
The Role of Clearance and Dissolution in Determining the Durability or Biopersistence of Mineral Fibers

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It is generally accepted that to cause pulmonary disease, mineral fibers must be relatively long and thin but also able to remain in the lung for long periods. This "biopersistence" of fibers is limited by two main mechanisms of fiber clearance: removal by macrophages after phagocytosis and, for some fibers, by actual dissolution. The relative importance of these mechanisms has not been properly evaluated for any type of fiber and will certainly vary with mineral type. The efficiency of macrophage clearance is greatest with short fibers (<5 μm long) and is reduced as fibers get longer. Fibers >50 μm long cannot be cleared by macrophages and for some mineral types they may remain in the lung permanently. Others may fracture into shorter lengths, perhaps aided by chemical dissolution, and thus become susceptible to macrophage clearance. However, for a number of areas relating to fiber removal from the lung parenchyma detailed information is still needed: Do dusts differ in their ability to attract macrophages and stimulate these cells to phagocytosis? Following dust uptake what controls the movement of macrophages? Some may penetrate to the interstitium, some phagocytosing fibers in interstitial sites may migrate back to the alveolar space. Some move to the mucociliary escalator and some to the lymphatics. Some, most importantly, move to the pleura. Fibers are found and phagocytosed in the interstitium during the early stages of disease development, but with time many fibers appear isolated in areas of fibrous tissue. Are such fibers subsequently ignored or can they reenter the disease process after years of isolation? Finally, can phagocytosis by macrophages effect dissolution of fibers? The pH of the macrophage phagolysosome system is acid, while tissue fluids are close to neutral. Some fiber formulations might dissolve faster in an acid environment while some might be more stable. — *Environ Health Perspect* 102(Suppl 5):113–117 (1994)

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Introduction

Some of the earliest experimental studies on the harmful effects of asbestos (1,2) demonstrated that fiber length was an important factor in the development of pulmonary fibrosis, long fibers being the most dangerous. By the 1970s it had also been shown that fiber dimensions were important in carcinogenicity, the most potent fibers being >8 μm in length and <0.25 μm in diameter (3–6).

More recently it has been appreciated that the longer any fibers can remain in lung tissue after inhalation, the greater the likelihood that they could cause disease. The terms durability or biopersistence have been used to describe this long-term retention, which applies to particulate dusts as well as to fibers. Several processes are involved in pulmonary clearance and, therefore, in determining biopersistence, but the relative importance of each is not

fully understood and probably varies with different types of mineral.

Pulmonary Clearance Estimated by Mass

Many animal experimental studies have examined the overall biopersistence of fibers following inhalation, calculating lung dust burdens at the end of a period of dust inhalation and at a series of subsequent timepoints. In most cases dust has been estimated by mass, which has limited the value of results; but some important differences have been noted in fiber behavior in the lung, particularly with different varieties of asbestos. A number of studies have demonstrated that chrysotile asbestos accumulates in lung tissue much more slowly than the amphiboles and on cessation of exposure is removed from the lung much faster (7–9). It has been found that at the end of a 1-year inhalation period at a dose level of 10 mg/m^3 , rats usually have retained approximately 10 times more amphibole than chrysotile. Timbrell (10) originally suggested that much of this difference was due to the curly chrysotile fibers penetrating less well into the pulmonary parenchyma than the straight amphiboles; this may be partly true. However, during the 6-month period after

inhalation in rats, it was demonstrated (9) that while only 14% of a long fiber amosite dust had been cleared, 50% of a long fiber chrysotile sample had been removed. When short fiber samples of the same materials were used, differences were even more marked. In this case, clearance over 6 months was 20% for amosite and 90% for chrysotile. With asbestos, lung dust mass usually has been estimated by calculation of retained silica (7) or by infrared absorption spectroscopy (8). While the latter method gives specific results for asbestos, it relies on the presence of elements that will produce recognizable and measurable peaks and is not suitable for the detection of many types of man-made mineral fibers (MMMMF) in lung tissue. Moreover, this technique allows no information to be obtained on changes in fiber dimensions that may occur in lung tissue and that may be important in the development of disease.

Pulmonary Clearance Estimated by Fiber Number

To overcome these difficulties, some workers have now evaluated lung fiber burdens by extracting fibrous dusts from lung tissue and counting and sizing the liberated fibers (11–15). This type of data has permitted a

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much more detailed understanding of the mechanisms of pulmonary clearance and therefore the biopersistence of fibers. In general it has been found that some, but not all, types of MMMF are cleared from lung tissue faster than chrysotile asbestos, although where the amphibole asbestos types were included in the studies, they were almost always the most durable. Le Bouffant et al. (14) reported that glasswool and rockwool specimens were removed faster from rat lungs following inhalation than chrysotile, but a specimen of glass microfiber (JM Code 100-475) was more persistent than chrysotile. Intratracheal injection was used to deposit fibers in rats (11), and data were given on the half-life clearance rates of a number of types of MMMF as well as of chrysotile and crocidolite. The chrysotile results were confused by the early splitting of fiber bundles to increase the fiber numbers in lung tissue; but samples of glasswool, rockwool, and glass microfiber (Code 104-753; Code 104 E glass) all cleared much faster than crocidolite. A sample of ceramic fiber cleared slightly faster than crocidolite and only glass microfiber (Code 104-475) was removed more slowly. Rats were treated by inhalation with a preparation of glass fibers as well as crocidolite and chrysotile (13). One year after the end of dusting, 35% of the glass by mass remained in the lung compared with 58% for crocidolite and only 10% for chrysotile. By fiber numbers, retained dust was 60% for the glass, 45% for the crocidolite and 64% for the chrysotile when all fibers were recorded, but 35% for the glass, 92% for the crocidolite and only 33% for the chrysotile when fibers >5 μm in length were reported.

Importance of Fiber Dimensions in Clearance by Macrophages

This differential clearance of fibers depending on dimensions was first reported by Morgan et al. (16), who injected sized glass fibers intratracheally into rats. For fibers approximately 5 μm in length, clearance was over 90% in 1 year. In contrast, few fibers of 30 and 60 μm in length were removed. This differential retention of long fibers was also demonstrated for crocidolite, chrysotile, a number of glass fiber preparations, and rockwool (11). It results mainly from the limitations of the main pulmonary clearance mechanism of fibers from the lung parenchyma, namely physical removal from lung tissue after phagocytosis in pulmonary macrophages. Fibers impacting on the surfaces of the larger airways are transported out of the tracheo-

bronchial system on the mucociliary escalator within a few hours. For fibers that deposit on the bifurcations of the terminal or respiratory bronchioles or within the alveoli themselves, clearance is more complicated. Most deposited fibers are phagocytosed by pulmonary macrophages and eventually many macrophages move onto the mucociliary escalator and out of the lung with their burden of fibers. This can be demonstrated by the presence of macrophages containing dust in the sputum of exposed humans as well as by direct examination of the tracheobronchial surfaces in experimental animals. What is uncertain, however, is the proportion of deposited fibers removed completely from the lung by this method, since there are alternative pathways. Brody (17) has suggested that some fibers that deposit on type 1 alveolar epithelial cells are transported directly through the cytoplasm of these cells to reach the interstitium. In this case phagocytosis may be delayed or may not occur at all, although a large population of interstitial macrophages exist in the lung. Many workers believe that some—and perhaps most—fibers reach the interstitium by being transported there within macrophages from the surfaces of the respiratory bronchioles or alveoli. From the interstitial space, macrophages that have phagocytosed dust may enter the lymphatic channels and end up in the pulmonary-associated lymph nodes where very high fiber burdens may accumulate. This clearance of fibers within macrophages explains the differential retention of long fibers. A single macrophage can probably completely engulf fibers of up to 25 μm , but obviously macrophages containing long fibers are less able to migrate with their fiber burden. While cells with fibers <5 μm in length are probably able to migrate normally, a progressive reduction may be expected as fiber length increases. For very long fibers, a single macrophage can only engulf part of the fiber length and although groups of macrophages can fuse to form foreign body giant cells that can enclose very long fibers, the ability of these cells to migrate would appear to be minimal.

Importance of Chemical Dissolution

From these considerations it would be expected that fibers too long to be removed by macrophages would never be cleared from the lung parenchyma; this will be true for many long fibers. However, changes in the fibers themselves can occur, which may permit eventual clearance. An important example is found with chrysotile asbestos,

although the process may occur to a lesser extent with the amphiboles. The bundles of chrysotile fibrils that constitute the inhaled fibers separate quite rapidly in the lung tissue to their individual subunits, probably under the influence of surface active chemicals. Initially this greatly increases the number of fiber units of dangerous length (11) and explains why chrysotile appears particularly damaging to tissues in the short term. The separated fiber subunits now with extremely high aspect ratios are, however, much more susceptible to fracture and shortening, which permits phagocytosis. This process probably is assisted by chemical changes in the fiber in the lung since magnesium is leached quite rapidly from chrysotile fibers both *in vitro* and *in vivo* (18,19).

Chemical reactions between fibers and tissue fluid may be particularly important with some varieties of man-made fibers, for in rat lung (16) both short glass fibers that are cleared rapidly and long fibers that are scarcely cleared at all showed an overall reduction in diameter, particularly at the fiber ends, which tended to become pointed. This process was particularly marked with the longest fibers where mean diameters could be reduced by 50% in 18 months. It appeared that the glass fibers were dissolving in the lung tissue fluids and that with longer time than the available lifespan of the rat, could be expected to dissolve completely. Fibers that could not be moved at all by macrophages would, in this way, be cleared from the lung. This dissolution process obviously varies, as would be expected with the chemical composition of fibers, for sized rockwool fibers showed little overall reduction in diameter over an 18-month period following intratracheal injection in rats (20). However, the ends of many of these fibers became thinner, showing that some dissolution was occurring. Other workers have now shown similar changes in MMMFs extracted from lung tissue (11,14), and relative rates of dissolution for a whole range of fiber types in physiological saline have been calculated (21,22). An unusual form of chemical breakdown has recently been demonstrated for aramid fibers (23). These fibers, which are flat, lathlike sheets, break into smaller fragments, probably by neat longitudinal or transverse fracture along the crystal planes. This results in populations of shorter and thinner fibers remaining in the lung tissue of exposed rats, in contrast to the general rule that the longest fibers are the most difficult to clear.

The process of fiber dissolution in lung tissue is obviously an alternative clearance pathway to macrophage phagocytosis and migration, but the relative importance of these two methods of fiber removal has not been calculated for any fiber type. It could be done, certainly for MMMF, if some products could be uniformly labeled with radioactive elements. The level of radioactivity present in animal feces after fiber administration would indicate macrophage clearance (24), while radioactivity in blood or urine would demonstrate the amount of material leached from fibers.

Fiber/Cell Interactions That May Affect Clearance

Results from this type of study probably would demonstrate that the processes of macrophage clearance and dissolution are intermingled, and it cannot be assumed that macrophage clearance of all fibers of equal dimensions will occur at the same rate. The chemical surface of fibers, which is important in determining dissolution rates, probably affects macrophage clearance by stimulating macrophage motility. There are a number of indications of this. In all short-term tests both *in vitro* and *in vivo*, chrysotile asbestos has been shown to be much more toxic, especially to macrophages, and much more inflammogenic than the amphibole dusts (25–27). Overall, pulmonary clearance of chrysotile is much faster than the clearance of amphiboles over a timescale that makes it unlikely that magnesium leaching is causing significant fiber dissolution. It is logical to suggest that the chemical activity that can either kill macrophages or attract them in high dose situations may stimulate movement at lower doses. With MMMF high levels of fiber dissolution may not only permit long fibers to break into shorter lengths but may also create the chemical environment where macrophage clearance of these shorter particles is much more active. This process would be expected to vary from fiber to fiber, and experimental data are badly needed.

If some particles and fibers can actually stimulate macrophage movement, others, at heavy dose levels, can actually inhibit movement. In animal inhalation studies with relatively innocuous particles or short fibers of little chemical activity such as amosite, it is common to find in the oldest animals many alveoli packed with macrophages all heavily laden with dust (28). In these areas there is usually no pathological change, so that it is unlikely that the alveolar entrances are blocked. It

appears that the heavily laden cells cannot move, and the phenomenon is described as macrophage overload. Presumably, the stimulus to phagocytosis of these short fibers has continued to the point where the cells cannot move further. The fibers in an overloaded macrophage are unlikely to be cleared until the cell dies and liberates its load, which may then be rephagocytosed by a number of new cells, which thus avoid overloading. With chrysotile asbestos, overloaded macrophages are not found in old animals. This may be due to the greater stimulus to macrophage activity provided by these fibers, which are removed from the lung before overload can occur; alternatively, the greater toxicity of chrysotile fibers may kill overloaded cells relatively quickly, so that the fibers are soon redistributed.

Directions of Fiber Clearance from the Pulmonary Parenchyma

There is obviously a range of possibilities for the rates and direction of fiber removal from the pulmonary parenchyma that may be very important in disease development. While chrysotile seems readily removed from the lung tissue, studies with coalminers (29) have shown that quartz is not only retained preferentially in the lung, but that a higher proportion of quartz is found in dust residues in the lung than in the dust originally inhaled. Vincent et al. (30) demonstrated that quartz is moved more rapidly than some other dusts to the lymph nodes, and it seems likely that this mineral stimulates macrophages to penetrate to the interstitium rather than move to the mucociliary escalator. Quartz penetrates the visceral pleural surface, at least in rats, and when inhaled with other dusts causes them to penetrate to the pleura as well (9). Penetration of the pleural surface by asbestos fibers probably is related to mesothelioma development; and quartz, an ubiquitous mineral, may facilitate this process in humans, as it certainly does in animal experiments (31). In addition, different fiber varieties may themselves have the ability to stimulate macrophages to move to the pleura rather than to be cleared from the lung. This could be a major reason for the harmful potential of crocidolite and, particularly, erionite.

Fate of Fibers That Remain

While most phagocytosis of inhaled fibers occurs on the lung surfaces where they are deposited, reactions of macrophages to fibers once they have entered the interstitium are also important. During the development of pulmonary fibrosis, asbestos

fibers frequently are found free among bundles of collagen. In the early stages of fibrosis at least, it has been demonstrated (32) that macrophages can seek out chrysotile fibers, phagocytose them, and perhaps move away to effect clearance. A similar process occurs following intrapleural injection of crocidolite (33). With time, the mean fiber length of this dust in granulomas increased, because the shorter material was removed. Whether this turnover of fibers in tissues is continuous or limited to the active phase of disease development is important and may vary with fiber type. Certainly, with chrysotile asbestos, old rats 18 months after the cessation of dusting have no chrysotile fibers still in contact with cells. The fibers that remain are either among collagen fibrils in old acellular fibrous tissue or contained within thickened basement membranes (34). There appears to be no stimulus for macrophages to find and move these fibers. No evidence is available regarding the long-term turnover or walling up of amphibole fibers, but if these were permanently able to attract macrophages, even to areas of old fibrosis and to be transported to where they could still react with other cell types, this could help explain their greater potential to cause disease in long-lived species.

Importance of Different Chemical Environments within the Lung

There is one way in which phagocytosis by macrophages and fiber dissolution may interact, either stimulating or impeding clearance. This would be where the rate of dissolution of phagocytosed fibers was different from dissolution in the extracellular tissue fluids. Dissolution will, of course, vary with the chemical formulation of the material in question, but it will also vary with the chemical environment in which a fiber finds itself. One of the most important variations in this environment will be pH. The pH of tissue fluids is nearly neutral but that of the macrophage phagolysosome system is quite acid. Thus a fiber that is acid soluble may dissolve faster once phagocytosed. One example of this is probably chrysotile, which is known to be acid-soluble, so that phagocytosis will aid dissolution and clearance. Conversely, fibers that are more soluble in an alkaline medium may be very stable once they are inside macrophages. This is the case for some glass formulations, where phagocytosis may actually reduce fiber clearance, particularly if macrophages become overloaded. An example could be found with

the variety of glass microfiber JM475. This material, in its code 100 size range, is similar to asbestos and appears to be durable (11,13,14). In one study, the glass microfiber was actually more durable than chrysotile (14). In spite of this and the presence of long fibers in the dust cloud, extensive animal inhalation studies have shown the microfiber to be harmless (35,36). The explanation of these combined data could be that once deposited in lung tissue, long microfibers, incompletely phagocytosed, will begin to dissolve and fragment into shorter lengths. These and the original short fibers may be very stable in macrophages although they are too short to cause disease. Obviously, studies on fiber durability in lung tissues can only be fully interpreted if complete fiber-size data of retained fibers are produced.

Different Timescales for Clearance in Humans and Experimental Animals

Counts and even complete size data alone may not be enough to interpret durability and the potential for disease production, when extrapolation from experimental animals to much longer lived humans is required. Some types of MMMF designed to be relatively soluble in lung tissue were found to show relatively little reduction in fiber numbers or overall dimensions after

inhalation in rats (Hesterberg, personal communication). Detailed analysis of the fibers, however, showed that their surfaces had been greatly changed with widespread leaching of major components. The extra years available for dissolution in humans most probably would have resulted in complete dissolution of these fibers.

Different timescales applied to constant rates of dissolution may produce apparently conflicting results, even where disease production occurs in both experimental animals and humans. This is found with chrysotile asbestos, which in numerous animal experiments has been shown to be as pathogenic as the amphiboles. However, epidemiological studies have shown that chrysotile is less likely to cause disease in humans. It appears likely that while dissolution has insufficient time to reduce the disease potential of chrysotile in short lived animals, this process does significantly reduce the retained chrysotile lung burden over the many years during which human disease develops.

Conclusions

In summary, it is believed that long thin fibers are the ones that cause pulmonary disease but to do so they probably have to remain in lung tissue for a considerable time. The potential for disease production

is reduced for all inhaled materials by the pulmonary clearance mechanisms which, at least for the deep lung parenchyma, rely on phagocytosis by macrophages and active removal of the inhaled material. Some toxic fibers or particles may well stimulate macrophage activity and movement and thus promote faster clearance than with inactive materials, but with quartz, and perhaps some fibers, there is evidence that macrophages are stimulated to enter the interstitium and the lymphatics rather than to move to the mucociliary escalator and thus out of the lung. Some fibers undergo leaching or dissolution in physiological fluids, which provides another mechanism for the removal of inhaled material from the lung. Dissolution may be particularly important in causing long, thin fibers to break down to short fragments that can be phagocytosed by a single macrophage, thus increasing clearance. However, because some materials may be more resistant at the acid pH of the macrophage phagolysosome system, phagocytosis might actually increase durability of materials that otherwise would have been soluble in the extracellular medium. Fortunately, since this would only apply to relatively short fibers, the process is unlikely to promote disease.

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