Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of Silica and Related Cosmetic Ingredients

September 25, 2009

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ABSTRACT

This is a safety assessment of silica and the related cosmetic ingredients: alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate. These ingredients are synthetic amorphous silicas and silicates; crystalline silica is not a cosmetic ingredient and is not used in cosmetics. These ingredients are used as bulking agents and for various other functions. The human and animal safety data relevant to the cosmetic use of these ingredients were assessed by the Cosmetic Ingredient Review (CIR) Expert Panel. The Panel concluded that these ingredients are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment when formulated to be non-respirable.

INTRODUCTION

There are 2 categories of silica, crystalline and amorphous. Only the amorphous forms of silica, and more specifically, synthetic amorphous silica and silicates, are used in cosmetics. Accordingly, this safety assessment addresses silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate.

Crystalline silica is not used in cosmetics.

Synthetic amorphous silica is created by 2 methods, wet and thermal. The wet process creates silica gel and hydrated silica (also known as precipitated silica). The thermal process creates fumed silica, which is also referred to as pyrogenic silica in recent publications. These are the only forms of silica addressed in this safety assessment. The fumed silica is not to be confused with silica fume, which is a crystalline form of silica, not used in cosmetics, and is not considered in this safety assessment. Figure 1 demonstrates the different polymorphs of silica.

In each summary of data, the term for the form of silica used by the author is used. When the type of silica is not made clear the term silica is used.

An earlier safety assessment 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, pryrophyllite, sodium metasilicate, sodium silicate, and zeolite. The CIR Expert Panel concluded that these ingredients were "...safe as used in cosmetic products...". The Panel also reviewed potassium silicate, sodium metasilicate, and sodium silicate and concluded that they were "...safe for use in cosmetic products in the practices of use and concentration described in this safety assessment, when formulated to avoid irritation..." (Andersen 2003, 2005).

CHEMISTRY

Definition and Structure

Silica

The CAS No. 7631-86-9 is the general CAS No. which includes all forms of Silicas including amorphous, crystalline, synthetic, and natural forms (United Nations Environmental Programme Chemicals Unit [UNEP] 2004). This safety assessment is limited to amorphous forms of silica.

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, anhydride;
- silicon dioxide; and
- silicon dioxide, fumed (Gottschalck and Bailey 2008).

The current terminology for “silicon dioxide, fumed” is “pyrogenic silica.”

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 an 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 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 production 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)
- colloidal silica (Arts et al. 2007).

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).

Amorphous Vs. Crystalline Silica

Silica is a silicon-oxygen tetrahedral unit 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. Amorphous silica has an irregular tetrahedral pattern. Crystalline silica is polymorphic where each variety has a characteristic regular 3-dimensional arrangement of the tetrahedral (Heppleston 1969). As would be predicted from these descriptions, crystalline silica has a well-defined x-ray diffraction pattern; whereas amorphous forms of silica do not (Villota and Hawkes 1986).

There are 3 classifications of amorphous 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. Microamorphous silica is a dense thermally unstable amorphous silica which converts to quartz at high temperatures. Microamorphous silica includes solutions, 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). Kaewamatawong et al. (2005) divides the microamorphous forms of amorphous silica

into fumed, colloidal, precipitated, diatomaceous earth, gel, and hydrous. The colloidal form ranges in size from 10 micrometers to <10 nanometers.

The different polymorphs of silica are shown in Figure 1. Again, only synthetic amorphous silica forms are used in cosmetics. Crystalline silica forms are not used in cosmetics.

Physical and Chemical Properties

Properties

Silica

The acidity of synthetic amorphous silica is related to the number and reactivity of the silanol groups present on the solid silica surface. Surface silanols (pKa = 7.1) are more acidic than monosilicic acid (pKa = 9.8). The acidity increases with the degree of polymerisation (Yates and Healy 1976).

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.

Amorphous silica is capable of rehydroxylating in aqueous systems to form a high ratio of silanol 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 (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 and Nutrition Board [FNB] 1996).

Cabot Corporatation (2004) stated that silica has thixotropic properties. The particles form a 3-dimensional network in a liquid system which 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 saturation concentrations for a set of analyzed Silicas ranged from 1.91 to 2.51 mmol/l (European Centre for Ecotoxicology and Toxicology of Chemicals [ECETOC] 2006). Saturation concentration increased with specific surface area. Surface treated, hydrophobic silica had a low solubility compared to hydrophilic silica. This was due to reduced wetting of the surface in aqueous systems. Wetting was increased by alcohol which decreased solubility.

Additional chemical and physical properties of silica are listed in Table 1. Physical properties by Brunauer, Emmett, and Teller (B.E.T.; a rule for the physical adsorption of gas molecules on a solid surface that serves as the basis for the

measurement of the specific surface area of a material) surface areas of 200, 325, or 380 m^2/g are shown in Table 2. Note that the properties are the same regardless of B.E.T. in this range.

Particle Size and Form

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).

Primary particles, or single particles, do not exist in isolation in fumed (pyrogenic) and precipitated silica; only in silica sol (colloidal). Aggregates assemble in chains (fumed) or clusters (precipitated and gel). Agglomerates are assemblies of aggregates, held together by strong physical adhesion forces and not in a dispersible nano size (< 100 nm) (ECETOC 2006; Gray and Muranko 2006).

Methods of Manufacture

All of the cosmetic ingredients in this assessment have mineral sources, but may be synthetically manufactured. 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).

Silica

Amorphous silicas used in cosmetics (silica gel, precipitated silica, and pyrogenic silica in Figure 1) are synthetically produced. A manufacturing process for amorphous pyrogenic silica is shown in Figure 2 (Villota and Hawkes 1986).

Lewinson et al. (1994) stated that silica may be produced by a vapor-phase process producing fumed (pyrogenic) silica or by a wet process producing precipitated silica (silica gel or precipitated silica in Figure 1). Fumed silica is produced in a relatively anhydrous state, whereas precipitated silica contains a larger amount of bound water.

Mean particle size, particle size distribution, and degree of aggregation and/or agglomeration can be determined by adjusting the process parameters (Hurd and Flower 1988).

ECETOC (2006) reports that the thermal synthesis process without liquid water results in the presence of fewer silanol groups on pyrogenic silica (Figure 1).

Silicas manufactured by the wet process (silica gel and precipitated silica) contain between 2% and 10% physically bonded water which can be removed by drying.

Amorphous pyrogenic silica (Figure 1) is manufactued by the hydrolysis of volatile silanes, usually silicon tetrachloride, in the flame of an oxygen-hydrogen burner. The silicon tetrachloride is continuously vaporized, mixed with dry air then hydrogen, and then hydrolysed. The silica is then grown (nucleation, condensation, coagulation) and aggregated.

Precipitated silica and silica gels (Figure 1) are produced from an alkali metal silicate dissolved in water (i.e., water

glass) and an acid, usually sulphuric acid. After the reaction of water glass with the acid, silica is precipitated. The properties of the silica can be influenced by the type of reactor and process parameters. The silica is filtered out and the resulting cake is 15% to 25% silica by weight. The cake is then dried and then milled.

Silica gels (Figure 1) are produced by the neutralization of an aqueous solution of alkali metal silicate with sulfphuric acid (gelation). Mixing continues until solidification begins; the gelation conditions dictate particle size in the hydrogel. The gel is then washed of excess salts; this procedure determines specific surface area. The silica gel is now a continuous structure with pores filled with water. The silica may be used as is, or dried. Xerogels are dried until water is still evaporating but the gel no longer shrinks. Aerogels are dried with negligible loss of pore volume.

Silica sols (colloidal silica; Figure 1) are dispersions of silica particles in a liquid, usually water, at 15% to 50%. These are sub-micron particles of silica and the sols flow like water. Silica sols are also produced by hydrolysis of monomeric $SiCl_4$ in aqueous solution followed by condensation of the original particles. Large particles are produced by hydrolysis of tetraethoxysilane in an alkaline solution of water and alcohol. Silica sols are also produced by redisperion of existing silicas (gels, precipitated or, occasionally pyrogenic). For any process, the dispersed silica particles are stabilized by the addition of KOH, NaOH, NH_3, or HCl (ECETOC 2006).

There was no information found on the manufacturing processes for the salts of silica in this report.

Analytical Methods

Silica

Surface silanols of silica may be analyzed by Fischer titration; chlorination with thionylchloride ($SOCl_2$); Zerewitinoff determination with methyl magnesium iodide (CH_3MgI) or methyl lithium (CH_3Li); infrared-spectroscopy; ^{29}Si cross-polarization magic-angle-spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy; titration with caustic soda (NaOH); and thermo-gravimetric analysis (loss on ignition) (ECETOC 2006).

Gas adsorption may be used to determine specific surface area and porosity of pyrogenic silica (ECETOC 2006). Infrared (IR) spectroscopy was used to analyze silanol groups, alkyl groups, and silonol groups. NMR spectroscopy was used to determine relative amounts of mono-, di-, and trialkylsilane groups, cross-linking of alkylsilanes, side chains, ethoxy and methoxy groups, extractable organic compounds, and Si-O-Si bond distribution. Electron spectroscopy was used to analyze composition and chemical state of the surface and concentration gradients and diffusion profiles of silica. Mass spectroscopy was used for trace analysis of the surface of silica. Atomic force microscopy (AFM) was used to analyze morphology and porosity. Gravimetry and titration was used to analyze silanol groups.

Particle size analysis may be done by sieving, cascade impactor, time-of-flight, dynamic light scattering, static light scattering, Fraunhofer diffraction with air disperison, and Fraunhofer diffraction with liquid disperison (ECETOC 2006).

Sayes et al. (2007) stated that the surface area of silica particles may be measured by the B.E.T. method; and size, size distribution, and surface charge by dynamic light-scattering (DLS) spectroscopy.

Impurities

Silica

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.

Pyrogenic silica was reported to be >99.8% pure with Al_2O_3 (< 0.05%), Fe_2O_3 (0.003%), TiO_2 (< 0.03%), Na_2O (<0.0009%), and chlorides as Cl (<0.025%). Precipitated silica and silica gel were reported to be ≥ 95% pure with Na_2O (0.2% to 2.4%), sulphates as SO_3 (0.2% to 3.0%), Fe_2O_3 (<0.05%), and trace oxides (<0.07%) (Roempp 2001).

The composition of colloidal silica was reported to be: SiO_2 (≥30%), Na_2O (0.1% to 0.4%), sulfates as $NaSO_4$ (0.01% to 0.03%), and aluminum oxide as a stabilizer (0.2%) (W.R. Grace & Co. 2003).

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 2009) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), silica was used in a total of 3,276 cosmetic products. Use concentrations ranged from 0.0000003 - 44% according to a survey of current use concentrations conducted by the Personal Care Products Council (Council 2008). Hydrated silica is reported to be used in 176 products in the VCRP, at use concentrations of 0.001 - 34% based on the Council survey. Alumina magnesium metasilicate was not reported to be used in any products in the VCRP, but use concentrations between 0.001 and 0.02% were reported in the Council survey. Aluminum calcium sodium silicate was reported to be used in 7 cosmetic products in the VCRP, at use concentrations of 0.4 - 6% in the Council survey. Sodium potassium aluminum silicate was reported to be used in 1 product in the VCRP, with a use concentration of 0.001 - 4% in the Council survey. There were no reported uses in the VCRP or concentration of use reported in the Council survey for Aluminum iron silicates. Available data for the number of uses and use concentrations as a function of cosmetic product type are given in Table 3.

Silica is used in 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, $\mathbf{d_a}$, 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 as 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

Aluminum calcium sodium silicate (sodium calcium aluminosilicate, hydrated) 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 (FDA

2007).

Hydrated aluminum calcium sodium silicate is used for countering the effects of aflatoxin (AF) in animal feed (Colvin et al 1989; Kubena et al. 1991; Chestnut et al. 1992; Harvey et al 1994; Smith et al. 1994; Abo-Norag et al 1995; Sarr et al. 1995; Edrington et al 1996; Abdel-Wahhab et al. 1998; Kubena et al. 1998; Ledoux et al. 1998; Mayura et al 1998; Bingham et al. 2004; Sehu et al 2007).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism and Excretion

Oral

Silica and Hydrated Silica

Sauer et al. (1959a,b) orally administered silica (80 mg/dose) in the form of sodium metasilicate, hydrated silica, 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 return to normal.

The urinary output of silica, in the form of silica solution 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. When orally administered, 63% of the silica was recovered. The authors suggested that all of the silica in the urine was in the soluble or molybdate reactive form and that the silica particles underwent depolymerization prior to excretion (Sauer et al. 1959a,b).

UNEP (2004) reported an unpublished oral study of silica (1500 mg/kg/d) using female rats (strain and n not specified). Silica was orally administered daily for 30 days. The rats were then killed and necropsied. 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 (n not specified) 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 µg), in the spleen was 5.5 µg (7.2 µg), and in the kidneys was 14.2 µg (7.8 µg) (UNEP 2004).

Parenteral

Silica and Hydrated Silica

Sauer et al. (1959a) also administered silica solution or precipitated silica by intraperitoneal (i.p.) injection to guinea pigs (n = 5; see ORAL section above for details). Urinary silica increased above normal for 16 days. The levels of silica as silicic acid increased above normal for 28 days. Recovery of silica was 48%. The authors suggest that conditions in the peritoneal cavity favor the depolymerization and subsequent excretion of silica.

Intratraceal

Silica and Hydrated Silica

Byers and Gage (1961) intratracheally injected 3 types of silica (25 mg; 2.5% in 1 ml suspension) into adult albino Wistar rats (n = 100; 50 male, 50 female). 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. Rats were killed and necropsied at 12, 24, and 52 weeks. The amounts of silica in the tissues are given in Table 4.

The 3 types of silica elicited the same type of response in the lungs between types and sexes. Distribution of silica particles 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 through 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 lesions decreased in size and number over time and as 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 and Hydrated Silica

Klosterkötter and Bünemann (1961, 1962) exposed female rats (strain and n not provided) to aerosolized pyrogenic or precipitated silica (concentration and particle size not provided) for up to 6 days. After 3 months of recovery, 73.8% of the inhaled silica had been eliminated from the lungs. Only small amounts of silica were observed in the mediastinal lymph nodes.

In a second similar study, female rats were exposed to aerosolized pyrogenic or precipitated silica (particle size not provided) to study elimination from the lungs. Elimination was only slightly influenced by particle size. The lymphnodes were moderately enlarged with a silica content of < 2% eliminated. Most of the silica was eliminated within 1 to 2 months. Small amounts were detected in the lymph nodes. The precipitated silica, which was less soluble, was more slowly eliminated than the more soluble pyrogenic types (Klosterkötter and Bünemann 1961,1962).

UNEP (2004) reported several unpublished studies. In an unpublished inhalation study of silica (0.050 to 0.055 mg/l; particle size not provided) 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. Corresponding to the respiration volume, 1% of the inhaled silica was retained in the lungs. After a recovery period 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; levels of deposited silica were low from 18 weeks to 12 months of exposure.

In another unpublished inhalation study, female inbred albino rats (strain not specified; n not provided) were exposed to aerosolized silica (dose and particle size 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 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 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; particle size not provided) 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 pyrogenic silica (55 mg/m^3; particle size not provided), 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 pyrogenic silica, the mean retention value was 0.130 mg/lung. For the precipitated silica, the mean silica-content of the lungs after 4 months recovery 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).

ECETOC (2006) reported an unpublished study in which rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (50 mg/m^3; particle size not provided) for 1 or 3 days. The rats were killed and necropsied after 20 h, 1 month, or 3 months. At 1 month recovery, elimination of silica was 78% (1 day exposure) and 75% (3 days exposure). After 3 months recovery, elimination was 87% and 92%, respectively. There was little silica in the mediastinal lymph nodes.

In another unpublished study, rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (200 mg/m^3; particle size not provided) for 5 h/d for 3 days. After 3 months recovery, 81% of the silica was eliminated. Elimination by the lymph nodes was marginal (ECETOC 2006).

Subcutaneous

Pyrogenic 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.

ECETOC (2006) reported an unpublished report where pyrogenic silica (10 mg in water) was subcutaneously injected

in rats (strain and n not specified). It was quickly removed from the injection site. Mean recovery was 6.90 mg at 24 h, 0.65 mg after 1 month, and 0.30 mg after 2 months.

In another study, pyrogenic silica (30, 40, or 50 mg in water) injected subcutaneously in rats was 95% to 97% recovered after 6 weeks (ECETOC 2006).

Cytotoxicity

Pyrogenic and Hydrated Silica

Mammalian Cells

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 pyrogenic or hydrated (precipitated) silica or silica gel (13.5, 25 or 50 µg/cm^3) with mouse macrophages for 18 h. Pyrogenic silica and silica gel were cytotoxic to mouse macrophages similarly to crystalline silica (also tested); precipitated silica was less cytotoxic. The author concluded that all 3 silica types were cytotoxic at 13.5 µg/cm^3.

Zimmerman et al. (1986) exposed macrophages and neutrophils from C57BL/6 x DBA/2)F_1 (DBF$_1$) mice to pyrogenic 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 phagocytize sheep red blood cells, less so on neutrophils. The ability of these cells to phagocytize *Listeria monocytogenes* was completely inhibited 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.

Nyberg et al. (1996) incubated macrophages from male Sprague-Dawley rats with silica particles (3.2 ± 0.4 µm) for 30 min. The number of ingested yeasts were higher than the control silica particles. Silica treated macrophages were similar to resting macrophages.

Pandurangi et al. (1990) incubated sheep blood erythrocytes (1%) with pyrogenic 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/ml 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), or saline (control) for 1 h. The effect of silica (0.6 to 600 µg/ml) on cell injury was examined using trypan blue exclusion. After 1 h, silica-induced injury 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.

Kim et al. (2002) exposed alveolar macrophages collected from the lungs of Sprague-Dawley rats to silica (0.5 - 10 µm particle size). 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 activation processes, protein kinases, and calcium transport.

Human Cell Lines

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 cyclooxygenase (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 of COX-1 was induced at all concentrations. Prostaglandin (PG)E_2 was increased in a dose and time dependent manner, ~7 times more potent than crystalline silica. There was no increase in interleukin (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, monocyte chemoattractant protein (MCP)-1, or transforming growth factor (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 and 3 days, respectively. By measuring both the 3-(4,5-dimethylthiazol-2-yI)-2,5-diphenyltetrazolium bromide (MTT)-conversion and DNA content, there were no effects on the cells by silica.

Sayes et al. (2007) incubated immortalized rat L2 lung epithelial cells, rat lung alveolar macrophages, or both of these cells combined with silica. In L2 cells, silica produced increases in LDH levels at 520 µg/cm^2 of cell culture at 4 h. At 24 and 48 h, LDH levels increased over controls at 5.2, 52, and 520 µg/cm^2 in L2 cells. In alveolar macrophages, silica produced no increase in LDH levels up to 48 h and 5200 µg/cm^2. The 2 cell types were cultured together; silica produced no increase in LDH except for 520 µg/cm^2 at 24 and 48 h.

Silica produced decreases in cellular MTT levels at doses of 5.3 and 52 µg/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 µg/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 µg/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 µg/cm^2. The authors concluded that there was little correlation between in vivo (see Intratracheal section below) and in vitro results (Sayes et al. 2007).

Bacterial Cells

Several strains of bacteria (0.15 ml bacterial suspension) were exposed to pressed and unpressed high purity silica (0.2 g). Rod-shaped gram-negative strains (*Escherichia coli*, *Bacterium proteus*, *Pseudomonas aeruginosa*, and *Aerobacter aerogenes*) died between 6 h and 3 days in contact with unpressed silica. Gram-positive strains (*Proteus* sp., *Micrococcus pyrogenes aureus*, *Streptococus faecalis*, *Streptococcus pyrogenes humanus*, *Corynebacterium diphtheriae*, *Candida albicans*, and *Bacillus subtilis*) were somewhat more resistant. Survival of bacteria exposed to unpressed silica was shorter than pressed silica (Keinholz 1970).

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}). ED_{100} ranged from 6 h to 22 days (UNEP 2004).

Sodium Potassium Aluminum Silicate

Alfaro Moreno et al. (1997) incubated thawed Balb 3T3 cells with Mexicali dust (sodium potassium aluminum silicate present as 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 cells allowed to incubate for

7 h. The cells were fixed. Between 220 and 280 anaphases for each concentrations were 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 cells exposed to Mexicali dust. The authors concluded that Mexicali dust is capable of inducing anaphasic alterations.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Silica

Hazelton Laboratories (1958a) administered a single oral dose of pyrogenic 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 reported to be >3.16 g/kg.

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

Lewinson et al (1994) orally administered pyrogenic silica (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 acute dosing with silica is virtually nontoxic by the oral route.

UNEP (2004) and ECETOC (2006) reported several unpublished studies of acute oral toxicity of silica. The results of these studies are summarized in Table 5. ECETOC (2006) reported that there were no signs of toxicity. There were no macroscopic findings at necropsy. Animals died at doses of 10,000 and 20,000 mg/kg. At doses of 5,620 mg/kg and higher, the feces in some animals were white.

Aluminum Calcium Sodium Silicate

Abbés et al. (2006a) orally administered a single dose of hydrated aluminum calcium sodium silicate (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 hydrated aluminum calcium sodium silicate alone had levels similar to control. The mid and high dose groups had increased cholesterol levels. Hydrated aluminum calcium sodium silicate mitigated the effects of ZEN at all dose levels. No adverse effects were reported for hydrated aluminum calcium sodium silicate alone at any dose.

Abbés et al. (2006b) orally administered a single dose of hydrated aluminum calcium sodium silicate (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. Hydrated aluminum calcium sodium silicate alone had no effect on these parameters.

Parenteral

Silica

Policard and Collet (1954) 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. Edema was observed that diminished with time. The lymph nodes were enlarged and contained large histocytes in various stages of degeneration. Lymphocytes were less numerous; the medullary sinuses were packed with clear cells. The thymus was atrophied. 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 than controls. 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 pyrogenic 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 no, 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.

UNEP (2004) reported an unpublished study that concluded that single i.p. injections of ≥50 mg silica caused death in rats.

Intravenous

Silica

Swensson et al. (1956) injected amorphous silica (0.01 to 0.1 μm diameter particles), in the form of commercial silica, or ground fused crystalline silica (0.15 to 0.45 μm), 0.05 mg at a time up to 0.1 ml in saline or all at once, into the tail vein of mice until the animals died or were in an unrecoverable condition. The mice survived larger quantities of silica if delivered in smaller doses. The toxicity decreased 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 .

In a study described previously, Byers and Gage (1961) injected various amounts of 3 types of silica into rats (strain and n not provided). 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 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 pyrogenic silica using rats. The LD_{50} was 15 mg/kg.

Intratracheal

Pyrogenic and Hydrated Silica

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). 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. 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 cytokines in BAL cells, macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (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.

Jones et al. (2002) explored the kinetics of lung macrophages by instilling silica (50 mg; 5 µm particle size) 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 scanned using positron emission tomography. 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.

Kaewamatawong et al. (2005) compared the effects of particle size of colloidal (hydrated) silica on female ICR mice. Ultrafine (14 nm particle size) 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 necropsied at 30 min and 2, 6, 12, and 24 h.

Both types of silica produced bronchiolar degeneration and necrosis, neutrophilic inflammation 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 of 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 damage than fine silica (Kaewamatawong et al. 2005).

Kaewamatawong et al. (2006) instilled ultrafine colloidal (hydrated) silica (0, 0.3, 3, 10, 30, or 100 µg; 120 mg/ml in water; 14 nm particle size) 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 showed 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 ultrafine silica of the same particle size. The groups were killed at 1, 3, 7, 15, and 30 days and the lungs examined. There was a transient increase in 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 then 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.

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 walls 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 terminal bronchioles and alveolar ducts. At day 15, inflammatory signs were decreased and almost or completely resolved. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) analyses showed an increase of the apoptotic index in lung parenchyma at all time points. 8-Hydroxy-2'-deoxyguanosine (8-OhdG) was detected in lung epithelial cells and activated macrophages and 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 (hydrated) 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 BALF 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.

Inhalation

Pyrogenic and Hydrated Silica

Lewinson et al. (1994) placed Wistar rats (n = 10; 5 males, 5 females) into an exposure chamber circulating pyrogenic silica (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 days 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 the rats gained weight normally. Necropsies were unremarkable.

Warheit et al (1995) exposed male CD rats (n = 24) to aerosolized precipitated (hydrated) 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. Recovery in the high dose group was similar to low dose group. The author concluded that low concentrations of silica induces a transient inflammatory tissue reaction.

UNEP (2004) reported an unpublished inhalation study (nose only exposure) of precipitated silica (< 5 µm particle size) in Wistar rats (n = 10; 5 male, 5 female). The LC_{50} was > 0.691 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 weight gain was normal. There were no remarkable findings at necropsy after a 14-day observation period.

An unpublished inhalation study of precipitated (hydrated) silica (< 5 µm particle size) 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 of the UNEP report considered this study invalid due to methodological deficiences and short length of exposure.

An unpublished inhalation study (full body exposure) of pyrogenic 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 weight gain was normal. One rat had discolored lungs at necropsy (UNEP 2004).

Unpublished studies of the acute inhalation toxicity of hydrophilic and hydrophobic silica are summarized in Table 6 (ECETOC 2006).

Intramuscular

Silica

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

Dermal

Pyrogenic and Hydrated Silica

UNEP (2004) reported an unpublished acute dermal toxicity test of precipitated (hydrated) 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 > 2 g/kg.

In another unpublished study, a single application of 4 different precipitated (hydrated) 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 and no effects by the fourth product (UNEP 2004).

ECETOC (2006) reported several unpublished studies on the acute dermal toxicity of silica to rabbits. Only slight erythema with intact skin and slight erythema and edema with abraded skin were observed. Precipitated silica had an LD_{50} of > 5 g/kg in 4 studies. Silica gel had an LD_{50} of > 2 g/kg in 1 study.

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 effect level (HNEL) for silica was 800 mg/kg/d. In another study using rats, the oral HNEL was 1 g/kg/d. For doses >2 g/kg/d, the rats had dirty fur, shyness, decreased motor activity, and hemorrhage of the mucous membrane of the eyes and nose. There was a decrease in body weights, feed consumption, hemorrhaging, and cellular atrophy in the liver epithelium.

Silica (8 g/kg/d) was incorporated into the feed of Beagle dogs and CD rats (n not provided) for 4 weeks. The animals were then killed and necropsied. There were no indications of any treatment-related effects observed in either species (Newberne and Wilson 1970).

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 effects or mortality reported. Gross necropsy findings were unremarkable (W.R. Grace & Co. 1981).

Lewinson et al. (1994) administered pyrogenic 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 4 g/kg/d after 14 days, to 8 g/kg/d after another 14 days, and finally to 16 g/kg/d. The rats were observed for clinical signs, weighed, and blood sampled before and at the end of the experiment. The rats were killed and necropsied.

Only the 16 g/kg/d dose (~25% of daily feed intake) caused any clinical signs, shyness, dirty fur, reduced activity, cachexia, and hemorrhage in the mucous membranes of the eyes and nose. Two males and 2 females died with severe cachexia 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 1 and 16 g/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 1 g/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 1 g/kg/d and the NOEL was 500 mg/kg/d (Lewinson et al. 1994).

UNEP (2004) and ECETOC (2006) reported unpublished short-term oral toxicity studies of silica. The studies are summarized in Table 7.

Dermal

Silica

ECETOC (2006) reported an unpublished dermal toxicity study of pyrogenic silica (0, 5, and 10 g/kg/d) using albino rabbits (n = 4; 2 male, 2 female). The silica was applied for 18 h/d, 5 d/week for 3 weeks to intact and abraded skin. There were no signs of systemic toxicity. There were no gross or microscopic pathological findings. The silica content of blood, urine, spleen, liver and kidney was similar among groups.

Inhalation

Pyrogenic and Hydrated Silica

Low et al. (1985) and Hemenway et al. (1986) exposed male Fischer 344 rats (n = 45) to aerosolized precipitated (hydrated) silica (30 mg/m^3; particle size not provided) for 6 h/d for 8 days. The recovery period was up to 112 days. During exposure, there was an early and transient influx of cells into 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, transient 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) to aerosolized silica (concentration unclear; particle size not provided) 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 (hydrated) silica (10.1, 50.5, and 154 mg/m^3; diluted 4:1 with deionized, distilled water; particle size not provided) 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 draining lymph nodes. There was a dose-dependent increase in mean lung weight and lung to body weight ratio after 4 weeks of exposure in the mid and high dose groups. The mean lung to body weight 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. Pulmonary 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 reticulo-epithelial (RE) 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 pyrogenic silica (17, 44, 164 mg/m^3; particle size not provided) 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 decreased body weights 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. The absolute and relative liver weights were decreased 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 decreased body weights and feed consumption in the mid and high dose groups. There were 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 were 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 silica in the 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 group (Reuzel et al 1991).

Lee and Kelly (1993) exposed male Crl:CD(SD)BR rats (n = 25) to aerosolized colloidal (hydrated) silica (0, 10, 50, 150 mg/m^3; particle size not provided) 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 were dose dependent lesions observed in the mid and high dose groups but not in the low dose group. Particles were mostly phagocytized 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. They were characterized by slender cytoplasmic processes, phagosomes, myelin figures, cholesterol clefts, and lipid droplets. Migrated particle-laden alveoli macrophages were observed to be necrotic and have released particles in the tracheo-bronchial lymph nodes.

At 3 months, the lungs of the low dose group were normal. The lungs of the mid dose group were 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 bronchioles. The high dose group had decreased numbers of particle-laden alveoli macrophages that 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 silicotic nodules in the perivascular region of the bronchioles (Lee and Kelly et al 1993).

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; particle size not provided) 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.

ECETOC (2006) reported several unpublished short-term inhalations studies of silica. These studies are summarized in Table 8.

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; particle sizes not provided): precipitated (hydrated) 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 tracheobronchial lymph nodes were below detection limits 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 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 at the higher exposure levels, were reversed during the recovery period. The low dose caused no adverse effects.

Intratracheal

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 was abscess formation associated with pneumonitis; accompanying cells were macrophages. Focal granulomatous inflammation was 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 granulomatous inflammation 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 were 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.

Granulomatous inflammation was observed 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 (Schepers et al. 1957d).

Intravenous

Silica

Schepers et al. (1957d) 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 70th day and the last 2 were killed on the 120th and 300th days. There was slight to moderate pleural effusion with pleuritits. The longest surviving rabbits had mediastinal abscesses. The lungs had moderate to marked congestion, which was most severe in the rabbits that died first. There were no foci consolidation but small areas of atelectasis. The lymph nodes were not markedly enlarged. The right ventricles were dilated and hypertrophic, most obviously 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 300th 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.

Subchronic Toxicity

Oral

Pyrogenic and Hydrated Silica

FDA (no date) reported the results of a 90-day rat feeding study of silica. There were no effects and the HNEL was 5 g/kg/d. In another study using rats for 180 days with a 3-week recovery period, the lowest effect 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 pyrogenic 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

silica 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 pyrogenic 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 the animals of both sexes which were kept on normal feed for an additional 3 weeks. The animals were observed daily; blood was sampled and the rats 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 (hydrated) 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 clinical signs; hematological, blood chemistry, and urinary parameters were normal. 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 (hydrated) 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 and the bone marrow analyzed. 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.

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).

ECETOC (2006) reported 2 sub-chronic oral toxicity studies where hydrophobic pyrogenic silica (500 mg/kg/d) was

administered by gavage to Wistar rats (n = 40; 20 male, 20 female) 5 d/week for 6 months. There were no clinical signs nor macroscopic findings at necropsy (ECETOC 2006).

Inhalation

Pyrogenic and Hydrated Silica

Schepers et al. (1957a) placed male and female Wistar rats (n = 25) in inhalation chambers to expose them to pyrogenic silica (average 1.5 mg/ft^3 [53 mg/m^3]; most measurements ranged from 0.7 to 2.4 mg/ft^3 [25 to 85 mg/m^3]; particle size not provided). 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) was 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 died from pulmonary vascular obstruction and emphysema beginning at the 4th 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 pulmonary emphysema after 4 months of exposure with lung distension and superficial alveoli dilation. 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 infiltration of large vacuolated cells within 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 parabronchiolar 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).

Rats (strain and n not provided) were exposed to aerosolized hydrophilic silica (40 to 50 mg/m^3; particle size not provided) for 4 h/d, 5 d/week for 2 to 12 weeks (Klosterkötter 1963). The overall elimination of silica was high without accumulation in the lungs. Equilibrium between retention and elimination was reached quickly. Only 5% to 6% of theoretical deposit of silica was observed after 120 days exposure. There was transfer of silica to the mediastinal lymph

nodes, ~31% of total deposited (1.5% to 2% theoretical deposition). The authors stated that involvement of the lymphatic elimination appeared not to be relevant up to 8 weeks of exposure. The silica particles were able to bypass the lymph nodes and were removed quickly.

Reuzel et al. (1991) reported an unpublished inhalation study of silica (1.3, 5.9, 31 mg/m^3; particle size not provided) 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 above control levels 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 have fibroblastic 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 and 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 of the effects 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 placed 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 mediastinal lymph nodes. Microscopic examination of the lungs revealed accumulation of

alveolar macrophages, intra-alveolar leukocytes, and increased septal 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 pyrogenic 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 activities (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 had 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).

ECETOC (2006) reported an unpublished study where Wistar rats (n = 20; 10 males, 10 females) were exposed to hydrophobic pyrogenic silica (0, 0.51, 2.05, and 10.01 mg/m^3; particle size not provided) for 6 h/d, 5 d/week for 13 weeks. A group of rats was allowed to recover for 13 weeks before being killed and necropsied. Silica was observed in the lungs in a concentration dependent manner at the end of exposure. Silica was observed in the tracheobronchial lymph nodes in 3 of 5 animals in the high dose group. After recovery, the amount of silica in the lungs was below detection limits in the low dose group and only a small amount was detected in the high dose group.

ECETOC (2006) reported an unpublished study in which Wistar rats (n = 20; 10 male, 10 female) were exposed to aerosolized hydrophobic pyrogenic silica (0, 0.51, 2.05, or 10.01 mg/m^3; particle size not provided) for 6 h/d, 5d/week for 13 weeks followed by a 13 week recovery. Most effects were in the high-dose group. There was an increase in asparate-amino-transferase level and alkaline phospatase activities in males. There was an increase in absolute and relative lung and tracheobronchial lymph node weights. The lungs had a red appearance with white spots. There was an accumulation of alveolar macrophges with few PMN cells accompanied by bronchiolar-alveolar epithelial hyperplasia and interstitial

inflammatory cell infiltrates in lungs. The lung draining mediastinal lymph nodes showed increased histiocytosis and macrophage aggregates in paracortex and/or germinal centers.

Chronic Toxicity

Oral

Pyrogenic and Hydrated Silica

Silica (3.2% or 10%) was incorporated into the feed of rats (n = 24; 12 male, 12 female) for 6 months (W.R. Grace & Co 1981). 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.

In another feeding study, rats (n = 24; 12 males, 12 females) were fed silica for 6 months. The low dose males and females consumed an average of 0.78 and 0.55 g silica/week, respectively. The high dose males and females consumed and average of 3.00 and 2.11 g silica/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 decreased serum calcium concentration in the female rats in a dose dependent manner. There were reduced absolute and reduced liver and prostate weights (W. R. Grace & Co. 1981).

Takizawa et al. (1988) fed precipitated (hydrated) 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. 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 parameters. 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

Pyrogenic and Hydrated Silica

Jötten and Klosterkötter (1951) reported that when rabbits were exposed to aerosolized silica (0.2 to 5.0 µm particle size) 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 SUBCHRONIC 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]; particle size not provided) for 8 h/d, 5 d/week for 12 months. Treatment related deaths were 26/35 (75%). After 6 months of exposure, aggregations of focal pigmentation visible as reddish-tan foci of dust were observed. There was also moderate, well-established generalized emphysema and lymph nodes that were greatly enlarged and firm. The majority of the rats died from pulmonary vascular obstruction and emphysema from the 4th to the 9th month. The authors concluded that high subchronic/chronic exposure to amorphous silica causes 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 pyrogenic 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.

Two guinea pigs died from non-experimental 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 observed histologically.

Histologically, the dominant response was bronchial and peribronchiolar intra-alveolar accumulations of 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 bronchioli, but at the expense of bronchioli 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 inflammation, 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 sequelae of the tissue reactions were emphysema, mural fibrosis, and bronchiolar and bronchial ectasia 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 pyrogenic 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 period. There was progressive functional incapacitation and increased hematocrits 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; particle size not provided) 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. Clinical signs 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 had dyspnea and 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 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 pneumonitis. The

LOAEL was 28 mg/m^3 (Schepers 1959).

Schepers (1962) exposed monkeys (*Macacus mulatta*; n = 5) to aerosolized synthetic silica (15 mg/m^3; particle size not provided) for up to 12 months. A monkey was killed and necropsied at 3 and 6 months. The remaining monkeys were killed and necropsied after 12 months of exposure. There were 15 untreated control monkeys. Body weight gains decreased and activity decreased during the initial exposures. At 3 months, emphysema was detectable. There was considerable cellular infiltration of the alveoli and alveolar septa was associated with distention of alveoli or accumulation of exudate and macrophages.

After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vascular occlusions, and cor pulmonale. Cor pulmonale was attributed to the emphysema and alveolar wall destruction. Tracheobrochial 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 pyrogenic silica (~45 mg/m^3; particle size not provided) 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 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 fibers, necrotic cells, desquamative catarrh and thickened alveolar septa.

After the recovery periods, the dust cell granulomas were fewer and reduced in size with only a few dust cells and fibers. The alveolar septa had not completely disappeared. After 3 months, the lymph nodes were enlarged; after 8 months, the size of lymph nodes and the extent of recovery 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 pyrogenic 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 mast 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 pyrogenic silica, but was a few were observed 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 the 3 types of silica for 6 h/d, 5 d/week, 13 or 18 months. The decrease in lung respiratory volume and ventilatory mechanics in the monkeys was more marked in the pyrogenic 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.

Cytoplasmic changes (increases in number of vacuoles) in macrophages in the lungs and tracheal lymph nodes were observed. Large numbers of macrophages and mononuclear cell aggregates (bronchioles, alveolar spaces venules, arterioles) were observed in the lungs. The frequency and size of the cell aggregates varied with the type of silica (precipitated > pyrogenic > gel). Reticulin fibers were present in the aggregates in all 3 groups. In 6/9 monkeys exposed to pyrogenic silica, collagen in varying quantities 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 pyrogenic silica is more detrimental than precipitated silica or silica gel. The smaller particle size of pyrogenic silica may be a contributing factor. UNEP (2004) reviewed this study and noted 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 (hydrated) silica (average of 126 mg/m^3 (3.57 mg/ft^3; particle size not provided) 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 in all species. 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. Mild deposition of reticulin fibers occurred in alveoli with no evidence of collagen formation. Bronchial and

tracheal epithelia remained intact. No epithelization or pleural changes were observed; no neoplasia occurred.

The emphysema was equally distributed between treated and control groups. It was dominated by the diffuse hypertrophic 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 possibly be due to aging and recurrent epizootic pneumontitis. There was complete reversibility of silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent in all species (Schepers 1981).

UNEP (2004) reported several unpublished chronic inhalation toxicity studies of silica. The studies are summarized in Table 9.

ECETOC (2006) reported a series of unpublished studies. Rats (strain and n not provided) were exposed to aerosolized hydrophilic silica (50 to 55 mg/m^3; 30 mg/m^3 respirable [sic]) for 12 months. Rats were killed and necropsied periodically and after 5 months recovery. At 3 days, there was 0.25 mg silica in the lung and 0.5 mg at 6 weeks. At 12 months, ~1% of the total administered respirable silica was observed in the lungs. Initial accumulation was rapid and dropped off between week 18 and 12 months (0.5 mg at 6 weeks; 1.2 mg at 18 weeks; 1.37 mg at 12 months). At 6 weeks, the mediastinal lymph nodes contained ~ 0.02 mg silica and 0.13 at 12 months. After 5 months of recovery, the silica in the lungs decreased to 0.16 mg/lung (88% reduction) and 0.047 mg/lymph node (> 50% reduction).

Rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (50 mg/m^3; particle size not provided) for 5 h/d, 2 d/week for 8 and 12 months. The lungs retained 1.448 mg silica (1.3% of exposure) and 1.759 mg (1.1%), respectively. The lymph nodes retained 0.05 and 0.113 mg, respectively. After 12 month exposure and 1 month recovery, the lungs contained 1.1 mg silica (37.5% elimination) and the lymph nodes contained 0.16 mg. After 3 months recovery, the lungs contained 0.43 mg and the lymph nodes 0.12 mg. After 5 months recovery, the lungs contained 0.41 mg (76.7% elimination) and the lymph nodes 0.13 mg.

Rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (100 mg/m^3; particle size not provided) for up to 1 year. Silica content of the lungs and the lymph nodes was 4.33 and 0.132 mg at 3 months, 6.71 and 0.214 mg at 5 months, and 11.46 and 0.378 mg, respectively. After 6 months of recovery, 55.5% of the silica was eliminated from the lungs. Lymph node elimination could not be observed.

Male rats (strain and n not provided) were exposed to aerosolized hydrophobic pyrogenic silica (0, 10, 50, or 150 mg/m^3; particle size not provided) for 6 h/d, 5 d/week for 12 months. The low dose had no effect. The mid- and high-dose groups had white foci on the lung surfaces and collections of foamy macrophages within the alveoli. The peribronchial lymph nodes were enlarged.

Male Cynomolgus monkeys (*Macaca fascicularis*; n not provided) were exposed to aerosolized hydrophobic pyrogenic silica (0, 10, 50, or 100 mg/m^3; particle size not provided) for 6 h/d, 5 d/week for 12 months. Recovery was 2 or 24 months. The low dose had no effect. The mid- and high-dose groups had interstitial fibrosis, which did not resolve or progress during recovery. Peribronchial lymph nodes were enlarged (ECETOC 2006).

Intratracheal

Silica

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-vinylpyridine-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 was treated 30 times. Two weeks after the last instillation, the rats were killed and the lungs examined. Rats treated with silica had moderate, but transient dyspnea that resolved in 1 to 4 h.

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. 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, PMNs, 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 decreased 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 stated that fibrotic lesions were 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 Zymosan. 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 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 suggested that these developed from acute alveolitis observed after a single administration of silica. The authors also suggested that the lesions resulted from acute epithelial damage at the sites of particle deposition with subsequent (granulomatous) inflammation and production of granulation tissue. Silica is not carcinogenic under these test conditions (Ernst et al. 2002).

Ocular Irritation

Precipitated and Hydrated Silica

Hazelton Laboratories (1958a) instilled a single dose of an aqueous solution of pyrogenic silica (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 mg) 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 strongly hydrophilic silica. There was no irritation when the eyes were rinsed or treated with an aqueous suspension.

In another study, silica (10 mg) was instilled into 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.

Lewinson et al. (1994) instilled pyrogenic or precipitated (hydrated) 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 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 pyrogenic silica. There were no clinical signs. The authors concluded that precipitated silica was slighly irritating to the eyes of rabbits and pyrogenic 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 several unpublished ocular studies. One irritation study of pyrogenic silica (100 mg) used rabbits (n = 3). The silica was instilled without rinsing. There were no signs of irritation up to 96 h after application.

In another ocular irritation study of pyrogenic 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 pyrogenic silica was non-irritating.

In another unpublished study of precipitated (hydrated) silica using rabbits, silica was found to be nonirritating.

In another unpublished study of precipitated silica gel (hydrated; 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 (hydrated) 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 (hydrated) silica (50% w/v in an aqueous slurry) was nonirritating to rabbits (UNEP 2004).

Unpublished ocular irritation studies are summarized in Table 10 (ECETOC 2006).

Dermal Irritation

Pyrogenic and Hydrated Silica

Hazelton Laboratories (1958b) applied pyrogenic 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 pyrogenic 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 pyrogenic 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 pyrogenic silica was non-irritating.

In another unpublished dermal irritation study, precipitated (hydrated) 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 rabbits for 24 h. There were no signs of irritation under either skin condition. The authors concluded that pyrogenic silica was non-irritating.

Another unpublished study reported a dermal irritation study of precipitated (hydrated) 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 (hydrated) 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 (hydrated) 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 (hydrated) 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).

ECETOC (2004) reported an unpublished dermal toxicity study of pyrogenic silica (0, 5,000, 10,000 mg/kg/d) using albino rabbits (n = 4; 2 male, 2 female). The silica was applied for 18 h/d, 5 d/week for 3 weeks to intact and abraded skin. There were no signs of systemic toxicity. There was no difference in dermal irritation between treated and control (cosmetic talc) groups as both produced mild dermal irritation consisting of erythema, atonia, and desquamation.

Unpublished studies of skin irritation of hydrophilic and hydrophobic silica on rabbits are summarized in Table 11 (ECETOC 2006).

Dermal Sensitization

Hydrated Silica

A guinea pig (n =10) sensitization study of hydrated silica (20% in distilled water) was conducted. No reactions were observed in either the test group or the control group (distilled water; n = 5) (Council 1984).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Pyrogenic 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 (specific gestation days not provided). 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 pyrogenic silica (500 mg/kg/day) to female Wistar rats (n not provided) in their feed. The female rats were mated with male rats consuming 500 mg/kg/day 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 matings were examined for abnormalities. At 6 months, the rats were killed and necropsied except for 5 rats which had a 3 week treatment-free period prior to being killed and necropsied.

There were no clinical signs during treatment. Body weights 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 oral NOEL was >500 mg/kg for developmental and reproductive toxicity (Lewinson et al. 1994).

GENOTOXICITY

Mammalian Mutagenicity

Pyrogenic and Hydrated Silica

Liu et al. (1996) performed an in vitro micronucleus test using Chinese hamster lung fibroblasts on silica (20, 40, 80, and 160 $\mu g/cm^3$; 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) and ECETOC (2006) reported several unpublished in vitro mutagenicity studies of silica in mammalian cells. Silica was not mutagenic. The studies are summarized in Table 12.

ECETOC (2006) reported several unpublished in vivo mutagenicity studies of silica gel. Silica gel was not mutagenic The studies are summarized in Table 13.

Microbial Mutagenicity

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 pyrogenic silica (5 to 1580 µg/plate) with and without metabolic activation. The toluene extract of pyrogenic 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.

UNEP (2004) and ECETOC (2006) reported several unpublished mutagenicity studies of silica. There was no evidence of mutagenic activity. The studies are summarized in Table 14.

Mutagenic Inhibition

Hydrated Aluminum Calcium Sodium Silicate

Abdel-Wahhab et al. (1998) incorporated aflatoxin (AF; 2.5 mg/kg feed) with or without hydrated aluminum calcium sodium silicate (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 aberrations of chromosomes, mainly chromatid breaks and chromatid gaps. Hydrated aluminum calcium sodium silicate decreased these effects for every category

of aberration except polyploidy. Hydrated aluminum calcium sodium silicate 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 hydrated aluminum calcium sodium silicate (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 hydrated aluminum calcium sodium silicate 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 hydrated aluminum calcium sodium silicate reduced malformations but not to the levels of the controls. The AF reduced the number of offspring ($p < .05, .01$). Hydrated aluminum calcium sodium silicate mitigated this effect but not to control levels. No ill effects were reported due to hydrated aluminum calcium sodium silicate, only protective effects.

CARCINOGENICITY

Oral

Pyrogenic and Hydrated Silica

In an experiment described earlier by Takizawa et al. (1988), the female mice were fed precipitated (hydrated) silica in feed, 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.

Lewinson et al. (1994) orally administered pyrogenic 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. The rates of tumors observed in the treated rats were comparable to historical controls. The authors concluded that there were no carcinogenic effects due to pyrogenic silica exposure.

Intratracheal

Pyrogenic Silica

Pott and Roller (2005) intratracheally instilled pyrogenic 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 30th month when they were killed and necropsied. A second group had silica at the same dose 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 treatment groups 13.0% in the control group.

Inhalation

Hydrated Silica

Campbell (1940) exposed 3 month old mice susceptible to tumors (n = 75) to aerosolized precipitated (hydrated) silica (0.5 g/d; ≤ 5 µg particle size) 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 in 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.

CLINICAL ASSESSMENT OF SAFETY

Oral

Pyrogenic and Hydrated Silica

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 of orally administered silica (1250 mg in apple juice) in the form of pyrogenic (n = 6; 5 males, 1 female) and precipitated (hydrated; 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 renal silica secretion were not observed. Daily silica increments in urine after ingestion ranged between 7 and 23 mg. For the pyrogenic 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. 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 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 concluded 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 and Sensitization

Hydrated Silica

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; and, from the allergic tests performed, there were little or no sensitizing properties of the powder.

Aqueous sodium lauryl sulfate (25%; ~0.05 ml) was applied to the upper outer arm, volar forearm, or the back of volunteers (n = 27; 18 males, 9 females) under occlusion for 24 h. This was followed by the application of a facial mask containing hydrated silica (0.05 ml; 17%) for 48 h (or 72 h over weekends), under occlusion. This was repeated for 5 inductions. After a 10-day rest, a challenge application was applied on a naive site (upper outer arm, volar forearm, or the back) for 48 h. There was no sensitization observed. The authors concluded that the test material was not likely to cause contact sensitivity under normal use (KGL, Inc. 2003).

Aqueous sodium lauryl sulfate (25%; ~0.05 ml) was applied to the upper outer arm, volar forearm, or the back of volunteers (n = 27; 18 males, 9 females) under occlusion for 24 h. This was followed by the application of a facial powder

containing hydrated silica (21.7436%) made into a 30% aqueous solution for 48 h (or 72 h over weekends), under occlusion. This was repeated for 5 inductions. After a 10-day rest, a challenge application was applied on a naive site (upper outer arm, volar forearm, or the back) for 48 h. There was no sensitization observed. The authors concluded that the test material was not likely to cause contact sensitivity under normal use (KGL, Inc. 2004).

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).

Immune Response

Hydrated Silica

Epstein et al. (1963) injected colloidal (hydrated) silica (1 to 4 mg in saline; ~15 µm particle size) 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 authors suggested that this was a particular type of foreign body response to a fibrogenic agent and not typical epithelioid cell nodules.

Occupational Exposure

Pyrogenic and Hydrated Silica

Volk (1960) studied workers (n = 215) with exposure to silica between 1947 and 1959 using 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 were no correlations between yearly change of pulmonary function and dose or 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 with 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 several unpublished studies of silica exposure. Workers (n = 200) with intensive and regular contact with silica from 1972 to 2000 were evaluated. There was no evidence of skin allergy caused by the silica. There were signs of irritation attributed to the desicative 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 was performed. Exposure ranged from 1 to 34 years. There were complaints of some disorder or exhibition of abnormalities in lung function or histology in 54/143 (36%) of the workers. Dry cough, expectoration or dyspnea was reported in 34/54 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 such as smoking.

In an unpublished occupational exposure study, x-rays were 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 to >20 years (mean 13.2 years). There were negative results in hematology, urine analysis, lung functions, and chest x-rays.

In 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).

ECETOC (2006) reported an unpublished study of workers exposed to pyrogenic (3 factories) or precipitated (2 factories) silica. There were 510 current workers with at least 1 month exposure studied. Complete data sets were collected from 397 workers. Data were also collected from 178 men who were no longer working in the factories. Unexposed workers totaled 210. Chronic bronchitis rates were within expected ranges for all group but somewhat higher in exposed subjects. Differences between factories were absent. The percentage of subjects with obstruction or restriction in breathing were similar between plants. Bronchial hyperresponsiveness was within expected levels. Chest radiographs showed no increased risk of pneumoconiosis in exposed subjects compared to controls. Relevant pleural thickening was not observed. The authors concluded that occupational exposure to silica was not a health risk.

In an unpublished study, 150 workers in a precipitated silica factory were examined by pulmonary function test and X-ray. The workers were exposed for ≥ 6 h/d for at least 5 continuous or discontinuous years. The mean duration was 12.2 years. The control group had been exposed for a maximum of 3 continuous or discontinuous months. The mean ages for the experimental and control groups were 43.1 and 44.3 years, respectively. There were no differences in the distributions and types of dysfunctional measurements observed between exposed and non-exposed groups. There were no differences in the mean percentage of predicted pulmonary function values between exposed and non-exposed groups. None of the X-rays showed signs of pneumoconiosis or fibrosis.

In an unpublished study, 29 workers in a silicone products manufacturing plant were surveyed. Silica exposure ranged from 0.15 to 10 mg/m^3 with a mean of 1.7 mg/m^3. Ten of 15 workers in the room temperature vulcanizing rubber area complained of upper respiratory tract irritation. In the heat curable rubber compounding area, the potential exposure to silica was greater, some of the workers complained about eye irritation, nausea, headaches, or rashes, none reported upper or lower respiratory problems (ECETOC 2006).

OTHER EVALUATION

The International Agency for Research on Cancer (IARC; 1996) concluded that amorphous silica is not classifiable as to its carcinogenicity to humans based on inadequate evidence in humans and inadequate evidence of increased tumors in animals.

OCCUPATIONAL EXPOSURE LIMITS

OSHA (2004) standard for exposure to amorphous silica is 80 mg/mm^3 or 20 mppcf air averaged over an 8-h work shift. NIOSH (2005) recommended that exposure to respirable silica be limited to 6 mg/m^3.

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 pyrogenic and hydrated amorphous silica and their salts are evaluated in this assessment; crystalline silicas are not included in this assessment.

Free silanol groups on the surface of silica particles influence the adsorption behavior. Silica has thixotropic properties. Amorphous silica does not exist as primary particles, except in solution-based forms, but as aggregates and agglomerates.

Amorphous pyrogenic silica (Figure 1) is manufactued by the hydrolysis of volatile silanes, usually silicon tetrachloride, in the flame of an oxygen-hydrogen burner. Precipitated silica and silica gels are produced from an alkali metal silicate dissolved in water (i.e., water glass) and an acid. Silica sols (colloidal silica) are dispersions of silica particles in a liquid, usually water. Impurities include calcium, sodium, potassium, antimony, barium, chromium, arsenic, lead, mercury, cadmium and selenium.

Analytical methods include colorimetric technique, electron microscopy, ICP-AES, CT-MAS, NMR, IR, BET, DLS, and AFM.

Silica was used in a total of 3,276 cosmetic products. Use concentrations ranged from 0.0000003% to 40%. Hydrated silica was reported to be used in 176 cosmetic products at 0.001% to 34%; aluminum calcium sodium silicate in 7 cosmetic products at 0.4% to 6%; and Sodium potassium aluminum silicate in 1 cosmetic product at 0.001% to 4%. Alumina magnesium metasilicate was not reported to be used by a voluntary FDA survey but was reported to be used at 0.002% to 0.001% in an industry survey. There were no reported uses or concentration of use reported for aluminum iron silicates.

Silica is used in food preparation as an anticaking agent, dispersion agent, suspending agent, and thickening agent. It is a defoaming and conditioning agent in malt beverages. Silica is a thickener in pastes and ointments.

Orally administered silica was mostly excreted through the urine in guinea pigs. Some studies showed accumulation of silica from oral exposure whereas others did not. Silica intraperitoneally injected into 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 into 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 up to 160 μg/l. Pyrogenic silica and silica gel were cytotoxic to macrophages at 13.5 $\mu g/cm^2$; precipitated silica was less cytotoxic. Micronuclei were induced in Chinese hamster cells when incubated with 80 and 160 mg/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. When incubated in pyrogenic silica, both macrophages and neutrophils were inhibited in their ability to phagocytose sheep red blood cells. Silica caused intracelluar alteration in calcium homeostasis 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.

Hydrated aluminum calcium sodium silicate countered the effects of aflatoxin in animal feed.

Silica was reported to have an oral LD_{50} up to > 40,000 mg/kg in rats and > 8,000 mg/kg in mice.

Orally administered aluminum calcium sodium silicate had no adverse effects up to 800 mg/kg in mice.

The acute dermal NOEL for silica is > 2,000 mg/kg for rabbits. When applied as an aqueous paste, there were no adverse effects. LD_{50} was > 5,000 mg/kg for precipitated (hydrated) Silcia and > 2,000 mg/kg for silica gel on intact and abraded rabbit skin with mild erythma.

Intraperitoneally injected silica was lethal to 20% to 30% of rats and rabbits at 100 mg/kg; all survived at 50 mg/kg. Peritoneal edema diminished and fibrosis increased over time. Silica injected i.p. was fatal to guinea pigs at 10%. Rats survived 5 days after an i.p. injection of 0.5 g silica, whereas another study reported 50 mg silica to be lethal in rats.

Silica injected in the veins of mice was 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 to 44.4 mg/kg in rats, depending on type and source.

The minimum lethal dose of acute 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. Silica particles (10 mg/kg; particle size range 2 to 3.5 µm) produced potent but transient pulmonary inflammation. Ultrafine silica induced more alveolar hemorrhage, compared to fine silica.

The acute inhalation of silica at 477 mg/m^3 by rats resulted in restlessness, droopy eyelids, lethargy, and dyspnea during treatment. Clinical signs resolved quickly after treatment and necropsies were unremarkable.

Silica at 200 µg 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 at 8,000 mg/kg/d produced no clinical effects in dogs. Short-term oral doses of silica produced no clinical effects for rats; HNELs were up to 1000 mg/kg/d. At 16,000 mg/kg clinical signs were observed: shyness, dirty fur, reduced activity, cachexia, hemorrhages of mucous membranes of the eyes and nose.

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 in one study. In other studies, the NOEL was 1 and 46 mg/m^3.

Intratracheal injections of 5% silica resulted in the death of 3 of 10 rats and 2 of 10 guinea pigs at 10%.

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 formation, and marked congestion of the lungs. 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 male and female rats, 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. There were no mortalities at 31 mg/m^3. The LOAEL was 53 mg/m^3. The NOEL was 1.3 mg/m^3.

Silica incorporated into the feed of rats at up to 10% for 6 months or more produced discolored stool and unremarkable necropsies. Females had increased leukocytes and males had increased eosinophils at 10% in feed. Females had reduced liver weights at 12 and 24 months at 5%. Mice treated with silica in their feed had similar results for up to 103 weeks.

Rabbits chronically exposed to 0.2 and 5.0 μm aerosolized silica had formation of nodular fibrotic or diffuse fibrotic changes in the lungs. 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 4th month.

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. In rabbits exposed to 360 mg/m^3 for a year, emphysema, 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 alveoli 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. At 50 and 100 mg/m^3, there was interstitial fibrosis which did not resolve after 24 months.

When monkeys were exposed to different types of silica, the precipitated (hydrated) 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 inflammatory cell aggregates varied with the type of silica.

Rats exposed to aerosolized silica at ~45 mg/m^3 for 1 year had 41/120 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 fibers, necrotic cells, desquamative catarrh and thickened alveolar septa. After 3 to 8 months recovery, the dust cell granuloma were fewer and reduced in size with only a few dust cells and fibers. The alveolar septa had not completely disappeared and lymph nodes were enlarged.

Rats exposed to aerosolized silica at 15 mg/m^3 for 12 months had a few macrophages aggregated in the lungs. The LOAEL was 6 to 9 mg/m^3. Rats exposed to aerosolized silica at 50 mg/m^3 for 12 months had 1.759 mg silica in the lungs, which decreased to 1.1, 0.43, and 0.41 mg after 1, 3, and 5 months recovery, respectively.

Intratracheal instillation of silica at 0.5 mg in rats every other week for 20 and 30 times resulted in 0.44 mg

silica/lung and associated lymph node.

Silica was a non- to mild ocular irritant in rabbits up to 100 mg.

Silica at 100% was nonirritating to the intact and abraded skin of rabbits.

A guinea pig sensitization study of 20% hydrated silica resulted in no reactions .

No reproductive or teratological effects were observed following the oral administration of silica in rabbits at 1600 mg/kg/d, hamsters at 1600 mg/kg/d, 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.

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 fibroblasts, there was an increase in DNA migration in a dose dependent manner. A chromosomal aberration test was negative up to 300 µl/ml without and 1000 µl/ml with metabolic activation. A HGPRT was negative up to to 250 µm/ml without and 500 µm/ml with metabolic activation. An unscheduled DNA synthesis test was negative up to 1000 µm/ml.

Silica was not mutagenic to CHO cells, hamster fibroblasts, rat hepatocytes, and human embryonic lung cells. Silica was not mutagenic to mice or rats.

A positive sister chromatid exchange test of AFB_1 showed inhibition 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.

A single intratracheal instillation of of 3 mg silica was not carcinogenic. Intratracheal 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.

Silica at 0.5 g/d instilled into mice for a year increased the incidence of overgrowth or hyperplasia of the tracheo-bronchial lymph nodes from 14.3% ot 29.5%.

The oral lethal dose of pyrogenic 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.

Silica was non-sensitizing at 45%. A powder containing silica up to 45% was non-irritating and 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. Exposure to hydrated silica also had no evidence of silicosis or pulmonary disease. There were signs of dermal irritation due to the desicative and defatting properties of silica.

DISCUSSION

The CIR Expert Panel emphasized that the silica considered in this safety assessment is synthetic amorphous silica (gel, hydrated, and fumed/pyrogenic) and does not include any form of crystalline silica.

The Panel recognizes that there are data gaps regarding use and concentration of these ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentrations indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

The Panel was concerned about the possibility of iron atoms reaching the lungs if aluminum iron silicates were to be used in a spray. In the absence of inhalation toxicity data, the Panel determined that aluminum iron silicates can be used safely in hair sprays, because the ingredient particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (38 μm) and pump hair sprays (>80 μm) is large compared to respirable particulate sizes (10 μm). The Panel recognizes that most of the formulations are not respirable and of the preparations that are so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation potential. Aluminum iron silicates is safe as a cosmetic ingredient because the particles for aggregates and agglomerates that are too large to be respirable.

The Panel determined that silicosis was not an issue since crystalline silica is not used in cosmetics.

CONCLUSION

Silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment.[1]

[1]Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

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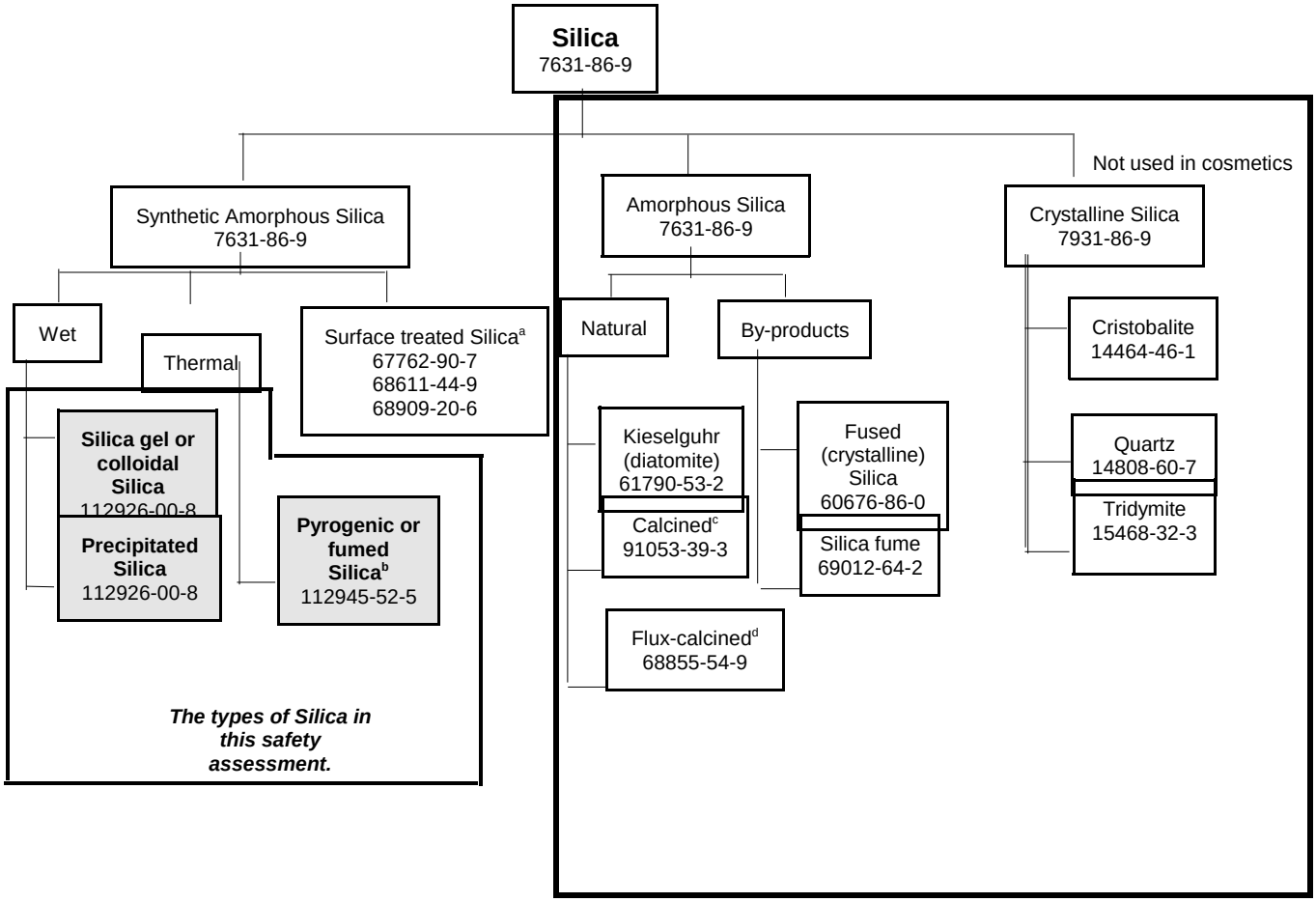

Figure 1. Different polymorphs of silica with CAS Numbers. Shaded boxes represent the types of silica covered in this report. a) All forms of synthetic amorphous silicas can be surface modified either physically or chemically; most common treating agents are organosilicon compounds. b) Pyrogenic silica is also known as fumed silica in English speaking countries and is not to be confused with silica fume, which is crystalline. Pyrogenic is used in recent publications. c) By-product from electrical furnace. d) Partial transformation into cristobalite (after Arts et al. 2007).

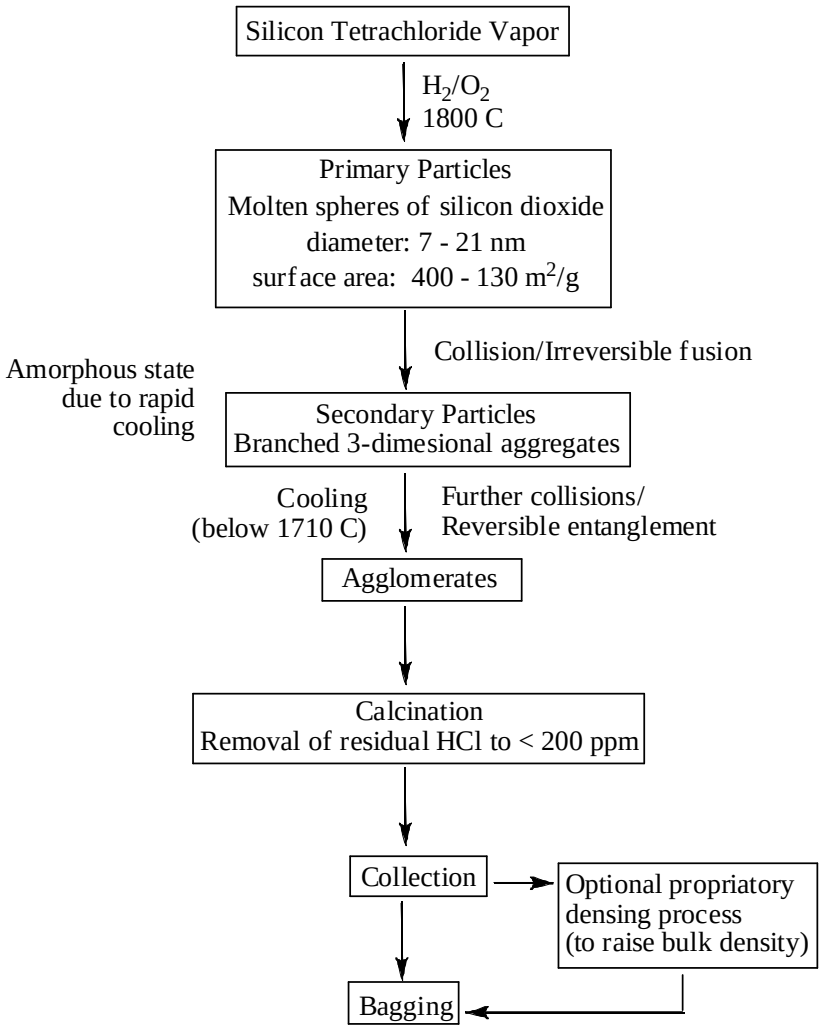

Figure 2. Process for the manufacture of pyrogenic silica (Villota and Hawkes 1986).

Table 1. Physical properties of fumed hydrophobic silica, precipitated hydrophobic silica, silica gel, and silica sol.

Property	Fumed hydrophobic silica	Precipitated hydrophobic silica	Gel[a]	Sol	Reference
Appearance	Fluffy powder	Fluffy powder			Lewinson et al. 1994
	White	White	White	White, milky	Ferch 1976
Molecular weight	60.1				NIOSH 2005
Particle size	1-350 µm				UNEP 2004
pH[b]	3.6-4.5	5-9	3-8	3-5; 8-11	Ferch 1976
	4-9				UNEP 2004
	~3.7 (4% aqueous slurry)				UNEP 2004
Boiling point	4046°F (2230°C)				NIOSH 2005
Melting point	3110°F (1710°C)				NIOSH 2005
	~1700 °C				UNEP 2004; OSHA 2004
BET surface area (m^2/g)	110-250	100			Lewinson et al. 1994
	50-400	30-500	250-1,000[c]	50-400	Ferch 1976
Tapped (bulk) density (g/l)	30-250	30-500	500-1,000	n/a[d]	Ferch 1976
	50-320 g/l				UNEP 2004
	40-60 g/l				American International Chemical, Inc. (no date)
Density	~2.2 at 20°C				UNEP 2004
Specific gravity (g/cm^3)	2.2	1.9-2.2	1.8-2.2	1.0-1.4	Ferch 1976
	2.65				OSHA 2004
Solubility	Insoluble				NIOSH 2005
Water solubility (saturation)	~15-68 mg/l at 20°C (pH 5.5-6.6)				UNEP 2004
	Insoluble				FNB 1996
Saturation	2.0 mmol/l(120 mg/l)				ECETOC 2006
Moisture (%)	<0.5	3			Lewinson et al. 1994
Stability in water	Stable; ion exchange processes possible				UNEP 2004
Loss on Ignition	1.0% max (2 hr @ 1,000°C)				UNEP 2004
Loss on drying (% by weight)	< 2.5	5-7	2-6	50-85	Ferch 1976
Loss on heating	1.0% max				American International Chemical, Inc. (no date)
Vapor pressure	~0 mm HG				NIOSH 2005; OSHA 2004
	None				UNEP 2004
Ignition loss (%)	<2	7			Lewinson et al. 1994
	<2	3-14	2-15	50-90	Ferch 1976
Temperature at ignition (°C)	400	400			Lewinson et al. 1994
Decomposition temperature of methyl groups (°C)	300	300			Lewinson et al. 1994
Purity of SiO_2 (%)	>99.8	>99.5			Lewinson et al. 1994
	> 99.8	> 95	> 95[e]	15-50	Ferch 1976
Carbon, bound	1	2			Lewinson et al. 1994
Al_2O_3 (%)	<0.05	0.1			Lewinson et al. 1994

Table 1. Physical properties of fumed hydrophobic silica, precipitated hydrophobic silica, silica gel, and silica sol.*(continued)*

Property	Fumed hydrophobic silica	Precipitated hydrophobic silica	Gel[a]	Sol	Reference
Fe_2O_3 (%)	<0.01	0.03			Lewinson et al. 1994
TiO_2 (%)	<0.03	0.03			Lewinson et al. 1994
HCl (%)	<0.025	<0.025			Lewinson et al. 1994
Dimethyldichlorosilane (%)	<0.1	<0.1			Lewinson et al. 1994
Primary particle size (µm)	0.005-0.05[f]	0.005-0.1[f]	0.001-0.01	0.005-0.02	Ferch 1976
Aggregate size (µm)	0.1-1	0.1-1	1-20	n/a	Ferch 1976
Agglomerate size (µm)	1-250	1-250	n/a	n/a	Ferch 1976
Mean pore size (µm)	None	> 0.03	0.0001-1	n/a	Ferch 1976
Pore size distribution	None	Very wide	Narrow	Wide	Ferch 1976
Structure, DBP[g] absorption (ml/100 g)	250-350	80-320	80-350	n/a	Ferch 1976

[a] After drying according to DIN 66131 or direct by titration with NaOH solution (Sears 1956).
[b] Hydrophilic grades.
[c] Porous surface.
[d] Not applicable.
[e] Dry product, no hydrogel.
[f] Primary particles do not normally exist as individual units.
[g] Dibutyl phthalate.

Table 2. 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 Wt./gallon	2.2 g/cm^3 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

B.E.T. - a rule for the physical adsorption of gas molecules on a solid surface that serves as the basis for the measurement of the specific surface area of a material

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilcate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate.

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	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)	22	0.02-10
Oils, tablets, and salts (257)	18	0.9-2
Capsules (4)	2	-
Other (239)	3	0.02
Eye products		
Eyebrow pencil (147)	42	0.01-6
Eyeliner (684)	89	0.3-19
Shadow (1196)	472	0.001-44
Lotion (177)	46	0.02-4
Makeup remover (131)	4	0.0004
Mascara (463)	135	0.2-10
Other (288)	79	0.04-3
Fragrance products		
Colognes and toilet waters (1288)	-	0.1
Perfumes (569)	6	1
Powders (278)	49	1-10
Sachets (28)	-	-
Other (399)	8	6-18[c]
Noncoloring hair care products		
Conditioners (1249)	12	0.002
Sprays/aerosol fixatives (371)	-	0.0005
Straighteners (144)	2	3[d]
Permanent waves (141)	1	-
Rinses (47)	-	0.003
Shampoos (1403)	7	0.02
Tonics, dressings, etc. (1097)	13	0.02-3
Other (716)	16	-
Hair coloring products		
Dyes and colors (2481)	80	0.002-0.3
Tints (58)	1	2
Color sprays/aerosol (8)	5	0.4
Hair lighteners with color (22)	3	-
Bleaches (152)	46	6[e]
Other (166)	14	1
Makeup		
Blushers (539)	122	2-20
Face powders (613)	198	1-26
Foundations (635)	268	0.01-40
Leg and body paints (29)	2	-
Lipstick (1912)	536	0.01-21
Makeup bases (164)	40	0.5-20
Rouges (99)	35	0.09-3

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilcate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. *(Continued)*

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
Silica (continued)		
Fixatives (38)	16	0.3-3
Other (406)	107	2-4[f]
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)	16	5
Oral hygiene products		
Dentifrices (59)	12	3-16
Other (48)	3	-
Personal hygiene products		
Underarm deodorants (540)	38	0.02-9
Feminine deodorants (21)	1	
Other (514)	27	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)	9	0.003
Skin care products		
Cleansing creams, lotions, liquids, and pads (1368)	47	0.002-5
Depilatories (62)	3	1
Face and neck creams, lotions, etc. (1195)	125	0.03-10
Body and hand creams, lotions, etc. (1513)	50	0.02-5[h]
Foot powders and sprays (48)	7	0.8
Moisturizers (2039)	163	0.008-8
Night creams, lotions, powder and sprays (343)	32	0.01-3
Paste masks/mud packs (418)	22	0.02-6
Fresheners (285)	5	0.00004-3
Other (1244)	97	0.04-11[i]
Suntan products		
Suntan gels, creams, liquids and sprays (156)	8	0.03-2
Indoor tanning preparations (200)	19	-
Other (62)	7	0.6
Total uses/ranges for Silica	**3276**	**0.0000003-44**
Hydrated Silica[j]		
Bath products		
Soaps and detergents (1329)	**13**	**0.05-4**
Oils, tablets, and salts (257)	**7**	**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)	**3**	**2**

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilcate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. *(Continued)*

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
Hydrated Silica (continued)		
Fragrance products		
Powders (278)	**10**	**2**
Noncoloring hair care products		
Conditioners (1249)	-	**0.04**
Shampoos (1403)	-	**0.05**
Tonics, dressings, etc. (1097)	-	**2**
Hair coloring products		
Bleaches (152)	**18**	**2**[l]
Other (166)	**2**	-
Makeup		
Blushers (539)	-	-
Face powders (613)	**23**	**4**
Foundations (635)	**4**	**3**
Makeup bases (164)	**1**	-
Lipstick (1912)	-	**0.003**
Other (406)	**3**	-
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)	**1**	-
Other (514)	**3**	**6**
Skin care products		
Cleansing creams, lotions, liquids, and pads (1368)	**5**	**3-17**
Depilatories (62)	**10**	-
Face and neck creams, lotions, etc. (1195)	**6**	**0.09**
Body and hand creams, lotions, etc. (1513)	**4**	**0.06-2**
Foot powders and sprays (48)	**1**	-
Moisturizers (2039)	**3**	**1-2**
Night creams, lotions, powder and sprays (343)	**1**	**0.04**
Paste masks/mud packs (418)	**2**	**0.01-10**
Fresheners (285)	**4**	-
Other (1244)	**6**	**0.001-0.004**
Suntan products		
Suntan gels, creams, liquids and sprays (156)	**1**	**0.2-2**
Other (62)	**1**	-
Total uses/ranges for Hydrated Silica	**176**	**0.001-34**

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilcate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. *(Continued)*

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
Alumina Magnesium Metasilicate		
Skin care products		
Face and neck creams, lotions, etc. (1195)	-	**0.01**
Body and hand creams, lotions, etc. (1513)	-	**0.002**
Total uses/ranges for Magnesium Aluminum Metacilicate	-	**0.002-0.01**
Aluminum Calcium Sodium Silicate		
Eye products		
Mascara (463)	-	**0.5**
Makeup		
Blushers (539)	**1**	-
Face powders (613)	**1**	-
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	**7**	**0.4-6**
Sodium Potassium Aluminum Silicate		
Nail care products		
Basecoats and undercoats (62)	-	**4**
Polish and enamel (419)	-	**0.001**
Skin care products		
Paste masks/mud packs (418)	**1**	-
Total uses/ranges for Sodium Potassium Aluminum Silicate	**1**	**0.001-4**

[a] Silica; silica, amorphous; silica, fumed; and silicon dioxide, colloidal 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] Hydrated silica and silicic acid were listed by the FDA. These data were combined.
[l] 6% in a body scrub.

Table 4. Retention of silica (25 mg/rat) in rat tissues after intratracheal injection (Byers and Gage 1961).

Sample (particle size)	Time after injection (weeks)	Lungs (µg/rat)	Liver (µg/rat)	Kidneys (µg/rat)	Spleen(µg/rat)
A1(19 µm)	12	1570	177	33	0
	24	755	20	12.4	1.4
	52	210	61	9.7	0
A2 (20 µm, 60 µm after storage)	12	5150	249	138	0
	24	1550	153	44	19
	52	720	153	56	0
B (25 µm)	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

Table 5. Unpublished acute oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance	Notes	LD_{50}
Sprague-Dawley rats (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 (10)	Precipitated silica	None	> 5110 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Precipitated silica	None	> 5000 mg/kg
Male rats (strain and n 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 not specified; 10; 5 male, 5 female)	Precipitated silica	No clinical signs	> 5000 mg/kg
Wistar rats (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 (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 (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 (strain and n not specified)	Aqueous colloidal silica (30%)	None	10,000 mg/kg
Rats (strain and n not specified)	Silica	None	40,000 mg/kg
Boltzman rats (5 male)	Hydrophilic fumed silica in 0.5% methylcellulose	None provided.	> 3,160 mg/kg
Sprague-Dawley (10; 5 male, 5 female)	Hydrophilic fumed silica in water	None provided.	> 5,000 mg/kg
Sprague-Dawley (10; 5 male 5 female)	Hydrophilic fumed silica in deionized water	None provided.	> 5,000 mg/kg

Table 5. Unpublished acute oral toxicity studies of Silica reported by UNEP (2004) and ECETOC (2006). *(continued)*

Species (n)	Test substance	Notes	LD_{50}
Swiss Mice (10 males)	Hydrophilic fumed silica in corn oil	None provided.	> 3,160 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in 1% aqueous gum arabic solution	None provided.	>31,800 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Hydrophilic precipitated silica in Aqueous carboxymethylcellulose	None provided.	5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in olive oil	None provided.	6,350 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in 10% aqueous arabic gum	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 10,000 mg/kg
Sprague-Dawley (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Male Sprague-Dawley rats (n not provided)	Hydrophilic silica gel in saline	None provided.	> 5,000 mg/kg
Male Sprague-Dawley rats (30)	Hydrophilic silica gel in distilled water	None provided.	> 5,620 mg/kg
Sprague-Dawle Rats (10; 5 male, 5 female)	Hydrophilic silica gel in water	24 h observation.	> 31,600 mg/kg
Male rats (strain and n not provided)	Hydrophilic silica sol in aqueous solution (colloidal)	None provided.	> 10,000 mg/kg
Male Sprague-Dawley rats (10)	Hydrophilic silica sol in aqueous solution (colloidal)	None provided.	> 40,000 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Hydrophobic fumed silica in peanut oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Male Sprague-Dawley (10)	Hydrophobic fumed silica in corn oil	None provided.	> 3,160 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in distilled water	None provided.	Males - 9,200 mg/kg Females - > 10,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Wistar rats (10; 5 males, 5 females)	Hydrophobic fumed silica in polyethylene glycol 400	None provided.	> 2,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic precipitated silica in olive oil	None provided.	> 7,900 mg/kg

Table 6. Acute inhalation toxicity studies of hydrophilic and hydrophobic silica (ECETOC 2006).

Species (n)	Test substance (surface area [m^2/g])	Notes	LC_{50} (mg/m^3)
		Hydrophilic	
Wistar rats (10; 5 male, 5 female)	Pyrogenic silica (200)	Exposure 4h, nose only. Particle size not provided, 56% of particles < 5 µm. No clinical signs observed and no organ abnormalities at necropsy.	> 139
Male albino rats (strain not specified; 10)	Pyrogenic silica (380)	Exposure for 1 h, nose only. No data on particle size. Vigorous cleansing, hypoactivity, abdominal respiration, gasping nasal exudation, closed eyes. Crust-like material around nose and mouth, fur chalky to touch for 2 days post exposure. Resolved quickly.	>207,000
Albino rats (strain, sex, and n not provided)	Pyrogenic silica (200)	Exposure for 1 h, nose only. No data on particle size.	> 191,300
Sprague-Dawley rats (10; 5 male, 5 female)	Pyrogenic silica (200)	Exposure for 4 h, nose only. Particle size 0.76 5 µm	> 2,080
Wistar rats (10; 5 male, 5 female)	Precipitated silica (190)	Exposure for 4 h, whole body. Particle size not provided, 45% of particles < 5 µm. Some decreased body weight in the females 2 days after exposure which resolved.	> 691
Male Sprague-Dawley rats (10)	Silica gel (not specified)	Exposure for 1 h, nose only. Particle size not provided. 1/10 rats died 2 h after exposure.	> 2,200
Male rats (strain not specified; 2)	Silica sol "solid" (not specified)	Exposure for 4 h, nose only. Particle size not provided.	> 3,100
Male rats (strain not specified; 2)	Silica sol (not specified)	Exposure for 2.5 and 6 h, mist, nose only, at 560 and 520 mg/m^3. Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 3.25 h, mist, whole body at 760 mg/m^3. Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 4.2 h, mist, whole body at 2,240 and 2500 mg/m3. Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 1.5 h, mist, whole body, at 3,300 mg/m^3. Particle size not provided.	No deaths
		Hydrophobic	
Wistar rats (10; 5 male, 5 female)	Fumed silica (80)	Exposure for 4 h, whole body. Particle size 1.4 - 1.8 µm.	2,863 - 3,730
Rats, strain not specified (10; 5 male, 5 females)	Fumed silica (130)	Exposure for 1 h, whole body. Particle size 0.15 µm.	> 2,280
Wistar rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body with 14 days recovery. Particle size 56% < 5 µm, ≥7.7 µm. Clinical signs transient body weight loss for 2 days. No abnormalities at necropsy.	> 477
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body. Particle size 0.36 µm.	< 4,900
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body. Particle size 0.54 µm. All died at 2,190 mg/m^3.	< 2,190

Table 6. Acute inhalation toxicity studies of hydrophilic and hydrophobic silica (ECETOC 2006).*(continued)*

Species (n)	Test substance (surface area [m^2/g])	Notes	LC_{50} (mg/m^3)
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 1 h, whole body. Particle size 0.48 µm.	1,260 - 2,830
Male Wistar rats (10); male Swiss mice (10); male English short hair guinea pigs (10)	Fumed silica (not specified)	Exposure for 6 h, whole body. Particle size not specified. All species preened. Rats hunched. Rats and mice had occasional prostration. No clinical signs in guinea pigs. Consolidation observed in 2/9 guinea pigs. Necropsies unremarkable.	> 250 for all species
Male albino rats (strain not specified; 10)	Fumed silica (not specified)	Exposure for 1 h, whole body. Particle size not specified.	> 3,150
Wistar rats (10; 5 male, 5 female)	Fumed silica (130)	Exposure for 4 h, whole body. Particle size 1.175-1.275 µm with 14 days recovery. At 2,100 mg/m^3, all rats died within 2.5 h. Clinical signs were few feces; closed eyes; wet, red staining on mouth/nose; labored breathing; respiratory distress; hunched position. Necropsy showed opaqued eyes, enlarged darkened lungs with red areas, white material in nasal turbinates, red areas in intestines. At 540 mg/m^3, 7/10 died. Clinical signs were closed eyes, red staining of mouth/nose, fur coated with silica, labored breathing, respiratory distress, hunched back. During recover, lethargy, ploerection, dyspnea, ptosis, few feces, eye crusting/lachrymation, unkempt appearance, anogenital wetness. Necropsy of survivors showed darkened lungs with red and white areas. At 210 mg/m^3, all rats survived. Clinical signs were closed eyes, labored breathing. Licking inside of mouth, laying on back. During recovery, sporadic instances of few feces, anorexia, chromodacryorrhea, labored breathin, wetness of nose/mout, diarhea. Transient decreases in body weight. After 14 days recovery, lungs darkened with white and red areas.	450
Wistar rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size 0.95-2.15 µm. Results similar to above.	90 - 840 [sic]
SD rats (10; 5 male, 5 female)	Fumed silica (130)	Exposure for 4 h, whole body. Particle size < 0.2 µm. All rats died at 2,530 and 5,300 mg/m^3. Severe red discoloration of the lungs of rats that died.	1,650
SD rats (10; 5 male, 5 female)	Fumed silica (130)	Exposed for 4 h, nose only. Particle size 7.2-7.7 µm. 4/10 died at 2,200 mg/m^3. Severe discoloration of the lung in rats that died. Surviving rats had normal lungs except 1 male and 2 females with trace discoloration.	> 2,200
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size < 0.1 µm.	90
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4, nose only. Particle size 7-7.1 µm.	500
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h; whole body. Particle size < 0.4 µm.	800

Species (n)	Test substance (surface area [m^2/g])	Notes	LC_{50} (mg/m^3)
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, nose only. Particle size 6.3-7.7 µm. Author concluded that number of particles (surface area) may be responsible for observed effects.	600
SD rats (6; 3 male, 3 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size 83% 1-5 µm, 17% 5-100.	660

Table 7. Short-term oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance; dose	Notes and results
Sprague-Dawley rats (5)	Precipitated silica gel; 16.5 mg/kg/d (10% w/w in feed), 5.8 g/kg/d for days 1-10 then 24.2 mg/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; 1.8, 7.2, and 7.8 µg in controls, respectively.
Charles river rats (30; 15 male 15 female)	Hydrophilic fumed silica;0, 1%, 3%, 5% (0, 1,000, 3,000, 5,000 mg/kg) in diet for 13 weeks	No gross signs of systemic toxicity. No effect on growth rate, feed consumption, or survival. No increase in silica content of the liver, kidney, spleen blood, or urine after 45 and 90 days. No gross or microscopic pathological changes.
Female rats (strain and not specified)	Hydrophilic precipitated silica; 0 or 1500 mg/kg by gavage for 4 weeks	No effects to body weight gain, feed consumption, and behavior.
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica; 0, 0.5%, 2%, or 8% (0, 250, 1,000, 4,000 mg/kg) in diet for 13 weeks	Increased feed intake associated with decreased feed efficiency in the high-dose group; mean absolute and relative weight of the cecum increased in the high-dose group. General condition, behavior, survival, body weights, water intake, and hematological and urinary parameters were not adversely affected. No gross or microscopic pathological abnormalities observed. NOEL = 4,000 mg/kg.
SD rats (10; 5 male, 5 female)	Hydrophilic silica gel; 0 or 10% (0, 16,500 mg/kg) for 2 weeks or 5% (5,800 mg/kg) for days 1-10 then 20% (24,200 mg/kg) for days 11 -14	No gross signs of systemic toxicity. No effect on growth rate, feed consumption, or survival. No gross or microscopic pathological changes.
Male rats (strain not specified; 6)	Hydrophilic silica sol; 7,500 mg/kg in diet, 5 d/week for 2 weeks	All animals lost weight during treatment but gained over the weekend and during recovery period. No effects on organs.
CD rats (n not specified; male and female)	Hydrophilic silica sol; 0 or 800 mg/kg in diet for 4 weeks	No treatment effects in clinical symptoms, urine and blood parameters, necropsy or histopathology.
Beagle dogs (17; 9 male, 8 female)	Hydrophilic silica sol; 0 or 800 mg/kg in diet for 4 weeks	No treatment effects in clinical symptoms, urine and blood parameters, necropsy or histopathology.

Table 7. Short-term oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance; dose	Notes and results
Wistar rats (10; 5 male, 5 female)	Hydrophobic fumed silica; 0, 500, or 1,000 mg/kg in diet for 5 week or 2,000 mg/kg increased stepwise every 2 weeks to 16,000 mg/kg (25% of daily feed intake) for 8 weeks.	At 2,000 mg/kg, pronounced reduction of body weight associated with reduced feed intake. No changes in biological parameters or macroscopic findings. As the rats reached the highest dose the last 2 weeks, clinical signs were observed: shyness, dirty fur, reduced activity, cachexia, hemorrhages of mucous membranes of the eyes and nose. 2 males and 2 females died in week 8. Microscopic evaluation of the liver showed severe atrophy in the epithelium. NOEL assumed to be 1,000 mg/kg.
Charles River rats (20; 10 male, 10 female)	Fumed silane-treated silica;1, 2%, 2%, 4% (0, 1,000, 2,000, 4,000 mg/kg) in diet for 13 weeks	No effect on appearance, behavior, growth, survival, clinical studies, or gross pathology. No cytopathological changes. Minimal change in thyroid gland morphology (smaller follicles lined by slightly taller epithelial cells) in males in mid- and high-dose groups.

Table 8. Unpublished short-term inhalation toxicity studies of silica (ECETOC 2006).

Species (n)	Test substance; dose	Notes and results
Male Wistar rats (10)	Hydrophilic fumed silica (0, 1.39, 5.41, 25.3 mg/m^3) for 6 h/d for 5 days. Recovery for 1 or 3 months.	There were no effects in the low-dose group. Mid- and high-dose groups had incidences of pulmonary inflammation after exposure. Effects diminished or disappeared during recovery.
Wistar rats (20; 10 male, 10 female)	Hydrophilic fumed silica (0, 17, 44, 164 mg/m^3) for 6 h/d, 5 d/week for 2 weeks.	Respiratory distress observed in all treatment groups. Reduced body weight gain and feed consumption in the mid- and high-dose groups. Hematological parameters were unremarkable. Increased absolute and relative lung weight in a dose-dependent manner. Lungs of several rats in each test group discolored, spotted, spongy, or irregular on the surface. Lungs increased septal cellularity, alveolar interstitial pneumonia, and early granulomata. Mediastinal lymph nodes of several rats were enlarged. Early granulomata observed in mediastinal lymph nosed in mid- and high-dose groups.
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica (0, 1.16, 5.39, or 25. mg/^{3}) for 6 h/d, 5 d/week. Recovery for 1 and 3 months.	No deaths. No adverse effects in low-dose group. There was a slight decrease in breathing. Incidence of pulmonary inflammation increased in mid- and high-dose groups. Effects lessened or resolved during recovery. NOEL = 1 mg/m^3.
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica (0, 46, 180, 668 mg/m^3) 6 h/d, 5 d/week for 2 weeks.	Signs of respiratory distress in all treatment groups. Males in mid- and high-dose groups had reduced body weight and feed consumption. Dose dependent increase in absolute and relative lung weights. In high-dose groups, several rats had spotted and swollen lungs with irregular surface. Males in mid- and high-dose groups had increased septal cellularity, alveolar macrophages and and particulates. Some of these changes were present in some mediastinal lymph nodes. Early granulomata in lungs in the high-dose group and mediatinal lymph nodes in the mid- and high-dose groups. NOEL < 46 mg/m^3.
Male Wistar rats (10)	Hydrophilic silica gel (0, 0.94, 5.13, 25.1 mg/m^3) 6 h/d for 5 days. Recovery for 1 and 3 months.	No effects in low-dose group. Pulmonary inflammation increased in the mid- and high-dose groups. Changes disappeared or lessened during recovery.
CD rat (80; 40 male, 40 female)	Hydrophobic fumed silica (0 or 60 mg/m^3 on day 1 then 30 mg/m^3 thereafter) 6 h/d, 5 d/week for 4 weeks. Recovery for 1, 2, 4, 6, and 12 weeks.	9 male rats died after first day due to acute pulmonary hemorrhage with bronchiolar plugs with emphysema. Active interstitial/alveolar inflammation that changed from diffuse to localized consolidation. After recovery, active inflammation was less prominent but some fibrosis and collagen apparent in the interstitium.
Wistar rats (20; 10 male, 10 female)	Hydrophobic fumed silica (0, 31, 87, or 209 mg/m^3) for 6 h/d, 5 d/week for 2 weeks.	Signs of respiratory distress in all test groups. Body weight and feed consumption reduced in mid- and high-dose groups. Hematological parameters were unremarkable. Increases in absolute and relative lung weights were dose-dependent. Lungs of several rats in all groups were pale, spotted, swollen and spongy. Lungs in all treatment groups had increased cellularity, accumulation of alveolar macrophages, alveolar edema, and early granulomata. NOAEL < 31 mg/m^3.

Table 9. 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 nodular fibrosis was observed in lungs or lymph nodes. 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. 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. 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. 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. 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. Microscopically visible dust foci under pulmonary pleura, mediastinal lymph nodes were moderately enlarged. Interior of alveoli: 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. 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 in 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 septae and perifocal emphysema. Silicatic nodules were not observed. Lymph nodes: increase of dusted cells and slight to moderate fibrosis, sporadic collagen fibrosis. 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 alveolas, edema, accumulation of macrophages in the lymph nodes and in the interstitium (perivascular, peribronchial, alveolar septea), granuloma of macrophages, dust cells, some thickening of the alveolar septas. Formation of connective tissue was minimal.

Table 10. Ocular irritation studies of hydrophilic and hydrophobic silica on rabbits (ECETOC 2006).

Type of silica	N	Description	Results
		Hydrophilic	
Fumed	8	Instilled 100 mg. Not rinsed (5) or rinsed after 5 min (3)	No signs of irritation.
Fumed	3	Instilled 3 mg	Slight to mild erythema; resolved at 48 h.
Fumed	6	Instilled 3.5 mg	Slight conjuctival erythema or chemosis in some animals at 24, 48, and 72 h. Mean score 0.6 and 0.1, respectively [sic]. Transient opacity in 2 animals at 4 h.
Fumed	9	Instilled 100 mg; not rinsed (6), rinsed (3)	No signs of irritation in washed eyes at 24, 48, and 72 h. Mean score 0.15; very slight conjuctival erythema up to 48 h.
Precipitated	3	Instilled 40 or 100 mg	No signs of irritation at 40 mg. At 100 mg, slight redness at 24, 48, and 72 h; mean score 0.7. Resolved by day 4.
Precipitated	8	Instilled 100 mg; not rinsed (5), rinsed (3)	No signs of irritation.
Precipitated	8	Instilled 0.1 ml of 50% dilution in olive oil; eyes not rinsed (5), rinsed in 5 min (3)	No signs of irritation in rinsed eyes. Very slight erythema (score 1) up to 24 h.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	6	Instilled 100 mg, 0.2 ml of 50% slurry	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Silica gel	9	Instilled 9 mg; eyes not rinsed (3), rinsed at 2 s (3) or 4 s (3)	No signs of irritation.
		Hydrophobic	
Fumed	8	Instilled 100 mg; eyes not rinsed (5), rinsed after 5 min (3)	No signs of irritation.
Fumed	9	Instilled 25 mg; eyes not rinsed (6), rinsed after 30 s (3)	No signs of irritation in washed eyes. 2 unwashed eyes had slight erythema for 24 h. Mean score 0.1 at 24, 48, and 72 h.
Fumed	9	Instilled 3 mg; eyes not rinsed (3), rinsed after 2 s (3) or 4 s (3)	Transient slight to moderate conjunctival erythema at 1 and 4 h. Resolved at 24 h.
Fumed	9	Installed 10 mg; eyes not rinsed (6), rinsed after 30 s (3)	No signs of irritation.
Fumed	9	Instilled 10 mg; eyes not rinsed (6), rinsed after 30 s	No signs of irritation.
Fumed	9	Installed ~10-20 mg; eyes not rinsed (6), rinsed after 30 s	No sign of irritation in washed eyes; 2 unwashed eyes had slight erythema for 24 h (mean score 1 at 24, 48, and 72 h).
Fumed	9	Installed 6 mg: eyes not rinsed (3), rinsed after 2 s (3) or 4 s (3)	No signs of irritation.
Fumed	3	Installed 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.

Table 11. Rabbit skin irritation studies of hydrophilic and hydrophobic silica (ECETOC 2006).

Type of silica	N	Description	Results
		Hydrophilic	
Fumed silica	6	Occlusive patch of 500 mg in 3 ml saline to intact and abraded skin for 24 h.	No signs of irritation to intact skin. Slight erythema on 3 abraded sites
Fumed silica	6	Occlusive patch of 500 mg moistened with saline to intact and abraded skin for 24 h.	Very slight erythema on 1 intact site at 24 h; very slight to well-defined erythema on abraded sites. No sign of erythema at 72 h.
Precipitated silica	3	Occlusive patch of 500 mg to intact skin for 4 h.	No signs of irritation.
Precipitated silica	12	Occlusive patch of 500 mg in methylelthyl cellulose to intact and abraded skin for 24 h.	No signs of irritation.
Precipitated silica	6	Occlusive patch of 23 mg to intact and abraded skin for 24 h.	Very slight erythema on 3 abraded sites and 5 intact sites at 24 h.
Precipitated silica	6	Occlusive patch of 190 mg to intact and abraded skin for 24 h.	Very slight erythema on 3 abraded and 4 intact sites at 24 h.
Precipitated silica	12	Occlusive patch of 500 mg in olive oil to intact and abraded skin for 24 h.	No signs of irritation
Precipitated silica	12	Occlusive patch of 500 mg to intact and abraded skin for 24 h.	No signs of irritation.
Precipitated silica	6	Occlusive patch of 500 mg to intact skin for 24 h.	No signs of irritation.
Silica gel	8	Occlusive patch of 20 mg to intact and abraded skin for 24 h.	No signs of irritation.
		Hydrophobic	
Fumed silica	12	Occlusive patch of 500 mg in methylethyl cellulose to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Occlusive patch of 500 mg moistened with PEG to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica silane treated	6	Occlusive patch of 500 mg moistened with corn oil to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Occlusive patch of 500 mg in 2 ml water to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Precipitated silica	12	Occlusive patch of 500 mg in olive oil to intact and abraded skin for 24 h.	No signs of irritation.

Table 12. Unpublished in vitro mutagenicity and chromosomal aberration tests of cultured mammalian cells (UNEP 2004; ECETOC 2006).

Test	Test system	Silica type (concentration)	Results
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 silica (0.3-1000 µg/ml)	Negative; cytotoxic at 260 - 500 µg/ml
6-Thioguanine resistance	CHO cells	Hydrophilic fumed silica(10-250 µg/ml without S9, 100-500 µg/ml with S9)	No significant mutagenic activity
Chromosome aberration	CHO cells	Hydrophilic fumed silica (38-1,000 µg/ml without S9, 250-1,000 µg/ml with S9)	No clastogenic activity
Unscheduled DNA synthesis	Primary rat hepatocytes	Hydrophilic fumed silica (0.3-1,000 µg/ml with and without S9)	No genotoxic activity
Chromosome aberration	Human embryonic lung cells (Wi-38)	Hydrophilic silica gel (1-1,000 µg/ml without S9)	Clastogenic activity not significant
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (42-333 µg/ml with and without S9)	No clastogenic activity
Chromosomal aberration test, with and without metabolic activation	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

Table 13. Unpublished in vivo mutagenicity studies of silica gel (ECETOC 2006).

Test	Test system	Protocol (dose)	Results
Gene mutation (host mediated)	Mice + *S. typhimurium* TA1530, G-46 (indicator)	i.p. injection of *S. typhimurium* cells collected 3 h after last administration (1 or 5 x 1.4-5,000 mg/kg)	No mutagenic activity
Mitotic recombination (host mediated)	Mice (host) + *S. cerevisiae* D3 (indicator)	i.p. injection of *S. cerevisiae* cells collected 3 h after last administration (1 or 5 x 1.4-5,000 mg/kg)	No genotoxic activity
Chromosome aberration	Male Sprague-Dawley rat bone marrow	Killed at 6, 24, and 48 h (1 x 1.4-5,000)	Negative
Chromosome aberration	Male Sprague-Dawley rat bone marrow	Killed 6 h after last administration (5 x 1.4-5,000 mg/kg)	Negative
Dominant lethal mutation	Female Sprague-Dawley rat	8 mated, killed 14 days after mating for uterus examination (1 x 1.4-5,000 mg/kg)	Negative
Dominant lethal mutation	Female Sprague-Dawley rat	8 mated, killed 14 days after mating for uterus examination (5 x 1.4-5,000 mg/kg)	Negative

Table 14. Unpublished in vitro mutagenicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Test	Test systems	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
Unscheduled DNA synthesis	Primary rat hepatocytes	Fumed silica (0.3 - 1000 µg/ml)	Negative; cytotoxic at 260 - 500 µg/ml
Ames, with metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic fumed silica (≤ 10,000 µg/plate)	Negative; not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic fumed silica (≤ 5,000 µg/plate	Negative; not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic silica gel (≤ 10,000 µg/plate)	Negative; not cytotoxic
Tryptophan reversion, with and without metabolic activation	*Escherichia coli* WP2	Hydrophilic silica gel (≤ 10,000 µg/plate)	Negative; not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA1530, G-46)	Hydrophilic silica gel (not provided)	Negative
Forward mutation, without metabolic activation	*Saccharomyces cerevisiae* (D3)	Hydrophilic silica gel (not provided)	Negative
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1537, TA98)	Hydrophobic fumed silica (1,580 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Tryptophan reversion, with and without metabolic activation	*E. coli* (WP2)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Triptophan reversion, with and without metabolic activation	*E. coli* (WP2)August 17, 2009	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic