FINAL DRAFT

Cardiovascular Effects Associated with Air Pollution: Potential Mechanisms and Methods of Testing

Mark J. Utell[1], Mark W. Frampton[1], Wojciech Zareba[2], Robert Devlin[3] and Wayne Casio[4]

[1] University of Rochester Medical Center, Departments of Medicine and Environmental Medicine, Rochester, New York, USA
[2] University of Rochester Medical Center, Department of Medicine, Rochester, New York, USA
[3] U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA
[4] University of North Carolina, Chapel Hill, North Carolina, USA

Abbreviated Title: Cardiovascular Effects and Particles

Address correspondence to: Mark J. Utell, M.D., Pulmonary Division – Box 692
University of Rochester Medical Center, Rochester, NY 14642, USA

There is no doubt that the dramatic air pollution events of 50 years ago, like the London Fog of December 1952, had a major impact upon health. The enforcement of laws and regulations in Europe and the United States has resulted in the reduction of major air pollution levels and there have been no major episodes since the 1970s. Nevertheless, a recent series of epidemiologic reports have shown associations between particulate matter (PM) levels and increased cardiovascular morbidity and mortality (Pope, 2000). Recently, elevated PM levels have been linked with serious cardiac arrhythmias (Peters et al., 2000). These findings have already spearheaded the decision of the U.S. EPA to recommend a new 24-hour and annual $PM_{2.5}$ standard while retaining the existing PM_{10} standard.

In 1999, diseases of the heart accounted for over 700,000 deaths in the United States or 30% of all deaths. Most of the deaths were precipitated by myocardial ischemia, infarction or the sudden onset of a serious arrhythmia. The factors triggering these events are of great interest and of public health importance as epidemiologic evidence now implicates ambient PM as a cardiovascular risk factor. For example, acute epidemiologic studies suggest that for each 5 $\mu g/m^3$ increase in $PM_{2.5}$, there is a 1.3% increase in cardiovascular mortality (Pope, 2000). Moreover, the epidemiologic findings linking ambient particles with cardiovascular events have provided a framework around which to build novel hypotheses to examine the biological mechanisms. Potential mechanisms include autonomic dysfunction, systemic and local inflammatory events, endothelial injury and alterations in the coagulation cascade. Air pollution investigators have recognized the unique opportunity to bring the technologies of non-invasive cardiology to the study of PM-induced cardiovascular events. In addition, the five EPA PM

Centers identified the need for a workshop to explore methodologic issues in cardiovascular research protocols.

WORKSHOP GOALS AND STRUCTURE

The American Petroleum Institute (API) and the U.S. Environmental Protection Agency together with the University of Rochester EPA Particle Center sponsored a workshop on March 7-8, 2001 in Rochester, New York to discuss the potential mechanisms and methods of testing that should be considered in developing a coherent approach to the investigation of cardiovascular effects associated with air pollution. The workshop brought together epidemiologists, cardiologists and toxicologists from academia, government and industry, all of whom share an interest in understanding the relationship between particulate matter and cardiovascular health (Appendix I). The workshop first reviewed the evidence from epidemiologic, animal and clinical studies linking PM with cardiovascular effects as well as the armamentarium of non-invasive tests available to examine these relationships. It then went on to examine the plausible mechanisms that could be responsible for such effects including neural mechanisms, plaque formation, and the role of cytokines and inflammation. In keeping with the objectives, the workshop participants were charged with reviewing the current protocols and methods used for detecting cardiovascular responses in epidemiologic and clinical studies, considering their robustness, and exploring new methodologies.

The structure of the workshop included three scientific sessions, each with three to five plenary speakers. At the end of each plenary session, multidisciplinary panels presented prepared comments focusing on mechanisms of response and potential testing methodologies.

SESSION I: Cardiovascular Effects Associated With Air Pollution

This session set the stage for the workshop, providing participants with an overview of the epidemiologic and clinical studies linking PM with adverse cardiac effects. A number of epidemiology studies have reported associations between PM and increased cardiovascular mortality and hospital admissions for cardiovascular diseases. These findings have stimulated further research using panel studies of elderly humans and controlled exposure of humans or animals to various kinds of PM.

In recent studies, tests of cardiac function (e.g. heart rate, heart rate variability) were done repeatedly for panels of elderly people over a period of several weeks, and these changes associated with exposure of the people to ambient PM. In general these studies report associations between PM levels and decreased heart rate variability, especially in the high frequency domain (Liao et al, 1999; Gold et al., 2000; Pope et al., 1999b). In one of these studies, conducted in Baltimore, the effects were observed only in elderly people with pre-existing cardiovascular disease (Liao et al., 1999). Associations have also been reported between exposure to PM and increased heart rate, although these data are not as consistent as the changes in heart rate variability. A significant increase in heart rate with PM was reported by Pope et al. (1999a) and in the Baltimore panel study; however, decreased heart rate was reported in the Boston panel study (Gold et al., 2000).

A recent epidemiology study of patients with implanted cardiac defibrillators used data on frequency of defibrillator discharges; these discharges occur when patients experience significant cardiac arrhythmias. Associations were reported between PM and increased defibrillator discharges (Peters et al., 2000). A second study examined several hundred patients with myocardial infarction (MI) and reported that the risk for MI onset increased in association with PM levels in the 2 hrs preceding the MI (Peters et al., 2001).

PM exposure has been linked with arrhythmia in recent toxicological studies. Changes in ECG patterns, including elevations of the ST segment, were reported in dogs exposed to concentrated ambient air particles (CAPS) (Godleski et al., 2000). Similarly, altered ECG patterns were observed in residual oil fly ash (ROFA) treated spontaneously hypertensive rats (Kodavanti et al., 2001). Increased arrhythmia was seen in rats exposed to ROFA or ambient PM, with rat models of cardiorespiratory disease having the most severe changes (Watkinson et al., 1998). A controlled exposure study reported that exposure of healthy elderly, but not young volunteers, resulted in decreased heart rate variability in both time and frequency domains (Devlin et al., 2001). Preliminary analyses of healthy volunteers exposed to ultrafine carbon particles revealed changes in the QT interval with Holter monitoring (Boscia et al., 2000; Frampton, 2001)

A number of studies have also reported PM-induced changes in blood components that may affect the heart. A cohort study conducted during a period that included an episode of unusually high PM levels reported an association between PM and levels of C reactive protein, an indicator of inflammation, tissue damage, and generally related to increased risk of coronary events (Peters et al., 2001). A similar finding of increased C reactive protein was reported in a panel study in the United Kingdom (Seaton et al., 1999). An earlier cohort study observed associations between increased plasma viscosity, associated with increased risk of heart attacks and levels of PM (Peters et al., 1997).

Young healthy volunteers exposed to CAPS have increased levels of blood fibrinogen, a clotting factor which has been reported to be a risk factor for ischemic heart disease (Ghio et al., 2000). Similarly a cohort study reported that people with high concentrations of blood fibrinogen had a higher likelihood of developing adverse cardiovascular outcomes related to PM (Prescott et al., 2000).

These reports of panel studies, controlled human exposure studies, and animal toxicology studies, though small in number, are generally coherent with findings of associations between PM and increased mortality or hospital admissions for cardiovascular diseases. Furthermore, they add support for specific hypotheses regarding the possible mechanisms by which PM exposure may be linked with adverse cardiac outcomes.

SESSION 2: Mechanisms of Cardiovascular Effects of Air Pollution

The epidemiological evidence associating increased cardiovascular morbidity and mortality with air pollution indicates several mechanisms by which air pollution may affect the

cardiovascular system. Possible mechanisms include (but are not exclusive to) neural mechanisms related to the response of the autonomic nervous system through direct reflexes from airways or through inflammatory response, chemical effects on ion channel function in myocardial cells, ischemic responses in the myocardium, or inflammatory responses triggering endothelial dysfunction, atherosclerosis, and thrombosis. The hypothetical pathways involved are depicted in Figure 1, along with some of the potentially measurable mediators.

Determining the mechanisms for the cardiovascular effects of particle exposure requires knowledge of the pathophysiology related to acute cardiac events, such as myocardial infarction, acute coronary syndromes, atherosclerotic plaque formation and rupture, disturbances of cardiac rhythm, and pathophysiology of the autonomic nervous system.

The earliest stages of atherosclerotic vascular disease are characterized by endothelial dysfunction, in which arterioles paradoxically constrict or fail to dilate in response to a physiologic stimulus such as exercise (Quyyumi, 1998). This has been attributed to a relative deficiency in nitric oxide (NO)-mediated smooth muscle relaxation and vasodilation. There may also be an excess of vasoconstrictive factors, such as endothelins and angiotensin II (Warner, 2001). A variety of factors involved in inflammation and infection appear to play a "pro-atherogenic" role through oxidant-mediated induction of the nuclear regulatory factor NF?B, with increased expression of various cytokines and adhesion molecules by endothelial cells and circulating leukocytes. These atherogenic factors include oxidized low-density lipoproteins, lipopolysaccharide, angiotensin II, inflammatory cytokines, and cytomegalovirus. Endothelial function can be measured using the forearm ischemia-hyperemia model, in which forearm vasodilation is measured by ultrasound following brief application of an arm tourniquet. Reduced or absent vasodilation is characteristic of endothelial dysfunction, and is seen in hypertension, hypercholesterolemia, smoking, congestive heart failure, and diabetes. Thus evidence for endothelial dysfunction can be demonstrated before the development of overt coronary artery disease. Genetic polymorphisms in the nitric oxide synthase gene have been associated with hypertension, myocardial infarction, and abnormal endothelial function.

It has become clear that atherosclerosis is an inflammatory disease (Sullivan et al., 2000). Development of the arterial plaque involves the classic mediators of inflammation, including chemotactic factors and inflammatory cells (Gerszten et al., 2000). Monocytes are recruited to sites of endothelial injury and fatty acid deposition, where they ingest oxidized LDL to become "foam cells" characteristic of the maturing plaque. Lymphocytes within the plaque demonstrate activation, with increased expression of the activation marker CD25. These lymphocytes may be responding to specific neo-antigens within the developing plaque. Engagement of CD40 on a variety of cells by CD40 ligand may contribute to cell activation (Laman et al., 1997); plaques are rich in $CD40^+$ cells and in CD40 ligand, and soluble CD40 ligand is increased in the blood of patients during acute coronary syndromes (Phipps, 2000). Polymorphonuclear leukocytes are also present in the lesion, and are thought to contribute to its development through release of reactive oxygen species and proteases. Smooth muscle cell migration causes the plaque to enlarge, but protects it from rupture.

In more advanced stages, the plaque may become destabilized through erosion or fracture of the fibrous cap, or hemorrhage within the plaque. Rupture exposes tissue factor and releases a variety of pro-coagulant substances, leading to adherence of platelets and activation of the coagulation cascade. Circulating pro-coagulant "microparticles" have been demonstrated in advanced atherosclerosis, which may represent apoptotic endothelial cells or platelets.

The autonomic nervous system has important influences on cardiac function in health and disease. These influences can be assessed by measurements of heart rate variability (Stys and Stys, 1998), baroreceptor sensitivity, and microneurography. Sympathetic stimulation shortens the cardiac PR interval, increases Purkinje automaticity, shortens the QT interval, decreases the fibrillation threshold, and increases the risk for T wave alternans. In contrast, vagal stimulation increases electrical stability and decreases the cardiac rate and energy of contraction, but may expose latent ventricular pacemakers. Autonomic imbalance often contributes importantly to the sequence of events culminating in cardiac arrhythmias. "Primary" ventricular fibrillation can occur in the absence of coronary artery disease, and recent findings from genetic testing and autonomous nervous system testing are providing insights into the pathogenesis of this cause of sudden death.

SESSION 3: Methodologies for Investigating Cardiovascular Effects of Air Pollution.

Designing clinical investigations to elucidate the mechanisms discussed above is a challenging task. It is important that such studies test specific cardiovascular hypotheses, rather than measure a collection of unrelated endpoints.

a) Predisposing Factors, Vulnerable Study Populations, and Study Protocols

Consideration of specific study designs must be preceded by recognition of factors contributing to increased morbidity and mortality in general, and by identifying the most vulnerable populations. Cardiovascular morbidity and mortality is influenced by: 1) inborn factors (including genetic predisposition, gender, and age), 2) underlying disease processes (e.g., ischemic heart disease, cardiomyopathy, COPD) or smoldering pathology (ongoing inflammatory process, endothelial dysfunction, increased thrombosis), and 3) environmental factors (e.g., diet, smoking, air pollution, weather). This complexity of conditions potentially leading to increased cardiovascular morbidity and mortality makes it difficult to identify the contribution of air pollution relative to other factors. For this reason, the population to be studied should be relatively homogenous, and the number of subjects large enough to identify potentially small effects of air pollution independently of other clinically relevant factors. Vulnerable subjects in such studies include elderly healthy individuals and patients with underlying cardiovascular or respiratory disorders with appropriate representation by gender and racial or ethnic groups.

Identifying and choosing vulnerable populations in air-pollution-related studies must be paralleled by choice of the exposure system; for example, concentrated ambient air, resuspended ambient air particles, or specific laboratory-generated particles. Using concentrated ambient air has the advantage of representing real-life air pollution conditions, but large variations in its composition and concentration frequently limit the statistical power. Laboratory-generated model particles can be used in clinical studies to test the role of specific particle characteristics and exposure-response relationships.

There is evidence for a time-delay between air pollution exposure and subsequent health effects. Therefore, studies focused on cardiovascular effects of air pollution should take into account this lag phenomenon and include monitoring over time. This should

include studying responses during sleep. Patients with COPD or obstructive sleep apnea, may be more vulnerable to cardiac complications during sleep. Air pollution could alter their susceptibility to cardiac arrhythmias or myocardial ischemia, which could be monitored using Holter ECG recordings.

b) Identifying Optimal Tests for the Study Protocol

A large armamentarium of tests is available to assess specific mechanistic pathways underlying the cardiovascular effects of air pollution. Some advantages and limitations of specific testing are highlighted below.

1. *Autonomic control of the cardiovascular system*

Analysis of heart rate variability (HRV) remains the most frequently used method to evaluate the influence of the autonomic nervous system on the human body (Malik and Camm, 1995). Assessment of baroreflex sensitivity using the phenylephrine test, or baroreflex response using heart rate turbulence and measurement of catecholamine levels, may complement information obtained from HRV analysis. The recent interest in air-pollution studies employing HRV analysis requires a more detailed analysis of this methodology.

The human body remains under the constant influence of both sympathetic and parasympathetic innervation from the autonomic nervous system, and monitoring beat-to-beat changes in heart rate (more precisely in heart period) provides insight into the balance between those two arms of the autonomic system (Schwartz et al., 1992; Malik and Camm, 1995; Tsuji et al., 1996). Various physiologic conditions (e.g., sleep, exercise), disease processes (myocardial infarction, diabetes, COPD), and drugs (beta-blockers, ACE-inhibitors) alter autonomic control of the cardiovascular system, therefore changing HRV parameters. A prolonged ECG recording (at least 5-10 minutes, but usually 24 hours) is needed to obtain full insight into the periodicity of heart rate changes. Variation in heart rate can be measured using time-domain statistical measures of variability and frequency-domain analysis of the power spectrum of the heart rate.

The time-domain HRV parameters, which are relatively stable over time and show good reproducibility, include SDNN (standard deviation of normal-to-normal sinus beat intervals), rMSSD (root mean square of successive differences in NN intervals), and pNN50 (percent of adjacent NN intervals differing by more than 50 ms). They reflect mainly the influence of the parasympathetic system on the heart. Additional time-domain parameters (SDANN and SDNNIX) are less frequently used especially since their physiologic meaning is less understood.

The frequency-domain HRV methods allow for identifying relative contribution of specific frequency bands reflecting oscillatory behavior of heart rate. The total power (TP) of the entire spectrum is usually measured from 0 to 0.40 Hz. Other components include high frequency (HF: 0.15-0.40 Hz), low frequency (LF: 0.04-0.15 Hz), very low frequency (VLF: 0.003-0.04 Hz), and ultra-low frequency (ULF: <0.003 Hz high frequency) bands. The HF power represents parasympathetic (respiratory) modulation of the heart whereas LF power reflects modulation of sympathetic and parasympathetic tone but with strong dominance of sympathetic influence. The physiologic background of VLF

and ULF is not well understood. The LF/HF ratio reflects sympatho-vagal tone. Frequency-domain HRV measures are less stable and less reproducible than time-domain HRV parameters and they also lack standardization of computing methodology. Standardization of protocols and algorithms among centers or centralized core lab analyses may overcome these limitations.

HRV parameters have been shown to be predictive for cardiac death in patients with myocardial infarction, nonischemic cardiomyopathy, diabetes, COPD and in healthy subjects (Zareba et al., 2001a). A few recent studies showed that air pollution may alter HRV parameters. Pope et al. (1999) found that elevation of PM_{10} was associated with increased heart rate and decreased HRV. Gold et al. (2000) showed that an increased level of $PM_{2.5}$ correlated significantly with decreased HRV. However, mechanisms linking inhalation of ambient particles and HRV changes remain unknown and could include airways-related reflexes, inflammatory responses, or both.

As mentioned in Session 2, there are several possible mechanisms linking exposure to particulate matter with changes in the autonomic nervous system. Direct input from the lungs to the autonomic nervous system via pulmonary afferent fibers might contribute to immediate changes in heart rate variability caused by air pollution (Seaton, 1995). This effect could also occur with some delay when an inflammatory process in the airways and lungs triggers signaling in autonomic fibers. Penetration of ultrafine particles or soluble components of particles to myocardial tissue might also contribute to changes in myocardial substrate and to secondary responses of the autonomic nervous system. Those changes could trigger cardiac arrhythmias and aggravate congestive heart failure in susceptible individuals. Sympathetic activation or parasympathetic withdrawal (impaired sympatho-vagal balance) induced by exposure to PM could influence homeostasis of other biological systems, including immunologic responses, the clotting system, and metabolism of glucose and lipids.

Measurement of heart rate turbulence, a novel ECG-based method reflecting response of baroreceptors to changing blood pressure due to ventricular premature beats, could be used in air pollution studies to complement HRV analyses. Similarly, catecholamine levels could be measured in specific protocols, reflecting neurohormonal activation in response to air pollution.

2. Myocardial substrate and vulnerability

Estimation of the ejection fraction and wall motion abnormalities in imaging studies (echocardiography, radionuclide imaging) are routine parameters describing myocardial substrate; however, they have not been used in air pollution studies. It is unlikely that air pollution exposure will lead to detectable changes in myocardial contractility. Nevertheless, with current Doppler technology allowing for noninvasive estimation of pressures (especially in the right ventricle and pulmonary artery) one could systematically evaluate the effects of air pollution on echocardiographic parameters reflecting hemodynamics of the circulatory system.

The ECG provides additional possibilities to evaluate changes in myocardial substrate and vulnerability in response to air pollution (Zareba et al., 2001b). Although the QRS duration and morphology is unlikely to be affected by air pollution, changes in the ST-T complex (and QT interval) duration and

morphology may reflect subtle changes in the state of myocardium. The ST segment monitoring during exercise testing or daily activities using Holter monitoring is broadly used in diagnosing patients with ischemic heart disease, and could be used in studies investigating effects of air pollution on the myocardium. In patients with ischemic heart disease, air pollution could trigger transient ischemia manifested by transient ST segment changes. However, ST segment monitoring probably will be of limited use in subjects with a nonischemic myocardium.

Repolarization duration and morphology are sensitive ECG parameters reflecting subtle changes in myocardial substrate and vulnerability governed by changes in ion channel function. There is vast evidence attesting to the clinical usefulness of QT duration, T wave morphology, QT and T wave variability, and T wave alternans in predicting the risk of sudden death (Zareba et al., 2001a). In particular, T wave alternans, illustrating increased heterogeneity of the action potential duration and repolarization, provides useful inflammation about arrhythmogenic substrate in the myocardium. Beat-to-beat variability of repolarization seems to represent similar phenomena and could be measured noninvasively from Holter recordings (Atiga et al., 1998).

However, these parameters have not been studied extensively in air pollution studies despite several novel signal processing methods allowing for detecting discrete changes in repolarization. Since repolarization parameters like QT duration, T wave amplitude or complexity, and T wave alternans can be affected by various drugs in vulnerable subjects, it is plausible that air pollution might also cause detectable changes in repolarization parameters. However, the mechanisms by which air pollution could affect ion channel function are unknown.

Frequency and complexity of ventricular premature beats are the oldest and the most standard measures of myocardial vulnerability. In particular, the presence of nonsustained ventricular tachycardia in patients with ischemic myocardium is considered an important harbinger of life-threatening cardiac arrhythmias. The association between increased air pollution and arrhythmia vulnerability has been demonstrated in the study of Peters et al. (2000) which included the analyses of arrhythmic episodes recorded by implantable cardioverter-defibrillators. Animal studies further support this clinical observation, indicating that arrhythmia monitoring should be included in the battery of tests aiming to determine deleterious effects of air pollution. Since cardiac arrhythmias are highly variable findings, prolonged ECG recordings should be performed in large numbers of studied subjects. Studying patients with implantable monitoring devices provides a unique opportunity to evaluate the risk.

3. *Endothelial function, atherosclerosis, thrombosis*

Acute coronary syndromes (including unstable angina, acute myocardial infarction, and sudden cardiac death) usually occur as a result of thrombus formation in the site of a ruptured atherosclerotic plaque (Maseri, 1997; Ross, 1999). Increased platelet aggregability and blood viscosity, and reduced fibrinolytic activity together with endothelial dysfunction promote a pro-coagulant state, increased inflammation, and lipid infiltration of the vessel wall. In a recent study by Peters et al. (2001) increased air pollution was found to be associated with increased risk of acute myocardial infarction, although the

mechanism underlying this association remains unknown. Increased blood viscosity in response to air pollution already identified in an epidemiological study by Peters et al. (1997) is one of the potential factors, but other mechanisms are likely to contribute to air-pollution-related changes in vascular function and structure. Platelet aggregability and several procoagulant factors (e.g., Factor VII, von Willebrand Factor, PAI-1, D-dimer) are measured in currently ongoing studies investigating effects of air pollution on thrombosis. There is no systematic analysis of levels of atherogenic lipids in air pollution studies, although based on smoking-related observations there is a justification to include lipid profiles in an armamentarium of tests included in such studies. Ongoing inflammatory processes have been shown to contribute to an increased risk of coronary artery disease, and high-sensitivity tests for C-reactive protein (CRP) and serum amyloid A (SAA) are used in current studies investigating mechanism of air pollution effects on biological systems. Quantifying levels of cytokines and interleukins may further elucidate the potential contribution of infection and inflammatory processes underlying responses to air pollution. The inflammatory process triggered by air pollution might affect the airways, but also may impact the vascular bed or the immune response.

Endothelial dysfunction may contribute to myocardial ischemia in patients without acute coronary syndromes and critical narrowing of coronary vessels (Quyyumi, 1998). Otherwise healthy subjects may have episodes of myocardial ischemia (silent or associated with chest pain) due to changes in coronary vasomotor tone leading to decreased coronary blood flow. The vascular endothelium secretes multiple factors that control vascular tone, modulate platelet activity, influence thrombogenicity, and contribute to vascular inflammation, cell migration and proliferation. These factors influence the progression of atherosclerosis. Levels of some of the factors can be measured directly in clinical studies (e.g. vasodilating prostacyclin or vasoconstricting endothelin). However, overall measurement of endothelial function using the forearm ischemia-hyperemia model, in which forearm vasodilation is measured by ultrasound following brief application of an arm tourniquet, could be recommended as clinically applicable for air pollution studies.

Future studies may also include investigating genetic predisposition to specific biological responses to air pollution. For example, polymorphisms in genes coding for the beta-2 adrenergic receptor or angiotensin converting enzyme have been associated with increased risk of cardiovascular disease, asthma, and altered responses to some medications. It is possible that polymorphisms in these and other genes might predispose to increased vulnerability to air pollution.

It is worth emphasizing that the large biological variability of many of the parameters discussed above may compromise the conclusiveness of studies, especially those with small sample sizes. In addition, several of the variables are non-specific and it is difficult to attribute their changes directly to the effects of air pollution. Drugs taken by study subjects may substantially modulate the results of some tests, as is the case with beta-blockers influencing heart rate variability parameters or lipid lowering drugs affecting endothelial function. These and several other factors have to be taken into account when designing a study aiming to elucidate cardiovascular effects of air pollution. Multicenter studies employing common

protocols, with core laboratories facilitating central analysis of tested parameters, might accomplish much more than a multitude of small independent studies.

What have we learned about the relationship between PM exposure and cardiovascular disease, and where should we go from here? The epidemiological studies suggest there are long term or chronic effects of PM exposure as well as acute effects, and it is possible that the long term burden of PM exposure may induce or cause more rapid progression of chronic cardiovascular disease, via direct or indirect effects. As shown in Figure 2, PM exposure may enhance progression of factors leading to chronic cardiovascular disease, while at the same time short-term elevations in PM may precipitate acute events in patients already susceptible from underlying disease. Research strategies are being developed to test these hypotheses.

SUMMARY OF RESEARCH RECOMMENDATIONS:

There was agreement among the participants that epidemiologic studies have consistently linked particles with adverse cardiovascular health effects, although the mechanisms of the presumed pathogenic process is just beginning to be defined. Participants were unanimous in recommending a broad-based approach to studying cardiovascular health effects of particulate matter. Basic immunologic, physiologic, molecular, genetic, and cellular studies; epidemiologic investigations; animal models; and controlled human exposures are all important parts of the research armamentarium.

Therefore, it is recommended that research on the adverse cardiovascular effects of particulate matter, particularly among individuals with cardiovascular diseases, be expanded. Based on existing epidemiologic and experimental data, mechanistic considerations should focus on alterations in the autonomic nervous system; ischemic responses in the myocardium; chemical effects on ion channel function in myocardial cells; and inflammatory responses triggering endothelial dysfunction, atherosclerosis, and thrombosis (Figure 1).

Intensive investigations are ongoing in animals to explain the epidemiologic findings. The potential role of rodents with targeted gene disruptions in better understanding the mechanisms of cardiovascular responses to particulate matter was emphasized. It may be possible in the future to determine genetic markers or define genetic polymorphisms that place individuals at high risk for particle-induced effects.

Finally, human clinical studies have become increasingly sophisticated and have access to a wide array of new investigative tools (Table 1). There was a great deal of discussion about some of the parameters, such as heart rate variability, as a marker for assessing potential cardiovascular effects of CAPs or laboratory generated particles. While HRV was recognized as an attractive study parameter because of the relative ease of measurement in the time domain, there remain important questions regarding the long-term clinical significance of changes in HRV in response to interventions such as CAPs. These uncertainties become compounded when one resorts to frequency domain analysis of HRV, which can lead to quite variable results depending upon how extrasystoles and signal artifact are handled in the computational algorithms. Such issues led to recommendations to either standardize the algorithms utilized or rely on a core laboratory that in effect provides such standardization. The success of air

pollution studies has always required involvement of multi-disciplinary teams; given the recent exciting cardiovascular observations, it is clear that these teams need to be expanded to include investigators in cardiovascular biology and medicine.

Acknowledgments:

The conference was supported in part by the University of Rochester EPA PM Center, EPA Center Grant #R827354. The authors acknowledge all the participants for their candid discussions throughout the meeting. We particularly thank Mike Terry for his administrative assistance and Ann Bauman for help with the manuscript.

Atiga, W., Calkins, H., Lawrence, J., Tomaselli, G., Smith, J., and Berger, R. 1998. Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. J. Cardiovasc. Electrophysiol. 9:899-908.

Boscia, J. A., Chalupa, D., Utell, M. J., Zareba, W., Konecki, J. A., Morrow, P. E., Gibb, F. R., Oberdörster, G., Azadniv, M., Frasier, L. M., Speers, D. M. and Frampton, M. W. 2000. Airway and cardiovascular effects of inhaled ultrafine carbon particles in resting healthy nonsmoking adults. Am. J. Respir. Crit. Care Med. A:239.

Devlin, R. B., Cascio, W., Kerhl, H., and Ghio, A. 2000. Changes in heart rate variability in young and elderly humans exposed to concentrated ambient air particles. Am. J. Respir. Crit. Care Med. 161:A239.

Frampton, M. W., 2001. Systemic and cardiovascular effects of airway injury and inflammation: Ultrafine particle exposure in humans. Environ. Health Perspect. 109(Suppl. 4):529-532.

Gerszten, R. E., Mach, F., Sauty, A., Rosenzweig, A., and Luster, A. D. 2000. Chemokines, leukocytes, and atherosclerosis. J. Lab. Clin. Med. 136:87-92.

Ghio, A. J., Kim, C., and Devlin, R. B. 2000. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. Am. J. Respir. Crit. Care Med. 162:981-988.

Godleski, J. J., Verrier, R. L., Koutrakis, P., and Catalano, P. 2000. Mechanisms of morbidity and mortality from exposure to ambient air particles. Res. Report Health Eff. Inst. 91:5-88.

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(11):1267-1273.

Kodavanti, U. P., Schladweiler, M. C., Richards, J. R. and Costa, D. L. 2001. Acute lung injury from intratracheal exposure to fugitive residual oil fly ash and its constituent metals in normo- and spontaneously hypertensive rats. Inhalation Toxicol. 13(1):37-54

Laman, J. D., de Smet, B. J., Schoneveld, A., and van Meurs, M. 1997. CD40-CD40L interactions in atherosclerosis. Immunol. Today. 18:272-7.

Liao, D., Greason, J., Shy, C., Williams, R., Watts, R. and Zweidinger, R. 1999. Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. Environ. Health Perspect. 107:521-525.

Malik, M., and Camm, A. J. 1995. Heart Rate Variability. Armonk, New York: Futura Publishing Company.

Maseri, A. 1997. Inflammation, artherosclerosis, and ischemic events: Exploring the hidden side of the moon. N. Engl. J. Med. 336:1014-1016.

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.

Peters, A., Döring, A., Wichmann, H-E., and Koenig, W. 1997. Increased plasma viscosity during an air pollution episode: A link to mortality? Lancet 349:1582-1587.

Peters, A., Fröhlich, O., Döring, A., Immervoll, T. Wichmann, H-E., Hutchinson, W. Pepys, M., and Koenig, J. 2001. Particulate air pollution is associated with an acute phase response in men. Results for the MONICA-Augsburg Study. Eur. Heart J. 22:1198-1204.

Peters, A., Liu, E. Verrier, R. L., Schwartz, J., Gold, D. R., Mittleman, M., Baliff, J., Oh, J. A., Allen, G., Monahan, K., and Dockery, D. W. 2000. Air Pollution and incidence of cardiac arrhythmia. Epidemiology 11:11-17.

Phipps, R. P. 2000. Atherosclerosis: the emerging role of inflammation and the CD40-CD40 ligand system. Proc. Natl. Acad. Sci. U S A. 97:6930-6932.

Pope, C. A., III, Dockery, D. W., Kanner, R. E., Villegas, G. M., and Schwartz, J. 1999a. Oxygen saturation, pulse rate, and particulate air pollution: A daily time-series panel study. Am. J. Respir. Crit. Care Med. 159:365-372.

Pope, C. A., III, Verrier, R. L., Lovett, E. G., Larson, A. C., Raizenne, M. E., Kanner, R. E., Schwartz, J., Villegas, G. M., Gold, D. R., and Dockery, D. W. 1999b. Heart rate variability associated with particulate air pollution. Am. Heart J. 138:890-899.

Pope, C. A., III. 2000. Epidemiology of fine particulate air pollution and human health: Biologic mechanisms and who's at risk. Environ. Health Perspect. 108(Suppl 4):713-723

Prescott, G. J., Lee, R. J., Cohen, G. R., Elton, R. H., Lee. A. J., Fowkes, F. G., and Agius, R. M. 2000. Investigation of factors which might indicate susceptibility to particulate air pollution. Occup. And Environ. Med. 57(1):53-57.

Quyyumi, A. A. (1998). Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. Am. J. Med. 105:32S-39S.

Ross, R., 1999. Atherosclerosis; an inflammatory disease. N. Engl. J. Med. 340:115-126.

Schwartz, P., La Rovere, M., and Vanoli, E. 1992. Autonomic nervous system and sudden cardiac death. Circulation 85(Suppl. 1):77-91.

Seaton, A. 1995. Particulate air pollution and acute health effects. Lancet 345:176-178.

Seaton, A., Soutar, A., Crawford, V., Elton, R., McNerlan, S. Cherrie, J., Watt, M., Agius, R., and Stout, R. 1999. Particulate air pollution and the blood. Thorax 54:1027-1032.

Stys, A. and Stys, T. (1998). Current clinical applications of heart rate variability. Clin. Cardiol. 21:719-724.

Sullivan, G. W., Sarembock, I. J., and Linden, J. (2000). The role of inflammation in vascular diseases. J. Leukoc. Biol. 67:591-602.

Tsuji, H., Larson, M. G., Venditti, F. J., Manders, E. S., Evans, J. C., Feldmann, C. L. and Levy, D. 1996. Impact of reduced heart rate variability on risk of cardiac events. The Framingham heart study. Circulation 94:2850-2855.

Warner, T. D. (2001). Endothelin and its inhibitors, Springer, Berlin.

Watkinson, W. P., Campen, M. J., Costa, D. L. 1998. Cardiac arrhythmia induction after exposure to residual oil fly ash particles in a rodent model of pulmonary hypertension. Toxicol. Sci. 41:209-216.

Zareba, W., Locati, E. and Maison-Blanche P. (eds.) 2001a. Noninvasive Electrocardiology in Clinical Practice. Armonk, New York: Futura Publishing Company.

Zareba, W., Nomura, A., and Couderc, J. P. 2000b. Cardiovascular effects of air pollution: What to measure in ECG? Environ. Health Perspect. 109(Suppl. 4):533-538.

Figure Legends

Figure 1. Complexity of potential pathophysiologic pathways for cardiovascular effects of particle inhalation. Examples of potential experimental markers are shown in parentheses. CRP: C-reactive protein; IL-6: interleukin-6; IL-8: interleukin-8; vWF: von-Willebrand factor; iNOS: inducible nitric oxide synthase; V_{NO}: airway production of nitric oxide.

Figure 2. Mechanisms for acute and chronic cardiovascular disease related to particulate matter exposure.

APPENDIX I: Conference Participants

Conference Organizers

Dr. Robert Devlin – U.S. Environmental Protection Agency
Dr. Mark W. Frampton – University of Rochester Medical Center
Dr. Mark J. Utell – University of Rochester Medical Center
Dr. Wojciech Zareba – University of Rochester Medical Center

Speaker and Panelists

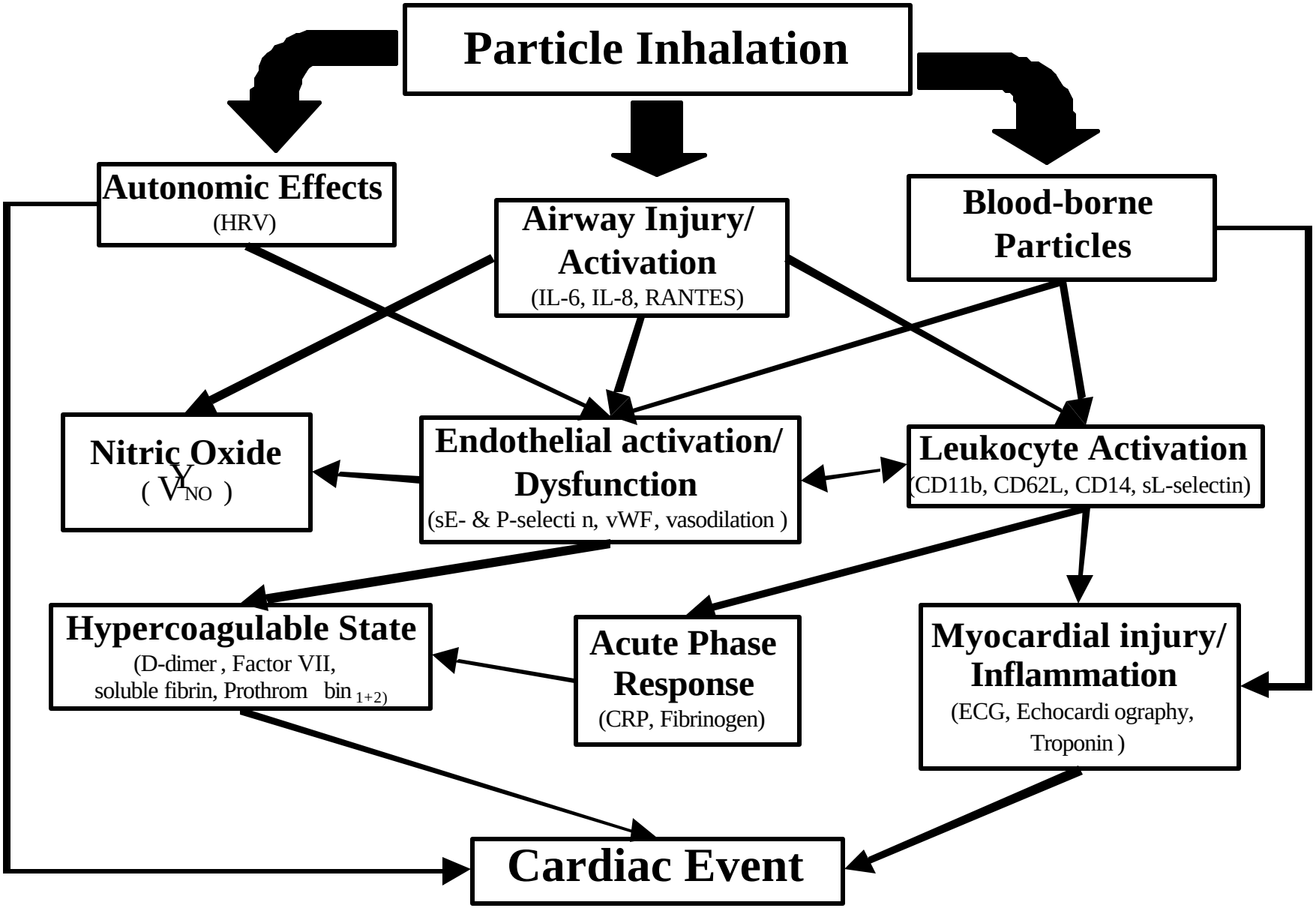
Dr. Bradford Berk – University of Rochester Medical Center
Dr. Wayne Cascio – University of North Carolina – Chapel Hill
Dr. Jean-Phillipe Couderc – University of Rochester Medical Center
Dr. Christopher Cox – University of Rochester Medical Center
Dr. Kevin Driscoll – Proctor and Gamble Company
Dr. John Godleski – Harvard School of Public Health
Dr. Diane Gold – Harvard Medical School
Dr. Henry Gong, Jr. – University of Southern California
Dr. Joel Kaufman – University of Washington Medical Center
Dr. Paul Kligfield – Cornell Medical Center
Dr. Michael Lehmann – University of Michigan Medical Center
Dr. Christine Nadziejko – New York University School of Medicine
Dr. Richard Phipps – University of Rochester Medical Center
Dr. Arden Pope – Brigham Young University
Dr. Arshed Quyyumi – NIH, National Heart Blood and Lung Institute
Dr. Richard Verrier – Harvard Medical School
Dr. Penn Watkinson – U.S. Environmental Protection Agency

Table 1.

Testing Recommended for Analysis in Studies Investigating the Effects of Air Pollution on the Cardiovascular System

Tests	Source of the Data	Assessed Mechanism
Heart rate variability	ECG	Autonomic nervous system
Heart rate turbulence	ECG	Baroreflex sensitivity
ST segment monitoring	ECG	Ischemic myocardium
QT and T wave morphology	ECG	Myocardial Substrate
T wave alternans and variability	ECG	Myocardial vulnerability
Cardiac arrhythmias	ECG, EGM	Myocardial vulnerability
Clotting factors, platelet function Blood viscosity	blood	Thrombosis
CRP, SAA, cytokines, interleukins	blood	Inflammatory process
Lipid profile	blood	Atherogenic potential
Forearm blood flow	USG	Endothelial function
Pulmonary artery pressures RV and LV function	ECHO	Hemodynamic changes

Definitions of Abbreviations:
ECG – includes standard, exercise, and Holter electrocardiographic recordings
EGM – electrocardiograms obtained from implantable devices
ECHO – echocardiographic evaluation
USG – Ultrasonographic evaluation


Particle Inhalation
Autonomic Effects
(HRV)
Airway Injury/
Activation
(IL-6, IL-8, RANTES)
Blood-borne
Particles
Nitric Oxide
(V_{NO})
Endothelial activation/
Dysfunction
(sE- & P-selecti n, vWF, vasodilation)
Leukocyte Activation
(CD11b, CD62L, CD14, sL-selectin)
Hypercoagulable State
(D-dimer , Factor VII,
soluble fibrin, Prothrom bin $_{1+2)}$
Acute Phase
Response
(CRP, Fibrinogen)
Myocardial injury/
Inflammation
(ECG, Echocardi ography,
Troponin)
Cardiac Event

PM Exposure and Cardiac Effects