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2.2 INGESTION HEALTH EFFECTS

3.0 REFERENCES
1.0 INTRODUCTION

Northshore Mining’s Silver Bay processing plant was formerly operated by Reserve Mining Company. In a landmark ruling in 1974 regarding the dumping of taconite tailings from the Silver Bay plant into Lake Superior, the United States District Court for the District of Minnesota found that evidence existed regarding the potential for exposure to amphibole mineral fibers to cause cancer and other health effects [United States v. Reserve Mining Company, 380 F. Supp. 11, 17 (D. Minn. 1974)]. This led to the construction of a tailings basin in 1980. Amphibole mineral fibers incorporate asbestos, as discussed below, as well as non-asbestos fibers. The Court found that since it can be difficult to tell the difference between asbestos and non-asbestos amphibole fibers under the microscope, these fibers, classified as asbestos or not, have the potential in the court’s ruling to produce health effects associated with asbestos exposure, such as asbestosis, mesothelioma, or other cancers (described below). Scientific work on the question of exposure to non-asbestos amphibole mineral fibers is still ongoing at the present time.

Iron mining operations have the potential to release mineral dust and fibers into the air including amphibole mineral fibers. Because the presence of amphibole minerals has been documented at the proposed mining site (Barr, 2007), and because the Minnesota Department of Health (MDH) has identified the occurrence of mesothelioma in iron mine workers in Minnesota (MDH, 2003), questions have been raised about the potential for exposure and health risks associated with these fibers at the proposed mining site. The purpose of this literature review is to provide an understanding of the current science on health risks from environmental exposure to long and short amphibole mineral fibers. This literature review will focus on asbestos-related diseases and risks from amphibole mineral fiber exposure and to assist in determining potential health impacts and needs for mitigation at the proposed mining site.

1.1 DEFINITIONS

The term “asbestos” is not a mineralogical definition; it is a regulatory and commercial term designating mineral products that possess high tensile strength, ability to be separated into long, thin, flexible fibers, low thermal and electrical conductivity, high mechanical and chemical durability, and high heat resistance. The fibers can be woven into various commercial products because of their flexibility. Asbestos refers to the fibrous variety of several naturally occurring silicate minerals. There are two groups of minerals that can crystallize as asbestos: serpentine and amphibole. There are approximately 100 minerals that may be found as asbestiform, (i.e., minerals in which the crystal growth is primarily one dimensional and the crystals form long, flexible fibers). The fibers
form in bundles and can be separated into smaller bundles and ultimately single fibers or fibrils. However, there are only six regulated types of asbestos, as shown in Table 1. Mineralogically, amphiboles are distinguished from each other by the amount of sodium, calcium, magnesium, and iron that they contain. Serpentine and amphibole minerals can have fibrous and nonfibrous structures.

Table 1. Regulated Asbestos Minerals

<table>
<thead>
<tr>
<th>Mineralogy</th>
<th>Amphibole</th>
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<tbody>
<tr>
<td>Serpentine</td>
<td>Chrysotile</td>
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<tr>
<td></td>
<td>Crocidolite (Reibeckite)</td>
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<tr>
<td></td>
<td>Amosite (Cummingtonite-grunerite)</td>
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<td></td>
<td>Anthophyllite Asbestos</td>
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<td>Tremolite Asbestos</td>
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<td>Actinolite Asbestos</td>
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Whether a mineral is asbestiform or non-asbestiform can be identified using a microscope on un-ground samples, however, the difference is much more difficult to identify with ground samples. Chrysotile asbestos is easily identified by microscopic examination because of its distinct particle shape. For amphiboles, the distinction between asbestiform and non-asbestiform varieties is much less clear under a microscope. Amphibole particles have a spectrum of shapes from blocky to prismatic to acicular to asbestiform. Amphiboles also can break (or cleave) into smaller fragments when finely ground. These smaller, non-natural structures are called cleavage fragments. Because these long, thin cleavage fragments resemble asbestos fibers, they are not easily distinguished from asbestiform amphibole fibers during analysis. An experienced analyst can usually compare amphibole particle shapes to asbestos reference materials and determine whether a sample is asbestiform with a fair degree of certainty. However, unless a fiber bundle has splaying ends, it is generally impossible to determine if a single long, thin particle is an asbestos fiber or is a cleavage fragment (USGS, 2001, Berman and Crump, 2003). It is more difficult to classify individual fibers as asbestiform or cleavage fragments because individual fibers do not exhibit all the characteristics of a population. Cleavage fragments tend to be roughly twice as thick as asbestos fibers (Addison and McConnell, 2008). The aspect-ratio distributions (i.e., length-to-width ratio) of a population of cleavage fragments and a population of asbestiform fibers can overlap, which adds to the difficulty in differentiating between fiber types. This overlap means that some fibers may be classified as either cleavage fragments or asbestos fibers (Millette, 2006). While the hazards of inhalation exposure to airborne asbestos fibers have

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1 A cleavage fragment is a particle formed by comminution (i.e., crushing, grinding or breaking) of minerals, often characterized by parallel sides. In contrast to fibers from an asbestos mineral, elongated mineral fibers in a population of cleavage fragments are generally wider and shorter, have generally lower aspect ratios, and do not exhibit fibrillar bundling.
been well documented, there is controversy about whether exposure to cleavage fragments from nonasbestiform analogs of the asbestos minerals is similarly hazardous.

1.1.1 Fiber Definitions

Asbestos is made up of fiber bundles. These bundles are composed of long, thin, and flexible fibers, called fibrils, which can easily be separated from each other. Bundles have splaying ends and are extremely flexible. The mean aspect ratio for asbestos fibers can range from 20:1 to 100:1 or higher for fibers longer than 5 micrometers (µm). In general, asbestos is characterized by fibrils often less than 0.5 µm in width, and two or more of the following features:

- Parallel fibers occurring in bundles
- Fiber bundles displaying splayed ends
- Matted masses of individual fibers
- Fibers showing curvature

As discussed in Section 1.1, it can be difficult to classify individual fibers as asbestiform or not. In addition, regulatory definitions for classifying asbestos fibers differ from agency to agency. The existence of inconsistent definitions of asbestos and similar minerals has created confusion. For example, different agencies have developed analytical-based definitions to use to identify a particle as a fiber. These regulatory definitions are generally counting strategies helpful for the microscopists and are meant to produce consistency (Addison and McConnell, 2008), but have little to do with the characteristics of asbestos. Another example is that the U.S. Environmental Protection Agency (USEPA) defines the dimensions of an asbestos fiber as one that is 5 µm in length or longer with an aspect ratio of at least 20:1 (USEPA, 1993), but the National Institute for Occupational Safety and Health (NIOSH) defines an occupational fiber as one that is 5 µm in length or longer with an aspect ratio of at least 3:1 (NIOSH, 1994).

As more information becomes available on the relationships between the dimensions of asbestos fibers and their ability to cause respiratory disease, interest has increased in exposure to other mineral fibers. It remains uncertain whether other elongated mineral particles, especially those with mineralogical compositions similar to asbestiform minerals warrant similar heath concerns. As a result, additional definitions for these types of minerals have become necessary and will continue to be developed in the future. However, for the present, the State of Minnesota defines a fiber (“MN-fiber”) as an amphibole or chrysotile mineral particle that has an aspect ratio of 3:1 or greater with no limit on length. Using these conventions means that any amphibole or chrysotile particle with an aspect ratio of greater than 3:1 would be counted as a fiber under one or more of
the above definitions. They may or may not actually be asbestos or asbestiform. Conversely, asbestos will produce asbestos dust particles that mostly have aspect ratios equal to or greater than 3:1, but will also produce particles with a lower aspect ratio. National Institute for Occupational Safety and Health (NIOSH) defines an elongated mineral particle as any particle or fragment of a mineral (e.g., fibril or bundle of fibrils acicular, prismatic, or cleavage fragment) with a minimum aspect ratio of 3:1, based on a microscopic analysis of an airborne sample.

1.2 DEFINITION OF DISEASES ATTRIBUTED TO ASBESTOS

Asbestos is the focus of the discussion on health effects because the impetus for regulation of amphibole mineral fibers and MN-fibers is due to the knowledge of health risks from exposure to asbestos. Also, the majority of health studies available are on asbestos. Asbestos exposure has been identified as the cause of both malignant and non-malignant diseases. The USEPA's Integrated Risk Information System (IRIS) has classified asbestos as a Group A, Human Carcinogen (USEPA, 2008). This classification means that there is sufficient human and animal carcinogenicity data to support the Group A, weight-of-evidence characterization as a human carcinogen for the inhalation route of exposure. The Group A classification is based on observations in occupationally-exposed workers of increased mortality and incidence of lung cancer, mesothelioma and gastrointestinal cancer. The incidences of lung cancer and mesothelioma are supported by studies on two different strains of rats. Evidence of carcinogenicity via the ingestion pathway was not supported in the animal studies reviewed for the USEPA IRIS classification in 1988 (USEPA, 2008).

1.2.1 Asbestosis

Asbestosis is a disease associated with occupational levels of exposure to asbestos (Atkinson, 2006). Most patients with asbestosis suffer from shortness of breath and a dry cough (Mossman and Churg, 1998). Asbestosis is characterized by chronic inflammation of the parenchymal tissue of the lungs and changes in the lung architecture. These changes include the deposition of collagen in the interstitial associated diagnostically with the presence of asbestos bodies (ferruginous bodies composed of an iron or protein coat deposited on a central asbestos core) (Atkinson, 2006). The increase of fibrous tissue reduces elasticity and gas diffusion, which reduces gas exchange in the lungs, decreasing oxygen transfer to the blood and removal of carbon dioxide from the blood.

Asbestosis appears to be associated with a high level of asbestos-containing dust exposure, either a very high level over a short period or a low level for an extended period (Atkinson, 2006). The level of exposure seems to be associated
with the length of the latency period, from initial exposure to the development of disease. Mossman and Churg opined that asbestosis requires a threshold level of exposure and that the lower the exposure, the longer it takes to reach the threshold (Mossman and Churg, 1998). Historically, asbestosis progresses even after workers are no longer exposed to asbestos dust (Atkinson, 2006).

1.2.2 Lung Cancer

The lung cancers caused by asbestos are mainly bronchial carcinomas and are indistinguishable from those caused by smoking or other agents (Doll and Peto, 1985). Carcinomas do not generally form until several years after the initial exposure.

1.2.3 Mesothelioma

Mesothelioma is a form of cancer almost always associated with a previous exposure to asbestos. The cancer forms in the mesothelium, most commonly in the pleura, which is the outer lining of the lungs and chest cavity. Symptoms take 15 to 50 years after exposure to appear and include shortness of breath and coughing. There is yet no cure for human mesothelioma (Suzuki and Yuen, 2002).

2.0 HUMAN HEALTH EFFECTS

2.1 INHALATION HEALTH EFFECTS

Many factors are suggested to contribute to carcinogenesis and disease from exposure to asbestos fibers via inhalation. USEPA established an inhalation unit risk factor for asbestos in 1988 of 0.23 per fibers/milliliter of air (USEPA, 2008). The unit risk factor is an estimate of the additional risk of cancer if a person was exposed to 1 fiber per milliliter of air of asbestos for his or her lifetime (assumed to be 70 years). However, this value does not take into consideration fiber type or dimensions. Although a risk assessment protocol for evaluating asbestos by type and dimensions has been developed for USEPA by Berman and Crump (2003), it may never be formally adopted. This model also does not consider fibers shorter than 10 micrometers in length. Fiber type, surface chemistry, fiber dimensions, and fiber burden are among the topics discussed in this section.

2.1.1 Fate of Fibers Following Inhalation into the Lung

The primary pulmonary defense against inspired fibers is their entrapment in the mucous layers of the upper airways or engulfment (phagocytosis) by alveolar macrophages in the lower airways. In either case, clearance occurs by way of the mucociliary escalator and elimination through the gastrointestinal tract (Atkinson, 2006). Uncleared fibers can translocate to lymph nodes, pleural and
peritoneal mesothelial tissues or other organs (Suzuki and Yuen, 2002). The translocation of asbestos fibers within the body appears to be a water-flow driven process and fibers will travel through the blood and lymphatic channels (Misericocchi et al., 2008).

### 2.1.2 Fiber Chemistry and Reactivity

Epidemiological, fiber burden and experimental data have shown that the lung has the ability to deal with a considerable number of fibers and particles without detectable molecular or pathogenic events or fibrosis development (Mossman and Churg, 1998; Quinlan et al., 1995). In groups of rats exposed to two dose levels of chrysotile asbestos, only rats exposed to the higher dose level showed the indicators of inflammation such as increased cell proliferation (Quinlan et al., 1995). Studies also indicate that exposure to lower levels of asbestos in humans and experimental animals often results in reversible inflammatory reactions in isolated areas accompanied by evidence of alveolar macrophages with heavy fiber burdens, but with normal lung histology (Quinlan et al., 1994; Quinlan et al., 1995; Mossman and Churg, 1998).

In contrast, higher levels of exposure result in a much more extensive and prolonged inflammatory reaction (Atkinson, 2006; Mossman and Churg, 1998). Mesenchymal cells deposit increased amounts of extracellular matrix including collagen. Alveolar macrophages secrete growth factors and oxidants, while other cells proliferate. Neutrophils, T-cells, and mast cells are recruited and accumulate in the lung interstitium. Interactions between these “defense” cells results in damage to specific lung cell populations. Injury to alveolar type I epithelial cells is an early event that results in proliferation of epithelial cells as a repair mechanism, but if unchecked the proliferation can lead to fibrosis and carcinogenesis (Atkinson, 2006; Mossman and Churg, 1998).

The proliferation of epithelial cells also has been found to occur after the upregulation of early response oncogenes, which may be critical to the disease development of both pulmonary fibrosis and lung cancer (Mossman and Churg, 1998). The expression of these early response genes has been linked to programmed cell death of mesothelial cells and alveolar macrophages (Mossman and Churg, 1998; Broaddus et al., 1996). Broaddus et al. (1996) found in particular that crocidolite, amosite, and chrysotile asbestos fibers induce programmed cell death in pleural mesothelial cells via reactive oxygen species (ROS) in both rabbit and human cells. A persistent inflammatory response may be linked to the development of cell injury, proliferation, cell death, and fibrogenesis. In the absence of fiber clearance this cycle continues year after year.
Asbestos fibers encountered in the lung are often found with an iron-rich coating on the fiber surface. These structures are called ferruginous bodies, or asbestos bodies, and when found in lung tissue are an indicator that alveolar macrophages, the defense cells of the lungs, have interacted with a particulate resulting in deposition of an iron-rich coating on its surface (Dodson, 2006).

Shorter fibers may be engulfed entirely by alveolar macrophages, which may facilitate their clearance by way of the mucociliary escalator. Long fibers do not physically “fit” within the macrophage. However, in instances of heavy exposure the system may become overloaded and the macrophages die in the terminal airways releasing not only the fibers but the macrophages’ cellular contents (hydrolytic enzymes, etc), which are known to have a role in chronic inflammatory response (Atkinson, 2006; Bignon and Jaurand, 1983; Oberdörster, 1995; and Morrow, 1988). The inability of alveolar macrophages to move dust from the alveolar regions of the lung to the mucociliary escalator can be correlated to an average composite particle volume per alveolar macrophage of 60 µm³/macrophage in the lung (Morrow, 1988). Oberdörster reported that the alveolar retention halftime for particles in rat lungs was approximately 70 days and for humans it may be 10 times longer (Oberdörster, 1995). Also, lung particle burden was found to increase until exposure ceased. After exposure ceased, alveolar retention halftimes significantly increased along with increased translocation of fibers to the pulmonary interstitium, which can contribute to adverse interstitial effects such as pulmonary fibrosis (Oberdörster, 1995).

Amphibole minerals are very resistant to dissolution by strong acids and bases (Addison and McConnell, 2008). Studies on chrysotile fibers have shown that magnesium is leached from the fibers in the lung and as a consequence are more readily dissolved than amphibole fibers (Atkinson, 2006; Churg 1994; Hume and Rimstidt, 1992). Therefore, chrysotile fibers are less persistent in the lungs than amphibole fibers. Continuous exposure to amphiboles tends to increase the fiber levels in the lung, while continuous exposure to chrysotile does not (Mossman and Churg, 1998). Amphibole exposure has a propensity to result in mesothelioma, which is likely a function of two properties of amphibole fibers; their greater resistance to dissolution in the lung and their iron content, which facilitates the production of the reactive oxygen radicals hydrogen peroxide (H₂O₂) and OH (hydroxyl radical) via a Haber-Weiss reaction. Both long and short crocidolite fibers increase the generation of H₂O₂ by macrophages in vitro with short fibers producing a greater concentration of peroxide (Goodglick and Kane, 1990). Goodglick and Kane also found that crocidolite fibers disrupt the macrophages’ mitochondrial membrane potential in vitro, which may reduce cell function (Goodglick and Kane, 1990).
2.1.3 Genetic Mutations

Multiple genetic changes in affected tissues are required for the development of tumors. Based on the current state of the science, the genetic changes caused by asbestos fibers in tissues are not completely understood with some studies conflicting with others. For example, short fibers taken up by dividing cells can interfere with spindle formation and chromosome separation and thus the separation of the daughter cells (Atkinson, 2006). On the other hand, Mossman suggested that persistent stimulation of cell division by asbestos fibers in mesothelial cells results in carcinogenesis (Mossman, 1993). In human mesotheliomas, the loss of chromosomes 4, 22, and 9p has been shown to occur, as well as an increase in chromosomes 5, 7 and 20 (Mossman, 1993). Popescu et al. (1988) also found deletions of the 3p chromosome, which is possibly related to the development of malignant mesothelioma.

Ghio found that even though amphibole fibers of crocidolite and amosite had less surface area than chrysotile fibers, they contained more silicon dioxide and complexed more iron to the surface (Ghio et al., 1994). In vitro experiments have demonstrated that increased surface complexed iron does increase DNA strand breaks indicating a carcinogenic potential. (Atkinson, 2006; Ghio et al., 1994).

2.1.4 Fiber Type

An editorial from Mossman (1993) in the British Journal of Industrial Medicine discussed findings that the prevalence of mesothelioma appears to vary with fiber type. Specifically, several studies indicate that exposure to amphibole fibers is much more likely to result in mesothelioma than exposure to chrysotile. Furthermore, it has been suggested that mesotheliomas resulting from exposure to Canadian chrysotile asbestos may, in fact, be due to the tremolite fibers existing as contaminants in chrysotile asbestos (Atkinson, 2006; McDonald and McDonald, 1996). Mossman indicated that the highest incidences of mesothelioma have been seen in workers exposed to crocidolite or mixtures of crocidolite and chrysotile (Mossman, 1993; Wagner, 1991). Exposures to amosite have not resulted in as sharp an increase of pleural or peritoneal mesotheliomas. This result is likely due to the needle-like configuration, durability, and increased iron content of crocidolite (Mossman, 1993). Iron-rich fibers are more likely to generate oxygen species caused by mobilization of the surface iron of the fiber during tumor development.

Although chrysotile was found to produce more fibrous collagen (i.e., fibrogenic) than amphiboles, these fibers were less active when 80% of the magnesium was leached from the fibers (Bignon and Jaurand, 1983). Chrysotile in the lung given by inhalation was found to be more fibrogenic than crocidolite, and crocidolite
more fibrogenic than amosite (Bignon and Jaurand, 1983). However, this study indicates that the differences may have been due to the prevalence of longer fibers in the chrysotile preparation used in the experiment (Bignon and Jaurand, 1983).

Bignon and Jaurand (1983) summarized several studies and found that chrysotile was more cytotoxic than amphibole. However, acid-treated chrysotile, which removed much of the magnesium from the fiber, was less cytotoxic than acid-treated amphibole (Bignon and Jaurand, 1983). Mossman and Churg (1998) concluded that this leaching of magnesium from chrysotile fibers in the lung changes the surface charge from positive to negative, and thereby reduces the fibers’ toxic potential. In contrast, acid-treatment of amphibole fibers increased the cytotoxicity over untreated amphibole (Bignon and Jaurand, 1983). Mossman and Churg concluded that on a fiber-by-fiber basis, amphibole fibers are more fibrogenic than chrysotile fibers (Mossman and Churg, 1998).

Several studies have been performed by Philip Cook, David Coffin, and Lalita Palekar, comparing various toxic responses including tumor production from amosite and ferroactinolite exposures. Ferroactinolite is not a regulated form of asbestos, but it is in the same mineralogical series as actinolite and tremolite and is found in the Iron Range of Minnesota. It is clear that in some of the Cook, Coffin, and Palekar studies that the ferroactinolite is asbestiform, but is not clear in others. The studies define a fiber as having an aspect ratio of 3:1, which is the definition of a MN-fiber (Cook et al., 1982; Coffin et al., 1982). Amosite was found to be more toxic in blood via hemolysis based on dose level (mass per volume), but ferroactinolite was more toxic based on number of fibers (fibers per volume) (Palekar et al., 1983). The ferroactinolite fibers ranged in length from 0.3-52.5 µm, with a mean length of 3.18 µm as measured by TEM. The amosite fibers ranged in length from 0.15-378 µm, with a mean length of 3.44 µm. The mean aspect ratios of the ferroactinolite and amosite fibers were 9 and 11.8, respectively. Amosite was also found to be more cytotoxic to Chinese hamster ovary cells (Palekar et al., 1983). An in vitro study using rabbit alveolar macrophages also found amosite more toxic than ferroactinolite (Palekar et al., 1983). Conversely, in vivo studies by the same researchers found ferroactinolite to be equally tumorigenic in intraplueral studies and more tumorigenic in intratracheal studies in rats (Coffin et al., 1982 and 1983). The ferroactinolite fibers were shorter and thinner than amosite fibers. The ferroactinolite fibers were found to split longitudinally in the lung, which increased the number of total fibers (Coffin et al., 1982 and 1983).
2.1.5 Fiber Physical Properties – Length and Width

The morphology of asbestos minerals can determine the potential for inhalation. Chrysotile fibers are structures that roll up on themselves and form hollow tubes or scrolls. In contrast, amphibole fibers tend to be straight even as fiber length increases. Therefore, while chrysotile fibers have a greater functional diameter than the actual diameter, amphibole fibers have a functional diameter that is similar to the actual diameter. Thus, it is easier to inhale longer fibers of amphiboles than equivalent length fibers of chrysotile (Dodson, 2006). In general, only fibers with diameters less than 0.25 µm are considered respirable (Gamble and Gibbs, 2008). Only a small percentage of cleavage fragments have diameters less than 0.25 µm (Gamble and Gibbs, 2008).

The “Stanton Hypothesis” is frequently quoted as “the carcinogenicity of fibers depends on dimension and durability rather than on physicochemical properties” (Stanton et al., 1981). However, this study also stated that the “probability of pleural sarcoma correlated best with numbers of fibers that measured 0.25 µm or less in diameter and more than 8 µm in length, but relative high correlations were also noted with fibers in other size categories having diameters up to 1.5 µm and lengths greater than 4 µm” (Stanton et al., 1981). Lippman (1990) used human data to conclude that asbestosis was most correlated to fibers longer than 2 µm and thicker than 0.15 µm; mesothelioma to fibers longer than approximately 5 µm and thinner than approximately 0.1 µm; and lung cancer to fibers longer than approximately 10 µm and thicker than approximately 0.15 µm. Churg and Vedal (1994) did not find mesothelioma associated with long fibers but indicate they are probably associated with lower aspect-ratio fibers. A study by McDonald et al. (2001) obtained lung, tissue and tumor samples from 69 men and 4 women who died of mesothelioma. The tissues showed a predominance of amosite and crocidolite fibers. The fibers were categorized by fiber length (<6 µm, 6-10 µm, and >10 µm). Short, medium and long amphibole fibers were all associated with increased risk, however, the longer the fiber the greater the risk (McDonald et al., 2001). The Coffin et al., (1982 and 1983) studies mentioned above found that shorter fibers (< 8 µm) of ferroactinolite contributed significantly to tumorigenesis. Coffin et al., (1982) indicates that if applying the Stanton Hypothesis, they would have expected many more tumors from the amosite. However, Cook et al. (1982) explains that the greater lung carcinogenicity of ferroactinolite is likely due to the longitudinal splitting of the ferroactinolite fibers in vivo which increased the aspect ratio and the dose in the lung.

Other studies that examined human tissues have found that the majority of asbestos fibers in mesothelial tissues were shorter than 5 µm in length, thus indicating the ability of shorter fibers to reach the tumor site, remain there, and
as a consequence thereby implicating their role in the development of disease (Lemen, 2006; Dodson et al., 1990; Suzuki and Yuen, 2002). Dodson et al. (1990) published a study comparing fiber burden in the lung, thoracic lymph nodes and pleural plaques in occupationally exposed workers. They found the average length to be longest in the lung for both chrysotile and amphibole fibers. However, the majority of asbestos fibers in all three sites were short (<5 µm). Suzuki and Yuen (2002) found that out of 168 human cases of mesothelioma approximately ninety percent of fibers found in the lungs, plaque and mesothelial tissues were less than 5 µm in length, however most were very thin (<0.04 µm geometric mean). In fact, Suzuki and Yuen (2002) suggested that short thin asbestos fibers should be considered carcinogenic because they are the principal asbestos fiber type found in the lung and mesothelial tissues in human mesothelioma cases.

Logic indicates inhalation of longer fibers would be eliminated less rapidly via the lung clearance mechanisms than an equivalent number of inhaled shorter fibers (Dodson, 2006). However, it is unlikely that only long fibers are present in the breathing zone. It is also logical that shorter fibers would be found in extrapulmonary sites as they are being removed from the lungs. The presence of shorter fibers in extrapulmonary sites where 10 to 15 percent of mesotheliomas occur compared to pulmonary sites was confirmed by Dodson et al. (2000). Dodson et al. (2000) also reported that the length of the longest fibers found at extrapulmonary sites (omentum and mesentery) in a cohort of mesothelioma patients were shorter than those in pulmonary sites, but that fibers up to 70 and 40 µm in length were found in omentum and mesentery, respectively. Short fibers reach extrapulmonary sites more readily than long fibers, including sites where mesotheliomas develop (Atkinson, 2006; Dodson et al., 2000). Thus, the potential contribution of these translocated short fibers to the development peritoneal mesotheliomas cannot be discounted (Dodson et al., 2000).

**Fiber Dimensions and Analysis Method**

Aside from the issue of respirability there remains a misconception that long asbestos fibers are more harmful than short fibers in part because they are less easily removed from the lung than short fibers. Part of this misconception arises from the size of fibers that are “counted” in regulatory count schemes. While the State of Minnesota analytical methods do not limit counts by fiber length, many methods put limitations on fiber length. It should be noted that these limitations stem from issues of standardization and instrument limitations rather than ones of potential pathogenicity. Dodson et al. (2003) published a review of studies on fiber length. They stated that it is imperative that any study must define its counting procedure. The differences shown using transmission electron microscopy (TEM) versus a phase contrast microscope (PCM) or similar light microscope are vast. TEM results are preferred. A Stanton definition fiber (> 8
µm in length and < 0.25 µm in diameter) count for amosite fibers using TEM found 48% were Stanton Fibers but a light microscope identified only 33% as Stanton Fibers. For chrysotile fibers, 39% were Stanton Fibers using TEM but only 1.4% would have been identified using a light microscope (Dodson et al., 2003).

**Fiber Dimensions and Reactivity**

The carcinogenicity of fibers depends on dimension and durability as well as the physicochemical properties (Dodson, 2006). There are certain physical and chemical differences between short and long fibers that may affect their relative toxicities. As already discussed, short fibers are more easily phagocytized by macrophages and consequently cleared. In circumstances where the fiber load is very high, short fibers may be preferentially relocated to extrapulmonary sites, presumably through the lymphatic system. The end result may be death of the macrophage releasing not only ingested fibers but also the macrophages’ cellular contents (e.g., hydrolytic enzymes), which can be harmful to surrounding tissues.

In the case of longer fibers, the phenomenon of frustrated phagocytosis leads to a state of chronic inflammation. Perhaps the most obvious difference between short and long fibers is their surface area per unit mass. Short fibers have a higher surface area per unit mass, so if surface reactive iron is the mediator of asbestos-related disease one would predict that short fibers would be more bioreactive and therefore more toxic than long fibers. However, bioreactivity is a function not only of the total amount of iron but, perhaps more critically, of the redox state of the surface iron and its availability to redox cycle and participation in ROS generation. Comparison of the oxidation state and coordination sites of iron on the surface of long and short fibers of amosite asbestos using infrared spectroscopy demonstrated that both types have more Fe$^{2+}$ iron than the more bioreactive Fe$^{3+}$ iron. However, long fibers have more iron in the bioreactive state than short fibers (Atkinson, 2006; Graham et al., 1999). This phenomenon suggests that longer fibers may have surface-available iron that is more redox active than short fibers.

Thus, while it may be argued that on a one-to-one basis long (> 8 µm) and thin (<0.25 µm) fibers (such as the “Stanton fiber”) possess a greater inherent carcinogenic risk than shorter fibers, the fact remains that shorter fibers can also cause pathological events through their multiple interactions between fibers and cells, cells and cells, clearance and retention, and retention and relocation that cumulatively lead to the causation of asbestos-related diseases. It should also be realized that shorter fibers when phagocytized offer a more direct interference with cellular processes such as cell division than longer fibers that do not “fit” inside cells or subcellular compartments (Atkinson, 2006).
Fiber Dimensions and Cytotoxicity

Goodglick and Kane examined the relative cytotoxicity of long and short crocidolite fibers both in vivo and in vitro (Goodglick and Kane, 1990). Short fibers ranged from 0.1-5.0 µm but were mostly less than 1 µm, while long fibers ranged from 0.1 µm to greater than 20.1 µm but were mostly between 1.1 and 5 µm in length. In in vitro systems, these researchers found that both long and short fibers were toxic to macrophages eliciting cell death. By mass, a dose of short fibers was found to be more toxic than long fibers. Goodglick and Kane (1990) reasoned an equivalent mass of long fibers contained fewer actual fibers, thus a lower dose. A 30 µg dose of short fibers was found to be equivalent in fiber number to a 120 µg dose of long fibers. Based on an equal number of fibers, long crocidolite fibers were found to be more toxic than short fibers. However, Goodglick and Kane (1990) found that short fibers took longer to settle onto macrophages compared to longer fibers, thereby resulting in a delayed toxic response compared to the long fibers. The researchers then allowed for a longer incubation time and found that both fiber groups were equally cytotoxic.

In an in vivo study, Goodglick and Kane (1990) injected fibers into the peritoneal cavity of mice. Single injections produced an inflammatory response on the surface of the diaphragm, but the response was less pronounced for shorter fibers. The short fibers were cleared from the surface of the diaphragm. They gave the mice five consecutive daily injections of short crocidolite fibers with the thought that it would saturate the lymphatic drainage system. Short fibers were not cleared efficiently and there was a continual recruitment of inflammatory cells. A large accumulation of inflammatory cells may also block lymphatic openings further reducing the amount of clearance. Reactive oxygen metabolites were also observed at the sites on the diaphragm where cell injury was found.

A study on amosite comparing fiber length in vivo found that long fibers (>10 µm) were significantly more toxic than short fibers (>5 µm). The study was performed in rats exposed separately by inhalation and intraperitoneal injection. Three pulmonary adenomas, eight pulmonary carcinomas and two pleural mesotheliomas, and one peritoneal mesothelioma were found in the group of rats treated with long fibers (Davis et al., 1986). Only one peritoneal mesothelioma was found in the rats exposed to the short fibers (Davis et al., 1986).

Cleavage Fragments

Cleavage fragments lack the high tensile strength found in their asbestiform counterparts. Amphibole cleavage fragments may share the same chemical structure of amphibole asbestos fibers; however, they do not share the same crystalline structure (Mossman, 2008). Also, they rarely have diameters less than 0.25 µm and aspect ratios exceed 15:1 only about six percent of the time (Gamble and Gibb, 2008). As stated in Section 1.1, it is currently difficult if not impossible
to distinguish individual asbestiform fibers from individual cleavage fragments because of the difficulty in determining whether or not an individual fiber exhibits all the characteristics of the source material.

Palekar et al. (1979) studied the cytotoxicity of asbestiform grunerite (amosite), acicular grunerite, semiasbestiform cummingtonite, and acicular cummingtonite. They reported the relative cytotoxicity to Chinese hamster ovary cells and hemolysis of sheep red blood cells of these fiber types to be (in decreasing order): asbestiform grunerite; semi-asbestiform cummingtonite; acicular cummingtonite; and acicular grunerite. Particle size of the acicular grunerite had an influence, with the larger particles being inert, while the smaller particles were cytotoxic and inert (Palekar et al., 1979). Additionally, Mossman used tracheal explant cultures to study nonasbestiform riebeckite and antigorite, which failed to form squamous metaplasia (Mossman, 2008; Woodworth et al., 1983). Squamous metaplasia is believed to be a precursor to carcinogenesis (Mossman, 2008). The fiber lengths for the riebeckite and antigorite were not given, however, the diameters were presented and were less than 5 µm (Woodworth et al., 1983). This seems to indicate that the riebeckite and antigorite may not have had an aspect ratio that would have classified it as a fiber under the MN-fiber definition. Mossman has also found nonasbestiform particles to be less cytotoxic and much less bioreactive than asbestiform fibers when phagocytized by alveolar macrophages (Mossman, 2008; Hansen and Mossman, 1987). However, this study also does not present fiber or particle lengths for the nonfibrous minerals and based on photos presented, very few of the nonfibrous mineral particles would be considered fibers (Hansen and Mossman, 1987).

Biopersistence appears to be important in inducing carcinogenic effects from asbestos exposure. No studies were identified concerning the biopersistence of cleavage fragments.

While Palekar et al. (1979) found non-asbestiform particles to be cytotoxic, epidemiological studies have found limited potential for carcinogenesis from cleavage fragments. Gamble and Gibbs (2008) provided a review of several epidemiological studies regarding exposure to cleavage fragments including several involving taconite miners. They found that there was no statistically significant increase in either lung cancer or mesothelioma from exposure to taconite mining. Ilgren (2004) reviewed animal and human studies and came to the same conclusion. Additionally, Gylseth et al. (1981) performed a study in which non-asbestiform amphibole dust in the lungs of taconite miners was examined. Whereas these researchers concluded that exposure to the miners constituted a minor carcinogenic risk, they could not exclude exposure to taconite as a contributing factor to the lung cancer found in the miners examined.
2.1.6 Other Attributing Factors (e.g. smoking)

It is well known that tobacco smoke exposure alters the cellular composition of the upper airways and reduces the function and effectiveness of the mucociliary escalator. Therefore, clearance of all types of dust and particles including asbestos is less efficient for a smoker than for a non-smoker. Mossman and Churg (1998) stated that smoking speeds the progression of asbestosis. Churg and Stevens (1995) found the concentrations of asbestos in the airway of smokers were significantly elevated over non-smokers, almost 6-fold for amosite and 50-fold for chrysotile. Churg and Stevens (1995) found that in the case of asbestos-exposed individuals, asbestos fibers recovered from the airway mucosa or parenchyma of smokers were shorter in length than that in nonsmokers. They concluded that cigarette smoking impairs clearance of short fibers and leads to enhanced fiber retention in macrophages and tissues (Dodson, 2006; Churg and Stevens, 1995; Churg et al., 1992). The effects of combined cigarette smoking and asbestos exposure likely reflect a number of synergistic effects including the impairment of pulmonary clearance mechanisms by the components of cigarette smoke and the adsorption of nitric oxide (NO) onto the fiber surface (Atkinson, 2006; Leanderson et al., 1997). The biological significance of this has not yet been determined, however, NO has several biological functions. NO is involved in the regulation of protein function and may affect gene regulation and expression (Leanderson et al., 1997). NO also makes glutathione peroxidase inactive, which due to the increased number of free radicals produced in the presence of bioreactive fibers, may increase toxicity (Leanderson et al., 1997).

2.2 INGESTION HEALTH EFFECTS

For the oral exposure route, the principal way humans are exposed to asbestos is through the ingestion of asbestos contaminated drinking water. Another pathway is the ingestion of fibers removed from the lungs by clearance mechanisms. The Agency for Toxic Substances Disease Registry (ATSDR) has provided the results of both human and animal studies that provide quantitative data on the effects of ingested asbestos (ATSDR, 2001). The results indicate that the ingestion of asbestos causes little or no risk of non-carcinogenic injury. A review done by Gamble (2008) found no or little evidence that ingestion of asbestos causes either stomach cancer, colorectal cancer, colon cancer, or rectal cancer.

3.0 REFERENCES


McDonald, JC, and AD McDonald. 1996. The Epidemiology of Mesothelioma in Historical Context. European Respiratory Journal. 9(9):1932-1942.


