# Scientific Literature Review of the Cosmetic Ingredient Review Expert Panel

# Safety Assessment of Silica, Alumina Magnesium Metasilicate, Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, And Sodium Potassium Aluminum Silicate

**March 23, 2009**

*All interested persons are provided 60 days from the above date to comment on this Scientific Literature Review and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.*

The 2009 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D. Curtis D. Klaassen, Ph.D.; Daniel C. Leibler, Ph.D. James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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## INTRODUCTION

This is a safety assessment of Silica, Alumina Magnesium Metasilicate, Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, and Sodium Potassium Aluminum Silicate. Another assessment reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel addressed the safety of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Potassium Silcate, Pyrophyllite, Sodium Metasilicate, Sodium Silicate, and Zeolite. The CIR Expert Panel concluded that these ingredients were "...safe as used in cosmetic products..." (Andersen 2003, 2005).

There are 2 categories of Silica, crystalline and amorphous. Only the amorphous forms of Silica are used in cosmetics. Below are descriptions of both types of Silica and their properties for informational purposes. Otherwise, only the safety of amorphous Silica is evaluated in this safety assessment and is referred to as Silica.

## CHEMISTRY

### DEFINITION AND STRUCTURE

#### AMORPHOUS VS. CRYSTALLINE SILICA

Silica is a silicon-oxygen tetrahedral where a silicon atom is central within 4 oxygen atoms that are shared with adjacent silicon atoms. Various physical forms of Silica are caused by differences in the spatial relationships of the tetrahedral that determine physical characteristics such as x-ray diffraction. Amorphous Silica has an irregular tetrahedral pattern and includes quartz glass (vitreous Silica). Crystalline silica is polymorphic where each variety has a characteristic regular 3-dimensional arrangement of the tetrahedral (Heppleston 1969). Crystalline Silica forms exhibit a well-defined patterns with x-ray diffraction; amorphous forms of Silica do not (Villota and Hawkes 1986).

There are 3 classifications of Silica. Vitreous Silica, or fused crystalline Silica, is formed by the supercooling of molten Silica. It has a low coefficient of thermal expansion, high thermal shock resistance and high ultraviolet transparency. Silica M is a dense thermally unstable amorphous Silica which converts to quartz at high temperatures. Microamorphous Silica includes sols, gels, powders, and porous glasses. This group has the subclasses amorphous Silica fibers, microscopic fibers; and microparticulate Silica, which includes precipitated

and fumed Silicas (Villota and Hawkes 1986).

Crystalline silica may be found in more than one form (polymorphism). The polymorphic forms of crystalline silica are alpha quartz, beta quartz, tridymite, cristobalite, keatite, coesite, stishovite, and moganite (Ampian and Virta 1992; Heaney 1994; Guthrie and Heaney 1995). Each polymorph is unique in its spacing, lattice structure, and angular relationship of the atoms.

There are multiple forms of amorphous Silica: fumed, colloidal, precipitated, diatomaceous earth, gel, and hydrous. The colloidal form ranges in size from 10 micrometers to <10 nanometers (Ewamatawong et al. 2005).

Burning of agricultural waste or products such as rice hulls may also cause amorphous Silica to become cristobalite (a crystalline form) (Rabovsky 1995).

The different polymorphs of Silica are demonstrated in Figure 1.

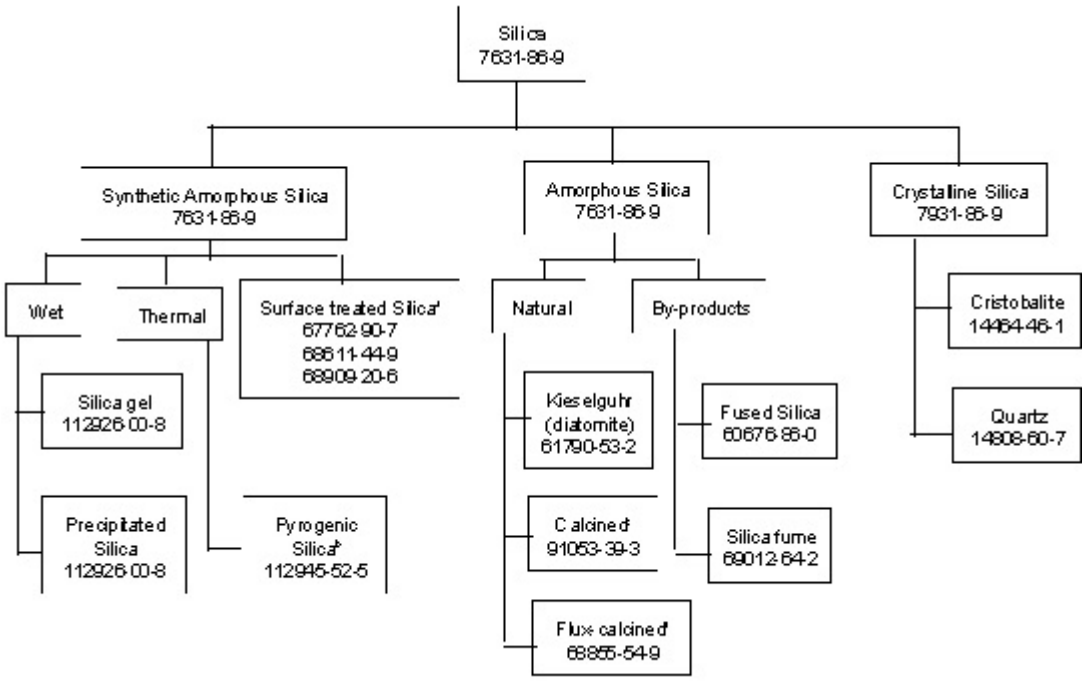


Figure 1. Different polymorphs of Silica with CAS Numbers. a) All forms of synthetic amorphous Silicas can be surface modified either physically or chemically; most common treating agents are organosilicon compounds. b) Pyrogenic Silica is alson known as fumed Silica in English speaking countries. c) By-product from electrical furnace. d) Partial transformation into cristobalite (Arts et al. 2007).

SILICA

The CAS No. 7631-86-9 is the general CAS No. which included all forms of Silicas including crystalline, synthetic, and natural forms (United Nations Environmental Programme Chemicals Unit [UNEP] 2004).

According to the *International Cosmetic Ingredient Dictionary and Handbook*, Silica (CAS Nos. 7631-86-9 [colloidal], 60676-86-0, 112945-52-5 [fumed]) is the inorganic oxide that conforms to the formula $SiO_2$. Silica functions as an abrasive, absorbent, anti-caking agent, bulking agent opacifying agent, and suspending agent - nonsurfactant. Other technical names for Silica are Amorphous Silica; Amorphous Silcon Oxide Hydrate; Silica, Amorphous; Silicon, Anhdride; Silicon Dioxide; Silicon Dioxide, Fumed; Spheron P-1000; and Spheron PL-700 (Gottschalck and Bailey 2008).

ALUMINA MAGNESIUM METASILICATE

Alumina Magnesium Metasilicate (no CAS No.) is the inorganic compound that conforms generally to the formula:

$$MgSiO_3 \cdot Al_2O_3.$$

Alumina Magnesium Metasilicate functions as and absorbent, bulking agent, and a viscosity increasing agent - nonaqueous. Another technical name is Magnesium Metasilicate/Aluminate (Gottschalck and Bailey 2008).

ALUMINUM CALCIUM SODIUM SILICATE

Aluminum Calcium Sodium Silicate (CAS No. 1344-01-0) is a complex silicate refined from naturally occurring minerals. Aluminum Calcium Sodium Silicate functions as a functions as a bulking agent (Gottschalck and Bailey 2008). Other technical names include Aluminosilicic Acid (Unspecified), Calcium Sodium Salt, Hydrate; Aluminosilicic Acid, Calcium Sodium Salt; Calcium Sodium Aluminosilicate; Sodium Calcium Aluminosilicate; Sodium Calcium Aluminosilicate, Hydrated; Sodium Calcium Silicoaluminate; and Sodium Calcium Silicoaluminate Hydrate (ChemIDplus Lite 2009).

ALUMINUM IRON SILICATES

Aluminum Iron Silicates (no CAS No.) is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, and ferric oxide. Aluminum Iron Silicates function as abrasives and bulking agents. Another technical name is Silica Aluminum Silicate Ceramics (Gottschalck and Bailey 2008).

HYDRATED SILICA

Hydrated Silica (CAS Nos. 1343-98-2 [Silicic Acid]; 10279-57-9; 63231-67-4; 112926-00-8) is the inorganic oxide that conforms generally to the formula

$SiO_2 \cdot xH_2O$

where x varies with the method of precipitation or gelation and extent of drying performed on the material. Hydrated Silica functions as an abrasive, absorbent, anti-caking agent, bulking agent, opacifying agent, oral care agent, skin-conditioning agent - miscellaneous, and viscosity increasing agent - aqueous. It is also referred to as Silicic Acid; Hydrosilicic Acid Precipitated Silica; Silica Gel; Silica Hydrate Silicic Acid Hydrate; and Silicon Dioxide Hydrate (Gottschalck and Bailey 2008).

### SODIUM POTASSIUM ALUMINUM SILICATE

Sodium Potassium Aluminum Silicate (CAS No. 12736-96-8 and 66402-68-4) is a complex silicate refined from naturally occurring minerals, or derived synthetically. It functions as a bulking agent (Gottschalck and Bailey 2008).

## PHYSICAL AND CHEMICAL PROPERTIES

### SILICA

Amorphous Silicas are composed of very fine particles (average of 20 μm) which tend to aggregate loosely in the air (Byers and Gage 1961).

Occupational Safety and Health Administration (OSHA; 1978) reported that there are no substances that contribute to the instability of Silica. Contact with fluorine, oxygen difluoride, and chlorine trifluoride will cause fire. Silica has no hazardous decompositional products.

Villota and Hawkes (1986) stated that the surface of Silica may be made up of free silanol groups (isolated hydroxyls), hydrogen-bonded silanol groups (hydroxyl groups on adjacent surface silicon atoms) and siloxane groups. An individual silicon atom on the Silica surface may be substituted with aluminum coordinated with 4 oxygen atoms as in the aluminate ion $Al(OH)_4^-$, introducing a negative charge to the silica and influencing the adsorption behavior of the Silica.

Amorphous Silica is capable of rehydroxylating in aqueous systems to form a high ratio of sianol to siloxane groups. Depending on the hydrophobic properties of the solvent, it may form a network-like structure through hydrogen bonding. This gives amorphous Silica gelling and thickening abilities in various solvent systems.

Oxygen electron donors of compounds such as ethers, alcohols, and ketones or the nitrogens of amides and amines may interact through hydrogen bonding due to the acid dissociation constant of the silanol groups on the Silica surface. Esterification has been reported with an Si-O-C-R structure. A totally dehydrated Silica or a fully hydrated Silica has little or no adsorption of hydrophobic organocompounds. With the addition of few isolated silanol groups, an optimal adsorption of the organocompounds takes place due to hydrogen bonding (Villota and

Hawkes 1986).

The *Food Chemicals Codex* states that Silica is a white, fluffy nongritty powder of extremely fine particle size that is hygroscopic. Silica absorbs moisture from the air in varying amounts (Food Nutrition Board [FNB] 1996).

Cabot Corporatation (2004) stated that Silica has thixotropic properties. The particles for a 3-dimensional network in a liquid system increases viscosity. When shear forces are applied (i.e., stirring), the material flows as a liquid. When the forces cease, the material gels again.

The particle size and Brunauer, Emmett, and Teller (B.E.T.) surface area of different Silica particles are listed in Table 1.

**Table 1.** B.E.T surface area and particle size of different Silica products (UNEP Chemicals Unit 2004).

| B.E.T surface area ($m^2/g$) | Particle size (µm) |
|---|---|
| 50 ± 15 | - |
| 90 ± 15 | - |
| 130 ± 25 | - |
| 150 ± 15 | - |
| 200 ± 25 | - |
| 380 ± 30 | - |
| 60 | 15 |
| 160 | 7 |
| 300 | 4.5 |
| 650 | 4 |
| 170 | 15 |
| 170 | 4 |
| 170 | 5 |
| 450 | 3.5 |
| 700 | 15 |
| No data | 4 |
| No data | 5 |
| No data | 4 |
| No data | 3 |
| 50 | 8 |
| 80 | 13 |
| 80 | 6 |
| 140 | 6 |
| 100 | 6 |
| 190 | 9 |
| 190 | 100 |
| 190 | 7 |
| 190 | 4.5 |
| 450 | 50 |
| 450 | 8 |
| 190 | 100 |

The National Institute for Occupational Safety and Health (NIOSH 2005) stated that fluorine, oxygen difluoride, and chlorine trifluoride are on the list of incompatibilities and reactivities.

Chemical and physical properties of Silica are listed in Table 2. The physical properties broken down into fumed and precipitated Silica are shown in Table 3. Physical properties by B.E.T. surface area are shown in Table 4.

## METHODS OF MANUFACTURE

All of the ingredients in this assessment have mineral sources (Gottschlack and Bailey 2008).

SILICA

Swensson et al. (1956) fused crystalline Silica material in a high-frequency furnace at 1750 to 1800 C to create amourphous Silica.

Byers and Gage (1961) stated that amorphous Silica is prepared by acid precipitation from an aqueous solution of sodium silictate.

**Table 2.** Chemical and physical properties of Silica.

| Property | Value | Reference |
|---|---|---|
| Molecular weight | 60.1 | NIOSH 2005 |
| Boiling point | 4046°F (2230°C) | NIOSH 2005 |
| Melting point | 3110°F (1710°C) | NIOSH 2005 |
| | ~1700 °C | UNEP 2004; OSHA 1978 |
| Specific gravity | 2.65 | OSHA 1978 |
| Solubility | Insoluble | NIOSH 2005 |
| Water solubility (saturation) | ~15-68 mg/l at 20°C (pH 5.5-6.6) | UNEP 2004 |
| | Insoluble | FNB 1996 |
| Vapor pressure | ~0 mm HG | NIOSH 2005; OSHA 1978 |
| | None | UNEP 2004 |
| Density | ~2.2 at 20°C | UNEP 2004 |
| Bulk density (tapped) | 50-320 g/l | UNEP 2004 |
| | 40-60 g/l | American International Chemical, Inc. (no date) |
| pH | 4-9 | UNEP 2004 |
| | ~3.7 (4% aqueous slurry) | American International Chemical, Inc. (no date) |
| Particle size | 1-350 µm | UNEP 2004 |
| Photodegradation | Stable in water and air | UNEP 2004 |
| Stability in water | Stable; ion exchange processes possible | UNEP 2004 |
| Loss on Ignition | 1.0% max (2 hr @ 1,000°C | American International Chemical, Inc. (no date) |
| Loss on heating | 1.0% max | American International Chemical, Inc. (no date) |

**Table 3.** Physical properties of fumed hydrophobic Silica and Precipitated hydrophobic Silica (Lewinson et al. 1994).

| | Fumed hydrophobic Silica | Precipitated hydrophobic Silica |
|---|---|---|
| Appearance | Fluffy powder | Fluffy powder |
| BET* surface area | 110-250 | 100 |
| Moisture (%) | <0.5 | 3 |
| Ignition loss (%) | <2 | 7 |
| Temperature at ignition (°C) | 400 | 400 |
| Decomposition temperature of methyl groups (°C) | 300 | 300 |
| $SiO_2$ (%) | >99.8 | >99.5 |
| Carbon, bound | 1 | 2 |
| $Al_2O_3$ (%) | <0.05 | 0.1 |
| $Fe_2O_3$ (%) | <0.01 | 0.03 |
| $TiO_2$ (%) | <0.03 | 0.03 |
| HCl (%) | <0.025 | <0.025 |
| Dimethyldichlorosilane (%) | <0.1 | <0.1 |

**Table 4.** Physical properties of Silica with B.E.T. surface areas of 200, 325, or 380 $m^2/g$ (Cabot Corporation 2006a,b,c).

| | Value |
|---|---|
| pH (4% aqueous slurry) | 3.7-4.3 |
| 325 Mesh residue (44 microns) | 0.02% max |
| Tamped density | 50 g/l |
| Loss on heating | < 1.5% |
| Specific Gravity<br>Wt./gallon | 2.2 g/cm3<br>18.3 lbs |
| Refractive index | 1.46 |
| X-ray form | Amorphous |
| Assay (% $SiO_2$) | >99.8 |
| Oil adsorption | ~350 g/100 g oil |
| Average particle (aggregate) length | 0.2-0.3 microns |

A manufacturing process for amorphous Silica is shown in Figure 2 (Villota and Hawkes 1986).

Lewinson et al. (1994) stated that Silica may be yielded by a vapor-phase process producing fumed Silica or by a wet process producing precipitated Silica. Fumed Silica is produced in a relatively anhydrous state, whereas precipitated Silica contains a larger amount of bound water.

Cabot Corporation (2004, 2006a,b,c, no date) stated that Silica particles are treated with other chemicals to give certain properties. Hexamethyldisilazane replaces many of the surface hydroxyl groups with trimethylsilyl groups, making the particles extremely hydrophobic, creating Silica Silylate. Dimethyldichlorosilane replaces many of the surface hydroxyl groups with methyl groups, making the particles less hydrophobic than untreated Silica, creating Silica Dimethyl Silylate. Silicon fluid replaces many of the surface hydroxyl groups with

polydimethyl-siloxane polymer, making the particles extremely hydrophobic, making Silica Dimethicone Silylate.

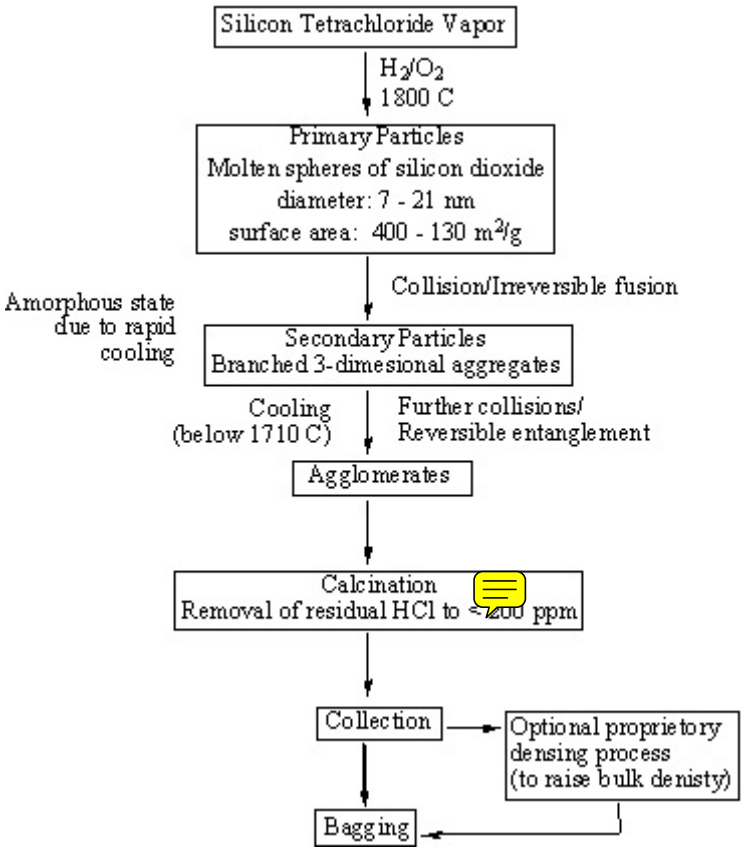


**Figure 2.** Process for the manufacture of Silica (Villota and Hawkes 1986).

Sodium Potassium Aluminum Silicate is refined from naturally occurring minerals or derived synthetically. Aluminum Calcium Sodium Silicate is refined from naturally occurring minerals (Gottschalck and Bailey 2008).

## ANALYTICAL METHODS

### SILICA

Particle size may be determined by an air permeability method and electron microscopy (Byers and Gage 1961).

Mollo et al. (1997) used electron paramagnetic resonace (EPR) to detect the presence of fluorescein isothiocyanate-labeled Silica in cells.

## IMPURITIES

### SILICA

Policard and Collet (1954) reported a sample of Silica being 99.9% pure with impurities of calcium, sodium,

and potassium. Amorphous Silica created by heating crystalline Silica contained less than 0.1% crystalline silica by x-ray diffraction tests (Swensson et al. 1956). Swensson et al. (1971) stated that in the production of fumed amorphous Silica, the composition of the fumes is ≥80% Silica and up to 6% to 8% is quartz. Cabot Corporation (2004) states that its Silica products are >99.8% pure. The moisture content of untreated Silica is < 1 wt%. Treated Silicas are susceptible to adsorbing chemical vapors.

UNEP (2004) reported Silica to be >95% pure. Possible impurities include: $Na_2O$ (0.2% to 2.1% wt.), sulfates as $SO_3$ (0.2% to 3.0% wt.) $Fe_2O_3$ (< 0.05% wt.), and trace oxides (<0.07% wt.). Heavy metal impurities include: antimony (<5 ppm), barium (<50 ppm), chromium (<10 ppm), arsenic (<3 ppm), lead (<10 ppm), mercury (<1 ppm), cadmium (<1ppm), and selenium (<1 ppm).

## USE

### COSMETIC

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Ingredient Reporting Program (VCRP), Silica was used in a total of 2406 cosmetic products. Use concentrations ranged from 0.0000003% to 44% according to a survey of current use concentrations conducted by the Personal Care Products Council (Council 2008). Hydrated Silica was reported to be used in 147 cosmetic products at 0.001% to 34%; Alumina Magnesium Silica in 732 products at 0.001% to 0.002%; Aluminum Calcium Sodium Silicate in 5 cosmetic products at 0.4% to 6%; and Sodium Potassium Aluminum Silicate in 2 products at 0.001% to 4%. There were no reported uses or concentration of use reported for Aluminum Iron Silicate. Uses are shown in Table 5.

Silica is used in 4 hair color sprays/aerosols. Jensen and O'Brien (1993) reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.

The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, $\mathbf{d_a}$,

**Table 5.** Currant cosmetic product uses and concentrations for Silica, Hydrated Silica, Alumina Magnesium Silcate, Aluminum Calcium Sodium Silicate, and Sodium Potassium Aluminum Silicate.

| Product Category (Total products in category - FDA 2008) | 2008 uses (FDA 2008) | 2008 concentrations (%) (Council 2008) |
|---|---|---|
| | ***Silica***[a] | |
| **Baby products** | | |
| Shampoos (55) | - | 0.003 |
| Lotions, oils, powders, etc. (132) | 1 | - |
| Other (138) | 1 | 10[b] |
| **Bath products** | | |
| Soaps and detergents (1329) | 16 | 0.02-10 |
| Oils, tablets, and salts (257) | 15 | 0.9-2 |
| Bubble baths (262) | 2 | - |
| Capsules (4) | 1 | - |
| Other (239) | 3 | 0.02 |
| **Eye products** | | |
| Eyebrow pencil (147) | 37 | 0.01-6 |
| Eyeliner (684) | 48 | 0.3-19 |
| Shadow 1196) | 325 | 0.001-44 |
| Lotion (177) | 29 | 0.02-4 |
| Makeup remover (131) | 4 | 0.0004 |
| Mascara (463) | 103 | 0.2-10 |
| Other (288) | 43 | 0.04-3 |
| **Fragrance products** | | |
| Colognes and toilet waters (1288) | 1 | 0.1 |
| Perfumes (569) | 5 | 1 |
| Powders (278) | 33 | 1-10 |
| Sachets (28) | - | - |
| Other (399) | 6 | 6-18[c] |
| **Noncoloring hair care products** | | |
| Conditioners (1249) | 9 | 0.002 |
| Sprays/aerosol fixatives (371) | - | 0.0005 |
| Straighteners (144) | 1 | 3[d] |
| Permanent waves (141) | 1 | - |
| Rinses (47) | - | 0.003 |
| Shampoos (1403) | 3 | 0.02 |
| Tonics, dressings, etc. (1097) | 4 | 0.02-3 |
| Other (716) | 11 | - |
| **Hair coloring products** | | |
| Dyes and colors (2481) | 77 | 0.002-0.3 |
| Tints (58) | 1 | 2 |
| Color sprays/aerosol (8) | 4 | 0.4 |
| Hair lighteners with color (22) | 3 | - |
| Bleaches (152) | 45 | 6[e] |
| Other (166) | 11 | 1 |
| **Makeup** | | |
| Blushers (539) | 95 | 2-20 |
| Face powders (613) | 164 | 1-26 |
| Foundations (635) | 220 | 0.01-40 |
| Leg and body paints (29) | 2 | - |
| Lipstick (1912) | 371 | 0.01-21 |
| Makeup bases (164) | 36 | 0.5-20 |
| Rouges (99) | 21 | 0.09-3 |
| Fixatives (38) | 12 | 0.3-3 |
| Other (406) | 80 | 2-4[f] |

**Table 5.** Historical and current cosmetic product uses and concentrations for Silica, Hydrated Silica, Alumina Magnesium Silcate, Aluminum Calcium Sodium Silicate, and Sodium Potassium Aluminum Silicate.

| Product Category (Total products in category - FDA 2008) | 2008 uses (FDA 2008) | 2008 concentrations (%) (Council 2008) |
|---|---|---|
| | ***Silica (continued)*** | |
| **Nail care products** | | |
| Basecoats and undercoats (62) | 5 | 5 |
| Creams and lotions (17) | 1 | 2-3 |
| Polish and enamel (419) | 70 | 0.3-9 |
| Other (124) | 13 | 5 |
| **Oral hygiene products** | | |
| Dentifrices (59) | 4 | 3-16 |
| Other (48) | 4 | - |
| **Personal hygiene products** | | |
| Underarm deodorants (540) | 20 | 0.02-9 |
| Douches (12) | 1 | - |
| Other (514) | 19 | 0.0000003-0.06[g] |
| **Shaving products** | | |
| Aftershave lotions (395) | 6 | 0.2-0.9 |
| Men's talcum (7) | 1 | - |
| Preshave lotions (27) | - | 5 |
| Shaving cream (162) | 1 | - |
| Other (107) | 8 | 0.003 |
| **Skin care products** | | |
| Cleansing creams, lotions, liquids, and pads (1368) | 34 | 0.002-5 |
| Depilatories (62) | - | 1 |
| Face and neck creams, lotions, etc. (1195) | 70 | 0.03-10 |
| Body and hand creams, lotions, etc. (1513) | 35 | 0.02-5[h] |
| Foot powders and sprays (48) | 6 | 0.8 |
| Moisturizers (2039) | 130 | 0.008-8 |
| Night creams, lotions, powder and sprays (343) | 19 | 0.01-3 |
| Paste masks/mud packs (418) | 13 | 0.02-6 |
| Fresheners (285) | 4 | 0.00004-3 |
| Other (1244) | 82 | 0.04-11[i] |
| **Suntan products** | | |
| Suntan gels, creams, liquids and sprays (156) | 5 | 0.03-2 |
| Indoor tanning preparations (200) | 6 | - |
| Other (62) | 5 | 0.6 |
| **Total uses/ranges for Silica** | **2406** | **0.0000003-44** |
| | ***Hydrated Silica*** | |
| **Bath products** | | |
| Soaps and detergents (1329) | 13 | 0.05-4 |
| Oils, tablets, and salts (257) | 6 | 0.4-2 |
| Other (239) | 1 | 4 |
| **Eye products** | | |
| Eyeliner (684) | 1 | - |
| Shadow 1196) | 2 | - |
| Lotion (177) | 1 | 0.06-1 |
| Mascara (463) | 1 | - |
| Other (288) | 2 | 2 |

**Table 5.** Historical and current cosmetic product uses and concentrations for Silica, Hydrated Silica, Alumina Magnesium Silcate, Aluminum Calcium Sodium Silicate, and Sodium Potassium Aluminum Silicate.

| Product Category (Total products in category - FDA 2008) | 2008 uses (FDA 2008) | 2008 concentrations (%) (Council 2008) |
|---|---|---|
| ***Hydrated Silica (continued)*** | | |
| **Fragrance products** | | |
| Powders (278) | 9 | 2 |
| **Noncoloring hair care products** | | |
| Conditioners (1249) | - | 0.04 |
| Shampoos (1403) | - | 0.05 |
| Tonics, dressings, etc. (1097) | - | 2 |
| **Hair coloring products** | | |
| Bleaches (152) | 19 | 2[j] |
| Other (166) | 1 | - |
| **Makeup** | | |
| Blushers (539) | - | - |
| Face powders (613) | 20 | 4 |
| Foundations (635) | 3 | 3 |
| Lipstick (1912) | - | 0.003 |
| Other (406) | 2 | - |
| **Nail care products** | | |
| Basecoats and undercoats (62) | 6 | - |
| Cuticle softeners (18) | 1 | - |
| Creams and lotions (17) | 1 | - |
| Polish and enamel (419) | 2 | 1-2 |
| Other (124) | 3 | - |
| **Oral hygiene products** | | |
| Dentifrices (59) | 23 | 7-34 |
| Mouthwashes and breath fresheners (85) | - | - |
| Other (48) | 2 | 0.2 |
| **Personal hygiene products** | | |
| Underarm deodorants (540) | - | 2 |
| Douches (12) | - | 0.03 |
| Feminine deodorants (21) | 2 | - |
| Other (514) | 2 | 6[k] |
| **Shaving products** | | |
| Men's talcum (7) | 1 | - |
| **Skin care products** | | |
| Cleansing creams, lotions, liquids, and pads (1368) | 3 | 3-17 |
| Face and neck creams, lotions, etc. (1195) | 4 | 0.09 |
| Body and hand creams, lotions, etc. (1513) | 4 | 0.06-2 |
| Foot powders and sprays (48) | 1 | - |
| Moisturizers (2039) | 2 | 1-2 |
| Night creams, lotions, powder and sprays (343) | - | 0.04 |
| Paste masks/mud packs (418) | 1 | 0.01-10 |
| Fresheners (285) | 4 | - |
| Other (1244) | 3 | 0.001-0.004 |
| **Suntan products** | | |
| Suntan gels, creams, liquids and sprays (156) | - | 0.2-2 |
| Other (62) | 1 | - |
| **Total uses/ranges for Hydrated Silica** | **147** | **0.001-34** |

**Table 5.** Historical and current cosmetic product uses and concentrations for Silica, Hydrated Silica, Alumina Magnesium Silcate, Aluminum Calcium Sodium Silicate, and Sodium Potassium Aluminum Silicate.

| Product Category (Total products in category - FDA 2008) | 2008 uses (FDA 2008) | 2008 concentrations (%) (Council 2008) |
|---|---|---|
| ***Alumina Magnesium Metasilicate*** | | |
| **Bath products** | | |
| Soaps and detergents (1329) | 3 | - |
| Other (239) | 2 | - |
| **Eye products** | | |
| Eyebrow pencil (147) | 6 | - |
| Eyeliner (684) | 31 | - |
| Shadow 1196) | 20 | - |
| Lotion (177) | 5 | - |
| Mascara (463) | 51 | - |
| Other (288) | 12 | - |
| **Fragrance products** | | |
| Colognes and toilet waters (1288) | **1** | **-** |
| Other (399) | **3** | **-** |
| **Noncoloring hair care products** | | |
| Straighteners (144) | 1 | - |
| Shampoos (1403) | 5 | - |
| Tonics, dressings, etc. (1097) | 2 | - |
| **Makeup** | | |
| Blushers (539) | 4 | - |
| Face powders (613) | 4 | - |
| Foundations (635) | 105 | - |
| Leg and body paints (29) | 2 | - |
| Lipstick (1912) | 3 | - |
| Makeup bases (164) | 18 | - |
| Rouges (99) | 1 | - |
| Fixatives (38) | 1 | - |
| Other (406) | 21 | - |
| **Personal hygiene products** | | |
| Underarm deodorants (540) | 13 | - |
| Other (514) | 12 | - |
| **Shaving products** | | |
| Aftershave lotions (395) | 8 | - |
| Shaving cream (162) | 2 | - |
| Other (107) | 2 | - |
| **Skin care products** | | |
| Cleansing creams, lotions, liquids, and pads (1368) | 62 | - |
| Face and neck creams, lotions, etc. (1195) | 39 | 0.001 |
| Body and hand creams, lotions, etc. (1513) | 78 | 0.002 |
| Foot powders and sprays (48) | 1 | - |
| Moisturizers (2039) | 103 | - |
| Night creams, lotions, powder and sprays (343) | 12 | - |
| Paste masks/mud packs (418) | 40 | - |
| Fresheners (285) | 3 | - |
| Other (1244) | 27 | - |

**Table 5.** Historical and current cosmetic product uses and concentrations for Silica, Hydrated Silica, Alumina Magnesium Silcate, Aluminum Calcium Sodium Silicate, and Sodium Potassium Aluminum Silicate.

| Product Category (Total products in category - FDA 2008) | 2008 uses (FDA 2008) | 2008 concentrations (%) (Council 2008) |
|---|---|---|
| ***Alumina Magnesium Metasilicate (continued)*** | | |
| **Suntan products** | | |
| Suntan gels, creams, liquids and sprays (156) | 6 | - |
| Indoor tanning preparations (200) | 22 | - |
| Other (62) | 1 | - |
| **Total uses/ranges for Alumina Magnesium Silicate** | **732** | **0.001-0.002** |
| ***Aluminum Calcium Sodium Silicate*** | | |
| **Eye products** | | |
| Mascara (463) | **-** | **0.5** |
| **Makeup** | | |
| Foundations (635) | **-** | **0.4-6** |
| Lipstick (1912) | **-** | **6** |
| **Nail care products** | | |
| Polish and enamel (419) | **4** | **0.5** |
| **Skin care products** | | |
| Moisturizers (2039) | **1** | **-** |
| **Total uses/ranges for Aluminum Calcium Sodium Silicate** | **5** | **0.4-6** |
| ***Sodium Potassium Aluminum Silicate*** | | |
| **Nail care products** | | |
| Basecoats and undercoats (62) | **-** | **4** |
| Polish and enamel (419) | **1** | **0.001** |
| **Skin care products** | | |
| Paste masks/mud packs (418) | **1** | **-** |
| **Total uses/ranges for Ingredient** | **2** | **0.001-4** |

[a] Both Silica and Amorphous Silica were listed by the FDA. These data were combined.
[b] 10% in a diaper liner.
[c] 10% and 18% in a solid perfume
[d] 1.5% after dilution.
[e] 3% after dilution.
[f] 2% in a concealer.
[g] 0.006% in a shower gel.
[h] 2% in body and hand sprays.
[i] 0.6% in a lip moisture cream/ 10% in a foot exfoliant.
[j] 1% after dilution.
[k] 6% in a body scrub.

defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of 4.25 ± 1.5 µm for respirable particles that could result in lung exposure (Jensen and O'Brien, 1993).

Bower (1999) reported diameters of anhydrous hair spray particles of 60 - 80 µm and pump hair sprays with particle diameters of ≥80 µm. Johnsen (2004) reported that the mean particle diameter is around 38 µm in a typical aerosol spray. In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 -

110 µm range.

## NON-COSMETIC

### SILICA

Silica is used as a filler in rubber formulations (Byers and Gage 1961).

Silica is used in food preparations as an anticaking agent in dry powders, dispersion agent for dry powders in liquids, antisettling or suspending agent, stabilizer in oil/water emulsions, thickening or thixotropic agent, gelling agent, flavor carrier, extrusion aid, clarification and separation aid, and support matrix for immobilization of enzymes. It is also a general excipient for pharmaceuticals (Villota and Hawkes 1986). It is also used as a defoaming agent, conditioning agent, a chillproofing agent in malt beverages, and a filter aid in foods (FNB 1996).

UNEP (2004) states that Silica is used in pharmaceuticals as a thickener in pastes and ointments to inhibit the separation of components and maintain flow properties in powder products. It is also used in foods. Silica can function as a carrier for fragrances or flavors. It is used in beer and wine clarification. Silica is used in animal feed as carriers and anticaking agents in vitamins and mineral premixes. Silica is used a reinforcing fillers for many non-staining and colored rubber and silicone products. It is used in “green tires”. Silica is also used in paints, lacquers, plastics, and paper. Silica is used as an insecticide by it sorption of the cuticular lipid layer causing dehydration.

The colloidal form of Silica is used in fiber, sizing, diazo paper manufacture, cellophane film, ceramics, grass fiber, paints, batteries, foods, and polishing (Kaewamatawong et al. 2005).

Javadzadeh et al. (2007) investigated the use of Silica in a drug delivery system.

### ALUMINUM CALCIUM SODIUM SILICATE

Under the name Sodium Calcium Aluminosilicate, Hydrated, the FDA (2007a) states that Alumina Cacium Sodium Silciate is generally recognized as safe (GRAS) in food for use at a level not exceeding 2% in accordance with good manufacturing processes as an anticaking agent.

### SODIUM SILICATE

Sodium Silicate is GRAS for use in dry food packaging made from cotton and cotton fibers and paper and paperboard porducts (FDA 2007b,c).

## GENERAL BIOLOGY

### ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

#### ORAL

SILICA

Sauer et al. (1959) orally administered Silica in the form of sodium metasilicate, precipitated silicic acid, and Silica solution (30%) to guinea pigs (n = 5) in a single dose or in 4 repeated doses every 48 h. Urine and feces were collected in 48-h increments after each dose and analyzed for Silica content.

The urinary output of Silica, in the form of sodium metasilicate, when orally administered peaked within 48 h and gradually returned to normal after 8 days. When administered 4 times, 48 hours apart, the peak was maintained, but not increased. Forty-eight h after the last dose the concentration of Silica in the urine began to reduce toward normal.

The urinary output of Silica, in the form of Silica sol and precipitated silicic acid, when orally administered peaked within 48 h and gradually returned to normal after 8 days. These peaks were much lower than those of sodium metasilicate. When administered 4 times, 48 hours apart, the Silica concentrations behaved similarly to the orally administered Silica with a lower peak. The authors suggested that all of the Silica in the urine was in the soluble or molybdate reactive form and it appears that the Silica particles underwent depolymerization prior to excretion (Sauer et al. 1959).

UNEP (2004) reported an unpublished oral study of Silica (1500 mg/kg/d) using female rats (strain not specified). Silica was orally administered daily for 30 days. The rats were then killed and necropsied. Body weight gain, food consumption and behavior were not influenced. The Silica content in the livers was 1.5 µg, in the kidneys was 6.4 µg, and in the spleen was 5.3 µg. The control values were 1.8, 7.2, and 7.8 µg Silica, respectively.

In another unpublished study, female Sprague-Dawley rats were orally administered Silica (100 mg/rat; ~500 mg/kg; aqueous suspension) 20 times over 1 month. No clinical signs were observed. The Silica content in the liver was 4.2 µg (control value = 1.8 ug), in the spleen was 5.5 µg (7.2 ug), and in the was kidneys 14.2 µg (7.8 µg) (UNEP 2004).

#### PARENTERAL

SILICA

When administered by intraperitoneal injection as Silica sol or precipitated Silica, urinary Silica increased

above normal for 16 days (see ORAL section above for details). As silicic acid, the levels increased above normal for 28 days. When orally administered, 63% of the Silica was recovered; 48% was recovered when intraperitoneally administered. The authors suggest that conditions in the peritoneal cavity favor the depolymerization and subsequent excretions of Silica (Sauer et al. 1959).

Byers and Gage (1961) intratracheally injected 3 types of Silica (see the ACUTE INTRAVENOUS section below; 25 mg; 2.5% in 1 ml suspension) into adult albino Wistar rats (n = 100; 50 male, 50 female). Rats were killed and necropsied at 12, 24, and 52 weeks. The amount of Silica in the tissues are given in Table 6.

**Table 6.** Retention of Silica in rat tissues after intratracheal injection (Byers and Gage 1961).

| Sample | Time after injection (weeks) | Lungs (µg/rat) | Liver (µg/rat) | Kidneys (µg/rat) | Spleen (µg/rat) |
|---|---|---|---|---|---|
| A1 | 12 | 1570 | 177 | 33 | 0 |
| | 24 | 755 | 20 | 12.4 | 1.4 |
| | 52 | 210 | 61 | 9.7 | 0 |
| A2 | 12 | 5150 | 249 | 138 | 0 |
| | 24 | 1550 | 153 | 44 | 19 |
| | 52 | 720 | 153 | 56 | 0 |
| B | 12 | 656 | 234 | 34 | 5.8 |
| | 24 | 324 | 43 | 8.9 | 1.4 |
| | 52 | 108 | 38 | 12 | 0 |
| Average found in normal rat tissue | | 28 | 37 | 11 | 5 |

The 3 types of Silica elicited the same type of response in the lungs between types and sexes. Distribution in the lungs was not uniform but aggregated in small areas throughout 1 or both lungs with the occasional large deposit. Silica particles were contained within macrophages, aggregated into foci around terminal and respiratory bronchioles. Some lymphocytes and fibroblasts surrounded these foci and intermingled within the macrophages with reticulin fibers woven though and around the lesion. Where dust deposits were heavy, the structure of the lung segments was completely obliterated by the lesions consisting of a group of macrophages with a few fibroblasts and lymphocytes that were contiguous and distinguished by the peripheral zone of fibroblasts and lymphocytes. These lesions were maximal at 12 weeks and gradually reduced with some contraction resulting in varying degrees of deformity of the lung. The number of lesion decreased in size and number over time and was a function of the amount of Silica present in the lungs.

Type B was the most quickly eliminated from the lungs; type A2, the largest particles, were the slowest. Type A2 also had larger lesions and induced a greater amount of fibroblastic proliferation. Lesions in the type B group were initially similar to A1 but the regression of the former was more rapid and final sections showed either

a complete resolution or small scattered dust foci with few reticulin fibers. There also remained a few areas of confluent lesions which were small and irregular with a light reticulin network and little retraction. Evidence of infection (mild emphysema, few large abscesses, foci of bronchiectasis, pneumonia and bronchopneumonia) was infrequent and similar to controls.

The authors concluded that the dust from these 3 samples of precipitated Silica do not aggregate sufficiently to be entirely retained by the upper respiratory passages and is still detectable in the lungs after 12 months. The lesions are different from those reported to be from quartz. The greater severity of the lesions from types A2 can be attributed to those surface properties which resulted in its greater tendency to aggregate (Byers and Gage 1961).

INHALATION

SILICA

UNEP (2004) reported an unpublished inhalation study of Silica (0.050 to 0.055 mg/l) using female Sprague-Dawley rats (n not provided). The rats were exposed to aerosolized Silica for 5 h/d for 5 d/weeks for 1 year. The rats had occurrences of bronchitis, putrid, lung inflammation, and pronounced cell reactions so exposure was reduced to 2 or 3 d/week; the exact time of the change was not provided. Rats in each group were killed and necropsied periodically during treatment and after treatment.

After 6 weeks of treatment, Silica was observed in the lungs (0.5 mg) and the mediastinal lymph node (0.02 mg); after 18 weeks these values were 1.2 mg and 0.11 mg; and after 12 months, 1.37 mg and 0.13 mg, respectively. Corresponded to the respiration volume, 1% of the inhaled Silica was retained in the lungs. After a post-observation of 5 months, there was 0.160 mg and 0.047 mg Silica observed in the lungs and mediastinal lymph node, respectively, a reduction of 88% in the lung and > 50% in the lymph nodes. The increase in lung deposition was rapid at the initial exposure, then low from 18 weeks to 12 months of exposure.

In another unpublished inhalation study, female inbred albino rats (breed not specified; n not provided) were exposed to aerosolized Silica (dose not provided) for 40 days. The amount was then increased to 40 to 50 mg/$m^3$ until day 120. A few of the rats were killed and necropsied periodically.

The average 1-day retention value was 28 µg/lung at the lower unspecified concentration. During the first 10 days, a steep linear increase was seen with ~28 µg/day as theoretically expected. Increments then became smaller. The author suggested that elimination increased and that an equilibrium between retention and elimination was established. After 40 exposures, the average 1-day retention value was 59 µg/lung at the high

concentration. After 120 exposures, the total deposit (lung and medistinal lymph nodes) was found to be only at 435 µg/lung, equivalent to 7.4 % of the theoretically deposited material (5840 µg/lung, based on the measured 1-day retention); more than 92% of the deposited Silica in the alveoli was eliminated during the exposure period. At that time, the mean retention of the lungs was only 300 µg/lung (~ 69% of the total). The deposition rate in the mediastinal lymph nodes was negligible during the first 40 days, but increased gradually. After 120 exposures, the retention there was substantial amounting to 135 µg (~ 31% of the total deposit). A test for the determination of free alveolar cells showed a decrease immediately after a single exposure and 24 hours later an increase of 100% was observed.

In another unpublished inhalation study, aerosolized Silica (0.05 mg/l) was administered for 5 h per day for 3 days to female Sprague-Dawley rats (n not specified). They were observed for up to 3 months. Twenty h after the last exposure, 0.25 mg Silica was found in the lungs. After 3 months, the Silica content was 0.018 mg. In the lymph node, 0.018 mg Silica was found after 1 month and 0.008 mg Silica after 3 months.

In an unpublished inhalation study of precipitated and fumed Silica (55 mg/$m^3$), rats (strain and n not specified) were exposed for 5 h to precipitated Silica. For the precipitated Silica, the mean retention value at 20 h was 0.138 mg/lung. For the fumed Silica, the mean retention value was 0.130 mg/lung. For the precipitated Silica, the mean Silcia-content of the lungs after 4 months was 1.022 mg, and 3.113 mg after 12 months. The corresponding values for the mediastinal lymphatic nodes were 0.033 mg and 0.069 mg, respectively. Five months after exposure, the average value for the lungs was only 0.457 mg (87% elimination rate) and 0.052 mg for the mediastinal lymphatic nodes (UNEP 2004).

SUBCUTANEOUS

SILICA

UNEP (2004) reported an unpublished subcutaneous (s.c.) study of a single dose of Silica (10 mg) using female Sprague-Dawley rats (n not provided). After 24 hours, 6.89 mg Silica was found in the tissue at the application site. After 1 month the amount was decreased to 0.646 mg; after 2 months 0.298 mg was found.

## MISCELLANEOUS STUDIES

LUNG AND LYMPH NODE EFFECTS

SILICA

Zimmerman et al. (1986) exposed macrophages and neutrophils from C57BL/6 x DBA/2)$F_1$ ($DBF_1$) mice to fumed Silica (0, 100, 300, or 500 µg) for 1 to 3 h. Incubation with mid or high concentrations killed 80% to 100% of

both types of phagocytes. An additional experiment showed that macrophages and neutrophils incubated with 10 or 30 µg Silica were 88% to 99% viable after 1 to 3 h. When incubated in Silica, both macrophages and neutrophils were inhibited in their ability to phagocytose sheep red blood cells, less so on neutrophils. The ability of these cells to phagocytose *Listeria monocytogenes* was complete at 300 µg and above; the inhibition was concentration dependent between 10 and 100 µg. Pre-incubation with Silica (100 to 500 µg) also inhibited bactericidal activity of the macrophages and neutrophils; there was no effect on bacterial growth. There was a concentration dependent inhibition of bactericidal activity when the phagocytes were pre-incubated in Silica at 10 to 300 µg.

Warheit et al. (1995) exposed CD rats (n = 24) to aerosolized Silica (10 or 100 $mg/m^3$; particle size 2.4 to 3.4 µm) for 3 days. Brochoalveolar lavage (BAL) was then performed periodically over 90 days and the recovered cells were identified, counted, and evaluated. Transient inflammatory response was observed at 24 h post-exposure which was resolved within 8 days. There was a transient increase in lactate dehydrogenase (LDH) in the rats for 3 days which resolved by day 8. LDH in the BAL fluid increased within 24 h after exposure but returned to control levels by day 8. There was also a transient increase in BAL fluid protein. N-Acetyl glucosaminidase did not increase in the BAL fluid. The authors suggested that the effects of inhalation exposure to Silica is transient and reversible.

Nyberg et al. (1996) incubated macrophages from male sprague-Dawley rats with Silica particles (3.2 ± 0.4 µm), heat killed *Candida albicans*, or heat killed *Saccharomyces cerevisiae* ($10 \times 10^6$ fungi or particles/ml) for 30 min. The number of ingested yeasts were higher than the control Silica particles. Both yeasts induced a 3- to 4-fold increase in the Nitroblue tetrazolium (NBT) reductions by the macrophages whereas the Silica treated macrophages were similar to resting macrophages. After 3 and 24 h incubation, there were no phagolysomes observed in the pH $\geq$ 6.5 range, 14 ± 15 in the $\geq$ 5.5 to 6.6 range, and 86 ± 15 in the < 5.5 range.

Yuen et al. (1996) intratracheally instilled male Crl:CD BR rats (n = 3; 7 to 9 weeks old) with Silica particles (10 mg/kg; particle size range 2 to 3.5 µm). Other mice were also exposed to crytalline silica and titanium dioxide. The mice were killed and examined 0.5, 2, and 5 h, and 2 and 10 days after exposure. Neutrophilic inflammation was induced as early as 5 h after exposure. Both Silicas induced higher degrees of pulmonary inflammation than titanium dioxide. Maximal infiltration of neutrophils into the lungs occurred at 5 to 6 h. The inflammatory response for Silica was transient, diminishing at 2 days and back to control levels at 10 days. Within 2 h, chemotactic activity for neutrophils was detected directly in BAL fluids with the influx and appearance of neutrophils into

alveolar regions of the lungs. The mRNA expression of 2 known neutrophil chemotactic cytodines in BAL cells, macrophage inflammatory protein-2 (MIP-2) and KC, correlated with chemotactic activity and acute pulmonary inflammatory responses. MIP-2 mRNA was expressed prior to detection of chemotactic activity in BAL fluids and was no longer detectable after 2 days. The authors stated that Silica produced a potent but trantient pulmonary inflammatory response.

Ernst et al. (2002) used female Wistar WU rats (Crl:WI(WU)BR) to test the carcinogenicity of Silica after intratracheal instillation in several experiments. The authors also tested the preventive effects of poly-2-vinylpryridine-N-oxide (PVNO) against Silica carcinogenicty. Starting at 8 weeks of age, the rats were anesthetized and treated by intratracheal instillation of a particle suspension of Silica. In the first experiment, the rats (n = 4) were treated 20 times at 2-week intervals with Silica (0.5 mg). A second set were treated 30 times. Two weeks after the last instillation, the rats were killed and the lungs examined. Rats treated with Silica showed moderate, but transient dyspnea that resolved in 1 to 4 h. In the former group, there was 0.18 ± 0.03 mg (1.8% of a single dose) retained in each lung. There was 0.035 ± 0.012 mg Silica/lung associated lymph node (LALN; 0.35% of a single dose). In the latter group, there was 0.026 ± 0.011 mg (0.17% of a single dose) retained in each lung. There was 0.044 ± 0.007 mg Silica/lung associated lymph node (LALN; 0.29% of a single dose).

The experiment was repeated with various doses and with the addition of PVNO measuring various parameters, comparing the results to the control saline solution. Silica administered twice at 0.3 mg at 7-day intervals increased lung weights compared to controls. At 1 and 3 mg, there were increased leukocytes, PMNs, lymphocytes, and lung weights. The similar results were observed when administered 3 times. When administered 4 times, there was also an increase in leukocytes and PMNs at 0.3 mg/dose. Rats administered 0.2 mg once had increased leukocytes and PMNs; at 2 mg, there was increased leukocytes, PMNs, and lung weight. PVNO administered with 2 mg of Silica reduced the number of leukocytes, PMNx, lymphocytes, and lung weight compared to Silica alone.

Using the data from the above experiments, the authors designed experiments lasting 3 and 9 months. The rats treated with Silica had lower body weights of ~5% after 9 months.

The particle-laden alveolar macrophages in the lungs of the Silica-treated groups appeared to be generally intact. In the 4-week experiment there was multifocal moderate to severe granulomatous alveolitis characterized by abundant macrophages, fewer fibroblasts, and T-lymphocytes and only a few granulocytes. Over time after instillation of Silica, the majority of these inflammatory foci had progressed to “scar-like” interstitial fibrotic

granulomas. This process was markedly augmented by additional treatment with PVNO. The authors state that fribrotic lesions are considered to represent chronic stages of alveolitis induced by Silica.

Cells lavaged from the lungs of the rats treated for 9 months had increased reactive nitrogen intermediates, reactive oxygen intermediates and TNF-α than controls when exposed to lipopolysaccharides or Zymonsan. The authors concluded that amorphous Silica is more toxic than quartz (also tested in this study) but recruitment of leukocytes and PMN concentration in the lavage seems to be lower and may decrease faster than for quartz. This may be due to amorphous Silica's rapid elimination from the lungs. Silica induced inflammation that persisted as long as there were repeated instillations.

Lesions in the lungs were characterized by a lack of alveolar lipoproteinosis and relatively low numbers of intra-alveolar macrophages. Most of the macrophages were foamy but not necrotizing. Silica also produced a pronounced but localized interstitial fibrosis (interstitial fibrotic granulomas). The authors suggest that these developed from acute alveolitis observed after a single administration of Silica. The authors also suggest that the lesions resulted from acute epithelial damage at the sites of particle deposition with subsequent (granulomatous) inflammation and production of granulation tissue. The authors concluded that Silica did not affect the normal ability of cells to respond to lipopolysaccharides (Ernst et al. 2002).

Jones et al. (2002) explored the kinetics of lung macorphages by instilling Silica (50 mg; 5 µm) into the right upper lobe of the lungs of New Zealand white rabbits (n = 12). At intervals, the rabbits were re-anesthetized and injected with [$^{11}$C]R-PK11195 and PET scanned. The rabbits were killed at different times and the lungs examined. All of the rabbits remained healthy throughout the study. Three and 6 days following instillation, the [$^{11}$C]R-PK11195 was localized to the challenged lung and observed caudally on the contralateral side. On day 1 post-instillation, there were many macrophages containing particles in airspace. On day 5, some particle-bearing macrophages had migrated into the interstitium. There were few neutrophils present. At week 2, most of the particle-bearing macrophages were found in the interstitium and the perivascular lymph vessels. The macrophages did not appear to be highly activated. The Silica was removed from the lungs in a highly organized manner and no fibrosis developed.

Kim et al. (2002) exposed alveolar macrophages collected from the lungs of Sprague-Dawley rats to Silica (0.5 - 10 µm). The macrophages released reactive oxygen species (ROS); when exposed to 100 µg/ml Silica for 6 h, 2.36 nM superoxide anion and 2.81 nM hydrogen peroxide were released. Ambroxol attenuated the ROS response in a concentration dependent manner. Staurosporine, a protein kinate C (PKC) inhibitor, and genistein,

a tyrosine kinate (PTK) inhibitor, depressed the respiratory burst of the macorphages. However, ambroxol and staurosporine did not have a potentiating effect. The effect was decreased by a calcium chelator (ethylene glycol tetraacetic acid [EGTA]) and a calmodulin inhibitor, triluoperazine. These 2 chemicals also reduced ROS production in macrophages not exposed to Silica, but staurosporine and genistein did not.

Incubation with Silica caused an increase in $NO_x$ production in alveolar macrophages, peaking at 50 µg/ml. The addition of ambroxol decreased $NO_x$ production, with and without Silica exposure. Staurosporine and genistein decreased Silica-induced $NO_x$ production, but not in macrophages not exposed to Silica. Macrophages exposed to Silica released acid phophatase and lysozyme, which was inhibited by ambroxol, staurosporine, genistein, EGTA, and trifluoperazine. Macrophages not exposed to Silica still produced granule enzyme when treated with ambroxol, EGTA, or trifluoperazine.

Macrophages exposed to Silica had increased protein kinases which were attenuated by exposure to staurosporine and genistein. Ambroxol decreased the Silica-induced increases in PKC and PTK in a concentration dependent manner. Macrophages exposed to Silica increased $Ca^{2+}$ production which was attenuated by ambroxol in a concentration dependent manner.

When macrophages were exposed to Silica, cell death occurred in a concentration dependent manner with ~60% viability in cells exposed to 100 to 200 µg/ml which was attenuated by ambroxol. The authors concluded that ambroxol had a depressant effect on the Silica-stimulated responses and cell death, which may be due to the inhibiton of cativation processed, protein kinases, and calcium transport (Kim et al 2002).

Kaewamatawong et al. (2005) compared the effects of particle size of colloidal Silica on female ICR mice. Ultrafine (14 nm) colloidal Silica (120 mg/ml in water) or fine (213 nm) colloidal Silica (239 mg/ml in water) were intratracheally administered to the mice (n = 3; 5 control groups, 10 exposure groups). The mice were killed and necropsies at 30 min and 2, 6, 12, and 24 h.

Both types of Silica produced bronchiolar degeneration and necrosis, neutrophilic infammation in alveoli with alveolar type II cell swelling, and particle-laden alveolar macrophage accumulation. Ultrafine Silica induced more alveolar hemorrhage, compared to fine Silica, from 30 min. There was also more severe broncholar epithelial cell necrosis and neutrophil influx in alveoli in the ultrafine-treated mice than in the fine Silica-treated mice at 12 and 24 h. Immunolabelling by Laminin in basement membranes of bronchioles and alveoli in the ultrafine Silica-treated groups was weaker than the fine Silica-treated groups at all time periods. Electron microscopy revealed both types of Silica on bronchiolar and alveolar wall surfaces as well as in the cytoplasm of

alveolar epithelial cells, alveolar macrophages, and neutrophils. Type I alveolar epithelial cell erosion with basement membrane damage was greater in the ultrafine Silica groups than in the fine Silica groups. Bronchiolar epithelial cells in the ultrafine Silica groups had more intense vacuolation and necrosis than in the fine Silica groups. The authors suggested that ultrafine Silica had greater ability to induce lung inflammation and tissue damages than fine Silica (Kaewamatawong et al. 2005).

Kaewamatawong et al. (2006) instilled ultrafine colloidal Silica (0, 0.3, 3, 10, 30, or 100 µg; 120 mg/ml in water; 14 nm) into the tracheas of male ICR mice (n = 10). After 3 days, the mice were killed and the lungs examined. The total cell counts in broncho alveolar lavage fluid (BALF) were increased for 10, 30 and 100 µg groups. Cell differential analysis of BALF of the 2 highest groups had increases in numbers of neutrophils and lymphocytes. All exposure groups had increased total protein values in BALF.

In a followup experiment, mice (n = 8) were instilled with 50 µl of 30 µg of Silica. The groups were killed at 1, 3, 7, 15, and 30 days and the lungs examined. There was a transient increase to the total numbers of cells, macrophages, neutrophils, and lymphoctyes in BALF. Total numbers of lung cells increased and persisted to day 15 and resolved by day 30. Alveolar macrophages were elevated at day 1 to day 7. Lymphocytes increased until day 7 the returned to control levels by day 30. Total protein in BALF was greater than control at day 1 and returned to control levels by day 15.

Histopathological examination revealed no lesions at all time points. On day 1 there were moderate increases of neutrophils sharply demarcated from normal alveoli. There were nodular aggregates of neutrophils and particle-laden alveolar macrophages in some alveolar regions adjacent to the bronchioles. Nodular lesions consisted of neutrophils, active alveolar macrophages, particle-laden alveolar macrophages, and cell debris. At day 3, moderate focal alveolitis was observed at the terminal abronchiolar and alveolar duct regions. Alveolar septal wals were thickened. At day 7, changes were only in the appearance of the aggregated foci consisting of particle-laden alvoelar macrophages, lymphocytes, and fibroblasts with occasional collagen fibers. Lesions were located around blood vessels adjacent to termainal bronchioles and alveolar ducts. At day 15, inflammatory signs were reduced and almost or completely recovered. TUNEL analyses showed an increase of the apoptotic index in lung parenchyma at all time points. 8-OhdG expression occured in lung epithelial cells and activated macrophages which corrolated with lung lesions. The authors suggested that small doses of ultrafine colloidal Silica caused transient, acute moderate lung inflammation and tissue damage. Oxidative stress and apoptosis may underlie the tissue injury induction (Kaewamatawong et al. 2006).

Sayes et al. (2007) instilled precipitated Silica (1 or 5 mg/kg; 1000 to 3000 nm), as well as other particles, intratracheally to male Crl:CD (SK)IGS BR rats (n = 20). Controls were administered phosphate-buffered saline (PBS). At 24 h, 1 week, and 1 and 3 months, the BAL fluid of 5 rats/group was analyzed. The number of cells at 24 h was higher than at other time points. Exposure to both doses produced transient and reversible neutrophilic lung inflammation responses at 24 h which was diminished at 1 week.

In an in vitro experiment, immortalized rat L2 lung epithelial cells, rat lung alveolar macrophages or both of these cells combined were incubated with Silica. In L2 cells, Silica produced increases in LDH levels at 520 $\mu g/cm^2$ at 4 h. At 24 and 48 h, LDH levels increased over controls at 5.2, 52, and 520 $\mu g/cm^2$ in L2 cells. In alveolar macrophages, Silica produced no increase in LDH levels up to 48 h and 5200 $\mu g/cm^2$. The 2 cell types were cultured together; Silica produced no increase in LDH except for 520 $\mu g/cm^2$ at 24 and 48 h.

In 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) studies, Silica produced decreases in cellular MTT levels at doses of 5.3 and 52 $\mu g/cm^2$ at 4 and 24 h in alveolar macrophages and no effect in L2 cells. In the combined cultures, there were no changes in MTT values due to Silica at 4 h but a decrease in MTT levels at 5.2 and 52 $\mu g/cm^2$. MIP-2 production did not increase due to L2 cells exposed to Silica but did in alveolar macrophages after 24 h. There was no increase in tumor necrosis factor (TNF)-α for either cell type when exposed to Silica but levels were increased at 0.52 and 5.2 $\mu g/cm^2$ after 24 h. Interleukin (IL)-6 levels were not increased for either type of cell at 24 h, however, when the cells were combined, IL-6 levels were increased at 0.52, 5.2, 52, and 520 $\mu g/cm^2$. The authors concluded that there was little correlation between in vivo and in vitro results (Sayes et al. 2007).

## CELLULAR EFFECTS

### SILICA

FDA (no date) reported the results of a cytotoxicity test using Chinese hamster V79 cells. There were no effects after 144 h of exposure to Silica.

Davies (1981) incubated fumed or precipitated Silica or Silica gel (13.5, 25 or 50 $\mu g/cm^3$) to macrophages from female Theillers original mice for 18 h. Fumed Silica and Silica gel were cytotoxic to macrophages similarly to crystalline Silica; precipitated Silica was less cytotoxic. The author concluded that all 3 Silica types were cytotoxic at 13.5 $\mu g/cm^3$,

Pandurangi et al. (1990) incubated sheep blood erythrocytes (1%) with fumed Silica (0.02 to 1.0 mg/ml) for 30 min. There was ~85% lysis for all concentrations tested. The authors concluded that the hemolytic activity

may be connected to surface free OH group concentration.

Liu et al. (1996) exposed Chinese hamster lung fibroblasts (V79 cells) to Silica (0, 20, 40, 80, or 160 µg/ml) for 24 h. Silica was cytotoxic at 80 µg/cm$^2$ and no effects were observed at 40 µg/ml.

Mollo et al. (1997) incubated rat pleural mesothelial cells in fluorescein isothiocyanate-labeled Silica (17, 33, and 66 µg/ml) for 6 and 24 h. The Silica was present in the cytoplasm and concentrated around the nucleus, suggesting particle uptake, at both observation times. There was evidence of Silica particles present in internalization vacuoles. The authors suggested that exposure to Silica elicited an immediate defense response from cells through release of oxidizing and/or radical annealing agents.

Cha et al. (1999) incubated 2 renal cell lines ($S_1$ and IMCT) from transgenic mice harboring the SV40 large T antigen gene with various concentrations of Silica (particle size 0.5 to 10 µm), a calcium channel blocker, or saline (control) for 1 h. Calcium uptake was measured using florescent dye. There was a rapid increase in calcium ($[Ca^{2+}]_i$) with a 5 to 20 sec peak responding time and maintained high level of sustained phase > 10 min in both cell lines. The mean resting $[Ca^{2+}]_i$ levels were 95 ± 8 and 98 ± 11nm for $S_1$ and IMCT cell lines, respectively. In $S_1$ cells, the $[Ca^{2+}]_i$ increase was observed at relatively high concentrations (>60 µg/ml Silica); $[Ca^{2+}]_i$ increase was observed at concentrations as low as 6 µg/ml Silica. At 600 µg/ml Silica, the increase in $[Ca^{2+}]_i$ were 559 ± 39% and 731 ± 34% over resting for $S_1$ and IMCT cells, respectively.

The experiment was repeated but with pretreatment with a calcium chelator and a phospholipase C inhibitor and with the delayed addition of calcium chloride. There was no increase in $[Ca^{2+}]$ before the addition of calcium chloride. Addition of calcium chloride resulted in the return to normal levels of $[Ca^{2+}]$ concentrations. Addition of the chelator alone had no effect on $[Ca^{2+}]$ concentration. There was no inhibitory effect of the phospholipase C inhibitor to Silica induced $[Ca^{2+}]_i$ concentration in both cell lines. The authors concluded that the source of calcium was across plasma membrane and not from internal stores and that $[Ca^{2+}]_i$ increase is an early response leading to cell damage.

The effect of Silica (0.6 to 600 µg/ml) on cell injury was also examined using trypan blue exclusion. Silica-induced injury after 1 h increased in a concentration-dependent manner in both cell lines. Cell injury increased at 60 µg/ml for $S_1$ cells and at 6 µg/ml for IMCT cells. At 600 µg/ml, cell injury was 28.0 ± 1.5% and 47.0 ± 2.2%, respectively. Cell injury was reduced with the addition of the chelator as well as a calcium channel blocker. Cells incubated for 1 h in Silica had reduced $[ATP]_i$ in a concentration-dependent manner in both cell lines but increased $[Ca^{2+}]$. The authors suggested that alteration of intracellular calcium homeostasis by Silica is closely related with

renal cell injury (Cha et al. 1999).

O'Reilly et al. (2005) exposed normal human primary fibroblasts to Silica (10 to 100 µg/ml) for 24 h to explore the sources of pulmonary inflammation from Silica inhalation. Silica exposure induced COX-2 in a dose dependent manner starting at 10 µg/ml (10-fold more than crystalline silica). COX-1 expression was not affected by Silica. Silica was nontoxic to the fibroblasts up to 100 µg/ml. COX-1 mRNA was unchanged by Silica exposure. When exposure time was increased to 6 h, expression was induced at all concentrations. $PGE_2$ was increased in a dose and time dependent manner, ~7 times more potent than crystalline Silica. There was no increase in IL-1β expression. Silica exposure increased mPGES expression and was persistent up to 72 h. Silica exposure stimulated production of $PGF_{2\alpha}$ in a dose dependent manner and was 10-fold lower than $PGE_2$. Silica did not induce IL-6, MCP-1, or TGF-β production but did strongly induce IL-8 production. The authors suggest that increased production of $PGE_2$ prevents the lung's transient inflammatory response from developing into fibrosis.

Brunner et al. (2006) tested the cytotoxicity of Silica. Human mesothelioma MSTO-211H and rodent 3T3 fibroblast cells were cultured with Silica (0 to 15 ppm and 0 to 30 ppm) for 6 days and 3 days, respectively. By measuring both the MTT-conversion and DNA content, there were no effects to the cells by Silica.

SODIUM POTASSIUM ALUMINUM SILICATE

Alfaro Moreno et al. (1997) incubated thawed Balb 3T3 cells with Mexicali dust (Potassium Aluminum Silicates [98%] and sodium dioxide [2%]; 20, 40, or 80 µg/ml), chrysotile asbestos (40 µg/ml; positive control), or nothing (negative control) for 12 h. The medium was then changed and the cell cycle allowed to incubate for 7 h. The cells were fixed. Between 220 and 280 anaphases for each concentrations was examined blind. Abnormal anaphases were observed in 27.42% of the cases in the low dose group, 29.60% in the mid dose group, and 37.10% in the high dose group. The asbestos induced abnormal anaphases in 34.78% of the cases and 11.62% in the control. The most frequent alterations were multipolar anaphases. An increase in anaphases with retarded chromosomes was observed in the test groups and the positive control. The frequency of anaphase bridges was lower in the treated groups than in the positive control group ($p < .05$). When comparing the mid dose group to the positive control group, there were more lagging chromosomes (23.95% vs 18.20%), fewer anaphase bridges (10.40% vs. 78.80%), and more anaphase bridges (10.40% vs. 1.51%). No changes were observed in the mitotic index of cell exposed to Mexicali dust. The authors concluded that Mexicali dust is capable of inducing anaphasic alterations.

## ANTI-AFLATOXIN EFFECTS

### HYDRATED ALUMINUM CALCIUM SODIUM SILICATE

There are many studies on the use of hydrated Aluminum Calcium Sodium Silicate (HACSS) for countering the effects of aflatoxin (AF) in animal feed. Mixed into feed at 0.5%, it reduces the effects of several strains of AF in multiple species of animals. None of the studies reported any clinical effects nor remarkable findings at necropsy. These studies are summarized in Table 7.

**Table 7.** Summaries of studies of the use of HACSS to counteract the effects of AF.

| Species (n) | AF strain | Protocol | Results | Reference |
|---|---|---|---|---|
| crossbred pigs (5) | Not specified | HACSS (0.5%) incorporated into feed for 5 weeks. | Weight gains and feed consumption similar to controls; no pathological differences at necropsy between the HACSS treated group and the control group. | Colvin et al. 1989 |
| Nicholas Large White turkey poults (6) | AF from *Aspergillus parasiticus* | HACSS (0.5%) incorporated into feed; AF at 0.5 or 1.0 mg/kg feed for 3 weeks. | HACSS reduced mortality in low dose AF group, not high dose group. No effect on parameters measured at necropsy (i.e., relative organ weights, hematology, serum biochemistry, enzyme activities). | Kubena et al. 1991 |
| Male Sprague-Dawley rats (6) | *Acremonium coenophialum* on tall fescue. AF not detected on feed. | HACSS (2%) on infested or not infested fescue for 28 days. | HACSS did not affect feed intake but reduced weight gain. HACSS did not affect testes, kidney, or liver weights. Infested fescue caused reduced testes weight that was not mitigated by HACSS. | Chestnut et al. 1992 |
| Crossbred wethers (4) | *Acremonium coenophialum* on tall fescue. AF not detected on feed. | HACSS (2%) on infested or not infested fescue for 17 days. | HACSS had no effect on elevated temperatures caused by infested fescue and did not affect temperature alone. Serum prolactin concentrations were greater with HACSS. HACSS mitigated increased sorbitol dehydrogenase activity. There were no effects to the ruminal fluid by HACSS. HACSS did not effect OM, N, Ca, P, Na, K, or Cu absorption but reduced Mg, Mn, and Zn absorption. | Chestnut et al. 1992 |
| Yorkshire x American Landrace x Hampshire Barrows (8) | Not specified. | HACSS (5 g/kg) incorporated into feed for 3 weeks; AF at 0.5 or 3 mg/kg feed for 28 days. Two “types” of HACSS (-1 and -3) tested, difference not defined. | Body weight gain lower for all AF groups, less for each group with HACSS. No decreased feed consumption in HACSS groups. Feed consumption was reduced for AF group only. Feed efficiency not affected by any treatment. Alkaline phophatase (ALP) and gamma glutamyltransferse (GGT) were increased in AF group, ALP was elevated in the AF + HACSS-3 group, and triglycerides were increased in both AF + HACSS groups. Urea nitrogen decreased AF and both AF plus HACSS groups. Both HACSSs prevented AF-induced decreases in cholesterol, albumin, and GGT; only HACSS-1 reduced increase in ALP. Lymphoblastogenic responses to phytohemagglutinin decreased in AF and both AF + HACSS groups compared to controls. The stimulation index was greater in AF + HACSS-1 group than AF alone. AF group livers were rubbery and tan. The livers were darker and less resistant to cutting AF | Harvey et al. 1994 |

**Table 7.** Summaries of studies of the use of HACSS to counteract the effects of AF *(continued)*.

| Species (n) | AF strain | Protocol | Results | Reference |
|---|---|---|---|---|
| | | | + HACSS groups. Liver weights in all 3 of these groups greater than controls with diffuse hepatocellular lipidosis. The severity of the lesions was greater in the AF alone group than the AF + HACSS. No clinical signs associated to either HACSS groups. | |
| Lactating Alpine goats (8) | AF from *A. parasiticus* NRRL 2999 | HACSS (0, 1%, 2%, or 4%) incorporated into feed; AF (200 µg/kg feed). Milk samples collected on days 2, 4, 6, 8. | Natural levels of AF $M_1$ in control (0.005 - 0.037 ppb). No AF detected with HACSS diets. $M_1$ with AF diet 1.477 - 1.046 ppb reduced to 0.163 - 0.189 ppb with HACSS. No clinical signs from exposure to HACSS. | Smith et al. 1994 |
| Lactating Alpine goats (3) | AF from *A. parasiticus* NRRL 2999 | HACSS (0, 1%, or 2%) incorporated into feed; AF (100 µg/kg feed). Milk samples collected on days 2, 4, 6, 10, 12. | Natural levels of AF $M_1$ in control (0.017 - 0.022 ppb). No AF detected with HACSS diets. $M_1$ with AF diet 1.046 - 1.477 ppb reduced to 0.094 - 0.111 ppb with HACSS (2%). No clinical signs from exposure to HACSS. | Smith et al. 1994 |
| Male broiler chicks (7) | AF from *A. parasiticus* | HACSS (0.5%) incorporated into feed; AF (3.5 mg/kg feed) with/without Virginiamycin (16.5 mg/kg feed) for 28 days. | No differences in body weights of chicks fed AF or AF + VM. diets. No differences in body weights of chicks fed diets without AF and those fed AF + HACSS or all 3 treatments. The authors stated that this indicates ~ 75% protection against decreased body weight gain by HACSS and 87% protection by HACSS and VM combined. Relative liver weights, and kidney and creatine kinase activity were increased and albumin, total protein, cholesterol, uric acid, and inorganic phosphorus concentrations were decreased in chicks in the AF group, which was kept at control levels in the HACSS with the exception of albumin levels. The authors concluded that HACSS, with and without VM, can counteract some of the toxic effects of AF in growing chicks. | Abo-Norag et al. 1995 |
| Male Fischer 244 rats (?) | AF $B_1$ | HACSS (0.5%) incorporated in feed for 1 week prior to dosing with AF (0.125, 0.25, 0.5, or 1 mg/kg). Urine was collected up to 48 h. | Metabolites $Q_1$, $M_1$, and $P_1$ were present in urine of AF dosed rats, reduced in HACSS-treated rats. No adverse effects of HACSS were reported. | Sarr et al. 1995 |
| Female Nicholas large white turkey poults (2 - 4) | $AFB_1$ | HACSS (0.5%) administered in capsule after colostomy. $AFB_1$ (0.75 mg/kg). Urine collected for 48 h. | AF metabolite was $M_1$. $M_1$ in urine reduced by 29% to 80% (average 52%) over the collection period compared to $AFB_1$ alone. | Edrington et al. 1996 |
| Sprague-Dawley rats (10) | AF from *A. parasiticus* NRRL 2999 | HACSS (0.5%) incorporated into feed with and without AF(2.5 mg/kg feed) for 15 days. | AF induced decreased feed intake, altered serum biochemical parameters of liver and kidney functions. HACSS did not alter parameters measured and caused no adverse effects. HACSS diminished the effects of AF. | Abdel-Wahhab et al. 1998 |
| Day old chicks (6) | AF rom *A. parasiticus* NRRL 2999 and T-2 toxin | HACSS (0.250% or 0.375%) incorporated into feed; AF (5.0 mg/kg feed); T-2 toxin (8.0 mg/kg feed) for 3 weeks. | Body weights, feed consumption, feed utilization, mortality, and lesion scores not affected by HACSS alone. HACSS at both doses reduced the decrease in body weight gain in AF but not for T-2 toxin groups. HACSS, at both doses, did not affect liver, kidney, heart, spleen, pancreas, or proventriculus weights. Addition of HACSS | Kubena et al. 1998 |

**Table 7.** Summaries of studies of the use of HACSS to counteract the effects of AF *(continued)*.

| Species (n) | AF strain | Protocol | Results | Reference |
|---|---|---|---|---|
| | | | with AF alleviated or diminished decreased organ weights; T-2 toxin did not alter organ weights. HACSS, at both doses, did not affect serum total protein, albumin, urea nitrogen, cholesterol, calcium or creatine kinase levels activity. The effects of AF on serum albumin and cholesterol and creatine kinase activity were decreased by both doses of HACSS. | |
| Day-old chicks (6) | From *A. parasiticus* NRRL 2999 and T-2 toxin | HACSS (0.250% or 0.375%) incorporated into feed; T-2 toxin (8.0 mg/kg feed) for 3 weeks. | HACSS group had similar body weight gains and feed consumption as controls. HACSS did not reduce the effects of T-2 toxin on the decreased weight gains and feed consumption. Authors concluded that HACSS is a viable treatment for AF but not T-2 toxin for chicks. | Kubena et al. 1998 |
| Day-old chicks (6) | From *A. parasiticus* NRRL 2999 and T-2 toxin | HACSS (0.80%) incorporated into feed; T-2 toxin (8.0 mg/kg feed) for 3 weeks. | HACSS group had similar body weight gains and feed consumption as controls. HACSS did not reduce the effects of T-2 toxin on the decreased weight gains and feed consumption. Authors concluded that HACSS is a viable treatment for AF but not T-2 toxin for chicks | Kubena et al. 1998 |
| Chicks (6) | $AFB_1$ | HACSS (1%) incorporated into feed; $AFB_1$ (4 mg/kg feed) for 21 days. | AF $B_1$ group had reduced feed intake and body weight gain; the 2 treatment groups were similar to control. The chicks fed $AFB_1$ had increased liver, heart, kidney, proventriculus, and pancreas weights; the other 2 treatment groups were similar to controls. Chicks fed HACSS had similar blood chemistry as the control group; chicks fed $AFB_1$ had decreased serum concentrations of calcium, phosphorus, cholesterol, glucose, albumen, total protein, and globulin. Chicks fed HACSS and $AFB_1$ had similar blood serum chemistry as the controls except for glucose and cholesterol, which were both increased. There were no nutritional deficiency-related signs. Necropsy of the chicks on the HACSS diet, with and without $AFB_1$ was unremarkable. Authors concluded that HACSS did not adversely affect the chicks and was effective against $AFB_1$. | Ledoux et al. 1998 |
| Sprague-Dawley Rats (10-11) | AF $B_1$ | HACSS (0.5%) incorporated into the feed of pregnant rats days 0 - 20 of pregnancy (also administered by oral gavage) with or without AF (2 mg/kg) on days 6 - 13. | No mortalities of dams associated with HACSS. No difference between controls, and HACSS treated groups. HACSS had no effect on feed intake. HACSS had no effect on number of implants, resorptions/dead fetuses, or live fetuses with or without AF. HACSS had no effect on fetal body weight and mitigated the effect of AF on body weight and abnormalities. Maternal kidneys and livers had lesions that were mitigated by HACSS. HACSS did not affect these organs. | Mayura et al. 1998 |
| Male Sprague-Dawley Rats (10 or 11) | AF $B_1$ | HACSS (0.5%) incorporated into feed for 1 week then orally | The metabolite $AFQ_1$ was present in all AF-treated groups, the least amount in the group treated with HACSS. No adverse | Mayura et al. 1998 |

**Table 7.** Summaries of studies of the use of HACSS to counteract the effects of AF *(continued)*.

| Species (n) | AF strain | Protocol | Results | Reference |
|---|---|---|---|---|
| | | administered AF (2 mg/kg). | effects were reported. | |
| Labrador and mixed-breed dogs (n =6) | AF $B_1$ | HACSS (0.5%) incorporated into the feed for 7 10 days in cross-over study then administered aflatoxin $B_1$. | Urinary aflatoxin $M_1$ was reduced when the dogs were treated with HACSS. No adverse effects were reported. | Bingham et al. 2004 |
| Male Fischer 344 rats (n = 3) | Aflatoxin $B_1$ | HACSS (0.5%) incorporated into feed for 1 week then administered aflatoxin $B_1$ | Urinary AF $M_1$ was reduced in HACSS-treated rats over 48 h. No adverse effects reported | Bingham et al. 2004 |
| Day-old quail (*Coturnix coturnix japonica*) chicks (45) | From AF (*from A. parasiticus*) | HACSS (0.5%) incorporated into feed; AF (2.4 mg/kg feed) for 3 weeks. | Body weight increased with the addition of HACSS to the AF diet compared to the addition of AF. None of the treatments affected the feed conversion ratio. Addition of HACSS partially decreased the fat deposition caused by AF and there were indications of a re-organization in the endopasmic reticulum and increase in the number of ribosomes and polisomes. | Sehu et al. 2007 |

Aly et al. (2004) tested the ability of HACSS to adsorb (0.5%, 1%, 2%, and 4%) to the AFs $AFB_1$ (5, 10 and 50 ppm) and $FB_1$ (5, 10, 50 ppm). The adsorption ratio of hydrated HACSS ranged from 95.3% to 99.1% for $AFB_1$ and 84.7% to 92.4% for $FB_1$.

## MICROBIAL EFFECTS

### SILICA

Various bacteria were incubated in Silica (0.2 g; dilution 1:50,000 for *A. Aerogenes*, *Proteus* sp., *P. Aeruginosa*, *E. coli*, and *S. aureus*, and 1:100,000 for *C. albicans* and *B. subtilis*) at 22°C or 37°C. The time until complete mortality was recorded up to 28 days ($EC_{100}$). The results are shown in Table 8 (UNEP 2004 ).

**Table 8.** $EC_{100}$ of microorganisms exposed to Silica at 2 temperatures (UNEP 2004).

| | $EC_{100}$ | |
|---|---|---|
| Microorganism | 22°C | 37°C |
| *P. putida* | 6 h | 3 d |
| *E. coli* | 2 d | 2 d |
| *A. aerogenes* | 3 d | 2 d |
| *Proteus* sp. | 2 d | 2 d |
| *S. aureus* | 18 d | 22 d |
| *C. albicans* | 10 d | 3 d |
| *B. subtilis* | 18 d | 2 d |

## ANIMAL TOXICOLOGY

### ACUTE TOXICITY

#### ORAL

SILICA

FDA (no date) reported an acute oral toxicity study of Silica using rats with an $LD_{50}$ of 3160 mg/kg.

Hazelton Laboratories (1958a) administered a single oral dose of fumed Silica (1.00, 2.15, or 3.16 g/kg) to male albino rats (n = 5). There were no gross signs of systemic toxicity observed and no mortalities. The $LD_{50}$ was >3.16 g/kg.

W.R. Grace & Co. (1981) reported that the $LD_{50}$ of fumed Silica was > 5.62 g/kg for male rats (n = 30). There were no toxic sign or deaths over the 2 weeks observation.

In a second study using male albino rats, the $LD_{50}$ of Silica in water was > 3.16 g/kg (W.R. Grace & Co. 1981).

Lewinson et al (1994) orally administered Silica (fumed; 5040, 6350, or 7900 mg/kg in olive oil or 2500 or 5000 mg/kg in peanut oil) to Sprague-Dawley rats (n = 10) after fasting. The rats were monitored for 4 weeks, then killed and necropsied. There was no mortality. There were no toxicological signs and the necropsies were unremarkable. The authors concluded that Silica is virtually acutely nontoxic by the oral route.

UNEP (2004) reported several unpublished studies of acute oral toxicity of Silica. The $LD_{50}$s of these studies are summarized in Table 9.

ALUMINUM CALCIUM SODIUM SILICATE

Abbés et al. (2006a) orally administered a single dose of HACSS (400, 600, or 800 mg/kg) to female Balb/c mice (n = 6) with or without Zearalenone (ZEN; a mycotoxin produced by fusarium genera; 40 mg/kg). After 48 h, blood samples were collected and the mice killed and examined. ZEN caused reduced total cholesterol, high denisty lipoprotien (HDL), low density lipoprotein (LDL), triglycerides, total protein, albumin, white blood cell count, immunoglobulin profile (Ig A and Ig G) and T-cell subtypes. ZEN increased uric acid and urea and induced degenerative changes in the spleen tissues. The low dose of HACSS alone had levels similar to control. The mid and high dose groups had increased cholesterol levels. HACSS mitigated the effects of ZEN at all dose levels. No adverse effects were reported for HACSS alone at any dose.

**Table 9.** Unpublished acute oral toxicity studies of Silica reported by UNEP (2004).

| Species (n) | Test substance | Notes | $LD_{50}$ |
|---|---|---|---|
| Sprague-Dawley rats (n = 20; 10 males, 10 females) | Fumed Silica in aqueous solution | There were no clinical signs or pathological observations at necropsy | > 3300 mg/kg |
| Wistar rats, male and female (n = 10) | Precipitated Silica | None | > 5110 mg/kg |
| Sprague-Dawley rats (n = 20; 10 male, 10 female) | Precipitated Silica | None | > 5000 mg/kg |
| Male rats (n and strain unspecified) | Precipitated Silica (10 to 5000 mg/kg) | Followed by a 10-day observation period. >100 mg/kg, distended stomachs with bloody patches at the pyloric end were observed at necropsy. At the highest dose, a vascular stomach and reddened intestinal lining were observed. The editors concluded that the test was questionable due to the non-lethality of Silica in other studies at similar doses. | 470 mg/kg |
| Male rats (strain and n unspecified) | Precipitated Silica in saline | No clinical signs in a 10 day observation period | > 5000 mg/kg |
| Rats (strain unspecified; n = 10; 5 male, 5 female) | Precipitated Silica | No clinical signs | > 5000 mg/kg |
| Wistar rats (n = 10) | Silica incorporated into a stock diet at a ratio of 1:4 (w/w)for 24 h | Most rats consumed the diet quantitatively. There were no clinical signs or remarkable findings at necropsy. Stool change color to grey with normal consistency but larger than normal pellets. | > 10,000 mg/kg |
| Male rats (strain and n unspecified) | Precipitated Silica in saline | No clinical signs in a 10 day observation period | > 5000 mg/kg |
| Male Sprague-Dawley rats (n = 30) | Precipitated Silica in water | No clinical signs in a 14-day observation period. The stool turned white for 2 days. | > 5620 mg/kg |
| Sprague-Dawley rats (n = 10; 5 male, 5 female) | Precipitated Silica in water | No clinical signs in a 14 day observation period. The stool turned white for 2 days. | > 20,000 mg/kg |
| Rats (n and strain not specified) | Aqueous colloidal Silica (30%) | None | 10,000 mg/kg |
| Rats (n and strain not specified) | Silica | None | 40,000 mg/kg |
| Male Swiss mice (n = 10) | Fumed Silica in corn oil | No clinical signs in the 14-day observation period. There were no remarkable findings at necropsy. | > 3160 mg/kg |

Abbés et al. (2006b) orally administered a single dose of HACSS (40 mg/kg or 500 mg/kg) to female Balb/c mice (n = 6) with or without ZEN (40 or 500 mg/kg). The high dose of ZEN is the reported $LD_{50}$. After 48 h, blood samples were collected and the mice were killed and the kidneys and livers dissected. ZEN increased hematocrit, hemaglobin, white blood cells, lymphocytes, eosinophils, neutrophils, monocytes, and most of the biochemical serum parameters. ZEN reduced platelets and induced degenerative changes in the hepatic and renal tissues. All of these parameters were similar to controls with the addition of HACSS at both dose levels. HACSS alone had no effect on these parameters.

DERMAL

SILICA

UNEP (2004) reported an unpublished acute dermal toxicity test of precipitated Silica using rabbits (n and strain not provided). Silica was applied to intact and abraded skin for 48 h with no effect. The no observed effect level (NOEL) was > 2000 mg/kg.

In another unpublished study, a single appreciation of 4 different precipitated Silica products (in an aqueous paste) was applied to the intact and abraded skin of New Zealand white rabbits (n = 16) under an occlusive patch (length of time not specified). The rabbits were observed for 14 days. There was very slight erythema that disappeared after 2, 4 or 5 days for 3 Silica products (UNEP 2004).

INTRAPERITONEAL

SILICA

Policard and Collet (1954) intraperitoneally (i.p.) injected Silica (30, 50, or 100 mg/kg in saline) to Wistar rats and rabbits. At 100 mg/kg, 20% to 30% of the animals died quickly. At 50 mg/kg, all the animals survived.

At necropsy, in the peritoneal cavity, vacuoles were observed in the cytoplasm and the nuclei were fragmented or destroyed. There were areas of damaged cells with normal or slightly altered histocytes at the periphery. Edematous was observed that diminished with time. The lymph nodes were enlarged and contained large histocytes in various stages of degeneration. Lymphocytes were less numerous and young. The medullary sinuses were packed with clear cells. The thymus was atrophied and inverted. The cortex was clearer than the medulla. The spleen was hypertropied and altered. The malpighian corpuscles were almost gone; the zones of blood sinusoids were disorganized. The liver was enlarged with many fat cells and clusters of cells, mainly histocytes. The adrenals were enlarged (50% to 97%). The lipids in the cortex had a change in distribution; there was a general increase of lipid cells throughout the cortex.

Surviving animals were killed after 8, 20, 30, and 60 days. Peritoneal edema diminished then disappeared over time. Small spherical nodules (1.5 to 2 mm) were observed on the omentum. Mesenteric and tracheobroncheal lymph nodes were 2- to 4-fold larger. Microscopic examination revealed granuloma in the peritoneal lesions. The center was degenerating and there were dead cells and histocytes. The outer edge was made up of histocytes. Connective tissue showed a fibrous reaction. At 20 and 30 days, the center of the granuloma had a few cells and irregular thickened collagen fibers; the periphery was packed with reticular fibers filling the intercellular spaces and surrounding the cells. The fibrosis gradually increased; after 30 and 60 days

there were extensive fibrous areas that were almost acellular; the lymph nodes were similar (Poicard and Collet 1954).

Schepers et al. (1957d) injected Silica (10% in saline; 2 ml) into the peritoneal cavity of 2 guinea pigs. Both animals died on day 2 of generalized acute peritoneal inflammatory reaction. The lungs were slightly congested and the spleen was swollen. There was a small amount of fluid and adhesions of the intestines and fibrin deposits on the liver. The remains of the Silica were near these reactions.

Kang et al. (1992) intraperitoneally injected female Wistar rats (n = 5) with a single dose of fumed Silica (0.02, 0.1, and 0.5 g). After 5 days, the rats were killed and necropsied. The control group was injected with saline. No adhesions, ascites, or other intra-abdominal pathology was observed in the control group. The rats in the low, mid, and high dose groups treated with Silica had 5 mild, 4 severe, and 4 severe adhesions, respectively. There were none, 4, and 5 occurrences of ascites and deposits of powder adherent to viscera and 5, 4, and 5 rats with ascites in the low, mid, and high dose groups, respectively. The authors ranked the powders tested for relative pathological potentials as: Silica > mica > talc > lycopodium > calcium carbonate > magnesium carbonate > Biosorb starch.

UNEP (2004) reported an unpublished study that concluded that single i.p. injections of $\geq$50 mg Silica caused death in rats.

INTRAVENOUS

SILICA

Swensson et al. (1956) injected amorphous Silica, in the form of commercial Silica or ground fused crystalline Silica, (0.05 at a time up to 0.1 ml in saline or all at once) into the tail vein of mice at 1 min intervals until the animals died or were in an unrecoverable condition. The mice survived larger quantities of Silica if delivered in smaller doses. The toxicity reduced with increasing particle size. Toxicity of amorphous Silica was lower than crystalline Silica. The lethal dose of commercial Silica ranged from 0.2 ± 0.01 to 0.5 ± 0.02 mg/30 g body weight depending on particle size. The lethal dose of fused Silica ranged from 2.1 ± 0.06 to 4.5 ± 0.39 mg/30 g (Table 10).

Byers and Gage (1961) injected various amounts of 3 types of Silica into rats (breed and n not provided). Type A1 had a particle size of 19 µm; type A2 had a particle size of 20 µm but after storage became 60 µm; and type B had a particle size of 25 µm. Types A1 and A2 were from the same manufacturer. Most deaths occurred within 2 h. Rats that survived for 24 h recovered fully. The $LD_{50}$ for types A1, A2, and B were 35.2 (confidence

**Table 10.** Toxicity of various particle sizes of Silica (commercial and ground fused crystalline) to mice (i.v.) (Swensson et al. 1965).

| Sample type | Average size (µm) | Concentration (mg/ml) | n | Mean lethal dose (mg/30 g) |
|---|---|---|---|---|
| Commercial amorphous Silica | 0.01 | 0.5 | 19 | 0.2 ± 0.01 |
| | 0.1 | 2.0 | 18 | 0.5 ± 0.02 |
| | 0.015 | 1.0 | 11 | 0.2 ± 0.01 |
| Ground fused crystalline Silica | 0.15 | 5 | 10 | 2.1 ± 0.06 |
| | 0.30 | 10 | 10 | 3.1 ± 0.22 |
| | 0.45 | 10 | 8 | 4.5 ± 0.39 |

interval [CI] 23 to 39, 41.2 (CI 34.5 to 42), and 44.4 (CI 40.5 to 49) mg/kg.

UNEP (2004) reported an unpublished acute i.v. toxicity study of fumed Silica using rats. The $LD_{50}$ was 15 mg/kg.

INTRATRACHEAL

SILICA

Klosterkötter (1952) reported that the minimum lethal dose of intratracheal administration of Silica was 1.8 $mg/cm^3$ in rats. Effects included serious reactions of the end-arteries with diapedesis of the plasma, erythrocytes, and leukocytes. Desquamative catarrh and parenchymal damages of the liver and kidneys were observed.

Policard and Collet (1954) intratracheally injected Silica (30, 50, or 100 mg/kg in saline) to Wistar rats and rabbits (n not provided). In rats at 30 and 50 mg/kg, the injections were fatal immediately or within a few hours for 80% to 90% of the rats and 100% at 100 mg/kg. The injections were nearly always fatal for rabbits. Death resulted from acute pulmonary edema, often with alveolar hemorrhage and dilation of the pulmonary blood vessels. The surviving rats were necropsied. There was formation of confluent nodules of histocytes, with subsequent fibrosis due to fibers then collagen. The histology of the pulmonary and peritoneal lesions was similar.

INHALATION

SILICA

Lewinson et al. (1994) placed Wistar rats (n = 10; 5 males, 5 females) into an exposure chamber circulating Silica (fumed; mean exposure 477 $mg/m^3$; lowest reading 342 $mg/m^3$) for 4 h. Approximately 56% of the particles were < 5 µm. The rats were observed for 2 h after exposure then daily for 14 d and periodically weighed. The rats were then killed and necropsied. The rats were restless and had drooping eyelids during exposure. There was no

mortality during exposure or during the observation period. Body weights decreased during the first 2 days after exposure then gained normally. Necropsies were unremarkable.

UNEP (2004) reported an unpublished acute inhalation study (nose only exposure) of fumed Silica (< 5 µm) using Wistar rats (n = 10; 5 male, 5 female). The medium lethal concentration ($LC_{50}$) was > 0.139 mg/l for 4 h. There were no clinical signs. After a 14-day observation period there were no remarkable findings at necropsy.

An unpublished inhalation study (nose only exposure) of precipitated Silica (< 5 µm) used Wistar rats (n = 10; 5 male, 5 female). The $LC_{50}$ was > 2.691 mg/l for 4 h. Clinical signs were restlessness and eye closing. There was no body weight gain in females the first 3 days after exposure and then was normal. There were no remarkable findings at necropsy after a 14-day observation period.

An unpublished inhalation study of precipitated Silica (< 5 µm) used Sprague-Dawley rats (n =10; 5). The $LC_{50}$ was > 2.2 mg/l for 1 h. Clinical signs were irritation and dyspnea in most of the rats. One rat died during the 14-day observation period. The editors considered this study invalid due to methodological deficiences and short length of exposure.

An unpublished inhalation study (full body exposure) of fumed Silica used Sprague-Dawley rats (n =10). Approximately 84% of the particles were ≤ 3 µm. The $LC_{50}$ was > 2.08 mg/l for 4 h. Clinical signs were nasal discharge during exposure and crusty eyes and nose, and alopecia during the 14 day observation period. There was no body weight gain in females the first 3 days after exposure and then was normal. One rat had discolored lungs at necropsy (UNEP 2004).

INTRAMUSCULAR

SILICA

W.R. Grace & Co. (1981) reported a study where Silica (200 g) was implanted into the paravertebral musculature of the lumbar region of rabbits (n = 6). The rabbits were killed and necropsied at 6, 12, 24 weeks. There were local inflammatory reactions up to 6 weeks. Then there was granulomatous scarring with necrotic muscle fibers and fatty degeneration of local macrophages.

## SHORT-TERM TOXICITY

ORAL

SILICA

FDA (no date) reported the results of a dog feeding study of 28 days. There were no effects and the highest no effects level (HNEL) for Silica was 800 mg/kg/d.

In another study using rats, the HNEL was 1000 mg/kg/d. For doses >2000 mg/kg/d, the rats had dirty fur, shyness, decrease of motor activity, and hemorrhage of the mucus membrane of the eyes and nose. There was a decrease in body weight, feed consumption, hemorrhaging, and cellular atrophy in the liver epithelium (FDA no date).

Silica (0.2%, 1.0%, or 2.5%) was incorporated into the feed of male rats (n = 10) for 28 days. There were no adverse effect or mortality reported. Gross necropsy findings were unremarkable (W.R. Grace & Co. 1981).

Lewinson et al. (1994) administered fumed Silica (0, 500, 1000, 2000 mg/kg/d) in the diet of Wistar rats (n= 20; 10 males, 10 females) for 8 weeks. Since the high dose was well tolerated, the dose was increased to 4000 mg/kg/d after 14 days, to 8000 mg/kg/d after another 14 days, and finally to 16,000 mg/kg/d. The rats were observed for clinical signs, weighed, and blood sampled before at the end of the experiment. The rats were killed and necropsied.

Only the 16,000 mg/kg/d dose (~25% of daily feed intake) caused any clinical signs, shyness, dirty fur, reduced activity, cachexia, and hemorrage in the mucous membranes of the eyes and nose. Two males and 2 females died with severe cacexia in week 8 (days 9 and 13 of the highest dose). This group had pronounced reduction in body weight and decreased feed intake. No changes were observed in hematological parameters. Microscopic evaluation revealed severe atrophy in the epithelium of the liver in the 2000 and 16,000 mg/kg dose groups; condensation of the cytoplasm, loss of basophilic structure, and hyperchromatic and contracted nuclei occurred in the liver cells. These findings were observed to a lesser extent in 2 females in the 1000 mg/kg dose group. There were no effects to the kidneys. There were no treatment-related effects observed in the 500 mg/kg dose group. The authors concluded that lowest observed effect level (LOEL) was 1000 mg/kg/d and the NOEL was 500 mg/kg/d (Lewinson et al. 1994).

UNEP (2004) reported unpublished short-term oral toxicity studies of Silica. The studies are summarized in Table 11.

DERMAL

SILICA

Hazelton Laboratories (1958b) applied fumed Silica to the intact and abraded skin of albino rabbits (n = 4) daily, 5 d/week, for 15 applications. Urine was collected at the end of day 14. The rabbits were killed and necropsied. Methyl cellulose and #1625 Cosmetic Talc were the positive and negative controls, respectively. There was no systemic toxicity observed. There were no gross or microscopic pathologic findings associated with

**Table 11.** Short-term oral toxicity studies of Silica reported by UNEP (2004).

| Species (n) | Test substance; dose | Notes and results |
|---|---|---|
| Sprague-Dawley rats (5) | Precipitated Silica gel; 16.5 g/kg/d (10% w/w in feed), 5.8 g/kg/d for days 1-10 then 24.2 g/kg/d for days 11-14 (20% in feed) | No observed adverse effects level (NOAEL) ≥24.2 mg/kg. No clinical signs observed. |
| Female inbred rat (not provided) | Precipitated Silica; 1500 mg/kg/d in aqueous solution by gavage daily for 1 month | No clinical signs. Silica content in liver, 1.5 µg; kidney, 6.4 µg, spleen 5.3 µg and 1.8, 7.2, and 7.8 µg in controls, respectively. |

the test product. Silica levels in the blood, urine, spleen, liver, and kidneys were consistent with negative controls.

INHALATION

SILICA

Low et al. (1985) and Hemenway et al. (1986) exposed male Fischer 344 rats (n = 45) to aerosolized precipitated Silica (30 mg/m$^3$) for 6 h/d for 8 days. The recovery period was up to 112 days. During exposure, there was an early and trasient influx of cells in to the lung tissue which returned to normal by day 12. At 5 days post-exposure, the number and differential counts of alveolar lavage-derived cells were similar to controls. The BAL protein, lipid phosphorus, and saturated dipalmitoyl phosphatidyl-choline levels increased immediately after exposure and were normal by day 5 post exposure. There were no differences between controls and treated lungs as to weight, DNA-, protein-, or hydroxyproline-content. The authors concluded that inhaled Silica caused an early, tranisent alveolar inflammatory response, without producing fibrosis. There was only a mild inflammatory response with no evidence of connective tissue response.

Hemenway et al. (1986) exposed Fischer 344 rats (n = 15) were exposed to aerosolized Silica (concentration on clear) for 8 days. Three of the rats were killed and necropsied at days 0, 5, 12, 60, and 120 after exposure. There was initial inflammation, predominantly alveolar, which subsided before day 12.

Warheit et al. (1990, 1991) exposed male CD BR rats (n not provided) to aerosolized colloidal Silica (10.1, 50.5, and 154 mg/m$^3$; diluted 4:1 with deionized, distilled water) for 6 h/d, 5 d/week, for 4 weeks followed by a 10 and 94 day recovery period. The controls were unexposed. Lesions were only observed in lungs and associated

drainage lymph nodes. There was a dose-dependent increase in mean lung weight and lung/body weight ratio after 4 weeks of exposure in the mid and high dose groups. The mean lung/body ratio continued to increase in the high dose group 10 days into recovery but was similar to controls after 3 months. There were dust laden alveolar macrophages, neutrophilic infiltration, and Type II pneumocyte hyperplasia observed in the alveolar duct region of the lungs. Plulmonary lesions progressively decreased in rats examined after the 10 day and 3 month recovery period.

At 3 months post-exposure, most dust laden alveolar macrophages were cleared from the lungs but small numbers of minute silicotic nodule-like lesions were present in the alveolar ducts and perivascular regions where dust laden alveolar macrophages had aggregated. There was minimal collagen deposition observed in the silicotic nodule-like lesions; the lesions did not increase in size or number over time. The lung clearance half-life was ~50 days for the mid and high dose groups. In the high dose group, there was an increase in mean neutrophil count and globulin concentration and a decrease in mean lymphocyte count at the end of the treatment. The increase in mean neutrophil count and decrease in mean lymphocyte count were still present after 3 months of recovery. The tracheal and mediastinal lymph nodes were enlarged with nodular aggregates of dust-laden alveolar machophages and hyperplastic R-E cells. The NOAEL was 10.1 mg/m$^3$ (Warheit et al. 1990,1991).

Reuzel et al. (1991) exposed Wistar rats (n = 80; 40 male, 40 female) to fumed Silica (17, 44, 164 mg/m$^3$) in a whole body exposure chamber for 6 h/d, 5 d/week for a total of 14 days. The control was 6 male and 6 female unexposed rats. There was respiratory distress in all groups. One female in the high dose group died. There was reduced body weight and feed consumption in the males in the mid and high dose groups. Hematological measurements were unremarkable. There was increased lung weights in both sexes (47%, 65%, and 86% for the low, mid, and high dose groups) compared to controls. There absolute and relative liver weights were lower in males, but not females. There were dose-dependent changes in the lungs (i.e., pale, spotted and/or spongy, occasionally irregular surface, alveolar interstitial pneumonia, early granulomata). The mediastinal lymph nodes were enlarged.

The above study was repeated with Silica (46, 180, and 668 mg/m$^3$) on Wistar rats (n = 60; 30 males, 30 females). There was respiratory distress in all groups. One male died in the high dose group. There was reduced body weight and feed consumption in the mid and high dose groups. There was increased lung weights in both sexes compared to controls (males 25%, 39%, and 68%; females 34%, 50%, and 86% in the low, mid, and high dose groups, respectively). There was decreased liver weights in all dose groups of the males and the high dose

group of the females. The lungs were spotted, swollen, and had irregular surfaces in the high dose groups as well as interstitial pneumonia and early granulomata. There was mediastinal lymph nodes in the mid and high dose groups and 1 rat in the low dose group. There was accumulation of alveolar macrophages and particulate material in the lungs of males in the mid and high dose grou euzel et al 1991).

Lee and Kelly (1993) exposed male Crl:CD(SD)BR rats (n = ) to aerosolized colloidal Silica (0, 10, 50, 150 mg/m$^3$) for 6 h/day, 5 days/week for 4 weeks. Some of the rats were killed at the end of the exposure period, at 10 days, or 3 months. There was dose dependent lesions observed at the mid and high dose groups but not in the low dose group. Particles were mostly phagocyized by alveolar macrophages in the alveolar duct region and a few free particles were observed in Type I pneumonocytes in the alveoli. Particle-laden alveolar macrophages directly penetrated into the brochiolar interstitium from alveoli and accumulated in bronchus-associate lymphoid tissue, peribronchial, or perivascular interstitium and accumulated in the tracheo-bronchial lymph nodes. Some particle-laden alveolar macrohages in the bronchus-associated lymphoid tissue transmigrated directly into bronchial lumen through the epithelium. The transmigrated particle-laden alveolar macrophages in the tracheo-bronchial lymph nodes were similar to those in the alveoli. There were described by slender cytoplasmic porcesses, phagosomes, myelin figures, cholesterol clefts, and lipid droplets. Migrated particle-laden alveoli macrophages were observed necrotic and released particles in the tracheo-bronchial lymph nodes.

At 3 months, the lungs of the low dose group were normal. The mid dose group was normal in appearance but a small number of tiny nodular aggregates of dust-laden alveoli macrophages and epithelioid cells were observed. One rat had a few silicotic nodules in perivascular regions adjacent to the bornchioles. The high dose group had a reduced number of particle-laden alveoli macrophages were sharply circumscribed in the alveoli. Some aggregates of particle-laden alveoli macrophages and epitheliod cells were closely apposed with alveolar walls and transformed into nodular aggregates without any collagen fiber deposition. Three of 10 rats had lilicotic nodules in the perivascular region of the bronchioles (Lee and Kelly et al 1993).

Warheit et al (1995) exposed male CD rats (n = 24) to aerosolized precipitated Silica (10 and 100 mg/m$^3$) 6 h/d for 3 days followed by recovery periods of 1, 8, 30, and 90 days. The low dose produced a transient inflammatory response which was present 24 h post-exposure and subsided within 8 days. This response reflected an increase and decrease in the number of granulocytes, extracellular LDH activity, protein levels, and N-acetyl-glucosaminidase (NAG) in the BAL. Recovery in the high dose group was similar to low dose group. The author concluded that low concentration of Silica induces a transient inflammatory tissue reaction.

UNEP (2004) reported an unpublished short-term inhalation toxicity study using female Wistar rats (n not clear) exposed to aerosolized Silica (8 and 40 mg/m$^3$) for 1 h/d, 5 d/week for up to 3 months. The rats were killed and necropsied at 7 d and 3 weeks after treatment. There were no macroscopic changes. Histopathologically, there was an occurrence of dust cells in the lungs which decreased during post-exposure. There was no fibrosis of the reticulo-cellular type and normal parenchma of the lungs. There was no emphysema. A decrease of Silica content in the lungs was observed 7 and 48 days after treatment termination. After 3 months, there was almost no Silica in the lungs.

Arts et al. (2007) exposed young adult Wistar (Crl:WI)WU BR) rats (n = 20; 10 male and 10 female) to 3 types of aerosolized Silica (1, 5, or 25 mg/m$^3$): precipitated Silica, Silica gel, and pyrogenic Silica for 6 h/d for 5 consecutive days followed by a 3-month recovery period. The rats were killed and necropsied. There were no clinical signs during exposure. The effects were limited to 1 day post exposure. Silica levels in the tracheonbronchial lymph nodes were below detectable limit in all 3 groups. Silica was found in the lungs at day 1 but had cleared by 3 months. All 3 types of Silica induced elevated biomarkers of cytotoxicity in BAL fluid, increases in lung and tracheobronchial lymph node weights, and histopathological lung changes in the high dose groups at day 1 post exposure. The mid dose only induced histopathological changes and changes in BAL fluid. The effects of all 3 types of Silica, with the exception of slight histopathological lung changes a the higher exposure levels, were reversed during the recovery period. The low dose caused no adverse effects.

INTRAVENOUS

SILICA

Schepers et al. (1957d) intratracheally injected rats (n = 10) with Silica (5%; 0.25 ml) once per week for 3 weeks. Two rats died after the first injection, 3 died before the third injection. Three rats survived the observation period (length not stated). Pleural effusion was observed in 4 rats, 2 responses were delayed, 1 supervened and the rat survived 220 days. Pulmonary congestion was observed in 3 rats until the ninth month. Tracheobronchial lymph nodes were moderately to markedly enlarged and firm for 5 months. There were abscess formation associated with pneumonitis; accompanying cells were macrophages. Focal granulomatous processes were observed in both lungs in rats that survived more than 2 days. Hyperemia of the alveolar walls later resolved. Infiltration of the alveolar walls was mostly by macrophages. Early collagen became profuse in the alveolar walls in relation to focal granulomata and cell necrosis.

This experiment was repeated with guinea pigs at double the dose of Silica. At 2 weeks, 2 guinea pigs died;

the rest survived to be killed and necropsied at intervals. Most of the effects were confined to the lungs. A few consolidated areas were palpable in a few animals. There was multiple foci of atelectasis in the lungs. Tracheobronchial lymph nodes were moderately to markedly enlarged. Cellular phenomena predominated early and resolved with residual fibrotic change. Granulomata occurred in the first month. There was a tendency toward cellular invasion. Slight to moderate atrophic vesicular emphysema was detectable during the second half of the year of observation. There was an absence of necrosis in the lymph nodes.

The authors intravenously injected Silica (1% in saline; 5 ml) into rabbits (n = 5) 20 times biweekly. One rabbit died after the second injection and another after half the injections. One died on the 70$^{th}$ day and the last 2 were killed on the 120$^{th}$ and 300$^{th}$ days. There was slight to moderate pleural effusion with pleuritits. The longest surviving rabbits had a mediastinal abscess. The lungs had moderate to marked congestion, greatest in the rabbits that died first. There was no foci consolidation but small areas of atelectasis. The lymph nodes were not markedly enlarged. The right ventricles were dilated and hypertrophic, most obvious in the rabbit that died first. The livers were moderately to markedly enlarged, turning pale and firm over time. However, the liver had returned to normal in the rabbit killed on the 300$^{th}$ day. There was some splenomegaly in the 2 rabbits that died during treatment. Atrophy of the spleen increased over time. The size of the kidneys increased over time, to almost double normal size. Histological changes included hyperemia with associated exudate into alveolar spaces, ischemia, dust-filled macrophages in alveolar spaces, distension of the proximal convoluted tubules with fibrosis, and small granulomata that diminished over time. The epithelial cells were well preserved. There was minimal collagenosis. The alveolar walls thickened then became thin (Schepers et al. 1957d).

## SUBCHRONIC TOXICITY

### ORAL

#### SILICA

FDA (No date) reported the results of a 90-day rat feeding study. There were no effects and the HNEL was 5000 mg/kg/d.

In another study using rats for 180 days with a 3-week recovery period, the lowest effects level (LEL) was 500 mg/kg/d. There was an increase in adrenal weight in the males. Adrenal weight also increased in the females but only during the recovery period. Histopathology showed an increase in lipid content in the adrenal glands; this resolved during the recovery period.

Hazelton Laboratories (1958c) incorporated fumed Silica (1.0%, 3.0%, or 5.0%: 10,000, 30,000, or 50,000

ppm) into the feed of male and female albino weanling rats (n = 30; 15 male, 15 female) for 90 days. Controls were fed the basal diet or 3.0% #1625 Cosmetic Talc. The rats were killed and necropsied at 45 and 90 days. There were no gross signs of toxicity. Growth rates, feed consumption, and survival were similar to controls. The Silica content of the liver, kidneys, spleen, blood, and urine was similar to controls. There were no gross, microscopic, nor pathological changes associated with Silcia consumption at any dose level.

Silica (50 mg/d) was fed to male and female rats (n = 30) by stomach tube for 3 months. No adverse effects on body weight gain or mortality were observed. The results of pathological examination were similar to controls (W. R. Grace & Co. 1981).

In another experiment, Silica (0, 1.0%, 3.0% 5.0%) was incorporated into the feed of male and female rats (n = 30) for 90 days. The positive control group was fed cosmetic talc. There was no systemic toxicity by Silica in terms of survival, weight gain, and feed consumption observed. There was no increase in deposition of Silica observed in the kidney, livers, spleen, blood, or urine (W. R. Grace & Co. 1981).

Lewinson et al. (1994) orally administered fumed Silica (500 mg/kg/day) in the feed of Wistar rats (n = 40; 20 males, 20 females) for 6 months. The animals were then killed and necropsied except for animals of both sexes which were kept on normal feed for and additional 3 weeks. The animals were observed daily, blood samples periodically, and weighed periodically.

There were no clinical signs during the treatment period. One male in the treatment group died; a lung infection was observed. Two rats in the control group died with enteritis and cachexia. There were no differences in weights or feed consumption. There were no differences in hematological parameters. Macroscopic evaluation at necropsy was unremarkable. Histopathological examination revealed increased lipid content in the fasciculata and zone fasciculata of the adrenal glands; this condition resolved after the recovery period. No other differences between treated and control animals were observed. The authors concluded that the NOEL was 500 mg/kg/day (Lewinson et al. 1994).

UNEP (2004) reported an unpublished feeding study of precipitated Silica (0.5%, 2%, and 6.7%; 300 to 330, 1200 to 1400, and 4000 to 4500 mg/kg/d) using Wistar rats (n = 20; 10 male, 10 female) for 13 weeks. There were no clincal signs including hematological, blood chemistry, and urinary parameters. Feed intake was slightly increased in the high dose females after 4 weeks. Gross and microscopic examinations were unremarkable.

In another unpublished feeding study precipitated Silica gel (3.2% and 10%) was fed to CD-1 rats (n = 24; 12 males, 12 females) for 6 months. Calculated doses were 2170 and 7950 mg/kg/d for the males and 2420 and

8980 mg/kg/d for females. At 6, 13, and 26 weeks, 4 rats of each sex in each group were killed and necropsied including bone marrow. There were no treatment-related findings. Behavior was normal. Body weights were not affected. There were no histopathological changes in the kidneys. The NOAEL was 8980 mg/kg/d.

In another unpublished feeding study, Charles River rats (n = 30; 15 males, 15 females) were fed fumed Silica (1%, 3%, 5%; 700, 2100, and 3500 mg/kg/d) for 13 weeks. Some rats were killed and necropsied at 45 and 90 days. There were no clinical signs observed. There were no gross pathological and histopathological changes that could be attributed to treatment. In the high-dose group, $SiO_2$ content of the liver, kidneys, spleen, blood, and urine at 45 and 90 days was similar to controls.

Another unpublished study in which Wistar rats (n = 40; 20 male, 20 female) were fed Silica (495 to 497 mg/kg/d) for 6 months resulted in an NOAEL of 497 mg/kg. No further information was provided (UNEP 2004).

INHALATION

SILICA

Schepers et al. (1957a) placed male and female Wistar rats (n = 25) in inhalations chambers to expose them to fumed Silica (average 1.5 mg/ft$^3$ [53 mg/m$^3$]; most measurements ranged 0.7 to 2.4 mg/ft$^3$ [25 to 85 mg/m$^3$]). The rats were exposed to aerated Silica for 8 h/d then had passive exposure (dust settling) for the remaining 16 h. The exposure was for 5 d/week for 6 months followed by 6 months of recovery. Rats were periodically killed and necropsied. The control group (n = 42) were in normal air and killed and necropsied at 6 and 12 months.

In the test group, 11/25 (44%) died, mostly during the Silica exposure. The death rate decreased during the recovery period. The majority of the rats spontaeously died from pulmonary vascular obstruction and emphysema beginning at the 4$^{th}$ month. Focal pigmentation was conspicuous after 3 months of exposure with profusely scattered small, dark-pink discrete but irregular subpleural foci of reaction. Congestion of the lungs was dominant after 3 months. There was lymph node enlargement after 3 months. There was an incipient tendency toward lung emphysema after 4 months of exposure with lung distension and superficial aveoli dialation. Atelectasis was noted in some rats after 4 to 5 months.

Histological examination revealed invasion of the lymphatic system of the lung by mononuclear macrophages forming clusters of plasma cells and lymphocytes. There was production of large vacuolated cells withing the alveolar spaces; the cytoplasm had a foamy appearance with macrophages fused to giant cells. There were large vacuolated cells within the alveolar spaces, with the cytoplasm having foamy appearance,

macrophages apparently fused to giant cells. There was progressive nodule formation in the lung parenchyma and peri- and paravascular, in some cases parabrochiolar distribution and accumulation, consisting of central macrophages and surrounding plasma cells, some nodules enveloped by an epithelial layer of cells. Some necrosis was noted in the central zone of the nodules; there was progressive tendency toward fibrosis in the nodules and evidence of progressive emphysematous processes around the nodules.

Average Silica load in the lung increased to 1.5 mg/lung after 3 months and remained at that level through exposure. At the end of recovery, the level reduced to 0.3 mg/lung. The authors concluded that the lowest observed adverse effects level (LOAEL) was 53 $mg/m^3$ (Schepers et al. 1957a).

Reuzel et al. (1991) reported an unpublished inhalation study of Silica (1.3, 5.9, 31 $mg/m^3$) using Wistar rats (n = 100; 50 male, 50 female). The rats were subjected to full body exposure for 6 h/d, 5 d/week, for 13 weeks. Ten rats of each sex were killed and necropsied at weeks 13, 26, 39, and 52.

There were no mortalities during treatment or recovery. Clinical signs were increased respiration rates in a dose dependent manner and body weight gains were depressed. Red blood cell (RBC) count was increased in males in the high-dose group. In the mid- and high-dose groups, white blood cells (WBC) were elevated in both males and females; the concentration-response relationship was poor. Blood cell counts returned to normal by week 39. Necropsy at 13 weeks revealed swollen and spotted lungs and enlarged mediatinal lymph nodes; the severity was dose dependent. All groups had increased lung weights and collagen content, less so in the low-dose group. All these effects reduced to control levels by the end of the study except for collagen content in males in the mid- and high-dose groups.

After treatment, Silica could be detected in the lungs of all the rats in relatively small amounts. In the high-dose group, the average Silica amount in the lungs was 0.2 mg. Silica was detected in 1 male in this group in the regional lymph node. At the end of the study, no Silica could be detected in any rat. Microscopic evaluation after treatment revealed accumulation of alveolar macrophages and granular material, cellular debris, polymorphonuclear leukocytes, increased septal cellularity, alveolar bronchialization, focal interstitial fibrosis, cholesterol clefts, and granuloma-like lesions in the lung. The lesions in the lung did not show fibroblasic activity or hyalinization and regressed during recovery. All types of pulmonary lesions were more marked in males than in females. Accumulation of macrophages was observed in the mediastinal lymph node at 13 and 26 weeks. Treatment-related, microscopic changes in the nasal region were occasionally found at week 13 such as focal necrosis slight atrophy of the olfactory epithelium. Interstitial fibrosis was not noted directly after the exposure

period, but was observed for the first time after 13 weeks postexposure, with increasing incidence especially in the high-dose group, and a few in the mid-dose group. There was decreased severity and frequency until the end of the study. The authors concluded that the NOEL was 1.3 mg/m$^3$.

In a second study, male and female Wistar rats were places in a whole body inhalation chamber 6 h/d, 5 d/week, for 13 weeks to be exposed to precipitated Silica at 35 mg/m$^3$ (particle and agglomerate/aggregage size 1 to ~120 µm). The rats were periodically killed and necropsied over the 52-week recovery period.

Slightly decreased body weight and increased lung and thymus weights were observed. Necropsy revealed swollen and spotted lungs and enlarged mediatinal lymph nodes. Microscopic examination of the lungs revealed accumulation of alveolar macrophages, intra-alveolar leuckocytes, and increased setal cellularity. There was also accumulation of macrophages in the lymph nodes. The collagen content in the lungs was slightly increased. During the recovery period, the effects of Silica exposure were mostly gone within 26 weeks. Accumulation of Silica and macrophages in the mediastinal lymph nodes were still present at the end of the recovery period (Reuzel et al. 1991).

Johnston et al. (2000) exposed male Fischer 344 rats ( n = 4) to aerosolized fumed Silica (50.4 ± 19 mg/m$^3$; mean diameter 0.81 µm) for 6 h/d, 5d/week, for 13 weeks. The control group was not treated. The Silica burden was determined after 6.5 and 13 weeks of exposure and after 3 and 8 months of recovery. The Silica load increased quickly during the first 6.5 weeks of exposure (0.76 mg/lung) but less so after 13 weeks (0.88 mg/lung). During recovery, the Silica burden disappeared rapidly from lung tissue (15% after 12 weeks; 6% after 32 weeks). BAL showed mean cell numbers in the lavage increased 5- to 15-fold compared to control. The cells comprised > 50% polymorphonuclear leukocytes (PMN) and some 2% lymphocytes whereas the control lavages only contained < 1% of either cell type. Protein content and enzyme activitys (LDH and glucuronidase) were markedly higher than under control conditions. All BAL markers approached normal levels after 13 weeks recovery in most rats, however, a few showed minimal increases.

There was invasion of neutrophils and macrophages into the alveoli after 6.5 weeks but this effect tended to decrease during recovery. Fibrosis was observed in alveolar septa which subsided during recovery. After 13 weeks of exposure, intensely stained TUNEL-positive cells were detected throughout the terminal bronchiolar epithelium and through the parenchyma of the lungs. The authors concluded that aerosolized Silica produced transient pulmonary inflammatory response and most biochemical markers return to control levels post exposure (Johnston et al. 2000).

## CHRONIC TOXICITY

### ORAL

#### SILICA

Silica (3.2% or 10%) was incorporated into the feed of rats (n = 24; 12 male, 12 female) for 6 months. There were no mortalities. The only clinical sign was discolored stools. Growth and development was normal and feed consumption similar to controls. Necropsy was unremarkable; organ weights, absolute and relative, were similar to controls. Histology and hemotology was unremarkable. There were no changes in clinical chemistry (W. R. Grace & Co. 1981).

In another study, the data from a feeding study was presented. Rats (n = 24; 12 males, 12 females) were fed Silica 6 months. The low dose males and females consumed an average of 0.78 and 0.55 g/week, respectively. The high dose males and females consumed and average of 3.00 and 2.11 g/week, respectively. There was no effect with regards to body weight gain, feed consumption, blood chemistry, or urinalysis. There was an increase in the number of leukocytes in the female high dose group and eosinophils in the male high dose group. There was a decrease in glucose concentration and AP activity in the male rats in a dose dependent manner. There was reduced serum calcium concentration in the female rats in a dose dependent manner. There were reduced absolute and reduced liver and prostate weights.

Takizawa et al. (1988) fed precipitated Silica gel (1.25, 2.5, or 5% incorporated into feed) to male and female Fischer 344 rats (n = 80; 40 males, 40 females) for 103 weeks. Twenty animals per group had a recovery period of 21 months. The mean cumulative intake of Silica was 143.46, 179.55, and 581.18 g/male rat and 107.25, 205.02, and 435.33 g/female rat for the low, mid and high dose groups, respectively. Survival for all treatment groups was similar to controls. There were no differences between treatment groups and controls with regards to body weight, feed intake, behavior, or in hematological or chemistry parameter. Liver weights in the females in the mid and high dose groups were lower at 12 to 24 months. There were no significant findings at the histopathological examinations.

The above experiment was repeated feeding B6C3F1 mice (n = 80; 40 male, 40 female) for 93 weeks. The mean cumulative intake of Silica was 38.45, 79.78, and 160 g/male mouse and 37.02, 72.46, and 157.59 g/female mouse for the low, mid, and high dose groups, respectively. There were no differences in survival between treatment groups and controls. Feed consumption was increased in the mid and high dose groups whereas there was reduced weight increase in the males during weeks 15 through 50 ($p < .01$) and weeks 30 through 50 for the

females ($p < .05$). There were no remarkable findings with regards to hematology or organ weights. There was no increase in the incidence of tumors (Takizawa et al. 1988).

INHALATION

SILICA

Jötten and Klosterkötter (1951) reported that when rabbits were exposed to aerosolized Silica (0.2 to 5.0 µm) there was formation of nodular fibrotic or diffuse fibrotic changes in the lungs. The authors concluded that the concentration of the dissolved Silica, the surface forces of the colloidal particles, mechanical and physiochemical condidtions were factors in the observed changes.

Schepers et al. (1957a) performed a parallel study (see SUB-CHRONIC above for more details) where the Wistar rats (n = 35) were exposed to aerosolized precipitated Silica (average 1.5 mg/ft$^3$ [53 mg/m$^3$]; most measurements ranged 0.7 to 2.4 mg/ft$^3$ [25 to 85 mg/m$^3$]) for 8 h/d, 5 d/week for 12 months. Treatment related deaths were 26/35 (75%). After 6 months of exposure, aggravation of focal pigmentation visible as reddish-tan foci of dust was observed. There was also moderate, well-established generalized emphysema and lymph nodes that were greatly enlarged with their firm consistency markedly increased. The majority of the rats spontaneously died from pulmonary vascular obstruction and emphysema from the 4$^{th}$ to the 9$^{th}$ month. The authors concluded that high subchronic/chronic exposure to amorphous Silica produces severe progressive pulmonary inflammation associated with increased mortality of the animals, primarily through partial obstruction of the pulmonary vasculature combined with pulmonary insufficiency due to emphysema.

Schepers et al. (1957b) exposed male and female albino guinea pigs to aerosolized fumed Silica (average concentration 1.5 mg/ft$^3$; ranging 0.7 to 2.4 mg/ft$^3$; 85% between 1 to 10 µm) in 3 experiments. The whole body exposure was for 8 h/d with the remaining 16 h as passive exposure (dust settling). In experiment 1, the guinea pigs (n = 40) were exposed to the Silica up to 24 months, with a few killed and necropsied every 2 months. In experiment 2, the guinea pigs were exposed for 12 (n = 15) or 24 (n = 18) months with variable recovery periods up to 12 months. In experiment 3, the guinea pigs (n = 17) were exposed for 12 months, followed by a 1 month recovery period, then re-exposure for 8 to 24 h. A control group of 80 guinea pigs were sampled and necropsied from 1 to 36 months.

Only 2 guinea pigs died from unrelated causes. Overall, the chronic reaction of the lung tissue was established by 4 months of exposure. There was focal pigmentation after 1 month. Lymph nodes were enlarged by 1 month and did not increase over time, including hepatic lymph nodes. There was a tendency for lung

emphysema after 4 to 8 months of exposure. Atelectasis was not pervasive macroscopically but observed histologically. There were no nodule development.

Histologically, the dominant repsonse was periductal and peribronchiolar intra-alveolar accumulations of the giant cells. At 8 to 12 months there was incipient atrophy of infiltrated alveoli which apparently led to compensatory expansion of adjacent alveoli. There was a combined effect of atelectasis and consolidation around brochioli, but at the expense of brochioli distortion. Incipient fibrosis around bronchioli and shrunken alveoli was noted at this stage. There was a marked tendency toward cuboidal epithelization of atelectactic alveoli by the end of the second year of exposure.

In the lymphoid tissue, medullary hyperplasia with the formation of slight amounts of reticulum was prominent during the second year of exposure. No peridenitis, sinus catarrh, nor fibrosis were noted in the lymph nodes.

In the recovery phase after 12 months of exposure, there was progressive recovery beginning almost immediately. There were no macroscopically visible anomalies after 1 year of recovery. Residual sequalae of the tissue reactions were emphysema, mural fibrosis, and bronchiolar and ductile stenosis. The authors concluded that chronic exposure to amorphous Silica was non-lethal to guinea pigs, but caused significant inflammatory reactions and pulmonary lesions, however, without apparent disability of the animals (Schepers et al. 1957b).

Schepers et al. (1957c) exposed New Zealand white rabbits ( n = 10) to aerosolized fumed Silica (1.5 $mg/ft^3$, 53 $mg/m^3$; ranging from 0.7 to 2.4 $mg/ft^3$, 25 to 85 $mg/m^3$; 1 to 10 µm) for 8 h/d for 12 months. There was a 6 and 12 month recovery periods. There was progressive functional incapacitation and elevation of hematocrit levels observed in the majority of the rabbits, possibly due to the combined effect of pulmonary vascular obstruction and emphysema. Blood pressure changes (both increases and decreases) were observed in the majority of the animals which partially recovered with discontinuation of treatment. Essential pulmonary changes included peribronchiolar cellular catarrh, mural cellular infiltration along with deposition of reticulum and some collagen, the formation of peri-vascular cellular nodules, ductal stenosis, and emphysema. During recovery, the cellular reactions and emphysema regressed but minor focal alveolar mural collagen persisted.

Schepers (1959) exposed New Zealand white rabbits (n not clear, ~16) to aerosolized precipitate Silica (0, 28, 134, and 360 $mg/m^3$) for 8 h/d, 5 d/week. The mid and high dose groups were exposed for 9 months, the low dose and the control groups were exposed for 27 months. The rabbits in the mid and high dose became distressed during exposure. Symptoms were fewer, commenced later, and receded more quickly in the lower

concentrations. There was increased body weight gain which corrected when exposure was terminated. The author suggested that this was due to edema. The body weights then decreased. The rabbits exhibited dyspnea, shortness of breath accompanied by cyanosis. Elevated right and left ventriclar pressures were concentration and time related.

In the high dose group, emphysema was observed which decreased after termination of treatment. Pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed. Lesions were observed in the liver, spleen and kidney.

After 6 months of exposure, the cardiac pressure of the low dose group increased and continued steadily. At 24 months, the elevation was 64% over pre-exposure pressure. This effect was partially reversed with termination of treatment (34% after 12 months). The author reported concomitant radiographic changes, electrocardagraphic deviations, modification of lung functions, hematolytic changes, anatomical cor pulmonale, congestive cardiac failure, emphysema, and chemical pheumonitis. The LOAEL was 28 mg/m$^3$ (Schepers et al. 1959).

Schepers (1962) exposed monkeys (*Macacus mulatta*; n = 5) to aerosolized synthetic Silica (15 mg/m$^3$) for up to 12 months. A monkey was killed and necropsied at 3 and 6 months. The rest were killed and necropsied after 12 months of exposure. The control was 15 untreated monkeys. Body weight gain decreased and activity decreased during initial exposure. At 3 months, emphysema was detectable. There was considerable cellular infiltration of the alveoli and alveolar septa was associated with distention of alvoli or accumulation of exudate and macrophages.

After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vacular oclusions, and cor pulmonale. Core pulmonale was attributed to the emphysema and alveolar wall destruction. Tracheobrachial lymph nodes were slightly enlarged but not fibrotic. The Silica content remained low and decreased over time (Schepers 1962).

Klosterkötter (1965) exposed female albino rats (n = 120) to aerosolized fumed Silica (~45 mg/m$^3$) for 4 h/d for up to 1 year followed by a 3 to 8 month recovery period.. Some of the rats were killed and necropsied periodically. There were 41/120 spontaneous deaths. At necropsy, there were small white foci under the pleura, enlarged and discolored lymph nodes with formation of collagen and local necrosis, perivascular and peribronchiolar dust cell granuloma with reticulin and collagen figbers, necrotic cells, desquamative catarrh and thickened alveolar septs.

After the recovery periods, the dust cell ganuloma were fewer and reduced in size with only a few dust cells and fibers. The alveolar septs had not completely disappeared. After 3 months, the lymph nodes were enlarged; after 8 months, the size, necrosis, and fibers were reduced. The mean Silica content of the lungs was 0.32 mg/lung or lymph node (0.6 mg maximum). At the end of exposure, 0.132 mg was found in the mediastinal lymph node (Klosterkötter 1965).

With continued exposure, the cellular reaction decreased and was replaced by degenerative processes (loss of septa with confluence of alveoli), followed by destructive emphysema. Circulatory continuity was extensively impaired by extensive rupture of alveolar septa. Collagen appeared in the alveolar septa.

Groth et al. (1981) exposed male Sprague Dawley rats (n = 80) to aerosolized fumed Silica, precipitated Silica, or Silica gel (15 mg/m$^3$; 6 to 9 mg/m$^3$ respirable $\leq$4.7 µm). Exposure was for 5.5 to 6 h/day, 5 d/week for up to 12 months. At maximum exposure, a few macrophage aggregates were found in the lungs. Interstitial fibrosis associated with dense collections of most cells appeared in some of the rats of the control and treatment groups, although there was a trend of a more frequent incidence in those exposed to fumed Silica, but was obscured by the presence in some control animals. The authors concluded that the LOAEL was 6 to 9 mg/m$^3$. Macrophage aggregation was less pronounced in rats than in monkeys under these test conditions (see below). Fibrosis was of minor importance as test and control groups were similar.

Another experiment was conducted using male monkeys (*Macaca fascicularis*; n = 10) with exposure to 3 types of Silica for 6 h/d, 5 d/week, 13 or 18 months. The reduction in lung respiratory volume and ventilatory mechanics in the monkeys was more marked in the fumed Silica group. Dynamic pulmonary compliance, forced vital capacity, inspiratory capacity, total lung capacity, and forced expiratory flow were decreased. Average flow resistance and closing volume were increased. In the precipitated Silica group, lower lung volumes were observed. There were no changes in lung volume parameters, but there were reductions in ventilatory performance and mechanical parameters, dynamic lung compliance, and forced expiratory flow when exposed to Silica gel.

Cytoplasmatic changes (increases in number of vacuoles) in macrophages inthe lungs and tracheal lymph nodes were observed. Large numbers of macrophages and monomuclear cell aggregates (bronchioles, alveolar ducts venules, arterioles) were observed in the lungs. The frequency and size of the cells aggregates varied with the type of Silica (precipitated > fumed > gel). Reticulin fibers were present in the aggregates in all 3 groups. In 6/9 monkeys exposed to fumed Silica, collagen in varying amount was found in 5 to 50% of the aggregates,with

signs of early nodular fibrosis. In 3/9 monkeys no or little collagen was present. No collagen fibers were observed in aggregates in the lung of monkeys exposed to Silica gel and only very few after exposure to precipitated Silica. The authors concluded that early nodular fibrosis indicated that fumed Silica is more detrimental than precipitated Silica or Silica gel. The smaller particle size of fumed Silica may be a contributing factor. UNEP (2004) reviewed this study and notes that the monkeys were transported in bags that had contained asbestos and that suspect particles in the lungs were identified as mica and kaolin. Quartz or asbestos fibers could not be ruled out.

In a third study, the authors exposed male Hartley guinea pigs (n = 20) to the 3 types of Silica for 5.5 to 6 h/d, 5 d/week for 12 months. A few macrophages containing particles of Silica were observed in the lungs and lymph nodes, similar to the findings in rats (see above) (Groth et al. 1981).

Schepers (1981) exposed rabbits (n = 50), rats (n = 84), and guinea pigs (n = 82) to aerosolized precipitated Silica (average of 126 mg/$m^3$ (3.57 mg/$ft^3$) for 8 h/d, 5 d/week, for 12, 15, and 24 months, respectively, followed by a recovery period of up to 12 months. Control groups were not treated. There were no treatment-related differences in mortality between treated and control groups. For the rats, most of the deaths were due to intercurrent infection.

Lung weights increased during exposure but returned to normal during recovery. Particle-phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue. Hilar lymph nodes were enlarged, mildly in rats and more evidently in the guinea pigs and rabbits; this disappeared with the termination of treatment. Epithelial proliferation was minimal. There was mild deposition of reticulin fibers occurred in alveoles with no evidence of collagen formation. Bronchial and tracheal epithelia remained intact. There were no epithelization or pleural changes were observed; no neoplasmia occurred.

The emphysema was dominated by the diffuse hypertrphic vesicular distention but apparently did not result from destructive effects on the mucosa or terminal bronchioles and disruption of the continuity of alveolar walls. The author stated that the emphysematous effect in the rats could possible be due to aging and recurrent epizootic pneumontitis. The tuberculogenic response in the guinea pigs was limited to a slight increase in size of some lesions and slightly longer persistence of the active cellular proliferation phase of the tubercles. No extrapulmonary spread of the tuberculosis occurred. There was complete reversibility of Silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent (Schepers 1981).

Pratt (1983) exposed albino guinea pigs (n not provided; starting at 200 to 300 g) to Silica dust (in the form

of diatomaceous earth) in dust rooms for 7 to 8 h/day, 5.5 days/week, for 24 months. Exposure ranged from 150 to 200 mp/ft$^3$ (mean of 171 mp/ft$^3$; 100 mg/m$^3$). Two guinea pigs were chosen randomly every 2 months the first year and every 3 months the second year to be killed and the lungs examined. Controls were housed in ambient air.

The amount of Silica in the lung ash increased until the 8$^{th}$ month at 41%, then fluctuated and reached ~50% at 24 months. The author suggests that the fibrotic response may be beginning when the Silica no longer increased, resulting in heavier lungs that might have diluted the Silica. The weight of Silica in the lungs of the guinea pigs increase over time to ~100 mg at 24 month. Lung ash was ~230 mg at 24 months, ~120 mg was not Silica. Fibrosis was noted at necropsy after 24 months. The author concluded that since amorphous Silica accumulated more than crystalline Silica (465 mg), dust that produces cell damage may be cleared more effectively than from the lungs than an innocuous dust (Pratt 1983).

UNEP (2004) reported several unpublished chronic inhalation toxicity studies of Silica. The studies are summarized in Table 12.

## OCULAR IRRITATION

### SILICA

Hazelton Laboratories (1958a) administered a single application of fumed Silcia (3 mg) to the eyes of albino rabbits (n = 3). The eyes were observed at 1, 4, and 24 h. There was mild eye irritation in the form of erythema and vascularization of the lower sclera and nictating membrane, which resolved within 48 h.

W. R. Grace & Co. (1981) reported a Draize test of Silica (9 g) using rabbits (n not provided). The dry material was a mild irritant (score of 2.4) in the unrinsed eyes. The authors suggested that was due to the strong hydrophilic Silica. There was no irritation when the eyes were rinsed or with an aqueous suspension.

In another study, Silica (10 g) was applied to the eye of rabbits and not rinsed or rinsed after 2 or 4 sec. There was faint irritation of the mucous tissues in the eyes not rinsed which resolved after 1 day. There was no irritation in the eyes that were rinsed. When the test was repeated with the same amount of Silica in aqueous solution there was no irritation (W. R. Grace & Co. 1981).

Lewinson et al. (1994) instilled fumed or precipitated Silica (0.1 g in olive oil) into the eyes of male New Zealand white rabbits (n = 8). After 5 min, the eyes of 5 rabbits in each group were rinsed. After 24 h, the rest of the rabbits' eyes were rinsed. The eyes were examined with a split lamp at 1, 24, 48 and 72 h (24-h exposure

**Table 12.** Chronic inhalation studies of Silica reported by UNEP (2004).

| Species (n) | Test substance; dose | Notes and results |
|---|---|---|
| Female, inbred white rats (n = 10) | Precipitated synthetic Silica; 55 mg/m$^3$ for 5 h/d, 5 d/week/ 3, 6, and 12 months. Post exposure 5 months. | At necropsy, some white-grey foci were observed subpleurally. Desquamation of alveolar cells with fine granula after 4 months. After 12 months, peribronchial and intra-alveolar small dust cell foci with few reticulin fibres were found. Small increased cell numbers and fibers were observed. The mediastinal lymph nodes were enlarged and contained dust cells with fine granules. Neither a diffuse nor nodula fibrosis was observed in lungs or lymph nodes.<br>At recovery, effects regressed. Lung weights were normal with a few foci left. There was no significant desquamation. Lymph nodes were slightly enlarged with some dust cells.<br>One day retention value of Silica was 0.138 mg/lung. Average Silica content was 1.022 mg/lung after 4 months and 3.443 mg after 12 months.<br>Conclusion: The lymphatic system appears to play a minor role in the elimination of Silica from the lung. Therefore, there is no evidence for a silicosis or a lymphatic-type pneumoconiosis to develop from exposure to synthetic Silica. |
| Female Sprague-Dawley Rats (n = 150) | Fumed Silica; 50 - 55 mg/m$^3$, ~30 mg/m$^3$ respirable for 5 h/d, 5x/week then 2 - 3x/week partway through the experiment for 12 months. Post exposure up to 5 months. | Frequency of exposure was reduced due to fatal cases caused by massive substance-related purulent bronchitis, focal pneumontitis, and massive cellular reactions.<br>After 12 months, ~1% of respirable dust was still retained in the lung. The increase in lung deposition was low from 18 weeks (1.2 mg) to 12 months (1.37 mg) of exposure. Mediastinal lymph nodes contained ~0.13 mg Silica after 12 months.<br>After 5 months post exposure, mean Silica load was 0.16 mg/lung and 0.047 mg/lymph node, a reduction of 88% in the lung and > 50% in the lymph nodes.<br>Microscopically visible dust foci under pulmonary pleura, mediastinal lymph nodes were moderately enlarged. Interior of alveoles: numerous macrophages accumulated, partially destroyed, associated with deposition of cell debris. Perivascular and peribronchiolar small dust foci of macrophages, associated with mild and moderate formation of connective tissue. Increased collagen formation in alveolar septa. Foci and clusters of phagocytes (partially normal, partially showing decay) and some collagenic fibrosis was observed in the mediastinal lymph nodes.<br>Conclusion: In some cases, Silica, at sites of highly concentrated deposits, caused a marked collagenic fibrosis, but without signs of typical silicosis. |
| Female albino rats (not provided) | Fumed Silica; 0.112 mg/l; 5 h/d, 5 d/week for 1 year followed by 4 month recovery. | At 4 months, 1.578 mg were in the lungs and 0.151 in the lymph nodes; at 12 months, 1.820 and 0.430 mg, respectively. At necropsy, white foci under the plasma were observed, the mediastinal lymph node was enlarged. Histological examination revealed desquamative catarrh, sporadic dust modules, and foci with minimal to moderate fibrosis, increased collagen, and sporadic diffuse fibrosis of the alveolar septes and perifocal emphysema. Silicatic nodules were not observed. Lymph nodes: increase of dusted cells and slight to moderate fibrosis, sporadic colagen fibrosis.<br>After recovery, Silica content of lungs, 0.92 mg and lymph nodes, 0.814 mg. Necropsy revealed subpleural dust foci and enlarged lymph nodes. Lung weight increased. Microscopically cell desquamation gone whereas there was no improvement in other parameters. |
| Rabbits (not provided) | Silica; dose not provided; 4-5 h/d, 5 d/week for ~ 3 years followed by 30 to 150 days recovery | No clinical signs during inhalation. Mortality relationship to treatment not clear. Macroscopic examination: emphysema of the lungs. Microscopic evaluation: bronchial and alveolar dequamative catarrh, lymphocityes and leukocytes increased in the alveoles, edema, accumulation of macrophages in the lymph nodes and in the interstitium (perivascular, peribronchial, alveolar septes), granuloma of macrophages, dust cells, some thickening of the alveolar septes. Formation of connective tissue was minimal. |

only).

The rabbits treated with precipitated Silica had slight redness of the conjuctiva at 1 and 24 h in the group rinsed after 5 min and at 1, 24, and 48 h in the group rinsed after 24 h. There were no signs of irritation in the rabbits treated with fumed Silica. There were no clinical signs. The authors concluded that precipitated Silica was slighly irritating to the eyes of rabbits and fumed Silica was not irritating. It is not clear whether the greater water solubility of precipitated Silica or the incomplete removal of olive oil caused the difference (Lewinson et al. 1994).

UNEP (2004) reported an unpublished ocular irritation study of fumed Silica (100 mg) using rabbits (n = 3).

The Silica was instilled without rinsing. There were no signs of irritation up to 96 h after application.

In another unpublished ocular irritation study of fumed Silica (100 mg) using rabbits (n = 3), the Silica was instilled without rinsing. There were weak irritating effects in the conjuctivae with a redness score of 2/4 in all rabbits at 1 and 2 h, 1 rabbit at 24 h, and non at 72 h. Chemosis and discharge were slight after 1 h. The authors concluded that fumed Silica was non-irritating.

In another unpublished ocular irritation study of precipitated Silica using rabbits, Silica was found to be nonirritating.

In another unpublished ocular irritation study of precipitated Silica gel (suspended in water), the eyes were unrinsed or rinsed after 2 or 4 sec. The authors concluded that precipitated Silica was nonirritating.

In another unpublished study, 4 products of precipitated Silica (100 mg) were instilled in the eyes of rabbits. All types had isolated cases of very slight and transient irritating effects on the conjunctiva with a redness score of 1/4. The authors concluded the precipitated Silica products were nonirritating.

In another unpublished ocular irritation study, precipitated Silica (50% w/v in an aqueous slurry) was nonirritating to rabbits (UNEP 2004).

## DERMAL IRRITATION

### SILICA

Hazelton Laboratories (1958b) applied fumed Silica (5 or 10 g as a paste in water) to the intact and abraded skin of albino rabbits (n = 4) daily, 5 d/week, for 15 applications. Mild dermal irritation consisting of erythema, atonia, and desquamation was observed for both doses. The abraded skin healed completely.

W. R. Grace & Co. (1981) reported a study where a US Department of Transportation test for skin irritation of Silica (assumed at 100%) was performed on rabbits (n = 8) on intact and abraded skin. One rabbit showed very mild reddening of the abraded skin. Silica was determined to be virtually non-irritating.

Lewinson et al. (1994) used a gauze patch to apply fumed Silica (0.5 g in olive oil) or precipitated Silica (0.5 g in aqueous methylhdroxyethyl cellulose gel 300 P [1%]) to the intact and abraded skin of New Zealand white rabbits (n = 6; 3 male, 3 female) for 24 h. The patch site was scored after removal and 48 h later. The rabbits were observed for clinical signs during exposure and for 14 d after exposure. No irritation was observed for either type of Silica on either intact or abraded skin. No effects were observed.

UNEP (2004) reported an unpublished dermal irritation study of fumed Silica (0.5 g; 12% suspension/gel in 1% methylhydroxyethyl cellulose in water) using rabbits. The Silica was applied under occlusion to the intact (n =

6) and scarified (n = 6) skin of the rabbits for 24 h. There were no signs of irritation under either skin condition. The authors concluded that fumed Silica was non-irritating.

In another unpublished dermal irritation study, precipitated Silica (0.5 g; 23% suspension/gel in 1% methylhydroxyethyl cellulose in water) using rabbits, Silica was applied under occlusion to the intact (n = 6) and scarified (n = 6) skin of the rabbits for 24 h. There were no signs of irritation under either skin condition. The authors concluded that fumed Silica was non-irritating.

Another unpublished study reported a dermal irritation study of precipitated Silica (0.5 g in 0.5 ml water) on rabbits (n = 3). The test substance was applied to the skin under occlusion for 4 h. There were no irritating effects.

Another unpublished study reported a dermal irritation study of precipitated Silica (0.5 g) on rabbits (n = 12). The test substance was applied to the skin under occlusion for 24 h. There were no irritating effects.

Another unpublished study reported a dermal irritation study of precipitated Silica (20 mg) on rabbits (n = 8). The test substance was applied to the skin under occlusion for 24 h. There were no irritating effects.

Another unpublished study reported that a patch test of Silica was non-irritating to rabbits. No further details were provided.

Another unpublished study reported a dermal irritation study of precipitated Silica (190 mg; 17% w/w; ~0.38 g/ml) on rabbits (n = 6). The test substance was applied to the skin under occlusion for 24 h. There was slight erythemas in 4/6 rabbits 0.5 h after removal. There were no irritating effects at 72 h. The authors concluded that precipitated Silica was non-irritating.

Another unpublished study reported a dermal irritation study of precipitated Silica (33 mg) on rabbits (n = 6). The test substance was applied to the skin under occlusion for 24 h. There was slight erythema at 24 h after removal. The authors concluded that precipitated Silica was non-irritating (UNEP 2004).

### DERMAL SENSITIZATION

There were no sensitization studies found on Silica or the related ingredients.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### SILICA

W. R. Grace & Co. (1981) reported a study in which Silica (500 mg/d) was fed to male and female rats (n = 40) for 6 months. After 4.5 months, 5 females were mated. There were no adverse effects observed for mortality,

body weight gain, hematology, and reproductive performance. Histology of the stomach, intestines, pancreas, liver, and kidneys were similar to controls. Litter size, birth weight, morphology, and development of the offspring were similar to controls.

In another study, pregnant female mice were fed up to 1340 mg/kg Silica for 10 days. There were no effects on nidation or on maternal or fetal survival. Fetal abnormalities were similar to controls.

The same results were reported for rats (up to 1350 mg/kg for 10 days), hamsters (up to 1600 mg/kg for 5 days) and rabbits (up to 1600 mg/kg for 13 days) (W. R. Grace & Co. 1981).

Lewinson et al. (1994) administered fumed Silica (500 mg/kg/day) to female Wistar rats in their feed. The female rats were mated with male rats from the subchronic study (above) at weeks 8 and 17. The rats were weighed periodically, blood sampled monthly (except during pregnancy), and observed daily. The progeny from both litters were examined for abnormalities. At 6 month, the rats were killed and necropsied except for 5 rats which had a 3 week treatment free period. Then they were killed and necropsied.

There were no clinical signs during treatment. Body weight and feed consumption were similar between treatment and control groups. Hematological parameters and organ weights were unremarkable. Reproductive performance was similar between groups. Pathological examination revealed no differences between the groups.

At the first mating, 6 control and 9 treatment dams became pregnant; 7 from each group became pregnant at the second mating. There were no treatment-related effects in litter size, birth weight, physical parameters, or behavior. Development of progeny during lactation was without adverse effects; weight gains were normal. No treatment related effects were found during gross pathology. The authors conclude that the NOEL was 500 mg/kg for developmental and reproductive toxicity (Lewinson et al. 1994).

## GENOTOXICITY

### SILICA

Kanematsu et al. (1980) performed a Rec assay and an Ames assay (using *Escherichia coli* TA98, TA100, TA1535, TA1538) on Silica. Both assays were negative at 0.001 to 10 M.

Prival et al. (1991) performed an Ames test on synthetic Silica (0.033 to 10 mg/plate in dimethylsulfoxide [DMSO]) using *Salmonella typhimuriun* (TA98, TA100, TA1535, TA1537, TA1538) and *E. coli* (WP 2) with and without metabolic activation. All results were negative.

Lewinson et al. (1994) exposed *S. typhimurium* (strains TA98, TA100, and TA1535) and *E. coli* (WP2uvrA)

to a toluene extract of fumed Silica (5 to 1580 µg/plate) with and without metabolic activation. The toluene extract of fumed Silica was not mutagenic at any concentration with or without activation. In an additional test, the extract was not mutagenic to *S. typhimurium* TA98 where the epoxide hydrolase inhibitor and glutathione depletor 1,1,1-trichloropropene-2,3-oxide was added to the activation mix to increase sensitivity of the test toward compounds that are activated to mutagenic epoxides.

Liu et al. (1996) performed an in vitro micronucleus test using Chinese hamster lung fibroblasts on Silica (20, 40, 80, and 160 µg/cm$^2$; 0.12, 0.23, 0.46, and 0.93 mg/ml). There was weak, but significant, dose dependent induction of micronuclei at cytotoxic concentrations with the results of the 2 highest dose groups (13.33 ± 1.77, $p < .05$; 18.00 ± 2.08, $p < .01$) being greater than controls (7.67 ± 2.33; correlation coefficient 0.96). No clastogenicity was observed in concentrations lower than cytotoxic levels.

Zhong et al. (1997) performed a single-cell gel/Comet assay using Chinese hamster fibroblasts (V79) and human embryonic lung fibroblasts (HEL 299) on Silica (68.9 and 137.9 µg/cm$^2$). There was a dose dependent increase in DNA migration in the gel in both cell types in a similar manner.

UNEP (2004) reported several unpublished mutagenicity studies of Silica. The studies are summarized in Table 13.

**Table 13.** Unpublished mutagenicity studies of Silica reported by UNEP (2004).

| Test | Test subjects | Silica type (concentration) | Results |
|---|---|---|---|
| Ames, with and without metabolic activation | *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) | Fumed Silica (667 - 10,000 µg/plate) | Negative |
| Chromosomal aberration test, with and without metabolic activation | Chinese hamster ovary (CHO) cells | Fumed Silica (19 - 300 µl/ml without S9, 250 - 1000 µl/ml with S9) | Negative |
| Hypoxanthine-guanine phosphoribosyl transferase test (HGPRT) | CHO cells | Fumed Silica (10 - 250 µg/ml without S9, 100 - 500 µg/ml with S9. | Negative |
| Unscheduled DNA synthesis | Primary rat hepatocytes | Fumed Silic (0.3 - 1000 µg/ml | Negative, cytotoxic at 260 - 500 µg/ml |

## MUTAGENIC INHIBITION

### HYDRATED ALUMINUM CALCIUM SODIUM SILICATE

Abdel-Wahhab et al. (1998) incorporated AF (2.5mg/kg feed) with or without HACSS (0.5%) into the feed of Sprague-Dawley rats (n = 10) for 15 days. The rats were killed and bone marrow samples were collected for

chromosomal analysis. AF caused structural and numerical aberration of chromosomes, mainly chromatid breaks and chromatid gaps. HACSS decreased these effects for every category of aberrations except polyploidy. HACSS alone did not cause an increase in aberrations.

Şişman (2006) incorporated AF $B_1$ (0, 0.2, 0.5, or 0.8 ppm) with and without HACSS (5.0 or 10.0 ppm) into the agar feed of adult Oregon-R wild type *Drosophilia melanogaster* flies. The flies were then paired and mated and the offspring observed. The low, mid, and high dose of AF caused the retardation of development of the $F_1$ adults by 1, 2, and 3 days, respectively. Both doses of HACSS prevented the delayed development. Malformations in the AF treated groups increased from 0.38% (control) to 7.35%, 9.10% and 11.11%, respectively. The low and high dose of HACSS reduced malformations but not to the levels of the controls. The AF reduced the number of offspring ($p < .05, .01$). HACSS mitigated this effect but not to control levels. No ill effects were reported due to HACSS, only protective effects.

Türkez and Şişman (2007) exposed human peripheral blood lymphocyte cultures to AF ($AFB_1$; 1, 5 or 10 µM) with or without HACSS ($5 \times 10^{-6}$ or $1 \times 10^{-5}$ M) and measured sister chromatid exchages (SCEs). Treated lymphocytes did not show any increases in SCE frequencies. SCE frequency increases by $AFB_1$ was completely inhibited by the high concentration of HACSS.

## CARCINOGENICITY

### ORAL

SILICA

Campbell (1940) exposed 3 month old mice susceptible to tumors (n = 75) to aerosolized precipitated Silica (0.5 g/d; ≤ 5 µg) once/h, 6h/d, 5 d/week for a year. The mice were allowed to live out their natural life span up to 917 days from the start of the experiment. Incidence of primary lung tumors was 7.9% in the control group and 21.3% in the treated group in mice living 10 months or longer. There was no obvious fibrosis in the lung tissue; there was fibrotic nodules in the tracheo-bronchial lymph nodes in > 50% of the mice. The author suggested that most of the Silica dust was removed by cilia action through the trachea and also through the lymphatics. Half of the treated mice had overgrowth of the mediastinal connective tissue covering the tracheo-bronchial nodes which occurred on only 10% of the controls. In the treated group, 29.5% had an increase in incidence of overgrowth or hyperplasia of the tracheo-bronchial lymph nodes compared to 14.3% of the controls.

In the female mice consuming precipitated Silica in feed (see ORAL CHRONIC TOXICITY for details), the

frequency of adenocarcinomas in the lungs was 1/16 (6.25%) for the control and 1/19 (5.3%), 0/20 (0%), and 1/20 (5%) for the low, mid, and high dose groups. In the males, the frequency of adenocarcinomas in the lungs was 1/16 (6.25%) for the control and 2/17 (11.8%), 3/14 (21.4%), and 3/16 (18.8%) for the low, mid, and high dose groups. There was low correlation of hyperplastic nodules/hepatocellular carcinoma/hemangioma/fibrosarcoma in the treatment groups compared to controls. The authors concluded that the non-neoplastic lesions were of no toxicological significance (Takizawa et al. 1988).

Lewinson et al. (1994) orally administered fumed Silica to Wistar rats (n = 40; 20 males, 20 females) in their feed (100 mg/kg) for 24 months. The rats were weighed before and after treatment. The rats were killed and necropsied. There were no clinical signs observed during the treatment period. Comparison of the rates of tumors observed in the treated rats were comparable to historical controls. The authors conclude that there were no carcinogenic effects due to Silica exposure.

INTRATRACHEAL

SILICA

Pott and Roller (2005) intratracheally instilled Silica (3 mg in 0.9% PBS; 0.01 to 0.03 µm) into female SPF Wistar rats (HsdCpb:WU) (n = 40; 8 to 9 weeks old) 5 times weekly. The rats were then followed until death or the 30$^{th}$ month when they were killed and necropsied. A second group had Silica instilled 10 times weekly. Controls (n = 48) were untreated. In the first group, 37 rats survived the entire experiment, 35 in the second group, and 46/48 in the control group. The period of time after the first treatment in which 50% of the rats died was 113 and 112 weeks in the first and second groups and 113 weeks in the control group. The percentage of rats with macroscopic lung tumor(s) was 13.5% in the first group, 2.9% in the second group, and 6.5% in the control group. The percentage of rats with macroscopic lung tumor(s) which are probably not a metastasis of other tumors located elsewhere was 8.1% in the first group, none in the second group, and none in the control group. The percentage of rats with benign tumors in the second group was 5.7% and there were none in the control group; this was not analyzed in the first experiment. Neither group had malignant tumors. The percentage of rats with tumors that were metatases of other tumors was 14.3% in the second experiment and 13.0 in the control group.

## CLINICAL ASSESSMENT OF SAFETY

### ORAL

SILICA

The oral lethal dose of fumed Silica in humans is 15 g/kg and 99% of the Silica ingested is secreted (FDA no date).

Worth and Campen (1951) assessed the Silica level in the blood of volunteers (n = 264) before and after the oral administration of colloidal Silica protein or silica acid, tetrayglycol ester (amount not provided). There was a rapid increase of Silica blood levels and a rapid elimination in the urine over 8 to 24 h. There was no influence of sex, age, employment, lung disease (dust lung), or other disease.

UNEP (2004) reported an unpublished study or orally administered Silica (1250 mg in apple juice) in the form of fumed (n = 6; 5 males, 1 female) and precipitated (n = 6; 5 males, 1 female) Silica, to volunteers. The solutions were consumed in 2 doses, morning and midday on the same day. The total urine was collected daily and analyzed. During the 4 days post-treatment, changes of the renal Silica secretion were not observed. Daily Silica increments in urine after ingestion ranged between 7 and 23 mg. For the fumed Silica, the individual baseline values of the pre-test phase were very variable and individually different; mean excretion rates ranged from 25 to 87 mg/day. In the post-treatment phase, individual mean excretion rates ranged from 32 to 61 mg/day. For the precipitated Silica, the individual baseline values of the pre-test phase were very variable and individually different; mean excretion rates ranged from 16 to 71 mg/day. In the post-treatment phase, individual mean excretion rates ranged from 20 to 81 mg/day. Overall, increases in excretion were not unequivocally detectable. The authors noted that the small apparent increases were in marked contrast to the high dose of 2500 mg Silica applied.

In an unpublished study on the effectiveness of Silica gel in the treatment of type II hyperlipoproteinemia, 6 adults (3 men, 3 women; 20 to 51 years old) were admitted to a metabolic unit for 3 weeks. Four subjects were on a liquid formula diet containing 100 mg cholesterol/d and a ratio of poly unsaturated to saturated fat (P/S) of 1.0. The rest were on a solid food diet containing 200 mg cholesterol/d and a P/S of 2.0. Silica was administered with the morning and evening meals starting with an oral dose of 1.0 g/d which increased by 1.0 g/d until 16 g/d was reached. There were no effects to plasma levels of total cholesterol, low density protein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, or total triglycerides observed. There were no increases in the serum or urinary levels of Silica. The number of white and red blood cells and platelets were unaffected. Two subjects had a decrease in serum iron levels, 1 had a decrease in hemoglobin concentration, 2 had a decrease in carotene, 1

had a decrease in serum folate, and 1 had a decrease in vitamin A. Clinical side effects included constipation in half the subjects and an unusual aftertaste in all subjects. One subject had gastritis. The authors judged that there were no adverse effects of the Silica on hepatic or renal function. Silica gel was not absorbed significantly from the intestine (UNEP 2004).

## DERMAL IRRITATION

### SILICA

Epstein et al. (1963) injected colloidal Silica (1 to 4 mg in saline; ~15 µm) subcutaneously 2 to 8 times in volunteers (n = 28). Biopsies were taken from day 1 to 6 months. Granulomatous inflammation was observed within 7 days and persisted for months. The cells invested blood vessels and did not organized into tubercles. The authors suggested that this was a particular type of foreign body response to a fibrogenic agent and not typical epithelioid cell nodules.

W. R. Grace & Co. (1981) reported a dermal study of a dusting powder that included micronized Silica gel (amount not provided) on patients (n = 300). The authors concluded that the powder was non-irritating and non-toxic and could be safely applied to babies, children, and adults. From the allergic tests performed, there were little or no sensitizing properties of the powder.

## DERMAL SENSITIZATION

### SILICA

UNEP (2004) reported an unpublished sensitization study of colloidal Silica (45%). Patches were applied to volunteers (n = 20; 10 men, 10 women) for 6 days. After 2 weeks, challenge patches were applied for 48 h. Skin under the patches was examined at 1, 2, 3, and 6 days after the first application and on removal of the challenge patch. No skin reactions were observed (UNEP 2004).

## OCCUPATIONAL EXPOSURE

### SILICA

Volk (1960) studied workers (n = 215) with exposure to Silica between 1947 and 1959 with chest x-rays. Exposure ranged from 15 to 100 mg/m$^3$, 2 to 6 mg/m$^3$, and 3 to 7 mg/m$^3$ , depending on workstation. Hairline actuation of the interlobar fissures, suggesting slight interlobar pleuritis, was the only remarkable sign. There were no signs of silicosis.

Plunkett and DeWitt (1962) examined 78 workers (aged 21 to 67 years; average 34.23 years) who had been occupationally exposed to precipitated Silica from 1941 to 1959. Dust concentrations ranged from 0.35 to 204

$mg/m^3$. There was no evidence of silicosis or other pulmonary disease.

Wilson et al. (1979,1981) examined workers (n = 165) exposed to precipitated Silica a mean of 8.6 years (44 workers had been exposed a mean of 18 years [10-35 years]). Dust levels varied from <1 to 10 $mg/m^3$ with some higher intermittent levels. Examination included spirograms, respiratory questionnaires, and chest radiographs. Cough and dyspnea correlated with level/time of smoking and not Silica exposure. There was no correlations between yearly change of pulmonary function and dose nor time of exposure. The workers with the mean exposure time of 18 years had pulmonary function similar to the rest of the group. There was radiographic evidence of minimal pneumoconiosis that was biased due to prior exposure to limestone. None of the 143 workers exposure only to Silica showed radiographic evidence of pneumoconiosis.

Choudat et al. (1990) examined workers (n = 41) exposed to precipitated Silica and compared them to a control group. The examination included blood gas analysis and chest radiographs. There was a reduction in forced expiratory flow in the exposed group. There was no correlation between the exposure index and pulmonary function. The authors concluded that smoking and exposure to Silica synergise to induce small airway disease.

UNEP (2004) reported an unpublished study of workers (n = 200) with intensive and regular contact with Silica from 1972 to 2000. Thre was no evidence of skin allergy caused by the Silica. There were signs of irritation due to the desiccative and defatting properties of Silica which resulted in skin dryness which could be controlled by regular use of skin-protection ointment.

In another unpublished study, an occupational study of workers (n = 143) exposed to Silica from 1959 to 1985. Exposure ranged from 1 to 34 years. There were complaints of some disorder or exhibition of abnormalities in lung function or histology in 54/142 (36%) of the workers. Dry cough, expectoration or dyspnea was reported in 34 of these workers. A total of 42/54 (78%) of these workers had some possible confounding factor (i.e., smoking). Radiological examination did not show any signs of fibrotic disease. Spirometric examination showed obstructive and/or restrictive ventilation disturbances in 24 workers. Most of the adverse findings were associated with confounding factors.

In an unpublished occupational exposure study, an x-ray was take of 99 workers who had manufactured Silica for various amounts of time. The x-rays revealed no evidence of any occupational disease including silicosis.

In an unpublished occupational study of workers in precipitated Silica factories (1952 to 1981), there was no silicosis in workers employed for 1 or >20 years (mean 13.2 years). There were negative results in hematology,

urine analysis, lung functions, and chest x-rays (UNEP 2004).

HYDRATED SILICA

UNEP (2004) reported an unpublished study of workers (n = 78) in a factory that manufactured Hydrated Silica pigment between 1941 and 1959. Dust concentrations ranged from 0.35 to 205 mg/m$^3$. No evidence of silicosis or other pulmonary disease was observed. The incidence of illness and injuries were similar to other workers in this plant (UNEP 2004).

## REGULATION

OSHA (1978) standard for exposure to amorphous Silica is 20 mppcf air averaged over an 8-h work shift.

## SUMMARY

This is a safety assessment of Silica, Alumina Magnesium Metasilicate, Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, and Sodium Potassium Aluminum Silicate. Silica is a silicon-oxygen tetrahedra where a silicon atom is central within 4 oxygen atoms that are shared with adjacent silicon atoms. There are many forms of Silica. Only the safety of amorphous Silica is evaluated in this assessment, not crystalline.

Free silanol groups on the surface of Silica particles influence the adsorption behavior. Silica has thixotropic properties.

Amorphous Silica is the product of a high heat process applied to crystalline Silica. Impurities include calcium, sodium, potassium, antimony, barium, chromium, arsenic, lead, mercury, cadmium and selenium. There may be some crystalline silica.

Silica was used in a total of 2406 cosmetic products. Use concentrations ranged from 0.0000003% to 40%. Hydrated Silica was reported to be used in 147 cosmetic products at 0.001% to 34%; Alumina Magnesium Silica in 732 products at 0.001% to 0.002%; Aluminum Calcium Sodium Silicate in 5 cosmetic products at 0.4% to 6%; and Sodium Potassium aluminum Silicate at 0.001% to 4%. There were no reported uses or concentration of use reported for Aluminum Iron Silicates.

Aluminum Calcium Sodium Silicate it is generally recognized as safe (GRAS) in food for use at a level not exceeding 2% and Sodium Silicate is GRAS for dry food packaging.

Orally administered Silica was mostly excreted through the urine in guinea pigs. Over time, Silica may or

may not accumulate in the liver, kidneys, or spleen. Intraperitoneal injection of Silica in guinea pigs was mostly excreted through the urine.

When Silica is inhaled by rats, it accumulates in the lungs and lymph nodes initially. The accumulated amount remains at a steady state with continued treatment. When treatment ceases, the Silica decreases. When subcutaneously injected into rats, Silica is absorbed over a few months.

When Silica is inhaled or intratracheally instilled by rats, mice, and rabbits, there is a transient inflammatory response that resolves in days. Incubated macrophages ingested fewer Silica particles than *C. albicans* or *S. cerevisiae*. Ultra-fine Silica (14 nm) did more damage to the lungs during the inflammation than did fine Silica (213 nm).

Silica is not cytotoxic to Chinese hamster V79 cells. Micronuclei were induced in Chinese hamster cells when incubated with 80 and 160 g/ml. Micronuclei were induced with the presence of Silica. There was ~85% lysis of sheep blood erythrocytes incubated with Silica. Mesothelial cells incubated in Silica were observed to accumulate Silica in the cytoplasm, around the nucleus, and vacuoles. Silica caused intacelluar alteration in calcium homeostatis in renal cells.

Alveolar macrophages exposed to Silica had increased protein kinases, $NO_x$ production, and cell death. Human primary fibroblasts exposed to Silica produced COX-2 and $PGE_2$ in a dose dependent manner. COX-1 was not affected. Silica was not cytotoxic to human mesothelioma and rodent fibroblast cells.

Sodium Potassium Aluminum Silicate, in the form of Mexicali dust, induced anaphasic alterations in Balb3T3 cells.

HACSS is used to counter the effects of aflatoxin in animal feed.

Silica was lethal to various bacteria (*A. aerogenes*, *P. putida*, *Proteus* sp., *E. coli*, *B. subtilis*, *S. aureus*, and *C. albicans*).

Silica was reported to have an oral $LD_{50}$ up to 5.62 g/kg in rats and > 8,000 mg/kg in mice.

Aluminum Calcium Sodium Silicate had no adverse effects up to 800 mg/kg in mice.

The dermal NOEL for Silica is > 2000 mg/kg for rabbits. When applied as an aqueous paste, there were no adverse effects.

Intraperitoneally injected Silica was lethal to 20% to 30% of rats and rabbits at 100 mg/kg; all survived at 50 mg/kg. Silica was fatal to guinea pigs at 10%. Rats suvived 5 days after injection of 0.5 g Silica.

Silica injected in the veins of mice were better tolerated in small doses than in 1 large dose. The lethal dose ranged from 0.2 to 0.5 mg/30 g body weight, depending on particle size. The intravenous $LD_{50}$ of Silica was 15 mg/kg in rats.

The minimum lethal dose of intratracheal administration of Silica was 1.8 mg/$cm^3$ in rats. In rats, Silica at 30 and 50 mg/kg was fatal to 80% to 90% immediately or within a few hours; these doses were nearly always fatal for rabbits. Intratracheally administered Silica at 5% killed 2 of 10 rats after 1 injection and 3 more before a third weekly injection. Two of 10 guinea pigs died under the same treatment. The effects on the lungs by Silica may depend on particle size and surface properties.

The acute inhalation of Silica by rats resulted in restlessness, droopy eyelids, lethargy, and dyspnea during treatment. Clinical signs resolved quickly after treatment and necropsies were unremarkable.

Silica instilled into the musculature induced local inflammation for up to 6 month with granulomatous scarring with necrotic muscle fibers and fatty degeneration of local macrophages.

Short-term oral doses of Silica produced no clinical effects for dogs. Highest no effects level was 800 mg/kg/d. Short-term oral doses of Silica produced no clinical effects for rats. HNELs were up to 1000 mg/kg/d.

Short-term dermal application of Silica to intact and abraded skin resulted in no dermal toxicity in rabbits.

Short-term inhalation of Silica up to 668 mg/$m^3$ resulted in respiratory distress during treatment and a short-term inflammation response in the lungs, which resolved quickly when treatment ceased in rats. The lung clearance half-life was ~50 day for 50.5 and 154 mg/$m^3$. The NOAEL was 10.1 mg/$m^3$.

Intravenous injections of 1% Silica biweekly for 20 weeks into 5 rabbits resulted in 2 deaths. One more died during recovery. There was pleural effusion with pleuritis, mediastinal abscess, and marked congestion. Right ventricles were dilated, the livers enlarged, and the spleen atrophied.

The oral subchronic HNEL was 5000 mg/kg/d, the NOEL was 500 mg/kg/d and the lowest effect level was 500 mg/kg/d for rats. There were no clinical signs up to 7950 and 8980 mg/kg/d for males and females, respectively. There were no gross findings of toxicity up to 50,000 ppm in feed.

Subchronic inhalation of Silica at 53 mg/$m^3$ caused 44% mortality in rats from pulmonary vascular obstruction and emphysema. There was increased respiration rates and decreased weight gain during treatment. Necropsy findings included congestion of the lungs, lymph node enlargement, emphysema, vacuolated cells within alveolar spaces, and increased lung weights and collagen content. NOEL was 1.3 mg/$m^3$.

Silica incorporated into the feed at up to 10% of rats and for 6 months or more produced discolored stool

and unremarkable necropsies. Mice had similar results for up to 103 weeks.

Mice bred to be susceptible to tumors exposed to aerosolized Silica at 0.5 g/d for a year had increased incidence of lung tumors with no obvious fibrosis of the lung tissue but fibrotic nodules in the tracheo-bronchial lymph nodes. At 53 mg/$m^3$ for a year, treatment related deaths were 75% in rats from pulmonary vascular obstruction and emphysema starting in the 4$^{th}$ month.

Exposure to aerosolized Silica at guinea pigs exposed to Silica at 1.5 mg/$ft^3$ for up to 24 months had no deaths. A chronic reaction of the lung tissue was established at 4 months and emphysema after 4 to 8 months. Histologically, there was periductal and peribronchiolar intra-alveolar accumulations of the giant cells. In the lymphoid tissue, medullary hyperplasia with the formation of slight amounts of reticulum was prominent during the second year of exposure. There were no macroscopically visible anomalies after 1 year of recovery.

At 126 mg/$m^3$ of Silica for up to 24 months, guinea pigs and rabbits had increased lung weights and particle-phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue. There was complete reversibility of Silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent.

Rabbits exposed to aerosolized Silica at 1.5 mg/$ft^3$ for 12 months had progressive functional incapacitation and elevation of hematocrit levels observed in the majority of the rabbits, possibly due to the combined effect of pulmonary vascular obstruction and emphysema. During recovery, the cellular reactions and emphysema regressed but minor focal alveolar mural collagen persisted. Rabbits exposed to 360 mg/$m^3$ for a year emphasema, pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed. Lesions were observed in the liver, spleen and kidney. The LOAEL was 28 mg/$m^3$.

Monkeys exposed to aerosolized Silica at 15 mg/$m^3$ for 12 months had initial decreased activity and body weight gain. There was emphysema at 3 months and considerable cellular infiltration of the alveoli and alveolar septa associated with distention of alvoli or accumulation of exudate and macrophages. After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vacular occlusions, and cor pulmonale. The Silica content remained low and decreased over time.

When monkeys were exposed to different types of Silica, the precipitated Silica group had lower lung volumes. There were no changes in lung volume parameters, but in ventilatory performance and mechanical

parameters, dynamic lung compliance, and forced expiratory flow when exposed to Silica gel. The frequency and size of the cells aggregates varied with the type of Silica (precipitated > fumed > gel).

Rats exposed to aerosolized Silica at ~45 mg/m$^3$ for 1 year had 41 of 120 deaths. There were small white foci under the pleura, enlarged and discolored lymph nodes with formation of collagen and local necrosis, perivascular and peribronchiolar dust cell granuloma with reticulin and collagen figbers, necrotic cells, desquamative catarrh and thickened alveolar septs. After 3 to 8 months recovery, the dust cell ganuloma were fewer and reduced in size with only a few dust cells and fibers. The alveolar septs had not completely disappeared and lymph nodes were enlarged.

Rats exposed to aerosolized Silica at 15 mg/m$^3$ for 12 months has a few macrophage aggregated in the lungs. The LOAEL was 6 to 9 mg/m$^3$.

Silica was a non- to mild ocular irritant in rabbits.

Silica was nonirritating to the intact and abraded skin of rabbits.

There were no sensitization studies found on Silica or the related ingredients.

No reproductive or teratological effects were observed for the oral administration of Silica for rabbits at 1600 mg/kg/d, for hamsters at 1600 mg/kg/d, for mice 1340 mg/kg/d, and rats up to 1350 mg/kg/d. An oral NOEL of 500 mg/kg/d was reported for rats; an oral NOAEL of 1350 mg/kg/d was also reported.

There were no teratological effects to *D. melanogaster* that were fed Hydrated Aluminum Calcium Silicate up to 0.8 ppm.

Silica was not mutagenic using the Rec or Ames test up to 10 M. Silica was not mutagenic in Ames test up to 1580 µg/plate. In a single-cell gel/Comet assay using Chinese hamster fibroblast, there was an increase in DNA migration in a dose dependent manner. A chromosomal aberration test was negative up to 300 l/ml without and 1000 l/ml with metabolic activation. An hypoxanthine-guanine phosphoribosyl transferase test (HGPRT) was negative up to to 250 l/ml without and 500 l/ml with metabolic activation. An unscheduled DNA sythesis test was negative up to 1000 /ml.

A positive sister chromatid exchange test of $AFB_1$ was inhibited by $10^{-5}$ M Aluminum Calcium Sodium Silicate. Hydrated Aluminum Calcium Sodium Silicate at 0.5% in the feed of rats inhibited the effects of AF.

Oral administration of Silica to rats for 24 months was not carcinogenic up to 100 mg/kg/d.

Intracheal instillation of Silica at 0.5 mg twice per week for 30 weeks to rats was not carcinogenic. Silica at 3 mg was not carcinogenic in rats intracheally instilled 5 times weekly for 30 months.

The oral lethal dose of fumed Silica in humans is 15 g/kg.

Oral ingestion of Silica up to 1250 mg resulted in a rapid increase of Silica blood levels and a rapid elimination in the urine over 8 to 24 h with no adverse effect reported.

Silica subcutaneously instilled in humans caused granulomatous inflammation within 7 days that persisted for months. The cells invested blood vessels and did not organized into tubercles.

A powder containing Silica was non-irritating Silica, up to 45%, was non-sensitizing in humans.

Workers in environments with aerosolized Silica had few signs of silicosis or pulmonary disease up to 100 $mg/m^3$. Smoking and exposure to Silica synergise to induce small airway disease. Hydrated Silica also had no evidence of silicosis or pulmonary disease. There were signs of dermal irritation due to the desiccative and defatting properties of Silica.

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