# FINAL

## Report on Carcinogens Background Document for

# Silica, Crystalline (Respirable Size)

**December 2 - 3, 1998**

**Meeting of the**
**NTP Board of Scientific Counselors**
**Report on Carcinogens Subcommittee**

Prepared for the:
**U.S. Department of Health and Human Services**
**Public Health Services**
**National Toxicology Program**
**Research Triangle Park, North Carolina 27709**

Prepared by:
**Technology Planning and Management Corporation**
**Canterbury Hall, Suite 310**
**4815 Emperor Boulevard**
**Durham, NC 27703**
**Contract Number NOI-ES-85421**

# Table of Contents

## List of Tables



## List of Figures

# Summary Statement

## Carcinogenicity

Respirable crystalline silica (RCS) is *known to be a human carcinogen*, based on findings of increased lung cancer rates in occupational groups exposed to crystalline silica dust (IARC, 1997; Brown *et al.*, 1997; Hnizdo *et al.*, 1997), and supporting animal [IARC, 1997] and mechanistic data. Cancer risks are associated with exposure to quartz and crystabolite but not to amorphous silica. The overall relative risk is about 1.3 to 1.5. Higher risks are found in groups with greater exposure or longer latency. Silicosis, a marker for exposures to silica dust, is associated with elevated lung cancer rates, with relative risks of 2.0 to 4.0. Elevated risks have been seen in studies that accounted for smoking or asbestos exposure, so confounding is unlikely to explain these results.

Results of animal experiments have shown consistent increases in lung cancers in rats, but not hamsters, chronically exposed to RCS by inhalation. Single intrapleural or intraperitoneal injections of various forms of RCS to rats resulted in lymphomas.

## Other Relevant Information

Crystalline silica is an abundant and commonly found natural material. Hazardous human exposure to RCS, primarily quartz dusts, occurs mainly in industrial and occupational settings. Respirable quartz levels exceeding 0.1 $mg/m^3$ are most frequently found in metal, nonmetal, and coal mines and mills; in granite quarrying and processing; in crushed stone and related industries; in foundries; in the ceramics industry; in construction; and in sandblasting operations.

RCS deposited in the lungs causes epithelial injury and macrophage activation, leading to inflammatory responses and cell proliferation of the epithelial and interstitial cells. In humans, RCS persists in the lungs, culminating in the development of chronic silicosis, emphysema, obstructive airways disease, and lymph node fibrosis. RCS stimulates (1) release of cytokines and growth factors from macrophages and epithelial cells; (2) release of reactive oxygen and nitrogen intermediates; and (3) oxidative stress in lungs. All these pathways contribute to lung disease. Marked and persistent inflammation, specifically inflammatory cell-derived oxidants, may provide a mechanism by which RCS exposure can result in genotoxic effects in the lung parenchyma. Humans exposed to RCS had increases in sister chromatid exchanges and chromosomal aberrations in peripheral blood lymphocytes. In vitro exposure to some quartz samples induced micronuclei or cell transformation in several cell types, including cells of human origin.

# 1 Physical and Chemical Properties

Crystalline Silica (CS) is the scientific name for a group of minerals composed of silicon (Si) and oxygen (O) (U.S. DOI 1992). The word crystalline implies that silicon and oxygen are arranged in a three dimensional repeating pattern. Silicon-oxygen tetrahedra (SiO4) are the basic units of CS. In a tetrahedron, each Si atom is surrounded by four O atoms. Each O atom is shared by two tetrahedra. CS has seven polymorphs. Four of these polymorphs (Coesite, Keatite, Stishovite, and Moganite) are extremely rare. The other three (α and β Cristobalite, α and β Quartz, and α, $\beta_1$ and $\beta_2$ Tridymite) are the most commonly found forms of free CS. These forms are interrelated and change forms depending upon the temperature and pressure. In comparison to tridymite and cristobalite, the quartz structure is the most compact. Quartz, which melts to glass, has the lowest heat of expansion of any substance. CS ($SiO_2$, Mol. Wt. = 60.08) is also known as:

Silicon dioxide
Cristobalite (CASRN 14464-46-1)
Tridymite (CASRN 15468-32-3)
Quartz (CASRN 14808-60-7)

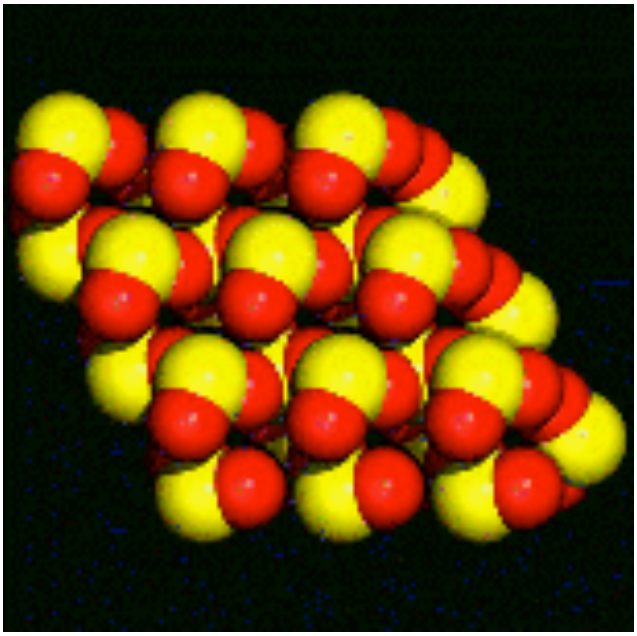
α-Quartz is the most stable form of CS under ambient conditions (α is the nomenclature for lower-temperature phase and β, the higher-temperature phase). Tridymite and cristobalite are formed at higher temperatures, while coesite and stishovite are formed at higher pressures. Keatite is not commonly found in nature. The physical and chemical properties and structure of α–Quartz are as follows:

**Table 1-1. Industrial sand and gravel use Physical - Chemical Properties (α–Quartz)**

| Property | Information | Reference |
|---|---|---|
| Molecular Weight | 60.08 | Budavari *et al.* (1996) |
| Color | Transparent | Budavari *et al.* (1996) |
| Physical State | Transparent solid crystals (noncombustible) | Budavari *et al.* (1996) |
| Melting Point °C | 573 transition to β–Quartz | CRC (1996) |
| Density, g/mL | 2.648 | Frondel (1962); Roberts *et al.* (1974); Smyth and Bish (1988) |
| Crystal system | Trigonal | Frondel (1962); Roberts *et al.* (1974); Smyth and Bish (1988) |
| Solubility: | | |
| Water at 20°C | Insoluble | Iler (1979) |
| Acids | Insoluble | Budavari *et al.* (1996) |
| Hydrofluoric acids | Soluble (produces gaseous silicon tetrafluoride) | |
| Alkaline aqueous solutions | Soluble | |

The α-Quartz structure is trigonal. Within it, the mean Si-O distance is 0.161-0.162 nm and the mean O-O distance is 0.264 nm (Florke and Martin 1993). It is stable up to 573°C and, above this temperature, converts to β-Quartz (Deer *et al*. 1966). The different polymorphs have different arrangements of tetrahedra, thus changing the density and distance between atoms. The structure of α–Quartz (3-D ball rendering) is shown in Figure 1.

**Figure 1. 3-D ball rendering of α–Quartz**



(http://vanish.science.gmu.edu/~ccruz1/c80397/mvsio2.html)

# 2 Human Exposure

## 2.1 Use

Owing to its unique physical and chemical properties, Crystalline Silica (CS) has many uses. Common, commercially produced silica products include quartzite, tripoli, gannister, chert, and novaculite. CS also occurs in nature as agate, amethyst, chalcedony, cristobalite, flint, quartz, tridymite, and, in its most common form, sand (IARC 1997).

### *2.1.1 Sand and Gravel*

Sand has been used for many products throughout human history, but its most common use is in the production of glass. Table 2-1 summarizes other uses for sand and gravel. In some instances, grinding of sand or gravel is required, increasing levels of dust containing respirable crystalline silica (RSC) (IARC 1997).

**Table 2-1. Industrial sand and gravel use**

| Product | Major End Use |
|---|---|
| **Sand** | |
| Glass Making | Containers, flat (plate and window), specialty, fiberglass (un-ground or ground) |
| Foundry | Molding and core, molding and core facing (ground) refractory |
| Metallurgical | Silicon carbide, flux for metal smelting |
| Abrasives | Blasting, scouring cleansers (ground), sawing and sanding, chemicals (ground and un-ground) |
| Fillers | Rubber, paints, putty, whole grain fillers/building products |
| Ceramic | Pottery, brick, tile, and refractory ceramics |
| Filtration | Water (municipal, county, local), swimming pool, others |
| Petroleum industry | Hydraulic fracturing, well packing, and cementing |
| Recreational | Golf courses, baseball, volleyball, play sands, beaches, traction (engine), roofing granules and fillers, other (ground silica or whole grain) |
| **Gravel** | Silica, ferrosilicon, filtration, nonmetallurgical flux, other |

U.S. DOI (1994; cited by IARC 1997)

### *2.1.2 Quartz*

Quartz was first used in crafting tools, weapons, and jewelry. It is the major component of the gemstones amethyst, tiger's eye, and onyx. Owing to its dielectric and piezoelectric properties, the electronic and optical components industries now use quartz. Electronic-grade quartz crystals are in demand because they can be used accurately to control frequency, timing, and filtering. High-purity, synthetic, and natural quartz crystals are used for special optical applications, such as fiber optics, and in the manufacture of watches, microcomputers, television equipment, and wireless communications equipment. Optical grade quartz is used to make lenses and windows in lasers and other devices (IARC 1997).

### *2.1.3 Diatomitous Earth (DE)*

DE, composed of fossilized diatoms, is used primarily as a substrate for filtration (60% of world production) because of their intricate microstructure and high pore-to-space volume. DE is amorphous silica, but becomes crystalline upon calcination. Calcined DE has been used to clarify or filter dry-cleaning solvents, pharmaceuticals, beer, wine, municipal and industrial water, fruit and vegetable juices, oils, and other chemical preparations. The next most important use of processed DE (25% of world production) is as fillers. It is used as a filler in paint, paper, and scouring powders. It is also used for their abrasive qualities in polishes, to provide flow and color qualities in paints, and to reinforce paper (Kadey 1975; cited by IARC 1987). DE is also used as a carrier for pesticides, fillers in synthetic rubber goods, laboratory absorbents, and in anti-caking agents (IARC 1997).

## 2.2 Production

Silica used in commercial products is obtained mainly from natural sources. U.S. production of silica sand was estimated at 25.8 million tons for 1990 and 27.9 million tons for 1994 (IARC 1997). The U.S. exports only about 4% of its production and, for the most part, does not import silica (IARC 1987). Quartz crystals are mined from the minor deposits found in the U.S. Synthetic quartz crystals (hydrothermally cultured quartz crystals) are becoming an increasingly important source of quartz production. The U.S. is one of three major countries (along with Russia and Japan) producing such crystals. The U.S. is also the major producing country of DE, with California being the most important commercial source (IARC 1997).

Fifteen suppliers for silicon/silicon dioxide have been identified: Quartz Unlimited, Inc.; 3M/Ceramic Fiber Products Division; Galtech, Inc.; TAM Ceramics, Inc.; Vesuvius McDanel Co.; Abar Ispen International, Inc.; C-E Minerals, Inc.; CEMCOM Corporation; Dentsply International, Inc.; Ferro Corp., Kyocera Industrial Ceramic Corp.; Malvern Minerals Co.; Oglebay Norton Industrial Sands; PPG Industries, Inc.; and PG Corporation (http://www.corptech.com/FSP/Suppliers.cfm?RequestTimeout=900&ProductCode=MAT-CE-CH).

## 2.3 Environmental Exposure

Silica-bearing deposits are found in every landmass and strata from every period of geologic time. Quartz, from which most silica deposits are derived, is hard, inert, and practically insoluble. Quartz survives numerous weathering processes and, as a result, constitutes approximately 12% of the continental landmass (IARC 1997). Granite may contain 25-30% silica, and shale, up to 30% silica. Sandstone is predominantly quartz, and limestone contains substantial amounts of silica. Silica is the major constituent of commercial sand. Metallic and nonmetallic ore bodies and fossil fuels may contain quartz (IARC 1987). Table 2-2 summarizes quartz concentration in different rocks.

**Table 2-2. Average Quartz composition of major igneous and sedimentary rocks**

| Rock Type | Quartz-containing Rock | % Quartz (by weight) |
|---|---|---|
| Igneous | Rhyolites | 33.2 |
| | Alkali granites | 32.2 |
| | Alkali rhyolites | 31.1 |
| | Granites | 29.2 |
| | Quartz latites | 26.1 |
| | Quartz monzonites | 24.8 |
| | Quartz diorites | 24.1 |
| | Granodiorites | 21.9 |
| | Rhyodacites | 20.8 |
| | Dacites | 19.6 |
| | Latite andesites | 7.2 |
| | Andesites | 5.7 |
| | Syenites | 2.0 |
| | Monozodiorites | 2.0 |
| | Alkali syenites | 1.7 |
| | Diorites | 0.3 |
| Sedimentary | Sandstones | 82 |
| | Greywackes | 37 |
| | Shales | 20 |

Carmichael (1989; cited by IARC 1997)

Owing to the ubiquity of silica in the natural environment and in various everyday products, environmental exposure is inevitable. Table 2-3 lists common products that could lead to human exposure to silica.

**Table 2-3. Common products containing ≥ 0.1% CS**

| At Home (as a consumer of the following items) | | Everywhere (exposure could be on the job or at home) | |
|---|---|---|---|
| **Product** | **Comment** | **Product** | **Comment** |
| Art clays and glazes | Contain clay and sometimes RCS | Caulk and putty | Contain clay as a filler |
| Cleansers | Contain pumice and feldspar as abrasives | Dust (whether household or industrial) | Contain RCS |
| Cosmetics | Contain talc and clay | Fill dirt and topsoil | Contain sand |
| Pet litter | Composed primarily of clay | Foam in furniture and on rug backings | Contain talc and silica |
| Talcum powder | Contains talc | Paint | Contains clay, talc, sand, and diatomite |
| Unwashed root vegetables (such as potatoes and carrots) | Coated with soil, which has a high RCS content | Paper and paper dust | Contain kaolin and clay |
| Pharmaceuticals | Contain clays and talc as filler | | |
| Sand | It is CS and amorphous silica | | |

U.S. DOI (1992)

## 2.4 Occupational Exposure

National Institute for Occupational Safety and Health (NIOSH) estimates that nearly two million workers are exposed to respirable silica, with over 100,000 of them being in high-risk environments. High-risk environments include sandblasters (including painters who sandblast), rock drillers, roof bolters, and high-risk foundry work (NIOSH 1994; cited by OSHA 1998: http://www.osha.gov/oshinfo/priorities/silica.html).

Respirable silica is so prevalent in the environment that, between 1980 and 1992, Occupational Safety and Health Administration (OSHA) found respirable quartz in 255 different industries, with 48% of these being above permissible exposure levels (PELs) (IARC 1997). NIOSH has compiled a list (summarized in Table 2-4) of industries for which respirable silica samples were found to be at least twice the PEL.

## Table 2-4. Respirable Silica exposure in U.S. industries, 1979-1982

| Industry | No. of Samples | Percentage of noncompliance (>Twice PEL) |
|---|---|---|
| Agriculture, Forestry, and Fishing | 43 | 63 |
| Mining | 43 | 57 |
| Construction | | |
| Building Construction—general contractors | 45 | 29 |
| Construction other than building construction—general contractors | 424 | 30 |
| Construction—special trade contractors | 289 | 10 |
| Manufacturing | | |
| Food and allied products | 187 | 52 |
| Textile mill products | 52 | 27 |
| Apparel and other finished products | 16 | 0 |
| Lumber and wood products, except furniture | 13 | 8 |
| Furniture and fixtures | 31 | 0 |
| Paper and allied products | 82 | 13 |
| Printing, publishing, and allied industries | 31 | 0 |
| Chemicals and allied products | 640 | 013 |
| Petroleum refining and related industries | 214 | 11 |
| Rubber and miscellaneous plastic products | 269 | 9 |
| Leather and leather products | 14 | 0 |
| Flat glass | 82 | 9 |
| Glassware, pressed or blown | 229 | 11 |
| Glass products from purchased glass | 37 | 11 |
| Hydraulic cement | 65 | 0 |
| Structured clay products | 635 | 26 |
| Pottery and related products | 945 | 23 |
| Concrete, gypsum, and plaster products | 347 | 12 |
| Cut stone and stone products | 270 | 27 |
| Abrasive, asbestos, and miscellaneous nonmetallic mineral products | 558 | 16 |
| Primary metal industries | | |
| Blast furnace, steel works, rolling and finishing mills | 639 | 32 |
| Iron and steel foundries | 10850 | 23 |
| Primary smelting and refining of nonferrous metals | 146 | 9 |
| Secondary smelting and refining of nonferrous metals | 39 | 0 |
| Rolling, drawing, and extruding of nonferrous metals | 23 | 22 |
| Nonferrous foundries (casting) | 2170 | 9 |
| Miscellaneous primary metal product | 68 | 46 |
| Fabricated metal products, except machinery and transportation equipment | 1265 | 22 |

| Industry | No. of Samples | Percentage of noncompliance (>Twice PEL) |
|---|---|---|
| Machinery except electrical | 1377 | 13 |
| Electrical machinery and supplies | 474 | 23 |
| Transportation equipment | 600 | 20 |
| Measuring, analyzing, and controlling instruments and photographic and medical instruments | 137 | 36 |
| Miscellaneous manufacturing | 211 | 9 |
| Other industries | 460 | 15 |

IARC (1987)

Table 2-5 summarizes occupational activities that lead to RCS exposure.

**Table 2-5. Main activities in which workers may be exposed to RCS**

| Industry/Activity | Specific Operation/Task | Source Material |
|---|---|---|
| Agriculture | Plowing, harvesting, use of machinery | Soil |
| Mining and related milling operations | Most occupations (underground, surface, mill) and mines (metal, nonmetal, coal) | Ores and associated rock |
| Quarry and related milling operations | Crushing stone, sand and gravel processing, monument stone cutting and abrasive blasting, slate work, diatomite calcination | Sandstone, granite, flint, sand, gravel, slate, diatomaceous earth |
| Construction | Abrasive blasting of structures, buildings<br>Highway and tunnel construction<br>Excavation and earth moving<br>Masonry, concrete work, demolition | Sand, concrete<br>Rock<br>Soil and rock<br>Concrete, mortar, plaster |
| Glass, including fiberglass and refractory ceramics | Raw material processing<br>Refractory installation and repair | Sand, crushed quartz<br>Refractory materials |
| Cement | Raw materials processing | Clay, sand, limestone, diatomaceous earth |
| Abrasives | Silicon carbide production | Sand |
| Ceramics, including bricks, tiles, sanitary ware, porcelain, pottery, refractories, vitreous enamels | Mixing, molding, 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 foundries (ferrous and nonferrous) | Casting, shaking out<br>Abrasive blasting, fettling<br>Furnace installation and repair | Sand<br>Sand<br>Sand |
| Metal products including structural metal, machinery, transportation equipment | Abrasive blasting | Sand |
| Shipbuilding and repair | Abrasive blasting | Sand |

| Industry/Activity | Specific Operation/Task | Source Material |
|---|---|---|
| Rubber and plastic | Raw materials 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 |
| Jewelry | 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 concentrations |

Kusnetz and Hutchison (1979); Corn (1980); Webster (1982); NIOSH (1983); Froines *et al*. (1986); Lauwerys (1990); U.S. Bureau of Mines (1992); Hilt (1993); Weill *et al*. (1994); and Burgess (1995); all cited by (IARC 1997)

## 2.5 RCS Analysis and Sampling

### *2.5.1 Air Sampling and Analysis*

Air sampling involves collecting samples of air (by instruments such as the konimeter, thermal precipitator, or impinger) and analyzing these by light microscopy. One NIOSH approved method is to use X-ray diffraction to analyze air samples (another method uses infrared [IR] for analysis). The detection limit for X-ray diffraction is 5 μg for quartz (which translates to 0.01-0.02 mg/m$^3$ for a 0.5 m$^3$ air sample) (IARC 1997). Cost and time are other factors in this type of analysis. Collection is very time-consuming and expensive. Faster, cheaper methods have been suggested, but because they do not return consistent results, the X-ray diffraction method suggested by NIOSH presently appears to be the most useful (OSHA 1998: http://www.osha-slc.gov/SLTC/silicacrystalline/smithdk/index.html).

### *2.5.2 Bulk Analysis*

Bulk analysis is used to analyze surfaces to determine silica concentrations. It does not seem to be effective in determining silica toxicity; instead, the exposed surface of silica is what predicts biological activity. Respirable silica concentrations may be determined by low-voltage scanning electron microscopy X-ray analysis. Laser microprobe mass analysis is also used to ionize a small volume of the material, which is analyzed by a time-of-flight mass spectrometer. There are other methods for analyzing bulk materials, however, these are too expensive and complicated for routine analysis. Certain physical properties of RCS have been found to be identical to the bulk properties (mostly surface properties such as hydrophilicity, surface radicals, and defects). Surface properties, therefore, can be obtained for smaller samples and applied to the bulk quantity (IARC 1997).

The major problems with bulk sampling are obtaining a representative sample and eliminating preferred orientation. Samples must be carefully extracted so that aliquots of samples have the same composition as the original material being observed. Particle separation, which is natural because of the different physical properties of the various phases, must be avoided. The side-

rifted method of loading sample holders can sometimes eliminate this orientation problem. Spheroidizing will eliminate the most severe orientation problems (Smith 1992: http://www.osha-slc.gov/SLTC/silicacrystalline/smithdk/index.html).

## 2.6 Regulations

RCS is federally regulated by EPA, FDA and OSHA as shown in the following tables:

### Table 2-6. EPA Regulations

| EPA Regulations | |
|---|---|
| 40 CFR 411—PART 411—Cement Manufacturing Point Source Category. Promulgated: 39 FR 6591, 02/20/74.U.S. Codes: 33 U.S.C. 1251, 1311, 1314 (b) and (c), 1316 (b) and (c), and 1317(c); 86 Stat. 816 et seq., Pub. L., 92-500; 91 Stat. 1567, Pub. L. 95-217. | The provisions of this subpart are applicable to discharges resulting from the process in which several mineral ingredients (including silica) are used in the manufacturing of cement. |
| 40 CFR 427—PART 427—Asbestos Manufacturing Point Source Category. Promulgated: 39 FR 7527, 02/26/74. U.S. Codes: 33 U.S.C. 1251, 1311, 1314 (b) and (c), 1316 (b) and (c), 1317(c); 86 Stat. 816 et seq.; Pub. L. 92-500. | The provisions of this subpart are applicable to discharges resulting from the process in which silica and other ingredients are used in the manufacturing of asbestos-cement pipe. |
| 40 CFR 469—Subpart B—Electrical and Electronic Components Point Source Category. Promulgated: 48 FR 45250, 10/04/83. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, 1318, and 1361; 86 Stat. 816, Pub. L. 92-500; 91 Stat. 1567, Pub. L. 95-217. | Any existing point source must achieve the following effluent limitations representing the degree of effluent reduction attainable by the application of the best practicable control technology currently available. |

### Table 2-7. OSHA Regulations

| OSHA Regulations | |
|---|---|
| 29 CFR 1910.1000—Subpart Z—Toxic and Hazardous Substances. Promulgated: 55 FR 9033 1/90. U.S. Codes: 29 U.S.C. 653, 655, and 657. PEL $\leq$ 0.1 mg crystalline quartz (respirable dust)/$m^3$ 8-hr TWA; PEL $\leq$ 0.05 mg crystalline cristobalite (respirable dust)/$m^3$ 8-hr TWA; PEL $\leq$ 0.05 mg crystalline tridymite (respirable dust)/$m^3$ 8-hr TWA. | Limits employee exposure to RCS based on respiratory effects. Where administrative or engineering control is not feasible for compliance, protective equipment or protective measures approved for particular use by industrial hygienist or other technically qualified person shall be used to keep employee exposure within PEL. |
| 29 CFR 1910.1450—Occupational exposure to hazardous chemicals in laboratories. Promulgated: 55 FR 3327, 01/31/90. U.S. Codes: 29 U.S.C. 653, 655, and 657. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories. | As a select carcinogen (IARC Group 2A), RCS is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and implement a Chemical Hygiene Plan. |
| 29 CFR 1918—SUBPART I—General Working Conditions. Promulgated: 61 FR 5509, 02/13/96. Hazard Communication. | The purpose of this section is to ensure that the hazards of all chemicals produced or imported are evaluated, and that information concerning their hazards is transmitted to employers and employees. This transmittal of information is to be accomplished by means of comprehensive hazard communication programs, which are to include container labeling and other forms of warning, material safety data sheets, and employee |

| OSHA Regulations | |
|---|---|
| | training. |

## Table 2-8. FDA Regulations

| FDA Regulations | |
|---|---|
| 21 CFR 582—Substances Generally Recognized as Safe. Promulgated: 41 FR 38657, 10/10/76. U.S. Codes: 21 U.S.C. 321, 342, 348, 371. | Silica aerogel, a finely powdered microcellular silica foam having a minimum silica content of 89.5%, is generally recognized as safe when used as a component of an anti-foaming agent in accordance with good manufacturing or feeding practice. |
| 21 CFR PART 872—Dental Devices. Promulgated: 52 FR 30097, 08/12/87. U.S. Codes: 21 U.S.C. 351, 360, 360c, 360e, 360j, 371. | Porcelain powder for clinical use, a device consisting of a mixture of kaolin, felspar, quartz, or other substances intended for use in the production of artificial teeth in fixed or removable dentures, of jacket crowns, facings, and veneers, may be safely used in prosthetic dentistry. |

# 3 Human Studies of Silica

Human cancer studies of silica before October 1996 were reviewed by International Agency for Research on Cancer (IARC) in Volume 68 (IARC 1997). Since the IARC review, seven studies of lung cancer and occupational exposures to silica dust have been published, as summarized in Table 3-1. Two of the cohort studies (Checkoway 1996b, 1997) and the nested case-control studies (Hnizdo 1997; de Klerk 1998) are extensions of earlier reports. Also, the association between silica and lung cancer was recently reviewed by Smith *et al.* (1995), Weill and McDonald (1996), Goldsmith (1997), and Steenland and Stayner (1997).

## 3.1 IARC (1997)

The IARC Working Group's 1996 evaluation divides silica into Crystalline Silica (CS) (quartz and cristobalite) and amorphous silica. For CS, the Working Group evaluated a large number of epidemiological studies. This evaluation included 13 cohort and five case-control studies of ore mining; six cohort studies of stone-cutters, quarry and granite production workers; 11 cohort (plus two that included nested-case-control analyses) and two case-control studies of the ceramic, pottery, refractory brick, and diatomaceous earth industries; and three cohort studies (plus one nested-case-control analysis) and one case-control study of foundry workers. There were also 23 studies evaluating cancer risk in cohorts of silicosis patients.

The IARC Working Group evaluated industry-based occupational cohorts and nested case-control studies, and one case-control study set in a pottery manufacturing region in central Italy. Community-based studies that relied on self-reported occupations and jobs were not included in the evaluation. The rationale for excluding these studies was that they would not add to the information on silica and cancer risks in the industry-based studies.

The IARC Working Group noted that the studies of miners and of foundry workers able to address the potential confounding effects of concurrent exposures (*e.g.*, radon, arsenic) and of smoking, provide only weak, inconsistent evidence of an association between silica dust exposure and lung cancer. However, based on the findings from the relatively large number of epidemiological studies of other exposures and populations (quarry and granite workers; ceramic, pottery, refractory brick, and diatomaceous earth industries; and silicosis patients from a variety of occupational settings), the IARC Working Group concluded that overall the epidemiological studies support increased lung cancer risk from inhaled CS resulting from occupational exposures. The IARC evaluation is that there is *sufficient* evidence in humans for the carcinogenicity of inhaled CS. The following studies were viewed by the IARC Working Group to be the least confounded:

1. Steenland and Brown (1995) followed a cohort study of 3,328 former gold miners in South Dakota. The standardized mortality ratio (SMR) for lung cancer, using the U.S. population rates as the reference, was 1.13 (95% CI [0.94-1.36]). For those with 30 years or more since first exposure the SMR was 1.27 (95% CI [1.02-1.55]), but there was no trend with duration or estimated cumulative dust exposure.

2. Guenel *et al.* (1989) reported on a cohort study of 2175 Danish stone workers. There were 2071 cancer cases identified through the Danish Cancer Registry from 1943-1984. The

standardized incidence ratio (SIR) for lung cancer was 1.38 (95% CI [1.00-1.89]) for skilled workers and 0.72 (95% CI [0.46-1.08]) for unskilled workers. Adjusting for region (to account for regional differences in smoking) potentiated the estimate for skilled workers (SIR 2.00, [1.49-2.69]) and substantially changed the estimate for unskilled workers (SIR 1.81, [1.16-2.70]).

3. Costello and Graham (1988) followed a cohort study of 5,414 male granite shed and quarry workers in Vermont. The risk of lung cancer mortality was increased among shed workers (SMR 1.27, 95% CI [1.03-1.55]) but not among quarry workers (SMR 0.82, 95% CI [0.50-1.27]). Risk increased with increasing number of years worked in the sheds and with year since first hire (latency).

4. Costello *et al*. (1995) reported a cohort study of 3,246 crushed stone workers in the U.S. Lung cancer mortality was increased with a SMR 1.19, 95% CI (0.85-1.62) for whites and SMR 1.85 95% CI (0.92-3.31) for nonwhites. Higher risks were seen among those with at least ten years of work and 20 or more years since first hire, particularly among granite workers (SMR 3.54, 95% CI [1.42-7.29]). There were also elevated values for limestone but not for traprock, SMR 1.50 95% CI (0.95-2.25) and 0.63 95% CI (0.13-1.84).

5. Checkoway *et al.* (1993, 1996a) studied a cohort study of 2,570 diatomaceous earth industry workers in California, but only 2,266 were included in the analyses that included asbestos exposure. Lung cancer mortality was increased (using U.S. population rates, SMR 1.43, 95% CI [1.09-1.84]; using local rates, SMR 1.59). There was evidence of increasing risk with increasing duration of employment and with a semiquantitative measure of cumulative dust exposure. This pattern was seen in the analysis that adjusted for asbestos exposure, and in the analysis, within the subgroup, without asbestos exposure.

6. Dong *et al*. (1995) reported a cohort study of 6,266 refractory brick workers in China (compared to 11,470 nonsilicotic steel workers). Lung cancer mortality risk was increased (Standardized Rate Ratios) (SRR 1.49 95% CI [1.15-1.90]), but this increased risk was only seen in workers with silicosis (SRR 2.10, 95% CI [1.46-2.92]). Smoking history did not affect the risk among silicotics (SRR 2.34, 95% CI [1.45-3.58] among smokers and 2.13, 95% CI [1.10-3.72] among nonsmokers).

7. Merlo *et al.* (1991) reported a cohort study of 1,022 refractory brick workers in Italy. Lung cancer mortality risk was increased (SMR 1.51, 95% CI [1.0-2.18]). The increased risk was seen in those employed before 1957, when exposures were higher (SMR 1.77, 95% CI [1.03-2.84]).

8. Winter *et al.* (1990), McDonald *et al*. (1995, 1997), Cherry *et al*. (1995a), and Burgess *et al.* (1997) presented cohort and nested case-control studies of pottery workers in the United Kingdom. There were 3,669 male and female workers under age 60 in 1970-1971 in the first cohort analysis through 1985 (Winter *et al*. 1990): the SMR was 1.40 (95% CI [1.07-1.80]) using national rates as the reference; 1.32 (95% CI [1.00-1.69]) using local rate comparisons. In another cohort of 5,115 pottery workers who had not worked in foundries or with asbestos or other dusts, the lung cancer SMR was 1.28 (95% CI [0.99-1.62 using local rates]) (Cherry

*et al.* 1995b). Intensity of exposure, rather than duration, appeared to have a stronger influence on risk.

9. Chen *et al*. (1992) and McLaughlin *et al.* (1992) reported cohort and nested case-control studies of pottery workers in China. Lung cancer risk among 13,719 pottery workers was not increased (SMR 0.58 [p< 0.05]). In the nested case-control analyses, there were higher risks with the highest levels of cumulative dust (odds ratio [OR] adjusting for age and smoking, 1.5) or cumulative respirable silica (OR 2.1, [0.8-4.12]), but the dose-trends were not significant.

10. Amandus *et al*. (1991, 1992, 1995) presented studies of silicosis patients in North Carolina, and Partanen *et al*. (1994) presented studies of silicosis patients in Finland. Most studies of silicosis patients have reported increased risks of lung cancer (mortality or incidence), with risk ratios between 0.5 and 4.0. These studies are among those that address potential diagnostic and self-selection biases.

Three case-control studies considered the possible associations of amorphous silica from biogenic amorphous silica fiber exposures in the sugar cane industry.

Rothschild and Mulvey (1982) studied 284 lung cancer deaths and 284 controls in Southern Louisiana. An association with sugar cane farming was found only among smokers (OR 2.6 (95% CI [1.8-4.0]) but not in nonsmokers (OR 0.9 [95% CI (0.2-3.9]). There was no suggestion of an association between lung cancer and silica fibers by the authors and no measurements of fiber concentrations were reported.

Brooks *et al*. (1992) studied 98 male lung cancer cases and 44 male mesothelioma cases and matched controls in Florida. No consistent association with either lung cancer or mesothelioma and residence near sugar cane fields was found. For employment in the sugar cane industry the odds ratio for lung cancer was 1.8 (95% CI [0.5-7.5]). There was one case of mesothelioma who worked in the industry but had previous asbestos exposures.

Sinks *et al.* (1994) compared work histories of 93 mesothelioma cases in Hawaii with matched controls. The odds ratio for employment as a sugar cane worker was 1.1 (95% CI [0.4-2.9]).

The IARC Working Group concluded that no association was detected with biogenic amorphous silica fibers in these three studies. The IARC evaluation is that there is *inadequate evidence* in humans for the carcinogenicity of amorphous silica.

## 3.2 Current Epidemiological Studies

Table 3-1 gives the details of seven studies (three occupational cohorts, two silicosis cohorts, and two nested-case control studies) published since the IARC Working Group meeting in October 1996 (IARC 1997). These studies concern occupational exposures to CS and lung cancer. There is no new information to add to the IARC report on amorphous silica.

The cohort study of workers in the diatomaceous earth industry has been extended by Checkoway *et al*. (1997) by an additional seven years of follow-up and quantitative dose-response analyses. Exposure assessments were based on air monitoring data that date back into

the 1940s. Asbestos exposures were also available and estimates were made for each cohort member. The relative risk for lung cancer was 2.15 (95% CI [1.08-4.28]) in the highest exposure category of ≥ 5 mg/m$^3$-years of respirable CS. Among workers without asbestos exposure, the relative risk was 2.03 (95% CI [0.93-4.45]) in the highest exposure category.

Rafnsson and Gunnarsdottir (1997) reported a study of 1,346 diatomaceous earth workers in Iceland. Five lung cancers occurred, compared to an expected 4.4 (SIR 1.14, 95% CI [0.37-2.65]). The plant began operation in 1967, so the maximum time of follow-up (and exposure) was 25 years; < 20% had worked for five or more years. Smoking data were available and the workers had lower smoking rates than the general population.

Checkoway *et al.* (1996b) updated a follow-up study of 22,992 Florida phosphate industry workers. Among whites, the SMR was 1.19 (95% CI [1.07-1.32]) using national population rates as the reference, and 0.98 (95% CI [0.88-1.09]) using local rates. Among nonwhites, the SMR was 1.13 (95% CI [0.92-1.37]) using national rates and 0.94 (95% CI [0.77-1.13]) using local rates. There was no dose-related association with estimated cumulative exposure to silica.

A large cohort (n=11,224) of men with pneumoconiosis from the Polish National Registry of Occupational Diseases was studied by Starzynski *et al*. (1996). The study divided the workers into subcohorts: a) coal miners (n=7,065), b) underground workers (n=924), c) metallurgical industry workers (n=1,796), and d) refractory materials (china, ceramics, and quarry) workers (n=1,439). Silica exposures were lowest among coal miners and highest among refractory workers. Lung cancer mortality was increased among metallurgical workers (SMR 1.59, 95% CI [1.24-2.01]), but not among refractory workers (SMR 1.02, 95% CI [0.72-1.4]). The metallurgical workers had the more complex exposures (including to polycyclic aromatic hydrocarbons) and smoked at a higher rate than the general public.

Cancer incidence and mortality was examined in a cohort of 1,295 silicosis patients identified from hospitalization registries in Sweden and Denmark (Brown *et al.* 1997). Incidence of lung cancer was increased (SIR 3.1, 95% CI (2.1-4.2) in Sweden and 2.9, 95% CI [1.5-5.2] in Denmark). Mortality was only assessed in Sweden, and there was also an increased risk in this measure (SMR 2.9, 95% CI [2.1-3.9]).

Hnizdo *et al*. (1997) conducted a nested case-control study of lung cancer among a cohort of South African gold miners. Lung cancer risk was associated with smoking (RR 1.0, 3.5, 5.7, and 13.2 for < 6.5, 6.5-20, 21-30, and > 30 pack years) and with silicosis (RR 2.45, 95% CI [1.2-5.2]). There was some evidence of a multiplicative interaction between smoking and silicosis.

A nested-case control study among 2,297 gold miners (138 cases) in western Australia was reported by de Klerk *et al.* (1998). The relative risk for lung cancer and silicosis was 1.59 (95% CI 1.1-2.28), but there were no other significant associations with measures of silica exposure.

Two additional studies of other types of cancers and occupational silica exposure have recently been published. A case-control study of salivary gland cancer in Shanghai included 41 cases and 414 controls (Zheng *et al.* 1996). The association between occupational exposure to silica dust and salivary gland cancer was 2.5 (95% CI [1.1-5.8]). Occupational history was based on a standardized interview, but information on the methods used to classify silica exposure was not

presented. Parent *et al.* (1997) conducted a case-control study of gastric cancer in Montreal, with 250 male patients and 2,289 controls. Occupational silica dust exposure was based on an evaluation of a patient's work history obtained from participant interviews. The evaluation was conducted by a panel of chemists and industrial hygienists. They reported an increased risk across levels of exposure (OR 1.7, 95% CI [1.1-2.7]) for substantial and OR 1.4 (95% CI [1.0-1.9]) for nonsubstantial, compared to none. Risk also increased with increased frequency and concentration of exposure, but not with duration.

## Table 3-1. Studies of Silica and Lung Cancer in Humans

| Study Type | Population Group | Exposure | Effects | Potential Confounders and Modifiers | Comments | References |
|---|---|---|---|---|---|---|
| Cohort (mortality) | 2,342 white male workers exposed to RCS in the diatomaceous earth industry during 1942-1994. Quantitative asbestos exposure was estimated for a sub-cohort of 2,266. | Estimated cumulative exposure to RCS (primarily cristobalite, asbestos, and chrysotile). | 77 lung cancer deaths; SMR 1.29 (95% CI (1.01-1.61)) using US rates; SMR 1.43 using local rates. The SMR for the highest level of silica exposure was 2.15 (95% CI [1.08-4.28]). For those with no asbestos exposure (47 lung cancer deaths) the SMRs were 1.0 (referent), 0.73, 0.73, 1.00, and 2.03 for increasing levels of cumulative RCS. | Adjusted for and stratified by asbestos. | Smoking information was incomplete. The previous suggestion of a possible synergism between silica and asbestos was not observed in this updated analysis. The previous reports followed the cohort from 1942-1987. | Checkoway *et al*. (1996a, 1997, 1993) |
| Cohort (inci-dence) | 920 men and 426 women from a diatomaceous plant in Iceland. | RCS with a high cristobalite content from heating diatomite. | Only five lung cancer cases were observed with 4.4 expected, SIR 1.14 (95% CI [0.37-2.65]). Among men with at least five years exposure there was an excess of lung cancer (three cases), SIR 2.34 (95% CI [0.48-6.85]). | Smoking was less than in the general population. | Small cohort, relatively short follow-up, and relatively short length of employment (< 20% had worked five or more years). | Rafnsson and Gunnarsdottir (1997) |
| Cohort (mortality) | 18,446 white and 4,546 nonwhite male workers in the Florida phosphate industry. Follow-up period 1949-1992. | RCS exposure from mining and processing of phosphate ores. | Among whites, 354 lung cancer deaths, (SMR 1.19 (95% CI [1.07-1.32]) using national reference; 0.98 (95% CI [0.88-1.09]) using local rates. Among nonwhites, the SMR was 1.13 (95% CI [0.92-1.37]) using national rates and 0.94 (95% CI [0.77-1.13]) using local rates. There was no dose-related association with estimated cumulative exposure to silica | | Lack of industrial hygiene and cigarette smoking data. Exposure to total dust, alpha and gamma radiation, and acid mists also assessed. | Checkoway *et al.* (1996b, 1985) |

| Study Type | Population Group | Exposure | Effects | Potential Confounders and Modifiers | Comments | References |
|---|---|---|---|---|---|---|
| | | | (relative risk 1.05 in highest compared to lowest category for whites, 0.68 for nonwhites). | | | |
| Cohort - silicotics (mortality) | 11,224 men with pneumoconiosis from the Polish National Registry of Occupational Diseases. | Analysis divided by type of work (coal miners; other underground, metallurgical, and refractory material workers). | Significantly elevated lung cancer risk in metallurgical industry and nonferrous foundry workers (SMR 1.59, 95% CI [1.24-2.01]), but not in miners (SMR 1.04, 95% CI [0.88-1.22]), other underground workers, (SMR 1.30, 95% CI [0.85-1.90]) or refractory materials workers (SMR 1.02, 95% CI [0.72-1.40]). | Smoking rates were higher in metallurgy and foundry workers (85% compared to 75% in the general male population). These workers also have complex exposures including polycyclic aromatic hydrocarbons and carcinogenic metals. | | Starzynski *et al*. (1996) |
| Cohort - silicotics (incidence and mortality) | 1,295 silicosis patients in Sweden (n-=1052) and Sweden (n=243). | | Lung cancer incidence increased (SIR 3.1, 95% CI [2.1-4.2]) in Sweden and 2.9, (95% CI [1.5-5.2]) in Denmark; lung cancer mortality increased (SMR 2.9, 95% CI [2.1-3.9]). | | Silicosis patients identified through hospital discharge diagnoses. Incident cases or deaths within 12 months of study entry excluded. | Brown *et al*. (1997) |
| Nested case-control | 78 lung cancer cases and 386 controls among a cohort of 2,260 South African gold miners followed from 1970 to 1986. | Mining in rock composed of quartz (70-90%), silicates (10-30%). | Lung cancer risk was associated with smoking (RR 1.0, 3.5, 5.7, and 13.2 for < 6.5, 6.5-20, 21-30, and > 30 pack years) and with silicosis RR 2.45 (95% CI [1.2-5.2]). There was some evidence of a multiplication interaction between smoking and silicosis: among those without silicosis, the association (relative risk) between smoking (measured by three levels of pack-years) and | Uranium exposure was also present in this cohort, but no association with lung cancer mortality was seen with the measures of uranium available in this analysis. | Smoking habits ascertained by questionnaire during medical examination. | Hnizdo *et al.* (1991, 1997) |

| Study Type | Population Group | Exposure | Effects | Potential Confounders and Modifiers | Comments | References |
|---|---|---|---|---|---|---|
| | | | lung cancer was 1.0, 5.1, and 11.7; among silicosis patients, corresponding associations were 4.1, 7.9, and 48.9. | | | |
| Nested case-control | 138 lung cancer cases among 2,297 western Australia gold miners (cases age-matched to controls). | | Relative risk for lung cancer and silicosis was 1.59 (95% CI 1.1-2.28). No significant associations with measures of silica exposure. | Adjusted for smoking. | | de Klerk *et al.* (1995, 1998) |
| Case-control | 41 incident salivary gland cancers and 414 controls in Shanghai. | Occupational exposures to silica dust based on work histories. | Ten cases and 61 controls were exposed to silica dust for an OR=2.5 (1.1-5.8). For males 18 cases and OR=3.1 (1.1-8.9) and for females 23 cases and OR=1.5 (0.3-7.7). | np | Among males there were eight cases and 52 controls exposed to silica dust and for females two cases and nine controls exposed. Smoking habits were assessed and no association was found with smoking or other exposures such as asbestos, coal dust, textile dust, or wood dust. An association was found with head x-ray examinations and exposure to kerosene in cooking. | Zheng *et al.* (1996) |
| Case-control | 250 male gastric cancer patents and | RCS from work as carpenters, | For nonsubstantial exposure OR=1.4 (1.0-1.9) and for | np | Exposures based on reviews of question- | Parent *et al.* (1997) |

| Study Type | Population Group | Exposure | Effects | Potential Confounders and Modifiers | Comments | References |
|---|---|---|---|---|---|---|
| | 2,289 controls. | cabinet and furniture makers, and construction laborers. | substantial exposure OR=1.7 (1.1-2.7). For levels of exposure concentration; low OR=1.3 (0.9-1.9), medium OR=1.6 (1.0-2.4) and high OR=1.7 (0.8-3.6). | | naires. 82 cases were associated with the silica occupations. There was not an association with duration of exposure. | |

## 3.3 Discussion

Several review papers and meta-analyses have been published recently. Goldsmith (1997) reviewed those lung cancer studies that had dose-response associations. He compared the extrapolated risk based on animal studies with risk estimates from the worker studies. A factor of two difference was reported, with the animals being more sensitive.

Weill and McDonald (1996) reviewed the epidemiological literature on the relationship between silica, silicosis, and lung cancer. They considered work reported after 1985 and found papers by Merlo *et al*. (1991) and Checkoway *et al*. (1993) particularly compelling. They pointed out that for many of these studies there were important confounders such as polycyclic aromatic hydrocarbons (PAH) in foundries and radon and arsenic in mines. They also discussed the selection bias of using registries of silicosis patients. For example, for many of the compensated cases there were likely to be respiratory problems due to smoking. They believed that the study by Amandus *et al*. (1991, 1992) was free of this problem.

Smith *et al*. (1995) carried out a formal meta-analysis of lung cancer studies among silicotics. They considered 29 studies and were able to use 23 in their analysis. The pooled relative risk for lung cancer was RR=2.2 (95% CI [2.1-2.4]), with RR=2.0 for cohort studies, and RR=2.5 for case-control studies. The authors conclude that the association between silicosis and lung cancer was causal, either due to silicosis itself or due to the effect of exposure to silica.

Steenland and Stayner (1997) carried out a meta-analysis using cohort and case-control studies (for 16 silica exposed workers and 19 silicotics workers) of the largest and pertinent occupational studies. They calculated a pooled relative risk of RR=1.3 (95% CI [1.2-1.4]) for the meta analysis using 16 occupational studies. They noted that the relative risk was greatest and most consistent among 19 studies of silicotics with a RR=2.3 (95% CI [2.2-2.4]).

## 3.4 Summary of Epidemiological Studies

The quantity and quality of cancer epidemiology studies (primarily lung cancer) of silica exposure have increased substantially in the past decade. Generally, these have focused on either specific occupations with potentially high exposures to silica (*e.g*., miners, quarry and stone workers) or on patients with silicosis. An important issue in evaluating these studies is the possibility of confounding or effect modification by smoking. Another issue for some of the occupational studies is the potential for confounding by other exposures. This is of particular concern for the studies of mining (*e.g*., arsenic and radon exposures in gold mines) and foundries (polycyclic aromatic hydrocarbons exposure).

Most studies of miners and of foundry workers did not assess the potential confounding effects of other exposures. The studies that addressed this issue in the analysis provide only weak and inconsistent evidence of an association between silica dust exposure and lung cancer.

Confounding exposures are less likely to influence studies of stone, pottery, brick, and ceramic workers. These studies have shown an increased risk of lung cancer (incidence or mortality), with overall risk ratios around 1.3. Some studies have suggested a latency of at least 15 years, increased risks with increased duration of exposure, or that the increased risk is most evident in workers exposed before dust control measures were undertaken. It is difficult to separate these different effects given the exposure distributions in these studies.

An increased risk of lung cancer among silicosis patients has been reported in more than 20 studies, with risk ratios generally between 0.5 and 4.0. This increased risk is seen in studies that adjust for smoking and in stratified analyses of smokers and nonsmokers. It is also seen in studies in a variety of occupational settings, so it is unlikely that silicosis is only acting as a marker for exposure to other carcinogens (*e.g.*, radon). One criticism raised about some studies is the potential for biased assessment of cancer (*i.e.*, greater surveillance or reporting of lung cancer among silicosis patients because of compensation issues). However, results from the studies that addressed these issues are similar to earlier studies that were more likely to be influenced by these potential biases.

# 4 Experimental Carcinogenesis

International Agency for Research on Cancer (IARC) conducted two evaluative reviews of silica-induced carcinogenesis (IARC 1987, 1997). In its most recent review, the IARC Working Group noted numerous animal experiments tested the carcinogenic potential Crystalline Silica (CS) by a variety of routes. Those studies were judged to have provided sufficient evidence that CS is carcinogenic in experimental animals.

Various types of quartz (see previous sections), with particle sizes in the respirable range, have been studied in four studies each of exposure by inhalation and intratracheal instillation. In these eight rat experiments, Respirable Crystalline Silica (RCS) exposure was associated with increased incidences of pulmonary adenocarcinoma and squamous cell carcinoma. These lesions were accompanied by dense pulmonary fibrosis which was considered to be an important aspect of the overall biological response.

Although pulmonary tumor incidence was not increased in hamsters after repeated intratracheal instillation of quartz dust (three experiments), pulmonary granulomatous inflammation, and slight- to moderate fibrosis of the alveolar septa was observed.

Neither a mouse lung adenoma assay (A/J mice), nor a limited inhalation study of quartz in mice, produced increased incidences of lung tumors. Quartz exposure caused silicotic granulomas and lymphoid cuffing around airways, but pulmonary fibrosis was not observed in quartz-exposed mice.

RCS was administered either intrapleurally or intraperitoneally to rats in several experiments. This treatment caused thoracic and abdominal malignant lymphomas, primarily of the histiocytic type.

## 4.1 Previously Reviewed Studies (IARC 1997)

### *4.1.1 Inhalationally administered RCS in rats*

Protocol outlines and results of inhalation studies, considered by IARC to be adequate for evaluation, are summarized in Table 4.1.

**Table 4-1. Summary of inhalation experiments conducted on RCS and reviewed in IARC (1997)**

| Sex/ Species/ Strain | N/group | Concen-tration | Crystalline silica characteristics | Inhalation parameters | Duration of experiment | Interim sacrifice | Results | Reference (Cited in IARC 1987) |
|---|---|---|---|---|---|---|---|---|
| Male/female F 344 rats | Not reported but pre-sumed to be 36 males and 36 females. | 0<br>51.6 mg/m3 | Quartz, median aerodynamic. diam = 1.7-2.5 μm | Whole body, 6 h/d, 5 d/wk | 24 mo. 10 rats/ group removed from exposure at 4, 8, 12, and 16 mo. and observed until 24 mo. | 5 of each sex after 4, 8, 12, and 16 mo. | Epidermoid carcinoma in 10/53 males and 1/47 females. No lung tumors in controls. 3/5 females exposed for four mo. had tumors. | Dagle *et al.* (1986) |
| Female F344 rats | 62 | 12±5 mg/m3 | Quartz, median aerodynamic. diam = 2.24±0.2 μm | Nose only, 6 h/d, 4 d/wk | Exposed for 83 weeks then observed until natural death. | No | 18/60 exposed had squamous cell carcinoma, adenocarcinoma, and/or adenomas. | Holland *et al.* (1983, 1986) |
| Male/female SPF F 344 rats | 50<br>50 | 50<br>50 | 0<br>1 mg/m3 | Silicon dioxide, aerodynamic. diam =1.3μm | Whole body, 6 h/d, 5 d/wk | 24 mo. then observed for additional 6 weeks. | No | 7/50 male and 12/50 female had lung adenoma, adenocarcinoma, cystic keratinizing squamous cell tumors, adenosquamous carcinoma, and/or squamous cell carcinoma. | Muhle *et al.* (1989, 1991, 1995) |
| Female Wistar rats | | 90<br>90<br>90 | 0<br>6.1±0.36 mg/m3<br>30.6±1.59 mg/m3 | Quartz, aerodynamic. diam =1.8 μm | Nose only, 6 h/d, 5 d/wk | 34 mo., exposed for 29 days. | 2-6 rats/ group at 6, 12, and 24 mo. | 8/37 low-dose, 43/82 high-dose rats with bronchio-/alveolor adenoma or carcinoma, squamous cell carcinoma, and or anaplastic carcinoma. Distant metastases were seen. | Spiethoff *et al.* (1992) |

Dagle *et al.* (1986; cited in IARC 1987) exposed male and female Fischer 344 rats to 0.0 or 51.6 mg/m$^3$ quartz (mass median aerodynamic diameter, 1.7-2.5 μm; geometric standard deviation, 1.9-2.1) in inhalation chambers. Animals were exposed 6h/d, 5 d/wk, for up to 24 months. After 4, 8, 12 or 16 months, ten animals of each sex were removed from exposure and five were sacrificed. The remaining five were observed and survivors were sacrificed at 24 months. The incidence of epidermoid carcinomas of the lungs in rats surviving for 494 days (*i.e.*, roughly 16 months) when the first tumor was detected, was 10/53 (19%) in females and 1/47 (2%) in males. Three of five females that received no further exposure to quartz after four months had epidermoid carcinomas. No lung tumors were found in controls of either gender.

Holland *et al.* (1983) and Holland *et al.* (1986; both cited in IARC 1987) exposed female Fischer 344 rats to quartz (mass median aerodynamic diameter, 2.24±0.2 μm; geometric standard deviation, 1.75±0.3, all particles were <5.0 μm). Rats were exposed by a nose-only technique for 6 h/d, 4 d/wk, for 83 weeks (*i.e.*, approximately 21 months) and observed for the remainder of their lives. Quartz exposure was associated with squamous cell carcinomas, adenocarcinomas, and/or adenomas of the lung. There were no lung tumors in sham exposed rats. Most quartz exposed rats that survived more than 400 days had pronounced pulmonary fibrosis, granulomatous and silicotic nodules, often with emphysema and alveolar proteinosis.

Muhle *et al.* (1989, 1991, 1995; all cited in IARC 1997) studied groups of 50 male and 50 female viral antibody-free, SPF Fischer 344 rats, 8 weeks of age at the start of the experiment. The animals were exposed, in inhalation chambers, to 0.0 or 1.0 mg/m$^3$ silica (silicon dioxide, mass median aerodynamic diameter about 1.3 μm, with a geometric standard deviation of 1.8). Animals were exposed 6 h/d, 5 d/wk, for 24 months. Animals that survived 24 months were maintained, without further exposure, for an additional six weeks. The incidences of primary lung tumors in RCS exposed rats were 7/50 in males and 12/50 in females. Lung tumors included adenoma, adenocarcinoma, benign cystic keratinizing squamous cell tumors, and adenosquamous carcinoma. Furthermore, nodular bronchoalveolar hyperplasia, interpreted as *borderline adenoma*, was found in 13/100 silica-exposed animals. No lung tumors were observed in controls. Non-neoplastic changes reported in silica-exposed animals included multifocal lipoproteinosis with adjacent fibrotic areas, fibrosis, and alveolar- and bronchiolar-type bronchoalveolar hyperplasia. The severity of these lesions was directly related to duration of exposure.

Spiethoff *et al.* (1992; cited in IARC 1997) exposed groups of female Wistar rats (6 to 8 weeks old) to 0.0, 6.1±0.36, or 30.6±1.59 mg/m$^3$ quartz (mass median aerodynamic diameter, 1.8 μm) 6 h/d, 5 d/wk, for 29 days (nose-only exposure). Two to six animals per group were sacrificed at 29 days and at 6, 12, and 24 months. The study was terminated at 34 months, when all surviving animals were sacrificed. The overall incidence of lung tumors in the low- and high-dose groups after the 24-month sacrifice was 8/37 (22%) and 13/43 (30%), respectively. Total lung tumor incidences, at termination of the study, were 37/82 (45%) and 43/82 (52%) in the low- and high-dose groups, respectively. No lung tumors were observed in control rats. Many animals exhibited lung tumor multiplicity, sometimes with the same tumor type, and sometimes with different tumor types. Tumor types included bronchiolo-alveolar adenomas, bronchiolo-alveolar carcinomas, squamous cell carcinomas, and anaplastic carcinomas. Metastases were observed in

the tracheobronchial lymph nodes and, occasionally, in the kidneys and heart. Non-neoplastic pulmonary lesions included fibrosis and alveolar and bronchiolar epithelial proliferation.

#### *4.1.2 Inhalationally administered RCS in mice*

Wilson *et al.* (1986; cited in IARC 1997) exposed BALB/cBYJ mice to RCS (>96% quartz) for 8 h/d, 5 d/wk in inhalation chambers. Animals were exposed 150, 300, or 570 days. Average exposure concentrations were 1,475; 1,800; or 1,950 mg/m$^3$ (diameter < 2.1 μm). Overall lung tumor incidences were 9/60 in exposed mice and 7/59 in controls. This difference was not statistically significant. In their review, the IARC working group noted small numbers of animals were used and that exposure and observation periods varied widely within the studies.

#### *4.1.3 Intratracheal administration in rats*

Holland *et al.* (1983; cited in IARC 1997) instilled 7 mg of quartz (mean particle size 1.71±1.86 μm; all particles < 5 μm) into the tracheas of Sprague-Dawley rats (gender not specified) once weekly for ten weeks. Animals were observed over their lifetimes. Lung tumors (adenomas or carcinomas) were observed in 6/36 (17%) of dosed animals, 0/40 saline controls, and 0/18 untreated controls. Focal and diffuse pulmonary fibrosis was observed in quartz dosed animals.

Male Fischer 344 rats were administered 20 mg of quartz (Min-U-Sil; particle size, 0.1% $\geq$ 5 μm; or novaculite, particle size, 2.2% $\geq$ 5 μm) as a single intratracheal/lung instillation. Interim sacrifices were conducted at 6, 12, and 18 months (ten animals at each interval) and terminal sacrifice was made at 22 months. The tumor incidences observed in this study are summarized in Table 4-2.

**Table 4-2. Lung tumor incidences in F 344 rats that received intratracheal instillations of RCS**

| Material Tested | Incidences of lung tumors at various intervals | | | | |
|---|---|---|---|---|---|
| | 12 mo. | 18 mo. | 12-22 mo. spontaneous deaths | 22 mo. | Total Lung tumors |
| Min-U-Sil | 1/10 | 5/10 | 5/17 | 19/30 | 30/67 (45%) |
| Novaculite | 1/10 | 2/10 | 2/17 | 16/35 | 21/72 (29%) |

Holland et al. (1993)

Animals dosed with Min-U-Sil had larger lung tumors and more extensive granulomatous and fibrotic lung lesions than did the novaculite-dosed group (Groth *et al.* 1986; cited in IARC 1997).

Saffiotti (1990, 1992) and Saffiotti *et al.* (1996; all cited in IARC 1997) instilled single, intratracheal doses of 12 or 20 mg of quartz (Min-U-Sil, 99% pure with 0.1% iron and 99% pure with no iron, and a particle size distribution between 0.5 and 2.0 μm) into male and female F344/NCr rats. Additional groups received 20 mg of ferric oxide ($Fe_2 O_3$, nonfibrogenic dust). Tumor incidences are summarized in Table 4-3.

**Table 4-3. Incidence of lung tumors in F344/NCr rats after single intratracheal instillation of quartz**

| Treatment | | Observation time | Lung tumors | |
|---|---|---|---|---|
| Material | Dose | | Incidence | Types |
| Males | | | | |
| Untreated | None | 17-26 mo | 0/32 | |
| Ferric oxide | 20 mg | 11-26 mo | 0/15 | |
| Quartz w iron | 12 mg | Killed at 11 mo | 3/18 (17%) | Adenomas and adenocarcinomas |
| | | Killed at 17 mo | 6/19 | Undiff carc., mixed carc. |
| | | 17-26 mo | 12/14 | Epidermoid carc. |
| Quartz w/o iron | 12 mg | Killed at 11 mo | 2/18 (11%) | Adenomas, adenocarc., mixed carc. |
| | | Killed at 17 mo | 7/19 (78%) | |
| | | 17-26 mo | 7/9 (78%) | |
| Females | | | | |
| Untreated | None | 17-26 mo | 1/20 (5%) | Adenoma |
| Ferric oxide | 20 mg | 11-26 mo | 0/18 ( | |
| Quartz w iron | 12 mg | Killed at 11 mo | 8/19 (42%) | Adenomas, adenocarcinomas, undiff carc. |
| | | Killed at 17 mo | 10/17 (59%) | |
| | | 17-26 mo | 8/9 (89%) | Mixed carc. Epidermoid carc. |
| | 20 mg | 17-26 mo | 6/8 (75%) | Adenoma, adenocarc. Mixed carc. Epidermoid carc. |
| Quartz w/o iron | 12 mg | Killed at 11 mo | 7/18 (39%) | Adenoma, adenocarc. |
| | | Killed at 17 mo | 13/16 (81%) | Adenoma, adenocarcinoma, mixed carc., epidermoid carc. |
| | | 17-26 mo | 8/8 (100%) | |

Saffiotti (1990, 1992) and Saffiotti *et al.* (1996)

Non-neoplastic changes observed in this study included interstitial fibrosis, hyperplasia of peribronchial lymphoid tissue, silicotic granulomas, hypertrophy, and finally, hyperplasia and adenomatoid proliferation of alveolar epithelium.

In a study by Pott *et al.* (1994; cited in IARC 1997) six groups of female Wistar rats received either one (45 mg), or 15 (3 mg), intratracheal instillations of one of three quartz preparations (DQ 12, Min-U-Sil, or Quartz Sykron F 600) in saline. Two of the groups received subcutaneous injections of polyvinylpyridine-N-oxide (PVNO) (seven total injections administered at four-month intervals) to retard the development of silicosis. Animals died spontaneously, were killed when moribund, or were terminated at 131 weeks. Tumor incidences are summarized in Table 4-4.

**Table 4-4. Incidence of lung tumors in female Wistar rats after intratracheal instillation of quartz**

| Test Material | No. of Instillations | Tumor Incidence |
|---|---|---|
| Quartz (DQ 12) | 1 x 45 mg | 9/40 (23%) |
| Quartz (DQ 12) | 15 x 3 mg | 14/37 (38%) |
| Quartz (DQ 12)+PVNO | 5 x 3 mg | 22/38 (58%) |
| Quartz Min-U-Sil | 15 x 3 mg | 21/39 (54%) |
| Quartz Min-U-Sil+PVNO | 15 x 3 mg | 20/35 (57%) |
| Quartz Sykron (F600) | 15 x 3 mg | 12/40 (30%) |

Pott *et al.* (1994)

RCS produced a variety of tumors in these studies (adenomas, adenocarcinomas, squamous cell carcinomas and benign cystic, keratinizing, squamous cell tumors, fibrosarcoma, lymphosarcoma, and mesotheliomas). Animals dosed with DQ 12 developed severe silicosis and had a median survival time of about 15 months. Survival of PVNO-dosed rats was not specified, but those animals developed more lung tumors. The authors attributed this difference to the protective effect against silicosis (Pott *et al.* 1994; cited in IARC 1997).

#### *4.1.4 Intratracheal administration in hamsters*

Intratracheal instillation of RCS in hamsters has not produced increased incidences of lung tumors. Three studies were reviewed by IARC as summarized in Table 4-5.

**Table 4-5. Summary of protocols and results of intratracheal RCS-instillation experiments conducted in hamsters**

| Material tested | Dose | Comments | Reference |
|---|---|---|---|
| Min-U-Sil | 3 or 7 mg once weekly for 10 weeks | Incidence and severity of pulmonary fibrosis was minimal. | Holland *et al.* (1983; cited in IARC 1997) |
| Min-U-Sil | 0.03, 0.33, 3.3 or 6.0 mg once weekly for 15 weeks | No animals developed nodular fibrosis or foci of dense fibrous tissue in lung. | Renne *et al.* (1985; cited in IARC 1997) |
| Sil-Co-Sil and Min-U-Sil | 1.1 mg weekly for 15 weeks | Bronchiolo-alveolar hyperplasia occasionally seen but no fibrosis. | Niemeier *et al.* (1986; cited in IARC 1997) |

Cited in IARC (1997)

#### *4.1.5 Intrapulmonary deposition*

Kahulau (1961; cited in IARC 1987) administered an unspecified amount of quartz (particle size about 2 μm) into the lung of seven rabbits. Two rabbits died after administration, but five survived for five to six years. Four of the survivors developed malignant lung tumors. Interpretation of this study is confounded by the absence of a vehicle control group.

#### *4.1.6 Intrapleural and intrathoracic administration*

Bryson *et al*. (1974; cited in IARC 1997) administered single, intrathoracic injections of 10 mg tridymite (20% of particles <3.3 μm) or 5 mg chrysotile (particle size not given) to mice. After 19 months (32-34 animals were surviving), one animal given tridymite had a lung adenocarcinoma and two had intrapleural lymphoid tumors. There were four lung adenocarcinomas and four lymphoid tumors in the chrysotile group. There was one lung adenocarcinoma and no lymphoid tumors in the control group.

Single, intrapleural, injections of 20 mg of quartz particles (<5μm) were carcinogenic in male and female SPF Wistar rats and standard Wistar rats. Malignant tumors of the reticuloendothelial system, involving the thoracic region, were observed in 39/95 (41%) SPF rats and in 31/94 (33%) standard rats (genders were combined in presentation of the data). These results were confirmed in a larger study involving groups of 80 male and 80 female SPF Wistar rats. The same studies were also conducted using other rat strains (Wagner and Berry 1969; Wagner 1970, 1976, Wagner *et al*. 1980; all cited in IARC 1997).

Jaurand *et al.* (1987; cited in IARC 1997) dosed Sprague-Dawley rats with 20 mg quartz (DQ 12) (in 1 mL saline) by intrapleural space injection. Animals were observed over their life spans and were sacrificed when moribund. Mean survival times were 769, 809 and 780 days for untreated controls, saline controls, and dosed animals, respectively. Six malignant histiocytic lymphomas and two malignant Schwannomas were found in RCS-dosed animals. Furthermore, one chronic lymphoid leukemia and one fibrosarcoma were observed in the saline and untreated controls groups, respectively.

#### *4.1.7 Other routes of administration*

When administered into the peritoneal cavity of male and female rats (single 20 mg injection ), quartz (Min-U-Sil) increased the incidence of malignant lymphomas (Wagner 1976; cited in IARC 1997). Intravenous administration of 1 mg quartz (average particle size, 1.6 μm) to mice produced no evidence of carcinogenicity (Shimkin and Leiter 1940; cited in IARC 1997). In a later study, Wagner (1976; cited in IARC 1997) administered 20 mg quartz intravenously to groups of 16 male and 16 female SPF Wistar rats. Four animals (sexes not specified) developed thymomas/lymphosarcomas. Whether these results are indicative of a carcinogenic effect can not be ascertained since vehicle control groups were not included.

Two groups of 40 female (C57xBALB/ $F_1$) mice received single, subcutaneous injections of 4 mg of *d*-, or 4 mg of *l*-, quartz (synthetic *d*- and *l*-quartz). A control group of 60 mice were injected with only saline. Survivors were killed at 18 months. Incidences of lymphomas/leukemias were 0/60, 1/40, and 12/40 for saline, *d*-quartz, and *l*-quartz dosed mice, respectively. In addition, 1/40 *d*-quartz and 3/40 *l*-quartz-dosed mice had liver adenomas while none of the controls had liver tumors. Fibrotic nodules were observed at the injection sites in 17/40 *d*-quartz- and 27/40 *l*-quartz-injected mice, but not in controls (Ebbesen 1991; cited in IARC 1997).

#### *4.1.8 Administration of RCS with known carcinogens*

In an inhalation study using Wistar rats, quartz (DQ 12) produced a pronounced pulmonary carcinogenic interaction with Thorotrast, an α-particle emitting material (Spiethoff *et al.* 1992; cited in IARC 1997).

Intratracheal instillation of quartz (Sil-Co-Sil and Min-U-Sil, 50 mg/animal) enhanced the respiratory tract carcinogenesis of benzo[a]pyrene (5 mg/animal) in hamsters (Niemeier *et al.* 1986; cited in IARC 1997). In two studies that administered mixtures of quartz and ferric oxide (1:1) to hamsters by intratracheal instillation, there were no increases in pulmonary tumor incidence (Saffiotti 1990, 1992, 1996; all cited in IARC 1997) (see Table 4-2).

## 4.2 Interpretations by Earlier Review Groups

The IARC Working Group concluded there is *sufficient evidence* for the carcinogenicity of CS in experimental animals.

## 4.3 Pertinent Information Developed Since Earlier Reviews

Williams and Knapton (1996) administered quartz (suspended in Eagle's minimum essential medium) subcutaneously or intraperitoneally to nude mice and Syrian golden hamsters. Mice received 3.5 g/kg and hamsters received 1.6 g/kg. Animals were killed, serially, and these authors presented descriptions of pathological changes produced in the liver by the treatment. Quartz-exposure produced granulomas, fibrosis, and cirrhosis, reaching 100% at 12 and 3 months in mice and hamsters, respectively. Hyperplasia and hypertrophy of nonparenchymal cells and foci of hyperplastic liver nodules were also found. Two of six mice (given quartz subcutaneously) killed between 13 and 17 months had liver cell carcinomas. One of these animals had a 1 cm diameter carcinoma and coexisting microscopic adenoma with cytoplasmic inclusion bodies. Control animals were reported to have no hepatic lesions.

# 5 Genotoxicity

## 5.1 Prokaryotic Systems

No data found.

## 5.2 Lower Eukaryotic Systems

No data found.

## 5.3 Mammalian Systems *in vitro*

### *5.3.1 Induction of micronuclei*

Several studies have demonstrated the ability of Respirable Crystalline Silica (RCS) to cause chromosomal damage in mammalian cells in culture. Hesterberg *et al*. (1986; cited by IARC 1997), treating Syrian hamster embryo cells with Min-U-Sil quartz, noted a significant increase in micronuclei. The same group (Oshimura *et al*. 1984; cited by IARC 1997), however, using another sample of quartz, failed to induce micronuclei, a modification of the number of chromosomal aberrations, aneuploid cells, or tetraploid cells.

Nagalakshmi *et al*. (1995), using Chinese hamster lung fibroblasts (V79) and human embryonic lung (Hel 299) cells, found a significant increase in micronuclei in both types of cells following treatment with Min-U-Sil 5 and Min-U-Sil 10 for 24 hours at several concentrations. However, the frequency of chromosome aberrations was not affected.

Liu *et al*. (1996) treated V79 cells with respirable silica particles. Two particle sizes of crystalline quartz and a noncrystalline form of silica were assayed for induction of micronuclei. Both forms of silica, dispersed in medium, induced micronuclei formation in dose-dependent manner. The RCS was more active, however, than the noncrystalline silica on a mass basis (as shown in Table 5-1). Treatment of cells with surfactant-coated silica was not significantly different from that of nontreated control cultures.

### *5.3.2 Direct interaction with DNA measured by infrared spectroscopy*

Using Fourier transform infrared spectroscopy, Daniel *et al*. (1995) showed *distinct alterations* in the DNA spectra of fetal rat aveolar epithelial cells following *in vitro* exposure to quartz. By means of electron microscopy and energy dispersive X-ray spectroscopy, they also demonstrated localization of quartz particles in the nuclei and mitotic spindles of the cells and related changes to the quartz spectra. As discussed in Section 6, they speculated that RCS particles in aqueous buffer produce oxygen radicals that can mediate DNA strand breakage. Estimating a hydroxyl radical reaction distance of 15 Angstoms, they described the likely hydrogen bonding between surface silanol groups of the quartz and the phosphate-sugar backbone of DNA. Finally, they discussed the means by which direct interaction of RCS with DNA may trigger carcinogenesis by interfering with DNA mitotic processes, replication, or repair.

### *5.3.3 DNA damage measured by SCG assay*

The single cell gel (SCG)/comet assay was used to compare DNA damage in cultured Chinese hamster lung fibroblasts (V79 cells) and human embryonic lung fibroblasts (Hel 299 cells) exposed to RCS, amorphous silica, and glass fibers (Zhong *et al*. 1997). RCS and glass fibers

caused a significant increase in DNA migration, measured as tail length in both cell lines, at almost all concentrations tested. The increase was much higher in the Chinese hamster lung fibroblasts than in human embryonic lung fibroblasts for RCS. These results indicate that silica and glass fibers induce DNA damage in mammalian cells. RCS was found to have a higher DNA-damaging activity than amorphous silica.

## *5.4* Mammalian Systems *in vivo*

### *5.4.1 Induction of hprt mutations*

Driscoll *et al*. (1995) were able to demonstrate that *in vivo* treatment of rats with α-quartz could induce mutations in the hypoxanthine guanine phosphoribosyl transferase (*hprt*) gene of aveolar epithelial lung cells. Seven months after exposure to 100 mg/kg body weight of intratracheally instilled α-quartz, female F344 rats were sacrificed and alveolar type II cells harvested and cultured to select for *hprt* mutants. Isolated cells showed a significant (greater than ten-fold) increase in *hprt* mutant frequency compared to cells isolated from saline instilled controls.

**Table 5-1. Frequency of micronuclei induced by silica particles in V79 cells**

| Compound | Concentration ($\mu g/cm^2$) | Frequency of MN ($\chi \pm SE$) |
|---|---|---|
| Min-U-Sil 5 | 0 | 6.67 ± 0.33 |
| | 20 | 9.33 ± 0.88 |
| | 40 | 15.67 ± 0.88[b] |
| | 80 | 20.67 ± 1.77[b] |
| | 160 | 25.67 ± 0.33[b] |
| Min-U-Sil 10 | 0 | 6.67 ± 0.33 |
| | 20 | 9.67 ± 0.67 |
| | 40 | 11.67 ± 0.67[a] |
| | 80 | 17.00 ± 1.00[b] |
| | 160 | 19.67 ± 0.67[b] |
| SSB1 | 0 | 7.67 ± 2.33 |
| | 20 | 10.33 ± 0.86 |
| | 40 | 8.67 ± 2.41 |
| | 80 | 13.33 ± 1.77[a] |
| | 160 | 18.00 ± 2.08[b] |
| MNNG[c] | 1 (μg/ml) | 96.67 ± 9.53b |

Liu *et al*. (1996)
3000 cells were scored for each treatment group; frequencies are mean values per 1000 cells.
[a]Compared with solvent control $p < 0.05$.
[b]Compared with solvent control $p < 0.01$.
[c]MNNG was used as a positive control in this experiment.

# 6 Other Information Relative to Evaluation of the Carcinogenicity RCS in Laboratory Animals

## 6.1 Particle Size, Deposition, and Disposition in the Respiratory Tract

The nature of the deposition of respired particles within the pulmonary system is a function of both species and the aerodynamic diameter of the particle in question.

In humans, large particles with aerodynamic diameters $\geq$ 10 μm, generally deposit in the upper respiratory tract and only smaller particles will deposit in the tracheobronchial region. Deposition in the alveolar region begins to be substantial only when aerodynamic diameters are well below 10 μm (Task Force Group on Lung Dynamic 1966; cited in IARC 1997). In small laboratory animals, such as the rat, deposition of particles of aerodynamic diameter > 6 μm is negligible. This species differential is attributable to differing modes of breathing (rodents are obligatory nose breathers, while humans are both mouth and nose breathers) and respiratory patterns, such as cycle period and tidal volume (Jones 1993; cited in IARC 1997).

Quartz particles with an aerodynamic diameter < 6 μm are likely to be the most damaging to rats. Substantial deposition of quartz particles with a mean aerodynamic diameter of 1.4 μm (range, 0.3-4.0) was reported on alveolar duct/terminal bronchiolar surfaces (Brody *et al.* 1982; cited in IARC 1997). More than 80% of particles deposited peripherally in the lungs (on the alveloar ducts, particularly at their bifurications, and on the distal terminal bronchioles) had an aerodynamic diameter of 3.7 μm (Warheit *et al.* 1991; cited in IARC 1997).

## 6.2 Distribution And Clearance Of Particles

After deposition of quartz on the surface of the respiratory tract, there is either rapid mucociliary clearance (from the upper airways), or phagocytosis by alveolar macrophages. Clearance tends to be slower if deposition is in the lung periphery (Brody *et al.* 1982; Warheit *et al.* 1991; both cited in IARC 1997). Clearance from the lungs of humans, dogs, and guinea pigs is slower than from the lungs of rats and hamsters (Oberdorster 1988; Jones 1993; both cited in IARC 1997). In general, clearance of particles by mucociliary mechanisms is considered to be efficient, while clearance from the lung periphery is slow and incomplete (Morgan 1984; Vacek *et al.* 1991; both cited in IARC 1997).

## 6.3 Quartz-Induced Inflammation

Exposure of rats to RCS results in a marked and persistent inflammatory response, characterized by a high percentage of neutrophils. This response has been repeatedly demonstrated after either inhalation or intratracheal instillation exposure (IARC 1997).

Henderson *et al.* (1995; cited in IARC 1997) exposed female Fischer 344 rats, by inhalation, to air or 0.1, 1.0, or 10 mg/m3 RCS (Min-U-Sil) 6 h/d, 5d/wk for 4 weeks. The mass median aerodynamic diameter of the aerosol was 1.3-2.0 μm. Lung responses were characterized by analysis of bronchoalveolar lavage (BAL) cell populations one, eight, and 24 weeks after exposure. Lung burdens of $SiO_2$ were determined one week after cessation of exposure. The results are summarized in Table 6-1.

**Table 6-1. BAL cell populations in rats exposed to RCS for four weeks**

| Treatment | Route | Cell differential (%) | | $SiO_2$ Burden μg/mg[1] | Comments |
|---|---|---|---|---|---|
| | | Macrophages | Neutrophils | | |
| Air (control) | Inhalation | 99 | 1 | | BAL results are for 24 weeks after cessation of either inhalation or intratracheal exposure. |
| 0.1 mg/m$^3$ | Inhalation | 99.5 | 0.5 | 43 | |
| 1 mg/m$^3$ | Inhalation | 97 | 2.5 | 190 | |
| 10 | Inhalation | 59 | 41 | 720 | |
| Saline (control) | Intratracheal | 98 | 2 | Not reported | |
| 750μg | Intratracheal | 38 | 62 | Not reported | |

[1] Lung burdens of $SiO_2$ were determined one week after cessation of inhalation exposure
Henderson *et al*. (1995)

Exposure to 10 mg/m$^3$ RCS (Min-U-Sil) caused lung injury and inflammation as evidenced by the pronounced increase in percent of neutrophils among the BAL cells. The authors reported that the 1 mg/m$^3$ dose caused a transient increase in BAL fluid neutrophils (however, no data were presented). Histopathological examination of lung tissues 24 weeks after exposure revealed an active-chronic inflammatory response in bronchial-associated lymphoid tissues, interstitium, and intrapleural regions of animals dosed with 10 mg/m$^3$. Intratracheal instillation of 750 μg of RCS produced an unequivocal increase in BAL fluid neutrophils.

## 6.4 Quartz-Induced Epithelial Injury and Proliferation in the Lung

After intratracheal instillation of quartz in mice, a wave of type II cell proliferation to regenerate damaged type I cells was reported by Adamson and Bowden (1984; cited in IARC 1997). This was accompanied by increasing hydroxyproline levels and sustained interstitial proliferation, interpreted to be evidence of mesenchymal cell proliferation and fibrosis. Miller and Hook (1988; cited in IARC 1997) reported a nearly two-fold increase in the number of type II epithelial cells in Min-U-Sil quartz-exposed lungs. These cells were hypertrophic with increased numbers of lamellar bodies. Warheit *et al.* (1991; cited in IARC 1997) reported that inhalation of Min-U-Sil quartz (but not carbonyl-iron) particles, caused proliferation in the lung parenchyma of rats. Type II cells, isolated from Min-U-Sil-exposed lungs, were shown to synthesize DNA *in vitro*, but they did not divide (Panos *et al.* 1990; cited in IARC 1997).

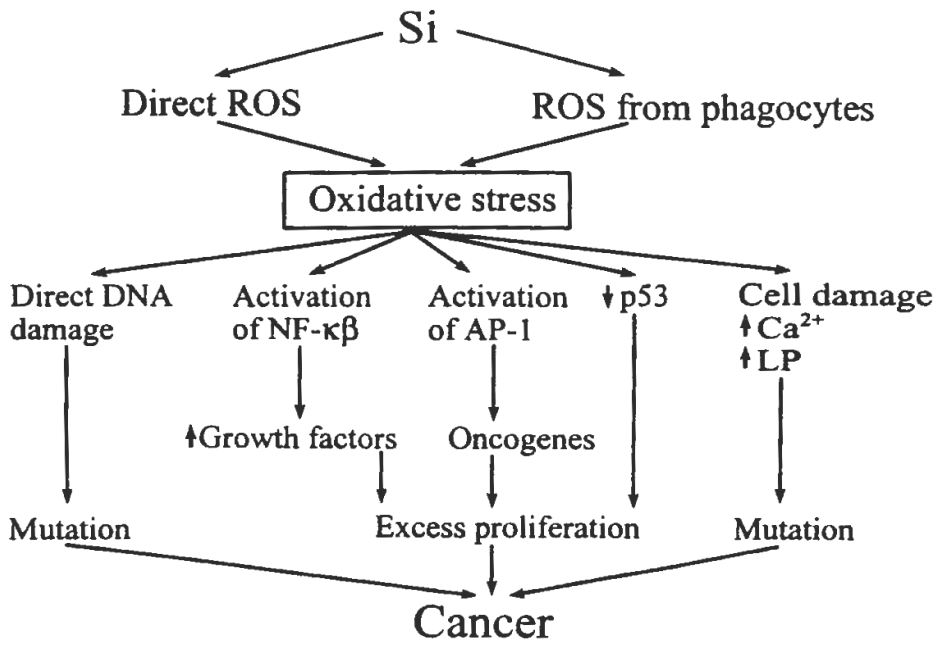
Lesur *et al.* (1992; cited in IARC 1997) reported that noncytotoxic concentrations of Min-U-Sil quartz caused proliferation and thymidine uptake in isolated rat epithelial cells. Macrophages, exposed to Min-U-Sil quartz *in vitro,* have been shown to release factors that stimulate growth of type II epithelial cells (Melloni *et al*. 1993; cited in IARC 1997). These results suggest that quartz may cause direct and indirect growth promotion of epithelial cells.

## 6.5 Oxidative Stress in Quartz-Exposed Lungs

Min-U-Sil quartz-exposed lungs have been shown to contain two- to three-fold more hydroxy radical activity than lungs exposed to either titanium dioxide or saline (Schapira *et al.* 1994; cited in IARC 1997). Both reactive oxygen and reactive nitrogen species (NO and peroxynitrite) are generated in Min-U-Sil quartz-induced inflammation (Blackford *et al.* 1994; Van Dyke *et al.* 1994; both cited in IARC 1997).

Shi *et al*. (1998) recently reviewed the scientific literature describing molecular level interactions between silica and biological materials. They have hypothesized that silica-mediated free radical reactions may cause oxidative stress in pulmonary tissue and thereby play a role in carcinogenesis. The Shi *et al*. model is presented schematically in Figure 2.

**Figure 2. Schematic representation of (hypothesized) silica-induced generation of reactive oxygen species (ROS) and possible role in carcinogenesis.**




Shi *et al.* (1998)

Fracturing silica crystals has been demonstrated to result in the generation of silicon-based free radicals which, upon reaction with water or $H_2O_2$, generate reactive oxygen species (ROS). ROS may also be generated from silica-stimulated phagocytes. It is hypothesized (Shi *et al*. 1998) that persistent oxidative stress possesses the potential to mediate carcinogenesis through mutations caused by direct DNA damage or via lipid peroxidation, activation of nuclear transcription and growth factors, oncogene expression, or inhibition of tumor suppressor genes.

### 6.6 Formation Of 8-Oxoguanine In Quartz-Exposed Rat Lungs

Nehls *et al.* (1997) exposed Wistar rats to quartz (DQ 12; 2.5 mg/rat) or corundum (2.5 mg/rat) suspended in saline, by intratracheal instillation. Animals were sacrificed 7, 21, or 90 days after dosing. Lung sections were examined for 8-Oxoguanine (8-oxoGua) level by immunocytological assay and proliferation was assessed by labeling with bromodeoxyuridine (doses administered two hours prior to sacrifice). The 8-oxoGua levels in quartz-exposed lung cells were markedly elevated, at all time intervals tested, relative to those saline- or corundum-exposed. Lung cell proliferation, in saline- or corundum-dosed animals, was unchanged, but exposure to quartz resulted in a three- to four-fold increase in cell proliferation at each of the time points.

These observations suggest that pulmonary inflammation (repeatedly demonstrated in rats), in conjunction with mutagenic DNA oxidation products in target cells (demonstrated by Nehls *et al.* 1997), and a quartz-induced proliferative response (demonstrated by earlier workers and Nehls *et al.* 1997), combine to play key roles in RCS-induced pulmonary carcinogenesis in rats. Nehls *et al*. (1997) further speculated that mutagenic events, triggered by ROS, may activate protoncogenes or inactivate tumor-suppessor genes to effect carcinogenesis. Shi *et al.* (1998) have suggested that differences in intra- and extracellular antioxidant levels between species may explain species' differences with respect to susceptibilities to silica-induced carcinogenesis.

# 7 References


1. Adamson, I.Y., and D. H. Bowden. 1984. Role of polymorphonuclear leukocytes in silica induced pulmonary fibrosis. *Am.J.Pathol.* 117:37-43.

2. Amandus, H.E., R.M. Castellan, C. Shy, E.F. Heineman, and A. Blair. 1992. Reevaluation of silicosis and lung cancer in North Carolina dusty trades workers. *Am.J.Ind.Med*. 22:147-153.

3. Amandus, H.E., C. Shy, R.M. Castellan, A. Blair, and E.F. Heineman. 1995. Silicosis and lung cancer among workers in North Carolina dusty trades. *Scand.J.Work.Environ.Health* 21 Suppl 2:81-83.

4. Amandus, H.E., C. Shy, S. Wing, A. Blair, and E.F. Heineman. 1991. Silicosis and lung cancer in North Carolina dusty trades workers. *Am.J.Ind.Med.* 20:57-70.

5. Blackford, J.A.J., J.M. Antonini, V. Castranova, and R.D. Dey. 1994. Intratracheal instillation of silica up-regulates inducible nitric oxide synthase gene expression and increases nitric oxide production in alveolar macrophages and neutrophils. *Am.J.Respir.Cell Mol.Biol.* 11:426-431.

6. Brody, A.R., M.W. Roe, J.N. Evans, and G.S. Davis. 1982. Deposition and translocation of inhaled silica in rats. Quantification of particle distribution, macrophage participation, and function. *Lab.Invest*. 47:533-542.

7. Brooks, S.M., H.G. Stockwell, P.A. Pinkham, A.W. Armstrong, and D.A. Witter. 1992. Sugarcane exposure and the risk of lung cancer and mesothelioma. *Environ.Res*. 58:195-203.

8. Brown, L.M., G. Gridley, J.H. Olsen, L. Mellemkjaer, M.S. Linet, and J.F.J. Fraumeni. 1997. Cancer risk and mortality patterns among silicotic men in Sweden and Denmark. *J.Occup.Environ.Med*. 39:633-638.

9. Bryson, G., F. Bischoff, and R. Stauffer. 1974. A comparison of chrystobalite and tridymite at the intrathoracic site in male Marsh mice (Abstract No. 22). *Proc.Am.Assoc.Cancer Res*. 15:6

10. Budavari, S., M.J. O'Neil, A. Smith, and P.E. Heckelman, eds. 1996. *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals.* Whitehouse Station: Merck & Co., Inc.

11. Burgess, G., S. Turner, J.C. McDonald, and N.M. Cherry. 1997. Cohort mortality study of Straffordshire pottery workers: radiographic validation of an exposure matrix for respirable crystalline silica (Abstract). In Inhaled particles VIII. *Occupational and Environmental Implications for Human Health*, Revised Final Programme and Abstracts, 26-30 August, 1996, Robinson College. N.M. Cherry and T.LK. Ogden, editors. Cambridge: Elsevier Science Ltd.

12. Burgess, W.A. 1995. *Recognition of Health Hazards in Industry*. New York: John Wiley & Sons.

13. Carmichael, R.S. 1989. *Practical Handbook of Physical Properties of Rocks and Minerals*. Boca Raton: CRC Press.

14. Checkoway, H., N.J. Heyer, and P.A. Demers. 1996a. An updated mortality follow-up study of Florida phosphate industry workers. *Am.J.Ind.Med*. 30:452-460.

15. Checkoway, H., N.J. Heyer, P.A. Demers, and N.E. Breslow. 1993. Mortality among workers in the diatomaceous earth industry. *Br.J.Ind.Med*. 50:586-597.

16. Checkoway, H., N.J. Heyer, P.A. Demers, and G.W. Gibbs. 1996b. Reanalysis of mortality from lung cancer among diatomaceous earth industry workers, with consideration of potential confounding by asbestos exposure. *Occup.Environ.Med.* 53:645-647.

17. Checkoway, H., N.J. Heyer, N.S. Seixas, E.A. Welp, P.A. Demers, J.M. Hughes, and H. Weill. 1997. Dose-response associations of silica with nonmalignant respiratory disease and lung cancer mortality in the diatomaceous earth industry. *Am.J.Epidemiol*. 145:680-688.

18. Checkoway, H., R.M. Mathew, J.L. Hickey, C.M. Shy, R.L.J. Harris, E.W. Hunt, and G.T. Waldman. 1985. Mortality among workers in the Florida phosphate industry. I. Industry-wide cause-specific mortality patterns. *J.Occup.Med*. 27:885-892.

19. Chen, J., J.K. McLaughlin, J.Y. Zhang, B.J. Stone, J.Luo, R.A. Chen, M. Dosemeci, S.H. Rexing, Z. Wu, and F.J. Hearl. 1992. Mortality among dust-exposed Chinese Mine and Pottery workers. *J.Occup.Med.* 34:311-316.

20. Cherry, N., G. Burgess, R. McNamee, S. Turner, and J.C. McDonald. 1995a. Initial findings from a cohort mortality study of British pottery workers. *Appl.occup.environ.Hyg.* 10:1042-1045.

21. Cherry, N., G. Burgess, R. McNamee, S. Turner, and J.C. McDonald. 1995b. Cohort mortality study on Staffordshire pottery workers: nested case referent analysis on lung cancer. In Inhaled particles VIII. *Occupational and Environmental Implications for Human Health*, Revised Final Programme and Abstracts, 26-30 August, 1996, Robinson College. N. Cherry and T.L. Ogden, editors. Elsevier Science Ltd., Cambridge, UK.

22. Corn, J.K. 1980. Historical aspects of industrial hygiene. II. Silicosis. *Am.Ind.Hyg.Assoc.J.* 41:125-133.

23. Corptech. 1998. http://www.corptech.com/FSP/Suppliers.cfm?requestTimeout=900&productCode=MAT-CE-CH.

24. Costello, J., R.M. Castellan, G.S. Swecker, and G.J. Kullman. 1995. Mortality of a cohort of U.S. workers employed in the crushed stone industry, 1940-1980. *Am.J.Ind.Med*. 27:625-640.

25. Costello, J. and W.G. Graham. 1988. Vermont granite workers' mortality study. *Am.J.Ind.Med.* 13:483-497.

26. CRC. 1996. *CRC Handbook of Chemistry and Physics*. New York: CRC Press. 4 pp.

27. Dagle, D., A. Wehner, M. Clark, and R. Buschbom. 1986. Chronic inhalation exposure of rats to quartz. In Silica, Silicosis and Cancer. *Controversy in Occupational Medicine.* D.W.D.&C. Goldsmith, editor. Praeger, New York. 255-266.

28. Daniel, L.N., Y. Mao , A.O. Williams, and U. Saffiotti. 1995. Direct interaction between crystalline silica and DNA - A proposed model for silica carcinogenesis. *Scand.J.Work.Environ.Health* 21 Suppl 2: 22-26.

29. Deer, W., R. Howie, and J. Zussman. 1966. *An Introduction to Rock-Forming Minerals.* New York: John Wiley & Sons. 340 pp.

30. de Klerk, N.H., and A.W. Musk. 1998. Silica, compensated silicosis, and lung cancer in Western Australian goldminers. *Occup.Environ.Med*. 55:243-248.

31. de Klerk, N.H., A.W. Musk, S. Tetlow, J. Hansen, and J.L. Eccles. 1995. Preliminary study of lung cancer mortality among Western Australian gold miners exposed to silica. *Scand.J.Work.Environ.Health* 21 Suppl 2:66-68.

32. Dong, D., G. Xu, Y. Sun, and P. Hu. 1995. Lung cancer among workers exposed to silica dust in Chinese refractory plants. *Scand.J.Work.Environ.Health* 21 Suppl 2:69-72.

33. Driscoll, K.E., L.C. Deyo, B.W. Howard, J. Poynter, and J.M. Carter. 1995. Characterizing mutagenesis in the hprt gene of rat alveolar epithelial cells. *Exp.Lung Res.* 21:941-956.

34. Ebbesen, P. 1991. Chirality of quartz. Fibrosis and tumour development in dust inoculated mice. *Eur.J.Cancer Prev*. 1:39-41.

35. Florke, O., and B. Martin. 1993. Silica Modifications and Product. *VCH Verlagsgesellschaft mbH, Weinheim*. 584 pp.

36. Froines, J.R., D.H. Wegman, and C.A. Dellenbaugh. 1986. An approach to the characterization of silica exposure in U.S. industry. *Am.J.Ind.Med.* 10:345-361.

37. Frondel, C. 1962. *The System of Mineralogy*, 7th Ed., Vol. III, Silica Minerals. New York: John Wiley and Sons.

38. Goldsmith, D.F. 1997. Evidence for silica's neoplastic risk among workers and derivation of cancer risk assessment. *J.Expo.Anal.Environ.Epidemiol.* 7:291-301.

39. Groth, D., L. Stellar, S. Platek, J. Lal, and J. Burg. 1986. Lung tumors in rats treated with quartz by intratracheal instillation. In Silica, Silicosis and cancer. *Controversy in Occupational Medicine.* D. Goldsmith, D. Winn, and C. Shy, editors. New York: Praeger. 243-253.

40. Guenel, P., G. Hojberg, and E. Lynge. 1989. Cancer incidence among Danish stone workers. S*cand.J.Work.Environ.Health* 15:265-270.

41. Henderson, R.F., K.E. Driscoll, J.R. Harkema, R.C. Lindenschmidt, I.Y. Chang, K.R. Maples, and E.B. Barr. 1995. A comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. *Fundam.Appl.Toxicol* 24:183-197.

42. Hesterberg, T.W., M. Oshimura, A.R. Brody, and J.C. Barrett. 1986. Asbestos and silica induce morpholgical transformation of mammalian cells in culture: a possible mechanism. In Silica, Silicosis, and Cancer. *Controversy in Occupational Medicine*. D. Goldsmith, D. Winn, and C. Shy, editors. New York: Praeger. 177-190.

43. Hilt, B. 1993. Crystallin Silica. In *Criteria Documents from the Nordic Expert Group*. B. Beije and P. Lundberg, editors. Arbete och Halsa, Solna, Sweden. 1-82.

44. Hnizdo, E., J. Murray, and S. Klempman. 1997. Lung cancer in relation to exposure to silica dust, silicosis and uranium production in South African gold miners. *Thorax.* 52:271-275.

45. Hnizdo, E. and G.K. Sluis-Cremer. 1991. Silica exposure, silicosis, and lung cancer: A mortality study of South African gold miners. *Br.J.Ind.Med.* 48:53-60.

46. Holland, L., M. Gonzales, J. Wilson, and M. Tillery. 1983. Pulmonary effects of shale dust in experimental animals. In: *Health Issues Related to Metal and Nonmetallic Mining*. W. Wagner, W. Rom, and J. Merchant, editors. Boston: Butterworths. 485-496.

47. Holland, L., J. Wilson, M. Tillery, and D. Smith. 1986. Lung cancer in rats exposed to fibrogenic dusts. In Silica, Silicosis and cancer. *Controversy in Occupational Medicine*. D. Goldsmith, D. Winn, and C. Shy, editors. New York: Praeger. 267-279.

48. IARC (International Agency for Research on Cancer). 1987. *Silica and some silicates*. Lyon, France. Vol 42:39 pp.

49. IARC (International Agency for Reseacrh on Cancer). 1997. *Silica, some silicates, coal dust and para-aramid fibrils.* Lyon, France. Vol 68:40 pp.

50. Iler, R. 1979. *The Chemistry of Silica.* New York: John Wiley & Sons.

51. Jaurand, M.C., J. Fleury, G. Monchaux, M. Nebut, and J. Bignon. 1987. Pleural carcinogenic potency of mineral fibers (asbestos, attapulgite) and their cytotoxicity on cultured cells. *J.Natl.Cancer Inst.* 79:797-804.

52. Jones, A.D. 1993. Respirable industrial fibres: deposition, clearance and dissolution in animal models. *Ann.Occup.Hyg*. 37:211-226.

53. Kadey, F.L. 1975. *Diatomite*. New York: American Insitute of Mining; Metallurgical and Petroleum Engineers. 605 pp.

54. Kahulau, G. 1961. Anatomo-pathological and animal experiments on the question of silicosis and lung cancer. *Frankfurt.Z.Pathol*. 71:3-13.

55. Kusnetz, S., and M.K. Hutchison. 1979. *A Guide to the Work-Relatedness of Disease* (NIOSH Pub. No. 79-116). Cincinnati: National Institute for Occupational Safety and Health. 155 pp.

56. Lauwerys, R.R. 1990. *Toxilcologe Industrielle et Intoxicantions Professionnelles (Industrial Toxicology and Occupational Intoxications)*. Masson, Paris.

57. Lesur, O., A.M. Cantin, A.K. Tanswell, B. Melloni, J.F. Beaulieu, and R. Begin. 1992. Silica exposure induces cytotoxicity and proliferative activity of type II pneumocytes. *Exp.Lung Res.* 18:173-190.

58. Liu, X., M.J. Keane, B.Z. Zhong, T.M. Ong, and W.E. Wallace. 1996. Micronucleus formation in V79 cells treated with respirable silica dispersed in medium and in simulated pulmonary surfactant. *Mutat.Res*. 361:89-94.

59. McDonald, J.C., G. Burgess, S. Turner, and N.M. Cheney. 1997. Cohort mortality study of Strffordshire pottery workers: Lung cancer, radiographic changes, silica exposure and smoking habit (Abstract). In Inhaled particles VIII. *Occupational and Environmental Implications for Human Health, Revised Final Programme and Abstracts*, 26-30 August, 1996, Robinson College. N.M. Cherry and T.L. Ogden, editors. Cambridge: Elsevier Science Ltd.

60. McDonald, J.C., N. Cherry, R. McNamee, G. Burgess, and S. Turner. 1995. Preliminary analysis of proportional mortality in a cohort of British pottery workers exposed to crystalline silica. *Scand.J.Work.Environ.Health* 21 Suppl 2:63-65.

61. McLaughlin, J.K., J.Q. Chen, M. Dosemeci, R.A. Chen, S.H. Rexing, Z. Wu, F.J. Hearl, M.A. McCawley, and W.J. Blot. 1992. A nested case-control study of lung cancer among silica exposed workers in China. *Br.J.Ind.Med*. 49:167-171.

62. Melloni, B., O. Lesur, A. Cantin, and R. Begin. 1993. Silica-exposed macrophages release a growth-promoting activity for type II pneumocytes. *J.Leukoc.Biol*. 53:327-335.

63. Merlo, F., M. Costantini, G. Reggiardo, M. Ceppi, and R. Puntoni. 1991. Lung cancer risk among refractory brick workers exposed to crystalline silica: a retrospective cohort study. *Epidemiology*. 2:299-305.

64. Miller, B.E., and G.E. Hook. 1988. Isolation and characterization of hypertrophic type II cells from the lungs of silica-treated rats. *Lab.Invest*. 58:565-575.

65. Morgan, W. 1984. *The deposition and clearance of dust from the lungs*. Philadelphia: Saunders. 77 pp.

66. Muhle, H., B. Bellmann, O. Creutzenberg, C. Dasenbrock, H. Ernst, R. Kilpper, J.C. MacKenzie, P. Morrow, U. Mohr, and S. Takenaka. 1991. Pulmonary response to toner upon chronic inhalation exposure in rats [published erratum appears in Fundam Appl Toxicol 1991 Nov;17(4):827]. *Fundam.Appl.Toxicol* 17:280-299.

67. Muhle, H., B. Kittel, H. Ernst, U. Mohr, and R. Mermelstein. 1995. Neoplastic lung lesions in rat after chronic exposure to crystalline silica. *Scand.J.Work.Environ.Health* 21 Suppl 2:27-29.

68. Muhle, H., S. Takenaka, U. Mohr, C. Dasenbrock, and R. Mermelstein. 1989. Lung tumor induction upon long-term low-level inhalation of crystalline silica. *Am.J.Ind.Med*. 15:343-346.

69. Nagalakshmi, R., J. Nath, T. Ong, and W.Z. Whong. 1995. Silica-induced micronuclei and chromosomal aberrations in Chinese hamster lung (V79) and human lung (Hel 299) cells. *Mutat.Res*. 335:27-33.

70. Nehls, P., F. Seiler, B. Rehn, R. Greferath, and J. Bruch. 1997. Formation and persistence of 8-oxoguanine in rat lung cells as an important determinant for tumor formation following particle exposure. *Environ.Health Perspect*. 105 Suppl 5:1291-1296.

71. Niemeier, R., L. Mulligan, and J. Rowland. 1986. *Cocarcinogenicity of foundry silica sand in hamsters*. New York: Praeger. 215 pp.

72. NIOSH (National Institute for Occupational Safety and Health). 1983. *Review of the Literature on Crystalline Silica (PB 83-238733)*. Cincinnati: United State National Institute for Occupational Safety and Health.

73. NIOSH (National Institute for Occupational Safety and Health). 1994. *Work-Related Lung disease Surveillance Report.* Center for Disease Control/National Institute for Occupational Safety and Health.

74. Oberdorster, G. 1988. Lung clearance of inhaled insoluble and soluble particles. *J.aerosol Med.* 1:289-330.

75. OSHA (Occupational Safety and Health Administration). 1998. http://www.osha.gov/oshinfo/priorities/silica.html.

76. Oshimura, M., T.W. Hesterberg, T. Tsutsui, and J.C. Barrett. 1984. Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. *Cancer Res*. 44:5017-5022.

77. Panos, R.J., A. Suwabe, C.C. Leslie, and R.J. Mason. 1990. Hypertrophic alveolar type II cells from silica-treated rats are committed to DNA synthesis in vitro. *Am.J.Respir.Cell Mol.Biol.* 3:51-59.

78. Parent, M., J. Siemiatycki, and L. Fritschi. 1997. Occupational exposures and gastric cancer. *Epidemiology*. 9:48-55.

79. Partanen, T., E. Pukkala, H. Vainio, K. Kurppa, and H. Koskinen. 1994. Increased incidence of lung and skin cancer in Finnish silicotic patients. *J.Occup.Med*. 36:616-622.

80. Pott, F., D. Dungworth, U. Heinrich, H. Muhle, K. Kamino, P.-G. Germann, M. Roller, R. Rippe, and U. Mohr. 1994. Lung tumours in rats after intratracheal instillation of dusts. *Ann.Occup.Hyg*. 38:357-363.

81. Rafnsson, V. and H. Gunnarsdottir. 1997. Lung cancer incidence among an Icelandic cohort exposed to diatomaceous earth and cristobalite. *Scand.J.Work.Environ.Health*. 23:187-192.

82. Renne, R.A., S.R. Eldridge, T.R. Lewis, and D.L. Stevens. 1985. Fibrogenic potential of intratracheally instilled quartz, ferric oxide, fibrous glass, and hydrated alumina in hamsters. *Toxicol Pathol*. 13:306-314.

83. Roberts, L., G. Rapp, Jr., and J. Weber. 1974. *Encyclopedia of Minerals.* New York: Van Nostrand Reinhold.

84. Rothschild, H., and J.J. Mulvey. 1982. An increased risk for lung cancer mortality associated with sugarcane farming. *J.Natl.Cancer Inst*. 68:755-760.

85. Saffiotti, U. 1990. Lung cancer induction by silica in rats, but not in mice and hamsters: Species differences in epithelial and granulomatous reactions. In *Environmental Hygiene II*. N. Seemayer and W. Hadnagy, editors. New York: Springer Verlag. 235-238.

86. Saffiotti, U. 1992. Lung cancer induction by crystalline silica. In *Relevance of animal studies to the evaluation of human cancer risk.* R. D'Amato, T. Slaga, W. Farland, and C. Henry, editors. New York: Wiley-Liss. 51-69.

87. Saffiotti, U., A.O. Williams, L. Daniel, M. Kaighn, Y. Mao, and X. Shi. 1996. Carcinogenesis by crystalline silica: Animal, cellular, and molecular studies. In *Silica and silica-induced lung diseases.* V. Castranova, V. Vallyathan, and W. Wallace, editors. Boca Raton: CRC Press. 345-381.

88. Schapira, R.M., A.J. Ghio, R.M. Effros, J. Morrisey, C.A. Dawson, and A.D. Hacker. 1994. Hydroxyl radicals are formed in the rat lung after asbestos instillation in vivo. *Am.J.Respir.Cell Mol.Biol*. 10:573-579.

89. Shimkin, M. and J. Leiter. 1940. Induced pulmonary tumors in mice. III. The role of chronic irritation in the production of pulmonary tumors in strain A mice. *J.Natl.cancer.Inst*. 1:241-254.

90. Shi, X., V. Castranova, B. Halliwell, and V. Vallyathan. 1998. Reactive Oxygen Species and Silica-Induced Carcinogenesis. *J. Toxicol. Environ. Health*, Part B. 1:181-197.

91. Sinks, T., M.T. Goodman, L.N. Kolonel, and B. Anderson. 1994. A case-control study of mesothelioma and employment in the Hawaii sugarcane industry. *Epidemiology*. 5:466-468.

92. Smith, A.K. 1992. http://www.osha-slc.gov/SLTC/silicacrystalline/smithdk/index.html.

93. Smith, A.H., P.A. Lopipero, and V.R. Barroga. 1995. Meta-analysis of studies of lung cancer among silicotics. *Epidemiology.* 6:617-624.

94. Smyth, J., and D. Bish. 1988. *Crystal Structures and Cation Sites of the Rock-forming Minerals.* Boston: Allen and Unwin.

95. Spiethoff, A., H. Wesch, K. Wegener, and H.J. Klimisch. 1992. The effects of thorotrast and quartz on the induction of lung tumors in rats. *Health Phys*. 63:101-110.

96. Starzynski, Z., K. Marek, A. Kujawska, and W. Szymczak. 1996. Mortality among different occupational groups of workers with pneumoconiosis: results from a register-based cohort study. *Am.J.Ind.Med*. 30:718-725.

97. Steenland, K. and D. Brown. 1995. Mortality study of gold miners exposed to silica and nonasbestiform amphibole minerals: an update with 14 more years of follow-up. *Am.J.Ind.Med*. 27:217-229.

98. Steenland, K. and L. Stayner. 1997. Silica, asbestos, man-made mineral fibers, and cancer. *Cancer Causes.Control.* 8:491-503.

99. Task Force Group on Lung Dynamics. 1966. Deposition and retention models for internal dosimetry of human respiratory tract. *Health Phys*. 12:173-207.

100. U.S. Bureau of Mines. 1992. *Crystalline Silica Primer*. Washington, DC.

101. U.S. DOI (Unites States Department of the Interior) *Crystalline Silica Primer.* U.S. Department of the Interior.U.S.Bureau of Mines Special Publication No.RC965.S5C79. 1992. (GENERIC)

102. U.S. DOI (Unites States Department of the Interior). 1994. *Mineral Industry Surveys, Industrial Sand and Gravel, Annual Review; Quartz Crystal Annual Review*. Washington, DC.

103. Vacek, P.M., D.R. Hemenway, M.P. Absher, and G.D. Goodwin. 1991. The translocation of inhaled silicon dioxide: an empirically derived compartmental model. *Fundam.Appl.Toxicol* 17:614-626.

104. VanDyke, K., J.M. Antonini, L. Wu, Z. Ye, and M.J. Reasor. 1994. The inhibition of silica-induced lung inflammation by dexamethasone as measured by bronchoalveolar lavage fluid parameters and peroxynitrite- dependent chemiluminescence. *Agents Actions* 41:44-49.

105. Wagner, J. and G. Berry. 1969. Mesotheliomas in rats following innoculation with asbestos. *Br.J.Cancer* 23:567-581.

106. Wagner, J. 1970. The pathogenesis of tumors following the intrapleural injection of asbestos and silica. In *Morphology of experimental respiratory carcinogenesis*. P. Nettesheim, M. Hanna, Jr., and J. Deatherage, Jr., editors. Oak Ridge: U. S. Atomic Energy Commission. 347-358.

107. Wagner, M.M. 1976. Pathogenesis of malignant histiocytic lymphoma induced by silica in a colony of specificpathogen-free Wistar rats. *J.Natl.Cancer Inst*. 57:509-518.

108. Wagner, M.M., J.C. Wagner, R. Davies, and D.M. Griffiths. 1980. Silica-induced malignant histiocytic lymphoma: incidence linked with strain of rat and type of silica. *Br.J.Cancer.* 41:908-917.

109. Warheit, D.B., M.C. Carakostas, M.A. Hartsky, and J.F. Hansen. 1991. Development of a short-term inhalation bioassay to assess pulmonary toxicity of inhaled particles: comparisons of pulmonary responses to carbonyl iron and silica. *Toxicol Appl.Pharmacol.* 107:350-368.

110. Webster, D.L. 1982. *Steel Production. In Industrial Hygiene Aspects of Plant Operations*, Volume 1, Process Flows. L.V. Crawley and L.J. Crawley, editors. 457-487.

111. Weill, H., R.N. Jones, and W. Parkes. 1994. Silcosis and Related Diseases. In *Occupational Lung Disorders.* W.R. Parkes, editor. Oxford: Butterworth Heineman. 457-487.

112. Weill, H. and J.C. McDonald. 1996. Exposure to crystalline silica and risk of lung cancer: The epidemiological evidence [see comments]. *Thorax*. 51:97-102.

113. Williams, A.O. and A.D. Knapton. 1996. Hepatic silicosis, cirrhosis, and liver tumors in mice and hamsters: studies of transforming growth factor beta expression. *Hepatology*. 23:1268-1275.

114. Wilson, T., W.J. Scheuchenzuber, M.L. Eskew, and A. Zarkower. 1986. Comparative pathological aspects of chronic olivine and silica inhalation in mice. *Environ.Res*. 39:331-344.

115. Winter, P.D., M.J. Gardner, A.C. Fletcher, and R.D. Jones. 1990. A mortality follow-up study of pottery workers: preliminary findings on lung cancer. *IARC.Sci.Publ.* 83-94.

116. Zheng, W., X.O. Shu, B.T. Ji, and Y.T. Gao. 1996. Diet and other risk factors for cancer of the salivary glands: A population-based case-control study. *Int.J.Cancer.* 67:194-198.

117. Zhong, B.Z., W.Z. Whong, and T.M. Ong. 1997. Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay. *Mutat.Res.* 393:181-187.

# Appendix A - IARC Monographs.1997. Vol 68:41-242.

***Silica, some silicates, coal dust and para-aramid fibrils.*** **Lyon, France.**