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RESEARCH NEEDS FOR RISK ASSESSMENT OF INHALED PARTICULATE MATTER

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PREFACE

Recent studies have shown that insoluble, biochemically inert particles, small enough to deposit in the deep lung, are capable of inducing carcinogenic as well as pathological effects. These findings have important implications for risk assessment, especially since particulate matter of this type, carbon black for example, has been considered in the past to be relatively benign. On March 10-11, 1992, the U.S. Environmental Protection Agency's (EPA) Office of Health and Environmental Assessment sponsored a workshop in McLean, Virginia, on "Research Needs for Risk Assessment of Inhaled Particulate Matter." During the 1½-day workshop, the 14 expert panelists discussed the current state of the art regarding pathological effects of inhalable particulate matter. They also developed a list of research recommendations aimed at improving risk assessment in this area. A number of observers also were present to witness and join in the discussion. This report summarizes the proceedings of the workshop.

1. EXECUTIVE SUMMARY

At a workshop sponsored by the U.S. Environmental Protection Agency's Office of Health and Environmental Assessment, expert panelists discussed research needed to support the development of improved risk assessment methodologies for inhalable, biochemically inert, insoluble particulate matter. During the 1½-day workshop, the panelists exchanged current data concerning the effects and mechanisms of action of particles, identified data gaps, and provided research recommendations. While both carcinogenic and noncarcinogenic pathological effects were discussed, the panel focused on carcinogenic endpoints.

The panelists agreed that the first step in the pathological and/or carcinogenic process is the result of particle ingestion by macrophages residing in the alveolar regions. Following particle uptake, the macrophages are induced to secrete a variety of mediators (growth factors, oxidants, proteolytic enzymes, etc.). These mediators diffuse to the target cells lining the alveoli, are taken up, and induce pathological effects. The panelists also agreed that the combination of mediators responsible for effects and mechanisms of action are still somewhat uncertain. There was less agreement about the lung burdens of particulate matter required to induce harmful effects. Some believed a minimal particle load is required to induce secretion of mediators. Others pointed out that, for any level of exposure, particle load varied among macrophages. Thus, no clear threshold for overload activation of particles is likely.

Other questions arose about the characteristics of particles responsible for activation of macrophages and the relevance of animal data to human responses. There appeared to be a general agreement that inflammatory and subsequent cellular proliferative responses may be the most important events leading to fixation of induced mutations and, finally, particle-induced lung tumors.

As a result of the discussions, a list of specific recommendations for research was developed. These included (1) determination of the ability of particles to induce tumor

promotion, (2) determination of the specific DNA adducts induced by particles, (3) determination as to whether deposited particles induce specific mutational changes or whether spontaneous mutations become "fixed" as a result of proliferative responses, (4) determination of dose-time response for radical production by macrophages, (5) determination of dose-time response for cell proliferation, (6) determination of linkages between macrophage release of cytokine/growth factors and mutational events, (7) use of archival tissue from previous animal experiments and autopsy material to aid in determining mechanisms of action, and (8) evaluation of particle-associated compromises in tumor defense mechanisms.

2. INTRODUCTION

During March 1992, a workshop sponsored by the U.S. Environmental Protection Agency's Office of Health and Environmental Assessment convened to discuss research needs supporting the development of risk assessment methods for inhaled particulate matter, with a primary focus on insoluble, biochemically inert particles. During the 1½-day workshop, 25 participants from academia, industry, and regulatory agencies reviewed current data on the toxicology, carcinogenicity, and mechanisms of action of inhaled particles; identified data gaps; and formulated general and specific research recommendations. It is expected that identification of knowledge gaps and research needs ultimately will benefit EPA's risk assessment process and also will improve our understanding of mechanistic links between particle exposure and lung injury, especially tumorigenesis.

The need for health risk assessment and regulation in this area is based on the results of a number of recent long-term inhalation studies in rats. These studies have shown that exposure to different particles, including coal dust, titanium dioxide (TiO_2), toner, carbon black, and diesel exhaust, can result in serious lung injury. The injuries are characterized by functional impairment of alveolar macrophages, chronic inflammation, cell proliferation, fibrotic reactions, and lung tumors. All of the particles used in these studies are highly insoluble and have a low intrinsic cytotoxicity. Apparent threshold levels varied with the agent tested, but effects generally occurred at lung particle burdens exceeding about 1-2 mg/g of lung. Since lung injury and impaired clearance appeared to be associated with excessive accumulation of particles in the lung macrophages, the term "particle overload of alveolar macrophages" was used subsequently to associate these effects with excessive particle accumulation in alveolar macrophages. It is uncertain at this time, however, whether lung injury occurs only with lung overload, or if lung overload is a necessary precursor of lung injury.

A number of questions arose from these studies, such as: Are these effects specific to the rat? Are these generic particle effects that will occur with any type of highly insoluble

particle of low cytotoxicity? Does potency vary with the particle? If effects vary qualitatively and quantitatively among particles, what are the physicochemical characteristics of a particle that are important for its effects? Are these studies relevant to human exposures? Can we extrapolate these results to humans? Can we extrapolate these results to low lung particle burdens due to low exposure concentrations or short exposures? Is there a threshold for toxic effects? If so, is it related to particle overload of macrophages? Can we identify biologically plausible mechanisms of action(s) to explain these results? What data gaps do we need to fill to gain a better understanding of mechanistic events? While these questions were addressed by the participants, time did not allow discussing each of them exhaustively.

Presentations were made by seven speakers who addressed issues of particle-induced lung tumors, the role of DNA adducts, the role of mediators (oxidants, cytokines, growth factors, chemotactic factors) released by alveolar macrophages and other cells, dosimetric considerations with respect to particle deposition and clearance, and particle-alveolar macrophage interactions as well as issues of risk extrapolation. The presentations were followed by intensive discussions. Short summaries of the presentations and some points of the discussions and individual recommendations are given in the pages that follow.

It was agreed that not enough data are available to improve currently inadequate procedures for risk assessment of particle-induced lung tumors. For such improvement, the most urgent need is for mechanistic data. The group, therefore, focused on mechanisms of particle-induced lung tumors.

3. SUMMARIES OF INDIVIDUAL PRESENTATIONS

3.1. Overview, Gunter Oberdörster

An overview of particle-induced pulmonary effects was presented, which included a discussion of the sequence of events originating from the particle-macrophage encounter and leading to chronic inflammatory, cell proliferative, fibrotic, and, in some cases, tumorigenic effects. The question was raised whether fibrotic alterations are a necessary prerequisite for subsequent induction of tumors. It was also pointed out that different pathophysiological changes due to particle "overload" effect appeared to correlate with different dose parameters. Alveolar macrophage functional changes might be correlated best with a dose expressed as the phagocytized particle volume in macrophages, whereas inflammatory and proliferative responses appeared to be better correlated with the particle dose expressed as surface area of the retained particles. The importance of the pulmonary inflammatory response in particle overload situations was emphasized, and several research needs were presented with the ultimate goal of replacing the present simplistic approach of dosimetric extrapolation modeling with a mechanistically oriented dosimetric model.

Needed mechanistic data on cellular and molecular mechanisms include cell proliferative responses, involvement of cytokines and growth factors, the development of fibrosis, and the formation of DNA and protein adducts. Such data can be collected by using results of new studies as well as by using archival tissues. It also would be useful to search for biomarkers or functional assays of "overload," for example, analyzing lavaged lung cells and fluid for biological mediators released by overloaded macrophages or perhaps measuring particle clearance in vivo. Known species differences between rats and hamsters, with respect to tumor induction and other long-term effects in the lung, also should be exploited to address the question of extrapolation to humans. Dosimetric mechanisms with respect to particle uptake and retention (e.g., why do alveolar macrophages migrate up the mucociliary ladder and what retards their movement when they are overloaded?) need further investigation.

3.2. DNA Adducts, James Bond

Data were presented and discussed indicating that although total adducts induced in the lungs of rats either by carbon black or by diesel exhaust are quantitatively similar, there might be differences in specific adduct formation. Preliminary data mentioned in the discussion showed that adducts elicited by exposure to carbon black may represent polar adducts, possibly including adducts induced by reactive oxygen species, whereas diesel exhaust-induced adducts are more specific for the organics present in diesel exhaust. The carbon core of diesel particles is similar to that of carbon black. However, whereas carbon black has no more than a trace of adsorbed organic material, numerous organic agents are adsorbed to the surface of the diesel particle, including carcinogens such as benzo(a)pyrene and nitropyrenes. These organic agents are thought to be taken up and to act directly on the target cells. Particles, on the other hand, induce macrophages to secrete mediators, which then migrate to and are taken up by the target cells. It is therefore not surprising that different adducts are formed.

It was also pointed out that DNA repair is an important issue and that repair of DNA adducts could be very different for different regional lung tissues. The specificity of DNA adducts is a very important issue that needs to be investigated.

Specific recommendations for future research to fill present data gaps include:

1. Ascertainment of the effect of carrier particles on the delivery of adsorbed compounds to specific regions of the respiratory tract.
2. Ascertainment of rates of desorption of compounds from particles after deposition.
3. Better elucidation of the actual toxic effects of increased lung retention of inhaled particles.
4. More complete characterization of respiratory tract metabolism of inhaled particle-associated xenobiotics (with emphasis on lung metabolism).
5. Identification of specific biomarkers of exposure to inhaled particles.

3.3. Particle-Induced Macrophage Function, Kevin Driscoll

In this presentation it was pointed out that releases of certain cytokines and growth factors by alveolar macrophages following particle uptake are important events in the particle-induced inflammatory reaction. Fibronectin, for example, may prove to be a biomarker of fibrotic responses occurring at a later time point. Fibronectin release could be determined in cultured lavaged macrophages after in vivo particle exposure. The importance of the inflammatory cytokine tumor necrosis factor (TNF-α) for the influx of polymorphonuclear leukocytes (PMN) was discussed, but it was also shown that the release of cytokines (IL-1, TNF, fibronectin) is not necessarily the same after in vivo or in vitro particle exposure of alveolar macrophages. Although the in vitro responses of cytokine release appear to be lower, alveolar macrophages can be primed by γ-interferon to release TNF also in vitro. Thus, important mechanistic interactions with respect to cytokine release are occurring and need further study. Research needs with respect to mechanisms of particle-induced inflammation were outlined, including development of biological markers; influence of the pulmonary extracellular environment in the lung's response to particles; the role of particle surface, mass, volume, and numbers in the inflammatory response; contribution of other macrophage populations to particle responses; species differences in responses; the role of macrophage interaction with other cells, e.g., structural cells; contribution of fibroblasts, epithelial cells, and endothelial cells, as initial effectors of response since these cells in turn can, via an intricate cytokine networking, interfere with each other in the overall inflammatory response.

3.4. Biology of Macrophages, Bruce Lehnert

An overview of the biology of macrophages was presented. Heterogeneity of the alveolar macrophages in different lung regions was discussed. The possibility was brought up that this heterogeneity could indicate development stages.

Macrophage-particle interactions in different compartments of the lung, including the redistribution of phagocytized particles within the macrophages of the alveolar space over time, were reviewed. As a result of either cell death or cell division, the particle load per

macrophage decreases following cessation of exposure. Presumably fewer of the cells are then overloaded. In spite of a decrease in the alveolar macrophage load, impaired clearance was still observed. The underlying mechanisms are still unclear. In this context, the importance of the pores of Kohn for alveolar clearance was mentioned, as preliminary evidence has suggested that these pores may be grown over by epithelial cells when lung burdens are excessive. A pathway is therefore occluded that might otherwise be important for clearance of macrophages.

Research needs were outlined with respect to basic pulmonary macrophage biology and the interaction of pulmonary macrophages and particles. Needs include the following:

- Origins, translocation pathways and transit times, proliferative characteristics, and lifetimes of pulmonary subpopulations.
- Factors controlling the steady-state regulation of the sizes of the pulmonary macrophage subpopulations.
- Bases of phenotype and functional heterogeneity and changes following particle deposition.
- Identification of macrophage-derived growth factors, their target cells, and cytokine networks.
- Influence of prior phagocyte history on subsequent chemotactic responsiveness and phagocyte function; in vivo factors that regulate macrophage-particle encounters and rates of phagocytosis.
- Mechanism(s) by which alveolar macrophages encounter and become coupled to the mucociliary apparatus for tracheobronchial transport.
- Roles of the airway intraluminal macrophages in particle clearance and airway disease.
- Factors that govern the fate of free particles that enter the lung's interstitial compartments.
- Functional characteristics and translocation/clearance pathways of pulmonary interstitial macrophages.

- Mechanism(s) responsible for the formation of aggregates of alveolar macrophages in the particle overload condition.

- Mechanisms underlying the "particle redistribution phenomenon" and how they are affected by differing lung burdens, particles of differing size, and particle cytotoxicity.

- Comparative studies of human alveolar macrophages and alveolar macrophages from other laboratory species as to their relative abilities to extract/metabolize procarcinogens and carcinogens from particles and the effect of particle load on this process.

- Characterizations of the abilities of other endocyte cell types, including polymorphonuclear leukocytes, interstitial macrophages, and type I epithelial cells to extract/metabolize procarcinogens and carcinogens from engulfed particles.

- Kinetics of particle endocytosis by airway/alveolar epithelial cells, and how particle size, numbers, and surface characteristics affect the endocytic process, and the subsequent transcellular passage of particles into subepithelial compartments.

- Measure of reactive oxygen species, proinflammatory mediators, and promitogenic cytokines as a result of cell particle interactions.

3.5. Clearance of Inhaled Particles, Timothy Gerrity

The first point made was a reminder of the tremendous species differences in retention half-times of highly insoluble particles deposited in the lungs. Underlying mechanisms for these differences in clearance from the alveolar region require further evaluation. It was also emphasized that retention of particles in the conducting airways may not be as short as generally thought, since newer studies seem to indicate that there is a long-term component involved, which leads to a significantly longer retention of particles in this region. Research needs include the study of very basic mechanisms of macrophage-mediated particle clearance, including the basic question--why do macrophages move toward the mucociliary ladder for effective clearance of phagocytized material? With respect to overload-induced retardation of clearance, we need to find out whether this is an irreversible event or to what degree recovery does occur.

3.6. Dosimetry Models, Chia Ping Yu

The deposition and clearance behavior of particles in humans was reviewed. It was pointed out that the relative total deposition of inhaled particles in humans of different ages is very similar. Across species, deposition in different airway regions can differ significantly. For example, deposition in the head region is significantly different in different animal species (e.g., rats vs. guinea pigs vs. man). With respect to research needs in the area of particle deposition, more details are needed on airway geometry and local deposition. With respect to particle clearance, the relationship between clearance rate and cell function needs to be evaluated. Deposition and clearance models likewise need to be refined to include and study regional nonuniformity of these events. With respect to dosimetry, models are needed to include children and diseased lungs, and a larger intersubject variability also has to be incorporated. Finally, the basis for an interspecies extrapolation method needs to be developed.

3.7. Risk Assessment Methodology for Particles, Roy Albert

The complexity of the problem was stressed when dealing with animal data and attempting to make an assessment of human risk. Furthermore, using possibly available data from human occupational studies to extrapolate to the environmental situation is a difficult undertaking, potentially involving orders of magnitude downward extrapolation. In rats, we are almost exclusively dealing with bronchoalveolar tumors, whereas in humans the observed tumors are more of bronchial origin. This represents an additional difficulty for using animal studies to assess human risks and requires a more detailed analysis of the exposure and dosimetry of airways and bronchi. The need to know more about the contribution of the alveolar particle dose to bronchial cells during subsequent clearance in order to assess the significance of bronchogenic carcinogenesis was also stressed. Recommendations for future research included the evaluation of the involvement of oxygen radicals in adduct formation. It was further suggested that use be made of human populations heavily exposed to particles, such as coal miners; possibly, pulmonary lavage could be used to assess DNA adducts in lavageable cells. Pulmonary tissues from animals exposed to different particles, such as diesel exhaust, carbon black, and TiO_2, could be used to examine more closely the

relationship of particle exposure and adduct formation, and alveolar macrophages could be used to determine if a correlation exists between tissue adducts and macrophage adducts. In vitro studies with alveolar macrophages to evaluate DNA adduct formation after different particle exposures also would be useful in this respect. The basic question concerning the usefulness of this marker needs to be addressed.

3.8. Additional Comments by the Workgroup

Several issues already presented were reemphasized, such as the necessity and methodology of extrapolating to low-dose levels, the question of human risk of exposure to particle "X," the biological plausibility of mechanisms, and the question of whether there are multiple mechanisms of action, some occurring at high levels as opposed to others operating at low levels of exposure. Extrapolation from laboratory animal data to man requires the incorporation of both extrapolation across materials, i.e., carbon black, TiO_2, diesel exhaust, as well as across exposure levels when multiple mechanisms of action may take place, i.e., at high exposure levels and others operating at low exposure levels. Exposure-dose-response relationships for a number of studies are needed, including in vitro cell/tissue culture studies, in vivo short-term studies, and in vivo long-term studies, to address the issues of high to low exposure, and possibly even of in vitro studies of human exposure. It might be useful to search for human data that may serve as a "gold standard" with which animal data could be compared (diesel studies, coal dust?). Other questions to be addressed relate to whether nonmutagenic materials have a threshold and what the basis is for a threshold generally believed to be present for mutagenic materials. In any type of research, dose-response relationships are important components, where the dose might be represented as particle burden or formation of adducts and the response in terms of tumors, mutational events, DNA damage, etc. Possible mechanisms for particle-induced lung injury include direct and indirect mutational events, cell proliferative responses, oxidative damage, and others. These mechanistic aspects were discussed with respect to the needs outlined in Figure 1 and detailed in the first part of the summary report.

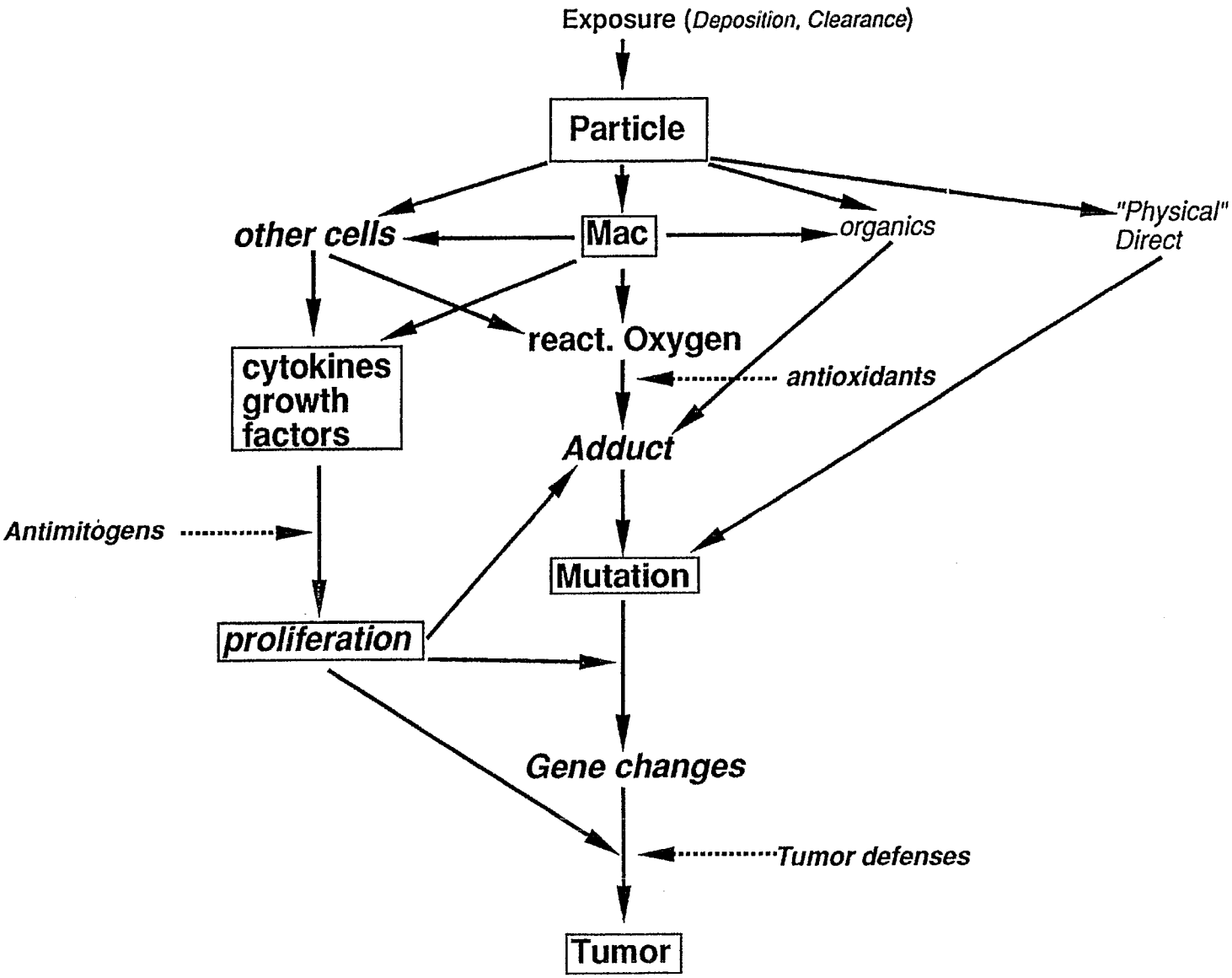

Figure 1. Hypothetical sequence for tumorigenic effects of inhaled particulate matter in the lungs.

4. WORKSHOP RECOMMENDATIONS

4.1. General Outline

As a result of these discussions, a scheme was developed during the last session of the workshop, which outlined a number of mechanisms of particle-induced effects in the lung and which served as a basis for discussing and recommending research needs. Figure 1 depicts this scheme, which tries to incorporate in a very general way major mechanistic pathways that may lead to particle-induced lung tumors. This scheme does not imply that all of the indicated intermediate steps occur with any particle, but rather it demonstrates some of the different interactions in the lung that may occur with highly insoluble particles. Although the group discussion in this last session focused exclusively on the issue of mechanisms of particle-induced tumorigenesis (see Figure 1), also discussed was the possibility that these tumors are the result of several intermediate events involving chronic inflammatory, proliferative, and possibly fibrotic reactions in the lung. Although fibrosis is a dominant and equally serious endpoint of particle-induced pulmonary toxicity, the workshop dealt primarily with tumorigenicity of insoluble particles. However, the interplay of several features of Figure 1 may be subject to incorporation into a comparable schema for pulmonary fibrosis.

The major pathways and events in Figure 1 are as follows: Particles deposited in the lung will mostly be phagocytized by alveolar macrophages, which in turn may be activated to release mediators including reactive oxygen species, cytokines, and growth factors. Inflammatory cells such as PMN elicited into the lung also will contribute to the release of mediators including reactive oxygen species. Reactive oxygen species could interact with DNA of target cells and induce specific DNA adducts such as 8-hydroxyguanine adducts, which may in turn lead to mutations and genetic changes, including activation of oncogenes or inactivation of suppressor genes, which may eventually result in an increased tumor incidence. Particles also may interact directly with other cells in the lung, such as epithelial cells and fibroblasts, which may then be stimulated to release mediators. One expected outcome of particle-cell interactions is fibrosis. Particles also may have a physical direct interaction during cell division, which, for example, has been demonstrated for asbestos

fibers interfering with the spindle apparatus of dividing cells, and may also lead to mutational events.

An important pathway derived from the interaction of particles with macrophages and other cell types involves the release of growth factors and cytokines, which leads to an intricate network of cell-cell interactions resulting in stimuli for proliferative responses that, as indicated in the scheme, can contribute to the manifestation of tumors at several crucial points. Specific particles such as diesel soot containing organic materials also may lead to direct organic-specific adduct formations, which are different from those induced by reactive oxygen species. The importance of these adducts in particle-induced lung tumor formation, however, needs further investigation. A conclusive pathogenic role could not be established for organic-induced DNA adducts or oxygen radical-induced DNA adducts.

At several points in the scheme of Figure 1, physiological defense mechanisms are indicated that may affect lung injury and reduce tumor formation. For example, in the first case, cell-particle interactions can lead to elaboration of many factors that affect proliferation and are important in long-term effects including fibrosis. In the latter case, antioxidant systems are present in most cell systems in the lung; antimitogenic factors in addition to promitogenic factors also can significantly alter the proliferative processes occurring at different levels during pathogenesis of tumor formation. Tumor defenses are also present, including natural killer cell activity released from alveolar macrophages. All these could become effective in this general scheme of particle-induced lung injury and tumor formation. It was generally agreed that we do not need more studies just to demonstrate that inhaled particles can induce lung tumors, but that research should focus on mechanistic links. This does not preclude the need for bioassays of additional materials to determine their carcinogenic potency.

4.2. Specific Recommendations

Several research recommendations were made aimed at filling knowledge gaps in our understanding of the different pathways outlined in the figure. Research may start at

different points of the scheme, involving a multidisciplinary approach. While it is recognized that particle-adsorbed organics may be important for certain particulate compounds (diesel), their contribution to tumor development via specific adduct formation and mutational events might only be minimal and was thought, therefore, not to be as important in contrast to other more generic effects of particles. Ongoing research in this area by several groups should clarify the significance of particle-adsorbed organics, but this should not be high on EPA's priority list of research needs for particle-induced tumors. Research involving insoluble particulate matter such as silica and freshly fractured coal dust, which are thought to have additional toxic properties due to the presence of oxygen radicals on the particle surfaces, also should be lower on EPA's priority list of research needs for inert particles. Mechanistic studies pertaining to the surface-associated radicals, however, may aid in our overall understanding of particle-induced toxicity.

The pathway in Figure 1, starting with direct particle-macrophage interactions, includes the most important events, and the group agreed on several recommendations to investigate specific mechanisms along this pathway.

4.2.1. *Particles Are Promoters*

Particles deposited in the lung may have a promoting effect that can result in increased proliferative responses. Research in this area should include initiation-promotion studies in which an initiating pulmonary carcinogen would be combined with administration of particles in the lung. However, since target cells may be different for the initiating agent on one hand and particles on the other (particles so far have induced peripheral lung tumors in rats whereas other initiators may target more central lung regions), one must be careful in interpreting observed effects. Study of proliferative responses induced by particles for different cell types in the lung would be of high importance in these studies.

4.2.2. *Differentiation of Adducts*

DNA adduct formation has been observed in the lung after inhalation of diesel exhaust (particles with adsorbed organic compounds) and after inhalation of carbon black (three

orders of magnitude less organic content). However, whereas inhaled diesel exhaust particles induced organic-specific adducts, preliminary evidence appears to suggest that inhalation of carbon black as well as of TiO_2 (ultrafine particle size) led to the formation of polar adducts (oxygen radical induced?), which have yet to be specified. Whether, indeed, diesel exhaust and carbon black induce different adducts needs to be verified. Since the tumor response was not different between the two particle types, the role of adduct formation for particle tumorigenesis needs to be established. Studies are needed to investigate the importance of the adducts and correlation of the adduct formation with the particle-induced tumors. The specificity of adducts in different regions of the lung and for different particle types needs to be studied. However, results on DNA adduct formation should be interpreted very cautiously, in particular with respect to linking them to specific mutations. DNA adduct formation also has been found in alveolar macrophages of humans who have been exposed to coal or smoke particles, and the question is whether and how these adducts, which could conveniently be determined from lavage samples, might be correlated to adducts induced in other cell types and regions of the lung.

4.2.3. *Mutation Spectra*

Mutational changes may occur at different gene locations, and nothing is known as to whether specific mutations are related to specific particle exposures or not. Thus, analysis of specific gene mutations needs to be performed in studies using different particle types and comparing those to spontaneously occurring mutations. The fact that chronic inhalation of particles at high concentrations can lead to lung tumors in rats implies that mutations have occurred. However, it needs to be investigated whether these are just spontaneous mutations that have become "fixed" due to a high proliferative response in the lung or whether these are new mutations with a very different spectrum.

4.2.4. *Dose-Time Response for Radical Production by Macrophages*

Oxygen-derived radicals may play an important role both for the formation of adducts (see paragraph 4.2.2.) and for causing cell injury leading to cell death. The lack of knowledge of dose-response curves and of the time course of production of oxygen-derived

radicals from inflammatory cells and other cells was seen as an area warranting further research.

4.2.5. *Dose-Time Response for Cell Proliferation*

Proliferative events are important aspects in the scheme outlined in Figure 1. There is an urgent need to know more about such proliferative responses induced by inhaled particles in an exposure/dose-dependent relationship pertinent to fibrosis and tumor development. Although proliferative lesions have been observed in essentially all of the chronic high-exposure particle inhalation studies in rats, no data to quantitate these responses have been reported pertinent to fibrosis and tumor development. It would be of particular importance to obtain such information in response to particle exposure during as well as after cessation of such exposures.

4.2.6. *Particle-Macrophage-Cytokine-Mutation Links*

This is perhaps the most important area for research needs--combining the particle-induced inflammatory responses of alveolar macrophages to the release of cytokines/growth factors and their linkages to both mutational events and fibrotic changes (upper left portion of Figure 1). Understanding the mechanisms that eventually lead to lung injury and mutations after the initial macrophage-particle encounter will give a better basis and justification for extrapolating results of animal studies to humans. Knowledge about the intricate networking of released cytokines and growth factors, not only from alveolar and interstitial macrophages but also from other cell types such as type 1 and type 2 epithelial cells and fibroblasts, is only slowly emerging now. We need more data related specifically to particle effects in this respect to better understand interactions of pro- and antimitogenic events in the lung and their implications for the fixation of mutations. The inflammatory response resulting from the activation of macrophages and the subsequent release of mediators from these cells and other cells is of crucial importance in the initial phase because this may eventually lead to the formation of tumors. Cellular proliferative responses as a consequence of inflammatory events were considered an important area requiring further research.

4.2.7. *Examination of Archival Tissue*

Techniques have been developed and are being developed further to use fixed tissues from previous animal experiments or from autopsy material for a detailed analysis of some of the mechanistic events outlined in Figure 1. For example, in situ hybridization techniques are available to determine whether and which types of growth factors or cytokines might have been involved in a specific response. Methodologies to examine cell proliferation events using proliferating cell nuclear antigens (PCNA) are being refined for use in archival tissues to quantitate proliferative responses of specific cell types after particle exposure. Therefore, archival tissues are an important resource for further investigations, thereby reducing the need for some new and costly experiments.

4.2.8. *Particle-Associated Compromises in Tumor Defense Mechanisms*

It is unknown whether excessive particle burdens actually initiate or promote the emergence of tumors or whether transformed cells, which may normally occur otherwise, successfully progress because of a failure in tumor surveillance and killing activities by cells such as macrophages. The "tumor defenses" shown in Figure 1 refer to this mechanism.

Many of the research needs outlined in this section can and should make use of both in vivo and in vitro studies. In vitro studies also should incorporate the use of human alveolar macrophages and possibly other human pulmonary cells and compare their responses with those of the respective cells of rats and other animal species; that is, studying species-specific responses is very important. The influence of culture conditions must be appropriately assessed. It was also emphasized that experimental studies using particles should be performed over a wide range of doses. There was a consensus that the research outlined above must include studies in different species, which might uncover important mechanistic differences in particle-induced lung tumors.

The different pathways proposed for carcinogenesis converge at the point of mutations in Figure 1. Thus, it was suggested that the basic underlying questions can be summarized in a "convergence" approach by examining (1) whether particles cause increased or different

mutations and (2) whether particle-induced changes in "cancer genes" are different from those occurring spontaneously.

Although prioritization of the research recommendations was not attempted at the workshop, there appeared to be a general consensus that inflammatory and subsequent cellular proliferative responses may be the most important events leading to the fixation of induced mutations and finally particle-induced lung tumors.

5. RESEARCH PLANS RESULTING FROM WORKSHOP RECOMMENDATIONS

5.1. Goals of the Research

EPA funding to support research in this area is limited. To provide answers to some of the most pressing questions, an experiment was designed that will be carried out with the aid and cooperation of other government and private institutions. The study is designed to focus on the following issues:

1. Species differences in responsiveness to inhaled particulate matter.
2. The relationship between lung particle burdens and release of mediators by pulmonary macrophages. Is there a threshold?
3. Relationship between the output of mediators and pathological responses in the lungs.
4. Relationship between the output of mediators and cell proliferation.
5. Relationship between the lung burden of particulate matter, production of mediators, and formation of DNA adducts.

5.2. Work Plan

Animals will be exposed for periods of up to 90 days to several concentrations of carbon black. Additional groups exposed to cristobalite and amorphous ultrafine silica will serve as positive controls along with one group of sham-exposed animals.

Fischer 344 rats will be used. Groups of animals will be sacrificed after 45 and 90 days of exposure as well as 6 months and 12 months post exposure. Exposure durations of 7 to 8 hours per day, 5 days per week, are planned. Whole animal exposure chambers will be used.

The following endpoints will be examined:

- Particle lung burdens
- Analysis of bronchoalveolar lavage fluid
- Cell proliferation assays
- Growth factor production by macrophages
- Cytokines and chemotactic mRNA
- Oxidant-induced DNA adducts in lung epithelium
- Mutation assay in alveolar epithelial cells
- Lung histology-morphology

5.3. Time Schedule

These studies are planned to begin during the third quarter of 1993 and are expected to be completed near the end of 1994.

5.4. Utilization of Research Data

The EPA and other regulatory agencies are charged with assessing health risk effects of a variety of types of inhalable particulate matter. The EPA, for example, is developing unit risk estimates for diesel exhaust. Although a large number of organic compounds, including some that are carcinogenic, are adsorbed to the surface of diesel exhaust particles, available evidence, nevertheless, indicates that pathological and carcinogenic effects of diesel exhaust are primarily a function of the insoluble core of the diesel particle. Certain manmade mineral fibers, which are insoluble as well as biochemically inert, also have been shown to be toxic and carcinogenic. Insoluble, biochemically inert polymers are regulated by EPA's Office of Prevention, Pesticides, and Toxic Substances. Other insoluble particulate matter of concern to EPA and other regulatory agencies includes silica and coal dust. Although the latter two agents have additional toxic properties due to the presence of oxygen radicals on the particle surface, particle effects may still contribute to responses. The research data that are obtained are expected to be used to develop updated risk estimates of exposure to particulate matter agents such as the ones mentioned above.

The results of planned research should allow the development of mechanistically based dose-response models, in the hope that a major uncertainty in quantitatively assessing risk of exposure to particles, the shape of the low-dose extrapolation curve, will be eliminated or at least decreased. It may be possible to partition the relative effects of particles, surface-adsorbed organics, particle-associated oxygen radicals, or vapor phase compounds. The ultimate goal in collecting these data is to reduce uncertainty in assessing risk of exposure to the agents listed above.

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