DRAFT PROPOSAL

Co-exposure to PM and O_3: Pulmonary C fiber and platelet activation in decreased HRV

Fern Tablin, VMD, PhD
Principal Investigator

Prepared For:
State of California Air Resources Board
Research Division
PO Bo 2815
Sacramento A 95812

University of California, Davis
One Shields Avenue
Davis, CA 95616
530-752-8259

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Check if applicable:
Animal Subjects X
Human Subjects

B) **Table of Contents**:

C) Abstract:

Exposure to ambient particulate matter (PM) and/or ozone (O_3) has been associated with increased cardiovascular events, particularly in people with ongoing cardiovascular disease. Many studies have demonstrated that pulmonary, vascular and neuronal actions are in part responsible for increased cardiovascular morbidity and mortality, however the mechanisms by which these three systems converge has not yet been defined. The studies proposed herein are designed to address this question. Our research has demonstrated increases in systemic pro-inflammatory cytokines as well as platelet activation and the production of platelet-monocyte aggregates in PM exposed mice. Additionally, new unpublished data demonstrate constriction of terminal pulmonary arterioles, potentially leading to increased pulmonary pressures, suggesting an additional mechanism for PM cardiac effects. Studies from the Schlegle lab have demonstrated a clear association between O_3 exposure and up-regulation of pulmonary C fiber afferents. Pulmonary C-fibers initiate central nervous system reflexes altering breathing pattern and parasympathetic input to the heart while releasing neoropeptides, including substance P, at the site of activation. Interestingly, substance P released from C-fiber endings is known to activate platelets forming microthrombi and releasing 5 hydroxy-tryptamine (5HT) and thromboxane (TXA_2) while 5HT and TXA_2 have been shown to activate pulmonary C fiber afferents. All of these findings suggest CNS mediated and direct hematogenous pathways converging on myocardial and pulmonary receptors regulating heart rate and contractility, as well as suggesting a mechanistic basis for synergistic interaction between O_3 and PM resulting in further increasing the effects of decreased HRV associated with PM.
We will evaluate the effects of flame generated PM and O_3, either alone or in combination, on the vascular, hemostatic, pulmonary and cardiovascular systems of normal and spontaneously hypertensive rats. Four sets of conditions will be examined for normal and SH rats: filtered air, PM alone, O_3 alone, PM +O_3. Animals will be exposed to the above conditions for 8 hours and heart rate variability (HRV) and breathing patterns recorded in awake unrestricted rats equipped with surgically implanted telemetry units.

At the end of the exposure, animals will be euthanized and the following endpoints determined. This study will focus on platelet activation, as well as the persistence of platelet-monocyte aggregates previously shown to persist for up to 30 days in patients post myocardial infarction. We also will measure levels of 5HT and TXA2 from blood directly taken from the left ventricle. To examine the role of endothelial activation, both in the terminal pulmonary arterioles as well as in the coronary vasculature. Additionally, we will measure the diameter and wall thickness of the pulmonary arteries, which, we believe will be altered upon multiple pollutant exposures, particularly in hypertensive animals. We will examine sections of heart and lung pathology, as well as determining the presence of platelet aggregates in the vessel walls. C fiber activation, reflected as increased 5HT3 receptors in both in the heart and the lung, will be examined by immunocytochemistry. There are no studies in the literature examining the role of ozone on platelet activation and the formation and persistence of platelet-monocyte aggregates.

D) **Introduction**:

Many observational and epidemiological studies have consistently demonstrated that exposure to fine PM 2.5 is associated with increased cardiovascular (CV) mobidity and mortality. This is particularly true for people with underlying CV disease including but not limited to: hypertension, atherosclerosis, angina and myocardial infarction. Furthermore, short term (acute) PM exposures have been associated with excess deaths from myocardial ischemia, heart failure and arrhythmias (Samet et al. 2000, Peters et al., 2001).

The pathways by which PM exerts cardiovascular (CV) effects can be divided into two different areas. In the first pathway, the pulmonary system has been shown to responds to PM through the production of systemic pro-inflammatory and pro-thrombotic cytokines. Thus, indirectly leading to downstream events, including production of acute phase proteins CRP and plasma fibrinogen. This pathway has been borne out by human clinical studies (refs) as well as numerous animal studies (us, nemmar and lots of others) including CAPs studies from our laboratory (Wilson et al., 2010; Tablin et al., 2012). The development of a prothrombotic and/or procoaguable state requires release of proinflammatory factors into the pulmonary vasculature leading to downstream activation of both the vascular endothelium and blood platelets. Human and animal studies of inhaled concentrated PM has been shown to result in increases in platelet activation, platelet-leukocyte and platelet-monocyte interactions (Lucking et al.,2008) as well as decreased fibrinolysis – all of which are predisposing factors for thrombus formation potentially resulting in myocardial ischemia.

A second pathway by which PM can exert their CV effects is the direct translocation of fine and ultrafine PM into the pulmonary circulation. This has been convincingly demonstrated in animal models (Oberdorster et al., 2002), but direct evidence in humans appears lacking. Movement of PM directly into the circulation allows for direct interactions with blood cells and the vascular endothelium. While a variety of factors determine which elements of PM are able to cross the alveolar wall, the end result can be similar to that of indirect pulmonary effects; increased systemic inflammation. There is considerable data to suggest that circulating PM also can interact with and destabilize atherosclerotic plaques leading to their rupture, the develop of thrombi, and acute coronary syndrome (Brook et al., 2004). Studies in $ApoE^{-/-}$ mice demonstrated increased number and sizes of aortic plaques, in association with ultrafine PM; rich in polycyclic aromatic hydrocarbons (PAHs)(Araujo et al., 2008 Circ Res).

Regardless of the pathway, PM can exert their effects directly or indirectly on the autonomic nervous system leading to decreased heart rate variability (HRV). There is a well documented relationship between the ability of the autonomic nervous system to regulate the cardiac cycle and cardiovascular mortality (Malik et al., 1996). Alterations in HRV have been demonstrated in both short term and chronic PM exposures. Studies by Fan and colleagues (2009) demonstrated that acute PM exposure in healthy older adults could result in acute decline in HRV. The elderly, with and without ongoing cardiovascular disease, are among the largest of the affected populations (Adar et al., 2007; Pope et al., 2004). A meta-analysis study by Pieters et al. (2012) concluded that there is “an inverse relationship between HRV, a marker for a worse cardiovascular prognosis, and particulate air pollution”.

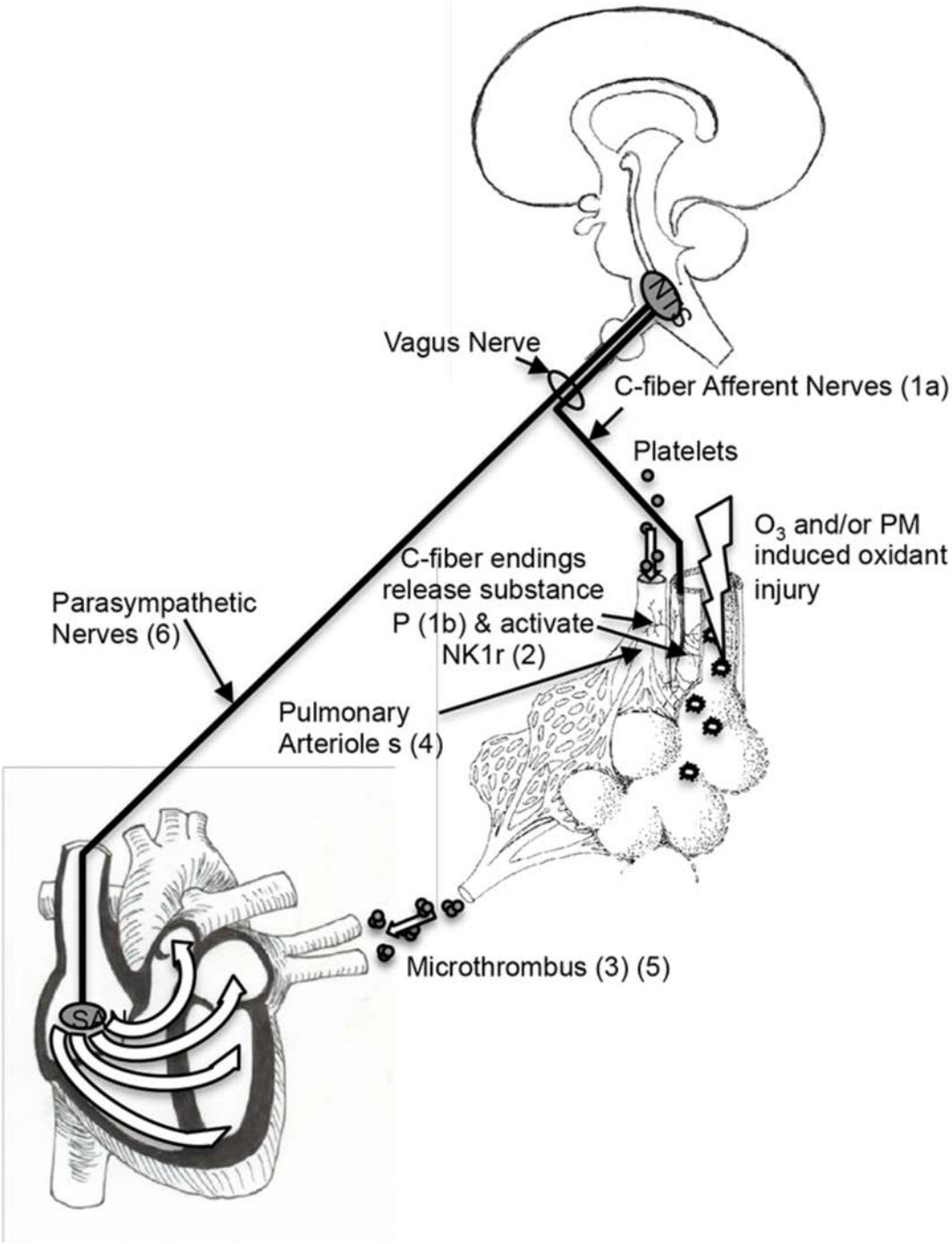
Human PM exposure studies provide clear indications of association with systemic inflammation, platelet activation, and the increase in acute phase proteins ((Delfino et al. 2008). Some of the most pronounced effects were seen with UF PM. Studies with wood smoke and cigarette smoke have also shown that these pollutants activate pulmonary C fibers, releasing neurokinins that affect both the systemic circulation and the autonomic nervous system, resulting in a reflex reaction that eventually leads to decreased HRV.

Due to the close association, both temporally and geographically, of O_3 and PM, it has been difficult to separate the effects of these two pollutants. Animal model studies have shown that O_3 acts through biochemical modification of airway surface liquid to form oxidative intermediates that injure airway epithelial cells, and selectively targets ciliated cells of the upper airways and terminal bronchiolar regions for degeneration and necrosis. O_3 airway injury has been shown to be associated with C fiber pathway activation (Schelegle et al., 2001). There is limited literature in this regard, despite the potential for this pathway to induce alterations in heart rate regulation. However, a recent experimental study of healthy human subjects by Devlin and colleagues (Devlin et al.2012) has demonstrated that O_3 can result in

increases in systemic inflammation, fibrinolysis and autonomic dysfunction markers, similar to those seen in association with PM exposure.

Despite the fact that air pollution is frequently a mixture of PM and O_3, there are few studies examining their individual and synergistic effects. Studies of co-pollutant exposures demonstrated decreased vagal tone (a short term autonomic imbalance) resulting in reduced HRV (Gold et al., 2000). Work by Brook and colleagues (Brook et al.,2002) in healthy adults, demonstrated that co-exposures resulted in acute arterial vasoconstriction, which could, particularly in patients with CV disease, promote myocardial ischemia.

The proposed studies are designed to addresses the overall hypothesis that: Co-exposure to PM and O_3 results in a synergistic activation of pulmonary C fibers and platelets leading to changes in the autonomic nervous system resulting in decreased HRV. Further, in a compromised heart, PM induced platelet activation and the production and persistence of platelet-monocyte aggregates leads to release of platelet derived neurotransmitters and bioactive lipids resulting in physiologic heart failure which is exacerbated by O_3 induced C fiber activation enhancing decreased HRV.



Schematic diagram shown above of hypothesized role of lung C-fibers in adverse cardiovascular responses induced by ozone and/or PM inhalation. (1) Ozone and PM activate pulmonary C- fibers resulting in (a) reflex rapid shallow breathing and increased parasympathetic nerve activity to the myocardium, and (b) release substance P into the surrounding tissue. (2) The binding of substance P activates neurokinin-1 (NK-1) receptors within terminal airways and pulmonary vasculature and on platelets modulating cell death and repair and pulmonary vasoconstriction. (3) NK-1 receptor mediated platelet activation results in microthrombus formation and the release of arachidonic acid metabolites and serotonin. (4) Serotonin further contributes to pulmonary vasoconstriction. (5) Increases in local arteriolar resistance increases shear stress that acts to further activate platelets traveling to the heart.

E) Objectives:

The objectives of this study are directly relevant to the mission of the Air Resources Board. The proposed work will lead to an improved scientific understanding of the relationship between PM, O_3 and cardiac events. Our hypothesis is that O_3 and PM exposures result in synergistic activation of pulmonary C fibers and platelets. Activation of these systems and signaling through the nodose ganglia to the nucleus tractus solitaries result in altered vagal tone and decrease in HRV. Furthermore, hypertensive animals are more likely to experience greater C fiber and platelet activation than normal animals. A diagram of this hypothesis is provided at the end of the objective section

Three weeks prior to the experimental procedures, both normal and spontaneously hypertensive rats will receive surgically implanted telemetry units, allowing us to evaluate the animals in an awake and non-constrained environment.

Normal and spontaneously hypertensive rats will be exposed to one of four conditions: 1) filtered air, 2) 0.50 ppm O_3, 3) 25 ug/m^3 flame generated PM or 4) a combination of O_3 and PM. Animals will be exposed for one of the 4 conditions for 6 hours.

1. Determine the effects of PM, O3 and PM+O3 on platelet activation, as well as platelet-monocyte and platelet-leukocyte aggregates, and the release of platelet bioactive lipids.
2. Determine the effects of the above exposures on pulmonary C fiber activation by examining breathing patterns and HRV.
3. Determine the effects of the above exposure conditions on the development of microthrombi within the pulmonary and cardiac circulation.
4. Determine pulmonary vascular vasoconstriction.

F) Technical Plan:

a) Experimental Rationale:

We propose short-term exposures of rats to a variety of conditions which have been associated with increases in cardiac deaths due to decreased HRV. We will evaluate breathing frequency, HRV, platelet activation and changes in the pulmonary and cardiac vasculature in response to these exposures. These studies were chosen to address the data from epidemiological studies indicating that people with pre-existing heart conditions such as angina, hypertension, and myocardial infarction are more likely to experience alterations in vagal tone resulting in decreased HRV putting them at increased risk for cardiac morbidity and mortality.

Two separate populations of Adult male Wistar rats (250-300 grams) will be used in this study: normal Wistar rats and Spontaneously Hypertensive (SH) Rats.

The proposed experiment will be conducted in two phases. Each phase is composed of four exposure regimens (6 hrs.) for each control and SH animal: (1) filtered air; (2) 0.50 ppm Ozone; (3) 25 ug/m^3 fine particulate matter, and (4) 0.50 ppm Ozone and 25 ug/m^3 fine particulate mater. Phase 1 will consist of exposure to these four atmospheres in Wistar rats. Phase 2 will consist of exposure to these four atmospheres in SH rats. This experimental design results in 8 groups of rats (8 groups = 2 phases x 4 exposure regimens). Each group will consist of 10 rats for a total of 80 rats to be studied.

Concentration and choice of synthetic particle. Despite a large body of epidemiologic data correlating adverse health effects from PM exposure mechanisms of cardiovascular morbidity and mortality, especially in susceptible populations, remain relatively unexplored. This is due to the complicated spatiotemporal variation in PM containing atmospheres as well as the dauntingly complex mixture of PM and gases that characterize these atmospheres. Sampling sites, seasonal variations, and time of day all contribute to the marked variability in ambient particle composition complicating the systematic study of PM health effects. Despite this, it is well established that vehicular exhaust from combustion of gasoline, diesel, and other petroleum fuels is the dominant contributor to the fine and ultrafine particulate ranges (Pey et al., 2009). Combustion in vehicle engines may be incomplete and lead to the emission into the atmosphere of carbonaceous particles and a variety of fused and free polycyclic aromatic hydrocarbons (PAHs). Due to the highly variable nature of outdoor ambient PM, researchers on the UC Davis campus have developed and used a premixed flame particulate (PFP) generating system (Lee et al., 2010) to create an exposure atmosphere for in vivo studies. This system allows for the generation of a highly reproducible PM atmosphere without potential confounders like temperature, weather, variations in air quality, allergens, endotoxins, or metals present in field samples, while providing the flexibility to make modifications of PM composition (i.e., PAH content, metals, gases, etc.).

The PFP to be used in this study are generated in a laminar fuel-rich flame resulting in fine and ultrafine particles. Typical morphologies of PFP were soot particles composed of 10–20 nm round primary particles that form larger fractal aggregates. A variety of PAH species are present in both the particulate and vapor phases (see Appendix 1 and 2). The exposure chamber mass concentration from a series of PFP exposures was determined to be 22.40 ± 5.60 μg/m^3 PFP (mean ± SD) based on gravimetric filter measurement. SMPS measurements showed a geometric mean mobility diameter of 70.56 nm with a geometric standard deviation of 1.51 nm. The mean particle number concentration was $9.37 \times 10^4 \pm 4.8 \times 10^3$ particles/cm^3 (mean ± SD) based on CPC measurements over duration of exposure. PFP exposure chamber CO levels were within 0.2 ppm of FA chamber levels, with quantification below 0.2 ppm limited by instrument accuracy. Chamber NO and NO_2 concentrations were within 0.01 ppm of FA levels. Particles were high in organic carbon and had an EC/OC ratio of 0.58. The amount of total PAH measured on PM was 54 ng/m^3 and the amount of gas phase PAH was 405 ng/m^3. In general, biphenyls and naphthalene compounds dominated the vapor phase, and non, mono, and poly substituted naphthalenes constituted the particulate phase. A single 6-h exposure of fuel-rich ultrafine PFPs results in gene expression consistent with oxidant stress and injury (Chan et al., 2011 and 2012) further supporting that PFP is a valid surrogate for ambient PM.

b) Experimental Design, Techniques and Major Tasks:

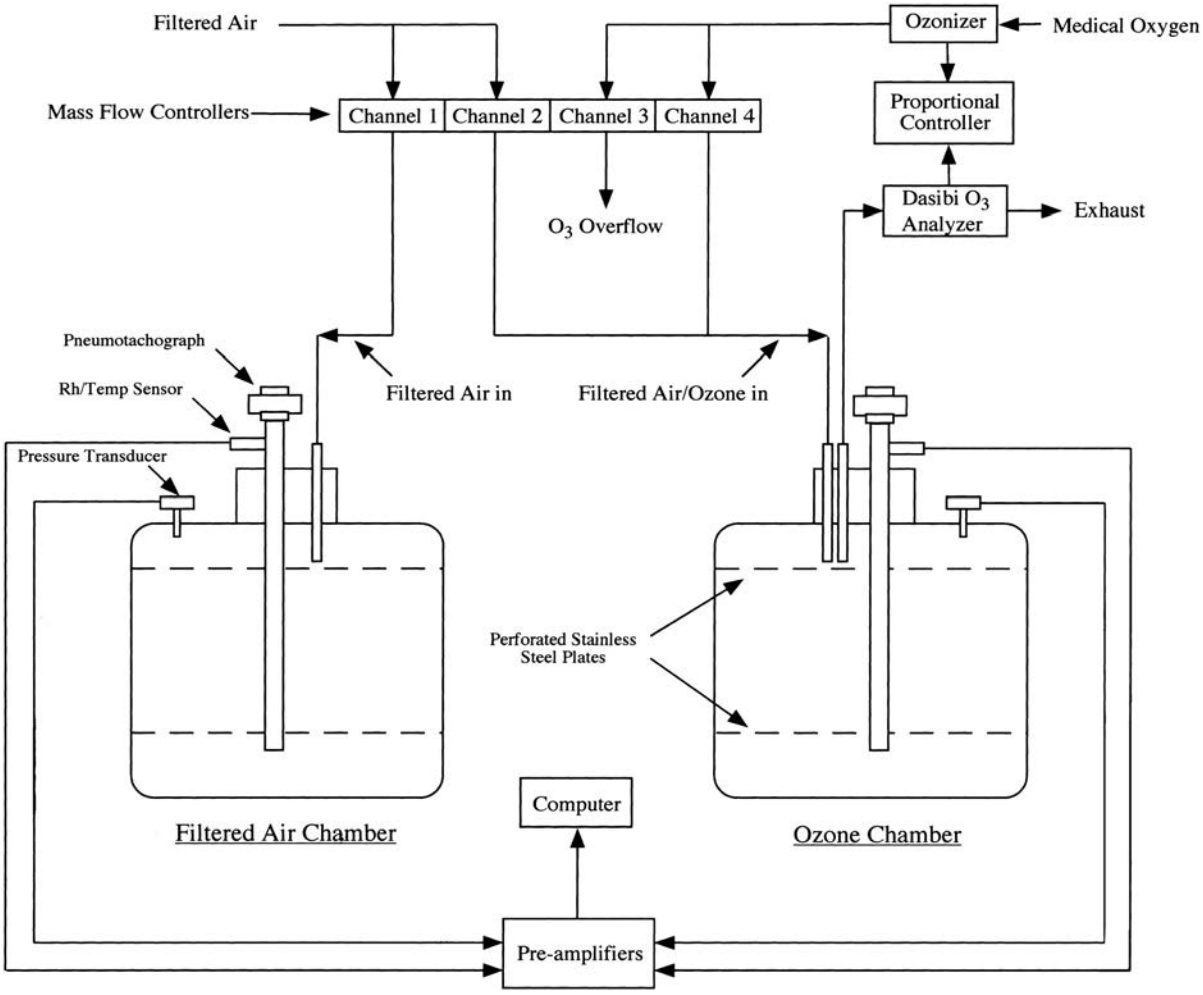
Rats will be delivered to the vivarium in Veterinary Medicine 3B on the University of California Davis campus and allowed to acclimate for 3-5 days. Ten to fouteen days before the beginning of exposure rats will be removed from the vivarium in which they will be housed and anesthetized intraperitoneally with a mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml). Subcutaneous prophylactic antibiotic and analgesic therapy will be administered before surgery penicillin G (50,000 IU) and ketoprofen (2mg/kg). A MLE0050B Telemetry Biopotential Telemeter (TR50B, Small, AD instruments) suitable for measuring ECG will be implanted using the technique described by Tontodonati et al. (2011). In brief, using sterile surgical techniques a small midline incision will be made in the abdominal wall. Once the transmitter is placed in the abdomen, the linea alba will be closed with appropriate, non-absorbable, suture at the same time securing the transmitter in its final position. The ECG leads will be placed in a Lead II configuration by placing the leads between the Pectoralis Superficialis Profundus and Scalenus muscles (Tontodonati et al., 2011). After placing the ECG leads all skin incisions will be sutured. Each surgical procedure lasts approximately 45 minutes.

Once the surgery is completed, the animals will be allowed to wake gently in a heated cage at approximately 37 °C. After their health condition has been assured animals will be individually-housed in standard rat cages. During the recovery period, the animals will be monitored twice daily and standard antibiotic and analgesic t

herapies will be administered. After a recovery period of 7-days (Tontodonati et al., 2011) the animals will be housed in standard rat cages equipped with a MLE0180 Telemetry SmartPad (TR180) below each cage.

On the day of study rats the will be placed one rat per chamber in 8-liter Pyrex glass chambers in which the test atmospheres will be delivered. Rats were given ~3 h in the chambers before data collection was initiated. The experimental time sequence to be followed is, (1) a 30 minute control period followed by (2) a 6 hour exposure period. At the end of the exposure period animals will be euthanized and the following techniques performed as described below.

Exposure Chambers: Exposure chambers are constructed of glass, stainless steel, and Teflon because of their non-reactivity with ozone. The exposure chambers and ozone generation system are illustrated in Fig.2. The two exposure chambers were designed using two 8-liter Pyrex glass desiccators. Two perforated stainless steel plates were incorporated into the chamber design: one inside the chamber itself on which the rat was placed and the other between the lid and the chamber compartment. A hole in the center of the chamber lid allowed air to flow into the top compartment, through the middle and bottom compartments, and to be exhausted from a low-resistance glass tube passing through the center of the chamber. All flows into the chamber were carried through Teflon tubing, and outflow traveled through the glass tube connected to a pneumotachograph (model 4700, Hans Rudolph, Kansas City, MO).

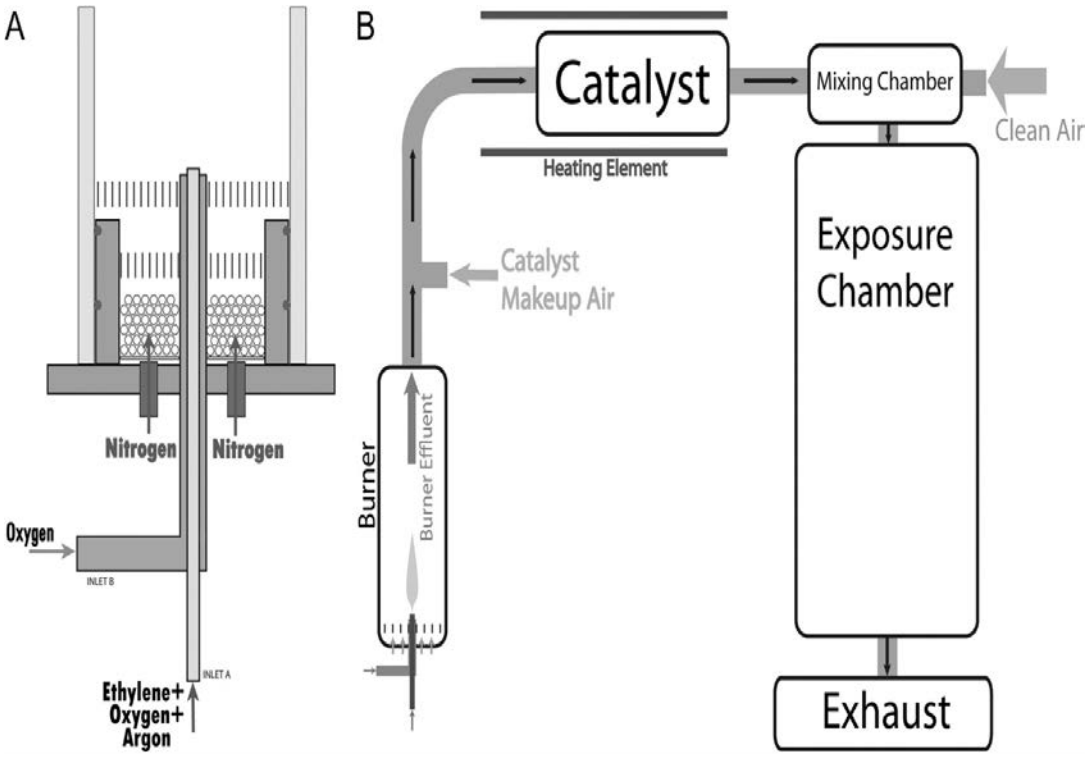


Schelegle E S et al. J Appl Physiol 2001;91:1611-1618

Chamber temperature (°C) and relative humidity (RH; %) will be measured between the chamber lid and the pneumotachograph using a temperature-RH probe (model 50Y, Vaisala, Irvine, CA). Chamber pressure will be measured using a solid-state differential pressure transducer (SenSym, Milpitas, CA) via a second port in the lid.

Ozone Generation: Ozone will be generated as described previously (Schelegle et al., 2001) by passing oxygen through an ozonizer (model 100, Sanders, Uetze-Eltze, Germany). After being mixed with filtered air, the gas will be delivered (10 l/min) to the top compartment of the exposure chamber. All flows will be controlled using mass flow controllers (Tylan General). The concentration of ozone was kept constant using a proportional controller (Inhalation Facility, University of California, Davis, California Regional Primate Research Center, Davis, CA) interfaced with an ultraviolet ozone analyzer (model 1003-AH, Dasibi Environmental, Glendale, CA). The ozone analyzer is routinely calibrated using the ultraviolet-absorption photometric method at the University of California, Davis, California Regional Primate Research Center.

Particulate Matter generation and characterization: PFPs will be generated using a coannular premixed flame burner (Fig. 3A). The burner consists of a 7.1-mm tube (inner diameter) surrounded by an 88.9-mm concentric outer tube (inner diameter). The burner is enclosed in a Pyrex tube to isolate the burner from ambient air. A mixture of ethylene, oxygen, and argon is metered through the inner tube at 212.4 cc/min, 289.2 cc/min, and 1499 cc/min, respectively, using mass flow controllers (model 647C flow control unit and model 1179A and M100B flow control valves, MKS Instruments, Andover, MA). A small flow rate of oxygen (52 cc/min) flows through the outer annulus to stabilize the flame. The flame is shielded from room air by a curtain flow of nitrogen metered at 10 l/min using a Fisher and Porter variable area flow meter (Andrews Glass, Vineland, NJ) and delivered around the circumference of the burner chamber. Filtered dried air is added to the flow downstream of the flame, and all burner effluent passes through a heated 3-way catalyst to remove NO_x and CO. PFP are diluted with clean HEPA and CBR (chemical/biological/radiological) FA and mixed into a mixing chamber before entering the inhalation exposure chamber (Fig. 3B).

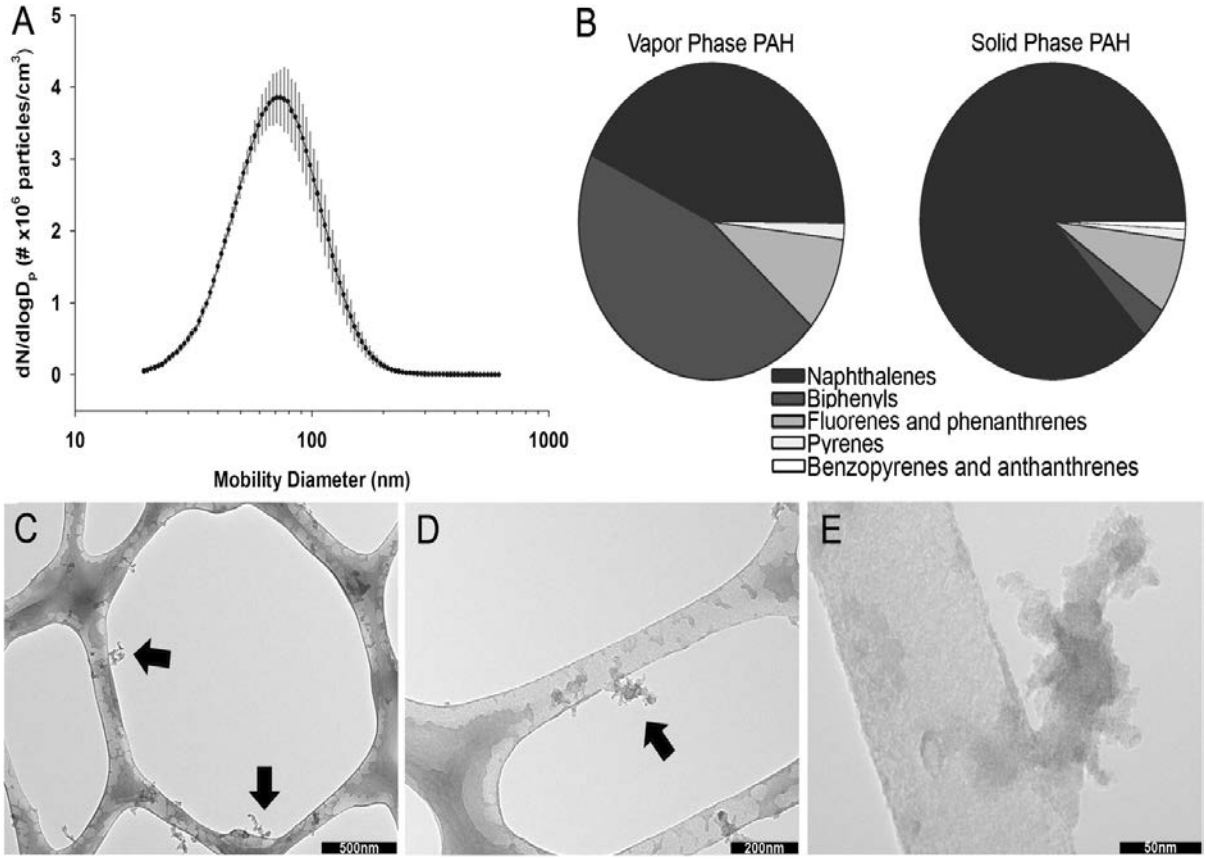


Chan J K W et al. Toxicol. Sci. 2011;124:472-486

Chamber CO levels will be monitored using a Teledyne-API Model 300E CO analyzer (San Diego, CA) calibrated with an NIST traceable span gas of 202.4 ppm CO diluted in ultrapure air to 10 ppm CO (Scott-

Marrin Inc., Riverside, CA). PFP will be collected directly from the exposure chamber for analysis though ports in the chamber wall. Particle number concentration will be determined using a condensation particle counter (CPC, TSI model 3775, Shoreview, MN). Particle size distribution will be determined using a scanning mobility particle sizer (SMPS) (model 3080 electrostatic classifier with model 3081 differential mobility analyzer) and a model 3020 CPC (TSI).

PFP mass concentration will be determined by collecting particles from the chamber on glass fiber filters (Pallflex Emfab 47-mm filters, Ann Arbor, MI) placed in a filter housing (BGI, Waltham, MA). The sampling flow rate will be set at 20 l/min air flow rate driven by a vacuum source downstream of the flow. Collection will be performed for the duration of the exposure. Total particulate mass will be determined



Chan J K W et al. Toxicol. Sci. 2011;124:472-486

gravimetrically (Sartorius AG MC5 microbalance, Goettingen, Germany). Particle samples will be collected on 47-mm glass fiber filters (Pallflex Tissuequartz, Ann Arbor, MI) for elemental carbon to organic carbon ratio (EC/OC) analysis.

Measurement of breathing pattern: Respiratory frequency (f) and an estimate of VT in the unrestrained, conscious rats during the experimental protocols will be obtained and averaged over each minute by monitoring the small variation in chamber pressure associated with heating and humidification of air during inspiration. An estimate of VT will be obtained using the equations of Schelegle et al. (2002). These equations used the integral of the variation in chamber pressure, chamber temperature, and RH while assuming a constant body temperature for the rats of 36.5°C. An estimate of minute ventilation ($\dot{V}E$) will be calculated post hoc by multiplying f and the estimate of VT. All data will be collected and analyzed using a digital data acquisition system (BioSystem XA, Buxco Electronics, Sharon, CT).

Measurement of HRV: Beat-to-beat variation in RR interval of the ECG recordings will be analyzed using

the MLS310/7 Heart Rate Variability Module for Macintosh (AD instruments). Beats will be automatically distinguished by the software and classified into three groups; normal, ectopic or artifact. Beats that are determined to be ectopic and artifact will be eliminated from the HRV analysis. The R component from each raw ECG waveform will be detected using a threshold detector to generate RR interval data. The RR interval data will be analyzed in the frequency domain using spectral analysis to determine the low-frequency (LF) band (0.3-0.6 Hz) and high-frequency (HF) band (0.6-2.5 Hz) power and calculate the LF-to-HF ratio (Scridon et al., 2012).

Hematologic Assessment: A complete blood count, including platelet count will be done for all animals at the end of the study. Those samples will be collected in EDTA and analyzed on an Advia Veterinary Hematology Analyzer. A white cell differential count also will be performed.

Platelet Activation Analysis: Additional blood will be collected in acid citrate and dextrose and platelet activation analysis will be completed by flow cytometry. We will analyze platelet shape by forward and side scatter, as activated platelets show a discrete and recognizable change in shape. We will also determine whether platelet alpha granules and lysosomal granules have been secreted by the presence of the platelet alpha granule membrane protein P-selectin, and the lysosomal granule protein LAMP-1 on the platelet surface. We will also label platelets with an antibody to the major platelet integrin, and evaluate integrin associated microparticles (an indicator of increased coaguability) by flow. All samples will be analyzed on a Beckman-Coulter FC500 Cytometer. All of these techniques are currently used in our laboratory, and we have demonstrated, in prior CARB funded studies, that when mice are exposed either to CAPs or instilled particles, there is clear and demonstrable platelet activation (Wilson et al., 2010; Tablin et al., 2012). Platelet cytometry data will be analyzed in FloJo (Treestar, Ashland, OR). If the data are normally distributed we will conduct ANOVA analysis of the data. If the data are not normally distributed we will use a Kruskal-Wallace analysis. For comparisons between normal and spontaneously hypertensive rats we will use a repeated measure analysis.

To evaluate the release of serotonin from platelet dense granules, we will conduct ELISAs on EDTA plasma for serotonin as well as for thromboxane B2 (a stable downstream product of the labile thromboxane A2 released from activated platelets). Data will be analyzed as described above.

Pulmonary and Cardiovascular Pathology: Pulmonary Methodology: The lung will be fixed by (intratracheal or intrabronchial) instillation of neutral buffered formalin with a controlled pressure of 20 cm H2O. Three standardized transverse sections of lung from the apex, middle and caudal portions of the left lobe will be selected using airway branch point landmarks. Standard H+E sections will be prepared and coded with a random sequencing for histologic scoring. Scoring will be performed b a treatment-blinded pathologist with every terminal bronchiolar region in each section individually scored. Separate severity scores will be recorded for 1) airway epithelial changes 2) extent of inflammation 3) relative component of PMNs in the inflammatory exudate 4) density of visible particulate mater 5) vascular wall changes in pulmonary arterioles and 6) density of platelets evident in arteriolar lumens.

The extent of pulmonary arteriolar contracture will be confirmed in selected experiments where its presence is evident by subjective scoring. In this approach, digitized images of cross sections of all arterioles in the three lung sections are collected. An average vascular wall thickness is calculated by measuring mean Ferret diameters of the internal and external wall using the Image J image analysis software platform. Statistical evaluation of subjective scores (ranked form 0-5) is done by nonparametric rank statistic using the Kruskal – Wallace test. Comparison of mean vascular wall thickness is a parametric endpoint and will be analyzed by a 2-way ANOVA with Bonferroni post-hoc tests between treatment groups.

The heart will be bisected from the base to apex in a plane including both right and left ventricles and atria. Standard H+E sections will be subjectively evaluated to identify any treatment associated myocyte or vascular lesions.

c) Major Tasks:

Task 1: Purchase of equipment and surgical Implantation of Telemetry Units in normal animals (1 month for surgery and recovery)

Tasks 2: Exposure of normal animals to one of 4 conditions: filtered air (FA), O3, PM, or PM+O3.

a. Data collection and initial analysis of breathing frequency and HRV
b. hematologic and flow cyomtetry assays of whole blood and platelet activation
c. histopathology analysis of lung and cardiac tissue
d. Data analysis of platelet activation and HRV
e. Data analysis of histopathology

Task 3: Surgical implantation of telemetry units in spontaneously hypertensive rats

Task 4: Exposure of spontaneously hypertensive rates to one of the 4 conditions listed in Task 2.

a. data collection of breathing frequency and HRV
b. hematologic and flow cytometry assays as for Task 2
c. histpathology studies as for Task 2
d. as for Task 2
e. as for Task 2

Task 5: Data analysis with statistician for all of the above parameters

Task 6: Draft Preparation of the final report

d) **Data Management** :

Each animal in the study will be given a number that will follow the tissues and fluids to be collected throughout the study. These animal numbers, and their associated treatment group will be maintained in laboratory notebooks but treatment group assignments will not be available to researchers until analysis of data set is complete to assure unbiased evaluation of data.

Sample size required to assure statistically valid data: a minimum of 10 animals will be used in all experimental and control groups to assure an adequate sample size for the generation of statistically valid data, given the biological variability present in any in vivo experimental system.

Methods of data handling, reduction and analyses: Data format and approaches to addressing quality assurance of data.

Quality Assurance: The University of California, Davis is committed to producing quality environmental exposure data and will implement a quality assurance program for all PM sampling, laboratory analysis and data analysis efforts. A key function of this program will be to document the methods used to collect and analyze the data so that the data produced will be scientifically valid and defensive.

Data collection activities: Data quality objectives and acceptance criteria, characterized in terms of accuracy, precision, detection limits (LODs), completeness and representivity will be set based on these intended uses (described below). We will have in place aquality system that complies with ANSI/ASQC: Specifications and Gidlines for Quality Systems for Environmental Data Collection and Environmental Data Collection and TechnologyPrograms.

Study Design: The proposed study will take place at the University of California Davis, Davis CA and will make use of inhalation facilities in the newly constructed Veterinary IIIB laboratory of Dr. Edward Schelegle. Replicates of each experiment will be performed and relative variability in outcome measurements will be determined. If, after two replications, background variability is substantially greater than anticipated, additional replications will be done.

Histological Samples. All tissues for morphological assessment are processed under rigid conditions. A record of each embedment procedure is maintained as well as acquisition numbers for each tissue specimen. These numbers are recorded in the acquisition numbering log book as a permanent record.
Biochemical assays: Biochemical assays are validated for each experiment. All biochemical assays will be performed in triplicate to assure accuracy and reproducibility. Laboratory notebooks are well maintained for each experiment and standard quality assurance and quality control procedures will be implemented and are currently practiced in our laboratories.
Instrument calibration: Standard operating procedures for all instruments, data processing, and quality control protocols will be documented in written manuals. All instrumentation will be calibrated according to fixed schedules, manufacturer's instructions and to the requirements of the analysis as dictated by the SOP.

Statistical Analysis: We will use statistical analysis that reflects our multi-factor design. Differences between groups will be assessed by repeated measures ANOVA. All comparisons with $p<0.05$ will be considered significant (Glantz, 1992). Platelet cytometry data will be analyzed in FloJo (Treestar, Ashland, OR). If the data are normally distributed we will conduct ANOVA analysis of the data. If the data are not normally distributed we will use a Kruskal-Wallace analysis. For comparisons between normal and spontaneously hypertensive rats we will use a repeated measure analysis. Statistical consulting is available fro Dr. Neil Willitis in the UCD department of statistics in a collaborative agreement with the Tablin laboratory. At each stage of the analysis, statistical significance will be defined as $p<0.0$.

Facilities:
Facilities: Laboratories for Drs. Tablin and Wilson are located in the recently constructed research facility (VMIIIA). Dr. Schelegle will be moving into the newly constructed research facility (VMIIIB) in March 2013. Dr. Schelegle's laboratory is equipped with exposure facilities for the proposed experiments.
Animal Facilities: Vet Med IIIB has animal surgeries and animal facilities under the AALAC accredited Animal Resources Service. Exposure Facilities: Dr. Schelegle is the leader of the California National Primate Research Center Inhalation Exposure Facility the currently houses the flame particulate generator and particulate characterization equipment.
Major Equipment: Laboratory or shared use instruments are available for this project and include: cell culture hoods and incubators, a Beckman-Coulter FC-500 multicolor flow cytometer, Olympus high resolution microscope with digital capture capabilities The Veterinary Medical Teaching Hospital of the School of Veterinary medicine maintains a full service histo-technology laboratory.

References:
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Brook, R.D, Brook, J.R., Urch,B., Vincent, R., Rajagopalan, and Silverman,R., 2002 Inhalation nof fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.

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Delfino, R.J., Staimer, N., Tjoa,T., Polidori,A., Arhami,M., Gillen,D.L. et al. 2008. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. Environ Health Perspect 116:8998-906.

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Gold, D.R., Litonjua, A., Schwartz, J., Lovett,E., Larson,A., Nearing, B., Allen,G., Verrier, M., Cherry, R. and Verrier, R. 2000. Ambient pollution and heart rate variability. Circulation 101:1267-1273.

Lee D, Wallis C, Wexler AS, Schelegle ES, Van Winkle LS,
Plopper CG, Fanucchi MV, Kumfer B, Kennedy IM, et al. 2010 Small particles disrupt postnatal airway development. J. Appl. Physiol. 109:1115-1124.

Lucking, A.J., Lundback,M., Mills, N.L., Faratian,D., Barath,S.F., Pourazar, J., Cassee, F.R., et Al., 2008 Diesel exhaust inhalation increases thrombus formation in man. European Heart J 29: 3043-3051.

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Oberdorster,G., Sharp,Z., Atudorei,V., Elder, A., Gelein,R., Lunts,A., Kreyling,W. and Cox, C. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J. Toxicol Environ Health A 65:1531-43

Peters,A., Dockery,D.W., Muller, J.E. and Mittleman, M.A. 2001 Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.

Pey J, Querol X, Alastuey A, Rodriguez S, Putaud JP, Van Dingenen R. 2009 Source apportionment of urban fine and ultra-fine particle number concentration in a Western Mediterranean city. Atmos. Environ.43:4407-4415.

Pieters,N., Plusquin,M., Cox, B., Kicinski, M., Vangronsveld, J., and Nawrot, T.S. 2012. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. Heart 98:11237-1135.

Pope,C.A., Buirnett,R.T., Thurston,G.D., Thun, M.J.M, Call,E., Krewski,D., and Godleski,J. 2004. Cardiovascular mortality and long term exposure to particulate air pollution. Circulation 109:71-77.

Samet,J.M., Dominici,F., Curriero,F.C., Coursac, I., Zeger, S.L. 2000. Fine particulate air pollution and mortality in 20 U.S. Cities, 1987-1994. N Engl J Med 343:1752-49..

Schelegle E. S., Alfaro, M. F., Putney,L., Stovall, M., Tyler, N., and Hyde, D.M. 2001 Effect of C-fiber mediated, ozone-induced rapid shallow breathing on airway epithelium in injury in rats. J Appl Physiol 91:1611-1618

Tablin,F., den Hartigh, L.J., Aung, H.H., Lame, M.W., Kleeman, M.J., Ham,W., Norris, N.J., Pombo, M., and Wilson, D.W. 2012 Seasonal influences on CAPs exposures: differential responses in platelet activation, serum cytokines and xenobiotic gene expression. Inhal Toxicol. 24:506-517.

Wilson, D.W., Aung,H.H., Lame, M.W., Plummer,L., Pinkerton,K.E., Ham, W., Kleeman, M., Norris, J.W. and Tablin, F. 2010 Exposure of mice to concentrated ambient particulate matter results in platelet and systemic cytokine activation. Inhal Toxicol 22:267-276.

G. Project Timetable:

Please note that we are proposing two large sets of exposures, each with multiple tasks, within which there are additional objectives listed under each task.

Task 1: Purchase equipment and surgical Implantation of Telemetry Units in normal animals (1 month for surgery and recovery)

Tasks 2: Exposure of normal animals to one of 4 conditions: filtered air (FA), O3, PM, or PM+O3.

a. Data collection and intiial analysis of breathing frequency and HRV
b. hematologic and flow cyomtetry assays of whole blood and platelet activation
c. histopathology analysis of lung and cardiac tissue
d. Data analysis of platelet activation and HRV
e. Data analysis of histopathology

Task 3: Surgical implantation of telemetry units in spontaneously hypertensive rats

Task 4: Exposure of spontaneously hypertensive rates to one of the 4 conditions listed in Task 2.

a. data collection of breathing frequency and HRV
b. hematologic and flow cytometry assays as for Task 2
c. histpathology studies as for Task 2
d. as for Task 2
e. as for Task 2

Task 5: Data analysis with statistician for all of the above parameters, publication preparation and submission

Task 6: Draft Preparation of the final report

	MO	1	2	4	6	8	10	12	14	16	18	20	22	24	28	30	36
TASK																	
1		x	x														
2			x	x	x	x	x										
3								x	x								
4									x	x	x	x	x				
5						x	x	x	x	x		x	x	x	x		
6																x	x
		m		p		m		p		m	p			p		d	F

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

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

NAME	POSITION TITLE
Fern Tablin	Professor

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

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Pennsylvania, Philadelphia,PA	BA	1973	History
University of Pennsylvania, Philadelphia,PA	VMD	1980	Veterinary Medicine
University of Pennsylvania, Philadelphia,PA	PhD	1984	Anatomy

A. **PERSONAL STATEMENT:** I currently direct a translational research program that examines how environmental air pollution affects hemostasis and coagulation in human and animal health. The majority of our platelet studies focus on animal models of disease and their response to PM. We have shown that exposure to PM results in up-regulation of platelets i.e. "platelet priming", such that these cells are more sensitive to agonist and more likely to develop micro-thrombi in the systemic circulation leading to increased incident of cardiac events.

B.**POSITIONS, PROFESSIONAL MEMBERSHIPS AND HONORS.**

Positions

1984-1985 Lecturer, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania
1985–1992 Assistant Professor, School of Veterinary Medicine, University of California, Davis
1992-1999 Associate Professor, School of Veterinary Medicine, University of California, Davis
1999-present Full Professor, School of Veterinary Medicine, University of California, Davis
2012 Department Chair

Honors

1980 Phi Zeta, Veterinary Honors Society
1980-83 NIH, National Research Service Award, AM-07185-06
1987 Frederik B. Bang Fellowship, Marine Biological Laboratory, Woods Hole, MA
2002 Elected Fellow, American Association for the Advancement of Science

Professional Memberships:
American Association for the Advancement of Science
American Society of Hematology
American Society for Cell Biology

C. SELECTED RECENT PEER-REVIEWED PUBLICATIONS 2002-2012 (IN CHRONOLOGICAL ORDER).

Tsvetkova, N.M., Phillips, B.L., Krishnan, V.V., Feeney R.E., Fink, W.H.,Crowe, J.H., Risbud, S.H., **Tablin**, F. and Y.Yeh. Dynamics of antifreeze glycoproteins in the presence of ice. Biophysical Journal 82:464-473, 2002.
Gousset, K., Wolkers, W.F., Tsvetkova, N.M., Oliver, A.E., Field, C.L., Walker, N.J., Crowe, J.H. and F. **Tablin**. Evidence for a physiological role for membrane rafts in human platelets. Journal of Cellular Physiology 190:117-128, 2002

Tomczak, M.M., Hincha, D.K., Estrada, S.D., Wolkers, W.F., Crowe, L.M., Feeney, R.E., **Tablin**, F. and J.H. Crowe. A mechanism for stabilization of membranes at low temperatures by an antifreeze protein. Biophysical Journal 82:874-881, 2002.
Wolkers, W.F., **Tablin**, F. and J.H. Crowe. From anhydrobiosis to freeze-drying of eukaryotic cells. Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology 131A:535-543, 2002.
Wolkers, W.F., Crowe, L.M., Tsvetekova, N.M., **Tablin,** F. and J.H. Crowe. In situ assessment of erythrocyte membrane properties during cold storage. Molecular Membrane Biology 19:59-65, 2002.
Luria, A., Vegelyte-Avery, V., Smith, B., Tsvetkova, N.M., Wolkers, W.F., Crowe, J.H., **Tablin**, F. and R. Nuccitelli. Detergent-free domain isolated from Xenopus egg plasma membrane with properties similar to those of detergent resistant membranes. Biochemistry 41:13189-13197, 2002.
Crowe, J.H., Oliver, A.E. and F. **Tablin**. Is there a single biochemical adaptation to anhydrobiosis? Integrative and Comparative Biology 42:497-503, 2002.
Crowe, J.H., **Tablin**, F., Wolkers, W.F., Gousset, K., Tsvetkova, N.M., and J. Ricker. Stabilization of membranes in human platelets freeze-dried with trehalose. Chemistry and Physics of Lipids 1122:41-52, 2003.
Tsvetkova, N.M., Horvath, I., Torok, Z., Wolkers, W.F., Balogi,Z., Shigapova,N., Crowe, L.M., **Tablin**, F., Vierling, E., Crowe, J.H. and L. Vigh. Small heat shock proteins regulation membrane lipid polymorphism. Proc. Natl. Acad. Sci. USA 99:13504-13509, 2003.
Wolkers, W.F., S.A. Looper, A.E. McKiernan, N.M. Tsvetkova, F. Tablin and J.H. Crowe. Membrane and protein properties of freeze-dried mouse platelets. Molecular Membrane Biology 19:201-210, 2003.
Wolkers, W.F., N.J. Walker, Y. Tamari, F. **Tablin** and J.H. Crowe. Towards a clinical application of freeze-dried platelets. Cell Preservation Technology 1:175-188, 2003.
Gousset, K., Tsvetkova, N.M., Crowe, J.H. and F. **Tablin**. Important role of raft aggregation in the signaling events of cold-induced platelet activation. Biochimica et Biophysica Acta 1660:7-15, 2004.
Wolkers, W.F., Oldenhof, H., Tablin, F. and J.H. Crowe. Preservation of dried liposomes in the presence of sugar and phosphate. Biochim. Biophys. Acta. 1661:125-134, 2004.
Shrimpton, C.N., Gousset, K., **Tablin**, F. and J.A. Lopez. Isolation and analysis of platelet lipid rafts. Methods Mol. Biol. 273:213-238, 2004
Ma, X., Jamil, K., Macrae, T.H., Clegg, J.S., Russell, J.M., Villeneuve, T.S., Euloth, M., Sun, Y., Crowe, J.H., Tablin, F. and A.E. Oliver. A small stress protein acts synergistically with trehalose to confer desiccation tolerance on mammalian cells. Cryobiology 51:15-28, 2005
Tang, M., Wolkers, W.F., Crowe, J.H. and **F. Tablin**. Freeze-dried rehydrated human blood platelets regulate intracellular pH. Transfusion, 46:1029-37, 2006
Norris, J.W., Pratt, S.M., Hunter, J.F., Gardner, I.A. and **F. Tablin**. Prevalence of reduced fibrinogen binding to platelets in a population of Thoroughbred horses. Am. J. Vet. Res. 68:716-721, 2007
Bali,R., Savino, L., Ramirez, D.A., Tsvetkova, N.M., Bagatolli, L., **Tablin, F**., Crowe, J.H. and C. Leidy. Macroscopic domain formation during cooling in the platelet plasma membrane: an issue of low cholesterol content. Biochim. Biophys. Acta. 1788:1229-1237, 2009
Wiilson,D.W., Aung, H.H., Lame, M.W., Plummer, L., Pinkerton, K.E., Ham, W., Kleeman, M, Norris, J.W., and **F. Tablin**. Exposure of mice to concentrated ambient particulate matter results in platelet and systemic cytokine activation. Inhalation Toxicology 22:267-76, 2010
Owens, S.D., Johns, J.L., Walker, N.J., Librach, F.A., Carrade, D.D., **Tablin, F**. and D.L. Borjesson. Use of an in vitro biotinylation technique for determination of posttransfusion survival of fresh and stored autologous red blood cells in Thoroughbreds. Am. J. Vet. Res. 71:960-966. 2010
den Hartigh, L.J., Lame, M.W., Ham, W., Kleeman, M.J. **Tablin, F** and D.W. Wilson Endotoxin and polycyclic aromatic hydrocarbons in ambient fine particulate matter from Fresno, California initiate human monocyte inflammatory responses mediated by reactive oxygen species. Toxicol. In Vitro. 24: 1993-2002, 2010
Guillaumin, J., Jandrey, K.E., Norris, J.W. and **F. Tablin**. Analysis of a commercial dimethyl-sulfoxide-stabilized frozen canine platelet concentrate by turbidimetric aggregometry. J. Vet. Emerg. Crit. Care 20:571-577, 2010.
Textor, J.A., Norris, J.W. and **F. Tablin**. Effects of preparation method, shear force, and exposure to collagen on release of growth factors from equine platelet-rich plasma. Am. J. Vet. Res. 72:271-278, 2011
Tablin, F. Proplatelet formation: flex required. Blood 118:1434-5, 2011
Field, C.L. and **F. Tablin**. Response of Northern Elephant Seal platelets to pressure and temperature changes: a comparison with human platelets. Comp Biochem Physiol A Integr Physiol. 162:289-295, 2012
Textor, J.A. and **F. Tablin**. Activation of equine platelet-rich plasma: comparison of methods and characterization of equine autologous thrombin. Vet. Surg. Jun 28 Epub ahead of print, 2012
Tablin, F., denHartigh, L.J., Aung,H.H., Lame, M.W., Kleeman,M.J., Ham.W., Norris, J.W., Pombo, M. and D.W. Wilson. Seasonal influences on CAPs exposures: differential responses in platelet activation, serum cytokines and xenobiotic gene expression. Inhal. Toxicol. 24:506-515.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Edward S. Schelegle	Associate Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Davis	B.S	1973-1977	Animal Physiology
University of California, Davis	M.A.	1977-1981	Exercise Physiology
University of California, Davis	Ph.D.	1981-1988	Cardiorespiratory, Physiology & Environmental Toxicology

A. Personal Statement

I currently direct a translational program that examines how environmental air pollutants and aeroallergens affect human health. This translational program includes rat, non-human primate and clinical human studies. The majority of studies using rats have focused on the role that lung vagal sensory nerves (including C-fibers) play in acute and chronic lung injury and repair. As director of the Inhalation Exposure Facility and the Pulmonary Function Testing Laboratory at the California National Primate Research Center I have extensive expertise in allergen challenge, pollutant generation and exposure and the evaluation of airway function, including specific and non-specific airway reactivity. I was leader of project 3 on the NIEHS program project entitled "Pulmonary effects of environmental oxidant pollutants" (P01 ES00628; PI - DM Hyde). Investigators in project 3 completed multiple studies to determine how the repeated episodic exposure to ozone and/or house dust mite allergen affects airway sensory nerve function and development and airway parasympathetic nerve innervation and function. I played a key role developing the initial allergen sensitization and challenge protocols used to generate asthmatic infant and adult rhesus monkeys. Human clinical studies have been a part of my research program since 1980 and have focused on the ozone exposure-response relationship and mechanisms of ozone-induced responses using pharmaceutical intervention.

B. Postions and Honors

Positions and Employment

1988 - 1990 NIH Postdoctoral Trainee, Department of Internal Medicine, UC Davis.
1990 - 1992 TRDRP Postdoctoral Fellowship, Department of Human Physiology, UC Davis.
1992 – 1994 Assistant Research Physiologist, Department of Human Physiology, UC Davis
1994 - 2000 Assistant Research Physiologist, Dept of Anatomy, Cell Biology and Physiology, UC Davis
2000 - 2006 Assistant Professor, Depart of Anatomy, Cell Biology and Physiology, UC Davis
2006-present Associate Professor, Depart of Anatomy, Cell Biology and Physiology, UC Davis

Honors and Awards

1979 Graduate Research Award, University of California, Davis.
1988 - 1990 NIH Postdoctoral Traineeship
1990 - 1992 TRDRP Postdoctoral Fellowship
1993 - 1998 National Institute of Health R-29 Award, “Lung afferents in chronic lung disease.”
1999 Invited Speaker: American Association of Anatomist Symposium, Experimental Biology Meetings, “The role of neurokinins in inflammation, immunoregulation and epithelial repair.”

B. Selected Peer-reviewed Publications (Selected from 91 peer-reviewed publications)

Most relevant to current application

1: Schelegle ES, Walby WF. Vagal afferents contribute to exacerbated airway responses following ozone and allergen challenge. Respir Physiol Neurobiol. 2012 Apr 12;181(3):277-285.
2: Moore BD, Hyde D, Miller L, Wong E, Frelinger J, Schelegle ES. Allergen and Ozone Exacerbate Serotonin-Induced Increases in Airway Smooth Muscle Contraction in a Model of Childhood Asthma. Respiration. 2012 Apr 13. [Epub ahead of print] PubMed PMID: 22507883.
3: Oslund KL, Hyde DM, Putney LF, Alfaro MF, Walby WF, Tyler NK, Schelegle ES. Activation of calcitonin gene-related peptide receptor during ozone inhalation contributes to airway epithelial injury and repair. Toxicol Pathol. 2009 Oct;37(6):805-13.
4: Lee D, Wallis C, Wexler AS, Schelegle ES, Van Winkle LS, Plopper CG, Fanucchi MV, Kumfer B, Kennedy IM, Chan JK. Small particles disrupt postnatal airway development. J Appl Physiol. 2010 Oct;109(4):1115-24.
5: Schelegle ES, Morales CA, Walby WF, Marion S, Allen RP. 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am J Respir Crit Care Med. 2009 Aug 1;180(3):265-72.
6: Oslund KL, Hyde DM, Putney LF, Alfaro MF, Walby WF, Tyler NK, Schelegle ES. Activation of neurokinin-1 receptors during ozone inhalation contributes to epithelial injury and repair. Am J Respir Cell Mol Biol. 2008 Sep;39(3):279-88.
7: Alfaro MF, Walby WF, Adams WC, Schelegle ES. Breath condensate levels of 8-isoprostane and leukotriene B4 after ozone inhalation are greater in sensitive versus nonsensitive subjects. Exp Lung Res. 2007 Apr-May;33(3-4):115-33.
8: Schelegle ES, Walby WF, Adams WC. Time course of ozone-induced changes in breathing pattern in healthy exercising humans. J Appl Physiol. 2007 Feb;102(2):688-97.
9: Fanucchi MV, Plopper CG, Evans MJ, Hyde DM, Van Winkle LS, Gershwin LJ, Schelegle ES. Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. Am J Physiol Lung Cell Mol Physiol. 2006 Oct;291(4):L644-50.
10: Kajekar R, Pieczarka EM, Smiley-Jewell SM, Schelegle ES, Fanucchi MV, Plopper CG. Early postnatal exposure to allergen and ozone leads to hyperinnervation of the pulmonary epithelium. Respir Physiol Neurobiol. 2007 Jan 15;155(1):55-63.
11: Van Winkle LS, Fanucchi MV, Miller LA, Baker GL, Gershwin LJ, Schelegle ES, Hyde DM, Evans MJ, Plopper CG. Epithelial cell distribution and abundance in rhesus monkey airways during postnatal lung growth and development. J Appl Physiol. 2004 Dec;97(6):2355-63.
12: Alfaro MF, Putney L, Tarkington BK, Hatch GE, Hyde DM, Schelegle ES. Effect of rapid shallow breathing on the distribution of 18O-labeled ozone reaction product in the respiratory tract of the rat. Inhal Toxicol. 2004 Feb;16(2):77-85.
13: Larson SD, Plopper CG, Baker G, Tarkington BK, Decile KC, Pinkerton K, Mansoor JK, Hyde DM, Schelegle ES. Proximal airway mucous cells of ovalbumin-sensitized and -challenged Brown Norway rats accumulate the neuropeptide calcitonin gene-related peptide. Am J Physiol Lung Cell Mol Physiol. 2004 Aug;287(2):L286-95.
14: Larson SD, Schelegle ES, Walby WF, Gershwin LJ, Fanuccihi MV, Evans MJ, Joad JP, Tarkington BK, Hyde DM, Plopper CG. Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. Toxicol Appl Pharmacol. 2004 Feb 1;194(3):211-20.
15: Schelegle ES, Miller LA, Gershwin LJ, Fanucchi MV, Van Winkle LS, GerrietsJE, Walby WF, Mitchell V, Tarkington BK, Wong VJ, Baker GL, Pantle LM, Joad JP, Pinkerton KE, Wu R, Evans MJ, Hyde DM, Plopper CG. Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. Toxicol Appl Pharmacol. 2003 Aug 15;191(1):74-85.
16: Larson SD, Schelegle ES, Hyde DM, Plopper CG. The three-dimensional distribution of nerves along the entire intrapulmonary airway tree of the adult rat and the anatomical relationship between nerves and neuroepithelial bodies. Am J Respir Cell Mol Biol. 2003 May;28(5):592-9.
17: Evans MJ, Fanucchi MV, Baker GL, Van Winkle LS, Pantle LM, Nishio SJ, Schelegle ES, Gershwin LJ, Miller LA, Hyde DM, Sannes PL, Plopper CG. Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. Am J Physiol Lung Cell Mol Physiol. 2003 Oct;285(4):L931-9.
18: Schelegle ES, Walby WF, Alfaro MF, Wong VJ, Putney L, Stovall MY, Sterner-Kock A, Hyde DM, Plopper CG. Repeated episodes of ozone inhalation attenuates airway injury/repair and release of substance P, but not adaptation. Toxicol Appl Pharmacol. 2003 Feb 1;186(3):127-42.
19: Schelegle ES, Alfaro MF, Putney L, Stovall M, Tyler N, Hyde DM. Effect of C-fiber-mediated, ozone-induced rapid shallow breathing on airway epithelial injury in rats. J Appl Physiol. 2001 Oct;91(4):1611-8.

20: Schelegle ES, Eldridge MW, Cross CE, Walby WF, Adams WC. Differential effects of airway anesthesia on ozone-induced pulmonary responses in human subjects. Am J Respir Crit Care Med. 2001 Apr;163(5):1121-7.
21: Vesely KR, Schelegle ES, Stovall MY, Harkema JR, Green JF, Hyde DM. Breathing pattern response and epithelial labeling in ozone-induced airway injury in neutrophil-depleted rats. Am J Respir Cell Mol Biol. 1999 Apr;20(4):699-709.
22: Vesely KR, Hyde DM, Stovall MY, Harkema JR, Green JF, Schelegle ES. Capsaicin-sensitive C-fiber-mediated protective responses in ozone inhalation in rats. J Appl Physiol. 1999 Mar;86(3):951-62.
23: Sterner-Kock A, Vesely KR, Stovall MY, Schelegle ES, Green JF, Hyde DM. Neonatal capsaicin treatment increases the severity of ozone-induced lung injury. Am J Respir Crit Care Med. 1996 Jan;153(1):436-43.
24: Coleridge JC, Coleridge HM, Schelegle ES, Green JF. Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs. J Appl Physiol. 1993 May;74(5):2345-52.

BIOGRAPHICAL SKETCH
Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Wilson, Dennis W.	POSITION TITLE Professor
eRA COMMONS USER NAME dwwilson	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing,*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Illinois	BS	1970	Biology
University of Illinois	DVM	1975	Veterinary Medicine
University of Illinois	MS	1979	Environmental Toxicology
University of California, Davis	PhD	1983	Comparative Pathology

A. Personal Statement

My long-term interest in cardiopulmonary pathology has developed expertise in pulmonary endothelial cell biology relative to endothelial signaling and stress responses. The first research focus in my laboratory is understanding the relationship between pulmonary vascular inflammation induced by inhaled environmental particulates and resultant cardiovascular disease. We demonstrate endothelial activation in response to ROS, PAH and endotoxin mediated pathways that result in systemic platelet activation. Secondly, In collaboration with Dr. J. Rutledge, we have investigated signal transduction and cellular stress responses to postprandial lipids and their breakdown products. Our findings implicate TGFb and oxidant stress responses in initiating pro-inflammatory activation of vascular endothelium. This work also identifies ATF3 as a key regulator of endothelial cell responses to lipolysis products in vitro and in vivo. Common mechanisms of endothelial activation related to both pulmonary and systemic inflammation are emerging from these research projects and will provide mechanistic research projects for trainees with significant translational applications.

B. Positions and Honors

Positions and Employment

1972-1975 Research Assistant in Veterinary Toxicology, University of Illinois, Urbana, IL
1975-1976 Research Associate in Veterinary Toxicology, University of Illinois, Urbana, IL
1976-1979 Private Veterinary Practice, Danville, IL
1979-1982 NIEHS Environmental Pathology Trainee, University of California, Davis, CA
1982-1985 Supervisor, Electron Microscopy Facility and Respiratory Diseases Morphology Unit, California Primate Research Center, Davis, CA
1983-1990 Assistant Professor, Department of Veterinary Pathology, University of California, Davis, CA
1990-1995 Associate Professor, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA
1995-present Professor, Department Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA
2003-2012 Chairman, Department Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA

Professional Memberships

1983- Diplomate and member, American College of Veterinary Pathologists

C. Selected Peer-Reviewed Publications (Selected from 111)

Most relevant to the current application

1. Martin-McNulty B, Tham DM, da Cunha V, Ho JJ, Wilson DW, Rutledge JC, Deng GG, Vergona R, Sullivan ME, Wang YX. 17β-Estradiol Attenuates Development of II-Induced Aortic Abdominal Aneurysm in Apolipoprotein E- Deficient Mice. Arterioscler Thromb Vasc Biol(23): 1627-1632, 2003.
2. da Cunha V, Tham DM, Martin-McNulty B, Deng G, Ho JJ, Wilson DW, Rutledge JC, Vergona R, Sullivan ME, Wang YX. Enalapril attenuates angiotensin II-induced atherosclerosis and vascular inflammation. Atherosclerosis, 178(1): 9-17, 2005.
3. Zhang LN, Wilson DW, da Cunha V, Sullivan ME, Vergona R, Rutledge JC, Wang YX. Endothelial NO synthase deficiency promotes smooth muscle progenitor cells in association with upregulation of stromal cell-derived factor-1alpha in a mouse model of carotid artery ligation. Arterioscler Thromb Vasc Biol, 26(4): 765-72, 2006.
4. Benton,J Powers, A. Eiselein, L. Fitch, R. Wilson, D. W. Villablanca, A.C. Rutledge, J.C.. Hyperglycemia and loss of ovarian hormones mediate atheroma formation through endothelial layer disruption and increased permeability.. Am J Physiol Regul Interg Comp Physiol, 292: R723-730, 2007.
5. Eiselein L, Wilson DW, Lamé MW, Rutledge JC. Lipolysis products from triglyceride-rich lipoproteins increase endothelial permeability, perturb zonula occludens-1 and F-actin, and induce apoptosis. Am J Physiol Heart Circ Physiol, 292(6): H2745-53, 2007.
6. Wilson, D.W., Aung, H.H. Ng, K. Rutledge, J.C.. TGRL: role in the microvascular and macrovascular complications of diabetic nephropathy. Nature Reviews Nephrology (6):361-70, 2010.
7. Den Hartigh, L.J. Lamé, M.W., Ham, W. Kleeman, M.J.F, Tablin, F., Wilson, D.W. Endotoxin and polycyclic aromatic hydrocarbons in ambient particulate matter from Fresno, California initiate human monocyte inflammatory responses mediated by reactive oxygen species. Toxicology in vitro, (7):1993-2002, 2010.
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Additional recent publications of importance to the field (in chronological order)

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2. da Cunha V, Martin-McNulty B, Vincelette J, Zhang L, Rutledge JC, Wilson DW, Vergona R, Sullivan ME, Wang YX. Interaction between mild hypercholesterolemia, HDL-cholesterol levels, and angiotensin II in intimal hyperplasia in mice. J Lipid Res, 47(3): 476-83, 2005.
3. Ramos M, Lamé MW, Segall HJ, Wilson DW. Monocrotaline pyrrole induces Smad nuclear accumulation and altered signaling expression in human pulmonary arterial endothelial cells. Vascul Pharmacol, 46(6): 439-48, 2007.
4. Ramos MF, Lamé MW, Segall HJ, Wilson DW. Smad signaling in the rat model of monocrotaline pulmonary hypertension. Toxicol Pathol, 36(2): 311-20, 2008.
5. Wilson, D.W., Aung, H.H., Lame, M.W., Plummer, L., Pinkerton K.E., Ham, W., Kleeman, M., Norris, J.W. and F. Tablin. Exposure of Mice to concentrated ambient particulate matter results in platelet and systemic cytokine activation. Inhalation Toxicology, 22: 267-276, 2010.
6. Wong, L.N., Lamé, M.W.; Jones, A.D.; Wilson, D.W. Differential cellular responses to protein adducts of Naphthoquinone and Monocrotaline Pyrrole. Chemical Research in Toxicology, 2010.
7. Tablin, F., Den Hartigh, L.J. Aung, H. H., Lame, M. W., Kleeman, M.J.F, Ham, W. Norris, J., Pombo, M, and Wilson, D. W. Seasonal influences on CAPs exposures: Differential responses in platelet activation, serum cytokines and xenobiotic gene expression. Inhalation Toxicology
8. 24(8):506-17
   Dennis W. Wilson DW, Karen L. Oslund, K.L. Bonnie Lyons,B. Oded Foreman, O. Lisa Burzenski, L. Karen L. Svenson, K Thomas H. Chase, T.H. and Leonard D. Shultz, L.D. Inflammatory Dilated Cardiomyopathy in *Abcg5* Deficient Mice,Toxicologic Pathology in press

Preliminary Cost Proposal

Task	Labor	Benefits	Equipment	Travel	Animal/care	Materials and supplies	Analyses	Overhead	Total
1			34,951		4,443				
2						14,000			
3				3,000	4,443				
3									
4						14,000			
6				3,000			6,000		
	308,730	128,518	34,951	6,000	8,886	28,000	6,000	52,108	573,193

Description of Personnel

Tablin, Fern	Professor	PI	10%
Schelegle, Ed	Assoc. Professor	Co-I	25%
Wilson, Dennis	Professor	Co-I	5%
Walker, Naomi	Staff Res. Assoc. III	Research Support	33%
Walby, William	Staff Res. Assoc. III	Research Support	40%