



Safety In Mines Research Advisory Committee

Facilitating safety and health research in the South African mining industry

Investigation of Crystalline Phases in Silica Fume

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Executive Summary

Silicosis is a debilitating lung disease, believed to be caused by the inhalation of crystalline silica. Despite intervention in exposure of workers over many decades, silicosis is still recognised internationally as a major occupational disease of special concern. Because of the ongoing health problems associated with silicosis, it appears that simply measuring airborne levels of crystalline silica using the standard occupational hygiene techniques may not in all situations provide the information necessary to assess and manage silicosis risks in industries. One of the issues that have been controversial for many years relates to the possible link between amorphous silica and silicosis.

Silica fume, generally described as amorphous silica, is likely to be associated with the development of fibrotic effects in exposed individuals. If microcrystalline phases were present in thermally generated silica fume, this might lead to the development of silicosis. Microcrystalline phases are however difficult to detect with routine X-ray diffraction in the ultrafine matrix. It may therefore be possible that silica fume that appears to be amorphous in occupational hygiene surveys, might have the potential to cause silicosis because it contains a small proportion of crystalline silica. In order to further clarify this issue, this SIMRAC study was initiated to confirm whether crystalline phases could in fact be present in silica fume and if so, at what levels. Furthermore, the role of the ultrafine nature of silica fume in its overall toxicity was considered to require better clarification.

Samples of airborne silica fume were collected on filters at various locations in a typical silicon smelter plant. Collected particles were transferred to carbon grids and used for transmission electron microscopic (TEM) analyses of particle sizes, crystallinity and of the composition of crystalline phases. The TEM techniques used were bright field (to count particles), conical dark field (to determine the crystallinity of the particles) and EDS (energy dispersive spectroscopy) to evaluate the composition of the crystalline particles.

The transmission electron microscopy evidence presented in this report leaves no doubt that crystalline particles are present in silica fume that forms when oxygen is bubbled through molten silicon. The concentration of crystalline particles was low (less than 1 per cent of silica particles were crystalline), but its potential impact on the development of lung fibrosis should not be dismissed. Particle sizes were not quantitatively characterised, but a general estimate could be made from evidence on the electron microscopy photographs. The needle-shaped crystalline particles were approximately 200 nm and less in length and approximately 20 nm wide. Amorphous silica fume particles in all of the samples were smoothly spherical in shape and the diameter appeared to be almost exclusively in the order of 100 nm and less. This places silica fume clearly in the category of ultrafine particles.

In humans, occupational exposure to amorphous silica generally did not show a silicotic effect. In most cases in the literature where silicosis was reported after exposure to amorphous silica, it was acknowledged that exposures were mixed, with both amorphous and crystalline silica being present in the dusts/fumes. A major limitation in most of the studies is that exposure levels were not known accurately and, where dust levels were measured, the crystalline content was uncertain. Furthermore, many of the studies considered other types of amorphous silica, and not silica fume. It is, however, clear that even small contaminations of crystalline silica in the order of 0.1 per cent of the total amorphous content, is known to result in fibrotic effects.

Clinical diagnoses of lung fibrosis and decreased lung function parameters were described for uncontaminated amorphous silica in the occupational setting. Inflammatory responses such as bronchitis, airway obstruction and emphysema were described, but the importance of confounders has not been sufficiently quantified.

More recent work on ultrafine particles has added to the evidence that ultrafine particles clearly have respiratory effects that include inflammatory responses, while amorphous silica exposure

can clearly result in lung fibrosis. The aetiology of silicosis involves both of these physiological processes. It is possible that co-exposure to ultrafine particles might sensitise the lung to silicotic effects by stimulating recruitment and activation of inflammatory cells and the amplified release of fibrogenic factors, preparing the scene for low concentrations of crystalline particles to trigger a silicotic effect. The dose-effect relationship for the development of silicosis under such conditions is not known, and it is therefore difficult to estimate an appropriate occupational exposure threshold level. It has now been confirmed unambiguously, however, that small concentrations of crystalline silica can be present in amorphous silica under certain process conditions, and this fact should affect the overall interpretation of the association between exposure to silica fume and the potential for development of silicosis.

The current scientific understanding of the fibrogenic effects of ultrafine amorphous silica fume, irrespective of the potential for development of silicosis, also suggests a re-assessment of the guidelines that have been promulgated in the interest of protection of occupational health. The fibrogenic effects associated with exposure to amorphous silica fume may be transient, but they still classify as adverse. Both the U.S. National Institute for Occupational Safety and Health (NIOSH) and the U.S. Occupational Safety and Health Administration (OSHA) have set guidelines for various amorphous forms of silica, but not for silica fume *per se*. The ACGIH occupational exposure guideline for silica fume is 2 mg/m³, but certain specialists have questioned this guideline. The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has set a guideline (MAK) value of 0.3 mg/m³ for fine dust for several forms of amorphous silica together. The guideline concentration used in South Africa for assessment of exposure to respirable amorphous SiO₂ is 3 mg/m³, which is actually at the level recommended for diatomaceous earth in the ACGIH TLVs. There appears to be insufficient justification to retain this guideline in view of the available evidence on adverse health effects of ultrafine silica fume. It is therefore apparent from this SIMRAC study that the occupational exposure guideline for amorphous silica fume should be reassessed. Setting a new guideline has to follow a specific regulatory process, and has to be initiated by the Department of Minerals and Energy.

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Glossary of abbreviations, symbols and terms

Abbreviations

Threshold limit value
National Institute for Occupational Safety and Health

TLV
NIOSH

Terminology

Aerodynamic diameter

The diameter of a unit density sphere with the same terminal settling velocity as the particle, regardless of its size, shape and density. The terminal settling velocity is the constant velocity at which a particle will settle in air medium when equilibrium is reached between the gravitational force and the sum of the particle's buoyancy and air resistance.

Alveoli

Sites of gas exchange with blood in the lungs. Alveoli take on the form of blind pockets at the terminal ends of the bronchial tree.

Alveolar macrophages

Phagocytic cells which lie free within the alveoli of the lungs and ingest dust particles and bacteria.

Amorphous particles

Non-crystalline, of no definite shape

Bronchioles

Refer to the finest divisions of the bronchial tubes.

Bronchial tubes

Also known as bronchus, the name used to describe the windpipe where it divides, one going to either lung.

Cardiovascular

Pertaining to the heart, blood and blood vessels.

Cardiorespiratory

Pertaining to the heart, blood and blood vessels and to the respiratory system.

Connective tissue

Connective tissue provides a structural framework for the body that stabilises the relative positions of the other tissue types. It includes connective tissue proper, cartilage, bone and blood.

Crystalline particles

Having the regular internal arrangement of atoms, ions, or molecules characteristic of crystals.

Endocytosis

The movement of relatively large volumes of extracellular material into the cytoplasm via the formation of a membranous vesicle at the cell surface.

Endothelium

The simple epithelium that lines blood and lymphatic vessels.

Endothelial system

Membranes lining various vessels and cavities of the body.

Epithelium

Layer of cells that forms a superficial covering or an internal lining of a body cavity or vessel.

Exertional dyspnoea

Difficulty in breathing provoked by exercise or physical effort.

Fibroblasts

Connective tissue cells that are responsible for the production of extracellular fibres.

Fibrosis

Formation of fibrous or scar tissue, usually due to either infection or deficient blood supply.

Fibrotic effects

Effects related to fibrosis.

Fume

Aerosol of solid particles resulting from condensation of the vapour given off from the heating of metals.

Granuloma

An imprecise term applied to an aggregation of inflammatory cells, initiated by various infectious or non-infectious agents.

Hypercoagulability

Tendency to form blood clots in otherwise normal and undamaged blood vessels.

Lung function test

Spirometry, which measures the volume of air inspired or expired over a period of time.

Lysosomal enzymes

Enzymes in the lysosomal sacs (see phagolysosome).

Macrophages

A large phagocyte that forms part of the reticuloendothelial system.

Mist

Finely divided liquid droplets suspended in air, formed by bubbling, boiling, foaming, spraying, splashing or otherwise agitating a liquid that contains heavy metals.

PAHs

Polynuclear aromatic hydrocarbons (found in coal tar pitch volatiles).

Phagolysosome

The lysosome is an intracellular organelle. It consists of a membrane-enclosed sac. The sac contains enzymes, such as lysosymes, that catalyse the digestion of most substances in living cells, including proteins, nucleic acids, some carbohydrates, and possibly fats. Disruption of the lysosomal membrane and release of the enzymes result in rapid digestion and dissolution of the cell. Lysosomes normally digest food stored in the cell or break down foreign particles engulfed by white blood cells (phagocytes).

Phagolysosomal membranes

Membranes relating to phagolysosome.

Pneumoconiosis

Permanent alteration of lung structure due to the inhalation of mineral dust; and the tissue reactions of the lung (usually a chronic form of inflammation) to the presence of inhaled dust.

Silicosis

A specific form of pneumoconiosis caused by the inhalation of free silica, producing lung fibrosis (scar tissue formation).

Response

The reaction of a body or organ to a chemical substance or other physical, chemical, or biological agent.

Surfactant

A surface-active agent lining the alveoli of the lungs, preventing the alveoli collapsing at the end of expiration.

Threshold

The lowest dose or exposure of a chemical at which a specified measurable effect is observed and below which such effect is not observed. *Threshold dose* is the minimum exposure dose of a chemical that will evoke a stipulated toxicological response. *Toxicological threshold* refers to the concentration at which a compound exhibits toxic effects.

Threshold limit

The concentration of a chemical above which adverse health and/or environmental effects may occur.

Toxic

Harmful, or deleterious with respect to the effects produced by exposure to a chemical substance.

Toxicant

Any synthetic or natural chemical with an ability to produce adverse health effects. It is a poisonous contaminant that may injure an exposed organism.

Toxicity

The harmful effects produced by a chemical substance. It is the quality or degree of being poisonous or harmful to human or ecological receptors. It represents the property of a substance to cause any adverse physiological effects (on living organisms).

Ultrafine particles

Particles with an **aerodynamic diameter** of less than 0.1 μm (100 nm).

Vagus nerve

Nerve with mixed sensory and motor function that is widely distributed in the thorax and abdomen.

Vapour

The gaseous form of a substance that is normally in the liquid or solid state at room temperature and pressure.

1 Introduction

Silicosis is a debilitating lung disease, believed to be caused by the inhalation of crystalline silica. Despite intervention in exposure of workers over many decades, silicosis is still recognised internationally as a major occupational disease of special concern. Because of the ongoing health problems associated with silicosis, it appears that simply measuring airborne levels of crystalline silica using the standard occupational hygiene techniques may not in all situations provide the information necessary to assess and manage silicosis risks in industries. One of the issues that have been controversial for many years relates to the possible link between amorphous silica and silicosis. Although amorphous silica does not appear to be associated unambiguously with silicosis, exposure characterisation in certain industrial scenarios is uncertain, and a generalised statement may be inappropriate. Silica fume, generally regarded as completely amorphous and not associated with silicosis, is of special interest in this regard. It is possible that routine X-ray diffraction analysis of silica fume, because of inherent limitations in the technique, could fail to identify crystalline silica that might be present as an impurity in silica fume, due to peak broadening in the matrix where the particles are ultra fine. The silica fume that appears to be completely amorphous from the analysis might therefore contain crystalline phases, and might have the potential to cause silicosis.

A previous SIMRAC study (Project Health 709) (Van Niekerk et al., 2000) was initiated to study possible causes for lung disease in silicon smelting plants, because of the wide concern about silicosis. The study highlighted several limitations in the current understanding of health risks in silicon smelting plants. One of the outcomes of the study was that it appears to be a fallacy to focus the assessment of silicosis in a smelter environment on exposure to crystalline silica per se while assuming that silica fume, which is assumed to be completely amorphous, has little adverse effect on the lung. Most of the inconsistencies in the interpretation of health effects relating to amorphous silica appear to be a result of:

- ? Generalisations between the different forms of amorphous silica;
- ? Disregarding the presence of microcrystalline phases in silica fume, and
- ? Underestimating the role of ultrafine particles in the overall particulate toxicity.

These aspects have led to the initiation of the current study.

1.1 Research problem statement

Silica fume, generally described as amorphous silica, is likely to be associated with the development of fibrotic effects in exposed individuals. If microcrystalline phases were present in thermally generated silica fume, this might lead to the development of silicosis. Microcrystalline phases are however difficult to detect with routine X-ray diffraction in the ultrafine matrix. It may therefore be possible that silica fume that appears to be amorphous in occupational hygiene surveys, might have the potential to cause silicosis because it contains a small proportion of crystalline silica. In order to further clarify this issue, it was considered important to confirm whether crystalline phases could in fact be present in silica fume and if so, at what levels. Furthermore, the role of the ultrafine nature of silica fume in its overall toxicity was considered to require better clarification.

1.2 Objectives and aims of this study

1.2.1 Main objective

The outcome of the study should indicate whether microcrystalline phases could be present in silica fume produced in thermal processes. The intention of the study was of a generic nature, not referring to health risks in a specific silicon producing company. Silicon smelter processes, where silicon is produced from quartzite, are known to produce silica fume, and therefore have the potential to expose workers to the fume. Silica fume samples were therefore taken in

various areas in a typical silicon smelter to produce the required analytical information. The study was however not designed with the intention to assess quantitative exposure to silica fume. On a scientific basis, the findings of the study should be relevant to any high-temperature process where silica fume could be produced.

1.2.2 Goals

The goals of the study were:

- ? The quantitative determination of microcrystalline phases in silica fume;
- ? Interpretation of the analytical information in terms of the potential for development of silicosis;
- ? Determination of particle size distributions of silica fume, and
- ? Updating of literature reviews on characteristics and health effects of amorphous silica fume and characteristics and health effects of ultrafine particles.

1.3 Research context and design

1.3.1 Research context

Historically, crystalline silica has been known as an agent responsible for pulmonary nodular fibrosis, but there are examples where the development of silicosis is unlikely to be attributable only to silica in the traditionally defined crystalline state. It is unlikely that extensive monitoring, as has been done over many years for crystalline silica in the occupational environment, would clarify the continuing doubt where exposure to ultrafine silica fume is the major issue. This SIMRAC project was therefore initiated to address uncertainties around silicosis at a more fundamental level where workers are exposed to silica fume, by investigating the possible presence of microcrystalline phases in thermally generated silica fume. These phases are difficult to detect with routine X-ray diffraction in the ultrafine matrix and a more specific and sophisticated analytical approach was therefore used in the study.

A short introduction to the structural aspects of silica and silicon compounds and to the silicon production process is given below, to clarify the physical context of the project.

1.3.2 Structural aspects of silica and silicon compounds

Especially to the layman, the term silica is not always unambiguous. The element silicon (Si) is readily oxidisable, especially to the dioxide, namely SiO_2 (silicon dioxide or silica). Many structural variations of silica are possible, both synthesised and naturally occurring. In addition, polymerisation of silica is possible under favourable conditions. In the case of silica, even the term polymerisation is confusing since, in the silica system, the monomer $\text{Si}(\text{OH})_4$ condenses to form a polymer that ultimately has the structure $(\text{SiO}_2)_n$. Regardless of the processes involved, the international convention is that the term silica implies the presence of SiO_2 , without considering the form in which it is actually present.

1.3.3 Classes of silica

1.3.3.1 Primary classification

The most prevalent form of silica is quartz, the main constituent of common sand. However, both in nature and in the laboratory, other forms may also be produced or occur and are divided into the following classes:

Crystalline silica

- ? Anhydrous crystalline silica, and
- ? Hydrated crystalline silica $\text{SiO}_2 \cdot x\text{H}_2\text{O}$.

Amorphous silica

- ? Anhydrous amorphous silica of microporous anisotropic forms such as fibres or sheets;
- ? Anhydrous and hydrous amorphous silica of colloiddally-subdivided or microporous isotropic form such as sols, gels and fine powders, and
- ? Massive dense amorphous silica glass.

The following classes are pertinent to the study of processes where silicon occurs in the molten state.

1.3.3.2 Anhydrous, crystalline silicas

Especially with respect to solubility, there are three main phases to be considered:

- ? Quartz;
- ? Tridymite, and
- ? Cristobalite.

The transformations between the above-mentioned three common forms and massive vitreous silica are as follows (Iler, 1979):



More recently, other metastable phases have also been identified, namely kealite, coesite, stishovite, silica W, melanophogite, silica O, silica X and silicalite. However, for the purpose of this study, these metastable phases will not be considered in detail.

1.3.3.3 Amorphous silica

Micro-amorphous silica includes sols, gels, powders and porous glasses, which generally consist of particles less than 1 μm in size. Amorphous silica may not be truly amorphous, but may consist of regions of local atomic order "crystals" of extremely small size (Vitums et al., 1977), which by careful X-ray diffraction studies appear to possess the cristobalite structure (Iler, 1979). Nevertheless, by ordinary X-ray diffraction procedures this material exhibits only a broad band with no multiple peaks as are ordinarily obtained with macroscopic crystals, and is therefore referred to as amorphous. Incidentally, it is known that heating of amorphous silica to 1 500 $^{\circ}\text{C}$ leads to the formation of cristobalite. This forms the basis of the NIOSH analytical method for amorphous silica (NIOSH, 2000).

Since micro-amorphous silica seems to be the most probable species to be encountered under silica smelter operating conditions, it is appropriate at this stage to concentrate more fully on this material.

Fibrous silica

A special type of micro-amorphous silica is obtained when silicon monoxide (SiO) is oxidised, and results in the formation of fibrous silica. This is typical of material condensed from the vapour from the reaction between silica and silicon metal. This material consists of a mat of hollow fibres and spiral fibres of amorphous silica of less than 0. 04 μm in diameter, and many microns in length.

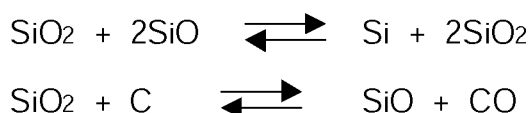
Fumed silica

This amorphous material is prepared by the high-temperature hydrolysis of volatile Si-species, such as SiCl_4 , SiHCl_3 and SiO_4 in a hydrogen/oxygen flame. The resultant silica consists of very fine spherical particles of an even size (typically in the 7 to 20 nm range). Primary particles are often joined together in aggregates or chains and, most importantly, hydroxyl groups populate the outer surface. This material finds a myriad of uses in the food, pharmaceutical, paint and cosmetics industries. It is generally considered to be innocuous to human health,

whether ingested or inhaled. Although not relevant in this study, fumed silica is mentioned here to avoid confusion with silica fume that is described below.

Microsilica or silica fume

This largely amorphous material is produced as a by-product during thermal treatment under reducing conditions of silica, quartzite, etc., to produce silicon "metal". The process occurs in silicon smelting plants and can be illustrated as follows:



The above reactions take place at above 1 700 °C, and as is evident, produce silica as a by-product, the main aim being to produce elemental silicon. In addition, the silica is only moderately pure (90 to 98 per cent), and as far as known, no commercial process for the sole production of SiO₂ via this route exists in the world. The small particle size of silica fume is believed to be one of the most significant factors that determine its toxic characteristics. Although several statements have been made in the literature about the small particle size distribution, not many comprehensive studies have been published. Vitums et al. (1977) described silica fume as *opaque, round, and smooth particles, ranging from less than 0.05 to 0.75 microns in diameter*.

1.3.4 Overview of the silicon production process

Exposure to silica fume can be expected to occur mostly in the silicon production process. Silicon is almost exclusively prepared by carbothermal reduction of quartzite in submerged-arc furnaces at temperatures above 1700 °C.

Quartzite ore is taken through a crushing, scrubbing, screening and grading process, and eventually to storage in silos. The feed to the furnace typically consists of crushed silica ore and reducing agents that may consist of coke (coal- or petroleum-derived), charcoal, low-ash coal, and wood chips. The feed material from the storage silos is continuously charged to the furnace where it covers the electrodes and molten silicon. This area is referred to as the charge level.

At the top of the furnaces, the pitch-containing electrode material is fed into electrode sheaths. This area is designated as the upper/electrode floor.

The upper part of the furnace is shielded by means of movable vertical metal covers that can be slid away to allow access to the charge bed for the stoking equipment. A fume hood is installed above the furnace for the discharge of gas and fumes to a bag-house. Molten silicon is tapped from the bottom of the furnace to a ladle for transport to the refining station, where oxygen is bubbled through the molten silicon. After the refining stage, the molten silicon is transferred to the casting bay where the silicon ingots are cast.

Various areas in a smelting plant may have different air contamination profiles, but the most prominent reaction is the formation of silicon monoxide under reducing conditions in the furnace. Once released into air, silicon monoxide rapidly condenses to silica fume. The reaction that produces silica fume at the refining station can be expected to be different, seeing that oxygen is bubbled through red-hot molten silicon.

1.3.5 Research design

Samples were collected at various locations (temperature zones) in the smelter plant and used for transmission electron microscopic (TEM) analyses of particle sizes (Thomas and Goringe, 1979). Since this was an exploratory study, only one sample was taken in each sampling location. The selected sampling locations listed below were representative of the most prominent areas of exposure to silica fume in the processing plant:

- ? Upper/electrode floor;
- ? Charge floor;
- ? Tap hole, and
- ? Refining station.

2 Research methodology

Representative samples were collected on air filters at the identified sites (one sample at each location). The sampling system is diagrammatically represented in Figure 2.1. The impactor assembly screens out particles larger than 10 μm . The 8- μm and 0.4- μm cellulose ester filters were used to collect particle sizes in the nanometer range. Quantitative collection cannot be expected, because the 8- μm filter would retain some proportion of particles smaller than 8- μm . Quantitative particulate collection was however not the primary objective in this investigation, but the intention was to investigate the potential presence and proportion of crystalline particles in silica fume. The samples were considered to be a good representation of the nature of the silica fume in various areas of the process.

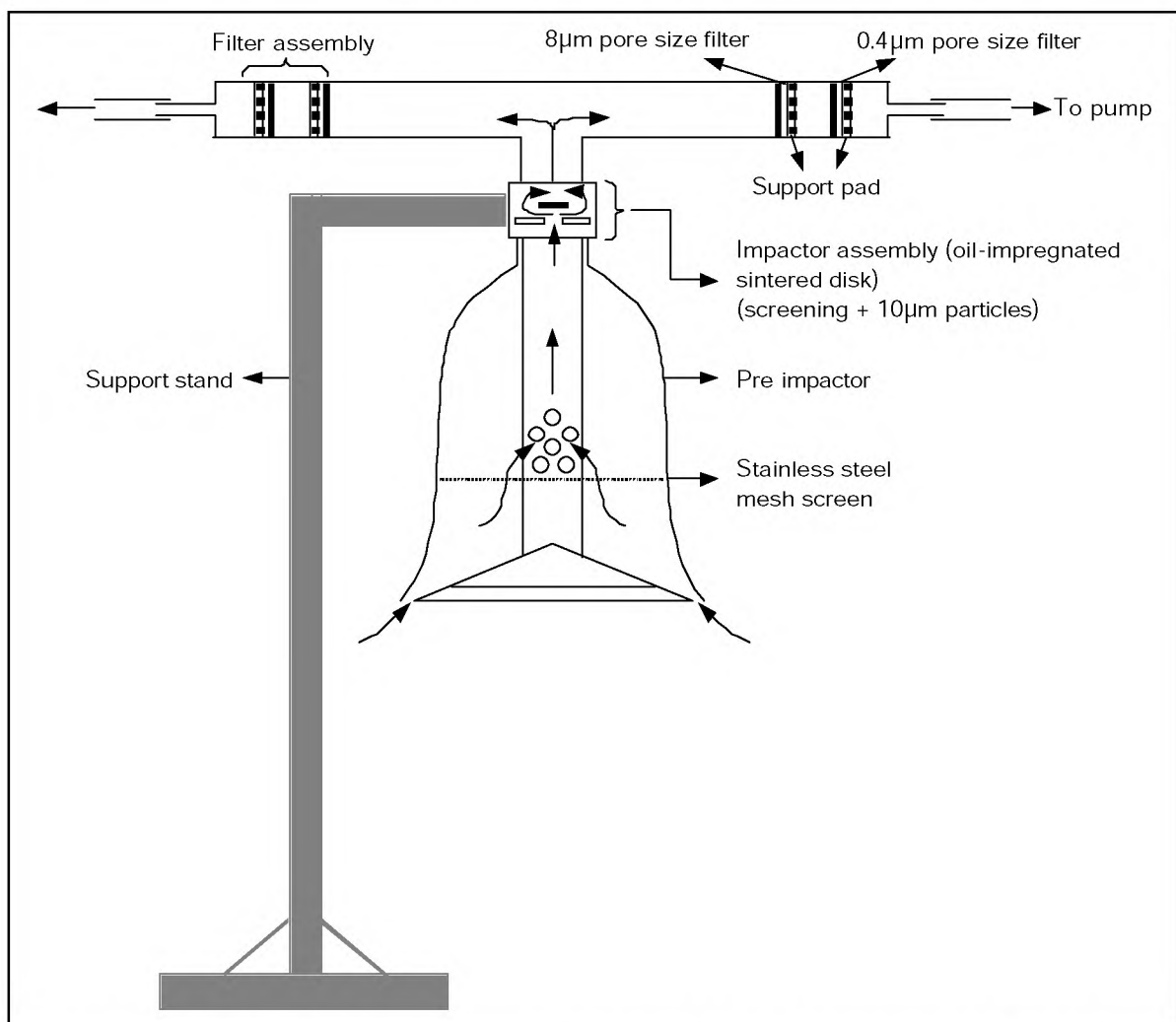


Figure 2.1: Sampling assembly for silica fume (DiAL Environmental Services).

Sample analyses were done at the Industrial Metals & Minerals Research Institute (IMMRI) of the University of Pretoria.



Collected particles were transferred to carbon grids and used for transmission electron microscopic (TEM) analyses of particle sizes and crystallinity and of the composition of crystalline phases.

The TEM techniques used were bright field (to count particles), conical dark field (to determine the crystallinity of the particles) and EDS (energy dispersive spectroscopy) to evaluate the composition of the crystalline particles.

The advantage of TEM over X-ray diffraction (XRD) is that it resolves into the nanometer range (500 to 1 nm), whereas XRD is effective in the micrometer range (>500nm). The advantage of TEM over scanning electron microscopy (SEM) is that the imaging can be coupled with diffraction information, which is of great importance for the detection of crystallinity.

Figure 2.2: *The transmission electron microscope that was used in the study.*

3 Results

3.1 The quantitative determination of microcrystalline phases in silica fume

3.1.1 Upper electrode floor

Figure 3.1.1 shows the transmission electron microscope photograph of the upper electrode-floor sample. No crystalline silica particles were observed, but the analysis of this sample was complicated by the presence of zinc crystals. Although crystalline silica particles were not identifiable, the complex nature of the sample does not allow a clear conclusion to be reached. The analysis could therefore not rule out the potential presence of crystalline phases. However, it can be stated with 95 per cent confidence that less than 1 per cent of crystalline silica particles would be present, if at all.

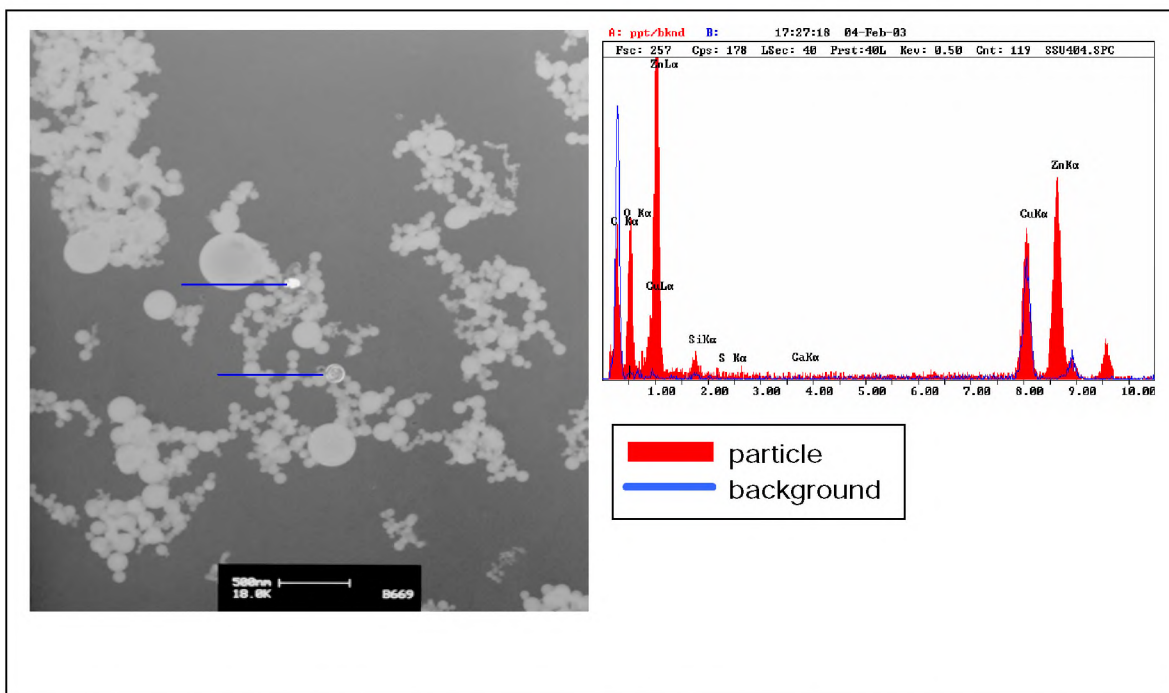


Figure 3.1.1: *Bright field TEM image of Zn particles and amorphous silica particles in the upper/electrode floor sample (arrows indicates the Zn particles).*

3.1.2 Charge-floor and tap-hole samples

The charge-floor and tap-hole samples were both characterised by the presence of crystalline phases, which were identified as carbonaceous material (carbon and sulphur), as well as inorganic salts. The presence of these substances complicated the analysis. Crystalline silica particles were not detected, but similar to the sample collected at the upper electrode floor, the analysis could not rule out the potential presence of crystalline particles. Again, for both the charge floor and tap hole samples it can be stated with 95 per cent confidence that less than 1 per cent of crystalline silica particles might have been present, if at all. Figure 3.1.2 shows the transmission electron microscope photographs of the sample from the charge floor. The sample from the tap hole area had a similar appearance.

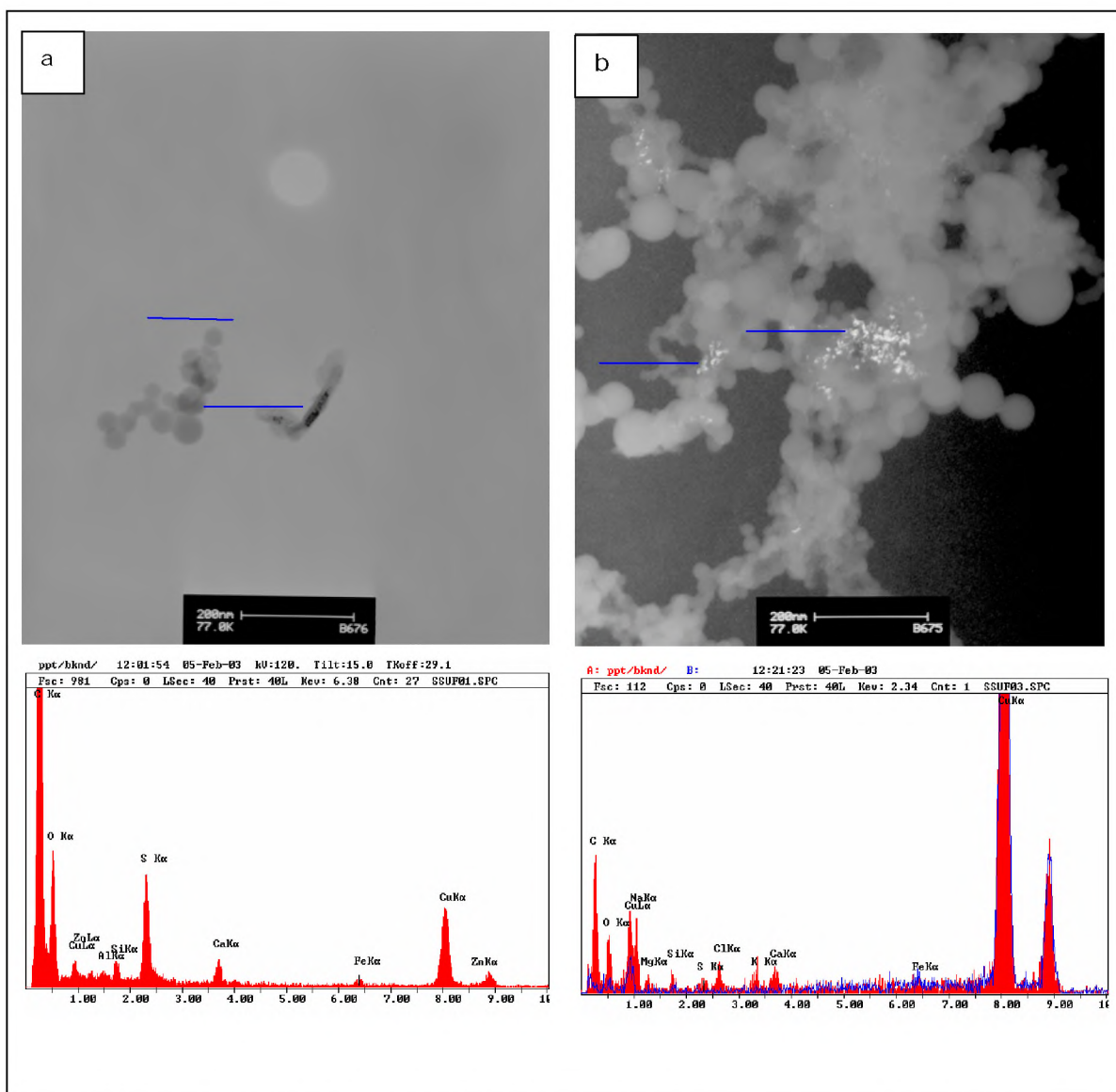


Figure 3.1.2: (a) Bright field TEM image and energy dispersive spectroscopy of carbonaceous crystals and amorphous silica, and

(b) Dark field TEM image and energy dispersive spectroscopy of salt crystals.

3.1.3 Refining station

A general image of the refining-station sample showed amorphous silica particles, as is illustrated in Figure 3.1.3 (A).

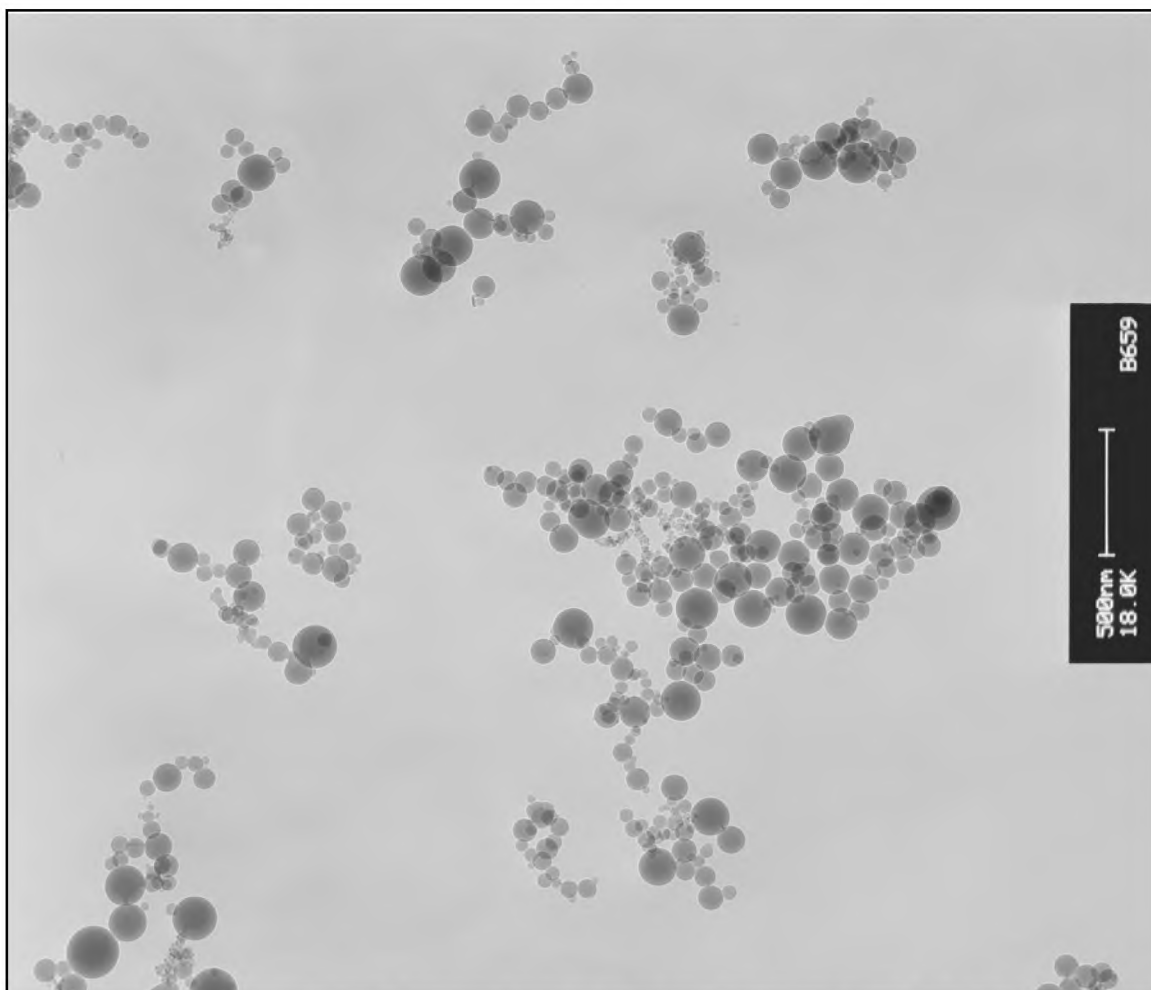


Figure 3.1.3 (A): General view of amorphous silica particles in the refining-station sample.

More intensive particle surveying however clearly identified crystalline silica particles, as is illustrated in Figure 3.1.3 (B). The illumination visible in the dark field image originates from a specific point in the back focal plane of the objective lens, corresponding to a unique set of crystalline planes. Energy dispersive spectroscopy of the needles confirmed that they exist of silicon and oxygen. The copper and carbon were from the carbon support and grid, respectively.

In the bright field image there is a crystalline particle as part of the bigger amorphous particle, appearing as partial crystallinity.

The percentage of crystalline particles was less than 1 per cent (that is, less than one particle in a hundred) and this can be stated with 99 per cent confidence. Therefore, there is no doubt that crystalline phases were present and it is 99 per cent certain that the concentration of crystalline phases is not higher than 1 per cent in this sample.

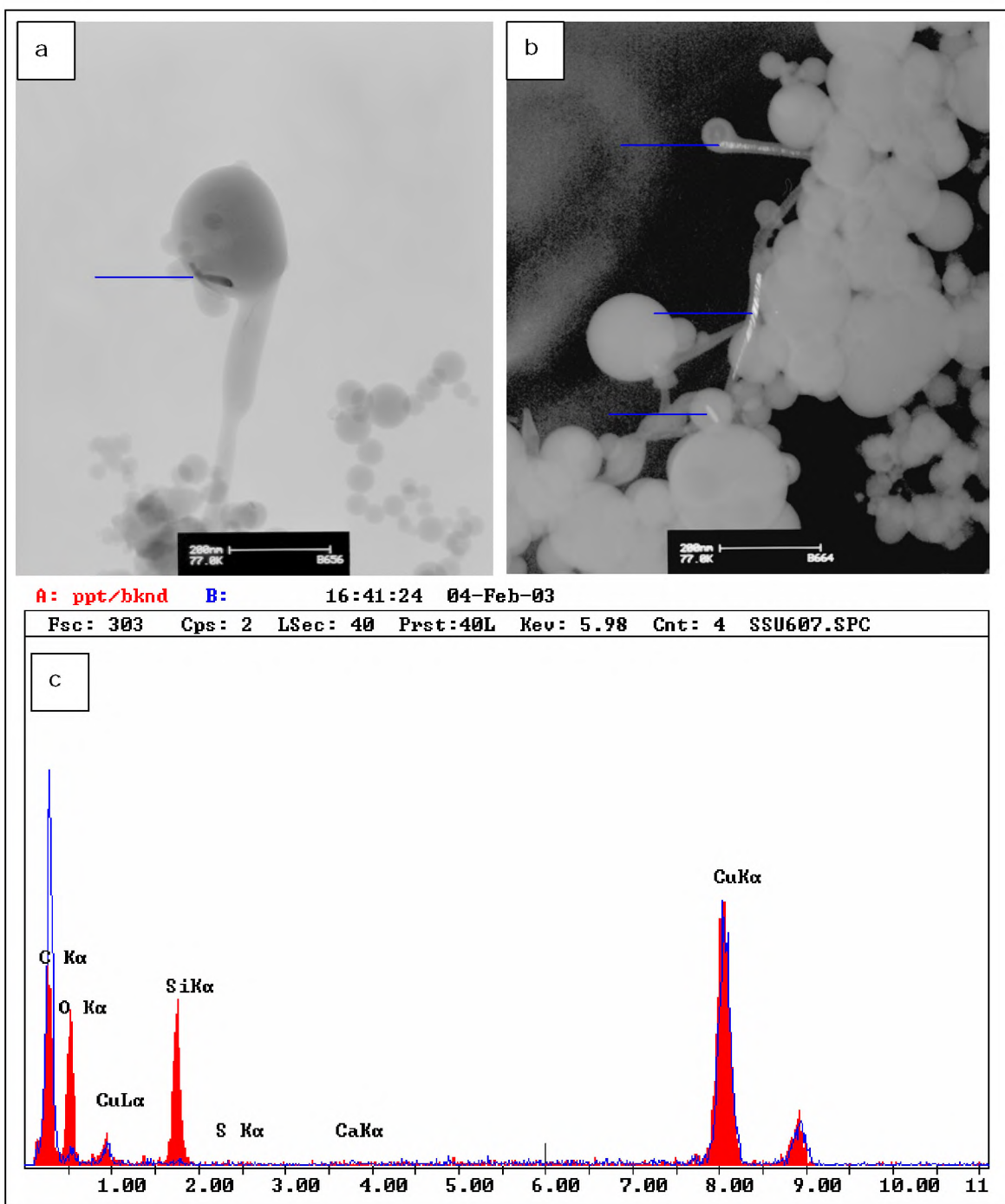


Figure 3.1.3 (B): (a) Bright field TEM of a partially crystalline silica particle in the refining-station sample (arrow indicates the crystalline portion), and

(b) Dark field image of needle-like, crystalline silica particles in the refining station sample (arrow indicates the crystalline portion).

(c) Energy dispersive spectroscopy of the silica needles.

3.2 Determination of particle size distributions of silica fume

Particle sizes were not quantitatively characterised, but a general estimate could be made from evidence on the electron microscopy photographs (Tuling, 2003). The needle-shaped crystalline particles observed in the refining-station sample were approximately 200 nm and less in length and approximately 20 nm in diameter (Figure 3.1.3). Amorphous silica fume particles in all of the samples were smoothly spherical in shape and the diameter appeared to be almost exclusively in the order of 100 nm and less (Figures 3.1.1 to 3.1.3). The physical diameter is related to the aerodynamic diameter and it may be generally estimated that most of the amorphous silica particles were in the ultrafine aerodynamic particle range.

The electron microscopy photographs showed aggregates of particles, but this is not an indication of the particle state in the original air samples, since air samples were subjected to processing prior to microscopic analyses. The aggregated appearance on the electron microscopy photographs could therefore be an artefact of sample preparation.

4 Updated literature reviews on the characteristics and health effects of amorphous silica fume and ultrafine particles

4.1 Background: the pathogenesis of silicosis

Crystal-induced lung diseases, such as silicosis, are believed to involve a series of complex processes, most of which are applicable to mineral dust exposure in general (*Wiessner et al., 1988 and Kumar et al., 1997*). Critical steps are as follows:

- ? Most inhaled dust is entrapped in the mucous blanket within the bronchioles and alveoli, coated with pulmonary proteins and surfactants and rapidly removed from the lung by ciliary movement;
- ? Some particles become impacted in the alveoli, where macrophages accumulate and endocytose the impacted particulates;
- ? The more reactive particulates trigger the macrophage to release a number of products. Some of these are directly toxic to the lung, initiating lung injury. Other pro-inflammatory factors initiate a cascading inflammatory response, by recruiting and activating additional inflammatory cells that in turn also release toxic factors mediating lung injury;
- ? Macrophages also release fibrogenic factors that recruit fibroblasts and induce collagen synthesis, resulting in the initiation of fibrogenesis. A summary of the factors is given in Table 4.1.1;
- ? Once the crystals are inside the phagolysosome, lysosomal enzymes strip the protein and surfactant coat off the crystals, creating clean crystal surfaces;
- ? The clean crystals interact with the interior of the phagolysosomal membranes, resulting in the rupture of the phagolysosome followed by cell death and the release of clean crystals, and
- ? The crystals are once more absorbed by other macrophages and the entire cycle is repeated in endless successions, even when inhalation exposure is terminated, perpetuating the typical progressive silicotic process.

Some mineralogical particles may be taken up by epithelial cells or cross the epithelial cell lining and interact directly with fibroblasts and interstitial macrophages. Some may reach the lymphatic nodes, initiating an immune response that leads to amplification and extension of the local reaction. Tobacco smoking worsens the effects of all inhaled mineral dusts (*Kumar et al., 1997*).

Table 4.1.1
Summary of factors released after endocytosis of particulates by alveolar macrophages.

Pro-inflammatory factors	Toxic factors	Fibrogenic factors
Leukotriene B ₄ Cytokines: Interleukins 6 and 8 (IL-6 and IL-8) Tumour necrosis factor ? (TNF-?)	Proteases O ₂ free radicals	Cytokines: Interleukin-1 (IL-1) Tumour necrosis factor ? (TNF-?) Platelet-derived growth factor (PDGF) Fibronectin Insulin-like growth factor 1 (IGF-1) Transforming growth factor-beta (TGF-?)
Effects: Recruitment and activation of inflammatory cells. ? Amplified release of toxic factors.	Effect: Lung injury	Effect: Induction of fibrogenesis.

Sources: Kumar *et al.*, 1997 and Olbrück *et al.*, 1998.

In order to fully appreciate the data presented below, a brief overview of the fibrotic process (fibrogenesis) resulting in the lung scarring typical of pneumoconiosis and specifically silicosis, is in order. Fibrosis occurs via the following steps (Kumar *et al.*, 1997; Mitchell and Cotran, 1997 and Prendergast and Ruoss, 2000):

- ? Alveolar macrophages release fibrogenic factors that stimulate migration, activation and proliferation of fibroblasts;
- ? Activated fibroblasts deposit matrix proteins, particularly collagen;
- ? The perpetuation of this pattern of fibroblast activation and proliferation, resulting in increased deposition of collagen and other tissue matrix components, occurs under the influence of inflammatory cells;
- ? If this process is not terminated, granulation tissue eventually develops, characterised by a proliferation of fibroblasts and new, thin-walled, delicate capillaries in a loose tissue matrix, and
- ? Granulation tissue progressively accumulates connective tissue matrix and eventually results in fibrosis (scarring).

In humans, silicosis manifests as four pathologically distinct outcomes, i.e.

- ? Acute silicosis (silicoliproteinosis);
- ? Accelerated silicosis;
- ? Chronic simple silicosis, and
- ? Complicated silicosis, which develops to progressive massive fibrosis by the conglomeration of nodular lesions.

Acute silicosis and accelerated silicosis have short latency periods (several months to several years) and are associated with intense, brief exposures. Chronic simple silicosis and complicated silicosis are more likely to occur a decade or more after first exposure, and are associated with lower levels of exposure over long periods of time.

Numerous studies have indicated that the crystalline state of inhaled silica plays a very important role in subsequent health issues (Iler, 1979 and Castranova *et al.*, 1996), but it has also been shown in various studies that silica fume generated in silicon smelting plants causes pulmonary illness in humans. Probably the most comprehensive review of documentation relating to exposure to silica fume has been published by Galton-Fenzi (1998). Having reviewed 24 primary research papers dating from 1937 to 1997, the author concluded that *there is evidence that thermally generated silica fume is causally associated with respiratory diseases*. An updated literature review of the characteristics and health effects of amorphous silica is presented below. Since silica fume could be accurately classified as an ultrafine aerosol and since it has been concluded that size distributions could be important in the

consideration of health effects from silica fume exposure (*Cunningham et al., 1996*), an updated literature review of the characteristics and health effects of ultrafine particles is included.

4.2 Updated literature review of the characteristics and health effects of amorphous silica

4.2.1 Animal and laboratory studies

Warheit (2001) conducted a review focussed on the toxicological profiles of amorphous silica particulates and, where available, comparisons of crystalline to amorphous silica. Silica fume was not specifically included, but the respiratory toxicology of several types of amorphous silica particles were reviewed. Those were fumed silica, colloidal amorphous silica, diatomaceous earth and precipitated amorphous silica. The studies were all experimental by design, exposing laboratory animals for various periods, extending from days to years, to a range of air concentrations. Some animals were followed up during a withdrawal period. As expected, crystalline forms of silica dust were more potent in producing pulmonary toxicity when compared to amorphous forms of silica particles. Another recurring and important difference was that amorphous silica produced transient pulmonary inflammation that regressed during the recovery period. In contrast, inflammation elicited by crystalline silica was sustained during the recovery period.

The pro-inflammatory potential of amorphous silica is particularly evident under low mass and low surface area of exposure. Under conditions of low mass concentrations of exposure, amorphous microsilica, compared to quartz, was a more potent inducer of the pro-inflammatory cytokine interleukin 6 (IL-6). Similarly, under conditions of low units of total surface area exposure, amorphous silica was a more potent inducer of IL-6. This pattern was not consistent for different cytokines, since, under similar experimental conditions, amorphous silica was a less potent inducer of IL-8. At higher mass concentrations, crystallinity seems to be more important than particle size and surface area with respect to cytokine release, as well as to cytotoxicity (*Hetland et al., 2001*).

A review by *Merget et al. (2002)* of several animal studies on amorphous silica exposure identified two important consistent findings. Firstly, emphysema or alveolar hyperinflation was present in many animal studies. Especially in rats, this was the cause of high mortality. This process was partially reversible after discontinuation of exposure. Secondly, inflammation and fibrogenic effects were less pronounced than following quartz inhalation. In addition, persistent or progressing silicotic nodules were not found after the discontinuation of exposure. Regression of granuloma (new growth of granulation tissue caused by various forms of chronic inflammation) and connective tissue formation after discontinuation of exposure was found in all animal species. Similarly, *Murphy et al. (1998)* reported an experimental animal study with single intratracheal instillations indicating that crystalline quartz causes progressive damage whilst a similar mass of amorphous ultrafine silica induces a transient lung epithelial damage, which then regresses.

The issue of lung clearance is probably an important factor that determines the occurrence of silicosis; *Merget et al. (2002)* also reviewed this aspect. In contrast to quartz, a number of amorphous silica products were almost completely eliminated from the lungs of various animal species within months after discontinuation of exposure. Amorphous silica accumulation in the lymph nodes was also at least partially reversible. The difference in clearance between crystalline and amorphous silica is not yet fully understood. Amorphous silica is accumulated in alveolar macrophages and transported to the lymph nodes, where it accumulates (*Lee and Kelly, 1993* and *Lehnert et al., 1986*). However, in contrast to crystalline silica, amorphous silica is eliminated much faster (*Pratt, 1983*).

4.2.2 Occupational studies

An occupational study in diatomite-exposed workers showed that exposure to natural diatomite (contaminated with between 0.1 and 4 per cent crystalline silica) was associated with simple fibrosis while exposure to calcined diatomite (up to 60 per cent crystalline silica) was associated with progressive pulmonary fibrosis (reviewed in *Merget et al., 2002*). These authors also reviewed the limited number of epidemiological studies on workers with long-term exposure to intentionally manufactured synthetic amorphous silica. No silicosis was found, except for one study (*Mohrmann and Kann, 1985*) in which silicosis caused by amorphous silica not obviously contaminated with quartz was found in 4 of 28 workers. However, Mohrmann and Kann could not exclude contamination by small amounts of cristobalite. *Philippou et al. (1992)* described histological examination of lung biopsies of two subjects with exposure to amorphous silica and a clinical diagnosis of lung fibrosis. The histological examination disclosed non-birefringent material in the vicinity of fibrotic lesions, and birefringent particles (shows luminous edge under light microscopy; probably glassy) were found to a much lesser degree.

In occupational settings, significant decreases in lung function parameters have been associated with exposure to amorphous silica (*Vitums et al., 1977* and *Choudat et al., 1990*). However, *Ferch et al. (1987, referenced in Merget et al, 2002)* could not find a significant association. *McLaughlin et al. (1997)* reviewed the health effects of amorphous silica in humans, covering a number of studies in occupational settings. It was concluded that occupational exposure to amorphous silica generally did not show a silicotic effect, and that there was no evidence of carcinogenic effects of amorphous silica in humans.

4.2.3 Conclusions on the status on knowledge about the toxicity of amorphous silica

Animal studies have shown that inflammatory responses, granuloma, collagen and connective tissue formation often occur in animals exposed to amorphous silica. These were consistently found to be at least partially reversible. Inflammation and fibrogenic effects were less pronounced with amorphous silica than following quartz inhalation. Silicotic nodules were sometimes found, but these were not persistent, even in long-term inhalation experiments with high concentrations of amorphous silicas that are probably not encountered in workplaces. This contrasts with inhalation experiments using crystalline silica that clearly demonstrated such effects. However, some studies describe a minor persistent interstitial collagen deposition (reviewed by *Merget et al., (2002)*).

In humans, occupational exposure to amorphous silica generally did not show a silicotic effect. In terms of the current study, it is important to note that there are indications that contamination of amorphous silica with even small amounts (between 0.1 and 4 per cent) of crystalline silica may result in simple fibrosis or silicosis.

Clinical diagnoses of lung fibrosis and decreased lung function parameters were described for uncontaminated amorphous silica in the occupational setting. Inflammatory responses such as bronchitis, airway obstruction and emphysema were described, but the importance of confounders has not been sufficiently quantified. These parameters have to be considered in further epidemiological studies as primary outcome variables and this will be of utmost importance for the regulation of amorphous silica exposure on the basis of the prevention of inflammatory responses.

4.3 Updated literature review of the characteristics and health effects of ultrafine particles

4.3.1 Introduction

Particulate matter (PM) may be classified by size as "total suspended particulates" (TSP), "coarse" particles less than 10 µm aerodynamic diameter (PM₁₀), "fine" particles less than 2.5

μm diameter ($\text{PM}_{2.5}$) and "ultrafine" particles less than $0.1 \mu\text{m}$ diameter (100 nm) ($\text{PM}_{0.1}$). In the biological context, particles with a diameter bigger than around $2 \mu\text{m}$ are called coarse mode particles, while the smaller fraction is called fine mode particles (*Wilson and Suh, 1997*).

A review of the literature makes it clear that the epidemiology of particulate matter has received considerable attention, but that environmental and occupational effects of ultrafine particle exposures are only in the early stages of exploration. This is in contrast with evidence from epidemiological studies on fine particle effects. The Harvard Six-City study showed that a $10 \mu\text{g}/\text{m}^3$ increment in $\text{PM}_{2.5}$ was associated with increased mortality from pneumonia (+ 4 per cent), chronic obstructive pulmonary disease (+ 3 per cent) and ischemic heart disease (+ 2 per cent) (*Dockery et al., 1993*).

However, information on the physiological effects of ultrafine particles and on the physical, chemical and cytological mechanisms that are involved are available and these are summarised below. Ultrafine particles induce respiratory inflammatory responses (*Ferin et al., 1992, Oberdörster et al., 1994, 1995 and 1996, Li et al., 1996, Gilmour et al., 1997, Donaldson and MacNee, 1998 and Churg et al., 1999*). Ultrafine particles are also implicated in the adverse effects of particulate air pollution on the cardiovascular system (*Utell and Frampton, 2000 and MacNee and Donaldson, 2000*).

The mechanisms of observed health effects have not been fully delineated, but significant progress has been made. Direct effects on tissue by toxic components residing on the surface of particles are important, but the cellular components of the immune system and their signalling and effect chemicals are obviously also involved. A central theme in cellular studies of the effects of ultrafine particles is the initiation of inflammatory reactions. These reactions result in the pathological manifestations noted in exposed individuals.

4.3.2 Pathogenic characteristics of ultrafine particles

4.3.2.1 Introduction

In general, the scientific literature indicates that ultrafine particles are more toxic to the respiratory system than coarse particles. The increased toxicity of ultrafine particles can be related firstly to their larger surface area per given mass. The increased surface area can act as a carrier for co-pollutants such as gases and chemicals, specifically transition metals that could form a coat on the particle surfaces during their formation (*Oberdörster, 2001*). Increased surface areas have also been associated with increased inflammatory responses, probably related to increased reactive oxygen species generation, independent of transition metal exposure (*Brown et al., 2001*).

Secondly, dosimetric aspects of the deposition and disposition of inhaled ultrafine particles differ from those of larger particles. Several plausible mechanisms have been proposed for both the initial pulmonary injury and the consequent systemic effects following ultrafine particle exposure. These mechanisms involve physical, chemical and biological characteristics of particulate matter.

4.3.2.2 Implications of the greater surface area per unit mass

A variety of studies have indicated that ultrafine particles are typically more pathogenic than larger particles of the same mineral type, even when the mineral involved, for example titanium dioxide or carbon black, is an insoluble dust of low toxicity (*Churg et al., 1999*). Indeed, the concept is emerging that surface area is the dose measure that predicts pulmonary response, rather than mass (*Donaldson et al., 2001*). The greater toxicity associated with greater surface area per unit mass, has been attributed to increased interaction between the lung tissue and the toxic components residing on the surface of the particles (*Harrison and Yin, 2000*). In the ultrafine hypothesis, Oberdörster and colleagues suggested that particles smaller than $0.02 \mu\text{m}$ might elicit a strong and persistent pulmonary inflammation (*Oberdörster et al., 1994 and 1996*). This hypothesis has been extended by other researchers and theorises that the very large

surface of absorption offered by this particle size may carry toxicants into the deep lung, and may cause inflammation, adhesion molecule expression, altered blood coagulation and cardiac electrical aberrations as mechanisms of disease (*Oberdörster et al., 1995, Utell and Frampton, 2000 and Dockery, 2001*).

Results of experimental studies with ultrafine particles composed of low-toxicity materials such as polystyrene have also shown an increased inflammatory response with increased total surface area, without any contribution from other factors such as co-pollutants or transition metals. This suggests that surface area drives inflammation in the short term and that ultrafine particles cause a greater response because of the greater surface area they possess (*Brown et al., 2001*).

4.3.2.3 The number of particles

Some epidemiological studies have found good associations between the number of ultrafine particles per unit volume of air and the occurrence of respiratory effects (*Peters et al., 1997*). *Oberdörster et al., (1994 and 1996)* have proposed that ultrafine particles, even at small mass concentrations, may have serious negative health effects because of their high number concentrations. It has also been suggested that high numbers of ultrafine particles in the alveolar region may overwhelm the alveolar macrophages, resulting in decreased clearing efficiency of ultrafines from the alveoli (*Oberdorster, 2001 and ILSI, 2000*).

4.3.2.4 Surface properties and chemical composition

The health effects observed after ultrafine particle exposure have been attributed to chemical agents that may be delivered to the airways and alveoli through the deposition of particles. These include acidity, hydrogen peroxides, nitrates, sulphates, organic carbon and acid aldehydes (*Kennedy et al., 1989 and Costa and Dreher, 1997*). Recent analyses have reported water-soluble species, including calcium, sodium, ammonium ion, nitrate, and sulphate in the ultrafine atmospheric particles. Other substances detected in the ultrafine range were potassium, iron, copper, zinc, and strontium (*Chung et al., 2001*). Reactive metal ions produce oxidative lung injury due to the formation of reactive oxygen and/or reactive nitrogen species (*Kennedy et al., 1989 and Costa and Dreher, 1997*). Metals that have been proposed to play a role include nickel (*Dreher et al., 1997*), vanadium and copper (*Kennedy et al., 1998*).

The complexity of the surface chemistry also impacts on the bioreactivity of ultrafine particles. *Murphy et al. (1999)* have shown that ultrafine particles with diverse surface chemical constituents attached more easily to the extracellular matrix than particles with uniform surface chemical compositions. Another indication of the importance of surface properties is the finding that hydrophobic and hydrophilic particles of the same size elicit pulmonary-inflammatory responses of different strengths, although conflicting relationships have been described in the literature (*Pott et al., 1998 and Oberdörster, 2001*).

Under certain circumstances, the electric charge on ultrafine particles can also determine the particle deposition pattern. Experimental studies conducted in vivo with humans and with airway models have all shown an increase in respiratory tract deposition due to particle charge. However, enhanced deposition by electrical forces was noted only if the charge on the particle exceeded a threshold value, which depended on the particle size as well as the airway size (*Melandri et al., 1983 and Cohen et al., 1998*).

Concerning specifically silica particles, a direct relationship was found between the surface intensity of free silanol groups and the experimental lysing of red blood cells. Although haemolytic activity is not definitive proof of fibrogenicity, monitoring of the surface intensity of free silanol groups might be useful to determine the cytotoxic activity of silica particles (*Pandurangi et al., 1990*).

4.3.2.5 The unique deposition of inhaled ultrafine particles

Ultrafine particles may exist as singlet particles or as aggregates. In the form of aggregates their deposition characteristics can change, as the aggregates would have a greater aerodynamic diameter than the singlet particles. The aerodynamic characteristics of the aggregates would, however, vary depending on their compactness. If the aggregate is more open with chains and extensions then, like thistledown, it will have greater aerodynamic resistance and the likelihood of settling will be less (*Donaldson et al., 2001*). According to the International Commission on Radiological Protection (ICRP) model for particle deposition in the human respiratory tract, alveolar deposition is highest for inhaled singlet (as opposed to aggregated) ultrafine particles of around 20 nm (0.020 µm) diameter (*ICRP, 1994*).

It was previously believed that ultrafine particles would not deposit in significant concentrations in the alveolar region, due to a mechanism referred to as Brownian diffusion. However, this impression has been shown to be incorrect. Several studies have indicated effects in laboratory animals exposed to ultrafine particles that could not be repeated in exposures to non-ultrafine particles of the same chemical composition in the same study (*Donaldson et al., 2001*). This may be indirect evidence, but it is difficult to imagine health effects without particle-to-cell contact in the epithelial interface of the lung. Some recent studies present direct evidence that inhaled ultrafine particles are deposited in the lungs (*Frampton et al., 2000*) and that the experimental deposition is consistent with deposition model predictions. *Jaques and Kim (2000)* reported that inhalation of decreasing ultrafine particle sizes result in increased total lung deposition. *Brown et al., (2002)* was able to demonstrate deposition of technetium-99m-labelled particles in volunteers and average 24-hour lung retention of deposited particles of 80 to 90 per cent.

4.3.3 Mechanisms of particle-induced respiratory health effects

4.3.3.1 General

The greater pathogenicity of ultrafine particles can be explained through experimental observations of more intense inflammatory responses, increased fibrogenic potential, and increased oxidant-generating abilities (*Ferin et al., 1992, Oberdörster et al., 1994, 1995 and 1996, Li et al., 1996, Gilmour et al., 1997, Donaldson and MacNee, 1998 and Churg et al., 1999*). The chemical, cellular, physiological and biochemical mechanisms that underlie these effects are discussed below.

4.3.3.2 Immunological mechanisms

Studies in rats have indicated that ultrafine carbon black particles show greater pro-inflammatory effects compared to fine particles (*Li, et al., 1996 and Brown et al., 2000*). Alveolar macrophages, lymphocytes and neutrophils can be stimulated to release a variety of cytokines including platelet activating factor and tumour necrosis factor (*Sibille and Reynolds, 1990, Agostini et al., 1993 and Sibille and Merchandisi, 1993*). Airway epithelial cells have been shown to act directly as immune effector cells by releasing cytokines, such as interleukin-8 (*Staniford et al., 1990*), granulocyte-macrophage colony stimulating factor (*Marini et al., 1992*) and platelet activating factor (*Salari and Wong, 1990*). The releases of these factors mediate the inflammatory and fibrogenic response of the lungs to the inhalation of ultrafine particles.

4.3.3.3 Reactive oxygen species generation

Ultrafine particle exposure induces oxidative stress as a result of reactive oxygen species production (*Li, et al., 1996 and Brown et al., 2001*). Increased generation of oxidant species, e.g. the hydroxyl radical, is an important finding, since these are known to have pro-inflammatory effects and to result in tissue damage (*Dreher et al., 1997 and Kennedy et al., 1989*). According to the transition metal hypothesis, mentioned previously, reactive metal ions that occur on the surface or in particles produce an oxidative lung injury due to the formation of reactive oxygen and/or reactive nitrogen species (*Kennedy et al., 1989 and Costa and Dreher, 1997*).

Metals that have been proposed to play a role include nickel (*Dreher et al., 1997*), vanadium and copper (*Kennedy et al., 1998*). However, reactive oxygen generation have also been shown to occur independent of transition metal exposure (*Brown et al., 2000*). *MacNee and Donaldson (2000)* reviewed the issue and concluded that preliminary data in vitro and in vivo suggest that local and systemic oxidative stress occur in response to ultrafine particles and that the effects of such oxidative stress on pro-inflammatory gene regulation and changes in blood coagulation may result in the adverse effects of particulate air pollution.

4.3.3.4 Differential gene expression

Chemically identical dusts of differing size can produce quite different patterns of gene expression in the airway wall. *Churg et al. (1999)* found that ultrafine particles (in this case, 0.021 μm diameter) were intrinsically able to induce pro-collagen expression, even in the absence of inflammatory cells. This would explain ultrafine particle-mediated increases in airway wall fibrosis. *MacNee and Donaldson (2000)* concluded that ultrafine particles stimulate both local and systemic oxidative stress that impacts on pro-inflammatory gene regulation, resulting in the adverse effects of particulate air pollution.

4.3.3.5 Interaction of gaseous and particulate pollutants in the respiratory tract

Biological responses to the inhalation of polluted atmospheres may depend upon the interplay between individual materials. *Schlesinger* has already proposed in 1995 that characterising effects from exposures to mixtures of air pollutants is necessary for adequate quantification of health risks. Mixtures may act synergistically, resulting in more-than-additive effects, or interactions may be antagonistic (*Schlesinger, 1995*). The occurrence and type of interaction depends on numerous factors, including the biological endpoint and the specific exposure conditions, such as concentration, duration, and the physico-chemical characteristics of the exposure atmosphere. Proposed mechanisms of interaction include physical adsorption, chemical reactions in the exposure atmosphere, or on a particle surface, and alteration of the pulmonary environment.

An example of synergism resulting from gas adsorption onto the particle surfaces is the exacerbation of the fibrogenicity of quartz by adsorbed NO_2 , presented in an early animal study by *Shevchenko (1971)*. With regard to alterations of the pulmonary environment, *Last (1989)* has hypothesised that synergism between oxidant gases and acidic sulphate particles would result from a shift in the local micro-environmental pH of the lung following deposition of the acidic particles, enhancing the effects of the co-inhaled gas by producing a change in their reactivity or residence time of reactants, such as free radicals, involved in oxidant-induced tissue injury. Under specific conditions, however, antagonism could also result (*Schlesinger, 1995*).

4.3.3.6 Inhibited disposal by macrophages

Ultrafine particles are probably more easily deposited in the lungs, as explained previously. In addition, the fate of ultrafine particles after their deposition may be very different from that of larger particles. Preliminary studies showed that decreasing particle size, down to the ultrafine size, was associated with decreased efficiency of phagocytosis by macrophages. It appears that deposited ultrafine particles are not as readily phagocytised by alveolar macrophages as are larger particles (*Oberdörster, 2001*).

Some studies demonstrated a direct deleterious effect of pre-exposure to ultrafine particles (about 20 nm in diameter) on the subsequent phagocytotic abilities of macrophages (measured with 2 μm diameter indicator beads). This effect was not seen with fine particle (about 200 nm diameter) exposure (*Donaldson et al., 2001*). It therefore seems that the adverse effects of ultrafine particles may be mediated in part by their ability to inhibit phagocytosis. Inhibition of phagocytosis would allow increased interaction between ultrafine particles and alveolar epithelial cells. Ultrafine particles could consequently penetrate much more rapidly to interstitial sites, possibly including the endothelium and even entering the blood circulation (*Oberdörster, 2001* and *Donaldson et al., 2001*).

4.3.4 Mechanisms of particle-induced systemic health effects

4.3.4.1 Particle penetration into the circulation

Recently, the hypothesis has evolved that ultrafine particles have a greater ability to penetrate the pulmonary interstitium (*Harrison and Yin, 2000*). The ultrafine hypothesis advanced by *Oberdörster et al. (1996)* suggests that ultrafine particles penetrate through the alveolar wall into the blood circulation and is deposited in the cardiac tissue, where it causes cardiac arrhythmia and death. Experimental animal studies indicated the penetration of ultrafine particles into the circulation and its deposition in several organs, including the heart, in relatively small percentages of the total inhaled dose (*Oberdörster, 2001*). *Nemmar et al. (2001)* described the clearance of denatured albumin from the lung into the blood circulation in hamsters and *Oberdörster (2000)* described the translocation of platinum particles (13 nm diameter) to the liver in rats.

Oberdörster et al. (2002) also demonstrated effective translocation of ultrafine elemental carbon particles to the liver of rats by 1 day after inhalation exposure. Translocation pathways included direct input into the blood compartment from ultrafine carbon particles deposited throughout the respiratory tract. However, it was concluded that input from ultrafine particles present in the gastrointestinal tract needs to be considered as well and that translocation to blood and extrapulmonary tissues may well be different between ultrafine carbon and other insoluble (metal) ultrafine particles. A recent human study showed translocation of inhaled isotope-labelled ultrafine carbon aerosols to the liver (*Nemmar et al., 2002*).

4.3.4.2 Ultrafine particles and cardiovascular effects

It has been suggested that the induction of airway inflammation, expression of leukocyte and endothelial adhesion molecules in blood, the alteration of blood coagulability and alteration in cardiac electrical activity may be involved in the exacerbation of underlying cardiorespiratory disease. It was hypothesised that airway inflammation may activate endothelium and circulating leukocytes, and induce a systemic acute phase response with transient hypercoagulability (*Utecht and Frampton et al., 2000*). *MacNee and Donaldson (2000)* reviewed the issue and concluded that preliminary data *in vitro* and *in vivo* suggest that both local and systemic oxidative stress occur in response to ultrafine particles and that the effects of such oxidative stress on changes in blood coagulation may result in the adverse effects of particulate air pollution on the cardiovascular system. The hypothesis of systemic effects is supported by a study by *Donaldson et al. (2001)* on carbon black (11 nm primary particle size) that reported global oxidative stress in the plasma and increased plasma factor VII, which is an independent risk factor for cardiovascular disease.

The cytokine hypothesis involves the release of secondary messengers (cytokines) by various pulmonary cells into the circulation, ultimately resulting in particulate-matter induced health effects (*Seaton et al., 1995*). Cytokines, including tumour necrosis factor and interleukins, have been linked to cardiac arrhythmias and increased levels of platelet-activating factor, a clotting factor implicated in atherosclerosis and cardiac thrombosis. The principal objection to the cytokine hypothesis as applied to cardiac and circulatory effects, is the short half-lives of cytokines in the blood (*Neas, 2000*).

The vagal nerve hypothesis involves the response of the autonomic nervous system to pulmonary irritants. The C-fibres in the lung and other receptors detect the initial irritation of airways and alveoli by particulate matter and the afferent arm of the vagus nerve transmits the signal to the respiratory centres. Signalling in this nerve is associated with measures of autonomic dysfunction, such as increased heart rate and decreased heart rate variability. This, in turn, is associated with adverse coronary events and death (*Neas, 2000*).

4.3.5 Host susceptibility factors

Factors such as advanced age; specific disease states, pre-existing inflammation in the lungs and sensitisation due to pre-exposures to sensitising agents appear to influence the susceptibility of individuals to the health effects of ultrafine particles. The implications of susceptibility is potentially so important that very low concentrations may have no noticeable effect on "normal" individuals, but may only become evident in susceptible persons (*Donaldson et al., 2001*).

Individuals with compromised respiratory tract function due to pre-existing disease states (e.g. emphysema and early stages of respiratory tract infection with gram negative bacteria) are more sensitive than healthy individuals. Aged individuals also seem to be at a higher risk of oxidative stress-induced lung injury (*Oberdörster, 2001* and *Elder et al., 2000*). A theoretical model of deposition of 20 nm particles in human alveoli showed increased deposition and retention of nanoparticles in pathological alveoli, that is alveoli with higher stiffness of the alveolar wall (*Graton et al., 2000*). This would contribute to the increased sensitivity noticed in individuals with pre-existing disease states. Other authors have also concluded that pathological changes in the lungs, such as the airway narrowing found in chronic obstructive pulmonary diseases (COPD) and asthma cause an increase in the efficiency of deposition of ultrafine particles (*Anderson, et al., 1990, Donaldson et al., 2001* and *Brown et al., 2002*).

4.3.6 Conclusions on the status on knowledge about the toxicity of ultrafine particles

Experimental studies indicate that ultrafine particles are more toxic than coarse particles with the same chemical composition. This greater toxicity has been linked to increased surface areas and increased particle numbers at the same mass concentrations. Absorbed chemicals on the surface may also play a role. Physiological considerations such as increased alveolar deposition of ultrafine particles and decreased efficiency of phagocytosis by macrophages are also influential in the health outcomes of ultrafine particle exposure.

Ultrafine particles have respiratory as well as systemic effects and several hypotheses for the mechanisms governing these effects have been forwarded. The biochemical, cytological and physiological reactions that are triggered by interactions between ultrafine particles and pulmonary cells are being delineated by experimental studies. This should develop a clearer picture of the molecular mechanisms underlying the pathological manifestations of inflammation and fibrogenesis. Knowledge of these mechanisms will hopefully provide an opportunity for more specific regulation of ultrafine particles. Finally, regulation of exposure to ultrafine particles should consider the host susceptibility of the receptor population, since important factors that influence the outcome of exposure have been identified.

5 Limitations and conclusions of the investigation

5.1 Limitations

This SIMRAC investigation has reaffirmed the difficulties in obtaining reliable data on the crystalline nature of ultrafine silica fume. Assessment of the samples collected at the upper electrode floor and the charge floor and tap hole locations in a typical smelter failed to confirm the presence of crystalline silica. The result is however inconclusive, because it cannot be said that crystalline phases are definitely absent. As stated, it was 95 per cent certain that any possible crystalline phases, if present, would represent less than 1 per cent of sampled particles. Increasing the confidence of stating that crystalline silica particles were absent from those samples in which they were not detected is impractical, because this would require an enormous increase in the number of particles that would have to be surveyed. This is a practical limitation of the transmission electron microscopy (TEM) technique.

Although crystalline structures were clearly visible in the electron micrographs of the refining station sample, only one sample was available for analysis. This does not place doubt on the finding, but it should add value if more samples could be characterised.

It should be noted that the process of formation of silica fume at the refining station, where crystalline silica was identified very clearly, is quite different from processes under conditions in the immediate furnace zones. Silicon monoxide forms at the furnace, which after release into the air condenses into silica fume. At the refining station, oxygen is bubbled through red-hot molten silicon, which provides different thermodynamic conditions for the formation of silica fume, and crystalline silica. It may therefore be possible that this is the only area in the silicon production process where crystalline silica could form together with amorphous silica fume. The current investigation could however not prove this unambiguously.

The electron microscopy evidence does not provide proof of the singlet or aggregated nature of the ultrafine amorphous crystalline particles. This may influence the relevance of ultrafine particle effects, since aggregated particles are likely to be absorbed in the upper airways and the bronchi, limiting alveolar exposure.

5.2 Conclusions

This SIMRAC study has produced clear evidence of crystalline silica in silica fume that forms where oxygen is bubbled through molten silicon. It can be stated at the 99-per cent level of confidence that the concentration of the needle-like crystals does not exceed 1 per cent of the number of particles that were surveyed. Transmission electron microscopy (TEM) coupled with energy dispersive spectroscopy (EDS) was a successful technique for evaluating the morphology and chemical composition of silica fume, although some practical limitations applied. It is clear that X-ray diffraction, widely used in occupational hygiene surveys, is not the technique of choice when detailed analysis of mostly amorphous ultrafine particles with very low concentrations of crystalline particles is required.

Assessment of the samples collected at the upper electrode floor and the charge floor and tap hole locations in a typical smelter failed to confirm the presence of crystalline silica, but it cannot be said that crystalline phases are definitely absent. As discussed above, increasing the confidence of stating that crystalline silica particles were absent from those samples in which they were not detected is impractical, because this would require an enormous increase in the number of particles that would have to be surveyed.

The concentration of crystalline particles in the sample from the refining station was very low (less than 1 per cent of silica particles were crystalline), but this does not exclude the possibility of health effects in occupationally exposed individuals. Occupational studies showed that exposures to natural diatomite (contaminated with between 0.1 and 4 per cent crystalline silica) were associated with simple fibrosis (reviewed in *Merget et al., 2002*). Animal studies indicate that such fibrotic effects might at least partially resolve after discontinuation of exposure. Ultrafine amorphous silica particles are distinct aetiological entities to crystalline silica particles, which differ in respect of their long-term health effects. However, in situations of exposure to amorphous ultrafine particles, which also involve exposure to low or very low levels of crystalline silica, the exposure context is different from that of isolated exposure to amorphous ultrafine particles.

6 Discussion and recommendations

In most cases where silicosis was observed after exposure to amorphous silica, it was acknowledged that exposures were mixed, with both amorphous and crystalline silica being present in the dusts/fumes (*Galton-Fenzi, 1998*). A major limitation in most of the studies is that exposure levels were not known accurately and, where dust levels were measured, the crystalline content was uncertain. Furthermore, many of the studies considered other types of amorphous silica, and not silica fume.

Under current guidance of the American Conference of Governmental Industrial Hygienists (ACGIH), silica fume has a threshold limit value (TLV) of 2 mg/m^3 . This value was changed in 1992 from 0.2 mg/m^3 to 2 mg/m^3 , a ten-fold increase. *Cunningham, Todd and Jablonski (1998)* reviewed the documentation used to support the initial decision in setting the 0.2 mg/m^3 guideline in 1989, and compared those with the data used to support the revised 2 mg/m^3 TLV. Additional information was also considered. A major consideration by *Cunningham et al. (1998)* was the fact that silica fume in a smelter is an ultrafine aerosol, which would have a very different and far more serious deposition pattern in the respiratory system than the substance described in the documentation considered by ACGIH in their revision. Furthermore, *Cunningham et al. (1988)* highlighted several other disagreements with the interpretations of ACGIH. Overall, the authors concluded that there was insufficient justification for the tenfold increase in the TLV for silica fume.

More recent work on ultrafine particles has added to the evidence that ultrafine particles clearly have respiratory effects that include inflammatory responses, while amorphous silica exposure can clearly result in lung fibrosis. The aetiology of silicosis involves both of these physiological processes. It is possible that co-exposure to ultrafine particles might sensitise the lung to silicotic effects by stimulating recruitment and activation of inflammatory cells and the amplified release of fibrogenic factors, preparing the scene for low concentrations of crystalline particles to trigger a silicotic effect. The dose-effect relationship for the development of silicosis under such conditions is not known, and it is therefore difficult to estimate an appropriate occupational exposure threshold level.

It has now been confirmed unambiguously, however, that small concentrations of crystalline silica can be present in amorphous silica under certain process conditions, and this fact should affect the overall interpretation of the association between exposure to silica fume and the potential for development of silicosis. The primary objectives should now be to obtain more information to quantify the crystalline silica content, and to establish which type of crystalline silica actually forms. This is an important aspect, because the current understanding is that alpha quartz is less fibrogenic than cristobalite and tridymite, and different exposure guidelines are followed by regulatory agencies for these forms.

Previous assessments of the prevalence of pneumoconiosis amongst workers in specific areas of South African smelter plants did not address the refining station *per se*. It is therefore, unfortunately, not possible to integrate the TEM findings with relevant health effects. However, South African health investigators have previously cautioned that "*thermally generated silica fume should be viewed as a respiratory health hazard*" (*White, 2002*). Retrospective information on cases and suspected cases of silicosis associated with exposure to silica fume may be available from these studies, and it should be a logical next step to integrate the understanding of that information with the work done so far in this investigation.

Because the TEM technique is very expensive and not suitable for routine determination of crystalline silica in occupational hygiene samples, it is recommended that the techniques of neutron diffraction, and possible refinement of X-ray diffraction and Fourier-transform infrared spectrometry be considered for feasibility as routine analytical techniques.

The current scientific understanding of the fibrogenic effects of ultrafine amorphous silica fume, irrespective of the potential for development of silicosis, also suggests a re-assessment of the guidelines that have been promulgated in the interest of protection of occupational health. The fibrogenic effects associated with exposure to amorphous silica fume may be transient, but they still classify as adverse. Both the U.S. National Institute for Occupational Safety and Health (NIOSH) and the U.S. Occupational Safety and Health Administration (OSHA) have set guidelines for various amorphous forms of silica, but not for silica fume *per se*. The ACGIH occupational exposure guideline for silica fume is 2 mg/m^3 , but that has been questioned convincingly by *Cunningham et al (1998)*. The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has set a guideline (MAK) value of 0.3 mg/m^3 for fine dust for several forms of amorphous silica together. The guideline

concentration used in South Africa for assessment of exposure to respirable amorphous SiO₂ is 3 mg/m³, which is actually at the level recommended for diatomaceous earth in the ACGIH TLVs. There appears to be insufficient justification to retain this guideline in view of the available evidence on adverse health effects of ultrafine silica fume. It is therefore apparent from this SIMRAC study that the occupational exposure guideline for amorphous silica fume should be revised. Setting a new guideline has to follow a specific regulatory process, and has to be initiated by the Department of Minerals and Energy.

7 References

Agostini, C., Chilosi, M., Zambello, R., Trentin, L. and Semenzato, G. 1993. Pulmonary immune cells in health and disease: lymphocytes. *European Respiratory Journal* 6:1378-1401.

Anderson, P.J., Wilson, J.D. & Hiller, FC. 1990. Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. *Chest*. 97:1115-1120.

Brown, D. M., Stone, V., Findlay, P., MacNee, W. & Donaldson, K. 2000. Increased inflammations and intracellular calcium caused by ultrafine carbon black is independent of transition metals or other soluble components. *Occupational and Environmental Medicine* 57:685-91.

Brown, D. M., Wilson, M. R., Stone, V., Findlay, P., MacNee, W. & Donaldson, K. 2001. Size-dependent pro-inflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology & Applied Pharmacology*. 175:191-199.

Brown J. S., Zeman, K. L. & Bennett, W. D. 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. *American Journal of Respiratory & Critical Care Medicine*. 166:1240-7.

Castranova, V., Vallyathan, V. & Wallace, W. E. 1996. *Silica and Silica-induced Lung Diseases*. Boca Raton: CRC Lewis Publishers, 417p.

Choudat, D., Frisch, C., Barrat, G., Kholti, E. I. & Conso, F. 1990. Occupational exposure to amorphous silica dust and pulmonary function. *British Journal of Industrial Medicine* 47:763-766.

Churg, A., Gilks, B. & Dai, J. 1999. Induction of fibrogenic mediators by fine and ultrafine titanium dioxide in rat tracheal explants. *American Journal of Physiology* 277:L975-L982.

Chung, A., Herner, J. D. & Kleeman, M. J. 2001. Detection of alkaline ultrafine atmospheric particles at Bakersfield, California. *Environmental Science & Technology*. 35:2184-90.

Cohen, B. S., Xiong, J. Q., Fang, C.-P. & Li, W. 1998. Deposition of charged particles on lung airways. *Health Physics* 74:554-560.

Costa, D. L. & Dreher, K. L. 1997. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animals. *Environmental Health Perspectives* 105 (Suppl 5):1053-1060.

Cunningham, E. A., Jablonski, W., & Todd, J. J. 1996. Electron Microscopy Studies of Silica Fume Emissions from a Silicon Smelter in Southern Tasmania, Australia. *American Industrial Hygiene Association Journal*, 57(11):1024-1034.

Cunningham, E. A., Todd, J. J. & Jablonski, W. & 1998. Was There Sufficient Justification for the 10-Fold Increase in the TLV for Silica Fume? A Critical Review. *American Journal of Industrial Medicine*, 33:212-223.

- Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G. & Speizer, F.E. 1993.** An association between air pollution and mortality in six U. S. cities. *New England Journal of Medicine* 329:1753-1759.
- Dockery, D.W. 2001.** Epidemiologic evidence of cardiovascular effects of particulate air pollution. *Environmental Health Perspectives* 109:483-486.
- Donaldson, K., Li, X. Y., MacNee, W. 1998.** Ultrafine (nanometre) particle mediated lung injury. *Journal of Aerosol Science* 29:553-560.
- Donaldson, K., Stone, V., Clouter, A., Renwick, L. and MacNee, W. 2001.** Ultrafine particles. *Occupational and Environmental Medicine* 58:211-216.
- Dreher, K. L., Jaskot, R. H., Lehmann, J. R., Richards, J. H., McGee, J. K., Ghio, A. J. & Costa, D. L. 1997.** Soluble transition metals mediate residual oil fly ash induced acute lung injury. *Journal of Toxicology and Environmental Health* 50:285-305.
- Elder, A. C. P., Gelein, R., Finkelstein, J. N., Cox, C. & Oberdörster, G. 2000.** Endotoxin priming affects the lung response to ultrafine particles and ozone in young and old rats. *Inhalation Toxicology* 12(Suppl 1):85-89.
- Ferin, J. G., Oberdörster, G. & Penney, D. P. 1992.** Pulmonary retention of ultrafine and fine particles in rats. *American Journal of Respiratory Cell Molecular Biology* 6:535-542.
- Frampton, M. W., Chalupa, D., Morrow, P. E., Gibb, F. R., Oberdörster, G., Boscia, J., Speers, D. M., & Utell, M. J. 2000.** Deposition of inhaled ultrafine carbon particles in resting healthy nonsmoking adults. *American Journal of Respiratory Critical Care Medicine* 161:A257 (Abstract).
- Ferch, H., Gerofke, H., Itzel, H., & Klebe, H. 1987.** Examination of workers with long-term occupational exposure to Aerosil (in German, referenced in *Merget et al., 2001*). *Arbeitsmed Sozialmed Präventivmed* 22:33-37.
- Galton-Fenzi, B. 1998.** Thermally generated silica fume – is it a non-malignant respiratory hazard? Western Australia, International Association for the Promotion of Silicon Metal, 50p plus Annexures 16p.
- Gilmour, P. S., Brown, D. M., Beswick, P. H., Benton, E., MacNee, W. & Donaldson, K. 1997.** Surface free radical activity of PM₁₀ and ultrafine titanium dioxide: a unifying factor in their toxicity? *Annals of Occupational Hygiene* 41(Suppl):32-38.
- Gradon, L., Orlicki, D & Podgorski, A. 2000.** Deposition and retention of ultrafine aerosol particles in the human respiratory system. Normal and pathological cases. *International Journal of Occupational Safety and Ergonomics* 6:189-207.
- Harrison, R. M & Yin, Y. 2000.** Particulate matter in the atmosphere: which particle properties are important for its effects on health? *The Science of the Total Environment* 249:85-101.
- Hetland, R. B., Schwarze, P. E., Johansen, B. V., Myran T., Uthus, N. & Refsnes, M. 2001.** Silica-induced cytokine release from A549 cells: importance of surface area versus size. *Human and Experimental Toxicology* 20:46-55.
- Iler, R. K. 1979.** The chemistry of silica. Van Nostrand Reinhold, 866p.

ILO, 1972. The definition of pneumoconiosis. International Labour Office Geneva. (*In: Encyclopedia of occupational health and safety*, 2, Appendix 7. New York, McGraw Hill Book Co., p.1158).

ILSI Workshop. 2000. The relevance of the rat lung response to particle overload for human risk assessment. *Inhalation Toxicology* 12:1-2.

International Commission on Radiological Protection (ICRP). 1994. Annals of the ICRP. Human Respiratory Tract Model for Radiological Protection. A report of the Task Group of the ICRP. *ICRP Publication 66*. Oxford: Pergamon Press.

Jaques, P. A. & Kim, C.S. 2000. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. *Inhalation Toxicology* 12:715-31.

Kennedy, T. P., Dodson, R., Rao, N. V., Ky, H., Hopkins, C., Baser, M., Tolley, E. & Hoidal, J. R. 1989. Dusts causing pneumoconiosis generate $\cdot\text{OH}$ and produce hemolysis by acting as Fenton catalysts. *Archives of Biochemistry and Biophysics* 269:359-364.

Kennedy, T., Ghio, A. J., Reed, W., Samet, J., Zagorski, J., Quay, J., Carter, J., Dailey, L., Hoidal, J. R. & Devlin R. B. 1998. Copper-dependent inflammation and nuclear factor- κB activation by particulate air pollution. *American Journal of Respiratory Cellular and Molecular Biology* 19:366-378.

Kumar, V., Cotran, R. S. & Robbins, S. L. 1997. Environmental Diseases. *In: V. Kumar, R. S. Cotran & S. L. Robbins (Ed.), Basic Pathology, 6th Ed.*, New York: W. B. Saunders Company, pp. 221 – 262.

Last, J. A. 1989. Effects of inhaled acids on lung biochemistry. *Environmental Health Perspectives* 79:115-119.

Lee, K. P. & Kelly, D. P. 1993. Translocation of particle-laden alveolar macrophages and intra-alveolar granuloma formation in rats exposed to Ludox colloidal amorphous silica by inhalation. *Toxicology* 77:205-222.

Lehnert, B. E., Valdez, Y. E., Stewart, C. C. 1986. Translocation of particles to the tracheobronchial lymph nodes after lung deposition: kinetics and particle-cell relationships. *Experimental Lung Research* 10:245-266.

Li, X. Y., Gilmour, P. S., Donaldson, K. & MacNee, W. 1996. Free radical and pro-inflammatory activity of particulate air pollution (PM_{10}) in vivo and in vitro. *Thorax* 51:1216-1222.

MacNee, W. & Donaldson, K. 2000. How can ultrafine particles be responsible for increased mortality? *Monaldi Archives for Chest Disease* 55:135-9.

Marini, M., Vittori, J., Hollemborg, J. & Mattoli, S. 1992. Expression of potent inflammatory cytokines, granulocyte-macrophage colony stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *Journal of Allergy and Clinical Immunology* 89:1001-1009.

McLaughlin, J. K., Chow, W.-H. & Levy, L. S. 1997. Amorphous silica: a review of health effects from inhalation exposure with particular reference to cancer. *Journal of Toxicology and Environmental Health* 50:553-566.

Melandri, C., Tarroni, G., Prodi, V., DeZaiacomo, T. G., Formignani, M. & Lombardi, C. C. 1983. Deposition of charged particles in the human airways. *Journal of Aerosol Science* 14:657-669.

Merget, R., Bauer, T., Küpper, H. U., Philippou, S., Bauer, H. D., Breitstadt, R. & Bruening, T. 2002. Health hazards due to the inhalation of amorphous silica. *Archives of toxicology*, 75:625-634.

Mitchell, R. N. & Cotran, R. S. 1997. Repair: Cell regeneration, fibrosis, and wound healing. In: V. Kumar, R. S. Cotran & S. L. Robbins (Ed.), *Basic Pathology*, 6th Ed., New York: W. B. Saunders Company, pp. 47 – 59.

Mohrmann, W. & Kann, J. 1985. Amorphous silica can cause silicosis. In: *Case reports* (in German). Stuttgart: Gentner Verlag, p. 587.

Murphy, S. A., Bérubé, K. A., Pooley, F. D. & Richards, R. J. 1998. The response of lung epithelium to well characterised fine particles. *Life Science* 62:1789-1799.

Murphy, S. A. M., Bérubé, K. A. & Richards, R. J. 1999. Bioreactivity of carbon black and diesel exhaust particles to primary Clara and type II epithelial cell cultures. *Occupational and Environmental Medicine* 56:813-819.

Neas, L. M. 2000. Fine particulate matter and cardiovascular disease. *Fuel Processing Technology* 65-66:55-67.

Nemmar, A., Vanbilloen, H., Hoylaerts, M. F., Hoet, P. H. M., Verbruggen, A. & Nemery, B. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *American Journal of Respiratory and Critical Care Medicine* 164:1665-1668.

NIOSH, 2000. Method 7501: Silica, amorphous. NIOSH Manual of Analytical Methods. Cincinnati, Ohio, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health.

Oberdörster, G., Ferin, J. & Lehnert, B.E. 1994. Correlation between particle size, in vivo particle persistence, and lung injury. *Environmental Health Perspectives* 102 (Suppl. 5):173-179.

Oberdörster, G., Gelein, R.-M., Ferin, J. & Weiss, B. 1995. Association of particle air pollution and acute mortality: involvement of ultrafine particles? *Inhalation Toxicology* 7:11-124.

Oberdörster, G., Finkelstein, J., Ferin, J., Godleski, J., Chang, L. Y., Gelein, R., Johnston, C. & Crapo, J.D. 1996. Ultrafine particles as a potential environmental health hazard. Studies with model particles. *Chest* 109:68S-69S.

Oberdörster, G., Finkelstein, J. N., Johnston, C., Gelein, R., Cox, C., Baggs, R. & Elder, A. 2000. *Investigator's Report: Acute pulmonary effects of ultrafine particles in rats and mice.* The Health Effects Institute Report 96. Cambridge, MA: Health Effects Institute.

Oberdörster, G. 2001. Pulmonary effects of inhaled ultrafine particles. *International Archive of Occupational and Environmental Health*, 74:1-8.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W. & Cox, C. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *Journal of Toxicology & Environmental Health Part A*. 65:1531-1543.

Olbrück, H., Seemayer, N. H., Voss, B. and Wilhelm, M. 1998. Supernatants from quartz dust treated human macrophages stimulate cell proliferation of different human lung cells as

well as collagen-synthesis of human diploid lung fibroblasts in vitro. *Toxicology Letters* 96: 97:85-95.

Pandurangi, R. S., Seehra, M. S., Razzaboni, B. L. & Bolsaitis, P. 1990. Surface and bulk infrared modes of crystalline and amorphous silica particles: a study of the relation of surface structure to cytotoxicity of respirable silica. *Environmental Health Perspectives* 86:327-336.

Peters, A., Wichmann, H. E., Tuch, T., Heinrich, J. & Heyder, J. 1997. Respiratory effects are associated with the number of ultrafine particles. *American Journal of Respiratory and Critical Care Medicine* 155:1376-1383.

Philippou, S., Teschler, H., Morgenroth, K. 1992. Lung fibrosis after inhalation of amorphous silica (in German). *Zentralblatt Pathologie* 138:41-46.

Pott, F., Althoff, G.-H., Roller, M., Hohr, D. & Fienmann, J. 1998. High acute toxicity of hydrophobic ultrafine titanium dioxide in an intratracheal study with several dusts in rats. In: Mohr, U. (editor in chief) *ILSI Monographs*. Dungworth, D. L. et al., (eds) *Relationships between respiratory diseases and exposure to air pollution*. Washington: ILSI Press, pp. 270-277

Pratt, P. C. 1983. Lung dust content and response in guinea pigs inhaling three forms of silica. *Archives of Environmental Health* 38:197-204.

Prendergast, T. J. & Ruoss, S. J. 2000. Pulmonary disease. In: S. J. McPhee, V. R. Lingappa, W. F. Ganong and J. D. Lange (Eds), *Pathophysiology of disease*, 3rd Ed., New York: Lange Medical Books/McGraw-Hill, pp. 184 – 221.

Salari, H. & Wong, A. 1990. Generation of platelet activating factor (PAF) by a human epithelial cell line. *European Journal of Pharmacology* 175:253-259.

Schlesinger, R.B. 1995. Interaction of gaseous and particulate pollutants in the respiratory tract: mechanisms and modulators. *Toxicology* 105:315 –325.

Seaton, A., MacNee, K., Donaldson, K. & Godden, D. 1995. Particulate air pollution and acute health effects. *Lancet* 345:176-178.

Shevchenko, A. M. 1971. The role of some physical and chemical properties of mine dust in the development of pneumoconiosis. In: W. H. Walton (Ed.), *Inhaled Particles III*, Surrey, U. K.: Unwin Bros.

Sibille, Y. & Reynolds, H. Y. 1990. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *American Review of Respiratory Disease* 141:471-501.

Sibille, Y. & Merchandisi, F.X. 1993. Pulmonary immune cells in health and disease: polymorphonuclear neutrophils. *European Respiratory Journal* 6:1529-1543.

Staniford, T. J., Kunkel, S. L., Basha, M. A., Chensue, S. W., Lynch, J. P., Toews, G. B., Westwick, J. and Strieter, R. M. 1990. Interleukin-8 gene expression by pulmonary epithelial cell line. A model for cytokine networks in the lung. *Journal of Clinical Investigation* 86:1945-1953.

Thomas, G. & Goringe, M. J., 1979. Transmission Electron Microscopy of Materials. Wiley Interscience Publication.

Tuling, A. 2003. *Report on the survey of SiO fume samples*. Report on analyses conducted at the IMMRI, University of Pretoria, for INFOTOX.

- Utell, M. J. & Frampton, M. W. 2000.** Acute health effects of ambient air pollution: the ultrafine particle hypothesis. *Journal of Aerosol Medicine* 13:355-359.
- Van Niekerk, W. C. A., Coombs, W. M., Simpson, S. F. & Retief, W. L. 2000.** Identification of health hazards relating to toxic substances in silicon smelters. *SIMRAC Project Health* 709.
- Vitums, V. C., Edwards, M J., Niles, N. R., Borman, J. O. & Lowry, R. D. 1977.** Pulmonary fibrosis from amorphous silica dust, a product of silica vapor. *Archives of Environmental Health* 32:62-68.
- Warheit, D. B. 2001.** Inhaled amorphous silica particulates: what do we know about their toxicological profiles? *Journal of Environmental Pathology, Toxicology and Oncology* 20 (Suppl 1):133-141.
- White, N. 2002.** Silicon Smelter Health Survey: Medical Recommendations. Presentation to INVENSIL.
- Wiessner, J H., Henderson J. D. Jr., Sohnle, P. G., Mandel N. S. and Mandel, G. S. 1988.** The Effect of Crystal Structure on Mouse Lung Inflammation and Fibrosis. *American Reviews of Respiratory Disease*, 138, (2), p. 445 450.
- Wilson, W. E. & Suh, H. H. 1997.** Fine particles and coarse particles: concentration relationships relevant to epidemiological studies. *Journal of Air and Waste Management Association* 47:1238-1249.