International Journal of Toxicology
2025, Vol. 44(Supplement 3) 5S–35S

DOI: 10.1177/10915818251356416
journals.sagepub.com/home/ijt
Sage

Amended Safety Assessment of Synthetically Manufactured Amorphous Silica and Hydrated Silica as Used in Cosmetics

Christina L. Burnett*, Wilma F. Bergfeld, Donald V. Belsito**, Curtis D. Klaassen**, Daniel C. Liebler***, James G. Marks Jr.***, Ronald C. Shank***, Thomas J. Slaga***, Paul W. Snyder**, Monice M. Fiume†, and Bart Heldreth††**

Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of synthetically manufactured amorphous Silica and Hydrated Silica. The Panel considered the method of manufacture of these ingredients to be of significant importance when reviewing safety. Thus, the current assessment is exclusive to amorphous Silica and Hydrated Silica when manufactured via synthetic methods. Both of these ingredients are reported to function as abrasives, absorbents, anticaking agents, bulking agents, and opacifying agents in cosmetic products. The Panel reviewed relevant data, including frequency and concentration of uses. The Panel concluded that synthetically manufactured amorphous Silica and Hydrated Silica are safe in the present practices of use and concentration when formulated to be non-irritating.

Keywords

Cosmetic Ingredient Review, Expert Panel for Cosmetic Ingredient Safety, Safety, Cosmetics, Synthetically Manufactured Amorphous Silica, Hydrated Silica

Introduction

This report assesses the safety of synthetically manufactured amorphous Silica and Hydrated Silica as used in cosmetics. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*; see Table 1), both of these ingredients are reported to function as abrasives, absorbents, anticaking agents, bulking agents, and opacifying agents in cosmetic products.[1] (Additional functions specific to each ingredient are described in Table 1.) These ingredients were previously reviewed in a report that was finalized in 2009; other ingredients from that report will be re-reviewed elsewhere. Therefore, the conclusion for this current report on synthetically manufactured amorphous Silica and Hydrated Silica supersedes that previous conclusion just for these two ingredients.

The Panel considered the method of manufacture of these ingredients (whether synthetic or mined) to be of significant importance when reviewing safety. Thus, the current assessment is exclusive to amorphous Silica and Hydrated Silica when manufactured via synthetic methods.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data were provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on the synthetically manufactured amorphous Silica and Hydrated Silica included in this safety assessment were obtained from assessments by the Organisation for Economic Co-Operation and Development Screening Information Data Sets (OECD SIDS)[2] and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC).[3] These data summaries are available

*Cosmetic Ingredient Review Senior Scientific Analyst/Writer
**Expert Panel for Cosmetic Ingredient Safety Member
***Expert Panel for Cosmetic Ingredient Safety Former Member
†Cosmetic Ingredient Review Senior Director
††Cosmetic Ingredient Review Executive Director

Corresponding Author:
Bart Heldreth, Executive Director, Cosmetic Ingredient Review, 555 13th St., NW, Suite 300W, Washington, DC 20004, USA.
Email: cirinfo@cir-safety.org

on the OECD SIDS and ECETOC websites, respectively, and when deemed appropriate, information from the summaries has been included in this report.

Chemistry

Definition

Silica and Hydrated Silica, or silicon dioxide, are solids that can be derived from naturally occurring minerals. However, in this safety assessment, only ingredients that are produced synthetically are being considered. Indeed, in the case of Silica and Hydrated Silica, these ingredients are more commonly prepared as such for commercial purposes. The definitions and functions of synthetically manufactured amorphous Silica and Hydrated Silica are provided in Table 1.[1]

Silica. Silica comprises silicon–oxygen tetrahedral units, in which a silicon atom is central within 4 oxygen atoms that are shared with adjacent silicon atoms.[4] 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 (the safety of which is not reviewed in this assessment) is polymorphic, where each variety has a characteristic regular 3-dimensional arrangement of the tetrahedral. As would be predicted from these descriptions, crystalline Silica has a well-defined X-ray diffraction pattern, whereas amorphous forms of Silica do not.

The CAS No. 7631-86-9 is a general CAS No. which includes all forms of silicas, including amorphous, crystalline, synthetic, and natural forms.[2] The amorphous forms of Silica may also be referred to as amorphous silicon oxide hydrate, silicic anhydride, silicon dioxide, and silicon dioxide, fumed.[1] Pyrogenic Silica is the current terminology for silicon dioxide, fumed.[5] The CAS No. 112945-52-5 has been reported to be associated with synthetic pyrogenic Silica, whereas the CAS Nos. 67762-90-7; 68611-44-9; and 68909-20-6 have been reported to be associated with synthetic surface treated Silica.[6]

Hydrated Silica. Hydrated Silica may also be referred to as hydrosilicic acid, precipitated silica, silica gel, silica hydrate, silicic acid, silicic acid hydrate, silicon dioxide hydrate, synthetic amorphous silicon dioxide, and colloidal silica.[1,7] The CAS No. 112926-00-8 has been reported to be associated with both synthetic precipitated silica and silica gel.[6]

Table 1. Definitions and Functions.[1]

Ingredient and CAS No.	Definition	Function(s)
Hydrated Silica 10279-57-9 112926-00-8 1343-98-2 (silicic acid) 63231-67-4 7631-86-9	Hydrated Silica is the inorganic oxide that conforms generally to the formula $SiO_2 \cdot xH_2O$.	Abrasives; absorbents; anticaking agents; bulking agents; opacifying agents; oral care agents; skin-conditioning agents—misc.; viscosity increasing agents—aqueous
Silica 112945-52-5 60676-86-0 7631-86-9	Silica is the inorganic oxide that conforms to the formula SiO_2.	Abrasives; absorbents; anticaking agents; bulking agents; dispersing agents—nonsurfactant; opacifying agents

Physical and Chemical Properties

Physical and chemical properties of synthetically manufactured amorphous Silica are provided in Table 2.[2,8-10] Silica has a melting point of approximately 1700°C and water solubility of 15-68 mg/l at 20°C.

Silica and Hydrated Silica. According to size distribution measurements taken by several manufacturers of various synthetic amorphous Silica raw materials, the median particle sizes are approximately between 6 and 682 µm.[11] Particle size was reported to range from as small as <1 µm to as large as 2060 µm; data submitted to CIR reported that for 11 out of 20 samples, 0.15 to 80.1% of the particle measured 10 µm or less. However, these measurements will change once these ingredients are formulated in cosmetic products due to aggregation of the particles. These manufacturers also reported the size distribution of various synthetic amorphous Silica materials are approximately between 8 and 65 µm, with particle size ranges of approximately <1-344 µm.

The *Food Chemicals Codex* states that Silica is a white, fluffy non-gritty powder of extremely fine particle size that is hygroscopic.[12] Silica absorbs moisture from the air in varying amounts. Amorphous silicas are composed of very fine particles (average of 20 µm) which tend to aggregate loosely in the air.[13] Primary particles, or single particles, exist only in the colloidal form of Hydrated Silica.[3,14] Aggregates assemble in chains (Silica; pyrogenic) or clusters (Hydrated Silica; precipitated and gel). Agglomerates are assemblies of aggregates,

Table 2. Physical and Chemical Properties of Silica.

Property	Value	Reference
Physical form	White fluffy powder	8
Formula weight (Da)	60.1	9
Density (g/ml @ 20°C)	2.2	2
Specific gravity (g/ml)	2.65	10
Vapor pressure (mmHg)	0	9,10
Melting point (oC)	~1700-1710	2,9,10
Boiling point (oC)	2230	9
Water solubility (mg/l @ 20°C)	15-68	2
pH	4-9	2

held together by strong physical adhesion forces and not in a dispersible nano-size (<100 nm).

The acidity of synthetic amorphous Silica is related to the number and reactivity of the silanol groups present on the solid Silica surface.[15] Surface silanols (pKa = 7.1) are more acidic than monosilicic acid (pKa = 9.8). The acidity increases with the degree of polymerization. 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 nitrogen 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 a Si-O-C-R structure. A totally dehydrated Silica or a fully hydrated Silica has little or no adsorption of hydrophobic organic compounds.

Method of Manufacturing

Silica and Hydrated Silica. Amorphous Silica and Hydrated Silica, as used in cosmetics, are produced synthetically.[2,3,6] A manufacturing process for Silica (pyrogenic form) is shown in Figure 1. Mean particle size, particle size distribution, and degree of aggregation and/or agglomeration can be determined by adjusting processing parameters.[16]

Silica may be produced by a vapor-phase process.[8] The pyrogenic form of Silica is produced in a relatively anhydrous state. Hydrated Silica is produced by a wet process and contains a large amount of bound water.

Composition/Impurities

Silica. Silica has been reported to be >95% to >99.6% pure.[2,11] Possible impurities include: sodium oxide (0.2 to 2.1% wt.), sulfur trioxide (0.2 to 3.0% wt.), iron (III) oxide (<0.05% wt.), and other trace oxides (<0.07% wt.). Heavy metal limits 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).

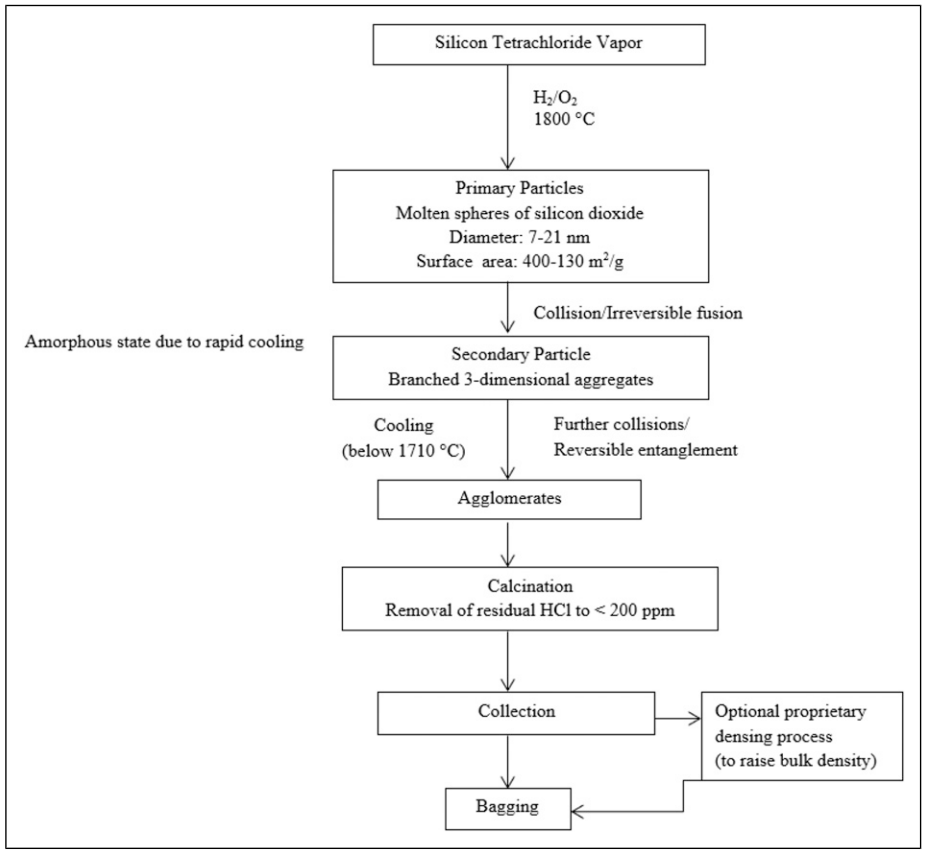

Figure 1. Process for the manufacture of Silica (pyrogenic form).

According to the *Food Chemicals Codex*, Silica (listed as silicon dioxide) may not contain more than 5 mg/kg lead.[17]

Use

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP data, Silica has a total of 8222 uses; the majority of the uses are in leave-on makeup preparations (e.g., eye makeup, lipsticks, foundations, and face powders; Table 3).[18] Hydrated Silica has a total of 462 uses; the majority of the uses are in rinse-off oral hygiene and personal cleanliness products. The frequencies of use for Silica and Hydrated Silica have greatly increased since the original safety assessments were finalized; in 2009, Silica was reported to have 3276 uses and Hydrated Silica was reported to have 176 uses.[5]

The results of the concentration of use surveys conducted in 2018 by the Council indicate Silica is used at up to 82% in face and neck products and 50% in mascaras and lipsticks.[19] Hydrated Silica is used at up to 33.8% in oral hygiene products and at up to 10% in leave-on skin care products. According to the original safety assessment, in 2008, the maximum use concentration reported for Silica was 44% in eye shadows,[5] and the maximum use concentration reported for Hydrated Silica was 34% in dentifrices, with a maximum leave-on concentration of 4% in face powders.

Silica and Hydrated Silica may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, Silica is reported to be used in lipsticks at up to 50%, and Hydrated Silica is reported to be in dentifrices at up to 17.1%.[19] Additionally, these ingredients have been reported to be used in products that may come into

Table 3. Current and Historical Frequency and Concentration According to Duration and Type of Exposure for Synthetically Manufactured Silica and Hydrated Silica.[5,18,19]

	Hydrated Silica				Silica[f]			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2019[e]	2009	2018	2008	2019	2009	2018	2008
Totals[e,g]	462	176	0.00001-33.8	0.001-34	8222	3276	0.000005-82	0.0000003-44
Leave-on	171	90	0.0002-10	0.001-4	7499	2937	0.0001-82	0.00004-44
Rinse-off	283	78	0.00001-33.8	0.01-34	669	316	0.000005-21	0.0000003-16
Diluted for (bath) use	8	8	0.3-12	0.4-4	54	23	0.1-4	0.02-2
Eye area	9	8	0.001-5.8	0.06-2	2348	867	0.00068-50	0.0004-44
Incidental ingestion	81	25	0.17-33.8	0.003-34	1565	551	0.014-50	0.01-21
Incidental inhalation-spray	16[a]; 10[b]	10[a]; 12[b]	0.45-0.9; 8.9-23.7[a]	0.04-2[a]; 0.06-2[b]	166; 516[a]; 419[b]	19; 247[a]; 183[b]	0.0001-2; 0.0042-14[a]; 0.5-1[b]	0.0005-6; 0.00004-8[a]; 0.02-10[b]
Incidental inhalation-powder	33; 10[b]	33; 12[b]	1; 0.0012-10[c]	2-4; 0.06-2[b]	520; 419[b]; 3[c]	248; 183[b]; 1[c]	0.016-66; 0.5-1[b]; 0.08-82[c]	1-26; 0.02-10[b]
Dermal contact	349	117	0.0002-16	0.001-17	5416	2298	0.0001-82	0.0000003-44
Deodorant (underarm)	1[a]	NR	0.066	2[a]	31[a]	38[a]	0.0001-10.4[d]	0.02-9[a]
Hair—non-coloring	4	NR	0.00001-8.9	0.04-2	142	51	0.000005-4	0.0005-3
Hair—coloring	10	20	1.9-8.9	2	233	149	0.0005-10	0.002-6
Nail	15	13	0.75-5.5	1-2	559	92	0.2-10	0.3-9
Mucous membrane	250	50	0.0051-33.8	0.003-34	1834	624	0.0005-50	0.0000003-21
Baby products	NR	NR	0.0041-0.005	NR	7	2	0.0006-3	0.003-10

NR = Not reported.
[a]It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[b]Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[c]It is possible these products may be powders, but it is not specified whether the reported uses are powders.
[d]Concentration of use in aerosol deodorants reported to be 0.0001% - 0.084%.
[e]Includes entries for Hydrated Silica and Silicic Acid from the VCRP database.
[f]Includes entries for Silica; Silica, Amorphous; Silica, Fumed; and Silicon Dioxide, Colloidal from the VCRP database.
[g]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

contact with the eyes, such as eye shadows, eye liners, and mascaras. Silica is reported to be used at up to 50% in mascaras, and Hydrated Silica at up to 5.8% in eyeliners.[19] Moreover, these ingredients are reported to be used in spray products that could possibly be inhaled; for example, Silica is reported to be used at up to 2% in aerosol hair spray and at up to 0.84% in aerosol deodorants.[19] Concerning final consumer product formulations (typically a mixture of ingredients), the Panel has noted that in practice, most droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump spray.[20-23] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[20,22] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[22] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Ingredients in this report are also used in powders, and these products could possibly be inhaled. For example, Silica is reported to be used at up to 66% in face powders.[19] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[24-26]

In regulations governing cosmetic products, Silica and Hydrated Silica are not restricted from use in any way in the European Union.[27]

Non-Cosmetic

According to Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Silica (as amorphous, fumed, crystalline-free; gel; gel-precipitated, crystalline-free; and vitreous) is a Tier I chemical (not considered to pose an unreasonable risk to the health of workers and public health).[28]

Hydrated Silica. Hydrated Silica (colloidal) is used in fiber, sizing, diazo paper manufacture, cellophane film, ceramics, glass fiber, paints, batteries, foods, and polishing.[29]

Silica. 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.[2] It is also a general excipient for pharmaceuticals.[30,31] Silica can function as a carrier for fragrances.[2] Silica is used in animal feed as carriers and anticaking agents in vitamins and mineral premixes. Silica is also used in paints, lacquers, plastics, paper, and in the production of "green tires." Silica is used as an insecticide by cuticular lipid layer dehydration. Silica is used as reinforcing fillers for many non-staining and colored rubber and silicone products.[2,13]

Silica has many uses in foods and food preparations.[2,12,31] These include use as an anticaking agent in dry powders, a dispersion agent for dry powders in liquids, an anti-settling or suspending agent, a stabilizer in oil/water emulsions, a thickening or thixotropic agent, a gelling agent, a flavor carrier, an extrusion aid, a clarification and separation aid, and a support matrix for immobilization of enzymes. It is also used as a defoaming agent, conditioning agent, a chill-proofing agent in malt beverages, and a filter aid in foods.

Toxicokinetics

Absorption, Distribution, Metabolism, Excretion (ADME)

Animal

Oral

Silica. In an oral study of Silica (synthetic amorphous; average particle size 15 μm) in an aqueous suspension, female rats (strain and number not specified) received 1500 mg/kg/d for 30 d via gavage.[2] Controls were not described. The rats were then killed and necropsied. The Silica content in the livers, kidneys, and spleen was 1.5 μg (control value = 1.8 μg), 6.4 μg (7.2 μg), and 5.3 μg (7.8 μg), respectively.

In a similar study, 20 female Sprague–Dawley rats received Silica (average particle size not reported) via gavage in an aqueous suspension (100 mg/rat; ~500 mg/kg) 20 times over 1 month.[2] Controls were not described. No clinical signs of toxicity were observed. The Silica content in the liver, spleen, and kidneys was 4.2 μg (control value = 1.8 μg), 5.5 μg (7.2 μg), and 14.2 μg (7.8 μg), respectively.

Silica and Hydrated Silica. In a dietary ADME study, five guinea pigs received Silica (0.8 mg/g feed) as three separate forms (sodium metasilicate, Hydrated Silica, and Silica solution (30%)) in single doses or in four repeated doses every 48 h.[32,33] Urine and feces were collected in 48-h increments after each dose of each form and analyzed for Silica content. For the sodium metasilicate doses, the urinary output of Silica peaked within 48 h and gradually returned to normal after 8 d. When administered four times, 48 h apart, the peak was maintained, but did not increase. Within 48 h after the last dose, the concentration of Silica in the urine began to return to normal. With the Silica solution and Hydrated Silica, the urinary output of Silica also peaked within 48 h and gradually returned to normal after 8 d, but the peaks were much lower than those observed with sodium metasilicate. When administered four times, 48 h apart, the Silica concentrations behaved similarly to the sodium metasilicate form, except with a lower peak. In this study, approximately 63% of the Silica was recovered. The authors of the study suggested that the Silica in the urine was in the soluble or molybdate reactive

form, and that the Silica particles underwent depolymerization prior to excretion.

Inhalation

Silica. The retention and elimination of aerosolized Silica (synthetic amorphous; initial dose and particle size not reported) was studied in female inbred albino rats (strain and number not reported).[2] The rats were exposed to the test material 4 h/d, 5 d/wk, for 40 d. The amount was then increased to 40 to 50 mg/m^3 until day 120. Groups of rats were killed and necropsied periodically through the test period.

The average 1-d retention value was 28 µg/lung at the lower unspecified concentration. During the first 10 d, a steep linear increase was seen with ~28 µg/d, 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-d retention value was 59 µg/lung at the high concentration. After 120 exposures, the total deposit (lung and mediastinal lymph nodes) was 435 µg/lung, equivalent to 7.4% of the theoretically deposited material (5840 µg/lung, based on the measured 1-d retention); more than 92% of the deposited Silica in the alveoli was eliminated during the exposure period. At that time, the mean retention in the lungs was 300 µg/lung (~69% of the total). The deposition rate in the mediastinal lymph nodes was negligible during the first 40 d, 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 h later an increase of 100% was observed.[2]

In another retention and elimination study, female Sprague–Dawley rats (number not reported) were exposed to aerosolized Silica (0.050 to 0.055 mg/l; particle size not provided) for 5 h/d for 5 d/wk for 1 yr.[2] Because the rats had occurrences of bronchitis, putrid lung inflammation, and pronounced cell reactions, the exposure incidences were reduced to 2 or 3 d/wk. Rats in each group were killed and necropsied periodically during treatment and after treatment.

After 6 wk of treatment, Silica was observed in the lungs (0.5 mg) and the mediastinal lymph node (0.02 mg); after 18 wk, these values were 1.2 mg and 0.11 mg, respectively, and after 12 mo, the values were 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 mo, there was 0.160 mg and 0.047 mg Silica observed in the lungs and mediastinal lymph node, respectively, with a reduction of 88% in the lung and >50% in the lymph nodes. The increase in lung deposition was rapid at the initial exposure; amounts of deposited Silica were low from 18 wk to 12 mo of exposure.[2]

Groups of 10 male and 10 female Wistar rats were exposed to Silica (0, 0.51, 2.05, or 10.01 mg/m^3; particle size not provided) for 6 h/d, 5 d/wk, for 13 wk.[3] A group of rats from each dose group was allowed to recover for 13 wk 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 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.

Rats (strain and number not provided) were exposed to aerosolized Silica (hydrophilic; 50 to 55 mg/m^3) for 12 mo.[3] Rats were killed and necropsied periodically and after 5-mo recovery. There was 0.25 mg Silica in the lung at day 3, and 0.5 mg at 6 wk. At 12 mo, ~1% of the total administered respirable Silica was observed in the lungs. Initial accumulation was rapid and dropped off between week 18 and 12 mo (0.5 mg at 6 wk; 1.2 mg at 18 wk; 1.37 mg at 12 mo). The mediastinal lymph nodes contained ~0.02 mg Silica at 6 wk and 0.13 at 12 mo. After 5 mo 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 number not provided) were exposed to aerosolized Silica (hydrophobic; 50 mg/m^3; particle size not provided) for 5 h/d, 2 d/wk, for 8 and 12 mo.[3] After 8 mo, the lungs retained 1.448 mg Silica (1.3% of exposure) and after 12 mo, 1.759 mg Silica (1.1%). The lymph nodes retained 0.05 and 0.113 mg, respectively. After a 12-mo exposure and 1-mo recovery, the lungs contained 1.1 mg Silica (37.5% elimination) and the lymph nodes contained 0.16 mg. After 3-mo recovery, the lungs contained 0.43 mg and the lymph nodes 0.12 mg Silica. After 5-mo recovery, the lungs contained 0.41 mg (76.7% elimination) and the lymph nodes 0.13 mg Silica.

Rats (strain and number not provided) were exposed to aerosolized Silica (hydrophobic; 100 mg/m^3; particle size not provided) for up to 1 yr.[3] Silica content of the lungs and the lymph nodes was 4.33 and 0.132 mg at 3 mo, 6.71 and 0.214 mg at 5 mo, and 11.46 and 0.378 mg at 12 mo, respectively. After 6 mo of recovery, 55.5% of the Silica was eliminated from the lungs. Lymph node elimination could not be observed.

In an elimination study, aerosolized Silica (0.05 mg/l; particle size not provided) was administered for 5 h/d for 3 d to female Sprague–Dawley rats (number not specified).[2] The rats were observed for up to 3 mo. Twenty hours after the last exposure, 0.25 mg Silica was found in the lungs. After 3 mo, the Silica content was 0.018 mg. In the lymph node, 0.018 mg Silica was found after 1 mo and 0.008 mg Silica after 3 mo.

An elimination study was performed on rats (details not provided) exposed to aerosolized Silica (hydrophobic; 50 mg/m^3; particle size not provided) for 1 or 3 d.[3] The rats were killed and necropsied after 20 h, 1 mo, or 3 mo. At 1-mo recovery, elimination of Silica was 78% (1 d exposure) and 75% (3 d exposure). After 3-mo recovery, elimination was 87% and 92%, respectively. There was little Silica in the mediastinal lymph nodes.

Rats (details not provided) were exposed to aerosolized Silica (hydrophobic; 200 mg/m^3; particle size not provided) in an elimination study for 5 h/d for 3 d.[3] After a 3-mo recovery period, 81% of the Silica was eliminated. Elimination by the lymph nodes was marginal.

Hydrated Silica. In an elimination study of Hydrated Silica (55 mg/m^3; average particle size 15 µm), rats (details not provided) were exposed to the test material for 5 h.[2] The mean retention value at 20 h was 0.138 mg/lung. The mean Silica content of the lungs for Hydrated Silica was 1.022 mg after 4-mo recovery and 3.113 mg after 12-mo recovery. 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.

Subcutaneous

Silica. In a subcutaneous study in female Sprague–Dawley rats (number not provided), 6.90 mg Silica was measured in the tissue 24 h after a single dose of 10 mg was injected.[2,3] One month after injection, the amount of Silica had decreased to 0.65 mg, and after 2 mo, the amount of Silica at the injection site was 0.30 mg.

Approximately 95% to 97% of Silica (30, 40, or 50 mg in water) injected subcutaneously in rats was recovered 6 wk after treatment (no further details).[3]

Human

Oral

Silica and Hydrated Silica. Excretion of orally administered Silica and Hydrated Silica (as 1250 mg of Silica in apple juice) was evaluated in 2 groups of 6 volunteers (5 males and 1 female in each group).[2] The solutions were consumed in two doses, morning and midday, on the same day. The total urine was collected daily and analyzed. During the 4 d post-treatment, changes of renal Silica secretion were not observed. Daily Silica increments in urine after ingestion ranged between 7 and 23 mg. For 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/d. In the post-treatment phase, individual mean excretion rates ranged from 32 to 61 mg/d. For Hydrated 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/d. In the post-treatment phase, individual mean excretion rates ranged from 20 to 81 mg/d. 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 ingested.

Toxicological Studies

Acute Toxicity Studies

Acute dermal, oral, and inhalation data are summarized in Table 4. Hydrated Silica in water had a dermal LD_{50} greater than 5 g/kg in rabbits.[2,3] In oral rat studies, the LD_{50}s were >2 g/kg for Silica (in polyethylene glycol 400).[3] For Hydrated Silica at 12.1% in saline and 26% in water, oral LD_{50}s in rats were >5 g/kg and 40 g/kg, respectively.[2,3] In inhalation studies that ranged in duration from 1 to 6 h, the LC_{50}s for Hydrated Silica (30% SiO_2) and Silica (concentration not reported) in rats were >560 mg/m^3 and >139 mg/m^3, respectively.[2,3]

Short-Term, Subchronic, and Chronic Toxicity Studies

Animal. Short-term, subchronic, and chronic toxicity studies for Hydrated Silica and Silica are summarized in Table 5.

No adverse effects were reported in a 3-wk dermal study of Silica (up to 10 g/kg/d) in rabbits.[3] In short-term oral studies, the no-observed-adverse-effect-level (NOAEL) for Hydrated Silica was $\geq$ 24.2 g/kg/d in a 14-d dietary study in rats.[2,3] The no-observed-effect-level (NOEL) was 500 mg/kg/d in a 5- to 8-wk dietary study in rats that were fed up to 16,000 mg/kg/d Silica.[8] No treatment-related effects were observed in a 4-wk dietary study of Silica (800 mg/kg/d) in rats or dogs.[34] In subchronic oral studies, the NOEL was 4000 mg/kg/d in a 13-wk dietary study in rats fed Hydrated Silica at up to 4000 mg/kg/d.[3] No clinical signs of toxicity or gross or microscopic changes were reported in a 13-wk dietary study in rats that received up to 3500 mg/kg/d Silica.[2,3] In oral chronic studies, lower liver weights in female rats, without significant findings at histopathological examinations, were observed in a 103-wk dietary study of up to 5% Hydrated Silica in rats.[35] No remarkable findings were observed by the same researchers of the same material in a 93-wk dietary study in mice.[35] The NOAEL in a 6-mo dietary rat study of up to 10% Hydrated Silica was 8980 mg/kg/d.[2,3] No remarkable findings were reported in 6-mo dietary studies of up to 10% Silica in rats, although there were increased numbers of leukocytes and eosinophils in female and male rats, respectively, and reduced liver and prostate weights in another 6-mo study at up to 3 g Silica/wk.[36]

In short-term inhalation studies with Hydrated Silica, inflammatory and pulmonary lesions were observed in rats at 30 mg/m^3.[7,37-42] Inflammatory responses were also observed in rats exposed to Silica in studies that lasted between 5 and 14 d.[7,38,43] No significant lung histopathological findings or adverse changes in inflammatory markers were observed in rats that were exposed to nanoparticle Silica (particle size 50-79 nm; concentrations 0.4-5.4 mg/m^3) for 4 wk.[44] In subchronic inhalation studies, inflammatory responses were noted in the lungs and lymph nodes along with pulmonary lesions after exposure to Hydrated Silica at 35 mg/m^3 (particle and agglomerate/aggregate size 1 to ~120 µm).[43] In a 13-wk inhalation study of Silica in rats, the NOEL was 1.3 mg/m^3.[43] Inflammation and pulmonary lesions, including fibrosis, were noted in this study and another 13-wk rat study (fibrosis subsided during recovery).[43,45] The lowest-observed-adverse-effect-concentration (LOAEC) in rabbits exposed for 9 mo to Hydrated Silica was 28 mg/m^3.[46] In inhalation studies of 9- to 12-mo duration, Hydrated Silica caused pulmonary inflammation and emphysema in rats exposed to 25 to 85 mg/m^3.[47]

Table 4. Acute Toxicity Studies.

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD_{50} or LC_{50}	Reference
Dermal				
Hydrated Silica; no further details	2000 mg/kg bw applied to intact and abraded skin for 24 h; 10 New Zealand white rabbits; no further details	Details not provided	>2000 mg/kg	[2,3]
Hydrated Silica; in water	2000, 3000, 4000, or 5000 mg/kg in groups of 4 New Zealand white rabbits; 2 rabbits in each group had abraded skin; test site was covered with occlusive patch for 24 h; no further details	Very slight erythema; no systemic signs of toxicity or organ toxicity	>5000 mg/kg	[2,3]
Oral				
Hydrated Silica; suspended (12.1% (w/v)) in 0.85% saline	Male rats; no further details	No clinical signs of toxicity; no treatment-related effects at necropsy	>5000 mg/kg	[2,3]
Hydrated Silica; 26% in water; pH 4.5	10 male Sprague–Dawley rats; no further details	Details not provided	40,000 mg/kg bw	[2,3]
Hydrated Silica; suspended in water (33% w/w)	10,000, 12,600, 15,800, or 20,000 mg/kg bw; 5 Sprague–Dawley rats per sex per dose via gavage	No clinical signs of toxicity; stools were white for 2 d	>20,000 mg/kg bw	[2,3]
Hydrated Silica; in water	5620 mg/kg; 30 male Sprague–Dawley rats via single gavage dose	No clinical signs of toxicity; stools were white for 2 d	>5620 mg/kg bw	[2,3]
Hydrated Silica; in water	10,000 mg/kg bw; 5 male and 5 female Sprague–Dawley rats; no further details	Details not provided	>10,000 mg/kg bw	[3]
Hydrated Silica; in water	31,600 mg/kg bw; 5 male and 5 female Sprague–Dawley rats; 24-h observation; no further details	Details not provided	>31,600 mg/kg bw	[3]
Hydrated Silica; in 0.85% saline	10 to 5000 mg/kg bw; male rats; no further details	Distended stomachs with bloody patches at the pyloric end were observed at necropsy in animals that received >100 mg/kg; at 5000 mg/kg, vascular stomach and reddened intestinal lining were observed	470 mg/kg	[2,3]
Hydrated Silica; in saline	5000 mg/kg bw; male Sprague–Dawley rats; no further details	Details not provided	>5000 mg/kg bw	[3]
Hydrated Silica; average particle size 100 μm; in aqueous suspension of 1% carboxymethylcellulose	2000 or 5000 mg/kg bw; 10 male and 10 female Sprague–Dawley rats per single dose via gavage	No clinical signs of toxicity; no treatment-related effects at necropsy	>5000 mg/kg	[2,3]
Hydrated Silica; average particle size 8 μm; in carboxymethylcellulose	5110 mg/kg; 5 male and 5 female Wistar rats via gavage	No clinical signs of toxicity; no treatment-related effects at necropsy	>5110 mg/kg	[2,3]
Hydrated Silica; in olive oil	4000, 5040, or 6350 mg/kg bw; 5 male and 5 female Sprague–Dawley rats per dose group; no further details	Details not provided	>6350 mg/kg bw	[3]
Hydrated Silica; in olive oil	5040, 6350, or 7900 mg/kg bw; 5 male and 5 female Sprague–Dawley rats per dose group; no further details	Details not provided	>7900 mg/kg bw	[3]
Hydrated Silica; in 1% aqueous gum arabic solution	20,000, 25,200, or 31,800 mg/kg bw; 5 male and 5 female Sprague–Dawley rats per dose group; no further details	Details not provided	>31,800 mg/kg bw	[3]

(continued)

Table 4. (continued)

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD_{50} or LC_{50}	Reference
Hydrated Silica; in dispersion of 10% gum arabic in water	5000 mg/kg; 5 male and 5 female rats; no further details	No clinical signs of toxicity; no treatment-related effects at necropsy	>5000 mg/kg	2,3
Hydrated Silica; 30% neutralized with HCl	Male rats; no further details	Details not provided	10,000 mg/kg bw	2,3
Silica (hydrophilic); in corn oil	178, 316, 562, 1000, 1780, or 3160 mg/kg bw; groups of 10 male Swiss mice; via gavage	No adverse signs of toxicity and no macroscopic lesions at necropsy	>3160 mg/kg bw	2,3
Silica; no further details	1000, 2150, or 3160 mg/kg bw in 5 male albino rats; no further details	No gross signs of systemic toxicity and no mortalities	>3160 mg/kg bw	65
Silica; no further details	30 male rats; no further details	No clinical signs of toxicity or mortalities during the 2-wk observation period	>5620 mg/kg bw	36
Silica; incorporated into a stock diet at a ratio of 1:4 (w/w)	10 Wistar male/female rats; dosing period was 24 h; no further details	No clinical signs of toxicity; no treatment-related effects at necropsy; stool grey in color with normal consistently but larger in size than normal	>10,000 mg/kg	2,3
Silica (hydrophilic); in water	5 male and 5 female Sprague–Dawley rats; no further details	Details not provided	>5000 mg/kg bw	3
Silica (hydrophobic); in distilled water	1000, 1590, 2510, 3980, 6310, or 10,000 mg/kg bw; groups of 5 male and 5 female Sprague–Dawley rats; no further details	Details not provided	9200 mg/kg bw males >10,000 mg/kg bw females	3
Silica (hydrophobic); in corn oil	178, 316, 562, 1000, 1780, or 3160 mg/kg bw; groups of 10 male Sprague–Dawley rats; no further details	Details not provided	>3160 mg/kg bw	3
Silica (hydrophobic); in corn oil	5000 mg/kg bw; 5 male and 5 female Sprague–Dawley rats; no further details	Details not provided	>5000 mg/kg bw	3
Silica (hydrophobic); in peanut oil	2500 or 5000 mg/kg bw; 10 male and 10 female Sprague–Dawley rats; no further details	Details not provided	>5000 mg/kg	3
Silica; in olive oil	5040, 6350, or 7900 mg/kg in olive oil or 2500 or 5000 mg/kg in peanut oil	No clinical signs of toxicity or unscheduled mortalities during the 4-wk observation period; no treatment-related effects at necropsy	>7900 mg/kg in olive oil	8
Silica; in aqueous suspension of 1% methylhydroxyethyl cellulose	2000 or 3300 mg/kg bw in 10 male and 10 female Sprague–Dawley rats per single dose via gavage	No clinical signs or gross macroscopic signs of toxicity observed	>3300 mg/kg	2,3
Silica (hydrophilic); in 0.5% methylcellulose	1000, 2750, or 3160 mg/kg bw; 5 male Boltzman rats per dose group; no further details	Details not provided	>3160 mg/kg	3
Silica (hydrophobic); in polyethylene glycol 400	2000 mg/kg bw; 5 male and 5 female Wistar rats; no further details	Details not provided	>2000 mg/kg bw	3
Inhalation				
Hydrated Silica (5% SiO_2); as mist; no further details	760 mg/m^3; male albino rats; 3.25-h whole body exposure; no further details	No deaths; no further details	>760 mg/m^3	3
Hydrated Silica (20% SiO_2); as mist; no further details	2240 or 2500 mg/m^3; male albino rats; 4.2-h whole body exposure; no further details	No deaths; no further details	>2500 mg/m^3	3

(continued)

Table 4. (continued)

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD_{50} or LC_{50}	Reference
Hydrated Silica (30% SiO_2); as mist; no further details	520 or 560 mg/m^3; 2 male rats; 2.5 or 6-h nose-only exposure; preliminary test; no further details	No deaths; no further details	>560 mg/m^3	3
Hydrated Silica (30% SiO_2); as mist; no further details	3300 mg/m^3; male albino rats; 1.5-h whole body exposure; no further details	No deaths; no further details	>3300 mg/m^3	3
Hydrated Silica; 45% of particles <5 μm; surface area (SA) = 190	691 mg/m^3; 5 male and 5 female Wistar rats; 4-h whole body exposure; no further details	Some decreased body weight gain in females 2 d post-exposure which resolved by day 14; no abnormalities observed at necropsy	>691 mg/m^3	2,3
Hydrated Silica; no further details	2200 mg/m^3; 10 male Sprague–Dawley rats; 1-h nose-only exposure; no further details	One rat died 2 h after exposure; irritation and dyspnea observed in most animals; no further details	>2200 mg/m^3	2,3
Hydrated Silica; no further details	3100 mg/m^3; 2 male rats; 4-h nose-only exposure; no further details	Details not provided	>3100 mg/m^3	3
Silica (hydrophobic); no further details	250 mg/m^3; groups of 10 male Swiss mice; 6-h whole body exposure; no further details	Clinical signs of toxicity included preening and occasional prostration; no significant findings at necropsy	>250 mg/m^3	3
Silica (hydrophobic); particle size <0.1 μm; SA = 300 m^2/g	90, 350, or 5000 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h whole body exposure; no further details	Details not provided	90 mg/m^3	3
Silica (hydrophobic); particle size = 0.15 μm; SA = 130 m^2/g	2280 mg/m^3; 5 male and 5 female rats; 1-h whole body exposure; no further details	Details not provided	>2280 mg/m^3	3
Silica (hydrophobic); particle size <0.2 μm; SA = 130 m^2/g	350, 770, 2530, or 5300 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h whole body exposure; no further details	All rats in 2530 and 5300 mg/m^3 dose groups died; severe red discoloration of the lungs was noted in the rats that died during the study; no further details	1650 mg/m^3	3
Silica (hydrophobic); particle size = 0.36 μm; SA = 200 m^2/g	0 or 4900 mg/m^3; groups of 5 male and 5 female Sprague–Dawley rats; 4-h whole body exposure; no further details	All animals of the test group died	<4900 mg/m^3	3
Silica (hydrophobic); particle size <0.4 μm; SA = 300 m^2/g	80, 340, 1200, or 5000 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h whole body exposure; no further details	Details not provided	800 mg/m^3	3
Silica (hydrophobic); particle size = 0.48 μm; SA = 200 m^2/g	0, 1260, 2830, or 6280 mg/m^3; groups of 5 male and 5 female Sprague–Dawley rats; 1-h whole body exposure; no further details	Details not provided	1260-2830 mg/m^3; no further details	3
Silica (hydrophobic); particle size = 0.54 μm; SA = 200 m^2/g	0 or 2190 mg/m^3; groups of 5 male and 5 female Sprague–Dawley rats; 4-h whole body exposure; no further details	All animals of the test group died	<2190 mg/m^3	3
Silica (hydrophilic); particle size = 0.76 μm; SA = 200 m^2/g	2080 mg/ m^3; 5 male and 5 female Sprague–Dawley rats; 4-h nose-only exposure; no further details	Details not provided	>2080 mg/m^3	2,3
Silica (hydrophobic); particle size = 0.95-2.15 μm; SA = 300 m^2/g	90 or 840 mg/m^3; groups of 5 male and 5 female Wistar rats; 4-h whole body exposure; no further details	Results similar as those listed below; no further details	90-840 mg/m^3	3

(continued)

Table 4. (continued)

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD_{50} or LC_{50}	Reference
Silica (hydrophobic); particle size = 1.175-1.275 μm; SA = 130 m^2/g	210, 540, or 2100 mg/m^3; groups of 5 male and 5 female Wistar rats; 4-h whole body exposure; no further details	All animals died in high-dose group within 2.5 h of exposure; necropsy of this group discovered eye opacity, lung enlargement with red areas, and white material in the nasal turbinates; in the mid-dose group, 7/10 animals died during exposure; necropsy of mid-dose group discovered opaque eyes, dark enlarged lungs with red areas, white material in nasal turbinates, and red areas in the intestines; all rats in low-dose group survived; at necropsy, low-dose group had dark lungs with white and red areas	540 mg/m^3	3
Silica (hydrophobic); particle size = 1.4-1.8 μm; SA = 80 m^2/g	1094, 2863, 3730, or 5382 mg/m^3; groups of 5 male and 5 female Wistar rats; 4-h whole body exposure; no further details	Details not provided	2863-3730 mg/m^3; no further details	3
Silica (hydrophobic); particle size = 1-5 μm (83%) and 5-100 μm (17%); SA = 300 m^2/g	120, 400, 1370, or 3360 mg/m^3; groups of 3 male and 3 female Sprague–Dawley rats; 4-h whole body exposure; no further details	Details not provided	660 mg/m^3	3
Silica; particle size ≤ 3 μm (84%); no further details	10 Sprague–Dawley rats; 4-h whole body exposure; no further details	Clinical signs included nasal discharge during exposure and crusty eyes and nose and alopecia during the 14-d observation period; reduced body weight gain observed in females in the first 3 d post-exposure and then returned to normal; discolored lungs observed in 1 rat at necropsy	>2.08 mg/m^3	2
Silica (hydrophilic); 56% of particles <5 μm; SA = 200 m^2/g	139 mg/m^3; 5 male and 5 female Wistar rats; 4-h nose-only exposure; no further details	No clinical signs of toxicity and no organ abnormalities at necropsy	>139 mg/m^3	2,3
Silica (hydrophobic); particle size <5 μm (56%) and > 7.7 μm (44%); SA = 200 m^2/g	477 mg/m^3; 5 male and 5 female Wistar rats; 4-h whole body exposure; rats were observed for 14 d post-exposure and periodically weighed; no further details	No mortalities during exposure or observation period; body weights decreased during the first 2 d after exposure before returning to normal; necropsies were unremarkable	>477 mg/m^3	8
Silica (hydrophobic); particle size = 6.3-7.7 μm; SA = 300 m^2/g	400, 700, or 2000 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h nose-only exposure; no further details	Details not provided	600 mg/m^3	3
Silica (hydrophobic); particle size = 7.0-7.1 μm; SA = 300 m^2/g	400 or 600 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h nose-only exposure; no further details	Details not provided	500 mg/m^3	3
Silica (hydrophobic); particle size = 7.2-7.7 μm; SA = 130 m^2/g	900 or 2200 mg/m^3; groups of 5 male and 5 females Sprague–Dawley rats; 4-h nose-only exposure; no further details	4/10 rats in high-dose group died; severe discoloration of the lungs was noted in the rats that died during the study; surviving rats had normal lungs except 1 male and 2 females with trace discoloration	>2200 mg/m^3	3
Silica (hydrophilic); SA = 200 m^2/g	0 or 191,300 mg/m^3; albino rats; 1-h nose-only exposure; no further details	Details not provided	>191,300 mg/m^3	3

(continued)

Table 4. (continued)

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD_{50} or LC_{50}	Reference
Silica (hydrophilic); SA = 380 m^2/g	0 or 207,000 mg/m^3; 10 male albino rats per dose group; 1-h nose-only exposure; no further details	Vigorous cleansing activity, hypoactivity, abdominal respiration, gasping, nasal exudation, closed eyes, crust-like material around nose and mouth, and chalky fur up to 2 d post-exposure	>207,000 mg/m^3	3
Silica (hydrophobic); no further details	250 mg/m^3; groups of 10 male Wistar rats; 6-h whole body exposure; no further details	Clinical signs of toxicity included preening, hunching, and occasional prostration; no significant findings at necropsy	>250 mg/m^3	3
Silica (hydrophobic); no further details	670, 690, 710, 1540, or 3150 mg/m^3; 10 male albino rats per group; 1-h exposure; no further details	Details not provided	>3150 mg/m^3	3
Silica (hydrophobic); no further details	250 mg/m^3; groups of 10 male English short hair guinea pigs; 6-h whole body exposure; no further details	Clinical signs of toxicity included preening; consolidation observed in the lungs of 2/9 animals; no significant findings at necropsy	>250 mg/m^3	3

No silicotic processes were noted in studies of rabbits, rats, and guinea pigs exposed to an average of 126 mg/m^3 Hydrated Silica for 12, 15, and 24 mo, respectively; neoplasia was not observed.[48] In a 12-mo study with Hydrated Silica and Silica in rats, the LOAEC was 6.9 mg/m^3 due to interstitial fibrosis (which was comparable between test and control groups).[49] The same test materials also were associated with nodular fibrosis in an 18-mo study with monkeys, although the animals may have been exposed to quartz or asbestos fibers. The LOAEC in a 6-mo rat inhalation study with Silica was 53 mg/m^3.[47] Emphysema and fibrosis were noted around 4 mo of exposure. Inflammatory responses and pulmonary lesions were noted in rat, guinea pigs, rabbits, and monkeys in studies up to 24 mo in duration.[3,50-52] More than half the studies summarized in this report included recovery periods of various durations; results in recovery animals demonstrated that observed lung effects did not worsen, and in some cases began to resolve, after exposure ceased.

Developmental and Reproductive Toxicity (DART) Studies

Silica

In a DART study, pregnant female CD-1 mice were fed up to 1340 mg/kg Silica for 10 d (specific gestation days not provided).[2,3] 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 fed up to 1350 mg/kg for 10 d, hamsters fed up to 1600 mg/kg for 5 d, and rabbits fed up to 1600 mg/kg for 13 d.

In a subchronic dietary study that also investigated reproductive effects, Silica (500 mg/kg/d) was administered to female Wistar rats (n = 20) for 6 mo.[2,3,8] A control group of 20 female rats received just diet. The female rats were mated with male rats twice: at weeks 8 and 17. The male rats (number not reported) were also consuming 500 mg/kg/d of Silica. 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 mo, the rats were killed and necropsied, except for 5 rats which had a 3-wk treatment-free period prior to being killed and necropsied.

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.[2,3,8]

Genotoxicity Studies

Genotoxicity data are summarized in Table 6. Hydrated Silica and Silica were not genotoxic in Ames tests, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene mutation assays, or chromosome aberration tests.[2,3,8,53-56] Chromosome aberration (oral dosing), dominant lethal mutation (oral dosing), gene mutation (intraperitoneal (i.p.) injection), and mitotic recombination (i.p. injection) studies of Hydrated Silica at up to 5000 mg/kg in mice and rats were negative.[3,57]

Table 5. Repeated Dose Toxicity Studies.

Ingredient/Concentration/Dose/Vehicle	Species/Strain/Cell	Method	Results	Reference
Dermal toxicity				
Silica; 0, 5, or 10 g/kg/d	2 male and 2 female albino rabbits per dose group; no further details	Test material applied for 18 h/g, 5 d/wk for 3 wk on intact and abraded skin; no further details	No signs of systemic toxicity and no gross or microscopic pathological findings; Silica content of blood, urine, spleen, liver, and kidney similar to controls	[3]
Oral toxicity				
Hydrated Silica; 38.45, 79.78, or 160 g/male and 37.02, 72.46, or 157.59 g/female (1.25%, 2.5%, or 5%); in feed	Groups of 40 male and 40 female $B_6C_3F_1$ mice	93-wk dietary study	No remarkable findings with regard to hematology or organ weights; no differences between treated groups and controls with mortality; feed consumption was increased in mid- and high-dose groups while weight increases in males weeks 15-50 and in females weeks 30-50 were reduced	[35]
Hydrated Silica; 7500 mg/kg/d; in feed	6 albino male rats; no further details	Dietary study where rats received test material in feed 5 times per week for 2 wk	All animals lost weight during treatment, but gained over the weekend and during post-observation period; no significant effects on the organs	[2,3]
Hydrated Silica; 16.5 g/kg/d (10% w/w) in group 1 and 5.8 g/kg/d (5% w/w) and 24.2 g/kg/d (20% w/w) in group 2; in feed	Two groups of 5 male and 5 female Sprague–Dawley rats	14-d dietary study; group 1 received 16.5 g/kg/d test material for 14 d and group 2 received 5.8 g/kg/d for days 1-10 and 24.2 g/kg/d for days 11-14; pathological exam not performed	NOAEL $\geq$ 24.2 g/kg/d; no clinical signs of toxicity or significant changes in feed/water consumption, body weight gains, or behavior	[2,3]
Hydrated Silica; average particle size = 15 μm; 1500 mg/kg/d; in aqueous solution	Female inbred rat; no further details	Daily gavage for 1 mo	No clinical signs of toxicity or significant changes in feed consumption, body weight gain, or behavior; Silica content in liver = 1.5 μg, in kidney = 6.4 μg, and in spleen = 5.3 μg	[2,3]
Hydrated Silica; 0, 250, 1000, or 4000 mg/kg/d (0%, 0.5%, 2%, or 8%); in feed	Groups of 10 male and 10 female Wistar rats	13-wk dietary study	NOEL = 4000 mg/kg/d; high-dose group had increased feed intake associated with a decreased feed efficiency; increased mean absolute and relative weight for the cecum in the high-dose group; no gross or microscopic pathological changes in any dose group	[3]
Hydrated Silica; 0, 2170, or 7950 mg/kg/d in males or 0, 2420, or 8980 mg/kg/d in females (0%, 3.2%, or 10%); in feed	Groups of 12 male and 12 female CD-1 rats	6-mo dietary study	NOAEL = 8980 mg/kg/d; no clinical signs of toxicity or significant changes in feed consumption, growth, hematology, clinical chemistry, or gross or microscopic pathology	[2,3]
Hydrated Silica; 143.46, 179.55, or 581.18 g/male and 107.25, 205.02, or 435.33 g/female (1.25%, 2.5%, or 5%); in feed	Groups of 40 male and 40 female Fischer 344 rats	103-wk dietary study	No differences between treated groups and controls with body weight, feed intake, behavior, or hematological or chemistry parameters; liver weights in females in the mid- and high-dose groups were lower at 12 to 24 mo; no significant histopathological findings	[35]
Silica; 0.2%, 1.0%, or 2.5% in feed	Groups of 10 male rats; no further details	Dietary study 28 d in length; no further details	No adverse effects or unscheduled mortalities; gross necropsy findings unremarkable	[36]
Silica; 0.8 g/kg/d in feed; no further details	15 male and 15 female CD rats	Dietary study 4 wk in length; no further details	No treatment-related effects observed	[34]
Silica; 0, 500, 1000, or 2000 mg/kg/d with a 2-wk stepwise increase to 16,000 mg/kg/d (approximately 25% feed intake)	Groups of 5 male and 5 female Wistar rats	Dietary study 5 wk in length for low- and mid-dose groups and 8 wk for high-dose group	LOEL = 1000 mg/kg/d; NOEL = 500 mg/kg/d; high-dose group had significant reduction in body weight associated with decreased feed intake; no significant changes in biological parameters or macroscopic findings; at microscopic examination, liver had severe atrophy in the epithelium	[8]
Silica (hydrophilic); 0, 700, 2100, or 3500 mg/kg/d (0%, 1%, 3%, or 5%); in feed	Groups of 15 male and 15 female Charles River rats	13-wk dietary study; interim necropsies of 3 males and 3 females performed after 45 d	NOAEL = 3500 mg/kg/d; no clinical signs of toxicity or significant changes in feed consumption or growth rate; no gross or microscopic pathological changes; no increase in Silica content in the liver, kidney, spleen, blood, or urine after 45 or 90 d in the high-dose group	[2,3]
Silica (hydrophobic); 0, 1000, 2000, or 4000 mg/kg/d (0%, 1%, 2%, or 4%); in feed	Groups of 10 male and 10 female Charles River rats	13-wk dietary study	No clinical signs of toxicity; no gross or microscopic pathological changes; no changes in behavior or growth; a minimal change in the thyroid gland morphology was observed in the mid- and high-dose males	[3]
Silica; 3.2% or 10%; in feed	12 male and 12 female rats; no further details provided	6-mo dietary study; no further details provided	No mortalities; only clinical sign was discolored stools; no remarkable findings with growth and development, feed consumption, histology, hematology, or at necropsy	[36]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/ Vehicle	Species/Strain/Cell	Method	Results	Reference
Silica; 0.78 or 3.00 g/wk males and 0.55 or 2.11 g/wk females; in feed	12 male and 12 female rats; no further details provided	6-mo dietary study; no further details provided	Increase in the number of leukocytes in high-dose females and of eosinophils in high-dose males; dose-dependent decrease in glucose concentration and AP activity in male rats; dose-dependent decrease in serum calcium concentration; reduced liver and prostate weights; no effects on body weight gain, feed consumption, blood chemistry, or urinalysis	[36]
Silica; 500 mg/kg/d	20 male and 20 female Wistar rats	6-mo gavage study; 5 times/wk	No clinical signs of toxicity and no macroscopic findings	[8]
Silica; 0.8 g/kg/d in feed; no further details	Male and female Beagle dogs; no further details	Dietary study 4 wk in length; no further details	No treatment-related effects observed	[34]
Inhalation toxicity				
Hydrated Silica; no further details	10 or 100 mg/m^3; 24 male CD rats; 6 h/d for 3 d followed by recovery periods of 1, 8, 30, or 90 d	Transient inflammatory tissue reaction observed in low-dose group at 24 h post-exposure that resolved within 8 d; recovery in high-dose group similar to that in low-dose group	Not reported	[42]
Hydrated Silica (precipitated and gel) and Silica, aerosolized; particle sizes not provided; 1, 5, or 25 mg/m^3	10 male and 10 female Wistar (Crl:WI)WU BR rats per dose group	5-d study with 3-mo recovery period; 6 h/d; nose-only exposure	No clinical signs of toxicity during 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 mo; all 3 test materials induced biomarkers of cytotoxicity in bronchoalveolar lavage (BAL) fluid, increases in lung and tracheobronchial lymph node weights, and histopathological lung changes in the high-dose groups at day 1 post-exposure; mid dose only induced histopathological changes and changes in BAL fluid; all effects except slight histopathological lung changes at the higher exposure levels reversed during the recovery period; low dose caused no adverse effects	[7]
Hydrated Silica, aerosolized; particle size not provided; 30 mg/m^3	45 male Fischer 344 rats	8-d study with a 112-d recovery; 6 h/d	Early and transient influx of cells into the lung tissue during exposure which returned to normal by day 12; BAL protein, lipid phosphorus, and saturated dipalmitoyl phosphatidyl-choline levels increased immediately after exposure but recovered day 5 post-exposure; no differences between controls and treated lungs as to weight, DNA-, protein-, or hydroxyproline-content.	[37,38]
Hydrated Silica, aerosolized; particle size not provided; 0, 10.1, 50.5, and 154 mg/m^3; diluted 4:1 with deionized, distilled water	Male CD BR rats; no further details provided	4-wk study with a 10- or 94-d recovery period; 6 h/d, 5 d/wk	NOAEL = 10.1 mg/m^3; dose-dependent increase in mean lung weight and lung to body weight ratio after 4 wk of exposure in the mid- and high-dose groups; mean lung to body weight ratio continued to increase in the high-dose group 10 d into recovery, but was similar to controls after 3 mo; 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 after the 10 d and 3 mo recovery period; most dust-laden alveolar macrophages were cleared from the lungs 3 mo post-exposure, 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; minimal collagen deposition observed in the silicotic nodule-like lesions but the lesions did not increase in size or number over time.; there was an increase in mean neutrophil count and globulin concentration and a decrease in mean lymphocyte count at the end of the treatment for the high-dose group which were both still present after 3 mo of recovery; tracheal and mediastinal lymph nodes were enlarged with nodular aggregates of dust-laden alveolar macrophages and hyperplastic reticulo-epithelial (RE) cells	[39,40]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/ Vehicle	Species/Strain/Cell	Method	Results	Reference
Hydrated Silica, aerosolized; particle size not provided; 0, 10, 50, or 150 mg/m^3	Groups of 25 male Crl: CD(SD)BR rats; no further details provided	4-wk study with 10-d or 3-mo recovery period; 6 h/d, 5 d/wk	Dose-dependent lesions observed in the mid- and high-dose groups but not in low-dose group; particles mostly phagocytized by alveolar macrophages in 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 tracheobronchial lymph nodes; some particle-laden alveolar macrophages in the bronchus-associated lymphoid tissue transmigrated directly into bronchial lumen through the epithelium; migrated particle-laden alveoli macrophages observed to be necrotic and released particles in the tracheobronchial lymph nodes; at 3 mo, lungs of the low-dose group were normal while lungs of the mid dose group had a small number of tiny nodular aggregates of dust-laden alveoli macrophages and epithelioid cells were observed with 1 rat observed with a few silicotic nodules in perivascular regions adjacent to the bronchioles; high-dose recovery group had decreased numbers of particle-laden alveoli macrophages that were sharply circumscribed in the alveoli; 3/10 rats had silicotic nodules in the perivascular region of the bronchioles	[41]
Hydrated Silica; particle and agglomerate/aggregate size 1 to ~120 μm; 35 mg/m^3	Male and female Wistar rats	13-wk study with a 52-wk recovery period; 6 h/d, 5 d/wk	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 revealed accumulation of alveolar macrophages, intra-alveolar leukocytes, and increased septal cellularity; accumulation of macrophages observed in the lymph nodes; collagen content in the lungs was slightly increased; effects of exposure mostly resolved within 26 wk of recovery although accumulations of Silica and macrophages in the mediastinal lymph nodes were still present	[43]
Silica, aerosolized; 0 or 6.9 mg/m^3	80 male Sprague–Dawley rats	12-mo study; 5.5 to 6 h/d, 5 d/wk	LOAEL = 6.9 mg/m^3; a few macrophage aggregates found in lungs; interstitial fibrosis associated with dense collections of mast cells was a trend in rats exposed to Silica, some incidences also occurred in some control animals; fibrosis was comparable between test and control groups	[49]
Hydrated Silica, aerosolized; particle size not provided; measurements ranged from 25 to 74 mg/ m^3	Groups of 35 Wistar rats; no further details provided	12-mo study; 8 h/d, 5 d/wk	Deaths occurred in 74% (26/35) and were treatment-related; majority of deaths from pulmonary vascular obstruction and emphysema from months 4-9; after 6 mo, aggregations of focal pigmentation visible as reddish-tan foci of dust; greatly enlarged and firm lymph nodes were observed	[47]
Hydrated Silica, aerosolized; particle size not provided; 126 mg/m$^{3.}$	84 rats; no further details provided	15-mo study with up to 12-mo recovery period; 8 h/d, 5 d/wk	No treatment-related differences between test and control groups, most deaths were due to intercurrent infection; lung weights increased during exposure but returned to normal during recovery; particle phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue; hilar lymph nodes were mildly enlarged but disappeared at treatment termination; epithelial proliferation was minimal; mild deposition of reticulin fibers occurred in alveoli without collagen formation; no epithelization or pleural changes and no neoplasia; emphysematous effects may have been due to aging and recurrent epizootic pneumonia; silicotic processes were absent	[48]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/ Vehicle	Species/Strain/Cell	Method	Results	Reference
Hydrated Silica (precipitated and gel) and Silica, aerosolized; particle size ≤4.7 μm; 0 or 15 mg/m^3	20 male Hartley guinea pigs	12-mo study; 5.5 to 6 h/d, 5 d/wk	Few macrophages containing particles of Silica were observed in the lungs and lymph nodes	[49]
Hydrated Silica, aerosolized; particle size not provided; 0 or 126 mg/m^3	82 guinea pigs; no further details provided	24-mo study; 8 h/d, 5 d/wk; recovery period of up to 12 mo	No treatment-related differences between test and control groups; lung weights increased during exposure but returned to normal during recovery; particle phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue; hilar lymph nodes were enlarged but disappeared at treatment termination; epithelial proliferation was minimal; mild deposition of reticulin fibers occurred in alveoli without collagen formation; no epithelization or pleural changes and no neoplasia; complete reversibility of Silica retention and inflammatory response with 6 mo of recovery; silicotic processes were absent	[48]
Hydrated Silica, aerosolized; particle size not provided; 0 and 126 mg/m^3	50 rabbits; no further details provided	12-mo study; 8 h/d, 5 d/wk; recovery period of up to 12 mo	No treatment-related differences between test and control groups; lung weights increased during exposure but returned to normal during recovery; particle phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue; hilar lymph nodes were enlarged but disappeared at treatment termination; epithelial proliferation was minimal; mild deposition of reticulin fibers occurred in alveoli without collagen formation; no epithelization or pleural changes and no neoplasia; silicotic processes were absent	[48]
Hydrated Silica, aerosolized; particle size not provided; 0, 28, 134, or 360 mg/m^3	65 New Zealand white rabbits; sex not reported	9-mo study for mid- and high-dose groups; 27-mo study for low-dose and control groups; 8 h/d, 5 d/wk; recovery period not described	LOAEL = 28 mg/ m^3; mid- and high-dose became distressed during exposure; fewer clinical signs that commenced later and receded more quickly were observed at lower concentrations: dyspnea and shortness of breath accompanied by cyanosis; elevated right and left ventricular pressures were concentration and time related; emphysema observed in high-dose group which decreased after treatment termination; pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed; lesions were observed in liver, spleen and kidney; after 6 mo of exposure, the cardiac pressure of the low-dose group increased steadily; at 24 mo, the elevation was 64% over pre-exposure pressure but effect was partially reversed with termination of treatment (34% after 12 mo); the researcher reported concomitant radiographic changes, electrocardiographic deviations, modification of lung functions, hematolytic changes, anatomical cor pulmonale, congestive cardiac failure, emphysema, and chemical pneumonitis	[46]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/ Vehicle	Species/Strain/Cell	Method	Results	Reference
Hydrated Silica (precipitated and gel) and Silica, aerosolized; particle size ≤4.7 μm; 0 or 15 mg/m^3	10 male *Macaca fascicularis* monkeys	13- or 18-mo study; 6 h/d, 5 d/wk	Decrease in lung respiratory volume and ventilatory mechanics more marked in the 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; lower lung volumes were observed in precipitated Hydrated Silica group; reductions in ventilatory performance and mechanical parameters, dynamic lung compliance, and forced expiratory flow in gel Hydrated Silica group; cytoplasmic changes in macrophages in the lungs and tracheal lymph nodes were observed; large numbers of macrophages and mononuclear cell aggregates were observed in the lungs; reticulin fibers were present in the aggregates in all 3 groups; in 6/10 monkeys exposed to Silica, collagen in varying quantities was found in 5 to 50% of the aggregates, with signs of early nodular fibrosis; in 3/10 monkeys no or little collagen was present; no or very few collagen fibers were observed in aggregates in the lung of Hydrated Silica groups; a review of this study noted that the monkeys may have been exposed to quartz or asbestos fibers during the course of the experiment	[2,49]
Silica, aerosolized; no further details provided	15 Fischer 344 rats; no further details provided	8-d study with up to 120-d recovery period	Initial alveolar inflammation subsided by recovery day 12	[38]
Silica; particle sizes not provided; 0, 17, 44, or 164 mg/m^3	Groups of 40 male and 40 female Wistar rats; 6 male and 6 female rats served as unexposed controls	14-d study; 6 h/d, 5 d/wk; whole body exposure chamber	Respiratory distress observed in all groups, and 1 female in the high-dose group died; body weights and feed consumption were decreased in the males in the mid- and high-dose groups; hematological measurements were unremarkable; lung weights were increased in both sexes (47%, 65%, and 86% for the low-, mid-, and high-dose groups, respectively) compared to controls; absolute and relative liver weights were decreased in males, but not females; dose-dependent changes observed in lungs (i.e., pale, spotted, and/or spongy, occasionally irregular surface, alveolar interstitial pneumonia, early granulomata); mediastinal lymph nodes were enlarged	[43]
Silica; particle sizes not provided; 0, 46, 180, or 668 mg/m^3	Groups of 30 male and 30 female Wistar rats; 6 male and 6 female rats served as unexposed controls	14-d study; 6 h/d, 5 d/wk; whole body exposure chamber	Respiratory distress was observed in all groups, and 1 male died in the high-dose group; body weights were decreased in male mid- and high-dose groups and in high-dose females; feed consumption was decreased in both sexes in the mid- and high-dose groups; lung weights were increased 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); decreased liver weights observed in males of all dose groups and the high-dose group females; lungs were spotted, swollen, and had irregular surfaces in the high-dose groups as well as interstitial pneumonia and early granulomata; silica was observed in the mediastinal lymph nodes in the mid- and high-dose groups and 1 rat in the low-dose group; an accumulation of alveolar macrophages and particulate material was observed in the lungs of males in the mid- and high-dose group	[43]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/Vehicle	Species/Strain/Cell	Method	Results	Reference
Silica; aerosolized; particle size 50-79 nm (nanoparticles); 0, 0.4 mg/m^3, 1.4 mg/m^3, or 5.4 mg/m^3	Groups of 15 male Sprague–Dawley rats	4-wk study with up to 28 d recovery; 6 h/d, 5 d/wk; nose-only inhalation system	Minimal toxic effects included temporary decrease in body weight (high-concentration group), increased levels of red blood cells (all concentration groups) and hemoglobin concentrations (low- and middle-concentration groups); no significant lung histopathological findings or adverse changes in inflammatory markers in bronchoalveolar lavage fluid; no significant toxicological or inflammatory changes in the lungs of the exposed groups during all recovery days	[44]
Silica; particle size not provided; 1.3, 5.9, or 31 mg/m^3	Groups of 50 male and 50 female Wistar rats	13-wk study with up to 39-wk recovery; 6 h/d, 5 d/wk; full body exposure	NOEL = 1.3 mg/m^3; no mortalities during treatment or recovery; dose-dependent increase in respiration rates; body weight gains were depressed; RBC count was increased in high-dose males; white blood cells (WBC) were elevated in both males and females of mid- and high-dose groups but the concentration-response relationship was poor; blood cell counts returned to normal by week 39; necropsy revealed swollen and spotted lungs and enlarged mediastinal lymph nodes at 13 wk with a dose-dependent severity; all groups had increased lung weights and collagen content, these effects were reduced to control levels by the end of recovery except for collagen content in males in the mid- and high-dose groups; in high-dose group post-treatment, the average Silica amount in the lungs was 0.2 mg; no Silica above control levels could be detected in any rat at the end of recovery; 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; no fibroblastic activity noted in lung lesions nor was there hyalinization; all pulmonary lesions types were more marked in males than in females; accumulation of macrophages was observed in the mediastinal lymph node at 13 and 26 wk; focal necrosis and slight atrophy of the olfactory epithelium noted at week 13; interstitial fibrosis was not observed until 13 wk post-exposure, with increasing incidence especially in the high-dose group, and a few in the mid-dose group	[43]
Silica, aerosolized; particle size not provided; 8 and 40 mg/m^3	Female Wistar rats; no further details provided	3-mo study with a 7-d or 3-wk recovery period; 1 h/d, 5 d/wk	No macroscopic changes noted; dust cells noted in the lungs which decreased post-exposure; no fibrosis of the reticulo-cellular type and normal parenchyma of the lungs; decrease of Silica content in the lungs was observed 7 and 48 d after treatment termination with almost no Silica in the lungs after 3 mo	[2]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/ Vehicle	Species/Strain/Cell	Method	Results	Reference
Silica, aerosolized; mean diameter 0.81 μm; 0 or 50.4±19 mg/m^3	4 male Fischer 344 rats; control group details not provided	13-wk study with up to 8 mo recovery period; 6 h/d, 5 d/wk	Silica load increased quickly during the first 6.5 wk of exposure (0.76 mg/lung) but less so after 13 wk (0.88 mg/lung); Silica burden disappeared rapidly from lung tissue during recovery (15% after 12 wk; 6% after 32 wk); BAL showed mean cell numbers in the lavage increased 5- to 15-fold compared to control; cells comprised >50% polymorphonuclear leukocytes (PMN) and some 2% lymphocytes whereas the control lavages only contained <1% of either cell type; protein content and LDH and glucuronidase activities were markedly higher than controls; all BAL markers approached normal levels after 13 wk of recovery in most rats; invasion of neutrophils and macrophages into the alveoli noted after 6.5 wk that decreased during recovery; fibrosis observed in alveolar septa which subsided during recovery; intensely stained TUNEL-positive cells were detected throughout the terminal bronchiolar epithelium and through the parenchyma of the lungs at exposure end	[45]
Silica; particle size not provided; 25 to 85 mg/m^3	25 Wistar rats, half males and half females; control group had 42 rats; no further details provided	6-mo study with 6-mo recovery period; rats were exposed in inhalation chambers to aerated Silica for 8 h/d with passive exposure to settling dust the remaining 16 h; exposures were 5 d/wk	LOAEL = 53 mg/m^3; 44% of rats died during exposure with most dying from pulmonary vascular obstruction and emphysema beginning at month 4; focal pigmentation was conspicuous after 3 mo of exposure with profusely scattered small, dark-pink discrete but irregular subpleural foci of reaction; congestion of the lungs and lymph node enlargement observed after 3 mo; an incipient tendency toward pulmonary emphysema observed after 4 mo of exposure with lung distension and superficial alveoli dilation; atelectasis noted in some rats after 4 to 5 mo; mononuclear macrophages forming clusters of plasma cells and lymphocytes observed in lung lymphatic system; alveolar space was infiltrated with large vacuolated cells; cytoplasm had a foamy appearance with macrophages fused to giant cells; 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; necrosis noted in the central zone of the nodules with tendency toward fibrosis in the nodules and evidence of progressive emphysematous processes around the nodules; average Silica load in the lung after 3 mo was 1.5 mg/lung and reduced to 0.3 mg/lung at the end of recovery	[47]
Silica, hydrophobic, and aerosolized; particle size not provided; 0, 10, 50, or 150 mg/ m^3	Male rats; no further details provided	12-mo study; 6 h/d, 5 d/wk	No effects observed at lowest concentration; peribronchial lymph nodes enlargement and white foci on the lung surfaces and collections of foamy macrophages within the alveoli were observed in 50 and 150 mg/m^3 groups	[3]

(continued)

Table 5. (continued)

Ingredient/Concentration/Dose/Vehicle	Species/Strain/Cell	Method	Results	Reference
Silica, aerosolized; 85% particles between 1 to 10 μm; 25 to 85 mg/m^3	Male and female albino guinea pigs, number per experiment described in Methods; 80 control animals	Up to 24 mo; whole body exposure for 8 h/d with 16 h passive exposure to settling dust; study conducted as 3 experiments: Experiment 1: 40 animals exposed for 24 mo, Experiment 2: 15 or 18 animals exposed for 12 or 24 mo, respectively, with variable recovery periods up to 12 mo, and Experiment 3: 17 animals exposed for 12 mo with a 1-mo recovery period and a re-exposure for 8 to 24 h	Focal pigmentation and lymph node enlargement after 1 mo; lung emphysema after 4 to 8 mo of exposure; atelectasis observed histologically with dominant response of bronchial and peribronchiolar intra-alveolar accumulations of giant cells; at 8 to 12 mo there was incipient atrophy of infiltrated alveoli with compensatory expansion of adjacent alveoli; a combined effect of atelectasis and consolidation around bronchiole was noted with bronchioli distortion, along with incipient fibrosis around bronchioli and shrunken alveoli; a marked tendency toward cuboidal epithelization of atelectatic alveoli was noted by the end of the second year of exposure; medullary hyperplasia with the formation of slight amounts of reticulum was prominent during the second year of exposure in the lymphatic system with no inflammation, sinus catarrh, or fibrosis were noted in the lymph nodes; in the recovery phase after 12 mo of exposure, a progressive recovery began almost immediately with 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	[50]
Silica, aerosolized; particles between 1 to 10 μm; 25 to 85 mg/m^3	10 New Zealand white rabbits; no further details provided	12-mo study with a 6- and 12-mo recovery period; 8 h/d	A 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) 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 perivascular cellular nodules, ductal stenosis, and emphysema; during recovery, the cellular reactions and emphysema regressed but minor focal alveolar mural collagen persisted.	[51]
Silica, aerosolized; particle size not provided; 15 mg/m^3	5 *Macacus mulatta* monkeys with 15 untreated control monkeys; no further details provided	12-mo study; a monkey was killed and necropsied at 3 and 6 mos	Body weight gains decreased and activity decreased during the initial exposures; at 3 mo, emphysema detectable with considerable cellular infiltration of the alveoli and alveolar septa associated with distention of alveoli or accumulation of exudate and macrophages; after 12 mo, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vascular occlusions, and cor pulmonale, which was attributed to the emphysema and alveolar wall destruction; tracheobronchial lymph nodes were slightly enlarged but not fibrotic	[52]
Silica, hydrophobic and aerosolized; particle size not provided; 0, 10, 50, or 100 mg/m^3	Male *Macaca fascicularis* monkeys	12-mo study with a 2- or 24-mo recovery; 6 h/d, 5 d/wk	No effects observed at the lowest concentration; mid and high groups had interstitial fibrosis, which did not resolve or progress during recovery; peribronchial lymph nodes were enlarged	[3]

Carcinogenicity Studies

Silica

The International Agency for Research on Cancer (IARC) 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.[58]

Oral

Hydrated Silica. In a carcinogenicity study, groups of 10 male and 10 female $B_6C_3F_1$ mice received Hydrated Silica (0%, 1.25%, 2.5%, or 5%) in their feed for 93 wk.[35] In the female mice, the frequencies of adenocarcinoma in the lungs were 1/16 (6.25%) for the control group and 1/19 (5.3%), 0/20 (0%), and 1/20 (5%) for the low-, mid-, and high-dose groups. In the

Table 6. Genotoxicity Studies.

Ingredient/Concentration/ Dose	Species/Strain/Cell	Method	Results	Reference
In vitro				
Hydrated Silica; up to 10,000 μg/plate with and without metabolic activation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test	Negative; not cytotoxic	[2,3]
Hydrated Silica; concentration not provided; without metabolic activation	*S. typhimurium* strain TA1530, G-46	Ames test	Negative	[2,3]
Hydrated Silica; up to 10,000 μg/plate with and without metabolic activation	*Escherichia coli* WP2	Tryptophan reversion	Negative; not cytotoxic	[2,3]
Hydrated Silica; concentration not provided; without metabolic activation	*Saccharomyces cerevisiae* (D3)	Forward mutation	Negative	[2,3]
Hydrated Silica; 1-1000 μl/ml without metabolic activation	Human embryonic lung cells (Wi-38)	Chromosome aberration	No significant clastogenic activity	[2,3]
Silica; up to 10 M; details not reported	*Bacillus subtilis*	Rec assay	Negative	[53]
Silica; up to 10 M; details not reported	*E. coli* and *S. typhimurium* strains TA98, TA100, TA1535, and TA1538	Ames test	Not genotoxic	[53]
Silica (hydrophobic); 1580 μg/plate with and without metabolic activation	*S. typhimurium* strains TA98, TA100, and TA1537	Ames test	Negative, not cytotoxic	[2,3]
Silica (hydrophilic); up to 5000 μg/plate with and without metabolic activation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test (7 studies with identical test methods and findings)	Negative; not cytotoxic	[2,3]
Silica (hydrophilic); up to 10,000 μg/plate with metabolic activation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test	Negative; not cytotoxic	[2,3]
Silica; up to 10,000 μg/plate in DMSO with and without metabolic activation	*E. coli* strain WP 2 and *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test	Not genotoxic	[54]
Silica in a toluene extract; up to 1580 μg/plate with and without metabolic activation	*E. coli* strain WP2uvrA and *S. typhimurium* strains TA98, TA100, and TA1535	Ames test; additional test performed with epoxide hydrolase inhibitor and glutathione depletor 1,1,1-trichloropropene-2,3-oxide added to the activation mix in strain TA98 to increase sensitivity	Not genotoxic	[8]
Silica (hydrophobic); 5000 μg/plate with and without metabolic activation	*E. coli* WP2	Tryptophan reversion	Negative; not cytotoxic	[2,3]

(continued)

Table 6. (continued)

Ingredient/Concentration/ Dose	Species/Strain/Cell	Method	Results	Reference
Silica (hydrophobic); 5000 μg/plate with and without metabolic activation	*E. coli* WP2	Tryptophan reversion	Negative; not cytotoxic	[2,3]
Silica; up to 160 μg/cm^3	Chinese hamster lung fibroblasts	Micronucleus test	Weak, but significant, dose-dependent induction of micronuclei at cytotoxic concentrations; no clastogenicity observed in concentrations lower than cytotoxic levels	[55]
Silica; 19-300 μl/ml without metabolic activation and 250-1000 μl.ml with metabolic activation	Chinese hamster ovary (CHO) cells	Chromosomal aberration test	Negative	[2]
Silica (hydrophilic); 38-300 μl/ml without metabolic activation and 250-1000 μl/ml with metabolic activation	CHO cells	Chromosome aberration	No clastogenic activity	[2,3]
Silica; 10-250 μl/ml without metabolic activation and 100-500 μl/ml with metabolic activation	CHO cells	HGPRT assay	Negative	[2]
Silica; 68.9 and 137.9 μg/cm^2	Chinese hamster fibroblasts (V79) and human embryonic lung fibroblasts (HEL 299)	Single-cell gel/Comet assay	Dose-dependent increase in DNA migration in the gel in both cell lines	[56]
Silica; 0.3-1000 μl/ml; with and without metabolic activation	Primary rat hepatocytes	Unscheduled DNA synthesis	Negative; cytotoxic at 260-500 μl/ml	[2,3]
Silica (hydrophilic); 10-250 μl/ml without metabolic activation and 100-500 μl/ml with metabolic activation	CHO cells	6-Thioguanine resistance	No significant mutagenic activity	[2,3]
Silica (hydrophobic); 63-500 μl/ml with and without metabolic activation	CHO cells	Clastogenic activity; no further details provided	No clastogenic activity	[2,3]
Silica (hydrophobic); 42-333 μl/ml with and without metabolic activation	CHO cells	Clastogenic activity; no further details provided	No clastogenic activity	[2,3]
In vivo				
Hydrated Silica; 1.4-5000 mg/kg	Mice (host) + *S. typhimurium* TA1530, G-46 (indicator)	Gene mutation (host mediated) method; a single or 5 intraperitoneal (i.p.) injections of *S. typhimurium*; cells collected 3 h after last administration	No mutagenic activity	[3]

(continued)

Table 6. (continued)

Ingredient/Concentration/ Dose	Species/Strain/Cell	Method	Results	Reference
Hydrated Silica; 1.4-5000 mg/kg	Mice (host) + S. cerevisiae D3 (indicator)	Mitotic recombination (host mediated); a single or 5 i.p. injections of S. cerevisiae; cells collected 3 h after last administration	No genotoxic activity	[3]
Hydrated Silica; 1.4-5000 mg/kg	Male Sprague–Dawley rats	Chromosome aberration study with rat bone marrow; animals were killed at 6, 24, or 48 h after oral dosing	Negative	[3]
Hydrated Silica; 1.4-5000 mg/kg	Male Sprague–Dawley rats	Chromosome aberration study with rat bone marrow; animals were killed at 6 h after oral dosing	Negative	[3]
Hydrated Silica; 1 × 1.4-5000 mg/kg	10 male Sprague–Dawley rats mated with 2 virgin female rats	Dominant lethal mutation assay; female animals were killed 14 d after mating for uterus examination; oral dosing	Negative	[3,57]
Hydrated Silica; 5 × 1.4-5000 mg/kg	10 male Sprague–Dawley rats mated with 2 virgin female rats	Dominant lethal mutation assay; female animals were killed 14 d after mating for uterus examination; oral dosing	Negative	[3,57]

males, the frequencies of adenocarcinoma in the lungs were 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 the controls. The researchers concluded that the non-neoplastic lesions were of no toxicological significance.

Silica. In a 2-yr dietary study, Wistar rats (n = 40; 20 males and 20 females) received 100 mg/kg Silica (pyrogenic) in their feed.[8] The rats were weighed before and after treatment. At the end of the treatment period, the rats were killed and necropsied. There were no clinical signs of toxicity observed during the treatment period. The rates of tumors observed in the treated rats were comparable to historical controls. The researchers concluded that there were no carcinogenic effects from the daily ingestion of Silica in this study.

Inhalation

Hydrated Silica. The potential carcinogenic effects of aerosolized Hydrated Silica (≤5 µg particle size) was studied in tumor-susceptible mice (n = 75) starting at 3 mo of age.[59] The mice received 0.5 g/d Hydrated Silica in a 600-l capacity respiratory chamber once per hour, 6 h/d for 5 d/wk for 1 yr. The mice were allowed to live out their natural life span for up to 917 d from the start of the experiment. The incidence of primary lung tumors was 7.9% in the control group and 21.3% in the treated group in mice that lived 10 mo or longer. There was no obvious fibrosis in the lung tissue; however, there were fibrotic nodules in the trachea-bronchial lymph nodes in >50% of the mice. The researchers suggested that most of the Silica dust was removed by cilia action through the trachea and also through the lymphatic system. Half of the treated mice had overgrowth of the mediastinal connective tissue covering the trachea-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 trachea-bronchial lymph nodes compared to 14.3% of the controls.

Intratracheal

Silica. The carcinogenic potential of Silica (3 mg in 0.9% phosphate-buffered saline; 0.01 to 0.03 µm) was studied in 40 female SPF Wistar rats.[60] The rats received the test material intratracheally 5 times weekly and were observed until death or month 30, at which time they were killed and necropsied. A second group of 40 rats had Silica instilled at the same dose 10 times weekly. Controls (n = 48) were untreated. The survival rates were 37/40 for group 1, 35/40 for group 2, and 46/48 for the controls. The period of time after the first treatment in which 50% of the rats died was 113 and 112 wk in the first and second groups, respectively, and 113 wk in the control group. The percentage of rats with macroscopic lung tumors 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 tumors 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 lung 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 the second group nor the control group had malignant tumors. The percentage of rats with lung tumors that were metastases of other primary site tumors was 14.3% in the treatment groups and 13.0% in the control group.

Other Relevant Studies

Immune Response

Human

Hydrated Silica. Hydrated Silica (1 to 4 mg in saline; ~15 µm particle size) was injected subcutaneously 2 to 8 times in 28 volunteers.[61] Biopsies were taken from day 1 to 6 mo. Granulomatous inflammation was observed within 7 d and persisted for months. The researchers suggested that this was a particular type of foreign body response to a fibrogenic agent and not typical epithelioid cell nodules.

Dermal Irritation and Sensitization Studies

Dermal irritation and sensitization data summarized below are detailed in Table 7. Very slight to no irritation was observed in dermal irritation studies in rabbits with Hydrated Silica (at up to 50% solution in olive oil) and Silica (up to 12% solution in methyl ethyl cellulose).[2,3] Hydrated Silica (20%) was not sensitizing in guinea pig sensitization tests.[62] Hydrated Silica (up to 45%) and Silica (21.74% in formulation) were not sensitizing in human repeat insult patch tests (HRIPTs).[2,36,63,64]

Ocular Irritation Studies

In vitro and animal ocular irritation data are summarized in Table 8. Hydrated Silica (concentration not provided) and Silica were both not irritating to slightly irritating in rabbit eyes.[3,8,36,65]

Clinical Studies

Occupational Exposure

Hydrated Silica. In an occupational study, 78 workers (aged 21 to 67 yr; average 34.23 yr) were examined who had been exposed to precipitated Silica from 1941 to 1959.[66] Dust concentrations ranged from 0.35 to 204 mg/m^3. There was no evidence of silicosis or other pulmonary disease.

Workers (n = 165) exposed to Hydrated Silica for a mean of 8.6 yr were examined for adverse effects.[67] 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 yr 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 Hydrated Silica showed radiographic evidence of pneumoconiosis.

Another study examined 41 workers exposed to Hydrated Silica and compared them to a control group.[68] 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 synergize to induce small airway disease.

In another unpublished occupational study of workers in Hydrated Silica factories (1952 to 1981), there was no silicosis in workers employed for 1 to >20 yr (mean 13.2 yr).[2] There were negative results in hematology, urine analysis, lung functions, and chest X-rays.

In an unpublished study, 150 workers in a Hydrated Silica factory were examined by pulmonary function test and X-ray.[3] 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 yr, 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.

Silica. The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) to amorphous Silica is 80 mg/m^3 or 20 million particles per cubic foot air averaged over an 8-h work shift.[10] The National Institute for Occupational Safety and Health recommended exposure limit (REL) for amorphous Silica is 6 mg/m^3.

Workers (n = 215) with exposure to Silica between 1947 and 1959 were studied using chest X-rays.[69] 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.

In an unpublished study, 29 workers in a silicone products manufacturing plant were surveyed.[3] 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. Some of the workers in the heat curable rubber compounding area, where the potential exposure to Silica was greater, complained about eye irritation, nausea, headaches, or rashes; none reported upper or lower respiratory problems.

Table 7. Dermal Irritation and Sensitization.

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
Irritation—Animal				
Hydrated Silica; 500 mg as a 23% solution in methyl ethyl cellulose	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Hydrated Silica; 20 mg	8 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Hydrated Silica; 33 mg	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	Very slight erythema on 4 abraded sites and 5 intact sites at 24 h	3
Hydrated Silica; 190 mg	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	Very slight erythema on 3 abraded sites and 4 intact sites at 24 h	3
Hydrated Silica; 500 mg	3 rabbits; no further details	Dermal irritation study; test site occluded for 4 h; skin intact	No signs of irritation	3
Hydrated Silica; 500 mg	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact	No signs of irritation	3
Hydrated Silica; 500 mg	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Hydrated Silica; 500 mg as a 50% solution in olive oil	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Hydrated Silica (hydrophobic); 500 mg as a 50% solution in olive oil	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Silica (hydrophobic); 500 mg as a 6% solution in methyl ethyl cellulose	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Silica (hydrophilic); 500 mg as a 12% solution in methyl ethyl cellulose	12 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	2,3
Silica (hydrophobic); 500 mg in 2 ml water	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Silica (hydrophilic); 500 mg in 3 ml saline	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation on intact skin; slight erythema on 3 abraded sites	3
Silica (hydrophilic); 500 mg moistened with saline	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	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 post-patch removal	3
Silica (hydrophobic); 500 mg	6 rabbits; no further details	Dermal irritation study; test site semi-occluded for 4 h; skin intact	No signs of irritation	3
Silica (hydrophobic); 500 mg moistened with polyethylene glycol	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3
Silica (hydrophobic); silane treated; 500 mg moistened with corn oil	6 rabbits; no further details	Dermal irritation study; test site occluded for 24 h; skin intact and abraded	No signs of irritation	3

(continued)

Table 7. (continued)

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
Sensitization—Animal				
Hydrated Silica; 10% at induction, and 1-20% at challenge; in distilled water	10 female Hartley albino guinea pigs treated; 5 guinea pigs control	Guinea pig maximization test	Not sensitizing	62
Sensitization—human				
17% Hydrated Silica in a facial mask (0.05 ml)	27 subjects (18 males, 9 females)	HRIPT; test sites pre-treated with 25% sodium lauryl sulfate (SLS; aq.; 0.05 ml) under occlusion for 24 h prior to induction; occluded	Not sensitizing	63
45% Hydrated Silica; no further details reported	20 subjects (10 males, 10 females)	HRIPT; details not reported	Not sensitizing	2
Hydrated Silica (micronized gel) in a dusting powder; concentration and dose not reported	300 patients	Dermal irritation and sensitization study; details not reported	Non-irritating and non-toxic; little or no sensitizing reactions observed	36
21.74% Silica in a facial powder in a 30% aq. solution	27 subjects (18 males, 9 females)	HRIPT; test sites pre-treated with 25% SLS aq. (0.05 ml) under occlusion for 24 h prior to induction; occluded	Not sensitizing	64

Workers (n = 200) with intensive and regular contact with Silica from 1972 to 2000 were evaluated.[2] There was no evidence of skin allergy caused by the Silica. There were signs of irritation attributed to the desiccative and defatting properties of Silica, which resulted in skin dryness; this effect could be controlled by regular use of skin-protection ointment.

An occupational study of 143 workers exposed to Silica from 1959 to 1985 was performed.[2] Exposure ranged from 1 to 34 yr. There were complaints of abnormalities in lung function or histology in 54/143 (36%) of the workers (no further details available). 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. Spirometry showed obstructive and/or restrictive ventilation disturbances in 24 workers. Most of the adverse findings were associated with the confounding factors.

In an unpublished occupational exposure study, X-rays were taken of 99 workers who had manufactured Silica for various amounts of time.[2] The X-rays revealed no evidence of any occupational disease, including silicosis.

Summary

This report assesses the safety of synthetically manufactured amorphous Silica and Hydrated Silica as used in cosmetics. These ingredients are both reported to function as abrasives, absorbents, anticaking agents, bulking agents, and opacifying agents in cosmetic products. The Panel considered the method of manufacture of these ingredients (synthetic and not mined) to be of significant importance when reviewing safety. Thus, the current assessment is exclusive to amorphous Silica and Hydrated Silica when manufactured via synthetic methods.

According to 2019 VCRP data, Silica has a total of 8222 uses; the majority of the uses are in leave-on makeup preparations and eye makeup preparations. Hydrated Silica has a total of 462 uses; the majority of the uses are in rinse-off oral hygiene and personal cleanliness products. The uses for both of these ingredients have increased since the original safety assessments were finalized: in 2009, Silica was reported to have 3276 uses and Hydrated Silica was reported to have 176 uses. The results of the concentration of use survey conducted in 2018 by the Council indicate Silica is used at up to 82% in face and neck products and 50% in mascaras. Hydrated Silica is used at up to 33.8% in oral hygiene products and at up to 10% in leave-on skin care products. According to the original safety assessment, the maximum use concentration in 2008 for Silica was 44% in eye shadows. The maximum use concentration for Hydrated Silica in 2008 was 34% in dentifrices; the maximum leave-on concentration was 4% in face powders.

Hydrated Silica in water had a dermal LD_{50} greater than 5 g/kg in rabbits. In oral rat studies, LD_{50}s of 40 g/kg Hydrated Silica (26% in water) and >10 g/kg Silica (in stock diet 1:4 w/w) were reported. In inhalation studies that ranged in duration from 1 to 6 h, the LC_{50}s for Hydrated Silica (30% SiO_2) and Silica (concentration not reported) in rats were >3300 mg/m^3 and >191,300 mg/m^3, respectively.

No adverse effects were reported in a 3-wk dermal study of Silica (up to 10 g/kg/d) in rabbits. In short-term oral studies,

Table 8. Ocular Irritation.

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
Animal				
Hydrated Silica; 0.1 ml of 50% dilution in olive oil	8 male New Zealand white rabbits	Ocular irritation study; eyes rinsed after 5 min in 3 rabbits or not rinsed in 5 rabbits	No signs of irritation in rinsed eyes; very slight erythema observed up to 24 h after instillation	[8]
Hydrated Silica; 100 mg instilled; 0.2 ml of 50% slurry	6 rabbits; no further details	Ocular irritation study; no further details	No signs of irritation	[3]
Hydrated Silica; 9 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes rinsed after 2 sec in 3 rabbits, 4 sec in 3 rabbits, or not rinsed in 3 rabbits	No signs of irritation	[3]
Hydrated Silica; 40 mg instilled	3 rabbits; no further details	Ocular irritation study; no further details	No signs of irritation	[3]
Hydrated Silica; 100 mg instilled	3 rabbits; no further details	Ocular irritation study; no further details	Slight redness at 24, 48, and 72 h that resolved by day 4; mean score = 0.7	[3]
Hydrated Silica; 100 mg instilled	8 rabbits; no further details	Ocular irritation study; eyes rinsed after 5 min in 3 rabbits or not rinsed in 5 rabbits	No signs of irritation	[3]
Hydrated Silica; 100 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes rinsed after 4 sec in 3 rabbits or not rinsed in 6 rabbits	No signs of irritation	[3]
Silica; 0.1 ml of 50% dilution in olive oil	8 male New Zealand white rabbits	Ocular irritation study; eyes rinsed after 5 min in 3 rabbits or not rinsed in 5 rabbits	No irritation	[8]
Silica (hydrophilic); 3 mg instilled	3 rabbits; no further details	Ocular irritation study; no further details	Slight to mild erythema that resolved by 48 h	[65]
Silica (hydrophobic); 3 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 3 rabbits, eyes rinsed after 2 sec in 3 rabbits, or after 4 sec in 3 rabbits	Transient slight to moderate conjunctival erythema observed and 1 and 4 h post-treatment that resolved within 24 h	[3]
Silica (hydrophilic); 3.5 mg instilled	6 rabbits; no further details	Ocular irritation study; no further details	Slight conjunctival erythema or chemosis in some animals at 24, 48, and 72 h; mean score 0.6 and 0.1, respectively; transient corneal opacity observed in 2 animals at 4 h	[3]
Silica (hydrophobic); 6 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 3 rabbits, eyes rinsed after 2 sec in 3 rabbits, or eyes rinsed after 4 sec in 3 rabbits	No signs of irritation	[3]
Silica (hydrophilic); 9 mg instilled; neat and in aqueous suspension; no further details	Rabbis; no further details	Draize ocular irritation study; rinsed and unrinsed eyes; no further details	Neat material was a mild irritant in unrinsed eyes (score = 2.4); no irritation in rinsed eyes or those treated with aqueous suspension	[36]
Silica (hydrophobic); 10 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 6 rabbits; eyes rinsed after 30 s in 3 rabbits	No signs of irritation	[3]
Silica; 10 mg instilled; neat and in aqueous solution; no further details	Rabbits; no further details	Ocular irritation study; some eyes rinsed after 2 s, 4 s, or not rinsed; no further details	Faint irritation in mucous tissues in eyes treated with neat material and not rinsed; no irritation in eyes that were rinsed and with aqueous solution	[36]

(continued)

Table 8. (continued)

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
Silica (hydrophobic); 10-20 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 6 rabbits; eyes rinsed after 30 sec in 3 rabbits	No signs of irritation in rinsed eyes; 2 unrinsed eyes had slight erythema for 24 h after instillation; mean score = 0.1 at 24, 48, and 72 h	[3]
Silica (hydrophobic); 25 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 6 rabbits; eyes rinsed after 30 sec in 3 rabbits	No signs of irritation in rinsed eyes; 2 unrinsed eyes had slight erythema for 24 h after instillation; mean score = 0.1 at 24, 48, and 72 h	[3]
Silica (hydrophobic); 100 mg instilled	8 rabbits; no further details	Ocular irritation study; eyes not rinsed in 5 rabbits; eyes rinsed after 5 min in 3 rabbits	No signs of irritation	[3]
Silica (hydrophobic); 100 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 6 rabbits; eyes rinsed after 4 sec in 3 rabbits	No signs of irritation	[3]
Silica (hydrophilic); 100 mg instilled	8 rabbits; no further details	Ocular irritation study; eyes not rinsed in 5 rabbits; eyes rinsed in 3 rabbits after 5 min	No signs of irritation	[3]
Silica (hydrophilic); 100 mg instilled	9 rabbits; no further details	Ocular irritation study; eyes not rinsed in 6 rabbits; eyes rinsed after 30 sec in 3 rabbits	No signs of irritation in rinsed eyes; mean score 0.15; very slight conjunctival erythema up to 48 h	[3]

the NOAEL for Hydrated Silica was > 24.2 g/kg/d in a 14-d dietary study in rats. The NOEL was 500 mg/kg/d in a 5- to 8-wk dietary study in rats that were fed up to 16,000 mg/kg/d Silica. In subchronic oral studies, the NOEL was 4000 mg/kg/d in a 13-wk dietary study in rats fed Hydrated Silica at up to 4000 mg/kg/d. No clinical signs of toxicity or gross or microscopic changes were reported in a 13-wk dietary study in rats that received up to 3500 mg/kg/d Silica. In oral chronic studies, lower liver weights in female rats without significant findings at histopathological examinations was observed in a 103-wk dietary study of up to 5% Hydrated Silica in rats, but no remarkable findings were observed by the same researchers of the same material in a 93-wk dietary study in mice. The NOAEL in a 6-mo dietary rat study of up to 10% Hydrated Silica was 8980 mg/kg/d. No remarkable findings were reported in 6-mo dietary studies of up to 10% Silica in rats, although there were reduced liver and prostate weights and increased numbers of leukocytes and eosinophils in female and male rats, respectively, in another 6-mo study at up to 3 g Silica/wk.

In short-term inhalation studies with Hydrated Silica, inflammatory and pulmonary lesions were observed in rats at 30 mg/m^3. Inflammatory responses were also observed in rats exposed to Silica in studies that lasted between 5 and 14 d. No significant lung histopathological findings or adverse changes in inflammatory markers were observed in rats that were exposed to nanoparticle Silica (particle size 50-79 nm; concentrations 0.4-5.4 mg/m^3) for 4 wk. In subchronic inhalation studies, inflammatory responses were noted in the lungs and lymph nodes along with pulmonary lesions after exposure to Hydrated Silica at 35 mg/m^3 (particle and agglomerate/aggregate size 1 to ~120 μm). In a 13-wk inhalation study of Silica in rats, the NOEL was 1.3 mg/m^3. Inflammation and pulmonary lesions, including fibrosis, were noted in this study and another 13-wk rat study (fibrosis subsided during recovery). In inhalation studies of 9- to 12-mo duration, Hydrated Silica caused pulmonary inflammation and emphysema in rats exposed to 25 to 85 mg/m^3. The LOAEC in rabbits exposed for 9 mo to Hydrated Silica was 28 mg/m^3. No silicotic processes were noted in studies of rabbits, rats, and guinea pigs exposed to an average of 126 mg/m^3 Hydrated Silica for 12, 15, and 24 mo, respectively. No neoplasia was observed. In a 12-mo study with Hydrated Silica and Silica in rats, the LOAEC was 6.9 mg/m^3 due to interstitial fibrosis (which was comparable between test and control groups). The same test materials also were associated with nodular fibrosis in an 18-mo study with monkeys, although the animals may have been exposed to quartz or asbestos fibers. The LOAEC in a 6-mo rat inhalation study with Silica was 53 mg/m^3. Emphysema and fibrosis were noted around 4 mo of exposure. Inflammatory responses and pulmonary lesions were noted in rat, guinea pigs, rabbits, and monkeys in studies up to 24 mo in duration. More than half of the studies summarized in this report included recovery periods of various durations that showed that observed lung effects began to resolve or did not worsen after exposure ceased.

Hydrated Silica and Silica were not genotoxic in Ames tests, HGPRT gene mutation assays, or chromosome aberration tests. Genotoxicity studies of Hydrated Silica at up to 5000 mg/kg in mice and rats were negative.

Carcinogenic effects were not reported in oral studies of Hydrated Silica (0, 1.25, 2.5, or 5%) in mice or Silica (100 mg/kg) in rats. An inhalation study of Hydrated Silica (≤5 µg particle size; 0.5 g/d) in mice and an intratracheal study of Silica (3 mg in 0.9% phosphate-buffered saline; 0.01 to 0.03 µm) in rats also were negative for carcinogenicity.

Very slight to no irritation was observed dermal irritation studies in rabbits with Hydrated Silica (at up to 50% solution in olive oil) and Silica (up to 12% solution in methyl ethyl cellulose). Hydrated Silica (20%) was not sensitizing in guinea pig sensitization tests. Hydrated Silica (up to 45%) and Silica (21.74% in formulation) were not sensitizing in HRIPT. Hydrated Silica (concentration not provided) and Silica were not irritating to slight irritating in rabbit eyes.

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 synergize 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 desiccative and defatting properties of Silica.

Discussion

The Panel assessed the safety of synthetically manufactured amorphous Silica and Hydrated Silica, and considered the method of manufacture of these ingredients (synthetic and not mined) to be of significant importance when reviewing safety. The Panel emphasized that this report reviews only the safety of synthetically manufactured amorphous Silica and Hydrated Silica. Crystalline silica and synthetic and mined silicates are not toxicologically similar to synthetically manufactured amorphous Silica and Hydrated Silica, and thus require separate reviews.

Data were sufficient to assess the safety of synthetically manufactured amorphous Silica and Hydrated Silica, and the Panel determined that these two ingredients do not pose an incidental inhalation safety risk, under conditions of cosmetic use. The exposures that were tested in inhalation studies were at much higher concentrations than those possible with cosmetic use, and had very few adverse effects. Aggregation and agglomeration of Silica and Hydrated Silica particles in cosmetic formulations reduces potential inhalation exposure. While the Panel noted the effects on tracheabronchial lymph nodes in mice, the carcinogenicity study used such high concentrations of Hydrated Silica that the effects were due to the overload of the animal system; therefore, concern over incidental inhalation of Silica in cosmetics was mitigated.

The Panel was concerned, however, that the potential exists for dermal and ocular irritation with the use of products formulated using Silica and Hydrated Silica. Therefore, the Panel specified that products containing these ingredients must be formulated to be non-irritating.

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded that synthetically manufactured amorphous Silica and Hydrated Silica are safe in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating.

Author's Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 555 13th St., NW, Suite 300W, Washington, DC 20004. cirinfo@cir-safety.org

Author Contributions

The articles in this supplement were sponsored by the Cosmetic Ingredient Review.

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. Nikitakis J, Lange B. *wINCI: International Cosmetic Ingredient Dictionary and Handbook*. Washington, DC: Personal Care Products Council. https://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Last Updated: 2019. Accessed: 4/5/2018.
2. OECD SIDS. Synthetic amorphous silica and silicates. Berlin, Germany 2004, 1-254. https://hpvchemicals.oecd.org/UI/handler.axd?id=4c05aa97-50de-4090-a1cb-70a5e8ed2c8d
3. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Synthetic amorphous silica (CAS No. 7631-86-9). Brussels 2006. JACC No. 51, 1-237. https://www.ecetoc.org/wp-content/uploads/2014/08/JACC-051.pdf
4. Heppleston A. The fibrogenic action of silica. *Br Med Bull*. 1969;25(3):282-287.
5. Becker L, Bergfeld W, Belsito D, et al. Safety assessment of silica and related cosmetic ingredients. Review CI, ed; 2009.
6. The Synthetic Amorphous Silica and Silicates Industry Association. Nanoscale Materials Stewardship Program (NMSP) voluntary submittal package for synthetic amorphous silica. Washington, DC. Submitted to CIR by the Synthetic Amorphous Silica and Silicate Industry Association on March 11, 2019; 2008.
7. Arts J, Muijser H, Duistermaat E, Junker K, Kuper C. Five-day inhalation toxicity study of three types of synthetic amorphous

silicas in Wistar rats and post-exposure evaluations for up to 3 months. *Food Chem Toxicol*. 2007;45(10):1856-1867.
8. Lewinson J, Mayr W, Wagner H. Characterization and toxicological behavior of synthetic amorphous hydrophobic silica. *Regul Toxicol Pharmacol*. 1994;20(1 Pt 1):37-57.
9. National Institute for Occupational Safety and Health (NIOSH). NIOSH pocket guide to chemical hazards: silica, amorphous. https://www.cdc.gov/niosh/npg/npgd0552.html. Last Updated: 2018. Accessed: 11/1/2018.
10. U. S. Department of Labor. Silica, amorphous, precipitated and gel. https://www.osha.gov/dts/chemicalsampling/data/CH_266700.html. Last Updated: 2018. Accessed: 6/1/2018.
11. Pavlich D. Comments from the synthetic amorphous silica and silicate industry association to cosmetic ingredient review. Unpublished data submitted to CIR by the Synthetic Amorphous Silica and Silicate Industry Association on March 11, 2019; 2019.
12. Council of Experts, United States Phamacopeial Convention. *Food Chemicals Codex*. 8th ed. Rockville, MD: United States Pharmacopeia (USP); 2012.
13. Byers P, Gage J. The toxicity of precipitated silica. *Br J Ind Med*. 1961;18(4):295-302.
14. Gray C, Muranko H. Studies of robustness of industrial aciniform aggregates and agglomerates - carbon black and amorphous silicas: a review amplified by new data. *J Occup Environ Med*. 2006;48(12):1279-1290.
15. Yates D, Healy T. The structure of the silica/electrolyte interface. *J Colloid Interface Sci*. 1976;55(1):9-19.
16. Hurd A, Flower W. In situ growth and structure of fractal silica aggregates in a flame. *J Colloid Interface Sci*. 1988;122(1):178-192.
17. U.S. Pharmacopeial Convention. *Food Chemicals Codex*. 8th ed. Baltimore, MD: United Book Press, Inc.; 2012.
18. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary cosmetic registration Program - frequency of use of cosmetic ingredients. College Park, MD, 2019. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3, 2019; received February 13, 2019).
19. Personal Care Products Council. Council concentration of use survey: silica ingredients. Unpublished data submitted by Personal Care Products Council on October 26, 2018; 2018.
20. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
21. Rothe H. Special aspects of cosmetic spray evaluation. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, D.C.
22. Bremmer H, Prud'homme de Lodder L, Engelen J. *Cosmetics Fact Sheet: To Assess the Risks for the Consumer; Updated Version for ConsExpo 4*. Bilthoven, Netherlands; 2006:1-77. RIVM 320104001/2006. https://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed 8/24/2011.
23. Johnsen M. The influence of particle size. *Spray Technol Mark*. 2004;14(11):24-27.
24. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). Cosmetic powder exposure. Unpublished data submitted by the Personal Care Products Council; 2015.
25. Aylott R, Byrne G, Middleton J, Roberts M. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci*. 1976;1(3):177-186.
26. Russell R, Merz R, Sherman W, Sivertson JN. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol*. 1979;17(2):117-122.
27. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products; 2009.
28. Australian Government Department of Health. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). https://www.nicnas.gov.au/chemical-information. Last Updated: Accessed: 5/1/2018.
29. Kaewamatawong T, Kawamura N, Okajima M, Sawada M, Morita T, Shimada A. Acute pulmonary toxicity caused by exposure to colloidal silica: particle size dependent pathological changes in mice. *Toxicol Pathol*. 2005;33(7):743-749.
30. Javadzadeh Y, Jafari-Navimipour B, Nokhodchi A. Liquisolid technique for dissolution rate enhancement of a high dose water-insoluble drug (carbamazepine). *Int J Pharm*. 2007;341(1-2):26-34.
31. Villota R, Hawkes J. Food applications and the toxicological and nutritional implications of amorphous silicon dioxide. *Crit Rev Food Sci Nutr*. 1986;23(4):289-321.
32. Sauer F, Laughland D, Davidson W. Silica metabolism in Guinea pigs. *Can J Biochem Physiol*. 1959;37(2):183-191.
33. Sauer F, Laughland D, Davidson W. The silica content of Guinea pig tissues as determined by chemical and isotopic techniques. *Can J Biochem Physiol*. 1959;37(10):1173-1181.
34. Newberne P, Wilson R. Renal damage associated with silicon compounds in dogs. *Proc Natl Acad Sci USA*. 1970;65(4):872-875.
35. Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H, Uesugi S. Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. *Acta Med Biol*. 1988;36(1):27-56.
36. W.R. Grace & Co. Supplement to GRAS affirmation petition No. 1G0270: silica gel for use as a carrier for flavors. Submitted by the US FDA in response to a FOI request; 1981; 189.
37. Low R, Absher P, Hemenway D, Giancola M. Bronchoalveolar lavage lipids in rats exposed to aerosolized silicon dioxide polymers. *Am Rev Resp Dis*. 1985;13:183.
38. Hemenway D, Abasher M, Landesman M, Trombley L, Emerson R. Differential lung response following silicon dioxide polymorph aerosol exposure. In: DF G, ed. *Silica, Silicosis and Cancer*. NY: Praeger Publ; 1986:105-116.
39. Warheit D, Carakostas M, Kelly D, Hartsky MA. Four-week inhalation toxicity study with Ludox colloidal silica in rats: pulmonary cellular responses. *Fundam Appl Toxicol*. 1991;16(3):590-601.
40. Warheit D, Achinko L, Carakostas M, Hartsky M. Testing the efficacy of biomarkers to predict pulmonary toxicity of inhaled materials. *Am Rev Resp Dis*. 1990;141A:419.

41. Lee K, Kelly D. Translocation of particle-laden alveolar macrophages and intra-alveolar granuloma formation in rats exposed to Ludox colloidal amorphous silica by inhalation. *Toxicol*. 1993;77(3):205-222.
42. Warheit D, McHugh T, Hartsky M. Differential pulmonary responses in rats inhaling crystalline, colloidal, or amorphous silica dusts. *Scand J Work Environ Health*. 1995;21 Suppl 2:19-21.
43. Reuzel P, Bruihintjes J, Reron V, Woutersen R. Subchronic inhalation toxicity of amorphous silicas and quartz dust in rats. *Fd Chem Toxic*. 1991;29(5):341-354.
44. Shin J, Jeon K, Kim J, et al. Subacute inhalation toxicity study of synthetic amorphous silica nanoparticles in Sprague-Dawley rats. *Inhal Toxicol*. 2017;29(12-14):567-576.
45. Johnston C, Driscoll K, Finkelstein J, et al. Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica. *Toxicol Sci*. 2000; 56(2):405-413.
46. Schepers G. Hypertension due to inhaled submicron amorphous silica. *Toxicol Appl Pharmacol*. 1959;1(5):487-500.
47. Schepers G, Durkan T, Delahant A, Creedon F, Redlin A. The biological action of Degussa submicron amorphous silica dust (Dow Corning silica). I. Inhalation studies on rats. *AMA Arch Ind Health*. 1957;16(2):125-146.
48. Schepers G. Biological action of precipitation-process submicron amorphous silica (HI-SIL 233). In: DD D, ed. *Health Effects of Synthetic Silica Particulates, ASTM STP 732*. Philadelphia: American Society for Testing and Materials; 1981:144-173.
49. Groth D, Moormann W, Lynch D, Stettler L, Wagner W, Hornung R. Chronic effects of inhaled amorphous silicas in animals. In: DD D, ed. *Health Effects of Synthetic Silica Particulates, ASTM STP 732*. Philadelphia: American Society for Testing and Materials; 1981:118-143.
50. Schepers G, Durkan T, Delahant A, Creedon F, Redlin A. The biological action of inhaled Degussa submicron amorphous silica dust (Dow Corning Silica): II. The pulmonary reaction in uninfected Guinea pigs. *AMA Arch Ind Health*. 1957;16(3): 203-224.
51. Schepers G, Delahant A, Schmidt J, Von Wecheln J, Creedon F, Clark R. The biological action of Degussa submicron amorphous silica dust (Dow Corning Silica): III. Inhalation studies in rabbits. *AMA Arch Ind Health*. 1957;16(4):280-301.
52. Schepers G. Reaction of monkey lung to siliceous dusts. *Arch Environ Health*. 1962;5(4):278-299.
53. Kanematsu N, Hara M, Kada T. Rec assay and mutagenicity studies on metal compounds. *Mutat Res*. 1980;77(2):109-116.
54. Prival M, Simmon V, Mortelmans K. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. *Mut Res*. 1991;260(4):321-329.
55. Liu X, Keane M, Zhong B-Z, Ong T, Wallace W. Micronucleus formation in V79 cells treated with respirable silica dispersed in medium and in simulated pulmonary surfactant. *Mutat Res*. 1996;361(2-3):89-94.
56. Zhong B, Ong T, Whong W. Studies on the relationship between treatment condition and micronucleus induction in V79 cells exposed to silica and glass fibers. *Mutat Res*. 1997;391(1-2): 111-116.
57. U.S. Food and Drug Administration (FDA). Mutagenic evaluation of compound FDA 71-48, silica aerogel. Unpublished report 2446 by Litton Bionetics, Kensington, MD. Washington, DC: Food and Drug Administration;1974. FDABF-GRAS-311. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB245467.xhtml
58. International Agency for Research on Cancer (IARC). *Silica, Some Silicates, Coal Dust and Para-Aramid Fibrils*, Vol 68. Lyon, France: World Health Organization; 1997.
59. Campbell J. Effects of precipitated silica and of iron oxide on the incidence of primary lung tumours in mice. *Br Med J*. 1940; 2(4156):275-280.
60. Pott F, Roller M. Carcinogenicity study with nineteen granular dusts in rats. *Eur J Oncol*. 2005;10(4):249-281.
61. Epstein W, Skahen J, Krasnobrod H. The organized epithelioid cell granuloma: differentiation of allergic (zirconium) from colloidal (silica) types. *Am J Pathol*. 1963;43(3):391-405.
62. Anonymous. Safety data of hydrated silica - contact allergenicity (Translated into English in 2009). Unpublished data submitted by the Personal Care Products Council; 1984.
63. KGL Inc. An evaluation of the contact-sensitization potential of a topical coded product (facial mask containing 17% Hydrated Silica) in human skin by means of the maximization assay. *KGL Protocol #5384*. Unpublished data submitted by the Personal Care Products Council; 2003.
64. KGL Inc. An evaluation of the contact-sensitization potential of a topical coded product (face powder containing 21.7436% Silica) in human skin by means of the maximization assay. *KGL Protocol #5632*. Unpublished data submitted by the Personal Care Products Council; 2004.
65. Hazelton Laboratories. Progress report no. 1; acute oral administration, acute eye application. Submitted by EPA in response to a FOI request in 2008; 1958, 66.
66. Plunkett E, DeWitt B. Occupational exposure to hil-sil and silene: report of an 18-year study. *Arch Environ Health*. 1962; 5(5):469-472.
67. Wilson R, Stevens P, Lovejoy H, Bell Z, Richie R. Effects of chronic amorphous silica exposure on sequential pulmonary function. *J Occup Med*. 1979;21(6):399-402.
68. Choudat D, Frisch C, Barrat G, el Kholti A, Conso F. Occupational exposure to amorphous silica dust and pulmonary function. *Br J Ind Med*. 1990;47(11):763-766.
69. Volk H. The health of workers in a plant making highly dispersed silica. *Arch Environ Health*. 1960;1(2):125-128.