A CRITICAL REVIEW OF THE PARTICULATE MATTER TOXICOLOGY LITERATURE FOR SENATE BILL 25 REVIEW OF THE PARTICULATE MATTER STANDARD

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ABSTRACT

The California Environmental Protection Agency has been charged with reviewing the ambient air quality standards for particulate matter (PM) and sulfates to ensure they are protective of children. Toxicology studies provide a critical component of the overall standards review process. Toxicology studies can provide information essential for identifying which components or characteristics of PM air pollution may be more harmful or more closely associated with the adverse health effects seen in epidemiological studies of human populations exposed to ambient levels of PM. Toxicology studies can also provide valuable information on the biological mechanisms involved in causing adverse health effects in animals and humans exposed to PM. This report provides a critical review of the peer reviewed toxicology literature as it pertains to PM and PM component exposure. It is not an exhaustive summary of all toxicology studies conducted using PM or PM components. This report reflects a focused effort to examine the results of toxicology studies that the authors believe will be most helpful in addressing the ongoing air quality standards review in California. As of the completion of this report, the majority of the toxicology studies reviewed found associations between exposure to PM of many different sizes and compositions resulted in direct effects on the respiratory tract. These effects include general as well as site specific cell and tissue injury, increased production of inflammatory biochemical species leading to increased pulmonary inflammation, increases in airway tissue reactivity leading to exacerbation of existing respiratory conditions, typically in compromised animal models. Changes have also been noted in immune cell populations or function that may lead to increased host susceptibility to respiratory infections. To date, few studies have provided much concrete information regarding the effects of PM exposure on other organs or systems in the body or on systemic effects that may result in biological events which will lead to mortality or morbidity in animals or humans. A very few carefully controlled studies do suggest that PM exposure to concentrated ambient particles, combustion particles or coarse particles containing endotoxin may result in systemic effects which could help explain the cardiorespiratory effects seen in epidemiological studies of human populations exposed to PM.

EXECUTIVE SUMMARY

Background

The critical toxicology literature review provided here is essential to assist ARB and OEHHA in completing the review of the particulate matter and sulfate standards by May 2002. Toxicological studies provide a critical tool to address both short-term and potential long-term effects of particulate matter exposure in humans. Although epidemiological studies can provide critical evidence of associations between human populations, air pollution exposure and adverse health impacts, only toxicological studies can provide the types of data needed to begin identifying which components or characteristics of PM may be most harmful or perhaps more directly related to observed adverse health effects. It is essential to identify which components or characteristics of PM are most harmful so that ambient air quality standards can be developed and/or revised as needed to protect public health. It is also highly desirable to use toxicology studies to identify which components or characteristics of PM are most harmful or more directly associated with health impacts so that effective source control strategies can be developed. The following presents a critical review of the PM related toxicology literature designed to assist in the ambient air quality standards review process.

Methods

The scope of work presented here includes a critical review of the most relevant toxicology literature pertaining to particulate matter (PM) and sulfate air pollutant species. Particulate matter includes the most commonly identified respirable particulate species found in ambient air. In conducting this critical review the investigators concentrated on the most relevant studies found in peer reviewed literature, especially those from or pertaining to California. Where possible careful attention has been paid to address the question of biological plausibility; however, the investigators in this effort have also addressed as much as possible the question of

particle specific toxicity, dose-response and other toxicity measures specific to risk assessment.

This critical literature review was not designed to be exhaustive. The focus of this effort was on the most relevant studies included in the recent PM_{10} Air Quality Standards Criteria Document review by the U.S. Environmental Protection Agency (U.S. EPA) as well as any new studies not included in the U.S. EPA document, including studies from California. Studies dealing specifically with coarse, fine and where possible ultrafine particles were also reviewed. Those studies that had particular relevance to children were also considered.

The critical literature review presented here also includes a summary and an analysis of critical factors which affect potential PM toxicity. A critical analysis of these factors may help explain the potential biological or physical mechanisms whereby children, the elderly and people with preexisting heart or lung conditions may be more susceptible and face greater health risks upon exposure to PM. Such an analysis may also help focus future California Environmental Protection Agency toxicology research efforts. Higher priority has been placed on those studies that may identify or directly relate observed adverse health effects to known constituents or components of California ambient PM. Higher priority was also placed on those studies which provide insight into the understanding and interpretation of the growing body of epidemiological evidence relating PM exposure to adverse health effects in sensitive populations. Where possible a comparison between the effects on children, physically compromised and healthy adults as well as aged animals has been provided.

Results

Epidemiological evidence of adverse health effects associated with particulate matter exposure drives in large measure virtually all toxicological studies currently found in the literature. Toxicological studies have begun to address factors affecting particle toxicity by examining the physical makeup of particles including size, number, mass, composition, and associations with other co-pollutants. No single study

is available which clearly distinguishes these important factors, but inroads are being established using the result of well-characterized laboratory-based studies.

Toxicological studies are beginning to provide useful insights for the biologic plausibility of potential mechanisms leading to adverse health effects by the separation and investigation of specific PM components and their composition. This work is based in exposure of humans and animals to laboratory generated particles, concentrated ambient particles, resuspended particles as well as *in vitro* cell culture work. However, the precise "silver bullet" continues to remain elusive. Whether acids, metals, bioaerosols, geologic dusts, or combustion-related products each act in a unique manner remains to be seen. Interaction between particle components as well as other co-pollutants is an area of active ongoing research facilitated in large measure by concentrator technology.

Host characteristics affecting the dosimetry of particles may have critical effects and serve to further direct the search for useful and sensitive biomarkers of effects to particle exposure. These include measures of cell death, inflammation, cell proliferation, the induction and/or activation of cell signaling pathways as well as markers of pathological change and tissue remodeling in respiratory, cardiovascular, neural and immune systems.

The effects of particulate matter on children are largely unknown. Children are undergoing rapid changes during critical periods of development that span from conception to adolescence. Our knowledge gap regarding the susceptibility of children to airborne particles is large and will require extensive, carefully planned research to better evaluate their actual risk. In a similar manner, the effects of particulate matter on the elderly are also poorly understood and will require further research to evaluate risk.

Conclusions

Toxicological studies provide a critical tool to address adverse effects of particulate matter exposure in humans, animals and cells in culture. These studies provide information essential to understanding: 1) the toxicity of known components of ambient particulate matter, 2) the toxicity of PM in combination with other air pollutants, 3) the identification of inherently susceptible sub-populations, and 4) the identification of potential mechanisms involved in the manifestation of adverse health effects following PM exposure. Although epidemiological studies can provide critical evidence of associations between human populations, air pollution exposure and adverse health impacts, only toxicological studies can provide the types of data needed to begin identifying which components or characteristics of PM may be most harmful or perhaps more directly related to observed adverse health effects.

Particle composition, including combustion, geologic and bioaerosol components, particle size and host health status remain important factors in explaining the role PM exposure plays in observed health effects. Combustion related particles appear to produce a variety of direct and indirect effects on the respiratory and cardiovascular systems in animals. Bioaerosols and geologic dusts are important components of the "PM soup" that should not be forgotten. Exposure to these components of both coarse and fine PM appear to be involved in direct effects on the respiratory system and in worsening the effects of asthma and bronchitis. Biological effects of exposure to airborne particles do not have to cause death to be important in setting PM standards. The potential for worsening pre-existing health conditions such as asthma, bronchitis, chronic obstructive pulmonary disease or cardiovascular disease should also be considered in the standards setting process. These in large part drive the need to further explore animal models that mimic the human condition. Studies to better elucidate the impact of exposure to airborne particles during aging as well as critical windows of development in children during pre- and postnatal periods of growth must be a priority for future research.

As of the completion of this report, the majority of the toxicology studies reviewed found associations between exposure to PM of many different sizes and compositions resulted in direct effects on the respiratory tract. These effects include general as well as site specific cell and tissue injury, increased production of inflammatory biochemical species leading to increased pulmonary inflammation, increases in airway tissue reactivity leading to exacerbation of existing respiratory conditions such as asthma like conditions in compromised animal models, and changes in immune cell populations or function that may lead to increased host susceptibility to respiratory infections. To date few studies have provided much concrete information regarding the effects of PM exposure on other organs or systems in the body or on systemic effects that may result in biological events which will lead to mortality or morbidity in animals or humans. A very few carefully controlled studies do suggest that PM exposure to concentrated ambient particles, combustion particles or coarse particles containing endotoxin may result in systemic effects which could help explain the cardiorespiratory effects seen in epidemiological studies of human populations exposed to PM.

In general a review of the toxicology literature clearly supports the conclusion that different particulate matter component species acting by different biological mechanisms cause a variety of short-term health effects in animals and humans. However, compared to the number of studies typically necessary to define the toxicity of other chemicals of known composition, the body of evidence to support this conclusion needs considerable improvement. Few studies to date have systematically explored the various but most likely factors responsible for PM toxicity. These include particle composition, particle mass, particle size, particle concentration and possible particle number. These also include differences in respiratory physiology and resulting dosimetry between both animals and humans, adult humans, children and ill or physiologically compromised animals and humans. Clearly more focused, controlled systematic toxicology studies are needed to conclusively identify which toxicity factors are most important in explaining the adverse health effects seen with ambient PM exposure and to more clearly define which biological mechanisms are involved.

1.0 INTRODUCTION

Under the Children's Environmental Health Protection Act (Senate Bill 25, authored by Senator Martha Escutia, 1999), as requested by the California Environmental protection Agency (Cal/EPA), the Air Resources Board (ARB) in consultation with the Office of Environmental Health Hazard Assessment (OEHHA) reviewed all California's ambient air quality standards for their adequacy to protect the health of children and infants. The review, completed in December by ARB, concluded that the air quality standards for particulate matter smaller than 10 microns in size (PM_{10}), ozone, and nitrogen dioxide may not be health protective for infants and children. At that time the ARB and OEHHA determined that the highest priority should be given to reviewing the PM_{10} and sulfate air quality standards. This review will be completed and a final document will be presented at the May 2002 Air Resources Board meeting. The first draft of the standards review document is tentatively scheduled for release in November 2001. As part of the review process and final document development effort, a focused and critical review of the particulate matter toxicology literature is needed.

The scope of work presented here includes a critical review of the most relevant toxicology literature pertaining to particulate matter (PM) and sulfate air pollutant species. Particulate matter includes the most commonly identified respirable particulate species found in ambient air. In conducting this critical review the investigators concentrated on the most relevant studies found in peer reviewed literature, especially those from or pertaining to California. Where possible careful attention has been paid to address the question of biological plausibility; however, the investigators conducting this effort have also addressed as much as possible the question of particle specific toxicity, dose-response and other toxicity measures specific to risk assessment.

This critical literature review was not designed to be exhaustive. The focus of this effort was on the most relevant studies included in the recent PM_{10} Air Quality

Standards Criteria Document review by the U.S. Environmental Protection Agency (USEPA) as well as any new studies not included in the U.S. EPA document, including studies from California. Studies dealing specifically with coarse, fine and where possible ultrafine particles were also reviewed. Those studies that had particular relevance to children were also considered.

This critical literature review also includes a summary and an analysis of critical factors which affect potential PM toxicity. A critical analysis of these factors may help explain the potential biological or physical mechanisms whereby children, the elderly and people with preexisting heart or lung conditions may be more susceptible and face greater health risks upon exposure to PM. Higher priority has been placed on those studies that may identify or directly relate observed adverse health effects to known constituents or components of California ambient PM. Higher priority was also placed on those studies which provide insight into the understanding and interpretation of the growing body of epidemiological evidence relating PM exposure to adverse health effects in sensitive populations. Where possible a comparison between the effects on children versus adults has been provided.

In this review the investigators undertook to address a number of questions that have been raised regarding the toxicology and biological effects associated with PM exposure. For example, what type of PM poses the biggest threat to children, adults, and the elderly? What characteristics of PM (i.e. particle size, composition, number, concentration, etc.) may play a greater role in PM toxicity? Why might children be at greater risk for adverse health effects from PM exposure than adults? In a similar manner, are the elderly at greater risk for adverse health effects from PM exposure, and if so, what factors are responsible for this outcome? What influence does cardiorespiratory development and disease have on how humans may respond when exposed to PM, especially in children and the elderly? How are children different from adults with regard to PM exposure? How are the elderly different with regard to PM exposure? What role does lung physiology, pulmonary detoxification, and metabolic function play in the response of a physically compromised person or possibly

due to age, i.e., a child or an elderly individual to PM exposure? How do these factors and responses vary compared to that seen in healthy people and adults? It is expected that the majority of the information to assist in answering these questions, if available, will be derived from animal toxicology studies.

1.1 Project Objectives

The objectives of this project were to: (1) prepare a focused review of the toxicology literature for PM, (2) provide an overview of what information from animal toxicology may be useful in the standard setting process, (3) examine the toxicology of fine versus coarse particles, and (4) explore the differences between children, adults and compromised individuals with regard to adverse health effects and toxicology of PM exposure.

1.2 Statement of Work

Funding for this project was provided by the Air Resources Board to support Dr. Kent E. Pinkerton in his efforts to prepare the critical toxicology literature review document for use by ARB and OEHHA in the PM_{10} standards review process. The original ARB/OEHHA Staff Report, "Adequacy of California Ambient Air Quality Standards: Senate Bill No. 25 – Children's Environmental Health Protection" did not provide a comprehensive review of the toxicology of PM. This project will seek to fill this deficit in the review process. In order to deliver a quality product in the time frame required, the work to complete this critical toxicology literature review was carried out in collaboration with individuals assisting Dr. Pinkerton.

1.3 Particulate Matter Air Pollution and Children

As evidenced by the California Senate Bill 25, considerable importance has recently been placed on reviewing existing environmental standards to ensure they adequately protect infants and children. While potential impacts on children were considered in the development of the original particulate matter and sulfate ambient air

quality standards, recent epidemiological evidence suggests that lung development in elementary school aged children exposed to elevated levels of PM may be adversely affected (Koenig, et al. 1993). Additional concern has been raised in response to more recent epidemiological studies which show a correlation between elevated ambient particulate matter air pollution and increased morbidity and possibly mortality in infants (Dejmek et al., 2001; Hertz-Picciotto et al., 2001).

In light of such evidence it has become essential to better understand the underlying biologic mechanisms involved in the development or manifestation of adverse health effects associated with PM air pollution exposure in humans, especially children, the elderly and persons with preexisting heart or lung disease. With respect to children, it is possible that children may be inherently more susceptible to effects of air pollution because their biological systems are developing and growing. It is also possible that during critical stages of growth and development children or more specifically, their various biological systems, may be more susceptible than at other times (Pinkerton and Joad, 2000; Dietert, et al., 2000; Selevan, et al. 2000; Pedem, et al., 2000; Jedrychowski, et al., 1999). Within this context where possible a comparison between children and adults has been provided throughout this document.

2.0 Health Effects Associated with Particulate Matter Exposure: Epidemiological Evidence Summary

A considerable, and growing, body of epidemiological evidence clearly supports the conclusion that human exposure to elevated levels of ambient particulate matter air pollution is associated with adverse health impacts [U.S. EPA 1996]. To date these health impacts include 1) increased incidence and severity of asthma, worsening of bronchitis symptoms, and increased incidence of respiratory infections which may result from direct effects on the respiratory system, and 2) premature death which may result from either direct or systemic effects on the cardiorespiratory system (Dockery, et

al, 1993; Pope III and Dockery, 1999). Short-term or "acute" exposures have been linked to adverse health impacts in people whose health is already compromised. These include people with pre-existing pulmonary or cardiac disease, especially the elderly. The vast majority of epidemiological studies have focused on short-term exposure effects largely because these studies are easier and less costly to carryout than studies that address long term health impacts (Pope 1998). However, many more individuals may be impacted as a result of long term exposures which are to date, largely uncharacterized. The long-term effects of particulate matter exposure in children may be more substantial than in adults (Koenig, et al. 1993).

Indirect effects of PM exposure can also be significant. The adverse health impacts most commonly associated with PM exposure also result in decreased life expectancy, lost work days, lost school days, and increased medical costs (Lipsett, et al., 1997; Morgan, et al., 1988; Jacobs, et al., 1995). These indirect effects of PM air pollution have the potential to seriously impact the quality of life of many Californians. In light of such epidemiological evidence it has become essential to better understand the underlying biologic mechanisms involved in the development or manifestation of adverse health effects associated with PM air pollution exposure in humans, especially children, the elderly and persons with preexisting heart or lung disease.

Toxicological studies provide a critical tool to address both short-term and potential long-term effects of particulate matter exposure in humans. Of the top 10 research priorities for airborne particulate matter recommended by the National Research Council in 1998, four research priorities require the use of carefully designed toxicology studies. These include the following: 1) an examination of the toxicity of known components of ambient particulate matter, 2) understanding the toxicity of PM in combination with other air pollutants such as ozone or carbon monoxide, 3) the identification of inherently susceptible sub-populations, such as possibly the elderly, infants or growing children, and 4) the identification of the mechanisms of injury which are involved in the manifestation of adverse health effects following PM exposure.

Although epidemiological studies can provide critical evidence of associations between human populations, air pollution exposure and adverse health impacts, only toxicological studies can provide the types of data needed to begin identifying which components or characteristics of PM may be most harmful or perhaps more directly related to observed adverse health effects. It is essential to identify which components or characteristics of PM are most harmful so that ambient air quality standards can be developed and/or revised as needed to protect public health. It is also highly desirable to identify which components or characteristics of PM are most harmful or more directly associated with health impacts so that effective source control strategies can be developed.

3.0 FACTORS AFFECTING THE TOXICITY OF PARTICULATE MATTER

Unlike other air pollutants such as ozone (O_3), nitrogen dioxide (NO_2) or carbon monoxide (CO) which are single species with known chemical structures, particulate matter air pollution in California is a highly complex mixture comprised of many chemically distinct species (California Air Resources Board, 1987; Dolislager and Motallebi, 1998; Chow et al, 1992; Chow et al, 1993; Christoforou et al, 2000). These chemically distinct species can be present in ambient air environments in several different physical forms. These forms include solid particles, liquid aerosol droplets or even solid particles with very fine liquid coatings. Particulate matter species or otherwise known as "components" may be comprised of seemingly inert material such as dust of geologic origin or solid carbon particles from combustion sources. Particulate matter may be comprised of highly chemically reactive species such as oxides of metals, volatile organic species and well known carcinogens such as polycyclic aromatic hydrocarbons (PAHs) (Rogge, R.F. et al., 1993). Complex ambient air PM mixtures can also contain particles of greatly varying size and number.

Because of its complexity, the toxicity of ambient PM is very likely highly variable. The toxicity of PM will also likely depend on a number of PM component physical and chemical factors. The most immediately obvious factors, which may affect inherent PM toxicity, include particle size, particle number, particle mass, particle concentration and particle composition. Less obvious but equally important—if not more important—factors that play a role in understanding PM toxicity are factors specific to the animal model or humans exposed. These factors include differences or variability in respiratory physiology, age, gender, and health status. Such differences can directly impact the amount of PM deposited in the lungs and/or the location it is deposited, i.e. the *dosimetry* (Schlesinger, 2000). These factors may also play a key role in defining who in our human populations may or may not be more sensitive or otherwise more susceptible to PM exposure. The presence of other air pollutants in combination with ambient PM component mixtures may play a role in PM toxicity as well. These factors, as they pertain to the relevant PM toxicology literature review, are discussed in greater detail in the following paragraphs.

3.1 Particle Size

Particle size is a very important factor in the toxicity of PM air pollution. Not only does size have the potential to affect where particles are deposited in the lungs, particle size is often directly related to the sources of PM release in the ambient environment. This means that PM size in the ambient environment is often directly related to PM component chemical composition. Depending on the sources contributing to ambient PM levels in any given area of California, there is often significant overlap between contributing PM sources—such as with geologic dusts and combustion related processes—particle size and PM component chemical composition (Rogers, et al., 1998). Because this factor is such an important element in addressing the question of particle toxicity, where possible the investigators on this effort have placed more emphasis on the studies presented in the toxicology literature to date which have been conducted in this fashion.

3.2 Particle Number

Particle number can be an important factor in the toxicity of PM air pollution. If an exposure concentration is fixed, regardless of particle size, if there are more total particles present in a given volume of air the particle surface area available for contact with the respiratory tract will be increased. As such, differences in particle numbers within a fixed volume of air inhaled can have a direct influence on the dose of PM delivered to the body. While the dose on a total body mass per unit volume of air breathed may remain the same, the dose per surface area of respiratory tract tissue could vary substantially based on the numbers of particles present in the exposure setting. Because of this, particle number as a factor in determining the toxicity of ambient PM has the potential to directly impact the toxicity of any given PM species or mixture. For any given fixed concentration of measured ambient PM, it will take fewer coarse particles in contrast to many more fine or ultrafine particles, in total number, to make up the same particle mass (i.e. measured concentration) in air (Oberdörster, et al., 1992). Where possible investigators on this effort have addressed this issue in their critical assessment of the toxicology literature.

3.3 Particle Mass and/or Concentration

Ambient air quality standards set by Cal/EPA for California and by U.S. EPA for the Nation are based on PM concentration as measured in units of PM mass per volume of air, i.e. $\mu g/m^3$. Although this may serve as a very practical measure of total ambient PM for air quality program compliance purposes, it can prove challenging from a toxicological assessment perspective. Particle concentrations in ambient environments can be directly related to the sizes of particles comprising the mixture, particle mass, particle number and composition of the particles present in a given volume of sampled air. In many cases; however, on a mass per volume of air basis particle concentration can remain the same even though the number, size, mass and/or composition of the measured particles varies. In light of this it is difficult to correlate particle concentration in general, on a mass per volume of air basis, with inherent PM toxicity. For these reasons the investigators on this critical PM literature review effort

have not focused on particle concentration as a specific toxicity factor. Particulate concentration has; however, been considered very carefully as a critical component in characterizing overall exposures in the various toxicological studies. Particle concentration has also been considered in those cases where studies have attempted to address particulate species or composition dose-response, i.e. address the absolute effect of varying particle concentration while fixing particle size and composition.

3.4 Particle Composition

Particle composition is most likely the single largest determinant of PM toxicity on a component by component single particle basis. In the ambient air quality standards setting process it is important to recognize that the observed response and subsequent "toxicity" of any given component of PM is highly dependent on the biological endpoint or adverse health effect of concern under study. The biological effects seen with exposure to PM of varying composition do not have to be grossly toxic, as in causing cancer or respiratory tissue cell death, to be important in the PM standards setting process. Particulate components other than combustion related particles may cause more subtle biochemical, cellular or systemic effects that result in more the worsening of asthma, allergic and bronchitic conditions. Effects such as these resulting from inherently "less toxic" PM components may be of considerable political and social importance in that they have to potential to impact the quality of life of many Californians. Where possible, the investigators on this effort have attempted to address the complex question of PM composition as a factor in their critical review of the toxicology literature.

3.5 Particles in Combination with Other Air Pollutants

The inherent toxicity of PM components in complex ambient environments may be altered by the presence of other air pollutants. The toxicity may be enhanced or reduced depending on the physical and chemical properties of the PM components of interest and the other air pollutants present (Mauderly, 1993). Rarely are individuals exposed to only PM air pollution. More commonly people are routinely exposed to

complex mixtures containing many different potentially harmful pollutants which vary greatly in concentration and composition throughout any given year of exposure. In light of this, the question of enhanced PM toxicity in the presence of other air pollutants remains an important factor to consider in the study of PM toxicology.

3.6 Respiratory Physiology and Dosimetry

Respiratory physiology is a central factor in determining total PM dose delivered to a person or laboratory animal. It is also the single largest source of variability between animal models used in laboratory studies and humans. Differences in respiratory physiology and resulting dosimetry are anticipated to be central to explaining the differences in adverse health effects associated with PM exposure in sensitive or susceptible human populations.

There are significant differences in respiratory physiology between the developing lungs of children and those of physiologically mature adults. These include differences in the size and shape of the respiratory tract conducting airways and the number and orientation of physiologically active gas exchanges regions which can lead to large differences in ventilatory patterns (Table 1). These differences will result in significant differences in the relative dose of a gas or particle delivered to the individual. Differences in these physiological factors result not only from differences in age and gender (Snodgrass 1992), but also as a result of disease or health status in physically compromised individuals, such the elderly or people with heart or lung conditions.

Differences in respiratory physiology coupled with related differences in individual activity patterns, such as with children who are typically more active out of doors and the elderly who if ill, may be less active and may tend to stay in doors more often, can lead to tissue level delivery of significantly different doses of PM components (Table 2) (Adams, 1993). Even if all individuals are in the same physical area breathing the same type of ambient PM, individual doses at both the whole body and tissue level can vary significantly based because of variations in respiratory physiology and activity patterns.

Particle dosimetry is based not only on host factors, but also on the characteristics of the particle. Particle deposition, translocation, clearance and retention of particles within the respiratory tract as well as non-pulmonary tissues are all important in understanding the relative effects of particles on animals and humans. Particle concentration as well as duration of particle exposure, coupled with respiratory tract anatomy and physiology influence the particle deposition in the lungs (Schlesinger, 2000). However, the actual dose of particles delivered to the respiratory tract is likely to be different for different species based on dissimilarities in anatomy and ventilation. However, these differences may also serve to more precisely define the actual toxicologic effects of particles on the respiratory and cardiovascular systems, based on the ability to better define the dosimetric relationship to the actual biological response observed (Lippman and Schlesinger, 2000).

Table 1: Differences in Ventilatory Rates

Parameter	Units	Infants	Adults
Respiratory Volume	ML/kg/breath	10	10
Alveolar Surface Area	m^2	3	75
Respiration Rate	Breaths/min	40	15
Minute Ventilation	mL/kg body weight/m^2 lung surface area/min	133	2

Adapted from Snodgrass, 1992.

Table 2: Percent Time Californians Spend in Different Environments

Age (yrs)	0-2	3-5	6-11	12+
Indoors Home	85	76	71	62
Indoors (other)	4	9	12	25
Inside Vehicles	4	5	4	7
Outdoors	6	10	13	6

From Adams, 1993.

4.0 BIOMARKERS OF EFFECT

In the study of the toxicity of any air pollutant species it is important to distinguish between physiologic or biologic effects that result from exposure to irritating atmospheres in general and physiological, biological or biochemical responses that represent adverse health effects that may result from exposure to air pollution in either humans or animals. Biological effects that represent the later are called biomarkers. There are many biomarkers of biological effect used in toxicology studies. The biomarkers investigated depend on the adverse health effect or anticipated biological mechanism suspected to be involved in causing the effect of interest. Some are markers of direct effects on cells or tissues. Others are representative of gross systemic effects. Still others are markers of more subtle effects while others may be markers of cancer causing or pre-cancer related biological events.

Biomarkers of effect are essential tools needed to measure respiratory, cardiovascular and other systemic responses to particle exposure. Biomarkers provide a measure of change to be used as an early sign of potentially adverse effects induced by particle exposure. A basic question yet to be resolved is whether particles at ambient concentrations can elicit direct toxic effects on cells and the organ systems they come into contact. If toxic effects do occur, what are the cellular mechanisms leading to injury and damage? If an inflammatory reaction is the basis for particle toxicity, how does exposure to PM initiate and/or exacerbate the response in the respiratory, cardiovascular or other organ system? In a similar manner, could PM induce and/or promulgate an allergic response by way of an immune-mediated pathway triggered by the presence of particulate matter coming into contact with cells of the respiratory tract?

Although particle-induced responses in animals and humans may impact on multiple organ systems, the respiratory tract is likely to be the first site of interaction and response to inhaled particles. Therefore, the majority of biomarkers of effect discussed in this section focus on the respiratory tract.

4.1 Cell Death

Death to the cell is a terminal event that can occur by a variety of processes. These include damage to the cell membrane leading to loss of cytoplasmic contents, depletion of key enzymes to protect the cell, loss of energy stores to maintain the function and integrity of the cell, or damage to cellular DNA by disruption or breakage. Techniques to detect these processes occurring in cells can serve as excellent biomarkers of effect. If the basic tenant that particles act to damage cells then measures of such events become critical to understanding the toxicity of PM.

An important tool used by the toxicologist to assess cell injury and death in the respiratory tract is to recover cells from the lung for study by bronchoavleolar lavage (BAL). This technique requires the instillation of a sterile, buffered solution into the lungs to collect cells found in the air spaces of the lungs, including airways and alveoli. These recovered cells can be examined microscopically. They can also be analyzed for a variety of cellular and biochemical functions. In a similar manner, the fluids recovered from the lungs by these lavage techniques can be analyzed using biochemical measures which reflect cell permeability, injury and loss of intracellular contents. Protein detected within the lavage supernatant is a simple measure of cell membrane disruption and/or cell death. Lactic dehydrogenase (LDH), a common intracellular enzyme, can also be used as an indication of cell injury. The relative amount of protein or LDH measured in the lavage fluid is a reasonable reflection of the extent of injury present in the lungs.

Cell membrane damage can also be assessed using malondialdehyde, a measure of lipid peroxidation or damage. A simple measure of cell viability can be carried out on cells recovered from the lungs using Evan's blue dye. Cells with an intact membrane will effectively prevent the dye from entering the cells. In contrast, those cells with permeable or leaky membranes cannot block the uptake of dye into the cells. This simple measure can be used as a powerful tool to detect particle-induced alterations on cells.

One potential mechanism leading to cell injury and death is the loss of the natural defenses of the cells. Antioxidants are thought to play a critical role in cell protection. Depletion of these antioxidants may be a precursor to the loss of cell viability and integrity. In fact, depletion of these enzymes is likely to be a leading cause of irreversible injury to the cell. Therefore, assays to measure antioxidant enzyme levels could serve as a sensitive measure of oxidative stress due to particle exposure. Glutathione levels and the ratio of oxidized and reduced forms of glutathione are excellent measures to assess oxidative stress. Total glutathione levels can be measured in BAL as well as homogenized tissues. Site-specific changes in the bronchial airways and lung parenchyma may also be useful in measuring subtle effects of particle exposure using microdissection techniques. Assays to measure total antioxidant reducing power are also available to further assess oxidative stress in BAL, lung tissues and blood plasma.

Apoptosis or programmed cell death can also be used as an effective indicator of cellular change. These assays can be applied to cells recovered by lavage, on lung tissue sections or directly on DNA isolated from the lungs. A variety of commercially available products can be used.

4.2 Inflammation

Inflammatory cells form an important component of the pulmonary and systemic defenses. They can also play a critical role in pathogenesis. The influx of inflammatory cells into the lungs can serve as a sensitive signal of an adverse response which occur under a wide range of conditions. Measures of such conditions include changes in the number of cells recovered by bronchoalveolar lavage and/or the relative proportion of inflammatory cell types present in BAL. The predominant cell present in the air spaces of the lungs which is recovered by BAL is the alveolar macrophage. An increase in alveolar macrophage number or a shift in cell types to neutrophils, lymphocytes and/or eosinophils can be easily measured in BAL following exposure to particles. This methodology can be applied to both animals and humans.

4.3 Cell Proliferation

Injury and death to cells are associated with the release of biochemical mediators. These mediators stimulate neighboring cells to undergo DNA synthesis and cell division. These proliferating cells replace cells lost during the process of injury. The proliferation of cells following acute or chronic injury is a natural event necessary to maintain the normal homeostasis of all organ systems. Nucleotide analogs can be used to measure active DNA synthesis as a indicator of cell proliferation. These analogs can be radiolabeled such as tritiated thymidine (^{3}H-thymidine) or use a nonradioactive tag such as bromodeoxyuridine (BrdU). Labeling can be done using a single intraperitoneal injection one to two hours prior to necropsy or through continuous infusion for a set period of time using a miniosmotic pump. Both methods are effective in determining the proportion of cells undergoing DNA synthesis during the period the nucleotide analog is present in the body as available as part of the nucleotide pool in the circulation.

Measures of cell proliferation are best employed in animal toxicology studies in which tissue processing of organs is included in the analysis. The detection of these nucleotide analogs is done on tissue sections prepared for microscopic analysis following autoradiography or immunohistochemistry to visualize its uptake into the DNA of the cell.

4.4 Cell Signaling

A number of biochemical processes occur as a consequence of cell or tissue injury and subsequent localized inflammation. One of these processes involves the elevation of cellular mediators, which serve to further regulate injury and repair. These biochemical mediators include cytokines, chemokines and growth factors. Detection of cytokines, chemokines, and growth factors within lavage fluids, tissues and blood elements (i.e., plasma) are a useful marker of the inflammatory response. Alterations in cytokine levels may also serve to identify the exacerbation of a pre-

existing condition in the lungs such as asthma, fibrosis, bronchitis or chronic obstructive pulmonary disease. Measurement of a variety of mediators may provide important keys to better understanding the plausibility of particle toxicity in healthy and compromised individuals both in humans and animals.

Gene expression is involved in inflammatory, immunoregulatory, stress response and cell survival. This gene expression results in the production of cytokines, chemokines, adhesion molecules, enzymes and acute phase reactants. NFκB is the pivotal transcription factor that regulates gene expression. An understanding of how inhaled particles impact on NFκB may provide a critical key to potential mechanisms leading to adverse effects in the respiratory and cardiovascular systems.

4.5 Cell/Tissue Level Morphology

The consequence of a toxic insult to macrophages lining the air spaces of the lungs or epithelial cells of the airways and alveoli can be multifactorial. Cell injury and/or death can initiate a cascade of events resulting in the release of pro-inflammatory markers including cytokines and prostaglandins, as well as the recruitment of inflammatory cells to sites of injury. If not resolved in the initial stages of these events, further biochemical and biological events will occur. These subsequent stages of the inflammation process can lead to cell proliferation and the release of fibrogenic factors. Recruitment of inflammatory cells including polymorphonuclear leucocytes (PMNS) may give rise to the release of oxidants and proteolytic enzymes. The action of onsite PMNS can further exacerbate cellular changes and the remodeling of normal lung structures.

Assessment of respiratory cell morphology and tissue structure may provide important information in the determination of long-term effects of particle exposure in animal toxicology studies. Morphometric approaches allow for the subtle detection of changes occurring in the lungs. Histochemical techniques can be applied to detect changes in specific cell types lining the airways and alveoli that might be

altered by exposure to particles. In compromised individuals such changes may be reflected by a significant shift in the number and distribution of mucous (Goblet) cells lining the airways. Immunohistochemical techniques may also be applied to examine the distribution and abundance of specific enzymes, cytokines and growth factors that play a role in inflammation and disease. Localization of inflammatory and immune effector cells can be readily assessed using microscopic analysis of tissue sections.

4.6 Systemic Physiologic

Epidemiologic as well as animal toxicology studies suggest subtle, but significant, systemic effects of particle exposure. These systemic effects include changes in blood pressure and heart rate. Although these systemic effects are not always seen, alterations appear to more likely to occur in individuals with pre-existing cardiopulmonary conditions or in compromised animal hosts. Therefore, measures of systemic effects may provide an additional measure for assessment of particle effects.

The measurement of blood clotting factors following particle exposure

5.0 TOXICITY OF PARTICULATE MATTER: RESULTS OF THE CRITICAL LITERATURE REVIEW FOR ANIMAL AND HUMAN STUDIES

Particulate matter air pollution is a very complex mixture comprised of a variety of chemically distinct components of varying physical state and particle size (California Air Resources Board, 1987; Dolislager and Motallebi, 1998; Chow et al, 1992; Chow et al, 1993; Christoforou et al, 2000). The composition of the particulate matter air pollution in California is highly dependent on the sources, both primary and secondary, contributing to the atmospheric PM burden in the ambient area. Sources in an area may directly emit primary particles such as carbon or geologic dusts. Sources may also emit reactive species that will vary in chemical composition over time as they are acted upon by atmospheric conditions, i.e. particle "aging". Sources may also emit chemically reactive species that go on to form secondary types of PM such as ammonium nitrate or ammonium sulfate. The chemical composition of PM and the relative ratio of the various components or sizes of toxicological importance vary based on the PM sources in the area, the topography and climate of the air shed or air basin of interest. As a consequence the ambient composition of PM can vary greatly throughout California and other parts of the United States (Dolislager and Motallebi, 1998; Pace, 1998). This makes the interpretation of PM related epidemiological studies complex. It also makes the design and development of carefully controlled PM toxicology studies especially challenging. In addition, inter-study comparison and ultimate interpretation of results from toxicology studies carried out by different investigators using varying PM exposure mixtures and/or ambient conditions representative of other parts of the United States and the world, is difficult.

Because the factors which potentially affect PM toxicity are so highly correlated in most ambient exposure environments, it is a challenge to test the independent effects of various PM factors experimentally in laboratory settings. It is equally challenging to interpret the relevance of the results obtained from such studies

to exposures seen in complex ambient environments. In designing toxicology studies to address the questions of mechanisms of action and biological plausibility—which may help explain the adverse health effects observed with PM exposures throughout California, the United States and other parts of the world—it is absolutely essential to characterize to the greatest extent possible the PM exposure setting and conditions. Particle composition, particle size distribution, particle number, etc. must be carefully defined. This in and of itself can be an extensive research effort and will vary depending on the type of exposure study employed. Despite study design challenges toxicology studies can provide a great deal of information essential to understanding the biological mechanisms involved in explaining the occurrence of adverse health effects seen with ambient PM exposure.

In the last several years there has been a significant increase in the number of studies reported in the toxicology literature which address the question of PM toxicity. There are a number of different types of toxicology study designs reflected in the PM toxicology literature. These typically fall into four categories: 1) laboratory generated particle inhalation exposures, 2) concentrated ambient particle inhalation exposures, 3) source particle resuspension instillation and/or inhalation laboratory exposures, and 4) *in vitro* laboratory PM studies. The strengths and weakness of each are discussed in greater detail below with specific examples of key studies provided in Section 5.2 of this document. In general, the relevance of results obtained from instillation toxicology studies and *in vitro* laboratory studies are given less emphasis in this critical review effort than results reported from controlled inhalation studies. Differences in particle delivery and deposition have the potential to affect the results of the exposure study and may make interpretation and extrapolation of experimental results to observed ambient exposure setting difficult. The main focus of this effort is to assist the Cal/EPA in its review of the California Ambient Air Quality Particulate Matter Standards. The investigators on this effort believe that the results from direct inhalation studies will be most helpful in accomplishing this task since other methods of exposure can significantly affect where particles are delivered and deposited in the respiratory tract (Driscoll et al., 2000). In their review of the relevant toxicology literature

investigators have focused on toxicology studies that were based on direct source inhalation study designs. However, the other types of toxicology study designs noted below can be very useful in the examination of biological effects and mechanisms of action of PM exposure in both animals and humans.

5.1 Types of Toxicology Studies Reviewed

5.1.1. Laboratory Generated Particle Exposures

A number of inhalation toxicology studies have been conducted and will continue to be conducted using laboratory generated particles, particulate matter mixtures alone and in combination with other pollutants (please refer to Sections 5.2.1, 5.2.3 and 5.2.7). Studies conducted using controlled laboratory generated particles offer a number of important advantages including the ability to directly control the exposure particle composition, concentration, particle size distribution as well as relative mixture of other air pollutants. This allows one to test the specific toxicity of PM components of interest with and without the presence of other air pollutants of concern. Inhalation studies conducted in this fashion also preserve natural inhalation pathways, particle deposition patterns and overall dosimetry parameters that can be directly compared with the same found in native ambient PM exposure settings. However, the primary drawback to conducting toxicology studies using laboratory generated particles and particle mixtures is the concern regarding exposure relevance and subsequent extrapolation to environmentally meaningful settings where the PM mixtures are much more complex. While studies using laboratory generated particles can be very useful in identifying direct biological effects, mechanisms of PM action in the respiratory tract and dose-response patterns for various types of laboratory generated PM, care should be used when extrapolating results directly from toxicology studies conducted using this method for use in identifying or explaining dose-response relationships in complex ambient PM mixtures in environmentally relevant exposure settings.

5.1.2. Concentrated Ambient Particle Exposures

Particle concentrator technologies provide unique opportunities for conducting inhalation toxicology studies using actual ambient particle mixtures. These techniques are relatively new and are still developing. However, the results of many studies conducted using particle concentrator technologies seem quite promising (please see Section 5.2.6 for additional details). In these toxicology studies it is possible to expose animals or humans, either independently or simultaneously, to identified ambient mixtures of PM. It is possible to concentrate ambient particles of specific size. And, by choosing the time when the studies are being conducted and where, it is also possible to manipulate to some degree the composition of the particles delivered to animal or human subjects. Perhaps most importantly, using ambient particle collection and delivery techniques it is possible to directly assess the potential toxicity of native ambient particles. Using these techniques it is also possible to test directly the effects of concentrating those same particles up to 90-fold greater than concentrations found in ambient environments. This eliminates the traditional criticisms and concerns regarding exposure relevance and subsequent extrapolation to environmentally meaningful settings which are inherent to laboratory derived exposure settings.

There is tremendous value in being able to conduct these kinds of studies. If conducted properly, under well characterized exposure settings, they offer a way to help bridge the gap between adverse health effects associated with complex ambient PM exposures observed in epidemiological studies and biological effects and mechanisms of action reproduced in the laboratory under less complex PM exposure settings. However, the drawbacks of conducting toxicology studies using particle concentrator technologies are important to consider. Although it is possible to fix particle concentrations, one of the most significant drawbacks to using these methods is the inability to deliver consistently and reproducibly exposures of varying particle composition over a fixed exposure time frame. There is little flexibility to predetermine and/or vary particle composition in the exposure efforts because it is completely determined by whatever is present in the ambient environment on any given exposure

day. There are also concerns about whether concentrating ambient particles may alter to some degree the physiochemical properties of some PM components. However, pulmonary effects of controlled exposures to concentrated ambient PM mixtures have been carried out successfully using particle concentrator technology in both humans and animals and the results are very promising (Sioutas et al., 1995; Gordon et al., 1998; Ghio, et al, 2000).

5.1.3. Source Particulate Resuspension & Instillation

Numerous toxicology studies are conducted using resuspended PM source material and specialized direct instillation techniques. There are a number of advantages to resuspending particles collected from known sources, such as residual oil fly ash from combustion processes, and then conducting toxicology studies via direct instillation into the respiratory tract (please see Section 5.2.3). Toxicology studies conducted in this fashion are typically less expensive. They can be easier to conduct than inhalation studies. They also require less PM source material. Most importantly they can deliver a very exact dose of PM of known composition, size, etc. directly to the respiratory tract of the host model. These types of studies can be very useful in identifying direct biological effects on specific cell types or tissues, mechanisms of PM action in the respiratory tract and dose-response patterns for various types of PM. However, the results from toxicology studies conducted using instillation techniques should not be directly extrapolated to dose-response relationships in ambient PM exposure settings. When source particles are resuspended, typically in saline solution, particle physiochemical properties, compositions and particle interactions may be altered or lost. Instillation techniques also bypass normal inhalation pathways. This results in alterations in deposition patterns and overall dosimetry such that toxicology studies conducted in this fashion should not be considered representative of actual ambient inhalation exposure conditions. In spite of this limitation, source particle resuspension and instillation toxicology studies can provide considerable insight into the potential mechanisms whereby PM exposure causes adverse health impacts in the body.

5.1.4. In vitro laboratory PM Studies

In vitro exposure techniques can be very useful in examining the discrete effects of PM components on specific cell or tissue types of interest. These techniques allow for the investigation of specific isolated biochemical pathways and effects on cell types that result directly from PM exposure independent of circulating systemic effects (please see Sections 5.2.3, 5.2.4, and 5.2.5 for specific examples). However, results from *in vitro* studies do not typically lend themselves to dose-response extrapolation from *in vitro* exposure to meaningful *in vivo* biological exposure settings for the purpose of standards setting. They are also limited in their applicability for use in ambient air quality standards setting because alterations in the physicochemical properties of the PM delivered in an in vitro setting ensure *in vitro* cell or tissue exposures do not represent ambient exposures in intact animals or humans. However, *in vitro* studies can provide important insight into the biological mechanisms involved in the development of observed adverse health effects, especially if they are used to examine effects in circulating systemic blood or lymph system cell populations such as macrophages, monocytes, lymphocytes and other immune system cells or to examine direct effects on respiratory tract cell types or tissues.

5.2 Effects of Specific PM Component Composition

5.2.1. Acid Aerosols

A large number of controlled laboratory particle generated exposure studies have been conducted to assess the effects of acidic aerosol inhalation in both humans (Frampton et al., 1992; Folinsbee et al., 1997 – review of literature) and animals (Schlesinger et al., 1992). Studies using acid aerosols have been done in large measure with sulfuric acid or derivatives of sulfate (ammonium sulfate). A limited number of studies have also examined the effects of nitric acid and associated derivatives (ammonium nitrate). Minimal effects have been found in both humans and animals following exposure to these compounds following exposures as high as 2000 $\mu g/m^3$ in young healthy adults (Avol et al., 1986; 1988) or greater than 6000 $\mu g/m^3$ in

rats (Lewkowski et al., 1979). Frampton and colleagues (1992) found acid aerosol exposure in humans (1000 $\mu g/m^3$) was not associated with airway inflammation or altered macrophage function. Similar results were found in rabbits and humans exposed to comparable levels of acid aerosol (1000 $\mu g/m^3$) by Zelikoff and colleagues (1997). Daily exposure to a high dose of acid aerosol (1.5 mg/m^3) over a period of 13 months in healthy beagle dogs did not affect pulmonary function or lung pathology (Heyder et al., 1999). In all instances concentrations of acid aerosols tested in the laboratory setting well exceeded ambient levels. However, particle size was well described in all instances.

The general consensus in the literature is that acidic aerosols containing ammonium nitrate and ammonium sulfate particles, nitric acid or even sulfuric acid vapors have minimal acute effects on healthy humans and laboratory animals and no long term effects on overall respiratory function. However, in some cases respiratory mucociliary clearance was impacted with exposure to acidic aerosols. In humans mucociliary clearance was increased at brief lower concentration exposures (approximately 100 $\mu g/m^3$ for less than 1 hour). In contrast, mucociliary clearance was depressed at much higher concentrations of greater than 100 $\mu g/m^3$ delivered over several hours or even months (U.S. EPA, 1989; Mautz, W.J. et al., 1996; Kleinman et al., 1999). The effects of altered mucociliary clearance in humans has the potential to impact incidence of respiratory infection in healthy as well as compromised subjects. In asthmatic subjects inhalation of acidic aerosols appears to somewhat impact the mechanical function of the airways by an increase in bronchoconstriction (Honma et al., 2000). However, the literature is not consistent with respect to acidic aerosol inhalation and affects on sensitive populations (Leduc et al., 1995; Linn et al, 1997). While exposure to acid aerosols at ambient concentrations may not significantly impact healthy individuals and may not result in long term health impacts, at this point in time we cannot discount the potential affects of exposure to ambient acid aerosols in enhancing the toxicity of other PM components, such as metals (Dreher et al., 1997).

5.2.2. Particulate Metals, Ultra Fine Metal Particles, and Metal Fumes

The effects of exposure to single constituent metal particles by inhalation have been examined in humans and animals. These metals include titanium, vanadium, manganese, zinc, and cadmium. Exposures were typically for short-term and at high concentrations. Titanium particles in the fine (250 nm) and ultrafine (21 num) size modes resulted in pulmonary inflammation and increased levels of macrophage inhibitory protein-2 (MIP-2) in lavage fluid, but less than that observed following instillation (Osier, 1997; Osier and Oberdorster, 1997). The effects of vanadium pentoxide (V_2O_5) exposure in plant workers under working conditions rather than a controlled laboratory setting demonstrated the incidence of asthma and bronchial hyperreactivity in 12 of 40 workers (Irsigler et al., 1999). Studies with zinc particle inhalation in human subjects resulted in the elevation of the pro-inflammatory cytokine IL-6 accompanied by myalgia, cough and fatigue (Fine et al., 1997). Exposure to cadmium fumes in mice demonstrated little effects, but did result in higher metallothionein levels in mice than found in rats (McKenna et al., 1998). Although each of these metals were typically examined under carefully controlled conditions, the relative concentrations were in excess of those present in the ambient environment.

Data from prior occupational and high concentration animal exposure studies indicate that both high level acute and low level chronic exposures to ultrafine and fine particle metals as well as PM mixtures containing metals can adversely impact the respiratory tract by increasing inflammation and bronchial hyperreactivity (Irsigler, et al., 1999; Fine, et al., 1997). These studies examined the effects of several metals and metal oxide species including ZnO, colloidal iron, fuel oil fly ash, V_2O_5, MgO, Fe_2O_3, MnO_2, TiO_2, CdO fume, and $NaVO_3$.

However, these studies were conducted at exposure levels more than 1000-fold higher (> 5 mg/m^3) than that expected for ambient PM environmental exposures which are on the order of < 1 µg/m^3. The majority of these studies were also conducted by direct instillation of aqueous mixtures containing metals. In general the

relevance of results obtained from instillation toxicology studies is given less emphasis in this critical review effort than results from controlled inhalation studies. It is difficult to comprehend the relevance of data obtained via instillation of high concentrations of metals and metal oxides in explaining potential adverse effects of ambient PM exposures which occur at considerably lower metals concentrations. In several cases, such as with exposures to metal fumes, ambient exposures in the general population are unlikely to occur at all.

Lambert et al. (2000) demonstrated that metals are responsible for the ROFA-enhanced allergic response of Brown Norway rats sensitized to house dust mite allergen. Goldsmith et al. (1999) compared the effect of inhalation of concentrated ambient PM multi-day exposure vs the effect of a single exposure to ROFA lecheate in ovalbumin sensitized mice and found that while ROFA exposure enhanced airway hyperresponsiveness, concentrated ambient PM did not.

Metals such as arsenic, iron, cadmium, copper, vanadium, and zinc as well as several metal oxide species are routinely found at detectable levels in ambient air PM samples in both the coarse and fine fractions (Eldred, et al., 1998). Iron and its various oxides are capable of catalyzing oxidant species generation both *in vivo* (Kadiiska et al., 1997) and *in vitro* (Smith et al., 1997; Halliwell and Gutteridge, 1999). Autopsy data collected in Mexico City during the 1980s shows an appreciable increase in metals content in body tissues compared to tissues examined in the 1950s (Fortoul, et al., 1996). In view of the marked pulmonary effects observed for residual oil fly ash (ROFA) following intratracheal instillation, the role of transition metals in particle toxicity deserves more extensive research to elucidate their role in cardiopulmonary toxicity.

Instillation studies have also clearly implicated adverse effects to both cardiovascular (bradycardia – Campen et al 2000; mild arrhythmias – Costa and Dreher, 1997; increased plasma fibrinogen – Gardner et al., 2000) and pulmonary (cell permeability, LDH increase, elevated inflammatory cell numbers and cytokines [IL-6, IL-10, MIP-2], histopathology – Kodavanti et al., 1999; Lambert et al., 1999) systems. Other investigators have found that transition metals may induce cytotoxicity and may

direct increases in inflammation independent of macrophage-derived cytokines (Monn and Beckert, 1999). Exposure to very high concentrations of PM fuel oil related combustion particles has been found to cause lung inflammation, cell and tissue injury, and changes in cell populations in a variety of laboratory settings in a variety of *in vivo* animal models (Costa and Dreher, 1997; Brain, et al., 1998) and a variety of *in vitro* studies (Li et al., 1996; Kennedy, et al. 1998). In many cases these effects appear to be related to the composition and concentration of soluble metals present in the exposure media (Kodavanti, et al., 1997b). It is reasonable to conclude that particle associated metals may play a role in PM toxicity. Additional work in this area may be warranted, especially in addressing exposures to ambient PM concentrations where metals content may be elevated.

5.2.3. Combustion Related Particles

Because of their known chemical composition, combustion related particles are suspected by most toxicologist and public health professionals to have the potential to be involved in a number of clearly identified adverse health effects. Careful and considerable attention has been paid in the toxicology literature to the examination of the toxic effects of PM derived from the combustion of diesel and other automotive/fossil fuel sources. Presumably this is a result of not only the inherently potential toxicity of these particulate species, but also the extent to which automotive emissions contribute to ambient PM ambient loadings in many areas of California, the United States and the world.

Studies in the experimental setting with combustion-generated particles as well as surrogate combustion-like particles have focused primarily on diesel exhaust and carbon black. The ultimate objective of the best-designed diesel and carbon black inhalation studies was to estimate cancer risk (Mauderly et al., 1994; Nikula et al., 1995). Therefore, the concentrations of diesel soot and carbon black were unusually high and for prolonged periods of time. However, these studies contained as a component of the experiments a dose-response analysis using three different concentrations of particles. The highest concentrations of diesel soot and carbon black

demonstrated marked pulmonary effects, including the presence of tumors in a subset of animals. These effects were attributed in part to the phenomenon of particle overload. The lowest concentration demonstrated minimal inflammatory or histopathological effects (Nikula et al., 1995).

A followup study using diesel exhaust and coal dust by inhalation in rats and monkeys demonstrated that monkeys differentially retain inhaled particles. In contrast to rats, monkeys internalize particles to the pulmonary interstitial space, while rats retain particles in the lumen of ducts and alveoli. This pattern of retention in rats was associated with greater inflammatory influx and hyperplastic epithelial changes as well as septal wall thickening, in large measure absent in the lungs of monkeys. These differences in tissue response of rats and monkeys to inhaled diesel particles may be indicative that rats may not be predictive of long-term particle retention and pulmonary

appear to be critical in driving many of the biological responses observed following exposure in animals by instillation or inhalation of this material (Kodavanti et al., 1996, 1997, 1998b, 1999, 2000a, 2000b; Kadiiska et al., 1997). A number of studies using ROFA in cell culture systems have also been published (Samet et al., 1996, 1997; Quay et al., 1998) to demonstrate the induction and/or disruption of signaling pathways leading to the expression of mediators involved in pro-inflammatory events.

For purposes of discussing applicability to the standards setting process, discussions of ROFA in this review are limited to aerosolization studies. Killingsworth et al (1997) exposed rats to ROFA for 6 hours/day for 3 days. Only rats pretreated with monocrotaline experienced death with exposure to ROFA. Neutrophils in BAL were significantly increased as well as an increase in macrophage inhibitory protein-2 (MIP-2) mRNA. Kodavanti et al. (2000b) found spontaneously hypertensive rats (SHR) to have an exacerbated ST segment depression in the electrocardiogram reading caused by exposure to ROFA. Muggenberg and colleagues (2000) found inhalation of ROFA in dogs for 3 hours/day for 3 days failed to demonstrate consistent changes in cardiac function. Subsequent experimental studies with carbon black have considered the importance of particle size in the ultrafine range on host toxicity. Studies of Li and colleagues (1996; 1997; 1999) have demonstrated that exposure to ultrafine particles at a concentration of 1000 $\mu g/m^3$ is associated with markers of inflammation and cellular injury.

In a variety of *in vivo* animal models (Costa and Dieher, 1997; Brain, et al., 1998) and a variety of *in vitro* studies (Li et al., 1996; Kennedy, et al. 1998) exposure to very high concentrations of PM fuel oil related combustion particles has been found to cause lung inflammation, cell and tissue injury, and changes in cell populations in a variety of laboratory settings. In many cases these effects appear to be related to the composition and concentration of soluble metals present in the exposure media (Kodavanti, et al., 1997b). However, results obtained from toxicology studies using high concentrations of PM with inherently elevated levels of metals, such as with ROFA, can

be difficult to extrapolate to ambient environmental settings for purposes of ambient air quality standards setting.

Combustion processes in general produce very complex mixtures of both complete and incomplete combustion products. The composition and concentration of these products vary greatly depending on the fuel consumed and the conditions under which the fuel is burned. To date there has been an inordinate emphasis placed on investigating the toxicity associated with the metals component of fuel oil combustion generated and combustion related particles and little or no emphasis in the literature placed on studying the potential toxicity of the many hundreds to thousands of other chemical constituents that may be involved in the toxicity of combustion PM.

It is important not to ignore PM derived from other combustion sources such as residential wood burning, forest biomass burning or second hand environmental tobacco smoke. Adverse health impacts from exposure to both primary and secondary tobacco smoke are well documented (Daisey, et al., 1994; Miller, et al. 1998). Adverse health effects have been observed in people such as firefighters who have been exposed to increased concentrations of wood smoke (Betchley, et al. 1997). Although particles from these combustion sources will fall to a great extent in the fine particle $PM_{2.5}$ size range of most measured ambient PM, particles derived from other combustion processes will vary to some degree in composition and may vary, as a consequence, in toxicity.

As of the development of this document, insufficient data on the toxicity of combustion related PM particles and particle mixtures in general exist to be able to extrapolate meaningful dose-response information for the purposes of revising or setting ambient air quality standards based on toxicology data alone. Important epidemiological evidence of combustion particle related effects may prove valuable in this effort. However, studies in toxicology which employ ambient PM concentrator technology offer new hope in bridging the gap between the complex exposures identified in epidemiological studies and the biological effects observed in controlled laboratory settings. Toxicology studies using ambient particle concentrator technology

in ambient environments where increased concentrations of combustion and combustion related particles from different sources are present may offer new insights and may be helpful in extrapolating from high concentration laboratory exposure settings to lower concentration ambient exposure settings.

5.2.4. Bioaerosols

Bioaerosols identified in ambient PM air samples include either whole or fragment portions of fungal spores, pollen, bacteria, viruses, endotoxins, plant and animal debris. Although they are routinely found in the coarse fraction of PM bioaerosols, including endotoxins, can also be found in fine particle fractions (Monn and Beckert, 1999). The health effects associated with bioaerosol exposure appear to be related more to *morbidity* effects rather than *mortality* effects in humans and animals. More than 20 allergen source materials were found in paved road dust and ambient PM collected in three urban areas of California (Miguel, A.G. et al., 1998). In this same study it was determined that on the order of 8% of the allergenicity of the PM_{10} samples examined was derived from paved road dust. In one study it was found that prior exposure to endotoxin can enhance the effects of exposure to fine and ultrafine PM in rats (Elder, 2000). Exposure of humans to endotoxin in largely occupational settings has resulted in increased lung inflammation (Michel, et al., 1997), increased broncho-responsiveness (Michel, et al., 1997), increases in systemic immune cell populations (Michel, et al., 1997), and significantly decreased forced expiratory volume and vital capacity (Michel, et al., 1997; Vogelzang, et al.,1998; Zock, et al., 1998).

Monn and Becker (1999) examined the importance of bioaerosols associated with particle size. Particles from both outdoor and indoor sources were collected by size-fractionation into coarse ($PM_{10-2.5}$) and fine ($PM_{2.5}$) mode particles. They found significant toxicity and cytokine production were induced by outdoor $PM_{10-2.5}$, but not by outdoor $PM_{2.5}$ or particles collected indoors. Pro-inflammatory cytokines, IL-6 and IL-8, were found to be 20 times more induced in human monocytes following treatment with coarse particles compared with fine particles or indoor particles. Lipopolysaccharide binding protein (LBP) completely inhibited cytokine induction by

$PM_{10-2.5}$, suggesting gram negative bacteria and/or endotoxins are biologically active components of coarse particles.

These findings highlight the importance and overall biological relevance of coarse particles (even of geologic origin) in identifying and explaining potential mechanisms which explain the adverse health effects seen with exposure to coarse particles. However, there seems to be little emphasis placed in the toxicology literature on understanding the role bioaerosols may play in explaining the adverse effects seen with ambient PM exposure. This may be due to the inherent national emphasis on understanding biological mechanisms to explain premature mortality and PM exposure. None-the-less, this is an area where considerable research may be needed to more fully explain the non-mortality effects observed in humans exposed to complex ambient PM mixtures. Bioaerosols have the potential to play a particularly significant role in understanding the adverse effects of PM exposure in asthmatic and allergic humans.

5.2.5. Geologic Dusts

Despite prior epidemiological evidence of adverse health effects associated with exposure to ambient PM concentrations primarily comprised of coarse fraction geologic dusts (Bar-Ziv and Goldberg, 1974) and evidence of adverse health effects in workers in occupational environments where high levels of geologic dust are present (Brambilla, et al. 1979; Sherwin et al., 1979), very little emphasis has been placed in the toxicology literature reviewed on examining the toxicity of geologic dusts alone or in combination with other PM or air pollutants of interest. This is somewhat troubling since geologic dusts may contain a variety of biologically active components, including bioaerosol endotoxins.

Recent studies of Becker and colleagues (Monn and Becker, 1999; Soukup and Becker, 2001) elegantly demonstrate the importance of insoluable components of ambient PM, including endotoxin, associated with the coarse particle fraction ($PM_{10-2.5}$) in the induction of pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor (TNF) α, and monocyte chemoattractant protein-1 (MCP-1). In

these studies coarse fraction PM induced levels 50 times higher than those seen with the soluble fractions of coarse PM or fine mode ($PM_{0.1-2.5}$) particles. Cytokine induction within the coarse mode fraction could be partially blocked by polymyxin B and lipopolysaccharide-binding protein (LBP). This is indicative of the role of endotoxin may play in increasing cytokine production in human alveolar macrophages following incubation with coarse particles (Soukop and Becker, 2001). It is interesting to note that a transition metal chelator deferoxamine did not alter cytokine production. This suggests that the cytokine production observed in these studies are independent of the presence of transition metals.

In a study of elderly cardio-compromised individuals living in the Coachella Valley in California (Ostro et al., 1999; 2000), investigators found increased incidence of mortality in populations exposed to PM comprised largely of geologic dust. A study conducted by Kleinman et al. (1995) demonstrated that phagocytosis and oxidant generation of respiratory macrophages can be inhibited by road dust coarse particle exposure. They also demonstrated that lung permeability, a measure of cell damage marked by changes in the protein albumin, was increased with road dust exposure in a dose-dependent fashion. This provides additional evidence of potential mechanisms by which coarse particles are associated with increased morbidity and mortality. Muggenburg and colleagues (2000) in a review of animal models of cardiac disease suggested a variety of possible pathways by which inhaled particles could induce cardiovascular morbidity and mortality. One potentially potent means could be through the induction of pulmonary inflammation and release of mediators. This could result in subsequent involvement of the myocardium or blood factors leading to coagulation and/or coronary vessel thrombosis (Muggenberg et al., 2000).

Geologic dusts may also play a role in increasing the dose delivered or affecting the site of delivery in the lungs of particles of potentially smaller size and greater inherent toxicity. Lastly, significant amounts of geologic dust can be found routinely in fine and ultra-fine particle fractions of California PM ambient air samples (California Air Resources Board, 1987; Rogers, et al., 1998). Further research on the

toxicity of geologic dust or the role they may play in affecting the toxicity of other PM components appears to remain warranted, especially in circumstances where they may be involved in the exacerbation of existing asthmatic, allergic or bronchitic conditions. It is premature at this point to discount the potential role geologic dusts and other coarse particles may play in explaining the adverse health effects seen with ambient PM exposure in the epidemiological literature. In general, exposure to coarse and fine particles containing PM of geologic origin, in a variety of experimental settings, has been shown to increase inflammation, increase tissue permeability, and decrease macrophage activity. These effects appear to be independent of the metals content of the PM exposure media (Monn and Becker, 1999; Soukop and Becker, 2001).

5.2.6. Concentrated Ambient Particles

A number of investigators have examined the effects of concentrated ambient particles (CAPs) in healthy and compromised animal models. Godleski (2000) and others examined the effects of concentrated ambient PM on healthy and coronary artery occluded mongrel dogs. The concentration of CAPs ranged from 69 to 828 $\mu g/m^3$ during the period of exposure for 6 hours/day for 3 days. Although direct pulmonary effects in healthy dogs were not seen in these studies, significant changes in heart rate variability, decreased heart rate, and an increase in the indices related to ventricular fibrillation sensitivity were observed. In coronary artery occluded dogs increases in ischemic stress of cardiac tissues from repeated exposure to ambient PM was also observed. Clarke and colleagues (2000) used a similar approach to expose dogs via a tracheotomy to CAPs for up to 3 days. They found that peripheral blood parameters were related to specific particle components. However, blood changes were not associated with increases in total CAP concentration (Clarke et al., 2000). Concentrated ambient particles in this experiment had a maximum average concentration of 360 $\mu g/m^3$.

Further studies have been done in animals with CAPs. Kodavanti and associates (2000a) have examined the effects of CAPs (650 $\mu g/m^3$) in rats made bronchitic through prior exposure to SO_2. However, these studies were carried out

using intratracheal instillation of the particles, thus removing some of the significance of their findings due to a dramatic difference in the route of exposure to CAPs. Increases in bronchoalveolar lavage fluid (BALF) protein and neutrophil influx were observed in these studies.

Others have investigated the effects of concentrated ambient PM exposure in both dogs (Muggenberg et al., 2000) and rodents (Gordon et al., 2000). The results of these studies appear to contradict those found in Godleski's work with dogs. In studies with rats and hamsters exposed to concentrated New York ambient PM, no animal deaths occurred and no changes in heart electrocardio parameters were observed (Gordon, et al., 2000). In these studies heart rate was increased rather than decreased. Although, peripheral neutrophils as well as blood platelets were elevated compared to controls. The neutrophil effects observed did not occur on all exposure days which suggests that daily variation in particle concentration and/or composition are likely important in understanding the health effects associated with ambient PM exposure. In other studies using dogs, no affects were observed as a result of concentrated ambient PM exposure (Muggenberg et al., 2000). These findings serve to highlight one of the fundamental limitations of concentrated ambient PM toxicology study designs which is the inability to control particle concentration and composition on a daily basis and/or over any given exposure time frame. Despite somewhat inconsistent findings in day to day exposures, studies such as these do suggest that exposure to concentrated ambient particles can have systemic effects in animals. Such effects include changes in heart rate and systemic blood parameters (Godleski et al., 2000).

Very few investigators have examined the effects of concentrated ambient PM in humans. In one study inhalation of concentrated ambient PM from Chapel Hill, NC caused increased levels of blood fibrinogen in healthy non-smokers (Ghio, et al, 2000). Although North Carolina is not a region of the country noted to have high PM levels or an area associated with PM-induced health effects to its inhabitants, the levels of CAPs generated during exposure were sufficient to lead to pulmonary and systemic

effects. Such studies may provide useful information to delineate the precise particle constituents resulting in alterations in cardiorespiratory function.

In general the results from toxicity studies conducted using concentrated ambient particulate technology are mixed. In some cases they are contradictory. Based on the literature available to date, it is difficult to determine at this time if the different results found in the various studies conducted using concentrated ambient particles in both humans and animals reflect differences in the ambient PM composition, differences in ambient concentrations on exposure days, inherent physiological differences between the exposed animal models and humans, differences in the exposure conditions or the inherent toxicity of the PM components themselves.

Despite the inherent challenges in interpreting toxicology studies conducted using concentrated ambient particle technology, particle concentrator technologies if used in concert with other toxicology research methods have the potential to provide an increasingly important bridge between epidemiological based studies and carefully controlled laboratory toxicology studies. The key to addressing these challenges lies in careful exposure and animal model characterization for every study conducted. If resources are available, large data sets derived from extensive studies conducted throughout California with different animal and as appropriate human model exposure studies, have the potential to provide valuable dose-response information necessary to adequately asses the current ambient air quality standards.

5.2.7. Particulate Matter Effects in Combination with other Pollutants

With respect to public health protection and the ambient air quality standards setting process, the greatest concern regarding particle effects in combination with other pollutants is the potential for other air pollutants to increase the toxicity of PM component species. The existence and nature of any pollutant interactions are expected to depend on the pollutant mixture, physical particle forms, the size and composition of particles present, the adverse health effect or biological

endpoint of concern and the atmospheric conditions under which the mixed pollutant exposure occurs.

At the time of this report, there are no clearly and consistently identified PM and co-pollutant interactions. In general, oxidant gases such as ozone and acidic vapors tend to potentiate the effects of fine and ultrafine PM on markers of inflammation (Elder et al., 2000), cell injury (Kleinman et al., 1999; Elder et al., 2000), oxidative stress (Elder et al., 2000) and immune system macrophage function (Jakab, et al.; 1996) in animal exposure studies using laboratory generated particle and gas mixtures. However, ozone alone or ozone combined with acidic particles have been shown to produce similar effects (Last and Pinkerton, 1997). In general, no long term changes in lung function have been associated with PM exposures in combination with other pollutants.

In animal inhalation studies using dogs exposed to ambient particulate mixtures containing other gaseous pollutants, no specific effects could be tied to the combined presence of PM and oxidant gases in some exposures settings, while effects on neutrophils and lymphocytes were observed in others (Vanda, et al., 1998). In ambient level animal inhalation studies using pigeons alveolar macrophage were increased and effects in epithelial cells were observed (Lorz and Lopez, 1997). In similar studies using lambs morphological changes in the epithelial lining and in mucous secretion was observed (Gulisano et al., 1997). In these studies the variability in ambient PM component of the air pollutant mixture and the potential differences in the animal models studies make it difficult to assess the direct effects of the presence of other pollutants and to extrapolate to potential human exposures.

Other studies have examined the effects of combined exposure of resuspended aerosolized particles with other criteria pollutants such as ozone. Although investigators have used very high concentrations of resuspended particles in the exposure studies, it is of significant interest that the toxic effects of exposure were enhanced with concomitant exposure to ozone (Vincent et al., 1997). These toxic effects were identified as an increase in alveolar septal wall cellularity and thickness

(Vincent et al., 1997). The elucidation of the potential mechanisms responsible for the interaction of gases with airborne particles such as the concentration, duration, and physicochemical characteristics of particle exposure conditions (Schlesinger, 1995) certainly will require more research in the future.

In general the results seen in the toxicology literature with respect to PM in combination with other air pollutants are varied. They appear to often depend on the animal models examined and the adverse health effect or biological endpoint studied. However, it is important to keep in mind that no matter whether the presence of other air pollutants directly enhances the toxicity of PM or not, the presence of additional pollutants in the ambient environment has the potential to increase the overall pollutant induced stress in any population. This enhanced pollutant stress may play a role in how animals or humans respond to complex ambient PM mixtures in biologically relevant settings. This may be of particular importance to the standards setting process when one considers the possible impacts on those in our populations who are physically compromised or somehow inherently susceptible.

5.3 Effects of Particle Size

A variety of investigators have attempted to address the question of relative toxicity and particle size but only a very few investigators have systematically investigated the effects of varying particle size while keeping the PM composition or components the same. Results of these efforts support the general finding that increasingly smaller particles of the same composition, i.e. ultra-fine vs fine, show a significantly greater biological response and/or toxicity at the same or similar mass dose delivered (Oberdörster, et al., 1992; Li, et al., 1999). This finding has been linked directly to surface area in both *in vivo* and *in vitro* studies (Lison et al., 1997; Oettinger et al, 1999). This has implications with respect to control of diesel particle sources. Current control technologies reduce total PM mass, but the size of the particles released are significantly smaller in size and may be greater in number. However, in cases where particle size has been fixed and directly compared to varying compositions

of metal fumes, the PM composition appears to play a greater role in determining overall toxicity (Kuschren, et al., 1997).

Recent work from the laboratory of Becker (Monn and Becker, 1999; Soukup and Becker, 2001) provide further evidence that particle composition is more important than particle size in explaining the biological mechanisms involved in some adverse health effects. In these studies ambient coarse particles produced stronger biological effects than did fine particles. In these studies investigators found that cellular cytotoxicity and inflammation were induced, while macrophage function was inhibited through endotoxin or endotoxin-like components associated with both insoluble and soluble coarse particle components. The effects induced by insoluble PM coarse components were much higher (>50 fold) than those seen with soluble PM coarse components. They were also greater than those observed with insoluble PM fine components. This biological activity was not present with soluble fine or ultrafine particle components. In these studies significant toxicity and cytokine production (20 times) was induced by coarse outdoor PM but not by outdoor fine $PM_{2.5}$ or by particles collected indoors. Cytotoxicity was inhibited by transition metal chelators but cytokine production was not. Lipopolyscharide binding protein completely inhibited cytokine induction by coarse PM. This suggests that coarse PM components such as gram negative bacteria and/or endotoxins may have been present. The results of this work suggest that proinflammatory effects of endotoxins in PM coarse fraction on macrophages may upset lung homeostatsis while metals-induced cytotoxicity in coarse PM may set up inflammation independent of macrophage-derived cytokines.

Coarse size-fractioned resuspended ambient particles were found by Ohtoshi 1998 and Takano et al, 1998 to induce upregulation of the immune system in mice by increasing the production of IL-8 and granulocyte macrophage colony stimulating factor. The investigators in these efforts suggest that diesel exhaust particles in the coarse fraction material may be responsible for these effects. Experimental studies with carbon black have considered the importance of particle size in the ultrafine range on host toxicity. A study by Brown and colleagues demonstrated

that intratracheal instillation of ultrafine particles (14 nm diameter) of carbon black (125 mg total mass) compared to an identical mass of fine (260 nm diameter) carbon black particles was associated with more significant markers of inflammation and oxidant stress (Brown et al., 2000). A more recent study by Renwick et al. (2001) demonstrated that ultrafine carbon black particles significantly impair macrophage phagocytosis compared to fine carbon black particles, thereby providing a functionally based biologically plausible mechanism for slowing particle clearance from the lungs.

5.4 Effects in Sensitive and/or Susceptible Animal Models

Information obtain from epidemiological studies suggests that there are members in human populations who may be more sensitive, for some reason, to the effects of PM exposure. Those who may be more sensitive include the elderly, especially those with chronic cardiopulmonary conditions, and people with asthma bronchitis or pneumonia. Although the majority of toxicology studies reviewed were carried out in healthy animals or humans, more recent work with animal models that more closely mimic the effects of the compromised human has been carried out.

5.4.1. Effects in Cardiopulmonary Compromised Models

Treatment with monocrotaline (MCT) has been used to produce animals with highly compromised lung function. In MCT treated rats Costa and Dreher (1997) found that instillation of higher concentrations of ROFA particles (0-2.5 mg/rat) caused a marked increase in neutrophilic inflammation and in come cases increased mortality. In some more recent studies Kodavanti et al. (1999) found that MCT rats treated with lower concentrations of instilled ROFA particles (0-3.3 mg/kg) and more concentrated ROFA particles (15 mg/kg) by direct inhalation resulted in direct lung effects including lesions, as evidenced by edema, increases in alveolar wall thickening and an increase

on the lungs and can increase mortality rates in MCT treated animals. However, investigators have reported that MCT appears to impair macrophage chemotaxis. Impaired macrophage function reduces particle clearance. In the case of MCT treated animals this may serve to increase the effective dose of particles delivered over time. This makes these studies much less valuable in establishing dose-response relationships. These studies are none-the-less helpful in identifying what biological mechanisms may be involved in these particular exposure settings.

Investigators have developed a number of animal models mimicking chronic bronchitis by exposing laboratory animals to sulfur dioxide (SO_2) prior to PM exposure. Clarke et al. (1999) exposed SO_2 treated Sprague Dawley rats to concentrated ambient particles for 5 hrs/day over a three-day period at an average concentration of 515 $\mu g/m^3$. Exposure to concentrated ambient particles under these conditions produced significant changes in both cellular and biochemical measures in bronchoalveolar lavage fluid. These included increased numbers of neutrophils and lymphocytes. No animal deaths occurred in these studies. Kodavanti and colleagues (1998) also examined the effects of concentrated ambient particles in normal as well as the SO_2 induced bronchitic rats. In these studies investigators found a significant increase in lavage fluid protein and increased neutrophil counts in both normal and bronchitic animals. These changes are reflective of direct inflammatory effects of concentrated ambient PM in the lungs of SO_2 treated rats.

Genetically predisposed spontaneously hypertensive rats have been used as a model of cardiovascular disease in PM toxicology studies. Spontaneously hypertensive rats have been found to be more susceptible to acute pulmonary injury from instilled ROFA (Kodavanti et al., 2001). Differential effects of metals constituents of ROFA (vanadium and nickel) were found in control and spontaneously hypertensive animals. In both models vanadium was less toxic than nickel although vanadium exposure in ROFA caused inflammatory responses in only the control Wistar Kyoto controls. Nickel exposure apparently caused inflammation in both the control and spontaneously hypertensive rats. When exposed to ROFA via inhalation the

spontaneously hypertensive rats exhibited a hemorrhagic response (Kodavanti et al., 2000b). The spontaneously hypertensive rats also demonstated increased oxidative stress and a compromised ability to increase essential protective biochemicals that counteract damaging cellular oxidative action. Evidence such as this supports the conclusion that cardiovascular disease models in animals may play a role in increased susceptibility to ROFA particle exposure.

5.4.2. Effects in Hyperreactive Airway Models of Allergy and Asthma

Investigators have developed a number of animal models mimicking hyperresponsive air ways, a central feature of asthma, by initially sensitizing animals to ovalbumin. Goldsmith et al. (1999) examined the effects of concentrated ambient particles on normal and ovalbumin-sensitized mice and found no effects of PM exposure to methylcholine challenge responsiveness in either group. The same investigators also examined the effect of exposure by inhalation to the soluble fraction of ROFA in normal and sensitized mice. In these studies investigators found that exposure to ROFA could produce nonspecific airway hyperresponsiveness to methylcholine challenge in both groups. Other investigators have found similar effects in other normal and sensitized rodents (Gavett et al., 1997, 1999; Hamada et al., 1999, 2000). Studies by Takano and colleagues (1998) have also found exposure of mice to diesel exhaust particles could enhance allergen-related eosinophil recruitment and airway hyperresponsiveness.

5.4.3. Effects in Animal Models of the Aged, Young and Developing Respiratory Systems

Studies by Clarke et al. (2000) demonstrated age-related differences in the respiratory response of F344 rats to CAPs. In this study, young rats (6-8 weeks old) compared to older rats (17 months of age) had significantly higher BAL cell counts as well as significant increases in neutrophil number following CAPs exposure. Although older rats also had a small, but significant increase in neutrophil number, their advanced age was associated with significant decrements in total BAL cell count, total white blood

cell count and the percent of blood lymphocytes. Therefore, young F344 rats may represent a sensitive strain of rat for the detection of pulmonary inflammatory response to CAPs. In contrast, the lack of a pulmonary inflammatory response in aged F344 rats despite a higher percent of circulating neutrophils may reflect a decreased sensitivity to inhaled particles (Clarke et al., 2000). Although such a finding may seem paradoxical in the face of a suspected increased sensitivity of older individuals to the effects of airborne particles, a reduction in immune and/or inflammatory response in the aged could lead to a persistence of particles in the lungs with potential long-term consequences. Such age-related differences should be explored further.

5.5 Observed Biomarkers of Effect

There are many biomarkers of biological effect used in toxicology studies. The biomarkers investigated depend on the adverse health effect or anticipated biological mechanism suspected to be involved in causing the effect of interest. Some are markers of direct effects on cells or tissues. Others are representative of gross systemic effects.

5.5.1. Direct Effects on the Respiratory Tract

A variety of studies conducted using both normal and compromised animal models exposed to concentrated ambient particles have shown little effect on overall pulmonary function (Godleski et al., 2000; Clark et al., 1999; Gordon et al., 2000). Kodavanti et al. (1996 and 1997a) have demonstrated direct effects in the lungs of three different strains of rats exposed to ROFA via instillation. Results from these studies show focal areas of lung damage reflecting inflammatory cell infiltration as well as alveolar, airway and interstitial thickening in all three rat strains. One of the isoforms of fibronectin mRNA was upregulated in ROFA exposed Sprague Dawley and Wistar rats but not in Fischer 344 animals. These studies suggest that rat strain as an indicator or genetic variability may play a role in fibrotic response to instilled ROFA. Other investigators have investigated the question of interstrain variability or susceptibility to ambient particles (Shukla et al., 2000) in mice. No interstrain

differences in response were observed although ambient particle exposure resulted in increases in inflammatory cytokines and mRNA cytokine expression in both strains exposed to ambient PM.

5.5.2. Immune System Effects

NFκB is the pivotal transcription factor to regulate gene expression involved in inflammatory, immunoregulatory, stress response and cell survival. These genes include cytokines, chemokines, adhesion molecules, enzymes and acute phase reactants. NFκB consists of five subunits, p65, p50, rel-c, p52, and rel-b. Different forms of NFκB may activate different sets of target genes. Activation of NFκB may play a key role in biological responses. Cytokines, lipopolysaccharide (LPS) and oxidants such as tetrachlorodibenzo-P-dioxin (TCDD) (Puga, et al., 2000), benzo(a)pyrene (Banerjee, et al., 2000), as well as particulate matter (Shukla et al, 2000; Kennedy, et al. 1998) have been shown to activate NFκB. In contrast, inhibition of NFκB will lead to the down regulation of gene expression which may result in immunosuppression (Beauparlant et al., 1996; Barnes et al., 1997). An understanding of how inhaled particles impact on NFκB may provide a critical key to potential mechanisms leading to adverse effects in the respiratory and cardiovascular systems. This signaling pathway may also have important implications on particle effects to alter fetal and early childhood development in neuro/immune/respiratory structure and function. Therefore, expression of the transcription factor NFκB during perinatal development correlated with changes in glutathione-S-transferase (GST) activity, oxidative stress, and cytokine levels may be critically linked as part of essential steps to bring about development of the pulmonary, immune and neural systems.

Diesel particle exposure may also act an as adjuvant to increase the immune response system to antigenic challenges in mice and humans (Takano et al., 1997; Diaz-Sanchez et al, 1996, 1997; Oshtoshi et al., 1998; Wheatly and Platts-Mills, 1996). In *in vitro* studies with transformed IgE-producing human B lymphocytes Tsien et al. (1997) found that treatment with the organic fraction of diesel PM had no effect on

cytokine poroduction but did increase IgE production. The investigators noted that the diesel PM appeard to be acting on cells already producing IgE which suggests a mechanism by which the organic fraction of combustion particles may directly affect B cells and influence human allergic asthma. Worked conducted by Sagai and colleagues in 1996 with mice exposed via instillation to diesel PM appears to confirm the results of Tsien et al. (1997).

However, only a very few studies have examined the effects of ambient particles on allergic asthma. Coarse size-fractioned resuspended ambient particles were found by Ohtoshi 1998 to induce upregulation of the immune system in mice by increasing the production of IL-8 and granulocyte macrophage colony stimulating factor. Takano et al, 1998 found increases in airway inflammation, hyperresponsiveness, and increased production of IL-8 and granulocyte macrophage colony stimulating factor in mice exposed to diesel exhaust. The investigators in these efforts suggest that diesel exhaust particles in the coarse fraction material may be responsible for these effects.

Other investigators have found alterations in the immune response, airway hyperresponsiveness and an increase in eosinophil numbers in ovalbumin sensitized mice exposed to ROFA via instillation (Gavett et al, 1999 and Hamada et al., 1999, 2000). Lambert and colleges (1999) examined the effects of ROFA exposure via instillation on rats sensitized with house dust mite allergen. In these studies investigators found increased eosinophils, intracellular enzyme and protein components as well as IL-10. Lambert et al. (2000) demonstrated that metals are responsible for the ROFA-enhanced allergic response of Brown Norway rats sensitized to house dust mite allergen. Goldsmith et al. (1999) compared the effect of inhalation of concentrated ambient PM multi-day exposure vs the effect of a single exposure to ROFA lecheate in ovalbumin sensitized mice and found that while ROFA exposure enhanced airway hyperresponsiveness, concentrated ambient PM did not. Studies such as these suggest that exposure to ROFA via instillation may enhance allergic response in animals previously sensitized by antigenic materials.

5.5.3. Systemic, Physiological, Cardiorespiratory and Cardiovascular Effects

Of the toxicology studies designed to address the question of systemic effects, the majority reviewed to date focus on systemic effects related to the cardiorespiratory or cardiovascular systems. In the majority of studies examined compromised animal models have been exposed either via inhalation or instillation to different types of resuspended PM source materials. The source materials used include residual oil fly ash (Kodavanti et al., 1998b; 1999; 2000b; 2001), ambient PM air samples (Costa and Dreher, 1997), and volcanic ash (Watkinson et al., 2000). Other studies have focused on the effects of concentrated ambient PM as well as unconcentrated ambient PM exposures (Goldsmith et al., 1999; Clark et al., 1999; 2000; Kodavanti et al., 2000a).

The compromised animal models examined in these studies include monocrotaline treated rats, which serve as a model for cardiorespiratory disease, rodents with pulmonary inflammation induced by ozone exposure, spontaneously hypertensive rats, and rodent models of aged animals. Occlusion of the left descending coronary artery in the dog may also mimic closely the human condition. This model may also be be useful to determine if subsequent exposure to particles further affect cardiac function. Effects observed under these exposure conditions include mild to severe arrhythmias (Costa and Dreher, 1997; Watkinson, et al., 1998; 2000), bradycardia (Campen et al., 2000), increases in plasma fibrinogen (a protein integral to blood clotting) (Gardner, et al., 2000), increases in pulmonary inflammation (Costa and Dreher, 1997; Killingsworth, et al., 1997), and even animal death (Killingsworth, et al., 1997; Watkinson, et al., 1998; 2000).

Other systemic effects have been observed in other animal models. Gordon et.al (2000) examined the response of rats and hamsters to concentrated New York ambient PM and found that peripheral neutrophils as well as blood platelets were elevated compared to controls. However, direct cardiac effects were not noted in these species (Gordon et.al, 2000). Other models that may offer additional insights include

hereditary cardiomyopathy in Syrian hamsters as well as murine (mouse) models of viral and mycoplasmal myocarditis (Muggenberg et al., 2000). One potential mechanism that may be involved which might explain adverse cardiac effects seen in the epidemiological literature is the induction of pulmonary inflammation and release of biochemical mediators. This could result in the subsequent involvement of the myocardium or blood factors leading to coagulation and/or coronary vessel thrombosis (Muggenberg et al., 2000).

Exposure levels used in the majority of these studies, especially of ROFA, were many times that expected to be found in ambient exposure settings. The most severe effects were seen only with instillation ROFA exposures in monocrotaline treated animals (Kodavanti et al., 1999). This is a significant finding since as a result of the initial treatment monocrotaline treated animals are typically severely compromised. Although results observed using these types of studies may not be directly representative of effects expected for typically compromised humans exposed to ambient levels of PM, these studies do provide valuable insight into possible mechanisms of PM action in animals. Although few other systemic effects of PM exposure have been investigated, in general it can be concluded that exposure to PM from combustion processes and both concentrated and unconcentrated ambient PM can result in systemic effects that may play a role in explaining the cardiorespiratory effects in compromised humans seen in the epidemiological literature.

6.0 Particulate Matter Exposure and Childhood Development

Development of the human respiratory system involves the formation of a highly ordered airway branching system with 25,000 distinct terminations giving rise to more than 300 million alveoli as well as the differentiation and proliferation of over 40 different cell types. The transition of the lungs from a simple protruding bud of tissue from the foregut into a highly organized, integrated, complex structure that is innervated, ventilated and vascularized is a multi-step process.

The development of the lungs begins with the evagination of an avascular epithelial bud and subsequent growth into surrounding mesenchymal tissues. Following embryogenesis, the fetal lungs in all mammalian species undergo three anatomically distinct stages of growth termed pseudoglandular, canalicular and saccular (Figure 1, Burri, 1999). Although the lungs have developed sufficiently to sustain life at birth, growth is far from complete. Approximately 80 percent of alveoli in the adult lung arise postnatally. In essence, lung development is a continuum from embryogenesis through early adolescence.

The stages of lung development are controlled by a variety of factors that modulate the timing and pattern of cellular proliferation and differentiation as well as branching morphogenesis. Recent reviews by Hackett and Gitlin (1997), Shannon and Deterding (1997) and Ramon (1998) stress the importance of a number of transcription factors, molecular signals and soluble factors in orchestrating the developmental process of the respiratory tract. These molecular signals are expressed in both a temporal and spatial pattern to facilitate normal lung development and to regulate epithelial-mesenchymal interactions, cellular proliferation, extracellular matrix deposition and composition, growth factor and receptor expression, as well as cell-to-cell interactions.

Exposure to a variety of toxicants and/or conditions during lung development has the potential to significantly affect the overall growth and function of the respiratory system in children. The target of a toxic insult to the lungs during development is likely to involve the disruption and/or alteration of a specific molecular signal or transcription factor, but to date, little information is available as to the precise effect of such exposures. However, timing of exposure during development appears to be critical in the subsequent effects observed. For example, maternal malnutrition during gestation may significantly retard fetal growth and the development of the lungs leading to compromised lung function throughout life. In contrast, exposure to environmental toxicants such as second-hand cigarette smoke may actually accelerate the maturation of specific cell types in the fetal lung Ramon (1998), but the effects of such a change on overall lung function in the newborn to the adult are unknown. In general little-to-nothing is known regarding the effects of maternal exposure of airborne particles on the fetus.

A number of studies suggest that the processes of cellular differentiation, branching morphogenesis and overall lung growth can be affected by exposure to chemicals and particles. Both embryogenesis and fetal gestation represent critical periods of cellular differentiation and branching morphogenesis. The effects of exposure, however, are likely to be different for each period of development. For example, during embryogenesis and fetal development, cell number, cell type and cell function of the airways and alveoli may be significantly affected by exposure to a diverse number of substances and/or conditions. However, since cells continue to differentiate and divide during the postnatal period, chemical exposure during the postnatal period is also likely to affect the respiratory system, but in a different manner based on changes in the process of differentiation and morphogenesis (Smiley-Jewell et al., 1998). Since growth is essentially complete by the end of adolescence, exposure to chemicals and other factors are likely to have completely different consequences in the adult compared to that found in children (Smiley-Jewell et al., 1998; Plopper et al., 1994; Fanucchi et al., 1997).

Exposure to substances during critical windows of development may have profound effects that would not be seen if the same exposure were to occur in the adult. Since lung development occurs over the entire perinatal period, exposure effects can have significant consequences whether they occur during the pre- or postnatal period of life. Although our understanding of these changes at this time is extremely limited, it makes sense that abnormal developmental changes which occur in the perinatal period due to exposure to a variety of toxic chemicals, such as PM, may have long-term effects persisting into adult life.

6.1 Potential Targets of Toxic Agents During Lung Development

The effects of many toxicants on the respiratory system have been well characterized in the adult. Although less is known about the effects of toxicants in the developing lung, a number of toxicants are known to affect the developing lungs. These include environmental tobacco smoke (Gebremichael et al., 1995; USEPA 1992; Joad et al., 1995, 1999), bioactivated compounds (Smiley-Jewell et al., 1998; Plopper et al., 1994; Fanucchi et al., 1997), and oxidant gases (Randell et al., 1989; Gunnison et al., 1992). The target for a number of these compounds are in large measure airway epithelial cells undergoing maturation and/or rapid proliferation. However, the precise mechanism leading to greater sensitivity of these cells in the neonate compared to the adult is still unknown. The impact particles may have on lung, cardiovascular, immune development is expected to vary from that of adult animals although the actual effects are not known.

6.2 Factors That Affect Lung Development

To better understand the potential effects of critical windows of exposure in children on the respiratory system, it is important to consider the following factors that characterize the process of lung development. First, lung development is a multi-event process that is not restricted to prenatal life. Although the lungs undergo dramatic

changes during the embryonic, pseudoglandular, canalicular and saccular stages, the majority of changes to the lungs continue postnatally during the process of alveolarization. Second, only a limited number of maturational events must be finished at birth for successful survival of the organism. Third, cellular differentiation, branching morphogenesis and overall growth define lung development during both the pre- and postnatal periods. Finally, all these developmental events occur in the presence of an increasing mass of total cells.

6.3 Knowledge Gaps and Susceptibility of Children

The susceptibility of children to exposure to a wide range of environmental toxicants cannot be based on studies in adults. Cellular differentiation, cellular proliferation and cellular physiological function of the lungs are continually changing during gestational and postnatal growth. The sensitivity of these cells and their response to environmental insults including ambient airborne particles are likely to be completely different compared to that found in the adult. The route of delivery of particulate matter as well as other environmental toxicants to the respiratory system is completely different during the fetal period compared to the postnatal period. Influences of passage through other organ systems and the vasculature as well as through maternal organ systems must be taken into consideration. Our knowledge base regarding perinatal exposure and critical windows is negligible (Figure 2). Without question, there is still much to be learned about the effects of toxicants on gene regulation, molecular signaling and growth factors during lung development. Without the careful control and proper timing of expression for these factors, growth could become misdirected and chaotic. Future studies must be designed to address these critical windows of exposure to airborne particles to provide meaningful answers for the benefit of healthy children into adulthood.

Figure 1: Stages of Lung Growth

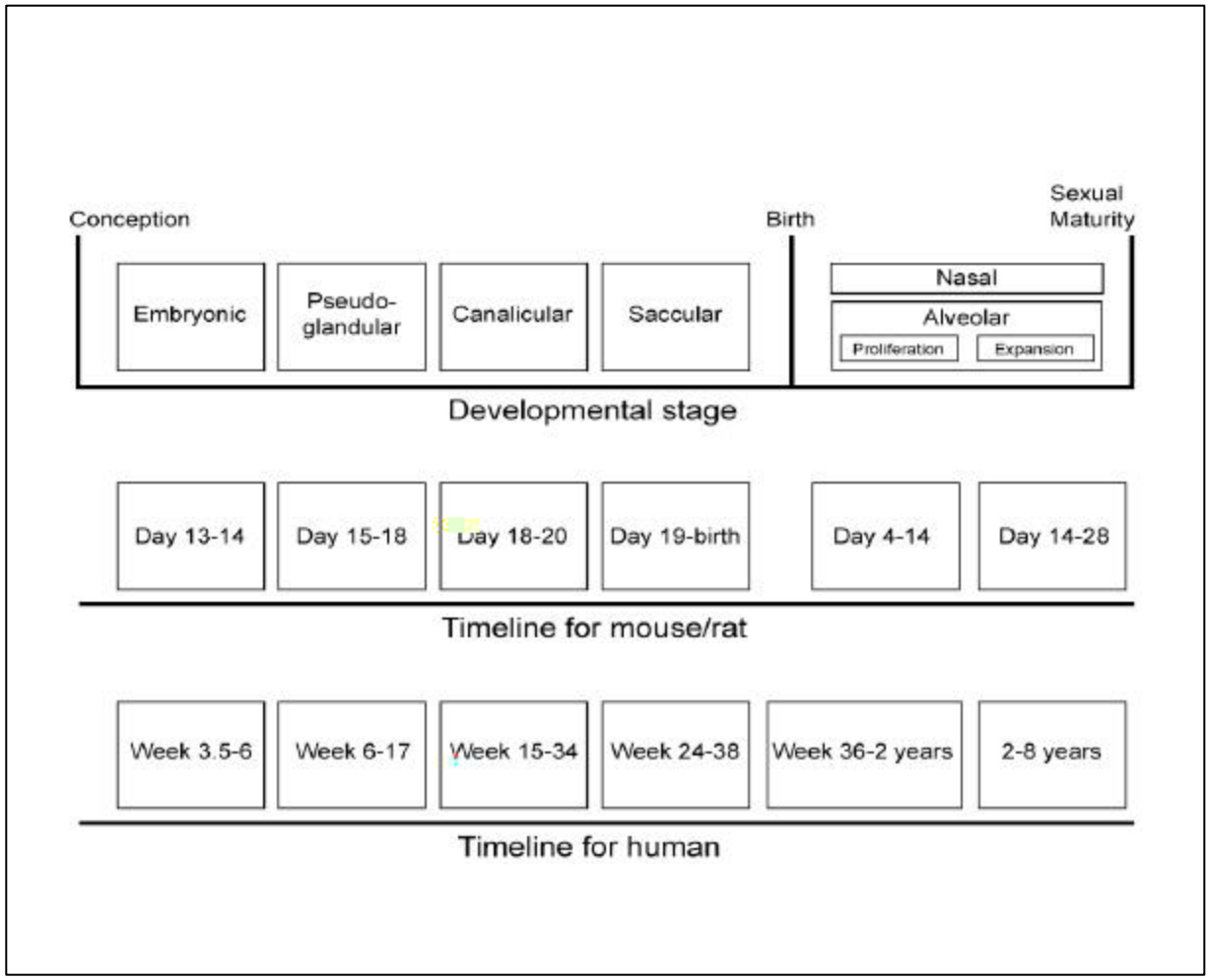
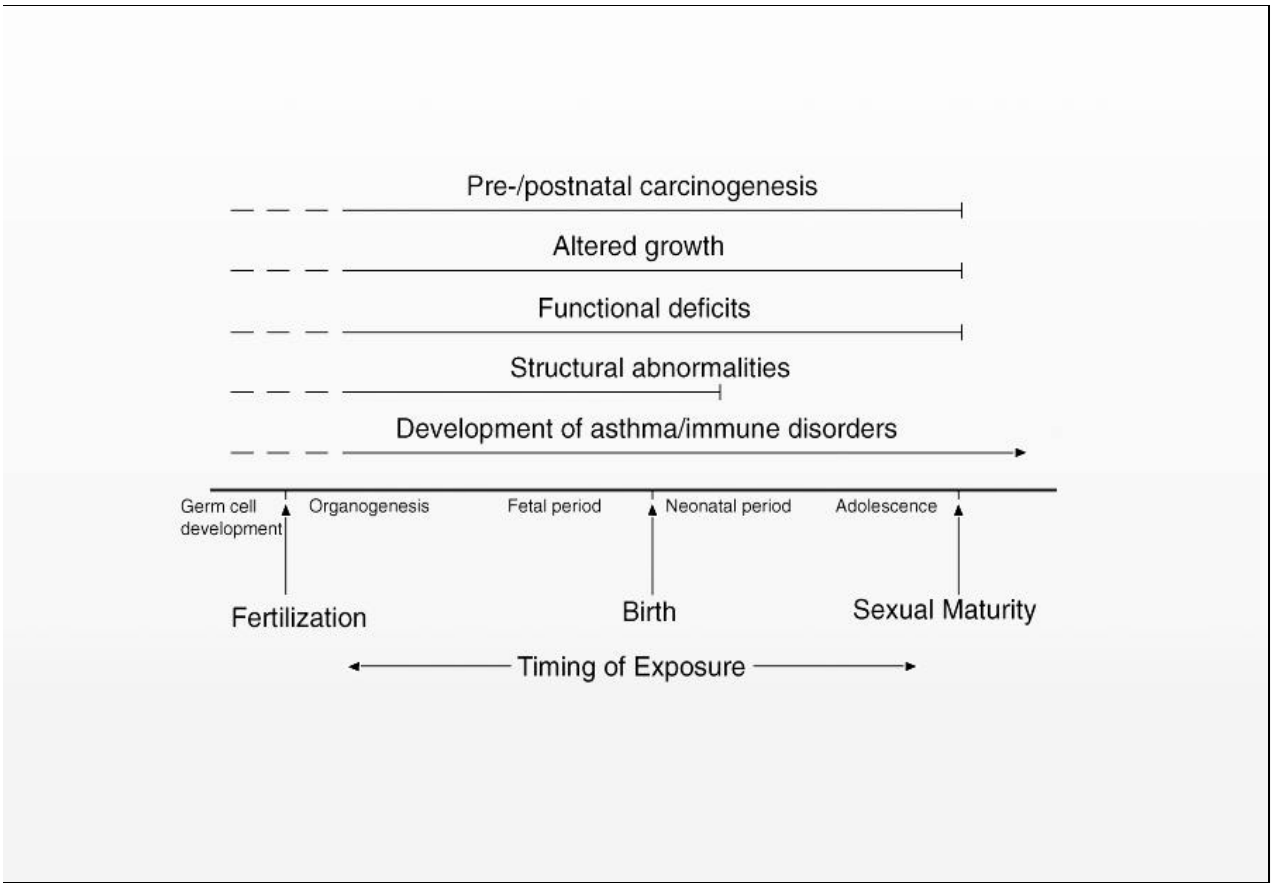


Figure 2: Critical Windows of Exposure



7.0 Potential Mechanisms of Particulate Matter Action and Biological Plausibility

Toxicological studies play an essential role in addressing the question of biological plausibility of PM health effects. However it is important to remember that associations between ambient particulate matter air pollution exposure and many different health effects, both short and long term, have been documented in the epidemiological literature. The key to assessing the validity and applicability of any toxicological study in addressing biological plausibility questions is to identify whether the PM component or components used in the study are: 1) reasonably expected to be found in related concentrations in the ambient air environment, and 2) realistically expected to be involved in mechanisms of biological action in the body which may be responsible for observed health effects in human populations. To date few toxicology studies have successfully addressed both criteria. Numerous studies have attempted to address the later.

7.1 Direct Effects on the Lung

Many investigators have reported direct effects of PM toxicity in the lung. The weight of evidence to support this premise has grown substantially in the last few years. A number of investigators employing a variety of PM exposure methods using chemically distinct PM components have documented direct pulmonary effects in several different animal species including humans. The direct pulmonary effects which have been observed include general as well as site specific cell and tissue injury (Brain, et al., 1998; Costa and Dieher, 1997; Clarke et al., 1999; Gulisano, et al., 1997; Jakab, et al., 1996; Kennedy, et al., 1998, Kleinman et al., 1999; Kodavanti et al., 1996, 1997, 1998, 1999, 2000b, 2001; Lambert et al., 1999; Mauderly, 1993) increased production of inflammatory chemical species leading to increased pulmonary inflammation (Brain, et al., 1998; Clarke et al., 1999; Costa and Dieher, 1997, Fine et al, 1997; Mauderly, 1993; Michel, et al., 1997; Monn and Beckert, 1999; Kennedy, et al., 1998; Kodavanti et

al., 2001; Li et al, 1997; Osier, 1997; Osier and Oberdorster, 1997; Soukop and Becker, 2001; Watkinson et al., 2000; Vogelzang, et al.,1998; Zock, et al., 1998), increases in airway tissue reactivity leading to exacerbation of existing respiratory conditions such as asthma (Diaz-Sanchez et al, 1996, 1997; Goldsmith et al., 1999; Gavett et al., 1997, 1999; Hamada et al., 1999, 2000; Irisgler et al., 1999; Lambert et aI., 2000; Michel, et al., 1997; Oshtoshi et al., 1998; Takano et al., 1997; Tsien et al., 1997; Vogelsang, et al., 1998; Wheatly and Platts-Mills, 1996; Zock et al., 1998), and decreased phagocytic activity of alveolar macrophages or other changes in immune cell populations leading to increased host susceptibility to respiratory infections (Beauparlant et al., 1996; Barnes et als., 1997; Clarke, et al., 2000; Kleinman et al, 1995; Lorz and Lopez, 1997; Ohtsuka et al., 2001; Vanada, et al., 1998). These effects in some cases have been increased by the presence of other air pollutant species such as ozone while in others they have not. To date no single component or chemically distinct group of components of particulate matter air pollution has been found to be solely responsible for these direct effects.

7.2 Direct Effects on Other Tissues or Organs

Direct effects on other target tissues or organs, such as the heart, have been document in a few studies in both animals and humans (Campen et al, 2000; Costa and Dreher, 1997; Godleski, et al, 2000; Gordon et al., 2000; Watkinsons et al., 2000). These effects include changes in heart rate, heart rate variability and electrical conductance. In some cases these effects have been documented in the absence of any direct pulmonary effects.

Possible mechanisms involved which may help explain these effects include: 1) the direct uptake of particles—especially those of smaller size—into the systemic circulation where they are translocated to the heart, 2) the release of soluble chemical species contained on particles into the systemic circulation where they may go on to directly impact the heart, and 3) direct activation of nerves in the lungs that directly

impact the cardiovascular system. However, the body of evidence for direct effects on the heart or other tissues and organs is not large and in many cases it is conflicting.

7.3 Systemic and/or Physiological Effects

Systemic effects resulting from PM exposure have been examined by several investigators. These effects include systemic changes in blood oxygen levels resulting from severe inflammatory damaged to lung tissues (Kleinman et al.,1998), changes in hemodynamic effects which may result from lung inflammation and reactive species production (Bouthillier et al., 1998), increased blood coagulability (Gardner et al., 2000; Ghio et al., 2000; Muggenberg et al., 2000) resulting from increased pulmonary inflammation which has the subsequent potential to significantly increase the incidence of heart attack and stroke, and changes in the release of important blood cell types from the bone marrow (Gordon et al, 1998, 2000). To date, however, few studies have been focused in these areas of investigation and the body of evidence to support or refute these possible mechanisms of PM action is limited. For the most part systemic effects studies have focused on cardiovascular or cardiopulmonary effects, presumably in an attempt to explain the epidemiological evidence supporting the association between PM exposure and premature mortality. However, systemic effects on other organ systems, such as neurological or endocrine, may also play a role in PM health effects. These should be explored as well.

In general a review of the toxicology literature clearly supports the conclusion that different particulate matter component species acting by different biological mechanisms cause a variety of short-term health effects in animals and humans. However, compared to the number of studies typically necessary to define the toxicity of other chemicals of known composition, the body of evidence to support this conclusion needs considerable improvement. Few studies to date have systematically explored the various but most likely factors responsible for PM toxicity. These include particle composition, particle mass, particle size, particle concentration and possible particle number. These also include differences in respiratory physiology and resulting

dosimetry between both animals and humans, adult humans, children and ill or physiologically compromised animals and humans. Clearly more focused, controlled systematic toxicology studies are needed to conclusively identify which toxicity factors are most important in explaining the adverse health effects seen with ambient PM exposure and to more clearly define which biological mechanisms are involved.

8.0 SUMMARY

To date very few controlled human exposure studies have been performed with particles other than acid aerosols. As a result the majority of information that is known about particle toxicity has been derived from animal toxicology studies. A brief summary of the factors suspected to be important in furthering our understanding the adverse health effects observed with exposure to PM in epidemiological study settings follows here.

8.1 Effects of Particle Size

Particle size is a very important factor in the toxicity of PM air pollution. Not only does size have the potential to affect where particles are deposited in the lungs, particle size is often directly related to the sources of PM release in the ambient environment. This means that PM size in the ambient environment is often directly related to PM component chemical composition. The only way to truly get at the question of PM toxicity as it relates to particle size is to conduct studies where the PM chemical composition and concentration area fixed and only the particle size is varied. The same is true with respect to examining the effects of varying PM composition while fixing PM size, and concentration.

A number of investigators have attempted to address the question of relative toxicity and particle size but only a very few investigators have systematically investigated the effects of varying particle size while keeping the PM composition or components the same (Brown et al., 2000). Results of these efforts support the general finding that increasingly smaller particles of the same composition, i.e. ultra-fine vs fine, show a significantly greater biological response and/or toxicity at the same or similar mass dose delivered. This finding has been linked directly to surface area in both *in vivo* and *in vitro* studies. As a consequence, if a given component of PM has a certain inherent toxicity, increasing the surface area by increasing the total number of smaller and smaller particles delivered can increase the potential toxicity of the PM

component as long as the overall concentration or whole body dose has not been equally reduced.

8.2 Effects of Particle Composition

Particle composition is most likely the single largest determinant of PM toxicity on a component by component particle basis. When one looks at particle size and composition distribution curves for ambient air samples it makes some intuitive sense to focus toxicology studies on those sized particles of suspected inherently greater potential toxicity. Such as is the case with the clear and continued focus on fine and ultrafine combustion related particles in the toxicology literature. However, in the ambient air quality standards setting process it is important to recognize that the observed response and subsequent "toxicity" of any given component of PM is highly dependent on the biological endpoint or adverse health effect of concern under study.

A review of the relevant toxicology literature confirms that biomarkers of obvious as well as potential adverse effects have been observed in animals and humans exposed via either through instillation or inhalation to many different PM components (Osier, 1997; Fine et al., 1997; Costa and Dreher, 1997; Kodavanti et al, 1999; 2000; Campen et al., 2000; Watkinson et al., 1998; 2000). The exposure components include acid aerosols, metal oxides, metal fumes, residual oil fly ash, mixtures of black carbon and other pollutants, volcanic ash, concentrated and unconcentrated ambient particles. The adverse effects vary from direct effects on respiratory tract cells and tissues (Kodavanti et al., 1999; Lambert et al., 1999) to systemic changes that may impact the cardiorespiratory system (Costa and Dreher, 1997; Campen et al., 2000; Gardner et al., 2000; Ghio et al., 2000). Although the relevance of some of these controlled exposures compared to PM compositions and concentrations seen with ambient California PM exposures could be debated, in general the results from toxicology studies pertaining to PM composition are varied. In some cases they are contradictory but overall, all exposures examined seemed to produce adverse affects or at least changes in important biomarkers of PM toxicity in one or more animal or human exposure settings.

The biological effects seen with exposure to PM of varying composition have not always been grossly toxic, as in causing cancer or respiratory tissue cell death. Particulate components other than combustion related particles appear to cause more subtle biochemical, cellular or systemic effects that result in the worsening of asthma, allergic and bronchitic conditions (Diaz-Sanchez et al., 1996; Tsien et al., 1997; Takano et al., 1997; 1998). Effects such as these resulting from inherently "less toxic" PM components, including geologic and bioaerosol endotoxins (Monn and Becker, 1999; Soukup and Becker, 2000), may be of considerable political and social importance in that they have to potential to impact the quality of life of many Californians, both healthy and physiologically compromised.

Complicated results such as this serve to highlight not only the inherent toxicity of various PM components, but also the apparently highly complex nature of PM toxicity. It is possible that particle composition alone cannot account for the varied adverse health effects seen with ambient PM exposures in numerous human populations. Physical parameters and interaction between particles of different chemical composition may play a significant role in PM toxicity. Particles of different chemical composition and size, and possibly of less inherent toxicity, may play a role in increasing the dose delivered or affect the site of delivery in the respiratory tract of particles of potentially smaller size and greater inherent toxicity.

8.3 Particle Effects in Children, Aged, and Compromised Individuals

It is essential to better understand the underlying biologic mechanisms involved in the development or manifestation of adverse health effects associated with PM air pollution exposure in humans, especially children, the elderly and persons with preexisting heart or lung disease. With respect to children, it is possible that children may be inherently more susceptible to effects of air pollution because their biological systems are developing and growing (Pinkerton and Joad, 2000). It is also possible that during critical stages of growth and development children or more specifically, their

various biological systems, may be more susceptible than at other times (Fanucchi et al., 1997; Pinkerton and Joad, 2000).

However, the susceptibility of children to exposure to a wide range of environmental toxicants cannot be based on studies in adults. The sensitivity of these cells and their response to environmental insults including ambient airborne particles are likely to be completely different compared to that found in the adult. Cellular differentiation, cellular proliferation and cellular physiological function of the lungs are continually changing during gestational and postnatal growth (Fanucchi et al., 1997).

Unfortunately our knowledge base regarding perinatal exposure and critical windows is negligible. Without question, there is still much to be learned about the effects of toxicants on gene regulation, molecular signaling and growth factors during lung development. The route of delivery of particulate matter as well as other environmental toxicants to the respiratory system is completely different during the fetal period compared to the postnatal period. Influences of passage through other organ systems and the vasculature as well as through maternal organ systems must be taken into consideration. Without the careful control and proper timing of expression for these factors, growth could become misdirected and chaotic. Future studies must be designed to address these critical windows of exposure to airborne particles to provide meaningful answers for the benefit of healthy children into adulthood.

Given this information it is not possible, at this time, to address the question of PM susceptibility in children based on the evidence provided in the toxicology peer reviewed literature. The one exception that may be warranted is in the case of allergic, asthmatic and bronchitic children who can be significantly impacted by exposure to PM. However, in these cases the adverse health impacts have largely been tied to health status and not to factors related to age.

The impact of aging in the absence of disease to be associated with greater susceptibility to PM exposure remains unclear (U.S. EPA, 1996). However, there have been a number of studies to suggest a positive association between living in

areas with historically high average PM levels and shortened life span, but this has not been documented for short-term mortality effects (Brunekreef, 1997; Abbey et al., 1999; U.S. EPA 1999). Lifetime exposure of rats and mice to whole diesel exhaust at a concentration of 350 $\mu g/m^3$ did not cause loss in lung function (Mauderly et al., 1988), lung tumors or shortening of life span (Mauderly et al., 1987a; 1987b; 1999).

The strongest evidence for PM effects in the older population comes from those individuals with preexisting cardiopulmonary disorders. These individuals by far have the highest risk with PM exposure and must be considered as a susceptible subpopulation with regard to the effects of ambient PM (U.S. EPA, 1999).

8.4 Particle Effects in Combination with Other Pollutants

The inherent toxicity of PM components in complex ambient environments may be altered by the presence of other air pollutants. The toxicity may be enhanced or reduced depending on the physical and chemical properties of the PM components of interest and the other air pollutants present (Schlesinger 1995). Rarely are individuals exposed to only PM air pollution. More commonly people are routinely exposed to complex mixtures containing many different potentially harmful pollutants which vary greatly in concentration and composition throughout any given year of exposure. In light of this the question of enhanced PM toxicity in the presence of other air pollutants remains an important factor to consider in the study of PM toxicology.

At the time this report was produced, there were no clearly and consistently identified PM and co-pollutant interactions. In general oxidant gases such as ozone and acidic vapors tend to potentiate the effects of fine and ultrafine PM on markers of inflammation, cell injury, oxidative stress and immune system macrophage function in animal exposure studies using laboratory generated particle and gas mixtures (Elder et al., 2000). However, both ozone alone and particles plus ozone also produce similar effects in a variety of independent studies (Last and Pinkerton, 1997; Kleinman et al., 1999). In general the particle effects seen in combination with other air

pollutants in the toxicology literature are varied. They often depend on the animal models examined and the adverse health effect or biological endpoint studied. The elucidation of those potential mechanisms responsible for the interaction of gases with airborne particles certainly will require more research in the future.

However, it is important to keep in mind that no matter whether the presence of other air pollutants directly enhances the toxicity of PM or not, the presence of additional pollutants in the ambient environment has the potential to increase the overall pollutant induced stress in any population. This enhanced pollutant stress may play a role in how animals or humans respond to complex ambient PM mixtures in biologically relevant settings. This may be of particular importance to the standards setting process when one considers the possible impacts on those in our populations who are physically compromised or somehow inherently susceptible.

9.0 CONCLUSIONS

1. Toxicological studies provide a critical tool to address both short-term and potential long-term effects of particulate matter exposure in humans. Toxicology studies can provide information essential to address: 1) the toxicity of known components of ambient particulate matter, 2) the toxicity of PM in combination with other air pollutants such as ozone or carbon monoxide, 3) the identification of inherently susceptible sub-populations, such as possibly the elderly, infants or growing children, and 4) the identification of the mechanisms of injury which are involved in the manifestation of adverse health effects following PM exposure.

2. Particle size is a very important factor in the toxicity of PM air pollution. Not only does size have the potential to affect where particles are deposited in the lungs, particle size is often directly related to the sources of PM release in the ambient environment (Schlesinger, 2000). The results of this critical review effort clearly support the general finding that increasingly smaller particles of the same composition, i.e. ultra-fine vs fine, show a significantly greater biological response and/or toxicity at the same or similar mass dose delivered in many experimental settings (Brown et al., 2000; Renwick et al., 2001). This finding has been linked directly to surface area in both *in vivo* and *in vitro* studies (Brown et al., 2000; Renwick et al., 2001). However, in cases where particle size has been fixed and directly compared to varying compositions of PM components with different inherent toxicity, PM composition appears to play a greater role in determining overall toxicity in many cases. The results of the Monn and Becker (1999) and Soukup and Becker (2001) work suggest proinflammatory effects of endotoxins in coarse fraction PM—but not fine PM—on macrophages may upset lung homeostasis, while metals-induced cytotoxicity in coarse PM and not fine PM may set up inflammation independent of macrophage-derived cytokines.

3. Combustion particles of varying size and from different sources appear to have both direct and indirect effects in both animal and human models. Exposure to high

concentrations of PM fuel oil related combustion particles has been found to cause lung inflammation, cell and tissue injury, and changes in cell populations in many different laboratory settings in a variety of *in vivo* animal models (Costa and Dreher, 1997; Brain et al., 1998) and *in vitro* studies (Li et al., 1996; Kennedy et al., 1998). In many cases these effects appear to be related to the composition and concentration of soluble metals present in the exposure media. However, investigators to date have yet to explore the potential toxic effects of the many 100's to 1,000's of other potentially harmful components of combustion PM. Although few other systemic effects of PM exposure have been investigated, in general it can be concluded that exposure to PM from combustion processes and both concentrated and unconcentrated ambient PM can result in systemic effects that may play a role in explaining the cardiorespiratory effects in compromised humans seen in the epidemiological literature (Killingsworth et al., 1997; Costa and Dreher, 1997; Watkinson et al., 1998; 2000; Campen et al., 2000; Gardner et al., 2000; Ghio et al. 2000). Results obtained from toxicology studies using high concentrations of PM with inherently elevated levels of metals, such as with ROFA, can be difficult to extrapolate to ambient environmental settings. As of the development of this document, a number of investigators have demonstrated direct effects of combustion particle exposure in animals (Nikula et al., 1995; 1997; Diaz-Sanchez et al., 1996). However, to date insufficient data on the toxicity of combustion related PM particles and particle mixtures in general exist to be able to extrapolate meaningful dose-response information for the purposes of revising or setting ambient air quality standards. Toxicology studies using ambient particle concentrator technology in ambient environments where increased concentrations of combustion and combustion related particles from different sources are present may offer new insights and may be helpful in extrapolating from high concentration laboratory exposure settings to lower concentration ambient exposure settings.

4. Bioaerosols have the potential to play an increasingly significant role in understanding the adverse effects of PM exposure, especially in asthmatic and allergic humans. Bioaerosols found in ambient PM air samples include either whole

or fragment portions of fungal spores, pollen, bacteria, viruses, endotoxins, plant and animal debris. Although they are routinely found in the coarse fraction of PM (Monn and Becker, 1999; Soukup and Becker, 2001) endotoxins can also be found in fine particle fractions. The health effects associated with bioaerosol exposure appear to be related more to *morbidity* effects and include allergergic response, increased sensitivity to subsequent particle effects, increased lung inflammation, increased broncho-responsiveness, increases in systemic immune cell populations, and significantly decreased lung function. Many of these effects appear to be related to the presence of endotoxins (Michel et al., 1997; Vogelzang et al., 1998; Zock et al., 1998). Although the majority of these effects were seen in humans in occupational settings, the potential for similar effects to occur in ambient exposure settings does exist. This is an area where considerable research may be needed to more fully explain the non-mortality effects observed in humans exposed to complex ambient PM mixtures.

5. Further research on the toxicity of geologic dusts, including that found in coarse PM, and the role they may play in affecting the toxicity of other PM components appears to remain warranted (Monn and Becker, 1999; Soukup and Becker, 2001), especially in circumstances where they may be involved in the exacerbation of existing asthmatic, allergic or bronchitic conditions. Significant amounts of geologic dust can be found routinely in fine and ultra-fine particle fractions of California PM ambient air samples. Geologic dusts can contain a variety of biologically active components, such as bioaersol endotoxins. The results of a number of *in vitro* studies examined suggest that proinflammatory effects of endotoxins in PM coarse fraction on macrophages may upset lung homeostasis while metals-induced cytotoxicity in coarse PM may set up inflammation independent of macrophage-derived cytokines (Monn and Becker, 1999; Soukup and Becker, 2001). Coarse particles, including those derived from geologic sources, may also play a role in increasing the dose delivered or affecting the site of delivery in the lungs of particles of potentially smaller size and greater inherent toxicity. This finding could be important from an ambient air quality standards setting perspective. These findings highlight the

importance and overall biological relevance of coarse particles (even of geologic origin) in identifying and explaining potential mechanisms which explain the adverse health effects seen with exposure to coarse particles. It is premature at this point to discount the potential role geologic dusts may play in explaining the adverse health effects seen with ambient PM exposure in the epidemiological literature.

6. The biological effects seen with exposure to PM of varying composition do not have to be grossly toxic, as in causing cancer or respiratory tissue cell death, to be important in the PM standards setting process. Particulate components other than combustion related particles may cause more subtle biochemical, cellular or systemic effects that result in more the worsening of asthma, allergic and bronchitic conditions (Diaz-Sanchez et al., 1996; Tsien et al., 1997; Takano et al., 1997; 1998). Effects such as these resulting from inherently "less toxic" PM components are of considerable political and social importance in that they have to potential to impact the quality of life of many Californians.

7. While exposure to acid aerosols at ambient concentrations may not significantly impact healthy individuals and may not result in long term health impacts, at this point in time we cannot discount the potential affects of exposure to ambient acid aerosols in enhancing the toxicity of other PM components, such as metals (Dreher et al., 1997).

8. In large measure, systemic effects studies have focused on cardiovascular or cardiopulmonary effects (Killingsworth et al., 1997; Costa and Dreher, 1997; Kodavanti et al., 1999; Watkinson et al., 1998; 2000; Campen et al., 2000; Gardner et al., 2000; Ghio et al. 2000), in an attempt to explain the epidemiological evidence supporting the association between PM exposure and premature mortality and the national emphasis on addressing fine combustion related particles. Despite somewhat inconsistent findings in day to day exposures, the results seen in studies using concentrated and unconcentrated ambient particles, including combuation particles, suggest that exposure to PM can have systemic effects in animals. Such effects include changes in heart rate and systemic blood parameters including

fibrinogen levels necessary for blood clotting (Gardner et al., 2000; Ghio et al., 2000). However, systemic effects on other organ systems, such as the neurological and endocrine systems, may also play a role in PM health effects. These should be explored as well.

9. In general, the results seen in the toxicology literature with respect to PM in combination with other air pollutants are varied (Vincent et al., 1997; Last and Pinkerton, 1997). They appear to often depend on the animal models examined and the adverse health effect or biological endpoint studied. However, it is important to keep in mind that no matter whether the presence of other air pollutants directly enhances the toxicity of PM or not, the presence of additional pollutants in the ambient environment has the potential to increase the overall pollutant induced stress in any population. This enhanced pollutant stress may play a role in how animals or humans respond to complex ambient PM mixtures in biologically relevant settings. This may be of particular importance to the standards setting process when one considers the possible impacts on those in our populations who are physically compromised or somehow inherently susceptible.

10. It is essential to better understand the underlying biologic mechanisms involved in the development or manifestation of adverse health effects associated with PM air pollution exposure in humans, especially children, the elderly and persons with preexisting heart or lung disease. Although considerable work has gone on in this area, additional research on animal models which mimic compromised or developing humans is needed to conduct controlled toxicology studies. There are several animal models that are quite promising, but some work is needed to characterize their applicability and use in different exposure settings.

11. A great deal of work needs to be carried out to address concerns regarding children and PM exposure. It is possible that children may be inherently more susceptible to effects of air pollution because their biological systems are developing and growing (Snodgrass 1992; Fanucchi et al., 1997). It is also possible that during critical stages of growth and development children or more specifically, their various biological

systems, may be more susceptible than at other times (Pinkerton and Joad, 2000). However, the susceptibility of children to exposure to a wide range of environmental toxicants cannot be based on studies in adults. The sensitivity of these cells and their response to environmental insults including ambient airborne particles are likely to be completely different compared to that found in the adult. Cellular differentiation, cellular proliferation and cellular physiological function of the lungs are continually changing during gestational and postnatal growth.

12. With respect to children and increased sensitvity or susceptibility to PM exposure, our knowledge base regarding perinatal exposure and critical windows is unfortunately negligible (Fanucchi et al., 1997; Pinkerton and Joad, 2000). Without question, there is still much to be learned about the effects of toxicants on gene regulation, molecular signaling and growth factors during lung development. The route of delivery of particulate matter as well as other environmental toxicants to the respiratory system is completely different during the fetal period compared to the postnatal period. Influences of passage through other organ systems and the vasculature as well as through maternal organ systems must be taken into consideration. Without the careful control and proper timing of expression for these factors, growth could become misdirected and chaotic. Future studies must be designed to address these critical windows of exposure to airborne particles to provide meaningful answers for the benefit of healthy children into adulthood.

13. From an ambient air quality standards setting perspective, the study of the toxicity of PM species not readily found in meaningful concentrations in ambient California air is not helpful in explaining adverse effects seen in epidemiological study settings. They are also of limited value in identifying and developing health protective PM ambient air quality standards for California.

14. In view of the marked pulmonary effects observed for residual oil fly ash (ROFA) following intratracheal instillation and/or inhalation, the role of transition metals in particle toxicity deserves more extensive research to elucidate their role in cardiopulmonary toxicity. Animal studies conducted by Kodavanti (1999; 2000) and

others provide evidence which supports the conclusion that cardiovascular disease models in animals may play a role in increased susceptibility to ROFA particle exposure. Although they can be helpful in identifying underlying mechanisms of potential PM action, considerable caution should be used in interpreting the results of studies using residual oil fly ash as the source of PM for exposure.

15. More research to determine particle dose and resulting dose-response effects is very clearly needed. The focus in the toxicology literature to date remains in the fact finding phase of "which particles cause what biological effects in what models under what conditions". Although considerable progress has been made in these areas in the past several years, only after these questions have been more clearly answered will functional dose-response studies be able to provide more information.

16. The emphasis in the toxicology literature to date has been placed on addressing the question of biological plausibility, presumably in an attempt to explain observed health effects in the epidemiological literature. There has been very little focused work on the fundamental aspects of particle toxicity, particle species or composition toxicity, particle interactions, complex particle dosimetry or particle dose-response. This makes the development of a PM standard based on the toxicology literature alone difficult to carryout at this time. What the body of toxicology literature can provide at this time are links between particle components of interest, mixtures or sources of known PM, potential biological mechanisms and observed health endpoints. The kinds of toxicological evidence presented in Sections 5.0, 6.0 and 7.0 of this report used in conjunction with results from prior epidemiological studies should prove helpful in addressing ambient air quality standards for PM.

17. Careful and considerable attention has been paid in the toxicology literature to the examination of the toxic effects of fine and ultrafine PM (Li et al., 1999; Brown et al., 2000; Renwick et al., 2001). Particles derived from the combustion of diesel and other automotive/fossil fuel sources have also been examined (Nikula et al. 1995; 1997; Diaz-Sanchez et al., 1996; Tankano et al., 1997; 1998). Presumably the findings in these studies are a result of not only the inherently potential toxicity of

these particulate species, but also the extent to which automotive emissions contribute to ambient PM ambient loadings in many areas of California, the United States and the world. It also likely results from the national emphasis placed on "fine" particles, which are typically assumed to be combustion related, and the setting of a national fine particle ambient air quality standard. As highly complex sources of ambient PM, combustion particles contain many known reactive and/or carcinogenic species. However, it is essential to understand that other components of ambient PM mixtures may be of equal or possibly even greater concern from a public health stand point.

18. From a toxicological perspective it is becoming increasingly important to define both particle size and composition when discussing or interpreting PM related study results. It is not adequate to refer simply to "fine" or "ultra-fine" particles and assume these terms are synonymous with fine or ultra-fine combustion particles. As has been clearly shown, significant portions of fine and ultrafine ambient California PM can be comprised of geologic dust and bioaerosols, including endotoxins which have been shown to be associated with adverse health effects in humans.

19. Differences in respiratory physiology and resulting dosimetry will continue to be important factors in understanding PM toxicity (Schlesinger 2000). Particle composition does play a role in understanding PM toxicity. However, no matter how inherently toxic a particular type of PM may be, if there is no exposure and resulting dose delivered to a specific biological host, target organ, system or cell type of concern, the risk of adverse affects from that type of PM is very low if not in fact zero. It is important to determine and describe differences between animals and humans in dosimetry and the effect other factors, such as exercise, age, gender, susceptibility or health status may have on particle dose parameters and resulting overall exposures (Lippman and Schlesinger, 2000). Clearly more focused controlled toxicology studies are needed in this area.

20. Although epidemiological studies can provide critical evidence of associations between human populations, air pollution exposure and adverse health impacts, only

toxicological studies can provide the types of data needed to begin identifying which components or characteristics of PM may be most harmful or perhaps more directly related to observed adverse health effects. It is essential to identify which components or characteristics of PM are most harmful so that ambient air quality standards can be developed and/or revised as needed to protect public health. It is also highly desirable to identify which components or characteristics of PM are most harmful or more directly associated with health impacts so that effective source control strategies can be developed. Clearly additional toxicology work is needed to more fully address these important questions.

21. Based on the literature available to date, it is difficult to determine at this time if the different results found in the various studies conducted using concentrated ambient particles in both humans and animals reflect differences in the ambient PM composition, differences in ambient concentrations on exposure days, inherent physiological differences between the exposed animal models and humans, differences in the exposure conditions or the inherent toxicity of the PM components themselves. Despite somewhat inconsistent findings in day to day exposures, studies such as these do suggest that exposure to concentrated ambient particles can have systemic effects in both healthy and compromised animals. Such effects include changes in heart rate, changes in blood parameters, including circulating blood platelet and fibrinogen levels which are necessary for blood clotting, as well as increases in peripheral neutrophils and lymphocytes (Killingsworth et al., 1997; Costa and Dreher, 1997; Watkinson et al., 1998; 2000; Campen et al., 2000; Gardner et al., 2000; Ghio et al. 2000). Particle concentrator technology offers a promising tool in the study of PM toxicity (Godleski et al., 2000; Clarke et al., 2000; Kodavanti et al., 2000a; Muggenberg et al., 2000; Gordon et al., 2000; Ghio et al., 2000). If used in concert with other toxicology research methods studies conducted using particle concentrator technologies have the potential to provide an increasingly important bridge between epidemiological based studies and carefully controlled laboratory toxicology studies.

10.0 RECOMMENDATIONS

1. Recommend supporting the use of carefully controlled toxicology and well designed epidemiological studies together to further address the questions of PM exposure and adverse health effects in humans, including both mortality and morbidity effects.

2. Recommend that the ambient air quality standards review process take into account adverse health effects associated with exposure to coarse as well as fine PM components found in California.

3. Recommend additional research be carried out to further address the questions of PM composition, size, health effects, biological mechanisms, and dose-response associated with the following: bioaerosols, geologic dusts, combustion PM from sources other than fossil fuel, PM in combination with other air pollutants, effects in children, effects in sensitive/compromised models that mimic human populations, maternal exposures, critical windows of exposure in development, and effects in the aged.

4. Recommend ambient particle concentrator technology in concert with well controlled laboratory studies be used to study some of the questions raised in this critical review effort. It is recommended that toxicology studies using ambient particle concentrator technology be designed to study effects of exposure to PM from California micro-environments where increased concentrations of PM components of particular interests, including size, composition or combination with other pollutants, are present. It is expected that these studies will offer new insights and may prove very helpful in extrapolating from high concentration laboratory exposure settings to lower concentration ambient exposure settings.

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12.0 GLOSSARY

^{3}H-thymidine	Tritiated Thymidine
ARB	Air Resources Board
BAL	Bronchoavleolar Lavage
BALF	Bronchoavleolar Lavage Fluid
BrdU	Bromodeoxyuridine
Cal/EPA	California Environmental Protection Agency
CAPs	Concentrated Ambient Particles
CdO	Cadmium Oxide
CO	Carbon Monoxide
Coarse PM	Coarse Particulate Matter
Fe_2O_3	Iron Oxide
Fine PM	Fine Particulate Matter
GST	Glutathione-S-Transferase
IL-6	Interleukin-6
kg	Kilogram
LDH	Lactic Dehydrogenase
LBP	Lipopolysaccharide Binding Protein
LPS	Lipopolysaccharide
MCP-1	Monocyte Chemoattractant Protein-1
MCT	Monocrotaline
mg	Milligrams

MgO	Magnesium Oxide
MIP-2	Macrophase Inhibitory Protein-2
MnO_2	Manganese Dioxide
m^3	Cubic Meters
ng	Nanogram
nm	Nanomolar
$NaVO_3$	Sodium Vanadate
NO_2	Nitrogen Dioxide
O_3	Ozone
OEHHA	Office of Environmental Health Hazard Assessment
PAHs	Polycyclin Aromatic Hydrocarbons
PM	Particulate Matter
$PM_{2.5}$	Particulate Matter 2.5 microns or less
PM_{10}	Particulate Matter 10 microns or less
PMNS	Polymorphonuclear Leucocytes
ROFA	Residual Oil Fly Ash
SHR	Spontaneously Hypertensive Rats
SO_2	Sulfur Dioxide
TCDD	Tetrachlorodibenzo-P-dioxin
TiO_2	Titanium Dioxide
TNFα	Tumor Necrosis Factor Alpha
$\mu g/m^3$	Microgram per Cubic Meter

μm	Micrometer
Ultra-Fine PM	Ultra-Fine Particulate Matter
U.S. EPA	United States Environmental Protection Agency
$V_2 O_5$	Vanadium Pentoxide
ZnO	Zinc Oxide