Chemical aspects of the toxicity of inhaled mineral dusts

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Some humans are often exposed to airborne mineral dusts at the workplace or in daily life. When inhaled, some kinds of mineral dusts can trigger a pathological response of the respiratory system. Silicosis (from silica dust) and asbestosis (from asbestos fibres) are the most commonly known diseases originating from inhaled mineral dusts; other examples are bronchogenic carcinoma and mesothelioma. Detailed knowledge of the chemical (and physical) factors underlying mineral dust toxicity is much needed in order to evaluate the relative risks from exposure to different kinds of materials, both natural and synthetic. These pathogenic factors have been reviewed, with a focus on the surface chemistry of mineral particles and interface phenomena. To facilitate understanding, an outline of the anatomy of the respiratory system and of the etiology of the main diseases involved is also given.

1 Introduction

Dating from antiquity, lung complaints related to inhaling mineral dusts are among the oldest occupational diseases known to mankind. Indeed, Pliny the Younger (61–113 AD) had already referred to the typical sickness of asbestos workers, and during the Middle Ages both Paracelsus (1493–1541) and Agricola (1494?-1555) extensively wrote about the *miner's disease*. By the turn of the century the first epidemiological studies on the health hazards associated with airborne mineral dusts, mainly silica and asbestos, began to appear and the medical community was rendered increasingly aware of the pathogenicity of these materials. This led to severe law regulations which now apply in most western countries. For instance, asbestos is today one of the most strictly regulated materials, and in the United States the EPA (Environmental Protection Agency) has required all schools and other public buildings to be inspected and analysed for any friable materials containing asbestos. Similar regulations apply in most western countries.

Besides silica and asbestos, many other kinds of airborne mineral dusts (particulates), both natural and synthetic, are today recognised as being potential health hazards: carbonaceous dust, glasswool and rockwool are only a few examples. These materials can be present in both the occupational and the general environment, and the resulting effects on health must be related to the quantity of particles inhaled as a whole, taking into account that different kinds of particulates may act not only additively but also in a synergistic (or perhaps sometimes inhibiting) way.

Diseases (mainly of the lung) related to inhaled mineral dusts and fibres are usually progressive and cumulative, and some of them have latencies of around 15 to 20 years. For these reasons, recent epidemiological studies have tracked groups of workers, mainly miners, over long time periods. These studies have shed some light on the etiology of the diseases, mainly relating the

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exposure to a particular kind of particulate to the corresponding illness. However, the mechanisms leading to pathogenicity of mineral dusts, and the underlying chemistry involved, are still poorly understood. And yet, knowledge of the chemical basis of particulate toxicity is much needed in order to evaluate the relative risks from exposure to different kinds of materials. This is so much the case at a time when popular concern has grown about the hazards of both natural materials (*e.g.* asbestos) and synthetic replacements, such as fibrous alumina or glasswool.

The available literature on the chemical aspects of the toxicity of mineral dusts is somewhat fragmentary and conflicting.[1,2] Comparative analysis of the results is complicated by the fact that many experiments (*in vivo* and *in vitro*) have been performed under non-equivalent conditions, and quite often the allegedly pathogenic materials have not been properly characterized. The long latency periods (already referred to) of several pathological conditions also hinder the evaluation of experimental results. However, short-term animal studies (over a period of days to weeks) following exposure to mineral dusts, shed some light on the initial stages of the pathological response,[1] including inflammation, pre-fibroplastic and pre-neoplastic changes. The increasing use of specific techniques[2,3] (FTIR spectroscopy, microcalorimetry, EDAX (Energy Dispersive X-ray Analysis), atomic force microscopy, *etc.*) for surface characterization is also contributing to our understanding of the pathogenicity of mineral dusts. Altogether some unifying ideas are beginning to emerge and we aim at reviewing them. For the benefit of the non-specialized reader, we first give an account of the anatomy of the lungs, the fate of particles that enter the respiratory system and the main health effects of inhaled particles.

2 Structure and function of the respiratory system: an outline

The main function of the lung is to act as a gas exchange system. Venous blood is pumped into the lungs and circulated through the pulmonary capillaries, which surround millions of air-containing alveoli. These alveoli, with a total surface of about 80 m^2, constitute the gas exchange area of the lungs. During circulation of venous blood through the pulmonary capillaries surrounding the alveoli, diffusion gradients favour exchange of oxygen and carbon dioxide between the inspired air and the blood; venous blood becomes thus oxygenated, and CO_2 is expelled.

The upper respiratory tract begins at the nose and mouth and continues through the larynx. After passing through the larynx, inspired air enters the trachea which divides into two main bronchi, each one leading into a lung. In the lung, the bronchus repeatedly divides into smaller and smaller air ducts. The narrowest and most numerous are the bronchioles, which have a lumen of about 0.5 mm. The terminal bronchioles end up forming alveolar ducts (0.2 mm in diameter) which open into the alveoli. This architecture and the final dimension of air passages have major implications for deposition and clearance of particulates entering the lung in inspired air. Particles having an aerodynamic size favouring the sedimentation or interception (mainly at bifurcations) have little chance of reaching the alveoli, and usually are removed by the mucociliary escalator (see later). Another important point to bear in mind is that the air in the lung is basically saturated with water vapour, as it becomes moistened on passing through the nose, bronchi and smaller air ducts to the alveoli. Therefore, inhaled particles necessarily become fully exposed to water vapour before reaching the distal parts of the lung.

To preserve the structural and functional integrity of the alveoli, specialized cells secrete a pulmonary surfactant rich in phospholipids. These surfactant lipids spread as a monolayer at the air–water interface lining the alveoli and prevent alveolar collapse (at low lung volumes) by decreasing the surface tension of the curved air–liquid interface. The pulmonary surfactant layer may also be involved in non-ciliary transport of inhaled particles from alveoli to bronchioles, thus facilitating mucociliary transport and clearance. Besides lipids, the lung surfactant contains, among other chemical species, antioxidants (ascorbate and glutathione) and proteins (mainly immunoglobulins) which can interact specifically with inhaled minerals.

Lining the inner surface of the air ducts, from the upper respiratory tract down to the terminal bronchioles, there is a structure composed of ciliated cells, mucus-secreting cells and glands; this mucociliary system (or escalator) plays a major role in removing solid particles from the lung. The cilia act as beaters which continuously transport mucus (and particles deposited on it) upward towards the trachea and larynx, where the mucus is then expectorated or swallowed. This is the main mechanism whereby particles deposited on the surface of air passages are removed. In addition, particles can also pass into the lymphatic system and be removed.

The lymphatic system is a network of thin-walled vessels found throughout all parts of the body (except the central nervous system) including the lung and pleura (see later). Along their course, small vessels merge to form larger lymphatic channels. The lymphatic system has the main purpose of removing excess interstitial fluid (the lymph) which is finally drained into the subclavian veins at the root of the neck. For this purpose, the lymphatic vessels have valves like those of veins, which prevent back flow. The lymph nodes filter off bacteria (a mechanism against infection) and foreign particles, and contribute leukocytes (a kind of white blood cell) and antibodies.

Foreign particles present in the lung can be engulfed by macrophages, which are migratory cells capable of transporting ingested materials through the lymphatic circulation towards the lymph nodes. Although primarily found at these nodes, the macrophages (or phagocytes) form part of a larger organisation, known as the reticuloendothelial system, which includes similar cells in other body tissues. Those present in the lung contribute to the clearance of inhaled particulates. However, upon ingestion of particulates, macrophages can also liberate fluids contributing to potentially dangerous free radical generation.

The lung is covered with two layers of a thin membrane: the pleura. The outer layer (parietal pleura) lines the inside of the thorax, the inner (visceral pleura) is directly attached to the outside of the lung, and is rich in lymphatic drainage channels. When considering the fate of inhaled particulates, it is important to realize that the lung alveoli are adjacent to the visceral pleura. Hence, particles reaching the distal structures of the lung can pass into the pleura, in addition to those that move by way of the lymphatic channels.

We shall now summarise the possible fate of inhaled mineral particles, and the most frequent pathological conditions they can originate. The interested reader is directed to comprehensive medical texts[4] for greater details on the respiratory system.

3 The fate of mineral particles that enter the respiratory system

To a large extent, the behaviour of particulates entering the respiratory system depends on their size and shape, which determine relevant aerodynamic characteristics. The anatomic structure of the respiratory system prevents large particles from reaching the distal parts of the lung; they tend to be intercepted along the passages of the nose or at branching points of bronchi. When particles show a more or less spherical shape, only those having a diameter smaller than 5 μm are likely to reach the alveoli; larger ones would be deposited primarily in the upper

air passages and removed by the mucociliary escalator. Fibres represent a more complex case, since their aerodynamic behaviour depends on length and diameter. Most airborne mineral fibres have a diameter of a few tenths of a μm, while length can go from a few μm to several hundreds. Although fibres as long as 200 μm have been found in pathological studies of lung tissue, the vast majority[5] are shorter than 50 μm. Besides affecting the rate of clearance from the lung, the relative dimensions of mineral fibres could also have some differential effect on pathogenicity. It has been suggested[6] that fine, long fibres (diameter smaller than 0.25 μm and length greater than 8 μm) are more carcinogenic than short and thick fibres. However, this hypothesis is not free from controversy,[7] and chemical factors are becoming ever more evident.

Airborne mineral particles which have reached the lung alveoli can undergo several different processes. First, they can simply remain free in the alveolus (some of them would be eventually removed *via* the mucociliary escalator). Secondly, they can be partially or completely dissolved over a period of time. Particles of limestone, marble or dolomite (composed of $CaCO_3$ containing variable amounts of other metal carbonates) are likely to have this fate since lysosomal fluids, released mainly by alveolar macrophages, have an acidic pH and can thus dissolve metal carbonates. Similarly, chrysotile (due to its higher magnesium content) is progressively leached by alveolar fluids while amphibole asbestos is more insoluble. This different behaviour could be correlated with the lower toxicity of chrysotile.

A third possible fate of inhaled mineral particles reaching the alveoli is to be ingested by macrophages. Alveolar macrophages have a life span averaging 50 days, although it can be shortened following fibre ingestion. When they die, ingested particles are discharged and can be reingested by other macrophages. This cycle can be repeated indefinitely. Macrophages have a diameter of about 10 μm, and are capable of engulfing particles smaller than 5 μm. Larger fibres cannot be completely engulfed, but they can pierce the plasma membrane of macrophages giving rise to the discharge of lysosomal fluids and enzymes.

A fourth fate involves migration of mineral particles, either naked or inside macrophages, across the alveolar membrane and into the interstitial lung tissue. They can either remain there or reach the lymphatic system. Particles entering the lymphatic ducts tend to be filtered off at lymph nodes, where they may stay indefinitely. However, some of them pass (by way of the lymph) into the blood circulation and can thus reach other organs of the body. Finally, inhaled mineral particles can pass through the alveolar membrane and into the pleura, although the mechanism of this translocation does not seem to be clear.

4 Main diseases related to inhaled mineral dusts

4.1 Pneumoconiosis

Pneumoconiosis has been defined[4b] as a non-neoplastic reaction of the lungs to inhaled minerals and the resultant alteration in their structure, excluding asthma, bronchitis and emphysema. Pneumoconioses can have several origins, and they are termed accordingly; those occurring more often are silicosis, asbestosis and coal worker's pneumoconiosis.

Inhaled silica dust can give rise to silicosis. Silica is widespread in nature; it occurs as a component of both the soil and many rocks, apart from forming quartz and several other mineral species. Exposure to finely divided silica occurs in a variety of jobs; high-risk workers include miners, sandblasters and people involved in drilling works. When the intensity and duration of exposure are high, silica particles accumulate in alveoli from where they spread into the lung and lymphoid tissue.

Crystalline silica dust causes an intense cellular reaction with local proliferation of macrophages, lymphocytes and fibroblasts. The characteristic histological lession in the lung is a silicotic nodule: the granuloma. When silicotic nodules are discrete, the condition is termed *simple silicosis* and is usually observed to develop only after ten or more years of exposure to silica dust. When the disease progresses, silicotic nodules tend to grow and coalesce into conglomerates, leading to massive fibrosis: a condition termed *conglomerate silicosis*.

Lung function is reasonably preserved in patients having simple silicosis, but after the onset of conglomeration lung function can become severely impaired. Shortness of breath (dyspnea), chronic cough and bronchitis are common symptoms. Advanced conglomerate silicosis can lead to a severe condition of the cardiorespiratory system.

Asbestosis is the form of lung fibrosis that results from inhaling asbestos fibres; it is frequently associated with pleural calcification (plaques). Exposure to asbestos can occur in many occupations; high-risk groups include asbestos miners and millers, asbestos textile workers, shipyard workers and labourers in various construction and insulation trades.

As in the case of silicosis, asbestosis is a disease which develops slowly over the years. The onset of substantial lung fibrosis needs exposure to a relatively high air concentration of asbestos fibres for at least 10 years. However, once initiated, fibrosis can progress even without further exposure. In contrast to the discrete nodular fibrosis observed in silicosis, the fibrogenic reaction typical of asbestosis usually spreads out along the supporting structures of the lung.

The mechanism of asbestos-induced fibrogenesis is not clear. The sequence of adverse health effects starts with alveolitis (inflammatory process at the alveoli) and is followed by a generalised fibrogenic response which leads to destruction of the alveolar architecture and adjacent vascular structures.[8] Long fibres seem to be more active than short ones. The reason for this size-dependent response seems to be that long fibres cannot be taken entirely by macrophages; as a result the pierced macrophage leaks and discharges fibrogenic factors. Note also that, because of their size, long fibres cannot be cleared from the alveoli as efficiently as smaller mineral particles.

Diffuse fibrosis generated by inhaled asbestos is often difficult to distinguish from other forms of lung fibrosis having a different etiology (*e.g.* idiopathic pulmonary fibrosis). A unique (and diagnostic) feature of asbestosis is the presence, in histological sections of the affected lung, of asbestos bodies.[9] Asbestos (or ferruginous) bodies is the name given to fibres which become coated with a thick layer of iron oxyhydroxide mixed with mucopolysaccharides and proteins. Long fibres tend to form asbestos bodies.

Heavy exposure to carbonaceous dust can lead to a condition known as coal miner's pneumoconiosis. The main occupations concerned are coal mining and processing, but the mining and processing of graphite and the fabrication of carbon black and carbon electrodes can also pose health hazards. Chimney sweeps also constitute a risk group.

Unlike silica or asbestos, pure coal or graphite dusts do not cause an intense cellular reaction in the lung, and they are less fibrogenic. Inhaled carbonaceous dust gives rise to localized nodules (in the area of alveoli and alveolar ducts) which are 1 to 2 mm in diameter and contain macrophages laden with carbon dust. Ingestion of carbon particles by macrophages does not prematurely kill them, but heavy and prolonged exposure to airborne carbonaceous dust overloads the cleansing mechanisms of the lung. At the stage of localized nodules, lung function is nearly normal and the resulting condition is less severe than simple silicosis. In some patients, however, conglomerate lesions develop, with an outcome similar to that found in conglomerate silicosis. It should also be noted that coal

in mines is often associated with silica. Therefore, a coal miner can develop silicosis.

4.2 Cancer

Exposure to respirable mineral dusts can be a risk factor for developing several forms of cancer. Among them, bronchogenic carcinoma and malignant mesothelioma are the most common forms. However, not all particulate minerals are carcinogenic. Table 1 lists the mineral carcinogens recognized by the International Agency for Research on Cancer (IARC).

Table 1 Carcinogens (according to the IARC) which often occur as mineral dusts. (*) Possibly carcinogenic

Material	Frequent occupational exposure
Asbestos	Asbestos mining, asbestos textile industry, insulation trades
Cadmium	Metal plating, zinc ore mining and processing
Carbon black (*)	Graphite processing, carbon black fabrication
Ceramic fibres (*)	
Chromium(VI) compounds	Tanning of leather
Coal tar pitches	
Erionite	Mining
Glasswool (*)	
Lead compounds (*)	
Nickel compounds	Metal plating
Nickel, metallic (*)	Metal plating
Silica, crystalline	Mining, rock drilling, sandblasting
Rockwool (*)	
Talc containing asbestiform fibres	

Bronchogenic carcinoma (lung cancer) arises from the epithelial lining of the lung airways. It usually has a latent period of at least 10 to 20 years between exposure to the carcinogen and clinical manifestation.[1] There is some evidence[10] that lung cancer is frequently associated with diffuse pulmonary fibrosis (asbestosis) rather than with other forms of pneumoconiosis.

Diffuse malignant mesothelioma is a cancer of the mesoderm, developing either at the pleura or the peritoneum (the lining membrane of the abdomen). Epidemiological studies[10] have shown an excess risk of developing mesothelioma among workers exposed to asbestos or to erionite (see below). It is also known[6,7] that asbestos fibres can induce mesothelial tumours in experimental animals.

4.3 Other adverse health effects of inhaled mineral dusts

Other lung conditions attributable to inhaled mineral dusts include chronic cough, chronic bronchitis, emphysema and thickening (calcification) of the pleura. Bronchitis (and alveolitis) results from inflammatory responses triggered by particle deposition in the respiratory tract. Inflammation is a defensive reaction to the irritation arising either from a direct effect of the inhaled dust or as a consequence of the activity of lymphocytes and macrophages. Alveolar macrophages can generate oxidizing free radicals during phagocytosis, and this activity can cause local tissue damage and inflammation. Impaired lung function, brought about by fibrosis, can cause over-inflation of the air spaces, thus leading to pulmonary emphysema. The condition is aggravated when conglomerate lesions occur. Finally, chronic infection (mainly due to anaerobic bacteria) occurs occasionally at the stage of conglomerate silicosis or diffuse fibrosis. There is also an increased risk of developing pulmonary tuberculosis.

There are reports in the literature[11] linking exposure to silica (or asbestos) with a variety of immunoregulatory disorders and autoimmune diseases. The best example of this link is the occasional coexistence of silicosis and rheumatoid arthritis (Caplan's syndrome). Other autoimmune diseases which have been implicated in the pathogenesis of silicosis (and to a lesser extent asbestosis) include systemic sclerosis, lupus, and chronic renal disease. The mechanisms of synergy between exposure to mineral dusts and autoimmune conditions are poorly understood. However, there may be a link between proliferation and destruction of macrophages (triggered by the presence of mineral dusts in the lung and lymphatic system) and immunoregulatory disorders.

5 Main materials of concern

The main inorganic materials of concern in relation to diseases caused by inhaled dusts can be divided into two categories: natural minerals and synthetic mineral fibres. Among natural minerals, silica, asbestos and erionite are those of main concern; coal can be cited in a second place.

Silica (SiO_2) is a widespread mineral which can occur in a large number of polymorphic forms (more than 20 have been described). The most common form of silica is quartz, cristobalite can be cited in second place. Quartz is a major constituent of a number of rocks, such as granite and sandstone. It also occurs alone, either in a highly crystalline form or forming poorly crystalline minerals (often referred to as being cryptocrystalline). Synthetic silicas include silica gel and pyrogenic silica.

Silica can also have a biogenic origin. Among biogenic silicas, diatomaceous earth (or kieselguhr) is mined in a large scale, and used mainly in filtration plants and as an insulation material. Calcination of kieselguhr yields cristobalite.

All silica minerals, both natural and synthetic, are formed by a framework of corner sharing SiO_4 tetrahedra. Different polymorphs arise from slightly different arrangements of these tetrahedra. The surface of the mineral contains mainly silanol (Si–OH) and siloxane (Si–O–Si) groups. In freshly crushed silica, some highly reactive chemical species can also be present (see Section 6.1).

Asbestos minerals are fibrous silicates which can be divided into two groups: serpentines and amphiboles. Chrysotile is the main representative of the serpentine group, it has the chemical composition $Mg_3Si_2O_5(OH)_4$. Amosite and crocidolite are the best known representatives of the amphibole group, their chemical formulae are $(Mg, Fe^{2+})_7Si_8O_{22}(OH)_2$ and $Na_2(Mg, Fe^{2+})_3Fe_2^{3+}Si_8O_{22}(OH)_2$, respectively.

Basically, the crystal structure of all the minerals in the serpentine group can be thought of as being formed by a double layer consisting of a tetrahedral (silicate) sheet of composition $(Si_2O_5)_n^{2n-}$ in which three of the oxygen atoms in each SiO_4 tetrahedron are shared by adjacent tetrahedra, and an octahedral (brucite) sheet of composition $[Mg_3O_2(OH)_4]_n^{2n+}$ formed by edge-sharing $MgO_2(OH)_4$ octahedra (iron can substitute for magnesium in this layer). The two sheets are bonded together forming a double layer in which the apical oxygens of the $(Si_2O_5)_n^{2n-}$ sheet are shared with the brucite layer, as shown in Fig. 1a. There is a slight misfit between the tetrahedral and octahedral sheets of the double layer which causes curling (like rolling up a carpet) to form concentric cylinders. Curling takes place keeping the silicate layer inside and the brucite layer on the outside of the curve (Fig. 1b). The average diameter of the cylinder thus formed (a chrysotile fibril) is about 25 nm.

The crystal structure of the amphiboles (Fig. 1c) can be described in terms of a basic structural unit formed by a double-tetrahedral chain (corner linked SiO_4 tetrahedra) of composition $(Si_4O_{11})_n^{6n-}$. These silicate double-chains share oxygen atoms with alternate layers of edge-sharing MO_6 octahedra, where M stands for a variety of cations: mostly Mg^{2+}, Ca^{2+}, Fe^{2+} or Fe^{3+}. Variations in the stacking of the tetrahedral and octahedral

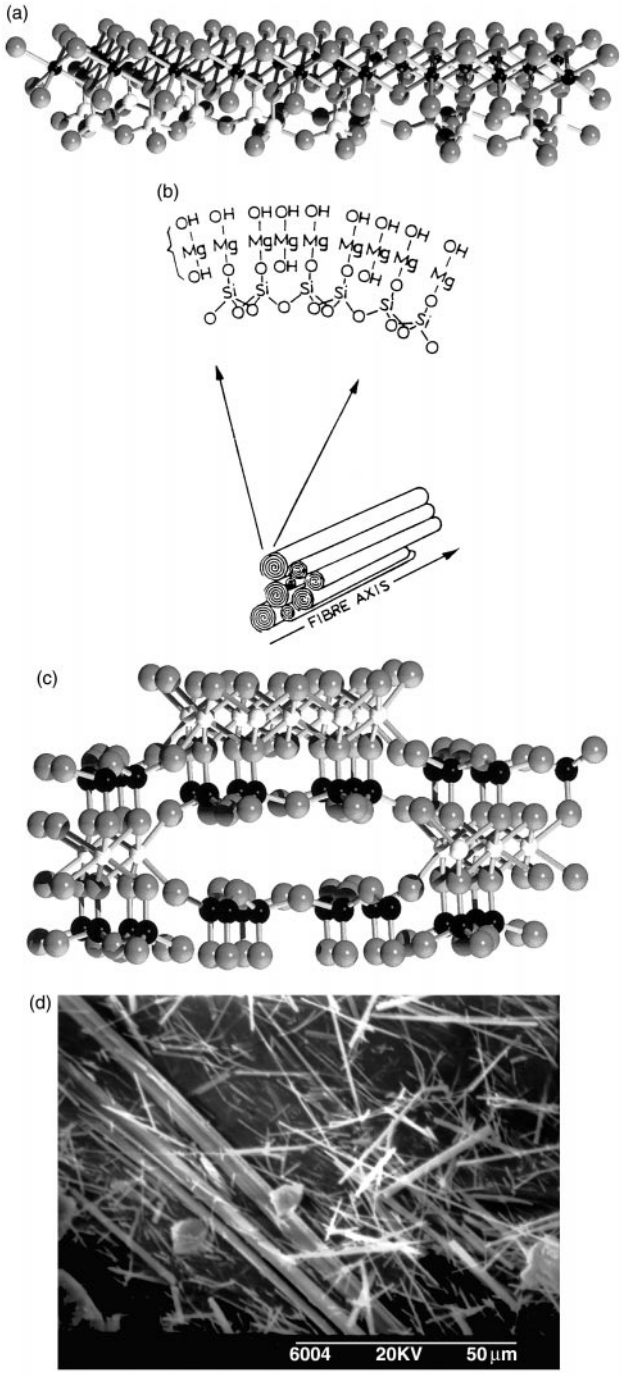

Fig. 1 (a) The double layer of the serpentine-type structure. (b) Schematic representation of chrysotile asbestos fibres. (c) The amphibole-type structure. (d) Typical morphology of amphibole fibres.

layers give rise to slightly different structural types. The amphiboles exhibit prismatic cleavage. When they are crushed or milled they fracture along the octahedral layers giving rise to acicular fragments having the typical morphology shown in Fig. 1d. Individual fibres are usually about 0.2 μm in diameter, and they tend to aggregate into bundles.

Serpentine and amphibole asbestos are the most typical fibrous minerals. However, some other silicates can present a fibrous habit. Zeolites (which are three-dimensional aluminosilicates) should be mentioned in this context, since one of them (erionite) is known to cause mesothelioma when inhaled. Erionite is one of the few fibrous zeolites; its typical composition can be described as $(K, Na)_5Ca_2[(AlO_2)_9(SiO_2)_{27}]\cdot 27H_2O$. It should be borne in mind, however, that the Na^+, K^+ and Ca^{2+} ions can be replaced by other cations.

Synthetic inorganic fibres are produced either to replace fibrous minerals or for more specific applications. Main uses of these synthetic materials are thermal and acoustic insulation, fireproofing, fibre optics and the manufacturing of fibre reinforced composites. Typical compositions of synthetic inorganic fibres are summarized in Table 2.

Table 2 Typical composition (weight%) of synthetic inorganic fibres

Component	Glasswool	Rockwool	Slagwool	Ceramic fibre
Al_2O_3	2.4–14.5	6.5–13.4	11.8–12.5	0–96
B_2O_3	3.5–8.5	—	—	0–14
CaO	5.5–22.0	10.8–30.3	37.5–40.0	0–0.7
FeO/Fe_2O_3	—	1.5–12.4	0.9–1.0	0–0.8
K_2O	0.5–3.5	1.0–1.6	0.3–0.4	0–0.1
MgO	3.0–5.5	—	—	0–0.5
Na_2O	0.5–16.0	2.3–2.5	0.2–1.5	0–0.2
PbO	0.0–59.0	—	—	—
SiO_2	34.0–73.0	45.5–52.9	40.6–41.0	0–53.9
TiO_2	0.0–8.0	0.5–2.0	0.4–0.5	0–1.6
Y_2O_3	—	—	—	0–8
ZrO_2	0.0–4.0	—	—	0–92

Fibrous glass includes glasswool and special-purpose fibre glass (*e.g.* optical fibre). It is an amorphous material made of fused silica to which other glass-forming oxides are added in variable concentration. Mineral wool includes rockwool, made from magma rock, and slagwool, derived from molten slag produced mainly in iron and steel metallurgical processes. Refractory ceramic fibres can be made from clays and other aluminosilicates, or from several metal oxides such as SiO_2, Al_2O_3 or ZrO_2. Whiskers constitute a less common type of refractory fibres; they are made mainly from metal carbides or boron nitride and frequently they are single crystal materials.

To a large extent, the possible pathological effects of synthetic mineral fibres in relation to respiratory diseases are as yet unknown. Some epidemiological studies[12] suggest that excess mortality from at least some types of synthetic mineral fibres is not high. However, because of limited knowledge, it would be prudent to treat these materials with similar precautions to those recommended for asbestos.

6 Chemical factors affecting pathogenicity

6.1 General background

The pathogenic effects of inhaled mineral dusts can be related to both physical and chemical factors. As already considered in Sections 2 and 3, the size and shape of inhaled particulates affect the rates at which deposition and clearance take place. Large airborne particles tend to be deposited in the air ducts of the respiratory system before reaching the terminal bronchioles and alveoli, and are efficiently removed by ciliated cells. Macrophages can ingest small particles and short fibres, thus helping to transport them out of the lung. Fibres of intermediate size, thin enough to reach the distal parts of the lung but too long to be entirely engulfed by macrophages may be a major culprit in causing lung disease. Indeed this notion, known as the Stanton hypothesis,[6,13] dominated early studies on the toxicity of inhaled mineral fibres. More recently, however, attention is being focused on the chemical aspects of particulate pathogenicity, since it has been recognized that morphological factors alone are unable to account for the differential toxicity of mineral dusts having different chemical composition and structure.[14] When dealing with solids which are (mostly) insoluble in biological fluids, the chemical aspects of their toxicity have to be related mainly to surface properties.

It is often considered that the chemical reactivity of a solid material is determined by its chemical composition and crystal structure. However, it should be realized that at the surface of a

(crystalline) solid the three-dimensional periodicity of the crystal lattice is lost. This fact affects electrostatic potentials and local electron distribution, giving rise to electronic states (surface states) which confer to the surface characteristic chemical and physical properties. Surface states can also arise from (or can be modified by) surface relaxation or reconstruction, and also from structural imperfections and adsorbed impurities (or surface segregation of chemical species from the bulk). The presence of surface states affects both the electrical properties of the surface and the chemical reactivity, by modifying the affinity of the surface for electrons.

When dealing with ionic solids, the presence at their surface of exposed cations and anions is of primary importance regarding chemical reactivity and interaction with biological molecules and living cells. Exposed, coordinatively unsaturated, cations act as electron acceptors (Lewis acid sites in the broad sense) and provide interaction points for electron donors. Similarly, surface exposed anions can interact with electron acceptors or dipolar molecules.

At the solid–liquid interface (between solid particles and biological fluids) the ionic composition of the surface layer can be altered by preferential transfer of cations, or anions, into the aqueous liquid phase. This phenomenon alters the electrical neutrality of the interface, and the solid surface becomes electrically charged. The corresponding electrokinetic potential thus generated, usually termed the zeta potential, can reach hundreds of millivolts and can critically affect the interaction of mineral particles with living cells. Indeed, Light and Wei[15] have found a strong (positive) correlation between the zeta potential of asbestos fibres and their hemolytic activity (ability to rupture the erythrocyte membrane).

The idealized surface of covalent solids (such as graphite or quartz) has a homopolar character, with no charge separation. However, as a result of mechanical fracture several reactive species can be generated, which represent localized surface states. As an example, let us consider silica.

In all silica polymorphs, SiO_4 tetrahedra share corners forming a three-dimensional network of Si–O–Si bonds which have mainly a covalent character. When these bonds are broken as a result of mechanical fracture two different situations can occur, as depicted in Fig. 2. Homolytic cleavage gives rise to

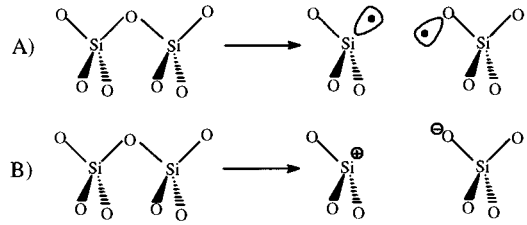

Fig. 2 Homolytic (A) and heterolytic (B) cleavage of Si–O–Si bonds in silica.

free radical •Si and Si–O• species, often referred to as dangling bonds. Heterolytic cleavage generates electrically charged surface species (Si^+ and SiO^-). Both types of surface states are highly reactive, and they are likely to be the main cause of the pathogenic effects induced by inhaled silica dust.[14e] Another factor to consider is that charge separation at the silica surface results in increased hydrophilicity of the mineral. This hydrophilicity can affect interaction with biological molecules.

6.2 Hydrophilicity and hydrophobicity

Solids presenting a heteropolar surface are hydrophilic, because exposed surface ions interact strongly with the electrical dipole of water molecules. Homopolar surfaces tend to be hydrophobic. Hydrophilic mineral particles show the strongest interaction with biological molecules, which are mainly polar or even contain charged groups. They can therefore act as electron donors or acceptors. Extremely hydrophobic surfaces can also strongly adsorb proteins, by a mechanism known as the hydrophobic interaction which involves a favourable entropic term arising from the release of water molecules forming the hydration sphere of the protein. However, such a high degree of hydrophobicity does not occur among mineral dusts.

Hydrophilic surfaces favour protein adsorption and denaturation. They also favour cell-surface adhesion which can lead to injury.[2,16] The outer cell membrane (the plasma membrane) is composed of amphiphilic phospholipids, proteins and steroids which form a thin bilayer. This bilayer is structured so as to have the hydrophobic cores of the biological molecules in each monolayer pointed inward while the hydrophilic head groups are pointed outward, towards the surrounding (aqueous) space and the internal cytoplasm. These hydrophilic head groups, which *in vivo* tend to be negatively charged may interact strongly with surface exposed cations of mineral particles (usually displacing adsorbed water molecules). Such an interaction can adversely affect membrane structure and dynamics leading to membranolysis.

As an example, Fig. 3 shows the hemolytic activity of a cristobalite powder as a function of hydrophilicity. When cristobalite was calcined at increasing temperature in order to

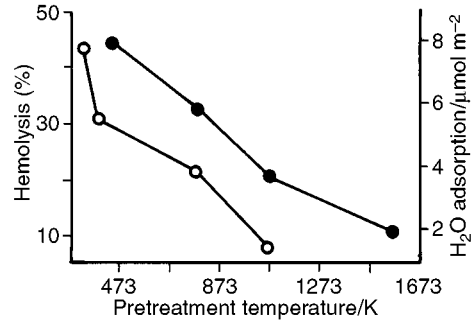

Fig. 3 Erythrocyte hemolysis (white circles) and water vapour adsorption (black circles) on cristobalite powder preheated at increasing temperature.

render it less hydrophilic, the hemolytic potential was also found to decrease.[17] Note that the degree of hydrophilicity was tested by measuring the extent of water vapour adsorption at an equilibrium pressure of 5 Torr. Although this experiment has to be interpreted with some caution, because heat treatment of cristobalite could also reduce the concentration of surface free radicals, it is tempting to think that the relative hydrophobicity of carbon dust (as compared to silica or asbestos) could be one factor determining its less intense cellular reaction in the lung (Section 4.1).

6.3 Mineral induced free radical generation

Many intracellular processes, including the mitochondrial respiratory chain, reduce molecular oxygen to the superoxide radical ($O_2^{\cdot -}$) or to hydrogen peroxide. In a low concentration, these chemical species are only moderately reactive towards biological molecules, and their potential adverse effects are minimized by the antioxidant defense mechanisms of the cell which include the action of the enzymes catalase, superoxide dismutase and glutathione peroxidase. However, in the presence of transition metal ions, the radical species $O_2^{\cdot -}$ and also free oxygen and H_2O_2 can generate the hydroxyl radical (•OH). This free radical is a highly reactive species capable of causing, among other deleterious effects, DNA damage, protein oxidation and lipid peroxidation.

Iron is the most ubiquitous transition metal in both natural minerals and synthetic mineral fibres. It can occur either as a major component (for example in many asbestos minerals) or as

an impurity. Exposed iron ions at the surface of mineral dusts can generate $^{\bullet}OH$ by the following (iron-catalysed) Haber–Weiss cycle:

$$\text{Reductant} + \text{Fe(III)} \rightarrow \text{oxidized reductant} + \text{Fe(II)} \quad (1)$$

$$\text{Fe(II)} + O_2 \rightarrow \text{Fe(III)} + O_2^{\bullet -} \quad (2)$$

$$O_2^{\bullet -} + 2H^+ + e^- \rightarrow H_2O_2 \quad (3)$$

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(III)} + OH^- + {}^{\bullet}OH \quad (4)$$

Eqn. (4) is known as the Fenton reaction. Several metabolites can act as the reductant species in eqn. (1); ascorbate, cysteine and glutathione are a few examples. Generation of hydroxyl radicals by the Haber–Weiss cycle needs iron only in catalytic (trace) amounts, and the turnover of free radicals can overload the antioxidant defence mechanisms of living cells.

In natural minerals (*e.g.* the amphibole group minerals amosite and crocidolite) and in some man-made fibres, iron can occur either as Fe(III) or as Fe(II), and some controversy has arisen as to which oxidation state can be more dangerous.[14,18–20] Note, however, that the oxidation state of iron in the bulk mineral could be of little concern for *in vivo* effects. It should be borne in mind that: (*i*) because of exposure to (humid) air, iron containing particulates would show Fe(III) ions at their surface regardless of the oxidation state of the metal in the bulk material, and (*ii*) once inhaled, mineral dusts come into contact with reductant chemical species (*e.g.* ascorbate, superoxide ions, *etc*.) capable of converting Fe(III) into Fe(II).

Iron-catalysed free radical generation is known to be an important factor enhancing acute lung inflammation, and it also appears to be a major carcinogenic factor.[1] Hobson *et al.*[21] have shown that $^{\bullet}OH$ radicals enhance asbestos fibre uptake by bronchial epithelial cells, and many pathologists agree that bronchogenic carcinoma (which originates at the epithelial cells of the lung airways) frequently occurs in association with exposure to iron containing asbestos.[22] Similarly, malignant mesotheliomas have often been associated with a history of occupational exposure to amphibole asbestos.[1]

The role of free radicals in asbestos-induced diseases has been recently reviewed.[14,23] Kane[1] has discussed in detail the possible mechanisms of mineral fibre carcinogenesis. Generation of free radicals (mainly $^{\bullet}OH$) constitutes one such mechanism. Free radicals are known to induce DNA damage which, according to Kane,[1] can lead to carcinogenesis by any one of the following routes: (*i*) alterations produced in oncogenes and growth factors, (*ii*) alterations in tumour suppressor genes, and (*iii*) alterations in growth regulatory genes.

The Haber–Weiss cycle is not the only mechanism involved in the generation of free radicals by inhaled mineral dusts. They are also produced as a consequence of macrophage activity, as stated in Section 4.3. Fig. 4 depicts a summarising scheme and a micrograph showing macrophage morphology. It should be noted that administration of antioxidants, such as superoxide dismutase or catalase, was found to result in amelioration of cell injury caused by asbestos in experimental animals.[7] This fact lends further support to the hypothesis linking free radical release with asbestos-induced pathogenesis.

6.4 Adsorption of endogenous chemical species

Inhaled mineral dusts have the potential to adsorb (and concentrate on their surface) endogenous molecules and metal ions which may influence pathogenicity. The extent to which adsorption takes place depends on such factors as specific surface area of the mineral dust, chemical composition, hydrophilicity or hydrophobicity, and zeta potential.

Endogenous iron is one of the metals known to concentrate on the surface of inhaled dusts. This accumulation of iron ions can boost free radical generation and consequent pathogenicity;

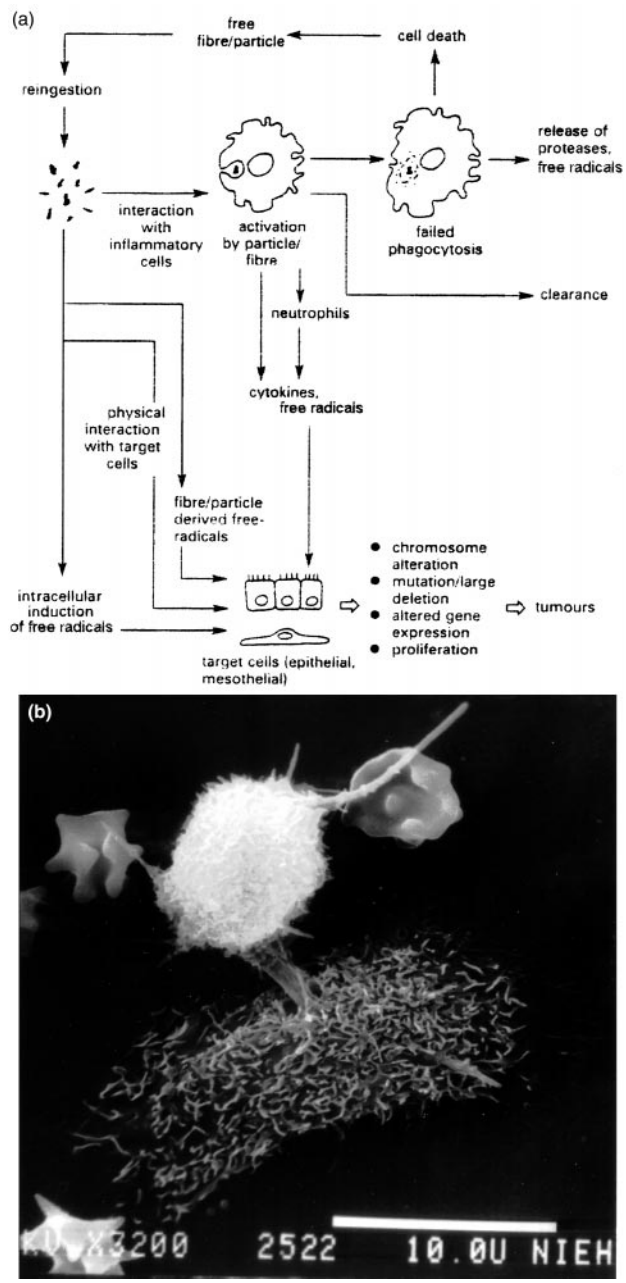

Fig. 4 (a) Scheme of processes relating to phagocytosis and free radical generation by mineral particulates. (b) Macrophage phagocytizing chrysotile asbestos fibres (courtesy of Dr D. B. Warheit).

it can also result in depressed resistance to infection. Thus, Ghio *et al.*[24] suggested that the increased incidence of tuberculosis observed among silicate workers may be explained by accumulation of iron complexed by inhaled dust particles and made available to dormant mycobacteria.

Endogenous iron deposited on inhaled mineral dusts can have several origins; the hemolytic activity of the mineral particles,[15] and the iron store proteins ferritin and hemosiderin are among the possible sources. Ferritin, in particular, was often found to be present in the coating of ferruginous bodies,[9] and it could constitute a local source of iron ions capable of supporting a Haber–Weiss cycle leading to radical damage to DNA and cell organelles.

In a recent *in vitro* study,[25,26] ferritin was found to adsorb strongly on the asbestos fibres amosite and crocidolite. When traces of ascorbic acid were added, these mineral fibres containing adsorbed ferritin were found to cause significant radical damage to DNA. These results are in agreement with the increased DNA damage found for amosite-core asbestos bodies when compared to the effect produced by the naked fibre.[27]

Macromolecules from the pulmonary surfactant can also be adsorbed onto inhaled mineral particles. Among these macromolecules, immunoglobulins are of some concern. They constitute a major component of the lung surfactant (*cf.* Section 2) and can trigger a pathogenic response when adsorbed on mineral particles. *In vitro* cell assays,[28] using alveolar macrophages from guinea pigs, have shown that immunoglobulin G (IgG) is strongly adsorbed on asbestos fibres, conferring to them antigenic determinants which enhance attack by macrophages with release of (potentially dangerous) superoxide anions. As the authors conclude, these *in vitro* studies may have significance for the interaction of asbestos with the alveolar macrophage *in vivo*. The human IgG subclasses have isoelectric points ranging from 7.2 to 8.6, so that they tend to be positively charged at physiological pH. Minerals such as crocidolite which show a negative zeta potential[15] in a physiological buffer are expected to be major adsorbents of IgG. Contrary to the effect produced by IgG adsorption, the pathogenic response to inhaled particles present at the alveoli can be mitigated when phospholipids (from the pulmonary surfactant) become adsorbed onto the mineral particle.[29]

6.5 Adsorption of exogenous substances

Airborne particulates can adsorb vapours from ambient air, some of which, *e.g.* polyaromatic hydrocarbons, are highly toxic. The solid particle may act as a carrier of these compounds into the lung, where they can damage cells and tissues. Equilibria at interfaces regulate some of these processes since the lung surfactant may dissolve adsorbed substances.

The increased risk of lung cancer among smokers exposed to silica and asbestos dust (as compared to non-smokers) has been related to adsorption of polyaromatics (from cigarette smoke) on the mineral particulates.[30] Cigarette smoke was also found to potentiate the damaging action of rockwool on isolated DNA.[31]

Nitric oxide is among the atmospheric contaminants which can be adsorbed onto airborne particulates. Its presence on inhaled mineral dusts can upset metabolic regulation of this biologically significant molecule. It can also result in increased free radical generation.[32]

6.6 Mechanical fracture and grinding

Many environmental dusts originate as a consequence of mechanical fracture and milling of bulk materials. In these cases the final state of the surface depends considerably upon the way the fracture occurred and the composition of the atmosphere during grinding.

Crystalline silica dusts in respirable size are usually generated during processes (mining, drilling, *etc.*) whereby large crystals become fractured. Since mechanical fracture does not usually follow crystal planes, the particles thus generated are very irregular, and show sharp edges and acute spikes. In a dry atmosphere mechanical cleavage of Si–O bonds can give rise to very reactive surface species (Fig. 2). When water vapour is present, surface SiOH species tend to be formed instead of dangling bonds.[14e] In experimental animals freshly ground silicas have shown a higher degree of toxicity than aged ones.[33] The cause of acute silicosis, which can occur among workers involved in sandblasting, drilling or grinding has to be sought in the peculiar properties of freshly cleaved crystals.[14e]

6.7 Synergic effects

Mixed dusts often contain several components which may interact in a synergistic (or sometimes inhibiting way) with each other.[2] Examples of synergic interaction are the enhanced toxicity of freshly fractured quartz containing traces of iron,[33] and that of metallic cobalt mixed with tungsten carbide.[34] In the latter case it was shown that WC/Co composite particles display a much higher cytotoxicity than either component alone, and this fact correlates with *in vitro* physicochemical studies showing that the composite material can generate free radicals in contact with (aerated) water. However, in similar conditions, tungsten carbide generates no free radicals, and cobalt shows little free radical activity.[2,35] Moreover, epidemiological and clinical studies suggest that association of cobalt to tungsten carbide is the determining factor causing lung disease when the composite dust is inhaled. This particular pathology has been termed *hard metal disease*.[34]

Examples of inhibiting effects are the decreased carcinogenicity of quartz dust when it comes into intimate contact with coal dust or with some metals, such as zinc, tungsten or gold.

Finally, it should be mentioned that simple adherence between different airborne particulates can modify the rates at which they are removed from the lung, or translocated into tissues adjacent to the lung alveoli. These effects are particularly relevant when dealing with association between fibrous and non-fibrous particulates.[1]

6.8 Biopersistence

Biopersistence can be defined as the retention in the lung, over time, of inhaled mineral dusts. Factors affecting biopersistence include particle size and shape (which affect the rate of clearance from the lung), chemical composition, surface area and structural parameters (which affect the rate of dissolution). Changes in any of these parameters may alter the toxicity of mineral dusts. Biopersistence is also dependent on the site and rate of deposition; a large increase in the rate of deposition in the alveoli can overwhelm macrophage clearance mechanisms. Regarding the rate of dissolution, the relationship between *in vivo* solubility and chemical composition has been demonstrated[36] for a number of different mineral fibres for which solubility was found to follow the order glass fiber > rockwool > ceramic fibre > chrysotile > amphibole asbestos. Significantly, their toxic potential appears to increase in the same order as decreasing solubility. The concept of biopersistence has gained importance in the last decade; clearly it should be taken into account when considering the toxicity of particulates. However, it is not easy to assess (in humans) the relative biopersistence of different mineral dusts, because of the many processes involved. For more details, the interested reader may consult the recent topical issue of *Environmental Health Perspectives.*[36]

7 Conclusions

It should be clear that toxicity of inhaled mineral dusts stems from a combination of (interrelated) factors which greatly complicates assessment of the role played by each individual process. However, our current knowledge about toxicity of inhaled particulates is being advanced by increasing awareness of the main physical and chemical parameters which determine adverse health effects.

Among physical factors, particle size and shape determine the rate of deposition of airborne particulates, and that of clearance from the lung. Surface roughness may mediate inflammatory processes and, to some extent, chemical behaviour and dissolution rate.

The main chemical factors determining particulate pathogenicity have been reviewed. They can all be related to processes occurring at the interface between the mineral particle and the pulmonary tissue. Therefore, surface chemical composition

(which may differ in several ways from that of the bulk material) and active surface states are the key chemical determinants of biological response.

It should be emphasized that a single chemical (or physical) factor is not likely to be the only pathogenic determinant for any kind of particulate. However, studies (both *in vivo* and *in vitro*) carefully designed to analyse the effect of each individual factor are very valuable, and they represent most of the current research work in the field of chemical toxicity of inhaled mineral dusts. Such studies should pave the path for further research concerning the interplay of the many factors affecting particulate toxicity which have already been identified.

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Review 8/05639K