Surface Reactivity in the Pathogenic Response to Particulates

Bice Fubini
Università di Torino, Facoltà di Farmacia, Dipartimento di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Torino, Italy

The peculiar characteristics of dust toxicity are discussed in relation to the processes taking place at the particle–biological medium interface. Because of surface reactivity, toxicity of solids is not merely predictable from chemical composition and molecular structure, as with water soluble compounds. With particles having the same bulk composition, micromorphology (the thermal and mechanical history of dust and adsorption from the environment) determines the kind and abundance of active surface sites, thus modulating reactivity toward cells and tissues. The quantitative evaluation of doses is discussed in comparisons of dose–response relationships obtained with different materials. Responses related to the surface of the particle are better compared on a per-unit surface than per-unit weight basis. The role of micromorphology, hydrophilicity, and reactive surface cations in determining the pathogenicity of inhaled particles is described with reference to silica and asbestos toxicity. Heating crystalline silica decreases hydrophilicity, with consequent modifications in membranotropic potential, retention, and transport. Transition metal ions exposed at the surface generate free radicals in aqueous suspensions. Continuous redox cycling of iron, with consequent activation–reactivation of the surface sites releasing free radicals, could account for the long-term pathogenicity caused by the inhalation of iron-containing fibers. In various pathogenicities caused by mixed dusts, the contact between components modifies toxicity. Hard metal lung disease is caused by exposure to mixtures of metals and carbides, typically cobalt (Co) and tungsten carbide (WC), but not to single components. Toxicity stems from reactive oxygen species generation in a mechanism involving both Co metal and WC in mutual contact. A relationship between the extent of water adsorption and biopersistence is proposed for vitreous fibers. Modifications of the surface taking place in vivo are described for ferruginous bodies and for the progressive comminution of chrysotile asbestos fibers. — Environ Health Perspect 105(Suppl 9):1013–1020 (1997)

Key words: fibers, particles, asbestos, silica, hard metals, free radicals, iron, chelators, mixed dusts, glass fibers

Introduction

The crucial role played by physicochemical characteristics in the pathogenic response to particulates has been recognized since the early research in dust toxicity. In some pioneering works the biological effects of different forms of the same mineral (mostly silica) or of chemically modified samples were compared and related to the physicochemical properties (1–4). Hemolysis of red blood cells was one of the first effects caused by mineral dusts to be extensively investigated over a large variety of specimens (5,6). Attention was then focused on the crystal faces exposed and on the chemical functionalities present at the surface (7–9). Few extensive reviews on the physicochemical properties of minerals relevant to biological activities appeared in the subsequent years (8–10); meanwhile, research proceeded much more on the biomedical side than on the chemical one. This paper addresses the role of surface reactivity in eliciting a given biological response and reports some of the most recent investigations on dust toxicity in which a relationship was envisaged between chemical properties and toxicity.

Inorganic Particles in Living Matter

When an inhaled particle comes in contact with biomolecules, cells, and tissues, reactions occur at the particle–biological medium interface that are determined by the reactivity of the particle surface. In this respect particle toxicity differs on many points from the toxicity originated by water-soluble toxic agents. Molecular structure determines the toxicity of a soluble compound. In the case of mineral particles, chemical nature, size, shape, surface roughness, exposure of crystal planes, and various surface functionalities all contribute to ultimate toxicity. Table 1 compares the characteristics of soluble and insoluble toxic agents. The mechanisms of action of particles is generally more complex because: a) the particle may interact at various stages with tissues; b) various surface functionalities may be implied; c) the in vivo durability, i.e., biopersistence, is also an important factor in determining overall pathogenicity.

In the early stages of pathogenic response, the function of the surface is mostly related to adsorption phenomena and cell–particle interactions. Adsorption may be either a primary step in toxicity or may inhibit adverse responses by blocking direct interactions between the particle surface and cell membrane.

Adsorption of xenobiotics prior to inhalation may transform the particle into a carrier of carcinogens in the lung, which may act synergistically with the particle itself (11). Polyaromatic hydrocarbons (PAHs) are adsorbed at the asbestos surface to a larger extent than on other solids such as silica or glass fibers (12–14). The surface concentration of PAHs attained is higher because on asbestos the PAH molecule is polarized and adsorbed vertically and not horizontally, as on silica. Multiple layers of vertically packed molecules build up and bind strongly to the surface (14). This may be related to the well-established synergistic effect of tobacco smoking and asbestos exposure on the risk of lung cancer; the relevant mechanism in still under debate (15).
Adsorption of endogenous material takes place in any body fluid and depends on the chemical characteristics of the surface such as charges, polarity, acidic–basic sites, and hydrogen-bonding potential (16).

By the time an inhaled fiber reaches the alveolar region, contact with body fluids has modified the external surface via physical adsorption of macromolecules. Surfactant treatments have been designed to model surface conditioning representative of initial events that occur when a particle deposits in the pulmonary alveolar space (17).

Interaction with cells may occur either with target cells that are directly damaged by the particle or with immune system cells attempting a defense against the foreign body, typically alveolar macrophage(s) (AM) and polymorphonuclear leucocytes. In the first case it is often hypothesized that DNA damage is caused by free radicals generated at the particle surface (11). The interaction with AM involves phagocytosis of the particle. In the lysosome the particle will experience a lower pH and a high concentration of oxidants, which may yield redox/radical reactions with consequent free radical release or chemical modifications at the surface of the particle itself.

Physicochemical Factors Influencing Surface Reactivity

Surface reactivity, hence pathogenicity, is not predictable simply from chemical composition of the bulk (Table 1). A typical example is the variety of biological responses to the simple compound, silicon dioxide (1–3,6–9,18). The various crystalline silica polymorphs exhibit remarkable differences in their pathogenic potential related to differences in crystal structure (1,3,8,19–21). Even specimens of the same silica polymorph, e.g., cristobalite dusts of different origin (22,23) or variously modified dusts (23,24), also elicit different biological effects, some inert and others very pathogenic. The differences in surface reactivity among specimens of minerals with the same chemical composition and crystal structure, which determine their different pathogenic potentials, are modified by physicochemical processes taking place at the surface.

Four major points must be stressed regarding any kind of particle:

- The surface is different from the bulk. Poorly coordinated atoms and ions, more abundant with finely divided materials, are present at the surface and constitute local centers of more pronounced reactivity (25,26). The surface may be enriched in some components with respect to the bulk. Following contact with atmospheric oxygen, transition metal ions exposed may be oxidized. Surface hydration and hydroxylation occurs in a moist atmosphere, with kinetics dependent on several factors, including crystallinity, fractal aspect of the surface, and thermal history of the sample.
- Fresh surfaces are different from aged ones. Freshly ground silica exhibit a peculiar reactivity, originated by dangling bonds and surface charges arising from the cleavage of the silicon–oxygen bonds (27), which has been related to acute damage (18,28). Freshly ground silica was recently reported to be more toxic than aged silica in vivo (29).
- Grinding and milling affect both form and surface composition of fibers. Crystalline fibers such as amphiboles retain a fibrous form even after comminution, whereas amorphous filaments, e.g., glass fibers, are progressively transformed into equant particles. In both cases, newly cleaved surfaces are brought in contact with air and react, sometimes very slowly, with atmospheric components. Asbestos is activated by mild grinding, probably because ferrous iron is exposed; prolonged grinding deeply modifies and inactivates chrysotile asbestos (30). A remarkable increase upon grinding in reactive oxygen species (ROS) release and in the formation of 8-hydroxydeoxyguanosine from deoxyguanosine was reported for various asbestos fibers (31).
- Chemical treatments may affect the surface without modifying the bulk of the particle. Acid washing eliminates contaminant surface ions with consequent modifications in the biological activity (32). Etching with hydrofluoric acid eliminates external amorphous layers in silicas, which inhibits or delays the fibrogenic response (2,18).
- Thermal treatments modify the state of the surface often without any modification in bulk structure. The reactivity of a silica surface depends greatly on dissociated and undissociated silanols (6–9). Silicas and some silicates are progressively rendered more hydrophobic by heating as a consequence of the transformation of silanols into siloxanes (22,33). Correspondingly, the memranolysis potential decreases (33,34). The reversible adsorption of water, reported in Figure 1 for cristobalite heated at different temperatures, measures the extent of silanols patches, i.e., the extent of hydrophilic sites (18). It is noteworthy that when reported as a function of the temperature of the thermal pretreatment (Figure 1), hydrophilicity paralleled memranolysis. The silica content of macrophages from bronchoalveolar lavage was much higher in the case of heated than in unheated cristobalite. This may be related to a decreased toxicity to macrophages, with consequent delay in macrophage death as a consequence of reduced membrane

### Table 1. Comparison between particulates and soluble toxic compounds.

| Water-soluble toxic agent | Toxic particulate |
|--------------------------|------------------|
| One well-defined target cell or molecule | Several particle–biological matter interactions contribute to the overall pathogenicity |
| The dose is easy to quantify for dose–effect relationships (per unit concentration in body fluids) | Quantification of the dose is not straightforward (per unit weight, per unit surface, per number of particles) |
| One mechanism of action at the molecular level | Several surface sites with different chemical properties may be involved |
| The molecular structure of the substance determines the reactivity | The reactivity is not fully predictable from chemical composition |
| The effect exists until the molecule is metabolized or excreted | The effect persists for long periods of time; the particle may be located in different compartments |

![Figure 1. Membraneolysis (hemolysis, % empty symbol) and adsorption of water vapor (number of moles adsorbed under H2O vapor pressure of 5 torr, full symbol) on cristobalite mineral dust preheated at different temperatures. Data replotted from Hemenway et al. (34).](image)
damage (34). When heated samples are cooled down and exposed to the atmosphere, only a part of the water lost in thermal treatment is adsorbed again by silica-based particles (25,33). The kinetics of this phenomenon may be very slow—from days to years—depending on chemical composition, impurities, and temperature attained during thermal treatment. As this temperature rises, further rehydration slows.

Any correct comparison between biologic responses to samples of the same material requires samples with the same chemical and mechanical and thermal history.

How Surface Properties Modulate the Fate of the Inhaled Particle

Once inhaled, the particle will be translocated into various compartments (alveoli, interstitium, mediastinal lymph nodes), internalized by cells, and finally may be partially cleared from the lung. Damage to DNA and lipid peroxidation found in in vitro tests may take place with the target cells contacted by the fiber or particle within these compartments. Table 2 illustrates possible relationships between some surface properties and the above processes, suggested by results from in vivo or in vitro tests.

### Surface Properties Relevant to Pathogenicity

#### Form, Micromorphology, and Surface Area

The form of a particle is of paramount importance in pathogenicity. In many cases, fibrous materials have been reported as more active than their nonfibrous counterparts (36).

With equant particles, the biological response is often related to the extent of exposed surface (37). Some particles are smooth at the atomic level so that their geometric surface may be calculated from the size distribution and approaches the true one. With indented particles evaluation of the true exposed surface may be performed only by means of physical adsorption of gases (Brunauer Emmet Teller [BET] method). When comparing the effects of various particles, the question of measurement arises. How should exposure in vivo or doses in vitro be expressed: by mass (current method), number of particles, number of sized particles, or unit surface area? The choice depends on the biological process investigated. If small molecules act as mediators of biochemical reactions, the true surface area is the most appropriate one. If cellular events such as the internalization of particles are involved, the number of sized particles should be considered. However, the mass is the most inappropriate measurement to use, particularly if comparing effects of materials with different specific weights or different surface per unit mass. Table 3 suggests the most appropriate parameters to adopt for dose evaluation by following the biological response investigated.

In fibers, expression is more complicated, as length, diameter, and aspect ratio also must be considered (11).

The fibrous habit has always been considered a physical property of the material unrelated to chemical ones. Recently, however, some authors have reported different surface reactivity among fibers of the same composition but different sizes (17,38,39), which would suggest a possible chemical fiber effect, based mainly on different surface composition and/or adsorptive capacity of long versus short fibers.

### Surface Hydrophobicity and Hydrophilicity

The degree of hydrophobicity of the surface regulates cell surface adhesion, protein denaturation at the interface, and selective adsorption of components from the liquid phase (16). A variation in hydrophilicity of the surface may involve a different translocation route in various biological compartments (Table 2), different coatings by endogenous materials, or different locations in close proximity to cells (16).

Heat treatment on crystalline silicas (cristobalite and quartz), which reduces the extent of hydrophilic sites (Figure 1), results in increased initial dust accumulation, increased recruitment of alveolar inflammatory cells, reduced short-term clearance, and enhanced long-term clearance. Heated quartz demonstrated a dramatic increase in particle retention in the lungs and in the rate of silica accumulation in the thymus and associated mediastinal lymph nodes, suggesting a relationship between the extent of hydrophobicity caused by thermal treatment and retention/transport in various biologic compartments (40).

Cristobalite fully hydrophobized by thermal treatment was inert both in vitro and in vivo (22,23). Chemically hydrophobized silica surfaces are also less pathogenic (41). This suggests that the pathogenic process is modulated by the extent of surface hydrophobicity, even if other surface functionalities are directly responsible for fibrogenic response to crystalline silicas (42).

### Transition Metals and Free Radical Release

A large set of data from in vivo and in vitro tests indicates that antioxidants, ROS scavengers, and strong iron chelators ameliorate or inhibit the biological response to asbestos, which supports the hypothesis that iron-derived ROS would mediate genetic damage and play some role in asbestos toxicity (11,43–45). This hypothesis, still controversial, leaves unexplained the pathogenicity of iron-free fibers such as tremolite or ceramic fibers. However, it must be pointed out that a few traces of iron are sufficient to catalyze free radical
generation; some iron may be deposited in vivo and originate catalytically active sites. ROS may be of endogenous origin, i.e., released following internalization or exogenous origin—the fiber itself generates ROS in biological fluids. There is evidence for both mechanisms occurring with some fibers, which implies radical release at the fiber surface ubiquitously, or, conversely, complex radical reactions during internalization between radicals of inorganic and biochemical origin (43). In both cases, the radical concentration attained may impair body defenses, and consequently, radicals may reach target cells.

The mechanism whereby a fiber promotes or catalyzes the abnormal release of radicals is still under debate. A crucial role in this reaction is played by the location of iron at the asbestos surface (43–45). Glass-, slag-, and rockwools also release free radicals in aqueous suspensions provided that iron is present in the fiber composition. (46–48).

The transforming potency on Syrian hamster embryo cells of various iron-containing solids was decreased by adding desferrioxamine, inferring that transforming potency is indeed related to iron (49). Not all iron was active, in agreement with a large number of cell-free tests indicating that only a small fraction of total iron present is capable of generating ROS (31,43,50).

Various hypotheses exist on the chemical nature of iron sites active at the surface and on their modifications in vivo following radical release (31,43–45,50–52). Reactive iron may be either at the solid surface linked in the crystalline matrix (31,38,50–52) or brought in solution by endogenous chelators (44,53). Single-strand DNA damage correlates with iron that may be mobilized from asbestos by low molecular weight chelators, which suggests a direct relationship between free radical generation and mobilized iron (44,53). However, a prolonged or catalytic mechanism is required to explain long-term pathogenicity, which may be sustained by an interplay between both surface and mobilized iron. Several recent papers indicate that iron complexed at the solid–liquid interface becomes very active (54–56).

Adachi et al. (55) prepared a deionized crocidolite by washing the original fibers with HCl and EDTA, which was more potent in oxidizing DNA in vitro and in the induction of mesotheliomas in vivo. This is likely due to the fact that redox potential of iron, and related potential for free radical release, is modified and often enhanced following chelation. All of the above data are consistent with a model where the active iron is a well-dispersed iron, loosely fixed at the surface, and partly coordinated by various ligands.

The iron involved in the reaction—originating ROS may be in the chemical composition of the fiber (amphibole asbestos) or present as an impurity (chrysotile and glass fibers) (31,48–52). There is evidence that endogenous iron is deposited on the fiber surface from macrophages or other cells (57) that might also become active. This latter hypothesis might explain the toxicity of fibers that do not contain any iron, e.g., ceramic fibers, but are carcinogenic in experimental animals.

Free radicals have been found in cell-free assays by different techniques, including direct spin trapping, oxygen consumption, lipid peroxidation, and DNA damage (43). Free radical release is not necessarily a catalytic reaction. The term "catalyzes" is often misused in place of the term "generates" in cases where no evidence was reported that free radical release was not just an outburst but a prolonged reaction. When the solid surface merely acts as a Fenton reagent:

\[ \text{particle} + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \cdot\text{OH}^- \]

the reaction is indeed catalytic (52). Catalysis would then be confined to the phagolysosomal medium, where hydrogen peroxide is released following phagocytosis of the particle. In vivo, however, the situation may be different, as in some cases the surface active sites may be regenerated through redox cycles (43,50).

**Interaction Between Components in Mixed Dusts**

In mixed dusts each component may undergo surface contamination to some extent from the other components, which may inhibit or enhance toxicity.

**Inhibition of Silica Activity by Clays**

The total amount of crystalline silica in coal mine dust does not correlate with pathogenicity. Several explanations have been proposed, including rank effect (relating toxicity to the geological strata of the coal deposit) and the role of clays in inhibiting the activity of quartz particles when in close contact with or actually covering the particle. Cytotoxicity of these dusts relates better to free (uncovered) quartz than to total quartz content (58).

Submicroscopic aluminosilicate coatings, recently evidenced on quartz, could also explain the rank effect in coal workers' pneumoconiosis (59).

**Hard Metal Lung Disease:**

**Activation of the Metal at the Carbide Surface**

In recent years, clinical, epidemiological, and experimental evidence has accumulated indicating that Co metal particles, when inhaled in association with other agents such as metallic carbides (hard metals) or diamond dust, may produce an interstitial lung disease termed hard metal lung disease (60–62). In vivo and in vitro tests indicate that the pure components, namely Co metallic particles and pure WC, are inert. The pathogenic response is also different from that elicited by Co salts (61,62). Toxicity originates only when contact occurs with mixed dusts. Cell-free assays reveal that mixed dusts, not the pure components, release ROS in a buffered aqueous suspension, while Co is progressively solubilized (63). Co was not toxic per se, as Co soluble salts in contact with carbide particles are also inert. All data are consistent with the model illustrated in Figure 2, where atmospheric oxygen is activated at the WC surface by electron migration from Co to the carbide. In this case it is the mere contact between the particles, i.e., the solid–solid interface, which causes the reactivity related to the pathogenic response (63).

**State of the Surface and Biopersistence**

The biodurability of a fiber or particle, one of the parameters relevant to biopersistence, depends mainly on dissolution and leaching in vivo (37). Chemical composition determines the solubility in equilibrium.
conditions, but the rate of dissolution also depends on extension and state of the surface. Selective leaching of ions exposed at the surface, removed by water molecules or endogenous chelators, facilitates penetration of solvent molecules in the solid, which allows solubility.

We compared the adsorptive capacities of water vapor molecules of some artificial fibers with those of pure vitreous silica. Figure 3 illustrates the amount of water adsorbed as a function of the equilibrium vapor pressure (adsorption isotherms) for man-made vitreous fiber (MMVF10), MMVF11, and MMVF21, and a ground pure silica glass (Suprasil). All glass fibers adsorb more water than pure vitreous silica under the same water vapor pressure because of coordination of water molecules with the exposed metal ions that are present in glass but not in silica. Their affinity for water, on a per-unit surface basis, is different, however, decreasing in the series

\[
\text{MMVF11} > \text{MMVF10} > \text{MMVF21} > \text{vitreous silica.}
\]

It is noteworthy that these fibers ranked in the same order when compared for biopersistence in vivo (64). If these findings could be validated on a much larger number of fibers, the number of water vapor molecules adsorbed by a given material might somehow predict, in a quick and simple way, the biopersistence of particles or fibers close in size and shape.

**Modification of the Surface in Vivo**

The surface is partly modified in vivo even in the most biopersistent particles such as amphibole asbestos or crystalline silica, whose form sometimes appears unchanged even after retention for decades in the body. Some fibers break apart; others, such as chrysotile, split into fibrils and are progressively leached. In addition to the obvious adsorption of biomolecules from extracellular fluids and cytoplasm, some ions will be selectively deposited on the particle and others extracted by endogenous chelators.

Iron chelators mobilize considerable amounts of iron from some asbestos, which modifies the surface and subsurface layers (44,50,53,65–67). Solubility in biological fluids containing molecules or anions that may chelate metal ions e.g., oxalate, phosphate, cysteine is much different from that in pure aqueous solution (66,67).

Scanning electron microscopy–energy dispersive spectroscopy investigations in our laboratory on ex-in vivo particles revealed that ion deposition is specific for each solid examined (unpublished results).

Major modifications take place in two cases: the formation of ferruginous bodies on asbestos (and few other kinds of fibers), and the progressive desegregation of chrysotile asbestos fibers.

Ferruginous bodies are the final product of a type of biomineralization that takes place on the surface of inhaled mineral fibers; endogenous iron deposits around the fiber, forming a thick, segmented coating of iron oxyhydroxides mixed with organic material (68–70). Long straight fibers are preferentially coated over short curled ones, but the percentage of coated fibers also depends on asbestos fiber type. In a recent Japanese study, a high number of asbestos bodies was found in a rather large cohort of patients. The number of coated fiber varied from 5% for chrysotile to 27% for amosite (71). This suggests that formation of asbestos bodies is related to both form and surface chemistry of the inhaled fiber. The biological significance of this process is still obscure (70). It is assumed to be a body defense mechanism attempting to isolate the fiber from direct contact with the lung. However, under some circumstances, the iron contained in the bodies may become redox active and trigger a series of deleterious reactions; amosite-coated asbestos bodies were responsible for the formation of single-strand breaks in DNA to a larger extent than uncoated fibers of similar size (72). If deposited iron is, or becomes, redox active, cycles of iron extraction and redeposition at the fiber surface may provide continuous activation of the surface, releasing free radicals via the above mechanisms, which may account for long-term pathogenicity (43,50).

As opposed to amphiboles, which persist within the body virtually unchanged in their crystal structure, chrysotile is progressively split into fibrils and is eventually cleared from the lung, which makes the assessment of exposure to chrysotile sometimes difficult. This is due to its typical serpentinite (layered silicate) structure, which is schematized in Figure 4. Chrysotile is a layered silicate; one [SiO₄]₄⁻ layer alternates with a [Mg(OH)₂] (brucitic) one. Because the latter is larger, the sheets curl up, assuming the familiar fibrous characteristics. When leached with oxalic acid solutions, which mimics what may happen in vivo over long periods of time, the brucitic layers are solubilized, which leaves, in extreme conditions, just the silica framework. Leaching of chrysotile also occurs in cell cultures. It is more pronounced with AM than with pleural mesothelial cells, most likely because of differences in pH within the cells (73).

Surface area is increased by the leaching process, both because of the formation of empty cavities within the solid, and the splitting of bundles of fibers into fibrils (Figure 4). The adsorptive capacity of different kinds of small molecules e.g., water, ammonia, tert-butanol, even when measured per unit surface area, is much higher on leached than on unleached fibers. Figure 5 illustrates the adsorption of ammonia, measured on a per-unit surface basis, which indicates the presence of acidic surface sites. The above data indicate that in addition to the increase in specific surface, leaching imparts a different chemical reactivity to the surface. On leached fibers, strong adsorption of proteinaceous material may take place, which eventually would protect the body from direct contact with the fiber. However, adsorption of PAHs is weaker with leached than with the original chrysotile, which suggests that solids with a basic character (asbestos, magnesia) have more affinity for PAHs than those with an acidic one (silica, alumina) (14).

As a consequence of surface differences, some biological responses to chrysotile fibers are also modified upon leaching: more cytoplasmic enzymes but fewer lysosomal enzymes were released with leached fibers than with unleached ones (74). The inactivation of human leukocyte elastase was greater on the leached fibers, probably in connection with their greater surface acidity (75), as was shown by its higher affinity for ammonia (Figure 5).
Surface Chemistry in Future Work

The molecular mechanisms by which particulates elicit a pathogenic response are still partly obscure even for well-established carcinogenic materials such as asbestos (11) or crystalline silica, which is one of the most fibrogenic dusts (7,18,76) and has been recently classified as carcinogenic to humans by the International Agency for Research on Cancer (42). This is partly because research in this field has proceeded in the following sequence, beginning with evidence of the association between exposure and pathogenicity of a given material: epidemiology $\rightarrow$ in vivo tests $\rightarrow$ in vitro tests $\rightarrow$ biochemical mechanisms $\rightarrow$ chemical roles played by the solid in the overall process.

An understanding of the last point for toxic particles and fibers whose toxicity is well established could be of great help in designing safer new materials to be used as substitutes for pathogenic ones. The above sequence could be reversed in the following way:

mineralogy (identification of properties of pathogenic minerals) $\rightarrow$ model solids (each bearing one of the identified properties) $\rightarrow$ in vitro tests (association between surface properties and cellular responses) $\rightarrow$ in vivo tests (validation of toxicity related to a given surface property)

Under these circumstances the knowledge of pathogenic minerals accumulated so far could be utilized to produce safer new materials and cell-free and in vitro tests could be used to prescreen new fibers and particles on the market and in the environment.

REFERENCES

1. King EJ, Mohanry GP, Harrison CV, Nagelschmidt G. The action of different forms of pure silica on the lungs of rats. Br J Ind Med 10:9-17 (1953).
2. Engelbrecht FM, Yoganathan M, King EJ, Nagelschmidt G. Fibrosis and collagen in rat's lung produced by etched and unetched free silica dusts. Arch Ind Health 17:287-294 (1958).
3. Nash T, Allison AC, Harington JS. Physico-chemical properties of silica in relation to its toxicity. Nature 210:259-261 (1966).
4. Harington JS, Allison AC, Badami DV. Mineral fibres chemical, physicochemical and biological properties. Adv Pharmacol Chemother 12:291-402 (1975).
5. Light WG, Wei ET. Surface charge and asbestos toxicity. Nature 26:537-539 (1977).
6. Nolan RP, Langer AM, Harington JS, Oster G, Selikoff IJ. Quartz haemolysis as related to its surface functionalities. Environ Res 1981;26:503–520.

7. Langer AM. Crystal faces and cleavage planes in quartz as templates in biological processes. Q Rev Biophys 1978;11:534–575.

8. Langer AM, Nolan RP. Physicochemical properties of minerals relevant to biological activities: state of the art. In: In Vitro Effects of Mineral Dusts. North Atlantic Treaty Organization Advanced Study Institute Ser C, Vol 3 (Beck DC, Bignon J, eds). Berlin:Springer-Verlag, 1985:9–24.

9. Langer AM, Nolan RP. Physicochemical properties of quartz controlling biological activity. In: Silica, Silicosis and Cancer (Goldsmith DF, Winn DM, Shy CM, eds). New York:Plaeger Publisher, 1986:125–135.

10. Jolicoeur C, Poisson D. Surface physico-chemical studies of chrysotile asbestos and related minerals. In: Asbestos Toxicity (Fisher GL, Gallo MA, eds). New York:Marcel Dekker, 1988:1–48.

11. Kane AB, Boffetta P, Saracci R, Willbourn JD, Mechanisms of Fibre Carcinogenesis. IARC Sci Publ No 140. Lyon:International Agency for Research on Cancer, 1996.

12. Fournier J, Pezetar H. Studies on surface properties of asbestos. III: Interaction between asbestos and polynuclear aromatic hydrocarbons. Environ Res 1986;41:276–295.

13. Gerde P, Scholander P. Adsorption of benzo[a]pyrene onto asbestos and man-made mineral fibres in an aqueous solution and in a biological model solution. Br J Ind Med 45:682–688.

14. Fournier J, Fubini B, Bolis V, Pezetar H. Thermodynamic aspects in the adsorption of polynuclear aromatic hydrocarbons on chrysotile and silica—possible relation to synergistic effects in lung toxicity. Can J Chem 67:289–296.

15. Davis JM. Mixed fibrous and non-fibrous dust exposures and interactions between agents in fibre carcinogenesis. In: Mechanisms of Fibre Carcinogenesis (Kane AB, Boffetta P, Saracci R, Willbourn JD, eds). IARC Sci Publ No 140. Lyon:International Agency for Research on Cancer, 1996:127–135.

16. Van Oss CJ. Interfacial Forces in Aqueous Media. New York:Marcel Dekker, 1994:308–332.

17. Lu L, Keane MJ, Ong T, Wallace WE. In vitro genotoxicity studies of chrysotile asbestos fibers dispersed in simulated pulmonary surfactant. Mutat Res 320:253–259.

18. Fubini B. Health effect of silica. In: The Surface Properties of Silicas (Legrand JP, ed). Chichester:Wiley and Sons (in press).

19. Wissner JW, Henderson JD Jr, Sohline PG, Mandel NS, Mandel GS. The effect of crystal structure on mouse lung inflammation and fibrosis. Am Rev Respir Dis 138:445–450.

20. Hemenway DR, Absher MP, Trombley L, Vacek P. Comparative clearance of quartz and cristobalite from the lung. Am Ind Hyg Assoc J 51:363–369.

21. Cerrato G, Fubini B, Barico M, Morterra C. Spectroscopic, structural and microcalorimetric study of Steinhovite, a non-pathogenic polymeric of SiO$_2$. J Mater Chem 5:1935–1941.

22. Fubini B, Bolis V, Cavenago A, Volante M. Physico-chemical properties of crystalline silica dusts and their possible implications in various biological responses. Scand J Work Environ Health 21(Suppl 2):9–14.

23. Elias Z, Poiriot MC, Daniere F, Terzetti F, Marande AM, Dwiguig S, Pezetar H, Fubini B. Unpublished data.

24. Daniel LN, Mao Y, Wang TL, Markay CJ Markay SP, Shi X, Saffiotti U. DNA strand breakage, thymine glycol production and hydroxyl radical generation induced by different samples of crystalline silica in vitro. Environ Res 71:60–73.

25. Fubini B. The possible role of surface chemistry in the toxicity of inhalated fibers. In: Fiber Toxicology, Vol 11 (Warheit DB, ed). San Diego CA:Academic Press, 1993:229–257.

26. Hochella MF. Surface chemistry, structure and reactivity of hazardous mineral dusts. In: Reviews in Mineralogy, Vol 28 (Guthrie GD, Mossman BT, eds). Chelsea, MI:Book Crafters, 1993:275–308.

27. Fubini B, Gamello E, Volante M, Bolis V. Chemical functionalities at the silica surface determining its reactivity when inhaled. Formation and reactivity of surface radicals. Toxicol Ind Health 6:571–594.

28. Castranova V, Dalal NS, Vallyathan V. Role of surface free radicals in the pathogenicity of silica. In: Silica and Silica-Induced Lung Diseases (Castranova V, Vallyathan V, Wallace WE, eds). Boca Raton, FL:CRC Press, 1996.

29. Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsay DM, Pretty JR, McLaurin JL et al. freshly fractured quartz inhalation leads to enhanced lung injury and inflammation in rats. Am J Respir Crit Care Med 152:1003–1009.

30. Langer AM, Wolf MS, Rohlf AN, Sedlikoff J. Variation of properties of chrysotile asbestos subjected to milling. J Toxicol Environ Health 4:173–188.

31. Nejari A, Fournier J, Pezetar H, Leanderson P. Mineral fibres: correlation between the oxidizing surface activity and the DNA base hydroxylation. Br J Ind Med 56:501–504.

32. Miles PR, Bowman L, Jones WG, Berry DS, Vallyathan V. Changes in alveolar lavage materials and lung immunological xenobiotic metabolism following exposures to HCl-washed or unwashed crystalline silica. Toxicol Appl Pharmacol 126:235–242.

33. Pandurangi RS, Seera MS, Razzaboni BL, Bolsaitis P. Surface and bulk infrared modes of crystalline and amorphous silica particles: a study on the relation of surface structure to cytotoxicity of respirable silica. Environ Health Perspect 86:327–336.

34. Hemenway DH, Absher MP, Fubini B, Bolis V. What is the relationship between hemolytic potential and fibrogenicity of mineral dusts? Arch Environ Health 48:343–347.

35. Hobson J, Wright JL, Churg A. Oxygen species mediate asbestos fibre uptake by tracheal epithelial cells. Fed Am Soc Exp Biol J 4:3134–3139.

36. Sesko A, Cabot M, Mossman BT. Hydrolysis of inositol phospholipids precedes cellular proliferation in asbestos-stimulated H tracheobronchial epithelial cells. Proc Natl Acad Sci USA 87:7385–7389.

37. Oberdoster G, Ferrin J, Lehnert BE. Correlation between particle size, in vivo particle persistence and lung injury. Environ Health Perspect 102(Suppl 5):173–179.

38. Gilmour PS, Beswick PH, Brown DM, Donaldson K. Detection of surface free radical activity of respirable industrial fibres using supercooled phi X174RFI plasmid DNA. Carcinogenesis 16:2973–2979.

39. Hill IM, Beswick PH, Donaldson K. Differential release of superoxide anions by crystalline fibres treated with long and short fibre amosite asbestos is a consequence of differential affinity for opsonin. Occup Environ Med 52(2):92–96.

40. Hemenway DR, Absher MP, Fubini B, Trombley L, Vacek P, Volante M, Cavenago A. Surface functionalities are related to biological response and transport of crystalline silica. Inhalated Particles VII, Ann Occup Hyg 38:447–454.

41. Wissner JW, Mandel NS, Sohline PG, Hasegawa A, Mandel GS. The effect of chemical modification of quartz surfaces on particulate-induced pulmonary inflammation and fibrosis in the mouse. Am Rev Respir Dis 141:1111–1116.

42. IARC. Silica, some silicates, coal dust and para-aramid fibres. In: Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 68. Lyon:International Agency for Research on Cancer, 1997:210–211.

43. Fubini B. Physico-chemical and cell free assays to evaluate the potential carcinogenicity of fibres. In: Mechanisms of Fibre Carcinogenesis. IARC Sci Publ No 140 (Kane AB, Boffetta P, Saracci R, Willbourn JD, eds). Lyon:International Agency for Research on Cancer, 1997.

44. Hardy JA, Aust AE. Iron in asbestos chemistry and carcinogenicity. Chem Rev 95:97–118.

45. Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 12:293–315.
46. Gulumian M, Van Wyk JA. Hydroxyl radical production in the presence of fibres by a Fenton-type reaction. Chem Biol Interact 62:89–97 (1987).
47. Leander P, Söderkvist P, Tagesson C, Axelson O. Formation of 8-hydroxydeoxyguanosine by asbestos and man-made mineral fibres. Brit J Ind Med 45:309–311 (1988).
48. Pézerat H, Guignard J and Cherrie JW. Man-made mineral fibers and lung cancer: an hypothesis. J Toxicol Ind Health 8:77–87 (1992).
49. Elias Z, Poizot O, Schneider O, Marande RA, Danıère MC, Terzetti F, Pézerat H, Fournier J, Zalma R. Cytotoxic and transforming effects of some iron-containing minerals in Syrian hamster embryo cells. Cancer Detect Prev 19:405–414 (1995).
50. Fabini B, Mollo L. Role of iron in the reactivity of mineral fibers. Toxicol Lett 82/83:951–960 (1995).
51. Pézerat H, Zalma R, Guignard J, Jaurand MC. Production of oxygen radicals by the reduction of oxygen arising from the surface activity of mineral fibers. In: Non-occupational Exposure to Mineral Fibres (Bignon J, Petó J, Saracci R, eds). IARC Sci Publ No 90. Lyon: International Agency for Research on Cancer, 1989;100–110.
52. Fabini B, Mollo L, Giamello E. Free radical generation at the solid/liquid interface in iron containing minerals. Free Radiuc Res 23:593–614 (1995).
53. Lund LG, Aust AE. Iron mobilization from crocidolite asbestos greatly enhances crocidolite-dependent formation of DNA single-strand breaks in phi X174 RFI DNA. Carcinogenesis 13:637–642 (1992).
54. Giulanelli C, Baeza-Squiban A, Boisvieux-Ulrich E, Houcine O, Zalma R, Guennou C, Pézerat H, Marano F. Effect of mineral particles containing iron on primary cultures of rabbit tracheal epithelial cells: possible implication of oxidative stress. Environ Health Perspect 101:436–442 (1993).
55. Adachi S, Yoshida S, Kawamura K, Takahashi M, Uchida H, Odagiri Y, Takemoto K. Induction of oxidative DNA damage and mesothelioma by crocidolite, with special reference to the presence of iron inside and outside of asbestos fiber. Carcinogenesis 15:753–758 (1994).
56. Ghio A, Kennedy TP, Stoneburner G, Crumbliss AL, Hoidal JR. DNA strand breaks following in vitro exposure to asbestos increase with surface-complexed [Fe]1++. Arch Biochem Biophys 311:13–18 (1994).
57. Sebastien P. Biopersistence of man-made vitreous silicate fibers in the human lung. Environ Health Perspect 102(Suppl 5):225–228 (1994).
58. Tourmann JL, Kaufmann RK. Laser microprobe mass spectrometric analysis (LAMMS) of quartz-related and non-quartz related factors of the specific harmfulness of coal mine dusts. Ann Occup Hyg 38(Suppl 1):455–467 (1994).
59. Wallace WE, Harrison JC, Grayson RL, Keane MJ, Bolsaitis P, Kennedy RD, Wearden AQ, Atfield MD. Aluminosilicate surface contamination of respirable quartz particles from coal mine dusts and from clay works dust. Ann Occup Hyg 38:439–445 (1994).
60. Lison D, Lauwereys R. The interaction of cobalt metal particles with different carbides on mouse peritoneal macrophages. Toxicol In Vitro 9:341–347 (1995).
61. Lison D. Human toxicity of cobalt-containing dust and experimental studies on the mechanism of interstitial lung disease (Hard Metal Disease). Crit Rev Toxicol 26:585–616 (1996).
62. Lison D, Lauwereys R, Demedts M, Nemery B. Experimental research into the pathogenesis of coal/iron lung disease. Eur Respir J 9:1024–1028 (1996).
63. Lison D, Carbonnelle P, Mollo L, Lauwereys R, Fabini B. Physicochemical mechanism of the interaction between cobalt metal and carbide particles to generate toxic activated oxygen species. Chem Res Toxicol 8:600–606 (1995).
64. Musselman RP, Miller WC, Eastes W, Hadley JG, Kamstrup O, Thevenaz P, Hersterberg T. Biopersistencies of man-made vitreous fibers and crocidolite fibers in rats lungs following short-term exposures. Environ Health Perspect 102(Suppl 5):139–143 (1994).
65. Mollo L, Merlo E, Giamello E, Volante M, Bolis V, Fabini B. Effect of chelators on the surface properties of asbestos. In: Cellular and Molecular Effects of Mineral and Synthetic Dusts and Fibres (Davis JM, Jaurand MC, eds). North Atlantic Treaty Organization Advanced Study Institute Series Vol H 85. Berlin:Springer-Verlag, 1994:425–432.
66. Werner AJ, Hochella MF, Guthrie GD, Hardy JA, Aust AE, Rimstidt JD. Asbestosform riebeckite (crocidolite) dissolution in the presence of Fe chelators: implications for mineral-induced disease. Am Mineralogist 80:1093–1103 (1995).
67. Gold J, Amanusson H, Krozer A, Kasemo B, Ericsson T, Zanetti G, Fabini B. Chemical characterization and reactivity. Environ Health Perspect 105(Suppl 5):1021–1030 (1997).
68. Governa M, Rosanda CA. A histochemical study of the asbestos body coating. Br J Ind Med 29:154–159 (1972).
69. Churg AM, Warnock ML. Asbestos and other ferruginous bodies, their formation and clinical significance. Am J Pathol 102:447–456 (1981).
70. Morgan A, Holmes A. The enigmatic asbestos body: its formation and significance in asbestosis-related disease. Environ Res 38:283–292 (1985).
71. Murai Y, Kitagawa M, Hiraoka T. Asbestos bodies formation in the human lung: distinctions, by type and size. Arch Environ Health 50(1):19–25 (1995).
72. Lund LG, Williams MG, Dodson RF, Aust AE. Iron associated with asbestos bodies is responsible for the formation of single strand breaks in phi X174 RFI DNA. Occup Environ Med 51:200–204 (1994).
73. Jaurand MC, Gaudichet A, Halpern S, Bignon J. In vitro biodegradation of chrysotile fibres by alveolar macrophages and mesothelial cells in culture: comparison with a pH effect. Brit J Ind Med 41:389–395 (1984).
74. Jaurand MC, Magne L, Boulier JL, Bignon J. In vitro reactivity of alveolar macrophages and red blood cells with asbestos fibres treated with oxalic acid, sulfur dioxide and benzo-3,4-pyrene. Toxicology 21:323–342 (1981).
75. Fabini B, Mollo L, Bodoardo S, Onida B, Oberson D, Lafuma C. Evaluation of the surface acidity of some phillipsilicates retatable to their inactivating activity towards the enzyme human leucocyte elastase. Langmuir 13:919–927 (1997).
76. Castranova V, Valleyathan V, Wallace WE, eds. Silica and Silica-induced Lung Diseases. Boca Raton, FL:CRCP Press, 1996.