and protein measurements in sera and tissue homogenates. The mobile exposure system, described under facilities, will be used for this study. Ozone, sulfur dioxide, nitrogen oxide and monitors for other gases are available to supplement those available at USC to support these proposed exposure studies. Calibrations for these instruments are traceable to primary or NBS standards. Analytical equipment includes a Cahn (Cerritos, CA) electronic microbalance, an Ion Chromatograph for anion and cation analyses, a high performance liquid chromatograph (HPLC) (LKB, Sweden) and a gas chromatograph (Hewlett Packard, Avondale, PA) for analyses of organic compounds.

3. PROJECT SCHEDULE

Task 1: Generation of atmospheres and exposure of apoE-/- mice to mixtures of O_3 and PM
Task 2: Physical and chemical characterization of ambient air and exposure atmospheres.
Task 3: Biological assays to determine adverse effects and mechanisms of action of exposures on the cardiovascular system.
Task 4: Data analysis and evaluation of acute and chronic cardiovascular inflammation and injury.
Task 5: Preparation of quarterly progress reports and manuscripts to be submitted for publication in the peer-reviewed literature.
Task 6: Preparation of the Final Reports for CARB submission at the end of the project

TASK	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																									
2																									
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TASK	Month	25	26	27	28	29	30	31	32	33	34	35	36
1													
2													
3													
4													
5													
6													

4. PROJECT PERSONNEL AND PROJECT MANAGEMENT

4.1 Overview

Overall management of the project will be under the direction of Dr. Kleinman. The study will be highly collaborative with close coordination between the atmospheric chemistry scientists at the AirUCI Center and the toxicology team at the Air Pollution Health Effects Laboratory. The research team will meet on a quarterly basis, or more frequently if needed, to discuss progress and problems, and any necessary adjustments to the data management and analysis protocols will be reviewed and implemented. The notes of these meetings will be incorporated into the quarterly reports of progress to the ARB.

4.2 Air Pollution Health Effects Laboratory Personnel

Michael T. Kleinman is a Professor in the Department of Medicine's Division of Occupational and Environmental Medicine at the University of California, Irvine. He has been studying the health effects of exposures to environmental contaminants found in ambient air for more than 30 years. He holds a MS in Chemistry from the Polytechnic Institute of Brooklyn and a Ph.D. in Environmental Health Sciences from New York University. He is the Co-Director of the Air Pollution Health Effects Laboratory. Prior to joining the faculty at U.C.I. in 1982, he directed the Aerosol Exposure and Analytical Laboratory at Rancho Los Amigos Hospital in Downey, CA. He has published more than 110 articles in peer-reviewed journals dealing with the uptake and dosimetry of inhaled pollutants in humans and laboratory animals, and effects on cardiopulmonary and immunological systems after controlled exposures to ozone and other photochemical oxidants, carbon monoxide, ambient or laboratory-generated aerosols and chemically or biologically reactive metals such as lead, cadmium, iron and manganese. He recently served on two National Academy committees to examine issues in protecting deployed US Forces from the effects of chemical and biological weapons; in one case as committee Chair. Dr. Kleinman's current studies focus on neurological and cardiopulmonary effects of inhaled particles, including nano-, ultrafine, fine and coarse particles in humans and laboratory animals. His current studies have demonstrated that inhalation of combustion-generated particles can promote airway allergies and accelerate the development of cardiovascular disease and that these effects may be associated with organic and elemental carbon components of the ultrafine fraction of the ambient aerosol. His studies have also demonstrated that inhalation of ambient particles is associated with persistent inflammation in the brain and that particles associated with manganese can alter dopamine and serotonin levels in the brain and can cause changes in nerve structure during brain development. Dr. Kleinman is a member of the U.S. EPA Science Advisory Board' Clean Air Scientific Advisory Committee (CASAC) Ozone panel and currently serves as the Chair of the California Air Quality Advisory Committee, which reviews California's air quality criteria documents.

Dr. Kleinman will be responsible for the overall conduct and direction of the proposed study in which genetically modified mice will be exposed to mixtures of ozone and inhaled ultrafine particles, with and without semivolatile organic constituents, to determine whether there are interactive or synergistic effects with respect to their

influence on the development and progression of cardiovascular diseases. Dr. Kleinman's primary responsibilities will be to:

1) Train the exposure team, and oversee setting up the exposure equipment;
2) Assure the timeliness of the overall project Tasks and data collection;
3) Coordinate exposure and atmospheric sampling/analysis activities with Dr. Wingen;
4) Coordinate quarterly meetings to review progress and plan analyses and presentation of results; and
5) Prepare major presentations, reports and publications of study results (Tasks 4,5 and 6)

Loyda Mendez Ph.D. (Co-Investigator)
Dr. Mendez will be responsible for training the Laboratory Assistant in performing exposures and bioassays and will assist in exposures, collect exposure and bioassay data, archive data, and perform data analyses.

Animal Technician (TBN)
This individual will be responsible for animal husbandry activities, maintenance of the onsite vivarium and will assist in performing exposures.

Laboratory Assistant I (TBN)
This individual will be responsible for performing exposures under the supervision of Drs. Mendez and Kleinman and will also perform regular equipment checks and calibrations between exposures to assure proper exposure system performance.

4.3 AirUCI Personnel
Lisa Wingen, Ph.D. (Co-Investigator) is

4.4 Resumes of the key scientific personnel

4.4.1 Principal Investigator/Program Director: Kleinman, Michael T.

NAME	POSITION TITLE
Michael T. Kleinman	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) michaelkleinman	

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
Brooklyn College, City University of New York	B.S.	1965	Chemistry
Polytechnic Institute of Brooklyn	M.S.	1971	Chemistry
New York University	Ph.D.	1977	Environmental Health Sciences

A. Personal Statement

Dr. Kleinman is a Professor of Occupational and Environmental Health and the Co-Director of the Air Pollution Health Effects Laboratory in the Department of Medicine at University of California with more than 30 years of experience in laboratory and field studies of environmental contaminants and their effects on health. Dr. Kleinman has published more than 100 peer-reviewed articles dealing with environmental contaminants and their effects on cardiopulmonary and immunological systems. He has directed more than 50 controlled exposure studies of human volunteers and laboratory animals to ozone, nitrogen oxides, carbon monoxide, ambient particulate matter and laboratory-generated aerosols containing chemically or biologically reactive metals such as lead, cadmium, iron and manganese, all of which are relevant to the current proposal on tobacco smoke health effects. Dr. Kleinman has extensive experience in the collection and analysis of contaminant aerosols and gases for organic constituents, including carbonyls and polycyclic organic compounds and for trace metals. His laboratory has an extensive history of performing exposure studies, using cell systems and animals, to ozone, nitrogen dioxide and ambient and laboratory-generated particulate matter (PM). His most recent studies have examined effects of chronic exposures to ambient air pollution on cardiopulmonary function and disease progression in animal models of cardiovascular diseases. These studies have identified, in addition to adverse effects on cardiac physiology, significant evidence of tissue inflammation and oxidative stress including evidence of lipid peroxidation and free radical generation in arteries that correlate with development of atherosclerotic plaques. In collaboration with Dr. Campbell we have also demonstrated activation of NFkB and Nrf2 transduction pathways in the central nervous systems of mice and rats after ambient PM exposures. His involvement in the current proposal will be in managing the project, supporting specific aims in which mice will be exposed to mixtures of ozone and ambient PM, participating in chemical and biological assays which will be performed to assess the degree to which these exposures mediate systemic inflammatory and antioxidant defenses that accelerate atherosclerosis and preparing reports and manuscripts.

B. Positions and Honors

Professional Positions:

1977-1982: Director, Aerosol and Analytical Laboratory, Environmental Health Service, Rancho Los Amigos Hospital, Downey, California.
1982-1989: Associate Adjunct Professor, Department of Community and Environmental Medicine, University of California, Irvine.
1992-Present: Adjunct Professor, Department of Medicine, School of Medicine, University of California, Irvine and Co-Director of the Air Pollution Health Effects Laboratory.

Awards and Other Professional Activities:

New York State Regents Scholarship (1959)
National Cancer Institute - Graduate Assistantship (1973-1977)
UCI Committee of 1000 Award for Research - (1997)
Board of Scientific Counselors, Center for Disease Control and Prevention Agency for Toxic Substances and Disease Registry
American Conference of Governmental Industrial Hygienists Threshold Limit Value Committee
USEPA Science Advisory Board: Health Effects Subcommittee (2000 to Present).
USEPA Science Advisory Board: Clean Air Scientific Advisory Committee, Ozone Panel (2004-Present); CO Panel (2008-Present).
Chair: State of California Environmental Protection Agency Air Quality Advisory Committee.
Director: Air and Waste Management Assoc. – Orange County Section
Chair: Publications Committee and Editorial Review Board – J. Air and Waste Management Association
Vice-chair: Inhalation and Respiratory Specialty Section, Society of Toxicology
Secretary: American Chemical Society, Orange County Section
Editorial Review Board – Air Quality and Environmental Health.
National Research Council/National Academy of Sciences-Principal Investigator Appointment (1998-2000).
Chair: Executive Committee – University of California Toxic Substance Teaching and Research Program (2000-2010)

C. Selected Peer-reviewed Publications (From a list of 114)

Kleinman, MT, Sioutas, C, Froines, J, Fanning, E, Hamade, A, Meacher, D and Oldham, M., Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice: relevance of particle composition and size, Inhal. Toxicol., 19 Suppl 1: 117-126, 2007 .

Simkhovich, B, **Kleinman, MT** and Kloner, RA, Young hearts are as susceptible as old hearts to the direct and acute cardiotoxic effects of ultrafine air pollutants, Basic Research in Cardiol. 102: 467-475, 2007.

Gong K, Zhao W, Li N, Barajas B, **Kleinman M**, Sioutas C, Horvath S, Lusis AJ, Nel A, Araujo J, Pro-oxidative air pollutant particle chemicals and oxidized LDL components exhibit genome wide synergistic effects on vascular endothelial cells, Genome Biology 8: R149, http://genomebiology.com/2007/8/7/R149.1-R149.13, 2007.

Araujo, J. A., Barajas, B., **Kleinman, M.**, Wang, X. P., Bennett, B. J., Gong, K. W., Navab, M., Harkema, J., Sioutas, C., Lusis, A. J., and Nel, A. E. (2008). Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circulation Research 102, 589-596, 2008.

Kleinman MT, Araujo J, Nel A, Sioutas C, Campbell A, Cong PQ, Li A, Bondy SC, Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways, Toxicol Lett 178, 127-130, 2008.

Delfino, R.J., Staimer, N., Tjoa, T., Polidori, A., Arhami, M., Gillen, D. L., **Kleinman, M.T**., Vaziri, N. D., Longhurst, J., Zaldivar, F., and Sioutas, C. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. Environ Health Perspect 116, 898-906, 2008.

Kleinman MT, Araujo J, Nel A, Sioutas C, Campbell A, Cong PQ, Li A, Bondy SC, Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways, Toxicol Lett 178, 127-130, 2008.

Simkhovich BZ, **Kleinman, MT**, Kloner RA, Air pollution and cardiovascular injury: epidemiology, toxicology and mechanisms, J. Amer. Coll. Cardiol. 52:719-726, 2008.

Simkhovich BZ, **Kleinman MT**, Kloner RA. 2009. Particulate air pollution and coronary heart disease. Current Opinion in Cardiology 24(6): 604-609.

Campbell A, Araujo JA, Li HH, Sioutas C, Kleinman M. 2009. Particulate Matter Induced Enhancement of Inflammatory Markers in the Brains of Apolipoprotein E Knockout Mice. Journal of Nanoscience and Nanotechnology 9(8): 5099-5104.

Li, N., Harkema, J. R., Lewandowski, R. P., Wang, M., Bramble, L. A., Gookin, G. R., Ning, Z., **Kleinman, M.** T., Sioutas, C. and Nel, A. E. 2010 Ambient ultrafine particles provide a strong adjuvant effect in the secondary immune response: implication for traffic-related asthma flares. *Am J Physiol Lung Cell Mol Physiol* **299**: L374-383.

Simkhovich, B.Z., **Kleinman, M.T**., Mehrian-Shai, R., Hsu, H., Meacher, D. Gookin, G., Mac Kinnon, M., Salazar, K. Willet, P. Feng, P., Lin, S.M. and Kloner, R.A. 2011 Chronic exposure to ambient particulate matter alters cardiac gene expression patterns and markers of oxidative stress in rats, Air Qual Atmos Health, DOI 10.1007/s11869-010-0089-0.

Richards, N. K., L. M. Wingen, **Kleinman, M.T.**,et al. 2011. "Nitrate ion photolysis in thin water films in the presence of bromide ions." The journal of physical chemistry. A 115(23): 5810-5821.

Verma, V., Cho A., **Kleinman M.T.**, Shafer M., Schauer J. and Sioutas S. 2011. Physicochemical and Oxidative Characteristics of Semi-Volatile Components of Quasi-Ultrafine Particles in an Urban Atmosphere. Atmospheric Environment **45**(4): 1025-1033.

Block ML, Elder A, Auten RL, Bilbo SD, Chen H, Chen JC, **Kleinman, MT**, et al. 2012. The outdoor air pollution and brain health workshop. Neurotoxicology 33(5): 972-984.

Program Director/Principal Investigator (Last, First, Middle):

4.4.2 Co-Investigator Loyda Mendez, Ph.D.

NAME Loyda B. Méndez	POSITION TITLE Assistant Specialist III
eRA COMMONS USER NAME (credential, e.g., agency login)	

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
University of Puerto Rico, Río Piedras	B.S.	2001	Biology
University of California, Irvine	Ph.D.	2006	Environmental Toxicology
University of Puerto Rico, Medical Sciences Campus	Postdoc	2007	Biochemistry/Toxicology
University of California, Irvine	Postdoc	2008	Microbiology/Aerosol dosimetry

A. Personal Statement

I have a broad background in both microbiology and inhalation toxicology, with specific training and expertise in key research areas necessary for conducting in-vitro & in-vivo studies with aerosols. In the area of microbiology I have over twelve years of experience in the sampling, enumeration, and characterization of bacteria and bacterial components (e.g. endotoxin) through conventional, biochemical, and molecular tools. In the inhalation toxicology field, I have over eight years of experience in the generation, sampling and characterization of aerosols, *in-vitro* and *in-vivo* exposures to aerosols, aerosol dosimetry, and animal models of pulmonary and systemic disease. As part of my doctoral dissertation I studied the interaction of bacterial components and particulate matter in both *in-vivo* and *in-vitro* models. In addition, as a postdoctoral researcher in the University of Puerto Rico, I was involved in studying molecular mechanisms involved in the induction of pro-inflammatory cytokines by particulate matter in an *in-vitro* model of human bronchial epithelial cells. Dr. Mendez will oversee exposure and animal husbandry activities and will participate in preparation of reports and manuscripts.

B. Positions and Honors

Positions:

1998-2001: Undergraduate Research Assistant. Environmental Microbiology Laboratory. University of Puerto Rico, Río Piedras.

2000: Undergraduate Research Assistant. Center for Environmental Biotechnology. Lawrence Berkeley National Laboratory, Berkeley, CA.

2001-2002: Graduate Research Assistant. Environmental Biotechnology Laboratory. University of California, Irvine.

2002-2006: Graduate Research Assistant. Air Pollution Health Effects Lab. University of California, Irvine.

2006-2007: Postdoctoral Researcher. Center for Environmental and Toxicological Studies. University of Puerto Rico, Medical Sciences Campus.

2008- 2010: Postdoctoral Reseacher. Pacific Southwest Regional Center of Excellence

for Biodefense and Emerging Infectious Diseases. University of California, Irvine.
2010- Present: Assistant Specialist III. Pacific Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases & Air Pollution Health Effects Lab. University of California, Irvine.

Honors:
1998 to 1999: Pre-Minority Access to Research Careers, NIH. University of Puerto Rico, Rio Piedras.
1999-2001: Minority Access to Research Careers, NIH. University of Puerto Rico, Rio Piedras.
2000: Energy Research Undergraduate Laboratory Fellowship, DOE. Lawrence Berkeley National Laboratory, Berkeley
2001-2006: Alliance for Graduate Education and the Professoriate, NSF. University of California, Irvine.

Professional Memberships:
American Society of Microbiology
Society of Toxicology

C. Selected Publications
Abstracts
1- Campbell A., Becaria A., **Méndez L.**, Bondy SC., Oldham M., Kleinman M. Airborne particulate matter upregulates inflammatory markers in the mouse brain: implications for neurodegeneration. The Toxicologist. 2005. 84 (S1): 295

2- Campbell A., **Méndez L.**, Becaria A., Kleinman M. Enhancement of innate immune responses and oxidative events after exposures to particulate matter present in urban air pollution. The Toxicologist. 2006. 90(1): 51

3- Rodríguez R.I., Méndez-Torres L., Jiménez-Vélez B.D. Studying Ambient PM2.5 Extracts from Saharan Dust Collected in Puerto Rico. Puerto Rico Health Sciences Journal. 2010. 29 (2): 153

4- Rivera-Ramírez E., **Méndez L.B.**, Jiménez-Vélez, B.D. Effects of $PM_{2.5}$ from Puerto Rico on the post-transcriptional control of pro-inflammatory cytokines. Puerto Rico Health Sciences Journal. 2010. 29(2):149

5- Rivera-Ramírez E., **Méndez L.**, Jiménez-Vélez B.D. Effects of $PM_{2.5}$ from Puerto Rico on the mRNA half-lives of pro-inflammatory cytokines. The Toxicologist. 2010. 114(1): 162

6- Ortiz-Martínez M., Rivera-Ramírez E., **Méndez L.**, Jiménez-Vélez B.D. Endotoxins in African Dust (PM_{10}): possible implication in Puerto Rican asthma exacerbation. The Toxicologist. 2010. 114(1): 161

7- **Mendez LB**, Phalen RF, Ramirez GJ. "Tracheobronchial Airway Morphometry for the C57BL/6 Mouse: Implications in Inhaled Dosimetry Predictions." The Toxicologist. 2011. 120 (S2): 127

Publications

1- Montañez J., **Méndez L.**, Chauhan S., Hazen T.C. Polynuclear aromatic hydrocarbons in situ bioremediation treatability test; focus on contaminant disappearance by HPLC analysis. U.S. Department of Energy Undergraduate Research Journal. 2001. 1:37

2- Campbell A., Oldham M., Becaria A., Bondy S.C., Meacher D., Sioutas C., Misra C.**, Méndez L.B.**, and Kleinman M. Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. Neurotoxicology. 2005. 26(1): 133-40. PMID: 15527881

3- Kleinman M.T., Sioutas C., Froines J.R., Fanning E., Hamade A., **Méndez L.**, Meacher D., Oldham M. Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice. Inhalation Toxicology. 2007. 19(S1): 117-26. PMID: 17886059

4- Hsu A., **Méndez L.**, Shah J., Sioutas C., Kleinman M., Campbell A. Nanoparticles in air pollution and CNS innate immne responses. International Journal of Neuroprotection and Neuroregeneration. 2007. 3(2): 107-113.

5- Phalen R.F., **Méndez L.B.** Aerosol Dosimetry Considerations for Animal Aerosol Inhalation Studies. Biomarkers. 2009. 14(S1): 63-66. PMCID: 19604062

6- Ortiz-Martínez M., Rivera-Ramirez E., **Méndez-Torres L.,** Jiménez-Vélez B.D. "Role of Chemical and Biological Constituents of Saharan Dust in the Exacerbation of Asthma in Puerto Rico". In: Biodiversity Science for Humanity. Ed. by *Michael Theophanides and Theophile Theophanides*. 2010. 2:101-118. ATINER, ISBN: 978-960-6672-41-5

7- **Méndez L.B.**, Gookin G., Phalen R.F. Inhaled Aerosol Dosimetry in Mice: A Review. Inhalation Toxicology. 2010. 22(S2): 15-20. PMID: 20879957

8- Phalen R.F., **Méndez L.B.**, Oldham MJ. New developments in aerosol dosimetry. Inhalation Toxicology. 2010. 22(S2):6-14. PMID: 20939685

D. Research Support

PHS-NIH Allergy and Infectious Diseases U54 AI065359 Barbour (PI): 5/1/05-4/30/14
Pacific-Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases
This is a multi-disciplinary, multi-institution research consortium for basic, applied, and clinical research on
arboviruses (dengue and West Nile virus), Nipah virus, arenaviruses (Lassa fever), botulinum toxin, Burkholderia pseudomallei, tularemia, plague, and coccidioidomycosis. There are 25 institutions participating
in California, Arizona, Nevada, and Hawaii (http://www.pswrce.uci.edu).
Role: Assistant Specialist

California EPA Air Resources Board ARB-08-306 Kleinman (PI): 11/17/09 -11/16/12
Neurotoxic Effects of Ambient Particulate Matter: The Role of Oxidative Stress and Inflammation
The goal of this project is to establish the association between exposure to ambient particulate matter and adverse CNS effects in an apolipoprotein E knockout mouse model.
Role: Assistant Specialist

4.4.3 Co-Investigator Lisa Wingen, Ph.D.

NAME	POSITION TITLE
Lisa M. Wingen	Project Scientist
eRA COMMONS USER NAME (credential, e.g., agency login)	

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
California State University, Fullerton, California	B.S.	1993	Chemistry
University of California, Irvine, California	M.S.	1996	Chemistry
University of California, Irvine, California	Ph.D.	1999	Chemistry

A. Personal Statement

Dr. Winger is part of the Finlayson-Pitts research group in the AirUCI,Center an organized research unit located at UC Irvine with several collaborators in the departments of chemistry, medicine, and engineering. Dr. Winger's specialization is the study of chemical reactivity on particle surfaces. In addition to her own projects, she advises post-doctoral researchers, graduate students and undergraduates in their research and academic activities. Her involvement in the current proposal will be in overseeing the chemical characterization of exposure atmospheres, examining seasonal changes in organic constituents and preparing reports and manuscripts.

B. Positions and Honors

Professional Positions:

2001 - present	University of California, Irvine	Project Scientist
1999-2000	University of California, Irvine	Post-doctoral researcher

C. Selected Peer-reviewed Publications

"A Semi-Blind Source Separation Method for Differential Optical Absorption Spectroscopy of Atmospheric Gas Mixtures," Y. Sun, L. M. Wingen, B. J. Finlayson-Pitts, and J. Xin, submitted to *Journal of Inverse Problems and Imaging*, November 2011.

"Rapid Formation of Molecular Bromine From Deliquesced NaBr Aerosol in the Presence of Ozone and UV Light," Paul Nissenson, Lisa M. Wingen, Sherri W. Hunt, Barbara J. Finlayson-Pitts, Donald Dabdub, *in preparation*.

"Nitrate Ion Photolysis in Thin Water Films in the Presence of Bromide Ions," Nicole K. Richards, Lisa M. Wingen, Karen M. Callahan, Noriko Nishino, Michael T. Kleinman, Douglas J. Tobias, and Barbara J. Finlayson-Pitts, *Journal of Physical Chemistry A*, 115, 5810-5821, **2011** (DOI: 10.1021/jp109560j).

"Enhanced Surface Photochemistry in Chloride-Nitrate Ion Mixtures," Lisa M. Wingen, Amy C. Moskun, Jennie L. Thomas, Martina Roeselová, Douglas J. Tobias and Barbara J. Finlayson-Pitts, *Physical Chemistry Chemical Physics*, 10, 5641-5788, **2008**. (Designated a "hot article" by *Phys. Chem. Chem. Phys.*)

"New Experimental and Theoretical Approach to the Heterogeneous Hydrolysis of NO_2: Key Role of Molecular Nitric Acid and Its Complexes," K. A. Ramazan, L. M. Wingen, Y. Miller, G. M. Chaban, R. B. Gerber, S. S. Xantheas, and B. J. Finlayson-Pitts, *Journal of Physical Chemistry A*, 110, 6886-6897, **2006** (DOI: 10.1021/jp056426n)

"Formation of Molecular Bromine from the Reaction of Ozone with Deliquesced NaBr Aerosol: Evidence for Interface Chemistry," S. W. Hunt, M. Roeselová, W. Wang, L. M. Wingen, E. M. Knipping, D. J. Tobias, D. Dabdub, and B. J. Finlayson-Pitts, *Journal of Physical Chemistry A*, 108, 11559-11572, **2004**.

"The Nature of Water on Surfaces of Laboratory Systems and Implications for Heterogeneous Chemistry in the Troposphere," Ann Louise Sumner, Erik. J. Menke, Yael Dubowski, John T. Newberg, Reginald M. Penner, John C. Hemminger, Lisa M. Wingen, Theo Brauers, and Barbara J. Finlayson-Pitts, *Physical Chemistry Chemical Physics*, 6, 604-613, **2004** (DOI: 10.1039/ b308125g).

"The Heterogeneous Hydrolysis of NO_2 in Laboratory Systems and in Outdoor and Indoor Atmospheres: An Integrated Mechanism," Barbara J. Finlayson-Pitts, Lisa M. Wingen, Ann Louise Sumner, Dennis Syomin, and Kevin A. Ramazan, *Physical Chemistry Chemical Physics*, 5, 223-242, **2003** (invited article).

Other Significant Publications:

"Heterogeneous NO_x Chemistry in Polluted Urban Atmospheres: Implications for the Formation of Particles and Ozone and Control Strategy Development," *Final Report Prepared for the California Air Resources Board and the California Environmental Protection Agency, Research Division, Contract 00-323*, B. J. Finlayson-Pitts, L. M. Wingen, Y. Dubowski, K. A. Ramazan, A. M. Rivera-Figueroa, A. L. Sumner, D. Syomin, D. Dabdub, A. Jimenez-Aranda, E. Knipping, January 2004.

"Heterogeneous Formation of Nitrous Acid in Laboratory Systems," L. M. Wingen, A. L. Sumner, D. Syomin, K. A. Ramazan, and B. J. Finlayson-Pitts, *Manuscript for the 5th Conference on Atmospheric Chemistry: Gases, Aerosols, and Clouds at the 83rd American Meteorological Society Meeting*, February 2003.

"The Formation of Gaseous Nitrous Acid (HONO): A Key Determinant of Tropospheric Ozone and Fine Particles," *Final Report prepared for the California Air Resources Board and the California Environmental Protection Agency, Research Division, Contract 97-311*, B. J. Finlayson-Pitts, N. A. Saliba, L. M. Wingen, W. S. Barney, M. Mochida, and H. Yang, July 2001.

PI NAME: Michael Kleinman

DETAILED BUDGET - DIRECT COSTS ONLY

FROM 7/1/2013 THROUGH 6/30/2014

PERSONNEL (UCI personnel only) NAME	TITLE	No. of Months	% Effort	Employee Benefit Rate	Current Monthly Salary	CoL	Merit	Monthly Salary for Year 1	DOLLAR AMOUNT REQUESTED (omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS	Hourly Billing Rate
Michael Kleinman	PI	12	10%	4%	$10,283	2%	0%	$10,489	12,587	564	13,151	$60
Lisa Wingen	Assoc Proj Sci, Step II	12	25%	35%	$5,783	2%	0%	$5,899	17,697	6,132	23,829	$34
Loyda Mendez	Assistant Specialist	12	25%	35%	$3,811	2%	5%	$4,078	12,233	4,239	16,472	$23
TBN, Animal Tech	Anim Tech	12	50%	35%	$2,984	2%	0%	$3,044	18,262	6,328	24,590	$17
TBN, Lab Assistant II	Lab Tech II	12	100%	35%	$2,927	2%	0%	$2,986	35,826	12,414	48,240	$17
		0	0%	0%	$0	0%	0%	$0	0	0	0	
		0	0%	0%	$0	0%	0%	$0	0	0	0	
		0	0%	0%	$0	0%	0%	$0	0	0	0	
		0	0%	0%	$0	0%	0%	$0	0	0	0	
		0	0%	0%	$0	0%	0%	$0	0	0	0	
		0	0%	0%	$0	0%	0%	$0	0	0	0	
TOTAL NUMBER OF PERSONNEL HOURS			3,342									
SUBTOTALS →									**96,605**	**29,677**	**126,282**	

CONSULTANT COSTS - *includes both fees and travel expense (if applicable)*

			0

EQUIPMENT *(Items with a unit value of $1500 or greater - items under $1500 each belong in the supply category)*

Particle denuder/monitor system	$20,000	$0	
		$0	
	$0	$0	
	$0	$0	20,000

SUPPLIES

DSI Implants and upgrades	$18,000		
Mice	$16,000		
Filters and analytical supplies	$4,000		
Fittings and hardware	$6,500		
	$0		44,500

TRAVEL

Travel and to annual meeting		$0	0

PATIENT CARE COSTS	INPATIENT	$0	0
	OUTPATIENT	$0	0

ALTERATIONS AND RENOVATIONS

	$0	0

OTHER EXPENSES

Animal Husbandry	$2,555		
Cage Washer Maint Contract	$750		
Pro-rated Cost-Equip and Fac. Maint	$1,250		
Publication, Photocopying and Printing	$250		
Mail, Telephone and FAX	$250		
Analyses for Air Contaminants	$5,000		
	$0		
	$0		10,055

SUBTOTAL DIRECT COSTS FOR BUDGET PERIOD		$	200,837
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS		
	F&A COSTS		
TOTAL DIRECT COSTS FOR BUDGET PERIOD		$	200,837
MODULAR BUDGET - ROUND TOTAL DIRECT COSTS UP TO NEAREST $25,000 →		$	

PI NAME: Michael Kleinman

DETAILED BUDGET - DIRECT COSTS ONLY

FROM 7/1/2014 THROUGH 6/30/2015

PERSONNEL *(UCI personnel only)* NAME	TITLE	No. of Months	% Effort	Employee Benefit Rate	Current Monthly Salary	CoL	Merit	Monthly Salary for Year 2	DOLLAR AMOUNT REQUESTED (omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Michael Kleinman	PI	12	10%	7%	$10,489	2%	0%	$10,699	12,839	877	13,716
Lisa Wingen	Assoc Proj Sci, Step II	12	25%	37%	$5,899	2%	5%	$6,312	18,936	7,006	25,942
Loyda Mendez	Assistant Specialist	12	25%	37%	$4,078	2%	0%	$4,159	12,478	4,617	17,095
TBN, Animal Tech	Anim Tech	12	50%	37%	$3,044	2%	5%	$3,257	19,540	7,230	26,770
TBN, Lab Assistant II	Lab Tech II	12	100%	37%	$2,986	2%	5%	$3,195	38,334	14,184	52,518
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	0%	$0	2%	0%	$0	0	0	0
0	0	0	0%	0%	$0	2%	5%	$0	0	0	0
0	0	0	0%	0%	$0	2%	0%	$0	0	0	0
TOTAL NUMBER OF PERSONNEL HOURS			4,385								
SUBTOTALS									**102,127**	**33,914**	**136,041**

CONSULTANT COSTS - *includes both fees and travel expense (if applicable)*				
			$0	0
EQUIPMENT *(Items with a unit value of $1500 or greater - items under $1500 each belong in the supply category)*				
		$0	$0	
		$0	$0	
		$0	$0	
		$0	$0	0
SUPPLIES				
DSI Implants and upgrades		$18,000		
Mice		$8,000		
Filters and analytical supplies		$1,250		
Fittings and hardware		$3,250		
0		$0		
		$0		30,500
TRAVEL				
Travel and registration for conferences			$1,098	1,098
PATIENT CARE COSTS	INPATIENT		$0	0
	OUTPATIENT		$0	0
ALTERATIONS AND RENOVATIONS				
			$0	0
OTHER EXPENSES				
Animal Husbandry		$1,278		
Cage Washer Maint Contract		$750		
Pro-rated Cost-Equip and Fac. Maint		$250		
Publication, Photocopying and Printing		$250		
Mail, Telephone and FAX		$250		
Analyses for Air Contaminants		$5,000		
		$0		
		$0		7,778
SUBTOTAL DIRECT COSTS FOR BUDGET PERIOD				$ 175,417
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS			
	F&A COSTS			
TOTAL DIRECT COSTS FOR BUDGET PERIOD				$ 175,417
MODULAR BUDGET - ROUND TOTAL DIRECT COSTS UP TO NEAREST $25,000 →				$

PI NAME: Michael Kleinman

DETAILED BUDGET - DIRECT COSTS ONLY

FROM 7/1/2015 THROUGH 6/30/2016

PERSONNEL (UCI personnel only) NAME	TITLE	No. of Months	% Effort	Employee Benefit Rate	Current Monthly Salary	CoL	Merit	Monthly Salary for Year 3	DOLLAR AMOUNT REQUESTED (omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Michael Kleinman	PI	12	20%	7%	$10,699	2%	0%	$10,913	26,191	1,789	27,980
Lisa Wingen	Assoc Proj Sci, Step II	12	25%	39%	$6,312	2%	5%	$6,754	20,262	7,862	28,124
Loyda Mendez	Assistant Specialist	12	25%	39%	$4,159	2%	0%	$4,242	12,727	4,938	17,665
TBN, Animal Tech	Anim Tech	12	25%	39%	$3,257	2%	5%	$3,485	10,455	4,057	14,512
TBN, Lab Assistant II	Lab Tech II	12	50%	39%	$3,195	2%	5%	$3,419	20,512	7,959	28,471
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	2%	$0	2%	5%	$0	0	0	0
0	0	0	0%	0%	$0	2%	0%	$0	0	0	0
0	0	0	0%	0%	$0	2%	5%	$0	0	0	0
0	0	0	0%	0%	$0	2%	0%	$0	0	0	0
TOTAL NUMBER OF PERSONNEL HOURS			3,028								
SUBTOTALS									90,147	26,605	116,752

CONSULTANT COSTS - *includes both fees and travel expense (if applicable)*

	$0	0

EQUIPMENT *(Items with a unit value of $1500 or greater - items under $1500 each belong in the supply category)*

$0	$0	
$0	$0	
$0	$0	
$0	$0	0

SUPPLIES

DSI Implants and upgrades	$18,000	
Mice	$8,000	
Filters and analytical supplies	$1,250	
Fittings and hardware	$3,250	
0	$0	
	$0	30,500

TRAVEL

Travel and registration for conferences	$1,098	1,098

PATIENT CARE COSTS	INPATIENT	$0	0
	OUTPATIENT	$0	0

ALTERATIONS AND RENOVATIONS

$0	0

OTHER EXPENSES

Animal Husbandry	$1,278	
Cage Washer Maint Contract	$750	
Pro-rated Cost-Equip and Fac. Maint	$250	
Publication, Photocopying and Printing	$250	
Mail, Telephone and FAX	$250	
Analyses for Air Contaminants	$5,000	
	$0	
	$0	7,778

SUBTOTAL DIRECT COSTS FOR BUDGET PERIOD	$	156,128
CONSORTIUM/CONTRACTUAL COSTS: DIRECT COSTS		
CONSORTIUM/CONTRACTUAL COSTS: F&A COSTS		
TOTAL DIRECT COSTS FOR BUDGET PERIOD	$	156,128
MODULAR BUDGET - ROUND TOTAL DIRECT COSTS UP TO NEAREST $25,000 →	$	

CALCULATION OF FACILITIES AND ADMINISTRATIVE COSTS (INDIRECT COSTS) FOR PROJECT PERIOD

F&A costs are calculated by multiplying the Modified Total Direct Costs (MTDC) base by the applicable F&A cost rate. The MTDC equals the Modular Direct Costs less the following exclusions: Equipment and capital expenditures; patient care costs for services provided by a hospital or clinic, including UCI Medical Center, but not the laboratories of academic departments or organized research units or salaries to personnel providing the services; rental, lease and maintenance costs of off-campus space; tuition and fee remission; financial aid paid directly to University students, but not as salaries and wages, when allowable under the terms of the award; and the portion of each subgrant, subcontract, and subaward modification that is in excess of $25,000.

	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5	TOTALS
Direct Costs	$200,837	$175,417	$156,128	$0	$0	$532,382
F&A (IDC) Exclusions:						
Fee Remission	$0	$0	$0	$0	$0	$0
Tuition Remission	$0	$0	$0	$0	$0	$0
Equipment	$20,000	$0	$0	$0	$0	$20,000
Patient Care	$0	$0	$0	$0	$0	$0
Alterations & Renovations	$0	$0	$0	$0	$0	$0
Rental of Space	$0	$0	$0	$0	$0	$0
Consortiums In Excess of $25000	$0	$0	$0	$0	$0	$0
Modified Total Direct Cost (MTDC) Base 1	$180,837	$175,417	$156,128	$0	$0	$512,382
Modified Total Direct Cost (MTDC) Base 2	$0	$0	$0	$0	$0	$0
F&A Cost Rate 1	10.0%	10.0%	10.0%	0.0%	0.0%	
F&A Cost Rate 2	0.0%	0.0%	0.0%	0.0%	0.0%	
F&A Costs 1	$18,084	$17,542	$15,613	$0	$0	$51,239
F&A Costs 2	$0	$0	$0	$0	$0	$0
TOTAL COSTS	$218,921	$192,959	$171,741	$0	$0	$583,621

Indirect Cost Calculation: http://www.rgs.uci.edu/rig/cgfa.htm

Task	Labor	Employee Fringe Benefits	Equip	Travel Subsist	EDP	Mail, Phone, Fax	Materials and Supplies	Analyses
1	$117,844	$36,794	$0	$0	$0	$0	$88,500	$0
2	$92,474	$28,873	$20,000	$0	$0	$0	$5,000	$10,000
3	$15,485	$4,835	$0	$0	$0	$0	$12,000	$5,000
4	$28,320	$8,842	$0	$1,696	$0	$450	$0	$0
5	$24,433	$7,629	$0	$500	$0	$300	$0	$0
6	$10,323	$3,223	$0	$0	$0	$0	$0	$0
	$288,879	$90,196	$20,000	$2,196		$750	$105,500	$15,000

288879 90196 20000 2196 0 750 105500 15000

PERCENTAGES

Task	Labor	Employee Fringe Benefits	Equip	Travel Subsist	EDP	Mail, Phone, Fax	Materials and Supplies	Analyses
1	41%	41%	0%	0%	0%	0%	84%	0%
2	32%	32%	100%	0%	0%	0%	5%	67%
3	5%	5%	0%	0%	0%	0%	11%	33%
4	10%	10%	0%	77%	0%	60%	0%	0%
5	8%	8%	0%	23%	0%	40%	0%	0%
6	4%	4%	0%	0%	0%	0%	0%	0%
	100%	100%	100%	100%	0%	100%	100%	100%

**Please only revise with the correct % (in green) for each task. Each category must
I am not sure how many tasks you have but the example listed 6 so that is what I used

Task	Description
1	Generation of atmospheres and exposure of mice to mixtures of ozone and PM
2	Physical and Chemical Characterization of atmospheres
3	Biological assays to determine adverse health effects
4	Data analysis and interpretation
5	Report and manuscript preparation
6	Final Report preparation

Misc	Overhead	Total
$3,000		
$3,800		
$3,061		
$0		
$0		
$0		
$9,861	$51,239	$583,621

9861 0 0

Misc	Overhead	Total
30%		
39%		
31%		
0%		
0%		
0%		
100%	0%	0%

add up to 100%.
l for the draft. I will delete out any unused task rows.