The Role of Environmental Agents in Cardiovascular Disease

August 6 and 7, 2002

Durham, NC

Workshop Summary

Sponsored by:
The National Institute of Environmental Health Sciences
The US Environmental Protection Agency
The National Heart, Lung, and Blood Institute
The American Heart Association's Council on Epidemiology and Prevention and Expert Panel on Population and Prevention Science
St. Jude Medical, Inc.

Cardiovascular disease represents the primary source of mortality in the industrialized world. According to the American Heart Association, 958,775 people died of cardiovascular disease (CVD) in 1999. This represents a death rate of 354.1 per 100,000 or 40% of all deaths. The rate was higher among blacks than whites. Coronary heart disease (CHD) accounted for 55% of the total death rate. The major risk factors include lifestyle (such as smoking, physical inactivity, serum lipids, and diet) and family history. Recent data, however, have established that exposure to environmental agents is also a risk factor. For instance, numerous epidemiologic studies have shown associations between exposure to ambient particulate matter (PM) and CVD mortality. Studies have also shown associations between PM exposure and pathophysiologic changes that are associated with CVD, such as heart rate variability (HRV) and changes in blood parameters. Cardiovascular malformations also represent an important public health concern. The March of Dimes estimates that one in every 125 to 150 infants is born with a heart defect, which is the most common form of birth defect. Maternal diet (especially deficiency in folic acid intake) has been associated with risk of birth defects, but the causes of most of these defects are not known, although there is evidence of an association between environmental exposures (to air pollution or solvents, for instance) and cardiovascular malformations.

Clearly, many questions remain as to the role that environmental agents play in CVD. The timely advances in the fields of signal transduction, genomics, proteomics, molecular and cellular biology, and epidemiology are complementing traditional investigational methods, thereby expanding research efforts to fill these gaps in knowledge. Some environmental health science and cardiovascular researchers have successfully bridged the gap between these disciplines, resulting in innovative approaches to the study of environmentally induced CVD. Therefore, enhanced collaboration between these disciplines is seen as vital to the success of these efforts.

On August 6 and 7, a workshop entitled, “The Role of Environmental Agents in Cardiovascular Disease” was held at the Durham Marriott at the Civic Center, in Durham, NC. The workshop was sponsored by the NIEHS and EPA who each provided funding and personnel effort, and by NHLBI and the American Heart Association Council on Epidemiology and Prevention and Expert Panel on Population and Prevention Science. In addition, St. Jude Medical provided funds for breaks. There were two main purposes of the workshop. The first was to bring together scientists from the environmental health science and cardiovascular research communities to discuss research questions and needs in the area of environmentally related cardiovascular disease, and to discuss what is needed to address these needs. The second purpose was to assess the need for interdisciplinary collaboration and to identify areas of potential collaboration between environmental health scientists and cardiovascular researchers.

The workshop was put together by an Organizing Committee consisting of representatives from NIEHS, EPA, and NHLBI, environmental health researchers with expertise in cardiovascular research, and cardiologists with research experience in environmental health. The list of the Organizing Committee members is included in Appendix 1.

The format of the workshop was a day-and-a-half program, consisting of a session of keynote speakers (to provide background on the topic and to help orient the diverse group), a session of breakout groups, and a final session to review the findings and recommendations of the breakout

groups. A final agenda for the workshop is included in Appendix 2. The main product of the workshop was to be ideas and recommendations developed by the breakout groups.

The Organizing Committee identified six topics for breakout sessions:

Session 1: *Cardiovascular Epidemiology: Risk Factors from Environmental and Occupational Exposure*

Session 2: *Particulate Air Pollution and Myocardial Infarction*

Session 3: *Environmental Stress and Vascular Disease*

Session 4: *Environmental Modulation of Myocardial Excitability*

Session 5: *Cardiovascular Oxidative Stress and Environmental Pollutants*

Session 6: *Environmental Toxicity and Cardiovascular Development*

A list of the breakout sessions, along with the moderators and other invited participants is included in Appendix 3.

The workshop was well attended, with approximately 115 people, including invited participants and those that attended on their own. The size of the breakout session groups ranged from about 30 in Session 1 to 11 in Session 4. At the end of the breakout sessions, each group was asked to prepare a list of areas of emphasis and recommendations to be presented to the workshop attendees as a whole the next morning (August 7).

The intent of the workshop was to cover as much of the topic of environmentally related cardiovascular disease as possible, looking not only at air pollution as a toxic agent, but at other agents as well. However, due to the extensive and rapidly growing body of data and the interests of many of the participants, issues related to exposure to air pollution, especially particulate matter, were discussed more than others. Likewise, because the topic of Session 6 was somewhat different from the other breakout sessions, dealing with prenatal exposures and diseases affecting primarily children, some of the recommendations were not very applicable to other sessions, although many clearly were.

Participants in the workshops were provided with lists of questions and issues as guides for discussion and were asked to address those they thought were most important, as well as other questions they saw as important but that were not on the lists. Although some questions that were discussed were specific to the breakout groups in which they were addressed, there were many questions and issues that were raised and discussed by several groups. The most commonly discussed of these are included below, along with some specific points. A summary of discussions of models, methodologies, and research recommendations for addressing these issues will follow that. The observations and recommendations of the individual breakout

groups is included in Appendix 4.

1. What environmental agents are associated with cardiovascular disease? Although certain environmental exposures, such as PM, have been clearly shown to be associated with CVD, there are other diseases, such as cardiac malformations, for which an environmental etiology is suggested, but little information on particular agents exists. Related to this is the question of what characteristics of environmental agents, such as chemical composition or physical characteristics (e.g., particle size), are most associated with CVD. This is of special interest for complex mixtures like air pollution. In the case of cardiovascular tetratogenesis, very few environmental agents have been linked with malformations, although there is evidence of an association with exposure.

2. What factors make individuals more susceptible to the effects of environmental agents? Research data have indicated that there are factors that make certain populations or members of a population more susceptible, such as age, gender, race, ethnicity, and socioeconomic status. The role of genetics in environmentally related CVD was also discussed, not only because of the family history risk factor for CVD, but because of the recognition of the importance of gene-environmental interactions in environmental health. Likewise, there is data to suggest that diseases, such as pre-existing cardiovascular or lung disease and diabetes, might also predispose individuals to the toxic effects of environmental agents. What other conditions might put a person at risk? Are young, healthy adults at risk from exposure to environmental agents?

3. What factors might interact with environmental exposure to increase the risk of CVD, or put another way, what confounding factors must be considered in designing research studies? Such factors might include co-exposures, such as exposures to gaseous pollutants along with PM, and lifestyle characteristics, such as diet, smoking history, and level of physical activity – things considered as "traditional" cardiovascular risk factors.

4. What diseases of the cardiovascular system are associated with exposure? Abundant data support, for instance, an association between PM and CVDs, and some data indicate which type, e.g., myocardial infarction. More data are needed, however, to better characterize the diseases and disease processes. For instance, if exposure to air pollution is associated with increased risk of arrhythmias, what type or types of arrhythmias? What are the target tissues for various environmental agents, for instance, myocardial versus vascular tissue, or the effects on different vascular beds? And related to this, what endpoints should researchers be looking at to identify and study these diseases, both in human and in animal studies? Another question is whether exposure to environmental agents is associated with chronic CVD, such as atherosclerosis, and if so, are the mechanisms the same as for the acute effects? What types of cardiovascular malformations might be induced by environmental exposures? The discussions of this issue were greatly facilitated by the presence of participants whose backgrounds were in cardiovascular research.

5. By what mechanisms do environmental agents cause or contribute to CVD; what are

their modes of actions? Epidemiologic evidence of an association between certain environmental agents, most notably PM and CVD is strong, but there are still large gaps in information about the toxicologic mechanisms. As a result, all of the breakout groups spent time discussing this topic. Numerous potential mechanisms were discussed, including those for which good data exists, such as oxidative stress and other inflammatory processes and neurogenic mechanisms, and others which are more speculative, such as alterations in cardiomyocyte membrane ion channels, alterations in endothelial function, and gene expression changes. Questions of how environmental agents might directly affect cardiovascular tissues, as opposed to indirectly via the central nervous system or systemic distribution of inflammatory mediators, were raised. Related to the issue of mechanisms was the fate of environmental agents in the body and this affects toxicity? For instance, can PM be transported to cardiac or vascular tissue where it might directly harm these tissues? What is the role of metabolism of organic pollutants in their toxicities?

The discussion of mechanisms was somewhat different in Session 6, "Environmental Toxicity and Cardiovascular Development," due to the nature of the science. The discussions were more focused on molecular mechanisms than in some of the other sessions, and included molecular targets, such as genes involved in development and growth of the components of the heart and outflow tract. Most participants in Session 6 agreed that, while numerous development genes have been identified, very few have been studied as targets of toxicologic agents, and that this represents an important and fertile area for future research.

The participants in the breakout sessions also discussed the types of studies that were needed to address some of these questions and issues, as well as the models and methodologies that could be used. Some of these are already being used in some form in environmental CVD research, while others are used in other areas of CVD research but not yet in the environment health area.

Human Studies

Although epidemiology has been the driving force in some areas of environmental CVD, especially in the PM field, the need for continued epidemiologic research was universally recognized. Such studies are needed to identify potential cardiovascular toxicants and to identify components of mixtures (such as air pollutants), to identify susceptible populations and risk factors which might interact with environmental exposures, and to provide information on potential mechanisms of environmentally related CVD. The need for more prospective studies was discussed, and the recommendation was made to try to work with on going studies, such as the NHANES CARDIA, and ARIC studies, to leverage funding. Community surveillance and community intervention studies were also recommended. Several groups noted a strong need for further research to identify and validate biomarkers of exposure and effect, to better classify exposure. The groups also recommended expanding the diseases studied to include, in addition to sudden cardiac death and myocardial infarct, congestive heart disease, stroke, peripheral vascular disease, cardiomyopathy, and congenital heart disease. They also recommended expanding the types of evaluations, including for subclinical endpoints, (beyond heart rate

variability) to include such things as measurements of intima-media thickness, markers of inflammation, endothelial function, and imaging techniques (e.g., MRI and echocardiography). A particular need for better death certificate classification and surveillance of disease outcomes was noted. Further international studies were recommended, and studies of occupational exposures were also seen as ways of studying exposure-related CVD in a situation of higher, more quantifiable exposure.

One area of controversy was the need for genetic epidemiological studies. Some participants felt that these studies are needed to address the role of genetics in susceptibility to environmental agents, while others argued that not enough is known about the role of environmental agents in these diseases to begin that work yet.

In addition to epidemiology, more studies of controlled human exposures are needed, for helping to identify physiologic mechanisms and for determining the role of pre-existing disease. Recommendations included the inclusion of asymptomatic cigarette smokers, hyperlipidemia and hypertensive patients, and if possible the study of genetic markers in research subjects. As with epidemiologic studies, the list of methodologies employed in these studies should be expanded. The expertise of the cardiologists and cardiovascular research was especially beneficial in identifying some of these methodologies.

Animal Studies

The importance of animal research in understanding the mechanisms of environmentally related CVD, in identifying environmental agents and components of environmental mixtures associated with CVD, and in determining susceptibility factors (especially pre-existing disease) was universally recognized. The presence of cardiovascular researchers in the sessions broadened the discussions of animal models and methodologies that could be of potential benefit in this field. Genetically manipulated models, such as ApoE and K/ATP channel knockouts and tissue-specific transgenics, and "natural" animal models, such as spontaneously hypertensive rats and diabetic mice, should be used to study genetic influences on CVD and the effect of pre-existing disease processes in the predisposition to toxic effects of environmental agents. Occlusion / ischemia models, including models of healed infarcts, have been used and should continue to be used as models of pre-existing disease in humans. The benefits of using large animal (e.g., pig) models in this kind of research were discussed. Denervation models can also be used for studying the effects of the central nervous system on system responses. Several groups also recommended studies to try and identify environmental agents (e.g., particulate matter) in cardiovascular tissues, such as the myocardium and atherosclerotic plaques, of exposed animals (as well as in human tissue samples, when available.)

Animal models were discussed at length in Session 6. The advantages and disadvantages of different mammalian and non-mammalian models, such as chick, frog, and zebrafish, were considered, such as the speed of development, the ease of making transgenics, and the similarity to human cardiovascular development. Knockout and transgenic animals are used extensively in the study of the normal development of the cardiovascular system, and many can be applied to teratogenesis studies. Animal models, based on solid mechanistic data, could also be used for less expensive and more efficient detection of tetratogens.

In vitro studies

In vitro studies are important in determining cellular and molecular mechanisms of environmentally related CVD. Models that might be of value in these kinds of studies include isolated, perfused heart models (Langendorf models), cultured myocytes, and isolated blood vessels.

In addition to those mentioned above, a number of other recommendations for future research were made. For instance, more research is needed on the chronic effects of air pollutants. To date most work has involved acute cardiovascular conditions, e.g., myocardial infarct and sudden cardiac death. The effects of exposure on atherosclerosis, for instance, needs further study. Several groups also pointed out the need for coordination and standardization of research efforts. For instance, there is a strong need for standard PM mixtures that can be made available to researchers. Likewise, standardization of methodologies that would allow study-to-study comparisons is important.

Other Recommendations

The most important recommendations were more general in nature.

> First, it was universally agreed that further researcher in the area of environmentally related cardiovascular disease is critical. This was evident not only in the recommendations by the breakout groups, but in the level of participation and the general enthusiasm of the attendees.
>
> Second, it was clear that a collaborative multidisciplinary approach is required to tackle these difficult questions, and that the inclusion of cardiovascular researchers and cardiologists in the discussions greatly expanded the range of discussions beyond what would be possible if they hadn't been there. Several environmental health scientists commented on the value of having this mixed group. Some participants also commented that studying the cardiovascular effects and mechanisms of environmental agents would also help to understand the basic mechanisms of cardiovascular disease. Potential collaborations were discussed among several attendees.
>
> Finally, the groups pointed out the need for funding support for these expanded activities, and strongly encouraged NIH and EPA to make more funding available for this research.

In summary, as gauged by the level of discussion, the enthusiasm of the attendees, and the feedback received by the Organizing Committee, the workshop was very successful and the need to continue work in this area is important. Numerous research issues were identified and recommendations for addressing these issues and expanding the list of currently used models and methodologies were made. Given that cardiovascular disease is the leading cause of death in the Western world and that clear links have been made between environmental exposures and CVD, it is important for NIH and EPA to continue, and to expand, its support of this research, and to engage in interagency coordination to leverage funds, reduce duplication of efforts, and assure that the important research issues are adequately addressed. Initiatives from governmental

agencies should try to insure that cross-disciplinary collaborations are encouraged and that innovative, state-of-the-art research is supported.

In meetings after the workshop, the Organizing Committee agreed that it is important to maintain the momentum that was generated at the workshop. The Committee recommended that a brief summary article be submitted to a high impact journal that is read by researchers in the cardiovascular field, and the editor of Circulation has been contacted about publishing such an article. In addition, the committee plans to publish a more in-depth summary of the workshop, perhaps in Environmental Health Perspectives. And finally, because the workshop was fairly short and covered a wide range of issues, the Committee proposes to try to organize or be involved in smaller meetings or symposia in the next one to two years that focus on narrower topics. One format that was suggested was smaller, more focused workshops that involve fewer attendees. These could perhaps be held at a university and cosponsored by NIH and / or EPA and the university. Drs. Bhatnagar and Cascio raised the possibility of having such workshops at their institutions. Another proposed approach is to organize and sponsor symposia at national meetings, such as the American Chemical Society, the Society of Toxicology, or the American Heart Association meetings in 2004.

Appendix 1

Organizing Committee

Aruni Bhatnagar, Ph.D.
University of Louisville

Wayne Cascio, M.D.
University of North Carolina

Robert Devlin, Ph.D.
U.S. Environmental Protection Agency

John Godleski, M.D.
Harvard University

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U.S. Environmental Protection Agency

J. Patrick Mastin, Ph.D.
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Murray Mittleman, M.D., Dr.P.H.
Beth Israel Deaconess Medical Center

Kenneth S. Ramos, Ph.D.
Texas A&M University

Gail Robarge, M.S.
U.S. Environmental Protection Agency

Eser Tolunay, Ph.D.
National Heart Lung and Blood Institute

Appendix 2

Agenda

August 6: *Morning*

8:15 - 8:45	**Registration**
8:45 - 9:00	**Opening** Dr. J. Patrick Mastin Program Administrator, NIEHS
9:00 - 9:15	**NIEHS Welcome** Dr. Anne Sassaman Director, Division of Extramural Research and Training, NIEHS
9:15 - 10:00	**Cardiovascular Effects Associated with Environmental Pollutants: A Pulmonologist's Prospective** Dr. Mark Utell Department of Medicine, University of Rochester
10:00 - 10:15	**Break**
10:15 - 11:00	**Molecular Insights into Redox-Regulated Transcription: Implications in Atherogenesis** Dr. Kenneth Ramos Department of Veterinary Physiology & Pharmacology Texas A&M University
11:00 - 11:30	**Particulate Matter Research: Building the Science Base for Regulatory Decisions** Dr. Paul Gilman Assistant Administrator for Research and Development, US. EPA

- Lunch -

August 6: *Afternoon*

1:00 - 5:30	**Break out Sessions** (with break as appropriate, around 2:30)

August 7: *Morning*

8:30 - 8:45	**Introduction**
8:45 - 12:00	**Presentations from Breakout Groups**
12:00 - 12:30	**Wrap up** Dr. Aruni Bhatnagar, University of Louisville

Appendix 3

Breakout Sessions

Session 1 - *Cardiovascular Epidemiology: Risk Factors from Environmental and Occupational Exposure*
Arden Pope - moderator (Brigham Young University)
Russell Luepker - moderator(University of Minnesota)
Robert Goldberg (University of Massachusetts)
Kim Gray (NIEHS)
David Siscovick (University of Washington)
Joel Schwartz (Harvard University)

Session 2 - *Particulate Air Pollution and Myocardial Infarction*
Robert Kloner - moderator (Good Samaritan Hospital, University of Southern California)
Robert Devlin - moderator (EPA)
Philip Adamson (University of Oklahoma)
Doug Dockery (Harvard University)
Murray Mittleman (Beth Israel Deaconess Medical Center)
Mark Utell (University of Rochester)

Session 3 - *Environmental Agents and Vascular Disease*
Ken Ramos - moderator (Texas A&M University)
Eser Tolunay - moderator (NHLBI)
Paul Boor (University of Texas Medical Branch)
Mark Frampton (University of Rochester)
Emily Wilson (Texas A&M)
David Gutterman (Med. College of Wisconsin)

Session 4 - *Environmental Modulation of Myocardial Dysfunction*
Wayne Cascio - moderator (University of North Carolina)
Aruni Bhatnagar - moderator (University of Louisville)
Penelope Boyden (Columbia University)
Robert Lux (University of Utah)
Richard Verrier (Harvard University)
Wojciech Zareba (University of Rochester)

Session 5 - *Cardiovascular Oxidative Stress, Environmental Pollutants*
Dan Costa - moderator (EPA)
Elizabeth Murphy - moderator (NIEHS)
Paige Anderson (Duke University)
Scott Ballinger (University of Texas Medical Branch)
Kevin Driscoll (Proctor and Gamble)
John Godleski (Harvard University)
James Kang (University of Louisville)

Session 6 - *Environmental Toxicity and Cardiovascular Development*
Mary Walker - moderator (University of New Mexico)
Roger Markwald- moderator (Medical University of South Carolina)
Seigo Izuma (Children's Hospital, Boston)
Richard Di Giulio (Duke University)
Stephen Fisher (Case Western Reserve University)

Appendix 4

Session 1: *Cardiovascular Epidemiology: Risk Factors from Environmental and Occupational Exposure*

Participants in this session identified a number of possible cardiovascular disease outcomes and endpoints that might be considered in epidemiologic research. Some of these include all-cause mortality and endpoints specific for cardiovascular mortality and morbidity (both for hospitalizations and out-patient visits), including

- Sudden out-of-hospital death
- Acute myocardial infarction
- Stroke
- Congestive heart failure
- Peripheral vascular disease
- Congenital heart disease
- Cardiomyopathy

In addition, they suggested that studies of pulmonary diseases that might be associated with cardiovascular disease, might be instructive. Finally, they recommended that studies evaluating subclinical disease endpoints, such as those listed below be carried out

- Intima-media thickness
- Lung Function
- ECG findings (e.g. QT interval, HRV)
- Markers of inflammation
- Endothelial Function
- Insulin resistance
- MRI
- Echocardiogram
- Measures of pulmonary inflammation
- Provocative testing
- General well-being
- Blood pressure

Participants also noted that there is a particular need for better death certificate classification and surveillance of disease outcomes.

In terms of research needs and recommended research approaches, the participants recommended the development of biologic measures of long-term and short-term individual exposures ("biomarkers"). They discussed the potential contribution various study methods which included

- Case-control studies, including studies of cases with known cardiovascular disease and of susceptible populations
- Prospective cohort studies with multiple measures over time, including studies based on

disease or markers of disease and using biologic and other measures of exposure
- Community surveillance studies, including multisite studies, studies of sentinel sites (e.g., where high exposures occur), and studies of susceptible populations
- Case-crossover Studies
- Community intervention studies

One important recommendation, to minimize costs of epidemiologic studies by leveraging resources, was to participate in ongoing studies, such as NHANES or one of the NHLBI studies (e.g. CARDIA, ARIC, CHS, MESA).

Session 2: *Particulate Air Pollution and Myocardial Infarction*

The participants in this workshop recommended that the following potential targets of air pollutants and endpoints that be studied:

Animal Models

1. Electrophysiologic studies: Arrhythmia, heart rate variability, QT dispersion, ST elevation
2. Left ventricular function: Global and regional assessment; echoangiography / echocardiography (which can be used in small animals), MRI, PET scanning
3. Right ventricular function: Indication of reduced lung function
4. Left and right ventricle hemodynamics: Heart rate, blood pressure, left ventricle pressure, changes in left ventricle pressure over time (dP/dT)
5. Myocardial blood flow: Regional myocardial blood flow (using e.g., radioactive or fluorescent microspheres), vascular reactivity
6. Baro-receptor reflex sensitivity
7. Structure of cells: Electron microscopy; pathology
8. Endothelial function: Vasoreactivity, plaque vulnerability, platelet aggregation, metalloproteinase activity in blood vessels
9. Myocardial infarct models: MI size, LV remodeling after MI (by echoangiography, MRI), LV dilation (a strong predictor of mortality), no reflow into small vessels of the myocardium
10. Foltz model: Cyclic flow variation in coronary stenosis model
11. Potential markers of disease: Mitochondrial DNA in aortas, VCAM, ICAM, iNOS, Brain Naturetic Peptide (marker for heart failure)

Human Studies

1. Electrophysiologic studies: Heart rate variability, T-wave alternans, QT dispersion, ST elevation, arrhythmias
2. For myocardial infarcts: Evaluation of potential triggers of MI; MI size; MI mortality; congestive heart failure; revascularization; arrhythmia; left ventricle ejection fraction (function); no reflow; remodeling (e.g., LV dilation)
3. Role of PM in development of atherosclerosis: Carotid intimal and medial thickness, carotid plaque area, carotid plaque characterization, pixel intensity
4. Endothelial function: Acute versus chronic; Brachial flow mediated dilation, brachial ultrasound, coronary PET, CD40, CD11, CD18, endothelins, adhesion molecules
5. Blood parameters
    a. Platelet aggregation, clotting factors, fibrinolytic fraction, iNOS
    b. Inflammatory mediators: C-reactive protein, tumor necrosis factor, IL-1
    c. Acute phase response: C-reactive protein
6. Electron beam computerized tomography: Calcium in coronary arteries

The participants also pointed out the need to differentiate between acute and chronic disease processes caused or exacerbated by exposure to environmental agents. Indications of acute processes might include changes in endothelial function, worsening of ischemia, and acute arrhythmias. Studies of the role of PM in chronic CVD would require serial followup studies. Indicators of chronic processes might include the following:

1. Progression of atherosclerosis (detected by carotid ultrasound, electron beam computed tomography, or MRI of carotids, aorta, coronary arteries)
2. Remodeling of left ventricle post MI
3. Left ventricle hypertrophy
4. Chronic heart damage (e.g., apoptosis)

Participants identified the need to study models of susceptibility to PM-induced cardiovascular disease and made the following recommendations for studies:

A. Human Studies
1. Subclinical Disorders, e.g., healthy smokers, hyperlipidemic subject, hypertensive subjects. This could potentially be a much larger population than those with overt CV disease.
2. Consideration of non-environmental factors: Genetic Factors, diet, protective factors, (such as statins, aspirin, beta-blockers). The question of whether databases could be screened for these factors for epidemiology studies was raised.
3. Panel and controlled exposure studies: patients from clinics or from screening potential subjects directly.

B. Animal Models
1. Genetically predisposed
    a. ApoE
    b. Ion channels transgenic and knockouts
    c. Other animals in the NIH database ??
2. "Natural": spontaneously hypertensive rats, hyperlipidemic rabbits
3. Large Animal Models, e.g., pigs and dogs: Conducive to manipulation (coronary occlusion, Foltz model); Can be made to mimic human disease

The group made recommendations for mechanistic studies to identify the "route of attack" of PM.
1. Neurogenic mechanisms: This could be studied using dennervation or ablation studies or using pharmacological intervention to interrupt nerve conduction.
2. Indirect Effects: These would include studies to measure potential mediators, such as inflammatory compounds (cytokines), acute phase reactants, and clotting coagulation factors. They suggested the use of models that block specific functions and the use of in vitro models, such as heart/lung perfused models.
3. Direct attack on the cardiovascular system: This would be particularly important for ultrafine particles and soluble components. This would require the identification of particles or components in the CV system (heart, EC, plaques) and measurement of the resultant damage to heart or vascular tissues. Again the value of isolated heart/lung and in vitro models to link to in vivo studies was noted.

Participant noted the importance of developing comparability among studies. This was seen as especially important for PM studies, since the effects are small and not always reproducible in multiple studies (in contrast to studies of ozone). They recommended, where possible the use of

- Common particles (as much as possible)
- Common animal models and human subpopulations
- Common endpoints assayed the same as much as possible (core labs)

Session 3: *Environmental Stress and Vascular Disease*

Participants in this group tried to address four specific questions:
1. Do environmental agents affect the coronary and peripheral vasculature?
2. If so, by what mechanisms?
3. What key in vivo and in vitro studies are needed to explore these mechanisms?
4. Is there a need for additional models for this research, or are existing models adequate? (The participants concluded that the models that exist are sufficient, so no recommendations were made in this area.)

The participants made five broad recommends:

Recommendation #1: Pursue more studies to understand the relationship between environmental toxicant exposure and vascular dysfunction.
Studies should look at the effects of acute versus chronic exposures. Studies of human and animal models, and studies using in vivo & ex vivo models (e.g., isolated vessels), are needed. In addition, studies of the different types of vascular beds, such glomerular vessels, should be done. Endpoints indicative of vascular dysfunction should include vessel reactivity, changes in leukocyte adhesion, thrombosis, proliferation / migration, and vascular remodeling**.**

Recommendation #2: Explore potential mechanisms by which environmental agents alter vascular function.
The use of genetic and functional genomics approaches should be encouraged. Important mechanisms and molecular targets that should be investigated include
- Oxidative stress
- Reactive nitrogen species
- Adhesion Molecules
- Inflammation
- Nitric Oxide

Although research should be driven by appropriate scientific questions, the use of stat-of-the art technologies, such as functional genomics, should be encouraged. Such studies may lead to the identification of much needed biomarkers of response.

Recommendation #3: Identify factors that modify vascular responses to environmental toxicants.
These factors might include
- Diet
- Age
- Genetic polymorphisms
- Exercise
- Drugs and dietary supplements
- Smoking
- Demographics
- Social stress

Recommendation #4: Define characteristics of environmental toxicants or mixtures of toxicants that determine outcomes of vascular toxicity.
This was considered one of the most important recommendations. Characterizations should include

- Determination of concentration-response relationships
- Proper dosing regimen (long- versus short-term)
- Evaluation of the toxicokinetics of the agent(s)
- Determination of the composition of the environmental agents

Recommendation #5: Encourage multi-disciplinary approaches to evaluate the role of environmental agents in vascular disease.

Disciplines that should be encouraged to participate in studies in this area include

- Cardiology
- Epidemiology
- Hematology
- Immunology
- Molecular biology
- Neurology
- Toxicology

Session 4: *Environmental Modulation of Myocardial Excitability*

Human studies are urgently needed to establish the link between environmental factors and electrocardiographic changes (e.g., bradycardia, tachycardia, or conduction block) and arrhythmias, and to identify the types of arrhythmias caused by environmental agents. Investigations should include evaluation of both atrial and ventricular arrhythmia through the use of panel studies, controlled human exposure studies, epidemiological studies, and occupational exposure studies. Studies must go beyond HRV and specifically define the arrhythmia and include more robust measures of the substrate, i.e., conduction and repolarization. Available tools to use in these studies might include

- Ambulatory electrocardiography
- Event monitoring
- High-resolution ECG (Sigma-averaged ECG)
- Measures of repolarization dispersion and restitution, QT, t-wave alternans

DNA banking should be done, when possible, for use then more genes related to cardiac disease have been identified.

Studies must focus initially on subjects at high risk for arrhythmia, that is, those with a conditional substrate, i.e., a remodeled myocardium. These would include patients with the following conditions:

- History of an arrhythmia
- Healed infarction, cardiomyopathy, and congestive heart failure
- Coronary artery disease
- Advanced age, diabetes mellitus and hypertension
- Left ventricular hypertrophy
- Congenital predisposition to arrhythmia, e.g., long QT and other catecholamine sensitivity ventricular tachycardias

Studies should consider interactions with these factors:

- Gender and race
- Inflammation
- Lipid status
- Concomitant membrane active drug use
- Concomitant use of other drugs, such as lipid lowering drugs, ACE inhibitors, and beta adrenergic blockers.

The participants also recommended that early studies of PM-induced heart disease should
1) establish the mechanism for changes in HRV: autonomic changes versus sinus node dysfunction, and
2) determine the which fraction of PM triggers the arrhythmias: ultra-fine versus fine versus course fractions.

As answers emerge from these studies more directed animal studies can be designed. Recommendations for these studies included

- Identification of sensitizing factors known to modify the electrophysiological substrate, such as lipid abnormalities, hypertension/hypertrophy, insulin resistance/diabetes, CAD, CHF, healed infarction
- Use of large animal models serving as surrogates of human disease to help define the mechanism of arrhythmia
- Development of genetic mouse models, that would follow once potential gene/protein targets were better understood

Conclusions

1. There is limited data on the role of environmental agents in the initiation and maintenance of arrhythmias in humans.
2. Environmental agents may remodel electrical properties directly or indirectly, and acutely or chronically.
3. Epidemiologic data showing increased arrhythmias with PM exposure is provocative and warrants further investigation.
4. In the absence of ECG data, ideas on mechanisms are only speculative.

Session 5: *Cardiovascular Oxidative Stress and Environmental Pollutants*

The participants identified numerous models for studying oxidative stress in the cardiovascular system, potential mechanisms of oxidative damage, technologies for studying the mechanisms, endpoints that should be evaluated, and methodologies for measuring reactive oxidative species (ROS). These are listed below.

Models

- Transgenics (cardiac specific): Mouse and rabbit models can be used to test hypotheses once they're generated. The down side of rodents (the typical transgenic models) is the differences between them and humans, e.g., in the action potential. Rabbits are more similar to humans in this regard.
- Disease models, e.g., diabetes and hypertension
- Aging
- In vivo exposure models
- Human studies
- Stems cells: The possibility that pollutants might affect stem cells involved in remodeling was raised.
- Cell models: These would be good for screening potential cardiovascular toxicants. Cardiomyocyte models can be useful and cost efficient.

Mechanisms

- Non oxidative processes: In addition to direct oxidant injury, at lower concentrations ROS can affect cell signaling. These affects should not be overlooked.
- Compartmentalization: Rapid reactivity versus diffusion of ROS
- Cardiac versus vascular events
- Time dependent responses (acute versus chronic)
- Endothelial dysfunction
- Gene-environment interactions
- Priming: Are there certain exposures that "set up" the organism for future exposure-related events?
- Loss of reserve and compensatory dysfunction, e.g., depletion of antioxidants due to pollutant exposure, leading to susceptibility to other oxidants, including exogenously generated ones.

In addition to these potential mechanisms, the participants discussed the importance of the sources of oxidants: direct oxidants, those created by membrane functions, mitochondrial-derived oxidants, etc. When looking at dose relationships, it would be useful to follow the path from source to target. The participants also discussed the possibility of interventions, such as diet.

Technologies

- Transgenics
- Genomics/proteomics
- Computational methods
- Measurements of ROS
- Electrophysiology/Echocardiology

- Separation of cells (e.g., via laser-capture microdissection)
- Novel statistical approaches and models
- Biophysical measurements of membrane properties
- Accessible exposure centers (to help standard exposure regimens)
- Non-invasive imaging

End Points
- Biomarkers for inflammation
- Vessel wall thickness
- Vascular function
- Oxidant-based transcription & other factors
- Genomics/proteomics
- Stress tests
- Protein modification
- Cardiac dysfunction over time
- Cardiac contractility
- Repair markers
- Clotting function
- Quantitative morphology

The value of having archived samples of cardiovascular tissues, similar to the NIOSH lung tissue bank, was raised, but it was not known if such a tissue bank exists.

Measurements of ROS
- Problems with fluorescent indicators
  - Lack of specificity for specific ROS
  - Leakage/calibration issues
  - Intracellular compartmentalization
- Validation
  - Transgenic animals with redox reporter, e.g., linked to a HOX gene
  - Spin trapping
  - Few in vivo methods
    - Luminescence
    - Validation issues
- Biomarkers
  - Modified protein
  - DNA adducts
  - Lipid modifications
  - Isoprostanes
  - Antioxidant metabolism levels
  - Aconitase activity
  - Transcription factors (gene profiling)

Other Issues
- Coordination of studies and collaborations: e.g., with NTP and the Health Effects Institute

- Communications/clearinghouse
- Susceptibility
- Oxidant subtleties vs. damage

The group identified to "tiers" of recommendations for future research, with Tier 1 representing more critical recommendations. These are listed below.

Recommendations (Tier 1)

1. Use of relevant disease models
2. Development of sensitive methods for ROS
3. Development of accessible exposure capabilities

Recommendations (Tier 2)

1. Studies of the links between oxidative species and cell/organ dysfunction and intermediaries
2. Effects of ROS in cardiac versus vascular tissues
3. Development of intervention studies and use of leveraging to save money
4. Assessment of acute markers for chronic outcomes
5. Influence of background oxidative environment
6. Development of non-invasive methods

Session 6: *Environmental Toxicity and Cardiovascular Development*

The first issue addressed by this group was the identification of potential cardiovascular teratogens. The participants suggest three important areas that need to be studied.

Epidemiology: The participants recommended that researchers expand their studies to include global assessments and to investigate high dose accidental exposures events where developmental abnormalities might be more common. They also noted that, if possible, early versus late in utero deaths differentiated. Finally, they recommended that, where possible, studies should combine epidemiological investigations with genetic analyses.

Screening of potential agents: In order to identify potential cardiovascular teratogens, the participants recommended exploring the use of stem cells and fish and avian models.

Fate and Transport: It was also recommended that the fate and transport of potential teratogens in experimental animals, especially mammals, be studied.

The participants discussed numerous animal models. Several of these are listed below along with the pros and cons of each.

Fish, e.g., Zebrafish and Fundulus

Pros

- Environmental Epidemiology
- Transgenic / microarrays available
- Fast development
- Small, easily imaged
- Large numbers – inexpensive

Cons

- No septation
- No true Outflow Tract

Xenopus

Pros & Cons: Similar to fish

- Simpler identification of transgenics
- Cell fate maps more easily established
- Embryo manipulation easier
- True Outflow Tract with 3-chambered heart

Chick or Quail

Pros

- Similarity to human heart
- Chimeras
- Embryo manipulation is easy
- Slow development, longer windows

- Inexpensive

Cons
- Lack genetic manipulation
- Lack cell markers
- No placenta

Mammalian (mouse, rat, rabbit, dogs)

Pros
- Genetics / genome database
- Placenta
- Pharmacology, dosing
- Imaging by confocal microscopy, MRI, and echo
- Known physiology

Cons
- Expensive
- More rapid development / shorter windows
- Viral vectors less effective
- More difficult to directly access
- Whole embryo cultures limited
- Cell / tissue fate mapping more difficult & less established
- Less representative of human heart development

In vitro models
- Explants
- Coronary vascular development
- Atrioventricular canal
- Cell lines: Initial screening
- Stem cells, teratocarcinoma
- Embryoid bodies

Septation was identified as an important morphogenetic targets that warranted further study. The steps in the process that should be looked at include

- Myocardialization
- Epicardialization
- Migration of extracardiac mesenchyme – OFT
- Outflow tract remodeling – apoptosis and other processes
- Conduction system
- Myocyte proliferation – Size abnormalities (puny hearts)

It was also recommended that factors that predispose to, and activities that increase risk of, increase risk of cardiovascular malformations. Tools for studying these could include disease models of increased susceptibility, such as obesity / diabetes models, models of pre-existing

cardiovascular disease (eg, hypertension, atherosclerosis), and models of chronic inflammation. Gene-specific models were recommended for dissecting developmental pathways that might be targets of environmental agents.

Possible mechanisms by which environmental agents might cause or increase the risk for cardiovascular malformations were identified. These include

- NMDA receptor
- Folic acid / homocystine
- Cytochrome P450s
- AhR / Arnt
- Oxidative Stress
- Heat Shock Proteins
- Retinoic acid signaling
- Inflammatory cytokines
- Neural active compounds
- Apoptosis control

The participants recommended an expansion of technologies to evaluate malformations and encouraged application of technologies that allow analysis of small tissue samples, such as microscale microarrays (including targeting microarrays to morphogenetic pathways), MRI pattern recognition of cardiovascular defects, echocardiography, and the use of in situ robots to increase sample throughput (3000 / year).

An important recommendation was the integration of development and toxicology databases, that would facilitate identification and study of potential cardiovascular teratogens.

In summary, the participants identified the following research needs:

- Use multiple models to identify cardiovascular teratogens
- Focus on key morphogenetic events
- Utilize models with predisposition
- Conduct more epidemiology studies to identify specific teratogens
- Develop collaborations between developmental biologists and toxicologists