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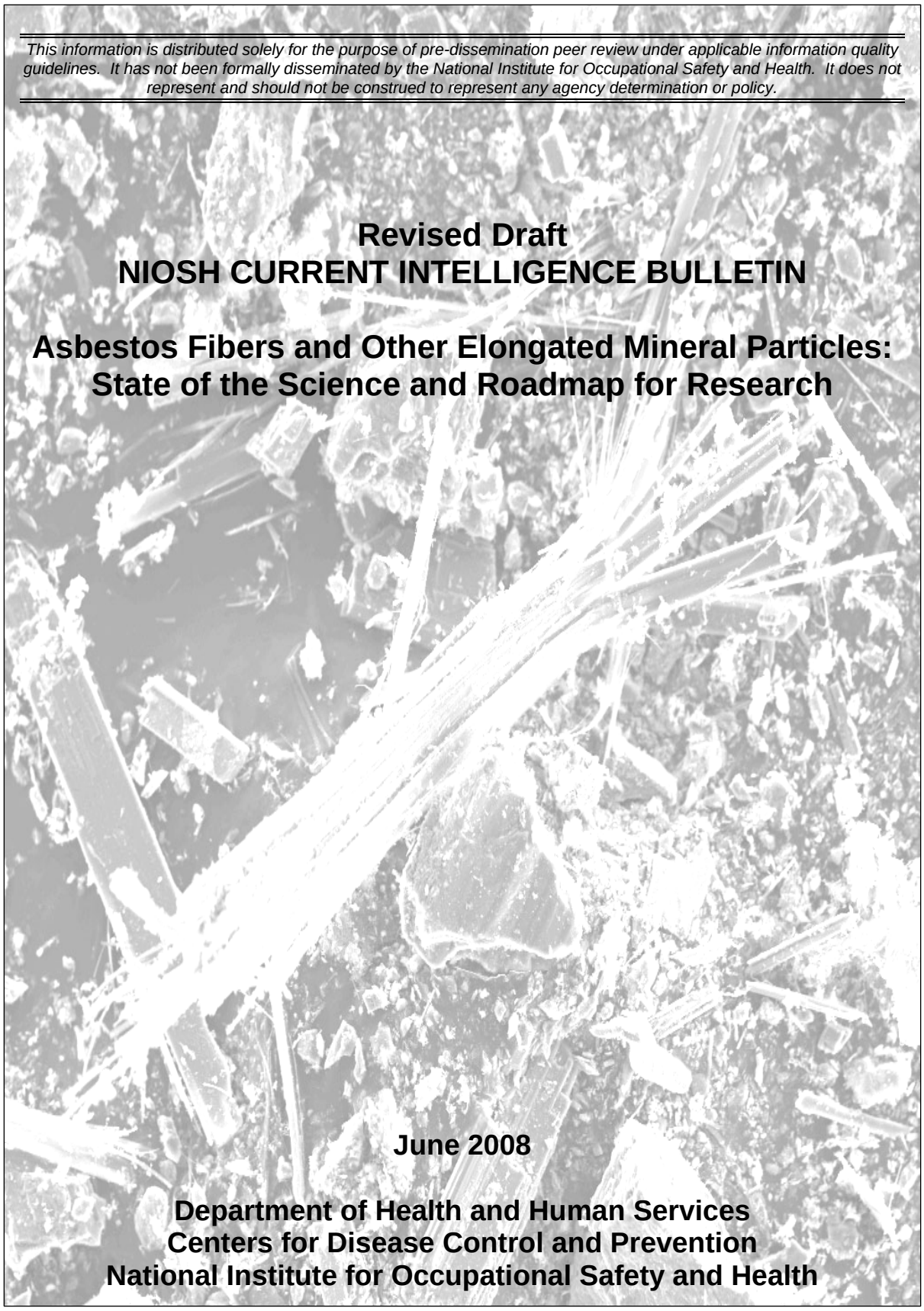

Revised Draft
NIOSH CURRENT INTELLIGENCE BULLETIN

Asbestos Fibers and Other Elongated Mineral Particles: State of the Science and Roadmap for Research

June 2008

Department of Health and Human Services
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

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Foreword

Asbestos has been a highly visible issue in public health for over three decades. During the mid- to late-20th century, many advances were made in the scientific understanding of worker health effects from exposure to asbestos and other elongated mineral particles (EMPs), and it is now well documented that fibers of asbestos minerals, when inhaled, can cause serious diseases in exposed workers. Yet, as we enter the 21st century, many questions and areas of scientific uncertainty remain. For instance, because of the complexity of the mineralogy, the scientific literature contains various inconsistencies in the definition and application of the term asbestos for health protection guidance and regulatory purposes.

As the federal agency responsible for conducting research and making recommendations for the prevention of worker injury and illness, the National Institute for Occupational Safety and Health (NIOSH) is undertaking a 21st century reappraisal of how to ensure adequate protection of workers from exposure to asbestos fibers and other EMPs. As a first step in this science reappraisal effort, NIOSH convened an internal work group to develop a framework for future scientific research and policy development. The NIOSH Mineral Fibers Work Group prepared an earlier draft of this *State of the Science and Roadmap for Scientific Research (Roadmap)*, which summarized NIOSH's understanding of occupational exposure and toxicity issues concerning asbestos and other EMPs.

NIOSH invited comments on the occupational health issues identified and the framework for research suggested in the first draft *Roadmap*. NIOSH sought other views about additional key issues that need to be identified, additional research that needs to be conducted, and suggested methods to conduct the research. In particular, NIOSH sought input from stakeholders concerning study designs, techniques for generating size-selected fibers, analytic approaches, sources of particular types of EMPs suitable for experimental studies, and worker populations suitable for epidemiological study. As a result of comments received during the public and expert peer review process, NIOSH has revised its first draft and is now disseminating a revised draft of the *Roadmap*. The purpose of the *Roadmap* is to outline a research agenda that will guide the development of specific research programs to be conducted by NIOSH and others, as well as within and across disciplines to provide answers to current scientific questions, reduce scientific uncertainties, and provide a sound scientific foundation for future policy development. NIOSH continues to be interested in available and forthcoming research results that can help answer the questions set forth in the *Roadmap*, as well as information on existing workplace exposure data, health effects, and control technologies.

NIOSH recognizes that results from toxicity research on asbestos and other EMPs may impact both occupational as well as environmental health policies and practices. Many of the issues that are important in the workplace are also important to communities and to

the general population. Therefore, NIOSH intends to continue to pursue partnerships with Federal agencies, including the Agency for Toxic Substances and Disease Registry (ATSDR), the Consumer Product Safety Commission (CPSC), the Environmental Protection Agency (EPA), the Mine Safety and Health Administration (MSHA), the National Institute of Environmental Health Sciences (NIEHS), the National Institute of Standards and Technology (NIST), the National Toxicology Program (NTP), the Occupational Safety and Health Administration (OSHA), and the United States Geological Survey (USGS), as well as with labor, industry, academia, practitioners, and other interested parties, including international groups. These partnerships will help to focus the scope of the research that will contribute to the scientific understanding of asbestos and other EMPs, to fund and conduct the research activities, and to develop and disseminate informational materials describing results from the research on EMPs and their implications for occupational and public health policies and practices.

John Howard, M.D.
Director
June 2008

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Executive Summary

In the 1970s, the U.S. developed occupational regulatory definitions and standards for exposure to airborne asbestos fibers based on human evidence of respiratory disease observed in workers exposed to the six commercially used asbestos minerals—chrysotile, and the amphibole minerals cummingtonite-grunerite asbestos (amosite), riebeckite asbestos (crocidolite), actinolite asbestos, anthophyllite asbestos, and tremolite asbestos. Since the promulgation of these standards, the use of asbestos in the U.S. has declined substantially and mining of asbestos in the U.S. ceased in 2002. Nevertheless, many asbestos products remain in use and new asbestos-containing products continue to be manufactured in or imported into the U.S.

As more information became available on the relationship between the dimensions of asbestos fibers and their ability to cause respiratory disease, interest increased in exposure to other "mineral fibers." The term "mineral fiber" has been frequently used to encompass thoracic-sized elongated mineral particles (EMPs) that grow either in an asbestiform habit (e.g., asbestos fibers) or a nonasbestiform habit (e.g., as needle-like (acicular) or prismatic crystals, as well as EMPs that result from the crushing or fracturing of non-fibrous minerals (e.g., cleavage fragments). EMPs that grow in asbestiform habits are clearly of health concern. It remains uncertain whether other thoracic-sized EMPs, especially those EMPs with mineralogical compositions similar to the asbestiform minerals, warrant similar health concern.

In 1990, NIOSH revised its recommendation concerning occupational exposure to airborne asbestos fibers. At issue were concerns about potential health risks associated with worker exposures to EMPs with mineralogical compositions similar to those of the asbestos minerals and the inability of the analytical method routinely used for airborne fibers (i.e., phase contrast microscopy (PCM)) to differentiate between these other EMPs and fibers from the asbestos minerals. To address this concern, the NIOSH defined "airborne asbestos fibers" to encompass not only fibers from the six previously listed asbestos minerals (chrysotile, crocidolite, amosite, anthophyllite asbestos, tremolite asbestos, and actinolite asbestos), but also EMPs from their nonasbestiform analogs. NIOSH retained the use of PCM for measuring airborne fiber concentrations and counting those EMPs having: (1) an aspect ratio of 3:1 or greater and (2) a length greater than 5 μm. NIOSH also retained its recommended exposure limit (REL) of 0.1 "airborne asbestos fibers" per cubic centimeter (f/cc).

Several concerns have been raised through the years about the revised NIOSH recommendation. These concerns include:

- NIOSH's explicit inclusion of EMPs from nonasbestiform amphiboles in its revised definition of "airborne asbestos fibers" contrasts with the regulatory

approach taken by OSHA and MSHA, and NIOSH's rationale for doing so is based on inconclusive science.

- The revised "airborne asbestos fibers" definition may be too restrictive because it does not explicitly encompass EMPs from other asbestiform amphiboles (e.g., winchite and richterite) or other fibrous minerals (e.g., erionite) that have been associated with health effects similar to those caused by asbestos.

- The specified dimensional criteria (length and aspect ratio) covered by the definition may not be optimal for protecting the health of exposed workers.

- Other physicochemical parameters, such as durability and surface activity, may be important toxicological parameters but are not reflected in the current definition.

- Use of the term "fiber" to describe all airborne EMPs covered by the NIOSH REL for asbestos is inconsistent with the way mineralogists use the term and this inconsistency leads to confusion about the toxicity of particles. (A clarification of the NIOSH recommendation that avoids this confusing use of the term "fiber" is presented in Section 1.8 of this document.)

NIOSH recognizes that its descriptions of the REL since 1990 have created confusion and caused many to infer that the additional covered minerals were included by NIOSH in its definition of "asbestos." NIOSH wishes to make clear that such nonasbestiform minerals are not "asbestos" or "asbestos minerals." NIOSH also wishes to minimize any potential future confusion by no longer referring to particles from the nonasbestiform analogs of the asbestos minerals as "asbestos fibers." In a clarified REL presented in this *Roadmap*, NIOSH avoids referring to particles from such nonasbestiform minerals as "asbestos fibers" and clarifies that particles meeting the specified dimensional criteria remain countable under the REL even if they are derived from nonasbestiform minerals.

PCM is the primary method specified by NIOSH, OSHA and MSHA for analysis of air samples for asbestos fibers, but it has several limitations including limited ability to resolve very thin fibers and to differentiate various types of EMPs. Occupational exposure limits derived from human risk assessments have been based on airborne asbestos fiber concentrations determined using PCM, or conversions from older impinger method-based particle concentrations to estimated PCM fiber concentrations. Current airborne asbestos fiber exposure risk estimates are based only on the subset of airborne fibers detected using PCM. The standard procedure for counting fibers using PCM includes only fibers longer than 5 µm and some fibers longer than 5 µm are too thin to be detected by PCM. Thus, this analytical method leaves an undetermined number of fibers collected on each sample uncounted. More sensitive analytical methods are currently available, but these methods will require standardization before they can be

recommended for routine analysis. In addition, any substantive change in analytical techniques used to evaluate samples and/or the criteria for determining exposure concentrations will necessitate a reassessment of current risk estimates derived from fiber concentrations based on PCM.

While epidemiological evidence clearly indicates a causal relationship between exposure to fibers from the asbestos minerals and various adverse health outcomes, including asbestosis, lung cancer, and mesothelioma, results from epidemiological studies do not provide entirely clear answers regarding potential toxicity of EMPs from the nonasbestiform analogs of the asbestos minerals. Due to various study limitations, NIOSH has viewed findings from relevant epidemiological studies as providing inconclusive, as opposed to either positive or negative, evidence regarding health hazards associated with exposures to EMPs from nonasbestiform amphiboles. Additional epidemiological studies are also warranted on other EMPs, such as winchite and richterite fibers (EMPs identified in vermiculite from a former mine near Libby, Montana) and zeolite fibers, among others. Populations of special interest include workers at talc mines in upstate New York and workers at taconite mines in northeastern Minnesota. Additional epidemiological studies are also warranted on other EMPs that have not been as well studied as fibers from the six asbestos varieties used commercially, such as winchite and richterite fibers (EMPs identified in vermiculite from a former mine near Libby, Montana) and zeolite fibers, among others.

Although opportunities for informative observational epidemiological studies are limited, there is considerable potential for experimental animal studies and *in vitro* studies to address specific scientific questions relating to the toxicity of EMPs. Short-term *in vivo* animal studies and *in vitro* studies have been conducted to variously examine cellular and tissue responses to EMPs, identify pathogenic mechanisms involved in those responses, and understand morphological and/or physicochemical EMP properties controlling those mechanisms. Long-term studies of animals exposed to EMPs have been conducted to assess the risk for adverse health outcomes (primarily lung cancer, mesothelioma, and lung fibrosis) associated with various types and dimensions of EMPs. Such studies have produced evidence demonstrating the importance of dimensional characteristics of mineral particles for determining carcinogenic potential of durable EMPs. In fact, NIOSH's policy to include the nonasbestiform analogs of the asbestos minerals as covered minerals under its definition of "airborne asbestos fibers" is largely based on evidence from these long-term animal studies. Although *in vitro* studies (which do not incorporate all *in vivo* conditions and processes) and animal studies (for which interspecies differences have been observed) are subject to uncertainties with respect to how their findings apply to humans, animal studies are warranted to systematically study and better understand the impacts of dimension, morphology, chemistry, and biopersistence of EMPs on malignant and nonmalignant respiratory disease outcomes.

To reduce existing uncertainties and to help resolve current controversies, strategic research endeavors are needed in toxicology, exposure assessment, epidemiology, and analytical methods. The objectives of such research will be to contribute to the development of new policies for exposure to airborne asbestos fibers and other EMPs with recommendations for exposure indices that are not only more effective in protecting workers' health, but are more firmly based on well-established risk estimates. To bridge existing uncertainties, this *Roadmap* proposes that research address the following three strategic goals: (1) develop a broader and clearer understanding of the important determinants of toxicity for EMPs; (2) develop information on occupational exposures to various EMPs and health risks associated with such exposures; and (3) develop improved sampling and analytical methods for asbestos fibers and other EMPs.

Developing a broader and clearer understanding of the important determinants of toxicity for EMPs will involve conducting *in vitro* and *in vivo* animal studies to ascertain what physical and chemical properties of EMPs influence their toxicity.

Developing information and knowledge on occupational exposures to various EMPs and potential health outcomes will involve: (1) collecting and analyzing available occupational exposure information to ascertain the characteristics and extent of exposure to various types of EMPs; (2) collecting and analyzing available information on health outcomes associated with exposures to various types of EMPs; (3) where scientifically warranted and technically feasible, conducting epidemiologic studies of workers exposed to various types of EMPs to better define the association between exposure and health effects; and (4) developing and validating methods for screening, diagnosis, and secondary prevention for diseases caused by exposure to asbestos fibers and other EMPs.

Developing improved sampling and analytical methods for EMPs will involve: (1) reducing inter-operator and inter-laboratory variability of currently used analytical methods; (2) developing a practical analytical method that will permit the counting, sizing, and identification of all EMPs deemed biologically relevant; (3) developing a practical analytical method that can assess the potential durability of EMPs as one determinant of biopersistence in the lung; and (4) developing and validating size-selective sampling methods for collecting and quantifying airborne thoracic-size asbestos fibers and other EMPs.

The primary anticipated outcomes of the research would be the identification of the physicochemical parameters such as chemical compositions, dimensional attributes (e.g., ranges of length, width, and aspect ratio), and durability as predictors of biopersistence, as well as particle surface characteristics or activities that determine the toxicity of asbestos fibers and other EMPs. The results of the research would also provide sampling and analytical methods that closely measure the important toxic characteristics. These results can then inform the development of appropriate recommendations for worker protection.

Another outcome of the proposed research framework could be the design of short-term studies and development of criteria that could be used to predict the relative toxicity of EMPs. Such criteria might include specific chemical compositions, dimensional attributes (e.g., ranges of length, width, and aspect ratio), and durability as predictors of biopersistence, as well as particle surface characteristics or activities (e.g., propensity for generating ROS). It would be particularly advantageous if toxicity of any particular type of EMP could be reliably predicted on the basis of results from *in vitro* and short-term *in vivo* studies. This would reduce the need for comprehensive toxicity testing with long-term *in vivo* studies in animals and/or epidemiological evaluation of each type of EMP. The results from such studies could possibly be extended beyond EMPs to encompass predictions of relative toxicities and adverse health outcomes associated with exposure to other elongated particles (EPs), including inorganic and organic manufactured particles. A coherent risk management approach that fully incorporates an understanding of the toxicity of particles could then be developed to minimize the potential for disease in exposed individuals and populations. Whether criteria can be developed to evaluate the potential toxicity of EMPs based on simple *in vitro* or short-term *in vivo* experiments is currently unclear, but the challenge to work toward such a goal could stimulate beneficial research and debate.

Asbestos Fibers and Other Elongated Mineral Particles: State of the Science and Roadmap for Scientific Research is intended to define the scientific and technical research issues that need to be addressed to insure that workers are adequately protected from exposure to asbestos fibers and other EMPs thought to pose a health risk. Achievement of the research goals framed in the *Roadmap* will require a significant investment of time, scientific talent, and resources by NIOSH and others. This investment, however, can result in a sound scientific basis for better occupational health protection policies for asbestos fibers and other EMPs.

Acknowledgements

Cover Photograph: Anthophyllite asbestos altering to talc, upstate New York. Photograph courtesy of USGS.

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Abbreviations

8-OHdG	8-hydroxydeoxyguanosine
AIHA	American Industrial Hygiene Association
AP-1	activator protein-1
ASTM	ASTM International
CI	confidence interval
COX-2	cyclooxygenase-2
DM	dark-medium microscopy
DNA	deoxyribonucleic acid
DPPC	dipalmitoyl phosphatidylcholine
ED	electron diffraction
EDS	energy dispersive x-ray spectroscopy
EGFR	epidermal growth factor receptor
EMP	elongated mineral particle
EP	elongated particle
EPA	US Environmental Protection Agency
ERK	extracellular signal-regulated kinases
f/cc	fibers per cubic centimeter
f/mL-yr	fibers per milliliter-year
ICD	International Classification of Diseases
IgG	Immunoglobulin G
IMA	International Mineralogical Association
IMIS	Integrated Management Information System
ISO	International Organization for Standardization
LDH	lactose dehydrogenase
LOQ	limit of quantification
MDH	Minnesota Department of Health
mg/m^3-d	milligrams per cubic meter-days
MAPK	mitogen-activated protein kinase
MMMF	man-made mineral fiber
MMVF	man-made vitreous fiber
MSHA	Mine Safety and Health Administration
NF-κB	nuclear factor kappa beta
NMRD	nonmalignant respiratory disease
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NORA	National Occupational Research Agenda
NORMS	National Occupational Respiratory Mortality System
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PCM	phase contrast microscopy
PEL	permissible exposure limit
RCF	refractory ceramic fiber

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Abbreviations (continued)

REL	recommended exposure limit
ROS	reactive oxygen species
RTV	RT Vanderbilt Company, Inc.
SEM	scanning electron microscopy
SOD	superoxide dismutase
SMR	standardized mortality ratio
SV40	simian virus 40
SVF	synthetic vitreous fiber
TEM	transmission electron microscopy
TF	tissue factor
TNF-α	tumor necrosis factor-alpha
TWA	time-weighted average
USGS	United States Geological Survey
XPS	X-ray photoelectron spectroscopy

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1 REVIEW OF CURRENT ISSUES

1.1 Introduction

Prior to the 1970s, attention concerning the health effects of exposure to airborne fibers was focused on six commercially exploited minerals termed "asbestos:" the serpentine mineral chrysotile and the amphibole minerals cummingtonite-grunerite asbestos (amosite), riebeckite asbestos (crocidolite), actinolite asbestos, anthophyllite asbestos, and tremolite asbestos. The realization that dimensional characteristics of asbestos fibers were important physical parameters in the initiation of respiratory disease broadened interest to man-made fibers (e.g., synthetic vitreous fibers [SVFs]) and to other elongated mineral particles (EMPs) of similar dimensions [Stanton et al. 1981]. To date, interest in the latter has been primarily in fibrous minerals exploited commercially (e.g., wollastonite, sepiolite, and attapulgite). Airborne exposure to thoracic-size EMPs (e.g., cleavage fragments) generated from the crushing and fracturing of nonasbestiform amphibole minerals is also of interest. Some of the asbestos minerals, as well as other types of fibrous minerals, are frequently associated with other minerals in geologic formations at various locations in the United States [van Gosen 2007]. The biological significance of occupational exposure to airborne particles of many of these minerals remains unknown and is difficult to ascertain given the mixed and sporadic nature of exposure in many work environments and the general lack of well-characterized exposure information.

The complex and evolving terminology used to name and describe the various minerals from which airborne EMPs are generated has led to much confusion and uncertainty in scientific and lay discourse related to asbestos fibers and other EMPs. To help minimize such confusion and uncertainty about the content of this *Roadmap*, key terms are defined in the Glossary (Section 5).

Although this *Roadmap* focuses on EMP exposures and their health effects, observed similarities and differences among wide-ranging types of elongated particles (EPs), including SVFs, might inform development of policy for asbestos fibers and other EMPs.

To address current controversies and uncertainties concerning exposure assessment and health effects relating to asbestos fibers and other EMPs, strategic research endeavors are needed in toxicology, exposure assessment, epidemiology, and analytical methods. The results of such research can inform the potential development of new policies for asbestos fibers and other EMPs with recommendations for exposure indices that are firmly based on well-established risk estimates and that effectively protect workers' health. What follows in the remainder of Section 1 is an overview of definitions and terms relevant to asbestos fibers and other EMPs; trends in production/use of asbestos, in occupational exposures to asbestos, and in asbestos-related diseases; sampling and analytical issues; and physicochemical properties associated with EMP toxicity.

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1.2 Minerals and Mineral Morphology

Minerals are naturally occurring inorganic compounds with a specific crystalline structure and elemental composition. They are defined by their distinctive structure and elemental composition. Asbestos is a term applied to select silicate minerals from the serpentine and amphibole groups that grow in a fibrous habit and have properties that have made them commercially valuable. The fibers of all varieties of asbestos are long, thin, and usually flexible when separated. One variety of asbestos, chrysotile, is a mineral in the serpentine group of sheet silicates. Five varieties of asbestos are minerals in the amphibole group of double chain silicates–riebeckite asbestos (crocidolite), cummingtonite-grunerite asbestos (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos.

Although a large amount of health information has been generated on workers occupationally exposed to asbestos, limited mineral characterization, use of non-mineralogical names for asbestos, and changing mineralogical nomenclature have resulted in uncertainty and confusion about the specific nature of exposures in many published studies. Over the past 50 years, several systems for naming amphibole minerals have been used. The current mineralogical nomenclature was unified by the International Mineralogical Association (IMA) under a single system in 1978 [Leake 1978] and later modified in 1997 [Leake et al. 1997]. For some amphibole minerals, the name assigned under the 1997 IMA system is different than the name originally assigned in 1978. In addition, common or commercial names have often been used instead of mineralogical nomenclature. The lack of consistency in nomenclature for asbestos and related minerals has contributed to frequent uncertainty in the specific identification of minerals reported in the literature.

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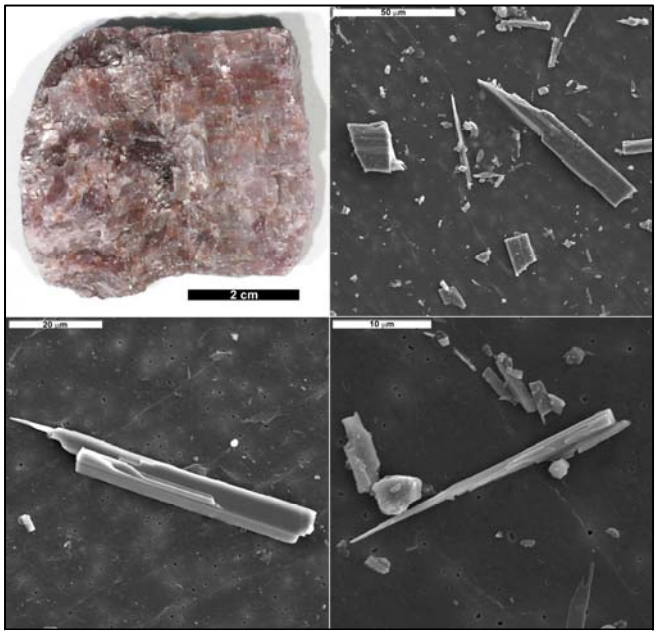

Figure 1. Massive tremolite (var. hexagonite) ground with a pestle in a mortar to produce cleavage fragments. Unground sample (upper left) and scanning electron micrographs at various magnifications are shown. Photograph courtesy of USGS.

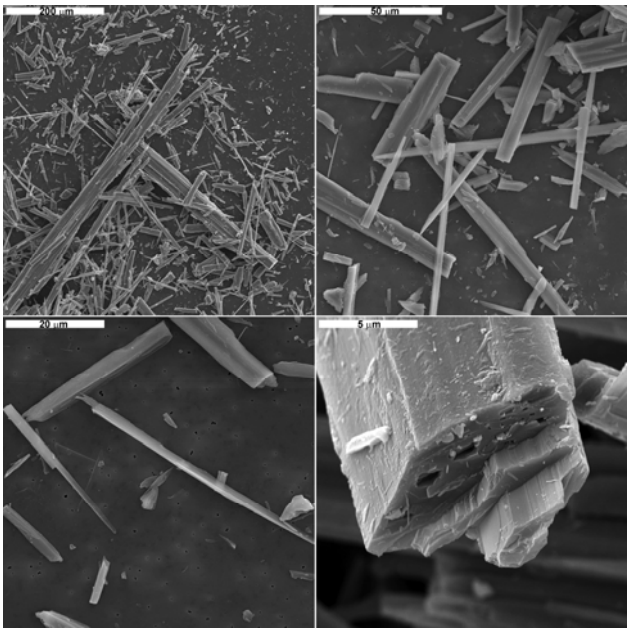

Figure 2. National Institute of Standards and Technology (NIST) Standard Reference Material 1867a "Commercial Asbestos-Tremolite". Photograph courtesy of USGS.

Trade names for mined asbestos minerals predated the development of rigorous scientific nomenclature. For example, amosite is the trade name for asbestiform cummingtonite-grunerite and crocidolite is the trade name for asbestiform riebeckite. Adding to the complexity of the nomenclature, serpentine and amphibole minerals typically develop through the alteration of other minerals. Consequently, they may exist as partially altered minerals having variations in elemental compositions. For example, the microscopic analysis of an elongated amphibole particle using energy dispersive x-ray spectroscopy (EDS) can reveal variations in elemental composition along the particle's length, making it difficult to identify the particle as a single specific amphibole mineral. In addition, a mineral may occur in different growth forms, or "habits," both sharing the same name, elemental composition, and chemical structure (see Figures 1 and 2).

Mineral habit results from the environmental conditions present during a mineral's formation. The mineralogical terms applied to habits are generally descriptive (e.g., fibrous, massive, prismatic, acicular, asbestiform, tabular, and platy). It is possible for both asbestiform (fibrous) and nonasbestiform (massive) versions (i.e., analogs) of the same mineral to occur in juxtaposition or matrixed together, so that both analogs of the same mineral can occur within a narrow geological formation (see Figure 3).

Figure 3. Fibrous to asbestiform richterite/magnesioriebeckite collected near Gem Park, Colorado. Hand sample (left) and scanning electron micrograph (right) are shown. Photograph Courtesy of USGS.

In the scientific literature, the term "mineral fibers" has often included not only particles that have grown in a fibrous or asbestiform habit, but also particles that have grown as needle-like (acicular) single crystals. The term "mineral fibers" has sometimes also encompassed other prismatic crystals and cleavage fragments that meet specified dimensional criteria. Cleavage fragments are generated by crushing and fracturing minerals, including the nonasbestiform analogs of the asbestos minerals. While the hazards of inhalational exposure to airborne asbestos fibers have been well documented, there is controversy about whether exposure to thoracic-size EMPs from nonasbestiform analogs of the asbestos minerals is similarly hazardous.

1.3.Trends in Asbestos Use, Occupational Exposures, and Disease

1.3.1 Trends in Asbestos Use

Over recent decades mining and use of asbestos have declined in the U.S. The mining of asbestos in the U.S. ceased in 2002. Consumption of raw asbestos continues to decline from a peak of 803,000 metric tons in 1973 [USGS 2006]. In 2006, 2000 metric tons of raw asbestos were imported, down from an estimated 35,000 metric tons in 1991 (see Figure 4) and a peak of 718,000 metric tons in 1973. Unlike information on the importation of raw asbestos, information is not readily available on the importation of asbestos-containing products. The primary recent uses for asbestos materials in the U.S. are estimated by the USGS [2007] as 55% for roofing products, 26% for coatings and compounds, and 19% for other applications, and more recently 84% for roofing products and 16% for other applications [USGS 2008].

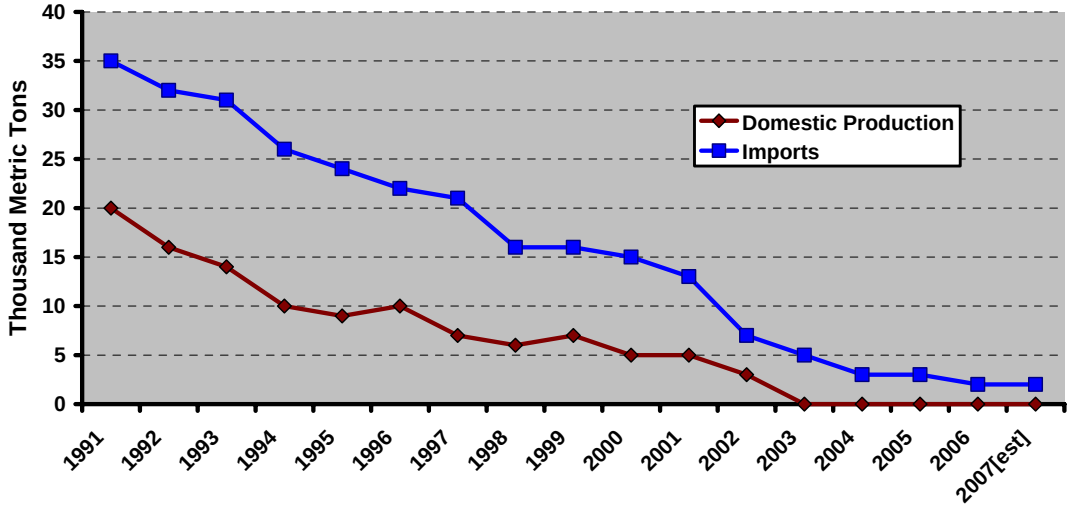

Figure 4. US Asbestos Production and Imports, 1991-2007. [USGS 2006]. Data are also found at: http://pubs.usgs.gov/circ/2006/1298/c1298.pdf

Worldwide, the use of asbestos has declined. Using the amount of asbestos mined as a surrogate for the amount used, worldwide use has declined from about 5 million metric tons in 1975 to about 2 million metric tons annually since 1999 [Taylor et al. 2006]. The European Union has banned imports and the use of asbestos with limited exceptions. In other regions of the world, there is a continued demand for inexpensive, durable construction materials. Consequently, markets remain strong in some countries for asbestos-cement products, such as asbestos-cement panels for construction of buildings and asbestos-cement pipe for water-supply lines. Currently over 70% of all mined asbestos is used in Eastern Europe and Asia [Tossavainen 2005].

Historically, chrysotile accounted for more than 90% of the world's mined asbestos; it presently accounts for over 99% [Ross and Virta 2001; Virta 2002]. Mining of crocidolite (asbestiform riebeckite) and amosite (asbestiform cummingtonite-grunerite) deposits have accounted for most of the remaining asbestos, although mining of amosite ceased in 1992 and mining of crocidolite ended in 1997. Small amounts of anthophyllite asbestos have been mined in Finland [Ross and Virta 2001] and are currently being mined in India [Ansari et al. 2007].

1.3.2 Trends in Occupational Exposure

Since 1986, in the U.S., the annual geometric means of occupational exposure concentrations to asbestos reported in the Occupational Safety and Health Administration's (OSHA) Integrated Management Information System (IMIS) and the

Mine Safety and Health Administration's (MSHA) database have been consistently below the NIOSH recommended exposure limit (REL) of 0.1 fibers per cubic centimeter of air (f/cm^3) for all major industry divisions in the U.S. (Figure 5). The number of occupational asbestos exposure samples that were measured and reported in IMIS decreased from an average of 890 per year during the 8-year period of 1987-1994 to 241 per year during the 5-year period of 1995-1999. The percentage exceeding the NIOSH REL decreased from 6.3% in 1987-1994 to 0.9% in 1995-1999. During the same two periods, the number of exposures measured and reported in MSHA's database decreased from an average of 47 per year during 1987-1994 to an average of 23 per year during 1995-1999. The percentage exceeding the NIOSH REL decreased from 11.1% in 1987-1994 to 2.6% in 1995-1999 [NIOSH 2002a].

The preceding summary of occupational exposures to asbestos is based on the OSHA and MSHA regulatory definitions relating to asbestos. Because of analytical limitations of the phase contrast microscopy (PCM) method and the variety of workplaces from which the data were obtained, it is unclear how much of these exposures were to EMPs from nonasbestiform analogs of the asbestos minerals, which are encompassed by the NIOSH REL for airborne asbestos fibers.

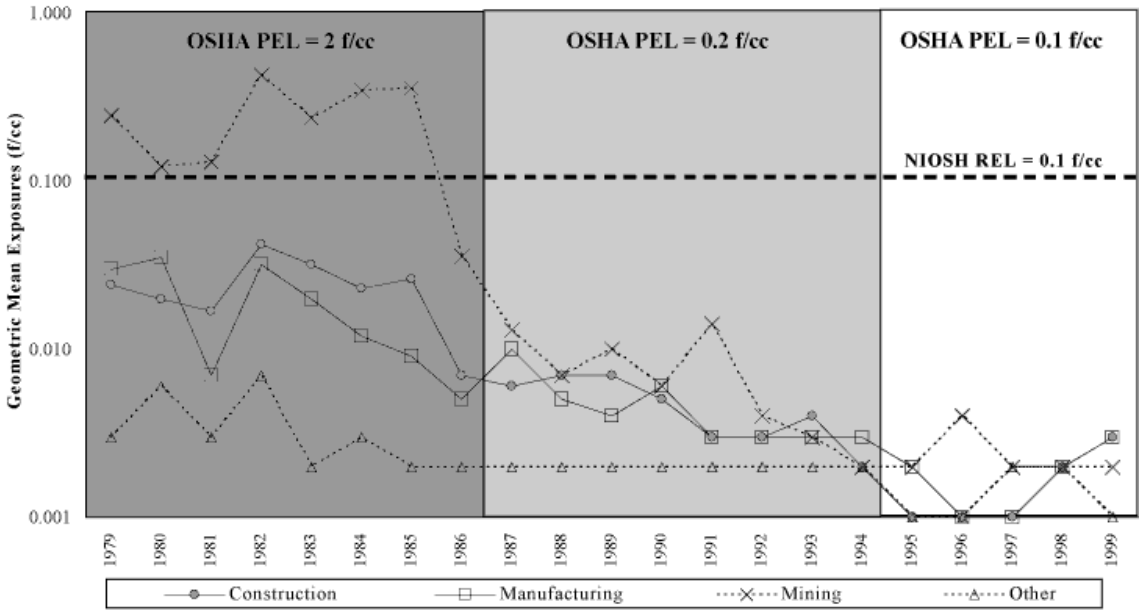

Figure 5. Asbestos: Annual geometric mean exposure concentrations by major industry division, MSHA and OSHA samples, 1979–1999. NIOSH [2002a]. Note: MSHA PEL for this time period was 2 f/cc. Data are also found at: http://www2a.cdc.gov/drds/WorldReportData/SectionDetails.asp?SectionTitleID=1.

Very limited information is available on the number of workers still exposed to asbestos. Based on MSHA [2002] mine employment data, an estimated 44,000 miners and other mine workers may be exposed to asbestos during the mining of some mineral

commodities in which asbestos may be a potential contaminant [NIOSH 2002b]. OSHA estimated in 1990 that about 568,000 workers in production and services industries and 114,000 in construction industries may be exposed to asbestos in the workplace [OSHA 1990]. More recently, OSHA has estimated that 1.3 million employees in construction and general industry face significant asbestos exposure on the job [OSHA 2008].

In addition to evidence from OSHA and MSHA that indicate a reduction in occupational exposures in the U.S. over the past several decades, other information compiled on workplace exposures to asbestos indicates that the nature of occupational exposures to asbestos has changed [Rice and Heineman 2003]. Once dominated by chronic exposures in manufacturing process such as textile mills, friction product manufacturing, and cement pipe fabrication, current occupational exposures to asbestos in the U.S. primarily occur during maintenance activities or remediation of buildings containing asbestos. These current occupational exposure scenarios frequently involve short-term, intermittent exposures.

1.3.3 Trends in Asbestos-related Disease

Epidemiological studies of workers occupationally exposed to asbestos have clearly documented the increased risk of several respiratory diseases, including lung cancer, mesothelioma, diffuse fibrosis of the lung, and non-malignant pleural abnormalities including acute pleuritis and chronic diffuse and localized thickening of the pleura. In addition, it has been determined that laryngeal cancer can be caused by exposure to asbestos [IOM 2006] and evidence suggests that asbestos may also cause other diseases (e.g., pharyngeal, stomach, and colorectal cancers [IOM 2006] and immune disorders [ATSDR 2001].

National surveillance data, showing trends over time, are available for two diseases with rather specific mineral fiber etiologies—asbestosis and malignant mesothelioma (see following sub-sections). Lung cancer is known to be caused in part by asbestos fiber exposure, but has multiple etiologies. Ongoing national surveillance for lung cancer caused by asbestos exposure has not been done. However, using various assumptions and methods, several researchers have projected the number of U.S. lung cancer deaths caused by asbestos. Examples of the projected number of asbestos-caused lung cancer deaths in the U.S. include 55,100 [Walker et al. 1983] and 76,700 [Lilienfeld et al. 1988], each of these projections representing the 30-year period from 1980 through 2009. However, in the absence of specific diagnostic criteria and a specific disease code for the subset of lung cancers caused by asbestos, ongoing surveillance has not been done for lung cancer caused by asbestos.

1.3.3.1 Asbestosis

NIOSH has annually tracked U.S. asbestosis deaths since 1968 and malignant mesothelioma deaths since 1999 using death certificate data in the National Occupational Respiratory Mortality System (NORMS). NORMS data, representing all deaths among U.S. residents, show that asbestosis deaths increased almost 20-fold from the late 1960s to the late 1990s (Figure 6) [NIOSH 2007a]. Trends in asbestosis mortality is expected to substantially trail trends in asbestos exposures (see Section 1.3.2) for two primary reasons: (1) the latency period between asbestos exposure and asbestosis onset is typically long, commonly one or two decades or more; and (2) asbestosis is a chronic disease, so affected individuals can live for many years with the disease before succumbing. In fact, asbestosis deaths have apparently plateaued (at nearly 1,500 per year) since 2000 (Figure 6) [NIOSH 2007a]. Ultimately, it is anticipated that the annual number of asbestosis deaths in the U.S. will decrease substantially as a result of documented reductions in exposure. However, asbestos usage has not been completely eliminated, and asbestos-containing materials remain in place in structural materials and machinery, so the potential for exposure remains. Thus, asbestosis deaths in the U.S. are anticipated to continue to occur for several decades.

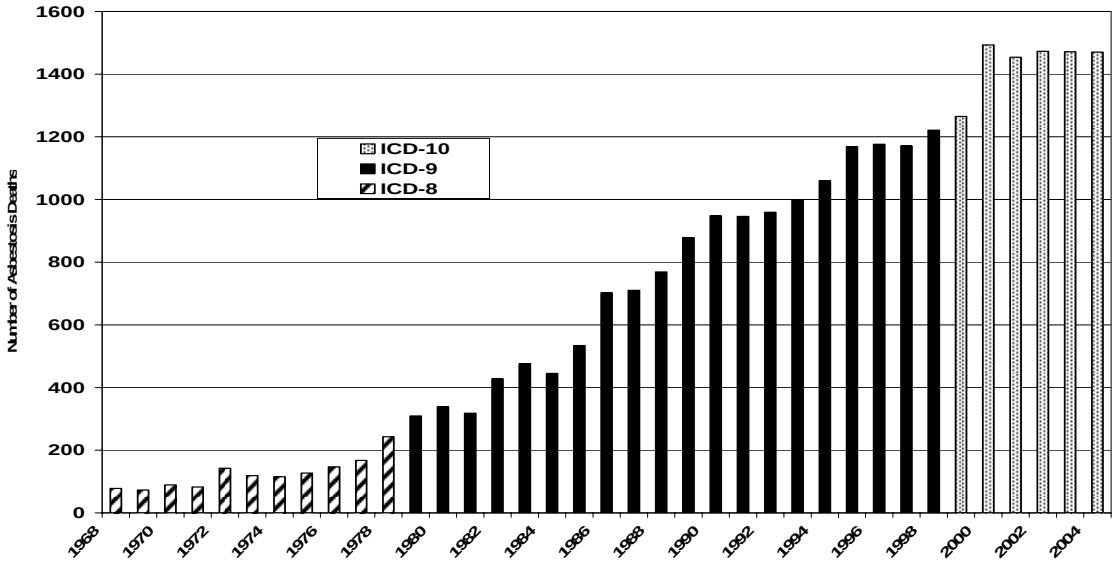

Figure 6. Number of asbestosis deaths, U.S. residents age 15 and over, 1968-2004. [NIOSH 2007a]. Source: National Occupational Respiratory Mortality System (NORMS), found at: http://webappa.cdc.gov/ords/norms.html.

1.3.3.2 Malignant Mesothelioma

Malignant mesothelioma, an aggressive disease that is nearly always fatal, is known to be caused by exposure to asbestos and some other mineral fibers [IOM 2006]. The

occurrence of mesothelioma has been strongly linked with occupational exposures to asbestos [Bang et al. 2006]. There had been no discrete International Classification of Disease (ICD) code for mesothelioma until its most recent 10th revision. Thus, only 6 years of NORMS data are available with a specific ICD code for mesothelioma (Figure 7); during this period, there was a 7% increase in annual mesothelioma deaths, from 2,484 in 1999 to 2,657 in 2004 [NIOSH 2007a]. A later peak for mesothelioma deaths than for asbestosis deaths would be entirely expected, given the longer latency for mesothelioma [Järvholm et al. 1999]. One analysis of malignant mesothelioma incidence based on the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program data found that an earlier steep increase in incidence had moderated and that mesothelioma incidence may have actually peaked sometime in the 1990s in SEER-covered areas [Weill et al. 2004]. In contrast to NORMS data, which represents a census of all deaths in the entire U.S., the analyzed SEER data was from areas in which reside a total of only about 15% of the U.S. population.

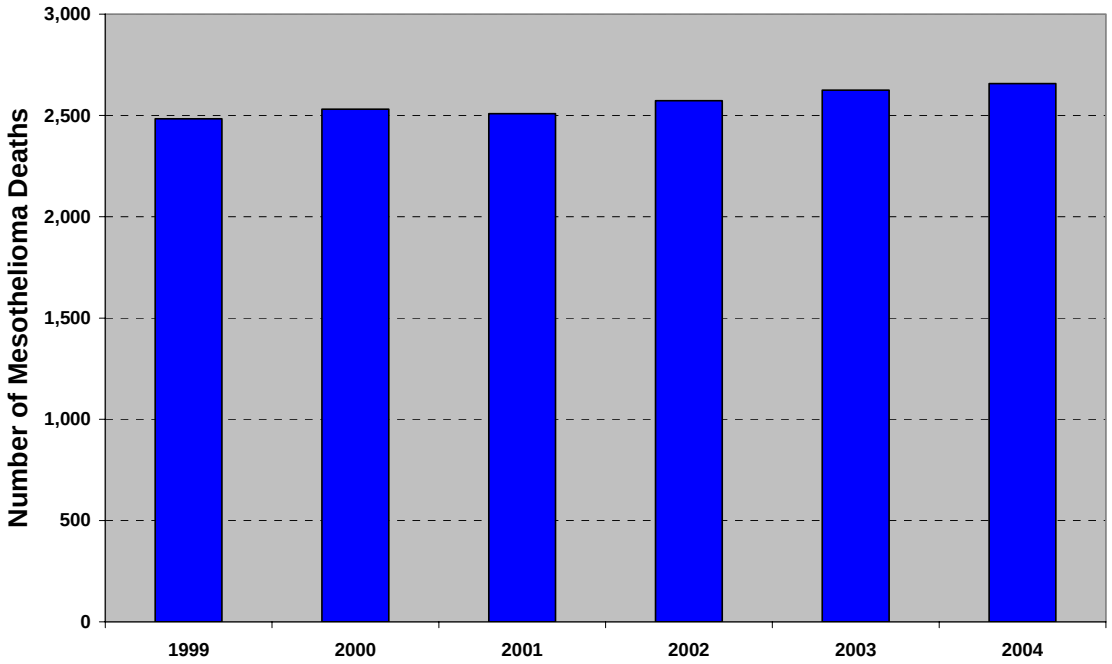

Figure 7. Number of malignant mesothelioma deaths, U.S. residents age 15 and over, 1999-2004. [NIOSH 2007a]. Source: National Occupational Respiratory Mortality System (NORMS), found at: http://webappa.cdc.gov/ords/norms.html.

1.4 Clinical Issues

A thorough review of how asbestos-related diseases are diagnosed is beyond the scope of this document, and authoritative guidance on the diagnosis and attribution of asbestos-

caused diseases has been published elsewhere [Anonymous 1997; British Thoracic Society Standards of Care Committee 2001; Henderson et al. 2004; ATS 2004].

The diagnosis of asbestos-caused malignancies (e.g., lung cancer and malignant mesothelioma) is almost always based on characteristic histology (or abnormal cytology in some cases). Despite research on other possible etiologies, genetic susceptibilities, and hypothesized co-factors such as simian virus 40, it is generally accepted that most cases of malignant mesothelioma are caused by exposure to asbestos or other mineral (e.g., erionite) fibers [Robinson and Lake 2005; Carbone 2006]. Of particular concern to patients diagnosed with malignant mesothelioma, as well as to individuals who remain at-risk due to past exposures, the disease currently is essentially incurable [British Thoracic Society Standards of Care Committee 2001]. Diagnosis may be relatively straightforward, but can be difficult due to a challenging differential diagnosis [Lee et al. 2002]. Advances have been made to improve diagnostic testing for malignant mesothelioma using immunochemical markers and other more sophisticated histopathological analyses, and additional research is aimed at improving treatment of the disease [Robinson and Lake 2005]. Notable recent research efforts have been directed towards the development of biomarkers for mesothelioma that can be assessed by noninvasive means. A long-term goal of the biomarker research is to enable screening of high-risk individuals with sufficiently sensitive and specific non-invasive biomarkers to identify disease at an early stage when therapeutic intervention might have a greater potential to slow the progression of the disease or to be curative. Other goals are to use non-invasive biomarkers for monitoring the disease in patients treated for mesothelioma and even for diagnosing the disease. Non-invasive biomarkers, including osteopontin and soluble mesothelin-related peptide, have been and continue to be evaluated, but none are ready for routine clinical application [Cullen 2005; Scherpereel and Lee 2007].

Non-malignant asbestos-related diseases are diagnosed by considering three major necessary criteria: (1) evidence of structural change consistent with asbestos-caused effect (e.g., abnormality on chest image; and/or tissue histology); (2) evidence of exposure to asbestos (e.g., history of occupational or environmental exposure with appropriate latency; and/or asbestos bodies identified in lung tissue, sputum, or bronchoalveolar lavage; and/or other concurrent marker of asbestos exposure such as pleural plaques); and (3) exclusion of alternative diagnoses [ATS 2004]. The specificity of an asbestosis diagnosis increases as the number of consistent clinical abnormalities increases [ATS 2004]. In practice, only a small proportion of cases are diagnosed on the basis of lung biopsy and tissue histopathology, as lung biopsy is an invasive procedure with inherent risks for the patient. Thus, following reasonable efforts to exclude other possible diagnoses, the diagnosis of asbestosis usually rests on chest imaging abnormalities that are consistent with asbestosis in an individual judged to have sufficient exposure and latency since first exposure.

Chest radiography remains the most commonly used imaging method for screening exposed individuals for asbestosis and for evaluating symptomatic patients.

Nevertheless, it is important to understand that, as with any screening tool for disease, in screening populations for asbestosis, the predictive value of a positive chest radiograph alone depends upon the underlying prevalence of asbestosis in the screened population [Ross 2003]. A widely accepted system for classifying radiographic abnormalities of the pneumoconioses was initially intended primarily for epidemiologic use, but has long been widely used for other purposes (e.g., to determine eligibility for compensation and for medicolegal purposes) [ILO 2002]. A NIOSH-administered "B Reader" Program trains and certifies physicians as proficient in the application of this system [NIOSH 2007b]. Certain problems with the use of chest radiography for pneumoconioses have long been recognized [Wagner et al. 1993] and recent abuses have garnered substantial attention [Miller 2007]. In response, NIOSH recently published guidance for B Readers [NIOSH 2007c] and for the use of B Readers and ILO classifications in various settings [NIOSH 2007d].

In developed countries, conventional film radiography is rapidly giving way to digital radiography, and some work is currently underway to develop digital standards and validate their use in classifying digital chest radiographs under the ILO system [Franzblau et al. 2006]. Computerized tomography, and especially high-resolution computed tomography (HRCT), has proven more sensitive and more specific than chest radiography for the diagnosis of asbestosis and is frequently used to help rule out other conditions [DeVuyst and Gevenois 2002]. Standardized systems for classifying pneumoconiotic abnormalities have been proposed for computed tomography, but have not yet been widely adopted [Kraus et al. 1996; Huuskonen et al. 2001].

In addition to documenting structural tissue changes consistent with asbestos-caused disease, usually assessed radiographically as discussed above, the diagnosis of asbestosis relies on documentation of exposure [ATS 2004]. In clinical practice, exposure is most often ascertained by the diagnosing physician from a careful occupational and environmental history, assessed with respect to intensity and duration. Such a history enables a judgment about whether the observed clinical abnormalities can be reasonably attributed to past asbestos exposure, recognizing that severity of lung fibrosis is related to dose and latency [ATS 2004]. The presence of characteristic pleural plaques, especially if calcified, can also be used as evidence of past asbestos exposure [ATS 2004]. In a small minority of cases, particularly when the exposure history is uncertain or vague or when additional clinical assessment is required to resolve a challenging differential diagnosis, past asbestos exposure is documented through mineralogical analysis of sputum, bronchoalveolar lavage fluid, or lung tissue. Light microscopy can be used to detect and count asbestos bodies (i.e., asbestos fibers that have become coated with iron-containing hemosiderin during residence in the body and more generically referred to as ferruginous bodies) in clinical samples. Electron microscopy can be used to detect and count uncoated asbestos fibers in clinical samples. Standards for such clinical mineralogical analyses often vary, valid background levels are difficult to establish, and the absence of asbestos bodies cannot be used to absolutely rule out past exposure,

particularly with chrysotile exposure (because chrysotile fibers are known to be less persistent in the lungs than amphibole asbestos fibers) [De Vuyst et al. 1998; ATS 2004].

1.5 Components of the NIOSH Recommendation for Asbestos

Evidence that asbestos causes lung cancer and mesothelioma in humans is well documented [NIOSH 1976; IARC 1977, 1987a, 1987b; EPA 1986; ATSDR 2001; HHS 2005a]. Based on the risk of disease from occupational exposure to airborne asbestos fibers, NIOSH established a REL of 100,000 asbestos fibers per cubic meter of air (100,000 fibers/m^3), which is equal to 0.1 f/cm^3 measured as an 8-hour time-weighted average (TWA) [NIOSH 1976][1]. The REL was set at the limit of quantification (LOQ) for the phase contrast microscopy (PCM) analytical method for a 400-L sample, but risk estimates indicated that exposure at 0.1 f/cc throughout a working lifetime would be associated with a residual risk for lung cancer. A risk-free level of exposure to airborne asbestos fibers has not been established.

In 1990, NIOSH [1990a] stated:

> *NIOSH has attempted to incorporate the appropriate mineralogic nomenclature in its recommended standard for asbestos and recommends the following to be adopted for regulating exposures to asbestos:*
>
> *The current NIOSH asbestos recommended exposure limit is 100,000 fibers greater than 5 micrometers in length per cubic meter of air, as determined in a sample collected over any 100-minute period at a flow rate of 4L/min using NIOSH Method 7400, or equivalent. In those cases when mixed fiber types occur in the same environment, then Method 7400 can be supplemented with electron microscopy, using electron diffraction and microchemical analyses to improve specificity of the fiber determination. NIOSH Method 7402 … provides a qualitative technique for assisting in the asbestos fiber determinations. Using these NIOSH microscopic methods, or equivalent, airborne asbestos fibers are defined, by reference, as those particles having (1) an aspect ratio of 3 to 1 or greater; and (2) the mineralogic characteristics (that is, the crystal structure and elemental composition) of the asbestos minerals and their nonasbestiform analogs. The asbestos minerals are defined as chrysotile, crocidolite, amosite (cummingtonite-grunerite), anthophyllite, tremolite, and actinolite. In addition, airborne cleavage fragments from the nonasbestiform habits of the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the*

[1] The averaging time for the REL was later changed to 100 minutes in accordance with NIOSH Analytical Method #7400 [NIOSH 1994a]. This change in sampling time was first noted in comments and testimony presented by NIOSH to OSHA [NIOSH 1990a; 1990b], and reaffirmed in comments to MSHA in 2002 with the explanation that the 100-minute averaging time would help "to identify and control sporadic exposures to asbestos and contribute to the overall reduction of exposure throughout the workshift" [NIOSH 2002b]

series cummingtonite-grunerite, tremolite-ferroactinolite, and glaucophane-riebeckite shall also be counted as fibers provided they meet the criteria for a fiber when viewed microscopically.

The NIOSH REL is comprised of a policy component, consisting of a statement of agency intent about what minerals should be covered by the REL, and an analytical component, describing the sampling and analytical methods to be used for collecting, characterizing, and quantifying exposure to airborne particles from the covered minerals. Each of these components of the NIOSH REL is discussed in detail in the following subsections.

1.5.1 Minerals Covered by the NIOSH REL

The minerals encompassed in the NIOSH REL include those having the crystal structure and elemental composition of the asbestos varieties [chrysotile, riebeckite asbestos (crocidolite), cummingtonite-grunerite asbestos (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos]. It also includes the nonasbestiform analogs of the asbestiform minerals (the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the cummingtonite-grunerite mineral series, the tremolite-ferroactinolite mineral series, and the glaucophane-riebeckite mineral series).

There is little doubt that fibers from all six of the regulated asbestos minerals can cause lung cancer and other diseases of the lung. As with most carcinogenic agents, there is a substantial latency period (10-40 years) between the onset of exposure to asbestos and the occurrence of lung cancer and risk increases in proportion to cumulative exposure. In spite of decades of research into the factors that influence the toxicity of asbestos, there remain several areas of continuing debate [Plumlee et al. 2006]. For example, a number of epidemiological, toxicological, and pathological studies indicate that fibers from amphibole asbestos minerals are more potent carcinogens than chrysotile. This greater potency has been postulated to be a result of slower dissolution of amphibole asbestos fibers in lung, interstitial, and phagolysosomal fluids than chrysotile fibers. This has been interpreted to suggest that amphibole asbestos fibers can persist for longer periods of time in the lungs and adjacent tissues, thereby imparting a greater potential to trigger fibrosis and cancer. However, others cite evidence, such as the presence of chrysotile fibers in mesothelioma tumors and the occurrence of chrysotile without amphibole asbestos in the lung fiber burden of some individuals with cancer and mesothelioma, to indicate that fibers from both chrysotile and amphibole asbestos are pathogenic.

While much is known about the health effects associated with exposure to asbestos fibers, much less information is available about the potential health effects of the other EMPs encompassed in the NIOSH REL for airborne asbestos fibers. Also, limited data are available about what effect exposure to asbestos fibers and other EMPs in a mixed-dust environment might have on the risk of respiratory disease [Plumlee and Ziegler 2006].

1.5.1.1 Chrysotile

Chrysotile fibers consist of aggregates of long, thin, flexible fibrils that resemble scrolls or cylinders, and the dimensions of individual chrysotile fibers depend on the extent to which the material has been manipulated. Chrysotile fibers split along the fiber length and undergo partial dissolution within the lungs after fibrillation [NRC 1984]. Longitudinal splitting of fibers after entering the lung represents one way that air sample PCM counts may underestimate the total dose of fibers in the lung.

Epidemiological studies of chrysotile in Quebec mines [McDonald and McDonald1997] and South Carolina textile mills [Dement et al. 1994; Hein et al. 2007] have produced very different estimates of the risk of cancer associated with exposure to chrysotile fibers. Several reasons for the differences in the lung cancer risks observed in these two different workplaces have been proposed. One suggested explanation is that the chrysotile in the textile mill was contaminated with tremolite asbestos; another is that the textile workers were exposed to mineral oil. However, neither of these explanations has satisfactorily explained the differences [Stayner et al. 1996]. Considering that the workers in textile mills were exposed to fibers considerably finer than those found in mines [Peto et al. 1982; Dement and Wallingford 1990], the most likely explanation is that the differences in risk may be due, at least in part, to dimensional differences in the particles to which workers were exposed. Stayner et al. [1997] also point out, in comparing a number of epidemiological studies, that the variation in relative risk for lung cancer is often greater within industry than between mineral type.

Some have argued that pure chrysotile may not be carcinogenic and that increased respiratory cancer among chrysotile workers can be explained by the presence of tremolite asbestos which is often found as a contaminant with chrysotile [McDonald and McDonald 1997]. This is referred to as the "amphibole hypothesis." However, several studies of workers using chrysotile with very little contamination by tremolite have demonstrated strong relationships between exposure to chrysotile and lung cancer. A study of asbestos workers in China [Yano et al. 2001] found an age- and smoking-adjusted relative risk of 8.1 for lung cancer among highly exposed workers compared to workers with low exposure to asbestos. The identified contamination of the chrysotile by tremolite was less than 0.001%. In the South Carolina textile mill study, a strong relation between lung cancer and chrysotile exposure has been demonstrated [Dement et al. 1994; Hein et al. 2007]. A recent reanalysis by transmission electron microscopy (TEM) of archived airborne dust exposure samples which had been selected using stratified random sampling identified only 2 amphibole fibers among 18,840 fiber structures (0.01%), while the remainder were identified as chrysotile based on morphology [Stayner et al. 2007].

A possible difference in risk for carcinogenicity between chrysotile and amphibole asbestos exposures has been investigated in animal model studies. In a one-year rat inhalation study, chrysotile samples were extremely fibrogenic and carcinogenic, with pulmonary carcinomas developing in approximately 25% of animals and advanced

interstitial fibrosis in lung tissue in 10% of all older animals, while intrapleural injection studies produced mesotheliomas in over 90% of animals. It was noted that very little chrysotile remained in the lungs of the animals that survived longest following dust inhalation. From this it was suggested that chrysotile is very potent in rodents but, except where exposure levels are very high and of long duration, may be less hazardous to man because it is removed from lung tissue quite rapidly [Davis et al. 1986]. In fiber lung burden studies of human malignant mesothelioma cases, chrysotile fibers were present in lungs even when amphiboles were not present [Suzuki and Yuen 2001; Suzuki et al. 2005]. Hodgson and Darnton [2000] reviewed the literature and estimated that, at exposure levels seen in occupational cohorts, the exposure-specific risk of mesothelioma from the three principal commercial asbestos types is broadly in the ratio 1:100:500 for chrysotile, amosite, and crocidolite, respectively, and the risk differential for lung cancer between chrysotile and the two types of amphibole asbestos fibers is between 1:10 and 1:50.

1.5.1.2 Amphibole Asbestos and Other Fibrous Minerals

There is little scientific debate that the asbestiform varieties of the five commercially important amphibole asbestos minerals are carcinogenic and should be covered in regulations to protect workers. However, concerns have been raised about whether the current OSHA and MSHA definition for asbestos (the asbestiform variety of the six commercially important asbestos minerals) provides sufficient worker protection from exposure to other fibrous minerals.

This concern is exemplified by exposures to winchite and richterite fibers at a vermiculite mine near Libby, MT, where exposures to the these fibers have resulted in high rates of lung fibrosis and cancer among exposed workers, similar to the occurrence of asbestos-related diseases among asbestos-exposed workers in other industries [Amandus et al. 1987a; Amandus et al. 1987b; Amandus and Wheeler 1987; McDonald et al. 2004; Sullivan 2007; Rohs et al. 2008]. Workers at the mine and residents of Libby were exposed to fibers identified (as defined using the 1997 IMA amphibole nomenclature) as the asbestiform amphiboles winchite and richterite as well as tremolite asbestos [Meeker et al. 2003]. Because winchite and richterite are not explicitly listed among the six commercial asbestos minerals, it is sometimes assumed that they are not included in the regulatory definition for asbestos. However, some of what is now referred to as asbestiform winchite and richterite using the 1997 IMA nomenclature would have been accurately referred to as tremolite asbestos using the 1978 IMA nomenclature [Meeker et al. 2003]. Furthermore, an even greater portion of richterite and winchite would have been identified as tremolite asbestos using the optical methods of identification used prior to 1978. In fact, over the years, amphibole minerals from the Libby mine that are now referred to as winchite and richterite have been identified by mineralogists as soda tremolite [Larsen 1942], soda-rich tremolite [Boettcher 1966b], and tremolite asbestos and richterite asbestos [Langer et al. 1991; Nolan et al. 1991]; they were similarly

identified as tremolite in reports of the Libby mine epidemiological studies conducted by NIOSH in the 1980s [Amandus et al. 1987a; Amandus and Wheeler 1987; Amandus et al. 1987b].

The progress of mineralogical science likely will continue to involve nomenclature changes, but workers should be protected against exposures to pathogenic asbestiform minerals without regard to nomenclature changes. The health and regulatory communities will need to carefully define the minerals covered by their policies and monitor the nomenclature changes to minimize the impact of these changes on worker protections.

Inhalational exposure to other fibrous minerals, such as erionite (a fibrous zeolite), have also been found to cause respiratory diseases similar to those caused by asbestos [HHS 2005b]. Thus, while these other fibrous minerals are not included in definitions for asbestos by Federal agencies, the significance of associated health risks warrant concern and possible future regulation of these other mineral fibers. NIOSH is considering methods to develop appropriate recommendations to protect workers from these other fibrous minerals.

1.5.1.3 Nonasbestiform Analogs of the Asbestos Varieties

The airborne EMPs encompassed by the current NIOSH REL for airborne asbestos fibers explicitly include particles from the nonasbestiform analogs of the asbestos minerals that meet the specified dimensional criteria as determined microscopically.

1.5.1.3.1 Rationale for NIOSH Policy

The rationale for recommending that nonasbestiform analogs of the asbestos minerals be encompassed within the policy definition of airborne asbestos fibers was first articulated in NIOSH comments and testimony to OSHA [NIOSH 1990a; 1990b]. In that testimony, NIOSH based its recommendation on three elements:

- The first element comprised results of epidemiologic studies of worker populations with mixed exposures to asbestos fibers and other EMPs from nonasbestiform mineral analogs of the asbestos minerals or with exposures solely to EMPs (e.g., cleavage fragments) from the nonasbestiform analogs. The testimony characterized the existing evidence as equivocal for excess lung cancer risk attributable to exposure to such nonasbestiform EMPs.

- The second element comprised results of animal carcinogenicity studies involving experimental intrapleural or intraperitoneal administration of various mineral particles. The testimony characterized the results of the studies as providing

strong evidence that carcinogenic potential depends on particle length and width and reasonable evidence that neither chemical composition nor mineralogic origin are critical factors in determining a mineral particle's carcinogenic potential.

- The third element comprised the inability to adequately distinguish between airborne exposures to particles from asbestiform and nonasbestiform minerals. The arguments were twofold. The first argument was that asbestiform and nonasbestiform minerals can occur in the same area and that determining the location and identification of tremolite asbestos, actinolite asbestos, and anthophyllite asbestos within deposits of their nonasbestiform mineral analogs can be difficult. The testimony expressed concern about resulting inadvertent contamination of some mined/quarried commodities by tremolite asbestos, actinolite asbestos, and/or anthophyllite asbestos. The second argument was that routine analytical methods for quantifying airborne exposures are not available that can be used to accurately differentiate between asbestos fibers and other nonasbestiform EMPs that meet the dimensional criteria of a countable particle when examined microscopically.

Based on inconclusive epidemiological evidence for lung cancer risk associated with exposure to cleavage fragments (see first bullet, above), NIOSH took a precautionary approach and relied upon the other two elements to recommend that the 0.1 f/cc REL for airborne asbestos fibers also include EMPs from the nonasbestiform analogs of the asbestos minerals.

Some scientists and others have questioned NIOSH's rationale for including EMPs from nonasbestiform amphibole minerals in its definition of airborne asbestos fibers. Mineralogists argue that these EMPs do not have the morphological characteristics to meet the mineralogical definition of "fibers"; acicular and prismatic amphibole crystals and cleavage fragments generated from the massive habits of the nonasbestiform analogs of the asbestos minerals are not true "fibers." Others have stated that the scientific literature does not demonstrate any health risks associated with exposure to the nonasbestiform EMPs covered by the NIOSH "airborne asbestos fiber" definition.

Whether or not to include EMPs from nonasbestiform analogs of the asbestos minerals in Federal regulatory asbestos policies has been the subject of long-standing debate. The impact of these different morphologies on the toxicology and health effects continues to be a central point in the debate. In 1986, OSHA revised its asbestos standard and included nonasbestiform anthophyllite, tremolite, and actinolite (ATA) as covered minerals within the scope of the revised standard. OSHA's decision to include nonasbestiform ATA proved controversial. In a 1990 proposal to reverse this revision, OSHA noted that there were "a number of studies which raise serious questions about the potential health hazard from occupational exposure to nonasbestiform tremolite, anthophyllite and actinolite," but that the "current evidence is not sufficiently adequate

for OSHA to conclude that these mineral types pose a health risk similar in magnitude or type to asbestos."

In 1992, in the preamble to the final rule removing nonasbestiform ATA from its asbestos standard, OSHA stated that:

> *various uncertainties in the data[2] and a body of data showing no carcinogenic effect, do not allow the Agency to perform qualitative or quantitative risk assessments concerning occupational exposures. Further, the subpopulations of nonasbestiform ATA which, based on mechanistic and toxicological data, may be associated with a carcinogenic effect, do not appear to present an occupational risk. Their presence in the workplace is not apparent from the record evidence [OSHA 1992].*

In its 2005 proposed rule for asbestos, MSHA stated that substantive changes to its asbestos definition were beyond the scope of the proposed rule and chose to retain its current definition of asbestos which "does not include nonfibrous or nonasbestiform minerals" [MSHA 2005]. These decisions are reflected in MSHA's final rule published in 2008 [MSHA 2008]. In formal comments during the rulemaking process, NIOSH agreed with MSHA's decision not to modify its asbestos definition in the current rulemaking, stating that "NIOSH is presently re-evaluating its definition of asbestos and nonasbestiform minerals, and will work with other agencies to assure consistency to the extent possible" [NIOSH 2005].

1.5.1.3.2 Epidemiological Studies

Epidemiologic studies of populations with exposures to EMPs that have been reported to be nonasbestiform have been conducted in the talc mining region of upstate New York, the Homestake gold mine in South Dakota, and the taconite mining region of northeastern Minnesota. A review of the findings from these investigations is presented below.

Studies of New York Talc Miners and Millers

Workers with exposure to talc have long been recognized to have an increased risk of developing pulmonary fibrosis, often referred to as talc pneumoconiosis [Siegel et al. 1943; Kleinfeld et al. 1955]. Talc-exposed workers have also been recognized to have an increased prevalence of pleural plaques [Siegel et al. 1943].

A number of more recent epidemiologic studies and reviews have been conducted of workers employed in talc mines and mills in upstate New York [Brown et al. 1979 and 1990; Gamble 1993; Kleinfeld et al. 1967 and 1974; Lamm et al. 1988; Stille and Tabershaw1982; Honda et al. 2002; Gamble and Gibbs 2007].

[2] OSHA was making reference to the scientific data on which NIOSH based its own carcinogenic health effect recommendation to OSHA.

Excessive rates of mesothelioma have been reported for Jefferson County, which (along with adjacent St Lawrence County), is a major site of the New York talc industry [Vianna et al. 1981; Enterline and Henderson 1987; Hull et al. 2002]. In a study of all histologically confirmed mesothelioma cases reported to New York State's tumor registry from 1973-1978, Vianna et al. [1981] reported 6 cases from Jefferson County, resulting in a mesothelioma rate for that county more than twice that of New York State (excluding New York City). In a national study of mesothelioma mortality from 1966 through 1981, Enterline and Henderson [1987] reported 4 mesothelioma cases in Jefferson County females (0.6 expected) and 7 cases in Jefferson County males (1.4 expected), giving that county mesothelioma rates that were the 2nd and 6th highest county-specific rates in the nation for females and males, respectively (both $p<0.01$). More recently, Hull et al. [2002] updated the Enterline and Henderson mesothelioma mortality analysis for Jefferson County, reporting 5 new male cases (2 expected) and 3 new female cases (0.5 expected) though 1997 and describing Jefferson County mesothelioma death rates as "5-10 times the background rate." A potential limitation of the Enterline and Henderson [1987] and Hull et al. [2002] mesothelioma death rates is that they relied on ICD code 163 ("malignant neoplasms of the pleura, mediastinum, and unspecified sites") as a surrogate for malignant mesothelioma. That code lacked specificity and sensitivity for mesothelioma; in a study of Massachusetts deaths, many non-mesothelioma malignancies involving the pleura were assigned code 163 and most mesotheliomas were not assigned code 163 [Davis et al. 1992]. The more recent ICD-10 system, which has been used since 1999 to code death certificate data in the U.S., includes a specific code for malignant mesothelioma. Based on that new ICD-10 code, the age-adjusted death rates (per million population) for 1999-2004 were 12.9 (based on 5 cases) for Jefferson County and 10.9 (based on 5 cases) for St. Lawrence County. These are similar to the overall U.S. mesothelioma death rates for this same period (based on a total of 15,379 mesothelioma deaths) of 11.39 per million [NIOSH 2007a].

The study by Hull et al. [2002] also examined particle content of lungs from 2 mesothelioma cases who had both worked in the talc mines in Jefferson and St. Lawrence counties and from a comparison group of 8 talc miners without mesothelioma from the same areas. A similar distribution of particle types was observed in the two groups, except for a high tremolite particle count in one of the two cases. The predominant type of EMP found in the lungs was talc, but anthophyllite, chrysotile, and tremolite/actinolite EMPs were also found in nearly all of the study subjects. Non-fibrous talc and silica were also detected in some of the study subjects. Notably, only one "commercial" amphibole fiber was found in all 10 individuals. This last finding suggests that the cause of these mesothelioma cases was unlikely to be due to asbestos exposures from employment in other (non-talc) jobs.

An excess of lung cancer has also been reported in several epidemiologic studies of New York talc mines and mills [Kleinfeld et al. 1967 and 1974; Brown et al. 1990; Lamm et

al. 1988; Stille et al. 1985; Honda et al. 2002]. The most extensive research has been conducted on workers at the talc mine and mills owned by RT Vanderbilt Company, Inc. (RTV), located in St. Lawrence County. A significant excess of mortality from nonmalignant respiratory disease (NMRD) has been consistently reported in these studies. These studies have also generally demonstrated an approximately two- to three-fold increase in lung cancer mortality among these workers [Brown et al. 1990; Honda et al. 2002; Lamm et al. 1988]. The lung cancer excess has been reported to be particularly high among workers with more than 20 years since their first exposure (latency), which is a pattern consistent with an occupational etiology [Brown et al. 1979 and 1990]. Authors of several studies have questioned whether the excess of lung cancer observed in these studies is due to employment at the RTV mines and mills or to other factors [Honda et al. 2002; Lamm et al. 1988; Stille and Tabershaw 1982]. A high smoking rate among the workers at the RTV mines and mills has been suggested as one possible explanation for the excess lung cancer mortality [Kelse 2005; Gamble 1993]. However, it is generally considered implausible that confounding by smoking in occupational cohort studies could explain such a large (i.e., ~2-3 fold) increase in lung cancer mortality [Axelson 1989].

The most persuasive argument against a causal interpretation of these findings is that the lung cancer excess in this study population did not increase with duration and measures of exposure to talc dust [Lamm et al. 1988; Stille and Tabershaw 1982; Honda et al. 2002]. Also, the excess of lung cancer in this cohort has been reported to be limited to workers with short employment (<1 year) [Lamm et al. 1988] and to workers who have been employed in other industries prior to working in the RTV mines and mills [Lamm et al. 1988; Stille and Tabershaw 1982]. The latter observation could be explained by there simply being too few workers and inadequate follow-up of workers who have only worked at RTV to provide the statistical power necessary to demonstrate an increased lung cancer risk. For example, in one of the studies only 10% of the decedents were reported to have not worked in other industries prior to their employment at RTV [Stille and Tabershaw 1982].

In the most recent study of RTV miners and millers, Honda et al. [2002] examined lung cancer mortality in relation to quantitative estimates of exposure to respirable talc dust [Oestenstad et al. 2002]. As in previous studies, mortality from lung cancer was found to be significantly elevated (standardized mortality ratio (SMR) = 2.32, 95% confidence interval (95%CI) = 1.57-3.29). However, the excess of lung cancer mortality was found to be most pronounced in short-term workers (< 5 years) and inversely related to cumulative exposure to respirable dust (mg/m^3-d).

A plausible explanation that has been offered for the lack of exposure-response in these studies is that the observed excess of lung cancer was a result of exposures from employment prior to starting work at RTV. It has been suggested that many of these workers may have had prior employment in neighboring talc mines in upstate New York with similar exposures to talc [NIOSH 1980]. Not considering exposures at these other mines could have substantially impacted results of exposure-response analyses.

Exposures to talc dust may also have been substantially higher in the neighboring mines than in RTV mines [Kelse 2005]. Because RTV workers may have had prior exposures to talc dust, their exposures may have been underestimated, which could explain the observed lack of an exposure-response relationship in the epidemiologic studies of RTV workers. There is also evidence to suggest that RTV workers may have been exposed to lung carcinogens from prior work in non-talc industries [Lamm et al. 1988].

Gamble [1993] conducted a nested lung cancer case-control study of the RTV cohort to further explore whether factors unrelated to exposures at RTV, such as smoking and exposures from prior employment, might be responsible for the observed excess of lung cancer among RTV workers. Cases and controls were identified from 710 workers who were employed between 1947 and 1958 and vital status was ascertained through 1983. All individuals with lung cancer as the underlying cause of death were included as cases (n=22). Three controls (n=66) for each case were selected from members of the cohort who had not died of NMRD or accidents, and were matched to cases based on dates of birth and hire. Controls were also required to have survived for as long as their matched case. Information on smoking and work histories was obtained by interviewing the case (if alive) or relatives. An attempt was made to verify information on previous employment by checking personnel records and by contacting previous employers. A panel of epidemiologists and industrial hygienists classified previous non-talc employment with regard to the probability of occupational exposure to a lung cancer risk.

As in previous investigations of the RTV cohort, the risk of lung cancer was found to decrease with increasing duration of employment at RTV. This was true among both smokers and non-smokers, and also when individuals with inadequate time since first exposure (<20 years) and short duration of employment were excluded. Lung cancer risk was also found to decrease with increasing probability of exposure to lung carcinogens from non-talc employment. A positive exposure-response relationship was evident when non-RTV talc exposures were included in the analysis, although this relationship was not statistically significant.

This study by Gamble [1993] does not provide support for the argument that prior employment in non-talc industries was responsible for the excess of lung cancer observed among RTV workers. The author interpreted his findings as providing support for the argument that the excess of lung cancer was due to confounding by smoking based on the fact that smoking was strongly associated with lung cancer risk and on the observation that the exposure-response relationship with talc was even more strongly negative (inverse) in analyses restricted to smokers than among all study subjects. However, it is no surprise that an association was observed between smoking and lung cancer and the fact that the negative (inverse) exposure trend was even stronger among smokers does not explain why the cohort as a whole experienced much higher lung cancer rates than expected.

Only two cases of pleural mesothelioma have been reported in the studies of RTV miners and millers [Honda et al. 2002]. It is unclear whether these cases are attributable to exposures to talc at the RTV mines and mills. One of the cases had only worked for a short time in a job with minimal talc exposure, had previously worked for many years in the construction of a talc mine, and had subsequently worked on repairing oil heating systems. The other case developed after only 15 years after first exposed (latency) to RTV talc. Mesothelioma has more often been observed to develop at least 20 to 40 years from the time of first exposure.

NIOSH [1980] reported that dust from these talc mines contains chrysotile, tremolite EMPs, and anthophyllite EMPs. However, the identification of these EMPs as asbestiform has been the subject of debate. In an industrial hygiene assessment conducted at RTV mines by NIOSH [1980], X-ray diffraction and petrographic microscopic analyses of talc product samples found them to contain 14-48% mineral talc, 37-59% tremolite, 4.5-15% anthophyllite, and 10-15% antigorite-lizardite. Based on airborne samples collected at the mine and mill and analyzed by TEM, 65% of the EMPs that were longer than 5 μm in length were anthophyllite and 7% were tremolite, with much of the tremolite determined to be from a non-fibrous habit. Median diameters were 0.13μm for the anthophyllite EMPs and 0.19 μm for the tremolite EMPs; median lengths were 1.5 μm for the anthophyllite EMPs and 1.6 μm for the tremolite EMPs. The mean time-weighted average exposure to respirable dust was reported to be 0.86 mg/m^3. In contrast, a paper prepared by Kelse [2005] reported the percentage by weight of talc from RTV mines in upstate New York as 20-40% talc, 40-60% nonasbestiform tremolite, 15-30% nonasbestiform antigorite-lizardite, and 1-5% nonasbestiform anthophyllite. Up to 5.6% of the total product was comprised of talc and talc/amphibole fibers, and up to 1.8% (typically about 0.5% by weight) of the total product was reported to be asbestiform amphibole fibers [Kelse 2005]. Serpentine and amphibole minerals typically develop through the alteration of other minerals. Consequently, they may exist as partially altered minerals having variations in elemental compositions. Minerals undergoing this alteration are often frequently called “transitional minerals”. Thus the elemental composition of individual mineral particles can vary within a mineral deposit containing transitional minerals, which could account for differences in the reported composition of talc from the RTV mines.

A major limitation of the epidemiology studies of RTV talc workers is the lack of an exposure-response analysis based on direct measurements of EMPs. Most of the studies used tenure as a surrogate for exposure, and the Honda [2002] study used respirable dust as the exposure metric. Although the Honda study was based on reconstructed exposures to respirable dust, these exposure estimates may not be correlated with exposure to EMPs.

Finally, a cohort study of Vermont talc miners and millers has some relevance for interpreting the findings from the studies of New York talc workers [Selevan et al. 1979]. The available evidence indicates that Vermont talc is free of asbestos fibers. A

statistically significant excess of NMRD mortality was observed among the millers (SMR=4.07, 95%CI=1.63-8.38), but not among the miners (SMR=1.63, 95%CI=0.20-9.57) in this study. In contrast, respiratory cancer was found to be significantly elevated among the miners (SMR=4.35, 95%CI=1.41-10.15) but not among the millers (SMR=1.02, 95%CI=0.12-3.96). The authors suggested that their respiratory cancer findings might be due to non-talc exposures, such as radon progeny, because exposures to talc dust were higher among millers than miners.

In summary, an excess of pulmonary fibrosis and pleural plaques is well recognized to have occurred among workers exposed to talc. Mesothelioma rates have been reported to be significantly elevated in Jefferson County, which is the site of much of the talc industry in New York. However, death data reported for 1999-2004 do not suggest particularly high rates in that county. Also, aspects of the few cases of mesothelioma that have been carefully evaluated in the studies of New York talc miners make it unclear whether the cases are attributable to employment in the talc industry. Lung cancer mortality has been consistently reported to be elevated in studies of New York talc miners. However, whether this excess is attributable to exposures to talc is questionable because the lung cancer excess was generally found to be most pronounced in short-term workers and did not increase with cumulative exposure to talc dust. Chance or confounding from smoking is highly unlikely to fully explain the large lung cancer excess observed in these studies. These findings may be at least in part explained by employment in other industries, including other talc mines in upstate New York.

Studies of Homestake Gold Miner

Three groups of investigators have conducted retrospective cohort studies of miners at the Homestake gold mine in South Dakota with somewhat different and overlapping cohort definitions. Gillam et al. [1976] studied 440 white males who were employed as of 1960 and who had worked underground for at least 5 years in the mine. McDonald et al. [1978] conducted a retrospective cohort study of 1,321 men who had retired and worked for at least 21 years in the mine as of 1973 and were followed for vital status until 1974. Brown et al. [1986] conducted a retrospective cohort study of 3,328 miners who had worked for at least 1 year between 1940 and 1965 with follow-up of vital status to 1977. This same cohort was subsequently updated to 1990 by Steenland and Brown [1995]. Exposures of potential concern at this mine include crystalline silica, radon progeny, arsenic, and nonasbestiform EMPs. The longer (>5 μm) nonasbestiform EMPs have been reported to be primarily cumingtonite-grunerite (69%), but tremolite-actinolite (15%) and other nonasbestiform amphibole varieties (16%) were also detected [Zumwalde et al. 1981]. Most of the EMPs observed by TEM (70-80 %) were shorter than 5 μm; for the entire population of EMPs, the geometric mean length was 3.2 μm and the geometric mean diameter was 0.4 μm.

There is very little evidence of an excess of mesothelioma in the studies of Homestake gold miners. One case of mediastinal mesothelioma with "low" dust exposure was reported in the study by McDonald et al. [1978]. Slight excesses of cancers of the

peritoneum (4 cases; SMR=2.81, 95%CI= 0.76-7.19) and other respiratory cancer (3 cases: SMR=2.54, 95%CI=0.52-7.43) were reported in the most recent study [Steenland and Brown 1995]. These categories might be expected to include cases of mesothelioma; however, mesothelioma was not mentioned on the death certificates for these cases.

Significant excesses in mortality from tuberculosis and pneumoconiosis (mainly silicosis) were observed in all of the studies. An excess of respiratory cancer (10 cases observed, SMR=3.7, 95%CI=1.78-6.71) was reported in the earliest study by Gillam et al. [1976]. Respiratory cancer mortality was not found to be elevated (34 cases, SMR=1.03, 95%CI=0.71-1.44) and there was only weak evidence that it increased with level of exposure in the study by McDonald et al. [1978]. A slight excess of lung cancer (115 cases, SMR=1.13, 95%CI=0.94-1.36) was reported in the most recent study based on comparison with U.S. mortality rates [Steenland and Brown 1995]. This lung cancer excess was more pronounced when county rates (SMR=1.25, 95%CI=1.03-1.51) and even more so when South Dakota state rates (SMR=1.59, 95%CI=1.31-1.92) were used as the referent. The excess was also increased (based on U.S. rates: SMR=1.27, 95%CI=1.02-1.55) when the analysis was restricted to individuals with at least 30 years of time since first exposure (latency). Lung cancer mortality was not found to increase with estimated cumulative exposure to dust in this study, though a clear exposure-response trend was observed for pneumoconiosis. The limited available data on smoking habits indicated that miners in this cohort smoked slightly more than the U.S. general population in a 1960 survey.

Taken together, the studies of Homestake gold miners provide at best weak evidence of an excess risk of lung cancer. These weak findings are particularly surprising because of the well documented exposures in the mine to crystalline silica, which has been recognized as a human lung carcinogen [IARC 1997], and because clear excesses of lung cancer has been reported in other studies of gold miners [e.g., Hnizdo and Sluis-Cremer 1991; Wyndham et al. 1986]. Although small excesses of lung cancer have been reported in the most recent studies of the Homestake gold miners, the increased mortality has not been found to increase with measures of cumulative dust exposure. However, because the vast majority of the airborne dust particles in this mine would not have been in the form of EMPs, it must be recognized that dust exposure is likely to be a very poor surrogate for measurement of exposure to EMPs in this mine. Thus the lack of exposure-response reported in these studies for lung cancer is largely uninformative with respect to the hypothesis that nonasbestiform EMPs are associated with increased risk of respiratory diseases in this population.

Studies of Taconite Miners

There has been a long history of concern about a potential association between exposures associated with the taconite iron ore industry in northeastern Minnesota and the risk of respiratory cancers and diseases. This concern started in 1973, when amphibole fibers were found in the Duluth water supply and were traced to tailings that had been disposed of in Lake Superior by the Reserve Mining Company. Extensive sampling and analysis

of areas of the Peter Mitchell taconite iron ore mines was recently reported by Ross et al. [2007]. While the authors reported finding "no asbestos fibers of any type" in the mines, they did find and describe fibrous ferroactinolite, fibrous ferrian sepiolite, fibrous grunerite-ferroactinolite, and fibrous actinolite in ore samples, some of which was very thin (<0.01 μm) with a very high aspect ratio. They estimated fibrous amphibole material to represent "a tiny fraction of one percent of the total rock mass of this taconite deposit" [Ross et al. 2007].

Several epidemiologic studies have examined mortality of miners working in the taconite mines and mills of Minnesota. Higgins et al. [1983] published the earliest study, which examined the mortality of approximately 5,700 workers who were employed at the Reserve Mining Company between 1952 and 1976 and were followed up to 1976. Overall mortality (SMR=0.87) and mortality from respiratory cancer (15 cases, SMR=0.84) was less than expected. Respiratory cancer mortality was not found to be increased among workers with at least 15 years since first exposure (latency) and did not increase with estimated cumulative exposure to dust. The maximum follow-up of this cohort was 24 years, which is probably too short to be able to detect increased mortality from lung cancer or mesothelioma.

Cooper et al. [1988 and 1992] have reported on the mortality experience of 3,431 miners and millers who were employed in either the Erie or Minntac mines and mills for at least 3 months between 1947 and 1958. In their most recent update [Cooper 1992], follow-up of the cohort was extended from their first study [Cooper et al. 1988] from 1983 to 1988. Comparisons were made with white male mortality rates for Minnesota and for the U.S. population. Mortality from respiratory cancer was found to be slightly less than expected in this study (106 cases, based on Minnesota rates: SMR=0.92, 95%CI=0.75-1.11). Respiratory cancer mortality was close to the expected value (46 cases, based on Minnesota rates: SMR=0.99, 95%CI=0.72-1.32) among workers with more than 20 years since first exposure (latency).

A statistically significant excess of mesothelioma has been reported in northeastern Minnesota, which is the area in which the taconite mining and milling industry is located [MDH 2007]. In its most recent report, the Minnesota Department of Health (MDH) reported that a total of 159 cases occurred in this region during the period of 1988 to 2006. The mesothelioma rate in males was approximately twice the expected rate based on the rest of the state (146 cases, rate ratio (RR) = 2.1, 95%CI = 1.79-2.49), while the rate in females was less than expected (RR = 0.72, 95%CI = 0.38-1.24). The fact that the excess of mesothelioma was only observed among males strongly suggests an occupational etiology. In addition to the taconite industry, a plant producing asbestos ceiling tiles (Conwed Corporation) was located in the northeastern Minnesota region. From 1958-1965 amosite was used at Conwed, and from 1966-1974 chrysotile was used [Mandel 2008]. The MDH has initiated epidemiologic studies of mesothelioma incidence among workers at the Conwed Corporation and at the iron mines in northeastern Minnesota. The records from a cohort of approximately 72,000 iron miners and from

5,700 Conwed workers have been linked with a mesothelioma data registry. Between 1988 and 2007, a total of 58 mesothelioma cases have been identified among the miners and 25 cases have been identified among the Conwed workers. Only 3 of the 58 mesothelioma cases identified in the miner cohort had also been employed at Conwed, and thus it does not appear likely that the mesothelioma excess in miners could be explained by asbestos exposures during employment at the Conwed ceiling tile facility [MDH 2007].

Brunner et al. [2007] have recently reported findings from a study of mesothelioma cases that have been reported by the MDH among iron miners between 1988 and 1996. The job histories of the cases were reviewed for evidence of exposure to commercial asbestos. Mining jobs were identified from company personnel files. Non-mining employment information was obtained from worker application files, worker compensation records, and obituaries. Potential asbestos exposures for jobs held in the mining industry were identified by conducting interviews of 350 workers representing 122 occupations and 7 different mining companies. An expert panel rated the potential for asbestos exposure based on these interviews, available job descriptions from the relevant time period, and their own knowledge of the mining environment to estimate the probability and intensity of potential exposure to commercial asbestos in each of the jobs. Fifteen of 17 iron miners known to have developed mesothelioma were judged to have sufficiently good work histories for the study. Eleven of the cases were reported to have had probable exposure, and 3 were reported to have possible exposure to commercial asbestos. The asbestos exposures were from non-mining jobs (4 cases), mining jobs (4 cases), or both (6 cases). The findings from this study suggest that the excess of mesothelioma observed among taconite miners might be explained by exposure to commercial chrysotile rather than from the nonasbestiform amphibole EMPs generated during processing of the iron ore. However, this being a case series, it was not possible to determine whether the commercial asbestos exposure was different in the cases than in the cohort as a whole or in a control group. This study also did not include the 41 additional mesothelioma cases that have been reported by the MDH since 1996 [MDH 2007].

In summary, the results from cohort mortality studies of taconite miners and millers in Minnesota have not provided any evidence of an increased risk of respiratory cancer or mesothelioma. This appears to be somewhat in conflict with reports from the MDH that mesothelioma incidence is significantly elevated among males (but not females) in northeastern Minnesota and that a large number of these cases were workers in the Minnesota taconite industry. There is some evidence that these cases could at least in part be related to exposures to commercial asbestos that occurred in or outside of the taconite mining industry, but further research on this question is needed. The MDH [2007] is currently working with researchers at the University of Minnesota, School of Public Health on a mesothelioma case-control study, a respiratory morbidity study, and a mortality study of the iron miners of northeastern Minnesota.

Summary of Epidemiological Studies of Cohorts Exposed to Nonasbestiform EMPs

The results from studies of populations reportedly exposed to nonasbestiform EMPs do not provide clear answers regarding the toxicity of these EMPs. There are a number of features of these studies that limit their usefulness for answering these questions. First, the populations in these studies were exposed to a complex mixture of particles. Nonasbestiform EMPs generally represented only a small percentage of airborne exposures, which included other minerals such as silica that are known to cause lung diseases. Thus, although an excess of pneumoconiosis has been observed in the studies of Homestake gold miners and New York talc workers, the extent to which these findings are attributable to their exposures to nonasbestiform EMPs cannot be determined. A potential limitation of the New York talc studies is that if the EMPs do include asbestiform minerals as found in the NIOSH [1980] study, it is difficult to determine whether the observed health effects are from asbestiform or other EMPs.

Another major limitation of these studies is that they lack adequate information on past exposure to EMPs. A relatively large excess of respiratory cancer was observed in the occupational studies of New York talc workers and a small excess was observed in the most recent study of Homestake gold miners. In both studies, the excess of respiratory cancer was not found to increase with cumulative exposure to dust. However, dust exposures are a very poor surrogate of exposure to nonasbestiform EMPs in these settings. Interpretation of findings from the New York talc studies has been further complicated by the employment of the workers elsewhere, including employment at other talc mines in the area. Lack of positive findings with respect to exposure-response analyses in the New York talc studies could have resulted from exposure misclassification caused by not including exposures at neighboring talc mines with similar exposures which may have resulted in an under-ascertainment of exposure to talc and other mineral particles in these studies

The reliability of death certificate information is another major limitation, particularly for the diagnosis of mesothelioma. Mesothelioma did not have a specific ICD code until the 10^{th} revision of the ICD, used for U.S. death certificate data only since 1999. This may explain the apparent contradiction between the lack of an excess of mesothelioma in the cohort studies of taconite miners, and the excess of mesothelioma that has been reported in the more recent studies based on a mesothelioma registry in northeastern Minnesota. Similarly, an excess of mesothelioma has been reported in the areas of New York State where the talc industry is located, but not in the occupational cohort mortality studies of talc miners and millers.

Finally, the lack of information on cigarette smoking habits of the studied workers is a major issue in interpreting the findings for respiratory cancer in these studies. Concerns about cigarette smoking in occupational cohort studies is generally based on the assumption that blue collar workers smoke more than the general population. However, the extent of this bias is generally not expected to be able to account for more than a 50% increase in lung cancer risk and is unlikely to explain the 2- to 3-fold risk reported in the New York talc studies. Confounding by smoking could conceivably explain the small

excess of lung cancer that has been reported in the most recent study of Homestake gold miners [Steenland and Brown 1995]. However, smoking may have introduced a negative bias in some of these studies. Cigarette smoking has been reported to have been banned in the Homestake gold mines [Brown et al. 1986] and in the underground taconite mines [Lawler et al. 1985]. Preventing workers from smoking at work could have negatively biased the lung cancer findings in these studies.

Because of the study limitations described above, the findings from these studies should best be viewed as providing inconclusive as opposed to negative evidence regarding the health hazards associated with exposures to nonasbestiform EMPs. To be more informative, additional studies of these populations would need improved characterizations of exposure to EMPs, diagnosis of mesothelioma, information on smoking, and exposures from prior employment. Additional studies of these populations should be attempted only if these improvements are deemed feasible.

1.5.1.3.3 Animal Studies

In NIOSH's rational for its 1990 recommendation that the REL for airborne asbestos fibers encompass cleavage fragments from the nonasbestiform analogs of the asbestos minerals, discussion of results of animal carcinogenicity studies cited several original studies and reviews [Stanton et al. 1977; Stanton et al. 1981; Wagner et al. 1982; Muhle et al. 1987; Pott et al. 1984; Pott et al. 1987; Lippmann et al. 1988]. NIOSH concluded that the cited papers provided evidence indicating that fiber dimension (and not fiber composition) was the major determinant of carcinogenicity for mineral fibers. NIOSH concluded that:

> *Literature reviews by Lippmann [1988] and Pott et al. [1987] enhance the hypothesis that any mineral particle can induce cancer and mesothelioma if it is sufficiently durable to be retained in the lung and if it has the appropriate aspect ratio and dimensions. Similarly, Wagner [1986] concluded that all mineral particles of a specific diameter and length size range may be associated with development of diffuse pleural and peritoneal mesotheliomas.* [NIOSH 1990a]

That general conclusion notwithstanding, a study by Smith et al. [1979] that was not cited by NIOSH in 1990 addressed the specific question of carcinogenicity of EMPs from nonasbestiform amphiboles. Pleural tumor induction by intrapleural (IP) injection challenge in hamsters was compared for various challenge materials including two asbestiform tremolites and two nonasbestiform (prismatic) tremolitic talcs. In contrast to the two asbestiform tremolites, which induced tumors in 22% and 42% of challenged hamsters at the higher dose, no tumors resulted following challenge with either of the two nonasbestiform tremolites [Smith et al 1979]. In its rule-making, OSHA noted several limitations the small number of animals in the study, the early death of many animals, and the lack of systematic characterization of fiber size and aspect ratio [OSHA 1992]. One of the nonasbestiform tremolitic talcs was later analyzed and confirmed to have

tremolitic chemical composition and 13% "fibers" as defined by a 3:1 aspect ratio [Wylie et al. 1993].

Since 1990, another carcinogenicity study of nonasbestiform amphibole minerals has been published. An IP injection study in rats used six samples of tremolite, including three asbestiform samples that induced mesothelioma in 100%, 97%, and 97% of challenged animals [Davis et al. 1991]. Two nonasbestiform tremolite samples resulted in mesotheliomas in 12% and 5% of the animals, at least the former incidence being above expected background levels. Another sample that was predominantly nonasbestiform but contained a small amount of asbestiform tremolite resulted in mesothelioma in 67% of animals. Of note, the nonasbestiform material associated with the 12% mesothelioma incidence and this latter material contained an approximately equal number of EMPs longer than 8 μm and thinner than 0.5 μm.

Studies of *in vitro* assays of various biological responses, some published before and some after 1990, have also found relative toxicities of asbestiform and nonasbestiform materials that generally parallel the differences observed in the *in vivo* animal IP injection studies of tumorigenicity [Wagner et al. 1982; Woodworth et al. 1983; Hansen and Mossman 1987; Marsh and Mossman 1988; Sesko and Mossman 1989; Janssen et al. 1994; Mossman and Sesko 1990], and a recent review of the literature concluded that cleavage fragments of amphiboles are less potent than asbestos fibers [Mossman 2007].

In summary, there is substantially more literature now than in 1990 pertaining to differential animal carcinogenicity and toxicity of EMPs from nonasbestiform amphiboles (i.e., cleavage fragments) in comparison with asbestos fibers. More detailed discussion of these studies, including discussion of important limitations of the studies, can be found in Section 1.6.4 of this document.

1.5.1.3.4 Analytical Limitations

The third element that served as a basis for NIOSH's current recommendation was the inability to adequately distinguish between airborne exposures to EMPs from asbestiform and nonasbestiform minerals. The first argument was that asbestiform and nonasbestiform minerals can occur in the same area and that determining the location and identification of tremolite asbestos, actinolite asbestos, and anthophyllite asbestos within deposits of their nonasbestiform mineral analogs can be difficult, and inadvertent contamination of some mined/quarried commodities by tremolite asbestos, actinolite asbestos, and/or anthophyllite asbestos was possible. These inherent factors of mineral deposits are not likely to change, and the potential for contamination remains.

The second argument was that routine analytical methods for quantifying airborne exposures are not available that can be used to accurately differentiate between fibers

from the asbestos minerals and EMPs from their nonasbestiform analogs that meet the dimensional criteria of a countable particle when examined microscopically.

Two analytical components of the NIOSH airborne asbestos fibers REL are applied to air samples, the microscopic methods and the counting rules. The microscopic methods include:

- *Phase contrast microscopy* (PCM) [NIOSH 1994a – Analytical Method 7400 "A rules" – Asbestos and Other Fibers by PCM] is used to count all particles that are longer than 5 μm and have a length-to-width ratio equal to or greater than 3:1.

- *Transmission electron microscopy* (TEM) [NIOSH 1994b – Analytical Method 7402 – Asbestos by TEM] is used as a supplement to the PCM method when there is uncertainty about the identification of elongated particles (EPs) that are counted. When TEM analysis is used for particle identification, only those EPs that are identified as "asbestos" and meet the dimensional criteria used by PCM (>0.25 μm width and > 5μm length) are counted and compared with PCM counts to yield corrected "asbestos" fiber counts.

There are several limitations with the use of PCM and TEM for asbestos analysis. PCM is stated to be limited to observing EPs with widths >0.25 μm and is not equipped for particle identification. TEM, while capable of resolving EPs with widths as small as 0.001 μm, frequently cannot differentiate nonasbestiform from asbestiform EMPs when the elemental composition is the same or when present in a heterogeneous mix of unknown particles. Important limitations of TEM are that partial lengths of long fibers that intersect grid bars are hidden, and the small TEM fields of view tend to bias the analyst towards only the thinnest of fibers. Another limitation of both methods is that high concentrations of background dust collected on samples may interfere with fiber counting with PCM and particle identification by TEM.

Thus, the current PCM and TEM methods used for routine exposure assessment continue to have the limitation of not being able to differentiate between the asbestiform and nonasbestiform EMPs. Further discussion of these methods and possible improvements that could lead to methods which differentiate between these varieties is provided in Section 1.7.

1.6 Determinants of Particle Toxicity and Health Effects

Current recommendations for assessing occupational and environmental exposures to asbestos fibers rely primarily on EMP dimensional and mineralogical characteristics. Dimension is an important determinant of toxicity in terms of where EMPs deposit in the lung as well as their impact on clearance mechanisms and retention time in the lung.

However, other particle characteristics have been identified, such as the durability of the particle in lung fluids, chemical composition, and particle surface activity, as possibly having an important role in causing respiratory disease. Research to elucidate what role these EMP characteristics may have in causing biological response may help to provide better evidence-based recommendations for asbestos fibers and other EMPs.

1.6.1. Deposition

Deposition of airborne particles in the respiratory system is defined as the loss of particles from the inspired air during respiration. Clearance pertains to the removal of these deposited particles by diverse processes over time, whereas retention is the temporal persistence of particles within the respiratory system [Morrow 1985]. The deposition of inhaled particles in the respiratory tract is a function of their physical characteristics (dimension and density) and of anatomical and physiological parameters of the airways [Yu et al. 1986]. While particle chemical composition does not play a role in deposition, respiratory clearance of all particle types is dependent on both physical and chemical characteristics of the particle. In addition, surface charge and hydrophilicity, as well as adsorbed materials (e.g., coatings on synthetic fibers) and other physical and chemical factors, determine whether small particles can be easily dispersed in the air or will agglomerate into larger, non-respirable masses [ILSI 2005].

Depending on their physical characteristics, inhaled particles are deposited in one of the following three respiratory system compartments: the extra-thoracic region consisting of the anterior and posterior nose, mouth, pharynx, and larynx; the bronchial region consisting of the trachea, bronchi, and bronchioles down to and including the terminal bronchioles; and the alveolar-interstitial region including respiratory bronchioles, alveolar ducts, and alveolar sacs.

Important parameters for the deposition of airborne particles are their aerodynamic and thermodynamic properties. Below a particle size of 0.5 μm aerodynamic equivalent diameter (AED), thermodynamic properties prevail. The aerodynamic equivalent diameter of EPs is mostly determined by their geometric diameter and density. Deposition of EPs in an airway is strongly related to the orientation of the particle with respect to the direction of the air flow [Asgharian and Yu 1988]. Deposition on airway surfaces typically occurs by diffusion, sedimentation, impaction, and interception. Interception is an important mechanism for the deposition of EPs, especially as the length of the particle increases. Diffusion deposition occurs mainly with small-width EPs which are subject to being moved by Brownian motion [Yu et al. 1986].

1.6.2 Clearance and Retention

A variety of mechanisms are associated with the removal of deposited particles from the respiratory tract [Warheit 1989]. Physical clearance of insoluble particles deposited in the lung is an important physiological defense mechanism that usually serves to moderate any risk that might otherwise be associated with exposure to particles. Inhaled particles that deposit on respiratory tract surfaces may be cleared by the tracheobronchial mucociliary escalator or nasal mucus flow to the throat and then may be either expectorated or swallowed. The role of clearance, as a pulmonary protective mechanism, depends upon the physicochemical properties of the inhaled particles, the sites of deposition, and respiratory anatomy and physiology. For example, inhaled insoluble particles with larger AEDs tend to be deposited on the nasopharyngeal mucus and are generally cleared by sneezing or blowing or by flow into the oropharynx where they are swallowed. Insoluble particles with smaller AEDs tend to deposit lower in the respiratory tract, with associated longer retention times. Those deposited in the alveolar region are subject to longer retention times than those deposited on the bronchial region [Lippmann and Esch 1988].

The most important process for removal of insoluble particles from the airways is mucociliary clearance, which involves a moving layer of mucus by the action of ciliated airway cells that line the trachea, bronchi, and terminal bronchioles [Warheit 1989]. The mucociliary transport system is sensitive to a variety of agents, including cigarette smoke and ozone [Vastag et al. 1985]. These toxicants affect the speed of mucus flow and consequent particle clearance by altering ciliary action and/or modifying the properties and/or amount of mucus. Chronic exposure to cigarette smoke has been shown to cause a prolonged impairment of particulate clearance from the bronchial region. This impaired clearance is associated with increased retention of asbestos fibers in the bronchi where they stimulate inflammatory processes in the bronchial epithelium [Churg et al. 1992; Churg and Stevens 1995].

Because the alveolar region of the lung does not possess mucociliary clearance capability, particles (generally <2 μm AED) deposited in this region are cleared at a much slower rate than particles deposited in the bronchial region. Particles that are soluble may dissolve and be absorbed into the pulmonary capillaries, while insoluble particles may physically translocate from the alveolar airspace [Lippmann et al. 1980; Lippmann and Schlesinger 1984; Schlesinger 1985]. Most insoluble EPs that deposit in the alveolar regions are phagocytized (i.e., engulfed) by alveolar macrophages. Macrophages contain lysosomes packed with digestive enzymes such as acid hydrolases at acidic pH levels. Lysosomal contents are capable of digesting many—though not all—types of phagocytized particles. Alveolar macrophages that have phagocytized particles tend to migrate to the bronchoalveolar junctions, where they enter onto the mucociliary escalator for subsequent removal from the lung [Green 1973]. It has been postulated by some investigators [Brain et al. 1994] that dissolution of particles within macrophages is a more important determinant of long-term clearance kinetics for many mineral dusts

than is mucociliary transport and the migratory potential of lung macrophages. However, there are circumstances which can disrupt the normal phagosomal function of alveolar macrophages. One such type of circumstance involves the toxic death of macrophages initiated by highly reactive particle surfaces (e.g., crystalline silica particles). Another such circumstance involves overwhelming the capacity of macrophages by an extreme burden of deposited particles, sometimes referred to as "overload", even by particles that would be considered "inert" at lower doses. A third type of circumstance, typified by asbestos fibers, involves EPs that, even though having a small enough AED (defined primarily by particle width) to permit deposition in the alveolar region, cannot be readily phagocytized because particle length exceeds macrophage capacity. When alveolar macrophages attempt to phagocytize such elongated particles, they cannot completely engulf them (sometimes referred to as "frustrated phagocytosis") and lysosomal contents are released into the alveolar space. "Frustrated phagocytosis" can initiate a process in which reactive oxygen species (ROS) are generated, stimulating the induction of tumor necrosis factor-alpha (TNF-α). TNF-α is considered to be an inflammatory and fibrogenic cytokine that plays an important role in the pathogenesis of pulmonary fibrosis [Blake et al. 1998].

All three types of disruption of normal macrophage function contribute to decreased particle clearance rates and can result in inflammation of the alveolar spaces. In addition, particles that are not phagocytized in the alveoli can translocate to the interstitium, where they may be phagocytized by interstitial macrophages or transported through the lymphatics to pulmonary lymph nodes [Lippmann et al. 1980; Lippmann and Schlesinger 1984; Schlesinger 1985; Oberdorster et al. 1988]. Tran and Buchanan [2000] have reported that for humans the sequestration of particles in the interstitial compartment is a more prominent feature than the retention of particles due to overload that is observed in animal studies. The importance of interstitialization in humans is consistent with the kinetic differences observed in lung clearance rates in humans and rats. The first-order rate coefficient for alveolar clearance is approximately 1 order of magnitude faster in rats than in humans [Snipes 1996], which may allow for greater interstitialization of particles in humans at all levels of lung dust burden. These findings indicate that adjustment of kinetic differences in particle clearance and retention is required when using rodent data to predict lung disease risks in humans and that current human lung models underestimate the working lifetime lung dust burdens in certain occupational populations [Kuempel et al. 2001].

Evidence from *in vitro* and *in vivo* studies in rodents indicate that EPs (vitreous glass and EMPs) with a length equal to or greater than the diameter of rodent lung macrophages (about 15 μm) are most closely linked to biological effects observed in the lung [Blake et al. 1998]. Alveolar macrophages appear to be capable of phagocytizing and removing EMPs shorter than approximately 15 μm, either by transport to the mucociliary system or to local lymph channels. With increasing length above approximately 15 μm, alveolar macrophages appear to be increasingly ineffective at physical removal, resulting in differential removal rates for EPs of different lengths. While EP lengths greater than 15

µm appear to be associated with toxicity in experimental animal studies, a "critical" length for toxicity in humans is probably greater than 15 µm [Zeidler-Erdely et al. 2006]. For long EPs that cannot be easily cleared by macrophages, biopersistence in the lung is influenced by the ease with which the EPs can break into shorter lengths.

1.6.3 Biopersistence and other Potentially Important Particle Characteristics

The differences in crystalline structure between amphibole asbestos fibers and amphibole cleavage fragments have been hypothesized to account for apparent differences in toxicological response to these particles. It has been observed that cleavage fragments which meet the dimensional criteria for countable particles under Federal regulatory policies for asbestos fibers tend to be shorter and wider than asbestos fibers [Siegrist and Wylie 1980; Wylie 1988]. This difference in dimension between populations of asbestos fibers and populations of cleavage fragments might contribute to a shorter biopersistence in the lung for cleavage fragments compared to asbestos fibers. Asbestos fibers also tend to separate longitudinally once deposited in the lung, thus increasing the total number of retained fibers without an accompanying reduction in lengths of the retained fibers [NRC 1984]. In contrast, cleavage fragments tend to break transversely due to dissolution of their weaker crystalline structure, resulting in shorter particles that can be more easily cleared through phagocytosis and mucociliary clearance [Zoltai 1981]. The impact of these structural differences on solubility in lung fluids warrants study, but if there are substantial differences in solubility in lung fluids between asbestos fibers and other EMPs (including amphibole cleavage fragments), toxicity could differ.

1.6.3.1 Biopersistence

Dissolution of EPs in the lung is a poorly understood process that is dependent on particle characteristics, biological processes, and concomitant exposure to other particulates. The ability of an EP to be retained and remain intact in the lung is considered to be an important factor in the process of an adverse biological response. EPs of sufficient length that remain intact and are retained in the lung are thought to pose the greatest risk for respiratory disease. The ability of an EP to reside long-term in the lung is generally referred to as "biopersistence." Biopersistence of EPs in the lung is a function of the site and rate of deposition, their rates of clearance by alveolar macrophages and mucociliary transport, their solubility in lung fluids, their breakage rate and breakage pattern (longitudinal or transverse), and their rates of translocation across biological membranes. The rates of some of these processes can affect the rates of other processes. For example, the rate of deposition in the alveolar region could potentially overwhelm macrophage clearance mechanisms and increase the rate of translocation to the lung interstitium.

The persistence of an EP in the lung is influenced by changes that may occur in its dimension, surface area, chemical composition, and surface chemistry. Differences in any

of these characteristics can potentially result in differences in clearance and retention and affect its pathologic potential. For example, EPs too long to be phagocytized by alveolar macrophages will tend to remain in the alveolar compartment and be subjected to other clearance mechanisms, including dissolution, breakage, and translocation to interstitial sites and subsequently to pleural and other sites.

The durability of EPs residing in the lung is considered an important characteristic which influences biopersistence. An EP's durability is generally measured by its ability to resist dissolution and mechanical disintegration after being subjected to lung extra-cellular fluid (approximately pH 7) and lysosomal fluids (approximately pH 5). EPs that are more soluble will be less biopersistent, and EPs with greater thickness may take longer to dissolve than thinner EPs, all else being equal. For example, long, thin EPs that are not very durable could dissolve and/or fragment into shorter EPs, increasing their probability of being cleared from the lung and thus potentially decreasing lung retention time and risk for neoplastic effects. Some EPs, such as certain types of glass fibers, are fairly soluble in lung fluid and are cleared from the lung in a matter of days or months; other EPs, such as amphibole asbestos, can remain in the lung for decades. It has been suggested that some types of EPs may alter the mobility of macrophages and the translocation of EPs towards the pleura or lymph nodes [Davis 1994]. No relationship has been established between biopersistence of EPs in the lung and the risk of induction of genetic and epigenetic changes that may lead to cancer [Barrett 1994]. While some evidence indicates that durability may be a determinant of toxicity for SVFs, EMPs need to be evaluated to determine whether they conform to this paradigm [ILSI 2005].

Measurement of the biopersistence of various EMPs has been suggested as a means for estimating their relative potential hazard. Short-term inhalation and intratracheal instillation studies have been used to determine the biopersistence of various SVFs and asbestos fibers. Animal inhalation studies are preferred over animal tracheal instillation studies to assess biopersistence because they more closely mimic typical human exposure. The European Commission has adopted specific testing criteria that permit the results from either short-term biopersistence studies or chronic animal studies to be used as a basis for determining carcinogenicity [European Commission 1997].

Several animal inhalation studies have indicated that oncogenic potential of long SVFs can be determined by their biopersistence [Mast et al. 2000; Bernstein et al. 2001; Moolgavkar et al. 2001]. It has been suggested that a certain minimum persistence of long fibers is necessary before even minute changes start to appear in the lungs of exposed animals [Bernstein et al. 2001]. Furthermore, Moolgavkar et al. [2001] have suggested that fiber-induced cancer risk, in addition to being a linear function of exposure concentration, is also a linear function of the weighted half-life of fibers observed in inhalation studies with rats. Furthermore, dosimetry models for rodents and humans indicate that, on a normalized basis, fiber deposition and clearance rates are lower in humans than in rats [Maxim and McConnell 2001]. Thus, results from chronic inhalation studies with rodents may underestimate lung cancer risks in humans.

Dissolution studies using *in vitro* assays have been conducted with various SVFs and silicate minerals to determine the dissolution rate in simulated lung and lysosomal fluids [Hume and Rimstidt 1992; Werner et al. 1995; Hesterberg and Hart 2000; Jurinski and Rimstidt 2001]. *In vitro* studies can provide a rapid and more controlled alternative to classical long-term toxicity testing in animals and could provide useful information when performed as companion experiments with *in vivo* studies if conditions of exposure and test agent can be made similar. The design of *in vitro* assays is intended to mimic the biological conditions that exist in the lung once the fiber comes into contact with lung tissue or macrophages. While uncertainties exist about the specific physiological processes that occur in the lung, results from *in vitro* assays can provide some insight into the chemical reactions that influence fiber dissolution. For example, it appears that fiber dissolution occurs more readily when the fiber is in contact with a fluid that is under-saturated with respect to the fiber's composition. The condition of under-saturation must be maintained at the fiber's surface for dissolution to continue. If a fiber is surrounded by a saturated or super-saturated solution (compared to the fiber composition), then no further dissolution occurs.

The results from many *in vitro* experiments demonstrate different patterns of dissolution for most of the tested fiber types under various test conditions. This effect was most notable in those experiments where different pH conditions were used. Fluid pH appears to influence the creation of complexes from the leached elements of the fiber, which in turn alters the rate of solubility. Chrysotile fibers tend to dissolve readily in acids because of the preferential leaching of Mg from the fiber. The leaching of Mg from tremolite and anthophyllite and Na from crocidolite also occurs more readily in acid conditions.

Rate of fiber dissolution has also been observed to be affected by differing internal and surface structures of the fiber. EMPs with porous or rough surfaces have larger surface areas compared to smooth fibers with the same gross dimensions. These larger surface areas interact more readily with the surrounding medium because of the greater number of sites where solute molecules can be absorbed. EMPs with cleavage plane surfaces will contain varying degrees of defects; the higher the number of surface defects, the greater the potential instability of the particle. Dissolution of these types of EMPs is typically initiated where surface vacancies or impurities are present [Searl 1994]. Chrysotile asbestos is an example of a sheet silicate made up of numerous fibrils comprised of tightly bound rolled layers of Mg hydroxide. These Mg hydroxide layers are readily leached by acid solutions within human tissues [Spurny 1983], causing disintegration of the fibril's crystalline structure. In contrast, the amphibole asbestos minerals are chain silicates with a crystalline structure comprised of alkali and alkali earth metals that are tightly bound, making the fibers less susceptible to dissolution. In contrast to the crystalline structure of the asbestos fibers, some high-temperature glass fibers are more stable than chrysotile because they are comprised of silicate chains, sheets, and frameworks [Searl 1994]. The absence of cleavage planes or structural defects in glass fibers limits the degree to which fluids can penetrate their interior to promote dissolution.

In some experiments chrysotile fibers were less durable in rat lungs than some high-temperature SVFs [Bellmann et al. 1987; Muhle et al. 1987] but more durable in physiological solutions than some refractory ceramic fibers (RCFs) [Scholze and Conradt 1987].

EMP surface characteristics (e.g., structural defects, porous surfaces) and composition not only influence the rate of dissolution, but also affect the manner in which dissolution occurs. In some instances, surface dissolution will cause alterations in internal structure sufficient to cause mechanical breakage. In some studies, slagwools and rockwools exposed to water developed irregular surfaces, creating stress fractures which caused transverse breakage [Bellmann et al.1987]. Similar occurrences of glass fiber breakage have been observed when there was leaching of alkaline elements [Searl 1994].

Results from *in vitro* and short-term *in vivo* studies conducted with various EMPs and SVFs provide some confirmation that persistence of EPs in the lung is influenced by particle durability [Bernstein et al.1996]. However, other evidence suggests that, because of the relatively short biodurability of chrysotile fibers, any damage to the lung tissue caused by chrysotile fibers must take place soon after exposure [Hume and Rimstidt 1992], suggesting that biopersistence of EPs in the lung may be one of many factors that contribute to biological response. A better understanding of the factors that determine the biological fate of EMPs deposited in the lung is critical to understanding the mechanisms underlying differences in toxic potential of various EMPs of different dimensions and compositions. Because biopersistence of EMPs is thought to play an important role in the development of disease, it may eventually prove to be an important characteristic to incorporate into occupational safety and health policies concerning exposures to EMPs.

1.6.3.2 Other Potentially Important Particle Characteristics

Fiber surface composition and surface-associated activities have been suggested as factors affecting the potential for disease induction [Bonneau et al. 1986; Kane 1991; Jaurand 1991; Fubini 1993]. For non-elongated respirable mineral particles, surface composition and surface interactions can directly and profoundly affect *in vitro* toxicities and *in vivo* pathogenicity; they can also directly cause membranolytic, cytotoxic, mutagenic or clastogenic damage to cells, and have been shown to induce fibrogenic activities in animals and humans. It does not necessarily follow that this will be the case for mineral fibers and other EMPs, but investigation is warranted. One strategy is to determine the effects of careful and well-characterized surface modification of different types of EMPs to determine cell-free interactions with biological materials, *in vitro* cellular cytotoxicities or genotoxicities, or pathology in animal models.

Surface properties of mineral fibers and other EMPs may be a direct factor in cytotoxic or genotoxic mechanisms responsible for fibrogenic or carcinogenic activity. Chemical surface modification of asbestos fibers has been shown to affect their cytotoxicity [Light

and Wei 1977a and 1977b; Jaurand et al. 1983; Vallyathan et al. 1985]. While asbestos fibers clearly can be carcinogenic, they are not consistently positive in genotoxicity assays; their principal damage is chromosomal rather than gene mutation or DNA damage [Jaurand 1991]. One study linked cytotoxicity with *in vitro* mammalian cell transformation [Hesterberg and Barrett 1984]; thus, surface factors affecting cytotoxicity might affect potential for inducing some genotoxic activities. However, surface modification of a well-characterized sample of chrysotile fibers to deplete surface Mg while retaining fiber length did not result in a significant quantitative difference for *in vitro* micronucleus induction between the native and surface-modified materials, both of which were positive in the assay [Keane et al. 1999].

Surfaces of mineral fibers and other EMPs also might be an indirect but critical factor in the manifestation of pathogenic activity: They may be principal determinants of EMP durability under conditions of *in vivo* dissolution in biological fluids. As such, they would be a controlling factor in biopersistence, critical to the suggested mechanisms of continuing irritation or inflammatory response as the source of fibrosis or neoplastic transformation.

1.6.4 Animal and In Vitro Toxicity Studies

Over the last half-century, *in vivo* animal model studies have explored cancer, mesothelioma, and pulmonary fibrosis induction by asbestos varieties and other EMPs following intrapleural, intraperitoneal, or inhalation challenge. Numerous cell-free, *in vitro* cellular, and *in vivo* short-term animal model studies have been pursued, attempting to: (1) examine tissue and cellular responses to EMPs and impact of EMP conditioning on these responses; (2) identify and evaluate interactions and mechanisms involved in pathogenesis; and (3) seek morphological or physicochemical EMP properties controlling those mechanisms. These short-term studies provide an evolving basis for design or interpretation of higher-tier chronic exposure studies of selected EMPs.

Some of the short-term studies have addressed:

- the general question of extrapolating human health effects from *in vivo* animal model studies;
- the physiological relevance of *in vitro* cellular studies of EMP toxicities;
- the association of EMP dimensions with pathology demonstrated in early animal model studies;
- the potential mechanisms and associated EMP properties responsible for initiating cell damage;
- the extensive information now available on a "central dogma" of subsequent intracellular biochemical pathway stimulation leading to toxicity or intercellular signaling in disease promotion; and
- the use of these mechanistic paradigms to explain specific questions of:

- differing activities of EMPs from asbestiform versus non-asbestiform habits of minerals;
- differences between the activities of erionite fibers versus amphibole asbestos fibers;
- seemingly anomalous differences between some *in vitro* and *in vivo* EMP activities; and
- the possibility of EMP-viral co-carcinogenesis.

Several reviews and recommendations for animal model and cellular studies on these issues have been developed by expert workshops and committees. Early studies on the carcinogenicity of asbestos and erionite fibers were reviewed by IARC [1977; 1987a; 1987b] and SVFs were reviewed more recently [IARC 2002]. Short-term *in vivo* and *in vitro* studies to elucidate mechanisms of fiber-induced genotoxicity and genetic mechanisms affecting fiber-induced lung fibrosis have been extensively reviewed. A review for the EPA by an international working group assembled in 2003 provides an update on short-term assay systems for fiber toxicity and carcinogenic potential [ILSI 2005] and two additional reviews discuss the fiber genotoxicity literature up to the current decade [Jaurand 1997; Schins 2002].

The paucity of human health effects information for some new synthetic EPs has led to renewed considerations of the value and limitations of animal model studies, and the question of the interpretability of intrapleural, intraperitoneal, or inhalation challenge methods of animal model tests to make predictions of human health effects [IARC 2002]. One analysis concluded that rat inhalation is not sufficiently sensitive for prediction of human carcinogenicity by fibers other than asbestos [Muhle and Pott 2000]. Another review concluded that there are significant interspecies differences between the mouse, hamster, rat, and human, with the available evidence suggesting that the rat is preferable as a model for the human, noting that rats develop fibrosis at comparable lung burdens, in fibers per gram of dry lung, to those that are associated with fibrosis in humans. The review suggested that, on a weight-of-evidence basis, there is no reason to conclude that humans are more sensitive to fibers than rats with respect to the development of lung cancer, and that rat inhalation studies may be more sensitive than human data in detecting carcinogenic potential of SVFs [Maxim and McConnell 2001]. However, others suggest that, because inhaled particles frequently sequester in the interstitial compartment of humans, alveolar clearance is approximately 1 order of magnitude faster in rats than humans [Snipes 1996; Tran and Buchanan 2000]. Those comparisons imply that results of inhalation studies with rats exposed to particles underestimate the risk for humans and that adjustment of kinetic differences in particle clearance and retention in rats is required to predict lung disease risks in humans [Kuempel et al. 2001].

How the results of *in vitro* tests which use cells or organ cultures apply to humans has been questioned because of differences in cell types and species-specific responses. It is difficult to isolate and maintain epithelial or mesothelial cells which are used as models for effects in the lung. Interpretation of results may be limited since *in vitro* models may

not consider all conditions and processes, such as clearance or surface conditioning, which are present *in vivo*. A major deficiency of *in vitro* systems is that fiber biopersistence is not easily addressed. And experimental design must consider exposure metrics of fiber mass, fiber number, or fiber surface area [Mossman 2007; Wylie et al. 1997].

Early animal inhalation studies found that chrysotile induced fibrosis, hyperplasia of lung epithelial cells, and carcinomas in mice [Nordman and Sorge 1941] and tumors in rats [Gross et al. 1967]. Another study found lung carcinomas and mesotheliomas in rat inhalation exposures to asbestos fiber samples of amosite, anthophyllite, crocidolite, and chrysotile [Wagner et al. 1974]. The effects of fiber length, width, and aspect ratio on carcinogenicity were addressed in a seminal study using a pleural surface implantation method of challenge in the rat [Stanton et al. 1977; 1981]. Tests were performed on 72 durable EPs: 13 crocidolite; 22 glasses; 8 aluminum oxide sapphire whiskers; 7 talcs; 7 dawsonites; 4 wollastonites; 2 asbestos tremolites; an amosite; 2 attapulgites; 2 halloysites; a silicon carbide whisker; and 3 titanates. The incidence of malignant mesenchymal neoplasms a year after implantation correlated best with EPs that were longer than 8 μm and no wider than 0.25 μm, with relatively high correlations with EPs longer than 4 μm and no wider than 1.5 μm. This suggested that carcinogenicity of durable EPs depends on dimension and durability rather than physicochemical properties. This is sometimes referred to as the "Stanton hypothesis" and has been the subject of continuing research. Reanalysis of the dimensions of seven of the crocidolite samples used in the 1981 study found that tumor probability was significantly correlated with the number of index particles (defined as particles longer than 8 μm and no wider than 0.25 μm), but the coefficient was low enough to suggest that factors other than size and shape play a role in carcinogenicity induced by durable EPs [Wylie et al. 1987]. Further analysis confirmed the number of such index particles as the primary dimensional predictor of tumor incidence, but the correlation was increased when the data were analyzed by separate mineral types [Oehlert 1991]. These analyses suggested that mineral type is important, which is counter to the "Stanton hypothesis".

Data from animal models exposed by instillation or inhalation to EMPs of defined size distributions have been reviewed, along with human lung fiber burden data and associated effects, to conclude that: (1) asbestosis was most closely associated with the surface area of retained EMPs; (2) mesothelioma was most closely associated with numbers of EMPs longer than about 5 μm and thinner than about 0.1 μm; and (3) lung cancer was most closely associated with EMPs longer than about 10 μm and thicker than about 0.15 μm [Lippmann 1988]. A more recent review of the response to asbestos fibers of various lengths in animal models, along with data from studies of human materials, concluded that asbestos fibers of all lengths induce pathological responses, and suggested caution when attempting to exclude any population of inhaled asbestos fibers, based on their length, from being contributors to the potential for development of asbestos-related diseases [Dodson et al. 2003].

A first question in seeking a full understanding of EMP properties and mechanisms responsible for fibrosis, cancer, or mesothelioma risks is the identity of initiating toxic interactions and the EMP morphological, physical, or chemical properties controlling them. Among proposed initiating mechanisms are: (1) EMP surfaces generate ROS (even *in vitro* in the absence of cells) which are the primary toxicants to cells; (2) EMP surfaces are directly membranolytic or otherwise directly cytotoxic or genotoxic to components of the cell, as are some non-elongated mineral particles, and that damage can cause necrosis, apoptosis, mutation, or transformation directly or by responsive cellular production of secondary reactive intermediates; and (3) EMP morphology itself results in "frustrated phagocytosis" with an anomalous stimulation or release of ROS or other toxic reactive species.

Asbestos fibers can generate reactive oxygen or nitrogen species in cell-free systems through direct aqueous-phase surface chemical reactions, as well as by stimulating secondary release of reactive species from cells. Electron spin resonance using spin trapping techniques found that crocidolite, chrysotile, and amosite asbestos fibers were all able to catalyze the generation of toxic hydroxyl radicals from hydrogen peroxide, a normal byproduct of tissue metabolism,and that the iron chelator desferroxamine inhibited the reaction, indicating a major role for iron in the catalytic process [Weitzman and Graceffa 1984]. ROS generated by some EMP surfaces in cell-free media may provide toxicants to initiate cell structural or functional damage, including chromosomal or DNA genetic damage or aneuploidy from spindle apparatus damage. They also may activate cellular signaling pathways that promote cell proliferation or transformation. Research has investigated the possible roles of iron in this reactivity and the roles of released- versus surface-borne iron.

Asbestos fibers can cause lipid peroxidation in mammalian cells and artificial membranes that can be prevented by removal of catalytic iron. Reduction of crocidolite asbestos cytotoxicity by certain antioxidants (including superoxide dismutase (SOD), a scavenger of superoxide (O2-.); catalase, a scavenger of H2O2; dimethylthiourea (DMTU), a scavenger of the hydroxyl radical (.OH); and desferroxamine, an iron chelator)) suggested that iron is involved in the generation of ROS through a modified Haber-Weiss Fenton-type reaction resulting in the production of hydroxyl radical (e.g., from superoxide anion and hydrogen peroxide generated during phagocytosis) [Goodglick and Kane 1986; Shatos et al. 1987]. Such scavenging or chelation prevented DNA strand breakage in cells *in vitro* by crocidolite [Mossman and Marsh 1989].

In a cell-free study of five natural and two synthetic fibers, erionite, JM code 100 glass fibers, and glass wool were the most effective initiators of hydroxyl radical formation, followed by crocidolite, amosite, and chrysotile fibers. Hydroxyl radical formation activity showed positive correlations with the tumor rates in rats challenged by intrapleural injection and with the human mesothelioma mortality rates, but not with the tumor rates in rats challenged by intraperitoneal injection [Maples and Johnson 1992]. SO-produced ROS then might induce DNA oxidative damage as determined by elevated

8-hydroxydeoxyguanosine (8-OHdG). In cell-free systems, crocidolite-induced increase of 8-OHdG in isolated DNA was enhanced by addition of H_2O_2 and diminished by addition of desferroxamine [Faux et al. 1994]. However, de-ironized crocidolite fibers incubated in a cell-free system induced twice the 8-OHdG oxidative damage to DNA as untreated crocidolite fibers. In parallel rat exposures, the combination of de-ironized crocidolite fibers plus Fe_2O_3 resulted in mesothelioma in all animals compared to half the animals injected with crocidolite fibers alone and none of the animals injected with Fe_2O_3 alone [Adachi et al. 1994]. Other research suggested that unreleased fiber-surface-bound iron is important to the reactivity: long fibers of amosite and crocidolite both caused significant dose-dependent free radical damage to cell-free phage DNA, supressible by the hydroxyl radical scavenger mannitol and by desferroxamine, but short RCFs and man-made vitreous fibers (MMVFs) did not, while releasing large quantities of Fe(III) iron [Gilmour et al. 1995]. Crocidolite fibers induced mutations in peritoneal tissue *in vivo* in rats, most prominently guanine-to-thymine (G to T) transversions known to be induced by 8-OHdG; this was interpreted as strong evidence for the involvement of reactive oxygen or nitrogen species in crocidolite-induced mutagenesis *in vivo*, consistent with *in vitro* and cell-freestudies [Unfried et al. 2002]. In contrast to glass fiber, crocidolite fiber intratracheal instillation in the rat, increased 8-OHdG levels in DNA at one day and in its repair enzyme activity at seven days. This *in vivo* activity is consistent with asbestos- and MMVF-induced increases of 8-OHdG oxidative damage *in vitro* [Yamaguchi et al. 1999].

Many mineral particles, elongated or not, can directly cause membranolysis or other cytotoxic responses without necessarily invoking extracellular generation of ROS. Mechanisms of cell damage by EMPs independent of reactive oxygen formation have been proposed to involve direct interactions of interactions of particle surface functional groups (e.g., silicon or aluminum or magnesium) with lipoproteins or glycoproteins of the cell membrane. It has been suggested that silica particle cytotoxicity to macrophages is due to distortion and disruption of secondary lysosomal membranes by phagocytosed particles whose surface silanol groups hydrogen bond to membrane lipid phosphates, but that chrysotile-induced cellular release of hydrolytic enzymes is due to surface magnesium interacting ionically with sialic acid residues of membrane glycoproteins, inducing cation leakage and osmotic lysis [Allison and Ferluga 1977]. Chrysotile fibers caused lysis of red blood cells. Electron microscopy indicated that cell membranes become wrapped around the fibers and that cell distortion and membrane deformation correlate with an increase in the intracellular ratio of sodium to potassium ions. Cell pretreatment with neuraminidase prevents the fiber-cell binding, suggesting mediation by cell membrane glycoproteins [Brody and Hill 1983]. However, chrysotile and crocidolite fibers both induced increased membrane rigidity in model unilamellar vesicles made of saturated dipalmitoyl phosphatidylcholine (DPPC), suggesting that lipid peroxidation is not involved in membrane rigidity induced by asbestos [Gendek and Brody 1990]. Silicate slate dust and chrysotile fibers both induced hemolysis of erythrocytes *in vitro* and peroxidation of polyunsaturated membrane lipids. However, poly(2-vinylpyridine N-oxide) (PVPNO) and DPPC surface prophylactic agents suppressed lysis but not

peroxidation, while SOD and catalase did the reverse; and lysis was much faster than peroxidation. This suggested that membrane lysis and peroxidation are independent processes [Sing and Rahman 1987]. However, either mechanism may be involved in membrane damage by EMPs; and seemingly disparate findings suggest uncharacterized details of EMP properties or of cellular or mineral conditioning under test conditions may be controlling.

As frequently performed, *in vitro* assays of mineral particle-induced damage, measured by cell death or cytosolic or lysosomal enzyme release, do not adequately model or predict the results of *in vivo* challenge or epidemiological findings. For example, respirable aluminosilicate clay dust is as cytotoxic as quartz dust in such *in vitro* assays, while quartz, but not clay, is strongly fibrogenic *in vivo* [Vallyathan et al. 1988]. Both quartz dust and chrysotile fibers induced loss of viability and release of lactate dehydrogenase (LDH) from alveolar macrophages *in vitro*. Diacyl phosphatidylcholine reduced these activities of the quartz but not of the asbestos [Schimmelpfeng et al. 1992]. DPPC is adsorbed from aqueous dispersion in approximately equal amounts on a surface area basis, about 5 mg phospholipid per square meter, by asbestos fibers [Jaurand et al. 1980] and by non-fibrous silicate particles [Wallace et al. 1992]; this is close to the value predicted by mathematical modeling of an adsorbed bilayer [Nagle 1993]. In the case of silica or clay membranolytic dusts, this adsorption fully suppresses their activity until toxicity is manifest as the prophylactic surfactant is digested from the particle surface by lysosomal phospholipase enzyme, with mineral-specific rates of the process suggesting a basis for differing fibrogenic potentials of different types of mineral particles [Wallace et al. 1992].

Samples of intermediate-length and short-length NIEHS chrysotile were compared, with and without DPPC lung surfactant pre-treatment, for micronucleus induction in Chinese hamster lung V79 cells *in vitro*. Increase in micronuclei frequency and multi-nuclear cell frequency were induced by all samples, with the greatest micronucleus induction by untreated intermediate-length chrysotile fibers and with greater activity for untreated versus treated short chrysotile fibers. Cell viability was greater for treated fibers [Lu et al. 1994]. NIEHS intermediate-length chrysotile was mildly acid treated to deplete surface-borne magnesium while only slightly affecting fiber length. Challenge of Chinese hamster lung fibroblast cells *in vitro* for micronucleus induction found no significant difference between the treated and untreated samples, supporting a model of chemically non-specific chromosomal and spindle damage effects [Keane et al. 1999]. Chrysotile asbestos induction of mucin secretion in a tracheal cell culture was inhibited by using lectins to block specific carbohydrate residues on the cell surface; and leached chrysotile was inactive, suggesting that the surface cationic magnesium of chrysotile was responsible for the surface interaction with cell surface glycolipids and glycoproteins [Mossman et al. 1983]. However, complete removal of accessible sialic acid residues from erythrocytes did not inhibit hemolysis by chrysotile asbestos, suggesting that chrysotile asbestos can induce lysis by interaction with some other component of the cell [Pele and Calvert 1983].

A third possible mechanism for damage by EMP is involved principally with morphology. The possibility of "frustrated phagocytosis" is suggested by the Stanton hypothesis of an over-riding significance of particle dimension for disease induction by durable EPs. A general concept is that EMPs longer than a phagocytic cell's linear dimensions can not be completely incorporated in a phagosome. Recruitment of membrane from the Golgi apparatus or endoplasmic reticulum may provide extensive addition to the plasma membrane for a cell's attempted invagination to accommodate a long EMP in a phagosomal membrane [Aderem 2002]. However, because of the length of the EMP relative to the dimensions of the cell, the final phagosomal structure is topologically an annulus extending fully through the cell, rather than an enclosed vacuole fully within the cell. Following uptake of non-elongated particles, there is a maturation of the phagosomal membrane: the initial phagosomal membrane is that of the cell's external plasma lemma, which cannot kill or digest phagocytosed material. After sealing of the fully invaginated phagosomal vesicle in the interior of the cell, there is a rapid and extensive change in the membrane composition [Scott et al. 2003]. This involves, in part, an association with lysosomal vesicles and exposure of particles within the secondary phagosome or phagolysosome to lytic enzymes and adjusted pH conditions. Failure to close the phagosome, as occurs in frustrated phagocytosis, is speculated to induce dysfunction of the system. Conventional phagocytosis of non-elongated particles can lead to a respiratory or oxidative burst of membrane-localized NADPH oxidase of superoxide radicals, which may be converted to hydrogen peroxide, hydroxyl radicals, and other toxic reactive products of oxygen. If these are released extracellularly in connection with frustrated phagocytosis, they are potentially harmful to the tissue [Bergstrand 1990].

Failure to complete normal phagocytosis may affect the duration or intensity of the phagocytic response. It may also affect the generation or release of reactive species or membranolytic digestive enzymes into the still exterior annulus. Another possible affect is to alter the maturation of the annular frustrated phagocytic membrane from the normal structural and functional evolution of a closed phagolysosomal vesicle fully interior to the cell. Even in the response to such a frustrated phagocytosis, there might be some mineral specificity beyond morphology alone for EMP-induced release of reactive species. Amosite fibers, MMVF, silicon carbide fibers, and RCF-1 fibers all stimulated modest release of superoxide which was not dose-dependent in isolated rat alveolar macrophages. However, when IgG, a normal component of lung lining fluid, was adsorbed onto the fiber surfaces, such release was strongly enhanced for all but the silicon carbide fibers. Superoxide release correlated with adsorptive capacity for IgG of the fibers, except for the amosite which required only poorly adsorbed IgG for strong activity, suggesting some mineral specificity beyond morphology alone for the EMP-induced cellular respiratory burst [Hill et al. 1996].

Subsequent to initiating damage, either by direct or induced ROS generation, or by direct membranolysis generated by interactions of mineral surface sites with membrane lipids

or glycoproteins, or by not-fully-defined toxic response to morphology-based frustrated phagocytosis, a standard model for subsequent complex cellular response has evolved and has been the subject of extensive and detailed analyses [Mossman et al. 1997]. EMP-generated primary toxic stimuli to the cell are subject to signal transduction by mitogen-activated protein kinase (MAPK), beginning an intracellular multiple kinase signal cascade which then induces transcription factors in the nucleus such as activator protein (AP)-1or nuclear factor kappa beta (NF-κB), which in turn regulate the transcription of mRNA from genes for TNF-α or other cytokines involved in cell proliferation or inflammation.

Fibers of the six asbestos minerals generate MAPK in lung epithelium *in vitro* and *in vivo*, increasing AP-1 transcription activation, cell proliferation, death, differentiation, or inflammation. This is synergistic with cigarette smoke [Mossman et al. 2006]. Macrophage release of oxidants or mitogenic factors through such a pathway could then cause cell proliferation or DNA damage [Driscoll et al.1998]. In contrast to MMVF-10 and RCF-4, amosite and two other carcinogenic fibers (silicon carbide and RCF-1) produced significant dose-dependent translocation of NF-κB to the nucleus in A549 lung epithelial cells. It was hypothesized that carcinogenic fibers have greater free radical activity, which produces greater oxidative stress and results in greater translocation of NF-κB to the nucleus for the transcription of pro-inflammatory genes (e.g., cytokines) [Brown et al. 1999]. Crocidolite induced AP-1 *in vitro* in JB6 cells and induced AP-1 transactivation in pulmonary and bronchial tissue after intratracheal instillation in transgenic mice, apparently mediated by activation of MAPK [Ding et al. 1999]. Chrysotile challenge to blood monocytes co-cultured with bronchial epithelial cells resulted in elevated levels in epithelial cells of protein-tyrosine kinase activity, NF-κB activity, and mRNA levels for IL-1β, IL-6, and TNF-α. Protein-tyrosine kinase activity, NF-κB activity, and mRNA synthesis were inhibited by antioxidants, suggesting ROS-dependent NF-κB-mediated transcription of inflammatory cytokines in bronchial epithelial cells [Drumm et al. 1999].

Chemokines known to be associated with particle-induced inflammation were found to be secreted by mesothelial cells after amosite challenge to cultured rat pleural mesothelial cells, and were found in pleural lavage of rats challenged *in vivo* [Hill et al. 2003].

Fibers from both crocidolite (asbestiform riebeckite) and nonfibrous milled riebeckite increased phosphorylation and activity of a MAPK cascade in association with induction of an inflammatory state of rat pleural mesothelial cells and progenitor cells of malignant mesothelioma. Amelioration by pre-incubation with vitamin E indicated this to be an oxidative stress effect [Swain et al. 2004]. Lung lysate, cells from bronchoalveolar lavage, and alveolar macrophages and bronchiolar epithelial cells from lung sections from rats exposed to crocidolite or chrysotile fibers contained nitrotyrosine and phosphorylated extracellular signal-regulated kinases (ERKs); nitrotyrosine is a marker for peroxynitrile which activates ERK signaling pathways, altering protein function [Iwagaki 2003]. *In vitro* challenge of human bronchiolar epithelial cells with crocidolite

or chrysotile fibers induced tissue factor (TF) mRNA expression and induced NF-κB and other transcription factors that bind the TF gene promoter. TF *in vivo* is involved in blood coagulation with inflammation and tissue remodeling [Iakhiaev et al. 2004]. Asbestos fibers activate an ERK pathway *in vitro* in mesothelial and epithelial cells. Crocidolite challenge to mice results in phosphorylation of ERK in bronchiolar and alveolar II epithelial cells, epithelial cell hyperplasia, and fibrotic lesions. Epithelial cell signals through the ERK pathway lead to tissue remodeling and fibrosis [Cummins et al. 2003].

Crocidolite and erionite fibers, but not milled riebeckite, up-regulated expression of epidermal growth factor receptor (EGFR) in rat pleural mesothelial cells *in vitro*. Cell proliferation was co-localized subsequent to EGFR, suggesting initiation of a cell-signaling cascade to cell proliferation and cancer [Faux et al. 2000]. "Long" amosite fibers were more active than "short" amosite fibers in causing: (1) damage to nude DNA; (2) *in vitro* cytotoxicity in a human lung epithelial cell line; (3) free radical reactions; (4) inhibition of glycerol-6-phosphate dehydrogenase and pentose phosphate pathways; (5) decrease in intracellular reduced glutathione; (6) increase in thiobarbituric acid reaction substances; and (7) leaking of LDH [Riganti et al. 2003].

An important paradox or seeming failure of *in vitro* studies concerns mesothelioma. While chrysotile or amphibole asbestos fibers clearly induce malignant mesothelioma *in vivo*, they do not transform primary human mesothelial cells *in vitro*, while erionite fibers do. Asbestos fibers can induce some genotoxic changes: crocidolite fibers induced cytogenotoxic effects, including increased polynucleated cells and formation of 8-OHdG in a phagocytic human mesothelial cell line, but did not induce cytogenotoxic effects in a non-phagocytic human promyelocytic leukemia cell line [Takeuchi et al. 1999]. Tremolite, erionite, RCF-1, and chrysotile fiber challenges of human-hamster hybrid A(L) cells found chrysotile fibers to be significantly more cytotoxic. Mutagenicity was not seen at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus for any of the fibers. Erionite and tremolite fibers induced dose-dependent mutations at the gene marker on the only human chromosome in the hybrid cell. Erionite was the most mutagenic type of fiber. RFC-1 fibers were not mutagenic, in seeming contrast to their known induction of mesothelioma in hamsters [Okayasu et al. 1999]. Crocidolite fibers induced significant but reversible DNA single-strand breaks in transformed human pleural mesothelial cells; TNF-α induced marginal increases; co-exposure to crocidolite fibers and TNF-α caused greater damage than fibers alone. Antioxidant enzymes did not reduce the DNA damage, suggesting a mechanism of damage other than by free radicals [Ollikainen et al. 1999]. Crocidolite fibers were also very cytotoxic to the cells; presumably cell death may prevent the observation of cell transformation. *In vitro* challenge to mesothelial cells and to fibroblast cells by crocidolite fibers, but not by glass wool SVF, induced dose-dependent cytotoxicity and increased DNA synthesis activity [Cardinali et al. 2006]. Crocidolite fibers were found to induce TNF-α secretion and receptors in human mesothelial cells; and TNF-α reduced cytotoxicity of crocidolite fibers by activating NF-κB and improving cell survival and permitting expression of cytogenetic activity [Yang et al. 2006]. Erionite fibers transformed immortalized non-

tumorigenic human mesothelial cells *in vitro* only when exposed in combination with IL-1β or TNF-α [Wang et al. 2004]. Erionite fibers were poorly cytotoxic but induced proliferation signals and high growth rate in hamster mesothelial cells. Long-term exposure to erionite fibers resulted in transformation of human mesothelial cells *in vitro* but exposure to asbestos fibers did not transform those cells [Bertino et al. 2007]. *In vitro* challenge of mesothelial cells to asbestos fibers induced cytotoxicity and apoptosis, but not transformation. *In vitro* challenge of human mesothelial cells to asbestos fibers induced the ferritin heavy chain of iron-binding protein, an anti-apoptotic protein, with decrease in hydrogen peroxide ROS and resistance to apoptosis [Aung et al. 2007]. This was seen also in a human malignant mesothelial cell line.

The question of a co-carcinogenic effect of asbestos fibers with a virus has been raised. Most malignant mesotheliomas are associated with asbestos exposures; but only a fraction of those exposed develop mesothelioma, indicating that other factors may play a role. It has been suggested that simian virus 40 (SV40) and asbestos fibers may be co-carcinogens. SV40 is a DNA tumor virus that causes mesothelioma in hamsters and has been detected in several human mesotheliomas. Asbestos fibers appear to increase SV40–mediated transformation of human mesothelial cells *in vitro* [Carbone et al. 2002]. In an *in vivo* demonstration of co-carcinogenicity of SV40 and asbestos fibers, mice containing high copy number of SV40 viral oncogene rapidly developed fast-growing mesothelioma following asbestos challenge. Transgenic copy number was proportional to cell survival and *in vitro* proliferation [Robinson et al. 2006].

Studies comparing EMPs from asbestiform versus nonasbestiform habit of an amphibole

Smith et al. [1979] compared tumor induction by IP injection in hamsters by two asbestiform tremolites, two nonasbestiform prismatic tremolitic talcs, and one tremolitic talc of uncertain asbestiform status. No tumors were observed following the non-asbestiform tremolite challenge, in contrast to the asbestiform tremolites. However, tumors were observed from the tremolitic talc of uncertain amphibole status.

In rule-making, OSHA [1992] noted the small number of animals in the study, the early death of many animals, and the lack of systematic characterization of fiber size and aspect ratio. Subsequent analyses performed on the nonasbestiform tremolitic (by chemical composition) talc from the study which was not associated with mesothelioma, found 13% fibers as defined by a 3:1 aspect ratio [Wylie et al. 1993]. A prismatic nonasbestiform tremolitic talc and an asbestiform tremolite from the study were analyzed for aspect ratio [Campbell et al. 1979]. They analyzed 200 particles of the asbestiform tremolite sample and found 17% had an aspect ratio of 3:1 or greater, and 9.5% had an aspect ratio greater than 10:1. Analysis of 200 particles of the prismatic tremolite found 2.5% had an aspect ratio of 3:1 or greater, and 0.5% (one particle) had an aspect ratio greater than10:1.

Wagner et al. [1982] challenged rats by IP injection using a tremolite asbestos, a prismatic non-asbestiform tremolite, or a tremolitic talc considered non-asbestiform

containing a limited number of long fibers. Only the tremolite asbestos produced tumors, and mesothelioma was found in 14/37 animals. On a per microgram of injected dose basis, the asbestiform sample contained 3.3 x 10^4 non-fibrous particles, 15.5 x 10^4 fibers, and 56.1 x 10^3 fibers >8 micrometers long and <1.5 micrometers wide. The prismatic amphibole corresponding values were 20.7 x 10^4, 4.8 x 10^4, and 0. Tremolitic talc values were 6.9 x 10^4, 5.1 x 10^4, and 1.7 x 10^3. Infection-reduced survival prevented evaluation of a crocidolite positive control.

Another IP injection study with the rat used six samples of tremolite of different morphological types [Davis et al. 1991]. For three asbestiform samples, mesothelioma occurred in 100%, 97%, and 97% of the animals, at corresponding injected doses of 13.4 x 10^9 fibers / 121 x 10^6 fibers with length >8 µm and diameter <0.25 µm; 2.1 x 10^9 / 8 x 10^6; and 7.8 x 10^9 / 48 x 10^6, respectively. For an Italian tremolite from a non-asbestos source and containing relatively few asbestiform fibers (1.0 x 10^9 / 1 x 10^6), mesothelioma was found in two-thirds of the animals, with delayed expression. For two nonasbestiform tremolites (0.9 x 10^9 / 0; 0.4 x 10^9 / 0), tumors were found in 12% or 5% of the animals; at least the former was above expected background levels. The Italian sample resulting in 67% mesothelioma contained only one-third the number of EMP's >8 µm long compared to the nonasbestiform sample associated with 12% mesothelioma; and those two samples contained an approximately equal number of fibers with length >8 µm and width <0.5 µm. The preparation of the three asbestiform samples and the Italian sample were essentially identical; however, the two nonasbestiform samples associated with low mesothelioma required significantly different pre-treatment, the first requiring multiple washing and sedimentation and the second grinding under water in a micronizing mill. It was noted that those two nonasbestiform samples and the Italian sample contained minor components of long thin asbestiform tremolite fibers. This study suggested that carcinogenicity may not depend simply on the number of EMPs and called for methods of distinguishing "carcinogenic tremolite fibers" from non-fibrous tremolite dusts that contain similar numbers of EMPs of similar aspect ratios [Davis et al. 1991]. It has been suggested that the response reported for the Italian nonasbestiform tremolite is of a pattern expected for a low dose of highly carcinogenic asbestos tremolite [Addison 2007].

A recent review of past studies of varieties of tremolite and the limitations of earlier studies (e.g., their use of injection or implantation versus inhalation) suggested that, based on observed differences in the carcinogenicity of tremolite asbestos and nonasbestiform prismatic tremolite, differences in carcinogenicity of amphibole asbestos fibers and nonasbestiform amphibole cleavage fragments are sufficiently large to be discernable even with the study limitations, and that there is evidence of a lower hazard associated with the shorter, thicker cleavage fragments of the non-asbestos amphiboles in comparison with the thinner asbestos fibers [Addison and McConnell 2007].

In summary, several types of animal studies have been conducted to assess the carcinogenicity and fibrogenicity of asbestiform and nonasbestiform tremolite fibers and

other EMPs. Tremolite asbestos was found to be both fibrogenic and carcinogenic in rats by inhalation. However, the data for other particle forms of tremolite and for other amphiboles in general is much more limited, and is based primarily on mesotheliomas produced by intrapleural administration studies in rats. These studies bypass the lung entirely, and thus provide no information on the test material's potential for causing lung tumors. In addition, they have often been criticized for employing a non-physiological route of administration. Some of the older studies [Smith et al. 1979, Wagner et al. 1982] are difficult to interpret due to inadequate characterization of the tremolite preparation that was used, although the studies do tend to show fewer tumors from prismatic tremolite than from asbestiform tremolite. Unfortunately, doses used in most animal studies are generally reported in terms of mass (e.g., 10, 25, or 40 mg/rat). Unless the test preparations are well characterized in terms of fiber counts and fiber size distributions it is difficult to relate the mass-based dose in the animals to the fiber count measurement used to assess human occupational exposures. Where semi-quantitative fiber count and size distribution data are given, as in the Davis et al. [1991] study, it is evident that the prismatic tremolite samples contain fewer countable fibers per 10mg dose than the asbestiform tremolite samples. Although the prismatic tremolite samples clearly generated fewer mesotheliomas than the asbestiform tremolite samples, it is not apparent whether the turmorigenic potency per fiber is lower for the nonasbestiform tremolites.

Cellular *in vitro* assays used LDH release, beta-glucuronidase release, cytotoxicity, and giant cell formation to compare two non-asbestiform and one asbestiform tremolites, finding relative toxicities parallel to the differences seen in an *in vivo* rat IP injection study of tumorigenicity using the same samples [Wagner et al. 1982]. *In vitro* cellular or organ tissue culture studies showed squamous metaplasia and increased DNA synthesis in tracheal explant cultures treated with long glass fibers or with crocidolite or chrysotile fibers, while cleavage fragments from their nonasbestiform analogues, riebeckite and antigorite, were not active [Woodworth et al. 1983]. For alveolar macrophages *in vitro*, crocidolite fibers induced the release of ROS an order of magnitude greater than cleavage fragments from nonasbestiform riebeckite [Hansen and Mossman 1987]. Similar differences were observed in hamster tracheal cells for:

- induction of ornithine decarboxylase, an enzyme associated with mouse skin cell proliferation and tumor promotion [Marsh and Mossman 1988];
- stimulating survival or proliferation in a colony-forming assay using those hamster tracheal epithelial cells [Sesko and Mossman 1989];
- activation of proto-oncogenes in tracheal as well as epithelial and mesothelial cells *in vitro* [Janssen et al. 1994]; and
- cytotoxicity [Mossman and Sesko 1990].

A recent review concludes that a large body of work shows that asbestos fibers have been most active in a number of *in vitro* bioassays comparing activities of a variety of asbestos fibers and other nonpathogenic fibers or particles, while cleavage fragments of amphiboles are less potent than asbestos fibers [Mossman 2007].

These are a fraction of the extensive number of studies that have provided detailed information on some of the biomolecular mechanisms induced in cells by EMP exposure, suggesting some bases underlying applied questions of relative toxicities and pathogenicities of asbestiform and nonasbestiform minerals. Seemingly contradictory implications of some of the different experiments suggest that new methods for preparation and characterization of EMPs may be needed. Also, careful attempts to identify *in vitro* and *in vivo* conditions which may unexpectedly influence the initiation or promotion of cell damage and progression to disease may aid the further elucidation of EMP properties and mechanisms and conditions of exposure determining disease risk.

There is a limited number of animal model *in vivo* studies of nonasbestiform amphibole dusts. To date this research has found generally significant differences between nonasbestiform and asbestiform amphibole pathogenicity. Within the studies there are few findings of biological effects or tumorigenicity induced by samples classified as nonasbestiform; and there are rational hypotheses as to the cause of those positive activities. There are general fundamental uncertainties of dust properties and biological mechanisms controlling for mineral dust toxicities and pathogenicities, and specifically in the similarities or differences in disease mechanisms between the asbestiform and nonasbestiform amphibole dusts. *In vitro* studies have generally found differences in specific toxic activities between some asbestiform and nonasbestiform amphibole, albeit that *in vitro* systems are not yet able to predict relative pathogenic risk for mineral fibers and other EMPs. This suggests a focus of research to identify if and when nonasbestiform amphibole dusts are active for tumorigenicity or other pathology, if there is a threshold for those activities, and if distinguishing conditions or properties for such pathogenicity can be found.

Research needs include the selection and storing of nonasbestiform amphibole samples and the selection of parallel asbestiform samples of the same mineral. This involves subsidiary questions of which properties to match and if such matches can be made (e.g., cleavage fragment dimensions versus fiber dimensions). To accomplish this research exhaustive characterization of the samples including contaminants is necessary. Detailed characterization of particles characteristics that may affect biological activities (e.g., surface composition, durability, morphology, and surface properties) are needed under conditions of incubation in pulmonary extracellular and intracellular media so they model *in vivo* conditions. This research focus would conform with the general strategies and tactics that have been recommended by several expert panels for clarifying the risks and causes of asbestos exposure-associated diseases, and with the current effort of the U.S. Federal Government Interagency Asbestos Working Group (IAWG), with the participation of the EPA, USGS, NIOSH, ATSDR, CPSC, OSHA, MSHA, and the NIEHS/NTP, to identify Federal research needs and possible actions regarding asbestos fibers and other durable EMPs of public health concerns [Vu et al. 1996; ILSI 2005; Schins 2002; Greim 2004; Mossman et al. 2007].

1.6.5 Thresholds

In the literature, discussions of thresholds for adverse health effects associated with exposure to asbestos fibers and related EMPs have focused on the characteristics of dimension, including length, width, and the derived aspect ratio, as well as concentration. Although other particle characteristics discussed above may impact these thresholds, or may have thresholds of their own that impact the toxicity of EMPs, they are not well represented or discussed in the literature. This discussion is focused on thresholds for dimension and concentration.

The seminal work of Stanton et al. [1981] has laid the foundation for much of the information on dimensional thresholds. Their analyses found that malignant neoplasms in exposed rats were best predicted by the number of EMPs longer than 8 μm and thinner than 0.25 μm. However, the number of EMPs in other size categories having lengths greater than 4 μm and widths up to 1.5 μm were also highly correlated with malignant neoplasms. Lippmann [1988; 1990] reviewed the literature and suggested that lung cancer is most closely associated with asbestos fibers longer than 10 μm with widths greater than 0.15 μm, while mesothelioma is most closely associated with asbestos fibers longer than 5 μm with widths less than 0.1 μm. Evidence from animal studies and some *in vitro* studies suggests that short asbestos fibers (e.g., <5 μm long) may play a role in fibrosis, but are of lesser concern than longer asbestos fibers for cancer development.

Berman et al. [1995] statistically analyzed aggregate data from 13 inhalation studies in which rats were exposed to 9 types of asbestos (4 chrysotiles, 3 amosites, a crocidolite, and a tremolite asbestos) to assess fiber dimension and mineralogy as predictors of lung tumor and mesothelioma risks. Archived samples from the studies were reanalyzed to provide detailed information on each asbestos structure, including mineralogy (i.e., chrysotile, amosite, crocidolite, or tremolite), size (i.e., length and width, each in 5 categories), type (i.e., fiber, bundle, cluster, or matrix), and complexity (i.e., number of identifiable components of a cluster or matrix). Multiple concentrations (each for asbestos structures with different specified characteristics) were calculated for the experimental exposures. While no univariate index of exposure adequately described lung tumor incidence observed across all inhalation studies, certain multivariate indices of exposure did adequately describe outcomes. Fibers and bundles longer than 5 μm and thinner than 0.4 μm contributed to lung tumor risk; very long (≥40 μm) and very thick (≥5 μm) complex clusters and matrices possibly contributed. While structures <5 μm long did not contribute to lung tumor risk, potency of thin (<0.4 μm) structures increased with increasing length above 5 μm and structures ≥40 μm long were estimated to be about 500 times more potent than structures between 5 and 40 μm long, With respect to lung tumor risk, there was no difference between chrysotile and amphibole asbestos. With respect to mesothelioma risk, chrysotile was found to be less potent than amphibole asbestos. While the Berman et al. [1995] analysis was limited to studies of asbestos

exposure, similar statistical approaches may be adaptable to assess outcomes from exposure to a broader range of EMPs beyond asbestos.

In addressing the issue of a length threshold, the Health Effects Institute [HEI 1991] concluded that asbestos fibers less than 5 μm in length appear to have much less carcinogenic activity than longer fibers and may be relatively inactive. A panel convened by the ATSDR [2003] concluded that "given findings from epidemiologic studies, laboratory animal studies, and *in vitro* genotoxicity studies, combined with the lung's ability to clear short fibers, the panelists agreed that there is a strong weight of evidence that asbestos and SVFs shorter than 5 μm are unlikely to cause cancer in humans." Also, an EPA [2003] peer consultant panel "agreed that the available data suggest that the risk for fibers less than 5 μm in length is very low and could be zero." They also generally agreed that the width cut-off should be between 0.5 and 1.5 μm, but deserved further analysis.

However, Dodson et al. [2003] have argued that it is difficult to rule out the involvement of short (<5 μm) asbestos fibers in causing disease because exposures to asbestos fibers are overwhelmingly composed of fibers less than 5 μm long and fibers observed in the lung and in extrapulmonary locations are also overwhelmingly less than 5 μm long. For example, in a study of malignant mesothelioma cases, Suzuki and Yuen [2002] found that the majority of asbestos fibers in lung and mesothelial tissues were shorter than 5 μm in length.

NIOSH investigators have recently evaluated the relationship between the dimensions (i.e., length and width) of airborne chrysotile fibers and the risk for developing lung cancer or asbestosis by updating the cohort of chrysotile-exposed textile workers previously studied by Dement et al. [1994], Stayner et al. [1997], and Hein et al. [2007]. Archived airborne samples collected at this chrysotile textile plant were re-analyzed by TEM to generate exposure estimates based on bivariate fiber-size distribution [Dement et al. 2007]. TEM analysis of samples found all fiber size-specific categories (35 categories were assigned based on combinations of fiber width and length) to be highly statistically significant predictors of lung cancer and asbestosis [Stayner et al. 2007]. The smallest fiber size-specific category was thinner than 0.25 μm and equal to or shorter than 1.5 μm. The largest size-specific category was thicker than 3.0 μm and longer than 40 μm. Both lung cancer and asbestosis were most strongly associated with exposures to thin fibers (<0.25 μm), and longer fibers (>10 μm) were found to be the strongest predictors of lung cancer. A limitation of the study is that cumulative exposures were highly correlated across all fiber-size categories for the cohort which complicates the interpretation of the study results.

In addition to the discussion of appropriate thresholds for length and width, an important parameter used to define EMPs is the aspect ratio. The use of the 3:1 length:width aspect ratio as the minimum to define an EMP was not established on scientific bases such as toxicity or exposure potential. Rather it was a decision based on the ability of the

microscopist to determine the elongated nature of a particle [Holmes 1965], and the practice has been carried through to this day. As the bivariate analyses are conducted, attention needs to be paid to assessing the impact of aspect ratio, as well as length and width, on toxicity and health outcomes.

As discussed in Section 1.3.2, the nature of occupational exposures to asbestos has changed over the last several decades. Once dominated by chronic exposures in textile mills, friction product manufacturing, and cement pipe fabrication, current occupational exposures to asbestos in the U.S. are primarily occurring during maintenance activities or remediation of buildings containing asbestos. These current occupational exposure scenarios frequently involve short-term, intermittent exposures. The generally lower current exposures give added significance to the question of whether or not there is an asbestos exposure threshold below which workers would incur no risk of adverse health outcomes.

Risk assessments of workers occupationally exposed to asbestos were reviewed by investigators sponsored by the Health Effects Institute [1991]. They found that dose-specific risk is highly dependent on how the measurement of dose (exposure) was determined. A common problem with many of the epidemiologic studies of workers exposed to asbestos was the quality of the exposure data. Few studies have good historical exposure data and those data which were available are mostly area samples with concentrations reported as millions of particles per cubic foot of air (mppcf). Although correction factors were used to convert exposures measured in mppcf to f/cm^3, the conversions were often based on more recent exposure measurements collected at concentrations lower than those prevalent in earlier years. In addition, a single conversion factor was typically used to estimate exposures throughout a facility, which may not accurately represent differences in particle sizes and counts at different processes in the facility.

More recently, the concept of a concentration threshold has been reviewed by Hodgson and Darnton [2000]. It is generally accepted that lung fibrosis requires relatively heavy exposure to asbestos and that the carcinogenic response of the lung may be an extension of the same inflammatory processes that produce lung fibrosis. Some evidence for a threshold is provided by an analysis of a chrysotile-exposed cohort which suggests a potential threshold dose of about 30 f/mL-yr to produce radiologically evident fibrosis [Weill 1994]. Another study of necropsy material from textile workers exposed to chrysotile shows a distinct step increase in fibrosis for exposures in the 20-30 f/mL-yr range [Green et al. 1997]. However, a study of textile mill workers exposed to chrysotile did not find evidence for significant concentration thresholds for either asbestosis or lung cancer [Stayner et al. 1997]. Hodgson and Darnton [2000] pointed out that any evidence suggesting a threshold for chrysotile would likely not apply to amphibole asbestos because radiologically evident fibrosis has been documented among workers exposed to amphibole asbestos at low levels (<5 f/mL-yr). They concluded that if a concentration threshold exists for amphiboles, it is very low.

For mesothelioma, Hodgson and Darnton [2000] identified cohorts with high rates of mesothelioma at levels of exposure below those at which lung cancer has been identified; in some studies, the proportion of mesothelioma cases with no likely asbestos exposure is much higher than expected. Hodgson and Darnton [2000] concluded that these studies support a non-zero risk, even from brief, low-level exposures.

Animal studies using intraperitoneal and intrapleural injection of asbestos fibers cited by Ilgren and Browne [1991] suggest a possible threshold concentration for mesothelioma. However, it is not clear how this would be useful to determine a threshold for inhalation exposure in humans.

1.7 Analytical Methods

Analytical methods are available which are capable of characterizing the size, morphology, elemental composition, crystal structure, and surface composition of individual particles of "thoracic" size. There are two separate paradigms for selecting among these methods for their use or further development for application to EMPs: one is for their support of standardized surveys or compliance assessments of workplace exposures to EMPs; another is for their support of research to identify physicochemical properties of EMPs that are critical to predicting toxicity or pathogenic potential for lung fibrosis, cancer, or mesothelioma.

Cost, time, availability, standardization requirements, and other pragmatic factors limit the selection of analytical methods for standardized analysis of field samples for the first set of uses. Additionally, those uses require methods with an historic established association with disease risk. Principal among these analyses for standardized industrial hygiene use is an optical microscopy method: PCM, (e.g., the NIOSH 7400 method or equivalent) [NIOSH 1994a]. Under the current NIOSH REL for airborne asbestos fibers, particles are counted if they are EMPs of the covered minerals and they have a length greater than 5 μm when viewed microscopically using NIOSH Analytical Method #7400 or its equivalent.

Care should be taken in developing or applying new analytical methods to the analysis of asbestos for standardized and compliance assessments. The use of new or different analytical methods to assess exposures must be carefully evaluated and validated to ensure that they measure exposures covered by the health protection standard.

1.7.1 NIOSH Sampling and Analytical Methods for Standardized Industrial Hygiene Surveys

The analytical components of NIOSH's REL for asbestos exposure take on substantial significance because the current REL was set on the basis of the limit of quantitation (LOQ) of the PCM method using a 400-L sample rather than solely on estimates of the health risk. Had a lower limit of quantification been possible, a lower REL may have been proposed to further reduce the risk of occupational cancer among asbestos-exposed workers. With the change from an 8-hour TWA to a 100-minute TWA, and the advances in sampling pump capabilities, using sampling pumps at the16 L/min maximum flow-rate of the method for 100 minutes provides a 1600-L sample which would allow quantitation of ~0.04 f/cc, provided there is not excessive interference from other dust.

PCM was designated as the principal analytical method for applying the REL because it was thought to be the most practical and reliable available method. The particle counting rules specified for PCM analysis of air samples result in an index of exposure which has been used with human health data for risk assessment. As an index of exposure for airborne asbestos fibers, PCM-based counts do not enumerate all EMPs because very thin particles, such as asbestos fibrils, are typically not visible by PCM.

Several fundamental difficulties are known in using the PCM method as an index for occupational exposure to asbestos. The ratio of countable EPs to the total number of EPs collected on air samples can vary for samples collected within the same workplace, as well as between different workplaces where the same or different asbestos materials are handled [Dement and Wallingford 1990]. The result of this is that equivalent PCM asbestos exposure concentrations determined at different work places would be considered to pose the same health risk, when, in fact, those risks may be substantially different due to unknown amounts of unobserved fibers on the samples.

It is commonly stated that particles thinner than about 0.25 μm typically cannot be observed with PCM because they are below the resolution limits of the microscope. However, the results for PCM counts may also vary depending on the index of refraction of the EMP (e.g., asbestos variety) being examined. When the index of refraction of the particle is similar to the filter substrate, the ability to resolve particles is less than when the refractive index of the particle differs from the substrate [Kenny and Rood 1987]. Also, particles with widths less than 0.25 μm can be resolved with high-quality microscopes; chrysotile fibers can be resolved as low as 0.15 μm [Rooker 1982]. Thus, "fiber" counts made with PCM may vary between microscopes and the differences may vary depending on the type of asbestos.

Another aspect of NIOSH Method 7400 is that two sets of counting rules are specified depending on the type of fiber analysis. The rules for counting particles for asbestos determination, referred to as the "A" rules, instruct the microscopist to count EPs of any width that have a 3:1 or larger aspect ratio and are longer than 5 μm. However, EPs with

a width greater than 3 μm are not likely to reach the thoracic region of the lung when inhaled. The "B" counting rules, which are used to evaluate airborne exposure to other fibers, specify that only EPs thinner than 3 μm and longer than 5 μm should be counted [NIOSH 1994a]. The European Union is moving toward a standardized PCM method for evaluating asbestos exposures using counting rules recommended by the World Health Organization (WHO), which specify counting only EPs thinner than 3 μm and with a 3:1 or larger aspect ratio [WHO 1997; European Parliament and Council 2003].

1.7.2 Analytical Methods for Research

For research purposes, it may be important for a more expansive set of analyses to be considered. Optical microscopes have a limit of spatial resolution of about 0.2 μm. However, fibers thinner than 0.2 μm are thought to be important etiologic agents for disease, so other detection and measurement methods must be used to investigate the relationship between fiber dimension (e.g., width) and disease outcomes.

TEM has much greater resolving power than optical microscopy, on the order of 0.001 μm. Additionally, TEM has the ability to semi-quantitatively determine elemental composition by using EDS. Incident electrons excite electronic states of atoms of the sample, and the atoms decay that excess energy either by emitting an X-ray of frequency specific to the element (X-ray spectroscopy) or by releasing a secondary electron with equivalent kinetic energy (an Auger electron). Furthermore, TEM can provide some level of electron diffraction (ED) analysis of fiber mineralogy by producing a mineral-specific diffraction pattern based on the regular arrangement of the fiber's crystal structure. [Egerton 2005].

The greater spatial resolving power and the crystallographic analysis abilities of TEM and TEM-ED are used in some cases for standardized workplace industrial hygiene characterizations. TEM methods (e.g., NIOSH 7402) are used to complement PCM in cases where there is apparent ambiguity in identifying EMPs [NIOSH 1994b] , and under the Asbestos Hazardous Emergency Response Act of 1986, the EPA requires that TEM analysis be used to assure the effective removal of asbestos from schools [EPA 1987]. Each of these methods employs specific criteria for defining and counting visualized fibers, and report different counts of fibers for a given sample. This can be addressed by using counting and recording criteria which retain a greater level of raw data. These data subsequently can be independently interpreted according to different definitional criteria, such as those developed by the International Organization for Standardization (ISO) which provides methods ISO 10312 and ISO 13794 [ISO 1995; 1999].

Improved analytical methods that have become widely available should be re-evaluated for complementary research applications or for ease of applicability to field samples. Scanning electron microscopy (SEM) is now generally available in research labs and commercial analytical service labs. SEM resolution is on the order of ten times that of

optical microscopy, and newly commercial Field Emission SEM (FESEM) can improve this resolution to the order of 0.01 μm or better, near that of TEM. SEM-EDS and SEM-Wavelength Dispersive Spectrometers (WDS) can identify the elemental composition of particles. It is not clear that SEM-backscatter electron diffraction analysis can be adapted to crystallinity analyses equivalent to TEM-ED capability. Ease of sample collection and preparation for SEM analysis compared to TEM, and some SEM advantage in visualizing fields of EMPs and EMP morphology suggest that SEM methods should be re-evaluated for EMP analyses both for field sample analyses and for research [Goldstein 2003].

Research on mechanisms of EMP toxicity includes concerns for surface-associated factors. To support this research, elemental surface analyses can be performed by scanning Auger spectroscopy on individual particles with widths near the upper end of SEM resolution. In scanning Auger spectroscopy, the Auger electrons stimulated by an incident electron beam are detected; the energy of these secondary electrons is low, which permits only secondary electrons from near-surface atoms to escape and be analyzed, thus analyzing the particle elemental composition to a depth of only one or a few atomic layers [Egerton 2005]. This method has been used in some pertinent research studies (e.g., assessing effects on toxicity of leaching Mg from chrysotile fiber surfaces) [Keane et al. 1999]. Currently, this form of analysis is time-consuming and not ideal for the routine analysis of samples collected from field studies.

Surface elemental composition and limited valence state information on surface-borne elements can be obtained by X-ray photoelectron spectroscopy (XPS or ESCA), albeit not for individual particles. XPS uses X-ray excitation of the sample, rather than electron excitation as used in SEM-EDS or TEM-EDS. The X-rays excite sample atom electrons to higher energy states, which then can decay by emission of photoelectrons. XPS detects these element-specific photoelectron energies, which are weak and therefore emitted only near the sample surface, similar to the case of Auger electron surface spectroscopy. In contrast to scanning Auger spectroscopy, XPS can in some cases provide not only elemental but also valence state information on atoms near the sample surface. However, in XPS the exciting X-rays cannot be finely focused on individual fibers, so analysis is made of a small area larger than single particle size [Watts and Wolstenholme 2003]. Thus, analysis of a mixed-composition dust sample would be confounded; XPS is applicable only to some selected or prepared homogeneous materials or to pure field samples.

1.7.3 Differential Counting and Other Proposed Analytical Approaches for Differentiating EMPs

The use of PCM to determine concentrations of airborne fibers from asbestos minerals cannot assure exclusion of EMPs from nonasbestiform minerals. Reliable and reproducible analytical methods are not available for air samples to distinguish fibers of asbestos minerals from EMPs generated from the nonasbestiform analogs of the asbestos

minerals. The lack of reliable and validated analytical methods that can make these distinctions in air samples is clearly a major limitation in applying airborne asbestos fiber definitions of Federal agencies.

A technique referred to as "differential counting," suggested as an approach to differentiate between asbestiform and nonasbestiform EMPs, is mentioned in a non-mandatory appendix to the OSHA asbestos standard. That appendix points out that the differential counting technique requires "a great deal of experience" and is "discouraged unless legally necessary." It relies heavily on subjective judgment and does not appear to be commonly used except for mine samples. In this technique, EMPs that the microscopist judges as nonasbestiform (e.g., having the appearance of cleavage fragments) are not counted; any EMPs not clearly distinguishable as either asbestos or nonasbestos using differential counting are to be counted as asbestos fibers. One effect of using differential counting is to introduce a source of variability in the particle counts because of different "reading" tendencies between microscopists. The technique has not been formally validated and has not been recommended by NIOSH.

For counting airborne asbestos fibers in mines and quarries, ASTM has proposed "discriminatory counting" that incorporates the concepts of differential counting. The proposed method uses PCM and TEM in a tiered scheme. Air samples are first analyzed by PCM and, if fiber concentrations are greater than one-half the OSHA or MSHA permissible exposure limit (PEL) but less than the PEL, discriminatory counting is then performed. Discriminatory counts are restricted to fiber bundles, fibers longer than 10 μm, and fibers thinner than 1.0 μm. If the discriminatory count is at least 50% of the initial fiber count, TEM is then performed to determine an equivalent PCM count of regulated asbestos fibers only. If the initial PCM count is greater than the PEL, then TEM is performed to determine an equivalent PCM count of regulated asbestos fibers only. These results are then compared to regulatory limits [ASTM 2006].

ASTM has begun an interlaboratory study (ILS#174) to determine the interlaboratory precision of "binning" fibers into different classes based on morphology. This is being carried out with NIOSH collaboration [Harper et al. 2007]. The first part of the validation process was to evaluate ground up samples of massive or coarsely crystalline amphiboles and samples from a taconite mine which have amphibole particulates characterized as cleavage fragments. Almost none of the observed particles met the Class 1 criteria (potentially asbestiform based on curved particles and/or bundle of fibrils). Many particles were classified as Class 2 (also potentially asbestiform with length >10 μm or width <1 μm), although their morphology suggested they were more likely cleavage fragments. Using alternative criteria for Class 2 (length >10 μm and width <1 μm), the number of Class 2 particles was greatly reduced. However, evidence from the literature [Dement et al. 1976; Griffis et al. 1983; Wylie et al. 1985; Siegrist and Wylie 1980; Beckett and Jarvis 1979; Myojo 1999] indicates that as much as 50% of airborne asbestos fibers are <10 μm long. The proportion of asbestos fibers in the length bracketed by 5 μm and 10 μm is also quite large, on the order of 30%, and the adoption of Class 2

criteria as length >10 µm and width <1 µm would cause this proportion of asbestos fibers to be classified as nonasbestiform [Harper et al. 2008a] .

Other procedures have been suggested with the intent of ensuring that the counts on air samples do not include cleavage fragments [IMA-NA 2005; NSSGA 2005]. These procedures include reviewing available geological information and/or results from analysis of bulk materials to establish that asbestos is present in the sampled environment, or specifying dimensional criteria to establish that airborne particulates have population characteristics typical of asbestos fibers (e.g., mean particle aspect ratios exceeding 20:1).

For research purposes, it is critically important that an analytical method that is able to clearly discriminate between asbestiform and nonasbestiform EMPs be developed, validated, and used. Whether any of these suggested procedures would assure adequate health protection for exposed workers is unclear, and the practical issues associated with implementing these supplemental procedures are also undetermined.

1.8 Basis for the Recommended Exposure Limit (REL)

The NIOSH REL for asbestos has been described in NIOSH publications and in formal comments and testimony submitted to the Department of Labor. The recommendation was based on the Institute's best understanding of potential hazards, the ability of the analytical methods to distinguish and count fibers, and the prevailing mineral definitions used to describe covered minerals.

1.8.1 1990 Recommendation

18.1.1 Comments to OSHA [NIOSH 1990a]

> *The NIOSH definition of minerals to be included in the regulatory standard for asbestos is as follows:*
>
> *Asbestos is defined as chrysotile, crocidolite, amosite (cummingtonite-grunerite), anthophyllite, tremolite, and actinolite. The nonasbestiform habits of the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the series cummingtonite-grunerite, tremolite-ferroactinolite, and glaucophane-riebeckite shall also be included provided they meet the criteria for a fiber as ascertained on a microscopic level. A fiber is defined as a particle with an aspect ratio of 3:l or larger and having a length >5 µm.*
>
> *The determinations of airborne fiber concentrations are made microscopically and can be determined using NIOSH Method 7400 [PCM], or its equivalent. In those cases when asbestos and other mineral fibers occur in the same*

environment, then Method 7400 can be supplemented by the use of NIOSH Method 7402 [TEM], or its equivalent, to improve specificity of the mineral determination.

1.8.1.2 Testimony at OSHA Public Meeting [NIOSH 1990b]

NIOSH has attempted to incorporate the appropriate mineralogical nomenclature in its recommended standard for asbestos and recommends the following to be adopted for regulating exposures to asbestos:

The current NIOSH asbestos recommended exposure limit is 100,000 fibers greater than 5 micrometers in length per cubic meter of air, as determined in a sample collected over any 100-minute period at a flow rate of 4L/min. This airborne fiber count can be determined using NIOSH Method 7400, or equivalent. In those cases when mixed fiber types occur in the same environment, then Method 7400 can be supplemented with electron microscopy, using electron diffraction and microchemical analyses to improve specificity of the fiber determination. NIOSH Method 7402 … provides a qualitative technique for assisting in the asbestos fiber determinations. Using these NIOSH microscopic methods, or equivalent, airborne asbestos fibers are defined, by reference, as those particles having (1) an aspect ratio of 3 to 1 or greater; and (2) the mineralogical characteristics (that is, the crystal structure and elemental composition) of the asbestos minerals and their nonasbestiform analogs. The asbestos minerals are defined as chrysotile, crocidolite, amosite (cummingtonite-grunerite), anthophyllite, tremolite, and actinolite. In addition, airborne cleavage fragments[3] from the nonasbestiform habits of the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the series cummingtonite-grunerite, tremolite-ferroactinolite, and glaucophane-riebeckite shall also be counted as fibers provided they meet the criteria for a fiber when viewed microscopically.

1.8.2 The NIOSH Recommended Exposure Limit

As described in the preceding sections, uncertainty remains concerning the adverse health effects that may be caused by elongated particles of the nonasbestiform minerals encompassed by NIOSH since 1990 in the REL for asbestos. In addition, current analytical methods still cannot reliably differentiate between fibers from the asbestos

[3] NIOSH intended the term "cleavage fragment" to include all elongated particles from the nonasbestiform habits of the specified serpentine minerals and amphibole minerals. This includes more particle types, such as acicular and prismatic crytstals, than the more restrictive meaning of "cleavage fragments" used by mineralogists.

minerals and other EMPs in mixed-dust environments. NIOSH recognizes that its descriptions of the REL since 1990 have created confusion and caused many to infer that the additional covered minerals were included by NIOSH in its definition of "asbestos." NIOSH wishes to make clear that such nonasbestiform minerals are not "asbestos" or "asbestos minerals." NIOSH also wishes to minimize any potential future confusion by no longer referring to particles from the nonasbestiform analogs of the asbestos minerals as "asbestos fibers." However, as the following clarified REL makes clear, particles that meet the specified dimensional criteria remain countable under the REL for the reasons stated above, even if they are derived from the nonasbestiform analogs of the asbestos minerals.

1.8.2.1 Clarification of the Recommended Exposure Limit

Using terms defined in this *Roadmap*, the NIOSH REL is now clarified as follows:

NIOSH's REL for airborne asbestos fibers and related elongated mineral particles (EMPs) is 0.1 EMPs from one or more covered minerals per cubic centimeter averaged over 100 minutes.

Where:

- An *elongated mineral particle (EMP)* is any fiber or fragment of a mineral longer than 5 μm when viewed microscopically using NIOSH Analytical Method #7400 ('A' rules) or its equivalent with a minimum aspect ratio of 3:1; and
- A c*overed mineral* is any mineral having the crystal structure and elemental composition of: one of the asbestos varieties (chrysotile, riebeckite asbestos [crocidolite], cummingtonite-grunerite asbestos [amosite], anthophyllite asbestos, tremolite asbestos, and actinolite asbestos) or one of their nonasbestiform analogs (the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the cummingtonite-grunerite mineral series, the tremolite-ferroactinolite mineral, and the glaucophane-riebeckite mineral series).

In evaluating occupational exposures against the REL, this clarification of the NIOSH REL for airborne asbestos fibers and related EMPs results in *no change* in the evaluated count. However, it clarifies definitionally that EMPs included in the count are not necessarily asbestos fibers.

The NIOSH REL remains subject to change as future research sheds new light on the toxicity of nonasbestiform amphibole EMPs currently covered by the REL and on the toxicity of other EMPs currently outside the range of those minerals covered by the REL. Also, due to the change from using optical methods for identification of minerals to a chemistry-based nomenclature, and subsequent changes in the specific nomenclature of amphibole minerals based on elemental ratios, a more extensive clarification of specific

minerals covered by the NIOSH REL is warranted. That more extensive clarification of covered minerals is beyond the scope of this Roadmap, but will be addressed through additional efforts by NIOSH to encompass contemporary mineralogical terminology.

1.9 Summary of Key Issues

For fibers from the asbestos minerals, an important question that remains unanswered is what are the important dimensional and physicochemical determinants of pathogenicity. Evidence from epidemiological and animal studies suggest that the potency of asbestos fibers is reduced as length decreases, but lung burden studies indicate the presence of short asbestos fibers at disease sites, and positive correlations between lung cancer and exposure to short asbestos fibers make it difficult to rule out a role for short asbestos fibers in the causation of disease.

Asbestos fibers are clearly carcinogenic and cause asbestosis. However, the biological relevance of other EMPs remains uncertain. The results of epidemiological studies remain inconclusive, but taken together with animal tests and *in vitro* test results, EMPs from nonasbestiform minerals have generally lower aspect ratios than asbestos fibers and appear to have generally lesser potential to produce lung pleural and lung disease. A question still to be answered satisfactorily is what particle characteristics (e.g., surface reactivity, ability to resist dissolution and breakage, etc.), in addition to EMP dimensions, influence toxicity.

Understanding the determinants of toxicity of EMPs from varieties of asbestos and of erionite, a fibrous zeolite, as well as non-elongated mineral particles such as quartz, may help to elucidate some of these issues. Also, the results of the human, animal, and *in vitro* studies performed on a limited number of nonasbestiform EMPs to date are not sufficient to conclude that exposure to EMPs from this large, highly variable group of minerals is not capable of causing substantial adverse health outcomes. Additional data are needed to develop risk assessments. There is a general lack of occupational exposure data on nonasbestiform EMPs, making it difficult to assess the range of particle characteristics, including dimension, in occupational settings with exposures to nonasbestiform EMPs. The limited number of studies that have assessed biopersistence or durability indicate that nonasbestiform EMPs are not as biopersistent as asbestiform fibers of the same dimension, but more information is needed to systematically assess the ranges and importance of biopersistence in determining toxicity. Any assessment of risk needs to address the influence of dimension, so studies that systematically compare effects of asbestiform and nonasbestiform particles of similar sizes from the same mineral for a variety of mineral types are needed.

An important need is to identify and develop methods of analysis that can be used or modified to assess exposures to EMPs that are capable of differentiating between EMPs based on particle characteristics that are important in causing disease. The current PCM

method is inadequate for assessing the mixed-dust types of exposures which are likely to predominate for the foreseeable future, and it does not have the capability to measure the important physical and chemical parameters of particles thought to be associated with toxicity. For routine use in assessing compliance with regulations, the limited availability, high relative cost, and long turnaround times associated with EM methods will need to be addressed to provide an alternative to the PCM method. Until these issues are addressed, improvements in PCM methodologies should be pursued. In epidemiological and toxicological research, EM methods will need to be used to carefully characterize the exposure materials. Also, the results of toxicological and epidemiological studies may identify additional determinants of particle toxicity which will need to be evaluated to determine whether they can be incorporated into sampling and analytical methods used to assess the health risks of exposure to EMPs.

To address these scientific issues and inform future NIOSH recommendations, a framework for proposed research is presented and discussed in Section 2 of this *Roadmap*.

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2 FRAMEWORK FOR RESEARCH

2.1 Strategic Research Goals and Objectives

Strategic goals and objectives for a multi-disciplinary research program on mineral fibers and other EMPs are identified below. Shown in brackets following each goal and objective is the number of the section of this *Roadmap* in which the goal or objective is subsequently discussed.

I. Develop a broader understanding of the important determinants of toxicity for mineral fibers and other EMPs [2.2].

- Conduct *in vitro* studies to ascertain what physical, chemical, and surface properties influence the toxicity of mineral fibers and other EMPs [2.2.1]; and
- Conduct animal studies to ascertain what physical and chemical properties influence the toxicity of mineral fibers and other EMPs [2.2.2].

II. Develop information and knowledge on occupational exposures to mineral fibers and other EMPs and related health outcomes [2.3].

- Assess available occupational exposure information relating to various types of mineral fibers and other EMPs [2.3.1];
- Collect and analyze available information on health outcomes associated with exposures to various types of mineral fibers and other EMPs[2.3.2];
- Conduct selective epidemiologic studies of workers exposed to various types of mineral fibers and other EMPs [2.3.3]; and
- Improve clinical tools and practices for screening, diagnosis, treatment, and secondary prevention of diseases caused by asbestos fibers and other EMPs [2.3.4].

III. Develop improved sampling and analytical methods for mineral fibers and other EMPs [2.4].

- Reduce inter-operator and inter-laboratory variability of the current analytical methods used for asbestos fibers [2.4.1];
- Develop analytical methods with improved sensitivity to visualize thinner EMPs to assure a more complete evaluation of airborne exposures [2.4.2];
- Develop a practical analytical method for air samples to differentiate between exposures to asbestiform fibers from the asbestos minerals and exposures to EMPs from their nonasbestiform analogs [2.4.3];
- Develop analytical methods to assess durability of EMPs [2.4.4]; and
- Develop and validate size-selective sampling methods for EMPs [2.4.5].

Research conducted to support these three research goals should be integrated to optimize resources, facilitate the simultaneous collection of data, and ensure, to the extent feasible, that the research builds toward a resolution of the key issues. Within each of the goals and objectives laid out in this framework, a more detailed research program will have to be developed. An objective of the research is to acquire a level of mechanistic understanding that can provide the basis for developing biologically-based models for extrapolating results of animal inhalation and other types of *in vivo* studies to exposure conditions typically encountered in the workplace. The information gained from such research can then be used by regulatory agencies and occupational health professionals to implement appropriate exposure limits and programs for monitoring worker exposure and health. Much of this research may be accomplished by NIOSH, other Federal agencies, or other stakeholders. Any research project that is undertaken should ensure that the results can be interpreted and applied within the context of other studies in the overall program and lead to outcomes useful for decision-making and policy-setting.

To support the needed research, a national reference repository of samples of asbestos and related minerals will be required. Minerals vary in composition and morphology by location and origin, and differences within the same mineral type can be significant. Currently, no national repository exists to retain, document, and distribute samples of asbestiform and nonasbestiform reference minerals for research and testing. These reference samples should be well-characterized research-grade materials that are made available to the research community so they can be used for testing and standardization.

The use of these samples in research would facilitate meaningful comparisons and reduce uncertainties in the interpretation of results between and among studies.

2.2 Develop a broader understanding of the important determinants of toxicity for mineral fibers and other EMPs

To address this objective, one of the first steps will be to identify the range of minerals and mineral habits needed to systematically address the mineral characteristics that may determine particle toxicity. Care must be taken to ensure that mineralogical issues in a study are as adequately addressed as biological issues. Information on both the crystalline lattice and composition are needed to define a mineral species because information on either alone is insufficient to describe the properties of a mineral. For example, nonasbestiform riebeckite and asbestiform riebeckite (crocidolite) share the same elemental composition but have different crystalline lattices. Crocidolite fibers generally have chain-width defects, which explain the flexibility of crocidolite fibers. (EMPs from nonasbestiform riebeckite are not flexible.) These chain-width defects also affect diffusion of cations and dissolution properties, both of which can explain greater release of iron into surrounding fluid by crocidolite than by nonasbestiform riebekite [Guthrie 1997]. In addition to elemental content and crystalline lattice, the particle characteristics identified by Hochella [1993] should be considered for particle characterization. Because of the many variations in elemental content, crystalline lattice, and other characteristics of these minerals, it will be impossible to study all variants. Therefore, a strategy will have to be developed for selecting the minerals for testing. Included in this strategy should be consideration of occupationally relevant minerals and habits, availability of appropriate and well-characterized specimens for testing, and practical relevance of the results to be achieved through testing.

EPA's Office of Pollution Prevention and Toxics, NIEHS, NIOSH, and OSHA assembled an expert panel workshop a decade ago to consider major issues in animal model chronic inhalation toxicity and carcinogenicity testing of thoracic-sized elongated particles. This included the design of chronic inhalation exposure of animals to EMPs; preliminary studies to guide them; parallel mechanistic studies to help interpret study results and to extrapolate findings to potential for human health effects; and available screening tests for identifying and assigning a priority for chronic inhalation study. There was general agreement that: (1) chronic inhalation studies of EMPs in the rat are the most appropriate tests for predicting inhalation hazard and risk of EMPs to humans; (2) no single assay and battery of short-term assays could predict the outcome of a chronic inhalation bioassay for carcinogenicity; and (3) several short-term *in vitro* and *in vivo* studies may be useful to assess the relative potential of various EMPs to cause lung toxicity or carcinogenicity [Vu et al. 1996].

Such short-term assays and strategies were considered by an expert working group assembled by the International Life Sciences Institute's Risk Science Institute to arrive at

a consensus on current useful short-term assays for screening EMPs for potential toxicity and carcinogenicity [ILSI 2005]. Dose, dimension, durability, and possibly surface reactivities were identified as critical parameters for study, while it was noted that no single physicochemical property or mechanism can now be used to predict carcinogenicity of all EMPs. The strategy for short-term animal model testing, defined as 3 months or less, included sample preparation and characterization (composition, crystallinity, habit, size-distribution); testing for biopersistence *in vivo* using a standard protocol such as that of the European Union [European Commission 1999]; and a sub-chronic inhalation or instillation challenge of the rat with evaluation of lung weight and fiber burden, bronchoalveolar lavage profile, cell proliferation, fibrosis, and histopathology. Additionally, other non-routine analyses for particle surface area and surface reactivities, and short-term *in vitro* cellular toxicological assays might be evaluated. The use of *in vitro* tests should be tempered by the observations that standard protocols fail to distinguish relative pathogenic potentials of even non-elongated silicates (i.e., quartz versus clay dusts) and treatment of particle surfaces (i.e., modeling their conditioning upon deposition on the lipoprotein rich aqueous hypophase surface of the deep lung) can greatly affect their expression of toxicities [ATSDR 2003].

EMPs encountered in any particular work environment are frequently heterogeneous, which limits the ability of epidemiological and other types of health assessment studies to evaluate the influence of EMP dimensions (length and width), chemical composition, biopersistence, and other characteristics on toxicity. Toxicological testing is needed to address some of the fundamental questions about EMP toxicity that cannot be determined through epidemiology or other types of health assessment studies. Irrespective of study type or design, the full characterization of all particulate material in a test sample is an essential step in understanding the mechanisms of EMP toxicity. The determination of EMP dimensions is important and best expressed as bivariate size distributions (i.e., width and length). Such determinations should be made using both relatively simple procedures (optical microscopy) and highly specialized techniques (e.g., TEM or SEM with EDS) because size-specific fractions of EMP exposures have both biological and regulatory significance.

The chemical composition (e.g., intrinsic chemical constituents and surface chemistry) of mineral fibers and other EMPs has been shown to have a direct effect on their ability to persist in the lung and to interact with surrounding tissue to cause DNA damage. For example, ferric and ferrous cations are major components of the crystalline lattice of amphibole asbestos fibers; iron may also be present as surface impurities on chrysotile asbestos fibers and other EMPs. The availability of iron at the surface of asbestos fibers and other EMPs has been shown to be a critical parameter in catalyzing the generation of ROS which may indirectly cause genetic damage [Kane 1996]. It has also been reported that the attempted clearance of long asbestos fibers from the lung causes frustrated phagocytosis, which stimulates the release of ROS [Mossman and Marsh 1989]. Individual adaptive responses to oxidant stress and the body's ability to repair damaged DNA are dependent on multiple exogenous and endogenous factors, but few experiments

have been attempted to evaluate these variables in animal or human model systems. Kane [1996] has suggested that the mechanisms responsible for the genotoxic effects of asbestos fibers are due to indirect DNA damage mediated by free radicals and to direct physical interference with the mitotic apparatus. Research to address the following questions would assist in validating these proposed mechanisms:

- Are *in vitro* genotoxicity assays relevant to carcinogenesis of asbestos fibers and other EMPs?
- Are *in vitro* doses relevant for *in vivo* exposures?
- Can genotoxic effects of asbestos fibers and other EMPs be assessed *in vivo*?

Macrophages are the initial target cells of EMPs and other particulates that deposit in the lungs or pleural and peritoneal spaces. Phagocytosis of asbestos fibers has been shown to be accompanied by the activation of macrophages, which results in the increased generation of ROS as well as a variety of chemical mediators and cytokines [Kane 1996]. These mediators amplify the local inflammatory reaction. Persistence of asbestos fibers in the interstitium of the lungs or in the sub-pleural connective tissue may lead to a sustained chronic inflammatory reaction accompanied by fibrosis [Oberdorster 1994]. The unregulated or persistent release of these inflammatory mediators may lead to tissue injury, scarring by fibrosis, and proliferation of epithelial and mesenchymal cells. In the lungs and pleural linings, chronic inflammation and fibrosis are common reactions following exposure to asbestos fibers; however, research is needed to understand the relationship between inflammation, fibrosis, and cancer induced by asbestos fibers and other EMPs.

It has been suggested that asbestos fibers and other EMPs may contribute to carcinogenesis by multiple mechanisms and that EMPs may act at multiple stages in neoplastic development depending on their physicochemical composition, surface reactivity, and biopersistence in the lung [Barrett 1994]. Animal inhalation studies are needed to investigate the biopersistence and toxicity of asbestos fibers and other EMPs representing a range of chemical compositions and morphological characteristics (including crystalline habits) and representing a range of discrete lengths and widths. Other factors which should be considered and evaluated for their influence on toxicity include accessory minerals, contaminants, matrix effects, biochemical events, and surface chemistry.

Much research has been focused on lung cancer and mesothelioma. If it is determined that EMPs from some minerals have low potency for causing cancer, then additional studies may be needed to investigate their potential for causing inflammation, fibrosis, and other nonmalignant respiratory effects. Also, the relationship between EMP dimension and fibrosis should be more fully investigated. The results of such research may allow currently used standard exposure indices to be modified by specifying different dimensional criteria (lengths and widths) relevant to each of the disease

outcomes associated with EMP exposures, and by determining whether biopersistence can be included as an additional criterion. However, this research is most likely dependent on developing new aerosol technology that can generate mineral fibers and other EMPs of specific dimensions in sufficient quantities to conduct animal inhalation experiments. Consequently, the development of revised exposure indices based on EMP dimension may not be possible in the short term.

Implicit in any new or revised policy for EMPs may be new risk assessments. Risk assessments for lung cancer and asbestosis have been conducted on worker populations exposed to fibers from various asbestos minerals. These risks have been qualitatively confirmed in animals, but no adequate quantitative dose-response inhalation studies with asbestos have been conducted in rats which would allow for comparisons between minerals. Given the availability of risk estimates for lung cancer in asbestos-exposed humans, chronic studies with rats exposed to asbestos (e.g., chrysotile) fibers would provide an assessment of the rat as a "predictor" for human lung cancer risks associated with exposure to asbestos fibers and other EMPs.

2.2.1 Conduct in vitro studies to ascertain what physical and chemical properties influence the toxicity of mineral fibers and other EMPs

In vitro studies may help to clarify the mechanisms by which some EMPs induce cancer, mesothelioma, or fibrosis, and the properties of EMPs and conditions of exposure that determine pathogenicity. Three of the phases of EMP interactions that should be addressed are:

- initial lesions evoking cell damage or response (e.g., direct or indirect cytotoxic or genotoxic events or induction of toxic reactive intermediate materials);
- subsequent multi-stage cellular response (e.g., intracellular signaling through a kinase cascade to nuclear transcription of factors for apoptosis, cell transformation, and cell or cell system proliferation or remodeling and initiation or promotion of neoplasia or fibrosis); and
- critical time-course events in those processes (e.g., cell-cycle-dependent EMP interactions or EMP durability under different phagocytic conditions).

Capabilities for these studies have improved in the last decade through:

- advancement in analytical methods for physicochemical characterization of EMP properties (e.g., for resolving small dimensions and nanoscale surface properties); and
- ability to prepare EMPs "monochromatic" in size or surface properties in quantities sufficient for well-controlled *in vitro* assays.

Identification of the initiating EMP-cell interactions calls for research on the mechanisms of:

- cell-free generation of toxic ROS by EMPs or EMP-induced cellular generation of toxic ROS; and
- direct membranolytic, cytotoxic, or genotoxic activities of the EMP surface in contact with cellular membranes or genetic material.

These investigations will require attention to the:

- effects of EMP surface composition (e.g., surface-borne iron species);
- effects of normal physiological conditioning of respired particles (e.g., *in vitro* modeling of *in vivo* initial conditioning of EMP surfaces by pulmonary surfactant);
- non-physiological conditioning of EMP under *in vitro* test conditions (e.g., by components of nutrient medium);
- cell type (e.g., phagocytic inflammatory cell, or phagocytic or non-phagocytic target cell); and
- EMP dimensions in relation to cell size (e.g., as a factor distinguishing total phagocytosis and partial "frustrated phagocytosis").

Cell generation of ROS is seen generally in phagocytic uptake of elongated or non-elongated particles (e.g., as a respiratory burst). In normal phagocytosis, there is a maturation of the phagosomal membrane with progress to a lysophagosomal structure for attempted lysosomal digestion. Anomalous behavior of this system may occur in frustrated phagocytosis of long EMPs. The "frustrated phagocytosis" hypothesis suggests that EMP length too great to permit full invagination may prompt a continuing stimulation of ROS by the cell or an anomalous release of lytic factors into the extracellular annulus rather than into a closed intracellular phagosome.

EMP surfaces may be tested for direct membranolytic or cytotoxic activities which are dependent on surface composition or structure. As a guide, membranolytic or cytotoxic activities of non-elongated particulate silicates are surface-property dependent. Non-elongated particulate silicates also provide an example of failure of *in vitro* cytotoxicity to relate with pathogenicity (e.g., respirable particles of quartz or kaolin clay significantly differ in disease risk for fibrosis, but are comparably cytotoxic *in vitro* unless they are pre-conditioned with pulmonary surfactants and subjected to phagolysosomal digestion). *In vitro* studies of direct versus indirect induction of genotoxic activities may consider factors affecting the bioavailability of the nuclear genetic material (e.g., the state of phagocytic activity of the cell or the stages in the cell cycle with collapse of the nuclear membrane in mitosis). These again suggest care in the preparation and manner of challenge of *in vitro* experiments on EMPs.

The two modes of primary damage, a release of reactive toxic agents induced by long particulates, or a surface-based membranolytic or genotoxic mechanism, may be involved singly or jointly in primary cell responses to EMPs. These may be investigated by differences between types of EMPs (e.g., relative potencies for erionite fibers versus amphibole asbestos fibers in *in vitro* cell transformation do not correspond with their relative potencies for *in vivo* induction of mesothelioma).

In the second phase of cellular response to EMPs, the central dogma of intracellular response is being well-researched as revealed by a review of animal and *in vitro* studies over the last decade or more. The initial extracellular primary damage induces intracellular signaling (e.g., by MAPK) which causes a cascade of kinase activities that stimulate selective nuclear transcription of mRNAs leading to production of TNF-α or other cytokines for extracellular signaling of target cells. Those cytokines may induce cell proliferation toward cancer or collagen synthesis toward fibrosis. Further definition of signaling mechanisms and analyses of their induction by different primary EMP-cellular interactions may better define the ultimate role of EMP properties in the overall process. That research, again, may be facilitated by using different specific types EMPs, each type with relatively homogeneous morphology and surface properties.

While full investigation of biopersistence of EMPs may require long-term animal model studies, *in vitro* systems coupled with advanced surface analytical tools (e.g., field emission scanning electron microscopy – energy dispersive X-ray spectroscopy or scanning Auger spectroscopy), may help guide *in vivo* studies. This could be done by detailing specific surface properties of EMPs and their modifications under cell-free or *in vitro* conditions representing the local pH and reactive species at the EMP surface under conditions of extracellular, intra-phagolysosomal, or frustrated annular phagocytic environments.

2.2.2 Conduct animal studies to ascertain what physical and chemical properties influence the toxicity of mineral fibers and other EMPs

A multi-animal testing approach has been recommended for short-term assays [ILSI 2005] and chronic inhalation studies [EPA 2000] that would provide solid scientific evidence on which to base human risk assessments for a variety of EMPs. To date, the most substantial base of human health data for estimating lung cancer risk exists for workers exposed to fibers from different types of asbestos minerals.

Interspecies differences have been identified in the clearance of inhaled particles. Variations in deposition patterns and airway cell morphology and distribution account for significant deposition and clearance differences among species. In addition, the efficacy of pulmonary macrophage functions differs among species. All these differences could affect particle clearance and retention. It has been suggested that the following species

differences should be considered in the design of experimental animal inhalation studies of elongated particles [Dai and Yu 1988; Warheit et al. 1988; Warheit 1989]:

- Due to differences in airway structure, airway size, and ventilation parameters, a greater fraction of larger AED particles are deposited in humans than in rodents.

- Alveolar deposition fraction in humans varies with workload. An increase in the workload reduces the deposition fraction in the alveolar region because more of the inhaled particulate is deposited in the extra-thoracic and bronchial regions.

- Mouth breathing by humans results in a greater upper bronchial deposition and enhanced particle penetration to the peripheral lung.

- For both animals and humans, the deposition rate of particles is greatest in the AED range between 1 and 2 μm. Alveolar deposition of EPs decreases as their aspect ratio increases when the width remains constant.

- For rats and hamsters, alveolar deposition becomes practically zero when particle AED exceeds 3.0 μm and aspect ratio exceeds 10. In contrast, considerable alveolar deposition is found for humans breathing at rest, even for EPs with AEDs approaching 5 μm and aspect ratio exceeding 10 μm.

- Rodents have smaller-diameter airways than humans, which increases the chance for particle deposition via contact with airway surfaces.

- Turbulent air flow, which enhances particle deposition via impaction, is common in human airways but rare in rodent airways.

- Variations in airway branching patterns may account for significant differences in deposition between humans and rodents. Human airways are characterized by symmetrical branching, wherein each bifurcation is located near the centerline of the parent airway. This symmetry favors deposition hotspots on carinal ridges at the bifurcations due to disrupted airstreams and local turbulence. Rodent airways are characterized by asymmetric branching, which results in a more diffuse deposition pattern because the bulk flow of inspired air follows the major airways with little change in velocity or direction.

- Human lung clearance is slower than rats, and human dosimetry models predict that a greater proportion of particles deposited in the alveolar region will be interstitialized and sequestered in humans than in rats at non-overloading exposure concentrations.

An important consideration in the conduct and interpretation of animal studies is the selection of well characterized (chemical and physical parameters) and appropriately sized EMPs that takes into account differences in EP deposition and clearance characteristics between rodents and humans. EMPs that are capable of being deposited in the bronchoalveolar region of humans cannot be completely evaluated in animal inhalation studies because the maximum thoracic size for rodents is an AED of approximately 2 µm, less than the maximum thoracic size of about 3 µm for humans [Timbrell 1982; Su and Cheng 2006].

2.2.2.1 Short-Term Animal Studies

There are advantages to conducting short-term animal studies in rats. The information gained (e.g., regarding overload and maximum tolerated dose [MTD]) from these studies can be used in designing chronic inhalation studies [ILSI 2005]. The objectives of these studies would be to:

- Evaluate EMP deposition, translocation, and clearance mechanisms;
- Compare the biopersistence of EMPs retained in the lung with results from *in vitro* durability experiments;
- Compare *in vivo* pulmonary responses to *in vitro* bioactivity for EMPs of different dimensions; and
- Compare cancer and noncancer toxicities of EMPs from asbestiform and nonasbestiform amphibole mineral varieties with varying shapes as well as within narrow length and width size ranges.

More fundamental studies should also be performed to:

- Identify biomarkers or tracer/imaging methods that could be used to predict or monitor active pulmonary inflammation, pulmonary fibrosis, and malignant transformation;
- Investigate mechanisms of EMP-induced pulmonary disease; and
- Determine whether cell proliferation in the lungs (terminal bronchioles and alveolar ducts) can be a predictive measure of pathogenicity following brief inhalation exposure using the BrdU assay [Cullen et al. 1997].

Exposure protocols for tracheal inhalation or instillation in an animal model for short-term *in vivo* or *ex-vivo* studies using field-collected or laboratory-generated EMPs should

address possible adulteration of EMP morphology (e.g., anomalous agglomeration of particles). This might be addressed in part by pre-conditioning EMPs in a delivery vehicle containing representative components of pulmonary hypophase fluids. Exposure protocols using pharyngeal aspiration as a delivery system should be considered given the observations in studies with single-walled carbon nanotubes that such a delivery system closely mimics animal inhalation studies [Shvedova et al. 2005].

Studies evaluating the roles of biopersistence and dimension in the development of non-cancer and cancer endpoints from exposure to EMPs are also needed. These studies should attempt to elucidate the physicochemical parameters that might affect bio-durability for EMPs of specific size dimensions. While short-term animal inhalation studies would be informative, companion *in vitro* assays should also be conducted to assess the viability of such assays for screening EMPs.

2.2.2.2 Long-Term Animal Studies

Chronic animal inhalation studies are required to address the impacts of dimension, morphology, chemistry, and biopersistence on critical disease endpoints of cancer induction and nonmalignant respiratory disease. The EPA's proposed testing guidelines should be used as the criteria for establishing the testing parameters for chronic studies [EPA 2000].

To date, chronic inhalation studies have been conducted with different animal species using different types of EPs. However, it is still uncertain which species of animal(s) best predict(s) the risk of respiratory disease(s) for workers exposed to different EPs. Chronic inhalation studies should be initiated to establish exposure/dose-response relationships for at least two animal species. The rat has historically been the animal of choice for chronic inhalation studies with EPs; however, the low incidence of lung tumors and mesotheliomas occurring in rats exposed to asbestos fibers suggests that rats may be less sensitive than humans. Therefore, any future consideration for conducting long-term animal inhalation studies should address the need for using a multi-animal testing approach to help provide solid scientific evidence on which to base human risk assessments for a variety of EMPs of different durabilities and dimensions. For example, some recent studies suggest that the hamster may be a more sensitive model for mesothelioma than the rat. Validation of appropriate animal models could reduce the resources needed to perform long-term experimental studies on other fiber types [EPA 2000].

Multi-dose animal inhalation studies with asbestos (probably a carefully selected and well-characterized chrysotile, because most of the estimates of human risk have been established from epidemiologic studies of chrysotile-exposed workers) are needed to provide an improved basis for comparing the potential cancer and non-cancer risks associated with other types of EMPs and various types of synthetic fibers. The asbestos

fibers administered in these animal studies should be comparable in dimension to those fibers found in the occupational environment. The results from these studies with asbestos (e.g., chrysotile) would provide a "gold standard" that could be used to validate the utility of long-term inhalation studies (in rats or other species) for predicting human risks of exposure to various fiber types.

2.3 Develop information and knowledge on occupational exposures to mineral fibers and other EMPs and related health outcomes

Many studies have been published concerning occupational exposures to asbestos and associated health effects. These studies have formed a knowledge base that has supported increased regulation of occupational asbestos exposures and marked reductions in asbestos use and asbestos exposures in the U.S. over the past several decades. But, as this *Roadmap* makes clear, much less is known about other types of mineral fibers and EMPs in terms of occupational exposures and potential health effects.

Research is needed to produce information on:

- current estimates and, where possible, future projections of numbers of U.S. workers exposed to asbestos fibers;
- levels of current exposures; and nature of the exposures (e.g., continuous, short-term or intermittent); and
- the nature of any concomitant dust exposures.

Similar research is needed to produce analogous information about occupational exposures to other mineral fibers and EMPs. Research is needed to assess and quantify potential human health risks associated with occupational exposures to other mineral fibers and EMPs, as well as to better understand and quantify the epidemiology of asbestos-related diseases using more refined indices of exposure. Research is also needed to produce improved methods and clinical guidance for screening, diagnosis, secondary prevention, and treatment of diseases caused by asbestos and other hazardous EMPs.

2.3.1 Assess available information on occupational exposures to various types of mineral fibers and other EMPs

A fully informed strategy for prioritizing research on EMPs should optimally be based on preliminary systematic collection and evaluation of available information on: (1) industries/occupations/job tasks/processes with exposure to various types of mineral fibers and other EMPs; (2) numbers of workers exposed; (3) characteristics and levels of

exposures to EMPs; and (4) associated concomitant particulate exposures. Such information could enable estimations of:

- the overall distribution and levels of occupational exposures to EMPs and the total number of workers exposed to EMPs currently, in the past, and an estimate of those at risk of exposure in the future; and

- specific distributions and levels of exposures to each particular type of EMP, as well as numbers of workers exposed to each type of EMP currently, in the past, and (projected) in the future.

Initial efforts should be made to collect, review, and summarize available occupational exposure information and to collect and analyze representative air samples relating to various types of EMPs. For example, systematic compilation of exposure data collected by OSHA, MSHA, NIOSH, state agencies, and private industry could contribute to an improved understanding of current occupational exposures to EMPs, particularly if there are opportunities to (re)analyze collected samples using enhanced analytical methods to better characterize the exposures (see Section 2.4). To help limit potential impact of sampling bias that may be inherent in the available EMP exposure data, these initial efforts should be supplemented with efforts to systematically identify, sample, and characterize EMP exposures throughout U.S. industry. These exposure assessments should include workplaces in which a fraction of the dust is comprised of EMPs (i.e., mixed-dust environments) (see Figure 8), and occupational environments in which EMPs may not meet the current regulatory criteria to be counted as fibers (i.e., "short" fibers). With appropriate planning and resources, such efforts could be designed and

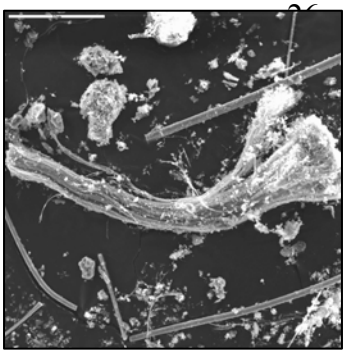

Figure 8. SEM image of a chrysotile bundle (center) and glass fibers from World Trade Center dust sample. Photograph courtesy of USGS

implemented as ongoing surveillance of occupational exposures to EMPs, with periodic summary reporting of findings. Representative EMP exposure data could help identify worker populations or particular types of EMPs that would warrant further study (i.e., more in-depth exposure assessment, medical surveillance; epidemiology studies of particular types of EMPs, processes, job tasks, occupations, or industries; toxicity studies of particular EMPs). New infrastructure, such as an occupational exposure database similar to that described in Marchant et al. [2002], should be developed to support these efforts. This could be accomplished in parallel with efforts to develop an occupational

exposure database for nanotechnology [Miller et al. 2007] or efforts to develop a national occupational exposure database [Middendorf et al. 2007].

2.3.2 Collect and analyze available information on health outcomes associated with exposures to various types of mineral fibers and other EMPs

The body of knowledge concerning human health effects from exposure to EMPs consists primarily of epidemiologic studies of workers exposed to asbestos fibers and several other types of EMPs (e.g., wollastonite, attapulgite, erionite). Additional information may be gleaned from the epidemiologic studies conducted on some SVFs (e.g., glass and mineral wool fibers, ceramic fibers). There is general agreement that workers exposed to fibers from any asbestiform mineral would be at risk of serious adverse health outcomes of the type caused by exposure to fibers from the six commercially exploited asbestos minerals. NIOSH commented on the recent MSHA proposed rule on asbestos (subsequently promulgated as a final rule), stating that "NIOSH remains concerned that the regulatory definition of asbestos should include asbestiform mineral fibers such as winchite and richterite, which were of major importance as contaminants in the Libby, MT vermiculite" [NIOSH 2005]. To assure a clear science base that might support a formal recommendation for control of occupational exposures to all asbestiform amphibole fibers, it would be reasonable to thoroughly review, assess, and summarize the available information on asbestiform amphiboles that have not been commercially exploited as asbestos. Publication of such a review could be done in the short term.

It will also be important to authoritatively determine whether EMPs from nonasbestiform amphiboles pose the same potential health risks as those observed in workers exposed to fibers from asbestiform amphiboles. Animal and *in vitro* studies have indicated a potential risk for exposed humans, and results from completed epidemiologic studies of workers exposed to amphibole cleavage fragments have to date failed to resolve this major area of current controversy. If nonasbestiform amphibole EMPs are, in fact, associated with some risk, a quantitative risk assessment would be needed to understand whether the risks are similar to the risk associated with exposures to asbestos fibers. An expert panel could be assembled and charged with ascertaining if the existing epidemiological evidence could support development of a likely maximum risk estimate associated with exposure to nonasbestiform amphibole EMPs. Such a risk estimate might inform the development of appropriate risk management policies. These results could also inform whether routine analytical methods to differentiate asbestiform from nonasbestiform particles on air sample filters are needed.

Surveillance and epidemiological studies generally have been circumscribed by the long multi-year or decades-long latency periods in manifestation of either cancer or pulmonary fibrosis associated with asbestos exposures (e.g., as detected by conventional chest radiography or pulmonary function tests). Modern medical pulmonary imaging techniques or bioassays of circulating levels of cytokines or other biochemical factors associated with disease processes might be adaptable to better define stages of asbestosis,

or to provide a new paradigm for early detection or grading of the active disease process. For example, positron emission tomographic imaging using tracers indicative of active collagen synthesis can detect fibrogenic response in a matter of weeks after quartz dust challenge in a rabbit animal model [Jones et al. 1997; Wallace et al. 2002].

2.3.3 Conduct selective epidemiologic studies of workers exposed to various types of mineral fibers and other EMPs

Statistically powerful and well designed epidemiological studies are typically very expensive and time consuming, but they have been invaluable for defining associations between human health outcomes and occupational exposures. In fact, the strongest human evidence indicating that, at a sufficient dose and with a sufficient latency, certain EMPs of thoracic dimension and high durability pose risks for malignant and nonmalignant respiratory disease has been provided by results from epidemiologic studies of workers exposed to fibers from asbestos minerals.

Results from epidemiologic studies of workers exposed to EMPs from nonasbestiform amphibole minerals have provided limited, if any, evidence in support of an association between occupational exposure and lung cancer or mesothelioma. To understand if occupational exposure to nonasbestiform amphibole EMPs is associated with insignificant risk, it will be important to identify the criteria for epidemiological studies or meta-analyses necessary to conclude that exposure is not associated with a risk that warrants preventive intervention. Clearly laying out these criteria and assessing the feasibility of conducting the necessary studies should be done by a panel of knowledgeable experts. Laboratory research will undoubtedly shed much light on the issue of potential human health risks associated with specific physicochemical characteristics of EMPs, including amphibole cleavage fragments. Still, there is reason to consider conducting, where not only feasible but also judged likely to be informative:

- Epidemiological studies of worker populations exposed to amphibole cleavage fragments (e.g., taconite miners in Minnesota, talc miners in New York, etc.) that are conducted *de novo,* or through updating prior studies for more complete follow-up of health outcomes and/or through re-analyses of archived exposure samples for development of more specific knowledge concerning etiologic determinants and quantitative risk assessment information;

- Epidemiological studies of populations incidentally exposed to EMPs from fibrous minerals, including asbestiform minerals (e.g., those associated with Libby vermiculite);

- Epidemiological studies of populations exposed to other less-well-studied EMPs (e.g., wollastonite, attapulgite, and erionite); and

- Meta-analyses of data from multiple epidemiological studies of various populations, each exposed to EMPs with somewhat different attributes (e.g., EMP type, dimensions, etc.) to better define specific determinants of EMP-associated adverse health outcomes for risk assessment purposes.

Outcomes from proposed research efforts outlined above in Section 2.3.2 may identify additional opportunities for informative epidemiological studies following the lead of NIOSH researchers who have recently undertaken a reanalysis of data from a prior epidemiological study of asbestos textile workers after having more thoroughly characterized exposures using sample filters archived from that study [Kuempel et al. 2006]. Outcomes from the approaches outlined above in Section 2.3.2 might also potentially identify opportunities for aggregate meta-analyses of data from multiple prior epidemiological studies, allowing an assessment of risks across various types of EMPs.

Large unstudied populations with sufficiently high exposure to asbestos fibers are unlikely to be identified in developed countries like the U.S., where asbestos use has been markedly curtailed and where occupational exposures have been strictly regulated in recent decades. Nevertheless, some developing countries (where asbestos use continues on a large scale and where exposures may be less regulated) may offer opportunities for *de novo* epidemiological studies that could contribute to a more refined understanding of the association of human health outcomes to occupational exposures to asbestos and other EMPs. Opportunities for epidemiological studies of exposed workers might be sought in other countries where medical registry data and historical or current workplace sampling data samples are available (e.g., in China, where epidemiological studies of another occupational dust disease, silicosis, have been collaboratively conducted by Chinese and NIOSH researchers [Chen et al. 2005]).

The following criteria should be considered in selecting and prioritizing possible populations for epidemiological study: (1) type of EMP exposure (e.g., mineral source, chemical composition, crystalline structure, surface characteristics, and durability); (2) adequate exposure information (e.g., EMP concentrations and (bivariate) EMP dimensions); (3) good work histories; (4) sufficient latency and number of workers to provide adequate statistical power to detect the health outcome(s) of interest; and (5) availability of data on other potentially confounding risk factors. Priority should be placed on epidemiological studies with potential to contribute to the understanding of EMP characteristics that determine toxicity, including type of mineral source (e.g., asbestiform mineral habit vs. other fibrous mineral habit vs. blocky mineral habit) and morphology and other aspects of the airborne EMPs (e.g., dimensions [length and width], chemical composition, crystalline structure, surface characteristics, and durability).

In addition to epidemiologic studies that address etiology and that quantify exposure-related risk, epidemiologic studies can be used to better understand the pathogenesis of lung diseases caused by asbestos fibers and other EMPs. For example, appropriately

designed epidemiological studies could be used to assess the relationship between lung fibrosis and lung cancer.

2.3.4 Improve clinical tools and practices for screening, diagnosis, treatment, and secondary prevention of diseases caused by asbestos fibers and other EMPs

Given the huge human and economic impact of asbestos-related disease and litigation, Congress has considered asbestos-related legislation on several occasions in recent years. Bills with provisions to require private industry to fund an asbestos victims trust fund have not succeeded in passing Congress. A "Ban Asbestos in America Act," which recently passed the U.S. Senate and is pending consideration in the House of Representatives, would authorize and fund a network of Asbestos-Related Disease Research and Treatment Centers to conduct research, including clinical trials, on effective treatment, early detection, and prevention [U.S. Senate 2007]. This bill also calls for the establishment of a mechanism for coordinating and providing data and specimens relating to asbestos-caused diseases from cancer registries and other centers, including a recently funded virtual biospecimen bank for mesothelioma [Mesothelioma Virtual Bank 2007].

Various research objectives relevant to clinical aspects of asbestos-related diseases are worthy of pursuit by NIOSH and other Federal agencies along with their partners to improve screening, diagnosis, secondary prevention, and treatment. These include, but are not limited to:

- Develop and validate approaches for standardized assessment of digital chest radiographs using the ILO classification system. The ILO system for classifying chest radiographs of the pneumoconioses is widely used as a standard throughout the world. While initially intended for use in epidemiological studies, the ILO system is now widely used as a basis for describing severity of disease in clinical care and for awarding compensation to individuals affected by non-malignant diseases of the chest caused by asbestos and other airborne dusts. The ongoing rapid displacement of traditional film radiography by digital radiography has raised concerns about whether and how the ILO system can be validly applied to digital chest images. Research is needed to describe specifications for classifying digital chest images using the ILO system.

- Develop and promote standardized assessment of non-malignant dust-induced diseases, including asbestos-related pleural and parenchymal disease, on computed tomography (CT) images of the chest. Over the past several decades, CT scanning of the chest has become increasingly used for assessing chest disease and high-resolution CT scanning is often done in clinical settings. While approaches for standardizing classifications of CT images for dust-related

diseases have been proposed, they have not yet been widely adopted or authoritatively promoted.

- Develop, validate, and promote standardization of approaches for assessment of past asbestos exposures by measurement of asbestos bodies and uncoated fibers, particularly in samples collected noninvasively such as sputum. Various approaches for quantifying fiber burden have been used for research and clinical purposes, but results are often difficult or impossible to compare across different studies due to lack of standardization and differential rates of biopersistence and translocation of various types of asbestos fibers.

- Develop and validate biomarkers for asbestosis, lung cancer, and mesothelioma to enable more specific identification of those at risk or early detection of disease in those previously exposed to asbestos. For example, non-invasive bioassays for mesothelioma warrant further research before they can be considered ready to apply routinely in clinical practice.

- Develop and/or adapt modern medical pulmonary imaging techniques to better define stages of asbestosis, or to provide a new paradigm for early detection or grading of the active disease process. For example, positron emission tomographic (PET) imaging using tracers indicative of active collagen synthesis can detect fibrogenic response in a matter of weeks after quartz dust challenge in a rabbit animal model [Jones et al. 1997; Wallace et al. 2002]. This holds promise for non-invasive approaches for earlier clinical detection and more sensitive surveillance and epidemiological studies, that to date have been circumscribed by the long multi-year or decades-long latency periods in manifestation of pulmonary fibrosis associated with asbestos exposures (e.g., as detected by conventional chest radiography or pulmonary function tests).

- Develop new treatment options to enhance the effectiveness of treatments for established disease and to reduce risk of malignant and nonmalignant disease among those previously exposed to asbestos. For example, many widely used anti-inflammatory drugs exert their effect by inhibiting cyclooxygenase-2 (COX-2), an enzyme that is induced in inflammatory and malignant (including pre-malignant) processes. Promising results of laboratory and case-control epidemiological studies have led to clinical trials of COX-2 inhibitors as adjuvant therapy to enhance treatments for various types of cancer. Research is warranted to determine whether these drugs can reduce the risk of asbestos-related malignancies in exposed individuals.

- Clear clinical guidance for practitioners, based on a synthesis of available literature, should be regularly updated and disseminated in an authoritative manner.

2.4 Develop improved sampling and analytical methods for mineral fibers and other EMPs

There are important scientific gaps in understanding the health impacts of exposure to EMPs. Changes in how EMPs are defined for regulatory purposes will likely have to be accompanied by improvements to currently used analytical methods or development and application of new analytical methods. The ability to differentiate between fibers from the asbestos minerals and EMPs from their nonasbestiform analogs is important, especially if recommendations (e.g., occupational exposure limits) are specific to the type of mineral. However, overcoming this obstacle may be difficult because of: (1) lack of standard criteria for the mineralogical identification of airborne EMPs; and (2) technical difficulties in generating test aerosols of size-specific EMPs representative of worker exposures so that sampling and analytical methods can be tested and validated.

Until new analytical methods are developed and applied, it will be necessary to investigate the various proposals that have been made to adjust current analytical methods, such as those discussed in Section 1.5.2, and additional modifications to the current analytical methods will have to be explored. Improvements in exposure assessment methods are needed to increase the accuracy of the methods used to identify, differentiate, and count EMPs captured in air-sampling filter media.

Some barriers to improving current analytical methods have been identified. Increasing the resolution used for performing PCM analysis may help to increase counts of thinner asbestos fibers. However, any increases in optical microscopy resolution will not be sufficient to detect all asbestos fibers. In addition, any improvements in counting EMPs (e.g., increase in the number of EMPs observed and counted) will need to be evaluated by comparing with counts made by the current PCM method. The use of electron microscopy (EM) would improve the capability to detect thin fibers and also provide a means to identify many types of minerals. However, the routine use of EM would:

(1) require the development of standardized analytical criteria for the identification of various EMPs;
(2) require specialized experience in microscopy and mineral identification;
(3) increase analytical costs; and
(4) potentially increase the lag time between collecting the sample and obtaining results.

In some workplace situations, such as in construction, increases in the time needed to analyze samples and identify EMPs could potentially delay the implementation of appropriate control measures to reduce exposures.

Several potential sampling and analytical improvements are currently under study. Some of the studies are aimed at improving the accuracy of current techniques used for monitoring exposures to asbestos. One such study is evaluating the use of thoracic samplers for the collection of airborne fibers and the use of gridded cover slips when performing PCM analysis. The proposed use of gridded cover slips for sample evaluation

can aid in the counting of EMPs and can provide a means for "recounting" fibers at specific points on the filter sample. Another study is evaluating the proposed ASTM method to determine whether inter-operator variability when performing differential counting (to distinguish fibers from asbestos minerals and other EMPs) is within an acceptable range.

Research into new method development is warranted. One such area would be the development of methods that would permit an assessment of the potential biopersistence (e.g., durability) of EMPs collected on air samples prior to their evaluation by PCM or other microscopic methods. If durability is deemed biologically relevant, then the assessment of only durable EMPs collected on samples would help to reduce possible interferences caused by other EMPs in the analysis. Another such area would be improvement in electron microscopy particle identification techniques, such as field emission SEM and the capability to determine the elemental composition of EMPs using an SEM-equipped with EDS.

Modifications of current analytical methods and development of new analytical methods will require an assessment of worker health implications (e.g., how do the results using improved or new methods relate to human risk estimates based on counts of EMPs made by PCM?). To ensure that relevant toxicological parameters (e.g., dimension, durability, and physicochemical parameters) are incorporated in the analysis and measurement, changes in analytical methods should be made in concert with changes in how asbestos fibers or other EMPs are defined.

2.4.1 Reduce inter-operator and inter-laboratory variability of the current analytical methods used for asbestos fibers

To ensure the validity of EMP counts made on air samples, it is important to ensure consistency in EMP counts between analysts. Microscopic counts of EMPs on air samples are made using only a small percentage of the surface area of the filter, and the counting procedures require the analysts to make decisions on whether each observed particle meets specified criteria that define a fiber. Interlaboratory sample exchange programs have been shown to be important for ensuring agreement in asbestos fiber counts between laboratories [Crawford et al. 1982]. Unfortunately, microscopists from different laboratories are unlikely to view exactly the same fields, resulting in some of the observed variation that exists in fiber counts between microscopists. A mechanism to allow recounts of fibers from the exact same field areas would remove this variable and allow a better assessment of the variation between microscopists in analyzing samples.

A technique is under development for improving the accuracy of PCM fiber-counting by allowing the same sample fields to be examined by multiple microscopists or the same microscopist on different occasions [Pang et al. 1984; Pang et al. 1989; Pang 2000]. The method involves the deposition of an almost transparent TEM grid onto the sample.

Included with the grid are coordinates allowing each grid opening to be relocated. Photomicrographs of typical grid openings superimposed on chrysotile and amosite samples have been published [Pang et al. 1989]. Slides prepared in this manner have been used in a Canadian proficiency test program for many years. The main errors affecting the counts of various types of fibers (e.g., chrysotile, amosite, and SVF) have been evaluated by examining large numbers of slides by large numbers of participants in this program. A recently developed scoring system for evaluating the performance of microscopists is based on errors compared with a reference value defined for each slide by the laboratory in which they were produced [Pang 2002]. A statistical analysis of the intragroup precision in this study was able to identify those analysts who were outliers [Harper and Bartolucci 2003]. In a pilot study, the pooled relative standard deviations, without the outliers, met the requirements for an unbiased air sampling method. Further study is needed to validate these findings and to identify other techniques that can reduce inter-laboratory and inter-operator variability in counting EMPs by PCM.

Reference slides made from proficiency test filters from the American Industrial Hygiene Association (AIHA) have been created and circulated to laboratories and individual microscopists recruited from the AIHAs laboratory quality programs. Initial results have been published [Pang and Harper 2008] and further results have been submitted for publication [Harper et al. 2008b]. The results illustrate clearly the greater discrimination possible between microscopists with proficiency test materials of more controlled composition. These reference slides have also been evaluated in Japan, the United Kingdom, and Europe. Further research will be useful in determining the value of these slides for training purposes.

2.4.2 Develop analytical methods with improved sensitivity to visualize thinner EMPs to ensure a more complete evaluation of airborne exposures

Most PCMs can visualize EMPs with widths >0.25 µm, which is the approximate lower resolution limit when the microscope is operated at a magnification of 400X and calibrated to NIOSH 7400 specifications [NIOSH 1994a]. However, higher-end optical microscopes can resolve thinner widths, and, depending on the variety of asbestos, they may resolve widths as small as 0.1 µm for crocidolite.

Improvement in the optical resolution may be possible using an oil-immersion 100X objective with a numerical aperture of 1.49. Also, the use of 15X eyepiece oculars would help improve the visibility of small particles and thin EMPs on samples. However, using oil immersion has several drawbacks. The oil dries on the slide when exposed to air for more than a few hours which changes the oil's optical properties. Also, the oil cannot be wiped off after looking at the slide because the cover slip is likely to be moved and ruin the sample. For these reasons using oil immersion does not permit recounts or further QC analysis and is not an attractive alternative.

Other methods may also allow for increased resolution using optical microscopes. Anecdotal information on the use of dark-medium microscopy (DM), presented at a meeting in November 2007, suggests that analysts using DM objectives could resolve all blocks of the HSL/NPL test slide (contrary to method requirements) and produced higher counts of chrysotile fibers than expected [Harper 2008c]. The implication is that using DM resolves thinner chrysotile fibers than does the accepted method. This methodology should be explored further to determine its resolution and potential application in asbestos exposure assessment.

However, because risk estimates for workers exposed to asbestos fibers have been based on fiber counts made by the current PCM method, fiber counts made with improved optical microscope resolution capabilities would not be directly comparable to current occupational exposure limits for asbestos. Additionally, the findings that asbestos fibers thinner than 0.1 μm are most associated with mesothelioma and that optical microscopes cannot resolve fibers <0.1 μm suggest that the PCM method should be used only as interim method until limitations relating to the cost, availability, and time for analysis issues of using EM methods are overcome, or other methods are identified, developed, and validated.

TEM can resolve asbestos fibers with widths <~0.01 μm, which is adequate for detecting the presence of asbestos fibers and other EMPs collected on airborne samples. TEM, as well as SEM, provide greater resolution for detecting and sizing EMPs. Both methods also provide capability for mineral identification using selected area x-ray diffraction (SAED) and/or elemental analysis (e.g., EDS and WDS). The cost of using TEM and/or SEM for routine sample analysis would be considerably higher than PCM analysis and the turnaround time for sample analysis would be increased substantially. In addition, any routine use of EM methods for counting and sizing fibers would require an evaluation of inter-operator and inter-laboratory variability.

SEM is now a generally available method which can routinely resolve features down to ~0.05 μm, an order of magnitude better than optical microscopes, and into the fiber size range implicated in disease. Field emission SEM (FE-SEM) is now commercially available and further increases this resolution. Laboratory *in vitro* or short-term or long-term animal model studies can now utilize these EM imaging technologies to characterize EMPs for studies of disease mechanism. For detailed laboratory studies of the role of EMP chemistry and surface composition in disease mechanism, EM analyses of EMP size and composition can be complemented with analysis of surface elemental composition by scanning Auger spectroscopy or X-ray photoelectron spectroscopy. Investigation is needed to determine whether SEM-backscatter electron diffraction analysis can be adapted to EMP crystallinity analyses equivalent to TEM-SAED capability. Ease of sample preparation and data collection for SEM analysis compared to TEM, along with some SEM advantage in visualizing EMP and EMP morphology (e.g., surface characteristics), suggests a re-evaluation of SEM methods for EMP characterization and mineral identification both for field and laboratory sample analysis.

2.4.3 Develop a practical analytical method for air samples to differentiate between exposures to asbestiform fibers from the asbestos minerals and exposures to EMPs from their nonasbestiform analogs

A recently published ASTM International method for distinguishing fiber-like particles from probable asbestos fibers uses PCM-determined morphologic features to differentiate asbestos fibers from other EMPs [ASTM 2006]. The proposed method has several points of deviation from existing PCM methodologies. It uses a new graticule that has not been tested for conformance with the traditional graticule used in PCM analysis of asbestos air samples. It specifies additional counting rules to classify particles, and there are few data to show these rules provide a consistently achievable or meaningful result. Also, only limited data are available to show inter- or intra-operator or inter-laboratory variation. These issues must be addressed before the methodology can be considered acceptable. NIOSH currently has a project addressing these issues. Specific aims of the project are:

- To determine the effect of using the traditional Walton-Beckett graticule and the new RIB graticule on the precision of measuring fiber dimensions; and

- To determine the inter-laboratory variation of the proposed method for determining particle identities through observation of morphological features.

The outcomes of these aims would include a measure of method precision, which will help to determine whether the method meets the requirements of regulatory and other agencies.

While EM may not be suitable for routine analysis of samples of airborne EMPs, current EM techniques used to characterize and identify minerals (e.g., differentiating between asbestos fibers and other EMPs) need to be further investigated and evaluated to determine whether the results can be reproduced by multiple microscopists and laboratories.

2.4.4 Develop analytical methods to assess fiber durability of EMPs

While some research has been conducted to determine the ability of biological assays to evaluate the biopersistence of EMPs in the lung, there is a need to consider how the assessment of EMP durability might be incorporated into the evaluation of air samples containing a heterogeneous mix of EMPs. Research with several types of glass fibers and some other SVFs indicate that they dissolve in media at different rates depending on the pH and that they dissolve more rapidly than chrysotile and amphibole asbestos fibers [Leineweber 1984]. Chrysotile fibers have been shown to dissolve at a rate which varies not only with the strength of the acid, but also with the type of acid. Amphibole asbestos fibers have been shown to be more resistant to dissolution than chrysotile fibers.

Research suggests that the rate of dissolution for most EMPs appears to be strongly dependent on their chemical composition, surface characteristics, and dimension.

The selective dissolution of EMPs might be a useful approach in eliminating specific types of EMPs or other particulates collected on air samples prior to analysis (e.g., microscopic counting). The removal of interfering EMPs prior to determining fiber concentrations could eliminate the need for additional analysis to identify EMPs on the sample and thereby reduce analytical time. Selective dissolution of samples to remove interferences is well established in NIOSH practice for other analytes. Method 5040 for diesel exhaust has an option for using acidification of the filter sample with hydrochloric acid to remove carbonate interference [NIOSH 2003a]. Silicate interferences for quartz by infra-red spectroscopic detection are removed by phosphoric acid digestion in Method 7603 [NIOSH 2003b]. Although selective dissolution might be accomplished, research will be necessary to develop and characterize a procedure that would correlate residual EMP counts to toxicity.

2.4.5 Develop and validate size-selective sampling methods for EMPs

For measuring concentrations of non-elongated dust in workplaces, conventions have been developed for sampling the aerosol fractions that penetrate to certain regions of the respiratory tract upon inhalation: the inhalable fraction of dust that enters into the nose or the mouth; the thoracic fraction of dust that penetrates into the thorax (i.e., beyond the larynx); and the respirable fraction of dust that reaches the alveolar lung. The thoracic convention is recognized by NIOSH and other organizations that recommend exposure limits, and NIOSH has begun to apply it in the derivation of RELs (e.g., the REL for metalworking fluid aerosols [NIOSH 1998]).

Asbestos fibers currently are collected for measurement using a standard sampling and analytical method, which is described in Method 7400 [NIOSH 1994a], in OSHA ID-160 [OSHA 1998] in Methods for the Determination of Hazardous Substances (MDHS) 39/4 [HSE 1995], and in ISO 8672 [ISO1993]. In these methods, air samples are taken using a membrane filter housed in a cassette with a cowled sampling head. Early studies [Walton 1954] showed that some exclusion of very coarse particles occurs due to elutriation in the vertical cowl, but its selection characteristics should have little effect on the collection efficiency for asbestos fibers. However, when Chen and Baron [1996] evaluated the sampling cassette with a conductive cowl used in sampling for asbestos fibers, they found inlet deposition was higher in field measurements than predicted by models.

Currently, NIOSH does not recommend an upper limit for asbestos fiber width since airborne asbestos fibers typically have diameters <3 μm. The absence of an upper diameter for the NIOSH Method 7400 A rules has generated some criticism that some fibers counted by this method may not be thoracic-sized. Others have recommended NIOSH Method 7400 B rules for the sampling and analysis of various types of fibers,

including asbestos fibers [Baron 1996], because the B rules specify an upper limit of 3 μm for fiber width. However, Method 7400 B rules have not been field-tested for the collection and analysis of occupational exposures to many types of EMPs or organic synthetic fibers.

Two separate but complementary projects have examined the performance of thoracic samplers for EMPs [Jones et al. 2005; Maynard 2002]. Thoracic samplers allow the collection of airborne particles that meet the aerodynamic definition of thoracic-sized fibers (i.e., with physical widths equal to or less than 3 μm for the typical length distributions of fibers of silicate composition), eliminating the deposition of large particles on the sample filter and collecting only those EMPs considered most pathogenic. The results of studies have indicated that penetration of some thoracic samplers is independent of EP length, at least up to 60 μm, indicating that the samplers' penetration characteristics for an EP aerosol should be no different than that of an isometric aerosol. In the Jones et al. [2005] study, the relative ability of the thoracic samplers to produce adequately uniform distributions of fibers on the surface of the membrane filter was also tested. Based on results of these studies, two samplers appeared to meet the criteria of minimal selection bias with respect to EP length and even distribution on the collection filters. However, neither of these samplers has been tested under conditions of field use. NIOSH is currently evaluating these two thoracic samplers and the traditional cowled sampler in three different mining environments. The results from the first of these environments have been published [Lee et al. 2008]. In this study, one sampler provided results as expected in comparison to the standard 25-mm cowled cassette, while the other did not. Additional results are required to clarify this conclusion.

2.5 How the proposed research framework could lead to improved public health policies for asbestos fibers and other EMPs

Section 2 of this document proposes several strategic goals and associated objectives for a multi-disciplinary research program to further elucidate the physicochemical properties of asbestos fibers and other EMPs that contribute to their pathogenicity. A major component of the proposed research will be aimed at improving existing analytical tools and developing new analytical tools for identifying and measuring exposures to EMPs using metrics that reflects the important determinants of toxicity (e.g., dimension, composition, etc.).

Results of many studies reported in the scientific literature offer some insight into possible physicochemical properties of asbestos fibers and biological mechanisms involved in asbestos-related human disease. Much of this evidence supports the important role of particle dimension as a determinant of lung deposition and retention and the concomitant role of particle composition and crystalline structure as a determinant of durability and biopersistence. Despite this body of research, several fundamental issues are not clearly understood and a broad systematic approach to further toxicological and

epidemiological research would help to reduce remaining uncertainties. Although long, thin asbestos fibers clearly cause respiratory disease, the role of unregulated short (i.e., <5μm) fibers is not entirely clear. It also remains unclear to what extent each of the various physicochemical parameters of asbestos fibers is responsible for respiratory disease outcomes (e.g., asbestosis, lung cancer, and mesothelioma) observed in asbestos-exposed individuals. Limited evidence from studies with other EMPs confirms the importance of particle dimension and biopersistence in causing a biological response. However, uncertainty remains as to whether the respiratory disease outcomes observed in workers exposed to asbestos fibers can be anticipated for workers exposed to other EMPs of thoracic-size and with mineral compositions similar to asbestos.

Results of much of the research to date, conducted on materials that are readily available or of specific interest, should be considered in developing the research program, including the specification of materials to be studied. Another important effort that can inform development of the research program will involve a systematic collection and review of available information on: (1) industries and occupations with exposure to EMPs; (2) airborne exposure data in these industries and occupations; and (3) numbers of workers potentially exposed in these industries and occupations. Any additional relevant minerals and mineral habits identified should also be considered. The minerals identified through these efforts should be carefully and comprehensively characterized with respect to both structure and composition. In the characterization of the mineral, consideration should also be given to: (1) purity of the mineral; (2) particle morphology (range of dimensions and sizes); (3) surface area; (4) surface chemistry; and (5) surface reactivity. Care must be taken to ensure that a sufficient amount of the studied material is available not only for the study, but also as reference material for possible future studies. The information developed from all of these efforts should be entered into a database which can serve as a tool for selection of minerals for testing and validation of toxicological tests, as well as to assist in identification of worker populations for epidemiological studies.

An objective of the proposed research is to achieve a level of mechanistic understanding that could provide a basis for developing biologically-based models for extrapolating results of animal inhalation and other types of *in vivo* studies to exposure conditions typically encountered in the workplace. Presently, little information exists on the mechanisms by which asbestos fibers and some other EMPs produce lung cancer, mesothelioma, and non-malignant respiratory disease. As these mechanisms become understood, biologically based models could be developed to extrapolate from exposure-dose-response relationships observed in animals to estimates of disease risk in exposed humans. In addition, such studies would provide: (1) an opportunity to measure molecular and cellular outcomes that can be used to determine why one animal species responds differently from another; and (2) information on EMP characteristics associated with eliciting or potentiating various biological effects. The outcomes of these studies can then be evaluated in subsequent experiments to provide: (1) risk assessors with the various disease mechanisms by which animals respond to EMP exposures; and (2)

regulatory agencies and industrial hygiene and occupational health professionals with information needed to implement appropriate exposure limits and programs for monitoring worker exposure and health.

It is anticipated that it will be difficult to find populations of workers with exposures to minerals with characteristics (e.g., dimension, composition) of interest, that are sufficiently large to provide adequate statistical power, and where exposures are unconfounded or where any confounding can be effectively controlled in the analysis. NIOSH has exposure information and, in some cases, personal samples collected and archived from past epidemiology studies of workers exposed to asbestos fibers and other EMPs. NIOSH intends to explore how such existing data might be used to update and extend findings from these studies. Where appropriately balanced epidemiological studies can be identified, it may be possible to conduct meta-analyses to investigate important EMP characteristics. The analysis of archived samples may help to elucidate how more detailed characteristics of exposure (e.g., fiber dimension) relate to disease outcomes. Any new epidemiologic (retrospective and prospective) studies should not undertaken unless appropriate feasibility studies (e.g., preliminary assessments of study population size, exposure latencies, records of exposure, confounders, etc.) have been appropriately considered.

Because of the limited opportunities for epidemiological studies, it will be necessary to complement them with toxicological testing, and an integrated approach to toxicological research will be needed to understand how these minerals might potentially induce disease. Where epidemiological studies are possible, or can be updated, attempts should be made to link their results with those of toxicological studies to assess the ability of various types of toxicological testing to predict health outcomes in humans. Toxicological testing should be done with attention to detailing more specific information, including: (1) physical characteristics (e.g., dimension); (2) chemical composition; (3) *in vitro* acellular data (dissolution, durability); and (4) *in vitro/in vivo* cellular data (e.g., cytotoxicity, phagocytosis, chromosomal damage, mediator release).

To help elucidate what physicochemical properties are important for inducing a biological effect, it may be necessary to generate exposures to EMPs of specific dimensions and composition. Several approaches are being pursued by NIOSH to overcome technological difficulties in generating sufficient quantities of well-characterized and dimensionally-restricted EMPs. NIOSH's efforts to grind test minerals to appropriate size ranges has met with some success, but has not been consistently able to generate EMPs in restricted size ranges of interest or in sufficient quantity to enable toxicity testing. Another approach has used a fiber size classifier developed at NIOSH [Deye et al. 1999], but this has not been able to provide quantities of EMPs large enough for long-term inhalational exposure studies in animals. NIOSH is currently evaluating the possibility of developing a fiber size classifier with increased output that may be able to generate much larger quantities of particles in restricted size-ranges for toxicological testing.

A goal of the research should be to develop an understanding of the relationships between and among the results of human observational studies and *in vitro*, short-term *in vivo*, and long-term *in vivo* experimental studies. Any research undertaken should be designed to ensure that results can be interpreted and applied within the context of other studies. For example, EMPs used in long-term animal inhalation studies should also be tested in *in vitro/in vivo* assay systems so that findings can be compared. The results of such experiments can help to develop and standardize *in vitro/in vivo* assay systems for use in predicting the potential toxicity of various types of EMPs.

Federal government agencies, organizations, and individual researchers have already recommended similar research strategies for evaluating the toxicity of mineral and synthetic fibers [Greim 2004; ILSI 2005; Mossman et al. 2007; Schins 2002; Vu et al. 1996]. These published strategies should be used as a foundation for developing a research program.

Some research and improvements in sampling and analytical methods used to routinely assess exposures to EMPs can be done in the short term, and as the results of the toxicological and epidemiological studies provide a clearer understanding of EMP characteristics that determine toxicity, it will be necessary to incorporate the results into improved sampling and analytical methods. Goals for these methods will be to: (1) reduce the subjectivity inherent in current methods of particle identification and counting; (2) to closely quantify EMPs based on the characteristics that are important to toxicity; and (3) to reduce cost and shorten turnaround times compared to current EM methods.

The toxicological, exposure assessment, and epidemiological research should be conducted with the overarching goal of developing information necessary for risk assessments. Risk assessments and available analytical methodology will inform the development of recommendations for occupational exposure limits and establish goals for any warranted controls for workplaces to comply with the recommended exposure limits.

3 THE PATH FORWARD

The framework for a research agenda proposed in *Asbestos Fibers and Other Elongated Mineral Particles: State of the Science and Roadmap for Research* will require a substantial investment of time, scientific talent, and resources by NIOSH and its partners to formulate research programs and prioritize research projects to achieve the proposed goals. However, achieving these goals will be well worth the investment because optimal occupational health protection policies for asbestos fibers and other EMPs will be based on the results of sound scientific research. As with any strategic approach, there may be unintended and unforeseen consequences that will require program adjustments as time goes on.

Some of the next steps will involve organizing study groups with representatives from Federal agencies, industry, academia, and workers' groups to identify the specific research to be done within this overarching research program. Study groups should be assembled to identify the specific research elements needed to address the information gaps and data needs outlined in this *Roadmap*. It may be appropriate to organize separate study groups around the scientific disciplines needed to conduct the research, such as epidemiology, toxicology, exposure assessment, analytical, and risk assessment. These study groups should be maintained over the lifetime of the research program to oversee and help guide the research. Also important will be coordination between study groups to ensure the efforts in the various research areas are complementary and move toward consistent goals and the eventual development of sufficient information for risk assessment. An independent group could also be included for oversight of the research programs to periodically review the research programs, help keep the research programs focused on the most appropriate research, and help ensure quality of the research.

The ideal outcome of a comprehensive research program for asbestos fibers and other EMPs would be use the results of this research to develop recommendations for thoracic-sized EMPs to protect workers' health that are based on unambiguous science. Ideal recommendations would specify criteria, such as a range of chemical composition, dimensional attributes (e.g., ranges of length, width, and aspect ratio), dissolution rate/fragility parameters, and other factors that can be used to indirectly assess the toxicity of EMPs. It would be particularly advantageous if criteria for inclusion of EMPs in the recommendations could be based primarily on results from validated *in vitro* or short-term *in vivo* assays. This would reduce the need for comprehensive toxicity testing and/or epidemiological evaluation of each material. Such an approach would have the advantage of identifying EMPs warranting concern based on their qualities and attributes, and newly identified EMPs (and even new synthetic fibers) could be compared to the criteria to determine a likelihood of toxicity. Coherent risk management approaches for EMPs that fully incorporates a clear understanding of the toxicity would then be developed to minimize the potential for disease.

The extent to which a policy concerning thoracic-size EMPs could be extended to SVFs and even to other manufactured materials such as engineered nanomaterials, would need to be explored. Although engineered nanomaterials are well beyond the scope of the *Roadmap*, it has been noted that elongated nanoscale particles (e.g., single-walled carbon nanotubes) have been shown to cause interstitial fibrosis in mice beginning within 7 days from the onset of exposure and progressing over a 60-day post-exposure period [Shvedova et al. 2005] In addition, peritoneal exposure of mice to carbon nanotubes has been reported to induce pathological responses similar to asbestos, suggesting potential for induction of mesothelioma [Poland et al. 2008]. Recommendations have been made to systematically investigate the health effects of these manufactured nanomaterials within the next five years [Maynard et al. 2006; NIOSH 2008]. Integrating the results of these nanoparticle toxicity investigations with the results of the research program developed as a result of this *Roadmap* may further a broader and more fundamental understanding of the determinants of toxicity of EPs.

Achieving the goals delineated in *Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research* is consonant with NIOSH's statutory mission to generate new knowledge in the field of occupational safety and health and to transfer that knowledge into practice for the benefit of workers. Advancing knowledge relevant for use in protecting workers from adverse health effects arising from exposure to asbestos fibers and other EMPs is the ultimate goal.

Though further scientific research conducted by NIOSH will continue to focus on the *occupational* environment, NIOSH intends to pursue partnerships to ensure that the results of any scientific research arising from the *Roadmap* can be extended to the general community and the general environment.

To ensure that the science arising from execution of the *Roadmap* is applied as broadly as possible, NIOSH plans to partner with other Federal agencies, including the Agency for Toxic Substances and Disease Registry (ATSDR), the Consumer Product Safety Commission (CPSC), the Environmental Protection Agency (EPA), the Mine Safety and Health Administration (MSHA), the National Institute of Standards and Technology (NIST), the National Institute of Environmental Health Sciences (NIEHS), the National Toxicology Program (NTP), the Occupational Safety and Health Administration (OSHA), and the United States Geological Survey (USGS), as well as with labor, industry, academia, practitioners, and other interested parties including international groups. Partnerships and collaborations will be used to help focus the scope of the research to be undertaken, enhance extramural research activities, and assist in the development and dissemination of educational materials describing the outcomes of the research and their implications for occupational and public health policies and practices.

NIOSH looks forward to integrating the research goals set forth in the *Roadmap* into the industry sector-based and research-to-practice-focused National Occupational Research

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Agenda (NORA). NORA is an agenda for the Nation and the goals and objectives of this *Roadmap* can be substantially advanced through robust public-private sector partnership.

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5 GLOSSARY

Acicular: The very long and very thin, often needle-like shape that characterizes some prismatic crystals. (Prismatic crystals have one elongated dimension and two other dimensions that are approximately equal.) Acicular crystals or fragments do not have the strength, flexibility, or other properties often associated with asbestiform fibers.

Actinolite: An amphibole mineral in the tremolite-ferroactinolite series. Actinolite can occur in both asbestiform and nonasbestiform mineral habits. The asbestiform variety is often referred to as actinolite asbestos.

Amphibole: A group of minerals composed of double chain SiO_4 tetrahedra linked at the vertices and generally containing ions of iron and/or magnesium in their structures. Amphibole minerals are of either igneous or metamorphic origin. Amphiboles can occur in a variety of mineral habits including asbestiform and nonasbestiform.

Amosite: An amphibole mineral in the cummingtonite-grunerite series that occurs in the asbestiform habit. The name amosite is a commercial term derived from the acronym for "Asbestos Mines of South Africa." Amosite is sometimes referred to as "brown asbestos."

Anthophyllite: An amphibole mineral that can occur in both the asbestiform and nonasbestiform mineral habits. The asbestiform variety is referred to as anthophyllite asbestos.

***Asbestiform*:** A specific type of mineral fibrosity in which crystal growth is primarily in one dimension and the crystals form as long, flexible fibers. In minerals occurring in asbestiform habit, fibers form in bundles that can be separated into smaller bundles and ultimately into fibrils.

Asbestos: A generic term for silicate minerals occurring in the asbestiform habit, usually used to refer to those minerals that have been commercially exploited as asbestos, including chrysotile in the serpentine mineral group and tremolite asbestos, actinolite asbestos, anthophyllite asbestos, cummingtonite-grunerite asbestos (amosite), and riebeckite asbestos (crocidolite) in the amphibole mineral group. See also *Covered Mineral*.

Aspect ratio: The ratio of the length of a particle to its diameter.

Biopersistence: The ability to remain in the lung or other tissue. Biopersistence of mineral fibers is a function of their fragility, solubility, and clearance.

Chrysotile: A mineral in the serpentine mineral group that occurs in the asbestiform habit. Chrysotile generally occurs segregated as parallel fibers in veins or veinlets and can be easily separated into individual fibers or bundles. Often referred to as "white asbestos," chrysotile is used commercially in cement or friction products and for its good spinnability in the making of textile products.

Cleavage fragment: A particle, formed by comminution (i.e., crushing, grinding or breaking) of minerals, often characterized by parallel sides. In contrast to a fibers from an asbestos mineral; EMPs in a population of cleavage fragments are generally wider and shorter, have generally lower aspect ratio, and do not exhibit fibrillar bundling at any level of examination.

Countable Particle: A particle that meets specified dimensional criteria and is (to be) counted according to an established protocol. A countable particle under the NIOSH asbestos fiber definition is any acicular crystal, asbestiform fiber, prismatic crystal, or cleavage fragment of a *Covered Mineral* which is longer than 5 μm and has a minimum aspect ratio of 3:1 based on a microscopic analysis of an airborne sample using NIOSH Method 7400 or an equivalent method.

Covered Mineral: Minerals encompassed under the current NIOSH REL for Asbestos Fibers and Related Elongated Mineral Particles which includes minerals having the crystal structure and elemental composition of the asbestos varieties [chrysotile, riebeckite asbestos (crocidolite), cummingtonite-grunerite asbestos (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos], or their nonasbestiform analogs (the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the cummingtonite-grunerite mineral series, the tremolite-ferroactinolite mineral series, and the glaucophane-riebeckite mineral series).

Crocidolite: An asbestiform amphibole mineral in the glaucophane-riebeckite series. Crocidolite, commonly referred to as "blue asbestos," is a varietal name for the asbestiform habit of the mineral riebeckite

Durability: The tendency of particles to resist degradation in lung fluids.

Elongated Mineral Particle (EMP): any particle or fragment of a mineral (e.g., fibril or bundle of fibrils acicular, prismatic, or cleavage fragment) with a minimum aspect ratio of 3:1, based on a microscopic analysis of an airborne sample using NIOSH Method 7400 or an equivalent method.

Elongated Particle (EP): A particle with a minimum aspect ratio of 3:1, based on a microscopic analysis of an airborne sample using NIOSH Method 7400 or an equivalent method.

Fiber: "Fiber" can be used in a regulatory context or in a mineralogical context.

In the regulatory context, a fiber is an elongated particle equal to or longer than 5 µm with a minimum aspect ratio of 3:1. The dimensional determination is made based on a microscopic analysis of an air sample using NIOSH Method 7400 or an equivalent method.

In the mineralogical context, a fiber is an elongated crystalline unit that resembles an organic fiber and that can be separated from a bundle or appears to have grown individually in that shape.

Fibril: A single fiber of asbestos which cannot be further separated longitudinally into thinner components without losing its fibrous properties or appearances.

Fibrous: A descriptive characteristic of a mineral composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable.

Fragility: The tendency of particles to break into smaller particles**.**

Nonasbestiform: Not having an asbestiform habit. The massive non-fibrous forms of the asbestos minerals have the same chemical formula and internal crystal structure as the asbestiform variety, but have crystal habits where growth is more equivalent in two or three dimensions instead of primarily one dimension. When milled or crushed, nonasbestiform minerals generally do not break into fibers/fibrils but rather into fragments resulting from cleavage along the two or three growth planes. Often cleavage fragments can appear fibrous.

Refractory ceramic fiber (RCF): An amorphous, synthetic fiber produced by melting and blowing or spinning calcined kaolin clay or a combination of alumina (Al_2O_3) and silicon dioxide (SiO_2). Oxides (such as zirconia, ferric oxide, titanium oxide, magnesium oxide, and calcium oxide) and alkalies may be added.

Solid solution series: A grouping of minerals that includes two or more minerals in which the cations in secondary structural position are similar in chemical properties and size and can be present in variable but frequently limited ratios.

Synthetic vitreous fiber (SVF): Any of a number of manufactured fibers produced by the melting and subsequent fiberization of kaolin clay, sand, rock, slag, etc. Fibrous glass, mineral wool, ceramic fibers, and alkaline earth silicate wools are the major types of SVF, also called man-made mineral fiber (MMMF) or man-made vitreous fiber (MMVF).

Thoracic-size particle: A particle with an aerodynamic equivalent diameter that enables it to be deposited in the airways of the lung or the gas exchange region of the lung when inhaled.

Tremolite: An amphibole mineral in the series tremolite-ferroactinolite. Tremolite can occur in both fibrous and non-fibrous mineral habits. The asbestiform variety is often referred to as tremolite asbestos. Due only to changes in the International Mineralogical Association's amphibole nomenclature, subsets of what was formerly referred to as tremolite asbestos are now mineralogically specified as asbestiform winchite and asbestiform richterite.