Research on the Mechanisms of PM Effects: Supplement to *Accomplishments of the Particulate Matter (PM) Centers (1999-2005)*

A. Nel[a], L. Chen[b], A. Elder[c], J. Godleski[d], T. Gordon[b], T. Larson[e], M. Frampton[c]
[a]*University of California, Los Angeles, California*
[b]*New York School of Medicine, Tuxedo, New York*
[c]*University of Rochester, Rochester, New York*
[d]*Harvard School of Public Health, Boston, Massachusetts*
[e]*University of Washington, Seattle, Washington*

This document summarizes additional mechanistic information obtained in the first 6 years of the PM Centers' research since their establishment in 1999. Research performed as part of the EPA-funded Particle Centers has increased the understanding of the mechanisms of PM health effects. Progress has been made in understanding the dosimetry and distribution of particles throughout the body, pulmonary inflammation and vascular effects, asthma and allergen responses, cardiac effects, vascular and systemic inflammatory effects, and possible impact on the central nervous system. These findings have increased our understanding of the mechanisms of injury at the organ, tissue, cellular, and subcellular levels, and have begun to shed light on systemic interactions in the whole organism that contribute to adverse health outcomes.

1. Introduction

A number of plausible mechanisms have been proposed to explain the adverse health impact of ambient air particulate matter (PM) (Table 1). Various aspects of the inflammatory response cascade are postulated to play key roles in PM effects. Data from a number of EPA Centers studies have shown that inhalation of PM can elicit pro-inflammatory effects in tissue culture cells, the lung, and the cardiovascular system (1-13). However, it remains unclear whether the systemic and cardiovascular responses are primarily driven by local (pulmonary) inflammation with production of pro-inflammatory cytokines and chemokines in the affected airways, or by translocation of particles into the pulmonary and systemic circulation, with more direct effects on the endothelium and other organs. In either case, evidence is building that reactive oxygen species (ROS) may play an important role as a mechanism of injury.

The major emphasis on the mechanistic report will be on the role of oxidative stress and inflammation in mediating the adverse effects of PM. This does not exclude the contribution of other mechanisms of injury (Table 1).

2. The Role of ROS and Inflammation in PM-induced Adverse Health Effects

A potential mechanistic link between PM exposures and inflammation involves the generation of ROS and oxidative stress (1, 6, 10, 12, 14-17). Several centers have demonstrated that ambient PM and DEP induce ROS production in target cells such as macrophages, bronchial epithelial and endothelial cells (2, 11, 12, 16-18). More direct evidence that ROS is involved in PM effects in vivo has been provided by chemiluminescence studies in rats, in parallel with a demonstration that antioxidants suppress these oxidative stress effects (17). In vivo chemiluminescence originating from H_2O_2 production in the lungs or heart, is a sensitive measure of inhaled CAPs effects,

including their impact on the lung (17). N-acetylcysteine (NAC) suppressed the chemiluminescence response (10) and was effective in suppressing ovalbumin (OVA) sensitization in mice co-challenged with aerosolized DEP, daily for 10 days (6, 10). NAC also interfered in the generation of carbonyl proteins in the lung of these animals; carbonylation is an oxidative modification of protein structure (6).

In order to explain the link between ROS production and tissue injury, a series of in vitro and in vivo experiments explored the link between oxidative stress and inflammation (6, 10-12, 16-18). This led to the characterization of a hierarchical oxidative stress model (Fig. 1), which posits that at the lower levels of oxidative stress (Tier 1), there is an induction of phase II enzymes by a genetic response pathway that involves the transcription factor, Nrf2 (2, 15, 19, 20). Diesel exhaust particles (DEP) and ambient ultrafine particles (UFP) increase the accumulation of Nrf2 in the nucleus, including their ability to activate the antioxidant response element (ARE) (21). Interestingly, the buildup of Nrf2 in the nucleus is dependent on a prolongation of protein half-life by interference in proteosomal degradation (21). Nrf2 drives the ARE in the promoter of phase II genes, leading to the expression of antioxidant and detoxification enzymes (21). Treatment with DEP, organic DEP extracts and ambient ultrafine particles induced the expression of heme oxygenase 1 (HO-1), glutathione-S- transferase (GST), NADPH quinone oxidoreductase, catalase, superoxide dismutase (SOD), glutathione peroxidase and glucoronosyltransferase (20, 21). It has also been demonstrated that source constituents of ambient particles and NIST ambient particulate can inactivate some of these protective enzymes. (22). The induction of phase II enzymes protect against oxidative stress injury (Tiers 2 and 3), and a reduced Tier 1 defense may promote PM susceptibility. In humans, this can result from phase II enzyme polymorphisms, e.g., the GST M1 null genotype which predisposes to the development of asthma and enhanced sensitization to common environmental allergens during nasal DEP challenge (23). Conversely, induction of a phase II response may be a key factor in adaptation to a polluted environment, and may explain why persistent inflammatory changes in the lung were not observed after repeated exposure to low CAPs levels in one study (24). Some phase II enzymes, such as HO-1, exert anti-inflammatory effects based on their antioxidant abilities.

If Tier 1 protection fails, a further increase in oxidative stress could lead to the generation of pro-inflammatory responses (Tier 2) or cytotoxic effects (Tier 3) (Fig. 1). Tier 2 responses are linked to the activation of intracellular signaling pathways which impact cytokine and chemokine production (1, 2, 25). An example is activation of the MAP kinase and NF-κB cascades (2, 25). This cascade is responsible for the expression and activation of AP-1 transcription factors (e.g., c-Jun and c-Fos), which in turn is responsible for the expression of pro-inflammatory genes, including those encoding for cytokines, chemokines and adhesion molecules. European investigators have recently confirmed that the Jun kinase and NF-κB cascades are activated in lung epithelial cells obtained from human subjects exposed to DEP inhalation (26). Tier 3 responses involve mitochondrial perturbation by pro-oxidative chemicals (15, 16, 27, 28). Although the *in vivo* significance of the mitochondrial pathway is uncertain, PM-induced interference in one electron transfers in the mitochondrial inner membrane and perturbation of the mitochondrial permeability transition (PT) pore can contribute to superoxide generation and the induction of cellular apoptosis (16, 28). These effects can be mimicked by organic extracts made from DEP as well as redox cycling quinones and functionalized aromatic hydrocarbons present in these particles (16, 27, 28). Each tier of oxidative stress is sensitive to the effects of NAC (16, 28).

The link between oxidative stress and inflammation has been confirmed by microarray and real-time PCR analyses (3, 21). Total lung RNA was collected to study the pulmonary response to CAPs in rats (3). This showed increases in pro-inflammatory mediators such as C-C chemokines,

IL-1, IL-6 and TNFα, in parallel with an overall decrease in immune enhancers such as IL-2 and interferon. (3). There is also evidence in microarray studies for increases in ROS activity, as well as evidence for activation of organic chemical metabolism and detoxification mechanisms (3). In another study in which exhaust aerosol and LPS were used, the expression of TNFα and the TNFα receptor were found to be consistently increased in the lung, heart, and olfactory bulb, although to different degrees in each tissue (29). Combining these stimuli, led to an additive response (29). A comparison of microarray results from the lung and heart showed that more genetic changes occur in the lung compared to the heart (30). There was a significant response at all dose levels in mice exposed to PM versus saline. The responding genes could be grouped into distinct functional entities such as inflammation, immune responsivity, oxidant responses, apoptosis, growth factors, transcription factors, kinase activity, protease activity, transport and cellular adhesion (30).

While there is still considerable debate about which particle components are responsible for the pro-oxidative and pro-inflammatory effects, there is accumulating evidence that transition metals, such as copper, vanadium, chromium, nickel, cobalt and iron, as well as aromatic and polar organic substances play a role in ROS production (1, 2). The particle backbone could play an important role in acting as a template for single electron transfers reactions, including electron transfer to molecular dioxygen (11, 14, 16, 20). This could involve redox cycling reactions, as demonstrated by the ability of the ambient PM collections to generate superoxide in the presence of dithiothreitol (DTT) (two, 14, 21). In fact, the DTT oxidation event can be assessed by a colorometric reaction to assay for the content of redox cycling chemicals in urban PM samples (14, 63). Biologically catalyzed oxidation-reduction reactions in the cell, as well as interference in one-electron transfers in the mitochondrial inner membrane, contribute to ROS generation. In addition to the ability of ROS to damage cellular proteins, DNA and cell membranes, electrophilic PM chemicals such as the quinones can modify cellular proteins by Michael acceptor reactions (2, 14, 64). It is likely that this type of reaction leads to Nrf2 release to the nucleus by the covalent modification of its cytosolic chaperone, Keap-I (21). The covalent modification of intracellular and tissue proteins was also confirmed by studying their tyrosylation and carbonylation (6). Much remains to be discovered about the mechanisms by which PM lead to ROS generation.

In order to characterize the redox cycling chemicals present in PM, silica gel chromatography was used to fractionate organic DEP extracts (11, 20, 21, 28). Increasing polar solvents eluted aliphatic, aromatic and polar chemical fractions. This demonstrated that the quinone-enriched polar material was more active than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction in the DTT assay, which, in turn, correlates with glutathione depletion in epithelial cells and macrophages (14, 20, 21). The aliphatic fraction was inactive in these assays.

The relationship between the organic chemical composition and the redox cycling potential of ambient PM was confirmed in a study where UFP were compared to coarse and fine particles collected in the Los Angeles basin (14). Ambient UFP were more active than coarse and fine particles in the DTT assay, and also more prone to generate oxidative stress in macrophages and epithelial cells (14). Both the in vitro and cellular responses showed an excellent correlation with the PAH content of UFP (14). Another important observation in this study was the ability of UFP to lodge in and disrupt the mitochondrial architecture (14). This involves cellular apoptosis and apo-necrosis by a pathway that requires opening of the mitochondrial permeability transition pore (PTP) (14, 16, 28). Functional effects on the PTP and inability to sustain one electron transductions in the mitochondrial inner membrane was confirmed in isolated mitochondrial preparations through the use of calcium-dependent swelling, calcium retention capacity and dissipation of the mitochondrial membrane potential (28). Moreover, UFP effects could be reproduced by polar and aromatic

chemicals fractionated from DEP, while commercial polystyrene nanoparticles were inactive (28). These data show differential particle toxicity based on size, composition, and subcellular localization.

Old age also predisposes to PM susceptibility (12, 29). This susceptibility could also be premised on the increased propensity of aged tissue to generate ROS and oxidative stress. Particle-induced cytokine gene expression is enhanced in macrophages from aged animals (12). Geriatric rats exposed to vehicular traffic on a freeway also showed changes in HRV, suggesting a shift in parasympathetic and sympathetic influences (29).

In summary, ROS generation by the particles themselves as well as their chemical constituents appear to be a major pathway of injury and could lead to oxidative stress and pro-inflammatory effects on the lung, heart and possibly also the CNS. Figure 2 summarizes the events that lead to ROS generation and the biological consequences of oxidative stress. Other important mechanisms of injury include endotoxin-mediated cellular and tissue responses, covalent modification of key regulatory proteins in the cell, vasoconstriction and altered blood coagulability.

3. Mechanisms of Cardiovascular Health Effects

3.1. Introduction to cardiovascular mechanisms

Ambient PM initiates adverse cardiovascular health effects in humans and animals. Evidence that ambient PM can affect cardiovascular health comes from studies that show: (i) associations between daily changes in PM concentration and cardiovascular deaths and hospitalization admissions, as well as (ii) increased adult cardiac and pulmonary mortality associated with spatial differences in PM concentrations. Pathways that have been suggested as potential mechanisms to explain these associations are shown schematically in Figure 3. The first pathway involves altered cardiac autonomic function resulting from particle inhalation. Studies have observed that changes in cardiac autonomic function, as measured by heart rate variability (HRV), are predictors of cardiovascular disease and mortality. Environmental epidemiological studies also report associations between the same HRV predictors and air pollution. The second mechanistic pathway involves lung and systemic inflammation leading to vascular dysfunction. Inhaled PM may provoke a low-grade inflammatory response in the lung, release potentially harmful cytokines into the blood, induce changes in platelets and blood coagulation, increase vascular reactivity, and trigger acute cardiovascular events specifically associated with ischemic heart disease.

3.2 Studies using CAPS exposure during or after coronary occlusion

The effect of inhalation exposure to CAPs on myocardial ischemia was assessed in a canine model of coronary artery occlusion (31). Exposure to CAPs significantly ($p = 0.007$) enhanced occlusion-induced peak ST-segment elevation in precordial leads. ST-segment elevation was significantly correlated with the Si concentration ($p = 0.003$) of the particles and other crustal elements. No associations were found with CAPs mass or number concentrations. Heart rate was not affected by CAPs exposures. These results suggest that exacerbation of myocardial ischemia during coronary artery events may be an important mechanism of environmentally related acute cardiac mortality. The suggested mechanism for these observations involves vasoconstriction of cardiac arteries as a result of PM exposure. Micro-array studies provide evidence of an increase in endothelin gene expression in the lung and a substantial decrease in endothelial nitric oxide synthase (eNOS) expression with CAPs exposure (3). Other studies in rats and mice (32, 33), showing morphometric

measures of vasoconstriction in the lung and heart, indicate that blood vessels are an important target of ambient PM health effects. Another recent study showed that diabetics have greater brachial artery diameter responses from increased exposure to ambient particles (34).

3.3 Chemiluminescence and pharmacological approaches to assess ROS production in the heart

To evaluate the ability of particulate air pollution to promote oxidative stress and tissue damage *in vivo,* a rat model of short-term CAPs exposure was used (17). CAPs exposure in the range of 300 $\mu g/m^3$ induced significant oxidative stress, determined as *in situ* chemiluminescence in the lungs and in the heart, but not in the liver. Increases in oxidant levels were also triggered by residual oil fly ash, but not by particle-free air nor by carbon black aerosols. Increases in chemiluminescence in the lung showed strong associations with the CAPs content of iron, manganese, copper, and zinc. Increases in cardiac chemiluminescence were associated with Fe, aluminum, silicon, and titanium. CAPs inhalation also led to tissue-specific increases in the activities of the antioxidant enzymes, SOD and catalase.

Pharmacological strategies were used to determine whether oxidants are implicated in PM-dependent cardiac dysfunction and whether a PM-induced increase in autonomic stimulation on the heart mediates cardiac oxidative stress and toxicity (35). In rats, intratracheal instillation of urban air particles (UAP 750 μg) or inhalation of CAPs (700 +/-180 $\mu g/m^3$) for 5 hr led to significant increases in heart oxidants measured as organ chemiluminescence or thiobarbituric acid reactive substances. Heart rate increased immediately after exposure and returned to basal levels over the next 30 min. HRV was unchanged immediately after exposure, but significantly increased during the recovery phase. To determine the role of ROS in the development of cardiac malfunction, rats were treated with 50 mg/kg N-acetylcysteine (NAC) 1 hr prior to UAP instillation or CAPs inhalation (10). NAC prevented changes in HRV in UAP-exposed rats. To investigate the role of the autonomic nervous system in PM-induced oxidative stress, rats were given 5 mg/kg atenolol (beta-1 receptor antagonist), 0.30 mg/kg glycopyrrolate (muscarinic receptor antagonist) or saline immediately before exposure to CAPs aerosols. Both atenolol and glycopyrrolate effectively prevented CAPs-induced cardiac oxidative stress (10). These data indicate that PM exposure increases cardiac oxidants via autonomic signals.

3.4 Studies on HRV and arrhythmias

If PM does cause serious cardiovascular effects shortly after exposure, one would expect to see some physiological change during exposure. In one study, spontaneously hypertensive rats with surgically implanted blood pressure transmitters were exposed to concentrated ambient PM (CAPs) for 4 hr to determine whether CAPs inhalation causes immediate effects (36). At other times, the rats were exposed to sulfuric acid aerosols because acid is one of the components of PM that could activate irritant receptors that cause effects. Exposure to CAPs or sulfuric acid aerosols caused a decrease in respiratory rate and heart rate that was apparent soon after the start of exposure and stopped when exposures ceased. The similarity between the effects of fine acid aerosol and CAPs suggests that CAPs activate airway-irritant receptors during exposure.

Pulmonary and systemic effects were assessed in 12 healthy human adult and 12 asthmatic volunteers exposed once for 2 hr in a whole-body chamber (37). The exposure dose was approximately 200 $\mu g/m^3$ CAPs in the fine size range. Each subject was also exposed once to

filtered air. Neither healthy nor asthmatic subjects showed significant changes in symptoms, spirometry, or routine hematologic measurements attributable to CAPs exposure compared with filtered air. Both groups showed CAPs-related (i) decreases of columnar cells in induced sputum after exposure, (ii) increases in certain blood mediators of inflammation (i.e., soluble intercellular ICAM-1 and IL-6), and (iii) parasympathetic stimulation of HRV. In the asthmatic group, systolic blood pressure modestly increased during filtered air exposure and decreased during CAPs exposure, whereas the pattern was reversed in the healthy group. Observed changes in some mediators of inflammation in blood and changes in HRV were consistent with PM-related effects reported from epidemiologic studies suggesting that exposure to concentrated $PM_{2.5}$ tends to elicit more systemic than pulmonary effects. (38).

UFP in ambient air may play a role in cardiovascular effects because of the possibility of penetrating the alveolar epithelium and entering the vascular space. The effects of inhalation of elemental carbon UFP were examined in healthy subjects using continuous ECG monitoring and measurement of HRV, markers of myocardial repolarization, ischemia, and arrhythmias (39). In two separate randomized, double-blinded, controlled studies, subjects were exposed to filtered air and to elemental carbon UFP at mass concentrations of 10 and 25 $\mu g/m^3$. The particle counts in these studies were approximately 2 and 7 x 10^6 particles/cm^3 respectively; particle median diameter was 25 nm. Exposures were for two hours with intermittent exercise. The observed changes were generally small and not clinically significant, although there were trends indicating an increase in parasympathetic tone. One of the studies indicated a dose-related shortening of the late-corrected QT interval with UFP exposure, compared with air. Overall, exposures of healthy subjects to carbon UFP at these concentrations did not cause clinically important changes in ECG-derived parameters.

An ECG telemetry study was done on aged (1-year) $ApoE^{-/-}$ mice (40) exposed intranasally to 50 µg of saline or 50 µg each of $PM_{2.5}$ or silica (Min-u-Sil 5). The mice were monitored for a one-day baseline prior to and for 4 days following exposure. After an initial increase in both heart rate and activity in all groups, there was delayed bradycardia with no change in activity of the animals in the PM and silica exposed groups. In addition, with PM and silica exposure, there was a decrease in HRV parameters suggesting a decrease in parasympathetic tone which may lead to cardiac arrhythmia (40).

Long-term exposure to $PM_{2.5}$ significantly decreased heart rate, body temperature, and physical activity for mice lacking apoliproprotein ($ApoE^{-/-}$) over 5 mo of exposure, with smaller and nonsignificant changes for C57BL/6 mice (41). The effect of subchronic CAPs exposure on HRV parameters that are sensitive to cardiac sympathetic and parasympathetic nerve activity was also studied (42). HRV in the late afternoon and overnight for the $ApoE^{-/-}$ mice showed a gradual increase for the first 6 wk, a decline for about 12 more wk, and a slight turn upward at the end of the study period. For C57BL/6 mice, there were no chronic changes in HRV in the late afternoon, and a slight increase after 6 wk for the overnight period. The response patterns of $ApoE^{-/-}$ mice implies a perturbation of the homeostatic function in the cardiovascular system (initial enhancement and later depression of the HRV parameters). These results complement the findings in human panel and controlled CAPs exposure studies in demonstrating that increased levels of particle pollution are able to perturb cardiac autonomic function, which may lead to adverse cardiovascular outcomes.

Ambient air pollution is a complex mixture of PM and gaseous pollutants such as CO. The effect of exposure to CO, alone or in combination with ambient PM, on arrhythmia incidence has been difficult to sort out in epidemiological studies. To evaluate these effects, left-ventricular myocardial infarction was induced in Sprague-Dawley rats by thermocoagulation, and 12-18 hr later the rats

were exposed (1 hr) to either filtered air (n = 40), CO (35 ppm), CAPs (median concentration = 350.5 µg/m^3), or CAPs and CO (CAPs median concentration = 318.2 µg/m^3) (43). CO exposure reduced ventricular premature beat (VPB) frequency by 60.4% (p = 0.012) during the exposure period compared to controls. CAPs exposure alone increased VPB frequency during the exposure period, and the response to CAPs plus CO was a decrease in arrhythmia similar to CO alone. No significant interactions were observed between the effects of CO and CAPs. Thus, in this animal model, the responses to CO and CAPs are distinctly different. Other studies have shown marked increases in post-MI arrhythmia in rats using PM surrogates (43, 44).

3.3 3.5 Systemic inflammation, acute phase reactions and effects on coagulation

Adverse effects of inhaled PM may be the indirect result of a PM-induced increase in blood coagulability. This explanation is biologically plausible because prospective studies have shown that increases in blood coagulation parameters are significantly associated with risk of adverse cardiovascular events. The hypothesis that acute exposure to elevated levels of PM causes prothrombotic changes in blood coagulation parameters was examined (45). Rats with indwelling jugular vein catheters were exposed for 6 hr to filtered air or CAPs in New York City air. Blood samples were taken at four time points: before and immediately after exposure and at 12 and 24 hr after the start of exposure. At each time point, six coagulation parameters (platelet count, fibrinogen level, factor VII activity, thrombin-antithrombin complex level, tissue plasminogen activator activity, and plasminogen activator inhibitor activity) were measured as well as all standard blood count parameters. Five concentrated-PM exposure experiments were performed over a period of 8 weeks. PM exposure concentrations ranged from 95 to 341 µg/m^3. There were no consistent exposure-related effects on any of the endpoints across the five experiments and no indication of any dose-dependent effects. The results do not indicate that exposure to ambient CAPs causes adverse effects on blood coagulation in healthy rats.

Systemic release of cytokines from the lung and vasculature may impact the production of clotting factors and anticoagulant enzymes in the liver. Laboratory-generated 30 nm elemental carbon UFP were intravenously administered to rats (46). It was shown that doses as low as 1 mg per rat were able to induce thrombus formation in the ear vein model (46). While the role of particle-adsorbed chemicals in these adverse cardiovascular events is uncertain, UFP gain access to the systemic circulation by alveolar penetration. This is illustrated by ear vein thrombus formation upon intratracheal instillation of UFP (46); even a low dose (0.2 µg per rat) was able to produce a significant effect.

Human studies failed to show an effect of UAP inhalation on coagulation and systemic inflammatory responses (47). Healthy and asthmatic subjects inhaled 10 to 50 µg/m^3 elemental carbon UFP (~25 nm) for 2 hr with intermittent exercise. There were no exposure-related increases in platelet count, serum fibrinogen, factor VII, or Von Willibrand's factor antigen in venous blood. There were no significant effects on measures of systemic inflammation, including serum amyloid A (SAA), IL-6, and soluble ICAM-1.

3.6 Accelerated atherosclerosis models (ApoE and variations)

Studies were conducted to determine whether PM can exacerbate atherosclerosis, which is a chronic inflammatory disease of the vessel wall. In one study, C57BL/6, ApoE$^{-/-}$ and ApoE$^{-/-}$/LDL-receptor (DK)-deficient mice were exposed to CAPs for 6 hr/5 days/wk, for up to 5 months (48). The overall mean exposure concentration for these groups of animals was 110 µg/m^3. The cross-sectional area

of the aorta root of DK mice was examined morphologically using confocal microscopy for the severity of lesion, extent of cellularity, and lipid contents. Aortas from the arch to the iliac bifurcations were also sectioned longitudinally and lesion areas were stained with Sudan IV. All DK mice, regardless of exposure, had developed extensive lesions in the aortic sinus regions, with lesion areas that covered > 79% of the total area. In male DK mice, the lesion areas in the aortic sinus regions appeared to be enhanced by CAPs, with changes approaching statistical significance ($p = 0.06$). In addition, plaque cellularity was increased by 28% ($p = 0.014$, combined) whereas there were no CAPs-associated changes in the lipid content in these mice. When examining the entire aorta opened longitudinally, both the $ApoE^{-/-}$ and DK mice had prominent areas of atherosclerosis covering 40% or more of the luminal surface. Visual examination of all images suggested that plaques tend to form in clusters concentrating near the aortic arch and the iliac bifurcations. Quantitative measurements showed that CAPs exposure increased the percentage of aortic intimal surface covered by grossly discernible atherosclerotic lesion by 57% in the $ApoE^{-/-}$ mice ($p = 0.03$). This study demonstrated in susceptible animals had a significant impact on the size, severity, and composition of aortic atherosclerotic plaques.

3.7 Implications of the cardiovascular mechanistic studies

Ischemic heart disease, arrhythmias, hypertension, and atherosclerosis are important outcomes that have been linked to ambient air pollution via epidemiological and experimental studies. The specific mechanisms involved are complex. It is clear that the autonomic nervous system, the responses of the endothelium, and ROS play important roles.

4. Mechanisms of Respiratory Effects of PM

4.1 Introduction to mechanisms of respiratory health effects

The respiratory epithelium is the first tissue to encounter, and respond to inhaled PM. Local responses include injury, inflammation, release of inflammatory mediators, alteration of allergen responsiveness, and activation of neuronal pathways. The respiratory tract is also the portal for distribution of PM to other organs. There are three potential pathways for PM effects: (i) local airway and respiratory effects: (ii) local mediator responses causing effects beyond the respiratory system, and (iii) effects from PM translocated beyond the respiratory tract. This section will focus on respiratory effects, including new findings on particle dosimetry, airway inflammation, asthma and allergen responsiveness, and the pulmonary vasculature.

4.2 Dosimetry and Particle distribution

The health effects of PM exposure are proportional to the locally deposited particle dose and this dose is influenced by the proportion of inhaled particles that are retained in the lung with each breath. Fractional particle deposition and distribution within compartments of the respiratory tract are markedly influenced by particle size. Some ultrafine particles have a high rate of deposition in the alveolar region of the lung because they deposit by diffusional mechanisms.

Human clinical studies with inhalation of ultrafine carbon particles were undertaken with a specially developed inhalation facility (49). In healthy subjects exposed at rest to 10 or 25 $\mu g/m^3$ UFP (~23 nm) for 2 hours, the number deposition fraction exceeded 0.6, and increased further during exercise (8). In subjects with mild asthma, UFP deposition was further enhanced, both at rest and with exercise (8). Remarkably, the number deposition fraction in asthmatics during exercise was 0.86

±0.04. Not only does exercise increase particle dose but the fraction of inhaled particles that deposits is increased as well.

4.3 Airway Inflammation

Airway inflammation can be measured (i) directly by histological examination or recovery of inflammatory cells from airway sampling such as bronchoalveolar lavage fluid or sputum, and (ii) indirectly via changes in inflammatory cytokine gene or protein expression, decrements in pulmonary function secondary to airway narrowing, increases in non-specific airways responsiveness, or increases in systemic markers of inflammation.

Until recently, there was little evidence that inhalation of ambient PM, even at relatively high concentrations, caused significant inflammation in the respiratory tract. Recent animal and human inhalation studies, using particle concentrators, have shown that short-term exposure to concentrated ambient fine particles may have pro-inflammatory effects. Similarly, exposure of dogs to CAPs in the Boston area showed no effects of total PM mass on indicators of airway or systemic inflammation (50). However, further analysis suggested that specific PM components were associated with specific inflammatory cell responses, both in the lung and the blood.

Pulmonary inflammatory responses to CAPs were assessed by exposing normal rats followed by collection of total lung RNA (3, 51). The RNA was pooled, labeled, and hybridized to multiple Affymetrix rat micro-array chips to explore the range of responses to CAPs exposure. Using the A-chip results, data from the sham-exposed group was subtracted from the CAPs group. Since these chips typically include multiple measurements of the same gene, cluster analyses of the results as well as biologic responder cluster assessments of these micro-array studies strongly support the pro-inflammatory potential of CAPs. Increases in pro-inflammatory mediators such as C-C chemokines, IL-1, IL-6 and TNFα are illustrated with an overall decrease in immune Th1 enhancers such as IL-2 and interferon. In addition to enhanced pro-inflammatory mediators, there is evidence of vascular endothelial responses to inhaled CAPs. There is also evidence in the microarray studies for increases in ROS activity, as well as evidence for activation of chemical metabolism and detoxification pathways.

In a human clinical exposure study, investigators in the Chapel Hill area, NC, found modest increases in inflammatory cells in BAL a fluid from healthy subjects after inhalation of concentrated ambient fine particles (52). In contrast, another group found no increases in inflammatory cells in induced sputum or changes in pulmonary function following concentrated fine PM inhalation in young healthy or asthmatic subjects (53), or in healthy elderly subjects with chronic obstructive pulmonary disease (54). Similarly, no inflammatory effects were observed with exposures to concentrated coarse PM (38). Pietropaoli et al. exposed subjects to 10 to 25 $\mu g/m^3$ elemental carbon ultrafine particles (UFP) for 2 hours, with intermittent exercise (47). They found no effects on sputum inflammatory cells, pulmonary function, or exhaled nitric oxide (NO), a noninvasive measure of airway inflammation. Additional studies at a higher concentration of 50 $\mu g/m^3$ also showed no changes in pulmonary function or exhaled NO, and no changes were seen in systemic markers of inflammation or coagulation (55). However, ambient UFP may have a greater pulmonary inflammatory potential than pure elemental carbon particles.

Mild pulmonary inflammatory responses have been seen in a study of “on-road” exposures to ambient particles in rats (56, 57, 58). In one experiment, a single 6 hr exposure to on-road aerosols was found to increase the total number of cells in BAL fluid 3 days after exposure in comparison to filtered air controls. In a separate experiment, the aerosols were found to induce a decrease in the percentage of circulating PMNs relative to filtered air controls after a single 6 hr exposure.

In summary, there is evidence that inhalation of PM at concentrations near ambient can initiate a mild acute inflammatory response in the lung. However, the absence of inflammatory cells or structural changes in the chronic CAPs inhalation studies suggests that pulmonary inflammation may resolve with chronic exposure in animals or humans without lung disease, perhaps due to upregulation of antioxidant and adaptive responses, as discussed in Section 2. The demonstration of long-term effects on the vascular endothelium and heart in the absence of pulmonary effects suggests the lung is better able to adapt to the effects of PM exposure than is the cardiovascular system. However, the effect of long-term PM exposures on the pulmonary inflammatory response in people with or without underlying lung disease, such as asthma or chronic obstructive lung disease, is unknown.

4.4 Asthma and Allergen Responsiveness

A number of human and animal studies have shown that DEP act as an adjuvant that can enhance the allergic inflammatory response to common environmental allergens. Exposure to aerosolized DEP can enhance antigen (OVA)-specific IgE production in a murine inhalation model (6). This adjuvant effect could be suppressed by NAC, in parallel with a decrease in carbonyl protein content in the lung (6). However, this route of sensitization does not lead to vigorous airway inflammation (2, 6), prompting the development of additional murine models to test the effect of DEP on the enhancement of airway inflammation and AHR (7). Two new protocols were developed (7). In the first, low-grade sensitization is achieved by injecting the antigen in BALB/c without alum, followed by challenge with aerosolized OVA ± DEP. This allows DEP to act as an adjuvant during secondary sensitization, with the ability to induce airway inflammation and AHR. In the second model, delivery of DEP post-OVA challenge in the classical sensitization model leads to a resurgence of airway inflammation and AHR (7).

4.5 Pulmonary Vascular Effects

There is evidence that inhalation of fine and ultrafine PM may alter the function of the vascular endothelium in the lung as well as systemically. In morphometric studies of the pulmonary vasculature in rats (32), exposure to CAPs produced a decrease in the lumen-to-wall ratio, indicating vasoconstriction in the pulmonary vascular bed. Sulfate and silicon were the chemical species most strongly associated with these vascular effects. Microarray studies showed a strong increase in mediators and receptors associated with vasoconstriction and endothelial injury with an inhibition of vasodilator mediator activity (57). The lungs and hearts of rats exposed to 3 days of CAPs, compared with sham exposures, showed morphologic evidence of both pulmonary and cardiac vascular endothelial activation. Similar effects were seen in human studies. Exposure to 50 $\mu g/m^3$ carbon UFP decreased the pulmonary diffusing capacity for carbon monoxide 21 hours after exposure (-1.76 ± 0.66 ml/min/mmHg (UFP) vs. -0.18 ± 0.41 ml/min/mmHg (air), p = 0.040) (55). This finding is consistent with decreased pulmonary capillary blood volume in response to UFP. Taken together, these findings provide evidence of endothelial cell changes with exposure to ambient particles.

In a series of randomized, double-blind studies, healthy and asthmatic subjects were exposed to elemental carbon UFP at concentrations of 10, 25 and 50 $\mu g/m^3$, and blood leukocytes were analyzed by flow cytometry for changes in surface expression of adhesion molecules (13). There were reductions in expression of adhesion molecules on blood monocytes and PMNs compared with control air exposures. For example, monocyte expression of ICAM-1 was decreased following inhalation of 10 and 25 $\mu g/m^3$ carbon UFP, in a concentration-related manner. This decrease could result from produced pulmonary capillary blood flow secondary to vasoconstriction.

In summary, the respiratory effects of exposure to ambient PM are influenced by the inhaled dose and airway distribution of the particles, which is determined both by particle characteristics and the presence or absence of airway obstruction. Inhaled fine and ultrafine particles appear to induce mild, possibly transient inflammatory responses. DEP appear to enhance the allergen response in an animal model of allergic airway inflammation. There is both direct and indirect evidence that inhalation of fine and UFP may affect the pulmonary vascular endothelium, leading to pulmonary vasoconstriction. Alteration of pulmonary vascular function may have effects on cardiac preload, with potential clinical consequences in patients with either pulmonary or cardiovascular disease.

5. Central Nervous System

EPA PM Center investigators have clearly demonstrated that inhalation of PM is associated with extrapulmonary effects. As discussed elsewhere in this report, it is unclear whether circulating mediators, such as cytokines, which originate in the lung, may elicit these extrapulmonary responses or whether particles themselves leave the respiratory tract and produce the toxic effects observed in other organs. While both mechanisms have merit, PM Center investigators have clearly demonstrated that the ultrafine fraction of PM can translocate from the respiratory tract to extrapulmonary tissues. In one study, animals were exposed to ultrafine elemental ^{13}C particles and isotope concentration was determined in lungs, cerebrum, cerebellum, and olfactory bulbs at 1, 3, 5, and 7 days postexposure (59). ^{13}C concentration in the olfactory bulb consistently increased from day 1 through day 7 postexposure, but not in cerebrum and cerebellum. In a second study, rats were exposed to ^{192}Ir-radiolabeled ultrafine iridium particles. Within the first week after exposure, radiolabel was measurable in the liver, spleen, heart, and brain. The radiolabeled particles were cleared from the body via excretion and the tissue concentrations decreased with time post-exposure (3 wk and 2 and 6 mo). Thus, the translocation of UFP from the respiratory tract could lead to extra-pulmonary effects.

PM Center investigators have examined the effect of exposure to CAPs on the central nervous system. UFP can be transported from the nasal mucosa to the brain via the olfactory bulb, and from these there are transynaptic routes to CNS targets such as hypothalamus, substantia nigra, and olfactory cortex (59, 60). Murine exposure to CAPs for 4 hr/day over a two-week time period resulted in increased pro-inflammatory responses in the brain tissue (61). Significant increases in NF-κB were observed in cortical tissue after exposure to concentrated UFP or fine + UFP. This increase was accompanied by increased TNFα and IL-1α levels in brain tissue and is compatible with the role of this prescription factor in oxidative stress and inflammation. Veronesi and colleagues have also observed a reaction in the brain tissue of $ApoE^{-/-}$ mice exposed to CAPs for 5 months (62). Dopamineric neurons were stained immunohistochemically and image analysis demonstrated that neurons in the substantia nigra nucleus compacta portion of the brain were significantly reduced in CAPs-exposed relative to air-exposed Apo E-/- control mice. This was accompanied by significant increases ($p < 0.05$) in staining for astrocytes. Because these mice are characterized by elevated levels of oxidative stress in the brain, these findings suggest that exposure to ambient PM could contribute to neurodegeneration in susceptible individuals.

Table:

Table 1: Plausible Mechanisms to Explain PM Adverse Health Effects

- **Local Inflammation →** asthma, COPD, adjuvancy
- **Systemic inflammation** (from lungs as well as circulation) **→** ↑ CRP, atherosclerosis, blood coagubility
- **Autonomic nervous system activity** → Cardiac arrhythmias
- ↑ **clotting factors, ↓ fibrinolytic activity →** ↑ blood coagulation → MI, stroke
- ↑ **bone marrow production of myeloid lineage cells** → ↑WBC, including neutrophils, DC
- **Endotoxin-mediated cellular responses & inflammation** → airway & systemic inflammation
- **Irritant-type receptors** (e.g., vanilloid receptors) → airway hypereactivity, asthma
  Covalent modification of intracellular enzymes, mitochondrial membrane → ROS generation, cellular apoptosis
- **Phagocytic particle overload** → ↓ phagocytosis → ↑ respiratory infections
- **Free oxygen Radicals and Oxidative Stress** → Oxidative stress

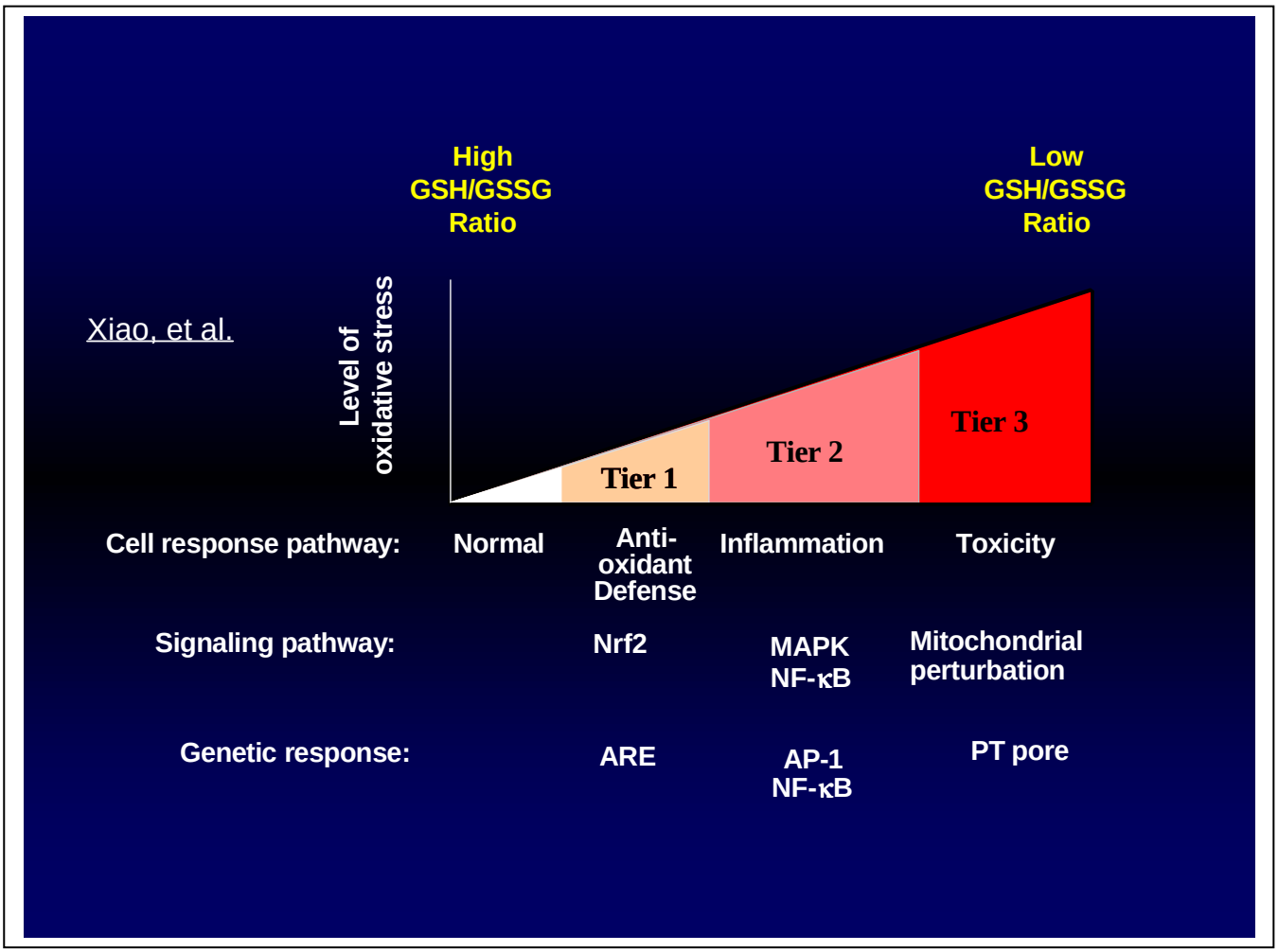

Figure 1: Hierarchical oxidative stress model in response to PM exposure. At a lower level of oxidative stress (tier 1), antioxidant enzymes are induced to restore cellular redox homeostasis. At an intermediate level of oxidative stress (tier 2), activation of MAPK cascades induce pro-inflammatory responses, e.g., cytokines and chemokines. At a high level of oxidative stress (tier 3), perturbation of the mitochondrial permeability transition pore and disruption of electron transfer result in cellular apoptosis or necrosis (adapted from 19).

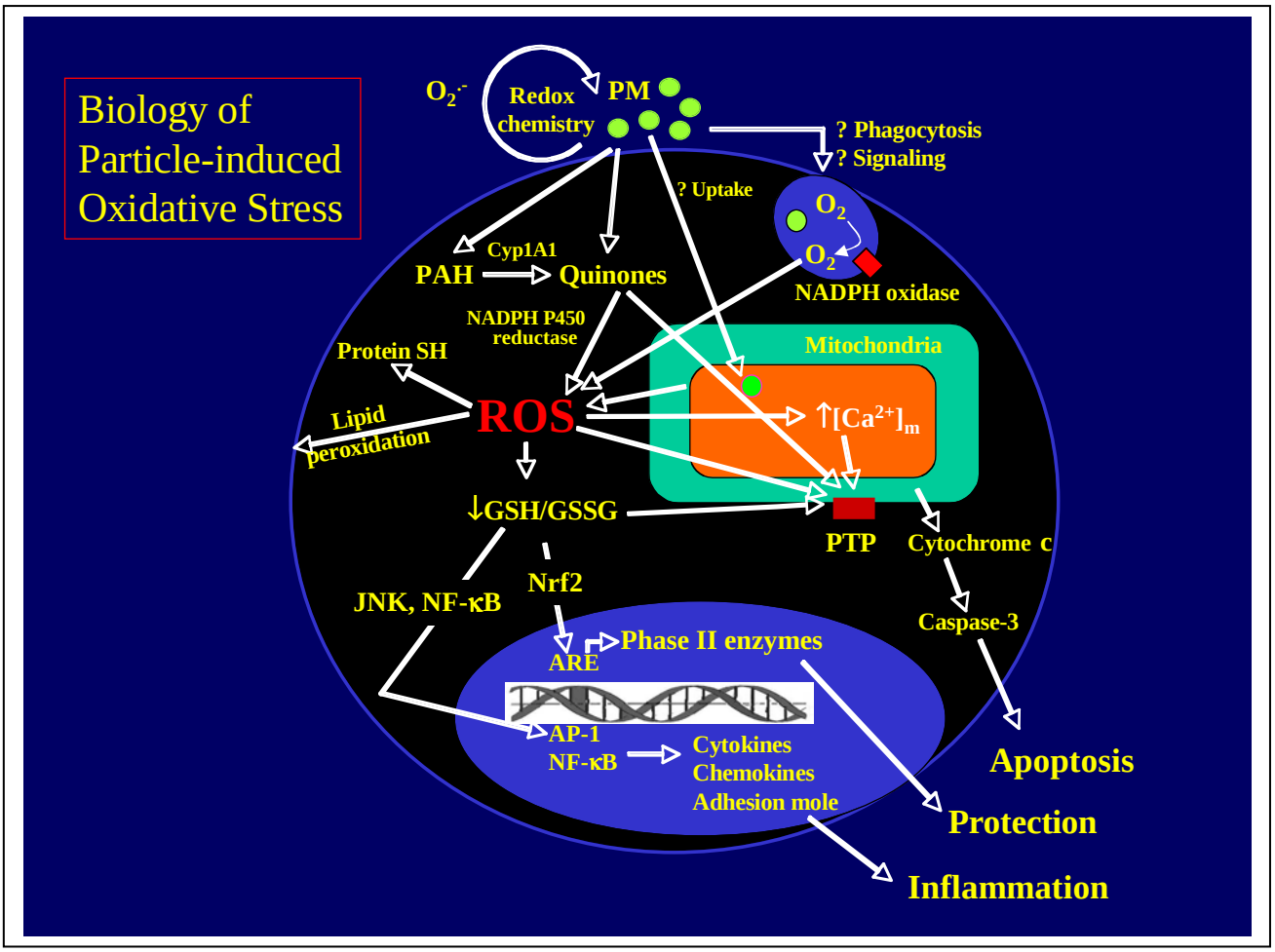

Figure 2: Mechanisms of particle-induced ROS generation, mitochondrial targeting and the biological effects of oxidative stress. Sources of ROS production and their effects on cells. Quinones can redox cycle to produce ROS in the endoplasmic reticulum under the catalytic influence of NADPH-cytochrome P450 reductase. Phygocytosis can induce the assembly and activation of NADPH oxidase to produce superoxide. PM can interfere in electron transduction in the mitochondrial inner membrane as well as in the perturbation of the PT pore to generate ROS. ROS leads to lipid peroxidation in the cell membrane can crosslink protein SH groups and induce redox equilibrium through a depletion of GSH. Depending on the level of oxidative stress this could induce Nrf2 release to the nucleus, activation of MAPK and NF-κB signaling cascades or cytotoxicity, Nrf2 interaction with the ARE leads to the expression HO-1 and other phase II enzymes at low level of oxidative stress, while MAPK and NF-κB signaling cascades lead to pro-inflammatory responses (e.g., cytokine production) at higher levels of oxidative stress. At the highest oxidative stress level, ROS can lead to opening of mitochondrial PT pore, followed by cytochrome c release, caspase-3 activation and apoptosis or necrosis.

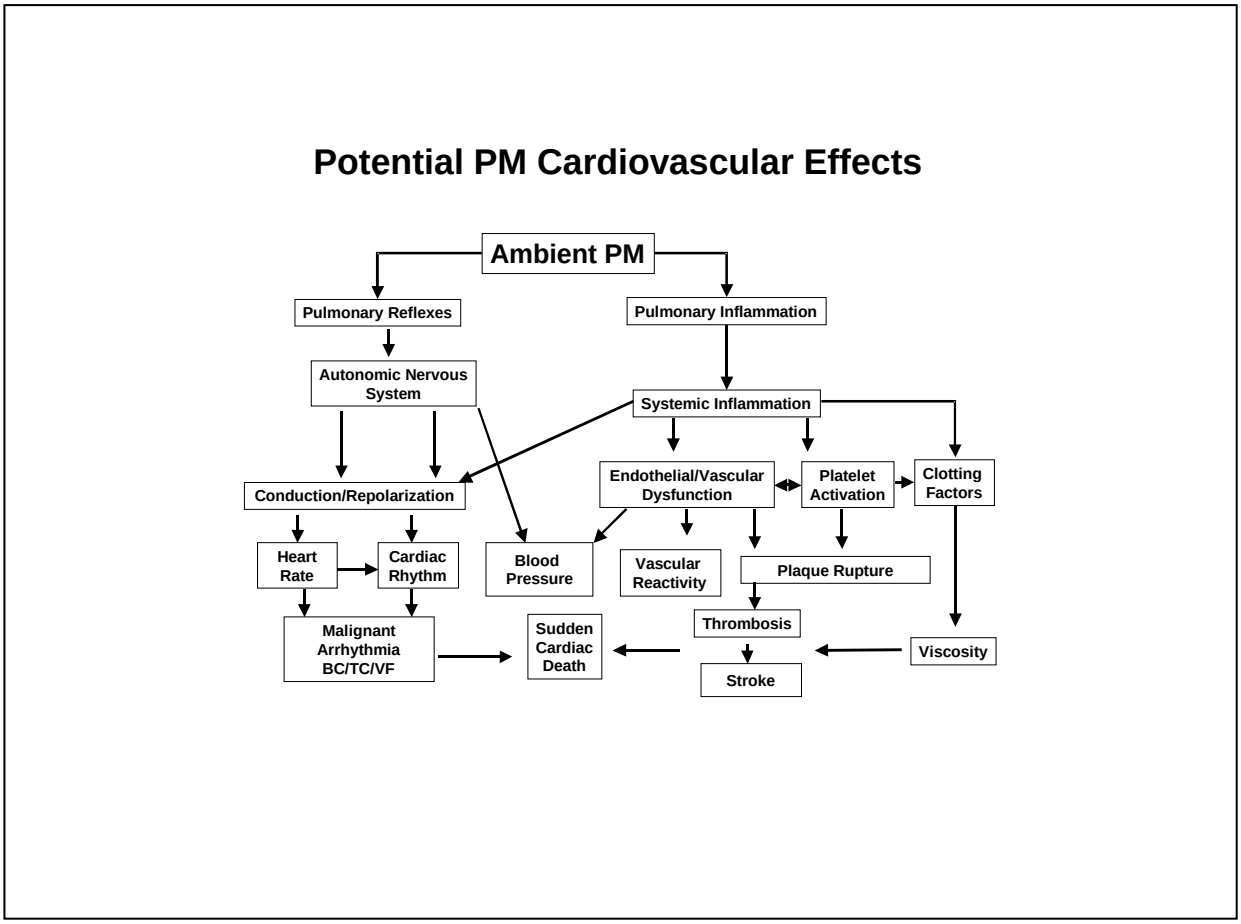

Figure 3: **Potential PM Cardiovascular Effects**

REFERENCES

1. Nel, A. 2005. Air pollution-related illness: Biomolecular effects of particles. Science 308: 804.

2. Li, N., Hao, M., Phalen, R.F., Hinds, W.C., Nel, A.E. 2003. Particulate air pollutants and asthma: A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clinical Immunology 3: 250-265.

3. Godleski, J.J., Clarke, R.W., Coull, B.A., Saldiva, P.H.N., Jiang, N.F., Lawrence, J., Koutrakis, P. 2002. Composition of inhaled urban air particles determines acute pulmonary responses. Annals of Occupational Hygiene 46 (Supplement 1): 419-424.

4. Saldiva, P.H.N., Clarke, R.W., Coull, B.A., Stearns, R., Lawrence, J., Koutrakis, P., Suh, H., Tsuda, A., Godleski, J.J. 2002. Acute pulmonary inflammation induced by concentrated ambient air particles is related to particle composition. American Journal of Respiratory and Critical Care Medicine 165: 1610-1617.

5. Nel, A.E., Diaz-Sanchez, D., Li, N. 2001. The role of particulate pollutants in pulmonary inflammation and asthma: Evidence for the involvement of organic chemicals and oxidative stress. Current Opinion in Pulmonary Medicine 7:20-26.

6. Whitekus, N., Li, M.J., Zhang, M., Wang, M, Horwitz, M., Nelson, S.K., Brechun, N., Diaz-Sanchez, D., Nel, A.E. 2002. Thiol antioxidants inhibit the adjuvant effects of aerosolized diesel exhaust particles in a murine model for ovalbumin sensitization. Journal of Immunology 168: 2560-2567.

7. Minqi, H., Comier, S., Wang, M., Lee, J., Nel, A. 2003. Diesel exhaust particles exert acute effects on airway inflammation and function in murine allergen provocation models. Journal of Allergy and Clinical Immunology 112: 905-14.

8. Chalupa, D.C., Morrow, P.E., Oberdörster, G., Utell, M.J., Frampton, M.W. 2004. Ultrafine particle deposition in subjects with asthma. Environmental Health Perspectives 112: 879-882.

9. Daigle, C.C., Chalupa, D.C., Gibb, F.R., Morrow, P.E., Oberdörster, G., Utell, M.J., Frampton, M.W. 2003. Ultrafine particle deposition in humans during rest and exercise. Inhalation Toxicology 15: 539-552.

10. Rhoden, C.R., Lawrence, J., Godleski, J.J., Gonzalez-Flecha, B. 2004. N-acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. Toxicological Science 79(2): 296-303.

11. Li, N., Wang, M., Oberley, T.D., Sempf, J.M., Nel, A.E., 2002. Comparison of the pro-oxidative and proinflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages. Journal of Immunology 169: 4531-4541.

12. Finkelstein, J.N., Reed, C., Johnston, C., Oberdorster, G. 2002. Age alters macrophage responses to endotoxin and particle - induced cytokine gene expression. American Journal of Respiratory and Critical Care Medicine 165 (abstract from ATS).

13. Frampton, M.W., Stewart, J.C., Oberdörster, G., Morrow, P.E., Chalupa, D., Pietropaoli, A.P., Frasier, L.M., Speers, D.M., Cox, C., Huang, L-S., Utell, M.J. 2006. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. Environmental Health Perspectives 114: 51-58.

14. Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Oberley, T., Froines, J., Nel, A. 2003. Particulate air pollutants, oxidative stress and mitochondrial damage. Environmental Health Perspectives 111: 455-460.

15. Li, N., Kim, S., Wang, M., Froines, J., Sioutas, C., Nel, A. 2002. Use of a stratified oxidative stress model to study the biological effects of ambient concentrated and diesel exhaust particulate matter. Inhalation Toxicology 14: 459-486.

16. Hiura, T.S., Kaszubowski, M.P., Li, N., Nel, A.E. 1999. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. Journal of Immunology 163: 5582-5591.

17. Gurgueira, S.A., Lawrence, J., Coull, B., Murthy, G.G., Gonzalez-Flecha, B. 2002. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environmental Health Perspectives 110(8): 749-755.

18. Elder, A.C.P., Gelein, R., Azadniv, M., Frampton, M., Finkelstein, J., Oberdörster, G. 2004. Systemic interactions between inhaled ultrafine particles and endotoxin in two rat strains. Inhalation Toxicology 16 (6-7): 461-471.

19. Gilmour, I.M., Jaakkola, M.S., London, S.J., Nel, A.E., and Rogers, C.A. 2006. How the indoor and outdoor environments influence the incidence and severity of asthma. Environmental Health Perspectives 114:627-633.

20. Li, N., Venkatesan, M.I., Miguel, A., Kaplan, R., Gujuluva, C., Alam, J., Nel, A. 2000. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. Journal of Immunology 165: 3393-3401.

21. Li, N., Alam, J., Venkatesan, M.I., Eiguren-Fernandez, A., Schmitz, D., Di Stefano, E. M., Slaughter, N., Killeen E., Wang, X., Huang, A., Wang, M., Miguel, A.H., Cho, A., Sioutas, C., Nel, A.E. 2004. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: Protecting against the pro-inflammatory and oxidizing effects of diesel exhaust chemicals. Journal of Immunology 173: 3467-3481.

22. Hatzis, C., Godleski, J.J., González-Flecha, B., Wolfson, J.M., Koutrakis, P. 2006. Ambient particulate matter exhibits direct inhibitory effects on oxidative stress enzymes. Environmental Science and Technology 40: 2805-2811.

23. Gilliland, F.D., Li, Y.F., Saxon, A., Diaz-Sanchez, D. 2004. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. Lancet 363(9403): 95-96.

24. Lippmann, M., Gordon, T., Chen, L.C. 2005. Effects of subchronic exposures to concentrated ambient particles in mice. IX. Integral assessment and human health implications of subchronic exposures of mice to CAPs. Inhalation Toxicology 17: 255-261.

25. Wang, M., Xiao, G.C., Li, N., Xie, Y., Loo, J.A., Nel, A.E. 2005. Phosphoproteome and cytokine array analysis show MAP kinases mediate inflammation by pro-oxidative diesel exhaust particle chemicals. Electrophoresis 26: 2092-2108.

26 Pourazar, J., Mudway, I.S., Samet, J.M., Helleday, R., Blomberg, A., Wilson, S.J., Frew, A.J., Kelly, F.J., Sandstrom, T. 2005. Diesel exhaust activates redox-sensitive transcription factors and kinases in human airways. American Journal of Physiology – Lung Cellular and Molecular Physiology 289(5): L724-30.

27. Hiura, T.S., Li, N., Kaplan, R., Horwitz, M., Seagrave, J.C., Nel, A.E. 2000. The role of a mitochondrial pathway in the induction of apoptosis by chemicals extracted from diesel exhaust particles. Journal of Immunology 165: 2703-2711.

28. Xia, T., Korge, P., Weiss, J.N., Li, N., Venkatesen, I., Sioutas, C., Nel, A. 2004. Quinones and aromatic chemical compounds in particulate matter (PM) induce mitochondrial dysfunction: Implications for PM-induced oxidative stress and toxicity. Environmental Health Perspectives 112(14): 1347-1358.

29. Elder, A., Gelein, R., Finkelstein, J., Phipps, R., Frampton, M., Utell, M., Kittleson, D.B., Watts, W.F., Hopke, P., Jeong, C.H., Kim, E., Liu, W., Zhao, W., Zhuo, L, Vincent, R., Kumarathasan, P., Oberdorster, G. 2004. On-road exposure to highway aerosols. 2. Exposures of aged, compromised rats. Inhal Toxicol 16 (Suppl. 1):41-53.

30. Corey, L.M., Baker, C., Luchtel, D.L. 2005. Genomic response of the ApoE-/- mouse to Seattle PM. Proceedings of the American Thoracic Society 2: A296.

31. Wellenius, G.A., Coull, B.A., Godleski, J.J., Koutrakis, P., Okabe, K., Savage, S.T., Lawrence, J.E., Krishna Murthy, G.G., Verrier, R.L. 2003. Inhalation of concentrated ambient air particles exacerbates myocardial ischemia in conscious dogs. Environmental Health Perspectives 111: 402-408.

32. Batalha, J.R.F., Saldiva, P.H.N., Clarke, R.W., Coull, B.A., Stearns, R.C., Lawrence, J., Krishna Murthy, G.G., Koutrakis, P., Godleski, J.J. 2002. Concentrated ambient air particles induce vasoconstriction of small pulmonary arteries in rats. Environmental Health Perspectives 110: 1191-1197.

33. Lemos, M., Mohallem, S., Macchione, M., Dolhnikoff, M., Assunção, J.V., Godleski, J.J., Koutrakis, P., Saldiva, P.N.H. 2006. Chronic exposure to urban air pollution induces structural alterations in murine coronary arteries. Inhalation Toxicology 18: 247-253.

34. O'Neill, M., Veves, L.A., Zanobetti, A., Gold, D.R., Economides, P.F., Horton, E., Schwartz, J. 2005. Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. Circulation 111(22): 2913-2920.

35. Rhoden, C.R., Wellenius, G.A., Ghelfi, E., Lawrence, J., Gonzalez-Flecha, B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. Biochimica et Biophysica Acta 1725 (3): 305-313.

36. Nadziejko, C., Fang, K., Nadziejko, E., Narciso, S.P., Zhong, M., Chen, L.C. 2002. Immediate effects of particulate air pollutants on heart rate and respiratory rate in hypertensive rats. Cardiovascular Toxicology 2(4): 245-252.

37. Gong, H. Jr, Sioutas, C., Linn, W.S. 2003. Controlled exposures of healthy and asthmatic volunteers to concentrated ambient particles in metropolitan Los Angeles. Res Rep Health Eff Inst. 118:1-36; discussion 37-47.

38. Gong, H. Jr, Linn, W.S., Terrell, S.L., Clark, K.W., Geller, M.D., Anderson, K.R., Cascio, W.E., Sioutas, C. 2004. Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. Inhalation Toxicology 16(6-7): 335-43.

39. Zareba W, Couderc JP, Oberdörster G, Chalupa D, Speers DM, Cox C, Huang L-S, Peters A, Utell MJ, Frampton MW. (In press). ECG parameters and exposure to carbon ultrafine particles in young healthy subjects. Inhalation Toxicology.

40. Corey, L.M., Baker, C., Luchtel, D.L. 2005. Heart rate variability in the apolipoprotein E knockout transgenic mouse following exposure to Seattle particulate matter. Journal of Toxicology and Environmental Health A 69(10): 953-965.

41. Hwang, J.S., Nadziejko, C., Chen, L.C. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. III. Acute and chronic effects of CAPs on heart rate, heart-rate fluctuation, and body temperature. Inhalation Toxicology 4-5: 199-207.

42. Chen, L.C., Hwang, J.S. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. IV. Characterization of acute and chronic effects of ambient air fine particulate matter exposures on heart-rate variability. Inhalation Toxicology 17(4-5): 209-216.

43. Wellenius, G.A., Batalha, J.R.F., Diaz, E.A., Lawrence, J., Coull, B.A., Katz, T., Verrier, R.L., Godleski, J.J. 2004. Cardiac effects of carbon monoxide and ambient particles in a rat model of myocardial infarction. Toxicological Sciences 80: 367-376.

44. Wellenius, G.A., Saldiva, P.H.N., Batalha, J.R.F., Krishna Murthy, G.G., Coull, B.A., Verrier, R.L., Godleski, J.J. 2002. Electrocardiographic changes during exposure to residual oil fly ash (ROFA) particles in a rat model of myocardial infarction. Toxicological Sciences 66: 327-335.

45. Nadziejko, C., Fang, K., Chen, L.C., Cohen, B., Karpatkin, M., Nadas, A. 2002. Effect of concentrated ambient particulate matter on blood coagulation parameters in rats. Res Rep Health Eff Inst. 111: 7-29; discussion 31-8.

46. Silva, V.M., Corson, N., Elder, A., Oberdörster, G. 2005. The rat ear vein model for investigating in vivo thrombogenicity of ultrafine particles (UFP). Toxicological Sciences 85: 983-989.

47. Pietropaoli, A.P., Frampton, M.W., Oberdörster, G., Cox, C., Huang, L-S., Marder, V.,Utell, M.J. 2004. Blood markers of coagulation and inflammation in healthy human subjects exposed to carbon ultrafine particles. In: Effects of Air Contaminants on the Respiratory Tract - Interpretations from Molecular to Meta Analysis. Stuttgart, Germany:INIS Monographs, Fraunhofer IRB Verlag, 181-194

48. Chen, L.C., Nadziejko, C. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. V. CAPs exacerbate aortic plaque development in hyperlipidemic mice. Inhalation Toxicology 17(4-5): 217-224.

49. Chalupa, D.F., Gibb, F.R., Morrow, P.E., Oberdörster, G., Riesenfeld, E., Gelein, R., Utell, M.J., Frampton, M.W. 2002. A facility for controlled human exposures to ultrafine particles. In: Crucial Issues in inhalation research - mechanistic, clinical and epidemiologic (Heinrich U, Mohr U, eds). Washington, DC:ILSI Press, 241-253.

50. Clarke, R.W., Coull, B., Reinisch, U., Catalano, P., Killingsworth, C.R., Koutrakis, P, Kavouras, I., Krishna Murthy, G.G., Lawrence, J., Lovett, E.G., Wolfson, J.M., Verrier, R.L., Godleski, J.J. 2000. Inhaled concentrated ambient particles are associated with hematologic and bronchoalveolar lavage changes in canines. Environmental Health Perspectives 108: 1179-1187.

51. Wellenius, G.A., Batalha, J.R.F., Diaz, E.A., Lawrence, J., Coull, B.A., Katz, T., Verrier, R.L., Godleski, J.J. 2004. Cardiac effects of carbon monoxide and ambient particles in a rat model of myocardial infarction. Toxicological Sciences 80: 367-376.

52. Ghio, A.J., Kim, C., Devlin, R.B. 2000. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. American Journal of Respiratory and Critical Care Medicine 162: 981-988.

53. Gong Jr., H., Linn, W.S., Sioutas, C., Terrell, S.L., Clark, K.W., Anderson, K.R., Terrell, L.L. 2003. Controlled exposures of healthy and asthmatic volunteers to concentrated ambient fine particles in Los Angeles. Inhalation Toxicology 15:305-325.

54. Gong Jr. H, Linn WS, Terrell SL, Anderson KR, Clark KW, Sioutas C, Cascio, W.E., Alexis, N., Devlin, R.B. 2004. Exposures of elderly volunteers with and without chronic obstructive pulmonary disease (COPD) to concentrated ambient fine particulate pollution. Inhalation Toxicology 16:731-744.

55. Pietropaoli AP, Frampton MW, Hyde RW, Morrow PE, Oberdörster G, Cox C, Speers, D.M., Frasier, L.M., Chalupa, D.C., Huang, L-S., Utell, M.J. 2004. Pulmonary function, diffusing capacity and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. Inhalation Toxicology 16 (Suppl. 1):59-72.

56. Gunnison, A., Chen, L.C. 2005. Effects of subchronic exposures to CAPs in mice: VI. Measurement of gene expression in heart and lung tissue following exposure. Inhalation Toxicology 17(4-5): 225-233.

57. Kittelson, D.B., Watts, W.F., Johnson, J.P., Remerowki, M.L., Ische, E.E., Oberdörster, G., Gelein, R.M., Elder, A.C.P., Hopke, P.K., Kim, E., Zhao, W., Zhou, L, Jeong, C-H. 2004. On-road exposure to highway aerosols. 1. Aerosol and gas measurements. Inhal Toxicol 16(supple. 1):31-39.

58. Elder, A.C.P., Gelein, R., Finkelstein, J., Frampton, M., Utell, M., Carter, J., Driscoll, K., Kittelson, Watts, W., Hopke, P., Vincent, R., Premkumari, K., Oberdorster, G. 2004. Effects of inhaled fine/ultrafine particles combined with other air pollutants. In: INIS Monographs, 9th Intl. Inhalation Symposium: Effects of Air Contaminants on the Respiratory Tract — Interpretations from Molecules to Meta Analysis. U. Heinrich, editor, pp. 53-68.

59. Corey, L.M., Baker, C., Luchtel, D.L. 2005. Genomic response of the ApoE-/- mouse to Seattle PM. Proceedings of the American Thoracic Society 2 A 296.62.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C. 2004. Translocation of inhaled ultrafinne particles to the brain. Inhal. Toxicol. 16:437-445.

60. Maciejczyk, P., Chen, L.C. 2005. Effects of subchronic exposures to CAPs in mice: VIII. Source-related daily variations in i*n vitro* responses to CAPs. Inhalation Toxicology 17(4-5): 243-253.

61. Campbell, A., Oldham, M., Becaria, A., Bondy, S.C., Meacher, D., Sioutas, C., Misra, C., Mendez, L.B., Kleinman, M. 2005. Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. Neurotoxicology 26(1): 133-140.

62. Veronesi, B., Makwana, O., Pooler, M., Chen, L.C. 2005. Effects of subchronic exposures to concentrated ambient particles. VII. Degeneration of dopaminergic neurons in Apo E-/- mice. Inhalation Toxicology 17(4-5): 235-241.

63. Cho, A.K., Sioutas, C., Miguel, A.H., Kumagai, Y., Schmitz, D.A., Misra, C., Singh, M., Eiguren-Fernandez, A., Froines, J.R. 2005. Redox activity of airborne particulate matter (PM) at different sites in the Los Angeles Basin. Environmental Research 99(1): 40-47.

64. Shinyashiki, M., Rodriguez, C.E., Di Stefano, E.W., Sioutas, C., Delfino, R.J., Kumagai, Y., Froines, J.R., Cho, A.K. 2008. On the interaction between glyceraldehyde-3-phosphate dehydrogenase and airborne particles: Evidence for electrophilic species. Atmospheric Environment 42: 517-529.