GreenScreen® Assessment for Amorphous Fumed Silica, nano *(112945-52-5)*

Method Version: GreenScreen® Version 1.2

Assessment Type: Authorized

GreenScreen® Assessment Prepared By:	**GreenScreen® Assessment Quality Control Performed By:**
Name: Nina Hwang and Jennifer Sass	Name: Alex Stone
Title: Scientist, Health Program	Title: Safer Chemical Alternative Chemist
Organization: NRDC	Organization: Washington State Dept. of Ecology
Date: **Feb 2016**	Date: December 2016
Assessor Type: Authorized GreenScreen Practitioner	

Confirm application of the *Disclosure and Assessment Rules and Best Practice*: No known contaminants based on theoretically pure substance, de minimus rule not applicable.

Chemical Name (CAS #): Amorphous fumed silica, nano (112945-52-5)

Also Called: This hazard assessment is for fumed (or pyrogenic) nanosilica, a synthetic amorphous silica (SAS) prepared by thermal or pyrogenic methods and assigned the specific CAS# 112945-52-5.

A common CAS number for all silicas is 7631-86-9. However, each different form of silica has its own polymorph specific CAS number. Crystalline silica, known for its carcinogenic properties, does not have a nanospecific structure, and is excluded from this assessment. Amorphous silica can be divided as follows:

- Synthetic amorphous silica (includes nanoparticles)
    - pyrogenic silica (fumed silica), produced by flame hydrolysis. This is the form that is the subject of this hazard assessment.
    - precipitated silica
    - colloidal silica (silica sol)
    - silica gel
- Natural amorphous silica (diatomaceous earth)
- Fused silica (quartz glass)
- Silica fume (by product of metal industry; includes nanoparticles)

SAS is further distinguished through the method of synthesis. In addition to fumed silica, other forms of SAS include colloidal nanosilica, sol-gel nanosilica, and precipitated nanosilica. These forms of SAS also vary with respect to primary particle size, porosity, and state of aggregation and agglomeration. Fumed nanosilica is hydrophilic, but can be made hydrophobic through surface treatments with chemicals such as hexamethyldisilazane, dimethyldichlorsilane, and polydimethylsiloxanes (European Centre for Ecotoxicology and Toxicology of Chemicals, 2006). While surface treatment does not change the particle's solid properties, such as particle size, it does change physico-chemical properties such as moisture uptake.

Both untreated (hydrophilic) silica and treated (hydrophobic) silica can be used as food additives, but only untreated silica is included in this assessment.

Trade names for food-grade untreated fumed nanosilica considered in this assessment include Aerosil 200F and 380F, and Cab-O-Sil M-5F and EH-5F. It is also sometimes referred to as "Cab-O-Sil Fluffy".

The following silicon dioxide substances are <u>outside the scope</u> of this GreenScreen:

- Amorphous silica, non-synthetic: CAS 7631-86-9.
- Crystalline silica: CAS 7631-86-9
- SAS, wet (silica gel, precipitated silica): CAS 112926-00-8
- Treated, hydrophobic SAS (silica dimethicone silylate, silica dimethyl silylate and silica silylate): CAS 67762-90-7, 68611-44-9 and 68909-20-6
- Precipitated aluminum or calcium silicates: 1344-00-9,1344-95-2
- Vitreous silica: 60676-86-0
- Silica, amorphous, precipitated and gel (CAS # 7699-41-40; synonym: silicic acid; listed in 40 CFR 180.950
- Silica gel (CAS # 632321-67-4); listed in 40 CFR 180.950
- Silica, hydrate (CAS # 10279-57-9); listed in 40 CFR 180.950

- Synonyms used in EFSA (2009) for food additive applications including silicon dioxide / silicic acid gel (CAS # 7631-86-9), precipitated silicon dioxide, hydrous silica, hydrated silicic acid, polysilicic acid gel, E551, food grade micron sized silica (an anticaking agent) and silica gel.

Due to the overlap in CAS numbers (for example SAS, Amorphous silica, and Crystalline silica all share a CASRN of 7631-86-9), studies were only included if the use of fumed SAS is explicitly stated. Another exception lays in E551, an authorized food additive for use as an anticaking agent and carrier. It may include fumed silica, but only studies explicitly stating the presence of fumed SAS are included in the assessment.

Primary particles of fumed nanosilica typically fall in the range of 2-50 nm in diameter, but nanosilica has a tendency to form aggregations which then can agglomerate into particles around 694 μm in diameter (Cab-O-Sil)(De Temmerman et al., 2012).[1] In the case of synthetic amorphous silica, biological activity is related to the particle shape and surface characteristics more so than to particle size or mass, since the SAS adsorbs to cellular surfaces and can affect membrane structures and integrity (Akbar, Mohamed, Whitehead, & Azzawi, 2011; Fruijtier-Pölloth, 2012)

Due to this behavior, there has been debate over whether fumed nanosilica is, in fact, a nanoscale material. The Cefic Sector Group Association of Synthetic Amorphous Silica Producers (ASASP) uses the ISO TS 80004-1 definition that the identifying feature of nanostructured materials is that their internal or surface structure is in the nanoscale, but their external dimensions are typically greater than the nanoscale range. Since the aggregate is the smallest individual unit and fall close to 100 nm, ASASP argues that fumed nanosilica should be considered a *nanostructured* material (ASASP, 2013).
Some argue that nanoparticles should be classified based on their volume specific surface area, which is an important parameter that is often related to an increase in reactivity, and potential toxicity more so than particle size or mass. The European Commission's definition of nanomaterial now includes an exception, considering particles with a specific surface area greater than 60 m^2/cm^3 (surface area by volume) to be a nanomaterial, even if the particles do not meet the size distribution requirement (Thomas et al., 2012). Considering the surface area attribute in the definition of nanomaterials as proposed by Kreyling et al. (2010), fumed silica, as typified by Aerosil 200 [>99.8 % (SiO2): CAS-Name: Silica, amorphous, fumed (precipitated), cryst.-free; CAS-No.: 112945-52-5] with a surface area (BET) of 150 - 200 m^2/g would clearly be considered a nanomaterial.

Technical definitions notwithstanding, nanosilica is often dispersed prior to use in order to maximize the benefits of nano-sized particles. Agglomerations can be broken apart via sonication or mechanical shearing. For example, in order to maintain transparency in coatings, silica particles must be smaller than 40nm[2]. Because viscosity increases with surface area, manufacturers recommend that nanosilica needs be properly dispersed in order to impart the desired rheology control.[3] Yang, et al found that dispersed nanosilica improved the flowability of corn starch more than agglomerated silica (500-nm) (Yang et al

[1] There is a general consensus within the scientific community that a nanomaterial contains unbound, aggregated or agglomerated particles, in which at least 50% of the particles in the number size distribution have at least one external dimension in the size range of one to 100 nm (De Temmerman et al., 2012). Agglomeration is a reversible process where primary particles assemble into larger units through weak physical interactions (e.g. Van der Waals, hydrogen bonds). Aggregates, on the other hand, develop when primary particles chemically (covalently) bond to one another (Sokolov, Tschulik, Batchelor-McAuley, Jurkschat, & Compton, 2015).
[2] Hielscher Ultrasound Technology. Ultrasonic Dispersing of Silica (SiO2).
https://www.hielscher.com/size_reduction_silica_01.htm
[3] Evonik Industries. Successful use of AEROSIL fumed silica in liquid systems. Technical Information 1279.
http://www.aerosil.com/sites/lists/IM/Documents/TI-1279-Successful-use-of-AEROSIL-in-liquid-systems-EN.pdf

2005). Treatment with creatin and bile salts also cause silica particles to deagglomerate into particles with a radii of about 109 nm (McCracken et al 2013).

Due to the uncertainty of the size of silica particles that workers and consumers may be exposed to, all studies on fumed silica will be included in this hazard assessment, and the particle size noted where that information is available.

Suitable analogs or moieties of chemicals used in this assessment (CAS #'s): NA

Chemical Structure(s):  Synthetic Amorphous Silica (SAS) is comprised of silicon and oxygen atoms connected in a random tetrahedral network.

Notes related to production specific attributes[4]: Fumed silica is a synthetic amorphous silica (SAS) prepared by thermal or pyrogenic methods. The method of SAS production can influence physico-chemical properties (additional details below).

For Inorganic Chemicals and relevant particulate organics, define Properties:
The following inorganic chemical characteristics were examined in each study and reported where available as part of the assessment of study quality and relevance:

The following additional nanoparticle characteristics (Card and Magnuson, 2010) were examined in each study and reported where available as part of the assessment of study quality and relevance:

- Particle Size: Primary particles range from 2-50nm in diameter.
- Surface Area: 200-280 m^2/g (Aerosil and Cab-O-Sil)
- Structure: Amorphous
- Mobility: Hydrophilic nanosilica particles are not volatile and are slightly soluble at ambient temperature and pH.
- Bioavailability: Hydrophilic nanosilica particles are unlikely to bioaccumulate. Absorption studies indicate that the ortho-silicic acid (which amorphous silica hydrolyzes into) is a main readily bioavailable source of silicon for humans, whereas its higher polymers (including amorphous silica) are not of significant absorbability (Jugdaohsingh et al., 2000)
- Agglomeration/Aggregation: Nanosilica has a tendency to form aggregations (1-250um in diameter) which then can agglomerate into particles around 694 um in diameter (CabOSil)(De Temmerman et al., 2012)
- Surface Charge: No net surface charge
- Shape: Spherical (http://www.tandfonline.com/doi/pdf/10.1080/02786820903499462)

The following additional particle characteristics (Card and Magnuson, 2010) were examined in each study and reported where available as part of the assessment of study quality and relevance:

1. Purity
2. Surface chemistry (including composition and reactivity)
3. Whether any characterization was conducted in the relevant experimental media.

Identify Applications/Functional Uses:

[4] Note any composition or hazard attributes of the chemical product relevant to how it is manufactured. For example, certain synthetic pathways or processes result in typical contaminants, by-products or transformation products. Explain any differences between the manufactured chemical product and the GreenScreen assessment of the generic chemical by CAS #.

The scope of this GreenScreen is restricted to use of fumed silica as a food additive. Desired properties of nanosilica by the food industry include its ability to adsorb moisture to prevent caking in dry powders, reduce evaporation rates of volatile compounds to prevent flavor change, and act as a thickening, emulsification, and anti-settling agent. For example, SAS is often added to powdered ingredients such as salt, spices, and powdered milk to prevent caking and improve flow.

GreenScreen Benchmark Score and Hazard Summary Table:[5,6,7,8] Fumed amorphous nanosilica was assigned a Preliminary Benchmark Score of 2 (Use – but Search for Safer Substitutes) based on very high persistence and moderate toxicity (Group I, II) and moderate toxicity (Group I). While fumed nanosilica is an inorganic compound, persistence is still factored into the final BM score if there is related repeat toxicity or chronic toxicity concerns. The **final score, after a data gap analysis, is Unspecified, U**, due to the large number of data gaps.

	Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
Assess & Classify Hazards	Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
							S	R*	S	R*	*	*								
	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
amorphous fumed silica Inorganic 112945-52-5	*M*	*M*	DG	DG	DG	L	*H*	*M*	DG	DG	DG	DG	L	L	*H*	DG	vH	L	L	L

S indicates single exposure, R or * indicates repeated exposure. Hazard levels in ITALICS reflect low confidence. Hazard levels in BOLD reflect values based on high confidence (See Guidance)*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M and L) instead of three (i.e., H, M and L), and are based on single exposures instead of repeated exposures.

Environmental Transformation Products and Ratings:
Identify feasible and relevant environmental transformation products (i.e., dissociation products, transformation products, valence states) and/or moieties of concern

Functional Use	Life Cycle Stage	Transformation Pathway	Transformation Products	CAS #	On CPA Red List[9]?	GS™ Rating
Flow agent	Production of fumed silica powders	Stable, no known transformation	N/A	N/A	N/A	N/A
Flow agent	Consumer use – ingestion of foods and use	Hydration	Orthosilicic acid $Si(OH)_4$	10193-36-9	No	LT-U

[5] See Appendix A for a glossary of hazard endpoint acronyms
[6] See Appendix B for alternative GreenScreen Hazard Summary Table (Classification presented by exposure route)
[7] For inorganic chemicals only, see GreenScreen Guidance V1.2 Section 14.4. (Exceptions for Persistence)
[8] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen Guidance V1.2 Section 9.3.
[9] The CPA "Red List" refers to chemicals 1. flagged as Benchmark 1 using the GreenScreen™ List Translator or 2. flagged as Benchmark 1 or 2 using the GreenScreen™ List Translator and further assessed and assigned as Benchmark 1. The most recent version of the GreenScreen™ List Translator should be used.

	of cosmetics containing fumed silica					
Flow agent	End of life – aquatic and terrestrial	Hydration, dissolution, aggregation, and agglomeration	Orthosilicic acid $Si(OH)_4$	10193-36-9	No	LT-U

Introduction

This GreenScreen assesses fumed hydrophilic nanosilica, a sub-type of synthetic amorphous silica (SAS). Fumed silica is a form of SAS (CAS No 71631-86-9) further distinguished by its method of production and with its own CAS No of 112945-52-5. Studies specifically on CAS No 112945-52-5 were included in this assessment. Studies that provided the general CAS No of 7631-86-9, were included if it was stated somewhere that the silica was fumed (pyrogenic or thermally-produced) and hydrophilic. OECD testing uses NM-202 and NM-203, both hydrophilic pyrogenic silica. Studies using NM-202 and 203 (or particles equivalent to NM-202 or 203) were included in this assessment.

Although crystalline and amorphous silica share a CAS No (7631-86-9), SAS has distinct physico-chemical properties compared to crystalline silica, a known carcinogen that can increase risk for pulmonary diseases such as silicosis and chronic obstructive pulmonary disease (COPD). To date, fewer health studies have been conducted on SAS, but its prevalence in food and other consumer products poses an increased potential for exposure.

This GreenScreen assessment seeks to identify all the health hazard studies publically available. With the field of nanotechnology rapidly growing, it is important to note that this assessment only captures the current understanding of the field as of February 2016.

Hazard Classification Summary Section:

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M or L): *M (low confidence*)

Fumed nanosilica was assigned a score of Moderate (low confidence) for carcinogenicity based on pre-cancer tissues. In one study (Kolling et al. 2011) statistically significant tumor response was reported following repeat exposure intratracheal administration of amorphous silica particles in rats. The tumor response correlated with inflammation in lungs. Most genotoxicity studies were negative (no mutagenicity observed). Confidence is low because there are only limited data available, and most relevant studies were poorly reported and underpowered.

- Authoritative and Screening Lists:
  - Authoritative: IARC determined it was not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1997)

The following studies were relied upon to inform the hazard determination:

Kolling et al. (2011) published a study in rats, in which they evaluated the induction of lung tumors after repeated intratracheal instillation of crystalline silica, **amorphous silica (pyrogenic Aerosil 150, particle size 14 nm)**, carbon black and coal dust at the dose levels, which were known to induce pulmonary inflammation. The positive control, crystalline silica (1 × 3 mg), elicited the highest inflammatory reactions in lungs, fibrosis, and the highest incidence of primary lung tumors (39.6%). **After repeated instillation of soluble, ultrafine amorphous silica (30 × 0.5 mg) a statistically significant tumor**

response (9.4%) was observed. Tumor responses correlated with inflammatory responses in lungs. Overall, the results showed a relationship between tumor responses and non-cancerous effects (like inflammation and fibrosis) in rats. For assessing these data, the different dose rate and distribution of material delivered by intratracheal instillation versus inhalation have to be taken into consideration. The frequent intratracheal instillation may have added to the development of the neoplasms (Kolling et al. 2011; Reported in Scaffold 2014).

In another study applying repeated intratracheal instillation, **a statistically significant increased incidence of lung tumours (7.9%) was observed in rats at a cumulative dose of 30 mg of amorphous silica particles (14 nm)** (Morfeld et al. 2006). The tumor response was significantly lower than observed with equal dose of low-solubility particles, carbon black (14 nm; 77%), diesel exhaust particles (41%) or titanium dioxide (30 nm; 62%). No increase of lung tumor incidence was observed with amorphous silica at a cumulative dose of 15 mg. (Reported in Scaffold 2014).

Exposure to fumed nanosilica (Aerosil 200, surface area 200 m2/g and 16-40 nm) **stimulated the proliferation of human colon carcinoma cells (HT29), depending on particle size and incubation time.** After 24h, significant growth stimulus observed at a concentration of 100 ug/mL and 300 ug/mL. At 48h and 72h after exposure, significant growth stimulus was only observed at 300 ug/mL. Mitochondrial activity was not affected, and ORS induction did not occur. Total glutathione content increased in a concentration–dependent manner. Exposure seemed to interfere with glutathione biosynthesis, indicating that effects may be mediated by interference with the MAPK/ERK1/2 and Nrf2/ARE signaling pathways. (Gehrke et al., 2013) Study is reliable and provides some information supporting a possible mechanism of toxicity.

Summary: The above studies provide reliable and relevant data on the carcinogenicity of amorphous silica. In one study, a statistically significant tumor response was observed after repeated intratracheal administration of amorphous silica particles in rats. Tumor responses correlated with inflammatory responses in lungs and mechanisms related to lung overloading may have played a role in the tumour response.

<u>The following studies were not relied upon</u> for this assessment due to methodological limitations and lack of reporting details that made them impossible to interpret. They are provided here for completeness of this report only:

Schepers 1959 as cited in ECETOC, 2006:
A pyrogenic SAS described as "non-crystalline, prepared from sodium silicate using alcohol; SA 145 m^2/g " was exposed to NZW rabbits 8 h/d, 5 days/wk, for up to 27 months at doses of 0, 28.2, 134, and 360 mg/m^3. The number of animals was not stated. There was a high spontaneous death rate reported but no details are reported. Dose-related elevated right and left ventricular pressure was partly reversible during recovery. Various radiographic / electrocardiographic changes were noted, including modified lung functions and hemolytic and electrolytic disturbances. Autopsy revealed congestive cardiac failure, emphysema and chemical pneumonitis prevalent in the high dose group. Lesions disappeared after exposure ceased. (Schepers, 1959 as cited in ECETOC, 2006).

Study review: Reporting of results lacks details, so it is not clear whether lesions would progress to cancer if treatments continued. Study provided for information only.

Schepers et al., 1957 as summarized in IUCLID, 2004: There are several whole animal studies in several species (guinea pigs, Wistar rats, White rabbits) that were all submitted by Schepers et al (1957), sponsored by the manufacturer of nanosilica for the purposes of supporting its regulatory approval. All the studies are single-doses studies lasting 12 months. For each of them, ECETOC (2006) notes the test

substance concentrations were extremely variable (± 24.7 – 84.7 mg/m^3), and the findings were based on limited number of animals available for examination. Also, the duration was too short to reliable observe the absence of cancer risk. For example, EPA guidelines require a rat bioassay to be at least 24 months of exposure to the test substance. All these limitation along with the fact that the studies pre-date standardized toxicity testing protocols and GLP standards makes them inadequate for drawing conclusions regarding the safety (non-hazard) of the material.

- One group of guinea pigs (n = 19) was exposed for 12 months and another group (n = 18) for 24 months. Cab-O-Sil dust was administered by whole-body inhalation 8 h/d at 53 mg/m^3. After variable times of recovery, animals were killed and their lungs evaluated. Size distribution was reported at 1-10 μm for 85% of the dust mass in the chamber**,** as determined by an electrostatic precipitator. Interim sacrifices were performed at 2 month intervals. **Histological findings included focal pigmentation, lymph node enlargement, lung emphysema, and atelectasis but no development of nodules. The lymphoid tissue was affected only to a low extent, although medullary hyperplasia and formation of slight amounts of reticulum was prominent during the second year of exposure. No periadenitis and sinus catarrh as well as fibrosis were noted in the lymph nodes.** The maximum SiO_2 content reached approximately 8 mg per lung (12% of lung ash) and decreased to 0.6 mg/lung after cessation of exposure. Recovery was progressive with no macroscopically visible anomalies after one year. Residual sequelae of the tissue reactions were emphysema, mural fibrosis, and bronchiolar and ductile stenosis. **LOAEC was 53 mg/m^3, the only dose tested** (Schepers et al., 1957 as summarized in IUCLID, 2004).
- Fumed silica dust (reported equivalent to Cab-O-Sil and different from Aerosil in polymorphous structure) was administered to groups of male and female Wistar rats by whole-body inhalation 8h/day, 5days/wk, for 6-12 months at 53 mg/m^3. Group A consisted of 35 animals exposed for 6 months with a 12-month recovery period, and Group B was 25 animals exposed for 12 months with no recovery period. The control group was 42 animals autopsied at 6-month intervals [pre-GLP and pre-guideline]. Death rates were very high with 75% (26/35) in Group A, and 44% (11/25) in Group B. Autopsy observations included focal pigmentation, lung congestion, lymph node enlargement, lung emphysema, atelectasis (folded lungs) which accelerated from 3 to 6 months exposure. There were no changes noted in other organs of the body. Observed histological changes **included invasion of the lung lymph system by mononuclear macrophages, plasma cells and lymphocytes; vacuolization of the alveolar spaces, cytoplasm had a foamy appearance, macrophages fused to giant cells, progressive nodule formation in lung parenchyma, necrosis of nodules, progressive tendency to fibrosis, and progressive emphysematous processes around the nodules.** The steady state lung burden was approximately 1.5 mg SiO_2 after 3 months (10% of lung ash), dropping to 0.3 mg per lung post-exposure. **With 6-12 months recovery, rat lung weights, emphysema, and lymph nodes decreased in size, by 12 months most effects were completely resolved. The LOAEC was 53 mg/m^3, only dose tested** (Schepers et al., 1957 as summarized in IUCLID, 2004).
- Cab-O-Sil-equivalent fumed silica dust was administered to 10 New Zealand White rabbits 8h/d for 12 months, with 6-12 months recovery period, at 53 mg/m^3. 10 animals were exposed, with 50 controls. The size frequency distribution was reported at 1-10 μm for 85% of the dust mass, based on measurement from an electrostatic precipitator. **Progressive functional incapacitation and elevation of hematocrit levels were reported with pulmonary vascular obstruction and emphysema in the majority of animals. Blood pressure changes were observed in the majority of animals. Essential pulmonary changes were peribronchiolar cellular catarrh, mural cellular infiltration along with deposition of reticulum and some collagen, formation of peri-vascular cellular nodules, ductal stenosis and emphysem**a. During recovery, cellular reactions and emphysema regressed but alveolar mural collagen persisted. **The LOAEC was 53 mg/m^3, the only dose tested** (Schepers, 1957 as summarized in IUCLID, 2004).

Groth et al (1981) are a series of study reports conducted to support the regulatory approval of nanosilica. In all cases, there is insufficient reporting detail to determine study reliability with any confidence. Studies are provided here for information only.

- Hartley guinea pigs were exposed to Cab-O-Sil 6 h/day, 5 days/week for 12 months at 0 or 6.9 mg/m^3 **respirable** fraction. Small numbers of macrophage cell aggregates were present (Groth et al., 1981 as cited in ECETOC, 2006). [No further details provided]..
- Cynomolgus monkeys were exposed to Cab-O-Sil 5.5 h/d, 5 days/wk, for 13 months at 0, 6, or 9 mg/m^3 **respirable** fraction (**approximately 46% < 4.7 µm**). Large numbers of macrophage cell aggregates were present. **Significant collagen and early nodular fibrosis was evident.** FVC, IC, TLC, CL, FEF75, FEF90 decreased and RL and CV increased. A LOAEC of 15 mg/m3, corresponding to 6-9 mg/m3 of respiraple particles, was reported, based on nodular fibrosis in the lungs and decreased lung function (Groth et al., 1981 cited in ECETOC, 2006; Reported in Scaffold 2014).
- Sprague-Dawley rats were exposed to Cab-O-Sil at 0, or 6.9 mg/m^3 respirable fraction, 6 h/day, 5 days/week for 12 months. Small numbers of macrophage cell aggregates were present. **Interstitial fibrosis was observed in test and control animals** (Groth et al., 1981 as cited in ECETOC, 2006). [No further details provided]..

Mutagenicity/Genotoxicity (M) Score (H, M or L): *M (low confidence)*
Fumed nanosilica was assigned a score of *Moderate (low confidence*) for mutagenicity based on multiple positive animal and human cell assays as well as one whole animal study. Confidence is low because most of the mutagenicity/genotoxicity studies on amorphous silica particles show no (negative) effects.

- Authoritative and Screening Lists: None

In vitro

- Chromosome aberration assays
  - Undifferentiated human cell line Caco-2 were exposed to 9.50256 ug/mL. **Chromosomal damage was observed in 2 out of 3 experiments, suggesting fumed nanosilica is genotoxic** at the highest *in vitro* doses. (OECD 487) (Norppa 2013 via OECD 2015)
  - Cab-O-Sil EH5 was tested in a chromosome aberration assay using Chinese hamster ovary cells. Test substance concentrations were 19-300 µL/mL without S9, and 250-1,000 µL/mL with S9. **Results were negative with and without metabolic activation**. Toxicity was reported at 92% without S9, and 63% with S9 (Cabot, 1990 unpublished report as cited in IUCLID, 2004). [The concentrations at which cytotoxicity was observed was not reported].
  - Chinese hamster ovary chromosome aberration assay was **negative** both with and without metabolic activation. Test concentrations ranged from 38-1000 ug/L of fumed nanosilica in DMSO. (OECD 473) (Putman 1990 via OECD 2015)
- Mammalian cell mutation assays
  - NM 202 and 203 evaluated for genotoxicity in human lymphocytes up to a concentration of 1250 ug/mL. Median aggregate size was 74 and 86 nm, respectively, and specific surface area was 200 and 226 m2/mg. **Exposure did not induce micronuclei** in human lymphocytes. (Tavares et al., 2014)
  - Human bronchial cells were exposed to 32-128 ug/mL fumed nanosilica. Exposure **did not induce aneugenic or clastogenic damage** in the cytokinesis-block micronucleus assay (OECD 487)(Norppa 2013 via OECD 2015)
  - Human alveolar epithelial cells were exposed to 32-512 ug/mL fumed nanosilica. There was an increase in the frequency of binucleated cells, indicating that fumed nanosilica **induces aneugenic/clastogenic damage in epithelial cells with the cytokinesis-block micronucleus assay**. (OECD 487) (Norppa 2013 via OECD 2015)
  - Human bronchial cells were exposed to 4-64 ug/mL fumed nanosilica. **Aneugenic or clastogenic**

damage was not induced. (OECD 487) (Norppa 2013 via OECD 2015)
  - Human primary peripheral blood lymphocytes were exposed to 64-1260 ug/mL. **Aneugenic or clastogenic damage was not induced**. (OECD 487) (Norppa 2013 via OECD 2015)
  - Balb/3T3 mouse fibroblasts were exposed to fumed nanosilica (NM 203 with mean particle diameter of 185 nm) at concentrations of 1, 10, 100 ug/L. Toxic effects were not observed after 72h exposure. Exposure also did not induce the formation of type-III foci, which indicates it is not a carcinogen. **Micronucleus test was also negative** (mutagenicity) (Uboldi et al., 2012)
  - Rat hepatocytes were exposed to 10, 30, 100, 300, or 1000 ug/mL fumed nanosilica in DMSO. **Results were negative**, both with and without activation. (OECD 482) (Curren 1989 via OECD 2015)
  - Mouse lymphoma cells were exposed to 32-5000 ug/mL of fumed nanosilica. **All results were negative.** (OECD 476) (Norppa 2013 via OECD 2015)
  - Cab-O-Sil EH5 was tested in a HGPRT assay using Chinese hamster ovary cells. Test substance concentrations were 10-250 μg/mL without S9, and 100-500 ug/mL with S9. **Results were negative** with and without activation. Cytotoxicity was not reported (Cabot, 1990 unpublished report as cited in IUCLID, 2004).
- DNA damage and repair
  - Single cell gel/comet assay in undifferentiated human intestinal Caco-2 cells found that nanosilica **induced equivocal DNA strand breaks in A 549 cells at both 3 h and 24 h at the tested dose** (2.56-512 ug/mL) with the alkaline comet assay. Fumed nanosilica also induced oxidative DNA damage at 3 h but not at 24 h with the FpG-modified comet assay. (Norppa 2013 via OECD 2015)
  - Fumed nanosilica (20nm) induced apoptosis in Chinese hamster lung fibroblasts (V79 cells) - at the highest concentration of 100 ug/cm^2, caspase activity increased 4.6 fold. DNA damage was assessed via an FPG-modified comet assay**. After 24h of exposure, comet assays were positive at all concentrations in FPG-modified assays and at the highest concentration without FPG.** No significant ROS induction or micronucleus formation was observed. (Guichard et al., 2015)
  - Fumed nanosilica **did not induce DNA strand breaks in 16-HBE cells** at both 3 h and 24 h at the tested dose with the alkaline comet assay. SAS NM-202 does not induce oxidative DNA damage at both 3 h and 24 h at the tested dose with the FpG-modified alkaline comet assay (Norppa 2013 via OECD 2015)
- Bacterial reverse mutation assays
  - Cab-O-Sil EH-5 tested **negative in a reverse mutation assay** in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with metabolic activation up to 10,000 μg/plate. Cytotoxicity was not observed (Cabot, 1989 as cited in ECETOC, 2006).
  - Cab-O-Sil EH-5 **tested negative** in a reverse mutation assay in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation up to 5,000 μg/plate. Cytotoxicity was not observed (Cabot, 1978 as cited in ECETOC, 2006).
- Unscheduled DNA synthesis
  - Cab-O-Sil EH5 was tested in an Unscheduled DNA Synthesis assay using primary rat hepatocytes. There were 5 test substance concentrations ranging from 0.3-1,000 μg/mL, and an exogenous metabolic activation system was not employed. **Results were negative**, and cytotoxicity was approximately 50% in at 260-500 μg/mL (Cabot, 1989 unpublished report as cited in IUCLID, 2004).
- *In vivo*
  - SD rats exposed via gavage three times (at 0, 24, and 45 h) to 5, 10 mg/kg fumed nanosilica. Sampling occurred 3 hours following the last administration. **Micronucleus assay in colon found a significant increase in the frequency of micronucleated cells observed in rats treated at the lowest dose**. (OECD Guideline 474) (Fessard 2012 via OECD 2015).
  - SD rats were exposed via gavage to 5, 10, or 20 mg/kg/day fumed nanosilica (NM 202 or 203) for

three days. DNA strand breaks and oxidative DNA damage were investigated in seven tissues (blood, bone marrow from femur, liver, spleen, kidney, duodenum, and colon) with the alkaline and the (Fpg)-modified comet assays, respectively. The results showed neither obvious DNA strand breaks nor oxidative damage with the comet assay, irrespective of the dose and the organ investigated. Similarly, no increases in chromosome damage in bone marrow or lipid peroxidation in plasma were detected. However, although the response was **not dose-dependent, a weak increase in the percentage of micronucleated cells was observed in the colon of rats treated with the two pyrogenic SAS at the lowest dose** (5 mg/kg b.w./day). (Tarantini et al., 2015)

- A 90-day subchronic inhalation study exposed Fischer 344 rats to 50 mg/m3 of fumed nanosilica (Aerosil 200). A positive control group was exposed to crystalline silica. Testing included immunohistochemistry for DNA damage, and mutagenesis in alveolar epithelial cells. A HPRT gene-mutation assay using alveolar type-II cells isolated from BAL **did not show increased mutation frequency compared to the control**. Viability in lung cell isolates was not impaired. (Johnston, 2000).
- In a single cell gel/comet assay, SD rats were exposed three times via gavage to 5, 10, or 20 mg/kg bw/d fumed nanosilica. Blood, bone marrow, liver, kidney, spleen, colon, and duodenum were examined. **No DNA damage was observed**. (Fessard 2012 via OECD 2015)
- In a single cell gel/comet assay, SD rats were exposed intratracheally three times (at 0, 24, 45 hours) to 3,6, or 12 mg/kg bw fumed nanosilica. Sampling occurred 3 hours following the last administration. BAL cells, lung, blood, liver, spleen, kidney, and bone marrow were examined. **No DNA damage was observed**. (Fessard 2012 via OECD 2015)
- SD rats exposed via gavage (at 0, 24, 45 hours) to 5, 10, 20 mg/kg bw fumed nanosilica. Sampling occurred 3 hours following the last administration. Micronucleus assay was conducted on bone marrow. PCE: NCE ratio did not significantly decrease in treated groups, suggesting that **fumed nanosilica is not genotoxic** in rats following short-term oral exposure. (OECD 474) (Fessard 2012 via OECD 2015)

Reproductive Toxicity (R) Score (H, M, or L): DG
Fumed nanosilica was assigned a score of Data Gap for reproductive toxicity. There are limited data on reproductive effects.

- Authoritative and Screening Lists**:** none

In a study of Wister rats, no effects on pregnancy were reported in rats exposed orally via feed to 500 mg/kg bw/day fumed nanosilica (Aerosil R972) for 8 to 17 weeks before mating with similarly exposed males. Reported NOAEL for toxicity was 500 mg/kg/day. (Lewinson et al 1994; Degussa 1963; OECD 2015)

Study review: Low sample size (only 1 male and 5 females), single dose. The study design is too limited to draw conclusions with confidence; single dose studies that fail to find an effect are uninformative because there is no way to know if the dosing was appropriate, or if the test system worked. For this reason the study is inadequate to support a hazard score for this endpoint.

Note that although there are limited data on fertility and reproductive effects of amorphous silica, no adverse effects to reproductive organs have been reported in the repeat dose toxicity tests with inhalation, oral or dermal exposures.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M or L): DG

Fumed nanosilica was assigned a score of Data Gap for developmental toxicity.

- Authoritative and Screening Lists**:** none

In a study of Wister rats, no effects on pregnancy were reported in rats exposed orally via feed to 500 mg/kg bw/day fumed nanosilica (Aerosil R972) for 8 to 17 weeks before mating with similarly exposed males. No behavioral, developmental, or structural abnormalities were noted in pups during lactation. Reported NOAEL for toxicity was 500 mg/kg/day. (Lewinson et al 1994; Degussa 1963; OECD 2015)

Study review: Low sample size (only 1 male and 5 females), single dose. The study design is too limited to draw conclusions with confidence; single dose studies that fail to find an effect are uninformative because there is no way to know if the dosing was appropriate, or if the test system worked. For this reason the study is inadequate to support a hazard score for this endpoint.

Yamashita et al. (2011) reported structural and functional defects in the placenta, increased resorption rate and decreased size of the foetus in pregnant BALB/c mice exposed intravenously (tail vein injection) to amorphous silica nanoparticles of 70 nm at two days (Gestational Day 16 and 17) during the pregnancy at a maternally toxic dose of 40 mg/kg bw/day. Due to the applied exposure route and the observed maternal toxicity (decreased bodyweight of the dams), no firm conclusions can be drawn from this study.(Reported in Scaffold 2014).

Although micronized silica gel is outside the scope of this report, it may be helpful to the reader to know that the developmental toxicity of micronized silica gel (Syloid 244) has been studied in rats, mice, rabbits and hamsters exposed orally during gestation (FDA 1973; ECETOC 2006). No embryotoxicity was observed, and the weight and number of external, visceral or skeletal abnormalities in the pups did not differ from controls. No maternal toxicity was seen even at the highest dose of 1600 mg/kg bw/day.(Reported in Scaffold 2014).

Endocrine Activity (E) Score (H, M or L): DG

Fumed nanosilica was assigned a score of Data Gap for endocrine activity.

- Authoritative and Screening Lists**:** none
- No information was found in any additional sources.

<u>Group II and II* Human Health Effects (Group II and II* Human)</u>

Note: Group II and Group II endpoints are distinguished in the v 1.2 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. When classifying hazard for Systemic Toxicity/Organ Effects and Neurotoxicity endpoints, repeated exposure results are required and preferred. Lacking repeated exposure results in a data gap. Lacking single exposure data does not result in a data gap when repeated exposure data are present (shade out the cell in the hazard table and make a note). If data are available for both single and repeated exposures, then the more conservative value is used.*

Acute Mammalian Toxicity (AT) Group II Score (vH, H, M or L): L

Fumed nanosilica was assigned a score of **Low** (high confidence) for acute mammalian toxicity based on high LC50 values obtained from animal studies. The acute toxicity of synthetic amorphous silicas has been extensively studied. The most conservative value for oral LC50 was still greater than 3,160 mg/kg bw, which is equivalent to a GS hazard score of low.

- Authoritative and Screening Lists**:** none

Acute oral toxicity studies:

- Oral LC50 for SD rats >3300 mg/kg bw, the highest dose tested (OECD 401). Surface area and particle size not provided. Aerosil 200. (Degussa 1977 via OECD 2015)
- Oral LC50 for mice > 3160 mg/kg bw, the highest dose tested (OECD 401). Surface area and particle size not provided. (Powers 1964 via OECD 2015)
- Oral LC50 for SD rats > 5,000 mg/kg bw, the only dose tested. (Cab-O-Sil M5) (Cabot 1981 via ECETOC)

In acute inhalation studies the highest technically achievable concentrations without causing death by suffocation from the extremely high number of respirable particles were below LC50:

- In the acute inhalation toxicity studies with hydrophilic silica reviewed in ECETOC (2006), no mortality or other signs of toxicity were observed in rats exposed to 140 mg/m3 of pyrogenic silica (respirable; Aerosil 200) for 4 hours. 4h LC50 for Wistar Rats >= 0.14 mg/L air (OECD 403). (Aerosil 200, Surface area 200 m2/g, 56% of particles <5um) (Degussa 1983 via OECD 2015)
- 4h LC50 for SD rats >2.08 mg/L air (OECD 403). MMAD= 0.76um (Cabot 1981 via ECETOC))
- No mortality occurred in rats exposed 4 hours to 2,080 mg/m3 (MMAD 0.27 um; Cab-OSil) or 1 hour to 191,300 or 207,000 mg/m3 (total dust) of pyrogenic silica (Cab-O-Sil). 1h LC50 for albino rats >2.07 and 1.91 mg/L air for Cab-O-Sil EH5 and M5, respectively (Cabot 1972 via ECETOC)
- 4h LC50 for SD rats >58.8 mg/L air (OECD 403). MMAD= 0.76um. (Toxigenics 1981 via OECD 2015)
- In rats exposed 4 hours to another type of hydrophobic pyrogenic silica (MMAD 1.2 μm; Cab-O-Sil TS610), the mortality was 0% at 210 mg/m3, 70% at 540 mg/m3 and at 100% at 2,100 mg/m3 (ECETOC 2006). The mortality observed in these studies is associated with suffocation caused by the extremely high number of respirable particles generated to meet the required test atmosphere. (Reported in Scaffold 2014)

In acute dermal toxicity studies, only slight erythema with intact skin and slight erythema and edema with abraded skin was observed in rabbits exposed to hydrophilic precipitated silica and silica gel at doses up to 5,000 mg/kg bw for 24 hours (ECETOC 2006). In the acute oral toxicity studies of hydrophilic and hydrophobic synthetic amorphous silicas, no signs of toxicity were observed at single doses of up to 5,000 mg/kg bw (ECETOC 2006). (Reported in Scaffold 2014)

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)
(ST-single) Group II Score (single dose: vH, H, M or L); *H (low confidence)*
Fumed nanosilica was assigned a score of *High* (low confidence) because of mechanistic indicators of inflammation and immunotoxicity. Confidence is low because the data is from cellular assays. There are some reports of toxicity from intratracheal/intravenous routes of exposure, but this is not an approved pathway for GreenScreen.

- Authoritative and Screening Lists**:** None
- Inhalation
  - SD Rats were exposed via inhalation for 4 hours to 58.8mg/L fumed nanosilica (MMAD 0.76 um). Discoloration of the lung in one test rat was observed**. Clinical signs included nasal discharge, crusty eyes, alopecia, and crusty nose**. (Toxigenics 1981 via OECD 2015)
  - During the 1-hour exposure to Cab-O-Sil EH5 and M5 at nominal concentrations of 193.3 and 207.0 mg/L, respectively, initial vigorous cleansing activity and **subsequent hypoactivity, abdominal respiration, gasping nasal exudation and closed eyes were observed.** During the first 2 days after exposure all rats had dried crust-like material around the nose and mouth, and the fur appeared chalky to the touch. These conditions subsequently disappeared and all rats

appeared normal throughout the remainder of the 14-day observation period (Cabot 1972 via ECETOC).

- Intratracheal/intravenous
  - SD Rats were treated with three consecutive intratracheal instillations of 3, 6, or 12 mg/kg of fumed nanosilica (NM 202 or 203) at 48, 24, and 3 hrs prior to tissue collection (cumulative doses of 9, 18, and 36 mg/kg). Although all of the fumed nanosilica caused increased **dose-dependent changes in lung inflammation as demonstrated by BALF neutrophilia**, they did not induce any significant DNA damage. Rats then received three consecutive intravenous injections of 5, 10, or 20 mg/kg of NM 203 at 48, 24, and 3 hrs prior to tissue collection. Despite the **hepatotoxicity, thrombocytopenia, and even animal death** induced, no significant increase in DNA damage or micronucleus frequency was observed in fumed nanosilica-exposed animals. (Guichard et al., 2015)
  - Single dose study that compared SiO_2 with Si nanoparticles. Single intravenous administration of 11–15 nm fumed nanosilica to SD rats at a dose of 7 mg/kg resulted in granuloma formation and mononuclear infiltration in the liver and spleen that persisted for 60 days post-injection.(Ivanov et al., 2012)
- *In vitro* assays
  - A549 human alveolar epithelial cells were exposed to fumed nanosilica (diameter 10 nm) (Aerosil 200 NPs). The no observed transcriptomic adverse effect level was 1 ug/cm^2, and the lowest observed transcriptional effect level was set at 1.5 ug/cm^2. **At 3.0 µg/cm^2, many inflammatory mediators were upregulated and the coagulation system pathway was triggered. Lastly, at 6.0 µg/cm^2, oxidative stress was initiated.** (Pisani et al., 2015)
  - Human bronchial epithelial cell lines (BEAS-2b), mouse macrophages cell lines, and human acute monocytic leukemia cells) were exposed to solutions of 0.4, 0.8, 1.6, 3.2, 6.3, 12.5, 25, 50, 100 and 200 µg/mL fumed nanosilica particles (Aerosil, 16nm). Cell death, cell viability and intracellular ATP levels were determined by LDH, MTS and ATP assays. **The study finds a positive correlation of fumed nanosilica toxicity with hydroxyl concentration and its potential to generate reactive oxygen species (ROS) and cause red blood cell hemolysis** (Zhang et al., 2012).
  - Human lung A549 cells were exposed to fumed nanosilica (7, 12, 14 nm with surface area of 395 and 200 mg2/g) ranging in concentration from 25-100 ug/mL for 48 hours. Elevation of ROS in treated cells was observed at the highest exposure concentration as early as 4h and at lower concentrations by 12h. However, the corresponding toxicity was not so significant as compared with the low concentrations as assessed by the LDH and MTT assays, suggesting that ROS increase was not the sole cause of cytotoxicity in A549 cells. **Non-dose dependent LDH leakage (membrane integrity) was also observed.** The **early cytotoxicity** could be due to the interaction of NPs with cells, leading to oxidative stress, damage to cell membrane, and mitochondria dysfunction; the later cytotoxicity could be mainly due to sedimentation of NP aggregates, leading to further mitochondrial dysfunction and loss of cell viability. (Irfan et al., 2014)
  - Fumed nanosilica (Aerosil 200- about 295nm in solution) **induced IL-1β release in a concentration-dependent manner in LPS-primed RAW264.7 macrophages and primary rat lung macrophages after a 6 hour exposure period**. Cells were exposed to concentrations ranging from 5-100ug/L. Cytotoxicity was measured by LDH and was less than 2.5-fold. (Sandberg et al., 2012)
  - Fumed nanosilica (Aerosil 200) was screened for toxic effects in A549 lung epithelial cells and RAW264.7 macrophages in the presence and absence of fetal calf serum. Cells were treated by 200 ug/mL. **Acute toxicity, measured using AlamarBlue and the LDH assay, occurred after exposure to fumed nanosilica.** Toxicity was suppressed in the presence of the calf serum. ROS generation was not observed**. Exposure increased the expression levels of HO-1 and gGCLC (anti-oxidative and pro-inflammatory)** in the absence of serum specifically in macrophages. The pre-coating of silicaNPs with serum proteins completely abrogated their toxicity and

inflammatory potential suggesting that the NP surface and its interaction with sensitive biomolecules is key for the observed biological effects. (Panas et al., 2013)

(ST-repeat) Group II* Score (repeated dose: H, M, L): *M*
Fumed nanosilica was assigned a score of *Moderate* (low confidence) for systemic toxicity/organ effects based on repeated exposure based on a single animal study with exposure via inhalation. Confidence is low because the study was pre-GLP, outdated, and have poor statistical power. The most conservative LOAEL observed was 0.03 mg/L via inhalation of fumed nanosilica dust. The LOAELs for inhalation of fumed nanosilica dust ranged from 0.03 mg/L to 0.06mg/L, which is equivalent to GHS category 2 or GS Moderate.

- Authoritative and Screening Lists**:** None
- Oral
  - The European Food Safety Authority (EFSA) reviewed the use of silica in food additives and concluded that an intake of up to 1500 mg/day of silicon dioxide as food supplements is not a safety concern (EFSA 2009).
  - Male and female Charles River rats were exposed to Cab-O-Sil fluffy via feed for 13 weeks at 1, 3, or 5% in the diet (estimated 700, 2,100, and 3,500 mg/kg-d). Macroscopic and microscopic examinations were performed. There were no clinical signs of toxicity, no changes in body weight, food consumption or survival. No gross pathological or histopathological findings were attributable to exposure. There was no appreciable accumulation of SiO_2 in the liver, kidney, spleen, blood, or urine compared to controls as measured at 45 and 90 days. The **NOAEL was reported at 3,500 mg/kg-d** (Cabot, 1958 unpublished report as summarized in IUCLID, 2004).
  - Subchronic (28-day) oral toxicity study exposed rats to either SAS (fumed nanosilica with particle size of 7 nm and specific surface area of 380 m2/g) or NM 202 (fumed nanosilica with particle size of 10-25nm with specific surface area of 200 m2/g). Rats were orally exposed (either via feed or chocolate milk) to 100, 1000 or 25000 mg/kg/day fumed nanosilica or 100, 500, or 1000 mg/kg bw/day NM 202. For the highest doses, rats were exposed for 84 days. After 84 days, SAS accumulated in the spleen and exposure to NM 202 **resulted in a significantly increased incidence of liver** fibrosis**. NM 202 LOAEL is 1000 mg/kg/day** (van der Zande et al., 2014)
- *Dermal*
  - Albino rabbits were exposed for 3 weeks to 0, 5000, or 10000 mg/kg bw/day fumed nanosilica (Cab-O-Sil fluffy- similar or same to M5). No evidence of systemic toxicity or of gross or microscopic pathology. (Cabot 1958 via ECETOC 2006)

- *Inhalation*
  - Wistar rats were exposed via inhalation to 0.001, 0.005, 0.025 mg/L fumed nanosilica (Cab-O-Sil M5) for 5 days and monitored for 3 months. MMAD was 1-4 um in the test atmosphere. In addition to negative controls, positive controls were exposed to 0.025 mg/L crystalline silica. **At the highest exposure, elevated biomarkers of cytotoxicity in bronchoalveolar lavage fluid, increased lung and tracheobronchial lymph node weight, and histopathological lung changes were observed 1-day post exposure. Effects were transient** and no longer observed at the end of the 3 month follow-up period, unlike the positive control, where effects became more severe with time. NOAEC of 1 mg/m3 and LOAEC of 5 mg/m3. (Arts et al 2007)
  - Fumed silica dust (reported equivalent to Cab-O-Sil and different from Aerosil in polymorphous structure) was administered to groups of male and female Wistar rats by whole-body inhalation 8h/day, 5 days/wk, for 6-12 months at 0.053 mg/L. Group A consisted of 35 animals exposed for 6 months with a 12-month recovery period, and Group B was 25 animals exposed for 12 months

with no recovery period. The control group was 42 animals autopsied at 6-month intervals [pre-GLP and pre-guideline]. **Death rates were very high with 75% (26/35) in Group A, and 44% (11/25) in Group B.** Autopsy observations included focal pigmentation, lung congestion, lymph node enlargement, lung emphysema, atelectasis (folded lungs) which accelerated from 3 to 6 months exposure. There were no changes noted in other organs of the body. Observed histological changes **included invasion of the lung lymph system by mononuclear macrophages, plasma cells and lymphocytes; vacuolization of the alveolar spaces, cytoplasm had a foamy appearance, macrophages fused to giant cells, progressive nodule formation in lung parenchyma, necrosis of nodules, progressive tendency to fibrosis, and progressive emphysematous processes around the nodules.** The steady state lung burden was approximately 1.5 mg SiO2 after 3 months (10% of lung ash), dropping to 0.3 mg per lung post-exposure. With 6-12 months recovery, rat lung weights, emphysema, and lymph nodes decreased in size, by 12 months most effects were completely resolved. **The LOAEC was 0.053 mg/L, only dose tested** (Schepers et al., 1957 as summarized in IUCLID, 2004).
Study review: ECETOC (2006) notes the test substance concentrations were extremely variable (± 24.7 – 84.7 mg/m^3), and the findings were based on limited number of animals available for examination.

- 12 month inhalation study exposed SD rats to **0.030 mg/L** of fumed nanosilica with an aerodynamic diameter <= 7 um. Exposure frequency had to be decreased from 5x/week to 2-3x/week due to **supporative bronchitis and severe inflammation.** In the lungs, small dust foci were observed under the pulmonary pleura, and mediastinal lymph nodes were enlarged. **No NOAEC was determined** (Klosterkoetter, 1969 via OECD 2015)
- 90-day subchronic inhalation study exposed Wistar rats to 1.3, 5.9, or 31 mg/m3 of fumed nanosilica (Aerosil 200) with surface area equivalent to 151 m^2/g and a size range of 1-120 um. Hematology, clinical chemistry, urinalysis, body weight measurement were conducted during the study, and gross pathology and histopathology results were collected. No data was collected for neurobehavioural changes, opthalmoscopic changes, or food and water consumption. **The reported NOAEC was 0.013 mg/L (1.3 mg/m3), based on slight and transient pulmonary response. LOAEC was 0.059 mg/L (6 mg/m3) due to elevated neutrophilic leukocytes.** There was not an observable dose-response and effects were transient. Mean increase in relative weight of lungs also occurred- lungs were swollen and spotted. Mediastinal lymph nodes were enlarged. Silica was detected in lungs in small and non-dose dependent amounts. **Other effects on the lungs included accumulation of alveolar macrophages and granular material, cellular debris, polymorphonuclear leucocytes, increased septal cellularity, as well as alveolar bronchialisation, focal interstitial fibrosis, cholesterol clefts and granuloma-like lesions in the lung.** The lesions were more severe in males and while there was improvement following after termination of exposure, the lesions were not completely healed within the 13 week monitoring period. **Interstitial fibrosis appeared after the exposure period during the 13 week monitoring period**. (OECD 413) (Reuzel et al 1991 via ECETOC 2006)
- A 90-day subchronic inhalation study exposed Fischer 344 rats to 0.050 mg/L of fumed nanosilica (Aerosil 200). Positive control group was exposed to crystalline silica. Testing included cellular and biochemical Bronchoalveolar Lavage Fluid Analysis (BLA) on inflammatory markers, histopathology, inflammatory cytokine gene expression, immunohistochemistry for DNA damage, and mutagenesis in alveolar epithelial cells. Changes in bronchio-alveolar lavage (BAL) fluid content compared to controls included increases in total cells, neutrophils, protein, lactate dehydrogenase (LDH) and β-glucuronidase. The percentage of alveolar macrophages decreased while the percentage of lymphocytes and viable cells remained unchanged compared to controls. Significant increase in terminal transferase dUTP nick-end-labelling (TUNEL)-staining was detected in macrophages and terminal bronchiolar epithelial cells indicating cytotoxicity. All parameters returned to control level after 8 months recovery period (Johnston, 2000).

- Male Wistar rats were exposed via inhalation to 1.3, 5.3, and 25.3 mg/m3 fumed nanosilica. MMAD= 1.2 or 2.2-3.5 um. Monitoring continued for 1 or 3 months. Gross pathology was conducted, as well as histopathology for kidney, lung, and lymph nodes. LOAEC was 5.41 mg/m3 due to significant body weight loss, significant increase in lung weight, hypertrophy of brochiolar epithelium, alveolar macrophage accumulation. Accumulation of macrophages were still present following 3 months of recovery, although lesions were not present. Dose-related stimulation of neutrophil granulocytes were observed, and were still significant after 1 month of recovery. Increase respiration was observed but disappeared following one month. NOEC was 1.39 mg/m3 based on an absence of a pulmonary response. (OECD 412) (Degussa 2003 via OECD 2015)
- Groth et al. (1981) reported early nodular fibrosis in the lungs and effects on the lung function in monkeys exposed for 13 or 18 months to hydrophilic pyrogenic silica, with a LOAEC of 15 mg/m3, corresponding to approximately 6–9 mg/m3 of respirable particles.(Reported in Scaffold 2014)

Neurotoxicity (N)
(N-single) Group II Score (single dose: vH, H, M or L): DG
Fumed nanosilica was assigned a score of Data Gap for neurotoxicity based on single exposure due to lack of neurotoxicity observed in single exposure studies. No single exposure studies included a neurobehavioral examination.

- Authoritative and Screening Lists**:** None
- No information was found in any additional sources.

(N-repeat) Group II* Score (repeated dose: H, M, L): DG
Fumed nanosilica was assigned a score of Data Gap for neurotoxicity based on repeated exposure due to lack of neurotoxicity observed in repeated exposure studies. No repeated exposure studies included a neurobehavioral examination.

- Authoritative and Screening Lists**:** None
- No information was found in any additional sources.

Skin Sensitization (SnS) Group II* Score (H, M or L): DG
Fumed nanosilica was assigned a score of Data Gap for skin sensitization.

- Authoritative and Screening Lists**:** none
- No information was found in any additional sources.

Respiratory Sensitization (SnR) Group II* Score (H, M or L): M
Fumed nanosilica was assigned a score of M (high confidence) for respiratory sensitization, based on evidence in a whole animal study showing elevated measurements of specific immunological parameters, demonstrating an immune response. Confidence is high because the hazard score is based on data from whole animals.

- Authoritative and Screening Lists**:** None

- Fumed nanosilica can aggravate certain aspects of TMA-induced respiratory allergy**.** Exposure to fumed nanosilica alone in Brown Norway rats at 27 mg/m3 for 6 days induced transient changes in breathing parameters, as well as nasal and alveolar inflammation with neutrophils and macrophages. The second exposure to trimetallic anhydride in challenged rats (resembling allergic individuals)

following nanosilica increased lymphocytes in bronchoalveolar lavage fluid. (Arts et al., 2007)

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M or L): Low
Fumed nanosilica was assigned a score of **Low** (high confidence) for skin irritation/corrosivity based on negative animal studies. Confidence is high because it is based on data from three whole animal studies (in rabbits) that were considered reliable.

- Authoritative and Screening Lists**:** none
- NZ rabbits exposed to 0.5 g fumed nanosilica (Aerosil 200) for 24h did not show signs of irritation (received a Draize score of 0 out of 8). (OECD 404) (Degussa 1978 via OECD 2015)
- NZ rabbits exposed to 0.5 g fumed nanosilica (Cab-O-Sil M5) for 24h received a score of 1 out of 8 (very slight erythema) on abraded sites, and no signs of irritation on intact sites. (Cabot 1978 via ECETOC)
- NZ rabbits exposed to 0.5 g fumed nanosilica (Cab-O-Sil M5) for 24h received a score of 1 (very slight erythema) at an intact site and very slight to well-defined erythema on abraded sites. Effects were transient, and no signs of irritation were observed at 72h. (Cabot 1981 via ECETOC)

Summary: Overall, three skin irritation studies reported no or slight signs of irritation or erythema in rabbits exposed dermally to hydrophilic pyrogenic (and also, but out of scope of this assessment, to precipitated silica, silica gel or hydrophobic pyrogenic or precipitated silica) for 24 hours. Studies were well-conducted according to standardized methods and results are considered reliable (ECETOC 2006).

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M or L): Low
Fumed nanosilica was assigned a score of **Low** (high confidence) for eye irritation/corrosivity based on a negative animal studies. Confidence is high because it is based on data from three whole animal studies in rabbits that are considered reliable.

- Authoritative and Screening Lists**:** none
- NZ rabbits exposed to 100 mg of fumed nanosilica (Aerosil 200) did not exhibit any irritating response. (Degussa 1978 via OECD)
- NZ rabbits exposed to 3.5 mg of fumed nanosilica (Cab-O-Sil M5) exhibited slight conjunctival erythema or chemosis at 24, 48 and 72 h (mean score 0.6 and 0.1, respectively); transient corneal opacity observed in 2/6 animals at 4h (Cabot 1958 via ECETOC)
- NZ rabbits were exposed to 100 mg of fumed nanosilica (Cab-O-Sil M5). 3/9 had eyes rinsed at 30s. No signs of irritation were observed in washed eyes. Unwashed eyes had a mean score of 0.15, with very slight conjunctival erythema up to 48h (Cabot 1981 via ECETOC)

Summary: In three eye irritation studies, no or very slight reversible irritation was seen in the eyes of rabbits exposed to hydrophilic pyrogenic (and also, but out of scope of this assessment, to or precipitated silica, silica gel or hydrophobic pyrogenic or precipitated silica) for 24 hours (ECETOC 2006).

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M or L): H
Fumed nanosilica was assigned a score of High (high confidence) based on the most conservative (i.e. protective) study, reporting mortality in response to exposures as low as 1 mg/L. Confidence is high because the hazard score is based on data from two species.

- Authoritative and Screening Lists**:** None
- 96h LC50 for Danio Rerio (OECD 203) > 10,000 mg /L, the highest tested concentration (Degussa,

1992 via OECD 2015).
- 24h EL50 for Daphnia Magna (OECD 202) > 1,000 mg/L, the highest tested concentration (Degussa, 1991 via OECD 2015)
- Daphnia: An increase in the mortality rate was observed after a 96 h-treatment with fumed silica (mortality rate 10 ± 8.16%). Surface area 349.71 m2/g and primary particle size of 7 nm. Only single dose tested (1 mg/L). (Lee et al, 2009)
- C. Riparius: An increase in the mortality rate was observed after a 96 h-treatment with fumed silica (mortality rate 10 ± 8.16%). Surface area 349.71 m2/g and primary particle size of 7 nm. Only single dose tested (1 mg/L). (Lee et al., 2009)

Summary: Acute toxicity to aquatic organisms is demonstrated in two species – Daphnia (a water flea), and C. Riparius (a midge) – using commonly accepted study designs. Results are considered reliable.

Chronic Aquatic Toxicity (CA) Score (vH, H, M or L): DG
Fumed nanosilica was assigned a score of Data Gap for chronic aquatic toxicity.
- Authoritative and Screening Lists**:** none
- No information was found in any additional sources.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vH
Fumed nanosilica was assigned a score of **very High** (high confidence) for persistence because it is inorganic, not hydrolyzable and will not biodegrade. Confidence is high because the hazard score is based on the known physical-chemical properties of the material.
- Authoritative and Screening Lists**:** none
- The Si-O bond is highly stable and no photo- or chemical degradation is expected. Furthermore, SAS are not volatile and have no lipophilic character, therefore they are expected to settle into soils/sediments and have low water solubility, similar to naturally occurring silicon dioxide and other forms of inorganic matter (OECD SIDS, 2004).

Bioaccumulation (B) Score (vH, H, M, L, or vL): L
Fumed nanosilica was assigned a score of **Low** (high confidence) for bioaccumulation based on results from rat studies (Klosterkoetter ,1969 and supporting studies) as well as physico-chemical properties (a low predicted bioconcentration factor based on its octanol-water partition coefficient, Kow) demonstrating low bioavailability. Confidence is high because it is based on data and consistent with the physical-chemical properties of the material.

- Authoritative and Screening Lists**:** None

- One month inhalation study (OECD 413) exposed SD rats orally via gavage to 500 mg/kg bw. The fumed nanosilica had surface area of 150 m^2/g. There was a small increase of silica in liver and kidney. In the same study, other SD rats were exposed via inhalation to 0.05 mg/L for 5 hours. Silica was found in the lungs and lymph nodes. SD rats were also exposed once via a single s.c. injection of 10 mg/animals. After 2 months, 0.298 mg of silica was measured. **The author reports that bioaccumulation is low.** (Klosterkoetter, **1969** via OECD 2015)
- 13-week inhalation study (OECD 413) exposed Wistar rats via inhalation to fumed nanosilica (Cab-O-Sil M5) at doses of 1.3, 5.9 or 31 mg/m^3. The fumed nanosilica had a surface area of 151 m^2/g and

geometric size distribution about 1-120 µm. Particles were only detected in lungs in small amounts one week after the end of the exposure period, on average 0.2 mg in all animals of the 30 mg group and some of the lower exposure groups. (Degussa 1987 via OECD 2015)

- Male and female Charles River rats were fed Cab-O-Sil for 13 weeks ad libitum at 1, 3, or 5% in the diet (estimated 700, 2,100, and 3,500 mg/kg-d). **There was no appreciable accumulation of SiO_2 in the liver, kidney, spleen, blood, or urine compared to controls as measured at 45 and 90 days** (Cabot, 1958 unpublished report as summarized in IUCLID, 2004).
- Cab-O-Sil M5 was administered to groups of 10 male Wistar rats by nose-only inhalation 6h/d, for **5 days** at aerosolized concentrations of 1, 5, or 25 mg/m^3, followed by a 1 or 3 month observation period. **One day after exposure the silicon content in lungs was measured at 43 µg/rat for high-dose rats, and was below the detection limit (15 µg/rat) by 1 month** (TNO, 2003 as summarized in IUCLID, 2004).
- Nanosilica with a CAS 7631-86-9 – which is being used here as a surrogate for the form of nanosilica in this assessment - has a predicted bioconcentration factor (BCF) of 3.62 based on the log K_{ow} of 0.5 (EPISuite 4.1). A substance is considered to be not bioaccumulative if it has a BCF less than 1000. These physico-chemical properties support a low bioaccumulation score.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M or L): L
Fumed nanosilica was assigned a score of **Low** (high confidence) for reactivity based on 2016 MSDS information provided by the manufacturer of Aerosil, Evonik. Metallic and organic compounds, which are combustible or contain combustible impurities, are more likely to cause dust explosions than materials such as silica (OSHA, 2009). Pure suspended amorphous silica is not expected to cause dust explosions.

- Authoritative and Screening Lists**:** none
- Hydrophilic, fumed synthetic amorphous silicas (SiO_2) is not flammable. A dust explosion of pure SiO_2 is not to be expected. (Aerosil website)
- Evonik MSDS (2016) for Aerosil 200 indicates no upper or lower explosivity limit (i.e. "not applicable").

Flammability (F) Score (vH, H, M or L): L
Fumed nanosilica was assigned a score of **Low** for flammability based on 2016 MSDS information from Evonik, the manufacturer of Aerosil 200.

Authoritative and Screening Lists**:** none

- Hydrophilic, fumed synthetic amorphous silica is present in the highest oxidation state and therefore not flammable. (Aerosil website)
- Evonik MSDS (2016) for Aerosil 200 indicates it is not flammable.

References

Akbar, N., Mohamed, T., Whitehead, D., & Azzawi, M. (2011). Biocompatibility of amorphous silica nanoparticles: Size and charge effect on vascular function, in vitro. *Biotechnology and Applied Biochemistry*, *58*(5), 353–62. http://doi.org/10.1002/bab.46

Arts, J. H. E., Muijser, H., Duistermaat, E., Junker, K., & Kuper, C. F. (2007). 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 and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *45*(10), 1856–67. http://doi.org/10.1016/j.fct.2007.04.001

ASASP. (2013). Synthetic Amorphous Silica – ASASP's current interpretation as a nanomaterial. Retrieved February 5, 2016, from http://www.asasp.eu/docs/ASASP Nano Statement 201304.pdf

De Temmerman, P.-J., Van Doren, E., Verleysen, E., Van der Stede, Y., Francisco, M. A. D., & Mast, J. (2012). Quantitative characterization of agglomerates and aggregates of pyrogenic and precipitated amorphous silica nanomaterials by transmission electron microscopy. *Journal of Nanobiotechnology*, *10*, 24. http://doi.org/10.1186/1477-3155-10-24

European Centre for Ecotoxicology and Toxicology of Chemicals. (2006). Synthetic Amorphous Silica, *JACC 051*(7631), -. Retrieved from http://members.ecetoc.org/Documents/Document/JACC 051.pdf

FDA (1973) Teratologic evaluation of FDA 71-48 (Syloid; silica aerogel) in rats. Final report 1574e. Morgareidge K, Food and Drug Research Laboratories, East Orange, New Jersey, USA. Report FDABF-GRAS-127. Washington DC, USA.

FDA (1973) Teratologic evaluation of FDA 71-48 (Syloid; silica aerogel) in mice. Final report 1573e. Morgareidge K, Food and Drug Research Laboratories, East Orange, New Jersey, USA. Report FDABF-GRAS-127. Food and Drug Administration, Washington DC, USA.

FDA (1973) Teratologic evaluation of FDA 71-48 (Syloid; silica aerogel) in rabbits. Final report 1576e. Morgareidge K, Food and Drug Research Laboratories, East Orange, New Jersey, USA. Report FDABF-GRAS-127. Food and Drug Administration, Washington DC, USA.

FDA (1973) Teratologic evaluation of FDA 71-48 (Syloid; silica aerogel) in hamsters. Unpublished report 1575e. Morgareidge K, Food and Drug Research Laboratories, East Orange, New Jersey, USA. Report FDABF-GRAS-127. Food and Drug Administration, Washington DC, USA.

FDA (1974) Mutagenic evaluation of compound FDA 71-48, silica aerogel. Unpublished report 2446 by Litton Bionetics, Kensington, Maryland, USA. Report FDABF-GRAS-311. Food and Drug Administration, Washington DC, USA.

Fruijtier-Pölloth, C. (2012). The toxicological mode of action and the safety of synthetic amorphous silica-a nanostructured material. *Toxicology*, *294*(2-3), 61–79. http://doi.org/10.1016/j.tox.2012.02.001

Gehrke, H., Frühmesser, A., Pelka, J., Esselen, M., Hecht, L. L., Blank, H., … Marko, D. (2013). In vitro toxicity of amorphous silica nanoparticles in human colon carcinoma cells. *Nanotoxicology*, *7*(3), 274–93. http://doi.org/10.3109/17435390.2011.652207

Guichard, Y., Maire, M.-A., Sébillaud, S., Fontana, C., Langlais, C., Micillino, J.-C., … Gaté, L. (2015). Genotoxicity of synthetic amorphous silica nanoparticles in rats following short-term exposure. Part 2: intratracheal instillation and intravenous injection. *Environmental and Molecular Mutagenesis*, *56*(2), 228–44. http://doi.org/10.1002/em.21928

IARC. (1997). *IARC monographs on the evaluation of carcinogenic risks to humans Vol 68: Silica, Some Silicates, Coal Dust and para-Aramid Fibrils*. Lyon, France. Retrieved from http://monographs.iarc.fr/ENG/Monographs/vol68/

Irfan, A., Cauchi, M., Edmands, W., Gooderham, N. J., Njuguna, J., & Zhu, H. (2014). Assessment of temporal dose-toxicity relationship of fumed silica nanoparticle in human lung A549 cells by conventional cytotoxicity and ^{1}H-NMR-based extracellular metabonomic assays. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *138*(2), 354–64. http://doi.org/10.1093/toxsci/kfu009

Ivanov, S., Zhuravsky, S., Yukina, G., Tomson, V., Korolev, D., & Galagudza, M. (2012). In Vivo Toxicity of Intravenously Administered Silica and Silicon Nanoparticles. *Materials*, *5*(12), 1873–1889. http://doi.org/10.3390/ma5101873

Johnston, C. J. (2000). Pulmonary Chemokine and Mutagenic Responses in Rats after Subchronic Inhalation of Amorphous and Crystalline Silica. *Toxicological Sciences*, *56*(2), 405–413. http://doi.org/10.1093/toxsci/56.2.405

Jugdaohsingh, R., Reffitt, D. M., Oldham, C., Day, J. P., Fifield, L. K., Thompson, R. P., & Powell, J. J. (2000). Oligomeric but not monomeric silica prevents aluminum absorption in humans. *The American Journal of Clinical Nutrition*, *71*(4), 944–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10731501

Lee, S.-W., Kim, S.-M., & Choi, J. (2009). Genotoxicity and ecotoxicity assays using the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius to screen the ecological risks of nanoparticle exposure. *Environmental Toxicology and Pharmacology*, *28*(1), 86–91. http://doi.org/10.1016/j.etap.2009.03.001

McCracken, C., Zane, A., Knight, D. a., Dutta, P. K., & Waldman, W. J. (2013). Minimal intestinal epithelial cell toxicity in response to short- and long-term food-relevant inorganic nanoparticle exposure. *Chemical Research in Toxicology*, *26*(10), 1514–1525. http://doi.org/10.1021/tx400231u

MSDS 2016. Aerosil 200 Safety Data Sheet. Evonik. https://www.freemansupply.com/MSDS/Combined/Fillers/Aerosil200.pdf

OSHA. (2009). *Hazard Communication Guidance for Combustible Dusts*. Retrieved from https://www.osha.gov/Publications/3371combustible-dust.pdf

Panas, A., Marquardt, C., Nalcaci, O., Bockhorn, H., Baumann, W., Paur, H.-R., … Weiss, C. (2013). Screening of different metal oxide nanoparticles reveals selective toxicity and inflammatory

potential of silica nanoparticles in lung epithelial cells and macrophages. *Nanotoxicology*, *7*(3), 259–73. http://doi.org/10.3109/17435390.2011.652206

Pisani, C., Gaillard, J.-C., Nouvel, V., Odorico, M., Armengaud, J., & Prat, O. (2015). High-throughput, quantitative assessment of the effects of low-dose silica nanoparticles on lung cells: grasping complex toxicity with a great depth of field. *BMC Genomics*, *16*, 315. http://doi.org/10.1186/s12864-015-1521-5

Sandberg, W. J., Låg, M., Holme, J. A., Friede, B., Gualtieri, M., Kruszewski, M., … Refsnes, M. (2012). Comparison of non-crystalline silica nanoparticles in IL-1β release from macrophages. *Particle and Fibre Toxicology*, *9*, 32. http://doi.org/10.1186/1743-8977-9-32

Scaffold 2014. Review on the toxicity of manufactured nanomaterials applied in the construction sector. Scaffold Public Documents - Ref: Scaffold SPD5. 03/04/2014. Available at http://scaffold.eu-vri.eu/filehandler.ashx?file=13721

Sokolov, S. V, Tschulik, K., Batchelor-McAuley, C., Jurkschat, K., & Compton, R. G. (2015). Reversible or not? Distinguishing agglomeration and aggregation at the nanoscale. *Analytical Chemistry*, *87*(19), 10033–9. http://doi.org/10.1021/acs.analchem.5b02639

Tarantini, A., Huet, S., Jarry, G., Lanceleur, R., Poul, M., Tavares, A., … Fessard, V. (2015). Genotoxicity of synthetic amorphous silica nanoparticles in rats following short-term exposure. Part 1: oral route. *Environmental and Molecular Mutagenesis*, *56*(2), 218–27. http://doi.org/10.1002/em.21935

Tavares, A. M., Louro, H., Antunes, S., Quarré, S., Simar, S., De Temmerman, P.-J., … Silva, M. J. (2014). Genotoxicity evaluation of nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *28*(1), 60–9. http://doi.org/10.1016/j.tiv.2013.06.009

Thomas, L., Gert, R., Douglas, G., LUIGI, C., Francois, R., Peter, G., & Christoph, K. (2012, July 27). Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial." Publications Office of the European Union. http://doi.org/10.2787/63490 (online)

Uboldi, C., Giudetti, G., Broggi, F., Gilliland, D., Ponti, J., & Rossi, F. (2012). Amorphous silica nanoparticles do not induce cytotoxicity, cell transformation or genotoxicity in Balb/3T3 mouse fibroblasts. *Mutation Research*, *745*(1-2), 11–20. http://doi.org/10.1016/j.mrgentox.2011.10.010

Van der Zande, M., Vandebriel, R. J., Groot, M. J., Kramer, E., Herrera Rivera, Z. E., Rasmussen, K., … Bouwmeester, H. (2014). Sub-chronic toxicity study in rats orally exposed to nanostructured silica. *Particle and Fibre Toxicology*, *11*(1), 8. http://doi.org/10.1186/1743-8977-11-8

Warheit DB, Carakostas MC, Kelly DB, Hartsky MA (1991) Four-week inhalation toxicity study with Ludox colloidal silica in rats: Pulmonary cellular responses. Fundam Appl Toxicol 16, 590-601.

Wilson RK, Stevens PM, Lovejoy HB, Bell ZG, Richie RC. 1979. Effects of chronic amorphous silica exposure on sequential pulmonary function. J Occup Med 21, 399-402.

Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima H, Shishido K, Kawai Y, Mayumi T, Tsunoda S-i, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y (2011) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 6, 321-328

Yang, J., Sliva, A., Banerjee, A., Dave, R. N., & Pfeffer, R. (2005). Dry particle coating for improving the flowability of cohesive powders. *Powder Technology*, *158*(1-3), 21–33. http://doi.org/10.1016/j.powtec.2005.04.032

Zhang, H., Dunphy, D. R., Jiang, X., Meng, H., Sun, B., Tarn, D., … Brinker, C. J. (2012). Processing pathway dependence of amorphous silica nanoparticle toxicity: colloidal vs pyrolytic. *Journal of the American Chemical Society*, *134*(38), 15790–804. http://doi.org/10.1021/ja304907c

APPENDIX A: Hazard Benchmark Acronyms

(alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(Cr) Corrosion/ Irritation (Skin/ Eye)

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

Appendix B
Optional Hazard Summary Table

Exposure Route	GreenScreen Hazard Ratings: [*Chemical Name*]																			
	Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
	C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
							single	repeate	single	repeated*										
oral																				
dermal																				
inhalation																				

Appendix C
Modeling Results

Attach:

- **EPISuite Results for Chemical Name (CAS #)**
- **ECOSAR Results for Chemical Name (CAS #)**
- **Other**