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**ASSESSMENT OF THE EFFECTS OF SILICA
EXPOSURE ON THE CLINICALLY AND
TOXICOLOGICALLY IMPORTANT BIOMARKERS**

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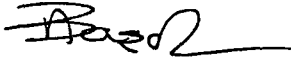
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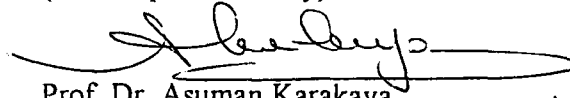
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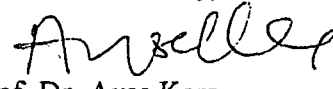
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ABSTRACT

Shubair, M., Assessment of the Effects of Silica Exposure on the Clinically and Toxicologically Important Biomarkers. Hacettepe University, Institute of Health Sciences Program of Pharmaceutical Toxicology Master of Sciences Thesis, Ankara, 2001. The abundance of silica in the environment is second to oxygen and it is one of the most documented workplace contaminants. Long term exposure to silica has been reported to be the principal cause of silicosis and other chronic respiratory abnormalities. Foundry work and making of pottery are some of the main industries in which workers may be exposed to silica. To assess the immune competence of workers occupationally exposed to silica, serum immunoglobulin and complement protein concentrations of 45 foundry and 22 pottery workers have been analysed and compared to that of 67 matched controls according to age, sex, and smoking habits. Significant decreases in serum IgG, IgA, and IgM levels of the foundry workers have been found compared to controls. In pottery workers although the levels were found to be low, the difference was not significant. No significant differences were observed in the serum complement C3 and C4 levels. To investigate the potential hazard associated with the occupational exposure to silica, DNA damage in the peripheral lymphocytes of 30 foundry and 22 pottery workers were examined by the alkaline single-cell gel electrophoresis 'comet' technique. The DNA damage observed in the lymphocytes of both foundry and pottery workers was significantly higher than that in their controls.

Key Words: Silica, Immunoglobulins, Complement Proteins, DNA Damage, Single-cell Gel Electrophoresis.

ÖZET

Shubair, M., Silika Maruziyetinin Klinik ve Toksikolojik Açidan Önemli Biyogöstergelere Etkisinin Değerlendirilmesi, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Farmasötik Toksikoloji Programı Yüksek Lisans Tezi, Ankara, 2001. Silika en iyi tanımlanmış iş yeri kirleticilerinden biri olup çevrede yaygınlığı oksijenden sonra ikinci sırada yer almaktadır. Uzun süren silika temasının silikozis ve diğer kronik solunum rahatsızlıklarının ana nedenlerinden biri olduğu bildirilmektedir. Döküm ve çömlek yapımı işçilerin silikaya temas ettikleri iş kollarının başında gelmektedir. Meslekleri nedeniyle silikaya maruz kalan işçilerin immün yeterliklerini değerlendirmek için 45 döküm ve 22 çömlek işçisinin serum immünoglobulin ve kompleman protein konsantrasyonları ölçülmüş ve yaş, cinsiyet ve sigara içme alışkanlıklarına göre eşdeştirilmiş 67 kontrol ile kıyaslanmıştır. Kontroller ile kıyaslandığında döküm işçilerinin serum IgG, IgA ve IgM düzeylerinde önemli azalmalar saptanmıştır. Çömlek işçilerinde düzeyler kontrollere göre düşükse de fark istatistiksel olarak önemli bulunmamıştır. Serum kompleman C3 ve C4 düzeylerinde önemli farklılıklar gözlenmemiştir.

Mesleki silika maruziyetiyle ilişkili olası tehlikeleri değerlendirmek için ayrıca 30 döküm, 22 çömlek işçisinin periferik lenfositlerindeki DNA hasarı alkali tek hücre jel elektroforez ya da 'comet' yöntemiyle değerlendirilmiştir. Hem döküm, hem de çömlek işçilerinde lenfositlerde gözlenen DNA hasarının kontrollerden önemli ölçüde yüksek olduğu gözlenmiştir.

Anahtar Kelimeler: Silika, İmmünoglobulinler, Kompleman Proteinleri, DNA Hasarı, Tek Hücre Jel Elektroforezi.

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ABBREVIATIONS

AA:	Arachidonic acid
AI-CFC:	Alveolar colony forming cells
BAL:	Bronchioalveolar lavage
CAT:	Catalase
IARC:	International Agency for Research on Cancer
IFN- γ :	Interferon-gamma
Ig:	Immunoglobulin
IL-1:	Interleukin 1
IL-2:	Interleukin 2
MDCF:	Monocyte-derived chemotactic factor
NAF:	Neutrophil-activating factor
NCF:	Neutrophil chemotactic factor
NF:	Nuclear factor
NIOSH:	National Institute for Occupational Safety and Health
NK:	Natural killer
NTM:	Nontuberculous mycobacteria
OSHA:	Occupational Safety and Health Administration
PEL:	Permissible Exposure Limit
PMN:	Polymorphonuclear cells
PPD:	Purified protein derivative
PSS:	Progressive systemic sclerosis
PWM:	Pokeweed mitogen
ROS:	Reactive oxygen species
SiO ₂ :	Silicon dioxide, silica
SLE:	Systemic lupus erythematosus
SMR:	Standardised mortality ratio
SOD:	Superoxide dismutase
TNF- α :	Tumour necrosis factor- α

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1. INTRODUCTION

Millions of workers in various occupational settings have the potential to be exposed to hazardous substances. Many of these substances are known to be genotoxic and cause genetic alterations in the target tissue of exposed workers. It has been also estimated that occupational exposure accounts for 4% of all human cancer.

On the other hand crystalline silica is probably one of the most documented workplace contaminants and its abundance in the environment is second to oxygen. Some of the industries in which workers may be exposed to silica are, foundry work, ceramics including the making of pottery, porcelain tiles, bricks etc., mining related milling operation, iron and steel milling, quarrying, glass and cement making and metal manufacturing. The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence in humans to show genotoxicity and carcinogenicity of inhaled crystalline silica from occupational sources. Long term exposure of silica sand has been considered to be the principal cause of silicosis and other chronic respiratory abnormalities. Furthermore, a variety of immune dysfunction related diseases has been reported in individuals exposed to silica.

In this study to assess the immune competence of workers occupationally exposed to silica serum immunoglobulin (Ig) and complement protein concentrations of workers from two silica-related workplaces have been analysed. Serum IgG, IgA, IgM and C3, C4 complement protein levels of 45 male foundry workers from small plants that were located in Ankara with a mean exposure period of 10 years and 22 male pottery workers from Nevşehir with a mean duration of exposure 14 years have been measured and compared to that of 67 matched controls according to age, sex and smoking habits.

To investigate the potential hazard associated with the exposure of silica and also to provide further data on the possible applicability of the new single-cell gel electrophoresis 'comet' technique in human biomonitoring studies, the eventual genetic damage in the peripheral lymphocytes of 30 foundry and 22 pottery workers were examined by the use of alkaline COMET assay and compared with their matched controls.



2. THEORITICAL PRINCIPLES

2.1. Structure, General Properties, Production, Use, Occurrence and Exposure

2.1.1. Structure

Silicon that is found in group IV A of the periodic table, beneath carbon, has a strong affinity for oxygen, and forms very stable silicon-oxygen bonds. In all naturally occurring silicon compounds ranging in form from the simple soluble orthosilicate $(\text{SiO}_4)_4$ to polymers having chain, planar, or sheet, or three-dimensional bulk structures, each silicon atom is attached to four oxygen atoms to form the tetrahedral SiO_4 unit. Silicon-oxygen tetrahedras are the basic units of all crystalline and amorphous forms. When silicon shares all of its oxygen atoms with other Si atoms; a three-dimensional framework is produced that has the empirical formula, SiO_2 , silicon dioxide, also called silica. All of the oxygen atoms serve as bridges between silica atoms in the three-dimensional polymer silica $(\text{SiO}_2)_x$. Silica is the end product in the complete polymerisation of the silicate monomeric anion [1, 2].

2.1.2. General Properties

Silica or silicon dioxide can be found in crystalline or amorphous forms. The natural types of crystalline forms are α , β quartz; α , β_1 , β_2 tridymite; α , β cristobalite; moganite etc. Whereas the synthetic types of crystalline forms are keatite, silica W, and porosils. The nomenclature used is that of α for a lower-temperature phase and β for a higher-temperature phase [2, 3]. The majority of natural crystalline silica exists as α -quartz, which is the thermodynamically stable form in ambient conditions. Amorphous forms of silica can be also natural and synthetic. Opal, biogenic silica, diatomaceous earths, silica fibres, and vitreous silica are natural types. Fused silica, pyrogenic silica, colloidal silica, and silica gel are synthetic amorphous forms [3, 4]. Opal is natural amorphous hydrous silica that may contain

cryptocrystalline cristobalite. Biogenic silica originates in living sources include bacteria, fungi, diatoms, sponges and plants. Diatomaceous earths are the geological products of decayed algae called diatoms. Vitreous silicas are volcanic glasses, and silica fibers are derived from plants. Amorphous silicas prepared for different uses differ in particle form and size, porous structure and residual water content. Fused silica is silica heated up to a liquid phase and cooled down without allowing it to crystallise. Pyrogenic silica is prepared by the combustion of a volatile silicon compound (usually SiCl_4). Silica gel is a coherent, rigid, spherical particles of colloidal microporous silica [2, 3, 5-7].

The stability of the polymorphs of silica is related to temperature and pressure. α -quartz is stable over most of the temperatures and pressures that characterise the earth's crust. Tridymite and cristobalite are formed at higher temperatures. The conversion from one crystalline structure to another requires the rupture of oxygen-silicon bonds and the reconstruction of new ones. This process requires very high activation energy. Although α -quartz is the only silica phase stable under ambient conditions, other silica polymorphs, namely α -tridymite, α -cristobalite, coesite and stishovite, exist with metastability at the earth's surface [7, 8].

Silica is poorly soluble in water and solubility is higher for the amorphous than for the crystalline forms. The solubility of the various forms of silicas is very complex and depends upon several factors. Solubility increases with temperature and pH and is affected by the presence of trace metals. Particle size also influences the rate of solubility [7].

Surface properties of silica are not only determined by the underlying crystalline structure but also by the origin and thermal and mechanical history of the dust and by the presence of contaminants. The surface of silica reacts with water vapour from the ambient air to form an external layer of silanol (SiOH). This process may be extremely low or very fast. The part of the surface covered with a dense layer of silanols is hydrophobic. The presence and extent of silanols at the surface of silica sample determines its hydrophilicity. Upon heating, silanols condense into siloxanes with elimination

of water: ($\text{SiOH} \rightarrow \text{Si} - \text{O} - \text{Si}$). Under the same conditions of humidity, the various polymorphs show a different degree of hydrophilicity quartz and stishovite being the most hydrophilic and pyrogenic amorphous silica the most hydrophobic [1-3, 9].

If grinding is performed in dry air, oxygen or hydrogen peroxide aqueous solutions, reactive oxygen species (ROS) are formed. Conversely, if grinding takes place in a wet atmosphere, silanols are formed rather than surface radicals [8]. In aqueous suspensions, freshly ground surfaces generates ROS [10]. Whether the ROS arise from the silica itself or from certain impurities exposed at the surface during the grinding procedure is still underdebate; acid washing decreases the radical yield [11]. Heating remove defects and radicals originated by grinding [12].

Etching with hydrofluoric acid, alkaline hydroxides, or catechol modifies the surface of silica sample. Etching also eliminates impurities that can modulate silica toxicity [13].

Major impurities in crystalline silica polymorphs include aluminium, iron, titanium, lithium, sodium, potassium and calcium. The concentrations of these impurities vary from specimen to specimen but are generally below 1.0% in weight as oxide. Metal impurities modify the surface reactivity of silica samples. Very pure quartz is rare. Very pure samples can be obtained from purification of the melt. Commercial products derived from silica sand; sandstone and quartzites are granular materials with a high silica content, mostly quartz. Impurities in this case may be up to 25% but are usually about 5% [2, 14, 15].

Synthetic amorphous silicas are generally of very high purity. Pyrogenic silica after drying is typically > 99.8% silica, with alkali and heavy metals in the low ppm range and the hydrochloric acid content < 100 ppm. Precipitated silicas initially contain residues from the salts formed in the production process and other metal oxides in trace amounts. Silica gels and special precipitated grades are subjected to washing steps, which reduce their contamination, by metal oxides [2, 15].

2.1.3. Production

Most silica in commercial use is obtained from naturally occurring sources. Several synthetic amorphous silicas such as fused silica, and silica gel are, however, prepared for various purposes, and cultured quartz monocrystals are used in particular applications [4, 15].

Sand and gravel

Silica- bearing deposits are found on every continent and geological era. The majority of deposits that are mined for silica sands consist of free quartz, quartzite and sedimentary deposits, such as sandstone. Industrial sand and gravel often referred to as ' silica sand ' and ' quartz sand ' includes high- silica – content sand and gravel. Processing operations depends both on the nature of the deposit and on the end product required. They generally include crushing and milling for refining particle size and wet / dry screening to separate very fine particles. USA, The Netherlands and Germany are the major producing countries of silica sand [15].

Quartz crystals

Two different kinds of quartz production may be distinguished: the processing of naturally occurring quartz; and the hydrothermal culturing of quartz. The largest reserves of highly pure quartz occur in Brazil. Minor deposits are found in Angola, India, Madagascar and the United States. Hydrothermally cultured quartz crystals are of major economic importance and their use is growing rapidly. Synthetic quartz crystal production is concentrated in Japan, Russia and the United States. Smaller production capacity exists in Belgium, Brazil, Bulgaria, China, France, Germany, the republic of South Africa and the United Kingdom [2, 15].

Refractory silica

Silica bricks are manufactured from mixtures of ground quartz arenite and quartzite and are fired at 1450- 1600°C. They are used in certain high-temperature processes and are generally produced in batches. Silica used in

refractories must contain > 93% in weight SiO_2 . Quartz is mostly transformed into cristobalite, but tridymite is also formed under the action of mineralisers. The brick consists of nearly equal amounts of cristobalite, tridymite, residual quartz and a glass phase [1, 2].

Diatomite

Diatomite is obtained from sedimentary rocks that are mainly composed of the skeletons of diatoms. These skeletons are composed of opal-like amorphous silica and exhibit a wide range of porous fine structures and shapes. Particle – size distribution, shape and fine structure vary from one deposit to another. The most notable commercial source of diatomite is in California, United States. Other major deposits that are mined occur in Algeria, Denmark, France, Iceland and Romania [2, 16].

Synthetic amorphous silicas

Commercial / synthetic amorphous silicas have been classified as 'wet process' silicas (including precipitated silicas and silica gel), pyrogenic (fumed) silicas and surface- modified silicas [15].

Wet process silicas

This manufacturing process is based mainly on the precipitation of amorphous SiO_2 particles from aqueous alkali metal silicate solutions by acid neutralisation. Usually, sulfuric acid is used, although carbon dioxide and hydrochloric acid can be used. Depending on the final pH of the solution, precipitated silicas-obtained in neutral or alkaline conditions or silica gels-obtained under acidic conditions can be gained [17].

Pyrogenic silicas

The manufacturing process for pyrogenic silicas is based mainly on the combustion of volatile silanes in an oxygen-hydrogen burner. Primary particles (7-50 nm particle size) of amorphous silica fuse together in the high-temperature flame to yield stable aggregates of between 100 and 500 nm in

diameter. The hydrochloric acid content of the product is commonly reduced to less than 100 ppm by desorbing the hydrochloric acid with air in a fluid-bed reactor [15].

Surface-modified synthetic amorphous silicas

All forms of synthetic amorphous silicas can be surface-modified either physically or chemically. Methods for chemical modification of the silica particle surface (e.g. silylation) are many and various. Most common treating agents are organosilicon compounds [17].

2.1.4. Use

Sand and gravel

Silica sand has been used for many different purposes for many years. Sands are used in manufacture of glass, ceramics, and foundry, abrasive, hydraulic fracturing applications and many other uses and several uses require the material to be ground. In some uses (e.g. sandblasting, abrasives), grinding also occurs during the use [2, 14, 15].

Quartz crystals

Quartz has been used for several thousand years in jewellery as a gemstone (e.g. amethyst, citrine). Large quantities of pure crystals were required when the application of pure quartz in the electronic industry was discovered. At present, the major demand comes from both electronics and optical components industries. An electronic-grade quartz crystal is single-crystal silica that is used in electronic circuits for accurate frequency control, timing and filtering. A smaller amount of optical-grade quartz crystal is used in windows and lenses in specialised devices, including some lasers. Cultured (synthetic) quartz has replaced natural crystal in most of these applications [2, 15].

Diatomites

The fine microstructure and high pore-space volume of diatomite have made it a major substrate for filtration. Diatomite has been used to filter or clarify dry- cleaning solvents, pharmaceuticals, beer, wine, municipal and industrial water, fruit and vegetable juices, oils and other chemical preparations. The next most important application of diatomite is as filler in paint, paper and scouring powders. It also used as a carrier for pesticides, filler in synthetic rubber goods, in laboratory absorbents and in anti-caking agents [15, 16].

Synthetic amorphous silicas

Consistent with their physico-chemical and morphological properties, the different classes of synthetic amorphous silicas find uses in very different areas of application. However, most of the applications are related to the reinforcement of various elastomers, the thickening of various liquid systems, the free-flow of powders or as a constituent of matting, absorbents and heat insulation material [15].

2.1.5. Occurrence

Natural occurrence

Silicon is the second most abundant chemical element, after oxygen, in the earth's crust accounting for 28.15% of its mass. Silicate minerals such as plagioclase, alkali feldspars, pyroxenes, amphiboles, micas, and clays comprise together 80% by volume of the earth's crust, while quartz, by far the most common form of silica in nature, comprises 12% by volume of the crust [1-3].

Crystalline silica

Quartz in its α form is abundant in most rock types, sands and soils. In igneous rocks, quartz is a common component of acid and intermediate plutonic rocks. However, quartz occurs at very low levels or is absent from

the basic and ultra-basic varieties. Quartz may also be present in a variety of volcanic tuffs. Quartz, being a hard, inert and insoluble mineral, endures through the various weathering processes and is found in trace to major amounts in a variety of sedimentary rocks. It is a major component of soils, composing 90- 95% of all sand and silt fractions in a soil. Sand is composed of quartz predominantly, while gravel is of variable composition. In coal, quartz constitutes typically up to 20% of the mineral matter. Diatomaceous earth typically contains 0.1-4.0% quartz. Limestones may contain a small proportion of quartz [18, 19].

Tridymite and cristobalite, formed during the devitrification of siliceous volcanic glass, can be found as fine – grained crystals in acid volcanic rocks. Furthermore, cristobalite is present in some bentonite clays and may be present as traces in diatomite [19].

Amorphous silica

Amorphous silica is widespread in nature as biogenic silica and non-biogenic silica glass. Silica glass forms as volcanic glass from extrusive magmas. Silica of biological origin is produced by diatoms, radiolarians and sponges that extract silica dissolved in water to form their structures or shells. Biogenic amorphous silica levels in diatoms vary with species and range from less than 1% to almost 50% by weight. Opaline materials characterise diatomaceous earth deposits and are also found in bentonite clays. Diatomaceous earth is typically 90% amorphous silica [14]. Biogenic silica is also produced by a variety of plants. Internal silicification of plant tissue promotes structural integrity and affords protection against plant pathogens and insects. The silica content is especially high in grasses, and silica can account for approximately 20% of the dry weight of rushes, rice and sugarcane. Plant biogenic silica is released to the soil through burning or normal decay; soil concentrations are typically in the range of < 1 to 3% [20, 21].

2.1.6. Exposure

2.1.6.1. Environmental Exposures

2.1.6.1.1. Air

There is no extensive data set on levels of silica in ambient air. Crystalline silica has been reported as a possible important constituent of volcanic ash collected at high altitude or as settled dust at ground level. Quartz is also the major mineral component of desert dust, which consists of fine particles smaller in size than 10 μm that can be transported by winds over thousands of kilometres and brought down by rainfall onto water or land surface. Exposure to quartz from dust storms has been suggested as a cause of non- occupational pneumoconiosis reported in certain regions of the world [15].

Ambient levels of quartz, based on inhalable particulate measurements taken in 1980 in 22 United States cities have been reported. Fine quartz levels (particles < 2.5 μm diameter) were from 0 to 1.9 $\mu\text{g}/\text{m}^3$, while coarse levels (from 2.5 to 15 μm) went from 1.0 to 8.0 $\mu\text{g}/\text{m}^3$. It has been estimated that crystalline silica concentrations in the range of 1- 10 $\mu\text{g}/\text{m}^3$ are common in urban and rural settings [22].

Non- occupational inhalation of crystalline silica may also occur during the use of a variety of consumer or hobby products, such as cleansers, cosmetics, art clays and glazes, pet litter, talcum powder, caulk and putty, paint, mortar and cement [15].

No data are available for ambient levels of amorphous silica, except for some measurements of silica fibres taken in the vicinity of farming operations. Amorphous silica fibres were identified as smoke constituents in three of seven area samples located near burning sugar cane fields in Hawaii [20, 23].

2.1.6.1.2. Water

Silica may be present in water as quartz particles and diatom fragments. No quantitative data on the levels of quartz or other silica forms in potable or other forms of water were available. Silica dissolves to a small extent in water as monomeric silicic acid. Levels range from 1ppm to almost 100 ppm depending on the climate, the depth and the activity of various biological processes [24].

2.1.6.1.3. Food

Amorphous silica (such as fumed silica) is incorporated in a variety of food products as anti-caking agent at levels up to 2% by weight. Such foods include beverage mixes, salad dressings, sauces, gravy mixes, seasoning mixes, soups, spices, snack foods, sugar substitutes and desserts. Amorphous silica is also used as an excipient in pharmaceuticals for various drug and vitamin preparations. Other possible uses include the following retention of volatiles, microencapsulation, dispersion agent, clarification of beverages, viscosity control, anti-foaming agent and dough modifier [25].

2.1.6.2. Occupational Exposures

2.1.6.2.1. Crystalline Silica

Because of the extensive natural occurrence of crystalline silica in the earth's crust and the wide uses of the materials in which it is a constituent, workers may be exposed to crystalline silica in a large variety of industries and occupations. The United States Occupational Safety and Health Administration (OSHA) found respirable quartz to be present in samples taken in 255 industries between 1980 and 1992 excluding mining. In 48% of those industries, average overall exposure exceeded permissible exposure levels [26].

Crystalline silica is probably one of the most documented workplace contaminants. Table 1 presents a number of industries, job or operations where occupational exposure to crystalline silica has been reported, together

TABLE 1. Main activities in which workers may be exposed to crystalline silica

Industry/activity	Specific operation/task	Source material
Agriculture	Ploughing, harvesting, use of machinery	Soil
Mining and related milling operations	Most occupations (underground, surface, mill) and mines (metal and non metal, coal)	Ores and associated rock
Quarrying and related milling operations	Crushing stone, sand and gravel processing, monumental stone cutting and abrasive blasting, slate work, diatomite calcination	Sandstone, granite, flint, sand, gravel, slate, diatomaceous earth
Construction	Abrasive blasting of structures, buildings Highway and tunnel construction Excavation and earth moving Masonry, concrete work, demolition	Sand, concrete Rock Soil and rock Concrete, mortar, plaster
Glass, including fibreglass	Raw material processing Refractory installation and repair	Sand, crushed quartz Refractory materials
Cement	Raw materials processing	Clay, sand, limestone, diatomaceous earth
Abrasives	Silicon carbide production Abrasive products fabrication	Sand Tripoli, sandstone
Ceramics, including bricks, tiles, sanitary ware, porcelain, pottery, refractories, vitreous enamels	Mixing, moulding, glaze or enamel spraying, finishing	Clay, shale, flint, sand, quartzite, diatomaceous earth
Iron and steel mills	Refractory preparation and furnace repair	Refractory material
Silicon and ferro-silicon	Raw materials handling	Sand
Foundries (ferrous and non-ferrous)	Casting, shaking out Abrasive blasting, fettling Furnace installation and repair	Sand Sand Sand Refractory material
Metal products including structural metal, machinery, transportation equipment	Abrasive blasting	Sand
Shipbuilding and repair	Abrasive blasting	Sand
Rubber and plastics	Raw material handling	Fillers (tripoli, diatomaceous earth)
Paint	Raw materials handling	Fillers (tripoli, diatomaceous earth, silica flour)
Soaps and cosmetics	Abrasive soaps, scouring powders	Silica flour
Asphalt and roofing felt	Filling and granule application	Sand and aggregate, diatomaceous earth
Agricultural chemicals	Raw material crushing, handling	Phosphate ores and rock
Jewellery	Cutting, grinding, polishing, buffing	Semi-precious gems or stones, abrasives
Dental material	Sand blasting, polishing	Sand, abrasives
Automobile repair	Abrasive blasting	Sand
Boiler scaling	Coal-fired boilers	Ash and concretions

with the source of the silica. The reporting of exposure levels to crystalline silica has changed considerably over the years with the evolution of the various sampling techniques and strategies, the development of improved analytical methods and the formulation of occupational exposure limits reflecting advances in the understanding of particle penetration and effects in the respiratory system [4, 27, 28]. Crystalline silica content has been found to be usually smaller in airborne than in settled dust and in respirable than in total airborne dust [29].

A number of factors remain to be taken into account when evaluating sampling and exposure data. There are uncertainties in the interpretation of analytical data for microcrystalline silica, or data taken in the presence of various interfering substances; in addition, in cases where the particle size distribution is widely different from that of the standard used, interpretation can be uncertain. More importantly, the representativeness of the data has to be evaluated in view of the sampling strategy used. A further complication results from the fact that respirable crystalline silica exposure levels are most often not reported directly in mg/m^3 but indirectly in terms of total respirable mass concentration. This total respirable mass concentration has to be compared (e.g. in the form of severity factor) with an occupational exposure limit that varies with the content of crystalline silica in the dust. It may be noted that industrial hygiene measurement practices do not take into account surface area of particles, which may well be a relevant indicator of exposure [28, 31].

Foundries

Occupational exposure to crystalline silica in foundries originates mainly in the use of sands in the making of moulds and cores. These sands have quartz contents of 5 to nearly 100%. Quartz and cristobalite, the latter being formed from quartz during the pouring of metal, may further contaminate the work environment during the knocking- out or shaking- out operations and during the removal of adherent sand from the castings by grinding or abrasive blasting operations. Other potential sources of crystalline

silica are parting powders such as silica flour applied on the moulds as well as the maintenance and repair of silica- rich refractory materials used in furnaces and ladles [3, 15].

In general, the various studies concur in identifying high- exposure jobs as being related to sand preparation and reclamation, knocking- out or shaking- out, cleaning of castings (fettling, grinding, sandblasting) furnace and ladle refractory relining and repair. Lower levels usually found in non-ferrous foundries compared to iron and steel foundries have been explained by the lower pouring temperatures of the metal, which results in lower sand contamination of the castings. Other factors may be related to the size of foundries, the size of castings and production rates [31].

Improvement in plant ventilation and work practices have been credited in a 10 - 20 – fold lowering in respirable crystalline silica exposure levels of fettlers and coremarkers between 1977 and 1983 in a United States grey iron foundry [32]. Effective controls include well- designed and maintained local ventilation, baffles and air jets on the ventilation equipment of grinding machines, good house keeping, use of vacuum systems and wet sweeping, as well as isolation to prevent cross- contamination [33, 34]. The substitution of siliceous sands with olivine which is a magnesium iron silicate that contains almost no free silica, sands results in decreased exposure levels, but contamination from processes using silica sand must be controlled. Silica flour parting powders can be replaced by low- silica powders such as those containing olivine or zircon [35].

Although crystalline silica represent a major potential air contaminant, the foundry environment is complex and several other exposures have been documented. For example, polycyclic aromatic hydrocarbons may originate from the thermal decomposition of organic material (such as coal- tar pitch, coal, mineral oils, synthetic resins, and vegetable matter) present as additives or binders in sands. Other exposures include various metal fumes and dusts depending on type of metal or alloy produced, carbon monoxide, sulfur dioxide, nitrogen oxides, formaldehyde, amines, phenols, furfural alcohol and aliphatic and aromatic hydrocarbons (e.g. benzene) [15, 36, 37].

Mines

Occupational exposure to crystalline silica in mines originates from the dust generated from the ore being extracted or its associated rock. Mines are usually classified as surface or underground, coal; metal or non- metal and may be associated with various milling operations [15].

The quartz content was determined in 2075 bulk settled dust samples collected from 1984 to 1989 in a representative sample of United States mines from 66 different mineral commodities, including coal. Approximately 50% of all samples had a percentage of quartz above 5%; the overall average was 14%. Commodities with an average quartz percentage above 40% were sand/ gravel and sandstone; those between 20 and 40% were copper, granite, lithium, mica, molybdenum, phosphate rock, shale, slate, stone, titanium and uranium- vanadium. For most commodities, wide variations were observed between the various samples. Surface labourers and bin pulley/ truck loader workers were potentially exposed to bulk dust containing the highest percentage quartz. These data are indicative of potential risk since settled dust composition may not be representative of inhalable or respirable dusts [18].

Beside crystalline silica, several other toxic hazards can be found in mines, such as carbon monoxide, and nitrogen dioxide from blasting and engine exhausts, nickel and arsenic, depending on rock composition, aldehydes and polycyclic aromatic hydrocarbons from diesel engine exhausts, various metallic and non- metallic compounds such as asbestos, and ionizing radiation from random daughters. The extent of such exposures is strongly dependent on work practices and varies with commodity and specific vein composition [15].

Ceramics, Cement and Glass Industries

In the manufacture of structural clay products (bricks, pipes, tiles), exposure to crystalline silica depends mainly on the quartz content of clay or shale that is the principal raw material. Refractory bricks are made with mineral of very high quartz content. In the case of pottery and sanitary ware,

flint (100% quartz) is added to clay as a raw material going into the manufacture of the slip. Sand, which may be used as a dusting powder, may also contribute to airborne silica in the ceramics industry, as well as the decorative material 'glaze' that may be added to the surface. Mixing, moulding, glaze spraying and finishing jobs have been associated with the higher exposure levels, often in the range of 0.1- 0.3 mg/m³. Successful reduction of exposure levels has been accomplished by simple control measures such as enclosure, use of moisture or water mist, use of non-siliceous dusting compounds, better housekeeping and ventilation [38, 39]. Even though crystalline silica constitutes the main health hazard in the ceramics industry, other exposures may be found in certain operations. For example, talc is sometimes used in the body of clay products and as a parting compound in sanitary ware manufacture; various metal compounds, such as chromates and lead compounds, are used as pigments in glazes [40].

In the cement industry, crystalline silica exposure may occur during the handling of raw materials that may contain some quartz, such as clay and volcanic tuff, as well as the sand dust may be added in the process [15].

Sand is the major raw material in the manufacture of glass, including fibreglass. When washed sand is used, airborne dust from the mixed batch commonly contains only 1-5% crystalline silica. In the manufacture of fibreglass, the silica is added to the batch as a finely divided powdered sand of 98.5% or higher silica content. In the glass industry in general, the manual unloading of dry sand and the use of crushed quartz are considered to be hazardous procedures. Hazards associated with hand filling of pots in the pot process, more common in the past, have been eliminated in the more modern tank process. Refractory blocks and bricks used in the construction of furnaces and tanks contain crystalline silica including cristobalite and tridymite and exposure may occur during their cutting, sawing and chipping to size [15, 41].

Construction

In the construction industry, rock drilling, sandblasting and the ubiquitous use of concrete are associated with opportunities for high-intensity silica exposure. The worst exposures were found for dry grinding or cutting in enclosed areas, which presented the potential for exposure to exceed 50 times the permissible exposure limit [41].

Granite Quarrying and Processing, Crushed Stone and Related Industries

Granite rock, containing from 10 to about 30% quartz, is obtained in quarries and further processed into structural stone or crushed for road materials. Other rocks rich in crystalline silica such as sandstone, flint and slate are also subjected to various quarrying, milling and processing operations to produce building or road materials [15].

Respirable crystalline silica levels related to the crystalline silica content of the rock being quarried or milled have been found to be higher with flint than with granite, and with granite or sandstone than with limestone or traprock. The higher exposure levels have usually been associated with the following jobs or operations: rock and stone drilling and cutting in quarries; dimensional stone cutting and finishing in sheds usually outside quarries; rock crushing; sieving and transport within or outside quarries [42]. In the United States granite quarries and sheds, control measures put in place during the late 1930s and the 1940s resulted in 10-100- fold reductions in what were very elevated dust levels. Granite stone cutting is now usually associated with mean levels of respirable quartz below $0.1\text{mg}/\text{m}^3$. In the industry as a whole, present measures include water-mist injection during drilling, local exhaust ventilation, wet methods for cutting granite and the use of control cabins [43, 44].

The presence of cristobalite has been reported in a limited number of samples in the road material industry in Denmark and traprock crushing operations in the United States. Asbestos fibers and other fibrous materials were found in one of 19 stone crushing facilities investigated in the United

States [42, 45]. Other constituents of the dust would depend on the mineral being mined or milled (e.g. silicates, carbonates) and would include abrasives such as silicon carbide and aluminium oxides [46].

Sandblasting of Metal Surfaces

Siliceous sands have been used in the past as abrasives in sandblasting operations designed to remove surface coatings, scale, rust and fused sand from metal surfaces in preparation for subsequent finishing operations. This includes indoor operations in metal fabrication facilities as well as outdoor operations on large equipments such as ships, trucks, trains, bridges, towers and water tanks. This practice is still current in some industries in several countries including the United States and Canada [15].

Miscellaneous Operations

In Iron and steel mills, occupational exposure to crystalline silica may occur during the installation and repair of refractory material in the lining of furnaces, ovens, troughs and runners. In the production of silicon, ferrosilicon and various silicon- containing alloys, quartz-containing materials are charged and melted in electric arc furnaces. Crystalline silica has been reported in proportions of 1-20% in airborne dust. It is recognised that farming operations may produce large quantities of dust, especially in dry and windy conditions and during the use of machinery [47].

In denture manufacturing workshops, crystalline silica may originate from refractory coatings, sanding products, polishing pastes and pumice. In Hong Kong gemstone workers, mean respirable quartz levels for grinder-polishers and buffers of 0.10 and 0.16 mg/m³, respectively, resulted mainly from the use of silica flour as an abrasive [48]. In India, agate workers have been found to be heavily exposed to respirable dust during grinding activities (186mg/m³, with 70% of free silica) [49].

Refractory plasters containing high proportions of quartz and/or cristobalite resulted in two out of four personal crystalline silica levels

measured in jewellery manufacturing workshop in France to exceed the occupational exposure limit [50].

Airborne respirable dust collected in grain elevators in Canada was found to range up to 76mg/m³, depending on work area, and to contain an average of 1.2-6.5% quartz, depending on grain type and stage of treatment. [51].

2.1.6.2.2. Amorphous Silica

Most varieties of amorphous silica have been considered to be of low toxicity compared to other occupational contaminants such as crystalline silica even though it may be present in a variety of work environments.

Diatomaceous Earth

Occupational exposure to amorphous silica dust contained in diatomaceous earth may occur during its extraction, its treatment by calcination and through the handling of the calcined product in a variety of end-use industries as filtration agent, mineral charge, refractory, abrasive, carrier or adsorbent. The use of respiratory protection and dust control by local ventilation and application of water serve to reduce worker exposure [52, 53].

Silica Fumes and Flies Ash

Silica fumes, generated unintentionally and emitted from electric arc furnaces may contaminate work environment in silicon, ferrosilicon and other silicon containing alloy production. Particles collected in this industry often contain crystalline silica as well as various metals. Fly ash from power stations and various manufacturing facilities (e.g. silicon, silicon carbide, silicon nitride, and ferrosilicon industries) may also contain significant amounts of amorphous and crystalline silica [15].

2.2. Biologic Fate of Silica

The primary route of silica entry into the body is through inhalation. Toxic response can mainly result from the absorption or deposition of silica particles in the lung. Ingestion of silica particles can occur from drinking or eating contaminated water and food or after mucociliary clearance from the lungs but the fate of ingested silica is still being debated. Silica particles may also lodge in the skin [2, 54].

Generally, only particles between 0.5 and 5 μm in diameter with a length-to-width ratio 3:1 will be deposited in the respiratory regions of the lung, i.e., alveoli and terminal bronchioles. Larger particles tend to be filtered out in the upper airway and nasopharynx. Smaller fibres tend to remain suspended in the inspired air, and the majority is exhaled. Fibers ranging from 5 to 10 μm in diameter can also penetrate to the lower respiratory regions of the lung, where they may have destructive effects [55].

The deposition of silica particles of compact shape in the human lungs is a function of particle diameter and breathing pattern, i.e., tidal volume, expiratory reserve volume, and frequency of breathing. The site of deposition of particles in the respiratory tract is also of great importance to their potential toxicity since the site of deposition affects the severity of consequences of tissue damage to the respiratory tract, and also the clearance mechanisms available for the ultimate removal of particles. The deposition is increased by breathing 80% He + 20% O₂ producing a larger interface between tidal and reserve air [55].

Silica particles may penetrate the respiratory epithelium directly to lodge in the interstitium where they remain for long periods of time. Silica particles remain at that site, or slowly be transported through interstitial planes and along lymphatic channels to regional lymph tissue, hilar lymph nodes, and subpleural lymphoid aggregates, where it may remain for long period of time. Clearance of silica particles, that deposited at alveolar level, from the lung may require months for significant removal of silica particles; much of the material probably remains in the lung permanently [55-57].

The silica particles render the lungs defence mechanisms. Smaller, nonfibrous particles are normally engulfed by macrophages and removed by lymphatic or mucocilliary mechanisms. Alveolar macrophages carrying silica particles are believed capable of penetrating the air space epithelium, carrying these particles to interstitial sites [4, 56, 58].

Mucocilliary clearance depends on bronchial and tracheal mucus transport velocities. It is depressed in asthmatic subjects, and may be increased by β -adrenergic agents. Transport of silica particles after they have been inhaled or ingested can result in their widespread dissemination. Silica may function as a biological cross-linking agent of connective tissue base membrane structures or in the microtubuler elements of cells. Silica was considered to be an integral component of glycosaminoglycan- protein complexes of connective tissue. It was also suggested a relation between an increase in silica in the human aortic intima and the occurrence and/or progression of arteriosclerosis. Silica particles have been demonstrated in brain and kidney. Silicotic nodules have been found in liver, spleen and bone marrow [2, 4].

2.3. Toxicity of Silica

Silica is the most intensively studied mineral dust. The main consequence of silica inhalation in man and both wildlife and experimental animals is pulmonary fibrosis. Whenever the earth's crust is disturbed and silica-containing rock and sand is used or processed, there are potential respiratory risks for workers. Clusters of new cases of silicosis in abrasive blasters continue to occur despite the development of respiratory protection more than 60 years ago, and silica sandblasting still carries high risk for excessive exposure to silica even when respiratory protection is used. Sandblasting has been banned in certain countries but new reports of silicosis in industries and work settings not previously recognized to be at risk still occur. Examples include tombstone sandblasters, silica flour mixers, construction workers, and surface strip miners operating drill rigs. Less

hazardous alternative abrasive materials without silica are available [4, 59-61].

2.3.1. Lung Diseases Caused by Silica

Lung diseases caused or contributed to by inhalation of crystalline silica include silicosis, pulmonary tuberculosis, and chronic bronchitis and airflow obstruction. The lung may also react to silica particles in such a way that a progressive segregation of the particle collections occur in the area. Particles become less subjected to removal, and the small mass increases in size with death of the cells and proliferations of others. This becomes beginning of the 'silicotic nodules' [2, 4, 60].

2.3.1.1. Silicosis

Silicosis is among the oldest pneumoconiosis known to be associated with occupational exposure to crystalline silica and the prevalence of the disease increases with increasing silica dust exposure. Silicosis is a lung disease characterized by chronic interstitial inflammation and subsequent fibrosis [2, 4, 55].

Acute silicosis or *silicoproteinosis* occur only in subjects exposed to a very high level of free silica small enough to be respirable over relatively short period, generally a few months to a few years, and may be seen in sandblasters. Acute silicosis resembles idiopathic alveolar proteinosis histologically, with the air spaces filled with material that is positive on periodic acid-Schiff staining. In patients with acute silicoproteinosis, x-ray films of the chest reveal diffuse air-space disease, and the findings may mimic those in pulmonary edema. These patients have worsening dyspnea, fever, cough, and weight loss. There is rapid progression of respiratory failure, usually ending in death within a few years. No known treatment modality influences the relentless course of acute silicosis [4, 61, 62].

Chronic silicosis, the most common form, results from low-to-moderate exposure for more than 20 years, i.e., it has a long latency period. Typical radiographic findings are nodular opacities, usually predominating in the

upper lung zone. In simple or uncomplicated silicosis, which is almost entirely asymptomatic, little alteration is shown on routine pulmonary functions tests even after the disease is radiographically demonstrable. The X-ray picture presents opacities which are discrete and range from 1-10 mm in diameter, generally in the apical portion of the lung. The hilar lymph nodes have peripheral calcifications known as eggshell calcifications. Simple silicosis may progress into complicated silicosis, which is caused by coalescence of silicotic nodules, usually in the upper zone, is diagnosed when the radiographic opacities greater than 1 cm in diameter. This condition, referred to as "progressive massive fibrosis", produces a restrictive pattern of pulmonary function, with decreased gas transfer, and may eventually result in cor pulmonale [2, 4, 60].

Accelerated silicosis is less well defined entity that is intermediate between chronic silicosis and acute silicosis. The feature of accelerated disease are identical to those of chronic silicosis but more rapidly progressive, requiring only 4 to 8 years of exposure [4, 60].

Three criteria are required for diagnosis of silicosis : an appropriate history of exposure, radiographic findings consistent with silicosis, and the absence of likely alternative explanation for the the clinical and radiographic findings [60, 63].

Controversy exists concerning the precise quantitative relationship between dust inhalation and disease. The United States Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for respirable crystalline silica in general industry is a respirable dust concentration of 10 mg/m^3 divided by $(\% \text{SiO}_2 + 2)$ or 250 million particles per cubic foot divided by $(\% \text{SiO}_2 + 5)$, averaged over an 8-h workshift which superseded the similar permissible exposure limit of 0.1 mg/m^3 respirable silica [60]. However, it is common for silicosis to be diagnosed after a worker has left the silica-exposed industry, and for it to continue to progress slowly over many decades of life after exposure has ended. Studies to establish the relationship between silica dust in air and the occurrence of silicosis are hampered by the long period between measurements of exposure and the

detection of the disease. Higher estimates of exposure risk have been published in studies including retired workers. The continuing controversy regarding quantitative exposure-response relationships casts some doubt on the efficacy in preventing silicosis at 0.1mg/m³ respirable quartz standard. By contrast, the standard recommended by the National Institute for Occupational Safety and Health (NIOSH) is 0.05mg/m³ [60, 64, 65].

Pathogenesis of Silicosis

Although many studies have documented the etiopathogenesis, the exact mechanism(s) by which silica can induce inflammatory and fibrogenic lung disease is obscure and remains to be elucidated [66].

The histopathology of silicosis provides clues to the mechanisms that produce the disease. A series of lesions evolves in humans and in experimental animals, with a sequence of immune-inflammatory events evident within them. The descriptions from animal as well as human histopathology studies suggest several key points: (i) silicosis is a very local disease; each focal lesion develops in very close proximity to a critical mass of the inciting agent, dust; (ii) clinical pulmonary dysfunction results from the summation of many isolated foci, not as a consequence of gradually worsening uniformly diffuse process; (iii) the cast of participants at each local site include macrophages with dust, macrophages/monocytes without dust, multinucleate giant cells, lymphocytes, neutrophils, fibroblasts and possibly type II alveolar epithelial cells. Interactions between these cell types presumably govern the evolution of the disease process; and (iv) the macrophage remains intimately associated with causal dust throughout the active evolution of silicosis. The interactions between silica and macrophages are probably fundamentally responsible for the chain of events which results in the characteristic disease [66, 67].

The cytotoxic effect of silica has been thought to be a major component in the development of fibrosis. According to this theory, silica-induced damage to the cell membrane kills the macrophage and initiates a chain reaction involving release of silica crystals and rephagocytosis by a

fresh crop of cells. So, it was hypothesized that injury, inflammation, and ultimately silicosis developed as a result of the release of toxic substances, proteolytic enzymes, and lysosomal hydrolases from dying macrophage. More recent studies suggest that this toxicity is more evident in vitro than in vivo, and that live cells secreting synthesized proinflammatory mediators are responsible for the disease, the macrophage seems to play a central role in this process [68, 69]. Coating quartz particles with surfactant lipids and other alveolar lining materials greatly reduced the toxicity of the dust for alveolar macrophages in vitro. It seems likely that quartz is toxic for macrophages in the lung, but that the time course of cell injury is more delayed than seen in cell culture models. Hypothetically, the actual dose received by each cell in vivo is probably lower than those most investigators have used in vitro, and the toxicity of the dust may be blunted by lipids that coat particles landing on the respiratory membrane. Silica probably exerts its effects on the macrophages which ingest them by altering their function while they are alive, rather than merely by disturbing them [70, 71].

Activated alveolar macrophages secrete a variety of proteases. These are connective tissue proteases such as elastase, collagenase which can act on connective tissue to generate fragments that are leukocyte chemotaxins. Proteases may also enhance inflammation by activating components of complement, and in the long term, are thought to have a major part in the remodeling of connective tissue components that is associated with pathological changes in the lung. Since collagenases are usually secreted in an inactive precursor form, the mechanism of activation is of some importance [70, 72].

Activated alveolar macrophages generate free radicals of which hydroxyl radical is particularly toxic to cells. The activated alveolar macrophages also release a potent inflammatory and fibrogenic mediators such as interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α). In addition, the activated alveolar macrophages secrete growth factors capable of inducing fibroblast proliferation. Soon after, inflammatory cells such as, lymphocytes, neutrophils, and monocytes are recruited to the site of immune

response to facilitate clearance of deposited particles and debridement of damaged tissues or stimulate cell proliferation and connective tissue synthesis. However, recruited and activated inflammatory cells can also contribute to the damage of respiratory structure through the release of various factors. Neutrophils are also capable of secreting ROS, which can damage cells and tissues. It has been demonstrated that polymorphonuclear cells (PMNs) incubated with quartz produce large amounts of ROS, which can contribute to the development of fibrosis. Increased numbers of PMNs has been observed in animal models of silicosis early in the evolution of the inflammation process. The balance of lung antioxidant and antiproteolytic factors may be overcome as neutrophils are recruited and the burden of their products increases. Thus, relatively small numbers of short-lived neutrophils might produce a significant tissue damage in silicosis, but still quite difficult to detect in histologic sections [73-76].

2.3.1.2. Pulmonary Tuberculosis

Mycobacterial infection must not be ruled out whenever a patient with silicosis has radiographically evident progression or a deterioration in respiratory function. The increased risk of tuberculosis and a typical mycobacterial infections in the patients with silicosis has long been recognized. Before the introduction of effective therapy, tuberculosis was the most important cause of death in workers with silicosis. *Aspergillus* species may also colonize tuberculosis or ischemic cavities in patients with silicosis [2, 4, 60].

A substantially higher tuberculosis mortality associated with silicosis was reported in the United States in the period 1979 to 1991 [77]. The occurrence of tuberculosis disease in silicotics is related to the underlying prevalence of prior latent tuberculous infection and risk of new exposure to tuberculosis of the population at risk for silicosis. Recent epidemiological studies have confirmed that those with chronic silicosis have a 3-fold increase in incidence of tuberculosis compared with a similarly aged, silica-exposed group without silicosis. The incidence of tuberculosis and

nontuberculous mycobacterial disease is highest in acute and accelerated silicosis [78, 79]. In chronic silicosis, the incidence of active tuberculosis increases in direct proportion with the increase in profusion of silicotic nodule. Some data suggest that subjects without silicosis but with long exposures to silica dust have an excess risk of developing tuberculosis compared with the non-exposed population [60, 79].

It is important to consider nontuberculous mycobacterial disease when investigating a subject with silicosis for "pulmonary tuberculosis". Nontuberculous mycobacteria (NTM) account for an increasing proportion of the mycobacterial disease in those with silicosis in the industrialized world. NTM disease is relatively uncommon in developing countries with a high prevalence of tuberculosis, but in a study of a working population of black South African gold miners with a first episode of pulmonary tuberculosis, NTM only were cultured from 16.9% of the men with silicosis [80]. These NTM have been associated with dusty occupations, including mining, and they are more prevalent in those with occupational and other lung disease [81].

The recommended management of tuberculosis in the setting of silicosis is the same as the management of tuberculosis. Because of increased risk of tuberculosis in silicotics, it is important to consider it in any patient with silicosis and to make sure the silicotic patient does not have active tuberculosis before giving isoniazid prophylaxis alone. The most effective way to prevent tuberculosis and NTM disease is to limit silica exposure. Those who already have silicosis and those without silicosis but with exposure to crystalline silica for 25 years or more should have a tuberculin test with an intradermal injection of 5 tuberculin unit of purified protein derivative (PPD). If they react with an area of induration greater than 9mm, irrespective of their status with regard to baccillus Calmette-Guerin (BCG), they should be offered tuberculosis chemoprophylaxis [81, 82].

2.3.1.3. Chronic Bronchitis and Airflow Obstruction

Chronic bronchitis, defined by chronic sputum production, is common among worker groups with exposure to dusty environments contaminated with silica. It can not be clinically distinguished from chronic bronchitis caused by tobacco smoking or other factors. Epidemiologically, it can be detected as bronchitis symptoms in excess of those expected from smoking alone in a cohort of workers exposed to dust, but who have no radiological evidence of silicosis. Increased frequency of chronic bronchitis has been reported in United States coal miners [96], German coal miners, South African and Australian gold miners [97, 98], Indonesian granite workers, and Indian agate workers [99]. In most studies chronic bronchitis was associated with airflow limitation [60, 83-85].

Studies from many different work environments suggest that exposure to working environments contaminated by silica at dust levels that appear not to cause radiographically visible simple silicosis, can cause chronic airflow limitation and/or mucus hypersecretion and/or pathologic emphysema. At low doses, this effect may not be substantial or disabling. In moderate to severe silicosis, nodules occur in close proximity to small and medium airways causing narrowing and distortion of the lumen. Hypertrophy and scarring in bronchial-associated lymphoid tissue and intrapulmonary lymph nodes may compress larger airways. In patients with more advanced silicosis, pulmonary function tests usually reflect a mixed pattern of nonirreversible airflow obstruction as well as the features of volume restriction and impaired gas exchange expected with diffuse interstitial lung disease [2, 4].

The data supporting quantitative relationships between silica exposure (or exposure to dusty environments containing silica dust) and airway disease are more limited than for silicosis and airway disease, although several studies have shown relationships for chronic airflow limitation, for chronic bronchitis, and for emphysema [60, 86, 87].

2.3.2. Effects of Silica on Other Organs and Systems

Transport of silica particles after they have been inhaled or ingested can result in their widespread dissemination. Silica particles have been demonstrated in brain and kidney. Silicotic nodules have been found in liver, spleen, and bone marrow. The silicon content of organs has sometimes been elevated in patients dying from silicosis. Silica may have systemic effects while remain in the lungs and regional lymph nodes [2, 4, 60].

Extrapulmonary effects are thus biologically plausible. Regional lymph node disease is the commonest extrapulmonary effect, common enough in silicosis to be considered part of the disease, but it occurs in some exposed workers who have no evidence of lung disease, even by histopathological examination. Extrathoracic nodes may be involved, including the anterior cervical and aortic chains. The process is a granulomatous inflammation leading to formation of typical silicotic nodules within lymph nodes. Capsular scarring can produce the radiographic pattern of peripheral or “eggshell” calcification of lymph nodes. The observation of other extrapulmonary conditions comes mainly from occupational settings in which exposures have been heavy enough to result in silicosis. No increased prevalence of these conditions has been convincingly described at low exposure levels [2, 88].

Scleroderma

Association between scleroderma with dusty work dates from 1914 description of “diffuse sclerodermia” in Scottish stonemasons. Subsequent reports have described increased prevalences of *acrosclerosis* (sclerodactyly), progressive systemic sclerosis (PSS), or both in patients from dusty trades or in patients with silicosis. The association is complicated by the occurrence of Raynaud’s phenomenon and acrosclerosis in vibration injury, to which many manual workers, including miners who drill rock, are subject. A recent view has concluded that “silica-associated systemic sclerosis” is indistinguishable from idiopathic PSS. Increased incidence of PSS has been found in South African miners. There is persuasive evidence

relating scleroderma to occupational silica exposures in settings where there is an appreciable silicosis risk [89, 91].

Rheumatoid Arthritis

The evidence of association between rheumatoid arthritis and silica exposure or silicosis is less clear, and the potential for misclassification is greater. It is often hard to be sure that an indolent polyarthritis is rheumatoid arthritis, and silicosis and most other pulmonary fibroses can produce positive serum tests for antinuclear antibody and rheumatoid factor [92]. South African miners with rheumatoid arthritis were more likely to have silicosis than were miners not exhibiting rheumatoid arthritis, and their silicosis was more progressive. The study design did not allow determination of whether silica or silicosis increased the risk of rheumatoid arthritis, but the investigators thought that if an association exists "it is unlikely to be a strong one". A causal association between rheumatoid arthritis and silica exposure thus plausible but unproved [93].

Renal Disease

An interest in the possible relationship between renal disease and silicosis dates from the finding in a study from England and Wales. There were 3158 deaths in those with occupations implying silica exposure, and the death rate from chronic nephritis was 45% greater than expected from age-specific rates in the reference population. A case-control study of men with end-stage renal disease found elevated odds ratios for "regular occupational exposures to solvents or silica" but characterized evidence of other silica-related renal disease as limited to case reports. Case reports have also shown an association between acute silicoproteinosis and a glomerular injury [60, 94, 95].

2.3.3. Carcinogenic Effects of Silica

Numerous Animal experiments have been carried out to investigate the association between silica exposure and cancer. Wanger et al [96]

observed lymphomas in different strains of rats when quartz, cristobalite, or tridymite were injected into the pleural or peritoneal cavities. This type of tumour as observed after intrapleural injection of quartz into Sprague-Dawley rats. In addition, pulmonary epithelium tumours were induced in Fisher 344 rats after inhalation and intratracheal administration of Min-U-Sil 5 and Min-U-Sil 10 quartz and lung tumours were seen in 16-45% of rats receiving silica intratracheally [97]. Mechanistic evidence suggests that the development of lung tumours in animals in response to crystalline silica is the result of marked and persistent inflammation and epithelial proliferation; however, a role for surface-generated oxidants or even a direct genotoxic effect can not be ruled out [98]. However, results of animal inhalation studies indicated that no significant differences in pulmonary adenomas in either silica or control groups of mice [99]. Intratracheal administration of silica did not induce tumours in hamsters [100].

Epidemiological studies that were considered relevant to assess the carcinogenic risk of crystalline silica for humans include studies on ore miners, quarry workers, granite and slate industry workers, workers in the ceramics, pottery, refractory brick and diatomaceous earths processing industries, and, in foundry workers. In addition, epidemiological studies were available on silicotic patients, many of them had been employed in the industries listed above which include autopsy studies,, cohort and case control studies drawn from workers with silicosis often presenting and diagnosed for compensation purposes, or from patients with cancer [101].

Elevated standardized mortality ratio (SMR), approximately 150 (1.5 times the expected rate of cancer deaths), were detected in large-population-based studies in Massachusetts and Canada. Workers exposed to silica in Italy, and in some, but not all Nordic countries showed 3-to 5-fold increased lung cancer [60, 102, 103]. Reports from many countries have identified lung cancer with increased frequency among workers compensated for silicosis, with a relative risk for lung cancer, compared with that in the general population, from 1.3 to 6.9 [104-106]. Metal ore miners with silica exposure in the United States, the United Kingdom, China, Canada, and Australia, and

South Dakota, experienced a significant increase in mortality because of lung cancer, with 2- to 5-fold increase in risk [107-110]. Some of these studies adjusted for smoking [108-111]. However, miners may also be exposed in the underground work environment to radon, arsenic, and diesel exhaust [112, 113].

In addition, an increased lung cancer risk (SMR, 150 to 200) has been demonstrated in a variety of other non-mining dusty trades. The following epidemiological studies provided the least confounded examinations of an association between silica exposure and lung cancer: a large cohort studies of Vermont granite shed and quarry workers, Finland, China, and German slate quarry workers, Danish stone industry workers, United states crushed-stone industry workers, Chinese refractory brick workers, Italian refractory brick workers, United Kingdom pottery workers, Chinese pottery workers [114-119]. Cohort studies in foundry workers provide further relatively consistent evidence of an association between silica exposure and increased lung cancer risk. These include mortality studies in British ferrous foundry workers, and in Danish foundry workers [101, 120].

However, there are some epidemiological studies that did not show an excess cancer mortality following exposure to silica. Thus, in appropriate mortality study on 969 granite workers, no association was shown between exposure to granite dust and an increased lung cancer risk, in spite of an exposure resulting in appreciable silicosis [121]. In contrast with many other studies of silicosis, Carta et al [122] who reported on the mortality of 724 subjects with silicosis, did not find an increased risk of lung cancer in association with either degree of exposure to silica dust or silicosis although they suggested that chronic airway obstruction could be an independent risk factor for lung cancer in subject with silicosis. A case control study of lung cancer deaths among South Africa gold miners carefully matched with controls especially for tobacco consumption showed no association between lung cancer and either silicosis or exposure to silica. The authors suggested that the association between silicosis and lung cancer seen in other studies

might have been the result of an association between silicosis and smoking [123].

Very little epidemiological evidence is available regarding the association between human exposure to amorphous silica and lung cancer. No association was detected for mesothelioma with biogenic amorphous silica fibres in the three community-based case-control studies. Separate analysis were not performed for cancer risks among a subset of diatomaceous earth industry workers exposed predominantly to amorphous silica [124, 125].

The available data support the conclusion that silicosis produces increased risk for lung cancer. Epidemiological studies provide convincing evidence for increased lung cancer risk among tobacco smokers with silicosis. Less information is available for never-smokers and for workers exposed to silica but who do not have silicosis. For workers with silicosis, the risks for lung cancer are relatively high and consistent among various countries and investigators [98].

The workers in the workplace are exposed to a combination of substances such as radon, polyaromatic hydrocarbons, sulfates and other known carcinogens. Evidence is abundant that under these conditions, an intense inflammatory response is involved. In the lung, the inflammatory response is crucial in the sequence of events from initial interaction of inhaled particles with alveolar macrophages and structural epithelial cells to subsequent development of fibrosis or neoplastic responses. The release of inflammatory mediators, reactive oxygen species (ROS), proteolytic enzymes, cytokines and growth-factors have been shown to be involved in local tissue damage and remodeling, and it has been demonstrated that ROS are essential in the activation of proto-oncogenes by carcinogenic fibers. Thus, cytokine release can affect a carcinogen by two routes: (i) Amplification pathways for secretion and effects of ROS by PMNs, via stimulation of chemoattractin, adherence (to epithelium) and activation of cell metabolism, (ii) Non-genotoxic pathways by stimulation or collaboration of cell replication and growth, apoptosis and necrosis, leading to remodeling of respiratory

epithelium. This data indicate that an inflammatory status of lung tissue significantly contributes to the formation of genotoxic metabolites in vivo and in vitro and can upregulate genes involved in cell proliferation. Apart from these effects, particles can act as "carriers" for carcinogens such as polyaromatic hydrocarbons, endotoxins, etc. The underlying hypothesis is that particle-associated carcinogens cause alterations in the toxicokinetic phase through an altered deposition, retention, translocation, and clearance pattern in the respiratory tract, which can affect their carcinogenic efficacy. It has been suggested, increased retention of the carcinogen, and the elution rate of the carcinogen from the particle as major co-carcinogenic mechanisms [126].

In 1996, IARC (International Agency for Research on Cancer) concluded that although carcinogenicity in humans was not detected in all industrial circumstances studied the epidemiological findings support increased lung cancer risks from inhaled crystalline silica (quartz and cristobalite) resulting from occupational exposure and the observed associations could not be explained by confounding or other biases. The overall evaluation by the IARC working group was that: (i) There is sufficient evidence in experimental animals for the carcinogenicity of quartz and cristobalite, (ii) There is limited evidence in experimental animals for the carcinogenicity of tridymite, (iii) There is sufficient evidence in humans for the carcinogenicity of inhaled crystalline silica in the form of quartz or cristobalite from occupational sources. Crystalline silica inhaled in the form of quartz or cristobalite is "carcinogenic" to humans (Group 1), (iv) There is inadequate evidence in experimental animals for the carcinogenicity of uncalcinated diatomaceous earths, (v) There is inadequate evidence in experimental animals for the carcinogenicity of synthetic amorphous silica, and (vi) There is inadequate evidence in humans for the carcinogenicity of amorphous silica. Amorphous silica is not classifiable as to its carcinogenicity to humans (Group 3) [15].

2.3.4. Effects of Silica on the Immune System

It has been shown that silica-related diseases such as silicosis are associated with alteration of cellular and humoral immune functions.

2.3.4.1. Effects of Silica on the Reticuloendothelial Cells

It was suggested that silica induces cytotoxicity to alveolar macrophages in a dose- and time-dependent manner. Early studies on the pathogenesis of silicosis suggested that the basic lesion was to alveolar macrophages and incubation with silica inhibited the ability of rabbit and guinea pig macrophages to degranulate and release lactic acid.

In vitro exposure of alveolar macrophages from rats to low concentrations of quartz, showed that cells remained viable, although increasing the concentration of quartz led to cell death [68, 127-130].

It was reported that silica injected intravenously and administered intratracheally decreased the ability of peritoneal and pulmonary macrophages to phagocytose bacteria and *E.coli*, respectively [127, 131]. Zimmerman et al. [132] showed that incubation with silica for 1-3 hours lessened viability of both murine inflammatory cells and reduced their ability to phagocytose erythrocytes and bacteria, and inhibited the ability of both cells to kill the facultative intracellular bacterium *Listeria monocytogens*.

Intratracheal administration of quartz leads to impaired adherence and chemotactic responsiveness in guinea pig alveolar macrophages [133]. Quartz inhalation causes impairment to macrophage chemotactic activity towards zymosan-activated serum. A marked loss of chemotactic ability in leukocytes from rats inhaling silica dust has been observed [134-136]. Silica administered intravenously to DAB/2 mice rapidly depresses the clearance of colloidal carbon by the reticuloendothelial system and reduces in vitro phagocytic activity of peritoneal macrophages harvested 3 days after silica injection [128].

The mechanisms by which silica can cause injury to alveolar macrophages are still unclear. It has been suggested that the cytotoxicity of silica on the alveolar macrophages may be due to the fact that silanol groups

are formed on the surface of quartz particles by the interaction with water. These silanol groups might act as sites for strong hydrogen bonding with the inner surface of the lysosomal membrane and it has been reported that stronger interactions are those between silanol groups and the phosphate ester groups of phospholipids and the reaction of the silanol groups with the lysosomal membrane results in damage of the lysosomes. The damaged lysosomes release destructive enzymes into the cytoplasm of the cells, resulting in breakdown and death of the alveolar macrophages [137, 138].

On the other hand, it was also shown that incubation of alveolar macrophages with silica causes increase in the intracellular free calcium, which was associated with injury of alveolar macrophages, and that silica causes rise in the intracellular free calcium in alveolar macrophages appears to involve at least two different mechanisms and is dose-dependent: (i) calcium influx through plasma membrane calcium channels, which results in a transient intracellular free calcium increases at low doses of silica, and (ii) non-specific membrane damage with sustained intracellular free calcium rise at high silica doses. It was suggested that alteration of calcium homeostasis of the alveolar macrophages could mediate several calcium-dependent cellular events including activation of phospholipases, proteases, endonucleases, and oxidases which catalyse the degradation of membrane phospholipids, cytoskeletal and membrane proteins. All of these responses may directly and indirectly impair cellular physiological functions and cause cellular injury of the alveolar macrophages leads finally to cell death [139].

It was also suggested that silica can generate hydroxy radicals and cause membrane lipid peroxidation. Among the mammalian cells, neutrophils, monocytes, and macrophages play important role in converting molecular oxygen to reactive oxygen metabolites; this is probably related to the phagocytosis and antimicrobial activities. Chemotactic and phagocytic activation processes result in substantial increase in the release of potent oxidants by stimulated phagocytes; these toxic oxygen radicals cause oxidative damage to the surrounding tissues. A key role for hydrogen peroxide as the mediator of extracellular cytotoxic mechanism of activated

phagocytes has been well documented. Phenomenon occurs at the phagocyte surface, such as those which may occur in endogenous lung phagocytes after exposure to dusts and toxic gases, or in the circulating phagocytes before their accumulation in the lung or after to their attachment to normal or damaged lung endothelium seem to be important in determining their degree of enhanced oxidative activity [138].

After the quartz particles are engulfed in phagosomes, both neutrophils and macrophages increase oxygen consumption in what is called the 'respiratory burst'. During this process, the hexose monophosphate shunt is activated, resulting in an increase in glucose oxidation. The increase in the oxygen uptake is not solely to provide ATP as a source of energy for phagocytosis, but also to form O_2^- and almost all the oxygen consumed during the respiratory burst is converted to H_2O_2 . The amount of O_2^- and H_2O_2 produced by neutrophils and macrophages play an important role because O_2^- facilitates killing of the engulfed substance such as bacteria. Superoxide dismutase (SOD) and catalase (CAT) can inhibit bacterial killing by the macrophages [140, 141]. Although the biochemical mechanisms underlying the bacterial killing by the macrophages are not yet fully understood, there is experimental evidence suggesting that the damaging effects are caused by H_2O_2 and not O_2^- [142]. Under normal conditions, the macrophages protect themselves by releasing SOD, glutathione peroxidase-glutathione reductase systems and CAT from potential oxidative damage by their own bactericidal products [134, 143].

When quartz is ground in air, silicon-based radicals are generated on the silica surface. These radicals subsequently react with atmospheric components and water to generate H_2O_2 , $OH^\bullet$, and possibly O_2^- . In addition, iron and other transition metals that are present as trace impurities or impregnated in the chemical structure of silica surface catalyze the generation of $OH^\bullet$ radicals from H_2O_2 which the lung itself supplies [10, 144]. These reactive oxygen species would further react with the cell membrane, leading to additional release of these species and to lipid peroxidation [14]. The reaction of quartz particles with H_2O produces silanol ($SiOH$) groups on

the particle surface. These silanol groups could form hydrogen bonds with the nitrogen or oxygen atomic sites on the cell membrane. This is supported by the report of a weak (hydrogen) bond formation between a secondary amide (peptide) of proteins and silanol moieties. Such hydrogen bonding bring the silica surface and cell membrane close enough to provide a favorable environment for the initiation of lipid peroxidation by the silicon-based radicals and their associated oxygenated radicals. The concentration of the reactive oxygenated species produced might be high enough to be beyond the cell defence [145, 146]. The oxidation of transmembrane proteins may cause the membrane permeability to increase, allowing more free-ion migration and breaking down transmembrane ion gradients in the cell, eventually resulting in cell death [145].

The concentrations of both silicon-based radicals and their associated $\text{OH}^\bullet$ radicals decrease with aging in air [144]. Dalal and coworkers [10] have shown that freshly ground silica is more biologically reactive than aged silica because freshly crushed silica activates a greater respiratory burst in alveolar macrophages than aged silica. For example, storage of freshly ground dust in air decreases silica-induced superoxide anion secretion, hydrogen peroxide release, and nitroblue tetrazolium (NBT) reduction by 25, 68, and 43%, respectively. Furthermore, compared to aged silica, freshly ground silica exhibits a greater cytotoxic effect on cellular membrane integrity. These results indicate that fractured-generated silicon-based radicals and their associated $\text{OH}^\bullet$ and possibly O_2^- radicals may play a significant role in the pathogenesis of silica.

Some studies have demonstrated that the toxic effects of silica on alveolar colony-forming cells (AL-CFC) may lead to an impairment of pulmonary alveolar macrophages, as intratracheal administration of silica as well as crocidolite to rats induced a significant depletion in the plating efficiency of AL-CFC and a decrease in pulmonary alveolar macrophages recovery compared to untreated or titanium dioxide-treated animals [147]. However, other investigators have suggested that silica-induced death of the alveolar macrophage is due to apoptosis rather than necrosis in *in vitro* and

in vivo studies. In vitro, it has been demonstrated that apoptotic cell fraction in silica-treated bronchial epithelial cell line (A549 cells) was significantly increased in comparison with untreated cells. Also genomic DNA from silica-treated A549 cells showed DNA ladder formation while control cells did not. Total cell numbers and apoptotic cell numbers of bronchoalveolar fluid from silica-instilled rats were significantly higher than control group [148]. In another in vitro study have established that treatment of human alveolar macrophages with silica and asbestos has resulted in apoptosis, while nonfibrogenic particulates did not induce apoptosis, which is correlated with the fibrogenicity of the particulate [149].

2.3.4.2. Effects of Silica on Host Resistance

Decrement in host lung defences against microorganisms may be partially responsible for the increase in respiratory illness observed in susceptible populations after silica exposure. It is clear that other diseases develop in the lungs along with silicosis and may progress more rapidly in the presence of silicotic nodules, for example, the incidence of tuberculosis is enhanced in silicotic humans. In addition to its long-standing reputation for enhancing susceptibility to tuberculosis, silica has been shown to increase susceptibility to other microorganisms [4, 55].

An increased evidence concern the decreased resistance of silica-treated animals towards infectious diseases has been accumulated. Scanning electron microscopy demonstrates that crystalline silica destroys liver kupffer cells but has no other obvious deleterious effects on the liver. Silica-treated livers still retain the ability to trap large numbers of bacteria perfused through the portal vein even though the rate of clearance is well below normal. In vivo, silica treatment decreases the rate of bacterial clearance from the blood, alters the in vivo organ distribution of cleared bacteria [150].

The susceptibility of F1 female mice to *Salmonella typhimurium* was assessed with silica. Silica given to mice 1 day before intraperitoneal challenge decreased the 50% lethal dose of *S.typhimurium* 100-fold. The

incidence of survival of mice given up to 14 days before infection with a sublethal dose of organisms was also decreased. The susceptibility could also be demonstrated when silica was given 10 days, but not 20 days, after *S.typhimurium* infection. Death among silica-treated mice followed uncontrolled multiplication of the organism in the spleen [150, 151].

It has also been demonstrated that mice given silica intravenously 1 day prior to challenge with liver yeast cells of *Histoplasma capsulatum* were more susceptible to infection than untreated controls. The increased susceptibility to *Histoplasma* was observed when mice were given silica at 1, 14, and 21 days prior to infection. Silica-treated mice that survived 30 days after challenge with a sublethal dose of *Histoplasma* had 23 times more viable organisms in their spleens than in those of untreated controls. The blastogenic response of spleen cells to concanavalin A and phytohemagglutinin was unaffected at 12 hours after silica injection but was significantly depressed between 1 and 21 days [152].

It has been demonstrated that silica administered to Balb/c mice presented with a reduced response to aerosolized *Escherichia coli* bacteria following a 570-day intermittent silica exposure [153].

2.3.4.3. Effects of Silica on Lymphocyte Populations

Increased number of activated lymphocytes have been found in both lung tissue and BAL fluid in a variety of pulmonary fibrotic disorders, including those induced by exposure to hazardous particles. Lymphocytes are frequently present in the interstitial inflammation associated with the development of pulmonary fibrosis and may participate in an immunologic response to alterations in the structural components of lung tissue induced by the fibrogenic stimuli [154, 155].

Perivascular and peribronchial aggregates of lymphocytes were described in mice exposed to silica dust by inhalation [152]. The presence of lymphocytes surrounding the granulomatous lesions in human silicosis has also been described [66]. Kumar et al [156] reported increased numbers of activated of lung paranchymal lymphocytes during pulmonary inflammation

induced by intratracheal injection of silica which was examined 2 weeks after administration of silica particles, while a significant degree of paranchymal fibrosis was demonstrable 8 weeks after administration of silica particles.

A marked influx of T lymphocytes in the silica-treated animals was observed in in vivo study. Cells expressing the CD4 antigens (T-helper cells) and CD8 antigens (T-suppressor cells) were increased in number, with the former accounting for approximately two-thirds of the T lymphocytes participating in the response. The T lymphocyte response, which preceeded the development of a significant fibrosis by 3 to 6 weeks, is sustained through the evolution of the pulmonary lesion. In the later stages, an increased number of B-lymphocytes were apparent [157].

Struhar et al. [158] reported that following administration of silica to rats by intratracheal injection, there was an early increase in the number of T lymphocytes obtained by BAL or by dissociation of lung tissue with collagenase with a predominance of T-helper (CD4+) cells relative to the T-suppressor cells (CD8+) during the development of fibrosis.

Newman et al. [159] studied blood natural killer (NK) cell number, percentage, and tumoricidal activity in 120 hard rock miners, 57 of whom had radiographic evidence of silicosis, and in 33 community controls. The results showed that NK cells count was normal in silicotics and those with silica dust exposure without silicosis. Although tumoricidal activity was elevated in silicotics, this was accounted for by their cigarette smoking.

2.3.4.4. Effects of Silica on Immunoglobulins and Complement Proteins

Experimentally, there is in vivo evidence for both an enhancement as well as a depression of humoral immune responses after exposure to silica. An intravenous or intraperitoneal injection of silica into animals is associated, under certain conditions, with an augmentation of immunoglobulin (Ig) production in response to soluble and particulate antigens [160-162]. In contrast, a depression of antibody responses after intravenous silica injection has also been reported [127].

In vitro studies by Thiele and Lipsky [163] demonstrated that immunoglobulin production by human peripheral mononuclear cells stimulated with pokeweed mitogen (PWM) is decreased after exposure to silica. But it was also found that silica added to PWM-stimulated high-density cultures significantly increased the number of immunoglobulin-secreting cells. Silica also significantly increased the numbers of immunoglobulin-secreting cells in high-density cultures stimulated with PPD and tetanus toxoid and augmented the proliferation of phytohemagglutinin-stimulated mononuclear cells. In contrast to high density cultures, silica added with PWM to low-density cultures reduced the numbers of immunoglobulin-secreting cells [164].

Complement proteins are known to mediate inflammatory responses and bacterial clearance in the lung of exposed animals. In particular, the fifth component of complement and its cleavage fragments, C5a and C5a des arg are present in biologically active concentrations on alveolar surfaces and facilitate pulmonary clearance responses through their opsonic (i.e phagocytosis) and chemotactic effects [166]. The role of complement in experimental silicosis has been studied in mice using intratracheal instillation exposures to silica. Callis and associates [166] reported that silica instillation produced a significant increase in cell numbers and protein content of lung lavage fluid in C5+ mice when compared to similarly exposed C5- congenic strains. These investigators concluded that complement may be responsible for mediating pulmonary inflammation, but not fibrosis elicited by silica exposure.

Warheit et al [167] assessed the role of complement proteins in modulating pulmonary inflammatory and macrophage phagocytic responses in rats exposed either to silica or carbonyl iron particles. The results showed that carbonyl iron exposure did not produce cellular or biochemical indices of pulmonary inflammation, either in normal or complement-depressed rats. However fewer phagocytic macrophages were recovered from the lungs of complement-depressed rats, carbonyl iron-exposed rats than from normal exposed animals. It was concluded that complement had little effect upon

sustained silica- induced pulmonary inflammatory parameters. The involvement of neutrophil has been identified as a prominent feature in complement and bacterial-induced lung inflammation in experimental animals. Free radicals elaborated by macrophages during the respiratory burst associated with phagocytosis of many types of particles could result in the formation of chemotactic factors upon reaction with complement proteins.

Some investigators have reported an increased complement levels in subjects exposed to silica. Increased C4 levels in silicotic patients from South African gold miners who all had been exposed to silica in gold mines for prolonged periods, and in subjects exposed to silica dust from quartz-crushing stone has been reported [165, 168].

The serum soluble FAS (sFAS) levels in 64 Japanese silicosis patients with no clinical symptoms of autoimmune diseases or malignant tumours were investigated. The results showed that serum sFAS levels in the silicotic patients were significantly higher than those in healthy volunteers. There was no significant difference in the expression of sFAS on peripheral blood lymphocytes between the patients with silicosis and age-matched healthy volunteers. To date, elevated sFAS levels have been detected only in patients with clinical symptoms of autoimmune diseases or malignant tumours. So, it remains to be clarified whether silicotic patients with elevated serum levels of sFAS have a tendency to develop autoimmune disease later, or whether some other distinct factor(s) is necessary to initiate the progression of autoimmune diseases [169].

It has been reported that stimulation of immunoglobulin production accompanies silicosis. Serum levels of IgG, IgA, and IgM were elevated in a majority of sandblasters with silicosis [162]. Healthy nonsmoking Vermont granite workers had increased concentrations of immunoglobulins in their bronchoalveolar lavage fluids, with normal serum immunoglobulin levels [66].

Nigam et al [165] examined the sera of a group of 19 subjects from the quartz-crushing industry. A significant increase in serum IgG was

recorded. An additional increase in serum IgA in radiographically proven silicotic cases was also observed, suggesting a local immune response.

2.3.4.5. Effects of Silica on Cytokines

Cytokines, also known as peptide growth factors or biological response modifiers, are extracellular signaling proteins secreted by specific (effector) cells and have their primary function as the ability to modify the behaviour of other closely adjacent cells. The cytokines, although first recognized for their capacity to modulate proliferation, can also affect other cellular functions such as motility, cytoskeletal arrangement and cell shape, contractility, and the production of extracellular matrix proteins. Many of the cytokines exhibit potent activity as chemotaxins for specific cell types and tissue homeostatic mechanisms are controlled by cytokine cascades and networks rather than by individual cytokines [170].

Interleukins

One well-established effect of silica on the functions of alveolar macrophages is to stimulate these cells to release the cytokine interleukin-1 (IL-1) which has wide-ranging biological effects on many cell types and organ system throughout the body and is important in the process of lymphocyte proliferation and the generation of immunoglobulin secreting cells. IL-1 may also attract lymphocytes from blood to site of immune response [164]. In vitro rat or rabbit alveolar macrophages secrete IL-1 on exposure to quartz [171, 172]. Inhalation exposure to silica has also been shown to increase the secretion of IL-1 by alveolar macrophages [173]. Several studies demonstrated the recruitment and activation of lung paranchymal lymphocytes during the pulmonary inflammation induced by intratracheal injection of silica, which was due to secretion of IL-1 by alveolar macrophages [174].

A critical component in the initiation of cell-mediated immunity is the presence of class II molecules (Ia) of the major histocompatibility complex on antigen-presenting cells (APC), and it has been reported that the increase of

aberrant expression of Ia antigens may be associated with autoimmunity and an amplification of inflammatory reactions. Strauhar et al. [175] have shown that the isolated macrophages and alveolar type II cells had a heightened expression of Ia, and that these cells produced increased amounts of IL-1 in the lungs of rats exposed to silica in vitro. Also, these authors have demonstrated that anti-Ia antibodies treatment of alveolar macrophages from silica-treated rats inhibits IL-1 secretion, which indicates a role of Ia molecules in the secretion of IL-1 from alveolar macrophages [176].

IL-8 is a recently described cytokine that previously was referred to as monocyte-derived chemotactic factor (MDCF), neutrophil-activating factor (NAF), neutrophil chemotactic factor (NCF), and more recently IL-8 and it is produced by a number of cell types, including monocytes and alveolar macrophages. IL-8 has a 10- to 100-fold increase in potency as a lymphocyte chemotaxin [177]. Human blood monocytes challenged by silica crystals in vitro, showed an increased secretion of IL-8, which is accompanied by increased influx of PMNs [178].

IL-6, is a cytokine which induces the final maturation of B cells, enhancing the secretion of immunoglobulins, and participates in the activation of resting T cells. It has been demonstrated by some investigators that alveolar macrophages treated with silica secrete IL-6 [179, 180]. The production of IL-6 by alveolar macrophages exposed to coal dust in vitro or in vivo suggests the involvement of TNF- α in the regulation of secretion of IL-6 from alveolar macrophages. And it can be postulated that these two cytokines may play an important role in that: (i) TNF- α -induced fibroblast proliferation and collagen synthesis might account for lung fibrosis, and (ii) IL-6-induced B cell maturation, immunoglobulin secretion, and T cell activation might explain the frequency of hypergammaglobulinemia and dysimmune anomalies occurring in pneumoconiosis [181].

Tumour Necrosis Factor (TNF)

Tumour necrosis factor- α (TNF- α) which was recognized because of its cytotoxic and antitumour properties, exerts a wide spectrum of biologic

activities, including modulation of endothelial cell homeostatic properties and surface antigens, enhancement of fibroblast growth, activation of neutrophils and macrophages. $\text{TNF-}\alpha$ can stimulate neutrophil phagocytosis and degranulation, prostaglandin E_2 and collagenase synthesis in a variety of tissues, bone resorption,, enhancement of eosinophils functional activity, IL-1 production, and be involved in granuloma formation, and is a potent inducer of fibroblast proliferation. Activated blood monocytes and tissue macrophages are known to secrete $\text{TNF-}\alpha$. It has also been proposed that some lymphocyte subpopulations can also produce $\text{TNF-}\alpha$ [182]. The role of $\text{TNF-}\alpha$ in silicosis has only recently received attention. Lassale and associates [183] observed an increased spontaneous secretion of $\text{TNF-}\alpha$ by BAL macrophages from dust-exposed miners and those with coal workers' pneumoconiosis, in contrast to unexposed control subjects. Borm et al [184] also observed spontaneous and stimulated release of $\text{TNF-}\alpha$ from blood monocytes of miners with coal workers pneumoconiosis.

Silica added to alveolar macrophages stimulated $\text{TNF-}\alpha$ secretion in vitro. Piguet and colleagues [179] reported that $\text{TNF-}\alpha$ mRNA levels in RNA extracted from whole lungs of mice were increased 15 days after intratracheal silica treatment. They also demonstrated that silica-induced collagen deposition is almost completely prevented by anti- $\text{TNF-}\alpha$ antibody. Their study provided clear evidence for the importance of $\text{TNF-}\alpha$ in animal model of silicosis. In a murine model of silicosis, it has been shown that silica stimulates alveolar macrophages to produce $\text{TNF-}\alpha$ [185]. In another study, it has been found an enhanced release of $\text{TNF-}\alpha$ from rat alveolar and peritoneal macrophages exposed to silica [186]. Different animal models of silicosis have shown that the inflammatory response induced by silica characterized by increased production of $\text{TNF-}\alpha$ is accompanied by a marked increase in alveolar macrophages as well as PMNs in these models [179, 185, 186].

The internalization of $\text{TNF-}\alpha$ by neutrophils stimulates them to release large quantities of ROS. Also, it has been demonstrated that $\text{TNF-}\alpha$ can

stimulate macrophage and neutrophil migration and production of H_2O_2 from these cells. There is evidence that the release of ROS and the production of $TNF-\alpha$ by alveolar macrophages occur during silicosis. Additionally, $TNF-\alpha$ cytotoxicity is inhibited by SOD and free radical scavengers [185].

Besides the wealth of evidence supporting a profibrotic role for $TNF-\alpha$, in silica-induced pulmonary inflammation and fibrosis, at least two experimental studies have shown that the lung fibrotic process may in some models be accompanied by a reduction of $TNF-\alpha$ activity. Ouellet and coworkers [187], using Wistar rats (a strain considered as relatively resistant to the fibrotic effect of silica), have shown a reduction in $TNF-\alpha$ production by BAL cells from silica-treated rats at all points considered up to 28 days after administration. The lung fibrotic response induced by silica in C3H/HeJ mice (also considered a relatively resistant strain to silica) was also associated with a reduction of $TNF-\alpha$ production by BAL cells [188].

Some investigators have observed that the administration of recombinant soluble TNF receptor to normal or silica-exposed mice markedly decreases the collagen content in the lung by limiting the activity of $TNF-\alpha$ in collagen synthesis [189]. Several reports have shown association between soluble TNF receptors and IL-10. IL-10 has been reported to regulate the cleavage of TNF receptors from monocytes/macrophages. When IL-10-deficient mice were exposed to silica, upregulation of $TNF-\alpha$ production was observed, indicating that IL-10 contributes to downregulation of $TNF-\alpha$ production, thus it was concluded that, in addition to its effects at the post-transcriptional level, IL-10 also controls $TNF-\alpha$ activity through an increased production of soluble TNF receptors. It has been reported that neutrophils stimulated in vivo by silica particles suppressed $TNF-\alpha$ release from naïve alveolar macrophage [180].

Eicosanoids

Another class of substances produced by alveolar macrophages and which is closely associated with inflammation and pulmonary disease is

composed of oxygenated metabolites of arachidonic acid (AA), collectively termed eicosanoids. Eicosanoids are synthesized and secreted by alveolar macrophages in response to various cellular stimuli as a result of the release of AA. Englen and associates [190, 191] investigated the in vitro production of AA metabolites in bovine alveolar macrophages incubated with varying quantities of crystalline silica particles. The results showed that there is a pronounced stimulation of AA metabolites, but the quantity and type released depended on the quantity of silica used to stimulate the bovine alveolar macrophages. At lowest silica dose, the released of cyclooxygenase metabolites, most notably TxB_2 , $\text{PGF}_{2\alpha}$, PGE_2 , and HHT. However, as the dose of silica was increased, the release of cyclooxygenase metabolites declined, and 5-lipoxygenase products, LTB_4 , and 5-HETE, were detected in greater quantities. Koren et al [192] investigated the cyclooxygenase and the lipoxygenase products produced by human alveolar macrophages obtained by BAL, incubated with different concentrations of silica. The results showed that silica causes an increased release of 5-lipoxygenase metabolites, LTB_4 , peptide leukotrienes C_4 , D_4 , E_4 , and 5-HETE after 3 hours and decreases in PGE_2 and TxB_2 production after 24 hours of exposure to silica. It has been suggested that stimulation of AA metabolism in alveolar macrophages by silica may result from an influx of extracellular calcium due to membrane damage or disruption caused by contact with silica particles [190, 191].

Suzuki and coworkers [193], examined, for the first time, the role of mast cell in mice model of silicosis. The results have shown that silica administration causes histologically less severe lung lesions in mast cell-deficient mice than in mast cell-intact or mast cell-reconstituted mice. The increases in neutrophil number and in protein and phospholipid content in BAL fluid after silica treatment were also significantly smaller in mast cell-deficient mice. Also, an increased number of lung mast cells as well as histamine content of the lung were observed in mice cell-intact mice as well as mast cell-reconstituted mice. These results suggest the involvement of mast cells in the development of silicosis. The release of histamine by mast cells may play a role in the pulmonary inflammatory inflammation as

histamine might cause an increase in exudative protein content, neutrophilia, and accumulation of inflammatory cells to form granulomata/nodules, all of which are characteristic of silica-induced lung disease [193].

There is also evidence supporting both the hypothesis that lymphocytes-derived lymphokines stimulate fibroblast proliferation and collagen synthesis, either directly or by activation of macrophages to secrete growth factor for fibroblasts [157]. Li et al. [194] have found that culture supernatant of lymphocytes isolated from the lungs of mice injected with silica dust 2 weeks previously were able to enhance the secretion of mitogenic activity for fibroblast cells by normal alveolar macrophages. Also, effective stimulation of normal alveolar macrophages to secrete mitogenic activity for fibroblasts, comparable to the maximal response elicited by interferon-gamma (IFN- γ), was induced by co-culture with comparatively small numbers of pulmonary lymphocytes from animals injected with silica.

2.3.5. Effects of Silica on Genetic Parameters

Several studies both *in vitro* and *in vivo* have been carried out to examine the probable effects of silica on genetic parameters. *In vitro* studies have shown a significant increases in binucleated and micronucleated cells in Syrian hamster embryo cells treated with Min-U-Sil quartz [15]. A significant and dose-dependent increase in the frequency of morphologically transformed Syrian hamster embryo cells was reported following treatment with Min-U-Sil 5 and other quartz samples [194]. Min-U-Sil 5 and Min-U-Sil 10 silica samples were also shown to induce a significant dose-related increase in micronuclei in Chinese hamster lung fibroblast (V79) cells and human embryo lung (HEL 299) cells, but not chromosomal aberrations in the same cell types [195].

In a study by Xiaorong et al. [196] Chinese hamsters lung fibroblasts (V79) were challenged with Min-U-Sil 5 and Min-U-Sil 10 and some of the silica particles used were pretreated with simulated pulmonary surfactant to model *in vivo* exposure conditions. The results have shown that both silica samples induced micronucleus formation in a dose-dependent manner when

compared to non-treated control cultures. The results also have shown that the pretreatment of silica particles with simulated pulmonary surfactant prevented micronucleus formation.

Gene expression using nine proto-oncogenes and the p53 tumor suppressor gene in a small number of cell lines suggested an increase in the mRNA expression of four proto-oncogenes (k-ras, H-ras, myc, abl) and of the p53 gene in some quartz-transformed cell lines [197]. Genomic instability has also been found in transformed BALB/c3T3 cells induced by silica, in vitro [198].

Epithelial cells from the lung of rats intratracheally exposed to quartz showed hprt gene mutations. Inflammatory cells from the quartz-exposed rat lungs caused mutations in the epithelial cells, while direct treatment of epithelial cells with quartz did not cause hprt mutations [199].

Pairon et al. [97] performed sister chromatid-exchange assays with human lymphocytes and monocytes using both quartz as well as tridymite. In these assays, tridymite induced sister chromatid exchanges in co-cultures of human lymphocytes and monocytes, but not in pure cultures of lymphocytes. However, quartz failed to induce sister-chromatid-exchanges either in co-cultures of human lymphocytes and monocytes or in pure cultures of lymphocytes.

Some investigators have examined the association between the effect of silica on DNA damage in alveolar macrophages. It was reported that there is a clear dose-and time-dependent relationships in silica induced DNA damage in alveolar macrophages assessed by single-cell gel electrophoresis (comet assay) [200, 201]. Shi X et al [202] have also reported silica-induced DNA strand breaks in alveolar macrophages. Also other investigators have demonstrated that silica induce oxidative DNA damage in alveolar macrophages in vitro [203, 204].

On the other hand, there are a few reports which have not provided an evidence for the genotoxic effects of silica. Silica has been shown to be not mutagenic to *Salmonella typhimurium*. Also quartz has been shown not to induce sister-chromatid exchanges in Chinese hamster lung fibroblast [205].

Oshimura et al. [206] reported that neither chromosomal aberration nor cellular transformation occurred in Syrian hamster embryo cells treated with quartz.

The effects of silica on genetic parameters have also been studied in silica-exposed workers. Sobti and Bhardwaj reported that the frequency of chromosomal aberrations in the peripheral lymphocytes of workers exposed to stone dust containing 50-60% silica in a stone crushing unit was higher than that of a control population [15].

IARC concluded that there is sufficient evidence in humans to show genotoxicity of inhaled crystalline silica from occupational sources [15]. However, the exact mechanism by which silica causes its genotoxic effect is less understood and needs further elucidation. Hesterberg et al. [194] have suggested a direct effect of silica particles on chromosomes during mitosis, when cells are capable of phagocytosis. This suggestion was further supported by Xiaorong et al [196], who have observed that pretreatment of silica particles with simulated pulmonary surfactants can prevent micronucleus induction and suggested that the micronucleus induction by silica might be due to direct interactions of particle surface with genetic materials or the spindle apparatus. However, other investigators have suggested an indirect mechanism for the genotoxicity of silica particles. Pairon et al [97], in their experimental model, observed that silica did not produce sister-chromatid exchanges in pure cultures of lymphocytes and induced the same effect in co-cultures of lymphocytes and monocytes. Accordingly, these investigators suggested that the phagocytosis of particles by monocytes/macrophages which is necessary to induce cytogenetic abnormalities, could be followed by monocyte/macrophage release of mediators such as IL-1, leukotrienes, ROS, AA metabolites, and possibly growth factors after stimulation. Growth factors and free radicals have been shown to induce sister chromatid-exchanges respectively in Chinese hamsters ovary fibroblasts and in lymphocytes. Recently, many investigators have provided convincing evidence that oxidative stress mediates the silica-induced genotoxicity by demonstrating that the addition of free radicals

scavengers (SOD and CAT) and antioxidants (glutathione) exhibited a protective effect against silica-induced genotoxicity in alveolar macrophages in vitro [200, 201].

2.3.6. Factors Affecting Toxicity of Silica

There are many factors which affect the fibrogenicity of silica, and hence the toxicity. However, the importance of each of these factors can be assessed in very general terms, but has not been studied in great detail for human populations. Physical and chemical properties may play an important role in the degree of exposure and subsequent toxicity of silica particles. Properties such as composition, particle diameter, particle surface area, shape, density, solubility, and hygroscopic and electrostatic properties may be important factors [15].

It is generally considered that the finer the particle, the farther it reaches the peripheral, functional respiratory units of the lung. Many studies have examined the relationship of silica particle size to its toxic effects and it appears to be about 1 μm . In animal experiments (rats, hamsters), the comparable values appear to be 1 to 2 μm . Particle size is usually a critical factor that determines the site of the respiratory tract in which a particle will be deposited in the lung. The solubility and the cytotoxicity of particles are influenced to a considerable extent by their size. Particulates can be eliminated from the lung either by dissolution or vascular transport. The rate of clearance, as well as toxicity, of particulates deposited in the respiratory tract can be predicted by their solubility in lung fluids. The lower the solubility the lower the removal rates. Thus, larger particles resist dissolution and so may persist within lung parenchyma for years. Surface area of the particle which is also related to its size, is of special importance when toxic materials, in the environment, are adsorbed on the surface of particles and thus are carried to the lung [207].

Hygroscopicity influences the increase in size of particles. Materials that are hygroscopic take on water and grow in size in the warm, saturated atmosphere of the lower respiratory tract [55].

The charge of the particles has been shown to be proportional to the severity of pulmonary damage caused by the inhaled particles. It was found that surfactants reduced the zeta potential, and measure of the charge, and also the toxicity of silica particles. The body appears to have low tolerance for positive charge or electron deficiency. Diseases of miners were related to the dryness of mines. It has been shown that silica particles carried higher values of positive charge when humidity was low in iron foundries, this suggest that keeping the humidity high may help in controlling the charge picked by the silica particles and thus their toxicity. Humidity would also help the coalescence of smaller particles to larger ones, possibly making them non-respirable [207].

The nature of the dust (e.g. silica polymorph, quartz, cristobalite etc.) also influences the toxicity of silica. The tetrahedral structure of silica is linked to its fibrogenic potential [66].

The composition of the dust have been shown to affect the fibrogenic action produced by silica. The term 'free silica' refers to the three important crystalline forms of silica, i.e., α -quartz, cristobalite, and tridymite to distinguish them from the silicates, minerals containing silica bound to one or more metallic cations. Such variations affect the biological activity of minerals. For example, silica polymorhs, including quartz and its varieties, can contain trace impurities that affect the biological activity of 'free silica'. Aluminium has been shown to decrease the fibrogenic activity of silica, while iron aggravates it [138].

It was found that the toxicity of quartz particles increased when the sample was ground in mill instead of a press. The reason may be that after crushing in the press, the particle has a smooth surface, whereas the milled particle has sharp egdes and a larger surface area [138].

The concentration of the dust particle in the inspired air is very important and the threshold concentration beyond which retention of particles occurs varies with the nature of the dust. The amount of dust retained in the lung is determined by the dust concentration in the ambient air and duration of exposure. In animal models, there appear to be a direct relationship

between the concentration of silica dust to which an animal is exposed and intensity and rapidity of the histologic reaction in the lung [207].

The importance of the concentration of dust in inspired air in the workplace as important factor affecting the extent of toxicity of silica became apparent when control measures have been taken in the workplace reduced the prevalence of silicosis. Despite much of the same level of exposure to silica dust, not all individuals develop a severe silicosis coupled with the lack of a uniform dose-response relation for the development of silicosis, has led to the suggestion that genetic factor(s) may be involved in the development of silicosis [66].

It has been demonstrated that cigarette smoking decreases the clearance of deposited particles, hence large amounts of the particles will be retained in the lung causing pulmonary damage. Other diseases, such as tuberculosis or rheumatoid arthritis, have been reported in subjects with silicosis. Whether these diseases complicate silicosis or silicosis complicates these diseases is not clear [66].

3. EXPERIMENTAL SECTION

3.1. Chemicals

Dimethylsulfoxide (DMSO)	Sigma
Disodium hydrogen phosphate (Na_2HPO_4)	Merck
Ethidium bromide (EtBr)	Sigma
Ethylene diamine tetraacetic acid disodium salt dihydrate (EDTA)	ICN
Ficoll (Histopaque)	Sigma
Heparine	Sigma
Low melting agarose (LMA)	Boehringer Mannheim
Normal melting point agarose (NMA)	Boehringer Mannheim
Phosphate buffered saline tablets (PBS)	Sigma
Sodium chloride (NaCl)	Merck
Sodium hydroxide (NaOH)	Merck
Sodium lauroyl sarcosinate	ICN
Tris	ICN
Triton-x 100	Sigma
Turbiquant-IgA (Anti-human IgA)	Behringwerke
Turbiquant-IgG (Anti-human IgG)	Behringwerke
Turbiquant-IgM (Anti-human IgM)	Behringwerke
Turbiquant-C3c (Anti-human C3c)	Behringwerke
Turbiquant-C4 (Anti-human C4)	Behringwerke

3.2. Apparatus

Balance	Sartorius
Centrifuge	MLW K 26 D
Coverslips (24x60mm)	Marienfield
Deep-freeze	Bosch
Electrophoresis	Biometra Analytic
Electrophoresis power supply	Power Pack P 25
Fluorescence microscopy	Zeiss

Fully frosted slides (26x76x1.0-1.2mm)	Surgipath
Hot plate	Stuart Scientific
Micropipettes	Gilson, Biohit
1-10µl, 0.5-50µl, 50-200µl, 200-1000µl, 1-5ml	
pH meter	NEL pH 890
Turbidimeter	Behring

3.3. Solutions

Electrophoresis Buffer

1705 ml cold distilled water, 52.8 ml 10 N NaOH, 8.8 ml 200 mM EDTA are mixed properly. It should be freshly prepared on the day of experiment.

EDTA 200mM

14.8 g ethylene diamine tetraacetic acid disodium salt dihydrate (EDTA) is dissolved in 200 ml distilled water. The pH is adjusted to 10 and the solution is kept at room temperature.

Ethidium Bromide Staining Solution

To prepare stock staining solution 5 mg ethidium bromide is dissolved in 25 ml distilled water, 1 ml of this solution is taken and the volume is adjusted to 10 ml with distilled water. Both solutions are kept at room temperature.

Low Melting Agarose (LMA) 0.5% Solution

125 mg LMA is dissolved in 25 ml PBS in hot water bath avoiding to boil the agarose. After the agarose is dissolved the solution has been divided into small amounts and kept in the refrigerator.

Lysing Solution

To prepare stock lysing solution, 146.1 g NaCl, 37.2 g EDTA and 1.2 g Tris are weighed and dissolved in 500 ml of distilled water. After adjusting pH

to 10 by 10 g of NaOH, 10 g of sodium lauroyl sarcosinate is added. The contents should be properly mixed, and then the volume adjusted to 1000 ml with distilled water. The stock lysing solution is kept at room temperature. To prepare lysing solution 178 ml of stock lysing solution, 2 ml Triton-x 100, and 20 ml dimethylsulfoxide, are mixed together properly. Cold lysing solution must be used so it is prepared and kept in the refrigerator for at least 1 hour before the experiment.

Neutralization Buffer

48.5 g Tris is dissolved in 750 ml distilled water, and the pH is set to 7.5. The volume is adjusted to 1000 ml with distilled water and the buffer is stored at room temperature.

Normal Melting Agarose (NMA) 0.5% Solution

125 mg NMA is dissolved in 25 ml PBS in hot water avoiding the agarose to boil. The solution is kept in small volumes in refrigerator.

Sodium Hydroxide (NaOH) 10N

200 g NaOH is dissolved in 500 ml distilled water, and the solution is kept at room temperature. The shelf life of the 10N NaOH stock solution is limited to 2 weeks.

Phosphate Buffered Saline (PBS) Buffer

1 PBS tablet is dissolved in 100 ml distilled water, and the buffer is sterilized, and stored at 4°C.

3.4. Exposed and Control Groups

Serum immunoglobulins (IgG, IgM, and IgA) and complement proteins (C3 and C4) levels of forty-five male foundry workers occupationally exposed to silica in the small plants that were located in Ankara, for at least 6 months (mean exposure period 9.8 ± 10.6 years, range 0.5-35 years) were determined. Serum immunoglobulins and complement levels of twenty-two

male pottery workers (mean duration of exposure 13.7 ± 12.1 years, range 0.5-45 years) from Nevşehir were also measured. Sixty-seven male healthy subjects of comparable age, and smoking habits, and with no history of silica exposure were chosen from the university staff as the control group. The age range of the whole workers was 17-65 years (mean 32.4 ± 10.5 years) and of the whole controls was 17-65 years (mean 32.3 ± 10.5 years). The age range of the foundry workers was 17-55 years (mean 32.6 ± 9.3 years) and of the pottery workers was 17-65 years (mean 31.5 ± 12.89 years).

DNA damage in the lymphocytes of the thirty foundry and the twenty-two pottery workers was investigated and compared with their fifty-two matched controls.

A questionnaire designed to yield information on age, occupational history, general health status, frequency of infections, smoking and dietary habits, and exposure to drugs and chemicals was used for each exposed and control subjects. Work characteristics of the workers such as the use of protective equipment (gloves, masks, eye glasses) and ventilation devices of the work places were also investigated.

3.5. Immunoglobulin and Complement Protein Determinations

Serum concentrations of IgG, IgA, IgM and the C3 and C4 components of complement proteins were determined by turbidimetry using anti-human IgG, IgA, IgM, C3 and C4 antibodies.

3.5.1. Blood Sampling

A 2-ml peripheral blood sample was taken from each individual and allowed to clot for the measurement of serum immunoglobulin, and complement concentrations. The serum samples were frozen the same day and were kept at -20°C until they were analysed.

The serum samples were diluted to 2ml with 0.9% NaCl solution before the experiments.

3.5.2. Determination of Immunoglobulin G Levels

20 μ l of diluted serum sample was put in a turbidimeter tube and 500 μ l of antihuman IgG antibody was added. The values were read as mg/dl by turbidimetry.

3.5.3. Determination of Immunoglobulin A Levels

500 μ l of antihuman IgA antibody was added to 50 μ l of diluted serum sample and in the turbidimeter the values were read as mg/dl.

3.5.4. Determination of Immunoglobulin M Levels

500 μ l antihuman IgM antibody was added to the 200 μ l of diluted serum sample and measured by turbidimetry.

3.5.5. Determination of C3 Complement Levels

50 μ l of diluted serum sample was taken in a turbidimeter tube and 500 μ l of antihuman C3 antibody was added. The levels were measured as mg/dl by turbidimetry.

3.5.6. Determination of C4 Complement Levels

500 μ l of antihuman C4 antibody was added to the 20 μ l of diluted serum sample and the levels were measured by turbidimetry.

3.6. Assessment of DNA Damage in the Peripheral Lymphocytes by the Alkaline COMET Assay

3.6.1. Blood Sampling and Lymphocyte Preparations

1. A 2 ml heparinized (50 units/mol sodium heparin) whole blood sample was taken by venipuncture from each exposed and control subject.
2. The whole blood which was diluted with 6 ml PBS was gently layered onto 2 ml Ficoll by a pasteur pipette in a centrifuge tube.

3. The mixture was centrifuged for 20 minutes at 1400 rpm and the lymphocytes were isolated by Ficoll-Hypaque density gradient [Boyum].
4. The cells on to the Ficoll layer in the interphase were carefully taken by a pasteur pipette after centrifugation.
5. To the lymphocytes PBS was added and mixed.
6. The cells were washed by centrifugation at 1800 rpm for 10 min.
7. The supernatant was poured off and the lymphocytes were washed with PBS again in the same manner.
8. After pouring off the supernatant the lymphocyte pellet was suspended in 75 μ l of 0.5% LMA for embedding on slides.

3.6.2. Slide Preparation

The basic alkaline technique of Singh et al. [208] as further described by Anderson et al. [209] was followed.

1. Fully frosted microscopic slides were each covered with 110 μ l of 0.5% NMA and they were immediately covered by a large no.1 coverslip and kept at room temperature for about 5 min to allow the agarose to solidify. This layer was used to promote the attachment of the second layer of 0.5% LMA.
2. After gently removing the coverslip the cell suspension in 75 μ l of 0.5% LMA was rapidly pipetted onto the first agarose layer, spread using a coverslip, and maintained on an ice-cold flat tray for 5 min to solidify.
3. The coverslip was gently removed and the third layer of 0.5% LMA (75 μ l) was added; spread using a coverslip, and again allowed to solidify on ice for 5 min.
4. After removal of the coverslip the slides were immersed in cold freshly made lysing solution for a minimum of 1 h at 4⁰C. Slides may be stored for extended periods of time in cold lysing solution.

3.6.3. Electrophoresis

1. The slides were removed from the lysing solution, drained and placed in a horizontal gel electrophoresis tank side by side, avoiding spaces and with the agarose ends facing each other, nearest the anode.
2. The tank was filled with fresh electrophoresis solution to a level approximately 0.25 cm above the slides.
3. Before electrophoresis, the slides were left in the solution for 20 min to allow the unwinding of the DNA and expression of alkali labile damage.
4. Electrophoresis was conducted at a low temperature (4°C) for 20 min using 24 V and adjusting the current to 300mA by raising or lowering the buffer level.
5. All of these steps were performed under dimmed light to prevent the occurrence of additional DNA damage and the tank was covered with a black cloth.
6. After electrophoresis, the slides were taken out of the tank.
7. Tris buffer was added dropwise and gently to neutralize the excess alkali and the slides were allowed to sit for 5 min. The neutralizing procedure was repeated three times.
8. To each slide 60µl ethidium bromide (20µg/ml) was added.
9. The slides were covered with a coverslip, placed in a humidified air-tight container to prevent drying of the gel, and analyzed within 3-4h.

3.7.4. Slide Scoring

For visualization of the DNA damage, the slides were examined at a x1000 magnification, using a 100x objective (oil immersion) on a fluorescence microscope. Images of 200 randomly selected lymphocytes, i.e., 100 cells from each of two replicate slides, were analysed from each sample and the DNA damage was scored visually [210, 211]. In principle comets are formed by the release of damaged DNA from the core of nucleus

with electrophoresis, at low damage levels, stretching of attached strands of DNA is likely to occur, and with increasing numbers of breaks, DNA pieces migrate freely into the tail of the comet. In the experiments, the cells were graded by eye into four categories on the basis of damage extent, i.e., undamaged (<5%), slightly damaged (5% to 20%), damaged (20-40%) and highly damaged (>40%).

3.6.4. Statistical Analysis

The results are expressed as mean $\pm$ SD. For immunotoxicological evaluation distribution of data was checked for normality by Kolmogorov-Smirnov test and for the statistical comparison of the mean values from exposed and non-exposed individuals Independent Samples Student's t-Test was performed. For the genotoxicological assessment the statistical comparison of the results from workers and controls was performed using the Ki-Square test. An analysis of linear regression was used to estimate the effects of the duration of occupational exposure of silica on the DNA damage [212].

4. RESULTS

4.1. Serum Immunoglobulin and Complement Levels of the Workers and Controls

The serum Ig and complement levels of foundry and pottery workers and their controls are presented in Table 2 and Table 3 respectively. The mean serum Ig and complement levels of workers and controls were given in Table 4, and Figures 1 and 2 respectively.

4.1.1. Serum Ig G Levels

Serum IgG levels of foundry workers (n=45) were found to be 868.8 ± 216.3 mg/dl (414-1530) whereas the IgG levels of their controls were 1107.3 ± 340.7 mg/dl (661-2310). As seen in Figure 1 a significant decrease in serum IgG levels was observed in foundry workers when compared to their controls ($p < 0.001$).

Serum IgG concentrations of pottery workers and their controls (n=22) were 1030.5 ± 199.1 mg/dl (728-1400), and 1107.3 ± 235.6 mg/dl (682-1570) respectively. No significant difference was observed in serum IgG levels of the pottery workers.

When serum IgG levels of foundry and pottery workers were evaluated all together, the serum IgG concentrations of the workers were 921.9 ± 222.9 mg/dl (414-1530). The serum IgG levels of the controls were 1107.3 ± 308.5 mg/dl (661-2310). A significant decrease in IgG concentrations in serum of the workers were found when compared to the controls ($p < 0.001$).

4.1.2. Serum IgA Levels

Serum IgA levels of the foundry workers and their controls were 170.1 ± 75.4 mg/dl (65.4-400) and 221 ± 94.8 mg/dl (82-384) respectively. A significant decrease in serum IgA levels in the foundry workers were found when compared with their controls ($p < 0.01$).

TABLE 2. Serum Immunoglobulin and Complement Levels of Foundry Workers and Controls

Ig A, Ig G, Ig M, C3, C4 Levels (mg/dl)										Ig A, Ig G, Ig M, C3, C4 Levels (mg/dl)									
Foundry Workers										Controls									
No	Age	Smoking	Duration of Exposure (year)	Ig A	Ig G	Ig M	C3	C4		No	Age	Smoking	Ig A	Ig G	Ig M	C3	C4		
1	33	-	3	235	1190	71.9	93.3	22.7		1	32	-	185	1280	53.5	83.5	19.5		
2	32	-	7	164	990	173	83.9	8.7		2	32	-	284	1690	121	118	19.2		
3	25	+	1.5	116	940	85.8	82.1	19.9		3	25	+	143	989	107	66	24		
4	29	-	3	250	1530	84.8	97.4	10.8		4	28	-	148	844	135	39	18		
5	25	+	2.5	182	890	52.2	84.8	17.1		5	26	+	132	987	88	46	12		
6	32	-	4.5	143	775	47.2	171	28.4		6	32	-	308	1300	77.6	109	19.1		
7	35	-	12	330	824	43.2	128	41.5		7	35	-	162	923	214	129	26.9		
8	27	+	3	103	889	151	114	26.2		8	28	+	273	947	240	80.9	20.7		
9	33	+	0.3	177	728	113	97	54.8		9	32	+	188	1530	110	49	30		
10	29	+	2	269	1110	166	156	45.4		10	29	+	684	1720	169	92	45		
11	30	+	0.5	195	987	50.9	111	21.6		11	30	+	294	1590	238	65	40		
12	28	+	2.5	193	1050	81.9	98.7	24.9		12	28	+	252	694	98.2	106	21.2		
13	37	+	0.2	92.6	576	105	137	28.1		13	37	+	201	805	77.8	88.7	20.9		
14	28	-	5	68	535	51.7	127	28		14	28	-	177	962	132	101	16		
15	43	-	11	216	1000	75.9	124	22.5		15	43	-	161	934	50.4	131	38.1		
16	39	-	17	265	1070	55.1	136	27		16	40	-	240	1520	108	154	33.4		
17	25	-	0.8	107	869	136	114	26.2		17	24	-	150	1200	63	62	14		
18	26	-	0.5	93.2	994	161	115	16.3		18	26	-	275	1170	65.4	72.4	10.3		
19	31	+	0.5	106	1040	111	152	32.6		19	31	+	153	989	81	62	24		
20	27	-	1.5	104	910	104	60.8	15.2		20	-	-	-	-	-	-	-		
21	24	-	1.5	131	1040	115	142	20.6		21	23	-	283	1030	58.5	114	22		
22	20	+	4.5	127	864	61.7	95.9	15.4		22	20	+	190	906	176	97.1	16.3		
23	28	+	14	270	1240	106	91.3	23.3		23	28	+	142	702	119	112	20.8		
24	55	+0.5	15	233	894	74	67.6	20.9		24	55	+0.5	227	1220	98	58			
25	45	+2	32	110	524	44.7	117	31.6		25	44	+2	184	827	53.3	10626	16.8		
26	29	+	15	141	715	53.9	116	29.9		26	30	+	211	896	67	75.5	18.3		
27	46	+	35	236	1050	182	114	30.8		27	47	+	290	939	63.2	156	44.5		
28	36	+0.5	22	179	849	92.3	119	30.6		28	36	+0.5	228	1340	154	124	21.8		
29	50	+	30	84.3	676	76.4	97.7	22		29	50	+	159	1240	135	87	24.2		
30	47	-	34	190	764	88.9	122	24.4		30	47	-	218	1290	129	110	17.3		
31	46	+	33	151	718	53	92.3	18.1		31	46	+	140	913	165	43	22		
32	18	-	03	65.4	639	102	67.8	13.4		32	19	-	285	1090	345	112	16.6		
33	34	+2	07	105	978	134	81.1	25.8		33	35	+2	154	729	82.6	122	30		
34	25	+	06	71.8	724	34.7	74.8	19.7		34	26	+	225	1220	159	95.6	16.8		
35	25	+	02	220	899	152	120	30.3		35	26	+	247	882	58	43.3	20.6		
36	47	-	30	251	1090	47.4	85.4	17.5		36	47	-	313	1440	198	163	31		
37	20	+0.5	01	93.2	600	19.2	72.1	14.8		37	20	+0.5	82	661	114	81.5	13.4		
38	34	+0.5	10	167	703	52.1	83.4	17.1		38	34	+0.5	365	2310	194	106	14		
39	17	+	1.5	131	821	54.2	76.4	15.7		39	17	+	181	746	73.1	82.5	15.2		
40	48	+	01	124	414	128	122	29.6		40	47	+	300	1100	104	140	38.5		
41	30	+0.3	17	400	1070	91.5	100	17.9		41	30	+0.3	160	1570	84	81	30		
42	41	+	15	104	607	40.7	97.2	24.7		42	42	+	231	929	85	59	16		
43	20	+	5	164	637	69.7	74.5	15.7		43	20	+	147	772	69.5	90.1	15		
44	32	-	20	222	977	151	101	23.8		44	31	-	163	713	199	116	32.5		

TABLE 3. Serum Immunoglobulin and Complement Levels of Pottery Workers and Controls

Ig A, Ig G, Ig M, C3, C4 Levels (mg/dl)										Ig A, Ig G, Ig M, C3, C4 Levels (mg/dl)									
Pottery Workers										Controls									
No	Name	Age	Smoking	Duration of Exposure (year)	Ig A	Ig G	Ig M	C3	C4	No	Name	Age	Smoking	Ig A	Ig G	Ig M	C3	C4	
1	K.A	65	+1	45	109	780	68.9	87.2	27.3	1	L.S	65	+1	287	1130	45.1	90.5	21.4	
2	P.C	51	-	13	197	1400	65.8	101	25.7	2	Y.C.	50	-	160	1220	83.6	105	31.6	
3	R.C	35	+1	20	194	910	78.4	113	21.3	3	N.K.	35	+1	207	981	173	126	22.3	
4	A.C	40	+1	20	105	1140	77.5	134	35.6	4	A.K	40	+1	153	1140	145	160	32.3	
5	S.V	17	-	1	106	1330	122	112	24.2	5	A.B	17	-	160	787	87.4	96.5	14.1	
6	O.C	29	+1.5	15	83.1	1140	87.2	110	20.7	6	H.G	28	+1.5	231	1410	222	65	17	
7	S.K	41	+2	19	174	1150	96.9	68.8	14	7	C.U	41	+2	221	1240	94.3	113	25.1	
8	Ü.K	35	-	19	393	1330	186	94.7	19.6	8	G.B	35	-	333	1570	87.4	141	27	
9	M.K	30	-	19	235	1360	142	118	18.9	9	D.F	30	-	<33	789	80.5	134	32	
10	M.S.G	27	+1.5	2	150	784	122	80.5	23.8	10	H.O	28	+1.5	283	1180	125	55	24	
11	F.K	23	+1	3	79.2	941	92.4	67	18.4	11	S.D	23	+1	224	1340	106	45	21	
12	M.Y	32	-	15	247	826	37.8	60.6	22.5	12	Ö.P.	32	-	44.2	1130	79.6	97.6	22.2	
13	H.K.B	19	+1	2	64.7	990	37.4	91.4	17.7	13	G.G	19	+1	166	761	109	110	21.2	
14	O.A	33	+1	15	212	875	43.6	82.7	18.3	14	F.O	33	+1	257	1100	106	108	21.6	
15	H.A	25	+1	10	113	1100	126	148	26.3	15	T.T	25	+1	156	1130	278	73	29	
16	G.E	23	+0.3	05	113	728	55.5	94.7	10.4	16	M.G	23	+0.3	184	1200	255	58	22	
17	G.Ö	27	-	10	175	965	159	82.4	36.6	17	O.K	27	-	141	682	105	114	21.2	
18	A.K	23	-	10	127	1070	185	75.9	23.7	18	A.E	22	-	235	1370	143	45	12	
19	M.A	19	+1	06	81.5	824	85.3	78	24.2	19	A.D	19	+1	126	1340	78.5	130	18.3	
20	O.B	24	+0.3	5-6	208	1080	196	94.7	13.6	20	H.P	24	+0.3	127	1020	37	85.1	19.3	
21	H.K	17	-	07	142	1010	62.9	96.2	27.9	21	I.I.	18	-	143	1100	96.7	91.6	16.6	
22	M.D	58	+1	40-45	179	938	54.5	95.9	24.1	22	M.E	58	+1	185	740	113	115	18.9	

TABLE 4. The Mean Serum Immunoglobulin and Complement Levels of Workers and Controls (Mean $\pm$ SD)

IgA, IgG, IgM, C3, C4 Levels (mg/dl)					
Groups	IgA	IgG	IgM	C3	C4
Foundry Workers (n=45)	170.1 ± 75.4**	868.8 ± 216.3***	89.1 ± 42.0**	104.5 ± 25.2	24.0 ± 8.3
Controls (n=45)	221.0 ± 94.8	1107.3 ± 340.7	120.3 ± 62.5	93.6 ± 31.3	22.9 ± 8.7
Pottery Workers (n=22)	158.5 ± 74.9	1030.5 ± 199.1	99.2 ± 49.1	94.9 ± 21.4	22.5 ± 6.3
Controls (n=22)	184.4 ± 72.2	1107.3 ± 235.6	120.5 ± 61.2	98.1 ± 31.2	22.3 ± 5.4
Whole of Workers (n=67)	166.3 ± 74.9**	921.9 ± 222.9***	92.4 ± 44.4**	101.4 ± 24.3	23.5 ± 8.0
Controls (n=67)	208.8 ± 89.2	1107.3 ± 308.5	120.3 ± 61.8	95.1 ± 31.2	22.7 ± 7.8

** p<0.01

*** p<0.001

Figure 1. The Mean Serum Immunoglobulin Levels of Workers and Controls

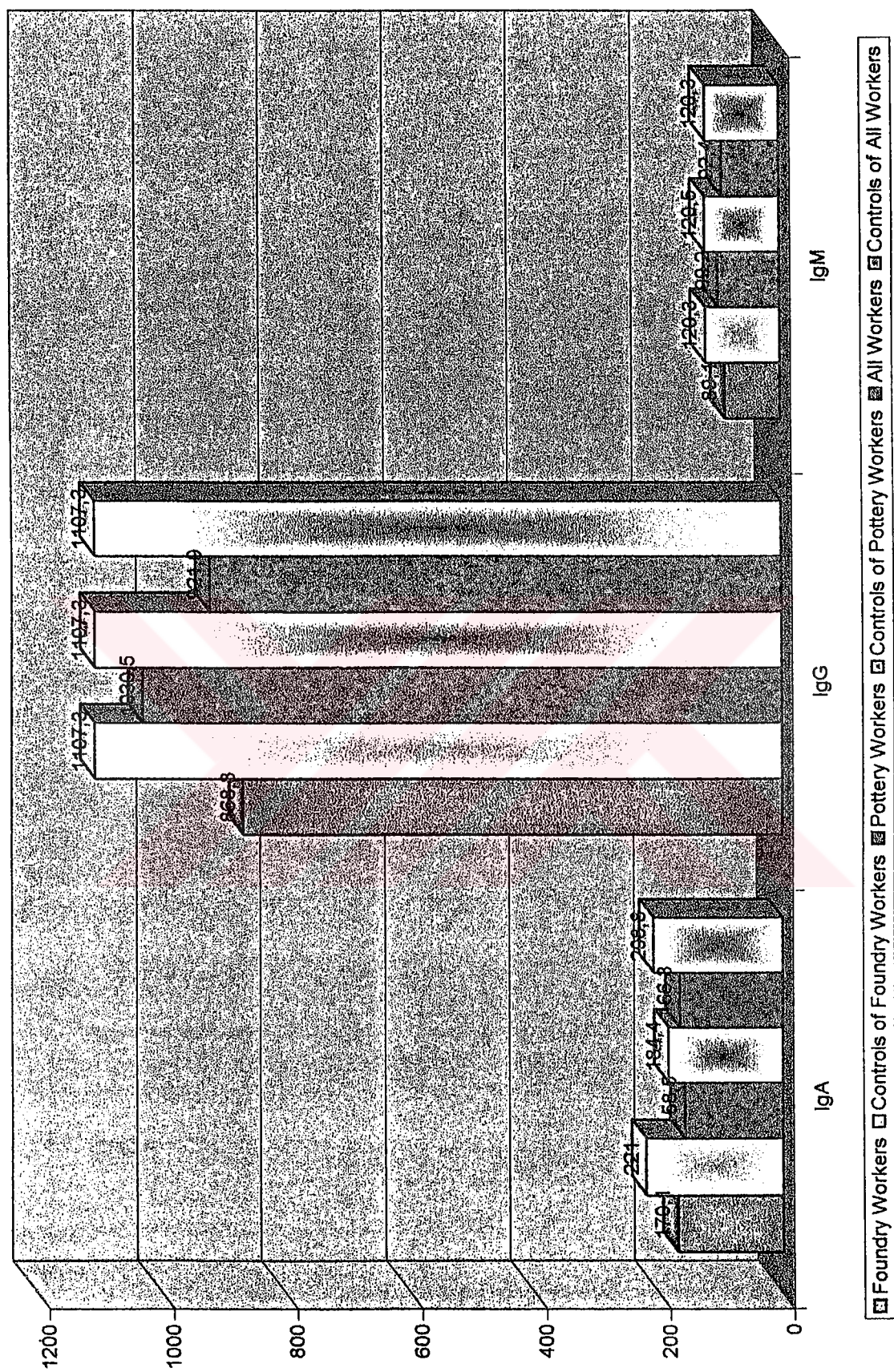
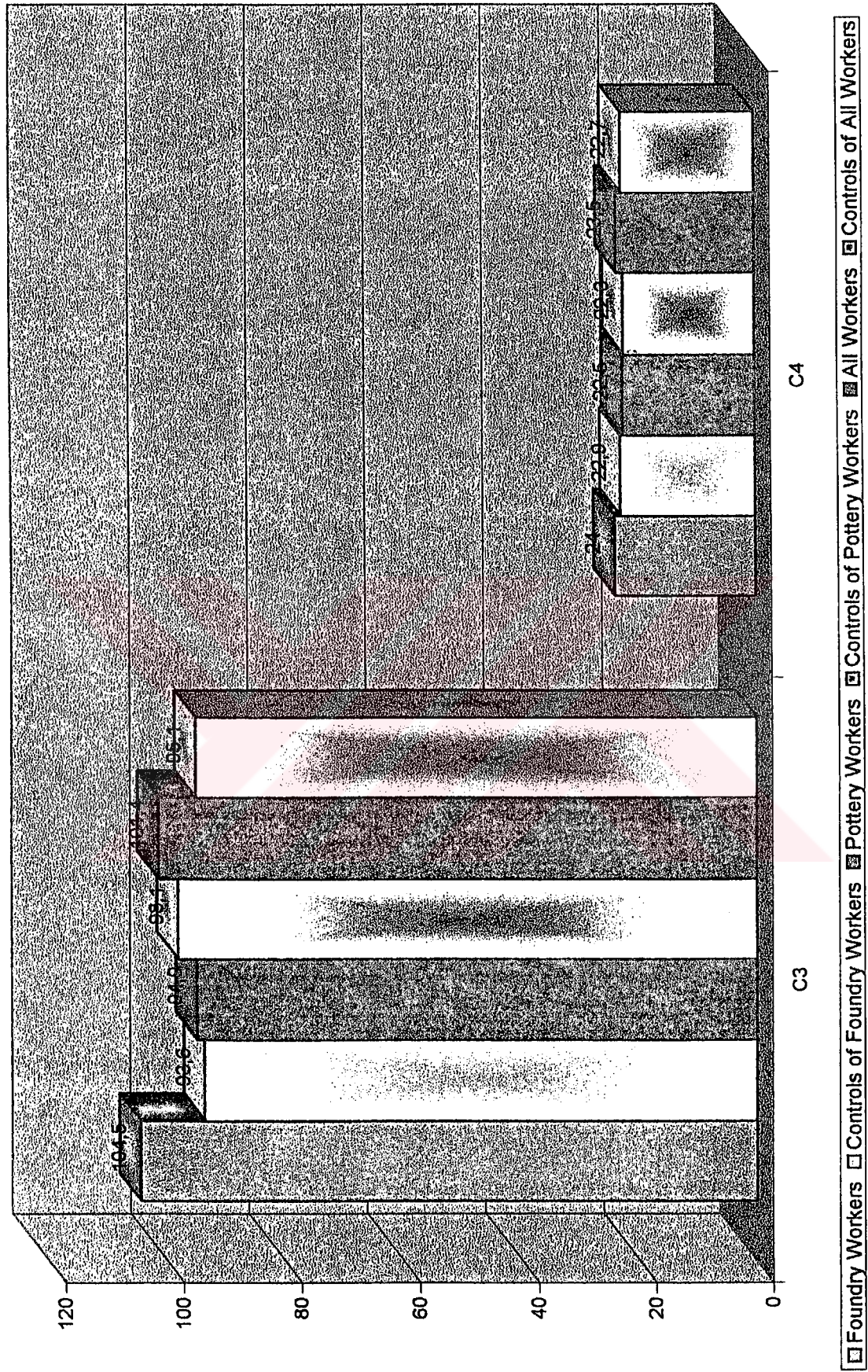


Figure 2. The mean Serum Complement Levels of Workers and Controls



Serum IgA concentrations of the pottery workers were found to be 158.5 ± 74.9 mg/dl (64.7-393) whereas the IgA levels were 184.4 ± 72.2 mg/dl (33-333) in the controls. No significant difference was observed.

Serum IgA levels of all workers were 166.3 ± 74.9 mg/dl (65.4-400) and the values in their controls were 208.8 ± 89.2 mg/dl (33-384). A significant decrease was seen in the workers when compared to the controls ($p < 0.01$).

4.1.3. Serum IgM Levels

Serum IgM concentrations of foundry workers were 89.1 ± 42 mg/dl (34.7-182) whereas the values were 120.3 ± 62.5 mg/dl (50.4-345) in the controls. A significant decrease was seen in IgM levels in the foundry workers ($p < 0.01$).

Serum IgM levels of the pottery workers were 99.2 ± 49.1 mg/dl (37.4-196) and the IgM levels of their controls were 120.5 ± 61.2 mg/dl (37-278). No significant difference was observed in serum IgM levels of the pottery workers.

But when compared all workers together with their controls, a significant decrease was found in the serum IgM levels of the workers ($p < 0.01$). Serum IgM levels of 92.4 ± 44.4 mg/dl for the workers, 120.3 ± 61.8 mg/dl for the controls were measured.

4.1.4. Serum C3 Complement Protein Levels

Serum C3 complement protein concentrations of the foundry workers and their controls were 104.5 ± 25.2 mg/dl (60.8-171) and 93.6 ± 31.3 mg/dl (39-163) respectively. No significant difference was observed in the serum C3 complement levels of the foundry workers when compared to controls.

Serum C3 complement protein concentrations of the pottery workers and their controls were 94.9 ± 21.4 mg/dl (60.6-148), and 98.1 ± 31.2 mg/dl (45-160) respectively. No significant difference was found in the pottery workers.

No significant difference was also observed in the serum C3 complement levels of the all workers when compared to all their controls.

4.1.5. Serum C4 Complement Protein Levels

Serum C4 complement protein concentrations of the foundry workers were found to be 24 ± 8.7 mg/dl (8.7-54.8) whereas the concentrations were 22.9 ± 8.8 mg/dl (10.3-44.5) in the controls. No significant difference was observed in the serum C4 complement levels of the foundry workers.

In the pottery workers, the serum C4 levels was found to be 22.5 ± 6.3 mg/dl (10.4-36.6). The serum C4 concentrations of the controls were 22.3 ± 5.4 mg/dl (12-32.3). No significant difference was observed between the pottery workers and their controls.

When serum C4 complement levels of all workers were compared with the controls no significant difference was also observed.

4.2. DNA Damage in the Peripheral Lymphocytes of the Workers and Controls

The results of the grades of DNA damage in the peripheral lymphocytes of the foundry and pottery workers with their controls are listed in Table 5 and 6 respectively. The mean DNA damage in the peripheral lymphocytes of the workers and controls were presented in Table 7, and also shown in Figure 3.

A significant increase ($\chi^2=253.8$ $p<0.001$) in the DNA damage was observed in foundry workers ($n=30$) when compared with their controls. The number of lymphocytes without DNA damage was significantly higher in the unexposed controls.

In the pottery workers ($n=22$) the number of lymphocytes with DNA damage was also found to be higher when compared to their controls ($\chi^2=261.9$ $p<0.001$).

When compared all foundry and pottery workers with their controls a significant increase ($\chi^2=491.3$ $p<0.001$) in the DNA damage was observed.

TABLE 5. Grades of DNA Damage in Peripheral Lymphocytes of the Foundry Workers and Controls

Grades of DNA Damage															
FoundryWorkers					Controls										
No	Age	Smoking	Duration of Exposure (year)	No Damaged	Slightly Damaged	Damaged	High Damaged	Σ	Age	Smoking	No Damaged	Slightly Damaged	Damaged	High Damaged	Σ
1	20	+1	4.5	169	11	7	13	200	20	+1	179	11	6	4	200
2	28	+1	14	153	12	8	27	200	28	+1	172	13	9	6	200
3	55	+0.5	15	145	10	12	33	200	55	+0.5	148	17	15	20	200
4	45	+2	32	131	18	10	41	200	44	+2	162	18	8	12	200
5	29	+1	15	142	16	14	28	200	29	+1	168	14	10	8	200
6	46	+1	35	138	12	13	37	200	47	+1	161	34	1	4	200
7	36	+0.5	22	159	8	10	23	200	36	+0.5	194	0	0	6	200
8	30	+1	20	158	4	14	24	200	30	+1	158	30	6	6	200
9	55	+2	41	129	15	20	36	200	55	+2	156	18	11	15	200
10	27	+1	3	172	12	6	10	200	27	+1	180	12	5	3	200
11	50	+1	30	136	21	9	34	200	50	+1	133	47	8	12	200
12	47	-	34	152	14	7	27	200	47	-	169	14	9	8	200
13	46	+1	33	143	15	10	32	200	47	+1	155	30	10	5	200
14	18	-	3	178	11	4	7	200	18	-	188	5	3	4	200
15	34	+2	7	160	20	13	7	200	35	+2	128	32	26	14	200
16	47	-	30	157	12	9	22	200	47	-	182	9	5	4	200
17	17	+1	1.5	180	6	6	8	200	17	+1	193	3	2	2	200
18	25	+1	2	170	8	7	15	200	26	+1	184	7	4	5	200
19	34	+0.5	10	160	15	10	15	200	34	+0.5	178	15	3	4	200
20	48	+1	1	169	16	7	8	200	48	+1	179	8	6	7	200
21	25	+1	6	162	14	11	13	200	25	+1	175	12	8	5	200
22	20	+0.5	1	188	6	2	4	200	20	+0.5	183	12	2	3	200
23	48	+0.3	33	137	14	10	39	200	48	+0.3	174	11	6	9	200
24	30	+0.3	17	153	13	12	22	200	30	+0.3	157	15	12	16	200
25	32	-	20	163	9	8	20	200	32	-	187	3	4	6	200
26	38	-	28	157	8	10	25	200	38	-	181	12	4	3	200
27	20	-	3	176	13	3	8	200	20	-	190	7	2	1	200
28	41	+1	15	140	24	16	20	200	42	+1	164	24	4	8	200
29	20	+1	5	172	10	7	11	200	20	+1	178	10	7	5	200
30	27	+2	14	149	15	11	25	200	28	+2	185	6	4	5	200

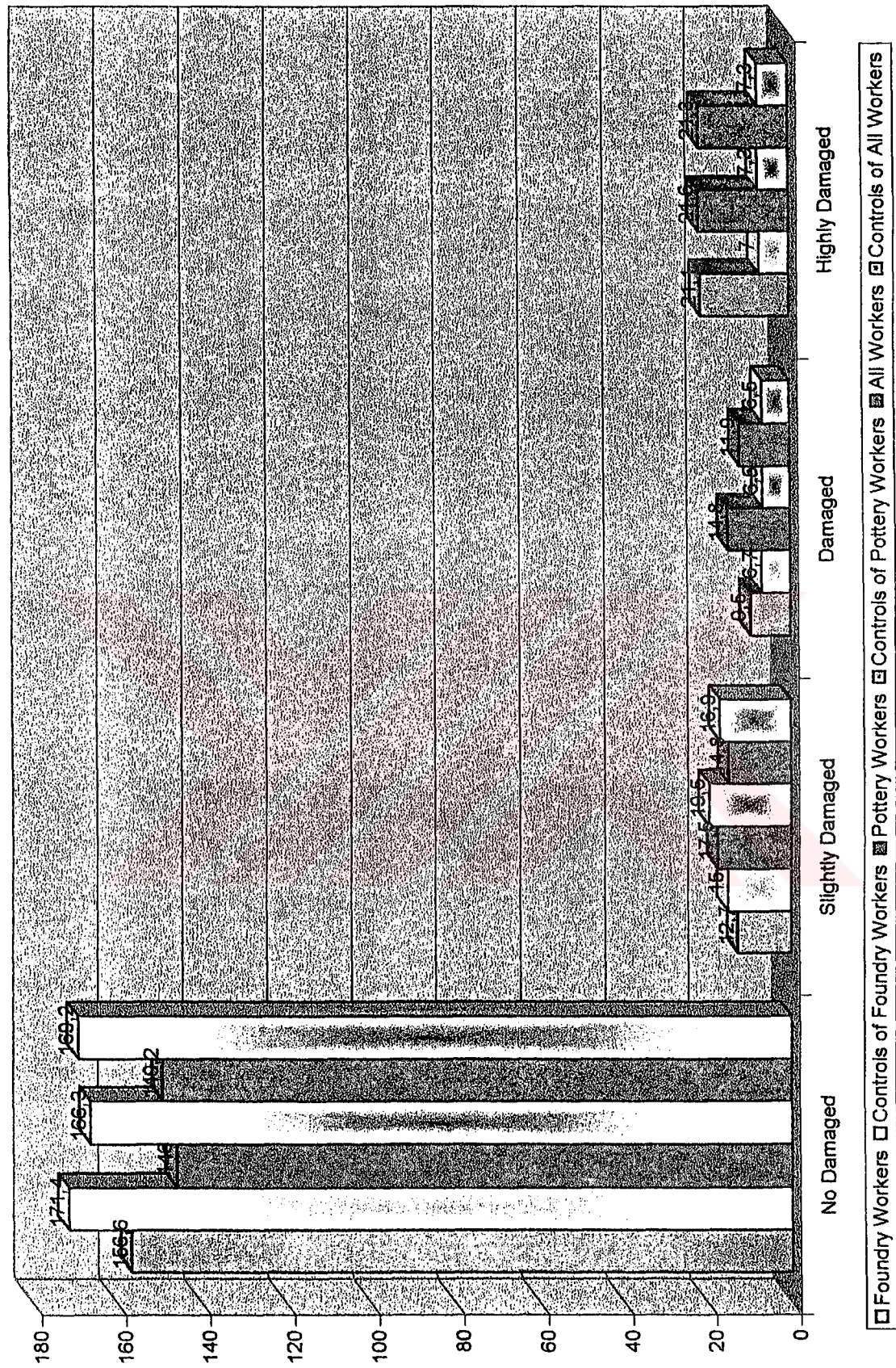
TABLE 6. Grades of DNA Damage in Peripheral Lymphocytes of the Pottery Workers and Controls

Grades of DNA Damage															
Pottery Workers					Controls										
No	Age	Smoking	Duration of Exposure (year)	No Damaged	Slightly Damaged	Damaged	High Damaged	Σ	Age	Smoking	No Damaged	Slightly Damaged	Damaged	High Damaged	Σ
1	65	+1	45	119	26	17	38	200	65	+1	155	16	12	17	200
2	51	-	13	150	19	17	14	200	50	-	190	3	2	5	200
3	35	+1	20	147	22	12	19	200	35	+1	131	50	12	7	200
4	40	+1	20	148	19	16	17	200	40	+1	183	10	4	3	200
5	17	-	1	184	9	4	3	200	18	-	192	3	3	2	200
6	29	+1.5	15	135	17	19	29	200	28	+1.5	180	10	8	2	200
7	41	+2	19	132	18	20	30	200	41	+2	132	39	19	10	200
8	35	-	19	154	15	12	19	200	35	-	179	14	1	6	200
9	30	-	19	156	18	10	16	200	30	-	149	36	4	11	200
10	27	+1.5	2	168	17	6	9	200	28	+1.5	165	27	2	6	200
11	23	+1	3	159	18	11	12	200	24	+1	106	54	17	23	200
12	32	-	15	149	17	21	13	200	31	-	165	4	7	24	200
13	19	+1	2	168	18	6	8	200	19	+1	184	7	5	4	200
14	33	+1	15	132	21	25	22	200	33	+1	162	24	6	8	200
15	25	+1	10	152	16	15	17	200	26	+1	164	16	14	6	200
16	23	+0.3	5	132	13	18	37	200	24	0.3	138	49	2	11	200
17	27	-	10	142	22	14	22	200	26	-	196	2	1	1	200
18	23	-	10	107	18	22	53	200	22	-	146	32	10	12	200
19	19	+1	6	150	20	13	19	200	19	+1	183	10	3	4	200
20	24	+0.3	5-6	154	14	15	17	200	24	+0.3	187	9	2	2	200
21	17	-	7	160	13	14	13	200	17	-	195	2	1	2	200
22	58	+1	40-45	114	16	22	48	200	58	+1	176	11	7	6	200

TABLE 7. The Mean DNA Damage in the Peripheral Lymphocytes of the Workers and Controls (Mean $\pm$ SD)

Groups	Grades of DNA Damage				χ^2	p
	No Damaged	Slightly Damaged	Damaged	Highly Damaged		
Foundry Workers (n=30)	156.6 $\pm$ 15.4***	12.7 $\pm$ 4.5***	9.5 $\pm$ 3.8***	21.1 $\pm$ 10.9***	253.831	<0.001
Controls (n=30)	171.4 $\pm$ 16.5	15.0 $\pm$ 10.6	6.7 $\pm$ 5.1	7.0 $\pm$ 4.5		
Pottery Workers (n=22)	146.0 $\pm$ 18.4***	17.5 $\pm$ 3.7***	14.8 $\pm$ 5.6***	21.6 $\pm$ 12.8***	261.900	<0.001
Controls (n=22)	166.3 $\pm$ 24.3	19.5 $\pm$ 16.8	6.5 $\pm$ 5.3	7.3 $\pm$ 5.4		
Whole of Workers (n=52)	149.2 $\pm$ 26.2***	14.8 $\pm$ 4.9***	11.9 $\pm$ 5.5***	21.3 $\pm$ 11.6***	491.318	<0.001
Controls (n=52)	169.2 $\pm$ 20.1	16.9 $\pm$ 13.6	6.5 $\pm$ 5.1	7.3 $\pm$ 5.4		

Figure 3. The Mean DNA Damage in the Peripheral Lymphocytes of the Workers and Controls



5. DISCUSSION

Millions of workers in various occupational settings have the potential to be exposed to hazardous substances. Most workers in foundry, pottery, manufacturing and mining industries are exposed to complex chemical mixtures mainly to silica. Many of these substances are known to be immunotoxic and genotoxic and have the potential to cause immune and genetic alterations in the target tissues of the exposed workers. Crystalline silica is probably one of the most documented work place contaminants. The severity and the widespread nature of exposure and the associated health effects have been well documented.

Experimentally, there is in vivo evidence for both enhancement and depression of immune responses after exposure to silica. For example, an intravenous or intraperitoneal injection of silica into animals is associated under certain conditions with an augmentation of immunoglobulin production in response to soluble and particulate antigens [160-162]. In contrast a depression of antibody responses after intravenous silica injection has also been reported [127]. Both enhancement and depression of immune responses have been also observed to occur following intrapulmonary injection of silica [131].

It has been reported that generally serum levels of immunoglobulins were elevated in workers exposed to silica [66, 162, 165]. Significant increases in serum IgG and IgA levels were recorded in quartz-crushing industry workers [165] whereas increased concentrations of immunoglobulins in the BAL fluids but not in serum samples were observed in granite workers [162]. Controversely in our study population in the foundry workers (n=45) significant decreases were examined in the serum IgG, IgA and IgM levels ($p<0.01$) when compared to their healthy matched controls. Although decreases in serum IgG, IgA and IgM levels were also observed in pottery workers (n=22) the differences were not significant. No correlation between the duration of silica exposure and the changes in serum immunoglobulin levels was observed. In foundry and pottery workers no studies on serum immunoglobulins and complement proteins have been reported to date, but

several studies have shown that foundry workers have an increased risk of lung cancer. On the other hand, serum and urinary neopterin, biopterin and dihydropteridine reductase levels have been found to increase in silica exposed foundry workers indicating an immune activation in these workers [213]. Although the workers in our study have not taken the necessary preventive measures in their workplace and besides of being heavy smokers they have not complained of any diseases including bronchitis, coughing, and other respiratory abnormalities. On the other hand it was estimated that a part from silica, several carcinogenic agents have been identified in contaminated foundry air, namely polycyclic aromatic hydrocarbons, formaldehyde, nickel, chromium, but levels of exposure to these chemicals through fume and dust are expected to be far below current threshold limit values [15, 36, 37]. The result of our study showing a depression in the serum immunoglobulin levels of the foundry workers can be a combination effect of all the chemicals exposed. Besides our results are in consistent with the in vitro findings of Thiele and Lipsky [163] who demonstrated that immunoglobulin production by human peripheral mononuclear cells stimulated with PWM is decreased after exposure to silica.

No significant differences were observed in C3 and C4 complement levels in both foundry and pottery workers although an increased complement levels in subjects exposed to silica have been reported in few studies [165, 168]. According to the interview done with these workers no respiratory failure has been existed yet and since the latency period of silicosis is long, and the mean duration of exposure of the workers in this study was around 10 years, an immunosuppression can be observed at the onset of the disease and later because of the inflammation an immune activation can take place. The follow-up of these workers together with the examination of the necessary cytokines and the other immune parameters must be done in order to have an idea about the progression of abnormalities induced by silica.

In conclusion of the immunologic parameters examined this study which indicated that industrial exposure to silica is associated with a

significant depression of serum IgG, IgA, and IgM levels, confirms that the immune system is a possible target for silica toxicity and elimination of silica hazard in working places and the necessary protective precautions are necessary.

On the other hand in different cell types a significant dose-related increases in micronucleus formation were observed although there are some reports indicating that in vitro silica exposure did not induce chromosomal aberrations and sister chromosomal exchanges in some cell types [123, 194-196]. The frequency of chromosomal aberrations and the sister chromatid exchanges in the peripheral lymphocytes of workers exposed to stone dust containing silica was found to be higher than that of a control population [214]. These increasing in vitro as well as in vivo evidence of silica-induced genotoxic effects, particularly in the crystalline form, has led the IARC to conclude that there is sufficient evidence in humans to show genotoxicity of inhaled crystalline silica and silica has been classified as a confirmed human carcinogen [15].

Silica-elicited DNA damage and changes in cell proliferation are the two key factors related to its carcinogenicity. It has been proposed that the silica-induced DNA damage is caused by two major mechanisms, free radical mediation and direct binding with DNA [203]. Silica-induced oxidative damage such as lipid peroxidation and oxidative damage such as lipid peroxidation and oxidative DNA damage in vitro and elevated levels of ROS, oxidative damage, and the upregulation of the antioxidant mechanisms in silicotic lungs under in vivo conditions have been well established [200, 201, 215, 216].

In this study, a significant increase in the DNA damage was observed both in (n=30) foundry and in pottery (n=22) workers when compared with their controls by alkaline single cell gel electrophoresis technique. By this technique in cultured rat alveolar macrophages silica-induced genotoxicity was evaluated only by Zhang et al. [200, 201] and it was found that silica led to a dose and time-dependent DNA strand breaks in alveolar macrophages and N-acetylcysteine increased intracellular glutathione levels and protected

against silica-induced reactive oxygen species formation. In contrast, buthionine sulfoximine pretreatment depleted cellular glutathione and enhanced the genotoxic effects of silica. No other genotoxicity studies evaluated by comet assay in vivo and in vitro have been reported. Our study is the first study evaluated the DNA damage by comet assay in a group of foundry and pottery workers that were occupationally exposed to silica. In the foundry workers there were 24 smokers and 6 non-smokers. Although there were more smokers in the study population it was observed that higher DNA damage was observed in smoking workers ($p < 0.001$). Also, when compared 14 smoking pottery workers to 8 non-smoking pottery workers, a significant increase in DNA damage was observed in the smoking group ($p < 0.001$). Higher DNA damage was observed in smoking silica-exposed workers ($n=38$), compared with the non-smoking 14 workers (both from pottery and foundry). These data are consistent with the studies that cigarette smoking cause additional DNA damage [211, 217]. On the other hand in the study it seemed that as the duration of exposure to silica increased, the number of highly damaged cells in the workers increased ($p < 0.05$).

In conclusion, the results of this study demonstrate the possibility of genetic risk to individuals who are occupationally exposed to silica. Our results also show that the single cell gel electrophoresis or 'comet' assay seems to be a reliable, rapid, and highly sensitive method of detecting DNA damage in individual cells and fulfils the requirements of a biological marker for silica exposure.

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BIOGRAPHY

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