Silica, amorphous, fumed, crystalline-free

SYNONYMS

Aquafil
Cab-O-grip II
Cab-O-sil
Cab-O-sperse
Cataloid
Dicalite
Dri-Die insecticide 67
ENT 25,550
Fumed silica, crystalline-free
Fumed synthetic amorphous silica
Ludox
Nalcoag
Nyacol
Nyacol 1430
Nyacol 830
Pyrogenic colloidal silica
Silica gel
Silicon dioxide
Silikill
Synthetic amorphous silica
Synthetic amorphous silica, fumed
Vulkasil

CHEMICAL STRUCTURE

CHEMICAL FORMULA

SiO_2

IDENTIFIER DETAILS

CAS Number	:	112945-52-5 (previously assessed by IARC as 7631-86-9), 112926-00-8 (silicon dioxide)
CoE Number	:	-
FEMA	:	-
EINECS Number	:	-
E Number	:	-

SPECIFICATIONS

Melting Point: approx. 1700ºC [OECD SIDS, 2004]

Boiling point: -

Polymorphs of Silica

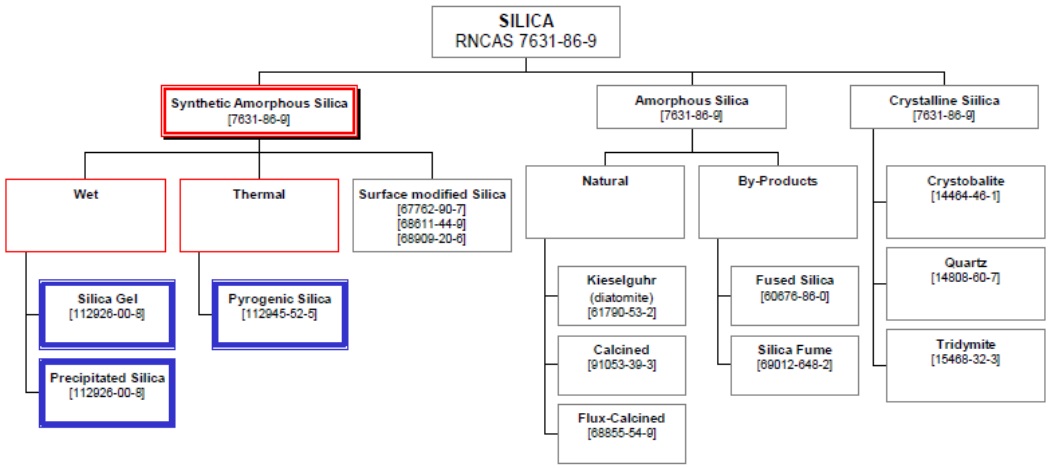

Diagram of the various polymorphs of silica, how they relate to one another and their respective CAS numbers [excerpt from OECD SIDS report, 2004]. Those highlighted are those that were relevant to the OECD SIDS report.

[OECD SIDS, 2004]

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
-	-	-	-

FDA Status: [CFR21]

Section Number	Comments
182.90	Generally regarded as safe, substances migrating to food from paper and paper board products.

HUMAN EXPOSURE

Natural Occurrence: Silica, silicic acid and the calcium, magnesium and aluminium salts occur ubiquitously in the environment and some have been used medically. Foods have been reported to contain various amounts of SiO2 in them including potatoes 10.1, milk 2.1, mineral water 22.5, drinking water 7.1 or beer 131 mg of SiO_2 per/g or cm^3. Very small quantities of silica are also reported to occur naturally in all of the body tissues, but does not appear to play any physiological role. The human tissue content of silica is reported to be between 10-200 mg/100 g of dry tissue [WHO 1974].

Amorphous silicates can form volcanic glass from extruded magma. Biological forms are found in plants (dry weight of grasses can have high levels up to 20 %) and also in diatoms and sponges that extract the silica from their surroundings to form their shells [IARC Monograph 68, 1997] .

Synthetic amorphous silica (silicon dioxide) is produced by a vapour phase hydrolysis process using chlorosilanes or substituted silanes such as, silicon tetrachloride in a flame of hydrogen and oxygen. This material is formed and collected in a dry state. This product contains no detectable crystalline silica [ToxNet, 2009].

Reported Uses: A luting cement [ToxNet, 2009]. Can be found in food as an anti-caking agent up to 2 %, and also found in cosmetics, pharmaceuticals, talcum powder, cleansers etc [IARC Monograph 68, 1997].

TOXICITY DATA

In Vivo Toxicity Status

Organism	Test Type	Route	Reported Dosage	Effect
Rat	LD50	intravenous	15 mg/kg (15 mg/kg)	LUNGS, THORAX, OR RESPIRATION: ACUTE PULMONARY EDEMA
Rat	LD50	oral	3160 mg/kg (3160 mg/kg)	
Rat	LDLo	intraperitoneal	50 mg/kg (50 mg/kg)	
Rat	LDLo	intratracheal	10 mg/kg (10 mg/kg)	

[ToxNet, 2009]

Rat	TC_{Lo}	inhalation	154 mg/m^3	

RTECS [17/05/04].

OSHA currently has a set limit of 20 mppcf (which is equivalent to 6 mg/m^3) for amorphous silica

Carcinogenicity and Mutagenicity

Amorphous silica was classified under IARC Group 3 – cannot be classified as to its carcinogenicity to humans [IARC Monograph 68, 1997]. There was insufficient evidence for a decision in both animals and humans.

Data on local genotoxicity after particle exposure are crucial to resolve mechanistic aspects such as the impact of chronic inflammation, types of DNA damage, and their role in lung carcinogenesis. We established immunohistochemical methods to quantify the DNA damage markers poly(ADP-ribose) (PAR), phosphorylated H2AX (γ-H2AX), 8-hydroxyguanosine (8-OH-dG), and 8-oxoguanine DNA glycosylase (OGG1) in paraffin-embedded tissue from particle-exposed rats. The study was based on lungs from a subchronic study that was part of an already published carcinogenicity study where rats had been intratracheally instilled with saline, quartz DQ12, amorphous silica (Aerosil(®) 150), or carbon black (Printex(®) 90) at monthly intervals for 3 months. Lung sections were stained immunohistochemically and markers were quantified in alveolar lining cells. Local genotoxicity was then correlated with already defined endpoints, i.e. mean inflammation score, bronchoalveolar lavage parameters, and carcinogenicity. Genotoxicity was most pronounced in quartz DQ12-treated rats, where all genotoxicity markers gave statistically significant positive results, indicating considerable genotoxic stress such as occurrence of DNA double-strand breaks (DSB), and oxidative damage with subsequent repair activity. Genotoxicity was less pronounced for Printex(®) 90, but significant increases in γ-H2AX- and 8-OH-dG-positive nuclei and OGG1-positive cytoplasm were nevertheless detected. In contrast, Aerosil(®) 150 significantly enhanced only 8-OH-dG-positive nuclei and oxidative damage-related repair activity (OGG1) in cytoplasm. In the present study, γ-H2AX was the most sensitive genotoxicity marker, differentiating best between the three types of particles. The mean number of 8-OH-dG-positive nuclei, however, correlated best with the mean inflammation score at the same time point. This methodological approach enables integration of local genotoxicity testing in subchronic inhalation studies and makes immunohistochemical detection, in particular of γ-H2AX and 8-hydroxyguanine, a very promising approach for local genotoxicity testing in lungs, with prognostic value for the long-term outcome of particle exposure [Rittinghausen et al., 2013].

Dermal Toxicity

No data identified

Reproductive and Developmental toxicity

A two regeneration reproductive study with the administration of 100 mg/kg of amorphous silica was conducted. A parent group of 1 male and 5 females produced 5 litters, totalling 25 offspring. From the offspring 1 male and 5 females were similarly mated, producing 21 second generation offspring. There was reported to be no adverse effects or malformation detected, however the samples sizes of the groups were small [WHO 1974].

Inhalation Toxicity

Goscicki et al., (1978) Measured the fibrogenic effect of two natural amorphous silica dusts-diatomite from deposits near Leszczawka (Poland) and silica earth (from the USA) were tested on the rats by intratracheal

introduction of 50 mg dust, as a single dose. Fibrogenic effect was assessed after 3, 6 and 9 months after introduction of dust. Analysis of diatomite dust carried out with X-ray diffraction method showed the presence of quartz in a quantity not exceeding 5%, while in silica earth dust from the USA no silica crystalline structures were found. The development of fibrogenic changes in lungs of the rats was assessed via hydroxyproline (collagen) increase and increase in the wet weight of the lungs. The increment of the indices was twice as high as compared to the lungs of control animals but lower than in the rats which were administered cristalline silica dusts. In histopathological examinations no progressive lung fibrosis was found though some signs of destruction and necrobiosis in some cells were noticed [Goscicki et al., 1978].

Warheit et al., (1995) examined pulmonary responses in rats after short-term inhalation exposure to polymorphs of silica dust. Groups of CD rats were exposed 6 h a day for 3 d to crystalline silica or amorphous silica. Another group was exposed to Ludox colloidal silica for 6 h a day, 5 d a week for two or four weeks. Thereafter the groups were killed, and the lungs washed at several post-exposure times. The crystalline silica produced persistent pulmonary inflammatory responses characterized by neutrophil recruitment and consistently elevated biomarkers of cytotoxicity in bronchoalveolar lavage fluids, and progressive histopathological lesions were observed within one month of the exposure. Amorphous silica produced a transient pulmonary inflammatory response, and Ludox elicited transient pulmonary inflammatory responses at 50 or 150 mg/m^3 but not at 10 mg/m^3. After three months most of the biochemical values of the Ludox-exposed animals had returned to the control level. These results demonstrated that crystalline silica dust is more potent in producing pulmonary toxicity when compared with amorphous or colloidal silica particles [Warheit et al., 1995].

Reuzel et al., (1991) compared the inhalation toxicity of three types of amorphous silica (Aerosil 200, Aerosil R 974 and Sipernat 22S) with that of quartz dust. Rats were exposed to 1, 6 or 30 mg Aerosil 200/m^3, 30 mg Aerosil R 974/m^3, 30 mg Sipernat 22S/m^3 or 60 mg quartz/m^3 for 6 hr/day, 5 days/wk for 13 wk. Some rats were killed at the end of the exposure period and some were killed 13, 26, 39 or 52 wk after the end of exposure. All test materials induced increases in lung weight, and pulmonary lesions such as the accumulation of alveolar macrophages, inflammation, alveolar bronchiolization and fibrosis. In addition, rats exposed to Aerosil 200, Aerosil R 974 or quartz developed granulomatous lesions. Silicosis was observed only in quartz-exposed animals. At the end of the exposure period, Aerosil 200 and quartz had induced the most severe changes. Quartz dust was hardly cleared from the lungs and the changes in the lungs progressed during the post-treatment period, and eventually resulted in lesions resembling silicotic nodules and in one rat squamous cell carcinoma. Although Aerosil 200 was very quickly cleared from the lungs and regional lymph nodes, the changes in these organs were only partly reversed during the post-exposure period in rats exposed to 30 mg/m^3. Aerosil R 974 and the lower levels of Aerosil 200 resulted in less severe, and mostly reversible, changes. The slightest changes were found after exposure to Sipernat 22S, notwithstanding the persistence of this silica in the lungs during the major part of the post-treatment period. The

authors concluded that only quartz induced progressive lesions in the lungs resembling silicotic nodules. Of the amorphous silicas examined Aerosil 200 induced the most severe changes in the lungs, which only partly recovered during the recovery period, whereas Sipernat 22S induced the least severe, completely reversible lung changes [Reuzel *et al.,* 1991].

Yuen *et al* (1996) examined the time course of neutrophil recruitment into the lung of rats, neutrophilic chemotactic activity, and the gene expression of neutrophilic chemokines by lavaged cells was determined after intratracheal instillation of various particles. Low-toxicity, low-solubility dusts such as titanium dioxide (TiO2) particles, as well as fibrogenic crystalline silica and nonfibrogenic amorphous silica particles were instilled into the lungs of rats. Results showed that all three dusts induced neutrophilic inflammation as early as 5 h after exposure. Both crystalline and amorphous silica elicited higher degrees of pulmonary inflammation when compared with TiO2 particles. Maximal infiltration of neutrophils into the lungs occurred 5 to 6 h after intratracheal instillation of the dusts. The inflammatory response was transient for TiO2 and amorphous silica, i.e., evident at 2 days after exposure but not different from controls at 10 days after exposure. In contrast, inflammatory effects were sustained through a 10-day period following exposures to crystalline silica. Chemotactic activity for neutrophils was detected directly in bronchoalveolar lavage (BAL) fluids of dust-exposed rats within 2 h after exposure, but not in the BAL fluids of saline- or unexposed rats. The chemotactic activity was correlated with the influx and disappearance of neutrophils into alveolar regions of the lung in TiO2- and amorphous silica-exposed rats. The mRNA expression of two known neutrophil chemotactic cytokines in BAL cells, macrophage inflammatory protein-2 (MIP-2) and KC, also correlated with chemotactic activity and acute and pulmonary inflammatory responses. MIP-2 mRNA was expressed prior to the detection of chemotactic activity in BAL fluids. However, the mRNA expressions of MIP-2 and KC were transient for rats that were exposed to these dusts as KC and MIP-2 message were no longer detectable in BAL cells after 2 days of recovery. Although both neutrophilic chemotactic activity and inflammation remained prominent 10 days after exposure to crystalline silica, MIP-2 expression could not be detected in BAL cells. Yuen et al., (1996) concluded that MIP-2 was likely to be only one of several cytokines involved in mediating neutrophilic inflammation following a single instillation of crystalline silica [Yuen *et al.,* 1996].

Groth *et al.,* (1981, as cited in Australian Government NOHSC 2004) reported an animal inhalation study where rats, guinea pigs, and monkey were exposed to fumed silica, silica gel or precipitated silica for 5.5 - 6 hours a day for 18 months at 15 mg/m^3 (6.9 - 9.9 mg/m^3 respirable fraction). It was reported that few or no macrophages containing silica were found in the lungs and lymph nodes of guinea pigs and rats. The most significant finding was that fumed silica induced early nodular fibrosis in the lungs of monkeys. However the toxic potential of the fumed silica was stated to possibly not be fully in the study due to the relatively short (10 - 18 months) exposure time compared to the life time of the animal. The researchers attributed the fibrogenic action of the fumed silica to a number of factors that included the

greater surface area, greater solubility and a higher content of Iron and aluminium compared to the other forms of amorphous silica [Groth *et al.,* 1981].

Chronic inhalation of crystalline silica can produce lung tumours in rats whereas this has not been demonstrated for amorphous silica. At present the mechanisms underlying this rat lung tumour response are unknown, although a significant role for chronic inflammation and cell proliferation has been postulated. In a study by Johnston *et al.,* (2000) rats were exposed for 6 h/day, on 5 days/week, for up to 13 weeks to 3 mg/m^3 crystalline or 50 mg/m^3 amorphous silica, at exposure concentrations selected to induce high pulmonary inflammatory-cell responses by both compounds. Endpoints characterized after silica exposure included mutation in the HPRT gene of isolated alveolar cells in an ex vivo assay, changes in bronchoalveolar lavage fluid markers of cellular and biochemical lung injury and inflammation, expression of mRNA for the chemokine MIP-2, and detection of oxidative DNA damage. After 13 weeks of exposure, lavage neutrophils were increased from 0.26 % (controls) to 47 and 55 % of total lavaged cells for crystalline and amorphous silica, with significantly greater lavage neutrophil numbers after amorphous silica (9.3 x 10^7 PMNs) compared to crystalline silica (6.5 x 10^7 PMNs). Lung burdens were 819 and 882 microg for crystalline and amorphous silica, respectively. BAL fluid levels of LDH as an indicator of cytotoxicity were twice as high for amorphous silica compared to those of crystalline silica, at the end of exposure. All parameters remained increased for crystalline silica and decreased rapidly for amorphous silica in the 8-month recovery period. After 8 months of recovery, those markers remained elevated in crystalline silica-exposed rats, whereas amorphous silica-exposed rats were not significantly different from controls. The authors commented that the observation that genotoxic effects in alveolar epithelial cells occurred only after crystalline but not amorphous silica exposure, despite a high degree of inflammatory-cell response after subchronic exposure to both types of silica, suggested that in addition to an inflammatory response, particle biopersistence, solubility, and direct or indirect epithelial cell cytotoxicity may be key factors for the induction of either mutagenic events or targeted cell death [Johnston *et al.,* 2000].

Arts *et al.,* (2007) performed a study in Wistar rats to assess whether pathological changes observed in 90 day studies could be observed at earlier time points. The authors exposed the rats to three different synthetic amorphous silicas (SAS); precipitated silica Zeosil 45, silica gel Syloid 74, and pyrogenic silica Cab-O-Sil M5. Exposure was by nose only, at doses of 1, 5 or 25 mg/m^3 of one of the SAS 6h a day for five consecutive days. Crystalline silica (quartz dust) at 25 mg/m^3 was used as a positive control, clean air was used as the negative control. Rats were sacrificed and necropsied the day after the last exposure or 1 or 3 months after. Exposures were well tolerated with differences in effects associated almost exclusively to the 1-day post-exposure time point. Silicon levels were measured in the tracheobronchial lymph nodes and lungs. Levels in lymph nodes were below detection. High levels were observed in the lungs at 1-day post-exposure but were cleared at 3-months post-exposure. Highest dose exposure induced markers of

cytotoxicity in bronchoalveolar lavage fluid (BALf), with increases in lung and tracheobronchial lymph node weight and histopathological lung changes 1-day post-exposure. Mid-dose exposure induced histopathological changes and changes in BAL fluid only. The effects of all SAS were transient and, with the exception of slight histopathological lung changes at higher doses, were reversible during the 3-month recovery period. No adverse changes were observed in animals exposed to any of the SAS at 1 mg/m^3. Positive control rats were found to exhibit persistent silicon presence in the lungs. The type and severity of the toxicological pathology as well as in the time-response profile was different to the SAS exposure. The authors suggest that the overall results are similar to others studying the 90-day time exposure period and both types of studies indicate that a lack of lung clearance as a key factor in silicosis development [Arts *et al,* 2007].

Choi *et al* (2008) exposed A/J mice to doses of 0, 2, 10 and 50 mg/kg of suspensions of ultrafine amorphous silica (UFAS) (approximately 50microl) (n=5 per group), and sacrificed at 24 h, 1, 4 and 14 weeks after exposure. Analysis of the lung tissues showed inflammatory mediator (cytokines (IL-4, IL-10, IL-13 and IFN-gamma), matrix metalloproteinases (MMP-2, MMP-9 and MMP-10) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1)) mRNA and protein were elevated at 24h and 1 week but returned to near normal thereafter (except IFN-gamma and MMP-2). Staining of the lung tissue by Gomori's trichrome showed that UFAS induced severe alveolar epithelial thickening and pulmonary fibrosis at 1 week, with recovery at 4 and 14 weeks. The authors suggest that amorphous silica produces pulmonary fibrosis similar to that by crystalline silica except that this effect is transient in nature [Choi *et al.,* 2008].

A rat inhalation study by Sayes *et al.,* (2010) investigated the effect of the size and particle number of amorphous silica nanoparticles (diameter d_{50} = 37 or 83 nm, and concentrations 3.1 x 10^7 to 1.8 x 10^8 particles/cm^3, 6h/day) over 1- or 3-day exposures. Male Crl:CD (SD)IGS BR rats were used. Lung toxicity was assessed by looking for lung inflammation via bronchiolar lavage fluid samples (assessed at 24 h, 1 wk, 1 mth and by histopathology at 2 months post-exposure), lung tissue samples were used to test for histological changes (assessed 2 months post-exposure) and cellular genotoxicity by using micronucleus assays (assessed 24h post-exposure). Regardless of duration of exposure no significant pulmonary inflammation, genotoxic or adverse lung histopathology was observed at high particles numbers (1.8 or 86 mg/m^3) [Sayes *et al.,* 2010].

Other Relevant Studies

Groups of 20 male and female rats were fed silica at 500 mg/kg for 6 months. After 4.5 months five of the females were mated. There was reported to be no effects between the treated and control rats for any of the parameters investigated. Groups of five male and female rats were fed at levels of 0, 500, 1000 and 2000 mg/kg/day for 5 weeks. The top dose was increased to 4000 mg/kg on day 15 and 8000 mg/kg on day 29. The top dose animals were reported to have atrophy of the liver, with regression of the basophilic

structures and the glycogen content. All other organs examined were considered normal when compared to the controls [WHO 1974].

Pure bred beagle dogs of about 6 months of age (6-9 per group) were fed silicon dioxide, sodium silicate, aluminium silicate or magnesium trisilicate at 800 mg/kg for four weeks. All haematological, urine and biochemical investigations were considered normal when compared to the controls. The administration of sodium silicate or magnesium trisilicate lead to characteristic kidney lesions visible grossly in most dogs. There were no treatment related effects in any of the other treatment groups [WHO 1974].

Twenty male and female rats were fed a pellet adjusted for bodyweight to supply 100 mg/kg/day of amorphous silica (> 98.3 % silica). At the end of the two year period there was 100 % survival of both treated group. There was reported to be no effect of treated upon any of the physiological or pathological parameters measured when compared to the controls. The administration of a single dose of 2.5 g of amorphous polymeric silicon dioxide to 12 humans, leads to a slight but not statistically significant increase in the urine production of silicon dioxide [WHO 1974].

An OECD SIDS report was compiled in 2004 on 'Synthetic amorphous silica and silicates'. A summary of the toxicological data was tabulated and is provided on the following page as a brief summary of the available information.

In brief, the OECD SIDS report assessed 'toxicokinetics, metabolism and distribution', 'acute toxicity', 'irritation', 'sensitisation' , 'repeated dose toxicity', 'mutagenicity', 'carcinogenicity' and 'toxicity for reproduction'. Details of individual references can be found in the original document but the OECD summaries of the information are provided here.

'Toxicokinetics, metabolism and distribution' – Animal and human inhalation data suggest that after an initial high deposition phase, there is a phase of low deposition. SAS is believed to be rapidly cleared from the lung (unlike crystallized silica), but there is no 'disproportionate deposition' in the lymph nodes. Oral administration was associated with no accumulation in body tissues, there was rapid clearance from the body and intestinal resorption 'appears to be insignificant'. Human urine increases were found to be low compared to high dose (2500mg SiO_2 exposure). Subcutaneous injection was associated with 'rapid dissolution and removal'.

'Acute toxicity' – Acute inhalation data were unavailable/unsuitable for interpretation, but it was said that 'inhalation of dust may cause discomfort and stress as well as sign of local irritation to nasal, bronchiolar and ocular mucous membranes'. In summary, it was stated that 'SAS dusts are considered as acutely non-toxic' Oral and dermal administration was said to produce 'no systemic toxicity'.

‘Irritation’ and ‘sensitisation’ – no sensitisation potential and no evidence of irritation to the skin and eyes although prolonged exposure may lead to skin dryness.

‘Repeated dose toxicity’ – Inhalation was associated with time- and dose-related inflammation. With doses such as 1.3 mg/m^3 over 13-weeks causing ‘mild reversible pro-inflammatory cell proliferation’, leading to a NOAEL and LOEL of 1.3 mg/m^3 being set. The LOAEL was set at 5.9mg/m^3 after adverse (although ‘reversible’) histopathological indicators were observed. A 5d LOAEL was set at 5.0 mg/m^3. Extrapolating SAS to encompass a range of amorphous silicates, the OECD report suggests that if the toxicity is based on particle size and morphology rather than composition, other amorphous silicates would not be expected to cause a more severe pulmonary reaction under the same test conditions. Oral administration over 2-years was not associated with adverse effects (only ‘occasional’ minor effects on growth depression or elevated organ weights at high doses). SAS and CS were given a NOAEL of approx. 2500mg/kg bw/d, with NAS showing no effects in a 14 day study at up to 10% inclusion in the diet,

‘Mutagenicity’ – no evidence of mutagenicity in a range of tests using a variety of amorphous silicates was found.

‘Carcinogenicity’ - no evidence of carcinogenicity was found in a rat intra-plueral study usinf NAS, in mice and rats at 5% SAS in the diet or in another rat study at up to 10% CS.

‘Toxicity for reproduction’ – tests looking at a range of endpoints and types of amorphous silicates suggest that there is no adverse effect on these endpoints, although some of the data has limited value.

A key statement within the summary of the report highlights the fact that;
‘Medical surveillance reports failed to reveal significant pathological lung effects attributable to occupational long-term exposure to SAS and/or synthetic amorphous silicates: in particular, no signs of pneumoconiosis, silicosis and fibrosis were evident’. [OECD SIDS, 2004].

Table: Truncated excerpt form an OECD SIDS report on 'Synthetic amorphous silica and silicates. Abbreviations for amorphous silicates: *SAS – synthetic amorphous silicate, NAS – sodium aluminium silicate and CS – calcium silicate.*

	SAS [7631-86-9]	NAS [1344-00-9]	CS [1344-95-2]
TOXICOLOGY			
Acute Oral Toxicity	LD_{50} >3300 mg/kg (limit test)	LD_{50} >5000 mg/kg	LD_{50} >5000 mg/kg
Acute Inhalation Toxicity	LC_{50} >0.14 - >2.0 mg/l (Maximum concentrations technically feasible)	no data: analogy	no data: analogy
Acute Dermal Toxicity	LD_{50} >5000 mg/kg (limit test)	LD_{50} >5000 mg/kg	no data: analogy
Primary Irritation (skin, eye)	not irritating	not irritating	no data: analogy
Sensitization	no data	no data	no data
Repeated Dose Toxicity (inhalation)	inflammatory reaction in the lung: NOEL(5 d) = 1.0 mg/m^3	no data: analogy	no data: analogy
Repeated Dose Toxicity (inhalation)	inflammatory reaction in the lung (rat) NOAEL(13 wks) = 1.3 mg/m^3	no data: analogy	no data: analogy
Repeated Dose Toxicity (oral)	no substance-related abnormalities in rat: NOAEL(6 months) = ~9000 mg/kgbw	Chronic: no data: analogy no gross signs of toxicity in rat and mouse, no death: NOAEL(14 d) >5000 mg/kgbw	No gross signs of toxicity in rat, no death NOAEL(2 years) = approx. 5000 mg/kgbw (Reliab. 4)
Genetic Toxicity *in Vitro*			
A. Bacterial Test (Gene mutation)	not mutagenic	no data: analogy	not mutagenic (Reliab. 4)
B. Non-Bacterial In-Vitro Test (Gene Mutation)	not mutagenic	no data: analogy	no data: analogy
C. Non-Bacterial In-Vitro Test (Chromosomal	not mutagenic	no data: analogy	not mutagenic (Reliab. 2)
Genetic Toxicity *in Vivo*	not mutagenic	no data: analogy	not mutagenic (Reliab. 2)
Carcinogenicity (inhalation)	inconclusive (Reliab. 3)	no data	no data
Carcinogenicity (oral)	not cancerogenic in rat and mouse	no data: analogy	not cancerogenic in rat (Reliab. 4)
Carcinogenicity (intrapleural)	no data: analogy	Not cancerogenic in rat	no data: analogy
Toxicity to Fertility	no effects in limited study in rat (Reliab. 3)	no data: analogy	no data: analogy
Developmental / Teratogenicity	no adverse effects in rat, mouse, rabbit and hamster	no adverse effects in rat, mouse, rabbit and hamster	no adverse effects in rat, mouse, and hamster

[OECD SIDS, 2004]

Behavioural Data

No data identified

***In Vitro* Toxicity Status**

Elias *et al.,* (2000) examined the effects of several crystalline and amorphous silica dusts (two quartz of natural origin, one cristobalite of natural and two of biogenic origin, three amorphous diatomite earths and one pyrogenic amorphous silica) were studied in the SHE cell transformation assay. The results showed that some quartz and cristobalite dusts (crystalline) as well as the diatomaceous earths (amorphous), but not the pyrogenic amorphous silica, were cytotoxic and induced morphological transformation of SHE cells in a concentration-dependent manner. The ranking in cytotoxicity was different from that in transforming potency, suggesting two separate molecular mechanisms for the two effects. The cytotoxic effects appeared to be related to the distribution and abundance of silanol groups and to the presence of trace amounts of iron on the silica surface. Silica particles with fractured surfaces and/or iron-active sites, were reported to be able to generate reactive oxygen species, that induced SHE cell transformation. The results showed that the activity of silica at the cellular level was sensitive to the composition and structure of surface functionalities and confirmed that the biological response to silica was a surface originated phenomenon [Elias *et al.,* 2000].

In a study by Iyer *et al.,* (1996) human alveolar macrophages were treated with fibrogenic, poorly fibrogenic, and nonfibrogenic model particulates, using silica (133 micrograms/ml), amorphous silica (80 micrograms/ml), and titanium dioxide (60 micrograms/ml), respectively. Cell were treated with these particulates in vitro for 6 and 24 hr and examined for apoptosis by morphological analysis. Treatment with silica resulted in morphological changes typical of apoptotic cells. In contrast, amorphous silica and titanium dioxide demonstrated no significant apoptotic potential. Results of experiments suggested that fibrogenic particulates, such as silica, caused apoptosis of alveolar macrophages and that the apoptotic potential of fibrogenic particulates may be a critical factor in initiating an inflammatory response resulting in fibrosis. The authors suggest that silica interacts with scavenger receptors on the macrophage cell surface, stimulating apoptosis via indirect activation of the interleukin converting enzyme (ICE) family of protein [Iyer *et al.,* 1996].

The inflammatory and cytotoxic potency of crystalline and amorphous silica in relation to particle size and surface area was assessed in. human epithelial lung cells. The cells (A549) were exposed to different size fractions of quartz (aerodynamic diameter 0.5, 2 and 10 micron) and amorphous silica (diameter 0.3 micron). All particles induced increased release of the proinflammatory cytokines interleukin (IL)-6 and IL-8. When cells were exposed to equal masses of quartz, the smallest size fraction was the most potent. These differences, however, disappeared when cytokine release was related to equal surface areas. When amorphous silica and quartz were compared, the amorphous silica was most potent to induce IL - 6 regardless of how exposure

was expressed, whereas the smallest size fraction of quartz was the most potent inducer of IL-8. Thus, the surface area was determined by the author to be the critical determinant when potency of different sizes of quartz was compared [Hetland *et al.,* 2001].

It has been estimated that over three million workers in the USA are potentially exposed to silica or other mineral dusts. The alkaline (pH > 13) single cell gel/comet (SCG) assay was used by Zhong et al., (1997) to determine and compare DNA damage in cultured Chinese hamster lung fibroblasts (V79 cells) and human embryonic lung fibroblasts (Hel 299 cells) exposed to crystalline silica (Min-U-Sil 5), amorphous silica (Spherisorb), carbon black, and glass fibres (AAA-10). V79 or Hel 299 cells were exposed to these mineral dusts for 3 h at various concentrations. Min-U-Sil 5 and AAA-10, at almost all concentrations tested, caused a significant increase in DNA migration measured as tail length in both V79 and Hel 299 exposed cells. However, the increase was much higher in V79 then in Hel 299 cells for Min-U-Sil 5. Tail length was also increased relative to controls after amorphous silica treatment, but not to the same extent as that induced by crystalline silica. Exposure to carbon black did not induce DNA migration at any of the concentrations tested. For glass fibers, induction of DNA damage in both V79 and Hel 299 cells was observed even at a concentration 10 times lower than silica and the response was similar in both cell lines [Zhong *et al.,* 1997].

In a study by Yang *et al*., (2010) the effects of amorphous silica micro- (365nm) and nano-particles (15nm and 30nm) on cell viability and protein expression associated with cell cycle and apoptosis in HaCaT cells. A dose-dependant decrease on cell viability was observed with both micro- and nano-sized particles, with IC_{50} values associated with particle size. A dose-dependant increase in apoptosis was also detected using flow cytometry, and smaller particles were associated with increased rate of apoptosis. Proteomic analysis showed 16 proteins were differentially expression upon exposure to silica exposure with a size dependant change in level of expression. The proteins differentially expressed where found to be associated with functions such as oxidative stress, apoptosis and tumour-associated proteins [Yang *et al.,* 2010].

A study by Waters *et al.,* (2009) in a macrophage model assessed macrophage responses to unopsonised amorphous silica nanoparticles after 24h exposure. Stimulated inflammatory protein secretion (customised multiplex ELISA system) and cytotoxicity (MTT assay) were found to correlate closely with total administered particle surface area (7-500nm). Affymetrix gene expression analysis also showed that the magnitude of change was correlated more with surface area than particle mass or number. Analysis of the gene expression changes however showed that overall biological processes were ‘nearly identical, irrespective of particle diameter [Waters *et al.,* 2009].

Carcinogenicity and Mutagenicity

A genotoxicity study was performed in 3T3-L1 fibroblasts using commercial colloidal and laboratory-synthesized silica nanoparticles. 3T3-L1 fibroblasts were incubated for 3, 6, and 24 h with doses of 4 or 40 microg/ml of silica nanoparticles. Comet assay showed no significant genotoxicity, and results were independently validated in two separate laboratories [Barnes *et al.,* 2008].

The aim of this study was to investigate the cytotoxic and genotoxic potential of silica particles of different sizes (250 and 500 nm) and structures (dense and mesoporous). Dense silica (DS) spheres were prepared by sol-gel synthesis, mesoporous silica particles (MCM-41) were prepared using hexadecyltrimethyl ammonium bromide as a structure-directing agent and tetraethylorthosilicate as silica source. Particles were accurately characterised by dynamic light scattering, nitrogen adsorption, X-ray diffraction and field emission scanning electron microscopy. Murine macrophages (RAW264.7) and human epithelial lung (A549) cell lines were selected for investigation. Genotoxicity was evaluated by Comet assay and micronucleus test. Cytotoxicity was tested by the trypan blue method. Cells were treated with 0, 5, 10, 20, 40 and 80 μg/cm^2 of different silica powders for 4 and 24 h. The intracellular localisation of silica was investigated by transmission electron microscopy. Amorphous particles penetrated into the cells, being compartmentalised within endocytic vacuoles. DS and MCM-41 particles induced cytotoxic and genotoxic effects in A549 and RAW264.7 although to different extent in the two cell lines. A549 were resistant in terms of cell viability, but showed a generalised induction of DNA strand breaks. RAW264.7 were susceptible to amorphous silica exposure, exhibiting both cytotoxic and genotoxic responses as DNA strand breaks and chromosomal alterations. The cytotoxic response of RAW264.7 was particularly relevant after MCM-41 exposure. The genotoxicity of amorphous silica highlights the need for a proper assessment of its potential hazard for human health [Guidi *et al*., 2012].

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