National Institute for Occupational Safety and Health
Occupational Fiber Exposures and Lung Disease
Research Strategy

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1.0 Introduction

1.1 Background and Goals of the Research Strategy

Chronic exposure to asbestos, both amphibole and serpentine varieties, can result in serious lung diseases including fibrosis (asbestosis), lung cancer, mesothelioma, and other pleural diseases. The morphology of the asbestiform minerals, elongated particles or "fibers," is generally thought to be a major factor in the toxicity/carcinogenicity of these materials. The surface properties of fibers may also play an important role. Because animal studies strongly associate mesothelioma and lung cancer with asbestos particle morphology, concern exists about the adverse health effects of any material of similar morphology.

The National Institute for Occupational Safety and Health (NIOSH) has a major responsibility to conduct and interpret research leading to the prevention of disease and injury in the workplace. To address research issues, NIOSH formed a Fiber Research Committee made up of scientists within the Institute. This document is the result of the Committee's work. It describes research needs and research strategies for identifying and preventing lung disease resulting from occupational fiber exposure. These materials of concern include synthetic minerals, other naturally occurring mineral fibers and non-asbestiform varieties of minerals that also exist as asbestos. The latter can form cleavage fragments that include elongated or acicular particles that are difficult to differentiate from asbestiform mineral fibers.

As is often the case in scientific endeavors, this document builds on the work of others. From July 10 to 12, 1989, the National Institute of Environmental Health Sciences (NIEHS) held a workshop entitled Fiber Toxicology Research Needs (Environmental Health Perspectives, 88:261-322, 1990). The goals of this workshop were to (1) critically review human and experimental data concerning fiber toxicology with an emphasis on biological mechanisms, (2) identify data gaps and research needs, and (3) suggest future research efforts. Many of the recommendations from the NIEHS workshop are consistent with those in this document.

This document focuses on data gaps and research needs related to occupational mineral fiber exposures. Also, it establishes research priorities and describes the relationships between the various types of research on fibers.

1.2 Nomenclature

Much of the debate over regulation of fibrous minerals (e.g. asbestos) is due to confusion over definitions and how these definitions have been used in the characterization of occupational exposures. For example, a major problem exists with the regulation of the non-asbestiform minerals which may fragment into elongated or acicular particles which resemble their asbestiform counterparts. It is difficult, if not impossible, to determine

whether amphibole particles of similar morphology are asbestiform or non-asbestiform with current environmental sampling methods. Regardless of the confusion over nomenclature, the primary issue is not so much what is or what is not an asbestos fiber in mineralogical terms. The important question is whether non-asbestiform cleavage fragments or any durable fiber having dimensions equivalent to asbestiform fibers are capable of inducing lung disease.

2.0 Research Areas

Although fibrous minerals (especially asbestos fibers) have been the subject of intense research during the past 20 years, a number of important issues have not been resolved. In addition, recent studies have generated new questions that need to be answered. In general, there is a need for further research on the properties and measurement methods for fibers, the diseases fibers produce, and the mechanisms by which any fibrous substance causes disease.

2.1 Fiber Characterization and Analysis

Fibers should be characterized as completely as possible when conducting any research. It is important to characterize not only all of the physical and chemical properties of fibers, but also to characterize their properties in biological systems, including solubility, phagocytosis, and translocation. For example, if a fiber cannot be deposited in the lung or if it is cleared rapidly with little or no damage to the lung, then it may present little or no hazard. On the other hand, for those fibers that are deposited and retained in the lungs, their durability or persistence in a biological system may be the property that best predicts disease production.

2.1.1 Fiber Characterization

Various fiber properties are associated with fiber toxicity, including fiber morphology (especially length, diameter, and surface area), and fiber durability. Other fiber properties, such as the potential for longitudinal and transverse splitting, trace chemical concentrations, absorbed or adsorbed materials, surface charge at physiological pH, surface reactivity, and fiber chemistry, need to be investigated as possible significant factors affecting fiber toxicity. Standardized laboratory tests need to be developed and validated for relevant properties.

When research into fiber toxicity is conducted, it is important that particle length and diameter data are completely reported and the study design includes sufficient fibers in each size range group. In addition, analysis of filter samples of airborne fibers requires that the sample on the filter be representative of the fiber concentration in the inhaled air.

2.1.1.1 Accuracy of Sampling Methods

Current sampling methods can be improved by accounting for possible losses on the sampler inlet walls and developing ways to correct for non-uniformity of particle deposition on the filter. In addition, the current sampler used for measuring occupational exposure to asbestos and other fibers uses a 25 mm diameter cassette with a 50 mm inlet cowl. This sampler was not designed to be selective for those fibers that reach the thoracic region of the respiratory tract, since this was not an issue when the sampler was originally designed. Research is needed to develop or improve samplers so they collect the biologically relevant fractions of the fibrous aerosol.

2.1.1.2 Standard Methods of Assessing Exposure

Problems still exist with non-uniform particle deposition on filters used to collect samples. In addition, it is not clear if particle aggregates containing clumps of fibers can separate in the lungs and affect the lung as several fibers or a single entity. While there are various measurement techniques available, a uniform method for measuring, characterizing, and reporting distributions of fiber length, diameter and other biologically relevant parameters needs to be developed, tested, and published. This will allow researchers performing toxicology and epidemiology studies to report comparable results using consistent methods.

2.1.1.3 Accuracy of Analytical Methods

The phase contrast microscope (PCM) method was developed to provide a readily available and inexpensive index of asbestos fiber concentration. The method is not specific, without subsequent back up analyses for specific fibers with electron microscopy and other techniques, and is limited to fibers with diameters larger than about 0.25 micrometers. This method is adequate for relatively high workplace fiber concentrations that existed at the time of the method's development, but its sensitivity is decreased at the current NIOSH recommended exposure limit (REL), and lower, for asbestos fibers concentrations (0.1 fiber/cc) when interfering dusts are present. This along with inadequate fiber counts can result in diminished method accuracy. Research is needed to determine the effect of these and other problems with the method, as well as to develop improved techniques for dealing with them.

2.1.1.4 Automated and Analytical Instruments and Techniques

Current microscopy methods for analyzing fibers require the manual counting and sizing of individual fibers. Automated analytical instruments to reduce the chance for human error and inter-observer differences are needed. In addition, direct reading instruments are needed to allow more rapid detection and control of hazardous exposures at the worksite.

2.1.2 Fiber Production and Generation

Techniques need to be developed for preparing adequate quantities of fibers of known length, diameter, and other relevant properties. These techniques need to be capable of yielding particles of the size(s) most appropriate for evaluating fiber toxicity, in both *in vitro* and *in vivo* inhalation studies. Standardized techniques for generating, characterizing, and controlling airborne fiber concentrations for inhalation experiments need to be developed:

2.1.3 Fiber Repository

2.1.3.1 Bulk Fiber Material Repository

In the past, UICC (Union Internationale Contre le Cancer) asbestos materials have been used for toxicity studies, but relatively small quantities of these materials remain. New and improved characterization techniques need to be applied to these reference materials.

Standardized reference materials of all relevant fiber types need to be collected for carrying out toxicologic experiments and for determining the comparability of different studies. Sufficient quantities of these materials must be selected, prepared, extensively characterized, and made available to researchers.

In order to elucidate the relationship between various naturally occurring mineral fibers, and toxicity, the bulk repository should include representative rock and mineral specimens which are composed of minerals of health concern. As many as possible of the common crystal habits of these minerals should be represented in the collection.

2.1.3.2 Fiber Sample Archive

Measurement methods evolve and change with time, but for epidemiologic studies it is important to preserve continuity in fiber measurements (Section 2.1.1.2). An archive of field bulk and air samples from various industries and other sources would provide a means of maintaining this continuity. Both filter samples and prepared samples should be analyzed by newly established techniques, and the results compared with results from classical techniques.

2.1.4 Biological Characterization of Fibers

Biological assays should be developed for studying fiber characteristics and toxicity. Specific research areas are discussed below.

2.1.4.1 Fiber Dimension and Toxicity

Questions about the relationship between fiber dimension and toxicity must be addressed. Research is needed to determine how fiber dimension affects a cell's normal function. The basic questions concern how the physical characteristics of fibers affect the uptake and transport (both intercellular and extracellular transport), the production and secretion of proteins and other substances, the proliferation of cells, and the structure and function of genetic material.

2.1.4.2 Surface Properties and Toxicity

It is generally assumed that to some extent the surface chemistry of particles affects biologic responses. The development of techniques to study particle surface chemistry effects on cell function may provide useful means of comparing different types of fibers, and defining surface chemistry features that are important in toxicity.

2.1.5 Aerodynamic Behavior and Fiber Deposition

The site of fiber deposition in the lung plays an important role in fiber toxicity. The aerodynamic properties related to fiber shape may influence deposition patterns. These patterns may, in turn, influence the type and extent of disease. Fiber deposition issues to be resolved are discussed in the following subsections.

2.1.5.1 Mathematical Models

Although mathematical models for particle deposition in human lungs have been developed, models for fiber deposition and translocation need to be developed and validated using animals. The effect of different fiber shapes and sizes (straight, curved, curly, etc.) on mathematical deposition and translocation models should be determined for specific animal species. Development of such models for animals would help identify fiber sizes that can reach and be deposited at specific sites in the lung. Such models would also be extremely valuable for developing suitable animal models to investigate mesothelioma production through the inhalation route of exposure.

2.1.5.2 Animal Models

Toxicological testing with different animal models often results in different disease endpoints even though exposures are similar. For example in rats, lung tumors are produced following exposure to several types of mineral fibers including refractory ceramic fibers (RCFs) but few mesotheliomas are observed. However in hamsters, lung cancer is not seen following similar exposure regimens but a much larger number of mesotheliomas are seen with exposure to RCFs. Since the ultimate use for animal data is human risk assessment, research needs to be conducted to determine the mechanisms for these species-specific differences, and how these mechanisms may relate to humans.

2.1.5.3 Fiber Quantitation

The ability to quantify the number of fibers deposited in tissue, as well as their two dimensional size (length/diameter) distribution, is important for developing fiber dose-response relationships and durability information. For research purposes, a standardized method to quantitate fibers by electron microscopy should be developed. All factors that influence counting and sizing results must be addressed when developing the method, including a means of extracting fibers from the tissue (drying and ashing versus digestion), filter preparation (use of sonication, type of filter, direct versus indirect preparation, etc.), counting statistics, choice of electron microscope (SEM/TEM), and choice of magnifications (low for quantitating larger fibers and high for smaller fibers).

2.1.5.4 Interference of Other Exposures

Excessive doses of almost any materials other than test fibers may complicate the interpretation of pathology in experimental animals. Specifically, test material that contain large amounts of other materials confound interpretation. Research is needed to address this problem, since an "overload dose" phenomenon may impair the ability to extrapolate animal data to humans.

2.1.6 Fiber Clearance and Retention

The long-term retention of fibers in the lung may result in pathological changes, including fibrosis, lung cancer, and mesothelioma, whereas clearance from the initial site of deposition may have either positive or negative health consequences. Elimination of fibers from the body through engulfment by macrophages and subsequent clearance by mucociliary transport is a non-pathogenic process, while translocation of fibers to the lung periphery may lead to mesothelioma production. Fibers may also completely dissolve while in the lung or disintegrate into fragments of smaller lengths and diameters that may generally be cleared more easily. A reduced fiber durability is frequently equated with decreased toxicity.

Because fiber durability in the lung has been suggested as a critical factor in fiber toxicity, a definition of fiber durability, and a standardized laboratory technique(s) for determining fiber durability needs to be developed. Additional research needs in fiber clearance and retention are discussed in the following subsections.

2.1.6.1 Quantitative *In Vivo* Methods

Quantitative *in vivo* methods to investigate fiber clearance should be developed. Methods which differentiate between clearance by dissolution versus translocation are needed. Factors which must be accounted for in these methods include both longitudinal and

transverse fiber splitting. Methods used to extract fibers from lung tissue should be validated to ensure that they do not change the fiber dimensions.

Quantitative data must be developed on the *in vivo* durability of fibers to which workers may be exposed. Research must be conducted to determine a time frame for toxicity. For example, how long must fibers persist before cancer (tumor) rates increase?

2.1.6.2 Longitudinal Splitting of Fibers *In Vivo*

For any study of biological activity there is a great need to establish a dose-response relationship. Data concerning longitudinal splitting of fibers *in vivo* is needed since this splitting may increase the effective dose of fibers received or conversely enhance clearance. These effects make dose-response relationships difficult to define.

2.1.6.3 Quantitative *In Vitro* Methods

To supplement quantitative *in vivo* clearance methodologies, quantitative *in vitro* cell culture methods for determining fiber durability should be developed. Attempts should be made to develop *in vitro* techniques to study the leaching of minerals or the fragmentation of particles. These methods should make it easier to determine *in vivo* changes in fiber size distribution over time.

2.1.6.4 Particle Size and Translocation

Only the thinnest fibers ($< 0.1\mu m$ in diameter) are thought to translocate to the pleura, where they can initiate mesothelioma. Thicker fibers are thought to remain in the lung where they may initiate other diseases such as lung cancer. Other data suggest that extra-pleural cancers (kidney) could be initiated as a result of fibers being translocated from the lung via the circulatory or lymphatic system. Animal research should be designed to quantify the size range of fibers that translocate to the pleura and other organ sites.

2.1.6.5 Relationship Between Fiber Deposition and Site of Lung Cancer

Most human asbestos-induced lung cancers originate in the bronchial wall, but nearly all asbestos fibers found in the lung are retained in the unciliated airways or interstitium. Research is needed to clarify which fibers are responsible for tumor induction -- the low number of fibers lodged in the bronchial wall or the much higher number of fibers retained in the alveoli and interstitium.

2.1.6.7 Fiber Retention

Workers who mine chrysotile asbestos in Canada have been reported to retain less chrysotile asbestos in their lungs than amphiboles, although amphiboles represent only a small portion of the ore. Research is needed to investigate the occurrence of mesothelioma with respect to fiber retention and exposure pattern (short-term high exposures versus long-term low exposures).

2.2 Biological Activity and Mechanisms of Disease

2.2.1 Need for Dose-Response Relationships

For any study of biological activity there is a need to establish a dose-response relationship. When studying the biologic activity of asbestos and other fiber substitutes, it is important to determine the most biologically appropriate unit for dose - number of particles in specific length and/or diameter ranges, surface area, or mass.

Factors other than fiber length and diameter may influence biologic responses. For example, erionite appears to be a more potent producer of mesotheliomas than amphiboles in humans and animals. Chrysotile is known to produce mesotheliomas in animals, but the incidence of mesothelioma in chrysotile workers is lower than may be predicted. Research is needed to explain the variability in biological responses. There is a need to develop *in vitro* assays to help identify the important variables and to identify materials for *in vivo* studies.

2.2.2 Role of Radicals in Pathogenesis

Many questions have been posed about the existence of radicals on the surface of particles and their role in pathogenesis. Research is needed to determine (1) the extent that radicals exist on the surface of asbestos fibers or are generated when fibers are broken or split, (2) how other fiber substitutes and non-asbestiform cleavage fragments compare in this regard, (3) whether fibers act to catalyze the formation of radicals in the presence of phagocytes, (4) whether the physical and chemical properties of fibers affect the ability to generate radicals, (5) how fiber-associated radicals affect the viability of pulmonary cells, and (6) whether such radicals cause DNA damage or compromise immune response.

2.2.3 Animal Short-term Exposure Studies

While a great deal has already been accomplished in this area, the results suffer from a lack of integration. Short-term exposure animal studies are needed to explore the induction of fibrosis and/or tumors. High dose, short-term inhalation studies and/or intratracheal instillation studies may prove useful in defining fiber characteristics responsible for fibrosis and/or tumors. Coupled with this is the need to develop assays that detect early

biochemical and/or genetic changes indicating whether fibrosis or carcinogenesis has been initiated, i.e., assessment of short-term exposures and responses.

2.2.4 Animal Long-term Exposure Studies

Long-term inhalation studies are needed to examine the relationships between fiber exposure and pulmonary and pleural fibrosis, lung cancer, and mesothelioma. The results of long- and short-term exposures should be compared to determine whether short-term responses are the precursors to long-term responses, or whether they are caused by separate mechanisms. Long-term studies should also be undertaken to identify biomarkers for chronic diseases.

2.2.4.1 Elements Leached from Fibers

Magnesium is leached from chrysotile during residence in the lung. Long-term studies are needed to determine how magnesium and/or other elements leaching from fibers affect lung disease processes.

2.2.4.2 Dose-Response for Asbestosis, Cancer, and Mesothelioma

Human and animal studies suggest that lower doses of fibers can cause mesothelioma than those that cause asbestosis and cancer. Further research is needed to validate these results and explore possible mechanisms for these differences. Information about the dose administered versus the dose received and retained in the lung parenchyma and pleura is essential for interpreting the results of these studies.

2.2.5 *In Vitro* Exposure Studies

The direct effects of fibers on cells and tissues, and the mechanisms of disease production can be more clearly determined by the use of *in vitro* methodologies. Questions ranging from the production of growth factors (which might relate to fibrosis) to cell surface marker changes (which might relate to carcinogenesis) should be addressed. *In vitro* studies have a number of advantages. They require much smaller quantities of materials, allow more precise control of exposure dose, and produce more quantitative measures of response. For materials in limited supply, such as highly purified samples of a specific size, *in vitro* techniques are of particular value.

Assays to determine the relative toxicity of asbestos and other fibers are needed. *In vitro* assays may be of value for such purposes. *In vitro* assays designed to mimic the physiologic conditions under which fiber-cell contact occurs *in vivo* should be evaluated. As stated earlier (Section 2.2.1), it is important to determine if an index of particle number, surface

area, or mass is the critical measure of dose. Some of the problems that need to be considered are as follows:

2.2.5.1 Cytotoxicity and Fiber Size

The relationship between fiber size and cytotoxicity needs further clarification. Research is needed to determine whether there are critical cutoff sizes (diameter and length) for cytotoxicity.

2.2.5.2 Cytotoxicity of Fibers Exposed to High Temperatures

Many fibers are used as refractory materials. The toxicity of these fibers after prolonged and repeated exposure to high temperatures encountered during commercial use needs to be determined.

2.2.5.3 Cell Factors Influencing Cytotoxicity

The type(s) of lung cells most susceptible to fiber-induced cytotoxicity need to be identified. If there is a range of susceptibility, the factors accounting for this range need to be understood.

2.2.5.4 Cytokines and Pathogenesis

The effects of fiber exposure on lung cell cytokine expression and secretion needs to be determined. Information concerning how these cytokines are related to the pathogenesis of asbestosis is also needed.

2.2.6 Genotoxicity

Exposure to asbestos fibers is known to increase the incidence of mesothelioma, lung cancer, and possibly other types of neoplasia. However, the mechanism of asbestos fiber carcinogenesis is not known. It is generally accepted that alterations or damage to DNA and/or chromosomes is involved in the initiation of carcinogenesis. Thus, analysis of the genotoxic effects of fibers may provide useful information on the potential mechanisms of carcinogenesis, and may help set priorities for those materials being considered for in depth carcinogenicity testing.

2.2.6.1 *In Vivo* and *In Vitro* Studies

In vivo and *in vitro* studies are needed to determine whether different types of fibers can induce DNA synthesis and cell division, clastogenic effects, or DNA damage in primary and/or cultured lung cells.

2.2.6.2 Morphological Cell Transformation Assay

The morphological cell transformation assay should be carried out for asbestos and other fibers. The cell transformation assay, which can be considered an *in vitro* carcinogenesis assay, is a sensitive short-term assay system for detecting potential carcinogens. The transformed foci induced by fibers should be studied for possible mechanism(s) of cell transformation by means of oncogene activation. There is need to develop such assays using human epithelial and mesothelial cell lines.

2.2.6.3 Synergistic Effects of Asbestos and Chemicals

In the occupational setting, workers may be simultaneously exposed to fibers and chemicals. Asbestos fibers and cigarette smoke are reported to have synergistic effects on carcinogenesis. *In vitro* studies (using genetic endpoints) should be conducted to determine whether there is any synergistic effect between fibers and chemicals found in the workplace.

2.2.6.4 Transfection Studies

Recent studies have suggested that asbestos particles may be able to transfect DNA segments from one cell to another. Transfection could play a role in the transfer, expression, or derepression of oncogenes. Additional research is needed to evaluate this phenomenon.

2.2.6.5 Genetic Analysis of Tumor Tissues

Human cancer tissue, especially mesothelioma and lung cancer tissue from fiber-exposed subjects, should be analyzed for the expression of oncogenes. Such studies could determine which, if any, of the known oncogenes or tumor suppressor genes are involved in these cancers.

2.2.7 Biomarkers

Biomarkers may be indicators of exposure, susceptibility, or adverse health effects. The identification and use of biomarkers as indicators of exposure and early health effects could enable effective intervention and thus potentially reduce carcinogenic outcomes resulting from fiber exposures. However, little research has been conducted to identify potential biomarkers for fiber exposure and response.

2.2.7.1 Cytogenetic Effects

Cytogenetic effects resulting from fiber exposures should be evaluated as biomarkers in both animal models and human subjects.

2.2.7.2 Oncogene Expression

Oncogene expression should be evaluated as a biomarker for pre-neoplastic states resulting from fiber exposures.

2.2.7.3 Immunological Biomarkers

Research in animal models is needed to identify whether there are specific immune system changes resulting from fiber exposures which can be used as biomarkers. Macrophage or other immunocyte phenotypic changes or changes in cytokine secretions may serve as indicators of exposure or early health effects. Follow-up studies with human samples are needed to validate any positive results seen in the animal work.

2.2.7.4 Bronchoalveolar Lavage and Sputum Cytology

Bronchoalveolar lavage or sputum samples need to be evaluated for their utility as biomarkers.

2.3 Epidemiological Investigations

Prospective and retrospective studies of workers exposed to fibers are needed because the types of fibrous substances in commerce are changing, fiber-induced diseases have long latency periods, and there remain unanswered questions about health effects of existing fibrous materials. Smaller cohorts and mixed exposures (which include exposures to other dusts and chemicals) require that new approaches, such as those incorporating markers of exposures and/or disease processes, be considered.

2.3.1 Characterization of Exposure

Accurate characterization of exposure in prospective and retrospective epidemiological research is critical to the success of a study. To adequately accomplish this objective, improved techniques are needed to estimate past exposures. Existing historical exposure data should be reexamined with respect to the development of process specific conversion factors between the diverse historical exposure measurements. Improved sampling and analytical techniques are also needed for assessing current and future exposures. These techniques should provide for, to the extent feasible, the identification of all fiber types, fiber morphology, and the physical, chemical, and biological parameters (dimension, solubility/durability,etc.) most relevant for adverse biological effects.

2.3.1.1 Assessment of Fiber Exposure

Exposures to asbestos and other fibers (e.g., glass, ceramic fibers) have not been well characterized in workers (e.g., construction workers) who handle these materials. Assessments of fiber exposures (peak and cumulative) are therefore needed in all stages of use (e.g., mining and milling, end use manufacturing, and consumer use) to understand risk and to select cohorts for future epidemiological studies.

2.3.1.2 Confounding or Synergistic Exposures

Exposure to quartz is common where amphiboles and serpentines are found. An Australian cohort of crocidolite miners has been shown to have concurrent silicosis and asbestos-related disease. Confounding or synergistic exposures can potentially alter dose-response relationships and should be individually defined and measured whenever possible. For example, binders, such as formaldehyde, used on synthetic vitreous fibers should be identified, measured, and taken into account.

2.3.2 Non-asbestiform Fibers

Although any durable fibers with an aspect ratio of greater than 3 to 1 may be hazardous, limited time and resources require that fibers most suspect initially receive the most attention. Therefore, a systematic survey should be conducted to determine the extent of exposure and/or the existence of cohorts with exposures to non-asbestiform varieties of amphiboles that are not contaminated with asbestos fibers and that have cleavage fragments that result in thoracic deposition (approximately less than 3 micrometer diameter) and have an aspect ratio greater than 3 to 1.

2.3.3 Mineral and Synthetic Fibers

Worker population studies are needed to determine fiber exposure, intermediary biological effects, and adverse health effects related to the following: (1) other mineral silicates such as zeolite, wollastonite, and attapulgite and (2) small diameter synthetic mineral fibers, ceramic fibers, and other long, thin, durable synthetic fibers.

2.3.4 Pulmonary Fibrosis and Carcinogenesis

Studies are needed to investigate the mechanism of fiber induced pulmonary fibrosis. These studies should include new evaluation techniques, such as high resolution computerized tomography (HRCT). The effect of pleural disease on lung function should be further explored. Studies are also needed to evaluate the possible role of pulmonary fibrosis (both parenchymal and pleural) in human pulmonary carcinogenesis. Lung cancer studies with data on fiber dose (peak and cumulative), presence and severity of fibrosis, and smoking, as well as other confounders, are needed to separate the effects of fibrosis and fiber dose.

2.3.5 Other Neoplasms

Research is needed to evaluate the relationships between fiber exposure and neoplasms at other sites (GI, upper respiratory tract, etc.).

2.3.6 Updated Studies

Existing cohort mortality studies should be updated with additional death information and person-years-at-risk, as well as further evaluation of the exposures (fiber type and physical, chemical, and biological parameters, and potential confounding factors) within the cohorts.

2.3.7 Archives

The following archives should be established to serve as a basis for future research: 1) bulk fiber materials and air samples (see Section 2.1.3) and 2) tissue specimens from exposed workers to assess fiber deposition and retention in the lung.

2.3.8 Biological Markers

As stated in Section 2.2.7, the identification and use of biomarkers, including lung fiber burden, should be evaluated (by fiber type, dimension, solubility/durability, etc.) as indicators of exposure (peak and cumulative) and/or early health effects. Prospective studies of cohorts exposed to asbestos or other mineral fibers offer a unique opportunity to assess the predictive value of various biological markers of fiber related exposure or disease.

2.4 Control Methods

2.4.1 Substitute Materials

Research is needed to develop non-toxic products as asbestos substitutes. If non-toxic substitutes are not possible, then new products and current materials need to be treated or modified to reduce the hazards associated with their use. For example, products that are nondusty or that do not generate fibers of respirable or thoracic fibers could be formulated.

2.4.2 New or Modified Controls

Control methods currently used need to be modified and new methods developed to prevent worker exposure to potentially toxic materials. Safe and reliable manufacturing methods should be promoted through the use of process hazard analysis procedures, e.g., to prevent system failures such as filter puncture or rupture that could generate a high hazard environment. The use of control technologies, such as isolation, automation, and remote processing in sealed equipment, need to be investigated in order to prevent or minimize

worker exposures to fibers or particulates. If worker interface is necessary (as in fabrication and installation operations), then effective, convenient, and portable local exhaust ventilation systems should be developed for field use.

2.4.3 Development of New Substitute Products

Some of the currently marketed materials readily release airborne particles when (1) they are cut or otherwise manipulated, (2) they become damaged through use or by accident, and (3) they are removed during repair, remodeling, or demolition. Research is needed to develop products that will not release fibers when they are applied, shaped, or removed from surfaces. These materials must also be resistant to decomposition when exposed to heat, cold, or exposure to water or solvents, and accidental damage. Application techniques need to be developed whereby these materials can be modified and shaped during installation, removal, or repair in the field, without releasing fibers.

2.4.4 Fiber Penetration of Protective Clothing

There are few published studies on fiber penetration of protective clothing and on the effectiveness of decontamination of protective clothing. Research is needed on fiber penetration of protective clothing and decontamination procedures with an initial emphasis on clothing used in the asbestos abatement industry.

3.0 Research Strategy

This section identifies fiber research priorities and recommends a strategy for addressing them. It shows how research needs can best be met through an interdisciplinary research approach.

3.1 Need for Disease Mechanism Research

There is a need to understand the basic biological mechanisms of disease production in order to discern the physical, chemical, and other characteristics of fibrous particles that are responsible for a given disease endpoint. The primary purpose of developing occupational health standards is disease prevention, which does not require an understanding of disease mechanisms (e.g., if one can identify and prevent the relevant exposure, the disease is prevented without the underlying mechanism being known). However, understanding the mechanisms of disease can help in the development of standards and, perhaps more importantly, helps in the development of methods to evaluate new substances to determine their potential toxicity. Thus, understanding mechanisms of fiber-induced diseases may improve our ability to predict toxicity of mineral fibers, including non-asbestiform minerals, synthetic mineral fibers, and other materials, including those not yet developed or

commercially introduced. In addition, the study of fiber-induced lung disease mechanisms may affect the overall understanding of pulmonary fibrosis and lung cancer of any etiology.

This goal of understanding disease mechanisms may not be completely obtainable by current technologies, but that does not make this an inappropriate goal. A number of basic questions about fiber-induced lung disease that remain can be addressed by current technology. For example, comparison of inhalation and implantation studies indicate that fibers must directly interact with the mesothelium to induce mesotheliomas, but how the fiber penetrates to the mesothelium of man is unclear. Understanding whether there is some active translocation of particles within the lung, or if this is a passive process related to the total number of particles inhaled, would be important to know in establishing appropriate workplace exposure criteria. In the same way, understanding whether it is the fiber morphology or chemistry that induces the cellular transformations leading to lung cancer or mesothelioma is of critical importance. Understanding the biological responses to inhaled particles, particularly those responses that are either unique to or determined by the morphology of fibrous minerals, will allow prevention strategies to be developed that protect workers and that are adaptable to changes in the workplace.

3.2 The Role of Epidemiological Studies in Assessing Human Risk

The main function of epidemiologic studies is to determine whether there is an association between specific exposures and disease endpoints such as fibrosis and cancer. Previous epidemiologic studies of occupational groups exposed to different types of mineral fibers have provided conclusive evidence of fibrosis and/or cancer for some fiber types (e.g., asbestos). However, many other studies of workers exposed to naturally occurring or synthetic mineral fibers have produced equivocal results, with some studies reporting no or small elevations in the relative risk of fibrosis and/or cancer. In epidemiologic studies, equivocal and/or contradictory results may be due to factors such as imprecise or inaccurate exposure characterization and categorization, inadequate sample size, insufficient latency to detect the disease of interest, lack of information on confounding factors, etc. A particular problem when conducting epidemiologic studies of fibers is that the types and quantities of fibers present, as well as the potentially confounding non-fibrous exposures, have not been well characterized, so it is unclear whether the exposures in one study are comparable with the exposures in another. Therefore, future epidemiologic studies should be conducted in populations which will allow adequate assessment of both the fibrous and non-fibrous exposures. The assessment should include, to the extent possible, an evaluation of the chemical, physical, and biological parameters of all the fibers present. An attempt should also be made to search out cohorts exposed to a single class of fiber with no, or limited, confounding non-fibrous exposures for epidemiologic study. In addition, future epidemiologic studies should consider the utilization of new approaches, such as those incorporating markers of exposure and/or disease processes. Use of such markers would be especially appropriate in epidemiological studies of new fibers, for which adequate

latency to detect disease outcome has not yet accrued. Based on such studies, as well as toxicologic information, it may be possible to further identify the fiber types and parameters of public health consequence.

3.3 The Role of *In Vivo* (Animal) Studies in Assessing Human Risk

The absence of human data has often necessitated the reliance on well-defined and well-conducted animal studies to identify and better characterize the risk of exposure to specific fibers. Ideally the animal species that responds most like humans should provide the data base for estimating human risk. Clearly, data should not be used from species known not to have a valid model human response. Therefore, it is important that the appropriate species be selected that is most similar to humans in their response to the hazardous agent (e.g., mineral fibers). Also, the most sensitive species should be used for determining risk estimates (e.g., exposure limits), assuming that human sensitivity may be as high or higher than the most sensitive animal species. Furthermore, one animal species may not provide suitable models for all of the fiber-related diseases that affect humans.

Chronic inhalation animal studies designed to reflect the characteristics of exposure in an occupational environment would provide the most useful data for estimating human risk and provide meaningful comparisons with other studies.

However, these studies may be very difficult to perform and require a long period of time to complete. Where inhalation studies are not feasible, chronic studies using alternate routes of exposure (e.g., intratracheal or intrapleural instillations) can offer the means to generate data concerning *in vivo* toxicity/carcinogenicity. However, extrapolation of these data to estimate human risk is difficult.

3.4 The Role of *In Vitro* (Cellular) Studies

In vitro studies are generally quick and inexpensive. These studies also offer the most practical approach to determining the underlying mechanisms of fiber toxicity and carcinogenicity. Parameters which can be investigated for cellular systems include cytotoxicity, changes in production of various chemicals, cell surface marker changes, alteration or damage to cellular DNA, etc. In general, *in vitro* data may provide useful information regarding a material's potential toxicity or carcinogenicity, the mechanism of toxicity or carcinogenicity, and the physical properties of a substance which affect toxicity or carcinogenicity. However, a single *in vitro* assay which can reliably predict toxicity or carcinogenicity remains to be developed. For purely mechanistic studies, a battery of *in vitro* tests as outlined below may be sufficient. However, a full-scale investigation of fiber toxicity/carcinogenicity would use both whole animal and cellular models. Initially, a complete battery of *in vitro* tests should be performed to ascertain various parameters such as (1) cytotoxicity; (2) ability of the material to generate active oxygen species; (3)

genotoxicity; (e.g., oncogene activation, clastogenic activity, DNA damage, etc.); (4) fiber durability; and (5) fiber-cell interactions under physiologic conditions. These studies could be followed by acute and/or chronic animal studies.

3.5 Integration of Epidemiological, In Vivo, and In Vitro Studies

In vivo (or animal exposure studies) and *in vitro* studies each have their own relative merits and limitation. As shown in Figure 1, the design of such studies should take into account the results and conclusions of clinical or epidemiologic studies, and other *in vivo* or *in vitro* studies. The research should be integrated to promote interdisciplinary dissemination of results. At the center of the research effort is the need to characterize the materials being studied (fibers) as completely as possible. Also depicted in Figure 1 is recognition of the fact that a given type of research is somewhat self-perpetuating. For example, an *in vitro* study will yield results that lead to additional *in vitro* studies. While this self-perpetuating aspect of research is natural and valuable, it is important that interdisciplinary communications be maintained.

3.6 Research Priorities

While there are several disease endpoints of concern, a high priority research effort should concentrate on the relationship of fibers to the more serious diseases of mesothelioma and lung cancer in a tiered approach described in Figure 2. In addition, this approach assumes that other diseases (i.e., fibrosis) will, for the most part, be controlled if we control carcinogenesis.

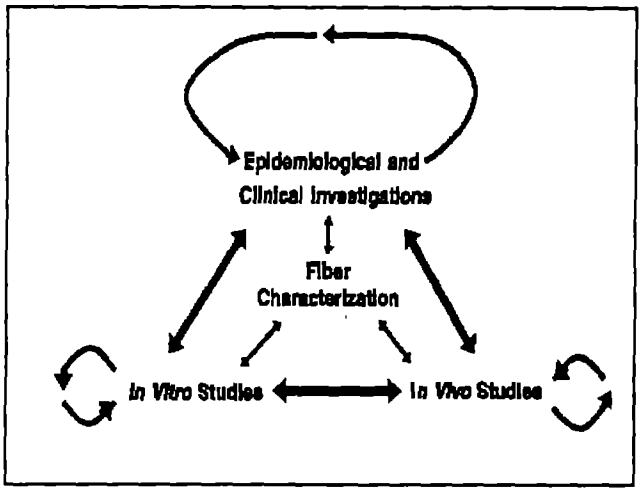

Figure 1. Relationship of various types of research studies.

One high research priority is to develop the means of generating fiber samples having specific, and preferably narrow, size distributions so that the relationships between physical dimension and toxicity/carcinogenicity can be completely defined. Given unlimited samples of fibers of varying type and size, a full battery of *in vitro* and *in vivo* tests can be performed to elucidate mechanisms and toxicity and/or carcinogenicity. These materials can also be used to perform the important task of validating *in vitro* and *in vivo* tests.

A repository of materials from a variety of mineral sources needs to be developed to provide comparable test materials for research. In addition, methods of reporting fiber distributions should be standardized.

New and more relevant tests of fiber durability are needed, particularly for establishing priorities for toxicity testing. For example, if a fiber cannot be deposited in the lung or if it is cleared rapidly with little or no damage to the lung, then it may have a lower priority for chronic inhalation studies. Assessing a fiber's chemical and physical properties as well as its solubility can be a useful first test to determine the need for further testing, but fiber durability tests need to go beyond fiber solubility.

Understanding the mechanisms of fiber induced diseases may lead to the development of methods to predict toxicity of durable fibers, including those not yet in commercial use. For example, the morphology of asbestiform fibers is generally thought to be a major factor in toxicity. Knowledge of the mechanisms involved may allow extension of these principles to other durable fibers. Therefore, research into disease mechanisms is a priority. However, this may not require a large number of new or separate research studies. When any *in vitro*, *in vivo*, or other research study is conducted, the goal of understanding disease mechanisms should be considered in the development of the study design. Therefore, all research elements shown in Figure 2 could have arrows leading to disease mechanisms.

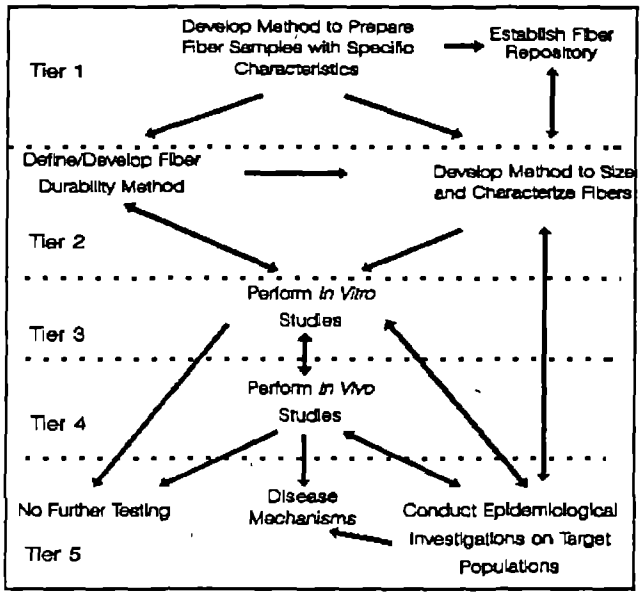

Figure 2. Research Priority Scheme

The schematic diagram in Figure 2 presents one approach to establishing research priorities, starting at the top of the figure and progressing downwards, with later research often being dependent on the successful completion of earlier research objectives. One possible application of this tiered model approach could be conducted using ceramic fibers (RCFs), perhaps as a National Toxicology Program (NTP) project. Recent extensive animal inhalation studies using ceramic fibers have provided valuable information on their toxicity. Other *in-vivo* and *in-vitro* assays system could be used to investigate ceramic fibers and the results from these studies compared. In addition, RCFs may provide a well characterized source of fibers for use in studying disease mechanisms.

It may not always be necessary to follow this tiered approach. For example, *in vivo* studies could be conducted without *in vitro* studies being performed first. However, this tiered approach should provide a more rapid and efficient means of identifying hazardous fibers, and is particularly suited for assessing the potential hazard of new fibers not yet in commercial production or for which adequate human data does not exist.