

FILE NAME: Talc (TALC)

DATE: 1992

DOC#: TALC173

DOCUMENT DESCRIPTION: Journal Article - Biological Effects of
Inhaled Minerals

Biological effects of inhaled minerals**GEORGE D. GUTHRIE, JR.**

Geology/Geochemistry, MS D469, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, U S A

ABSTRACT

Numerous studies present data on the biological effects of inhaled minerals, but these data are often disseminated within reports that primarily address the asbestos minerals. Furthermore, these reports are normally published in journals that are unfamiliar to most minerals scientists. This review compiles these data in order to facilitate an understanding of the known biological effects of minerals. An introduction to the types of studies from which the data were drawn is given so that those unfamiliar with such studies can assess the data critically.

In general, minerals exhibit a range of biological activities from apparently inactive or slightly active, such as hematite, to highly fibrogenic and carcinogenic, such as fibrous brucite ("nemalite"). The zeolites also exhibit such a range, with some mordenite being slightly active and erionite being highly active; however, erionite is the only zeolite that has been studied extensively.

Although several mechanisms have been proposed to explain how minerals induce disease, it is still unclear why minerals exhibit a range in biological activity. The diversity of mineral species holds great potential for probing these mechanisms, especially when mineralogical data are integrated with biological data. Unfortunately, many of the studies reporting data on the biological effects of inhaled minerals fail to report detailed mineralogical information; hence, it is difficult at present to interpret the biological activities of minerals in terms of their physical and chemical properties. More collaboration between minerals scientists and health scientists would benefit this area of research by enabling an integration of mineralogical and biological data. Important mineralogical data that are only rarely considered in biological research include exact mineral content of the specimen (i.e., identification and abundance of contaminants), physical and chemical properties of minerals, and surface properties of minerals.

INTRODUCTION

Because of their potential to induce a number of lung diseases (e.g., fibrosis, lung cancer, and mesothelioma), the asbestos minerals, fibrous serpentine (chrysotile) and fibrous amphiboles, have been the focus of numerous experimental studies, governmental regulations, and extensive public concern. Early studies of riebeckite-asbestos miners (Wagner et al., 1960) revealed an association between exposure to riebeckite asbestos and mesothelioma, a rare cancer with an extremely high morbidity rate. Subsequently, a higher than expected incidence of mesothelioma was found in American asbestos workers who were exposed primarily to chrysotile (Selikoff et al., 1964, 1965) but also to amphiboles (Ross, 1984), which occurred more frequently in the lungs of these workers (Langer and Nolan, 1989). The widespread use of the asbestos minerals meant that a large population was exposed and potentially at risk, and so began the proliferation of research on the biological effects of asbestos.

Fear of asbestos exposure led to a replacement of the asbestos minerals with substitutes and a skewing of the research on the biological effects of minerals toward the

asbestos minerals. Nevertheless, a wide range of fibrous and nonfibrous minerals has been studied to some extent for potential health hazards, although such data are frequently hidden within reports that focus on asbestos. These data are important, however, for several reasons. First, all humans are exposed to mineral dusts from both anthropogenic and natural sources. For example, in a 45-yr study on the mineral contents of lungs from residents of Tokyo, Shishido et al. (1989) found that by the early 1980s over 80% of Tokyo's residents had been exposed to mineral dusts. Similar observations have been made in a variety of urban environments, and nonoccupational exposure to mineral dusts can also result from living in a rural environment (e.g., Sébastien et al., 1981, 1984).

Second, various minerals are used both as replacements for asbestos and in numerous other commercial products, and the health risks associated with these minerals may differ from those associated with serpentine asbestos or with amphibole asbestos. If minerals are to be used and regulated properly, it is important to assess accurately the risk from exposure to each mineral and not simply assume that all fibrous minerals are equally hazardous.

Third, the mechanisms by which minerals in general (including asbestos) are toxic and carcinogenic can be more readily elucidated if differences in the toxicity and carcinogenicity of various minerals can be related to fundamental differences in crystal structure and crystal chemistry. Collaborative efforts between minerals scientists and life scientists would be extremely effective at achieving this. Unfortunately, very few minerals scientists are involved directly in such research, primarily because of scientific language barriers and a lack of familiarity with current issues in health-related mineral-dust research.

In order to address the latter problem, this paper will review the health risks associated with a variety of minerals. Ross (1981, 1984) reviewed the human health hazards associated with the asbestos minerals in a form accessible to geoscientists, and Mossman et al. (1990) presented a review of the current issues in this research field. These papers are useful starting points for anyone interested in the extensive literature available on asbestos. Likewise, the research on the biological effects of silica is extensive, and Heppleston (1984) provides an introduction to this literature. However, no such compilation exists for the literature on numerous other fine-grained minerals. The intentions of this paper are (1) to compile the available data on the biological effects of clays (exclusive of chrysotile), zeolites, and several other fine-grained minerals; (2) to facilitate a better understanding of the data by introducing the research techniques used in the studies from which the data were drawn; and (3) to provide a foundation that can be used by minerals scientists interested in pursuing this area of research.

The intentions of this paper are to avoid any discussion of mineral regulation or the regulatory implications of the material reviewed. Though many of the minerals covered in this review are toxic, carcinogenic, or both in some tests, the risk to humans exposed under normal conditions may be minimal. As will be noted, many of the experiments used to assess the pathogenicity of a mineral investigate the mechanisms of pathogenicity or evaluate the pathogenicity of a specific mineral relative to other minerals. These experiments do not necessarily emulate typical conditions of human exposure; hence the results may not reflect the exact response that would be expected in humans. The assessment of risk from exposure to a specific mineral is an extremely involved task. The interested reader is directed to the article by Mossman et al. (1990) for an introduction to the problem and a list of pertinent references. Current federal regulations, including those for minerals, can be found in *Codes of Federal Regulations* (CFR 29, part 1910.1000). CFR 29 is revised annually on July 1 and is found in most libraries.

INTRODUCTION TO BIOLOGICAL REPORTS

Determination of a substance's biological activity

Exposure to mineral dusts has been linked with a variety of lung diseases. Exposure to a fibrogenic mineral

can result in fibrosis (production of scar tissue) in the lung, which can impair the function of the lung. Exposure to a tumorigenic (or carcinogenic) mineral can result in cancer, such as lung cancer or mesothelioma. Lung cancer is associated with exposure to a variety of substances (e.g., cigarette smoke) in addition to minerals, but mesothelioma, or cancer of the mesothelium (lining of the abdominal wall), is commonly associated exclusively with exposure to fibrous minerals, predominantly the asbestos minerals. The potential for a specific mineral to induce these diseases can be evaluated by numerous techniques, each of which provides different information and has different factors that complicate interpretation. As pointed out by Rall (1988), there are four basic groups of methods used to determine the carcinogenic potential of a substance: epidemiological studies, in vitro studies, in vivo studies, and prediction of biological activity by comparison with a similar mineral (structure-activity relationship).

Epidemiological studies. In an epidemiological study, a substance's health risks are evaluated by determining the relationships between human exposure to a substance and the potential health effects. One approach to epidemiological studies uses cohorts, or groups of individuals whose lifestyles are similar, to monitor the incidence of disease in response to exposure to a substance. Ideally, two groups are chosen such that their difference is only in the exposure to a specific substance; thus one group serves as a control against the health effects from other agents. In many studies, however, no explicit control group is used, but the study group is compared with national averages. Epidemiological data are commonly reported using a standardized mortality ratio (SMR) that compares the observed death rate from a disease in the study group with the rate expected based on the control group. Another approach to epidemiological studies uses case studies to determine lifestyle patterns of individuals afflicted with a disease. In both approaches it can be difficult to assess the effect of other harmful substances to which individuals were exposed, such as other mineral dusts or tobacco. In other words, an epidemiological study looks for patterns in the incidence of a disease and, thus, permits only an indirect determination of the cause of that disease. An obvious advantage of an epidemiological study, however, is that it attempts to determine the actual effect of mineral-dust exposure on humans exposed under typical conditions.

An important aspect of epidemiological studies is the characterization of exposure, which can be achieved by a number of techniques. A dust's mineral content, particle size and shape, and areal distribution can be measured directly in an environment by collecting air samples or soil or dust samples. Such measurements are useful for providing detailed information concerning current exposure conditions. However, onset of disease can occur 20–30 yr after exposure to a mineral dust, and current exposure conditions may differ significantly from previous exposure conditions. Furthermore, dust characteris-

tics may fluctuate in some environments, causing an incorrect estimation of exposure.

A direct characterization of the dusts to which an individual was exposed can be made by analyzing lung tissue or expectorated sputum. A variety of processes occur in the lung following exposure to dust (Lehnert, 1990; Schlesinger and Driscoll, 1989), and many of these result in a clearance of dust particles via the mucociliary escalator within the lung and trachea. Thus, sputum provides a means of directly sampling some of the dust particles being cleared from the respiratory system. This process can continue over a prolonged period after exposure. Frequently, these particles become coated with ferruginous material believed to be derived from proteins; such coated particles are referred to as ferruginous bodies. Particle concentration in the sputum has been used as an indication of current particle content in the lung (Sébastien et al., 1984). Analysis of sputum samples is comparatively simple and inexpensive. However, high lung burdens—i.e., ~10000 ferruginous bodies per gram of dry lung (Sébastien et al., 1984)—are required before particles are detected, and exposure estimates are biased to the extent that the sample includes only those particles being cleared. An accurate assessment of the mineral content of lungs can be obtained even for lower lung burdens by analyzing lung tissue obtained during surgery or a post-mortem examination (e.g., Churg et al., 1984). However, biopsy of lung tissue is complicated by the heterogeneous variation of particle deposition and retention at different sites within the lung, so lung samples are needed from several locations to estimate an average lung burden.

Incidence of disease is typically estimated by several methods. Clinical examination of at-risk individuals combined with chest X-rays can often detect early indications of disease, but to assess the subjective factors associated with the grading of a chest X-ray, the same set of X-rays must be read by several individuals. Death certificates provide another means of estimating the incidence of disease. However, this method can result in incorrect estimates, since the accurate classification of a specific disease often requires more extensive analysis than is commonly performed during a postmortem examination. Finally, in some cases, biopsies can be performed on lung tissue from diseased individuals, and lungs and other organs can be removed and examined after an individual from an exposed group has died, as is done with asbestos workers in South Africa, for example. Even so, there is some concern that neoplasias (malignant tumors) can be classified incorrectly, resulting in either an overestimation or underestimation of the incidence of disease.

In vivo studies. Animal models are used extensively to study the effects of exposure to mineral dusts. Ideally, an animal species is chosen such that its response to a specific substance closely resembles the response observed in humans under similar conditions. With such an animal model, the complete biological effect of a substance can be studied under various exposure conditions. In practice, however, responses observed in animal models are

not identical to responses observed in humans, so prediction of a human response using results from an *in vivo* experiment is not always straightforward. In mineral-dust research, rats and mice are the most commonly used animal models; however, guinea pigs, sheep, dogs, hamsters, monkeys, and rabbits have also been used. Pott (1980) reviewed some of the *in vivo* experiments concerning the biological effects of mineral fibers and discussed the differences in response among species.

Because disease must be induced more rapidly in animals than it is induced in humans, *in vivo* experiments commonly use exposure methods that differ significantly from exposure conditions experienced by humans. Disease in humans often occurs up to 20–30 yr after initial exposure to a dust; however, most lab animals live less than 20–30 yr. Hence, disease is induced more rapidly in an *in vivo* experiment than would be expected under natural exposure conditions. Typical *in vivo* experiments employ one of three exposure methods: (1) intratracheal injection of a dust-saline solution into the target organ; (2) direct application of the dust to the target organ (e.g., intrapleural and intraperitoneal instillations), or (3) inhalation in a dust-rich environment (e.g., 1–50 mg/m³ or ~500–2500 fibers/mL). (The current regulatory standard for occupational asbestos exposure in the U.S. is <0.2 fibers/mL; however, 50% of all asbestos exposure levels in U.S. schools lie within 10⁻⁶–10⁻³ fibers/mL, according to the Environmental Protection Agency, 1986.) The route of entry for the dusts in such experiments clearly differs substantially from the typical route of entry in humans (inhalation in a comparatively dust-poor environment). However, even for inhalation experiments conducted with reasonable dust levels, exposure would differ from that expected in humans, since the differences between the respiratory systems of laboratory animals and humans (e.g., airway size and shape, breathing patterns, clearance mechanisms) introduce a sampling bias on the particles reaching the lungs (e.g., Oberdörster and Lehnert, 1990). Short-term (2–3 yr) animal experiments also inadequately model human exposure in that short-term experiments do not consider the long-term (10–30 yr) alteration of mineral particles (such as dissolution or surface modification) by the biological medium.

In vivo studies can provide important information, such as (1) the effect of mineral dusts on a living organism including types, incubation periods, and severity of diseases, translocation (migration) and clearance rates of particles from the site of initial exposure, and cellular responses to exposure; (2) the effect of various exposure conditions; (3) an evaluation of the risk to humans, (4) the elucidation of pathogenic (disease causing) mechanisms; and (5) the identification of potential treatment methods. However, such experiments are time consuming (up to several years duration), expensive, and difficult to interpret (unless a very strong or very weak effect is found) (Rall, 1988). Furthermore, the use of data from *in vivo* experiments to predict human response can be complicated by a variety of experimental factors, including

differences between human and animal response to exposure, the degree and method of exposure, and the use of animal strains particularly susceptible or resistant to disease. These two last factors additionally make comparison of results from different studies difficult unless identical experimental procedures were used. When provided in the original report, animal strains will be included here in the review of *in vivo* data to allow comparisons to be made between studies.

In vitro studies. *In vitro* experiments use specific cells to determine a mineral's biological activity and are commonly used because they are rapid and relatively inexpensive. One of the more commonly used *in vitro* methods is the Ames test (Ames et al., 1975), which uses mutation rates in bacteria as a measure of carcinogenic potential. However, the asbestos minerals are one of only two suspected carcinogens (the other being conjugated estrogen) that do not appear mutagenic in a bacterial assay (Chamberlain and Tarmy, 1977; Shelby, 1988). The implications of this remain incompletely understood.

Eukaryotic mammalian cells are also used for *in vitro* experiments to test a mineral's biological activity, with red blood cells (RBCs), macrophages, and epithelial cells being the most commonly used cell types. These cell types are also found in the lung where they can potentially interact with inhaled dusts. Hemolysis experiments test the ability of a substance to destroy or lyse RBCs by incubating RBCs in contact with the substance and then measuring cell viability (the release of hemoglobin is an index of cell destruction). *In vitro* experiments with other cell lines also test for cytotoxicity (the ability of a substance to kill a cell) normally either by (1) the cellular exclusion of a vital dye (where dead cells allow penetration of the dye but living cells do not); (2) the reduction in the ability of the cells to develop into colonies; or (3) the release of enzymes indicative of an increase in cell-membrane permeability or cell death, where membranes of healthy cells are impermeable to the enzymes. Heppleston (1984) presented an excellent discussion of information that can be derived from cytotoxicity experiments.

In addition to their use in determining cytotoxicity, *in vitro* experiments can be used to determine the genotoxicity (ability to affect genetic material) or mutagenicity (ability to cause mutations) of a substance. As mentioned above, the Ames test is one such technique. Other methods involve a quantification of mutation by measuring processes involving chromosomes or DNA directly, such as sister chromatid exchange (SCE) or unscheduled DNA synthesis. O radicals, such as the superoxide anion, are believed by some investigators to participate in both fibrosis and carcinogenesis (Mossman et al., 1989; Mossman and Marsh, 1989), and the measurement of the generation of such anions by a catalytic reaction involving a mineral surface (Pezerat et al., 1989) is another potential indicator of a particle's mutagenic activity.

Structure-activity relationship. The simplest and least expensive method to determine the potential health hazards of a mineral dust may eventually prove to be the

prediction of a mineral's biological activity by comparison with known structure-activity relationships. However, an accurate knowledge of the mechanisms by which a mineral is toxic is essential for this method to be effective. Several mechanisms are currently proposed to explain the biological activities of minerals (Table 1 and Fig. 1). However, these mechanisms remain too poorly understood to allow an accurate prediction based on a mineral's structure. Nevertheless, such predictions are made, primarily based upon the observed correlation between biological activity and particle shape and size (e.g., Stanton et al., 1981).

It is interesting to note that many of the proposed mechanisms for mineral-induced disease involve chemical reactions (e.g., oxidation/reduction) that are similar to reactions that occur in a geological environment. A geochemist's approach to the study of such reactions differs greatly from the approach taken by most biological scientists. Consequently, geochemists can contribute enormously to the study of mineral-induced diseases. One role for the geochemist is the identification and characterization of active sites on mineral surfaces (the results of which would be of interest to both geologists and biologists). By characterizing the appropriate physical and chemical aspects of each sample, biological activity can be related to a measurable parameter (e.g., surface area, Lewis-acid/base sites per surface area, Brønsted-acid sites per surface area) and a model for biological activity can be developed. Furthermore, a sound mineralogical approach would facilitate the design of biological experiments that control most potentially active mineralogical characteristics while allowing the active site of interest to be studied. For example, numerous experiments have been conducted to test the biological activity of fibrous amphiboles. As shown in Figure 1, however, amphiboles have numerous potentially active sites. Hence, the results of a study on the toxicity of amphibole cannot be associated with one particular active site. Conversely, if experiments were conducted using two amphiboles that differed only in the composition of the octahedral site, then the activity of polyvalent cations in amphiboles (active site 3 on Fig. 1) could be determined. The diversity of mineral species offers a unique potential for characterizing the activity of numerous mineralogical characteristics. In addition to natural samples, synthetic minerals could be used effectively, e.g., by growing zeolites with identical framework topologies but with various amounts of tetrahedral Al.

Mineralogical aspects

Mineral names. In principle, usage of nomenclature to describe a mineral should follow strict guidelines. For species names, these guidelines are widely accepted and have been developed so that a mineral name provides important information concerning both structure and composition. For example, the nomenclature for amphiboles is extremely complex and relies on a knowledge of the composition and structure of a given amphibole spec-

TABLE 1. Mechanisms of mineral-induced disease

Proposed mineralogical mechanisms	Proposed biological mechanisms
Brønsted-acid sites proton-donor sites associated with underbonded O atoms resulting from either cation substitutions or broken bonds at a mineral surface	Oxidative stress resulting from the catalytic production of oxygen radicals at a mineral surface
Lewis-acid/base sites electron-acceptor/donor sites associated with polyvalent cations	Genetic alteration by a number of mechanisms, including transfection of intercellular DNA and DNA-particle interaction during mitosis
Alkali-cation sites easily exchangeable cations, e.g., amphibole A site or zeolite cage site	Incomplete phagocytosis resulting in the release of cytotoxic enzymes by the cell
Specific surface periodicities periodicities that promote a specific interaction with a particular molecule, e.g., DNA-particle interactions reported by Appel et al (1988)	

Note. There is no implied correlation between opposing mineralogical and biological mechanisms.

imen (Leake, 1978). Hence, use of the species name riebeckite defines a mineral as a clinoamphibole (i.e., a double-chain silicate with a specific stacking order) with a composition of $\text{Na}_2\text{Fe}_3\text{Si}_8\text{O}_{22}(\text{OH})_2$. Varietal names, however, are not so strictly defined. So, although the varietal name "crocidolite" is commonly used to describe asbestiform riebeckite, the identification can be based on the blue color and asbestiform habit but not on compositional or structural information.

Numerous examples illustrate the problems that can result from inaccurate usage of mineral-species nomenclature. "Amosite" is a term used to describe brown asbestos. The term originates from an acronym for the Asbestos Miners of South Africa. Although the term is frequently used to describe asbestiform cummingtonite-grunerite, it has also been used to describe ores containing mixtures of asbestiform amphiboles. This ambiguity has led to conflicting definitions of "amosite," i.e., "amosite" has been defined as a varietal name for asbestiform ferroggedrite by some (e.g., Roberts et al., 1990). Hence, the term "amosite" provides limited information pertaining to the mineral content of the sample.

In reports on the effects of zeolites, erionite and mordenite are often referred to as fibrous and nonfibrous equivalents, despite the fact that both are normally fibrous and have different structures and composition. Furthermore, the identification of one or the other is typically made using qualitative analytical TEM (ATEM) based on the presence or absence of specific exchangeable cations. Similar examples of the incorrect usage of mineral names occur throughout the literature on the health effects of dusts. Hence, the reported mineral content is potentially suspect, unless adequate data were used for identification.

The lack of interest in the importance of a mineral's structure and composition is further demonstrated by data

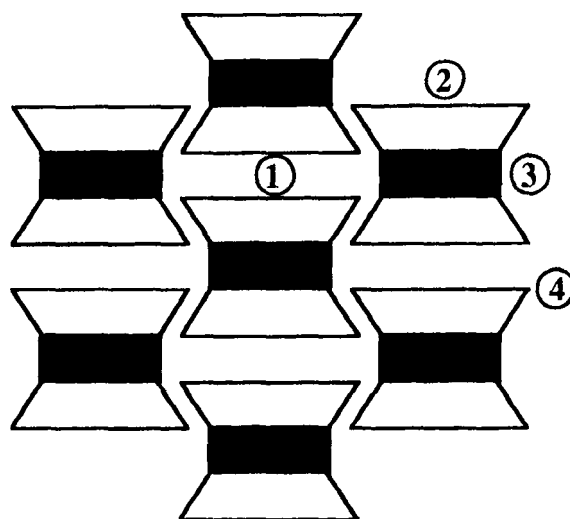


Fig. 1. Schematic diagram of the amphibole structure viewed along the c axis. Numbers indicate possible active sites (1) A-site cations, (2) protons associated with Al substitution in the tetrahedral sites, (3) polyvalent cations in the octahedral sites, (4) protons associated with underbonded O atoms

sheets for the asbestos minerals. Both the National Institute for Occupational Safety and Health (NIOSH) and at least one major supplier of asbestos for biological research provide data sheets listing incorrect mineral formulae for the asbestos minerals. Such incorrect information may form the basis of mineral identification or interpretation of results used in some studies on the health effects of asbestos.

Because of this casual usage of mineral-species nomenclature, it is difficult to interpret the results of a study in terms of mineralogical properties, such as structure and composition. The improper use of mineral-species names is comparable to an incorrect usage of nomenclature for cell type or animal species. If a report stated that "rodents were used in the experiments" or, worse, "rats were used in the experiments" when in fact the experiments were conducted on mice, the data would be extremely difficult to interpret. Most reports on the *in vivo* effects of minerals are extremely specific in describing animal species and cell types, yet they often fail to identify mineral species correctly. Unfortunately, the advantages of a strict usage of mineral-species nomenclature are not fully appreciated by many scientists involved in health-related mineral research, including both minerals scientists and nonminerals scientists.

Sample purity. Another source of uncertainty in these studies concerns purity of the mineral dust. Detailed description of the mineral content is rarely given, and identification of the minerals generally relies upon that made by the supplier. Even when obtained from reliable sources, however, the exact mineral content of a dust is suspect. Most suppliers provide mineral samples that contain mixtures of minerals, even though one mineral may be

the dominant constituent. Mineral impurities, even in small quantities, may have a significant effect on a biological response. For example, recent studies (e.g., Churg et al., 1984) show that the lungs of workers exposed to chrysotile contain abundant amounts of fibrous tremolite, a minor contaminant of chrysotile ores. Churg et al. (1989) found that the rate of mesothelioma is strongly correlated with tremolite but much less so with chrysotile. Continuing with the analogy above, the use of impure mineral samples is equivalent to running an *in vitro* assay with lung cells, which would consist of several types of cells (e.g., macrophages and epithelial cells). This would never be done in an *in vitro* experiment, as each cell type responds differently.

Mineral identification. Most of the studies reviewed below generally characterized samples by light microscopy or transmission electron microscopy (TEM) and less commonly by ATEM. Electron diffraction analysis is rarely used to determine mineral content, despite the fact that most of the particles cannot be identified uniquely based on morphological and compositional data alone. Particle identification by TEM can be time consuming when large numbers of particles are included, especially when ATEM and electron diffraction are used. Hence, results are potentially affected by poor counting statistics and incorrect identification of the particles (when electron diffraction is not used). Minerals present in dust can also be determined by quantitative X-ray diffraction (XRD). Davis (1990) measured reference intensity ratios (RIRs) for chrysotile and amphibole asbestoses and demonstrated that the minerals in asbestos mixtures can be determined with a lower detection limit of ~0.5–2.0 wt%. Puledra and Marconi (1990) also reported a low detection limit (2 μ g) for chrysotile in various matrices. Chipera and Bish (1989) and Bish and Chipera (1991) used RIRs to determine erionite concentrations in dusts and reported detection limits as low as 100 ppm. Quantitative analysis of mineral content using the Rietveld method with X-ray diffraction data (Snyder and Bish, 1989) may prove to be even more successful than the use of RIRs, since the Rietveld method effectively addresses problems associated with peak overlap and compensates somewhat for the high degree of preferred orientation exhibited by fibrous and platy minerals. However, no biological studies using this approach have been reported to date.

Assessing human risk

As indicated above, many of the results of experiments on mineral-induced pathogenesis do not relate directly to risks to humans. For example, though a mineral may be highly active in an *in vitro* assay, it may pose little risk to humans under normal conditions (e.g., kaolinite). Human response relates to a number of factors, including the pathogenicity of the sample, residence time *in vivo*, dose, and variations in individual response (e.g., physical condition of the person, smoking history, propensity toward a specific disease). Hence, a mineral such as kaolinite may be highly active at the cellular level, but it does

not reside long enough in the lungs to induce disease. Alternatively, a mineral may show a positive response in a given assay, but the mechanism tested by the assay is not involved in pathogenesis in humans.

REVIEW OF DATA

Numerous data are reported that describe the biological effects of mineral dusts. However, most such studies provide only limited mineralogical detail. Hence, the results from these studies may not reflect results one would anticipate from a mineralogically pure sample. Studies that reported only limited mineralogical data may have used samples that were (1) mineralogically homogeneous, containing the mineral described; (2) mineralogically heterogeneous, containing numerous minerals; or (3) mineralogically homogeneous, containing a mineral different from the one described. In general, it is not possible based on the published data to determine to which category a sample belongs. In the absence of data to the contrary, I believe it should be assumed that the mineral content of a sample is as described by the original investigators. Nevertheless, this adds uncertainty to any interpretation based on these data, and this uncertainty should be recognized. In order to clarify these uncertainties, some investigators have supplied me with their samples, and these samples are being characterized using quantitative X-ray diffraction analysis (Guthrie and Bish, unpublished data). Where preliminary results are available, these will be given. Those investigators that have supplied samples are commended for their desire to augment their studies with detailed mineralogical information.

For those studies that did present mineralogical information (ranging from a generalized locality to complete descriptions, including compositional analysis), the mineralogical detail will be included with the summary of results. When no mineralogical information was given, only a mineral or material name will be listed.

Related to the poor mineralogical detail is potentially inaccurate usage of mineral-species names. The use of unaccepted mineral names, rock names, and compound names is rampant in the biological literature. Some assumptions, therefore, were made in order to categorize these studies with respect to mineralogy. For instance, for the purpose of categorizing it was assumed that "Fe₂O₃" and "iron-ore dust" refer to hematite, "bentonite" refers to montmorillonite, and "attapulgitite" refers to palygorskite; the quoted terms are retained in the review to allow the reader to recognize assumptions by the author. Furthermore, when mineral group terms (such as carbonate or mica) were used, these are also retained in the review. This is not meant to imply that either the quoted terms or the mineral group names are mineral-species names, but rather it serves to categorize the study as appropriately as possible based on the probable major mineral.

Oxides and hydroxides

Most studies on oxide- and hydroxide-bearing dusts suggest that some samples can produce fibrosis *in vivo*

but are generally relatively inactive minerals. Exceptions may include oxides containing Cr (not necessarily mineral samples) and fibrous brucite. Fibrous brucite may, in fact, be extremely active, with exposure resulting in fibrosis, carcinogenesis, or both.

Hematite. Epidemiological studies suggest that exposure to hematite-bearing dust alone under modern mining conditions—i.e., low dust exposure, ventilation, wet drilling—does not increase the risk of lung cancer. However, individuals exposed under unfavorable mining conditions—i.e., high exposures to dusts (especially when contaminated with silica), radon, and tobacco smoke—do show a higher than expected incidence of respiratory disease.

Lawler et al. (1985) found no overall excess of mortality due to lung cancer (SMR = 0.97 for all cancers and SMR = 0.94 for lung cancer; SMRs based on U.S. white males) among 10403 underground miners in Minnesota who were exposed to iron-ore dust (hematite + “limonite,” silica, phosphates, and other oxides, as given by Lawler et al., 1985). In fact, they found a lower than expected mortality for respiratory disease overall (SMR = 0.79). However, they reported no information about exposure conditions, i.e., quantitative mineral content of the dusts, particle sizes, or mass concentrations. Lawler et al. agreed with suggestions by previous workers (e.g., Boyd et al., 1970; Radford and Renard, 1984) that observed excesses in mortality due to lung cancer in iron-ore miners reflect exposures to other factors, such as radon and radon daughter products (RRDs), silica, tobacco smoke, and diesel fuel, since exposure to these factors was minimal in their cohort compared with cohorts of iron-ore miners studied previously.

Chen et al. (1990) also suggested that exposure to RRDs and silica could explain excesses of mortality due to lung cancer in hematite miners. They studied 6444 male workers associated with hematite mining in China, 5406 of whom were involved in underground operations, and reported SMRs (based on age-specific death rates for Chinese males) for lung cancer ranging from ~1.0 at the 95% confidence level for zero-to-medium dust exposure to >2.7 for heavy dust exposures. However, exposure to RRDs is highly correlated with dust exposure; hence, the individual effects of the two could not be separated. Incidences of other respiratory diseases, such as silicosis and tuberculosis, are also correlated with the incidence of lung cancer, but no data on silica exposure were given. Cigarette smoking shows a positive relationship with lung cancer as well, as indicated by an SMR for lung cancer in smokers of 2.7–6.3 (95% confidence interval or CI). In the cohort of Chen et al. the use of modern mining conditions (wet drilling and ventilation) has lowered airborne dust exposure significantly (from >100 mg/m³ to <5 mg/m³), and workers exposed only under the improved conditions may show a lower SMR for lung cancer (1.1–4.6, 95% CI) than those first exposed prior to the use of wet drilling and ventilation (2.9–7.4, 95% CI).

In vivo experiments indicate that hematite-bearing

samples are biologically inactive. Pott and coworkers (Pott and Friedrichs, 1972; Pott et al., 1974) found no fibrosis or tumors in 80 Wistar rats 530 d after intraperitoneal injections of hematite (source not given), whereas in the same study, Wistar rats showed fibrosis and up to a 40% incidence of tumors when injected with chrysotile and a 55% incidence of tumors following the injection of silica glass. Vorwald and Karr (1938) found that hematite dust induces no tumors in guinea pigs or rats following inhalation of the dust; no information was given concerning the source of dusts or exposure levels. However, asbestos (type not given) also failed to induce tumors in guinea pigs in the same experiment. Finally, Mossman and Craighead (1982) found that hematite (IIT Research Institute, Chicago) does not induce tumors in golden Syrian hamsters following subcutaneous implantation of in vitro-exposed tracheas; however, hematite is nearly as effective a cocarcinogen as riebeckite asbestos [Union Internationale Contre le Cancer (UICC) standard] when pretreated with a polycyclic aromatic hydrocarbon.

In vitro experiments suggest that hematite-bearing samples are noncytotoxic and nongenotoxic. Dubes and Mack (1988) used an in vitro technique to test the ability of a variety of materials to mediate the transfection of mammalian cell cultures; transfection of cells is the process of introducing foreign genetic material into a cell and is one proposed mechanism for the carcinogenicity of mineral dusts (e.g., Appel et al., 1988). Their data show that Fe₂O₃ (reagent grade from Matheson, Coleman, and Bell Company) is only slightly effective as a mediator. For comparison, asbestos (from a variety of sources) and Cr₂O₃ (from Matheson, Coleman, and Bell) were ~3–7 times more effective mediators. Witmer and Cooper (1983) also reported that Fe₂O₃ is nonmutagenic whereas Cr₂O₃ is mutagenic, as determined in a modified Ames test.

Boehmite, goethite, and lepidocrocite. In vivo experiments suggest that samples containing iron and aluminum hydroxides may be slightly active in the lung. Inhalation experiments by Gardner et al. (1944) showed no effect of boehmite laths [measuring ~7.5 × 30.0 nm; mineral identification confirmed by King et al. (1955) using TEM and XRD] on the lungs of guinea pigs. However, using the same material, King et al. (1955) observed that severe and rapid pulmonary fibrosis develops in rats after a direct injection of boehmite-saline solution into the lungs. Stacy et al. (1959) extended the study by King et al. (1955) to include an additional sample of boehmite (better crystallized and having a mean size of ~3 μm) and samples of goethite and “lepidocrocite” (presumably lepidocrocite) measuring <0.5 μm and ~0.5 × 2 μm, respectively. They demonstrated that the boehmite sample used in the experiments by Gardner et al. (1944) produces a dose-dependent fibrogenic response, whereas the larger-grained, better-crystallized boehmite is much less fibrogenic. Goethite- and lepidocrocite-bearing samples, however, are only slightly fibrogenic, even at higher doses than boehmite. Inhalation experiments by Campbell

(1940) using "the precipitated brown oxide of iron (Fe_2O_3 , H_2O), British Drug Houses" produced a 22.7% incidence of lung tumors in mice, compared with 6.8% in the control group and 17.6% in a group exposed to "precipitated silica" dust; tumors did not develop until after 300 d, with most developing 600–900 d after first exposure. Animals were exposed to 0.5 g of dust per hour, 6 h per day, 5 d per week for 1 yr.

Brucite. In vivo experiments suggest that samples containing fibrous brucite (described as "nemalite" in most studies) are both fibrogenic and carcinogenic. Pott and coworkers (Pott and Friedrichs, 1972; Pott et al., 1974) found that fibrous brucite, when intrapleurally injected in Wistar rats, produces fibrosis comparable to that produced by silica or chrysotile injections and exhibits a tumor rate (62.5%) exceeding those of silica (55%) and chrysotile (40%); palygorskite, however, exhibits a 65% tumor rate in the same test. Wagner et al. (1973) found that 20 mg of a sample containing fibrous brucite administered to Fischer 344 rats by intrapleural injection induces mesotheliomas at a rate of 56%. Their sample was obtained from a Canadian mine and contained chrysotile, though no estimate for the amount of chrysotile contamination was given. It should also be noted that chrysotile from this same mine induces mesotheliomas under the same conditions at a slightly higher rate (61%).

In vitro experiments further indicate that samples containing fibrous brucite are cytotoxic to a variety of cell lines. Chamberlain and Brown (1978) showed that fibrous brucite (same material as used by Wagner et al. 1973) reduces the colony-forming efficiency of Chinese-hamster lung cells at doses roughly equivalent to those for a comparable cytotoxic response in experiments with either amphibole or serpentine asbestos. The dose required to reduce cloning efficiency to 50% is 12 $\mu\text{g}/\text{mL}$ for fibrous brucite compared with 9 $\mu\text{g}/\text{mL}$ for riebeckite asbestos and 17–26 $\mu\text{g}/\text{mL}$ for chrysotile; talc is nontoxic at 50 $\mu\text{g}/\text{mL}$, the highest dose used. Jaurand et al. (1980) found that fibrous brucite is cytotoxic to human RBCs and rabbit alveolar macrophages. They demonstrated that both the hemolytic and cytotoxic activities of fibrous brucite are intermediate to those of chrysotile or riebeckite asbestos. Finally, Pezerat et al. (1989) found that fibrous brucite is comparable to chrysotile and much more effective than amphibole asbestos in its ability to catalyze the production of O radicals, a step of potential importance in both fibrogenesis and carcinogenesis.

The 1:1 layer silicates and chlorite

Most studies of samples containing 1:1 layer silicates or chlorite suggest that some samples can produce fibrosis or tumors in vivo and can be highly active in vitro. However, epidemiological data on exposure to kaolinite-bearing dusts suggest that fibrosis is induced only in extraordinary conditions, i.e., high exposures or in the presence of other pulmonary complications. Although these minerals may be cleared rapidly from the lung (and hence are not pathogenic in humans), their in vivo and in vitro

activities may provide clues to the mechanisms of mineral-induced pathogenesis.

Kaolinite and halloysite. Epidemiological studies suggest that kaolinite-bearing dust is fibrogenic only under extraordinary conditions, i.e., high dust conditions or exposure combined with another respiratory disease, such as tuberculosis. Hale et al. (1956) reported case studies of seven kaolinite workers (primarily baggers exposed to extremely high dust conditions) who showed indications of respiratory disease, as determined by clinical examinations including chest X-rays. Autopsies of two of the men revealed fibrosis associated with large amounts of kaolinite, mica, and amorphous silica. One of the autopsies, however, also noted tuberculosis; dead tuberculosis bacilli enhance the fibrogenic effect of kaolin dust in animals (Kettle, 1934; Attygalle et al., 1954). Similar observations were reported in a case study by Lynch and McIver (1954). In a cohort study, Sheers (1964) found fibrosis in up to 13% of kaolinite workers exposed to high dust levels. Tuberculosis was uncommon in his cohort. However, fibrosis was highly correlated with high dust exposures and length of employment. More recent studies confirm that exposure to high dust levels (particularly silica-bearing dusts) during kaolin mining can be correlated with abnormalities in chest X-rays (e.g., Kennedy et al., 1983; Oldham, 1983; Sepulveda et al., 1983; Ogle et al., 1989). However, some of these abnormalities may not reflect the onset of fibrosis (Oldham, 1983). Lapenas et al. (1984) confirmed the presence of kaolinite in pulmonary tissue from five kaolin workers with pneumoconiosis; silica was not present in the lung samples.

In vivo experiments reported thus far on the fibrogenic potential of kaolinite-bearing dusts are inconclusive. Kettle (1934) observed no fibrosis in guinea pigs following intratracheal injection of kaolinite (British Drug Houses; the sample contained quartz and "very numerous" sericite fibers), though, as indicated above, he did find that exposure to kaolinite and dead tuberculosis bacilli does result in fibrosis. King and Harrison (1948) used direct injection into the lung to study the effects of two kaolinite samples on rats. Unfortunately, one of the experiments used kaolinite samples containing 35.68 wt% carbonate minerals (species not given), whereas in the other experiment, which used a comparatively pure sample of kaolinite, only two rats survived more than 10 d after exposure. Neither of these rats developed fibrosis. Mossman and Craighead (1982) found that kaolinite (3–5 μm in diameter, Georgia Kaolin Company) does not induce tumors in golden Syrian hamsters following subcutaneous implantation of in vitro-exposed tracheas and is a slightly less effective cocarcinogen than UICC crocidolite when pretreated with a polycyclic aromatic hydrocarbon. Inhalation experiments by Wagner (1990) produced no lung tumors in 20 rats (probably from the Wistar strain) exposed over a period of 3–24 months, but a slight fibrogenic response was observed. His samples contained 85–95% kaolinite, with the remainder consisting of mica, feldspar, and quartz. For comparison, a "nonfibrous ze-

olite" and a "long attapulgite" produced more severe fibrogenic responses. Wastiaux and Daniel (1990) also used inhalation-exposure methods to assess the fibrogenicity of kaolin. They reported that their kaolin sample (Cornish kaolin dust) induces a moderate fibrogenic response in Wistar rats. Long-term experiments currently in progress by Maltoni and coworkers (Maltoni et al., 1982; Maltoni and Minardi, 1989) may provide additional information on the *in vivo* activity of kaolinite.

In vivo studies using halloysite-bearing samples, however, suggest that this kaolin-group mineral may be carcinogenic. Stanton et al. (1981) found that two samples of halloysite (obtained from the water supply of Hong Kong) induce a tumor rate of ~20% in Osborne-Mendel rats exposed by direct application of the dust to the pleural surface. For comparison, in the same experiments, amphibole asbestoses induce tumors at rates ranging from 0 to 100%. Wagner (1982) also reported *in vivo* data on halloysite. He observed no mesotheliomas in 40 Fischer 344 rats treated by intrapleural inoculation, whereas chrysotile (UICC standard B; derived from Canadian deposits) induces 22.5% mesotheliomas by the same technique. Whether the observed difference in the pathogenicities of kaolinite and halloysite is related to particle morphology or other mineralogical properties (e.g., surface characteristics) is not known.

In vitro experiments show that kaolinite-bearing samples are cytotoxic to most cell types studied, though some materials are noncytotoxic. Low et al. (1980) found that kaolinite is cytotoxic to rabbit alveolar macrophages; their sample was a 99% pure kaolinite (as determined by XRD and energy-dispersive spectrometry) obtained from the Georgia Kaolin Company. Davies (1983) showed that kaolinite is also cytotoxic to mouse peritoneal macrophages but that treatment of the dust with poly(2-vinylpyridine *N* oxide) (PVPNO), a class of polymers that inhibit the cytotoxicity of quartz (Holt et al., 1970), almost completely eliminates kaolinite's cytotoxicity. Davies noted that his sample contained 2% mica, as determined by XRD. Dubes and Mack (1988) found that kaolinite (J. T. Baker Chemical Company) is ~4–5 times more effective than asbestos in mediating transfection of mammalian cell cultures. Gormley and Addison (1983) also found that the kaolinite standards of the CMS Clay Mineral Repository, KGa-1 and KGa-2, are cytotoxic to a macrophagelike mouse cell line only at high doses. In contrast to the above studies, however, Marks and Nagelschmidt (1959) found that kaolinite is much less cytotoxic to guinea pig peritoneal macrophages than silica minerals. Woodworth et al. (1982) used the release of ^{51}Cr to monitor changes in cell-membrane permeability and cell death in Syrian hamster tracheal epithelial cells. They found that kaolinite (Georgia Kaolin Company) will cause the release of ^{51}Cr . Kaolinite is less effective than chrysotile and montmorillonite but more effective than silica.

There is some indication that kaolinite's cytotoxicity is in part related to broken Si-O bonds at the crystallite edges. As noted above, Davies (1983) found that the

treatment of kaolinite with PVPNO reduces kaolinite's cytotoxicity at amounts less than the total amount of polymer that can be adsorbed, implying that only some of the polymer-binding sites may be related to kaolinite's cytotoxic activity. Furthermore, PVPNO is effective at inhibiting the cytotoxic activity of quartz (Holt et al., 1970), suggesting that the mechanisms by which quartz and kaolinite exert their cytotoxic effects are related. Steel and Anderson (1972) found that the addition of a bacterium, *Staphylococcus aureus*, to a kaolinite-NaCl solution at low NaCl concentrations (14 mM) inhibits flocculation, possibly because of an interaction between the bacterium and the kaolinite crystal edges. Others (Kennedy et al., 1989; Ghio et al., 1990) have shown that kaolinite (noncalcined Georgian sample) and other pneumoconiosis-causing minerals function as Fenton catalysts (electron transfer by $\text{Fe}^{2+} = \text{Fe}^{3+} + e^-$), possibly as a result of Fe^{3+} adsorbed on its surface. Their studies illustrate that biochemical mechanisms can be probed effectively if mineral samples are selected carefully.

Serpentine, berthierine, and chlorite. Chrysotile is the serpentine mineral that has been studied in greatest detail as a potential health hazard. As indicated above, because of the enormous body of literature on the health effects of chrysotile, this mineral is not addressed directly in this paper. The interested reader, however, is directed to the recent article by Mossman et al. (1990) and papers by Ross (1981, 1984) for reviews of the research on chrysotile.

With respect to other serpentine minerals, Woodworth et al. (1983) found that antigorite (Ward's Scientific; sample from Arizona) does not induce metaplasia (proliferation of cells) in tracheal mucosa of the golden Syrian hamster *in vitro*, whereas riebeckite asbestos does, the tracheal mucosa, or lining of the trachea, is a part of the respiratory tract with which inhaled dusts interact. Using the same material, Mossman and Sesko (1990) found that antigorite does not cause the release of ^{51}Cr from hamster tracheal epithelial cells, whereas riebeckite asbestos and chrysotile do. Hence, if antigorite is cytotoxic, it is much less active than chrysotile.

A berthierine-rich iron ore (40% berthierine; sample from Lorraine, France) and two Fe-rich chlorites (Pyrénées and Anjou) were studied by Costa et al. (1990), using a chemical assay to measure the production of activated O species. Production of activated O species is a mechanism by which a material can induce a toxic response. They found that the samples were highly active, and they associated this activity with the high Fe content. Fe contents (reported as FeO) were in the range 12.5–30.0% for the three samples, but they did not show that the Fe was directly responsible for the observed activity. For comparison, they found that kaolinite (St Austelle) and quartz (DQ 12) are inactive in the assay. Despite the poor quality of the specimens used, their study is a good example of the type of mineralogical-based research that can benefit the field of mineral-induced pathogenesis. The biochemical mechanisms they investigated with their assay

involved an electron-transfer process at the mineral surface, a process similar to many geochemical processes.

The 2:1 layer silicates

Most studies of samples containing 2:1 layer silicates suggest that some samples can produce fibrosis *in vivo* and can be highly active *in vitro*. However, epidemiological data suggest that fibrosis may not be a problem in modern mining conditions. As with the 1:1 layer silicates, though 2:1 layer silicates may be cleared rapidly from the lung (and, hence, are not pathogenic in humans), their activity may provide clues to the mechanisms of mineral-induced pathogenesis.

Talc. In general, epidemiological studies suggest that exposure to talc-bearing dusts elicits a dose-dependent (albeit minor) response. Kleinfeld et al. (1967, 1974) studied the mortality in a group of 220 talc miners employed for at least 15 yr between 1940 and 1965. Dust exposures in this group were very high before 1945 (10^2 – 10^5 particles/mL) but dropped substantially after 1945 ($\sim 10^1$ – 10^3 particles/mL); furthermore, miners were exposed to a variety of dusts, including talc, serpentine, tremolite, carbonates, and silica. During 1945–1959, the mortality rate due to lung cancer was 3.4 times the rate expected based on U.S. white males in 1957, but the rate dropped to near normal during 1960–1969, possibly because of lower exposure levels. However, because of the exposure to dusts other than talc (particularly tremolite), it is not possible to assign this effect to talc exposure alone. Other studies (Selevan et al., 1979; Brown and Wagoner, 1980; Leophonte and Didier, 1990) of talc miners and millers in New England reported similar observations, but one study (Stille and Tabershaw, 1982) found no increases in mortality from lung cancers among workers at one New York mine who had no prior work exposure.

Coexposure to amphiboles is likely in many studies of talc-exposed workers. Cullinan and McDonald (1990) separated seven studies of talc workers on the basis of suspected amphibole exposure. Among the three studies with no suspected amphibole exposure, no mesotheliomas were reported out of a total of 2540 workers, though a slight increase in other respiratory malignancies was observed in two studies (Cullinan and McDonald, 1990).

In vivo experiments on talc-bearing dusts suggest that talc is nonfibrogenic and noncarcinogenic. Pott and Friedrichs (1972) observed no fibrosis or abdominal tumors following intraperitoneal injection of talc in Wistar rats. In a later experiment using the same technique, however, Pott et al. (1974) observed a slight incidence of tumors (2.5%) with a latency period (587 d) twice that observed for chrysotile or fibrous brucite. Wehner (1980) observed no significant changes in golden Syrian hamsters exposed to talc baby powder (presumably obtained from the funding agency, Johnson and Johnson); hamsters exposed to asbestos cement (mineral content not described) exhibited a response similar to the talc response at comparable exposures. Wehner used asbestos

dust (mineral content not detailed) as a positive control, but comparison is hindered because the control experiments used exposures 8 times those used in the talc experiments. Stanton et al. (1981) observed a statistically insignificant tumor rate in Osborne-Mendel rats exposed by direct application of talc to the pleural surface. Wagner et al. (1979) and Wagner (1990) found no lung tumors among 96 Wistar rats exposed to talc by inhalation for 3–12 months. Their sample contained 8% impurities including silica, chlorite, and carbonate. Endo-Capron et al. (1990) induced no pleural tumors after intrapleural injection of 20-mg talc (Luzenac, France).

In vitro experiments are inconclusive regarding the cytotoxic activity of talc-bearing dusts. Chamberlain and Brown (1978) found that Italian talc (commercial cosmetic grade; source not given) is noncytotoxic to Chinese-hamster lung cells at concentrations up to 50 $\mu\text{g/mL}$. However, using the same assay, Pigott and Pinto (1983) reported that talc (source not given) is slightly cytotoxic at the same concentration, for comparison, Pigott and Pinto found that riebeckite asbestos is highly cytotoxic and calcium carbonate is noncytotoxic. Talc is much less hemolytic than kaolinite or montmorillonite (Woodworth et al., 1982; Brown et al., 1980). Despite its weak cytotoxicity, talc is an effective mediator in transfection. Dubes and Mack (1988) showed that talc (talcum powder, Mallinckrodt Chemical Works) is ~ 2 times more effective than asbestos but ~ 2 times less effective than kaolinite in mediating transfection of mammalian cell cultures. Woodworth et al. (1982) found that talc (Cyprus Industrial Minerals Company, Los Angeles) will affect cell membrane (in Syrian-hamster tracheal epithelial cells) as monitored by the release of ^{51}Cr . Talc is approximately as active as kaolinite in this assay. However, Endo-Capron et al. (1990) found that talc (Luzenac, France) produces no SCEs in rat pleural mesothelial cells.

Phlogopite, muscovite, illite, smectite, and vermiculite. Only a few epidemiological studies of respiratory disease resulting from exposure to dusts containing micas or mica-like clays have been published, and some of these suggest that such samples can elicit a mild, dose-dependent fibrogenic response at high exposure levels (e.g., Vestal et al., 1943). Exposure to mica-like minerals is generally accompanied by an exposure to other minerals (e.g., silica and amphiboles), and the response to these minerals complicates the interpretation of the data (e.g., Heimann et al., 1953; McDonald et al., 1988). For example, some cases of vermiculite-related mesothelioma may be correlated with amphibole contamination (see Cullinan and McDonald, 1990, for a review of the studies).

In vivo experiments suggest that samples containing micas or mica-like clays are slightly fibrogenic. King et al. (1947) found injection of 50 mg of illite dust (separated from shales in southern Wales) into the lungs of rats produces no fibrosis unless the clay is pretreated in an HCl solution. Policard (1934) used exposure by inhalation to study the short-term effects (3–30 d) of ground white mica (from Madagascar; light microscopy showed

the dust to contain both fibrous and polyhedral particles) on the lungs of rats, ground white mica induces a cellular response similar to that observed with quartz. Pott et al. (1974) found that biotite is inactive following intraperitoneal injection in Wistar rats. Sykes et al. (1982) used intratracheal instillation to study the short-term (<7 d) and medium-term (<100 d) effects of "bentonite" on Alderley-Park-derived rats (strain 1; specific pathogen free). Though these results show that "bentonite" induces a greater pulmonary response than quartz in the short term, medium-term effects indicate that "bentonite" induces a response similar to a saline control. Rosmanith et al. (1990) studied the relationship between surface area and activity using intratracheal installation of a well-characterized muscovite sample in SPF-Wistar rats (Rosmanith et al., 1990; Schyma, 1990). They found that the finest material elicits the greatest fibrogenic response. Brambilla et al. (1979) reported mild pulmonary lesions in zoo animals exposed to mica dusts. Mineralogical analysis of lung contents indicated the presence of muscovite and illite.

In vitro experiments suggest that samples containing micas and mica-like clays may be slightly cytotoxic, though some studies suggest that phlogopite and montmorillonite may be highly cytotoxic. Pigott and Pinto (1983) studied the cytotoxicity of phlogopite, "hydrophlogopite" (?), and biotite (distinction not explained) and muscovite using Chinese-hamster lung cells. All four micas are slightly cytotoxic, with muscovite showing the greatest effect and being comparable to talc in activity. As mentioned above, riebeckite asbestos is highly cytotoxic in the same study.

Gormley and Addison (1983) found that samples SAz-1 and STx-1 (calcium montmorillonites), SWy-1 (sodium montmorillonite), and SHCa-1 (hectorite) from the CMS Clay Repository exhibit a range in toxicities, with SHCa-1 being slightly cytotoxic (roughly comparable to kaolinite samples KGa-1 and KGA-2) and STx-1 being highly cytotoxic (more cytotoxic than their positive control, quartz). It should be noted, however, that they reported 10% cristobalite in STx-1, as determined by XRD, and cristobalite is even more cytotoxic than quartz (Marks and Nagelschmidt, 1959).

Adamis and Timár (1978) used peritoneal macrophages from Sprague-Dawley rats to show that both quartz and "bentonite" (Istenmezeje, Hungary; obtained from Z. Juhász) are cytotoxic, but their modes of action are different. Although quartz alters the permeability of cell membranes to the enzyme lactate dehydrogenase (LDH), "bentonite" does not. However, "bentonite" does significantly lower the intracellular activity of LDH.

Costa et al. (1990) used a chemical assay to determine the role of Fe^{2+} in the production of activated O species. As found for Fe-rich chlorite and berthierine, Fe-rich biotite (Razés) is an effective catalyst in this assay, whereas an Fe-poor montmorillonite (Maroc) is an ineffective catalyst. In light of the purity of other samples used in the study (i.e., iron ore to test berthierine and granite to test

biotite and muscovite), the mineralogical purity of these specimens may be of some concern.

Woodworth et al. (1982) found that montmorillonite (American Colloid Company, Skokie, Illinois) will affect cell membrane (in Syrian-hamster tracheal epithelial cells) as monitored by the release of ^{51}Cr . Montmorillonite is roughly as active as chrysotile in this assay. However, Dubes and Mack (1988) found that "bentonite" (obtained from Fisher Scientific Company) is approximately one-tenth as effective as asbestos at mediating transfection of mammalian cell cultures.

In contrast, Holopainen et al. (1990) found that phlogopite ($\text{phl}_{87}\text{ann}_{13}$) is almost twice as hemolytic as quartz and as cytotoxic as quartz to rat alveolar macrophages (as determined by the release of LDH). After treatment with nitric and sulfuric acids, the phlogopite is more hemolytic but less cytotoxic. In their assay, the hemolytic and cytotoxic activities of muscovite are comparable to those of rutile (a negative control).

Modulated 2:1 layer silicates

Most studies on samples containing modulated 2:1 layer silicates suggest that some samples can produce fibrosis or tumors in vivo and can be highly active in vitro. However, epidemiological data suggest these minerals are at most mildly active in humans.

Sepiolite. One epidemiological study suggests that exposure to sepiolite-bearing dust does not increase the risk of pulmonary disease. Baris et al. (1980) studied 63 sepiolite workers in Turkey involved in trimming, cleaning, and polishing sepiolitic stones. Ten of the 63 showed signs of pulmonary fibrosis, but no relationship was established between exposure to sepiolite and fibrosis. Sputum was analyzed from one of the ten, but no ferruginous bodies were observed.

In vivo experiments by Wagner (1982) using Fischer 344 rats exposed for 1 yr through inhalation showed that sepiolite (termed by Wagner as "European sepiolite," possessing a fibrous morphology) is as fibrogenic as riebeckite asbestos. However, sepiolite induces no mesotheliomas in Fischer 344 rats exposed by intrapleural inoculation, whereas chrysotile (UICC standard B) induces mesotheliomas at a rate of 22.5%. Pott et al. (1990) found that the response elicited by sepiolite is highly sample dependent. The two sepiolite samples studied by Pott et al. (1990) showed tumor rates of 6% (Finland) and 67% (Uicaluaro) following intrapleural injection in female Wistar rats. Preliminary results of a powder X-ray diffraction study indicate that these samples contain significant amounts of other minerals (Guthrie and Bish, unpublished data); quantitative mineral content data are not available yet, so it is not possible to correlate purity with biological activity.

In vitro experiments by Hansen, Mossman, and co-workers (Hansen and Mossman, 1987; Mossman et al., 1989) indicate that sepiolite (Minerals Research) is capable of inducing the release of the superoxide anion from both hamster and rat alveolar macrophages in a dose-

dependent manner. In hamster alveolar macrophages, the release induced by sepiolite is comparable to the release induced by erionite and riebeckite asbestos; however, in rat alveolar macrophages, sepiolite is less active than erionite or riebeckite asbestos in eliciting a response. Chamberlain et al. (1982) found that long-fiber sepiolite (source not given) is as cytotoxic as riebeckite asbestos (UICC standard) to mouse peritoneal macrophages (as determined by release of LDH) and human type II alveolar cells (as determined by the formation of giant cells), but less cytotoxic than riebeckite asbestos to Chinese-hamster lung cells (as determined by reduction in cloning efficiency); short-fiber sepiolite (source not given), however, was determined to be noncytotoxic in the same experiments.

Palygorskite ("attapulgit"). Epidemiological data suggest that exposure to palygorskite-bearing dusts may increase the risk of lung cancer among whites (Waxweiler et al., 1988). Waxweiler et al. studied a cohort of 2302 miners and millers from an "attapulgit" company in the United States. They reported SMRs of ≤ 1.0 (based on U.S. males) for nonmalignant respiratory disease in all races (0.23–0.76, 90% CI) and race-specific SMRs for lung cancer (1.21–2.93 in whites, 90% CI; 0.21–1.12 in non-whites, 90% CI). Respirable dust exposures were $< 5 \text{ mg/m}^3$, but no information concerning mineral content was given except to note that the only fibrous mineral observed is "attapulgit clay." No mineral content is reported for the dust to which their cohort was exposed. Instead, reference was made to the "typical" mineral content of "attapulgit" clay mined in the United States as reported by Haden and Schwint (1967); a "typical" dust would thus consist of "70–80% attapulgit; 1–15% montmorillonite, sepiolite, and other clays; 4–8% quartz; and 1–5% calcite or dolomite" (Waxweiler et al., 1988). Sors et al. (1979) reported a case study of a mining engineer who exhibited signs of respiratory disease following a 2-yr exposure to "attapulgit." Lung lavage fluids suggested heavy particle burdens; XRD gave a pattern "similar to those of mineral attapulgit."

In vivo experiments have suggested that palygorskite-bearing dusts are mildly active in the lung, though some samples can be very active. Stanton et al. (1981) showed that "attapulgit" is slightly tumorigenic in Osborne-Mendel rats following direct application of the dust to the lungs. Experiments using two different samples resulted in tumor rates of $8 \pm 5.3\%$ and $11 \pm 7.5\%$; samples were from Attapulgit, Georgia, and $\geq 90\%$ pure, the remainder consisting of quartz. Jaurand et al. (1987) found that "attapulgit" (French; obtained from a deposit in Mormoiron) is nontumorigenic following intrapleural injection in specific pathogen-free Sprague-Dawley rats, whereas in the same experiments, chrysotile induces tumors at a rate of 19–52%, depending on particle size. However, Wagner (1982) observed mesothelioma rates of 12.5–25% (depending on specimen preparation method) for "attapulgit" (Spanish) following intrapleural inoculation of Fischer 344 rats; chrysotile (UICC standard B) exhibits a

comparable mesothelioma rate (22.5%). In his inhalation experiments, Wagner (1982) showed that "attapulgit" (Spanish) is as fibrogenic as riebeckite asbestos, but he reported negative results in the two experiments for another "attapulgit" (American). Bégin et al. (1987, 1990) used a bronchoalveolar lavage technique to monitor the cellular response in lungs of sheep exposed to "attapulgit" (from northern Florida). Exposure results in increases in cell numbers and enzyme levels, comparable to those observed after similar experiments using the UICC asbestos standards. No fibrosis was observed at the end of the study, but the elevated levels of enzymes indicate that the "attapulgit" is cytotoxic in vivo. Coffin et al. (1989a) reported a 1.4% incidence of mesotheliomas in rats injected intrapleurally with "attapulgit" from Georgia and Florida compared with a 1.3% incidence in the control group. Pott et al. (1974, 1990) found that "attapulgit" is carcinogenic at rates from 3.5–40% following intrapleural injection in Wistar rats, i.e., the response is sample dependent. Preliminary results of a powder X-ray diffraction study indicate that these samples contain significant amounts of other minerals (Guthrie and Bish, unpublished data).

In vitro experiments have indicated that palygorskite is as hemolytic as chrysotile, but in other nonerythrocyte cell types palygorskite is at most slightly cytotoxic and is nongenotoxic. Bignon et al. (1980) showed that "attapulgit" (Spanish) was ~ 8 times more hemolytic to human red blood cells than chrysotile (UICC standard A; derived from Rhodesian deposits). Perderiset et al. (1989) found that "attapulgit" (Senegalese; obtained from Rhône Poulenc) is hemolytic but that pretreatment of the dust with lipids or proteins (material similar to cell membranes or extracellular lung fluid) reduces the hemolytic activity. Nadeau et al. (1983) reported that "attapulgit" is as hemolytic as chrysotile (UICC standard B) and more hemolytic than sepiolite or erionite.

In contrast, in vitro experiments using cells other than RBCs have suggested that palygorskite-bearing dusts are generally inactive, although the activity varies greatly as a function of the surface characteristics of the sample. Woodworth et al. (1983) found that palygorskite (CMS Clay Repository sample from Nevada) might have a slight effect on cultured hamster trachea, but the effect is not statistically different from the control group, riebeckite asbestos and fiber glass, however, test positive statistically in the same assay. Jaurand et al. (1987) found that "attapulgit" (French) may be cytotoxic to rat pleural mesothelial cells only at high doses, whereas chrysotile is generally much more cytotoxic. Reiss et al. (1980) demonstrated that palygorskite (Attapulgit, Georgia) is much less cytotoxic than "amosite" asbestos to human-embryonic, intestine-derived epithelial cells. Pezerat et al. (1989) found that "attapulgit" (Senegalese) is inactive in catalyzing the production of O radicals, and Achard et al. (1987) found that the same material does not induce SCEs in rat pleural mesothelial cells. Renier et al. (1990) found no unscheduled DNA repair synthesis in rat pleural me-

sothelial cells following treatment with "attapulgit" (Mormoiron region, France). Chamberlain et al. (1982) found that long-fiber "attapulgit" (source not given) is more cytotoxic than riebeckite asbestos (UICC standard) to mouse peritoneal macrophages (see description of assays in the section on sepiolite) but less cytotoxic than riebeckite asbestos to Chinese-hamster lung cells; short-fiber "attapulgit" (source not given), however, is slightly cytotoxic to mouse peritoneal macrophages and noncytotoxic to Chinese-hamster lung cells. Nolan et al. (1991) further demonstrated that the *in vitro* activity of palygorskite varies between samples by showing that among nine palygorskites that possess different surface characteristics there is a corresponding range in hemolytic activity.

Zeolites

The biological activity of erionite has been studied extensively, and all data indicate that it is extremely active in humans, *in vivo*, and *in vitro*. Data on other zeolites are less conclusive, particularly in light of the poor quality of the samples studied.

Erionite. Epidemiological data suggest that exposure to erionite-bearing dusts increases the risk of mesothelioma in humans, even at much lower exposure levels than required for amphibole asbestos-induced mesothelioma. Earlier epidemiological studies in the Cappadocian region of Turkey revealed outbreaks of asbestos-related respiratory diseases, including mesothelioma (Baris et al., 1979). Initially, it was assumed that asbestos present in the stucco used in that region was the cause of these diseases; however, Baris et al. (1979) found outbreaks in villages in which the stucco does not contain asbestos. Later studies focused on exposure to erionite as the cause. Baris et al. (1987) found zeolite fibers (as determined by ATEM) in air samples from affected villages, although air samples from some of these villages additionally contain fibrous tremolite. Lung contents as determined from sputum samples (Sébastien et al., 1981, 1984; identification by ATEM and electron diffraction) and biopsies (Baris et al., 1987; identification by ATEM) also indicate that individuals from these regions have been exposed to both erionite and asbestos (chrysotile, tremolite asbestos, and riebeckite asbestos), though the amount of zeolite exceeded the combined amounts of asbestos (on a per-fiber basis) in the two samples of human lung contents.

Mumpton (1979) investigated the mineral content of dusts in the Cappadocian region. He found that erionite was present in samples from two villages in which mesothelioma rates are high, although erionite is abundant only in one of those villages. He also found that erionite is abundant in a third village, Sarihidir, which at that time had no reported cases of mesothelioma. Mumpton concluded, therefore, that the geographic distribution of erionite is inconsistent with the distribution of mesothelioma. Subsequently, however, Baris et al. (1987) surveyed Sarihidir and reported three cases of mesothelioma. Baris et al. also reported fiber characteristics from

the affected villages, indicating that zeolite fibers were present ubiquitously but other types of fibers varied between villages.

The most disturbing implication of the observations in Turkey is that if erionite is indeed the cause of the high rates of mesothelioma, then erionite is capable of inducing mesothelioma in humans at low exposures. Baris et al. (1987) reported total fiber levels in the villages from 0.004 to 0.175 fibers/mL, and these measurements included other dusts in addition to zeolite. Simonato et al. (1989) reported newer estimates of fiber characteristics in Karain and Sarihidir (two of the affected villages) and found levels to be 0.002–0.010 (~80% zeolite) and 0.001–0.029 (~60% zeolite), respectively.

In vivo experiments have further demonstrated the high fibrogenic and carcinogenic potential of erionite-bearing dusts. Suzuki and Kohyama (Suzuki, 1982; Suzuki and Kohyama, 1984, 1988) studied the effects on mice of intrapleural injections of two erionite samples, one from Needle Peak, Nevada (Minerals Research; listed by Suzuki and Kohyama as "Needle Park"), and one from an unknown locality (Resource International Company). In mice injected intrapleurally with 2 mg of dust, Needle Peak erionite induces tumors at a rate of 54.5%, compared with 0–25% for chrysotile and 40.5% for "amosite" asbestos; fibrosis also develops after injection of any of the dusts. They also performed experiments using higher doses, but a low percentage of mice survived long enough to develop tumors (>7 months). Maltoni et al. (1982) are investigating the effect of method of exposure to erionite on the induction of mesotheliomas in rats. For their initial results, they reported that erionite (sedimentary, obtained from G. Gottardi, University of Modena, Italy) induces mesotheliomas by intrapleural injection at a rate of 90% (nine of ten rats), whereas riebeckite asbestos induces mesothelioma by intraperitoneal injection at a rate of 100% (12 of 12).

Wagner and coworkers have also studied the *in vivo* effects of exposure to erionite using specific pathogen-free Sprague-Dawley rats (Wagner, 1982) and Fischer 344 rats (Wagner et al., 1985). They used intrapleural injection (20 mg/rat) and inhalation to study the carcinogenic potential of four erionite samples: Oregonian erionite (from F. Mumpton, Minerals Research), nonfibrous synthetic zeolite (chemically identical to erionite, from R. Taylor, Laporte Industries), a New Zealand erionite (similar size distribution to the Oregonian sample, though fibers are slightly thicker), and a Turkish (Karain) rock determined to consist of "poorly consolidated rock . . . [made of] incompletely formed erionite . . . in an amorphous matrix which has the same composition as erionite" (Wagner et al., 1985). In the rats intrapleurally injected with Oregonian erionite and Turkish rock, 40 of 40 and 38 of 40 developed mesotheliomas, respectively, with mean survival times of 390 and 435 d; the New Zealand erionite was ~1/4 as potent as the Oregonian and Turkish erionites. For comparison, in the same experiment, chrysotile induced 19 mesotheliomas in 40 rats with a mean sur-

vival time of 678 d. The same effect was observed in the inhalation experiments: 27 of 28 rats exposed to Oregonian erionite developed mesotheliomas with a mean survival time of 580 d compared with one of 28 rats exposed to riebeckite asbestos (UICC standard) with a mean survival time of 917 d. The synthetic zeolite was also tested in the inhalation experiments and induced two tumors in 28 rats with a mean survival time of 784 d. With respect to the erionite sample, they stated "No other dusts we have investigated have produced this high incidence of tumours particularly following inhalation" (Wagner et al., 1985). Coffin et al. (1989a) confirmed the observation that erionite-treated rats develop mesotheliomas at a higher rate and in a shorter time than chrysotile asbestos- or riebeckite asbestos-treated rats; the erionite used was from Rome, Oregon (Minerals Research), and was prepared by either H₂O sedimentation or air elutriation.

In two later studies, the Wagner group confirmed their original finding. Johnson and Wagner (1989) exposed Fischer 344 rats to erionite from Rome, Oregon (obtained from Minerals Research), by inhalation in a dust-rich environment (10 mg/m³) and found that erionite exposure produces both fibrosis and mesothelioma; three of the three rats exposed to dust for 12 weeks and allowed to recover for 12 months developed mesothelioma. Hill et al. (1990) used intrapleural injection of Oregonian erionite in Porton rats to determine the dose-response relationship for induction of mesotheliomas. They found a sharp rise in mesothelioma rate from 0% mesotheliomas at 0.01 mg/rat to ~90% at 1.0 mg/rat; they stated "erionite is over 200 times more tumorigenic than crocidolite."

In vitro studies have demonstrated that erionite-bearing dusts are both cytotoxic and genotoxic. Poole et al. (1983b) studied the genotoxic effects of Oregonian erionite (Minerals Research) by monitoring morphological transformations and unscheduled DNA repair in mouse-embryo fibroblasts (cells that reside in the connective tissue and that are responsible for collagen production, i.e., that are involved in fibrosis). Erionite was found to be active in both of these tests, whereas amphibole asbestos does not cause morphological transformations (Poole et al., 1983a). Numerous studies have also demonstrated that erionite is cytotoxic (Palekar et al., 1988; Brown et al., 1989) and genotoxic (Palekar et al., 1987; 1989a, 1989b) to Chinese-hamster lung cells. Brown et al. (1989) found that the cytotoxic effects are related to long, thin fibers, since milling of the sample to reduce the fiber lengths also reduces its activity. The cytotoxic and genotoxic activities of erionite are slightly less than those of asbestos when compared on a mass basis, but comparison on a per-fiber basis shows that the activities of erionite are much greater than those of asbestos (Palekar et al., 1988; Brown et al., 1989).

In other tests for genotoxicity, Hansen, Mossman, and coworkers (Hansen and Mossman, 1987; Mossman et al., 1989; Mossman and Scsko, 1990) have shown that erionite (Rome, Oregon; from R. Davies) is as effective as

riebeckite asbestos in catalyzing the production of the superoxide anion from hamster and rat alveolar macrophages. In contrast, however, Pezerat et al. (1989) found that though Oregonian erionite is nearly as effective as most types of asbestos at catalyzing the production of O radicals from an aqueous medium, it is inactive when compared with chrysotile (UICC standard B) or fibrous brucite. Kelsey et al. (1986) found that erionite (Rome, Oregon; obtained from V. Timbrell) induces SCEs slightly in Chinese-hamster ovary cells, whereas riebeckite asbestos (UICC standard) does not; ultraviolet light, however, is much more effective at inducing SCEs. Both Oregonian erionite and riebeckite asbestos (UICC standard) induce low levels of chromosomal aberrations in the Chinese-hamster ovary cells (Kelsey et al., 1986). Coffin et al. are attempting to relate the biological activity of erionite to its mineralogical characteristics (Coffin et al., 1989b).

Mordenite and other zeolites. In vivo experiments by Suzuki and Kohyama (Suzuki, 1982; Suzuki and Kohyama, 1984, 1988) suggest that a mordenite-bearing dust and zeolite 4A are fibrogenic but noncarcinogenic. Suzuki and Kohyama used intraperitoneal injection in mice to test the biological response to various zeolites. Included in their experiments was a mordenite-bearing sample (Resource International Company, Denver) that contained both granular and fibrous morphologies and a synthetic zeolite, 4A (Union Carbide Corporation); quantitative X-ray diffraction of the mordenite-bearing sample has shown that it contains ~63.5% impurities, including clinoptilolite, feldspar, opal-CT, and gypsum (Guthrie and Bish, unpublished data). In fact, mordenite was used in these experiments to test the relationship between particle shape and activity among zeolites. The mordenite sample was described as a mixture of fibrous and nonfibrous forms, despite the fact that mordenite is uniquely fibrous. Hence, it should be recognized that their results apply to an impure mordenite sample. Their experiments show that 10-mg doses of either the mordenite-bearing sample or zeolite 4A induce no tumors in mice for experiments up to 23 months in duration; fibrosis, however, does result from the exposure. On the other hand, the same experiments showed that 0.5- and 2.0-mg doses of erionite (Needle Peak, Nevada) induce tumors at 33.3 and 54.5%, respectively; also, fibrosis in erionite-exposed mice is more pronounced. In their group exposed to a 10-mg dose of erionite, a 37.5% rate of tumor induction was observed, but only eight rats survived to >7 months, i.e., exposure to erionite may elicit a dose-dependent response in the lungs of mice, but this relationship cannot be tested because of the poor statistical significance of the results from the group exposed to high concentrations of erionite.

Maltoni and Minardi (1988, 1989) studied the biological activity of synthetic zeolites used in detergents (MS 4A and MS 5A, Na and Ca rich, respectively; source not given) by intraperitoneal, intrapleural, and subcutaneous injection in rats. For both zeolites and all routes of ex-

posure, tumors appeared but not at rates significantly different from those observed in the control groups that were injected with H₂O (~25–50%). In the same assay, however, riebeckite asbestos induces tumors following injection into the abdominal cavity at a rate of 97.5%.

In vitro experiments suggest that mordenite-bearing dust is much less active than erionite. Hansen, Mossman, and coworkers (Hansen and Mossman, 1987; Mossman et al., 1989; Mossman and Sesko, 1990) found that a mordenite-bearing sample (source not given) is less effective than sepiolite and much less effective than erionite at stimulating the release of the superoxide anion from rat alveolar macrophages. Palekar et al. (1988) found that this same mordenite is noncytotoxic to Chinese-hamster lung cells. Quantitative X-ray diffraction of this mordenite-bearing sample has shown that it contains ~50.5% impurities, including clinoptilolite, feldspar, and opal-CT (Guthrie and Bish, unpublished data). As was the case for the in vivo experiments of Suzuki and Kohyama, mordenite was used as a nonfibrous-zeolite control in the in vitro assays, despite that mordenite is uniquely fibrous.

Rom et al. (1983) discussed the implications of fibrous zeolite health hazards with respect to the western United States and stressed the need for epidemiological studies in this region.

DISCUSSION

The wide range of minerals that have been studied by various techniques offers the potential for revealing the causes of a mineral's biological activity. Indeed, it is clear from the dusts studied already that minerals exhibit different activities and elicit different biological responses (Table 2). In fact, differences in biological response can be found both between mineral species and between different samples of the same mineral species. The variations in response likely reflect variations in the interactions between the mineral surfaces and biological components (i.e., cell surfaces, enzymes, proteins, DNA, etc.). Ideally, if this observed variation can be related to differences in the physical and chemical properties of the minerals, then the mechanisms of mineral toxicity may be elucidated. Unfortunately, several mineralogical problems are present in the studies reviewed above that make such inferences difficult if not impossible. Generally, the primary mineralogical aspects that are controlled in most biological experiments are the particle shape and size distribution and mass concentration or dose employed, since these parameters appear to relate to the material's biological activity as determined by in vivo methods (e.g., Stanton et al., 1981). The exact mineral content of the dusts, however, is rarely characterized. In other words, little attention is generally given to the identification and amount of contaminants in the dust sample. Instead, it is assumed that the mineral content of the sample matches the information provided by the supplier. However, samples obtained from most suppliers potentially contain a mixture of minerals and often are simply a different mineral from the one listed on the label. The mordenite

TABLE 2. Summary of data on the biological activities of clays and zeolites

Mineral	Epidemiological	In vivo	In vitro
Hematite	—	—	—
Goethite	n.d.	+ to ++ (f)	n.d.
Lepidocrocite	n.d.	+ to ++ (f)	n.d.
Boehmite	n.d.	— to ++ (f)	n.d.
Fibrous brucite	n.d.	+++ (t)	++
Kaolinite	+	— to ++ (f)	+ to +++
Halloysite	n.d.	— to + (t)	n.d.
Antigorite	n.d.	n.d.	—
Berthierine	n.d.	n.d.	+++
Chlorite (Fe-rich)	n.d.	n.d.	+++
Talc	— to +	—	— to +++
Mica/mica-like clays	+	— to + (f)	— to +
Sepiolite	—	+++ (f)	— to +++
Palygorskite	— to +	— to +++ (f)	— to +++
Erionite	+++	+++ (f)	+++
Mordenite	n.d.	+++ (f)	— to +
Zeolites 4A and 5A	n.d.	— (f)	n.d.

Note: Symbols: — indicates inactive; + indicates active, n.d. indicates no data available, f and t indicate fibrogenic and tumorigenic, respectively

samples used in both the in vitro experiments (Hansen and Mossman, 1987; Palekar et al., 1988; Mossman et al., 1989; Mossman and Sesko, 1990) and the in vivo experiments (Suzuki, 1982; Suzuki and Kohyama, 1984, 1988) illustrate this well. Each sample actually contains a mixture of mordenite, clinoptilolite, feldspar, opal-CT, and gypsum (Guthrie and Bish, unpublished data). The published mordenite-toxicity data actually apply to a mixture of minerals that is ≤50% mordenite.

Another mineralogical problem in biological studies is that the surface properties of the samples are generally not adequately characterized. Recent work (e.g., Pezerat, 1990) has suggested that although the fibrous shape of a material may be important in maintaining the particle in the target organ or in enhancing surface area, the mechanisms by which minerals are toxic relate to their surface properties, such as active oxidation/reduction sites. Indeed, the activity of a mineral varies with surface state (Nolan et al., 1991) and surface area (Gormley and Addison, 1983), which in turn can vary substantially between samples. Thus, it is not only important to control the surface aspects of a mineral during an experiment, but it is important to characterize these properties so that they can be related to biological activity.

These mineralogical deficiencies in biomedical research can be rectified through collaborative efforts between minerals scientists and health scientists. Such collaboration should involve both characterization of the mineralogical aspects of the experiment and design of experiments that will allow mechanistic questions to be addressed. For example, an amphibole has numerous prop-

erties that might contribute to its activity (e.g., broken Si-O bonds, "exchangeable" cations in the A site, polyvalent cations in the octahedral sites, underbonded O resulting from Al substitution in the tetrahedral sites, specific surface periodicities). Hence, the results of a study on amphibole-induced pathogenesis may record effects from several mineralogical properties. On the other hand, it is possible to isolate the effects of a specific mineralogical characteristic by an appropriate choice of mineral pairs. For instance, the role of polyvalent cations in the octahedral site can be determined by comparing the activities between two minerals that differ only in the composition of the octahedral site (e.g., tremolite and ferroactinolite; annite and phlogopite). This type of approach could be extremely effective for determining mineralogical mechanisms of mineral-induced pathogenesis. Such information will lead to both a better understanding of diseases such as cancer and more effective regulation of minerals, since regulations can be based on mineralogical properties additional to particle size and shape.

Furthermore, it should be recognized that though a mineral is active in a particular assay, it may pose limited risk to humans. A complete understanding of the numerous factors that contribute to mineral-induced pathogenesis is essential before the results of any one assay can be used to predict risk to humans.

ACKNOWLEDGMENTS

I would like to thank D.L. Bish, G.D. Guthrie, Sr., and C.S. Nicholson-Guthrie for extensive discussions and encouragement during the preparation of this manuscript. I also benefited from discussions with F. Mumpton and B. Lehnert. Thoughtful reviews of the manuscript were provided by D.L. Bish, J. Hughes, B. Lehnert, W. Moll, B.T. Mossman, M. Ross, and D. Vaniman. My time was supported by a postdoctoral fellowship from the Director's office at the Los Alamos National Laboratory.

REFERENCES CITED

- Achard, S., Perderset, M., and Jaurand, M.-C. (1987) Sister chromatid exchanges in rat pleural mesothelial cells treated with crocidolite, attapulgite, or benzo 3-4 pyrene. *British Journal of Industrial Medicine*, 44, 281-283.
- Adamis, Z., and Timár, M. (1978) Studies on the effect of quartz, bentonite and coal dust mixtures on macrophages *in vitro*. *British Journal of Experimental Pathology*, 59, 411-415.
- Ames, B.N., McCann, J., and Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research*, 31, 347-364.
- Appel, J.D., Fasy, T.M., Kohtz, D.S., Kohtz, J.D., and Johnson, E.M. (1988) Asbestos fibers mediate transformation of monkey cells by exogenous plasmid DNA. *Proceedings of the National Academy of Sciences U.S.A.*, 85, 7670-7674.
- Attygalle, D., Harrison, C.V., King, E.J., and Mohanty, G.P. (1954) Infective pneumoconiosis. *British Journal of Industrial Medicine*, 11, 245-259.
- Baris, I., Simonato, L., Artvinli, M., Pooley, F., Saracci, R., Skidmore, J., and Wagner, C. (1987) Epidemiological and environmental evidence of the health effects of exposure to erionite fibres. A four-year study in the Cappadocian region of Turkey. *International Journal of Cancer*, 39, 10-17.
- Baris, Y.I., Artvinli, M., and Sahin, A.A. (1979) Environmental mesothelioma in Turkey. *Annals of the New York Academy of Science*, 330, 423-432.
- Baris, Y.I., Sahin, A.A., and Erkan, M.L. (1980) Clinical and radiological study in sepiolite workers. *Archives of Environmental Health*, 35, 343-346.
- Bégin, R., Massé, S., Rola-Pleszczynski, M., Geoffroy, M., Martel, M., Desmarais, Y., and Sébastien, P. (1987) The lung biological activity of American attapulgite. *Environmental Research*, 42, 328-339.
- Bégin, R., Massé, S., and Sébastien, P. (1990) Assessment of the fibrogenicity of attapulgite. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 395-403. Springer-Verlag, Berlin.
- Bignon, J., Sébastien, P., Gaudichet, A., and Jaurand, M.C. (1980) Biological effects of attapulgite. In J.C. Wagner, Ed., *Biological effects of mineral fibres*, vol. 1, IARC Scientific Publication 30, p. 163-181. World Health Organization, Lyon, France.
- Bish, D.L., and Chipera, S.J. (1991) Detection of trace erionite using X-ray powder diffraction. Erionite in tufts of Yucca Mountain, Nevada, and central Turkey. *Clays and Clay Minerals*, 39, 437-445.
- Boyd, J.T., Doll, R., Faulds, J.S., and Leiper, J. (1970) Cancer of the lung in iron ore (haematite) miners. *British Journal of Industrial Medicine*, 27, 97-105.
- Brambilla, C., Abraham, J., Brambilla, E., Benirschke, K., and Bloor, C. (1979) Comparative pathology of silicate pneumoconiosis. *American Journal of Pathology*, 96, 149-163.
- Brown, D.P., and Wagoner, J.K. (1980) Occupational exposure to talc containing asbestos: III. Retrospective cohort study of mortality. U.S. Department of HEW (NIOSH) Publication 80-115, 31-33.
- Brown, R.C., Chamberlain, M., Davies, R., Morgan, D.M.L., Pooley, F.D., and Richards, R.J. (1980) A comparison of 4 *in vitro* systems applied to 21 dusts. In E.G. Beck and J. Bignon, Eds., *In vitro effects of mineral dusts*, NATO ASI Series, p. 47-52. Springer-Verlag, Berlin.
- Brown, R.C., Davies, R., and Rood, A.P. (1989) Modification of fibrous Oregon erionite and its effects on *in vitro* activity. In J. Bignon, J. Peto and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 74-80. International Agency for Research on Cancer, Lyon, France.
- Campbell, J.A. (1940) Effects of precipitated silica and of iron oxide on the incidence of primary lung tumours in mice. *British Medical Journal*, 2, 275-280.
- Chamberlain, M., and Brown, R.C. (1978) The cytotoxic effects of asbestos and other mineral dust in tissue culture cell lines. *British Journal of Experimental Pathology*, 59, 183-189.
- Chamberlain, M., and Tarmy, E.M. (1977) Asbestos and glass fibers in bacterial mutation tests. *Mutation Research*, 43, 159-164.
- Chamberlain, M., Davies, R., Brown, R.C., and Griffiths, D.M. (1982) *In vitro* tests for the pathogenicity of mineral dusts. *Annals of Occupational Hygiene*, 26, 583-592.
- Chen, S.Y., Hayes, R.B., Liang, S.R., Li, Q.G., Stewart, P.A., and Blair, A. (1990) Mortality experience of haematite mine workers in China. *British Journal of Industrial Medicine*, 47, 175-181.
- Chipera, S.J., and Bish, D.L. (1989) The occurrence and distribution of erionite at Yucca Mountain, Nevada. Los Alamos National Laboratory Report LA-11663-MS, 20 p.
- Churg, A., Wiggs, B., Depaoli, L., Kampe, B., and Stevens, B. (1984) Lung asbestos content in chrysotile workers with mesothelioma. *American Reviews of Respiratory Disease*, 130, 1042-1045.
- Churg, A., Wright, J.L., Depaoli, L., and Wiggs, B. (1989) Mineralogic correlates of fibrosis in chrysotile miners and millers. *American Reviews of Respiratory Disease*, 139, 891-896.
- Coffin, D.L., Palekar, L.D., Cook, P.M., and Creason, J.P. (1989a) Comparison of mesothelioma induction in rats by asbestos and nonasbestos mineral fibers: Possible correlation with human exposure data. In A.P. Wehner, Ed., *Biological interaction of inhaled mineral fibers and cigarette smoke*, p. 347-354. Battelle Press, Seattle.
- Coffin, D.L., Peters, S.E., Palekar, L.D., and Stahel, E.P. (1989b) A study of the biological activity of erionite in relation to its chemical and structural characteristics. In A.P. Wehner, Ed., *Biological interaction of inhaled mineral fibers and cigarette smoke*, p. 355-372. Battelle Press, Seattle.
- Costa, D., Guignard, J., and Pezerat, H. (1990) Oxidizing surface properties of divalent iron-rich phyllosilicates in relation to their toxicity by oxidative stress mechanism. In J. Bignon, Ed., *Health related effects*

- of phyllosilicates, NATO ASI Series, vol. G21, p. 129-134. Springer-Verlag, Berlin
- Cullinan, P., and McDonald, J.C. (1990) Respiratory disease from occupational exposure to non-fibrous phyllosilicates. In J. Bignon, Ed., Health related effects of phyllosilicates, NATO ASI Series, vol. G21, p. 161-178. Springer-Verlag, Berlin.
- Davies, R. (1983) Factors involved in the cytotoxicity of kaolinite towards macrophages *in vitro*. Environmental Health Perspectives, 51, 249-252.
- Davis, B.L. (1990) Quantitative analysis of asbestos minerals by the reference intensity X-ray diffraction procedure. Journal of the American Industrial Hygiene Association, 51, 297-303.
- Dubey, G.R., and Mack, L.R. (1988) Asbestos-mediated transfection of mammalian cell cultures. In Vitro Cellular and Developmental Biology, 24, 175-182.
- Endo-Capron, S., Fleury-Feith, J., Nebut, M., De Neef, R., and Jaurand, M.C. (1990) Some *in vivo* and *in vitro* studies carried out with talc samples. In J. Bignon, Ed., Health related effects of phyllosilicates, NATO ASI Series, vol. G21, p. 369-375. Springer-Verlag, Berlin.
- Environmental Protection Agency (1986) Airborne asbestos health assessment update, EPA/600/8-84/003F.
- Gardner, L.U., Dworski, M., and Delahant, A.B. (1944) Aluminum therapy in silicosis. Journal of Industrial Hygiene and Toxicology, 26, 211-223.
- Ghio, A., Kennedy, T., Baser, M., Rawlings, W.R., Rao, N.V., and Hoidal, J.R. (1990) Kaolin generates H_2O_2 and causes hemolysis by acting as a Fenton reagent. In J. Bignon, Ed., Health related effects of phyllosilicates, NATO ASI Series, vol. G21, p. 361-368. Springer-Verlag, Berlin.
- Gormley, I.P., and Addison, J. (1983) The *in vitro* cytotoxicity of some standard clay mineral dusts of respirable size. Clay Minerals, 18, 153-163.
- Haden, W.L., Jr., and Schwint, I.A. (1967) Attapulgit: Its properties and applications. Industrial and Engineering Chemistry Research, 59, 58-69.
- Hale, L.W., Gough, J., King, E.J., and Nagelschmidt, G. (1956) Pneumoconiosis of kaolin workers. British Journal of Industrial Medicine, 13, 251-259.
- Hansen, K., and Mossman, B.T. (1987) Generation of superoxide (O_2^-) from alveolar macrophages exposed to asbestiform and nonfibrous particles. Cancer Research, 47, 1681-1686.
- Heimann, H., Moskowitz, S., Harihara, C.R., Gupta, M.N., and Manki, N.S. (1953) Silicosis in mica mining in Bihar, India. Archives of Industrial Hygiene and Occupational Medicine, 8, 420-435.
- Heppleston, A.G. (1984) Pulmonary toxicology of silica, coal and asbestos. Environmental Health Perspectives, 55, 111-127.
- Hill, R.J., Edwards, R.E., and Carthew, P. (1990) Early changes in the pleural mesothelium following intrapleural inoculation of the mineral fibre erionite and the subsequent development of mesotheliomas. Journal of Experimental Pathology, 71, 105-118.
- Holopainen, M., Vallyathan, V., Hedenborg, M., and Klockars, M. (1990) Toxicity of phlogopite and muscovite *in vitro*. In J. Bignon, Ed., Health related effects of phyllosilicates, NATO ASI Series, vol. G21, p. 349-360. Springer-Verlag, Berlin.
- Holt, P.F., Lindsay, H., and Beck, E.G. (1970) Some derivatives of polyvinylpyridine 1-oxides and their effect on the cytotoxicity of quartz in macrophage cultures. British Journal of Pharmacology, 38, 192-201.
- Jaurand, M.C., Magne, L., and Bignon, J. (1980) Effects of well-defined fibres on red blood cells and alveolar macrophages. In J.C. Wagner, Ed., Biological effects of mineral fibres, vol. 1, IARC Scientific Publication 30, p. 441-450. World Health Organization, Lyon, France.
- Jaurand, M.-C., Fleury, J., Monchaux, G., Michelne, N., and Bignon, J. (1987) Pleural carcinogenic potency of mineral fibers (asbestos, attapulgit) and their cytotoxicity on cultured cells. Journal of the National Cancer Institute, 79, 797-804.
- Johnson, N.F., and Wagner, J.C. (1989) Effect of erionite inhalation on the lungs of rats. In A.P. Wehner, Ed., Biological interaction of inhaled mineral fibers and cigarette smoke, p. 355-372. Battelle Press, Seattle.
- Kelsey, K.T., Yano, E., Liber, H.L., and Little, J.B. (1986) The *in vitro* genetic effects of fibrous erionite and crocidolite asbestos. British Journal of Cancer, 54, 107-114.
- Kennedy, T.P., Rawlings, W., Jr., Baser, M., and Tockman, M. (1983) Pneumoconiosis in Georgia kaolin workers. American Reviews of Respiratory Disease, 127, 215-220.
- Kennedy, T.P., Dodson, R., Rao, N.V., Ky, H., Hopkins, C., Baser, M., Tolley, E., and Hoidal, J.R. (1989) Dusts causing pneumoconiosis generate H_2O_2 and produce hemolysis by acting as Fenton catalysts. Archives of Biochemistry and Biophysics, 269, 359-364.
- Kettle, E.H. (1934) Experimental pneumoconiosis. Infective silicosis. Journal of Pathology and Bacteriology, 38, 201-208.
- King, E.J., and Harrison, C.V. (1948) The effects of kaolin on the lungs of rats. Journal of Pathology and Bacteriology, 60, 435-440.
- King, E.J., Gilchrist, M., and Rae, M.V. (1947) Tissue reaction to sericite and shale dusts treated with hydrochloric acid. An experimental investigation on the lungs of rats. Journal of Pathology and Bacteriology, 59, 325-327.
- King, E.J., Harrison, C.V., and Mohanty, G.P. (1955) The effect of various forms of alumina on the lungs of rats. Journal of Pathology and Bacteriology, 69, 81-93.
- Kleinfeld, M., Messite, J., Kooyman, O., and Zaki, M.H. (1967) Mortality among talc miners and millers in New York State. Archives of Environmental Health, 14, 663-667.
- Kleinfeld, M., Messite, J., and Zaki, M.H. (1974) Mortality experiences among talc workers: A follow-up study. Journal of Occupational Medicine, 16, 345-349.
- Langer, A.M., and Nolan, R.P. (1989) Fibre type and burden in parenchymal tissues of workers occupationally exposed to asbestos in the United States. In J. Bignon, J. Peto, and R. Saracci, Eds., Non-occupational exposure to mineral fibres, p. 330-335. International Agency for Research on Cancer, Lyon, France.
- Lapenas, D., Gale, P., Kennedy, T., Rawlings, W., Jr., and Dietrich, P. (1984) Kaolin pneumoconiosis. American Reviews of Respiratory Disease, 130, 282-288.
- Lawler, A.B., Mandel, J.S., Schuman, L.M., and Lubin, J.H. (1985) A retrospective cohort mortality study of iron ore (hematite) miners in Minnesota. Journal of Occupational Medicine, 27, 507-517.
- Leake, B.E. (1978) Nomenclature of amphiboles. Canadian Mineralogist, 16, 501-520.
- Lehnert, B.E. (1990) Lung defense mechanisms against deposited dusts. Problems in Respiratory Care, 3, 130-162.
- Leophonte, P., and Didier, A. (1990) French talc pneumoconiosis. In J. Bignon, Ed., Health related effects of phyllosilicates, NATO ASI Series, vol. G21, p. 203-209. Springer-Verlag, Berlin.
- Low, R.B., Leffingwell, C.M., and Bulman, C.A. (1980) Effects of kaolinite on amino acid transport and incorporation into protein by rabbit pulmonary alveolar macrophages. Archives of Environmental Health, 35, 217-223.
- Lynch, K.M., and McIver, F.A. (1954) Pneumoconiosis from exposure to kaolin dust. Kaolinosis. American Journal of Pathology, 30, 1117-1122.
- Maltoni, C., and Minardi, F. (1988) First available results of long-term carcinogenicity bioassay on detergency zeolites (MS 4A and MS 5A). Annals of the New York Academy of Science, 534, 978-985.
- (1989) Recent results of carcinogenicity bioassays of fibres and other particulate materials. In J. Bignon, J. Peto, and R. Saracci, Eds., Non-occupational exposure to mineral fibres, p. 46-53. International Agency for Research on Cancer, Lyon, France.
- Maltoni, C., Minardi, F., and Morisi, L. (1982) Pleural mesothelioma in Sprague-Dawley rats by erionite. First experimental evidence. Environmental Research, 29, 238-244.
- Marks, J., and Nagelschmidt, G. (1959) Study of the toxicity of dust with use of the *in vitro* dehydrogenase technique. American Medical Association Archives of Industrial Health, 20, 37/383-43/389.
- McDonald, J.C., McDonald, A.D., Sébastien, P., and May, K. (1988) Health of vermiculite miners exposed to trace amounts of fibrous tremolite. British Journal of Industrial Medicine, 45, 630-634.
- Mossman, B.T., and Craighead, J.E. (1982) Comparative cocarcinogenic effects of crocidolite asbestos, hematite, kaolin and carbon in implanted tracheal organ cultures. Annals of Occupational Hygiene, 26, 553-567.
- Mossman, B.T., and Marsh, J.P. (1989) Evidence supporting a role for active oxygen species in asbestos-induced toxicity and lung disease. Environmental Health Perspectives, 81, 91-94.

- Mossman, B.T., and Sesko, A.M. (1990) *In vitro* assays to predict the pathogenicity of mineral fibers. *Toxicology*, 60, 53-61.
- Mossman, B.T., Hansen, K., Marsh, J.P., Brew, M.E., and Petruska, J. (1989) Mechanisms of fibre-induced superoxide release from alveolar macrophages and induction of superoxide dismutase in the lungs of rats inhaling crocidolite. In J. Bignon, J. Peto, and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 81-92. International Agency for Research on Cancer, Lyon, France.
- Mossman, B.T., Bignon, J., Corn, M., Seaton, A., and Gee, J.B.L. (1990) Asbestos: Scientific developments and implications for public policy. *Science*, 247, 294-301.
- Mumpton, F.A. (1979) A reconnaissance study of the association of zeolites with mesothelioma occurrences in central Turkey. U.S. Geological Survey, Open-file Report 79-954, 55 p.
- Nadeau, D., Paradis, D., Gaudreau, A., Pele, J.P., and Calvert, R. (1983) Biological evaluation of various natural and man-made mineral fibers. Cytotoxicity, hemolytic activity and chemiluminescence study. *Environmental Health Perspectives*, 51, 374.
- Nolan, R.P., Langer, A.M., and Herson, G.B. (1991) Characterisation of palygorskite specimens from different geological locales for health hazard evaluation. *British Journal of Industrial Medicine*, 48, 463-475.
- Oberdörster, G., and Lehnert, B.E. (1990) Particle properties, tumors and fibrosis (abs.). Mechanisms in fibre carcinogenesis, p. 57. NATO Advanced Research Workshop, Albuquerque, New Mexico.
- Ogle, C.J., Rundle, E.M., and Sugar, E.T. (1989) China clay workers in the south west of England: Analysis of chest radiograph readings, ventilating capacity and respiratory symptoms in relation to type and duration of occupation. *British Journal of Industrial Medicine*, 46, 261-270.
- Oldham, P.D. (1983) Pneumoconiosis in Cornish china clay workers. *British Journal of Industrial Medicine*, 40, 131-137.
- Palekar, L.D., Eyre, J.F., Most, B.M., and Coffin, D.L. (1987) Metaphase and anaphase analysis of V79 cells exposed to erionite, UICC chrysotile and UICC crocidolite. *Carcinogenesis*, 8, 553-560.
- Palekar, L.D., Most, B.M., and Coffin, D.L. (1988) Significance of mass and number of fibers in the correlation of V79 cytotoxicity with tumorigenic potential of mineral fibers. *Environmental Research*, 46, 142-152.
- Palekar, L.D., Eyre, J.F., and Coffin, D. (1989a) Transplantation and chromosomal analysis of cell lines derived from mesotheliomas induced in rats with erionite and UICC chrysotile asbestos. In J. Bignon, J. Peto, and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 161-172. International Agency for Research on Cancer, Lyon, France.
- (1989b) Chromosomal changes associated with tumorigenic mineral fibers. In A.P. Wehner, Ed., *Biological interaction of inhaled mineral fibers and cigarette smoke*, p. 355-372. Battelle Press, Seattle.
- Perderset, M., Saint Etienne, L., Bignon, J., and Jaurand, M.C. (1989) Interactions of attapulgite (fibrous clay) with human red blood cells. *Toxicology Letters*, 47, 303-309.
- Pezzerat, H. (1990) Surface activity of mineral dusts and processes of oxidative stress (abs.). Mechanisms in fibre carcinogenesis, p. 60. NATO Advanced Research Workshop, Albuquerque, New Mexico.
- Pezzerat, H., Zalma, R., and Guignard, J. (1989) Production of oxygen radicals by the reduction of oxygen arising from the surface activity of mineral fibres. In J. Bignon, J. Peto, and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 100-111. International Agency for Research on Cancer, Lyon, France.
- Piggott, G.H., and Pinto, P.J. (1983) Effects of nonfibrous minerals in the V79-4 cytotoxicity test. *Environmental Health Perspectives*, 51, 173-179.
- Polcard, A. (1934) The action of mica dust on pulmonary tissue. *Journal of Industrial Hygiene*, 16, 160-164.
- Poole, A., Brown, R.C., and Fleming, G.T.A. (1983a) Study of the cell-transforming ability of amosite and crocidolite asbestos and the ability to induce changes in the metabolism and macromolecular binding of benzo(a)pyrene in C3H10T½ cells. *Environmental Health Perspectives*, 51, 319-324.
- Poole, A., Brown, R.C., Turver, C.J., Skidmore, J.W., and Griffiths, D.M. (1983b) *In vitro* genotoxic activities of fibrous erionite. *British Journal of Cancer*, 47, 697-705.
- Pott, F. (1980) Animal experiments on biological effects of mineral fibres. In J.C. Wagner, Ed., *Biological effects of mineral fibres*, vol. 1, IARC Scientific Publication 30, p. 261-272. World Health Organization, Lyon, France.
- Pott, F., and Friedrichs, K.H. (1972) Tumoren der ratte nach i.p.-injektion faserförmiger Stäube. *Die Naturwissenschaften*, 59, 318.
- Pott, F., Huth, F., and Friedrichs, K.H. (1974) Tumorigenic effect of fibrous dusts in experimental animals. *Environmental Health Perspectives*, 9, 313-315.
- Pott, F., Bellmann, B., Muhle, H., Rödelberger, K., Rippe, R.M., Roller, M., and Rosenbruch, M. (1990) Intraperitoneal injection studies for the evaluation of the carcinogenicity of fibrous phyllosilicates. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 319-329. Springer-Verlag, Berlin.
- Puledda, S., and Marconi, A. (1990) Quantitative X-ray diffraction analysis of asbestos by the silver membrane filter method. Application to chrysotile. *Journal of the American Industrial Hygiene Association*, 51, 107-114.
- Radford, E.P., and Renard, K.G.St.C. (1984) Lung cancer in Swedish iron miners exposed to low doses of radon daughters. *New England Journal of Medicine*, 310, 1485-1494.
- Rall, D.P. (1988) Laboratory animal toxicity and carcinogenesis testing. Underlying concepts, advantages, and constraints. *Annals of the New York Academy of Science*, 534, 78-83.
- Reiss, B., Millette, J.R., and Williams, G.M. (1980) The activity of environmental samples in a cell culture test for asbestos toxicity. *Environmental Research*, 22, 315-321.
- Renier, A., Levy, F., Pilliere, F., and Jaurand, M.C. (1990) Unscheduled DNA synthesis in rat pleural mesothelial cells treated with mineral fibres. *Mutation Research*, 241, 361-367.
- Roberts, W.L., Campbell, T.J., and Rapp, G.R., Jr. (1990) *Encyclopedia of minerals* (2nd edition), 979 p. Van Nostrand Reinhold, New York.
- Rom, W.N., Casey, K.R., Parry, W.T., Mjaatvedt, C.H., and Moatamed, F. (1983) Health implications of natural fibrous zeolites for the intermountain west. *Environmental Research*, 30, 1-8.
- Rosmanith, J., Hilscher, W., and Schyma, S.B. (1990) The effect of the surface quality on the fibrogenicity of the phyllosilicates muscovite and kaolinite. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 123-128. Springer-Verlag, Berlin.
- Ross, M. (1981) The geologic occurrences and health hazards of amphibole and serpentine asbestos. In *Mineralogical Society of America Reviews in Mineralogy*, 9A, 279-323.
- (1984) A survey of asbestos-related disease in trades and mining occupations and in factory and mining communities as a means of predicting health risks of nonoccupational exposure to fibrous minerals. In B. Levadie, Ed., *Definitions for asbestos and other health-related silicates*, ASTM STP 834, p. 51-104. American Society for Testing and Materials, Philadelphia.
- Schlesinger, R.B., and Driscoll, K.E. (1989) Respiratory tract defense mechanisms and their interaction with air pollutants. In M.A. Hollinger, Ed., *Focus on pulmonary pharmacology and toxicology*, p. 37-72. CRC Press, Boca Raton, Florida.
- Schyma, S.B. (1990) The physical characterization of muscovite and kaolinite dusts. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 97-105. Springer-Verlag, Berlin.
- Sébastien, P., Gaudichet, A., Bignon, J., and Bars, Y.I. (1981) Zeolite bodies in human lungs from Turkey. *Laboratory Investigations*, 44, 420-425.
- Sébastien, P., Bignon, J., Barris [sic], Y.I., Awad, L., and Petit, G. (1984) Ferruginous bodies in sputum as an indication of exposure to airborne mineral fibers in the mesothelioma villages of Cappadocia. *Archives of Environmental Health*, 39, 18-23.
- Selevan, S.G., Dement, J.M., Wagoner, J.K., and Froines, J.R. (1979) Mortality patterns among miners and millers of non-asbestiform talc. Preliminary report. *Journal of Environmental Pathology and Toxicology*, 2, 273-284.
- Selkoff, I.J., Churg, J., and Hammond, E.C. (1964) Asbestos exposure and neoplasia. *Journal of the American Medical Association*, 188, 22-26.
- (1965) Relation between exposure to asbestos and mesothelioma. *New England Journal of Medicine*, 272, 560-565.

- Sepulveda, M.H., Vallyathan, V., Attfield, M.D., Piacitelli, L., Tucker, J.H. (1983) Pneumoconiosis and lung function in a group of kaolin workers. *American Reviews of Respiratory Disease*, 127, 231-235.
- Sheers, G. (1964) Prevalence of pneumoconiosis in Cornish kaolin workers. *British Journal of Industrial Medicine*, 21, 218-225.
- Shelby, M.D. (1988) The genetic toxicity of human carcinogens and its implications. *Mutation Research*, 204, 3-15.
- Shishido, S., Iwai, K., and Tudagoshi, K. (1989) Incidence of ferruginous bodies in the lungs during a 45-year period and mineralogical analysis of the core fibres and uncoated fibres. In J. Bignon, J. Peto, and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 229-238. International Agency for Research on Cancer, Lyon, France.
- Simonato, L., Bars, R., Saracci, R., Skidmore, J., and Winkelmann, R. (1989) Relation of environmental exposure to erionite fibres to risk of respiratory cancer. In J. Bignon, J. Peto, and R. Saracci, Eds., *Non-occupational exposure to mineral fibres*, p. 398-405. International Agency for Research on Cancer, Lyon, France.
- Snyder, R.L., and Bish, D.L. (1989) Quantitative analysis. In *Mineralogical Society of America Reviews in Mineralogy*, 20, 101-144.
- Sors, H., Gaudichet, A., Sébastien, P., Bignon, J., and Even, P. (1979) Lung fibrosis after inhalation of fibrous attapulgite. *Thorax*, 34, 695.
- Stacy, B.D., King, E.J., and Harrison, C.V. (1959) Tissue changes in rats' lungs caused by hydroxides, oxides, and phosphates of aluminium and iron. *Journal of Pathology and Bacteriology*, 77, 417-426.
- Stanton, M.F., Layard, M., Tegens, A., Miller, E., May, M., Morgan, E., and Smith, A. (1981) Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *Journal of the National Cancer Institute*, 67, 965-975.
- Steel, R.F., and Anderson, W. (1972) The interaction between kaolinite and *Staphylococcus aureus*. *Journal of Pharmacy and Pharmacology*, 24, 129.
- Stille, W.T., and Tabershaw, I.R. (1982) The mortality experience of upstate New York talc workers. *Journal of Occupational Medicine*, 24, 480-484.
- Suzuki, Y. (1982) Carcinogenic and fibrogenic effects of zeolites. Preliminary observations. *Environmental Research*, 27, 433-445.
- Suzuki, Y., and Kohyama, N. (1984) Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. *Environmental Research*, 35, 277-292.
- (1988) Carcinogenic and fibrogenic effects of erionite, mordenite, and synthetic zeolite 4A. In D. Kalló and H.S. Sherry, Eds., *Occurrence, properties and utilization of natural zeolites*, p. 829-840. Akadémiai Kiadó, Budapest.
- Sykes, S.E., Morgan, A., Evans, J.C., Evans, N., Holmes, A., and Moores, S.R. (1982) Use of an *in vivo* test system to investigate the acute and sub-acute responses of the rat lung to mineral dusts. *Annals of Occupational Hygiene*, 26, 593-605.
- Vestal, T.F., Winstead, J.A., and Johet, P.V. (1943) Pneumoconiosis among mica and pegmatite workers. *Industrial Medicine*, 12, 1-14.
- Vorwald, A.J., and Karr, J.W. (1938) Pneumoconiosis and pulmonary carcinoma. *American Journal of Pathology*, 14, 49-57.
- Wagner, J.C. (1982) Health hazards of substitutes. *World Symposium on Asbestos, Proceedings, May 1982, Montreal*, p. 244-266. Canadian Asbestos Information Centre, Montreal.
- (1990) Review on pulmonary effects of phyllosilicates after inhalation. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 309-318. Springer-Verlag, Berlin.
- Wagner, J.C., Sleggs, C.A., and Marchand, P. (1960) Diffuse pleural mesothelioma and asbestos exposure in the north western Cape Province. *British Journal of Industrial Medicine*, 17, 260-271.
- Wagner, J.C., Berry, G., and Timbrell, V. (1973) Mesotheliomata in rats after inoculation with asbestos and other materials. *British Journal of Cancer*, 28, 173-185.
- Wagner, J.C., Berry, G., Hill, R.J., Skidmore, J.W., and Pooley, F.D. (1979) An animal model for inhalation exposure to talc. In R. Lemen and J.M. Dement, Eds., *Dusts and disease*, p. 389-392. Pathotox Publishers, Park Forest South, Illinois.
- Wagner, J.C., Skidmore, J.W., Hill, R.J., and Griffiths, D.M. (1985) Erionite exposure and mesotheliomas in rats. *British Journal of Cancer*, 51, 727-730.
- Wastiaux, A., and Daniel, H. (1990) Pulmonary toxicity of kaolin in rats exposed by inhalation. In J. Bignon, Ed., *Health related effects of phyllosilicates*, NATO ASI Series, vol. G21, p. 405-414. Springer-Verlag, Berlin.
- Waxweiler, R., Zumwalde, R.D., Ness, G.O., and Brown, D.P. (1988) A retrospective cohort mortality study of males mining and milling attapulgite clay. *American Journal of Industrial Medicine*, 13, 305-315.
- Wehner, A.P. (1980) Effects of inhaled asbestos, asbestos plus cigarette smoke, asbestos-cement and talc baby powder in hamsters. In J.C. Wagner, Ed., *Biological effects of mineral fibres*, vol. 1, IARC Scientific Publication 30, p. 373-375. World Health Organization, Lyon, France.
- Witmer, C., and Cooper, K. (1983) Lack of mutagenicity of baghouse samples in a modified Ames plate assay. *Environmental Health Perspectives*, 51, 373.
- Woodworth, C.D., Mossman, B.T., and Craighead, J.E. (1982) Comparative effects of fibrous and nonfibrous minerals on cells and liposomes. *Environmental Research*, 27, 190-205.
- (1983) Induction of squamous metaplasia in organ cultures of hamster trachea by naturally occurring and synthetic fibers. *Cancer Research*, 43, 4906-4912.

MANUSCRIPT RECEIVED AUGUST 14, 1991

MANUSCRIPT ACCEPTED NOVEMBER 4, 1991