MCL Report for:

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

EC Number: 272-697-1

CAS Number: 68909-20-6

Date: August 2024

Contents

Identity of the substance

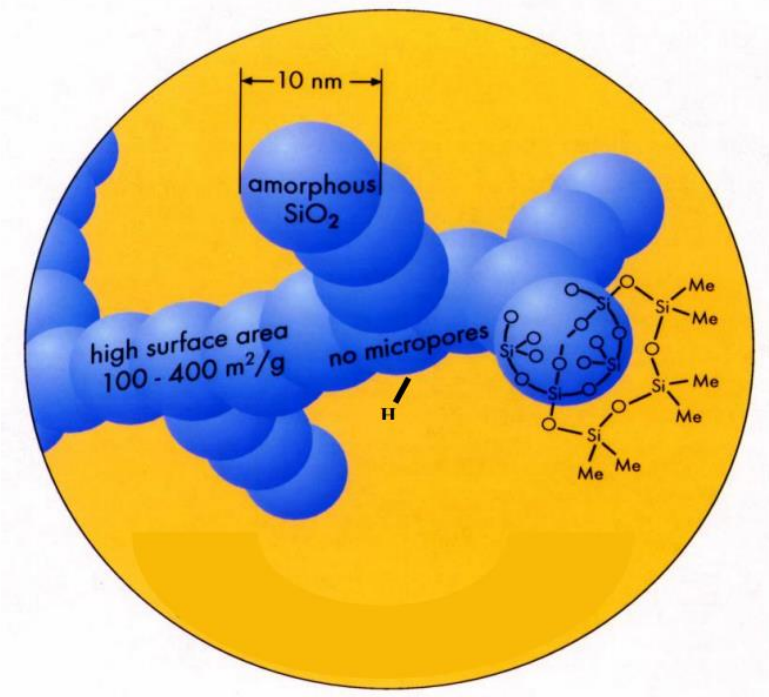
Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide
Other names (usual name, trade name, abbreviation)	surface treated synthetic amorphous silica, surface treated amorphous silicon dioxide Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)- ,hydrolysis products with silica Reaction products of 1,1,1-trimethyl-N-(trimethylsilyl)-silanamine with silica
ISO common name (if available and appropriate)	Pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide
EC number (if available and appropriate)	272-697-1
EC name (if available and appropriate)	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica
CAS number (if available)	68909-20-6
Other identity code (if available)	Aerosil R 812 S Aerosil R 812
Molecular formula	$[SiO_2]_n$-$[OSi(CH_3)_3]_m$ with n>m m corresponds to the surface treatment of silica with methyl groups.
Structural formula	Please see Figure 1 below.
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	Approx 60.08 g/mol (which is the molecular weight of one unit of SiO_2) The surface modification does not significantly affect the molecular weight of the substance which is

	slightly higher than the SiO_2 molecular weight (carbon content actually only represents from 0.6 to 4% w/w).
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	surface treatment of pyrogenic synthetic amorphous silica (nano) with 1,1,1-trimethyl-N-(trimethylsilyl)-silanamine)
Degree of purity (%) (if relevant for the entry in Annex VI)	Purity of silica : ≥ 99.8 % (w/w) for pyrogenic (fumed) silica before and after the surface modification
Primary particle size (TEM)	Experimental data : 6.9-8.6 nm Range covered by this report: 6.9-8.6 nm
Shape of primary particles (TEM)	spherical

Figure 1 - Treated hydrophobic amorphous silica: here with dichlorodimethylsilane (CLH, 2018)



1.1 Composition of the substance

Table 1: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL on GB MCL list (if applicable)
Purity of silica	≥ 99.8 % (w/w) for pyrogenic (fumed) silica before and after the surface modification	STOT RE 2

Table 2: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The impurity contributes to the classification and labelling?
Crystalline silica	<0.1%	**None**	No

2. Proposed mandatory classification and labelling

Table 5: Proposed mandatory classification and labelling according to the GB CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current GB MCL list entry	014-052-00-7	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-,hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide	272-697-1	68909-20-6	STOT RE 2	H373 (lungs, inhalation)	GHS08 Wng	H373 (lungs, inhalation)	EUH066		
GB proposal	014-052-00-7	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-,hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide	272-697-1	68909-20-6	No Additional Classification						
Resulting entry on GB MCL list	014-052-00-7	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-,hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide	272-697-1	68909-20-6	STOT RE 2	H373 (lungs, inhalation)	GHS08 Wng	H373 (lungs, inhalation)	EUH066		

Table 6: Reason for not proposing mandatory classification and status under public consultation

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Explosives	Not assessed in this dossier.	N/A
Flammable gases (including chemically unstable gases)	Not assessed in this dossier.	N/A
Oxidising gases	Not assessed in this dossier.	N/A
Gases under pressure	Not assessed in this dossier.	N/A
Flammable liquids	Not assessed in this dossier.	N/A
Flammable solids	Not assessed in this dossier.	N/A
Self-reactive substances	Not assessed in this dossier.	N/A
Pyrophoric liquids	Not assessed in this dossier.	N/A
Pyrophoric solids	Not assessed in this dossier.	N/A
Self-heating substances	Not assessed in this dossier.	N/A
Substances which in contact with water emit flammable gases	Not assessed in this dossier.	N/A
Oxidising liquids	Not assessed in this dossier.	N/A
Oxidising solids	Not assessed in this dossier.	N/A
Organic peroxides	Not assessed in this dossier.	N/A
Corrosive to metals	Not assessed in this dossier.	N/A
Acute toxicity via oral route	Not assessed in this dossier.	N/A
Acute toxicity via dermal route	Not assessed in this dossier.	N/A
Acute toxicity via inhalation route	***Data conclusive but not sufficient for classification***	Yes
Skin corrosion/irritation	Not assessed in this dossier.	N/A
Serious eye damage/eye irritation	Not assessed in this dossier.	N/A
Respiratory sensitisation	Not assessed in this dossier.	N/A
Skin sensitisation	Not assessed in this dossier.	N/A
Germ cell mutagenicity	Not assessed in this dossier.	N/A
Carcinogenicity	Not assessed in this dossier.	N/A
Reproductive toxicity	Not assessed in this dossier.	N/A
Specific target organ toxicity-single exposure	Not assessed in this dossier.	N/A
Specific target organ toxicity-repeated exposure	Not assessed in this dossier.	N/A
Aspiration hazard	Not assessed in this dossier.	N/A
Hazardous to the aquatic environment	Not assessed in this dossier.	N/A
Hazardous to the ozone layer	Not assessed in this dossier.	N/A

3. History of the classification and labelling

In the EU, an initial CLH dossier for silanamine, 1,1,1-trimethyl-N- (trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (EC: 272-697-1) was submitted by France in December 2018. The dossier submitter proposed a classification of Specific Target Organ Toxicity – Repeat Exposure (STOT-RE) in category 2 for effects on the lung via inhalation, but did not propose a classification for acute toxicity. In December 2019, the EU Committee for risk assessment (RAC) adopted their opinion, with RAC including additional studies from open literature. They considered that the forms of hydrophobic, synthetic amorphous silica (SAS) described were having acute inhalation effects on rats. In addition, a key study with DDS (dichlorodimethylsilane)-treated SAS (Anonymous 1994a) led RAC to conclude that the substance should be classified as Acute Toxicity Category 2 via inhalation, with an ATE of 0.45 mg/L.

During a targeted consultation, stakeholders suggested that the lethality observed was due to suffocation, based on the agglomerative properties of SAS. RAC had previously considered this mechanism, but did not have any reported findings to support this statement. In addition, histopathological findings in the studies were more indicative of acute respiratory distress syndrome, rather than suffocation.

In GB, the RAC Opinion was considered under Article 37 of GB CLP. The conclusion of the GB technical report (HSE, 2021) was to agree with the classification for STOT RE 2 and acute inhalation toxicity proposed by RAC, however the report identified various uncertainties regarding acute inhalation toxicity and noted that the ASASP (Association of Synthetic Amorphous Silica Producers) had commissioned a mechanistic study to address these. The GB Agency Opinion (HSE, 2022) concluded that the GB MCL list should be updated to include STOT RE 2; H373 (lungs; inhalation) and the additional label EUH066, but that the classification for acute inhalation toxicity should not be included in the GB MCL list at that time, pending assessment of the new data.

ASASP subsequently submitted the results of the mechanistic study to the EU Meeting of the Competent Authorities for REACH and CLP (CARACAL), and the EU Commission asked RAC (via an Article 77 (3)(c) Executive Director’s request) to reconsider the classification for acute inhalation toxicity in light of this new information. The new data were subject to a targeted public consultation. Following their reassessment, RAC concluded that no classification for acute inhalation toxicity was warranted (ECHA, 2023).

This GB MCL report assesses the results of the new mechanistic study, plus the information that was considered in the latest RAC Opinion (e.g., results of the targeted public consultation), to determine whether a classification for acute inhalation toxicity is warranted.

4. Justification that action is needed

Silanamine, 1,1,1-trimethyl-N- (trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide has been assessed by the Agency under Article 37 of GB CLP. The Agency opinion (HSE, 2022) stated the following:

“In the case of silanamine, HSE, as the GB CLP Agency, is aware of additional information that may alter the classification of silanamine. This meets the criteria for triggering Article 37A and therefore the acute inhalation toxicity classification requires further assessment under the Article 37A process of the GB CLP Regulation”.

This MCL report (prepared under Article 37A of GB CLP) documents the further assessment of acute inhalation toxicity.

5. Identified uses

According to the EU CLH report (CLH, 2018) “pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide” is approved as an existing active substance for use in biocidal products of product-type 18 (Insecticides, Acaricides and Products to Control Other Arthropods).

The main intended use assessed is the control of fowl-infesting ectoparasites in poultry houses, by professional operators.

6. Data sources

The information used in this report has been sourced from the original CLH report (CLH, 2018), as well as the RAC opinions (ECHA, 2019; 2023) (including Annexes) and minority opinion.

7. Physicochemical properties

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid / form: powder	Degussa SDS 2005b-e, 2006i	
Melting/freezing point	Not relevant, as the substance is an inorganic solid of mineral character with extreme melting point (of SiO2).	Degussa SDS 2005b-e, 2006i	
Boiling point	Not relevant, as the substance is an inorganic solid of mineral character with extreme melting point (of SiO2).	Degussa SDS 2005b-e, 2006i	
Relative density	Density of Bulk material: approx. 50 – 70 g/L density of particles: approx. 2 (20°C)	Degussa SDS 2005b-e, 2006i	measured
Vapour pressure	Not measurable	Degussa SDS 2005b-e, 2006i	
Surface tension	Not applicable / inorganic solid with mineral character	-	
Water solubility	Silica particles are not soluble. They form a suspension of particles in water. Due to the functionalisation of the surface it is expected that functionalised aerosol is not susceptible to hydrolyse in monosilicic acid.	Expert assessment	Statement
Partition coefficient n-octanol/water	Not applicable as the substance is insoluble in water and octanol.	-	
Flash point	Not relevant	-	
Flammability	Not flammable	AQura 2007c	

Property	Value	Reference	Comment (e.g., measured or estimated)
Explosive properties	The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for ignition (silica derivative).	-	
Self-ignition temperature	Not relevant, as the substance is an inorganic solid of mineral character with extreme melting point (of SiO2).	-	
Oxidising properties	The substance is an inorganic, inert solid with mineral character and no oxidising, reactive chemical structures (silica derivative).	-	
Granulometry	See table 8 below		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Not applicable	-	
Viscosity	Not applicable	-	

Substance characteristics

Silica, an alternate name for silicon dioxide (SiO_2), can be found in crystalline or amorphous forms. Both the crystalline and amorphous forms can be of synthetic or natural origin. Synthetic, amorphous silica (SAS) may be prepared through various processes, such as via a ‘wet route’ in a liquid phase, or by flame hydrolysis where fumed (pyrogenic) silicas are produced.

Flame hydrolysis can produce silica droplets that coalesce and when entering colder areas of the flame, will partially solidify. These droplets will collide and merge, forming larger 3-dimensional chain-like aggregates, of around 100-1000 nm. After solidification, aggregates can cluster to form agglomerates that are held together by weak molecular interactions. As a result, the agglomerates are relatively fragile and can be split into aggregates easily. For instance, shear forces may allow for split, before re-agglomeration in the absence of these forces. The shape of these aggregates contain empty space, giving the test material a low bulk density. Due to the low density, the geometric particle size is substantially larger than the aerodynamic diameter (defined as the diameter of a sphere of unit density that has the same gravitational settling velocity in air as the particle in question), the latter being a key determinant of deposition in the respiratory tract of particles with an aerodynamic diameter above ≈ 0.5 µm.

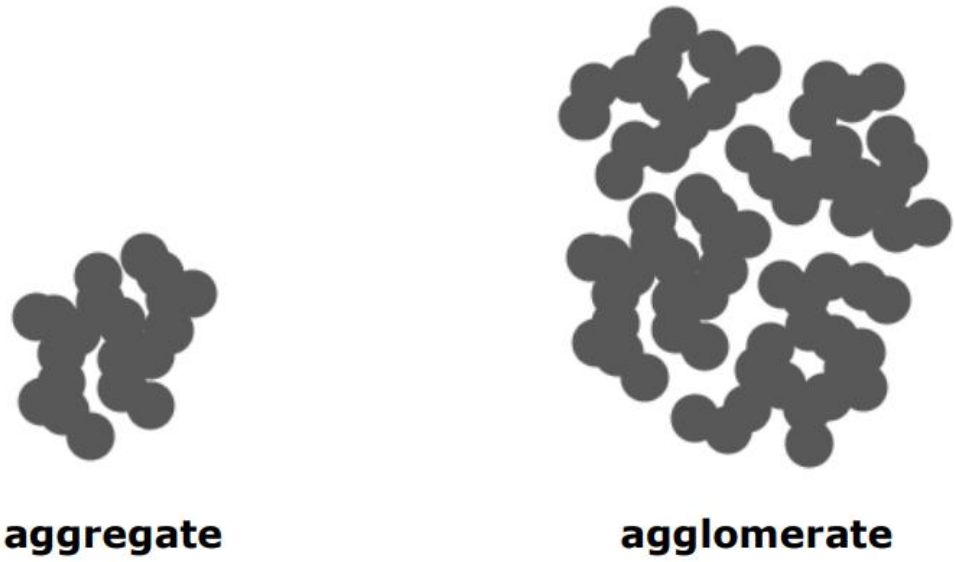
The different amorphous silicas produced by these methods each have differing surface chemistry. For instance, colloidal silica, precipitated silica, and silica gel (produced by 'wet route processes') have high hydrophilicity, because of a higher density of silanol groups on their surface. In comparison, fumed silica has a lower density of these silanol groups owing to dehydration of siloxane bonds; this results in a lower hydrophilicity. Although pyrophoric SAS has a less hydrophilic surface compared with silicas produced via wet processes, the hydrophobicity of fumed silicas can be increased by treatment with reagents such as hexamethyldisilazane (HMDZ), dichlorodimethylsilane (DDS) or polydimethylsiloxane (PDMS). The hydrophobicity of a substance can be determined by its contact angle with water when placed upon the test material, creating a tangent between the liquid surface and solid surface. The surface chemistries of HMDZ-, DDS-, and PDMS-treated SAS were found to be similar.

The substance in scope of this assessment (i.e. silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (EC: 272-697-1; CAS: 68909-20-6)) is the result of the reaction of synthetic amorphous silica treated with hexamethylsilazane (HMDS), leading to a nano-form of silica. The substance is clearly defined as a nanomaterial and due to its similarities with other (DDS- and PDMS treated SAS) forms of silica, the Agency considers that read across is acceptable.

Particle size distribution:

Silica is produced as very small particles called primary particles that have the potential to aggregate. Aggregates are particles comprising of strongly bound or fused particles. Under conditions of normal handling and use, it is considered that aggregates are the smallest stable particles. These aggregates can form agglomerates (see Figure 2 below, taken from RAC opinion, ECHA 2023).

Figure 2: Diagram of aggregates and agglomerates.



Different studies were submitted to the DS on different shear forces to characterize the active substances. Two products (AEROSIL® R812S and Indispron® D110) were analysed; AEROSIL® R812S is a HMDS-treated fumed silica, whereas Indispron® D110 is a ready-to-use aqueous dispersion containing 3% (w/v) of silanamine. The curves were submitted but raw data were not submitted on each volume fraction.

Table 8: Particle size distribution of silicon dioxide surface treated under different conditions (CLH, 2018)

Volume fraction (%)	d_{10}	d_{50}	d_{90}	Proportion < 10 µm
AEROSIL® R812S (Dynamic light scattering)				
20101222-001-6 , Perlet 2011 ***high shear force in ethanol, 1 batch***	95 nm	150 nm	190 nm	100%
A060009416 AQura 2006 ***Dispersion in ethanol, 1 batch***	3.7 µm	11.2 µm	25.7 µm	45%
AN-ASB 0638, anonymous 2014 ***In air stream, 5 batches***	5.1 µm	13.6 µm	32 µm	38%

Indispron® D110 (Dynamic light scattering)				
in 50/50 ethanol/water dispersion	15.9 µm	53.6 µm	93 µm	7%

The DS considered in the initial CLH proposal (CLH, 2018) that the AQura 2006 and test report AN-ASB 0638 (2014) studies measure agglomerate form (d_{50} around 10-15 µm) while Perlet 2011 measure aggregate form (d_{50} around 150 nm) of Aerosil R812S. This last value can be confirmed by TEM pictures demonstrating packs of 100-200 nm aggregates linked together with small chains of primary particles.

When the active substance was formulated in Indispron D110, the measured size of particles increased. Additional microscopy data were submitted to confirm the particle size distribution in Indispron D110. No data were submitted on particle size distribution of biocidal product under shear force to clarify if this particle size distribution changes when the biocidal product is sprayed.

Specific surface area:

Specific surface area was tested on Aerosil R812S using BET method. The range on 5 batches was found to be 217-225 m^2/g. These values can be converted to volume-specific surface area using absolute density of silicon dioxide 2.229 as given in Handbook of chemistry and physics (D. R. Lide 2005-2006): Range of volume specific surface area on 5 batches: 483-501 m^2/m^3 .

8. Evaluation of physical hazards

Not assessed.

9. Toxicokinetics (absorption, metabolism, distribution and elimination)

There is no experimental information regarding the toxicokinetic profile of silanamine. No systemic effects have been reported in toxicity studies, which may be due to a lack of systemic absorption.

10. Evaluation of health hazards

10.1 Acute toxicity – oral route

Not assessed.

10.2 Acute toxicity – dermal route

Not assessed.

10.3 Acute toxicity – inhalation route

Table 9: Summary of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Concentrations levels, duration of exposure**	**Value** **LC_{50}**	**Effects**	**Reference**
Acute inhalation toxicity study, nose only exposure OECD TG 436, with additional investigations GLP Additional investigations included histopathological examination	Rat, Wistar (Crl:WI (Han) Male and female 3/sex	Aerosil R812 (HMDZ-treated SAS) MMAD ± GSD : 0.50 ± 1.31 µm	517 mg/m^3 (0.517 mg/L) 3 hours 12 minutes, shortened from 4 hours owing to mortalities	**Not calculated**	100% mortality Reduced respiratory rate and respiratory distress Partial or full blockage of the nasal cavities	Anonymous 2022; also referred to as Krueger *et al*., 2022
Acute inhalation toxicity study, whole body exposure No guideline or GLP information available	Rat, species not detailed Male and female 5/sex	Cabot Cab-O-Sil N70TS (PDMS-treated SAS) MMAD 0.36 µm measured	4900 mg/m^3 4 hours	**Not calculated in this study, but in follow up studies with the same**	100% mortality Histopathology findings: acute diffuse foreign body bronchitis, bronchiolitis and alveolitis (severe), and	Anonymous 1981a; Anonymous 1982a (pathology report)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Concentrations levels, duration of exposure**	**Value** **LC_{50}**	**Effects**	**Reference**
Only males examined during histopathology		via cascade impactor		**materials LC_{50} was 0.5 mg/L (500 mg/m^3)**	multifocal alveolar emphysema (mostly moderate)	
Acute inhalation toxicity study, nose-only exposure No guideline or GLP information available	Rat, species not detailed 5/sex/dose, with exception of 1120 mg/m^3 group at 7/sex	Cab-O-Sil TS610 (DDS-treated SAS) MMAD (measured at 1120 mg/m^3 by aerodynamic particle sizer) : 0.8 µm	520, 1120 and 2790 mg/m^3 4 hours	**Between 520-1120 mg/m^3**	Mortality: 0/10, 14/14, 10/10 for each dose group respectively Haemorrhagic lungs of reduced elasticity, white powder in nasal cavity Histopathology: alveolar erythrocytes, eosinophilic oedema, bronchiolar lumina with several nucleated cells and eosinophilic material, slight bronchiolar epithelial erosion.	Anonymous 2000
Acute inhalation toxicity study, nose only exposure No guideline or GLP information available	Rat, SD 5/sex/dose	HDK SKS 300VI (SAS treated with vinyl-modified HMDZ) MMAD: 6.9 µm, measured	400, 700 and 2000 mg/m^3 4 hours	**500 mg/m^3**	Mortality: 2/10, 10/10 and 10/10 for each dose group respectively Red discoloration of lungs	Anonymous 1996c

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Concentrations levels, duration of exposure	Value LC_{50}	Effects	Reference
Additional investigations of lung silica content		by cascade impactor			Histopathology: Alveolar haemorrhage, interstitial oedema, inflammation Lung silica content increased with concentration, ranging from 4.0 mg/lung to 12.7 mg/lung.	

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

As detailed in the justification for action, new information has become available in the assessment for silanamine. An acute inhalation study (Anonymous 2022; Krueger *et al.* 2022) was submitted by industry with the aim of assessing the potential mode of action by which the deaths were caused.

In addition to new data, twelve acute inhalation toxicity studies were identified in the re-assessment conducted by RAC. Details of these studies can be found in Appendix I of the RAC re-assessment. Briefly, these studies used a range of DDS-, HMDZ- and PDMS-treated SAS. Mortality was observed in all studies with the exception of Anonymous (1983) and Anonymous (1982c). Clinical signs observed included respiratory distress, irregular or laboured breathing. As the purpose of this report is to assess the potential mode of action of lethality, the most relevant studies from the existing twelve were considered to be those with additional histopathological examination of the respiratory tract. Based on this, three previous studies are considered relevant in this re-assessment: Anonymous (1981a), Anonymous (2000) and Anonymous (1996c).

Anonymous (2022); Krueger *et al.* (2022)

In a guideline (OECD TG 436) and GLP acute inhalation toxicity study (Anonymous 2022; Krueger *et al.,* 2022), Wistar rats (3/sex) were exposed nose-only to Aerosil R812 at a mean concentration of 517 mg/m^3 (0.517 mg/L). Aerosil R812 is a form of HMDZ-treated

SAS, which had an MMAD ± GSD of 0.50 ± 1.31 µm (cascade impactor). The intended exposure duration was set at 4 hours, however, this was shortened to 3 hours, 12 minutes due to mortality.

Within the exposure period, 2 males and 1 female were found dead, each with reduced respiratory rate prior to death. The remaining 3 animals post exposure also presented with signs of respiratory distress, with 1 male and 1 female dying at 4 hours and 10 minutes, and the remaining female killed in a moribund condition. The study measured blood oxygen level in the two females that survived after exposure termination; the saturations were 75% and 124% approximately 1 hour post-exposure. No further testing at 0.05 mg/L was carried out, which is a recommendation when 100% mortality is observed at 0.5 mg/L. The justification to cease testing was that the study was not designed for determination of an LC_{50}, but instead for proving a hypothesis that the substance blocks the airways. For animal welfare reasons, further testing may have been inappropriate. However, RAC noted that testing additional concentrations may have proved useful in determining further information about the mode of action.

All animals were necropsied ~10 minutes after death. Animals were found to have dark red lungs, with some foamy contents in the trachea. The study authors noted that lung weights exceeded historical control means (no further details). Macroscopic changes were described as congestion, oedema, acute emphysema and petechiae, whilst histopathology examination showed alveolar haemorrhage, alveolar fibrin, acute emphysema and alveolar macrophages in most or all animals, with severity ranging from minimal to slight. No histopathology findings were identified in other tissues, with the exception of thymus haemorrhages, representing petechiae.

Examination of the nasal cavity of animals which died during exposure was carried out, with sections frozen, dried, sectioned and examined by digital microscopy. Upon examination, 1 animal had complete blockage of the nasal cavity, 1 had partial blockage, and another presented with multisite deposition but no obstruction. Large accumulations of silicon were detected in all 3 animals via scanning electron microscopy/energy dispersive X-ray analysis.

The remaining animals (post-exposure deaths) also underwent nasal cavity examination, with a different method of freezing, fixation in ethanol, before being embedded in methylmethacrylate resin prior to sawing. Silicon was not characterised by the energy dispersive X-ray analysis, likely because the resin prevented detection; however, partial or total blockage of the nasal cavities was observed through digital microscopy. RAC questioned this method in the RAC-65 CLH working group discussion. Industry clarified that this method was used to avoid the removal of substance deposits during fixation of tissues, as usual methods using formalin might have led to dissolution or removal of material. Industry suggested that the fixation process with ethanol may have partially

washed or dissolved the test material during the embedding process. RAC was unclear on why a liquid fixative was used, but noted the clarification.

A summary of the deposition of the test substance (RAC, 2023) can be found in table 10 below:

Table 10: Deposition of the test substance in the nasal cavity in Anonymous (2022), adapted from ECHA (2023)

Anonymous (2022): deposition of test substance in nasal cavities (as documented by photographs in the study report and in the additional information dated 16 May 2023)				
Animal no.	**Mode and time of death**	**Method of fixation of nasal cavity**	**Deposits in nasal cavity**	
			Level 3	**Level 4**
1101	Found dead after exposure	Embedded in resin	Partial blockage	
1102	Found dead during exposure	Frozen and dried		Partial blockage
1103	Found dead during exposure	Frozen and dried	Multifocal deposition, minimal	
1201	Found dead after exposure	Embedded in resin	Full or partial blockage	
1202	Found dead during exposure	Frozen and dried		Almost complete blockage
1203	Killed moribund after exposure	Embedded in resin	Partial blockage	

Industry also noted that experiments of the same design, with the same concentration (500 mg/m^3), and with 6 other test items (i.e. particles) were performed in parallel to Anonymous (2022). Lung histopathology findings included increased presence of reactive alveolar histiocytes, macrophage type II proliferation, and mixed inflammatory infiltrate or interstitial inflammation in 5/7 test materials.

The study by Anonymous (2022) demonstrated partial or almost complete obstruction of the nasal cavity (5/6 animals), with 5/6 animals dying as a result of exposure and 1 animal killed in a moribund state. No significant deposits were detected in the larynx, trachea or lungs. Lung histopathology revealed haemorrhages, focal emphysema and alveolar macrophages (severity grade 1). These are considered to be typical findings of oro-nasal occlusion, with some minor inflammation observed. As rats are obligate nasal breathers, it is likely that the mortalities observed were due to obstruction, causing suffocation via deposited test material in the nasal cavity.

Anonymous (1981a)

In a study by Anonymous (1981a), rats (5/sex) were exposed whole-body to PDMS-treated SAS at a concentration of 4900 mg/m^3 (MMAD 0.36 µm, cascade impactor). This study was designed as a limit test, with a target concentration of 5 mg/L. The reported mortality was 100% during exposure, with lungs failing to collapse on necropsy. Histopathological examination was carried out on all males, as reported in an addendum to another study report (Anonymous 1982a). Histopathology revealed the following main findings in all animals: severe acute diffuse foreign body bronchitis, bronchiolitis and alveolitis, as well as mostly moderate multifocal alveolar emphysema. The pathologist report describes that the deaths were a result of foreign body reactions in the lung. The test material was able to reach the bronchi (as observed by staining), cause complete occlusion of some bronchioles and in some cases, was found in alveoli spaces. The blockage and irritation of the respiratory tree described is interpreted by the pathologist as a result of the physical presence of the material rather than a toxic effect of the substance. Overall, the test material was able to cause serious effects at this concentration. However, it should be noted that compared to other studies with similar effects and mortality, the relevance of these findings may be questionable due to the high test concentration.

Anonymous (2000)

In a study by Anonymous (2000), rats (5/sex/group with exception of 1120 mg/m^3 group, 7/sex) were exposed nose-only to DDS-treated SAS at concentrations of 520, 1120 and 2790 mg/m^3 for 4 hours. It is noted that the MMAD was measured by an aerodynamic particle sizer at 1120 mg/m^3 (around 0.8µm throughout exposure) rather than a cascade impactor, as the cascade impactor resulted in all test material collecting in the back filter, leading to an MMAD below 0.1 µm.

Deaths were observed in 0/10, 14/14 and 10/10 rats at each concentration, respectively. Histopathological examination was only carried out on the 1120 mg/m^3 concentration group, in which 13 of the animals died during exposure and 1 animal was killed in extremis post-exposure. The Agency notes that the concentration group chosen for examination is higher than the guideline study by Anonymous (2022) and just above the LC_{50} (0.45 mg/L) set previously by RAC. Gross examination of the lung showed reduced elasticity and pulmonary haemorrhages, as well as the presence of white powder in the nasal cavity.

Two males and two females underwent histopathological examination of the nasal cavity, larynx, trachea and lungs (no rationale on how these particular animals were selected for examination was given). Findings in the nasal cavity included luminal, slight pale eosinophilic material, erythrocytes, and few nucleated cells (2/4 animals). The larynx featured a luminal plug of pale eosinophilic material, few nucleated cells and erythrocytes in 3 of 4 animals. One animal was found with epiglottal epithelial erosion. Lung findings in all animals included intra-alveolar erythrocytes (slight to severe), bronchiolar lumina with several nucleated cells and little eosinophilic material (2/4 with focal occlusion).

Eosinophilic oedema (predominantly perivascular) and slight bronchiolar epithelial erosion with alternating areas of flattened epithelium were observed in 3/4 animals.

Overall, the effects observed in this study were indicative of occlusion within the airways when the test material was administered under these conditions. The histopathology findings indicated some involvement of inflammatory processes, but it is likely that the occlusion was responsible for the deaths.

Anonymous (1996c)

In a study by Anonymous (1996c), rats (5/sex/group) were exposed nose-only to a SAS surface treated with vinyl-modified HMDZ, at concentrations of 400, 700 and 2000 mg/m^3 (MMAD 6.9µm, cascade impactor) for 4 hours. The test material featured < 1% vinyl function in HMDZ, which may have impacted the physico-chemical properties of the material; namely, agglomeration tendency. As a result, this study was considered by RAC to be supporting information.

Mortality was reported in 2/10, 10/10 and 10/10 animals at each concentration, respectively. Gross pathology showed red discoloration of the lungs. Histopathological examination was carried out on 5 animals from each group (2/3 of each sex), with remaining animals used in determination of silica content in lung homogenate.

Histopathology findings were similar across all groups, including alveolar haemorrhage (mostly mild), interstitial oedema (trace to moderate) and inflammation (mostly mild). Severity of these lung findings were considered to be low overall. No significant differences were observed between those animals that died during exposure and those which died post-exposure. The test substance was not observed in the lungs when undergoing standard histopathology examination, despite a confirmed presence by chemical analysis. No examination of other constituents of the respiratory tract were performed. The histopathology findings are summarised in table 11 below, and are considered to be inconclusive with regards to mortality.

Table 11: Lung histopathology and silica content results from Anonymous (1996c), adapted from RAC (2023)

Anonymous (1996c): lung histopathology and silica content			
Concentration (mg/m³)	**400**	**700**	**2000**
Number of examined animals that died as a result of exposure	2	5	5
Congestion, multifocal to diffuse	2 (1 +, 1 ++)	5 (1 +, 3 ++, 1 +++)	5 (1 +, 4 ++)
Haemorrhage, alveoli, multifocal to diffuse	2 (2 ++)	5 (1 +, 4 ++)	5 (2 +, 3 ++)
Oedema, alveoli, interstitial, multifocal to diffuse	2 (1 ++, 1 +++)	5 (3 +, 2 ++)	5 (5 +)
Vasculitis, acute, focal to multifocal	1 (1 ++)	5 (3 +, 2 ++)	3 (3 ++)
Inflammation, acute, alveoli, interstitial, diffuse	2 (2 ++)	2 (2 +)	0
Number of animals examined for silica content in the lungs	5	5	5
Mean silica content (mg/lung)	4.0	8.9	12.7

Severity: +, trace; ++, mild; +++, moderate; ++++, severe

The content of silica in the lungs increased in a concentration-dependent manner; control animals ranged between 0.3 and 1.6 mg/lung, whereas silica content was 4.0 mg/lung in the 400 mg/m^3 group and 12.7 mg/lung in the 2000 mg/m^3 group. The MMAD was measured as 6.9µm by cascade impactor, with an expectation that the actual MMAD may have been higher. RAC estimated that approximately 10% of the inhaled dose was deposited in the lungs, which is comparable to typical lung deposition fractions for a particle size of 1 and 4 µm. A substantial pulmonary deposition of surface-modified SAS may occur at MMADs above 4 µm.

Agency discussion of potential mode of action

HMDZ-treated SAS and its read-across substances have been previously assessed across oral and dermal acute toxicity, each showing low potential for toxicity. In addition, there is limited evidence of skin or eye irritation. No changes to non-respiratory tissues were observed in the histopathology analysis carried out in Anonymous (2022).

The respiratory system is usually affected by either obstruction, or inflammation. The study by Anonymous (2022) seems to indicate obstruction of the nasal cavity, yet other studies (Anonymous 2000) report obstruction of the airways, along with some lung inflammation. As speculated by RAC, this may be due to the smaller particle size used in Anonymous (2000). Despite the lung inflammation observed, the Agency considers that it is likely that obstruction drives lethality. As rats are nasal obligate breathers, whereas humans are not, the mode of action appropriate for human relevance is limited to obstruction of the upper and lower airways.

As mentioned above, the deposition pattern of particles within the airways is affected by the particle size. OECD guidelines for this test (OECD TG 436) recommend a MMAD of 1-4 µm with a geometric standard deviation of 1.5 to 3. This size is likely to deposit within all regions of the rat respiratory tract. When generating aerosols of this size, it is recommended by the CLP guidance (3.1.2.3.2) that the aerosol is fine enough to avoid overloading the airways of rats.

Breathing patterns and respiratory structures of rats differ from humans. Rats have complicated nasal turbinates which deviate into various chambers, with these branches allowing for a greater, more efficient surface area for exchange. However, the complexity of this pathway means there is higher likelihood for clogging. In comparison, humans have a relatively simple nasal turbinate structure, as well as the ability to utilise nasal and oral breathing. Obstruction in rodents may not translate to obstruction in humans.

OECD Guidance Document (39) suggests that at high concentrations, dry powder aerosols tend to form conglomerates which lead to physical obstruction of the airways, alongside impaired respiration. This is consistent with the effects observed in SAS, and implies that occlusion of the nasal cavity is not expected when the particle size distribution is met.

RAC noted that for hydrophobic, surface-treated fumed silica this may be an exception, as even in OECD compliant atmospheres, obstruction of the upper airways occurred. SAS may differ because of the low density of the test material (0.05 g/cm^3), where the deposition pattern could differ when compared to materials with density of 1g/cm^3.

Hydrophobicity may also impact the deposition in airways. RAC noted that another study (Hofmann *et al.* 2018) tested various organic pigments for acute inhalation toxicity in rats at a limit concentration of 5mg/L. Three substances led to 100% mortality, with deposition in various regions of the respiratory tract, and histopathology findings of lung obstruction and diffuse emphysema. The substances which led to mortality correlated with the pigments which were the most hydrophobic. In the case of fumed silicas, a combination of hydrophobicity, low solubility and low density may block the upper airways of rodents. Owing to the differences in respiration between rats and humans, obstruction of the nasal cavity is less relevant to humans.

In the case of the lower airways (bronchi, bronchioles and alveoli), there is evidence of substance deposition. Rats have a higher filtering capacity for particles compared to human nasal passages, meaning that the fraction which reaches post-nasal regions is much lower compared to humans. Studies on human filtration rates of nasal vs oral breathing suggest that there is a lower capacity for filtration in the nose, and higher particle deposition in the lower airways when oral breathing (Heyder *et al.,* 1986). However, human airways are larger than rats, making lungs more resistant to overload of deposited material. Industry also highlighted the difference in tracheobronchial branching pattern (monopodial in rats vs dichotomous in humans), which may lead to different deposition patterns. However, RAC considered that it is not obvious whether this could modify the level of concern, but noted that it is plausible that deposited material in the larynx could be removed by coughing. The Agency considers that interspecies differences are not sufficient in dismissing relevance of the lower airways to humans.

10.3.2 Comparison with the GB CLP criteria

The previous classification was based on a LC_{50} corresponding to Category 2 (0.05 mg/L < ATE ≤ 0.5 mg/L). However, when considering the study from Anonymous (2022), there is evidence of suffocation and obstruction being the cause of death. This study has some limitations; for instance, testing of a single concentration means any possible dose response relationship cannot be identified. In addition, it is unclear whether the study by Anonymous (1994a), which was initially used for classification by RAC, has the same mode of action (i.e. suffocation). Despite this, other relevant studies (Anonymous 1981a, Anonymous 2000), indicated that obstruction of the nose, airways and inflammation were involved in the deaths.

Industry comments noted that the obstruction of airways with test material is not an intrinsic property of SAS, but a physical effect that can be expected for materials with similar density, hydrophobicity and particle size. The available information indicates that HMDZ-treated SAS can cause obstruction and death which are related to the physico-chemical properties, rather than an inherently toxic effect.

Industry also pointed out that in the CLP Regulation, classification information should relate to the forms or physical states that the substance is placed on the market, and for which it should be expected to be used. HMDZ-treated SAS is used commercially as a non-respirable agglomerate, with a reported typical MMAD of 80 µm. Significant shear stress is required to break down the agglomerates into the respirable range of 10 µm. The CLP guidance (section 1.2.3.2) recommends that normally, testing should be performed on the smallest available particle size, although "*in some cases, substances or mixtures have to be transformed into specific forms not mirroring 'real-life' exposure in order that an animal test can be performed*". In this case, the exposure of the studies does not necessarily reflect real conditions. However, the products containing HMDZ-treated SAS

can vary in particle size, as well as variance in how MMAD is measured. For example, the study by Anonymous (1996c) on pyrogenic silica surface-treated with vinyl-modified HMDZ had an MMAD of 7 µm, as measured by cascade impactor. As a result, the particle size distribution of how products are used is not considered to be relevant to classification.

Based on a weight of evidence assessment, the Agency concludes that it is likely that suffocation was the cause of death. As this results from a physical property of the substance, no classification is warranted.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification warranted for acute inhalation toxicity- data conclusive but not sufficient for classification.

10.4 Specific target organ toxicity – single exposure (STOT SE)

Not assessed.

10.5 Skin corrosion/irritation

Not assessed.

10.6 Serious eye damage/eye irritation

Not assessed.

10.7 Respiratory sensitisation

Not assessed.

10.8 Skin sensitisation

Not assessed.

10.9 Specific target organ toxicity – repeated exposure (STOT RE).

Not assessed.

10.10 Germ cell mutagenicity

Not assessed.

10.11 Carcinogenicity

Not assessed.

10.12 Reproductive toxicity

Not assessed.

10.13 Aspiration hazard

Not assessed.

11. Evaluation of environmental hazards

Not assessed.

12. Evaluation of additional hazards

Not assessed.

13. Additional labelling

Not applicable.

14. References

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15. Glossary

CLH – Classification and Labelling

DS – Dossier Submitter

DDS – dichlorodimethylsilane

GLP – Good Laboratory Practice

GSD – geometric standard deviation

HMDZ – hexamethyldisilazane

MMAD – mass median aerodynamic diameter

PDMS – polydimethylsiloxane

RAC – Risk Assessment Committee

SAS – synthetic amorphous silica

STOT-RE – Specific Target Organ Toxicity – Repeated Exposure

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